

Chapter 1

Sperm Chemotaxis: The First Authentication Events Between Conspecific Gametes Before Fertilization

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Abstract Sperm chemotaxis toward eggs before fertilization has been observed in many living organisms. Sperm chemotaxis is the first communication or signaling event between male and female gametes in the process of fertilization, and species-specific events occur in many cases. Thus, sperm chemotaxis may act as a safety process for authenticating that fertilization occurs between conspecific egg and sperm and helps to prevent crossbreeding. Here, we introduce mechanisms of sperm chemotaxis, focusing on cross-talk between gametes and species specificity. Furthermore, we discuss the interactions between sperm-activating and sperm-attracting factors (SAAFs) in the ascidian species and that SAAF receptors on sperm cells are not all-or-none responses. The SAAF receptors may accept SAAF of related species (closely related molecules), with different affinities.

Keywords Fertilization • Species specificity • Sperm chemotaxis

1.1 Introduction

In all living organisms, male gametes are activated, with increase in their motility, and are subsequently attracted toward a female gamete in response to certain factors released from the female gametes or reproductive organs. Chemotactic behavior of male gametes toward the ovule was first described in Kingdom Plantae, bracken fern (Pfeffer 1884). Brown algae have also developed chemoattractants for male gametes, known as sexual pheromones (Maier and Müller 1986). In flowering plants, peptidic factors called LUREs attract the pollen tube toward the ovules, resulting in

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guiding the sperm cell to the ovule (Okuda et al. 2009). Gamete chemotaxis was also observed in Kingdom Fungi, and the aquatic fungus *Allomyces macrogynus* shows gamete chemotaxis (Machlis 1973).

In Kingdom Animalia, sperm chemotaxis toward the egg was first observed in the hydrozoan *Spirocodon saltatrix* (Dan 1950), and such an ability is now widely recognized in marine invertebrates, from cnidarians to ascidians (Miller 1966, 1985b; Cosson 1990), and in vertebrates, from fish to humans (Oda et al. 1995; Pillai et al. 1993; Suzuki 1958, 1959; Eisenbach 1999; Yanagimachi et al. 2013). In nematodes, spermatozoa are unflagellated but use an amoeboid movement to move from the bursa through the uterus to the spermatheca (Ward and Carrel 1979). A sperm-guiding factor present in the micropyle area of the egg of the teleost rosy barb has also been described (Amanze and Iyengar 1990).

In many cases, species specificity of sperm chemotaxis is present. Thus, these phenomena constitute the first communication event between the gametes during fertilization and prevent crossbreeding among different species. In this chapter, we review sperm chemotaxis and focus on the species specificity of this phenomenon.

1.2 Chemical Nature of Sperm Chemoattractants

Chemoattractant molecules for sperm in plants are low molecular weight organic compounds such as the bimalate ions in the bracken fern (Brokaw 1957, 1958) and unsaturated cyclic or linear hydrocarbons, such as ectocarpene, in the brown algae (Maier and Müller 1986). In the aquatic fungus *A. macrogynus*, the female gametes release a sesquiterpene “sirenin” as a attractant for male gametes (Machlis 1973), and interestingly, a different compound called “parisin” released by the male gametes is able to attract flagellated female gametes of the same species (Pommerville and Olson 1987).

In animals, sperm chemoattractants have been identified in several species, and most of these chemoattractant molecules are proteins or peptides. Chemoattractants such as “resact” in sea urchins (Ward et al. 1985; Guerrero et al. 2010), “sepsap” in cuttlefish (Zatylny et al. 2002), and “asterosap” in starfish (Böhmer et al. 2005) are peptides. A 21-kDa protein named “allurin” in the amphibian *Xenopus laevis* (Olson et al. 2001) and tryptophan in abalone (Riffell et al. 2002) act as sperm chemoattractants. In the hydrozoan *Hippopodius hippopus*, the attractant has not yet been identified but has been characterized as a small and thermoresistant protein with a molecular mass of 25 kDa and an isoelectric point of 3.5 (Cosson et al. 1986). Recently, a Coomassie Blue-affinity glycoprotein, “Micropyle Sperm Attractant” (MSA), around the opening and inside of the micropyle of herring and flounder eggs has been identified that guides (“attract”) the spermatozoa into the micropyle (Yanagimachi et al. 2013).

