Structural biology

Sperm ion channel swims into sharper focus

David E. Clapham & Raymond E. Hulse

Activation of the CatSper membrane ion channel triggers enhanced sperm motility, and this protein complex is required for male fertility. Structural data now provide insights into the channel, and offer a few surprises, too. **See p.746**

Many details about the structure of a key membrane ion channel required for normal sperm function have long remained mysterious. Lin *et al.*[1] present data on page 746 that fill some of the gaps in our knowledge.

Sperm and eggs are the vessels of our past and future, and they must overcome daunting challenges for the exchange of their genes to occur. A sperm cell enters a competitive race with millions of other sperm that are also trying to fertilize an egg cell. Each sperm has a long way to swim in the female reproductive tract, and this journey requires dramatic changes in the type of motion used, including a vigorous type of swimming movement that is triggered on encountering high pH. The fusion of a single sperm and egg during fertilization is another complex process that, after countless remarkable transformations, propagates a new genome in every cell of what will become an amazing, trillion-cell baby.

Not unlike anxious parents expecting their first child, scientists have also anticipated the arrival of structural insights into a sperm ion channel called CatSper (Fig. 1), which governs the hyperactive motility of sperm and is necessary for fertility[2]. Since its discovery 20 years ago[3,4], efforts to determine the composition and structure of CatSper have been evolving. Early attempts at structure determination using high-resolution X-ray crystallography were stymied by the large number of protein subunits that CatSper contains (more than ten), and by problems encountered when, in structural investigations, these proteins are expressed in cells in which they are not normally found.

An ancestral version of this channel first arose in single-celled organisms called uniflagellates[5]. The strong evolutionary pressures exerted on CatSper over time have driven its complexity – the CatSper complex is arguably the most elaborate ion channel known. The proteins CatSper1, CatSper2, CatSper3 and CatSper4 form a tetramer that is the ion channel's pore, and at least six other proteins are known to be associated with this complex (CatSperβ, CatSperγ, CatSperδ, CatSperε, CatSperζ and EFCAB9)[2,6]. CatSper responds to high pH, and perhaps to other factors as well, when sperm reach the upper cervix, or utero-tubal junction, of the female reproductive tract. The activation of CatSper, which is associated with the entry of calcium ions into sperm[7], completely transforms the motion of the sperm's 'tail' (flagellum), generating a robust, asymmetrical beating pattern called hyperactivated motility. CatSper activity has a weak voltage dependence[7] (sensitivity to the voltage across the cell membrane), perhaps due to the different number of positively charged amino acids in each of the pore-forming unit's voltage sensors.

Lin and colleagues now report structural data for CatSper. The authors generated mice that had a version of CatSper1 tagged with green fluorescent protein, together with copies of a peptide called FLAG. They used this tagged version of CatSper1 to isolate the CatSper complex from mouse sperm cells, and now present the first structure of CatSper to be generated using cryogenic electron microscopy.

The structure reveals that four accessory transmembrane proteins (CatSperβ, CatSperγ, CatSperδ and CatSperε) form a tent-like 'pavilion' over the pore (Fig. 1), with their pole-like, transmembrane-spanning supports positioned outside the pore, flanking the voltage sensors of the adjacent pore subunit. CatSperβ and CatSperε are in contact with their voltage sensors, CatSper4 and CatSper2, respectively. The pavilion's large interface, and particularly the presence of certain characteristic protein domains (for example, seven-bladed β-propeller domains), suggests regions that might bind to soluble ligand molecules. Notably, the multiple accessory components perturb the symmetry of the core tetrameric pore-forming subunits. This differs from the symmetry typically observed for related ion channels, and the discovery might reveal insights into the mechanisms involved in channel function.

