Escherichia coli dnaJ Deletion Mutation Results in Loss of Stability of a Positive Regulator, CRP*

(Received for publication, April 16, 1991)

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The dnaJ deletion mutant K7052(λdnaK) has a temperature-sensitive defect in the synthesis of β-galactosidase. We confirmed this operon-specific and temperature-sensitive defect in cell-free extracts prepared from the mutant cells and found that the missing factor was CRP. In the mutant, the cellular concentration of CRP was too low to allow the expression of lac operon at a nonpermissive temperature. Introduction of a CRP over-producing plasmid into the dnaJ deletion mutant suppressed the defect of β-galactosidase synthesis. The lower content of CRP in the mutant was found to result from extreme instability of the protein. These results strongly suggested that the heat shock protein dnaJ is involved in the stabilization (or degradation) of CRP.

The dnaJ gene of Escherichia coli was originally found as a cellular gene essential for the replication of bacteriophage λ (1, 2). Genetic experiments provided evidence for the involvement of the dnaJ protein in the replication of both mini-P and mini-F plasmids (3, 4). The dnaJ gene product has also been shown to be essential for cell growth, at least at 43 °C (2, 5).

Recently we have reported novel aspects of the phenotype that results from the dnaJ mutation (6). The mutant K7052 in which the whole dnaK-dnaJ operon is deleted (7) and its lysogenic derivative K7052(λdnak) show a temperature-sensitive defect in the synthesis of β-galactosidase. The synthesis of lac mRNA is reduced at the restrictive temperature (6). The mutants are also conditionally defective in the synthesis of a group of proteins whose synthesis occurred at a specific stage of a cell cycle (8), whereas the synthesis of anthranilate synthetase, encoded by trpED, and other cellular proteins is normal. Lysogenization of the transducing phage containing the wild type dnaJ gene restored the defects (6). From these findings, we proposed that the dnaJ gene product was involved in regulation of the synthesis of these proteins.

In order to elucidate the precise molecular mechanism of the regulation, we attempted to confirm the temperature-sensitive defect of β-galactosidase synthesis in a cell-free protein synthesizing system from the dnaJ deletion mutant and to identify the factor that is missing from an extract of the mutant cells grown at 43 °C. In this study, we found that the stability of CRP was decreased in the dnaJ deletion mutant and that the cellular concentration of CRP was lower than the threshold concentration necessary for the expression of the lac operon at a nonpermissive temperature.

These findings suggested that the heat shock protein, dnaJ, is involved in stabilization or degradation of the positive regulatory protein CRP.

MATERIALS AND METHODS

Bacterial Strains, Bacteriophages, and Plasmids—Bacterial strain K7052 and the transducing phages λdnaK and λdnaKdnaV have been described (5, 9). K7052(λdnak) was used as a dnaJ deletion mutant (7). K7052(λdnaKdnaV), which is lysogenized with λdnaKdnaV instead of with λdnaK, was used as an isogenic strain carrying the wild type dnaJ gene. lacZ derivatives of these strains were obtained by nitosoguanidine mutagenesis and used to prepare cell-free extracts for in vitro β-galactosidase synthesis. The parent lacZ strain was used in all other experiments throughout this work. CSH73 cells (HfrH, Δlac Δ ara-leu thi) (10) were used as wild type cells for purification of a complementing factor. Strain CSH44 (F- tonA Δ(lac λh80cl857868 thi (λh80cl857868Δlac)) (10) was used for λh80-clacDNA preparation. ME5466(Δlcrp Δlac thi StR+) (11) was used for cloning the crp gene. Plasmids pHA7 and pHA5, and a mini-F plasmid, pKPI013, were kindly donated by Alba (12) and Miki (13), respectively.

Gene fusion plasmid pTR10 was constructed as follows. A 220-base pair Alal-HinfI fragment (nucleotides -40 to +183), which contains the whole control region of the tryptophan operon and the first seven codons of trpE, was isolated from plasmid pMM778 constructed by Hopkins et al. (14). The fragment was inserted at the EcoRI site of plasmid pMCI403 reported by Casadaban et al. (15). In transformed cells, the fusion protein was expressed under the control of the tryptophan promoter; its synthesis was repressed by the presence of tryptophan and induced by the addition of indole acrylate.

