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Review

Macromolecular crowding effects on macromolecular interactions: some implications for genome structure and function

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Contents

I. Introduction

Living systems function at high concentrations of macromolecules [1,2]. Such concentrations must cause large macromolecular crowding effects that can affect the structure and function of cellular macromolecules in a number of ways. I will emphasize one such way, namely enhancement of macromolecular association, which seems to be particularly and directly relevant to questions of genome structure and function. Other aspects of crowding such as effects on conformation or solubility, which may well be relevant, have been discussed in general terms [3–5].

The potential for enhanced association between macromolecules in crowded solutions was recognized some years ago [6–9]. This effect of crowding is now a firmly based experimental observation; among the dozens of in vitro crowding studies, there is not one of which I am aware which serves as a counter-example. Since many regions of cells are crowded with high

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Abbreviations: PEG, polyethylene glycol; PVA, polyvinyl alcohol.

concentrations of macromolecules, there is good reason to think that this tendency toward increased interaction of macromolecules will not only occur in model systems, but will also be widespread in living systems. These premises have given rise to the general thesis of this review, namely that *crowding effects on interactions between cellular macromolecules will tend to increase the rates and extents of associations of those macromolecules*. Note that it is the interactions between *large* molecules that are particularly susceptible to crowding effects. It is exactly because the major components of the genome are large molecules that we expect many structural and functional aspects of the genome to be sensitive to crowding.

The suggested crowding-enhancement of association of cellular macromolecules may have wide-spread consequences in the cell. In this review, I will pursue some of these ideas as they impact on the genome; although most of the examples are from prokaryotes, their application is clearly more general.

II. The source of crowding effects

II-A. Terminology

Macromolecular crowding or simply *crowding* will refer to excluded volume effects, i.e., to non-specific steric effects, on a *test particle* (a macromolecule) due to the presence of a *background* of other particles (also macromolecules), often present at high concentrations (Fig. 1). A solution containing a high total concentration of macromolecules will be termed a *crowded solution*.

II-B. Macromolecular crowding

Crowding effects arise because two objects cannot occupy the same space at the same time. The volume excluded to one object by a second object can be much larger than their combined physical volumes because their peripheries must bump into each other before their volumes start to overlap [1]. The situation at higher concentrations of spheres becomes complex as the excluded volumes overlap [10]. Crowding effects in any given system will be a function of the shapes, sizes and numbers of both the test particles and the background particles.

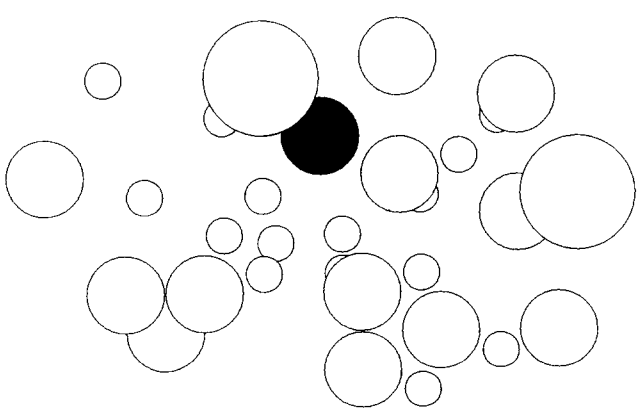

Fig. 1. A test particle in a background distribution of particles.

Calculation of these crowding effects generally supports the crowding-enhancement of association reactions that was suggested in the introduction [3,4,8]. Based upon such calculations, Minton has predicted that crowded conditions will indeed increase interactions between particles, irrespective of particle shape [3,4]. The calculated effects can be very large. For example, the equilibrium constant for dimerization of a protein of 40 kDa is calculated to increase by approx. 8- to 40-fold for the crowding estimated for *Escherichia coli* cytoplasm, while the constant for tetramer formation increases by approx. 10^3- to 10^5-fold [2]!

Berg [11] has proposed a modified form of scaled particle theory calculations in which solvent is introduced as an explicit component. His results differ from Minton's in two important respects. First, he predicts greatly reduced (but still very large) shifts due to crowding under many circumstances. Second, he predicts a shape dependence of the crowding effect, which in one case (a model for dimerization in which two spherical monomers are in close apposition) actually favors dissociation.

As noted in the Introduction, both rates and extents of macromolecular association reactions can be enhanced by crowding. Reaction rates must ultimately decrease at some level of crowding due to decreased rates of diffusion (the 'sieving effect' of Laurent [6]; Refs. 5,12). It is not obvious what concentrations of background molecules will cause this decrease in a given system. Because of these various caveats and complications, it is crucial to examine experimental results. The experimental results reviewed in the next section indicate a general response of macromolecular systems to crowding.

III. Crowded solutions increase interactions between macromolecules in model studies

III-A. Criteria for crowding in model systems

Experimental analysis of crowded reaction mixtures is generally more difficult and more prone to artifact than is analysis of the corresponding dilute solution

[1] For example, consider dilute mixtures of uniform impenetrable spheres of volume $V = 4\pi R^3/3$. The centers of any two of these spheres cannot get closer to each other than the sum of their radii, $2R$, so that the presence of one sphere makes a volume of $4\pi(2R)^3/3$, or $8V$, unavailable to the center of a second sphere [10].

mixtures. In crowding studies, effects are often elicited by addition of very high concentrations of macromolecules, frequently exceeding 200–300 mg/ml. It is fortunate that the effects of crowding are commonly large enough (often one or more orders of magnitude) that analytical difficulties do not obscure the results.

