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Review

Molecular mechanisms of allorecognition in a basal chordate

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ABSTRACT

Allorecognition has been described in many metazoan phyla, from the sponges to the mammals. In vertebrates, allorecognition is a result of a MHC-based recognition event central to adaptive immunity. However, the origin of the adaptive immune system and the potential relationship to more primitive allorecognition systems is unclear. The colonial ascidian, *Botryllus schlosseri*, has been used as a model organism for the study of allorecognition for over a century, as it undergoes a natural transplantation reaction controlled by a single, highly polymorphic locus. Herein we will summarize our current understanding of the molecular mechanisms that underlie this innate allorecognition reaction.

The ability to discriminate self from non-self is a process found throughout the metazoa and is the basis of immune function. In the vertebrates, allorecognition is carried out mainly via the adaptive immune system, which is characterized by its capacity for somatic recombination of germ-line encoded immune receptors coupled to recognition of highly polymorphic MHC proteins. As one of the most complex processes that occur within the vertebrate body, it has been thought that the origins of the adaptive immune system may reside within the more primitive organisms. In fact, highly polymorphic allorecognition systems have been discovered across a wide evolutionary spectra of taxa, including sponges, cnidarians, echinoderms, tunicates and the jawless fishes [1], suggesting the presence of complex and discriminatory recognition systems in non-vertebrate organisms. However, to date the ligands and receptors used within these systems are unrelated to those in the adaptive immunity, [2–4], thus the evolutionary precursor to the adaptive immune system remains elusive.

The colonial ascidian, *Botryllus schlosseri*, belongs to the chordate subphylum, *Tunicata*, and occupies a key phylogenetic position in vertebrate evolution. *Botryllus* has also been used as model organism for the study of allorecognition for over a century, as it undergoes a natural transplantation reaction controlled by a single, highly polymorphic locus (the *fuhc*), semantically similar to the MHC [5–10]. The allorecognition reaction in B. schlosseri is highly specific and capable of discriminating between 100 s of naturally occurring allelic variants, but recent molecular evidence has shown that the *fuhc* is not the ancestral MHC [11,13]. Nevertheless, as the closest living invertebrate relatives to the vertebrate line [12,14], allorecognition in the ascidians represents a basal form of self/non-self recognition, and properties of this system may be the building blocks for the evolution of chordate immunity. We are just beginning to dissect and understand the underlying genetic architecture of this exquisitely specific allorecognition system, which evolved nearly 500 million years before the emergence of adaptive immunity. Here we will summarize our current understanding of the molecular mechanisms which underlie this innate allorecognition reaction.

1. Biology of *B. schlosseri*

The life history of *B. schlosseri* cycles between two distinct body plans, beginning with a sexually derived, free-swimming chordate tadpole larvae which hatches, swims away from its parents and searches for a suitable substratum, then settles, usually within 12–24 h. The larva then undergoes metamorphosis, resorbing most of its chordate characteristics and transforming to an asexually propagating invertebrate bauplan, called an oozoid [13]. The oozoid immediately begins a budding process called blastogenesis, eventually giving rise to a colony of genetically identical individuals (zooids) encased in a common tunic and sharing a common blood supply [14]. Each individual is autonomous, possessing a gut, heart, gonads, etc., and can be manually dissected from the colony and transferred to a new substratum, where it will continue to bud. Thus multiple naïve *subclones* can be generated for any particular genotype. A connecting, extracorporeal vasculature permeates throughout the entire colony and terminates at structures called ampullae, which are oval-shaped protrusions found throughout the periphery of the tunic. Functionally, ampullae adhere the animal to the substratum and are also the location of the allorecognition phenomenon we study.

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2. Allorecognition phenotype in *B. schlosseri*

B. schlosseri are typically found on man-made structures in shallow subtidal environments around the world, and competition for space in these habitats can be fierce. Since the colonies are continuously undergoing asexual reproduction and expanding outwards, they often come into physical contact with other *Botryllus* colonies. The first part of the colonies to touch each other are the ampullae, and two outcomes can result from this interaction: either the two ampullae will initiate a blood-based inflammatory rejection reaction causing the two colonies to no longer interact, or they will undergo vascular reorganization, fusing to form a chimeric colony which shares a common blood supply (Fig. 1) [6,7,15].

The reaction begins when the ampullae of two adjacent colonies come into physical contact. The initial contact is typically tip to side, where the ampullar tip of one colony penetrates through the tunic matrix and touches the side of the ampullae on the adjacent individual. Following this, a characteristic set of morphological changes occur which eventually lead to fusion or rejection.

A rejection response is an active, blood-based inflammatory reaction that begins with the migration of a refractive cell called a morula [16] into the tips of the ampullae, causing swelling and the retreat of pigmented blood cells. Next, the epithelia of the ampullae lose their integrity and the morula cells leak into the periphery where they discharge their vacuoles, initiating a prophenoloxidase cascade which eventually forms dark melanin scars called points of rejection (PORs; Fig. 1b, red arrows). The formation of PORs prevents any further blood exchange between the two colonies and often causes the ampullae to disintegrate and the colonies to no longer interact. In contrast, if two colonies are set to fuse, the tip of the ampullae rapidly penetrate through the tunic and come into contact with base of the peripheral blood vessel. The pigmented cells retreat and the ampullae fill with an unknown population of cells, which causes the ampullae to swell and pulse and eventually adjoin with the neighboring blood vessel (Fig. 1b, black arrow).

