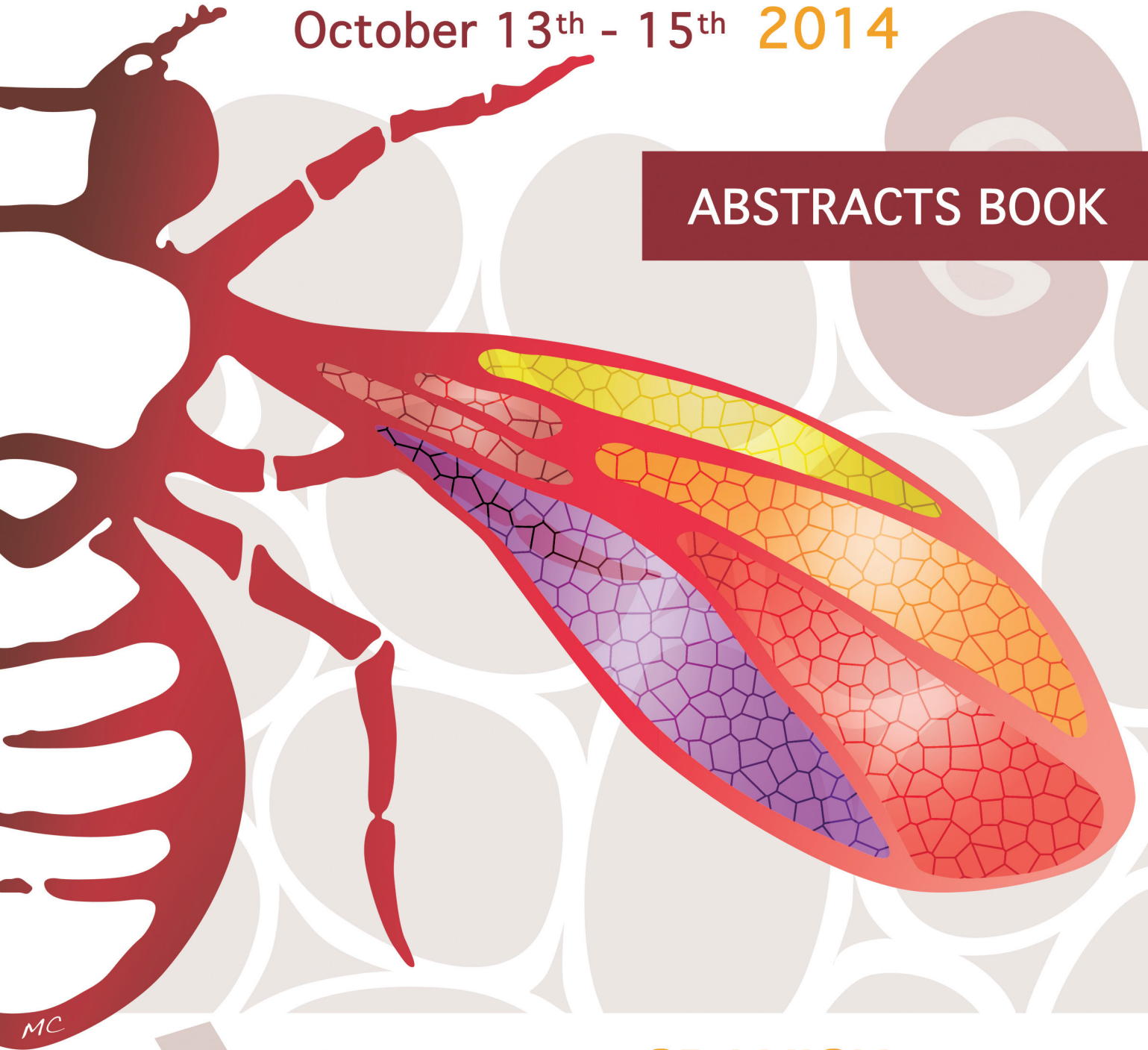


MADRID

October 13th - 15th 2014

ABSTRACTS BOOK



MEETING

SPANISH
SOCIETY FOR
DEVELOPMENTAL
BIOLOGY



Venue:

Hotel Rafael Atocha

www.sebd2014.com

Technical Secretariat:



C/ Londres, 17 - 1º - 28028 Madrid
Telf.: +34 91 361 2600 - Fax: +34 91 355 9208
E-mail: sebd2014@kenes.com

COMMITTEES

SEBD BOARD OF DIRECTORS

President: Ángela Nieto. *IN. Alicante*

Secretary: Miguel Manzanares. *CNIC. Madrid*

Treasurer: Paola Bovolenta. *CBMSO. Madrid*

Members: Amelia Aránega. *University of Jaén*
James C-G Hombría. *CABD. Sevilla*
Jordi García-Fernández. *UB, Barcelona*
Acaimo González-Reyes. *CABD. Sevilla*
María Ángeles Ros. *IBBTEC. Santander*

LOCAL ORGANIZER COMMITTEE

Miguel Manzanares. *CNIC. Madrid*

Paola Bovolenta. *CBM. Madrid*

Amelia Aránega. *University of Jaén*

Solveig Thorsteinsdattir. *University of Lisbon. Portugal*

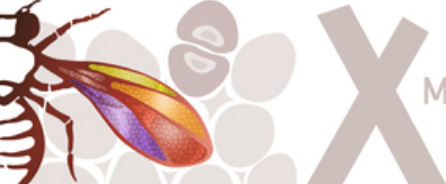
SPONSORS

Fundación **BBVA**



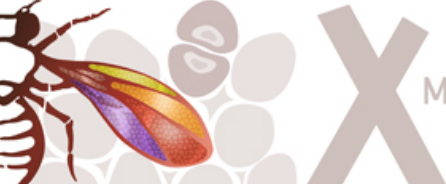


Poster Sessions



1. Morphogenesis & Organogenesis

- WNT/B-CATENIN PATHWAY CONTROLS DENTAL DEVELOPMENT AT ALL STAGES OF ODONTOGENESIS AND IS RELATED TO EPIPROFIN/SP6 TRANSCRIPTION FACTOR** 20
Maitane Aurrekoetxea¹, Igor Irastorza¹, Gaskon Ibarretxe¹, Fernando Unda¹
University Of The Basque Country, UPV/EHU
- COORDINATION OF MUSCLES AND PIGMENT CELL DURING THE DEVELOPMENT OF THE MALE REPRODUCTIVE SYSTEM OF DROSOPHILA MELANOGASTER.** 21
Inés Olivera Crego¹
CBMSO
- BIOPHYSICAL MECHANISMS DRIVING CELL SHAPE PULSATIONS DURING MORPHOGENESIS** 22
Angughali Sumi¹, Kai Dierkes¹, Guillaume Salbreux², Jerome Solon¹
*Centre For Genomic Regulation
MPI for the Physics of Complex Systems, Dresden*
- DLK 1 REGULATES BRANCHING MORPHOGENESIS AND PARASYMPATHETIC STIMULATION OF THE SALIVARY GLAND THROUGH INHIBITION OF NOTCH SIGNALING** 23
Patricia Garcia-Gallastegui¹, Jose Javier Garcia-Ramirez², Victor Baladron², Jorge Laborda², Gakon Ibarretxe¹, Fernando Unda¹
*Faculty of Medicine and Dentistry. University of the Basque Country, UPV/EHU. Bizkaia. Spain
Regional Center for Biomedical Research. University of Castilla-La Mancha. Albacete. Spain*
- EXPRESSION OR NOT DPP TWO DIFFERENT POPULATIONS OF GROWING CELLS IN DROSOPHILA** 24
Ana Macias¹, Carolina Arias¹, Gimena Fussero¹, Marcelo Zacharonok¹
FCEFYU-UNC
- PERIPODIAL EPITHELIUM EXPRESSION OF THE IROQUOIS COMPLEX GENES IS REQUIRED FOR VENTRAL ADULT HEAD MORPHOGENESIS** 25
Esther González-Pérez¹, Sonsoles Campuzano¹
Centro de Biología Molecular Severo Ochoa / CSIC-UAM
- COORDINATION OF PATTERNING AND MORPHOGENESIS DURING OPTIC CUP FOLDING.** 26
Florencia Cavodeassi¹, Sergio Salguero, Mario Ledesma, Paola Bovolenta
Centro De Biología Molecular Severo Ochoa, Madrid, Spain
- RECIPROCAL REPRESSION BETWEEN PAX2 AND SNAIL CONTROLS EPITHELIAL PLASTICITY DURING EMBRYONIC DEVELOPMENT** 27
Oscar Ocaña¹, Juan Manuel Fons¹, Hakan Coskun¹, Diana Abad¹, Maria Angela Nieto¹
Instituto De Neurociencias CSIC-UMH
- A COMBINATORIAL CODE OF MORPHOGENETIC SIGNALS CONTROLS NASO-TEMPORAL PATTERNING IN THE VERTEBRATE RETINA** 28
Maria Hernández-Bejarano¹, Alexander Picker³, Paola Bovolenta¹, Stephen W. Wilson², Gaia Gestri², Florencia Cavodeassi¹
Centro De Biología Molecular Severo Ochoa, Madrid, Spain



POSTER SESSION I

Page

University College London, London, UK
LIFE Biosystems GmbH, Heidelberg, Germany

THE BHLH TRANSCRIPTION FACTOR DYSFUSION REGULATES TARSAL JOINT FORMATION IN RESPONSE TO NOTCH ACTIVITY DURING DROSOPHILA LEG DEVELOPMENT

Sergio Córdoba¹, Carlos Estella¹

Centro De Biología Molecular Severo Ochoa, Universidad Autónoma De Madrid (UAM)

29

PITX2B ISOFORM IS DYNAMICALLY EXPRESSED DURING HEART DEVELOPMENT

Francisco Hernandez-Torres¹, Diego Franco¹, Francisco Navarro¹, Amelia E. Aranega

Department of Experimental Biology, Faculty of Experimental Sciences, University of Jaén

30

REQUIREMENT OF FOI DURING DROSOPHILA MYOGENESIS

Marta Carrasco-Rando¹, Alexandra Atienza-Manuel¹, Paloma Martín¹, Mar Ruiz-Gómez¹

Centro De Biología Molecular Severo Ochoa

31

A ROLE FOR NOTCH IN TROPHECTODERM SPECIFICATION IN THE EARLY MOUSE EMBRYO

Sergio Menchero¹, Teresa Rayon¹, Anna-Katerina Hadjantonakis², Jose Luis de la Pompa¹, Miguel Manzanares¹

Centro Nacional De Investigaciones Cardiovasculares, Madrid, Spain

Sloan-Kettering Institute, New York, USA

32

THE ABSENCE OF MYF5 AND MYOD IN EPAXIAL MUSCLE PROGENITORS CAUSES VERTEBRAL COLUMN CURVATURE ABNORMALITIES

Macarena López-Mayorga¹, Natalia Moncaut², Lydia Teboul³, Rosa M. Giráldez-Pérez¹, Cristina R. Bernal-Lozano¹, Ana M. Castro-Cañal¹, Peter W.J. Rigby², Jaime J. Carvajal¹

Centro Andaluz de Biología del Desarrollo, Seville, Spain

The Institute of Cancer Research, London, UK

MRC Harwell, Oxfordshire, UK

33

SPATIOTEMPORAL CONTROL OF NEUROSENSORY DEVELOPMENT

Laura Fargas¹, Esteban Hoijman¹, Berta Alsina¹

Universitat Pompeu Fabra

34

NETWORK DEVELOPMENT INTO THE GASTROINTESTINAL TRACT

Andreia Margarido¹, Ludovic Le Guen¹, Sólveig Thorsteinsdóttir², de Santa Barbara Pascal¹

InsERM U1046, Montpellier FRANCE

Centro de Biologia Ambiental, Faculdade de Ciências, Universidade de Lisboa, Portugal

35

FIBRONECTIN ASSEMBLY DYNAMICS DURING CHICK EMBRYOGENESIS – IS FIBRONECTIN A PARACRINE SIGNAL?

Patrícia Almeida¹, Gonçalo Pinheiro¹, Sólveig Thorsteinsdóttir^{1, 2}

Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

Instituto Gulbenkian de Ciência, Oeiras, Portugal

36

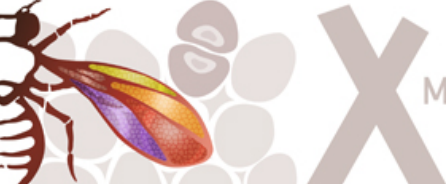
DOES TISSUE TENSION PLAY A ROLE IN SOMITOGENESIS IN THE CHICK EMBRYO?

Gonçalo Pinheiro¹, Patrícia Gomes¹, Pedro Rifes², Sólveig Thorsteinsdóttir¹

Faculdade De Ciências Da Universidade De Lisboa

DanStem, Kobenhavns Universitet, Kobenhavn, Denmark

37



POSTER SESSION I

Page

HIGHLY EFFICIENT TARGETED MUTAGENESIS IN ONE-CELL MOUSE EMBRYOS MEDIATED BY TALEN AND CRISPR/CAS SYSTEMS

Akihiro Yasue¹, Silvia N. Mitsui¹, Takahito Watanabe¹, Tetsushi Sakuma², Seiichi Oyadomari¹, Takashi Yamamoto², Sumihare Noji¹, Taro Mito¹, Eiji Tanaka¹

*The University Of Tokushima
Hiroshima University*

38

QUANTIFICATION AND MODELLING OF THE GENE REGULATORY NETWORK THAT CONTROLS AND SPECIFIES THE DROSOPHILA RETINA.

Máximo Sánchez-Aragón, Fernando Casares

*CABD (Andalusian Center for Developmental Biology), CSIC
Universidad Pablo de Olavide - Junta de Andalucía, Seville, SPAIN*

39

FUNCTIONAL PARTNERSHIP BETWEEN ABDOMINAL-B AND EXTRADENTICLE-HOMOTHORAX IN THE ABDOMEN AND FEMALE GENITALIA

Jesús R. Curt¹, Nagraj Sambrani², Bruno Hudry², Samir Merabet², Andrew Saurin², Yacine Graba², Ernesto Sánchez-Herrero¹,

*CBMSO, CSIC, Madrid, Spain
IBDML, CNRS, Marseille, France*

40

DEVELOPMENTAL RESPONSE TO BLOCKING GROWTH IN DIFFERENT GENETIC DOMAINS OF THE DROSOPHILA WING DISC

Antonio José Montes Ruiz¹, Ginés Morata Pérez¹
Centro De Biología Molecular Severo Ochoa (CBMSO)

41

DISTINCT TISSUE-SPECIFIC REQUIREMENTS FOR THE ZEBRAFISH TBX5 GENES DURING HEART, RETINA AND PECTORAL FIN DEVELOPMENT

Aina Pi-Roig^{1,2}, Enrique Martín-Blanco¹, Carolina Minguillón¹, Jordi Garcia-Fernández²

*Facultat de Biologia, UB (Universitat de Barcelona)
CSIC-Institut de Biologia Molecular de Barcelona*

42

NON-CANONICAL WNT PATHWAY, WOUND HEALING AND NEURAL TUBE CLOSURE IN MOUSE EMBRYOS

Patricia Ybot-Gonzalez¹, Laura Mañas-García¹, Cecilia Lazarini-Suarez¹, Beatriz Lopez-Escobar¹

Instituto De Biomedicina De Sevilla (IBiS), Hospital Universitario Virgen Del Rocío/CSIC/Universidad De Sevilla

43

VALIDATION OF CABUT TARGET GENES IDENTIFIED BY CHIP-ON-CHIP AND MICROARRAY EXPRESSION ANALYSES

Verónica Muñoz-Soriano^{1,2}, Sandra Lópe-Domenech^{1,2}, Nuria Paricio^{1,2}

*Universidad De Valencia, Burjassot, Valencia, Spain
ERI de Biotecnología y Biomedicina, Universidad de Valencia, Burjassot, Valencia, Spain*

44

INTERACTION BETWEEN BMP2 AND MIR-133 DURING EARLY CHICK CARDIAC DEVELOPMENT

Carmen Lopez-Sanchez¹, Diego Franco², Amelia Aranega², Ana Ortiz¹, Virginio Garcia-Lopez¹, Fernando Bonet², Virginio Garcia-Martinez¹

*University of Extremadura. Badajoz. Spain
University of Jaen. Jaén. Spain*

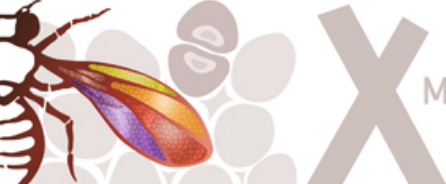
45

THE EGFR PATHWAY REGULATES GUT BRANCHING MORPHOGENESIS IN INTACT AND REGENERATING PLANARIANS

Sara Barberán¹, Francesc Cebrià¹

University Of Barcelona

46



POSTER SESSION I

Page

THE CONSERVED TRANSMEMBRANE PROTEOGLYCAN PERDIDO IS ESSENTIAL FOR MYOFIBRILLOGENESIS

Juan José Pérez Moreno¹, Marcus Bischoff², María Dolores Martín Bermudo¹, Beatriz Estrada¹
Centro Andaluz de Biología del Desarrollo (UPO-CSIC), Seville, Spain
University of Cambridge, Cambridge, UK

47

THE MYOTOME IS NECESSARY FOR NORMAL EPAXIAL MUSCLE DIFFERENTIATION.

André Gonçalves¹, Marianne Deries¹, Andreia Nunes¹, Marta Luz¹, Sólveig Thorsteinsdóttir^{1,2}, Shahragim Tajbakhsh³, Patrícia Ybot-Gonzalez⁴
Centro De Biologia Ambiental / Faculdade De Ciências Da Universidade De Lisboa
Instituto Gulbenkian de Ciência, Oeiras, Portugal
Cellules souches et développement, Institut Pasteur, Paris, France
Hospital Universitario Virgen del Rocío / Instituto de Biomedicina de Sevilla, Sevilla, Spain

48

THE CATECHOLAMINERGIC PATHWAY IS REQUIRED FOR NORMAL DEVELOPMENT OF BETA-CELLS IN THE MOUSE PANCREAS

Patricia Vázquez^{1,2}, Ana María Robles¹, Flora DePablo^{1,2}, Catalina Hernández^{1,2}
Centro de Investigaciones Biológicas (CIB, CSIC)
CIBERDEM (ISCIII)

72

MEMBRANE TYPE-4 MATRIX METALLOPROTEINASE (MT4-MMP) EXPRESSION DURING MOUSE EMBRYONIC DEVELOPMENT

Cristina Sánchez-Camacho^{1,2}, María José Blanco¹, Mara Martín-Alonso², Motoharu Seiki³, Alicia G. Arroyo²
Universidad Europea de Madrid (UEM), Villaviciosa de Odón, Madrid, Spain
Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain
Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo, Japan

51

THE BASEMENT MEMBRANE AS A REGULATOR OF “GLOBAL TISSUE ROTATION” DURING DROSOPHILA MELANOGASTER OOGENESIS.

María del Carmen Díaz de la Loza¹, Alfonsa Díaz Torres¹, María Dolores Martín Bermudo¹, Acaimo González Reyes¹
Centro Andaluz De Biología Del Desarrollo

53

LAMININS ARE REQUIRED FOR PROPER MIGRATION OF EMBRYONIC HAEMOCYTES IN DROSOPHILA

Besaid J. Sánchez Sánchez¹, José M. Urbano², Kate Comber³, Will Wood³, María D. Martín Bermudo¹
Centro Andaluz De Biología Del Desarrollo (CABD) /Sevilla/Sevilla/Spain
PDN Department, University of Cambridge, UK.
Department of Developmental Biology, University of Bristol, UK.

54

INTEGRINS POSITIVELY REGULATE CELL SURVIVAL IN THE WING IMAGINAL DISC IN DROSOPHILA MELANOGASTER

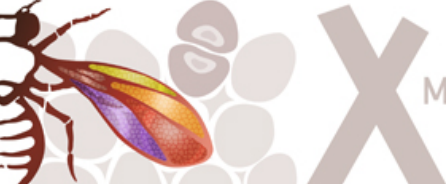
Andrea Valencia Expósito¹, María Jesús Gómez Lamarca², Thomas Widmann³, María D. Martín Bermudo¹
Centro Andaluz De Biología Del Desarrollo (CABD)/Sevilla/Spain
University of Cambridge, Downing Street Cambridge CB2 3EJ, UK
Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Granada, Spain

55

THE DUAL-SPECIFICITY PHOSPHATASE CG10089 REGULATES NEGATIVELY THE ACTIVITY OF THE EGFR SIGNALLING PATHWAY IN THE DROSOPHILA WING

Cristina Martínez Ostalé¹, Cristina Molnar¹, Jose Felix de Celis¹
Cbmso

56



POSTER SESSION I

Page

A BALANCED NOTCH LEVEL – AN ESSENTIAL REQUISITE FOR HEART REGENERATIONJuliane Münch¹, Dimitrios Grivas¹, Álvaro González-Rajal¹, José Luis de la Pompa¹
Centro Nacional De Investigaciones Cardiovasculares (CNIC)

57

A ROLE FOR MIR-15, MIR-23, MIR-106 AND MIR-199 DURING CARDIOGENESIS AND SOMITOGENESIS IN THE DEVELOPING CHICKDiego Franco¹, Fernando Bonet¹, Carmen Lopez-Sanchez², V Garcia-Lopez², A Ortiz², Amelia Aranega¹,
Virginio Garcia-Martinez²
University Of Jaen
University of Extremadura

58

MEIS FUNCTION IN LIMB INITIATION AND PROXIMO-DISTAL PATTERNING. A STUDY THROUGH MEIS1;2 KO ANALYSISIrene Delgado¹, Alberto Roselló, Miguel Torres
Centro Nacional De Investigaciones Cardiovasculares (CNIC). Madrid. Spain
Memorial Sloan-Kettering Cancer Center. New York. USA
Centro Nacional De Investigaciones Cardiovasculares (CNIC). Madrid. Spain

60

A CHICKEN EMBRYONIC MODEL FOR HEART REGENERATIONOSCAR MAURICIO FERNÁNDEZ¹, AMELIA ARANEGA JIMENEZ¹, DIEGO FRANCO JAIME¹, Jorge N
Dominguez Macias¹
University Of Jaen

61

GATA4-EXPRESSING LINEAGE CELLS CONTRIBUTE TO DEVELOPMENTAL AND ADULT HEMATOPOIESISRamon Muñoz-Chápuli^{1,2}, Elena Cano^{1,2}, Rita Carmona^{1,2}, Ana Cañete^{1,2}, Laura Ariza^{1,2}, Irene Delgado³, Anabel
Rojas³
Universidad De Málaga
BIONAND, Málaga
CABIMER, Sevilla

62

IDENTIFICATION OF NEW PLAYERS INVOLVED IN OPTIC CUP FOLDINGJoaquín Letelier¹, Marta San Martín-Alonso¹, Cristina González-Aguilera¹, Juan Ramón Martínez-Morales¹
Centro Andaluz De Biología Del Desarrollo

63

CYTONE-MEDIATED CONTACT-DEPENDENT HEDGEHOG SIGNALLINGLaura González Méndez¹, Irene Seijo¹, Paloma Ozores¹, Isabel Guerrero¹
Centro de Biología Molecular "Severo Ochoa"

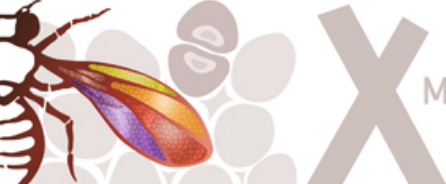
64

A-CATENIN MEDIATES THE EMERGENCE OF AN ELASTIC RESTORING FORCE DRIVING PULSATILE APICAL CONTRACTIONJaime Jurado-Gómez¹, Joaquín de Navascués², Nicole Gorfinkiel¹
Centro de Biología Molecular "Severo Ochoa", Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid, Spain
European Cancer Stem Cell Research Institute, School of Biosciences, Cardiff University, UK.

65

THE TRANSMEMBRANE PROTEIN CDON REGULATES DELAMINATION OF NEURAL CREST CELLSLucía Fanlo¹, Marcos Julián Cardozo^{2,3}, Susana Usieto¹, África Sandonis^{2,3}, Paola Bovolenta^{2,3}, Elisa Martí¹
Instituto de Biología Molecular De Barcelona-Consejo Superior De Investigaciones Científicas
Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas -Universidad Autónoma de Madrid
Ciber de Enfermedades Raras (CIBERER), ISCIII, Madrid, Spain

66



POSTER SESSION I

Page

A ROLE FOR SHH IN ESTABLISHING SYMMETRY AND LUMEN FORMATION IN THE ZEBRAFISH SPINAL CORD

Irene Gutiérrez-Vallejo¹, Elena González-Gobart¹
IbmB-CSIC

67

ASYMMETRIC DISTRIBUTION OF SHH SIGNALLING COMPONENTS DIRECTS THE MODE OF CELL DIVISION IN THE DEVELOPING NERVOUS SYSTEM

Murielle Saade¹, Rene Escalona¹, Elena Gonzalez-Gobart¹, Elisa Marti Gorostiza¹
Instituto De Biologia Molecular De Barcelona

68

THE INTERPLAY BETWEEN GROWTH PROMOTING PATHWAYS AND DPP ACTIVITY IN DROSOPHILA

Ana Ferreira¹, Marco Milán^{1,2}
IRB Barcelona, Spain
ICREA, Barcelona, Spain

69

ASSESSING THE LONG RANGE ACTION OF WNT SIGNALING WITH GENOMIC ENGINEERING

Luis ALberto Baena-Lopez¹, Cyrille Alexandre¹, Jean-Paul Vincent¹
MRC. National Institute For Medical Research

70

HOXA2/ZIC2 CONTROL THE EXPRESSION OF ROBO3 IN ATHO1-POSITIVE DERIVATIVES OF THE DORSAL NEURAL TUBE.

Francisco Javier Nieto Lopez¹, Thomas Di Meglio¹, Filippo M. Rijli², Eloisa Herrera³, Paola Bovolenta¹
Centro De Biología Molecular Severo Ochoa
Friedrich Miescher Institute
Instituto de Neurociencias

71

IS LINE-1 RETROTRANSPOSITION A SOURCE FOR DNA BREAKS IN MOUSE RETINAL DEVELOPMENT?

Noemí L. Álvarez-Lindo¹, Jimena Baleriola¹, Luis Blanco², Antonio Bernad³, Teresa Suarez¹, Enrique J. de la Rosa¹
Centro De Investigaciones Biológicas CSIC
Centro de Biología Molecular CSIC-UAM
Centro Nacional de Biotecnología CSIC

73

2. Systems Biology & Genomics

A TISSUE-SPECIFIC TRANSCRIPTIONAL PROGRAM CONTROLS REMODELING OF THE HEART CIRCULATION

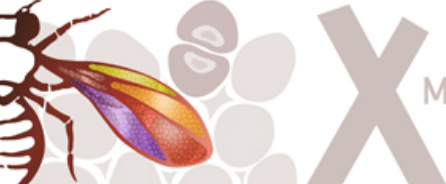
Marta Losa¹, Shilu Amin¹, Ian J. Donaldson¹, Leo Zeef¹, James Hensman², Magnus Rattray¹, Nicoletta Bobola¹
The University Of Manchester
Univeristy of Sheffield

74

LONG-RANGE INTERACTIONS IN THE REGULATION OF THE MOUSE MRF4/MYF5 LOCUS

Cristina Vicente Garcia¹, Angela Bella Carreño¹, Ana Fernandez Miñan¹, Juan J. Tena¹, Jose Luis Gomez Skarmeta¹, Jaime J. Carvajal¹
Centro Andaluz de Biología

75



POSTER SESSION I

Page

REGULATION OF GENOME ARCHITECTURE DURING HEART DEVELOPMENT

Melisa Gomez-Velazquez¹, Eva Fernandez, Claudio Badia-Careaga, Juan Tena, Isabel Rollan-Delgado, M. Eva Alonso, Niels Galjart, Jose Luis Gomez-Skarmeta Miguel Manzanares

National Center Of Developmental And Cardiovascular Repair

Centro Andaluz de Biología del Desarrollo (CSIC-UPO), Seville, Spain

Erasmus Medical Centre, Rotterdam, the Netherlands.

Present address: Centro Andaluz de Secuenciación Genómica Humana, Seville, Spain.

76

EXPRESSION PROFILE OF THE SEX-RELATED GENE DMRT1 DURING TEMPERATURE SEX DETERMINATION IN GONADS OF THE SEA TURTLE LEPIDOCHELYS OLIVACEA

Rogelio Montiel Manríquez¹, Daniela Venegas Suarez Peredo¹, Félix Recillas Targa², Jose Alejandro Marmolejo Valencia¹, Horacio Merchant Larios¹

Instituto De Investigaciones Biomédicas, UNAM

Instituto de Fisiología Celular, UNAM

77

CABUT/DTIEG ASSOCIATES WITH THE TRANSCRIPTION FACTOR YORKIE FOR GROWTH CONTROL

Marina Ruiz-Romero, Serras Florenci, Corominas Montserrat

Universitat De Barcelona, Barcelona, Spain

78

CHARACTERIZATION OF THE MOUSE SNAIL1 LOCUS

Juan Galceran¹, Eva Rodríguez-Aznar¹, Fabiana Oliveira¹, Miguel Manzanares², Jose Luis Gomez-Skarmeta³, M. Angela Nieto¹

Instituto De Neurociencias. CSIC-UMH

CNIC, CABD

79

DECODING BOUNDARIES: FROM GENOMIC LANDSCAPE TO CELLULAR FUNCTION.

Javier Terriente¹, Joaquin Letelier², Juan Ramon Martinez-Morales², Cristina Pujades¹

Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona

Centro Andaluz de Biología del Desarrollo (CSIC/UPO), Sevilla

80

POSTER SESSION II

Page

3. Evolution & Development

- NON-CANONICAL DORSOVENTRAL PATTERNING IN THE MOTH MIDGE CLOGMIA ALBIPUNCTATA.**
Eva Jimenez-Guri¹, Anna Alcaine¹, Johannes Jaeger¹, Karl Richard Wotton¹
Center For Genomic Regulation 81
- EVOLUTIONARY ANALYSIS OF THE PAIRED-RELATED HOMEBOX GENE FAMILY**
Aida Arcas¹, Ángela M. Nieto¹
Instituto De Neurociencias (CSIC-UMH) 82
- MULTIPLE DEVELOPMENTAL ROLES OF A TISSUE-SPECIFIC ALTERNATIVE SPLICING FACTOR ACROSS DEUTEROSTOMES: ESRP GENE FAMILY IS A MASTER REGULATOR OF DIVERSE EPITHELIAL FUNCTIONS**
Demian Burguera¹, Enrique Navas¹, Claudia Cuomo², Ylenia D'Agostino², Claudia Racioppi², Rosaria Esposito², Carlos Herrera¹, Beatriz Albuixech¹, Manuel Irimia³, Jordi Garcia-Fernández²
University Of Barcelona, Spain
Stazione Zoologica Anton Dohrn, Napoli, Italy
CRG, Barcelona, Spain 83
- EVOLUTION AND DEVELOPMENT OF NOVEL DENTITIONS IN TETREODONTIFORMES**
Takanori Shono¹, Alex Thiery¹, Gareth Fraser¹
University Of Sheffield 84
- THE SIX3 GENE OPTIX PATTERNS THE DROSOPHILA HEAD THROUGH AN ANTI-REPRESSOR MECHANISM WITHIN THE HH-SIGNALING PATHWAY.**
María Angeles Domínguez-Cejudo¹, Fernando Casares¹
Centro Andaluz De Biología Del Desarrollo 85
- MECHANISMS OF CELL DECISION-MAKING: USING THE OCELLUS TO UNDERSTAND HOW CELLS BECOME NEURONS AND HOW THESE NEURONS ESTABLISH SPECIFIC CONNECTIONS.**
Diana Garcia Morales¹, Fernando Casares Fernandez
Centro Andaluz De Biología Del Desarrollo (CABD) 86
- SELECTING THE EYE ARCHITECTURE IN DROSOPHILA: A WNT CHOICE.**
Marta Magri¹, María Ángeles Domínguez-Cejudo, Fernando Casares
Cabd 87
- HOX GENE FUNCTION AND REGULATION DURING AP AXIS ELONGATION IN THE CRICKET GRYLLUS BIMACULATUS**
Yuji Matsuoka¹, Tetsuya Bando², Takahito Watanabe¹, Sumihare Noji¹, Taro Mito¹
The University Of Tokushima
The University Of Okayama 88
- TARGETED GENOME EDITING USING CRISPR/CAS9 SYSTEM IN THE CRICKET GRYLLUS BIMACULATUS**
Taro Mito¹, Yuji Matsuoka¹, Takahito Watanabe¹, Sumihare Noji¹
The University Of Tokyo 89
- ORIGINS AND REGULATION OF AN EUTHERIAN NOVELTY: THE BGW CLUSTER**
Enrique Pérez¹, Salvatore d'Aniello², Jordi Garcia-Fernández¹
University Of Barcelona, Barcelona, Spain
Stazione Zoologica Anton Dohrn, Napoli, Italy 90
- HOX GENES AND THE EVOLUTION OF VERTEBRATE LIMBS**
João Castro¹, Renata Freitas¹
IBMC- Institute For Molecular And Cell Biology 91

POSTER SESSION II

Page

TRASLOCATION OF TORSO-LIKE FROM THE EGG SHELL TO THE OOCYTE PLASMA MEMBRANE

Alessandro Mineo^{1,2}, Marc Furriols^{1,2}, Jordi Casanova^{1,2}

Institute for Biomedical Research (IRB)

Institute of Molecular Biology of Barcelona (IBMB)

92

NEW INSIGHTS INTO VERTEBRAL CENTRUM FORMATION: AN EVO-DEVO PERSPECTIVE

Tomás Pais De Azevedo¹, Ann Huyseune², Paul Witten², Isabel Palmeirim¹

Univeristy of Algarve, Faro, Portugal

Univeristy of Gent, Gent, Belgium

93

ASYMMETRY VERSUS SYMMETRY: THE ROLE OF DMRT2A IN THE FORMATION OF THE VERTEBRATE BODY PLAN

Rita Pinto², José Almeida-Santos², Leonor Saúde²

Instituto de Medicina Molecular e Instituto de Histologia e Biologia do Desenvolvimento, Faculdade de Medicina da Universidade de Lisboa, 1649-028 Lisboa, Portugal

Instituto Gulbenkian de Ciência, P-2780-156 Oeiras, Portugal

94

FUNCTIONAL CHARACTERIZATION IN TRANSGENIC ZEBRAFISH OF REGULATORY SEQUENCES TARGETED BY THE TRANSCRIPTION FACTOR SOX2, IDENTIFIED BY STUDIES OF LONG-RANGE CHROMATIN INTERACTIONS IN BRAIN-DERIVED NEURAL STEM CELLS

Jessica Armida Bertolini¹, Rebecca Favaro¹, Yubo Zhang², Ben Martynoga³, Paul Robson⁴, Francois Guillemot³, Giulio Pavesi⁵, Chia-Lin Wei², Paola Bovolenta⁶, Silvia Kirsten Nicolis¹

University Of Milano-Bicocca, Milano, Italy

DOE Joint Genome Institute, Walnut Creek, CA, USA

MRC National Institute for Medical Research, London, UK

Genome Institute of Singapore

University of Milano, Milano, Italy

Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain

95

THE ROLE OF DIRECT NEUROGENESIS IN THE DEVELOPMENT OF THE OLFACTORY BULB

Adrián Cárdenas¹, Meaghan Cogswell², Camino De Juan Romero¹, Athanasia Tzika³, Michel Milinkovitch³, Luis Miguel Martínez¹, Marc Tessier-Lavigne⁴, Shelley Russek², Víctor Borrell¹

Instituto De Neurociencias CSIC-UMH

Boston University School of Medicine, Boston, United States

Université de Genève, Geneva, Switzerland

The Rockefeller University, New York, United States

96

DEEPLY CONSERVED TOPOLOGICAL ASSOCIATING DOMAINS CONTRIBUTE TO EVOLUTIONARY AND DEVELOPMENTAL REGULATORY CONSTRAINTS

Carlos Gómez Marín¹, Juan Jesus Tena¹, Rafael Rodríguez Acemel¹, Carmen Hidalgo¹, Silvia Naranjo¹, Elisa de la Calle-Mustienes¹, Paola Bovolenta^{2,3}, Eric H. Davidson⁴, Jaime J. Carvajal¹, Jose Luis Gomez Skarmeta¹

Centro Andaluz De Biología Del Desarrollo, Sevilla, Spain

Centro de Biología Molecular Severo Ochoa, Madrid, Spain

CIBER de enfermedades raras, Madrid, Spain.

