100 years of sperm chemotaxis

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Exactly 100 years ago, F.R. Lillie reported chemotaxis in animal sperm for the first time (Lillie, 1912). Chemotaxis refers to the directed movement of a cell or organism to the source of a chemical gradient; the chemical agent that elicits this movement is called a chemoattractant. Chemotaxis had previously been shown by Pfeffer (1884) for fern spermatozoids; however, attempts to demonstrate chemotaxis in animal sperm had failed. Lillie was an eminent scientist of the time; he was professor of embryology at the University of Chicago, president of the National Academy of Sciences of the USA, and a towering figure of the scientific community at the Marine Biological Laboratory (MBL) in Woods Hole, MA. From 1908 to 1925, he served as director of the MBL.

In a report entitled "The production of sperm isoagglutinins by ova" (Lillie, 1912), Lillie described an agglutination reaction when drops of seawater that had been conditioned with unfertilized eggs were injected into a milky suspension of sperm from the sea urchin *Arbacia punctulata*. The sperm agglutinated—or clumped—into small masses, or beads. The reaction was reversible; after some time, the sperm freed themselves and regained full mobility. Lillie called the ova-derived factor an iso-agglutinin because it acted on sperm of the same species. Because Lillie thought that the iso-agglutinin represented a key factor for fertilization, he dubbed it "fertilizin" (Lillie, 1913). Lillie interpreted the agglutination as physical cross-linking of sperm by egg jelly molecules analogous to the antigen–antibody reaction. The spontaneous reversal was later explained by fragmentation of fertilizin by a sperm enzyme called "lysin" (Tyler, 1941).

At the end of his manuscript (Lillie, 1912), Lillie reported almost in passing that egg extracts also contain a chemotactically active agent distinct from the iso-agglutinin. The short passage about the chemotactic behavior is worth quoting for its clarity and succinct style:

"The egg-extracts contain not only an agglutinin for the spermatozoa, but also an aggregative agent, i.e., a substance towards which the spermatozoa are positively chemotactic. This may be readily demonstrated by the form of the reaction when a drop of the fluid to be tested is injected into a sperm suspension beneath a raised cover glass. If an aggregative agent be present, a ring of spermatozoa forms at or within the margin of the drop, depending on the strength of the agent, and a clear zone arises between this ring and the general sperm suspension. The clear zone is produced by migration of the spermatozoa to the ring; in case the agent is very strong the ring expands, owing to immigration of spermatozoa, but the clear zone is never obliterated, no matter how much the ring may expand. In the case of Nereis, which has unusually large spermatozoa, the passage of spermatozoa across the clear zone to the ring may be readily studied under a low power of the microscope, and it gives the impression of a regular rain falling on the ring."

Lillie believed that the two reactions—agglutination and chemotaxis—served distinct functions and were caused by different substances. In the following years, Lillie was more interested in the agglutination reaction (summarized in his book "Problems of Fertilization"; Lillie, 1919), and he did not follow up on his study of the chemotactic response, perhaps because another Woods Hole luminary and founder of *The Journal of General Physiology*, Jacques Loeb, took a decided stand against chemotropism of sperm in animals (Loeb, 1914, 1916, 1918). In general, for the next 50 years, studies of sperm chemotaxis produced mixed results. As late as 1951 and 1952, Lord Rothschild concluded that "in the animal kingdom, spermatozoa probably meet or collide with eggs by chance" (Rothschild, 1952), and that "chemotaxis of spermatozoa toward eggs has never been observed with certainty" (Rothschild, 1951). It was not until the experiments of R.L. Miller in the late 1960s and 1970s that chemotaxis in animal sperm was firmly established (Miller, 1966, 1970, 1985). To this day, chemotaxis in mammalian sperm has proved to be exceedingly difficult to study (Eisenbach and Giojalas, 2006; Armon et al., 2012). Numerous molecules—from odorants to gases—have been proposed to attract sperm. However, their function as chemoattractants has not been as firmly established as the chemoattractants of sperm from marine invertebrates.

Ironically, the fertilizin hypothesis turned out to be incorrect, whereas Lillie's observation of sperm chemotaxis stood the test of time. Indeed, *A. punctulata* has

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become the most valuable model of sperm chemotaxis research. The sea urchin agglutination phenomenon requires freely moving sperm and is probably produced by the swarming of sperm to a common focus (Collins, 1976). Moreover, at high density, sperm of a related sea urchin, *Strongylocentrotus purpuratus*, form large-scale patterns solely mediated by hydrodynamic interactions (Riedel et al., 2005). At planar surfaces, sperm display hexagonal arrays of vortices. Thus, the agglutination reaction is probably caused by vigorous chemotactic swimming of sperm toward the drop of chemoattractant combined with hydrodynamic interactions. The dispersal of sperm clumps after some time is probably caused by dissipation of the chemical gradient and sperm adaptation to the chemoattractant.

