Published Online: 30 September, 2002 | Supp Info: http://doi.org/10.1083/jcb1587iti3

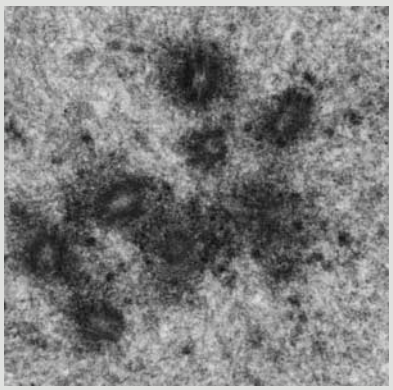
In This Issue

Birth control for centrioles

Omnis centriolus e centriolo—every centriole comes from a centriole. This statement underlies the standard model of centrosome duplication, in which daughter centrioles that will form the foundation of a new centrosome must be patterned on some kind of template provided by a mother centriole. On page 1171, Khodjakov et al. now demonstrate that vertebrate cells are fully capable of assembling centrioles de novo, overturning this long-held belief and suggesting a different function for the mother centrioles.

A few specialized cell types, such as clam zygotes and rabbit blastomeres, previously have been shown to form centrioles de novo, but these examples were thought to be exceptions. The new work argues otherwise. The authors arrested CHO cells in S phase, and then destroyed their centrosomes with a laser. In this system, a loose cloud of pericentriolar material (PCM) forms, and within 24 h this PCM cloud becomes more compact, correlating with the appearance of new centrioles. The formation of the PCM clouds does not require microtubules, but centriole assembly does.

The de novo centriole synthesis occurred in every cell, indicating that it is very efficient, but the process produces a random number of centrioles—as many as 14 in one cell. This suggests that in normal cells, a mother centriole may act to limit the number of centrioles produced and prevent multipolar mitosis, rather than providing a necessary template for centriole formation. Preliminary analysis of other vertebrate cell lines suggests that the de novo pathway is a general mechanism, raising the possibility that it could be involved in cancerous transformation.



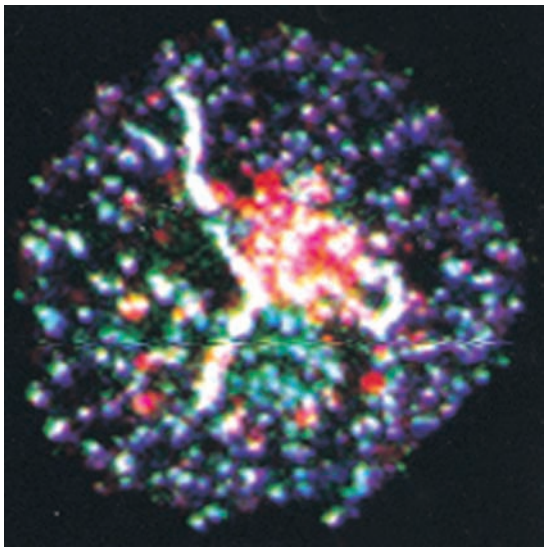
Centrioles can form de novo.

On page 1183, Dupuis-Williams et al. used a different model system to analyze the function of ε-tubulin, which specifically associates with centrosomes in human cells. The authors cloned the ε-tubulin gene of *Paramecium*, and found that it is required to stabilize the microtubule triplets that assemble into basal bodies in the protozoan. ■

Signaling clusters caught in the act

Biochemical studies have provided a detailed accounting of the molecules present in the T cell receptor (TCR) complex during T cell activation, but relatively little is known about the maturation and dynamic composition of these complexes. Now Bunnell et al., reporting on page 1263, have developed a novel assay that allows them to visualize the formation of these complexes in live cells.

The authors dropped live Jurkat leukemic T cells onto coverslips coated with anti-TCR antibodies, and then tracked chimeric signaling proteins as they associated with and dissociated from signaling complexes. TCR-signaling complexes develop within 15 s, and undergo dynamic assembly and disassembly throughout the process of contact formation. Initial clustering of the TCRs does not require phosphotyrosine-dependent interactions, but the formation of a functional signaling complex does. A lipid raft marker does not localize to the complexes, a result that should fan the controversy surrounding raft function in signaling.



Signaling complexes assemble soon after TCR ligation.

Molecules that are recruited to the complexes depart by distinct mechanisms. The adaptor protein SLP-76 exhibits a particularly striking pattern, departing TCR-rich signaling complexes in large structures that are transported along microtubules to accumulate in a novel perinuclear structure. Bunnell et al. suggest that signaling complexes are functioning as sorting structures, as molecules are recruited to the complexes from different compartments, then sent from the complexes in different ways.

