provides crucial niche signals to induce fast expansion of HSCs.

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Selected Reading

Baron, M.H. (2003). Exp. Hematol. *31*, 1160–1169.

Choi, K. (2002). J. Hematother. Stem Cell Res. *11*, 91–101.

Dzierzak, E. (2003). Curr. Opin. Hematol. *10*, 229–234.

Gekas, C., Dieterlen-Lievre, F., Orkin, S.T., and Millola, H.K.A. (2005). Dev. Cell *8*, 1–20.

Gering, M., and Patient, R. (2005). Dev. Cell *8*, 1–20.

Kumaravelu, P., Hook, L., Morrison, A.M., Ure, J., Zhao, S., Zuyev, S., Ansell, J., and Medvinsky, A. (2002). Development *129*, 4891–4899.

Matsuoka, S., Tsuji, K., Hisakawa, H., Xu, M., Ebihara, Y., Ishii, T., Sugiyama, D., Manabe, A., Tanaka, R., Ikeda, Y., et al. (2001). Blood *98*, 6–12.

North, T.E., de Bruijn, M.F., Stacy, T., Talebian, L., Lind, E., Robin, C., Binder, M., Dzierzak, E., and Speck, N.A. (2002). Immunity *16*, 661–672.

Ottersbach, K., and Dzierzak, E. (2005). Dev. Cell *8*, 1–20.

Yoder, M.C., Hiatt, K., and Mukherjee, P. (1997). Proc. Natl. Acad. Sci. USA *94*, 6776–6780.

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Morphology of Mitochondria During Apoptosis: Worms-to-Beetles in Worms

Although mitochondria are crucial for most pathways of mammalian cell apoptosis, evidence for their role in classic invertebrate models of programmed cell death has been frustratingly scant. New work showing that inhibition of mitochondrial fragmentation during *C. elegans* development inhibits programmed cell death bridges this gap and should advance a more detailed understanding of the role of mitochondria in caspase activation.

Mitochondria are important mediators of apoptosis in mammals (Green and Kroemer, 2004). The strength of various survival and death signals from simultaneous environmental stimuli are integrated and relayed by BH3-only proteins in the Bcl-2 family from the cell surface, cytosol, and nucleus to other Bcl-2 family members anchored into the outer mitochondrial membrane. At the mitochondria, these BH3-only proteins are thought to either activate proapoptotic members of the Bcl-2 family, Bax and Bak, or to inhibit the death repressive affects of Bcl-2, BclxL, and Bcl-w (Letai et al., 2002). Activated Bax and Bak effect the release of cytochrome c across the mitochondrial outer membrane into the cytosol, which, in turn, can activate caspases by binding APAF1 in the apoptosome. Alternative pathways of caspase activation are also regulated by Bcl-2 family members (Marsden et al., 2002). Recent work in mammalian cells shows that during apoptosis, mitochondria undergo morphological remodeling and fragmentation into more numerous and smaller units, called a worm-to-beetle transition, to reflect the change in their appearance. This change in mitochondrial morphology occurs in many cell types with many inducers of apoptosis upstream of caspase activation and close in time to cytochrome c release.

Insects and round worms display abundant apoptosis during development and have provided powerful genetic models to reveal gene products important for cell death. Surprisingly, considering the extensive studies in mammalian cells, a role for mitochondria in apoptosis in invertebrates has been lacking prior to a recent paper from the Conradt lab (Jagasia et al., 2005). This new work shows that Drp1, a protein required for mitochondrial fission, is important during developmental programmed cell death in *C. elegans*.

Classic genetic studies in *C. elegans* have shown that four gene products form a central pathway of apoptosis (Hengartner and Horvitz, 1994). Egl-1, a BH3-only protein, is induced specifically in cells destined to die. It binds to Ced-9, a Bcl-2 family member, and relieves Ced-9 suppression of Ced-4. Ced-4, a homolog of APAF1, proceeds to activate the caspase Ced-3, thereby initiating efficient cell disintegration and engulfment (Figure 1, classic model). Conradt and colleagues show that mitochondria fragment during physiological programmed cell death in *C. elegans* (Jagasia et al., 2005). Inhibition of Drp1 activity with a dominant-negative inhibitor prevents this mitochondrial fragmentation and the apoptosis of some of the cells destined to die during development. Interestingly, the mitochondrial fragmentation process mediated by Drp1 occurs downstream of Ced-9 but upstream of Ced-4, in between two proteins that bind one another on the mitochondrial outer membrane.

Required for normal mitochondrial fission in healthy eukaryotic cells, Drp1 is a large GTPase homologous