Preparation of Cell-free Extracts—Cell-free extracts, S-30s, were prepared as described by Zubay (16). Wild type CSH73 cells were grown in nutrient broth (8 g/liter) at 30 °C, while cells of the lacZ derivative of the dnaJ deletion mutant, K7052(ΔdnaK)lacZ were grown in Tris-AY medium (0.1 M Tris-HCl, pH 7.5, 1% tryptone, 0.1% yeast extract, 0.25% NaCl, 20 μg/ml of thymine, and 2 mM CaCl2). S-30 extracts prepared from cells grown at 30 °C were named 30'-S-30, and S-30 extracts prepared from cells grown at 30 °C and then further grown at 43 °C for 30 min were named 43'-S-30.

In Vitro Protein Synthesis—Synthesis of β-galactosidase in vitro using an S-30 extract and a template DNA was carried out as described by Zubay (16). Where indicated, in vitro β-galactosidase synthesis was separated into two stages, transcription and translation (17).

DNA Preparation—λh80-clacDNA was prepared from strain CSH44 as described by Zubay (16). Plasmid DNA was prepared described by Maniatis et al. (18).

Enzyme Assay—β-galactosidase was assayed as described previously (19). One unit of enzyme was defined as the amount hydrolyzing 1 μmol of 2-nitrophenyl-β-d-galactopyranoside/h. Binding of [1H]cAMP was measured by the ammonium sulfate precipitation method (20). One unit of CRP was defined as that binding 1 pmol of cAMP.

Electrophoresis and Western Blotting Analysis—SDS-polyacrylamide gel electrophoresis was carried out as described by Ames (21). Following SDS-polyacrylamide gel electrophoresis, proteins were electrophoretically transferred to nitrocellulose membrane, and the CRP band was detected with polyclonal anti-CRP serum, biotinylated rabbit anti-

1 The abbreviation used is: SDS, sodium dodecyl sulfate.
body, streptavidine alkaline phosphatase conjugate, and 5-bromo-4-chloro-3-indolyl phosphate with nitro-blue tetrazolium according to the manufacturer’s instruction (Amersham).

**Measurement of the Rate of Synthesis and Stability of CRP**—Strains K7052(ΔdnaK) and K7052(ΔdnaKΔdnaJ) were grown to 2 x 10^9 cells/ml at 30°C in Tris-glucose medium containing necessary supplements (6). For measurement of the rate of synthesis of CRP, cultures were divided into two parts. Incubation of one part was continued at 30°C, while the other part was shifted to 43°C. After 15 min, the cultures were pulse-labeled with [35S]methionine (436 μCi/ml, 50 μCi/ml, Amersham) for 5 min. Samples were then promptly transferred to tubes containing sodium azide (20 mM, final concentration). For measurement of the stability of CRP, cells were labeled with [3H]leucine for 3 min and then chased with excess unlabeled leucine. The cultures were then divided into two parts, which were incubated at 30°C and 43°C, respectively. After various times of chase, 10-ml aliquots were transferred to tubes containing sodium azide. The cells were washed twice with 50 mM Tris-HCl, pH 7.4, containing 0.5 M NaCl and lysed with 1 ml of RIPA buffer (1% deoxycholate, 1% Triton X-100, 0.3 M NaCl, 0.1% SDS, 0.1 M Tris-HCl, pH 7.5, 1 mM phenylmethylsulfonyl fluoride) for 2 h on ice. The supernatants obtained by centrifugation (20,000 × g, 60 min) were supplemented with 20 μl of anti-CRP serum and 5 μg of purified CRP and incubated overnight at 4°C. Then 20 μl of protein A (400 μg/ml) was added, and incubation was continued for an additional 2 h. The immunoprecipitates were washed four times with RIPA buffer and boiled in SDS loading buffer for 30 min, and samples were subjected to electrophoresis in 14% SDS-polyacrylamide gel. The amount of CRP remaining after different chase times were measured with a bioimager analyzer (Fujix BAS 2000).

**Cloning of the crp Gene of K7052(ΔdnaK)—**For cloning the crp gene of K7052(ΔdnaK), genomic libraries were constructed with λD69 as a cloning vector (22). Chromosomal DNA was digested with BamHI and ligated with λD69 phage DNA digested with BamHI. The ligated DNA sample was packaged into phage particles by use of Gigapack gold-10 (Stratagene). The phages were screened by plaque hybridization with a 3.6-kilobase BamHI fragment containing the wild type crp gene isolated from plasmid pHA5 as a probe (12). Recombinant phage DNA containing the crp gene of the mutant was cloned into the unique BamHI site of a single copy plasmid pKPi013 (13), and the recombinant plasmid was named pKPi014. As a control, a 3.6-kilobase BamHI fragment containing the wild type crp gene (obtained from pHA5) was cloned into pKPi013 and the resulting plasmid was named pKPi015.

