A titinic extension

In a homage to reductionism, Hongbin Li, Julio Fernandez (Columbia University, New York, NY), and colleagues show that the properties of individual domains of titin, a giant muscle protein, can explain the elasticity of intact muscle.

The pulling in muscle is done by actin and myosin, but stretching is resisted by the elasticity of titin. Individual titin molecules of up to 3 MDa span over an entire half sarcomere—the unit of contraction in muscle. But only one region of titin confers elasticity, and this region can be broken down into discrete domains.

Fernandez and colleagues stretch various combinations of these domains by single molecule atomic force microscopy. They find that, under increasing force, proximal Ig domains undergo little passive stretching before giving way to a wholesale unfolding.

The result is a sawtooth pattern of extension, with each peak representing the resistance of a single Ig domain.

In contrast, the N2B domain can be stretched over a long distance with relatively little force. “N2B is behaving as a simple entropic spring,” says Fernandez. This suggests that N2B does not have any significant fixed structural elements to resist stretching. “It’s very hard to design a protein that will not attain some [fixed] three-dimensional structure,” said Fernandez. “It’s clearly something that is not accidental and was meant to be this way.”

The extension of N2B and the similarly elastic PEVK domain explains most of the elastic behavior of titin. But at higher extensions some of the proximal Ig domains also unfold. “They serve as a gearbox,” says Fernandez. Unfolding of an Ig domain creates a longer spring. This flexibility may allow muscle to operate at various levels of extension without the danger of breaking apart the sarcomere. Adding this effect to the calculations, and multiplying by the number of titin molecules present in a sarcomere, yields a curve that fits the behavior of intact muscle.

Reference: Li, H., et al. 2002. Nature. 418:998–1002.

Channeling hedgehogs

The Hedgehog (Hh) signaling pathway strikes again. The framework of the pathway is simple enough: Hh binds to its receptor Patched (Ptc), thus relieving Ptc inhibition of Smo (Smo) signaling. But now Jussi Taipale, Philip Beachy, and colleagues (Johns Hopkins University, Baltimore, MD) have found that Ptc may act as a channel.

This is not the first strange episode in Hh biology. Hh looks like a bacterial cell wall protein, and uses a bizarre mechanism related to that of self-splicing proteins to attach cholesterol to itself. “This signaling pathway, which has very profound roles in multicellular organisms, was very clearly put together with bits of this and that,” says Beachy. “At this point, nothing surprises us.”

Ptc was thought to be a conventional receptor that gripped Smo in an inhibitory embrace. But Taipale suspected that the original coimmunoprecipitation data were tainted by overexpression and the hydrophobicity of Ptc and Smo. “We thought we should start from scratch,” he says.

Taipale failed to find a significant interaction between the two proteins, and like others found that the proteins were present in different parts of the cell. Furthermore, free Ptc (in excess to Smo) affected pathway activity, and substoichiometric Ptc (1:45 of Ptc:Smo) resulted in 80% reduction of Smo activity, suggesting that Ptc acts catalytically.

Ptc is similar to bacterial proton-driven transporters. Beachy’s group found that mutation of two channel-conserved residues led to a dramatic defect in Ptc activity. Thus, Hh may block Ptc from shipping in a Smo inhibitor or shipping out a Smo activator.

The relevant inhibitor or activator is unknown, but it may resemble cyclopamine. This steroidal alkaloid was first discovered when some Idaho sheep munched a maize lily and had malformed cyclopic offspring. The chemical culprit, cyclopamine, was isolated. Some thirty years later, Beachy found that mice with Hh defects suffered a similar fate, and that cyclopamine inhibits Smo.

Beachy has now found that cyclopamine regresses murine medulloblastomas, probably because these brain tumors result from Hh-dependent proliferation of stem cells. If other similarly aggressive but more common tumor types are found to be dependent on Hh, then cyclopamine may be the basis for an important cancer drug.

References: Berman, D.M., et al. 2002. Science. 297:1559–1561. Taipale, J., et al. 2002. Nature. 418:892–897.