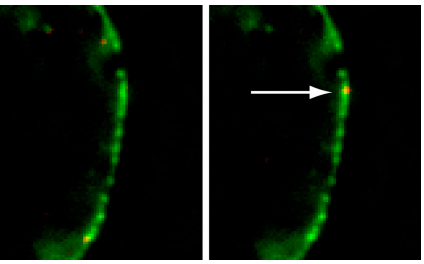
In This Issue



A single molecule of labeled transport receptor (red, arrow) binds to the nuclear pore complex (green).

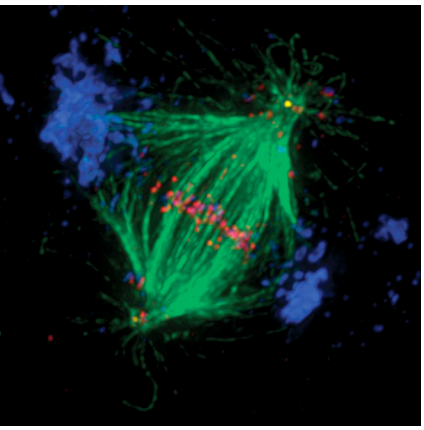
No waiting at the nuclear pore complex

Airport security checkpoints might take a lesson from the nuclear pore complex (NPC), where, according to Dange et al., there are no long lines and no slowdown despite the hubbub around them.

The group used single-molecule video microscopy, with a localization precision of about the width of a protein, to track single molecules of labeled, cargo-carrying receptors entering the NPC in vivo. They had previously used the same technique to look at the NPC in lifeless cells, but thought that the lack of vital activity—the absence of ion gradients in the cytoplasm, transcription in the nucleus—might affect the receptor's behavior. Instead, they found that the spatial and temporal details of NPC transport for the three receptors they studied was essentially the same: Once a receptor encountered the NPC, it was largely confined to a narrow central channel, and moved quickly through the pore to deliver its cargo. "There was no waiting at the gate," says corresponding author David Grünwald.

The similarity between their in vivo data and their previous experiments suggests that nuclear import dynamics are determined mainly by cargo–receptor–pore interactions, and are mostly free of influence from other cell processes and other transported molecules, including, surprisingly, RNA. Grünwald suggests that perhaps RNA has its own export pathway through the NPC, a possibility the team intends to study in the future.

Dange, T., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200806173.



Kinetochores (red) attach to the spindle (green), whereas chromatin (blue) is largely excluded.

No stretch for spindle assembly checkpoint

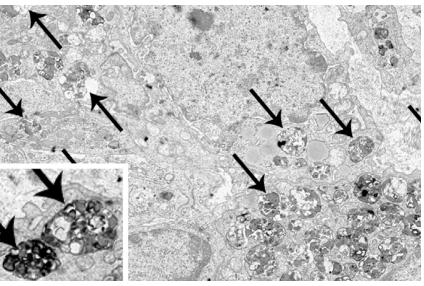
Dividing cells aren't quite as obsessed with quality control as previously thought, according to results from O'Connell and colleagues.

The spindle assembly checkpoint assures that chromosomes segregate properly during mitosis. This checkpoint has been thought to monitor two separate events: the attachment of kinetochores to spindle fibers, and the stretch force across the centromere as paired sister chromatid kinetochores start to pull apart. But whether detection of stretch is required to satisfy the checkpoint has never been conclusively demonstrated. To test this, the authors treated cells with hydroxyurea to inhibit replication, creating cells that undergo mitosis with unreplicated genomes (MUG). Such cells contain normal centrosomes and form bipolar spindles, but each kinetochore is unreplicated; thus, inner centromere stretch is not possible.

MUG cells weren't normal in every respect—their kinetochores were separated from the bulk of chromatin, unattached chromatin was largely excluded from the spindle apparatus, and the duration of mitosis was more variable and longer on average than in normal cells. Nonetheless, a normal bipolar spindle formed, and kinetochores attached to spindle microtubules, aligned at the equator, and moved to the poles at anaphase—even without centromeric stretch. In contrast, when the team induced microtubule depolymerization, mitosis was arrested, indicating MUG cells possess a spindle assembly checkpoint that relies on kinetochores attaching to intact spindle fibers.

These results don't necessarily imply that stretch is unimportant or unmonitored, says lead investigator Christopher O'Connell, only that its absence cannot halt anaphase. If stretch is monitored, he says, the correction mechanism is likely to be so rapid that delaying mitosis is unnecessary to resolve the problem.

O'Connell, C.B., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200801038.



Numerous dense and enlarged autophagic vesicles (arrows) accumulate in tumor cells treated with chloroquine and Akt inhibitor.

Autophagy inhibitors deliver a knock-out blow

If you want to kill a tumor cell, prompting apoptosis is a good place to start. But don't stop there, say Degtyarev et al.: Wait a while, and then hit it again in the autophagosome.

