Efficient modification of *E. coli* RNA polymerase *in vitro* by the *N* gene transcription antitermination protein of bacteriophage lambda

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ABSTRACT

The N gene protein of bacteriophage lambda prevents termination of transcription by *E. coli* RNA polymerase. We describe here the conditions of a cell-free reaction system in which pure N stimulates net transcription up to tenfold and therefore nearly stoichiometrically modifies transcribing RNA polymerase molecules. The reaction contains micrococcal nuclease-treated S100 extract derived from *E. coli* and a plasmid template DNA containing the lambda early promoter P_L , the N utilization site nut, and the Rho-dependent terminator t_{L1} . Stimulation by N in this system is specific and biologically relevant since it is absent with vector pBR322 DNA and with extracts derived from *E. coli* strains bearing the nusA1 and nusE71 mutations known to block N function in vivo. We use the system to provide further evidence that ribosomes are not necessary for N function and to demonstrate the direct involvement in N function of the NusA protein of *E. coli*.

INTRODUCTION

The N gene protein of bacteriophage lambda prevents termination of transcription by molecules of *E. coli* RNA polymerase that have initiated transcription at the lambda early promoters p_L and p_R (1-3). The ability of N to antiterminate transcription at the terminators of an operon requires the presence of an N utilization site (nut site) within the operon upstream of the first terminator (4,5). A nut site can be regarded as a locus at which N modifies RNA polymerase in such a way that it becomes termination resistant (6-8). There is evidence that at least part of the functional form of the nut site is RNA rather than DNA (9).

Several host proteins other than RNA polymerase are involved in antitermination by N. Each has been identified by a mutation ("nus") which abolishes the ability of N to function: the nusA gene (10) codes for a transcription termination factor (11) which binds directly to N and to the core component of RNA polymerase (12,13) and may serve as an adaptor to couple N to RNA polymerase; the nusB gene (14,15) codes for a 15 kD protein (16,17) whose involvement in N function is as yet unexplained; and the nusE71

mutation is an allele of rpsJ, the gene for ribosomal protein S10 (18). In this latter case, it has not been established whether S10 functions directly or indirectly in transcriptional antitermination, or even whether it does so as part of the ribosome.

Several types of cell-free reaction have been described in which the lambda N protein stimulates gene expression in vitro (19-22). The reactions were sufficiently specific to enable active N to be purified to homogeneity (23,24). Since N is thought to function by coordinating the formation of a multiprotein complex on RNA polymerase (13,25), we thought it wise to develop a cell-free system in which modification of RNA polymerase by N was essentially stoichiometric. Such a system should then enable us ultimately to identify the components of the N-modified transcriptional complex. We describe here the development of such a system, and use the system to demonstrate the direct involvement of the NusA protein in N function.

MATERIALS AND METHODS

Bacterial Strains

JG148 is lac^Δ74 ara Δ 498 leu Δ 498 (26). JG466 is a nusE71 zhb511::Tn10 derivative of JG148 constructed by D.I. Friedman. JG358 is strain K37: str^R galK2 obtained from M. Pearson. JG359 is a nusA1 derivative of JG358 constructed by D.I. Friedman and obtained from M. Pearson.

Materials

Active NusA was purified by a method and activity assay already described (11) using as source a NusA overproducer strain P047 constructed by P. Olins (27). Active N was purified by a slight modification (25) of our original purification scheme (23) and was assayed as previously described (19). Active NusB was purified by a modification of a method developed by J. Swindle and C. Georgopoulos (personal communication) using as source a NusB overproducer strain supplied by C. Georgopoulos (28). Our activity assay for NusB involved its ability to restore lambda N-dependent endolysin synthesis in a reaction programmed with λ N⁻ DNA (29) and containing S30 extract derived from the nusB5 strain JG449 (J. Greenblatt and J. Li, unpublished data). NusA, NusB, and N were all greater than 99% pure as judged by polyacrylamide gel electrophoresis in the presence of SDS and by HPLC on a TSK-2000 SW column.

pp_L λ (PL Biochemicals) and pBR322 (30) DNAs were prepared by the alkaline lysis method (31) followed by centrifugation to equilibrium on CsCl gradients containing ethidium bromide.

