equivalent and competitive priming efficiency with divergent sequences, primers incorporated inosine residues at the few positions where divergence from the conserved consensus sequence has been reported (*13, 16*). For QC-PCR analysis, two plasmids were prepared, one containing the target sequence (pQP1) and the other containing the identical sequence except for an 80-bp internal deletion (pQP1Δ80), sufficient to allow the derived PCR products to be readily resolved by electrophoresis (*13*). In vitro RNA transcripts were prepared with commercially available kits. Final preparations in water were determined to be essentially free of degradation products by Northern (RNA) blot analysis and were quantified by measurement of absorbance at 260 nm. Portions were stored at −70°C until needed. PCR reaction conditions and protocols were generally similar to those found in commercially available kits (Perkin-Elmer, Norwalk, CT). Each test sample was divided into eight replicate portions and analyzed in the presence of 0 to 50,000 copies per reaction of competitive template. The initial reaction was performed in a total volume of 30 μl and contained 5 μl of test RNA (corresponding to 5% or less of the total specimen), 5 μl of competing RNA preparation or water, and 30 U of cloned Moloney virus reverse transcriptase (BRL, Bethesda, MD). One portion from each specimen was analyzed without reverse transcription and in the absence of competitive template. After 10 min at room temperature to allow for partial extension and stabilization of random hexamer primers, conversion of RNA into cDNA was allowed to continue for 30 min at 42°C. This reaction was then adjusted to contain primers and additional buffer in a total volume of 60 μl. Amplification was performed as described (*13*), with 45 cycles (94°C for 1 min, 50°C for 2 min, and 72°C for 1 min), followed by a final incubation at 5°C for 5 min. After amplification, approximately 7% of each reaction product mixture was separated by electrophoresis in composite 2% Synergel (Diversified Biotech, Newton Center, MA)–1% agarose (FMC Bioproducts, Rockport, ME) gels in 20 mM tris acetate (pH 7.8) and 1 mM EDTA. Gels were stained with ethidium bromide for visualization under ultraviolet illumination. Quantitation of fluorescence of both wild-type and competitive template product bands was performed on a Lynx 4000 molecular biology workstation with matched custom software (Applied Imaging, Santa Clara, CA), as described (*13*). Competition equivalence points were determined by interpolation on plots of the logarithm of the calculated ratio of signal for the competitive template–derived product over the signal for the wild-type target sequence–derived product (corrected for molar ratio) versus the logarithm of the copy number of added competitive template (Fig. 1) (*13*).

