Minireview

Phagosome proteomes open the way to a better understanding of phagosome function

Gareth Griffiths* and Luis Mayorga†

Addresses: *Cell Biology Program, EMBL, Meyerhofstrasse, 69117 Heidelberg, Germany. †IHEM (U.N. Cuyo-CONICET), Casilla de Correo, Mendoza, 5500, Argentina.

Correspondence: Gareth Griffiths. Email: gareth.griffiths@embl-heidelberg.de

Published: 15 March 2007

Genome ***Biology*** 2007, **8:**207 (doi:10.1186/gb-2007-8-3-207)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2007/8/3/207

Abstract

Phagocytic cells take up microbes and other particles into membrane-bounded organelles called phagosomes. Studies on the protein and lipid composition of model phagosomes containing latex beads are the first step in a systems-biology approach to understanding how these organelles function.

Early in their evolution, eukaryotic cells acquired the capacity to take up microbes by phagocytosis as a source of food. Since then, phagocytosis has evolved into a highly complex and regulated process, and is one of the main ways in which multicellular animals clear the body of pathogenic microbes and cellular debris. Microbes and other particles are taken up by phagocytosis into an intracellular membrane-bounded organelle called a phagosome (Figure 1). This eventually fuses with other organelles, notably endosomes and lysosomes, resulting in a gradual alteration of the composition and function of the phagosome, a process referred to as phagosome maturation. After full maturation, the phagosome will contain a battery of hydrolytic enzymes and have an internal pH as low as 4-4.5 (Figure 1).

In the vast majority of cases, the microbe inside the phagosome is killed and digested, but a number of important pathogens, including the bacterium *Mycobacterium tuberculosis*, which kills around two million people each year, have acquired the ability to survive, and even replicate, in this hostile environment. Each type of pathogen that exploits intracellular vesicles seems to have evolved a different survival strategy. Phagosome maturation follows a defined biochemical program, and different pathogens probably redirect this program in a unique fashion. Pathogen proteins and/or lipids released inside phagosomes alter signaling pathways in the phagosomal membrane or in the cytoplasm.

A pathogen-containing phagosome in, for example, a macrophage, has three distinct 'compartments'. These are the pathogen itself; the luminal contents, which are enriched in hydrolases, protons, and ions such as Ca^{2+}, and have a still poorly defined redox state; and the phagosomal membrane, the boundary between the pathogen and the cytoplasm. This last controls most phagosome functions, including their fusion, recycling, and interactions with the cytoskeleton. Determining the molecular composition of the phagosome membrane and phagosomal contents is essential if we are to understand in detail how these organelles function.

Knowing how a 'normal' phagosome works would provide a strong foundation for understanding how pathogens alter phagosome maturation. This could lead to the development of drugs that block pathogen-induced alteration of phagosome signaling. That might appear a tall order, but a simple model system of phagocytosis involving the uptake of latex beads has recently opened up this problem to molecular dissection. In the most recent study of this sort, a proteomic analysis of latex bead phagosomes (LBPs) in cultured *Drosophila melanogaster* S2 cells, Stuart *et al.* [1] have identified more than 600 phagosome-associated proteins. Of the 140 proteins identified in mouse LBPs in earlier studies [2], 70% have orthologs in the *Drosophila* phagosome, indicating a high degree of conservation. Recent