Transcription Reactions

Our standard reaction contained, in 20 μ l, 40 mM Tris acetate, pH 7.3, 20 mM magnesium acetate, 20 mM ammonium acetate, 20 mM potassium acetate, 1 mM EGTA, 1 mM dithiothreitol, 21 mM phosphoenol pyruvate, 2 mM ATP, 500 μ M CTP, 500 μ M GTP, 500 μ M [3 H]UTP at 5 mCi/mmol, 50 μ g/ml supercoiled pp $_{\text{L}}$ DNA, 6 μ l of S100 extract, and, where indicated, 200 ng of pure N protein (10,000 U/mg). Reaction mixtures were assembled on ice, incubated for 15 min at 37 $^\circ$, chilled and diluted with 100 μ l of cold water. 100 μ l of this was spotted onto a 25 mm DE81 filter (Whatman) and the filters were washed with 5% NaH $_2$ PO $_4$, 10 mM sodium pyrophosphate, followed by water and ethanol, then dried and counted in toluene-omnifluor (New England Nuclear).

Sucrose Gradient Centrifugation

RNA synthesized in various reactions was extracted with phenol/chloroform, precipitated with ethanol, and analyzed on 5-20% linear sucrose gradients containing 40mM Tris acetate, pH8.2, 20mM potassium acetate, 20mM ammonium acetate, 20mM magnesium acetate, 1mM EGTA, 1mM dithiothreitol. Centrifugation was carried out for 1 hr at 4 $^\circ$ in a Beckman SW50.1 rotor at 45,000 rpm. Fractions were collected from the bottom, and the RNA present in each fraction was determined as described above.

Preparation of S100 Extracts

Cells were grown, harvested, and washed as previously described for the preparation of S30 extracts (23). The cell pellet was ground with 1.5 gm alumina/gm cells and then resuspended in 1.5 ml buffer B (10 mM Tris-acetate, pH 7.8, 60 mM KCl, 14 mM Mg acetate, 1 mM dithiothreitol) per gm cells. This was centrifuged for 30 min at 30,000 g and the supernatant was brought to 300 mM in KCl and centrifuged for 2 hr at 40,000 rpm in a Beckman type 65 rotor with the tube one-third full. The final supernatant was dialyzed for 5 hr against buffer C (10 mM Tris acetate pH 7.8, 60 mM K acetate, 14 mM Mg acetate, 1 mM dithiothreitol) and stored frozen in aliquots at -70 $^\circ$.

Before its use in an in vitro transcription reaction, each aliquot of S100 was brought to 1 mM CaCl $_2$ and treated for 30 min at 23 $^\circ$ with 500 units/ml micrococcal nuclease (Boehringer). The micrococcal nuclease reaction was terminated by bringing the reaction to 2 mM with EGTA.

RESULTS

A Reaction in which N Functions Efficiently

Our previous N assay reaction (19) contained an E. coli S30 extract as a source of RNA polymerase, Nus proteins, and other factors for transcription

and translation. However, a high speed supernatant S100 extract allows much more efficient N function in vitro than a lower speed S30 extract (data not shown). Because of the known ionic interactions involved in N function (12,13), this extract was prepared and centrifuged at moderately high ionic strength, and its salt concentration was lowered subsequently by dialysis.

The nucleic acids left in such an S100 caused a significant and variable background rate of transcription in the absence of added template DNA. Pre-incubation of the S100 at 37° to destroy the endogenous nucleic acids was not practical because one of the host factors required for N function is quite unstable at 37° (R. Horwitz, unpublished data) and because N-independent transcription, presumably terminator read-through, greatly increased after such a pre-incubation step (data not shown). We have therefore treated the extract with micrococcal nuclease at 23° to destroy the endogenous nucleic acids and have included EGTA in the final transcription reactions. Since micrococcal nuclease is a Ca^{++} -dependent enzyme, the DNA template and the RNA product in the final reaction were protected from the nuclease by the EGTA. In practice, micrococcal nuclease treatment of the extract always reduced background transcription while leaving template-dependent transcription completely unaffected (data not shown).

