The molecular basis of allorecognition in ascidians

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Summary
The process of allorecognition consists of an ability to discriminate self from non-self. This discrimination is used either to identify non-self cells and reject them (“non-self histocompatibility”) or to identify self cells and reject them (as in the avoidance of self-fertilization by hermaphrodites (“self incompatibility”)). The molecular basis governing these two distinct systems has been studied recently in hermaphroditic ascidian urochordates. Harada et al. postulated two highly polymorphic self-incompatibility loci, Themis (A and B), that are transcribed from both strands, forward to yield sperm (∆) trans-membrane antigen, and reverse to yield the egg vitelline coat (ν) receptor. De Tomaso et al. characterized a candidate histocompatibility locus, encoding a highly variable immunoglobulin. Nyholm et al. isolated its candidate allorecognition receptor, fester. Only a minute similarity was found in the structure of the genes involved. It appears that ascidian harbor two very separate types of labeling and recognition genetic systems: one for self and the other for non-self.

Introduction
Allorecognition is the ability of a multicellular organism to distinguish between self and alloantigens carried by foreign cells. The components of allorecognition consist of a non-self (pathogen) negative detection and response system. A related and not less significant process is the self negative response detection ability. For example, some hermaphroditic plants and animals have a self-incompatibility (SI) capacity preventing self fertilization. The SI system is based on self recognition rather than recognition of nonself. The underlying basis for identification of and response to self/nonself is currently a major topic in immunology.

Any molecular self/non-self identification scheme is required to integrate: (i) labeling molecules (antigens), (ii) recognizing molecules (receptors) and (iii) reaction pathway (either negative or positive response). The identification capability requires high specificity and, thus, if coded genetically, it should consist of highly variable genetic systems, resulting in the production of considerable allelic diversity. Such a hypervariable allelic assortment would probably be the outcome of high mutation and recombination rates at these loci. The recognition system should select and classify this genetic array as self or non-self. Consequently, in case of high mutation rates, the recognition system should instantly be reorganized to identify new mutations (alleles). Any recognition errors should be subjected to intense selection pressure. Such a frequent novel action and reaction (i.e. mutation and recognition) necessitates close association between different genetic traits.

The molecular basis governing both types of allorecognition, self incompatibility and non-self negative response, has been studied recently in several species of ascidian urochordates. The subphylum Urochordata represents invertebrate chordates, considered as the ancestor of vertebrates, and thus may present an insight to the evolution of the vertebrate immune response.

The Asciidiacea urochordata
The invertebrate group that is most closely related to vertebrates is the tunicates. The class Asciidiacea of the phylum Chordata (Tunicata) is considered to be related to higher chordates mainly because of the tadpolelike larvae that possess a notochord in the tail and a tubular dorsal nerve cord. Among tunicates the class Asciidiacea consists of two different types of ascidian: (i) the large solitary type that aggregates in a gelatinous colony and (ii) the compound type that aggregates in a gelatinous colony. In both forms, the adults are sessile, while the larval stage is dispersive as free swimming tadpole. Ascidians are hermaphrodites; hence, they produce both male and female gametes. Self-fertilization is generally prevented through sperm–ova incompatibility or by different maturation times for male and female gametes.

Self-incompatibility in ascidian
The male and female gametes of hermaphroditic organisms are produced through meiosis. Self fertilization (siling) can occur when the production of gametes overlaps at least partly in time. The genetic consequence of selfing are an increase in the likelihood of inbreeding depression (i.e. reduction in fitness accompanied inbreeding), and a reduction in level of heterozygosity. Therefore processes preventing inbreeding and promoting outcrossing would have a selective advantage.
Outcrossing in this case is based on the ability of a species to distinguish between self and nonself gametes and to single out genetic relatives.

The solitary ascidian *Ciona intestinalis* possesses a well-developed SI system. The male SI factor(s) is probably expressed on the sperm surface. The female discrimination site, i.e. the primary sperm receptors, is located in the vitelline coat (VC) — the matrix surrounding the egg. Self sterility probably results from the failure of the sperm to bind to the VC of the autologous eggs. However, self-sterility is not absolute and some are self-fertile.

