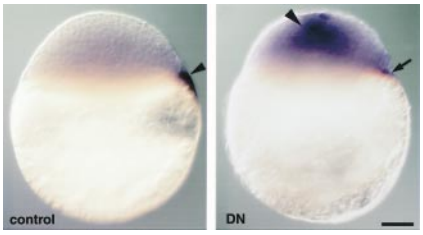
Asymmetric Symmetry

On page 1335, Fujii et al. report that p38 MAP kinase is activated asymmetrically in the zebrafish embryo. The p38 is activated and, based on the effects of a dominant-negative construct, necessary for cleavage only on the future dorsal side, even though normal early embryos display symmetric cleavage.



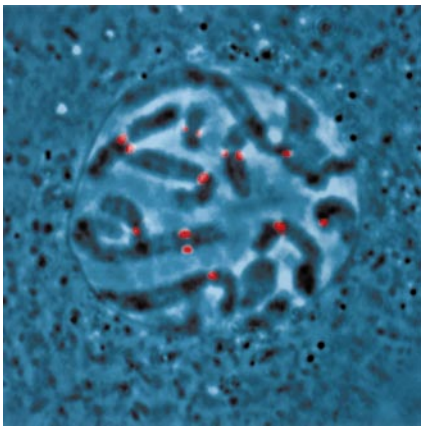
When p38 is inhibited, the affected blastomeres still replicate their DNA, and still express the dorsal side-specific gene, *dharma*. Dharma expression is eliminated by treatments (UV irradiation and yolk-mass removal) that disrupt transport of dorsal determinants to the future dorsal side via a microtubule-transport pathway in the embryo; the same treatments interfere with p38 activation. The p38 activation occurs far earlier, however, so it may be triggered by lower concentrations of the same dorsal determinant, or by another determinant altogether.

After the UV treatment, what should be the dorsal side has neither dorsal determinants nor activated p38, and yet cleavage is normal. This leads Fujii et al. to suggest that something associated with dorsal determination is inhibitory for cleavage, and that activated p38 compensates for that inhibition. This complicated double-negative may reflect the fact that vertebrates, in an attempt to increase cell mass early, evolved symmetric cleavage from the asymmetric development patterns that were already established by invertebrates.

Mad2 Sends a Message

The spindle checkpoint delays anaphase onset if even a single kinetochore fails to attach to the mitotic spindle. This suggests that the free kinetochore must be generating a diffusible signal. On page 1233, Howell et al. describe the first strong evidence in support of this hypothesis.

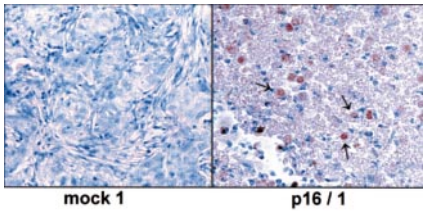
Mad2 is one of the proteins identified in budding yeast as being necessary for the spindle checkpoint. By binding to Cdc20, Mad2 prevents Cdc20 from activating the anaphase promoting complex (APC) that drives mitotic exit. Mad2 has been localized to unattached kinetochores, and now Howell et al. report that Mad2 cycles on and off the kinetochores of tissue culture cells with a half-life of ~24–28 s. Thus, the free kinetochore may be a catalytic site that loads Cdc20 onto Mad2 in an inactive form. If cross-linking of Mad2 to the kinetochore can prevent Mad2 release and the generation of a checkpoint signal, the catalytic model would be confirmed.



Howell et al. also observe Mad2 that is transported down spindle microtubules to the spindle poles. They suggest that Mad2 binding sites may be pulled off during the process of kinetochore remodeling by microtubule interactions, and then transported along spindle fibers to the spindle poles. This would turn off the kinetochore signal, but ensure that Mad2 catalytic sites are spread to the sites in the spindle that control cyclin degradation and anaphase onset. Once these distributed Mad complexes decay, mitosis can finally proceed.

A Cell Cycle Inhibitor Induces Apoptosis

The tumor suppressing activity of $p16^{INK4a}$ would seem to be well accounted for by its G1 cell cycle inhibitory activity. Once $p16^{INK4a}$ has bound to complexes of cyclin D with cdk4 or cdk6, the kinase complexes can no longer phosphorylate Rb to release Rb's inhibitory grip on S phase transcription factors. But $p16^{INK4a}$ has been turning up in a number of non-cell-cycle processes, and now Plath et al. find on page 1467 that $p16^{INK4a}$ is necessary for anoikis, the apoptosis that follows when nontransformed epithelial cells lose their matrix contact. Loss of anoikis in cancer cells allows the cells to grow without a dependence on contact with a particular matrix.

