Flagellar Motor-switch Binding Face of CheY and the Biochemical Basis of Suppression by CheY Mutants That Compensate for Motor-switch Defects in *Escherichia coli*

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CheY is a response regulator protein of *Escherichia coli* that interacts with the flagellar motor-switch complex to modulate flagellar rotation during chemotaxis. The switch complex is composed of three proteins, FliG, FliM, and FliN. Recent biochemical data suggest a direct interaction of CheY with FliM. In order to determine the FliM binding face of CheY, we isolated dominant suppressors of fliM mutations in cheY with limited allele specificity. The protein products of suppressor cheY alleles were purified and assayed for FliM binding. Six out of nine CheY mutants were defective in FliM binding. Suppressor amino acid substitutions were mapped on the crystal structure of CheY showing clustering of reduced binding mutations on a solvent-accessible face of CheY, thus revealing a FliM binding face of CheY. To examine the basis of genetic suppression, we cloned, purified, and tested FliM mutants for CheY binding. Like the wild-type FliM, the mutants were also defective in binding to various CheY suppressor mutants. This was not expected if CheY suppressors were compensatory conformational suppressors. Furthermore, a comparison of flagellar rotation patterns indicated that the CheY suppressors had readjusted the clockwise bias of the fliM strains. However, a chemotaxis assay revealed that the readjustment of the clockwise bias was not sufficient to make cells chemotactic. Although the suppressors did not restore chemotaxis, they did increase swarming on motility plates by a process called “pseudotaxis.” Therefore, our genetic selection scheme generated suppressors of pseudotaxis or switch bias adjustment. The binding results suggest that the mechanism for this adjustment is the reduction in binding affinity of activated CheY. Therefore, these suppressors identified the switch-binding surface of CheY by loss-of-function defects rather than gain-of-function compensatory conformational changes.

Chemotaxis in flagellated bacteria like *Escherichia coli* and *Salmonella typhimurium* occurs in response to environmental changes, when the cells swim toward chemical attractants and away from repellents (1, 2). A proper balance between tumbling and smooth swimming allows cells to move toward a more favorable and away from a less favorable environment (3, 4). Chemotaxis requires a complex signaling mechanism to regulate the frequencies of smooth swimming and tumbling. Tumbling and smooth swimming are both needed for taxis, and mutants that exclusively use one or the other mode fail to respond to environmental stimuli (5–7). Smooth swimming is caused by a counterclockwise (CCW)\(^1\) rotation of flagella, resulting in the formation of a flagellar bundle. Disruption of this bundle, caused by a clockwise (CW) rotation of flagella, results in tumbling. A motor-switch complex, composed of multiple numbers of three proteins, FliG, FliM, and FliN (5, 8–11), controls flagellar rotation. In turn, a small cytoplasmic protein, CheY (1), regulates the motor-switch complex.

CheY is a regulatory protein that couples flagellar rotation to the environment by transducing the chemotactic signals from the transmembrane chemoreceptors to the flagellar switch. Three other Che (chemotaxis) proteins, CheA, CheW, and CheZ, also contribute to this process (1, 6, 12–15). CheA, a histidine autokinase, and CheY belong to the family of two-component sensory transduction proteins (4). In this family of proteins, CheY represents 50 or more homologous proteins, called response regulators (3, 4). The three-dimensional structure of CheY is known (16–18), indicating that CheY is a single domain protein that folds into a (β/α)\(^5\) topology, with five β-sheets forming the hydrophobic core, surrounded by five α-helices. Three aspartate residues Asp-12, -13, and -57 form the active site of the molecule (3, 19). The CheA and CheZ binding faces of CheY have been identified by binding studies (28, 45, 47), and the FliG binding face has been predicted by genetic studies (27). Similar to that of other response regulators, phosphorylated CheY appears to be the active form required for CW flagellar rotation (4, 20, 21). A phosphotransfer from phospho-CheA to CheY occurs rapidly in *vitro* (22–24). CheY and CheA mutations that disrupt phosphotransfer reactions also result in a smooth swimming phenotype, suggesting that phospho-CheY is the active CW generator (25, 26). With a well defined structure and function, CheY is a model for understanding the structure-function aspects of response regulators in other bacterial sensory transduction systems.

