Sperm chemotaxis and regulation of flagellar movement by Ca\(^{2+}\)

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ABSTRACT: The chemotaxis of sperm towards eggs is a widespread phenomenon that occurs in most forms of life from lower plants to mammals and plays important roles in ensuring fertilization. In spermatozoa, the attractants act as beacons, indicating the path leading to the eggs from the same species. The existence of species-specific sperm chemotaxis has been demonstrated in marine invertebrates; thus, sperm chemotaxis may be involved in preventing crossbreeding, especially in marine invertebrates with external fertilization. However, the mechanisms of sperm chemotaxis in mammalian species differ from those of marine invertebrates. In mammals, the attractant source is not the egg, but follicular fluids or cumulus cells and chemotactic behaviour is shown only in small populations of sperm. Nevertheless, the fundamental mechanisms underlying sperm chemotaxis are likely to be common among all species. Among these mechanisms, intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) is an important factor for the regulation of chemotactic behaviour in spermatozoa. Sperm attractants induce the entry of extracellular Ca\(^{2+}\), resulting in [Ca\(^{2+}\)]\(_i\) increase in the sperm cells. Furthermore, [Ca\(^{2+}\)]\(_i\) modulates sperm flagellar movement. However, the relationship between [Ca\(^{2+}\)]\(_i\) and the chemotactic response of a sperm flagellum is not well known. Investigation of the dynamic responses of sperm cells to their attractants is important for our understanding of the regulation of fertilization. Here, we reviewed sperm chemotaxis focusing on the mechanisms that regulate sperm flagellar beating during the chemotactic response.

Key words: Ca\(^{2+}\) / chemotaxis / fertilization / sperm flagella

Introduction

From plants to humans, the spermatozoa of many species exhibit chemotactic behaviour towards eggs. Sperm chemotaxis has been extensively studied and is considered a species-specific phenomenon in marine invertebrates. Although this phenomenon is well understood in many phyla of animals and plants, sperm attractants have been identified only in some invertebrate species (Fig. 1). Furthermore, despite the importance of the dynamic changes that occur in spermatozoa in response to specific attractants, the molecular mechanism of sperm chemotactic responses has not been fully elucidated, and our understanding of the regulation of sperm chemotaxis, especially in mammals. The present study reviews the mechanisms that regulate attractant-induced sperm chemotaxis. A summary of sperm behaviour during chemotaxis and the nature of sperm attractants are followed by a description of the known mechanisms of regulation of flagellar movement induced by intracellular Ca\(^{2+}\). In addition, the putative signalling mechanisms mediated by sperm attractants in spermatozoa are discussed.

Chemical nature of sperm attractants

The first sperm attractants were identified in plants (Brokaw, 1958). In animals, sperm attractants have been identified in eight non-mammalian species; an ascidian (Yoshida et al., 2002), a coral (Coll et al., 1994), sea urchins (Ward et al., 1985; Guerrero et al., 2010), a starfish (Böhmer et al., 2005), a cuttlefish (Zatylny et al., 2002), an abalone (Riffell et al., 2002) and an amphibian (Olson et al., 2001) (Fig. 1). Six of these attractants are proteins, peptides or amino acids, and two of them are low-molecular-weight compounds. Among mammals, sperm chemotaxis towards female-derived attractants was observed in humans (Ralt et al., 1991), mice (Oliveira et al., 1999), rabbits (Fabro et al., 2002), horses (Navarro et al., 1998), pigs (Serrano et al., 2001) and bulls (Gil et al., 2008). Sperm chemotaxis towards factors in follicular fluid has been demonstrated in humans, and several candidate sperm attractants have been proposed in follicular fluid such as...
N-formylated peptides (Iqbal et al., 1980), atrial natriuretic peptide (Zamir et al., 1993), progesterone (Villanueva-Díaz et al., 1995) and regulated on activation, normal T cell expressed and secreted (RANTES) (Isobe et al., 2002) (Fig. 1). Recently, progesterone released from the cumulus oophorus was postulated as the mammalian sperm attractant (Teves et al., 2006; Guidobaldi et al., 2008), although progesterone is present in other places in the female reproductive tract. Mammalian spermatozoa are also known to contain many G-protein-coupled odorant receptors (Parmentier et al., 1992; Vanderhaeghen et al., 1993), and human olfactory receptor 17-4 (hOR17-4), one of these receptors, seems to be involved in the chemotaxis of human spermatozoa (Spehr et al., 2003). Bourgeonal, an aromatic aldehyde used in perfumery, is a potent ligand of hOR17-4 and acts as a chemoattractant (Spehr et al., 2003) (Fig. 1). Similar results have been reported in studies on murine sperm chemotaxis, and the odorant lyral was found to act as an attractant of murine spermatozoa (Fukuda et al., 2004). However, these odorants have not been identified in the female reproductive tract yet, and native ligands for the odorant receptors are as yet unknown.

