Christmas in July

First there was green fluorescent protein (GFP) from jellyfish, then came red fluorescent protein (DsRed) from coral. Now researchers from the University of California at Irvine show that DsRed may become equally indispensable for tracking cells, organelles, and fusion proteins by turning them... green?

Ian Parker says he and his colleagues made the discovery quite by accident while exploring the use of DsRed for multiphoton confocal imaging. Brief exposure to a femtosecond-pulsed laser beam turned DsRed’s fluorescence from red to green when viewed with a conventional one-photon microscope. The excitation, they explain, selectively bleaches the mature, red-emitting form of DsRed, which enhances emission from the immature green form.

The color change persists for hours or even days, and appears to do the cells no damage. Parker likens the technique to using a highlighter pen to mark important bits of text. The color change, he says, provides a powerful tool for picking out individual cells, fusion proteins, or even small regions of cells, by bleaching them green and leaving red everywhere else. Thus, the technique is suitable for studying cell lineages, organelle dynamics, and protein trafficking, as well as for selective retrieval of cells from a population.

Another method for labeling part of a cell has been described by Shuichi Takayama and colleagues (Harvard University, Cambridge, MA). They developed a chip that uses multiple laminar streams in a microfluidic channel; it can deliver membrane-permeable molecules to selected bits of a cell without causing major disruptions.

Keeping the lid on destruction

Proteins slated for destruction enter the proteasome core particle (CP) through a channel that usually remains closed for safety’s sake. It opens when a CP associates with the proteasome regulatory particle (RP), which recognizes the substrates and ushers them into the channel. What persuades the channel to open? A ring of six ATPases straddles the channel, and those enzymes have been thought to figure in this process. Now, Alwin Köhler and his colleagues in the laboratories of Daniel Finley and Alfred Goldberg (Harvard Medical School, Boston, MA) report, surprisingly, that just one of the six ATPases, Rpt2, is the key that unlocks the channel. In addition, the products of protein degradation exit the proteasome the same way that they came in, pointing to some possible traffic problems.

The paper also reports on one reason why the proteasome might want to keep tight control over gating. With the help of yeast CP mutants that stay open, the authors compared the products of the proteasome when it was kept in a closed or an open state. The median length of peptides produced by the mutant CP was 40% larger than those produced by the wild type. The results, Finley says, confirmed their suspicions that the size of products is determined by competition between their ongoing degradation and their exit from the internal chamber of the proteasome.

The size of degradation products is not an idle question, because in mammals such a product may be incorporated into a class I histocompatibility molecule to be presented to the immune system as a potential antigen. Therefore, proteasome efficiency is likely to be important for immune system function. That makes the state of the channel important too, and may explain why the immune system churns out channel-opening proteins as it is revving up, Finley points out. “That’s very consistent with our data, that you would want to open the channel” he says. “Because if the channel were closed and the peptides couldn’t get out efficiently, then they would be overdigested, and when overdigested they would no longer be competent to be presented to the immune system.”

Stay tuned. Finley says there are reasons to believe that there is a second mechanism for gating, which may be revealed by the use of full-length proteins rather than peptides in the in vitro proteasome assays.

Reference: Köhler, A., et al. 2001. Molecular Cell. 7:1143–1152.