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GUEST COMMENTARY

Why Spherical *Escherichia coli* Dies: the Inside Story[▽]

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It is remarkable how often what we think must be true is not. In this issue, Bendezú and de Boer (1) tackle a phenomenon that seemed to have a perfectly good explanation, only to find that another, more satisfying and more interesting mechanism is at work. The short story is that several methods used to force *Escherichia coli* to grow as spheres share a common mode of bacterial killing in which cell division is impeded because FtsZ is mislocalized. The mechanism by which this occurs is surprisingly different. The inner membrane grows faster than is required by the reduced surface area, and the excess membrane folds inward and may pinch off to form intracytoplasmic vesicles. These extraneous membrane surfaces compete for FtsZ and hinder it from initiating normal division. This description is satisfying because it unites several disparate observations and replaces a previous, more simplistic explanation. It is interesting because it identifies an unusual capability of the bacterial membrane and because it implies that syntheses of membrane and cell wall are not coregulated as strictly as previously believed.

At issue is a simple observation. *E. coli* normally grows as a uniform, straight rod. However, after deletion or inhibition of one of a few genes or proteins, *E. coli* loses its rod shape and takes on a spherical form that continues to enlarge until the cell eventually lyses (Fig. 1). The process can be triggered (i) by deleting one or more of the *mreBCD* genes or by inhibiting the MreB protein with compound A22; (ii) by deleting the *mrdA* gene that encodes PBP 2, inactivating a temperature-sensitive version of this protein, or inactivating PBP 2 with the β-lactam antibiotic amdinocillin; or (iii) by inactivating a temperature-sensitive version of the RodA protein, encoded by the *mrdB* gene (Fig. 1A) (see references in reference 1). In all these cases, *E. coli* becomes spherical and dies. Nonetheless, long-lived cultures of coccoidal *E. coli* can be derived after each of these treatments by manipulating the growth conditions or genetic background (Fig. 1B).

The older observation that elevated FtsZ levels suppress death in most of these cases led to the following commonly invoked and straightforward explanation. For a given volume, a rod shape will have a smaller circumference than will a sphere. Thus, a rod requires less FtsZ to form an unbroken septal ring and, by this reasoning, when *E. coli* loses the ability to grow as a rod there is too little FtsZ to create a functional ring after the transition to a spherical shape. Spherical cells may grow larger but cannot divide, and the cells eventually die.

Bendezú and de Boer poke a neat and simple hole in the preceding argument. They note that if a particular FtsZ-to-circumference ratio is all that is required for proper cell division, then this ratio can be reestablished in a slightly larger spherical cell in less than a single generation. So then, they ask, why does *E. coli* not simply reestablish this ratio so that each cell continues to grow as a sphere with a slightly increased mass? The answer must be that "something else" is responsible for the inability of these cells to divide.

In search of this "something else," Bendezú and de Boer first show that the classical methods for creating spherical *E. coli* produce cells with essentially the same characteristics. In particular, the lethal effects of any of the treatments are suppressed by overexpression of FtsZ, by increasing the concentration of ppGpp, or by reducing the growth rate of the culture (Fig. 1B). These results suggest that a common mechanism undergirds all the sphere-inducing treatments. In addition, MreBCD, RodA, and PBP 2 act as though they belong to a common pathway, since the loss of any one produces an equivalent phenotype. This finding is in line with current thinking about the relationships among these proteins. The results also remove the confusion about whether the MreBCD proteins are essential. The first *mreBCD* mutants grew as spherical cells, implying that they were dispensable (7, 8), though other investigators came to the opposite conclusion (4). Bendezú and de Boer clarify the situation by showing that the original strains of Wachi et al. (8) contain about twice the normal amount of FtsZ, which suppresses the effects of deleting *mreBCD* and explains the previous discrepancies.

Secondly, Bendezú and de Boer observe that FtsZ forms aberrant structures in spherical cells, including patches, arcs, and branched or folded ribbons. This odd behavior is explained by the fact that synthesis of inner membrane continues unabated even though expansion of the cell surface slows, meaning that "extra" membrane must accumulate. The additional surface area is accommodated by membrane folding and the creation of internal vesicles, which may float free in the cytoplasm. Because FtsZ assembles on membrane surfaces, this extra mass of membrane siphons off FtsZ that would otherwise form a uniform ring to mark the site of septation. Hence, a modest increase in the amount of FtsZ is not sufficient to reestablish the FtsZ-to-circumference ratio, and the cells do not divide or else they divide abnormally. The explanation of cell death, then, is not that there is insufficient FtsZ but that it is distributed haphazardly.

