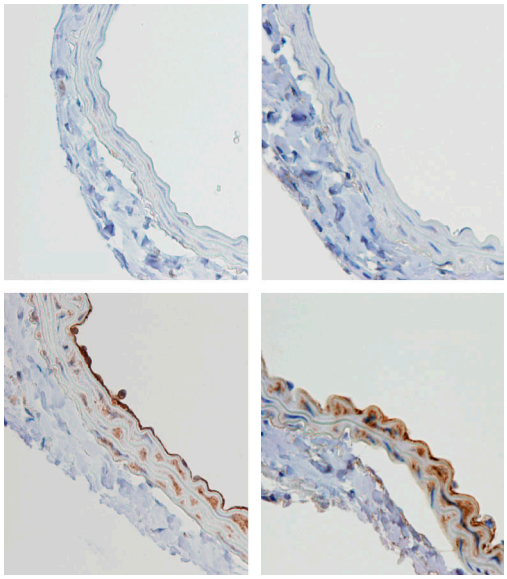
In This Issue



Atherosclerotic arterial regions (bottom) show more PAK activation (left) and fibronectin deposition (right) compared with healthy regions (top).

Vascular permeability: the flow factor

Rivers rarely run a smooth course. Blood vessels, just like rivers, have turns, tributaries and other obstacles that create eddies and other irregularities in the flow. In vessels, such areas of disturbed flow are more permeable and more prone to atherosclerotic plaques. Orr et al. (page 719) now reveal that a matrix protein that is abundant at such sites might be the trigger for this increased permeability.

Vessel permeability is increased by the activation of endothelial cell p21-activated kinase (PAK), which promotes the cytoskeletal contraction that opens pores between cells. The team now shows that PAK is activated by the onset of laminar flow and that this activation is enhanced in atherosclerosis-prone sites in arteries.

Flow alone was not enough to induce permeability, however. PAK was strongly activated in cells adhered to fibronectin, which is made during injury and remodeling and found in atherosclerosis-prone regions. In contrast, normal basement membrane, which contains mostly laminin and collagen, did not support flow-mediated permeability. In mice, PAK activation and vessel permeability were high in atherosclerosis-prone, fibronectin-abundant regions. Inhibiting PAK reduced permeability in atherosclerotic mice.

General, long-term PAK inhibition is not a feasible means of atherosclerosis prevention, as PAK function is important in many cell types. Indeed pan-PAK inhibition was recently shown to induce Alzheimer-like symptoms in mice. Inhibiting the fibronectin-dependent pathway to PAK activation, however, might provide a more specific target for treatment. JCB

Early and late recombination roadblocks

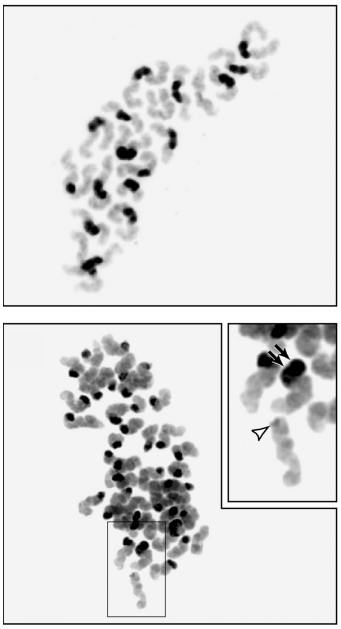
A defective recombination protein messes up meiosis at different points in sperm and eggs. Kuznetsov et al., on page 581, indicate that RAD51C is important for two different steps of homologous recombination.

Homologous recombination during prophase I of meiosis ensures genetic variability in gamete genomes. Seven members of the RecA/RAD51 family are each essential for homologous recombination. All are thought to be important for early recombination events—such as forming the RAD51 foci that jump-start the process—but in vitro evidence suggested that one member, RAD51C, might also be important for the much later step of Holliday junction resolution.

Mutant female mice now further support this in vitro evidence. The team found that mice with too little RAD51C are frequently infertile. In infertile males, the majority of spermatocytes arrested at prophase I and their chromosomes had fewer RAD51 foci. Some spermatocytes progressed to metaphase I but had fewer chiasmata. Both of these defects are consistent with early recombination failure.

In infertile females, however, meiosis progressed through to metaphase II before problems appeared. These oocytes seemed to fail to resolve Holliday junctions, as suggested by the appearance of aneuploidy, broken chromosomes, and precocious separation of sister chromatids. Unlike sperm, eggs have a long lag between prophase I and metaphase II. This could provide the RAD51C-deficient eggs with time to correct the metaphase I defect or to be deleted if they don't. Indeed the sterile females had fewer eggs. It is this fortuitous feature of egg development—their tolerance to infidelities—that allowed the later stage defects to be revealed.

RAD51C does not itself possess the endonuclease activity required for resolving Holliday junctions. Determining its interaction partners and mechanism of action at this late stage of recombination is the team's next step. JCB



RAD51C mutant oocytes (bottom), but not wild-type oocytes (top), show broken chromosomes (inset) and abnormal sister chromatid cohesion.

