WEB WATCH

• http://npd.hgu.mrc.ac.uk/

Centre of attention

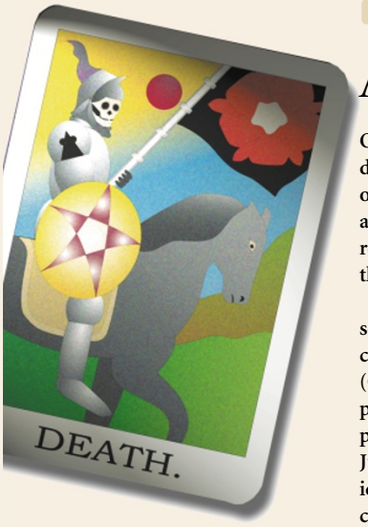
Proteins that localize to the nucleus are the centre of attention in the Nuclear Protein Database (NPD). This searchable database contains information on more than 1,000 vertebrate nuclear proteins, mainly from mice and humans.

The web site was an initiative undertaken by Wendy Bickmore's Group at the MRC-Human Genetics Unit, Edinburgh, United Kingdom, with the aim of making data available on new nuclear proteins. However, it soon became clear that such a database would be valuable to the entire community, so it was expanded to include published data on nuclear proteins. Knowledge of the subnuclear localization of proteins can be important in understanding the regulation and function of the genome, and can also provide clues to protein function.

You can search the entire database, or you can browse by subnuclear compartment or by domain/motif. The compartment browsing option takes you to a visually appealing, 'clickable' graphic of the nucleus, and gives you information on each compartment and the associated proteins. There are also useful links to nuclear structure and function resources, other nuclear protein databases and bioinformatics resources.

For each protein, the subnuclear compartment is reported, if known, together with information on the isoelectric point, protein size and sequence (including any repeats, motifs or domains). Protein functions are described using Gene Ontology™ terms, and links to other databases — for example, SwissProt and PubMed — are included where possible. In the future, the Bickmore Group would like to include more data from other groups, and hope to develop partnerships with other database projects.

Rachel Smallridge



APOPTOSIS

A marked CARD

One characteristic of apoptotic cell death is the extensive fragmentation of nuclear DNA, which depends on a DNase called CAD. According to a report in *Current Biology*, however, this might not be the whole story.

The twist in the tale began with a screen for proteins that contain the caspase-recruitment domain (CARD), which mediates protein–protein interactions in pro-apoptotic signalling pathways. Jürg Tschopp and colleagues identified a new protein that contains two amino-terminal CARD domains and a predicted helicase domain, hence its name — Helicard.

Transient transfection of 293T cells with Helicard led to the expression of full-length Helicard, but also to the appearance of a 45-kDa fragment. Then, when FasL was used to induce apoptosis in these cells, two further fragments were generated. The authors calculated that these processing events occurred in the region between the CARD and helicase domains.

Confocal microscopy showed that, on cleavage of Helicard in apoptotic cells, the CARD-containing fragment remains in the cytoplasm, whereas the helicase

APOPTOSIS

Getting rid of obstacles

The activation of caspases — which are essential effectors of apoptosis — is regulated by IAP ('inhibitor of apoptosis') proteins. In *Drosophila*, cell death and caspase activation require proteins of the Reaper, Hid, Grim and Sickle family (referred to here as RHG proteins). A series of reports published in *Nature Cell Biology* now sheds light on the relationship between these essential components of the apoptotic pathway.

The reports show that regulation of the levels of DIAP1 (*Drosophila* IAP1) is important for the initiation of apoptosis by RHG proteins *in vivo*. All six groups saw that RHG proteins reduce DIAP1 levels. In mammals, it has been reported that IAPs can be degraded by the proteasome after treatment with an apoptotic stimulus. Also, the RING-finger domain of IAPs has E3 ligase activity, which allows the polymerization of polyubiquitin chains on target proteins, and thereby targets them for degradation by the proteasome.

So, each group checked whether RHG proteins could induce ubiquitylation of DIAP1 and its degradation by the proteasome. They found that RHG proteins can indeed induce the autoubiquitylation of DIAP1 and its degradation in a proteasome-dependent manner. This requires the presence of the RING-finger domain of DIAP1, which is also essential for RHG-induced killing.

