been found for pre-gastrula embryos, the beta4 gain and loss-of-function phenotypes suggest potentially novel roles for the beta4 subunit that may involve the cytoskeleton.

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Program/Abstract # 298
Patterning the sea urchin skeleton: A role for calcium signaling

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Many experiments have demonstrated that patterning information in the sea urchin embryo is distributed throughout the ectoderm. These spatial cues are passed onto the mesoderm to correctly pattern the bilaterally symmetric larval skeleton. Previous data have shown that exposure to metals (such as nickel and zinc) can disrupt patterning information within the ectoderm, presumably due to their ability to interfere with calcium transport, resulting in mispatterned embryos with radialized skeletons. To test the hypothesis that the proper distribution of skeletal patterning information within the sea urchin ectoderm is dependent on calcium signaling, we treated embryos with an inhibitor of IP3-mediated calcium release, Xestospongin C. Our data show that Xestospongin C exposure disrupts ectodermal patterning, producing embryos with radially symmetric skeletons. Normal skeletal patterning in radialized embryos can be rescued by treatment with the calcium ionophore ionomycin. More intriguingly, radialized embryos can also be rescued by surgical bisection along the animal–vegetal axis at the mesenchyme blastula stage, followed by recovery in the presence of calcium. However, simply removing calcium from the environment during recovery was sufficient to block the rescue of skeletal patterning in bisected radialized embryos. These results suggest that it is not the wound healing process itself, but rather the presence of calcium, which is responsible for the rescue of patterning. Together, these data reveal a critical role for calcium signaling, not directly for skeletogenesis but rather for the distribution of skeletal patterning information throughout the sea urchin ectoderm.

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Program/Abstract # 299
Long-range signalling of TGF-β type morphogens in the *Xenopus* embryo

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Across a range of species much of embryonic development is dependent upon signals that act at a distance from their source. Many of these signals are thought to be morphogens, secreted molecules that form concentration gradients across tissues. At a given position in the gradient, the cellular response depends upon the morphogen concentration at that given position. In vertebrates such as the frog *Xenopus laevis*, much work on morphogens has focused on how members of the TGF-b superfamily, including Activin and Xnr1, 2, 4, 5 and 6, can act as morphogens in mesoderm induction. In such early embryonic tissue, these ligands have been shown to induce mesodermal response genes in a concentration-dependent manner. In order to study the progression of TGF-b morphogens we took advantage of a recently published technique called "bimolecular fluorescence complementation", BiFC, which was designed to analyse protein interaction *in vivo*. This technique was adapted in our lab to the frog system to enable the observation of Smad-signalling pathway activation as a direct response to Activin like ligand binding. We were able to visualize in real time the expected long-range activation of the Smad pathway in response to Activin and Xnr1 but not for Xnr2 signalling. At present we are investigating the role of several possible players in TGF-b morphogen progression and signalling. The quantity of ligand–receptor bindings as well as endocytosis seems to be important for the morphogen signalling range.

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Program/Abstract # 300
A mathematical basis for the clock and wavefront model for somitogenesis

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Somites arise as the result of a complex process that takes place in the early vertebrate embryo: a seemingly uniform field of cells is organised into discrete blocks via a mechanism which is tightly regulated both in space and time. Various experimental results have shown the existence of a wavefront of gene signalling along the vertebrate embryo, which, coupled with a segmentation clock, is able to gate cells through to the final stages of somitogenesis. We use a signalling based approach, with cues from the wavefront and the segmentation clock, to mathematically model somite formation. Our model produces a somite pre-pattern that is tightly controlled both spatially and temporally. Moreover, it is capable of reproducing the results observed in vivo when progression of the wavefront is disturbed by local FGF8 application. We are also able to use the model to make predictions about the somites arising with other types of perturbation, such as down-regulation of FGF signalling.

