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Review

Self/non-self recognition mechanisms in sexual reproduction: New insight into the self-incompatibility system shared by flowering plants and hermaphroditic animals

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ABSTRACT

Sexual reproduction is an essential process for generating a genetic variety in the next generation. However, most flowering plants and hermaphroditic animals potentially allow self-fertilization. Approximately 60% of angiosperms possess a self-incompatibility (SI) system to avoid inbreeding. The SI system functions at a process of interaction between pollen (or pollen tube) and the pistil. These SI-responsible factors (*S*-determinants) in pollen and the pistil are encoded by highly polymorphic multiallelic genes in the *S*-locus, which are tightly linked making a single haplotype. Different taxonomic families utilize different types of *S*-determinant proteins.

In contrast to the plant system, the mechanisms of SI in simultaneously hermaphroditic animals are largely unknown. Among them, promising candidates for SI in ascidians (primitive chordates) were recently identified. The SI system in the ascidian *Ciona intestinalis* was found to be very similar to those in flowering plants: The products of sperm- and egg-side multiallelic SI genes, which are tight linked and highly polymorphic, appear to be responsible for the SI system as revealed by genetic analysis. These findings led us to speculate that the SI systems in plants and animals evolved in a manner of convergent evolution. Here, we review the current understanding of the molecular mechanisms of the SI system in flowering plants, particularly Brassicacea, and in ascidians from the viewpoint of common mechanisms shared by plants and animals.

Contents

Abbreviations: SI, self-incompatibility; VC, vitelline coat.

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1. Introduction

Most living organisms undergo sexual reproduction as a reproductive strategy. This enables them to make a genetic variety in the next generation. In contrast to most animals, flowering plants and hermaphroditic marine animals releasing gametes may allow self-fertilization under natural conditions. In order to avoid inbreeding, many flowering plants possess a self-incompatibility (SI) system, which eventually prevents self-fertilization. There are several SI systems to block self-fertilization depending on the taxonomic family [1–3]. In this review, the SI systems of flowering plants, particularly that in Brassicaceae, are described in detail.

Most terrestrial and aquatic hermaphroditic animals exhibit reproductive behaviors. Therefore, a self-gamete recognition system is not necessarily required to avoid inbreeding. However, in hermaphroditic marine animals that release sperm and eggs to the surrounding seawater, a self/non-self-recognition system between sperm and eggs (or the egg investments) is indispensable to avoid self-fertilization. There are several gamete proteins involved in species specificity and in self/non-self-recognition (or SI) during fertilization. The molecular mechanism of SI, which is also referred to as self-sterility, was a long-standing enigma in animals. However, a recent discovery in the ascidian SI system provided a new insight: the SI system in *Ciona intestinalis* appears to be very similar to those in flowering plants [4–6]. This discovery was surprising, since it had long been believed that the SI system in ascidians might be similar to that of adaptive immunity. Before describing the SI system in ascidians and other animals, we will summarize the current understanding in flowering plants.

2. Self-incompatibility in flowering plants

A SI system in angiosperms has been found in more than 250 of the 600 genera, which occupy about 60% of angiosperms [7]. In many angiosperms, self/non-self recognition of SI is controlled by pollen-*S* determinant and pistil-*S* determinant encoded at the *S* loci. Recent genetic, molecular biological and biochemical analyses of Brassicaceae, Papaveraceae and Solanaceae have suggested that angiosperms have developed diverse SI systems, which can be classified into two fundamentally different systems, self-recognition and non-self recognition systems: the SI systems in Brassicaceae and Papaveraceae are classified into a self-recognition system, while the SI system in Solanaceae is classified into a non-self recognition system (see Table 1) [1,2]. In Papaveraceae, self-pollen undergoes apoptosis elicited by an intracellular increase in Ca^{2+} concentration in pollination after being recognized as self [1–3]. On the other hand, pollen tube elongation is inhibited by degrading RNA with *S*-RNase of the self-pistil in Solanaceae, resulting in the avoidance of self-fertilization. This is a non-self recognition system, in which non-self S-RNase in the pistil is recognized by a ubiquitin ligase E3 (SLF/SBP) and then degraded by the ubiquitin–proteasome system in a pollen tube (Table 1) [1–3]. For Brassicaceae, we will describe recent progress made forward elucidation of the mechanism of the SI system.

In Brassicaceae, SI is controlled by a self-recognition system. The stigma is covered by a layer of epithelial cells, called papilla cells. When a cross pollen grain lands on a papilla cell, the pollen grain hydrates and geminates a pollen tube. The pollen tube then invades the papilla cell wall and grows toward the ovule cells to achieve fertilization. On the other hand, when a self pollen grain lands on a papilla cell, pollen hydration and germination are inhibited, and then self-fertilization is arrested.

The pistil-*S* determinant encoded at the *S* locus is *S*-locus receptor kinase (SRK), which localizes to the plasma membrane of stigmatic papilla cells [8]. The pollen-*S* determinant is *S*-locus protein 11 (SP11, or *S*-locus cysteine-rich protein: SCR), which is a small basic protein secreted from the anther tapetum and transferred to the pollen coat during pollen maturation [9,10]. *S*-haplotype-specific binding of SP11 to SRK induces autophosphorylation of SRK to elicit a self-incompatible reaction for rejecting self-pollen (Fig. 1) [11,12].

