The *Caulobacter* divisome: parts list, assembly, and mechanism of action

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The cell division apparatus (divisome) in bacteria mediates the constriction of the cell membranes and the inward growth of the cell wall in coordination with cell growth and chromosome segregation. Despite decades of study, however, relatively little is known about the structure and assembly of the divisome or the molecular functions of its components. The tubulin relative FtsZ is the best characterized and most highly conserved divisome protein. It is a GTPase that polymerizes near midcell, defining the site of cell division. FtsZ serves as a scaffold for assembly of the divisome and is hypothesized to generate constrictive forces. As the structure, dynamics and function of FtsZ are likely to be regulated by interacting partners, we sought to identify all FtsZ-binding proteins in *Caulobacter*. To do this, we developed an assay in which we overproduce a GTPase-defective mutant of FtsZ, causing cells to adopt a distinct morphology wherein long, slender constrictions containing FtsZ separate the cell bodies. We found that fluorescent fusions to *Caulobacter* homologs of all known FtsZ-binding proteins co-localize with FtsZ in the constrictions of mutant cells, whereas divisome proteins that do not bind to FtsZ are diffuse. To identify novel FtsZ-binding proteins we used this assay to probe the library of *Caulobacter* strains bearing fluorescent fusions to 442 different localized proteins generated by the Gitai lab at Princeton. From this screen, we identified six proteins that clearly localized to the constrictions, four of which are previously uncharacterized. To date, we have confirmed that three of these proteins bind directly to FtsZ *in vitro*. Surprisingly, we discovered an additional set of proteins in our screen that were specifically excluded from the FtsZ-rich constrictions, indicating a second mode of FtsZ-directed protein localization in *Caulobacter*. We are now taking genetic, cytological, and biochemical approaches aimed at uncovering the mechanisms by which FtsZ controls the localization of these factors and determining their cellular functions.

In addition to those new FtsZ-binding proteins identified in our screen, at least sixteen other proteins are recruited to form the divisome downstream of FtsZ. To gain molecular insight into their functions, we have cloned the *Caulobacter* homologs of the known cell division proteins and have so far confirmed that thirteen of these are essential for cytokinesis in this organism. Moreover, we find that fluorescent fusions to each of these proteins localize to the division site and we are in the midst of a careful study following the timing of their localization to better understand the process of divisome assembly. Finally, we have made significant progress in understanding the role of the Tol-Pal complex in *Caulobacter* cytokinesis. Using a combination of genetic, cell biological, and high resolution microscopy techniques (in collaboration with the Downing lab at LBNL) we have shown that Pal is specifically required for outer membrane integrity and for invagination of the outer membrane during cytokinesis. Current and future efforts are directed at similarly detailed structural and functional analyses of the other *Caulobacter* divisome proteins.

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