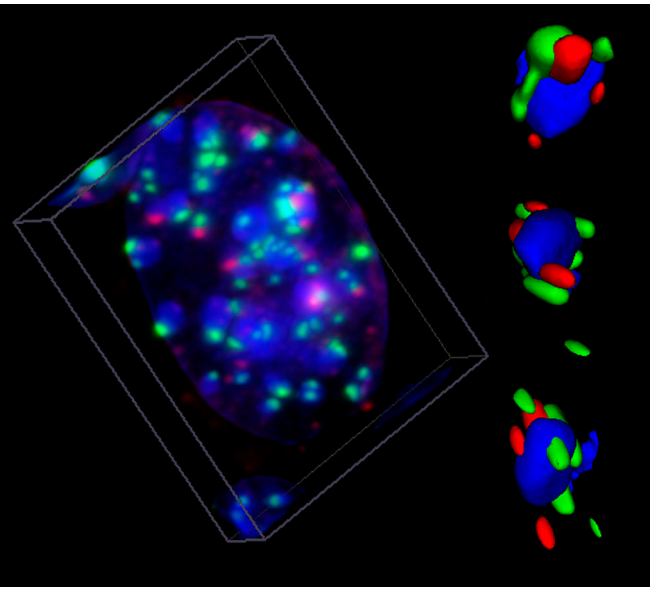
In This Issue



BRCA1 (red) is found with replicating heterochromatic DNA (green) in mouse cells. Three views of a single cluster of centromeres are shown on the right.

BRCA1 in replication

On page 693, BRCA1 branches out from its DNA repair duties. Images from Pageau and Lawrence suggest that this tumor suppressor also helps out during heterochromatin replication.

Repair is the function usually ascribed to BRCA1, which accumulates at damaged DNA in irradiated cells. But undamaged cells also have nuclear BRCA1 foci. They appear mostly during S phase, but because the foci do not have a clear-cut overlap with the majority of replicating DNA, their function has been difficult to explain.

The new results reveal an association of nuclear BRCA1 foci specifically with subsets of replicating heterochromatin. In mouse cells, highly heterochromatic centromeres gather in easily stained clusters known as chromocenters. In cells that are replicating these bright blocks, the BRCA1 association was readily apparent. The authors also noted the same association in human cells.

This isn't the first time BRCA1 has been linked with heterochromatin. A previous report suggested an association of BRCA1 with the Xist RNA along the inactive X chromosome. But the 3D images in this issue show that BRCA1 is only adjacent to Xist territories and that it also abuts the constitutive heterochromatin of autosomal chromosomes.

The authors hope now to better characterize the timing of BRCA1's arrival relative to replication. If BRCA1 arrives before DNA polymerase, it might help to open up tightly wound heterochromatin, perhaps by interacting with chromatin-remodeling factors. But if it arrives later, BRCA1 might be helping to reassemble and unkink replicated chromatin, possibly through its reported ability to activate topoisomerase II.

Breast cancer cells that lacked BRCA1 were more prone to mitotic defects, including lagging DNA bridges containing centromeric sequences between daughter cells. BRCA1 might thus be needed for the maintenance of centromere-linked heterochromatin. Mitotic problems and the potential for widespread expression of normally silent heterochromatin are efficient ways in which BRCA1 mutant cells could acquire cancerous traits even beyond any failures in DNA repair. JCB

Peroxide detection system

Homes are safeguarded by smoke detectors and carbon monoxide detectors. On page 779, Phalen et al. find that cells are likewise protected by a peroxide-monitoring system. Instead of beeping, this detector forms oligomers to alert cells to dangerous levels of H_2O_2.

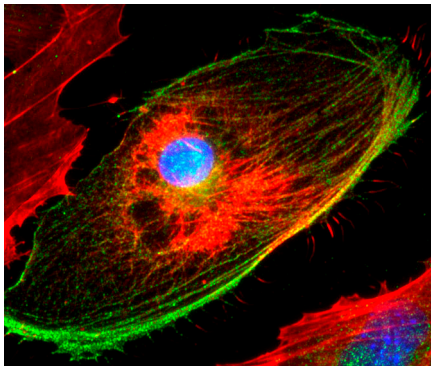
H_2O_2 is a helpful signaling molecule, but at high concentrations it can also be a damaging reactive oxygen species. One family of antioxidant enzymes that hydrolyze—and thus neutralize—H_2O_2 is the peroxiredoxins (Prxs). The 2-Cys class of Prxs are inhibited by H_2O_2-mediated hyperoxidation. Scientists originally thought this negative feedback permitted H_2O_2 to accumulate to signaling-competent concentrations.

The new results show that signaling occurs at H_2O_2 concentrations that are too low to inactivate Prxs. Here, signaling was measured as growth factor–induced mitotic progression, which depends on H_2O_2 production. Cells responded to growth factors just fine without Prx inactivation.

At higher H_2O_2 levels, however, PrxII underwent a sudden shift in conformation. As PrxII became hyperoxidized, it formed cytoplasmic filaments. Progression through the cell cycle halted until PrxII was reduced and the filaments had dissolved.

The authors have more recently identified several PrxII-interacting proteins, one of which is known to relocate from the cytoplasm to the nucleus during H_2O_2 stress. They hypothesize that hyperoxidation and oligomerization of PrxII might liberate its interacting factors, which are then free to activate stress responses that stop the cell cycle and start recovery programs, eventually culminating in PrxII reduction. The group now plans to test the response of the interacting proteins to changes in the PrxII oxidation state.