California Institute of Technology, Pasadena, California, USA.

97

ROLE OF MIRNAS IN OLFACTORY BULB FORMATION

Virginia Fernández¹, María Ángeles Martínez¹, Ugo Tomasello¹, Víctor Borrell¹

Instituto De Neurociencias (CSIC&UMH)

98

GENETIC BASIS OF THE EVOLUTION OF DIFFERENCES IN EYE SIZE BETWEEN DROSOPHILA SIMULANS AND DROSOPHILA MAURITIANA

Isabel Almudi¹, Maria D.S. Nunes¹, Montserrat Torres², Saad Arif¹, Nico Posnien², Alistair P.

McGregor¹

Oxford Brookes University

Georg-August-University Goettingen

99

POSTER SESSION II

Page

MATURE BLOOD CELL CLUSTERS CONSTITUTE A TRUE HEMATOPOIETIC TISSUE THAT REGULATES BLOOD CELL DIFFERENTIATION IN DROSOPHILA

Alexandre Leitão¹, Élio Sucena^{1,2}

Instituto Gulbenkian De Ciência

Faculdade de Ciências da Universidade de Lisboa

100

THE AMPHIOXUS HOX CLUSTER REGULATORY LANDSCAPE AND THE ORIGIN OF VERTEBRATE REGULATORY INNOVATIONS

afael D. Acemel¹, Juan Jesús Tena¹, Ferdinand Marletaz², Ignacio Maeso¹, Daniel Aldea³, Carlos Gómez-Marín³, Stéphanie Bertrand³, Héctor Escrivà³, José Luis Gómez-Skarmeta¹

Centro Andaluz de Biología del Desarrollo CSIC/UPO, Seville, Spain

University of Oxford, Oxford, United Kingdom

Observatoire Oceanologique de Banyuls-sur-Mer CNRS-UMR7232 Université Pierre et Marie Curie, Banyuls-sur-Mer, France

101

EXPLORING THE ROLE OF MATERNAL GENES IN THE FORMATION OF TRANSCRIPTION BORDER IN THE BICOID SYSTEM

José M De Las Heras¹

Institut Curie

102

PROBLEMS AND SOLUTIONS IN VERTEBRATE SKULL DEVELOPMENT

Rui Castanhinha¹, Élio Sucena², Joaquín Leon²

Instituto Gulbenkian De Ciência And Museu Da Lourinhã

Instituto Gulbenkian De Ciência

103

4. Development & Disease

THE DEVELOPMENTAL TRANSCRIPTION FACTOR PITX2 DIMINISHES AFTER BIRTH, BECOMES RE-ACTIVATED IN HEART FAILURE AND STIMULATES MYF5 EXPRESSION IN CARDIOMYOCYTES

Alexander Mikhailov¹

University of La Coruna

104

SNAIL1 CONTROLS BONE LENGHT

Sonia Vega¹, Cristina López-Blau¹, Cristina A. de Frutos^{1,3}, Joan Galcerán¹, Stephen J Weiss², M.

Angela Nieto¹

Instituto Neurociencias Alicante. Spain University

of Michigan Ann Harbour. USA Ecole Normale

Supérieure Paris. France

105

SEARCHING FOR NOVEL COMPONENTS/REGULATORS OF THE DROSOPHILA NEPHROCYTE SLIT DIAPHRAGM.

Antonio S. Tutor^{1,2}, Mar Ruiz-Gómez¹

Centro De Biología Molecular Severo Ochoa

Universidad Europea de Madrid

106

REGULATION OF CYTONEMES FORMATION IN DROSOPHILA

Sheila Jordán-Álvarez¹, Isabel Guerrero¹

Centro De Biología Molecular Severo Ochoa

107

THE REGULATION OF ATOH1 DURING OTIC DEVELOPMENT

Héctor Gálvez García¹, Jelena Petrovic¹, Joana Neves¹, Fernando Giraldez¹, Gina Abelló¹

Universitat Pompeu Fabra, Barcelona, Spain

108

DEFECTS IN EMBRYONIC LAMININ DEPOSITION AND MYOGENESIS CONTRIBUTE TO DISEASE PROGRESSION IN A MOUSE MODEL OF CONGENITAL MUSCULAR DYSTROPHY

A. M. Nunes^{1,2}, A. B. Gonçalves¹, P. Ybot-Gonzalez³, M. Deries¹, D. J. Burkin², S. Thorsteinsdóttir¹

Centro De Biologia Ambiental, Faculdade De Ciências Da Universidade De Lisboa, Lisbon, Portugal

Center for Molecular Medicine, University of Nevada School of Medicine, Reno, USA Hospital Infantil

Virgen del Rocío, Instituto de Biomedicina de Sevilla (IBIS), Sevilla, Spain

109

POSTER SESSION II

Page

TISSUE OVERGROWTH INDUCED BY COEXPRESSION OF THE PROGENITOR GENES HTH/MEIS1 AND TSH/TSHZ RESULTS FROM AN IMBALANCE IN THE ESTROGEN RESPONSE PATHWAY IN DROSOPHILA.

Marta Neto^{1,2}, Marina Naval-Sánchez³, Delphine Potier³, Paulo S. Pereira², Dirk Geerts⁴, Stein Aerts³, Fernando Casares¹

CABD (Centro Andaluz de Biología del Desarrollo), CSIC-Universidad Pablo de Olavide-Junta de Andalucía, Seville, Spain

IBMC (Institute for Molecular and Cell Biology), Universidade do Porto, Porto, Portugal

Center for Human Genetics, University of Leuven, Leuven, Belgium

Department of Pediatric Oncology and Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands

110

DRUG SCREENING IN DROSOPHILA MODELS OF FRIEDREICH ATAXIA AND PARKINSON'S DISEASE

Francisco José Sanz¹, Pablo Calap-Quintana¹, Verónica Muñoz-Soriano^{1, 2}, Rubén Pavia¹, Sandra López-Domenech^{1, 2}, María Dolores Moltó^{1, 3, 4}, María José Martínez-Sebastián¹, Nuria Paricio^{1, 2}

Universidad De Valencia, Burjassot, Valencia, Spain

ERI de Biotecnología y Biomedicina, Universidad de Valencia, Burjasot, Valencia, Spain

Red de Salud Mental, CIBERSAM

Fundación Investigación Clínico de Valencia, INCLIVA, Valencia, Spain

111

THE ROLE OF JNK PATHWAY IN TUMOR FORMATION AND PROGRESSION IN THE ABSENCE OF APOPTOSIS.

Noelia Pinal¹, Gines Morata¹

Centro De Biología Molecular Severo Ochoa

112

REGENERATIVE POTENTIAL OF DIFFERENT REGIONS OF THE DROSOPHILA WING IMAGINAL DISC

Raquel Martín¹, Ginés Morata¹

Centro De Biología Molecular Severo Ochoa

113

ROLE AND MECHANISM OF CELL COMPETITION IN TUMOUR PROGRESSION IN DROSOPHILA

Luna Ballesteros-Arias¹, Gines Morata¹

Centro De Biología Molecular Severo Ochoa

114

EPIGENETIC CHARACTERIZATION AND IDENTIFICATION OF GENETIC MODIFIERS OF TRANSCRIPTIONAL REPRESSION IN A FRIEDREICH ATAXIA MODEL

Lucía Benito-Jardón¹, Pablo Calap-Quintana¹, José Vicente Llorens¹, Sirena Soriano¹, María José Martínez-Sebastián¹, María Dolores Moltó^{1,2,3}

University Of Valencia, Valencia, Spain

Network on Mental Health, CIBERSAM, Valencia, Spain

Clinical Research Foundation of Valencia, Valencia, Spain

115

MORPHOMETRICS DETECT ALTERED GENE EXPRESSION PATTERNS AFFECTING EARLY LIMB DEVELOPMENT IN APERT SYNDROME

Jaume Sastre¹, Lucia Russo¹, Joan Richtsmeier², James Sharpe¹, Neus Martínez-Abadías

Center For Genomic Regulation

Pennsylvania State University

116

REGULATORY VARIATION OF THE PITX2 LOCUS IN ATRIAL FIBRILLATION

Raquel Rouco¹, Luis A. Aguirre¹, Claudio Badia-Careaga¹, Melisa Gomez-Velazquez¹, Juan J. Tena², Eva Alonso¹, Ana Fernandez-Minan², Amelia Aranega³, Jose Luis Gomez-Skarmeta², Diego Franco³, Miguel Manzanares¹

Centro Nacional De Investigaciones Cardiovasculares (CNIC), Madrid, Spain

Centro Andaluz de Biología del Desarrollo (CSIC-UPO), Seville, Spain

Department of Experimental Biology, University of Jaen, Spain

117

HOX MEDIATED CONTROL OF NEURONAL DIVERSITY WITHIN THE RHOMBIC LIP LINEAGE AND ITS POSSIBLE CONSEQUENCES IN MEDULLOBLASTOMA FORMATION

Thomas Di Meglio¹, Francisco Javier Nieto-Lopez¹, Dominik Kraus², Ariel Di Nardo³, Alain Prochiantz³,

118

POSTER SESSION II

Page

Filippo Rijli², Paola Bovolenta¹

Centro De Biología Molecular Severo Ochoa, Madrid, Spain

Friedrich Miescher Institute, Basel, Switzerland

Collège de France, Paris, France

MEIS FUNCTION IN CARDIAC FIELDS IS ESSENTIAL FOR HEART DEVELOPMENT

119

Laura Carramolino¹, Mónica González, Alejandra López-Delgado, Vanessa Cadenas, Miguel Torres

Centro Nacional de Investigaciones Cardiovasculares

NEW MARKERS FOR ASTROCYTE IDENTITY AND FUNCTION

121

Alejandra Quiroga¹, William D. Richardson¹, Huiliang Li¹

Wolfson Institute For Biomedical Research, University College London

RANDOM RETROSPECTIVE CLONAL ANALYSIS OF THE DEVELOPING MOUSE HEART

122

Ghislaine Lioux¹, Susana Temiño¹, Miguel Torres¹

Cnic

ENDOTHELIAL JAG1/NOTCH1 AND ITS SIGNALING EFFECTOR EFNB2 ARE ESSENTIAL FOR PROPER MYOCARDIAL COMPACTION AND CORONARY VESSELS FORMATION

123

Stanislao Igor Travisano¹, Donal MacGrogan¹, José Luis de la Pompa¹

Cnic

5. Stem Cells & Reprogramming

THE CALCINEURIN SPLICING ISOFORM CNAB1 IS IMPLICATED IN EARLY CARDIAC DIFFERENTIATION.

124

Jesús María Gómez-Salinerio¹, Marina Mercedes-Olañeta¹, Paula Ortiz-Sánchez¹, José Javier Larrasa-Alonso¹, Enrique Lara-Pezzi¹

Fundación Centro Nacional De Investigaciones Cardiovasculares Carlos III

A PITX2-MIRNAS PATHWAY REGULATES SATELLITE CELL PROLIFERATION AND SKELETAL MUSCLE REGENERATION

125

Estefanía Lozano-Velasco¹, Daniel Vallejo¹, Felicitas Ramírez¹, Francisco Hernández-Torres¹, Diego Franco¹, Amelia Aránega¹

University Of Jaén

CHARACTERIZATION OF MOLECULAR PATHWAYS INVOLVED IN CELL COMPETITION IN MAMMALIAN EMBRYONIC STEM CELLS

126

Covadonga Díaz¹, Cristina Clavería¹, Giovanna Giovinzio¹, Miguel Torres¹

Centro Nacional De Investigaciones Cardiovasculares, Madrid, Spain.

REGULATION OF THE TROPHOBLAST FATE IN VITRO

127

Teresa Rayon¹, Sergio Menchero¹, Inmaculada Ors¹, Janet Rossant², Miguel Manza¹

Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

University of Toronto. Toronto, Canada

FUNCTION OF PERLECAN IN THE DROSOPHILA OVARY

128

Alfonsa Díaz-Torres¹, John Pearson¹, Acaimo González Reyes¹

Centro Andaluz De Biología Del Desarrollo

EARLY SIGNALS THAT TRIGGER EPITHELIAL REGENERATION IN DROSOPHILA IMAGINAL DISCS

129

Paula Santa Bárbara Ruiz¹, Florenci Serras Rigalt¹

Departamento De Genética, Universidad Barcelona

POSTER SESSION II

Page

DINAMIC CHARACTERIZATION OF EC, CIRC AND CC IN UC-HMSCS OVER TIME

Alexandra Filipe¹

DCBM, CBME, Universidade Do Algarve

130

CHARACTERIZATION OF THE PLURIPOTENCY OF MOUSE TRANSMITOCHONDRIAL EMBRYONIC STEM CELLS

Rocio Nieto-Arellano¹, Rebeca Acín-Pérez¹, Patricia Meade-Huerta², Patricio Fernandez-Silva², Jose Antonio Enriquez^{1,2}

Cnic

Universidad de Zaragoza

131

BMP SIGNALLING PROMOTES STEM CELL MAINTENANCE IN THE DEVELOPING CHICK DORSAL PALLIUM.

Gwenvael Le Dréau¹, Elisa Martí¹

Instituto De Biologia Molecular De Barcelona (IBMB-CSIC)

133

TAMOXIFEN INDUCIBLE SYSTEMS FOR MODULATING POSTMITOTIC BRAIN PLASTICITY

Carlos García Briz¹, Noelia Sofía De León Reyes¹, Marta Nieto López¹

Centro Nacional De Biotecnología. CSIC.

134

THE STRESS-ACTIVATED JNK COORDINATES PLANARIAN REGENERATION AND RESCALING BY TRIGGERING APOPTOSIS AND MODULATING THE CELL CYCLE

Emili Saló¹

Dept. Genètica, IBUB & UB

135

6. Miscellanea

DECIPHERING THE ROLE OF EMT-TFS IN DIFFERENT STEPS OF THE METASTATIC CASCADE BY USING A ZEBRAFISH MODEL

Berta Sanchez-Laorden¹, Oscar Ocaña², Rebeca Córcoles³, M Angela Nieto⁴

Instituto De Neurociencias CSIC-UMH

136

PROTEIN PROFILE OF WILD PLANTS OF SOTOL (DASYLIRION LEIOPHYLLUM ENGELM. EX TREL) IN CHIHUAHUA, MÉXICO

Juencio González¹, Quintín Rascón¹, Andrés Vargas¹

Universidad Autónoma De Chihuahua

137

THE STEROID HORMONE ECDYSONE PROMOTES GROWTH OF IMAGINAL DISCS IN DROSOPHILA MELANOGASTER

Leire Herboso¹, Marisa Oliveira², Ana Talamillo¹, Coralía Pérez¹, Monika González¹, David Martín³, James Sutherland¹, Christen Mirth², Rosa Barrio¹

CIC bioGUNE, Derio, Bizkaia, Spain

Instituto Gulbenkian de Ciência, Oerias, Portugal

Institute de Biología Evolutiva-CSIC-Universitat Pompeu Fabra, Barcelona, Spain

138

SOXD GENES CONTROLS PROLIFERATION AND DORSAL SPECIFICATION IN THE SPINAL CORD

Aixa V. Morales¹, Alejandra C. Quiroga¹, Claus C. Stolt², Ruth Diez del Corral¹, Spas Dimitrov¹, Elisabeth Sock², Michael Wegner²,

Instituto Cajal (CSIC)

Institut für Biochemie, Universität Erlangen-Nürnberg

139

ENHANCEMENT OF A SONIC HEDGEHOG NEGATIVE FEEDBACK LOOP BY FGF SIGNALLING CONTROLS INITIATION OF SPINAL CORD VENTRAL PATTERNING

Aixa V. Morales¹, Sergio Espeso-Gil¹, Inmaculada Ocaña^{1,3}, Francisco Nieto-Lopez^{1,2,3}, Paola Bovolenta^{1,2,3}, Mark Lewandoski⁴, Ruth Diez Del Corral¹

Instituto Cajal, CSIC, Madrid, Spain

140



POSTER SESSION II

Page

*Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Madrid, Spain
CIBER de Enfermedades Raras, Spain
Center for Cancer Research, National Cancer Institute, Frederick, USA*

P27KIP1 PARTICIPATES IN THE REGULATION OF ENDOREDUPPLICATIVE CYCLES IN DIFFERENTIATING CHICK RETINAL GANGLION CELLS

141

José María Frade¹, María Carmen Ovejero-Benito¹
Cajal Institute, CSIC

APP MRNA LOCALIZES WITHIN A SUBSET OF AXONS OF THE TECTUM IN CHICK EMBRYOS

142

Nozomi Onodera¹, Ryuji Nanayama¹, Keiko Okudaira¹, Shiho Nozaki¹, Isato Araki¹
Iwate Univ

VARIATION IN SPERM FUNCTION AMONG MOUSE SPECIES DURING PREPARATION FOR FERTILIZATION

143

Ester Sansegundo-Hernando¹, Maximiliano Tourmente¹, Eduardo Roldan¹
Museo Nacional De Ciencias Naturales (CSIC)

JAK/STAT AND HOX DYNAMIC INTERACTIONS IN AN ORGANOGENETIC GENE CASCADE

144

P. B. Pinto^{1,2}, J. M. Espinosa-Vázquez¹, M.L. Rivas¹, J. Castelli-Gair Hombría¹
*CABD, CSIC/JA/Universidad Pablo De Olavide, Seville, Spain
Current Address: Centre For Organismal Studies (COS) Heidelberg, Heidelberg, Germany*

Abstracts Book

WNT/B-CATENIN PATHWAY CONTROLS DENTAL DEVELOPMENT AT ALL STAGES OF ODONTOGENESIS AND IS RELATED TO EPIPROFIN/SP6 TRANSCRIPTION FACTOR

M. Aurrekoetxea, I. Irastorza, G. Ibarretxe, F. Unda

University of the Basque Country, UPV/EHU, Bizkaia, Spain

Mouse tooth is a good model for the study of organogenesis and regulatory pathways involved in cell differentiation and proliferation. Extracellular signals of BMP, FGF, Hedgehog and Wnt families have been involved in the correct patterning and formation of mammalian teeth. Particularly, Wnt/ β -catenin signaling is highly conserved pathway, and the precise control of this pathway is required for normal tooth development.

The aim of this work was to further study the effects of overactivation of the Wnt signaling in each stage of odontogenesis (initiation, morphogenesis and cell differentiation), in the absence of biological interferences related to inhibition or overactivation of the route at other times of odontogenesis.

To activate Wnt/ β -catenin pathway we used 6-bromoindirubin-3'-oxime (BIO), a specific pharmacological inhibitor of glycogen synthase kinase-3 IX (GSK-3).

Our results indicated that the overactivation of the Wnt/ β -catenin pathway, in the initiation stages, promoted the formation of supernumerary regulatory centers extra teeth generating. Furthermore, in the morphogenesis and cell differentiation stages we observed alterations of tooth developmental pattern and dental cusps, when embryo first molars were treated with BIO. These disorders were related with changes in the expression of some odontogenic target genes, as Dkk1, Bmp4, Wnt10b and Shh. In addition, at morphogenesis stage we observed an increased expression of Epiprofin/Sp6, Bmp4 and Nestin genes and high levels of alkaline phosphatase, in the dental mesenchyme. These results seem to be related with the ectopic mesenchymal differentiation detected in our tooth BIO-cultures. The presence of ectopic dentin and differentiated odontoblasts in dental pulp in this condition is associated with a human pathology called dental pulp calcification.

In summary, the results of this study stress the importance of the right balance in the activity of Wnt/ β -catenin pathway. Moreover, we deepen in the study about the relationship between the signaling pathway Wnt/ β -catenin, Epiprofin transcription factor and Bmp4 during odontogenesis.

MUSCLES AND PIGMENT CELLS COORDINATION DURING THE DEVELOPMENT OF THE REPRODUCTIVE SYSTEM OF DROSOPHILA MELANOGASTER

I. Olivera¹, E. Sánchez-Herrero¹

¹. CBMSO, Madrid, Spain

The development of the reproductive system in *Drosophila* requires the fusion of two structures: the genital disc and the gonads. The fusion takes place during pupal stages when the genital disc evaginates from the abdominal segment A8 towards the abdominal segment A5 to contact the gonads. We have found that derivatives from the genital disc move towards the position of the gonads even if these are absent. We have focused in males, where muscle tissue coming from the genital disc surrounds the testes, and a group of cells known as pigment cells surrounds, in turn, the muscles. When muscles contact testes, these elongate and spiralize. Mutations that impair muscle development, or changes in the sex of the muscles, from male to female, prevent testes elongation. The JNK and JAK-STAT pathways are active in the pigment cells, and inhibition of the JNK pathway, or a change in their sex also prevents testes elongation. Finally, a reduction in laminins changes testes morphology, suggesting that the extracellular matrix may play a role in the communication between pigment cells, muscles in the testes.

ww 179

BIOPHYSICAL MECHANISMS DRIVING CELL SHAPE PULSATIONS DURING MORPHOGENESIS

A. Sumi¹, K. Dierkes¹, G. Salbreux², J. Solon¹

¹ Centre for Genomic Regulation, Barcelona

² MPI for the Physics of Complex Systems, Dresden

Epithelial tissues are known to possess remarkable plasticity and undergo extensive remodeling during embryogenesis. Recent studies have revealed that in a number of developmental processes, these tissues exhibit pulsed contractions of their apical surface areas. We are investigating the biophysical mechanisms that drive these pulsations and their possible roles in tissue remodeling.

Our model is the amnioserosa (AS), a tissue covering the dorsal side of a developing *Drosophila* embryo that shows contractile pulsations.

We combine experimental and theoretical approaches to reveal that intrinsic properties of the acto-myosin cytoskeleton such as turnover play a major role in the generation of the contractile pulses. Simple biophysical model developed in the laboratory indicates that contractile pulses can be generated when turnover time is similar to the time scale of a contractile pulse. With high resolution imaging of myosin and cadherin, we have shown that AS cell cortex is turning over in about 100 seconds, which is similar to that of contractile pulse and consistent with our biophysical description.

Additionally, with laser dissection of the acto-myosin cortex, we assessed the effect of myosin concentration on cortical tension. We found a non-linear relationship that is consistent with our model's prediction.

Next, with controlled application of mechanical pressure, we are able to arrest the AS pulsations, and also to synchronize all the cells upon pressure release. We show that the timescale of the mechanical perturbation is critical for such synchronization to occur. Our theoretical approach recapitulates both the arrest of the contractile pulses and the synchronization of the cell pulses following pressure release. Our study highlights the role of acto-myosin dynamics in the regulation of cell shape during development and the influence of external mechanical forces on cell shape remodeling.

DLK 1 REGULATES BRANCHING MORPHOGENESIS AND PARASYMPATHETIC STIMULATION OF THE SALIVARY GLAND THROUGH INHIBITION OF NOTCH SIGNALING

P. García-Gallastegui¹, JJ García-Ramírez², V Baladrón², J Laborda², G. Ibarretxe¹, F. Unda¹

¹. Faculty of Medicine and Dentistry, University of the Basque Country, UPV/EHU, Bizkaia, Spain

². Regional Center for Biomedical Research, University of Castilla-La Mancha, Albacete, Spain

Morphogenesis of submandibular salivary gland (SMG) is dependent on branching process. This process requires events such as cell-cell and cell-matrix interactions, extracellular matrix components expressed at specific times and locations and epithelial-axonal interactions, since parasympathetic innervation occurs in parallel with salivary gland outgrowth.

The goal of our experiments was to investigate the function of DLK1 and DLK2 in SMG branching and innervation development.

We firstly localized DLK1, DLK2 and N1ICD (active form of NOTCH 1 receptor) during the development of the SMG. We performed a luciferase assay in HSG salivary gland cell line, which revealed that DLK1 and DLK2 inhibited NOTCH signaling *in vitro*. Thereafter, we cultured SMGs during 48h with a recombinant soluble form of DLK1 (sDLK1). We found a developmental growth delay and a reduction of the branching on the SMGs, associated to NOTCH signaling inhibition. In addition, by β -III Tubulin we detected a reduction on the innervation when compared to the control, which could be directly involved in the outgrowth delay.

Our next step was to culture SMGs with sDLK1 and Carbachol (CCh), an analog of acetylcholine used as an agent to mimic parasympathetic stimulation. SMGs cultured with sDLK1 and CCh at the same time, shown a branching morphogenesis not restored but innervation was partially recovered. However, when we removed the sDLK1 from the medium and CCh was added for 48h, branching of the SMGs increased when compared to SMG control, which received no CCh.

In conclusion, NOTCH ligands DLK1 and DLK2 are inhibitors of NOTCH signaling. DLK1 is a negative regulator of the morphogenesis of the salivary gland and its parasympathetic innervation.

Key words: DLK, SMG, NOTCH signaling pathway

EXPRESSION OR NOT DPP TWO DIFFERENT POPULATIONS OF GROWING CELLS IN *DROSOPHILA*

A. Macías, C. Arias, G. Fussero, M. Zacharonok

Cátedra “Genética”, Facultad de Ciencias Exactas Físicas y Naturales de la Universidad Nacional de Córdoba, Argentina

Cell competition is a mechanism whereby slow-growing cells confronted with fast-growing cells are eliminated by apoptosis. The factors that regulate growth can create cell competition based on differences in their expression levels among cells. Here, we analyzed the role of JNK, RGH and caspases in growth in the genital disc under physiological conditions. For this analysis, we tested the regulatory relationships among Dpp, JNK, RGH and caspases as well as tested their functions by overexpressing these genes and using genetic mosaics approaches. We determined there is an external signal and four internal conditions (the levels of Dpp, RH, Dronc and Drice) in the activation of JNK. In the genital disc, we identified two different growing population of cells that either express or do not express Dpp, leading to boundary cell competition. This discontinuity in growth is also marked by the upregulation of JNK, RGH and caspases and the occurrence of cell divisions that are presumably compensatory proliferation caused by competitive death. Although RGH appeared to be growth regulators and factors that mediate cell competition, we did not exclude JNK as acting in this same role.

PERIPODIAL EPITHELIUM EXPRESSION OF THE IROQUOIS COMPLEX GENES IS REQUIRED FOR VENTRAL ADULT HEAD MORPHOGENESIS

E. González-Pérez, S. Campuzano

Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain

Imaginal discs are sacs of epithelial cells formed by two opposing epithelial cell layers, one of them formed by columnar cells (the disc proper, DP) and another one (the peripodial epithelium, PE), formed mostly by squamous cells save at specific locations where they are of cuboidal shape. Although most of the adult cuticle derives from the DP region of the imaginal discs, it has been recently shown that in the eye-antennal disc PE cells contribute to the patterning and growth of the DP region of the imaginal discs during larval development and to the formation of certain adult structures. In the eye-antennal disc, the three genes of the Iroquois (Iro) complex *araucan*, *caupolican* and *mirror* are expressed in the dorsal region of both the DP and the PE. In the antennal domain of the PE, *iro*-expressing cells show a characteristic morphology, different from that of the rest of the PE cells. We have generated a novel regulatory *iro* mutation, *iro*^{EGP1}, which removes *caupolican* transcription unit and prevents *araucan* expression in the PE of the eye-antennal discs by interfering with the action of specific enhancers. The phenotypic analysis of the *iro*^{EGP1} flies shows the cell-autonomous requirement of *araucan* / *caupolican* expression at the PE for the proper development of the upper part of the ventral head capsule. Furthermore, albeit indirectly, the *iro* genes are also required for the normal development of the rest of the PE and the derived adult structures: the remaining of the ventral head capsule and the maxillary palps. Gene expression analysis in *iro*^{EGP1} antennal discs indicates that those phenotypic effects are not associated to misspecification of the maxillary palp territory since expression of the involved genes, *Deformed*, *Labial* and *decapentaplegic*, is unaffected.

COORDINATION OF PATTERNING AND MORPHOGENESIS DURING OPTIC CUP FOLDING

F. Cavodeassi, S. Salgüero, M. Ledesma and P. Bovolenta
Centro de Biología Molecular Severo Ochoa, Madrid, Spain

The optic vesicles constitute the first morphological manifestation of vertebrate eye formation. The optic stalk, neural retina and retinal pigmented epithelium (RPE) derive from the optic vesicle. Concomitant to its specification, the optic vesicle undergoes a complex morphogenetic transformation and folds to give rise to the optic cup. RPE specification occurs just prior to optic cup folding, and is followed by a dramatic change in the shape of the tissue. RPE cells spread over the back of the optic vesicle and give rise to a very cohesive and rigid squamous epithelium. The mechanical properties of the RPE have been proposed to contribute to optic cup folding, a hypothesis that up to date has not been tested. One of the reasons for the scarcity in data regarding this process is the lack of appropriate *in vivo* markers for the RPE.

We have made use of the regulatory sequences directing the expression of *bhlhe40*, one of the earliest markers of the RPE, to generate a transgenic line that labels the RPE primordium. We are currently using this new tool to undertake a dynamic analysis of the changes in RPE cell shape and organisation that accompany optic cup folding and to determine how they impact on optic cup morphogenesis. Here, we will present our preliminary data and our working model for the future.

RECIPROCAL REPRESSION BETWEEN PAX2 AND SNAIL CONTROLS EPITHELIAL PLASTICITY DURING EMBRYONIC DEVELOPMENT

O. Ocaña, J.M. Fons, H. Coskun, D. Abad, M.A. Nieto

Instituto de Neurociencias CSIC-UMH, San Juan de Alicante, Spain

During embryonic development, most tissues arise through several rounds of EMT/MET. In particular, the renal system, generated from the intermediate mesoderm (IM), is a paradigmatic example of epithelial plasticity. The first MET that takes place in the IM occurs during the formation of the pronephros, an epithelial tubule that grows caudally and symmetrically on both sides of the embryo. At the molecular level, the *Snail* genes are expressed in the IM and its disappearance correlates with the MET of these mesodermal cells. In contrast, the transcription factor *Pax2* is expressed in the emerging epithelial structures and its expression correlates with the necessary MET for pronephros differentiation. Here, we describe an antagonistic relationship between these two transcription factors during the development of the renal system. Using the chick embryo as a model we demonstrate that *Snail* represses *Pax2* in mesenchymal precursors and *Pax2* represses *Snail* during the epithelialization of the IM. In addition, we have found that BMP7 induces MET during pronephros differentiation by repressing *Snail1* and activating *Pax2* transcription at axial levels corresponding to the transition zone (TZ). These data indicate that the TZ, in addition to regulating neurogenesis and somitogenesis, also controls the differentiation of the IM in time and in space by regulating the expression of the *Pax2* and *Snail* genes. Therefore, the TZ behaves as a morphogenetic field that extends along the medio lateral embryonic axis of the embryo. Preliminary data indicate that the relationship between *Snail* and *Pax2* genes that we have described for the IM can be extended to other developmental processes. In summary, our results indicate that the reciprocal repression between *Snail* and *Pax2* genes may be used several times during embryonic development to control the epithelial plasticity. Keywords: *Snail*, *Pax2*, BMP7, EMT/MET, Mesoderm.

A COMBINATORIAL CODE OF MORPHOGENETIC SIGNALS CONTROLS NASO-TEMPORAL PATTERNING IN THE VERTEBRATE RETINA

M. Hernández-Bejarano¹, Alexander Picker³, P. Bovolenta¹, S. W. Wilson², G. Gestri², F. Cavodeassi¹

¹. Centro de Biología Molecular Severo Ochoa, Madrid, Spain

². University College London, London, UK

³. Current address: LIFE Biosystems GmbH, Heidelberg, Germany

Naso-temporal (NT) fates in the vertebrate retina are specified at the onset of optic vesicle (OV) evagination along the dorso-ventral axis of the nascent primordium. The earliest NT determinants activated in the retina are the transcriptional regulators *Foxg1* and *Foxd1*, which show complementary patterns of expression in the OV - *foxg1* is expressed in the dorsal (future nasal) half of the OV, while *foxd1* is expressed in the ventral (future temporal) half of the primordium. Fgf signals from the dorsal forebrain are required for specification of nasal identity in the dorsal half of the optic vesicle. In the absence of Fgf signalling, nasal markers are not expressed and the whole retina develops with temporal character. Conversely, ectopic Fgfs promote the expansion of nasal markers but the retina maintains some temporal identity. This suggests that, besides Fgfs, other signals likely influence NT regionalization. One of such candidates is Sonic-Hedgehog (Shh), which is expressed along the ventral midline of the embryo. Here we show that Shh activity is required to activate *foxd1* expression and to promote temporal fate in the OV. Conditions in which Shh activity is lost result in the downregulation of *foxd1* and the partial loss of temporal fate. Conversely, ectopic Shh activity in the dorsal OV results in the ectopic activation of *foxd1* and the repression of *foxg1*. These changes in *foxd1/foxg1* expression upon manipulations of the Shh pathway are independent of changes in the expression and/or activity of Fgfs. Once established, the complementary expression of *foxg1* and *foxd1* is maintained by mutual repression. With our results, we propose a model in which Fgfs and Shh, emanating from dorsal (Fgf) and ventral (Shh) sources of the forebrain primordium, work in concert to establish nasal and temporal fates in the nascent OVs.

THE BHLH TRANSCRIPTION FACTOR DYSFUSION REGULATES TARSAL JOINT FORMATION IN RESPONSE TO NOTCH ACTIVITY DURING *DROSOPHILA* LEG DEVELOPMENT

S. Córdoba, C. Estella

Departamento de Biología Molecular and Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid (UAM), Madrid, Spain

A characteristic of all arthropods is the presence of flexible structures called joints that connect all leg segments. *Drosophila* legs include two types of joints: the proximal or “true” joints that are motile due to the presence of muscle attachment and the distal joints that lack musculature. These joints are not only morphological, functional and evolutionary different, but also the morphogenetic program that forms them is distinct. Development of both proximal and distal joints requires Notch activity; however, it is still unknown how this pathway can control the development of such homologous although distinct structures. Here we show that the bHLH transcription factor encoded by the gene *dysfusion* (*dys*), is expressed and absolutely required for tarsal joint development while it is dispensable for proximal joints. In the presumptive tarsal joints, Dys regulates the expression of the pro-apoptotic genes *reaper* and *head involution defective* and the expression of the RhoGTPases modulators, *RhoGEf2* and *RhoGap71E*, thus directing key morphogenetic events required for tarsal joint development. When ectopically expressed, *dys* is able to induce some aspects of the morphogenetic program necessary for distal joint development such as fold formation and programmed cell death. This novel Dys function depends on its obligated partner Tango to activate the transcription of target genes. We also identified a dedicated *dys cis*-regulatory module that regulates *dys* expression in the tarsal presumptive leg joints through direct Su(H) binding. All these data place *dys* as a key player downstream of Notch, directing distal *versus* proximal joint morphogenesis.

PITX2B ISOFORM IS DYNAMICALLY EXPRESSED DURING HEART DEVELOPMENT

Francisco Hernández-Torres¹, Diego Franco¹, Francisco Navarro¹ and Amelia E. Aránega¹.

¹Department of Experimental Biology, Faculty of Experimental Sciences, University of Jaén, 23071, Jaén, Spain.

The Pitx2 gene is a member of the Bicoid-like homeobox family involved in the establishment of vertebrate left-right axis with an important role in the subsequent heart organogenesis. Mutations in Pitx2 gene have been associated with Axenfeld-Rieger syndrome, which is characterized by ocular, craniofacial, and umbilical anomalies, as well as cardiac defects, such as atrial septal defects, atrio-ventricular valve defects, and conduction abnormalities. In addition, recent data has unravelled a molecular link between PITX2 loss of function and atrial fibrillation (AF), supporting an important role of Pitx2 not only in development but also in heart homeostasis.

