Based on the authors' data, one would predict that RAG complexes associate with bulk cellular chromatin in a tissue-specific fashion (e.g., with IgH but not TCR gene segments in pro-B cells) and will not be brought down with chromatin displaying repressive marks (e.g., H3K9me3). Future studies should also address whether the transcriptional function of promoters is required for recombinase accessibility above and beyond promoter-directed H3K4 methylation. As the authors point out, the link between H3K4me3 and RAG suggests at face value that recombinase may be targeted to almost any active gene in precursor lymphocytes. Clearly this is not the case. An obvious source of additional specificity is the RSS, which is bound by RAG1 and may synergize with RAG2-H3K4me3 interactions to generate a productive recombinase-RSS complex. Alternatively, additional chromatin modifications may contribute to a "histone code" for recombinase targeting.

Lastly, Liu et al. (2007) show that a PHD point mutation in the noncore region of RAG2 (W453A) abrogates DH→JH recombination in pro-B cells. These data are in seeming contradiction with prior studies showing that core RAG2, which lacks the entire C terminus including the PHD, mediates a normal degree of DH→JH recombination but is defective for VH→DHJH rearrangement. The authors propose a highly testable model to explain this apparent discrepancy. They hypothesize that other parts of the noncore RAG2 region impose an inhibitory function on recombinase that is counteracted by PHD binding to H3K4me3-marked chromatin. In this model, the PHD mutation would fail to relieve inhibition by the noncore module, and recombination of chromatinized substrates would be blocked. In contrast, loss of the entire RAG2 C terminus would generate an active recombinase capable of rearranging proximal (DH→JH) but perhaps not distant (VH→DHJH) gene segments. The latter, less efficient process may benefit from the additional punch provided by PHD-H3K4me3 interactions. Notwithstanding, Liu et al. (2007) have contributed an important step in our quest to understand how genetic elements coordinate the dynamic changes in transcription and chromatin that drive stepwise assembly of antigen receptor genes to diversify our adaptive immune repertoire.

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Ubiquitin-Proteasome: Pallbearer Carries the Deceased to the Grave

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Phagocytosis is a complex process that involves multiple cellular functions. In this issue of *Immunity*, Silva et al. (2007) report that a protein ubiquitylation complex and the proteasome are required for the clearance of apoptotic cells in *Drosophila*.

The denomination "phagocyte" comes from the Greek *phagein*—to eat—and *kytos*—cell—and was coined by Metchnikoff (and Claus) when he discovered phagocytosis while investigating digestion in the starfish larva. This mechanism is evolutionary very ancient; it was possibly selected prior to the invention of multicellularity by early eukaryotic cells to ingest nutrients. Indeed, amoeba such as *Dictyostelium discoideum* feed on microorganisms, some of which have evolved strategies to elude or hamper phagocytosis. As a result, it is likely that

amoebae have developed countermeasures to deal with such pathogens. Thus, a link with immunity is possibly already present in unicellular eucaryotes. As initially discovered by Metchnikoff, phagocytosis plays an essential role in host defense in higher eucaryotes. Phagocytosis also fulfills other essential functions like homeostasis in multicellular organisms and morphogenesis during development by disposing of cells undergoing programmed cell death or necrosis. This phenomenon concerns relatively few cells in simple organisms such as the nematode *Caenorhabditis elegans* in which bystander neighboring cells quickly engulf cells as soon as they have implemented programmed cell death (PCD) (Yu et al., 2006, and references therein). Indeed, engulfment promotes apoptosis in this model organism. By contrast, PCD is quantitatively much more important in other organisms, for example in *Drosophila melanogaster*, where many neurons are eliminated during the maturation of the central nervous system (CNS), or in mammals, where up to 10^{10} cells are disposed of every day. As a result, phagocytosis is often performed by professional phagocytes such as macrophages, although other cells such as glial cells may also be involved in this function in the CNS. The challenge is to prevent corpses from releasing cytotoxic molecules or from inducing an excessive inflammatory reaction. A failure to achieve a rapid clearance of effete cells may result in autoimmune disease such as systemic lupus erythematosus in humans or improper formation of the CNS in *Drosophila*. In this issue of *Immunity*, Silva et al. (2007) cast a new light on the regulation of engulfment of dying cells. They report that the ubiquitin-proteasome machinery for protein degradation is required for the efficient clearance of apoptotic cells in *Drosophila*.

