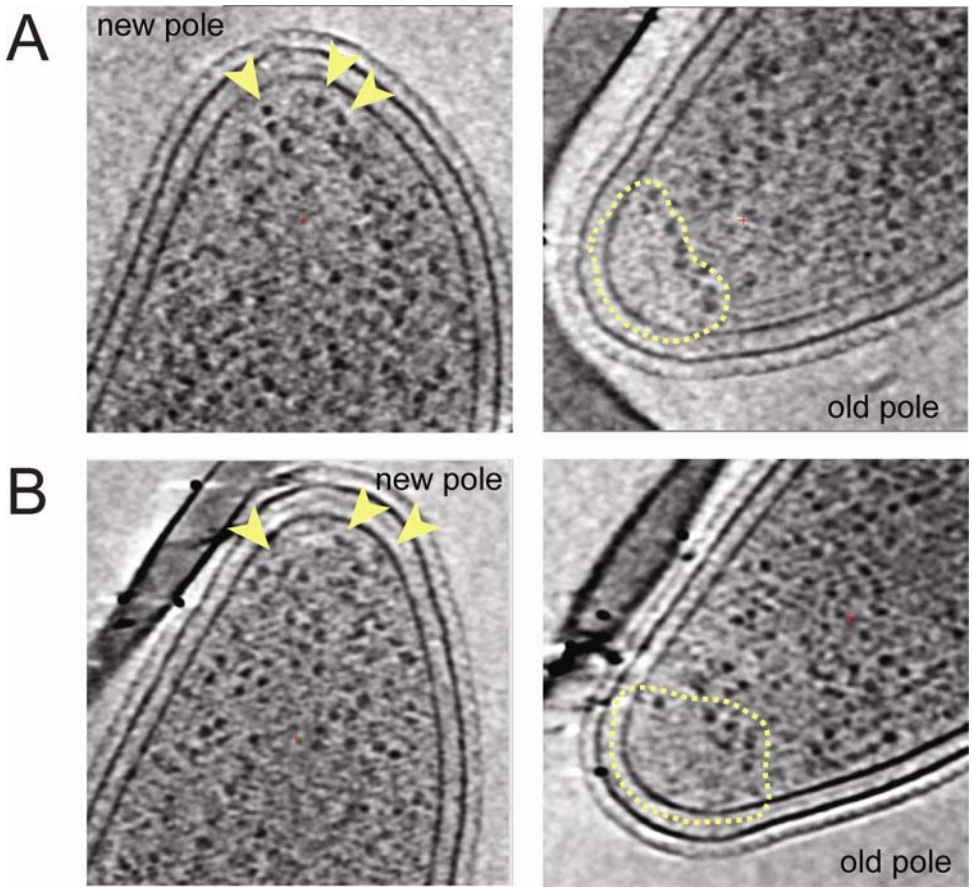
Supplementary Data

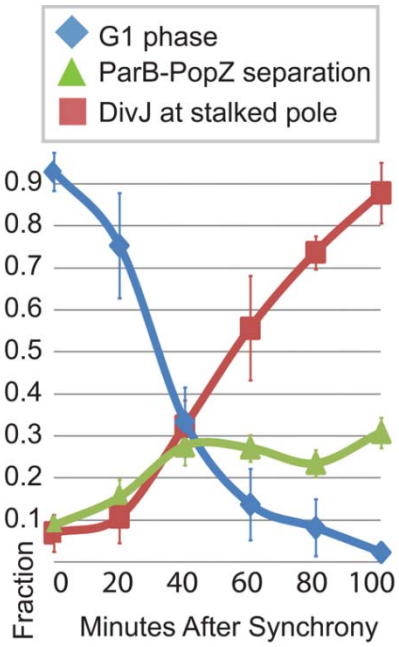
Caulobacter PopZ forms a polar sub-domain dictating sequential changes in pole composition and function

Grant R. Bowman[1], Luis R. Comolli[2], Guido M. Gaietta[3], Mike Fero[1], Sun-Hae Hong[5], Ying Jones[3], Julie H. Lee [3], Kenneth H Downing[2], Mark H. Ellisman[3,4], Harley H. McAdams[1], and Lucy Shapiro[1*].

