Meeting Report

Holding on for life

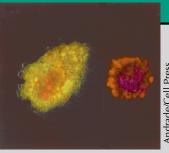
A denoviruses have co-opted one of their important structural proteins to inhibit apoptosis, according to recent results. The inhibition comes about because the protein binds its protease target so tightly that cleavage takes, on average, three hours.

Felipe Andrade, Antony Rosen (both at the Johns Hopkins University School of Medicine, Baltimore, MD) and colleagues found that adenovirus L4-100K protein is a specific inhibitor of granzyme B (GrB), an apoptosis-inducing protease produced by cytotoxic T and natural killer cells in response to viral infection. Other adenovirus pro-

teins interfere with apoptosis induced by the action of Fas ligand on immune cells, but no inhibitor of granzyme B activity was previously known.

Andrade reported that the rate of binding of 100K to GrB was diffusion-limited, and that the proteins bound with high affinity. But cleavage occurred so slowly that the half-life of the complex averaged 3 hours. If a critical 100K aspartate was mutated, 100K lost its ability to inhibit, and instead was cleaved at an alternative site.

As 100K is an important structural protein for the virus, it is present at significant molar excess over GrB. Andrade stated that, in a typical infected cell, this molar excess would ensure an average lag of 141 hours before any



A cell with 100K (green, at left), is protected from granzyme-mediated apoptosis (red blebs at right).

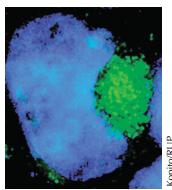
GrB activity was free to cleave other substrates. By this time the adenovirus will have replicated and departed from the cell.

Further reading: Andrade, F., et al. 2001. *Immunity*. 14:751–762.

Aggregation inhibits destruction

peposits of aggregated proteins have long been associated with many neurodegenerative diseases. But this correlation leaves unanswered the most important questions: is the aggregation a cause or an effect of the disease, and how might aggregates exert a toxic gain of function to cause disease?

Now Neil Bence, Ron Kopito and colleagues from Stanford University (Stanford, CA) have found that protein aggregation impairs the function of the ubiquitin–proteasome system (UPS). Reduced UPS-mediated degradation of certain key substrates could lead to cell-cycle abnormalities and cell death, and indeed Bence et al. find evidence for a cell-cycle delay in cells with aggregates.

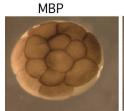


Aggresome (green) forming next to DNA (blue).

Their reporter for UPS function is an unstable form of GFP. Expression of aggregation-prone proteins leads to an increase in GFP reporter fluorescence, particularly in cells with large inclusions called aggresomes. In 1998, Kopito and colleagues discovered that cells collect large excesses of aggregated protein into aggresomes. These structures may sequester or jam UPS com-

ponents. The resulting reduction in UPS function would increase accumulation of unfolded proteins, initiating a positive-feedback loop and a rapid decline in cell viability.

Further reading: Bence, N., et al. 2001. Science. 292:1552-1555.



MBP-Emi1

Injection of Emi1 into frog cells (right) blocks mitosis.

Delaying degradation

An inhibitor of protein-destruction machinery may explain the delay between cyclin synthesis and degradation. The delay, say Julie Reimann and Peter Jackson of Stanford University School of Medicine (Stanford, California), is essential if mitosis is to occur correctly.

Mitosis has a nice symmetry: accumulation of active cyclin/cdk complexes initiates mitotic events, but the induced events include destruction of the cyclins themselves. Somehow a delay must be built in such that cyclin destruction is one of the last events to occur. The spindle checkpoint helps out—it delays cyclin destruction if there are chromosomes not yet attached to the spindle. But a similar inhibitor of cyclin destruction has not yet been identified for the earlier stages of mitosis.

Reimann, Jackson and colleagues believe that Emi1 is that inhibitor. Excess Emi1 prevents destruction of both mitotic cyclins (cyclin A and B), thus blocking cells in prometaphase. The block appears to occur via Emi1's binding of Cdc20, an activator of the machinery that destroys cyclins. Without Emi1 destruction isn't inhibited, so cells never accumulate cyclins and never enter mitosis.

