Hypothesis

Macromolecular crowding and the mandatory condensation of DNA in bacteria

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Abstract Cellular DNA in bacteria is localized into nucleoids enclosed by cytoplasm. The forces which cause condensation of the DNA into nucleoids are poorly understood. We suggest that direct and indirect macromolecular crowding forces from the surrounding cytoplasm are critical factors for nucleoid condensation, and that within a bacterial cell these crowding forces are always present at such high levels that the DNA is maintained in a condensed state. The DNA affected includes not only the preexisting genomic DNA but also DNA that is newly introduced by viral infection, replication or other means.

Key words: Cytoplasmic macromolecule; DNA condensation; *Escherichia coli*; Macromolecular crowding; Mandatory condensation; Nucleoid

1. DNA condensation in bacteria: general considerations

The genomic DNA of bacteria is condensed into one or a few nucleoids per cell [1] that are in direct contact with a surrounding cytoplasm containing very high concentrations of macromolecules (e.g. ~340 mg/ml of total RNA and protein [2]). The term 'condensation' is used to indicate adoption of a relatively concentrated, compact state occupying a fraction of the volume available. The DNA of the bacterial nucleoid is estimated to have a local concentration of ~50–100 mg DNA/ml (see footnote 6 of [3]) and to occupy 1/8 to 1/5 of the volume within the cell envelope [1].

Condensation of DNA within bacterial cells has been observed by light microscopy of both living and fixed cells, as well as by electron microscopy [4–8] (reviewed in [9,10]). Condensation of DNA in isolated nucleoids is indicated by microscopy as well as by hydrodynamic properties [11–13]. Condensation of DNA in model in vitro systems has been assayed by aggregation, as described below.

2. Condensing forces

The origins and magnitudes of the forces which cause DNA condensation are poorly understood. Supercoiling, macromolecular crowding and the binding of histone-like proteins and of polyamines have all been suggested to contribute to DNA condensation. It is unclear how important each of these factors is in causing DNA condensation in vivo. Model studies of the binding of histone-like proteins under dilute solution conditions indicate that the cellular amounts of DNA-binding proteins in bacteria are 5–10-fold lower than those required for condensation (see [14] for references and discussion). The polyamines putrescine and spermidine can occur in large amounts in *E. coli* [15]. Spermidine but not putrescine can condense DNA from aqueous solution [16], although the binding reaction is relatively salt-sensitive [17,18] and so may make a restricted contribution over much of the range of intracellular salt concentrations observed in growing cells [19,20]. Enzymatic supercoiling directly yields more compact forms [21].

Macromolecular crowding ('crowding', reviewed in [22,23]) can cause DNA condensation by two distinctly different mechanisms. The importance of *direct* macromolecular crowding (i.e. excluded volume effects favoring compact molecular conformations) by the cytoplasm upon condensation of cellular DNA was suggested many years ago, after the discovery of DNA collapse in crowded media [24,25]. We have recently provided experimental evidence for a second, *indirect* mode of crowding-enhancement of DNA condensation. Indirect crowding effects arise from the increased binding under crowded conditions of condensing ligands such as the histone-like proteins; the effects are large enough to account for the 5–10-fold discrepancy noted above between cellular amounts of the histone-like proteins and the amounts needed for condensation [14]. Indeed, the crowding potential of the cytoplasm of *E. coli* appears to be at least several fold higher than is required for DNA condensation, based on the model studies in cytoplasmic extracts described below.

Crowding is basically different from the other condensing forces. Enzymatic supercoiling and DNA condensation by polyamines or the histone-like proteins depend on binding interactions of DNA with the respective agents and are therefore sensitive to changes in local environment (salts, pH, etc.) or the presence of materials already bound to the DNA. In contrast, crowding forces do not act through binding interactions and are relatively indifferent to ionic or other characteristics of the internal medium or to the presence of bound ligands. The non-crowding forces are caused by the products of one or a few genes, and are more or less subject to genetic change. In contrast, crowding is an expression of the overall macromolecular composition of the cytoplasm and should be highly buffered. In this regard, we note that the crowding potentials of the cytoplasmic fraction from logarithmic phase and stationary phase cells appear very similar based upon experimental analysis [2] despite the enormous differences in functional activity of growing and nongrowing cells.

