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## Protein Localization: Reach out and Touch the Forespore

**Bacterial proteins are typically sorted to subcellular regions with distinct physical characteristics that serve as cellular ‘addresses’, but many proteins are evidently sorted to specific areas that lack any apparent unique identity. Recent work in *Bacillus subtilis* suggests that such proteins may be localized by interacting with extracellular domains of proteins in an adjacent cellular compartment.**

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The proper localization of a protein depends on two parameters. First, the protein itself must harbor a localization signal that specifies its ultimate destination. Second, this destination must harbor a chemical landmark that distinguishes it from other regions of the cell [1]. Some proteins, however, are targeted to specific subcellular locations with no obvious unique physical characteristic. In bacteria, these regions are often patches of membrane, either at the cell poles or at recently created cell division septa. To date, it is unclear how a cell distinguishes these subcellular sites from other sites in the cell. Two recent studies [2,3] have revealed a mechanism by which this protein localization occurs during the process of sporulation in the Gram-positive bacterium *Bacillus subtilis*.

A hallmark of sporulation is the formation of an asymmetrically positioned division septum (the polar septum) which divides the developing cell into two adjacent, but unequal-sized compartments called the forespore (the smaller cell) and the mother cell [4,5] (Figure 1). Surrounding the cells is the bacterium’s cell wall, which

keeps the forespore and the mother cell adjoined. The polar septum that separates the two cells initially contains a layer of peptidoglycan, but this cell wall material is degraded shortly after its formation, leaving the septal membranes of the mother-cell and forespore in close proximity.

The mother cell is known to elaborate a large number of proteins that come to localize on the mother-cell face of the division septum [6–8]. Evidence indicates that these proteins are initially inserted indiscriminately into the membrane surrounding the mother cell and are rapidly recruited to the polar septum by a diffusion-and-capture mechanism [9].

In subsequent development, the septal membrane of the mother cell migrates around the forespore in a phagocytic-like process that eventually results in complete engulfment of the forespore within the cytoplasm of the mother cell. Proteins that have been deposited on the mother-cell face of the septum remain associated with the septal membrane during this encapsulation process such that when engulfment is complete the forespore is fully enveloped by membrane decorated with proteins that had originally been localized to the polar septum.

Prior to engulfment, the septal membrane is contiguous with the

remainder of the plasma membrane and delineates the outer boundary of the mother cell. What then is special about the septal membrane that provides a unique chemical environment for the capture of specific sporulation proteins? Perhaps the septal membrane is embedded with certain proteins that provide a landmark for the recruitment of other proteins. But if such landmark proteins exist — and, as we shall see, at least one such landmark protein has been identified — this merely begs the question: how do septal landmark proteins come to localize specifically to one patch of membrane in the mother cell?

The reports by Blaylock *et al.* [2] and by Doan *et al.* [3] indicate that the answer lies in the fact that the septal membrane is adjacent to the forespore, whereas the remainder of the plasma membrane faces cell wall. Remarkably, the extracellular domain of an integral membrane protein called SpoIIQ (henceforth simply Q) produced in the forespore directly contacts, and thereby anchors, the extracellular domain of an integral membrane protein called SpoIIIAH (henceforth simply AH) from the mother cell. (Indeed, the recognition that Q has an extracellular domain prompted the suggestion some years ago that it might be able to interact with proteins in the mother cell [10].) In other words, AH and Q reach out to each other across the two cells, thereby anchoring AH specifically in the patch of membrane that is within contact with its counterpart in the forespore.

Blaylock *et al.* [2] found that, in the absence of Q, AH does not localize to the septum, but is