Thus far, two candidate molecules, *M*-locus protein kinase (MLPK) and Arm-Repeat Containing 1 (ARC1), have been identified as direct downstream factors of the SRK signaling pathway in *Brassica* species. MLPK is a membrane-anchored cytoplasmic protein kinase that co-localizes and interacts with SRK on the papilla cell membrane [13,14]. Recent studies have suggested that MLPK is also related to intra-species unilateral incompatibility of *Brassica rapa* [15], but it remains controversial whether MLPK is required for SI throughout the Brassicaceae [16]. ARC1 is a U-box protein with E3 ubiquitin ligase activity, phosphorylated by the kinase domain of SRK in *Brassica napus* [17–19], and interacts with Exo70A1, a putative component of the exocyst complex [20,21]. Suppression of *ARC1* expression results in incomplete breakdown of SI in both *B. napus* and *Arabidopsis lyrata* [18,22]. However, the precise role of ARC1 in the signaling pathway downstream of SRK that leads to self-pollen rejection remains controversial [16].

Physiological studies during cross- and self-pollination in *B. rapa* showed that actin bundles were concentrated at the cross-pollen attachment site, while actin reorganization occurs at the self-pollen attachment site. Additionally, electron tomography revealed attachment of the actin cytoskeleton with an apical vacuole network. Self-pollination disrupted the vacuole network, while cross-pollination led to vacuolar rearrangements toward the site of pollen attachment. These findings suggested that self-pollination and cross-pollination differently affect the dynamics of the actin cytoskeleton, leading to changes in vacuolar structure that might be associated with hydration and germination.

A recent biological assay using water-soluble Calcium Green showed that Ca^{2+} is exported from the papilla cell during cross-pollination and that the cross pollen coat alone elicits the Ca^{2+} transport [23]. In contrast, neither a self-pollen grain nor the self-pollen coat in *B. rapa* induced Ca^{2+} transport. These findings led us to speculate that Ca^{2+} is transported from the papilla cell to the cell wall and then transferred from the cell wall to the pollen

Table 1
Male and female determinants in self-incompatibility of plants and animals.

Family	Female determinant	Male determinant	Self or non-self recognition
Flowering plants (Angiosperms)			
Brassicaceae	SRK	SP11/SCR	Self
Papaveraceae	PrsS	PrpS	Self
Solanaceae/Rosaceae	S-RNase	SLF/SBP	Non-self
Animals (Ascidians)			
Cionidae (*C. intestinalis*)	v-Themis-A, -B	s-Themis-A, -B	Self
Pyuridae (*H. roretzi*)	VC70	(TTSP1, Urabin)?	Non-self ?

See Refs. [1–6], [25], [27–28].

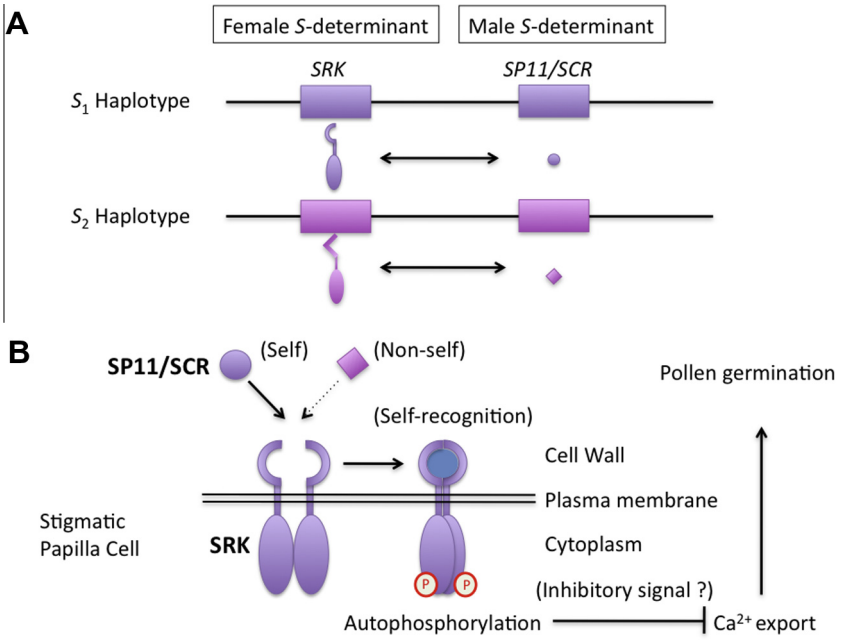

Fig. 1. Self-incompatibility system in Brassicaceae. Male S-determinant SP11/SCR and female S-determinant SRK (S-locus receptor kinase) reside in the same chromosome. The same allelic SP11/SCR can bind to SRK on papilla cells. When SP11 is recognized as self, autophosphorylation takes place, triggering SI responses including prevention of Ca^{2+} export in the papilla cell, which is involved in germination. Modified from [2].