Three Pitx2 isoforms have been described in mice: Pitx2a, Pitx2b, and Pitx2c. During organogenesis Pitx2c seems to play a determinant role in left-right signalling from early somitogenesis onwards. However the participation of Pitx2a and/or Pitx2b isoforms during cardiogenesis is controversial. Here we report, for the first time, the coexpression of Pitx2a, Pitx2b and Pitx2c isoforms during heart development. Interestingly, Pitx2b and Pitx2c isoforms display similar expression profiles during cardiogenesis, decreasing with further development but maintaining its expression until adult stages. Moreover, a detailed analysis of Pitx2b protein during cardiac development show that Pitx2b is dynamically expressed in the developing ventricular septum and asymmetrically expressed in the tricuspid valves primordia suggesting an putative role of Pitx2b isoform during ventricular septation as well as in the maturation of the right portion of the atrioventricular canal.

REQUIREMENT OF FOI DURING *DROSOPHILA* MYOGENESIS

M. Carrasco-Rando, A. Atienza-Manuel, P. Martín, M. Ruiz-Gómez

Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain

During *Drosophila* myogenesis multinucleated muscles are generated through a fusion process between two kinds of myoblasts: the founder and fusion competent myoblasts. The segregation and the execution of the differentiation programme of these two populations of cells are essential to achieve a normal muscle pattern. We are interested in the characterization of novel genes controlling distinct aspects of myogenesis regulation.

Here we present data about the functional analysis of *fear of intimacy (foi)* during *Drosophila* myogenesis. Foi is a Zn transporter required for gonad formation, tracheal branch fusion and glial cell migration. We have found that Foi is also required for the proper development of the somatic and visceral muscles. The regulation of the concentration of cellular Zinc by Foi in mesodermal cells is essential for the correct function of different Zinc-Finger transcription factors, which have important roles during myogenesis.

A ROLE FOR NOTCH IN TROPHECTODERM SPECIFICATION IN THE EARLY MOUSE EMBRYO

S. Menchero¹, T. Rayon¹, A.K. Hadjantonakis², J.L. de la Pompa¹, M. Manzanares¹

¹. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

². Sloan-Kettering Institute, New York, USA

Cells generated from a zygote restrict their potential gradually to follow a specific lineage. The first cell fate specification event that takes place in the mammalian embryo results in the segregation of the trophectoderm (extraembryonic population) and the inner cell mass (embryonic population) at blastocyst stage. The position of the cells that define the early embryo before the establishment of these lineages is key to their specification. The differential activity of the Hippo pathway in inner or outer cells is also believed to result in the activation of lineage-specific transcriptional programmes. Recently, we have shown a role of Notch signalling in regulating trophectoderm specific expression of *Cdx2* which strengthen the idea that lineage determination at early stages is conferred through combinatorial inputs. Now, we are dissecting how the main components of the Notch pathway are modulated in order to integrate the interplay of different cues and to better understand how lineage-specific regulators are expressed in an initial stochastic manner, to then be restricted to specific populations of the blastocyst.

THE ABSENCE OF MYF5 AND MYOD IN EPAXIAL MUSCLE PROGENITORS CAUSES VERTEBRAL COLUMN CURVATURE ABNORMALITIES

M. López-Mayorga¹, N. Moncaut², L. Teboul³, M. R. Giráldez-Pérez¹, C. R. Bernal-Lozano¹, A. M. Castro-Cañal, P. W. J. Rigby², J. J. Carvajal¹

¹. Centro Andaluz de Biología del Desarrollo, Seville, Spain

². The Institute of Cancer Research, London, UK

³. MRC Harwell, Oxfordshire, UK

The determination and specification of skeletal muscle in vertebrates is orchestrated by the myogenic regulatory factors (MRFs): Myf5, Mrf4, MyoD and Myogenin. Myf5 is the first to be expressed in the embryo, initiating and co-ordinating the myogenic cascade. In absence of Myf5, progenitors fail to be specified; activation of MyoD rescues the phenotype and myogenesis progresses. In Myf5/MyoD KO animals rescue does not take place and animals lack all skeletal muscles. Transcription of the *Mrf4/Myf5* locus is controlled by over 25 regulatory elements. The Early Epaxial Enhancer (EEE) operates in the dermomyotomal dorsomedial lip (DML) and is the first to activate *Myf5* at E8.5. Although the contribution of the different regulatory elements to the expression pattern is well defined we lack an understanding on the contribution of different subpopulations of progenitors to adult musculature. Furthermore, there is still no connection between the spatiotemporal *Myf5* activation and its function within the different myogenic precursors.

To address these questions we are following two strategies: 1/ Generation of transgenic strains to analyse EEE function by lineage tracing and cell ablation methods, and 2/ Generation of an enhancer-specific KO strain in which the EEE has been targeted.

RNA-seq from somites 1-5 in *Myf5*^{EEE-/-} and WT embryos reveals several genes downregulated. We are now validating this data by qPCR and In Situ Hybridisation (ISH). Importantly we show that the muscle specific Mef2C transcription factor is downregulated in the new KO.

We observed that double *Myf5*^{EEE}/MyoD KO die perinatally and have started an in-depth analyses of possible abnormalities in these embryos. As some of the downregulated genes identified are involved in chondrogenesis, we are analysing bone and cartilage structures in these animals by Alizarin/Alcian staining as well as contrast tomography (μCT). In addition, we are performing Magnetic Resonance Imaging (MRI) analyses to detect possible muscular abnormalities.

SPATIOTEMPORAL CONTROL OF NEUROSENSORY DEVELOPMENT

L. Fargas, E. Hoijman, B. Alsina

Universitat Pompeu Fabra, Barcelona, Spain

The hair cells and sensory neurons of the inner ear are responsible for transmitting hearing and balance sensory information to the brain. The development of sensory neurons is highly spatio-temporally regulated. This process involves, first the specification of neuronal progenitors in the otic epithelium and secondly their delamination outside the epithelium, coalescence into the statoacoustic ganglion (SAG) and differentiation of axonal tracts that project to the hindbrain. A dynamical view of how signals are coordinated with cell specification, growth and morphogenesis is still missing. Using the zebrafish as a model system we have followed by time-lapse imaging the process of neurogenesis. Based on previous work in the vertebrate retina and in *Drosophila* optic lobe, we hypothesize that FGF generates a wave of proneural activity in the inner ear. We find that there is a progression of *neurogenin1* (*neurog1*) activation in the epithelia from anterolateral to posteromedial. However, this spatiotemporal wave does not match perfectly the one of FGF signaling activation reported by *dusp6* activity. High spatiotemporal resolution of the delamination process also shows that *neurog1* positive cells of the epithelium display fast and dynamic cellular remodelling processes in their apical and basal domains. At the apical domain, we detect a constriction and elongation of the apical side that, together with a physical pushing and competition event with neighbouring cells, causes the break of the apical contact of neuronal cells with the lumen. In addition, dynamical imaging of *pard3*-GFP localization shows that this process of apical constriction generates a transient mechanical deformation of the lumen that could contribute to the expansion and shaping of the inner ear cavity. On the other hand, at the basal membrane, highly dynamic foot-like protrusions are observed followed by the exit of sensory neurons from the epithelia.

NETWORK DEVELOPMENT INTO THE GASTROINTESTINAL TRACT

A. Margarido¹, L. Le Guen¹, S. Thorsteinsdóttir², P. de Santa Barbara¹

¹Inserm U1046, Montpellier, France

²Centro de Biologia Ambiental, Faculdade de Ciências, Universidade de Lisboa, Portugal

The gastrointestinal tract (gut) is a complex organ whose major functions are nutrients and water absorption. Its functioning relies on the coordinated work of many different tissue types such as smooth muscles innervation by the enteric nervous system (ENS), which allow the motility of food intake, while intestine's epithelium and the lymphatic system (LS) play a key role in nutrient absorption. Thus gut's development through embryogenesis requires precise fine-tuning for the establishment and differentiation of all its components. Interestingly, LS and ENS, two components of the gut that will form networks, do not develop intrinsically but rather colonize the gut from adjacent tissues. The LS develops from venous endothelial cells inside the cardinal vein that migrate towards a VEGFC gradient coming from the mesenchyme. The ENS differentiates from two distinct populations of neural crest cells: vagal and sacral. The vagal migrate in an anterior-posterior direction, giving rise to most of the cells composing the ENS, while the sacral migrate inversely, colonizing the post-umbilical part of the gut.

We found that although these two systems migrate towards the gut from very different locations and at different stages of development, they both require the expression of the same key regulatory gene (KRG). At initial stages (around E6) KRG is expressed in the nerve of Remak, a structure formed from sacral NCCs. Interestingly, colonization of the intestine by this population of cells correlates with KRG downregulation, around E9. At later stages (E18) KRG expression is turned on again within the submucosa layer and between circular and longitudinal muscles, this time labeling the lymphatic system.

Using *in ovo* electroporation technique we are now misexpressing KRG in the gut in order to address its function during ENS development.

FIBRONECTIN ASSEMBLY DYNAMICS DURING CHICK EMBRYOGENESIS – IS FIBRONECTIN A PARACRINE

SIGNAL? P. Gomes de Almeida¹, G. Pinheiro¹, S. Thorsteinsdóttir^{1,2}

¹. Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

². Instituto Gulbenkian de Ciência, Oeiras, Portugal

The extracellular matrix (ECM) is a key regulator of vertebrate development, controlling cell physiology, morphology, and differentiation. One of the most abundant ECM molecules during development is fibronectin. Fibronectin-deficient *Xenopus*, zebrafish and mouse embryos present various developmental defects, including *cardia bifida*, shortened anterior-posterior axis, and no somites, establishing fibronectin as essential for normal vertebrate development.

In the chick embryo, fibronectin is also crucial for somitogenesis. The presomitic mesoderm (PSM) produces integrin $\alpha 5$, a fibronectin assembly receptor, and is surrounded by a complex fibronectin matrix; however, *Fn1* is expressed by the overlying ectoderm. This suggests that fibronectin acts like a paracrine factors in this context, with one tissue producing and the other building the matrix. Intriguingly, fibronectin produced by the Wolffian duct was recently described as crucial for epithelialization of intermediate mesoderm tubes, being transferred from one epithelium to the other.

Here we address which tissues produce fibronectin throughout chick embryo development to better understand the dynamics of its production vs localization. Using *in situ* hybridization and immunohistochemistry, we characterized fibronectin expression and localization in various tissues in chick embryos until day 4 of incubation. We found evidence supporting a paracrine system in fibronectin matrix assembly in several tissues. For example, the non-neural ectoderm is a major site of *Fn1*-expression at several stages of development, both in the trunk and limbs, while the underlying mesenchyme presents an abundant fibronectin matrix. Furthermore, muscle progenitors delaminating from the dermomyotome seem to produce the fibronectin matrix observed in the mature myotome. Another example is the notochord which does not express *Fn1*, but has a thick fibronectin matrix, presumably coming from the *Fn1*-expressing sclerotome.

We aim to further characterize the assembly of fibronectin matrices during development, not only addressing their role as a supportive scaffold, but also determining whether fibronectin signals in a paracrine fashion.

DOES TISSUE TENSION PLAY A ROLE IN SOMITOGENESIS IN THE CHICK EMBRYO?

G. Pinheiro¹, P. Rifes², P. Gomes de Almeida¹, S. Thorsteinsdóttir¹

¹. Centro de Biologia Ambiental/Departamento de Biologia Animal, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

². DanStem, Kobenhavns Universitet, Kobenhavn, Denmark

Somitogenesis is morphogenetic process all the skeletomuscular components of the vertebrate. During embryogenesis in vertebrates, the cell types composing this system must arise, in the correct order and within tightly regulated time-frames to allow for the proper assembly and organization of this system. Therefore, it is of no wonder that somitogenesis itself would be a highly regulated process. To explain the potential regulation of this process, in 1976, Cooke and Zeeman presented the “Clock and Wavefront” model. In the chick embryo, cyclic expression of genes of the Notch signaling pathway have been associated with the “clock” part of the model, where as Fgf8 and retinoic acid gradients are thought to establish the “wavefront” (see Andrade et al., 2007). Besides this regulatory network, other cell-cell communication pathways have been found to influence somitogenesis. One example is Sonic hedgehog from the notochord (Resende et al., 2010) and another is fibronectin protein from the ectoderm (Rifes et al., 2007). Fibronectin protein is essential for the assembly of a fibronectin extracellular matrix around the presomitic mesoderm (PSM) which is crucial for somite formation.

Here we use tension-inhibiting drugs to test whether tissue tension sensed by PSM cells is one of the factors contributing to its correct and timely maturation.

We cultured bisected explants of posterior regions of chicken embryos in a tension inhibiting drug (RockOut) for 6 or 12 hours and assessed the effect of the drug on gene expression, cell morphology and fibronectin organization. We find that treatment with RockOut leads to a delay in the expression of *hairy2* and also to a delay in somite formation, but does not significantly affect *fgf8* or *raldh2* expression, both markers of the wavefront. These results suggest that tissue tension may play specific roles in the preparation of the PSM for somite formation.

HIGHLY EFFICIENT TARGETED MUTAGENESIS IN ONE-CELL MOUSE EMBRYOS MEDIATED BY TALEN AND CRISPR/CAS SYSTEMS

A. Yasue¹, S. N. Mitsui¹, T. Watanabe¹, T. Sakuma², S. Oyadomari¹, T. Yamamoto², S. Noji¹, T. Mito¹, E. Tanaka¹

¹. The University of Tokushima, Tokushima, Japan

². Hiroshima University, Hiroshima, Japan

Recent development of TALEN and CRISPR/Cas enables to induce site-specific mutations for various species. Although gene targeting technology using ES cells have been employed for a long time, the application of TALEN and CRISPR/Cas techniques to mice also attracts attention. In this study, we attempted to establish gene targeting technologies of TALEN and CRISPR/Cas systems by RNA microinjection into one-cell mouse embryos.

TALEN pairs and guide RNA target sites were designed in mouse *Fgf10* gene. This gene was selected according to the simply identified limb defects phenotype of homozygous mutants. RNAs of TALENs, guide RNA and Cas9 were generated by in vitro transcription. Then, the mRNAs were microinjected into fertilized mouse eggs. After culturing overnight, oviducts implantation of resulting two-cell embryos into pseudopregnant females was carried out. To detect *Fgf10* TALEN- and CRISPR/Cas-induced mutations, both Surveyor (Cel-I) nuclease assay and DNA sequencing were used.

In the embryos and neonatal mice created by TALEN RNA injection, up to 48% of mutation induction was identified. Two of 129 embryos (2%) showed limb defect phenotype. Mosaicism was detected in both embryos and newborns. All of the examined TALEN-modified alleles were confirmed to be transmitted through the germline. For the embryos produced by CRISPR/Cas system, a strikingly higher rate of limb defect phenotypes (40 - 100%) were obtained. Mosaicism and abnormal limb development were occasionally observed in embryos created by CRISPR/Cas system.

Gene disruptions at the target sites were induced by TALEN and CRISPR/Cas technologies through the RNA microinjection at one-cell stage of mouse embryo, however, the CRISPR/Cas system more effectively elicited single-step biallelic mutations.

QUANTIFICATION AND MODELLING OF THE GENE REGULATORY NETWORK THAT CONTROLS AND SPECIFIES THE *DROSOPHILA* RETINA

M. Sánchez-Aragón, F. Casares

CABD (Andalusian Center for Developmental Biology), CSIC

Universidad Pablo de Olavide - Junta de Andalucía, Seville, Spain

Drosophila imaginal discs are excellent models to study developmental processes, including the spatial and temporal regulation of gene expression by intercellular signals, the coupling of these expression changes to specific proliferation/death programs and the global regulation of organ size and shape. Our work focuses on understanding the regulatory logic that control the differential expression of key transcription factors during the process of specification of the *Drosophila* retina. This logic is based on quantitative activation and repression interactions among transcription factors and signaling pathways. However, obtaining quantitative information on gene expression from imaginal discs in 3D is not a trivial task, and current standard methods based in watersheds do not perform well. To improve the 3D segmentation methods of Laser Scanning Confocal Microscopy (LSCM) data, we have developed a new method, combining graphs and hierarchical clustering, which outperforms current watershed-based methods in retrieving nuclei segments from standard LSCM data. This method has been implemented in a software, iFLIC, which then is used to obtain quantitative data, with single cell resolution, from fluorescence signal of the LSCM image stacks. We tested its precision by applying this software to a biological problem: determining the effect of preventing the normal cell cycle stop at the morphogenetic furrow (MF) over atonal expression, a gene that is known to be required for photoreceptor specification. This cell cycle stop leads to an observable phenotype. We could demonstrate that, whereas the average levels of atonal are normal, the cell-to-cell variability of atonal expression is higher posterior to the MF, explaining in part the observed phenotype in the *Drosophila* eye.

Using iFLIC and modeling methods we are currently investigating the quantitative aspects of the retinal determination network, as a particularly tractable organ-specification network, with the hope that this work will throw light on general principles of gene network function.

FUNCTIONAL PARTNERSHIP BETWEEN ABDOMINAL-B AND EXTRADENTICLE-HOMOTHORAX IN THE ABDOMEN

JR. Curt¹, N. Sambrani², B. Hudry², S. Merabet², A. Saurin², Y. Graba², E. Sánchez-Herrero¹

¹. CBMSO, CSIC, Madrid, Spain

². IBDML, CNRS, Marseille, France

The Hox genes specify different structures in the fly with the aid of cofactors like those encoded by the genes extradenticle (exd) and homothorax (hth). We are interested in studying the role of exd and hth in modifying the activity of the Hox gene Abdominal-B (Abd-B) in a sexually dimorphic structure, the seventh abdominal segment (A7), which is repressed in males. We analyzed the transcriptional regulation between Abd-B and exd-hth and the regulation of several Abd-B targets, such as Dsx, Wg, bab, tsh, and the EGFR pathway. We have found that a reduction in exd or hth expression ectopically activates wg and that increasing hth expression suppresses wg expression in an Abd-B mutant background. We have also tested several residues from the Abd-B protein that are likely candidates of being interaction sites with the Exd protein with an co-immunoprecipitation assay in vitro. A protein mutant in the tryptophane close (and N-terminal) to the homeodomain significantly reduces the interaction between the two proteins in vitro. We are analyzing the effect of this mutant protein on Abd-B activity. This set of experiments will allow us to differentiate between the role of hth-exd as Abd-B cofactors and their independent role in the development of the A7 segment.

DEVELOPMENTAL RESPONSE TO BLOCKING GROWTH IN DIFFERENT GENETIC DOMAINS OF THE DROSOPHILA WING DISC

A.J. Montes-Ruiz, G. Morata

Centro de Biología Molecular Severo Ochoa, Madrid, Spain

We are studying the mechanisms of growth control in the wing disc. To this effect we are analyzing the response of the disc to blocking the growth of different genetic domains or compartments. This is achieved by compromising, by RNA interference, the activity of the CDK1 gene, necessary for cell division

In our experiments we block the growth of either open lineage domains like the Rotund (Rn), or closed lineage domains like the posterior (P) or the dorsal (D) wing compartment.

The Rn domain covers approximately 40% of the wing disc, and we find that blocking cell division for most of larval period in the entire region has little, or minor, effect on the size, shape and identity of the domain. Pulse and chase experiments using BrdU incorporation as marker of cell able to divide indicate that under these conditions the Rn domain is built by the continuous incorporation of cells migrating from outside. This observation demonstrates a powerful homeostatic mechanism controlling size and identity.

The experiments blocking growth of P or D compartments yield different results. After short term (up to 48hrs) CDK1 inactivation, compartments are able to regulate their size by increasing the size of individual cells. However, this control mechanism cannot cope with longer (72 hrs) inactivation of CDK1 and then the affected compartments become very small. Probably reflecting a proliferative response to the growth arrest, these compartments show high levels Yorkie activity and of its targets diap1, expanded and bantam. Interestingly, the up regulation of Yki targets in the arrested P or D compartments extends non autonomously to the Anterior and Ventral compartments respectively, which exhibit an increase of cell proliferation in the vicinity of the compartment boundaries. We also find that clones of cells near the boundaries show abnormal shape, indicating alterations in cell orientation, which are reflected in the expression of planar polarity genes like four jointed and dachous. These observations suggest the existence of crosstalk between compartments that has not been observed before.

Finally, we have also studied how growth arrest of the Rn and P and D domains is reflected at the organismic level by examining expression of the secreted dilp8 peptide. We find that there is strong dilp8 activation when the P or D compartments cannot compensate size. If there is size compensation, like in the arrested Rn domain, dilp8 is not activated.

DISTINCT TISSUE-SPECIFIC REQUIREMENTS FOR THE ZEBRAFISH TBX5 GENES DURING HEART, RETINA AND PECTORAL FIN DEVELOPMENT

A. Pi-Roig^{1,2}, E. Martín-Blanco¹, J. Garcia-Fernández², C. Minguillón¹

¹CSIC-Institut de Biologia Molecular de Barcelona

²Facultat de Biologia, Universitat de Barcelona

The transcription factor Tbx5 is expressed in the developing heart, eyes and anterior appendages. Mutations in human TBX5 cause Holt–Oram syndrome, a condition characterized by heart and upper limb malformations. Tbx5-knockout mouse embryos have severely impaired forelimb and heart morphogenesis from the earliest stages of their development. However, zebrafish embryos with compromised *tbx5* function show a complete absence of pectoral fins, while heart development is disturbed at significantly later developmental stages and eye development remains to be thoroughly analysed. We identified a novel *tbx5* gene in zebrafish—*tbx5b*—that is co-expressed with its paralogue, *tbx5a*, in the developing eye and heart and hypothesized that functional redundancy could be occurring in these organs in embryos with impaired *tbx5a* function. We have now investigated the consequences of *tbx5a* and/or *tbx5b* downregulation in zebrafish to reveal that *tbx5* genes have essential roles in the establishment of cardiac laterality, dorsoventral retina axis organization and pectoral fin development. Our data show that distinct relationships between *tbx5* paralogues are required in a tissue-specific manner to ensure the proper morphogenesis of the three organs in which they are expressed. Furthermore, we uncover a novel role for *tbx5* genes in the establishment of correct heart asymmetry in zebrafish embryos.

NON-CANONICAL WNT PATHWAY, WOUND HEALING AND NEURAL TUBE CLOSURE IN MOUSE EMBRYO

P. Ybot-González, L. Mañas-García, C. Lazarini-Suarez, and B. Lopez-Escobar

Grupo de Neurodesarrollo, Unidad de Pediatría, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, Spain

Neural tube closure is a complex developmental process that takes place early during embryogenesis and is a key step in neurulation. Neurulation is regulated to a great extent by the non-canonical Wnt signalling pathway. Mutation of genes in this pathway, such as *Vangl2*, *Celsr* and *disheveled*, yields embryos that exhibit severe neural tube defects owing to failure of initiation of neural tube closure. However, there is an increasing evidence for the non-canonical Wnt signalling pathway to be involved in the final step in neurulation, the closure of the posterior neuropore. The non-canonical Wnt signalling leads to the reorganization of actin cytoskeleton via the activation of the Rho family of small GTPases (RhoA and Rac1) and their downstream protein kinases (JNK or Rho associated kinase, ROCK). This signalling mechanism regulates polarized cell movement and establishes planar cell polarity (PCP) that are essential as well as for neural tube closure for other processes like epidermal wound repair. Interestingly, mice mutants for genes implicated in embryonic wound repair, like *Grainy head-like 3* and *PDGFr-alpha* present a phenotype of spina bifida, demonstrating a clear interaction between wound healing and neurulation. Moreover blocking assembly of the actin cable in chick and mouse embryos, by drugs or by inactivation of the small GTPase Rho, severely hinders the re-epithelialization process as well as neural tube closure. Our preliminary results demonstrate the presence of the wound healing machinery at the time of final step of the posterior neuropore closure. In addition, chemically blocking wound healing processes leads to an inhibition of neural tube closure. Thus, embryonic wound re-epithelialization machinery recapitulates that used during naturally occurring morphogenetic episodes as typified posterior neuropore closure, suggesting that in order to “heal”, the embryo uses general mechanisms that regulate natural cell and tissue movements in the embryo.

VALIDATION OF CABUT TARGET GENES IDENTIFIED BY CHIP-ON-CHIP AND MICROARRAY EXPRESSION ANALYSES

V. Muñoz-Soriano^{1,2}, S. López-Domenech^{1,2}, Y. Belacortu¹, N. Paricio^{1,2}

¹. Universidad de Valencia, Valencia, Spain

². ERI de Biotecnología y Biomedicina, Universidad de Valencia, Valencia, Spain

Cbt is the *Drosophila* ortholog of the mammalian TGF- β -inducible early-response genes (TIEG) proteins, which belong to Sp1-like/KLF family of transcription factors. It is involved in dorsal closure (DC) during embryogenesis, but is also required for other processes such as neuroendocrine cell remodeling, epithelial regeneration or circadian rhythms. DC is a morphogenetic movement in which the lateral epidermal sheets migrate and fuse over the amnioserosa to form a continuous epithelium. *cbt* mutant embryos present anterior holes and defects in the elongation of the dorsal-most epidermal cells as well as in the actomyosin cable assembly at the leading edge. Cbt functions downstream of JNK signaling during DC, regulating *dpp* expression in the leading edge cells. To identify direct transcriptional Cbt targets, ChIP-on-chip and microarray expression analyses were performed in embryos during DC. We have selected several candidates to validate their functional relationship with *cbt* and the DC process. RT-qPCR expression assays as well as immunostaining experiments, genetic interactions and phenotypic analyses of mutants, suggest a functional relationship between *cbt* and some of the candidate genes. We will present these results and discuss the possible role of these genes in the DC process.

INTERACTION BETWEEN *Bmp2* AND miR-133 DURING EARLY CHICK CARDIAC DEVELOPMENT

C. Lopez-Sanchez¹, D. Franco², A. Aranega², A. Ortiz¹, V. Garcia-Lopez¹, F. Bonet², V. Garcia-Martinez¹

¹. University of Extremadura, Badajoz, Spain

². University of Jaen, Jaen, Spain

In the last few years, several authors have revealed that microRNAs play essential roles in distinct and diverse biological processes, including embryology and pathology. In our laboratory, we have recently investigated the role of several microRNAs in cardiac development. In this work we will show a detailed analysis of miR-133 expression during early cardiogenesis. By using *in situ* hybridization with microRNA-specific LNA-probe miR-133, from early gastrula (HH2; PS2) to early organogenesis (HH12) stages in chicken embryos, we observed its expression at the levels of primitive streak and epiblast, being observed subsequently in precardiac mesoderm and primitive endocardial tubes.

In order to determine the role of miR-133 during cardiogenesis, we conducted gain-of-function experiments with miR-133, by means of *in vitro* electroporation at the primitive streak level (where the precardiac cells are initially located) by using premiR-133. Our results show that *Fgf8* expression decreases, while *Bmp2* expression increases after electroporation, both relevant genes involved in cardiac induction. Moreover, the specific cardiac markers *cNkx-2.5* and *GATA4* present an increased expression, while *AMHC1* and *Tbx5* expressions are diminished. Additionally, we observed that *Bmp2* overexpression induces similar effects: induction of *cNkx-2.5* and *GATA4*, and suppression of *Fgf8*, *AMHC1* and *Tbx5*. Interestingly, our *Bmp2* overexpression experiments proved to induce miR-133.

All these data together suggest that there must be a *Bmp2*-miR-133 interaction. Therefore, miR-133 would regulate RAS/MAPK pathway, by binding to specific sequences in the 3'UTR of target gene *Fgfr1*, as well as retinoic acid signaling, by linking to specific sequences in the 3'UTR of target gene *RARB*.

This work has been partially supported by Grants GR10067 (to VGM) from the Junta de Extremadura, with FEDER co-financing, and CVI-6556 (to DF) from the Junta de Andalucía Regional Council.

THE EGFR PATHWAY REGULATES GUT BRANCHING MORPHOGENESIS IN INTACT AND REGENERATING PLANARIANS

F. Cebrià, S. Barberán

University of Barcelona, Catalunya, Spain

Branched structures occur in a wide range of organs and systems within different animal groups. Although their final shape and function differ greatly, the EGFR signalling pathway has been shown to play important roles during key steps of branching morphogenesis in all studied model systems. The regeneration model planarian *Schmidtea mediterranea* has two branched organs: the gut and the excretory system. Among the six EGF receptors (EGFR) identified, *egfr-5* is expressed in and essential for the branching of the excretory system; on the other side, *egfr-1* and *egfr-2* are expressed in the gut, but their possible function in gut morphogenesis is still unknown. Here we show that *egfr-1* is required for proper gut branching during planarian regeneration and homeostasis. *egfr-1* (RNAi) animals have shorter primary gut branches, fewer and shorter secondary and no tertiary and quaternary branches. Moreover, their gut has a very reduced lumen, aberrant tissue organization, and less gastrodermal cells. We have looked further into the *egfr-1* function by studying upstream and downstream elements. We have identified six putative EGF ligands and are currently addressing their functional relation with *egfr-1*. A comparative transcriptomic approach has helped us to identify putative target genes missregulated after *egfr-1* (RNAi). Among them we have found several genes involved in branching and tissue morphogenesis, as well as regulators of cell shape and proliferation. At this time, we are deciphering the implications of a selection of them in the progression of the *egfr-1* phenotype. Altogether, our results support a function of the EGFR signalling pathway in the development of branched structures in the planarian *S. mediterranea*. Our data together with the role of EGFR pathway in branching morphogenesis in *Drosophila* and mammals suggest that the diversity of animal branched structures evolved convergently using similar molecular mechanisms.

THE CONSERVED TRANSMEMBRANE PROTEOGLYCAN PERDIDO IS ESSENTIAL FOR MYOFIBRILLOGENESIS

J. J. Pérez-Moreno¹, M. Bischoff², M. D. Martín-Bermudo¹ and B. Estrada¹

¹. Centro Andaluz de Biología del Desarrollo (UPO-CSIC), Seville, Spain

². University of Cambridge, Cambridge, UK

Muscle differentiation requires the assembly of high-order structures called myofibrils, composed of sarcomeres. Even though the molecular organization of sarcomeres is well known, the mechanisms underlying myofibrillogenesis are poorly understood. It has been proposed that integrin-dependent adhesion nucleates myofibrils at the periphery of the muscle cell to sustain sarcomere assembly. Here, we report a role for the gene *perdido* (*perd*, also known as *kon-tiki*, a transmembrane chondroitin proteoglycan) in myofibrillogenesis. Expression of *perd* RNAi in muscles, prior to adult myogenesis, can induce misorientation and detachment of *Drosophila* adult abdominal muscles. In comparison to controls, *perd*-depleted muscles contain fewer myofibrils, which are localized at the cell periphery. These myofibrils are detached from each other and display a defective sarcomeric structure. Our results demonstrate that the extracellular matrix receptor Perd has a specific role in the assembly of myofibrils and in sarcomeric organization. We suggest that Perd acts downstream or in parallel to integrins to enable the connection of nascent myofibrils to the Z-bands. Our work identifies the *Drosophila* adult abdominal muscles as a model to investigate in vivo the mechanisms behind myofibrillogenesis.

THE MYOTOME IS NECESSARY FOR NORMAL EPAXIAL MUSCLE DIFFERENTIATION

A. Gonçalves¹, M. Deries¹, A. Nunes¹, M. Luz¹, S. Tajbakhsh³, P. Ybot-Gonzalez⁴, S. Thorsteinsdóttir^{1,2}

¹. Centro de Biologia Ambiental, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

². Instituto Gulbenkian de Ciência, Oeiras, Portugal

³. Cellules souches et développement, Institut Pasteur, Paris, France

⁴. Hospital Universitario Virgen del Rocío / Instituto de Biomedicina de Sevilla, Sevilla, Spain.

Skeletal muscle plays a crucial role for the fitness of all animals including humans. Acting in a well-coordinated genetic hierarchy, Myf5, Mrf4, MyoD and Myogenin are four myogenic regulatory factors involved in the specification and differentiation of muscle cells in the myotome of all vertebrate embryos. This myogenic programme is also influenced by the upstream transcription factors Pax3 and Pax7, both markers of the mesoderm-derived myogenic precursor cells (MPCs). Although much knowledge exists about how these genetic hierarchies control the muscle differentiation programme, much less is known about how they translate into the spatial organization of MPCs, myoblasts and differentiated cells into the correct positioning of muscle groups.

To address this issue, we are studying epaxial muscle morphogenesis in *Myf5^{nLacZ/nLacZ}* mutant embryos, lacking both *Myf5* and *Mrf4* (Tajbakhsh et al. Nature. 266:270, 1996), in which the myotome is absent, and epaxial myogenesis arises with a delay in MyoD expression. We find that two of the three epaxial muscle groups are missing in the mutants and that MPCs are absent in the areas of the missing muscle groups. Moreover, MyoD is not upregulated throughout the whole segment, as occurs during normal myotome development, but is restricted to epaxial and hypaxial dermomyotomal lips raising the hypothesis that signals from the myotome are necessary for the differentiation of these two muscle groups.

Together these results suggest that MPCs from the central dermomyotome do not delaminate and upregulate MyoD. The environment created by the myotome therefore seems to be essential for the recruitment of the central dermomyotome into the myogenic lineage. We are currently investigating what combination of cues released and organized by the myotome are involved in triggering the differentiation of these epaxial muscle groups.

SPATIO-TEMPORAL CONTROL OF ACTO-MYOSIN CONTRACTILITY IN DROSOPHILA FOLLICULAR EPITHELIUM

M. D. Martín Bermudo¹, I. Grosheva¹, D. Gómez Mígues², A. González Reyes¹

¹. Centro Andaluz de Biología del Desarrollo, Sevilla, Spain

². Universidad Autónoma de Madrid, Madrid, Spain

Coordinated acto-myosin driven cell contraction underlies organ shape generation during development. An excellent model for understanding genetic control of contractility in organogenesis is provided by the *Drosophila* follicular epithelium, which surrounds developing eggs in the fly ovary. Squeezing force required for egg elongation is produced by periodic contraction of acto-myosin stress fibers formed in the follicular epithelium at late stages of oogenesis. Mechanisms spatially and temporally regulating these contractile structures are currently unknown. In the course of this work we have described involvement of negative contractility regulator Myosin Light Chain Phosphates (MLCP) in the control of contractile structures assembly and activity.

Visualization of endogenous MLCP revealed its direct localization to basal domain of follicle cells and association with actin filaments in order to restrict acto-myosin interaction. MLCP release from the basal surface happens concomitantly with formation of acto-myosin arrays providing cell contraction. Moreover, removal of MLCP from follicle cells results in premature formation of basal acto-myosin structures. Thus, MLCP functions as an intracellular timer controlling onset of contractile activity. Intriguing feature of follicle cell behavior is their periodic contraction driven by cycles of basal myosin recruitment and release. We have shown that MLCP knock-out cells loose characteristic periodicity and myosin pulsation becomes stochastic. This suggests that in addition to temporary control of contractile array's formation MLCP is also required for maintenance of their oscillatory behavior. In order to better understand nature of cell oscillation, we performed mathematical modeling of basal myosin dynamics in wild type and mutant conditions. The model we generated not only reproduced *in silico* experimentally observed cell behavior, but also produced some insights into mechanosensitive feedback circuit operating during cell pulsation. We are currently testing in living cells prediction made by the model.

CELL COMPETITION IN HEART DEVELOPMENT AND HOMEOSTASIS

C. Villa del Campo, C. Claveria, R. Sierra, M. Torres

Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

Heterogeneous anabolic capacity in cell populations can trigger a phenomenon known as cell competition, through which less active cells are eliminated. Cell competition has been induced experimentally in stem/precursor cell populations in insects and mammals and takes place endogenously in early mouse embryonic cells. Here we show that cell competition can be efficiently induced in mouse cardiomyocytes by mosaic overexpression of *Myc* both during gestation and adult life. The expansion of the *Myc*-overexpressing cardiomyocyte population is driven by the elimination of wild type cardiomyocytes. Importantly, this cardiomyocyte replacement is phenotypically silent and does not affect heart anatomy or function. These results show that capacity for cell competition in mammals is not restricted to stem cell populations and suggest that stimulated cell competition has potential as a cardiomyocyte replacement strategy.

