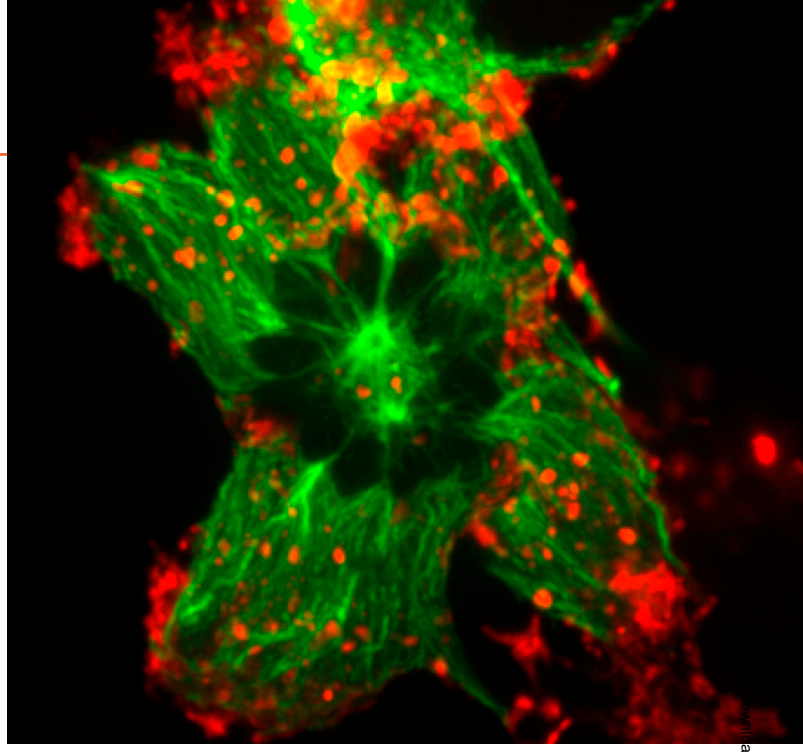
In This Issue



Monopolar HeLa cells fail to generate a single polarity axis for cytokinesis when actin filaments are disrupted.

Polarizing for furrows

Splitting mitotic cells in two is not the one-way signaling road it once seemed, based on evidence from Hu et al. The group identifies a positive feedback loop that creates a furrow at cytokinesis. The findings also throw a wrench in the well-accepted dogma of microtubule dynamic instability.

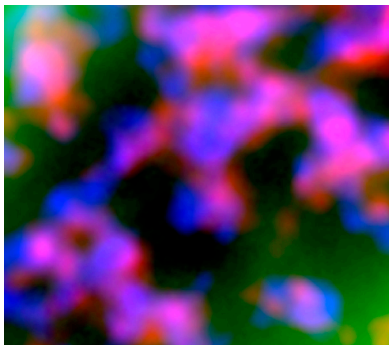
Hu and colleagues devised a monopolar HeLa system to determine how the cytokinetic furrow is created. Monopolar spindles are useful because they can be forced into cytokinesis synchronously, but the chromosomes don't get in the way as they would in bipolar cells. In the round, monopolar HeLa cells, the spindle and its chromosomes were organized in a radially symmetric manner. Gradually, however, this symmetry waned, and microtubules and cortical furrow components began to polarize toward one side of the cell.

The de novo creation of this asymmetry implies that a positive feedback loop exists. Previous models proposed that a one-way signal is sent from spindle microtubules to the cortex to create a furrow. But the new results indicate that the cortex must talk back to microtubules, to stabilize them and further ensure polarization. Polarization required microtubules, Aurora B kinase activity, actin, myosin, and RhoA, which activates cortical contraction.

Because the monopolar spindles lacked a spindle midzone, where plus ends of microtubules from opposite poles overlap, their plus ends were easily identified. The group was stunned to note that these microtubule tips appeared nondynamic and terminated evenly at a distance from the growing, polarizing cortex. Their behavior contradicts popular dynamic instability models and suggests that plus ends may be capped in monopolar spindles and perhaps even at the midzone of bipolar spindles.

Within the gap between the plus ends and the expanding cortex, Aurora B colocalized with actin filaments. In bipolar spindles, Aurora B shifts from the kinetochore to microtubule plus ends. The new results suggest that it then travels along actin filaments to signal from the spindle to the cortex and possibly back again. JCB

Hu, C.-K., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200711105.



Lamins (green) position PCNA (red) on chromatin (blue) for the elongation phase of DNA replication.

Lamins position PCNA

Nuclear lamins help out the chain elongation phase of DNA replication, according to new results from Shumaker et al. A conserved lamin domain, the group shows, positions a DNA polymerase processivity factor on chromatin.

The lamins are self-assembling proteins that create a network of intermediate filaments around the inner membrane of the nuclear envelope. Lamins are also found in the nucleoplasm, where their structure is uncertain. The new findings suggest that some of these nonenvelope lamins create a docking site for PCNA, which helps clamp DNA polymerase onto the genome.

PCNA and lamin B were previously shown to colocalize during S phase. The authors now reveal that PCNA hooks on via lamin's highly conserved Ig-fold motif. In vitro, formation of a lamin network preceded PCNA localization to chromatin. Flooding the cell with lamin Ig fragments hindered DNA replication in HeLa cells and in nuclei assembled in vitro, most likely by displacing PCNA from the chromatin. Whether the interaction with lamins also activates PCNA is not yet clear.

Some lamin isoforms held more tightly to PCNA than others, apparently due to the presence of longer C-terminal tail regions just past the Ig-fold. The authors suggest that the tail may fold back and stabilize any Ig–PCNA interactions.