grain during cross-pollination, while the pollen *S*-determinant SP11/SCR prevents Ca^{2+} transport to the cell wall, leading to the rejection of self-pollen. Furthermore, by transcriptome analysis and reverse genetic analysis in *Arabidopsis thaliana*, *autoinhibited Ca^{2+}-ATPase 13* (*ACA13*) has been identified as a Ca^{2+} pump functioning during cross-pollination. ACA13 localized to the plasma membrane and to vesicles near the Golgi body and accumulated at the pollen tube penetration site after pollination [23]. Collectively, these findings suggest that the secretory pathway functions during compatible pollination to direct the accumulation of molecules such as ACA13 to regions of the plasma membrane near the pollen attachment site and around the germinated pollen tube and that molecules that have accumulated at these sites effectively export Ca^{2+} and water from papilla cells to support successful pollination. Above-described ARC1 interacts with Exo70A1 and may be involved in the polarized secretion pathway during cross-pollination [20,21]. Taken together, it has been hypothesized that a secretion pathway regulates pollen hydration and germination not only during cross-pollination but also during self-pollination *via* the actin cytoskeleton, exocyst component, and SNARE proteins.

3. Self-incompatibility in Stolidobranch ascidians

Ascidians (primitive chordates, urochordates) are simultaneously hermaphroditic marine invertebrates, which release sperm and eggs to the surrounding seawater almost simultaneously. At least several species, including *Halocynthia roretzi* and *C. intestinalis,* show strict self-sterility. Therefore, these two species have been used for studies on the mechanism of SI. Generally, in contrast to intact eggs, vitelline coat (VC)-fee eggs and immature oocytes allow self-fertilization. Therefore, it is thought that self/non-self-recognition (or allorecognition) molecules play a key role in the process of interaction between sperm and the VC of the egg and that allorecognition molecules or systems are acquired during oocyte maturation [4–6].

In the Stolidobranch ascidian *H. roretzi*, a 70-kDa main component of the VC named HrVC70 appears to be a self/non-self recognition protein on the VC for the following reasons. First, the amount of HrVC70 on the VC was shown to significantly increase during oocyte maturation [24,25]. Secondly, the mature egg becomes self-fertile by treatment with acidic seawater (pH 2–3) for 1 min, which is closely related to the fact that HrVC70 is efficiently and almost specifically extracted from the isolated VC by 1–10 mM HCl (~pH 2–3) [24]. Thirdly, non-self sperm can bind to HrVC70-immobilized agarose more efficiently than can self-sperm [25]. Fourthly, sperm pretreated with HrVC70 of non-self eggs more effectively inhibited fertilization than did sperm pretreated with that of self-eggs [25]. Fifthly, HrVC70 consists of 12 epidermal growth factor (EGF)-like repeats, which is not at variance with the fact that EGF-like-repeat-containing proteins such as Notch and Delta are involved in cell–cell interaction. Lastly, HrVC70 showed several synonymous and non-synonymous substitutions among individuals, which might be sufficient to cause a drastic change in the protein–protein interaction, since EGF-like repeat-containing proteins such as Notch cause Notch signaling diseases even in a single amino acid substitution [26].

As sperm-side binding partners of HrVC70, HrTTSP-1 (type-II transmembrane serine protease) and HrUrabin (*u*nique *RA*FT-derived *bin*ding partner for HrVC70: a GPI-anchored CRISP-family protein) have been identified by yeast two-hybrid screening [27] and Far-Western blotting, respectively [28]. HrTTSP1 has an estimated molecular mass of 337 kDa and consists of 23 CCP/SCP/Sushi-domains, 3 ricin B domains and 1 CUB domain in its extracellular region. Whereas HrTTSP-1 is able to interact with HrVC70, it is still unclear whether HrTTSP-1 is able to distinguish the polymorphism of HrVC70. HrUrabin has been reported to play a key role in fertilization, since anti-HrUrabin antibody is able to strongly inhibit the fertilization and also the self/non-self-recognizable binding of sperm to HrVC70-agarose beads. However, HrUrabin *per se* had little polymorphism among individuals and showed no apparent difference in its binding ability to HrVC70 isolated from self- and non-self-eggs. Thus, it is thought that HrUrabin is unable

to directly discriminate self- and non-self-HrVC70, although HrUrabin must play a role in a certain process of allorecognition during sperm-egg interaction [28].

There is an ortholog of HrVC120, a precursor of HrVC70, in another ascidian species, *Halocynthia aurantium*, referred to as HaVC130. The mature protein HaVC80 appears to have 13 EGF-like repeats [29]. The amino acid identity between HrVC120 and HaVC130 was very high (83.4%), and the 8th EGF domain of the HrVC120 gene appears to have been duplicated during molecular evolution. HaVC80 is also highly polymorphic among individuals, and the substituted regions of HaVC80 are restricted similarly to those of HrVC70 [29]. It seems likely that EGF-like repeat-containing VC proteins play a key role in self/non-self recognition during fertilization of the genus *Halocynthia* or in Pyuridae or Stolidobranch ascidians.

