

Emerging Model Organisms

The Demosponge *Amphimedon queenslandica*: Reconstructing the Ancestral Metazoan Genome and Deciphering the Origin of Animal Multicellularity

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INTRODUCTION

Sponges are one of the earliest branching metazoans. In addition to undergoing complex development and differentiation, they can regenerate via stem cells and can discern self from nonself (“allorecognition”), making them a useful comparative model for a range of metazoan-specific processes. Molecular analyses of these processes have the potential to reveal ancient homologies shared among all living animals and critical genomic innovations that underpin metazoan multicellularity. *Amphimedon queenslandica* (Porifera, Demospongiae, Haplosclerida, Niphatidae) is the first poriferan representative to have its genome sequenced, assembled, and annotated. *Amphimedon* exemplifies many sessile and sedentary marine invertebrates (e.g., corals, ascidians, bryozoans): They disperse during a planktonic larval phase, settle in the vicinity of conspecifics, ward off potential competitors (including incompatible genotypes), and ensure that brooded eggs are fertilized by conspecific sperm. Using genomic and expressed sequence tag (EST) resources from *Amphimedon*, functional genomic approaches can be applied to a wide range of ecological and population genetic processes, including fertilization, dispersal, and colonization dynamics, host-symbiont interactions, and secondary metabolite production. Unlike most other sponges, *Amphimedon* produce hundreds of asynchronously developing embryos and larvae year-round in distinct, easily accessible brood chambers. Embryogenesis gives rise to larvae with at least a dozen cell types that are segregated into three layers and patterned along the body axis. In this article, we describe some of the methods currently available for studying *A. queenslandica*, focusing on the analysis of embryos, larvae, and post-larvae.

RELATED INFORMATION

The Australian Faunal Directory (<http://www.environment.gov.au/cgi-bin/abrs/fauna/tree.pl?pstrVol=PORIFERA&pintMode=1>) is an online version of the Zoological Catalogue of Australia. The Porifera section is compiled and updated by J.N.A. Hooper and F. Wiedenmayer.

BACKGROUND INFORMATION

Sponges (phylum Porifera) are sessile, aquatic (largely marine) animals with external pores connected to a flowthrough system of canals and chambers through which water is pumped to extract food.

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Passage of water through the body is driven by a single layer of specialized flagellated cells called “choanocytes.” Unlike most other metazoans, sponges do not construct true tissues, lack a centralized gut, and do not possess conventional nerves and muscle. Nonetheless, they have a range of other cell types, including a population of totipotent stem cells and skeletogenic cells that form siliceous or calcitic spicules (Hooper and Van Soest 2002). Although many have not yet been described or named, there are an estimated 15,000 sponge species alive today. Their evolution and ecology are associated tightly with a range of microbial symbionts and the ability of these sponge-microbe communities to synthesize and deploy unique bioactive compounds with a variety of ecological roles (Taylor et al. 2007).

A. queenslandica is named for Queensland and was described originally from individuals found at Heron and One Tree Island Reefs in the Capricorn-Bunker Group, southern Great Barrier Reef (GBR) (Hooper and Van Soest 2006). Its discovery off Magnetic Island on the central/northern GBR suggests a pan-GBR distribution. Recent analysis of an *Amphimedon* species collected from Dahab, Egypt revealed identical external characteristics and brood chambers with identically colored and sized embryos and larvae, suggesting a possibly wider distribution, with Red Sea specimens having 93% sequence identity with the *A. queenslandica* 28S rRNA gene. Anecdotal evidence from southern Japan suggests that a species similar to *A. queenslandica*, as well as closely related sister species, lives there, and thus it might be distributed beyond the South Pacific Ocean.

A. queenslandica growths range from thin to thick encrustings over coral or other substrata. The latter form has massive lobate or digitate bulbs arising from the base, measuring no more than several centimeters in diameter. Live sponges are gray-blue to green, with a lighter shade of gray around the rim of the oscules. They have large brood chambers (up to 1 cm in diameter) containing as many as 200 embryos at any time (Leys and Degnan 2001). Hooper and Van Soest (2006) provide a full description of the species and comparison with similar species in the Indo-West Pacific.