MEMBRANE TYPE-4 MATRIX METALLOPROTEINASE (MT4-MMP) EXPRESSION DURING MOUSE EMBRYONIC DEVELOPMENT

C. Sánchez-Camacho^{1,2}, M. J. Blanco¹, M. Martín-Alonso², M. Seiki³, A. G. Arroyo²

¹. Universidad Europea de Madrid (UEM), Villaviciosa de Odón, Madrid, Spain

². Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

³. Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo, Japan

Matrix metalloproteinases (MMPs) constitute a large group of endoproteases that play essential functions during embryonic development, tumor metastasis and vasculogenesis by degrading components of the extracellular matrix. Within this family, there is a distinct subset of MMPs that has received less attention, those that are tethered to the cell membrane (MT-MMPs). We have focused our study on MT4-MMP (MMP17) that is anchored to the cell surface via a glycosylphosphatidylinositol (GPI) moiety and with the catalytic site exposed to the extracellular space. Information about its function and substrates is very limited to date, and almost nothing has been reported on its role in the developing embryo. Here, we report a detailed expression analysis of Mmp17 during mouse embryonic development by using a LacZ reporter transgenic mouse line (Rikimaru et al., 2007). We showed that MT4-MMP is highly expressed from early stages of development to postnatal stages following a very dynamic and restricted pattern. MT4-MMP was first detected at E8.5 restricted to the intersomitic vascularization, the endocardial endothelium and the dorsal aorta. Mt4-mmp^{LacZ/+} cells were also observed in the somites, floor plate and notochord at early stages. From E10.5, expression was detected in the limb buds and persists during limb development. Stronger expression in the brain begins at E14.5 and continues to postnatal stages. It was observed particularly in the olfactory bulb, the cerebral cortex, the hippocampus, the retina and the spinal cord. Our data suggest important roles of this metalloproteinase during embryonic development, and in particular during brain formation, vasculogenesis and limb development.

THE ROLE OF THYROID HORMONE SIGNALING IN PANCREATIC β CELL MATURATION IN ZEBRAFISH

H. Matsuda¹, D. Hesselson², D. Stainier¹

¹. Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany

². Gravan Institute, Sydney, Australia

Organ development in vertebrates, including mammals, often involves a two-step process: the formation of an immature but somewhat functional organ during embryogenesis followed by the maturation into the adult form. Often this second step takes place during the so-called post-embryonic developmental stage, when plasma thyroid hormone (TH) concentrations are high. The pancreas is one of these vertebrate organs that undergoes functional and morphological changes during postembryonic development. We are especially interested in the molecular mechanism underlying how the glucose-sensing, fully functional mature β cell emerges during postembryonic development. Here we investigated the role of TH, which is known as one of the key factors for tissue maturation/adult organ development, in the pancreas during postembryonic development using zebrafish as a model system.

To understand the roles of TH in pancreas development, we first generated TH reporter fish and visualized the target tissues of TH. Identified TH targets in the pancreas during postembryonic development comprise the endocrine lineage, including β cells. Next, we investigated the effect of TH on β cell development in larval zebrafish treated with or without TH. These results indicated that TH enhances insulin expression levels. In addition, we found that glucose levels in TH-treated larvae were reduced, correlating with upregulation of insulin expression.

These results suggest that TH is involved in generating more functional mature β cells during postembryonic development, at least in part through enhancing insulin expression. We will discuss how TH might enhance insulin expression, as well as how TH might also affect other endocrine lineages.

THE BASEMENT MEMBRANE AS A REGULATOR OF “GLOBAL TISSUE ROTATION” DURING *DROSOPHILA MELANOGASTER* OOGENESIS

MC. Díaz de la Loza, A. Díaz-Torres, MD. Martín-Bermudo, A. González Reyes
Centro Andaluz de Biología del Desarrollo (CABD), Seville, Spain

Elongation of *Drosophila* eggs depends on a particular type of morphogenetic movement during oogenesis termed “global tissue rotation”. This consists in the rotation of the egg chambers (developing eggs) along their anterior-posterior axis during mid-oogenesis. Similarly to the traditional migration of an epithelial sheet, interactions between the follicular epithelium of the egg chamber and the basement membrane (BM) are essential for the above rotation movement. We have used live imaging to clarify the role that the interaction between the BM and the epithelial follicle cells plays in the regulation of egg chamber rotation. We report that egg chambers from mutant females showing low levels of Laminins, an essential component of BMs, undergo premature rotation. We also find that proper oocyte positioning in the egg chamber depends on a regulated rotation, as mutant egg chambers display misplaced oocytes. Finally, we have characterized the formation of highly dynamic filopodia-like protrusions at the basal side of the follicle cells that seem to control the direction of rotation. We propose that BM composition regulates the timing of rotation by supporting the formation of basal filopodia-like projections in follicle cells.

LAMININS ARE REQUIRED FOR PROPER MIGRATION OF EMBRYONIC HAEMOCYTES IN *DROSOPHILA MELANOGASTER*

B. J. Sánchez-Sánchez¹, J. M. Urbano², K. Comber³, W. Wood³, M. D. Martín-Bermudo¹

1. Centro Andalúz de Biología del Desarrollo (CABD), Sevilla, Spain

2. PDN Department, University of Cambridge, UK

3. Department of Developmental Biology, University of Bristol, UK

The extracellular matrix (ECM) covers the basal side of all epithelia and endothelia and surrounds muscles, peripheral nerves and other tissues providing physical support. In addition, the ECM has been implicated in many processes such as cell differentiation, shape, adhesion, survival and migration. The main producers of ECM in the embryo are fat body and macrophages. Macrophages (haemocytes) not only constitute the first line of defence against infection but also help to sculpt organs and tissues of the embryo by removing dead cells and secreting ECM components, such as Perlecan, Laminin, Collagen IV and Nidogen. Key to their function is the ability of embryonic macrophages to migrate and disperse throughout the embryo. Yet despite these important developmental functions, little is known about the molecular mechanisms underlying embryonic macrophage migration in vivo. Using *Drosophila Melanogaster* macrophages as a model system, we have analysed the role of different ECM components, such as Laminins, Collagen IV and Perlecan, during haemocyte migration. Our results show that Laminins are the main ECM components supporting haemocyte migration. Among the two Laminin trimers present in *Drosophila*, we have identified the Laminin $\alpha 1,2; \beta 1; \gamma 1$, as the main Laminin required in this process. We show that Laminins are required at different stages of the migratory process, during both early phases of migration, such as the migration into the tail, and late migratory events, such as random and lateral migration over the ventral nerve cord. In addition, laminins are required for the contact-repulsion events that keep an even distribution of macrophages and for lamellipodia formation and stability. Furthermore, our results support the idea that haemocytes secrete the Laminin necessary for their migration as they move.

INTEGRINS POSITIVELY REGULATE CELL SURVIVAL IN THE WING IMAGINAL DISC IN *DROSOPHILA MELANOGASTER*

A. Valencia-Expósito¹, M. J. Gómez-Lamarca², T.J. Widmann³, M.D. Martín-Bermudo¹

¹. Centro Andaluz de Biología del Desarrollo (CABD), Sevilla, Spain

². University of Cambridge, Cambridge, UK

³. Pfizer-University of Granada-Junta de Andalucía Centre for Genomics and Oncological Research (GENYO), Granada, Spain

Integrins are a widely expressed family of transmembrane receptors that binds preferentially extracellular matrix components. Integrins can provide mechanical links and activate different pathways, thus regulating various cellular processes, including cell survival. In fact, disruption of integrin function results in a class of apoptosis called anoikis. During development, anoikis is an important surveillance mechanism, ensuring that any cell that loses its appropriate position within a tissue is targeted for death. However, although there is a lot of information about the role of integrins in promoting cell survival in cell culture experiments, little is known about its role during morphogenesis. Thus, in this study we aim to better understand the role of integrins in regulating cell survival during development using *Drosophila* wing imaginal disc as a model system. We show that loss of integrin function in the wing disc results in caspase dependent anoikis. We also show that removal of integrins results in ectopic activation of JNK pathway. Moreover, we can rescue cell death phenotype due to loss of integrins by downregulating JNK activity. We also found that removal of integrins leads to increase expression of the proapoptotic gene *hid*. Again, downregulation of *hid* expression rescues cell death due to absence of integrins. Altogether, our results suggest that integrins regulate cell survival by inhibiting *hid* expression through downregulation of JNK pathway. In addition, our data show that loss of integrin function results in an increase in myosin tension. Interestingly, we found that an increase in tension per se induces cell death. Furthermore, we can partially rescue the cell death phenotype observed in integrin mutant cells by reducing tension levels. These results lead us to propose that in a physiological context integrins regulate cell survival in part by controlling cellular tension.

THE DUAL-SPECIFICITY PHOSPHATASE CG10089 REGULATES NEGATIVELY THE ACTIVITY OF THE EGFR SIGNALLING PATHWAY IN THE DROSOPHILA WING

C. M. Ostalé, C. Molnar, J. F. de Celis

Centro de Biología Molecular Severo Ochoa, Madrid, Spain

The EGFR/MAPK signalling pathway is required in a variety of developmental contexts for the regulation of cell proliferation and differentiation during multicellular development. A key component of the pathway is the mitogen activated protein kinase Erk (Extracellular regulated kinase). Erk is localised in the nucleus and cytoplasm, and its activity and subcellular localization is regulated by the pathway through phosphorylation. Erk phosphorylation relies on Mek, whereas Erk inactivation depends on the activity of several phosphatase proteins. The best-characterized Erk phosphatase is Mkp3, which localization is mostly cytoplasmic. Because Erk phosphorylation causes its nuclear accumulation, it is expected that some Erk phosphatase might reside predominantly in the nucleus. Furthermore, loss of Mkp3 only results in moderate excess of EGFR/MAPK signalling, suggesting the existence of additional negative regulators. In this work we identified and present a preliminary characterization of the *Drosophila melanogaster* gene CG10089. The gene CG10089 encodes a protein with a phosphatase catalytic domain with homology to dual-specificity phosphatases. We find that reduction of CG10089 expression causes the formation of ectopic veins in the wing. This phenotype is reminiscent of those caused by a moderate excess of EGFR signalling, suggesting that CG10089 participates in the negative regulation of the pathway activity. Accordingly, the accumulation of phospho-Erk is increased in cells with reduced expression of CG10089. The gene CG10089 encodes six protein isoforms that share the same catalytic domain. Intriguingly the longer isoform (CG10089-PD) is predominantly localised in the nucleus, whereas the shorter isoform (CG10089-PC) is mostly cytoplasmic. We propose that CG10089 is a novel component of the EGFR signalling pathway that participates in the negative regulation of Erk through the dephosphorylation of both nuclear and cytoplasmic dP-Erk.

A BALANCED NOTCH LEVEL – AN ESSENTIAL REQUISITE FOR HEART REGENERATION

J. Münch, D. Grivas, Á. González-Rajal, J.L. de la Pompa

Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

The zebrafish has been shown to fully regenerate the heart after experimental cardiac injury (ci), a process, which implies the formation of a scar, its regression and the dedifferentiation and proliferation of cardiomyocytes. The Notch pathway is one of the key players during heart development in various species. We observed an up-regulation of Notch signaling components shortly after ci in the endocardium, lining the proliferating myocardium and penetrating the injured area of the heart. By using a cardiac enhancer trap line (ET33mi60A), which GFP-labels the endocardium, we imaged these cells in 3D. Their coherent structure points out a possible function in separating the injured area from the rest of the heart.

Functional studies show, that chemical Notch inhibition reduces cardiomyocyte proliferation and impairs regeneration in long-term experiments. Further genetically induced Notch over-activation increases cardiomyocyte proliferation. However transgenic hearts retain big amounts of fibrin after cryoinjury comparing to control hearts and fail to regenerate, too.

To decipher the molecular mechanism, which makes Notch signaling indispensable for heart regeneration, we performed RNAseq of regenerating hearts following Notch inhibition. The data indicate that Notch pathway inhibition does not only affect genes related to proliferation and differentiation, but that also genes, involved in extracellular matrix remodeling and inflammation, were differentially expressed in treated animals. Indeed we found that Notch positive endocardial cells secrete collagens and thereby may contribute to the formation of the scar from the inner side of the wound.

Altogether our results indicate that the endocardium represents an important interface separating the injured area from the rest of the heart. Further Notch signaling in these cells essentially modulates key processes, that occur during regeneration like fibrosis and inflammation. However a tightly regulation of Notch activation seems to be crucial as both loss- and gain of function experiments result in abnormal regeneration.

A ROLE FOR MIR-15, MIR-23, MIR-106 AND MIR-199 DURING CARDIOGENESIS AND SOMITOGENESIS IN THE DEVELOPING CHICK

D. Franco¹, F. Bonet¹, C. Lopez-Sanchez², V. Garcia-Lopez², A. Ortiz², A. Aranega¹, V. Garcia-Martinez²

¹. University of Jaen, Jaen, Spain

². University of Extremadura, Badajoz, Spain

microRNAs have been reported to play essential roles in distinct and diverse biological contexts, including stem cell determination, cell fate acquisition morphogenesis as well as pathogenesis. We have recently reported that multiple microRNAs display a differential expression during ventricular heart development. We have also demonstrated that miR-27 plays an essential role in regulating Mef2c expression, in this context. In this work we proved, in detail, the developmental expression profile of three microRNAs -miR-15, miR-23 and miR-106- which display increased expression levels during ventricular maturation. In addition, a fourth microRNA -miR-199-, not differentially expressed in this context, was also explored. In situ hybridization with microRNA-specific LNA-probes was performed from early gastrula (HH3; PS3) to early organogenesis (HH20) stages in chicken embryos.

miR-15, miR-23b and miR-106 are already expressed at early gastrula stage in the developing primitive streak and subsequently in the cardiogenic plate. At early cardiac tubular stages, expression of these miRNAs is mostly confined to the venous pole. In addition to the heart, miR-15 and miR-23b have been also observed at the intersomitic level, mainly in a vascular distribution appearance. Importantly, as expected, miR-199 expression is absent from early gastrula to the early tubular heart formation stages. However, a discrete pattern in the developing somites is observed later in development.

Overall, the expression pattern of these microRNAs reveals a putative role in cardiogenesis and angiogenesis for miR-15, miR-23 and miR-106. In addition, a role in myotome formation, including miR-199, is also suggested. We are currently dissecting the molecular pathways in which these microRNAs might be involved in the context of these biological processes.

This work has been partially supported by Grants GR10067 (to VGM) from the Junta de Extremadura, with FEDER co-financing, and CVI-6556 (to DF) from the Junta de Andalucía Regional Council.

THE ORGANOGENESIS OF THE DROSOPHILA RING GLAND, A NEW MODEL TO STUDY IN VIVO COLLECTIVE CELL MIGRATION

C. Sánchez-Higueras, J. Castelli-Gair Hombria

CABD, CSIC/JA/Universidad Pablo de Olavide, Seville, Spain

We present the morphogenesis of the ring glands of *Drosophila* as a powerful system for the genetic analysis of in vivo collective cell migration, Epithelial to Mesenchymal Transition (EMT) and interaction of a migratory organ with changing heterogeneous substrates.

The Ring Gland is composed by three different endocrine glands: the corpora allata, the prothoracic gland and the corpora cardiaca. These glands originate in different embryonic locations converging around the anterior aorta after a long migration. The corpora cardiaca is of cephalic mesodermal origin, while we show that the corpora allata and the prothoracic gland, originate respectively from two groups of ectodermal cells in the maxillary and labial segments. The ectodermal glands are homologous to the cells forming the trachea in more posterior segments and are specified at equivalent positions. Initially, the corpora allata and the prothoracic gland primordia have epithelial characteristics but, after invagination, activation of Snail (*sna*) induces an EMT. A *sna*-ring gland enhancer allows following *in vivo* the migration of these cells. The two ectodermal primordia migrate posteriorly and dorsally coalescing into single cluster after which, they move dorsally where they meet the corpora cardiaca, surround the aorta, and fuse to the contralateral corpora allata to form the ring gland.

We show that after coalescence, the corpora allata/prothoracic gland precursors, attach to the most anterior part of the aorta primordium, undergoing a positional reorganization, giving rise at late embryonic stages to the ring gland. Laser ablation of the aorta or transformation of the cardiac mesoderm into visceral mesoderm, blocks the dorsal migration of the gland precursors. On the other hand, we have found that the cephalo-pharyngeal tracheal branch helps the endocrine precursors to reach the cardiac mesoderm. Our work shows that the migration of the glands is achieved by following a sequence of stepwise processes.

MEIS FUNCTION IN LIMB INITIATION AND PROXIMO-DISTAL PATTERNING: A STUDY THROUGH MEIS1;2 KO ANALYSIS

I. Delgado¹, A. Roselló^{1,2}, M. Torres¹

¹. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Instituto de Salud Carlos III, Madrid, Spain

². Memorial Sloan-Kettering Cancer Center, New York, USA

Meis 1 and *2* are members of the TALE homeobox Transcription factor (TF) family. These TFs are expressed in the lateral plate in the early embryo and then, as the limb bud grows, their expression becomes restricted to a proximal domain. *Meis* genes have been proposed to regulate proximo-distal patterning along the limb bud, however the mechanism is barely known. To better understand this mechanism, *Meis1* and *Meis2* were conditionally knocked-out by using the *HoxB6-CreER^T* deleter line. *Meis1*Het; *Meis2*KO embryos showed reduction in size of proximal elements, the posterior element of the zeugopod is altered and they show a reduction in the number of digits. These phenotypes reflect *Meis* function in P-D specification and a possible role in antero-posterior patterning. Moreover, the lack of *Fgf8* and *Shh* expression in double *Meis1*; 2KO embryos reveals a function in the establishment of the three main signalling centers of the developing limb. In order to further study these aspects of *Meis* function, RNAseq experiments have been performed.

A CHICKEN EMBRYONIC MODEL FOR HEART REGENERATION

O. M. Fernández, A. Aránega, D. Franco, J. N. Domínguez
University of Jaén, Jaén, Spain

Cardiac regeneration potential has been extensively studied in lower vertebrates such as zebrafish or amphibian. Recently, it has been described that this repairing capacity is already present during lower vertebrate developmental stages. However, whether higher vertebrate embryos show similar features is still unknown.

Here, we describe for the first time the cardiac regeneration potential of embryonic chicken hearts. Chicken eggs were opened at 5 days post-fertilization (HH27) to carry on a micro-cauterization on the right ventricle of the developing heart. After micro-surgery, eggs were closed with adhesive tape and re-incubated during 2, 24, 48, 72 and 96 hours.

Hearts at different times of post-cauterization were firstly process to stain with Triphenyltetrazolium chloride (TTC), a routinely used method to determine infarct volume and area. Secondly, we embedded the thorax of non-injured and injured embryos at different times of post-microsurgery to perform Mallory staining and immunohystological analysis.

Our data reveal that after 24 hours of the microcauterization, hearts still display some damaged area. However, after 48 hours of recovering from the surgery, most of embryos did not show any injury at cardiac level revealed by TTC. With further development, 72 and 96 hours post-surgery, no evidence of wound was observed in the heart.

These data demonstrate that the embryonic chicken heart has a high potential to repair an injury during development. Compared with the technically arduous and demanding mammals model for heart regeneration, this chicken embryonic heart regeneration model is an easy and cheaper way to study cellular and molecular mechanism underlying cardiac regeneration, which seems to be absent in higher vertebrates adult hearts.

GATA4-EXPRESSING LINEAGE CELLS CONTRIBUTE TO DEVELOPMENTAL AND ADULT HEMATOPOIESIS

E. Cano^{1,2}, R. Carmona^{1,2}, A. Cañete^{1,2}, L. Ariza^{1,2}, I. Delgado³, A. Rojas³, R. Muñoz-Chápuli^{1,2}

¹. University of Malaga, Málaga (Spain)

². Andalusian Institute of Nanomedicine and Biotechnology (BIONAND), Campanillas, Spain

³. Andalusian Center of Molecular Biology and Regenerative Medicine (CABIMER), Sevilla, Spain

GATA4 is a zinc finger transcription factor widely expressed in mesoderm and endoderm during development. Whereas other members of the GATA family such as GATA1,2 and 3, are critically involved in hematopoiesis, GATA4 has not been previously related with the hematopoietic process. Previous studies of the mouse *Gata4* gene identified a distal regulatory element, named as enhancer G2, which is sufficient to direct expression throughout the lateral mesoderm and the allantois of the mouse embryo, beginning at E7.5 (Rojas et al., *Development*, 2005, 132:3405-17). G2-driven *Gata4* expression become restricted to the septum transversum mesenchyme by E10.5, and it disappears by midgestation. We have followed the developmental fate of the GATA+ mesodermal cells driven by this enhancer, using a G2-*Gata4*^{Cre} mouse line crossed with a R26R-YFP reporter mice. We found substantial number of YFP+ hematopoietic cells of myeloid and erythroid lineages in the fetal liver and peripheral blood of embryos at midgestation. Surprisingly, YFP+ cells were also found in bone marrow and in all blood cell lineages in the adult mice, accounting for about 25% of all the hematopoietic system. This observation strongly suggests that a significant fraction of the definitive hematopoietic stem cells originates in the lateral mesoderm from *Gata4*-expressing progenitors. Furthermore, G2-*Gata4*^{Cre} x *Gata4*-flox embryos show an anemic phenotype (Delgado et al., *Hepatology*, 2014,596:2358-70), reinforcing the idea that GATA4 might play an important role in the differentiation of a subset of the embryonic and adult hematopoietic cells..

IDENTIFICATION OF NEW PLAYERS INVOLVED IN OPTIC CUP FOLDING

J. Letelier, C. Gonzalez-Aguilar, M. San Martín Alonso, J.R. Martínez-Morales
Centro Andaluz de Biología del Desarrollo, Seville, Spain

One of the long-term objectives in our group is to identify novel components of the molecular machinery driving the inward folding of the retina epithelium during early development. To overcome the limitations of classical forward genetic approaches, we have performed transcriptomic analyses using zebrafish embryos. RNA-seq experiments have been carried out to explore the collection of transcripts expressed immediately before (16hpf), or during the active folding of the optic cup (20hpf). Through this approach we have identified 286 up-regulated and 59 down-regulated genes (FDR<0.05, log FC 1.5), which may act as regulators of optic cup morphogenesis. Using a combination of ontology annotations and expression pattern profiles, we have filtered this collection to select those differentially expressed genes in the retina that have a predicted role as direct regulators of cytoskeletal assembly, cell adhesion or polarized trafficking. To investigate the role of the most promising candidates, we are currently generating chromosomal lesions in their respective loci using either TILLING or CRISPR/Cas9 technologies. *In vivo* imaging experiments will be carried out in the next future to record the morphogenesis of the optic cup in the mutant models generated.

CYTONEME-MEDIATED CONTACT-DEPENDENT HEDGEHOG SIGNALLING

L. González-Méndez, I. Seijo, P. Ozores, I. Guerrero

Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid, Spain

From *Drosophila* to humans, Hedgehog (Hh) signalling pathway is crucial for both development and homeostasis in adult tissues. In several systems, Hh morphogen is secreted from a defined group of cells and distributed to create a concentration gradient that drives differential genes expression in target cells. The mechanism responsible for keeping Hh signalling under precise spatial and temporal control during development is still not fully understood. Our research group recently demonstrated that Hh transport along highly dynamic specialized filopodia, also called cytonemes, emanating from Hh producing cells is essential for the restricted distribution of Hh during gradient formation in *Drosophila* epithelia. Currently, we are focusing on the contribution of the cytonemes emerging from Hh-responding cells in gradient establishment during *Drosophila* wing disc patterning. We are also studying the cytoneme-mediated crosstalk between Hh producing and receiving cells, based on the hypothesis that Hh signalling takes place by cytonemes-mediated cell-to-cell contacts in a process similar to neuronal synapses. In order to identify the physical interaction sites along cytonemes between Hh producing and target cells and specifically visualize the membrane contacts between these groups of cells, we are using CD4-based GRASP technique combined with LexA/lexO and Gal4/UAS binary expression systems. We are analysing the involvement of proteins directly implicated in Hh presentation and/or reception, such as Ihog/Boi and Patched, in cytoneme formation/dynamics. Additionally, we are testing a set of proteins involved on cell-to-cell and cell-extracellular matrix interactions responsible for crucial events during development and adulthood, such as those implicated in cell migration and axon guidance, where Hh signalling plays a role in generation of filopodia. We believe that our research will provide new insights into the role of cytonemes in Hh transport and reception, which can help to know the molecular and cellular mechanisms that govern Hh signalling in tissue patterning, developmental disorders, and cancer.

α -CATENIN MEDIATES THE EMERGENCE OF AN ELASTIC RESTORING FORCE DRIVING PULSATILE APICAL CONTRACTION

J. Jurado-Gómez¹, J. de Navascués², N. Gorfinkel¹

¹. Centro de Biología Molecular “Severo Ochoa”, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid, Spain

²European Cancer Stem Cell Research Institute, School of Biosciences, Cardiff University, UK

Apical contraction is a common cell shape change that drives different morphogenetic processes in the context of embryonic development. During Dorsal Closure (DC) in *Drosophila*, amnioserosa (AS) cells apically contract and generate one of the major forces to close a discontinuity in the dorsal region of the embryo. Apical contraction in these cells is pulsatile and driven by the oscillating activity of the medial actomyosin cytoskeleton. The frequency and amplitude of apical cell area fluctuations evolves in a stereotyped manner during DC as apical cell area reduces. Theoretical models of actomyosin and cell area oscillations predict the existence of an elastic restoring force whose stiffness modulates the frequency of oscillations. In this context, we have analysed the function of α -Catenin as a physical linker between the actin cytoskeleton and the apical membrane. Using genetics, quantitative live imaging and mechanical perturbation, we have analysed an α -Catenin allelic series in which the C-terminal/actin-binding region of the protein has been progressively eliminated. All the alleles tested are embryonic lethal and develop head and dorsal-open phenotypes in cuticle preparations. The quantitative analysis of the dynamics of DC shows that in these embryos the overall rate of DC is slower, and importantly, this is accompanied by a decrease in the frequency of oscillations, greater in alleles with a truncated actin-binding domain. Cell and tissue tension does not develop as inferred from the corrugated appearance of the membranes and laser ablation experiments. Finally, the onset of net contraction is more heterogeneous across the tissue than in wild type embryos suggesting that the coordination of cell contraction is perturbed. Altogether, our results suggest that the binding of α -catenin to the actin cytoskeleton provides a molecular basis for an elastic restoring force modulating the frequency of cell area oscillations and mediating the emergence of tissue tension.

THE TRANSMEMBRANE PROTEIN CDON REGULATES DELAMINATION OF NEURAL CREST CELLS

L. Fanlo¹, M. J. Cardozo^{2,3}, S. Usieto¹, Á. Sardonis^{2,3}, P. Bovolenta^{2,3}, E. Martí¹

¹. Instituto de Biología Molecular de Barcelona, CSIC, Parc Científic de Barcelona, Barcelona, Spain

². Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas - Universidad Autónoma de Madrid and ³Ciber de Enfermedades Raras (CIBERER), ISCIII, Madrid, Spain

Cdon (cell adhesion molecule-related, down-regulated by oncogenes) is an evolutionary conserved transmembrane glycoprotein belonging to a subgroup of the immunoglobulin superfamily of cell adhesion molecules (CAMs). Initially isolated in vertebrates as a CAM that links N-cadherin function with MAPK signalling, Cdon has thereafter been shown to act as an essential receptor for the Hedgehog (Hh) family of secreted proteins by associating with both ligand and other Hh receptor components, including Ptc and Gas1. Cdon interactions with N-cadherin and Hh signalling are mediated by the three fibronectin III (FNIII) repeats present in its ectodomain: N-cadherin (FnIII-1), Ptc (FnIII-1-2) and Hh (FnIII-3).

Neural crest (NC) cells are a population of cells that forms at the neural plate border of all vertebrate embryos, which produce multiple derivatives including the peripheral nervous system. In a transcriptome screen designed to search for a genomic signature associated to NC cells in the developing chick embryo, we retrieved Cdon among the positive candidates. In line with this result, here we show that Cdon is expressed by cephalic and trunk premigratory NC cells in both *zebrafish* and chick embryos. Expression however is soon down regulated as cells migrate away from the neural tube borders. Morpholino mediated knock-down of Cdon in zebrafish embryos impaired NC formation, whereas gain of Cdon function (by electroporation) in the chick embryo resulted in reduction of NC delamination from the dorsal NT. This effect likely depends on Cdon interaction with N-Cadherin and Ptc/Hh because expression of Cdon versions, which lacked the FnII (1-2) or FnIII (3) domains, resulted in a reduced effect on NC migration. We will present data aimed at elucidating whether Cdon function in NC development involve interaction with both pathways.

A ROLE FOR SHH IN ESTABLISHING SYMMETRY AND LUMEN FORMATION IN THE ZEBRAFISH SPINAL CORD

I. Gutiérrez-Vallejo, E. Gonzalez-Gobartt, E. Martí

Instituto de Biología Molecular de Barcelona, CSIC, Parc Científic de Barcelona, Barcelona, Spain

Development of the spinal cord in human embryos involves both primary and secondary neurulation. Although the developmental events occurring in each process differ considerably, they lead to essentially the same end-product, a hollow neural tube composed of a pseudostratified columnar epithelium. Here we propose to take advantage of the zebrafish embryo to gain understanding in the cellular and molecular events of neural rod cavitation, and to apply these findings to identify the signals and mechanisms controlling cavitation in secondary neurulation in amniotes, a poorly understood process.

We show that over activation of the Shh pathway at early developmental stages strongly affects spinal cord morphogenesis. We will present data aimed at elucidating the cell biology processes and gene-regulatory networks that Shh might control during zebrafish neurulation. High-resolution time-lapse images of embryos, analyzed by means of Particle Image Velocimetry (PIV), revealed that Shh over-activation cause a delay in neural plate convergence. In search for new Shh targets involved in this process, we performed a transcriptome analysis in which we manipulated levels of Shh-signalling to identify regulators of neuroepithelial cell polarity, cell adhesion and trafficking. The expression of well-known proteins such as N-Cadherin, Laminin or ZO-2 appeared to be regulated by Shh. Furthermore we obtained novel targets for which we are currently analyzing the roles in the zebrafish spinal cord morphogenesis.

ASYMMETRIC DISTRIBUTION OF SHH SIGNALLING COMPONENTS DIRECTS THE MODE OF CELL DIVISION IN THE DEVELOPING NERVOUS SYSTEM

M. Saade, R. Escalona, E. Gonzalez-Gobartt, E. Marti

Instituto de Biología Molecular de Barcelona, CSIC, Parc Científic de Barcelona, Barcelona, Spain

Three distinct modes of divisions occur during spinal cord development: self-expanding (symmetric proliferative, PP) divisions ensure the expansion of the progenitor pool by generating two daughter cells with identical progenitor potential; self-renewing (asymmetric, PN) divisions generate one daughter cell with a developmental potential indistinguishable from that of the parental cell and another with a more restricted potential; self-consuming (terminal symmetric neurogenic, NN) divisions generate two cells committed to differentiation, thereby depleting the progenitor pool. We recently developed markers that provided the single cell resolution necessary to identify these three modes of division. Using these markers and a mathematical model that predicts the dynamics of motor neuron progenitor division, we showed that Sonic hedgehog (Shh) signaling drives cell fate choices upstream of the re-arrangement of cell cycle parameters. Functional *in vivo* experiments showed that by artificially maintaining Shh activity high by introducing a dominant active form of the Hh receptor Smoothed (SmoM2), the rate of PP divisions increased significantly. The increase in PP divisions takes place at the expense of both types of neurogenic divisions, such that PN and NN divisions were reduced. Moreover, *in vivo* experiments strongly support the idea that the distinct modes of progenitor division are correlated to different levels of endogenous canonical Shh activity during primary neurogenesis. We will present data aimed at elucidating how Shh-signalling might directly control the mode of division in neuroepithelial cells.

THE INTERPLAY BETWEEN GROWTH PROMOTING PATHWAYS AND DPP ACTIVITY IN DROSOPHILA

A. Ferreira¹, M. Milán^{1,2}

¹. Institute for Research in Biomedicine, Barcelona, Spain

². Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Coordination of growth between and within organs contributes to the generation of well-proportioned organs and functional adults. The Dpp pathway plays a major role in the coordination of growth and patterning in the wing primordium of *Drosophila*, and its activity gradient activates target genes in a concentration-dependent manner. Moreover, competition for the Dpp ligand has been proposed to be a fundamental mechanism to determine the fitness of a cell during the process of cell competition. We analyze how an organ responds when a growth advantage has been provided to a specific territory during wing development. Interestingly, when growth is induced in one compartment, the adjacent domain responds by reducing its size. We found evidence that competition for the Dpp ligand contributes to the coordination of growth between adjacent cell populations.

ASSESSING THE LONG RANGE ACTION OF WNT SIGNALING WITH GENOMIC ENGINEERING

L.A. Baena-López¹, C. Alexandre¹, JP. Vincent¹.

¹. MRC. National Institute for Medical Research. London. UK

Wnts are secreted proteins that control cell fate determination, cell proliferation and stem cell maintenance. Misregulation of wingless signalling leads to developmental abnormalities and other diseases, including cancer. It is therefore important to elucidate the mode of action of Wnts. We are particularly interested in the question of how Wnt act at a distance.

We have used genome engineering to express membrane tethered Wingless under physiological control in the absence of endogenous Wingless protein. Unexpectedly flies expressing this non-spreadable form of Wingless hatched into properly patterned adults, albeit slightly smaller than their wildtype siblings. We showed that normal patterning of these flies can be explained by the combined effect of two processes. One involves extended transcriptional activation of *wingless* in presumptive receiving cells. The other, akin to cellular memory, ensures that target gene expression can persist in the absence of continuous signalling.

Our results show that the spread of Wingless is not essential in specific experimental conditions. Yet, extensive work by others has show that, in the wildtype, Wingless does spread. We suggest that signaling activity throughout the prospective wing results from the combination of extended transcription, persistent expression of target genes and spreading. The involvement on several biological processes could ensure the reproducibility of pattern formation and size of appendages.

HOXA2/ZIC2 CONTROL THE EXPRESSION OF ROBO3 IN ATHO1-POSITIVE DERIVATIVES OF THE DORSAL NEURAL TUBE.

F. Nieto-Lopez¹, T. Di Meglio¹, F.M. Rijli², E. Herrera³, P. Bovolenta¹

¹. Centro de Biología Molecular Severo Ochoa, Madrid; Spain

².Friedrich Miescher Institute, Basel; Switzerland

³.Instituto de Neurociencias, Alicante; Spain

Progenitors of the dorsal neural tube, characterized by the expression of the transcription factor *Atho1* give rise, with a precise spatio-temporal sequence, to neurons that project to the contralateral side of the neural tube (commissural neurons) and to “non-crossing” ipsilateral neurons. Robo3, an axonal guidance receptor, is critical for the proper establishment of commissural projections. Here, we have investigated the transcriptional mechanism by which *Robo3* expression is regulated in *Atho1*-positive dorsal progenitor lineages. *Robo3* is not expressed in proliferating dorsal progenitors, but its expression is activated by the transcription factor *Hoxa2* in all *Atho1* positive dorsal derivatives that follow a commissural program. Notably, the *Hoxa2*-negative anterior dorsal cerebellar lineage lacks *Robo3* expression. Supporting the idea that *Hoxa2* regulates positively Robo3, we have forced expression of *Hoxa2* in this population and Robo3 was induced. The activation of Robo3 occurs only in *Zic2*-negative neurons and is efficiently blocked by the ectopic expression of *zic2*. Notably, *Zic2* expression appears in *Atho1*-positive progenitor after E13, when *Robo3*-negative ipsilateral projecting neurons are produced. This late *Zic2* inductions occurs under modulation of the Shh binding protein Boc and correlates with the down regulation of Robo3 expression. We thus propose that a dynamic regulation of Robo3 expression under the opposite influence of *Hoxa2* and *zic2* plays an important role in establishing the axonal behaviour of neural tube dorsal neurons.

