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MicroReview

Regulatory proteins with a sense of direction: cell cycle signalling network in *Caulobacter*

Christine Jacobs-Wagner
Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520, USA.

Summary

Localization of kinases and other signalling molecules at discrete cellular locations is often an essential component of signal transduction in eukaryotes. *Caulobacter crescentus* is a small, single-celled bacterium that presumably lacks intracellular organelles. Yet in *Caulobacter*, the subcellular distribution of several two-component signal transduction proteins involved in the control of polar morphogenesis and cell cycle progression changes from a fairly dispersed distribution to a tight accumulation at one or both poles in a spatial and temporal pattern that is reproduced during each cell cycle. This cell cycle-dependent choreography suggests that similarly to what happens in eukaryotes, protein localization provides a means of modulating signal transduction in bacteria. Recent studies have provided important insights into the biological role and the mechanisms for the differential localization of these bacterial signalling proteins during the *Caulobacter* cell cycle.

Introduction

The recent progress of fluorescence microscopy techniques and related tools has opened the door to a new era in which the complex spatial organization of the bacterial cell is finally recognized at its molecular level (reviewed in Shapiro *et al*., 2002). Some bacterial proteins [for instance, the components of the cell division machinery (Buddelmeijer and Beckwith, 2002)] are recruited at discrete subcellular locations where their function is needed. Regions of chromosomal and plasmidic DNA as well as proteins involved in DNA replication and segregation have also been shown to reside at preferential cellular sites in bacteria (Gordon and Wright, 2000; Draper and Gober, 2002; Marczynski and Shapiro, 2002; Pogliano, 2002). Perhaps one of the most thought-provoking discoveries in this rapidly evolving field of bacterial cell biology was the observation that some bacterial signal transduction proteins accumulate at discrete subcellular locations in a temporally regulated fashion (Arigoni *et al*., 1995; Duncan *et al*., 1995; Jacobs *et al*., 1999; 2001; Wheeler and Shapiro, 1999; Boyd, 2000), suggesting that protein localization may play a regulatory role in bacterial signalling processes. In eukaryotes, the localization of kinases and phosphatases is commonly used to regulate signal transduction. For example, the phospholipid and calcium activated protein kinase C (PKC) localizes to discrete subcellular compartments. Protein kinase C is present in multiple isoforms and the localization of each isoform is unique and changes upon activation (Kraft *et al*., 1982; Disatnik *et al*., 1995). This compartmentalization process alters the access of the isoforms to their substrates and thereby modulates signal transduction. But what purpose may the differential localization of signalling proteins serve in a small bacterial cell with presumably no intracellular compartmentalization? What are the mechanisms by which localization and trafficking of signalling molecules occur in bacteria? *Caulobacter crescentus* constitutes an excellent model system to address these questions because in this bacterium, most signal transduction components that coordinate polar morphogenesis with cell cycle progression exhibit a remarkable, dynamic localization at the pole(s) during the cell cycle (Jacobs *et al*., 1999; 2001; Wheeler and Shapiro, 1999).

A cell cycle signalling network under spatial control

To discuss the *Caulobacter* cell cycle signalling network and its spatial regulation, the cell cycle of this bacterium needs to be introduced (Fig. 1). In *Caulobacter*, a motile 'swarmer' (SW) cell, which has a single flagellum and several pili at one pole, differentiates into a non-motile 'stalked' (ST) cell during each cell cycle. During this obligate differentiation process, the flagellum is shed, the pili are lost and a stalk tipped by a holdfast is synthesized at

Accepted 15 September, 2003. *For correspondence. E-mail christine.jacobs-wagner@yale.edu; Tel. (+1) .203 432 5170; Fax (+1) 413 803 9808.

