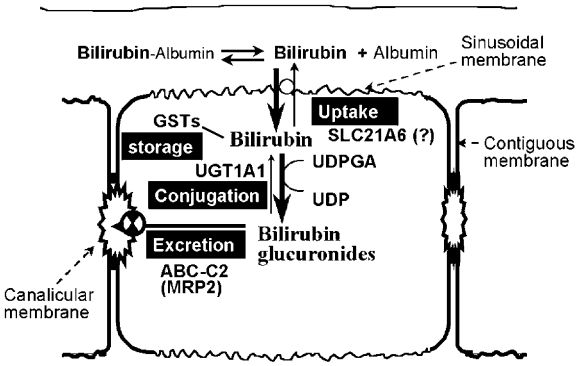

Figure 1. Bilirubin Metabolism

Bilirubin uptake, intracellular storage, conjugation with glucuronic acid, and biliary excretion are mediated by SLC21A6, GSTs, UGT1A1, and ABC-C2 (MRP2), respectively. The genes for each step of this pathway are coordinately induced by activated CAR.

expressing only human CAR, but not CAR null mice, showed enhancement of bilirubin clearance by phenobarbital. Surprisingly, they also showed induction of UGT1A1 by bilirubin, although the effect of human CAR was less pronounced. The results suggest that bilirubin and phenobarbital induce UGT1A1 through a common mechanism, activating CAR via nuclear translocation. However, patients with CN2 or Gilbert syndrome have hyperbilirubinemia that is markedly ameliorated by phenobarbital therapy. Thus bilirubin alone is an insufficient activator and must be potentiated by phenobarbital. Finally, Huang et al. show that the liver concentration of CAR, like UGTA1, is low in neonates, which may explain their tendency to develop hyperbilirubinemia. However, since phenobarbital effectively induces UGT1A1 in neonates, CAR levels must still be adequate for induction, or else phenobarbital can also act by another mechanism.

The two papers show a new level of regulation for bilirubin metabolism. They also highlight the importance of further research to find drugs that are ligands for human CAR and to explain how phenobarbital activates this receptor.

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Caspase Activation Finds Fertile Ground

Cytochrome c is a critical regulator of apoptosome assembly, caspase activation, and programmed cell death. Recent work demonstrates that cytochrome c and caspases function in *Drosophila* sperm cell differentiation and indicates that caspase activity can be regulated in a subcellular manner in cells that live.

Caspases are a family of cysteine proteases that function in programmed cell death (apoptosis) during animal development (Baehrecke, 2002). The activation of initiator caspases can be deadly, as these proteases trigger activation of executioner caspases, and the latter proteases cleave substrates, destroying the cell. Caspases are residents in cells as inactive proenzymes and become activated by multiple mechanisms (Martin, 2002). For example, the release of cytochrome c from mitochondria stimulates the assembly of an initiator caspase activation complex called the apoptosome. In mammalian cells, Apaf-1 binds cytochrome c and dATP, and this complex recruits caspase-9, forming the apoptotsome. Negative regulators also oppose caspase activation; the inhibitor of apoptosis proteins (IAPs) restrain proteolysis by physically interacting with caspases. Studies of caspases have emphasized their role in apoptosis, as caspase activity is generally considered synonymous with cell death. In this issue of *Developmental Cell*, Steller and colleagues present contrasting data that caspases function in *Drosophila* sperm differentiation (Arama et al., 2003).

The paradox that killer proteases may have functions in cells that live is not a novel concept, as it has been suggested that the loss of organelles from differentiating cells, such as the nuclei of red blood cells, may be controlled by cell death regulators (Jacobson et al., 1997). Significantly, the work of Arama et al. provides in vivo evidence that caspase activation is required for bulk degradation of cytosolic proteins during sperm differentiation and that caspases must be actively prevented from cleaving substrates, which would lead to gamete cell death. Furthermore, cytochrome c is required for sperm differentiation, providing a new link between this mitochondrial protein and caspases in flies.

