PROKARYOTIC DNA IN NUCLEOID STRUCTURE

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I. INTRODUCTION

One of the primary characteristics distinguishing prokaryotic from eukaryotic cells is the absence of a nucleus with a clearly defined nuclear membrane. In prokaryotic cells the DNA is condensed into a structure called the nucleoid. This structure has also been referred to at times as the nuclear body, prokaryotic nucleus, bacterial chromosome, folded genome, or folded bacterial chromosome. The nomenclature sometimes becomes confusing because unfolded bacterial DNA free of other components of the nucleoid has also been referred to as the bacterial chromosome. To avoid such confusion, it would be preferable to reserve the terms nucleoid or bacterial chromosome to describe the condensed prokaryotic DNA structures which have some features analogous to the eukaryotic metaphase chromosome and condensed interphase chromatin. If this convention is followed, the terms "folded chromosome" or "folded genome" become ambiguous because they could equally mean "folded nucleoid." These latter terms will, therefore, be avoided throughout this article.

Since there are no membranes separating the nucleoid from other components of the cytoplasm, it appears that interactions within the structure must define its shape and size. In this sense the nucleoid bears a superficial resemblance to the metaphase chromosome of eukaryotic cells. The structure of the nucleoid is of considerable interest to biochemists and cell biologists for a number of reasons.

1. As complicated as the nucleoid is, its components and size are simple compared to eukaryotic chromosomes; therefore, one might hope that its structure could be more easily determined.

2. Very little is known about the three-dimensional structure of packaged DNA in any chromosome. It seems likely that the interactions organizing tertiary structure in packaged DNAs may have common functional elements which may first be elucidated in simpler systems.

3. The influence of the tertiary structure of DNA on the genetic expression and replication of genes is poorly understood. Some general understanding may emerge from studies of the simpler chromosomes where the genetics has been better determined.

In the past 6 years there has been a substantial increase in research directed at the structure of

bacterial nucleoids. Much of this has utilized isolated nucleoids from *Escherichia coli*. There have been significant developments in elucidating the nucleoid structure as well as in the technology for purifying and characterizing large-packaged DNA molecules. In this article findings will be reviewed which, in the author's opinion, are representative of these developments. No attempt will be made to exhaustively cover the field. An emphasis will be placed on analysis of the results and the interpretations which may be drawn from them. One major section of the review will be concerned with the methodology of nucleoid purification. Although parts of that discussion involve technical detail, it is believed that such a discussion is critical to the field at this time. I will argue that some of the controversies that exist probably are derived from structural or compositional differences in the isolated nucleoids, some of which result from differences in isolation procedures. For readers who merely seek an analysis and overview of the findings, the sections on isolation of nucleoids and membrane-associated nucleoids may be omitted without breaking the continuity.

II. MICROSCOPIC OBSERVATIONS OF THE INTRACELLULAR NUCLEOID

Studies of the nucleoid in the cell with optical or electron microscopes have been essential in defining the existence, gross structure and the membrane association of the nucleoid. Although some early investigations with the light microscope concluded that bacteria had nuclear-like regions (for examples, see References 1 and 2), at that time it was not possible to unequivocally distinguish the observed structures from other granular bodies; the existence of a bacterial nucleus was not generally accepted at the time (1941) of Lewis' review.[3] In the late 1930s and early 1940s, researchers using Feulgen's stain clearly observed nuclear structures which divided and segregated during cell division in the manner expected of a chromosome (for examples, see References 4 to 6). Piekarsky seems to have originated the term *nucleoid* (see Reference 3). Later it was found that similar dividing and segregating structures could be seen in living cells via phase microscopy, demonstrating that the nucleoid is not merely some artifact of fixation, dehydration, or staining.[7,8]

Examination of bacterial sections in the electron microscope has revealed the nucleoid structure in more detail. Bundles of fibers with dimensions similar to those of DNA can be seen transversing the sections.[9,10] Examples of such micrographs are shown in Figure 1. Regions of attachment of the nucleoid to the cell membrane-wall complex are also seen in appropriate sections at sites of mesosome association.[10-12] The structure of the nucleoid in vivo and its membrane attachment as examined with the electron microscope have been reviewed by Ryter[11] and recently by Leibowitz and Schaechter.[12] Since few fundamentally new findings have emerged by this approach, subsequent to these reviews, extensive additional discussion is not required here. It will suffice to summarize a few important findings.

The size and shape of nucleoids vary in different prokaryotic organisms and in the same organism growing under different conditions. For example, the nucleoids of *Bacillus subtilis* (particularly in some strains) are smaller and more compact than those of *E. coli*[10,11,14] (see Figure 1). This difference seems to be characteristic of most Gram-positive and Gram-negative bacteria.[11] In part it comes about because there is less DNA per genome in *B. subtilis*, but this does not entirely account for the difference, and it seems that the DNA may be more densely packed in the nucleoids of *B. subtilis* as seen by electron microscopy.[14] Nucleoids in other prokaryotes such as the blue-green algae also appear to vary in size and density.[15] In *E. coli* the nucleoids change their shape, and the apparent "surface" of the structures appears more convoluted and uneven when cells are grown in richer medium promoting faster growth.[14] By contrast, nucleoids in *B. subtilis* are small in rapidly growing cells and, if anything, somewhat larger when cells are grown in media resulting in slower growth rates. In some cases inhibition of cellular protein or RNA synthesis can, depending on the inhibitor, result in changes of shape or size of the nucleoid[9,14,16,17,85] (see also Figure 1).

The picture that emerges from these studies is *not* that of a static nucleoid organized in a rigid crystalline-like structure; rather, they suggest a chromosomal organization that is dynamic in its response to different metabolic states of the cell. The electron micrographs provide few clues about the molecular nature of these rearrangements. However, they do indicate that there may *not* be a

simple, fundamental unit of *long-range* chromosomal condensation that is monotonically identical in all prokaryotic chromosomes. At least the number per unit of DNA and the size of these hypothetical units of condensation may vary in different prokaryotes (since the chromosomes seem to vary in DNA packing density). It should also be kept in mind that the fixation, dehydration, and other manipulations essential for electron microscopy may themselves have

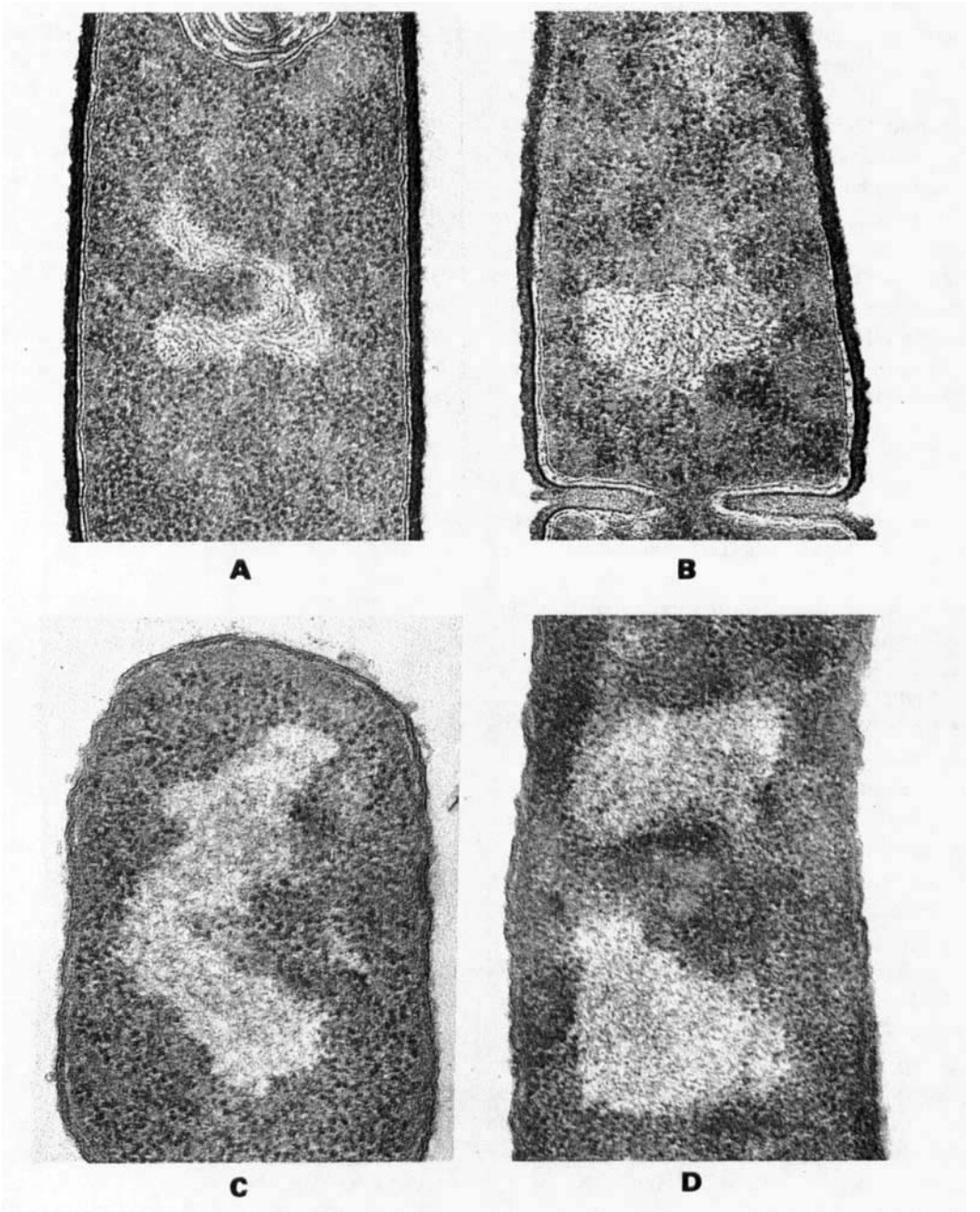

FIGURE 1. Thin sections showing nucleoid of *B. subtilis* and *E. coli* in cells grown under different conditions. (a) *B. subtilis* grown in complex medium. Magnification × 100,000. (b) *B. subtilis* after addition of puromycin. Magnification × 90,000. (c) *E. coli* grown in complex medium. Magnification × 90,000. (d) *E. coli* grown in synthetic medium. Magnification × 80,000. (From Ryter, A. and Chang, A., *J. Mol. Biol.*, 98, 797, 1975. With permission.)

influenced the appearance of the nucleoids. This possibility has previously been the subject of discussion (for examples, see References 12 and 18).

While electron microscopic studies of the intracellular nucleoid have provided quite informative descriptions of the chromosome, this approach has not been particularly successful (and with present techniques it is not likely to be) in delineating the tertiary structure of the DNA. The dense packaging of the DNA and the dense background due to other cytoplasmic components preclude a detailed examination of the DNA conformation and its molecular interactions in the chromosomes. As will be described below, electron microscopy of *purified* nucleoids is presently proving more fruitful for studies of DNA conformation.

Further review of electron microscopic and autoradiographic studies of the intracellular nucleoid are deferred to the section on transcription of the nucleoid.

III. ISOLATION OF NUCLEOIDS

Detailed biochemical and biophysical investigations of nucleoid structure first required the development of methods for isolating the nucleoids. A useful procedure should maintain the state of condensation of the DNA similar to its organization in the cell. In the 1950s methods were first developed for isolating "nuclear material" or "nuclear bodies" from both *E. coli*[19] and *Bacillus megaterium*.[20] The methods were rather rough by today's standards, in the case of *E. coli* involving disruption of the cells by sonication. The procedure developed for *B. megaterium* was more gentle, and the structure separated by centrifuging into a pellet had several properties expected of condensed DNA in nucleoids.[21,22] The "nuclear gel" in the pellets was greatly enriched for pulse-labeled mRNA,[21] and condensed DNA structures at various degrees of aggregation could be observed in the electron microscope.[22] Although the stabilizing influence of high concentrations of cations was recognized in this early work, the isolated material in gel-like pellets was "highly viscous,"[22] indicative of at least some DNA unfolding.