**RESULTS**

**In Vitro Synthesis of β-Galactosidase with an Extract of the dnaJ Deletion Mutant—**As shown in Table I, the S-30 fraction prepared from the lacZ derivative of the mutant cells, grown at the permissive temperature (30°C-30) showed normal activity for in vitro β-galactosidase synthesis with XgalDNA as a template. But after shift of the dnaJ mutant to 43°C for 30 min, the activity for β-galactosidase synthesis of the resulting cell-free extract (43°C-S-30) was 8% of that of 30°C-S-30. The addition of a small amount of S-30 from the wild type CSH73 cells to 43°C-S-30 restored the activity to almost the same level as that of 50°C-S-30.

The plasmid pTR10 contains a trp-lacZ fusion gene in which the promoter region of the lac operon is replaced by that of the tryptophan operon, and a chimeric β-galactosidase can be synthesized under the control of the tryptophan promoter. As shown in Table I, when this plasmid DNA was used as a template, the activity of the 43°C-S-30 was 36% of that of 30°C-S-30, and addition of a small amount of wild type S-30 did not increase the synthetic activity of 43°C-S-30 or 30°C-S-30. The reduced synthetic activity of the 43°C-S-30 was due to the effect of temperature shift from 30 to 43°C, not to a defect caused by the dnaJ deletion mutation, because a similar decrease was observed with S-30 prepared from wild type cells subjected to the same temperature shift (data not shown). These results demonstrate that the cell-free extract from the mutant showed a promoter-specific defect in β-galactosidase biosynthesis. An S-30 extract prepared from lacZ’ parent strain showed a basal β-galactosidase activity without the addition of template DNA which is due to the β-galactosidase present in the parent lacZ strain as those shown in Table I.

To determine whether the defect of 43°C-S-30 prepared from the mutant is in the transcription or translation step, we separated the overall reaction into transcription and translation steps by synthesizing lac mRNA in the absence of added amino acids and then adding rifampicin and 20 amino acids simultaneously to achieve translation without further initiation of mRNA synthesis. As shown in Table II, a stimulatory effect was observed only when a small amount of the wild type S-30 was added at the step of transcription. These in vitro results are entirely consistent with in vivo results and indicate that transcription of the lacZ gene is blocked at the restrictive temperature.

**Identification of the Complementing Factor in the Wild Type S-30 Extract as CRP—**During the experiments to purify the complementing factor from S-30 of the wild type cells, we found that the chromatographic behavior of the factor resembled that of CRP, a well-known positive regulator of the lac operon. This finding, together with the observations that both factors function in the transcription step, led us to test the possibility that the two factors might be identical. We meas-

**TABLE I**

| Gene | Template DNA | Wild type S-30 | β-Galactosidase activity %(units x 10^9) |
|------|--------------|----------------|------------------------------------------|
| 30°C-S-30 | Xgal DNA | 475 | 100 |
| 43°C-S-30 | Xgal DNA | 778 | 164 |
| 30°C-S-30 | pTR10 | 39 | 8 |
| 43°C-S-30 | pTR10 | 589 | 124 |

**TABLE II**

| Gene | Template DNA | Wild type S-30 added |
|------|--------------|---------------------|
| 30°C-S-30 | Xgal DNA | 6309 | 100 |
| 43°C-S-30 | Xgal DNA | 6687 | 106 |
| 43°C-S-30 | pTR10 | 2267 | 36 |
| 43°C-S-30 | pTR10 | 2153 | 34 |

**Differential effects of a complementing factor in the process of DNA-directed in vitro β-galactosidase synthesis**

In vitro β-galactosidase synthesis was separated into transcription and translation steps as described under "Materials and Methods." Transcription was carried out at 30°C for 7 min, and translation at 30°C for 60 min after addition of 2 μg of rifampicin and 20 amino acids. Protein concentrations (μg/0.1 ml); K7052(ΔdnaK) 43°C-S-30, 990; wild type S-30, 130. XgalDNA, 8 μg.
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ured the cAMP binding activity at each step of purification and found that cAMP binding activity was copurified with the complementing activity monitored by the ability to restore in vitro β-galactosidase synthesis by 43°-S-30 prepared from the mutant. Moreover, the electrophoretic mobility of the factor on SDS-polyacrylamide gel coincided with that of CRP purified by the method described by Ellen et al. (24), indicating that the complementing factor was CRP.