About 70 years after Lillie's discovery, the chemoattractant resact, a short peptide isolated from jelly of *Arbacia* eggs (Hansbrough and Garbers, 1981), was identified and the chemotactic response of *Arbacia* sperm to resact was unequivocally identified (Ward et al., 1985).

We repeated Lillie's experiment using modern microscopy techniques and a caged form of resact. We did not inject resact into a sperm suspension by a pipette; instead, we created a gradient of resact concentration by releasing resact from the caged compound with a brief flash of UV light (Video 1). The intensity of the light flash formed a circular distribution. The results of this experiment recapitulate Lillie's original observations, as did those of Ward et al. (1985): Sperm swarm to the site where the light intensity is highest and form clumps; an annulus of low sperm density forms that separates the area in the center of the gradient that is densely populated by sperm from the surrounding sperm suspension. After some time, the sperm clumps come apart. Thus, Lillie's observations were as precise as they were correct.

We also made use of Lillie's egg-extract experiment to determine the amount of resact released by an egg; the equivalent concentration in the egg is 50 µM (Kashikar et al., 2012). This figure combined with a lower boundary of 0.8 fM μm^{-1} for the gradient sensitivity of *A. punctulata* sperm allows us to estimate the maximal effective range of a chemical gradient that forms by radial diffusion of resact from the egg (~0.4 cm) (Kashikar et al., 2012).

After the seminal discoveries of resact, and its cognate receptor, guanylyl cyclase (GC) (Suzuki et al., 1984; Shimomura et al., 1986; Singh et al., 1988), an ever-increasing number of signaling molecules have been identified in the chemotactic pathway of sea urchin sperm (Fig. 1). Rapid kinetic techniques combined with flash photolysis of caged compounds have enabled dissection of the sequence of signaling events from the receptor to the voltage-dependent Ca_v channels that mediate the Ca^{2+} response. Of note, this signaling pathway endows *Arbacia* sperm with sensitivity to chemoattractant at the physical limit: they can respond to binding of a single resact molecule (Kaupp et al., 2003; Strünker et al., 2006; Kashikar et al., 2012). The identity and function of some signaling components have been firmly established, although the physiological roles of others continue to be vague. Notably, the identity of the Ca_v channels is unknown, and the function of the rise in pH_i and the workings of the underlying Na^+–H^+ exchanger (NHE) are still enigmatic. Finally, the mechanisms underlying

