document spotty hyperplasia of neural stem cells and a parallel loss of epithelium. This neural stem cell phenotype resembles the Notch-Delta neurogenic phenotype, so we have conducted genetic interactions with this pathway. Results imply that aqz interacts with this pathway.

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Program/Abstract # 268
Neural crest and ectodermal contributions to the development of the nasal placode
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The lineage of nasal placode derivatives such as olfactory ensheathing cells and GnRH-1 neurons has been debated for several decades. To analyzed neural crest and ectodermal contributions to the development of the nasal placode, cell fate tracing was performed using Wnt1Cre/ Rosa and ectodermal Crect/Rosa mouse lines. Wnt1Cre recombination was found in cells commingled with the superficial ectoderm at E8.5 and in the developing placode starting from E9.5. Wnt1Cre recombination was found in the entire population of olfactory ensheathing cells and in subpopulations of GnRH-1 neurons, olfactory and vomeronasal cells. No ectopic Wnt1Cre expression was detected in the superficial ectoderm and in the developing placode. Analyzing the olfactory and GnRH-1 system in Crect/Rosa mutants established that the majority of sensory neurons and GnRH-1 cells were ectodermal derivatives and that subpopulations of both cell types were negative for ectodermal recombination. The Crect negative sensory cells and GnRH-1 cells were found in similar number to those positive for Wnt1Cre neural crest tracing. In addition, analysis after ectodermal tracing confirmed the exclusive neural crest origin of the olfactory ensheathing cells in mammals. These data indicate that the olfactory and GnRH-1 systems are composed of cells from both neural crest and ectodermal origin that mix in the developing nasal placode.

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Program/Abstract # 269
A Bmp-Id2a-Twist1-Fli1a network specifies ectomesenchyme from cranial neural crest
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Vertebrate cranial neural crest cells (CNCCs) contribute not only to ectodermal lineages like neurons, glia and pigment but also to "ectomesenchymal" lineages like cartilage and bone. Whereas it has been previously proposed that signaling from the pharyngeal endoderm induces ectomesenchyme fates, we find that CNCCs express the ectomesenchyme-specific marker dx2a in the absence of endoderm. Further, we show that downregulation of Bmp signaling in CNCCs as they migrate away from the neural tube is essential for ectomesenchyme formation. We find that in zebrafish, forced expression of Bmp4 in migratory CNCCs inhibits expression of the ectomesenchyme genes dx2a and fli1a and prolongs expression of early CNCC genes sox10. Previous studies have shown a role for the bHLH transcription factor Twist1 in ectomesenchyme specification in mouse, and here we show in zebrafish that Bmp signaling functions to induce expression of id2a, an inhibitor of Twist1. id2a expression is restricted to the non-ectome-senchyme population, and forced expression of id2a or reduction of Twist1 in migratory CNCCs results in loss of fli1a expression and prolonged expression of sox10. Moreover, we find that increasing Bmp4, id2a or decreasing Twist1 or Fli1a, results in similar losses of ectomesenchyme derived head skeleton and concomitant increases in glial fates. Therefore, we have identified a Twist1-binding element in the enhancer of fli1a and suggest that fli1a is a likely direct target of Twist1. Hence, we propose a novel model of ectomesenchyme specification in which migration away from a Bmp signaling source at the neural-plate border is critical for CNCCs to downregulate id2a and activate the ectomesenchyme genes Twist1 and Fli1a.

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Program/Abstract # 270
Understanding neural crest cell development using Gcnf−/− mutant mice as a model system
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The neural crest (NC) is a multipotent, migratory cell population that is a hallmark of vertebrate evolution and gives rise to a diverse array of tissues including peripheral nervous system and craniofacial skeleton. Formation of the NC encompasses several steps including induction and specification from a precursor pool, epithelial to mesenchymal transition (EMT) and emigration from the neural tube, migration and differentiation into diverse cell types. Previous work in aquatic and avian model systems has revealed that a gene regulatory network mediated by Wnt, BMP and FGF signaling drives NC formation. However, knockout mouse models have failed to recapitulate a role for these pathways in mammalian NC cell induction. We are currently using a mouse mutant in germ cell nuclear factor (Gcnf)/Nr6a1 to study mammalian NC formation. Analysis of Nr6a1+/−/− mutant embryos in our laboratory has revealed an absence of NC cells, which results from a failure of neural progenitors to differentiate into NC and undergo EMT. Along with our global gene expression and protein interaction analysis, our results revealed that Gcnf regulates two key processes during NC formation: stem cell maintenance and EMT. Therefore, we hypothesize that Gcnf acts as a bimodal switch (i) repressing pluripotency genes and activating NC-specific genes during NCC formation and (ii) regulating EMT during NCC delamination. This is the first example of a complete absence of NC in a mammalian system. We will use Nr6a1+/−/− mice to identify the global gene and protein networks that regulate NC formation, which will further our understanding of the causes of neurocraniopathies including craniofacial, cardiac and enteric congenital malformations.

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Program/Abstract # 271
Characterization of downstream targets of Pax3 and Zic1 in the developing neural crest
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In Xenopus, the neural plate border gives rise to at least three cell populations: the neural crest, the pre-placodal ectoderm and the hatching gland. The fate of these cells is largely dependent on the activity of two transcription factors, Pax3 and Zic1. Using gain of function and knockdown approaches in whole embryos we have previously shown that Pax3 and Zic1 are necessary and sufficient to promote hatching gland and pre-placodal fates, respectively, while the combined activity of Pax3 and Zic1 is essential to specify the
neural crest. By manipulating the levels of Pax3 and Zic1 in isolated animal explants, we can generate a fairly pure population of neural crest progenitors independently of the induction of other neural plate border cell types. Taking advantage of this explant-system we conducted a microarray screen in Xenopus to isolate genes synergistically activated by Pax3 and Zic1. The characterization of these targets is expected to expand our understanding of the gene regulatory network underlying neural crest formation. We identified over 100 genes that were upregulated two folds or more, among which are a number of well-studied neural crest specifiers, including Foxd3, Twist, Snai1, Sox8, Sox9 and Inca, which fully validates our approach. The rest of the genes are either novel or had no known function in neural crest formation. They represent potential regulators of gene transcription, signal transduction and cell signaling, as well as genes with yet uncharacterized function.