18. Endpoint dilution cultures of plasma for cell-free infectious virus were determined for fresh specimens, generally in duplicate or quadruplicate, as described (*6, 7*). Plasma samples were not filtered (to eliminate the possibility of inadvertent loss of virus).
19. Regular and ICD p24 antigen determinations were performed in duplicate with the Coulter Diagnostics kit assay, according to the manufacturer's recommendations (Coulter, Hialeah, FL).
20. M. Piatak *et al.*, unpublished observations.
21. Analysis of variance, Duncan's multiple range test, and Tukey's Studentized range (HSD) test as calculated by the SAS statistical analysis software package (SAS Institute, Cary, NC). Plasma RNA data were subjected to logarithm transformation before statistical analysis.
22. Spearman correlation coefficients as calculated by the SAS statistical analysis software package. Plasma RNA data were subjected to logarithm transformation before statistical analysis.
23. M. Tersmette *et al.*, *Lancet* **335**, 983 (1989); M. Tersmette *et al.*, *J. Virol.* **63**, 2118 (1989); D. S. Stein, J. A. Korvick, S. H. Vermund, *J. Infect. Dis.* **165**, 352 (1992).
24. Paired *t* test analysis on logarithm-transformed data set.
25. S. P. Layne *et al.*, *Virology* **189**, 695 (1992); A. S. Bourinbaiar, *Nature* **349**, 111 (1991); J. A. McKeating, J. P. Moore, *ibid.*, p. 660.
26. F. Barré-Sinoussi *et al.*, *Science* **220**, 868 (1983); M. Popovic, M. G. Sarngadharan, E. Read, R. C. Gallo, *ibid.* **224**, 497 (1984); J. A. Levy *et al.*, *ibid.* **225**, 840 (1984).
27. To calculate ratios of total virions determined by RNA QC-PCR analysis to culturable virus, we assigned specimens that did not contain culturable virus a TCID value of 1 to avoid denominators of 0. The ratios calculated therefore represent a lower estimate of the true ratios of total circulating virions to culturable virus.
28. R. A. Weiss *et al.*, *Nature* **316**, 69 (1985); M. Robert-Guroff, M. Brown, R. C. Gallo, *ibid.*, p. 72; P. L. Nara, R. R. Garrity, J. Goudsmit, *FASEB J.* **5**, 2437 (1991).
29. J. P. Moore, J. A. McKeating, R. A. Weiss, Q. J. Sattentau, *Science* **250**, 1139 (1990).
30. Y. Li *et al.*, *J. Virol.* **65**, 3973 (1991); Y. Li *et al.*, *ibid.* **66**, 6587 (1992); J. Meyerhans *et al.*, *Cell* **58**, 901 (1989).
31. S. M. Schnittman, J. J. Greenhouse, H. C. Lane, P. F. Pierce, A. S. Fauci, *AIDS Res. Hum. Retroviruses* **7**, 361 (1991).
32. N. L. Michael, M. Vahey, D. Burke, R. R. Redfield, *J. Virol.* **66**, 310 (1992).
33. J. Overbaugh, P. R. Donahue, S. L. Quackenbush, E. A. Hoover, J. I. Mullins, *Science* **239**, 906 (1988); D. C. Aziz, Z. Hanna, P. Jolicoeur, *Nature* **338**, 505 (1989); S. K. Chattopadhyay, H. C. Morse III, M. Makino, S. K. Ruscetti, J. W. Hartley, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3862 (1989).
34. J. B. Margolick, D. J. Volkman, T. M. Folks, A. S. Fauci, *J. Immunol.* **138**, 1719 (1987); D. L. Mann *et al.*, *ibid.*, p. 2640; M. R. Shalaby *et al.*, *Cell. Immunol.* **110**, 140 (1987); L. O. Arthur *et al.*, *Science* **258**, 1935 (1992); L. Imberti, A. Sottini, A. Bettinardi, M. Puoti, D. Primi, *ibid.* **254**, 860 (1991); C. Terai, R. S. Kornbluth, C. D. Pauza, D. D. Richman, D. A. Carson, *J. Clin. Invest.* **87**, 1710 (1991); A. G. Laurent-Crawford *et al.*, *Virology* **185**, 829 (1991); J. C. Ameisen and A. Capron, *Immunol. Today* **12**, 102 (1991); H. Groux *et al.*, *J. Exp. Med.* **175**, 331 (1992); M. K. Newell, L. J. Haughn, C. R. Maroun, M. H. Julius, *Nature* **347**, 286 (1990); L. Meyaard *et al.*, *Science* **257**, 217 (1992); R. S. Mittler and M. K. Hoffman, *ibid.* **245**, 1380 (1989); G. P. Linette, R. J. Hartzman, J. A. Ledbetter, C. H. June, *ibid.* **241**, 573 (1988).
35. M. Clerici, J. A. Berzofsky, G. M. Shearer, C. O. Tacket, *J. Infect. Dis.* **164**, 178 (1991); M. Clerici *et al.*, *ibid.* **165**, 1012 (1992).
36. J. Cohen, *Science* **258**, 388 (1992).

37. We thank the patients who provided specimens for this study; R. Whitley for helpful discussions; A. Fauci for sharing work before publication; S. Campbell-Hill and P. Chopra for technical assistance; S. Chen for assistance with statistical analysis; and R. Cuevas and A. Chapman for assistance with preparation of the manuscript. Supported in part by grants AI25922 (to J.D.L.), AI27290 (to G.M.S.), and AI32775 (to M.S.S.) from the National Institutes of Health; by grant DAMD 17-90-C-0064 (to G.M.S.) from the Department of Defense; and by the core research facilities of the University of Alabama, Birmingham, Center for AIDS Research, the Birmingham Veterans Administration Medical Center, and the General Clinical Research Center.

