Sporoptosis: Sowing the Seeds of Nuclear Destruction

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What are the origins of programmed cell death (PCD)? In this issue of *Developmental Cell*, Eastwood et al. (2012) uncover an ancient developmental program of nuclear destruction in yeast, implying that some PCD mechanisms could have emerged from nonlethal processes before the divergence of fungi and metazoan.

The ability to "commit suicide" in response to various developmental signals and damage-induced stresses, a process often called programmed cell death (PCD), is a fundamental genetic trait of virtually every metazoan cell and is essential for the wellbeing of the organism (Fuchs and Steller, 2011). Furthermore, ample evidence exists that under certain (usually stressful) conditions, unicellular organisms can also trigger a cell death program, which is clearly counterproductive for an individual microbe but which might be advantageous for a whole-cell population (Engelberg-Kulka et al., 2006; Carmona-Gutierrez et al., 2010). An intriguing question is how such allegedly "altruistic" conduct of an individual cell has evolved, because it opposes the basic propensity of cells to survive. One logical hypothesis is that prospective cell death mechanisms were co-opted from basic cellular processes originally unrelated to cell death. In this issue of *Developmental Cell*, Meneghini and colleagues describe a developmental event of programmed nuclear destruction (PND) in yeast, which may represent a "living fossil" of a prominent PCD module (Eastwood et al., 2012).

Apoptosis is a major, morphologically distinct, form of PCD in metazoans, executed by unique cysteine proteases called caspases (Fuchs and Steller, 2011). Upon induction of apoptosis, caspases are activated in a proteolytic cascade and, in turn, cleave hundreds of cellular proteins, bringing about the biochemical and morphological changes that culminate in cell death (Salvesen and Riedl, 2008). These highly conserved changes include shrinkage and fragmentation of cells and their nuclei, cytoskeletal rearrangement, cell membrane disruption and blebbing, and extensive degradation of chromosomal DNA (Fuchs and Steller, 2011). However, several studies have reported similar phenomena during other vital cellular processes, such as the enucleation that occurs during terminal differentiation of lens fiber cells and erythrocytes in mammals (Feinstein-Rotkopf and Arama, 2009). Conversely, cellular processes that retain intact nuclei but involve the shedding of cytoplasm and other organelles, such as spermatid individualization and neurite pruning in *Drosophila*, have been also reported (Feinstein-Rotkopf and Arama, 2009). Finally, in addition to apoptosis, alternative, caspase-independent, cell death pathways, sometimes referred to as type II (autophagic) and type III (necrosis) cell death, have been also characterized and have been found to involve the proteolytic activity of the lysosomes, albeit through distinct mechanisms (Turk and Turk, 2009).

The budding yeast, *S. cerevisiae*, enters meiosis in response to nitrogen starvation in the presence of a poor carbon source, and the four haploid nuclei are then packaged into spores, giving rise to an ascus with four spores (also termed "tetrad"). It has been noted, though, that when the carbon source in the medium is further restricted, the yeast respond by limiting the number of spores that are formed in a process called "spore number control" (Neiman, 2011). In the past decades, some of the mechanisms underlying the selective aspect of this process have been uncovered; however, the fate of the unselected nuclei remained unclear. Eastwood et al. (2012) have now solved the mystery by developing a robust protocol for monitoring sporogenesis in yeast treated to produce 3- and 2-spore asci. Using this approach, the authors show that soon after completion of meiosis, unselected haploid nuclei undergo active disintegration executed by two apparently nonoverlapping pathways emanating from the vacuole and the mitochondria.