Ubiquitylation of a protein substrate requires three successive enzymatic reactions that are controlled by an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme and an E3 ubiquitin protein ligase. As mentioned before, DIAP1 has E3-ligase activity, and Hermann Steller's group identified an E2 enzyme, UBCD1, that is required for Reaper- and Grim-induced DIAP1 ubiquitylation and degradation, and for Reaper- and Grim-induced killing. The groups of Ross Cagan and John Nambu identified another protein, Morgue, that is required for the ubiquitin-conjugating reaction. Morgue is related to E2 enzymes, yet lacks the consensus active site, so it could work in association with another E2 enzyme. John Nambu's lab provides preliminary evidence that it might bind SkpA, a component of the SCF–E3 ubiquitin ligase complex.

Sally Kornbluth and Bruce Hay made another interesting observation: they saw that RHG proteins could reduce levels of general protein translation, which would contribute further to reducing DIAP1 levels. And finally, Pascal Meier's lab found that DIAP1 also induces the ubiquitylation of the *Drosophila* caspase Dronc and

domain translocates to the nucleus. Tschopp and colleagues then showed that supernatants that contain recombinant Helicard sensitized non-apoptotic extracts to DNA fragmentation.

Although Helicard has no intrinsic DNase activity, the authors speculate that it might facilitate CAD-mediated cleavage. It probably does this through its helicase activity, which opens up the DNA to allow easier access for CAD — a clever twist indeed.

Alison Mitchell

References and links

ORIGINAL RESEARCH PAPER Kovacsovics, M. *et al*. Overexpression of Helicard, a CARD-containing helicase cleaved during apoptosis, accelerates DNA degradation. *Curr. Biol*. **12**, 838–843 (2002)

its inactivation. It will be interesting to establish how DIAP1-mediated ubiquitylation of Dronc leads to its inactivation, as the authors have preliminary evidence is that it does not occur by proteasome degradation.

These findings put RHG protein signalling into a new perspective, but they also raise many questions about the link between Morgue and E3 ligase complexes, and about the mechanism by which RHG proteins regulate translation.

Valerie Ferrier, Associate Editor,
Nature Cell Biology

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ORIGINAL RESEARCH PAPERS Yoo, S. J. *et al.* Hid, Rpr and Grim negatively regulate DIAP1 levels through distinct mechanisms. *Nature Cell Biol.* **4**, 416–424 (2002) | Hays, R., Wickline, L. & Cagan, R. Morgue mediates apoptosis in the *Drosophila melanogaster* retina by promoting degradation of DIAP1. *Nature Cell Biol.* **4**, 425–431 (2002) | Ryoo, H. D., Bergmann, A., Gonen, H., Ciechanover, A. & Steller, H. Regulation of *Drosophila* IAP1 degradation and apoptosis by *reaper* and *ubcD1*. *Nature Cell Biol.* **4**, 432–438 (2002) | Holley, C. L., Olson, M. R., Colón-Ramos, D. A. & Kornbluth, S. Reaper eliminates IAP proteins through stimulated IAP degradation and generalized translational inhibition. *Nature Cell Biol.* **4**, 439–444 (2002) | Wilson, R. *et al.* The DIAP1 RING finger mediates ubiquitination of Dronc and is indispensable for regulating apoptosis. *Nature Cell Biol.* **4**, 445–450 (2002) | Wing, J. P. *et al. Drosophila* Morgue is an F box/ubiquitin conjugase domain protein important for *grim-reaper* mediated apoptosis. *Nature Cell Biol.* **4**, 451–456 (2002)

WEB SITES

Hermann Steller's laboratory: http://www.rockefeller.edu/labheads/steller/steller.html

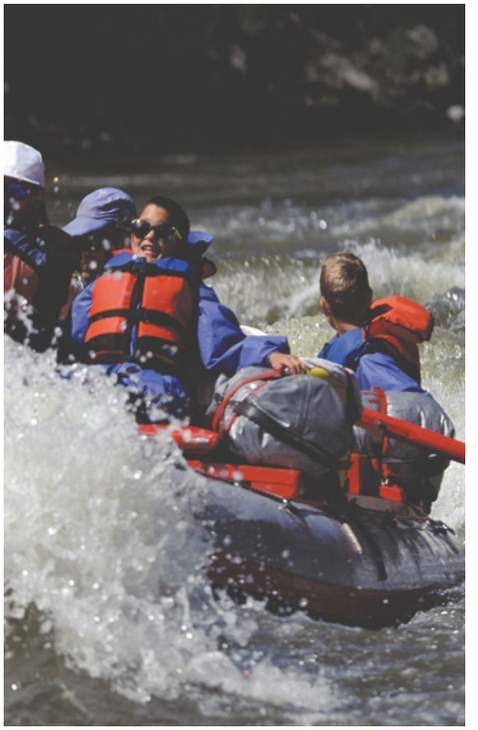
Ross Cagan's laboratory: http://molecool.wustl.edu/caganlab.html

