In This Issue

Cross-linking can be good

Protein cross-linking by radical reactions is normally associated with aging-related tissue damage, but on page 879 Edens et al. report that worms specifically use cross-linking of collagen to make a strong cuticle. Without the combined oxidase/peroxidase that does the cross-linking, the cuticle separates into distinct layers, resulting in translucent worms that often suffer from massive blisters and defective movement.

The clue that the cross-linking enzyme (called Duox for Dual Oxidase) might exist came from studies of phagocyte NADPH-oxidase. The latter enzyme generates bursts of superoxide to effect killing of engulfed cells. But other cell types, some of which lack phagocyte NADPH-oxidase, generate lower levels of reactive oxygen species.

Worms without Duox (left) are translucent and often burst.

Duox may be one of the sources of this oxidative activity. Edens et al. isolated genes for human and worm Duox enzymes based on similarity to phagocyte NADPH-oxidase, then investigated the function of the two worm genes by RNAi. The RNAi animals appeared similar to collagen mutants, and lacked di- and tri-tyrosine cross-links normally present in cuticular collagen. Furthermore, the peroxidase domains of both the human and worm Duox enzymes (which set Duox apart from phagocyte NADPH-oxidase) could cross-link tyrosine ethyl esters in a bacterial lysate.

The combined biochemical activities suggest the following model for Duox action. First the intracellular flavoprotein domain pulls electrons off NADPH. After passing through the membrane, these electrons transform molecular oxygen to superoxide, which spontaneously decays to hydrogen peroxide. The hydrogen peroxide reacts with the heme iron in Duox's peroxidase domain to form a powerful oxidant that can then oxidize the tyrosine residues, creating tyrosyl radicals that react to form a cross-link between two protein chains.

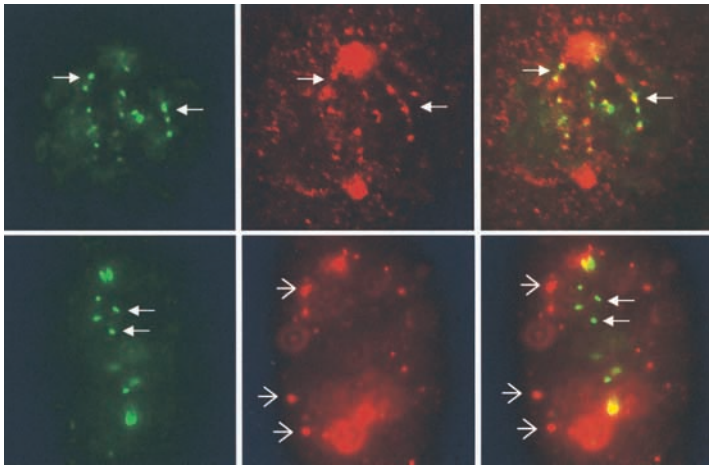
Such an activity is likely to hit any protein that is nearby. Worms minimize the danger of unwanted cross-links by using Duox primarily or perhaps solely in the cuticle. Humans express Duox in a number of tissues, notably lung, where cross-linking of elastin may help create the unique extracellular matrix that makes lungs so resilient to stretching. ■

CENP-E falls off

On page 707, Topper et al. report that an excess of Cdc34, a ubiquitin ligase, prevents the association of CENP-E with kinetochores. A reduction in the levels of kinetochore-localized CENP-E occurs normally during the progression from prometaphase to metaphase (Hoffman, D.B., et al. 2001. *Mol. Biol. Cell.* 12:1995–2009), as kinetochores reduce in size while switching from microtubule-capture to microtubule-maintenance mode. Overexpression of Cdc34 may be accelerating and exaggerating this reduction process to the extent that kinetochores cannot set up proper connections to the spindle. The result is a failure to get beyond prometaphase.

Progression from prometaphase to metaphase is also inhibited by the recently discovered protein Emi1. One simple model—that excess Cdc34 triggers premature destruction of Emi1and thus premature loss of CENP-E—does not appear to be tenable. Although a ubiquitin ligase is exerting the (possibly indirect) effect on CENP-E, Topper et al. show that ubiquitin-mediated proteolysis is not required. In their hands, excess Cdc34 plus a proteasome inhibitor still results in CENP-E loss and prometaphase arrest. This is yet another case where ubiquitin conjugation is acting as a regulator of protein function rather than as a marker for degradation.

Any link from the overexpression result to a physiologically relevant process remains speculative. But with new antibodies to Cdc34, Topper et al. hope to address the true function of Cdc34 in mitosis. ■



CENP-E (red) no longer localizes to kinteochores (green) in cells with excess Cdc34 (bottom).