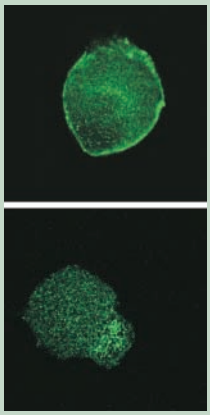
Intracellular calcium elevations in the T cells begin within 12 s of contact initiation, suggesting that a single TCR cluster may be sufficient to produce calcium elevation. TCR clusters may therefore constitute "proto-synapses" capable of directing T cell activation, a model that may explain how some types of T cells are activated without the formation of well-defined immune synapses. The authors are now extending their assay to include more markers to characterize the process of complex formation in greater detail. ■

Text By Alan W. Dove
alanwdove@earthlink.net

Herpes spoils excitement

People infected with the cold sore–causing herpes simplex virus 1 (HSV-1) often complain of abnormal sensations around the site of initial infection, a symptom apparently caused by the virus's ability to reduce the excitability of infected neurons. On page 1251, Storey et al. describe the molecular mechanism responsible for this phenomenon. Their findings may also be relevant to the normal rearrangements of voltage-dependent sodium channels that occur during neuronal development.

The authors found that neurons from the rat dorsal root ganglion show a profound and rapid loss of voltage-dependent sodium currents ~24 h after infection with HSV-1. Loss of excitability in these neurons correlates with the loss of sodium channels from the cell surface. Blocking endocytosis or preventing the production of HSV-1 late proteins prevents the loss of excitability, and a mutant virus lacking the neurovirulence factor ICP 34.5 does not cause the loss of sodium channels seen in wild-type viral infections.



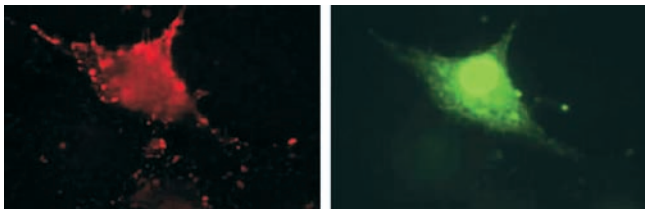
HSV-1 infection (bottom) prompts a loss of sodium channels (green).

Previous work has shown that HSV-1 modifies the ubiquitin–proteasome pathway, leading to the destruction of many cellular proteins, and this may also explain the internalization of sodium channels. If the virus is tapping into a normal cellular pathway, then the same ubiquitin–proteasome pathway may also explain the rapid disappearance of sodium channels at nodes of Ranvier during development. ■

Finding a heat-shock absorber

Load the heat-shock protein Hsp70 with a peptide from a tumor-specific antigen, then inject it into a mouse bearing a tumor with the same antigen, and the mouse will mount a vigorous immune response that can significantly reduce tumor progression. The result is promising, but how does it work? Studying human Hsp70, Becker et al. (page 1277) now show that the cell surface protein CD40 binds specifically to peptide-loaded Hsp70, apparently mediating the uptake of the bound peptide into antigen-presenting cells.

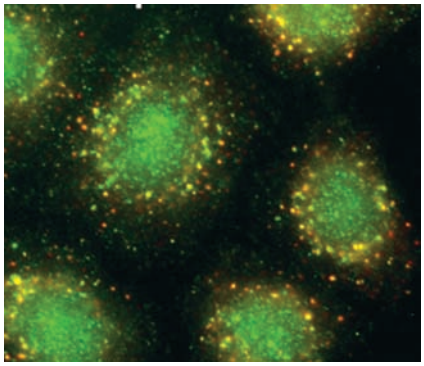
The binding of Hsp70 to CD40 occurs at the ATPase domain of Hsp70, and is strongly dependent on the presence of a peptide substrate on the heat-shock protein. This could explain why previous efforts, where Hsp70 binding was examined in the absence of nucleotides and peptide substrate, failed to identify the interaction. As the cochaperone protein Hip competes with CD40 for Hsp70 binding, CD40 and Hip apparently recognize overlapping binding sites. The authors propose that the peptide-binding domain of Hsp70, when free of peptide, masks the Hsp70 ATPase domain, thus preventing recognition by CD40. Peptide binding exposes the ATPase domain and allows CD40 to bind, ensuring that only the peptide-bound form of Hsp70 is taken up by antigen-presenting cells for processing. ■



Peptide-bound Hsp70 (red) binds cells expressing CD40 (green).

An endosomal switchboard

Studies on the internalization of signaling receptors generally suggest that receptor endocytosis attenuates signaling. On page 1239, Hayes et al. turn this concept on its head, showing that inhibiting endocytosis actually impairs TGFβ receptor signaling. The findings suggest that endosomes may in fact serve as specialized signaling compartments that amplify and propagate certain types of cellular signals.



TGFβ signaling (green) takes place in endosomes (red).

The authors found that the endogenous TGFβ receptor in cultured cells localizes to endosomes containing the early endosomal marker EEA1. SARA, a soluble cytoplasmic protein required for TGFβ function, is found almost exclusively in the same EEA1-enriched endosomes. Disrupting TGFβ receptor endocytosis impairs downstream signaling events normally mediated by the receptor. This result is reminiscent of the disruption of anti-apoptotic signaling recently noted in cells that lack clathrin function (Wettey, F.R., et al. 2002. *Science* 297:1521–1525).

This is the first time membrane trafficking has been shown to be required for the productive association of two components of a signaling pathway, and it suggests that the function of the endosome in signaling may need to be reevaluated. Hayes et al. propose two models to explain their results: either SARA and other signaling components are restricted to the endosome, requiring TGFβ receptor internalization to form the signaling complex, or the TGFβ receptor signaling complex forms at the plasma membrane, but requires the biochemical conditions of the endosome to produce a signal. ■