There are several differences in sperm chemotaxis between mammals and marine invertebrates. One of these differences is species specificity. Sperm chemotaxis in marine invertebrates is a species- or genera-specific phenomenon (Miller, 1985), but sperm chemotaxis in mammals appears to have no species-specificity: the follicular fluids of humans, rabbits and cows attract both rabbit and human spermatozoa (Sun et al., 2003). Moreover, progesterone can act as an attractant for human and rabbit spermatozoa (Teves et al., 2006; Guidobaldi et al., 2008). However, the potent human sperm attractant bourgeonal (Spehr et al., 2003) does not attract murine sperm (Fukuda et al., 2004). A more precise analysis of the species-specificity of mammalian sperm chemotaxis is necessary, although progesterone is a good candidate as the attractant in some mammalian species.

Another significant difference in sperm chemotaxis between mammals and marine invertebrates is the heterogeneity of the

Figure 1  Identified sperm chemoattractants. (A) Attractants in invertebrate sperm. Invertebrate sperm attractants have been identified in eight species: an ascidian (1) (Yoshida et al., 2002), a coral (2) (Coll et al., 1994), sea urchins (3,4) (Ward et al., 1985; Guerrero et al., 2010), a starfish (5) (Bohmer et al., 2005), a cuttlefish (6) (Zatylny et al., 2002), an amphibian (7) (Olson et al., 2001) and an abalone (8) (Riffell et al., 2002). (B) Candidate sperm attractants in mammals are N-formylated peptides (9) (Iqbal et al., 1980), atrial natriuretic peptide (10) (Zamir et al., 1993), progesterone (11) (Villanueva-Díaz et al., 1995; Teves et al., 2006; Guidobaldi et al., 2008) and RANTES (12) (Isobe et al., 2002), which were found in follicular fluids. Certain artificial odorants act as mammalian sperm attractants: bourgeonal (13) (Spehr et al., 2003) and lyral (14) (Fukuda et al., 2004).
sperm chemotactic response. In marine invertebrates, a very large population of spermatozoa shows chemotactic behaviour in response to the attractant. On the contrary, a chemotactic response was shown in only a small subpopulation of mammalian spermatozoa. In humans, a fraction of 2–12% of spermatozoa is chemotactically responsive to progesterone, and this subpopulation is considered as the capacitated spermatozoa (Cohen-Dayag et al., 1994, 1995). Furthermore, 36% of human spermatozoa (Sphër et al., 2003) and ≈10% of murine spermatozoa (Fukuda et al., 2004) exhibited increases in Ca\(^{2+}\) levels in response to bourgeonal and lyral, respectively. Murine olfactory receptor 23, the odorant receptor for lyral, is expressed in only ≈30% of the seminiferous tubules in murine testes. Therefore, only some ejaculated spermatozoon can respond to the attractant. In humans, the diversity in genetic backgrounds could cause variation in the proportion of fertile spermatozoa, which could therefore be low in certain cases. On the other hand, experiments in mice are performed with inbred lines, and the genetic background of each mouse is therefore the same. These results suggest that a subset of cells with the complement of receptors required for fertilization is predetermined during spermatogenesis. This is surprising since predetermined ‘losing’ cells should be excluded during evolution. Thus, if the heterogeneous expression of chemotaxis-related molecules in the mammalian spermatozoa is a true reflection of chemotactic sensitivity, sperm chemotaxis is not essential for mammalian fertilization. Alternatively, other molecules may be responsible for mammalian sperm chemotaxis. In human sperm, chemotaxis towards progesterone is directly correlated to capacitation (Eisenbach and Giojalas, 2006), and heterogeneity of sperm chemotaxis may be due to low levels of capacitation rather than to the heterogeneous expression of chemotaxis-related molecules. However, if the hypothesis is true, we must consider heterogeneity of sperm capacitation. Furthermore, in vitro capacitated mouse spermatozoa also show the heterogeneity in their chemotactic response (Fukuda et al., 2004), and progesterone seems not to attract mouse sperm. Further studies are required for understanding the heterogeneous sperm response and the role of chemotaxis in selection of spermatozoa.