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Requirement of the Carboxyl Terminus of a Bacterial Chemoreceptor for Its Targeted Proteolysis

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The bacterium *Caulobacter crescentus* yields two different progeny at each cell division; a chemotactically competent swarmer cell and a sessile stalked cell. The chemotaxis proteins are synthesized in the predivisional cell and then partition only to the swarmer cell upon division. The chemoreceptors that were newly synthesized were located at the nascent swarmer pole of the predivisional cell, an indication that asymmetry was established prior to cell division. When the swarmer cell differentiated into a stalked cell, the chemoreceptor was specifically degraded by virtue of an amino acid sequence located at its carboxyl terminus. Thus, a temporally and spatially restricted proteolytic event was a component of this differentiation process.

Cell divisions that yield two different progeny cells are fundamental to developmental programs in all organisms (*1*). In *Caulobacter crescentus*, the generation of two distinct cell types at each cell division is due, in part, to the asymmetric distribution of proteins in the cell before it has divided. The predivisional cell assembles a flagellum and several pili at one pole, which are then partitioned to the swarmer cell progeny. In addition to bearing a flagellum and polar pili, the swarmer cell is chemotactically competent. The chemoreceptor McpA, which is one of the family of MCPs (methyl accepting chemotaxis protein), is synthesized only in the predivisional cell (*2*). Thus, the swarmer cell inherits McpA from the predivisional cell (*3*).

We now describe our studies of the spatial distribution of the newly synthesized chemoreceptor in the predivisional cell and its subsequent fate throughout the cell cycle. Assays in vitro have shown that the methyl-accepting activity of the chemoreceptors, and the activities of the methyltransferase and methylesterase are lost during the transition of a swarmer cell into a stalked cell (*4*). The McpA chemoreceptor is positioned at the flagellated pole of the swarmer cell (*3*), and we now show that it is degraded during the transi-

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tion from the swarmer to the stalked cell.

To investigate the role of proteolysis in the cellular distribution of the chemoreceptors during the cell cycle, we synchronized cultures of C. *crescentus* and at intervals tested for the presence of the McpA chemoreceptor by immunoblotting with antiserum to McpA (*3*). McpA was present in swarmer cells (Fig. 1), but began to rapidly disappear 15 minutes after the start of the cell cycle, as swarmer cells differentiated into stalked cells. Later in the cell cycle McpA reappeared after its synthesis was initiated in the predivisional cell. The McpA chemoreceptor had a half-life of more than 90 minutes when measured by pulse-chase labeling experiments with [^{35}S]methionine (*5*); however, the receptor was rapidly degraded when swarmer cells differentiated into stalked cells (Fig. 1). These data suggest that proteolysis of McpA was associated with the transition from swarmer to stalk cells. This proteolysis coincides with specific morphological events, such as the loss of the single polar flagellum and polar pili.

We used immunoelectron microscopy to determine the spatial distribution of McpA throughout the cell cycle (Fig. 2). The McpA chemoreceptor tagged with the M2 epitope (6) was located at one pole of the swarmer cell (Fig. 2A1). McpA was then lost as the swarmer cell pole developed into the stalked cell pole (Fig. 2A2), and McpA reappeared at the nascent swarmer pole of the predivisional cell after the initiation of its synthesis (Fig. 2A3). The newly synthesized McpA chemoreceptor was thus targeted to the flagellated pole of the predivisional cell where it remained in the swarmer cell progeny. The cellular distribution of the epitope-tagged McpA behaved identically to the wild-type McpA chemoreceptor as shown below.

