Towards Understanding the Molecular Mechanism of Sperm Chemotaxis

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The awareness that, in most animal species, the prospects of the male's spermatozoa to reach the female's eggs are very slim in the absence of some guidance mechanism (usually chemical in nature) has been acquired gradually; first, in marine species, where both types of gametes are released into sea water (for review see Miller, 1985), and ultimately, in the last decade or so, in mammals (for review see Eisenbach, 1999). Such chemical guidance, sperm chemotaxis, is now recognized in many marine invertebrates, fish, amphibians, and a few mammals (humans included) (Eisenbach, 2004). This suggests that sperm chemotaxis is a general guidance mechanism, irrespective of whether the fertilization is external, like in most marine species, or whether it is internal, as in mammals.

In spite of this generality, there are some basic differences between sperm chemotaxis of mammals and that of marine invertebrates, the most pronounced one being the fractional chemotactic response in the former; namely, the restriction of the chemotactic response to a subpopulation of the spermatozoa. Unlike spermatozoa of marine species, spermatozoa of mammals must undergo a process of maturation, termed capacitation, for acquiring the ability to bind to the egg and penetrate it (for review see Jaiswal and Eisenbach, 2002). At any given time, the percentage of capacitated spermatozoa is small due to both the limited time window of the capacitated stage and the continuous replacement of capacitated cells in the sperm population (Cohen-Dayag et al., 1995). Since the role of sperm chemotaxis is to bring to the egg spermatozoa that are capable of fertilizing it, it is not surprising that, unlike in marine invertebrates where most, if not all, the spermatozoa appear to be chemotactically responsive, in mammals only the small fraction of capacitated spermatozoa are chemotactic (Cohen-Dayag et al., 1994, 1995; Fabro et al., 2002). Because of the massive chemotactic response in marine invertebrates and its much earlier discovery, most of our, limited, knowledge about the molecular mechanism of sperm chemotaxis is in these species.

The "*Escherichia coli*" of spermatozoa, namely, the system in which sperm chemotaxis has been most investigated, is the sea urchin, primarily the species *Arbacia punctulata*. First, Ward et al. (1985) demonstrated the

occurrence of Ca^{2+} -dependent sperm chemotaxis to resact, a 14-mer peptide that belongs to the family of sperm-activating peptides (Suzuki, 1995), isolated from the egg jelly layer of *A. punctulata*. Thereafter, the receptor for resact was identified as a guanylyl cyclase (Singh et al., 1988). Resact binding to this receptor turns on the guanylyl cyclase activity of the latter (Garbers, 1989), and the resulting rise in cGMP apparently triggers a cascade of signal transduction events, one of which is elevation of the intracellular concentration of Ca^{2+} ($\text{Ca}^{2+}_{\text{in}}$) (Cook et al., 1994).

It was assumed that additional information about the signaling cascade in sperm chemotaxis also could be obtained from signal transduction pathways triggered by different sperm-activating peptides in other sea urchins. For example, relatively much information has been accumulated about signaling in spermatozoa of *Strongylocentrotus purpuratus*, a sea urchin that is evolutionary ~200 million years apart from *A. punctulata* (Smith, 1988) and whose sperm-activating peptide is speract, a decamer. Although sperm chemotaxis of *S. purpuratus* to speract has not been demonstrated, Cook et al. (1994) found similarities between the resact- and speract-induced responses of spermatozoa of *A. punctulata* and *S. purpuratus*, respectively: both resact and speract raised $\text{Ca}^{2+}_{\text{in}}$, and a phosphodiesterase inhibitor prolonged these responses and similarly increased the flagellar waveform asymmetry of both types of spermatozoa with resultant more circular swimming paths. Cook et al. (1994), therefore, suggested that the resact- and speract-triggered signaling cascade for controlling $\text{Ca}^{2+}_{\text{in}}$ and the flagellar response is conserved in these species. Models, based primarily on studies with *S. purpuratus*, were proposed for chemotactic signaling in sea urchin spermatozoa (Cook et al., 1994; Darszon et al., 2001). According to these models, chemoattractant binding to its receptor activates a guanylyl cyclase. The resulting rise in the cGMP concentration promotes K^{+} efflux through a cGMP-dependent K^{+} channel, causing hyperpolarization; this hyperpolarization activates $\text{Na}^{+}/\text{H}^{+}$ exchange (with a consequent rise in the intracellular pH, pH_{in} , and Na^{+} influx), adenylyl cyclase (with a consequent elevation of the intracellular concentration of cAMP and then a cAMP-mediated rise in $\text{Ca}^{2+}_{\text{in}}$), a K^{+} channel, and, possibly,