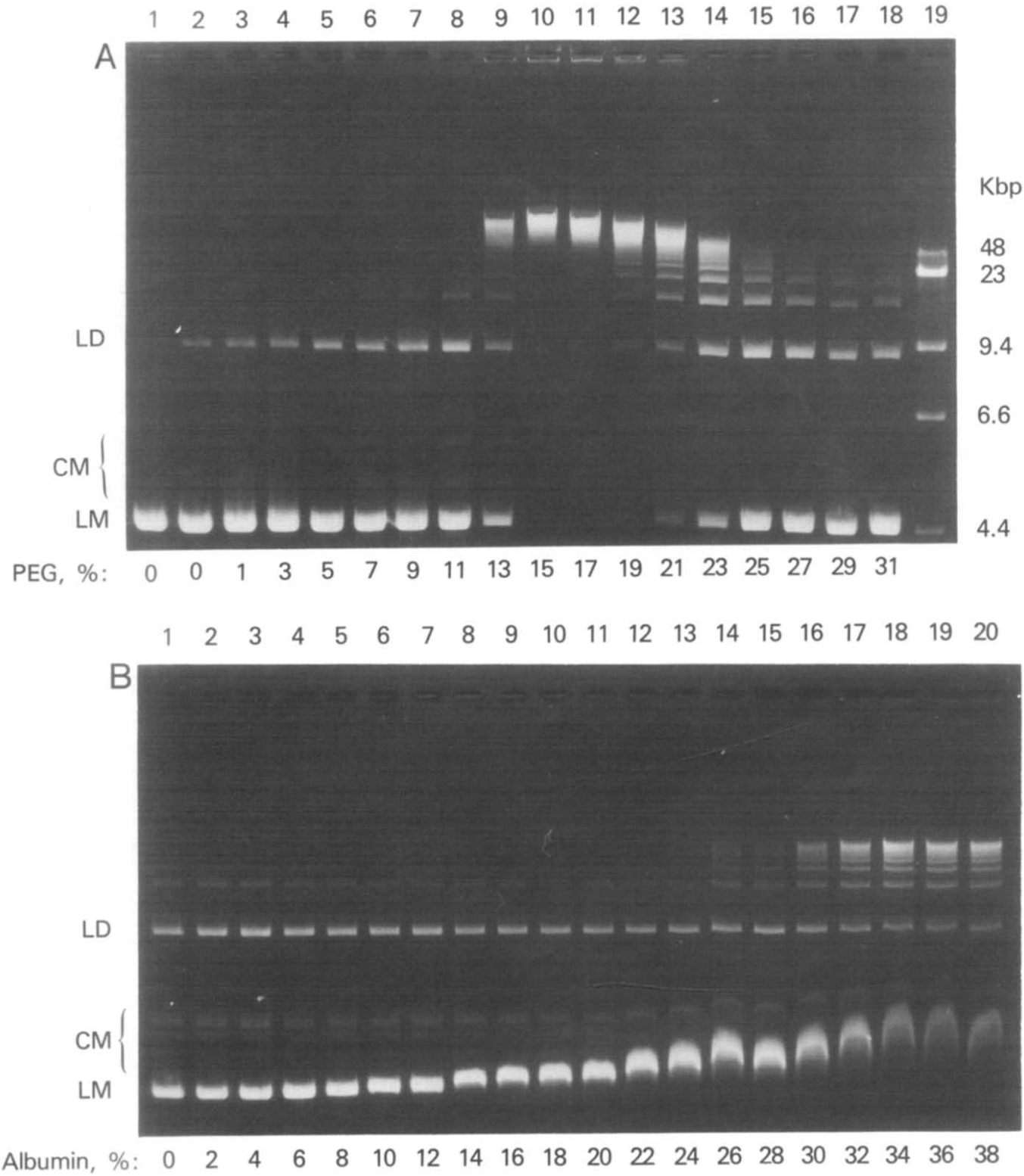

Fig. 2. Effects of PEG or serum albumin on blunt-end ligation of DNA by T4 DNA ligase. (A) Crowding with PEG 8000; (B) crowding with bovine plasma albumin. T4 DNA ligase (1.25 units) is present except for lanes 1 and 19 of (A). DNA standards are in lane 19 of (A). The DNA substrate being ligated is *Pvu*II nuclease-digested pBR322. The substrate is indicated as linear monomer (LM); ligation products include circular monomers (CM), linear dimer (LD) and larger species. From Ref. 26.

Even if analyses indicate large changes in rates or equilibria due to added macromolecules, it is not a trivial problem to determine if these effects come from crowding or from specific interactions between the test particles and the background particles. The following criteria for crowding in model systems may be useful in this regard:

(1) The same phenomenon should be elicited by more than one crowding agent. Crowding is in principle a non-specific effect, so a variety of background macromolecules should yield similar effects; compare, for example, Figs. 2A and B [2]. There is no immediately obvious way of deciding if the effect of a high concentration of a single type of background molecule is due to crowding or to a specific interaction of the background with the test particle (or even due to interaction with an impurity in the background materials, which may become of importance because of the very large amounts of background materials generally added). Further, background molecules are subject themselves to crowding effects which may increase their association with each other and thus diminish their crowding potential (see 3 below). The use of contrasting types of background materials such as hydrophilic polymers (e.g., polyethylene glycol, polyvinyl alcohol), polysaccharides (e.g., dextran, Ficoll, glycogen), or various purified proteins may be helpful in the recognition of such effects [3].

(2) Increasing the concentration of the background should cause disproportionately larger increases in the phenomenon.

(3) The effect of the size of the background particles should be consistent with crowding: big particles should cause a bigger effect than small particles when compared at the same number density, but the reverse should hold when compared at the same weight concentration. Appropriate monomers or oligomers provide valuable controls.

III-B. Experimental studies of crowding in vitro

Minton and I recently tabulated some 31 model systems in which concentrated macromolecular backgrounds were tested for effects on rates or equilibria [5]. The systems reflect interactions between proteins, between proteins and nucleic acids, or between nucleic acids. In all cases, the addition of macromolecular backgrounds appears to cause increased interactions as judged either by increased rates of reaction, by shifts of equilibrium positions in the direction of association, or by increased processivity [4].

In the following paragraphs, I have listed and commented on those examples which involved nucleic acids as well as on several new citations. The wide variety of enhanced interactions should be noted.

Renaturation and stability of double-stranded nucleic acids

Rates of renaturation of DNA are increased by 1–2 orders of magnitude under crowded conditions [14,15].

Backgrounds of dextrans or PEG raise the T_m values of poly(dAT) by ~3–10°C [16] and poly(rI)·poly(rC) ~1–2°C [17].

Nonenzymatic cohesion of DNA

The rate of nonenzymatic cohesion between the 'sticky' ends of λ DNA is increased by 1–3 orders of magnitude by crowding with albumin or Ficoll [18] or with PEG [18,19].

