Histocompatibility: Clarifying Fusion Confusion

In the colonial tunicate *Botryllus schlosseri*, a co-dominant trait determines the capacity of adjacent colonies to fuse or reject. An innovative RNA sequencing approach has now identified the gene that predicts the outcomes of this naturally occurring allograft.

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Transplantation immunology has its foundations in over a half century of intensive laboratory investigations that initially were based on bovine dizygotic twins [1] and later inbred and congenic strains of mice. Transplantation of human organs, bone marrow and specific cell populations [2] as well as pregnancy (the most common histocompatibility–incompatibility scenario) [3,4] have further expanded our understanding of the genetic basis for allorecognition, defined as the ability of an organism to distinguish its own tissues from those of another of the same species. Whether or not a graft is accepted or rejected depends in jawed vertebrates on highly polymorphic and broadly expressed genes encoded within the major histocompatibility system, the primary function of which is to respond to viral and bacterial antigens [5]; the outcomes of transplantation are also dependent on minor histocompatibility antigens. Allograft responses are not unique to vertebrates and have been characterized in several other systems, including the sessile tunicate *Botryllus schlosseri*, a distant relative of vertebrates. A recent study by Voskoboynik *et al.* [6] identifies the gene governing allorecognition in *Botryllus*, thereby clarifying much about the molecular basis of allorecognition in this system.

The evolutionary origins of histocompatibility have long interested immunologists [7], but like tracking the origins of immunoglobulin-mediated antibody response of the adaptive immune system [8], the trail runs cold at the time of the divergence of the jawed vertebrates. However, a histocompatibility reaction that at face value bears a striking resemblance to graft acceptance and rejection phenomena characteristic of tissue transplantation in mammals has been described in basal chordates [9,10]. Histocompatibility in colonial tunicates is a naturally occurring phenomenon that involves fusion or rejection of adjacent animals (Figure 1). This process protects against stem cell parasitism (stem cells of one genotypic background overtaking the colonial tissue of the adjacent animal), which could severely compromise population diversity and thereby the organism's capacity for adaptation or survival [11,12]. From a genetic perspective, histocompatibility in these colonial organisms behaves as a co-dominant autosomal trait. If two distinct colonies share one or both 'histocompatibility' alleles, the colonies fuse leading to the sharing of their blood supply; if colonies do not share alleles, they reject fusion through the formation of inflammatory lesions.

Over three decades, the senior author, Irv Weissman, his students and other collaborators have developed the colonial tunicate *Botryllus schlosseri* into a well-recognized model of genetically predictable histocompatibility [13–16]. In previous studies, it was shown that the genetic segregation of a highly polymorphic candidate fusibility histocompatibility gene (cFuHC) correlates strongly but not perfectly with transplantation outcomes [14]. cFuHC encodes both membrane-bound and secreted forms, which recently were shown to be encoded by independent gene loci separated by ~250 base pairs [17]. In a system, where direct approaches to ascertain gene function are not readily applicable, the lingering question has been whether or not cFuHC encodes a true determinant of allorejection or whether the actual determinant is a closely linked locus. In response to a recent report that cast doubt on the role of cFuHC in allorecognition [18], definitive studies have been carried out and it now seems that the latter possibility is the case.

The elegance of the new work reported by Voskoboynik *et al.* [6] lies in the use of high-throughput, deep coverage RNA sequencing to approach the identity of the histocompatibility determinant from a functional perspective, which is neither based on nor biased by earlier work [6]. Phased, paired-end RNA sequencing analysis of both laboratory-reared and wild-type colonies of *Botryllus* was used to define haplotypes in such a manner whereby computational approaches could be used to determine whether a particular transcript stratifies a known fusion outcome. In other words, extraordinarily large numbers of expressed sequences were queried to determine whether or not observed sequence polymorphisms correlated with transplantation outcomes. In every instance of colony fusion, no sequence differences could be identified [6]. Candidate sequences also were entirely reliable in predicting outcomes of wild-type pairings with laboratory strains. The interpretation of the earlier results [14] was compromised by an incomplete sequence across the histocompatibility determinant region. Using the recently completed sequence of the *Botryllus* genome [19], the authors show that the cFuHC locus is located ~62,000 base pairs from the new gene reported here. Finally, it seems that the molecular basis of urochordate histocompatibility is at hand.