From contact to a fusion or rejection reactions typically takes 24–48 h and is limited to the ampullae that are interacting. As shown in Fig. 1b, a colony can simultaneously fuse and reject. The two colonies positioned on the right (labeled b1 and b2) are fusing, genetically identical subclones that are simultaneously rejecting with the colony on the left (labeled a). At the interface between colonies b1 and b2, the ampullae have migrated much further into the neighboring colony than the interface between the rejecting pairs. This observation suggests that allorecognition is due to spatially restricted interactions between juxtaposed ampullae and is not globally activated within the colony.

3. The self-ligand: fuhc

Fusion or rejection is governed by a single, highly polymorphic locus called the *fuhc* [11,17,18]. Several criteria were put forth during the identification of the *fuhc*, the first being that the candidate must be highly polymorphic. Next, these polymorphisms must correlate to fusion and rejection outcomes in both wild-type and lab-reared colonies with fusing individuals sharing at least on *fuhc* allele and rejecting colonies none. Finally, the segregation of these polymorphisms in the crosses must also correlate 100% with histocompatibility outcomes. Genetic mapping of the *fuhc* locus in five independent crosses identified several candidate histocompatibility ligands, however only one satisfied all the genetic criteria to be the putative *fuhc* [13].

This candidate *fuhc* gene encodes a large (1008 aa) type I transmembrane protein containing a signal sequence, two and potentially three extracellular Ig domains and two EGF domains of about 750aa, followed by a TM domain and an intracellular tail which had no known signaling or other domains (Fig. 2). Besides the full-length form, we identified two alternative splice variants. One removed the last half of the protein and added three new exons creating a putative secreted protein that consisted of about 1/2 the ectodomain. Another added a new exon within the tail. This was seen in about 5% of the TM containing clones but added no known domains. There is no clear homolog covering the length of this protein in any other genome, including three related ascidian genomes that have been sequenced (although they are all in different Orders). However, homology is seen with the vertebrates over the Ig domains by sequence similarity (similar to the protein IgSF4). 3D predictions of the Ig domain followed by a structural comparison done by PSSM revealed that the predicted Ig domains strongly resembled those of the poliovirus receptor, CD155. Finally, SMART searches reveal that this domain architecture (extracellular Ig and EGF domains) are only on the proteins Tie-1 and Tie-2 in the vertebrates, although the *fuhc* does not have fibronectin domains also found on these genes [13]. Wu-Kabat analysis of the distribution of polymorphisms revealed that the majority of alleles were different by about 25–50 amino acids spread throughout the ectodomain. At this point, we have not identified any hypervariable regions, and this result lends some insight into how *fuhc* polymorphism might be interpreted by its effector system (discussed below). In addition, results of *in situ* hybridization of the *fuhc* found that this transcript is expressed on ampullae in both adults and juveniles and at the anterior portion of the tadpole. Thus, the *fuhc* is found on all tissues known to be intimately involved in the allorecognition response. The expression profile in combination with the high degree of polymorphism and the 100% correlation with fusion rejection outcomes,

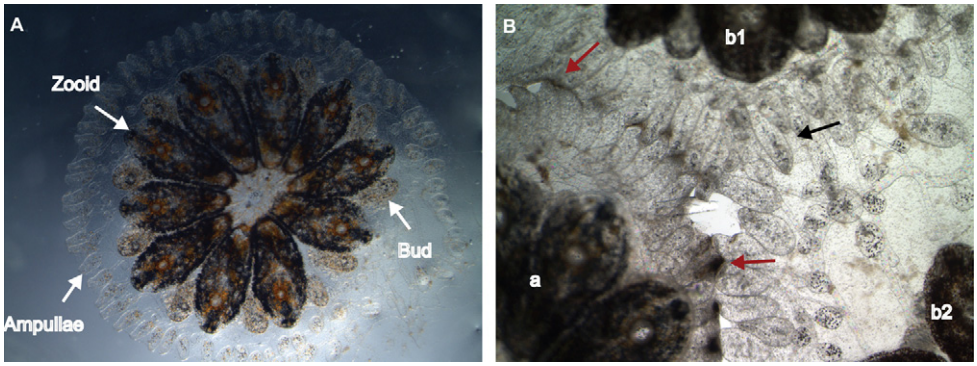

Fig. 1. (A) A dorsal view of a *Botryllus schlosseri* colony as described in the text. Adult individuals (zooids), the asexually developing progeny (buds) and the structures involved in allorecognition (ampullae) are highlighted. (B) An allorecognition reaction occurring between three distinct colonies (a, b1 and b2). Colonies b1 and b2 are genetically identical subclones that are undergoing vascular reorganization (black arrow) which will eventually lead to a fusion event. Simultaneously, both colonies b1 and b2 are rejecting colony a, and have developed several points of rejection (red arrows) which prevent blood transfer.