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# Spermatogenesis: Borrowing the Apoptotic Machinery

Dispatch

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**The apoptotic machinery is utilized for a wide variety of tasks during development. Recent work has uncovered a new, non-apoptotic role for these factors during the individualization process of maturing spermatids.**

It was inevitable. Each metazoan cell contains a number of enzymes that are capable of provoking apoptosis; why not use them for other means? In an elegant and detailed demonstration of this multiple-uses-for-one-cassette idea, Arama *et al.* [1] used *Drosophila* to provide support for the emerging proposal that maturing spermatids utilize components of the apoptotic machinery to remove unneeded cytoplasmic contents during the process of individualization.

## Spermatogenesis and Apoptosis in Mammals

Researchers in the spermatogenesis field have known for some time that apoptosis plays an important role in, for example, removing abnormal sperm (reviewed in [2,3]). In addition, the idea that spermatogenesis invokes some sort of modified apoptosis for non-apoptotic ends has been percolating for a few years. For example, Blanco-Rodriguez and Martinez-Garcia [4] have shown that spermatids display many of the histological and molecular fingerprints of apoptosis. Maturing spermatids form darkly staining basophilic bodies and express multiple caspases within these 'residual bodies' — typical hallmarks of dying cells. In addition, these bodies contain proteins linked to the regulation of cell death such as FLIP, Fas, p21, p53, and c-Jun [4–6]. The cytoplasm of maturing spermatids is collected and removed by residual bodies, which express annexin V: this probably accounts for the ability of neighboring Sertoli cells to recognize and phagocytose them as they are shed. All of this has led to the idea that developing spermatozoa use the apoptotic machinery to selectively dissipate unneeded portions of their cytoplasm. In this view, apoptotic factors are somehow segregated to the cytoplasm — away from the nucleus — and this segregation permits the emerging sperm to utilize the apoptotic machinery without dying [4].

## *Drosophila* Spermatogenesis

Steller and colleagues [1] have now strongly bolstered this idea with *in situ* evidence that regulators of the apoptosis machinery direct a non-apoptotic event. Similar to mammals, fly spermatogenesis occurs within bundles ('cysts') of spermatids that develop in a coordinated fashion [7,8]. The 64 spermatids within each cyst coordinate their development by retaining meiotic cytoplasmic bridges. The final step in differentiation is termed 'individualization': spermatids form an initial 'individualization complex' near the nucleus that travels caudally down the spermatid within the 'cystic bulge', gathering the bulk of the cytoplasm, and eventually shedding this unneeded cytoplasm into a 'waste bag' at the base of the cyst (Figure 1). The emerging sperm, disconnected from their neighbors, are lean, mobile, and ready to rock. The similarities to mammals are striking.

Arama *et al.* [1] provide several lines of molecular evidence that individualization is under the control of the apoptotic complex. They demonstrate the presence of the caspase-9 ortholog Dronc and, importantly, presence of the activated form of the caspase-3 ortholog Drice. By targeting the caspase inhibitors zVAD or p35 to the male gonads, this group demonstrated a function for both these caspases: blocking caspase activity prevented proper caudal movement and gathering of cytoplasm by the individualization complex. The consequence was abnormally thick spermatids and male sterility. These results indicated that developing cysts utilize caspase activity to propagate a normal cystic bulge and to properly rid themselves of cytoplasm. Studies of mutants blocked at progressive steps in sperm maturation revealed that activation of Drice did not depend specifically on formation of the individualization complex, but rather on the overall maturation of the spermatids. Another interesting prospect worth exploring is whether late features, for example removal of the waste bag, show features of apoptotic engulfment such as presentation of annexin V.

At what step do caspases act? This is less clear, but the authors' data offer some intriguing clues. The dispensed cytoplasm may be altered by caspases — perhaps degraded in a manner reminiscent of apoptotic cells — and this alteration may permit the cytoplasm to enter the cystic bulge. Alternatively, perhaps the role of caspase activity is to 'un-tether' the individualization complex — comprising actin, myosin, and a number of associated proteins [8,9] — allowing it to travel caudally and scoop out cytoplasm along the way. Consistent with either of these possibilities, activated Drice is found both within the cystic bulge and within the cytoplasm in its path.