We constructed a series of McpA COOH-terminal deletions (*7*) in order to identify the region necessary for McpA degradation during the transition from swarmer cells to stalked cells (Fig. 3). Both the epitope-tagged McpA (pRCM22) and the wild-type protein encoded by the plasmid pRCH9 were degraded in a manner similar to the chromosomally encoded McpA (Fig. 4). The degradation of the COOH-terminal epitope-tagged McpA, pRCM22, implied that a free COOH-terminus was not required. When a large COOH-terminal deletion of *mcpA*, pRCM223 (Fig. 3, top), was tested, it was not degraded during

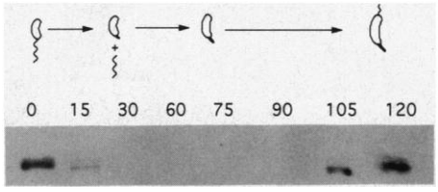


Fig. 1. Cell cycle degradation of the McpA chemoreceptor. *Caulobacter crescentus* NA1000 was grown in M2G minimal medium (*17*) at 32°C to an A_{660} of 1.0 and centrifuged at 11,300*g* for 15 minutes at 4°C. The sedimented pellet was suspended in ice-cold M2 salts, and the swarmer cells were isolated by Ludox density centrifugation (*18*). The swarmer cells were allowed to proceed through the cell cycle. At the times indicated, samples (20 μl) were removed (*19*), added to an equal volume of buffer [125 mM tris-HCl, pH 7.0, 4 percent (w/v) SDS, 1.46 M 2-mercaptoethanol, 20 percent (v/v) glycerol], and the diluted samples were frozen at −20°C. As needed, the frozen samples were boiled for 5 minutes and then subjected to electrophoresis on an SDS–10 percent polyacrylamide gel (*20*). The separated proteins were transferred to nitrocellulose (*21*) and were processed (*2*) with antiserum to McpA (*3*). Time is shown in minutes with a generation time of 180 minutes.

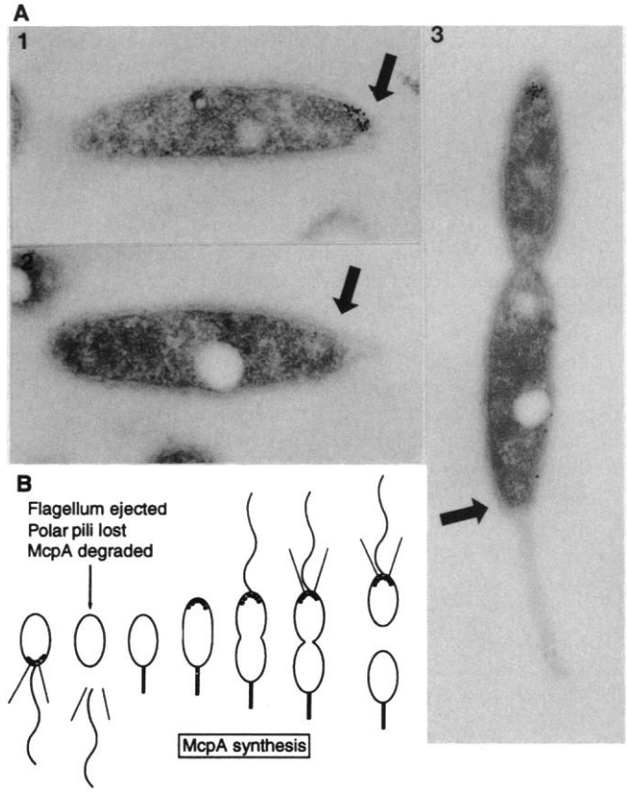


Fig. 2. Cell cycle localization of the McpA chemoreceptor. (**A**) Immunoelectron microscopic localization (*3*) of a full-length epitope-tagged McpA derivative, pRCM22 in the strain SC1130N (*22*), with antiserum to McpA and goat antiserum to rabbit immunoglobulin G conjugated with 10 nM colloidal gold particles. (Panel 1) McpA-M2 localization in a swarmer cell; (panel 2) a representative stalk cell at 45 minutes from the start of the cell cycle; (panel 3) a typical predivisional cell at 150 minutes from the start of the cell cycle. Arrows indicate the flagellated pole of the swarmer cell and the same cell pole as the swarmer cell differentiates into a stalked cell and then a predivisional cell. (**B**) Wild-type *C. crescentus* cell cycle showing the location throughout the cell cycle of the single polar flagellum, polar pili, and McpA (dots).