On the other hand, nonproteinaceous chemoattractants have been identified in coral and ascidians: the chemoattractant of the coral *Montipora digitata* is an unsaturated fatty alcohol (Coll et al. 1994), and those of the ascidians *Ciona intestinalis*

and *Ascidia sydneiensis* (Yoshida et al. 2002; Matsumori et al. 2013) are sulfated hydroxysterols. Mammalian spermatozoa also show chemotactic behavior, and many candidate chemoattractants for spermatozoa have been proposed (Eisenbach and Giojalas 2006). Recently, progesterone released from the cumulus oophorus was considered as a candidate of sperm attractant for human sperm (Guidobaldi et al. 2008). On the other hand, odorants such as bourgeonal (Spehr et al. 2003) and lylal (Fukuda et al. 2004), which are aromatic aldehydes used in perfumes, could also act as chemoattractants in human and mouse sperm, respectively.

Where are the sperm chemoattractants released? Fern sperms show a chemotactic response to secretions from the female reproductive structures (Pfeffer 1884). Sperm attractants of sea urchins and sea stars (starfish) are derived from the egg jelly (Ward et al. 1985; Nishigaki et al. 1996), and the source of sperm attractant of the hydrozoan, the siphonophore, is a cupule, the extracellular structure of the egg (Carré and Sardet 1981). Therefore, sperm attractants are released from the egg accessory organs or female gametes in these species. In contrast, in ascidians, sperm-attracting activity does not originate from the overall egg coat as a layer of jelly surrounding the eggs, but originates from the egg (Yoshida et al. 1993), indicating that the eggs themselves release the chemoattractant for the sperm.

1.3 Ca^{2+} Changes Mediate Sperm Chemotaxis

In all examples of well-characterized chemotaxis, the intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) appears to be a common element of absolute necessity in the attraction mechanism (Kaupp et al. 2008; Yoshida and Yoshida 2011). Ca^{2+} plays a key role in the regulation of flagellar beating, and in the case of sea urchin spermatozoa, the sperm attractant triggers $[\text{Ca}^{2+}]_i$ fluctuations (Böhmer et al. 2005; Wood et al. 2005) that appear to correlate with the asymmetrical beating of sperm flagella (Brokaw et al. 1974; Brokaw 1979). In the hydrozoan siphonophores, the diameters of the sperm trajectories decrease on approach of the sperm to the cupule (a sperm-attracting accessory organ of the egg), but the sperm trajectories are unchanged in the absence of Ca^{2+} (Cosson et al. 1984). A similar role for extracellular Ca^{2+} in mediating flagellar asymmetry of the spermatozoon during chemotactic behavior has been reported in hydrozoa (Miller and Brokaw 1970; Cosson et al. 1984). In ascidians, the spermatozoa normally exhibit circular movements, as just described, and maintain $[\text{Ca}^{2+}]_i$ at very low levels (Shiba et al. 2008). During chemotactic behavior the spermatozoa produce frequent and transient increases of $[\text{Ca}^{2+}]_i$ in the flagella (Ca^{2+} bursts) (Shiba et al. 2008). Interestingly, the Ca^{2+} bursts are consistently evoked at points at which the spermatozoon is around a temporally minimal value for a given sperm-activating and sperm-attracting factor (SAAF) concentration (Shiba et al. 2008) and to trigger a sequence of “turn-and-straight” movements. These data suggest that sperm attractants induce Ca^{2+} entry from extracellular spaces into the sperm cell, and the resultant increase in $[\text{Ca}^{2+}]_i$ mediates the beating of sperm flagella, resulting in chemotactic “turn-and-straight” movements.

