3rd Australia-Brazil-Chile (ABC) Regenerative Medicine and Developmental Biology Symposium

FeSBE Federação de Sociedades de Biologia Experimental

Symposium Program

Timing and presentation titles are subject to change

Day 1: Monday 9 September 2019

9:00-9:15 **Opening Ceremony**
Hernandes Carvalho (director FeSBE) and Silvio Tiziani (director External Strategy and Planning, Australian Regenerative Medicine Institute)

Session I: *Genetic and molecular control of development and regeneration*

Chair: Lucia Elvira Alvares (Unicamp, Brazil)

9:15-9:45 ***"Telling a tale of tails: The Hox13 genes and the specification of the posterior vertebrae and tail in the zebrafish"***
Miguel Allende (FONDAP Center for Genome Regulation (CGR), Universidad de Chile, Chile)

9:45-10:15 ***"Insights from Developmental Pathways on the Evolution of Snakelike phenotypes in Tetrapoda"***
Tiana Kohlsdorf (University of Sao Paulo - Ribeirao Preto, Brazil)

10:15-10:45 ***"Deciphering the cardiac gene regulatory network"***
Mirana Ramialison (Australian Regenerative Medicine Institute, Australia)

10:45-11:15 *Coffee Break*

11:15-11:45 ***"Gene regulation in early cell fate decisions and digit regeneration in mice"***
Henrique Marques Souza – University of Campinas (Unicamp)

11:45-12:15 ***"When the inhibitor also helps: Cactus/IkB protein function in Drosophila embryogenesis and immunity"***
Helena Araujo (Federal University of Rio de Janeiro, Brazil)

12:15-12:45 ***"Effect of tRNA composition in ORF selection and proteostasis in Drosophila"***
Alvaro Glavic (Universidad de Chile, Chile)

12:45-14:30 **Lunch**

Session II: *Nervous System development and regeneration*

Chair: Alvaro Glavic (Universidad de Chile, Chile)

14:30-15:00 ***"New insights on AP axis and neural induction"***
Jose Garcia Abreu Junior (Federal University of Rio de Janeiro, Brazil)

15:00-15:30 ***"Pre and pos-transcriptional regulation of cSCRATCH2 gene expression in neural embryogenesis"***
Irene Yan (University of Sao Paulo, Brazil)

15:30-16:00 *Coffee Break*

16:00-16:30 ***"Make do and make new: how zebrafish rapidly regenerates CNS injury"***
Jan Kaslin (Australian Regenerative Medicine Institute, Australia)

16:30-17:00 ***"Cellular and Molecular Mechanisms of Zebrafish Spinal Cord Regeneration"***
Hozana Castillo (Brazilian Biosciences National Laboratory (LNBio/CNPEM), Brazil)

18:00-18:30 **FeSBE Opening Ceremony**

18:30-19:30 **Plenary Conference**
"Understanding skeletal muscle regeneration using zebrafish models"
Peter Currie (Australian Regenerative Medicine Institute, Australia)

Day 2: Tuesday 10 September 2019

Session III: *Stem cells and disease*

Chair: Laura Galvis (Institut NeuroMyoGène. France and Australian Regenerative Medicine Institute, Australia)

9:00-9:30 ***"Can you model breast cancer using human induced pluripotent stem cells?"***
Andrew Laslett (Australian Regenerative Medicine Institute and Commonwealth Scientific and Industrial Research Organisation, Australia)

9:30-10:00 ***"Stem cell therapy in lung diseases"***
Patricia Rocco (Federal University of Rio de Janeiro, Brazil)

10:00-10:30 ***"JAM-A is critical for HSC trafficking and maintenance of their quiescence in the stem cell niche"***
Susie Nilsson (Australian Regenerative Medicine Institute and Commonwealth Scientific and Industrial Research Organisation, Australia)

10:30-11:00 ***"iPSC: a powerful modeling tool to study diseases"***
Bruno Torres (Brazilian Biosciences National Laboratory (LNBio/CNPEM), Brazil

11:00-11:30 *Coffee Break*

11:30-12:30 **FeSBE Plenary Conference**
"What lies ahead of the frontier in cardiac development and evolution?"
José Xavier Neto – Federal University of Ceara (UFC)

12:30-14:30 **Lunch**

Session IV: *Molecular control of muscle development*

Chair: Hozana Castillo (Brazilian Biosciences National Laboratory, Brazil)

