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Editorial

Death in the nervous system: JNK signaling in junk clearance

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Dying cells, dead cells and debris are normally cleared effectively by 'amateur' and 'professional' phagocytes, most notably by macrophages, particularly when damage or death occurs at high rate. The mechanisms underlying this process feature prominently in development, in tissue homeostasis and in responses to damage or infection, and their exquisite efficiency is exemplified by the speed at which the overt evidence of cell death disappears from tissues. Conversely, impairment of clearance mechanisms can have pathological consequences, best known being those contributing to autoimmune disease.[1] Given the increasing awareness of the broadening implications of cell-clearance responses of the host, it seems likely that many disease processes will prove to be linked to the molecular mechanisms underlying clearance of dying cells and cell-derived debris.

In the '3Rs' approach to clearance,[2] illustrated well in apoptosis, these molecular mechanisms can be divided mainly for convenience into three overlapping phases (Figure 1a): (1) *Recognition*—physiological realization that cell death is occurring and active engagement of phagocytes with the process through receptor-ligand interactions, (2) *Response*—activation of signaling pathways in cells that recognize that cell death or damage is in progress, including anti-inflammatory responses, cell-fate decisions and pathways that culminate in, (3) *Removal*—including internalization and degradation of engulfed cargo. Of these, the *Response* phase is perhaps the most intriguing, as it encompasses the signaling processes that engender the broadening cellular and organismal effects of cell corpse clearance. Dissecting these signaling pathways presents significant challenges and, although a good deal of progress has been made in recent years, especially guided by the two main genetically tractable clearance pathways following programed cell death in the nematode *Caenorhabditis elegans*, substantial gaps in our knowledge remain. In this issue, Macdonald *et al.*[3] elegantly identify the c-Jun kinase (JNK) signaling cascade as a novel clearance *Response* pathway in phagocytes triggered by neuronal injury and death.

In the central nervous system (CNS) of mammals, neuronal damage and death caused by injury or disease is accompanied by clearance responses of glial cells, microglia (CNS macrophages) and astrocytes, which rapidly accumulate and remove the dying/dead cell bodies and debris arising from damaged and degraded axons, dendrites and synapses. This reactive gliosis is important in CNS well-being, repair and regeneration. Furthermore, such efficient clearance of axonal and synaptic debris is required for effective development and remodeling of the nervous system.[4,5] Working in the fruit fly, *Drosophila melanogaster*, Freeman's group had previously demonstrated that the *ced-1* ortholog, *draper* is required for neuronal corpse clearance by glial cells during embryonic development[6] and subsequently the importance of the Draper protein as an engulfment receptor became apparent in all aspects of glial responses to neural degeneration, including injury in adult flies. Signaling pathways downstream of Draper are activated via Src family kinase-mediated phosphorylation of its intracellular ITAM domain[7] (Figure 1b). Specifically, Src42a phosphorylation of the ITAM has been shown to induce recruitment of the non-receptor tyrosine kinase, Shark, ultimately to activate Rac-1-dependent engulfment. In keeping with its place in the *C. elegans ced-1/-6/-7* cell corpse clearance pathway, Draper can also activate downstream signaling for engulfment via the dCed-6 adapter protein. Details of additional signaling components downstream of Draper have remained unclear until now.

In this latest work, Freeman and colleagues began to unearth clues as to the linkage between glial responses to axonal injury and the JNK signaling cascade using an RNAi-based screening approach. They first identified a critical role for *basket* (*bsk*), which encodes the *Drosophila* c-Jun Kinase (dJNK), in glial engulfment of axonal debris that occurs rapidly (within 5 days) after axotomy; in bsk^{RNAi} animals, almost all the axonal debris remained uncleared, and could persist for extended time periods approximating the lifespan of adult flies. By overexpressing *puckered* (*puc*), which encodes a phosphatase known to negatively regulate dJNK activity, they were able to demonstrate a virtually identical phenocopy to bsk^{RNAi} in *UAS-puc* animals.

Having ruled out a conceivable role for dJNK in glial development, the authors next turned their attention to the possibility that dJNK signaling in clearance of degenerating axons occurred at the level of the ensheathing glia. This indeed proved to be the case and it was also noted that dJNK signaling in astrocytes played no part in the response. By analyzing known components of dJNK signaling pathways, it was further shown that the MAPKKKs, Slipper and Tak1

