

The Dynami(n)cs of Cell Corpse Engulfment

Engulfment of dying cells plays an important role during animal development and homeostasis, and several proteins involved in this process are known. However, the cell biology underlying phagocyte arm extension and cell corpse degradation is not well understood. A study published in this issue of *Developmental Cell* (Yu et al., 2006) now demonstrates an important role for the GTPase dynamin in these events.

Cell death is a major cell fate during the development of metazoans. In the mammalian nervous system, for example, it is estimated that 80% of the neurons generated undergo cell death as a normal consequence of development. Ninety-five percent of T cells generated in the immune system are also thought to encounter a similar fate. It is no surprise, therefore, that the clearance of cells that have died either due to injury or as a normal course of animal development is of great importance. In mammals, engulfment of dying cells allows phagocytes to initiate immune responses toward viral or bacterial pathogens, and is involved in controlling homeostasis of photoreceptor cells in the retina. In *Drosophila*, engulfment plays an important role in morphogenesis of the central nervous system. Mutations that block engulfment in the fruit fly lead to the accumulation of a large number of cell corpses, forcing the mispositioning of glial cells and leading to defects in axon projection patterns (Sears et al., 2003). In *C. elegans*, engulfment seems to play a role not only in the clearance of dying cells, but also in the cell death process itself; inappropriate survival of cells destined to die in animals carrying a weak mutation in the caspase-encoding gene *ced-3* is enhanced by mutations in genes that promote engulfment (Hoepfner et al., 2001; Reddien et al., 2001).

A host of proteins implicated in the control of the various stages of cell corpse engulfment have been isolated in mammals, *Drosophila*, and *C. elegans* (Zhou et al., 2004). The sequences of these proteins have led to hypotheses about how phagocytic targets are recognized and about the mechanics of engulfment. However, despite this progress, much remains to be clarified. For example, although some entities displayed by dying cells to promote their engulfment are known, it is clear that these only represent a partial set of “eat-me” signals. Furthermore, the cell biological events that mediate extension of phagocytic arms to surround dying cells, as well as the events that occur within the phagocyte to degrade the engulfed cell corpse, are only understood superficially.

To uncover the details of the cell corpse engulfment process, genetic approaches in the nematode *Caenorhabditis elegans* have been used to identify components involved in engulfment (Zhou et al., 2004). These studies led to the identification of seven genes,

functioning in two partially redundant pathways, which promote engulfment. The proteins encoded by the *ced-2/CrklI*, *ced-5/Dock180*, and *ced-12/Elmo1* genes function in a pathway within engulfing cells to control cytoskeletal events required for the extension of phagocytic arms. These proteins control cytoskeleton dynamics by regulating the activity of the small Rac GTPase protein CED-10. CED-10 protein may also be regulated by the second pathway, consisting of the CED-1, CED-6, and CED-7 proteins (Kinchen et al., 2005). CED-7 is a plasma membrane ABC transporter whose function is required in both dying and engulfing cells for proper engulfment. CED-7 may function to present an “eat-me” signal generated by the dying cell. CED-1 is a single-pass transmembrane protein that clusters at the region of the engulfing cell involved in corpse phagocytosis. CED-6 protein contains phosphotyrosine binding domains, and interacts with CED-1 (Su et al., 2002), perhaps mediating a signaling event from this membrane protein.

In this issue of *Developmental Cell*, Yu et al. (2006) report the identification and characterization of another gene, *dyn-1*, involved in the phagocytosis of cell corpses in *C. elegans*. *dyn-1* encodes a homolog of the GTPase dynamin, and is expressed in many *C. elegans* cells. Dynamin proteins are thought to assemble in a ring structure around the neck of endocytic vesicles, using their GTPase activity to facilitate fission of the vesicles from the plasma membrane. How precisely dynamin promotes membrane separation is highly debated.

To identify genes involved in cell corpse engulfment in *C. elegans*, Yu et al. screened for mutants displaying a block in the process. Previous genetic screens aimed at identifying engulfment-defective mutants were designed to isolate viable mutants, able to produce progeny. However, such screens would fail to identify mutations in genes that have essential roles in other processes. To bypass this limitation, Yu et al. sought mutants that both prevented engulfment and exhibited an arrest during embryonic development. Fourteen such mutants had lesions in the *dyn-1* gene, supporting a critical role for this gene in both engulfment and other essential processes.