14:30-15:00 ***"Sonic hedgehog signaling during chick myogenesis"***
Claudia Mermelstein (Federal University of Rio de Janeiro, Brazil)

15:00-15:30 ***"Elucidating the role of Fgfr4 in skeletal muscle development and metabolism"***
Laura Galvis (Institut NeuroMyoGène, Université Claude Bernard, France and Australian Regenerative Medicine Institute, Australia)

15:30-16:00 ***"DACT1 is a nucleocytoplasmic protein expressed during amniote myogenesis and modulated in human skeletal muscle disease"***
Lucia Alvares University of Campinas (Unicamp)

16:00-18:00 ***Poster Session***

18:00-18:30 **Poster Prize Announcement and Closing Remarks**

Session I: *Genetic and molecular control of development and regeneration*

1

Telling a tale of tails: The Hox13 genes and the specification of the posterior vertebrae and tail in the zebrafish

Nicolás Cumplido[1], Salomé Muñoz-Sánchez[1], Gloria Arratia[2] and Miguel L. Allende[1]
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The highly variable shape of the caudal fin in ray-finned fishes is a textbook example on the interplay between development and evolution. Over evolutionary time, there have been major changes in the terminal portion of the axial skeleton, providing anatomical features regarded as highly important for the classification of modern teleosts. Despite this, little is known about the underlying genetic mechanisms involved in caudal fin development, or how they differ from the other fins or appendages. In paired fins and appendages in vertebrates, the HoxA/D clusters play a major role in specification of the axes. We asked if, likewise, a particular subset of Hox genes could be involved in the shaping of the zebrafish caudal fin. We focused on the Hox13 paralogous group, since their members are known to be expressed in the posterior portion of the body in vertebrates, and we studied the effects of specific mutations on them. First, we examined hox gene expression patterns in zebrafish embryos and larvae by in situ hybridization. Next, we selected a subset of them found to be expressed at the posterior end of the body. The selected genes were then mutated by using CRISPR/Cas9 gene editing, and we studied the resulting phenotypes by examining the skeleton in mutant compared to wild type fish. We find that a distinct and previously unknown module in development, comprising members of the HoxC cluster (hoxc13a and hoxc13b), are required for the normal patterning of the caudal fin. Observed phenotypes incude alteration in the number of caudal vertebrae and in the number of fin rays in the caudal fin. Our findings suggest that the teleostean caudal fin represents a distinct and unique morphogenetic module, opening new possible interpretations for the evolution of fins in teleosts, and the evolution of appendages in vertebrates.

2

Insights from Developmental Pathways on the Evolution of Snakelike phenotypes in Tetrapoda

Tiana Kohlsdorf
University of Sao Paulo - Ribeirao Preto, Brazil)

TBA

Session I: *Genetic and molecular control of development and regeneration*

3

Deciphering the cardiac gene regulatory network

Mirana Ramialison
Australian Regenerative Medicine Institute, Australia

Predicting novel genetic determinants for heart development and disease 1% of babies are born with Congenital Heart Disease (CHD), manifesting as anatomical heart defects which often require treatment in the form of open-heart surgery within the first year of life. The causes of CHD have been attributed to environment and genetic factors but the aetiology of more than 80% of CHD cases remains unknown, making diagnosis and evaluation of the risk of the disease inheritance difficult. Our team has a long-standing interest in identifying the specific gene sets required for the formation of a healthy heart based on the principle that perturbations in these genes will impair normal development, resulting in cardiac defects. Thousands of genes are expressed in the heart at any given time point during development, but which of these genes are critical for heart formation and play a significant role in CHD? To answer this question, we performed a genome-wide investigation of the regulatory properties of genes that are known to be involved in heart development and disease by performing DamID-chip, ChIP-seq and RNA-seq in cardiac cells. Our bioinformatic investigations followed by in vivo functional validation led us to identify novel genes and regulatory elements essential for heart development and disease. Here we propose to present novel genetic components of the cardiac gene regulatory network, including novel cardiac transcription factors and heart-specific enhancers.