A previous study from our laboratory employed genetic suppression as a tool for identifying a signaling face of CheY (27). It was achieved by mapping the locations of the cheY suppressor mutations of fliG mutations on the crystal structure of CheY. We had further extended this work by using an ELISA-based binding assay to identify the CheA binding face of CheY (28). In the present study, we have combined the two approaches by using genetic suppression to identify mutations in cheY that suppress fliM mutations and by biochemically analyzing the binding abilities of the CheY suppressor mutants for FliM. Our results reveal the face of CheY that is involved in FliM binding, and also the results provide biochemical evidence

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\(^1\) The abbreviations used are: CCW, counterclockwise; CW, clockwise; ELISA, enzyme-linked immunosorbent assay; AcP, acetyl phosphate.
for the mechanism by which cheY mutations act to suppress switch mutations.

**MATERIALS AND METHODS**

**Chemicals**—Bacto-tryptone, Bacto-agar, and Bacto-yeast extract were from Difco. Electrophoresis grade agarose, penicillin, kanamycin, 3-β-indoleacrylic acid, Sigma 104 phosphate substrate, and anti-rabbit IgG (Fc-specific) were obtained from Sigma. Sequenase version II was obtained from U. S. Biochemical Corp. 

**Bacterial Strains and Media**—Table I lists all bacterial strains and plasmids used in this study. Cultures were routinely grown in Luria Broth (LB), which contains 1% tryptone, 1% NaCl, and 0.5% yeast extract. Penicillin (100 μg/ml) was used if the cells contained antibiotic-resistant plasmids. Swarm assays were performed on tryptone swarm agar plates. Those transformants, recognized by rapid swarming away from the point of inoculation, were chosen for plasmid isolation and purification. The purified plasmids were analyzed by restriction enzyme digestion. These plasmids were then used to retransform the original cheY strain (to confirm that suppression was plasmid-encoded) and for complementation assays in strains RP4079 (cheY 216) and RP4139 (cheZ 280). Only those plasmids that complemented cheZ, but not cheY, were used in subsequent studies. Analysis of allele specificity was made by measuring the final swarm diameter for eight isolates of each plasmid-strain combination, grown under identical conditions, to yield mean values relative to the wild-type controls (RP437 containing pMM1).

**Binding Assays**—The previously published binding assay (29) was modified to assay CheY binding to Flg and Flim. Wells of a microtiter plate were incubated overnight at 4 °C with 100 μl of 2-fold serially diluted protein solutions (starting with 300 ng of Flg or Flim in 50 μl Tris, pH 7.9). On the 2nd day, wells were washed one time with blocking buffer (Buffy B, 1% dry skim milk and 0.1% sodium azide in 50 μl Tris, pH 7.9) and incubated again overnight at 4 °C with Buffer Br in a block.day unoccupied sites. On the 3rd day, the wells were washed two times with Buffer B and each of the following proteins was added sequentially in 100 μl of a buffer (10 mM MgCl₂, 50 mM Tris, pH 7.9): (i) purified CheY (2 μg/well), (ii) polyclonal anti-CheY antibody (50 ng/well), and (iii) IgG-specific antibody linked to alkaline phosphatase (1:1000 dilution).

**Isolation of Suppressor cheY Mutants and Analysis of Allele Specificity**—Five different flim strains were transformed with hydroxylation-mutagenized low copy number plasmids bearing putative suppressor mutations. Transformants were tested for restoration of swarming on soft agar plates. Those transformants, recognized by rapid swarming away from the point of inoculation, were chosen for plasmid isolation and purification. The purified plasmids were analyzed by restriction enzyme digestion. These plasmids were then used to retransform the original flim strain (to confirm that suppression was plasmid-encoded) and for complementation assays in strains RP4079 (cheY 216) and RP4139 (cheZ 280). Only those plasmids that complemented cheZ, but not cheY, were used in subsequent studies. Analysis of allele specificity was made by measuring the final swarm diameter for eight isolates of each plasmid-strain combination, grown under identical conditions, to yield mean values relative to the wild-type controls (RP437 containing pMM1).