In the early 1970s it was found that the DNA in lysates of *E. coli* maintains nonviscous, particle-like properties if the cells are gently opened in solutions containing high concentrations of counterions.[23,24] Any cation that was tried (Li^+, Na^+, K^+,NH_4^+,Mg^{++}, or polyamines such as spermidine) enhanced the stability of the condensed DNA.[32] It was then possible to separate the rapidly sedimenting nucleoids from other components of the lysate by centrifugation in sucrose gradients. The sedimentation profile of the DNA, while broader than that of a monodisperse species, was sufficiently narrow to suggest that the particles had a limited size range similar to that of the in vivo nucleoid[24,25] (see Figure 2 for representative sedimentation profiles). Subsequently it was demonstrated that the heterogeneity was attributable to variation in the size of nucleoids having different DNA contents because they are at different stages of chromosome replication.[25] When nucleoids were isolated from cells which were aligned in their DNA replication cycle by amino acid starvation, the sedimentation profile became nearly monodisperse. It should be mentioned, however, that this latter finding may

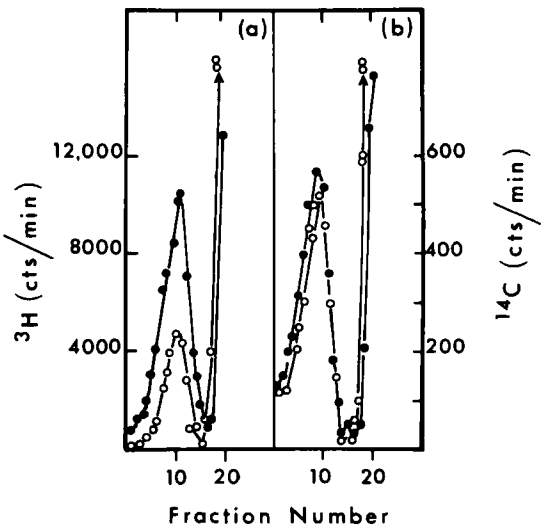

FIGURE 2. Nucleoid isolation by sedimentation on sucrose gradients. A culture of *E. coli* strain D-10 growing exponentially was incubated for 30 min with [^{14}C] thymidine to label the DNA for 3 min with [5-^{3}H] uridine just before harvesting to label nascent RNA. Cells were lysed with lysozyme and the detergents Brij®, Sarkosyl®, and deoxycholate in the presence of 1.0 *M* NaCl using methods already described.[30] Half of the cells (a) were incubated for 5 min at 24°C during lysis and the other half (b) at 4°C for 30 min. The lysates were then layered on sucrose gradients containing 1.0 *M* NaCl. (a) The membrane-free nucleoids centrifuged for 30 min at 17,000 rpm at 4°C. (b) The membrane-associated nucleoids centrifuged for 7 min at 17,000 rpm at 4°C. (•) [^{14}C] DNA; (o) [^{3}H] RNA.

have to be reexamined in light of the recently discovered effects of amino acid starvation on nucleoid isolation (see Section VI). The nucleoids isolated from growing cells and observed by fluorescent microscopy had sizes and DNA contents similar to the nucleoids observed *in vivo*.[26] This finding suggests that at least the gross DNA packaging in the nucleoid was preserved during isolation.

The stabilizing effect of the counterions is believed to be attributable to shielding of charge repulsions of the densely packaged phosphate groups in the condensed RNA and DNA of the nucleoid, although this interpretation has not been established.[24] The stabilization is quite apparent in recent studies of thermally induced unfolding of the nucleoid DNA.[27,28] Nucleoids isolated in the presence of high salt concentrations are sensitive to high temperatures.[24] The transition temperature for DNA unfolding, estimated from changes in sedimentation rate[27] or specific viscosity,[28] varies with the ionic strength of the solvents. For example, at low salt concentrations (<0.10 *M* NaCl) the DNA is substantially unfolded at temperatures <40°C, while unfolding occurs at 50 to 65°C in the presence of 1 *M* NaCl as measured by sedimentation changes. Small concentrations of multivalent counterions such as Mg^{++} or spermidine greatly enhance the stability of the structure.[26] The use of these counterions has permitted nucleoid isolation in solvents of much lower ionic strength.[29] Likewise, spermidine has been used as a stabilizing counterion during transcription *in vitro* (which requires lower ionic strengths) of the nucleoid.[30] It would be expected that nucleoids isolated in these lower ionic strength solvents would retain many DNA-bound proteins that normally are dissociated at higher salt concentrations.

It was observed that while high counterion concentrations are required during bacterial lysis, once isolated the nucleoid is stable for a time in solvents of lower ionic strength.[24-28] Observations of this kind raised the possibility that the high salt during lysis is required to promote a metastable molecular interaction into a more stable state. For example, the essential RNA-DNA interactions (to be described below) which stabilized the isolated nucleoid may achieve their most stable state only in the presence of appropriate counterion concentrations; this possibility will be discussed in more detail later.

The methods initially devised for isolation of the *E. coli* nucleoid utilized a precentrifugation of the crude lysate to eliminate cell debris and unlysed cells.[24] Nucleoids remaining in the supernates were then purified on sucrose gradients. Losses of the nucleoids with the cell debris were often substantial (40 to 80%), especially during isolation of the more rapidly sedimenting membrane-associated nucleoids (see Section IV). This loss was reduced when the sedimentation rate of the nucleoids was less (when wall-membrane complex was removed using lysis at higher temperatures);[25,39,40] however, there was still an incomplete recovery of nucleoids after precentrifugation. The loss of nucleoids introduced uncertainties in some of the subsequent studies, especially when the analysis assumes that the purified nucleoids are representative of the total population. For experiments where maximal recoveries are essential, it is recommended that precentrifugation steps be eliminated, as has been done in more recent studies.[31-33] Recoveries then are generally from 70 to 100%. Although recoveries are much better after this modification, contamination of the nucleoids with membrane-wall complex, nucleases, and other proteins which dissociate from the debris may be more extensive.[73] In this connection it should be emphasized that where the purity of isolated nucleoids is relevant to interpretation of an experiment, it is essential that protein analysis of preparations be conducted. The amounts of contaminating protein sometimes greatly exceed the published values obtained with the most carefully purified nucleoids.[24,34]

Recently it was found that membrane-associated nucleoids can be isolated without the use of multivalent counterions or high concentrations of monovalent counterions.[35] The concentrations of nonionic detergent used in this procedure are much lower than those used in the older procedure. It is argued that this preserves nucleic acid-protein associations which stabilize the nucleoid and which can be dissociated by higher concentrations of the detergents. The properties of nucleoids isolated by this method are distinct from those isolated in the presence of high salt, although there may be more similarity to the Mg^{++}-spermidine stabilized nucleoids;[29] their properties will be discussed below.

Methods similar to those developed for *E. coli* have also been applied for isolating nucleoids from

Mycoplasma.[74,75] It is of considerable interest to compare the conformational organization of the DNA in these smaller nucleoids with that of the *E. coli* nucleoid. Recently a procedure was also described[36] for isolating nucleoids from mitochondria with DNA which has properties similar to that of bacterial nucleoids. When the procedures for isolating *E. coli* nucleoids are applied to bacterial cells infected with phage T4, the replicating pool of T4 DNA can also be isolated in a compact structure with size and DNA content similar to the intracellular vegetative T4 DNA pool.[37] Thus, the approaches developed for isolating nucleoids from *E. coli* seem to be generally applicable to other prokaryotes.

At present the methods for nucleoid isolation have elements of empiricism and are very much an art. The procedures that work are essentially compromises between two extremes. If methods are too harsh, the DNA unfolds or the chromosome is broken; if methods are too mild, the cell wall-membrane complex is not sufficiently disrupted to permit separation of the nucleoid from other cellular components. While the techniques are reproducible and rationale for each step is available, the interactions stabilizing the nucleoid are not completely understood; therefore, one must proceed empirically.

IV. ISOLATION OF MEMBRANE-ASSOCIATED AND MEMBRANE-FREE NUCLEOIDS

During lysis of bacteria the degree of disruption of the cell wall-membrane complex is dependent on the concentrations and activity of lysozyme and detergents, the kinds of detergents, the solvent, and the time and temperature of the lysis reaction. When the lysis reaction is limited in these factors, a portion of the wall-membrane complex remains intact and copurifies with the nucleoid.[32-35,37,38] The sedimentation rate of these membrane-associated nucleoids is greater than that of the "membrane-free" nucleoid (see Figure 2). The amount of bound membrane is dependent on the extent of reaction with the reagents; nucleoids sedimenting at different rates, generally in the range 7000 to 1600S, can be obtained, depending on how much membrane-wall complex remains bound.[29,32-35,38] When the reactions are allowed to proceed for some time at elevated temperatures, the amount of associated membrane becomes minimal and the median sedimentation rate of the nucleoids isolated from rapidly growing cells is about 1600S (Figure 2a). In Figure 2b it can be seen that nucleoids prepared from cells that were reacted at lower temperatures which do not completely disrupt the membrane-wall complex require fourfold less centrifugation time to reach a similar position in the gradient. These membrane-associated nucleoids have a sedimentation rate of about 6000S.

The sedimentation rates described here were determined at 17,000 rpm and have not been corrected for rotor speed effect.[53] Recently it was found that the sedimentation rates of isolated nucleoids depend on the centrifugal force. When extrapolated to zero rotor speed, the sedimentation rate of the membrane-free nucleoid is 1900S.[76] Throughout this review the membrane-free nucleoid will frequently be referred to as the 1600S nucleoid. It must be remembered that this is an uncorrected sedimentation rate determined under the condition in which nucleoids have most frequently been studied.

Nucleoids stabilized during lysis and isolation by multivalent counterions are membrane-associated,[29] as are the ones isolated at low ionic strengths using reduced concentrations of detergents and lysozyme.[35] It has not been possible so far to use these latter methods to prepare membrane-free nucleoids. Apparently, the associations between the nucleoid and the wall-membrane complex are comparatively stable at low ionic strengths and in the presence of multivalent counterions. When detergent concentrations or incubation temperatures are increased to disrupt the membrane, the DNA usually unfolds.[29,35] The major proteins which copurify with the membrane-associated nucleoids isolated by these methods are similar to those observed with earlier methods.[29,32-34]

The amount of protein cosedimenting with the membrane-associated nucleoid is dependent on how much membrane was dissociated during lysis, but is always much greater than in the membrane-free nucleoid (Figure 3). The additional protein is primarily due to cell membrane-wall proteins[32,34] (see also Figure 4). The so-called "membrane-free" nucleoids have very little associated membrane-wall protein compared to the more rapidly sedimenting membrane-associated nucleoids, yet small amounts of these proteins are always detected sedimenting with the nucleoids (see Figure 4).

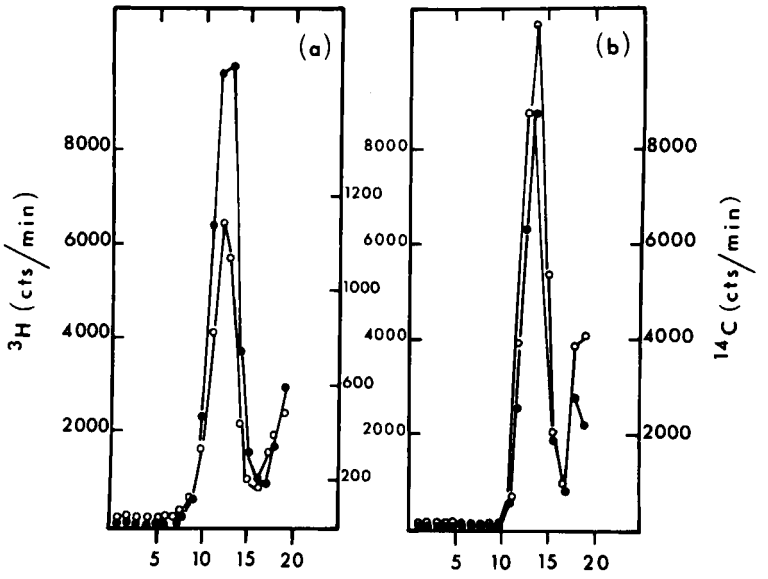

FIGURE 3. Sedimentation of nucleoids with labelled protein components. A culture of *E.coli* strain B_{s-1} was grown with [^{14}C] amino acid mixture to label proteins and with [^{3}H] thymidine to label DNA. Half of the culture was lysed at 24°C, half at 4°C, and membrane-free and membrane-associated nucleoids were purified as in Figure 2. Peak fractions were pooled, diluted, and resedimented on similar gradients. (a) The "membrane-free" nucleoids centrifuged 30 min at 17,000 rpm, 4°C. (b) The membrane-associated nucleoids centrifuged 7 min at 17,000 rpm, 4°C. (•) [^{3}H] DNA; (○) [^{14}C] protein. (Note change in ^{14}C scales.)