All of this raises the further question of how caspase activity is kept away from the nucleus. Activated Drice is found within the individualization complex and the cytoplasm it targets (the location of pro-Drice expression is not known); how is this localization achieved? Is Dronc activation similarly regulated and, if so, how? The answer may prove complex, as whatever factor regulates caspase activity will need to be localized to discrete regions of the spermatid. The authors do not

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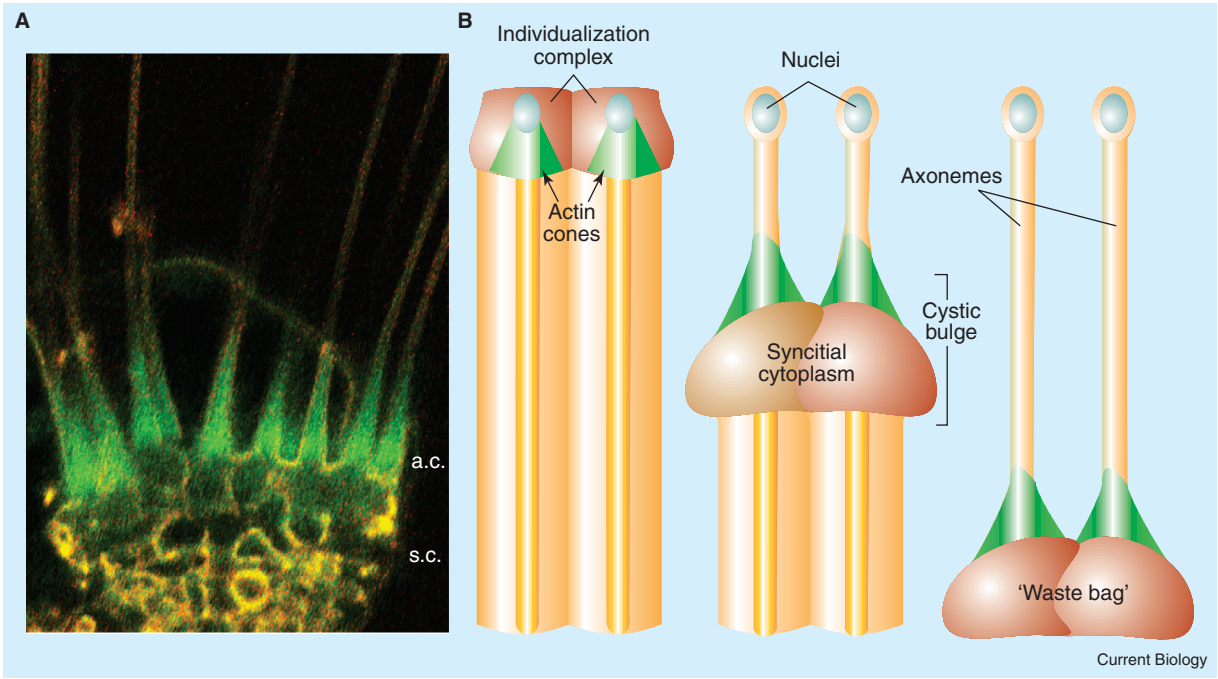


Figure 1. *Drosophila* spermatogenesis.

(A) Live cystic bulges in culture (courtesy of T. Noguchi and K. Miller). Membrane (yellow) is visualized with FM1-43; GFP–actin is green. The top half shows the thin individualized spermatids, which have been released by the removal of the enwrapping epithelial cyst cap. In the lower half, the actin cones (a.c.) and the syncitial cytoplasm (s.c.) create the cystic bulge. (B) A schematic showing three stages in the descending cystic bulge, which pushes cytoplasm into the waste bag. As cytoplasm is removed from the axoneme, activated caspase is lost as well.

solve this problem, but they offer a plausible candidate. dBruce is an unconventional ubiquitin-conjugating enzyme that contains a consensus domain — the BIR domain — known in other proteins to bind directly to caspases (reviewed in [10]). The authors report that mutations in dBruce result in nuclear hypercondensation and degeneration in spermatids. A simple interpretation of these experiments is that dBruce acts to oppose caspase activation in spermatid nuclei, protecting them from the scurrilous effects of Drice and/or Dronc. Testing this possibility will involve determining whether dBruce directly targets caspases for degradation, whether dBruce is localized to the nuclear area, and whether loss of dBruce function leads to a spatial expansion in activated caspases and caspase targets.