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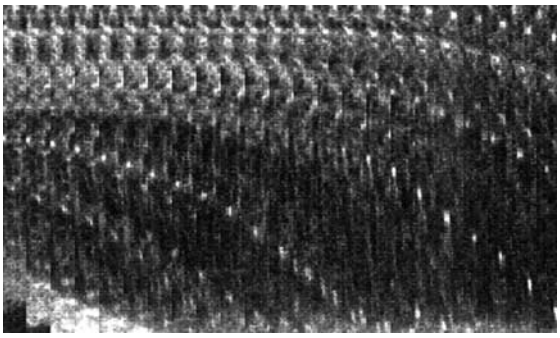
Zones and rings in wound healing

Cells sustain rips and tears during processes such as migration. The plasma membrane is repaired within milliseconds, but resealing of the underlying actomyosin cortex can take several minutes. On page 785, Mandato and Bement investigate this process in frog oocytes and find that a polymerization zone and a contractile ring work together to close wounds.

Mandato and Bement find that the structure surrounding a wound is clearly contractile—square wounds round up, elongated wounds shorten fastest along their long axis, and rewounding causes nearby areas to spring open further. But contractility seems to be restricted to a narrow ring, whereas actin and myosin accumulate in a broader zone around this ring. Actin filaments can be tracked as they flow and accelerate into this zone, thus depleting the surrounding area. This process of cortical flow should create a positive feedback loop, as incoming actin increases contractility, thus increasing flow.

Flow is probably not, however, how things get started. In the absence of actin, several proteins implicated in actin polymerization accumulate around a wound. De novo polymerization triggered by these proteins would normally provide the initial signal that sets up a zone of actin and myosin accumulation. Mandato and Bement show that the zone can be established in the absence of any flow, although the zone is unstable without contraction and does not move to heal the wound. Presumably the zone normally triggers enough contraction to initiate flow, and the flow then results in the formation of a contractile ring and healing. ■

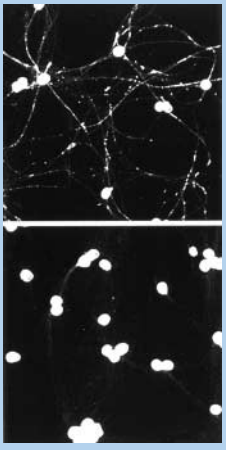


Actin (white) accelerates toward a wound (bottom) over time (left to right)

Making virus in the axon

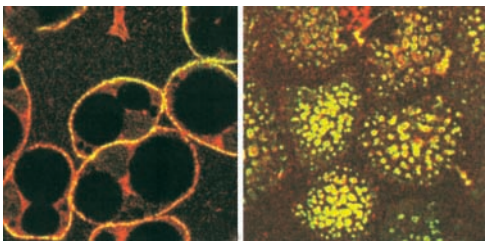
Reactivated herpes viruses make their way from the nerve cell body to the axon terminal, where they are either shed or transferred across the synapse to another neuron. Tomishima and Enquist report on page 741 that, during this process, viral membrane proteins are transported into the axon independently of viral capsids, suggesting that viral assembly must occur at or near the synapse.

Viral assembly was thought to occur only in the cell body, with subsequent transport of fully assembled virions to axon terminals for release. Indeed, a pseudorabies virus mutant for Us9 appeared to be defective only in the second step, as it still makes infectious viral particles in the cell body but is defective for viral shedding from axons. But Tomishima and Enquist find that in the Us9 mutant some of the viral capsids are transported into the axon, leaving the viral membrane proteins behind in the cell body.



Only cells with Us9 (top) get viral membrane proteins into axons.

Us9 has motifs suggesting that it could interact with a neuron-specific adaptor, thus creating an axonal transport vesicle for viral membrane proteins. Segregating viral membrane proteins from viral capsids during axonal transport may ensure that virus components can reach the axon terminal without prematurely uniting and being shed as assembled virus. Perhaps when the components reach the synapse they encounter a synapse-specific protein that finally allows them to join together to form infectious particles. ■



TC10 (green) colocalizes with caveolin (red) to signal.

Compartmentalized insulin signaling

On page 829, Watson et al. find that the small GTP-binding protein TC10 must be in a lipid raft compartment to impact insulin signaling. Such spatial control may be one way in which cells create a distinct reponse from activation of a common set of signal transduction proteins.

Insulin acts on the insulin receptor to trigger translocation of the GLUT4 transporter to the plasma membrane, resulting in increased glucose uptake. In previous studies, activation of both PI 3-kinase and TC10 were implicated in transducing a signal from activated receptor to GLUT4.

Now Watson et al. show that TC10 localizes to lipid raft domains, and that inhibition of this localization (using a dominant-interfering caveolin mutant) prevents TC10 activation by insulin. Although the current authors and others have worked out a complex series of links from the activated receptor to TC10, it seems that these links can only be set up productively when the relevant proteins are concentrated in a specific domain. ■