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PLANT SCIENCE

Sexual attraction channelled in moss

An analysis reveals that both sexual reproduction and early-embryo development in the moss *Physcomitrella patens* are controlled by cellular calcium influxes through ion-channel proteins.

LEONIE STEINHORST & JÖRG KUDLA

The attraction of free-swimming sperm to a stationary egg is a widespread phenomenon, occurring in organisms from plants to mammals. The mechanisms underlying this process, which is called chemotaxis, involve gradients of chemical signals that are perceived by sperm and used to direct their locomotion. But many aspects of sperm chemotaxis, including the identity of some of the major components involved, have remained unclear — especially in mosses and ferns. In a paper online in *Nature*, Ortiz-Ramírez *et al.*[1] identify two membrane-spanning, glutamate-receptor-like proteins (GLRs) that are indispensable for sperm orientation in the moss *Physcomitrella patens*. In addition, the authors provide evidence that these channel proteins target the transcription factor BELL1 to control early embryonic development more generally.

There is considerable interest among plant researchers in analysing the function of GLRs, which control the passage of calcium ions (Ca^{2+}) across plant-cell membranes. However, such analysis has proved difficult, because the gene family that encodes these proteins is typically large[2]. By contrast, the genome of *P. patens* encodes only two GLRs, *Pp*GLR1 and *Pp*GLR2, a low level of complexity that makes it possible to modify the *GLR* genes and explore their function.

Ortiz-Ramírez *et al.* generated *P. patens* mosses that lacked *PpGLR1* or *PpGLR2*, or both. They found that these mosses showed severe defects in fertility in crossing experiments in this usually self-fertilizing species. The authors therefore developed a neat *in vitro* sperm-navigation assay to analyse sperm chemotaxis in the mutant plants and in wild-type controls.

They observed that, following release from the male sex organ (the antheridium), wild-type sperm moved in a spiral motion at an average speed of 16.7 micrometres per second. Approximately 1 in 50 sperm successfully contacted the opening of the female sexual organ, the archegonium. Of these, half managed to enter the organ.

Sperm lacking both GLRs were much less efficient at targeting and entering the archegonium opening. Interestingly, however, the mutants moved faster than wild-type sperm, with an average speed of 23.2 $\mu m\,s^{-1}$. The authors suggest that this difference arises because the loss of GLRs prevents the sperm from detecting or responding to chemoattractant signals from the archegonium; these signals could cause changes in direction that decrease speed.

This finding parallels observations in marine invertebrates[3,4], in which a reduction in extracellular Ca^{2+} concentration rendered sperm unable to change direction but had little effect on straight swimming. In agreement with a role for Ca^{2+} signalling in sperm orientation in mosses, Ortiz-Ramírez *et al.* found that the mutant moss sperm had lower cytoplasmic Ca^{2+} concentrations than their wild-type counterparts.

Next, the authors analysed the passage of Ca^{2+} across cell membranes in wild-type and GLR-deficient cells, and in cells that overexpressed GLRs. Moreover, to exclude the possibility that unidentified moss proteins contribute to Ca^{2+} fluxes, the authors overexpressed the moss GLRs in human cells (which lack GLRs) and studied their Ca^{2+} accumulation. Collectively, these approaches provided compelling evidence that GLRs enable Ca^{2+} flux into cells, establishing appropriate ion concentrations for efficient fertilization (Fig. 1). It is tempting to speculate that a threshold Ca^{2+} concentration must be reached or exceeded in sperm to activate mechanisms that facilitate an active change in swimming direction.

Probably owing to technical limitations of image resolution, combined with the fact that sperm motility disturbs microscopic observations, Ortiz-Ramírez and colleagues did not provide further insights into the dynamics and subcellular distribution of Ca^{2+} in sperm. But alterations in Ca^{2+} concentration can directly affect, for instance, the beating and bending of hair-like extensions, called flagella, on the sperm body that control the cell's trajectory and motility[5–7]. This

