

TB or not TB: Calcium Regulation in Mycobacterial Survival

William S. Trimble¹ and Sergio Grinstein^{1,*}

¹Program in Cell Biology, Hospital for Sick Children, and Department of Biochemistry, University of Toronto, 555 University Avenue, Toronto, Canada M5G 1X8

*Correspondence: sga@sickkids.ca

DOI 10.1016/j.cell.2007.06.039

Mycobacterium tuberculosis (*Mtb*)—the bacterium that causes tuberculosis—resides in phagosomes inside macrophages. This bacterium evades destruction by preventing phagosome maturation, which involves the fusion of phagosomes with lysosomes. In this issue of *Cell*, Jayachandran et al. (2007) suggest that mycobacteria co-opt the actin-binding protein coronin 1 to activate the phosphatase calcineurin, thereby preventing phagosomal maturation.

Tuberculosis, a disease caused by active infection with *Mycobacterium tuberculosis* (*Mtb*), is a major health threat. The World Health Organization estimates that one in three humans is a carrier infected with dormant *Mtb* and that 1.6 million will die annually from the disease (<http://www.who.int/tb/en/>). The recent appearance of highly drug-resistant strains poses an even greater threat, as these are refractory to most treatments. Thus, there is an urgent need to devise alternative

therapeutic strategies aimed at bolstering the host immune response to *Mtb*, which will require a better understanding of how *Mtb* survives in host macrophages. The report by Pieters and colleagues (Jayachandran et al., 2007) in this issue of *Cell* offers a timely step in this direction.

Mtb becomes internalized into macrophages via phagocytosis and resides in phagosomes, where it interferes with the fusion of the phagosomes with lysosomes (i.e., phagosomal maturation) to escape

destruction (Figure 1A). Jayachandran et al. (2007) expose links between mycobacterial survival and coronin 1-dependent activation of the calcium-dependent protein phosphatase 2B, calcineurin, in macrophages. Pieters and colleagues had previously shown that coronin 1 (an actin-binding protein formerly termed “TACO”) was persistently recruited to phagosomes containing mycobacteria. They speculated that coronin 1 functions as a physical barrier that blocks

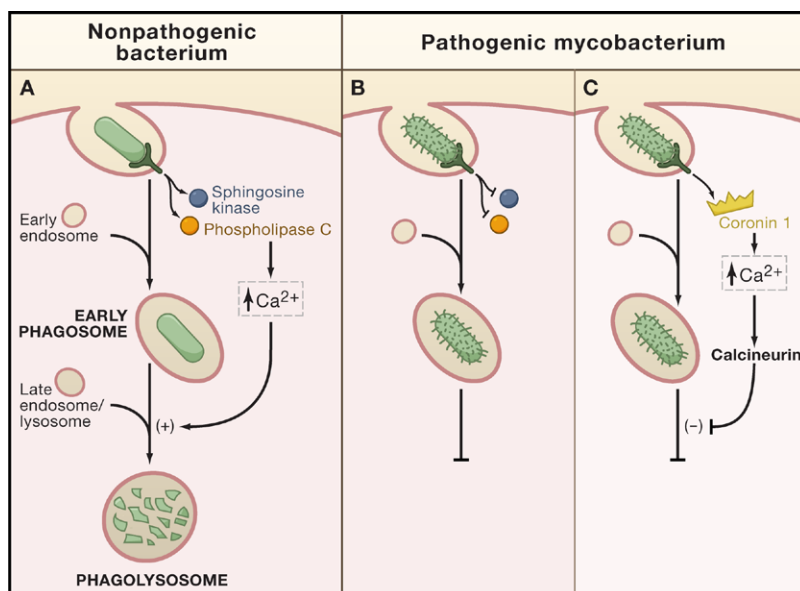


Figure 1. How Mycobacteria Evade Destruction

Mechanisms of maturation of phagosomes containing nonpathogenic bacteria (A) or pathogenic mycobacteria (B and C). (A) Receptors that engage the bacteria or molecules on its surface activate sphingosine kinase and/or phospholipase C (PLC), which induce an increase in the amount of cytosolic free calcium ions (Ca^{2+}). Nascent phagosomes fuse with early endosomes to generate early phagosomes, which in turn fuse with late endosomes/lysosomes to become phagolysosomes. The latter step is thought to require elevated Ca^{2+} . Lysosomal enzymes in the phagolysosome destroy the bacterium.

(B) In the presence of pathogenic mycobacteria, such as *Mycobacterium tuberculosis*, the conventional model states that bacterial components, possibly lipoarabinomannan, preclude the elevation of Ca^{2+} and thereby prevent fusion with late endosomes/lysosomes, arresting maturation at the early phagosome stage.

