Chapter 3
Sperm Activation and Chemotaxis in Invertebrates

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Abstract The gametes of aqua-living animals are equipped with several systems to ensure fertilization. Sperm activation and chemotaxis toward the conspecific eggs—some factors from the eggs or female organs activate and attract conspecific sperm—are the first two steps of authentication between sperm and eggs. Phenomena and molecular mechanisms underlying sperm activation and chemotaxis are varied, even in the same taxa. Considering species specificity of sperm chemotaxis, the system may prevent crossbreeding. It is an interesting point why the system of sperm activation and chemotaxis has been evolved with such a high diversity. In this chapter, we reviewed the sperm activation and chemotaxis in aquatic invertebrates.

Keywords Sperm chemotaxis · Fertilization · Species specificity

3.1 Introduction

In cases of aqua-living animals, many of them perform external fertilization. Even in animals undergoing internal fertilization, many of their males spawn spermatozoa into surrounding water without mating (see Chap. 1 for details). Thus, it is challenging for spermatozoa to find conspecific eggs, and they are equipped with several processes to overcome difficulties in the aquatic circumstances such as seawater. First, the spermatozoa start swimming when spawned into water around the male body (initiation). When the spermatozoa get close to the egg, the motility of the sperm is activated by chemicals from the egg or the female reproductive organs (activation). In many cases, the spermatozoa change their swimming pattern due to chemicals from the egg and are guided toward the egg (chemotaxis). Even in mammals, the process of changing sperm motility (initiation, activation, and chemotaxis)

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has been observed. In this chapter, we reviewed the sperm activation and chemotaxis in aquatic invertebrates.

3.2 Sperm Activation

Spermatozoa are usually immotile while stored in the male body and become motile when they are ejaculated or spawned from the male. Since most invertebrates living in aquatic circumstances are external fertilizers or internal fertilizers without mating, sperm motility is activated in aquatic conditions. Initiation of sperm motility in aquatic vertebrates showing external fertilization, such as fishes and amphibians, is mediated by changes of osmolarity (cf. Chapters 2, 4, and 11). On the other hand, in marine invertebrates, there is no difference in osmolarity between the seminal plasma and the aquatic circumstances around the male body, and inducer of sperm motility is almost unknown. The spermatozoa of several other animals are almost quiescent when they are spawned, and some factors released from eggs or female organs activate their motility. These female-derived "sperm-activating factors" are proposed in many marine invertebrates, but identification of these factors have been performed in only a few animals. Thus, the molecular mechanisms of sperm activation are almost unknown.

3.3 Sperm Chemotaxis

In many animals and plants, it is widely observed that spermatozoa sense substances released from the eggs or female organs and are led toward the direction to the egg. This phenomenon is called "sperm chemotaxis" and guarantees that conspecific gametes successfully come together. Sperm chemotaxis was already observed in the nineteenth century in ferns and mosses (Pfeffer 1884), and in animals, when J.C. Dan first observed it in the hydrozoan *Spirocodon saltatrix* (Dan 1950). Similar to the studies on sperm activation, sperm chemotaxis has been studied in marine invertebrates, taking advantage of external fertilization. In fact, Miller studied sperm chemotaxis extensively in many metazoan animals such as Cnidaria, Mollusca, and Echinodermata (see review; Miller 1985b). For now, the mechanisms of sperm chemotaxis are particularly researched in sea urchins and ascidians (see Sect. 3.4).

Although sperm chemotaxis in mammals does not seem to be species-specific, sperm chemotaxis of many other animals, especially marine invertebrates, is species-specific (Table 3.1). Thus, sperm chemotaxis may prevent crossbreeding, even though little is known about the molecular mechanisms of species specificity in sperm chemotaxis.

