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Condensation and cohesion of λ DNA in cell extracts and other media: Implications for the structure and function of DNA in prokaryotes

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Abstract

DNA added to concentrated extracts of *Escherichia coli* undergoes a reversible transition to a readily-sedimentable ('condensed') form. The transition occurs over a relatively small increment in extract concentration.

The extract appears to play two roles in this transition, supplying both DNA-binding protein(s) and a crowded environment that increases protein binding and favors compact DNA conformations. The two roles of the extract are suggested by properties of fractions prepared by absorption of extracts with DNA-cellulose. The DNA-binding fraction and the DNA-nonbinding fractions from these columns are separately poorer condensing agents than the original extract, but when rejoined are similar to the original extract in the amount required for condensation. The dual role for the extract is supported by model studies of condensation with combinations of purified DNA-binding materials (protein HU or spermidine) and concentrated solutions of crowding agents (albumin or polyethylene glycol 8000); in each case, crowding agents and DNA-binding materials jointly reduce the amounts of each other required for condensation. The condensation reaction as studied in extracts or in the purified systems may be a useful approach to the forces which stabilize the compact form of DNA within the bacterial nucleoid.

The effect of condensation on the reactivity of the DNA was measured by changes in the rate of cohesion between duplex DNA molecules bearing the complementary single-strand termini of λ DNA. Condensation caused large increases in the rates of cohesion of both λ DNA and of restriction fragments of λ DNA bearing the cohesive termini. Cohesion products of λ DNA made in vitro are a mixture of linear and circular aggregates, whereas those made in vivo are cyclic monomers. We suggest a simple mechanism based upon condensation at the site of viral injection which may explain this discrepancy.

Keywords: Nucleoid; *Escherichia coli*; Macromolecular crowding; Protein HU; DNA-binding proteins; Spermidine

1. Introduction

Bacterial cytoplasm is a poor solvent for DNA. Far from being uniformly distributed within the nominally available volume, the bulk of the cell's DNA occurs as one or a few highly compacted, morphologically-distinct bodies (nucleoids), immersed in a cytoplasmic background [1-4]. Unlike its eukaryotic counterpart, DNA-packaging in nucleoids seems to be dependent upon the integrity of the cell. Subnucleoid particles containing bacterial DNA rapidly

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disappear upon cell lysis [5]; in vitro binding of bacterial 'histone-like proteins' to the DNA is weak and greatly reduced at even low salt concentrations [6]. Such studies suggest that the surrounding cytoplasm may play an active role in compaction of the genomic DNA in bacteria. It was therefore of great interest to us to find that DNA added to concentrated extracts of *Escherichia coli* spontaneously underwent a form of condensation [1] accompanied by strongly correlated increases of > 30-fold in the reactivity of the DNA as measured by its rate of cohesion. We have analyzed this system as a model for certain aspects of the condensation of bacterial DNA in vivo.

A number of factors probably contribute to the cellular compaction of DNA in bacteria. Binding to DNA of cellular components, particularly polyamines [10] and the 'histone-like' proteins [11] can favor compaction by decreasing repulsive electrostatic interactions between DNA segments, and may also bend or crosslink the DNA. Relatively large amounts of two 'histone-like' DNA-binding proteins, HU and H-NS, occur in *E. coli*; both of these proteins can cause folding or compaction of DNA in vitro [12–14], but only at ratios to added DNA that are much higher than the ratio occurring intracellularly [15]. The polyamines spermidine and putrescine can also occur at high intracellular concentrations in *E. coli*, depending on growth conditions [10]. In vitro, binding of spermidine but not putrescine has been shown to condense DNA from aqueous media [16,17].

Macromolecular crowding (excluded volume) effects can also compact DNA [18,19]; crowding effects are reviewed in Refs. [20–23]. Significant crowding effects are expected in bacterial cytoplasm [24,25] because of the enormous concentrations of macromolecules (e.g., ~ 340 mg/ml of total RNA and protein [25]). Two mechanisms of crowding-enhancement of DNA compaction have been suggested. Excluded volume interactions with the macromolecular background in crowded media favor more compact DNA conformations, which we will refer to as a direct effect of crowding. This type of crowding effect on the cellular conformation of DNA was proposed earlier [18,19,26], based upon formation of compacted DNA ('psi' DNA) in the presence of high concentrations of polymers and suitable concentrations of monovalent or polyvalent cations [18,19,27,28]. We have recently suggested a second, indirect mechanism by which crowding can enhance compaction, namely by increasing the binding of certain proteins to the DNA. The binding of protein HU to DNA serves as an example [15]: large quantities of this prokaryotic 'histone-like' protein can condense DNA in the absence of crowding. However, increasing the volume occupancy of this system with polyethylene glycol (PEG) allows condensation by decreased levels of HU that are compatible with the cellular abundance of HU. Finally, we note supercoiling as an additional means of compaction of DNA [29].

Hence, there are a number of factors which can each compact DNA in vitro. We have examined interactions between certain of these factors by assaying condensation of DNA added to concentrated bacterial extracts or to more purified systems. A sedimentation assay for condensation is used that is based on the aggregation of added DNA; the relevance of DNA aggregation to DNA compaction is considered in the Discussion.

In addition, we have assayed the effects of condensation on the reactivity of the DNA by measuring changes in the rate of cohesion between double-stranded DNA molecules bearing the complementary terminal sequences of λ DNA. The rate of this reaction is known to be increased by crowding by purified macromolecules [30,31]; at elevated temperatures the rate of cohesion of λ DNA with crowding by PEG is comparable to that in vivo [31]. We show here that concentrated cell extracts at a physiological temperature also greatly increase the rate of cohesion. The products of the in vitro systems are mixtures of linear aggregates and circular monomers rather than the circular monomers recovered in vivo. Condensation at the site of viral injection provides a simple explanation for this discrepancy (see Discussion).

[1] We use the term condensation in the generic sense of Post and Zimm [7] to include both the intermolecular association or aggregation of DNA molecules as well as the individual collapse of DNA molecules (cf. [8]). Aggregation will be used to include both the association between molecules to make particles as well as further associations between particles [9]. See Discussion.

2. Materials and methods

Materials and methods are as before [15,25] where not specified.

2.1. Materials

λ DNA (N^6-methyl-adenine-free, New England BioLabs) was used directly or was digested to a limit with NruI restriction nuclease (NE BioLabs). The NruI digest was treated with phenol-$CHCl_3$, ethanol-precipitated and washed, and redissolved in 10 mM Tris–HCl buffer (pH 8.0)–1 mM EDTA. The concentration of λ DNA and of NruI nuclease-digested λ DNA was based upon an absorbance at 260 mμ = 20 for 1 mg/ml.

Dialysis tubing (dialysis membrane, Union Carbide) was boiled 20 min in 0.6 M Na_2CO_3 and then washed thoroughly in 0.1 mM EDTA. Crystallized bovine plasma albumin was from ICN Immunobiologicals, spermidine and T7 DNA from Sigma, λ DNA concatemers (Promega-Markers) from Promega Corp., acrylamide/bis-acrylamide mixture from International Biotechnologies Inc.

2.2. Methods

Extract preparation and reconcentration procedures to minimize interfering reactions

This section summarizes extensive trials of the manipulation of cell extracts to yield fractions that have macromolecular compositions relatively similar to those of bacterial cells, and that allow the assay of certain reactions of DNA.

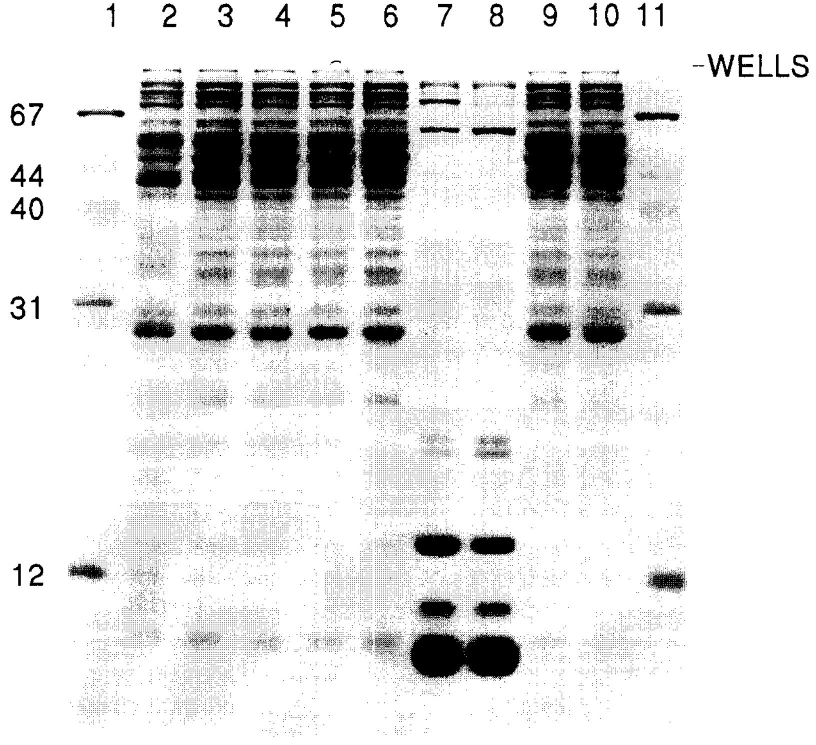

Fig. 1. SDS-acrylamide gel electrophoresis of proteins of cell fractions from *Escherichia coli*. Molecular weight standards in lanes 1 and 11: cytochrome *c* (12.4k), carbonic anhydrase (31k), aldolase (40k), ovalbumin (43.5k), bovine plasma albumin (67k). Samples (13 μg protein in lane 2, 10 μg protein in lanes 3–10); lane 2, whole stationary phase cells of *E. coli*; lanes 3 and 4, high-speed extract of stationary phase cells, before and after the reconcentration procedure described in Methods; lanes 5 and 6, DNA-nonbinding fraction (from high-speed extract used for lanes 3 and 4), before and after reconcentration; lanes 7 and 8, DNA-binding fraction (from high-speed extract used for lanes 3 and 4), before and after reconcentration; lanes 9 and 10, pool of DNA-binding and DNA-nonbinding fractions, before and after reconcentration.

