

Collagen reveals a hidden talent

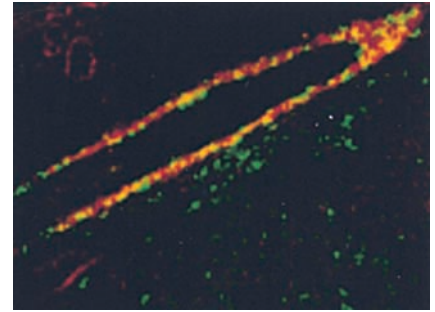
Inject monoclonal antibodies into an animal with a tumor, the treatment inhibits angiogenesis, and tumor growth stops. Sound familiar?

This time, however, there is a twist: the antibodies described by Xu et al. (page 1069) target a cryptic epitope in the extracellular matrix rather than a cellular growth factor, and the results suggest an important activity for

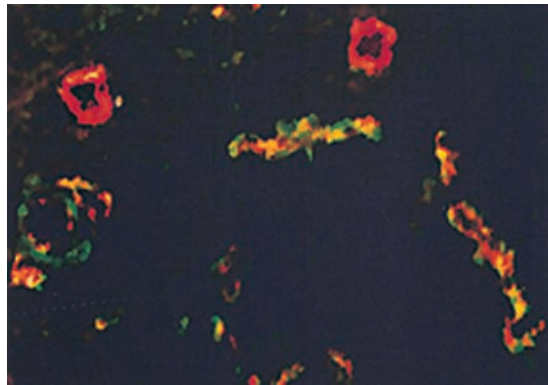
collagen type IV in directing the growth of new blood vessels.

Recent work has shown that proteolytic enzymes like the matrix metalloproteinases are required for normal angiogenesis, but the significance of these findings remained unclear. In the new work, the authors analyzed a monoclonal antibody that binds to proteolyzed collagen-IV, but not to the intact triple helical form of the protein.

The cryptic epitope, HUIV26, is not seen in the basement membranes of normal blood vessels, but becomes exposed in angiogenic and tumor-associated blood vessels. HUIV26 epitope exposure correlates with a loss of $\alpha 1\beta 1$ integrin binding and a gain of $\alpha v\beta 3$ binding, and the monoclonal antibody can strongly inhibit angiogenesis and tumor growth in several animal models.



The collagen epitope (green) is associated with matrix metalloproteinase-2 (red).



A cryptic collagen epitope (green) is exposed on some tumor blood vessels.

The new results suggest that proteolysis of the extracellular matrix exposes cryptic sites, which then transduce signals required for angiogenesis. The authors are now searching for additional cryptic signaling sites in other extracellular matrix proteins, and have already found additional monoclonal antibodies that inhibit angiogenesis and tumor growth. They have also discovered that the HUIV26 epitope may be able to regulate other types of behavior in neuronal, epithelial, and tumor cells. ■

The spindle assembly checkpoint gets a complex

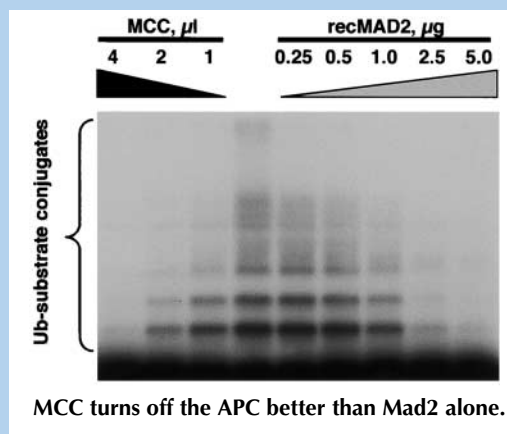
Once a cell has successfully replicated its DNA, it must correctly segregate its chromosomes, a process guarded by the spindle assembly checkpoint. Sudakin et al. (page 925) have isolated and characterized a protein complex that appears to transmit an important signal in this checkpoint, findings that necessitate revising an earlier model. Tang et al. (*Developmental Cell*, 1:227–237) also examined the regulation of this checkpoint; the two papers are compared and discussed in a Comment by Hoyt (page 909).

Previous work suggested that unattached kinetochores caused the conversion of the Mad2 checkpoint protein to an activated form, which then bound to the anaphase-promoting complex or cyclosome (APC). In this model, interaction with activated Mad2 blocked the ubiquitin ligase activity of the APC, which is required for complete mitosis. In the new work, Sudakin and colleagues purified an

APC inhibitory factor, the MCC (mitotic checkpoint complex), from HeLa cells. The MCC, which includes Mad2 as well as BubR1, Bub3, and Cdc20, inhibits APC activity $\sim 3,000$ -fold more effectively than purified Mad2 alone, apparently by binding stoichiometrically to the APC. Tang and colleagues found that recombinant BubR1 alone is also a more potent inhibitor of the APC than Mad2 alone. Interestingly, Sudakin and colleagues found that active MCC is present in interphase cells as well as mitotic cells, but MCC only inhibits APC from mitotic cells.

The results suggest a new model in which unattached kinetochores sensitize the APC to inhibition by the MCC, rather than directly

generating the inhibitor of the APC as previously thought. The nature of the signal generated by unattached kinetochores remains to be determined. ■

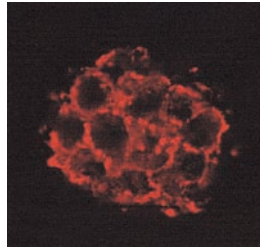


MCC turns off the APC better than Mad2 alone.