Sperm movement and its flagellar beating during chemotactic response have been observed in several marine invertebrate species. On the surface of a glass slide, the spermatozoon usually swims in a circular track with low asymmetric flagellar

Table 3.1 Observed sperm activation and sperm chemotaxis

Phylum	Class	Style of fertilization	Sperm activation	Sperm chemotaxis and species-specificity[a]
Cnidaria	Anthozoa	External	+ (Morita et al. 2006)	+/+ (Coll et al. 1994; Coll et al. 1995; Morita et al. 2006)
	Hydrozoa	External	n.d.	+/+ (Carré and Sardet 1981; Dan 1950; Miller 1966; Miller 1973; Miller 1979a; Noda and Kanai 1981)
	Staurozoa	External	n.d.	+? (Miller 1985b)
Mollusca	Bivalvia	External	+ (Alavi et al. 2014)	n.d.
	Polyplacophora	External	n.d.	+/− (Miller 1977)
	Gastropoda	External	n.d.	+ (Riffell et al. 2002)
	Cephalopoda	Internal (mating)	+ (Tosti et al. 2001)	+ (Hirohashi et al. 2013; Zatylny et al. 2002)
Annelida		External	+ (Lillie 1913b)	+? (Miller 1985b)
Bryozoa		Internal	n.d.	+? (Miller 1985b)
Arthropoda		Internal (mating)	n.d.	− (Miller 1985b)
Echinodermata	Echinoidea	External	+ (Hansbrough and Garbers 1981; Lillie 1913a; Ohtake 1976a; Suzuki 1990)	+ (Guerrero et al. 2010; Ward et al. 1985)
	Asteroidea	External	+ (Nishigaki et al. 1996)	+/+ (Miller 1985a)
	Holothuroidea	External	+ (Morita et al. 2009)	+/+ (Miller 1985a; Miller 1997; Morita et al. 2009)
	Ophiuroidea	External	n.d.	+/+ (Miller 1985a; Miller 1997)
Chordata	Ascidiacea	External	+ (Minganti 1951; Yoshida et al. 1994)	+/+ (Matsumori et al. 2013; Miller 1975; Miller 1982; Minganti 1951; Yoshida et al. 2013; Yoshida et al. 1993; Yoshida et al. 2002)
	Larvacea	External	n.d.	+ (Miller and King 1983)

[a]Initial plus or minus means existence of sperm chemotaxis and second plus or minus means that of species specificity. Even in class in which species specificity is "+", not all species show species specificity. "+?" means that the group has species specificity but less evidence

beating in the absence of eggs nor sperm attractants. On the other hand, when the spermatozoon shows chemotactic behavior, it draws a distinctive track: periodical quick turn and straight swimming (see review; Yoshida and Yoshida 2011). In the

pathway, the spermatozoon dynamically regulates its flagellar beating pattern. This regulates sperm direction, resulting in the spermatozoon's approach toward the egg.

In the regulation of flagellar beating, transient Ca^{2+} increase is a cue signal (see Sect. 3.5.1 for details).

3.4 Knowledge of Sperm Activation and Chemotaxis in Aquatic Invertebrates

As described in Sects. 3.2 and 3.3, phenomena of sperm activation and chemotaxis have been observed in many animals. We show an overview of sperm activation and chemotaxis in Table 3.1, and detailed descriptions are shown in Sects. 3.4.1–3.4.6.

3.4.1 Cnidarians

Studies on sperm activation and chemotaxis in animals were initially performed on Cnidarian species. The first observation of sperm chemotaxis was on the sperm of hydrozoan, *Spirocodon saltatrix* (Dan 1950), and Miller had studied extensively on hydrozoan species (Freeman and Miller 1982; Miller 1966, 1970, 1973, 1979a, 1979b). Sperm chemotaxis was observed in many hydrozoan species, even though they are sessile or planktonic, or internal or external fertilizers (Miller 1985b). The sperm chemotaxis in hydrozoan is highly species-specific: Miller examined chemotaxis in 32 species of marine hydromedusae, and only 13 heterospecific cross-reactions were found (Miller 1979a). The attractant seems to be released from the egg itself (Miller 1985b) or from an extracellular structure localized around the animal pole of the egg called the cupule (Cosson et al. 1984).

