In This Issue

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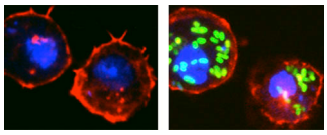
Mutant phagosomes bite off more than they can chew



E. coli **(green) accumulate in macrophages from flies lacking Vps16B (right) but not in wild-type cells (left).**

Akbar et al. reveal that the fly homologue of a gene mutated in human disease encodes a protein required for phagosome maturation and digestion of pathogens.

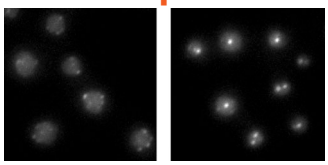
The HOPS complex is a multi-subunit tethering factor that regulates vacuole fusion in budding yeast. Metazoans express two versions of the HOPS subunits Vps16p and Vps33p. Vps16A and Vps33A promote the delivery of endosomes and autophagosomes to lysosomes in flies. The function of Vps16B and Vps33B is less clear, but mutations in the human homologues of these proteins cause a fatal multisystem disease called arthrogryposis, renal dysfunction, and cholestasis (ARC) syndrome.

Akbar et al. generated flies lacking Vps16B and found that they were surprisingly healthy and had no defects in autophagosome or endocytic trafficking. Knowing that ARC syndrome patients are susceptible to recurrent bacterial infections, however, the researchers injected the flies with *E. coli* and found that Vps16B mutants died much faster than wild-type strains. Mutant fly macrophages engulfed the bacteria normally but failed to digest them, as phagosomes failed to fully mature and fuse with lysosomes. This caused the bacteria to accumulate in intermediate phagocytic compartments, prompting Akbar et al. to name the *Drosophila* Vps16B gene *full-of-bacteria*. Similar effects were seen after knocking down Vps33B in flies.

It remains to be seen whether human Vps16B and Vps33B also control phagosome maturation and whether defects in this process account for other symptoms of ARC syndrome. Senior author Helmut Krämer now wants to investigate how the different HOPS subunit homologues contribute to distinct trafficking pathways.

Akbar, M.A., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201008119.

Sir3 helps telomeres stick together



Compared with wild-type cells (left), telomeres cluster into larger foci in yeast overexpressing Sir3 (right).

The silencing factor Sir3 clusters together the ends of yeast chromosomes, Ruault et al. report.

Yeast telomeres gather into distinct subcompartments near the nuclear periphery, thereby concentrating the gene-silencing factors that bind them. This improves the silencing of genes lying near chromosome ends, while avoiding inappropriate repression of genes located elsewhere in the genome. Silencing may in turn regulate clustering, because deleting components of the Sir2–Sir3–Sir4 silencing complex disrupts telomere organization.

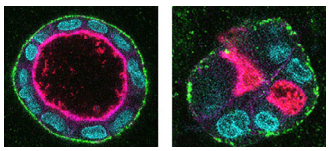
Ruault et al. overexpressed individual subunits of the Sir2–Sir3–Sir4 complex to investigate which of them was responsible for aggregating telomeres. Sir3 overexpression bunched telomeres into larger foci than those found in wild-type cells and repressed genes in subtelomeric regions more stably. Silencing wasn't required for telomere clustering to occur, however. Overexpression of a Sir3 mutant lacking silencing activity still induced large telomere clusters, even in the absence of Sir2 and Sir4. But deleting the telomeric protein Rap1 blocked the Sir3 mutant's recruitment to chromosome ends and prevented it from inducing telomere hyperclustering.

Because Sir3 can oligomerize with itself, Ruault et al. think that neighboring telomeres are held together by arrays of the protein at chromosome ends. Mammalian telomeres normally don't cluster, but different chromatin proteins may act similarly to organize other parts of the genome.

Although silencing isn't required for clustering, Sir3's involvement in both processes ensures that telomere organization and function are tightly linked. Senior author Angela Taddei now wants to investigate whether environmental changes that affect gene silencing also regulate telomere aggregation.

Ruault, M., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201008007.

IRSp53 keeps ECM signaling up to Par



Compared with wild-type cells (left), cells lacking IRSp53 (right) form cysts with multiple, misoriented lumens (magenta) and fail to deposit laminin (green) evenly around their basal surface.

Par1b controls cell polarity by phosphorylating an actin-regulatory protein to inhibit cells' interactions with the extracellular matrix, Cohen et al. reveal.

Par1b is a kinase that regulates the polarity of many different cell types. MDCK kidney epithelial cells, for example, fail to adhere to each other or polarize correctly in the absence of the kinase. Par1b overexpression, on the other hand, causes kidney cells to relocalize their lumens from their apical domains to the lateral membranes between neighboring cells, an orientation normally found only in liver epithelia.

Cohen et al. screened for new Par1b substrates and identified IRSp53, an adaptor protein that links Rho GTPases to downstream promoters of actin polymerization. MDCK cells lacking IRSp53 formed lateral lumens similar to cells overexpressing Par1b. Yet IRSp53 knockdown had no effect on intercellular adhesion. Instead, IRSp53 depletion inhibited cell–matrix interactions, reducing cell spreading, focal adhesion formation, and basal lamina deposition. Par1b overexpression also inhibited cell spreading but a nonphosphorylatable form of IRSp53 reversed this phenotype and restored lumen formation to the apical surface.

Par1b thus regulates cell polarity by inhibiting IRSp53's control of cell–extracellular matrix signaling. Par1b phosphorylation prompted IRSp53 to preferentially bind 14-3-3 adaptor proteins instead of actin regulators, potentially inhibiting the Rho GTPase signaling pathways that control the interactions of cells with their surrounding matrix. Senior author Anne Müsch now wants to identify the precise pathways blocked by Par1b and to understand how this results in lumen repositioning.

Cohen, D., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201007002.