ON THE ORGANIZATION OF DNA IN ISOLATED BACTERIAL CHROMOSOMES

(bacterial DNA, folded chromosomes, EM, RNA)

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SUMMARY

We review physical studies and electron microscopy of membrane-free folded chromosomes from E. coli [*Kavenoff and Bowen, Chromosoma 59, 89 (1977)*], *and we consider the apparent relation between the organization of the DNA and the association of nascent RNA.*

INTRODUCTION

We want to know what determines the structure of a chromosome. From chromosome banding and *in situ* hybridization studies of eukaryotic chromosomes, we have evidence of fairly specific organization of DNA in particular chromosomes. And from physical measurements of DNA size and chromosomal DNA content, we have evidence that most chromosomes contain relatively long DNA molecules; for example, in *Drosophila melanogaster* there are DNA molecules one to two centimeters long approximating the DNA content of chromosomes only a few microns long (Kavenoff, Klotz and Zimm, 1973). How is so much DNA organized in such small structures?

To learn how large DNA molecules are organized in small chromosomes, we have used the electron microscope to examine isolated bacterial chromosomes (Fig. 1). These chromosomes are less than a micron in diameter and contain about 2 mm of DNA, that is, about one-tenth as much DNA as *Drosophila* chromosomes, one-hundredth as much DNA as mammalian chromosomes, and one-thousandth as much as some plant chromosomes. This size difference is important because it reduces the technical difficulties which increase radically with chromosome size.

Here we review work showing that bacterial DNA is highly organized in the isolated chromosomes, and we discuss the role of RNA in this organization.

FOLDED CHROMOSOMES FROM *Escherichia coli*

The work we will consider was done with "membrane-free folded chromosomes" isolated from *E. coli* by the method of Stonington and Pettijohn (1971) (Fig. 1). Growing cells are treated with lysozyme to degrade their cell walls, and then they are lysed with mild detergents in 1 M salt at room temperature. These lysates are sedimented on sucrose gradients to purify the chromosomes. The chromosomes have a sedimentation coefficient of about 1900 S (Fig. 2, Bowen, unpublished; Hecht 1976). They contain about 60% (dry weight) DNA, 30% RNA, including most of the nascent RNA, 5 to 10% protein, most of which is RNA polymerase, and less than 1% lipid (Pettijohn 1976).

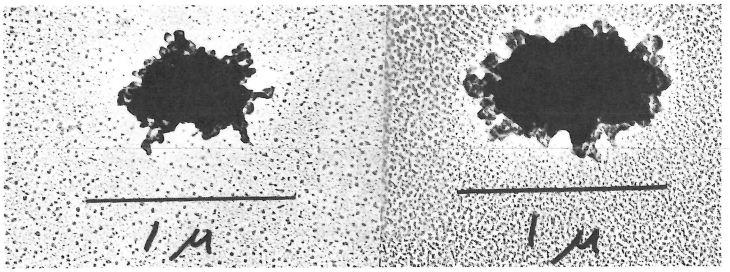


Figure 1. Electron micrograph of a highly condensed folded chromosome. Prepared as discussed in the text, except that the hypophase contained 0.15 M salt and more formaldehyde (0.1%) and spreading was performed in a room at 4°C.

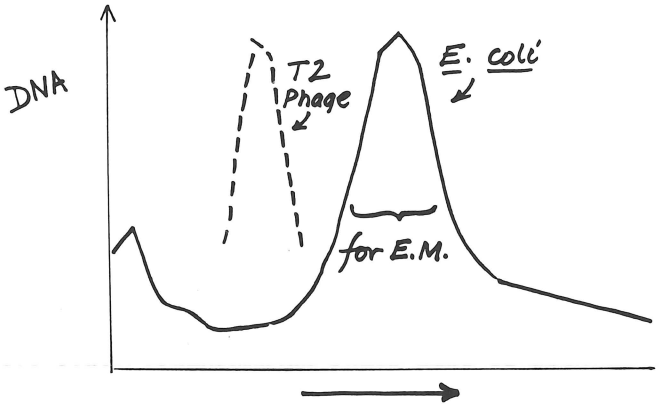


Figure 2. Sedimentation pattern of folded chromosomes prepared as described in the text and, in this particular case, co-sedimented with T4 phage as a sedimentation marker.

PHYSICAL STUDIES

Several studies indicated that in these folded chromosomes the DNA is essentially intact. Accurate measurements of DNA size are difficult with DNA's the size of bacterial DNA (or larger), but the measurements indicated that at least some of the single strands of DNA (from denatured chromosomes) are at least as long as the haploid length (Worcel and Burgi 1972; Pettijohn and Hecht 1973; Drlica and Worcel 1975; Bowen, unpublished).