Low- and high-speed extracts of whole cells of logarithmic or stationary phase *E. coli* C600 were made as before [25]. These extracts rapidly degrade added DNA, due partially to the DNase I (1 μg/ml) added in the extraction buffer, but largely to endogenous DNase activities. Additions of high concentrations of EDTA (14 or 28 mM) prevented DNase I activity and reduced endogenous activities to a level allowing partial recovery of added DNA and the detection of the cohesion reaction. These conditions were unsatisfactory for the current studies for several reasons: (a) The presence of residual nuclease activity. (b) The formation of significant amounts of cohered DNA product which was not dissociated by heating at 65°C; this non-dissociable product was not formed if the terminal 5'-phosphate groups of the DNA were removed with phosphatase [32], implying covalent joining by DNA ligase. (c) Dialysis of extracts against a variety of media caused the appearance of overwhelming levels of DNA-degrading activity. (d) The concentration of macromolecules in the extracts was below the level expected to yield significant crowding effects.

These problems prompted development of the following procedures that greatly reduce interference by nuclease(s), prevent the covalent joining reaction, allow extensive dialysis without concomitant activation of DNase(s), and reconcentrate the extracts under mild conditions to > 200 mg/ml of protein without apparent changes in protein composition. EDTA (0.2 volume of 0.2 M solution) was added to high-speed extracts, which were then dialyzed for several days at 5°C against two changes of 50 volumes of a medium (TNE) composed of 50 mM Tris–HCl buffer, pH 7.8–100 mM NaCl–1 mM EDTA, followed by a final dialysis into 0.3-strength TNE. Nuclease activity in dialyzed preparations was greatly reduced, even when assayed in the presence of excess Mg^{2+} (TNE + 5 mM $MgCl_2$). The TNE-dialyzed extracts were reconcentrated by dialysis for 4–8 h versus dry Sephadex G-200 (Superfine, Pharmacia) at room temperature in closed containers; the extracts were slowly rocked during the concentration procedure and were transferred once to smaller casing as the volume decreased. Salts and water pass through the dialysis membrane at similar rates based upon conductivity measurements. Redialysis against TNE after the concentration procedure did not appreciably change the condensation and cohesion-stimulating effects. The samples were finally centrifuged (15 min at $12\,000 \times g$ at 20°C) to remove small amounts of insoluble protein, yielding a yellow, rather viscous solution of reconcentrated high-speed extract containing 200 to 300 mg/ml of protein, corresponding to 40 to 60% of the initial protein of the high-speed extract. The initial high-speed extract and the final reconcentrated extract are indistinguishable (a) with respect to protein components visualized by Coomassie Blue staining after SDS-acrylamide gel electrophoresis (Fig. 1, lane 3 vs. 4), and (b) by Sephacryl S-300 gel filtration [25] (unpublished results of the authors). Where not specified, the extract used is a reconcentrated high-speed extract prepared from stationary phase whole cells by the above procedure.

Application of this procedure to ribosome-containing, low-speed extracts destabilizes the ribosomes, allowing degradation of ribosomal RNA by endogenous RNase(s). Application to extracts of spheroplasts (made as [25]) resulted in a precipitate during reconcentration that was at least partially composed of aggregates of the egg white lysozyme added in the preparation of the spheroplasts; after removal of the precipitate by low speed centrifugation and reconcentration as above, spheroplast extracts were similar to whole cell extracts in their condensation of DNA and their stimulation of cohesion. Preparations from logarithmic phase cells or spheroplasts were also similar to stationary phase material, but their higher nuclease levels allow only qualitative comparisons.

DNA-cellulose chromatography

DNA-binding fractions were prepared from dialyzed extracts by absorption to and elution from DNA-cellulose [15] except that the DNA-cellulose was prepared according to Litman [33] using CF 11 cellulose (Whatman; acid-washed as [33]) and *E. coli* DNA, and the column was alternately washed with the low and high salt elutrients several times immediately before use.

Aliquots were taken from the dialyzed extract used for the column, from the fraction not retained by column, and from the fraction eluted with 2 M NaCl solution; in addition, half of the non-retained fraction and half of the fraction eluted at 2 M NaCl

were pooled. Each of these four samples were extensively dialyzed at 5°C versus 15 mM Tris-HCl, pH 7.8-30 mM NaCl-0.3 mM EDTA, concentrated by dialysis against dry Sephadex G-200 as above, and insoluble material ($\leqslant$ 10% of protein) removed by centrifugation to yield the final reconcentrated fractions. Overall recoveries after dialysis, concentration and centrifugation were 40-60% of the protein of the corresponding column fraction. Most of the losses occurred in the handling of small volumes of highly concentrated fractions. The patterns of the final reconcentrated fractions on SDS-acrylamide gels were similar to those of samples before dialysis and reconcentration (Fig. 1). Conductivity of the final samples corresponds to 0.7-1.1 times that of TNE.

Cohesion and condensation assays

Condensation and cohesion of DNA are assayed in the same reaction mixture (7-14 μl, in 0.65 ml polypropylene tubes). Where not specified, a solution of 0.55 mg/ml of NruI-digested λ DNA in 10 mM Tris-HCl, pH 8.0-1 mM EDTA was heated 10 min at 65°C to dissociate cohered termini, quenched at 0°C, and aliquots diluted 7-fold into otherwise complete reaction mixtures in TNE containing additions that had been either dialyzed into or dissolved in TNE. Where specified, T7 or λ DNA was used in place of the λ DNA digest. After incubation (50 min at 20°C where not specified), reaction mixtures were remixed and an aliquot was removed. A second aliquot was removed after centrifugation at 20°C for 5 min at 12000 $\times g$; centrifugation for 10 min at 19000 $\times g$ gave identical results. The aliquots taken before and after centrifugation were diluted in 1.66% SDS-8.5% glycerol-0.023% bromphenol blue-6.6 mM EDTA (mix L), and 6 μl samples (21 ng DNA) were analyzed by SDS-agarose gel electrophoresis followed by ethidium bromide staining and densitometry [15].

Condensation of DNA is expressed as the fraction of the DNA that is lost by sedimentation based upon comparison of aliquots taken before and after centrifugation; areas of peaks (or sum of areas for 3.7, 4.6, 6.7, 9.4, 11.3, and 23.5 kbp fragments for the NruI digest of λ DNA) from densitometer scans of ethidium-bromide stained gels were used for this purpose. There was generally quantitative recovery of added DNA in the aliquot taken before centrifugation. In a few reaction media, especially at high spermidine concentrations, recovery was diminished, apparently by sticking to surfaces; there was invariably total condensation in these samples.

The extent of cohesion in NruI nuclease-digests of λ DNA is based upon the ratio of the observed amount of 11.3 kbp cohesion product to the amount of that product at full cohesion, determined for each reaction mixture using Eq. 1 of Ref. [34]. The extent of cohesion of NruI digest fragments was > 98% after annealing as Ref. [30], and 90-100% after 50 min incubation at 30°C, 37°C, or 45°C in the presence of 17% PEG 8000.

Rate constants for cohesion of 4.6 and 6.7 kbp fragments in the NruI nuclease digest of λ DNA were calculated with an equation derived by Wang and Davidson [35] for the analogous reaction involving left and right halves of λ DNA under conditions where the reverse reaction is not important:

$$\frac{1}{1-f} = \frac{PN_{\text{Avogadro}}k_f}{1000 \cdot n}t + \frac{1}{1-f^0} \qquad (1)$$

where f and f^0 = fractions of cohesion at times t and 0 (using observed amount of cohesion corrected for the maximal obtainable cohesion), P = total moles of nucleotide residues/liter of the combined 4.6, 6.7, and 11.3 kbp fragments, $n = 2(11284) = 22568$, and t = time in seconds. The rate constant, k_f was obtained by dividing the slope of a plot of $(1/1-f)$ vs. time by $PN_{\text{Avogadro}}/1000n = 1.05 \times 10^{12}$ molecules/cm^3 (corresponding to a maximal extent of cohesion = 0.71).

Two-dimensional gel electrophoresis of λ DNA cohesion products

A two-dimensional gel protocol was used to characterize the products of λ DNA cohesion, with field inversion gel electrophoresis (FIGE) in the first dimension to partially resolve various circular and linear species, followed by conventional agarose gel electrophoresis at a relatively high voltage gradient that moves linear aggregates of λ DNA at similar speeds but prevents migration of circular λ DNA molecules. This combination of gel protocols is similar to that of Fig. 4 of Louie and Serwer [31] but uses FIGE instead of pulsed field gel electrophoresis.

Incubation mixtures (28 μl, in TNE) containing 6

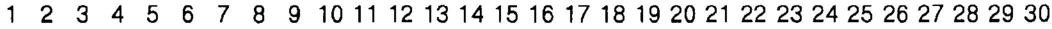

Fig. 2. Agarose gel electrophoresis patterns of cohesion and condensation of NruI nuclease restriction fragments of λ DNA as a function of the concentration of stationary phase extract of *E. coli*. NruI nuclease digestion mixtures of λ DNA were added to reaction mixtures 1–10 containing 0, 35, 70, 90, 110, 130, 150, 170, 190 or 210 mg/ml of extract protein. Lanes 1–10, 11–20, and 21–30 are aliquots of the whole reaction mixtures prior to centrifugation, of the redissolved pellets, and of the supernatant fractions, respectively. Sizes of DNA fragments in kbp are indicated in the margin. The 11.3 kbp fragment is the product of cohesion of the 6.7 and 4.6 kbp fragments.