$\text{Na}^+/\text{Ca}^{2+}$ exchange to maintain low $\text{Ca}^{2+}_{\text{in}}$. Finally, this low concentration of Ca^{2+} results in linear swimming up the chemoattractant gradient. However, because sperm chemotaxis to speract has not been demonstrated in *S. purpuratus* and because, unlike the case of *A. punctulata*, the receptor of *S. purpuratus* spermatozoa is not a guanylyl cyclase but rather a protein that, in response to speract binding, activates the guanylyl cyclase (Garbers, 1989), it is not at all clear to what extent the information obtained for speract-activated signaling in *S. purpuratus* is relevant to chemotactic signaling in *A. punctulata* by resact.

A breakthrough in revealing the molecular mechanism of sperm chemotaxis of sea urchin was made when, in a beautiful study that involved rapid mixing techniques and novel caged compounds of cyclic nucleotides and of the chemoattractant resact, Kaupp et al. (2003) demonstrated in *A. punctulata* that the first event following resact stimulation is, as expected, a rapid and transient rise in the cGMP concentration, followed by a transient increase in the Ca^{2+} concentration. Resact also stimulated a smaller and slower rise in cAMP. Interestingly, resact triggered two distinct Ca^{2+} responses: an early and a late response. The cGMP response and the early Ca^{2+} response were very sensitive; the binding of a single resact molecule could elicit a measurable response, and 50–100 bound molecules already saturated the response. These results suggested that binding of resact to guanylyl cyclase results in rapid rise of cGMP, and that this rise, perhaps indirectly, opens Ca^{2+} channels with a resultant increase in $\text{Ca}^{2+}_{\text{in}}$, affecting the asymmetry of the sperm flagellum. Kaupp et al. (2003) further suggested that the slower rise of cAMP and the second rise of Ca^{2+} might be involved in adaptation of the cells to the chemotactic response.

These results were inconsistent with some aspects of the models described above for speract-triggered signaling cascade in *S. purpuratus*, primarily with their prediction that the stimulant should cause an initial decrease in $\text{Ca}^{2+}_{\text{in}}$. Does this mean that resact-induced signaling in *A. punctulata* is different from speract-induced signaling in *S. purpuratus*? In a study that is published in this issue of the *Journal of General Physiology*, Solzin et al. (2004) addressed this question. A key feature of the speract-based model is that a rise in pH_{in} precedes Ca^{2+} entry and that the former is a prerequisite for the latter (Cook and Babcock, 1993; Cook et al., 1994). Using rapid mixing techniques with *A. punctulata*, Solzin et al. (2004) found that the opposite happens: the resact-stimulated rise in $\text{Ca}^{2+}_{\text{in}}$ precedes the rise in pH_{in} . Furthermore, imidazole, a membrane-permeant proton buffer, abolished the resact-stimulated changes in pH_{in} , but it had no effect on the $\text{Ca}^{2+}_{\text{in}}$ change and on the swimming response of *A. punctulata* to resact. Rapid

photorelease of cGMP intracellularly from a caged compound, known to cause a rapid and transient rise in $\text{Ca}^{2+}_{\text{in}}$ (Kaupp et al., 2003), as well as photorelease of cAMP, did not affect pH_{in} (Solzin et al., 2004). All these results suggest that the resact-stimulated signaling cascade in chemotaxis of *A. punctulata* is not dependent on pH_{in} . To determine whether the observed differences between these results and the models for the speract-triggered signaling cascade in *S. purpuratus* are due to the species difference, Solzin et al. (2004) repeated in *S. purpuratus* the same series of experiments, with speract substituting for resact. The results were similar, suggesting that the difference in species is not the cause. Solzin et al. (2004) further suggest that the rise in pH_{in} is likely due to the proton-consuming process of replenishment of the GTP and ATP pools.

The above findings made in Kaupp's group, are a leap advance toward revealing the molecular mechanism of sperm chemotaxis of sea urchin and, possibly, in sperm in general. However, the road to complete understanding of the molecular mechanism is still at its onset. We probably still do not recognize all the players in chemotactic signaling and we do not know how they integrate into a signaling network. Even less is known about the correlation between the molecular events and the swimming behavior of the spermatozoa and about how single cells respond to a chemoattractant gradient. For example, the linearity of swimming generally decreases only when spermatozoa swim down, not up, a chemoattractant gradient (Miller and Brokaw, 1970). In contrast, $\text{Ca}^{2+}_{\text{in}}$ increases rather than decreases in response to resact stimulation (Cook et al., 1994; Kaupp et al., 2003), and elevation of $\text{Ca}^{2+}_{\text{in}}$ is known to increase flagellar asymmetry and reduce linearity of swimming (Brokaw et al., 1974; Cook et al., 1994). Furthermore, photorelease of resact affects the swimming path of the spermatozoa only after a lag period of several hundreds of milliseconds (Kaupp et al., 2003), suggesting that there is a mechanism which delays the sperm's swimming response to the initial rise in Ca^{2+} . This delay may allow spermatozoa swimming up a chemoattractant gradient to continue in the same direction for as long as the chemoattractant concentration increases.