Analysis of the chemotactic behaviour of spermatozoa

The analysis of sperm chemotaxis in invertebrates is commonly performed using the micropipette assay. In this activity assay for sperm chemotaxis, the attractant is enclosed at the tip of a glass micropipette, the micropipette is placed in the sperm suspension and the sperm trajectories are observed. The advantage of using caged attractants is that the timing of the release of the attractant to spermatozoa can be precisely controlled; however, in this method, the maintenance of a continuous gradient field of attractants is difficult because uncaged attractants by brief light flash are easily and quickly dispersed. The quantitative evaluation of sperm chemotaxis has been performed using various methods. As described later, during the chemotactic response, spermatozoa often exhibit quick turning movements with highly asymmetric flagellar waveforms (Miller, 1985). The curvature of sperm trajectories and/or an asymmetry in sperm flagellar movements are used as indices for evaluating the chemotactic response (Miller, 1982; Cosson et al., 1984; Yoshida et al., 2002; Fukuda et al., 2004; Böhmer et al., 2005; Shiba et al., 2005; Wood et al., 2007; Guerrero et al., 2010). The linear equation chemotaxis index has been proposed as an index for the measurement of sperm chemotaxis based on sperm trajectories (Yoshida et al., 2002; Yoshida, 2004). These methods for the evaluation of sperm chemotaxis are linked to sperm flagellar movement and can be applicable to the analyses of when and where a spermatozoon responds to the attractant.

In mammals, the most commonly used method for evaluating chemotaxis is the accumulation assay (see review, Eisenbach, 1999). In this assay, the attractant is placed in one of two reservoirs of a chemotaxis-analysing apparatus that are connected by a micro porous filter or a connecting channel, which results in the formation of a gradient of analyte by diffusion. Spermatozoa are introduced into the other reservoir, and if they accumulate in the reservoir that contains the analyte, the analyte is considered a sperm attractant. Although this method provides a simple and easily administered assay, it cannot distinguish chemotaxis from sperm accumulation caused by trapping or chemokinetics (Eisenbach, 1999) because the method is based on sperm concentration. An improved method based on the direction of sperm movement was therefore proposed (Fabro et al., 2002). This method uses the Zigmund chamber to measure cell accumulation (Zigmund, 1977), but the movement of sperm in the channel between two reservoirs is recorded, and the net distances that spermatozoa travel per unit time both parallel to the attractant gradient (\(\Delta X\)) and perpendicular to it (\(\Delta Y\)) are measured. Although this method is more accurate than the accumulation assay, the analysis of the results cannot be linked to sperm flagellar movement, and it is therefore difficult to establish when and where the spermatozoon responds to the attractant.

Chemotactic behaviour of spermatozoa

The chemotactic responses of spermatozoa have been precisely determined in several marine invertebrate species. In the absence of sperm attractants, spermatozoa of many marine invertebrates show circular movement with low asymmetric flagellar beating, while mammalian spermatozoa exhibit linear movement with symmetric flagellar beating. In the hydroid siphonophore Muggiae kochi, the radius of curvature of the sperm trajectory is reduced as the spermatozoon approaches the cupule, which is the source of sperm attractant (Cosson et al., 1984). In contrast, quick turning movements of spermatozoa exhibiting chemotactic behaviour are observed in other hydrozoans. The turning movement of sperm is a typical phenotype of the sperm during chemotactic behaviour, and during the turning movements, the spermatozoon demonstrates a temporarily increased asymmetrical flagellar beating (Miller, 1966, 1982; Miller and Brokaw, 1970).