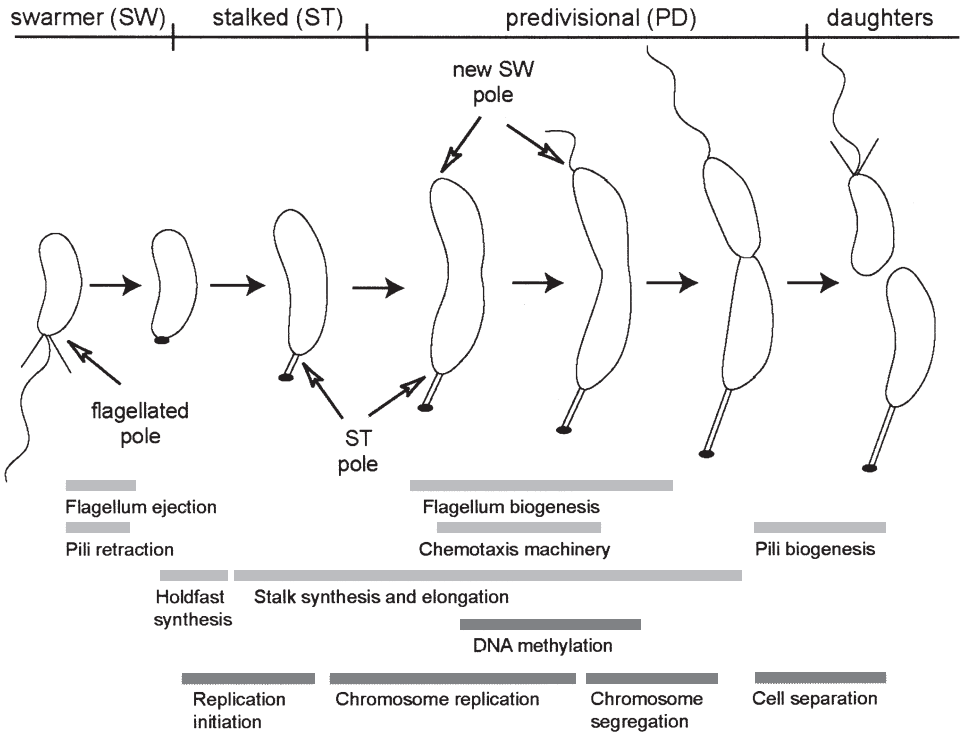

Fig. 1. The *Caulobacter* cell cycle. The motile, replication-incompetent swarmer cell has a polar flagellum and pili. After a period of G1 phase, the swarmer cell differentiates into a stalked cell in a succession of events involving the ejection of the flagellum, the retraction of the pili, the synthesis of the holdfast, the elongation of the stalk and the initiation of DNA replication. The stalked cell elongates into a predivisional cell in which DNA methylation and the biosynthesis of the flagellum and the chemotaxis machinery are initiated. After chromosome segregration, the late predivisional cell divides asymmetrically to yield two different progenies: a swarmer cell and a stalked cell. The timing of several morphogenic and cell cycle events is shown by the light and dark grey bars respectively. The flagellated, stalked (ST) and new swarmer (SW) poles are indicated.

the pole where the flagellum and pili were originally located. The ST cell then elongates into a predivisional (PD) cell in which a chemotaxis apparatus and a flagellum are assembled at the pole opposite the stalk (the new SW pole). This yields a polarized PD cell, which divides asymmetrically to give rise to a SW daughter cell and a ST daughter cell.

A regulatory network orchestrates the coupling between essential cell cycle processes (such as chromosome replication, DNA methylation and cell division) and the morphogenic events at the poles that result in the cell cycle-dependent formation and disappearance of organelles (the pili, the flagellum and the stalk) (Fig. 1). This network is composed of several two-component signal transduction pathways. The hybrid histidine kinase, CckA, which is essential for viability, participates in one pathway (Jacobs *et al*., 1999; 2003). Another pathway is composed of two non-essential histidine kinases, PleC and DivJ, and an essential single domain response regulator, DivK (Sommer and Newton, 1991; Ohta *et al*., 1992; Wang *et al*., 1993; Hecht *et al*., 1995; Burton *et al*., 1997; Wu *et al*., 1998). A third pathway involves a kinase, DivL (Sommer and Newton, 1991; Wu *et al*., 1999), which will not be discussed here because the subcellular location of DivL is unknown at present time. The nature of the signals that activate these three pathways remain mysterious. Upon activation, these signalling pathways ultimately affect the activity of a downstream effector, CtrA (Wu *et al*., 1998; 1999; Jacobs *et al*., 1999), which in turn controls the initiation of DNA replication and the expression of many cell cycle-regulated genes such as those involved in DNA methylation, cell division and the biogenesis of polar organelles (Quon *et al*., 1996; 1998; Kelly *et al*., 1998; Reisenauer *et al*., 1999; Skerker and Shapiro, 2000; Laub *et al*., 2002). The mechanisms by which these signalling pathways affect CtrA activity are not completely understood. They probably involve multi-component phosphorelays for which phospho-intermediates remain to be identified. Because of space limitation, the regulation of CtrA activity will not be addressed further; if interested, the reader is invited to consult recent reviews on the topic (Martin and Brun, 2000; Jenal and Stephens, 2002). This review will focus on one of the most intriguing aspects of the CtrA signalling network; that is, the differential localization of its components during the cell cycle.