4. Self-incompatibility in Phlebobranch ascidians

The Phlebobranch ascidian *C. intestinalis* is cosmopolitan and very useful for many biological studies since the genome database is available [30]. Several candidate molecules involved in SI in *C. intestinalis* have been proposed. Kawamura et al. [31] reported that acid extract of the VC is capable of inhibiting the binding of non-self-sperm to the VC but not the binding of self-sperm to the VC. They reported that there are a non-allorecognizable inhibitor and peptide-modulators, which serve as acceptors of non-self-sperm, in the acid extract and that certain combinations of these factors exhibit inhibitory ability toward the binding of non-self-sperm [31]. On the other hand, De Santis and Pinto showed that SI becomes effective several hours after germinal vesicle breakdown [32]. Since removal of follicle cells arrested the onset of self-sterility, they proposed that follicle cells release an SI factor(s) that binds to the VC [32]. By analogy to the mammalian cellular immune system, Marino et al. [33,34] proposed that the peptides produced by proteasome are loaded onto Cihsp70, which is a molecular chaperone assumed to be an ancestor protein of MHC class I and II molecules in lower vertebrates or invertebrates, and delivered to the surface of the VC. Since the proteasome inhibitor clasto-lactacystin β-lacton and anti-HSP70 antibody inhibited the onset of SI, they proposed that Cihsp70 and a self-peptide produced by proteasome might be responsible for SI in *C. intestinalis* [33,34]. However, the antigenic peptide fragments on Cihsp70 on the VC have not yet been identified. Another approach was carried out by Khalturin et al. [35], who performed PCR-based subtraction experiments and compared gonad cDNAs between genetically unrelated individuals. They identified several candidate genes that are expressed in developing oocytes or/and follicle cells and are polymorphic among individuals, including CiS7 (EGF-like repeat-containing gene) and vCRL1 (Sushi (or SCR)-domain-containing gene) [35–37]. However, they recently reported that vCRL1 genes are not related to the SI system in *C. intestinalis*, but that the s/v-Themis-A and -B system, which was proposed by Harada et al. [4] as described below, plays a key role in SI under their experimental conditions [38]. It has also been reported that the terminal fucose-residue on VC glycoproteins [39] and sperm-side fucosidase may make an enzyme-substrate complex, allowing interaction between sperm and the VC of the eggs, although it is unclear whether these proteins are involved in SI [40].

Extensive studies on the mechanism of self/non-self recognition during fertilization of *C. intestinalis* were carried out by Thomas H. Morgan in the early part of the 20th century [41–44]. He reported that the VC is a barrier against self-fertilization and that the SI system is abolished by treatment of eggs with acidic seawater or protease [42]. By acid-induced self-fertilization, he raised many selfed F1 siblings and examined cross-fertility and cross-sterility among them [43,44]. Cross-sterility is hardly observed in wild populations, but cross-sterile combinations were sometimes observed in selfed or experimentally cross-fertilized siblings, suggesting that self-sterility is genetically controlled. Among cross-sterile combinations in selfed F1 siblings, he identified two types of cross-sterility, *i.e.,* bi-directional and one-way combinations [43,44]. Morgan proposed a "haploid sperm hypothesis" to explain the phenomenon of one-way cross-sterility [43,44]. According to his hypothesis, a parent heterozygous at the SI locus (A/a) produces two populations of sperm (A- and a-expressing sperm), either of which can fertilize both types of homozygous eggs (A/A and a/a eggs). In contrast, sperm (A- or a-expressing sperm) from two types of homozygotes (A/A or a/a individual) cannot fertilize the heterozygous eggs (A/a eggs), since heterozygous eggs express both types of female SI gene products on the VC. Thus, once a one-way cross-sterile pair of individuals was found, egg-donating individuals should be a heterozygote in the *S*-locus, whereas sperm-donating individuals should be a homozygote in the *S*-locus.

Based on these criteria, Harada et al. [4] surveyed a candidate *S*-locus by determining the DNA sequence at about 70 genetic markers in 14 chromosomes, and they found that two loci, loci A and B, located in chromosomes 2q and 7q, respectively, are involved in SI in this species (Fig. 2) [4]. Among the proteins encoded in locus A, a fibrinogen C-terminus-like protein, referred to as v-Themis-A, with a high degree of polymorphism among individuals was detected on the VC by proteome analysis. On the other hand, among genes encoded in locus A, four genes were found to be expressed in the testis, among which a PKD-1-like protein, referred to as s-Themis-A, was thought to be a candidate sperm-side receptor protein with a hyper-variable region in its N-terminal region (Themis is a Greek goddess who prohibited incest.). Although there is no overall synteny between loci A and B, a similar gene pair of proteins (v-Themis-B and s-Themis-B) was identified in locus B [4]. Interestingly, *v-Themis-A/B* genes were located in the first intron of *s-Themis-A/B* genes, respectively, in the opposite direction in both cases (Fig. 2). These features indicate a tight linkage between *s-Themis* and *v-Themis* genes. By genetic analysis, it has been proposed that when sperm-side s-Themis-A and s-Themis-B interact with the same allelic v-Themis-A and v-Themis-B, respectively, on the VC, sperm must recognize the VC as self-egg, resulting in prevention of self-fertilization.