Two new ways to eat beans

The seedlings of higher plants must orchestrate an ordered degradation of their cotyledon cells, which store carbohydrates and proteins, in order to germinate. Toyooka et al. (page 973) sought to characterize the breakdown of starch by α -amylase in these cells, and managed to identify two distinct autophagic pathways in *Vigna mungo* seedlings. These two types of autophagy may be general components of programmed cell death in senescing plant cells.

After determining that α -amylase localizes to protein storage vacuoles and lytic vacuoles, the authors found that starch granules enter the lytic vacuoles through autophagy. The starch granules



There are two ways to chew up cotyledon cells.

are wrapped with an acidic cell compartment in a process similar to micropexophagy observed in yeast cells. In addition, cotyledon cells degrade mitochondria and cytoplasm in a process mediated by autophagosomes, similar to yeast macroautophagy. In contrast to yeast autophagy, however, the degradation of cotyledon cells is part of a normal developmental process rather than a response to nutritional stress. The two *V. mungo* autophagic pathways can be uncoupled by removing the embryo axis, indicating that they are controlled by separate cellular mechanisms.

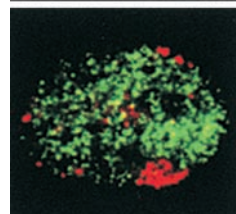
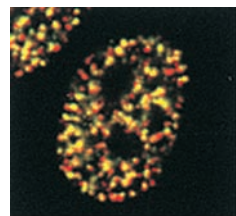
The degradation of cotyledon cells is structurally and biochemically distinct from the apoptotic cell death seen in plants defending themselves against pathogens, presumably reflecting the need to extract nutrients from the senescing cotyledons. In recent work, the authors have also observed autophagy in the cells of senescing leaves, in which chloroplasts are engulfed by vacuoles before degradation. ■

Chk1, Chk2, is the amplifier on?

When DNA replication goes awry, it is critical for a cell to detect and correct the error as soon as possible. Thus, when replication from an early-firing origin is blocked, an intra-S-phase checkpoint prevents the initiation of replication from late-firing origins. The molecular machinery of this checkpoint has remained poorly understood. Now, Feijoo et al. (page

913) describe the activities of the kinases Chk1 and Chk2 in mammalian cells during replication arrest, and demonstrate that Chk1 is required for this intra-S-phase checkpoint.

Arresting DNA replication in HeLa cells with hydroxyurea causes both Chk1 and Chk2 to be phosphorylated and activated, whereas neither protein is activated during normal S phase progression, suggesting that the proteins are phosphorylated in response to stalled or slowed replication. When the replication block is released, Chk1 is rapidly inactivated, and DNA synthesis resumes, but Chk2 remains activated. In arrested cells, blocking Chk1 activation causes destabilization of stalled replication forks and allows late replication origins to fire, indicating that Chk1 is required



Inhibiting a checkpoint (bottom) allows both early (green) and late (red) replication origins to fire.

for this intra-S-phase checkpoint.

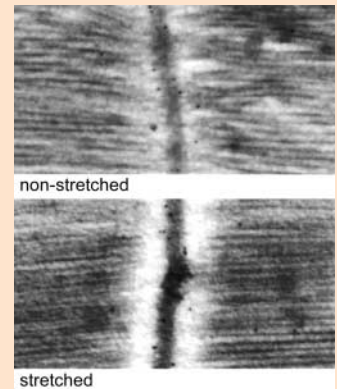
The results suggest that stalled replication forks in S phase cause the phosphorylation and activation of both Chk1 and Chk2, and that Chk1 is primarily responsible for blocking the activation of late replication origins. Since DNA synthesis can proceed in the presence of activated Chk2, it is possible that Chk2 controls a later mitotic checkpoint. ■

Flight of the titins

To execute the rapid wingbeats that allow them to fly, many small insects rely on an elastic-filament system that makes their flight muscles extremely stiff. Although the I-band of indirect flight muscle sarcomeres has been shown to contain projectin and kettin, two large titin-like proteins, it was not known whether these proteins functioned like titin, which in vertebrates provides elasticity in striated muscle. Kulke et al. (page 1045) now provide strong evidence that kettin is functionally equivalent to titin, and suggest that this protein and projectin are the main contributors to the high stiffness of myofibrils in insects.

Using a novel technique to analyze the mechanical properties of individual myofibrils from *Drosophila* indirect flight muscle, the authors show that disrupting kettin immediately decreases myofibril stiffness. Additional analysis demonstrates that kettin in myofibrils is attached to both actin and the myosin thick filament, and suggests that kettin and projectin are the major constituents of the elastic-filament system.

Different isoforms of kettin may be alternatively spliced products of the large D-titin gene. Large kettin isoforms are associated with muscle tissues that are more extensible and less stiff, leading the authors to propose that insect myofibril stiffness is determined by the specific isoforms of projectin and kettin expressed in different muscle types. This closely parallels current models for the activity of titin in mammals, in which different titin isoforms exhibit muscle type-specific expression.



Kettin determines stretchability.