Attempts to determine the rules governing self-incompatibility in *C. intestinalis* were initiated in the 1930s by T.H. Morgan who conducted various selfing and sibling crosses experiments. Morgan noticed two types of cross-sterility: a bidirectional cross-sterility (both reciprocal crosses were sterile), and one way cross-sterility (the egg is fertilized by the sperm in one cross, but the reciprocal cross is sterile). Morgan suggested the “haploid-sperm hypothesis” to explain the phenomenon of one-way cross-sterility. He suggested that distinctiveness is determined by haploid expression in the sperm and diploid expression in the egg. Thus, homoyzogotes show one type of sperm, while heterozygotes produce different types. Sperm cannot fertilize eggs that share one allele with them. For example: the cross of $A_1A_1 \times A_2A_2$. In the case of homoyzogote egg ($A_1A_1$) crossed by heterozygote originated sperm ($A_1A_2$), the egg is sterile to $A_1$ sperm but is fertile to $A_2$. In reciprocal cross, the heterozygote egg ($A_1A_2$) is sterile to both type of sperm ($A_1$ or $A_2$). From the results of the different crossing, Morgan concluded that at least four independent loci code for incompatibility.

Subsequently, considerable detail about the possible location of antigens and receptors has been collected. Natural populations of *C. intestinalis* were found to produce highly genetically diverse gametes, and cross-sterile combinations are rarely established in any wild populations. Nonetheless, the specific loci governed the inheritance of SI trait have not been found by classical genetic analyses. However *Ciona* is one of the model organisms for which the genome has been sequenced; hence, recently, Harada et al. attempted to identify the SI loci by finding linked markers and by tracing synteny via chromosome walking and positional cloning.

Harada et al. repeated Morgan’s sibling crosses and self-fertilization experiments, in matrix of 24 pairwise fertility/sterility outcomes (576 combinations). The outcome of these crosses revealed six distinct clusters of cross-sterility alternatives (see Figs 1c and S1 in Harada et al., 2008). Within each group, gametes were reciprocally sterile, but bidirectional, and one-way cross-sterility outcomes varied between groups. The minimum number of loci that can explain these six clusters under the assumption of “haploid-sperm hypothesis” is two polymorphic SI loci. Harada et al. than found two genetic markers that segregated in similar manner, one on chromosome 2q and the other on 7q. Fine mapping narrowed the search regions and, among the transcription units situated in these regions of the chromosomes, they suggested two *polycystin*-T-like (*PDKT*) genes that code for transmembrane receptors, as the putative SI genes. The focus on these two genes comes from the fact that signaling molecules on the sperm may involve transmembrane receptors and an association between the *polycystin* gene family and fertilization has been previously suggested for other species like the sea urchin and *Drosophila* (for review see refs 20,21).

Harada et al. called these putative SI loci *Themis* (A and B), after the Greek Goddess of divine law and order. The molecular specificity of these loci is intriguing. *Themis*-A codes for five transmembrane domains and *Themis*-B codes for 11. Each of the two loci is transcribed from both strands, forward (+) to yield sperm (s-) trans-membrane antigen; and reverse (−) to yield the egg VC (ν-) receptor. All loci and reading frames include hyper-variable region, as is theoretically expected for SI loci. The putative s-*Themis* transcripts code for *PDKT*-like (*polycystin*) trans-membrane receptors. The putative ν-*Themis* transcripts code for a fibrinogen-like ligand.

Fibrinogen domain has been found to be associated with innate immunology and pathogen intolerance. A diverse family of fibrinogen-related proteins consisting of amino-terminal immunoglobulin domain and C-terminal fibrinogen domain were found in mollusks. Fibrinogen-related proteins have also been thought to be associated with either induced or repressed response to parasitic infection in several species of insects (summarized in Ref. 23). High levels of sequence diversity suggested their involvement in internal defense and invertebrate non-self recognition. Fibrinogen-related proteins have also been associated with pathogen recognition in vertebrates like catfish and mammals.

Fibrinogen domains are thus implicated in two contrasting allorecognition systems: (1) identification and rejection of self in the SI systems of ascidians as suggested by Hamada et al.; and (2) detection and elimination of non-self in the innate immune system as described above. Both processes have an intolerance outcome. It is also possible, therefore, that the loci having fibrinogen domains are involved in the second phase of incompatibility, the intolerance process, rather than the recognition per se. Clearly, considerably more study is required before this process is fully understood. However, an indication of the specific process involved may come from previously reported putative loci involved in non-self-incompatibility in the colonial ascidian *Botryllus schlosseri*.

