In Brief

Suggestive Homologies

A WASP-related Protein in Dictyostelium

The Wiskott-Aldrich Syndrome protein (WASP) binds both Cdc42 and actin, and mutation of the human WASP gene leads to actin disorganization and defects in immune cell chemotaxis and antigen recognition. But it has proven difficult to define other members of the WASP pathway by biochemistry, as WASP overexpression results in a mess of aggregated filamentous actin.

Bear et al. (page 1325) have tackled this problem with genetics. They started with *Dictyostelium* cells mutant in cAR2, one of four cyclic AMP receptors. These G protein–coupled receptors transduce chemotactic and morphogenetic signals. Loss of cAR2 function arrests the cells after they have aggregated but before the aggregate has formed a single tip, the precursor to the slug form. Deletion of the gene for SCAR (suppressor of cAR2) allowed cAR2 mutant cells to form a single tip; the same deletion caused each aggregate of otherwise wild-type cells to form multiple tips and multiple small slugs. Cells lacking SCAR had reduced filamentous actin but appeared to chemotax.

SCAR shares similarity with important WASP domains—including a characteristic acidic domain and domains that bind actin and profilin—but SCAR has a unique domain in place of WASP's Cdc42-binding site.

These are the first experimental data that link, albeit indirectly, a WASP-related protein with a seven-transmembrane receptor. The data also define a new gene family, distinct from WASP. A *Dictyostelium* WASP is already known, and Bear et al. identify SCAR homologues in humans, mice, and worms. Budding yeast, an organism that does not chemotax, has a WASP but no SCAR.

Erv14p Loss Causes a Specific Transport Defect

On page 1209, Powers and Barlowe report the discovery of Erv14p. This integral membrane protein is a component of COPII vesicles, which transport proteins from the ER to the Golgi. Erv14p is not needed for general transport; deletion mutants are viable. But the haploid mutants often fail to place their bud site in the normal axial position, next to the site of the previous cell division. The errors arise because Axl2p, an integral membrane protein that helps define the axial bud site, gets stuck in the ER.

A handful of other proteins have been linked to the transport of specific secretory cargo from the ER, so Erv14p's function is not unprecedented. But the next step has not been taken: no one has detected a physical interaction between a putative cargo receptor and its cargo. Such a link is predicted, given that secretory proteins can be concentrated into transport vesicles.

Erv14p is related to *cornichon*, a *Drosophila* protein needed for the posterior localization of the determinant *gurken*. But is there a fly Axl2p? If so, suggests Barlowe, *cornichon* could help transport the fly Axl2p to the oocyte posterior, where a delivery system for *gurken* could use it as a landmark.

Migration Mechanisms Double Up on Function

Selectins That Signal

Movement of polymorphonuclear leukocytes (PMNs) from the blood to tissues starts with inflammatory mediators such as histamine, which induce the expression of various adhesion molecules on the endothelial cells (ECs) that line blood vessels. But there is more to transendothelial migration than adhesion. PMN migration is accompanied by a rise in intracellular calcium levels in ECs and is prevented by inhibition of this calcium transient.

Candidates for initiating this PMN-to-EC signaling event include the binding of the integrin CD11/CD18 to its EC partner intercellular cell adhesion molecule-1 (ICAM-1), and the homotypic binding of platelet/endothelial cell adhesion molecule-1 (PECAM-1). All these molecules are needed for transendothelial migration. But on page 1381, Lorenzon et al. find that it is the binding of PMN carbohydrates to their selectin partners that is necessary to generate the signal.

They find that monoclonal antibodies to vascular cell adhesion molecule-1 (VCAM-1) and E- and P-selectin induce the calcium transient in activated ECs; antibodies to ICAM-1 and PECAM-1 have no effect. PMN adherence induces the calcium transient, but not when the PMNs are pre-treated with antibodies to sialyl Lewis x (sLe^x), the counter-receptor for E- and P-selectins. Monocyte adhesion experiments give similar results, although interfering with VCAM-1 function also prevents the transient. Interfering with CD18 and PECAM-1 function has no effect on calcium transients in PMN or monocyte assays.

Treatments that induce the calcium transient also cause a rearrangement of EC actin from a circumferential ring to stress fibers. These stress fibers may help ECs retract from each other to aid PMN migration.

ZAP-70 in T Cell Migration

Activated T cells also migrate into tissues, notably in autoimmune diseases. T cell migration is dependent on the integrin LFA-1 (CD11a/CD18) on T cells.

Roos and colleagues (page 1371) use the Syk kinase inhibitor piceatannol and a dominant-negative ZAP-70 kinase to show that ZAP-70 is required for LFA-1–dependent T cell migration. This is true both in vitro (for cell migration through filters) and in vivo (for metastatic spread of hybridoma cells). ZAP-70 is known for its binding to the T cell receptor, an event that is essential for T cell activation, but in the hybridoma used by Roos, there is no T cell receptor.

Roos notes that ZAP-70–inhibiting drugs, designed to block immune responses, may have unexpected additional benefits. "If you have an autoimmune disease you already

have activated T cells," he says. "But the drugs would still have an effect on autoimmune disorders because they would prevent migration of T cells into tissues."

How Life Deals with Excess

Starting S in M

The early cell cycles of *Xenopus* embryos take just 30 min each, but the dimensions of the egg are large, meaning that chromosome segregation takes longer in the earliest cycles. LeMaitre et al. report that *Xenopus* keeps up with the cell cycle clock by starting DNA replication before chromosome segregation has been completed (see page 1159). By early telophase, when the division furrow is just starting to form and the spindle hasn't totally disappeared, replication is well underway, as evidenced by the incorporation of bromodeoxyuridine and the presence of replication protein A (RPA) and the processivity factor PCNA.

Xenopus does this by forming karyomeres. These structures had been characterized previously as single chromosomes encased in lipid. LeMaitre et al. find that karyomeres form in late anaphase, while chromosomes are still segregating, and before the chromosomes are close enough to each other to be enclosed in a single nuclear envelope (although eventually the karyomeres fuse to form a short-lived nuclear envelope). The karyomeres include nuclear lamins, which are essential for the initiation of DNA replication. Karyomere formation is probably made possible by the vast store of proteins and membrane vesicles in the egg. This supply runs out as embryos approach the midblastula transition.

The karyomeres make each chromosome an independent replication unit. Senior author Marcel Méchali says that the need for an enclosure—be it a nuclear envelope or a karyomere—fits with the licensing factor hypothesis. "Once you have formed the karyomere you are safe," he says. "No more licensing factor can get in, and you can start replication without worrying about getting rereplication."

Nucleoli Organize De Novo Using Pre-rRNA, Not Transcription

The nucleolus appears to be a self-organizing structure: its components end up in one place by binding to rDNA and to each other, rather than by being transported to a specific site. The main activity of the nucleolus—transcription of the genes for ribosomal RNAs—is therefore a reasonable candidate for bringing together components and acting as a nucleolus organizer. Indeed, rDNA transcription coincides with the reassembly of the nucleolus at the end of mitosis, and inhibition of this transcription blocks reassembly.

Verheggen et al. (page 1167) set out to see if the same is true in *Xenopus* embryos, which make nucleoli de novo after 12 cell cycles, at the midblastula transition. They find that nucleolin, fibrillarin, and unprocessed pre-rRNA are gradually recruited to sites that take on the appearance of nucleoli. This occurs some 2.5 h before rDNA transcription starts. It seems that, at least in the presence of the large amount of pre-rRNA supplied by the *Xenopus* oocyte, pre-rRNA can be used in nucleolus formation.

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