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Proteins from the prokaryotic nucleoid

Interaction of nucleic acids with the 15 kDa *Escherichia coli* histone-like protein H-NS

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The interaction between nucleic acids and *Escherichia coli* H-NS, an abundant 15 kDa histone-like protein, has been studied by affinity chromatography, nitrocellulose filtration and fluorescence spectroscopy. Intrinsic fluorescence studies showed that the single Trp residue of H-NS (position 108) has a restricted mobility and is located within an hydrophobic region inaccessible to both anionic and cationic quenchers. Binding of H-NS to nucleic acids, however, results in a change of the microenvironment of the Trp residue and fluorescence quenching; from the titration curves obtained with addition of increasing amounts of poly(dA)-poly(dT) and poly(dC)-poly(dG) it can be estimated that an H-NS dimer in 1.5 × SSC binds DNA with an apparent $K_a \simeq 1.1 \times 10^4\ M^{-1} \cdot bp^{-1}$. H-NS binds to double-stranded DNA with a higher affinity than the more abundant histone-like protein NS(HU) and, unlike NS, prefers double-stranded to single-stranded DNA and DNA to RNA; both monovalent and divalent cations are required for optimal binding.

Bacterial chromatin; Protein-nucleic acid interaction; Fluorescence spectroscopy

1. INTRODUCTION

Various lines of evidence suggest that DNA-binding proteins, functionally similar to histones, may play an important role in the structural organization of the bacterial nucleoid (review [1–3]). Among these proteins the most abundant is NS (HU). This is predominantly a tetramer composed of two types of subunits (NS1 and NS2), each of approx. 9 kDa, for which extensive spectroscopic, biochemical and genetic characterization data are available [1–6]. In addition to NS, several other DNA-binding proteins have been reported. One of these, H-NS, is an abundant (~20000 copies/*Escherichia coli* cell), neutral (p*I* = 7.3), heat-stable protein with a molecular mass of 15.4 kDa [7]. It is likely that H-NS corresponds to the 17 kDa protein B1 found by Varshavsky et al. [8] in a 1:2 molar ratio with B2 (i.e. NS, HU) every 150–200 bp of DNA in the bacterial nucleoid; H-NS is also perhaps related to one of the proteins (H1a, H1b, H1c) reported by Spassky et al. [9] to cause inhibition of transcription and a strong condensation of DNA without unwinding it. The primary structure of H-NS has been elucidated and found to consist of 136 amino acid residues [10]. Owing to a very strong hydrophobic protein-protein interaction, H-NS exists predominantly as a dimer even at very low concentrations ($\leqq 10^{-9}$ M); however, at higher concentrations (~10^{-4} M), presumably comparable to its intracellular concentration, H-NS can form tetramers [10]. The *hns* gene encoding H-NS has recently been identified by reverse genetics, cloned, sequenced and mapped at 6.1 min on the *E. coli* chromosome [11]. Like eukaryotic histones, H-NS

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has no affinity for hydroxyapatite, increases the thermal stability of DNA and inhibits transcription ([7] and unpublished).

In the present paper we have characterized the interaction between H-NS and nucleic acids and compared the nucleic acid binding properties of H-NS with those of NS.

2. MATERIALS AND METHODS

2.1. *Purification of H-NS*

H-NS was purified as described [10] starting from the 30000 × *g* supernatant of *E. coli* MRE600 cells [12]. In short, this purification procedure involves ionic-exchange chromatography on phosphocellulose followed by CM cellulose and affinity chromatography on DNA cellulose and yields a protein homogeneous by several electrophoretic criteria [10].

2.2. *Fluorescence spectroscopy*

Spectrofluorimetric measurements were carried out essentially as described by Steinhäuser et al. [13] using an SLM 8000 DS spectrofluorimeter. The data were processed using a Hewlett Packard 9825 A computer with software kindly provided by Dr P. Woolley.