Enzymatic ligation of DNA or RNA

The rate of enzymatic joining of DNA can be enormously increased by the addition of a macromolecular background. The stimulation varies with the DNA structure being ligated, from relatively minor effects on the rate of *intramolecular* joining at a 'nick' in one strand of double-stranded DNA [20,21], to orders-of-magnitude stimulation of *intermolecular* ligation of double-stranded DNA molecules [20–25]. Crowding effects on intermolecular ligation of blunt-end DNA duplexes are particularly large, the rate being increased from a weak reaction to a strong reaction for T4 DNA ligase (Fig. 2 A,B) or from a non-detectable reaction for *E. coli* or rat liver DNA ligases to easily detectable reactions [20,26].

The effects of high concentrations of monovalent cations or of temperature changes on the PEG-stimulated ligation of T4 DNA ligase and *E. coli* DNA

[2] Polyethylene glycol (PEG) is very commonly used to cause crowding effects, sometimes without comparison with other background macromolecules. This wide usage reflects the many favorable attributes of PEG such as its non-charged structure, relative stability, commercial availability in a range of molecular weights, and a high solubility with relatively low viscosity. Users of PEG with DNA should be alert to potential artifacts from the tendency of solutions containing PEG and similar polymers to form a second, DNA-rich phase of 'psi DNA' [13].

[3] We have recently also employed very concentrated cytoplasmic extracts from *E. coli* as backgrounds for studies of several reactions involving DNA (Murphy and Zimmerman, unpublished results).

[4] In several cases, systems were inhibited by higher background concentrations, apparently becoming diffusion-limited (section II-B). For example, Laurent [6] found the reaction of hyaluronate lyase to be inhibited at higher PEG concentrations; however, at lower PEG concentrations, the K_m for hyaluronic acid showed a small decrease, a change in the direction expected for increased association. Another possible example of diffusion-limitation can be seen in the decreased enzymatic ligation of DNA at high concentrations of PEG in Fig. 2A; note the massive stimulation at somewhat lower PEG concentrations.

ligase [27,28] are examples of extension of the range of conditions under which an enzyme can function because of crowding, a homeostatic effect of crowding further discussed in section V-C.

A number of joining reactions on single-stranded oligodeoxyribonucleotides or oligoribonucleotides by T4 RNA ligase are increased by one or more orders of magnitude under crowded conditions [29,30].

Enzymatic catenation of DNA circles

Topoisomerase I of *E. coli* catalyzes rapid and extensive catenation in mixtures of supercoiled and gapped circular DNA in the presence of 6–8% PVA or PEG [31]. Removal of polymer led to a reversal of catenation by the enzyme.

Enzymatic supercoiling of DNA

The 'reverse gyrase' from *Sulfolobus acidocaldarius* forms positive supercoils more rapidly, to a higher limit, and in a more processive manner in the presence of 10% PEG 6000 [32].

Enzymatic DNA transposition

The rate of in vitro transposition of Mu-ended plasmids is increased by more than two orders of magnitude by a hydrophilic polymer [33]. A requirement for a polymer was also noted for the in vitro reaction of transposon Tn3 [34].

RecA protein-promoted DNA strand exchange

An acceleration of *recA*-protein promoted strand exchange at lower, presumed more physiological Mg^{2+} concentrations occurs in the presence of PEG or PVA; the apparent affinity of *recA*-protein for single-stranded DNA is also increased under such conditions [35].

T4 polynucleotide kinase

An orders-of-magnitude crowding enhancement of the reaction rate of T4 polynucleotide kinase occurs, depending upon the nature of the DNA substrate and the crowding agent; a stabilization of oligomeric enzyme under crowded conditions was inferred but not demonstrated [36,37].

DNA polymerase activity

A variety of DNA polymerase-associated reactions are stimulated by the presence of macromolecules such as albumin, dextran, Ficoll or PEG [38]; reactions included nick-translation and gap-filling by DNA polymerase I of *E. coli*, nuclease and polymerase activities of the large fragment of that enzyme, and polymerization by T4 DNA polymerase. The stimulation increases the activities over a range of otherwise inhibitory salt concentrations, in essence greatly increasing the range of salt concentration in which high activity is obtainable (Fig. 3). The mechanism of increased activity was

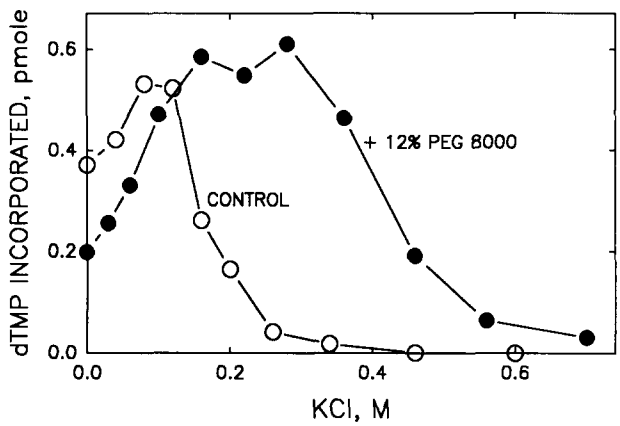

Fig. 3. Crowding by PEG 8000 extends the range of salt concentrations for nick-translation activity by *E. coli* DNA polymerase I. Based upon Ref. 38.

shown to result from increased binding of polymerase to DNA. The nick-translation reaction of *E. coli* polymerase I was subsequently used as a model to show the ability of crowding to extend the range of activity of an enzyme in the presence of a variety of inhibitors (ethidium bromide, urea, formamide) or under inhibitory conditions of temperature or pH [39]. These studies suggested a general concept of crowding as a source of homeostasis that is discussed further in section V-C.

DNA replication systems

Kornberg and his colleagues [40] provided an early demonstration of the stimulatory power of crowding by addition of hydrophilic polymers to a complex in vitro DNA replication system (see also Refs. 41,42). The need for the hydrophilic polymer in several such systems could be removed by supplying higher concentrations of constituent proteins [43,44], fully consistent with a role of crowding in enhancing macromolecular interactions in these systems.

