Knockdown of alpha-1-microglobulin bikunin precursor (AMBP) causes ocular, and craniofacial defects
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We, as part of a of a large-scale morpholino-based screen of commonly translated and transported proteins (transcripts with signal peptides), identified the zebrafish homologue of human alpha-1-microglobulin bikunin precursor (AMBP). Knockdown of AMBP with 2 nonoverlapping translational blocking morpholinos results in a quantifiable large pupil and microphthalmic eye. Transverse sections of 3-day post-fertilization larvae demonstrate a protruding lens and reduced globe size when compared to uninjected controls. Hematoxylin and eosin staining of eye sections show a considerable disruption of retinal layering and a nearly absent photoreceptor layer. The larvae also exhibit jaw and branchial arch anomalies with smaller pectoral fins. Lastly, we see a disruption in expression of an enhancer trap line in the 4th ventricle choroid plexus. We are currently pursuing the hypothesis that the bikunin subunit of the protein functions as a mitogen.

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N terminal variation in zebrafish calcium channel beta subunit genes
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In many organisms, the embryonic heart circulates blood before the organ itself is fully formed. From the outset, calcium coordinates excitation–contraction coupling and may indirectly affect growth and morphology of the heart as well. L-type calcium channels comprise the primary mode of calcium entry into cardiac myocytes. Cardiac L-type calcium channels are oligomeric complexes composed of a pore-forming alpha1C subunit, and auxiliary beta and alpha2-delta subunits. The beta subunits fine-tune L-type calcium channel function by modulating their gating properties and enabling the cell-surface expression of the alpha subunit. We report the cloning of four new zebrafish calcium channel beta (CACNB) subunit genes. All four genes show alternative splicing of N terminal exons. In humans, alternative N terminal CACNB4 isoforms are functionally significant, in that the encoded subunits differentially affect calcium channel gating in Xenopus oocytes. Zebrafish CACNB N terminal exons are short, separated by large genomic distances, and likely subject to individual regulatory control. All four genes are expressed in the embryonic heart, but isoforms show different temporal patterns of expression in embryonic development and adults. Morpholino data indicate that three CACNB genes are essential for cardiac function, but suggest an earlier, pre-gastrulation requirement for one gene. We hypothesize that heterogeneity in CACNB protein composition during development provides a mechanism to modulate L-type calcium current as the heart grows and to facilitate changes in contractile properties of the developing heart.


Adhesion ligand nanopatterning influences differentiation of preosteoblast cells: A combined experimental and computational approach
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We combine experimental and computational approaches to study the role that RGD presentation plays in regulation of osteogenic differentiation. An artificial extracellular matrix, alginate hydrogel, is used to present nanopatterns of RGD. Specifically, RGD is patterned into multivalent islands that are distributed throughout the matrix to promote integrin clustering and drive osteogenic differentiation. The nanopatterns are characterized using a multi-scale Monte Carlo modeling approach to predict both ligand presentation within islands and island distribution throughout the matrix. To test specific nanopatterns, MC3T3 preosteoblast cells are cultured in the hydrogel and assayed for osteocalcin secretion. Our computational work predicts that island distributions must be dense enough to include groups of islands and 5–25 RGD/island are necessary to observe an impact on osteogenic differentiation. These patterns can then be tested experimentally. For example, when MC3T3 cells are cultured both in nanopatterned matrix (using patterns suggested by the models) and in non-patterned matrix with the same bulk RGD density, cells in the patterned gels show a two-fold increase in osteocalcin secretion on day 10 and a 25% increase on day 21. Both the valency of the islands and the island distribution appear to impact osteogenic differentiation. These studies suggest that osteogenesis can be manipulated by the nanoscale organization of synthetic ECM.

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Nonautonomous effects that follow from disrupted signaling center regulation. Furthermore many of the skeletal and molecular phenotypes are encompassed by the sum of Twist1 target mutant phenotypes.

Our data have begun to reveal how balanced networks of antagonistic transcription factors control multiple aspects of limb patterning.

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