Developmental Insights into the Origin of Complex Colonial Hydrozoans

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SYNOPSIS. Colonial hydrozoans represent some of the most diverse and complex body plans within the Metazoa. Complex hydrozoans colonies are more physiologically and structurally integrated than their simple colonial relatives. Colonial integration is commonly associated with the regulation of the general structural plan of the colony, the division of labor, and the physiological integration of the colony. In the hydrozoan Hydractinia, these features are manifested through evolutionary innovations involving the spatial regulation of polyps within the colony, the development of polyp polymorphs, and the acquisition of a stolonal mat. These innovations all involve evolutionary changes in the regulation of polyp and colony-wide patterning systems. In Hydractinia, the ParaHox gene, Cnox-2, is expressed in a spatially restricted manner along the axes of stolons and polyps, suggesting that changes in the regulation of this gene may be in part responsible for the evolutionary innovations important for colonial complexity.

INTRODUCTION

A prominent feature in the history of colonial metazoans has been a trend towards increased colonial complexity. The evolution of complex colonial metazoans has been accompanied by increased colonial integration. Trends toward increased colonial complexity and integration are nowhere more apparent than within the cnidianarian Class Hydrozoa. Hydrozoan colonies display a diverse array of forms. The simplest type of hydrozoan is exemplified in Podocoryne (Fig. 1A), which possesses an array of identical polyps, loosely interconnected by structures called stolons. Hydractinia, a close relative of Podocoryne, possesses a higher degree of complexity and integration (Fig. 1B). Hydractinia displays four functionally and morphologically distinct polyp types, arranged at particular areas of the colony. Communication between polyps occurs via their common gastrovascular cavity and an innervated epithelial layer called a stolonal mat. The pinnacle of hydrozoan colonial complexity can be found in members of the Family Siphonophora (Fig. 1C). Siphonophores are complex, free-living colonies, displaying a division of labor through behaviorally coordinated and well-placed polymorphic polyps and medusae. The siphonophores have achieved a degree of integration such that they are often regarded as solitary organisms in spite of their colonial organization (Winsor, 1976; Gould, 1982).

Colonial complexity has evolved multiple times within the Metazoa. Despite their independent evolution, however, complex integrated colonies share a number of common attributes that include: (1) the regulation of the general structural plan of the colony; (2) the division of labor through polyp polymorphism; and (3) the physiological integration of the colony (Beklemishev, 1969). Insight into the developmental mechanisms responsible for these features is key to understanding the evolution of complex colonies.

The colonial hydrozoan Hydractinia is a common experimental organism amongst hydrozoan developmental biologists because its entire life cycle can be easily cultured, manipulated and studied in the laboratory. Hydractinia possesses those features outlined above that are considered important for colonial complexity and integration and thus makes it an ideal candidate for studying the developmental mechanisms underlying the evolution of colonial complexity.

Hydractinia life cycle

Hydractinia colonies in the North Atlantic are frequently found encrusting gastropod shells inhabited by hermit crabs. Hydractinia colonies are either male or female, and reproductive colonies shed gametes into the surrounding water. Following fertilization, cleavage gives rise to a crawling, non-feeding, planula larva (Fig. 2A–C). The planula larva settles on a hermit crab shell and metamorphoses into a primary polyp (Fig. 2D).

The base of the polyp becomes fixed to the substrate and stolons emanate from the aboral pole of the primary polyp (Fig. 2D). Stolons are fluid-filled tubes in which the lumen and tissue layers are continuous with the gastric cavity and tissue layers of the polyp. Stolons lie adherent to the substrate, elongate, branch, reunite, and bud polyps at their apical surface (Fig. 2E). As the polyps grow, they undergo characteristic axial differentiation into oral and aboral regions.

When stolon branching becomes dense, a stolonal mat will form. The stolonal mat consists of an upper and lower epithelial layer encasing the endodermal stolonal canals (Fig. 2F). The continuous upper ectodermal layer of the stolonal mat possesses a nerve net, which is lacking in stolons. The nerve net has been implicated in the behavioral coordination amongst polyps in the colony (Josephson, 1961; Stokes, 1974).

Hydractinia polyp polymorphs

The first type of polyps to develop in a young Hydractinia colony are the gastrozooids, which function...
primarily for feeding and consist of an aboral region comprising the body column and an oral region comprising the hypostome, mouth and tentacles (Figs. 2D and 3A). When the limits of the substrate have been reached, a Hydractinia colony will bud reproductive polyps called gonozooids from the stolonal mat. These bear gonophores on their body column (Fig. 3B). Another specialized polyp, the dactylozooid, develops only on the aperture of the gastropod shell, and specializes in capturing eggs from its hermit crab host (Fig. 3C). The gonozooid and dactylozooid both lack the hypostome and tentacles found in the gastrozooid, and instead consist of a body column with clusters of stinging cells, called nematocysts, at their oral end. The fourth polyp type, the tentaculozooid, functions in defense and develops only when induced by a foreign organism. The tentaculozooid resembles a single tentacle of a gastrozooid except that the tentaculozooid is the approximate size of a gastrozooid polyp (Fig. 3D) (e.g., Muller, 1964).