Interestingly, Yu et al. (2006) found that embryos carrying a specific *dyn-1* lesion exhibited profound defects in the engulfment of dying cells, yet were still able to concentrate yolk particles in intestinal cells by endocytosis. These observations suggest that *dyn-1* must be required for a function other than endocytosis to promote engulfment. Support for this hypothesis came from examination of *dyn-1* mutants by electron microscopy, which revealed the presence of abnormal vesicles not associated with the plasma membrane within engulfing cells.

To study the function of DYN-1 protein in greater detail, the authors examined its localization. They observed that DYN-1 clusters on extending phagocytic arms of engulfing cells in a manner highly reminiscent of CED-1 clustering. Moreover, the authors demonstrated

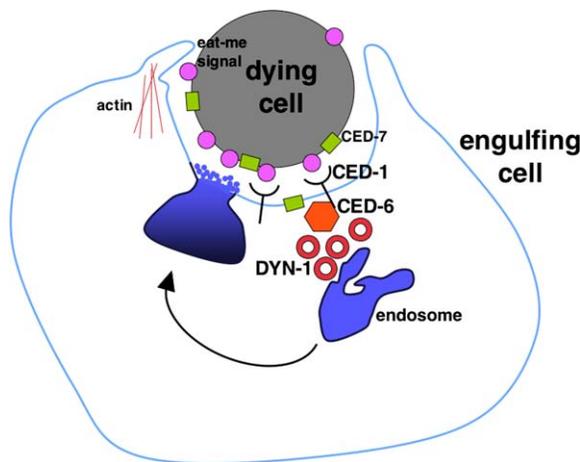


Figure 1. A Possible Model for the Role of Dynamin in Regulating Cell Corpse Engulfment in *C. elegans*

A dying cell presents a signal promoting a neighboring cell to engulf it, perhaps through the CED-7 ABC transporter. The CED-1 protein on the engulfing cell may recognize this signal. CED-1, in turn, activates CED-6, which is required for recruitment of DYN-1 and endosome-derived vesicles to sites of engulfment. These vesicles may then fuse with the plasma membrane, allowing extension of phagocytic arms, and deposition of material involved in cell corpse degradation. Actin dynamics also control the extension of phagocytic arms using the CED-2, 5, 10, and 12 proteins (not shown).

that DYN-1 and CED-1 colocalize in extending phagocytic arms. Double mutant analyses suggested that *dyn-1* functions in the *ced-1, 6, 7* pathway of engulfment. Consistent with this result, recruitment of DYN-1 to sites of engulfment was dependent on these genes.

How, then, does *dyn-1* function to regulate engulfment? One hypothesis put forth by the authors is that DYN-1 protein may actively recruit intracellular vesicles to sites of engulfment, promoting fusion with the plasma membrane. The vesicles would contribute membrane, to allow extension of phagocytic arms, and their contents may promote degradation of the dying cell. In support of this mechanism, the authors show that endosomes, which they normally find to be clustered at engulfment sites, fail to cluster in *dyn-1* mutants. Furthermore, Yu et al. demonstrate that degradation of engulfed corpses in *dyn-1* mutants is delayed. Although consistent with the data, the idea that *dyn-1* promotes vesicle fusion is difficult to reconcile with the large

number of studies demonstrating a very specific role for *dyn-1* in vesicle fission. Although *dyn-1* has been proposed to regulate at least some aspects of vesicle fusion, the experiments supporting this view are few, and suggest mechanistic details different from those described for fission (Peters et al., 2004; Praefcke and McMahon, 2004). One possibility worth considering is that DYN-1 protein is involved in the generation of intracellular vesicles, perhaps endosome-derived, that are recruited to sites of engulfment. In such a model, DYN-1 would not play a role in targeting per se, but in the generation of a vesicle that is targeted by other means (Figure 1). Such a model would be consistent with the well-studied topological activity of dynamins in membrane fission.

The success of identifying a specific role in engulfment for an essential cellular protein suggests that basic cell biological processes, operating in many cellular contexts, but also controlling engulfment can be studied genetically in *C. elegans*. The door is now open for deeper exploration of this important and intricate process.

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Selected Reading

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