The DNA template for the reaction was $\text{pp}_L\lambda$, a pBR322-derived plasmid containing a 1.2 kb lambda insert with the early promoter p_L , the nut_L site 50-100 bp downstream from p_L , the N gene, and the Rho-dependent terminator t_{L1} 1.0 kb downstream from p_L . The response of the system to $\text{pp}_L\lambda$ DNA was linear with DNA concentration to at least 50 ug/ml both in the presence and in the absence of N (data not shown).

The N-dependence of $\text{pp}_L\lambda$ -programmed transcription is shown in Fig. 1. Maximum stimulation, about tenfold, was obtained with 10 ug/ml pure N protein. This stimulation was specific since no stimulation was observed when the template was vector pBR322 DNA (Fig. 1). The ability of N to stimulate transcription tenfold implied that at least 90% of the transcribing RNA polymerase molecules in such a reaction saturated with N must be N-modified.

Stimulation of transcription by N was clearly associated with a failure of transcriptional termination. In the experiment shown in Fig.2 RNA molecules which accumulated in reactions containing or lacking N were subjected to sucrose gradient sedimentation. The RNA molecules which accumulated in reactions containing N were far larger, on the average, than those which accumulated in reactions lacking N (Fig.2). Therefore, chain

Figure 1. Stimulation of Transcription in vitro by N. Standard transcription reactions contained the indicated concentrations of pure N, S100 extract from strain JG148, and either pp_L DNA (●---●) or pBR322 DNA (o---o). Total RNA synthesis was determined.

termination and transcript release must be inhibited in PP_L-templated reactions containing N, as would be expected for a transcriptional antitermination factor.

Conditions for Efficient N Function

Our original N assay (19) made use of reaction conditions developed by

Figure 2. Effect of N on chain elongation and transcript release. Standard transcription reactions (100ul) containing 2uCi [³H]UTP, S100 extract from JG148, and 50 ug/ml pp_L DNA were carried out in the absence (o---o) or presence (●---●) of 10ug/ml N. The RNA made in the reactions was extracted with phenol and chloroform, precipitated with ethanol, and subjected to sucrose density gradient sedimentation.

Table 1. Comparison of Conditions for N Function in vitro

Reagent	Stimulation of endolysin synthesis in reaction with S30 (22)	Stimulation of transcription in reaction with S100 (this work)
[Tris-acetate] (mM)	44	40
pH	8.2	7.3
[K-acetate] (mM)	55	20
[NH ₄ -acetate] (mM)	27	27
[Mg-acetate] (mM)	11	20
[CaCl ₂] (mM)	7	-
[dithiothreitol] (mM)	1	1
[Na ₂ -phosphoenol pyruvate] (mM)	21	21
[tRNA] (ug/ml)	100	-
[20 amino acids] (uM)	220 each	-
[GTP, CTP, UTP] (uM)	550 each	550 each
[ATP] (mM)	2.2	2.2
[FAD, TPN, pyridoxine-HCl, folinic acid] (ug/ml)	27 each	-
[p-aminobenzoic acid] (ug/ml)	11	-
[protein] (mg/ml)	6	3
[DNA] (ug/ml)	100	up to 100
template DNA	λ	pp _L λ

Zubay and his collaborators (32). Reaction conditions required for efficient stimulation of transcription by pure N were considerably different (Table 1). The amino acids, vitamins, and Ca⁺⁺ in the original assay were omitted from reaction. In addition, the concentrations of most of the other ions in the reaction have been altered. The optimal Mg⁺⁺ concentration increased from 10-12 mM to 20-25 mM (Fig. 3a), probably at least in part because 7 mM Ca⁺⁺ was omitted from the reaction. Lower concentrations of Mg⁺⁺ reduced stimulation by N while higher concentrations increased transcription observed in the absence of N. The optimal K⁺ concentration was reduced from 55 mM to 20 mM or less (Fig. 3b) while the optimal NH₄⁺ concentration was unaltered at 20-40 mM (Fig. 3c). Lower concentrations of NH₄⁺ reduced somewhat the ability of N to stimulate transcription while higher concentrations of both monovalent cations greatly increased transcription observed in the absence of N. Finally, the pH of the reaction was reduced from 8.2 to 7.3 (Fig. 3d). The pH curve for N-dependent pp_Lλ transcription had a distinct optimum at 7.3 even though pH had almost no effect on N-independent transcription in the same range.

