CELL POLARITY

Taking sides

Which cell type starts off as a diploid spermatogonium, spends some time as a round, haploid spermatid, but ends up being elongated and haploid with a powerful flagellum? A spermatozoan, of course. What regulates the differentiation of spermatids into spermatazoa is less obvious, but Gliki *et al.* have uncovered a role for the junctional adhesion molecule (JAM)-C in the polarization of round spermatids, by virtue of its ability to recruit a cell-polarity complex.

To ascertain the function of JAM-C directly, the authors disrupted the *Jam-C* gene by homologous recombination in mice. Of the surviving ~60% of the offspring, the males had testes that were half the size of those of their littermates, and they couldn't make mature sperm cells; there were no differentiated, elongated spermatids. Analysis of the testes of wild-type mice showed JAM-C expression throughout most of the round spermatogenic cells, although some JAM-C was concentrated at the front (the presumptive head). As spermiogenesis proceeded, JAM-C was restricted to specialized adhesion structures — junctional plaques — that anchor spermatids to the signal- and nutrient-providing Sertoli cells. Closer inspection showed that another isoform — JAM-B — was present on Sertoli cells, so that, in principle, *trans*-interactions could occur between these two immunoglobulin-domain-containing proteins.

Having excluded a role for JAM-C in the maintenance of the blood–testes barrier (defects in which cause infertility), the authors noticed that morphological signs of polarization, such as the formation of an acrosome (the specialized penetrating vesicular organelle), were absent in the round *Jam-C*$^{-/-}$ spermatids. However, although cytoskeletal defects occurred in *Jam-C*$^{-/-}$ spermatids, these, along with defects in acrosome formation, weren't thought to be responsible for impaired polarization in spermatids.

Gliki *et al.* therefore looked at known mediators of cell polarization — specifically, Par6, Cdc42 and protein kinase Cλ (PKCλ; also referred to as atypical PKC (aPKC)). In wild-type spermatids, these proteins colocalized with JAM-C in the head regions, whereas their distribution was not polarized in *Jam-C*$^{-/-}$ spermatids. Another protein, PATJ, through interactions with PALS1 and crumbs3 (CRB3), can also regulate polarity, and the Par6–Cdc42–aPKC and CRB–PALS1–PATJ complexes can directly interact. PATJ also showed some colocalization with JAM-C at the junctional plaques.

How, then, might JAM-C affect these polarity proteins? To study this, the authors mimicked the binding of spermatids to Sertoli cells by providing cultured primary spermatids with soluble versions of JAM-B or JAM-C fused to immunoglobulin (Ig) domains. Before the addition of these fusion proteins, some PATJ was polarized and Cdc42 and PKCλ were broadly distributed in both wild-type and *Jam-C*$^{-/-}$ cells. But only in wild-type spermatids did the addition of the JAM-B and JAM-C fusion proteins cause Cdc42, PKCλ and PATJ to redistribute to the fusion-protein-binding sites. The authors also coimmunoprecipitated complexes containing JAM-B and JAM-C from testes, as well as complexes of JAM-B, Par6, Cdc42, PKCλ and PATJ with JAM-C. The polarized distribution of Par6 has been shown to require an interaction with Cdc42, and the authors showed that adding JAM-C–Ig fusion proteins to JAM-C-overexpressing cells increased activated Cdc42 — a potential way to recruit Par6 and the rest of the polarity proteins.

So JAM-C has a key role in mouse spermiogenesis during the polarization of spermatids by recruiting a polarity complex to junctional plaques. Because the proteins that are involved also show a similar distribution and behaviour in human spermatids, further investigation of JAM-C could uncover important information for the treatment of human infertility.

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Gliki, G. *et al.* Spermatid differentiation requires the assembly of a cell polarity complex downstream of junctional adhesion molecule-C. *Nature* **431**, 320–324 (2004)

STRUCTURE WATCH

A structural relay

Integrins are adhesion receptors that are important for processes such as cell migration and vascular homeostasis, and they signal bidirectionally across the plasma membrane. They are composed of an α and β subunit that form a 'head' with two long 'legs' in the extracellular region. This region is known to adopt three conformations with different ligand-binding affinities: a low-affinity, bent conformation; an intermediate-affinity, extended, 'closed' conformation; and a high-affinity, extended, 'open' conformation. The lack of an atomic structure for the latter state has meant that it has been unclear how allostery is relayed within integrins, but, in *Nature*, Springer and colleagues now provide the missing structural data.

They describe the crystal structure of the high-affinity open conformation of the head and upper-leg regions of integrin $\alpha_{IIb}\beta_3$. By comparing their data to previous crystal structures, they have identified the atomic basis for allostery between the ligand-binding site in the β_3 I domain, which is positioned at the top of the head, and the other integrin domains. Ligand binding induces a conformational change in the β_3 I domain, which results in a 62° pivot at the interface of this domain and the adjacent hybrid domain. The hybrid domain is rigidly connected to a domain of the upper β_3 leg, so, through this rigid link, the conformational change is transmitted to the 'knees' of the α and β legs. As a result, the knees separate by ~70 Å. Conformational changes in an integrin head can therefore disrupt interactions between the legs and favour leg extension, which positions the high-affinity head far above the cell surface.

REFERENCE Xiao, T. *et al.* Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature* 19 Sep 2004 (doi:10.1038/nature02976)

The missing piece of the puzzle

P-type ATPases are ATP pumps that establish ion-concentration gradients by transporting cations across membranes. The best-studied P-type ATPase is the sarcoplasmic reticulum (SR) Ca^{2+}-ATPase, which has three cytoplasmic domains — the actuator (A), nucleotide-binding (N) and phosphorylation (P) domains — and ten transmembrane helices. Crystal structures of this ATPase in three of the four principal states of its reaction cycle have been described previously and now, in *Nature*, Toyoshima and co-workers present a crystal structure that completes our view of these states.

They determined the 2.3-Å-resolution structure of the SR Ca^{2+}-ATPase bound to a phosphate analogue in the absence of Ca^{2+}. A comparison of these data with previous structures indicates that ADP release from the interface of the N and P domains induces a rotation in the A domain, which ultimately triggers the opening of the lumenal gate and disrupts Ca^{2+} binding, causing Ca^{2+} release into the lumen. Phosphate release triggers gate closure, and this lumenal gating mechanism is mediated mainly by movements of the A domain. This work has therefore shown how lumenal gating can be mediated by events some 50 Å away, and has allowed the authors to propose a complete model for ion pumping.

REFERENCE Toyoshima, C. *et al.* Lumenal gating mechanism revealed in calcium pump crystal structures with phosphate analogues. *Nature* 26 Sep 2004 (doi:10.1038/nature02981)