PROTEIN DEGRADATION

The workings of a machine

Protease-linked AAA+ proteins ('ATPases associated with various cellular activities' proteins) unfold substrate proteins and deliver them to their associated proteases for degradation. For example, the bacterial hexameric ring-shaped AAA+ machine ClpX catalyses the denaturation and translocation of substrates into the protease ClpP in a process that requires hundreds of cycles of ATP hydrolysis. But how exactly do AAA+ machines work? Martin, Baker and Sauer provide new insights in *Nature*.

They covalently linked different combinations of active and inactive subunits of ClpX to form hexamers, and studied how these variants affected the ClpP-mediated degradation of a denatured substrate. Their first conclusion was that models in which all six ClpX subunits bind and hydrolyse ATP in a concerted fashion cannot be correct, because a ClpX variant with alternating active and inactive subunits had an activity that was roughly proportional to the number of active subunits.

They then looked at asymmetrical hexamer variants of ClpX that contained three, four or five active subunits positioned consecutively. As before, these variants had ATPase activities and produced protein-degradation activities that were proportional to the number of active subunits. A symmetrical arrangement of active subunits is therefore not required for ClpX function.

Significantly, a ClpX variant containing a single active subunit could also drive ClpP-mediated protein degradation. Conformational changes in just one ClpX subunit that are the result of ATP binding and hydrolysis therefore represent the basic power stroke of ClpX and are sufficient to drive ClpP-dependent protein degradation.

Next, the authors showed that the ClpX variants could power the ClpP-mediated degradation of native substrates, and that ATP hydrolysis in just a single active subunit of a ClpX hexamer could drive substrate unfolding as well as translocation.

To conclude, Martin and colleagues have shown that diverse geometric arrangements of ClpX subunits can support substrate

FERTILITY

Food for life

Infertility is a growing concern for a large segment of the population. Many cases are attributable to a loss of oocyte competence — a gradual process that occurs as a woman ages. Although it is well known that oocytes are depleted by apoptosis, the molecular pathways that determine the timing of the death of these cells are not fully understood. Nutt and colleagues, reporting in *Cell*, now show a novel link between a specific oocyte metabolic pathway and the calcium/calmodulin-dependent protein kinase II (CaMKII)-mediated regulation of caspase-2, an essential cell-death effector protease.

The authors proposed that nutrient stores in the oocyte might contribute to cell survival by modulating metabolic pathways. To test this theory, they added glucose-6-phosphate (G6P) — which is used for glycogen deposition, or is metabolized through the pentose-phosphate pathway — directly to *Xenopus laevis* egg extracts *in vitro*, and found that apoptosis is inhibited, and that the block occurs either at the level, or upstream, of the mitochondria. Because they found that stimulation of the pentose-phosphate pathway or NADPH (nicotinamide-adenine dinucleotide phosphate (reduced)) could substitute for G6P to inhibit apoptosis in the egg extracts, Nutt *et al*. reasoned that it is in fact the generation of NADPH — a key metabolic by-product of the pentose-phosphate pathway — that transmits the anti-apoptotic signal. The authors also showed that inhibition of the pentose-phosphate pathway rapidly promotes the demise of *X. laevis* oocytes, highlighting the importance of this pathway in oocyte viability.

Having identified intermediates of the oocyte metabolic pathway as apoptosis inhibitors, the authors next set out to find the target of this inhibition. They focused on caspase-2, which is known to be an important constituent of oocyte apoptosis pathways. As well as confirming that caspase-2 is indeed a target for NADPH-mediated blocking of programmed cell death, they showed that this inhibition can be effected directly at the level of caspase-2 activation, rather than at an upstream signalling component.

Although the mechanism for caspase-2 activation in oocytes is unclear, Nutt and colleagues proposed that it might occur by the binding of an endogenous adaptor protein to the caspase-2 prodomain. It followed, therefore, that phosphorylation of this prodomain could block access of the adaptor, thereby preventing activation and subsequent cell death. The authors identified CaMKII as the phosphorylating kinase, and subsequently showed that G6P and NADPH promote caspase-2 phosphorylation by CaMKII, promoting cell survival. Accordingly, abrogation of caspase-2 phosphorylation prevents oocyte survival mediated by the pentose-phosphate-pathway metabolites.

The authors concede that there could be other pathways involved in inhibiting oocyte apoptosis. They have, however, succeeded in identifying a novel pathway that links oocyte metabolism to caspase-2 through the action of CaMKII. Their work might provide necessary clues as to why oocyte apoptotic death occurs as females age, and could have important therapeutic potential for infertility treatment.

Sharon Ahmad

References and links

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unfolding and translocation into ClpP for degradation. It seems that the power stroke is generated by ATP hydrolysis in a single ClpX subunit, and the results obtained here rule out a concerted model and a strict sequential model for ATP hydrolysis by ClpX. Instead, these data indicate a probabilistic sequence of nucleotide hydrolysis. This mechanism would allow any ClpX subunit that contacts a translocating polypeptide to hydrolyse ATP to drive the substrate into ClpP. It would also prevent substrate stalling if a particular subunit was unsuccessful in binding or hydrolysing ATP. Such a probabilistic mechanism might be especially important for molecular machines that function on diverse substrates.

