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Hypothesis: the RNase-sensitive restraint to unfolding of spermidine nucleoids from *Escherichia coli* is composed of cotranslational insertion linkages

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Abstract

The genomic DNA of bacteria is highly localized in one or a few bodies known as nucleoids. A number of restraints to the unfolding of the DNA of spermidine nucleoids from *Escherichia coli* were previously associated with characteristic urea concentrations (U_m values). The dominant restraint to unfolding was sensitive to pancreatic RNase and underwent a cooperative transition at $U_m = 3.2$ M urea. The losses of the RNase-sensitive restraint caused by urea or pancreatic RNase appear to result from breakage of cotranslational insertion linkages which joined the nucleoid to the cell envelope in growing cells. This conclusion is based upon effects from exposures of cells to antibiotics (chloramphenicol, rifampicin, streptomycin), treatment of nucleoid preparations with formaldehyde or concentrated NaCl solutions, and effects of urea on purified ribosomes. The specific RNase-sensitive and urea-sensitive components of the spermidine nucleoids are suggested to be the mRNA and ribosomes, respectively, of cotranslational insertion linkages.

Keywords: DNA; Nucleoid; *Escherichia coli*; Ribonuclease; Urea; Spermidine

1. Introduction

Bacterial DNA is largely confined to a fraction of the cell volume, in one or a few compact bodies known as nucleoids. The state of nucleoid compaction is a balance of compacting and decompacting forces [1,2]. Forces favoring compaction include macromolecular crowding by the surrounding cytoplasm [2–6], the binding of proteins [7–10] or of polyamines [11–16], and DNA supercoiling [17]. Forces favoring decompaction include entropy and electrostatic repulsion that are exacerbated by the enclosure of a millimeter-long piece of DNA within a cell envelope that is only a few microns in any dimension. A different, localized type of decompacting force was suggested [1] to arise from cotranslational insertion linkages that might exert pulling forces between the genomic DNA and the cell envelope. These link-

Abbreviations: U_m, urea concentration at 50% unfolding of the nucleoid.

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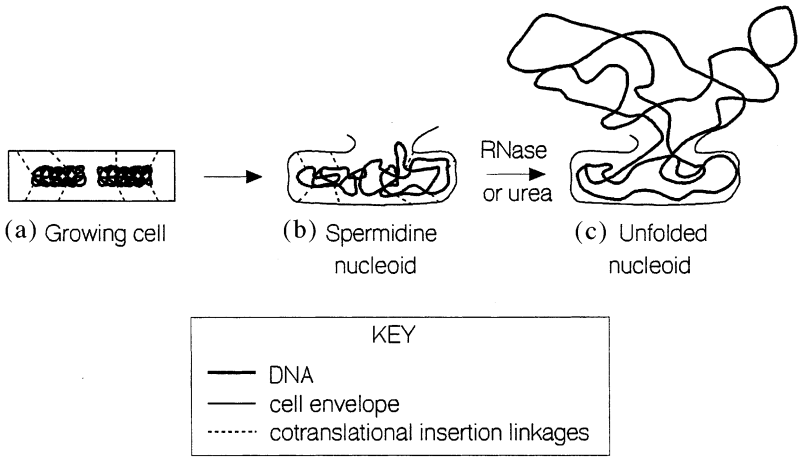

Fig. 1. A role of cotranslational insertion linkages in maintaining partial DNA compaction in lysed cells. The drawings illustrate concepts and interpretations described in detail in the text. A few of the thousands of cotranslational insertion linkages in a growing cell are represented between the nucleoid DNA and the cell envelope. Most of the DNA of the growing cell (a) is present in compact regions because of the macromolecular crowding of the cytoplasm of the cell, ligand binding, and supercoiling; linkages at the surfaces of these compact regions of DNA or to extensions of the DNA into the cytoplasm bind the DNA to the envelope. Upon lysis, the loss of macromolecular crowding allows a limited expansion of the DNA (b), but more complete unfolding is prevented by the cotranslational insertion linkages. If the linkages are broken by treatment of spermidine nucleoids with RNase or urea, extensive unfolding of the DNA occurs (c).

ages are formed in large numbers in growing cells by coordinate translation, transcription, and insertion of nascent polypeptides into cell membranes [1,18–21]. Vos-Scheperkeuter and Witholt [19] have estimated that the thousands of cotranslational insertion linkages in a growing cell will result in the inner surface of the cytoplasmic membrane being largely covered by the involved ribosomes.

As an experimental approach to nucleoid compaction and to the largely unknown local structure in the nucleoid, we have been attempting a systematic dissection of a type of 'isolated' bacterial nucleoid, the spermidine nucleoid from *Escherichia coli*, first described by Kornberg et al. [22]. The effects of cotranslational insertion linkages on DNA compaction in these or any other in vitro nucleoid preparations have not previously been addressed, to our knowledge.

Spermidine nucleoids are made by detergent lysis of lysozyme-treated cells [22,23]. The DNA in these preparations is maintained in a compact form by a series of restraints [24]. We here consider the possibility that one of these restraints, the dominant RNase-sensitive restraint to unfolding, is a direct result of the carryover into spermidine nucleoids of cotranslational insertion linkages made in the cell. This proposal is diagramed in Fig. 1: linkages that tie the nucleoid DNA to the envelope of the growing cell (a) would continue to tie the DNA to the residual envelope of the spermidine nucleoid (b); however, destruction of the linkages (e.g. by RNase or urea) would allow extensive unfolding of the relatively compact DNA of spermidine nucleoids (c).