An inhibitor of apoptosis called Akt is overactive and contributes to uncontrolled growth in a wide variety of tumors. Akt inhibition is thus a leading anti-cancer strategy. But reducing Akt doesn't always increase apoptosis, suggesting there may be another side to its pro-survival activity. Recently, it was shown that Akt also suppresses autophagy, an alternative cell-death pathway. In the present study, the authors knocked down Akt in cancer cells and found that slowing of cell growth correlated with cell cycle delay and an increase in autophagy, supporting Akt's influence on this pathway. Nonetheless, as often observed in cancer treatment, many cells survived. "The cells seemed to hunker down," says Kui Lin, who led the study—the increased autophagy appeared to allow the cells to cope with the metabolic stress induced by Akt suppression. Tricks that autophagy uses to slow down cell death include recycling of nutrients and scavenging of reactive oxygen species.

The authors then asked whether inhibiting autophagy at the same time might deliver the knock-

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out blow. They found that inhibitors of the earliest stages of autophagosome formation had no effect, but the anti-malaria drug chloroquine, which inhibits the degradative function of autolysosomes, dramatically increased cell death. Affected cells accumulated grossly distorted autophagic vacuoles, lost their mitochondrial membrane potential almost completely, and suffered a large increase in reactive oxygen species within the cell, both a consequence of incomplete organelle destruction.

The authors suggest that preventing digestion of autophagosome contents might help kill the cancer cells because the accumulating toxic debris could leak out. Whatever the reason, when tumors received this one-two punch of Akt suppression and chloroquine-induced autophagy disruption, tumor remission was substantially increased.

Degtyarev, M., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200801099.

Tre1 says when, and where, to go

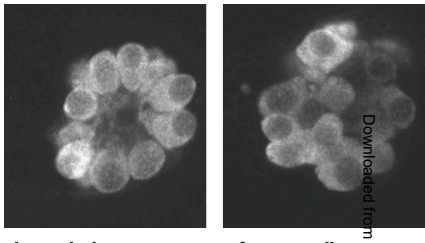
For migrants heading out on a journey, it's not enough to just pull up their stakes—they need to know where they're going. According to Kunwar et al., migrating germ cells take their cues from the G protein–coupled receptor Tre1, which not only loosens the cells' ties to home, but also points them in the right direction.

Drosophila germ cells arise next to the endoderm, but must migrate through the developing midgut to reach their final destination in the gonads. The researchers had previously shown that Tre1 ("trapped in endoderm") was essential for this transepithelial migration, but not for general cell motility, suggesting it might play a specific role at the start of migration.

Here, using live cell imaging, the team showed that when Tre1 function was lost, the cells remained in a disorganized clump at the endoderm and failed to assume the polarized shape characteristic of normal migrating cells. This mutant phenotype could be recapitulated by mutating G proteins, confirming that Tre1's role in migration was through its known function as a canonical G protein–coupled receptor.

So, was Tre1's role to start migration or to set up polarization? In fact, it was both. Tre1 signaling led to concentration of certain proteins towards the tail end of the cell, including Rho1, whose relocation had been implicated in germ cell migration, and E-cadherin. In Tre1 mutant cells E-cadherin remained at the cell periphery and kept germ cells attached to their neighbors. The authors wondered if this failure to separate from neighboring cells might account by itself for the loss of migratory ability. But by disabling cadherin in the mutant cells to loosen cell–cell adhesion, the team observed that germ cells dispersed randomly, but did not to move in an orderly way through the epithelium. "There is more to migration than the down-regulation of cadherin," concludes Ruth Lehmann, who led the study. "It requires polarization, and Tre1 appears to organize it."

Kunwar, P.S., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200807049.



The radial organization of germ cells is lost in the Tre1 mutant (right).

Random, fast, and out of control

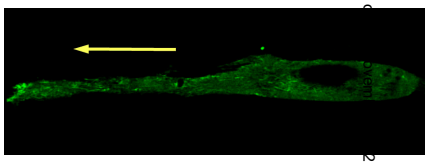
Caswell et al. report how the altered behavior of integrins can prompt metastatic movement in tumor cells.

On 2D surfaces, cells may migrate randomly, or be strongly unidirectional. Integrins, which link the cell to the extracellular matrix, are known to influence the mode of migration, but exactly how has been unclear. Recent work has suggested that an integrin called α5β1 drives random movement, while an integrin called αvβ3 has been associated with unidirectional migration—the balance of activity between the two determining the type of movement. To further explore the contribution of α5β1 to random migration, the authors thus blocked αvβ3.

The treated cells changed their mode of migration from unidirectional to random, and their ability to invade 3D gels increased. The changed behavior correlated with an increase in trafficking of α5β1 from intracellular compartments to anterior membrane protrusions. But this increase in trafficking did not significantly alter α5β1's contribution to cell adhesion—the ease with which cells were dislodged from a spinning disk increased as the amount of αvβ3 was reduced, but was not correlated with any change in α5β1. This suggested that the cells' increased invasive ability was due to alteration in some other property. That property turned out to be activation of a proinvasive pathway headed by a kinase called Akt.

In αvβ3-blocked cells, α5β1 became associated with epidermal growth factor receptor 1 (EGFR1), which increased EGFR1's abundance at the membrane protrusions, as well as its autophosphorylation. Because EGFR1 is an activator of the Akt pathway, hey presto, the cells took on some new moves.

Caswell, P.T., et al. 2008. *J. Cell Biol.* doi:10.1083/jcb.200804140.



An increase in α5β1 recycling promotes extension of long pseudopods at the cell front (arrow shows direction) in a 3D matrix.