The sperm attractants in the hydrozoan species seem to be proteins or peptides: the sperm attractant of *Hippopodius hippopus* seemed to be a protein with a molecular mass of 25 kDa and pI 3.5, even though it was still not identified (Cosson et al. 1986). On the other hand, the sperm attractant in the scleractinian coral *Montipora digitata* was found to be the unsaturated fatty alcohol dodeca-2,4-diynol (Coll et al. 1994). In the species, three fatty alcohols were isolated as sperm activating substances, but only dodeca-2,4,-diynol had sperm-attracting activity (Coll et al. 1994). Coll and his colleagues also found that the native sperm attractant of the soft coral *Lobophytum crissum* is the macrocyclic diterpene alcohol (-)-epi-thunbergol (Coll et al. 1995). Other coral species in the genus *Acropora* also showed sperm chemotaxis toward the egg (Morita et al. 2006), even though the sperm attractants were still not elucidated.

In addition to that in the hydorozoan and anthozoan species, it is thought that sperm chemotaxis is present in the Schyphozoan species.

Like in other animals, the chemotactic movement of the Cnidarian spermatozoa is mediated by Ca^{2+} (Cosson et al. 1983, 1984; Morita et al. 2006).

3.4.2 Mollusks

In protostome animals, fertilization of mollusks, especially in bivalves, has been studied well due to their economic importance. Alavi and his co-workers studied sperm activation of some bivalves (Bivalvia): Manila clam (*Ruditapes philippinarum*), Pacific oyster (*Crassostrea gigas*), and Japanese scallop (*Patinopecten yessoensis*) (Alavi et al. 2014). They showed that the sperm of the bivalves initiate its motility when released into sea water, and in addition, the movement of sperm is activated by 5-hydroxytryptamine (5-HT), one of the physiologically active substances inducing spawning and oocyte maturation in bivalves (effects of 5-HT on reproduction of mollusks are described in Chap. 7, Sect. 7.3 for details). During the initiation of sperm motility, 5-HT induces a Ca^{2+} influx via voltage-dependent ion channels associated with K^+ efflux, resulting in the activation of CaM-dependent flagellar beating (Alavi et al. 2014) (see Fig. 3.1). On the other hand, there is no report showing the chemotactic response of the bivalve sperm.

Sperm chemotaxis has been observed in the primitive mollusk chitons (Polyplacophora): Miller showed that the ethanol extracts of the eggs of several chitons attract the spermatozoa (Miller 1977). Interestingly, sperm chemotaxis in chitons is not species-specific (Miller 1977, 1985b). Sperm activation and chemotaxis is also observed in cephalopods. Spermatozoa of the octopus *Octopus vulgaris* are stored in a female genital tract prior to fertilization and seem to be pre-activated by progesterone. Progesterone-treated sperm show a breakdown of outer membrane around the acrosomal region, which is like a process of acrosome reaction in mammals (Tosti et al. 2001). Sperm chemotaxis in cephalopods is observed in *Octopus vulgaris* and the cuttlefish *Sepia officinalis*, and their sperm attractants are identified as the peptides named Octo-SAP (De Lisa et al. 2013) and SepSAP (Zatylny et al. 2002), respectively.

Concerning snails (Gastropoda), one of the major groups of mollusks, little is known about their sperm activation and chemotaxis since most of them are internal fertilizers. However, abalone, a primitive snail, is an external fertilizer, and its sperm shows chemotactic behavior (Riffell et al. 2002, 2004). The sperm attractant of the red abalone (*Haliotis rufescens*) is identified as the amino acid L-tryptophan (Riffell et al. 2004).