Colony complexity through changes in the regulation of axial structures

Hydractinia possesses many features associated with colonial integration and complexity. The develop-

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**Fig. 1.** Three hydrozoan species displaying diverse colonial forms. A) Podocoryne possesses an array of identical polyps connected by stolons. B) Hydractinia has distinct polyp types which bud from an epithelial mat. C) Algalma, a siphonophore colony, superficially resembles a solitary animal, but is composed of various modified polyps and medusae. Modified from Allman, 1872; Blackstone and Buss, 1993; Haeckel, 1888.

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**Fig. 2.** Hydractinia colony ontogeny. A) Fertilization. B) Cleavage. C) Planula larva. D) Primary polyp of Hydractinia, with stolons (st) beginning to elongate over substrate. E) A later stage, showing the development of stolons and polyps arising from elongating stolons. F) A later stage in colony ontogeny, where the basal ectoderm of the polyps has extended over the stolonal nexus to form a stolonal mat (m), and gonozooids (gn) bud from the stolonal mat. Modified from Berking, 1998.

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**Fig. 3.** Polymorphic polyps of Hydractinia. A) gastrozooid, B) gonozooid, C) dactylozooid, and D) tentaculozooid. Modified from Stokes (1974).
determination of these evolutionary innovations, in large part, involves evolutionary changes in the regulation of stolon branching, polyp budding along the axes of stolons, and changes in the regulation of the aboral/oral axes of polyps.

The regulation of the general structural plan of the colony

Complex, highly integrated colonies, such as siphonophores, display a well-defined colony shape with determinate growth. This implies that a colony-wide patterning mechanism exists to regulate colony form. Siphonophore development is tightly regulated and colony form and polyp placement is predictable and occurs along growth zones (Carré and Carré, 1987; Mackie et al., 1987; Pugh, 1999).

In Hydractinia, overall colony form is largely dictated by the distance between stolon branching, the distance between the placement of polyps, and the relative investment of stolonal mat relative to free stolons. In contrast to siphonophores, overall colony form can vary between individual colonies. Colony form has been shown to have a genetic component (e.g., Blackstone and Buss, 1991; Dudgeon and Buss, 1996) and can be experimentally altered by changing aspects of the colony’s gastrovascular architecture and gastrovascular flow (e.g., Blackstone and Buss, 1992, 1993; Blackstone, 1998; Dudgeon and Buss, 1996). These observations suggest the existence of a regulatory mechanism that can respond to variations in gastrovascular flow along the axes of stolons to signal stolon tip formation, polyp buds, and the outgrowth of the stolonal mat.

The division of labor through polyp polymorphism.

It has long been asserted that the division of labor in hydrozoan colonies arose via evolutionary modifications in the axial patterning of the gastrozooid to produce morphologically distinct polyp types (e.g., Agassiz, 1865; Huxley, 1859; Brooks, 1886; Metschnikoff, 1874; Haeckel, 1888). The gonozooid and dactylozooid both lack the oral structures found in the basic polyp (the gastrozooid), suggesting that they represent an expansion of the body column to the exclusion of the oral regions of the gastrozooid. The tentaculozooid resembles a single, polyp sized tentacle of a gastrozooid, suggesting an expansion of a gastrozooid tentacle to the exclusion of the rest of the polyp (Fig. 3). Changes in the regulation of oral/aboral axial patterning in evolution could result in the development of the different polyp morphs.

The physiological integration of the colony.

In Hydractinia the stolonal mat plays an important role in the physiological integration of the colony because its continuous epithelial layer with nerve net aids in the conduction of signals throughout the colony for the behavioral coordination of the polyps (Josephson, 1961; Stokes, 1974). The development of the stolonal mat can be explained, in part, as an extension of the innervated outer ectoderm from the polyp body column over the stolonal network. This implies that the extent of polyp base growth must be regulated along the polyp axis.

Those features associated with increased colonial integration in Hydractinia all involve specification of positional information along the axes of the stolons and the aboral/oral axes of the polyps. Axial patterning information in metazoans has been shown to be under the control of Hox and Hox-like genes. One particular Hox-like gene in Hydractinia appears to be involved in the designation of the developmental axes involved in colonial complexity.

Results and Discussion

The cnidarian ParaHox gene, Cnox-2

One of the best characterized developmental regulatory genes in cnidarians is Cnox-2, which has been identified in several cnidarian species (e.g., Schummer et al., 1992; Naito et al., 1993; Shenk et al., 1993; Kuhn et al., 1996; Finnerty and Martindale, 1997; Schierwater and Kuhn, 1998; Cartwright et al., 1999). Cnox-2 has been classified as an ortholog of the ParaHox gene GSX (Finnerty and Martindale, 1999). ParaHox genes are believed to be the sister group to the Hox genes (Brook et al., 1998). Their function is not as well characterized as the Hox genes, but it has been documented that ParaHox genes are expressed in an anterior-posterior co-linear pattern of expression, characteristic of Hox genes, in chordates (e.g., Hsieh-Li et al., 1995; Li et al., 1996; Brook et al., 1998). In Drosophila, the GSX homolog has a spatially restricted pattern of expression and appears to play a role in dorsoventral neural patterning (Cornell and Ohlen, 2000).

