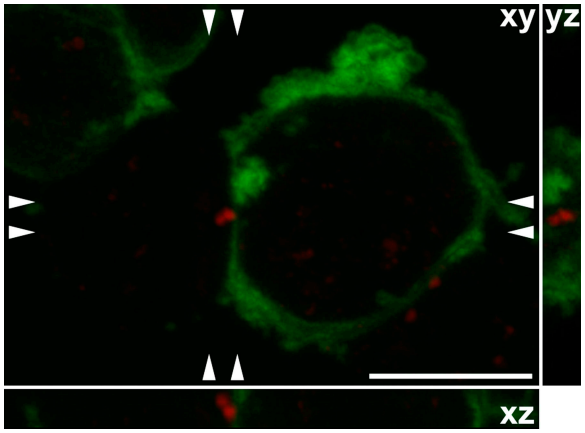
Research Roundup

Centrosomes deliver the death blow

Just as a deadly martial arts master channels his inner chi to deliver a fatal strike, cytotoxic T cells (CTLs) channel their toxic secretory granules to strike an infected target cell. New work by Jane Stinchcombe, Gillian Griffiths, and colleagues (Sir William Dunn School of Pathology, Oxford, UK) reveals that centrosomes do this channeling, going right to the plasma membrane to deliver their secretory granule death blows.



GRIFFITHS/MACMILLAN

Centrosomes (red) go right to the membrane (green) to deliver toxic granules at the synapse

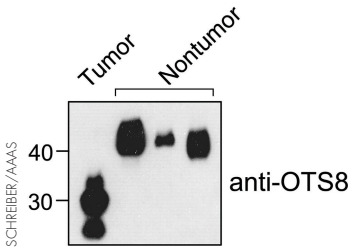
A CTL targets an infected cell by making transitory contact via an immunological synapse. Lytic protein–containing secretory granules are then released at the synapse to kill the target. Trafficking of the granules to the synapse was known to require transport along microtubules, but just how granules were delivered was unknown.

The general assumption was that CTLs would deliver their secretory granules in the same way that melanocytes deliver pigment for secretion—by transporting it along microtubules, transferring it to the actin cytoskeleton, and then delivering it to the membrane. Griffiths's group thus looked at actin in CTLs, but found that it is completely cleared away from the synapse.

The authors found that secretory granule movement toward the minus ends of microtubules was sufficient for killing target cells. Granules thus move toward the centrosome, which associates with microtubule minus ends, not toward plus ends at the plasma membrane.

The team observed that the centrosome itself associates with the membrane in a large number of synapses. They hypothesize that the action of clearing the actin might, via actins' attachment to microtubules, pull the centrosome close enough to the synapse membrane to send out the granules directly. Griffiths proposes that such direct delivery, without the need for transfer to the actin cytoskeleton, might also explain how the same CTL can engage and disengage synapses rapidly to kill multiple target cells, much like Bruce Lee rapidly defeats multiple opponents when surrounded. JCB

Reference: Stinchcombe, J.C., et al. 2006. *Nat. Cell Biol.* doi:10.1038/nature05071.



SCHREIBER/AAAS

Tumor-derived OTS8 is not mutated but is a lower molecular weight due to aberrant glycosylation.

Cosmc converter

Cancer biologists are constantly hunting for tumor-specific antigens that can be targeted for monoclonal antibody therapy. Work by Andrea Schietinger, Mary Philip, Hans Schreiber, and colleagues (University of Chicago, IL) reveals that you don't need a mutation in the antigen itself to make it tumor specific. Incorrect posttranslational modification will do.

The group identified a mutation-free antigen in a spontaneous murine fibrosarcoma, called Ag104A. Mice with this tumor produce a high-affinity, highly specific antibody against what the group now identifies as a transmembrane protein called OTS8. Although OTS8 is widely expressed, the antibody was only reactive to OTS8 from the Ag104A tumor.

Tumor-derived OTS8 was a different molecular weight than its nonantigenic counterpart yet, to the group's surprise, was not mutated. The different molecular weight was instead due to altered glycosylation. Whereas normal OTS8 contained large sugar moieties, tumor-derived OTS8 predominantly contained a smaller monosaccharide called Tn. This novel combination of Tn and OTS8 created the tumor-specific antigen. The defective glycosylation was due to a mutation in a chaperone protein called Cosmc, which controls the activity of a galactosyltransferase enzyme.

A single mutation that leads to defective glycosylation provides the potential for multiple possible antigens, and, encouragingly, Cosmc mutations do not appear to be limited to just one type of tumor. In addition to previously identified Cosmc mutations in a human colon cancer and a T cell leukemia cell line, the team discovered that Cosmc was also mutated in a murine neuroblastoma that also overexpressed Tn.

Certain human cancers are known to overexpress Tn. If patients with these cancers have Cosmc mutations, it would strongly suggest that the tumor cells express many incorrectly glycosylated proteins that are potential tumor-specific targets for therapy. JCB

Reference: Schietinger, A., et al. 2006. *Science.* 314:304–308.