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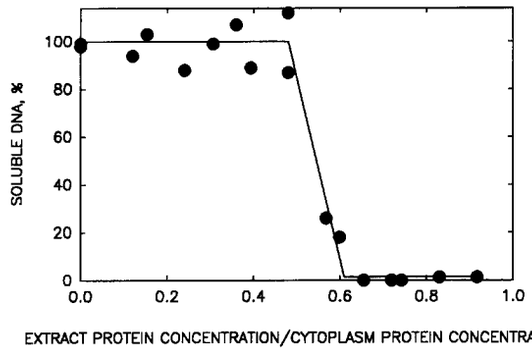

Fig. 1. Aggregation of DNA added to cytoplasmic extracts of *E. coli*. Based upon data in Fig. 3 of [3]. Extracts of stationary-phase cells were prepared by high-speed centrifugation; a concentration of ~230 mg/ml of protein corresponds to the in vivo protein concentration determined earlier [2].

3. A hypothesis of mandatory DNA condensation in prokaryotes

The powerful and invariant crowding forces for DNA condensation within bacterial cells have led us to propose a hypothesis of mandatory DNA condensation in bacteria which simply states that the DNA in a prokaryotic cell rapidly adopts and maintains a condensed state. This condensation is therefore uniform across the cell and invariant with time. DNA which is affected by mandatory condensation includes the cell's genomic DNA (both previously existing and newly synthesized) as well as any exogenous DNA introduced into the cell by viral sources, transformation, conjugation or other means[(1)].

4. Experimental evidence for a persistent state of condensation of DNA in bacteria

There are a number of indications that the condensing forces in bacteria may be more than sufficient to condense cellular DNA:

4.1. A condensed nucleoid is observed in situ

A condensed nucleoid is characteristic of *E. coli* as studied by a variety of electron and light microscopy techniques including observations on living cells, as cited above.

Electron microscope studies of infection of *E. coli* by bacteriophage T4 (reviewed in [26]) are fully consistent with the predictions of mandatory condensation. T4 infection causes a series of changes in the host cell DNA accompanied by the formation of pools of DNA which are ultimately incorporated into mature viral particles. Within the first few minutes of T4 infection, the host nucleoid undergoes 'nuclear disruption', a redistribution from its normally centralized localization to a more peripheral but still condensed distribution along the inner cell membrane or, in certain T4 mutants, to a scattered but still condensed distribution within the cytoplasm [27]. A pool of DNA accumulates during the infection, appearing as a centralized condensed body much like the host nucleoid. As mature bacteriophage appear in the cytoplasm, this centralized pool becomes more scattered but retains a condensed appearance [28]. Hence, the undegraded host DNA and the newly synthesized viral DNA present a similar compact appearance. In a normal infection, the host DNA is eventually degraded; the rate of degradation is exactly the same in wild type infections where the nuclear disruption has occurred vs infection with a viral mutant where no disruption occurs [29] – suggesting that the state of condensation is maintained unchanged in these two situations, again consistent with a putative constant background of crowding.

4.2. Nucleoid shape

Inhibition of protein synthesis in *E. coli* by chloramphenicol or related drugs causes the rapid conversion of the normally rather irregularly shaped nucleoids into more compact and more spherical structures [4,30], apparently due to disruptions of linkages between the nucleoid and the cell envelope (see partition studies below). Adoption of a shape approaching the lowest possible volume under these circumstances is consistent with mandatory condensation caused by a direct crowding mechanism. It is not obvious how supercoiling or ligand binding would form such relatively spherical bodies under noncrowded conditions.

4.3. DNA condensation in concentrated cellular extracts

Cytoplasmic extracts from *E. coli* cause quantitative reversible DNA aggregation at extract concentrations corresponding to about 1/2 the cellular concentration (Fig. 1). Similar forces and interactions are responsible for both aggregation and condensation [31], so that aggregation serves as a predictor for condensation [3]. The nonlinear concentration dependence of crowding ensures that DNA surrounded by the several-fold higher macromolecule concentrations in vivo will be exposed to forces far beyond those required for condensation.