Sperm behavior and intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in response to self/non-self-recognition have also been studied [45]. It has been reported that sperm motility markedly decreased within 5 min after attachment to the VC of self-eggs but not after attachment to the VC of non-self-eggs and that the apparent decrease in sperm motility was suppressed in low (~5 μM) Ca^{2+} seawater. It was also revealed that sperm detach from the self-VC or stop motility within 5 min after binding to the self-VC. Since s-Themis-B contains a cation channel domain in its C terminus (see Fig. 2C) [4], sperm $[Ca^{2+}]_i$ was monitored by real-time $[Ca^{2+}]_i$ imaging using Fluo-8H-AM, a cell-permeable Ca^{2+} indicator. Interestingly, sperm $[Ca^{2+}]_i$ rapidly and dramatically increased and was maintained at a high level in the head and flagellar regions when the sperm interacted with the self-VC but not when the sperm interacted with the non-self-VC [45]. The increase in $[Ca^{2+}]_i$ was also suppressed by low-Ca^{2+} seawater. These results indicate that the sperm self-recognition signal triggers $[Ca^{2+}]_i$ increase and/or Ca^{2+} influx, which induce an SI response to reject self-fertilization [45].

According to a previous report, a non-self-sperm-recognizing factor must be extracted from the VC by acidic conditions in *C. intestinalis* [31]. However, v-Themis-A and -B were hardly solubilized from the VC by acid treatment. In contrast, a novel factor, designated as Ci-v-Themis-like, is a major acid-extractable VC protein [46]. This protein consists of a coiled coil domain and C-terminal fibrinogen-like domain, similarly to those of v-Themis-A and -B

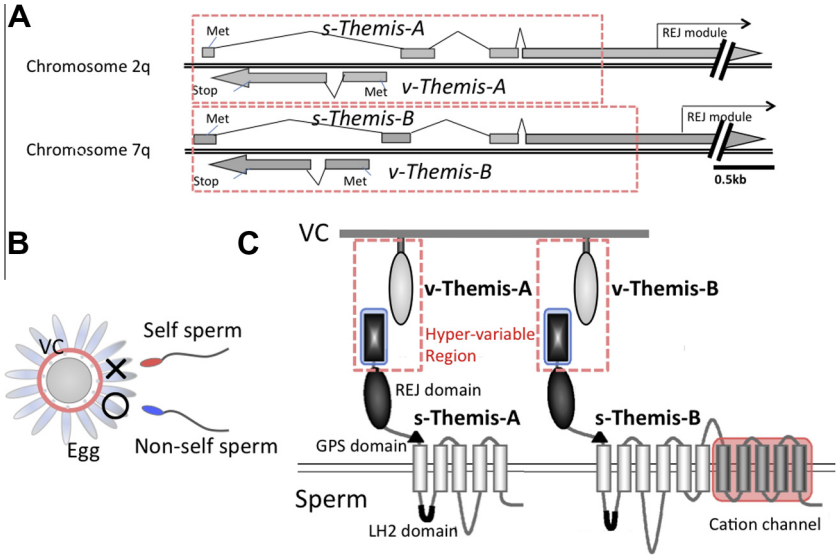

Fig. 2. Self-incompatibility system in the ascidian *Ciona intestinalis*. (A) Sperm-side SI genes (*s-Themis-A* and *s-Themis-B)* and egg-side SI genes (*v-Themis-A* and *v-Themis-B*) reside in the same loci in chromosomes 2q and 7q, respectively. The genes of *v-Themis-A* and *v-Themis-B* reside in the first introns of *s-Themis-A* and *s-Themis-B*, in an opposite direction, respectively. There is a hyper-variable region in the N-termini of s-Themis-A and -B, while v-Themis-A and -B are polymorphic in their entire regions. (B) Genetic analysis suggested that sperm recognize the VC of the eggs as self when both alleles of s-Themis-A and s-Themis-B recognize v-Themis-A and v-Themis-B as the same alleles. Only non-self sperm are allowed to penetrate through the VC, whereas self-sperm detach from the VC or stop motility. (C) There is a cation channel in the C-terminal region of s-Themis-B. When sperm bind to the VC of self-egg, intracellular Ca^{2+} concentration increases, the increase being elicited by Ca^{2+} influx. It is assumed that Ca^{2+} influx is mediated by the cation channel in s-Themis-B. Modified from [4].

except for having no apparent polymorphism. This seems to be an ancestral protein of v-Themis-A/B. Yeast two-hybrid screening showed that sperm protease is a potential binding protein [46]. Therefore, the interaction between Ci-v-Themis-like on the VC and sperm protease may participate in gamete interaction to support the Themis-mediated allorecognition system.

5. Mode of self-incompatibility in corals

Besides ascidians, the molecular mechanisms of self/non-self recognition during fertilization in hermaphroditic animals have not been studied well. As described above, since most terrestrial and several aquatic hermaphroditic animals exhibit reproductive behaviors, a self/non-self recognition system would not be required to avoid inbreeding. Therefore, hermaphroditic marine animals that release sperm and eggs to the surrounding seawater appear to be not so many. Among these animals, the coral reproduction strategy is very interesting, since they are endangered animals and undergo asexual and sexual reproduction. Therefore, in the present review, we briefly introduce the mode of coral reproduction, although the precise mechanisms are not known.

