Cell

A Molecular Beacon Defines Bacterial Cell Asymmetry

Melanie L. Lawler[1] and Yves V. Brun[1,*]
[1]Department of Biology, Indiana University, Bloomington, Indiana 47405, USA
*Contact: ybrun@indiana.edu
DOI 10.1016/j.cell.2006.02.027

Many cells divide asymmetrically by generating two different cell ends or poles prior to cell division, but the mechanisms by which cells distinguish one pole from the other is poorly understood. In this issue of *Cell*, Huitema et al. (2006) and Lam et al. (2006) describe a protein that defines one specific pole of a bacterial cell by localizing to the site of cell division to be inherited by both progeny at the resulting new poles.

Asymmetric cell division is often used during development by multicellular organisms to produce progeny cells with different fates. Unicellular organisms also use asymmetric cell division to differentiate. Sporulation in the bacterium *Bacillus subtilis* occurs only at one end of the cell (Ryan and Shapiro, 2003), and budding in the yeast *Saccharomyces cerevisiae* occurs at specific sites adjacent to the previous sites of budding (Chang and Peter, 2003). Even bacteria that appear to divide symmetrically have asymmetric localization of proteins. For example, the *Escherichia coli* chemoreceptor complex and the *Agrobacterium tumefaciens* type IV secretion apparatus localize to a single pole, and the *Shigella flexneri* IcsA protein localizes exclusively to the "old pole" of the cell (Janakiraman and Goldberg, 2004).

How can proteins distinguish between the two cell poles and localize exclusively to one of them? Part of the answer is now known for the dimorphic bacterium *Caulobacter crescentus*, a powerful model organism to study the mechanisms that control the generation of asymmetry. During each cell cycle, an asymmetric *C. crescentus* predivisional cell, with a flagellum at one pole and a stalk at the other, divides into two morphologically and functionally different bacterial cells (Figure 1). The sessile-stalked cell progeny is com-

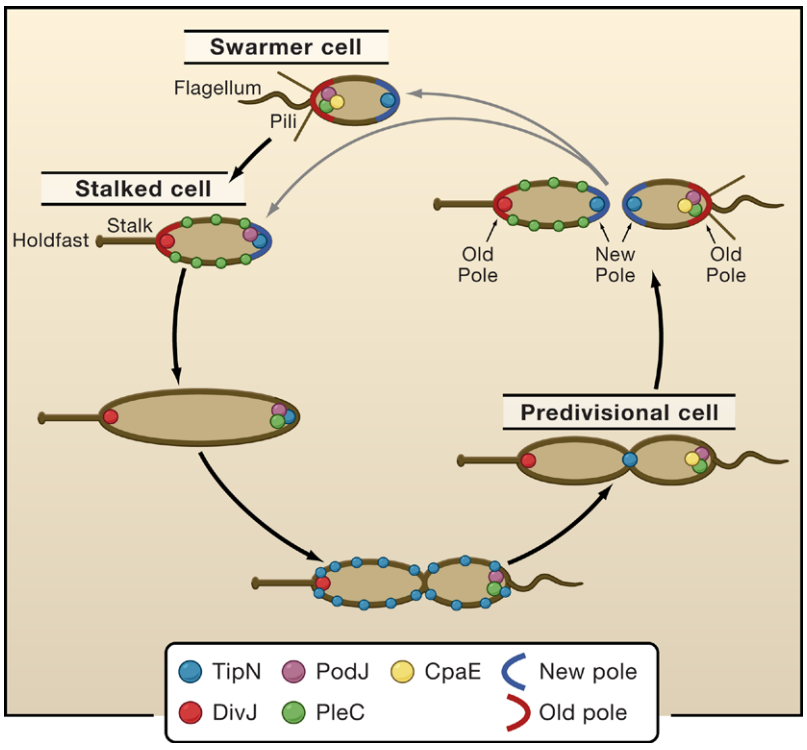

Figure 1. Life Cycle of the Bacterium *Caulobacter crescentus*
Asymmetric localization of proteins to the cell poles is crucial for development of *C. crescentus*. The "new poles" that are the result of the most recent cell division are shaded blue, and the "old poles" from a previous division are shaded red. Large colored dots represent the specific subcellular localization of proteins during the cell cycle; small colored dots around the cell represent diffuse protein localization. TipN localizes to the midcell following constriction by the cell division machinery—it is then inherited at the "new pole" of daughter cells. Localization of the developmental regulator DivJ to the "old pole" is independent of TipN, whereas localization of another developmental regulator, PleC, and the pili assembly factor CpaE to the new pole is partially dependent on TipN. The polar organelle development protein PodJ is also important for the proper localization of PleC and CpaE to the new pole during the cell cycle. PodJ is processed from a full-length ~100 kDa form (PodJL) to an ~90 kDa apparent molecular weight form (PodJS) at the end of the cell division cycle. The precise localization of these various proteins is important to establishing the different fate of the two daughter cells.

