Timing is everything—in love, in war, in sports, and in politics—but what about during multicellular development of *Myxococcus xanthus* bacteria? In this issue, Higgs et al. (9) report that loss of a single protein kinase can radically change the timing of aggregation and sporulation of 100,000 cells as they race to build a fruiting body when faced with starvation. Key transcription factors accumulate earlier in the kinase mutant, accelerating subsequent developmental events. What are the consequences of development in the fast lane? Spores form in normal numbers, but in smaller or deformed fruiting bodies or outside of fruiting bodies. Timing is not everything, but it probably has a profound impact on the ecology and evolution of social bacteria.

*M. xanthus* is the most studied of the myxobacteria, a group of gram-negative, deltaproteobacteria that move by gliding and inhabit soils globally (32). They are predators in the microbial world, hunting in packs and secreting antibiotics and enzymes to kill and devour their prey. This cooperative feeding behavior presumably provided selective pressure to evolve multicellular development. When food becomes scarce, cells in a swarm of *M. xanthus* aggregate into mounds, each containing about 100,000 cells (Fig. 1). In the nascent fruiting body, rod-shaped cells differentiate into spherical spores. Outside of fruiting bodies, some cells remain as peripheral rods. During the aggregation phase, many cells undergo programmed cell death, perhaps facilitating formation of spores and persistence of peripheral rods. The mature fruiting body is a cohesive, albeit perhaps facilitating formation of spores and persistence of peripheral rods. The mature fruiting body is a cohesive, albeit perhaps allowing for increased C-signaling to begin. Also needed for aggregation is the signals are unknown, with one exception. FruA is believed to sense population density (11, 16). If sufficiently high, A-signaling generates the next wave of gene expression and is necessary for aggregation to begin. Also needed for aggregation is a cascade of transcription factors that includes several enhancer-binding proteins (N. B. Caberoy and A. G. Garza, personal communication), MrpC, and FruA (reviewed in reference 13). All of these transcription factors likely respond to signals, but no predicted response regulator is encoded nearby in the genome. EspA is an orphan kinase whose output is unknown. Despite not knowing EspA’s input or output, Higgs et al. (9) sought a clue about its output by examining molecular markers of development in the *espA*-null mutant. By identifying the earliest change in the developmental program, the authors could begin to understand which step is regulated by EspA to control the timing of development.

Molecular events that drive *M. xanthus* development have been revealed by classical and molecular genetic approaches, as well as biochemical and, recently, genomic approaches (Fig. 1). Nutrient limitation triggers production of the intracellular signal (pppGpp) and expression of early developmental genes (6, 26). The (pppGpp) signal leads to elaboration of the extracellular A-signal, a mixture of amino acids and peptides used to sense population density (11, 16). If sufficiently high, A-signaling generates the next wave of gene expression and is necessary for aggregation to begin. Also needed for aggregation is a cascade of transcription factors that includes several enhancer-binding proteins (N. B. Caberoy and A. G. Garza, personal communication), MrpC, and FruA (reviewed in reference 13). All of these transcription factors likely respond to signals, but the signals are unknown, with one exception. FruA is believed to be phosphorylated in response to C-signaling from another cell (3). This signaling requires CsgA and possibly end-to-end contact of cells (reviewed in reference 27). As cells increasingly make contacts during aggregation, it has been proposed that increased C-signaling leads to a high level of phosphorylated FruA that activates expression of genes required for sporulation. According to this model, C-signaling coordinates aggregation with sporulation, ensuring that spores form within fruiting bodies.

Higgs et al. (9) discovered that the *fruA* transcript and FruA protein concentrations are elevated in the *espA* mutant compared with the wild type during aggregation. Genes required
for sporulation were expressed earlier in the espA mutant, presumably due to a higher concentration of phosphorylated FruA. This could explain the observed early sporulation of the espA mutant (2).

Why is the fruA transcript concentration elevated in the espA mutant? Higgs et al. (9) found that the MrpC concentration rose earlier in the espA mutant than in the wild type during development. MrpC binds to the fruA promoter region and very likely activates transcription (31), although activation has not been demonstrated in vitro since it not known which sigma factor directs fruA transcription. Nevertheless, early accumulation of MrpC provides a plausible explanation for early accumulation of FruA in the espA mutant.

Importantly, Higgs et al. (9) found that the mrpC transcript concentration was indistinguishable in the espA mutant compared with the wild type during aggregation. This implies that early events like (p)ppGpp signaling and A-signaling, on which mrpC transcription depends in part (30), are normal in the espA mutant. In agreement, Higgs et al. (9) found that spi transcription, which depends very strongly on (p)ppGpp signaling and A-signaling, was unimpaired in the espA mutant. Moreover, early signaling events that presumably lead to phos-
phorylation of enhancer-binding proteins required for mrpC transcription are like­ly normal in the espA mutant.