μg/ml of λ DNA (preheated 10 min at 70°C and quenched in ice water) and additions (either dissolved in or dialyzed into TNE) were incubated at 20°C for 50 min or as specified. After addition of 10.5 μl of 6.9% SDS–6.1% glycerol–0.016% bromphenol blue–4.7 mM EDTA (mix M), 14.5 μl were added to alternate wells of an SDS-agarose gel (15 cm × 15 cm tray, 105 ml of 1% SeaKem GTG agarose (FMC Bioproducts) in gel buffer of 0.1% SDS–45 mM Tris–45 mM boric acid–1.25 mM EDTA; 1.5 mm thick wells). Electrophoresis for both dimensions was at room temperature without buffer recirculation in a horizontal apparatus (Hoefer model HE99). The first dimension was controlled by a Hoefer Switchback Pulse Controller model PC500. The parameters were based on optimization of earlier procedures [36–39] for the current samples: after 10 min at 89 volts, a ramped pulse was applied for 14 h (forward pulse time 1.8 to 11 s with ratio of forward pulse time to reverse pulse time of 3); final buffer temperature was ⩽ 26°C. After the first electrophoresis, the gel was trimmed, rotated 90 degrees in the gel holder, and returned to the gel apparatus for a second electrophoresis of 35 min at a constant voltage (107 volts); final buffer temperature ⩽ 26°C. The gel was washed with rocking for 0.5 h in 1 l of water, stained in 0.5 l of 0.2 μg/ml of ethidium bromide for 1 h, destained with two changes of 1 l of water for 2.5 h and photographed through a red filter on Polaroid 665 film using an ultraviolet light source.

The linear products of cohesion of λ DNA are readily destroyed by the mechanical forces involved in conventional analyses [31,40] [2]. Mixtures containing λ DNA were therefore applied to gels without any mixing after the incubation except for that involved in withdrawing aliquots of the reaction mixtures with a wide-bore pipetting device (Microman, Rainin Instrument Co.), gently mixing a dilution into mix M with the pipette tip, and pipetting with a wide-bore device into the well of the gel; these procedures gave high recoveries of circular monomer,

[2] In contrast, the 11.3 kbp cohesion product in the standard assay with the NruI digest of λ DNA (see Methods) is not significantly dissociated by vortexing, tapping, or pipetting.

linear monomer, and linear dimer of mixtures prepared by incubating λ DNA at 0.5 to 200 μg/ml as described by Serwer and Allen [41].

Miscellaneous

Protein concentration was generally determined by the method of Lowry et al. [42] on samples after precipitation in cold 5% trichloroacetic acid, using bovine plasma albumin as a standard. HU concentration was determined on non-precipitated aliquots by the method of Sedmak and Grossberg [43] using the same albumin standard.

Samples for SDS-acrylamide gel electrophoresis were acid-precipitated [25]. Gel samples made without precipitation gave very similar gel patterns to those shown here except for distortions in some cases due to salt content.

3. Results

3.1. Condensation and cohesion of λ DNA restriction fragments in concentrated cell extracts

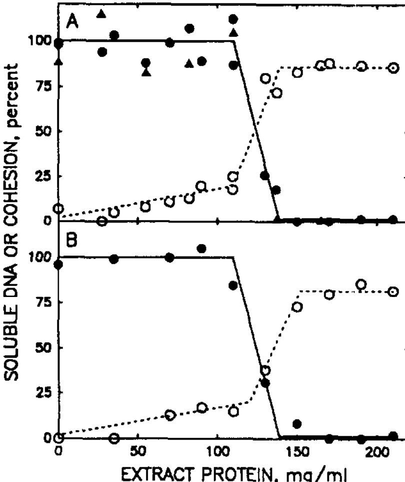

Fig. 3. Condensation and cohesion of NruI nuclease digests of λ DNA by extracts of stationary phase *E. coli*. (A) Filled circles, condensation assays as in Methods; filled triangles, condensation of reaction mixtures (initially containing 165 mg/ml of extract protein, preincubated 50 min at 20°C) that were diluted with TNE to the indicated protein concentrations and further incubated for 10 min at 20°C; open circles, cohesion assays as in Methods. (B) 1/3 DNA concentration. Symbols as (A).

Restriction fragments of λ DNA added to concentrated extracts of *E. coli* undergo two striking changes: the DNA becomes readily sedimentable and, coordinately, the rate of noncovalent cohesion between the two fragments with complementary 12-base terminal sequences from the λ DNA increases > 30-fold.

An example of the condensation and the enhanced cohesion rates is shown in Fig. 2. DNA was added to reaction mixtures containing increasing amounts of cell extract. After incubation, aliquots of the reaction mixtures were removed and diluted for lanes 1–10 of the gel of Fig. 2. The remaining portions of the reaction mixtures were then centrifuged 5 min at $12\,000 \times g$, and aliquots of the redissolved pellets and of the supernatant fluids were added to lanes 11–20 and 21–30, respectively, of this gel. Both condensation and an enhanced rate of cohesion appear at extract concentrations ⩾ 130 mg/ml protein. Condensation is indicated by the loss of DNA from the supernatant fluids (lanes 26–30) and by the appearance of DNA in the corresponding redissolved pellet fractions (lanes 16–20). Very low centrifugal forces are sufficient to sediment DNA at the higher extract concentrations: DNA added to mixtures containing 150 mg/ml extract protein is completely sedimented by 5 min at $\geq 800 \times g$, but not by centrifugation for 5 min at $80 \times g$. Increased rates of DNA cohesion are shown by the appearance of large amounts of the 11.3 kbp cohesion product at extract concentrations of ⩾ 130 mg/ml protein, in both the total reaction mixtures (lanes 6–10) and the redissolved pellets (lanes 16–20). Measurements of condensation and cohesion obtained from the gel of Fig. 2 and from other similar assays are shown in Fig. 3A by the filled and open circles, respectively. The concentration of extract causing half-maximal condensation and cohesion effects has been between 100 and 150 mg/ml of extract protein in over 20 preparations, including extracts of both spheroplasts and whole cells. The condensation assay is not well suited for kinetic measurements, requiring ca. 10 min for centrifugation and sampling; incubations of 5–50 min at 20°C before centrifugation gave similar extents of condensation at several extract concentrations (data not shown).

Condensation is not peculiar to the restriction

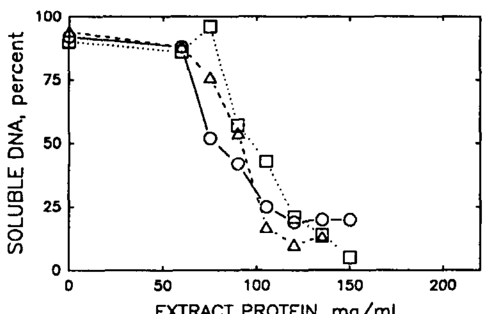

Fig. 4. Condensation of various DNA preparations by extracts of *E. coli*. Circles, T7 DNA; triangles, lambda DNA; squares, NruI-digest of lambda DNA. Incubation period 5 min.

nuclease fragments of λ DNA used in Figs. 2 and 3. Both T7 DNA and λ DNA itself had a similar extract-dependence for condensation (Fig. 4). The viral DNAs (T7, 40 kbp; λ DNA, 48.5 kbp) reproducibly condense at a slightly lower concentration of a given extract than do the restriction digest fragments of λ DNA (Fig. 4), and the condensation of the larger DNA fragments of the digest (6.7, 9.4, 11.3 and 23 kbp) occurs at somewhat lower extract concentrations than those required for the smaller fragments (3.7 and 4.6 kbp) (data not shown) indicating a small dependence on DNA size. A decrease in DNA concentration of 3-fold had slight effects on the extract-dependence of condensation and cohesion (Fig. 3B; see below also).

An increase of 20–30% in extract concentration converts the added DNA from a soluble form to a form that is totally sedimented by low centrifugal forces (Figs. 3 and 4). To help distinguish between binding and crowding effects of the extract on this conversion, condensation by a constant total amount of extract was assayed in varying reaction volumes (Table 1). A decrease in extract concentration from 125 to 100 mg/ml of extract protein completely prevented condensation over protracted incubations although the total amount of extract was unchanged. This result suggests that the transition of added DNA to a readily-sedimented form in concentrated extracts is not solely dependent on a strong noncooperative binding interaction of the DNA with extract component(s), since such a non-crowding interaction should be little changed by small variations in extract concentration.

Both condensation and cohesion can be reversed under appropriate conditions. Condensation is reversed simply by diluting reaction mixtures to extract concentrations slightly below those causing condensation (closed triangles, Fig. 3A). Cohesion is not significantly changed by such dilution, but the 11.3 kbp cohered product is fully dissociated by heating 5 min at 65°C (after dilution in Mix L), indicating that the cohesion product is not enzymatically joined (see Methods).

Condensation is inhibited by relatively low salt concentrations. Addition of 0.1 M or 0.2 M NaCl to assays in TNE (0.1 M NaCl–50 mM Tris buffer, pH 7.8–1 mM EDTA) raised the extract concentrations required for condensation and rapid cohesion by 30% or > 170%, respectively. Addition of an excess of Mg^{2+} (TNE + 5 mM $MgCl_2$) allowed significant nuclease degradation of the DNA substrate at the usual assay temperature; incubation at 0°C decreased the nuclease activity and gave condensation similar to that at 20°C in the absence of Mg^{2+}.

The magnitude of stimulation of the cohesion reaction in concentrated extracts in TNE was determined from plots of cohesion kinetics according to a

Table 1
Effect of extract concentration on DNA condensation at constant total amounts of extract in varying reaction volumes

Extract protein		Volume	DNA in supernatant fluid (percent)		
(mg/ml)	(mg)	(μl)	0 min	15 min	50 min
75	3.15	42.0	100	100	100
100	3.15	31.5	100	103	97
125	3.15	25.2	29	27	8
150	3.15	21.0	44	21	6

A constant amount of high speed extract in the indicated total volumes was incubated for the indicated times at 20°C in TNE with the standard concentration of NruI nuclease restriction digest of λ DNA and analyzed for DNA condensation as in Methods.