Using functional fusions to GFP, it has been shown that the membrane-bound histidine kinases CckA, DivJ and

PleC, and the soluble response regulator DivK undergo temporal regulation of their subcellular distribution during the cell cycle (Fig. 2). CckA is found dispersed throughout the cytoplasmic membrane in the first half of the cell cycle, but during the PD stage, CckA accumulates predominantly at the new SW pole and to a lesser extent at the ST pole (Jacobs *et al*., 1999). At cell division, CckA is released from the poles, yielding SW and ST progenies with CckA distributed throughout the membrane. Components of the DivK pathway (DivJ, PleC and DivK) also display a distinct localization pattern during the cell cycle (Fig. 2). DivJ, which is mostly absent in the SW cell, is synthesized in the ST cell where it accumulates at the ST pole (Ohta *et al*., 1992; Wheeler and Shapiro, 1999). The localization of DivJ remains polar throughout the rest of the cell cycle, resulting in segregation of the protein into the ST progeny at division (Wheeler and Shapiro, 1999). High resolution analysis by immunogold electron microscopy revealed that DivJ proteins tightly cluster at the base of the stalk (Sciochetti *et al*., 2002). PleC, on the other hand, is localized at the flagellated pole in the SW cell, but during the SW-to-ST cell transition, PleC delocalizes and remains dispersed around the cytoplasmic membrane for a certain period of time until it relocates to the new SW pole in the PD cell (Wheeler and Shapiro, 1999). The single domain response regulator DivK also has a complex pattern of localization during the cell cycle (Jacobs *et al*., 2001). DivK is dispersed in the cytoplasm of the SW cell. In the ST cell, DivK first accumulates at the ST pole. Soon after, DivK also localizes at the new SW pole, resulting in a bipolar distribution that lasts throughout most of the PD stage. Before cell separation, DivK is specifically released from the new SW pole, but remains anchored at the ST pole, culminating in the generation of two daughter cells with a distinct localization of DivK (Jacobs *et al*., 2001).

