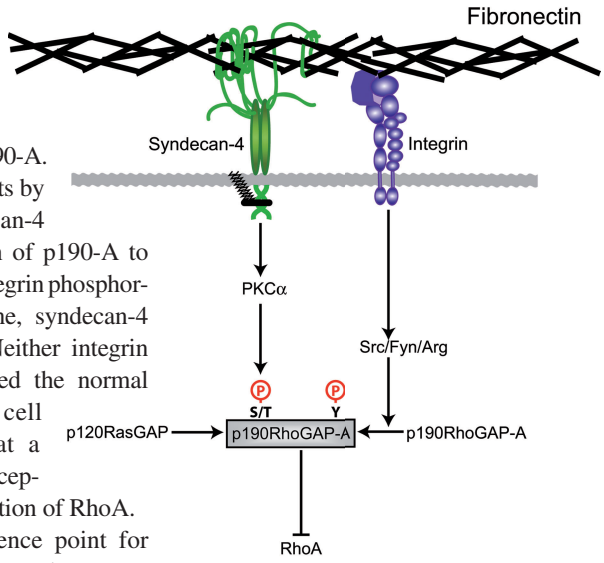
In This Issue

Complementary receptors control RhoA

A pair of adhesion receptors work together to fine-tune suppression of RhoA and promote membrane protrusion, say Bass et al.

During cell migration, the actin cytoskeleton regulator, RhoA, must be alternately inhibited (to allow the leading membrane edge to protrude) and reactivated (to pull up the trailing end of the cell from behind). Inhibition occurs when a receptor called α5β1 integrin, which binds to the extracellular matrix protein, fibronectin, triggers phosphorylation of a RhoA inhibitor called p190RhoGAP-A (p190-A). Another fibronectin receptor, syndecan-4, colocalizes with integrin during RhoA inhibition, but whether this second receptor contributes to inhibition was unknown.

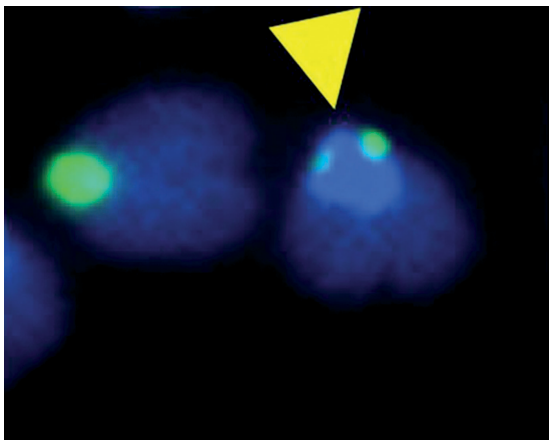
By treating cells with fragments of fibronectin that bound to syndecan-4 but not integrin, the authors showed that syndecan-4 triggered transient relocation of p190-A to the membrane, which correlated with reduced RhoA activity. Syndecan-4 is known to activate PKCα, and exogenous activation of PKCα is known to trigger phosphorylation and membrane recruitment of p190-A. Bass et al. connected the dots by showing that loss of syndecan-4 function reduced relocation of p190-A to the membrane. Although integrin phosphorylates p190-A at a tyrosine, syndecan-4 phosphorylated a serine. Neither integrin nor syndecan alone induced the normal Rho-related pattern of cell spreading, suggesting that a combination of these two receptors is needed for full inhibition of RhoA.



Syndecan-4 and integrin both contribute to RhoA regulation.

p190-A is the convergence point for signaling from these two independent adhesion receptors, the authors conclude. Although the two receptors colocalize during leading edge protrusion, syndecan-4, a lengthy protein, can stretch itself up to 500 nm from the cell. Syndecan-4 might therefore act as an exploratory receptor, whereas the shorter integrin may anchor the leading edge. In this model, p190-A serves to integrate information from both near and far. JCB

Bass, M.D., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200711129.



Two signals indicate a single fluorescent probe has been split by recombination.

Centromeres cross over, a lot

Recombination at centromeres is higher than anywhere else on the chromosome, even though methyltransferases do their best to prevent it, say Jaco et al.

Centromeric recombination has been hard to study because the DNA at centromeres is so repetitive—it's hard to see when a segment has switched chromatids. Jaco et al. have now addressed this challenge by using CO-FISH (chromosome orientation fluorescence in situ hybridization). After replication, the two new strands are digested away, leaving the two old strands. Because the strands are complementary in sequence, they can be tagged with strand-specific fluorescent probes. Using just one probe, only one chromatid would show a signal if no recombination had occurred.

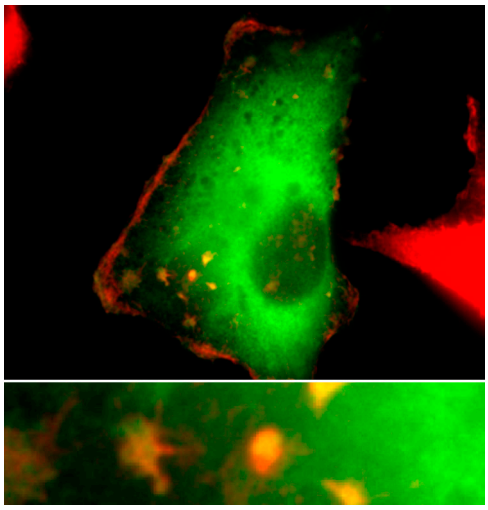
Instead, the authors found that both chromatids fluoresced. And not just at one point—on average, the authors counted, centromeres had undergone 15 recombination events. This is about six times the rate of recombination as that seen for an equal length of telomeric DNA, and 175 times the rate for genomic DNA as a whole.

