on the synaptotagmin-SNARE interactions, but some data have suggested that membrane binding can alter these interactions[14].

These issues do not diminish the importance of the work described by Vrljic *et al.*[6] and Choi *et al.*[5]. The new crystal structure of synaptotagmin-3 represents the first structure of a synaptotagmin determined in the presence of SNAREs, and the smFRET model built from the synaptotagmin-1 data constitutes the first model of a synaptotagmin–SNARE complex determined with experimentally derived geometric restraints. The self-consistency of the data obtained for synaptotagmin-1 and synaptotagmin-3 by different techniques gives confidence that these structural models represent true functional states of synaptotagmins. This conclusion is further supported by the fact that the smFRET model correlates with abundant biochemical and functional data on synaptotagmins[5]. Note also that the combined results obtained for synaptotagmin-1 and synaptotagmin-3 have important implications for understanding the function of synaptotagmin-3, which has remained enigmatic; the similarities observed in these two studies suggest that the role of synaptotagmin-3 likely resembles that of synaptotagmin-1. Finally, it is also worth noting that these studies illustrate the power of single-molecule microscopy to study macromolecular assemblies and yield useful methodology to handle dynamic assemblies such as the synaptotagmin–SNARE complex. As a scientist who has been working for a long time on synaptotagmins and SNAREs, I feel that these two papers represent big accomplishments in our field and bring us closer to a detailed understanding of how these proteins function.

COMPETING INTERESTS STATEMENT
The author declares no competing financial interests.

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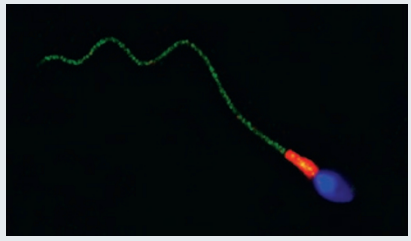
Channeling sperm activation

Regulation of intracellular pH has been known for some time to be a key molecular aspect of sperm regulation. In particular, male reproductive fluids help to keep sperm cytoplasmic pH acidic (remarkably, lower than pH 6.5), but initiation of motility in the female reproductive tract and hyperactivation in preparation for movement through the mucus of the oviduct and the protective vestments of the oocyte require a progressive change to an alkaline intracellular pH. This pH increase has been linked to stimulation of metabolism as well as to an influx of Ca^{2+} via a flagellar pH-sensitive CatSper Ca^{2+} channel, which intensifies the beating of the sperm flagellum. The molecular basis of this critical pH increase has been unclear, though it is likely that the mechanism involves the ejection of protons from the sperm cytoplasm.



Kirichok and colleagues (*Cell* **140**, 327–337, 2010) have now extended a patch-clamp technique, previously available only for quasi-mature epididymal mouse spermatozoa, to probe fully mature ejaculated human spermatozoa electrophysiology—a technological advance given the small size and volume of these cells. Using this technique, the authors provide evidence that alkalinization likely occurs via the extrusion of protons through the Hv1 channel, a four-pass transmembrane voltage-gated proton channel related to the voltage-sensing regions of voltage-gated cation channels. Whole-cell patch clamp was carried out by targeting the cytoplasmic droplet, a region of the sperm plasma membrane that is loosely attached to intracellular structures and is able to form a tight seal with a glass microelectrode, thus allowing access to the cell. Increasing transmembrane voltage (membrane depolarization) under these conditions elicited a robust outward current mediated by protons and sensitive to the transmembrane pH gradient (with the current becoming greater when intracellular pH was more acidic). This indicates the presence of a voltage-gated proton channel (initially referred to as "HSper" in the paper) in the plasma membrane of the human sperm cell.

The sensitivity of this channel to the membrane voltage and transmembrane proton gradient, as well as the slow kinetics of activation and deactivation, is reminiscent of the characteristics defined for Hv1. The authors therefore went on to test whether the pharmacological properties of HSper also match those of Hv1. Indeed, they found that HSper is inhibited by Zn^{2+} and sensitive to hanatoxin, a toxin from the Chilean rose tarantula that can act on Hv1. In addition, the observed sperm currents are enhanced by certain fatty acids, which is characteristic of Hv1. The authors argue that the powerful enhancing effect of the endocannabinoid anandamide may be directly through Hv1, in that cannabinoid receptor inhibitors (which would inhibit some of the alternative receptors that anandamide could be influencing) do not diminish the effects of anandamide. Anandamide is known to be present in the male and female reproductive tracts; thus, the possible physiological relevance of its effect in these experiments invites further investigation.

Hv1 is also expressed in spermatozoa, with both the mRNA and protein detected in sperm and the latter observed in the flagellum in particular (see image, which shows Hv1 (green), mitochondria (red) and DAPI (blue)). Activity of Hv1 correlates with sperm capacitation (functional maturation), increasing in capacitated compared to noncapacitated cells as judged by the amplitude of Hv1 current. These findings link Hv1 to the long-sought-after mechanism of pH increase and provide a molecular counterpart for a key physiological event. Hv1 can be studied in other cells that are more amenable to experimentation, but the development of the patch-clamp technique for human sperm will also allow further study of the mechanism of sperm activation as well as other processes critical to sperm development and fertilization.

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