**Analysis of Flagellar Rotation Patterns**—Bacterial flagellar rotation phase, and then the temperature was shifted to 42 °C for 30 min to induce the expression of phase RNA polymerase. The temperature was immediately lowered back to 30 °C, and the culture was grown for an additional 2 h allowing sufficient transcription and hence overexpression of Flim. The purification of Flim to near homogeneity was achieved by using a previously published protocol (10).
Biochemical Basis of Allele-specific Suppression

Fig. 1. Analysis of suppressor cheY allele specificity. Low copy number plasmids bearing suppressor cheY alleles were transformed into five fliM tester strains and assayed for swarming on semi-solid agar plates. The numbers along the x axis represent suppressor cheY alleles arranged from the best to worst restoration of the swarm phenotype in fliM 1002 (strain RP4493) background, in which the greatest level of suppression was observed. Bar 1, E117K; bar 2, V108M; bar 3, T112I; bar 4, A90V; bar 5, F111V; bar 6, I96S; bar 7, T115A; bar 8, A13N; bar 9, wild-type CheY. Vertical bars represent the swarm diameters of cell colonies normalized to the swarming of a wild-type strain, RP437, under identical conditions.

patterns were analyzed by cell tethering with anti-flagellin antibody, using the protocol described by Amsler et al. (31). Briefly, cells were grown in tryptone broth at 30 °C to 80 Klett units. Tethered cells, which were actively spinning, were video-taped with the aid of a light microscope. The direction of flagellar rotation was analyzed from the video images of 75 cells of each kind, and the ratio of CW to CCW rotations was determined.

Chemotaxis Assay—The chemotactic abilities of various strains were assayed by using a spectrophotometric assay as described by Zhu et al. (41). The assay is based on the principle that optical density, which is measured as the measure of cell density close to a piece of a gel containing an attractant (aspartate), increases with time if the cells perform chemotaxis (41). The assay was performed using the protocol described by Amsler et al. (31). Briefly, cells were grown in TB to early postexponential phase. Three milliliters of the cell cultures were harvested by gentle centrifugation. The cell pellet was resuspended in a buffer containing 10 mM potassium phosphate, pH 7.0, 0.1 mM EDTA, and 0.1 mM L-methionine. After incubation at 30 °C for 5 min, the cell suspension was transferred into a cuvette with a piece of agarose gel containing 5 mM serine, at the bottom of the cuvette. The cuvette was jacketed by 30 °C water to maintain a constant temperature. The cell density changes at the interface between the gel and the medium were determined over time, by using a spectrophotometer.

RESULTS

Isolation of Dominant cheY Suppressors of fliM Mutations—The fliM strain, RP4493, was transformed with randomly mutagenized tar operon plasmid, pMM1, that contains cheY under its native promoter. The transformants were screened for restoration of swarming on swarm plates. The plasmid pMM1, which is a low copy number plasmid, was used to recover the dominant cheY suppressors of the fliM mutation in the presence of the wild-type copy of cheY on the chromosome. This procedure yielded 13 suppressor isolates as follows; 7 had mutations in cheY and 6 had mutations in cheZ. This result is consistent with some previous studies (32–34) that suggested genetic interactions between cheY, cheZ, and switch genes. The dominant effects in our system are not due to the overproduction of the CheY mutant proteins since cheY expression is controlled by its native promoter on a vector that is maintained at one or two copies in the cell (35).

The suppressor cheY alleles were subcloned into a high copy number expression plasmid, pRL22ΔZ, and sequenced. All cheY suppressor alleles were found to contain single point mutations. The following mutations, reported here with residue numbers and inferred amino acid changes, were found: D13N, D57N, A90V, I96S, T112I, and T115A. Interestingly, A90V and T112I were also isolated as suppressors to fliG mutations (27). Therefore, the four other known fliG suppressor cheY mutations, E27K, V108M, F111V, and E117K, were also included for further characterization (27).

Suppressor cheY Mutations Demonstrate Limited Allele Specificity—Genetic suppressors may arise due to a variety of reasons. We aimed to isolate the intergenic conformational suppressors (36). In theory, these suppressors can occur if two proteins make stereospecific contact. The mutations in one protein that disrupt this interaction may be suppressed by compensatory mutations in the other protein that restores the interaction. True conformational suppressors should be highly allele-specific, since they reflect the precise stereospecific contact between the interacting proteins. Consequently, the locations of the allele-specific mutations should define the regions of physical contact between the proteins.