Quenching titrations were carried out by adding increasing microliter amounts of a 1 M stock solution of either KCl, CsCl or KI (containing 20 μM sodium thiosulfate as stabilizer) to 405 μl of 10 μM protein solution. The signal of a reference-containing buffer and quencher was subtracted from each point. For comparative purposes, the quenching of a solution of $NAcTrp-NH_2$ as a model for free tryptophan was determined. The results were analyzed according to the Stern-Volmer equation [13]. For protein denaturation experiments the temperature of the sample was increased by 0.3 K/min in approx. 5 K increments using a Haake PG10 thermostat programmer. Vertical and horizontal emission signals (F_{VV} and F_{VH}) were measured at an excitation wavelength of 295 nm.

DNA titration experiments were carried out with a (monomeric) protein concentration of 10.3 μM in 320 μl of 1.5 × SSC (1 × SSC = 15 mM Na citrate, pH 7, 150 mM NaCl). Increasing amounts of poly(dA)-poly(dT) or poly(dC)-poly(dG) were added up to a maximum of 120 μg/sample. To minimize the internal filter effect due to the absorption of the polydeoxynucleotides, excitation was performed at 306 nm with a bandwidth of 8 nm and the emission signal (λ_{em} = 327 nm, bandwidth = 8 nm) was corrected for both dilution of the sample and absorption at 306 nm.

2.3. *DNA binding experiments*

2.3.1. Affinity chromatography

Pasteur pipettes were filled up to 2 cm with matrix-bound nucleic acids as indicated in the figures and equilibrated with 20 mM Tris-HCl, pH 7.2, buffer containing 25 mM NaCl and 5 mM β-mercaptoethanol. Each column was loaded with approx. 50 μg of H-NS or NS labelled in vitro under very mild conditions (pH 8.5) [14]. The samples were applied to the columns in the above buffer and eluted with a 50 ml (25–700 mM) NaCl gradient in 20 mM Tris-HCl (pH 7.2) buffer containing β-mercaptoethanol. Fractions of 0.58 ml were collected and the radioactivity contained in 0.3 ml of each fraction was determined by liquid scintillation counting. The elution profiles were aligned based on the conductivity values determined for the indicated fractions.

2.3.2. Nitrocellulose filtration

E. coli [^{14}C]thymidine-labelled chromosomal DNA (650 cpm/μg) was incubated with purified H-NS in 0.3 ml of 0.1 × SSC in the presence of monovalent and divalent cations as indicated. After 15 min incubation at 37°C the samples were filtered through nitrocellulose (Millipore HA 0.45 μm) pretreated by soaking for 60 min at room temperature in a 20 mM Tris-HCl, pH 7.2, buffer containing 100 mM KCl, 5 mM $MgCl_2$, 1 mM NaEDTA, 5 mM β-mercaptoethanol and 50 μg/ml bovine serum albumin. Before use the filters were washed once with the same buffer containing no bovine serum albumin and then once with a buffer having the same composition as that used in the binding reaction.

3. RESULTS

The interaction between H-NS and nucleic acids has been studied by affinity chromatography, nitrocellulose filtration and fluorescence spectroscopy.

3.1. *Intrinsic fluorescence spectroscopy*

The intrinsic fluorescence emission spectrum of H-NS is shown in fig.1. Subtraction of the emission spectrum obtained when only Trp is excited at 295 nm from that obtained when Trp and Tyr residues are excited at 265 nm yields the normalized emission spectra of the three Tyr residues (positions 60, 96, 98) and that of the single Trp residue (position 108). The emission maximum of the latter is considerably blue-shifted (327 nm) with respect to free tryptophan (348 nm) and undergoes a progressive red shift as the temperature is increased; in fig.2A,B this effect is superimposed on the expected temperature-dependent decrease of the fluorescence intensity. The red shift begins around the physiological temperature (i.e. 35°C) and is virtually complete at 65°C. The fluorescence anisotropy of Trp-108 has a high value (0.15 at λ_{em} = 340 nm) in the native protein at physiological temperature (30–37°C) but diminishes as the temperature is increased (fig.2C,D). The intrinsic Trp-108 fluorescence is also insensitive to the addition of either cationic or anionic quenchers (fig.3A,B).