(C) In the model proposed by Jayachandran et al. (2007), receptors induce an elevation of Ca^{2+} by a process that requires the actin-

binding protein coronin 1. The rise in Ca^{2+} activates calcineurin, which blocks fusion of early phagosomes with late endosomes/lysosomes by an unknown mechanism. Both models would allow the bacterium to persist in macrophages.

fusion with lysosomes, thereby preventing phagosome maturation (Ferrari et al., 1999). However, as reported in the present study macrophages from coronin 1-deficient mice are capable of killing *M. bovis* (*Mb*), a close relative of *Mtb*, despite having actin dynamics and cell migratory properties that are indistinguishable from those of wild-type mice. Given that abnormal actin dynamics and migratory properties of T cells were previously observed in coronin 1-deficient mice, the latter findings are somewhat surprising (Foger et al., 2006). Jayachandran et al. further show that *Mb* infection in normal macrophages causes a persistent rise in the concentration of intracellular calcium ions leading to the activation of calcineurin, and that these events are absent in coronin 1-deficient macrophages. Furthermore, they report that inhibiting calcineurin with pharmacological agents also leads to *Mb* killing, suggesting that under normal circumstances activation of calcineurin, mediated by coronin 1-dependent calcium elevation, prevents *Mb* phagosome maturation, thereby allowing persistent infection.

This is not the first study to suggest a link between calcium signaling and mycobacterial infection. Indeed, other groups had shown that calcium signaling is altered in macrophages infected by mycobacteria. However, in most reported cases the elevation of calcium ions that normally accompanies phagocytosis of other bacteria or inanimate particles was reduced or absent when mycobacteria were the target (Majeed et al., 1998; Malik et al., 2000; Vergne et al., 2003) (Figure 1B). The inhibitory effect of mycobacteria has been attributed to the glycolipid mannosylated lipoarabinomannan (Vergne et al., 2003 and references therein). Lipoarabinomannan presumably impairs signaling by the sphingosine kinase that is thought to mediate the transient increase in calcium ions normally associated with phagocytosis of nonvirulent particles (Malik et al., 2000). In stark contrast to all the

preceding studies, Jayachandran et al. find that the amount of cytosolic free calcium ions undergoes a marked and persistent increase in cells infected by mycobacteria and that the increase is blunted in coronin 1-deficient cells. The observations of Jayachandran et al. seem to imply that elevation of calcium ion levels is an essential component of the mechanism whereby the mycobacteria arrest phagosomal maturation. They attribute the inhibitory effect of calcium ions on phagosome maturation to calcineurin, because in vitro assays show that calcineurin is activated in coronin 1-expressing cells exposed to *Mb* (Figure 1C). This conclusion was buttressed by the observation that cyclosporin A and FK506, potent inhibitors of calcineurin, bypassed the arrest in phagosome maturation.

When compared to all the preceding reports, the calcium responses reported by Jayachandran et al. are perplexing. The differences cannot be attributed to the use of murine cells or unopsonized bacteria, as reduced responses to calcium ions were reported by other groups using phagocytes from various sources and both mycobacteria that were coated or uncoated by opsonins. The conclusion that calcium ions play an inhibitory role in phagosome maturation is equally unprecedented. Based on the known effects of calcium on membrane fusion in a large variety of systems, it was generally assumed that completion of phagolysosome formation was similarly calcium dependent. This assumption was validated experimentally by Kusner's group (Malik et al., 2000), who found that maturation was arrested when changes in cytosolic calcium ion levels were precluded by buffering agents. That synaptotagmin VII, a calcium-dependent effector, is required for phagolysosome formation further supports the contention that maturation requires calcium ions (Czibener et al., 2006).

Thus, a profound conceptual chasm appears to exist between the earlier observations and those

published here by Jayachandran and colleagues. In the former studies the bacteria are seen as impairing both the normal increase in the amount of calcium ions and alternative methods of elevating cytosolic calcium levels—such as the stimulation of purine receptors or the use of ionophores—promote phagosome acidification and bacterial killing (Kusner and Barton, 2001; Malik et al., 2000; Stober et al., 2001). In marked contrast Jayachandran et al. find that the calcium ionophore calcimycin (commonly known as A23187) restores the infectivity of *Mb* in coronin 1-deficient cells. The implication that calcium inhibits phagosome maturation is remarkable in that mycobacterial infections last for months. For inhibition to be sustained, the elevated cytosolic concentration of the calcium cation would need to persist throughout the entire infection period. It is unclear what mechanism(s) sustain the calcium elevation for such an inordinately long period and how macrophages avoid undergoing apoptosis, a common consequence of persistent calcium elevation in other cell types.

The report of Jayachandran et al. presents a radically new viewpoint of mycobacterial infection. Additional work will be required to better establish the relationship between coronin 1 and calcium homeostasis and to define the mode of action of calcineurin in phago-lysosome formation. Such studies may reconcile the apparent discrepancies with the preceding literature and may also explain why in other systems coronin 1 was not found to have a role in the arrest of phagosomal maturation produced by mycobacteria (Schuller et al., 2001; Solomon et al., 2003).