The RNase-sensitive restraint was defined by its high sensitivity to pancreatic RNase. Recognition of the RNase-sensitive restraint was crucial to the study of the other restraints; it was only after the RNase-sensitive system had been removed that independent, RNase-resistant restraints to unfolding could be detected. The nucleoids remained folded after removal of this restraint with pancreatic RNase because of the presence of the RNase-resistant restraint(s), but had become much more urea sensitive [24]. The RNase-sensitive restraint was also sensitive to urea; nucleoids underwent a cooperative unfolding at a characteristic urea concentration (midpoint of urea transition or U_m

value = 3.2 ± 0.19 M urea). Our results suggest that cotranslational insertion linkages are responsible for the RNase-sensitive restraint and that its urea-sensitivity arises from the urea-sensitivity of the ribosomes involved.

2. Materials and methods

Materials and methods were as previously described [23,24] where not specified.

2.1. Materials

Rifampicin and streptomycin were purchased from Sigma Chemical Co.

2.2. Nucleoid preparations

Spermidine nucleoids were freshly prepared for each experiment from *E. coli* C600 as before [23]. Where indicated, logarithmically growing cells ($A_{600nm} = 0.25$) were treated with the specified concentrations of chloramphenicol, streptomycin, or rifampicin for 30 min at 37 °C under growth conditions and then the cultures were chilled and used for nucleoid isolation as before [23]. RNase-treatment of the spermidine nucleoids was 30 min at 0 °C with 100 ng/ml of pancreatic RNase (i.e. as used to prepare 'RNased nucleoids' [24]).

Where indicated, nucleoids were extracted with 1 M NaCl by gentle mixture of one volume of spermidine nucleoids with 0.43 volume of a solution containing 3.33 M NaCl–1.33% polyethylene glycol–10 mM Na diethylmalonate buffer, pH 7.1–5 mM $MgCl_2$–1 mM 2-mercaptoethanol. After 30 min at 0 °C, the mixture was centrifuged for 5 min at 12 000 × *g*, 5 °C. The pellet was resuspended in 1 vol. of Solution A (Solution A = 10 mM Na diethylmalonate buffer, pH 7.1–5 mM $MgCl_2$–1 mM 2-mercaptoethanol) by periodic, gentle stirring with a glass rod for 40 min at 0 °C.

2.3. Dilatancy assay for nucleoid unfolding

Nucleoid unfolding was assayed by the dilatancy assay. The dilatancy assay utilizes the tendency of unfolded nucleoids to undergo macroscopic clumping in centrifugal fields; it provides rapid quantitation of DNA unfolding in the presence of urea and other materials, and detects DNA degradation to the extent that the degradation interferes with clumping [24,25]. 'Unfolding' of isolated nucleoids is used to indicate the formation of less compact arrangements of the DNA and does not imply separation of the strands of the double helix or their degradation. The urea transitions were characterized in the dilatancy assay by the urea concentrations at their midpoints (U_m values), that is, points at which there were equal amounts of DNA in the pellet and supernatant fluid [25].

2.4. Urea-treatment and electrophoresis of ribosomes

Ribosomes were purified as before [26] from *E. coli* C600; ribosome concentration was determined assuming $E^{1\%} = 148$ at 260 mμ. A series of reaction mixtures were prepared containing ribosomes (10 μl of 4 mg/ml ribosomes in Solution A containing 28% sucrose). The aliquots were preincubated for 30 min with 5 μl of either Solution A, or, where indicated, with Solution A containing 200 ng/ml pancreatic RNase. Solutions (45 μl) of increasing concentrations of urea in Solution A were added; after 30 min at 0 °C, the mixtures were applied to 3% polyacrylamide–0.5% agarose composite gels [27,28]; gels and gel buffer contained 10 mM $MgCl_2$. Electrophoresis was for 2.5 h at 125 V at 0 °C without buffer circulation.

3. Results

3.1. An RNase-sensitive restraint to nucleoid unfolding

The RNase-sensitive restraint to DNA unfolding in spermidine nucleoids from *E. coli* C600 underwent a cooperative transition in urea solutions at 0 °C which released the restraint and allowed unfolding of the DNA of the nucleoids; the midpoint of the urea transition (U_m value), occurred at 3.2 ± 0.19 M urea [24]. Pretreatment of the nucleoids with low levels of pancreatic RNase removed this restraint and revealed the next most urea-stable restraint (with $U_m \leqslant 1.8$ M urea). Examples of the nucleoid transitions before and

