To overcome these limitations, the authors combined their hydrogel with other materials, each of which conferred a different benefit (Fig. 1).

The polymer backbone of the hydrogel was made up of hyaluronic acid, a naturally occurring polysaccharide that has mechanical properties similar to those of soft tissues. Hyaluronic acid is known to promote tissue regeneration[6] – it is abundant during fetal development[7] – and it is useful because other molecules can be attached to the chemical groups on its backbone[8]. Yet, hyaluronic acid is not conductive, so Jin *et al.* covalently linked it to compounds containing hexagonal rings with electron clouds that can hold gold nanoparticles. Gold is a conductive, tissue-friendly and inherently inert material.

The structural weakness of hydrogels is especially problematic when they are applied to tissues that undergo repeated strain, such as skeletal muscle. Simply introducing more irreversible covalent bonds to the hyaluronic acid backbone does not help, because this makes the hydrogel too stiff to flow. Instead, the authors introduced reversible bonds, such as those supplied by the hexagonal rings[9], or by hydrogen and ionic interactions, which break under the 'shear' force produced when the gel is pushed through a needle. This allows the gel to be injected easily, but also means that the bonds can be quickly re-established after the hydrogel sets in the wounded muscle, enabling long-term stability.

Owing to this innovative chemistry, Jin *et al.* were able to support the regeneration of an injured skeletal muscle in a rat's back leg by injecting their hydrogel prosthesis, which degraded almost completely in four weeks. Importantly, the prosthesis did not cause overactivation of the rat's immune system, or the formation of scar-like fibrous tissue.

The authors also showed that their hydrogel could adhere stably to the peripheral nerve in the rat's back leg and that it could interface with conventional electric wires. This enabled the researchers to use the hydrogel to record sensory neural signals that resulted from brushing or tapping the rat's foot. And this connection worked in reverse. Jin *et al.* could activate the muscle by applying electrical stimulation through the hydrogel while it was wrapped around the nerve. Although conventional electrodes damaged the nerve after prolonged stimulation, applying the same voltage through the hydrogel did not.

Finally, the team brought all these advances together, by applying the hydrogel both around the nerve and around the muscle and connecting it to a robotic system, which supports rehabilitation of animals shortly after injury, while the rats were awake. The robot was programmed to lift the animal's foot when the electrical signal from the muscle reached a certain threshold. The system then triggered feedback stimulation through the nerve; this could be adjusted depending on the magnitude of the electrical signal coming from the muscle.

Without such nerve stimulation, Jin and colleagues found that injured animals' muscles could not produce signals strong enough to activate the robot. However, with nerve electrical stimulation through the hydrogel, the muscle signals were robust enough to engage the robotic assistance, allowing the animals to walk soon after injury.

The authors also showed that the hydrogel's ability to enable the transmission of electrical signals by increasing the conductivity around the injury was crucial. A control group without the conductive hydrogel was not able to activate the robot, owing to the absence of electrical signal transmission. Simply increasing the stimulation voltage is not a viable option because this can lead to nerve damage.

This is an impressive demonstration of an injectable conductive hydrogel as a soft tissue prosthesis, but its applicability to humans is yet to be tested. Rats' injuries are smaller than those of humans. Further studies are needed in large animals to determine whether the hydrogel can conduct efficiently over longer distances[10]. Such studies would also better qualify the regenerative potential of Jin and colleagues' hydrogel and the immune response it invokes.

The timescales for muscle regeneration in people might also be longer than those in rats, necessitating further optimization of the rate at which the hydrogel degrades. Ultimately, regulatory approval will be required for this therapy to reach humans, and it might be difficult to obtain for such a complex prosthesis. Nonetheless, Jin and colleagues' work provides a potential strategy for the development of injectable prostheses that can rapidly restore injured muscle function both by electrical stimulation and by triggering tissue regeneration.

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Structural biology

An ion transporter with features of a channel

John Orlowski

A membrane-transport protein in sperm exchanges sodium and hydrogen ions. Its activations by voltage and by cyclic nucleotide molecules are usually only features of ion channels. Structural data shed light on this protein. **See p.193 & 202**

Sperm are highly responsive to the pH level of their external and internal environments. Writing in *Nature*, Yeo *et al.*[1] (page 193) and Kalienkova *et al.*[2] (page 202) describe unusual structural features of a sperm-specific transport protein for pH regulation called SLC9C1, which exchanges extracellular sodium ions (Na^+) for intracellular hydrogen ions (H^+). The protein also has regulatory sensors, typically associated with ion channels, that are controlled by voltage across the cell membrane and by molecules called cyclic nucleotides. These characteristics make SLC9C1 well suited to regulating the intracellular pH (pH_i) of sperm, along with their motility and ultimately, fertility.

