Tuning sperm chemotaxis

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Abstract

Sperm chemotaxis is a long-term puzzle and most of our knowledge comes from studying marine animals that are external fertilizers. Sperm are attracted by diffusible chemical factors (chemoattractants) released from the egg which redirect their swimming paths towards their source. This redirection is driven by increases in flagellar curvature that correlate with transient flagellar Ca^{2+} increases. Recent experimental and modelling results provide insights into the signal flow underlying the translation of an external chemical gradient into an intracellular molecular and motor response. A fundamental element of sea-urchin sperm chemotaxis lies in the ability of these cells to suppress Ca^{2+}-mediated increases in flagellar curvature while experiencing an increasing chemoattractant gradient. The article considers this new evidence and summarizes the known underlying cellular mechanisms and behavioural strategies that sperm use to locate and fertilize the oocyte.

Introduction

In many species, including mammals, spermatozoa are guided in their journey towards the egg or oocyte by gradients of chemical signals released by the female gamete or its associated structures, a mechanism known as chemotaxis (reviewed in [1]). Sperm chemotaxis has principally been studied in marine animals with external fertilization [2]. Their spermatozoa redirect themselves towards the centre of a chemoattractant concentration gradient through stereotypical sequences of turns interspersed by periods of straighter swimming (the 'turn-and-run' pattern) [3,4]. This redirection is driven by increases in flagellar curvature during turns, and decreases in curvature during the episodes of straighter swimming [4].

Sea-urchin sperm motility is modulated by SAPs (spermatozoa-activating peptides), a diverse group of peptides from the egg investments [5]. The first characterized and most widely studied member of the SAP family is speract, isolated from the Californian purple sea urchin *Strongylocentrotus purpuratus* [6,7]. After binding to its receptor, located in the flagellar membrane, speract triggers a train of fluctuations in $[Ca^{2+}]_i$ (intracellular Ca^{2+} concentration) that promote the stereotypical turn-and-run episodes (Figure 1A). Current models propose that the train of $[Ca^{2+}]_i$ fluctuations is controlled by changes in membrane potential through the co-ordinated and iterative opening and closure of Cavs (voltage-gated Ca^{2+} channels (Figure 1B; reviewed in [8]).

How these Ca^{2+}-stimulated motility responses are integrated to produce chemotaxis in a chemoattractant gradient of particular shape and form has become a key question in the field of sperm motility. Ample evidence indicates that the Ca^{2+}-dependent turning episodes are essential elements of a chemotactic motility response and that extracellular Ca^{2+} is a strict requirement for sperm chemotaxis, from bracken to mammals [1,2,9]. Recently, we discovered that *Lytechinus pictus* (painted sea urchin) spermatozoa undergo chemotaxis in response to speract gradients [10]. Interestingly, even though speract stimulates *S. purpuratus* spermatozoa to redirect their swimming paths with the stereotypical turn-and-run pattern, it does not induce chemotaxis uder tested experimental conditions [8,10]. Thus the Ca^{2+}-dependent turning episodes and the interspersed periods of straighter swimming trajectories are necessary, but not sufficient, for chemotaxis, which requires more subtle aspects of the timing and thus positioning of the turn-and-run pattern.

Spatial and temporal regulation of sperm chemotaxis

As spermatozoa swim in a chemoattractant gradient, they continuously sample the concentration field. The rate of chemoattractant binding depends on sperm velocity and the direction of movement relative to the gradient. It was proposed that the $[Ca^{2+}]_i$ of sperm may increase when bound chemoattractants dissociate from their receptors once the spermatozoon enters a descending gradient [2]. This seems unlikely for speract, as its binding is essentially irreversible ($k_{off} \sim 10^{-4}$–10^{-6} s^{-1}; $k_{on} \sim 10^{7}$ $M^{-1} \cdot s^{-1}$), therefore receptor occupancy is practically unaltered while spermatozoa swim down gradient [11,12]. Marine spermatozoa can detect a dynamic range of chemoattractant concentrations (10^{-12}–10^{-6}) before becoming saturated [3,4,11,13]. Presumably to prevent the saturation of the signal transduction mechanisms that drive chemotactic

Key words: calcium signalling, chemotaxis, fertilization, flagellum, sea-urchin sperm, sperm motility.

