structures. The insulin-signaling pathway is known to regulate growth with respect to nutrition, and suppression of the insulin receptor has less of an effect on the size of the genitals than it does on the wing, the maxillary palp, and the leg. Genetic and micro-array data suggest that the unusual allometric relationship between the genitals and the body is explained by differential expression of insulin-pathway genes in the genitals relative to other body parts. Organ-specific regulation of the insulin-signaling pathway may therefore be a general method by which animals maintain the size of key structures under variable nutritional conditions.

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Cardiac arterial pole development is conserved in vertebrates

Margaret L. Kirby [1], Adrian Grimes [2]
[1] *Duke University, Durham, NC, USA*
[2] *Medical University of South Carolina, Charleston, SC, USA*

The arterial pole of the heart consists of the myocardium and smooth muscle that meet at the ventriculoarterial junction. Both cell types are generated by splanchnic mesoderm caudal to the outflow tract. The outflow tract moves caudally across this field as cells from the splanchnic mesoderm are added. Following the addition of myocardial cells to the prospective arterial pole, a second wave of smooth muscle cells is added. Even though the zebrafish has an undivided outflow tract, the arterial pole is formed at the ventriculoarterial junction where ventricular myocardium joins arterial smooth muscle at the base of a structure commonly called the bulbus arteriosus in teleosts. Our study was designed to determine whether development of the arterial pole of the zebrafish heart is conserved. Single cell zebrafish embryos were injected with caged rhodamine and cells were uncaged just prior to gastrulation. The embryos were allowed to develop to 72 hpf. The arterial pole was marked with DAF-2DA and tropomyosin to visualize the smooth muscle and myocardium. Both myocardial and smooth muscle cells in the arterial pole were found to arise from common progenitors located in the blastoderm in the vicinity of, but distinct from, ventricular progenitors. As in chick and mouse, normal arterial pole development is dependent on tbx1 (van gogh mutant), and FGF8 signaling (acerebellar mutant). These data provide the first evidence for evolutionarily conserved origin and development of cells that form the arterial pole of the vertebrate heart.

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Hox genes and development of paired fins in teleost: An alternative view

Dae-gwon Ahn, Robert K. Ho
Univ. of Chicago, Chicago, IL, USA

Recent advances in molecular genetics have shown that pectoral and pelvic fins of teleost fishes are homologous structures to the fore- and hindlimbs of tetrapods, which utilize common sets of highly conserved developmental regulator genes for their growth and patterning. However, in contrast to the expression of many of the genes including Shh, Fgf8, Tbx4/5, which is mostly identical between limbs and fins, expression of Hox genes has been hypothesized to be quite different between fins and limbs. In particular, in contrast to the dynamic tri-phasic expression of Hox genes seen in tetrapod limbs, expression of hox genes in teleost fins has often been described as recapitulation of only the early parts of Hox expression within tetrapod limbs. Here, we show that a more detailed examination of the expression of posterior hox genes during pectoral fin development in zebrafish indicates the presence of at least three phases of hox gene expression which are similar but not completely identical to those seen in tetrapod Hox genes. Our results suggest that conservation of developmental mechanisms underlying formation and patterning of paired appendages between teleosts and tetrapods might be more extensive than previously thought.

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Differential partitioning of paralog group 2 *Hox* gene expression within the *Osteichthyii*

Jean-Luc Scemama, Pierre Le Pabic, Edmund J. Stellwag
East Carolina University, Greenville, NC, USA

Hox cluster evolution, particularly those events related to an actinopterygian-specific genome duplication, have generated variation in the complement of *Hox* paralogous group 2 (PG2) genes within the *Osteichthyii*. Both the tetrapods and the ostariophysans, as represented by zebrafish, possess only two *Hox* PG2 genes, *hoxa2* and *b2* (tetrapods), and *hoxa2b* and *b2a* (zebrafish). In the acanthopterygians, however, all species characterized to date have three *Hox* PG2 genes (*hoxa2a*, *a2b*, and *b2a*). We have previously demonstrated that striped bass *hoxb2a* gene fails to be expressed in the pharyngeal arches, indicative of an evolutionary divergence between striped bass and other osteichthyians. Here, we report the expression analysis of striped bass *hoxa2a* and *hoxa2b* genes. We demonstrate that both genes are expressed in the developing hindbrain and the pharyngeal arches albeit with different spatio-temporal distributions relative to one another. We show that the striped bass *hoxa2a* gene expression pattern is similar to the overall expression pattern described for the *hoxa2* genes in the tetrapod lineage and for the *hoxa2b* gene from zebrafish. It is notable that the pharyngeal arch expression pattern of the striped bass *hoxa2a* gene is more divergent from its sister paralog, *hoxa2b* than from the zebrafish *hoxa2b* gene. These results associated with a comparative genomic analysis of the promoter region of these PG2 genes suggest that evolution-

ary changes after the divergence of the striped bass and zebrafish lineages may be responsible for the differential partitioning of activities among the *Hox* PG2 genes in those divergent lineages.