Von Hippel and collaborators [45] analyzed crowding effects on in vitro synthesis of DNA by a T4 replication complex containing the DNA polymerase, single-stranded DNA binding protein and 3 'accessory proteins' (gene 44,45, and 62 proteins). Dilute solution studies demonstrate a critical dependence of assembly of the replication complex on the concentration of one of the accessory proteins, the gene 45 protein. This dependence, which reflects the weak binding of gene 45 protein to the complex under non-crowded conditions, emphasizes an interesting discrepancy between dilute solution studies and in vivo properties, namely that the cellular concentrations of gene 45 protein are insufficient to provide efficient rates of DNA synthesis under dilute solution (non-crowded) conditions [45]. However, addition of PEG or dextrans to the in vitro system increases binding of gene 45 protein by as much

as 50-fold, resulting in essentially stoichiometric binding of gene 45 protein to the complex. This increased binding under crowded conditions removes the discrepancy; the calculated rates of DNA synthesis under crowded conditions are in agreement with in vivo observations. In vivo crowding thus may be a way of reducing the required cellular inventory of gene 45 protein.

A most interesting role for crowding in enhancing the processivity of DNA synthesis by the T4 replication system has been suggested by Reddy et al. [46]. Those authors find that the enhanced binding of gene 45 protein to the T4 DNA polymerase, caused either by crowded conditions or by high concentrations of the gene 45 protein, greatly increases processivity. A mechanism is proposed, in part by analogy with the role of the β subunit of the *E. coli* DNA polymerase III holoenzyme in the processivity of that enzyme [47]: a trimer of the gene 45 protein is postulated to act as a sliding clamp on the DNA; crowding-enhancement of the binding of the polymerase to this sliding clamp is suggested to introduce a high degree of processivity to DNA synthesis. The role of the other two accessory proteins of the T4 replication complex is suggested to be in the ATP-dependent formation of that clamp [5].

Association of ribosomal subunits

Crowding shifts both the equilibrium for formation of 70S ribosomes from 30S and 50S particles and the equilibrium for dimerization of the 70S ribosomes in the direction of association [48].

Condensation of DNA by binding of 'histone-like' proteins

The condensation of DNA due to binding of either purified protein HU or of a fraction of DNA-binding proteins isolated from *E. coli* extracts by chromatography on DNA-cellulose is greatly accentuated by relatively low amounts of hydrophilic polymers. For example, addition of 8% PEG 8000 reduces the amounts of both HU and the DNA-binding protein fraction that are required for DNA condensation by about 10-fold (Murphy and Zimmerman, unpublished experiments). An analogous effect in vivo would serve to reduce the inventory of HU and the other DNA-binding proteins required by the cell.

[5] As part of their study of the T4 DNA replication system, Reddy et al. [46] introduce a technique of affinity chromatography under crowded conditions, as had been suggested earlier by Alberts [9]. Such manipulations under crowded conditions may be particularly useful in future studies of genome-related systems to characterize them not only for their response to crowding and not only for their response to DNA-binding proteins, but as an attempt to characterize the systems under appropriate crowding conditions in the presence of appropriate DNA-binding proteins.

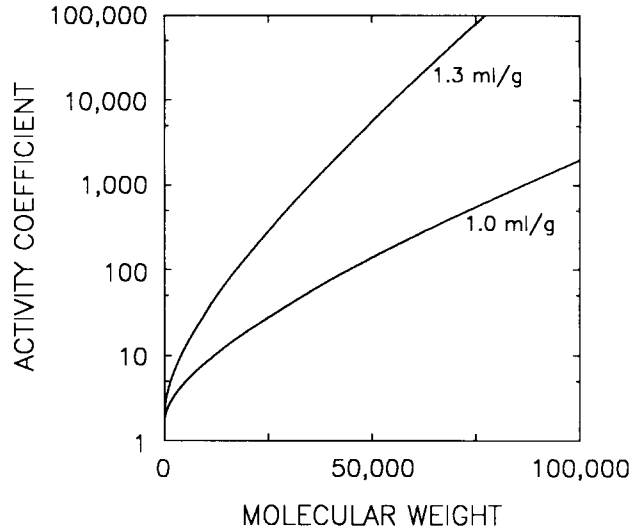

Fig. 4. Estimated activity coefficients for spherical test particles in the cytoplasm of *E. coli*. Based upon Ref. 2; the two curves correspond to a range of effective specific volumes which characterize crowding interactions.

IV. Crowded conditions occur in cells

IV-A. The cytoplasm of Escherichia coli is crowded

It is widely assumed that cells are 'crowded' with high concentrations of soluble macromolecules [1]. However, it is only recently that analyses have been made of an actual cellular material (the cytoplasm of *E. coli*) to estimate its crowding potential. These studies were done by two groups using quite different techniques and assumptions [2,49]. The groups measure or estimate very high concentrations of nominally soluble macromolecules in *E. coli* cytoplasm (e.g., 340 mg/ml of total RNA and protein [2]). Given the complexity of the systems and the dissimilar premises of the two studies (particularly the questions of the proper effective specific volume and the state of aggregation for the background), it is not surprising that there are many differences in the detailed conclusions. However, both groups predict very large crowding effects based upon scaled particle theory calculations for measured or estimated distributions of macromolecules (further discussed in Ref. 5). Estimated activity coefficients for spherical test particles in *E. coli* cytoplasm are shown in Fig. 4; the two curves indicate a range for those effective specific volumes (1.0–1.3 ml/g) which appear to characterize crowding interactions [2]. The activity coefficient, a parameter which acts as a correction to concentration terms, reaches very large values in response to crowded conditions.

IV-B. Crowding and confinement in eukaryotic cells

Similar evaluations of crowding potential have not as yet been done for the various compartments within

eukaryotic cells, although high concentrations of macromolecules characterize spaces such as the mitochondrial matrix [50] and possibly others. Minton [51] has suggested that the enormous numbers of filaments within eukaryotic cytoplasm [52] may engender a related state of *confinement* of test particles, rather than crowding in the generally used sense. Confinement is also expected to increase macromolecular associations, with the interesting difference that the resulting aggregates may tend to be relatively elongated rather than occurring in the compact aggregates favored by crowding [51].