REFERENCES

- Czibener, C., Sherer, N.M., Becker, S.M., Pypaert, M., Hui, E., Chapman, E.R., Mothes, W., and Andrews, N.W. (2006). *J. Cell Biol.* 174, 997–1007.
- Ferrari, G., Langen, H., Naito, M., and Pieters, J. (1999). *Cell* 97, 435–447.

Foger, N., Rangell, L., Danilenko, D.M., and Chan, A.C. (2006). *Science* 313, 839–842.

Jayachandran, R., Sundaramurthy, V., Combaluzier, B., Korf, H., Huygen, K., Miyazaki, T., Albrecht, I., Massner, J., and Pieters, J. (2007). *Cell*, this issue.

Kusner, D.J., and Barton, J.A. (2001). *J. Immunol.* 167, 3308–3315.

Majeed, M., Perskvist, N., Ernst, J.D., Orselius, K., and Stendahl, O. (1998). *Microb. Pathog.* 24, 309–320.

Malik, Z.A., Denning, G.M., and Kusner, D.J. (2000). *J. Exp. Med.* 191, 287–302.

Schuller, S., Neefjes, J., Ottenhoff, T., Thole, J., and Young, D. (2001). *Cell. Microbiol.* 3, 785–793.

Solomon, J.M., Leung, G.S., and Isberg, R.R. (2003). *Infect. Immun.* 71, 3578–3586.

Stober, C.B., Lammas, D.A., Li, C.M., Kumaratne, D.S., Lightman, S.L., and McArdle, C.A. (2001). *J. Immunol.* 166, 6276–6286.

Vergne, I., Chua, J., and Deretic, V. (2003). *J. Exp. Med.* 198, 653–659.

Bicoid by the Numbers: Quantifying a Morphogen Gradient

Matthew C. Gibson^{1,*}

¹Stowers Institute for Medical Research, 1000 East 50th Street, Kansas City, MO 64110, USA

*Correspondence: mg2@stowers-institute.org

DOI 10.1016/j.cell.2007.06.036

Morphogen gradients are typically analyzed from static images of fixed embryonic tissues. Two papers in this issue of *Cell* now report live imaging of the Bicoid gradient in developing fruit fly embryos (Gregor et al., 2007a, 2007b). Their findings indicate that the gradient is highly reproducible from embryo to embryo and reveal that the nuclear dynamics of Bicoid are critical for maintaining precision within the gradient.

The existence of morphogens, diffusible substances that can induce differential gene expression in a concentration-dependent manner, was suspected well before any molecular basis for their action was identified (e.g., Crick, 1970). “One is acutely conscious of the absence of the physiological and molecular basis of positional information and polarity,” wrote Lewis Wolpert in the closing section of his seminal 1969 exposition on positional information. “But unless the correct questions are asked one has little hope of finding out how genetic information is interpreted in terms of spatial patterns” (Wolpert, 1969). In the intervening years, many of the correct questions have been asked and answered, primarily through the marriage of genetics with qualitative methods for the visualization of mRNA and proteins in situ. The analysis of spatial patterning is now moved another important step forward thanks to a pair of papers in this issue that employ quantitative live imaging to establish

the temporal and spatial dynamics of a morphogen gradient in embryos of the fruit fly *Drosophila melanogaster* (Gregor et al., 2007a, 2007b).

During early development of the *Drosophila* embryo, a maternally supplied anterior cache of mRNA encoding the homeodomain transcription factor Bicoid (Bcd) is translated and gives rise to a morphogen gradient of Bcd protein along the embryonic anterior-posterior (A/P) axis (Driever and Nüsslein-Volhard, 1988a, 1988b). This transcription factor gradient is interpreted with remarkable precision such that target genes like Hunchback (Hb) are switched on in highly reproducible domains and trigger a subsequent developmental cascade that varies minimally from embryo to embryo (Driever and Nüsslein-Volhard, 1989; Struhl et al., 1989).

The spectacular initial discovery of the Bicoid gradient conferred molecular genetic respectability upon morphogens as critical regulators of embryonic pattern formation (see

Ephrussi and St. Johnston, 2004) and sparked a sustained period of intense interest in identifying other morphogens and understanding their roles in development and disease. Today, although genetics and imaging have certainly advanced our understanding of morphogen gradients, sizable gaps in knowledge still remain, even in the case of the venerable Bicoid. One fundamental limitation is that genetic analysis and qualitative imaging of fixed tissue do not always foster a quantitative understanding of the fine-scale dynamics and precise concentration thresholds central to the morphogen concept, leaving some critical questions unanswered. For example, given the rapid pace of early development and the heterogeneity of the embryonic milieu, how rapidly and how reproducibly is the Bcd gradient established? How can the Bcd profile accommodate both rapid proliferation (an increase in the number of nuclei) and a gen-