showed a reduction in tissue stiffness when contraction was suppressed. Moreover, microdissection experiments indicated tension around the AIP, and finite element simulations of these experiments suggested the endoderm as the primary contractile tissue layer. Taken together, these results indicate an active mechanical role for the endoderm during HT formation.

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Program/Abstract # 160
The effects of simvastatin in zebrafish development
Laise Campos, Eduardo Morris,
Claudia Mermelstein, Manoel Luis Costa
Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Striated muscle differentiation is based on the construction of a three-dimensional contractile apparatus. Myogenesis has been studied in cell cultures, because of the simpler observation in vitro. But in these cultures, cells are forced to adapt to the two-dimensional environment, and are induced to form structures that they rarely form in vivo, such as stress fibers. To overcome the culture limitations, we have been using the three-dimensional model of zebrafish embryos to study structural aspects of myogenesis. We studied the spatial distribution of myofibrils using Alpha-actinin, Desmin, Troponin, Titin, Actin, the distribution of cell adhesion sites using Paxillin, Dystrophin, Vinculin, and the extracellular proteins Laminin and Fibronectin. Our previous results in cell culture models showed that cholesterol is important to myogenesis. We therefore decided to study the effects of simvastatin, which impairs the synthesis of cholesterol. The embryos at 6 hpf were immersed in embryonic water at a concentration ranging from 0.003 to 12 μM of simvastatin for 18 h. In treated groups, we detected the formation of the same numbers of somites, like in control, but a significant dose-dependent somite and septum damage phenotype. We used conventional Zeiss optical microscope, but more commonly we used Zeiss multiphoton laser scanning, and Olympus spinning disk confocal microscope. The three-dimensional analysis, reconstructions and projections were made with ImageJ and we confirm that there are significant differences between the myogenesis in vitro, in two-dimensional cultures, from in situ and in three-dimensional models. The characterization of the consequences of simvastatin is important not only to understand myogenesis, but also to explain the collateral effects of its use as a treatment for cholesterol problems in humans.

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Program/Abstract # 161
Effect of the lipid-raft disorganization on the muscular differentiation in zebrafish model
Eduardo Ríos, Laise Campos, Claudia Mermelstein, Manoel Luis Costa
UFRJ, Rio de Janeiro, Brazil

The muscular differentiation begins with the myoblast cells aligned each other guided by different recognition signals which are inducing fusion of the cells forming the multinucleated myotubes. This process involves the plasma membrane and some other components, which are linked to the fusion event occurs. Some of these components are the “lipid-rafts”, cholestherol-enriched microdomains which are involved in several processes. Not much is known about the development of lipid-rafts, but some works in vitro show that this may have a relation in the activation of the muscular differentiation. Using zebrafish as a model, we use different strategies to disorganize the membrane microdomains. The first was the use of β-methyl-cyclodextrin, a molecule which removes the lipid-raft's cholesterol specifically. The second strategy is disturbance of the proteins involved in the organization of the lipid-raft and the transduction of the signal. For this we analyze the failure of function of the Reggie protein, which is an important component and marker of the lipid-rafts. The function of Reggie is not clear, but it is known that is associated to the lipid-rafts like anchorage molecule to the interaction with the some signaling complexes proteins and cytoskeleton components. This study shows the importance of the lipid-rafts in the fusion of myoblast cells and open a door to analyze the possible signaling pathways involved with the membrane microdomains and the muscular differentiation.

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Program/Abstract # 162
Analysis of the long non-coding RNA, MHM, in avian embryonic development
Kelly Roeszler
Murdoch Children's Research Institute, Parkville, VIC, Australia

The chicken embryo has a ZZ male: ZW female sex chromosome system. Although there is no large scale dosage compensation in chickens, a small percentage of genes are compensated. It has been proposed that compensated gene expression from the single Z chromosome of females might involve up-regulation mediated by a Z linked long non-coding RNA called MHM (Male-HyperMethylated) 1. MHM is methylated and silenced in males (ZZ). In contrast it is hypomethylated and expressed in females, in which it coats the Z-chromosome at the site of transcription (analogous to Xist in mammals). Hence, MHM may play a role in dosage compensation. Alternatively, MHM may play a role in avian sex determination, influencing the neighbouring DMRT1 gene, reducing expression in females. We have explored the possible role of MHM in dosage compensation and chicken sex determination via over-expression analysis. In chicken embryos, whole-mount in situ hybridisation demonstrated lowly expressed MHM in female brain, limbs and gonads. Intriguingly, the antisense strand was more strongly expressed and also detected in these sites. No expression was detected in males. Sectioned in situ's revealed a punctate nuclear localisation throughout the ovary, up-regulated around the timing of gonadal sex determination. Using viral-meditated over-expression techniques, MHM sense over-expression induced developmental abnormalities in the brain and gonads, marked by over-proliferation and failure of typical asymmetry. The antisense strand also had a strong effect on ovary development. 1. Teranishi M, Shimada Y, Hori T, Nakabayashi O, Kikuchi T, Macleod T, Pym R, Sheldon B, Solovei I, Macgregor H, Mizuno S. Transcripts of the MHM region on the chicken Z chromosome accumulate as non-coding RNA in the nucleus of female cells adjacent to the DMRT1 locus. Chromosome Res. 2001.

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Program/Abstract # 163
TCOF1 mutation affects the susceptibility to Hirschsprung's disease
Amanda J. Barlow, Paul Trainor
Stowers Institute for Medical Research, Kansas City, MO, USA

Hirschsprung's disease (HSCR), is a digestive tract disorder affecting 1:5000 live births. HSCR results from an absence of ganglia