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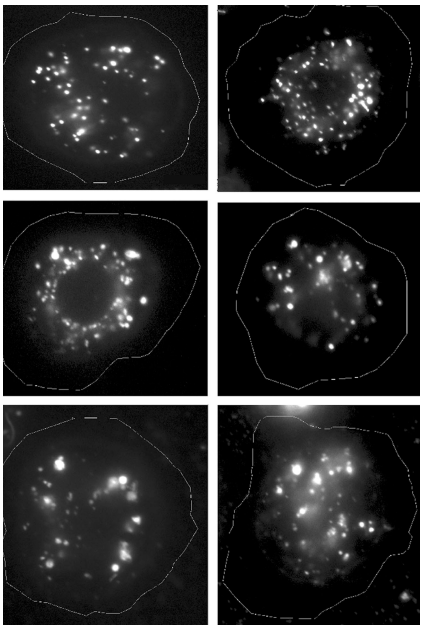


Organelle cargo distribution is similar in wild-type cells (left) and cells expressing nonmicrotubule-binding dynactin (right), indicating that transport remains intact.

Dynactin drives dynein without microtubule binding

Dynactin might bind to and organize microtubules. But it doesn't need to bind microtubules to jump-start dynein motoring, Kim et al. report on page 641.

Dynactin retrieves microtubule motors such as dynein from the cytoplasm and docks them onto their cargo. Dynactin also anchors the minus ends of microtubules on the centrosome.

One dynactin isoform that is found in human neurons lacks its microtubule-binding domain (MBD). Kim and colleagues thus supposed that dynactin might be functional even without locking onto microtubules. They found that indeed cargo transport does not rely on the MBD.

With or without a functional MBD, dynactin helped move four different physiological cargos, including vesicles and proteins, just as far along microtubules and at the same velocity and frequency.

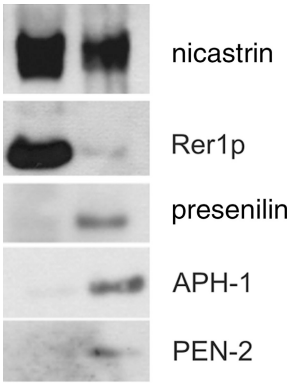
Dynactin's MBD was previously thought to increase dynein's processivity by giving it a second hand to hang onto microtubules. But this idea was based largely on studies of the transport of dynein-coated beads. This artificial cargo, or the way dynein and dynactin were attached to it, might have altered the normal relations between dynein and dynactin observed in a cell.

Not all dynactin activities were unaffected by the loss of the MBD, however. Cells expressing the MBD-deleted form commonly arrested in prometaphase with multipolar spindles, suggesting a failure of microtubules to correctly conjoin in centrosomes and thus a role of dynactin in latching together microtubules. JCB

Stalling secretase assembly

Alzheimer's disease might be slowed by inhibiting γ-secretase, the membrane protease complex that cleaves amyloid precursor protein (APP). Spasic et al. (page 629) now identify an endogenous inhibitor that prevents γ-secretase complex assembly and activity and thus might be targeted for therapy.

APP cleavage by γ-secretase leads to amyloid plaque deposition, one possible cause of Alzheimer's symptoms. γ-secretase is composed of four proteins—presenilin, nicastrin, PEN-2, and APH-1—which must come together for cleavage activity.



Nicastrin binds other γ-secretase components when it is not binding Rer1p (right).

Although all four components are present in the ER, their assembly into functional γ-secretase is somehow restricted; most of the active enzyme is found close to the cell surface. Assembly of γ-secretase begins with the binding of nicastrin to APH-1. This binding, Spasic and colleagues now find, is prevented early in the secretion pathway by Rer1p, a membrane receptor that retrieves proteins from the Golgi back to the ER. Rer1p binds to nicastrin, thus interfering with nicastrin's ability to bind APH-1. Decreasing the amount of Rer1p led to an increase in γ-secretase activity.

Exactly what triggers Rer1p to release nicastrin and allow it to bind to APH-1, and subsequently to the other γ-secretase components, remains to be determined. Preventing this release might provide a means to reduce γ-secretase activity and thus amyloid plaque formation. JCB

Glycosylation PERKs

Glycosylation is crucial for the folding and function of many proteins. How the cell deals with a lapse in glycosylation was unknown. Shang et al. (page 605) now show that a reduction in available glycans prompts an ER stress protein called PERK to put protein synthesis in a lower gear, allowing the glycan resource to restock.

Glycans are transferred to new peptides in the ER from a lipid-linked oligosaccharide (LLO). This LLO pool is often defective in patients who have congenital glycosylation disorders. Shang et al. mimicked these defects by culturing mammalian cells in low glucose medium. One LLO molecule normally has 14 sugars available for transfer to peptides, but low glucose dropped the number to as few as four.

This sugar defect, the group finds, can be fixed by limiting translation. An ER stress response factor called PERK senses the glycosylation problems via the accumulation of incorrectly folded proteins and reduces the activity of a translation initiation factor called eIF2α.

The team showed that PERK's ability to slow down translation reduced the demand on the glycosylation machinery, which in turn allowed more time for the biosynthesis of LLO forms carrying the correct quota of sugars. JCB

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