Over a half century following the discovery that colonial histocompatibility in *Botryllus* is determined genetically, we now know that it is mediated by *Botryllus* histocompatibility factor (BHF), a three exon protein, encoding 252 residues, that exhibits significant relatedness only to genes found in other tunicates [6]. The outcomes of transplantation can be predicted from sequences in the amino-terminal portion of BHF. In addition to the absolute genetic predictability of BHF as the

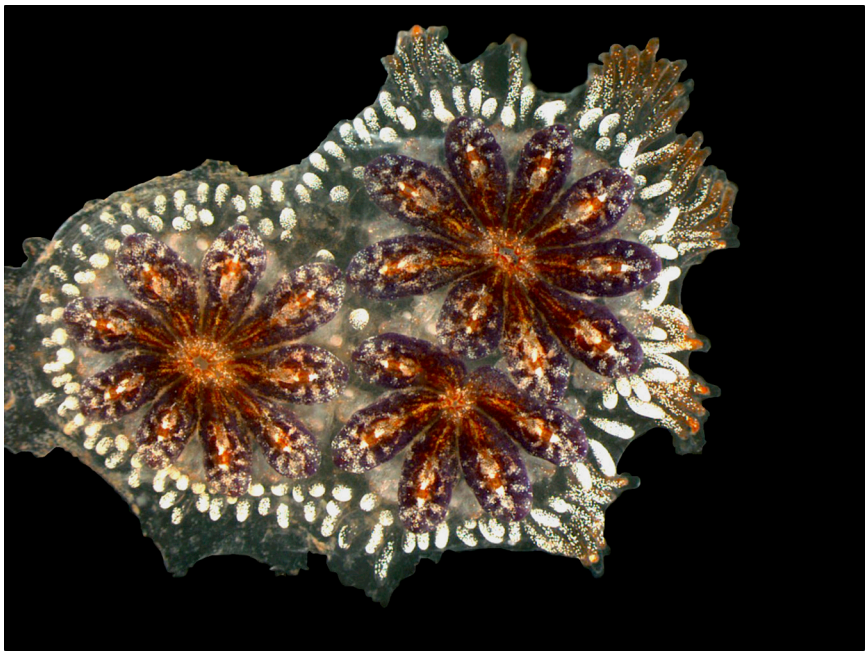

Figure 1. Colonial fusion in *Botryllus schlosseri*.

The fusion or rejection of adjacent colonies of *Botryllus schlosseri* represents a naturally occurring allograft recognition reaction. The fusion reaction shown here is between genetically identical colonies and includes the formation of a common tunic. All colonies sharing one co-dominant histocompatibility allele undergo fusion. Those pairs lacking an allele in common exhibit rejection. The work described here identifies highly polymorphic BHF (*Botryllus* histocompatiblity factor) as the gene governing this physiologically significant reaction. (Image: Michelle M. Osovitz.)

histocompatibility determinant, its patterns of expression during experimental transplantation generally are consistent with the sites of colony fusion or rejection. The rejection reaction can be inhibited with translation-blocking morpholinos based on BHF. Given the relationship between the polymorphic MHC and functions of the jawed vertebrate adaptive immune system, the lack of relatedness of BHF to the primary determinants of allorecognition in jawed vertebrates comes as no surprise. Interestingly, BHF also bears no relatedness to the determinants of histocompatibility in *Hydractinia* [20], a cnidarian, suggesting that this form of self–nonself recognition has multiple independent origins.

Although the lack of relatedness of BHF to previously described transplantation determinants may disappoint those focusing on transplantation in humans, what would the findings of a molecular homolog, for example an MHC-like molecule, really tell us? It is far more significant that the naturally occurring transplantation setting that confronts *Botryllus* sp. is mediated by a unique class of molecule, one that lacks the structural features of a cell surface-recognition protein. Future investigations of BHF function have the potential to afford us far more important information about the underlying commonality of integrated networks and signaling processes involved in self–nonself recognition as well as what most certainly represents an important mechanism for preserving population diversity.

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