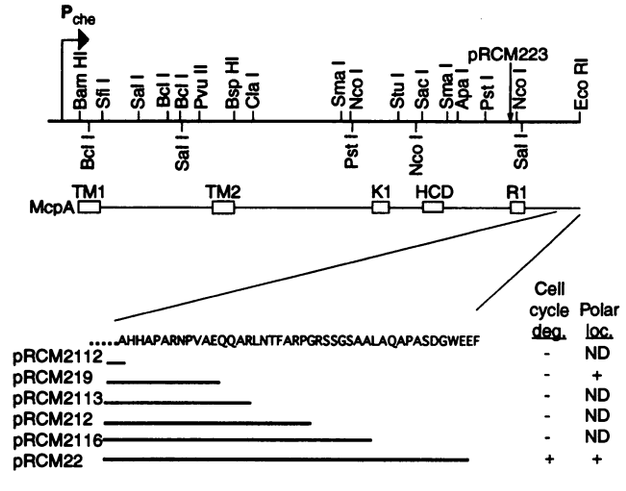


Fig. 3. Deletion constructs of *mcpA* and a restriction map of *mcpA* and the COOH-terminal sequence of McpA. TM1, transmembrane domain 1; TM2, transmembrane domain 2; K1 and R1, methylation domains; HCD, highly conserved domain. The COOH-terminal deletions of *mcpA* (*7*) are shown underneath the COOH-terminal sequence of McpA with the exception of pRCM223, which is indicated by an arrow on the restriction map. The black lines show the extent of McpA that is present in each deletion. Cell cycle deg., the presence (+) or absence (−) of cell cycle degradation of the McpA deletions; Polar loc., polar localization; ND, not determined. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

the cell cycle. However, in the same cells the chromosomally encoded wild-type McpA protein was degraded (Fig. 4). Therefore a portion of the McpA COOH-terminus was required for its proteolysis.

Further COOH-terminal deletions were constructed (Fig. 3), and cell cycle immunoblots were performed with strains bearing these plasmid-borne COOH-terminal McpA deletions (Fig. 4). The smallest COOH-terminal deletion, lacking only 14 amino acids, pRCM2116, was not degraded during the transition from the swarmer to the stalked cell. The COOH-terminal region of McpA (Fig. 3) was not conserved in the *Escherichia coli* chemoreceptors Tsr (8) and Tar (9), except for the last four amino acids. The last four amino acids are identical in Tsr (*10*) and Tar, but are similar to those in McpA. The conservation of the four COOH-terminal amino acids suggests that they may have a role in the structure or function of the chemoreceptors; but these amino acids are not solely responsible for the cell cycle controlled proteolysis of McpA protein. When the *E. coli* chemoreceptor gene *tsr* is expressed in C. *crescentus*, it is not degraded during the transition from the swarmer to the stalked cell (*11*). Thus, the COOH-terminal sequence that is present in the *Caulobacter* McpA, but absent in the *E. coli* chemoreceptor Tsr, is required for proteolysis in stalked cells.

Tsr is located at both poles in C. *crescentus* (*12*) and *E. coli* (*13*), even though it lacks the signal for cell cycle dependent proteolysis. This result does not support the model that proteolysis of randomly distributed chemoreceptors results in polar localization. We used immunoelectron microscopy to examine a strain bearing the plasmid pRCM219 (Fig. 3) to directly demonstrate that proteolysis of the chemoreceptor is not required for polar localization. This nondegraded deletion derivative of McpA localized to the swarmer cell pole (Fig. 5A1). However, it remained at the pole as the cell differentiated into a stalked cell (Fig. 5A2). Upon renewed synthesis of McpA, gold particles were detected at both the nascent swarmer pole and the old stalked pole of the predivisional cell (Fig. 5A3).