The Ig-fold motif is ideal for protein–protein interactions and seems to harbor additional sites for many binding partners, including nucleic acids and other lamins. PCNA that has bound to lamin might thus encounter other partners important for DNA synthesis, such as replication factor C.

Although it is just ~100–amino acids long, the lamin Ig-fold is a hotspot for mutations that cause a class of human diseases known as laminopathies. One such mutation, which causes Emery-Dreifuss muscular dystrophy, weakened the lamin–PCNA interactions. Whether this disease stems from DNA replication defects and why such defects would mainly affect muscle cells remain to be seen. JCB

Shumaker, D.K., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200708155.

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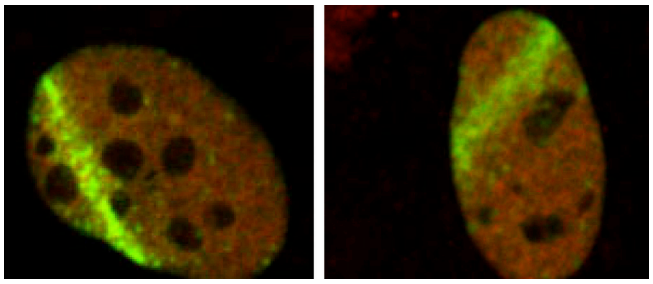
Phosphorylation for break repair

A constitutive, phosphate-based interaction between two repair proteins may help provide instant, well-coordinated repair of double-stranded DNA (dsDNA) breaks, according to two articles in this issue from Melander et al. and Spycher et al.

Nearly every step in dsDNA break repair requires MRN, from recognizing the break to activating signaling pathways to the mechanics of repair—or apoptosis, if repair fails. MRN can bind directly to DNA, but past experiments revealed that it also associates with the histones wrapped around damaged sequences, through an adaptor protein called MDC1.

Both groups now dissect this chromatin association. The findings reveal that the connection depends on a heavily phosphorylated domain within MDC1, which docks to a subunit of MRN called NBS1. In both articles, MRN was displaced from dsDNA breaks in cells containing mutant versions of MDC1 that lacked the phosphorylation sites.



The retention of MRN (red) at dsDNA breaks (green) is lost when MDC1 phosphorylation sites are mutated (right).

The link between MRN and MDC1 did not depend on the presence of damaged DNA, however; even undamaged cells had phosphorylated MDC1. This phosphorylation depended at least in part on the constitutive and ubiquitous kinase, casein kinase 2 (CK2). Depletion of CK2 blocked the interaction between the repair proteins.

Repair aficionados often think that events taking place on the DNA itself are more important. But cells spend a great deal of energy coordinating the comings and goings of repair proteins on the chromatin, suggesting that these events might have evolved to improve repair precision. MDC1 is one of the earliest proteins to recognize histones at damage sites; by forming a constitutive link with MDC1, MRN ensures a rapid arrival to those same sites.

Previous results showed that MDC1 stays on damaged chromatin longer than MRN, indicating that the MDC1-MRN link is dynamic. This freedom may allow MRN to travel from the damage to the sundry other sites where it is needed, including the broken ends of the DNA.

The groups now hope to uncouple MRN and MDC1 without interfering with MDC1's other binding partners by mutating only its CK2 target sites. They can then determine which of MRN's sundry repair duties rely on its association with chromatin. JCB

Melander, F., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200708210.
Spycher, C., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200709008.

An eIF for proliferation

Proliferation gets its own translation initiation factor, based on findings from Ramírez-Valle et al. The results help explain why that eIF is commonly overexpressed in breast cancers.

The same group previously showed that malignancy in human breast cancers is correlated with high levels of eIF4GI, a scaffolding protein within the translation initiation complex. In the new work, the authors block production of this protein to understand its effects on cells. In its absence, overall protein synthesis rates in an epithelial cell line were only partially reduced, but the cells were small and replicated slowly, similar to cells undergoing nutrient starvation.

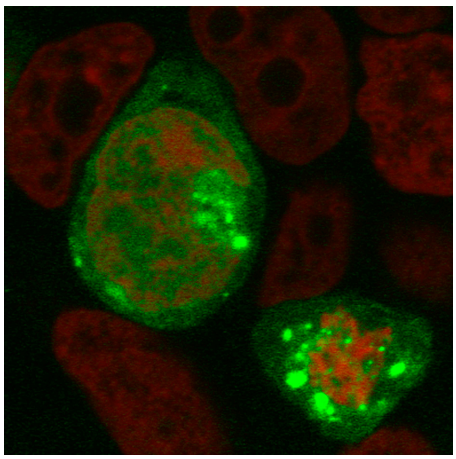
Like starved cells, those lacking eIF4GI had lethargic mitochondria and thus low ATP levels. In an attempt to boost ATP levels, the cells began to cannibalize their own contents via autophagy.

Some mRNAs were affected more than others by the absence of eIF4GI. By identifying transcripts on polysomes, the group showed that loss of eIF4GI specifically blocked the translation of mRNAs necessary for proliferation and energy production.

These transcript-specific effects might explain how cancer cells with extra eIF4GI thrive in the dense tumor environment, where cells should be starved for oxygen and nutrients. The initiation factor might therefore be a good target for cancer therapeutics.

According to the authors, the findings suggest that translation initiation requires individualized factors for classes of transcripts, much as transcription factors differentially activate promoters. The molecular basis for eIF4GI's preference for certain mRNAs is unknown. Many of the transcripts that depended on eIF4GI were in very low abundance or had additional upstream open reading frames. eIF4GI is known to help load the extra ribosomes needed to translate downstream reading frames. JCB

Ramírez-Valle, F., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200710215.



Breast cancer cells fill with autophagosomes (green) when eIF4GI is silenced.