The amount of S100 extract added to the reaction was also critical (Fig. 4). Lower than optimal volumes reduced stimulation of transcription by

Figure 3. Optimal conditions for stimulation of transcription by N. Standard transcription reactions with (●---●) or without (○---○) 10 µg/ml pure N and programmed with pp_λ DNA contained S100 extract from strain JG148 and variable concentrations of the indicated reagents. Total RNA synthesis was determined.

Figure 4. Effect of S100 extract concentration on N-dependent transcription *in vitro*. Standard 20 µl transcription reactions with (●---●) or without (○---○) 10 µg/ml pure N and programmed with pp_λ DNA contained the indicated volumes of micrococcal nuclease-treated S100 extract from strain JG148. Total RNA synthesis was determined.

N while higher than optimal volumes greatly stimulated N-independent transcription. The optimal amount of extract in this reaction (30% of the final reaction volume, about 3 mg/ml final protein concentration) was essentially the same, on a volume basis, as the optimal amount of extract in the original N assay (19).

The transcription rate in the reaction under optimal conditions was very substantial: the mass of RNA made in 15 min, about 150 ug/ml, was three times the mass of the template DNA. Since we have determined, by polyacrylamide gel electrophoresis in the presence of SDS and comparison with standard purified RNA polymerase, that the concentration of RNA polymerase in our transcriptional reactions was 30 ug/ml (data not shown), the average transcription rate was 8 nucleotides per RNA polymerase molecule per second. Since we have also determined that, after 15 min, only one-third to one-half the RNA polymerase molecules were present in rapidly sedimenting transcription complexes (data not shown), the active RNA polymerase molecules were transcribing at least half the time at physiological elongation rates. The reaction time was limited to 15 min because the half-life of the RNA product made in vitro was typically 15-30 min (data not shown).

Biological Relevance of N-Mediated Stimulation of Transcription

The nusA, nusB, and nusE gene products are required, directly or indirectly, for N function in vivo (10,14,15,18). The abilities of mutations in these genes to abolish N function in vivo therefore provided a convenient way for us to test whether the ability of N to stimulate transcription in vitro was relevant to the normal biological function of N protein. S100 extracts were prepared in parallel from nus⁺ strains and from isogenic nusA1 and nusE71 strains. The responses to N in reactions containing such extracts were then tested.

Reactions containing nusA1 mutant S100 extract were completely unresponsive to N at 37° (Fig. 5b), whereas reactions containing extract from an isogenic nus⁺ strain responded normally to N (Fig. 5a). Reactions containing nusA1 extract did respond slightly to N at 30° (data not shown), as would be expected from the fact that the nusA1 mutation is temperature-sensitive with regard to N function in vivo (10). The inability to respond to N at all at 37° in the nusA1 mutant extract was truly related to the nusA1 mutation and not to the conditions of cell growth or extract preparation because N function in the mutant extract could be completely restored by addition to the reaction of pure wild type NusA protein (Fig. 5b). Therefore, stimulation of transcription by N in this reaction directly

Figure 5. Dependence on NusA of transcriptional stimulation by N. Standard transcription reactions with (●---●) or without (○---○) 10 μg/ml pure N and programmed with pp_λ DNA contained either *nusA*⁺ extract derived from strain JG358 (a) or *nusA*¹ extract derived from strain JG359 (b). Each reaction was supplemented with the indicated amount of pure wild type NusA protein. Total transcription was determined.

requires NusA.