The spermatozoon of the ascidian Ciona intestinalis also exhibit quick turning movements during chemotaxis towards sperm-activating and attracting factor (SAAF), the attractant for ascidian sperm.
This movement has been named the ‘chemotactic turn’. The activated Ciona spermatozoon in seawater exhibits stable asymmetric flagellar beating, resulting in circular movement with near-constant swimming path curvatures (≈0.02 m m \(^{-2}\)). In contrast, the spermatozoon around the tip of the micropipette containing SAAF often exhibits the chemotactic turn; the flagellar waveform of the spermatozoon becomes highly asymmetric, and the swimming path curvature suddenly increases (Yoshida et al., 2002; Shiba et al., 2008) (Fig. 2). After the chemotactic turn, the spermatozoon changes its swimming pattern and swims in a straight line with low path curvatures (≈0 μm \(^{-1}\)) and symmetric flagellar waveforms (‘straight-swimming’ state). The spermatozoon then recovers its stable circular movement, which indicates that it is again in a ‘resting’ state (Shiba et al., 2008) (Fig. 2). These dynamic changes in swimming paths and asymmetry of flagellar waveforms are generally repeated, and the spermatozoa finally approach the micropipette tip. In the presence of a gradient of attractant, the chemotactic behaviour of the ascidian spermatozoa is characterized by a transient switch in the swimming patterns from ‘resting’ states to ‘turn-and-straight’ movements with quick regulation of flagellar asymmetry.

In sea urchin, on the other hand, a recent study showed that the Lytechinus pictus spermatozoa displayed dissimilar motility responses depending on their position relative to the source of the attractant uncaged by the photolysis of caged speract, the sperm attractant of L. pictus (Guerrero et al., 2010). The responses of spermatozoa in a position proximal to stimulation are biphasic: the first phase of the response of spermatozoa is the ‘turn-and-straight’ movement, swimming along negative speract gradients, and the second phase of the response is characterized by the absence of turning events and by increase in the size of the circular movements.

In contrast, mammalian spermatozoa usually exhibit straight swimming, and their flagellar beating is almost symmetric (Spehr et al., 2004). There are few studies on the sperm trajectories and flagellar movements during the chemotactic responses of mammalian spermatozoa, mostly because many studies were performed by using the accumulation assay as described earlier. However, two studies investigated the trajectories and flagellar beating of mammalian spermatozoa during chemotaxis (Fukuda et al., 2004; Spehr et al., 2004). Both studies demonstrated that the chemotactic behaviour of mammalian spermatozoa is oriented towards the source of the attractant, and the cells sometimes show highly asymmetrical flagellar beating similar to the ‘chemotactic turn’. However, frequency of the ‘chemotactic turn’-like movement in the mammalian spermatozoa is low: many sperm did not show the turn-like movement but simply orient to the attractant, and even in the sperm showing the movement, it was observed only one time during their pathway to the attractant. Thus, the mechanisms regulating the movement of mammalian spermatozoa during chemotaxis may be different from those of marine invertebrates.