Taken together, the results of Higgs et al. (9) indicate that EspA inhibits mrpC expression posttranscriptionally (Fig. 1). Failure to do so in the espA mutant causes premature accumulation of MrpC. This in turn causes premature accumulation of FruA, which accelerates sporulation. Aggregation is also somewhat accelerated but fails to keep pace with sporulation. The early aggregation of the espA mutant may also be due to premature accumulation of phosphorylated FruA, which has been proposed to influence the Frz chemosensory system (28). The Frz system affects the frequency of reversal of gliding motility and therefore aggregation (reviewed in references 10 and 33). The methylation pattern of FrzCD changes during development, providing a molecular marker related to activity of the Frz system (19). Higgs et al. (9) found that changes in the pattern of FrzCD methylation occurred earlier in the espA mutant than in the wild type. Hence, premature accumulation of phosphorylated FruA appears to prematurely stimulate aggregation, as well as sporulation, but coordination between the two processes is partly lost. Premature accumulation of CsgA has similar effects (4, 14), consistent with the model that CsgA mediates C-signaling and that FruA is phosphorylated in response to C-signaling (reviewed in reference 27). Interestingly though, an espA csgA double mutant aggregates and sporulates much better than a csgA mutant (9). In the double mutant, perhaps the elevated FruA concentration allows it to become partially active independent of C-signaling.

How might EspA inhibit mrpC expression posttranscriptionally? A two-step cascade of serine/threonine protein kinases leads to phosphorylation of MrpC during growth (Fig. 1) (22). Phosphorylated MrpC has lower affinity than MrpC for its own promoter, so phosphorylation inhibits positive autoregulation (23). Phosphorylation of MrpC also inhibits its processing to MrpC2, a form lacking the N-terminal 25 residues, which binds to the mrpC and fruA promoters with higher affinity than MrpC (23, 31). A mutation in either kinase of the two-step cascade that leads to phosphorylation of MrpC elevates the concentra­tions of MrpC and MrpC2 in growing cells and accelerates development (22, 23). Higgs et al. (9) did not observe elevated MrpC or MrpC2 during growth of the espA mutant, so it is unlikely that EspA affects the kinase cascade. During development of the espA mutant, early accumulation of FruA correlated with early accumulation of MrpC, not MrpC2. (Although MrpC2 did accumulate earlier in the espA mutant than in the wild type, it was undetectable when FruA began to accumulate.) This suggests that MrpC can activate fruA transcription and that EspA’s primary effect is on MrpC accumulation rather than its processing to MrpC2 (Fig. 1). Recently, it was discovered that MrpC functions as an antitoxin by interacting with MazF, an mRNA interferase that mediates programmed cell death during development (21). This antitoxin-toxin interaction might influence access of MrpC to degradative proteases. Whether or not MazF influences it, MrpC turnover is one possible target of EspA-mediated regulation. Another is translation of mrpC mRNA. Higgs et al. (9) speculate that EspA might function in a phosphorylase analogous to that of Lux proteins in Vibrio species. It is tempting to take the analogy further and speculate that the output of the EspA phosphore-

lay is production of small RNAs that inhibit translation of mrpC mRNA.

The ability of the espA mutant to undergo development in the fast lane is not unique. Mutations in other histidine protein kinases (8, 17, 24), serine/threonine protein kinases (20, 22), a sigma factor (1), an enhancer-binding protein (5), and a chemosensory system (12) accelerate development. The study by Higgs et al. (9) illustrates a systematic strategy for pinpointing where the brakes are applied during fruiting body development. Such checkpoints are observed in other examples of bacterial and eukaryotic development (reviewed in references 7, 13, 18, and 25). Typically, mutations in checkpoint genes have severe consequences. The espA mutant forms normal spore numbers, but they are not as well organized into large mounds as for wild-type M. xanthus. Presumably, large fruiting bodies favor dispersal to, and exploitation of, new environments, so development in the fast lane has been selected against evolutionarily, at least for some M. xanthus strains. In particular niches, rapidly changing conditions might favor faster development. These conditions might be sensed by EspA and other components of the complex signaling and gene regulatory network that governs coordination between aggregation and sporulation. Mutations in network components like espA would provide variation in aggregate size and shape and the timing of sporulation, which could be selected evolutionarily, so it is not surprising that some natural isolates of M. xanthus undergo development in the fast lane (S. Kramer and G. J. Velicer, personal communication).

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REFERENCES


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