Sperm chemotaxis in mollusks plays roles not only in the success of conspecific fertilization but also in reproductive strategy. Males of some squids (Cephalopoda) contend on females for mating, and the winner acts as a consort male, the loser a sneaker male. In other words, the consort male accompanies the female (see the Chap. 13). The consort male deposits his spermatophore around the opening of the oviduct on the female mantle cavity, and the sperm will get first access to the egg capsules. On the other hand, the sneaker male gets close to the female and scatters

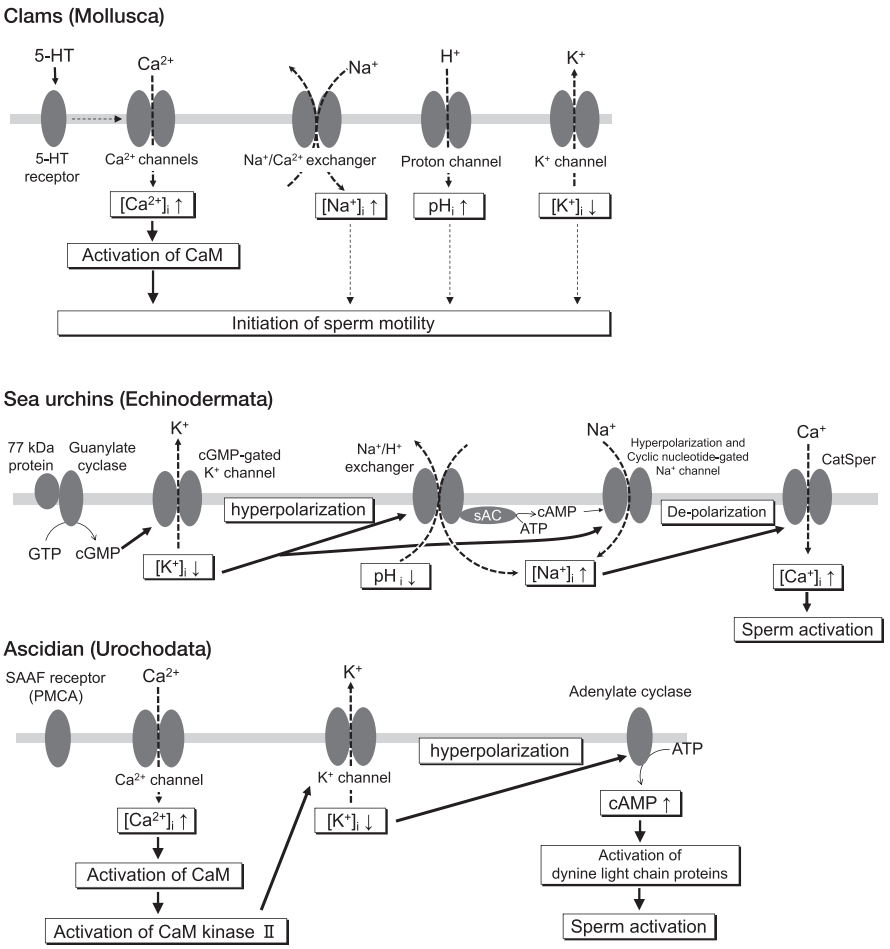

Fig. 3.1 Signaling cascades of sperm activation in animals: clams, sea urchins, and ascidians

its sperm when the female holds the egg capsules on her arm to lay it on the substrate, i.e., the male is "sneaking" to fertilize the eggs. Interestingly, in the squid *Heterololigo bleekeri*, spermatozoa from sneaker males make a cluster after spawning, whereas spermatozoa from consort males do not (Hirohashi et al. 2013). The clustering of sperm is caused by sperm chemotaxis toward CO_2 (Hirohashi et al. 2013). Higher sensitivity of the sneaker male sperm to pH seems to cause a chemotactic response only in the sneaker sperm (Hirohashi et al. 2013; Iida et al. 2017). For details, see Chap. 13, Sect. 13.3.4.