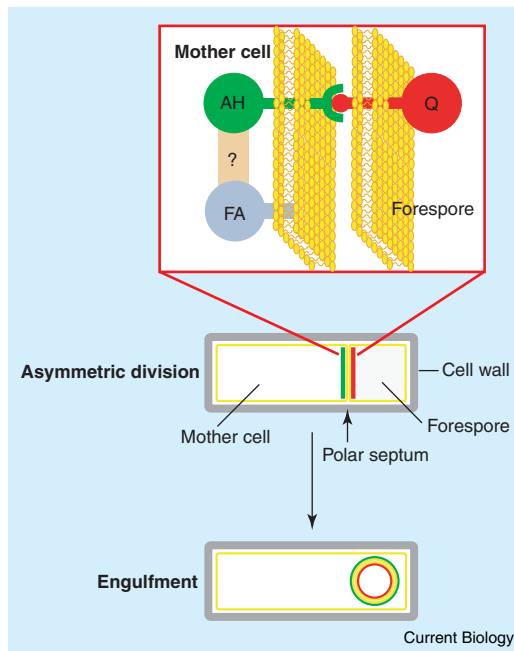


Figure 1. Protein localization is mediated by protein–protein interaction in the space between the forespore and the mother cell.

The center cartoon (asymmetric division) depicts a developing cell shortly after the start of sporulation which has been divided into adjacent mother-cell and forespore compartments by the formation of a polar septum. The restricted localization of the mother-cell membrane protein AH (SpoIIAH) to the mother-cell face of the septum is indicated in green, and the localization of the forespore membrane protein Q (SpoIIQ) to the forespore side of the septum is indicated in red; the plasma membrane is indicated in yellow. The bottom cartoon depicts the next stage of

development, when the forespore has been pinched off as a free protoplast within the mother cell cytoplasm as a result of the process of engulfment. The cartoon at the top is an expansion of the polar septum from the center cartoon. Shown in yellow are the adjacent septal membranes from the forespore and the mother cell. The cartoon depicts the interaction between the extracellular domains of AH and Q. The mother-cell membrane protein FA (SpoIVFA) is also anchored to the mother-cell face of the septum (at least in part) through an indirect interaction with AH that is mediated by an unknown protein labeled with a question mark.

instead uniformly distributed in the membrane surrounding the mother cell. Both proteins are thought to be oriented in their respective membranes, such that their amino termini reside in the cytosol, whereas their carboxyl termini extend into the space adjoining the two cells. Yeast two-hybrid analysis suggested that these extracellular domains display an affinity for each other, and copurification experiments confirmed biochemically that Q and AH do in fact interact [2,3].

It thus became plausible that AH identifies the septal membrane by its ability to bind to Q on the opposite side of the septum. As a further test of whether such an interaction is spatially possible, Blaylock *et al.* [2] examined AH localization in a mutant that is defective in the degradation of the layer of peptidoglycan that initially separates mother cell from the forespore. They reasoned that the continued presence of the cell wall between the two daughter cells might sterically hinder interaction between AH and Q. Consistent

with this hypothesis, they discovered that AH was largely mislocalized in the mutant, but continued to interact with the septum only at those points where the cell wall had been removed. Taken together, the data indicate that the polar septum is indeed a unique location in the cell. As the only region of the mother-cell membrane proximal to the forespore, the polar septum alone is able to allow interaction with proteins in the forespore, providing a chemical landmark by which proteins may identify a unique patch of membrane (Figure 1).

Might AH, in turn, serve as a landmark for the recruitment of other proteins in the mother cell to the septum? Doan *et al.* [3] addressed this question by investigating the basis for the septal localization of another mother cell protein called SpoIVFA (henceforth FA). They systematically surveyed a library of strains mutant for other genes expressed in the mother cell for those in which localization of FA was impaired. The most prominent

localization defect was observed in a mutant lacking AH. They also observed that, in the absence of the forespore protein Q, FA no longer localizes exclusively in the septal membrane. Thus, Q appears to mediate the localization of the mother-cell protein FA. In this case, however, the role of Q appears to be indirect: neither Q nor AH reportedly display an affinity for FA. The simplest interpretation of these results is that FA indirectly contacts AH through one or more yet-to-be identified proteins in the mother cell and that AH, in turn, contacts Q.

AH may not be the only landmark protein that anchors FA to the septum; the absence of AH impairs, but does not eliminate, preferential localization of FA to the septum. Doan *et al.* [3] suggest that multiple, somewhat redundant pathways likely participate in sorting proteins to the polar septum, and that numerous protein–protein interactions that span the space separating the forespore from the mother cell form a complex septal tether. Nonetheless, the contribution of the forespore protein Q in the sorting of mother-cell proteins appears to be paramount, and the observation that it either directly or indirectly affects the localization of at least two mother-cell proteins suggests that this may be a general phenomenon.

