A Sweet Sensor for Size-Conscious Bacteria

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Bacteria, like eukaryotic cells, regulate their size by coordinating cell growth and division, growing faster and becoming larger when nutrients are more plentiful. Weart et al. (2007) now identify an enzyme in a glucolipid pathway that inhibits assembly of the key cell division protein FtsZ, but only during high nutrient conditions. Delaying cell division during rapid growth allows bacterial cells to become larger.

The size of many cells, including bacteria, is homeostatically controlled to ensure maximum fitness. For bacteria capable of rapid growth, such as Escherichia coli or Bacillus subtilis, cell size increases considerably during fast growth rates (Schaechter et al., 1958). This is probably because faster growing cells need space for more ribosomes and other macromolecules, such as DNA, than slower growing cells. Indeed, when bacteria such as E. coli or B. subtilis are grown in nutrient-rich media, they initiate multiple rounds of DNA replication per cell division cycle. As a result, newborn fast-growing bacteria are transiently polyploid, and larger cells may be needed to contain this extra DNA. In fact, the size of bacteria and many eukaryotic cells is proportional to their DNA content (Jorgensen and Tyers, 2004). However, faster-growing bacterial cells divide more frequently than slower-growing cells, resulting in a shorter generation time. Therefore, to become larger cells, the rate of division must lag behind growth so that cells divide at a larger size (Figure 1).

One way to achieve this would be to transduce growth rate information to the cell division apparatus. The likely target of this regulation is FtsZ, a tubulin-like protein that assembles into a cytoskeletal structure called the Z ring (Bi and Lutkenhaus, 1991). This ring, in turn, recruits and assembles the machine that synthesizes the division septum. Because FtsZ is the first known protein to arrive at the future site of cell division and self-assembles into protofilaments, it is not surprising that FtsZ assembly is a target for cell division inhibitors. One type of inhibitor protein is induced upon DNA damage and delays Z ring assembly by directly binding to FtsZ until the damage can be repaired. Other factors inhibit Z ring assembly at inappropriate locations in the cell, such as close to the cell poles or on top of unpartitioned chromosomes. Therefore, regulating the timing of Z ring assembly or activity might be involved in growth rate-mediated control of cell size.

In this issue, Weart et al. (2007) reveal a new regulatory link between nutritional status and Z ring assembly. This link is a sugar utilization pathway that in the presence of high levels of glucose converts an enzyme in this pathway into an inhibitor of FtsZ activity. These authors first observed that wild-type B. subtilis cells have a constant ratio of the number of Z rings to cell length over.

Figure 1. Nutrients Control Cell Size in Bacillus subtilis
Three typical time courses of cell growth and division are shown: (left) the slow growth rate of B. subtilis in low nutrients; (middle and right) the faster growth rates of this bacterium in high nutrients. In cells with an intact UDP-glucose pathway that acts as part of a nutrient sensor, the average number of Z rings per cell length is constant and independent of growth rate (cells in box), resulting in Z-ring formation soon after the birth of daughter cells in fast growing cells. Z rings assemble much later in the cell cycle in slow-growing cells (gray cells, no Z rings). In high nutrients, UgpP—which transduces the nutrient signal to the Z ring—delays the action of the Z ring while growth continues; this results in larger cells at division compared with low nutrient conditions. However, under high nutrient conditions in the absence of UgpP, assembly and action of Z rings is no longer inhibited, resulting in smaller cells at division and formation of new Z rings in daughter cells at a shorter than normal cell length.
a wide range of growth rates. (Cell length in this organism is an excellent measure of cell size because cell diameters are constant over a range of growth rates.) This finding suggests that a Z ring forms only at a specific threshold cell length, which may be controlled by the cell cycle. However, when a gene called pgcA was deleted, Z rings were placed normally at the medial division site, but the Z-ring-to-cell-length ratio increased as growth rate increased. This had little effect on cell length in poor growth medium but caused cells in rich medium to divide at a length considerably shorter (35%) than normal (Figure 1). This is reminiscent of the wee1 mutation in fission yeast that knocks out negative regulation of mitosis (Russell and Nurse, 1987). The new finding suggests the presence of a key nutritional regulator of bacterial cell division.