The peroxide alarm might be silenced in cancerous cells, some of which have abnormally low PrxII levels. This deficiency might help to explain how these cells avoid mitotic arrest and apoptosis upon oxidative damage. JCB



Filaments of hyperoxidized PrxII (green) warn of high H_2O_2 levels and halt the cell cycle.

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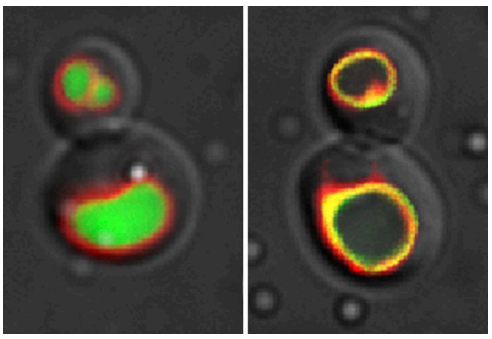


Dumping of MVB cargo (green) into the vacuole (red) fails in mutants (right) that cannot oligomerize ESCRT-I.

Promoting ESCRT-I narcissism

A new endosomal protein keeps ESCRT-I too wrapped up in itself to be attracted to anyone else, Chu et al. reveal on page 815. This matchbreaker prevents the premature assembly of the machinery that forms multivesicular bodies (MVBs).

MVBs are endosomes with internal vesicles. The vesicles contain membrane proteins bound for fusion with lysosomes or viral particles hoping to escape from the cell. Vesicles bud internally from the endosomal membrane with help from three ESCRT complexes (I, II, and III) that are sequentially recruited from the cytosol to the endosome. Chu et al. found a new component that helps keep this recruitment in proper order.

The helper is a small protein, which the authors call Mvb12, that binds to ESCRT-I and regulates its associations. With Mvb12, most ESCRT-I was found in stable cytoplasmic oligomers (probably trimers). Exactly how Mvb12 stabilizes the oligomers is not clear.

The oligomerization seems to mask ESCRT-I's binding site for ESCRT-II; in the absence of Mvb12, ESCRT-I was instead locked together with ESCRT-II, both in the cytoplasm and on endosomes. Their premature joining resulted in inefficient sorting into MVBs; much of the MVB cargo remained on the endosomal surface.

The authors now want to understand how Mvb12 release is prompted to allow timely ESCRT-II binding. Encountering PI3P and ubiquitinated cargo on the endosome might release Mvb12, resulting in oligomer disassembly and ESCRT-II binding. The final stages of MVB formation, including ESCRT-III recruitment, can then follow. JCB

Do-it-yourself insertion

The membrane insertion of eukaryotic proteins generally requires the assistance of other membrane proteins. But on page 767, Brambillasca et al. show that surprisingly long protein stretches get across membranes unassisted, as long as their transmembrane domains (TMD) are not too hydrophobic. Ancestral membrane proteins may have similarly self-inserted.

The group started with a variant of cytochrome b(5), whose 28-residue luminal domain was known to push its way through protein-free liposomes. They now find that b5 can push luminal domains of up to 85 residues through liposomes and into the ER of yeast cells lacking the normal translocation machinery. Unassisted translocation might also pick up the slack in normal eukaryotic cells when ER translocons are congested.

A membrane protein of similar topology, called Syb2, had no such power. The authors traced the disparity to the TMD: the lower hydrophobicity of b5's TMD was unexpectedly advantageous for insertion into liposomes. The authors imagine that more hydrophobic TMDs might aggregate in the cytoplasm as they are released from ribosomes, creating an unsuitable conformation for ER insertion. In vivo, however, chaperones might prevent this aggregation.

Based on its low TMD hydrophobicity, the authors identified the PTP1B phosphatase as another self-inserting protein. Unassisted insertion only works in low-cholesterol membranes, such as the ER and mitochondria, whose lipids are free to move around while proteins push through. The Golgi and plasma membrane, by contrast, are probably too rigid. JCB

Damage stops bud growth

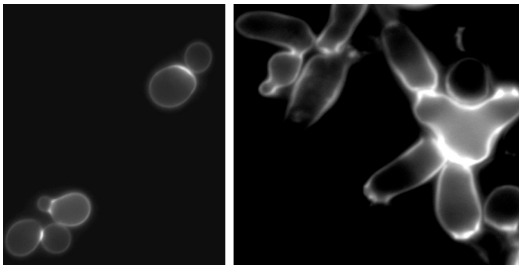
DNA damage checkpoint pathways reach out of the nucleus to control cell morphology, according to Smolka et al. (page 743) and Enserink et al. (page 729).

In budding yeast, DNA replication occurs in conjunction with the formation of the bud, which will receive the new set of chromosomes. DNA damage and other stresses activate checkpoint pathways that stall replication forks and start repair. The new papers show that these pathways also stall bud growth to maintain the synchrony between replication and morphology.

Both groups found that mutants of the Rad53 checkpoint kinase continued to elongate their buds during replication stress, when wild-type cells stopped bud growth. Enserink et al. suggest that checkpoint pathways activate a Cdk1-dependent switch, which turns off polar bud growth.

As buds grow, Cdk1 is normally inhibited by Swe1. But checkpoint activation caused Swe1 degradation and thus Cdk1 activation. Checkpoint mutants, however, were unable to degrade Swe1. They maintained bud-localized growth machinery and a polarized actin network.

Swe1 degradation depends on its localization to septins at the bud neck, where it is phosphorylated. Swe1 localization was normal in checkpoint kinase mutants. Smolka et al. found, however, that Rad53 binds to septins at the bud neck and phosphorylates septins in vitro. Perhaps this modification is a prerequisite to Swe1 phosphorylation. JCB



Checkpoint mutants (right) are abnormally shaped.