Although there is no evidence at this time that a small amount of membrane-wall complex is essential for stability of the nucleoid, it is difficult to exclude this possibility since no one has yet isolated nucleoids completely free of membrane-wall proteins. At least a portion of these proteins sedimenting with the 1600S nucleoid is attributable to contaminating membrane-wall fragments not bound to the 1600S nucleoid.[77,78] Whether or not very minor amounts are actually bound to the nucleoid is uncertain.

By adjusting lysis temperatures to intermediate ranges, it has been possible to produce the 1600S nucleoids in the same lysate as the more rapidly sedimenting membrane-associated nucleoids.[34, 38,40] When these structures are sedimented, nearly complete resolution between the two species is observed. There is no evidence that a continuum of structures having progressively less associated cell envelope is obtained, as would be expected if the mechanism of membrane-wall dissociation were exclusively by a progressive solubilization of the membrane. The results suggest that the chromosomes exist in two different states: either they have membrane and are more rapidly sedimenting or they do not have membrane and have attained a constant mass and frictional coefficient (1600S nucleoids). The most likely interpretation of these results is that the membrane can be stripped away from the nucleoid by two different processes. One comes about from dissociation of the attachments between the DNA and the cell envelope; the other comes about when the size of the associated membrane-wall patch(es) is decreased by solubilization with the lysozyme and detergents. Although the evidence reviewed above does not rigorously prove that the 1600S nucleoid is completely free of associated membrane, I will advisedly refer to it as the "membrane-free" nucleoid.

The possibility should be considered that the membrane of the membrane-associated nucleoid may form a vesicle about the nucleoid. Properties of the membrane-associated nucleoid to be described below indicate that the vesicle formation around the entire nucleoid is not likely.

V. COMPONENTS OF THE ISOLATED NUCLEOID

Table 1 provides a summary of the properties and molecular composition of the isolated membrane-free and membrane-associated nucleoids. The components of the membrane-associated

TABLE 1

Components and Properties of Isolated Nucleoids from *Escherichia coli* (30-min Generation Time)

	Membrane-free nucleoid	Membrane-associated nucleoid
DNA content (weight fraction)[a]	0.6	~0.4
RNA content (weight fraction)[a]	0.3	0.15–0.35
Protein content (weight fraction)[a]	0.05–0.1	~0.4
Lipid content (fraction of total labeled lipid)	<1%	~20%
Sedimentation rate (weight average)	1600S[c]	3200S[c]
Buoyant density in CsCl[41]	1.69 ± 0.02 g/cm^3	1.46 ± 0.02 g/cm^3
DNA mass per singlet[26] nucleoid	9 ± 1.0 × 10^{-9} μg	–
DNA mass per doublet[26] nucleoid	15 ± 2 × 10^{-9} μg	–
Genome equivalents per singlet[26]	2.2	–
Genome equivalents per doublet[26]	3.6	–
Fraction of total cellular membrane bound[32]	<0.01	~0.2
Bound proteins		
RNA polymerase	Yes	Yes
Envelope proteins	Little or none	Yes

[a]Weight fraction means fraction of total nucleoid dry weight.
[b]Lipids labeled with ^{14}C-oleic acid.[40]
[c]Determined at 17,000 rpm and uncorrected for rotor speed dependence. When extrapolated to zero rotor speed, the rates are approximately 20% greater.[84]

nucleoid are those determined for a 3200S complex purified in the presence of 1.0 *M* NaCl.[32,46] Similar analyses of nucleoids isolated in low ionic strength solvents are not available at the time of this review. The RNA and protein components of the highly purified membrane-free nucleoid are relatively constant for nucleoids isolated from cells grown in similar media. However, it should be emphasized that the protein and RNA components of the membrane-associated nucleoid are *not* fixed constants. As described above, these figures depend on how much membrane has been dissociated during lysis from the nucleoid. The reduction in fractional DNA and RNA content in the membrane-associated nucleoid (Table 1) does not represent an absolute decrease in the amount of the nucleic acids; it occurs only because the total mass of the membrane-associated nucleoid is greater due to the associated cell envelope. It can be seen that the amount of protein bound to the 3200S membrane-associated nucleoid is about tenfold greater than the membrane-free nucleoid (see Figure 3). Since the membrane-associated nucleoid can also have more associated RNA (see Figure 2), the difference in sedimentation rate between the two forms of the nucleoid can be accounted for by the mass difference between the two. It is not necessary to hypothesize a greater level of compaction of the membrane-associated nucleoid.

The isolated nucleoids have been studied by equilibrium centrifugation in CsCl density gradients.[41] When the chromosomes were fixed with formaldehyde, they had reproducible and characteristic buoyant densities. The near homogeneity in density of each form of the isolated nucleoid suggests a rather remarkable constancy in the gross composition of the particles. Any significant variations in protein, RNA, or lipid contents among different chromosomes would be expected to produce a greater density heterogeneity than was observed. Moreover, the density of different sized nucleoids from the heterogeneous sedimentation profile was the same, suggesting that the ratios of RNA-DNA-protein-lipid do not change significantly in nucleoids at different stages of chromosome replication.

A. DNA

The isolated nucleoids seem to contain the entire DNA complement of the in vivo nucleoid.[26] The average DNA content per singlet or doublet

nucleoid (see Figure 5 for definitions) is equivalent to about 2.2 or 3.6 genome equivalents of DNA per structure, respectively. The latter figure is in close agreement with the average DNA content per *E. coli* cell grown in similar media.[42] The nucleoids were isolated from exponentially growing cells, so that the DNA is expected to be at intermediate stages of replication in theta structures. Even so, the amount of DNA per doublet nucleoid is in excess of what would be expected for a single replicating chromosome, suggesting that segregation of replicated chromosomes may be incomplete in these nucleoids. Sedimentation studies of the DNA of the nucleoid after it was unfolded by treatment with strong ionic detergents suggest that its molecular weight is similar to that expected for the intact bacterial chromosomal DNA.[24,25,28] Moreover, the sedimentation rate of the denatured DNA on alkaline sucrose gradients indicates that some of the strands are covalently continuous, lacking even one nick per chromosome, while on the average there are only a few nicks per chromosome.[25,31]

B. Proteins

The proteins of the membrane-free (1600S) nucleoid are composed primarily of the subunits of core RNA polymerase.[23,24,34,43] For example, in the electrophoretic analyses shown in Figure 4B the major protein band (A) has a mobility indistinguishable from that of the $\beta\beta'$ subunits of RNA polymerase.[24,34] The more minor band (C) was previously shown to be that of the major cell envelope protein.[32,34,43] Bands C and D, attributable to cell wall-membrane proteins, are much more prominent in the proteins of the membrane-associated nucleoid (Figure 4C) and also in proteins associated with condensed T4 DNA (Figure 4A) isolated from T4 infected *E. coli* cells by a procedure analogous to the nucleoid isolation.[37] Band A, the RNA polymerase subunits, can also be seen among the proteins of the membrane-associated nucleoid and the condensed T4 DNA. Comparison with the analysis of total *E. coli* proteins (Figure 4B) shows that the proteins of the nucleoids are a highly selected fraction of the total cellular protein. Although the envelope proteins and RNA polymerase subunits are the only ones clearly observable in the analysis of membrane-free nucleoids, very minor bands corresponding to other proteins can be more clearly seen when higher concentrations of protein are analyzed. The identity of these more minor protein components is unknown. Recent investigations of the proteins of the membrane-associated nucleoid have indicated that both inner and outer membrane proteins are present.[44] These studies have also provided evidence for specific proteins involved in the DNA-nucleoid linkage; this will be discussed below.

C. RNA

Nascent RNA chains are attached to the DNA of the nucleoid by their associated RNA polymerase molecules.[23,39] Apparently, the number and distribution of these chains on the DNA is similar to their association *in vivo*. For example, hybridization analyses of the nascent chains has indicated that there are about 1000 to 3000 such chains bound per nucleoid, of which about 40% are rRNA sequences.[39] These figures are similar to those estimated from *in vivo* studies.[39] When cells are pulse-labeled with ^{3}H-uridine for 10 sec or less, nearly all of the labeled RNA cosediments with the nucleoid, suggesting that nearly all of the nascent chains of the cell remain attached to the nucleoid.[23] Labeling periods of 30 sec or greater resulted in substantial release of labeled RNA from the isolated chromosomes. In careful isolations there was no detectable degradation of the nascent RNA, since nascent rRNA chains could be elongated *in vitro* into homogeneous 30S rRNA precursors.[29] As shown in Figure 2, there is more ^{3}H-RNA bound per membrane-associated nucleoid than per membrane-free nucleoid. Electrophoretic analyses of the labeled RNA have shown that the additional RNA in the membrane-associated nucleoids is made up mostly of the rRNA precursors p16 and p23 + 23S.[79] Previous studies have shown that newly synthesized rRNA precursor molecules in *E. coli* are preferentially bound to membrane in cell lysates.[45] Therefore, it is likely that the additional RNA in the membrane-associated nucleoid is bound to the membrane fragment rather than to the DNA. Worcel and Burgi found the same amount of RNA bound to the membrane-free and membrane-associated nucleoids.[34] The amount of bound RNA is probably dependent on the amount of wall-membrane complexed with the nucleoid. As estimated from sedimentation rates, the membrane-associated nucleoids of Figure 2b have

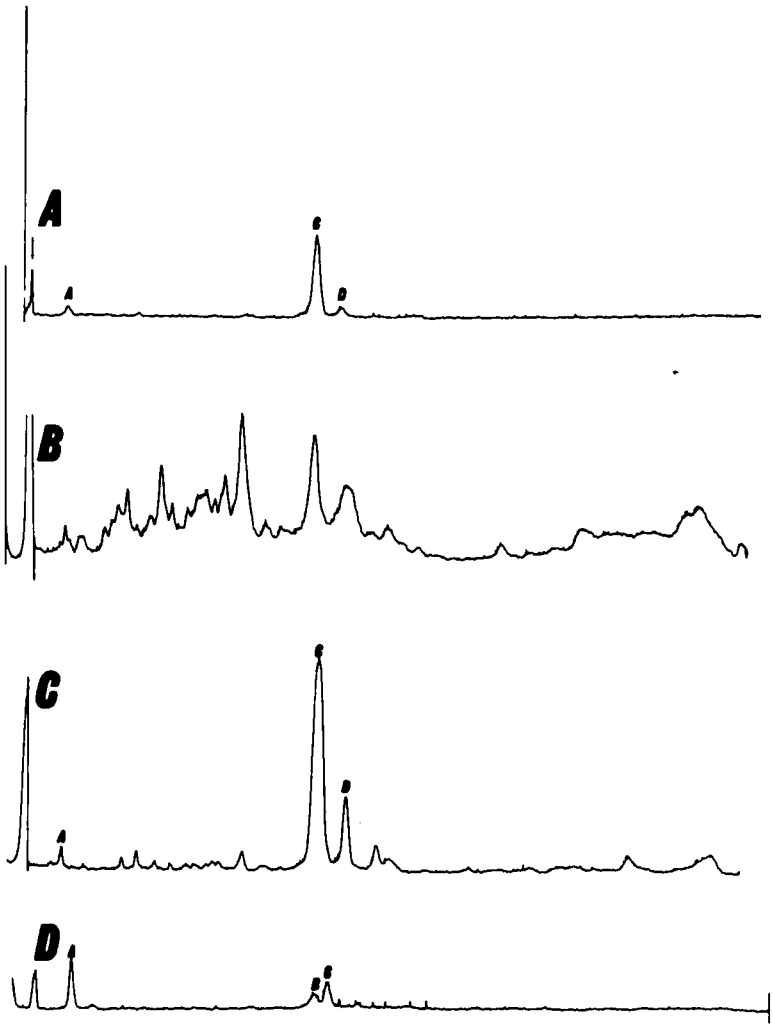

FIGURE 4. Electrophoretic analyses on SDS containing polyacrylamide gels of proteins of the isolated nucleoids. The above are densitometer scans of autoradiograms of the gels which contained ^{14}C-labeled proteins. (A) Proteins associated with condensed T4 DNA isolated from T4 infected cells.[37] (B) Total proteins of *E. coli* B. (C) Proteins from isolated membrane-associated nucleoids. (D) Proteins from isolated membrane-free nucleoids. Direction of electrophoresis was left to right. Band A has the mobility of RNA polymerase subunits $\beta\beta'$; bands C and D had molecular weights of 36K and 31K determined by comparison with T4 protein markers.

more wall-membrane complex than those analyzed in Worcel's laboratory.