The term phagocytosis encompasses distinct forms of engulfment depending on the nature of the ingested particle and that of the phagocyte. There are, however, common steps in this complex multistage process. An initial step of recognition mediated by receptors triggers intracellular signaling to recruit additional membrane material and the actin cytoskeleton needed to form engulfing pseudopods. Internalization then ensues and leads to the formation of a phagosomal vacuole that fuses with early endosomes during maturation and ultimately fuses with lysosomes to form phagolysosome. Phagosome maturation is accompanied by acidification of the vacuole. Hydrolytic enzymes delivered by vesicle fusion degrade the phagosome contents. In professional phagocytes, microbe killing is also achieved by the release of antimicrobial peptides, reactive oxygen, and nitrogen species.

Decades of study of phagocytosis led to the concept of "elegant complexity" mirrored in the identification of multiple receptors, opsonins, and signaling pathways (Stuart and Ezekowitz, 2005). Genetic model organisms offer an approach to dissect this complexity by highlighting the components required for this process. A limitation that should be kept in mind, however, is that mutations affecting genes that are also required in other functions may lead to other phenotypes that potentially mask their role in phagocytosis. The clearance of apoptotic corpses has been investigated extensively in *C. elegans*, and its failure leads to the persistence of cellular corpses. Studies have delineated two distinct pathways that act in partially redundant mechanisms (Yu et al., 2006, and references therein). The CED (cell death abnormal) genes CED-2 (CrkII), CED-12 (ELMO), and CED-5 (DOCK180) act downstream of the MIG-2 (RhoG) GTPase and its associated activator UNC-73 (TRIO). They activate CED-10 (Rac), which controls actin remodelling for pseudopod extension. The receptor that triggers this conserved pathway has not been identified in the worm, although the ortholog of the phosphatidylserine receptor (PSR) may play a minor role in this pathway. It has been proposed that the second pathway promotes vesicle recruitment and fusion to maturing phagosomes and to extending pseudopods by focal exocytosis (Yu et al., 2006). This pathway uses the CED-1 receptor and the CED-7 ATP-binding cassette transporter, which is perhaps required to promote phosphatidyl serine exposure on the outer membrane of both engulfed and dying cells. The CED-6 (GULP) adaptor functions downstream of CED-10 and upstream of dynamin. CED-10 may also mediate the action of the CED-1 pathway on cytoskeletal reorganization. By contrast, our understanding of apoptotic cell clearance in *Drosophila* is less extensive. One receptor of the CD36 family, Croquemort, has been shown to be required for effete cell engulfment by macrophages during embryogenesis (Franc et al., 1999), but surprisingly does not appear to be necessary in a cell culture system (Manaka et al., 2004). In the absence of apoptotic cells, Croquemort is much less expressed in embryonic macrophages. Another receptor, Draper, is required for clearance of corpses in macrophages and glial cells (Manaka et al., 2004). This CED-1-related protein, together with DmelCED-6, is also required in glial cells for disposal of pruned or severed axons. The nature of the "eat me" signals displayed by cells initiating PCD remains elusive and does not involve phosphatidylserine, at least in cell culture experiments (Manaka et al., 2004).

Silva et al. have embarked on a program of forward genetics to further identify genes involved in apoptotic cell clearance (Silva et al., 2007). To this end, they screened a collection of lines carrying large genetic deletions that collectively uncover most of the *Drosophila* genome. A quarter of the embryos laid by flies of a given line are homozygous for one deficiency. It is relatively straightforward to identify lines in which clearance does not take place by identifying the pattern of apoptotic cells stained by acridine orange. Nonphagocytosed corpses are not clustered within macrophages and thus appear dispersed throughout the embryo. In this way, it is possible to screen a large number of genes rapidly. One limitation is that gene products can be supplied by the heterozygous mother in the egg prior to fertilization and may provide (in some cases) enough function to compensate a zygotic defect. Once an interesting genetic deficiency has been identified, smaller deletions and mutants in the region are then tested. Thus, a P element transposon inserted