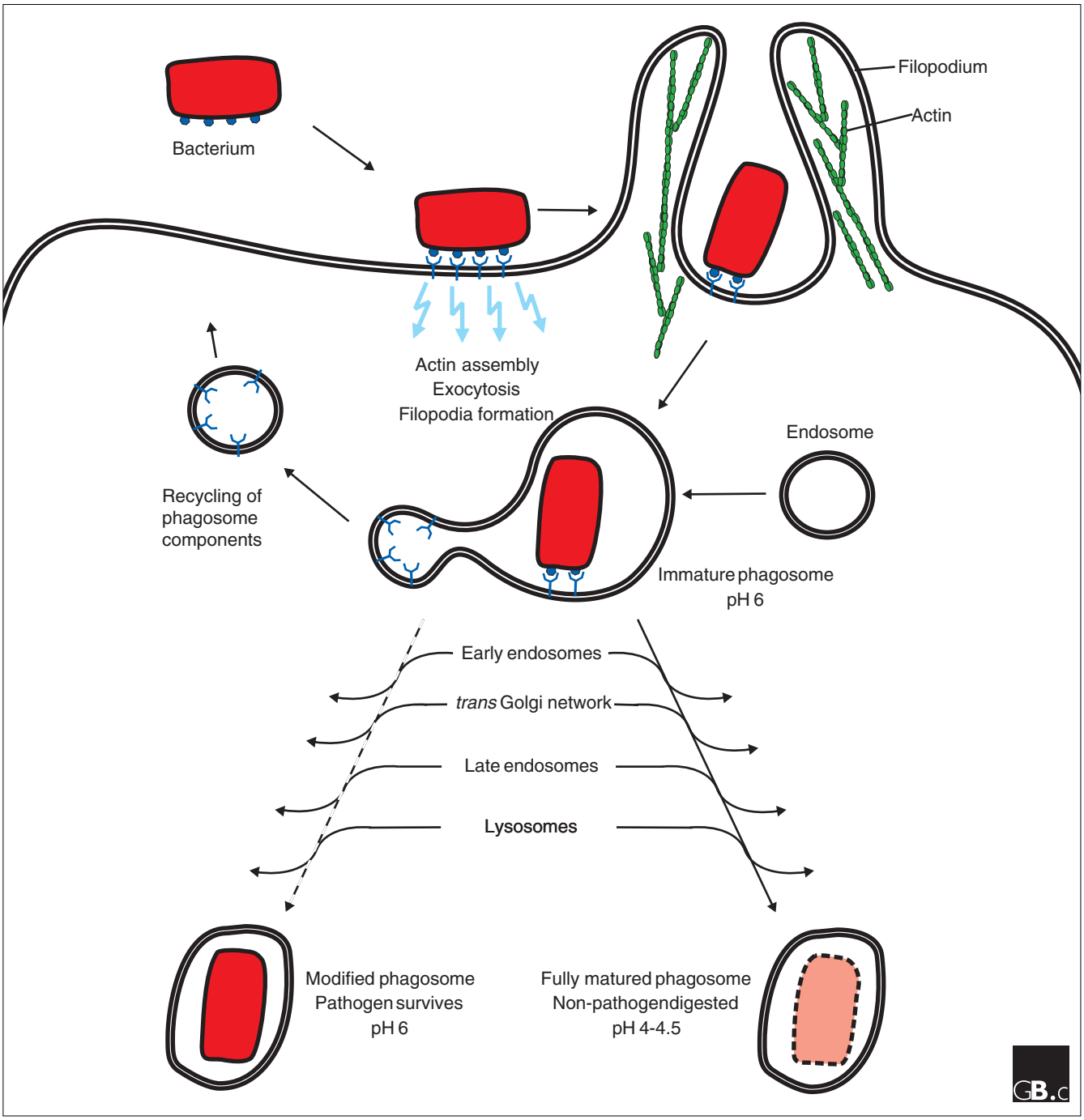

Figure 1
The key stages in phagocytosis and phagosome maturation. A microbe initially binds via molecules on its surface to receptors on the plasma membrane of the phagocyte. This activates the receptors, causing the initiation of intracellular signaling pathways, most prominently those leading to the membrane-dependent assembly of actin filaments and the exocytosis of various membrane compartments. These poorly understood processes in turn lead to the outgrowth of membrane-bounded projections, filopodia, that engulf the pathogen to form the phagosome, a cytoplasmic compartment containing the pathogen and bounded by a single membrane. Subsequent actin- and microtubule-dependent transport leads to the sequential fusion of the phagosome with other membrane-bounded compartments such as endosomes, vesicles of the *trans* Golgi network, and lysosomes. This phagosome maturation process results in alterations in the composition of phagosome contents and membrane as the phagosome acquires molecules delivered by fusion events and loses molecules by recycling of selected components via vesicular or tubular budding. The lower right-hand side of the diagram shows the 'normal' maturation pathway of a phagosome containing a non-pathogen, which is driven by fusion and recycling events involving the organelles listed. In this phagosome, the pathogen has been killed and digested by enzymes that are active at the low pH of the mature phagosome. The lower left-hand side of the diagram shows the maturation of a phagosome containing a persisting pathogen. The dotted line indicates that some of the compartments, most notably the late endosomes and lysosomes, fail to deliver their microbicidal contents into the phagosome, and the pathogen is not killed.

analyses of LBPs in *Dictyostelium discoideum* by Gotthard *et al.* [3,4] have revealed around 1,380 proteins, of which 179 have been identified.

Latex bead phagosomes

As first shown by Wetzel and Korn in 1969 [5], phagosomes enclosing latex beads (usually 0.5-3 μm in diameter) can be easily and cleanly isolated by flotation in a sucrose gradient. The enclosed beads float upwards against a strong centrifugal force, which enables LBPs to be purified to a level of contaminants of less than a few percent [3,6]. LBPs are isolated in one step, whereas all other membrane-bounded organelles require multiple steps of purification.

