

Research Roundup

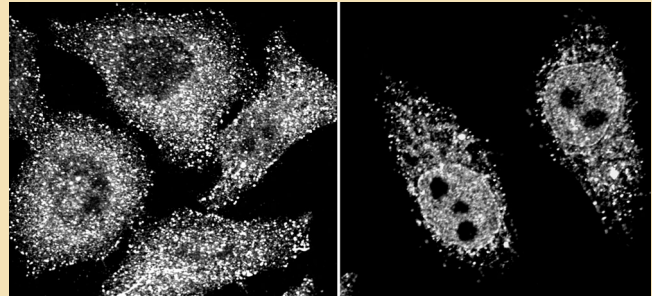
Endosomes are ready and APPL to signal

Endocytosis is a multifaceted regulator of cell surface-to-nucleus communication. It can dampen signaling by sending receptors to lysosomes for degradation. However, recent studies suggest that some receptors continue to signal from within endosomes. Now, Marta Miaczynska, Marino Zerial (Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany), and colleagues show that endosomes themselves are needed to transduce certain proliferation signals to the nucleus.

Endosome trafficking relies on a small GTPase called Rab5. Activation of Rab5 by binding of extracellular factors such as EGF to their receptors stimulates endocytosis. Zerial's group shows that some EGF-containing endosomes trigger nuclear responses via two newly identified Rab effectors, APPL1 and APPL2. Thus, EGF can elicit signal transduction cascades from both endosomes and the plasma membrane.

The APPL proteins were found in a unique subset of Rab5- and EGF-containing endosomes. GTP hydrolysis by Rab5 then released APPLs from the membranes so that they could enter the nucleus. There, APPLs interacted with a histone deacetylase and chromatin-remodeling proteins that are needed for cell cycle progression—and thus EGF's proliferative effects. Loss of APPL or its interaction with Rab5 blocked DNA synthesis and cell proliferation.

Besides EGF, oxidative stress also relocated APPLs to the



Zerial/Elsevier

Endosomal APPL (white) enters the nucleus after EGF treatment (right).

nucleus, and the authors believe that other growth factors will have the same effect. Miaczynska is now interested in determining whether the APPL-containing membranes are simply transport vesicles, subcompartments of early endosomes, or bona fide organelles. "If an organelle linked to endocytosis exists that is dedicated to signaling, it would increase the possibilities of regulation in the cell," says Zerial. "It would explain why different cells respond so differently to growth factors, because the amount of this pathway differs. In essence, the ability of a cell to respond to growth factors depends not only on the set of receptors and signaling molecules but also on their trafficking pathways." ■

Reference: Miaczynska, M., et al. 2004. *Cell*. 116:445–456.

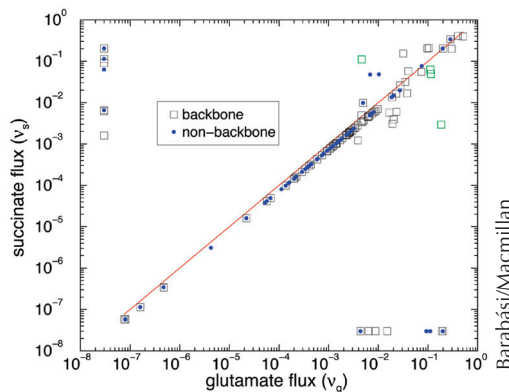
Feudal system in metabolic flux

Metabolism flows much like city traffic, according to an analysis by Eivind Almaas, Albert-László Barabási (University of Notre Dame, Notre Dame, IN), and colleagues. Although the vast majority of the reactions are less-traveled side roads, a few vital highways carry most of the metabolic traffic.

The organization of the numerous reactions that produces or consumes key metabolites is well-established in *E. coli*. Metabolites are arranged in a power-law distribution: a large number are made or consumed by only a few reactions, whereas a few metabolites are made or consumed by many more reactions. Barabási's group took a mathematical approach to examine the flux, or the frequency of use, through all of these reactions. Using flux-balance analysis, in which mass conservation, cellular equilibrium, and maximum growth rate constrain

the possible flow through each reaction, the authors identified all possible flux states through every reaction for a given growth condition.

They find that, like the metabolite network, flux is also a power-law distribution. Nearly all reactions show low flux. A few, however, channeled the majority of



Barabási/Macmillan

Flux changes are handled mostly by high flux reactions (squares).

metabolic flux. A similar pattern was found on a local level—when several reactions produced or consumed a given metabolite, most were low flux, whereas one carried most of the burden.

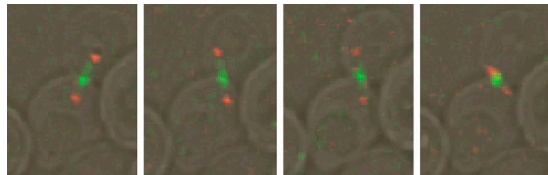
The inequality held true even if maximum growth rate was not assumed or growth conditions changed. "The distribution of flux is the same no matter what environment you have been dumped into," says Almaas. Traffic is somewhat shifted by new conditions, however, with some high flux pathways changing their flow by orders of magnitude. Low flux pathways change little, if at all. Almaas supposes that flux patterns will be similar for most species since metabolic networks of organisms ranging from yeast to man also show power-law distributions. ■

Reference: Almaas, E., et al. 2004. *Nature*. 427:839–843.