Abbreviations used: $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; Cav, voltage-activated Ca^{2+} channel; HCN channel, hyperpolarization-activated and cyclic-nucleotide-gated channel; SAP, spermatozoa-activating peptide; TetraKCNG, tetrameric cGMP-regulated K^+ channel.

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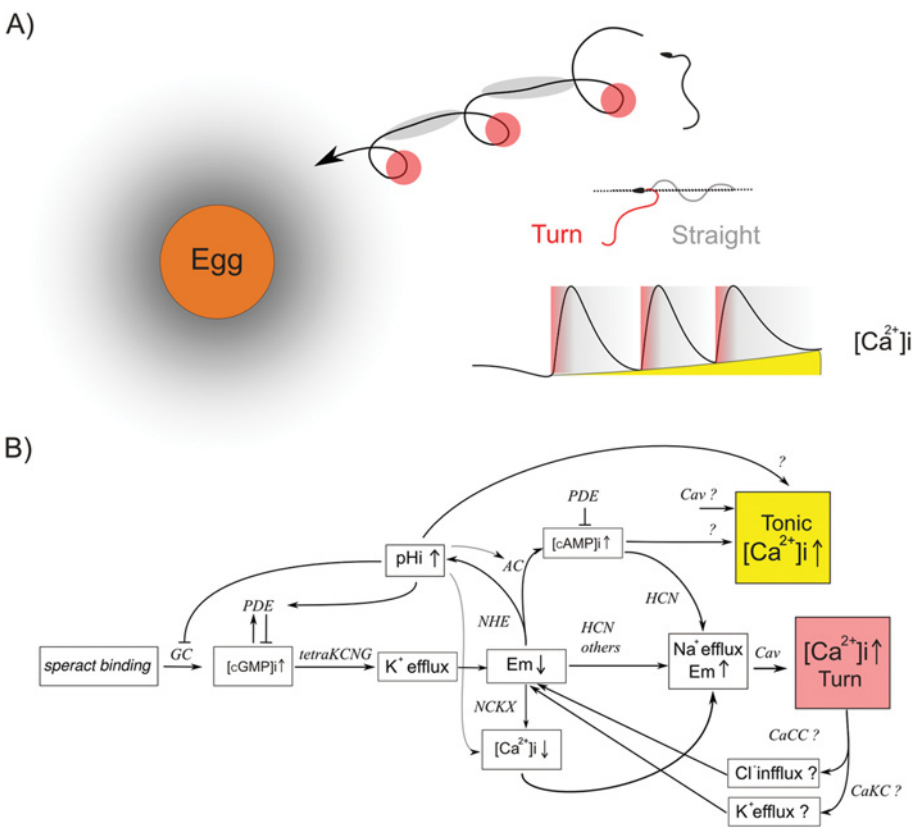
Figure 1 | Speract diffusion from the egg establishes a concentration gradient that spermatozoa detect resulting in motility changes that enhance their fertilizing capacity