**Ca\(^{2+}\) changes in sperm during chemotactic behaviour**

Ca\(^{2+}\) plays a key role in the regulation of flagellar beating. In sea urchin spermatozoa, Ca\(^{2+}\) concentration in the reactivation medium is correlated with the asymmetric flagellar beating of reactivated
demembranated sperm models (Brokaw et al., 1974; Brokaw, 1979), and sperm-activating peptides (SAPs) trigger increase in the intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) in the sperm (Cook and Babcock, 1993; Cook et al., 1994). The effect of extracellular Ca\(^{2+}\) concentration on flagellar asymmetry of the spermatozoon during chemotactic behaviour has been reported in hydrozoan (Miller and Brokaw, 1970; Cosson et al., 1984) and ascidian (Miller, 1982) spermatozoa. During the last decade, Ca\(^{2+}\) imaging techniques have improved significantly, and Ca\(^{2+}\) concentration in moving spermatozoa has been studied in sea urchins and ascidians. In sea urchins, [Ca\(^{2+}\)] increases are closely related to the chemotactic turn and flagellar asymmetry (Böhmer et al., 2005; Wood et al., 2005; Guerrero et al., 2010). In the ascidian, the spermatozoa normally exhibit circular movements as described earlier and maintain [Ca\(^{2+}\)], at very low levels, while frequently showing transient [Ca\(^{2+}\)] increases in the flagellum (Ca\(^{2+}\) bursts) during chemotactic behaviour (Shiba et al., 2008) (Fig. 3). Interestingly, the Ca\(^{2+}\) bursts are consistently evoked at a local minimal value for a given attractant concentration (Shiba et al., 2008) (Fig. 3). Similar Ca\(^{2+}\) transients in the sperm during movement down the attractant gradient are also observed in sea urchin (Böhmer et al., 2005; Guerrero et al., 2010). Therefore, sperm attractants appear to induce Ca\(^{2+}\) entry from extracellular spaces into the sperm cell, and the increase in [Ca\(^{2+}\)] mediates the beating of sperm flagella, resulting in the chemotactic turn and ‘turn-and-straight’ movements.

In mammals, transient [Ca\(^{2+}\)] increases were also induced by the candidate attractants: bourgeonal and progesterone induce [Ca\(^{2+}\)] increases in human spermatozoa (Spehr et al., 2003, 2004; Teves et al., 2009), while lyral affects mouse spermatozoa (Fukuda et al., 2004). However, a relationship between the [Ca\(^{2+}\)] increases in the sperm cells and flagellar beating has not yet been found, possibly because there are few studies on sperm movement during chemotactic behaviour as described already.

Signalling mechanisms of sperm chemotaxis: from attractant to [Ca\(^{2+}\)] increase

Despite the identification of eight sperm attractants, the receptors for these attractants have only been unequivocally identified in echinoderms among marine invertebrates. In the sea urchin, many SAPs have been identified (Suzuki and Yoshino, 1992), and among them, the peptides resact and speract have been found to act as sperm attractants (Ward et al., 1985; Guerrero et al., 2010). The receptor for resact was identified and characterized as a transmembrane-type guanylylcyclase (GC) (Singh et al., 1988). Cyclic GMP (cGMP)

**Figure 3** The [Ca\(^{2+}\)] signal and flagellar bending in the sperm of the ascidian C. intestinids during chemotactic behaviour. (A) Time-course of Ca\(^{2+}\) changes in the ascidian spermatozoa during chemotactic behaviour. The pseudo-coloured [Ca\(^{2+}\)] signal images of the sperm around the tip of a capillary containing egg-derived attractant at 20-ms intervals are shown. Bar: 10 μm. (B) Data points taken from (A). Dots show the sperm head points from the image, and pseudo-colours show the average intensity of the [Ca\(^{2+}\)] signal in the flagellum. Arrowheads 1, 2 and 3 indicate the signals obtained at instances 1, 2 and 3 shown in (A), respectively. The arrows indicate the swimming direction. Interestingly, Ca\(^{2+}\) levels in spermatozoa increased during turns and leading into straight swimming. (C) Top: Changes in [Ca\(^{2+}\)] signals derived from the head (blue line) and flagellum (orange line). Bottom: Changes in the swimming-path curvature (black line) and the asymmetric index (red dots) of the spermatozoon shown in (A). The grey bar indicates the period during which the images shown in (A) were captured. The numbered dots indicate signals obtained from the corresponding images in (A). [Ca\(^{2+}\)] in the flagellum of the ascidian sperm is poorly correlated with flagellar asymmetry. (Modified Figure from: Shiba et al., 2008).
The signaling of mammalian sperm chemotaxis stimulated by odorants is mediated by G-protein-coupled odorant receptors (Spehr et al., 2003; Fukuda et al., 2004). The human sperm odorant receptor hOR17-4, which is a potential candidate for mediating human sperm chemotaxis, activates membrane-associated adenylyl cyclases (mACs) (Spehr et al., 2004) and may result in increases in the [Ca\(^{2+}\)]\(_i\), and cAMP concentrations. Furthermore, a recent study suggested that the cAMP-dependent protein kinase and cGMP-dependent protein kinase pathways are involved in human sperm chemotaxis induced by progesterone (Teves et al., 2009), although the receptor for progesterone is still unknown. In both echinoderm and mammalian cases, the cyclic nucleotide is the first signal, and [Ca\(^{2+}\)]\(_i\) increases follow. On the other hand, other recent studies showed that progesterone directly activates the sperm-specific cation channel, CatSper, without the increase of cAMP levels (Lishko et al., 2011; Strünker et al., 2011). They also proposed that CatSper is a receptor for progesterone, though there is currently no direct evidence of interaction between progesterone and CatSper. Progress of the studies should lead breakthrough of the study on mechanism of mammalian sperm chemotaxis.