Sally Kornbluth's laboratory: http://cmb.duke.edu/faculty/kornbluth.html

Bruce Hay's laboratory: http://www.its.caltech.edu/~biology/brochure/faculty/hay.html

MEMBRANE DYNAMICS

Destination lipid rafts



The cell is a mêlée of reactions, so dividing some of these reactions into specific subcompartments should help increase their specificity and efficiency. One such compartment is the plasma membrane, which is further subdivided into lipid rafts and caveolae — microdomains that are enriched in cholesterol, sphingolipids and signalling proteins. But how do proteins reach these destinations? According to a report in *Science* by Roger Tsien's group, acylation is sufficient to preferentially localize fluorescent test proteins to lipid rafts.

Their studies involved the fluorescence resonance energy transfer (FRET) technique using the cyan and yellow fluorescent protein (CFP and YFP, respectively) variants of green fluorescent protein (GFP). As these proteins lack endogenous targeting sequences, the aim was to study the effect of adding consensus sequences for acylation — the addition of myristoyl and/or palmitoyl moieties — or prenylation — the addition of farnesyl or geranylgeranyl moieties — on the localization of these proteins in membrane microdomains of live cells. Individual microdomains are so small that they are beyond the resolution of standard light microscopy, so the authors relied on the greater spatial resolution that is provided by FRET to determine which lipid-modified fluorescent proteins were selectively sequestered within them. The hallmark of this clustering is that there is an association — as measured by FRET — even when very little of the protein is expressed on the plasma membrane.

Initially, Tsien and colleagues used the acylation substrate sequence from the kinase Lyn to promote the attachment of myristoyl and palmitoyl chains to the amino termini of CFP and YFP. The FRET efficiencies were high, which indicates that the proteins were close to each other and therefore, they presumed, in the microdomains. But as treatment with 5-methyl-β-cyclodextrin (MβCD) — a compound that depletes cholesterol and therefore disrupts lipid rafts and caveolae — did not reduce the interactions, they suspected that the fluorophores were themselves dimerizing, and giving a false impression of clustering. So, they changed the hydrophobic residues that are found at the dimerization interface of CFP and YFP to positively charged residues, which prevented dimer formation.

Adding the amino terminus of Lyn and expressing these 'monomeric' (m) variants in cells resulted in increased FRET efficiency that, this time, was destroyed by MβCD, which indicates that the clustering can be attributed to the localization of these proteins in microdomains. Furthermore, the authors showed that acylation promoted the association of both of the monomeric variants with caveolin, a marker for caveolae.

By contrast, geranylgeranylated versions of mCFP or mYFP — studied by fusing the CAAX box of Rho to their carboxyl termini — clustered with themselves, but not with acylated versions or with caveolin, which indicates that prenylation induces clustering, but not into the same membrane microdomain as the acylated fluorescent proteins or caveolae.

To confirm these findings, the authors reverted to traditional biochemical fractionation assays using the mYFP variant. Myristoylated and palmitoylated YFP separated mainly into detergent-soluble fractions with low-density caveolae-rich membranes — in the same manner as endogenous caveolin — which indicates that it had separated with caveolae. Geranylgeranylated YFP, on the other hand, was excluded from these fractions. Proof, then, that acylation, but not prenylation, will target you to a lipid raft!

Katrin Bussell

References and links

ORIGINAL RESEARCH PAPER Zacharias, D. A., Violin, J. D., Newton, A. C. & Tsien, R. Y. Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells. *Science* **296**, 913–916 (2002)

FURTHER READING Simons, K. & Toomre, D. Lipid rafts and signal transduction. *Nature Rev. Mol. Cell Biol.* **1**, 31–39 (2000) | Lippincott-Schwartz, J. *et al.* Studying protein dynamics in living cells. *Nature Rev. Mol. Cell Biol.* **2**, 444–456 (2001)

WEB SITES

Encyclopedia of Life Sciences: http://www.els.net
Green fluorescent protein

Roger Tsien's laboratory: http://www.tsienlab.ucsd.edu