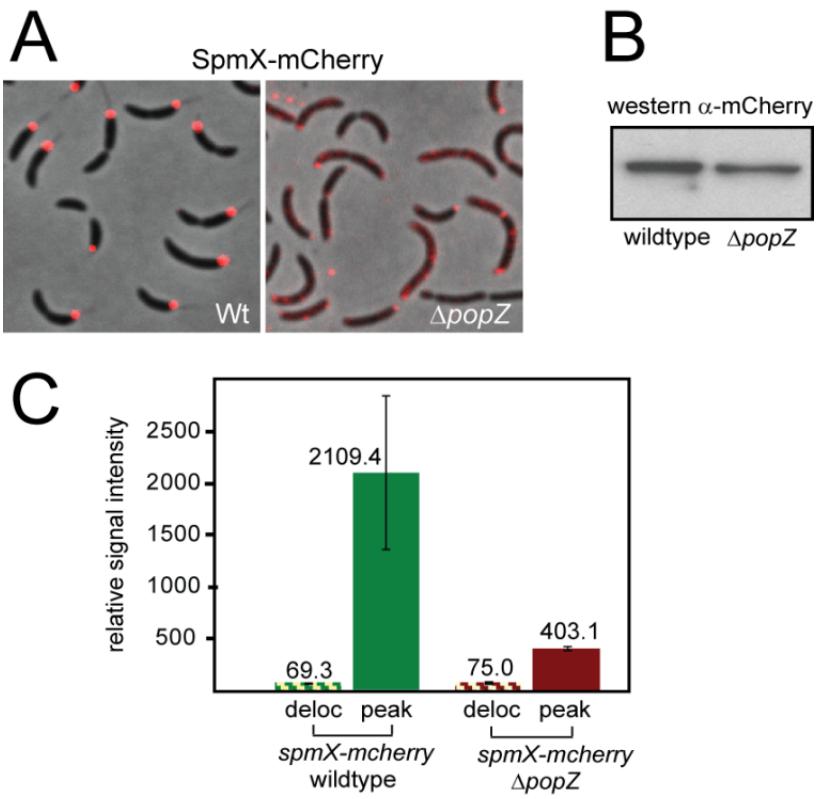
[1]Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305, USA
[2]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, USA
[3]National Center for Microscopy and Imaging Research, Center for Research on Biological Systems, and [4]Department of Neurosciences, Department Code 0608, University of California, San Diego, 92093, USA
[5]Department of Physics, Stanford University School of Humanities and Sciences, Stanford, CA 94305, USA



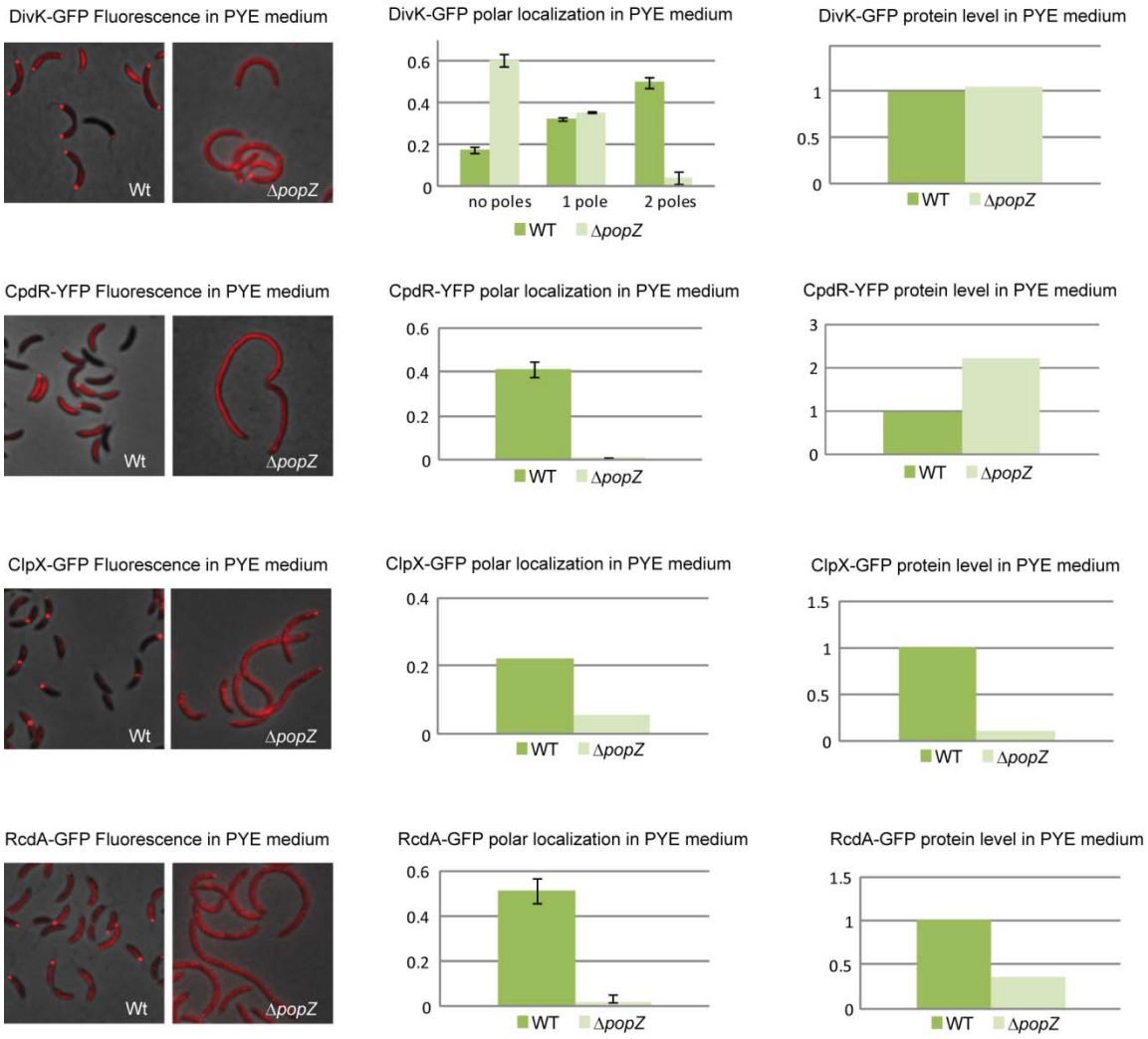
Supplementary Figure S1: Two representative examples of swarmer cells, as observed by cryo-EM. The old and new poles were distinguished by the flagellar basal body (not visible in these sections), which is found only at the old pole. Magnified views of the indicated poles from each cell are shown in these panels. Polar ribosomes are labeled with yellow arrowheads and ribosome-free poles are highlighted with a dotted yellow line. The three dimensional data sets that correspond to these images are presented in Supplementary Movies M1-M4.