The pgcA gene is widely conserved and encodes a phosphoglucomutase, which reversibly transforms glucose 1-phosphate into glucose 6-phosphate for glycolysis. Interestingly, a previous study of a deletion of the analogous gene in E. coli (pgm) noted that cells grown in rich medium were ~30% shorter than normal (Lu and Kleckner, 1994). Moreover, in B. subtilis deletion of another gene called ugtP also resulted in markedly shorter cells (Price et al., 1997). UgtP acts downstream of PgcA in a pathway in which glucose 6-phosphate is converted to glucose 1-phosphate by PgcA, then into UDP-glucose (via GtaB), and finally, via UgtP, into lipoteichoic acid, a major component of the B. subtilis cell wall. Inactivating pgcA, gtaB, or ugtP resulted in shorter cells, suggesting that the entire pathway is involved in nutritional regulation of cell length.

These results suggest that inhibiting lipoteichoic acid synthesis might result in short cells. However, inactivation of other parallel pathways leading to lipoteichoic acid production had no effect on cell length, pointing instead specifically to the PgcA-GtaB-UgtP pathway. Inactivating pgcA, gtaB, and ugtP in combination had the same effect as the single mutants, indicating that UgtP itself, possibly along with its UDP-glucose substrate, is responsible for making rapidly growing cells divide at longer cell lengths than slower growing cells. If this were true, then overproduction of UgtP protein might have the opposite effect of the mutant: cells would divide less frequently, becoming longer than wild-type cells under the same conditions. Weart et al. (2007) demonstrate that this is indeed the case, focusing their attention on UgtP and its possible interaction with FtsZ.

The authors make two key findings. First, UgtP is localized to seemingly random punctate spots during slow growth, but upon shifting to fast growth these spots disappear, with UgtP instead being found throughout the cell, often concentrating wherever FtsZ is localized. Localization of UgtP to division septa has been reported (Nishibori et al., 2005), also suggesting that UgtP interacts directly with FtsZ. To test this, Weart et al. (2007) purify UgtP protein and show that it strongly inhibits assembly of FtsZ in vitro at physiological concentrations. Moreover, addition of the UDP-glucose substrate modestly enhanced this inhibition. Therefore, UgtP seems to directly inhibit FtsZ assembly, but only during growth in high nutrients when it is released from sequestration.

How does UgtP become specifically activated to inhibit FtsZ assembly during rapid growth? When UgtP localizes to Z rings, perhaps it delays assembly of a stable ring structure. UgtP may also delay contraction of the ring, and nonring-associated UgtP may help by sequestering FtsZ subunits, which would inhibit Z-ring dynamics. How is UgtP released from the presumably inactive cytoplasmic foci? Blocking the flow of carbon upstream in the pathway by inactivating PgcA and GtaB also results in loss of cell size control. Thus, it is likely that increased nutrients during rapid growth induce increased flow of carbon through the pathway resulting in higher levels of UDP-glucose, which then titrate UgtP away from the cytoplasmic foci. The increase in cell length with growth rate is linear, suggesting that this activation of UgtP must be tightly regulated. Interestingly, Weart et al. (2007) demonstrate that cellular levels of UgtP protein are several-fold higher during rapid growth compared to slow growth. However, these levels are equally high in the short cells of pgcA or gtaB mutants during rapid growth when Z rings form proficiently, suggesting that changes in UgtP activity, not protein levels, inhibit FtsZ and make cells longer. It is also possible that other factors sensitive to UDP-glucose levels contribute to inhibition of cell division.

