APOPTOSIS

Fragments of death

The study of apoptosis is synonymous with the nematode *Caenorhabditis elegans*, as many of the important molecular components in this conserved biological process have been identified through genetic studies in this model organism. Now, reporting in *Nature*, Barbara Conradt and co-workers show that mitochondrial fragmentation — previously thought to be an exclusive characteristic of apoptotic mammalian cells — is also a feature of apoptosis in *C. elegans*. And by mutating the tractable genome of this useful organism, the researchers explain how mitochondrial break-up might contribute to the apoptotic process.

Although mitochondria had not previously been shown to have a crucial role in apoptosis in nematodes, it seemed unlikely that this organelle — such an enthusiastic participant in apoptosis in mammals — would be a mere 'innocent bystander' in the *C. elegans* cell-death programme. So Conradt and her team examined live *C. elegans* embryos using time-lapse confocal microscopy and noted the morphology of mitochondria in cells that were undergoing programmed cell death. They observed that, in non-apoptotic cells, mitochondria formed a cohesive network of tubules. However, soon after the induction of apoptosis, the network started to break up until only mitochondrial fragments remained in the cell.

But is the fragmentation of mitochondria a causal event in apoptosis, or simply a morphological phenomenon in a dying cell? Conradt and co-workers tackled this question by expressing a dominant-negative mutant of the mitochondrial-fission-promoting protein DRP-1 in *C. elegans* embryos, thereby inhibiting mitochondrial break-up. This inhibition of mitochondrial fragmentation prevented apoptosis in ~20% of cells in these transgenic animals. Importantly, the overexpression of wild-type *drp-1* during embryogenesis increased mitochondrial disintegration and also increased the number of cells that died by apoptosis.

Using loss-of-function and gain-of-function mutant embryos, the

DEVELOPMENT

Youth is overrated

It has long been thought that regenerating cells undergo a rejuvenation process to achieve pluripotency. However, a new study in *Cell* by Sustar and Schubiger challenges this view by showing that regenerating cells in *Drosophila melanogaster* imaginal discs do not revert to a 'younger', faster cell cycle — but instead have a unique cell cycle profile with characteristics of both younger and older cells.

Imaginal discs are small groups of epithelial cells that become determined late in larval development to form specific structures, such as wings and legs. The process of transdetermination in *D. melanogaster* imaginal discs is a well-characterized model system for cell plasticity. Transdetermination can be induced by disc injury ('disc fragmentation') or activation of the Wnt-family *wingless* (*wg*) gene, which causes a change in cell fate — for example, from leg to wing — of a small subset of regenerating cells in a region of the disc known as the 'weak point'.

To find out whether regenerating cells in fragmented imaginal discs rejuvenate, Sustar and Schubiger analysed their cell cycle profile and doubling time. The cells did not revert to a 'younger' cell cycle with a shorter doubling time, and maintained a profile that was similar to that of unfragmented disc cells of the same age.

Induction of the Wg signalling pathway activates the enhancer of the *vestigial* (*vg*) selector gene, which is necessary for wing development and the induction of leg-to-wing transdetermination. To visualize leg-to-wing transdetermination, the authors used the *vg* regulatory element to make a fluorescent reporter construct. Following Wg overexpression, disc cells initially divided asynchronously, but after ~2 days, cells in S phase were exclusively localized in the weak point region. This change in the cell cycle occurred before the reporter gene was visibly expressed and, therefore, before transdetermination. In the early phase of transdetermination, a greater proportion of transdetermining cells were in S phase compared with non-transdetermining cells and later, the cell cycle profile reverted to that of non-transdetermining cells. Strikingly, the cell cycle profile in the early phase of transdetermination was unique and did not match that of any specific developmental stage.

So, Wg induction seems to trigger an alteration in the cell cycle of transdetermining cells in the weak point. In addition, these cells are initially considerably larger than non-regenerating cells. So, might cell cycle induction or the activation of cell growth be sufficient to induce transdetermination? Sustar and Schubiger overexpressed several cell cycle and cell growth genes. They found that overexpression of the insulin receptor gene or *Ras*, but not other genes, mimicked the effects of Wg induction. The authors propose, however, that transdetermination probably requires more than a growth signal, and they suggest that Wg expression in the weak point has multiple functions — growth activation and an increase in developmental plasticity.

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authors deciphered which of the molecular components of the apoptotic pathway affected the break-up of mitochondria, and they incorporate mitochondrial disruption into a new working model of apoptosis in *C. elegans*. They propose that, in cells that are destined to die, the pro-apoptotic protein EGL-1 binds to, and activates, CED-9. CED-9 then activates DRP-1, which mediates mitochondrial disruption. How exactly mitochondrial fragmentation contributes to cell death in *C. elegans* remains unclear, but the authors suggest that, as these organelles fragment, a pro-apoptotic molecule might be liberated that potentiates the activity of the adaptor protein CED-4. CED-4 activates the caspase CED-3, and the enhanced activity of this enzyme results in efficient programmed cell death.