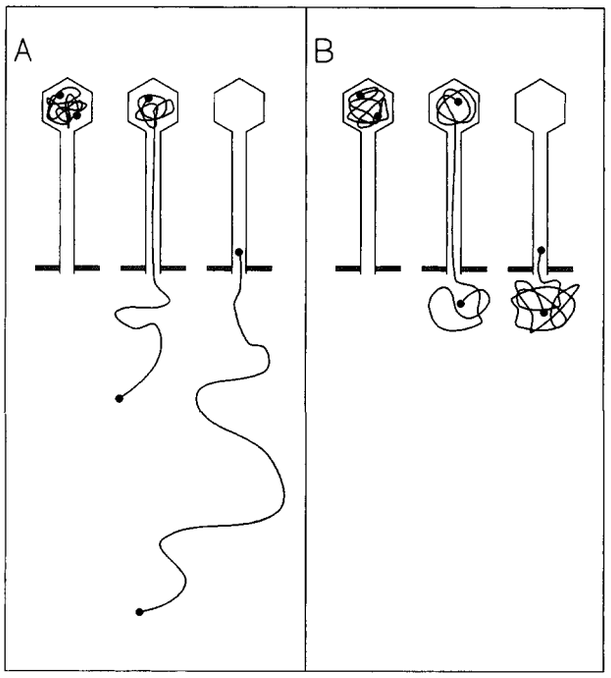

Fig. 2. Injection of viral DNA (A) without, and (B) with condensation at the site of injection. Reprinted from [3] with permission from Elsevier Science BV, The Netherlands.

(1) Mandatory condensation may have wider application in bacteria, potentially extending to non-ribosomal species of RNA and to RNA-DNA hybrids as well as to DNA. Application to non-nucleosomal DNA (e.g. viral DNAs) in eukaryotic cells seems a speculative possibility.

The DNA aggregation caused by the extracts in these experiments appears to be a result of both direct crowding effects favoring compact macromolecular conformations and indirect crowding effects causing increased binding of proteins to the DNA; supercoiling effects were not tested [3].

4.4. Cyclization of lambda DNA

The nature of the products formed and the rate of their formation upon DNA injection into a host cell by bacteriophage lambda are consistent with mandatory condensation. The sticky ends of lambda DNA tend to cohere in solution, forming both concatemers (linear dimers, trimers, etc.) and circular species (circular monomer, dimer, etc.). The relative amount of linear vs. circular products formed in vitro has the dependence on DNA concentration expected on a statistical basis [32]: at low DNA concentrations the ends of a DNA molecule tend to find each other rather than the ends of other molecules, so that circles are formed. At higher DNA concentrations, intermolecular reaction is favored and linear aggregates predominate [33–35].

If cells are infected with a single virus particle, the viral DNA is rapidly converted to a circular form [36–38], that being the only cohesion product possible for the newly entered DNA. However, if two or more phage particles inject their DNA into one cell, the concentration of viral DNA within the cell is in the range where concatemers would form in vitro – yet the actual product formed upon injection at such multiplicities is the circular monomer. Mandatory condensation provides a natural explanation: if viral DNA is condensed as it enters the cell, the first end into the cell remains close to the injection site and preferentially reacts with its other end as that end enters the cell (Fig. 2B).

Experiments under crowded conditions are consistent with the preceding mechanism. Cohesion in concentrated extracts at physiological temperatures [3] or cohesion in polymer solutions at elevated temperatures [39] forms significant amounts of both circular forms and linear aggregates. It would be interesting to test the effect of crowded solutions (extracts or polymers) on the proportion of circles formed upon in vitro ejection of DNA from the bacteriophage.

The rate of circularization of lambda DNA is much faster in vivo than in model studies under non-crowded conditions. The experimental demonstration of accelerated rates under crowded conditions [3,39,40] is again consistent with condensation of the viral DNA caused by cellular crowding.