Sperm development involves complex changes in cell structure. In *Drosophila*, spermatogenesis occurs within a cyst containing 64 spermatids, and differentiation of individual sperm involves the movement of most of the cytoplasm in a "cystic bulge" toward the spermatid tails (Tokuyasu et al., 1972). When the cystic bulge reaches

the sperm tail, it is detached in a membrane-bound structure, called the "waste bag," where protein degradation presumably occurs. Filamentous actin, nuclear lamin, and the activated form of the executioner caspase Drice are localized in the cystic bulge and waste bags during sperm differentiation, and this suggests that caspases function in sperm differentiation by degrading cytoplasmic substrates (Arama et al., 2003). To test this hypothesis, Arama et al. inhibited caspases by expressing the baculovirus inhibitor of caspases p35 in vivo and culturing testes with the caspase inhibitor Z-VAD in vitro, and both treatments prevented sperm differentiation. Although the presence of components of the apoptosome, including the caspase-9 ortholog Dronc and the Apaf-1 ortholog Ark (Dark, Hac-1, Dapaf-1), suggested a mechanism for Drice activation in sperm (Arama et al., 2003), the mechanism was unclear.

While apoptosome activation in mammals requires cytochrome c, the role of this mitochondrial protein in caspase activation has been a subject of significant debate in *Drosophila*. Since cytochrome c does not appear to be required for apoptosome activation in worms, it remains a formal possibility that cytochrome c is not required for caspase activation in flies. Although Ark interacts with cytochrome c in vitro (Rodriguez et al., 1999), cytochrome c was neither released from mitochondria nor required under conditions that trigger Ark-dependent apoptosis of *Drosophia* cell lines (Dorstyn et al., 2002; Zimmermann et al., 2002). To evaluate the role of cytochrome c in spermatogenesis, mutations were identified in the two *Drosophila* cytochrome c genes, named *cyt-c-p* and *cyt-c-d*. Homozygous mutations in *cyt-c-p* were lethal, while animals with mutations in *cyt-c-d* were viable, but male sterile. Furthermore, homozygous *cyt-c-d* mutants lack activity of the executioner caspase Drice in sperm, and sperm differentiation fails to occur (Arama et al., 2003). Combined, these data suggest that apoptosome assembly and caspase activation require cytochrome c during fly sperm development.

Compelling data indicates that assembly of the apoptosome and initiator caspase activation is sufficient to demolish a cell. If so, why don't sperm die, and why would killer proteases be used in the germline during development? Arama et al. provide a possible explanation by investigating the function of dBruce, the *Drosophila* ortholog of the giant E2 ubiquitin-conjugating enzyme Bruce. Homozygous *dBruce* mutants are viable, but males are sterile. While wild-type sperm nuclei are needle-like, *dBruce* mutant nuclei are condensed and appear to degenerate (Arama et al., 2003). These results suggest that, in the context of sperm differentiation, caspases are regulated in a subcellular manner. Although it is known that the expression of dBruce suppresses cell death that is activated by expression of the proapoptotic regulators Rpr and Grim (Vernooy et al., 2002), the specific mechanism by which this E2 ubiquitin-conjugating enzyme modulates caspase activity is not clear.

The discovery of a genetic requirement for cytochrome c and caspases in differentiating sperm provides a significant advance in our understanding of cell life and death during animal development, but many unanswered questions remain. Is cytochrome c directly involved in caspase activation through the regulation of apoptosome assembly, and is this really restricted to developing sperm? What are the caspase substrates in sperm, and why don't sperm die by apoptosis? Is the association of actin with the cystic bulge due to caspase cleavage, or is the assembly of actin driving the movement of the cystic bulge and its cytoplasmic content toward degradation? One of the most intriguing aspects of this story is that caspases may be regulated in a subcellular manner—how is this achieved? Since dBruce does not appear to directly interact with Rpr, Hid, or Grim-like proteins (Vernooy et al., 2002), it is possible that dBruce may directly interact with caspases preventing their activity. Like IAPs, dBruce encodes a BIR domain that could be involved in regulating caspase activity. If so, the interaction between dBruce and caspases would need to be spatially restricted within the cell, presenting a complex, but intriguing, possibility for modulating caspase activity. In closing, Steller and colleagues note the similarities between fly and mammalian sperm development (Blanco-Rodriguez and Martinez-Garcia, 1999) and suggest that caspases may also function in these gametes in higher animals, including humans. If this is the case, then defects associated with infertility could be associated with caspase activities. Clearly, studies of caspase function in cell life and death during development will be a fertile field of future research.

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