A. queenslandica's taxonomic history is checkered by misidentification and misallocation. This is the result of the state of poriferan taxonomy (partly remedied by a phylum synopsis by an international consortium of taxonomists) (Hooper and Van Soest 2002), the difficulty in identifying haplosclerid sponges in general and Niphatidae in particular (see, e.g., Desqueyroux-Faúndez and Valentine 2002), and the limited knowledge of Australian sponge fauna. Indeed, many species that initially appear to be new have been described previously in the largely ancient Australian sponge literature (see, e.g., Hooper and Wiedenmayer 1994; <http://www.environment.gov.au/cgi-bin/abrs/fauna/tree.pl?pstrVol=PORIFERA&pintMode=1>). *A. queenslandica*, for example, was initially identified as a species of *Reniera* (Haplosclerida, Chalinidae) based on a fragment of a single specimen (Leys and Degnan 2001). Its eventual allocation as a new species of *Amphimedon* in the Niphatidae family was confirmed by collection of several more specimens from different habitats within Heron and One Tree Islands.

A. queenslandica was discovered in 1998 on Heron Island Reef by S.P. Leys of B.M. Degnan's group during a survey to identify a sponge species with which to study development. Unlike most sponges, *A. queenslandica* broods embryos in large chambers year-round. Larvae are relatively large (~0.6 mm) and overtly negatively phototactic, moving quickly to the darker side of any collecting vessel. These attributes led to detailed morphological studies of its embryogenesis (Leys and Degnan 2002) and behavioral studies of its photoresponsiveness (Leys and Degnan 2001; Leys et al. 2002). These studies established nondestructive methods in which adult animals are maintained while attached to boulders in corals on the reef flat (i.e., “ranching”), allowing larvae to be harvested from the same adults over time. Methods developed subsequently in the Degnan laboratory include in vitro cultivation of individual late-stage embryos and post-larvae and cultivation of transplanted embryos in situ.

As with most marine invertebrates, *A. queenslandica* has a biphasic life cycle, including a planktonic larval phase and benthic juvenile and adult phases (Degnan and Degnan 2006). Eggs are fertilized internally, and embryos are brooded until they hatch as parenchymella larvae. For the first 4 h after emergence, larvae appear to be unable to settle, and undergo metamorphosis. During this period (up to 24 h post-emergence), larvae are conspicuously negatively phototactic, although this diminishes in older larvae (Leys and Degnan 2002). Negative phototaxis apparently results from the sum of responses to changing light intensity by individual ciliated cells in the posterior ring. Rotating larvae expose some ciliated cells to light, but shade others. Shaded cilia cease beating, causing the animal to swim into darker crevices among the coral boulders on the reef flat. Similar phototaxis has been documented in other parenchymella larvae (for review, see Maldonado 2006), confirming the widespread use of this mechanism.

SOURCES AND HUSBANDRY

A. queenslandica is reasonably common on coral reefs of the southern GBR. They or sibling species have been found as far away as the Red Sea, suggesting that these populations as model organisms are widely available as sources throughout the Indo-Pacific and possibly beyond. They live on the shallow subtidal reef flat and crest, mainly under boulders, in crevices, among coral rubble, in sand patches, and sometimes on hard algal pavement, partially exposed during low tide. Distribution on a given reef tends toward the patchy, often with adults found in areas of low current. In addition to easy accessibility, it is a robust sponge that tolerates being pried off the substratum. *Amphimedon* can be collected from the field and maintained in local aquaria under ambient conditions (i.e., in flowthrough systems). Alternatively, collected *Amphimedon* can be ranched in a local embayment where they can be readily accessed. During collection, care must be taken not to touch the sponge directly or expose it to air at any time. Ranched *Amphimedon* can live for more than 1 year.

Although adult *Amphimedon* can be transported in small volumes of seawater (for a maximum transport time of up to 10 h) and maintained in closed, seawater aquaria (24°C–27°C) for months, there is no evidence that they flourish in captivity. They can be maintained by being fed liquid foods developed for filter-feeding marine invertebrates twice per week. However, the number of viable embryos in captivity appears to diminish with time. Ideally, *Amphimedon* are best studied in a marine research laboratory near the point of collection, with access to high-quality flowing seawater. This ensures that adults, embryos, larvae, and experimentally manipulated cultures are maintained under ambient conditions. Most studies on the southern GBR populations are based at the University of Queensland Heron Island Research Station (<http://www.marine.uq.edu.au/index.html?page=54940>) and the University of Sydney One Tree Island Research Station (<http://www.bio.usyd.edu.au/OTI/>).