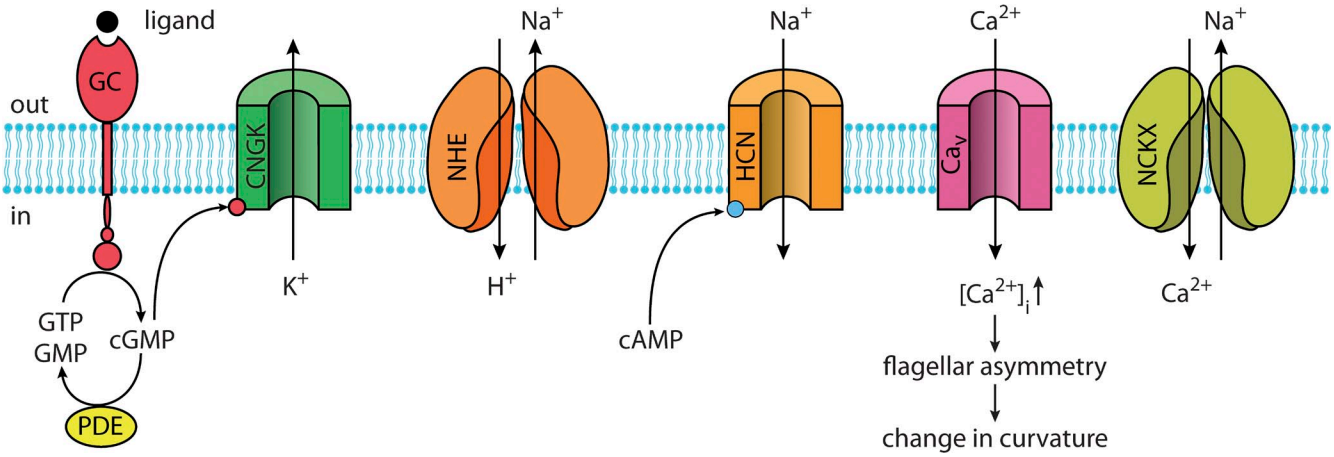

Figure 1. Chemotactic signaling pathway in sea urchin sperm. Resact, the chemoattractant peptide, binds to receptor GC and, thereby, stimulates the rapid synthesis of cGMP. The ensuing surge in cGMP opens K^+-selective cyclic nucleotide–gated (CNGK) (Strünker et al., 2006; Galindo et al., 2007; Bönigk et al., 2009) channels to produce a brief hyperpolarization of the cell membrane. This hyperpolarization activates two other signaling components: an NHE and a hyperpolarization-activated cyclic nucleotide–gated (HCN) channel (Gauss et al., 1998; Galindo et al., 2005). NHE activity causes a rapid alkalinization of the cytosol (Lee, 1984; Lee and Garbers, 1986). Upon opening of HCN channels, the ensuing Na^+ inward current depolarizes the cell and leads to the opening of voltage-dependent Ca^{2+} channels (Ca_v). Recovery from stimulation involves restoration of resting $[Ca^{2+}]_i$ by a Na^+–Ca^{2+}–K^+ exchanger (NCKX) (Su and Vacquier, 2002) and hydrolysis of cGMP by a phosphodiesterase (PDE) (Su and Vacquier, 2006). The physiological role of a soluble adenylate cyclase (not depicted) (Nomura et al., 2005) and of the second messenger cAMP is not known.

the sperm's recovery from stimulation and the regulation of its sensitivity to chemoattractant are largely unknown. Sperm from *S. purpuratus* sperm harbor a similar signaling pathway (Darszon et al., 2008); however, in the shallow recording chambers frequently used to study chemotactic behavior under the microscope, *S. purpuratus* sperm do not display chemotaxis (Guerrero et al., 2010).

Caged resact and caged cGMP (Hagen et al., 2001; Nishigaki et al., 2004) have also been instrumental in deciphering the swimming pattern of sperm in a chemical gradient and in defining the cellular algorithms that transduce chemoattractant binding into a change in flagellar beat and, thereby, the swimming path. When confined to the glass–water interface in a narrow recording chamber, sea urchin sperm swim in regular circles. In a chemical gradient, the circles start drifting toward the chemoattractant source, giving rise to looping swimming paths (Kaupp et al., 2003; Friedrich and Jülicher, 2007). To do so, sperm are equipped with high school calculus. Sperm can detect and count single molecules, integrate, and differentiate. Sperm temporally sample chemoattractant molecules impinging on their flagella and integrate the binding events to produce a summed response (Kashikar et al., 2012; Leslie, 2012). Because of the circular movement, sperm in a gradient are periodically exposed to higher and lower concentrations of chemoattractant. This periodic stimulation triggers Ca^{2+} spikes that initiate a change in the asymmetry of the flagellar beat and, thereby, the swimming path (Kaupp et al., 2003; Böhmer et al., 2005). Studies on detergent-treated and reactivated sea urchin sperm show that the flagellar beat is more asymmetrical at high $[Ca^{2+}]_i$ and more symmetrical at low $[Ca^{2+}]_i$ (Brokaw, 1979; Lindemann and Goltz, 1988). In intact sperm, however, it is the time derivative of the changes in Ca^{2+} concentration $d[Ca^{2+}]_i/dt$ rather than absolute $[Ca^{2+}]_i$ that determines the curvature of the swimming path (Alvarez et al., 2012).

Arbacia sperm hold great promise for a profound understanding of chemotaxis at the molecular, systems, and behavioral level. In the future, I anticipate major advances in three areas: first, identification of all signaling molecules, quantitative description of their properties, and modeling of the entire chemotactic signaling pathway. For example: What is the density of the GC on the flagellum, its ligand affinity, and capture efficacy? What is the turnover number of cGMP synthesis? Does the GC inactivate and, if so, how fast and through what mechanisms? Does the GC in the flagellar membrane form oligomers, a supramolecular architecture, or a complex with other signaling molecules?

The second area in which I anticipate an advance is in overcoming the technical challenge of experimentally reconstructing the 3-D flagellar beat in freely swimming sperm, and characterizing its modulation by Ca^{2+} with spatiotemporal precision. The last area of advance is in clarification of how the modulation of the 3-D flagellar beat shapes the sperm swimming path in 3-D (Su et al., 2012). Time-resolved electron tomography of ultrathin cryo-sections of the flagellum will open the door to a new 3-D world of ciliary beat mechanics (Nicastro et al., 2006; Ishikawa, 2012; Pigino et al., 2012).

Chemotactic signaling and swimming behavior in sperm of marine invertebrates might differ from those of mammals. However, the tools and concepts developed for the study of chemotaxis in sea urchin sperm will be invaluable to advance insights into chemotaxis of human sperm.

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