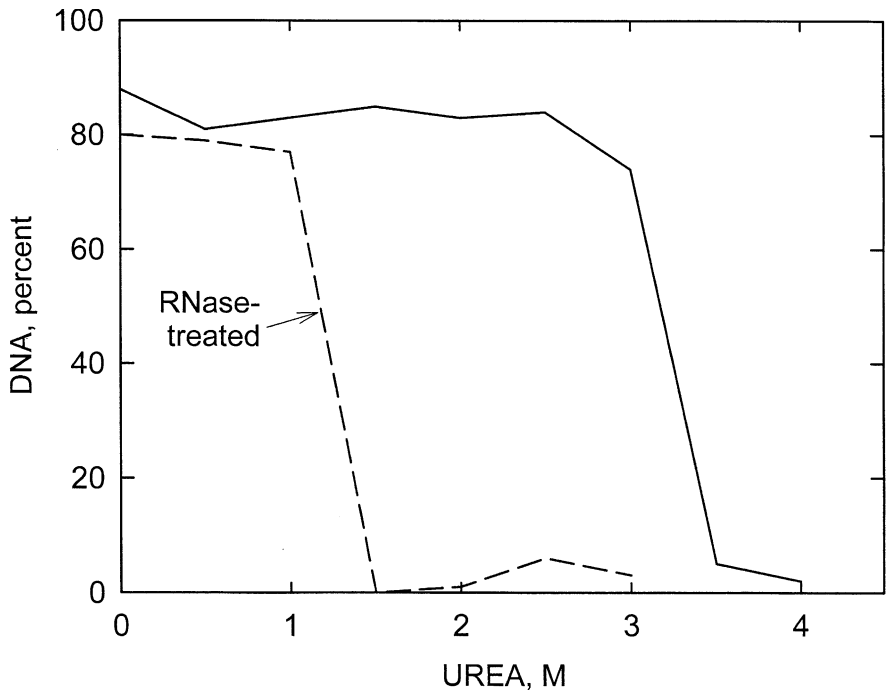

Fig. 2. Effects of pancreatic RNase on the urea transition of spermidine nucleoids. Representative urea transitions of spermidine nucleoids before (solid line) and after (dashed line) treatment with 100 ng/ml of pancreatic RNase for 30 min at 0 °C as measured by the dilatancy assay.

after RNase treatment are shown in Fig. 2 by the solid and dashed lines, respectively. These profiles indicate the presence of RNA-containing material with $U_m = 3.2$ M urea which prevented nucleoid unfolding. In the following sections, we describe properties of the RNase-sensitive restraint which suggest that the RNase-sensitive component and the urea-sensitive component are distinctly different, but that they are both integral parts of complex cotranslational insertion linkages that join the DNA to the cell envelope. These relationships are shown in Fig. 3. Note that loss of integrity of either of two putative links in the chain (i.e. the RNase-sensitive mRNA or the urea-sensitive ribosomes) removes the connection between the DNA and the cell envelope and allows unfolding of the DNA (cf. Fig. 1).

3.2. Effects of salt-extraction of spermidine nucleoids on the RNase-sensitive restraint

If cells of *E. coli* are lysed in the absence of some type of protective medium, their nucleoids expand due to internal repulsions and/or autolytic degradation and rapidly lose their characteristic appearance. This decompaction can be prevented to varying extents by the presence during cell lysis of either high NaCl concentrations [29], spermidine [22,23], or polylysine–spermidine mixtures [30].

The nucleoids used here were made in the presence of spermidine. We tested for effects of removal of the spermidine by extraction of the nucleoids with 1 M NaCl.[1] NaCl-extracted nucleoids no longer showed a cooperative transition at ~3.2 M urea. Instead, a gradual transition occurred at a lower urea concentration, similar to that found in intentionally unfolded or degraded nucleoids (0 mM spermidine curve of Fig. 4; figure 6B of Ref. [25]). Since spermidine is not

[1] Salt-extractions were done in the presence of 0.4% polyethylene glycol (average $M_r \sim 8$ kDa) for reasons unrelated to the present experiments. No effects on U_m values or protein content have been found in the absence of the polyethylene glycol in a limited set of similar experiments.

