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Evolution of Developmental Control Mechanisms

Juvenile skeletogenesis in anciently diverged sea urchin clades

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ABSTRACT

Mechanistic understanding of evolutionary divergence in animal body plans devolves from analysis of those developmental processes that, in forms descendant from a common ancestor, are responsible for their morphological differences. The last common ancestor of the two extant subclasses of sea urchins, i.e., euechinoids and cidaroids, existed well before the Permian/Triassic extinction (252 mya). Subsequent evolutionary divergence of these clades offers in principle a rare opportunity to solve the developmental regulatory events underlying a defined evolutionary divergence process. Thus (i) there is an excellent and fairly dense (if yet incompletely analyzed) fossil record; (ii) cladistically confined features of the skeletal structures of modern euechinoid and cidaroid sea urchins are preserved in fossils of ancestral forms; (iii) euechinoids and cidaroids are among current laboratory model systems in molecular developmental biology (here *Strongylocentrotus purpuratus* [*Sp*] and *Eucidaris tribuloides* [*Et*]); (iv) skeletogenic specification in sea urchins is uncommonly well understood at the causal level of interactions of regulatory genes with one another, and with known skeletogenic effector genes, providing a ready arsenal of available molecular tools. Here we focus on differences in test and perignathic girdle skeletal morphology that distinguish all modern euechinoid from all modern cidaroid sea urchins. We demonstrate distinct canonical test and girdle morphologies in juveniles of both species by use of SEM and X-ray microtomography. Among the sharply distinct morphological features of these clades are the internal skeletal structures of the perignathic girdle to which attach homologous muscles utilized for retraction and protraction of Aristotles' lantern and its teeth. We demonstrate that these structures develop de novo between one and four weeks after metamorphosis. In order to study the underlying developmental processes, a method of section whole mount in situ hybridization was adapted. This method displays current gene expression in the developing test and perignathic girdle skeletal elements of both *Sp* and *Et* juveniles. Active, specific expression of the *sm37* biomineralization gene in these muscle attachment structures accompanies morphogenetic development of these clade-specific features in juveniles of both species. Skeletogenesis at these clade-specific muscle attachment structures displays molecular earmarks of the well understood embryonic skeletogenic GRN: thus the upstream regulatory gene *alx1* and the gene encoding the *vegfR* signaling receptor are both expressed at the sites where they are formed. This work opens the way to analysis of the alternative spatial specification processes that were installed at the evolutionary divergence of the two extant subclasses of sea urchins.

Introduction

This is our initial report on an evolutionary research project, the specific objective of which is to determine the developmental programs that underlie divergent morphogenetic processes distinguishing cidaroid and euechinoid sea urchins. We are interested in characters that can be tracked in the fossil record, so that paleontological evidence can be used to establish the polarity, and the plesiomorphy vs. novelty, of characters in each lineage. Fortunately, the growing fossil record is providing high-resolution evidence of skeletal structures in Paleozoic and Triassic sea urchin clades that is directly relevant to the emergence of the modern euechinoid and cidaroid subclasses. To attain our ultimate goals it would be necessary to gain experimental access to the developmental processes by which distinct euechinoid vs. cidaroid skeletal morphologies arise in their respective adult body plans. We have discovered such processes taking place in juveniles in the weeks immediately following metamorphosis. However, specific developmental mechanisms have rarely if ever been

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studied at the molecular level in juvenile sea urchins, and an initial suite of methodological problems had first to be overcome. Our objectives in this work were (i) to identify divergent aspects of juvenile skeletogenesis that are specifically canonical to either of the two echinoid subclasses, using as laboratory sea urchin models the cidaroid *Eucidaris tribuloides* and the euechinoid *Strongylocentrotus purpuratus*; (ii) to characterize the morphogenesis of these features and determine when this occurs; (iii) to learn how to visualize gene expression in the relevant spatial phases of juvenile skeletogenesis; (iv) to obtain initial evidence that might relate these processes to the well known mechanisms of skeletogenesis in echinoid embryogenesis.

