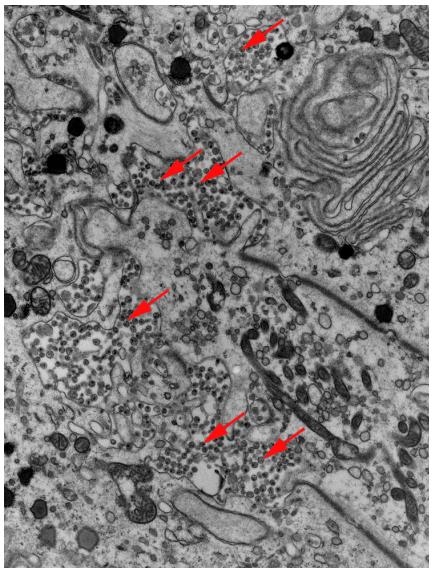
# In This Issue

## The macrophage home of HIV

HIV assembles in a compartment that lies deep within macrophages yet is continuous with the extracellular environment, report Deneka et al. (page 329).



**HIV (red arrows; tiny circles) occupies vacuoles that are continuous with but separated from the extracellular space.**

HIV forms membrane-encapsulated particles that assemble at, and bud off from, the surface of infected T cells. In macrophages, however, the majority of virus particles are found intracellularly and have been reported to associate with structures containing an endosomal marker called CD63.

Deneka and colleagues' data, however, indicate that HIV does not enter macrophages by endocytosis. Upon HIV infection, the virus did not colocalize with any other endosome markers besides CD63. It instead associated with a newly identified vesicular structure marked by the transmembrane receptors CD81, CD9, and CD53.

The CD81/9/53 structures were shown by electron microscopy to be connected to the outside of the cell by narrow channels that are too small for HIV to pass through. By being essentially outside and inside at the same time, HIV would be simultaneously protected from the acidic digestive environment of endosomes and lysosomes and from the humoral immune system.

The authors propose that these structures might also allow for a rapid release of virus particles for their transmission to other cells. They are currently trying to determine the function of the CD81/9/53 compartments in uninfected macrophages.

In uninfected macrophages, endosomal CD63 did not colocalize with these CD81/9/53 compartments. So why does it accumulate there upon HIV infection? CD63 can be incorporated into the membrane capsule of HIV. The authors suggest that CD63 might normally traffic in small amounts throughout internal membranes and get swept up by HIV into CD81/9/53 structures.

Two other recent reports agree that HIV particles bud off from plasma membrane in macrophages rather than being associated with endosomes (Jouvenet et al. 2006. *PLoS Biol*. 4:e435; Welsch et al. 2007. *PLoS Pathog*. 3:e36). Now it is shown that this plasma membrane region is both intracellular and extracellular at the same time. JCB

## Recruitment but no repair

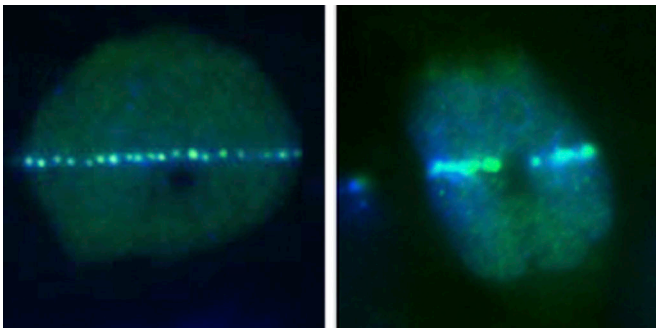
A DNA repair protein turns up at the job site even without its tool kit, according to Uematsu et al. (page 219). Its visit is then prolonged by its inefficiency.

Fixing double strand breaks in DNA by nonhomologous end joining (NHEJ) requires a DNA-dependent protein kinase (DNA-PK) to bind to the loose ends of broken DNA and a ligase to do the gluing. This protein machinery must turn up and fix the ends rapidly to minimize the chance that DNA diffusion causes the wrong partners to be glued back together.

Very little is known, however, about the in vivo dynamics of NHEJ. Here, Uematsu et al. describe the dynamics of DNA–PK recruitment in vivo. DNA-PK is composed of two subunits: Ku70/80 and DNA-PK$_{CS}$. They found that both subunits accumulated at damaged sites within two seconds after targeted DNA breakage. This repair site recruitment was dependent on the Ku70/80 subunit.

DNA-PK$_{CS}$ has a kinase domain and a cluster of phosphorylation sites, both of which are needed for mending DNA. Mutation of either, however, did not impair the enzyme's recruitment speed.

Despite turning up for work as usual, the mutant repair proteins didn't leave as quickly as the wild type. DNA-PK$_{CS}$ is well-known for performing autophosphorylation, and this ability is necessary for DNA repair. Since recruitment of the mutant proteins was normal but repair was deficient, the authors suggest that the main target of DNA-PK$_{CS}$ kinase activity is itself. Autophosphorylation might be the signal that the two loose DNA ends have come together. It most likely triggers the release of DNA-PK and allows access for the ligase to do its gluing. JCB



**A repair protein (green) that lacks its kinase activity (left) or phosphorylation sites (right) still turns up at the repair site (blue).**

