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Nucleus 'hallowed ground' no more

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Autophagy, from the Greek for 'self-eating,' was recently observed in a realm of the cell that scientists once considered off-limits for this process: the nucleus. A recent study by David Goldfarb and co-workers at the University of Rochester, New York (http://www.rochester.edu) showed that even this command centre of the cell is not safe from digestion in the vacuole, the degratory compartment of yeast cells [1]. As astonishing as this is, however, the work also takes a close look at how cells actually 'nibble off the little bites', finding hints of a regulated and finely tuned process, termed piecemeal microautophagy of the nucleus (PMN).

Autophagy versus PMN

Autophagy is the intracellular version of phagocytosis, in which outside materials are brought into the cell to be 'digested'. In autophagy [2,3], the targets are 'huge bites of cytoplasm, even whole organelles,' that are delivered to the vacuole to be degraded by that acidified compartment's array of hydrolytic enzymes, in what Goldfarb, Professor of Biology at Rochester, calls 'a mass slaughter of cytoplasm'.

By contrast, PMN appears to be more elegant: it occurs at specific velcro-like junctions between the vacuole and the nucleus. This leads to bulging of the nucleus into the vacuole and then 'blebbing', with a scission event that releases the new intravacuolar vesicle. Goldfarb suggests imagining sticking your thumb – a piece of nucleus – into a blown-up balloon – the vacuole – from the side, and then having it pinch off inside.

Interorganellar connections

These nuclear–vacuolar junctions are cause for excitement in themselves – they represent the first well-defined interorganellar connections, formed between the vacuolar protein Vac8 and its nuclear counterpart Nvj1 (see Ref. [4]). Although Vac8 has other defined roles, this appears to be the only known role for Nvj1. Such specialization suggests a role in cell regulation, aside from simply providing more nourishment. Although it is primarily seen in starvation conditions [5], Goldfarb says autophagy can be 'used for any reason you can think of to recycle parts of the cell,' and is 'really a set or family of pathways that deliver cargo to the lysozomes'. What those specific reasons are in the process of PMN he can only guess at, but ongoing studies of PMN in apoptosis might provide clues to its purpose and answers to other questions.

The sacred nucleus

Before now, there has been 'no indication that the nucleus is subject to degradation in the vacuole,' says Daniel Klionsky, Professor of Molecular, Cellular and Developmental Biology at the University of Michigan (http://www.umich.edu). It is no wonder then that 'people were reluctant to believe the nucleus could be degraded. We think of it as a sacred organelle… of course you're not going to be degrading that, but [in this case] you clearly are'. After all, autophagy often does away with whole organelles, notes Goldfarb, and you can not lose the nucleus. This, says Klionsky, presented Goldfarb with 'an extra burden to demonstrate this is a true process'. This was accomplished 'not just by looking in a microscope and counting,' but also by quantification with a biochemical assay, says Goldfarb. The assay was a 'pulse-chase' experiment and showed that microautophagy is not dependent on the same proteins that are involved in macroautophagy but is a distinct process that depends on the Vac8 and Nvj1 proteins.

The nucleus is certainly hallowed ground in that it contains the most vital material to the survival of the cell – chromatin – yet this crucial material is neatly excluded from PMN vesicles. How does the cell know which part of the nucleus to send off for breakdown? That remains to be seen, and is one of the mysteries also still held by macroautophagy. Another question, says Klionsky, is the source of the membrane that engulfs cargo for delivery to the vacuole. Of that, 'people have speculated about virtually every known membrane in the cell.' In any case, a double layer of membrane forms around the doomed material, which adds surface area to the vacuolar membrane after fusion releases the material into the vacuole. The vesicle scission of PMN, in contrast, results in a loss of vacuolar membrane. This, speculates Klionsky, could end up being 'the raison d'être of microautophagy'. He hints at the possibility of a regulatory function, saying 'you have to protect that vacuolar membrane'. If the destructive environment of the vacuole were turned loose, it would wreak havoc on the cell.

Biodegradation and biogenesis

Although macroautophagy is universal among eukaryotic cells, a feature of yeast cells could make PMN unique to them: a closed mitosis. The nuclear envelope never breaks down; therefore, the nucleus undergoes a scission during

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cell division that is much like the blebbing of PMN. Goldfarb notes that, although homologous genes have not been identified, 'it doesn't mean an autologous process does not occur' in mammalian cells. A similar blebbing event has been observed in Bloom's disease – a genetic disease that results from a mutation in a DNA helicase gene – in which 'nuclear microvesicles' are released into the cytoplasm [6]. Regardless, Klionsky finds the study of biodegradation 'fascinating overall'. Although most research has focused on biogenesis, he says 'there is always this homeostasis. You have to have both [processes], you can't have just one'.

Although the research undertaken here might not lead to the development of novel therapeutic agents, it brings us a step closer to understanding the ways in which eukaryotic cells recycle materials in specific and refined ways.

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Scientists expand the genetic code

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Ever wonder whether mankind would be better off with a genetic code that uses 21 amino acids, one more than the standard 20, to make proteins? It soon might be possible to find out. Scientists at The Scripps Research Institute (http://www.scripps.edu/) have created a bacterium that synthesizes an unnatural amino acid, *p*-aminophenylalanine (*p*AF), and incorporates it into proteins with a fidelity and efficiency rivaling that of the 20 natural amino acids [1].

'They've really redesigned *Escherichia coli*,' said Michael Ibba, Assistant Professor of Microbiology at The Ohio State University (http://www.osu.edu/). 'They chose what amino acid to add to the genetic code of this organism and they gave the organism the machinery to make the amino acid and use it.'

Although there is nothing special about *p*AF, a known synthetic amino acid with a structure similar to tyrosine, this study demonstrates the potential to make changes that are more interesting, both scientifically and therapeutically. 'The nice thing about their approach is that it's modular,' said Virginia Cornish, Assistant Professor of Chemistry at Columbia University (http://www.columbia.edu/). 'While they can only have 21 total amino acids, what that 21st amino acid is they can vary fairly easily.'

A complete system

The challenge facing the Scripps scientists was to put all the pieces necessary for the biosynthesis of *p*AF and its incorporation into protein in *E. coli* and ensure they worked together. 'In order to go from 20 to 21 amino acids, we had to add a biosynthetic pathway, add a synthetase, add a tRNA and add a codon for that new amino acid,' said Ryan A. Mehl, now an Assistant Professor of Chemistry at Franklin & Marshall College (http://www.fandm.edu/). 'We stole the machinery from other organisms and modified it.'

First, a biosynthetic pathway that enabled *E. coli* to make *p*AF from simple carbon sources was needed. Three genes from *Streptomyces venezuelae*, which lead to the production of *p*-aminophenylpyruvic acid as an intermediate metabolite in the conversion of chorismate to chloramphenicol, were introduced into *E. coli* (Fig. 1). A native *E. coli* enzyme, aromatic aminotransferase, completes the biosynthesis to *p*AF.

To incorporate the resulting 21st amino acid into protein, a tRNA–synthetase pair from *Methanococcus jannaschii* was introduced into the same bacterium. The tRNA was chosen because of its specificity for TAG, a nonsense codon that normally tells the cell machinery to stop making protein. However, in this system, the synthetase was altered to recognize *p*AF and load it onto the tRNA. Because of that modification, the unnatural amino acid is incorporated into protein in response to the TAG stop codon.

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