RELATED SPECIES

Sponge systematics are undergoing a major revision, based on recent morphological data (Hooper and Van Soest 2002) and a range of studies using molecular data (for review, see Wörheide et al. 2005). Traditionally, sponges were divided into three classes: Demospongiae, Hexactinellida, and Calcarea (Hooper and Van Soest 2002). However, there is debate about the monophyly of phylum Porifera and class Demospongiae (see, e.g., Borchiellini et al. 2004). The order of branching of “basal” metazoan phyla—Porifera, Placozoa, Ctenophora, and Cnidaria—and whether sponges are monophyletic or paraphyletic has a direct impact on the reconstruction of the ancestor from which all living metazoans stem. Undertaking comparative molecular, cellular, and developmental analyses within the Porifera will yield significant insights into the early metazoan genome and its role in the evolution of the first multicellular animals.

Most modern analyses of sponge genomics and development focus on demosponges, with the *A. queenslandica* genome being the only one sequenced to date. Many developmental and structural genes have been isolated and characterized in several demosponges, including *Ephydatia*, *Geodia*, *Spongilla*, and *Suberites* (Segawa et al. 2006; Wiens et al. 2007). Emerging models include the homoscleromorph *Oscarella* (Nichols et al. 2006), the calcareous sponges *Sycon* and *Leucetta* (Manuel and Le Parco 2000), and the hexactinellid *Oopsacas* (Leys et al. 2006). Detailed studies of these species will allow for a more comprehensive view of sponge genomics, evolution, and development, and thus a more mature view of the earliest metazoans.

USE OF THE *A. QUEENSLANDICA* MODEL SYSTEM

Amphimedon is a demosponge and, as such, represents one of the most (if not the most) ancient phyla of multicellular animals alive today. Sponges lack many fundamental metazoan attributes, including true tissues, yet comprehensive molecular phylogenies place them firmly within the Metazoa. They are usually considered to be an example of the earliest extant branching metazoan lineage, although two recent hypotheses have suggested that placozoans or ctenophores could be more basal (Dellaporta et al. 2006; Dunn et al. 2008).

Knowledge of the formation and patterning of cell types via embryogenesis, skeletogenesis, or the cellular and molecular basis of sponge behavior is rudimentary at best. The ancestor from which all extant metazoans stemmed was likely more sophisticated than is widely appreciated, and the morphogenetic tools used by all modern animals evolved well before the Cambrian explosion. A detailed

understanding of these features in sponges will contribute to the reconstruction of the ancestral metazoan genome and elucidate its role in directing the construction and maintenance of the first multicellular animals (Larroux et al. 2006, 2007, 2008a; Fahey et al. 2008).

Amphimedon larval morphogenesis provides a framework within which to address gastrulation and tissue formation in early animals. Embryogenic studies in poriferans have been largely descriptive, but many parallels can be drawn with other sponge larvae, of both parenchymella and other types, from studies on *Amphimedon*. The expression of “polarity” genes during *Amphimedon* larval morphogenesis (Adamska et al. 2007a) suggests that similar mechanisms function more broadly within the Porifera. Expression of a suite of genes involved in post-synaptic signaling (Sakarya et al. 2007) similarly hints at complex signaling systems in sponges.

Amphimedon embryos are large, yolky spheres numbering several hundred to a single chamber. Oogenesis has not been studied, but oocytes presumably arise from amoeboid cells within the maternal tissue, because feeding chambers with choanocytes (an alternative source of gametes in other sponges) are not always adjacent to chambers. Spermatocysts were encountered in only one of every 50 sponges examined in early surveys (Leys and Degnan 2001). Cleavage is irregular because of the yolky cytoplasm; early in development, unequal cleavages begin, and small and large cells (micromeres and macromeres) are produced throughout the embryo. During cellular differentiation, micromeres produce cilia and other features characteristic of differentiated cells (e.g., pigmentation in cells fated to form the pigment ring), whereas macromeres retain lipid and yolk reserves. The two cell types gradually sort, with micromeres localizing at the embryo periphery. Sclerocytes (i.e., skeletogenic cells) differentiate and start to form incipient spicules early in development. At the same time, a subpopulation of cells with dark pigment granules begins to migrate toward the future posterior pole. *Wnt* gene expression in this region preempts pigment cell migration (Adamska et al. 2007a). Cellular differentiation and sorting continue as the pigment cells form first a dense spot at the pole and subsequently tighten into a discrete ring of pigment that designates the larval posterior pole. Sclerocytes, with fully differentiated spicules secreted intracellularly, aggregate toward the posterior pole with spicules largely oriented in an anterior-posterior direction. The fully differentiated larva has distinct anterior-posterior polarity with a ciliated columnar epithelium, but it also has bare anterior and posterior poles, spicules in sclerocytes at the posterior pole, and a ring of pigment adjacent and posterior to a ring of cells with very long cilia. Interspersed among the columnar epithelial-like cells are globular cells (formerly called “mucous cells”) and flask cells. Current evidence suggests that globular cells express genes found in eumetazoan neurons and they have a sensory role (Richards et al. 2008).

