the wetland, and leaving the wetland. Water was collected at various times of the year and experiments were repeated. Surprisingly, water entering the wetland and midway through had no effect on early development but water leaving the wetland was largely lethal. This suggests that unidentified environmental contaminants are leaching out of the reclaimed wetland and entering surface water supplies. Far more lethality and developmental abnormalities were observed in the dejellied embryos versus those with their jelly coat intact, which suggests that the protein matrix of the jelly coat provides some protection from environmental contaminants. Subsequently, we found that a known estrogenic environmental contaminant (nonylphenol) showed similar effects on dejellied versus jelly coated embryos. We hope this work highlights the need to not only reclaim wetlands from existing farmland, but also to monitor these sites after they have been established.

doi:10.1016/j.ydbio.2006.04.322

295

Edn1/Ednra pathway in *Xenopus* neural crest development

M. Bonano [1], C. Tribulo [1], T. Aguero [1], J. DeCalisto [3], R. Mayor [3], S. Sanchez [1], Aybar [1]
[1] *Dept. Biol. Desarrollo, INSIBIO-UNT, Tucuman, Argentina*
[2] *MNDB, Fac. Cs., Univ. Chile, Chile*
[3] *Dept. Anat. and Dev. Biology, UCL, UK*

The neural crest (NC) develops at the border between neural plate and the prospective epidermis in vertebrate embryos. NC cells are highly migratory and generate a number of derivatives including neurons, pigment cells, craniofacial cartilage, endocrine cells, etc. Numerous studies have demonstrated that many signals (BMP4, Wnt, FGF, etc.) are involved in the induction of this tissue. However, the participation in neural crest specification of other cell signaling pathways has not been established yet. In this work, we have analyzed the expression and participation of the Endothelin1/Endothelin receptor A cell signaling pathway during *Xenopus laevis* development. We report the cloning and expression pattern of Ednra cDNA. Ednra is expressed at the neural plate border from early neurula stage, in the NC cells during migration, and in branchial arches and the otic vesicle. We analyzed the role of Edn1/Ednra pathway in NC development by conditional gain- and loss-of-function approaches using mRNA microinjection, morpholino-oligonucleotides, and the specific inhibitor of Ednra BQ123. We also present embryological evidence showing Edn1/Ednra pathway is also involved in the maintenance of NC specification and cell survival. Our results show that Edn1/Ednra cell signaling pathway is required for the induction and migration of neural crest cells in *Xenopus* embryos. Funding: CIUNT, PICT10623, ICM P02-050, PICTOUNT367, PIP6278, and UNSTA.

doi:10.1016/j.ydbio.2006.04.323

296

Banded hedgehog and Gli intracellular factors control *Xenopus* neural crest specification

T. Aguero, G. Vega Lopez, S. Sanchez, M. Aybar
Dept. Biol. Desarrollo, INSIBIO-UNT, Tucuman, Argentina
MNDB, Fac. Cs., Univ. Chile, Chile

Secreted morphogens of the Hedgehog (Hh) family in *Xenopus* are Sonic hedgehog, Cephalic hedgehog, and Banded hedgehog. They regulate a wide range of developmental processes such as nervous system and limb patterning. In this work, we revised the expression pattern of Banded hedgehog (Bhh) and components of the intracellular signaling cascade during early development of *Xenopus laevis* embryos. The double in situ hybridization analysis showed that Bhh is expressed during neurulation at the lateral border of neural plate in territories that overlap with the expression of Gli transcription factors and neural crest markers. In order to evaluate the participation of Bhh pathway in neural crest development, we carried out gain- and loss-of-function approaches by directed microinjection of Bhh mRNA, its dominant-negative and morpholino oligonucleotides. Results showed that the overexpression of Bhh leads to an increased expression of neural crest markers and Gli transcription factors. On the other hand, the dominant-negative construct of Bhh and the morpholino oligonucleotide reduced the expression of neural crest markers, indicating Bhh signaling is required for neural crest specification. Additionally, the overexpression of Gli3 produced an expansion in neural crest territory mimicking the effect produced by Bhh gain of function. Our results show that Bhh signaling and Gli transcription factors are participating in the early neural crest development. Funding: CIUNT, PICT10623, ICM P02-050, PICTOUNT367, PIP6278, and UNSTA.

doi:10.1016/j.ydbio.2006.04.324

297

A mutant with defective temporal coordination of uterine and vulval development in *C. elegans* is associated with reciprocal signaling defects

Wendy Hanna-Rose, Li Huang
Department of Biochemistry and Molecular Biology,
Penn State, University Park, PA, USA

Many biological functions require interactions between different organs whose development must be coordinated both spatially and temporally. For example, egg laying in *C. elegans* requires that a connection form between the lumens of the uterus in the gonad and the vulva in the extra-gonadal epithelium during organogenesis. A *cog-3(ku212)* mutant appears to form no connection between the vulval and the uterine lumens because the uterine lumen develops with a temporal delay relative to the vulva and thus is not present when the connection normally forms. The lack of temporal synchronization between the vulva and the uterus is not due to precocious or accelerated vulval development. Instead, gonadogenesis is delayed relative to

development of extra-gonadal tissue. *ku212* mutants also have a uterine fate defect. Normally, 4 cells of the ventral uterine pi lineage respond via their EGF receptor-like LET-23 receptors to a vulval derived EGF-like LIN-3 signal and adopt the uterine vulval 1 (uv1) fate. In *ku212* mutants, these 4 pi progeny cells are set aside as a pre-uv1 population, but they die prior to differentiation. A gain-of-function mutation in the LET-23 receptor can rescue the uv1 defect, suggesting that a lack of signaling, perhaps due to the temporal delay, is at fault. In support of this model, lack of vulval–uterine coordination due to precocious vulval development also leads to uv1 cell differentiation defects. We have achieved transformation rescue of *cog-3(ku212)* with a YAC clone and will report on our molecular and phenotypic analysis.