The vacuole is a membrane-bound organelle filled with hydrolytic enzymes and is functionally equivalent to the metazoan lysosome. Eastwood et al. (2012) provide evidence that the vacuole functions in the degradation of nuclear proteins during PND. More specifically, they show that after meiosis completion, the chromatin packaging protein, histone H2A, is specifically degraded in yeast undergoing spore number control, as opposed to sporulating cells that produce tetrads. They then demonstrate that inactivation of the major vacuolar protease, Prb1, strongly attenuates the degradation of the unselected nuclei. However, how are nuclear proteins actually exposed to proteases that reside in the vacuolar lumen, and how are proteins of the selected nuclei protected against this destructive activity? The answers for these questions are less clear, but observations made by the authors and others offer some intriguing clues. The authors first negate possible involvement of the autophagic machinery in lysosomal sequestration of nuclear proteins. Then, by carefully monitoring the dynamics of luminal and membranal vacuolar proteins during sporogenesis, the authors show that the vacuole starts losing its integrity at the time when spore maturation of the selected nuclei becomes apparent. Furthermore, the disintegration kinetics of the vacuole remarkably coincides with the deteriorating morphology of the unselected nuclei and the degradation of

histone H2A. Therefore, proteins in post-meiotic nuclei may become accessible for proteases emanating from the rupturing and/or leaking vacuole. Because vacuolar disintegration also occurs under the conditions that produce tetrads, but with no apparent nuclear protein degradation, the authors hypothesize that the spore package, per se, may constitute a physical barrier that can protect the selected sporulating nuclei from this destructive activity. Indeed, concomitant with meiosis completion, each haploid nucleus is invested by a membrane, but only the selected nuclei are further encapsulated by prospore membranes, which eventually constitute the plasma membrane of mature spores. Significantly, leakage or rupture of the vacuole (a process sometimes also termed mega-autophagy) and the lysosome (i.e., lysosomal membrane permeabilization, or LMP) has been described to mediate the execution of some forms of caspase-independent cell death pathways in plants and mammals, respectively (Turk and Turk, 2009). Therefore, the phenomenon in which lysosomes act as ''suicide bags'' is an ancient property of eukaryotic cells, predating the emergence of the apoptotic caspases.

In the course of exploring the intactness of the nuclei during PND, Eastwood et al. (2012) discovered a second, mitochondrial, pathway. They show that the unselected nuclei, but not their selected sibling nuclei, undergo DNA fragmentation reminiscent of apoptosis. Two major apoptotic nucleases, CAD and endonuclease G (EndoG), have been discovered and are activated by direct caspase-dependent and -independent mechanisms, respectively (Widlak and Garrard, 2005). Consistent with the fact that true caspases do not exist in unicellular organisms, an EndoG, but not CAD, ortholog, called Nuc1, has been identified in yeast. EndoG activation involves its release from the mitochondria to the nucleus after mitochondrial outer-membrane permeabilization (MOMP) (Widlak and Garrard, 2005). Interestingly, Nuc1 was also implicated in (cytotoxic-induced) yeast cell death and is similarly translocating from the mitochondria to the nucleus (Carmona-Gutierrez et al., 2010). Now, Eastwood et al. (2012) demonstrate that in the absence of *nuc1*, nucleosomal DNA ladders and TUNEL-positive nuclei are no longer detected in yeast undergoing spore number control, indicating Nuc1 as a major nuclease involved in fragmentation of the genomic DNA during PND.

Why do sporulating yeast cells undergo spore number control and PND in response to limited carbon? One possibility is that by reducing the number of nuclei, these cells may ensure that sufficient biosynthetic capacity is available to fully complete sporogenesis of the selected ones. Along this line, the authors hypothesize that PND products may be recycled and used by the ''starving'' neighboring cells. Indeed, by monitoring a yeast strain from the wild, they show that as the colony grows, more cells execute spore number control, suggesting that they may sense microchanges in the levels of carbon in their microenvironment. Furthermore, after a few days of constant increase in the number of cells producing triads and dyads, a mild but significant elevation in the number of tetrads was indicated, suggesting that the levels of recycled PND products may have passed a certain threshold that allowed this recovery.

To conclude, whether certain cells may have harnessed and refined PCD mechanisms for special cellular remodeling or whether PCD may represent a collection of several distinct cellular processes integrated through a common regulatory pathway is still a matter of debate (Fernando and Megeney, 2007). The work of Eastwood et al. (2012) provides an important validation to the theory that PCD could have evolved from nonlethal cellular processes that were taken to the extreme.

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