Research Roundup

RNAi takes on DNA

RNAi may carry out direct silencing not only of RNAs but also of DNA, according to new results from Tom Volpe, Shiv Grewal, Rob Martienssen, and colleagues (Cold Spring Harbor Laboratory (CSHL), Cold Spring Harbor, NY), and Brenda Reinhart and David Bartel (Whitehead Institute, Cambridge, MA). Another group, led by Kazufumi Mochizuki and Martin Gorovsky (University of Rochester, Rochester, NY), has found that similar machinery may be used to chop out DNA segments in *Tetrahymena*.

Both systems illustrate a solution to a puzzling problem in chromatin biology. How can such a wide variety of sequences be recognized and directed toward a single fate—either silencing in heterochromatin or processing in *Tetrahymena*? RNAi may provide the answer.

The story begins with transposons. These DNA invaders are characterized by little other than their overactive transcription from either end, leading to complementary transcripts that can anneal to each other. The resulting double-stranded RNA (dsRNA) "is the one thing a cell can grab onto," says Gorovsky. The RNAi machinery chops up dsRNAs, and the resulting small RNAs target any further transcripts for destruction.

Now, the CSHL and Whitehead groups have found that a similar process may be occurring at the repeats flanking the fission yeast centromere. The Whitehead group found small RNAs complementary to these repeats, with the CSHL group characterizing forward and reverse transcripts and centromere-localized RNA-dependent RNA polymerase that are probably the source of the small RNAs. The CSHL group also found that forward transcription, centromeric silencing, and histone modifications at the centromere are RNAi dependent. Thus, chromodomain proteins may bind the dsRNAs and methylate histone H3, leading to recruitment of chromatin silencing proteins such as Swi6.

The proposal in *Tetrahymena* is similar, but here the small RNAs are used to mark the chromatin for elimination. Widespread transcripts from one nucleus are matched against the DNA of another, and anything left over is characterized as a recent invader that should be purged. Gorovsky's group found small RNAs and movement of RNAi machinery between nuclei that fits such a model, and a group led by David Allis (University of Virginia, Charlottesville, VA) made the connection between histone H3 modification and DNA elimination.

Suspicions about a DNA connection are not new—both transposons and dsRNA constructed from untranscribed regions can cause chromatin changes. But the new work provides a direct connection. It does not, however, indicate whether RNAi causes silencing of noncentromeric heterochromatin. Heterochromatin is packed with repeats that could yield dsRNA transcripts, but worms and plants lacking RNAi components, although reported as embryonic lethal, have not been examined for heterochromatin defects.

A more imponderable question involves evolution. Did a system for shutting down parasitic transposons get coopted to build centromeres, mark *Tetrahymena* sequences for deletion, and stabilize repetitive, rearrangement-prone areas of the mammalian genome? Or did evolution run that sequence in reverse? Either way, RNAi has been a key determinant of genome dynamics. ■
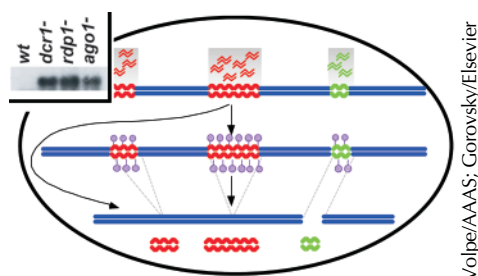

RNAi machinery directs silencing near centromeres (top inset) and elimination of *Tetrahymena* sequences.

Volpe/AAAS; Gorovsky/Elsevier

References: Mochizuki, K., et al. 2002. *Cell.* 10.1016/S0092867402009091.
Reinhart, B., and D. Bartel. 2002. *Science.* 10.1126/science.1077183.
Taverna, S.D., et al. 2002. *Cell.* 10.1016/S00092867402009418.
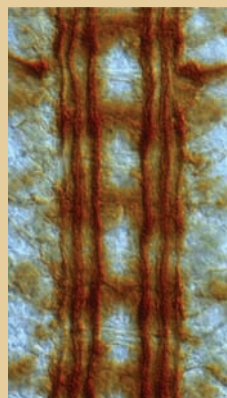Volpe, T., et al. 2002. *Science.* 10.1126/science.1074973.

Comm with me

Rapid rerouting of intracellular traffic is responsible for the axon guidance of certain fly neurons, according to Krystyna Keleman, Barry Dickson (Research Institute of Molecular Pathology, Vienna, Austria), and colleagues.

The neurons do not spend their whole time crossing and recrossing the midline of the fly because the midline repellent Slit binds the Robo receptor. Now, Dickson and colleagues show that crossing events require that the guidance protein Comm grab onto Robo in the Golgi and send it on a suicide journey to late endosomes and lysosomes. This eliminates the influence of Slit, allowing neurons to cross. After crossing, Comm is rapidly turned off to reestablish Robo signaling and prevent recrossing.

In the few cases studied, Comm transcription turns on just as neurons initiate their turn toward the midline. This is probably controlled by cell fate determinants that, in turn, are determined by the time and place that the neuron is born. The shutting down of Comm, post-crossing, may be controlled by either a midline-derived signal or a timing mechanism.

Comm's activity relies only on a minimal sorting motif, so there may well be Comm-like proteins in mammals. Furthermore, the existence of multiple Comm proteins suggest that these proteins may take many different victims to their deaths. "I guarantee you Comm is doing a lot more than sorting Robo receptors," says Dickson. ■

Midline crossing requires Comm's redirecting of Robo.

Dickson/Elsevier

Reference: Keleman, K., et al. 2002. *Cell.* 110:415–427.

TEXT BY WILLIAM A. WELLS
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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
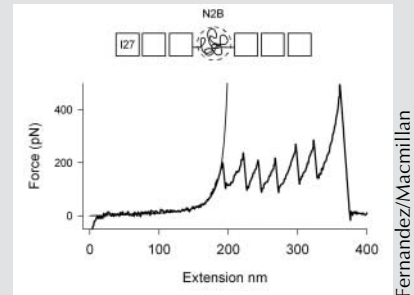
Titin extends first via N2B unravelling (smooth curve) before Ig domains give way (peaks).

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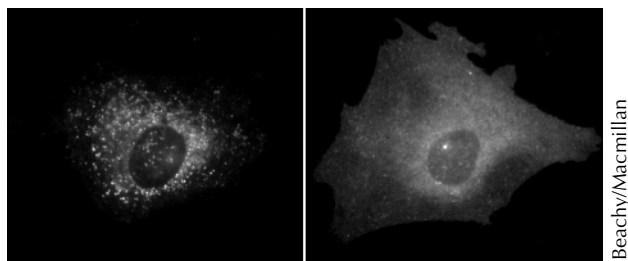
Reference: Li, H., et al. 2002. *Nature.* 418: 998–1002.

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 Smoothened (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. ∎

Ptc (left) regulates Smo (right) despite their different locations.

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