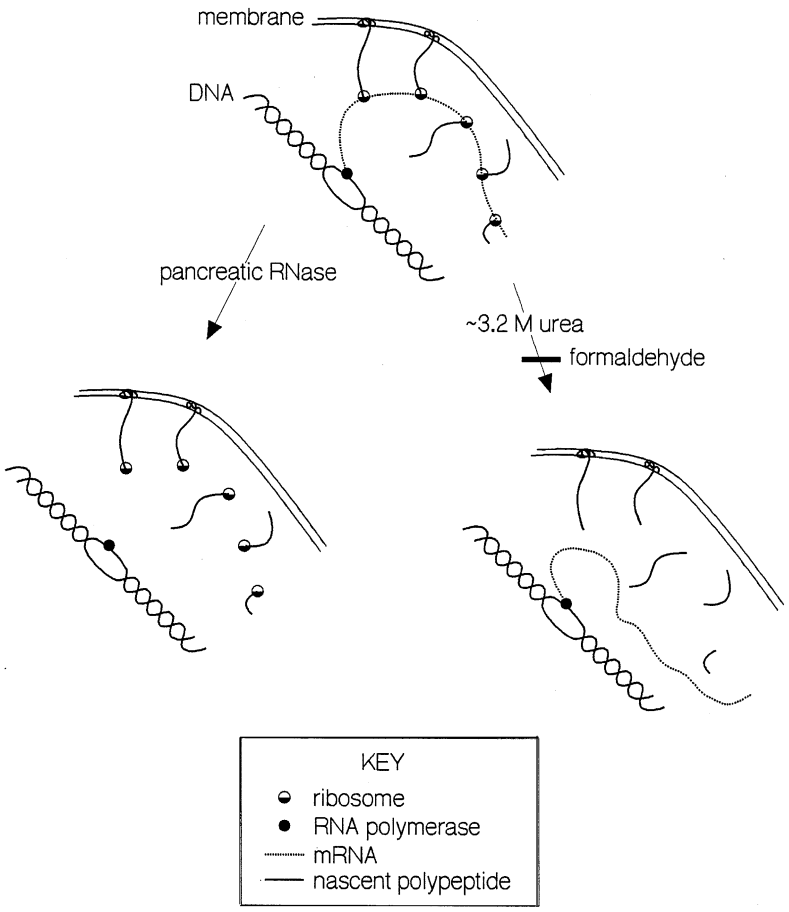

Fig. 3. Cotranslational insertion linkages. The diagram indicates the components of cotranslational insertion linkages, the suggested mechanisms of RNase and urea in disrupting those linkages, and the inhibition of polyribosome dissociation by formaldehyde discussed in the text.

bound to nucleic acids in the presence of 1 M NaCl [31], we tested the possibility that the loss of the transition was due to the loss of spermidine. Readdition of spermidine caused a dramatic reestablishment of a cooperative transition (Fig. 4), indicating that the structure responsible for the RNase-sensitive transition had not been removed by the extraction.[2]

Addition of spermidine to unextracted nucleoid preparations did not significantly change their U_m values; total omission of spermidine from the lysis procedure resulted in preparations with a very broad non-cooperative transition (data not shown).

3.3. Effects of antibiotic treatments of cells on the RNase-sensitive restraint in spermidine nucleoids from those cells

Spermidine nucleoids prepared from cells that had been treated with antibiotics that inhibit protein or RNA syntheses had altered stabilities in urea solutions.

3.3.1. Rifampicin

Rifampicin inhibits the productive initiation of RNA chains by the RNA polymerase of *E. coli*

[2] The RNA polymerase that is involved in the cotranslational insertion linkages discussed here is expected to resist extraction by high salt concentrations (‘normal complexes’, [32]).

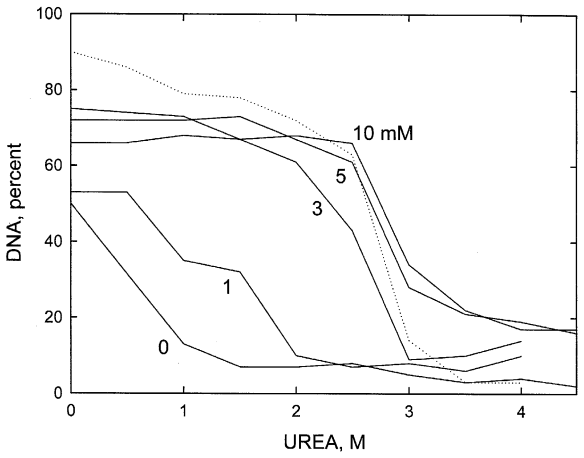

Fig. 4. Effects of added spermidine on the urea transition of 1 M NaCl-extracted spermidine nucleoids. One molar NaCl-extracted spermidine nucleoids were prepared as in Section 2. Spermidine was added to the dilatancy assay mixtures to the final concentrations indicated on the figure, and incubated for 60 min at 0 °C before urea addition. Dotted line is a spermidine nucleoid preparation which was extracted with a medium lacking the 1 M NaCl, but was otherwise treated as was the 1 M NaCl-extracted sample without further spermidine addition; this sample represents the behavior expected for full recovery of the transition lost due to salt extraction.

[33]. Exposure of cells to 40 μg/ml of rifampicin for 30 min caused a loss of $\sim\frac{1}{2}$ of the DNA into an aggregated form; such aggregates typically occur in partially or fully unfolded preparations of nucleoids [23]. The compact, non-aggregated fraction of the spermidine nucleoids from rifampicin-treated cells was much less stable to unfolding by urea than were such nucleoids from untreated cells (Table 1). This destabilizing effect of rifampicin was consistent with the involvement of an RNA-containing material with $U_m = 3.2$ M urea as part of the RNase-sensitive restraint to nucleoid unfolding, as suggested above.

3.3.2. Chloramphenicol and streptomycin

Both chloramphenicol and streptomycin inhibit protein synthesis [33]. Chloramphenicol inhibits peptide bond formation catalyzed in the 50S ribosomal subunit by peptidyl transferase. Streptomycin binds to the 30S ribosomal subunit causing inhibition of the initiation of polypeptide chains. Exposure of cells to either chloramphenicol [34–36] or streptomycin [37,38] has been shown to stabilize their polyribosomes.

Compact spermidine nucleoids were readily prepared from chloramphenicol- or streptomycin-treated cells. In both cases, the RNase-sensitive restraint of the nucleoids had become much more stable to urea solutions than were the nucleoids from untreated cells (Table 1 and [24]).[3] This correlation between the increased stability of the RNase-sensitive restraint in nucleoids from cells exposed to these two antibiotics with the increased stability of polyribosomes after exposure to the antibiotics cited above, suggested a role of ribosomes as the urea-sensitive component of the RNase-sensitive restraint.