The CAMP binding activity of 43°-S-30 of the dnaJ deletion mutant was negligible. Analysis of the Bio-Rex 70 column eluate by SDS-polyacrylamide gel electrophoresis also revealed no detectable 22.5-kDa protein band in the fractions in which it was detected in the preparation of wild type cells. Thus, the absence of CRP was responsible for the decrease in the synthesis of β-galactosidase with 43°-S-30 of the dnaJ deletion mutant.

Suppression of the Defect in β-Galactosidase Synthesis by Introduction of a CRP-overproducing Plasmid into the dnaJ Deletion Mutant—The plasmid pH7A contains a 910-base pair fragment which includes the entire structural gene for CRP (12). Strains containing pH7A overproduce CRP because it is under the control of the bla promoter originally present in pBR322. If the defect of β-galactosidase synthesis in the mutant at the restrictive temperature is due to the absence of CRP, introduction of this plasmid into the mutant should restore the synthesis of β-galactosidase at the restrictive temperature. Fig. 1 shows the time courses of synthesis of β-galactosidase in the mutant and the mutant harboring pH7A at 30 and 43°C. The rate of synthesis in K7052(ΔdnaK) was much lower at 43 than at 30°C (Fig. 1A) as already reported, but similar in the transformant at the two temperatures (Fig. 1B). These findings confirm the conclusion of the in vitro experiments that the defect in the dnaJ deletion mutant in the synthesis of β-galactosidase at 43°C was due to marked reduction in the cellular concentration of CRP.

Transformation of K7052(ΔdnaK) with pH7A did not complement the defect of λ phage multiplicity at either temperature or cellular growth at the nonpermissive temperature.

Instability of CRP in the dnaJ Deletion Mutant—We have shown that the synthesis of CRP-mRNA in K7052(ΔdnaK) is almost normal even in cultures at 43°C for 40 min (6). This finding suggests that the cellular concentration of CRP is controlled at a post-transcriptional level. Therefore, we compared the rates of synthesis of CRP at 30 and 43°C in the dnaJ mutant. For this, the mutant cells were pulse-labeled with [3H]leucine for 3 min at 30 or 43°C, and the labeled proteins were immunoprecipitated with anti-CRP serum. The precipitated proteins were then separated by SDS-polyacrylamide gel electrophoresis. Autoradiograms of the gels showed significant synthesis of CRP at 43°C, although it was slightly less than at 30°C (Fig. 2). However, we found that the amount of CRP labeled at 43°C relative to that at 30°C decreased markedly with an increase in the pulse time (data not shown). This finding suggested that the stability of CRP may be lower at the restrictive temperature than at the permissive temperature. The mutant cells were pulse labeled at 30°C for 3 min with [3H]leucine and chased at 30 or 43°C for various times with an excess of unlabeled leucine. The decay rates quantified by autoradiographic analysis of the gel showed that the half-life of CRP was 40 s at 43°C and 180 s at 30°C (Fig. 3A). Introduction of a transducing phage dnaKΔdnaJ into K7052 partially restored the stability of CRP at both temperatures (Fig. 3B), increasing the half-life 2.5-fold (from 40 to 100 s) at the restrictive temperature and 27-fold (from 3 to 80 min) at the permissive temperature. Thus, the lower content of CRP in the dnaJ deletion mutant can mainly be explained by instability of the protein. In the wild type genetic background, no detectable decrease in the amount of CRP was seen during the 60 min chase at either 30 or 43°C.

Fig. 1. Syntheses of β-galactosidase in K7052(ΔdnaK) and a transformant harboring CRP-overproducing plasmid pH7A. K7052(ΔdnaK) and the transformant, K7052(ΔdnaK)/pH7A were grown in Tris-XY medium to a density of A600 = 0.15–0.2. Ampicillin (30 μg/ml) was added to cultures of the pH7A transformant. Immediately after addition of 4 mM isopropyl-1-thio-β-D-galactopyranoside, cultures were separated into two parts, which were incubated at 30°C (C) and 43°C (●). Aliquots were taken during induction and their β-galactosidase activity was measured as described under “Materials and Methods.” Growth was monitored as increase in absorbance at 530 nm. Panel A, K7052(ΔdnaK); panel B, K7052(ΔdnaK)/pH7A.