The discovery that a protein in the forespore directs the sorting of proteins in the mother cell provides an attractive mechanism by which mother-cell proteins identify the polar septum. An outstanding question remains, however: how does Q specifically localize to the forespore face of the septum? Surprisingly, localization of Q in the forespore is not appreciably impaired by the absence of AH in the mother cell [2]. If Q does indeed recruit multiple mother-cell proteins to the septum, it is conceivable that these interactions may reciprocally anchor Q to the septum as well [11]. Accordingly, the absence of a single mother-cell protein, such as AH, may not result in a drastic mislocalization of Q. In any case, with respect to the sorting of

mother-cell proteins to the sporulation septum, a physical uniqueness that distinguishes the septal membrane from other regions of the cell seems to have been discovered. After insertion into the plasma membrane, proteins destined to reside in the polar septum know that they've arrived at their correct address when they can reach out and touch the forespore.

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## Virology: Gulliver among the Lilliputians

The discovery and genome sequencing of the mimivirus, a parasite of *Acanthamoeba*, blurs the boundary between viruses and cells: the 1.2 Mb genome of the mimivirus is predicted to contain 1262 genes and is much bigger than the genomes of many parasitic bacteria.

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The major discoveries of modern biology have come mostly through detailed molecular studies and comparative genomics. It is not common anymore, as it used to be in the 18<sup>th</sup> and 19<sup>th</sup> centuries, to discover marvelous creatures no one has ever seen before. Of course, in virology, which by definition deals with tiny intracellular parasites, the era of descriptive discoveries was delayed until the 20<sup>th</sup> century, and reports of new, sometimes unusual families of viruses continued into the new millennium [1]. Even so, the recent discovery [2] of the mimivirus, a parasite of the protozoan *Acanthamoeba polyphaga*, was entirely unexpected.

The mimivirus, the genome sequence of which has now been reported by Raoult *et al.* [3], is a true giant among viruses. Most strikingly, mimivirus crosses the boundary between viruses and cells that was considered more or less self-evident: viruses are

assumed to be tiny and to have (much) smaller genomes than cellular life forms. At 1.2 Mb and with an estimated 1262 genes, the mimivirus genome is larger than the genomes of numerous parasitic bacteria and the single known parasitic archaeon, and only slightly smaller than the genomes of the simplest free-living prokaryotes (Figure 1).

The mimivirus genome has about 2.5 times as many genes as the smallest known prokaryotic genomes, those of the bacterium *Mycoplasma genitalium* and the archaeon *Nanoarchaeon equitans*. So it does not just nudge up to the virus–cell boundary, it leaps right across it. The physical dimensions of the virion are equally impressive: the icosahedral capsid of the mimivirus is at least 400 nm in diameter, about the same size as a small bacterial cell such as *Mycoplasma* [3].

These are the dramatic numbers, but what about the actual genetic content of the giant virus genome? The first thing to note is that, despite careful computational

analysis, Raoult *et al.* [3] were able to assign homology-based functions to only 298 of the 1262 predicted genes (less than 25%). Most likely, extensive searches for subtle sequence and structural similarities will lead to additional functional assignments, but the current numbers are notably different from the typical results of analysing newly sequenced prokaryotic genomes. These days, at least for smaller bacterial and archaeal genomes, about 70% of the predicted genes have homologs with known functions [4].

Compared to prokaryotic genomes, therefore, the similar-sized genome of the mimivirus is almost like *terra incognita*. However, analysis of the evolutionary affinities and predicted functions of those genes that do have well-characterized homologs clearly shows that mimivirus did not originate from Mars, but has a lot in common with other viruses. These genes can be classified into two major categories: genes shared with all or some nucleocytoplasmic large DNA viruses (NCLDVs); and genes with prokaryotic and/or eukaryotic homologs not represented in other NCLDVs.

Earlier comparative analysis showed that the NCLDVs – which include poxviruses, iridoviruses, asfarviruses and phycodnaviruses – share a core set of conserved