Taken together, these results indicate that Trp-108 is situated in an internal, hydrophobic

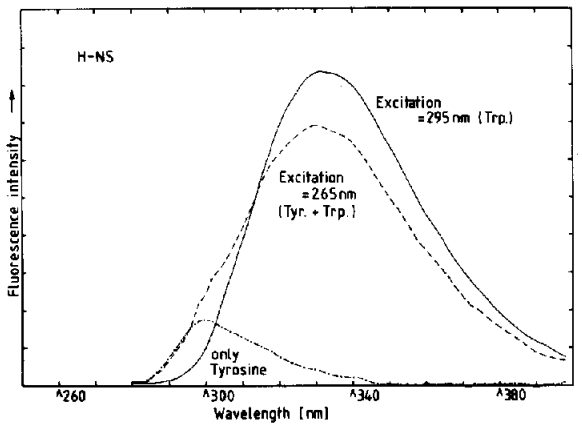

Fig.1. Intrinsic fluorescence emission spectra of H-NS. Fluorescence measurements were carried out at 20 ± 0.2°C as described in section 2. Excitation was either at 265 or 295 nm as indicated, with a bandwidth of 4 nm. The emission bandwidth was 2 nm.

region inaccessible to the quenchers and has a strongly restricted mobility. These properties are due to a tertiary or quaternary structure which unfolds or dissociates at approx. 60°C progressively exposing the tryptophan ring to the solvent and increasing its mobility. This conclusion is in full agreement with previous results of ^{1}H-NMR spectroscopy. In fact, from the linewidths and chemical shifts of the aromatic protons in the native protein and from the spectral changes obtained upon heating, it was concluded that the aromatic residues of H-NS are buried within the tertiary and/or quaternary structure of the protein and endowed with little mobility [2].

Addition of synthetic polydeoxyribonucleotides to H-NS decreases the intensity of the protein intrinsic fluorescence; this effect is not accompanied by any alteration of the emission maximum. Saturation is reached at approx. 40 or 60% fluorescence quenching upon addition of poly-