between the *Uch-L3* and *CG3654* genes displayed the phenotype found in a larger deletion, a decreased number of engulfed corpses per macrophage. *CG3654* was an obvious candidate because it encodes a fly ortholog of PSR. However, it is the neighboring gene *CG3428*, which is also affected by the insertion, that causes the phenotype. The expression of a wild-type *CG3428* transgene in macrophages is sufficient to rescue the apoptotic clearance phenotype to a *CG3428* mutant. *CG3428*, also called *pallbearer (pall)*, encodes an F box protein. F box proteins are thought to provide specificity to SCF (Skp-cullin-F box) E3 ligase complexes that form part of the ubiquitinylation machinery. Silva et al. (2007) went on to identify SkpA and Cul1 (LIN19) as components of the PALL-SCF complex, which is thus distinct from the SLIMB-SCF complex that negatively regulates an immune signaling pathway in *Drosophila*. Mutations in the genes encoding the E2 conjugating enzyme Effete (UbcD1) and dominant suppressor mutations for two proteasome subunits genes display the *pall* mutant phenotype and also interact genetically with *pall*. Taken together, these data establish a role for ubiquitin-dependent proteasome degradation in the clearance of apoptotic cells.

The link between phagocytosis and proteasome function is poorly known at present. In mammals, a transient association between the proteasome and phagosomes has been reported in macrophages and is thought to be involved in the process of crosspresentation of exogenous antigens by class I MHC (Houde et al., 2003). However, this association is unlikely to be required for phagocytosis itself. Interestingly, proteasome subunits have been identified in phagosomes containing a latex bead by a proteomics approach in *Drosophila* (Stuart et al., 2007). Yet, these proteins did not appear to be necessary for the phagocytosis of *Staphyloccocus aureus* or *Escherichia coli* in a cell culture model. Genome-wide RNAi screens have been performed in *Drosophila* cell culture to identify the genes that affect infection by the intracellular pathogens *Listeria monocytogenes* and *Mycobacterium fortuitum* (Ayres and Schneider, 2006, and references therein). These screens failed to reveal a role for SCF and the proteasome in controlling these infections, with the exception of the proteasome being necessary to limit the noxious effects of listeriolysin on the host cell. Thus, the ubiquitin-proteasome system appears to be required only for the clearance of apoptotic cells, or large particles, although further experimental evidence is required to confirm this conclusion.

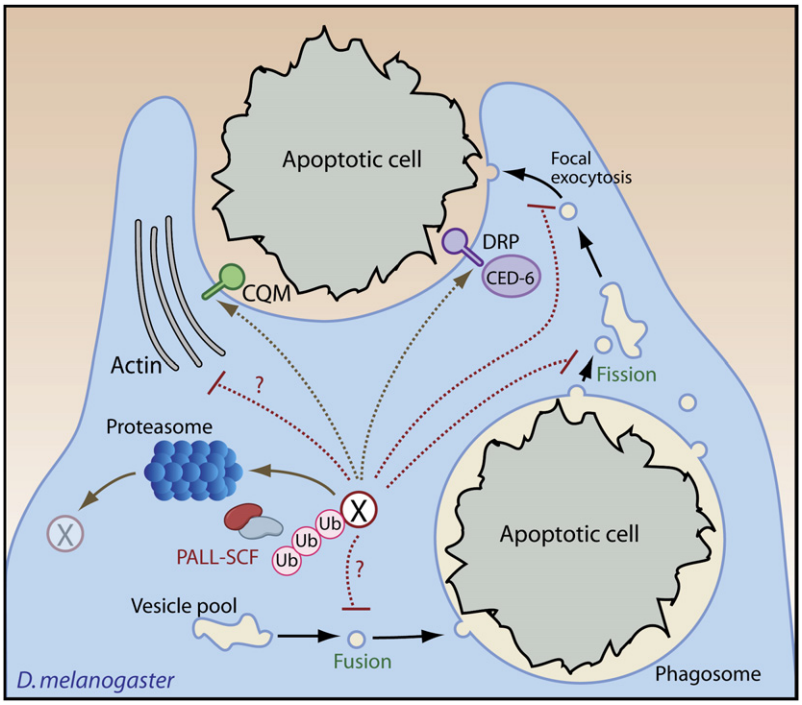

Figure 1. Model of Apoptotic Cell Clearance in *D. melanogaster*
The ubiquitin-proteasome system may control the stability of an inhibitor, X, that may regulate one or multiple steps required for efficient engulfment of apoptotic cells. See text for further discussion. CQM, Croquemort; DRP, Draper; Ub, ubiquitin.

