Comment

Quo Vadis: Polarized Membrane Recycling in Motility and Phagocytosis

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The colloquial expression "let's go out to eat" implies a relationship between movement and ingestion that can also be found at the cellular level. Phagocytic cells, such as neutrophils and *Dictyostelium discoideum* amoebae, have a marked capacity to move towards their intended meals by detecting and orienting towards chemoattractants released by bacteria and other microorganisms. In the case of neutrophils, subsequent phagocytosis of bacteria is for the purpose of host defense (as opposed to nutrition), but the relationship remains nevertheless.

There is also a mechanistic relationship between motility and phagocytosis. Both processes often involve the directed elaboration of cell extensions, either in the form of pseudopods that envelop particulate substrates during phagocytosis or the pronounced membrane ruffles that are found at the leading edge of a motile cell. Both processes are also well known to be highly dependent on the localized polymerization of actin filaments and activity of Rho family GTPases that help organize actin polymerization. The actin-binding protein coronin is also found either at a moving cell's leading edge or at the site of particle uptake (Gerisch et al., 1995; Maniak et al., 1995). Conceivably, motility and phagocytosis are reflections of the same, or at least tightly linked, functions which emerged together in evolution: competition for ingestion would be enhanced by the capacity to move towards the bacterial meal as opposed to waiting for the meal to be delivered, as it were. In this issue of *The Journal of Cell Biology*, Grinstein and colleagues present data that may help resolve the uncertainty over the mechanism of these processes (Bajno et al., 2000).

The problem of cell motility is one of the oldest in modern cell biology, dating back to studies in the early 1970's by Abercrombie, Raff, de Petris, and others who noted that cell-bound particles or cross-linked antibodies exhibited directed movement on the plasma membrane. As articulated by Mark Bretscher (Bretscher, 1996; Bretscher and Aguado-Velasco, 1998), such work gave rise to two views of how cells move. The first proposes that directed actin polymerization provides a physical force from within the cell that effectively pushes it forward. As the filamentous actin is pushed rearward, surface-bound particles would be similarly swept to the cell's trailing edge. The alternative view suggests that there is polarized insertion of membrane at the leading edge of a cell. Most likely, the inserted membrane would be derived by the recycling of cell surface components internalized via endocytosis. Over the years, ample indirect evidence has been invoked to support both views.

In the case of phagocytosis, it has long been presumed that pseudopod extension, which must precede particle ingestion, is an entirely actin-driven event, driven at least in part by a "zippering" of the forming pseudopod across the particle surface by progressive interactions with receptors on the phagocyte plasma membrane (Griffin et al., 1977). Indeed, a role for actin in phagocytosis is exceedingly well established (Aderem and Underhill, 1999). Some apparent discrepancies with this simple view have long been evident, however. Pioneering work from Werb and Cohn (1972) established that cells such as macrophages can internalize enormous areas of plasma membrane during particle uptake, up to 50% of a cell's entire surface area. Yet, when measured quantitatively, it was apparent even from our own group's early efforts that at best only a small fraction of a macrophages plasma membrane proteins were removed as a consequence of phagocytosis, and even these disappeared only transiently (Mellman et al., 1983). More recent work from Grinstein and colleagues demonstrated not only that the macrophage surface area does not decrease as a result of phagocytosis, but actually increases (Hackam et al., 1998). The source of compensatory membrane was most likely derived from one or more intracellular compartments. Indeed, injection of macrophages with the SNARE-active neurotoxin tetanus toxin was found to cause a significant decrease in phagocytosis, consistent with an inhibition of fusion of intracellular vesicles with the plasma membrane.