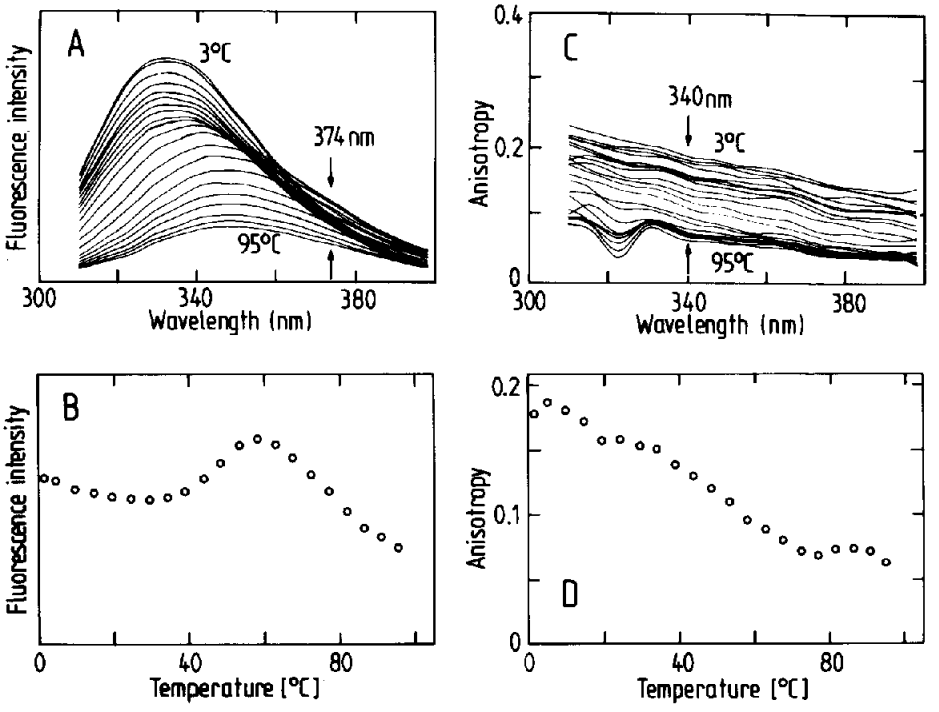

Fig.2. Thermal denaturation of H-NS monitored by changes of Trp-108 fluorescence. (A) Emission spectra (λ_{ex} = 295 nm) were recorded between 3 and 95°C at approx. 5°C intervals in 20 mM Tris-HCl, pH 7.2, 500 mM KCl, 0.5 mM DTE. (B) The fluorescence intensities at λ_{em} = 374 nm, indicated by the arrow in panel A, are plotted against the temperatures at which they were recorded. (C) Trp fluorescence anisotropy between 3 and 95°C. The trough at ~320 nm is due to a measurement artifact. (D) Plot of fluorescence anisotropy at λ_{em} = 340 nm vs temperature. The slight rise in anisotropy around 80°C can be attributed to protein aggregation.

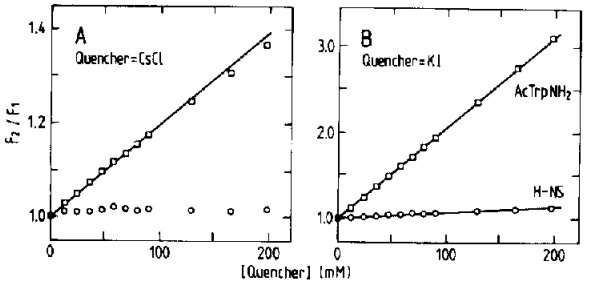

Fig.3. Inaccessibility of Trp-108 to both cationic and anionic fluorescence quenchers. Stern-Volmer plots for acetyltryptophanamide ($AcTrpNH_2$) (□) and H-NS (○) quenching by (A) CsCl and (B) KI. $F_2/F_1 = \tau_2/\tau_1 = 1 + K_{sv}[Q] = 1 + K_q\tau_2[Q]$ where F_2 and F_1, fluorescence with and without quencher, respectively; τ_2 and τ_1, fluorescence lifetime with and without quencher, respectively; [Q], quencher concentration; K_{sv}, Stern-Volmer constant; K_q, collisional rate constant.

(dA)-poly(dT) (fig.4A) or poly(dC)-poly(dG) (fig.4B), respectively. From the titration curves and assuming that all the protein molecules are active in DNA binding, one can estimate that in 1.5 × SSC at 20°C H-NS binds to DNA with an apparent $K_a = 1.1 \times 10^4\ M^{-1}$ based on base pair concentration. A qualitatively similar quenching effect was also observed upon addition of fragmented genomic *E. coli* DNA (not shown). In this case, however, possibly due to the heterogeneity of the DNA, the titration curves are irregular and cannot be straightforwardly evaluated.

3.2. *Affinity chromatography*

As seen in fig.5A, H-NS is eluted from columns containing cellulose-bound double-stranded DNA at a much higher salt concentration, thereby

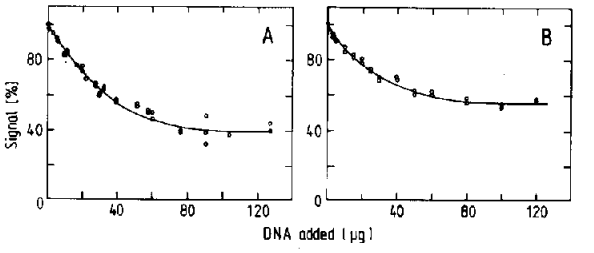

Fig.4. H-NS–polynucleotides interaction monitored by fluorescence quenching. The fluorescence intensity of a 10.3 µM solution of H-NS (monomer) in 0.32 ml of 1.5 × SSC was recorded before (100% signal) and after addition of the indicated amounts of (A) poly(dA)·poly(dT) and (B) poly(dC)·poly(dG). Further details are given in section 2.