D. Lipid

Lipid associated with the membrane-free nucleoid, estimated from incorporated [^{14}C] oleic acid, could not be detected. The amounts were less than 1% of the total incorporated ^{14}C-oleic acid and may even be zero.[40] Labeled lipid with the membrane-associated nucleoid was readily detectable (Table 1).

VI. "ATTACHMENT" OF THE NUCLEOID TO THE CELL MEMBRANE-WALL COMPLEX

It is of great interest to elucidate the molecular nature of the linkages between the nucleoid and the cell envelope and to determine the relationship of these linkages to DNA replication. Controversy exists over the changes that may occur during the DNA replication cycle in the observed associations between nucleoid and membrane. It was first

reported that only membrane-free nucleoids could be isolated from amino acid-starved cells which have completed their DNA replication cycles.[34,43] This led to the proposal that the chromosome was released from its membrane attachment sites at the completion of a round of DNA replication. Partial confirmation of this result also came from studies in which protein synthesis was inhibited with chloramphenicol, although in this case it was claimed that limited additional protein synthesis after termination of DNA replication was required to complete the release of nucleoids.[46] In amino acid-starved cells the additional protein synthesis comes about from protein turnover, which is blocked in the presence of chloramphenicol. All of these analyses were of nucleoids that were isolated after a precentrifugation step, known to result in losses of nucleoids (see above section on nucleoid isolation).

In contrast to these results, it was found that when the nucleoids were isolated by a procedure giving essentially complete recovery, nearly all of the nucleoids were membrane associated when isolated from cells which had completed their DNA replication cycle.[33,47] This was true whether DNA replication was brought to termination by amino acid starvation or by using a mutant temperature sensitive for initiation of DNA synthesis (dna C mutant). Rather than releasing from the membrane after inhibition of DNA replication, the nucleoids become much more rapid in their sedimentation than the control membrane-associated nucleoids. It was proposed that these more rapidly sedimenting nucleoids had been preferentially lost during precentrifugation steps in the prior experiments and that this gave rise to an artifactual enrichment of the membrane-free nucleoids. However, in reviewing data no evidence for such preferential losses could be found.[80]

Some recent work has done a good deal to resolve the controversy.[38] It was found that bacteria starved for amino acids became more refractory to lysozyme. The rapidly sedimenting nucleoids[33] obtained from starved cells occur because they have larger amounts of associated cell envelope. When amino acid-starved cells were more extensively treated with lysozyme so that the rate of lysis by the detergents was the same as in exponentially growing cells, nucleoids isolated from exponential or starved cells had similar sedimentation rates.[38] Also, the amount of membrane-associated nucleoids was independent of whether or not DNA synthesis had run to completion. In addition, nucleoids were isolated from amino acid-starved cells so that both membrane-free and membrane-associated nucleoids were obtained from the same lysate (see section above on nucleoid isolation). Pulse label with ^{3}H-thymidine given just prior to isolation appeared equally in the membrane-associated and membrane-free nucleoids. Although it was not proven that the incorporated pulse label was due to semiconservative DNA replication (and not to repair), the fact that the incorporation was equal in both kinds of nucleoids (some of which were known to be undergoing semiconservative replication) suggests that the membrane-free chromosomes are *not* those that stopped DNA replication. The results show no correlation between the synthesis of DNA and the association of nucleoids to membrane.[38]

In analyzing these results it seems apparent that the chromosomes are not generally released from membrane association at termination of DNA replication. Observations to the contrary[34,43,46] are probably attributable to changes in the resistance of the wall-membrane complex to lysis in nongrowing cells. The changes in sedimentation rate of the isolated nucleoids after interruption of DNA synthesis[34,47] are probably due to changes in the amount of associated membrane and not primarily from structural changes of the chromosomes. For example, in examining some of these data it does seem that the ratio of protein: DNA is increased in the more rapidly sedimenting nucleoids obtained from cells blocked in DNA initiation.[47] A resistance to lysis may also explain the nucleoid differences observed in *rep*$^+$ and *rep*$^-$ strains.[64,81] In making these assessments it should be kept in mind that the sites of association between the isolated nucleoid and membrane are poorly understood, but probably involve diverse interactions.[12] In addition to membrane protein-mediated linkages[44,48,49] there are probably linkages between nascent RNA chains bound to DNA at their 3′ end and to membrane at other regions of the chain.[12,17,45] It is not certain whether all of these linkages are biologically significant. In this circumstance it is difficult to confidently interpret changes that may or may not occur in membrane-DNA association. It is possible, for example, that changes in nucleoid-membrane attachments *could* occur during the DNA repli-

cation cycle yet be obscured by other membrane-DNA associations. It is apparent that the amount of RNA bound to the membrane-associated nucleoid can vary depending on how much membrane is left associated (see Figure 2 and discussion in Section V). If nascent RNA chains can link the DNA to membrane, the number of such interactions may depend on how the membrane-associated nucleoids are isolated. Until the molecular basis of the membrane-linkage is better understood, the use of the term membrane-associated nucleoid is recommended rather than a more specific term such as membrane-attached nucleoid.

Recent results indicate that identification of the proteins involved in linking the nucleoid to the DNA may be close at hand. DNA fragments linked to a specific 80 to 90K protein have been isolated from *E. coli* by free-flow electrophoresis.[48] Inactivation of this protein has been correlated with disruptions of the DNA, cell envelope complex. An 80K and a 56K protein in membrane-associated nucleoids from *E. coli* can be cross-linked by ultraviolet irradiation to 5-bromodeoxyuridine-labeled DNA of the nucleoid.[44] These were shown to probably be inner membrane proteins not present in the membrane-free nucleoids. A functionally similar protein has also been observed in *B. subtilis*.[49] Although these studies implicate certain proteins in the membrane-DNA link, they also illustrate the difficulties implicit in unambiguously identifying the nucleoid-membrane linking protein. What they do identify is a protein that is enriched in membrane-DNA complexes which in some cases is bound to the DNA and in some cases to the membrane. If disruption or degradation of the protein lends to release of the complex, it does not in itself prove the hypothesized role of the protein, since reagents for inactivation or cross-linking of the protein are not specific for that protein. What will be ultimately required to establish the role of a specific protein is a genetics approach in which mutants in the critical protein are obtained. Conversely, an in vitro reassembly of the defined components of the system could be more convincing.

VII. MEASURING UNFOLDING OF THE NUCLEOID DNA

One approach to investigating the molecular interactions which stabilize the structure of the nucleoid is to define conditions and reactions which promote unfolding of the DNA. To this end, methods for observing the unfolding process have been explored. The state of condensation of the DNA in the nucleoids can be visualized by fluorescence or electron microscopy (Figures 5 and 10). The fluorescence microscopy technique has the advantage of maintaining an aqueous environment, while dehydration (which may lead to DNA spreading or aggregation) is necessary in electron microscopy. Conversely, greater resolution is available using electron microscopy. When spreading conditions are avoided, either technique shows particles having dimensions roughly similar to those of the nucleoid observed in vivo.[26,32,37] If DNA spreading techniques (such as the Kleinschmidt method) are used, the DNA becomes more extended and the dimensions of the observed structures are greater than those of the cells from which they were isolated.[50-53] The microscopy techniques can be used to examine the state of condensation of the nucleoid as the DNA is unfolded.

Unfolding of the DNA is also indicated by a very large increase in the viscosity of solutions containing the nucleoids.[24,28,30] This technique provides a sensitive assay for changes in the condensation of the DNA; however, there are certain anomalies in the measurements which are not understood. Gel-like behavior in the unfolded DNA has been observed,[82] and anomalously large viscosities are obtained.[28] It appears that a DNA cross-linking reaction occurs in the viscometers while the DNA is unfolding. Until these effects are better understood, viscosity measurements of the bacterial nucleoid should be interpreted cautiously and at most used as qualitative indications of the state of condensation of the DNA (see also Section VIII).

Sedimentation studies have also been used to follow the unfolding of the DNA.[24-28,31,32] As the DNA unfolds, its frictional coefficient increases and its rate of sedimentation falls (Figure 6). It is necessary to distinguish between sedimentation changes that are derived from changes in mass from those that are attributable to conformation change. Because the changes due to unfolding are so large, this is not frequently a problem.

With all of these methods, certain precautions are required. While packaged in the nucleoid the

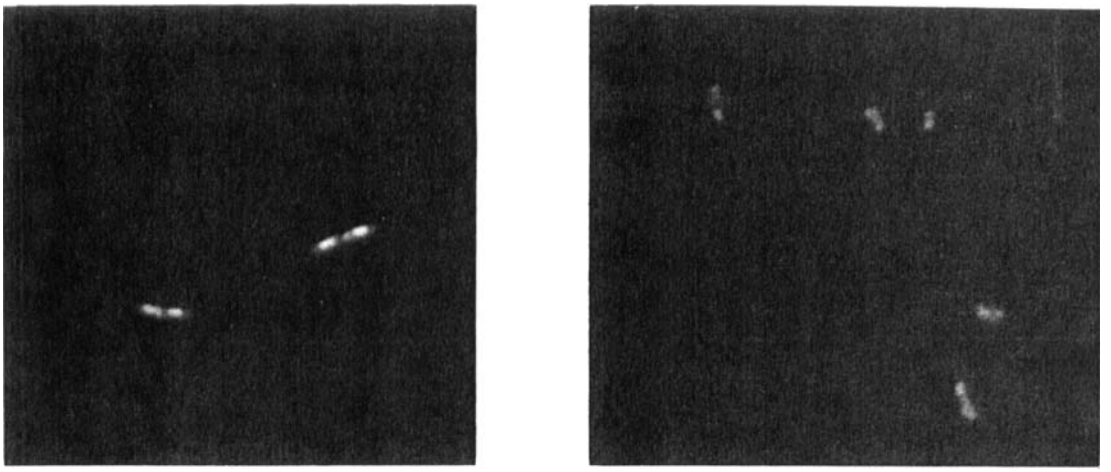

FIGURE 5. Fluorescent microscopy of the isolated membrane-free nucleoid and of intact bacteria. Ethidium bromide was the fluorescent chromophore. (left) Bacteria; (right) isolated nucleoids. Many of the nucleoids appeared to be doublets consisting of two closely linked particles. Singlets were defined as half of a doublet or particles not containing two observable parts.[26]

DNA is reasonably resistant to shear and can be handled with normal precautions. Once unfolded or partially unfolded, the DNA becomes very fragile. The above measurements on unfolding can be seriously influenced by DNA breakage. Currently there is no method available for measuring DNA folding in a quantitative manner, and all of the above techniques provide at best relative indications on the state of condensation of the DNA.

VIII. REACTIONS WHICH UNFOLD THE DNA OF NUCLEOIDS

As described above, the DNA of nucleoids isolated in the presence of high concentrations of salts unfolds at elevated temperatures. Counterions stabilize this unfolding. At low salt concentrations the DNA gradually unfolds, even at 0°C; while at higher concentrations the nucleoids are more stable.[27]

Incubation of the nucleoid with reagents that dissociate or denature proteins causes the DNA to unfold, suggesting that certain protein components of the isolated chromosome stabilize the condensed DNA.[24,25,28,32,40] Incubation with proteolytic enzymes, such as pronase or trypsin, did not result in DNA unfolding; however, it was observed that proteins such as RNA polymerase in the nucleoid were not inactivated by pronase and therefore are not readily accessible to this enzyme.[24,32] Recently it was found that trypsin can attack proteins on the DNA if the nucleoid is first partially unfolded by heating.[28] The proteins which may stabilize long-range DNA conformation in the nucleoids have not yet been isolated or characterized. For purposes of discussion, it is useful to categorize the kinds of interactions in which such proteins may participate: (1) proteins which may bind the DNA to the membrane-wall complex; (2) proteins which may participate in DNA-DNA linkages; and (3) proteins which may stabilize RNA-DNA interactions which fix DNA folds. Any of these reactions could stabilize DNA folds as well as restrict rotation of the DNA double helix. To account for a significant amount of DNA condensation, many separate sites on the DNA would have to be bound by such proteins. This is compatible with estimates of the number of sites of membrane-DNA association which have been measured (for review, see Reference 12), although it should be emphasized that many membrane-DNA linkages in isolated systems seem to involve nascent RNA molecules.[12,17] Potentially, the proteins could be involved either directly by linking DNA to membrane or indirectly by linking DNA to an RNA molecule which in turn is associated with membrane. Future research will have to sort out these possibilities (also see Section IX).