**Non-self-incompatibility**

The most-investigated non-self-incompatibility system is the major histocompatibility complex (MHC) multigene family of vertebrates, which is involved in T-cell self tolerance and allorecognition. This adaptive immune system of the
MHC is involved in the development of immune responses against pathogens. There is no evidence for the existence of MHC orthologs in older, pre-vertebrate species. Genomewide sequence analysis of C. intestinalis has not revealed any of the pivotal orthologs of adaptive immunity. Thus, understanding the evolution of pre-MHC molecules remains speculative. Recent findings, however, suggest a possibility of non-self recognition molecules and adaptive immunity also in invertebrates and plants.

**Non-self-incompatibility in ascidian**

In nature, the colonial ascidian *B. schlosseri* settle in aggregations of kin. When two *B. schlosseri* colonies come into contact, the result may be either development of an inflammatory reaction (colony rejection), or, alternatively, natural tissue transplantsations. In the latter process, a single entity is formed through peripheral blood vessels (fusions and chimera formation: Refs 36–38). This allorecognition is controlled by a single, highly polymorphic fusibility locus, termed Fu/HC (fusibility/histocompatibility: Refs 39, 40).

Recently, De Tomaso et al. isolated and characterized a candidate histocompatibility locus, cFu/HC, encoding a putative highly variable immunoglobulin. The group of Weissman also isolated its candidate allorecognition receptor, *fester*, which was found to be chromosomally linked to Fu/HC. Theoretical considerations, supported by molecular analyses, suggest that the Fu/HC locus is a ligand accounting for both stimulatory and inhibitory responses. *fester* did not show any recognizable intracellular signaling domain(s) but may include domains that have specificity for signaling molecules.

Taking the finding of the putative molecular component of allorecognition in ascidian collectively, to date, there is no one mechanism that can comprehensively describe either self or non-self allorecognition or incompatibility in ascidians. A comparison of the assumed molecular basis of the two traits (Table 1), shows only minute similarity in the structure of the genes involved and the candidate proteins. Thus, from what is known to date, it seems that ascidians harbor two very separate types of labeling and recognition genetic systems: one for self and the other for non-self. Nonetheless, both systems comprise signaling molecules that are tightly linked to their recognition molecules. *B. schlosseri* may show both types of allorecognition since, in addition to the FuHC system described above, it is hermaphrodite and may also harbor the SI system. Thus, looking for *Themis* orthologues in *B. schlosseri* should be a logical next step.

All loci involved in both types of allorecognition have been found to be highly variable. The ability to distinguish between self and non-self in both SI and FuHC systems is based on high levels of genetic diversity on the one hand and extraordinary precision in recognition on the other. Hence, both labeling and recognition are part of a co-evolving and highly mutable system. Studying the molecular basis and the natural selection pressure operating to mold this evolutionary important process will continue to be a challenging endeavor.

**Table 1.** Comparison between self incompatibility trait in *Ciona intestinalis* and non-self histocompatibility in *Botryllus schlosseri*

<table>
<thead>
<tr>
<th>Molecular specifications</th>
<th><em>Ciona intestinalis</em></th>
<th><em>Botryllus schlosseri</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Labeling ligand</strong></td>
<td>Two genes: <em>s-Themis</em> (A,B)</td>
<td>FuHC</td>
</tr>
<tr>
<td><strong>Type of protein</strong></td>
<td>Transmembrane protein</td>
<td>Immunoglobulin</td>
</tr>
<tr>
<td><strong>Recognition receptor</strong></td>
<td>Two genes: <em>v-Themis</em> (A,B)</td>
<td>Transmembrane protein</td>
</tr>
<tr>
<td><strong>Type of protein</strong></td>
<td>Fibrinogen-like domain</td>
<td>Also suggested alternatively spliced secreted forms</td>
</tr>
<tr>
<td><strong>Association between genes</strong></td>
<td>Transcribed from both strands of the same loci</td>
<td><em>fester</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Putative extracellular domain, intracellular tail and three predicted transmembrane domains</td>
</tr>
</tbody>
</table>

**References**