3.4.3 Arthropods and Other Protostomes

Knowledge of sperm activation and chemotaxis in protostomes other than mollusks is minimal, even in Arthropoda. One of the few studies on arthropod sperm was done in the horseshoe crab (Xiphosura) *Limulus polyphemus*. The *Limulus* spermatozoa are almost immotile when spawned in seawater and are activated if they encounter an egg-derived factor (Clapper and Brown 1980a). The egg-derived factor activating sperm release (also called a sperm motility initiating factor (SMI)) is still not identified, but it appears to be a hydrophobic peptide with the molecular mass of 500–2000 (Clapper and Brown 1980b; Clapper and Epel 1982). Fertilization of arthropods are reviewed in Chap. 7, Sect. 7.2.3.

In other protostomes, existence of sperm activation and chemotaxis is suggested in Annelida and Bryozoa (Miller 1985b), but these should be confirmed.

3.4.4 Sea Urchins (Echinodermata, Echinoidea)

Sea urchins, a group of echinoderms, have been studied well for a century, and their sperm activation and chemotaxis are also well known. Activation of sea urchin spermatozoa by some factors associated with the eggs was observed a century ago (Lillie 1913b). The sperm of the sea urchin is a traditional model for research on sperm activation, although sea urchin spermatozoa are usually highly activated after spawning in sea water. First, Ohtake showed that the jelly layer of eggs of *Hemicentrotus pulcherrimus* contains sperm-activating substances, which activate sperm motility and respiratory in acidic sea water (Ohtake 1976b). After that, the decapeptide named speract and another 14-amino-acid peptide named resact were identified as the sperm-activating substances from *Strongylocentrotus purpuratus* (Hansbrough and Garbers 1981) and *Arbacia punctulata* (Suzuki et al. 1984), respectively. Moreover, resact was found to be not only the sperm-activating substance but also the sperm attractant (Ward et al. 1985). Suzuki and his co-workers expanded the work in the various sea urchin species and finally found 74 sperm-activating peptides from 17 species distributed over five orders (Suzuki 1995).

Receptors and signaling mechanisms of the sperm-activating peptides, especially speract and resact, have been investigated for many years. Speract and resact bind to their receptors on the sperm membrane and activate guanylyl cyclase, which is the enzyme for the production of cGMP (Ramarao and Garbers 1985). The receptor of resact is a membrane-type guanylate cyclase (Shimomura et al. 1986), and as such, resact directly activates the guanylyl cyclase and induces cGMP production. On the other hand, the speract receptor is not a guanylyl cyclase: Speract binds to the 77 kDa membrane protein that seems to be associated with a guanylate cyclase (Bentley et al. 1988; Dangott and Garbers 1984; Dangott et al. 1989). In any case, increase of cGMP induces the activation of cyclic nucleotide-gated K^+-selective channel and K^+ efflux, resulting in the hyperpolarization of membrane potential

(Babcock et al. 1992; Galindo et al. 2000). Then, Ca^{2+} efflux and Na^{+} influx via a Na^{+}/Ca^{2+} exchanger and alkalization via a Na^{+}/H^{+} exchanger occur (Lee and Garbers 1986; Nishigaki et al. 2004). Finally, increase of Ca^{2+} in the sperm head and flagella is observed (Böhmer et al. 2005; Guerrero et al. 2010; Kaupp et al. 2003; Wood et al. 2005) (see Fig. 3.1). An additional pathway, cAMP can induce Ca^{2+} increase (Cook and Babcock 1993). In the sperm of *S. purpuratus*, the Na^{+}/H^{+} exchanger seems to associate with a soluble adenylate cyclase, which is the enzyme for the production of cAMP (Nomura and Vacquier 2006). Thus, this may be activated by the signal pathway initiated from speract.