Coffee break

4

Gene regulation in early cell fate decisions and digit regeneration in mice

Henrique Marques Souza
Department of Biochemistry and Tissue Biology, University of Campinas - Unicamp, Brazil

Gene regulatory network controlling cell fate decisions have been a major theme in cell biology and regeneration. Our laboratory explores mouse embryonic stem cells as a model to study embryonic development and mouse digit tip regeneration as a model to study the balance between tissue repair and tissue regeneration in mammals. In our study of mouse embryonic stem cell differentiation, we have identified a nuclear factor hemispherically localized in embryoid bodies and have established knockout lines using CRISPR-Cas9 to study its function in the context of germ layer specification. In our study of tissue dynamics after digit tip amputations in mice, our group is proposing a novel hypothesis for the field in which the process of regeneration after digit tip distal amputations is in fact a normal tissue-specific response to lesion instead of a process of global tissue organization resulting in the regrowth of a structure or organ after tissue losses, as known in salamanders. We propose that sources of osteoprogenitor cells and osteogenic signals are eliminated in proximal amputations while preserved in distal amputations, resulting in the contrasting phenotypes observed after both types of amputations.

Session I: *Genetic and molecular control of development and regeneration*

5

When the inhibitor also helps: Cactus/IkB protein function in Drosophila embryogenesis and immunity

Maira Cardoso, Alison Julio, Paloma Duarte and Helena Araujo
Federal University of Rio de Janeiro, Brazil

The Toll pathway is widely recognized for its function in innate immunity, performing an evolutionarily conserved role to activate NFkappaB family proteins and the consequent production of anti-fungal and anti-bacterial peptides (AMPs). However, Toll was initially identified in Drosophila for its role in embryonic dorsal-ventral patterning. A ventral-to-dorsal gradient of Toll activation induces proteasomal degradation of the flies' sole IkappaB inhibitor, Cactus, releasing the NFkappaB protein Dorsal for nuclear translocation. Threshold-dependent activation of target genes ensues, subdividing the embryo in several dorsal-ventral gene expression territories. Although a central element in this process, there is scarce data on alternative mechanisms that control Cactus function in the embryo and immune system. We have undertaken a genetic, molecular and modeling approach to inquire Cactus function in both contexts. Quantitative data from embryos carrying *cact* loss- and gain-of-function alleles shows that Cactus performs an additional role to favor Toll signals. By mathematical modeling the Toll pathway and simulating different mutant conditions, we find that Cactus impacts basal levels of Dorsal nuclear translocation by a Toll-independent mechanism. We observe comparable effects in the immune system, particularly in the fat body, home of AMP production. These results may have great impact on vertebrate innate immunity, since many of the elements we describe regulating Cactus have been suggested to control IkappaB. Therefore, analysis of the simple Drosophila system, where one sole IkappaB protein controls several NFkappaB activation outcomes, as compared to vertebrates that display more than five different IkappaBs, enables to reveal previously unknown mechanisms that regulate innate immunity.

6

Effect of tRNA composition in ORF selection and proteostasis in *Drosophila*

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Genome expression strongly relies in the accurate flux of information from DNA to proteins. In this process tRNA composition plays an instructive role decoding the transcriptome of the cell. Consequently, how isodecoder abundance and tRNA modifications influence protein translation is a relevant question that only recently has begun to be addressed. Furthermore, the importance of this layer of regulation in the development of multicellular organisms is almost completely unknown. We have explored the expression of tRNA loci in different cell types of *Drosophila*, and also investigated the impact of t6A and m6t6A modifications in ANN decoding tRNAs in open reading frame (ORF) selection and proteostasis. Our previous results show that levels of t6A, particularly in the initiator tRNA, determine cellular and animal growth by regulating mTOR activity. Also, t6A deficiencies impair translation inducing the unfolded protein response (UPR). Here we show that similar results are obtained decreasing the levels of TrmO, enzyme responsible of the m6t6 modification of ACT-Thr decoder. Expression and functional analyses of the enzymes that synthetize t6A and m6t6 reveal that they are not transcribed and required equally in different cell types. Additionally, *in vivo* experiments show that levels of t6A affect ORF selection in a Kozak dependent manner and this selectivity could be modulated by functional interactions with eIF1 and eIF5. Together our results highlights the variations in tRNA composition in different cell types and suggest how the integration of this layer of translational control in multicellular organisms could be part of the expression program during development as it is in response to environmental conditions.