When sperm are released from the testes, they undergo an intricate, pH-dependent maturation process as they 'awaken' from a dormant state. After release, they accumulate in the adjoining epididymis tube, which contains acidic fluid (at a pH of between around 6.5 and 6.8) that renders the sperm relatively immobile. However, when sperm become a component of semen and encounter fluids in the uterus and oviducts of the female reproductive system, they enter an alkaline environment that is enriched in bicarbonate ions (HCO_3^-) and

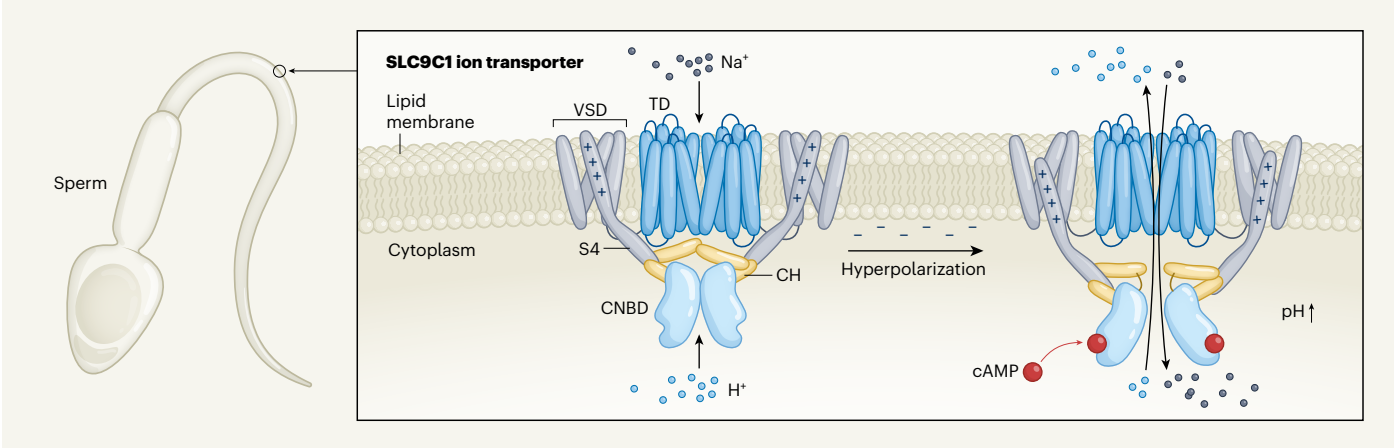

Figure 1 | The SLC9C1 ion transporter in the sperm tail. Yeo *et al.*[1] and Kalienkova *et al.*[2] present the structure of inactive SLC9C1 protein from the sea urchin *Strongylocentrotus purpuratus*, obtained using a method known as cryogenic electron microscopy. SLC9C1 functions in a pH-sensitive signalling cascade that triggers sperm motility and aids fertility. This protein exchanges sodium ions (Na^+) and hydrogen ions (H^+) and has three distinct domains: an ion-transport domain (TD); a voltage-sensor domain (VSD), of which the S4 segment is the key voltage sensor; and a cyclic-nucleotide-binding domain (CNBD), which has high affinity for the signalling molecule cAMP. The VSD and CNBD are joined by intervening coupling helices (CH). Both studies[1,2] propose how SLC9C1 might be activated. As the membrane's cytoplasmic side gains a negative charge (hyperpolarization) owing to ion movements (not shown), S4 rapidly moves down, displacing coupling helices and opening the CNBD. The binding of cAMP stabilizes the CNBD, enabling Na^+ and H^+ exchange and making the cytoplasmic pH more alkaline.

calcium ions (Ca^{2+}), and the pH reaches between about 7.6 and 7.8. This pH change, along with other factors (depending on the species), triggers sperm to transition to a 'hyperactive' motile state in a process called capacitation as they navigate energetically towards the egg[3]. During this conversion, the sperm's pH_i increases by up to 0.4 pH units, regulated by an ion carrier[4].

As the sperm reaches the egg, a specialized secretory vesicle called the acrosome — located in the uppermost region of the sperm's 'head' — fuses with the sperm plasma membrane. This aids fertilization by releasing adhesion molecules and enzymes that can break down particular external macromolecules, allowing sperm to attach to and penetrate the egg. Disrupting alkalinization severely curtails sperm motility and fertility[5].

The pH-dependent mechanisms governing sperm function have been investigated for decades. Although sperm from various species exhibit differences, such efforts have revealed a suite of unique ion channels, transporters and soluble cytoplasmic enzymes that are relatively evolutionarily conserved. These are functionally coupled in a pH_i-dependent manner and localized to the motile 'tail' (flagellum) that propels the sperm[5]. Essentially, the sperm's pH_i acts as a control switch that regulates its behaviour.

An evolutionarily conserved transporter protein in the cell membrane called the SLC9C1 Na^+/H^+ exchanger is a central player in this control switch. Its unusual features were recognized almost 40 years ago in sperm from the sea urchin *Strongylocentrotus purpuratus* (Sp), an established model system for studying fertilization[6].