V. Potential crowding effects on the structure and function of the genome

In this section I will consider some implications drawn from the model crowding studies.

V-A. Crowding and the stabilization of condensed regions of DNA

Genomic DNA in both prokaryotes and eukaryotes is restricted in its cellular localization. In eukaryotes, the mechanisms resulting in compaction and localization are relatively well understood: strong binding of the DNA to core particles composed of histones yields linear arrays of nucleosomes which undergo higher order supercoiling, and ultimately are restrained in chromosomes and by nuclear membranes [53]. In prokaryotes, genomic DNA is localized into one or a few bodies called nucleoids [54] without the assistance of membranes or chromosomes. While there are indications of nucleosome-like particles in prokaryotes, the factor(s) which stabilize these or other structural features are dynamic and tend to be lost upon lysis of the cells [55,56]. A variety of studies suggest a structure containing dozens of topologically-separated loops of DNA [54,57–60]. The DNA of the loops is negatively supercoiled, and about half of this supercoiling is restrained, i.e., bound into a supercoiled configuration, presumably by interaction with one or more of the histone-like bacterial proteins.

The basis for the stabilization of the condensed DNA of the nucleoid is uncertain, but either or both of two crowding-associated effects may help to stabilize the compacted DNA within nucleoids: (1) condensation of the DNA due to incompatibility of DNA and added macromolecules, yielding a second DNA-rich phase ('psi DNA' [13]). (2) Crowding-enhanced binding of histone-like DNA-binding proteins (which can cause compaction either directly, or indirectly by favoring supercoiling).

Both Lerman [13] and Pettijohn [61] suggested many years ago that crowding might be involved in the stabilization of nucleoids. Pettijohn [61], referring to the 'psi' DNA described by Lerman [13,62,63], wrote: "It has been observed that repulsive interactions between certain neutral or negatively charged polymers in the solvent and purified DNA can result in collapse of the DNA into an ordered compact state – the ψ-transition... It remains to be seen whether or not analogous interactions between polymers in the cytoplasm and the nucleoid are important in maintaining the nucleoid structure."

A certain level of mono- or divalent salt is required for psi DNA formation, presumably acting at least in part to reduce charge repulsion between DNA; another way to accomplish such a reduction is by binding to the DNA of proteins such as the known 'histone-like' proteins that are enriched in nucleoids [64–68]. These proteins (primarily HU and H1 in *E. coli*) have often been suggested to participate in or actually cause condensation of DNA, but the estimates of the amounts of the proteins available for such a reaction fall short by 5- to 10-fold [56,60,66,69–71]. Our recent studies (Zimmerman and Murphy, unpublished results; see section III-B) indicate that binding of HU and perhaps others of the 'histone-like proteins' is very sensitive to crowding; the amounts of protein required for condensation of DNA can be reduced by an order of magnitude by crowding in model studies. Such crowding effects on DNA-binding proteins may have a multitude of consequences given the many roles of their binding in DNA structure (DNA bending [72]; DNA supercoiling, particle formation [56,69,73]; DNA looping [74] [6].

Condensation of DNA has major implications for cellular DNA function as well as structure. Sikorav and Church [15] provide an interesting theoretical introduction to DNA condensation as well as a number of experimental examples of very large effects of condensation on reaction rates occurring in systems of DNA condensed by various methods. They describe DNA condensation in a multicomponent system as a critical phenomenon, i.e., one in which infinitesimal changes in concentrations or other parameters can cause a transition in conformation of a macromolecule such as that between a random coil and a compact, globular form. They note the ability of crowding effects to cause such critical phenomena and cite the formation of psi DNA by PEG or other polymers [13] as an example. Sikorav and Church measured rates of renaturation of single-stranded DNA and rates of strand-exchange of double-stranded DNA molecules under a number of conditions that condense DNA, including crowding

[6] Looping of DNA with resulting changes in effective *lac* repressor concentration is accentuated by HU [74]. Law et al. [74] note that only a small effect of HU on the looping of DNA is expected at in vivo concentrations of HU; however, crowding may increase the effect of HU in this system also.

with PEG. In all of the cases that they studied, reaction rates increased dramatically under condensing conditions [7]. They conclude that the functional form of DNA in vivo may well be analogous to that present in condensed DNA.

V-B. Crowding and interactions between DNA and proteins

lac repressor-operator interaction

The study of Richey et al. [75] provides a valuable comparison of in vivo results with previous dilute solution properties for two reactions involving DNA-protein interactions, namely *lac* repressor-operator binding and *E. coli* RNA polymerase binding to the λ P_R promoter. Richey et al. found a striking discrepancy: in dilute (i.e., non-crowded) solutions, both of these reactions are exquisitely sensitive to inhibition by added salt [76–80], whereas in vivo both reactions are virtually insensitive to such intracellular salt levels [75].

Our earlier model studies of the reversal of salt-inhibition of DNA polymerase by crowding [38] (section III-B) suggested that crowding effects might be involved in a resolution of this discrepancy. We accordingly calculated the effect of the parameters estimated for cytoplasmic crowding in *E. coli* (section IV-A) on the fraction of exposed *lac* operator, using a modified form of the equilibrium model for *lac* repressor binding described by von Hippel et al. [81] (see Ref. 2 for details of calculation). Such calculated values are compared in Fig. 5 with in vivo observations made over a range of monovalent salt concentrations expressed as KCl. The three panels in Fig. 5 are for wild-type *lac* operator and the two operator mutants analyzed by Richey et al. [75]. Values calculated for the absence of crowding are shown as solid curves and those for the limits of the expected range of crowding are shown as dashed and dotted curves. The in vivo observations are shown as solid points; these observations show the lack of change in *lac* expression as salt concentration is increased within the cells. The in vivo points are clearly in disagreement with the behavior calculated for the uncrowded system (solid lines) but are in reasonable agreement with the behavior calculated for crowding within *E. coli* cytoplasm (broken lines). Given the limits and assumptions of the model, it is only the trends of such calculations which are meaningful. It is therefore most gratifying that the effect of the estimated crowding environment in *E. coli* appears to rationalize this discrepancy between in vitro and in vivo