THE CATECHOLAMINERGIC PATHWAY IS REQUIRED FOR NORMAL DEVELOPMENT OF BETA-CELLS IN THE MOUSE PANCREAS

P. Vázquez^{1,2}, A.M. Robles¹, F. DePablo^{1,2}, C. Hernández^{1,2}

¹ Centro de Investigaciones Biológicas (CSIC), Madrid, Spain

² CIBERDEM (ISCIII), Madrid, Spain

Apart from transcription factors, little is known about the molecules that modulate the proliferation and differentiation of pancreatic endocrine cells. The early expression of Tyrosine hydroxylase (TH), during development, in a subset of glucagon⁺ cells, led us to investigate whether catecholamines have a role in beta-cell development. TH is the enzyme that catalyzes the conversion of L-tyrosine to L-DOPA, the rate-limiting step in the biosynthesis of catecholamines.

We studied the immunohistochemical characteristics of TH-expressing cells in wild-type (*Th*^{+/+}) mice during early pancreas development, and analyzed the endocrine pancreas phenotype of TH-deficient (*Th*^{-/-}) mice. We also analyzed the effect of dopamine addition and TH-inhibition on insulin producing cells in explant cultures.

We found, in the E12.5-E13.5 mouse pancreas, that the TH-expressing cells appear to be a subpopulation of the early glucagon endocrine cells; they rarely proliferated and did not express the precursor marker neurogenin 3 (NGN3) at E13.5. The number of insulin⁺ cells in the *Th*^{-/-} embryonic pancreas was reduced as compared with wild-type embryos at E13.5. Moreover, the decrease in the number of insulin⁺ cells was sustained in E13.5 pancreatic explants after five days in culture. While no changes in PDX1⁺-progenitor cell number were observed between groups at E12.5, the number of NGN3 and NKX2.2-expressing cells was reduced in *Th*^{-/-} embryonic pancreas, an effect that was paralleled by increased expression of the transcriptional repressor *Hes1*.

The potential role of dopamine as a pro-beta-cell stimulus was tested by treating pancreas explants with this catecholamine. The treatment with dopamine for five days resulted in an increase in total insulin⁺ cells relative to control explants. Conversely, inhibition of TH activity decreased the number of insulin⁺ cells in pancreatic explants.

In conclusion, a non-neural catecholaminergic pathway appears to modulate the pancreatic endocrine precursor and insulin producing cell neogenesis.

IS LINE-1 RETROTRANSPOSITION A SOURCE FOR DNA BREAKS IN MOUSE RETINAL DEVELOPMENT?

N. Alvarez-Lindo¹, J. Baleriola¹, L. Blanco², A. Bernad³, T. Suarez¹, E. J. de la Rosa¹

¹. Centro de Investigaciones Biológicas, CSIC, Madrid, Spain

². Centro de Biología Molecular CSIC-UAM, Madrid, Spain

³. Centro Nacional de Biotecnología, CSIC, Madrid, Spain

The debate on the existence of somatic mosaicism in the nervous system started long time ago, but only recently experimental observations have provided some evidence on it. During neural development, several phases of apoptosis help to sculpt the complex cytoarchitecture and connectivity of the nervous system, including an early one whose function is not well understood. Several genetically-modified mouse model systems defective in DNA double-strand break repair present a dramatic phenotype during neural development, suggesting a possible function of DNA break generation and repair in the process. LINE-1 retrotransposition has been suggested to act as a necessary source of DNA breaks during neural embryonic development that plays a crucial role in the generation of neural diversity. Hereby, we study the requirement of proteins involved in DNA double-strand break repair for proper neural development and their relationship with LINE-1 retrotransposition.

We have analyzed retinal development in two murine models defective for DNA repair: the SCID (DNA-PK truncated) and the Polμ deficient mice. Neural retina in both models presented more unresolved DNA breaks than their control strains as well as increased apoptosis. Axonal growth was also altered, and both mutants presented higher LINE-1 retrotransposition. However, LINE-1 was increased both in neural and non neural tissues.

We observed that retinal neurons require correct DSB repair for their survival and differentiation. Although retrotransposons are a source for DSBs, LINE-1 sole presence doesn't account the neuronal phenotype observed in DNA repair mutants, as its increment was non neural-specific. Either LINE-1 may also use previously generated DSBs as transposition sites, or DNA breaks have different impact on different tissues. Further work is required to integrate DSB generation and repair during retinal neurogenesis and proper retinal function.

A TISSUE-SPECIFIC TRANSCRIPTIONAL PROGRAM CONTROLS REMODELING OF THE HEART CIRCULATION

M. Losa¹, S. Amin¹, IJ. Donaldson¹, L. Zeef¹, J. Hensman², M. Rattray¹, N. Bobola¹

¹. The University of Manchester, Manchester, UK

². University of Sheffield, Sheffield, UK

Control of gene expression is crucial for embryonic development. The heart circulation derives from remodeling of a series of aortic arches that run through the branchial arches (BA) and connect the heart to the dorsal aorta in the embryo. During this remodelling, the first and second aortic arches regress, while the posterior aortic arches remain connected to the heart and will form part of the adult cardiovascular system. Here, we compare the second BA (IIBA) and the posterior BA (PBA) tissues in order to identify the gene regulatory network responsible for the persistence or regression of the aortic arches.

Using microarrays and epigenomic profiling at progressive developmental time points, we identified a subset of genes differentially expressed between the IIBA and the PBA. PBA-specific genes showed significant association with GO terms related to “cardiovascular development” and “blood vessel morphogenesis”. H3K27ac ChIP-seq shows different acetylation profiles in promoter regions and potential enhancers of PBA-specific genes between the IIBA and the PBA. Additionally, we clustered genes based on their temporal expression profiles to identify co-regulated candidate genes and potential upstream transcription factors involved in aortic arches remodeling. Our analysis reveals that the activation of a smooth muscle differentiation program restricted to the PBAs could allow the stabilization of the posterior aortic arches, while the aortic arches regress in the IIBA (where this transcriptional program is not active). In summary, this high-throughput analysis reveals for the first time a different transcriptional program between IIBA and PBA that could be responsible for the regression or persistence of the aortic arches.

LONG-RANGE INTERACTIONS IN THE REGULATION OF THE MOUSE *MRF4/MYF5* LOCUS

C. Vicente-García, Á. Bella-Carreño, A. Fernández-Miñán, J. Tena, J. L. Gómez-Skarmet, J. J. Carvajal
Centro Andaluz de Biología del Desarrollo, Seville, Spain

Myf5 and Mrf4 are two members of the myogenic regulatory factor (MRFs) family of transcription factors. Their function is to initiate the myogenic cascade and thus control the determination, specification and differentiation of skeletal muscle during embryonic development. The spatiotemporal regulation of these genes, which are syntenic in all vertebrates analyzed, involves the action of multiple interdigitated enhancers that are scattered throughout the locus. We wanted to understand how specificity of the different enhancers for their cognate promoters is established and we have defined the existence of a new type of element involved in transcriptional regulation, the transcription balancing sequences (TRABS) that create a series of equilibria states in which the correct long-range interactions between enhancers and the *Myf5* and *Mrf4* promoters are established. Circularized chromosome conformation capture (4C) experiments are being carried out in order to reveal the three-dimensional organization of the locus in several scenarios: in undifferentiated vs. differentiated C2C12 muscle cells, and in whole embryos (wild type vs. a *Mrf4* knockout allele which lacks the *Mrf4* promoter and a *Myf5* knockout allele that lacks the *Myf5* promoter and the TRABS) at different developmental stages. In addition, several CTCF binding sites exist in the locus, and may be also participating in its global regulation. This possibility is being explored using ChIP in C2C12 cells, in different embryonic tissues and developmental stages. These experiments will extend our understanding of the regulation of the *Mrf4/Myf5* locus during embryonic myogenesis and the mechanism behind TRABS function.

REGULATION OF GENOME ARCHITECTURE DURING HEART DEVELOPMENT

M. Gómez-Velázquez¹, E. Fernández-Caceres^{1,4}, C. Badía-Careaga¹, J. Tena², I. Rollan-Delgado³, M. Alonoso¹, N. Galjart⁴, J. L. Gómez-Skarmeta², M. Manzanares¹

¹. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

². Centro Andaluz de Biología del Desarrollo (CSIC-UPO), Seville, Spain

³. Erasmus Medical Centre, Rotterdam, the Netherlands

⁴. Centro Andaluz de Secuenciación Genómica Humana, Seville, Spain.

We are interested in understanding how genome architecture is involved in the control of spatially and temporally restricted gene expression during heart development. CTCF is an 11 zinc finger protein that has been established as a crucial genome organizer: it can act as an insulator factor, promote chromatin looping and facilitate enhancer-promoter interactions. To decipher the role that CTCF plays in this process, we are specifically deleting the gene in cardiac tissue by using a conditional *Ctcf* allele and the tissue-specific Nkx2.5-Cre driver. When doing so, embryos die at stage E13 with gross cardiac malformations. To analyze the global impact on gene expression of CTCF depletion in the developing heart, we have identified by RNAseq differentially expressed genes between wild type and KO hearts, and studied their relation with described CTCF binding sites and regulatory elements. We have found examples of mis-regulation of several tandem pairs of genes that, in the wild type, show divergent expression in the developing heart and are separated by stable CTCF binding sites. Among these is the *IrxA* cluster, which plays an important role in heart patterning. Analysis of chromatin conformation reveals changes in the pattern of long-range contacts that are established upon CTCF loss. Our data shows that genome architecture plays a key in gene regulation during heart development.

EXPRESSION PROFILE OF THE SEX-RELATED GENE *DMRT1* DURING TEMPERATURE SEX DETERMINATION IN GONADS OF THE SEA TURTLE *LEPIDOCHELYS OLIVACEA*

R. Montiel¹, D. Venegas¹, F. Recillas², A. Marmolejo¹, H. Merchant-Larios¹

¹. Instituto de Investigaciones Biomédicas

². Instituto de Fisiología Celular, UNAM, Ciudad Universitaria, Mexico, D.F.

Sexual reproduction involves the existence of individuals with dimorphic phenotypic characteristics. There are two general processes of sex determination, genetic sex determination (GSD) and environmental sex determination (ESD). The sea turtle *Lepidochelys olivacea*, is a species with temperature sex determination (TSD). Expression profiles of some genes involved in sex determination have been analyzed in gonads from embryos incubated at male- or female promoting temperatures (MPT or FPT). In bipotential gonads the gene *Sox9* remains expressed in the testes but it is down-regulated in ovaries at the onset of differentiation. Another important gene for male sex determination is *Dmrt1* (Doublesex and Mab-3 Related Transcription Factor, 1) which encode a transcription factor with a DM domain, a DNA-binding motif related with sex determination and differentiation. *Dmrt1* is a conserved gene highly expressed in testis of diverse species with GSD and TSD. Here we have analyzed the expression profile of *Dmrt1* in developing gonads of *L. olivacea* from embryos incubated at MPT or FPT. Sequences of *Dmrt1* in other vertebrates were aligned, and primers were generated with the most conserved sequences. Fragments of exon 2 were amplified by PCR and cloned with a plasmid vector. After sequencing, the identity of exon 2 of *Dmrt1* in *L. olivacea* was confirmed. Then, the expression profile of this gene was analyzed using qPCR. We found that *Dmrt1* is up-regulated in bipotential and differentiated gonads at MPT while it remains down-regulated at FPT. Furthermore, *Dmrt1* up-regulation precedes the dimorphic expression of *Sox9* in gonads of *L. olivacea*. Thus, we propose that *Dmrt1* is an upstream gene candidate whose dimorphic regulation mediates the pathway of temperature sex determination in this species. Supported by grant PAPIIT-IN205213.

CABUT/DTIEG ASSOCIATES WITH THE TRANSCRIPTION FACTOR YORKIE FOR GROWTH CONTROL

M. Ruiz- Romero, F. Serras, M. Corominas

Departament de Genètica i Institut de Biomedicina (IBUB) de la Universitat de Barcelona, Barcelona, Catalonia, Spain

The transcription factor Cabut (Cbt)/dTIEG is the fly ortholog of TGF- β inducible early genes 1 and 2 (TIEG1 and TIEG2) in mammals. TIEG proteins can function as either activators or repressors and participate in a wide variety of cellular processes, from development to cancer, and regulate genes that control proliferation, apoptosis, regeneration or differentiation. In *Drosophila*, it is known that Cbt/dTIEG is required during dorsal closure downstream of JNK signaling and is a modulator of different signaling pathways involved in wing patterning and proliferation. We determined Cbt/dTIEG association with chromatin and identified Yorkie (Yki), the transcriptional co-activator of the Hippo (Hpo) pathway as its partner. The interaction of these factors has been confirmed by co-immunoprecipitation experiments and ChIP-reChIP analyses corroborate co-binding of Cbt/dTIEG and Yki on the same DNA promoter regions. Cbt/dTIEG and Yki co-localize on common gene promoters and the expression of target genes varies according to changes in Cbt/dTIEG levels. Down-regulation of Cbt/dTIEG suppresses the overgrowth phenotypes caused by mutations in *expanded (ex)* and *yki* overexpression, whereas its up-regulation correlates with an increase in nuclear Yki. Our results imply that Cbt/dTIEG is a novel partner of Yki that is required as a transcriptional co-activator in growth control.

CHARACTERIZATION OF THE MOUSE *SNAIL1* LOCUS

J. Galceran¹, E. Rodriguez-Aznar¹, F. Oliveira¹, M. Manzanares², JL Gomez-Skarmeta³, MA Nieto¹

¹. Instituto de Neurociencias CSIC-UMH, Sant Joan d'Alacant, Spain

². CNIC, Madrid, Spain

³. CABD, Seville, Spain

The *Snail1* gene is a member of the Snail/Scratch family of Zn finger transcription factors. The members of this family have crucial roles in metazoan development and disease. The Snail members regulate cell-cell adhesion and trigger epithelial-to-mesenchymal transition (EMT). Snail proteins are expressed during development at all the places that undergo EMT; in the adult stage however, expression of the Snail proteins is selectively prevented. The only detectable *Snail* expression in adults corresponds to responses to insult or oncogenic processes. We have started the characterization of the mouse *Snail* locus in order to identify the control elements that can explain this sophisticated expression control.

By combining inter-species comparisons and zebrafish transgenesis we have identified regions with insulator/boundary activity that have allowed us to identify the limits of the mouse gene.

We have performed whole gene analysis for active and inactive epigenetic marks and combined it with conservation analysis to identify potential regulatory regions. These results combined with 4C analyses of different cell lines and tissues with differential *Snail* expression has allowed us to generate a map of potential regulatory regions in the mouse *Snail1* gene.

Taking advantage of mouse and zebrafish transgenesis we have been able to test the function of some selected regions of the mouse *Snail* gene. These mouse regions can be decoded by the zebrafish developmental programs and drive expression in the endogenous domains of the zebrafish *snail1a* and *snail1b* genes.

The identification of the mouse regulatory elements might contribute to our understanding of the *Snail1* gene regulation in development and disease.

DECODING BOUNDARIES: FROM GENOMIC LANDSCAPE TO CELLULAR FUNCTION

J. Terriente¹, J. Letelier², J.R. Martinez-Morales², C. Pujades¹

¹. Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, España

². Centro Andaluz de Biología del Desarrollo (CSIC/UPO), Sevilla, España

The existence of compartments in the vertebrate Central Nervous System was first demonstrated in the hindbrain, which is subdivided into segments called rhombomeres. At the interface between rhombomeres arises a specialized cell population – the rhombomere boundary cell population (rBCP) – that has specific cellular behavior in terms of gene expression profile, differentiation state, morphology and function. In previous works, we have described how the rBCP acts as a signalling center for patterning neurogenesis in the hindbrain and, thanks to the assembly of actomyosin cables at every boundary, as a physical barrier necessary for keeping rhombomere cells segregated during development. To further understand the specification of the rBCP and its specific cell behavior and cell fate, we have taken a genomic approach. We have dissected the possible non-coding regulatory elements that drive the expression of a set of genes to the rBCP. Particularly, in zebrafish we have found a genomic region that hosts a given number of genes, whose expression is specifically enriched at the rBCP; these genes are located in synteny in several species (from medaka to mice). These findings suggest the existence of a common regulatory element responsible for gene expression at the rBCP. With this hypothesis in mind, we combined data from gene expression studies in different species, “open chromatin” Chip-Seq analysis, functional enhancer activity and 4C analysis, and isolated a region of 2.5 kb displaying the features of a *bona fide* rBCP enhancer. These results provide a very novel example of genetic coregulation with interesting evolutionary implications. In addition, they shed light into the cis-regulatory logic of the rBCP fate and opens the opportunity to develop new tools for assessing the rBCP behavior and lineage.

NON-CANONICAL DORSOVENTRAL PATTERNING IN THE MOTH MIDGE *CLOGMIA ALBIPUNCTATA*.

A. Alcaine, K. R. Wotton, J. Jaeger, E. Jimenez-Guri
Center for Genomic Regulation, Barcelona, Spain

Étienne Geoffroy Saint-Hilaire famously proposed that invertebrates and vertebrates had very similar body patterns but with an inversion in the dorsoventral (DV) axis. This idea is nowadays supported by molecular evidence showing that key factors for DV patterning (such as BMP morphogens) are expressed with opposite polarity in these two groups of animals. We have recently reported surprising evidence that the situation may not be quite as clear-cut. The insect BMP homologue *dpp* is expressed ventrally, around the anterior and posterior poles, in the blastoderm of the nematoceran moth midge *Clogmia albipunctata*. How this arrangement of gene expression in *Clogmia* is able to function as a dorsal morphogen gradient is unknown. We are currently systematically characterising other components of the DV patterning pathway to elucidate the mechanism of Dpp gradient formation in this fly. Our analysis suggests that a shuttling mechanism like that proposed for *Drosophila* is improbable in *Clogmia*. We are using RNAi, previously unavailable in this species, to elucidate the interactions between these genes and improve our understanding of the variety of patterning mechanisms available to specify the DV axis.

EVOLUTIONARY ANALYSIS OF THE PAIRED-RELATED HOMEBOX GENE FAMILY

A. Arcas, M. Ángela Nieto

Instituto de Neurociencias de Alicante (CSIC-UMH), San Juan de Alicante, Spain

The Paired homeobox gene family are transcription factors that play essential roles during embryonic development. Significant members in vertebrates are the Paired mesoderm homeobox proteins 1 (PRRX1) and 2 (PRRX2), which are implicated in developmental processes, mainly of the mesoderm and neural crest-derived mesenchyme. Besides, PRRX1 was recently found to be an Epithelial-Mesenchymal Transition (EMT) inducer both in embryos and in cancer cells (1).

While alternative splicing yields two isoforms in *Prrx1*, the *Prrx2* gene has only one splice variant. The Prrx family contains a paired type DNA-binding homeodomain and a C-terminal OAR domain proposed to function as an interface for cofactor interactions (2).

Although extensive work has been conducted in particular homeobox proteins, few comprehensive evolutionary studies (3) have been done to understand the origin of the Paired-related homeobox transcription factor gene family.

Our objective is to study the origin and evolution of the Prrx family among taxa covering all lineages that can help understand its ancestral and co-opted functions when complemented with functional analyses.

We have identified Prrx orthologous proteins in a comprehensive set of species, analysed the sequence and domain conservation along evolution, compared the results with the evolution of other Paired homeobox protein families and studied the evolutionary relationships among the different families.

Our results show that Prrx homologous sequences can be identified down to the Lophotrochozoa superphylum, and while the Prrx1 long isoform is of ancient origin, the short isoform is found only in amniotes. Interestingly, the regions proposed to be important for the function of the TFs in humans (2) are clearly conserved only down to Gnathostomata. These data suggest that new functions might have been co-opted in evolution. Functional analyses are underway to dissect out the contribution of the different family members and protein domains to EMT.

References

- (1) Ocaña et al. *Cancer Cell*. 22(6):709-24. 2012
- (2) Norris and Kern. *J Biol Chem*. 276(29):26829-3. 2001
- (3) Braasch et al. *Comp Biochem Physiol C Toxicol Pharmacol*. 163:24-36. 2014

MULTIPLE DEVELOPMENTAL ROLES OF A TISSUE-SPECIFIC ALTERNATIVE SPLICING FACTOR ACROSS DEUTEROSTOMES: ESRP GENE FAMILY IS A MASTER REGULATOR OF DIVERSE EPITHELIAL FUNCTIONS

D. Burguera¹, E. Navas¹, C. Cuomo², Y. D'Agostino², C. Racioppi², R. Esposito², C. Herrera¹, B. Albuixech¹, S. D'Aniello², A. Spagnuolo², F. Ristoratore², M. Arnone², M. Irimia³, J. Garcia-Fernández¹

¹. Universitat de Barcelona, Spain

². Stazione Zoologica Anton Dohrn, Napoli, Italy

³. CRG, Barcelona, Spain

Alternative splicing (AS) – the process by which different pairs of splice sites in precursor RNAs are joined to create multiple mRNA variants – greatly expands the proteomic and regulatory complexity of vertebrate genomes. A significant fraction of this AS is tightly regulated in a cell and tissue manner, due to the action of tissue-specific RNA-binding proteins that regulate large networks of alternative exons. However, alternative spliced exons are highly evolvable and frequently not conserved, even between mammalian species. Thus, understanding the evolution of the biological roles of AS factors and how their regulated target networks are assembled is a pending task in EvoDevo.

The Epithelial Splicing Regulatory Protein (ESRP) gene family has been shown to be a master regulator of Epithelial to Mesenchymal Transition (EMT) in cell culture experiments, where it controls a network of alternative exons from genes involved in cell adhesion. Here, we combine expression and functional data of ESRP genes in various species, including sea urchin, amphioxus, vase tunicate and zebrafish. We show that ESRP expression is restricted to various types of epithelial cells in all studied species. At the functional level, this gene family seems to be necessary for a set of developmental processes, involving epithelia, within the deuterostome clade. For example, in zebrafish, ESRP1 knock-down causes defects in neural tube closure. In the urochordate *Ciona*, ESRP seems to counteract Twist2-like, a transcription factor linked with migration of mesenchyme cells in this species. In amphioxus, ESRP is expressed in the epidermal cells adjacent to the neural plate border during neurula stage. And finally, outside chordates, in sea urchin, we demonstrate that ESRP is involved in proper specification of the aboral ectoderm.

In summary, our results exemplify a master regulator of AS with generally conserved tissue type expression (i.e. epithelia) that evolved to regulate different developmental programs during the diversification of the deuterostome clade.

EVOLUTION AND DEVELOPMENT OF NOVEL DENTITIONS IN TETRAODONTIFORMES

T. Shono, A. Thiery, G. Fraser

University of Sheffield, Sheffield, United Kingdom

The range of morphological diversity represented in the teleost fishes is remarkable. Here we present information on the evolutionary and developmental origins of a unique suite of dentitions observed in the order Tetraodontiformes, which include several families with distinct and divergent dental morphologies. We present data on representative tetraodontiformes including the families of pufferfish (tetraodontidae). We observed the ontogenetic transition from a conserved dental structure and pattern early in pufferfish (*Monotretes* and *Takifugu* spp.) development to a novel modification of the dentition in juveniles and adults through the mechanism of tooth replacement. Our initial hypothesis was that the pufferfish 'beak', a sutured beak consisting of just two fused teeth on both the upper and lower jaw, represented a *de novo* morphological novelty. Observations of developmental stages, however, show that the 'beak' of adult pufferfish is a secondary structure that appears as a product of extreme modification and respecification of the tooth replacement phase. Tooth replacement and beak initiation takes place after the first generation teeth are formed. This pattern of novel ontogenetic modification through continuous tooth replacement is a common character of derived tetraodontiformes and is also observed in more basal families. We illustrate how the initial tooth pattern develops via gene expression and cell lineage labeling, and how this first phase is ordered as in osteichthyan tooth programmes and subsequently replaced by a suite of novel dental morphologies in this order of teleosts. These observations offer insights into how derived teleost fishes can develop extreme morphological novelty from conserved developmental patterns through ontogenetic modifications that may offer clues as to how certain vertebrate groups evolve divergent adult structures from a common bauplan.

THE SIX3 GENE *OPTIX* PATTERNS THE *DROSOPHILA* HEAD THROUGH AN ANTI-REPRESSOR MECHANISM WITHIN THE *HH*-SIGNALING PATHWAY

M.A. Domínguez-Cejudo, F. Casares

CABD (Centro Andaluz de Biología del Desarrollo) CSIC-Universidad Pablo de Olavide-Junta de Andalucía, Sevilla, Spain

Organ specification and patterning is controlled by the dynamic regulatory interactions between signaling pathways and transcription factors. Here we describe work in *Drosophila* aimed at understanding in detail how this regulatory interplay specifies and patterns part of the visual system in insects, the ocelli. The three ocelli, or simple eyes, are located at the vertices of a triangular patch of cuticle on the fly's forehead. The set of ocelli (OC) plus the intervening cuticle (the interocellar cuticle, IOC) is collectively called ocellar complex (OCx). Its structural simplicity and the fact that it forms part of the visual system makes the OCx an ideal model to study eye-related patterning mechanisms.

Previous work has described how *wg/Wnt-1*, *hh* and *otd* (an Otx family member) specify the OCx. Once specified, *hh* is responsible for the partition of the OCx into two ocellar placodes (the precursor region of the anterior and posterior ocellar retinas) separated by the prospective IOC. Although *hh* could in principle specify these two fates (OC and IOC) acting as a morphogen, we found that *hh* function most likely depends on dynamic changes of *hh*'s spatial expression pattern. Part of these changes is due to two additional transcription factors: *engrailed (en)* and *Six3/optix*. Our work indicates that the final *hh* expression pattern depends on a positive feedback between *hh*'s target *engrailed (en)*, which acts as a *hh* pathway repressor, and on the action of *Six3/optix*. *Six3/optix* limits *en* expression and by doing so sets the final *hh* expression domain. Otherwise, in *Six3/optix* mutants *en* extends anteriorly and the development of the anterior ocellus is blocked. In summary, our work identifies *Six3/optix* as necessary for ocellar development, acting as an "anti-repressor" within the *hh* signaling pathway.

MECHANISMS OF CELL DECISION-MAKING: USING THE OCELLUS TO UNDERSTAND HOW CELLS BECOME NEURONS AND HOW THESE NEURONS ESTABLISH SPECIFIC CONNECTIONS

D. García-Morales, F. Casares

CABD (Centro Andaluz de Biología del Desarrollo), CSIC-Universidad Pablo de Olavide-Junta de Andalucía, Seville, Spain

Insect eyes come in two types: compound and simple. Compound eyes detect color, polarization and motion, while the simple eyes –called *ocelli* and usually present as three on the insect's forehead – are involved in flight stabilization and in detecting sudden light intensity changes to trigger the escape response. The ocellus is structurally simple: comprises a single retinal cup covered by a large corneal lens. Each retina has about eighty photoreceptors in *Drosophila*, but this number is much higher in other insects, such as dragonflies. The three ocellar retinæ converge on just a few connecting interneurons. This large convergence must therefore result in an equally simple retinotopic map.

Due to its structural simplicity, we are setting up the ocellus as a model to address two questions: first, how cells within the ocellar placode decide upon two cell fates: photoreceptor and lens-cell. This question is linked to the issue of cell number, as during development the number of photoreceptors must match the number of lens-cells. The second question is how the three ocellar retinæ establish specific contacts with their few connecting interneurons, and how this wiring is then used to convey information important for navigation and escape.

We will present our progress on both issues. First, we will describe the kinetics of ocellar photoreceptor differentiation, and address the role played by the hedgehog (hh) pathway in the initiation and propagation of photoreceptor differentiation, and in the control of photoreceptor cell number. Second, we will characterize the early steps in the formation of the ocellar nerve, discriminating the paths taken by the axons derived from the anterior and posterior ocellar retinas.

SELECTING THE EYE ARCHITECTURE IN *DROSOPHILA*: A WNT CHOICE

M. Magri, M.A. Domínguez-Cejudo, F. Casares

CABD (Centro Andaluz de Biología del Desarrollo), CSIC-Universidad Pablo de Olavide-Junta de Andalucía, Seville, Spain

Evolution is Life's engine. Understanding the mechanics of evolution requires the understanding of how changes in the genotype shape phenotypes. Morphology is regulated during embryonic development by transcription factors and signaling pathways organized into functional units, called gene regulatory networks (GRNs). Therefore, evolutionary mechanics can be reduced, to some extent, to finding out the relevant changes in organ-specific GRNs. We have set to address this issue by asking what changes in the "eye" GRN would allow the transitions between two insect eye types, the compound eye and the ocellus – if this transition were at all possible. These two eye types have very different structure: the compound eye is large, and made up of hundreds of unit eyes or ommatidia, while the ocellus is small and formed by a single, cup-shaped retina. And yet, their development is controlled by a similar set of genes. We have found that the inactivation of the Wnt signaling pathway specifically in the prospective ocellar region leads to a transformation of the ocellus into compound eye. I will describe our work to find how the Wnt pathway controls the eye GRN in order to select one of the two eye architectures in *Drosophila*.

HOX GENE FUNCTION AND REGULATION DURING AP AXIS ELONGATION IN THE CRICKET *GRYLLUS BIMACULATUS*

Y. Matsuoka¹, T. Bando², T. Watanabe¹, S. Noji¹, T. Mito¹

¹. The University of Tokushima, Tokushima, Japan

². The University of Okayama, Okayama, Japan

Homeotic (Hox) genes determine each segment identity along AP axis. Extensive studies in a long germ insect, fruit fly, revealed that, in regulation of Hox genes, at first gap genes determine an anterior border of a Hox gene expression, and then Polycomb group (PcG) genes maintain anterior silencing in an epigenetic manner. On the other hand, in intermediate germ insects, in which modes of segmentation and Hox gene expression differ from long germ insects, how PcG genes act for regulating Hox gene expression has been poorly understood.

To address this issue, we performed RNAi-based functional analyses of the *Enhancer of zeste* (*E(z)*) gene, one of PcG genes, in the cricket *Gryllus bimaculatus*, an intermediate germ insect. In embryos depleted for *E(z)*, embryonic appendages excluding mandible showed characteristics of the thoracic leg. Concordantly, the Hox genes, *Antp*, *Ubx*, *abd-A*, and *Abd-B*, were ectopically expressed in the anterior embryo. From detailed analyses of Hox gene expression in the RNAi embryos, we found that the middle Hox genes first appear in their normal pattern, then ectopic expressions appear in the anterior region. On the other hand, posterior Hox genes showed abnormal patterns without showing normal pattern. These results suggest that, in the cricket, PcG genes are not only involved in maintenance of anterior Hox silencing similar to *Drosophila*, but also may play a role in establishment of expression domains in the initiation of posterior Hox gene expression. We will discuss an evolutionary transition of the Hox regulatory machinery from the intermediate germ type to the long germ type.

TARGETED GENOME EDITING USING CRISPR/CAS9 SYSTEM IN THE CRICKET *GRYLLUS BIMACULATUS*

T. Mito, Y. Matsuoka, S. Tomonari, T. Watanabe, S. Noji

The University of Tokushima, Tokushima, Japan

An orthopteran insect, the cricket *Gryllus bimaculatus*, has recently become a hemimetabolous insect model system owing to the successes of RNAi-based gene-functional analyses and transgenic technology, whereas target mutagenesis was intractable in this species. Recently, a gene-knockout technique by genome editing using the artificial restriction enzymes, ZFNs/TALENs, has been established, providing a new way to analyzing the cricket genome function.

To develop a simpler and more efficient genome-editing technology in the cricket, we focused on a method using the CRISPR/Cas9 system, an immune system against viruses in bacteria. In the CRISPR/Cas9 system, a target sequence is specifically recognized by a guide RNA (gRNA) and digested by the Cas9 nuclease to induce a double-strand break. We designed gRNAs for some *Gryllus* genes, such as *laccase2* and *Ubx*, and microinjected them into cricket eggs with Cas9 nuclease mRNA. The efficiency of introducing targeted mutations by the CRISPR/Cas9 system was significantly higher than by ZFNs/TALENs. Via selection of G₁ heterozygous mutants with SURVEYOR-based mutation assays and subsequent sibling crosses, we obtained homozygous mutants exhibiting knockout phenotypes. Furthermore, we also succeeded CRISPR/Cas9-mediated knock-in of a DNA cassette into the cricket genome by NHEJ-based repair pathway. We will report new results on CRISPR/Cas9-based genome editing in the cricket for developmental regulatory genes and discuss its potential in functional genomics.

ORIGINS AND REGULATION OF AN EUTHERIAN NOVELTY: THE BGW CLUSTER

E. Navas-Pérez¹, S. d'Aniello², J. Garcia-Fernández¹

¹. University of Barcelona, Barcelona, Spain

². Stazione Zoologica Anton Dohrn, Napoli, Italy

Two related gene subfamilies known as BEX and TCEAL map to a genomic region specific to Eutheria, located on the X chromosome. These families are part of a gene cluster, named “BGW cluster”, together with the ARMCX family and HNRNPH2. Some of the BEX/TCEAL genes have been related to control the balance between proliferation and differentiation, while others promote apoptosis in a p75-dependent manner, but most of them remain poorly studied.

The ARMCX family and HNRNPH2 are derived from autosomal retrocopies, whereas no orthologs have been found for the BEX/TCEAL family outside of Eutheria. However, all these genes share an intriguing feature: a sequence motif in their proximal promoter region that appears to be crucial for their expression, the BGW motif. To further understand the evolution of this gene cluster, we investigated the origin of the BEX/TCEAL genes and traced it to an atypical formation in the ancestor of eutherians. Furthermore, novel features associated with BEX/TCEAL suggest a more complete scenario for the origin of the cluster: the BGW motif was already present at the HNRNPH2 locus in the ancestor of therian mammals, being subsequently duplicated and coopted in the eutherian lineage by the BEX/TCEAL ancestor and, posteriorly, by the ARMCX ancestral gene. Finally, we also studied the expression of the BEX/TCEAL genes during mouse development. We found that they are highly expressed in the brain and placenta, structures that require a well-tuned control of cell cycle during their development in eutherian mammals.

Here we propose a scenario for the origin of the BEX/TCEAL family and for the formation of the BGW cluster where they belong. Their uncommon origin, their pattern of expression, and their putative biological function during development makes these genes an interesting subject of study to understand how lineage-specific genes could contribute to mammalian evolution.

HOX GENES AND THE EVOLUTION OF VERTEBRATE LIMBS

J. Castro^{1,2}, R. Freitas^{1,2}

¹. Porto, Portugal

². IBMC - Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal

Fossil data suggests that limbs evolved from fish fins by sequential elaboration of their distal endoskeleton, giving rise to the autopod close to the tetrapod origin. This elaboration occurred concomitantly with the reduction of the ectodermal fold present in the distal end of fish fins. Modulation of 5'HoxD gene transcription has long been suggested as a possible evolutionary mechanism involved in these morphological transformations. Recently, gain of *hoxd13a* function during zebrafish fin development was shown to cause increased proliferation and distal expansion of chondrogenic tissue, accompanied by finfold reduction. Here we will explore how overexpression of *hoxd13a* affects the expression of its downstream targets. For our analyses, we selected genes described to be strongly involved in limb development and shown to bind to Hoxd13 in Chip-to-Chip assays. The role that these genes might play in the overall picture of the fin-to-limb transition will be discussed.

TRASLOCATION OF TORSO-LIKE FROM THE EGG SHELL TO THE OOCYTE PLASMA MEMBRANE

A. Mineo^{1,2}, M. Furriols^{1,2}, J. Casanova^{1,2}

¹. Institute for Biomedical Research (IRB)

². Institute for Molecular Biology of Barcelona (IBMB), Barcelona, Spain

The specification of the terminal and dorsoventral axes in the *Drosophila* embryo rely on asymmetries among the ovarian follicle cells that are maintained until early embryogenesis to ensure the uneven activation of their receptors. In the case of the terminal regions of the embryo, they are specified by the Torso (Tor) tyrosine kinase receptor, which is uniformly present over the entire blastoderm membrane but activated exclusively at the embryonic poles. The precise mechanism of Tor activation is still unknown but it relies on the restricted expression of *torso-like* (*tsl*) in the egg chamber by subpopulations of follicle cells located at both ends of the oocyte. Tsl accumulates at the internal side of the vitelline membrane, the innermost layer of the eggshell, thus holding the initial asymmetric cue generated in the egg chamber. However, how this asymmetry from the eggshell would then feed into the embryo remained an open question. We have found that, during oogenesis, Tsl colocalises with eggshell components but it is associated with the oocyte plasma membrane only after egg activation. These results suggest a two-step mechanism: an initial anchoring of Tsl at the vitelline membrane, as it is secreted by the follicle cells, followed by its later translocation to the plasma membrane to enable the restricted Tor activation. We are now studying whether a similar translocation mechanism could take place in the dorsoventral axis specification.