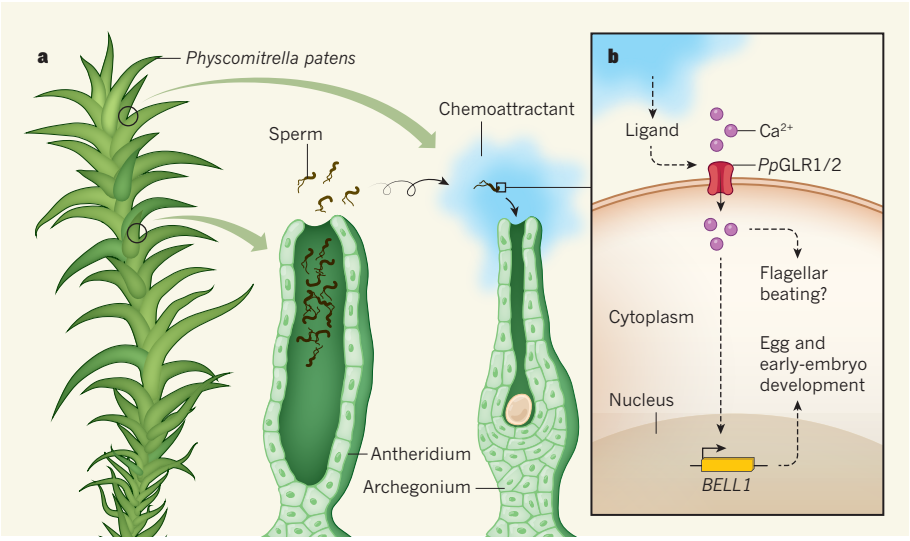

Figure 1 | Calcium signalling regulates reproduction in moss. **a**, In the moss *Physcomitrella patens*, sperm released from the antheridium are attracted to plant's female sexual organ (the archegonium) by an unknown chemoattractant molecule. **b**, Ortiz-Ramírez *et al.*[1] provide evidence that two glutamate-receptor-like ion channels (GLRs) on the sperm membrane, *Pp*GLR1 and *Pp*GLR2, are activated in response to this chemoattractant, either directly (not shown) or indirectly by an unknown ligand. This leads to an influx of calcium ions (Ca^{2+}) into the cell cytoplasm. Changes in intracellular Ca^{2+} levels modulate sperm orientation (perhaps through changes in the beating of hair-like extensions called flagella), promoting movement towards the archegonium. The authors also show that changes in GLR activity lead to altered transcription of the gene *BELL1* and related transcription factors, which are involved in regulating the development of the fertilized egg and early embryo. (Dashed lines indicate steps not known to be direct).

is an imaging challenge for the future.

Sperm chemotaxis has probably evolved many times. Consequently, the sperm-luring chemoattractant signal at its heart can take many forms — from protein fragments to hormones — and is often species-specific[6]. Previous work has identified[8] the amino acid D-serine, which is released by the female sexual organs of plants such as the model organism *Arabidopsis thaliana*, as an activator of GLRs. But moss fertilization occurs in water, where sperm of different species could be present in a single droplet, and cross-fertilization of different species would be unfavourable. As such, it seems unlikely that a substance as common as D-serine would function as a chemoattractant for moss-sperm guidance.

Instead, perhaps chemoattractant perception and GLR activation are separate processes. Chemoattraction might be conferred by species-specific signals such as protein fragments, and subsequent GLR activation could rely on a more evolutionarily conserved ligand. Identification of these factors could provide insights into the evolution of sexual demarcation and speciation in plants.

Ortiz-Ramírez *et al.* made another striking finding — that GLR-modulated Ca^{2+} concentration also regulates the development of *P. patens* embryos and sporophytes (the stage of the life cycle at which the plant produces spores). Mutant sporophytes lacking *PpGLR1* and *PpGLR2* produced smaller and fewer spores than their wild-type counterparts. Gene-expression analyses revealed that *BELL1* was among the genes downregulated in the double mutants, suggesting that the transcription of this gene depends on GLR-mediated Ca^{2+} influx.

Members of the BELL1 family of transcription factors control the development of egg cells and early embryos in other plants, including *Arabidopsis*[9]. When Ortiz-Ramírez *et al.* artificially restored the expression of *BELL1* in the immature sporophytes and reproductive organs of their *P. patens GLR* mutants, sporophyte development was restored, but chemoattractant responsiveness was not. This finding indicates that the two roles for the GLRs are distinct and clearly separable.

The discovery that GLR-mediated Ca^{2+} influx affects BELL1 during embryonic development could have an impact far beyond its implications for fertilization. GLR-dependent regulation of BELL1-family transcription factors points to the possibility that development of the fertilized *P. patens* egg and early embryo are under Ca^{2+} control. Notably, the authors' analysis of gene-expression networks revealed that *PpGLR2* transcription was associated not only with transcriptional regulation of *BELL1*-family genes, but also with the transcription of genes encoding protein kinase enzymes, which phosphorylate proteins. This allows for the speculative but exciting hypothesis that Ca^{2+}-mediated phosphorylation, perhaps triggered by GLR activity, brings about post-transcriptional regulation of the BELL1 protein and related transcription factors. The conversion of Ca^{2+} signals into reversible protein modifications would provide fine-tuned regulation of BELL1 activity to faithfully adjust development in response to internal and external cues. If such a regulatory mechanism is evolutionarily conserved, Ca^{2+} signalling could mediate development not just in mosses, but in plant embryos in general. ■

Leonie Steinhorst *and* **Jörg Kudla** *are in the Department of Biology, Institute of Plant Biology and Biotechnology, University of Münster, 48149 Münster, Germany.*
e-mails: leonie.st@uni-muenster.de; jkudla@uni-muenster.de

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