1.4 Specificity of Sperm Chemotaxis in Species Other Than Ascidians

As described here, the molecular structures of sperm chemoattractants are different in different species, and factors from one species cannot attract the sperm of another species. This specificity ensures species-specific fertilization by preventing cross-breeding. Species or genus specificity in sperm chemotaxis has been observed in hydrozoa (Miller 1979) and in echinoderms, other than sea urchins (Miller 1985a, 1997). However, no chemotactic cross-reactivity exists in siphonophore species examined, and contact with seawater without attractants is enough to activate sperm motility, although the presence of Ca^{2+} ions in seawater is involved in the chemoattraction process (Cosson 1990). Mammalian species also seem to share a common sperm attractant molecule (Sun et al. 2003; Guidobaldi et al. 2008; Teves et al. 2006), suggesting the lack of species specificity. In Mollusca, even though the abalone species seem to show species specificity in sperm chemotaxis (Riffell et al. 2004), there is a lack of species specificity of sperm chemotaxis among chitons (Miller 1977).

1.5 Species Specificity of Sperm Chemotaxis in Ascidians

In ascidians, species-specific sperm agglutination was reported in the early 1950s in five Mediterranean ascidians (Minganti 1951), and precise species-specificity tests of sperm attractants in egg ethanol extracts were also described in many ascidian species (Miller 1975, 1982) (Table 1.1). In these studies, ascidian sperm chemotaxis or agglutination tend to be species specific, but cross-reactivity among many species was also observed (Table 1.1). In particular, a lack of specificity was evident within the genus *Styela* (Miller 1975, 1982) (Table 1.1). However, the study on species specificity of ascidians contained both the order Phlebobranchiata, including the genus *Ciona*, and the order Stolidobranchiata, including the genus *Styela*, which are genetically distant, as per recent taxonomic data (Zeng et al. 2006; Tsagkogeorga et al. 2009).

We have previously identified the sperm chemoattractant released from the eggs of *Ciona intestinalis* as (25*S*)-3 α ,4 β ,7 α ,26-tetrahydroxy-5 α -cholestane-3,26-disulfate, which was designated as the *Ciona* sperm-activating and -attracting factor (Ci-SAAF) (Yoshida et al. 2002; Oishi et al. 2004). The synthesized Ci-SAAF molecule possesses abilities to both activate motility and attract sperm (Yoshida et al. 2008; Oishi et al. 2004). The SAAF of another *Ciona* species, *C. savignyi* (Cs-SAAF), seems to be identical with Ci-SAAF and presents no specificity for the sperm activation of *C. savignyi* and vice versa (Yoshida et al. 1993, 2002). We have also recently identified As-SAAF from another phlebobranchian species, *A. sydneyensis*, as 3 α ,7 α ,8 β ,26-tetrahydroxy-5 α -cholest-22-ene-3,26-disulfate (Matsumori et al. 2013); this was the first study leading to the identification of the chemoattractants of related species in Kingdom Animalia. Unexpectedly, Ci-SAAF and

Table 1.1 Species-specificity tests of sperm chemotaxis or agglutination in ascidians

		Sperm											
		1 ^a	2	3	4	5	6	7	8	9	10	11	12
Egg extracts	<i>Ciona intestinalis</i>	1	++	++	- /	-	-	-	-	-	-	-	-
	<i>Ascidia callosa</i>	2	+	++	/ /	++	- /	/ /	/ /	/ /	/ /	/ /	/ /
	<i>Corella inflata</i>	3	-	-	-	-	- /	/ /	-	-	-	-	-
	<i>Corella willmeriana</i>	4	±	+	±	++	++	/ /	/ /	/ /	/ /	/ /	/ /
	<i>Chelyosoma productum</i>	5	/	-	/ /	++	/ /	/ /	/ /	/ /	/ /	/ /	/ /
	<i>Pyura haustor</i>	6	-	-	/ /	-	±	/ /	/ /	/ /	/ /	/ /	/ /
	<i>Styela plicata</i>	7	-	/ /	/ /	/ /	-	++	-	-	/ /	/ /	/ /
	<i>Styela clava</i>	8	-	/ /	- /	-	-	±	-	/ /	/ /	/ /	/ /
	<i>Styela montereyensis</i>	9	-	-	-	-	+	+	+	++	+	+	-
	<i>Styela gibbsii</i>	10	-	-	-	-	+	+	/ /	-	++	/ /	/ /
	<i>Boltenia villosa</i>	11	-	-	-	-	/	±	/ /	/ /	/ /	++	/ /
	<i>Halocynthia igaboja</i>	12	-	-	-	+	+	- /	/ /	/ /	/ /	+	++

