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Current Opinion in Cell Biology

How to build a phagosome: new concepts for an old process

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Phagocytosis is a complex and elegant mechanism requiring finely coordinated deformation and restructuring of the membrane and the underlying cytoskeleton. Here we discuss the early events of receptor clustering and engagement required for signal transduction and actin remodeling. In addition, we summarize recent studies of the mechanisms whereby the nascent phagosome seals and evolves into a degradative phagolysosome by a process that seemingly involves the autophagic machinery. These studies provided new insights of the molecular basis of this long-appreciated, essential homeostatic process.

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Current Opinion in Cell Biology 2018, **50**:57–63

This review comes from a themed issue on **Cell architecture**

Edited by **Celeste Nelson** and **Franck Perez**

https://doi.org/10.1016/j.ceb.2018.01.009

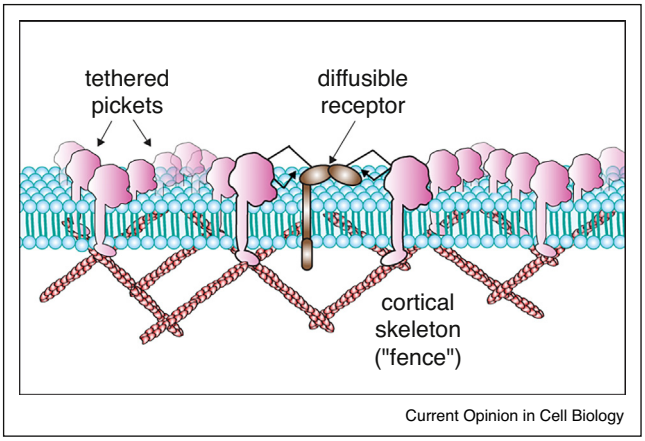
Phagocytosis is the mechanism of internalization of large (≥0.5 μm) particles, such as microorganisms and cellular debris. It has a nutritional function in protozoans, plays a crucial role during metazoan development and tissue homeostasis by removing dead cells, and allows cells of the innate immune system to effectively eliminate pathogenic bacteria and fungi. This entails the entrapment of the particulate material in an intracellular membrane-bound vacuole where a vast arsenal of microbicidal weapons and degradative tools is deployed. Here we review recent advances that shine light on the signaling and membrane remodeling events required for phagosome formation and maturation into a microbicidal and degradative organelle.

Early events: receptor engagement and clustering

Phagocytosis is a receptor-mediated process; a remarkable variety of ligands can be recognized on the surface of the phagocytic targets by an array of receptors that differ in structure and composition, and that generate distinct signaling patterns [1,2]. Their mode of activation is not known in all cases, but in those instances that have been studied in most detail — for example, the Fcγ receptors that recognize IgG — activation results from the clustering of several receptors upon binding to multiple vicinal ligands on the surface of the target particle. Such clustering requires the lateral displacement of individual receptors in the plane of the membrane. For clustering to occur rapidly, as required for effective signaling of phagocytosis, the rate of receptor diffusion must be substantial. Considering the fluid nature of the lipid bilayer that constitutes the plasma membrane [3], the requirement for rapid lateral diffusion was not initially regarded as an obstacle to successful clustering and phagocytosis. However, this paradigm shifted as a result of the observations made initially by Sheetz *et al.* [4], who found that membrane glycoproteins diffused ≈50 times faster in spherocytic red cells — that lack the major components of the submembranous 'matrix' — compared to normal red cells. These findings were refined and rationalized subsequently by Kusumi and his team [5,6], who proposed that the cortical actin skeleton that lines the plasma membrane poses a considerable obstacle to the lateral translocation of transmembrane proteins whose tails extend into the cytosol. Kusumi *et al.* found that actin filaments lining the plasmalemma assemble into partitions or 'corrals' [7] that constrain the movement of proteins, including Fc receptors [8]. The interweaving actin meshwork or 'fence' is attached to the membrane by 'pickets', transmembrane (glyco)proteins that are directly or indirectly tethered to the submembranous actin filaments.

A number of transmembrane proteins could conceivably serve as pickets. One such candidate, CD44, possesses a single transmembrane domain and can link to actin via ezrin and potentially also ankyrin. CD44, which is highly abundant (≈10^6 copies/cell) in phagocytes such as macrophages, was in fact observed to become immobilized for extended periods of time upon binding to the cytoskeleton [9••], thus fulfilling an essential requirement for a picket.