(**A**) A speract gradient induces spermatozoa to undergo a series of $[Ca^{2+}]_i$ fluctuations and turns intercalated with periods of straighter swimming that direct them towards the egg. (**B**) After receptor binding, speract induces synthesis of cGMP that activates TetraKCNGs, leading to membrane potential (Em) hyperpolarization. This Em change stimulates hyperpolarization-activated and cyclic-nucleotide-gated channels (HCN), removes inactivation from Cavs, facilitates the Ca^{2+} extrusion activity of K^+-dependent Na^+/Ca^{2+} exchangers (NCKX) and activates Na^+/H^+ exchangers (NHE) and adenylate cyclases (AC). HCN opening and Na+ influx contribute to Em depolarization, and increases in $[Ca^{2+}]_i$ and Na^+ further depolarize the Em. The increase in Ca^{2+} levels enhances flagellar bending and causes the spermatozoon to turn. Possibly, the $[Ca^{2+}]_i$ increase also opens Ca^{2+}-regulated Cl^--channels (CaCC) and/or Ca^{2+}-regulated K^+-channels (CaKC) which then contribute to hyperpolarize the Em again, removing inactivation from Cavs and opening HCN channels. The previous mechanism is then cyclically repeated to generate a train of Ca^{2+} increases that produce a repetitive sequence of turns. The sequence continues until one or more of the molecular components in the pathway are down-regulated. cAMP activates a poorly characterized Ca^{2+} influx pathway, which may contribute to a tonic $[Ca^{2+}]_i$ increase. Grey arrows indicate partially known interaction were the molecular elements have not been identified.



responses, sea-urchin spermatozoa have evolved high chemoattractant receptor densities: 10^{-4}–10^{-6} receptors per cell, according to species [3,4,11,12]. The latter observation indicates that marine spermatozoa register relative, and not absolute, changes in chemoattractant concentrations [3,4,11–13].

In 2005, Bohmer et al. [3] proposed that chemotactic spermatozoa of the sea urchin *Arbacia punctulata*, when swimming in thigmotactic circles typically observed close to a surface (e.g. a coverslip), will sense periodic chemoattractant concentration changes in a gradient that synchronize $[Ca^{2+}]_i$ fluctuations to periodically modulated flagellar waveforms [3]. A more recent study demonstrated that sperm of the ascidian *Ciona intestinalis* evoke $[Ca^{2+}]_i$ fluctuations as spermatozoa encounter chemoattractant gradient minima [13]. We investigated if this synchronization determines chemotactic behaviour in sea-urchin spermatozoa. Our findings indicated that approx. 80% of the $[Ca^{2+}]_i$ fluctuations in *L. pictus* chemotactic sperm occur while they swim down a negative speract gradient (Figure 2) [10]. In contrast, the non-chemotactic *S. purpuratus* sperm generate $[Ca^{2+}]_i$ fluctuations in both descending and ascending speract gradients with almost the same frequency [10]. This suggests that $[Ca^{2+}]_i$ fluctuations that occur

Figure 2 | Chemotactic behaviour of a single *L. pictus* sperm

A spermatozoon (green) swimming on a circular trajectory in a chemoattractant gradient (background) is periodically stimulated due to changes in the rate of chemoattractant capture. When swimming in an ascending gradient, the onset of $[Ca^{2+}]_i$ fluctuations is suppressed until the spermatozoon senses an ascending-to-descending gradient inversion (red circle). After an approx. 200 ms delay, the spermatozoon undergoes a $[Ca^{2+}]_i$ fluctuation just before reaching the gradient minima (white circles). Consequently, the spermatozoon experiments with a turn-and-run episode that promotes movement towards the chemoattractant source (black arrow).