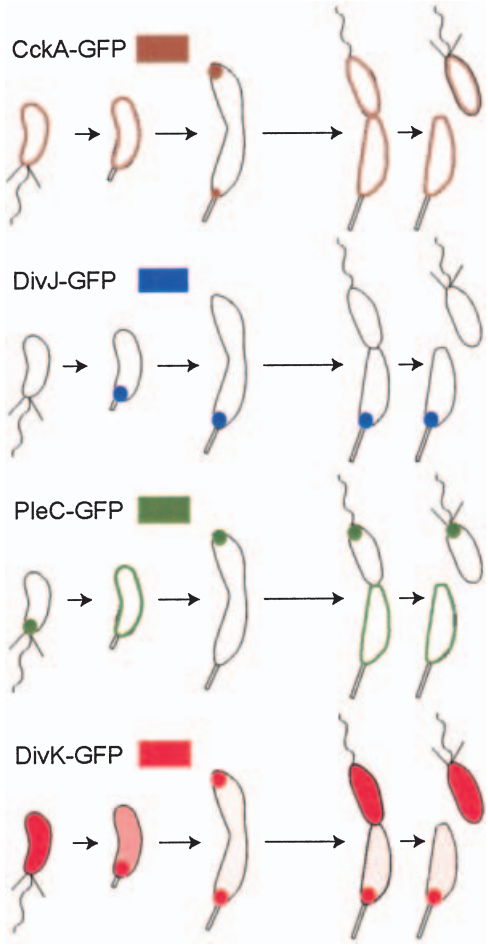

Fig. 2. Localization of CckA-GFP, DivJ-GFP, PleC-GFP and DivK-GFP during the cell cycle. The membrane-bound CckA-GFP (in brown) is mostly dispersed around the cytoplasmic membrane of the SW and ST cells. In the early PD cell, CckA-GFP localizes at the poles until just before cell division when CckA-GFP is released from the poles. Upon synthesis in the ST cell, DivJ-GFP (in blue) accumulates at the ST pole and maintains this localization thereafter, resulting in the segregation of DivJ-GFP in the ST progeny after cell division. The membrane-bound PleC-GFP (in green) is localized at the flagellated pole in the SW cell, but is released at the SW-to-ST cell transition and relocates at the opposite pole (new SW pole) in the PD cell. In the SW cell, DivK-GFP (in red) is evenly distributed in the cytoplasm. During the SW-to-ST cell differentiation process, DivK-GFP accumulates at the future ST pole, then soon after localizes at the new SW pole as well, resulting in a bipolar localization of DivK-GFP during most of the PD stage. However, before cell division, DivK-GFP is specifically released from the new SW pole, yielding progenies with a different subcellular distribution of DivK-GFP: a SW cell with diffuse DivK-GFP and a ST cell with polarly localized DivK-GFP. For each protein, the differential localization pattern between progenies is predetermined before cell division. The length of the arrows illustrates the time spent between each cell transition.

What is the role for the differential localization of these signalling proteins during the cell cycle?

In analogy to eukaryotic systems, the dynamic changes in the cellular locations of CckA, DivJ, PleC and DivK during the cell cycle may constitute novel regulation of signal transduction in bacteria. Supportive of a regulatory function for protein localization, the dynamic localization of each of these *Caulobacter* signalling proteins follows a temporal pattern that is consistent with the stage of the cell cycle in which the protein plays a role. Polar localization of CckA coincides with the peak of CckA phosphorylation during the cell cycle (Jacobs *et al*., 2003). As autophosphorylation of histidine kinases is often a measure of their activity, CckA appears to be most active when the protein is polarly localized. Although the phosphorylation patterns of PleC and DivJ during the cell cycle are unknown, genetic evidence suggests that the time of PleC and DivJ function during the cell cycle correlates with the time of their localization at their respective pole. DivJ controls an early cell division step at a time that is concomitant with its localization at the ST pole (Ohta *et al*.,

1992; Wheeler and Shapiro, 1999). PleC is required for motility and chemotaxis (Sommer and Newton, 1989; Wang *et al*., 1993) at times when PleC is localized at the pole where the chemotaxis and flagellar apparatus are located (Wheeler and Shapiro, 1999). In the case of the DivK response regulator, the coupling between activity and polar localization is evident by the fact that phosphorylation of DivK is required for both the function and the polar localization of DivK (Lam *et al*., 2003). DivK is essential for viability (Hecht *et al*., 1995) and non-phosphorylatable mutants of DivK do not support viability (Lam *et al*., 2003). These mutants also fail to polarly localize when present in cells that contain wild-type, untagged DivK to support life (Lam *et al*., 2003). Thus, it is the active, phosphorylated form of DivK (DivK~P) that preferentially binds at the poles.