The ability of a reaction containing extract derived from a *nusE71* mutant to respond to N was also defective (Fig. 6a,b). However, although there was no response to N in the *nusE71* extract at 40° (Fig. 6b), there was a small, but significant, response to N in the *nusE71* extract at 30° (Fig. 6a). This phenomenon corresponds well to the known temperature-

Figure 6. Effect of the *nusE71* mutation on stimulation of transcription by N. Standard transcription reactions programmed with pp_λ DNA and containing either *nus*⁺ S100 extract from strain JG148 (●---●) or *nusE71* S100 extract from strain JG466 (○---○) were incubated for 15 min at 30° (a) or 40° (b) with the indicated concentrations of pure N. Total transcription was determined.

Table 2. Complementation in vitro of the nusA1 and nusE71 Mutations

Source of S100 extract(s)	RNA synthesis in the absence of N (A) (cpm)	RNA synthesis in the presence of N (B) (cpm)	Effect of N (A/B)
JG358 (nus+)	2553	10946	4.3
JG148 (nus+)	2181	5671	2.6
JG359 (nusA1)	2296	1939	0.9
JG466 (nusE71)	662	848	1.3
JG359 + JG466	836	1971	2.4

Standard transcription reactions (20 μ l) on pp λ DNA lacking N (A) or with 10 μ g/ml N (B) were carried out at 37 $^{\circ}$ with 6 μ l of the indicated S100 extracts or with 3 μ l S100 from JG359 and 3 μ l S100 from JG466. Total transcription was determined.

sensitivity of the nusE71 mutation for N function in vivo (18) and suggests that our nusE71 extract was indeed properly prepared.

Further evidence that the nusE71 extract was properly prepared is shown in Table 2. The nusE71 and nusA1 S100 extracts were able to complement each other for N function. This implied that the nusE71 S100 was at least not deficient in NusA. In fact, the nusE71 extract had defects other than NusA or NusB because N function in a nusE71 extract could not be restored either by pure active NusA or by pure active NusB (R. Horwitz, unpublished data). We are currently purifying from a wild type extract the activities required to restore N function in a reaction containing extract from a nusE71 strain.

Although we have tried several times, we have not been able to make a transcriptionally active S100 extract from a strain with the nusB5 mutation (14). We have therefore not been able to test the NusB-dependence of N function in these reactions even though we possess pure NusB that has activity in an assay (19) containing S30 extract derived from an identical nusB5 strain. Our difficulty in preparing an active S100 from a nusB5 strain may be related to the inability of a nusB⁻ strain to grow well at 30 $^{\circ}$ (33).

Translation is not required for N Function

It has been suggested that ribosomes participate directly in transcriptional antitermination mediated by the lambda N protein (18,22,34). However, in the work described here, we have demonstrated N function in reactions containing a high speed supernatant fraction (S100) that is greatly depleted of ribosomes. In addition, this S100 extract was dialyzed, which ought to have removed the amino acids, and treated with micrococcal nuclease, which ought to have destroyed the tRNA and crippled the ribosomes.

Nevertheless, to show directly that translation is not required for N

Table 3. Effects of protein synthesis inhibitors on N function in vitro

Protein synthesis inhibitor	Concentration (uM)	RNA synthesis in absence of N (cpm)	RNA synthesis in presence of N (cpm)	Inhibition of protein synthesis (%)
-	-	2128	16690	0 (30445cpm)
chloramphenicol	500	2001	14942	95
puromycin	850	2154	16791	100
sparsomycin	27	2389	18647	99
fusidic acid	500	2057	18274	84
streptomycin	500	2171	17353	75
kanamycin	500	2660	19647	67
erythromycin	500	2480	16521	77
tetracycline	500	2457	17686	35
gougerotin	500	2441	17976	95
geneticin	100	2258	17174	51
-	-	2126	12365	0 (26481cpm)
amikacin	500	2177	13514	63
lincomycin	500	2146	13914	96
neomycin	500	5094	13182	89
oleandomycin	500	2098	14249	75
spectinomycin	500	2202	14039	73
tobramycin	500	3081	16039	73
kirromycin	10	2134	13525	53

Standard transcription reactions (20ul) contained 50ug/ml pp_λ DNA, S100 extract from JG148, the indicated antibiotics, and, where indicated, 10ug/ml N. The effects of the same antibiotics on protein synthesis were determined in parallel coupled reactions (23) containing [³H]leucine, S30 extract from JG148, and 100ug/ml λ DNA as template.

function, we tested the effects of 17 protein synthesis inhibitors on N function in vitro (Table 3). Although each of them was used at a concentration high enough to inhibit protein synthesis in a parallel reaction (see Table 3), not one of them had a significant effect on total transcription in the presence of N. N function was not even affected by the presence of high enough concentrations of puromycin or sparsomycin to inhibit protein synthesis more than 99%.