Many coral species are simultaneous hermaphrodites, which produce both sperm and eggs. In general, self-fertilization (selfing) is not favored, because self-fertilized progeny would exhibit lower fitness than out-crossed progeny [47]. Therefore, it is thought that the mechanisms to avoid selfing have developed also in coral [48]. However, it is known that several sessile marine invertebrates select selfing because of the complete absence of mates and/or of gamete limitation, although inbreeding depression might occur [49–51]. Taken together, many hermaphroditic marine invertebrates may have evolved mixed-mating systems that undergo facultative self-fertilization.

The mating system of hermaphroditic coral is variable. Some coral species show selfing, but others do not (see [52]). Results of many studies have suggested that selfing might occur under sperm-limited condition or in the absence of mates and that outcrossing occurs when sperm from other individuals is available. Allorecognition is crucial to avoid self-fertilization. To investigate a variety of allorecognition system in marine invertebrates, cnidarian corals appear to be good examples. Most species are simultaneous hermaphrodites and their breeding patterns are diverse such as broadcast spawning and brooding [53]. Approximately three quarters of coral species undergo broadcast spawning, in which sperm and eggs are released to the surrounding seawater and external fertilization takes place. On the other hand, the remaining one quarter of coral species undergo brooding, in which only sperm released from males or hermaphrodites is incorporated into females or hermaphrodites, resulting in internal fertilization [53].

The sessile corals *Acropora*, *Montipora*, and *Montastrea* are simultaneous hermaphrodites that release their gametes as a packaged sperm and eggs named "bundles" [54]. These bundles are filled with many eggs surrounded by sperm [54]. Although their gametes interact in the bundles, self-fertilization never takes place [52,55,56] and sperm motility is suppressed [56]. In another sessile coral, *Goniastrea favulus*, self-fertilization occurs 7 h after insemination [52,57] but self-fertilization rarely take place in the species of the same genus, *Goniastrea aspera* [52]. The difference in occurrence of self-fertilization appears to be related to mating success with others.

The brooding corals *Seriatopora hystrix*, *Favia fragum* and *Porites astreoides* show selfing [51,58]. In brooding corals, availability of sperm from another is not easy, and thus self-fertilization may be selected. These species are genetically heterogeneous, and thus out-crossing also takes place.

Although the annual spawning season of coral is very short under full moon light in the early summer in Okinawa, Japan, it is worthy to investigate the sexual reproduction mechanisms to maintain these endangered corals from the viewpoint of environmental biology.

6. Conclusion

Our current understanding of the mechanism of self/non-self recognition in ascidians is that it is very similar to the SI system in flowering plants: male-side *S*-determinant located in sperm head surface in animals and in the pollen surface in plants and female-side *S*-determinant located in the VC of animal eggs and

in papilla cells in the plant pistil recognize each other. When it is recognized as the same haplotypic allele, fertilization should be blocked. Although the *S*-determinant proteins are different in animals and plants and also among taxonomic families in plants, the basic strategies or mechanisms to block self-fertilization seem to be very similar. As is in the case of flowering plants, ascidians may utilize different *S*-determinants depending on taxonomic families or orders. It is also very interesting or rather surprising that living organisms, regardless of the taxonomic kingdom, adopt similar mechanisms to prevent self-fertilization and, in other words, that the SI system has evolved in a manner of convergent evolution. The mechanisms of SI in other hermaphroditic animals might be clarified in the future by analogy of plant SI systems and *vice versa*.