doi:10.1016/j.ydbio.2006.04.325

298

The chemokine Sdf1 and its receptor Cxcr4 are involved in the formation of fast muscle

Shang-Wei Chong [1], Nguyet Le-Minh [1], Yun-Jin Jiang [1], Vladimir Korzh [2]
[1] *Laboratory of Developmental Signaling and Patterning, Institute of Molecular and Cell Biology, Singapore*
[2] *Laboratory of Fish Developmental Biology, Institute of Molecular and Cell Biology, Singapore*

Somitic myogenesis involves various myogenic regulatory factors (MRFs), and myoblasts proceed through various steps before differentiating into muscle fibers. Here, we demonstrate a role for cxcr4 and sdf1a signaling in the presomitic mesoderm during fast fiber formation. We have investigated the role of these proteins by employing the technique of antisense oligonucleotide knockdown. Disrupting the chemokine signal caused a reduction in myoD and myf5 resulting in reduced fast fiber formation. In addition, we demonstrated that cxcr4a and sdf1a could be involved in the regulation of genes encoding these myogenic factors and vice versa. In effect, we propose the presence of a feedback mechanism between these molecules and suggest that cxcr4a-sdf1a has a role in myogenic maintenance.

doi:10.1016/j.ydbio.2006.04.326

299

Lrp4 is required for neuromuscular junction formation and differentiation

Scott D. Weatherbee [1], Kathryn V. Anderson [1], Lee A. Niswander [2]
[1] *Memorial Sloan Kettering, New York, NY, USA*
[2] *HHMI, Univ. of Colorado Health Sci. Ctr., CO, USA*

The formation of the neuromuscular junction (NMJ) is the best-understood example of synaptogenesis. NMJ initiation and maintenance require a complex exchange of signals between the developing motor neuron and skeletal muscle cell, leading to a nerve terminal that is specialized for the release of neurotransmitter and a postsynaptic muscle cell membrane that contains an abundance of neurotransmitter receptors. Previous studies have defined a series of events that lead to postsynaptic differentiation. A receptor tyrosine kinase, MuSK, plays a key role in all aspects of postsynaptic differentiation, including nerve-independent pre-patterning of neurotransmitter (acetylcholine) receptors (AChRs) as well as the subsequent refinement of AChRs to synaptic sites. However, the MuSK-dependent, neuron-independent events that initiate NMJ formation are not understood. Here we show that the mouse *Low-density lipoprotein receptor-related 4* (*Lrp4*) gene is required to form neuromuscular synapses. *Lrp4* mutant mice have defects in both presynaptic and postsynaptic differentiation, including aberrant motor axon growth and branching, a lack of AChR and postsynaptic protein clustering, and a failure to express postsynaptic genes selectively by myofiber synaptic nuclei. The similar phenotypes of *Lrp4* and *MuSK* mutants and the discovery that Lrp4 is required for the localization of MuSK suggest that Lrp4 is a critical factor for MuSK-mediated postsynaptic differentiation and that Lrp4 acts in the early, nerve-independent steps of NMJ assembly.

doi:10.1016/j.ydbio.2006.04.327

300

Cilia/IFT in mammalian limb patterning

Courtney J. Haycraft [1], Qihong Zhang [2], Buer Song [1], Rosa Serra [1], Bradley K. Yoder [1]
[1] *University of Alabama at Birmingham, Birmingham, AL, USA*
[2] *University of Iowa, Iowa City, IA, USA*

Cilia are microtubule-based organelles expressed on most mammalian cells. They are formed and maintained by a process termed intraflagellar transport (IFT) and disruption of any IFT protein results in defects in cilia formation and subsequent signaling activities. While they were previously considered to be evolutionary remnants, cilia have recently been shown to be required for development and function of many tissues. Recent work from several labs has indicated a requirement for proteins required for cilia and flagella formation, IFT proteins, in both the activation and repression of Shh signal transduction. In the developing limb, cilia are present on cells of the ectoderm and underlying mesenchyme and congenital loss of the IFT protein polaris results in expansion of the autopod, ectopic digit formation and loss of Shh signal transduction. We have begun using conditional alleles of IFT genes to bypass the midgestation lethality of IFT null mutants and investigate the function of cilia/IFT in specific cell populations in the developing limb. Similar to congenital loss of polaris, loss of cilia on the mesenchymal cells of the limb bud using prx1 promoter driven Cre expression results in expansion of the autopod and extensive polydactyly. In addition to polydactyly, the limbs of prx1cre;IFT conditional mutants are shortened relative to control littermates, suggesting a possible role in Ihh signaling during cartilage and bone development. We are also using msx2cre transgenic mice to