DISCUSSION

A Reaction in which N Efficiently Modifies RNA Polymerase

We have described a cell-free reaction system in which total transcription by *E. coli* RNA polymerase was greatly stimulated by the lambda N protein. N function in this system was template-specific and biologically relevant since it was abolished by two of the same mutations in genes coding

for host factors that abolish N function in vivo. Since the template for the reaction contained an N utilization site (nut site) and a strong Rho-dependent terminator, stimulation by N presumably occurred because of the ability of N to antiterminate transcription in operons containing nut sites (1-5). We have indeed found that the RNA molecules which accumulate in reactions containing N are far larger than those which accumulate in reactions lacking N (Fig.2).

Since N can stimulate transcription up to tenfold in this system, at least 90% of the transcribing RNA polymerase molecules in a reaction saturated with N must be N-modified. This system should therefore be extremely useful for physical analysis of the transcriptional complex made termination-resistant by N. Complicated models (25) developed from the results of indirect experiments should now be testable.

Translation is not needed for N Function

N function in this reaction does not require amino acids and probably does not require ribosomes since the S100 extract used in the reaction was dialyzed and centrifuged hard enough to theoretically pellet all particles with a sedimentation velocity greater than 20. The lack of a requirement for amino acids and the apparent lack of a requirement for ribosomes have been observed before (21,35). In addition, there is strong genetic evidence that the presence of a ribosome on the messenger RNA too close to the nut site can interfere with N function (9). In the work described here the few ribosomes remaining in the S100 should have been incapable of translation because micrococcal nuclease treatment of the S100 should have crippled the ribosomes and destroyed the tRNA. Furthermore, not one of 17 different protein synthesis inhibitors had any effect on N function (Table 3). Therefore translating ribosomes are clearly not required for N function. The only remaining strong reason to think that ribosomes may be involved at all in N function (34) is the phenotypes of nusE mutations in rpsJ, the gene that codes for ribosomal protein S10 (18). S100 extracts prepared from nusE71 strains are indeed unable to support N function in vitro (Fig. 6). However, even if S10 participates directly in the control of transcription termination, and other work currently underway in our laboratory suggests that it does (R. Horwitz, unpublished data), we think it is unlikely to do so as part of the ribosome.

Nus Proteins and N Function

It has been inferred from the phenotype of the nusA1 mutation (10) and from the phenotypes of punA mutations in the N gene that suppress it (36), as

well as from the ability of N to bind directly to NusA (13), that NusA participates directly in antitermination of transcription by N. We have shown here (Fig. 5) in a direct way that this is indeed true. A similar conclusion regarding NusA has recently been reached independently by Das and Wolska (22). It is also known that NusB participates directly in N function (28), and we have inferred from the data shown in Fig. 6 and from unpublished data that two other proteins are also directly required for N function. Antitermination of transcription by N therefore requires the active collaboration of at least four E. coli proteins.