Any which way but loose

A back-to-back arrangement of kinetochores is not needed for correct alignment on the mitotic spindle, as shown by Hilary Dewar, Tomoyuki Tanaka (University of Dundee, UK), and colleagues.

Sister chromatids are glued together by cohesin such that their kinetochores face opposing poles. This arrangement might thus prevent both chromatids from attaching to spindle microtubules from the same pole. But Tanaka's group shows that even when geometry fails, a tension-sensitive mechanism fixes any mistakes.



Dicentric chromosomes (green) reveal that tension is enough for attachment to both poles (red).

The authors messed with the usual geometry in two ways. First, they confronted yeast cells with a nonreplicating dicentric minichromosome. Its two kinetochores are not held in any fixed relative orientation, yet were efficiently attached to opposing poles. Second, normal chromosomes in cohesin mutants (which have attachment defects), were roughly linked by inhibiting topoisomerase II. This restored bipolar attachments. Thus, any connection that can produce tension is enough to ensure biorientation.

The tension-sensitive correction depends on the Ipl1 kinase, whose mammalian homologue, Aurora B, prevents monopolar attachments. This suggests that Ipl1 activity knocks off attachments until tension somehow stops it—perhaps by turning off the kinase, turning on a counteracting phosphatase, or pulling substrates away from the kinase. ■

Reference: Dewar, H., et al. 2004. *Nature*. 10.1038/nature02328.

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A dynamite intercellular highway

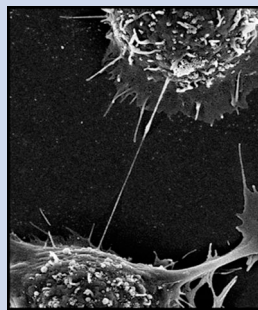
Long, delicate tubules are a new trade route for the intercellular exchange of goods, as shown by Amin Rustom, Hans-Hermann Gerdes (University of Heidelberg, Germany), and colleagues.

Rustom noticed these actin-rich extensions, which the group calls tunneling nanotubes (TNTs), while looking for secretory granules near the plasma membrane. The membrane dye that he used revealed long thin tubes, up to several cell diameters long but only ~100 nm wide, linking some of the plated cells. "They are extremely sensitive," says Gerdes. "Even light exposure disrupts them. Maybe because of [this sensitivity], this is the first time we realized these unique structures are there." The group has seen the connectors in kidney and neurosecretory cell lines and primary neuroendocrine cultures, but other cell types could be similarly linked.

The TNTs are made when filopodia contact a distant cell. Once it becomes contiguous with the new partner, the extension establishes a one-way conveyor belt-like system through which one cell (probably the one that sent out the filopodia) dispatches endosomal-like vesicles to the other.

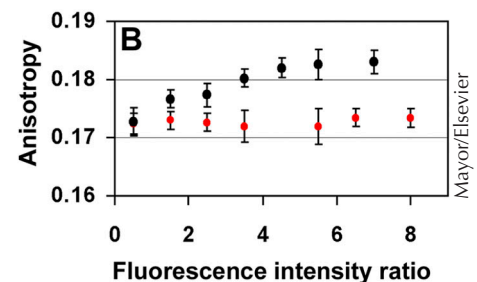
Smaller soluble molecules, however, are blocked from entry into the TNTs. The actin bundle at the base of the TNT, wrapped tightly by the surrounding membrane, may act like a plug to prevent passive diffusion of small molecules. If TNTs exist in vivo, then theoretically anything packaged into endosomes, such as morphogens or immunogenic components, could be used to signal between otherwise unconnected cells. ■

Reference: Rustom, A., et al. 2004. *Science*. 303:1007–1010.



Actin tubules called TNTs connect distant cells.

Teeny tiny rafts



Native clusters (black circles) reorganize (red circles) after cross-linking removes one type of GPI-AP.

Tiny but dynamic domains define the elusive lipid raft, based on results from Madan Rao, Satyajit Mayor (National Centre for Biological Science, Bangalore, India), and colleagues.

Describing the structure and components of rafts—membrane domains enriched in specific lipids and proteins—has been a long-standing challenge for cell biologists. In this new report, the authors use FRET, photobleaching, and theoretical modeling to get the closest look yet at raft components called GPI-APs (GPI-anchored proteins). They show that lipid rafts contain small clusters (four or fewer molecules) of very tight-knit GPI-APs packed into an ~4-nm-wide space.

About a third of any given GPI-AP species is found in rafts. The rest remain as monomers. This percentage holds true across multiple expression levels, which is inconsistent with equilibrium-based formation. "It means rafts have to be actively maintained," says Mayor. "And within these regions, the GPI proteins form clusters."

Different types of GPI-APs are found within a cluster, and the clusters are dynamic—cross-linking of one species removes it from the cluster, and another species readily takes its place. The cross-linked GPI-APs formed larger groups that were endocytosed by the clathrin-mediated pathway, rather than the route responsible for uptake of raft GPI-APs. Thus, ligation of a receptor could change its fate by altering its lipid environment. ■

Reference: Sharma, P., et al. 2004. *Cell*. 116:577–589.