3.4. Effects of formaldehyde-treatment on the urea transition

Formaldehyde-treatment prevents the dissociation of ribosomes into subunits [39–41]. If the unfolding of the nucleoids by urea ($U_m = 3.2$ M urea) was caused by dissociation of ribosomes, we would anticipate that formaldehyde would stabilize the nucleoids to unfolding in urea solutions. Indeed, stabilization by exposure to formaldehyde was readily demonstrable (Table 2). Significantly, formaldehyde treatment of *RNase-treated* nucleoids had a much reduced effect on the nucleoids (Table 2). These results suggested the

Table 1
Effects of in vivo exposure to antibiotics on the urea transitions of isolated nucleoids

Antibiotic added to cells	Urea concentration at mid-point of transition, M	
	Control	RNase-treated
None	3.2 ± 0.19[a]	1.8 ± 0.50[a]
Rifampicin, 40 μg/ml	0.7	0.2
Streptomycin, 50 μg/ml	5.2	$\geqslant 5.5$
Chloramphenicol, 100 μg/ml	>5.5	2.0

[a] Mean ± standard deviation; data from Ref. [24].

[3] The stabilizing effects of streptomycin on the spermidine nucleoids were not a result of carryover of the cationic streptomycin; direct addition of either 5 or 50 μg/ml of streptomycin to the dilatancy assays had no affect on the U_m of the nucleoids.

Table 2
Effects of formaldehyde on the RNase-sensitive transition

Nucleoid treatment	Urea concentration at midpoint of transition, M	
	Control	RNase-treated
None	3.0	0.8
Formaldehyde	5.4	1.2

Nucleoids were incubated at 0 °C for 30 min with RNase as in Section 2, where indicated. Aliquots of control or RNase-treated nucleoids were then treated with 0.45% formaldehyde for 30 min at 0 °C. Finally, all samples were treated with increasing concentrations of urea to determine their U_m values with the dilatancy assay.

presence of two different kinds of sites in the RNase-sensitive restraint, a urea-sensitive site ($U_m = 3.2$ M urea) that was protected by formaldehyde treatment, and a RNase-sensitive site that was not protected by formaldehyde treatment (cf. Fig. 3). The urea-sensitive site of the RNase-sensitive restraint is suggested to be the ribosomes engaged in polysomal units of cotranslational insertion linkages. This proposal requires that the integrity of the ribosomes not be destroyed by pancreatic RNase, for which there is considerable evidence; although the RNAs within ribosomes are degraded to limited extents by pancreatic RNase, the ribosomes do not unfold as a result [42–44]. Addition of formaldehyde *after* urea had little effect on U_m values (data not shown).

3.5. Effects of urea on ribosomes

The results of antibiotic treatments of cells and of formaldehyde treatment of nucleoid preparations supported a role of ribosomes in the RNase-sensitive restraint, and suggested that it was disruption of the ribosomes that initiated the urea-sensitive unfolding. Urea can promote the Mg^{++}-dependent dissociation of both prokaryotic and eukaryotic ribosomes into subunits [45,46]. The cited studies each contained only one or a few urea concentrations under very different conditions than used here, so that the cooperativity and midpoint of the transition were not defined. We tested the effects on ribosomes purified from *E. coli* C600 of a graded set of urea concentrations under the conditions used with the spermidine nucleoids. The effects were assayed on composite agarose–acrylamide gels [27,28]. A cooperative dissociation and unfolding of purified ribosomes occurred at 3.2 M urea, the characteristic concentration for loss of the RNase-sensitive restraint (Fig. 5, upper gel). Addition of pancreatic RNase before the urea solutions had little effect on the urea concentration at which the unfolding occurred (Fig. 5, lower gel). RNase was highly active in the presence of urea and degraded the RNA after unfolding (Fig. 5, lower gel). The unfolding of the 30S and 50S ribosomal subunits at a similar urea concentration suggested that the 70S particle was more stable to unfolding in urea solutions than was either of its free subunits.

Urea exposure of spermidine nucleoids gave patterns on composite gels similar to those shown in Fig. 5 with purified ribosomes. However, only a small fraction of the ribosomes present were in a form which entered the composite gels, with or without a DNase treatment (unpublished results of the authors).

4. Discussion

4.1. DNA–membrane associations and the RNase-sensitive restraint

There is a long history of association between the DNA of growing cells and their cell membranes, both in *E. coli* and in other bacteria [47–53]. The preparations used in those studies were made by various lysis procedures using lysozyme-treated cells, and contained a large part of the transcription and translation machinery of the cells, including mRNA, RNA polymerase, and polysomes, as well as residual cell envelope components [48–50]. Those studies defined in whole or in part what were subsequently conceptualized as cotranslational insertion linkages [18–21]. These early DNA-membrane preparations were similar to the ‘isolated nucleoid’ preparations being made at that time [22,29], one of which [22] is used here. The major difference was the inclusion in the nucleoid preparations of high salt concentrations [29] or spermidine [22] to maintain a partial degree of DNA compaction. There is no obvious reason why preexisting cotranslational insertion linkages