The increase in [Ca\(^{2+}\)]\(_i\) in spermatozoa is mediated by Ca\(^{2+}\) channels on the sperm plasmamembrane. In the sea urchin A. punctulata, resact induces an increase in [Ca\(^{2+}\)]\(_i\); through cGMP and cAMP (Cook et al., 1994), and the [Ca\(^{2+}\)]\(_i\) increase is regulated by K\(^+\)-selective cyclic nucleotide-gated cation channels (Strünker et al., 2006; Galindo et al., 2007; Bönik et al., 2009). Fluctuations in [Ca\(^{2+}\)]\(_i\) have been observed in the swimming spermatozoa of sea urchins, and these [Ca\(^{2+}\)]\(_i\) fluctuations could be induced by caged cGMP (Böhmer et al., 2005; Wood et al., 2005). In the ascidian, store-operated Ca\(^{2+}\) channels mediate the asymmetric flagellar waveform of spermatozoa and result in chemotactic behaviour (Yoshida et al., 2003). In mammalian sperm, although the Ca\(^{2+}\) channels that mediate sperm chemotaxis have not been identified, many Ca\(^{2+}\) channels are found in the sperm head and flagella (see reviews, Darszon et al., 2005, 2006). In particular, the voltage-gated Ca\(^{2+}\) channel Ca\(_{\text{v}}\)2.3 (Sakato et al., 2002), transient receptor potential channels (Castellano et al., 2003) and CatSper1–4 (Ren et al., 2001; Carlson et al., 2003, 2005; Quill et al., 2003) are thought to play a role in the swimming behaviour of mammalian sperm and may be involved in sperm chemotaxis.

**How do Ca\(^{2+}\) levels mediate flagellar beating?**

As described already, sperm flagellar beating modulates the chemotactic response of spermatozoa. The asymmetry of sperm flagellar bending is generally believed to be closely correlated with [Ca\(^{2+}\)]\(_i\), because the pattern of microtubule sliding and flagellar bending in demembranated sperm models have been shown to be correlated with Ca\(^{2+}\) concentrations in the medium. In sea urchins (Brokaw et al., 1974; Brokaw, 1979; Bannai et al., 2000) and ascidians (Brokaw, 1997), the Ca\(^{2+}\) levels in reactivated solutions are correlated with the flagellar asymmetry of the reactivated models. Thus, it has been postulated that [Ca\(^{2+}\)]\(_i\) fluctuates during sperm chemotaxis and that high and low [Ca\(^{2+}\)]\(_i\) induce turning and straight swimming with asymmetric and symmetric flagellar bending, respectively (Cook et al., 1994; Nishigaki et al., 2004). However, in the flagellum of the ascidian sperm, [Ca\(^{2+}\)]\(_i\) is poorly correlated with flagellar asymmetry, despite the observation that stereotypic chemotactic responses comprising turning and straight swimming always occur during Ca\(^{2+}\) bursts (Shiba et al., 2008) (Fig. 3). Poor correlation between [Ca\(^{2+}\)]\(_i\) and flagellar asymmetry was also observed in the sea urchin sperm (Böhmer et al., 2005; Wood et al., 2005), and the magnitude and duration of the SAP-induced Ca\(^{2+}\) fluctuations in the sea urchin spermatozoa affect the properties of sperm turning (Wood et al., 2007). Thus, [Ca\(^{2+}\)]\(_i\) in the flagellum alone does not determine the magnitude of beat asymmetry of sperm flagella, but some unknown mechanisms activated by Ca\(^{2+}\) are likely involved in this case.