Recently, Joseleau-Petit et al. manipulated *E. coli* to prolif-

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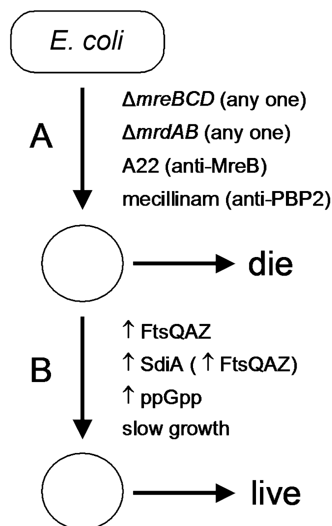

FIG. 1. Treatments that cause *E. coli* to become spherical and die (A) and those that allow the resulting cells to survive (B). See text for details.

erate as spherical L forms, whose growth requires FtsZ but not MreB (3). These cells grow and divide as spheres, and although they retain a tiny amount of peptidoglycan, the inner membrane is not confined by a continuous cell wall, so there is no need for the cells to accommodate excess membrane by involution. Thus, these cells represent an interesting test of the idea that spherical cells should propagate if only there were no internal membranes to confuse the correct localization of FtsZ. If all else were equal, then according to the calculations of Bendezú and de Boer these cells should stabilize as spheres with a slightly larger volume than rod shaped *E. coli*. Instead, the L forms propagate as spheres with diameters from 0.6 to 1.8 μm and volumes ranging from 0.11 to 3.0 μm^3 (3). Therefore, either all things are not equal (because of the absence of the cell wall) or the FtsZ-to-circumference ratio is not the only important parameter that determines the volume at which cell division can occur.

Of course, new insights generate new questions. One of the biggest is how FtsZ by itself suppresses the lethality associated with the presence of extraneous membrane. Because FtsA and ZipA are thought to bring FtsZ to the inner surface of the cytoplasmic membrane (5, 6), why are increased levels of these proteins not also required to organize the increased amounts of FtsZ? Also, since the FtsZ/FtsA ratio is important for proper cell division (2), why does an increase in this ratio in spherical cells not have an obvious deleterious effect?

Another interesting question addresses the rate of peptidoglycan synthesis in the absence of MreBCD. Bendezú and de Boer show that the rate of phospholipid synthesis remains constant in wild-type *E. coli* and in spherical mutants, leading to an excess of inner membrane compared to peptidoglycan surface. The implication is that the rate of peptidoglycan synthesis falls below that of phospholipid. If this can be shown, then one implication is that the Mre proteins are not just inanimate tethers that position peptidoglycan synthases in the periplasm. Instead, one or more of the Mre or Mrd proteins may stimulate synthase activity. There is still a large gap between the synthesis rate that must occur in vivo and that which can be achieved in vitro, so perhaps a more complex group of proteins is required for full activity.

A question related to the above is this: why does *E. coli* not regulate the rate of phospholipid synthesis to match that of cell wall growth? Are the two rates normally balanced by coincidence? For we who believe strongly in the ingenuity of *E. coli*, this seems too hard to swallow. An alternative is that the rates are maintained at a proper ratio by a common signal (e.g., osmotic pressure? something else?) but that this mechanism is broken in spherical cells. In any case, the results of Bendezú and de Boer indicate that phospholipid insertion does not itself control the rate of peptidoglycan incorporation, nor does the opposite control exist.

Finally, a bevy of smaller questions flutter about. Do the involuted vesicles contain peptidoglycan? If not, why? Since the vesicles contain periplasmic material, should the peptidoglycan synthases not be active in these compartments? Do the intracellular vesicles attract other membrane or periplasmic proteins, or can the cell distinguish between vesicles and the normal tripartite envelope? And are such vesicles associated only with abnormal growth, or might there be some circumstance under which this phenomenon has a physiological function? Whatever the answers may be, it is exciting to know that, at least with regard to our understanding of bacterial biology, "the end is not near!"

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