Because *Amphimedon* adults produce many embryos year-round, parenchymella larvae can be harvested continually. In addition, the larvae are large and easy to study, both for morphology and behavior. *Amphimedon* development and developmental gene structure and expression have been studied extensively (Leys and Degnan 2001, 2002; Degnan et al. 2005; Larroux et al. 2006, 2007, 2008a; Adamska et al. 2007a,b; Sakarya et al. 2007; Fahey et al. 2008; Gauthier and Degnan 2008a,b). Excellent recent reviews on sponge embryos and larvae are also available (Leys and Ereskovsky 2006; Maldonado 2006).

GENETICS, GENOMICS, AND ASSOCIATED RESOURCES

A. queenslandica is not amenable to classical genetic studies and currently has no available genetic markers, maps, or mutants. Given the inability to culture this species, it is unlikely that such resources will become available in the near future. All genes isolated and characterized in *A. queenslandica* are given a name with the prefix “*Amq*” followed by a descriptor based on its metazoan/eukaryotic gene family. Ideally, names are based on orthology to known eumetazoan genes, for example, *AmqNF-κB* (Gauthier and Degnan 2008a) or *AmqSix1/2* (Larroux et al. 2008a). Sequence divergence often makes such precise naming difficult. In such cases, a more generic name is applied (e.g., *AmqbHLH1*) (Simionato et al. 2007; Richards et al. 2008). The *Amphimedon* genome also contains genes with novel domain architectures. Names are created for these genes that reflect their relation to known eumetazoan genes. For example, *Amq-hedgling* encodes a large transmembrane protein with the Hedgehog amino-terminal signaling domain (Adamska et al. 2007b). Novel genes are named based on contig number and location; these may be revised in the future if homologs are identified.

The *A. queenslandica* genome has been sequenced by the United States Department of Energy Joint Genome Institute (<http://www.jgi.doe.gov/>). Genomic resources currently include genomic traces (~14-fold coverage) and more than 75,000 ESTs. These sequences are available in the National

Center for Biotechnology Information Trace archive (<http://www.ncbi.nlm.nih.gov/Traces/trace.cgi?>) and can be retrieved by searching with species_code = "RENIERA SP. JGI-2005" (reflecting the incorrect original designation of *Amphimedon* as *Reniera* sp.). Release of the annotated draft assembly by the Joint Genome Institute is scheduled for late 2008.

Biological material (fixed specimens, RNA, cDNA, genomic DNA, etc.) can generally be obtained by direct collection from field sites or by contacting the corresponding investigator. Natural populations are managed sustainably, and collections of adults might be restricted periodically. Permits are required to collect animals from the GBR.

TECHNICAL APPROACHES

Protocols are available that describe methods to obtain *Amphimedon* tissues at various stages of development (with particular emphasis on embryonic, larval, and juvenile samples; see **Isolation of *Amphimedon* Developmental Material** [Leys et al. 2008]) and to process them for studies such as in situ hybridization (**Whole-Mount In Situ Hybridization in *Amphimedon*** [Larroux et al. 2008b]) and genotyping (**Genotyping Individual *Amphimedon* Embryos, Larvae, and Adults** [Degnan et al. 2008]). Methods for microinjection of individual embryos to study cell migration during development are described in **Analysis of Cell Movement in *Amphimedon* Embryos by Injection of Fluorescent Tracers** (Adamska and Degnan 2008).

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