We determined the number of gold particle clusters at the pole of swarmer cells, and followed the fate of these clusters at that pole as the cells proceeded through the cell cycle (Fig. 6). In the strain bearing the plasmid pRCM219, approximately 50 percent of the swarmer cell sections had visible polar clusters and these clusters were observed at that pole throughout the cell cycle. In wild-type cells, or cells with a plasmid-borne *mcpA* gene, a significant number of clustered colloidal gold particles were observed only at the pole of swarmer cells, and were lost during the transition from swarmer to stalked cells. The presence of gold particles at the stalked pole in strains bearing pRCM219 supports the argument that proteolysis is not a requirement for polar localization and that stalked poles are

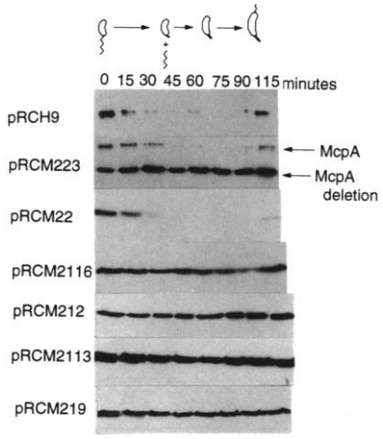


Fig. 4. Cell cycle immunoblots of McpA COOH-terminal deletions. Cell cycle immunoblots were performed as described in the legend to Fig. 1. The plasmid pRCH9 encodes the entire *mcpA* operon. Immunoblots of *C. crescentus* NA1000 strains containing pRCH9 and pRCM223 (*23*) were performed with antiserum to McpA. Immunoblots of all the other *C. crescentus* NA1000 strains containing the plasmids pRCM22, pRCM2116, pRCM212, pRCM2113, and pRCM219 were performed with the monoclonal M2 antibody (IBI, Inc.). The immunoblots were incubated with M2 monoclonal antibody because the strains contained the wild-type McpA as well as the McpA deletion derivatives, and the McpA deletions were not electrophoretically distinct from the wild-type protein. A schematic of the cell cycle is shown to indicate the cell types assayed at the times indicated.

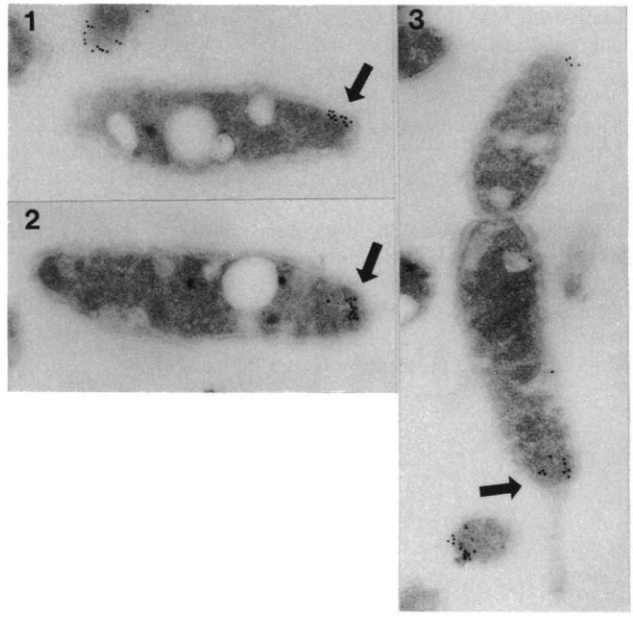
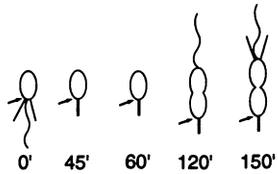


Fig. 5. Cell cycle localization of a nondegraded McpA derivative. Cell cycle immunoelectron micrographs of the strain SC1130N bearing the plasmid pRCM219. The intracellular distribution of the nondegraded McpA derivative was detected using antibodies to McpA as described in Fig. 2. (Panel 1) Swarmer cell; (panel 2) A stalked cell 60 minutes from the start of the cell cycle; (panel 3) A typical predivisional cell at 150 minutes. Arrows indicate clusters of gold particles at the same poles.



