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APOPTOSIS

WAH-1 and SCRM-1 make lipids flip

...a new mitochondria to plasma-membrane signalling pathway that promotes cell-surface changes in apoptotic cells.

In healthy mammalian cells, the lipid phosphatidylserine (PS) is localized to the inner leaflet of the plasma membrane. In apoptotic cells, however, PS is externalized to the outer leaflet where it can function as an 'eat-me' signal to induce phagocytosis. Xue and colleagues now show that this phenomenon is conserved in *Caenorhabditis elegans*, and that the apoptogenic factor WAH-1 (which is released from mitochondria during apoptosis) activates phospholipid scramblase-1 (SCRM-1) to effect PS externalization. The authors thereby establish "a new mitochondria to plasma-membrane signalling pathway that promotes cell-surface changes in apoptotic cells."

The authors first developed a gentle surgical procedure that allowed them to stain apoptotic cells on the surface of the gonads of *C. elegans*. Subsequent fluorescent staining revealed that, as in mammalian cells, the outer surfaces of a significant proportion of apoptotic cells were labelled with annexin V, a specific PS-binding protein; the process of PS externalization is therefore conserved.

As an indirect link had already been established between PS externalization and apoptosis-inducing factor (AIF), the authors examined whether WAH-1, the *C. elegans* homologue of AIF, affects PS externalization in apoptotic cells. RNA interference (RNAi) knockdown of *wah-1* expression resulted in reduced annexin V labelling of apoptotic cells, whereas overexpression of *wah-1* resulted in increased labelling (but not in increased apoptosis).

However, WAH-1 does not contain any domains or biochemical properties that would enable it to externalize PS directly. Using human phospholipid scramblases as a starting point, the authors identified eight *C. elegans* scramblase proteins through which WAH-1 might function. Of these, only the reduction of SCRM-1 activity affected PS externalization. Also, consistent with a role for SCRM-1 in PS externalization, SCRM-1 was shown to localize to the plasma membrane.

Further annexin V labelling experiments showed that the inactivation of WAH-1 (by RNAi knockdown) and SCRM-1 (by mutation) together did not have additive effects on reducing PS externalization, which suggests that WAH-1 and SCRM-1 function in the same pathway. In addition, an *in vitro* pull-down assay showed that WAH-1 interacts with SCRM-1. Indeed, using an *in vitro* phospholipid-scrambling assay, Xue and co-workers demonstrated that whereas SCRM-1 has only low lipid-scramblase activity, the presence of WAH-1 increases the scramblase activity of SCRM-1 by tenfold (WAH-1 on its own has no scramblase activity).

So, WAH-1, after it is released from mitochondria, activates the scramblase activity of SCRM-1 to promote PS externalization. As the authors point out, "How phospholipid asymmetry on the lipid bilayer is generated, maintained and altered ... is a fundamental but poorly understood issue in cell biology." Through their demonstration and dissection of PS externalization during apoptosis in *C. elegans*, Xue and co-workers have made strides towards understanding this crucial process.

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ORIGINAL RESEARCH PAPER Wang, X. et al. *C. elegans* mitochondrial factor WAH-1 promotes phosphatidylserine externalization in apoptotic cells through phospholipid scramblase SCRM-1. *Nature Cell Biol.* 1 Apr 2007 (doi:10.1038/ncb1574)