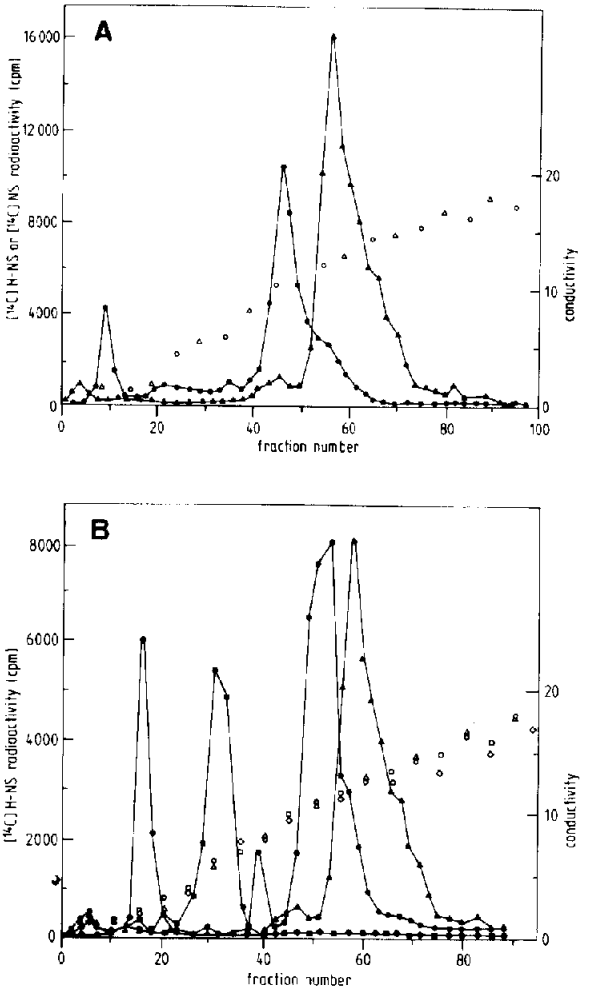

Fig.5. Affinity of H-NS and NS (HU) for dsDNA and of H-NS for different nucleic acids. (A) Elution of radioactive NS (●) and H-NS (▲) from affinity-chromatography columns containing matrix-bound dsDNA. (B) Elution of H-NS from affinity-chromatography columns containing dsDNA (▲), ssDNA (●), tRNA (■) and rRNA (♦). The conductivity measured for the indicated fractions of each eluate are indicated by the corresponding open symbols.

displaying substantially greater affinity for dsDNA than the better characterized histone-like protein NS (HU). The relative affinity of H-NS for various nucleic acids was also studied by comparison of its elution profiles from columns containing matrix-bound rRNA, tRNA, ssDNA or dsDNA. The affinity of H-NS decreases in the order: dsDNA > ssDNA > tRNA > rRNA (fig.5B). This order of preference for nucleic acids is also at variance with