This research was supported by FONDECYT grant 1190119 and FONDAP grant 15090007

Session II: *Nervous system development and regeneration*

1

New insights on AP axis and neural induction

Jose Garcia Abreu Junior
Federal University of Rio de Janeiro, Brazil

TBA

2

Pre and pos-transcriptional regulation of cSCRATCH2 gene expression in neural embryogenesis

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Scratch2 (Scrt2) is a transcription factor that has an evolutionarily conserved role in embryonic neural development. Scrt2 is expressed in a very restricted population of post-mitotic neural precursors in the inner layer of the intermediate zone of the chick neural tube, and this pattern remains conserved in vertebrates. Identification of the mechanisms that regulate its expression levels could contribute towards to our understanding of gene regulation during neural differentiation. Our preliminary in silico analysis identified a potential genomic regulatory element 13kb upstream of Scrt2 TSS and the promoter, which we called E1 and potential binding sites in the Scrt2 3'UTR for miR-125b, -200b. We tested the biological function of the genomic regulatory region and miRNAs through electroporation and CRISPR-mediated genomic editing in chick embryos. The genomic E1 element interacted with Scrt2 promoter as seen through 3C assays. Also, it drove mRFP expression in all of the neural tube. Conversely, its repression through epigenetic manipulation reduced Scrt2 expression. At the pos-transcriptional level, our results show that miR-125 and -200b temporal and spatial expression complements that of Scrt2. Further, Scrt2 3'UTR can regulate luciferase levels in the neural tube. Finally, removal of miR-125 target site increased Scrt2 expression. We propose that the genomic element E1 promotes Scrt2 transcription in the neural tube and miR-125b and -200b may refine further the expression field of Scrt2 post-transcriptionally.
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Coffee break

Session II: *Nervous system development and regeneration*

3

Make do and make new: how zebrafish rapidly regenerates CNS injury

Jan Kaslin
Australian Regenerative Medicine Institute, Australia

Zebrafish have a remarkable capacity to regenerate following spinal cord injury. While many factors controlling neurogenesis have been identified, the cellular mechanisms regulating global neural regeneration are largely unknown. We used in vivo imaging to pin-point specific cells and signals that control CNS regeneration in zebrafish. Surprisingly, we identified two temporally and mechanistically distinct waves of cellular regeneration in the spinal cord. The initial wave of regeneration relying on cell migration of neural precursors to the lesion site, enabling rapid functional recovery, and the activation of quiescent neural stem and progenitor cells (NCSs). This is then followed by the second wave of regeneration which largely driven by regenerative neurogenesis. Neurogenesis compensates for both the loss of tissue at injury site as well as the cells depleted from proximal areas due to early migration. Furthermore, we find that inflammation and leukocytes play a critical role in differentially regulating cell recruitment and activation of NSCs after injury. The two waves of regeneration demonstrate how the zebrafish are able to rapidly regain motor function after complete ablation, but also gradually replenish lost tissue over time. Taken together, our data suggest that inflammation driven recruitment of neural precursors play an unanticipated role in neural repair.

4

Cellular and molecular mechanisms of zebrafish spinal cord regeneration

Paula P. Morão [1], Isabel S. A. B. Vidal [1], Carlos B. Sato [2], Carlos Perez [2], Jan Kaslin [3], Hozana A. Castillo [1,3]
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Mammals cannot regenerate their Central Nervous System, resulting in permanent sensory-motor loss after spinal cord (SC) injury. Remarkably, zebrafish have high capacity of regenerating neurons after SC injury, a process accompanied by tissue restoration and functional recovery. The cellular and molecular mechanisms regulating this notable process remain unclear. However, it is known that the activation of dormant ependymal stem cells is required to initiate neural regeneration in zebrafish SC. In mammals, ependymal cells also act as stem cells after SC injury, but they are unable to undergo neurogenesis in loco. Here we describe the cellular and molecular mechanisms that are involved in the activation of ependymal cells and in the recruitment of multipotent progenitors in the zebrafish SC. During early embryonic development most if not all neural progenitors in the spinal cord express nestin but the expression is downregulated and not detected in ependymal cells at larval stages. Upon injury, nesting expression is upregulated and detected at high levels in the neural progenitors. Genetically ablating the nestin cell lineage significantly blocks neural regeneration demonstrating a requirement of this cell lineage in spinal cord regeneration. Next, we searched for signals controlling the activation of ependymal. We showed that leptin and retinoic acid signaling, have a role in the control of stem/progenitor cells proliferation and neural progenitors' formation, suggesting that these signaling pathways are important drivers of spinal cord regeneration. To understand the cellular mechanisms of adult SC regeneration in zebrafish, we are developing tools to label and image newborn neurons and axonal process using imaging techniques with Synchrotron Radiation, such as X-ray Fluorescence Microscopy, recent findings in labeling specific cell types will be presented. The identification of the cellular process and signaling pathways that control zebrafish neural stem cells activation and neurogenesis, can offer basis for stimulation of mammal endogenous stem cells through the similar processes.