For the most part, the physiological role for the differential localization of these signalling proteins has been inferred from the analyses of mutants in which protein localization is perturbed during the cell cycle. In Δ*divJ* cells, the subcellular distribution of DivK remains cytoplasmic throughout the cell cycle (Jacobs *et al*., 2001); Δ*divJ* mutants have a cell division defect, and long and sometimes misplaced stalks (Wheeler and Shapiro, 1999). In the absence of PleC, DivK fails to delocalize from the new SW pole at cell division, yielding two daughter cells with polarly localized DivK (Jacobs *et al*., 2001); such *pleC* mutants are defective in motility and in the formation of the polar stalk and pili (Sommer and Newton, 1989; Wang *et al*., 1993; Wheeler and Shapiro, 1999). Another example is the Δ*podJ* mutant in which PleC is unable to polarly localize (Viollier *et al*., 2002a; Hinz *et al*., 2003); this mutant is defective in chemotaxis and fails to assemble the adhesive holdfast and to release the flagellum during the SW-to-ST cell transition (Wang *et al*., 1993; Crymes *et al*., 1999; Viollier *et al*., 2002a; Hinz *et al*., 2003). Thus, in each case, the loss of temporal or spatial regulation of protein localization is accompanied with various defects in cell cycle-controlled morphogenetic events, suggesting that the localization of these signalling proteins regulates the coordination between polar morphogenesis and cell cycle progression. However, some caveats remain with this interpretation as the defects observed in the *divJ*, *pleC* and *podJ* mutants may not result solely from the protein localization defect described. It will be important to disrupt protein localization by creating point mutations in CckA, DivJ, PleC and DivK to see how these mutations affect protein activity and cell function. To this end, the *cis*-acting determinants involved in protein localization must be determined. A recent study has made a crucial step in this direction by showing that a small region of 62 residues in the cytoplasmic linker of the sensor domain of DivJ (domain which presumably senses the signal that activates the autokinase activity of the protein) is necessary for the accumulation of DivJ at the ST pole (Sciochetti *et al*., 2002).

Although further experimentation will be necessary to pinpoint the specific function for the cell cycle-dependent localization of each signalling protein, it is safe to conclude from the available data that the differential localization of these proteins plays a regulatory role. This is certainly an exciting result as it uncovers a novel level of regulation in bacterial signal transduction. How does it work? In the compartmentalized and often bigger eukaryotic cell, differential localization of signalling proteins can fulfill at least three purposes. First, asymmetric protein localization can provide a means to produce daughters of distinct fate by segregating cell fate determinants preferentially to one daughter (Doe and Bowerman, 2001). Second, targeting signalling proteins to specific locations (such as intracellular organelles, the plasma membrane, lipid domains or cytoskeletal filaments) can regulate activation or substrate specificity by limiting the access of signalling proteins to localized signals or subtrates (Dorn and Mochly Rosen, 2002). Third, it is likely that the formation of signalling complexes at discrete subcellular sites increases the number of interactions between cognate signalling proteins and thereby augments the extent of activation of downstream processes. All three scenarios are conceivable for the cell cycle signalling system of *Caulobacter*. For instance, the fate of the ST progeny may be specified by the asymmetric subcellular localization of DivJ, which ensures that DivJ is inherited only by the ST daughter cell. The relationship between PleC, DivJ and DivK provides support for the ideas of signalling complexes and the presence of a localized effector and/or signal. DivJ controls cell division and the length and placement of the stalk (Ohta *et al*., 1992; Wheeler and Shapiro, 1999) whereas PleC regulates cellular asymmetry, motility, chemotaxis and pili assembly (Sommer and Newton, 1989; Wang *et al*., 1993; Burton *et al*., 1997). Clearly, these two histidine kinases activate different downstream processes in response to distinct signals. Yet, genetic and biochemical evidence indicates that DivJ and PleC are both cognate histidine kinases of DivK (Sommer and Newton, 1991; Hecht *et al*., 1995; Ohta and Newton, 2003). The formation of distinct signalling complexes at opposite poles, DivJ-DivK at the ST pole and PleC-DivK at the new SW pole in the PD cell, may limit cross-talk and hence provide some of the specificity of the responses mediated by PleC and DivJ. The signals sensed by DivJ and PleC may also be polarly localized given the involvement of DivJ and PleC in stalk biogenesis and motility respectively.

How is polar localization accomplished and regulated?