Figure 1



The cytoskeletal 'picket fence' restricts phagocytic receptor mobility. The cortical actin skeleton (or fence), composed primarily of formin-induced linear actin filaments, serves to tether and immobilize transmembrane proteins (pickets) like CD44. Molecular crowding by pickets, which are immobilized (at least a fraction of the time) by association with F-actin, obstructs and constrains the diffusion of bystander molecules such as phagocytic (e.g. Fcγ) receptors.

Importantly, in addition to fastening the cytoskeletal fence to the membrane, the pickets are themselves physical obstacles to the movement of unattached proteins. Membrane proteins lacking a cytosolic domain, and even lipids, experience reduced mobility as a result of collisions with the dense forest of immobile transmembrane pickets (Figure 1). Accordingly, unattached, diffusible proteins such as the Fcγ phagocytic receptors are more mobile in macrophages lacking CD44 than in their wildtype counterparts [9••]. Thus, it appears that the role of the actin fence is to maintain the pickets in place, rather than the other way around, as is the case for a garden-variety fence! The molecular crowding caused by the immobile/poorly mobile pickets and their tightly associated proteins and lipids is likely the main contributor to the reduced mobility of unattached, diffusible molecules, including phagocytic receptors (Figure 1), in good agreement with Kusumi's picket-fence model [5,6].

Since diffusion of transmembrane proteins can be severely restricted by the cortical skeleton, how do phagocytic receptors manage to travel sufficiently fast to cluster and generate a phagocytic signal? One possibility is that, as proposed by Kusumi and colleagues, proteins can escape the corrals by 'hopping' over the fence [6]. Transient openings in the actin fence and/or detachment of the mesh from the pickets could facilitate such hopping, enabling long-range displacement of the receptors. Alternatively, the actin meshwork may not be homogeneous. We have obtained evidence that receptor mobility is much greater at the leading edge of migrating macrophages than in the trailing uropod [9••]. We attribute this to the differential distribution of ezrin, which appears to associate more avidly with linear F-actin — such as that generated by formins — than with branched actin, which is nucleated by Arp2/3 and propels the extension of lamellipodia at the leading edge of migrating cells. In this manner, phagocytic receptors would be preferentially active at the front of the cells, where encounters with phagocytic targets are more likely to occur.

Signal generation and amplification

Clustering of receptors initiates signaling by activating Src-family kinases (SFKs). The mode of activation of SFKs is complex. All family members contain two critical tyrosine residues plus an SH2 domain. The tyrosine closest to the C-terminus is continuously phosphorylated by Csk, a constitutively active kinase. When phosphorylated, the C-terminal tyrosine binds intramolecularly to the SH2 domain, occluding the kinase site and rendering the SFK catalytically inactive (see Figure 4a in [10]). Restoration of activity is brought about by CD45/CD148, two structurally related transmembrane tyrosine phosphatases. It is only in this 'primed' state, that is, when the C-terminal tyrosine is dephosphorylated, that SFKs can become activated by receptor cross-linking. Activation results from phosphorylation of the second, more N-terminal tyrosine by another SFK or by Syk. Paradoxically, this phosphorylation event can also be reversed by CD45/CD148. How then can sustained activation be accomplished?

The secret was revealed by Goodridge *et al.* [11], who noted that the phosphatases, which are present throughout the macrophage surface before phagocytosis, become excluded from the site where receptors engage the phagocytic target, as reported for the immunological synapse [12]. In this manner, CD45 and CD148 can prime the receptors for activation, without also terminating the response prematurely.

The mechanism whereby the phosphatases are excluded is conceptually simple, yet requires the sophisticated coordination of signaling, tethering and cytoskeletal components. Both CD45 and CD148 are long, rigid molecules that extend perpendicularly from the surface of the plasmalemma [13]. Ultimately, their exclusion from the phagocytic cup is caused by the tight apposition of the macrophage membrane to the target particle. The resulting space between the two surfaces is smaller than the length of the exofacial domain of the phosphatases, resulting in their physical extrusion. It is noteworthy that the mobility of CD45 increases markedly when the phagocytic receptors are engaged [14••]. This is suggestive of loosening of the cortical fence in the vicinity of the nascent phagocytic cup, conceivably due to dephosphorylation of ezrin and/or other mechanisms that would lead to detachment of the pickets or would sever the actin mesh. Regardless of the