Rachel Smallridge

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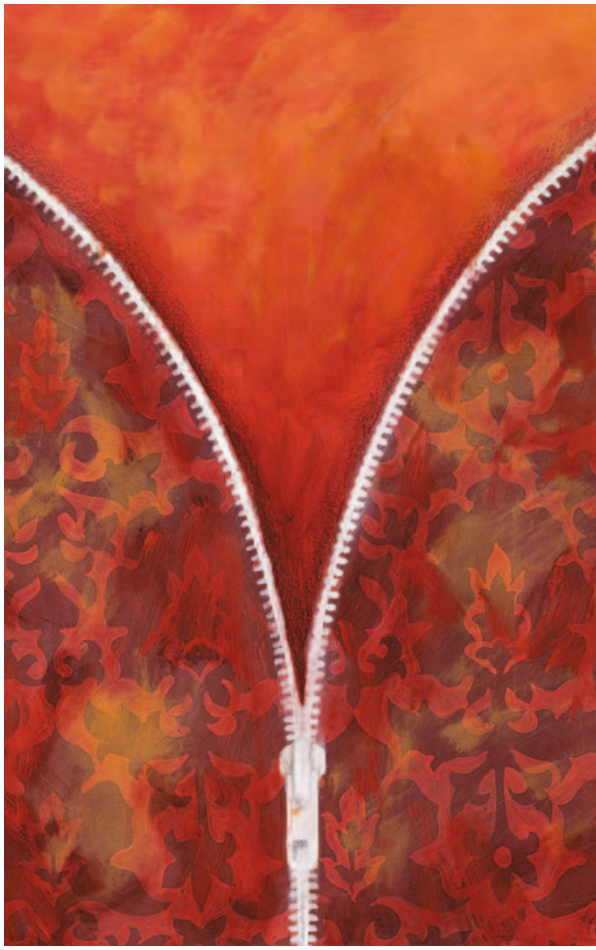
WEB SITE
Bob Sauer's laboratory: http://web.mit.edu/biology/www/facultyareas/facresearch/sauer.shtml

CELL SIGNALLING

Divided divisions



The activation of heterotrimeric G-protein signalling is essential during asymmetric cell division because this signalling is required for correct spindle position and/or orientation. An important component of this cascade, RIC-8 has been studied in *Caenorhabditis elegans* and mammals. Using *Drosophila melanogaster,* three reports now show that RIC-8 is essential for plasma-membrane localization of G-protein subunits and provide further insights into the RIC-8-mediated regulation of heterotrimeric G-protein signalling.

Heterotrimeric G proteins are composed of non-identical α, β and γ subunits. In most cases, when a Gα subunit binds GDP, it forms a complex with a Gβ and a Gγ subunit, and it functionally dissociates from the Gβγ complex when it binds GTP. The pathway is activated by two types of Gα-binding proteins, GDP-dissociation inhibitors (GDI) and RIC-8, which is thought to be a non-receptor guanine nucleotide-exchange factor (GEF) for Gα.

After studies in *C. elegans* and mammals, three different groups set out to find the role of RIC-8 in *D. melanogaster*. Wang *et al.* and Hampoelz *et al.* both investigated the role of RIC-8 in the asymmetric division of the neuroblast, whereas David and colleagues studied RIC-8 in sensory-organ precursor cells. All three groups show that *ric-8* mutants have several defects — asymmetric localization of cell-fate determinants is not maintained, mitotic spindles are misorientated and the sizes of the two daughter cells become almost equal. These observations indicate that RIC-8 is essential for proper spindle orientation and for controlling daughter-cell size. RIC-8 was also found to be important for gastrulation, a process that is known to rely on G-protein signalling and requires the highly coordinated movement of cells.

In the absence of RIC-8, the G-protein subunits, $G\alpha_i$ and $G\beta_{13F}$, seem to be cytoplasmic, which makes *ric-8* mutants an attractive model in which to study the regulation of heterotrimeric G-protein signalling during asymmetric cell division. Hampoelz and colleagues found that the *ric-8* mutant phenotype resembled *Gβ* but was different from the one described for *Gα* mutants. By contrast, Wang *et al.* showed that both the *ric-8* mutant and the *ric-8 Gβ* double mutant exhibited similar phenotypes to *Gα* mutants in terms of their daughter-cell size phenotype, and concluded that Gα–GDI functions downstream of Gβγ. Even more intriguing, Hampoelz and colleagues also showed that RIC-8 does not function as a GEF for Gα, and that RIC-8 binds both the GDP and GTP forms of Gα — a result that contradicts previous data from *C. elegans.* Whether the observed differences reveal a new function for RIC-8, or whether they are due to mutant variations or limitations of the experimental approaches, remains to be seen.

However, these studies represent important steps in understanding how asymmetric cell division is regulated in *D. melanogaster*, as they all indicate that RIC-8 is required for plasma-membrane localization of G proteins — a much more general role for this protein compared to that originally proposed in *C. elegans*. Although these findings will not end the debate, they are important pieces in the complicated puzzle of spindle position and orientation.

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