In the presence of ATP and other necessary components, isolated LBPs have been shown to be able to carry out most phagosome functions. They will fuse with endosomes and lysosomes, bind microtubules, move along microtubules, promote the assembly of actin filaments and bind to them, and become acidified [7,8]. Phagosomes containing non-pathogenic *M. smegmatis*, but not those containing the pathogens *M. tuberculosis* and *M. avium*, have also been shown to assemble actin [9], confirming that LBPs are a good model for providing insights into the behavior of phagosomes containing non-pathogenic bacteria.

Proteomic analyses of LBPs

One of the first proteomic studies using LBPs was that of Garin *et al.* [2], who determined a partial proteome of LBPs in the mouse J774 macrophage cell line 2 hours after internalization and identified 171 phagosome proteins. A continuation of this analysis has since identified more than 800 of the estimated 1,000 proteins in mouse macrophage phagosomes (M Desjardins, personal communication). Burlak *et al.* [10] identified about 200 proteins in a proteomic analysis of LBPs from human neutrophils. As well as mammalian studies, LBPs have been used to analyze phagocytosis in other organisms. Marion *et al.* [11] carried out a proteomic analysis on phagosomes isolated from the human protozoan pathogen *Entamoeba histolytica* using magnetic beads coated with human serum. Around 150 proteins were identified, including myosins and other actin-binding proteins. LBPs have also been used in extensive proteomics analyses of phagosomes from *Drosophila* [1] and *Dictyostelium* [3,4,12], which are described in more detail below.

Proteins of similar function are consistently detected in all the phagosomes studied. In mature phagosomes, major classes of luminal proteins include hydrolases and other bacteriocidal proteins. In the phagosome membrane are found the various subunits of the proton transporter H^+-ATPase, other transporters and ion channels, heterotrimeric G proteins, monomeric GTPases of the Rab and Rho families, SNARE fusion machinery, actin-binding and microtubule-binding proteins, clathrin and COP proteins of vesicle coats, and a spectrum of signaling proteins such as protein kinase C and phospholipase D (PLD). PLD is only one of many lipid-converting enzymes that are active in the LBP membrane [8,9]. Collectively, these analyses leave no doubt that the phagosome, even when it contains only an inert bead, is a complex signaling machine.

A systems approach to understanding phagosome function and phagocytosis

In *Dictyostelium,* phagocytic uptake of latex beads can be highly synchronized, enabling a detailed kinetic analysis. In contrast to phagocytosis in mammalian cells, in which the particles, or their remains, usually stay within the cells, *Dictyostelium* phagosomes synchronously exocytose their contents about one hour after uptake. This is a clear signal that the maturation is complete. In their most recent proteomic analysis of *Dictyostelium* LBPs, Gotthardt *et al.* [4] made a detailed analysis of six different phagosome maturation stages, differentiating a total of 1,388 phagosome protein spots on two-dimensional gel electrophoresis. The analysis revealed a fascinating, and hitherto unexpected, dynamic record of phagosome maturation. Sets of phagosome proteins were identified that were up- or downregulated on phagosomes at well-defined times in the maturation cycle. For example, a comparison of LBPs isolated after 5 minutes with those isolated after 15 minutes revealed that 469 protein spots present at the earlier time had disappeared from the 15-minute phagosome (presumably by recycling or degradation) whereas 130 proteins had appeared at 15 minutes that were absent earlier. Identification of the complete phagosome proteome is still in progress.

In their impressive study of LBPs in *Drosophila* cells, Stuart *et al.* [1] also took a systems-biology approach. Having first identified 617 LBP proteins, they extended the analysis using both RNA interference (RNAi), to knock down protein expression, and bioinformatics. Bioinformatic approaches were used to identify proteins that had been shown to interact with the 617 identified LBP proteins. The rationale was that this 'interactome' would identify phagosome proteins that interact only transiently or weakly with identified phagosome proteins. Such proteins do not co-purify with phagosomes, but might be functionally very important. The interaction map shows an impressive set of linked proteins, with a number of functional classes that one would not have expected on phagosomes, although some were suggested in the earlier proteomic analyses, such as components of the spliceosome and of protein translation machinery, whose role in phagosomes remains to be demonstrated. Less surprising was the presence of proteasome and chaperone proteins, which fitted with earlier functional analyses [13,14]. One protein complex found by Stuart *et al.* [1] that had not been noticed on phagosomes previously was

the exocyst complex, which controls some exocytic docking and fusion events.

Extensive RNAi screening was used to selectively knock down the 617 LBP proteins, and 220 additional proteins predicted from the interactome to test their potential roles in the phagocytosis of the Gram-negative bacterium *Escherichia coli* and the Gram-positive *Staphylococcus aureus* [1]. The fact that 28% of the RNAs tested affected the process of uptake, either increasing or decreasing bacterial uptake, strongly validates the initial screening with LBPs and the interactome analysis. RNAi also confirmed a role in phagocytosis for several proteins of the exocyst complex. Interestingly, there was considerable divergence between the sets of interfering RNAs that affected phagocytosis of *S. aureus* and *E. coli*, respectively. Both positive and negative regulators of phagocytosis were identified, a number of which were specific to one of the two pathogens. Some of the genes identified and their effects were unexpected. For example, the knock down of a ribosomal protein increased the phagocytosis of both bacteria. The power of this kind of analysis is that it gives rise to a rich spectrum of molecular hypotheses that can drive the entire field.