Telomeric recombination is inhibited by protein complexes called shelterins and by DNA methylation. The centromere has no shelterin, but it is methylated. Knockdown of DNA methyltransferases increased recombination at the centromere by about 50%, and decreased centromere length, possibly because of misalignment between repeated segments during recombination, a common problem with repetitive DNA. How methylation limits recombination, and why centromeres didn't lengthen as well as shorten, are unknown.

Their repetitive structure makes centromeres recombinogenic by nature, and the authors suggest that epigenetic regulation may ensure the continued stability of essential binding regions for proteins that link to the centromere. JCB

Jaco, I., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200803042.

Text by Richard Robinson
rrobinson@nasw.org



IQGAP1 and exocyst component Sec8 colocalize at invadopodia.

Partner up to invade

A secretory complex and a cytoskeletal polarity protein cooperate to make tumor cells more invasive, according to new work from Sakurai-Yageta et al.

To burrow into nearby tissue, tumor cells create membrane protrusions called invadopodia. Within invadopodia, vesicles accumulate that contain metalloproteinase enzymes needed to degrade the extracellular matrix. Because a secretory complex called the exocyst is known to link exocytic vesicles to growing membranes, the team tested the effect of depleting particular exocyst subunits in breast cancer cells. They showed that the cells were less able to degrade the matrix on which they were cultured and became less invasive.

The same subunits bound to a protein called IQGAP1, which links actin and microtubules at the leading edge of migrating cells, accumulate in invadopodia and have been implicated in tumor invasion. Linkage of IQGAP1 and the exocyst was needed for metalloproteinase accumulation at the invadopodia, though it remains to be determined whether the exocyst directly targets the vesicles. Two important promoters of actin cytoskeletal assembly, Cdc42 and RhoA, promoted the association of IQGAP1 with the exocyst, through direct contact with exocyst subunits. Knockdown of either Cdc42 or RhoA also reduced matrix degradation and tumor invasiveness.

The authors suggest that by linking IQGAP1 to the exocyst, the cell ensures coordination of two central features of tumor invasion: actin reorganization at the invadopodia and exocytosis of protein-degrading enzymes. JCB

Sakurai-Yageta, M., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200709076.

A better way to see splicing partners

Splicing factor interactions have been well-characterized in vitro, but less so in vivo. Ellis et al. have now used live cell imaging techniques to study these interactions in vivo and to discover where in the nucleus they occur.

Coimmunoprecipitation can't capture the dynamic nature of protein interactions, and the resolution of conventional fluorescence microscopy is too poor to distinguish truly interacting proteins from ones that are merely close to each other. In contrast, a positive FRET (fluorescence resonance energy transfer) signal implies two proteins are close enough to interact, and photobleaching or FLIM (fluorescence lifetime imaging microscopy) can detect changes in an interacting pair over time.

Using these techniques, the authors confirmed that the splice site protein, SF2/ASF, and the 5′ splice site factor, U1 70K, interact in a type of nuclear body called a "speckle" and, to a lesser extent, in the nucleoplasm. When transcription was blocked, nucleoplasmic, but not speckle-localized, interactions were diminished. This finding bolsters the case for speckles as storage or assembly sites for splicing factors rather than sites of transcription-associated splicing.

The same localization and response to transcription inhibition was seen for interactions between SF2/ASF and the 3′ splice site binder, U2AF35. But apparently not all splicing factors accumulate in speckles: interactions between HCC1 and either U2AF35 or U2AF65 were most prominent in the nucleoplasm, even when transcription was blocked.

There is clearly still much to uncover about splicing factor interactions in vivo, but by using FLIM-FRET, the authors have a powerful tool to dig deeper. JCB

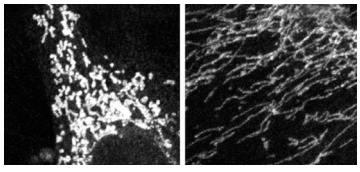
Ellis, J.D., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200710051.

The mitochondrial connection

Add two, and perhaps three, more skills to the portfolio of multitalented plectin, say Winter et al.: one isoform of the protein anchors mitochondria to the cytoskeleton and keeps these organelles in shape, and may also create a platform for signaling.

Plectin is a "cytolinker" protein responsible for connecting intermediate filaments with, among other things, actin microfilaments, microtubules, and myosin motors. Its versatility arises from alternative splicing—almost a dozen isoforms are known, which differ at their N termini, affecting the substrates to which they can attach. One isoform, P1b, has been found targeted to mitochondria, but the significance of this linkage has been unclear.

The authors have now found that P1b links mitochondria to the intermediate filament protein, vimentin. P1b colocalized with vimentin, and loss of P1b reduced the amount of vimentin in mitochondrial extracts. Loss of P1b also dramatically altered mitochondrial shape, increasing the number of mitochondria that were highly elongated. Elongation was not caused by alterations in mitochondrial membrane potential, mass, or fusion dynamics. But it may have resulted from a reduction in protein kinase C-δ activation, as this was apparent in cells that lacked P1b, through unknown mechanisms. PKC-δ promotes mitochondrial fission, and the authors suggest that intermediate filament–linked P1b may serve as a scaffold for recruiting PKC-δ, and perhaps other signaling molecules, to regulate mitochondrial fission. JCB



In the absence of P1b, mitochondrial networks become more tubular (right).

Winter, L., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200710151.