In order to assess the allele specificity of our suppressors, we tested their ability to restore swarming in different fliM strains. The suppressor cheY allele showed variable degrees of suppression in a given fliM strain, but the order of suppression
Suppressor Mutations with Defective FliM Binding Cluster on a Face of CheY—A high resolution crystal structure of CheY has been determined (16). The suppressor cheY mutations that demonstrated reduced FliM binding when phosphorylated were mapped onto this structure. Fig. 3 depicts the CheY amino acid residues we identified as being involved in FliM binding. The residues that affect the binding are located on the same face of CheY. They are solvent-accessible, and also, their side chains extend out to the surrounding solvent where they can interact with FliM (except Phe-111). Clearly, the substitutions at these positions could change the topology of the proposed FliM-binding surface and cause reduced binding. These residues are located on the bottom of α-1 (Asp-27), on the loop region between β-4 and α-4 (Ala-90), β-4 (Val-108), α-5 (Glu-117), and the loop region between β-5 and α-5 (Phe-111 and Thr-112). The observed amino acid substitutions at these locations can produce electrostatic (E117K), hydrophobic (T112I), or steric (A90V, V108M) alterations for the proposed protein-protein interactions. The E27K mutant was also assayed because it was previously identified as a suppressor of fliG (27).
Two of the suppressor cheY alleles, D13K and D57N (data not shown), are part of the phosphorylation site. These phosphorylation sites or catalytic mutants appear to act as suppressors by a mechanism that is dependent on their inability to become phosphorylated (see “Discussion”).

CheY Suppressors Tend to Reset the Clockwise Bias of fliM Strains—The mechanism by which a cheY suppresses a given fliM mutation is very much dependent on the nature of fliM defect. The fliM strains are motile but show aberrant swimming patterns that are characterized by an extremely high rate of spontaneous directional changes or tumbles (33). In order to assess the possible switch defects, patterns of flagellar rotations were examined by cell tethering by anti-flagellin antibody. Cells that do not contain cheY exclusively rotate their flagella in a counterclockwise manner (Fig. 4B). The fliM mutant strains we used were shown to have a slight clockwise bias in their flagellar rotation pattern, in the absence of cheY (33). This bias becomes highly pronounced when the wild-type copy of cheY is present in the chromosome (Fig. 4C). The presence of an extra copy of cheY from plasmid pMM1 further enhances the bias (Fig. 4D); however, when the cheY suppressor alleles were introduced into two fliM strains (with wild-type cheY in their chromosomes), the flagellar rotation patterns were found to be similar to that of the wild-type cells (Fig. 4, E and F). Since CheY is known to generate a signal for tumbling, one way CheY mutants can bring the flagellar rotation patterns back to normal is by reducing the wild-type CheY activity (arising from the chromosomal copy of cheY) at the switch. Therefore, it is very likely that the CheY suppressor mutants are loss-of-function mutants that despite being defective in FliM binding are still capable of phosphorylation by CheA (see “Discussion”). These loss-of-function mutants thus act as “phosphate sinks” that do not interact with the switch efficiently and, therefore, reduce clockwise bias of fliM strains close to the wild-type level.

Resetting of Clockwise Bias Is Not Sufficient to Restore Chemotaxis—Readjustment of the switch bias may result in swarming, which may not necessarily be due to the restoration of chemotaxis. It has been previously reported that non-chemotactic cells that retain the ability to tumble and run can produce swarms similar to that of the wild-type cells (39). In order to test the chemotactic abilities of suppressor-mutant strains, a chemotaxis assay was performed with two fliM strains transformed with a cheY allele, E117K, that showed the maximum restoration of swarm phenotype (Fig. 1). Both of these suppressor-mutant strains showed little or no chemotaxis as compared with a wild-type strain (Fig. 5). The observed restoration of swarming (suppression) was due to the resetting of the clockwise bias and not due to the restoration of chemotaxis.