Going back in deep time, the fossil record shows that organization of the radial test endoskeletons of the various clades of Paleozoic echinoids varied enormously in respect to the absolute and relative numbers of columns of ambulacral vs. interambulacral plates (ambulacral plates are those containing perforations through which tube feet extend) (Kier, 1965). In contrast, the pentaradial tests of all modern echinoids, i.e., including both cidaroid and all regular euechinoid sea urchins, display a constant alternation of two ambulacral columns with two interambulacral columns of test plates. Many shared characters identify the Mississippian to Permian echinoid stem group *Archaeocidaris* as the closest known common ancestor of cidaroids and euechinoids (Kroh and Smith, 2010), though *Archaeocidaris* displays two columns of ambulacral plates alternating with four columns of interambulacral plates. The first well preserved forms of cidaroid and euechinoid lineages, known from the Permian and Triassic respectively (Kier, 1977; Smith and Hollingworth, 1990), display the crown group feature of two columns of ambulacral plates offset with two columns of interambulacral plates. However, the detailed evolutionary steps intervening between *Archaeocidaris* and the earliest crown group Mesozoic cidaroids and euechinoids remain obscure, and, as we report elsewhere, additional paleontological evidence is now leading to significant revision of current scenarios. It is clear (contrary to the conventional description of cidaroids as “primitive”) that both modern echinoid subclasses retain some plesiomorphic characters, such as their large spines and their tubercular support structures, plus a number of features relating to their coronal plating (Smith, 2005), and it is these features which lead to the conclusion that the last common ancestor was derived from the *Archaeocidaris* stem lineage. However, the paleontological record is likely missing intermediates between the *Archaeocidaris* stem lineage and crown group euechinoids and cidaroids. Each subclass also presents features that are derived with respect to the *Archaeocidaris* common ancestor as well as plesiomorphic characters shared with *Archaeocidaris*. Two prominent derived features of the endoskeleton distinguish cidaroid from euechinoid sea urchins. The first of these is the organization of their ambulacral test plates. The second is the entirely distinct morphology of the bony protrusions from the radial perignathic girdle which serve as attachment anchors for the powerful paired muscles that retract Aristotle's lantern and the five teeth suspended within from the extruded position (Wilkie et al., 1998; Kroh and Smith, 2010). These skeletal features are illustrated below. Lantern and dental morphology, and the presence or absence of buccal notches, provide an additional sets of distinguishing characters (Smith and Hollingworth, 1990), but we have not addressed these more difficult features as they are less frequently preserved paleontologically and more difficult to study developmentally.

Results and discussion

Morphological differences in the skeletal structures of cidaroid vs. euechinoid adult body plans

In modern echinoids the endoskeletal test plates develop essentially in the following manner. The dorsal-most or apical plates, that is, the 10 plates surrounding the anus, including the five that contain the gonopores, and the other five (ocular) plates are present in very young metamorphosed juveniles. Formation of these plates is initiated in larval life, prior to metamorphosis. In young juveniles, circular rings consisting of horizontal rows of the body wall test plates, ambulacral and interambulacral, are delaminated downward from a generative zone immediately surrounding the apical plates. This process continues in juveniles for the first few weeks after metamorphosis, until the adult number of plate rows is produced (e.g., 14), such that the most adoral plate rows (furthest down) are developmentally the oldest, and the most adapical, adjacent to the apical plates, are the youngest. Thus, as rows are added, the form of the juvenile gradually changes from an almost flat pancake-like structure containing only a very few lateral plate rows to a globular one. Again in contrast to Paleozoic forms, the growth of the animal in post-juvenile life occurs by continuing accretion of biomineral to the periphery of pre-existing plates, rather than by continuing formation of numerous additional plates (Smith, 2005).

A phyletically distributed endoskeletal character sharply distinguishes the ambulacral test plates of cidaroid and euechinoid sea urchins. This is that cidaroid plates each bear a single pair of pores and the initial plate boundaries are also the final plate boundaries, while in euchinoids, the initially formed plates (consisting of a primary plate and numerous demiplates) progressively fuse, so that in the aggregate the resulting compound plates contain many pore pairs (Kroh and Smith, 2010). In the *Archaeocidaris* stem group, ambulacral plates are exclusively simple, and thus the simple plating in cidaroids, as opposed to compound plating, is the plesiomorphic character. The comparison is shown graphically and photographically for *S. purpuratus* and *E. tribuloides* in Fig. 1. The mechanism of fusion involves overgrowth of the tuberclear biomineral mounds onto adjacent demiplates. In our observations of test formation the earliest plate fusions could be observed only towards the end of the several week period we studied. Thus plate fusion is a relatively later event in body wall test formation, following delamination of all the plate rows and development of the initial sets of spines, tube feet and other external organs. Though a valuable subclass diagnostic, the progressive nature and relatively late process of ambulacral plate fusion did not recommend itself as a likely target for developmental investigation. Furthermore, plate fusion is a character that is present in one clade, the euechinoids, and entirely absent in the other, the cidaroids, rather than a character that develops differently in the two clades, but which, since it exists in both, might lend itself to differential developmental comparison.

A second, and for us more exciting distinction, is in the five pairs of muscle attachment structures of the perignathic girdle (Wilkie et al., 1998). These muscles mobilize the jaw of the sea urchin, which comprise the pentaradial Aristotle's lantern structure in which the teeth are mounted. The muscle attachment mechanism motivates the physical deployment of the teeth, which can be extruded during feeding. Euechinoid sea urchins produce 10 erect structures known as auricles which are located exactly on the interior edges of the perignathic ambulacral plates, extending upward into the interior of the animal (Fig. 2A–C). In some clades, such as the Strongylocentrotidae, the two auricles present in each ambulacral area merge above the ambulacral plates forming an inverted “V”. In contrast, cidaroid sea urchins develop for this purpose five pairs of broader double-pointed protrusions known as apophyses, which grow out of the inner edges of the interambulacral adoral test plates (Fig. 2D–F). Though as we see in the following the functions of auricle and apophysis are similar, in that they both anchor the retractor muscles, these structures are strikingly different in form. In addition, they develop 180° out of phase with one another spatially, as they are ambulacral in the

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