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GUEST COMMENTARY

New Insights into the Developmental History of the Bacterial Cell Division Site

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Cell division in *Escherichia coli* involves several sequential stages: identification of the correct site for septum formation, differentiation of this site in preparation for the septation event, and fabrication of the division septum by the coordinated ingrowth of all of the cell envelope layers. Over the past decade, differentiation of the bacterial cell division site has been studied extensively by examining the assembly of the proteins that comprise the division machinery, using fluorescence-based techniques to localize the proteins within intact cells. These studies have shown that the proteins assemble into a narrow FtsZ-based cytokinetic ring at an early stage of differentiation of the future division site (reviewed in reference 10). However, little is known about other changes that occur at the division site as part of its developmental history.

During the past few years, Miguel de Pedro and his collaborators have taken a different approach, using an ingenious new method to examine the cellular pattern of localized murein synthesis and turnover in normal cells and in cells blocked at various stages of division site differentiation. The present issue of the *Journal of Bacteriology* contains the latest (3a) of a series of papers (2, 3) describing these studies, which have provided evidence that changes in the local pattern of murein synthesis occur at a very early stage of differentiation of the future division site. The studies have defined sharply demarcated regions of murein synthesis at division sites. The preseptal murein in these zones persists for many generations, probably permanently, even in the absence of septum formation. As shown in this paper, these zones of murein sequestration also identify positions where branches grow out along the cell cylinder or at the poles of *E. coli* cells when the DacA protein (penicillin-binding protein 5 [PBP 5]) is absent, suggesting that the changes in murein organization at potential division sites may play a role in the genesis of branching patterns of bacterial growth under these conditions.

Murein has always been a difficult structure to study because of its enormous size (one molecule of murein surrounds the entire bacterial cell) and because of its complex peptidoglycan structure (9). In recent years, aside from studies relevant to antibiotic action and resistance, only a few laboratories have been bold enough to tackle basic problems in the cellular biology of murein. Nevertheless, because of its central role in determining cell shape, an understanding of the pattern of insertion of new material into the murein sacculus is essential to an understanding of cellular morphogenesis.

De Pedro and his collaborators have exploited the ability of the cell to incorporate trace amounts of the "abnormal" amino acid D-cysteine into murein during its biosynthesis, thereby providing a marker for regions of new murein synthesis. The reactivity of the cysteine residues with biotin made it possible to use antibiotin antibody to define the topology of new murein synthesis and turnover by immunofluorescence or autoradiographic immunolabeling in pulse-chase experiments (3).

Previous autoradiographic studies with the murein precursor [^{3}H]diaminopimelate have shown that cell elongation is associated with the diffuse incorporation of new murein subunits over the entire length of the cell cylinder (12, 13). When septation begins (or shortly before, as discussed below), there is a shift in the topology of peptidoglycan assembly into the sacculus. Incorporation along the body of the cell falls dramatically, and new synthesis becomes largely restricted to a narrow zone that defines the future division site at midcell. Following division, the septal murein remains at the cell pole as an inert part of the sacculus. This and other evidence led to the idea that a switch from an "elongation" mode to a "septal" mode of new murein synthesis occurs at the future division site at a specific time in the division cycle (6). The mechanism responsible for this shift is unknown.

Using the D-cysteine labeling technique, de Pedro et al. confirmed that new murein synthesis occurs in a narrow zone at the future division site (3). After septation and cell separation, the septal murein is retained at the poles for many generations. This distinguishes septal murein from murein over the body of the cell, which is progressively diluted as new murein incorporation and turnover occur during succeeding cell cycles.

Strikingly, de Pedro et al. found that the sharply localized zones of murein synthesis at division sites were formed even in the absence of proteins required for septal ingrowth. This was accomplished by studying cells in which penicillin-binding protein 3 (PBP3), the enzyme required for septal ingrowth, was inactivated, leading to formation of nonseptate filaments (3). The D-cysteine labeling experiments revealed sharply demarcated zones of new murein synthesis that were regularly spaced along the filaments at positions corresponding to potential division sites, implying that the zones were formed periodically at the appropriate place and appropriate time in each cell cycle. This appears to identify a distinct stage of differentiation of the division site, the stage of preseptal murein assembly,

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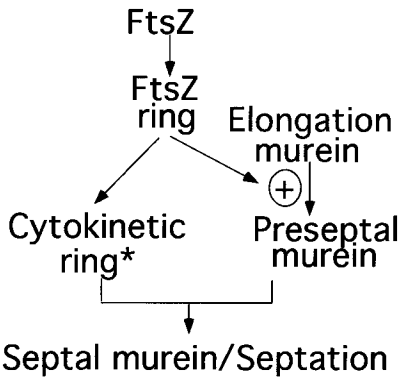

FIG. 1. Proposed outline of division site development. * indicates the addition of ZipA, FtsA, FtsK, FtsQ, FtsL, FtsI, FtsN, and FtsW (10).

when the switch from elongation murein synthesis to preseptal murein synthesis takes place at the future division site in preparation for later septal ingrowth.