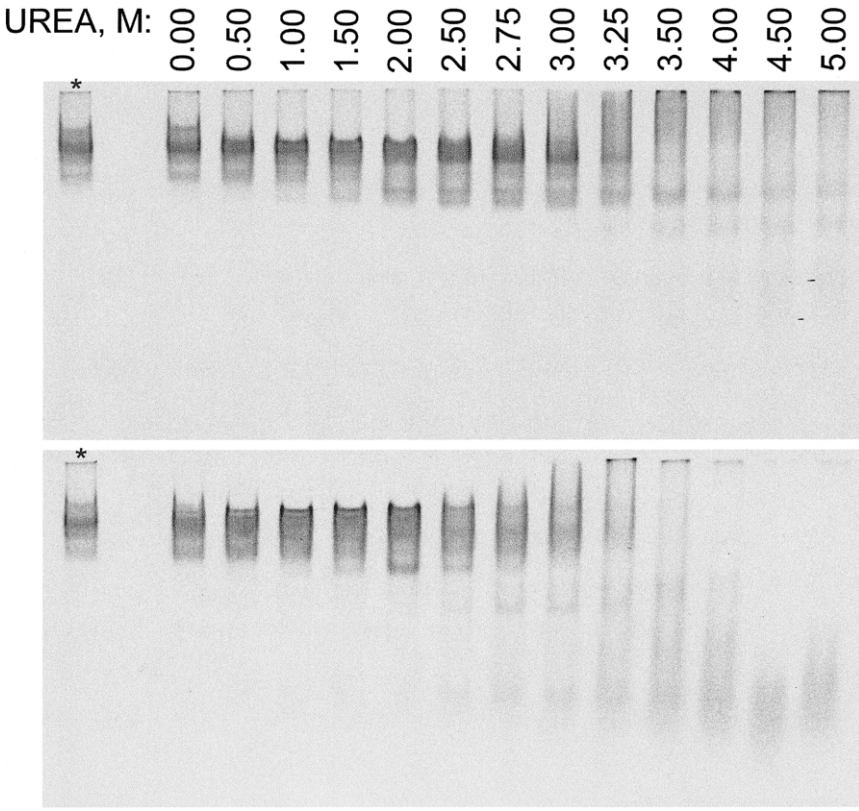

Fig. 5. Composite gel of purified ribosomes as a function of urea exposure. Upper gel, no RNase; lower gel, samples treated with RNase as in Section 2. Gel was stained with ethidium bromide; intensity is inverted. Lanes marked with asterisks contained a sample of 70S ribosomes.

in the cells would not be preserved in these high-salt or spermidine nucleoid preparations. We propose that these linkages do survive and are represented by the RNase-sensitive restraint to DNA unfolding.

The RNase-sensitive restraint is a robust and widespread phenomenon. It occurs in high-salt nucleoids (see below) as well as in spermidine nucleoids. All of the known properties of the RNase-sensitive restraint are consistent with its identification as cotranslational insertion linkages that had been formed in the cells from which the nucleoids were isolated; however, the involvement of all parts of those linkages has not been demonstrated. The ribosomes appear to be the urea-sensitive site of that restraint; the RNase-sensitive site is presumably the mRNA of those linkages.

The proposed role of cotranslational insertion linkages in maintaining partial DNA compaction in vitro reflects the same multiple connections between DNA and cell envelope that the linkages have been suggested to play in growing cells. The presence of cotranslational insertion linkages in spermidine nucleoids provides a rationalization for the association of partially compacted DNA with residual cell envelope fragments (Fig. 1).

Polyamines have been repeatedly suggested to be involved in the compaction of the genomic DNA of bacteria. Our observations of the effects of spermidine on the RNase-sensitive restraint of spermidine nucleoids provide an example of an effect of a polyamine on a specific restraint to unfolding; the mechanism of this effect of spermidine is unknown, but may be related to the

ability of spermidine to stabilize ribosomes to dissociation [41].

4.2. Comparison with high-salt nucleoids

Studies of nucleoids isolated in the presence of 1 M NaCl ('high-salt nucleoids'; [29], reviewed in Refs. [54] and [55]) indicated a role for RNA in maintaining nucleoid compaction, primarily based upon the unfolding of nucleoid DNA caused by a preexposure of cells to rifampicin or by treatment of isolated nucleoids with RNase. One or more species of nascent RNA was implicated in the stabilization. Cotranslational insertion linkages have apparently only been considered previously for high-salt nucleoids as a potential means of separating nucleoidal domains [54,55].

4.3. Application to nucleoid isolation

Finally, we note the application of these results to the central problem in the isolation of nucleoids, namely how to separate nucleoids fully from cell envelope and other non-nucleoid material without damaging the nucleoids. The arguments made here suggest that cotranslational insertion linkages will have to be broken or prevented from forming before such a goal can be achieved.

We are currently testing the effects of breaking these linkages during the course of isolation of compact nucleoids with characteristic shapes from growing or antibiotic-treated cells [30]. The fraction of completely released nucleoids from growing cells was relatively low, and there was a tendency to retain fragments of envelope in those nucleoids. Short exposures of the cells to puromycin, designed to break cotranslational insertion linkages by release of the nascent polypeptides from polyribosomes [33], have significantly increased the yield of released nucleoids and increased the regularity of the shapes of the nucleoids without causing nucleoid coalescence (unpublished results of the authors).

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References

[1] C.L. Woldringh, P.R. Jensen, H.V. Westerhoff, Structure and partitioning of bacterial DNA: determined by a balance of compaction and expansion forces?, FEMS Lett. 131 (1995) 235–242.

[2] S.B. Zimmerman, L.D. Murphy, Macromolecular crowding and the mandatory condensation of DNA in bacteria, FEBS Lett. 390 (1996) 245–248.