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References

[1] S. Takayama, A. Isogai, Self-incompatibility in plants, Ann. Rev. Plant Biol. 56 (2005) 467–489.
[2] M. Iwano, S. Takayama, Self/non-self discrimination in angiosperm self-incompatibility, Curr. Opin. Plant Biol. 15 (2012) 78–83.
[3] H. Sawada, N. Inoue, M. Iwano, Sexual Reproduction in Animals and Plants, Springer, 2014.
[4] Y. Harada, Y. Takagaki, M. Sunagawa, et al., Mechanism of self-sterility in a hermaphroditic chordate, Science 320 (2008) 548–550.
[5] Y. Harada, H. Sawada, Allorecognition mechanisms during ascidian fertilization, Int. J. Dev. Biol. 52 (2008) 637–645.
[6] H. Sawada, K. Yamamoto, K. Otsuka, et al., Allorecognition and lysin sytems during ascidian fertilization, in: H. Sawada, N. Inoue, M. Iwano (Eds.), Sexual Reproduction in Animals and Plants, Springer, 2014, pp. 231–239.
[7] D. de Nettancourt, Incompatibility in angiosperms, Sex. Plant Reprod. 10 (1997) 185–199.
[8] T. Takasaki, K. Hatakeyama, G. Suzuki, et al., The S receptor kinase determines self-incompatibility in *Brassica stigma*, Nature 403 (2000) 913–916.
[9] C.R. Schopfer, M.E. Nasrallah, J.B. Nasrallah, The male determinant of self-incompatibility in Brassica, Science 286 (1999) 1697–1700.
[10] S. Takayama, H. Shiba, M. Iwano, et al., The pollen determinant of self-incompatibility in *Brassica campestris*, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 1920–1925.
[11] S. Takayama, H. Shimosato, H. Shiba, et al., Direct ligand–receptor complex interaction controls *Brassica* self-incompatibility, Nature 413 (2001) 534–538.
[12] H. Shimosato, N. Yokota, H. Shiba, et al., Characterization of the SP11/SCR high-affinity binding site involved in self/nonself recognition in brassica self-incompatibility, Plant Cell 19 (2007) 107–117.
[13] K. Murase, H. Shiba, M. Iwano, et al., A membrane-anchored protein kinase involved in *Brassica* self-incompatibility signalling, Science 303 (2004) 1516–1519.
[14] M. Kakita, K. Murase, M. Iwano, et al., Two distinct forms of *M*-locus protein kinase localise to the plasma membrane and interact directly with *S*-locus receptor kinase to transduce self-incompatibility signalling in *Brassica rapa*, Plant Cell 19 (2007) 3961–3973.
[15] Y. Takada, T. Sato, G. Suzuki, et al., Involvement of MLPK pathway in intraspecies unilateral incompatibility regulated by a single locus with stigma and pollen factors, G3 3 (2013) 719–726.
[16] H. Kitashiba, P. Liu, T. Nishio, et al., Functional test of Brassica self-incompatibility modifiers in *Arabidopsis thaliana*, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 18173–18178.
[17] T. Gu, M. Mazzurco, W. Sulaman, et al., Binding of an arm repeat protein to the kinase domain of the S-locus receptor kinase, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 382–387.
[18] S.L. Stone, M. Arnoldo, D.R. Goring, A breakdown of Brassica self-incompatibility in ARC1 antisense transgenic plants, Science 286 (1999) 1729–1731.
[19] S.L. Stone, E.M. Anderson, R.T. Mullen, D.R. Goring, ARC1 is an E3 ubiquitin ligase and promotes the ubiquitination of proteins during the rejection of self-incompatible *Brassica* pollen, Plant Cell 15 (2003) 885–898.
[20] S.C. Hsu, D. TerBush, M. Abraham, W. Guo, The exocyst complex in polarized exocytosis, Int. Rev. Cytol. 233 (2004) 243–265.
[21] M.A. Samuel, Y.T. Chong, K.E. Haasen, et al., Cellular pathways regulating responses to compatible and self-incompatible pollen in *Brassica* and *Arabidopsis* stigmas intersect at Exo70A1, a putative component of the exocyst complex, Plant Cell 21 (2009) 2655–2671.
[22] E. Indriolo, P. Tharmapalan, S.I. Wright, D.R. Goring, The ARC1 E3 ligase gene is frequently deleted in self-compatible Brassicaceae species and has a conserved role in *Arabidopsis lyrata* self-pollen rejection, Plant Cell 24 (2013) 4607–4620.
[23] M. Iwano, M. Igarashi, Y. Tarutani, et al., Pollen coat-inducible autoinhibited Ca^{2+}-ATPase expressed in stigmatic papilla cells is required for compatible pollination in the Brassicaceae, Plant Cell 26 (2014) 636–649.
[24] H. Sawada, N. Sakai, Y. Abe, et al., Extracellular ubiquitination and proteasome-mediated degradation of the ascidian sperm receptor, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 1223–1228.
[25] H. Sawada, E. Tanaka, S. Ban, et al., Self/nonself recognition in ascidian fertilization: vitelline coat protein HrVC70 is a candidate allorecognition molecule, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 15615–15620.
[26] S. Artavanis-Tsakonas, K. Matsumoto, M.E. Fortini, Notch signaling, Science 268 (1995) 225–232.
[27] Y. Harada, H. Sawada, Proteins interacting with the ascian vitelline-coat sperm receptor HrVC70 as revealed by yeast two-hybrid screening, Mol. Reprod. Dev. 74 (2007) 1178–1187.
[28] S. Urayama, Y. Harada, Y. Nakagawa, et al., Ascidian sperm glycosylphosphatidylinositol-anchored CRISP-like protein as a binding partner for an allorecognizable sperm receptor on the vitelline coat, J. Biol. Chem. 283 (2008) 21725–21733.
[29] S. Ban, Y. Harada, H. Yokosawa, H. Sawada, Highly polymorphic vitelline-coat protein HaVC80 from the ascidian, *Halocynthia aurantium:* structural analysis and involvement in self/nonself recognition during fertilization, Dev. Biol. 286 (2005) 440–451.