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Genetic analyses of adult pigment pattern development reveal homology and evolutionary novelty in *Danio* fishes

Margaret G. Mills, Richard J. Nuckels, David M. Parichy
Department of Biology, University of Washington, Seattle, WA, USA

Pigment patterns of *Danio* fishes are a convenient system for studying the evolution of development. In zebrafish, *D. rerio*, stripes form by migration and differentiation of distinct populations of melanophores: early metamorphic (EM) melanophores arise widely dispersed and then migrate into stripes, whereas late metamorphic (LM) melanophores arise already within stripes. EM melanophores require the kit receptor tyrosine kinase, as kit mutants lack these cells but retain LM melanophores that form a residual stripe pattern. To see if similar requirements are present in other species, we examined *D. albolineatus*, which has relatively few, uniformly dispersed melanophores. We isolated a null allele of *D. albolineatus* kit and asked whether residual, LM melanophores develop, as in *D. rerio*. We find that kit mutant *D. albolineatus* lack EM melanophores, yet retain LM melanophores. Interestingly, kit mutant *D. albolineatus* also develop a striped pattern similar to kit mutant *D. rerio*, indicating that (i) latent stripe-forming potential remains in this species, despite its uniform pattern; and (ii) evolutionary differences between *D. rerio* and *D. albolineatus* reflect changes in the behavior of kit-dependent melanophores, which migrate into stripes in *D. rerio* but fail to do so in *D. albolineatus*. Our results show how genetic analyses of closely related species can reveal both conservatism and innovation in developmental mechanisms, and the cellular processes underlying evolutionary changes in adult form.

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Characterization of zebrafish Deltex homologues

Stephanie, Chia-Chia Tan, Haoying Xu, Yun-Jin Jiang
Laboratory of Developmental Signalling and Patterning, Institute of Molecular and Cell Biology, 61 Biopolis Drive, Singapore

It is widely known that Deltex is a cytoplasmic protein that binds to Notch and may mediate CSL-independent Notch signaling. Deltex proteins share three functional domains: the WWE domain that binds to Notch ankyrin repeats, the Ring Finger (RF) domain that is often found in a subset of E3 ubiquitin ligases and a proline-rich region. We have cloned three full-length zebrafish *deltex* homologues, namely *deltex1*, *deltex2* and *deltex3*. In silico domain analysis revealed structural similarities as well as differences among the zebrafish *deltex* homologues. The most interesting difference is the lack of a WWE domain in Deltex3, which indicates that it may not interact with Notch receptors directly. Whole mount in situ hybridization assay demonstrated zebrafish *deltex1* expression in many tissues, including neural and sensory structures, raising the possibility that it may be involved in neurogenesis via the Notch signaling pathway. Increasing evidence suggests that Deltex possesses an E3 ligase activity and is responsible for endosomal trafficking of Notch through interaction with the Notch ankyrin repeats. The E3 ligase activity of zebrafish Deltex1 was carried out by in vitro ubiquitylation assay. Our result confirms that Deltex1 has an E3 autoligase activity in the presence of an E2, UbcH5a. Our characterization provides the first description of expression pattern of Deltex homologue and demonstration of its E3 ligase activity in zebrafish that would help in determining the molecular function of Deltex in the context of Notch signaling.

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A zebrafish Pax6a reporter BAC recapitulates Pax6 expression in the mouse

Jorn Lakowski, James D. Lauderdale
Univ. of Georgia, Athens, GA, USA

Pax6 is a highly conserved transcription factor which is crucial to the development of the central nervous system, eye and pancreas. Pax6 transcription is complex and subject to very strict regulatory mechanisms, which include a large number of tissue specific regulatory elements as well a differential promoter usage. Unlike most vertebrates, zebrafish have two Pax6 genes, designated Pax6a and Pax6b, which are located on different chromosomes, and likely arose from a genome duplication that occurred after the split between the tetrapod and teleost lineages. The Pax6 proteins encoded by Pax6a and Pax6b share 95% amino acid identity over their entire length and both generate ectopic eyes in *Drosophila* suggesting that the two proteins have retained similar biochemical functions. Furthermore, it has been postulated that both genes have been retained due to a partitioning of certain tissue specific, regulatory elements, crucial to proper Pax6 function. In order to test the degree of evolutionary conservation of the mechanisms governing Pax6 expression as well as to further investigate the basis for the retention of two Pax6 genes in fish, we took advantage of BAC modification technology. We have tested a dual reporter BAC containing the zebrafish Pax6a transcription unit (200 kb) in both mouse and zebrafish and demonstrate that the teleost regulatory elements can in fact direct reporter gene expression in the mouse. Our findings directly show that the factors occupying distinct regulatory elements can induce proper Pax6 transcription despite the vast