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REFERENCES

1. Roberts, J.W. (1969) *Nature* 224, 1168-1174.
2. Luzzati, D. (1970) *J. Mol. Biol.* 49, 515-517.
3. Lozeron, H.A., Anevski, P.J. and Apirion, D. (1977) *J. Mol. Biol.* 109, 359-365.
4. Salstrom, J.S. and Szybalski, W. (1978) *J. Mol. Biol.* 124, 195-221.
5. de Crombrughe, B., Mudryj, M., DiLauro, R. and Gottesman, M. (1979) *Cell* 18, 1145-1151.
6. Friedman, D.I., Wilgus, G.S. and Mural, R.J. (1973) *J. Mol. Biol.* 81, 505-516.
7. Adhya, S., Gottesman, M. and de Crombrughe, B. (1974) *Proc. Nat. Acad. Sci. USA* 71, 2534-2538.
8. Franklin, N.C. (1974) *J. Mol. Biol.* 89, 33-48.
9. Olson, E.R., Flamm, E.L. and Friedman, D.I. (1982) *Cell* 31, 61-70.
10. Friedman, D. (1971) in *The Bacteriophage Lambda*, Hershey, A.D. Ed., pp.733-738, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. Greenblatt, J., McLimont, M. and Hanly, S. (1981) *Nature* 292, 215-220.
12. Greenblatt, J. and Li, J. (1981) *Cell* 24, 421-428.
13. Greenblatt, J. and Li, J. (1981) *J. Mol. Biol.* 147, 11-23.
14. Friedman, D.I., Baumann, M. and Baron, L.S. (1976) *Virology*, 73, 119-127.
15. Keppel, F., Georgopoulos, C. and Eisen, H. (1974) *Biochimie* 56, 1503-1509.
16. Swindle, J., Ajioka, J. and Georgopoulos, C. (1981) *Mol. Gen. Genet.* 182, 409-413.
17. Strauch, M. and Friedman, D.I. (1981) *Mol. Gen. Genet.* 182, 498-501.
18. Friedman, D.I., Schauer, A.T., Baumann, M.R., Baron, L.S. and Adhya, S.L. (1981) *Proc. Nat. Acad. Sci. USA* 78, 1114-1118.
19. Greenblatt, J. (1973) *Proc. Nat. Acad. Sci. USA* 70, 421-424.
20. Dottin, R.P. and Pearson, M.L. (1973) *Proc. Nat. Acad. Sci. USA* 70, 1078-1082.
21. Ishii, S., Salstrom, J.S., Sugino, Y., Szybalski, W. and Imamoto, F. (1980) *Gene* 10, 17-25.
22. Das, A. and Wolska, K. (1984) *Cell* 38, 165-173.
23. Greenblatt, J. and Li, J. (1980) *J. Biol. Chem.* 255, 1465-1470.
24. Ishii, S., Sugino, Y. and Imamoto, F. (1980) *Gene* 10, 291-300.

25. Greenblatt, J. (1984) *Can. J. Biochem. Cell Biol.* 62, 79-88.
26. Greenblatt, J. and Schleif, R. (1971) *Nature New Biol.* 233, 166-170.
27. Olins, P.O., Erickson, B.D. and Burgess, R.R. (1983) *Gene* 26, 11-18.
28. Swindle, J., Ajioka, J. and Georgopoulos, C. (1983) in *Microbiology 1983*, Schlessinger, D. Ed., pp.63-65, American Society for Microbiology, Washington, D.C.
29. Greenblatt, J. (1972) *Proc. Nat. Acad. Sci. USA* 69, 3606-3610.
30. Bolivar, F., Rodriguez, R.L., Greene, P.J. Betlach, M.C., Henecker, H.L., Boyer, H.W., Crosa, J.H. and Falkow, S. (1977) *Gene* 2, 95-113.
31. Birnboim, H.C. and Doly, J. (1979) *Nucleic Acids Res.* 7, 1513-1523.
32. Zubay, G., Chambers, D.A. and Cheong, L.C. (1970) in *The Lactose Operon*, Beckwith, J.R. and Zipser, D. Eds., pp.375-391, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. Georgopoulos, C.P., Swindle, J., Keppel, F., Ballivet, M., Bisig, R. and Eisen, H. (1980) *Mol. Gen. Genet.* 179, 55-61.
34. Adhya, S. and Gottesman, M. (1978) *Ann. Rev. Biochem.* 47, 967-996.
35. Greenblatt, J. (1982) *Ann. Microbiol. (Inst. Pasteur)* 133A, 225-233.
36. Friedman, D.I. and Ponce-Campos, R. (1975) *J. Mol. Biol.* 98, 537-549.