petent for DNA replication and cell division, whereas the motile swarmer cell progeny (which also has pili at the flagellar pole) is unable to initiate a new cell cycle until it differentiates into a stalked cell (Figure 1). The stalk has an adhesive holdfast at its tip, which mediates attachment to surfaces. The generation of asymmetry and progression through the cell cycle are controlled by an elaborate regulatory network of proteins, many of which localize to a specific pole of the cell (Ryan and Shapiro, 2003). As a result, synthesis of polar organelles follows a strict temporal order during the cell cycle. The flagellum is the first external structure to assemble at the new poles created by cell division, followed by the synthesis of pili, the holdfast, and the stalk. One early suggestion for the mechanism that generates asymmetry in *C. crescentus* came from the observation that cells blocked early in division are unable to assemble flagella or bacteriophage receptor sites at the future site of cell division but are able to assemble these structures if cell division is blocked at a late stage (Huguenel and Newton, 1982). Huguenel and Newton therefore proposed that "organizational centers" are laid down late in cell division and serve as markers for the new cell poles. The identity of these organizational centers had remained a mystery until work by the Viollier and Jacobs-Wagner laboratories, reported in this issue of *Cell*, identified a protein involved in marking the new poles created by division. Huitema et al. (2006) reasoned that because the flagellum is the first structure to be synthesized at the new poles, a protein marker remaining at the new poles after division might be required for flagellum synthesis at these poles. They screened for mutants with impaired motility accompanied by defects in flagellum localization or synthesis. Lam et al. (2006) scanned the genome for proteins with transmembrane regions that could help anchor the protein in place and interaction domains that could help it establish or maintain a complex at the new pole.

Both approaches identified the protein encoded by *CC1485*, named TipN (for *tip* of *n*ew pole). TipN is a 94 kDa protein with two putative transmembrane domains and an extended region predicted to form coiled coils, which are often involved in protein-protein interactions. TipN localizes to the site of cell division after constriction begins, and is retained at the new pole after division (Figure 1). As the newborn cells proceed through the cell cycle, TipN relocalizes to the new division site. In the absence of TipN, flagella are often aberrantly formed at the stalked pole of the cell, rather than at the pole opposite the stalk. Huitema et al. (2006) also identified a protein, TipF, which requires TipN for localization. TipF is a 50 kDa protein with two transmembrane domains, a coiled-coil region, and a DUF2/EAL domain likely to have phosphodiesterase activity for cyclic di-guanosine monophosphate (c-di-GMP), a bacterial secondary messenger involved in regulating the transition from the planktonic to the sessile state. *tipF* mutants are devoid of flagella, suggesting that c-di-GMP degradation by TipF, localized to the new pole by TipN, plays a role in flagellum biosynthesis.

Localization of TipN is dependent on cell division; this protein does not localize to the division site in cells depleted of the division initiation protein FtsZ (Huitema et al., 2006; Lam et al., 2006) or in cells in which the division site peptidoglycan synthesis protein FtsI has been inactivated (Huitema et al., 2006). When additional cell constrictions are produced by overexpression of FtsZ, TipN localizes to these sites (Huitema et al., 2006). In contrast, recruitment of the *S. flexneri* IcsA protein to future sites of cell division occurs independently of FtsZ (Janakiraman and Goldberg, 2004), indicating that other mechanisms can generate asymmetry. Lam et al. (2006) found that release of TipN from the old pole is not dependent on cell division, however, and appears to be regulated by cell size.

Investigating the mechanisms by which cell size is sensed by or transduced to TipN should prove interesting, especially as TipN is also required for proper cell division (Huitema et al., 2006; Lam et al., 2006). *C. crescentus* predivisional cells normally divide slightly off center, so that the daughter swarmer cell is approximately 40% of the predivisional cell length. How the location of the division site is faithfully established off-center is not known, as *C. crescentus* does not have clear homologs of Min proteins, known to regulate the location of FtsZ in *E. coli* (Ryan and Shapiro, 2003). Yet, in the absence of TipN, the swarmer compartment of predivisional cells is larger than the stalked compartment in a majority of cells (Lam et al., 2006). Consecutive mislocalized divisions lead to slightly longer cells in the population. Furthermore, mild overexpression of TipN results in abnormalities at the site of constriction, which give rise to daughter cells with bulbous structures at the pole (Huitema et al., 2006).

TipN is clearly not the only factor controlling cellular asymmetry. For example, despite the fact that the localization of the pili assembly factor CpaE at the new pole is less efficient in a *tipN* mutant (Lam et al., 2006), pili are localized properly in this mutant (Huitema et al., 2006). This indicates that there are different factors involved in the formation of pili and flagella at the pole opposite the stalk. Neither study examined whether TipN regulates the asymmetric localization of the holdfast.