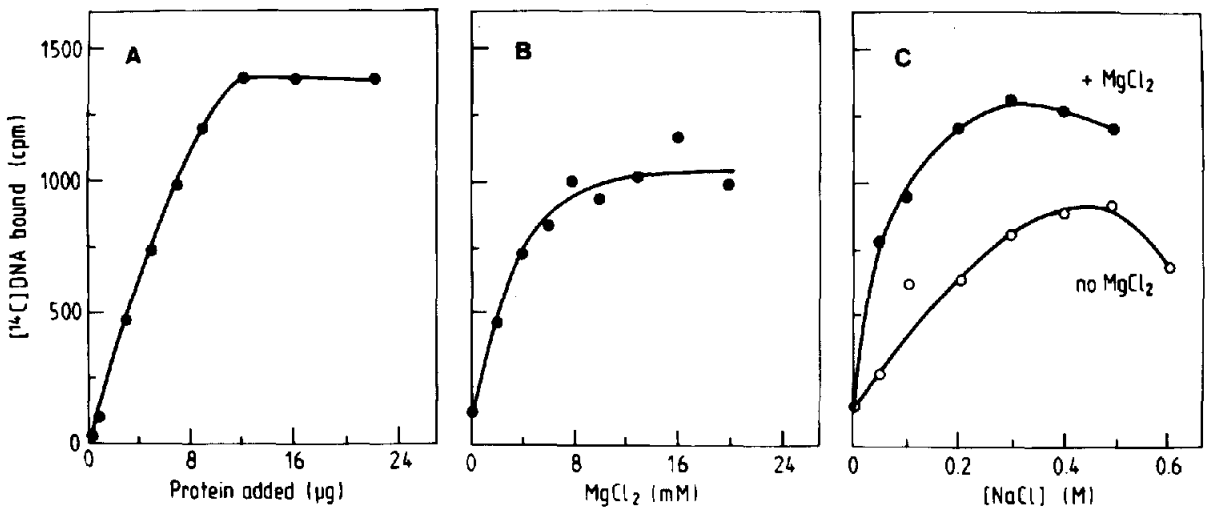

Fig.6. H-NS–DNA interaction as monitored by nitrocellulose filtration. (A) Dose response curve of DNA binding by H-NS. 2.5 μg [^{14}C]thymidine-labelled *E. coli* chromosomal DNA (650 cpm/μg) were incubated with the indicated amounts of H-NS in 0.3 ml of 0.1 × SSC containing 150 mM NH_4Cl. (B) Effect of $MgCl_2$ concentration on the retention of radioactive DNA by 3 μg H-NS. The binding was performed as in A, but without added NH_4Cl. (C) Effect of NaCl and $MgCl_2$ on the retention of DNA by H-NS. The binding conditions were the same as in B with the exceptions that the reaction volume was 0.4 ml and the NaCl concentration was varied as indicated: (○) no $MgCl_2$ present; (●) 10 mM $MgCl_2$ present.

that found for protein NS (HU) which was reported to bind better to ssDNA than to dsDNA and, overall, better to RNA than to DNA [15].

3.3. *Millipore filtration*

The retention of radioactive DNA on nitrocellulose filters by H-NS is linearly proportional to the amount of H-NS added; saturation is reached at a protein:DNA ratio of approx. 4 (fig.6A) which corresponds to approx. 12 bp DNA/H-NS dimer. This figure should represent the minimum estimated size of the DNA binding site. Addition of monovalent and divalent cations (fig.6B,C) increases the retention of DNA on the filters. In the presence of 8 mM $MgCl_2$, optimum binding is observed at about 300 mM NaCl; if Mg^{2+} is omitted, however, the optimum concentration of NaCl is shifted to higher values.

4. DISCUSSION

A structural and genetic characterization of protein H-NS from *E. coli* has been presented elsewhere [10,11]. It is very likely that this protein corresponds to B1 found in association with bacterial chromatin [8] for which, however, a thorough characterization is lacking and to one of the three histone-like proteins H1 described by Spassky et al. [9]. Indeed, the sequence of the first 20 amino acids is the same for H-NS and H1a [10,16]. The latter, however, was found to accumulate in stationary phase cells while H-NS is the only protein with similar properties which can be isolated from exponentially growing cells. We have no evidence, however, for the existence of related genes encoding proteins similar to H-NS [11].

The primary structure of H-NS bears no resemblance to that of the more abundant and so far better studied 9 kDa histone-like proteins NS-1 and NS-2 (HU) [10,17]. Here we have shown that H-NS binds more tightly to DNA than NS and, unlike the latter [15], prefers dsDNA to ssDNA, and DNA to RNA. Furthermore, we have found that, unlike that of NS [7], binding of H-NS to DNA does not show any indication of cooperativity and is stimulated by magnesium as well as by monovalent cations.