[30] P. Dehal, Y. Satou, R.K. Campbell, et al., The draft genome of *Ciona intestinalis:* insights into chordate and vertebrate origins, Science 298 (2008) 2156–2167.
[31] K. Kawamura, M. Nomura, T. Kameda, et al., Self-nonself recognition activity extracted from self-sterile eggs of the ascidian, *Ciona intestinalis*, Dev. Growth Differ. 33 (1991) 139–148.
[32] R. De Santis, M.R. Pinto, Gamete self-discrimination in ascidians: a role for the follicle cells, Mol. Reprod. Dev. 29 (1991) 47–50.
[33] R. Marino, M.R. Pinto, F. Cotelli, et al., The hsp70 protein is involved in the acquisition of gamete self-sterility in the ascidian *Ciona intestinalis*, Development 125 (1998) 899–907.
[34] R. Marino, R. De Santis, P. Giuliano, M.R. Pinto, Follicle cell proteasome activity and acid extract from the egg vitelline coat prompt the onset of self-sterility in *Ciona intestinalis* oocytes, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 9633–9636.
[35] K. Khalturin, U. Kürn, N. Pinnow, T.C. Bosch, Towards a molecular code for individuality in the absence of MHC: screening for individually variable genes in the urochordate *Ciona intestinalis*, Dev. Comp. Immunol. 29 (2005) 759–773.
[36] U. Kürn, F. Sommer, T.C. Bosch, K. Khalturin, In the urochordate *Ciona intestinalis* zona pellucida domain proteins vary among individuals, Dev. Comp. Immunol. 31 (2007) 1242–1254.
[37] U. Kürn, F. Sommer, G. Hemmrich, et al., Allorecognition in urochordates: identification of a highly variable complement receptor-like protein expressed in follicle cells of Ciona, Dev. Comp. Immunol. 31 (2007) 360–371.
[38] F. Sommer, S. Awazu, F. Anton-Erxleben, et al., Blood system formation in the urochordate *Ciona intestinalis* requires the variable receptor vCRL1, Mol. Biol. Evol. 29 (2012) 3081–3093.
[39] F. Rosati, R. De Santis, Role of the surface carbohydrates in sperm–egg interaction in *Ciona intestinalis*, Nature 283 (1980) 762–764.
[40] M. Hoshi, Sperm glycosidase as a plausible mediator of sperm binding to the vitelline envelope in ascidians, Adv. Exp. Med. Biol. 207 (1986) 251–260.
[41] T.H. Morgan, Cross- and self-fertilization in *Ciona intestinalis*, Roux Arch. Entwicklungsmech. 30 (1911) 206–235.
[42] T.H. Morgan, The genetic and the physiological problems of self-sterility in *Ciona*. III. Induced self-fertilization, J. Exp. Zool. 80 (1939) 19–54.
[43] T.H. Morgan, The genetic and the physiological problems of self-sterility in *Ciona*. V. The genetic problem, J. Exp. Zool. 90 (1942) 199–228.
[44] T.H. Morgan, The genetic and the physiological problems of self-sterility in Ciona. VI. Theoretical discussion of genetic data, J. Exp. Zool. 95 (1944) 37–59.
[45] T. Saito, K. Shiba, K. Inaba, et al., Self-incompatiblity response induced by calcium increase in sperm of the ascidian *Ciona intestinalis*, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 4158–4162.
[46] K. Otsuka, L. Yamada, H. Sawada, cDNA cloning, localization and candidate binding partners of acid-extractable vitelline-coat protein Ci-v-Themis-like in the ascidian *Ciona intestinalis*, Mol. Reprod. Dev. 80 (2013) 840–848.
[47] N. Knowlton, J.B.C. Jackson, Inbreeding and outbreeding in marine invertebrates, in: N. Thornhill (Ed.), The Natural History of Inbreeding and Outbreeding, University of Chicago, 1993, pp. 200–249.
[48] M. Feldgarden, P.O. Yund, Allorecognition in colonial marine-invertebrates – does selection favor fusion with kin or fusion with self, Biol. Bull. 182 (1992) 155–158.
[49] P.O. Yund, M.A. MacCartny, Male reproductive success in sessile invertebrates: competition for fertilizations, Ecology 75 (1994) 2151–2167.
[50] D.R. Levitan, C. Petersen, Sperm limitation in the sea, Trend Ecol. Evol. 10 (1995) 228–231.
[51] D.A. Brazeau, D.F. Gleason, M.E. Morgan, Self-fertilization in brooding hermaphroditic Caribbean corals: evidence from molecular markers, J. Exp. Mar. Biol. Ecol. 231 (1998) 225–238.
[52] A.J. Heyward, R.C. Babcock, Self- and cross-fertilization in scleractinian corals, Mar. Biol. 90 (1986) 191–195.

[53] A.H. Baird, J.R. Guest, B.L. Willis, Systematic and biogeographical patterns in the reproductive biology of scleractinian corals, Ann. Rev. Ecol. Evol. Syst. 40 (2009) 551–571.
[54] C.C. Wallace, Staghorn Corals of the World: A Revision of the Genus Acropora, CSIRO Publishing, Collingwood, Australia, 1999.
[55] A.M. Szmant, E. Weil, M.W. Miller, D.E. Colon, Hybridization within the species complex of the scleractinan coral *Montastraea annularis*, Mar. Biol. 129 (1997) 561–572.
[56] M. Morita, A. Nishikawa, A. Nakajima, et al., Eggs regulate sperm flagellar motility initiation, chemotaxis and inhibition in the coral *Acropora digitifera*, *A. gemmifera* and *A. tenuis*, J. Exp. Biol. 209 (2006) 4574–4579.
[57] J.A. Stoddart, R.C. Babcock, A.J. Heyward, Self-fertilization and maternal enzymes in the planulae of the coral *Goniastrea favulus*, Mar. Biol. 99 (1988) 489–494.
[58] C.D. Sherman, Mating system variation in the hermaphroditic brooding coral, *Seriatopora hystrix*, Heredity 100 (2008) 296–303.