# In Brief

## *A Potential Flippase in Trafficking*

Adding material to one leaflet of the lipid bilayer, at the expense of the other, can do work and bend the membrane. The findings of Chen et al. (page 1223) are the first hint that such a mechanism may be used in protein trafficking, in this case in the budding yeast TGN. Doubts remain, however, about the ability of Drs2p (the trafficking protein studied by Chen et al.) to move lipids from one side of a membrane to the other.

Chen et al. identify a *drs2* mutation as being synthetically lethal with a deletion in ADP-ribosylation factor 1 (ARF1). ARF1 is one of two redundant ARFs. Both recruit two coat proteins: COPI for Golgi to ER transport, and clathrin, possibly for TGN to endosome transport. Drs2p appears to function in the clathrin pathway, as a *drs2* deletion interacts only with mutations in clathrin and a protein from clathrin-coated pits, and the deletion strain accumulates excess Golgi membranes and fewer clathrin-coated vesicles. Drs2p localizes to the TGN.

Drs2p is most similar to the bovine P-type ATPase II, a possible aminophospholipid translocase (flippase). ATPase II was originally thought to be responsible for flipping phosphatidylserine from the external leaflet of the lipid bilayer to prevent recognition of the cell by the apoptotic apparatus. Drs2p seemed to fit this bill for yeast, as the mutant apparently failed to flip a lipid derivative across the plasma membrane, and an earlier study localized Drs2p by fractionation to the plasma membrane.

But in a more recent study, the *drs2* mutant did not show differences in lipid transport across the plasma membrane. The new localization results of Chen et al. suggest that any possible Drs2p flippase activity would be found in the TGN, not at the plasma membrane.

The ATPase activity of Drs2p is necessary for its trafficking function, but Todd Graham says he must now confirm that the enzyme is a flippase, and see if Drs2p activity has any effect in vitro on the tubulation or ARF recruitment of purified Golgi membranes.

## *Balancing Forces in Meiosis*

On page 1137, Matthies et al. demonstrate that balanced forces in the metaphase spindle allow the correct segregation of chromosomes that lack recombinational linkages.

The linkages, or chiasmata, are the products of recombination that normally hold chromosome pairs together in the first meiotic division. A fly's fourth chromosomes are small and packed with poorly recombining heterochromatin, so they must do without chiasmata. They resort instead to a weak heterochromatin-based affinity that usually breaks apart soon after spindle formation, and well before the extended metaphase arrest is released in the oocyte. Up to 10% of fly X chromosomes also fail to recombine and follow a similar pathway of achiasmate segregation, and many errors in human chromosome segregation involve chromosomes that show no signs of prior recombination.

The first of the balanced forces is directed polewards. It may be generated by microtubule flux or a motor at the kinetochore; in these experiments it is eliminated by a mutation in the *tub67C* gene, which encodes a divergent α tubulin. Chromatin in the mutant is no longer stretched along the spindle during metaphase arrest, and centromeres are not distributed to the polewards ends of the chromatin mass. The result is an increase in achiasmate non-disjunction.

These phenotypes are reversed by reducing the dosage of the Nod kinesin, the source of the opposing force. Nod attaches to DNA, and has been postulated to provide a force pushing chromosomes back into the spindle. Some Nod is needed to keep chromosomes from flying out of the spindle, but too much Nod pushes the achiasmate chromosomes together even after the heterochromatin linkage has dissolved, thus confounding any heterochromatin-directed segregations.

## *Linking Actin and Microtubules*

A protein dubbed microtubule actin cross-linking factor (MACF) is described by Leung et al. on page 1275. As its name suggests, MACF is capable of binding both actin filaments and microtubules in vitro, and it colocalizes with both cytoskeletal systems in vivo. Expression of the protein, especially a truncated version, results in the close apposition of actin filaments and microtubules.

MACF is a hybrid protein: it has an actin-binding amino terminus resembling some of the plakins (which link actin and intermediate filaments), whereas its central stem and part of the carboxy-terminal tail resemble proteins from the spectrin superfamily. The third, novel, component is a unique microtubule-binding domain at the carboxyl terminus.

MACF is a homologue of the *Drosophila* protein Kakapo, which was described in three papers in the November 30, 1998 issue of this journal. An actin filament–microtubule linking function would explain the varied structural and neuronal defects in *kakapo* mutants. In other cells, an actin filament–microtubule linker could impart rigidity, anchor and position the spindle, and transfer neuronal vesicles from microtubules to actin.

## *PKA Anchors and Lets Go*

### *An Anchoring Complex for Chromatin Condensation*

The large condensin complex that condenses chromatin in mitosis has a natural affinity for DNA, but it binds to chromatin only during mitosis, and it is not clear if it can do so by itself. Collas et al. (page 1167) indicate that AKAP95, an anchoring protein for the cyclic AMP (cAMP)-activated protein kinase A (PKA), may be one factor that tethers condensins to chromatin.

Collas et al. find that PKA activity is needed for maintenance of chromatin condensation. As AKAP95 anchors

the regulatory subunits of PKA, the catalytic subunits may phosphorylate chromatin substrates in a burst as the catalytic subunits are released, before they cycle back onto the chromatin, possibly in a preformed complex with the regulatory subunits and AKAP95.

AKAP95, but not PKA, is needed for the establishment of chromatin condensation. AKAP95 antibodies prevent the recruitment of the condensin component Eg7 to chromatin, and AKAP95 and Eg7 coprecipitate. This suggests that, like other AKAPs, AKAP95 may anchor more than PKA.

### *Letting Go of MAP Kinase*

The cyclic AMP (cAMP)-activated PKA is known to influence the MAP kinase pathway, for example at the level of Ras action. Blanco-Aparicio et al. (page 1129) and Saxena et al. (Saxena, M., S. Williams, K. Taskén and T. Mustelin. 1999. *Nat. Cell Biol.* 1:305–311) now show that PKA can also activate MAP kinases by disabling their association with protein tyrosine phosphatases that contain a MAP kinase interaction motif (KIM).

Blanco-Aparicio et al. find that, both in vitro and in vivo, PKA phosphorylates a serine within the KIM of the brain-specific phosphatase PTP-SL. This causes PTP-SL to release the MAP kinases from its grasp, thus allowing the MAP kinases to activate and enter the nucleus. Saxena et al. define a similar mechanism used by the hematopoietic tyrosine phosphatase HePTP, and demonstrate that it is sufficient to induce downstream events such as c-*fos* transcription.

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