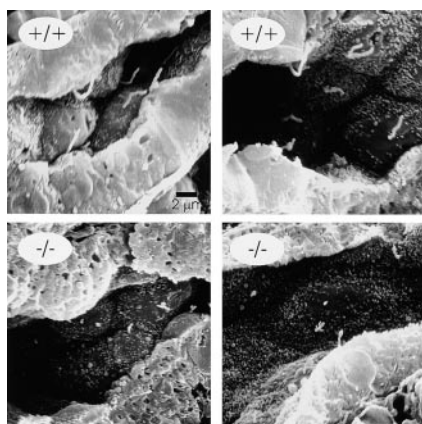


Assembly of Cilia in *Chlamydomonas* and Mouse

After identifying a protein in *Chlamydomonas* that is required for the assembly of flagella, Pazour et al. (page 709) discovered that the murine homologue of the gene plays a critical role in the development of cilia in the kidney. The findings add to a body of data supporting a highly conserved mechanism for cilia assembly in eukaryotes, and suggest that defects in this process may underlie several genetic diseases.

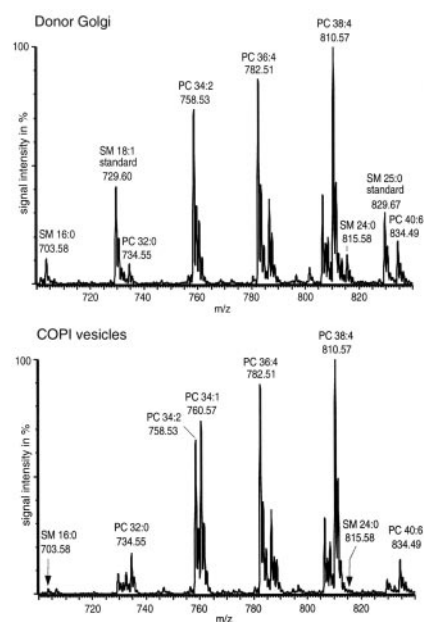


The authors cloned the *Chlamydomonas* gene for IFT88, a subunit of a multiprotein complex involved in intraflagellar transport (IFT), the process that builds and maintains cilia and flagella. Cells lacking IFT88 fail to assemble flagella, making IFT88 the first IFT particle subunit shown to be required for this process. The sequence of IFT88 is homologous to that of the murine gene Tg737. It has been shown previously that mice with defects in Tg737 show kidney and liver defects similar to those seen in humans with autosomal recessive polycystic kidney disease. In the new work, Pazour et al. show that Tg737 mutant mice have shorter than normal primary cilia in their kidneys, consistent with an essential role for Tg737 in cilia assembly in mammals. The results also indicate that primary cilia have an important function in the kidney, although that function remains to be determined. Defects in IFT may be implicated in

additional genetic diseases, including retinitis pigmentosa and primary ciliary dyskinesia.

Lipid Segregation in Vesicle Formation

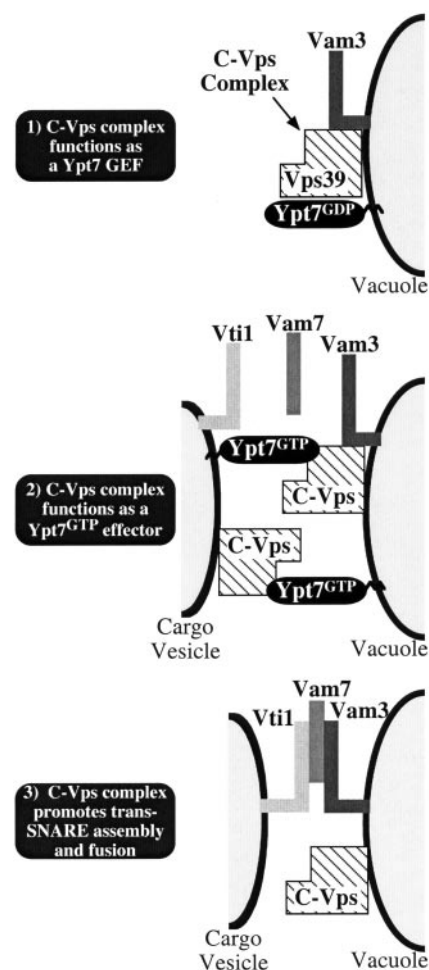
Using nano-electrospray ionization tandem mass spectrometry, Brügger et al. (page 507) have performed the first qualitative and quantitative analysis of lipids in mammalian Golgi-derived coat protein I (COPI)-coated transport vesicles. The results show that in comparison to the parent Golgi membranes, the COPI-coated vesicles contain significantly lower levels of sphingomyelin and cholesterol, suggesting that the lipids are segregated during membrane budding.