In mammals, ubiquitylation and proteasomal function is required for the formation of an acidic intraphagosomal multivesicular compartment that may play a role in clearing the FcγRII receptor from the limiting membrane of the phagosome (Lee et al., 2005). Nevertheless, fusion of the phagosome to lysosomes proceeds normally when proteasomal function is impaired. Thus, it may be worth determining whether Croquemort or Draper are targeted by the PALL-SCF complex to regulate the stability and phagocytic activity of these apoptotic cell receptors.

Having potentially ruled out a role for the proteasome in the basic mechanisms of phagocytosis, it is conceivable that the SCF-proteasome axis controls the half-life of an inhibitor targeting a specific step of phagocytosis (Figure 1). Indeed, such inhibitors have been identified in *Drosophila* (Stuart et al., 2007). What could be this step? *pall* mutant macrophages are able to degrade apoptotic corpses almost completely. They contain an average of one apoptotic corpse. These observations suggest that phagocytosis is able to proceed to completion, but much less efficiently. It could be that a partially redundant mechanism compensates a defective PALL-SCF complex. Alternatively, apoptotic corpses are large particles that likely require massive membrane trafficking for the extension of pseudopods and the formation of the phagosome membrane. This may be a step that limits the efficiency of engulfment and that could be finely regulated by a degradable inhibitor. However, neither the PALL-SCF nor the proteasome were apparently required for the uptake of yeasts

in cell culture (Stroschein-Stevenson et al., 2006), suggesting that size of the engulfed particle may not be the relevant parameter that distinguishes engulfment of corpses from other forms of phagocytosis. Thus, the determination of the processs regulated by the PALL-SCF complex is a priority for further investigations. Importantly, it will be interesting to determine whether SCF complexes and the proteasome are required for the clearance of apoptotic cells in amoebes, nematodes, and mammals.

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Dendritic Cells Break Bonds to Tolerize

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Typically, dendritic cells (DCs) induce peripheral tolerance under steady state but immunity during inflammation or infection. In this issue of *Immunity*, Jiang et al. (2007) identify disruption of E-cadherin interactions as a unique maturation pathway by which DCs are capable of mediating tolerance.

The induction of an efficient and protective immune response depends on the interaction between naive antigen-specific T cells and professional antigen-presenting cells (APCs). Because of their unique features, such as migratory capacity and expression of costimulatory molecules, dendritic cells (DCs) are considered the prototypic professional APCs. DCs are present as sentinels in peripheral tissues, where they capture antigens that may be presented to $CD4^+$ and $CD8^+$ T cells. DCs undergo a maturation process after sensing pathogen-derived structures through pattern recognition receptors such as Toll-like receptors (TLRs), exposure to proinflammatory cytokines, or after ligation of the surface receptor CD40. Upon maturation, DCs stop taking up antigens, change their pattern of homing receptors (e.g., upregulation of CCR7; Roake et al., 1995), which allows them to migrate into the T cell areas of secondary lymphoid organs, and upregulate costimulatory molecules such as CD86. These changes enable efficient priming of naive antigen-specific T cells.

Under steady state, most DCs in peripheral tissues have an immature phenotype. They efficiently take up antigens but lack high expression of costimulatory molecules and CCR7 and thus can't productively activate naive T cells to develop into effectors. Rather, it was thought that the interaction of naive T cells with immature DCs results in the induction of peripheral T cell tolerance (Probst et al., 2005; Steinman et al., 2003) in a T cell-intrinsic (e.g., anergy, deletion) or -extrinsic (e.g., via T regulatory [Treg] cells or cytokines) fashion (Probst et al., 2005; Sakaguchi, 2004; Steinman et al., 2003). The contact between naive T cells and DCs is thought to take place in T cell areas of secondary lymhoid organs, which requires the DCs in the peripheral tissues to move there. However, although mature DCs with upregulated expression of CCR7 could migrate to the secondary lymphoid organs (Roake et al., 1995), how immature DCs reach these organs is less clear.

Langerhans cells (LCs), a subset of DCs that reside in mucosal epithelia and epidermis, form a 3-dimensional network and adhere to surrounding keratinocytes through the homophilic adhesion molecule E-cadherin. It has been shown that LCs migrate into the cutaneous lymph nodes under steady-state conditions, albeit much slower than after mechanical trauma (Kissenpfennig et al., 2005). Along the same line, E-cadherin was found to be markedly downregulated on LCs upon their maturation, which may allow LCs to more efficiently leave the epidermis and migrate into cutaneous lymph nodes, where they (in)directly