Cnox-2 expression was originally described for the solitary hydrozoan polyp, Hydra, where it was found to be expressed strongly in the body column and only weakly in the oral region (Shenk et al., 1993). In addition, Shenk et al. (1993) found that Cnox-2 expression declines when oral regions are experimentally induced along the body column, suggesting that Cnox-2 may be a negative regulator of the oral region in Hydra.

Cnox-2 expression in Hydractinia

Evidence from Hydra suggests that Cnox-2 is involved in the specification of the oral/aboral axis of the polyp. Since no gene had previously been implicated in colonial development we investigated the expression of Cnox-2 in Hydractinia. Using whole mount immunolocalization, we examined Cnox-2 expression in developing stolons, the stolonal mat, budding and mature gastrozooids, and mature gonozooids, dactylozooids and tentaculozooids.

Cnox-2 expression was investigated in developing gastrozooids as they were budded from stolons. Cnox-2 is expressed at the first detectable indication of polyp bud formation (Fig. 4A), and continues to be expressed at high intensities throughout the epithelia of the elongating polyp bud until well after the aboral/oral axis is fully formed (Fig. 4B). In mature polyps (3–4 days
old), the level of Cnox-2 expression decreases in the oral region, but continues to be expressed at high levels in the polyp body column (Fig. 4C) (Cartwright et al., 1999). The pattern of expression in mature polyps is consistent with that previously described for Hydra (Shenk et al., 1993).

In the gonozooid and dactylozooid, Cnox-2 displays strong, uniform expression throughout the entire length of the polyp, at levels approximately equivalent to that seen in the polyp body column (Fig. 4D–E) (Cartwright et al., 1999). Conversely, the tentaculozooid displays very weak Cnox-2 expression from its basal region to the tip, at approximately the same levels seen in the tentacles of the gastrozooids (Fig. 5F) (Cartwright et al., 1999). The expression of Cnox-2 in the polyp polymorphs is consistent with the differences in aboral/oral patterning between polyp types. This suggests that changes in the regulation of Cnox-2 along the aboral/oral axis may be, in part, responsible for the evolution of polyp polymorphs and the subsequent division of labor found in Hydractinia.

The expression of Cnox-2 in the body column and base of the gastrozooid suggests that this gene may also be involved in stolonal mat specification, since the mat is thought to develop, in part, as an extension of the polyp body column. Cnox-2 is expressed at uniform and high levels throughout the stolonal mat (Fig. 5). The level of expression in the mat is comparable to that found in the polyp body column and base. Cnox-2 expression is continuous from the ectoderm of the polyp base to the upper ectodermal layer of the mat, with no apparent boundary between the base of the polyp and the stolonal mat (Fig. 5) (Cartwright and Buss, 1999). This suggests that expansion of Cnox-2 expression boundaries beyond the base of the polyp may play a role in the evolution and development of the stolonal mat.

At the periphery of the column, where stolonal mat has not developed, Cnox-2 is expressed differentially along the longitudinal axes of the stolons. Cnox-2 expression is only found on the apical surface at the regions where polyps bud (Fig. 4A), at the tips of stolons (Fig. 5) and in regions where stolons branch (not shown). The spatially restricted pattern of expression along the longitudinal axes of the stolons suggests that Cnox-2 is involved in specification of positional information for stolonal growth, branching and polyp budding. These are all features important in shaping Hydractinia colony form.

Conclusions

Trends in colonial complexity share a common theme of increased colonial integration. Evolutionary developmental innovations important for colonial integration have evolved independently in many colonial hydrozoans, and are displayed both in Hydractinia and in more advanced forms of siphonophores. In Hydractinia, these features involve changes in the regulation
of axial patterning along stolons and polyps. The spatially restricted expression of Cnox-2 along the longitudinal axes of stolons is consistent with it having a role in specifying positional information along the stolonal axes. Thus, it may play a key role in the regulation of colony form. Likewise, the continuous pattern of Cnox-2 expression at the polyp base and stolonal mat contrasts with the sharp expression boundary at the region of free stolons from which polyps bud, supporting the suggestion that the stolonal mat represents an expansion of the polyp base to encase the endodermal canals. This implies that Cnox-2 may be involved in the development of the stolonal mat, a structure important for the physiological integration of the colony. In addition, differences in Cnox-2 expression between polyp polymorphs are consistent with their differences in aboral/oral patterning, implicating Cnox-2 in the evolution of polyp polymorphs and the division of labor in *Hydractinia*.

The evolution of complex colonial hydroids has been accompanied by axial patterning changes in both polyps and stolons. The spatially restricted pattern of expression of the ParaHox gene *Cnox-2* along the axes of stolons and polyps suggests that changes in the regulation of *Cnox-2* and other axial patterning genes in evolution may be in large part responsible for the remarkable diversity of forms that characterize colonial hydroids.

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**REFERENCES**