In eukaryotes, several mechanisms participate in protein

localization, including mRNA localization, localized translation, localized degradation, active protein transport along cytoskeletal elements using molecular motors, and diffusion with protein trapping by anchors or scaffolds. Although mRNA localization and localized synthesis or proteolysis may still occur, several findings indicate that in the *Caulobacter* cell cycle signalling system, the localized protein itself contains temporal and spatial information for its cell cycle-dependent localization. For example, the abundance of CckA and DivK is fairly constant during the cell cycle and the half-life of these proteins well exceeds the length of the cell cycle (Jacobs *et al*., 1999; 2001), indicating that it is the same population of CckA and DivK proteins that changes location during the cell cycle. The cellular level of DivJ and PleC varies during the cell cycle (Wheeler and Shapiro, 1999; Viollier *et al*., 2002b). However, inducing the presence of DivJ or of a functional PleC derivative throughout the cell cycle does not affect the cell cycle-dependent localization profile of the protein (Wheeler and Shapiro, 1999), indicating that the localization of the protein is inherently dynamic.

What characteristics of these proteins make them competent for polar localization? As mentioned above, phosphorylation of the DivK response regulator on its conserved aspartate residue (D53) is essential for the polar localization of DivK (Lam *et al*., 2003). Further evidence suggests that the binding of phosphorylated DivK (DivK~P) at the distinct poles during the cell cycle is controlled by the asymmetric localization of DivJ and PleC and the antagonistic activities of these two proteins on the cellular concentration of DivK~P (Lam *et al*., 2003).

In a Δ*divJ* mutant, the cellular concentration of DivK~P is decreased relative to wild-type, indicating that DivJ primarily functions as a kinase for DivK *in vivo* (Wheeler and Shapiro, 1999). It should be noted that the DivK~P level is not abolished in the Δ*divJ* mutant (or in the Δ*divJ pleC*::Tn*5* double mutant), suggesting that another kinase contributes to this residual level of DivK~P (Wheeler and Shapiro, 1999). Using a kinase-inactive DivJ mutant that has retained its ability to localize at the ST pole, it has been shown that the mere presence of DivJ at the ST pole recruits to that site the low level of DivK~P present in the cell. The kinase activity of DivJ promotes the subsequent binding of DivK~P at the new SW pole, presumably by increasing the concentration of DivK~P in the cell (Lam *et al*., 2003). Because the cellular level of DivK~P is lower in the SW stage relative to later stages of the cell cycle (Jacobs *et al*., 2001), these results suggest a model in which the polar location and kinase activity of DivJ coordinates the localization of DivK~P at the distinct poles.

PleC, on the other hand, mediates the pole-specific release of DivK~P in the late PD cell. In the absence of functional PleC, DivK~P fails to delocalize from the new SW pole at cell division, yielding two daughter cells with polarly localized DivK~P (Jacobs *et al*., 2001; Lam *et al*., 2003). In these *pleC* mutants, the level of DivK~P is higher than in wild-type cells (Wheeler and Shapiro, 1999; Lam *et al*., 2003), arguing that PleC primarily acts as a phosphatase for DivK~P *in vivo*. It is common for histidine kinases to have phosphatase activity toward their cognate response regulators (Shinagawa *et al*., 1994; Hsing and Silhavy, 1997; Hsing *et al*., 1998; Kramer and Weiss, 1999). Together, the data suggest that the asymmetric location of PleC in the late PD cell (Fig. 2) favours the release of DivK~P from the new SW pole by decreasing the overall DivK~P concentration in the SW compartment (Lam *et al*., 2003). Thus, the asymmetric location and antagonistic activity of DivJ and PleC on the cellular DivK~P levels regulates the differential localization of DivK during the cell cycle.