DISCUSSION

Genetic Suppression Has Defined Residues of CheY Critical for FliM Binding—Our goal in this study was to identify the motor-switch binding face of CheY. We originally intended to use genetic suppression as a tool to isolate the conformational suppressors in cheY to fliM mutations. To increase the possibility of isolating conformational suppressors, we selected for dominant suppressors. We initially reasoned that by requiring dominance we would select for suppressors that function by restoring protein interactions, i.e. gain of function mutants. By using this approach, we were able to characterize a set of cheY...
alleles that suppress fliM mutations, in the presence of wild-type cheY. However, these mutations are not compensatory conformational suppressors, as we had initially predicted. When assayed for FliM binding, the products of the suppressor cheY mutations did show reduced binding to wild-type FliM, but the binding was not restored with the FliM mutants. Therefore, CheY mutants were not interacting conformational suppressors of FliM mutants as we had predicted. However, our genetic approach was successful in leading us to mutations that truly affected FliM binding (Fig. 2, A and B).

An Overlap in Docking Sites for CheA and FliM—An NMR study by Swanson et al. (45) has identified the possible residues on CheY that bind to CheA. Many of these residues lie in the region that had been identified as the CheA binding face of CheY (28). Interestingly, this region overlaps with the FliM binding face of CheY that we identified in this study. This surface is utilized as a docking surface for both CheA and FliM. Upon phosphorylation, affinity for CheA is decreased (13) and enhanced for FliM (10). The three-dimensional structure of phospho-CheY has not been determined due to its very short half-life (about 10 s). However, another NMR study by Lowry et al. (43) does indicate that a major subset of those residues that undergo large chemical shifts changes upon phosphorylation lie on our proposed CheA-FliM docking face of CheY. This is consistent with the idea that the proposed CheA-FliM docking site undergoes a conformational change upon phosphorylation, acquiring an increased affinity for FliM.

Mechanism of Suppression—The restoration of swarming observed in our suppressor-mutant combinations is due to the adjustment of the balance between CW and CCW rotation of flagella and is not due to the restoration of chemotaxis (Figs 4 and 5). However, our results are consistent with Wolfe and Berg (39) who showed that some Che mutants can swim and swarm, but chemotaxis requires a complete signaling system. In this regard, it is useful to suggest that true conformational suppression may occur in the CheY-FliM interaction, but it is difficult to distinguish against the background from the more prominent bias correction phenomenon. This is where the limitation of our genetic screen becomes apparent. However, combined with other techniques, genetics provides an explanation and understanding of this biological phenomenon.

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REFERENCES
1. Blair, D. F. (1995) Annu. Rev. Microbiol. 49, 489–522
2. Hazelbauer, G. L., Berg, H. C., and Matsumura, P. (1993) Cell 73, 15–22
3. Volz, K. (1993) Biochemistry 32, 11741–11753
4. Parkinson, J. S. (1995) Cell 73, 857–871
5. Macnab, R. (1995) Two Component Signal Transduction (Hoch, J. A., and Silhavy, T. J., eds pp. 181–199, American Society for Microbiology, Washington, D. C.
6. Amster, C. D., and Matsumura, P. (1995) Two Component Signal Transduction (Hoch, J. A., and Silhavy, T. J., eds.) pp. 89–103, American Society for Microbiology, Washington, D. C.
7. Zhu, X., Amster, C. D., Volz, K., and Matsumura, P. (1996) J. Bacteriol. 176, 4208–4215
8. Yamaguchi, S., Aizawa, S., Kihara, M., Isomura, M., and Macnab, R. M. (1986) J. Bacteriol. 168, 1172–1179
9. Yamaguchi, S., Fujita, H., Ishihara, A., Aizawa, S., and Macnab, R. M. (1986) J. Bacteriol. 166, 187–193
10. Welch, M., Oosawa, K., Aizawa, S.-I., and Eisenbach, M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6787–6791
11. Welch, M., Oosawa, K., Aizawa, S.-I., and Eisenbach, M. (1994) Biochemistry 33, 10270–10476
12. McNally, D. F., and Matsumura, P. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 6269–6273
13. Schuster, S. C., Swanson, R., Alex, L., Bowers, J. D., and Simon, M. I. (1993) Nature 365, 343–347
14. Gnegner, J. A., Graham, D., Roth, A., and Dahlquist, F. (1992) Cell 70, 975–982
15. Blat, Y., and Eisenbach, M. (1994) Biochemistry 33, 902–906
16. Volk, K., and Matsumura, P. (1991) J. Biol. Chem. 266, 15511–15519
17. Stock, A., Mottonen J., Stock, J. B., and Schult, C. E. (1989) Nature 340, 745–749
18. Kar, L., Matsumura, P., and Johnson, M. E. (1992) Biochem. J. 287, 521–531
19. Lukat, G. S., Lee, B., Mottonen, J., Stock, A., and Stock, J. (1991) J. Biol. Chem. 266, 8348–8354