FESBE Opening Ceremony

Plenary Conference

1

Understanding skeletal muscle regeneration using zebrafish models

Peter D. Currie
Australian Regenerative Medicine Institute. Monash University

Skeletal muscle deploys a self-renewing stem cell, the satellite cell, to effect regeneration. Recent *in vitro* studies have highlighted a role for asymmetric divisions in renewing rare "immortal" stem cells and generating a clonal population of differentiation-competent myoblasts. However, this model has lacked *in vivo* validation. We have defined a zebrafish muscle stem cell population analogous to the mammalian satellite cell and image the entire process of muscle regeneration from injury to fiber replacement *in vivo*. This analysis reveals complex interactions between satellite cells and both injured and uninjured fibers and provides *in vivo* evidence for the asymmetric division of satellite cells driving both self-renewal and regeneration via a clonally restricted progenitor pool. Our latest observations reveal that wound dwelling macrophages provide an obligate transient stem cell niche that directs muscle stem cell division and we discuss the implications of these findings for muscle stem cell biology.

Session III: *Stem cells and disease*

1

Can you model breast cancer using human induced pluripotent stem cells?

Andrew L. Laslett[1,2]
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Breast cancer is one of the most common cancers affecting women, with an expected lifetime risk of 12%. While breast cancer is predominantly caused by sporadic mutations, up to 10% of all cases can be attributed to mutations in the BRCA gene family. Carriers of BRCA mutations have an expected lifetime risk of 45% (BRCA2) to 65% (BCRA1) of developing breast cancer. The only currently available prophylactic therapies are drugs that are not suitable for all women, or complete mastectomy. The aim of this project is to develop a differentiation protocol to drive cells to mammary epithelial cell types from induced pluripotent stem cell (iPSC) lines derived from women with a BRCA mutation. These cells can then be used to screen for novel therapeutics to prevent malignant transformation of breast epithelial cells carrying BRCA family mutations. iPSC lines were generated from tissue samples donated by women undergoing either prophylactic mastectomy or breast reduction surgery and who were either BRCA 1 or 2 carriers or controls. All cell lines generated were characterised for pluripotency markers, karyotypic normality and ability to form teratomas. The iPSC lines are differentiated using a substrate/media driven approach to drive cells into a putative mammary epithelial fate. Differentiated cells have been generated that express the breast luminal progenitor cell markers EpCAM, CD49f, cytokeratin 8/18 and are negative for the basal cell marker cytokeratin 14 and pluripotency markers OCT4 and PCDH1. These cells also exhibit luminal morphology in colony formation assays, and further functional characterisation is underway. We have shown that this protocol gives rise to putative mammary epithelial cells of human origin. We anticipate using these cells to search for novel compounds to prevent malignant transformation of breast epithelial cells carrying BRCA family mutations as well as providing a tool to assess the mechanisms leading to transformation.

2

Stem cell therapy in lung diseases

Patricia R. M. Rocco, MD, PhD
Laboratory of Pulmonary Investigation, Carlos Chagas Filho Biophysics Institute,
Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Mesenchymal stem/stromal cells (MSCs) have been shown to improve lung function
and survival in acute and chronic inflammatory lung diseases, including acute respiratory distress syndrome, asthma, chronic obstructive pulmonary disease (COPD), and silicosis. MSC therapy holds promise for the treatment of acute and chronic lung diseases, given their demonstrated therapeutic benefits: anti-inflammatory and anti-apoptotic effects, enhanced epithelial and endothelial cell recovery, as well as microbial and alveolar fluid clearance. The benefits of MSC-based therapies appeared to be induced by complex, well-orchestrated signaling pathways rather than by any one (or few) mechanisms. Key mechanisms of action include secretion of paracrine factors and transfer of cellular contents via extracellular vesicles or cell-to-cell contact. Safety results from phase I and II clinical trials are encouraging, but the safety and efficacy profile has yet to be proven in large-scale trials. In an ideal clinical scenario, MSCs would be promptly available and obtained through well-standardized procedures, but some barriers still pose challenges to the feasibility of MSC therapy.

