DOI:
10.1038/nrm2397

MEMBRANE TRAFFICKING

A maturing influence

> …a model for the regulation of RAB5 activity during phagocytosis…

Not only is the small GTPase RAB5 a key regulator of the early endocytic pathway, it has also been implicated in apoptotic cell engulfment, or phagocytosis. But the exact role of RAB5 in phagocytosis has so far remained uncharacterized.

Using a fluorescence resonance energy transfer (FRET) probe, Kitano *et al.* studied the activity of RAB5 — an increase in RAB5–GTP correlates with an increase in FRET efficiency. Increased RAB5 activity was observed during the engulfment of apoptotic thymocytes, which coincided with disassembly of the actin coat that encapsulates phagosomes. The duration of RAB5 activation varied significantly between phagosomes. Some phagosomes showed continuous RAB5 activity during a period of up to 10 min, whereas other phagosomes showed various patterns of cessation and reactivation of RAB5 activity. Final RAB5 inactivation preceded the collapse and breakdown of engulfed apoptotic cells.

Expression of a dominant-negative RAB5 mutant delayed the breakdown of apoptotic thymocytes, which suggests that RAB5 activation has a function in phagosome maturation. The authors speculated that RAB5 may mediate the fusion of phagosomes with early endosomes. Active RAB5 is also needed for the recruitment of RAB7, which regulates the fusion of phagosomes with late endosomes to initiate lysis of cell corpses.

Disruption of microtubules (following treatment with nocodazole) decreased RAB5 activity at the engulfment site, which suggests that microtubules are somehow required for RAB5 activation. By knocking down expression of various candidate RAB5 guanine nucleotide exchange factors (GEFs), the authors found that depletion of Gapex5 inhibits RAB5 activation at engulfment sites, whereas depleting other GEFs has no effect. Gapex5-depleted cells showed a delay in breakdown of the engulfed cells.

But how do microtubules deliver Gapex5 to phagosomes? Rather than directly binding to microtubules, Gapex5 associates with a microtubule-associating protein, EB1. Indeed, knockdown of EB1 expression reduced RAB5 activation during phagocytosis.

On the basis of their imaging studies, the authors proposed a model for the regulation of RAB5 activity during phagocytosis whereby the engulfment of apoptotic cells and the accumulation of actin filaments around the phagosomes is followed by actin disassembly and invasion of a microtubule network, which recruits Gapex5 to phagosomes and induces transient RAB5 activation during engulfment — when it functions in phagosome maturation.

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ORIGINAL RESEARCH PAPER Kitano, M. *et al.* Imaging of Rab5 activity identifies essential regulators for phagosome maturation. *Nature* 2 April 2008 (doi:10.1038/nature06857)