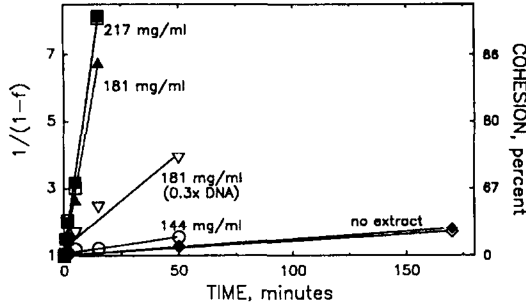

Fig. 5. Effect of concentration of extract of stationary phase *E. coli* on the kinetics of cohesion of fragments of λ DNA. DNA substrate is a NruI nuclease digest of λ DNA (79 μg/ml), as in Methods. Concentrations are extract protein in reaction mixtures. Where indicated, 0.32-fold lower DNA concentration was used. See Methods for details.

rate law derived by Wang and Davidson [35] for the analogous cohesion reaction of half-molecules of λ DNA (Fig. 5). Rate constants calculated from these plots indicate extract-dependent increases in cohesion rate of 30- to 80-fold (Table 2); a 3-fold decrease in DNA concentration caused a decrease in cohesion rate that was consistent with the rate law. Addition of an excess of Mg^{2+} to assays in TNE at the usual assay temperature of 20°C also yielded > 30-fold stimulations in cohesion rate in concentrated extracts, but was accompanied by partial degradation of the DNA substrate (data not shown).

3.2. Comparison of the effects of the DNA-binding and DNA-nonbinding fractions from extracts

Extracts were fractionated by absorption to DNA-cellulose to help distinguish between binding and crowding contributions to DNA condensation from the extracts. TNE-dialyzed extracts were applied to DNA-cellulose columns equilibrated with TNE. Most extract protein is not bound to the column and is recovered as a 'DNA-nonbinding fraction'; the protein composition of this fraction determined by SDS/acrylamide electrophoresis (lane 5 of Fig. 1) is similar to that of the original cells (lane 2) or extract (lane 3). About 0.7% (0.51, 0.81, and 0.86% in three preparations) of the extract protein is bound to the DNA-cellulose in TNE and subsequently eluted by 2 M NaCl–50 mM Tris–HCl, pH 7.8–1 mM EDTA as a 'DNA-binding fraction'. This DNA-binding fraction (lane 7) is highly concentrated in two proteins, HU and H-NS [15]; these proteins are not obvious in the extract patterns of lane 3 because they are each < 1% of the total extract protein.

The original extract and the two fractions obtained from the DNA-cellulose column were individually dialyzed and reconcentrated. In addition, equal aliquots of the DNA-binding and DNA-nonbinding fractions were rejoined to serve as a control for the loss of active component(s) in the chromatography and reconcentration steps; this rejoined material was also dialyzed and reconcentrated. SDS-acrylamide gel electrophoresis indicated very similar compositions before and after reconcentration of each of these four samples (Fig. 1, lane 3 vs. 4, 5 vs. 6, 7 vs. 8, 9 vs. 10).

The DNA-nonbinding fraction caused little or no condensation over the concentration range that could be tested (Fig. 6, closed symbols). The first and second halves of the DNA-nonbinding fraction to flow through the DNA-cellulose column were similar in this respect (closed triangles and closed inverse triangles, respectively, in Fig. 6). Condensation by the rejoined DNA-binding and DNA-nonbinding fractions (open triangles) was indistinguishable from that of the original extract (open circles), indicating that fractionation on DNA-cellulose and the reconcentration procedures did not inactivate major factor(s) involved in condensation.

Not only did the DNA-nonbinding fraction have

Table 2
Effect of concentrated extracts on rate constants for cohesion in NruI nuclease-λ DNA digests

Extract (mg/ml of protein)	Slope (min^{-1})	$k_f \times 10^{15}$ (cm^3 $molec^{-1}$ sec^{-1}) [a]
0	0.006	0.10
145	0.010	0.15
181	0.056 [b]	2.8 [b]
181	0.176	2.8
181	0.382	6.0
217	0.463	7.2
217	0.465	7.3

[a] A value of $f_{max} = 0.71$ is assumed; see Methods.
[b] Determined at 0.32-fold lower DNA concentration.

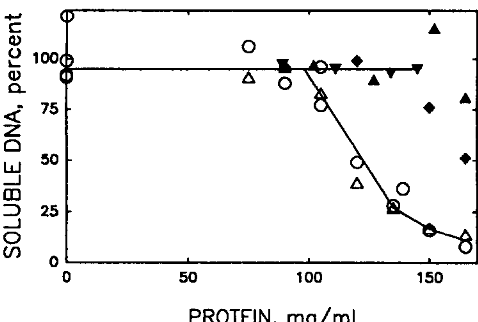

Fig. 6. Comparison of condensation of NruI nuclease digests of λ DNA by the fraction of cell extract not bound to DNA-cellulose with condensation by the original extract or by pooled DNA-cellulose fractions. Closed symbols, DNA-nonbinding fraction (closed triangles and inverse triangles refer to first and second halves of DNA-nonbinding fraction, respectively; closed diamonds is a separate preparation collected as a single fraction); open circles, original extract; open triangles, pooled DNA-nonbinding and -binding fractions. Incubation period 5 min.

diminished capacity to cause condensation, but, in addition, dilution with high concentrations of this fraction (145 mg/ml protein) allowed reversal of condensation (Fig. 7, squares), similar to dilution in the solvent, TNE (triangles); in contrast, dilution into extract (at 145 mg/ml protein) prevented reversal of condensation (circles).

The DNA-binding fraction, also, was relatively inactive in causing condensation of added DNA. Concentrations up to 3.3 mg/ml of DNA-binding fraction protein did not cause significant DNA condensation in the standard condensation assay. This concentration is nominally equivalent to an extract concentration of about $(3.3/0.007) = 471$ mg/ml protein. Hence, the DNA-binding protein fraction when assayed by itself was inactive in condensing DNA at protein concentrations equivalent to more than three times the extract concentration causing full DNA condensation.

3.3. Condensation and cohesion by mixtures containing purified components

The preceding results suggested two roles for cellular components in promoting condensation, namely as a source of DNA-binding materials (DNA-binding fraction) and as a source of high volume occupancy (DNA-nonbinding fraction). Both functions appeared to be necessary for efficient condensation. If this interpretation is correct, it should be possible to reproduce certain aspects of the extract-induced condensation with appropriate mixtures of purified DNA-binding materials with purified macromolecules that can contribute a high volume occupancy. We tested two purified DNA-binding materials, HU, a major DNA-binding protein of *E. coli* [11,44] and spermidine, a major polyamine of *E. coli* [10]. Two macromolecules that have often been used in crowding studies were used as sources of high volume occupancy: the random coil polymer, PEG 8000, and the globular protein, bovine plasma albumin. Certain combinations of these materials and of the DNA-cellulose fractions were assayed.

DNA added to very concentrated solutions of plasma albumin (≤ 280 mg/ml protein) in the absence of DNA-binding materials remains non-sedimentable (filled circles in Fig. 8A); the cohesion rate of the DNA undergoes only a small, gradual increase. Addition of DNA-binding materials greatly changes both properties: DNA-binding fraction, HU, or spermidine (Fig. 8B, C, or D, respectively) all allow condensation at concentrations of albumin of ca. 100–200 mg/ml. In each case, the rate of cohesion of the added DNA was also greatly increased, as seen in Fig. 8 by comparing the concentration depen-

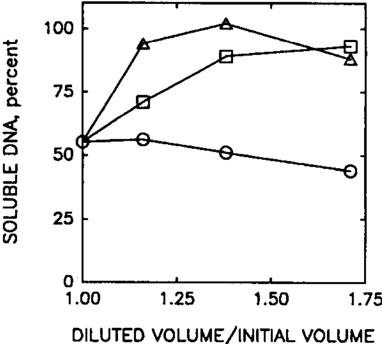

Fig. 7. Reversal of condensation by dilution into cell fractions. Condensation assay mixtures containing stationary phase extract at 145 mg/ml were incubated 5 min at 20°C and then diluted as indicated with TNE (triangles) or with a solution of DNA-nonbinding fraction at 145 mg/ml protein (squares), or with the initial extract at 145 mg/ml protein (circles). After 5 min at 20°C, the mixtures were analyzed as described for the condensation assay.

dence of condensation (closed symbols) with that for enhanced cohesion (open symbols). These same levels of DNA-binding materials did not cause condensation in the absence of the albumin (Fig. 8B–D), although the basal rate of cohesion was increased, particularly by spermidine (Fig. 8D and 9D).