Session III: *Stem cells and disease*

3

JAM-A is critical for HSC trafficking and maintenance of stem cell quiescence in the niche

Susie Nilsson
Australian Regenerative Medicine Institute and Commonwealth Scientific and Industrial Research Organisation, Australia

The success of clinical hematopoietic stem cell (HSC) transplants is highly dependent on the ability of HSC to home to bone marrow (BM). Although it is appreciated that hematopoietic recovery post-transplant is correlated to the number of infused HSC, greater understanding of homing mechanisms should allow us to identify improved transplant strategies that are not solely reliant on HSC number but also HSC quality. We recently identified the importance of junctional adhesion molecule A (JAM-A) in HSC regulation during steady state and stem cell mobilization. Specifically, JAM-A is critical for HSC homing and engraftment post-transplant and contributes to the preferential maintenance of HSC quiescence in the endosteal niche (region closely associated with the bone/BM interface). Furthermore, granulocyte-colony stimulating factor (G-CSF) dependent HSC mobilization significantly reduces JAM-A expression on mobilized HSC which contributes to impaired homing potential when compared to cells mobilized using the small molecule combination of the $\alpha^4\beta^1/\alpha^9\beta^1$integrin antagonist BOP and the CXCR4 antagonist AMD3100. Consequently, we show that a single injection of BOP in combination with AMD3100 for 1 hour was capable of mobilizing HSC and progenitors with significantly greater long-term multi-lineage engraftment potential compared to a 4-day G-CSF approach. Together, our results identify JAM-A as a critical regulator of stem and progenitor trafficking to and maintenance in BM and suggests the use of G-CSF independent stem cell mobilization strategies that do not abrogate JAM-A expression or function should result in improved clinical stem cell transplants.

4

iPSC: a powerful modeling tool to study diseases

Bruno Torres
Brazilian Biosciences National Laboratory (LNBio/CNPEM), Brazil

Neurobiological mechanisms of brain disorders can be investigated with a variety of approaches in humans and model organisms. Much of what we know today about how different brain diseases impact the development, electrophysiological and morphological patterns of the central nervous system came from studies in animal models. However, the cellular reprogramming experiments led by the group of Shinya Yamanaka have provided a new experimental approach, allowing the development of human brain diseases “in a dish”. During the last decade, an immense advance in protocols to differentiate induced pluripotent stem cells (iPSC) in neurons and glia cells has provided a better understanding of several diseases. Lastly, the bioengineering of stem cell-derived, self-organizing, three-dimensional cell cultures, also known as organoids, introduces more degrees of developmental freedom and models brain structure in remarkable and surprisingly complex ways. During my talk I will describe the impact of iPSC technology on the investigation of disease with a focus on neurobiological mechanisms and different based experimental strategies used to uncover them.

Coffee break

FESBE Plenary Conference

1

What lies ahead of the frontier in cardiac development and evolution?

José Xavier Neto – Federal University of Ceara (UFC)

Optimal cardiac work requires appropriate contractile proteins in heart chambers. Atria require slow myosins to act as variable reservoirs, while ventricles demand fast myosins for swift pump function. How was myosin expression matched to atria and ventricles during development and evolution? We used the quail Slow Myosin Heavy Chain 3 (SMyHC3) paradigm to show that chamber expression is controlled in a dualistic manner according to the context: activation in atria and repression in ventricles. The switch between SMyHC3 gene states is autonomously orchestrated by a complex nuclear receptor element (cNRE), a 32-bp sequence with overlapping hexanucleotide binding repeats. Unliganded, widely expressed, glucocorticoid receptor (GR) associates with the cNRE in ventricular repression, while the switch to active states in atria requires protein-to-protein interaction between two repressors: the sino-atrial-restricted orphan nuclear receptor COUPTF-II and the pervasive, unliganded androgen receptor (AR). We provide evidence that the cNRE is an endogenous viral element derived from an unidentified virus with limited homology to HSV-1. Comparative genomic studies suggest that the cNRE associated to the SMyHC3 gene through infection of an ancestral host germline and further recombination into the genome of a Galliform bird ancestor at the root of the Galliformes radiation in the Cretaceous, about 100 million years ago. The cNRE example suggests a pathway to selective cardiac chamber expression through nuclear receptor signalling and underscores the haphazard nature of regulatory genome innovation.