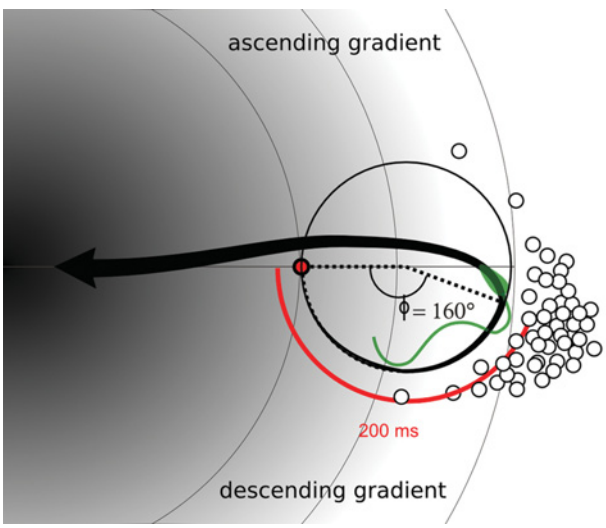
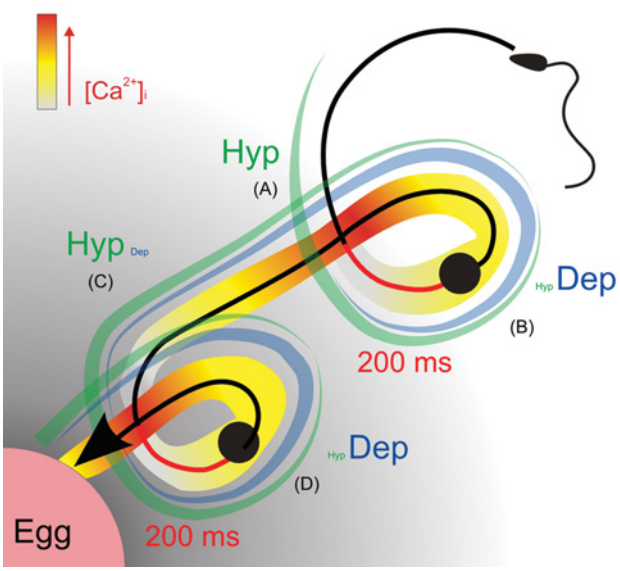


Figure 3 | Model of molecular mechanisms that drive chemotaxis in marine spermatozoa

Chemotactic spermatozoon swimming in a chemoattractant gradient (background) undergoing cyclic changes in membrane potential from resting to an hyperpolarized state (Hyp, green shadow) and then to a depolarized state (Dep, blue shadow) that control Cav activity. The spermatozoon path is depicted as a black arrow during the journey to the egg (pink). The red line indicates the 200 ms delay between the point of positive-to-negative gradient inversion and the onset of each $[Ca^{2+}]_i$ fluctuation (black circles). The pseudo-colour bar represent the kinetics of $[Ca^{2+}]_i$ changes in the flagella; red and grey indicate low and high $[Ca^{2+}]_i$ concentrations respectively. Note that the straight swimming periods coincide with an interval of elevated $[Ca^{2+}]_i$.



in an ascending chemoattractant gradient do not favour chemotaxis, and, conversely, selectively undergoing $[Ca^{2+}]_i$ fluctuations in descending chemoattractant gradients is a feature of chemotaxis.

It is likely that spermatozoa are sensitive to the ascending-to-descending gradient inflection which, when crossed, initiates the sequence of signalling events, leading to a flagellar $[Ca^{2+}]_i$ fluctuation and a chemotactic turning event. We found that *L. pictus* spermatozoa are able to suppress the onset of $[Ca^{2+}]_i$ fluctuations while swimming in ascending chemoattractant gradients [10]. After crossing the positive-to-negative gradient inversion point, *L. pictus* spermatozoa still require approx. 200 ms [10] to carry out signalling events that lead to the opening of Ca^{2+} entry pathways [4,11,14,15]. These events are likely to involve hyperpolarization/depolarization cycles that result in Cav openings [15]. The $[Ca^{2+}]_i$ fluctuations occur just before the speract gradient minima, ~160° from the preceding gradient inflection point (Figure 2) [10].

In summary, the ability to selectively inhibit increases in flagellar Ca^{2+} while experiencing an ascending chemoattractant gradient is an essential component that characterizes chemotaxis in sea-urchin spermatozoa, and possibly chemotactic sperm motility in general.