One of the earliest and most intriguing observations was the finding that incubation of the

nucleoids with RNase resulted in DNA unfolding.[24,25,28,31,32] This effect was attributable to the RNase activity of the enzyme and not to either the physical properties of the protein or contaminants. No direct effects of the RNase on the DNA or proteins of the nucleoid could be seen;[31,32] other proteins having physical-chemical properties similar to RNase did not elicit the unfolding,[24] and different highly purified RNases caused unfolding.[24,28]

One study of the RNase-mediated unfolding reaction indicated that the unfolding was an all-or-none process.[25] That is, nucleoids at intermediate stages of unfolding were not observed, and it was claimed that only completely folded or completely unfolded DNA could be obtained after limited reactions with RNase. This result led to the proposal that a single "core" RNA molecule stabilized the nucleoid.[25] Other experiments using nucleoids isolated from different bacterial strains and after a variety of different growth conditions have not supported this conclusion. Isolated nucleoids subjected to progressively more hydrolysis of their associated RNA components sedimented at progressively smaller sedimentation rates, and a continuous spectrum of partially unfolded chromosomes was observed[32] (Figure 6). The changes were attributable to conformational relaxation of the DNA and not to mass losses.[32] Thus, it seems that many different stages of partially unfolded DNA can be obtained in nucleoids with partially hydrolyzed RNA components, supporting the conclusion that many RNA molecules per nucleoid stabilized the condensed DNA. This result was confirmed in the laboratory where the initial findings were made.[28]

Recently, it was observed that the DNA of isolated nucleoids treated with RNase does not attain a minimal sedimentation rate or maximum "specific viscosity" until proteins are also removed from the DNA.[28] Thus, it was suggested that the DNA in membrane-free nucleoids treated with RNase cannot be completely unfolded and that proteins of the structure maintain DNA folds independently of the RNA-DNA interactions. While this conclusion may be correct, there are uncertainties in this interpretation that should be noted. The difficulties in interpreting viscosity changes of nucleoid DNA have already been discussed (see Section VIII). Viscosities of the unfolded and relaxed nucleoid DNA measured in

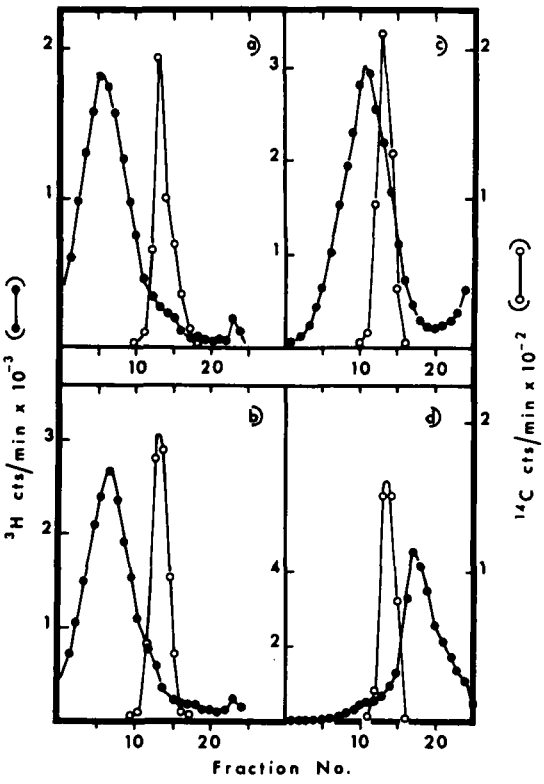

FIGURE 6. Sedimentation on sucrose gradients of nucleoids reacted to various extents with pancreatic RNase. Identical solutions containing constant amounts of nucleoids with ^{3}H-labeled DNA were incubated for 3 min at 15°C with (a) 0 RNase, (b) 2 μg/ml RNase, (c) 20 μg/ml RNase, and (d) 50 μg/ml RNase. After inactivating the RNase with diethylpyrocarbonate, the solutions were centrifuged on separate identical sucrose gradients, 19,000 rpm at 4°C for 40 min. When mixed, with each solution was a ^{14}C-labeled T4 phage sedimentation marker. The changes in sedimentation rate are primarily attributable to unfolding of the DNA, since mass losses cannot account for the differences.[31] (From Pettijohn, D. and Hecht, R., *Cold Spring Harbor Symp. Quant. Biol.*, 38, 31, 1973. With permission.)

this work are apparently attributable to a DNA aggregation phenomenon which is poorly understood. To accept the conclusions of the viscosity data, one must accept the assumption that the viscosity changes due to DNA aggregation accurately amplify the true reduced viscosity of the unfolded DNA. In this regard, it was observed that the measured viscosities of the deproteinized DNA aggregates depend strongly on the salt concentrations present when DNA unfolding and aggregation occur. At low salt concentrations the measured viscosity of the DNA unfolded by denaturation of proteins was only two- to three-fold greater than the viscosity of DNA unfolded

with RNase. It seems possible that more precise extrapolations in the lowest salt range would indicate even less conformational difference between the deproteinized and RNased forms of the unfolded DNA. While it is clear from this study that there are differences between the deproteinized and RNase-treated nucleoid DNA, it is not clear whether these differences come about from differences in the folding of the DNA or its aggregation.

The sedimentation measurements show that after what appeared to be a limit digest with RNase, the unfolded DNA of the nucleoid sedimented at about 400 to 500S.[28] If these structures were heated at 67°C (presumably to remove proteins), the average sedimentation rate became 130S at 7000 rpm. A similar sedimentation rate (120 to 130S at 7000 rpm) was also noted when the proteins were removed from the DNA by sodium dodecylsulfate or trypsin treatment of partially unfolded, heated nucleoids. These findings do suggest that the difference between the 400 to 500S and the 130 to 200S structures may be due to DNA-bound proteins maintaining some DNA folding independent of the RNA-DNA interactions. However, the data do not conclusively rule out the possibility that some or all of the observed transition could be due to changes in the mass of the structures occurring as a result of the deproteinizations. As discussed above (Section V), isolated nucleoids seem to be composed of the entire cellular complement of DNA. Cells grown in minimal media (as in this study) would be expected to yield nucleoids having an average of about two genome equivalents of DNA.[42] Cells containing pairs of nucleoids can apparently yield double nucleoids after isolation.[26] The interactions holding the pairs together are not well understood, but it seems possible that after dissociation of proteins there may be a separation of the DNA components of each. Although many of the cells grown in minimal media do not have double nucleoids, some do. A mass change such as this could occur after deproteinization, independent of any additional DNA unfolding. The deproteinized DNA is very large and apparently contains few if any double-strand breaks; however, from this study it is difficult to rule out the possibility that one or two double-strand breaks were introduced into each circular DNA molecule. If the DNA fragments were held together by proteins, the dissociation of the proteins could amplify a minimal amount of unfolding into a more conspicuous sedimentation change. In addition, mass effects from dissociation of the proteins themselves are not clearly defined in this study. It is known that the most highly purified membrane-free nucleoids have only about 10% by weight bound protein, but that the mass of protein is frequently much greater in less stringently purified preparations. Apparently, in this study at least, some of the nucleoids prior to reaction with RNase had sedimentation rates of 2000S or greater (see Figure 4 of Reference 28), suggesting a significantly greater-than-normal protein content. It should also be pointed out that the finding that a proteolytic enzyme like trypsin can result in DNA unfolding does not by itself prove that the trypsin-sensitive protein stabilized different folds than the RNA-DNA interactions. If the trypsin-sensitive proteins bind RNA molecules to DNA (as RNA polymerase does), trypsin may release nascent RNA chains that stabilize the folds. The results of this investigation,[28] as well as others (see above), implicate the proteins of the membrane-free nucleoid as being important in maintaining DNA folds in the structure. To establish whether or not they stabilize different folds than the RNA-DNA interactions will require additional confirmation.

The molecular nature of the RNA-DNA interactions stabilizing the isolated nucleoid are not yet thoroughly understood. As discussed above, studies of the effects of inhibitors of RNA synthesis indicate that the essential RNA molecules are probably nascent RNA chains. These chains would be bound to the DNA at their 3′ ends at ternary complexes involving associated RNA polyermase molecules. Strong interactions of the critical nascent RNA chains with one or more additional regions of the DNA would be required to stabilize DNA folds. A search for RNA molecules bound to the nucleoid DNA, independent of the ternary complexes, has revealed that 74 ± 14 RNA chains per nucleoid remain associated with the DNA after RNA polymerase molecules are dissociated.[54] This is a small fraction (~2.5%) of the total nascent RNA chains of the nucleoid. Sensitive assays on the amount of remnant RNA polymerase indicate that less than 0.02 RNA polymerase molecules remain per bound RNA molecule. The RNA could be released from the DNA by heating, indicating a hydrogen-bonded

association. Portions of a few (as few as one to two chains per nucleoid) of the chains were bound in an RNase resistant form containing sequences averaging about 300 nucleotides in length. These became sensitive to RNase after heating the complex. The chains average 1200 bases in length and are heterogeneous in size. Hybridization analysis indicates that they are composed of known mRNA and nascent rRNA sequences. The amount of this DNA-bound RNA was greatly reduced in cells grown briefly with rifampicin. Thus, these molecules have many of the properties expected for the nucleoid stabilizing RNA, although it has not been established that they actually are the stabilizing RNA.

Evidence has recently appeared indicating that many of the nascent RNA chains of the nucleoid may have multiple sites of association with the DNA of the nucleoid.[55] Isolated nucleoids with ^{3}H-labeled RNA components were incubated briefly with RNase so that the nucleoid was only slightly unfolded, as revealed by the small reduction in its sedimentation rate. The amount of labeled RNA released from the nucleoids was less than 15% of the total, yet it was determined that the average nascent RNA chain had received at least four cuts per molecule. If the only site of attachment of the RNA chains were at their 3′ ends, much more released RNA would have been expected. If an RNA chain had extensive amounts of secondary structure, it might be possible to break a chain four times at random positions yet not release fragments from one another. However, the mRNAs of *E. coli* are not known to have extensive secondary structures. The results hint at the possibility of extensive interactions between the nucleic acids of the nucleoid.

While it has been established that the RNA chains of the nucleoid isolated by the high salt methods are essential for the stability of the *isolated* chromosome, it is not clear that these same interactions are important in vivo. The possibility has not been excluded that the stabilization of the chromosome by RNA is the result of some fortuitous association or tangling of the nascent RNA occurring during isolation of the packaged DNA. Even if this is the case, however, the methods are still of some interest. At a minimum they permit the isolation of DNA which appears to have reproducible periodic structures and which most likely bears some resemblance to the natural chromosomal structure (see Section XI).

Nucleoids isolated in solutions of low ionic strength using reduced concentrations of detergents and cetyl trimethylammonium bromide (CETAB) to lyse the cells have different properties with respect to stabilization by RNA.[35,36] The condensed folded DNA can be isolated from cells grown with rifampicin; however, it was found that these structures are still sensitive to RNase. At certain salt concentrations it was observed that the nucleoids isolated from growing cells are resistant to RNase, while at other salt concentrations they are not. It was proposed that the reduced concentrations of detergents preserve certain protein-nucleic acid interactions in the nucleoid which are dissociated using other more aggressive methods. These interactions may be important to the stability of the linking RNA molecules and other protein-mediated cross-connections which preserve the condensed state of the DNA. In this regard, one additional observation is in order. It has been observed that the CETAB seems to exert a stabilizing effect on nucleoids isolated from rifampicin-treated bacteria.[83] Condensed DNA in lysates from cells grown with rifampicin and lysed by the Dworsky procedure[56] are quite unstable, unless the CETAB is present. It seems possible that the CETAB, a known DNA precipitating agent, may stabilize the chromosome in a state similar to the Ψ-condensed DNA transition.[57]

After reading this section, it must be clear to the reader that considerable work is still required before there can be any detailed understanding of the interactions which stabilize and organize the structure of the isolated nucleoid.