McpA source	Cells with clusters at pole (%)				
Chromosome	46	0		4	0
pRCM22	73	2		1	
pRCM219	53		51		52

Fig. 6. Localization of McpA at the flagellated pole of the swarmer cell and the subsequent stalked pole. Cells at the indicated times (minutes) in the cell cycle were prepared for immunoelectron microscopy as described in Fig. 2. The wild-type McpA (chromosomal copy) distribution was determined in sections of a synchronizable wild-type strain, NA1000. Both plasmids pRCM22, which encodes an epitope-tagged McpA, and pRCM219, which encodes a nondegraded McpA derivative, were in the strain SC1130N. The distribution of McpA at the flagellated pole of the swarmer cell was followed throughout the cell cycle as indicated by the arrows in the diagram of the cell cycle. Sections of swarmer cells that appeared to contain both poles were identified and the presence of McpA (≥ four gold particles tightly associated with the indicated polar region) was determined. Stalked poles of stalked cells were similarly identified and counted as were stalked poles of predivisional cells. In each strain more than 100 sections were examined, and the percentage of sections of each cell type with polar gold particles are shown.

competent for retention of McpA. Although the presence of the McpA chemoreceptors at the stalked pole is due to lack of turnover, it may be that under normal circumstances the newly synthesized McpA is targeted to the stalked pole as well as to the flagellated pole, but is degraded at the stalked pole soon after synthesis. We know that in *E. coli* the chemoreceptors can be targeted to both cell poles (*13*). Thus, proteolysis could play a role in the spatial distribution of McpA in C. *crescentus* by removing McpA from the stalked pole of the predivisional cell. Perhaps the presence of protease at the stalked cell pole prevents the deposition of other proteins that are used for the assembly of the flagellum and pili. There is evidence for spatially restricted proteolysis in eukaryotic cells. Localized proteolysis has been shown to be involved in setting up restricted protein distribution in polarized epithelial cells, resulting in the removal of proteins from one membrane domain and not the other upon induction of polarization (*14*).

The specific degradation of McpA could be mediated by a localized activity that modifies the protein, rendering the polypeptide susceptible to degradation by a protease that is present in all cells. Alternatively, the protease could be present or specifically activated only in the stalked cell. A stalked cell-specific protease is likely to be cytoplasmic or possibly associated with the inner membrane, because the COOH-terminus of the chemoreceptor is in the cytoplasm. There is evidence that the cytoplasmic Lon protease is involved in *Myxococcus xanthus* fruiting body formation (*15*). It has been shown that the C. *crescentus* homologue of the Lon protease preferentially segregates to the stalked cell upon division of the predivisional cell (*16*). The fact that Lon segregates to the stalked cell, and not to the swarmer cell, suggests that it might be involved in degradation of any McpA that ends up in the stalked cell portion of the predivisional cell. It is not yet known whether Lon recognizes McpA and whether Lon is specifically targeted to the stalk pole.