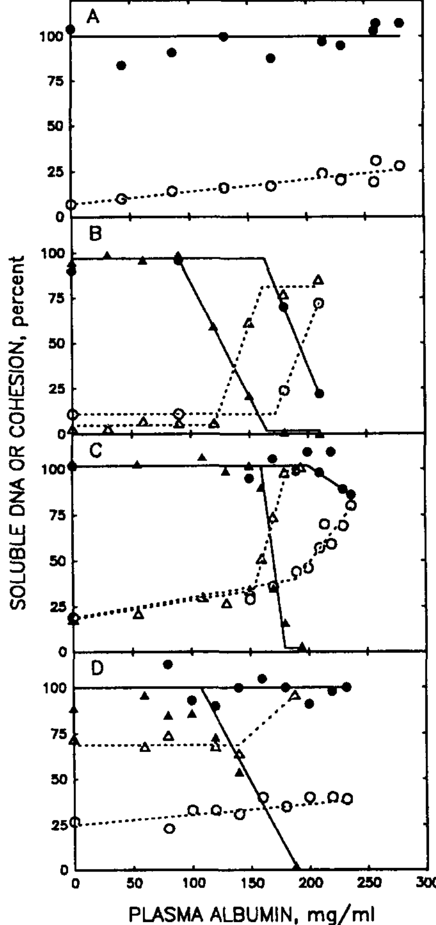

Fig. 8. Effect of various additions on condensation and cohesion of NruI nuclease digests of λ DNA in solutions of bovine plasma albumin. Filled symbols and solid lines, condensation assays; open symbols and dashed lines, cohesion assays. (A) No additions. (B) Circles or triangles, DNA-binding fraction at 138 or 276 μg/ml, respectively; the DNA-binding fraction contains 52% HU based upon densitometry of Coomassie Brilliant Blue-stained SDS/acrylamide gels. (C) Circles or triangles, HU at 58 or 116 μg/ml, respectively. (D) Circles or triangles, spermidine at 5 or 10 mM, respectively; aliquots taken before centrifugation of 10 mM spermidine mixtures had only partial recovery of added DNA.

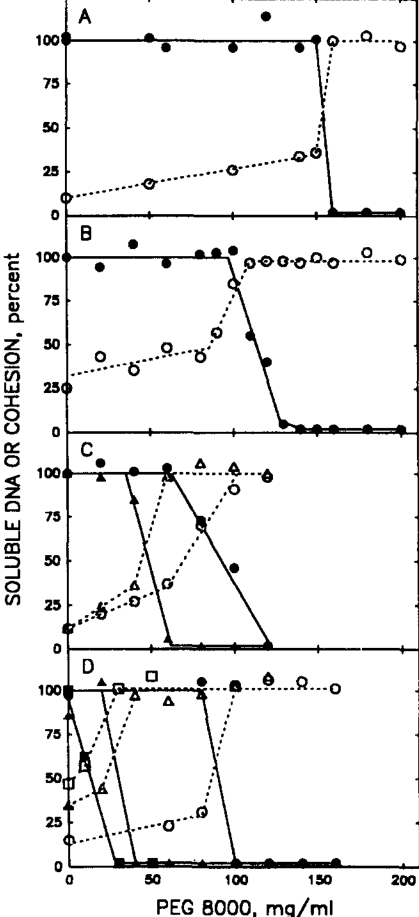

Fig. 9. Effects of various additions on condensation and cohesion of NruI nuclease digests of λ DNA in PEG 8000 solutions. Filled symbols and solid lines, condensation assays; open symbols and dashed lines, cohesion assays. (A) No additions. (B) 5 mM $MgCl_2$. (C) Circles or triangles, HU at 39 or 78 μg/ml, respectively. (D) Circles, triangles, or squares, spermidine at 1, 5, or 10 mM.

A similar pattern of effects of DNA-binding materials occurred with the second crowding agent, PEG 8000 (Fig. 9; see also Ref. [15]). In contrast to albumin, the attainable concentrations of PEG 8000 are readily able to cause condensation and enhanced cohesion in the absence of DNA-binding materials (Fig. 9A). However, addition of DNA-binding materials such as Mg^{2+}, HU, or spermidine can greatly decrease the concentrations of PEG 8000 required

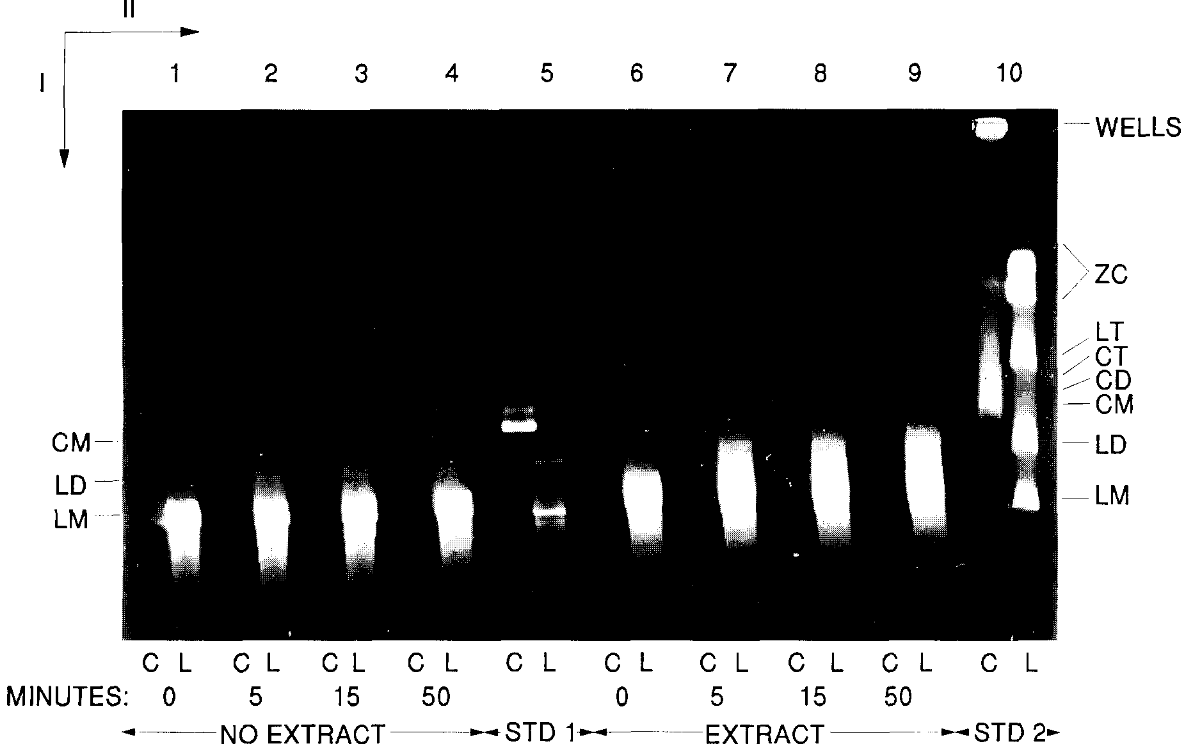

Fig. 10. Kinetics of cohesion of λ DNA in the presence or absence of concentrated extract from *E. coli* assayed on two-dimensional agarose gel electrophoresis. Samples 1, 2, 3 and 4 are incubation mixtures containing 6 μg/ml λ DNA in TNE without added extract, incubated at 20°C for 0, 5, 15 and 50 min, respectively; samples 6–9 are similar mixtures containing 150 mg/ml extract from stationary phase cells. Sample 5 is a cohesion standard made by incubating λ DNA at 6 μg/ml for 7 h at 45°C [41]. Sample 10 is a commercial preparation of concatemerized λ DNA. Directions of the first and second electrophoreses are indicated by arrows I and II, respectively. CM, CD, and CT, circular monomer, dimer and trimer; LM, LD, and LT, linear monomer, dimer, and trimer; ZC, zone of compression; C and L refer to lanes enriched in circular and linear species, respectively, as described in text. Identification of DNA species based upon gel patterns of mixtures prepared over a range of DNA concentrations and by comparison with the λ DNA 'ladder' in lane 10.

for condensation (Fig. 9B, C, or D, respectively) [3]; similar effects occurred with putrescine (e.g., 50% condensation in 7% PEG 8000 at 10 mM putrescine).

In each combination tested in Fig. 9, there again is a correlation between the PEG 8000 concentration causing condensation (closed symbols) and that causing enhanced cohesion (open symbols) [4].

These results indicate that condensation and cohesion behavior in these relatively well-defined sys-

[3] Even relatively high concentrations of spermidine (1–10 mM) do not cause condensation of DNA in the standard assay medium (TNE) (Figs. 8D or 9D) or that medium with excess Mg^{2+} ion (TNE + 5 mM Mg^{2+}) (data not shown). To judge the magnitude of the crowding effect on spermidine-dependent condensation, we repeated the measurements at lower salt concentrations (50 mM Tris-HCl, pH 7.8–1 mM EDTA, or that medium with 5 mM $MgCl_2$). In either medium, condensation occurred at ca. 2–3 mM spermidine in the absence of PEG and ca. 0.5 mM spermidine in the presence of 8% PEG 8000, indicating a ca. 5–10 fold reduction in spermidine concentration required for condensation under crowded conditions.

[4] We have previously demonstrated condensation by relatively low concentrations of DNA-binding fraction from either stationary or exponential growth phase cells and 30–120 mg/ml of PEG 8000 or polyvinylpyrrolidone 10000 or 40000 [15]. Assays of mixtures of HU or spermidine with the DNA-nonbinding fraction have been ambiguous because of a significant level of nuclease degradation of the DNA substrate even in the absence of added divalent ions (unpublished results).

tems is generally similar to that which occurs in concentrated extracts.

3.4. Condensation and cohesion of λ DNA by cell fractions

In the experiments described to this point, a NruI restriction digest of λ DNA was chosen as the DNA substrate because of the simplicity inherent in a single, linear, small (11.3 kbp) product of cohesion and the availability of the non-cohesive fragments in the digest to act as internal standards for estimating cohesion [34]. We next extend those observations to reactions with λ DNA itself.