IX. SUPERCOILED DNA IN ISOLATED NUCLEOIDS

Worcel and Burgi (1972) first demonstrated that the effects of intercalated ethidium bromide on the isolated nucleoid were characteristic of supercoiled DNA.[25] A biphasic transition in the sedimentation rate of the nucleoid was observed when its sedimentation rates in different concentrations of ethidium were compared (Figure 7). The transition was similar to that observed with other simpler DNA molecules containing negative superhelical turns.[58] The sedimentation rate was minimal at a critical concentration of ethidium bromide (2 μg/ml) where the supercoils are removed. At concentrations above and below this critical concentration, negative or positive

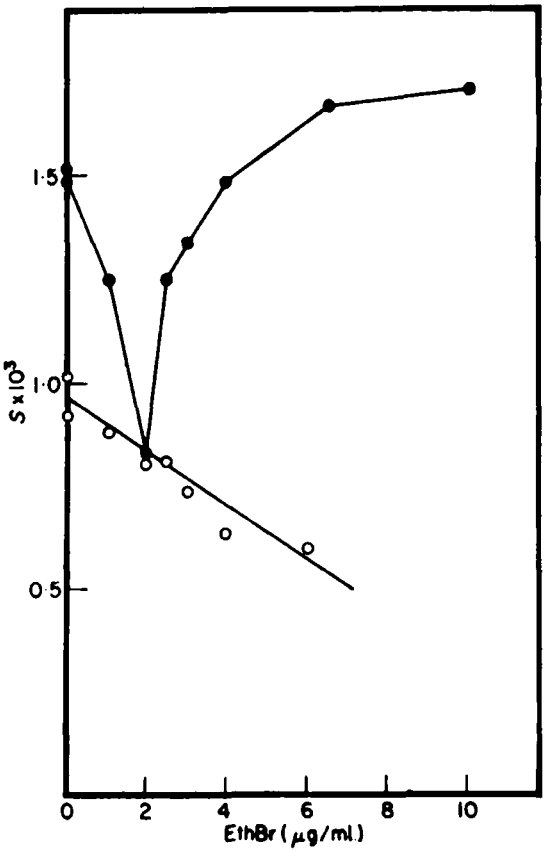

FIGURE 7. Sedimentation coefficients of isolated nucleoids sedimented in different concentrations of ethidium bromide (EthBr). (●–●) Untreated isolated nucleoids; (O–O) nucleoid with supercoiling relaxed after introduction of many single-strand breaks with DNase. (From Worcel, A. and Burgi, E., *J. Mol. Biol.*, 71, 127, 1972. With permission.)

supercoils exist, making the structure more compact and therefore more rapidly sedimenting. The number of supercoils in the DNA could be estimated from the amount of ethidium required to just remove the supercoils. It was observed that the critical concentration of ethidium was close to that required with several circular phage or plasmid DNAs, suggesting that the superhelical density in all of these DNAs was similar. It should be noted, however, that in this study only total ethidium bromide concentrations were measured;[25] the amount of ethidium actually bound to the nucleoid DNA was not determined. Earlier studies with simple circular DNAs were done in solvents usually containing 3.0 *M* CsCl, while the studies of nucleoids were done in solvents containing 1.0 *M* NaCl. Since the association constant of ethidium is strongly dependent on salt concentration,[59] there was initially some uncertainty as to whether the two sets of data could be directly compared without correction for salt effects. It appears, however, that the association constants in 1.0 *M* NaCl and 3.0 *M* CsCl do not differ greatly (for example, see References 58 and 59) so that the required corrections are not large. Thus, it seems that the superhelical densities of the nucleoid DNA and many simple circular DNAs are similar. The measured numbers are near $\sigma = -0.05$ or about 1 negative superhelical turn per 200 base pairs (measured in 1.0 *M* NaCl and assuming an unwinding angle of -26° for ethidium bromide). In 0.1 *M* NaCl the average rotational angle between base pairs of the DNA double helix is less, and the superhelical density should be about 20% less.[59,60]

When single-strand breaks were introduced into the DNA of the nucleoid with DNase, the breaks provided swivels which relaxed the supercoiling. It can be seen in Figure 7 that at the equivalence point (2 μg/ml ethidium bromide) the sedimentation rate of the "native" nucleoid and the nucleoid relaxed with single-strand breaks were identical, since both have lost their supercoils (although for different reasons). It was found that many single-strand breaks per chromosome were required to relax all of the supercoiling.[25,31] This finding suggests that the chromosome is segregated into separate domains, so that sites occur in the nucleoid which restrain the rotation of the double helix (see Figure 9). A nick in one domain relaxes the supercoiling only in that domain, since rotation about the nick cannot be propagated to adjacent domains.

The term "loop" was originally proposed to denote the DNA units which were relaxed by one single-strand break.[25] The author prefers the term "domain" since it is a functionally descriptive word rather than a topological term. There is at this time no proof that the DNA loops seen by electron microscopy of nucleoids have a one-to-one equivalence with the domains of supercoiling determined from the number of single-strand breaks required to relax supercoiling. A domain of supercoiling is defined as a region of a DNA double helix bounded by two sites which restrict the rotation of the double helix.[31]

Nucleoids partially unfolded by reaction with RNase exhibited biphasic transitions similar to the untreated nucleoids when sedimented in the presence of varied concentrations of ethidium bromide.[31] The equivalence point was at 2 μg/ml

ethidium bromide, and the fractional reduction in sedimentation rate at this point was similar for the folded and partially unfolded DNA. This finding indicates that some unfolding of the DNA could occur without detectably relaxing the supercoiling. Likewise, it appears that partial or complete relaxation of supercoiling could occur without affecting the DNA folding. Nucleoids reacted to various extents with DNase so that only a part of the supercoiling was relaxed, sedimented at different rates intermediate to that of completely relaxed and completely supercoiled structures. In the presence of 2 μg/ml ethidium bromide the nucleoids, nicked or unnicked, sedimented at the same rate, suggesting that the extent of DNA folding of these partially relaxed chromosomes was the same. When the number of single-strand breaks was sufficient to relax all detectable supercoiling, the relaxed chromosome attained a constant sedimentation rate.[25,31] Additional nicks could be introduced without affecting the sedimentation rate, also indicating that the nicks relax supercoiling but do not influence the DNA folding. Thus, considerable independence in the relaxation of supercoiling and the unfolding of the DNA was possible.

It was also demonstrated that nucleoids which were partially unfolded by RNase required fewer single-strand breaks in the DNA to relax their supercoiling.[31] Thus, even though the partial unfolding achieved with RNase did not relax the supercoiling, it did affect the manner in which the supercoiling was stabilized or segregated in the isolated chromosome. The findings suggest that there is no loss of supercoiling in the partially unfolded chromosomes, but the number of domains of supercoiling is reduced. When the RNA of the nucleoids was more completely degraded with RNase, all detectable supercoiling was relaxed, even in the absence of added single-strand breaks.[28] These findings can be explained by a model of the chromosome to be discussed below (Section X).

The reason why DNA in the nucleoid is supercoiled is not yet clear, but some recent findings indicate a likely explanation.[61] Loops of DNA-containing fibers emanating from partially disrupted *E. coli* cells were observed by electron microscopy (see Figure 8). The cells were finally disrupted with Triton® X-100; if processed immediately thereafter, fibers with diameters of 120 Å were observed with a 130 Å repeating beaded structure (Figures 8A, B, and D). If the fibers were exposed to the Triton for longer periods, the beaded structures disappeared and the fibers became more extended, twisted, and thinner (Figure 8C). The beaded structure resembles similar beaded fibers observed when chromatin from eukaryotic cells is visualized by electron microscopy (for review, see Reference 62). The DNA in a 120-Å chromatin fiber is about seven times longer than the fiber itself, indicating that this beaded fiber may represent the primary unit of DNA condensation in chromatin. It has also been proposed that toroidal superhelices in the DNA are wound around each of the beaded nu bodies, which in eukaryotes are composed of an octamer of four histone subunits.[62] If the prokaryotic DNA in the observed 120-Å fibers were similarly associated in toroidal superhelices, many of the properties of the isolated nucleoids and the 120-Å fibers could be reconciled. The 120-Å fibers do not appear to have interwound superhelices; that is, the observed loops are open without twisting (Figure 8). However, after more extended exposure to Triton, other detergents, or high salt concentrations the beaded structure disappeared and the fibers elongated and appeared twisted (Figure 8C). This is what is expected if the 120-Å fiber holding toroidal superhelices in the DNA is disrupted so that the DNA can lengthen and toroidal superhelices can be expressed as interwound superhelices. The observed sensitivity of the beaded structure to high salt concentrations and detergents would lead one to anticipate that these 120-Å fibers have been disrupted in the isolated nucleoids. The toroidal superhelices would therefore be expected to appear as interwound supercoils in the isolated nucleoid DNA, which is what is observed.

While it seems clear in eukaryotic cells that the nu bodies are composed of histones,[62] the component(s) organizing equivalent structures in prokaryotic DNA have not yet been elucidated. A histone-like protein was recently isolated from *E. coli*,[63] but the amount of this protein per cell does not seem sufficient to allow one or more proteins to be associated with each 200 base pairs of the DNA. In rapidly growing cells this would require about 800,000 copies per cell. Additional studies are required to confirm the existence of the chromatin-like fibers seen in bacteria and to establish their structure.

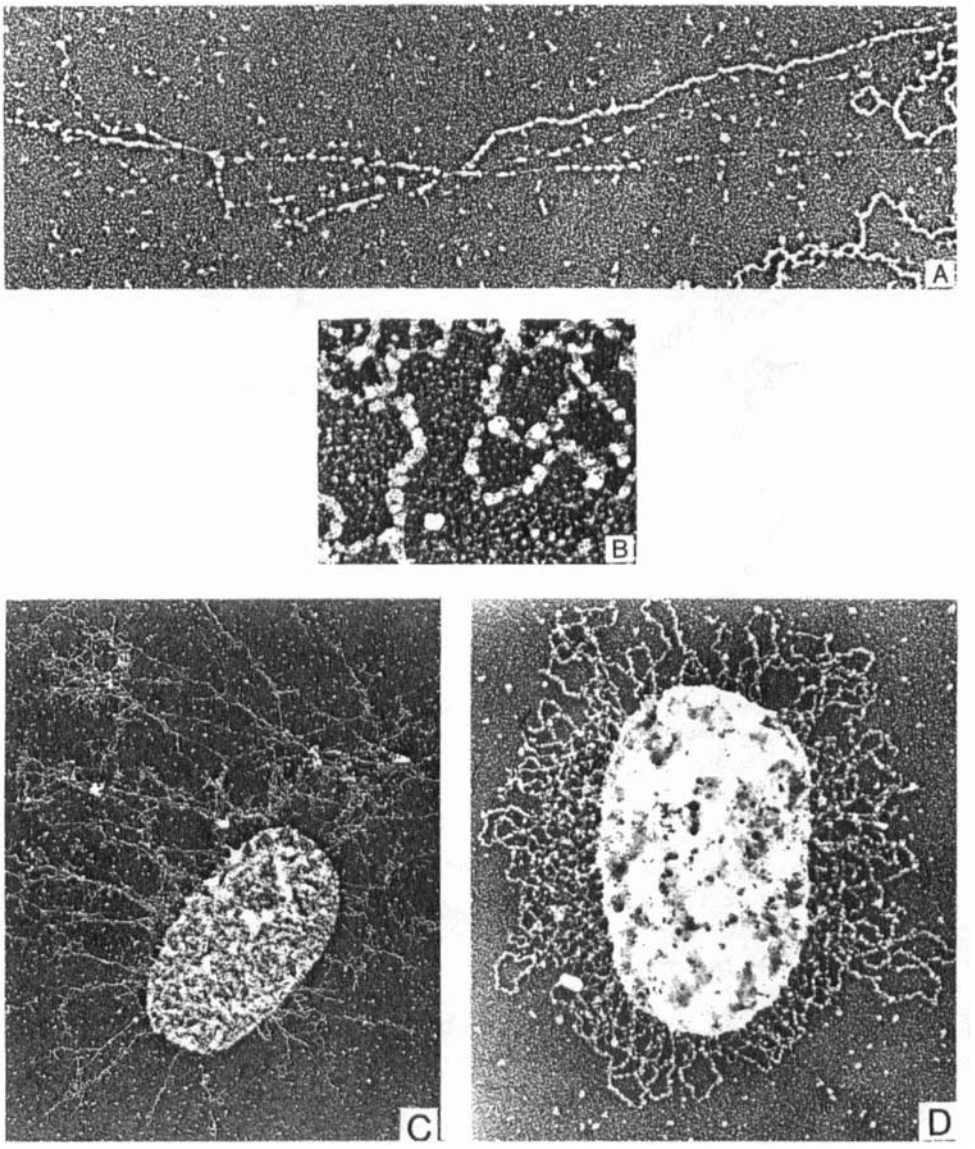

FIGURE 8. Electron microscopy of "chromatin-like" fibers emanating from partially disrupted cells of *E. coli*. (A) A selected section of one fiber apparently stretched during mounting. (B) A more typical fiber clearly showing the beaded-like structure, an enlargement of one section of panel D. (C) The fibers after holding for 4 min in Triton® X-100. They are now thinner and more supercoiled. (D) The fibers after 2 min in Triton X-100. Bar equals 1 μm. (From Griffith, J., *Proc. Natl. Acad. Sci. U.S.A.*, 73, 563, 1976. With permission.)