Interestingly, sperm chemotaxis in sea urchins other than *Arbacia* is unclear. Chemotaxis of *A. punctulata* sperm has been observed in 1985 (Ward et al. 1985) and that of *A. lixula* has also been detected (Yoshida, unpublished data). On the other hand, spermatozoa of other sea urchins, including *S. purpuratus* and *H. pulcherrimus*, have been considered not to show chemotactic behavior toward the sperm-activating peptides for a long time (Cosson 1990; Darszon et al. 2008; Miller 1985b). However, Guerrero and his collaborators have shown that the *Lytechinus pictus* spermatozoa displayed Ca^{2+} responses similar to a sperm showing chemotactic behavior, and finally the sperm has shown chemotactic behavior toward speract even though the phenomenon is not obvious (Guerrero et al. 2010) (see Chap. 12 for details).

Regardless, chemotactic response of the sea urchin sperm other than *Arbacia* is not obvious. Why do only a small number of sea urchin species conserve sperm chemotaxis? This is a difficult and unsolved question. A possible hint to solve the question is that resact is the only 14-amino-acid peptide, while other sperm-activating peptides, including speract, are mostly 10-amino-acid peptides (Suzuki 1995). In addition, the receptor of resact is different from that of other sperm-activating peptides.

3.4.5 Echinoderms Other than Sea Urchin (Starfish, Sea Cucumber, and Brittle Star)

Like in the sea urchin, the spermatozoa of many other echinoderms have their motility in sea water. Thus, study on sperm activation is little. In exceptional cases, the sperm of the sea cucumber *Holothuria atra* is quiescent in sea water and activated by substances from the egg (Morita et al. 2009).

Sperm chemotaxis in echinoderms other than Echinoidea are observed in starfishes (Asteroidea), sea cucumbers (Holothuroidea), and brittle stars (Ophiuroidea) (Miller 1985a). In the experiments, species specificity of sperm chemotaxis has been observed between the used species in the brittle stars, but some hetero-specific cross reactivities were observed in starfishes (Miller 1985a). Moreover, in sea cucumbers, hetero-specific cross-reactivities were observed in broad genus and specificity of sperm chemotaxis was observed in limited genus (Miller 1985a). In

the sea cucumber, sperm attractants seem to be released from the egg cell and retained at the vitelline membrane (Morita et al. 2009). In the starfish, sperm attractants seem to exist in the jelly layer: the sperm-activating peptides named Asterosaps that was purified from the jelly layer of the starfish *Asterias amurensis* (Nishigaki et al. 1996) has sperm-attracting activity (Böhmer et al. 2005). Asterosaps are 3.8-kDa glutamine-rich polypeptides and have an intramolecular disulfide linkage between 8C and 32C (Nishigaki et al. 1996). In another starfish, *Pycnopodia helianthoides*, the sperm attractant was identified as the 12-kDa protein named Startrak, and the sequence of N-terminal 34 amino acids has been decoded (Miller and Vogt 1996; Punnett et al. 1992). Interestingly, the synthetic 32-amino-acid peptide, which is part of the decoded region of Startrak, has a stronger sperm-attracting activity than the purified Startrak (Miller and Vogt 1996). Furthermore, the N-terminus sequence of Startrak (xxAELGLCIARVRQQNQGQDDVSIYQAIMSQCQS) has a high degree of homology with the sequences of Asterosaps (e.g., sequence of Asterosap P15: GGTQFGVCIARVRQQHQGQDEASIFQAILSQCQS) (Böhmer et al. 2005). Therefore, the region is important for the chemotaxis of starfish sperm.

Like resact in the sea urchin *A. punctulata*, Asterosaps bind to a membrane-type guanylyl cyclase on the sperm membrane and increase $[Ca^{2+}]_i$ via the increase in cGMP (Matsumoto et al. 2003; Nishigaki et al. 2000).

