rise to kidneys, gonads, aorta and red blood cells. Very little is known about how this tissue is initially patterned. Previous work from our lab has shown that Bmp signaling can induce the earlist IM gene – odd1 (also known as Osr1) – in a dose-dependent manner. However, this effect is indirect, implying the existence of other genes in the pathway downstream of Bmp signaling. A microarray screen for genes regulated by Bmp revealed several candidates for genes mediating this mesodermal specification. A subset of these genes consisted of transcription factors such as Gata4, Gata5, Id1–4, Msx1, Msx2, Tbx6 and MesO1. We are currently characterizing the function of these genes with respect to mesodermal patterning using in vivo electroporation, whole mount in situ hybridization and RT–PCR techniques. These genes may not only induce odd1 expression, but also repress somite genes, in this way ultimately defining the border between IM and somitic mesoderm in response to Bmp.

Program/Abstract # 207
A proliferative role for Pax3 and Pax7 in the chick somite
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Pax3 and Pax7 are closely related members of the paired-box transcription factor family that are important in muscle development in the vertebrate embryo. Our lab previously showed that signals such as Wnt-3a that maintain or induce Pax3 and Pax7 expression in the dermomyotome also cause an increase in proliferation. Other labs have suggested that Pax3 and Pax7 have redundant roles in the early embryo. We therefore hypothesized that Pax3 and Pax7 function as positive regulators of proliferation during myogenesis. Immunostaining for endogenous Pax3 and Pax7 localization that are established early. To determine if Pax3 and Pax7 have proliferative roles in vivo, we electroporated somites with Pax3 constructs, RNAi targeted to Pax3, and Pax7 constructs. Evidence from the overexpression and knockdown of Pax3 in developing somites indicated Pax3 is sufficient and required to increase proliferation. Conversely, overexpression of Pax7 caused a decrease in proliferative cells. These results strongly suggested divergent roles for Pax3 and Pax7 with respect to proliferation. Because these results were unexpected, we investigated if the effects of Pax3 and Pax7 on proliferation could be due to the presence of alternative transcripts. We identified alternative transcripts of Pax3 and Pax7, including a novel transcript of Pax7. When the Pax7 isoform was overexpressed in somites, there was increased proliferation, similar to the effect of Pax3. Cumulatively, our results indicate that both Pax3 and Pax7 have a positive effect on proliferation in somites, but this effect is dependent on which isoform of Pax7 is expressed.

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Program/Abstract # 208
The transcription factors Foxa2 and Noto pattern three distinct rostrocaudal regions of the axial midline in mouse
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The organizer is a highly conserved signaling centre in the vertebrate embryo that is required to establish the basic body plan. The mouse organizer or node is a transient and mixed population of cells that creates different tissues in the axial midline. We present time-lapse movies that support the idea that axial mesoderm, or notochord, has distinct morphogenetic origins in the anterior, trunk and tail regions. The anterior notochord arises by condensation of dispersed progenitors, the trunk notochord arises by convergent extension, and the tail notochord arises from posterior migration of node-derived cells. The genetic hierarchy of conserved regulators known to pattern the notochord along the rostrocaudal axis mirrors these morphogenetic distinctions. Our group and others have reported that Foxa2 is necessary for notochord formation in all regions of the midline. This study reveals patterning of the axial midline is also tightly linked to Foxa2 gene dosage. The Noto null mouse was reported as having only partial penetrance of tail notochord defects. We found that Noto null embryos that also lack one copy of Foxa2 have severe defects in trunk notochord. Our data also shows that Noto, like its zebrafish homologue, has a role in controlling the fate decision of organizer cells to adopt either an axial or paraxial mesoderm fate. It will be interesting to explore how the early rostrocaudal patterning of the axial midline correlates with anterior–posterior domains in the overlying central nervous system.

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Program/Abstract # 210
Keratin expression during the development of mouth
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The mouth, the entrance to the digestive tube is formed at the boundary between ectoderm- and endoderm-like anus during development. Except for the teeth, the mouth is lined by a stratified squamous epithelium, with a submucosa present only in certain regions. The aim of this study was to analyze the

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differentiation profile of the intermediate filaments of the cytoskeleton such as cytokeratins during the development of mouse lips and palate, so to define the specific expression patterns during the oral cavity formation. Along the antero-posterior axis, morphological changes with HE staining, immunohistochemical analyses with specific antibody against the keratins, gap junction proteins, section in situ hybridizations with shh, Wnts, Bmps and Fgfs at E12, E17 and PN5 developmental stages were examined.

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Program/Abstract # 211
stuck on you (soy) participates in Dlx-mediated patterning of the pharyngeal arch intermediate domains in zebrafish
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We are interested in understanding the molecular patterning of the zebrafish jaw and jaw supporting skeleton, which are derived from the first and second pharyngeal arches respectively. Here we describe a zebrafish mutant, stuck on you (soy−), which shows dorsal–ventral (DV) cartilage fusions in intermediate regions of both arches, near the joints. In both mouse and fish, Dlx genes have been identified as strong candidate effectors of pharyngeal arch DV patterning. We find that dlx3b, dlx4b, and dlx4a are specifically expressed in the intermediate domains, while dlx5a and dlx6a expression includes both the intermediate and more ventral regions. Combinatorial studies of dlx3b-MO, dlx5a-MO and dlx5a− suggest that dlx3b and dlx5a function redundantly in intermediate patterning. Co-injection of dlx3b-MO and dlx4b-MO into WT fish did not lead to pronounced intermediate domain defects. In contrast, we injected soy mutants with dlx3b-MO and dlx4b-MO, singly and combined, and found synergistic enhancement of the soy− phenotype. This synergism indicates that soy- and Dlx-mediated jaw patterning are intimately connected. Identification of the gene mutated in soy− may reveal the nature of these connections.

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Program/Abstract # 212
A dominant negative form of p63 is regulated by BMP4 and participates in Xenopus epidermis development
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The ectodermal cells have to decide between two possible fates: the cells in the ventral side follow an epidermal fate while dorsal cells adopt a neural fate. The ectoderm patterning has been suggested to be under the control of BMP4 that induces epidermal fate and inhibits the formation of neural tissue. However, target genes regulated by BMP4 and their roles during the specification of epidermis are less understood. p63, a member of the p53 gene family, is required for mammalian epidermal development. In Zebrafish, the ΔNp63 isoform is a direct target of BMP and is required for epidermal proliferation. In Xenopus, only a cDNA corresponding to mammal ΔNp63g has been identified and its role in development remains unknown. We analyzed the participation of ΔNp63 in Xenopus epidermal development and whether it is regulated by BMP4. First, the expression pattern of ΔNp63 was analyzed and compared with the expression of FoxD3 and Sox2. By neurula stage, the main expression of ΔNp63 is located in the epidermis and also detected as a defined line in the limit between neural folds and epidermis. Then, we overexpressed a dominant negative form of BMP4 (CM-BMP4) in whole embryos and in animal caps. The decrease in BMP4 levels leaded to a lower expression of ΔNp63. Finally, gain of function experiments of ΔNp63 produced an increase of the epidermal marker XK81A and a decrease of the neural crest and neural plate markers FoxD3 and Sox2. Taken together, our results suggest that ΔNp63 participates in the regulation of ectodermal fates by promoting the epidermis specification under the control of BMP4.

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