Fig. 2. Synthesis of CRP in K7052(ΔdnaK) at 30 and 43°C. Cells were pulse-labeled for 3 min with [3H]leucine at 30 and 43°C. The labeled proteins were immunoprecipitated with anti-CRP serum as described under “Materials and Methods,” and precipitates were analyzed by autoradiography after polyacrylamide gel electrophoresis.

Fig. 3. Stability of CRP in the dnaJ deletion mutant K7052(ΔdnaK) and its dnaJ+ lysogenic derivative. Samples obtained at various chasing times were subjected to SDS-polyacrylamide gel electrophoresis. The amounts of radioactivity remaining in the CRP band are measured by autoradiography and presented relative to that at 0 min of chase. Panel A, K7052(ΔdnaK); panel B, K7052(ΔdnaKΔdnaJ). Open circles, at 30°C; closed circles, at 43°C.
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The present study demonstrated that CRP, a well-known positive regulator of catabolite-sensitive operons, was very unstable in the dnaJ deletion mutant K7052ΔdnaK: its half-life in the mutant was 40 s at 43°C and 180 s at 30°C (Fig. 3). The temperature sensitivity of the synthesis of β-galactosidase in the mutant can be explained as follows. The cellular concentration of CRP in the cells grown at 30°C is lower than that in the wild type cells, but sufficient to support transcription of the lac operon, whereas in cells incubated at 43°C, its concentration becomes lower than the threshold for supporting expression of the lac operon. The temperature-sensitive defects in the synthesis of other cell cycle-dependent proteins in the mutant (6) can also be explained by CRP instability because almost all the genes for these proteins belong to the catabolite-sensitive operons.

Lysogenization of a transducing phage containing the dnaJ gene derived from wild type cells into the dnaJ mutant increased the stability of CRP 27-fold (from 3 to 80 min) at 30°C and 2.5-fold (from 40 to 100 s) at 43°C (Fig. 3). On the other hand, no detectable degradation of CRP was observed during incubation of wild type cells at 43°C for 60 min. To exclude the possibility that the amount of dnaJ protein synthesized from ΔdnaKdnaJ was not sufficient to fully complement the deletion, we compared the cellular levels of dnaJ protein in K7052ΔdnaJ, Western blotting analysis shows that the cellular concentration of dnaJ protein in the wild type parent strain HR9 and K7052ΔdnaJ were almost the same as that in HR9 both at 30°C and 43°C (data not shown).

The reason that wild type level of stability was not restored in dnaJ mutant genetic background is not clear. Mutant K7052 was obtained from nitrosoguanidine treated cells by a method designed to select E. coli mutants showing temperature-sensitive growth and a defect in supporting replication of phage λ (5). The mutant was considered to have temperature-sensitive defects of both the dnaK and dnaJ genes but recently has been shown to be a deletion mutant lacking the whole dnaK-dnaJ operon (7). Bukau and Walker (4) reported that dnaJ deletion mutant was genetically unstable at 30°C and frequently acquired secondary mutations to grow normally at 30°C. They showed that the dnaK gene is essential for growth at 30°C as well as at 43°C. It is therefore likely that K7052 also has such a suppressor mutation which circumvents the normal requirement of the dnaK gene at 30°C and significantly contributes to instability of CRP especially at 43°C.

Although the lysogenization of ΔdnaKdnaJ results in the almost complete restoration of β-galactosidase synthesis at 43°C in vivo, the in vitro synthetic activity of 43°C-S-30 prepared from K7052ΔdnaKdnaJ is dependent on the addition of CRP. This can be explained by the fact that CRP is less stable in K7052ΔdnaKdnaJ than in wild type cells, and the concentration of CRP in 43°C-S-30 was not sufficient for transcription from the lac promoter.

How do the dnaJ protein and a putative suppressor gene product participate in stabilization or degradation of CRP? A protease responsible for proteolytic degradation of CRP might be induced or activated in the mutant K7052ΔdnaK, and this protease might be a heat shock protein since CRP was less stable at 43°C than at 30°C. In this case, the putative degradation system should be specific for CRP or a certain class of proteins since the stabilities of the proteins studied other than CRP were unaffected in the mutant. Another interesting possibility is that the dnaJ protein might interact with CRP and maintain a suitable configuration that protects CRP from the attack by a responsive protease. This possibility is consistent with the idea that heat shock proteins in general act as molecular chaperons to control the higher order conformations of various proteins (25-29).