NEW INSIGHTS INTO VERTEBRAL CENTRUM FORMATION: AN EVO-DEVO PERSPECTIVE

T. Pais de Azevedo¹, A. Huysseune², P. Witten², I. Palmeirim¹

1. University of Algarve, Faro, Portugal

2. University of Gent, Gent, Belgium

Vertebrates have their name derived from one of its most conspicuous characteristics, a segmented body plan with a vertebral column composed of vertebrae. In both anamniotes and amniotes, each one of these structures is composed of a core designated centrum and arches located in its periphery. Although similar in composition, the process of forming segmented vertebrae differs in these two groups especially in the structure that contains and confers the segmented pattern information to the vertebral centrum. More specifically, in the zebrafish, it is the mineralization of the notochordal sheath that sets up the segmented pattern, while in the chick, this information is located in the most ventromedial sclerotome cells that migrate to the space surrounding the notochord. In this system, one vertebrae is formed by the posterior half of one sclerotome and the anterior half of the next, with each sclerotome being separated by Von Ebner's fissure. In the chick, however, notochord removal experiments done by Strudel in 1955 resulting in formation of fused vertebral centra, suggest that the notochord may have some of the information for vertebrae segmentation.

Both the role of the notochord in the chick embryo and the overall process on zebrafish are still questions with a lot of missing information. As so, we set out to explore these processes in both animal models starting with thorough morphological study of the structures that contribute to the formation of the vertebral centra: the sclerotome and the notochord. This was accomplished by performing coronal 1µm sections of both chick and zebrafish embryos of said stages. At the same time, we performed microsurgical notochord removal experiments in chick embryos to clarify the role of the notochord in vertebral centrum segmentation.

ASYMMETRY VERSUS SYMMETRY: THE ROLE OF DMRT2A IN THE FORMATION OF THE VERTEBRATE BODY PLAN

R.A. Pinto^{1,2}, J. Almeida-Santos^{1,2}, L. Saúde^{1,2}

¹. Instituto de Medicina Molecular e Instituto de Histologia e Biologia do Desenvolvimento, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

². Instituto Gulbenkian de Ciência, Oeiras, Portugal

To design the vertebrate body plan it is fundamental to create asymmetry between the left and the right side of the lateral plate mesoderm (LPM), in order to correctly position the asymmetric internal organs. Also, it is crucial to maintain symmetry between the left and the right side of the presomitic mesoderm (PSM) to ensure the perfect allocation of symmetric body structures such as the axial skeleton, skeletal muscles and peripheral nerves. We have previously identified the zinc finger-like transcription factor Dmrt2a/Terra as the first coordinator of these two key processes that set up the vertebrate body plan: left-right asymmetry in the LPM and bilateral symmetry in the PSM.

In order to understand the dual function of Dmrt2a/Terra, it is crucial to uncover its mechanism of action. To achieve this goal, we are building two heat-shock inducible transgenic lines that will overexpress Dmrt2a/Terra (Hsp70:Venus-*dmrt2a* and Hsp70:HA-*dmrt2a*), and a *dmrt2a* mutant line resorting to the TALEN technology. Following their characterization, we will use these lines in a microarray approach to expose the transcriptional regulatory network where Dmrt2a/Terra operates and in a ChIP-seq assay to identify Dmrt2a/Terra consensus binding site(s). From the genes identified, we will select the ones expressed in relevant places at the correct developmental stages. The function of the selected genes as left-right regulators downstream of Dmrt2a/Terra, will be characterized in gain and loss-of-function assays. Finally, according to our main goal, we will organize the newly formed pathway downstream of Dmrt2a/Terra.

FUNCTIONAL CHARACTERIZATION IN TRANSGENIC ZEBRAFISH OF REGULATORY SEQUENCES TARGETED BY THE TRANSCRIPTION FACTOR SOX2, IDENTIFIED BY STUDIES OF LONG-RANGE CHROMATIN INTERACTIONS IN BRAIN-DERIVED NEURAL STEM CELLS

J.A. Bertolini¹, R. Favaro¹, Y. Zhang², B. Martynoga³, C. Barone¹, S. Delvecchio¹, S. Mercurio¹, P. Robson⁴, F. Guillemot³, G. Pavesi⁵, M.J. Cardozo⁶, C.-L. Wei², P. Bovolenta⁶, S.K. Nicolis¹

¹. University of Milano-Bicocca, Milano, Italy

². DOE Joint Genome Institute, Walnut Creek, CA, USA

³. MRC National Institute for Medical Research, London, UK

⁴. Genome Institute of Singapore

⁵. University of Milano, Milano, Italy

⁶. Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain

Sox2 encodes a transcription factor required for embryonic stem cell pluripotency. Heterozygous *Sox2* mutations in humans cause neurological defects, and *Sox2*-conditional knock-out in mouse causes abnormal telencephalic development and impairs neural stem cells maintenance. We compared long-range DNA interactions in chromatin of wild-type mouse neural stem/precursor cells (NPCs) and *Sox2*-deleted cells, using the ChIA-PET technique: out of a total of 7000 long-range interactions mapped in wild-type NPCs, 2700 were lost in *Sox2*-deleted cells. We also determined differentially expressed genes in wild-type *versus Sox2*-deleted NPCs, by RNA-seq, and the genome-wide map of SOX2 binding sites in chromatin of wild-type NPCs, by ChIP-seq. We are functionally characterizing a selected number (13) of putative regulatory elements involved in SOX2-dependent ChIA-PET interactions with the promoter of genes important for neural development and deregulated following *Sox2* deletion. The selected regulatory elements contain SOX2-binding sites, demonstrated by ChIP-seq, and are localized at several kilobases of distance from the associated gene. We wish to determine if these distal sequences, associated in a SOX2-dependent way to genes that were deregulated following *Sox2* loss, represent transcriptional regulatory elements active during embryonic brain development and, if so, if their activity is regulated by SOX2. We cloned the 13 putative regulatory elements selected upstream of a minimal promoter, driving GFP expression, in a Zebrafish Enhancer Detection vector. We found that 12 out of 13 putative regulatory elements drive reproducible GFP expression in the developing forebrain and/or in more posterior neural regions, matching the expression pattern of the putative associated gene. This indicates that the selected elements alone are able to guide reporter gene expression. We have obtained the stable lines from these transgenic fishes that we intend to use to modulate SOX2 levels with experiments of gain and loss of function.

THE ROLE OF DIRECT NEUROGENESIS IN THE DEVELOPMENT OF THE OLFACTORY BULB

A. Cárdenas¹, M. Cogswell², C. De Juan Romero¹, A. Tzika³, M. Milinkovitch³, L.M. Martínez¹, M. Tessier-Lavigne⁴, S. Russek², V. Borrell¹

¹. Instituto de Neurociencias CSIC-UMH, San Juan de Alicante, Alicante, España

². Boston University School of Medicine, Boston, United States

³. Université de Genève, Geneva, Switzerland

⁴. The Rockefeller University, New York, United States

The olfactory bulb (OB) develops as a unique specialization of the rostral pallium (OB primordium) from early stages of development. The onset of OB development coincides with changes in progenitor cell cycle parameters and early neurogenesis locally within the OB primordium, which have been suggested to be key for its initial evagination and growth. Here we show that the distinction between OB and neocortical development starts at the onset of neurogenesis, with changes in progenitor cell cycle parameters such as cycle lengthening and increased cycle exit, producing within a short developmental period a prominent accumulation of neurons. We find that these changes in progenitor cell dynamics involve mainly apical progenitors, and are linked to an equally important accumulation of newborn pallial neurons within the VZ of the OB primordium, virtually absent in the neocortex. Time-course and time-lapse analyses of progenitor cell lineages demonstrate that these changes are preceded by significant OB progenitor cell dynamics, including the abundant occurrence of direct neurogenesis from Radial Glia cells, which we show is nearly anecdotic in the neocortex at those stages. Hence, whereas in the neocortex the majority of neurons are born from intermediate progenitor cells through indirect neurogenesis, in the OB we find that a significant fraction of neurons are produced directly from apical progenitors, while maintaining similar rates of indirect neurogenesis as in the neocortex. Direct neurogenesis in the OB is paralleled by a reduced self-amplification of RGCs, coupling an increased neurogenesis with a reduction in apical surface, which leads to OB evagination. In summary, the development of the OB is initiated by a transient peak of neurogenesis in a short developmental period driven by a high incidence of direct neurogenesis from RGCs. We are currently searching for candidate genes differentially expressed between OB and neocortex that may regulate cell cycle dynamics.

DEEPLY CONSERVED TOPOLOGICAL ASSOCIATING DOMAINS CONTRIBUTE TO EVOLUTIONARY AND DEVELOPMENTAL REGULATORY CONSTRAINTS

C. Gómez-Marín¹, J.J. Tena¹, R.D. Acemel^{1,2}, C. Hidalgo¹, S. Naranjo¹, E. de la Calle-Mustienes¹, L. Beccari^{2,3}, E. Vielmas⁴, M. Lopez-Mayorga¹, P. Bovolenta^{2,3}, E.H. Davidson⁴, J.J. Carvajal¹, J.L. Gomez-Skarmeta¹

¹. Centro Andaluz de Biología del Desarrollo (CABD), Consejo Superior de Investigaciones Científicas/Universidad Pablo de Olavide, Sevilla, Spain

². Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, Madrid, Spain

³. CIBER de Enfermedades Raras, Madrid, Spain

⁴. Division of Biology, California Institute of Technology, Pasadena, CA, USA

Cis-regulatory elements (CREs) can act at very long distances contacting target promoters through chromatin loops, implying that, genome wide, thousands of highly specific CREs-promoter interactions must be tightly regulated to ensure proper gene expression. As a result, chromatin is compartmentalized into large Topological Associated Domains (TADs) that define megabase regions within which contacts preferentially occur. Although TADs boundaries are extremely conserved in vertebrates, the functional significance and regulatory impact of these boundaries is largely unexplored and their conservation at deeper evolutionary distances unknown. Here, we analyze the 3D regulatory architecture of Six genes (*six3/6*, *six1/2* and *six4/5*), homeobox transcription factors with crucial developmental roles that have been maintained in genomic clusters in all studied deuterostomes species. First, using 4C-seq in sea urchin, amphioxus and vertebrates, we identify a broad topological architecture strongly conserved across deuterostome phyla: Six genes are divided into two different TADs by a boundary isolating *six3/6* from its two clustered paralogs *six1/2* and *six4/5*. Second, we determine the functional contribution of this conserved TAD boundary to the zebrafish *six3a/2a* cluster chromatin architecture designing a mutant version of a *six2a/six3a*-containing BAC with the conserved boundary deleted and the Six coding sequences replaced by fluorescence reporters. After transgenesis, we observe that specific 3D topological contacts are clearly shifted from *six3a* to *six2a* promoter, including an enhancer region driving expression in *six3a* domains. These abnormal interactions result into dramatic changes in gene expression, with the *six2a* reporter incorporating *six3a*-like expression domains that in turn vanished from the *six3a* reporter. We conclude that this deeply conserved TAD boundary plays a critical role preventing competition between *six* promoters for enhancers located in opposite regulatory landscapes and that this crucial modulation may have been the major constraint for the astonishing conservation of this chromatin architecture for more than 540 of deuterostome evolution.

ROLE OF miRNAS IN OLFACTORY BULB FORMATION

V. Fernández-Martínez, M^a A. Martínez-Martínez, U. Tomasello, V. Borrell

Instituto de Neurociencias, CSIC & Universidad Miguel Hernández, Sant Joan d'Alacant, Alicante, Spain

During early telencephalic development a small domain of progenitors in the rostral pallium initiates a developmental program different from the nearby pallium causing a marked tissue growth and evagination, leading to the eventual formation of the olfactory bulb (OB). The cellular and molecular mechanisms regulating this specialization of rostral pallial progenitors into generating the OB, as opposed to the neocortex, remain largely unknown. Here we tested the potential role of miRNAs in controlling the behavior of OB progenitor cells using a *Dicer*^{flox/flox};Rx-Cre mouse, deficient in Dicer-dependent miRNAs since the onset of telencephalic development. We find that the absence of miRNAs from very early stages of brain development causes deficits in OB formation and growth, which is much smaller than in WT littermates. Rx-Dicer mutants display a significant reduction in progenitor cell proliferation and a high frequency of cell death from early stages (E10.5-E12.5), predominantly affecting Pax6+ progenitor cells and coincident with the peak of OB neurogenesis. At later stages (E13.5-E17.5) these deficits are gradually compensated by hyper-proliferation and the formation of rosette-like structures in the basal telencephalon. We are currently screening for genes differentially-expressed as a result of miRNA loss, and that may underlie the onset of these phenotypes. Our results demonstrate a crucial role of Dicer-dependent miRNAs during OB development, not only promoting cell survival but also limiting the proliferation of telencephalic progenitors.

GENETIC BASIS OF THE EVOLUTION OF DIFFERENCES IN EYE SIZE BETWEEN *DROSOPHILA SIMULANS* AND *DROSOPHILA MAURITIANA*

I. Almudi¹, MDS. Nunes¹, M. Torres², S. Arif¹, N. Posnien², AP. McGregor¹

¹. Oxford Brookes University, Oxford, United Kingdom

². Georg-August-University Goettingen, Goettingen, Germany

In the last decade, the genetic basis for the evolution of particular traits have been identified but, nevertheless, our understanding of the evolution of complex morphological features, and how their underlying genetic changes arose and spread in populations is still limited. We have found considerable variation in eye size within and among species of the *Drosophila melanogaster* subgroup. In particular, *D. mauritiana* has larger eyes than its sibling species, *D. simulans*, mainly due to differences in ommatidia size.

Here, we identified the major loci responsible for the *D. simulans* and *D. mauritiana* eye size differences on the X chromosome by QTL mapping. Using independent introgressions of the QTL region from *D. mauritiana* into *D. simulans* genome we refined this mapping and restricted it to a region of 1Mb. To further investigate the functional and developmental bases of eye size variation, we performed transcriptome profiling by RNA-Seq of the eye-antenna imaginal discs of the two species at different developmental points. By combining our high-resolution mapping data with our transcriptome datasets, we identified differentially expressed genes that lie in the QTL. Finally, our functionally characterization of these candidate genes suggests that changes in *orthodenticle* expression underlie natural variation in ommatidia size, shedding light into the genetic basis responsible for *Drosophila* eye evolution.

MATURE BLOOD CELL CLUSTERS CONSTITUTE A TRUE HEMATOPOIETIC TISSUE THAT REGULATES BLOOD CELL DIFFERENTIATION IN *DROSOPHILA*

A. Leitão¹, É. Sucena^{1,2}

¹. Instituto Gulbenkian de Ciência, Oeiras, Portugal

². Universidade de Lisboa, Faculdade de Ciências, Lisboa, Portugal

Blood cells can be found in virtually all species of coelomate animals. Their functions are usually compartmentalized into different cell types for which the correct establishment of proper numbers and ratios is essential for homeostasis. This depends upon a regulated balance between proliferation and differentiation mostly carried out in the hematopoietic organs. In *Drosophila melanogaster*, the larval hematopoietic organ (lymph gland) produces two types of mature hemocytes (blood cells), plasmatocytes and crystal cells. Strikingly, in homeostatic conditions, hemocytes produced in the lymph gland are not released until pupariation. Yet, as larval development proceeds, the numbers of circulating hemocytes of both types increase, an observation difficult to reconcile with the post-mitotic character of crystal cells. In this light, it has been proposed that hematopoietic properties must reside elsewhere, namely within the clusters of hemocytes found in close association with epidermal and neuronal cells along the larval body axis. Here, we show that hemocyte clusters function as a *bona fide* hematopoietic tissue as their structure is necessary for Notch-dependent differentiation of crystal cells. Moreover our results suggest that, contrarily to the lymph gland, crystal cells formed in clusters do not derive from prohemocytes but from the transdifferentiation of plasmatocytes. The existence of this novel hematopoietic tissue, relying on structure-dependent signaling events to promote blood homeostasis, creates a new paradigm for addressing outstanding questions in *Drosophila* hematopoiesis and establish further parallels with vertebrate systems.

THE AMPHIOXUS HOX CLUSTER REGULATORY LANDSCAPE AND THE ORIGIN OF VERTEBRATE REGULATORY INNOVATIONS

R.D. Acemel^{1*}, J.J. Tena^{1*}, F. Marletaz^{2*}, I. Maeso¹, D. Aldea³, C. Gómez-Marín¹, S. Bertrand³, H. Escrivà³, J.-L. Gómez-Skarmeta¹.

¹. Centro Andaluz de Biología del Desarrollo CSIC/UPO, Seville, Spain

². University of Oxford, Oxford, United Kingdom

³. Observatoire Oceanologique de Banyuls-sur-Mer CNRS-UMR7232 Université Pierre et Marie Curie, Banyuls-sur-Mer, France

Hox genes are essential for the antero-posterior patterning of bilaterian animals, establishing a collinear expression code that mirrors the position of Hox genes within the clusters. In vertebrates, this precise spatio-temporal expression relies upon a conserved bimodal 3D architecture based on a set regulatory inputs located in two genomic deserts flanking both ends of the cluster. Elements located in the 3'-anterior desert, along with others within the cluster itself, are important for the determination of the main body axis. In turn, the regulatory islands of the 5'-posterior desert are devoted, among other functions, to the patterning of one of the major vertebrate novelties: paired appendages. To shed light on how this bimodal regulation evolved, we have studied the topological architecture of the single Hox cluster of amphioxus, a basal slow-evolving invertebrate chordate. To this end, we perform 4C-seq in early neurula stage embryos from eight different viewpoints spanning the full 450 kb-long cluster using the promoter regions of anterior, medial and posterior genes. Contrary to vertebrates, most Hox interactions are internal to the cluster, although we also identify some contacts with distant upstream and downstream regions. Strikingly, whereas distant 3'-anterior interactions show perfect syntenic conservation with the 3' desert of vertebrates, the 5'-posterior contacts are located in a region that, beyond *Evx* and *Lunapark* genes, has no syntenic similarity with vertebrates. Instead, in amphioxus as well as in other lineages, regions orthologous to the vertebrate 5'-posterior desert are positioned in a different genomic location. Thus, whereas part of the regulatory landscape responsible for the ancestral antero-posterior role of Hox genes has been conserved since the last common chordate ancestor, the origin of the vertebrate posterior gene desert and its associated bimodal regulation controlling limb development could have been triggered by a single major genomic rearrangement in the vertebrate lineage.

EXPLORING THE ROLE OF MATERNAL GENES IN THE FORMATION OF TRANSCRIPTION BORDER IN THE BICOID SYSTEM

JM De las Heras and N Dostatni
CNRS/Institut Curie, Paris, France

The Bicoid system of *Drosophila* has been widely used as a model to understand how a gradually distributed transcription factor, can activate the expression of target genes in a discrete and precise manner (Porcher and Dostatni, 2010). One of the most studied Bicoid target genes is *hunchback* (*hb*), which is expressed at the onset of zygotic transcription in the anterior half of the embryo. *hb* expression has been analyzed by FISH on fixed embryos, which allowed precise access to parameters such as efficiency of transcription at each individual locus or position of the expression border but provided poor information about the dynamics of the process generating the expression pattern (Porcher et al., 2010).

To access the dynamics of the transcription process in the Bicoid system, we have recently used the MS2 system (which allows the fluorescent tagging of RNA in living cells), to monitor ongoing transcription of the canonical *hunchback* promoter in living embryos (Lucas et al., 2013). This approach revealed that the canonical *hunchback* promoter (500 bp) is not able to recapitulate the endogenous expression at early stages of development, as ectopic MS2 signals are also detected in the posterior region of the embryo up to nuclear cycle 12. This indicates that maternal factors are likely repressing *hb* expression in the posterior of the embryo prior to nuclear cycle 12 and that specific sequences are likely missing in the reporter construct to avoid expression in the posterior region of the embryo. Using FISH, genetics and the MS2 system, we have observed that the reduction of caudal (*cad*) dose leads to the extension of anterior *hb* expression domain to the posterior of the embryo. These data suggest that two opposite gradients with opposite functions collaborate to define a specific pattern of expression in the early *Drosophila* embryo.

PROBLEMS AND SOLUTIONS IN VERTEBRATE SKULL DEVELOPMENT

R. Castanhinha^{1,2}, E. Sucena¹, J. Leon¹

¹. Instituto Gulbenkian de Ciência, Oeiras, Portugal

². Museu da Lourinhã, Lourinhã, Portugal

The *Gallus gallus* (chicken) embryo is a central model organism in evolutionary developmental biology. Its anatomy and developmental genetics have been extensively studied and many relevant evolutionary implications have been made so far. However, important questions regarding the developmental origin of the chicken skull bones are still unresolved such that no solid homology can be established across organisms. This precludes evolutionary comparisons between this model and other avian systems in which skull anatomy has evolved significantly. A classical example is the disputed double origin of the frontal bone. Different lineage tracing studies present dissimilar results. The first hypothesis claims that a population of cells exclusively derived from neural crest forms this bone. Other authors advocate for a double contribution from neural crest and paraxial mesoderm derived cells. In mice the results are unanimous attributing the origin of the entire frontal bone to cells derived from neural crest, while the posteriorly contiguous bone (the parietal) is formed exclusively by paraxial mesoderm derived cells. At the same time the posterior region of bird's adult skull misses one bone when compared with other archosauria and mammals. This absence has been traditionally interpreted as an evolutionary loss of the interparietal bone in the bird lineage. Nevertheless, it is not obvious whether the bird's frontal is homologous to one (frontal), or to a fusion of two skull bones (frontal + parietal). Here, we present data combining new gene expression studies, fate mapping using GFP chicken embryos and several comparative anatomy techniques that establish different levels of homology relationships between the bones of Aves and Mammalia, shading new light to our understanding of the evolution of development of the vertebrate skull.

THE DEVELOPMENTAL TRANSCRIPTION FACTOR PITX2 DIMINISHES AFTER BIRTH, BECOMES RE-ACTIVATED IN HEART FAILURE AND STIMULATES MYF5 EXPRESSION IN CARDIOMYOCYTES

M. Torrado¹, D. Franco², F. Hernández-Torres², A.T. Mikhailov¹

¹, University of La Coruña, La Coruña, Spain

², University of Jaén, Jaén, Spain

Background: PITX2 (paired-like homeodomain 2 transcription factor) is crucial for cardiac embryogenesis, but its role in postnatal heart development and disease remains largely uncertain.

Aim: To unravel the involvement of PITX2 in both normal and pathological conditions of early-postnatal heart.

Methods: Neonatal piglets were used as model animals. Heart failure (HF) was induced in 6-day-old piglets by a single doxorubicin injection. A variety of molecular, cell-based, and immunochemical assays were used to evaluate: (1) the expression of *Pitx2* and a subset of *Pitx2* target genes in normal and failing myocardium and (2) molecular consequences of *Pitx2* expression manipulation in cardiomyocytes *in vitro*.

Results: *Pitx2c* is the only transcript detected in the porcine left ventricular (LV) myocardium. In neonatal piglets, the LV wall thickness was approximately 2-fold higher in 30-day-old piglets than in newborns. Comparative qRT-PCR analysis of the LV-samples from these animals revealed that *Pitx2c* mRNA levels at birth are 16-fold higher than are those detected in 30-day-old animals. The expression of *Pitx2c*, physiologically downregulated in the postnatal heart, is 8-fold re-activated in LV failing myocardium which, in turn, is associated with increased expression of a restrictive set of *Pitx2* target genes as revealed by microarray analysis. Among these, *Myf5* was identified as the top upregulated gene. Transient transfections of *Pitx2c* to HL-1 cardiomyocytes strongly enhanced, in a dose-dependent manner, *Myf5* expression at both transcript and protein levels, but no stimulation of *Myf5* expression was observed in *Pitx2c*-transfected Sol8 myoblasts. The inhibition of *Pitx2c* overexpression, using *Pitx2*-siRNAs, led to a decrease of *Pitx2c* activation of the *Myf5* gene in co-transfected HL-1 cardiomyoblasts, whereas did not affect the level of *Myf5* expression in Sol8 myoblasts.

Conclusions: *Pitx2c* is reactivated in postnatal heart at HF that in turn is associated with modulation of *Myf5* expression in failing myocardium.

SNAIL1 CONTROLS BONE LENGTH

S. Vega¹, C. López-Blau¹, C. A. de Frutos^{1,3}, J. Galcerán¹, S.J. Weiss², M.A. Nieto¹

¹. Instituto de Neurociencias CSIC-UMH, San Juan de Alicante, Spain

². University of Michigan, Ann Harbour, USA

³. École Normale Supérieure, Institut de Biologie de l'ENS, Paris, France

Non-endocrine dwarfism is caused by different activating mutations of FGFR3 signalling pathway. This aberrant activation leads to a decrease in bone length and defects in chondrocyte differentiation that give rise to several phenotypes (from hypochondroplasia to achondroplasia and the most severe, thanatophoric dysplasia) (1). We previously found that the mediator of the FGFR3 pathway in the developing bone is the Snail1 transcription factor. Snail1 overexpression is sufficient to induce achondroplasia in mice, and its expression is highly increased in bones from thanatophoric fetuses (2). We have now assessed whether deregulated Snail1 is not only sufficient but also required to generate the achondroplastic phenotype. To directly test it, we have generated an inducible mouse model where Snail1 expression can be specifically attenuated in chondrocytes (Col2CreERT2:Snail1^{flox/flox}). We will present data showing that indeed, Snail1 levels seem to control the final length of the long bones. This suggests that Snail may behave as a good therapeutic target in achondroplasia and our next aim is to examine whether achondroplasia caused by mutations in the *Fgfr3* gene can be rescued in mice by inhibiting Snail1 function.

References

- (1) Horton WA, Hall JG, Hecht JT. (2007) Achondroplasia. Lancet; 370: 162-72.
- (2) de Frutos CA, Vega S, Manzanares M, Flores JM, Huertas H, Martínez-Frías ML, Nieto MA. (2007). Snail1 is a transcriptional effector of FGFR3 signaling during chondrogenesis and achondroplasias. Dev Cell; 13: 872-83.

SEARCHING FOR NOVEL COMPONENTS/REGULATORS OF THE *DROSOPHILA* NEPHROCYTE SLIT DIAPHRAGM

A. S. Tutor^{1,2}, M. Ruiz-Gómez¹

¹. Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain

². Universidad Europea, Madrid, Spain

Previous work of the laboratory has demonstrated that the cells involved in the filtration of the haemolymph in *Drosophila melanogaster*, the nephrocytes, have filtration diaphragms that are structurally, molecularly and functionally homologous to the slit diaphragm of vertebrate podocytes (1). Our data suggest that the nephrocytes are an ideal experimental model to study the formation, maintenance and function of the slit diaphragm under physiological conditions and for the study of pathologies resulting from their dysfunction. This is so, because the mechanisms that regulate slit diaphragm dynamics in response to injury are conserved between vertebrates and flies (2).

To further investigate nephrocyte slit diaphragm assembly and function, we are interested in the identification of novel genes that somehow could be implicated in these processes. In this context, we have performed an in vivo RNAi screening using the Gal4-UAS system to specific knockdown the expression of several candidate genes. We will present the result of this analysis.

References:

- (1) Weavers, H*, Prieto-Sánchez, S*, Grawe, F., Garcia-López, A., Artero, R., Wilsch-Bräuninger, M., Ruiz-Gómez, M., Skaer, H. and Denholm, B. (2009). The insect nephrocyte is a podocyte-like cell with a filtration slit diaphragm. *Nature*, 457: 322-326.
- (2) AS. Tutor, S. Prieto-Sánchez and M. Ruiz-Gomez. (2014). Src64B phosphorylates Dumbfounded and regulates slit diaphragm dynamics: *Drosophila*, a model to study nephropathies. *Development*, 141 (2): 367-376

REGULATION OF CYTONEMES FORMATION IN *DROSOPHILA*

S. Jordán-Álvarez, I. Guerrero

Centro de Biología Molecular “Severo Ochoa” (CSIC-UAM), Madrid, Spain

We would like to understand signalling dynamics by exploring the role of specialized cytoplasmic projections (cytonemes) in the crosstalk between signalling pathways during development and also in some diseases like cancer. Signalling between cells is fundamental for proper development and functioning of multicellular systems. Cooperation between different pathways achieves a new level of functional diversity: the understanding this cooperation is essential for knowing how the development of different tissues is achieved and also to understand the progress of some diseases. For example, misregulation of some pathway leads to cancer. The signalling molecules Hedgehog (Hh), Epidermal Growth Factor (EGF) and other proteins like PI3K and Ras are vital for tissue patterning and organ development, but their cooperation has been poorly explored. Collaboration of these proteins is implicated in tumour development, and crosstalk between them is necessary for signal co-activation in individual cells, but mechanisms for their intercellular communication are not known.

We study cooperation between these pathways through EGF regulation of Hedgehog (Hh) cytonemes, where activation of one pathway affects the signal outcome of another at a distance. Our goal is to study cytoneme's establishment and dynamics, combining genetics, cell biology and confocal imaging. We will achieve these goals by characterizing the signalling pathways and protein interactions involved in cytoneme formation during *Drosophila* development.

In addition, due to the similarities between this type of cell-cell communication mediated by cytonemes and neuronal synapse, proteins regulating synaptogenesis could control also cytonemes dynamics. We are screening for proteins that could be involved in these two processes.

The results of our research will give us new insights into molecular mechanisms of cytoneme formation and signalling cooperation during the formation of tissues in animals. These data could improve the knowledge of how cancer is developed and lead to new therapies against this disease.

THE REGULATION OF ATOH1 DURING OTIC DEVELOPMENT

H. Gálvez, J. Petrovic, J. Neves, F. Giraldez, G. Abelló

Universitat Pompeu Fabra, Barcelona, Spain

The proneural gene *Atoh1* is crucial for the development and regeneration of hair cells (HCs). *Atoh1* expression is recapitulated by an enhancer situated 3.5kb downstream the coding region. Notch signaling plays an important role in ear development and in the regulation of *Atoh1* expression. Notch ligands and targets are expressed during ear development: *Jag1* and *Hey1* are involved in sensory patch specification, whereas *Dll1* and *Hes5* are related to HC determination. *Sox2* promotes sensory competence and self-renewal of otic progenitors, and it activates *Atoh1* through direct binding to the 3'*Atoh1*-enhancer. In parallel, *Sox2* induces also the expression of several bHLH factors like Notch target genes *Hes5* and *Hey1*, neurogenic genes *Neurog1*, *NeuroD*, and BMP targets like *Ids*. Therefore, *Sox2* triggers an incoherent response that both promotes and counteracts *Atoh1* expression. We are currently analyzing the interactions between *Sox2*, Notch and other factors with the 3'*Atoh1*-enhancer. One specific aim of this project is to understand how repressors interact with *Atoh1* and prevent the onset of HC differentiation during development. This question has a direct link with HC regeneration that relies on the re-activation of *Atoh1* and is facilitated by Notch blockade. The 3'*Atoh1*-enhancer contains putative Ebox binding sites for bHLH factors. We have studied the behaviour of the 3'*Atoh1*-enhancer in the presence of the *Sox2* bHLH target genes during early chick inner ear development. The results show that *Hes5*, *Hey1* and *Neurogenin1*, are able to repress the activity of the 3'*Atoh1*-enhancer *in vivo*, while *Atoh1* and *Sox2* activate it. These and other experiments suggest that the repression of *Atoh1* is rather robust and tightly regulated by complex interactions among different repressor factors.

DEFECTS IN EMBRYONIC LAMININ DEPOSITION AND MYOGENESIS CONTRIBUTE TO DISEASE PROGRESSION IN A MOUSE MODEL OF CONGENITAL MUSCULAR DYSTROPHY

A. M. Nunes^{1,2}, A. B. Gonçalves¹, P. Ybot-Gonzalez³, M. Deries¹, D. J. Burkin², S. Thorsteinsdóttir¹

¹. Centro de Biología Ambiental, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal

². Center for Molecular Medicine, University of Nevada School of Medicine, Reno, USA

³. Unidad de Gestión de Pediatría, Hospital Infantil Virgen del Rocío, Instituto de Biomedicina de Sevilla (IBIS), Sevilla, Spain

Skeletal muscle development is a complex process which starts early in embryogenesis, and progressively builds up skeletal muscle. Laminins are glycoproteins which compose the extracellular matrix around muscle cells and are essential for muscle development and function. Laminin 111 and 511 are thought to be essential for early stages of myogenesis, while laminin 211/221 are crucial for later stages. When no alpha2-laminins are present, as in Merosin deficient congenital muscular dystrophy type 1A (MDC1A), crippling muscle weakness is evident from birth.

We undertook a detailed analysis of laminin isoforms distribution during epaxial myogenesis in normal mouse embryos. We found that laminin alpha2 deposition correlates with myotome development and the onset of secondary myogenesis, while most of primary myogenesis occurs in the absence of assembled laminins. Surprisingly, 3D reconstruction of laminin immunolabeling at fetal stages reveals the laminin matrix may not only be important for myofibers, but also for Pax3/Pax7-positive muscle precursors and myoblasts. We are presently comparing this normal developmental pattern of laminin deposition with the pattern observed in *dy*^{W/-} embryos, a mouse model for MDC1A. Preliminary data show that the myotome is reduced in size at E10.5 and there is increased apoptosis in the dermomyotome, the source of Pax3/Pax7-positive muscle precursors. These results indicate that *dy*^{W/-} embryos have a defect in the earliest stages of muscle development. At fetal stages, the *dy*^{W/-} muscles are morphologically similar to wild-type muscles, but at E17.5 they exhibit increased levels of embryonic myosin, a marker of regeneration, and fewer Pax7-positive cells than controls. Our results indicate that laminin alpha2 is critical to early muscle development and suggest that its absence leads to an abnormal activation of the muscle regeneration machinery.

TISSUE OVERGROWTH INDUCED BY COEXPRESSION OF THE PROGENITOR GENES *HTH/MEIS1* AND *TSH/TSHZ* RESULTS FROM AN IMBALANCE IN THE ESTROGEN RESPONSE PATHWAY IN *DROSOPHILA*

M. Neto^{1,2}, M. Naval-Sánchez³, D. Potier³, P.S. Pereira², D. Geerts⁴, S. Aerts³, F. Casares¹

¹. CABD (Centro Andaluz de Biología del Desarrollo), CSIC-Universidad Pablo de Olavide-Junta de Andalucía, Seville, Spain

². IBMC (Institute for Molecular and Cell Biology), Universidade do Porto, Porto, Portugal

³. Center for Human Genetics, University of Leuven, Leuven, Belgium

⁴. Department of Pediatric Oncology and Hematology, Erasmus University Medical Center, Rotterdam, The Netherlands

During normal development, transcription factors (TFs) control the state of cells, including properties such as cell proliferation, death or differentiation. Altered expression of TFs is often associated to human disease. This is the case for the TFs of the Meis1 gene family. Meis TFs are usually expressed in progenitors and it has been shown that abnormal maintenance of Meis1 in progenitors of the myeloid lineage is oncogenic. However, the exact mechanisms that are triggered by Meis1 during oncogenesis are still very poorly understood. In *Drosophila*, the Meis1 homologue *homothorax* (*hth*) is also associated with the maintenance of progenitors during eye development. Interestingly, forced co-expression of Hth and another progenitor TF, Teashirt (*Tsh*), produces hyperplastic growth. We have found a highly significant association between the co-expression of the human *hth* and *tsh* homologues (MEIS1 and TSHZ family genes) in specific tumor types. This fact highlights a broader role of Hth/Meis and Tsh/TSHZ genes beyond development in tumorigenesis. Using *Drosophila* as model organism, we have found that Hth+Tsh expressing cells show transcriptional changes in the ecdysone receptor (EcR) pathway, cell cycle and DNA repair-related genes. Ecdysone is the major insect steroid hormone and plays key roles in controlling developmental timing, apoptosis and cell proliferation and growth in insects. We found that two components of this pathway, the nuclear receptors Ftz-f1 and DHR3, play an important role in promoting the Hth+Tsh-specific overgrowth. Furthermore, the transcriptional changes in EcR cascade genes correlate with those in cell cycle genes, and cis-regulatory regions associated to these genes show a significant enrichment in DHR3/Ftz-f1 potential binding sites. Together, our results uncover a mechanism by which the Hth+Tsh-induced overgrowths would partly result from the expression imbalance of nuclear receptor expression and the consequent downstream regulation of cell cycle related genes.