Cohesion of λ DNA (48.5 kbp) can yield complex mixtures of linear and circular aggregates containing different numbers of λ DNA molecules. Louie and Serwer [31] resolved such mixtures made over a range of temperatures in the presence of PEG, using both pulsed-field gel electrophoresis and the characteristic immobilization of circular DNA on agarose gels at higher voltage gradients. We have used a two-dimensional gel protocol for separation of the products of cohesion of λ DNA in cell extracts or in PEG 8000-HU mixtures that is similar to that for Fig. 4 of Louie and Serwer [31], but with field inversion gel electrophoresis (FIGE) instead of pulsed field gel electrophoresis in the first dimension. We adopt their use of electrophoresis at a constant, higher voltage in the second dimension to prevent migration of circular species while allowing migration of linear aggregates at a size-independent velocity. Samples are loaded in alternate wells. The FIGE in the first dimension (arrow I in Fig. 10) distributes species along the lanes to which the samples were applied, separating both on the basis of circular vs. linear nature and size. Circular and linear products overlap after the first electrophoresis, but are separated by a second electrophoresis applied at a right angle to the first. This second electrophoresis causes the migration of the linear species (in the direction marked by arrow II in Fig. 10) by ca. one lane's width to the right, to the zones marked 'L' on the figure. Circular species do not migrate apprecia-

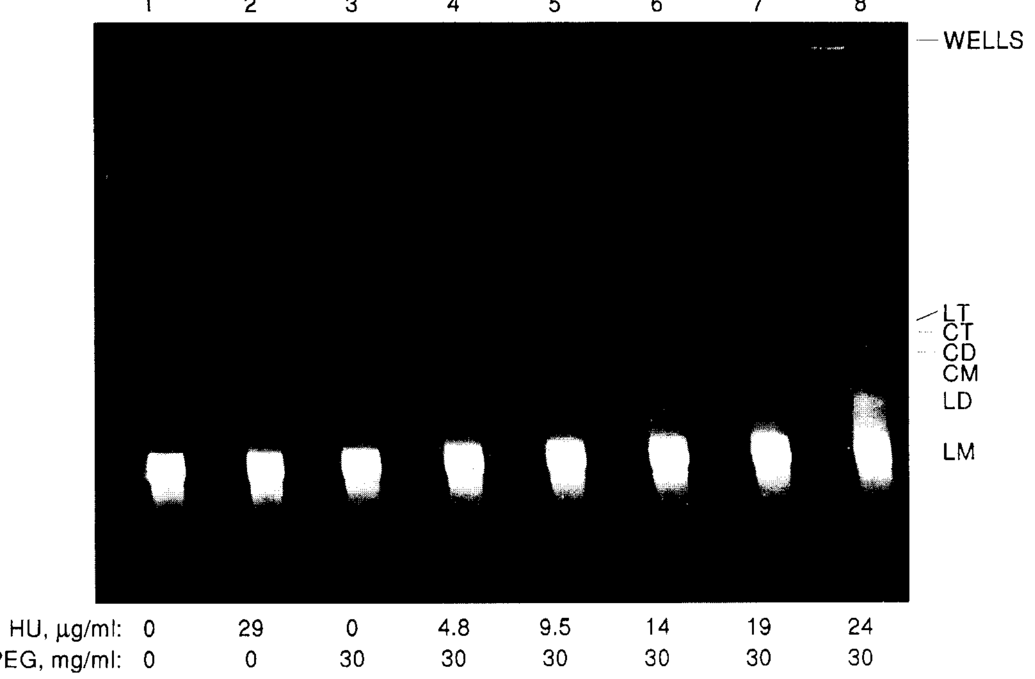

Fig. 11. Effect of PEG 8000 and protein HU on the cohesion of λ DNA assayed on two-dimensional agarose gel electrophoresis. Labels as Fig. 10.

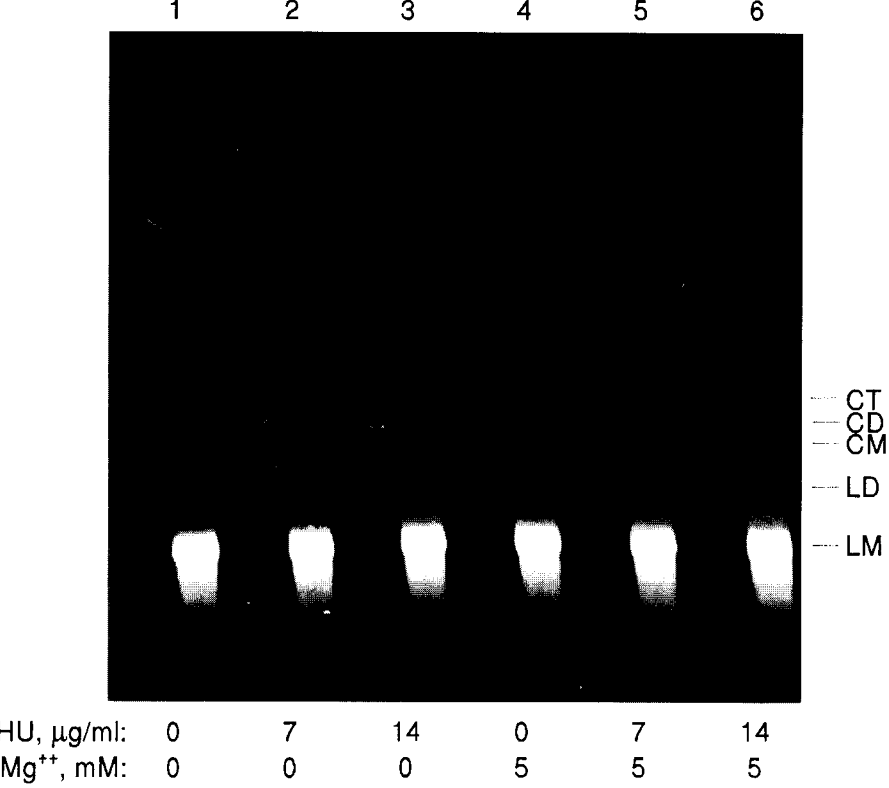

Fig. 12. Effect of Mg^{2+} on the cohesion of λ DNA in PEG 8000/protein HU mixtures assayed on two-dimensional agarose gel electrophoresis. All reaction mixtures contained 30 mg/ml PEG 8000. Labels as Fig. 10.

bly during the second electrophoresis, and so remain in their original lanes, marked 'C'. The DNA standard in sample 5 illustrates the resolution of circular monomer and dimer and linear monomer and dimer bands.

Lambda DNA added to a concentrated cell extract very rapidly formed a small amount of circular products as well as larger amounts of linear aggregates (Fig. 10). Traces of circular monomer and linear dimer present in the λ DNA substrate before incubation did not change significantly on prolonged incubation in the absence of extract (lane 4 vs. lane 1) nor did the DNA become sedimentable. However, in the presence of concentrated extract (150 mg/ml extract protein), a small amount of circular species was rapidly formed (halftime for the conversion shown in Fig. 10 is < 5 min, and is < 2 min in similar experiments not shown); the amount of circular species (ca. 1% of the input DNA) did not change appreciably on further incubation (lane 7 vs. lanes 8,9). Further incubation resulted in increased amounts of the linear dimer, reaching 15–40% of input DNA at 50 min (lane 9 and similar assays). Assays not shown indicate that formation of circular species occurs only at extract concentrations that also cause condensation of the λ DNA ($\geqslant 90$ mg/ml extract protein), and that formation of circular and linear dimers occurs at higher extract concentrations ($\geqslant 120$ mg/ml extract protein) [5].

[5] The DNA-binding and DNA-nonbinding fractions from the DNA-cellulose chromatography described earlier did not change control patterns such as lanes 1–4 of Fig. 10 when assayed separately at concentrations equivalent to 150 mg/ml extract protein.

3.5. *Condensation and cohesion of λ DNA by protein HU and PEG*

Lambda DNA added to mixtures of 3% PEG 8000 and relatively low concentrations of protein HU rapidly formed distributions of circular DNA products similar to those made in concentrated extracts. For example, a mixture of 3% PEG 8000 and 14 or 19 μg/ml HU converted 4–6% of the added DNA to circular species (lanes 6 or 7, Fig. 11). Slightly higher concentrations of HU (lane 8, Fig. 11) (or a PEG 8000 concentration of 8%, data not shown) caused an abrupt change in the pattern to yield large amounts of DNA retained in the wells or converted to linear dimers, with only traces of monomer and dimer circles and linear trimers. A HU- and PEG-dependent conversion of λ DNA to circular species also occurred in the presence of Mg^{2+} (Fig. 12).

4. Discussion

The nucleoid of *E. coli* contains one or a few enormous circular duplex DNA molecule(s) condensed into a fraction of the cellular volume [1–4] [6]. Despite extensive characterization, it is not obvious what forces stabilize this compact conformation. The condensation of DNA added to cell extracts is studied here as an approach to this problem.

4.1. *DNA aggregation as an assay for DNA compaction*

Direct study of the monomolecular collapse of DNA is difficult because of the tendency of the collapsed macromolecules to undergo aggregation [7,49–51]. The theoretical analyses of Post and Zimm [7,52,53], however, suggest that the aggregation process itself provides an assay for important aspects of collapse. High DNA segment density is the common denominator of both collapse and aggregation: whether DNA occurs as isolated, collapsed molecules or as aggregates of many molecules is a function of ambient DNA concentration.

Post and Zimm [7] compared the effects of good and poor solvents on the interactions of DNA with solvent or with itself. (In this context, solvent includes solutes other than DNA as well as the solvent per se.) The changes in DNA-solvent interaction were expressed by the Flory–Huggins interaction parameter, χ, proportional to the difference in free energy between unlike contacts (DNA–solvent contacts) and like contacts (DNA–DNA contacts). In good solvents ($\chi < 1/2$), DNA prefers to interact with the solvent rather than with itself and does not tend to condense or form more than one phase. In poor solvents ($\chi > 1/2$), DNA segments tend to associate with each other rather than with solvent, with results that depend upon the ambient concentration of DNA molecules. At relatively low DNA concentrations, of the order of 1 μg/ml or less, association of DNA segments tends to be intramolecular, leading to a collapse of the DNA molecules. If the concentration of DNA is considerably higher, the tendency of DNA segments to associate results in intermolecular interactions and the separation of the DNA as a second more concentrated phase. The predicted conformation of the DNA in the concentrated phase is as an extended random coil structure rather than a collapsed structure. Post and Zimm [7] cite two examples of solutes which form poor solvents for DNA in vitro, causing condensation: spermidine, which binds to DNA and thereby decreases electrostatic repulsion between DNA segments, and PEG, which increases the repulsion between DNA segments and 'solvent' so that the interaction between DNA segments is favored. Effects analogous to these examples appear to be of importance in the compaction of DNA by cellular extracts, as discussed further below.