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Program/Abstract # 272
Cell cycle control of NOTCH signalling during *C. elegans* vulval development
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The egg-laying organ of *C. elegans*, the vulva, is an excellent model to study cell–cell communication during development. The interplay between conserved signalling pathways such as DELTA/NOTCH and EGFR/RAS/MAPK is crucial to establish the proper cell fate pattern during vulval development. The detailed knowledge of the components constituting these signalling pathways permits us to create computational models of vulval fate specification (Fisher et al., 2007, the PANACEA project). By comparing the predictions made by computational modelling with the actual alterations in the vulval cell fate patterns observed in different mutants, we can identify gaps in our understanding and make novel predictions. One insight gained by computational modelling suggests that a global timing mechanism must exist in order to synchronise the differentiation status of the vulval precursor cells (VPCs) within certain limits. We thus investigated the influence of the VPC cell cycle on vulval cell fate specification. We discovered that the degradation of the NOTCH receptor (LIN-12) is tightly linked to cell cycle progression. When the VPCs are arrested in the G1 or S phase using tissue–specific expression of the CDK inhibitor CKI-1 or by hydroxyurea treatment, the NOTCH reporter fails to be expressed in cells of the 1st lineage and instead accumulated in the nucleus and cytoplasm. Moreover, a downstream target of LIN-12 NOTCH, which is normally not expressed in the 1st lineage, continues to be expressed in the arrested VPCs. We will present the results of a mutant analysis of different cell cycle components used to identify the link between cell cycle progression and NOTCH degradation.

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Program/Abstract # 273
Cooperative activity of nogin and gremlin in the development of the axial skeleton
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Cooperative activity of nogin and gremlin in the development of the axial skeleton
Cooperative activity of nogin and gremlin in the development of the axial skeleton

Inductive signals from adjacent tissues mediate initial pattern formation within the somite. Here we focused on how the Hh and BMP pathways interact during sclerotome specification. We confirmed the absence of somitic Pax1 and Pax9 expression in embryos mutant for the BMP antagonists Noggin and Gremlin1, consistent with the loss of sclerotome. Furthermore, Noggin mutants also lacking one allele of Gremlin1 exhibited a dramatic reduction in axial skeleton relative to embryos mutant for Noggin alone. In contrast, expression of Pax3, Myf5 and Lbx1 indicated dermomyotome induction persists in double mutants. Conditional Bmpr1a mutation or BMP type-1 receptor (BMPR1) inhibition did not alter sclerotome marker expression, suggesting that BMP antagonists lack an instructive function in sclerotome specification. We found that BMPR1 inhibition restored sclerotome induction in Noggin:Gremlin1 double mutants, demonstrating that elevated BMP blocks sclerotome formation. However, this treatment did not rescue Pax1 expression in Smoothened mutants, suggesting that inhibition of BMP-signaling is not sufficient to induce sclerotome in the absence of Hh-signaling. Furthermore, Smoothened activation did not rescue Pax1 expression in Noggin:Gremlin1 double mutants, demonstrating aberrant Hh-signal transduction. We conclude that Noggin and Gremlin1 cooperate to maintain a BMP signaling free zone that is a critical prerequisite for Hh-mediated sclerotome specification. We are currently using the chick system examine how BMP may alter the conformation of Gli transcription factors and have initiated tissue engineering studies to develop in vitro models to dissect the mechanism by which BMP affects the outcome of Hh signaling.

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Program/Abstract # 274
Retaionic acid signaling preferentially activates Pod1 and WT1 expression and inhibits smooth muscle differentiation in epicardium-derived cells
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During embryonic development, epicardial cells undergo epithelial-to-mesenchymal transition to form epicardium-derived cells (EPDCs), which invade the myocardium and differentiate into fibroblasts, smooth muscle, and endothelial cells. Immunofluorescence studies demonstrate that the transcription factors (TF) Pod1, WT1, Tbx18, and NFATc1 are expressed in overlapping and distinct EPDC subpopulations in chicken and mouse hearts. Multiple inductive factors including retinoic acid (RA), Wnt, BMP, and FGF influence EPDC development. The upstream regulation of TF expression in EPDCs was examined in isolated chick embryonic day 7 (E7) EPDCs. Expression of Pod1 and WT1, but not Tbx18 or NFATc1, is upregulated with all-trans-RA treatment, as determined by quantitative RT-PCR. BMP2, Wnt3a, or FGF2 treatment does not significantly affect TF gene expression levels in isolated EPDCs. Similar induction of Pod1 and WT1 expression in EPDCs occurs with RA treatment of intact CE7 hearts. In addition, RA treatment inhibits smooth muscle differentiation as indicated by SM22α expression, while treatment with the RALDH2 inhibitor DEAB increases SM22α in CE7 hearts in vitro. Current experiments are investigating if Pod1 is a direct downstream target of RA receptors and if Pod1 is required to inhibit EPDC differentiation into smooth muscle in vivo in mice. These data support our hypothesis that RA promotes Pod1 and WT1 expression while inhibiting smooth muscle differentiation in EPDCs.

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