REFERENCES AND NOTES

1. H. R. Horvitz and I. Herskowitz, *Cell* **68**, 237 (1992).
2. M. R. K. Alley, S. L. Gomes, W. Alexander, L. Shapiro, *Genetics* **129**, 333 (1991).
3. M. R. K. Alley, J. R. Maddock, L. Shapiro, *Genes Dev.* **6**, 825 (1992).
4. S. L. Gomes and L. Shapiro, *J. Mol. Biol.* **178**, 551 (1984).
5. The half-life of McpA was determined in a mixed population of cells (M. R. K. Alley and L. Shapiro, unpublished data).
6. T. P. Hopp *et al.*, *BioTechnology* **6**, 1204 (1988).
7. Deletions of *mcpA* were obtained by digestion with exonuclease III and S1 nuclease (*3*) and were then ligated to the M2 epitope (*6*) and an in-frame stop codon present in the vectors pJM21, pJM22, and pJM23. These vectors have the M2 epitope in three different reading frames with respect to the polylinker. They also contain an in-frame stop codon with respect to the M2 epitope, and this codon is followed by the site for the restriction enzyme Spe I. These constructs were transferred on Eco RI–Spe I fragments from the M2 epitope vectors into a plasmid capable of replication in *C. crescentus*, pRK290KS1 (*2*). There was no effect attributable to the copy number of the plasmid, because the McpA protein derived from the plasmid-borne *mcpA* gene on pRCH9 (three to five copies per cell) showed a pattern of cell-cycle turnover similar to that of a single chromosomal copy of *mcpA* (Fig. 1).
8. A. Boyd, K. Kendall, M. I. Simon, *Nature* **301**, 623 (1983).
9. A. Krikos, N. Mutoh, A. Boyd, M. I. Simon, *Cell* **33**, 615 (1983).
10. The Tsr sequence originally submitted to GenBank (*9*) is incorrect due to a frameshift near the COOH-terminus. The corrected COOH-terminus of Tsr resembles that of Tar (J. S. Parkinson, personal communication).
11. P. Frederikse and L. Shapiro, unpublished data.
12. J. R. Maddock, M. R. K. Alley, L. Shapiro, unpublished data.
13. J. R. Maddock and L. Shapiro, *Science* **259**, 1717 (1993).
14. R. W. Hammerton *et al.*, *ibid.* **254**, 847 (1991); D. A. Wollner, K. A. Krzeminski, W. J. Nelson, *J. Cell Biol.* **116**, 889 (1992).
15. R. E. Gill, personal communication.
16. S. H. Reuter and L. Shapiro, *J. Mol. Biol.* **194**, 653 (1987).
17. R. C. Johnson and B. Ely, *Genetics* **86**, 25 (1977).
18. M. Evinger and N. Agabian, *J. Bacteriol.* **132**, 294 (1977).
19. McpA is not synthesized in swarmer cells but is synthesized later in the cell cycle (*2*). Total protein synthesis continues during transition from the swarmer to stalked cell. Therefore the ratio of McpA to total protein will decrease prior to McpA synthesis later in the cell cycle. By loading an equal volume of culture in each lane, we avoided observing a decrease in McpA because of the lack of its synthesis during the early part of the cell cycle.
20. U. K. Laemmli, *Nature* **227**, 680 (1970).
21. H. T. Towbin, T. Staehelin, J. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350 (1979).
22. The parental strain used in these experiments was SC1130N (*2*). This strain has a Tn5 insertion in *mcpA* and therefore has no McpA present, and therefore the antiserum to McpA could be used. The strain SC1130N is unable to carry out chemotaxis because the Tn5 insertion is in the first gene in the *mcpA* operon and thus is polar on the downstream genes that are required for chemotaxis (*2*). Although this strain is incapable of methylating McpA, it still degrades McpA, when it is provided in trans.
23. The *mcpA* deletion carried on plasmid pRCM223 (Fig. 3) was introduced into a synchronizable derivative of *C. crescentus* CB15, NA1000, and cell cycle immunoblots were performed on this strain with antiserum to McpA, as described in the legend of Fig. 1. Therefore, a direct comparison of the stability of the wild-type and deleted proteins could be performed in the same immunoblot.

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Microbial Competition: *Escherichia coli* Mutants That Take Over Stationary Phase Cultures

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Many microorganisms, including *Escherichia coli*, can survive extended periods of starvation. The properties of cells that survived prolonged incubation in stationary phase were studied by mixture of 10-day-old (aged) cultures with 1-day-old (young) cultures of the same strain of *Escherichia coli*. Mutants from the aged cultures that could grow eventually took over the population, which resulted in the death of the cells from the young cultures. This phenotype was conferred by mutations in *rpoS*, which encodes a putative stationary phase–specific sigma factor. These rapid population shifts have implications for the studies of microbial evolution and ecology.

Bacteria can remain viable under conditions of poor nutrient availability. Many microorganisms respond to starvation by forming dormant spores, which are generally resistant to extreme environments (*1*). Nonsporulating Gram-negative bacteria, among them *Escherichia coli*, remain metabolically active but also develop increased resistance to a variety of environmental stresses after exponential growth has stopped and cells enter stationary phase (*2*). In Gram-negative bacteria, the overall rate of protein synthesis decreases, but distinct sets of proteins are induced upon entry into stationary phase (*3*, *4*). Some of these proteins protect the cell against environmental challenges such as oxidative damage; others are necessary to maintain viability (*5*, *6*). The molecular mechanism of this response involves the induction of at least one regulon, defined by the genes whose expression depends on the putative stationary phase–specific sigma factor σ^S, the product of the *rpoS* gene also known as *katF* (*4*, *7*). In this report we show that mutations in *rpoS* can have profound effects on the ability of cells to compete and

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