4.2. *Assay and characterization of condensation*

We have used the formation of readily-sedimented DNA as a simple assay for DNA condensa-

[6] Estimates of DNA concentrations in nucleoids vary widely. The average DNA concentration in *E. coli* is ca. 7 mg/ml based upon total DNA and water contents [45]; if the nucleoid volume is ca. 1/4–1/7 of the cytoplasmic volume [1], an estimate of 26–46 mg/ml DNA in nucleoids is obtained. An early estimate of ca. 15 mg/ml DNA was made by Fuhs [46]. Woldringh and Nanninga [1] calculate ca. 57 mg/ml DNA for nucleoids of slowly growing *E. coli* B/r H. Kellenberger [47] estimated 14–34 mg/ml DNA in the ribosome-free regions of *E. coli* and more recently, ca. 100 mg/ml by an electron microscope technique and ca. 69–92 mg/ml based upon composition and condensation by a factor of ca. 3 to 4 [48].

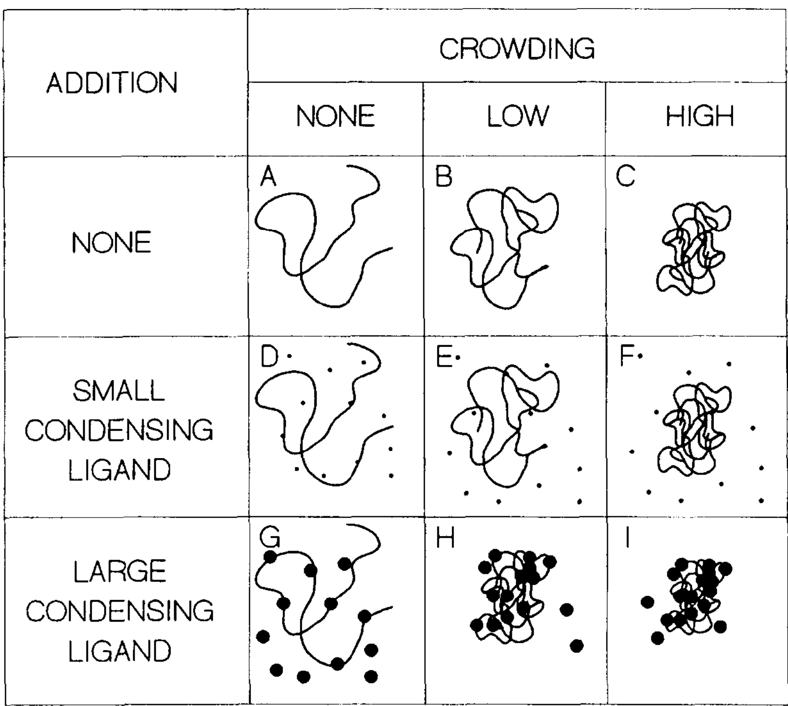

Fig. 13. Direct and indirect crowding effects on DNA condensation by weakly-bound ligands. The solid line represents a segment of double-stranded DNA. In A–C, increased crowding results in increasingly compacted DNA in the absence of condensing ligands ('direct-crowding effect'). In D–F, binding of a small, weakly-bound condensing ligand is relatively unaffected by crowding because of its small size [20,21], so that DNA condensation at relatively low ligand levels is due to direct crowding effects similar to those in the absence of ligand, A–C. In G–I, binding of a large, weakly-bound condensing ligand is increased by crowding ('indirect crowding effect') to the point where condensation occurs (H) at levels of direct crowding that would be insufficient to condense DNA in the absence of the enhanced binding of ligand.

tion [15,54–57] that is applicable to samples containing very high concentrations of macromolecules — often over 200 mg/ml of extract protein or other macromolecules [7]. The DNA species studied here are too small to sediment from simple solution as individual molecules under the conditions of the condensation assay. Their ready sedimentation in the presence of extract indicates either precipitation due to aggregation (i.e., joining into hydrodynamic units much larger than the individual DNA molecules by entanglement or by direct attractive interactions mediated by extract components) and/or preferential partition into a second, liquid phase which is readily separated by low centrifugal forces [58–61].

We have chosen TNE (50 mM Tris buffer, pH 7.8–100 mM NaCl–1 mM EDTA) as the standard assay medium. A number of alternative media give qualitatively similar condensation and cohesion results but at somewhat different concentrations of extract or of purified additions and sometimes (notably in potassium glutamate solutions) allowed significant nuclease levels in assays of extracts (unpublished experiments; see also Methods). Addition of an excess of Mg^{2+} caused similar transitions at 0–30% lower concentrations of background macromolecules or DNA-binding materials where its presence could be tested without an overwhelming amount of nuclease activity.

[7] Krasnow and Cozzarelli [55] have suggested the use of aggregated DNA as a model for compacted DNA in vivo, based upon their study of topoisomerase activity in spermidine-induced DNA aggregates.

4.3. Condensation of DNA by cell extracts

Crowded conditions provided by high concentrations of synthetic polymers can increase the binding to DNA of proteins from *E. coli* to the point where the cellular abundance of the binding proteins causes condensation of added DNA [15]. Extrapolation of such results to a cellular milieu is always problematical. We have used extracts as crowding agents in vitro as an approach to this problem. Although the crowding potential of the macromolecules in prokaryotic cytoplasm is obviously large [24,25], cellular fractions have never been experimentally tested for crowding effects in any system, to our knowledge. It was therefore unclear whether the macromolecules in cellular extracts would cause condensation of added DNA in the manner observed earlier using PEG and other macromolecules as crowding agents. This appears to be the case, but in addition, the study of this system has suggested a broader involvement of crowding in cellular DNA condensation.

Specifically, our results suggest that condensation of the genomic DNA of bacteria into nucleoids may be a response to at least three distinctly different functions of the surrounding cytoplasm: (i) cytoplasm as a source of materials (e.g., histone-like proteins, polyamines) whose binding decreases the net charge of the DNA; (ii) cytoplasm as a source of crowding effects which *directly* favor compact conformations of the DNA; (iii) cytoplasm as a source of crowding effects which *indirectly* favor compact conformations of the DNA by increasing the binding of materials which make compact complexes with DNA. It is anticipated that these indirect crowding effects will be a major factor in promoting the binding to the DNA of large ligands such as proteins, but of lesser importance in controlling binding of small ligands such as polyamines because of the size-dependence of the crowding effect [20] [8]. Fig. 13 is a pictorial representation of these effects.

[8] Condensation of DNA from non-crowded aqueous solutions by polycations requires polycations bearing at least three charges [17,62]. Crowding may alter this requirement to the point where putrescine or Mg^{2+}, for example, may be effective condensing agents at cellular concentrations.

Support for these multiple roles of the cytoplasm comes in part from experiments in which cell extracts were separated into DNA-binding and DNA-nonbinding fractions by affinity chromatography on DNA-cellulose; neither of these fractions condensed DNA as efficiently as did a combination of the fractions. Model systems composed of a DNA-binding material (HU or spermidine) and high concentrations of a macromolecule (PEG 8000 or albumin) caused condensation similar to that found in the presence of extract or of the reunited DNA-cellulose fractions. A crowding role for the macromolecular background is suggested both by the nonspecificity of the requirement and the ready reversibility of condensation, implying a lack of strong attractive interactions.

The inhibition of condensation at relatively low salt concentrations may be due to inhibition of the binding to DNA of extract protein(s), as inferred from the weak, salt-inhibited binding of protein HU [6], the major DNA-binding protein of *E. coli*. The ability of higher extract concentrations to overcome inhibition by at least certain levels of salt suggests a homeostatic role of the crowding effects being described here; larger effects may occur at the higher concentrations of macromolecules in vivo. Homeostasis is used here to indicate the maintenance of a relatively stable internal environment with no connotations of negative feedback mechanisms [63,64].

4.4. Supercoiling

A large fraction of natural DNA is supercoiled, either as topologically separate molecules or as topologically separated domains. Supercoils may be restrained by interactions with ligands or particles, e.g., binding of DNA to nucleosomes in eukaryotes, or they may be free to change the local conformations of the DNA, as by the in vitro extrusion of cruciforms or by localized melting [65]. Supercoiling favors relatively compact conformations [29], and should be evaluated experimentally as a condensing force in concert with direct and indirect crowding effects and ligand binding. Such measurements are, however, beyond the scope of the present study.

The media used here may be useful experimentally in defining the forces which affect supercoiling

within cells [66]. Although it is often assumed that those forces arise directly from ligand binding, both direct and indirect crowding effects may make important contributions to cellular supercoiling phenomena and may help our understanding of certain differences between DNA under in vivo versus in vitro conditions [67–70].

4.5. DNA condensation and the rate of cohesion of DNA in vitro

The rate of cohesion of double-stranded DNA with complementary single-strand termini of λ DNA increased under conditions causing condensation. These increases occurred both with λ DNA itself (48.5 kbp) and with the two complementary NruI nuclease-fragments of λ DNA (4.6 and 6.7 kbp) that have the cohesive termini of λ DNA. The products of cohesion are enriched in the readily-sedimented fraction, consistent with their formation within this fraction. Sikorav and Church [57] made similar observations on renaturation of single-stranded DNA that was condensed by a variety of agents, including PEG.

The high concentrations of DNA in the condensed phase should tend to increase the rate of reactions in the condensed phase due to mass action [57]. For example, if the initial rate of product (P) formation in a bimolecular reaction is given by $dP/dt = k_f(\mathrm{DNA})^2$, then a 100-fold increase in rate, which is approximately the largest we have observed, corresponds to a 10-fold increase in DNA concentration; such an increase would still require only a relatively low DNA concentration (< 1 mg/ml of DNA) in the concentrated phase. Increases in interaction between separate regions on a single large DNA molecule seem likely as well [9].