Supplementary Figure S2: The experiment and quantitation in Figure 3A-B was repeated for strain GB563, expressing CFP-ParB, mCherry-PopZ and DivJ-YFP. The genotype for GB563 is *parB*::*cfp-parB ; vanA*::P_{vanA}-*mCherry-popZ* ; *divJ*::*divJ-yfp*. To make this strain, strain MT190 was transformed by plasmid pGB539 and by transduction with Kan^R from CJW858 (Matroule, J. Y., H. Lam, D. T. Burnette & C. Jacobs-Wagner, (2004) Cytokinesis monitoring during development; rapid pole-to-pole shuttling of a signaling protein by localized kinase and phosphatase in Caulobacter. *Cell* **118**: 579-590.)



Supplementary Figure S3: PopZ facilitates SpmX-mCherry localization when cells are grown in minimal medium. A) Localization of SpmX-mCherry in wildtype (strain GB378) and Δ*popZ* (strain GB387) backgrounds when cells are grown in M2G medium. B) A western blot showing the cellular levels of SpmX-mCherry protein in native *popZ* and Δ*popZ* backgrounds when cells are grown in M2G medium. The amount of SpmX-mCherry protein in the Δ*popZ* strain is 65% of the level in wildtype cells. C) Quantitation of the data in A, as described in Materials and Methods. Peak refers to the intensity of the localized signal; Deloc refers to the median intensity of the delocalized signal across the cell body.



Supplementary Figure S4: PopZ mediates multiprotein complex assembly at the developing stalked pole when cells are grown in rich medium. A) Localization of DivK-GFP, CpdR-YFP, ClpX-GFP, and RcdA-GFP in wildtype and Δ*popZ* cells grown in PYE medium. For each image set, representative images of fluorescence intensity (red) are overlayed on the corresponding phase contrast background (gray). The middle panel shows quantified data from at least two independent experiments, each involving more than 200 cells, with error bars representing the standard deviation between trials. The right panel shows the cellular levels of the fluorescently tagged protein in PYE medium, as determined by western blot, shown as a bar graph depicting the quantitation and normalization of band intensities, with wildtype level set to 1. The strains used were the same as in Figure 5.

Legends for Supplementary Movies:

Supplementary Movie M1: A three dimensional cryo-EM tomographic data set corresponding to the new pole of the swarmer cell presented in the upper panels of Supplementary Figure S1. Ribosomal particles fill the cytoplasm, including the area adjacent to the inner membrane at the pole.

Supplementary Movie M2: A three dimensional cryo-EM tomographic data set corresponding to the old pole of the swarmer cell presented in the upper panels of Supplementary Figure S1. A distinct ribosome-free area is visible at the cell pole.

Supplementary Movie M3: A three dimensional cryo-EM tomographic data set corresponding to the new pole of the swarmer cell presented in the lower panels of Supplementary Figure S1. Ribosomal particles fill the cytoplasm, including the area adjacent to the inner membrane at the pole.

Supplementary Movie M4: A three dimensional cryo-EM tomographic data set corresponding to the old pole of the swarmer cell presented in the lower panels of Supplementary Figure S1. A distinct ribosome-free area is visible at the cell pole, and outlined in yellow in select frames.

Supplementary Movie M5: A time-lapse movie of strain MT190 cells on an agarose pad in M2G medium. Five frames were collected at four minute intervals. CFP-ParB fluorescence is colored green and overlayed on the corresponding phase contrast image (gray shading). Stalked poles are marked by cyan squares.

Supplementary Movie M6: A time-lapse movie of strain GB529 cells on an agarose pad in M2G medium. Five frames were collected at four minute intervals. CFP-ParB fluorescence is colored green, mCherry-PopZ fluorescence is colored red, and these layers are superimposed on the corresponding phase contrast image (gray shading). Stalked poles are marked by cyan squares.

Supplementary Movie M7: A time-lapse movie of strain GB301 cells on an agarose pad in M2G medium. Six frames were collected at four minute intervals. CFP-ParB fluorescence is colored green, PopZ-YFP fluorescence is colored red, and these layers are superimposed on the corresponding phase contrast image (gray shading). Stalked poles are marked by cyan squares.