**Program/Abstract # 160**
**Investigating the role of the argonautes during mouse embryogenesis**
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A combination of genetic, biochemical and structural studies has helped us dissect components of the RNA Induced Silencing Complex (RISC) and how they orchestrate different populations of small RNAs to regulate the genome. However, very little is known about the biological role of the RISC components. We have shown that Dicer and Argonaute2 are essential during mouse embryogenesis. Dicer deficient mice die around gastrulation and Ago2 deficient mice develop up to midgestion with heart and neural tube malformations. In mammals, the Argonautes are classified into two subfamilies: a family that is germline specific and a family that is more ubiquitously expressed. Ago2 belongs to the latter family and has been very well characterized biochemically. It associates with microRNAs and has been demonstrated to be the slicer enzyme responsible for cleaving target messenger RNAs. However, Ago2's role in regulating gene expression at the transcriptional and translational level is still unclear. We propose that the catalytic function of Ago2 is essential for its developmental role and we are testing this by generating a catalytically dead Ago2 mouse. Even less is known about the remaining family members, Argonaute1, Argonaute3 and Argonaute 4. We have generated mice deficient in Ago1, Ago2, Ago3 and Ago4 as well as Ago1/3 double and Ago1/3/4 triple knockout mice. So far, we have shown that the action of Ago1, Ago3, Ago4 or Ago1/3 is not required for completion of embryogenesis. Dissecting the argonautes genetic mutants together with unraveling their biochemical function, will help us understand their role in regulating gene expression during development.

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**Program/Abstract # 161**
**The role of Fgf8 in head myogenesis**
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Muscle diseases such as muscular dystrophies pose a challenge to medicine and society since no cure has been found to date. Recent studies suggested that head and trunk muscles use different programs for their embryonic development. However, the program for craniofacial muscle development is not understood. Fgf8 is a regulator for skull and heart development. As it is expressed next to the branchial arch muscle, Fgf8 may control the patterning and differentiation of head muscles. Grafting Fgf8-beads, it was found that MyoR, a marker for undifferentiated and proliferating myoblasts was upregulated. Conversely, markers for differentiating cells such as Myf5 were downregulated. This suggests that Fgf8 may stimulate head myoblast proliferation and inhibit their differentiation. Genes indicative of muscle pattern were also affected: En2, which labels the developing jaw muscles in the mandibular arch, was upregulated, while Paraxis, a marker for the lateral rectus eye muscle, was downregulated. This finding suggests a role of Fgf8 in the promotion of branchiomeric and the suppression of extraocular muscle development. We showed that a signal from the neural tube positively regulates extraocular muscle development and negatively regulates branchiomeric muscles development. We propose a model by which antagonising signals from the neural tube and branchial arches pattern the craniofacial musculature. Fgf8 agonists and antagonists are expressed in the mesodermal core of the arches, which suggest that Fgf8, expressed in the endoderm, may signal directly to the mesoderm and thereby control its downstream target genes. Supported by Marie Curie EST

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**Program/Abstract # 162**
**Sox9 negatively regulates the expression of the muscle specific gene alpha sarcoglycan during myogenesis in C2C12 cells culture**
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The sarcoglycan sarcospan complex is composed of the transmembranal proteins a, b, g, d y e SG as well sarcospan. Mutations in a, b, g, d SG cause autosomal recessive limb girdle muscular dystrophies. a SG is expressed exclusively in striated muscle. Due to this observations, a tissue specific a SG gene expression regulation has been suggested. In this regard it has been demonstrated that the a SG mRNA is positively regulated during myogenesis, which implicate the myogenic factors participation on the expression of this protein. Recently, our group has demonstrated the interaction of MyoD with the transcription factors TFIIB and TFIID in the a SG promoter region. In order to know the mechanisms involved in the negative regulation of this gene, we analyzed the sox9 participation. We analyzed the luciferase activity driven by a SG promoter C2C12 cells cotransfected with cDNA of sox9, the a SG promoter is down-regulated in 80% in C2C12 myoblasts, but not in myotubes where the endogenous sox9 expression level is undetectable. We analyzed the endogenous a SG mRNA level in a C2C12 stable line that overexpresses Sox9. The transcript was reduced in 60% with respect to wild type cells, as well as the a SG protein. By immunofluorescence analysis we find the colocalization of Sox9 and MyoD in myoblasts cell nucleus and we are analyzing the possibility of p-Smad3 interacting with sox9 and MyoD, which suggests a myostatin dependent mechanism of regulation. In this moment we are