X. MODELS FOR THE CONFORMATIONAL ORGANIZATION OF DNA IN ISOLATED NUCLEOIDS

Several models for the structure of the bacterial nucleoid have been proposed (for examples, see References 14, 25, 31, and 65). These have been formulated to account for the various properties of the nucleoid as described in the previous sections of this review. The model shown in Figure 9 attempts to conserve certain elements in prior models (see particularly Reference 25) and still account in more detail for the observed RNA-DNA and protein-DNA interactions which stabilize the isolated nucleoid. The model was formulated to satisfy properties of the nucleoid observed in vitro.

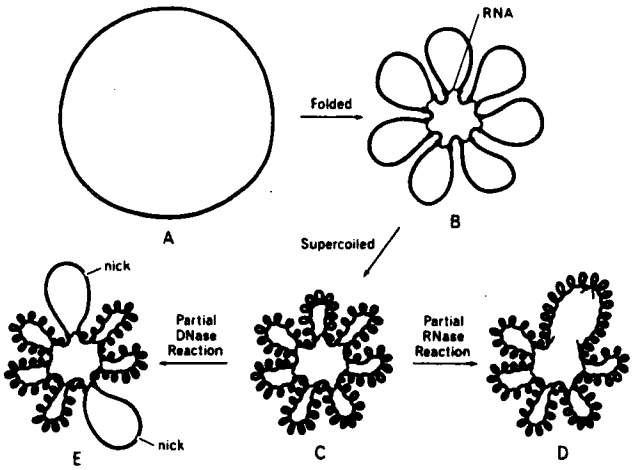

FIGURE 9. A model for the organization of DNA in the bacterial membrane-free nucleoid. (A) The circular completely unfolded, relaxed DNA. (B) The DNA containing only folds restrained by RNA-DNA interactions; seven domains are thus defined (the actual number is more than 100). (C) Folded and supercoiled DNA in the nucleoid. (D) The partially unfolded DNA of the nucleoid obtained by hydrolysing some of the RNA. (E) Chromosome with two single-strand breaks in the DNA, relaxing only the domains in which the nicks occur. See text for details. Certain elements of this model were first proposed in Reference 25. (From Pettijohn, D. and Hecht, R., *Cold Spring Harbor Symp. Quant. Biol.*, 38, 31, 1973. With permission.)

Its relationship to the intracellular nucleoid is not defined. As was discussed above, in a structure as complicated as the nucleoid, there is a definite possibility that structural rearrangements in the DNA and other components of the chromosome could occur during its isolation.

It is proposed that an RNA molecule bound to two or more separate sites on the isolated chromosome can define the position of a DNA fold and also restrain the rotation of the double helix at the sites of attachment. Rotation of the DNA would be limited by the possible coiling of the RNA about the DNA or would require breaking the RNA-DNA interaction. The DNA restrained by many different RNA molecules in this fashion would be segregated into a series of domains defined by the sites of RNA binding. Nicks in the DNA would relax only the domains in which they occur, since the rotation of the double helix could not be propagated into adjacent domains[25] (Figure 9E). Thus, many single-strand breaks per chromosome would be required to relax all of the supercoiling. Partial hydrolysis of the linking RNA molecules with RNase would result in partial unfolding of the DNA as the boundaries between adjacent domains are broken (Figure 9D). This results in a coalescence of adjacent domains, so that many domains become larger. A single nick in the DNA of an expanded domain will now relax a greater portion of the total supercoiling, explaining the finding that fewer nicks are required to relax the partially unfolded DNA. Complete hydrolysis of the RNA would result in a single circular supercoiled molecule, which could be relaxed by a single break or nick in the entire molecule. With this model, independence in the relaxation of folding and supercoiling is also possible. Nicks in the DNA relax supercoiling but should not affect folding since they need not influence the RNA-DNA interactions. Nucleoids partially unfolded after reaction with RNase would lose no supercoiling, since neighboring RNA-DNA sites would continue to limit the rotation of the DNA.

To account for the properties of the stabilizing RNA chains (see Section VIII), it is proposed that they are nascent RNAs under continuous synthesis in the nucleoid in vivo. Therefore, one of the sites of attachment of the stabilizing RNA to the folded DNA would be via its associated RNA polymerase

at the 3′ end of the nascent RNA. The nature of the other site(s) of attachment cannot be specified. It is possible that it may involve the formation of an RNA-DNA hybrid between a region(s) of the nascent RNA chain closer to its 5′ end. Evidence that RNA molecules may be bound to the DNA in this fashion was reviewed (Section VIII). Studies of nascent RNA molecules synthesized on supercoiled DNA templates indicate that the RNA may form a stable hybrid with the supercoiled DNA.[66,67] Apparently, the hybrid formation is energetically favored, as it provides a mechanism for relieving the strain in the underwound double helix. Although it is not known that such hybrids can form on DNA sequences removed from sites of RNA synthesis, extensive hybrids containing up to 600 RNA bases have been found.[67]

The model of Figure 9 as it stands would account for the DNA unfolding which occurs when proteins are denatured or dissociated. The release of RNA polymerase molecules could destabilize the 3′ attachment sites of the nascent RNA chains. Growth of cells in the presence of rifampicin or other inhibitors of RNA synthesis would also remove most of RNA polymerase and nascent RNA chains from the nucleoid, which could then lead to unfolding of the DNA. It is also possible that other proteins could be involved in stabilizing critical RNA-DNA interactions. As discussed above (Section VIII), there may be proteins which directly stabilize DNA folds by linking DNA to DNA in the membrane-free nucleoid.[28] For the reasons given in that section, these hypothetical proteins are not included in the model. If strong protein-mediated linkages such as these do occur, they would be expected to segregate the chromosome into separate domains. In this state the DNA of the chromosome, free of breaks or nicks, would be expected to maintain its supercoiling even when the RNA-DNA linkages are removed. When the chromosomes were treated exhaustively with RNase, no remnant supercoiling could be detected.[28] This suggests that either the DNA is not segregated into domains by protein-DNA linkages independent of RNA or that the chromosomes were nicked or broken extensively.

The model described in Figure 9 is intended to deal with the membrane-free nucleoid. Many of the properties of the membrane-associated nucleoid are similar, and one would expect to find extensive similarities in the two forms of the nucleoid. However, the sites of association of the nucleoid with the membrane should bring in additional constraints on the DNA. Multiple sites of strong attachment should also segregate the chromosome into domains and could stabilize folding patterns on the DNA independent of other interactions in the chromosome.

Potentially, the number of domains in a chromosome can be calculated from the number of single-strand breaks required to relax all of the supercoiling in the chromosome. Application of Poisson statistics to this problem requires knowledge of the size distribution of the domains and the assumption that the nicks occur randomly in the DNA. Assuming equally sized domains and random distribution, the number of domains relaxed by DNase hits has been estimated in the range 12 to 100 per genome equivalent of DNA[25,31] (a genome equivalent is the mass of the DNA in a single nonreplicating chromosome). The wide range is attributable to uncertainties in measuring precisely the single-strand molecular weights of the nicked DNA, difficulties in measuring simultaneously the supercoiling and number of nicks in nucleoids (since the rate of nicking is not totally reproducible from time to time), and uncertainties in the above assumptions concerning randomness of the nicking.

The use of gamma irradiation to introduce single-strand breaks into the DNA can alleviate most of these uncertainties. The rate of nicking the DNA is constant in time and reproducible, so that numbers of nicks can be more accurately measured at any dose. Since there is no diffusion problem (as there is with DNase), the assumption of random nicking seems more justified. Indeed, the measured single-strand molecular weights show random distributions.[68] Recent measurements using gamma irradiation to relax supercoiling in the nucleoid have indicated 100 ± 40 domains per genome equivalent of DNA (assuming equal-sized domains).[68] This approach also enables one to introduce single-strand breaks into the nucleoid DNA in vivo. Therefore, it should be possible to compare the restraints on the DNA in the intracellular nucleoid with those present in the isolated nucleoid. Studies of this kind are required to investigate any changes thay may come about in the structure of the chromosome during isolation.

XI. ELECTRON MICROSCOPIC OBSERVATIONS OF ISOLATED NUCLEOIDS

The structure of the isolated nucleoids observed in the electron microscope confirms several of the features deduced from physical-chemical properties and also suggests additional facets of the chromosomal structure not previously described. Some early studies of spread protoplasts showed the DNA of nucleoids with considerable clarity.[69] The authors identified supercoiled loops of DNA and even commented on possible participation of RNA molecules in the observed structure. Another observation on partially purified nucleoids of *B. megaterium* identified masses of DNA fibers "tangled" in structures having dimensions similar to that of the in vivo nucleoids.[22] More recent studies of nucleoids isolated by the high-salt methods have observed supercoiled loops of DNA radiating from a central dense mass.[50-52] In the case of the membrane-associated nucleoid, the central mass includes a patch of membrane[51] (Figure 10). The amount of twisting seen in the DNA loops was markedly varied by the addition of ethidium bromide,[50] in the manner expected from the physical-chemical characterization of the chromosome. In preparations where the DNA was more spread, occasional RNA molecules linking DNA-double helices were seen.[50]

In the most recent studies it has been found that the appearance of the nucleoids is dependent on salt concentrations present during the spreading on hypophases.[51,52] In many cases a strikingly clear picture emerges of the spread DNA of the nucleoids (Figure 10). The loops of DNA seem to occur within a limited size range and are resolved enough that some estimates of their number could be made. The membrane-associated nucleoids seemed to have fewer loops in the range of 65 to 130 per nucleoid, while the membrane-free nucleoids had 98 to 194 per nucleoid, with an average of 144. It was suggested that some of the loops in the membrane-associated nucleoids may have remained condensed in the central region and thereby were obscured by the envelope patch(es). The average number of loops per membrane-free nucleoid is close to the number of domains of supercoiling per genome equivalent of DNA – 100 ± 40 (see Section X). However, the nucleoids are composed of more than one genome equivalent of DNA so that the agreement is probably only within about a factor of two. There could be as many as two domains for each observed loop. It is fairly certain that most of the DNA of the membrane-free nucleoids appears in loops since estimates of the amount of DNA in loops is about 5×10^9 daltons per nucleoid or about two genome equivalents.[52]

The central "core" region of the membrane-associated nucleoid is obscured by the membrane patch, but this region can be seen more clearly in the micrographs of the membrane-free nucleoid (Figure 10). The dense material at the center appeared to be condensed RNA; this observation was supported by the finding that the material was greatly depleted when nucleoids on the monolayers were exposed briefly to RNase.[52] The results generally supported previous proposals (see Sections VIII and X) that there are many RNA molecules near the chromosome center stabilizing the condensed DNA. However, the micrographs suggest that most of the RNA of the isolated nucleoid may be tightly condensed in the central core regions. The reason for this is not clear.

The nucleoid structure shown in micrographs such as those of Figure 10 has been substantially spread out from its natural state in the cell. It should be noted that the maximal dimensions of the structures are much greater than the size of cells of *E. coli*. Possibly the conversion from fibers having toroidal DNA superhelices to loops having interwound superhelices accounts for some of the expansion; however, it also appears that the DNA has been partially unfolded. Approximately one half of the nucleoids observed in this study had more tightly condensed structures in which the unfolded loops could not be seen so easily.[51,52] It is likely that these more compact nucleoids more closely resemble the nucleoids in solution.