Shannon Amoils

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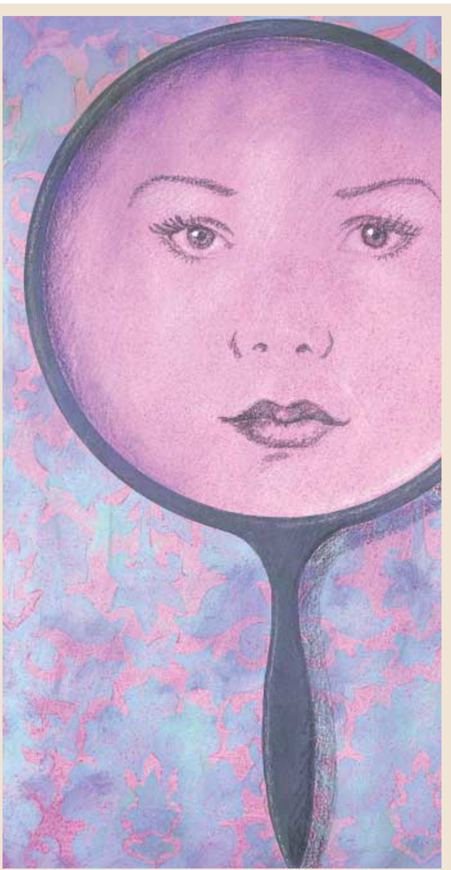

MEMBRANE TRAFFICKING

Controlling the flow

There are various hypotheses regarding the flow of material within the Golgi stack. For example, some researchers believe that anterograde movement occurs through the maturation of the Golgi cisternae and that retrograde transport is mediated by coatomer protein complex-I (COPI) vesicles, whereas others believe that vesicles mediate both anterograde and retrograde transport. However, the various hypotheses are not mutually exclusive, but they do require the strict control of the flow pattern of COPI vesicles. So, how might COPI vesicles be targeted to particular cisternae?

Golgin-family proteins have been proposed to be involved in intra-Golgi transport by functioning as tethers for COPI vesicles, and the well-characterized golgins giantin and GM130 link COPI vesicles to p115 on the *cis*-Golgi network (CGN). In *Science*, Warren and colleagues now describe the characterization of the golgin-84–CASP tether, and their results indicate that subpopulations of COPI vesicles are defined by their golgin tethers.

First, they showed that the golgin-84–CASP tether is asymmetric — that is, golgin-84 was found on COPI vesicles, whereas CASP was located in Golgi membranes. Next, they determined the protein composition of the COPI vesicles that bind CASP. Surprisingly, they lacked any p24-family members, proteins that are thought to be involved in COPI-vesicle biogenesis. Other proteins must therefore nucleate the budding of COPI vesicles that use the golgin-84–CASP tether. Interestingly, the vesicles were enriched for mannosidase-I and -II, which reside in *cis* and *medial* cisternae, but not for a representative cargo protein destined for the plasma membrane. These data therefore indicate that the vesicles are involved in retrograde transport.

Warren and co-workers then looked at the protein composition of COPI vesicles that bind p115. They found that they contained all of the p24-family members and a representative cargo protein, but were depleted of mannosidase-I and -II. This indicates that these vesicles bud from the CGN, where p24 proteins are localized, and move in an anterograde direction. However, this movement must be limited to the early part of the Golgi stack (for example, from the CGN to the *cis*-cisternae), because p115 is limited to these membranes.

Finally, the authors confirmed the role of the golgin-84–CASP tether in retrograde transport by studying the cycling of a Golgi enzyme through the endoplasmic reticulum (ER). They inhibited the export of a labelled Golgi enzyme from the ER, and observed that it was gradually relocated to the ER. The injection of soluble golgin-84 or CASP during this time substantially inhibited this retrograde movement (agents that disrupted the p115 tether had only a modest effect). However, they also showed that soluble golgin-84 or CASP did not affect the retrograde movement of COPI vesicles (carrying a recycling protein, ERGIC53) from the CGN to the ER.

It therefore seems that the retrograde movement of CASP-binding COPI vesicles occurs within the Golgi, and not from the Golgi to the ER. A two-step retrograde pathway — "…the first step mediated by intra-Golgi COPI transport to the CGN, the second by a COPI-independent pathway to the ER" — might explain why earlier work did not identify a role for COPI vesicles in the retrograde transport of Golgi enzymes to the ER despite the presence of such enzymes in these vesicles. Furthermore, this study has shown that golgin tethers define subpopulations of COPI vesicles, and this information will help us to further our understanding of the intra-Golgi flow of material in the future.

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ORIGINAL RESEARCH PAPER Malsam, J. *et al.* Golgin tethers define subpopulations of COPI vesicles. *Science* **307**, 1095–1098 (2005)

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