DRUG SCREENING IN *DROSOPHILA* MODELS OF FRIEDREICH ATAXIA AND PARKINSON'S DISEASE

V. Muñoz-Soriano^{1,2}, F. J. Sanz¹, P. Calap-Quintana¹, R. Pavia¹, S. López-Domenech^{1,2}, M. D. Moltó^{1,3,4}, M. J. Martínez-Sebastián¹, N. Paricio^{1,2}

¹. Universidad de Valencia, Valencia, Spain

². ERI de Biotecnología y Biomedicina, Universidad de Valencia, Valencia, Spain

³. Red de Salud Mental, CIBERSAM

⁴. Fundación Investigación Clínico de Valencia, INCLIVA, Valencia, Spain

Friedreich ataxia (FRDA) is the most common inherited ataxia in the western population and is caused by a reduction in the level of the mitochondrial protein frataxin. Parkinson's disease (PD) is the second most common neurodegenerative disease in the world and is caused by the selective loss of dopaminergic neurons in the substantia nigra pars compacta and the decrease of the striatal dopamine levels. Oxidative stress and mitochondrial alterations seem to have an important role in the pathogenesis of both neurodegenerative diseases. Many groups are trying to identify possible therapeutic molecules able to alleviate the symptoms of both disorders. For FRDA, the most studied drug candidates are antioxidants, iron chelators and compounds that increase frataxin synthesis. Current therapies for PD, based on a dopamine replacement strategy, are effective in the first stages of the disease, but fail to retard, stop or reverse neurodegeneration. In this work, we use a *Drosophila* FRDA model in which downregulation of the *fh* gene (that encodes *Drosophila* frataxin) is obtained by an iRNA ubiquitously expressed with the GAL4-UAS system. Regarding PD, mutations in *DJ-1*, a gene closely linked to oxidative stress response, are associated to recessive forms of PD. Therefore, flies carrying a loss-of-function mutation in the *DJ-1 β* gene (the *Drosophila DJ-1* ortholog) are used as PD model. Individuals of both models show a decrease of their motor performance. Using this phenotype, we are performing a screening of molecules that could recover the motor ability of these flies. So far, we have identified some possible candidates that belong to different functional categories. Among these compounds, there are antioxidants, chelators of different metals and metabolism modulators. Interestingly, some of them are able to improve motor performance in flies of both disease models, while others seem to have a specific effect over either FRDA or PD flies.

THE ROLE OF JNK PATHWAY IN TUMOR FORMATION AND PROGRESSION IN THE ABSENCE OF APOPTOSIS

N. Pinal, G. Morata

Centro de Biología Molecular Severo Ochoa, Madrid, Spain

The JNK pathway is implicated in many different processes; among these, in the developing eye, it is activated in tumours formed by the over-activation of the Ras pathway together with mutations in the polarity genes, leading to overgrowth and invasion, acting as an pro-oncogenic pathway. In contrast, activation of this pathway in the wing tissue leads to apoptosis, a function that could be viewed as tumour suppression. This apparent contradiction led us to investigate what it is downstream of the JNK pathway to drive such different outcomes in these different contexts.

To address this question we over-activate the JNK pathway using different drivers in a *dronc* mutant background to avoid apoptosis. Over-expression of *hep* constitutively active (*hep^{CA}*) leads to different phenotypes depending on the wing disc domain where it is expressed. Expression of *hep^{CA}* in the wing pouch induces overgrowth and loss of the tissue architecture. In contrast, over- expression in the posterior compartment for 48h induces cell detachment from the tissue. And finally over- expression in the notum region has the weakest effect. We think that these different outcomes may be due to differential gene expression downstream of JNK; therefore we are studying known candidate genes downstream of JNK that could be differentially expressed in these contexts and also want to search for new targets through transcriptomic analysis.

REGENERATIVE POTENTIAL OF DIFFERENT REGIONS OF THE DROSOPHILA WING IMAGINAL DISC

R. Martín, G. Morata

Centro de Biología Molecular Severo Ochoa, Madrid, Spain

Classical transplantation experiments performed with *Drosophila* imaginal discs, the precursors of adult cuticular structures, demonstrated that disc fragments have the capacity to regenerate the entire disc. Current experiments use genetic methods to induce ablation and regeneration, and are mainly focused to test the recovery of the presumptive wing cells after massive damage. However, little is known about the regenerative capacity of the disc when the region of the wing imaginal disc that gives rise to the adult thorax (notum) is eliminated.

In this project, we aim to examine: 1) the regenerative potential or plasticity of the cells from the presumptive wing to reconstruct a damaged notum and 2) whether notum cells are able to reconstruct wing pouch structures. We have induced genetic ablation in the notum or in the presumptive wing by the expression of a pro-apoptotic gene driven by two combinations of gene enhancers specific for one or the other wing disc region. We observe different effects and regenerative potential depending on the pro-apoptotic gene used to induce ablation. The entire disc reacts to the ablation of the presumptive wing induced by *hid*, which results in a reduced notum. However, the notum proliferation rate is not affected, suggesting putative cell rearrangements and remodelling of the entire disc to reconstruct the missing region. In some aspects, these processes remind the morphallaxis displayed by *Hydra* after amputation, a regeneration type in which the remaining tissue reorganizes to reconstruct a complete organism without proliferation. By contrast, an intact notum is not able to regenerate a damaged presumptive wing after *reaper*-induced ablation; surprisingly it produces an additional notum in mirror-image. We are using the Raeppli technique to visualize cell movements and rearrangements and a LexA/LexO inducible system to trace the lineage of cells participating in the regeneration event.

ROLE AND MECHANISM OF CELL COMPETITION IN TUMOUR PROGRESSION IN DROSOPHILA

L. Ballesteros-Arias, G. Morata

Centro de Biología Molecular Severo Ochoa, Madrid, Spain

Cell competition was described in *Drosophila* as a phenomenon occurring during normal development, which consists on the interaction between normal and abnormal or unhealthy cells. This interaction results in the elimination of the later from the tissue through apoptosis. Nevertheless, both interacting types of cells are viable on their own. Recently, cell competition has been linked to tumour progression. It has been described that clones of cells deficient for several tumour suppressor genes (TSGs) within a normal tissue, die through apoptosis. However, when a whole organism is mutant for a TSG, it develops neoplastic overgrowths, indicating that mutant cells are viable on their own. This led to the idea that cell competition acts as a tumour suppressor mechanism, both in *Drosophila* and vertebrates, by eliminating malignant cells that may appear during development. However, we have recently found that the apoptosis associated with cell competition may in some circumstances function as a tumour-stimulating factor, thus reversing its normal role. Other studies in vertebrates agree with our conclusions indicating that this could be a general mechanism.

We are presently investigating the mechanisms behind the interactions needed for cell competition between normal and tumour cells. To this effect we are trying to identify new genes involved in the recognition process. We have performed a genetic screen for siRNAs that promote the tumorigenic potential of *lgl* (*lethal giant larvae*, a TSG) mutant cells growing within a normal tissue in *Drosophila*. These genes were pre-selected in a ChIP-seq assay to detect methylations and acetylations in the genome of cancer vs control tissues. We have selected and analysed those that allow the cancer cells to develop, thus overcoming the interaction that otherwise would have eliminated the tumour.

EPIGENETIC CHARACTERIZATION AND IDENTIFICATION OF GENETIC MODIFIERS OF TRANSCRIPTIONAL REPRESSION IN A FRIEDREICH ATAXIA MODEL

L. Benito-Jardón¹, P. Calap-Quintana¹, JV. Llorens¹, S. Soriano¹, MJ Martínez-Sebastián¹, MD. Moltó^{1,2,3}

¹. University of Valencia, Valencia, Spain

². Network on Mental Health, CIBERSAM, Valencia, Spain

³. Clinical Research Foundation of Valencia, Valencia, Spain

Friedreich ataxia (FA; OMIM 229300) is a neurodegenerative disorder with recessive autosomic inheritance, being the most common inherited ataxia in Caucasian population (prevalence 2-4:100000). It is characterized by several neurological symptoms, like gait and limb ataxia, and non-neurological symptoms, such as hypertrophic cardiomyopathy.

This disease is caused by an unstable GAA repeat expansion within the first intron of *FXN* gene, which leads to a reduction in the level of the mitochondrial protein frataxin. The most accepted hypothesis to explain the transcriptional repression caused by the pathological expansion is the heterocromatinization of the *FXN* locus. However, the underlying molecular mechanism of this process is not completely understood yet. In order to identify the potential factors involved in this process, we are carrying out the following study.

We developed a new *Drosophila melanogaster* model that consists in two strains expressing the reporter gene firefly luciferase preceded by 9 GAA repeats (normal expansion) in one strain and 300 GAA repeats in the other (pathological expansion).

We checked that the 300 GAA repeat expansion represses the firefly luciferase expression, similarly to transcriptional silencing of the *FXN* gene in FA patients, and also produces a reduction in the luminiscence generated by the reporter protein. It was also observed a higher level of chromatin compaction in the luciferase construct of the 300 GAA line and an increase in DNA methylation levels in the region upstream of the expanded GAA repeats compared to the 9GAA line.

Next, we started a genetic screen by crossing both model lines with other *Drosophila* strains carrying alleles of genes involved in postranslational histone modifications, heterochromatin formation and maintenance and transcriptional activation or repression. So far, we have identified some potential regulators of the repression mediated by the pathological expansion, like *Su(var)2-5*.

MORPHOMETRICS DETECT ALTERED GENE EXPRESSION PATTERNS AFFECTING EARLY LIMB DEVELOPMENT IN APERT SYNDROME

J. Sastre¹, L. Russo¹, J. Richtsmeier², J. Sharpe¹, N. Martínez-Abadías¹

¹. Center for Genomic Regulation (CRG), Barcelona, Spain

². Pennsylvania State University, University Park (PA), USA

Apert syndrome is a rare congenital disorder characterized by cranial, neural, limb and visceral malformations. Over 98% of Apert cases are caused by two FGFR2 mutations, Ser252Trp and Pro253Arg, which alter the ligand-binding specificity of the receptors. Patients carrying the P253R mutation show more severe limb malformations, such as syndactyly and symphalangism. Here we explored limb morphogenesis in early development using an inbred mouse model of Apert syndrome, *Fgfr2*^{+/P253R}, to assess whether the P253R mutation induces changes in the expression pattern of *Dusp6*, a downstream gene of the FGF/FGFR signaling pathway, and whether these genetic changes can be associated with limb malformations in mutant littermates. We performed Geometric Morphometric (GM) analyses of 3D landmark-based data recorded on optical projection tomography (OPT) images of 11.5 embryonic day (E11.5) embryos labeled for *Dusp6* expression using whole-mount *in situ* hybridization. Comparative quantitative analysis of large samples of limbs and their corresponding *Dusp6* gene expression pattern show differences in limb size and shape between mutant and unaffected littermates. At E11.5, mutant mice are significantly smaller and appear underdeveloped in comparison with unaffected littermates. Results show that size explains more than 80% of limb shape variation, but when this allometric effect is minimized the limbs of mutant mice, especially hindlimbs, remain different from the limbs of unaffected littermates. Shape differences are also found at the gene expression level, suggesting that altered FGF/FGFR signaling due to the FGFR2 mutation alters *Dusp6* gene expression patterns and contributes to limb malformations as early as E11.5. Our morphometric assessment of gene expression patterns by combining OPT and GM is a novel and useful tool to compare normal and disease-altered patterns of variation. Precise embryo phenotyping of Apert syndrome mice will allow us to identify the origins of abnormal processes that can have a damaging effect on the developing limbs.

REGULATORY VARIATION OF THE *PITX2* LOCUS IN ATRIAL FIBRILLATION

R. Rouco¹, L.A. Aguirre Pérez¹, C. Badia-Careaga¹, M. Gómez-Velázquez¹, J.J. Tena², M.E. Alonso¹, A. Fernández-Minan², A. Aranega³, J.L. Gómez-Skarmeta², D. Franco³, M. Manzanares¹

¹. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

². Centro Andaluz de Biología del Desarrollo (CSIC-UPO), Seville, Spain

³. Department of Experimental Biology, University of Jaen, Spain

PITX2 encodes a transcription factor involved in the establishment of the left-right axis and the vertebrate heart, during development, being *PITX2c* isoform the main expressed in the embryonic heart. Recent genome-wide association studies (GWAS) have revealed atrial fibrillation (AF, the most common cardiac arrhythmia in humans) associated variants on chromosome 4q25, around 100 kb upstream *PITX2*. Atrial specific deletion of *Pitx2c* in mouse hearts modifies the regulation of genes encoding ion channels subunits and signalling molecules involved in AF. Our aim is to establish whether this GWAS region harbours cis- regulatory elements that could be acting on neighbouring genes activity, such as *PITX2*. For this purpose, we have tested the regulatory activity of several genomic fragments from 4q25 in tissue cultures and transgenic mouse embryos. We have also analysed the chromatin architecture of the region by studying its physical interactions by means of Chromosome Conformation Capture (3C) and Circular Chromosome Conformation Capture combined with high-throughput sequencing (4C-seq). We have found that a genomic fragment containing rs2200733 (the lead variant associated with AF) and some of its sub-fragments, had regulatory activity in both models, although they did not show tissue or cell-type specificity. Therefore, they could be acting as accessory elements that potentiate the activity of tissue-specific enhancers located elsewhere in the locus. Furthermore, 3C and 4C-seq analyses show a complex pattern of long range interactions in the region, unveiling expected and novel interactions. Our work shows how regulatory variations acting on developmental genes can underlie the increased risk of common human diseases.

HOX MEDIATED CONTROL OF NEURONAL DIVERSITY WITHIN THE RHOMBIC LIP LINEAGE AND ITS POSSIBLE CONSEQUENCES IN MEDULLOBLASTOMA FORMATION

T. Di Meglio¹, F. Nieto-Lopez¹, D. Kraus², A. Di Nardo³, A. Prochiantz³, F. Rijli², P. Bovolenta¹

¹. Centro de Biología Molecular Severo Ochoa, Madrid, Spain

². Friedrich Miescher Institute, Basel, Switzerland

³. Collège de France, Paris, France

Medulloblastomas (MBs) are the most common malignant posterior tumors of the central nervous system. MBs mostly derive from neuronal precursors generated in the rhombic lip (RL) -a dorsal germinal epithelium of the developing hindbrain-, which fail to exit the cell cycle and differentiate. The majority of lethal MBs falls into two subgroups characterized either by high expression of the transcription factor *Otx2* or by expression of *Shh* related genes. Recent studies in mice suggest that this second type of *Shh* dependent MBs arises from precursors emerging from the most anterior cerebellar and cochlear RL, which proliferate in response to *Shh* during development. Noteworthy, the simultaneously generated precerebellar RL derivatives do not divide in response to *Shh* and do not form *Shh* dependent MB. We have combined expression analysis to gain and loss of function experiments in order to identify the mechanisms responsible for these heterogeneous proliferative properties within the RL lineages. We demonstrate that cross-functional interactions between *Otx2* and *Hoxa2* transcription factors directly influence the distinct response of RL derivatives to *Shh*. In cerebellar and cochlear RL derivatives, the induction of *Shh* signaling related genes and the proliferative response appears after the onset of *Otx2* expression in RL progenitors. Notably, in the cochlear progenitors characterized by the initial expression of *Hoxa2*, *Otx2* induction occurs only after *Hoxa2* down-regulation, which in turn is a pre-requisite for the emergence of cochlear RL derivatives mitogenic response to *Shh*. This observation may explain why *Hoxa2*-positive but *Otx2*-negative precerebellar progenitors do not respond to *Shh* signaling. We thus postulate that such developmental mechanisms could contribute to the etiological heterogeneity of MBs; accordingly *Hoxa2* should counteract *Otx2* and *Shh* dependent MB formation.

MEIS FUNCTION IN CARDIAC FIELDS IS ESSENTIAL FOR HEART DEVELOPMENT

L. Carramolino, M. González, A. López-Delgado, V. Cadenas, M. Torres
Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

The heart is the first functional organ that forms during embryonic development. The developmental complexity of heart formation is reflected in the high incidence of congenital heart defects (~1%), with 20% to 30% of these defects affecting the outflow tract (OFT). Cardiac development arises from two sources of mesoderm progenitors, the first (FHF) and the second heart field (SHF). The SHF contributes to the right ventricle, the venous pole, the OFT and part of the atria.

Meis1 and *2* are transcription factors that belong to the TALE family of homeodomain proteins. *Meis* genes show extremely complex transcriptional and spatiotemporal expression patterns and are implicated in several functions during embryogenesis, including hematopoiesis, eye, limb and heart development. *Meis1* and *2* have very similar expression patterns and can functionally replace each other *in vivo*, so the understanding of their function frequently requires elimination of both genes.

We have generated *Meis1*-constitutive and *Meis2*-conditional mutant mice and studied their involvement in heart development. Both genes are expressed in early cardiac precursors, transiently in the FHF and more stably in the SHF, and are downregulated during cardiomyocyte differentiation. Individual elimination of *Meis1* or *Meis2* produces mid-gestation lethality and provokes inter-ventricular septum and OFT defects. The combination of *Meis1* and *Meis2* mutations leads to aggravated defects in correlation with the reduction of *Meis* alleles dosage. In the complete elimination of *Meis* function, cardiac morphogenesis is severely disrupted with agenesis of cardiac chambers, inflow and outflow tracts. Embryos with one functional *Meis* allele show severely disrupted cardiac morphogenesis with defective chamber formation and cardiac looping. Outflow tract defects are present in *Mesp1Cre*- and *Nkx2.5Cre*-recombined embryos, suggesting that neural crest function is not involved in the phenotypes observed. In all cases differentiation of cardiomyocytes was observed, indicating that *Meis* function is not essential for cardiac specification or differentiation. Our results identify *Meis* function in cardiac fields as essential for cardiac morphogenesis.

CELL BEHAVIOUR ASSOCIATED WITH DIFFERENT EPITHELIAL-MESENCHYMAL INDUCERS (EMT-TFS) IN EMBRYOS AND CANCER CELLS

Rebeca Córcoles, Oscar Ocaña, Verona Villar-Cerviño, Berta Sánchez-Laorden, Jose M. Mingot, Sonia Vega and M. Angela Nieto

Instituto de Neurociencias de Alicante CSIC-UMH, 03550 San Juan de Alicante, Spain

The epithelial to mesenchymal transition (EMT) converts adherent and polarized epithelial cells into mesenchymal cells with migratory and invasive properties. During embryonic development, rounds of EMT and the reverse process, mesenchymal to epithelial transition (MET) are crucial for the formation of many tissues and organs. The reactivation of the EMT programme in the adult promotes tumour progression and organ fibrosis, and can also confer stem cell properties (Nieto, M.A. *Science.*, 2013). Similar to the situation in embryos, a reversion of the EMT (MET) seems to be necessary for metastatic colonization once malignant cells extravasate and find their niche in distant organs.

The main inducers of the EMT are transcription factors of the Snail, Zeb and Twist families (EMT-TFs). Recently, we have identified Prrx1, another transcription factor that can trigger EMT in embryos and in cancer cells (Ocaña et al. *Cancer Cell.*, 2012). Importantly, the loss of Prrx1 in mesenchymal cancer cells induces a complete reversion to the epithelial phenotype.

One question that emerges is why the organism needs so many EMT inducers and whether there are differences in the EMT triggered by each of them. Therefore, our aim is to characterize the cells that have undergone EMT triggered by each individual factor or by a combination of them, as different developing tissues and human tumors usually express several inducers. We have found that PRRX expression in embryos correlates with stages of cell migration towards different destinations, in particular, associated with areas in which cells disperse to occupy final territories. Compatible with this, we find that the loss of Prrx1 is sufficient to revert cells to the epithelial phenotype while inducing (i) increased proliferation and (ii) inhibition of cell dispersion, all favouring metastatic colonization and compatible with our functional analyses during embryonic development.

NEW MARKERS FOR ASTROCYTE IDENTITY AND FUNCTION

A. Quiroga, W. D. Richardson, H. Li.

Wolfson Institute for Biomedical Research, University College London, London, United Kingdom.

In central nervous system (CNS), despite the predominant research attention on various neuron subtypes, astrocytes are dominant in number. A diversity of astrocyte morphology across various regions of the CNS has been described suggesting that different astrocyte populations may be underlain by molecular heterogeneity. Furthermore, astrocytes have been implicated in a wide variety of physiological roles such as synaptogenesis, neurotransmission and trophic regulation. However the molecular mechanisms that specify different subpopulations responsible for these diverse functions are still poorly understood.

Using different histological techniques (e.g. in situ hybridization and immunohistochemistry) we have newly identified two potential specific markers, both transcription factors, for astrocyte subtypes. We will further investigate the functions of these two transcription factors with various approaches including gene knockout, regional astrocyte ablation and electrophysiological analysis to reveal their roles in synaptic function and in the interaction between astrocytes and neurons.

RANDOM RETROSPECTIVE CLONAL ANALYSIS OF THE DEVELOPING MOUSE HEART

G. Lioux, S. Temiño, M. Torres
CNIC, Madrid, Spain.

Mammalian heart function relies on a complex arrangement of cardiomyocytes and others. Cardiac cell diversity is the result of lineage establishment through specification of cardiac progenitors. So far early sources of heart lineages such as the cardiogenic mesoderm, proepicardium and cardiac neural crest cells, have been identified. However their exact cell potentiality is still unclear and conflicting reports on lineage contributions appeared in the literature. One possible approach to systematically interrogate precursor cell potentiality, avoiding confounding effects of unreliable lineage tracers, is the use of single-cell random labeling.

In the laboratory, we address the question of heart lineage segregation using random retrospective clonal analysis. We are targeting heart precursors around Embryonic day 9 (E9), when the primary heart tube has been allocated and the second heart field precursors are contributing to complete heart development. Results are analyzed at E14.5, when the organization plan of the heart is established and the different heart lineages are advanced in differentiation. Heart progenitors are labelled using a ubiquitous low-level expression Tamoxifen-inducible Cre recombinase mouse line (RERT). Moreover a double-reporter strategy, combining simultaneously Rosa26R:LacZ and Rosa26R:EYFP reporters, allows us to assess the clonality of the lineage labels induced, taking into account that clonally related cells are identically labelled.

Up to now hundreds of hearts were isolated and stained for both markers. The information they contain is currently being processed. We are classifying cell clusters expressing the same reporter according to their cell type/s, the heart layer they contribute to and their anatomical position. Statistical analysis is being used to establish the clonality of identified clusters. As a result, some well-established lineage relationships were confirmed by our strategy and new associations were identified. The new associations found, establish interesting hypotheses, which may help to complete our understanding of heart development.

ENDOTHELIAL JAG1/NOTCH1 AND ITS SIGNALING EFFECTOR *EFNB2* ARE ESSENTIAL FOR PROPER MYOCARDIAL COMPACTION AND CORONARY VESSELS FORMATION

Stanislao Igor Travisano, Donal MacGrogan and José Luis de la Pompa
Cardiovascular Development Biology Program, Cardiovascular Development and Repair Departement,
Centro Nacional de Investigaciones Cardiovasculares (CNIC), Melchor Fernández Almagro 3, 28029
Madrid SPAIN.

Notch signalling plays critical roles in vertebrate cardiac development and is required for the formation of ventricular chambers, cardiac valves and coronary vessels. This requirement is underscored by the fact that mutations in Notch pathway components cause multiple congenital heart defects in humans. We examined the role of Notch ligand *Jagged1* during coronary vessels development. *Jagged1* is strongly expressed in coronary vessels, from early developmental stages. Endothelial-specific deletion of *Jagged1* using the *Nfatc1-Cre* driver line resulted in embryonic lethality at E13.5. *Jagged1*-deleted mutants displayed thinner compact myocardium decreased myocardial proliferation and reduced coronary arteries formation. This latter phenotype was associated with reduced Notch1 intracellular domain expression in emerging coronary vessels and reduced expression of downstream Notch1 effector *Efnb2*, a key marker of arterial vessels identity. Endothelially-deleted *Efnb2* mutants survived up to 2 weeks post-natally and exhibited thinner myocardial wall, reduced compact zone, aberrant coronary artery formation, and/or enlarged semi-lunar valves with variable penetrance. Moreover coronary vessels number was increased by detection *in situ* of artery (*Hey1*, *Hey3*) and vein (*EphB4* and *CouptFII*) marker transcripts, suggesting that *Efnb2* is required for remodeling of the coronary plexus. These data suggest that a hierarchical network in the endothelium involving Jag1/Notch1 signaling upstream of *Efnb2* is essential for myocardial compaction and coronary formation.

THE CALCINEURIN SPLICING ISOFORM CnA β 1 IS IMPLICATED IN EARLY CARDIAC DIFFERENTIATION.

Jesús M^a Gómez-Salinero, Marina Mercedes López-Olañeta, Paula Ortiz-Sánchez, José Javier Larrasa-Alonso, Enrique Lara-Pezzi

Fundación Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain.

Embryonic stem cells (ESCs) have the ability to proliferate indefinitely in culture, and to differentiate into all embryonic lineages. Although the transcriptional program that coordinates pluripotency has been progressively unveiled during the past few years, the signalling pathways that regulate early differentiation events are not completely understood. We have recently described that CnA β 1, an alternative splicing variant of the phosphatase calcineurin, enhances muscle regeneration and improves cardiac function after myocardial infarction. CnA β 1 lacks the autoinhibitory domain typical of calcineurins, and instead has a unique C-terminal domain, with no similarity to any known protein. Unlike other calcineurin isoforms, CnA β 1 has no impact on NFAT-regulated genes and instead activates the Akt pathway. Interestingly, we have currently determined that CnA β 1 is specifically located at the Golgi contrary to other calcineurin isoforms, and that this localization is mediated by its unique C-terminal domain. CnA β 1 is strongly expressed in stem and progenitor cells, although its role in these cells is unknown. We found that CnA β 1 downregulation had no effect on ESC pluripotency. However, CnA β 1 depletion in mESCs during the first 48 hours of differentiation specifically affected differentiation towards the cardiac mesoderm lineage. In contrast, CnA β 1 overexpression promoted differentiation towards the cardiac mesoderm lineage. To further investigate the role of this isoform in mESCs differentiation we are currently characterising a CnA β 1 KO mouse line. In summary, we propose a new and specific role for the calcineurin splicing isoform CnA β 1 in the control of ESC differentiation to the cardiac mesoderm lineage.

A PITX2-MIRNAS PATHWAY REGULATES SATELLITE CELL PROLIFERATION AND SKELETAL MUSCLE REGENERATION

A. Aránega, E. Lozano-Velasco, D. Vallejo, F. Ramírez, F. Hernández-Torres, D. Franco

Department of Experimental Biology, Faculty of Experimental Sciences, University of Jaén, Spain

Regeneration of skeletal muscle mainly depends on adult muscle stem cells, named satellite cells. Pitx2 expression has been detected in putative migrating myoblasts during development as well as in adult satellite cells. We have previously documented that c-isoform of Pitx2 plays a pivotal role modulating proliferation vs differentiation during *in vitro* and *in vivo* myogenesis and regulating key myogenic transcription factors such as Pax3 by repressing miR-27. Now, by analyses of gene expression profiling of microRNA microarrays in a myogenic cell line, we have identified a set of microRNAs that are differentially regulated in Pitx2c-overexpressing myoblasts. *In vitro* and *in vivo* experiments lead us to find out a subset of microRNAs regulated by Pitx2c, with previous unknown functions on myogenic cells, which have profound effects on myoblast proliferation. We have observed that this Pitx2-microRNAs pathway regulating cell proliferation is conserved in isolated satellite cells, providing developmental cues that enhance the commitment of satellite cells to the myogenic lineage differentiation by down-regulating miR-106 expression. Interestingly, we have observed that Pitx2c is up-regulated after muscle injury indicating a putative involvement of this transcription factor in muscle regeneration. To investigate the role of Pitx2c during muscle regeneration we have carried out a “*in vivo*” cell transplantation approach that allowed us to shown that Pitx2c overexpression enhance the process of muscle regeneration in the mouse model for Duchene Muscular Dystrophy. The results obtained demonstrate that cell transplantation of Pitx2c-overexpressing dystrophic satellite cells enhance the number of myofibers, repress miR-31 reaching dystrophin restoration, improving finally muscle regeneration in MDX mice. Furthermore, we have also obtained additional evidences that the Pitx2-microRNAs pathway controlling cell proliferation is also presence during muscle regeneration. These results place Pitx2 as a new player on skeletal muscle satellite cell biology and identify unknown functions of microRNAs regulated by Pitx2 during regenerative myogenesis.

CHARACTERIZATION OF MOLECULAR PATHWAYS INVOLVED IN CELL COMPETITION IN MAMMALIAN EMBRYONIC STEM CELLS

C. Díaz Díaz, C. Clavería, G. Giovino, M. Torres

Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

Cell competition is a type of cell-cell interaction in which cells with different metabolic rates compare each other's anabolic ability and the less fit ones are eliminated from the tissue (loser) by the fitter cells that proliferate at their expense (winner). This process has been proposed to control cellular fitness and tissue homeostasis through the elimination of suboptimal cells. Although primarily studied in *Drosophila*, recent works have shown that this mechanism is a universal feature of metazoans. In our laboratory, an inducible mosaic system that leads to an imbalance of Myc expression has been generated. Using this model *in vivo*, it has been shown that c-Myc overexpression in cell populations in a mosaic fashion is able to induce competition allowing these cells to become "supercompetitors". Moreover, it has been found that during normal development Myc levels are heterogeneous among epiblast pluripotent cells giving rise to endogenous cell competition, in which cells with higher Myc levels are selected to be part of the final embryo. However, the mechanism by which neighbouring cells are able to compare each other remains elusive. Identifying cell competition mechanisms in the mammalian embryo is a challenging task; therefore, we have generated a powerful *in vitro* model that will allow us to perform a deeper analysis of the molecular pathways involved in cell competition. The *in vitro* model has been established in mouse embryonic stem cells and allows accessible handling and modification procedures difficult to establish in a whole organism. We are currently using microscopy, live-imaging and -omics techniques, to study the molecular and cellular mechanisms involved in the comparison of different anabolic capacity between neighbouring cells and how this leads to the death of the less fit ones.

REGULATION OF THE TROPHOBLAST FATE IN VITRO

T. Rayon¹, S. Menchero¹, I. Ors¹, J. Rossant², M. Manzanara¹

¹Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

²Hospital for Sick Children Research Institute, Toronto, Canada

The first differentiated tissue in mammalian development is the trophectoderm (TE), the outermost layer of the blastocyst that surrounds the pluripotent inner cell mass (ICM) and the fluid-filled blastocoel. At this stage of development, different stem cell populations can be obtained. Embryonic stem (ES) cells can be derived from the ICM, and trophoblast stem (TS) cells from the TE. These populations retain *in vitro* the capacity to differentiate into the same tissues as the cells they come from, as well as self-renew indefinitely in culture. Previously in the lab, we have identified a TE-specific enhancer that faithfully reproduces early expression of the trophectoderm transcription factor *Cdx2* in the blastocyst. Strikingly, the enhancer is not able to reproduce *Cdx2* expression after implantation or in TS cells. To understand the differential regulation of *Cdx2*, we are taking advantage of blastocyst derived stem cells, both trophoblast (TS) and embryonic (ES) stem cells, to understand the molecular differences that may arise upon implantation and within different contexts. In this way, we have found that specific cis-regulatory elements are involved in different aspects of *Cdx2* expression suggestive of a two-stage regulation model.

FUNCTION OF PERLECAN IN THE DROSOPHILA OVARY

A. Díaz-Torres, J. Pearson, A. González Reyes

Centro Andaluz de Biología del Desarrollo, Sevilla, España

The *Drosophila* female develops two ovaries, each composed of 16-20 egg-producing tubes called ovarioles. Eggs chambers are generated in the germarium, formed at the anterior end of each ovariole and home to two or three Germline Stem Cells (GSCs). Stem cells often reside in specialised cellular microenvironments or niches that offer stem cells structural support. In addition, signalling between support cells and stem cells is essential to regulate stem cell proliferation and to prevent their differentiation. The extracellular matrix plays a key role in controlling the homeostasis of stem cell niches, as it provides physical support and conveys extracellular signals. Perlecan, a heparan sulphate proteoglycan component of the extracellular matrix, has recently attracted much interest as it has been shown to act as a modulator of intercellular signals in development and morphogenesis. We will present our studies on the role of Perlecan in the maintenance of the *Drosophila* female germline stem cell niche and ovariole architecture. In addition, we will report the presence of different splicing isoforms of Perlecan in the basement membrane and in the interstitial matrix deposited in the GSC niche.

EARLY SIGNALS THAT TRIGGER EPITHELIAL REGENERATION IN DROSOPHILA IMAGINAL DISCS

P. SantaBárbara-Ruiz, F. Serras

Universitat de Barcelona, Institut de Biomedicina de la Universitat de Barcelona (IBUB), Barcelona, Spain

Drosophila imaginal discs are able to regenerate by compensatory proliferation after injury or cell death. It has been reported that one of the pathways required for wing imaginal disc regeneration is the *Jun N-terminal Kinase* (JNK). This pathway is activated during regeneration. However, the nature of the molecules upstream and downstream of JNK is poorly understood. In this work we found that immediately after injury or cell death a burst of Reactive Oxygen Species (ROS) propagates from dead cells to the adjacent living cells. This boost of ROS is previous to JNK. Treatment with antioxidants inhibits regeneration, demonstrating the requirement of this oxidative stress signal. In addition to that, we found that another early signal is the transcriptional activation of the cytokines unpaired (Upd), which act as ligands of the JAK/STAT pathway. Upd's were activated in dead cells and also in the nearby living cells after tissue damage. We found that Upd's are also essential for regeneration, as inhibition of *upd* or of JAK/STAT genes interfere with regenerative growth. Strikingly, mutants of JNK do not showed activation of the JAK/STAT reporter. In summary, we demonstrate that JNK leads to activation of JAK/STAT and that JAK/STAT promotes compensatory proliferation for tissue recovery. Eventually we found that ROS are necessary for JNK activation. We present a model in which cell damage results in a ROS burst which activates the stress-activated protein kinase JNK and consequently JNK activates the cytokines that will trigger regenerative growth. We are currently investigating whether ROS can also activate the cytokines Upd to backup growth.