The next obvious question is what controls the localization of DivJ and PleC? The polar localization of DivJ and PleC is not controlled by phosphorylation as mutants of DivJ or PleC that lack the phosphorylation site are still able to localize polarly (Sciochetti *et al*., 2002; Viollier *et al*., 2002b; Lam *et al*., 2003). In analogy to eukaryotic systems, PleC and DivJ may diffuse in the cytoplasmic membrane to reach their specific destination where localized anchor or scaffold molecules may trap them to form localized signalling complexes. A good candidate for the anchoring or scaffolding role is the polar organelle development protein, PodJ, which is involved in the timed ejection of the flagellum and in the development of the pili and holdfast (Crymes *et al*., 1999; Viollier *et al*., 2002a; Hinz *et al*., 2003; Smith *et al*., 2003). In the absence of PodJ, the abundance of PleC is unaffected but the dynamic localization of PleC during the cell cycle is completely impaired such that PleC is found dispersed around the cytoplasmic membrane in all cell types (Viollier *et al*., 2002a; Hinz *et al*., 2003). PodJ has two forms with differential abundance and localization during the cell cycle: a full-length form ($PodJ_L$) and a truncated form ($PodJ_S$) that derives from a C-terminal processing of $PodJ_L$ (Viollier *et al*., 2002a; Hinz *et al*., 2003). $PodJ_S$ is sufficient to carry out the developmental functions involved in chemotaxis and holdfast assembly whereas pili assembly requires the function of Pod_L (Viollier *et al*., 2002a). $PodJ_L$ is synthesized in the PD cell where it accumulates at the new SW pole (Fig. 3). Before cell separation, a proteolytic cleavage converts $PodJ_L$ into its shorter form, $PodJ_S$, producing a SW cell with polarly localized $PodJ_S$ after cell division. During the SW-to-ST cell transition, $PodJ_S$ is degraded and $PodJ_L$ reappears by *de novo* synthesis in the early PD cell. Thus, the polar localization of PodJ isoforms during the cell cycle strikingly resembles that of PleC, suggesting that the differential abundance and localization of PodJ isoforms provide temporal and positional informa-

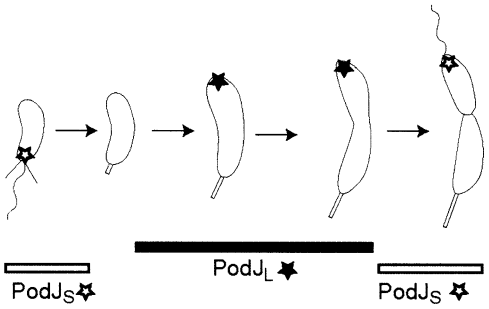

Fig. 3. Differential abundance and localization of PodJ isoforms. The full-length form of PodJ, $PodJ_L$ (closed star), is synthesized in the PD cell where it accumulates at the new SW pole. Before cell division, $PodJ_L$ is processed, yielding a truncated form, $PodJ_S$ (open star). The asymmetric localization of $PodJ_S$ results in the segregation of $PodJ_S$ into the SW progeny. During the SW-to-ST cell transition, $PodJ_S$ is degraded. The times during which $PodJ_L$ and $PodJ_S$ are present are indicated by the closed and open bars respectively.

tion for the localization of PleC during the cell cycle (Viollier *et al*., 2002a; Hinz *et al*., 2003).

Perspective

It is now evident that the dynamic localization of signalling proteins that orchestrate the *Caulobacter* cell cycle provides an important means of regulation. Unless we consider the cellular location of these proteins, their interaction at the subcellular level, and the characteristics of their movement, our understanding of the cell cycle regulatory network will be incomplete. It will also be important to pinpoint the specific residues in these proteins critical for localization and to identify factors that interact with these residues. Recently, a functional DivK homologue in *Sinorhizobium meliloti* was shown to exhibit a cyclical localization, alternating between polar localization and dispersed cytoplasmic distribution during the *S. meliloti* cell cycle (Lam *et al*., 2003). This observation in a seemingly symmetric bacterium that has no polar organelles suggests that the modulation of localization of signalling proteins may be a common bacterial strategy to regulate signal transduction. Clearly, much remains to be discovered and this new odyssey promises to be full of exciting surprises.

Acknowledgements

Because of space constraints, literature citations have been sometimes limited to recent relevant reviews; I apologise to those authors whose important work has not been cited directly. I would like to thank Greg Marczynski and Bert Ely for their helpful suggestions on the manuscript. I thank Yves Brun and the members of the Jacobs-Wagner laboratory for critical reading of the manuscript. A portion of the work discussed in this review was supported by the NIH Grant GM065835.

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