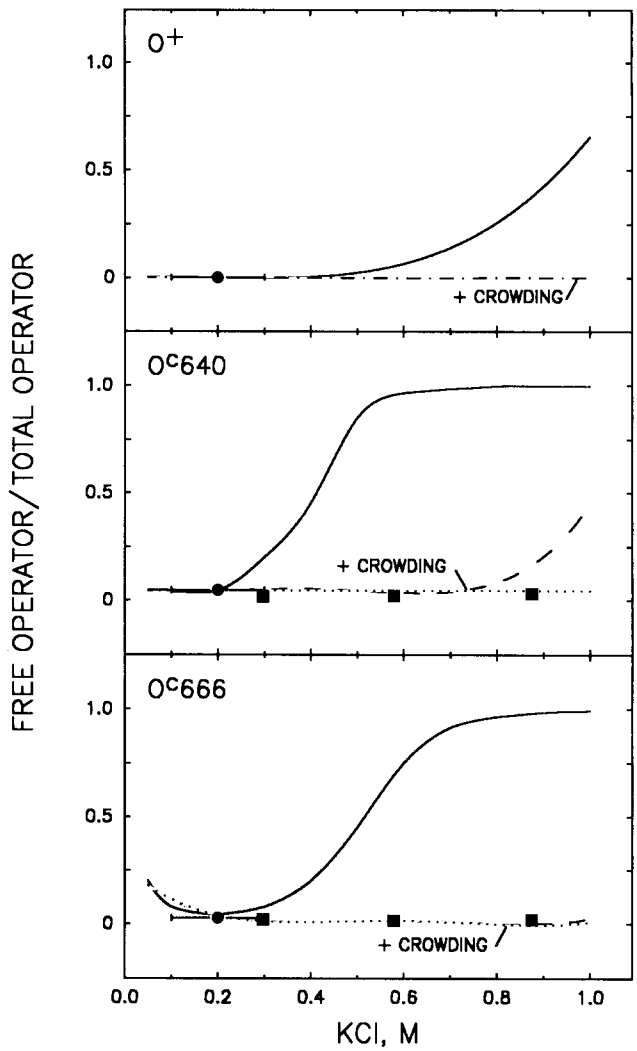

Fig. 5. Crowding and salt effects on the *lac* operator-repressor equilibrium. Points are experimental values from in vivo studies: filled circles, values from Jobe et al. [89] (averaged *P* values plotted arbitrarily at 0.2 M KCl with error bar indicating estimated range of intracellular salt concentrations; filled squares from Richey et al. [75]. Solid lines are calculated assuming no crowding effects. The two broken lines in each panel indicate the range of crowding effects estimated for *E. coli* cytoplasm [2]. The broken lines overlap almost completely in the top and bottom panels (for wild type and O^c666 operators) but less completely for the middle panel (O^c640 operator). See Ref. 2 for details.

results. Certain homeostatic implications of these results are discussed in section V-C.

As noted in section IV-A above, a concurrent study of cytoplasmic crowding effects in *E. coli* was made by Cayley et al. [49] using very different assumptions, experimental techniques, and computational parameters. Despite areas of disagreement, there seems to be major agreement in the important role ascribed to crowding effects as a force that opposes salt-induced dissociation of proteins from DNA.

Crowding and transcription of DNA

RNA polymerase interaction at the λP_R promoter is one of two interactions found by Richey et al. [75] to

[7] We also find a greatly increased rate of a different type of DNA-association reaction, the cohesion of double-stranded DNA molecules bearing the complementary terminal sequences of λ DNA, under conditions causing DNA condensation (Murphy and Zimmerman, unpublished results).

be insensitive to salt inhibition in vivo although exquisitely sensitive to salt inhibition in dilute (non-crowded) solution studies in vitro (see above). Cayley et al. [49] have estimated the in vivo effects on this system, using their crowding parameters for *E. coli* cytoplasm. Their model assumes that increasing the intracellular salt concentration causes two counterbalancing effects: (1) an increased interaction between promoter and polymerase because of increased crowding from decreased cytoplasmic volumes, and (2) decreased association of promoter and polymerase due to the dissociative effects of high intracellular salt concentrations. Various extents of aggregation were assumed for the inferred background distribution in their calculation. Cayley et al. [49] conclude that "No other thermodynamic effect besides crowding appears large enough to compensate for the large polyelectrolyte effect of increasing $[K^+]$ on protein-nucleic acid interactions."

Ishihama [82] has emphasized the importance of the interaction of a variety of transcription factors and sigma factors with RNA polymerase for determining which genes will be expressed under particular environmental conditions. It would be most interesting to test the effects of crowding on such interactions.

DNA-binding proteins

The histone-like DNA-binding protein HU interacts with a large number of systems; HU can differentially facilitate binding of proteins [83], binds to 30S subribosomal particles [84] and affects transposition, replication, and transcription (for references, see Refs. 67,85); interactions in these systems generally involve a number of other macromolecules and can be very complex. We have recently observed a strong crowding effect on the binding of HU to DNA (see section III-B). Given the general tendency of macromolecular associations to be affected by crowding as emphasized here, as well as the tendency of HU to oligomerize [84,86], it seems likely that the parameters of at least some of these systems will be very different when measured in crowded circumstances, either in vivo or in vitro, instead of under non-crowded dilute solution conditions. Interactions of other non-sequence specific DNA-binding proteins, both eukaryotic and prokaryotic [87], may well be crowding-sensitive, with widespread structural and functional consequences.

V-C. Crowding and homeostasis

Virtually all cellular reactions are affected by macromolecular interactions. If one accepts the general thesis of this review, namely that crowding favors association between macromolecules, then crowding will shift cellular reactions in ways that reflect such increased association. In some cases, the shifts may make the reactions more resistant to change by various perturbants. The DNA polymerase-DNA interaction (section III-B) and the *lac* repressor-operator interaction (section V-B) are examples. In the former case, crowding tends to keep the polymerase bound to its template-primer DNA, maintaining polymerase activity in the presence of otherwise inhibitory levels of salt or other perturbants. In the case of the *lac* system, crowding is suggested to maintain the relative binding to specific vs. nonspecific DNA sites, making the level of expression of the gene less sensitive to perturbations such as increased salt concentrations.