As to the actual function of H-NS in vivo, its properties described here as well as other characteristics to be reported in detail elsewhere (e.g. the inhibition of transcription and increase of the thermal stability of DNA) are compatible with a DNA-packaging function.

From the recovery of H-NS upon purification, we estimate that each cell should contain approx. 20000 copies (i.e. 10000 dimers). Thus, with the binding site assumed to be 40 bp/dimer, the amount of H-NS in the cell is hardly sufficient to cover 10% of the total genomic DNA. Therefore, when thinking of a possible function of H-NS in the organization of bacterial chromatin, one can hypothesize that this protein, which has a higher affinity for DNA than NS (HU), might be responsible for condensing portions of the chromosome into tight cores from which topologically independent, negatively supercoiled domains of DNA may loop out [18]. Gene replacement experiments with modified *hns* and direct intracellular localization of the protein by immune electron microscopy should allow us to test this hypothesis.

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REFERENCES

[1] Gualerzi, C.O., Losso, M.A., Lammi, M., Friedrich, K., Pawlik, R.T., Canonaco, M.A., Gianfranceschi, G., Pingoud, A. and Pon, C.L. (1986) in: Bacterial Chromatin (Gualerzi, C.O. and Pon, C.L. eds) pp.101–134, Springer, Heidelberg.

[2] Paci, M., Pon, C.L., Canonaco, M.A. and Gualerzi, C.O. (1986) in: Bacterial Chromatin (Gualerzi, C.O. and Pon, C.L. eds) pp.135–154, Springer, Heidelberg.

[3] Drlica, K. and Rouviere-Yaniv, J. (1987) Microbiol. Rev. 51, 301–319.

[4] Kano, Y., Yoshino, S., Wada, M., Yokoyama, K., Nobuhara, M. and Imamoto, F. (1985) Mol. Gen. Genet. 201, 360–362.

[5] Kano, Y., Wada, M., Nagase, T. and Imamoto, F. (1986) Gene 45, 37–44.

[6] Kano, Y., Osato, K., Wada, M. and Imamoto, F. (1987) Mol. Gen. Genet. 209, 408–410.

[7] Lammi, M., Paci, M., Pon, C.L., Losso, M.A., Miano, A., Pawlik, R.T., Gianfranceschi, G. and Gualerzi, C.O. (1984) in: Proteins Involved in DNA Replication (Hübscher, U. and Spadari, S. eds) pp.467–477, Plenum, New York.

[8] Varshavsky, A.J., Nedospasov, S.A., Bakayev, V.V., Bakayeva, T.G. and Georgiev, G.P. (1977) Nucleic Acids Res. 4, 2725–2745.

[9] Spassky, A., Rimsky, S., Garreau, H. and Buc, H. (1984) Nucleic Acids Res. 12, 5321–5340.

[10] Falconi, M., Gualtieri, M.T., La Teana, A., Losso, M.A. and Pon, C.L. (1988) Mol. Microbiol., in press.

[11] Pon, C.L., Calogero, R.A. and Gualerzi, C.O. (1988) Mol. Gen. Genet., in press.

[12] Pawlik, R.T., Littlechild, J., Pon, C.L. and Gualerzi, C. (1981) Biochem. Int. 2, 421–428.

[13] Steinhäuser, K.G., Woolley, P., Epe, B. and Dijk, J. (1982) Eur. J. Biochem. 127, 587–595.

[14] Gualerzi, C. and Pon, C.L. (1979) Methods Enzymol. 59, 782–795.

[15] Holk, A. and Kleppe, K. (1985) FEBS Lett. 185, 121–124.

[16] Laine, B., Sautière, P., Spassky, A. and Rimsky, S. (1984) Biochem. Biophys. Res. Commun. 119, 1147–1153.

[17] Mende, L., Timm, B. and Subramanian, A.R. (1978) FEBS Lett. 96, 395–398.

[18] Pettijohn, D.E. (1982) Cell 30, 667–669.