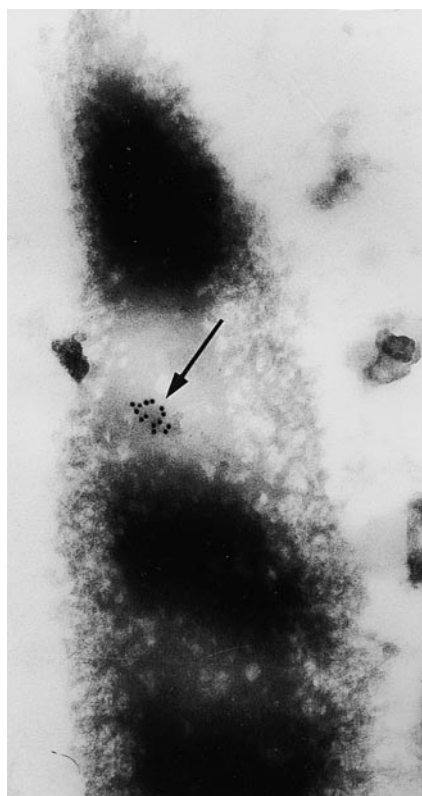


### Reverse Transcriptase in Mature Spermatozoa

Though the spermatozoa of many animal species can take up DNA molecules and internalize them into nuclei, Giordano and colleagues (page 1107) present the first evidence that murine spermatozoa can take up and retrotranscribe RNA. Since the resulting cDNA molecules are subsequently transferred into eggs, the reverse transcriptase (RT) activity of mammalian spermatozoa could play a role in embryonic development.



In previous work, the same lab found that nuclease-hypersensitive DNA from murine spermatozoa contains a high proportion of sequences of retrotransposon origin. Reasoning that the RT activity expressed by these sequences might have persisted, the researchers incubated mouse spermatozoa with purified human poliovirus RNA, and then tested for the production of cDNA by PCR amplification. The poliovirus RNA is reverse transcribed by spermatozoa, and anal-

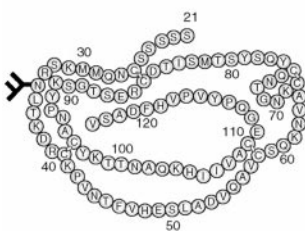
ysis of two-cell embryos produced by *in vitro* fertilization shows that RNA-incubated spermatozoa transfer the retrotranscribed cDNA into eggs. Immunogold electron microscopy reveals RT molecules on sperm nuclear scaffolds.

Though the physiological role of RT in spermatozoa remains unclear, the researchers suggest that RT may be involved in the reshuffling of genetic material in sperm chromatin, an activity which would have important implications for both evolutionary and developmental biology.

### Requirements for Reglucosylation of Glycoproteins

Using a panel of model substrates with defined conformations, Trombetta and Helenius (page 1123) have begun to dissect the molecular mechanisms responsible for reglucosylation, a process carried out on most glycoproteins in the ER. During folding and quality control in the ER, monoglucosylated oligosaccharides interact with lectins, an interaction regulated by glucosidase II and UDP-Glc:glycoprotein:glucosyltransferase (GT), which remove and reattach glucose residues on N-linked oligosaccharides. GT selectively reglucosylates misfolded glycoproteins, but the signals responsible for GT recognition of proteins have not been characterized.

RNaseB-Prot



Using defined conformers of RNaseB to probe the specificity of GT recognition, the researchers found that fully unfolded conformers were poorly recognized. Substrates

with very slight structural perturbations were also poorly recognized, but partially structured nonnative forms of RNaseB were recognized efficiently by GT. Results from this *in vitro* system, which agree well with available *in vivo* evidence, show that GT can distinguish between different nonnative conformations and has a marked preference for partially structured conformers, suggesting that reglucosylation is a selective process targeting specific subpopulations of misfolded proteins. The availability of defined protein conformers that are recognized differentially by GT should facilitate further characterization of this pathway.

### A New Cascade of Trafficking Protein Interactions

In a pair of papers (page 1223 and page 1231), Price and colleagues find a novel order of interactions among Rab/Ypt, Rab/Ypt effectors, SNAREs, and NSF during the homotypic fusion of yeast vacuoles. Homotypic vacuole fusion occurs in three steps: priming, docking, and bilayer fusion. Priming prepares the SNARE proteins on a vesicle surface to bind *in trans* with SNARE proteins of another vesicle, rather than binding *in cis* on the same vacuole. The *trans* binding of SNAREs is a central event in docking, but the molecular mechanism linking priming and docking has remained obscure.

The researchers found that Vam2/Vps41p, a protein previously shown to be necessary for transport vesicle budding from the Golgi apparatus, is also required for homotypic vacuole fusion. Vam2p and its partner, Vam6/Vps39p, are part of a large complex that is initially associated with vacuolar SNAREs. During priming, ATP hydrolysis by Sec18p/NSF disassembles this complex and allows Vam2p and Vam6p to associate with Ypt7p, thereby turning on the tethering stage of docking.

The results reveal a new order and causal relationship for these central trafficking proteins. For vacuole fusion, large *cis*-SNARE complexes con-

tain chaperones, Ypt/Rab effectors, as well as SNAREs. The action of Sec18p/NSF has a novel signaling role, as it not only liberates SNAREs from cis associations, in preparation for their later association in trans on apposed vacuoles, but also frees the Vam2/6p Rab effector to bind to Ypt7p and turn on tethering. Studies in other trafficking reactions will be necessary to test the generality of this new order of events.

### **Rap1 Mediates CD31-induced Integrin Adhesion**

Beginning on page 1151, Reedquist and colleagues demonstrate that CD31 specifically activates the small Ras-related GTPase, Rap1, to induce integrin-mediated T cell adhesion. The results suggest that Rap1 may play a general role in adhesion-dependent signaling during leukocyte migration and extravasation.

Though CD31 is known to stimulate integrin-dependent adhesion through a process called adhesion amplification, the molecular basis of this phenomenon has remained poorly understood. By stably expressing mutant and truncated forms of CD31 in Jurkat cells, the team found that CD31-stimulated integrin-dependent adhesion is regulated by signaling through the cytoplasmic tail of CD31. When cells are stimulated with anti-CD31 antibodies, Rap1 (but not Rap2, Ras, or R-Ras) is rapidly converted to its active, GTP-bound state. Expression of an interfering mutant of Rap1 abolished CD31-dependent increases in adhesion.

The researchers suggest that Rap1 may regulate the ability of LFA-1 to undergo ligand-induced conformational changes, either by binding directly to integrins or by stimulating effector pathways that regulate integrins. The report is the first to demonstrate a requirement for Rap1 in coupling integrin-mediated adhesion to cell-surface receptor stimulation, and the specificity of the coupling between Rap1 and CD31 suggests that Rap1 may be involved in other integrin-dependent events in vascular cells.

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