3.4.6 Urochordate

Urochordate (tunicate) is one of the primitive groups (subphylum) of chordates, and it consists of ascidians (Ascidiacea), salps (Thaliacea), and larvaceans (Larvacea). In tunicates, ascidian species have been used as materials for developmental biology for a century, and fertilization of ascidians has been well investigated. The first observation of sperm behavior around the egg was observed in the ascidian *Styela partita* a century ago: Conklin observed that the spermatozoa accumulated at the vegetal pole of the egg (Conklin 1905). Several decades later, Miller studied sperm activation and chemotaxis in the ascidians (Miller 1975, 1982; Minganti 1951). In ascidians, the sperm activators and attractants are released from egg cells and pass through the vitelline membranes (Yoshida et al. 1993). In ascidians, species specificity is observed in not only sperm chemotaxis but also sperm activation (Yoshida 2014; Yoshida et al. 2013), probably because the sperm attractant has both sperm-activating and sperm-attracting activities (Yoshida et al. 1994, 2002). Thus, the attractant of ascidians was called sperm-activating and -attracting factor (SAAF) (Yoshida et al. 1994). Species specificity of sperm chemotaxis is not so strict: spermatozoa of some species show chemotactic behavior toward egg extracts from congeneric species (Miller 1982, 1985b; Yoshida et al. 2013). Actually, the SAAF in the ascidians *Ciona intestinalis* (type A; also called *C. robusta*) and *Ciona savignyi* is the same molecule: the polyhydroxysterol sulfoconjugate (25S)-3α,4β,7α,26-tetrahydroxy-5α-cholestane-3,26-disulfate (*Ciona*-SAAF) (Oishi et al. 2004;

Yoshida et al. 2002). Furthermore, we identified the SAAF of another ascidian, *Ascidia sydneiensis,* as 3α,7α,8β,26-tetrahydroxy-5α-cholest-22-ene-3,26-disulfate (*Assydn*-SAAF) (Matsumori et al. 2013; Watanabe et al. 2018). Differences of SAAF between the two genus (*Ciona* and *Ascidia*) comprise only of the position of the OH group and the double bond. Such small differences in the SAAFs may be sufficient for species-specific responses.

In the activation of the *Ciona* sperm, SAAF binds to the receptor, and Ca^{2+} channel is activated followed by Ca^{2+} influx (Yoshida et al. 1994). Then, Ca^{2+} binds to CaM and activation of CaM/CaM kinase pathway occurs (Nomura et al. 2004). Through the regulation of K^+ channel by CaM kinase, hyperpolarization occurs, and adenylated cyclase is activated followed by an increase in the concentration of cAMP (Izumi et al. 1999). Finally, dynein light chain and axonemal protein are phosphorylated, triggering the sperm activation (Nomura et al. 2000) (see Fig. 3.1).

On the other hand, in spermatozoa showing chemotactic behavior, transient increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is observed periodically (Ca^{2+} burst), and the Ca^{2+} bursts play a key role in the regulation of flagellar beating (Shiba et al. 2008). Calaxin, a Ca^{2+}-sensor protein associating axonemal dynein (Mizuno et al. 2009), mediates the pattern of the sperm flagellar beating during chemotactic behavior. Calaxin and Ca^{2+} may regulate dynein-mediated microtubule sliding in the axonemes, resulting in the control of the propagation of asymmetric flagellar bending (Mizuno et al. 2012).

Recently, the SAAF receptor in the sperm of *C. intestinalis* was identified as a plasma membrane Ca^{2+}-ATPase (PMCA) (Yoshida et al. 2018). PMCA may probably be continuously activated by SAAF to keep $[Ca^{2+}]_i$ at low levels, and when the spermatozoon detects a decrease in SAAF, SAAF may detach from PMCA and become inactivated, resulting in the Ca^{2+} bursts (Yoshida et al. 2018).

Sperm chemotaxis of tunicates other than ascidians has also been observed in the larvacean *Oikopleula dioica* (Miller and King 1983).