The LBP has emerged as an excellent model for studying the biogenesis of a membrane organelle. It is discrete and easily defined, unlike, for example, endosomes, and is straightforward to isolate. It has additional advantages, including the ease with which phylogenetic comparisons can be made, as exemplified by the ongoing proteomic analyses of *Dictyostelium*, *Entamoeba*, *Drosophila*, mouse and human phagosomes. Phagosomes can also be compared from host cells of different genetic background. Because of the distinct sequence of phagosome maturation, it is much easier to analyze phagosomes in different functional states than it is for other organelles. Finally, given that the type of ligand that induces phagocytosis helps determine the final fate of the phagosome, LBPs can be used to study the effect of different ligands (such as IgG, complement, or mannose) and different receptors on phagosome behavior.

References

1. Stuart LM, Boulais J, Charriere GM, Hennessy EJ, Brunet S, Jutras I, Goyette G, Rondeau C, Letarte S, Huang H, *et al.*: **A systems biology analysis of the *Drosophila* phagosome.** *Nature* 2007, **445:**95-101.
2. Garin J, Diez R, Kieffer S, Dermine JF, Duclos S, Gagnon E, Sadoul R, Rondeau C, Desjardins M: **The phagosome proteome: insight into phagosome functions.** *J Cell Biol* 2001, **152:**165-180.
3. Gotthardt D, Dieckmann R, Blancheteau V, Kistler C, Reichardt F, Soldati T: **Preparation of intact, highly purified phagosomes from *Dictyostelium*.** *Methods Mol Biol* 2006, **346:**439-448.
4. Gotthardt D, Blancheteau V, Bosserhoff A, Ruppert T, Delorenzi M, Soldati T: **Proteomics fingerprinting of phagosome maturation and evidence for the role of a Galpha during uptake.** *Mol Cell Proteomics* 2006, **5:**2228-2243.
5. Wetzel MG, Korn ED: **Phagocytosis of latex beads by *Acanthamoeba castellanii* (Neff). 3. Isolation of the phagocytic vesicles and their membranes.** *J Cell Biol* 1969, **43:**90-104.
6. Desjardins M, Huber LA, Parton RG, Griffiths G: **Biogenesis of phagosomes proceeds through a sequential series of interactions with the endocytic apparatus.** *J Cell Biol* 1994, **124:**677-688.
7. Desjardins M, Griffiths G: **Phagocytosis: latex leads the way.** *Curr Opin Cell Biol* 2003, **15:**498-503.
8. Defacque H, Bos E, Garvalov B, Barret C, Roy C, Mangeat P, Shin HW, Rybin V, Griffiths G: **Phosphoinositides regulate membrane-dependent actin assembly by latex bead phagosomes.** *Mol Biol Cell* 2002, **13:**1190-1202.
9. Anes E, Kühnel MP, Bos E, Moniz-Pereira J, Habermann A, Griffiths G: **Selected lipids activate phagosome actin assembly and maturation leading to killing of pathogenic mycobacteria.** *Nature Cell Biol* 2003, **5:**793-802.
10. Burlak C, Whitney AR, Mead DJ, Hackstadt T, Deleo FR: **Maturation of human neutrophil phagosomes includes incorporation of molecular chaperones and endoplasmic reticulum quality control machinery.** *Mol Cell Proteomics* 2005, **5:**620-634.
11. Marion S, Laurent C, Guillen N: **Signalization and cytoskeleton activity through myosin IB during the early steps of phagocytosis in *Entamoeba histolytica*: a proteomic approach.** *Cell Microbiol* 2005, **7:**1504-1518.
12. Gotthardt D, Warnatz HJ, Henschel O, Bruckert F, Schleicher M, Soldati T: **High-resolution dissection of phagosome maturation reveals distinct membrane trafficking phases.** *Mol Biol Cell* 2002, **13:**3508-3520.
13. Houde M, Bertholet S, Gagnon E, Brunet S, Goyette G, Laplante A, Princiotta MF, Thibault P, Sacks D, Desjardins M: **Phagosomes are competent organelles for antigen cross-presentation.** *Nature* 2003, **425:**402-406.
14. Lee WL, Kim MK, Schreiber AD, Grinstein S: **Role of ubiquitin and proteasomes in phagosome maturation.** *Mol Biol Cell* 2005, **16:**2077-2090.