STRUCTURE WATCH

A constricting girdle

Electrical signalling between nerve and muscle cells is controlled by the acetylcholine (ACh) receptor (AChR) — a transmitter-gated ion channel. AChR has five subunits (α, α, β, γ or ε, δ), which each contain four predicted transmembrane segments (M1–M4). When ACh binds to the ligand-binding regions of the two α subunits, the cation-selective pore opens. But how does this gating occur? In *Nature*, Unwin and colleagues now provide insights by describing a 4-Å-resolution model of the closed pore, which was obtained using electron microscopy and crystalline postsynaptic membranes.

The authors found that the pentameric pore has two, mainly separate, structural components: an inner circle of M2 helices that create a tapering path for ions; and an outer shell of M1, M3 and M4 helices that shield the inner circle from the lipids. They also found that the ion-conduction path has three negatively charged rings, which might influence ion transport. Furthermore, they were able to identify the gate of the pore — a constricting hydrophobic girdle in the middle of the lipid bilayer that is formed by weak interactions between adjacent M2 helices. Finally, this work allowed Unwin and co-workers to propose a model for the AChR gating mechanism. In this model, ACh-induced rotations in the ligand-binding domain of the AChR are transmitted to the girdle through the M2 helices, and these rotations open the pore by breaking the girdle apart.

REFERENCE Miyazawa, A., Fujiyoshi, Y. & Unwin, N. Structure and gating mechanism of the acetylcholine receptor pore. *Nature* **423**, 949–955 (2003)

Straight to the core

Muscle contraction is regulated by intracellular Ca^{2+} concentration, and troponin is essential in this regulation. Troponin is composed of three different subunits — a Ca^{2+}-binding subunit (TnC), an inhibitory subunit (TnI) and a tropomyosin-binding subunit (TnT) — and, together with tropomyosin, it is located on the actin backbone of muscle thin filaments. Although changes in TnC–TnI interactions are thought to be important for muscle contraction, how troponin–tropomyosin regulates the actomyosin ATPase has remained unclear, because high-resolution structures have only been obtained for small sections of the troponin–tropomyosin–actin complex. However, in *Nature*, Maéda and colleagues now present the high-resolution crystal structures of Ca^{2+}-saturated core domains of human cardiac troponin.

These structures revealed the overall architecture of troponin, and showed that it can be divided into structurally distinct subdomains, the boundaries of which do not coincide with the boundaries of TnC, TnI and TnT. These subdomains are connected by flexible linkers, which make the entire molecule very flexible. However, a coiled-coil that is made up of α-helical regions of TnI and TnT forms part of one rigid asymmetric subdomain called the IT arm, and this subdomain bridges putative tropomyosin-anchoring regions. The structures also indicated that Ca^{2+} binding to the regulatory site of TnC might result in the carboxy-terminal region of TnI being detached from actin — a conformational change that could alter the mobility and/or flexibility of troponin and tropomyosin on actin.

REFERENCE Takeda, S., Yamashita, A., Maeda, K. & Maéda, Y. Structure of the core domain of human cardiac troponin in the Ca^{2+}-saturated form. *Nature* **424**, 35–41 (2003)

METASTASIS

Keep moving

Tumour-cell metastasis is complex, but the most basic feature is cell movement — away from the primary tumour through the extracellular matrix (ECM). Cell motility involves remodelling of the cytoskeleton. As Rho-family GTPases regulate cytoskeletal proteins — through the Rho kinase (ROCK) family — and are often overexpressed in tumours, Sahai and Marshall studied Rho–ROCK signalling in the motility of tumour-cell lines.

The ability of some cell lines (such as A375m2 metastatic melanoma) to invade a three-dimensional ECM-like matrix was almost completely blocked by TAT-C3, which inactivates Rho proteins, or Y27632, which inhibits ROCK proteins; other cell lines (such as BE colon carcinoma) were unaffected. The effects of these inhibitors correlated with the morphology of the invading cells in the matrix. TAT-C3- and Y27632-susceptible cell lines were rounded, with membrane blebs, whereas non-susceptible cells such as BE were elongated, with membrane spikes. To show a causal relationship, the effects of modulating Rho–ROCK signalling on tumour-cell morphology were examined. A375m2 cells treated with TAT-C3 or Y27632 lost their membrane blebs and developed membrane protrusions, whereas activating Rho–ROCK signalling in their less rounded, parental non-metastatic counterparts or in BE cells increased cell rounding. So Rho–ROCK signalling is necessary and sufficient for a round morphology.

Finally, Sahai and Marshall looked at the mechanism of cell motility. Whereas cells that move in an elongated manner localized phosphatidylinositol-3,4,5-trisphosphate to the leading edge and had a polarized Golgi apparatus, rounded A375m2 cells had localized membrane patches of ezrin orientated in the direction of cell movement. Interfering with ezrin function only inhibited the invasive ability of cells that use rounded movement. Ezrin links the ECM and the cytoskeleton, so movement occurs in response to polarized cell adhesions that 'drag' the cell in one direction.

Interestingly, A375m2 cell movement didn't depend on proteolytic degradation of the matrix. Furthermore, when extracellular proteolysis was blocked, cells that would normally move in an elongated manner (such as BE cells) — with no requirement for Rho–ROCK signalling — started moving in a rounded, Rho–ROCK-dependent way. For some tumour cells, therefore, invasion is only blocked when both proteolysis and Rho–ROCK signalling are inhibited.

Kirsty Minton, Assistant Editor, Nature Reviews

References and links

ORIGINAL RESEARCH PAPER Sahai, E. & Marshall, C. J. Differing modes of tumour-cell invasion have distinct requirements for Rho/ROCK signalling and extracellular proteolysis. *Nature Cell Biol.* **5**, 711–719 (2003)