[3] L.S. Lerman, A transition to a compact form of DNA in polymer solutions, Proc. Natl. Acad. Sci. USA 68 (1971) 1886–1890.

[4] Y.M. Evdokimov, A.L. Platonov, A.S. Tikhonenko, Y.M. Varshavsky, A compact form of double-stranded DNA in solution, FEBS Lett. 23 (1972) 180–184.

[5] S.B. Zimmerman, S.O. Trach, Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli*, J. Mol. Biol. 222 (1991) 599–620.

[6] L.D. Murphy, S.B. Zimmerman, A limited loss of DNA compaction accompanying the release of cytoplasm from cells of *Escherichia coli*, J. Struct. Biol. 133 (2001) 75–86.

[7] L.D. Murphy, S.B. Zimmerman, Macromolecular crowding effects on the interaction of DNA with *Escherichia coli* DNA-binding proteins: a model for bacterial nucleoid stabilization, Biochim. Biophys. Acta 1219 (1994) 277–284.

[8] L.D. Murphy, S.B. Zimmerman, Condensation and cohesion of lambda DNA in cell extracts and other media: implications for the structure and function of DNA in prokaryotes, Biophys. Chem. 57 (1995) 71–92.

[9] T.A. Azam, A. Ishihama, Twelve species of the nucleoid-associated protein from *Escherichia coli*. Sequence recognition specificity and DNA binding affinity, J. Biol. Chem. 274 (1999) 33105–33113.

[10] S.M. McLeod, R.C. Johnson, Control of transcription by nucleoid proteins, Curr. Opin. Microbiol. 4 (2001) 152–159.

[11] I. Flink, D.E. Pettijohn, Polyamines stabilise DNA folds, Nature (London) 253 (1975) 62–63.

[12] L.C. Gosule, J.A. Schellman, Compact form of DNA induced by spermidine, Nature (London) 259 (1976) 333–335.

[13] L.C. Gosule, J.A. Schellman, DNA condensation with polyamines. I, spectroscopic studies, J. Mol. Biol. 121 (1978) 311–326.

[14] R.W. Wilson, V.A. Bloomfield, Counter-ion induced condensation of deoxyribonucleic acid. A light scattering study, Biochemistry 18 (1979) 2192–2196.