In this issue of the *JCB*, Grinstein's group appears to have provided a critical element to the story by focussing on a tetanus toxin-sensitive v-SNARE, VAMP3/Cellubrevin, as the possible relevant site of action (Bajno et al., 2000). In many mammalian cells, VAMP3/Cellubrevin is localized to a specific endosome subpopulation designated "recycling endosomes" (McMahon et al., 1993; Daro et al., 1996). These enigmatic vesicles function as intermediates on the receptor recycling pathway, but seem to host the transit of only a small fraction of internalized receptors. Passage through recycling endosomes appears to delay their rate of their recycling, perhaps causing the accumulation of an intracellular pool of receptors and other plasma membrane components (Sheff et al., 1999). Whereas recycling endosomes have been implicated in polarized sorting of receptors in epithelial cells, their function, particularly

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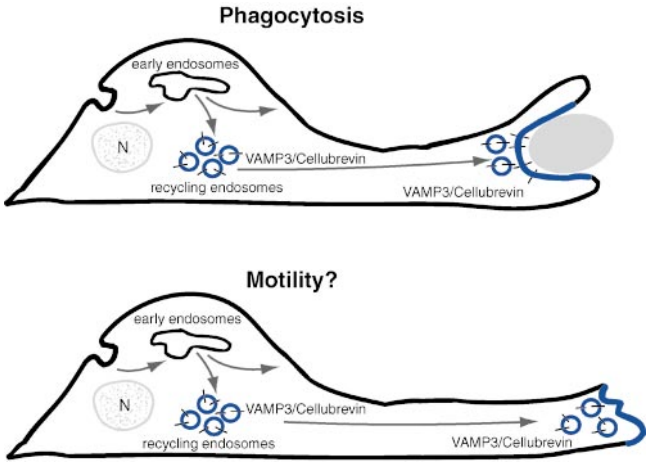

Figure 1. Proposed polarized insertion of recycling plasma membrane components during phagocytosis and cell motility. Plasma membrane internalized during clathrin-mediated endocytosis is first delivered to peripheral early endosomes from which the majority of membrane components recycles rapidly back to the cell surface. A fraction is delivered, however, to a perinuclear population of recycling endosomes enriched in the v-SNARE VAMP3/Cellubrevin (blue). During phagocytosis, membrane from this recycling endosome population must be recruited, in a tetanus toxin–sensitive VAMP3/Cellubrevin-dependent fashion, to the site of particle uptake to allow for pseudopod extension. Conceivably, much the same may occur during the process of cell migration, namely that recycling endosomes fuse at a cell's leading edge providing membrane to allow forward extension of the migrating cell.

in nonpolarized cells such as fibroblasts and macrophages, has remained unknown. Grinstein and colleagues (Bajno et al., 2000) now suggest that they are involved in a form of polarity here as well, namely polarized insertion of new membrane at sites of particle uptake (Fig. 1).

By expressing GFP-fusions of VAMP3/Cellubrevin in phagocytic cells, Bajno et al. (2000) find that elements of the VAMP3/Cellubrevin-positive recycling endosome population seem to fuse with the plasma membrane, quite possibly providing the estra membrane required for pseudopod extension. Importantly, fusion seems to occur in a polarized fashion, being concentrated at and within the newly formed phagocytic vacuole, with VAMP3/Cellubrevin then rapidly removed from the plasma membrane by endocytosis. Since tetanus toxin treatment cleaves VAMP3/Cellubrevin and was previously found to inhibit phagocytosis, it seems reasonable to propose that phagocytosis requires the vectorial insertion of recycling endosomes.

Although much more work will be needed before it is possible to propose that a similar mechanism is also responsible for inserting new membrane at the leading edge of a migrating cell, Bajno et al.'s work provides some easily testable hypotheses. Indeed, it is already known that recycling receptors, even ones known to reside in recycling endosomes, seem to appear preferentially at the forward margins of moving cells (Pierini et al., 2000). The identification of a recycling endosome-associated v-SNARE, which may control the actual insertion process may finally provide a path to resolving the long standing debate over the mechanism of cell motility. Is a VAMP3/Cellubrevin-dependent insertion of recycling endosome membrane at the leading edge of a motile cell required for sustained mobility? As an added bonus, this work, although targeted at understanding the mechanism of phagocytosis, may also provide a welcome explanation for why recycling endosomes exist at all.

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