Like cyclins, Emi1 is destroyed by ubiquitylation during mitosis, but its destruction occurs earlier and is controlled by a different activator. This destruction event may be dependent on the completion of certain prophase events.

Further reading: Reimann, J., et al. 2001. Cell. 105:645-655.

Combinatorial proteomics

In the final talk at the conference, Matthew Bogyo (University of California, San Francisco) described an exciting approach that can be used both to profile the activity of entire classes of proteases, and to identify specific inhibitors of individual proteases. Activity profiling is particularly important for proteases, which are often inactive until after the cleavage of a pro-peptide. This results in a poor correlation between mRNA or protein levels and activity levels.

Bogyo's starting point is a natural product, E-64, that binds to and covalently modifies the active site of cysteine proteases. Labeling of an E-64 variant allows Bogyo to visualize many active

cysteine proteases in gels. Bogyo has made a library of E-64 variants, and tested their ability to compete with the binding of the original labeled compound.

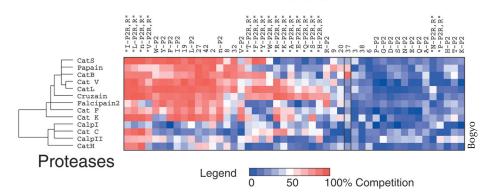
He expresses his results as a matrix with inhibitor structures arrayed along the x axis, and individual proteases arrayed along the y axis. Red denotes strong binding, and green weak binding. Thus columns with only one or a few red spots identify specific inhibitors. Bogyo then tests whether these specific inhibitors have phenotypic

effects on whole cells. If they do, the relevant protease can be purified by affinity purification using a tag on the inhibitor. The labeled specific inhibitor can also be used to detect the location of active enzyme within the cell.

Bogyo is using this new system to investigate a series of proteases belonging to the malaria parasite *Plasmodium falciparum*. "Malaria is such a great example [for this technique] because it is so difficult to manipulate genetically," he says. After isolating specific inhibitors of the proteases, Bogyo will test which proteases are necessary for different parts of the *Plasmodium* infection cycle.

Further reading: Greenbaum, D., et al. 2000. Chem. Biol. 7:569–581.

Inhibitors



An activity matrix for proteases (y axis) with different inhibitor substrates (x axis).

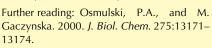
Wave of mutilation

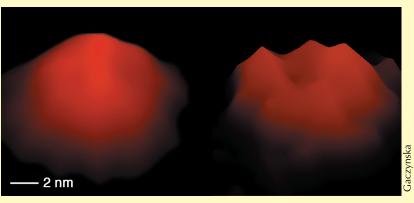
An organized wave of activity may underlie the complicated interactions of proteasome active sites, according to Maria Gaczynska of the University of Texas Health Science Center at San Antonio. She believes that the gate to the proteasome is a tuner that provides substrates at a specific, controllable frequency, such that the substrates can be cleaved in the most efficient way.

In Gaczynska's earlier work she used atomic force microscopy (AFM) to see direct evidence for a gating of proteasomes from closed to open states. Addition of substrates favored the open conformation, as long as the

relevant active site (trypsin-like, chymotrypsin-like, or caspase-like) was not mutated.

She now reports that a permanently open gate (resulting from the removal of the protein domain that normally forms the gate) increases the efficiency of each of the proteolytic activities, when the activities are measured individually. But coordination between the activities appears to be lost. Gaczynska has used individual site mutants and the addition of different substrates to derive a pattern of allosteric effects between the three types of sites, and she finds that this allosterism is disrupted when the gate is missing. She suggests that the gate normally feeds substrates into the proteasome at a specific frequency such that the substrate will always be processed along the active sites in an orderly wave of catalytic events.





AFM can detect closed (left) and open (right) proteasome conformations.