Cell Host & Microbe
Previews

Regulation of Protein Secretion by … Protein Secretion?

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DOI 10.1016/j.chom.2008.08.009

Mycobacterium tuberculosis (Mtb) requires an alternative protein secretion system, ESX1, for virulence. Recently, Raghavan et al. (2008) reported a new regulatory circuit that may explain how ESX1 activity is controlled during infection. *Mtb* appears to regulate ESX1 by modulating transcription of associated genes rather than structural components of the secretion system itself.

All bacterial pathogens face similar challenges within the infected host. They must survive the onslaught of innate and adaptive immunity, acquire nutrients, multiply, and be transmitted. In a diverse microbial universe, such similar problems might be solved in very different ways. Yet many pathogens seem to have converged on a few solutions. For example, many bacterial pathogens use specialized secretion systems to translocate effector molecules into their host cells in order to manipulate host cell targets. The type III and type IV secretion systems are found in a variety of gram-negative pathogens and are required for the virulence of these organisms. In both systems, bacteria secrete and assemble components of a complex extracellular machine. They then use these machines to inject, in needle-like fashion, effectors across host cell membranes into privileged host cell compartments.

Mycobacterium tuberculosis, which causes the clinical disease tuberculosis, is an intracellular pathogen of macrophages and like other intracellular pathogens, *Mtb* modulates many host cell functions to its own advantage. Because *Mtb* alters various macrophage processes while growing within a phagolysosomal vacuole, it seems reasonable to expect that *Mtb* might contain a functional ortholog of the type III and type IV secretion systems. While no such system has been identified, there is a contender. The ESX1 secretion system is an alternative protein secretion system that is required for *Mtb* to survive and grow in macrophages and animals. The biologic importance of ESX1 is highlighted by the finding that the primary attenuating deletion in BCG, the vaccine strain of *Mtb*, is the loss of nine genes from the heart of the ESX1 locus (Lewis et al., 2003; Mahairas et al., 1996). While obviously of biologic importance, the molecular details of ESX1 function are not clear and major questions remain. Do ESX1 components assemble into a needle-like translocon? Does the system secrete effector molecules into the host cell and if so, what are these effectors and what are their targets? Or are we imposing an appealing model on a system that functions in a fundamentally different way?

Indeed ESX1 differs in some ways from the better-studied type III and IV secretion systems. ESX1 is only one of five homologous ESX secretion systems in the *Mtb* genome (Gey Van Pittius et al., 2001). Like the other ESX genes, genes in the ESX1 locus are constitutively expressed under all studied growth conditions, and there is no evidence that ESX1 expression is induced in vivo (Schnappinger et al., 2003; Talaat et al., 2007). Furthermore, it is striking that ESX1 seems constitutively active under in vitro conditions, whereas effectors of the type III and type IV systems are produced and secreted in a carefully orchestrated manner specifically during infection. These observations raise the possibility that since *Mtb* is an obligate human pathogen, it may not have acquired a pathway to switch on and off its virulence systems.

However, this month in *Nature*, Raghavan and colleagues identify an intriguing new pathway that at least partially explains how ESX1 activity might be regulated during infection (Raghavan et al., 2008).

The key to this regulatory circuit is a novel transcription factor, EspR, which positively regulates the expression of a five gene operon, Rv3616c-3612c, which is unlinked to the ESX1 locus. Two genes in the locus, Rv3615c and Rv3614c, are independently required for ESX1-mediated secretion (MacGurn et al., 2005). One of the gene products, Rv3616c (also known as EspA), is one of the four known substrates of the apparatus. Since all four of these proteins are secreted in a codependent fashion (Fortune et al., 2005), titration of the Rv3616c-14c operon is sufficient to regulate ESX1 activity—at least as we understand it. These observations complement recent data showing that a two-component regulatory system, PhoP/R, also regulates ESX1 function by controlling the expression of genes in the Rv3616-Rv3612c locus (Frigui et al., 2008).

Thus, *M. tuberculosis* appears to regulate the function of ESX1 by modulating Rv3616c-3612c transcription instead of the structural components of the secretion system itself. Perhaps regulation of this relatively small number of ESX1-associated proteins allows dynamic regulation of ESX1 activity without requiring the bacterium to rapidly assemble and disassemble the core secretion machine, which could be a relatively difficult and costly undertaking given the complexity of the mycobacterial cell wall. It is also possible, however, that Rv3616c-Rv3612c serves a more specific virulence function than the ESX1 secretion system as a whole. In *Mycobacterium marinum,* a waterborne pathogenic mycobacterium whose natural hosts range from amoebae to frogs and fish, there has been a dramatic reduplication of Rv3616c homologs without expansion of any of the other ESX1 genes, raising the possibility that the eighteen homologs of Rv3616c provide host, tissue, or milieu specificity to ESX1 secretion (Stinear et al., 2008).

Interestingly, Raghavan et al. also show that the regulation of Rv3616c-Rv3612c expression is directly linked to ESX1 function in an unusual way. Not only is EspR a DNA-binding protein that positively regulates Rv3616c-Rv3612c transcription, but it is also a substrate of the ESX1 apparatus. Thus, when ESX1 is active, it secretes EspR and shuts itself down. It is notable that other bacteria use a similar strategy to regulate alternative secretion systems. In both flagellar assembly and the evolutionarily related type III secretion system of *Pseudomonas*, the apparatus secretes negative regulators of substrate expression allowing the bacteria to rapidly increase the amount of substrate when secretion is triggered (Brutinel and Yahr, 2008).

Some observations suggest that in *Mtb* this regulatory loop is likely to be more complex and involve other proteins and interactions. First, deletion of the core secretion apparatus, which should lead to cytosolic accumulation of EspR and concomitant transcriptional changes, has been shown to have a minimal effect on gene expression. Second, in contrast to the regulatory loops associated with type III secretion and flagellar assembly, *Mtb* secretes a positive transcriptional regulator. This creates a negative feedback loop in which a burst of ESX1 secretion is presumably followed by ESX1 inactivation. The predicted pattern of oscillating secretion is somewhat at odds with the observation that ESX1 is constitutively active, at least in vitro. There are a couple of possible explanations. First, the discrepancy may come from studying a population of bacteria where secretion in each individual bacterium is oscillating in an unsynchronized fashion. Alternatively, other factors in vivo may affect EspR expression such that after a period of ESX1 activity, secretion is permanently turned off.

And why might the bacteria turn off ESX1 secretion during infection, especially when ESX1 seems required for bacteria to survive in macrophages? Ragahavan et al. point out that many ESX1 substrates are strong T cell antigens and that downregulation of ESX1-mediated secretion may allow the bacteria to become antigenically silent (Raghavan et al., 2008). If so, this could explain why some bacteria survive in the face of an apparently robust antigen-specific immune response. Alternatively, ESX1-mediated secretion might be important at certain times during infection (e.g., for survival in the phagolysosome) but deleterious at other times (e.g., growth in caseum). This again would suggest that individual cells might be turning secretion on and off even when population analysis suggests that expression is ongoing. In either case, single-cell studies might be required to establish the conditions of regulation.

The big question remains—what is the function of ESX1? While studies such as this do not elucidate the reason that ESX1 is required for virulence, they can help to determine the circumstances under which these proteins are required. Thus, while we do not yet know the answer, we can begin to establish guilt by association.

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