There are several reports that heat shock proteins are involved in protein degradation in E. coli. Straus et al. (30) reported that mutations in the heat shock genes, dnaK, dnaJ, grpE, and groEL result in defective proteolysis of abnormal proteins such as puromycin peptide or a nonsense fragment of β-galactosidase and that overproduction of heat shock proteins increases the rate of decay of the puromycin fragment. Keller and Simons (23) have also found that a dnaK mutant is defective in the activity to degrade canavanyl proteins and puromycin peptides but that a temperature-sensitive lacI gene product is degraded more rapidly in the mutant. Studies on the mechanism of the CRP instability in the dnaJ deletion mutant K7052ΔdnaK should provide new information on heat shock proteins.

REFERENCES

1. Saito, H., and Uchida, H. (1977) J. Mol. Biol. 113, 1-25
2. Sunshine, M., Feiss, M., Stuart, J., and Yochem, J. (1977) Mol. Gen. Genet. 151, 27-34
3. Tilly, K., and Varmolinsky, M. (1989) J. Bacteriol. 171, 6025-6029
4. Bukau, B., and Walker, G. (1989) J. Bacteriol. 171, 6030-6038
5. Saito, H., and Uchida, H. (1978) Mol. Gen. Genet. 164, 1-8
6. Ohki, M., Uchida, H., Tamura, F., Ohki, R., and Nishimura, S. (1987) J. Bacteriol. 169, 1917-1922
7. Ohki, R., Morita, R., Kawamura, T., Uchida, H., and Ohki, M. (1988) Biochim. Biophys. Acta 1009, 94-98
8. Ohki, M. (1979) in Bacterial Outer Membrane (Inouye, M., ed) pp. 289-315, John Wiley & Sons, Inc., New York
9. Yochem, J., Uchida, H., Sunshine, M., Saito, H., Georgopoulos, C. P., and Feiss, M. (1978) Mol. Gen. Genet. 164, 9-14
10. Miller, J. H. (1972) Experiments in Molecular Genetics, pp. 419-424, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
11. Sabourin, D., and Beckwith, J. (1975) J. Bacteriol. 122, 338-340
12. Aiba, H., Fujimoto, S., and Ozaki, N. (1982) Nucleic Acids Res. 10, 1345-1361
13. Maki, S., Kuribayashi, M., Miki, T., and Horiiuchi, T. (1983) Mol. Gen. Genet. 189, 211-237
14. Hopkins, A. S., Murray, N. E., and Brammer, W. J. (1976) J. Biol. Chem. 250, 589-589
15. Casadaban, M. J., Chou, J., and Cohen, S. N. (1980) J. Bacteriol. 143, 971-980
16. Zubay, G. (1973) Annu. Rev. Gen. 7, 267-287
17. Kung, H-FU, Brot, N., Spears, C., Chen, B., and Weissbach, H. (1974) Arch. Biochem. Biophys. 160, 168-174
18. Maniatis, T., Fritsch, E. E., and Sambrook, J. S. (1982) Molecular
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Cloning: A Laboratory Manual, pp. 76–85, 86–96, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

19. Ohki, R. (1975) J. Bacteriol. 123, 815–823
20. Krakow, J. S., and Pastan, I. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 2529–2533
21. Ames, G. F. L. (1974) J. Biol. Chem. 249, 634–644
22. Mizusawa, S., and Ward, D. F. (1982) Gene (Amst.) 20, 317–322
23. Keller, J. A., and Simons, L. D. (1988) Mol. Microbiol. 2, 31–41
24. Eilen, E., Pampeno, C., and Krakow, S. (1978) Biochemistry 17, 2469–2473
25. Pelham, H. R. B. (1986) Cell 46, 959–961
26. Ellis, R. J., and Hemmingsen, S. M. (1989) Trends. Biochem. Sci. 14, 339–342
27. Dyk, T. K. V., Gatenby, A. A., and LaRossa, R. A. (1989) Nature 342, 451–453
28. Phillips, G. J., and Silhavy, T. J. (1990) Nature 344, 882–884
29. Lecker, S., Lill, R., Ziegelhoffer, T., Georgopoulos, C., Bassford, P. J., Jr., Kumamoto, C. A., and Wickner, W. (1989) EMBO J. 8, 2703–2709
30. Straus, D. B., Walter, W. A., and Gross, C. A. (1988) Genes & Dev. 2, 1851–1858