

[i]In vitro[/i] myotubes derived from zebrafish myogenic precursor cells upregulate Pax-3 and-7 following starvation

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The origin and developmental underpinnings of craniofacial divergence between crocodiles and birds, the two great archosaurian lineages

Crocodylians represent one of the two crown radiations of Archosauria, the ruling reptiles. Although much has been described regarding the series of transitions from the ancestral archosaur to the ancestral bird, somewhat less has been made of the gradual assembly of the similarly specialized crocodylian body and head. I use new CT data and new specimens from the stem of Archosauria and the stem of Crocodylia to trace the gradual assembly of the crocodile form, focusing on the skull. The divergence between the avian and crocodylian lineages was quickly marked by a distinctive lightening of the skull in the former and reinforcement in the latter, culminating in the heavy, solid crania of Crocodyliformes. The wide crocodylian facial region is a relatively recent innovation, which can be pinpointed with an expanded fossil record of Jurassic and Cretaceous taxa. Some of the molecular developmental underpinnings of the divergence between crocodile and bird faces are also now coming to light. New data implicate very early molecular patterning differences in the morphological divergence of the premaxillary region at the tip of the snout, the maxillary region in the middle of the head, and the brain and skull roof at the back of the cranium. This work, however, represents only a beginning. With the release of several crocodylian genomes, it might now be hoped that the upstream regulatory changes leading to crocodylian- or bird-specific gene expression and phenotype are within reach.

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Cellular Mechanisms of Cnidarian Bleaching

Several possible mechanisms of bleaching (loss of symbiotic dinoflagellates) have been reported in corals and sea anemones. These include in situ degradation of algal cells, exocytosis of algal cells, detachment of host cells containing algae, and death of host cells containing algae (by either apoptosis or necrosis). However, these mechanisms have not been studied in parallel, quantitative studies, and the observations were made in different species under different stress conditions. In addition, the molecular mechanisms underlying these four possible bleaching mechanisms remain unclear. We have developed assays to monitor all four possible bleaching mechanisms in parallel, using animals from a clonal population of the small sea anemone Aiptasia that have been exposed to a variety of precisely controlled temperature-stress and light-stress conditions. The overall bleaching responses are assessed by counting the numbers of algae remaining in the hosts using a flow cytometer that allows precise and rapid counting of a large number of samples. To distinguish exocytosis from host-cell detachment, cells in the seawater surrounding the stressed anemones are collected and examined by fluorescence and electron microscopy. To look at host-cell death and in situ degradation, we are using western blotting, immunohistochemistry, protease-activity assays, and qPCR to study the possible roles of different cellular pathways including apoptosis and autophagy. Under the several stress conditions tested (heat, cold, high light, and heat plus high light), it seems that host-cell detachment, host-cell death, and in situ degradation of algae contribute little to the overall bleaching of Aiptasia, and expulsion of algae appears to be the major cause of bleaching.

P2.105 BIERMAN, HS*; BOUSLOG, C; STREETS, A; ZHANG, G; CARR, CE; Univ. of Maryland, College Park; hilaryb@umd.edu
Behavioral evidence for sound localization in Alligator
mississippiensis

Alligator mississippiensis vocalizations and sound localization are thought to be important for maternal care and group cohesion in young animals (Hunt and Watanabe 1982), and neurophysiological and acoustical studies have demonstrated that these animals are capable of sound localization (Carr et al. 2009; Bierman et al. 2014). Behavioral evidence of localization is, however, lacking. We therefore used psychophysical techniques to determine if juvenile A. mississippiensis could localize sound in the laboratory. We trained and tested the alligators in a large tank with speakers and food sources placed on opposite sides. The alligators learned an association between a 1,000Hz tone with a food reward at the tone source. Super–threshold tones were played at random, sparse intervals to prevent habituation to the sound. Every tone represented a trial. Responses were scored if they occurred within 3 seconds of the offset of the tone. Consistently, across animals, trials, and speakers, most movements were towards the side of the tank with the speaker playing the tone.

85.6 BIGA, PR*; FROEHLICH, JM; GABILLARD, JC; SEILIEZ, I; Univ. Alabama at Birmingham , INRA, Rennes, INRA, St–Pee; pegbiga@uab.edu

In vitro myotubes derived from zebrafish myogenic precursor cells upregulate Pax-3 and -7 following starvation.

The zebrafish (*Danio rerio*) remains the teleost fish of choice for biological investigations due to the plethora of molecular tools available for use in this system. However, its somatic growth is not representative of other teleost fishes, most of which are able to grow throughout their lives (termed indeterminate growth) while the zebrafish possesses a rather limited growth potential (determinate growth). In vertebrate skeletal muscle, growth is largely regulated by protein turnover, with the balance between protein synthesis and degradation governing whether myofibers hypertrophy or atrophy. To better describe the potentially divergent mechanisms of skeletal muscle atrophy in this species, we developed a primary myotube culture system generated from isolated myogenic precursor cells (MPCs) from adult zebrafish. Using a media devoid of serum and amino acids, we induced the expression of many genes associated with autophagy. Interestingly, paired—box transcription factor (*Pax3/7*) expression increased concurrently with myocyte enhancing factor—2ca (*Mef2c*) upregulation when *de novo* myotubes were subjected to starvation. From these results, it suggests that zebrafish myotubes, cultured from primary myoblasts, can be induced to express early myogenic biomarkers using nutrient alterations.