The molecular mechanisms connecting [Ca\(^{2+}\)]\(_i\) increases to sperm flagellar asymmetry remain obscure. The light chain of the outer arm dynein, which is the motor protein in the sperm flagellum, has a Ca\(^{2+}\)-binding site (King and Patel-King, 1995) and might regulate dynein motor activity in response to Ca\(^{2+}\)-binding (Sakato et al., 2007) in the Chlamydomonas flagellum. Recently, the sperm flagellar-specific Ca\(^{2+}\)-binding protein calaxin was found to regulate the inner arm dynein during the chemotactic behaviour of ascidian sperm (Mizuno et al., 2009). Further investigation is required to understand how Ca\(^{2+}\) levels modulate flagellar beating.

**Conclusions: problems and future aspects of sperm chemotaxis research**

In this paper, the mechanisms involved in the regulation of sperm flagellar beating during the chemotactic response were reviewed. The review was based mainly on studies of marine invertebrates because although mammalian sperm chemotaxis has been studied extensively, few studies have specifically addressed the mechanisms of flagellar beating. Furthermore, the study of mammalian sperm chemotaxis is complex and has been limited by discrepancies, in part because mammalian sperm movement is closely linked to other phenomena that occur in the female reproductive tract, such as semen liquefaction, capacitation, hyperactivation and acrosome reaction. One series of studies reported that the chemotactic behaviour of human sperm is observed only in capacitated spermatozoa (Eisenbach and Giojalas, 2006), while a different series of studies made no reference to capacitation (Spehr et al., 2003, 2004). Several candidate sources and chemical compositions of sperm attractants exist, as described already, and these different forms are disputed in the literature. The integration and verification of the discrepant studies is necessary to improve our understanding of mammalian sperm chemotaxis.
Confusingly, most of the sperm attractants can induce phenomena other than chemotaxis not only in mammalian but also in marine invertebrate sperm, and most of these functions involve Ca\(^{2+}\) signalling. For example, SAAF and resact can induce the activation of sperm motility in the ascidian and sea urchin spermatozoa, respectively; progesterone can induce capacitation, hyperactivation and acrosome reaction in the mammalian sperm. Therefore, it is inadequate to use Ca\(^{2+}\) responses as the basis for evaluating attractant-induced chemotactic responses, although many studies have used this assay because it is easier to observe the Ca\(^{2+}\) response than the chemotactic behaviour of sperm. Although the Ca\(^{2+}\) response plays an important role in the chemotactic response, it should be observed concomitantly with sperm flagellar movement.

Another important question that remains unanswered is when and where the spermatozoa detect the attractant. Furthermore, the wide dynamic range of detection possessed by sperm is intriguing, as sperm can respond to concentrations of attractants from 10\(^{-12}\) to 10\(^{-6}\) M. Technical advances in research are necessary to shed light on some of the issues associated with sperm chemotaxis and to overcome the difficulties associated with its study, such as the fact that the chemotactic response is too fast to analyse biochemically, and the maintenance of the gradient field of attractant during experimentation is difficult.

Finally, the species specificity of sperm chemotaxis is interesting from the point of view of the evolution of fertilization. In marine sperm, the reproductive season is reached simultaneously by many animals, and species-specific sperm attractants ensure that the spermatozoa find eggs from the same species and may thus participate in the prevention of crossbreeding. In mammals, sperm chemotaxis has been reported to have no species specificity (Sun et al., 2003; Teves et al., 2006; Guidobaldi et al., 2008), although another study on odorous attractants reported species-specific sperm attraction (Fukuda et al., 2004). The species-specificity of mammalian sperm chemotaxis should be examined more accurately, even though progesterone is a potent candidate as an attractant in some mammalian species. Further studies would provide new insights into the diversity and generality of the mechanisms that regulate sperm chemotaxis.

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