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Program/Abstract # 301
Fgf4 and Fgf8 are required for maintenance of the primitive streak and somitic clock
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Fibroblast growth factor (FGF) activity has been implicated in mesoderm induction and migration, as well as somitogenesis. To study the role of FGF signaling in the emerging mesoderm we inactivated both *Fgf4* and *Fgf8* in the primitive streak (PS), utilizing TCre, a transgene in which Cre is controlled by Brachyurany regulatory elements active in the PS. *Fgf4/8* inactivation results in embryonic lethality due to a severe caudal truncation such that development of all germ layers ceases posterior to the eighth somite due to loss of the PS by E8.5. Prior to PS loss at E8.0, *Fgf4/8* are required to maintain *Brachyura* and *Wnt* gene expression. Preliminary data eliminate changes in apoptosis and proliferation as causes of loss of the PS. TCre/Fgf4/Fgf8 mutant embryos have defects in anterior–posterior patterning of somites as indicated by a shift in the expression of *Mesp2*, which marks the initiation of somitogenesis and a reduction in *Hes7*, an oscillatory gene and part of the somitic clock. Retinoic acid (RA) activity, which is has been postulated to antagonize the caudal–rostral FGF gradient along the anterior–posterior axis of the embryo, is up-regulated in TCre/Fgf4/Fgf8 mutant mice. Our data indicate that *Fgf4* and *Fgf8* play fully redundant roles in maintaining the primitive streak stem cell population, play a role in the maintenance of the clock genes required for somitogenesis and are active in maintaining a balance between FGF and RA signaling.

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Program/Abstract # 302
***Dkk1* and *Wnt3* interaction is critical for head morphogenesis in the mouse**
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Dkk1 is essential for head formation and acts by inhibiting canonical Wnt signalling. Loss of *Dkk1* function leads to ectopic WNT signals in anterior germ layer tissues of the gastrulating mouse embryo and head truncation. A study of compound *Dkk1;Wnt3* mutant embryos reveals an interaction between the activity of these two genes is required for embryonic patterning. Compound *Dkk1;Wnt3* heterozygotes display a range of abnormalities in head and trunk morphology, suggesting that an apparently balanced gene dosage of *Wnt3* and *Dkk1* may not achieve a proper level of signalling. However, by reducing the gene dosage of *Wnt3* over the *Dkk1*−/− genotype, the *Dkk1*-null truncated head phenotype can be rescued, showing that head development is sensitive to the level of signalling. The spatial overlap of the expression domains of *Wnt3* and *Dkk1* before and at gastrulation strongly implicates that genetic interaction during early embryogenesis is crucial for head morphogenesis.

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Program/Abstract # 303
Regulation of a novel skeletal muscle signaling center at the occipitocervical somite boundary
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Skeletal muscle groups located in the neck region have evolved along with the and cartilage to control the rotation of the skull, swallowing, respiration and vocalization. Vocalization defects (dysphonia) and swallowing defects (dysphagia) have been implicated in several congenital diseases. Laryngeal, tongue, tracheal, diaphragm and cervical muscles are derived from the occipital and cervical somites. However, little is known about the embryonic events that lead to the development of these muscles. We have used a muscle-specific lacZ transgene to perform a detailed study of myotome formation in this region of the mouse embryo. Our analysis revealed that myogenesis is initiated at the occipitocervical boundary and progresses both rostrally and caudally. Myotome formation as described by the site of myoblast entry into the myotome and the direction of myocyte elongation occurs in a mirror image on either side of the boundary. This indicates that early events in myogenesis are regulated by positional information along the rostrocaudal axis. Members of the Hox 3 gene family are expressed in somites at the occipitocervical boundary. We found that compound Hox 3 mutant neonatal mice exhibit muscle defects in the intrinsic laryngeal muscles, pharyngeal constrictor muscles and deep cervical muscles. Our current observations on the regulation of this novel signaling center will be discussed.

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Program/Abstract # 304
Bone morphogenetic proteins (BMPs) direct formation of a signaling center that regulates facial development
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