In these examples, the tendency to maintain a relatively stable condition amounts to a homeostatic effect of crowding [8]. We have suggested that such effects may be general within cells, reducing otherwise inhibitory chemical or physical interactions [38,39], a kind of 'metabolic buffering'.

Similar effects seem likely in the interactions of a wide variety of DNA-binding proteins. In particular, the binding of protein HU is relatively salt sensitive [56]; crowding effects on this material (see section III-B) may act to decrease salt effects in the many systems in which it is a participant.

VI. Concluding comments

I conclude by suggesting the following generalizations, based upon material reviewed here:

(i) Crowding tends to be a synthetic force in macromolecular systems, in the sense of favoring macromolecular associations.
(ii) Crowding is a way of reducing the required cellular inventory of components of crowding-enhanced reactions.
(iii) Crowding will have widespread structural, functional, and homeostatic consequences in vivo.

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References

1 Fulton, A.B. (1982) Cell 30, 345–347.
2 Zimmerman, S.B. and Trach, S.O. (1991) J. Mol. Biol. 222, 599–620.
3 Minton, A.P. (1981) Biopolymers 20, 2093–2120.
4 Minton, A.P. (1983) Mol. Cell. Biochem. 55, 119–140.

[8] The term homeostasis is used here in the general sense of maintenance of a relatively stable internal environment [88] and has no implications of negative feedback mechanisms like those which commonly control homeostatic systems.

5 Zimmerman, S.B. and Minton, A.P. (1993) Annu. Rev. Biophys. Biomol. Struct. 22, 27–65.
6 Laurent, T.C. (1971) Eur. J. Biochem. 21, 498–506.
7 Comper, W.D. and Laurent, T.C. (1978) Physiol. Rev. 58, 255–315.
8 Nichol, L.W., Ogston, A.G. and Wills, P.R. (1981) FEBS Lett. 126, 18–20.
9 Alberts, B.M. (1984) Cold Spring Harb. Symp. Quant. Biol. 49, 1–12.
10 Tanford, C. (1961) Physical Chemistry of Macromolecules, pp. 192–196, Wiley & Sons, New York.
11 Berg, O.G. (1990) Biopolymers 30, 1027–1037.
12 Minton, A.P. (1990) in Structural and Organizational Aspects of Metabolic Regulation (Srere, P.A., Jones, M.E. and Mathews, C.K., eds.), pp. 291–306, Wiley-Liss, New York.
13 Lerman, L.S. (1971) Proc. Natl. Acad. Sci. USA 68, 1886–1890.
14 Wieder, R. and Wetmur, J.G. (1981) Biopolymers 20, 1537–1547.
15 Sikorav, J.-L. and Church, G.M. (1991) J. Mol. Biol. 222, 1085–1108.
16 Laurent, T.C., Preston, B.N. and Carlsson, B. (1974) Eur. J. Biochem. 43, 231–235.
17 Woolley, P. and Wills, P.R. (1985) Biophys. Chem. 22, 89–94.
18 Zimmerman, S.B. and Harrison, B. (1985) Nucleic Acids Res. 13, 2241–2249.
19 Louie, D. and Serwer, P. (1991) Nucleic Acids Res. 19, 3047–3054.
20 Zimmerman, S.B. and Pheiffer, B.H. (1983) Proc. Natl. Acad. Sci. USA 80, 5852–5856.
21 Teraoka, H. and Tsukada, K. (1987) J. Biochem. (Tokyo) 101, 225–231.
22 Arrand, J.E., Willis, A.E., Goldsmith, I. and Lindahl, T. (1986) J. Biol. Chem. 261, 9079–9082.
23 Rabin, B.A. and Chase, J.W. (1987) J. Biol. Chem. 262, 14105–14111.
24 Aoufouchi, S., Hardy, S., Prigent, C., Philippe, M. and Thiebaud, P. (1991) Nucleic Acids Res. 19, 4395–4398.
25 Takahashi, M. and Uchida, T. (1986) J. Biochem. (Tokyo) 100, 123–131.
26 Pheiffer, B.H. and Zimmerman, S.B. (1983) Nucleic Acids Res. 11, 7853–7871.
27 Hayashi, K., Nakazawa, M., Ishizaki, Y., Hiraoka, N. and Obayashi, A. (1985) Nucleic Acids Res. 13, 7979–7992.
28 Hayashi, K., Nakazawa, M., Ishizaki, Y. and Obayashi, A. (1985) Nucleic Acids Res. 13, 3261–3271.
29 Harrison, B. and Zimmerman, S.B. (1984) Nucleic Acids Res. 12, 8235–8251.
30 Tessier, D.C., Brousseau, R. and Vernet, T. (1986) Anal. Biochem. 158, 171–178.
31 Low, R.L., Kaguni, J.M. and Kornberg, A. (1984) J. Biol. Chem. 259, 4576–4581.
32 Forterre, P., Mirambeau, G., Jaxel, C., Nadal, M. and Duguet, M. (1985) EMBO J. 4, 2123–2128.
33 Mizuuchi, K. (1983) Cell 35, 785–794.
34 Ichikawa, H. and Ohtsubo, E. (1990) J. Biol. Chem. 265, 18829–18832
35 Lavery, P.E. and Kowalczykowski, S.C. (1992) J. Biol. Chem. 267, 9307–9314.
36 Harrison, B. and Zimmerman, S.B. (1986) Anal. Biochem. 158, 307–315.
37 Harrison, B. and Zimmerman, S.B. (1986) Nucleic Acids Res. 14, 1863–1870.
38 Zimmerman, S.B. and Harrison, B. (1987) Proc. Natl. Acad. Sci. USA 84, 1871–1875.
39 Zimmerman, S.B. and Trach, S.O. (1988) Biochim. Biophys. Acta 949, 297–304.
40 Fuller, R.S., Kaguni, J.M. and Kornberg, A. (1981) Proc. Natl. Acad. Sci. USA 78, 7370–7374.
41 Tsurimoto, T. and Matsubara, K. (1982) Proc. Natl Acad. Sci. USA 79, 7639–7643.
42 LeBowitz, J.H. and McMacken, R. (1984) Nucleic Acids Res. 12, 3069–3088.
43 Kaguni, J.M. and Kornberg, A. (1984) Cell 38, 183–190.
44 LeBowitz, J.H., Zylicz, M., Georgopoulos, C. and McMacken, R. (1985) Proc. Natl. Acad. Sci. USA 82, 3988–3992.
45 Jarvis, T.C., Ring, D.M., Daube, S.S. and Von Hippel, P.H. (1990) J. Biol. Chem. 265, 15160–15167.
46 Reddy, M.K., Weitzel, S.E. and Von Hippel, P.H. (1993) Proc. Natl. Acad. Sci. USA 90, 3211–3215.
47 Kong, X.-P., Onrust, R., O'Donnell, M. and Kuriyan, J. (1992) Cell 69, 425–437.
48 Zimmerman, S.B. and Trach, S.O. (1988) Nucleic Acids Res. 16, 6309–6326; erratum: Nucleic Acids Res. 16, 9892.
49 Cayley, S., Lewis, B.A., Guttman, H.J. and Record, M.T., Jr. (1991) J. Mol. Biol. 222, 281–300.
50 Srere, P.A. (1980) Trends Biochem. Sci. 5, 120–121.
51 Minton, A.P. (1992) Biophys. J. 63,1090–1100.
52 Gershon, N.D., Porter, K.R. and Trus, B.L. (1985) Proc. Natl. Acad. Sci. USA 82, 5030–5034.
53 Van Holde, K.E. (1989) Chromatin, Springer-Verlag, New York.
54 Drlica, K. (1987) in *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology (Neidhardt, F.C., ed.), Vol. 1, pp. 91–103, American Society for Microbiology, Washington.
55 Griffith, J.D. (1976) Proc. Natl. Acad. Sci. USA 73, 563–567.
56 Broyles, S.S. and Pettijohn, D.E. (1986) J. Mol. Biol. 187, 47–60.
57 Worcel, A. and Burgi, E. (1972) J. Mol. Biol. 71, 127–147.
58 Pettijohn, D.E. and Hecht, R. (1973) Cold Spring Harb. Symp. Quant. Biol. 38, 31–41.
59 Pettijohn, D.E. and Sinden, R.R. (1985) in Molecular Cytology of *Escherichia coli* (Nanninga, N., ed.), pp. 199–227, Academic Press, New York.
60 Pettijohn, D.E. (1990) Nucleic Acids Mol. Biol. 4, 152–162.
61 Pettijohn, D.E. (1976) Crit. Rev. Biochem. 4, 175–202.
62 Lerman, L.S. (1973) Cold Spring Harb. Symp. Quant. Biol. 38, 59–73.
63 Lerman, L.S. (1973) in Physico-Chemical Properties of Nucleic Acids (Duchesne, J., ed.), Vol. 3, pp. 59–76, Academic Press, New York.
64 Varshavsky, A.J., Nedospasov, S.A., Bakayev, V.V., Bakayeva, T.G. and Georgiev, G.P. (1977) Nucleic Acids Res. 4, 2725–2745.
65 Yamazaki, K., Nagata, A., Kano, Y. and Imamoto, F. (1984) Mol. Gen. Genet. 196, 217–224.
66 Drlica, K. and Rouvière-Yaniv, J. (1987) Microbiol. Rev. 51, 301–319.
67 Pettijohn, D.E. (1988) J. Biol. Chem. 263, 12793–12796.
68 Schmid, M.B. (1990) Cell, 63, 451–453.
69 Rouvière-Yaniv, J., Yaniv, M. and Germond, J.-E. (1979) Cell 17, 265–274.
70 Geider, K. and Hoffmann-Berling, H. (1981) Annu. Rev. Biochem. 50, 233–260.
71 Friedrich, K., Gualerzi, C.O., Lammi, M., Losso, M.A. and Pon, C.L. (1988) FEBS Lett. 229, 197–202.
72 Hodges-Garcia, Y., Hagerman, P.J. and Pettijohn, D.E. (1989) J. Biol. Chem. 264, 14621–14623.
73 Rouvière-Yaniv, J. (1977) Cold Spring Harb. Symp. Quant. Biol. 42, 439–447.
74 Law, S.M., Bellomy, G.R., Schlax, P.J. and Record, M.T., Jr. (1993) J. Mol. Biol. 230, 161–173.
75 Richey, B., Cayley, D.S., Mossing, M.C., Kolka, C., Anderson, C.F, Farrar, T.C. and Record, M.T., Jr. (1987) J. Biol. Chem. 262, 7157–7164.
76 Riggs, A.D., Bourgeois, S. and Cohn, M. (1970) J. Mol. Biol. 53, 401–417.
77 Barkley, M.D. (1981) Biochemistry 20, 3833–3842.