The structure of CatSper brings several surprises. Structural modelling (and supporting evidence obtained using mass spectrometry) reveals that a protein called SLCO6C1, which is a type of transporter known as an organic
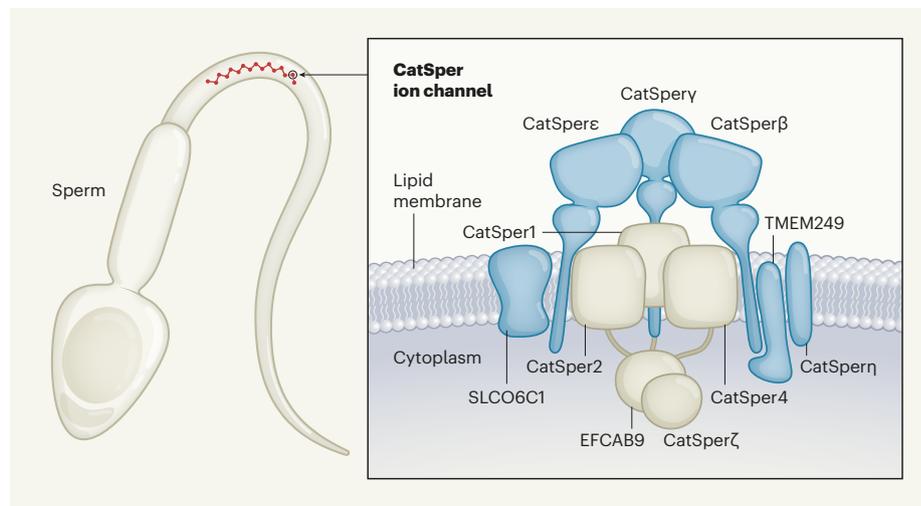

Figure 1 | Structure of the CatSper ion channel. Lin *et al.*[1] present structural data obtained using cryogenic electron microscopy. This channel, arranged in a 'racing-stripe' pattern on the sperm tail, aids fertility by facilitating the entry of cations (positively charged calcium ions) needed for the hyperactive swimming motion of sperm. The channel's central ion-conducting pore is formed from four subunits: CatSper1, CatSper2, CatSper3 and CatSper4 (CatSper3 is not shown to aid the visibility of other components). The authors report that some of the other proteins in the complex – CatSperβ, CatSperγ, CatSperδ and CatSperε – form a tent-like 'pavilion' over the pore (CatSperδ is not shown to aid visibility of other components). One of the proteins shown to be associated with the complex is SLCO6C1. Having a transporter of anions (negatively charged ions) associated with a cation channel is surprising. The newly discovered proteins associated with the channel include CatSperη and TMEM249. The structure also indicates the location of EFCAB9 and CatSperζ, which are proteins already known to be associated with the complex.

anion transporter, links to CatSperε and to the voltage-sensing domain of CatSper2. This observation is consistent with other work[8,9]. Another newly discovered protein association with CatSper is that of the transmembrane protein CatSperη − another previously unknown interactor − with the protein TMEM249 and an as-yet-unidentified cytoplasmic protein.

The cytoplasmic components are the least clearly visualized part of the structure, with the subcomplex of CatSperζ and EFCAB9 revealing an unknown protein (consisting of α-helices) and another unknown entity sandwiched between the elongated S6 segments of CatSper2 and CatSper3. Two lobes of EFCAB9 containing calcium-binding motifs (termed EF-hands) fit into the region where EFCAB9 and CatSperζ interact. These domains might bind to calcium[8], thereby linking calcium entry into sperm by means of intermediates to downstream proteins, such as the motor protein dynein. A major unanswered question is how the calcium signal results in an increased frequency and degree of bending of the sperm flagellar tail during swimming motions.

Exciting complementary data from other research groups, obtained using cryo-electron tomography and microscopy, show that the CatSper complex is assembled in a zigzag pattern[9] in each of the four quadrilateral 'racing stripes' along the sperm tail[10]. An EFCAB9–CatSperζ pair bridges two staggered channel units (rotated by 180°) as a building block for the zigzag assembly, and it was proposed[9] that this longitudinal nanodomain allows the simultaneous opening of the array of CatSper channels along the flagellum to allow rapid signalling in the sperm tail.