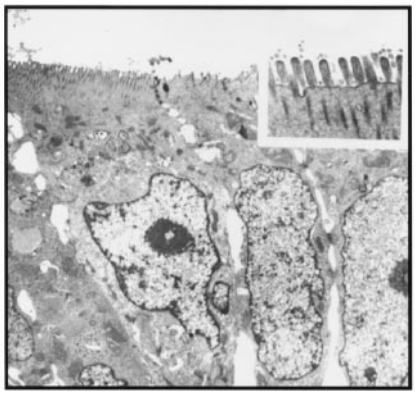


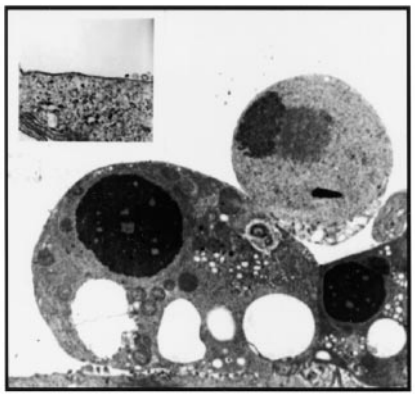
Plath et al. find that expressing $p16^{INK4a}$ in a number of different cancer cell lines sharply reduces the ability of the cells to form colonies in soft agar, in the absence of extracellular matrix. In nude mice, the $p16^{INK4a}$-expressing cells apoptose instead of forming tumors, and apoptosis also results when the cells are cultured in vitro on a substrate that doesn't allow attachment or matrix deposition. Of the integrins expressed in the cells, the transcription of α_5 is specifically upregulated in response to $p16^{INK4a}$ expression. Anoikis can be avoided either by addition of soluble fibronectin (and thus ligation of $\alpha_5\beta_1$) or by antisense treatment to eliminate α_5 overexpression. Thus, unligated $\alpha_5\beta_1$ must be transmitting a death signal to the cell to prevent it from growing where it should not.

Invasion and Death

Inflammatory bowel diseases (IBDs) are characterized by trans-epithelial

migration of polymorphonuclear leukocytes (PMNL) and extensive cell death in the affected areas of the colon. Le'Negrate et al. tie these two phenomena together with their finding that PMNL transmigration can induce epithelial apoptosis (page 1479).



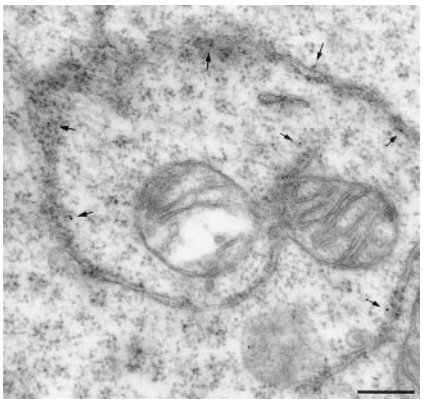


Little apoptosis occurs if the PMNL are in contact with, but not migrating through, epithelial monolayers growing in vitro. Even when extensive death does occur, the death ligand Fas and its receptor are not involved, although they are both present. Death can be successfully triggered, however, by depolymerization of actin, and the resulting caspase activation and death follow the same time course as is seen after transmigration. Furthermore, transmigration-associated apoptosis can be eliminated by adding the actin-stabilizing drug phallicidin.

Epithelial cell actin is known to be disrupted during transmigration. Le'Negrate et al. are now determining whether the critical disruption results from the physical force of transmigration, from factors released locally by the PMNL during transmigration, or from both effects.

Calcium Regulates ER–Mitochondria Association

Calcium release from the ER, resulting from receptor activation and generation of inositol 1,4,5-triphosphate (IP_3), is concentrated at sites of contact between the ER and mitochondria. On page 1489, Wang et al. identify the autocrine motility factor receptor (AMF-R) as a marker for



the subdomain of the smooth ER that associates with mitochondria, and find that the association is sensitive to the presence of cytosol and calcium.

Permeabilized cells in buffer show high ER–mitochondrial association, but addition of cytosol with physiological levels of calcium leads to ~50% dissociation. Higher concentrations of calcium prevent this dissociation, however, suggesting that increased association may follow calcium release induced by receptor activation. Subsequent passive uptake by mitochondria of the calcium concentrated at these contact points may help in both the homeostasis of cytosolic calcium levels and the generation of calcium oscillations. Whether or not AMF-R is involved in any of these processes is unknown.

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