5. Potential effects of mandatory condensation

5.1. Reaction rates involving DNA

Intuitively, it might seem that condensation of DNA would decrease its reactivity. Experimental results, however, provide several examples of very large increases in reaction rates due to condensation. Krasnow and Cozzarelli [17] found virtually an absolute dependence of the rates of enzymatic DNA catenation on polyamine-induced DNA aggregation. In a comprehensive experimental and theoretical study of DNA condensation, Sikorav and Church [41] demonstrated 10–100-fold increases in reaction rates in DNA renaturation reactions under several condensing conditions, including condensation by crowding. Similarly large increases in cohesion rates between sticky-ended DNA fragments occur under condensing conditions [3]. A number of other DNA reactions which were accelerated under crowded conditions in our previous studies may have occurred under condensing conditions, particularly those in Mg^{2+}-containing media in which PEG was used as a background (e.g. [42–44]).

Sikorav and Church [41] suggest that condensed DNA may be the functional form of DNA in vivo; their conclusion is consistent with our hypothesis that the prokaryotic cell is basically designed to operate under a state of continual DNA condensation.

The results of Hildebrandt and Cozzarelli [45] may be an exception to increased DNA reaction rates under condensing conditions. Those authors found the rate of a plasmid recombination reaction to be slower under in vivo (presumably condensing) conditions than under (non-condensing) in vitro conditions; a direct comparison of in vitro rates under condensing and non-condensing conditions would be of interest.

5.2. Nucleoid partitioning

Partitioning of newly synthesized chromosomes into daughter cells has been hypothesized to occur continuously as the chromosome is replicated: as each domain is synthesized, it becomes condensed. In the model of Wake and Errington [46], the continuous condensation occurs due to supercoiling and the association of histone-like proteins with the DNA. Mandatory condensation predicts continuous condensation as replication proceeds, but we would obviously view the relative importance of the various condensing forces differently than did those authors. In the partition model proposed by Løbner-Olesen and Kuempel [47], as each domain is replicated, a DNA-gyrase site is also replicated which allows the gyrase to supercoil that domain and thereby promote its folding up. Mandatory condensation predicts that the newly synthesized domain would condense as it is synthesized independent of supercoiling by gyrase; gyrase action may, of course, modulate the structure of the domains.

Woldringh et al. [48] suggest that chromosome partitioning and condensation are critically dependent on the balance of DNA compaction and expansion forces; compaction is suggested to result from effects of DNA supercoiling and from the binding of histone-like proteins, whereas expansion is suggested to arise from coupled transcription-translation-translocation of plasma membrane and cell wall proteins. Our proposal would modify that of Woldringh et al. [48] in two important respects: (1) Crowding effects are central to our hypothesis but were not included by Woldringh et al. (2) Mandatory condensation would not allow decondensation under physiological conditions. Woldringh et al. suggest that coupled transcription-translation-translocation forces change the state of condensation of the DNA, allowing decondensation under certain circumstances. We suggest that decondensation cannot occur and it is *condensed* parts or entireties of the nucleoid that are moved by the transcription-translation-translocation forces.

5.3. Metabolic buffering/cellular homeostasis

We have previously argued that macromolecular crowding is a significant contributor to cellular homeostasis[2]. The sta-

[2] The term homeostasis is used in the general sense of helping to maintain a relatively stable internal environment with no implications of negative feedback mechanisms like those which commonly control homeostatic systems.

bilizing effect was suggested to arise from such massive shifts in reactions between macromolecules under crowded conditions that the reactions would be little affected by changes in local environment, an effect we termed 'metabolic buffering' [44,49] and used to rationalize certain experimental discrepancies between in vitro and in vivo properties of the *lac* system in *E. coli* [2]. Mandatory condensation is a similar concept in that it is also a result of macromolecular crowding and it is also suggested to decrease the response of cellular components to changes in the internal cellular environment, but is different in that a cellular structure, the nucleoid, can be affected rather than a cellular reaction. It is proposed that mandatory condensation will stabilize nucleoid structure and thereby increase cell survival over a wide range of cellular conditions and environmental changes.

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