DINAMIC CHARACTERIZATION OF EC, CIRC AND CC IN UC-HMSCS OVER TIME

A. Filipe, J. Bragança, I. Palmeirim

Universidade do Algarve, Campus de Gambelas, Faro, Portugal

Multiple molecular oscillators have been characterized and revealed to be crucial timekeeping mechanisms. Palmeirim et al. unveiled an Embryonic Clock (EC) capable of timing embryonic development through gene expression cycles. Other findings unveiled a crosstalk between the Cell Cycle (CC) and both Circadian Clock (CirC) and EC. Preliminary data from our laboratory points to a CirC-EC interplay. Could these three oscillators be intertwined? This project aims to uncover/characterize the interplay between three different molecular mechanisms, EC, CC and CirC, driving temporal control of biological events. First we intend to characterize the clock's oscillation by monitoring EC/CC/CirC periodic promoter's activation in human umbilical cord mesenchymal stem cells (UC-hMSC) over time, using reporter vectors. To monitor CirC activation, a 1.5-Kb promoter region of *Per1*, already described as being able to follow the promoter activation cycles, was cloned upstream the codifying region for a destabilized variant of a far-red fluorescent protein (pPer1-HcRed). The CC will be monitored using the FUCCI system, a two color reporter system that enables visualizing the cell cycle phases. Regarding EC, comparative *in silico* analysis was performed to address for the potential of a 2.5-kb mouse *Hes1* promoter region, from pHes1-d2EGFP, to follow the human *Hes1* promoter activation. Using stringent conditions, dot plot analysis identified three overlapping regions, including a region of 200bp with 91% of identity and containing the transcription start site and four N-boxes, known to be required for the negative auto-regulation of *Hes1*. Transfection assays revealed that pHes1-d2EGFP is able to follow the human *Hes1* promoter cyclic activation in UC-hMSC. Currently, stable transfected UC-hMSC with pHes1-d2EGFP and hPer1-HcRed are being obtained to monitor simultaneously and *in vivo* the periodic gene oscillations of EC and CirC, respectively. In parallel, transduction with FUCCI is being optimized to follow CC oscillations.

CHARACTERIZATION OF THE PLURIPOTENCY OF MOUSE TRANSMITOCHONDRIAL EMBRYONIC STEM CELLS

R. Nieto-Arellano¹, R. Acín-Pérez¹, P. Meade-Huerta², P. Fernández-Silva² and JA. Enriquez^{1,2}

¹. Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

². Universidad de Zaragoza. Zaragoza (Spain)

Mitochondria are important organelles in stem cell biology as regulators of stem cell pluripotency and differentiation. Different studies have demonstrated that pluripotent stem cells show low mitochondrial mass consistent with their metabolism of non-oxidative glycolysis as a major energy source. Therefore, it is very interesting to build tools that allow us to study the role of mitochondria in this context. We have generated mouse transmitochondrial embryonic stem (ES) lines carrying mutations in different genes of the mtDNA that could lead to the generation of mouse models for mitochondrial diseases. Some of these ES cells have been used in the generation of chimaeras however no germline transmission has been achieved. For that reason, we have decided to carefully study the pluripotency status of these ES cells and their ability to differentiate into several lineages. We have analyzed ES cells, both WT and mutant, for different components of electron transport chain encoded by mtDNA. So far we have not detected significant changes in the initial pluripotency between them, despite the mutation. Likewise, the transmitochondrial ES cells are able to differentiate into beating cardiomyocytes when using a more specific differentiation protocol.

THE CONTENT FUNCTIONAL EXPRESSION OF PLASMALEMMAL SYNTAXINS ON GERM-LAYER DIFFERENTIATION EXECUTED BY THEIR EXTRACELLULAR LOCALIZATION

N. Hagiwara¹, Y. Hirai¹

¹. Kwansei Gakuin university, Hyogo, Japan

The proteins in the syntaxin family are known to mediate fusion of cytoplasmic vesicles to the target membrane, yet subpopulations of certain plasmalemmal syntaxins, including epimorphin (syntaxin2) and syntaxin4, translocate across the cell membrane in response to external stimuli so as to function as signaling molecules. Here, we show that extracellularly supplied syntaxin impacted cell behaviours and regulated the differentiation direction in teratocarcinoma (EC) cells. We found that undifferentiated F9 cells extruded a small subpopulation of extracellular syntaxin4 at the lateral cell membrane, while this polarized expression pattern was abolished by the differentiation induction with all-trans retinoic acid. The forced apolar expression of extracellular syntaxin4, but not syntaxin2, in F9 cells resulted in the dramatic enhancement of cell-substrate interaction and suppression of cell-cell adhesion, with an alternation in the expression profile of vinculin, a regulator of both cell-substrate and cell-cell interactions. F9 cells expressing extracellular syntaxin4 up-regulated several differentiation markers for endodermal lineages, together with another family member syntaxin3 in these cells. The exogenous over-expression of cytoplasmic syntaxin3 elicited cell responses similar to the stimulation with extracellular syntaxin4, indicating that syntaxin4's effects were attributed, at least in part, to the up-regulation of syntaxin3. In contrast to the clear effects on F9 cells, another EC cell line P19 did not clearly respond to the extracellular syntaxin4. On the other hand, an artificial expression of extracellular syntaxin2 conferred the substrate-adherent property upon P19 cells, along with the expression induction of several mesodermal markers including that of cardiomyocyte. When P19-CL6 cells, P19-derivative cells, that have been introduced with *GFP* reporter gene connected with myosin promoter were treated with extracellular syntaxin2, green fluorescence became detectable within a few days even without any differentiation stimuli. Taken these results together, plasmalemmal syntaxins emerged as potential initiators and/or regulators of differentiation of anaplastic EC cells.

BMP SIGNALLING PROMOTES STEM CELL MAINTENANCE IN THE DEVELOPING CHICK DORSAL PALLIUM

G. Le Dréau, E. Martí

Instituto de Biología Molecular de Barcelona (IBMB-CSIC), Barcelona, Spain

The different modes of stem cell division are tightly regulated to balance growth and differentiation during organ development and homeostasis. However, the mechanisms controlling such events are not fully understood. Bone morphogenetic proteins (BMPs) are emerging as crucial external cues controlling such a balance in the developing vertebrate central nervous system.

We recently demonstrated that BMP signaling favors stem cell expansion during spinal cord development in a graded manner. We indeed reported that the level of activity of SMAD1/5, two canonical effectors of the BMP pathway, dictates the mode of division that neural progenitors undergo during spinal interneuron generation. More specifically, our results proposed that high, intermediate and low levels of SMAD1/5 activity instruct respectively symmetric proliferative (PP), asymmetric (PN) and symmetric neurogenic (NN) divisions.

Here we decided to investigate whether the BMP pathway plays a similar role during development of the chick dorsal pallium, the region homologous to the mammalian neocortex. The results obtained so far by gain- and loss-of-function strategies suggest that SMAD1/5 fulfill a similar function in the developing chick dorsal pallium. Their activity appears to be required not only to avoid a premature commitment of neural progenitors towards differentiation, but also to restrain the transition from the neural stem cell-like apical progenitors to the more fate-restricted basal progenitors. Hence, these results point to a general function of BMP signaling in the maintenance of stem cell identity during neural development.

TAMOXIFEN INDUCIBLE SYSTEMS FOR MODULATING POSTMITOTIC BRAIN PLASTICITY

C.G. Briz, N.S. de León-Reyes, M. Nieto

Centro Nacional de Biotecnología, CSIC, Madrid , Spain

Agenesis of the corpus callosum (CC) includes a number of rare diseases characterized by the absence of axonal tract connecting the two cerebral hemispheres and also accompanies many diseases of the nervous system. CC connectivity is also affected in neurodegeneration. CC connections have shown a remarkable plasticity and, in mice, can be regenerated at early postnatal stages. Cux1 is a transcription factor selectively expressed in layer II-III callosal projecting neurons. Loss of Cux1 blocks contralateral synapses of CC axons. This is due to down-regulation of the expression of Kv1 potassium channels, abnormal excitability and irregular firing response. We have shown that restoring expression of Cux1 or Kv1 channels in Cux1 embryonic deficient neurons rescue CC formation. We still do not know if postnatal activation of Cux1 or Kv1 expression allows CC repair once the circuit have been incorrectly formed. Using tamoxifen inducible constructs and in utero electroporation to reprogram layer II-III neurons, we will evaluate rescue of axonal phenotypes. We will analyze the capacity of callosal neurons to reconnect, branch and innervate at different times of development. This will determine the temporal window in which the circuit is still plastic. In addition, we will test if normal Cux1 expression is required for maintenance of axonal terminals after correct branching and innervation has occurred during development. We will implement for the first time the CRISPR mediated knock-out of genes in layer II-III neurons by in utero electroporation. Thereafter we will create a tamoxifen inducible Cas9 construct to conditionally knock-out genes. These innovative experiments will define the dependence of callosal axons on Cux1 and normal firing pattern and excitability to maintain contralateral innervation. These results will open new avenues for pharmacological and genetic intervention and compensation of the symptoms of patients with agenesis of the CC and of diseases involving axonal degeneration.

THE STRESS-ACTIVATED JNK COORDINATES PLANARIAN REGENERATION AND RESCALING BY TRIGGERING APOPTOSIS AND MODULATING THE CELL CYCLE

E. Saló, M. Almuedo-Castillo, T. Adell

Departament de Genètica, Facultat de Biologia and Institut de Biomedicina de la Universitat de Barcelona (IBUB), Catalunya, Spain

Adult planarians enjoy a formidable morphological plasticity, which allows them to regenerate any body part in two weeks and to continuously modulate their size, growth or de-growth, depending on the availability of food, maintaining always the functionality of their organs. The presence of pluripotent stem cells, the neoblasts, and the maintenance of active cell signalling pathways allows such unusual behavior. Both, during regeneration and during homeostasis neoblasts proliferation but also cell death should be tightly balanced in order to rebuild and maintain proportioned animals.

Here we report the essential role of JNK (c-Jun N-terminal kinase), a stress-activated protein kinase well-preserved in metazoan evolution, in the control of the cell cycle progression and in the triggering of apoptotic cell death during planarians regeneration and homeostasis.

Loss of function of the *Schmidtea mediterranea* JNK (*Smed-JNK*) after RNA interference (RNAi) decreases the expression of wound-induced genes, generates a severe attenuation of the two apoptotic waves and accelerates the G2- to M-phase transition of neoblasts, altogether preventing the regeneration of missing structures. JNK silencing in shrinking starved adult organisms interfered with the maintenance of body proportions. However, it was not so in fed growing planarians, since shrink but not growth depends on the activation of apoptosis. Altogether, these results point to JNK as an essential stress response element required for the integration and coordination of the apoptotic and proliferative responses triggered by tissue loss or starvation to ensure successful regeneration and tissue remodeling.

DECIPHERING THE ROLE OF EMT-TFS IN DIFFERENT STEPS OF THE METASTATIC CASCADE BY USING A ZEBRAFISH MODEL

B. Sanchez-Laorden, O. Ocaña, R. Corcoles and M. A. Nieto

Instituto de Neurociencias CSIC-UMH, San Juan de Alicante, Spain

Metastasis is a challenging clinical problem as it is the cause of over 90% of deaths from cancer. Metastasis is a complex biological process whereby tumour cells overcome the many barriers that prevent the primary tumour from spreading throughout the body. To achieve metastasis, tumour cells undergo changes associated with a multiple step program, which starts by facilitating cell escape from the primary tumor, followed by cell intravasation and extravasation from the blood vessels and ends with the colonization of distant organs. Increasing evidence has revealed the importance in tumour metastasis of genes normally involved in embryonic development, especially the epithelial to mesenchymal transition transcription factors (EMT-TFs). Despite previous findings, several questions related to the role of these genes in the different steps of the metastatic cascade are still pending. We use the zebrafish as a model to investigate this issue. We perform injections of cancer cell lines into the blood circulation of zebrafish embryos in order to address the impact of specific EMT-TFs in both extravasation and metastatic colonization. The advantages of this system include the conservation of molecular and cellular components in embryonic development and tumour progression and the transparency and the small size of the fish that allows imaging the entire living animal. We will present the characterization of the putative role of specific EMT-TFs in extravasation, as well as our findings on the impact of these factors in metastatic colonization.

PROTEIN PROFILE OF WILD PLANTS OF SOTOL (*DASYLIRION LEIOPHYLLUM ENGELM. EX TREL*) IN CHIHUAHUA, MÉXICO

J. González-García, Q. Rascón-Cruz, A. Vargas-García

Universidad Autónoma de Chihuahua, Chihuahua, México

The alcoholic beverage Sotol is product of the fermentation of the carbohydrates in the pineapple of the plant *Dasyllirion spp.* Vinomex and Garza *et al.* (2008) have shown that the concentration of carbohydrates in the plant decreases in the months of summer resulting in a less efficient production of alcohol. The adaptation to the environment of *Dasyllirion spp.* is responsible for the change in concentration of carbohydrates, as the habitat of the plant sotol of the Chihuahua desert is very extreme with variations up to 30 °C between different seasons, with moderated precipitations and few constant restricted to the months of summer.

It is difficult to explain the change in the concentration of carbohydrates in *Dasyllirion spp.* due to the environment conditions, which is why this investigation provides information of which proteins are regulated to the high or low seasons when the plant has greater concentration of carbohydrates. The results indicate that the protein profile of non photosynthetic tissue of the plant *Dasyllirion leiophyllum Engelm. ex Trel* in gels of polyacrylamide (SDS-PAGE) changes depending on the environment conditions, mainly the proteins with molecular weight in the range of 80 to 23 kDa, that may belong to the group of fructosyltransferases and exo-hydrolases enzymes. In the month of October the proteins within that range have more relevant activity, according to the bibliography, to a stage of increase in the concentration of carbohydrates; the months of December and February have practically the same protein profile, but less enzymatic activity that the month of October, which corresponds to a latent stage of maximum concentration of carbohydrates; and the month of June with the least concentration of carbohydrates, it also presented the least intensity in the corresponding bands of the analysed proteins.

THE STEROID HORMONE ECDYSONE PROMOTES GROWTH OF IMAGINAL DISCS IN *DROSOPHILA MELANOGASTER*

R. Barrio¹, L. Herboso¹, M. Oliveira², A. Talamillo¹, C. Pérez¹, M. González¹, D. Martín³, J. D. Sutherland¹, C. Mirth²

¹. CIC bioGUNE, Derio, Bizkaia, Spain

². Instituto Gulbenkian de Ciência, Fundação Calouste Gulbenkian, Oerias, Portugal

³. Institute of Evolutionary Biology, Consejo Superior de Investigaciones Científicas–Universitat Pompeu Fabra, Barcelona, Spain

Animals have a determined species-specific body size. This is regulated by steroid hormones, such is ecdysone in insects, which are involved in the regulation of the growth period extension, while the insulin/insulin-like and Tor signalling pathways are implicated in the control of the growth rate. In addition, organs need to coordinate their growth to maintain a correct proportion. In *Drosophila*, ecdysone regulates developmental transitions by controlling moulting and metamorphosis. The size achieved by the end of the third instar prior to pupariation, defines the final size of the adult fly. The entry in metamorphosis is determined by a peak of ecdysone that has a negative effect on organ growth rate and promotes tissue differentiation. However, here we show that ecdysone is also needed to promote tissue growth at mid third instar larvae, as imaginal discs not exposed to the increasing ecdysone levels corresponding to this period, are smaller than wild type. These organs have fewer and smaller cells than the controls, parameters that are recovered after exogenous ecdysone administration. Results obtained in the less derived hemimetabolous insect *Blattella germanica* indicate that regulation of organ growth by ecdysone is an evolutionary conserved feature in insects. We also show that insulin signalling is being produced and secreted normally by the insulin producing cells, and it is received in the imaginal discs even with low levels of circulating ecdysone. However, we observed that ecdysone is necessary to downregulate Thor in the imaginal discs, a growth inhibitor acting downstream of the insulin/insulin-like/Tor signalling pathways. Our data provide new insights in the relationship between insulin/insulin-like/Tor and ecdysone signalling pathways in the control of organ growth.

SOXD GENES CONTROLS PROLIFERATION AND DORSAL SPECIFICATION IN THE SPINAL CORD

A.V. Morales¹, A.C. Quiroga¹, C.C. Stolt², R. Diez del Corral¹, S. Dimitrov¹, E. Sock², M. Wegner²

¹. Instituto Cajal, CSIC, Madrid, 28002, Spain

². Institut für Biochemie, Universität Erlangen-Nürnberg, Erlangen, D-91054, Germany

The basic organization of somatosensory circuits in the spinal cord is already set up during the initial patterning of the dorsal neural tube. Extrinsic signals, such as Wnt and TGF- β pathways, activate combinatorial codes of transcription factors that are responsible for generating a pattern of discrete domains of dorsal progenitors (dp). These progenitors will give rise to distinct dorsal interneurons (dI). The Wnt/ β catenin signaling pathway controls specification of dp/dI1-3 progenitors and interneurons. According to the current model in the field, Wnt/ β catenin activity seems to act in a graded fashion in the spinal cord, as different relative levels determine the identity of adjacent progenitors. However, it is not clear how this activity gradient is controlled and how the identities of dI1-3 are differentially regulated by Wnt signalling. We have determined that two SoxD transcription factors, Sox5 and Sox6, are expressed in restricted domains of dorsal progenitors in the neural tube. Using gain- and loss-of function approaches in chicken embryos, we have established that Sox5 controls cell fate specification of progenitors dp2 and dp3 and, as a result, controls the correct number of the corresponding dorsal interneurons (dI2 and dI3). Furthermore, Sox5 exerts its function by restricting dorsally Wnt signaling activity via direct transcriptional induction of the negative Wnt pathway regulator *Axin2*. By that way, Sox5 acts as a Wnt pathway modulator that contributes to sharpen the dorsal gradient of Wnt/ β catenin activity to control the distinction of two functionally distinct types of interneurons, dI2 and dI3 involved in the somatosensory relay.

ENHANCEMENT OF A SONIC HEDGEHOG NEGATIVE FEEDBACK LOOP BY FGF SIGNALLING CONTROLS INITIATION OF SPINAL CORD VENTRAL PATTERNING

R. Díez del Corral¹, A. V. Morales¹, S. Espeso-Gil¹, I. Ocaña^{1,3}, F. Nieto-Lopez^{1,2,3}, P. Bovolenta^{1,2,3}, M. Lewandoski⁴

¹. Instituto Cajal, CSIC, Madrid, Spain

². Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Madrid, Spain

³. CIBER de Enfermedades Raras, Spain

⁴. Center for Cancer Research, National Cancer Institute, Frederick, USA

A prevalent developmental mechanism for the assignment of cell identities is the production of spatio-temporal concentration gradients of extracellular signalling molecules that are interpreted by the responding cells to activate specific developmental programs. One of such signalling systems is the Shh gradient that controls neuronal subtype identity in the ventral spinal cord. Using loss and gain of function approaches in chick and mouse embryos, we show here that the Fibroblast Growth Factor (FGF) signalling pathway is required to limit the activity of the Sonic Hedgehog (Shh) pathway and to restrict the domains of ventral gene expression as neuroepithelial cells become exposed to Shh during caudal extension of the embryo. FGF signalling activates the expression of the Shh receptor and negative pathway regulator Patched 2 (Ptch2) and therefore enhances a negative feedback loop that restrains the activity of the pathway. Thus, we identify the mechanism by which FGF signalling acts as an essential modulator of the onset of Shh signalling activity in the context of coordination of ventral patterning and caudal axis extension. Similar mechanisms of negative feedback enhancement may be operating in other developmental contexts as well as in pathological conditions and we propose that this may represent a common gene regulatory toolbox used in living organisms.

p27^{Kip1} PARTICIPATES IN THE REGULATION OF ENDOREDUPPLICATIVE CYCLES IN DIFFERENTIATING CHICK RETINAL GANGLION CELLS

M.C. Ovejero-Benito, J.M. Frade

Cajal Institute, CSIC, Madrid, Spain

Endoreduplication is a process characterized by genomic DNA duplication in the absence of cell division that leads to polyploidy in eukaryotic cells. This mechanism has been shown to be independent of cyclinD-cdk4/6 activity. Endoreduplicative cycles are observed in some neuronal populations from invertebrates. A paradigmatic case are giant neurons from *Aplysia*, whose nuclei may contain up to 200,000-fold the normal haploid DNA amount (C). In contrast, nuclei from polyploid neurons in higher vertebrates show only 4C DNA content, indicating that a mechanism should exist to prevent extra rounds of DNA synthesis in these cells. Focusing on the chick retina, we show that the expression of p27^{Kip1} in differentiating chick retinal ganglion cells (RGCs) as they become layered could be crucial for this mechanism. Indeed, many of the p27^{Kip1}-positive, differentiating RGCs express the neurotrophic receptor p75 while lacking retinoblastoma protein expression, a feature of RGCs that become tetraploid in the chick retina. We show that two different interfering RNAs (RNAi) directed against the *Cdkn1b* transcript, which significantly downregulate p27^{Kip1} expression, are both able to facilitate DNA synthesis and to increase ploidy in isolated chick RGCs. Forced DNA synthesis triggered by p27^{Kip1}-specific RNAi constructs could not be prevented by cdk4/6 inhibition, thus indicating that in the absence of p27^{Kip1}-mediated repression, DNA synthesis in layered RGCs may be triggered by a mechanism similar to endoreduplication. We propose that a molecular mechanism involving p27^{Kip1} is used by neurons from high vertebrates to prevent endoreduplicative rounds of DNA synthesis. This mechanism may prevent tetraploid RGCs from increasing their DNA amount.

APP mRNA LOCALIZES WITHIN A SUBSET OF AXONS OF THE TECTUM IN CHICK EMBRYOS

N. Onodera, R. Nanayama, K. Okudaira, S. Nozaki & I. Araki
Iwate University, Morioka, Japan

Amyloid precursor protein (APP) is a transmembrane protein whose inappropriate processing is hypothesized to lead the pathogenesis of Alzheimer's disease. APP gene is expressed in the tectum of chick embryos in layer specific manner, and APPs α , the cleaved ectodomain of APP, has been suggested to modulate NgCAM-dependent retinal axon outgrowth together with contactin 4 in vitro (Osterfield et al, 2008). On the other hand, APP is linked to other cellular functions including cell migration and cell survival in other systems. As a first step to investigate possible other APP functions in the tectal development, we have started the detailed analysis of the expression of APP gene in the developing tectum. During the process we detected APP transcripts in the axons in the late stage chick embryos. In the immunofluorescence microscopy we observed the staining for APP protein in the the cell bodies but not in the axons in the same stage embryos. We also detected antisense transcript(s) from APP locus in the axons. Northern hybridization confirmed the existence of the antisense transcript in the late-stage embryonic brain. According to Ensembl database multiple antisense transcripts are assigned for APP locus in the human and mouse genomes. Such antisense transcripts might be involved in the regulation of APP gene.

VARIATION IN SPERM FUNCTION AMONG MOUSE SPECIES DURING PREPARATION FOR FERTILIZATION

E. Sansegundo-Hernando¹, M. Tourmente¹, E. R. S. Roldan¹

¹Grupo de Ecología y Biología de la Reproducción, Museo Nacional de Ciencias Naturales (CSIC), Madrid, Spain.

Mammalian spermatozoa must undergo a series of cellular and molecular changes before being able to participate in fertilization. This physiological "switching-on" (known as "capacitation") is essential for exocytosis of the sperm acrosomal granule and includes modifications in the pattern of sperm movement. Upon capacitation, spermatozoa change from a linear and symmetrical trajectory to an asymmetrical, vigorous and non-linear movement ("hyperactivation"). We compared changes associated to capacitation in spermatozoa of three mouse species (*M. musculus*, *M. spretus* and *M. spicilegus*) that differ in their levels of postcopulatory sexual selection (PCSS). Spermatozoa were incubated in capacitating (mT-BH) and control (mT-H) media, and several parameters were quantified over time: percentages of sperm that were motile, capacitated, acrosome-reacted, and hyperactivated, along with descriptors of sperm swimming velocity, and sperm ATP content. Timing of capacitation differed between the three species: the species with higher PCSS levels achieved a higher percentage of capacitated cells in a shorter period of time. In all species, hyperactivation was characterized by an increase in curvilinear velocity and lateral head displacement, and a decrease in straight-line velocity and linearity. However, no clear pattern of association was seen between hyperactivation patterns and PCSS levels. Therefore, PCSS may act on certain sperm features (capacitation), whereas other sperm traits could be selected by cryptic female choice in the female tract or by male-female coevolution.

JAK/STAT AND HOX DYNAMIC INTERACTIONS IN AN ORGANOGENETIC GENE CASCADE

P. B. Pinto^{1,2}, J. M. Espinosa-Vázquez¹, M.L. Rivas¹ and J. Castelli-Gair Hombria¹

¹. CABD, CSIC/JA/Universidad Pablo de Olavide, Seville, Spain

². Current address: Centre for Organismal Studies (COS) Heidelberg, Heidelberg, Germany

Organogenesis is controlled by complex gene networks activated by upstream regulatory master genes. The posterior spiracles of *Drosophila* are activated by the Hox gene Abd-B. Among several targets, Abd-B activates in the spiracles the transcription of *unpaired* (*upd*), the main JAK/STAT pathway ligand. STAT is required for the upregulation of spiracle targets, like the crumbs (*crb*) apical determinant. To address how the organogenetic gene network evolves at later stages of organogenesis we analysed the activation of *crb*.

Dissection of the *crb* spiracle enhancer shows it integrates STAT and Abd-B input. The enhancer is composed of three elements, one responsible for the spiracle specificity, another having a repressor function and a third module binding STAT.

Using ChIP and EMSA we show that Abd-B and STAT bind the *crb*-spiracle enhancer and that both are required for its activation. We find that STAT activity is not required as a transcriptional collaborator of Abd-B, but it acts by blocking the effect of the repressive element over the specificity element. In the absence of the repressor element, the STAT binding module is not required. This uncovers a novel function for the STAT protein, where it contributes to gene expression working as a “counter-repressor” rather than as a direct transcription.

Our results show that Abd-B is required directly and indirectly for *crb* expression. Indirectly by activating STAT in the spiracle, and directly by binding to the specificity element. STAT is also required directly and indirectly for *crb* expression. Directly by binding to the *crb* enhancer and preventing the function of the repressor, and indirectly because it is required for the specificity module expression without the direct binding to the enhancer.

Surprisingly we find that STAT activity feeds back into Abd-B, showing that what started as a linear Hox cascade is evolving into a gene network.

Participants List

Updated 10th October 2014

PARTICIPANTS LIST		
Last Name	First Name	Country
Agata	Kiyokazu	JAPAN
Albuxech-Crespo	Beatriz	SPAIN
Almeida	Patrícia	PORTUGAL
Almudi	Isabel	UNITED KINGDOM
Alsina	Berta	SPAIN
Alvarez	Noemi L	SPAIN
Alves	Filipa	PORTUGAL
Andrés-Delgado	Laura	SPAIN
Araki	Isato	JAPAN
Aranega	Amelia	SPAIN
Arcas	Aida	SPAIN
Atienza Manuel	Alexandra	SPAIN
Aznar-Benitah	Salvador	SPAIN
Azpiazu	Natalia	SPAIN
Badía	Claudio	SPAIN
Baena-Lopez	Luis ALberto	UNITED KINGDOM
Baguña	Jaume	SPAIN
Ballesteros Arias	Luna	SPAIN
Barlow	Linda	USA
Barrio	Rosa	SPAIN
Benito	Lucía	SPAIN
Bertolini	Jessica Armida	ITALY
Borrell	Victor	SPAIN
Bovolenta	Paola	SPAIN
Burguera	Demian	SPAIN
Calap	Pablo	SPAIN
Calleja Requena	Manuel	SPAIN
Campuzano Corrales	Sonsoles	SPAIN
Cardenas	Adrián	SPAIN
Carramolino	Laura	SPAIN
Carrasco Rando	Marta	SPAIN
Carvajal	Jaime	SPAIN
Casal	Jose	UNITED KINGDOM
Casanova	Jordi	SPAIN
Casares	Fernando	SPAIN
Casas Tinto	Sergio	SPAIN
Castelli-Gair Hombría	James	SPAIN
Castro	João	PORTUGAL
Cavodeassi	Florencia	SPAIN
Cebrià	Francesc	SPAIN
Chaouiya	Claudine	PORTUGAL
Córdoba	Sergio	SPAIN
Corominas Guiu	Montserrat	SPAIN

Last Name	First Name	Country
Coskun	Hakan	SPAIN
Cosma	María Pía	SPAIN
Cubas	Pilar	SPAIN
De La Cruz	Ana Carmena	SPAIN
De Las Heras	José M	FRANCE
Del Saz Soler	Delia	SPAIN
Delgado	Irene	SPAIN
Di Croce	Luciano	SPAIN
Di Meglio	Thomas	FRANCE
Díaz	Covadonga	SPAIN
Díaz de la Loza	M. Carmen	SPAIN
Díaz Torres	Alfonsa	SPAIN
Diego	Xavier	SPAIN
Diez Del Corral	Ruth	SPAIN
Dominguez	María	SPAIN
Domínguez Acemel	Rafael	SPAIN
Dominguez-Cejudo	Maria Angeles	SPAIN
Duboule	Denis	SWITZERLAND
Escalona	Jose Rene	SPAIN
Espinosa-Vásquez	José Manuel	SPAIN
Estella	Carlos	SPAIN
Estrada	Beatriz	SPAIN
Fanlo Escudero	Lucía	SPAIN
Fargas Madriles	Laura	SPAIN
Fernández	Virginia	SPAIN
Ferrandiz	Cristina	SPAIN
Ferreira	Ana	SPAIN
Filipe	Alexandra	PORTUGAL
Frade	José María	SPAIN
Galceran	Juan	SPAIN
Gálvez García	Héctor	SPAIN
García Morales	Diana	SPAIN
Garcia poyatos	Carolina	SPAIN
García-Fernandez	Jordi	SPAIN
Garcia-Martinez	Virginio	SPAIN
Germann	Philipp	SPAIN
Giovinazzo	Giovanna	SPAIN
Giraldez	Fernando	SPAIN
Gómez	Melisa	SPAIN
Gomez Apiñaniz	Paula	SPAIN
Gómez Marín	Carlos	SPAIN
Gomez Miguez	David	SPAIN
Gómez Salinero	Jesús	SPAIN
Gomez Skarmeta	José Luis	SPAIN

Last Name	First Name	Country
Gonçalves	André	PORTUGAL
González	Sara	SPAIN
González Gobbart	Elena	SPAIN
González Méndez	Laura	SPAIN
González Pérez	Esther	SPAIN
González Sainz de Aja	Julio	SPAIN
González-García	Juencio	MEXICO
Gonzalez-Reyes	Acaimo	SPAIN
Gorfinkiel	Nicole	SPAIN
Gutiérrez Vallejo	Irene	SPAIN
Hagiwara	Natsumi	JAPAN
Hernández	Catalina	SPAIN
Hernández Bejarano	Maria	SPAIN
Hernández-Torres	Francisco	SPAIN
Irastorza	Igor	SPAIN
Irie	Naoki	JAPAN
Isern	Joan	SPAIN
Ivanovitch	Kenzo David	SPAIN
Jacinto	Ana Raquel	PORTUGAL
Jiménez	Alba	SPAIN
Jimenez-Guri	Eva	SPAIN
Jordán-Álvarez	Sheila	SPAIN
Juarez Uribe	Rafael	SPAIN
Jurado-Gómez	Jaime	SPAIN
Kass Youssef	Khalil	SPAIN
Kondo	Takefumi	JAPAN
Kornberg	Tom	USA
Kuranaga	Erina	JAPAN
Kuriyama	Sei	JAPAN
Lawrence	Peter	UNITED KINGDOM
Le Dréau	Gwenvael	SPAIN
Lehner	Ben	SPAIN
Letelier	Joaquín	SPAIN
Lioux	Ghislaine	SPAIN
Lobo Pecellin	María	SPAIN
Lopes	Susana	PORTUGAL
López	Alejandra Cristina	SPAIN
López Sánchez-Laorden	Berta	SPAIN
López-Mayorga	Macarena	SPAIN
Lopez-Sanchez	Carmen	SPAIN
Losa	Marta	UNITED KINGDOM
Lozano-Velasco	Estefania	SPAIN
Macias	Ana	ARGENTIN
Maeso	Ignacio	SPAIN

Last Name	First Name	Country
Magri	Marta	SPAIN
Manzanares	Miguel	SPAIN
Margarido	Andreia	PORTUGAL
Martí Gorostiza	Elisa	SPAIN
Martin	Francisco A.	FRANCE
Martín Bermudo	María Dolores	SPAIN
Martin Palomeque	Raquel	SPAIN
Martínez -Morales	Juan Ramón	SPAIN
Martínez Ostalé	Cristina	SPAIN
Matsuda	Hiroki	GERMANY
Menchero	Sergio	SPAIN
Mendez-Ferrer	Simon	SPAIN
Mercader	Nadia	SPAIN
Mikhailov	Alexander	SPAIN
Milán	Marco	SPAIN
Mingot Acencao	Jose Manuel	SPAIN
Montes Ruiz	Antonio Jose	SPAIN
Montiel Manríquez	Rogelio	MEXICO
Morales	Aixa V.	SPAIN
Morata	Gines	SPAIN
Moreno	Eduardo	SWITZERLAND
Münch	Juliane	SPAIN
Muñoz	Pura	SPAIN
Muñoz-Chápuli	Ramon	SPAIN
Muñoz-Soriano	Veronica	SPAIN
Nava	M. Paz	SPAIN
Navas	Enrique	SPAIN
Neto	Marta	SPAIN
Neto	Goncalo	FRANCE
Nieto	Rocio	SPAIN
Nieto	Angela	SPAIN
Nieto Lopez	Francisco Javier	SPAIN
Nishiyama	Koichi	JAPAN
Nunes	Andreia	PORTUGAL
Ocaña	Oscar	SPAIN
Olivera Crego	Inés	SPAIN
Onimaru	Koh	SPAIN
Onodera	Nozomi	JAPAN
Pais De Azevedo	Tomás	PORTUGAL
Pérez Moreno	Juan José	SPAIN
Perez-Garijo	Ainhua	USA
Pinal	Noelia	SPAIN
Pinheiro	Gonçalo	PORTUGAL
Pinto	Rita	PORTUGAL

Last Name	First Name	Country
Pi-Roig	Aina	SPAIN
Pontes	Samuel	SPAIN
Prieto Hueso	Nuria	SPAIN
Pujades	Cristina	SPAIN
Quiroga	Alejandra	UNITED KINGDOM
Rada-Iglesias	Alvaro	GERMANY
Rago	Luciano	SPAIN
Rayón	Teresa	SPAIN
Recasens	Carles	SPAIN
Rodrigues	Inês	PORTUGAL
Rodríguez Curt	Jesús	SPAIN
Ros	Marian	SPAIN
Rouco	Raquel	SPAIN
Rubbini	Davide	SPAIN
Ruíz	Mar	SPAIN
Ruiz-Romero	Marina	SPAIN
Ruiz-Trillo	Iñaki	SPAIN
Saade	Murielle	SPAIN
Saló	Emili	SPAIN
Sanchez Arrones	Luisa	SPAIN
Sanchez Sanchez	Besaid	SPAIN
Sánchez-Aragón	Máximo	SPAIN
Sanchez-Arrones	Luisa	SPAIN
Sánchez-Camacho	Cristina	SPAIN
Sanchez-Herrero	Ernesto	SPAIN
Sansegundo Hernando	Ester	SPAIN
Santa Bárbara Ruiz	Paula	SPAIN
Saude	Leonor	PORTUGAL
Serrano	Manuel	SPAIN
Serras Rigalt	Florenci	SPAIN
Sharpe	James	SPAIN
Shirae-Kurabayashi	Maki	JAPAN
Shono	Takanori	UNITED KINGDOM
Simpson	Pat	USA
Slakov	Ivica	SPAIN
Sobrado	Antonio	SPAIN
Struhl	Gary	USA
Suarez	Teresa	SPAIN
Sumi	Anghugali	SPAIN
Takahashi	Yoshiko	JAPAN
Takeda	Hiroyuki	JAPAN
Tanaka	Kohtaro	PORTUGAL
Terriente	Javier	SPAIN
Thorsteinsdattir	Solveig	PORTUGAL

Last Name	First Name	Country
Torres	Miguel	SPAIN
Travisano	Stanislao	SPAIN
Unda	Fernando	SPAIN
Usieto Camín	Susana	SPAIN
Uzkudun	Manu	SPAIN
Valencia Expósito	Andrea	SPAIN
Vásquez	Patricia	SPAIN
Vega	Sonia	SPAIN
Veiga Fernandes	Henrique	PORTUGAL
Vicente Garcia	Cristina	SPAIN
Villa	Cristina	SPAIN
Villar Cerviño	Verona	SPAIN
Wotton	Karl	SPAIN
Ybot-Gonzalez	Patricia	SPAIN