As the authors note, the defects they observe when blocking caspase function are not only of academic interest. Defective sperm is the most common cause of male infertility. The authors point to an intriguing similarity between the arrested defects observed in caspase-inhibited fly sperm and mammalian syndromes such as 'cytoplasmic droplet sperm', in which cytoplasmic removal is incomplete. The authors' point is clear: mutations or environmental factors that alter the function of apoptotic factors may contribute to male infertility. As our knowledge of these factors increase, new attractive therapeutic targets will come to the fore.

## The Special Case of Cytochrome c

In the course of exploring the role of apoptotic factors on spermatid maturation, Arama *et al.* [1] address another point that has provoked extensive discussion in the cell death field: the role of cytochrome c in *Drosophila*. The impact of this issue goes beyond the importance of a single molecule, so some background is in order. Cytochrome c has two apparently separable roles in a cell. As you may recall from high school biology, it acts in the respiratory chain to regulate energy metabolism within the mitochondria. More recently, cytochrome c has been shown to be an important regulator of the 'intrinsic' apoptotic pathway. Death stimulation triggered by pro-apoptotic members of the Bcl-2/Bax family commonly leads to release of mitochondrial factors including cytochrome c; once released, cytochrome c forms part of a wheel-like, macromolecular complex that includes caspase-9 and Apaf-1 (reviewed in [11,12]). This 'apoptosome' then gathers downstream caspases, directing their cleavage and activation. In most cells, this means death.

*Caenorhabditis elegans* makes use of this intrinsic pathway, although it has some differences (most notably, the Apaf-1 ortholog Ced-4 does not contain a cytochrome c binding site). Flies also have at least most of the pieces of the intrinsic apoptotic pathway, and their Apaf-1 ortholog, Dark/Hac-1/Dapaf-1, does contain a consensus cytochrome c binding site [13,14]. It would appear reasonable, therefore, to assume that flies use the standard 'intrinsic' cell death pathway in a manner similar to their furry cousins. But the best evidence to date suggests this is not the case. For example, multiple studies have failed to confirm a role for cytochrome c during apoptotic death [15,16]. Work

to date, provoked by the results of genetic screens for factors that regulate cell death, has focused instead on the role of the inhibitors of apoptosis (IAPs) as central regulators of caspase activity. IAPs are cytoplasmic proteins that bind directly to caspases to promote their degradation and cell death [13]. Reaper, Grim, Hid, Sickle, Morgue, and perhaps even Dark/Hac-1/Dapaf-1, are all thought to act at least in part by regulating the *Drosophila* IAP ortholog Diap-1. By contrast, reducing mammalian IAP function has yielded minimal phenotypic consequences (for example, see [17]). All of this has led to the view that flies use primarily IAPs to regulate cell death, whereas mammals use the Bcl-2/Bax mitochondrial 'system'.

So what exactly is the role of cytochrome c in cell death and, for that matter, why does Dark/Hac-1/Dapaf-1 contain a consensus cytochrome c binding site? In short, Arama *et al.* [1] provide evidence that loss of the cytochrome c isoform Cyt-C-d leads to sterile males whose spermatids show defects similar to caspase-inhibited spermatids. Mutants show no other phenotype. Together with the previous work outlined above, this work suggests that Cyt-C-d acts in the 'apoptotic' pathway that regulates spermatid maturation, though probably does not act in instances of cell death. From an evolutionary perspective, this provides an interesting example of how different organisms evolved to emphasize different pathways; tweaking these pathways permits them to travel different molecular routes to the same end. It will be interesting to determine whether mammals also make use of a cytochrome c isoform during spermatid maturation, and whether it is dedicated specifically for this purpose in a manner similar to flies.

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