[15] J.A. Schellman, N. Parthasarathy, X-ray diffraction studies on cation-collapsed DNA, J. Mol. Biol. 175 (1984) 313–329.
[16] V.A. Bloomfield, DNA condensation, Curr. Opin. Struct. Biol. 6 (1996) 334–341.
[17] A.V. Vologodskii, N.R. Cozzarelli, Conformational and thermodynamic properties of supercoiled DNA, Annu. Rev. Biophys. Biomol. Struct. 23 (1994) 609–643.
[18] K. Kleppe, S. Överbö, I. Lossius, The bacterial nucleoid, J. Gen. Microb. 112 (1979) 1–13.
[19] G.H. Vos-Scheperkeuter, B. Witholt, Co-translational insertion of envelope proteins: theoretical considerations and implications, Ann. Inst. Pasteur Mic. 133A (1982) 129–138.
[20] A.S. Lynch, J.C. Wang, Anchoring of DNA to the bacterial cytoplasmic membrane through cotranscriptional synthesis of polypeptides encoding membrane proteins or proteins for export: a mechanism of plasmid hypernegative supercoiling in mutants deficient in DNA topoisomerase I, J. Bacteriol. 175 (1993) 1645–1655.
[21] V. Norris, Hypothesis: chromosome separation in *Escherichia coli* involves autocatalytic gene expression, transertion, and membrane-domain formation, Mol. Microbiol. 16 (1995) 1051–1057.
[22] T. Kornberg, A. Lockwood, A. Worcel, Replication of the *Escherichia coli* chromosome with a soluble enzyme system, Proc. Natl. Acad. Sci. USA 71 (1974) 3189–3193.
[23] L.D. Murphy, S.B. Zimmerman, Isolation and characterization of spermidine nucleoids from *Escherichia coli*, J. Struct. Biol. 119 (1997) 321–335.
[24] L.D. Murphy, S.B. Zimmerman, Multiple restraints to the unfolding of spermidine nucleoids from *Escherichia coli*, J. Struct. Biol. 132 (2000) 46–62.
[25] L.D. Murphy, S.B. Zimmerman, A dilatancy assay for nucleoid denaturation: the centrifugation-dependent clumping of denatured spermidine nucleoids, Anal. Biochem. 266 (1999) 16–22.
[26] S.B. Zimmerman, S.O. Trach, Effects of macromolecular crowding on the association of *E. coli* ribosomal particles, Nucleic Acids Res. 16 (1988) 6309–6326.
[27] A.E. Dahlberg, C.W. Dingman, A.C. Peacock, Electrophoretic characterization of bacteria polyribosomes in agarose–acrylamide composite gels, J. Mol. Biol. 41 (1969) 139–147.
[28] A.E. Dahlberg, P.J. Grabowski, Gel electrophoresis of ribonucleoproteinseds, in: D. Rickwood, B.D. Hames, Gel Electrophoresis of Nucleic Acids: A Practical Approach, second ed., IRL Press, Oxford, 1990, pp. 275–289.
[29] O.G. Stonington, D.E. Pettijohn, The folded genome of *Escherichia coli* isolated in a protein–DNA–RNA complex, Proc. Natl. Acad. Sci. USA 68 (1971) 6–9.
[30] S.B. Zimmerman, L.D. Murphy, Release of compact nucleoids with characteristic shapes from *Escherichia coli*, J. Bacteriol. 183 (2001) 5041–5049.
[31] R.L. Rubin, Spermidine–deoxyribonucleic acid interaction in vitro and in *Escherichia coli*, J. Bacteriol. 129 (1977) 916–925.
[32] K.M. Arndt, M.J. Chamberlin, RNA chain elongation by *Escherichia coli* RNA polymerase. Factors affecting the stability of elongating ternary complexes, J. Mol. Biol. 213 (1990) 79–108.
[33] E.F. Gale, E. Cundliffe, P.E. Reynolds, M.H. Richmond, M.J. Waring, The Molecular Basis of Antibiotic Action, second ed., Wiley, London, 1981.
[34] G. Mangiarotti, D. Schlessinger, Polyribosome metabolism in *Escherichia coli* I. Extraction of polyribosomes and ribosomal subunits from fragile, growing *Escherichia coli*, J. Mol. Biol. 20 (1966) 123–143.
[35] P.H. van Knippenberg, G.A.H. Duijts, Polyribosomes of *E. coli*: the distribution of free 70S particles and subunits in the presence of K^+ and Na^+ ions, FEBS Lett. 13 (1971) 243–246.
[36] C.P. Flessel, Chloramphenicol protects polyribosomes, Biochem. Biophys. Res. Commun. 32 (1968) 438–446.
[37] A. Herzog, An effect of streptomycin on the dissociation of *Escherichia coli* 70S ribosomes, Biochem. Biophys. Res. Commun. 15 (1964) 172–176.
[38] A.E. Dahlberg, E. Lund, N.O. Kjeldgaard, Some effects of antibiotics on bacterial polyribosomes as studied by gel electrophoresis, J. Mol. Biol. 78 (1973) 627–636.
[39] A.S. Spirin, M.Y. Sofronova, B. Sabo, Dissociation of 70S monoribosomes of *Escherichia coli* in the presence of a reduced medium magnesium-ion concentration, Molec. Biol. (Engl. Transl.) 4 (1970) 501–508.
[40] R. Amons, W. Möller, On the mode of reaction of formaldehyde with ribosomes, Biochim. Biophys. Acta 272 (1972) 95–107.
[41] R.S. Zitomer, J.G. Flaks, Magnesium dependence and equilibrium of the *Escherichia coli* ribosomal subunit association, J. Mol. Biol. 71 (1972) 263–279.
[42] A. Tissières, J.D. Watson, Ribonucleoprotein particles from *Escherichia coli*, Nature (London) 182 (1958) 778–780.
[43] M. Santer, J.R. Smith, Ribonuclease sensitivity of *Escherichia coli* ribosomes, J. Bacteriol. 92 (1966) 1099–1110.
[44] N. Delihas, Effect of ribonuclease on *Escherichia coli* ribosomes, Biochem. Biophys. Res. Commun. 39 (1970) 905–910.
[45] A.S. Spirin, E.B. Lishnevskaya, Effect of non-ionic agents on the stability of association of ribosomal subparticles, FEBS Lett. 14 (1971) 114–116.
[46] M.L. Petermann, A. Pavlovec, Dissociation of rat liver ribosomes to active subunits by urea, Biochemistry 10 (1971) 2770–2775.
[47] G.Y. Tremblay, M.J. Daniels, M. Schaechter, Isolation of a cell membrane–DNA–nascent RNA complex from bacteria, J. Mol. Biol. 40 (1969) 65–76.
[48] J. Rouvière, S. Lederberg, P. Granboulan, F. Gros, Structural sites of RNA synthesis in *Escherichia coli*, J. Mol. Biol. 46 (1969) (1969) 413–430.

[49] P.H. van Knippenberg, G.A.H. Duijts, M.S.T. Euwe, Polyribosomes of *Escherichia coli* I. Isolation of polysomes from a complex of DNA and membrane, Molec. Gen. Genet. 112 (1971) 197–207.

[50] P.H. van Knippenberg, G.A.H. Duijts, Polyribosomes of *Escherichia coli* II. In vivo and in vitro studies on DNA bound and cytoplasmic polysomes, Molec. Gen. Genet. 112 (1971) 208–220.

[51] P. Dworsky, Membrane attachment of folded chromosomes of *Escherichia coli*, Biochem. J. 154 (1976) 239–241.

[52] V. Weisshaüpl, P. Dworsky, Studies on the deoxyribonucleic acid-bearing portion of the cell envelope of *Escherichia coli*, Z. Allg. Mikrobiol. 18 (1978) 681–689.

[53] B. Porter, D.K. Fraser, Involvement of protein and ribonucleic acid in the association of deoxyribonucleic acid with other cell components of *Escherichia coli*, J. Bacteriol. 96 (1968) 98–104.

[54] D.E. Pettijohn, R.R. Sinden, in: N. Nanninga (Ed.), Structure of the Isolated Nucleoid, Molecular Cytology of *Escherichia coli*, Academic Press, London, 1985, pp. 199–227.

[55] D.E. Pettijohn, in: F.C. Neidhardt (Ed.), The Nucleoid, In *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, vol. I, second ed., ASM Press, Washington DC, 1996, pp. 158–166.