A mechanistic model for sperm chemotaxis

In 1994, Cook et al. [16] proposed that shallow or decreasing chemoattractant gradients elevate $[Ca^{2+}]_i$ to generate chemotactic turns, yet sufficiently steep increasing gradients maintain $[Ca^{2+}]_i$ low and swimming trajectories as linear until the egg is reached. Single-cell measurements demonstrating that the straighter swimming episodes that intersperse the Ca^{2+} fluctuations often occur while $[Ca^{2+}]_i$ is still elevated throw some aspects of this proposal into question [3,13,17]. However, after an adjustment to fit this more recent data, we propose a unified mechanistic model for sperm chemotaxis. At the heart of the model lies a negative-feedback loop, in which SAP receptor binding activates guanylate cyclase to elevate cGMP, which leads to a membrane potential hyperpolarization due to cGMP-mediated activation of TetraKCNGs (tetrameric cGMP-regulated K^+ channels) (Figure 3A). For spermatozoa undergoing chemotaxis in ascending gradients the incremental activation of speract receptors leads to extended hyperpolarization which accounts for the observed suppression of the Ca^{2+} fluctuations [15,18,19]. The hyperpolarization reverses once sperm enter a negative speract gradient, which, after a typical ~200 ms

delay, leads to generation of a chemotactic turn that optimally reorients the sperm into swimming once more towards the source of the gradient (Figure 3B). This re-polarization that leads to the opening of Cavs, possibly of the T- and L-types [15,20], could be attributed, in part, to inactivation of guanylate cyclase, reduction of cGMP levels by degradation, Na^+ influx through HCN (hyperpolarization-activated and cyclic-nucleotide-gated) channels and other unknown depolarizing elements (Figure 3B). At some point during the subsequent straighter swimming phase in the positive speract gradient, a hyperpolarized membrane potential is re-established and extended by continuous speract receptor recruitment (Figure 3C), which once again reverts to depolarized membrane potentials as sperm leave the positive gradient (Figure 3D). This sets up a sequence of chemotactic turns, triggered by hyperpolarization/depolarization cycles that serve as the primary translators of the state of the extracellular chemoattractant gradient. How the hyperpolarization/depolarization cycles and Ca^{2+} transients are translated into motor responses in flagella is currently unknown, although a number of Ca^{2+}-binding proteins have been identified in the axoneme [21].

Additional searching strategies, besides chemotaxis, enhance the ability of sperm to locate the egg

Evidence indicates that a 1 mm radial distance from the oocytes is the limiting distance over which chemotaxis functions in marine species during a restricted temporal window (seconds) [2,10,22,23]. Individual species evolutionarily hone their reproductive strategies to the hydrodynamic properties of their environment [24]. Sea-urchin spermatozoa and eggs must conjoin in a turbulent ocean where chemical gradients are shaped by eddies, convection and gamete drifting, rather than by homogeneous diffusion of chemoattractants. It has been shown that the laminar shear acting on the oocyte is of primary importance in determining whether chemotaxis is a viable strategy to enhance reproductive success in marine animals. As laminar shear values increase, chemoattractant plumes contract and begin to fragment, and eventually cease to carry any interpretable information of the location of the oocyte [25]. In the case of the red abalone (*Haliotis rufescens*), whose spermatozoa undergo chemotaxis, the fertilization efficiency peaks sharply at levels of laminar shear similar to those found in its natural environment [25]. This suggests a natural physical limit on the degree of laminar shear compatible with long-range gamete communication.

The searcher's probability of finding discontinuous chemoattractant gradients or patches (plumes) decays exponentially with the distance from their source [26]. Thus, under these circumstances and at significant shear values, other exploration tactics become appealing, such as random or biased walks, which would allow spermatozoa to jump between irregular concentration fields [26,27]. Once spermatozoa are swimming inside a chemoattractant plume close to the egg's neighbourhood, the trains of turns-and-runs may increase the probability of gamete encounter. Furthermore, searching strategies need to be robust to preserve the main swimming and signalling mechanisms [27–30], and must have the capability of adapting to the surroundings. Combining searching strategies to locate the egg seems beneficial to improve the probability that spermatozoa accomplish fertilization.

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