Lin and colleagues' structural data, together with these contributions, greatly increase our knowledge of CatSper, and suggest potential answers to, or ways of addressing, long-standing questions. For example, to what, if anything, do the extracellular pavilion structures bind? If binding partners exist for these structures, does their binding affect CatSper voltage sensitivity or calcium entry, or cause structural changes that propagate along CatSper and affect cytoplasmic components of the complex? And does CatSper activation alter the activity of the anion transporter?

These seminal contributions will speed up the identification of molecules that could form targets for the development of male-specific contraceptives (and species-specific forms of such contraceptives). Finally, the discoveries relating to CatSper might point the way to the treatment of male infertility associated with mutations in the genes encoding components of this complex.

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Earth science

A model of seismicity at a tectonically active oil field

Mirko van der Baan

An analysis of the Val d'Agri oil field in Italy provides insight into how processes associated with wastewater disposal trigger earthquakes − and how such effects can be reduced to maintain the economic viability of mature oil fields. **See p.684**

Gas and oil extraction generates wealth − it can significantly boost the gross domestic product of a country. But water is also extracted with the hydrocarbons, and is often reinjected into the ground for disposal. Unfortunately, large-scale fluid injection can induce earthquakes[1], potentially leading to the termination of extraction before the full economic potential of an oil field has been realized. On page 684, Hager *et al.*[2] describe a multidisciplinary process to manage earthquake hazard in an active oil field, maintaining the economic viability of a field that uses fluid injection for water disposal, while minimizing the likelihood of seismic activity that is sufficiently strong to be felt by humans.

Worldwide hydrocarbon extraction of both natural gas and petroleum liquids has increased steadily (see go.nature.com/3hnqdat) since the mid-1980s (although the COVID-19 pandemic has interrupted this long-term trend). The water produced during the process must be treated, recycled or disposed of, because it is salty and contaminated by hydrocarbons and other organic and inorganic compounds[3]. Moreover, wastewater production tends to increase with the maturity of an oil field. This effect, combined with the sustained increase in hydrocarbon extraction, means that the disposal of wastewater is a growing global challenge.

Although most earthquakes are caused by tectonic forces, they can also be triggered by fluid injection into bedrock, most commonly when fluids penetrate pre-existing faults. The associated increase in fluid pressures reduces frictional resistance to slip, which, in turn, can reactivate the fault and trigger an earthquake (Fig. 1). Fluid injection and hydrocarbon extraction can also cause large changes in volume or mass underground that exert stresses on nearby, pre-existing faults, resulting in seismic activity[1,4]. In the past few years, various regions have undergone significant changes in earthquake-recurrence patterns owing to large-scale fluid injection − including Oklahoma in the United States[5], the Sichuan Basin in China[6] and the Western Canadian Sedimentary Basin[7]. Such changes have been observed for fluid injection associated both with water disposal[1,5] and with hydraulic fracturing (fracking)[8].

Fluid-flow simulations are typically used to investigate correlations between earthquake patterns and fluid injection[9]. This approach provides insight into the underlying drivers of earthquake occurrence in tectonically quiet areas. However, fluid simulations alone are probably insufficient for developing strategies to manage seismicity, particularly in areas in which tectonic earthquakes are common, because the dominant cause in such regions is explicitly ignored.

Hager *et al.* have developed a multidisciplinary approach to earthquake mitigation in Italy's Val d'Agri field, which is located in a tectonically active area. Val d'Agri is the largest onshore oil field in Western Europe. Extraction started in 1993, and the field now accounts for more than half of Italy's oil production. Wastewater disposal started in 2006 and led to about 300 small seismic events (maximum local magnitude 2.2, which is too small to be felt). Historically, an average of