78 Winter, R.B., Berg, O.G. and Von Hippel, P.H. (1981) Biochemistry 20, 6961–6977.
79 Roe, J.-H., Burgess, R.R. and Record, M.T., Jr. (1984) J. Mol. Biol. 176, 495–521.
80 Roe, J.-H. and Record, M.T., Jr. (1985) Biochemistry 24, 4721–4726.
81 Von Hippel, P.H., Revzin, A., Gross, C.A. and Wang, A.C. (1974) Proc. Natl. Acad. Sci. USA 71,4808–4812.
82 Ishihama, A. (1993) J. Bacteriol. 175, 2483–2489.
83 Flashner, Y. and Gralla, J.D. (1988) Cell 54, 713–721.
84 Suryanarayana, T. and Subramanian, A.-R. (1978) Biochim. Biophys. Acta 520, 342–357.
85 Kornberg, A. and Baker, T.A. (1992) DNA Replication, 2nd Edn., Freeman, New York.
86 Rouvière-Yaniv, J. and Kjeldgaard, N.O. (1979) FEBS Lett. 106, 297–300.
87 Serrano, M., Salas, M. and Hermoso, J.M. (1993) Trends Biochem. Sci. 18, 202–206.
88 Sherwood, L. (1993) Human Physiology: From Cells to Systems, p. 6, West Publishing Co., Minneapolis.
89 Jobe, A., Sadler, J.R. and Bourgeois, S. (1974) J. Mol. Biol. 85, 231–248.