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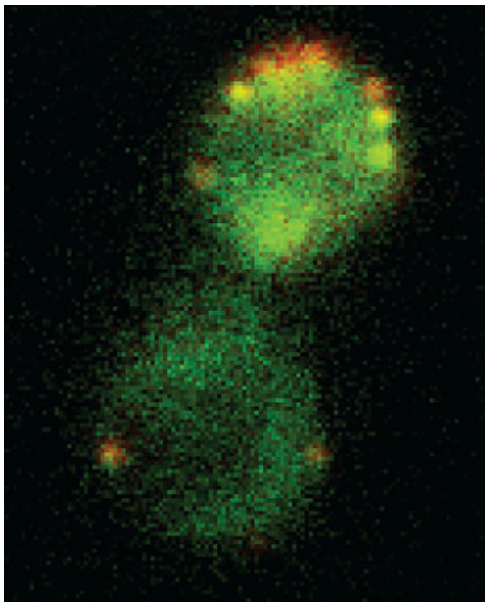


**The breakdown of PI(4,5)$P_2$ by a phosphatase (green) allows endocytosis (red) to complete.**

## PIP$_2$ in endocytosis

Sites of endocytosis are enriched in PI(4,5)$P_2$, but its removal is required for endocytosis to be completed, report Sun et al. (page 355).

The membrane minor phospholipid PI(4,5)$P_2$ has long been implicated in endocytosis. Many endocytotic proteins contain binding sites for it, and, without this binding, endocytosis is impaired. Evidence for the enrichment of PI(4,5)$P_2$ at sites of endocytosis, however, has been lacking. The team used a PI(4,5)$P_2$- binding domain, tagged with a fluorescent protein, to follow the dynamics of PI(4,5)$P_2$ during endocytosis in live cells.

They show that PI(4,5)$P_2$ is present in patches on the membrane and that these patches move into the cell over time and disappear. To confirm this inward movement was indeed endocytosis, the team used a second fluorescently labeled endocytosis coat protein and showed that the two colors colocalized.

The disappearance of PI(4,5)$P_2$ coincided with the recruitment of phosphoinositide phosphatase, which is known to break down PI(4,5)$P_2$. In cells in which PI(4,5)$P_2$ breakdown was impaired, the team saw abnormal membrane invaginations that are thought to be the sites of multiple attempts and failures at endocytosis.

Indeed, without PI(4,5)$P_2$ turnover, the endocytotic machinery still arrived at the membrane patches but hung around for longer without completing their job. It thus appears that the breakdown of PI(4,5)$P_2$ is required for the scission of endocytotic vesicles. JCB

## How ERK5 prompts proliferation

Overactive ERK5 drives cell proliferation and transformation and is associated with highly aggressive forms of breast and prostate cancer. Now, Cude et al. (page 253) reveal that this MAP kinase pushes the cell cycle forward by promoting entry into M phase.

ERK5 is activated by various growth factors. Its known targets include cyclin D and NFκB, both of which help cells enter S phase. It had been suggested, therefore, that growth factor–induced ERK5 might promote cell proliferation by kick-starting S phase. No one had yet looked, however, at how ERK5 activity normally changes during the cell cycle.

Cude et al. have now done just that. They found that ERK5 activity peaked at G2/M phase, not S phase. Suppressing this activation reduced the number of cells entering mitosis, while overactivating ERK5 drove more cells into mitosis. The ERK5-driven entry into M phase was dependent on the activity of the transcription factor NFκB, which the team found up-regulated a number of mitosis-promoting genes.

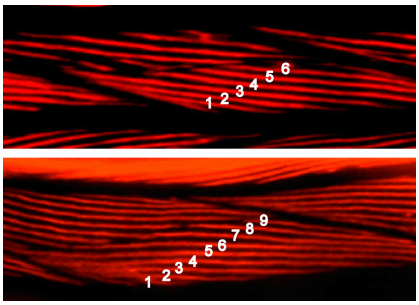
It's unclear yet whether high levels of ERK5 activity are a direct cause of cancer. But if high ERK5 is enough to overcome G2/M phase DNA damage checkpoints, as the team now plans to investigate, then mutations might accumulate over subsequent cell divisions. Given that ERK5 might also promote S phase entry, and that high ERK5 activity suppresses apoptosis, it's plausible that mutations causing ERK5 overactivity might be enough to drive aggressive tumor development. JCB

## The critical chaperone balance

Chaperones protect proteins during their vulnerable folding stages. But too much chaperoning is counterproductive, as it keeps proteins in this vulnerable stage for too long, suggest Landsverk et al. (page 205).

The UNC-45 chaperone protects immature myosin from degradation while it folds into a form compatible with assembly into myofilaments. Without UNC-45, worms have fewer myofilaments in their muscle cells and are, as a result, severely paralyzed. The team now shows, however, that too much UNC-45 also results in myosin degradation, reduced myofilament numbers, and decreased mobility.

Myosin degradation caused by the loss of UNC-45 occurs via ubiquitination-mediated targeting to the proteasome. This same pathway was also the fate of myosin in the presence of too much UNC-45. Inhibiting the proteasome restored myosin levels and worm mobility.




**Worms have fewer filaments in their muscles when there's too much UNC-45 chaperone (top).**

The authors propose that, when there's too much UNC-45, it holds more myosin proteins in their immature, nonmyofilament form. Because most chaperone–substrate interactions are highly dynamic, UNC-45 and myosin are probably constantly binding and releasing. During these moments of release, all this immature myosin would be available for ubiquitination.

Other chaperones have been similarly reported to have optimal concentration ranges. Ensuring that this optimal range is not exceeded might, itself, be achieved by the ubiquitination–proteosome pathway. Indeed, the team has previously shown UNC-45 to be degraded by this route. JCB