3.5 Molecular Mechanisms of Sperm Activation and Chemotaxis

As described in Sect. 3.4, sperm activation and chemotaxis are highly diverse systems in animals. Molecular mechanisms controlling sperm motility are well investigated in mammalian species, especially in mouse and human, but those in aquatic animals have been studied only in a few species. Since there are many review papers describing molecular mechanisms in sperm activation and chemotaxis (Darszon et al. 2008; Kaupp et al. 2008; Kaupp and Strunker 2017; Nishigaki et al. 2014; Yoshida et al. 2008; Yoshida and Yoshida 2011, 2018), in this section we describe only the essence of common mechanisms. Summary of the signaling cascades of sperm activation in the three animal groups—clams, sea urchins, and ascidians—was shown in Fig. 3.1.

3.5.1 Intracellular Ca^{2+} Concentration Is the Most Important Factor in the Sperm Activation and Chemotaxis

The most important player for regulating sperm activation and chemotaxis is Ca^{2+}. In a spermatozoon showing chemotactic responses, requirement of extracellular Ca^{2+} is seen in many animals (Yoshida and Yoshida 2011), and transient Ca^{2+} increases have been observed in the sea urchins and the ascidian (Böhmer et al. 2005; Guerrero et al. 2010; Shiba et al. 2008; Wood et al. 2003). Thus, Ca^{2+} mediates sperm flagellar movement during chemotactic behavior. However, the precise role of Ca^{2+} in flagellar movement during sperm chemotaxis remains poorly understood. In the ascidian, Calaxin, which is a Ca^{2+}-sensor protein associating axonemal dynein, appears to mediate Ca^{2+}-induced asymmetrical beating of the sperm flagellum (Mizuno et al. 2009, 2012), and the protein may be a global player in spermatozoa of other animals.

How are the Ca^{2+} transients controlled by the sperm attractants? In mammals, it is known that a sperm-specific Ca^{2+} channel, CatSper, plays a crucial role in regulating sperm function (Lishko and Mannowetz 2018). Sperm chemotaxis of the sea urchin sperm also seems to be mediated by CatSper (Seifert et al. 2015). On the other hand, some taxon in deuterostomes, including bony fishes and amphibians, and all protostomes seems to lack CatSper (Cai and Clapham 2008). Furthermore, in the ascidian sperm chemotaxis, the sperm attractant seems to mediate intracellular Ca^{2+} via the Ca^{2+} pump, even though the role of CatSper in the ascidian sperm is not elucidated (Yoshida et al. 2018). Interestingly, the Ca^{2+} transients in the sperm are observed when the spermatozoon swims away from the egg (Böhmer et al. 2005; Guerrero et al. 2010; Shiba et al. 2008). The model in the ascidian easily explains the phenomena. On the other hand, in the sea urchin sperm, the Ca^{2+} transients seem to be induced by increases in the attractant after an appropriate delay (Böhmer et al. 2005; Kashikar et al. 2012). It seems that the sensing system of the sperm attractant may be diverse in the animals.

3.5.2 Other Factors

Changes in membrane potential and pH are also important events in signaling in sperm activation and chemotaxis even though these are not molecules; these two events modulate the Ca^{2+} signals.

Change of intracellular pH—in many cases alkalization was observed in spermatozoa—appears to involve a sperm-specific Na^+/H^+ exchanger (Wang et al. 2007), and alkalization drives the CatSper channel (Kirichok et al. 2006). In contrast, molecules regulating membrane potential in spermatozoa are varied, although hyperpolarization of the sperm membrane is induced by an efflux of K^+. In the activation of sea urchin sperm, hyperpolarization of the sperm was induced by cyclic nucleotide-gated K^+-selective channel (Babcock et al. 1992; Galindo et al. 2000). Although

other players mediating K^+ efflux have not been identified in aquatic invertebrates, it is known in mammalian sperm that some K^+ channels, such as Slo3, are involved in hyperpolarization and Ca^{2+} regulation (Chavez et al. 2014; Schreiber et al. 1998). For details, see the reviews (Nishigaki et al. 2014; Ritagliati et al. 2018; Yoshida and Yoshida 2018).

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