Fig. 5. Chemotactic abilities of suppressor cheY mutants in fliM strains versus the wild-type cheY strain based on spectrophotometric assay. fliM strains carrying cheY suppressor mutation (E117K) are compared with that of the wild-type strain RP437 and cheY mutant strain RP4079.
20. Parkinson, J. S. (1976) J. Bacteriol. 126, 758–770
21. Parkinson, J. S. (1978) J. Bacteriol. 135, 45–53
22. Hess, J. F., Oosawa, K., Matsumura, P., and Simon, M. I. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7699–7713
23. Hess, J. F., Oosawa, K., Kaplan, N., and Simon, M. I. (1988) Cell 53, 79–87
24. Hess, J. F., Bourret, R., and Simon, M. I. (1988) Nature 336, 139–14225
25. Bourret, R. B., Hess, J., and Simon, M. I. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 41–45
26. Oosawa, K., Hess, J. F., and Simon, M. I. (1988) Cell 53, 89–96
27. Roman, S. J., Meyers, M., Volz, K., and Matsumura, P. (1992) J. Bacteriol. 176, 6247–6255
28. Shukla, D., and Matsumura, P. (1995) J. Biol. Chem. 270, 24414–24419
29. Matsumura, P., Rydel, J. J., Linzmeier, R., and Vacante, D. (1994) J. Bacteriol. 169, 46–46
30. Tabor, S., and Richardson, C. C. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 1074–1078
31. Amsler, C. D., Cho, M., and Matsumura, P. (1993) J. Bacteriol. 175, 6238–6244
32. Parkinson, J. S., and Parker, S. R. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 2390–2394
33. Parkinson, J. S., Parker, S. R., Talbot, P. B., and Houts, S. E. (1983) J. Bacteriol. 155, 265–274
34. Sockett, H., Yamaguchi, S., Kihara, M., Irikura, V. M., and Macnab, R. M. (1992) J. Bacteriol. 174, 793–806
35. Koop, A. H., Hartley, M. E., and Bourgeois, M. E. (1987) Gene (Amst.) 52, 245–256
36. Treptow, N. A., and Shuman, H. A. (1988) J. Mol. Biol. 202, 809–822
37. Ravid, S., Matsumura, P., and Eisenbach, M. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 7357–7361
38. Ames, P., Yu, Y. A., and Parkinson, J. S. (1996) Mol. Microbiol. 19, 737–746
39. Wolfe, A. J., and Berg, H. C. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 6973–6977
40. Bourret, R. B., Hess, J. F., and Simon, M. I. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 41–45
41. Zhulin, I. B., Gibel, I. B., and Ignatiev, V. V. (1995) Curr. Microbiol. 22, 307–309
42. Drake, S. K., Bourret, R. B., Luck, L. A., Simon, M. I., and Falke, J. J. (1993) J. Biol. Chem. 268, 13081–13088
43. Lowry, D. F., Roth, A. F., Rupert, P. B., Dahlquist, P. W., and Falke, J. J. (1994) J. Biol. Chem. 269, 26358–26362
44. Magariyama, Y., Yamaguchi, S., and Aizawa, S. (1990) J. Bacteriol. 172, 4359–4368
45. Swanon, R. V., Lowry, D. F., Matsumura, P., McEvoy, M. M., Simon, M. I., and Dahlquist, P. W. (1995) Nat. Struct. Biol. 2, 986–991
46. Belloso, P., Cronet, P., Majolero, M., Serrano, L., and Coll, M. (1996) J. Mol. Biol. 257, 116–128
47. Parkinson, J. S., and Houts, S. E. (1982) J. Bacteriol. 151, 106–113
48. Nichols, B. P., and Yanofsky, C. (1983) Methods Enzymol. 101, 155–164
49. Parkinson, J. S. (1993) Cell 337, 745–749