This scanty knowledge about the molecular mechanism of sperm chemotaxis may seem astonishing in view of the fact that, in another system, in bacteria, chemotaxis is perhaps the best-understood system among all signal transduction systems (Eisenbach, 2004). The reason for this striking gap between bacteria and spermatozoa is probably the availability of specific mutants in the former. This situation may change with the gradually increasing availability of knockout mice defective in specific genes with consequent fertilization defects. One such prominent example is the recent finding of

FIGURE 1. A scheme, simplified to a large extent, demonstrating two potential signal transduction pathways in mammalian sperm chemotaxis. (A) A pathway initiated with a guanylyl cyclase receptor, based on the molecular events found by Kaupp et al. (2003). (B) A pathway starting with a G protein-coupled receptor that belongs to the family of olfactory receptors. A basic difference between the pathways is that, in A, the excitatory rise in $\text{Ca}^{2+}_{\text{in}}$ (distinct from the $\text{Ca}^{2+}_{\text{in}}$ increase during the presumed adaptation) is triggered by cGMP elevation, whereas in B, it is triggered by cAMP increase. An upward arrow indicates an increase.

Quill et al. (2003) that mouse spermatozoa, in which the gene for the protein that belongs to the family of sperm-specific cation channels, CatSper2, was disrupted, are solely defective in their hyperactivated motility, a motility form restricted to capacitated spermatozoa. Examination of whether the mutated spermatozoa are defective in chemotaxis is likely to yield an insight into the molecular mechanism of sperm chemotaxis in mammals.

What do we know about the molecular mechanism of sperm chemotaxis in mammals? Can we assume that the molecular mechanisms of sperm chemotaxis are similar in all species whose spermatozoa swim (distinct from species, like nematodes, whose spermatozoa crawl rather than swim; Bottino et al., 2002)? In other words, are the findings made in spermatozoa of sea urchins and other marine species with respect to the molecular mechanism of sperm chemotaxis relevant to mammalian spermatozoa? The answer to these questions is neither trivial nor obvious. On the one hand, assuming universality, the molecular mechanisms may be similar. The finding in human spermatozoa that atrial natriuretic peptide (ANP, a known activator of particulate guanylyl cyclase) is chemotactically active and the consequent suggestion that ANP may directly affect guanylyl cyclase in a manner similar to that caused by the physiological attractant (Zamir et al., 1993) are in line with this possibility. On the other hand, the recent identification of the odorant receptor hOR17-4 on human spermatozoa and the demonstration of sperm chemotaxis to its agonist bourgeonal (Spehr et al., 2003) suggest that mammalian sperm chemotaxis involves a signal transduction pathway similar to that of the olfactory system. This may seem a valid possibility in view of

the finding that male germ cells appear to contain all the elements of the signaling cascade present in olfactory cells (Defer et al., 1998) and the observation that bourgeonal induces a transient Ca^{2+} influx in about one third of the cells of human spermatozoa, a response that is inhibited by an adenylyl cyclase inhibitor (Spehr et al., 2003). The differences between both potential pathways are schematically shown in Fig. 1. The possibility that mammalian spermatozoa possess both signal transduction systems is valid as well. For example, it is possible that there are two complementary systems, each serving as a backup for the other, that different chemoattractants trigger different signal transduction systems, or that both pathways merge into a single signaling pathway. The time is ripe to determine whether mammalian spermatozoa possess one or more signal transduction systems for chemotaxis and, in the latter case, to establish whether each system operates in response to a different chemoattractant or whether each of them functions in parallel. I have no doubt that the exciting field of sperm chemotaxis still conceals a few surprises.

M. Eisenbach is an incumbent of the Jack and Simon Djanogly Professorial Chair in Biochemistry. Work in M. Eisenbach's laboratory is supported by the Horowitz Foundation, the Philip M. Klutznick Research Fund, the Woman's Health Research Center, and the Gerald Bamberger research grant.

Olaf S. Andersen served as editor.

Submitted: 6 July 2004

Accepted: 6 July 2004

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