XII. TRANSCRIPTION OF NUCLEOIDS

The question of how DNA tightly packed in chromosomes can function as a template has intrigued molecular biologists for some time. Analysis of the DNA packing densities in bacterial nucleoids led some researchers to conclude that the limited space between double helices would restrict diffusion of large proteins and that transcription would be limited to DNA sequences on or beyond the nucleoid surface.[65] In support of this idea, it was noted that ribosomes are not

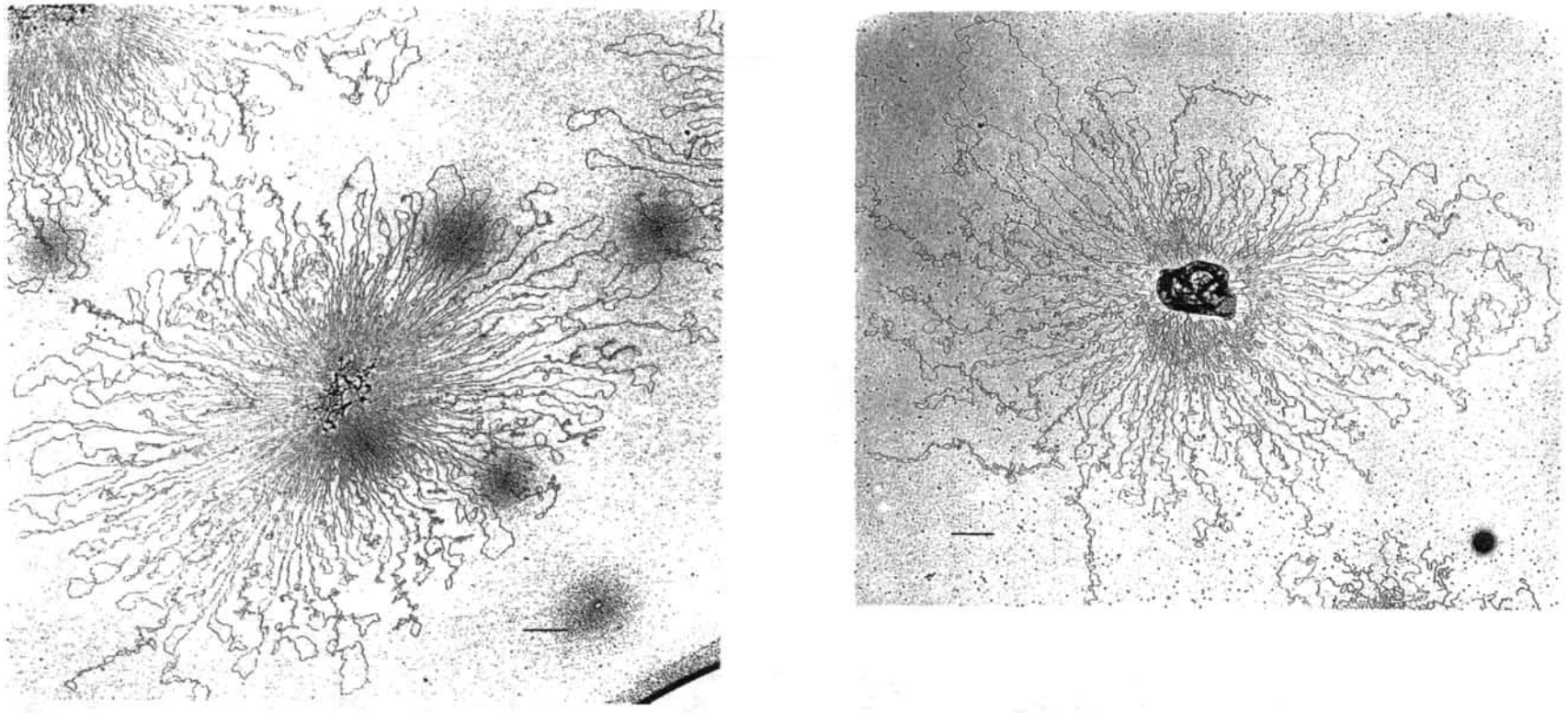

FIGURE 10. Electron micrographs of the membrane-associated and "membrane-free" nucleoids. The bars represent 1 μm. The nucleoids were surface spread with cytochrome C stained with uranyl acetate and rotary shadowed with platinum-palladium. (left) A "membrane-free" nucleoid; (right) a membrane-associated nucleoid. (From Kavenoff, R. and Ryder, O., *Chromasoma,* 55, 13, 1976. With permission. Micrographs courtesy of Drs. Ruth Kavenoff, B. Bowen, and O. Ryder.)

observed inside nucleoids; yet it is known that transcription and translation are closely coupled in prokaryotic cells. Ribosomes are observed on nascent RNA chains attached at positions close to the point of synthesis of the RNA.[71]

Ryter has proposed that DNA sequences containing active genes are looped out of the nucleoid into the cytoplasm where they can be available for interactions with RNA polymerase and ribosomes.[14] To test this hypothesis, high-resolution autoradiographic methods were used to examine the intracellular distribution of DNA with respect to the nucleoid surface. This experiment is technically difficult because the mean free path of β particles from tritium is comparable in length to the dimensions of nucleoids. Therefore, statistical distributions of the autoradiographic grains were required. It was found that in rapidly growing cells where transcription is extensive, the distribution of grains from ^{3}H-labeled DNA was more external to the nucleoid surface, while in slow growing or nongrowing cells the frequency of grains removed from the nucleoid surface was reduced. The distribution of grains from pulse ^{3}H-uracil-labeled RNA was also examined to investigate the positions of nascent RNA chains in the cell.[14] Again, the distribution was indicative of locations external to the nucleoid surface. From these results it was suggested that nuclear regions seen in thin sections of the cell correspond to genetically inactive DNA, while the active DNA is more removed from the visible nucleoid and cannot be visualized directly. This interpretation is certainly compatible with the microscopic observations summarized above (Section II) in which the nucleoid appears to be a dynamic structure undergoing shape and size variations in response to cellular metabolism. There are certain details of this interesting study that should be carefully considered.

The data do support the conclusion that there are significant amounts of DNA in the cytoplasm external to the visible nucleoid. Also, it seems convincing that the amount of this "looped-out" DNA varies in rapidly growing and slowly growing cells. It is not quite so clear, however, whether the sites of RNA synthesis are removed from the nucleoid. Considering the length of an average transcriptional unit in *E. coli* and the rate of RNA chain propagation, one would expect most of the RNA labeled in a 30-sec pulse to be in nascent chains, while in a 3-min labeling time most of the labeled RNA should have completed its synthesis. Therefore, one might expect the distribution of labeled RNA in the cell to be quite different after labeling times of 30 sec and 3 min; instead, the distributions were indistinguishable.[14] It does not seem that the similarity can be accounted for by limited diffusion of polysomes released from the DNA. The diffusion distances in the cell are very tiny, on the order of a micron or less, and many of the labeled mRNA molecules should have been at intermediate stages of degradation at this time. It is not clear from the description[14] whether or not RNA synthesis had been completely blocked following the labeling of RNA chains. Continued metabolism of RNA could explain similarities in the two distributions. It will be of considerable interest to conduct additional experiments confirming the conclusions of this important and provocative study.

Potentially, many of the questions concerning the transcription of genes in folded DNAs could be probed in vitro using the isolated bacterial nucleoids as a model system. The endogenous RNA polymerase molecules isolated with the nucleoid are active in RNA synthesis, and one can readily study chain elongation in vitro of nascent RNA chains which were initiated in the cell. This approach has been used to evaluate the number of RNA polymerase molecules on the rRNA genes of the nucleoid and to study the synthesis of rRNA in the absence of posttranscriptional modifications (for example, see References 23 and 39). Likewise, it has been demonstrated that the isolated nucleoid is an active template for added RNA polymerase.[30] The folded DNA of the nucleoid supports a more rapid rate of RNA chain initiation than the unfolded DNA from the same chromosomes. The maximum number of RNA polymerase molecules which can simultaneously synthesize RNA is greater on the nucleoid than on the unfolded DNA.[30] As estimated from the rates of rRNA synthesis in vitro relative to the rates of synthesis of all other RNA species, the availability of rRNA genes to interact with RNA polymerase was nearly identical in isolated nucleoids and unfolded DNA. From this kind of study no evidence emerged to support the conclusion that extensive amounts of potential template in the isolated nucleoid was unavailable for transcription. However, this approach indirectly estimates the amounts of available template from the maximal rates of RNA synthesis that can occur on the

DNA. As noted in this study,[30] unfolding the DNA could affect its template capacity in several ways, and it is difficult to separate the contribution due to exposing the DNA of the nucleoid's interior. Also, as noted above, it is not certain how closely the structure of the isolated nucleoids resembles its structure in vivo. Questions of this kind will have to be resolved before the isolated chromosomes can be fully exploited in the studies of in vitro transcription.

XIII. ADDENDUM AND SPECULATION

It is apparent that research on the structure of the nucleoid has just begun; there is much still to be learned. The major questions concerning details of the DNA structure in the chromosome, definition of the elements which organize the DNA conformation, and relationships between the structure of the chromosome and gene expression are unanswered.

While the evidence reviewed here strongly suggests that the nucleoid structure is dynamic in the organization of the DNA, this idea has not been proven; the mechanisms by which the chromosomal structure could be reorganized during growth are also not indicated by the studies. Rather strong restrictions would be imposed on the organization of the chromosome if it is true that DNA within the interior of nucleoids is transcriptionally silent. It would be required either that all promoters be permanently maintained on the surface or that the dynamics of the chromosomal rearrangements be so rapid that all promoters are frequently brought to the surface. There is not evidence that repressed operators and promoters are unavailable in the chromosome. To the contrary, induction of repressed genes is known to be a very rapid process. A likely alternative is that RNA polymerase molecules can diffuse into the interior of the nucleoid, bind to promoters, and initiate transcription; however, when transient rearrangements of the chromosome carry that DNA to the outside of the nucleoid, a looping-out of the DNA is stabilized by interactions of the nascent mRNA with ribosomes.

In such a model the organization of coordinately regulated genes into operons is strongly favored. A single loop of DNA could maintain the entire operon in a position for transcription. The coordinate expression of genes separated on the linear genetic map (such as the arginine system) would require multiple loop-outs. Any periodicities in the folds or loops of the DNA in the nucleoids could be used in the periodic spacing of separated coordinately controlled genes. Location of one gene in position for transcription would then favor a similar positioning for the other genes. Periodic spacing of the known genes on the genetic map of *E. coli* has already been noted.[72] However, the observed periodicities are over a much longer range than any of the domains or loops observed in nucleoids.

In the near future it should be possible to determine more explicitly how much freedom there is in the chromosome to permit long-range conformational transitions of the DNA. It will also be of interest to attempt to relate "loop-outs" of the nucleoid DNA with the expression of specific genes and operons.

The domains of supercoiling indicated by physical studies of nucleoids, the DNA loops visualized by electron microscopy of the isolated chromosomes, and the DNA loops indicated from the autoradiographic analysis of cell sections may be equivalent. The dimensions of the loops seen by electron microscopy are too large to fit into a cell; however, it should be remembered that the DNA in isolated nucleoids seems to have interwound rather than toroidal superhelices. If toroidal superhelices exist in the cell (as might be expected if the prokaryotic equivalent of nu bodies exist), the loops would be compressed about sevenfold in vivo. The question of what defines the limits of the DNA loops in vivo has not been answered. It is clear from studies of the isolated nucleoids that the domains of supercoiling (which in the simplest models of chromosome structure would be equivalent to loops) are defined by interactions of the DNA with certain RNA and protein components of the isolated nucleoid. The essential RNA molecules seem to be nascent RNA chains at various stages of synthesis. While there is no reason why some nascent RNA chains could not also function in this manner in the cell, there is as yet no proof that they do. If this were the case, the limits on the domains would also be in a constant state of flux in vivo.

A final thought should be mentioned before closing. All of the discussion throughout this review has been oriented around the idea that the nucleoid structure, whatever its degree of flexibility, is organized by molecules bound to the DNA of the chromosome. Indeed, a good deal of

the evidence from studies of isolated nucleoids supports this view. There is also another alternative which may play some role in maintaining the state of condensation of the chromosome. 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 (see Reference 57 for example). 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.

XIV. ACKNOWLEDGMENTS

I would like to thank Dr. Susan Hamilton for providing the data described in Figures 2, 3, and 4 (from Reference 70). Drs. J. Griffith, A. Ryter, and A. Worcel generously permitted me to use some of their published data to illustrate this review and also supplied photographs. Special thanks are due Drs. R. Kavenoff, O. Ryder, and B. Bowen for providing the electron micrographs shown in Figure 10. I would also like to express my gratitude to the scientists throughout the world, too numerous to mention here by name, who supplied copies of manuscripts and descriptions of unpublished results. The research from the author's laboratory described here has been supported by research grants from the U.S. Public Health Service, Grant No. GM18243, the U.S. National Science Foundation, Grant No. GB43358; and U.S. Public Health Service Training Grant No. GM00781. This is publication No. 678 of the Department of Biophysics and Genetics, University of Colorado Medical Center, Denver, Colorado.

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