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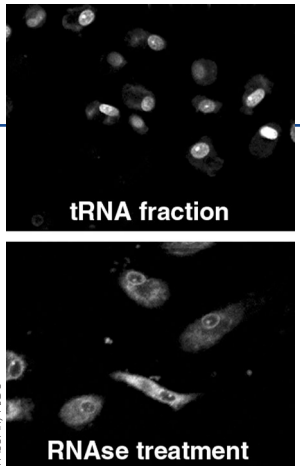
tRNA transport to the nucleus



FASSATI/PLOS

HIV RTC accumulation in the nucleus (top) fails without tRNA (bottom).

HIV rides the tRNA train to the nucleus, according to Lyubov Zaitseva, Richard Myers, and Ariberto Fassati (University College, London, UK).

For HIV to propagate inside cells, it must first enter the nucleus and, using its reverse transcription complex (RTC), integrate into the host genome. Fassati and his team previously showed that a nuclear import protein called importin 7 was partly responsible for HIV RTC transport to the nucleus.

The team has now set about an unbiased task to find other nuclear import factors. Through multiple cell fractionation steps, they identified an RNA component capable of supporting nuclear import of HIV RTC. Sequencing revealed that the component was tRNA. Sure that tRNAs—instrumental for cytoplasmic protein translation—could not be responsible, Fassati thought, "Oh no! It's all wrong, we've got to start all over again." Only after multiple repetitions was the team convinced that the tRNA was not merely a contaminant.

Virtually all the tRNAs isolated from the active fraction (the one capable of nuclear import) had defective 3′ ends and were thus incapable of supporting translation. Wild-type tRNAs, on the other hand, had very little nuclear import activity.

Fassati speculates that perhaps the defective tRNAs get shuttled back to the nucleus for repair or degradation. Nuclear import costs energy, but the expense is probably worthwhile to ensure undisturbed protein translation. Whatever the host cell's cost, HIV enjoys a free ride. JCB

Reference: Zaitseva, L., et al. 2006. *PLoS.* 4:1689–1706.

Ubp6 delays destruction

The destructive force of the proteasome is tempered by one of its own components, report John Hanna, Daniel Finley, and colleagues (Harvard Medical School, Boston, MA).

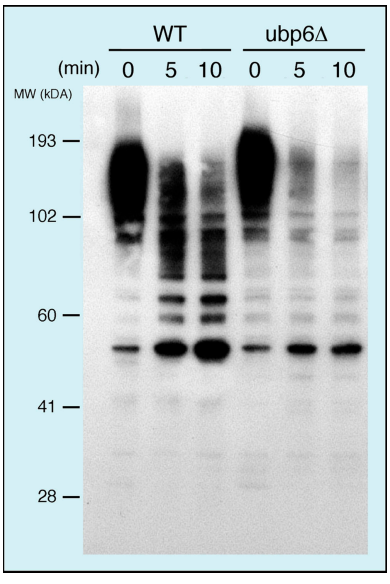
The traditional view of the proteasome was, says Finley, "like a pencil sharpener," mindlessly chewing away at ubiquitinated proteins. The characterization of one proteasome-associated factor now changes that view. Ubp6, the team shows, actually delays the rate of protein destruction. Proteasomes purified from yeast Ubp6 deletion mutants degraded ubiquitinated cyclin B protein faster than did those from their wild-type counterparts.

Ubp6 is a deubiquitinase, but this activity was not responsible for delaying degradation. Inhibiting its deubiquitinase active site did not speed up degradation. Ubp6 also needed to be bound to the proteasome to delay degradation, which would not be necessary if the delay tactic was simply to prevent targeting of proteins to the proteasome by removing ubiquitin moieties.

Instead, Ubp6 seems to delay degradation, at least in part, by inhibiting the action of a second proteasome component, called Rpn11. Rpn11 is itself a deubiquitinase whose activity is strictly coupled with degradation, unlike Ubp6.

Though Rpn11 inhibition might not be the sole cause for the degradation delay, it is clear that the proteasome is a more finely self-tuning machine than was first thought. JCB

Reference: Hanna, J., et al. 2006. *Cell.* 127:99–111.



FINLEY/ELSEVIER

With Ubp6, degradation of a ubiquitinated protein is delayed.

Point of no return for senescence

Senescent cells live on but can never again divide. This proliferative block is thought to safeguard against rampant oncogenic cell division. Akiko Takahashi, Eiji Hara, and colleagues (University of Tokushima, Japan) now show that, to enforce this irreversible stasis, cells switch on a self-perpetuating loop that suppresses cytokinesis.

Stable cell cycle arrest is induced by activating the retinoblastoma (RB) tumor suppressor. In senescent human cells, unlike nonsenescent cells, subsequent inactivation of RB leads to the reinitiation of DNA replication but no proliferation, suggesting that a second safety mechanism arrests senescent cells in G2 or M phase.

This second arrest only works in cells that are grown in mitogen-rich (and thus tumor friendly) conditions, the authors now find. The mitogens induce reactive oxygen species (ROS), which then start a self-perpetuating loop. The team observed that ROS remained high in senescent cells even after RB inactivation.

ROS, which are thought to induce senescence, are known to activate PKCδ, which studies suggest in turn activates ROS production. Consistent with this positive feedback, levels of PKCδ were also high in the permanently arrested cells.

High PKCδ, the team showed, suppressed the WARTS cytokinesis activator. Inhibiting PKCδ released this suppression and enabled senescent cells that managed to escape the early RB-induced block to proliferate. JCB

Reference: Takahasi, A., et al. 2006. *Nat. Cell Biol.* doi:10.1038/ncb1491.