[9] High concentration of macromolecules in the condensed phase could *decrease* cohesion by slowing diffusion of the reactants. Although there are no obvious indications of such an effect in the present results, it is unknown what would occur at concentrations of extract between those tested here ($\leq$ 200 mg/ml extract protein) and those which have been estimated for the cell (e.g., ca. 340 mg/ml extract protein and RNA [25]).

4.6. DNA condensation and the cohesion of λ DNA in vivo

The wealth of information on bacteriophage λ and its DNA allow comparison between certain properties determined in vitro (under both uncrowded and crowded conditions) and those in vivo.

Rates of cohesion between termini of λ DNA.
The linear DNA injected into the cytoplasm of *E. coli* by bacteriophage λ is rapidly converted into circular species [71–74]; the halftime for conversion is less than 2 min at 37°C (based upon the kinetics of DNA inactivation in Fig. 1 of Bode and Kaiser [72]. At least 90% of the injected DNA can be recovered as either open or closed circular monomers. Closed circular monomers appear to be formed in two stages: first, the complementary termini on the two ends of a duplex molecule undergo hydrogen-bonding to yield open, circular monomers, followed by enzymatic ligation to yield closed circles [75]. Under typical non-crowded in vitro conditions, the rate of open circle formation is > 50-fold slower than circle formation in vivo. The in vitro rate increases dramatically in response to crowding. Previous studies employed purified macromolecules as background materials at physiological temperatures [30] or at higher temperatures [31]. The current studies at 20°C with concentrated cell extracts from *E. coli* give high initial rates of open circle formation, comparable to in vivo rates of circle formation; the extent of circle formation at these rates is, however, very limited. (Crowding also increases the enzymatic ligation reaction which presumably converts Hershey circles to closed circular DNA [76,77].)

Products of cohesion of λ DNA: a model for condensation at the site of viral injection.
In vitro studies of the products of cohesion of λ DNA under non-crowded conditions demonstrate a characteristic dependence on the initial DNA concentration [35,40,78]. At DNA concentrations below 2 μg/ml, for example, the products are mainly circular monomers, whereas at DNA concentrations above 50 μg/ml, the products are mainly linear aggregates (concatemers); intermediate DNA concentrations yield mixtures of these products. Jacobson and

Stockmayer [79] have presented a statistical basis for such a concentration-dependence of linear and circular products. The in vitro concentration-dependence predicts that the in vivo product of multiply-infected bacteria will be linear aggregates, given that the average DNA concentration in the host cytoplasm resulting from the injection of the DNA from even a single λ virus is of the order of 50 μg/ml [10]. However, in disagreement with this prediction, circular products are recovered after injection of viral DNA into the host cytoplasm at a multiplicity of infection of 9 [72] or 5 [71]; circular products are also inferred from studies of the eclipse reaction at a multiplicity of infection of 5 [74] [11].

The rapid conversion to circular species of a fraction of the λ DNA added to concentrated extracts or added to mixtures of PEG and HU suggests a natural explanation for the high fraction of circular viral DNA made upon in vivo injection of DNA by bacteriophage λ. Specifically, we propose that injected viral DNA does not disperse evenly throughout the bacterial cytoplasm (Fig. 14A), but is immediately condensed where it first encounters the cytoplasm (Fig. 14B). Because of this condensation, the first end of the viral DNA molecule to enter the cytoplasm will tend to stay in the vicinity of the site of injection and will therefore be much more likely to react with the other end of the same DNA molecule than with ends on other DNA molecules entering from other injection sites in the same cell. This model predicts (a) a high rate of cohesion and (b) increased probability of circular monomer formation relative to formation of linear aggregates. This mechanism is related to two much earlier suggestions. Tomizawa and Ogawa [80] proposed that the infecting DNA is oriented in such a way that the molecules circularize immediately upon injection into the host; rapid cyclization suggested the presence of a special mechanism to facilitate circularization. Hogness (cited in Dove [73]) suggested that the first end of the λ DNA to be injected might be fixed to the cellular membrane at the site of injection, thus increasing the probability of that end reacting with the other end of the same molecule to form cyclic monomers. In our model, the first end injected is localized to the site of injection by decreasing the end-to-end length of the DNA; the first end of the DNA to enter the cell is not covalently fixed, but is relatively free to undergo diffusion and thereby to encounter and cohere to the last end of the DNA to enter the cell. An alternative or additional effect of the crowded nature of cytoplasm is the slowing of diffusion of the leading DNA end away from the site of its injection into the cytoplasm; if this diffusion is slow relative to the rate of injection, the two ends will have an additional tendency to cohere. These ideas may be testable by artificially-induced injection of λ virus DNA into crowded solutions.

Cyclization at the site of injection may also tend to reduce the amounts of catenanes formed [81].

4.7. *Implications of a general tendency for DNA to condense in prokaryotic cytoplasm*

A large number of variables can change the energy difference between compact and expanded conformations of DNA. Some of these factors are sufficient by themselves to condense DNA in vitro. All of the factors considered here are of that type and all are characteristic of the bacterial cytoplasm, suggesting that condensation in vivo will be a resultant of many forces — as proposed earlier by Gosule and Schellman [16,82]. Even the highest extract concentration tested here is only ca. 60% of a nominal cellular concentration of macromolecules, implying much larger crowding effects in vivo because of the nonlinear concentration dependence of excluded volume effects [20,21]. While it is possible that variations in the contributions from various factors might modulate the extents of compaction or exposure of the DNA, the variety of forces may have actually evolved to ensure that the bulk of DNA stays condensed despite changes in internal milieux or losses of specific components. An excess of crowding beyond that needed for condensation may provide a safeguard to the genomic DNA for the eventualities of life, such as fluctuations in internal salt concentra-

[10] Based upon injection of a single molecule of lambda DNA of M_r ca. 32×10^6 into a cell of volume ca. 1 μm^3 to yield ca. 5.3×10^{-11} μg of DNA/1×10^{-12} cm^3 or ca. 53 μg/ml).

[11] The prediction assumes that two or more non-cohered λ DNA molecules coexist in the cytoplasm of a multiply-infected cell. Asynchrony in injection may decrease such a temporal overlap, as suggested to us by M. Gellert (personal communication).

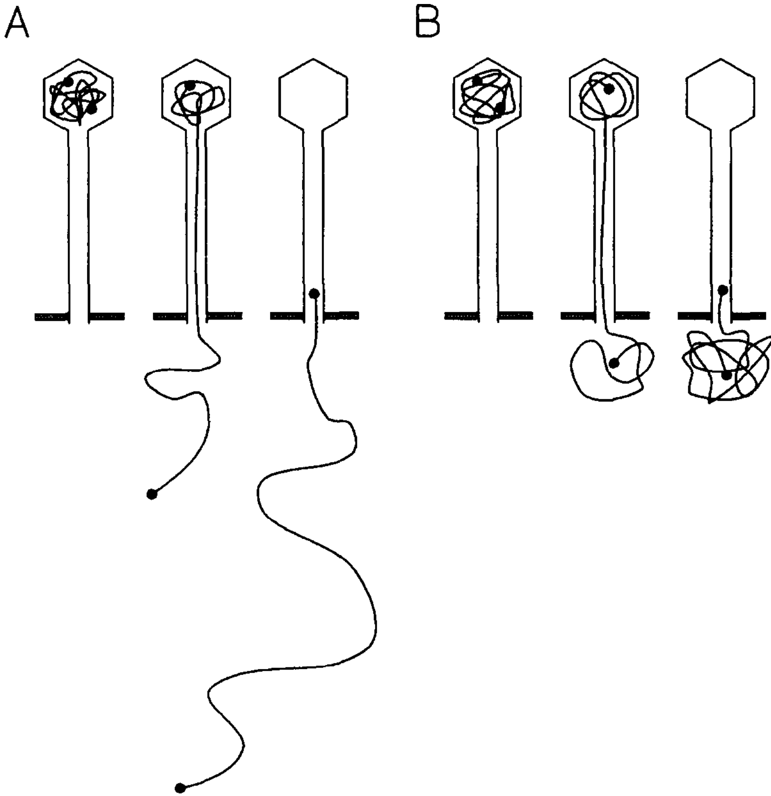

Fig. 14. Injection of viral DNA (A) without, and (B) with condensation at the site of injection. Crosshatched areas represent the boundary of the bacterial cytoplasm.

tions, temperature, etc. The need for multiple forces that can cause condensation may also reflect the inhomogeneity of cellular DNA environments. It has been suggested [47,83] that only DNA near the cytoplasmic interface is complexed with HU in *E. coli*, leaving condensation of the bulk of the cellular DNA to an unspecified mechanism (cf. [84]).

We propose that cellular forces will tend to condense DNA whenever and wherever it is introduced. Such 'condensation at the site of introduction' suggests that in prokaryotes and probably in eukaryotes as well:

(a) Condensation of DNA at sites of entry into cells or at sites of synthesis within the cells may play controlling roles in the function of various DNA species, notably with a phenomenon such as transcription that is known to respond to DNA curvature [85]. Condensation effects upon RNA and DNA–RNA hybrid species as well as DNA species also seem likely.
(b) Condensation may control the types of cohered products made between termini of nucleic acids, including not only those that cohere by means of longer single-strand interactions as studied here and discussed earlier for λ DNA cyclization, but also the cohesion of DNA species with shorter termini which only form stable products by enzymatic ligation. Effects on recombination may be significant.
(c) Extrachromosomal DNA (plasmids, viral genomes) may tend to be physically aggregated with the genomic DNA, with implications for the distribution of such DNAs upon cell division.
(d) While our immediate focus is on prokaryotic cytoplasm, similar phenomena may occur upon introduction of nucleic acids into eukaryotic cells.

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