The concentration of lipids, like cholesterol and sphingomyelin, varies between organelles, but it has remained unclear how a cell maintains these specific lipid concentrations in the presence of a steady flow of vesicular traffic. By comparing the lipid composition of Golgi membranes with COPI-coated vesicles, which are involved in the early secretory pathway, the authors determined that cholesterol and sphingomyelin in the parent Golgi membrane are segregated away from COPI-coated vesicles. Al-

though the mechanism for this segregation is unclear, the researchers envision two possible models: a dynamic segregation of lipids could occur during COPI-coated vesicle formation, or lipids could be segregated within the Golgi membrane before vesicle budding, after which COPI-coated vesicles could form from membrane subdomains low in sphingomyelin and cholesterol. Brügger et al. are now testing the predictions of these two models and examining the lipid compositions of other types of vesicles.

Additional Components in C-Vps Complex

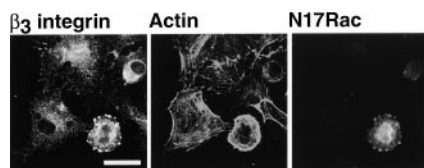


Beginning on page 551, Wurmser et al. demonstrate that the yeast vacuolar protein sorting (VPS) gene products Vps39 and Vps41 are part of the

C-Vps complex that previously was shown to contain Vps 11, 16, 18, and 33. Vps39 appears to regulate the fusion of transport intermediates with the vacuole by stimulating nucleotide exchange on the Rab GTPase Ypt7. Previous work has shown that the C-Vps complex also functions as a downstream effector of Ypt7. The new findings unify these results and provide mechanistic insight into the fusion of vesicles with the vacuole.

The authors demonstrate that disrupting Vps39 interactions with the complex causes severe defects in vesicle sorting. In vitro, purified Vps39 stimulates nucleotide exchange on Ypt7, but not on other GTPases. Based on their results, Wurmser et al. propose a model in which Vps39 association with the C-Vps complex leads to the conversion of inactive GDP-Ypt7 to its active GTP-bound form. After activation, Ypt7 could then associate with effector proteins in the C-Vps complex, tethering the vesicle to the vacuole. The C-Vps complex could then allow trans-SNARE complexes to form, driving the membranes to fuse. A separate paper published by the same group (Sato, T.K., P. Rehling, M.R. Peterson, and S.D. Emr. 2000. *Mol. Cell.* 6:661–671) provides additional support for this model.

Integrin-induced Rac Activation



In findings that suggest a novel pathway for the activation of Rho-family GTPases, Bialkowska et al. (page 685) describe integrin clusters that form

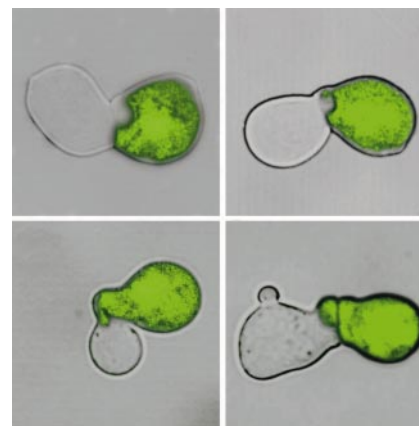
upstream of integrin-induced Rac activation. The clusters, formed by the action of calpain, are capable of binding Rac, but have a different protein composition than previously described Rac-induced focal complexes or Rho-induced focal adhesions.

Though Rac and Rho are known to play important roles in the formation of integrin signaling complexes, the mechanism by which integrin-ligand interaction initially activates these GTPases has remained obscure. In the current work, the authors identified a new type of integrin complex upstream of Rac activation. Unlike focal adhesions and focal complexes, these integrin clusters contain calpain and calpain-cleaved β 3-integrin subunit. Formation of the integrin clusters requires calpain activity, but constitutively active Rac and Rho can induce the formation of focal adhesions and focal complexes even when calpain is inhibited.

The results are consistent with a model in which integrin–ligand interactions lead to the transient formation of the newly described integrin clusters, which then activate Rac. Rac-induced focal complexes and Rho-induced focal adhesions could then form by calpain-independent mechanisms.

Data Mining Yields a Plasma Membrane Fusion Protein

By using a clever informatics-based approach that could be broadly applicable, Heiman and Walter (page 719) identified a yeast gene involved in plasma membrane fusion, a target that has eluded more traditional genetic screens. Yeast mating has considerable potential as a system for studying cell fusion, but previously discovered genes involved in this pro-



cess have acted at or before cell wall breakdown.

To identify proteins that mediate plasma membrane fusion, the authors compiled published databases of *Saccharomyces cerevisiae* gene expression data and gene properties into a common format and developed software to search the database. Using several criteria expected to identify fusion proteins, the researchers identified 20 candidate proteins, 10 of which have been described previously. One of the remaining 10, dubbed *PRMI* by the authors, was singled out for further study. *PRMI* encodes a protein that is expressed only in response to mating pheromone and localizes to regions of the cell membrane that are involved in fusion. *PRMI* deletion mutants have a defect in mating at the step of plasma membrane fusion, an intermediate that has not been trapped in previous genetic screens. The informatics-based gene hunting technique should be applicable to an increasing number of problems as genomic and gene-expression databases grow.

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