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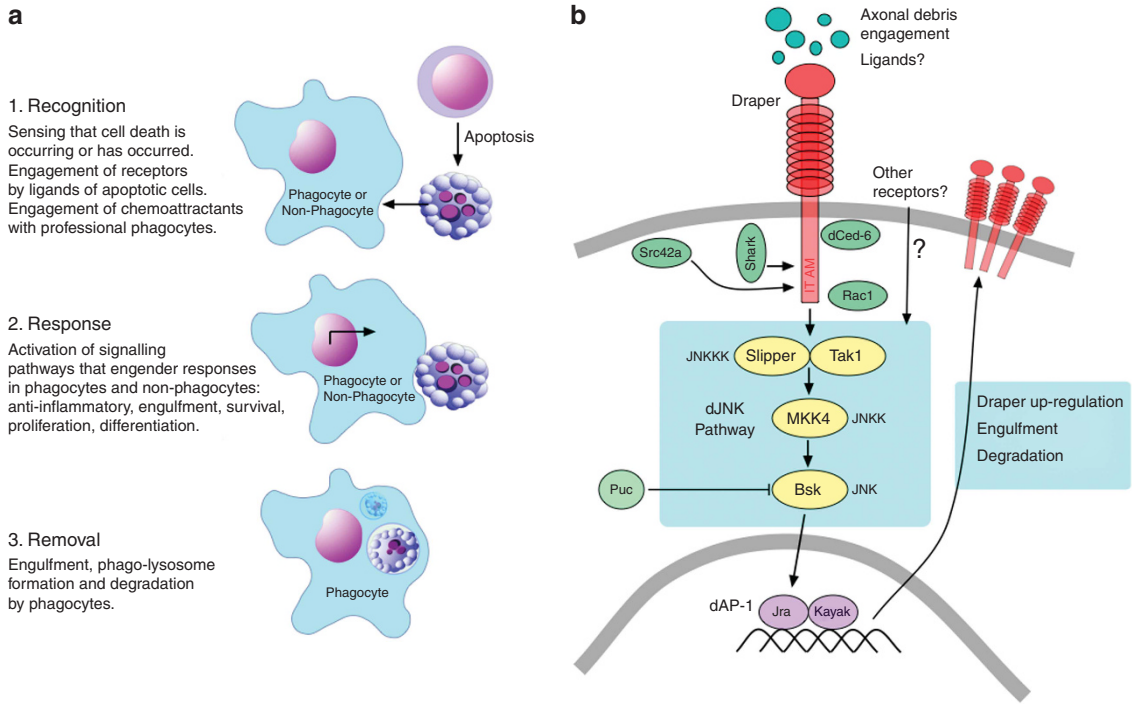

Figure 1 Schematic outline of (**a**) conceptual phases in clearance of dying cells and (**b**) *Drosophila* clearance signaling in the CNS in light of the findings by Macdonald *et al*.[3] See text for details

operated in the axonal debris-clearance response, along with the MAPKK, MKK4 and dAP-1 (which is composed of *Drosophila* c-Jun and c-Fos, aka Jra and Kayak, respectively). Intriguingly, Slipper and Tak1 appear to play a redundant role as double, but not single, mutants suppressed clearance of axonal debris. These results indicate that a signaling cascade involving Slipper/Tak1 → MKK4 → Bsk → dAP-1 induces gene expression required for efficient glial clearance responses (Figure 1b). Furthermore, as ensheathing glia were already known to undergo Draper-dependent engulfment of degenerating axons, these results strongly favored a close link between dJNK/dAP-1 signaling and Draper in this clearance context.

Investigating this link further, the authors subsequently showed that, in response to axonal injury, glial dJNK signaling markedly upregulated Draper expression. Indeed, expression of full-length Draper-I (of the three isoforms of Draper, the only isoform shown to be functional in clearance of axonal debris), was found to be sufficient to rescue the clearance defect in bsk^{RNAi} and *UAS-puc* animals, thus showing that Draper upregulation and engulfment is likely to be a primary consequence of dJNK signaling following axonal injury. Furthermore, dJNK signaling was found to be necessary for the extension of glial membranes to engulfment targets and for lysosomal activation during phagocytosis by glial cells responding to axonal injury. By contrast, glial recruitment to degenerating axons of basal Draper—Draper that is present prior to injury—does not appear to depend upon dJNK signaling. These results argue convincingly that, while increases in glial levels of Draper and effective axon engulfment are critically dependent upon dJNK, sensation of injury and accumulation of Draper molecules at the injured loci are dJNK-independent. In other words, *Recognition* in this context is dJNK-independent, whereas aspects of *Response* and *Removal* are critically dJNK-dependent. Notably, in keeping with an additional role in negative feedback in these glial activities, dJNK signaling was also found to be important in terminating the responses to axonal injury.

These results firmly place dJNK/AP-1 as an important pathway in the clearance of dead cells and debris, at least in the CNS of the fly. Given the general principle that the cellular and molecular mechanisms underlying cell-death biology are highly conserved from worms to mammals, it is an exciting possibility that this clearance *Response/Removal* pathway will prove to extend not only to the mammalian CNS but also to the clearance of dead and dying cells in other tissue contexts. It is noteworthy that glia responding to CNS injury in mammals activate JNK and upregulate MEGF10, a close mammalian sequence ortholog of Draper. Furthermore, JNK signaling has been shown to be activated in mammalian macrophages responding to apoptotic cells *in vitro*.[8] It therefore seems highly likely that the dJNK/AP1 signaling pathway that is central to clearance in the CNS in flies is evolutionarily conserved and has wide-ranging relevance in the apoptotic cell clearance arena. Inevitably this raises many questions: What phagocyte receptors and ligands activate this pathway (a front-runner for the latter is Draper and its mammalian orthologs; Draper may engage multiple ligands including phosphatidylserine in apoptotic cell removal[9])? Can cell death activate the pathway in non-phagocytes as well as phagocytes? Is this pathway a key to response outcomes in addition to engulfment, such as cell-fate decision-making that

can occur consequent to cell death in the tissue neighborhood? No doubt, answers to these and other questions will be forthcoming in time and an understanding of the potential therapeutic applications of this mechanism will be founded.

Conflict of Interest

The author declares no conflict of interest.

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