In an important observation, de Pedro et al. further showed that formation of the local zones of murein synthesis requires a functional FtsZ protein but does not require several other components of the cytokinetic ring (FtsA, FtsI, and FtsQ) (3). This implies that FtsZ is required for the local switch from elongation to preseptal murein synthesis at potential division sites and adds an additional function for FtsZ in division site development beyond its clearly established role in assembly of the cytokinetic ring (Fig. 1). Since formation of the FtsZ ring is the first detectable event in differentiation of the future division site, these results show that the switch from elongation synthesis to preseptal synthesis occurs at a very early stage in development of the mature division site.

There had been other indications that cell wall changes occur at an early stage in division site development. This was shown by the presence of blunt constrictions at regular intervals along the nonseptate filaments of *ftsA*, *ftsI*, and *ftsQ* mutants (11). Significantly, the blunt constrictions did not form in the absence of a functional FtsZ protein. The observation that the blunt constrictions do not require PBP3 (and hence are independent of septal murein synthesis) or the *ftsA* or *ftsQ* gene product but do require a functional FtsZ protein suggests that their formation may well correspond to the stage of preseptal murein synthesis discussed above. The idea that FtsZ may play a direct or indirect role in regulating the murein assembly pattern is also consistent with studies of glycan chain lengths within the murein sacculus of cell division mutants (5). These studies showed that inactivation of FtsZ is associated with a significant change in the length of the glycan chains within the sacculus, whereas inactivation of PBP3 has no detectable effect. These effects mimic the difference in the effects of FtsZ and PBP3 inactivation in the studies of de Pedro et al. (3).

Many questions about the role of FtsZ in the switch from elongation murein to preseptal murein assembly remain to be answered. Of immediate interest is to determine whether other proteins are involved in transducing the signal from FtsZ to the machinery for preseptal murein synthesis. The first proteins to be added to the FtsZ ring are FtsA and ZipA (10). The local zones of murein assembly (and formation of the blunt constrictions) at potential division sites do not require FtsA. However, ZipA does seem to be required for formation of the blunt constrictions (P. de Boer, personal communication), suggesting a possible role for ZipA in the switch. It therefore will be of interest to see whether ZipA is also required for the local zones of murein deposition at potential division sites.

In their article in the current issue of the *Journal of Bacteriology* (3a), de Pedro et al. present evidence that the potential division site may play a role in the generation of branching patterns of bacterial growth. It had previously been shown that deletion of the *dacA* gene, coding for PBP5, leads to a branching pattern of growth in *E. coli* in which long branches grow out from positions along the body of the cell or from the cell pole (7, 8). Branching growth can occur in the absence of functional PBP3 and therefore in the absence of septum formation. The D-cysteine labeling experiments of de Pedro et al. (3a) showed that the branches originate at or adjacent to the localized sites of inert murein that identify potential division sites along the length of the cell. Strikingly, even when the branches were formed in the absence of PBP3, and hence in the absence of septation, the murein at the tips of the outgrowing branches was stable and inert.

In this regard, the murein at the tips (poles) of the branches resembled the murein at the cell poles of normally dividing cells, which represents septal murein that originated from previous divisions. An explanation for the remarkable parallelism between these aspects of the behavior of preseptal and septal murein and of the branching pattern of growth will require further work. However, several points remain to be clarified. Most strikingly, it has been reported that branching can be provoked at high frequency in some strains by certain growth conditions, even in the absence of a functional FtsZ protein (1, 4). Since FtsZ function appears to be required for assembly of the zones of preseptal murein synthesis (3), this implies that branching in those cases does not require the preseptal zones.

It is possible that the genesis of the branching growth pattern that can be induced in some strains by specific growth conditions differs from branching due to PBP5 deficiency and that different local perturbations of the murein sacculus are capable of providing the sites for branching growth. It therefore will be of interest to determine the patterns of murein assembly at the origins and poles of the branches that are induced under these different conditions.

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