Unlike other Na^+/H^+ exchangers or other known transporters, the activity of SpSLC9C1 requires previous activation of the sperm-specific potassium channel SpKCNG1 (known as KCNU1 or SLO3 in mammals[7]), in which the voltage across the flagellar membrane becomes more negative (a process called hyperpolarization). This requirement suggests that SpSLC9C1 has voltage-detection abilities[6]. A previous study[8] used the techniques of molecular cloning and computer modelling to analyse the theoretical structure of the mouse version of SLC9C1 (mSLC9C1). This revealed that four transmembrane helices (S1–S4) form a proposed voltage-sensing domain (VSD) that is similar to those found in voltage-gated ion channels[8].

Unexpectedly, mSLC9C1 is also predicted to contain a domain that can bind to molecules called cyclic nucleotides, and such cyclic-nucleotide-binding domains (CNBDs) are found in cyclic-nucleotide-gated ion channels[9]. This is intriguing, because the important intracellular signalling molecule cyclic adenosine monophosphate (cAMP) — a cyclic nucleotide that is produced in sperm by the HCO_3^--regulated enzyme ADCY10 — is also a key determinant of sperm motility and fertility[10]. This indicates a possible link between cAMP production and SLC9C1 activity. Indeed, mSLC9C1 is required for the expression of ADCY10 and physically interacts with it[11].

Expressing SpSLC9C1 in hamster cells *in vitro* confirmed the functionality of these channel-like regulatory modules[12]. Ion-transport activity was increased by hyperpolarization, whereas cAMP brought the activation voltage closer to a non-hyperpolarized state (the resting membrane potential), which allowed for prolonged activity of SLC9C1 (ref. 12).

Yeo *et al.*[1] and Kalienkova *et al.*[2] now link form to function, using the method of cryogenic electron microscopy to reveal the atomic-level architectural details of inactive SpSLC9C1 (Fig. 1). The protein assembles from two identical subunits similar to those of other Na^+/H^+ exchangers. The structure also contains a membrane VSD and a cytoplasmic CNBD, and has some interesting differences from voltage-gated ion channels. In those channels, the VSD is physically connected to the ion-permeation pore. Movement of the main voltage-sensing S4 transmembrane segment (which is enriched in positively charged amino-acid residues that respond to the membrane's electric field) imparts mechanical force on the adjoining S5–S6 segments that form the channel pore, causing the pore to open or close.

By contrast, for SpSLC9C1, the VSD is located on either side of the membrane-embedded ion-transport domain (TD), with the S4 segment making no direct contacts with the TD. This explains why SpSLC9C1 mutations that eliminate transport activity do not impair the gating currents generated by voltage-dependent movement of the VSD S4 segment[12] — these elements can operate independently, yet are functionally coupled.

The VSD of SpSLC9C1 is further distinguished from those in typical voltage-gated channels by an unusually long S4 segment (of roughly 90 ångströms) that indirectly connects the VSD to the CNBD through intermediary cytoplasmic coupling segments (helices). In the resting inactive state, the CNBD and coupling helices in each subunit form a cytoplasmic interface (a dimer of the two domains) that seems to block and possibly constrain the catalytic domain. However, in structures

obtained with cAMP, the CNBDs have separated from the dimer interface, exposing the ion-transport domains to the cytoplasm.

These data led both groups to propose a previously unknown gating mechanism for SLC9C1. This is that, on activation of the sperm-specific potassium channel SLO3 (or its equivalent in other species), the resulting membrane hyperpolarization elicits a rapid and transient downward displacement of the VSD's positively charged S4 segment. In turn, this shifts the adjacent coupling helices and disrupts the CNBD's dimeric cytoplasmic interface. The CNBD is stabilized by cAMP binding, weakening the dimer interface and enabling the movement of the S4 segment at lower membrane voltages, although how this occurs remains unclear.

These structural movements are thought to release the exchanger from a locked, inactive state, allowing sustained Na^+ and H^+ exchange at lower membrane voltages. Validating this model will require resolving the transporter's structure in its active, hyperpolarized state, and using the approach of mutagenesis to investigate the coupling interactions. The physical interaction of ADCY10 with SLC9C1 also remains to be determined.

How does activation of SLC9C1 aid in sperm capacitation? Although differences exist between species, in general, SLC9C1-induced alkalinization stimulates the entry of Ca^{2+} into sperm through the sperm-specific channel CATSPER, which is voltage-gated and also responsive to high pH (ref. 13). The rise in intracellular Ca^{2+} triggers downstream events that drive vigorous asymmetrical flagellar beating and accelerated sperm motility. Deletion of the mouse genes encoding any of SLO3 (ref. 14), ADCY10 (ref. 15), SLC9C1 (ref. 8) or CATSPER (ref. 16) strikingly decrease sperm motility and render male mice infertile. Curiously, emerging evidence suggests that human SLC9C1 might be regulated differently, because voltage and cAMP do not seem to gate this transporter[17]. Nevertheless, a mutation that disrupts the structure of human SLC9C1 impairs sperm motility and causes infertility[18]. More work needs to be done.

"SLC9C1 functions in a pH-sensitive signalling cascade that triggers sperm motility and aids fertility."

Collectively, these data provide notable structural and functional insights into an unusual transporter with a specialized role that is crucial for sperm motility and fertilization. These studies might also open up fresh avenues in the search to develop male contraceptives.

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