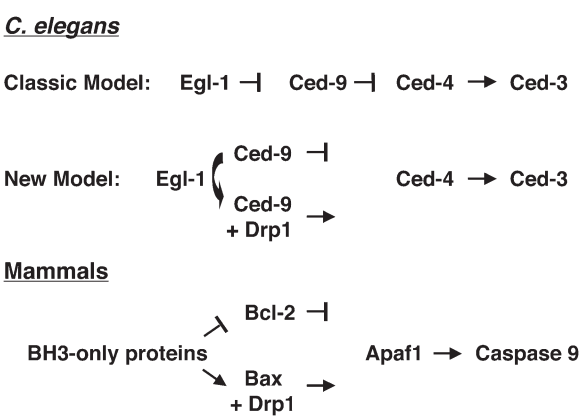

Figure 1. Models of Programmed Cell Death Pathways

to dynamin, a protein that forms spirals around the necks of endocytic vesicles and mediates their scission from the plasma membrane. Mitochondria recruit Drp1 from the cytosol into foci on the surface of the outer membrane at sites primed for organelle division (Labrousse et al., 1999). Drp1-mediated fission is balanced by organelle fusion to yield the normal, yet dynamic, reticulum of elongated and interconnected mitochondria in healthy cells (Shaw and Nunnari, 2002). Mutation of an amino acid in the GTPase enzyme active site of Drp1 yields a dominant-negative inhibitor that blocks mitochondrial fission and yields abnormally long and interconnected organelles due to an imbalance of fission with an active fusion machinery. Inhibition of Drp1 activity with the dominant-negative inhibitor during apoptosis in mammalian cells inhibits mitochondrial fragmentation, cytochrome c release, and the rate of cell death (Frank et al., 2001), putting Drp1 upstream of APAF1, consistent with the genetic studies in *C. elegans* (Jagasia et al., 2005) (Figure 1).

Ectopic expression of wild-type Drp1 in *C. elegans* induced mitochondrial fragmentation and cell death (Jagasia et al., 2005). Unexpectedly, Drp1-mediated fragmentation was inhibited in cells lacking expression of Ced-9, indicating that Ced-9 is actually required for the mitochondrial fragmentation in apoptotic cells (Figure 1, new model). This is consistent with the previous indication that Ced-9 not only inhibits cell death but can also change conformation to promote cell death (Hengartner and Horvitz, 1994). Jagasia et al. (Jagasia et al., 2005) extend this hypothesis to suggest that the proapoptotic activity of Ced-9 correlates with the mitochondrial fragmentation activity and requires Drp1. A Ced-9 gain-of-function mutation also inhibited mitochondrial fragmentation induced by ectopic Drp1 expression, suggesting that this mutant Ced-9 protein maintains cell death inhibition activity but lacks cell death induction activity. These results are compatible with the hypothesis that mammalian Bcl-2 family members can convert between anti- and proapoptotic conformations (Fannjiang et al., 2003). Binding of the BH3-only Egl-1 protein to Ced-9 could throw this conformational switch to activate the mitochondrial fission activity (Figure 1, new model).

Further linking mitochondrial fragmentation to the activation step of Ced-9 are results in mammalian cells showing that Bax and Bak colocalize specifically with Drp1 at the sites of mitochondrial fission. Prior to apoptosis, Bax resides either in the cytosol or, like Bak, evenly circumscribing the outer mitochondrial membrane. During apoptosis, both Bax and Bak change conformation and coalesce upon the mitochondrial outer membrane into concentrated foci with the mitochondrial division apparatus at mitochondrial fission sites (Karbowski et al., 2002). Inhibition of Drp1 does not inhibit Bax translocation to mitochondria or the coalescence into foci, putting Drp1 downstream of or at the same level as Bax, consistent with the *C. elegans* results (Figure 1). Interestingly, in cells under apoptotic stress lacking Drp1, the mitochondria constrict but fail to fragment and Bax still coalesces at the constriction sites, indicating that Drp1 is not directly binding or recruiting Bax into these regions.

Although it makes sense that mitochondria would be directed to fragment during the programmed dismantling of a cell, it seems surprising that this step is actually required for cell death, and it remains unknown how this process may lead to caspase activation. Nevertheless, these new results showing a requirement for mitochondria during *C. elegans* apoptosis provide some unification to a previously fragmented field.

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Selected Reading

Fannjiang, Y., Kim, C.H., Huganir, R.L., Zou, S., Lindsten, T., Thompson, C.B., Mito, T., Traystman, R.J., Larsen, T., Griffin, D.E., et al. (2003). Dev. Cell *4*, 575–585.

Frank, S., Gaume, B., Bergmann-Leitner, E.S., Leitner, W.W., Robert, E.G., Catez, F., Smith, C.L., and Youle, R.J. (2001). Dev. Cell *1*, 515–525.

Green, D.R., and Kroemer, G. (2004). Science *305*, 626–629.

Hengartner, M.O., and Horvitz, H.R. (1994). Nature *369*, 318–320.

Jagasia, R., Grote, P., Westermann, B., and Conradt, B. (2005). Nature *433*, 754–760.

Karbowski, M., Lee, Y.-J., Gaume, B., Jeong, S.-Y., Frank, S., Nechushtan, A., Santel, A., Fuller, M., Smith, C.L., and Youle, R.J. (2002). J. Cell Biol. *159*, 931–938.

Labrousse, A.M., Zappaterra, M.D., Rube, D.A., and van der Bliek, A.M. (1999). Mol. Cell *4*, 815–826.

Letai, A., Bassik, M.C., Walensky, L.D., Sorcinelli, M.D., Weiler, S., and Korsmeyer, S.J. (2002). Cancer Cell *2*, 183–192.

Marsden, V.S., O'Connor, L., O'Reilly, L.A., Silke, J., Metcalf, D., Ekert, P.G., Huang, D.C., Cecconi, F., Kuida, K., Tomaselli, K.J., et al. (2002). Nature *419*, 634–637.

Shaw, J.M., and Nunnari, J. (2002). Trends Cell Biol. *12*, 178–184.