The asymmetric localization of the developmental regulators PleC to the flagellar pole of predivisional cells and DivJ to the old, stalked pole is important to set up different programs of gene expression in each daughter cell (Ryan and Shapiro, 2003). Localization of DivJ at the old pole is not dependent on TipN, but the localization of PleC at the new pole is somewhat disrupted in a *tipN* mutant (Huitema et al., 2006; Lam et al., 2006). The localization of both CpaE and PleC to the flagellar pole depends on the developmental

regulator PodJ, which is required for swarming motility and synthesis of pili and holdfasts (Hinz et al., 2003; Viollier et al., 2002). As PodJ also contains a coiled-coil region (Lawler et al., 2006), perhaps PodJ interacts with TipN to establish a localization complex at the pole.

Overexpression of TipN-GFP causes the formation of new poles through cell branching; these new poles are competent for flagellum assembly and localization of polar proteins (Lam et al., 2006). This phenotype is reminiscent of the phenotype obtained when the expression of the actin-like cytoskeletal protein MreB is perturbed (Gitai et al., 2004; Wagner et al., 2005), suggesting an interaction between TipN and MreB. Indeed, TipN is required for localization of MreB to the site of cell division (Lam et al., 2006), suggesting the possibility that TipN regulates asymmetry and cell division through MreB. Given that MreB coordinates the synthesis of the cell wall peptidoglycan, which is critical for the generation and maintenance of bacterial cell shape, the role of TipN in controlling polarity may ultimately be mediated through changes in the peptidoglycan. It has been proposed that the differences in peptidoglycan composition and growth at the septum could help direct proteins involved in generating polarity (reviewed in Janakiraman and Goldberg, 2004).

Many different bacterial species utilize polar localization for functions as diverse as chemoreception, adhesion to surfaces, and translocation of effector proteins during pathogenesis. Determining whether proteins similar to TipN regulate polarity in these bacteria becomes of obvious interest. The discovery of TipN brings the field closer to understanding the regulation of polarity, whose importance in bacterial cell biology is becoming more obvious with the finding that many proteins localize to the bacterial cell pole.

REFERENCES

Chang, F., and Peter, M. (2003). Nat. Cell Biol. *5*, 294–299.

Gitai, Z., Dye, N., and Shapiro, L. (2004). Proc. Natl. Acad. Sci. USA *101*, 8643–8648.

Hinz, A.J., Larson, D.E., Smith, C.S., and Brun, Y.V. (2003). Mol. Microbiol. *47*, 929–941.

Huguenel, E.D., and Newton, A. (1982). Differentiation *21*, 71–78.

Huitema, E., Pritchard, S., Matteson, D., Radhakrishnan, S.K., and Viollier, P.H. (2006). Cell, this issue.

Janakiraman, A., and Goldberg, M.B. (2004). Trends Microbiol. *12*, 518–525.

Lam, H., Schofield, W.B., and Jacobs-Wagner, C. (2006). Cell, this issue.

Lawler, M.L., Larson, D.E., Hinz, A.J., Klein, D., and Brun, Y.V. (2006). Mol. Microbiol. *59*, 301–316.

Ryan, K.R., and Shapiro, L. (2003). Annu. Rev. Biochem. *72*, 367–394.

Viollier, P.H., Sternheim, N., and Shapiro, L. (2002). Proc. Natl. Acad. Sci. USA *99*, 13831–13836.

Wagner, J.K., Galvani, C.D., and Brun, Y.V. (2005). J. Bacteriol. *187*, 544–553.

TGF-β Regulation by Emilin1: New Links in the Etiology of Hypertension

Malavika Raman[1] and Melanie H. Cobb[1,*]

[1]Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA

*Contact: melanie.cobb@utsouthwestern.edu

DOI 10.1016/j.cell.2006.02.031

Hypertension is a complex disease influenced by multiple genetic and environmental factors. The TGF-β signaling pathway has a long recognized role in blood pressure homeostasis. In this issue of *Cell*, Zacchigna et al. (2006) report that the secreted protein Emilin1 is a negative regulator of TGF-β signaling. Emilin1 knockout mice display elevated blood pressure due to increased TGF-β signaling in the vasculature.

Hypertension, generally an increase in arterial blood pressure, is a major health concern and risk factor for other diseases such as myocardial infarction and kidney failure. Despite its prevalence, the pathogenesis of hypertension is poorly understood. What is known is that hypertension is multifactorial; both genetic determinants, such as allelic variation in genes involved in renal salt absorption, and environmental factors including diet are implicated in the development of this disease (Lifton et al., 2001).

Arterial blood pressure is a function of both the cardiac output—the amount of blood pumped out by