Is this control mechanism likely to be conserved? There are other reports of bacterial cells dividing at abnormally small sizes—for example, in E. coli when FtsZ activity is stimulated by another cell division protein FtsA (Geissler et al., 2007). E. coli lacks a UgtP homolog, so it will be interesting to know whether this effect is dependent on pathways that involve the pgcA homolog pgm. It is also likely that the cell division apparatus senses nutritional cues other than carbon flow, such as the pace of ribosome biogenesis (Jorgensen and Tyers, 2004).

Despite the important new insights gained from the Weart et al. (2007) study, the question of how bacterial cells time their cell divisions and maintain their lengths remains to be answered. In the abnormally short B. subtilis cells described by Weart et al. (2007), the timing of early cell cycle events, including DNA replication and chromosome segregation, are not significantly changed. In fact, Z rings often become localized aberrantly over unpartitioned chromosomes in pgcA mutants, indicating that the rings assemble prematurely relative to the rest of the cell cycle. But the formation of Z rings is still being regulated, particularly under slow growth conditions, to maintain a consistent cell size. Understanding how this works will be yet another piece in the long-standing puzzle of how cell growth and division are controlled.
REFERENCES


Listeria InlB Takes a Different Route to Met

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InlB, a surface protein of the human bacterial pathogen Listeria monocytogenes, interacts with the receptor tyrosine kinase Met on host cells to enable bacterial invasion. In this issue, Niemann et al. (2007) provide the first structural evidence that InlB does not compete for the same interaction site on Met as the natural ligand HGF.

Met is a receptor tyrosine kinase that is activated by the binding of hepatocyte growth factor (HGF) to its ectodomain. The Met ectodomain has an N-terminal Semaphorin (Sema) domain, a small PSI domain (named for its presence in plexins, semaphorins, and integrins), and four immunoglobulin (Ig)-like domains (Figure 1). Activation of Met is thought to require receptor dimerization, as evidenced by experiments showing that crosslinking by antibodies directed at the extracellular region triggers receptor activation (Prat et al., 1998). The Met receptor also serves as a target for the human pathogen Listeria monocytogenes surface protein InlB that binds to and activates Met. Met activation promotes the invasion of host cells by the pathogen. Despite an abundance of structural information concerning Met and InlB, the molecular mechanism by which InlB binding triggers Met dimerization has remained elusive. Niemann et al. (2007) now clarify these issues by cocrystallizing the domain of InlB that interacts with Met and a large extracellular portion of the human Met receptor.

Previous work has provided clues to the function of individual domains of InlB. The InlB protein has an N-terminal cap region, a leucine-rich repeat (LRR) domain, an interrepeat Ig-like (IR) region, a B-repeat, and three C-terminal GW modules (which are 80 amino acid repeats that start with the amino acid sequence GW). Latex beads coated with the InlB LRR region can be internalized by nonphagocytic cells, whereas uncoated beads are not internalized (Braun et al., 1999). This experiment showed that the LRR domain alone can promote entry when present at high local concentrations. However, the minimal region of InlB necessary to activate Met includes both the LRR and the adjacent IR region (Banerjee et al., 2004). In contrast, an LRR homodimer formed by a disulfide bridge between two LRR molecules is able to activate Met without the presence of the IR regions, most likely by forcing the dimerization of Met (Banerjee et al., 2004). However, this InlB dimer is unlikely to be physiologically relevant, as the cysteine that forms the disulfide is buried and unavailable in full-length InlB.

In addition, the C-terminal GW domains of InlB also impact Met activation. They allow noncovalent attachment of InlB to the lipoteichoic acids of the bacterial cell wall and interact with a host coreceptor of InlB (gC1qR) and with glycosaminoglycans (Braun et al., 2000; Jonquieres et al., 2001). InlB containing the GW modules induces stronger activation of Met than recombinant proteins containing only the LRR and IR domains, confirming that the recruitment of additional molecules is necessary for full activation of Met by InlB (Banerjee et al., 2004; Niemann et al., 2007).

The structure now presented by Niemann et al. (2007) shows that the LRR domain of InlB binds strongly to the first Ig-like domain of Met while maintaining the flexibility in the Met N-terminal Sema domain. The authors confirm the importance of the amino