Species 1–5 are Phlebobranchia; species 6–12 are Stolidobranchia
 ++ strong activity, + weak activity, ± uncertain response, – negative response, / test not done
^aNumbers show the same species shown in egg extracts
 Source: Miller (1982)

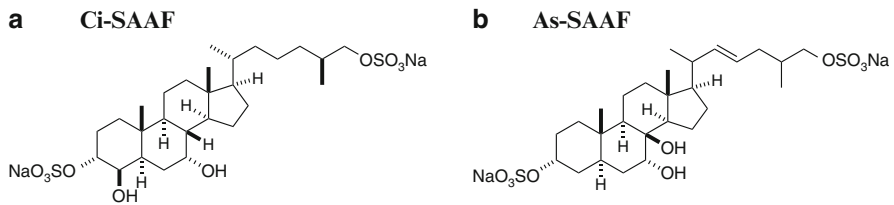


Fig. 1.1 Molecular structure of ascidian sperm attractants: *Ciona intestinalis* (Ci-SAAF) (a); *Ascidia sydneiensis* (As-SAAF) (b)

As-SAAF vary only by one double bond and the position of the OH group (Fig. 1.1). Even such a small difference in the sperm attractant molecules is enough to result in species-specific responses.

The cross-reactivity data of sperm chemotaxis for several ascidian species belonging to order Phlebobranchia show some specificity in the cross-reactivity between egg-conditioned seawater (ESW) and sperm response when comparing *Ciona* versus *Phallusia* and *Phallusia* versus *Ascidia*. However, this does not seem to be true in all cases in terms of “species” or “genus” specificity. For example, there is a “one-way” (no reciprocity) cross-reaction between *C. savignyi* and *A. sydneiensis* (Table 1.2) (Yoshida et al. 2013). Furthermore, even when a cross-reaction is observed, the level of activity is different. The interactions between the SAAFs in the ascidian species and the SAAF receptors on the sperm cells are not all-or-none responses. The SAAF receptor may accept SAAFs of related species, which are closely related molecules, with different affinity. Hence, sperm chemotaxis is neither a “species”- nor a “genus”-specific phenomenon among ascidians.

Table 1.2 Cross-reactivity in sperm chemotaxis elicited by egg-conditioned seawater (ESW) from different ascidian species

		Sperm					
		1 ^a	2	3	4	5	
Egg-conditioned seawater	<i>Ciona intestinalis</i>	1	++	++	/	/	/
	<i>Ciona savignyi</i>	2	++	++	-	/	-
	<i>Phallusia nigra</i>	3	/	-	++	/	-
	<i>Phallusia mammillata</i>	4	/	±	+	++	-
	<i>Ascidia sydneiensis</i>	5	/	+	-	/	++

++ active, + weakly active, ± uncertain response, - negative, / not examined

^aNumbers show the same species shown in egg extracts

Source: Yoshida et al. (2013)

1.6 Conclusion

Sperm chemotaxis appears to be a much more specific phenomenon at the species or genus level in many animal species: cnidarians (Miller 1979), echinoderms other than sea urchins (Miller 1985a, 1997), and ascidians (Miller 1982; Yoshida et al. 2013). These results indicate that the specificity of sperm chemotaxis participates in the prevention of crossbreeding at fertilization. It is hypothesized that the interaction between sperm attractants from egg and attractant receptors on the sperm does not result in all-or-none responses, and that attractant receptors may accept some heterospecific sperm attractants having related chemical structures but with different binding or dissociation constants. Research into the precise chemical nature of sperm attractants and their corresponding receptors in different species may provide new horizons for studies of the fertilization system, especially on the mechanisms by which authentic interactions between conspecific eggs and spermatozoa occur.

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