Session VI: *Molecular control of skeletal muscle development*

1

Sonic hedgehog signaling during chick myogenesis

Claudia Mermelstein
Federal University of Rio de Janeiro, Brazil

The Sonic Hedgehog signaling (Shh) pathway has been implicated in both proliferation of myoblast cells and terminal differentiation of muscle fibers, and contradictory results of these effects have been described. To clarify the role of Shh during myogenesis, we decided to study the effects of recombinant Shh and the distribution of Gli-1 during in vitro and in situ embryonic chick skeletal muscle differentiation at later stages of development. Gli-1 was found in small aggregates near the nucleus in mononucleated myoblasts and in multinucleated myotubes both in vitro and in situ chick muscle cells. Some Gli-1 aggregates colocalized with gamma-tubulin positive-centrosomes. Gli-1 was also found in striations and at the subsarcolemmal membrane in muscle fibers in situ. Recombinant Shh added to in vitro grown muscle cells induced the nuclear translocation of Gli-1, as well as an increase in the number of myoblasts and in the number of nuclei within myotubes. We suggest that Gli-1 aggregates observed in chick muscle cells near the nuclei of myoblasts and myotubes could be a storage site for the rapid cellular redistribution of Gli-1 upon specific signals during muscle differentiation.

2

Elucidating the role of FGFR-4 in skeletal muscle homeostasis and regeneration

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Fibroblast growth factor receptor 4 (FGFR-4) is specifically expressed in skeletal muscle development and adult satellite cells. However, its exact role in skeletal muscle remains unknown. We aim to better understand the function of this receptor in skeletal muscle by studying different models of *Fgfr4* knockouts during development, regeneration and homeostasis. Analysis of *Fgfr4* knockout mice shows loss of muscle mass, increase of scapular brown adipose tissue (BAT) and changes in lipid compositions in the muscle. In contrast, analysis of mice which are specifically knocked out for *Fgfr4* in *Pax7*-positive cells postnatally (postnatal muscle progenitors), do not show the same effects on muscle mass and BAT, suggesting the effects of *Fgfr4* deletion are determined during development. This further indicates that FGFR-4 may play a key role in the cell fate determination between BAT and skeletal muscle; a process which remains largely uncharacterised given the recent identification of an early common progenitor. Additionally, both the full *Fgfr4* knockout and the *Pax7*-specific *Fgfr4* knockout display a faster glucose uptake in glucose tolerance tests compared to controls, highlighting an intriguing function for FGFR-4 in skeletal muscle glucose regulation. Together, the data suggests FGFR-4 may have novel roles in cell fate decision during development and metabolic regulation of skeletal muscle in adulthood.

Session VI: *Molecular control of skeletal muscle development*

3

DACT1 is a nucleocytoplasmic protein expressed during amniote myogenesis and modulated in human skeletal muscle disease

Renata E. Contriciani, Fernanda C. da Veiga, Bianca G. Castelucci, Carla B. Collares-Buzato, Marcelo B. de Jesus and Lúcia E. Alvares
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Dact1 is a multifunctional adaptor protein, which plays several functions during embryonic development as well as for homeostasis after birth. Since this molecule is expressed in somites of different vertebrates, in this work we investigated whether Dact1 participates on skeletal myogenesis and a possible modulation of its activity in human muscular diseases. Our results showed that in chicken Dact1 is expressed in the fetal pectoral muscle and in primary myogenic cultures. Its transcription is induced at later myogenesis, with mRNAs being found mainly in myotubes and myoblasts-myotube contact regions. Dact1 acts as a nucleocytoplasmic protein that displays an enhancement in its labeling as myogenesis progress. In myotubes, its distribution is markedly sarcomeric. In mouse, similar but not identical expression patterns were found in fetal skeletal muscle and C2C12 cultures. Finally, differential gene expression analysis of human muscle diseases profiles by array showed that Dact1 is modulated in patients with Duchenne Muscular Dystrophy, Limb-Girdle Muscular Dystrophy type 2A and Acute Quadriplegic Myopathy. Therefore, we conclude that Dact1 is expressed during amniote myogenesis, pointing to several possible functions for this protein. The modulation of Dact1 in human muscle diseases suggests that it can be a new therapeutic target for the control of these pathologies.

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