In view of the genetic and autoradiographic evidence for the circularity of the *E. coli* chromosome and in view of the fact that many circular DNA's are supercoiled, Worcel and Burgi asked whether a folded chromosome contains a closed covalent circle of DNA and whether the DNA is supercoiled. What they found was a novel type of supercoiling (Worcel and Burgi 1972). [Whether the chromosome contains a closed covalent circle of DNA and not a "relaxation complex", that is, a circle closed by a non-DNA link such as RNA or protein, has still not been established, although the balance of evidence available (Drlica and Worcel 1975; Dworsky 1975 a and b) suggests to us, at least, that the folded chromosome may contain one or two such non-DNA links.]

To test for supercoiling of the DNA, Worcel and Burgi examined the effect of the dye ethidium bromide on the velocity sedimentation behavior of folded chromosomes. The effect of this dye on the sedimentation behavior of supercoiled DNA is unique--it differs from its effects on other, non-supercoiled DNA's. Worcel and Burgi found the dye's effect with folded chromosomes to be like the effect on supercoiled circles, with one difference. Whereas supercoiled circles require only one DNase break to abolish the dye's unique effect, the folded chromosomes required many breaks!

To explain their data, Worcel and Burgi proposed a model (Fig. 3). They wrote, "The chromosome shown is made up of seven loops (although the actual number is much larger...) and the DNA of each loop is coiled into a 'slinky' (this coiling will result in super helices when the DNA is extended) [as on the right]. The black rod holding the loops together (both in their supercoiled and relaxed conformation) represent the RNA core" (Worcel and Burgi 1972, p. 143).

The reason that Worcel and Burgi proposed an RNA core holding together the loops of DNA was that the folded chromosomes were very sensitive to RNase and insensitive to proteases. With RNase, Stonington and Pettijohn found a large increase in viscosity (Stonington and Pettijohn 1971), and Worcel and Burgi found a large decrease in sedimentation coefficient (Worcel and Burgi 1972). Worcel and Burgi speculated that the core was a single RNA molecule because the effect of RNase appeared to be all or nothing; however, Pettijohn and Hecht showed that the effect of RNase was progressive, not all or nothing (Pettijohn and Hecht 1973). In this way they refined the Worcel-Burgi model.

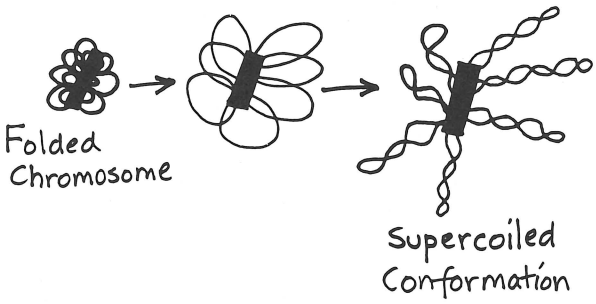


Figure 3. Schematic representation of Worcel and Burgi's model for the folded *E. coli* chromosome. Thick bar represents RNA; all other lines represent DNA.

ELECTRON MICROSCOPY

We have been able to confirm the models of Worcel and Burgi (1972) and Pettijohn and Hecht (1973) by electron microscopy. For example, consider the following electron micrograph (Fig. 4, Untreated). This shows a folded chromosome that has been "spread out" for visualization by the Kleinschmidt and Zahn technique. (For exact experimental details, see Kavenoff and Bowen 1977). This is typical of our results when we spread the chromosomes in 1 M salt and cytochrome c on a hypophase of 0.4 M salt and very dilute formaldehyde. You can see many supercoiled loops of DNA radiating from a central core.

There is rough agreement between the number of loops we observe and the numbers estimated from biophysical studies. With Robin Cole's help, we counted the number of loops per chromosome in 39 different chromosomes, and we found 98 to 194, with an average of 144. The numbers estimated from biophysical studies range from 12 to 220 (Worcell and Burgi 1972; Pettijohn and Hecht 1973; Hecht, Taggart and Pettijohn 1975; Lydersen and Pettijohn 1977).

Two lines of evidence show that the core contains RNA. The first is that the core is degraded by RNase. For example, compare the central region typical of chromosomes treated with RNase (Fig. 4, RNased) with the central region typical of the controls (Fig. 4, Washed). (In these experiments, the chromosomes were spread before the RNase treatment, so that the organization of the loops of DNA was stablized by the cytochrome c and formaldehyde). The second line of evidence that the core contains RNA is that the core is dissociated by lowering the salt concentration in the hypophase, and this also results in the appearance of RNA concentrated in the central region. For example, consider this example of the results with a hypophase of 0.15 M salt (Fig. 5, Washed). Under these spreading conditions free RNA appears very bush-like, and you can see bush-like material concentrated in the central region (Fig. 5, Washed). You can see that this material is degraded by RNase (Fig. 5, RNased),

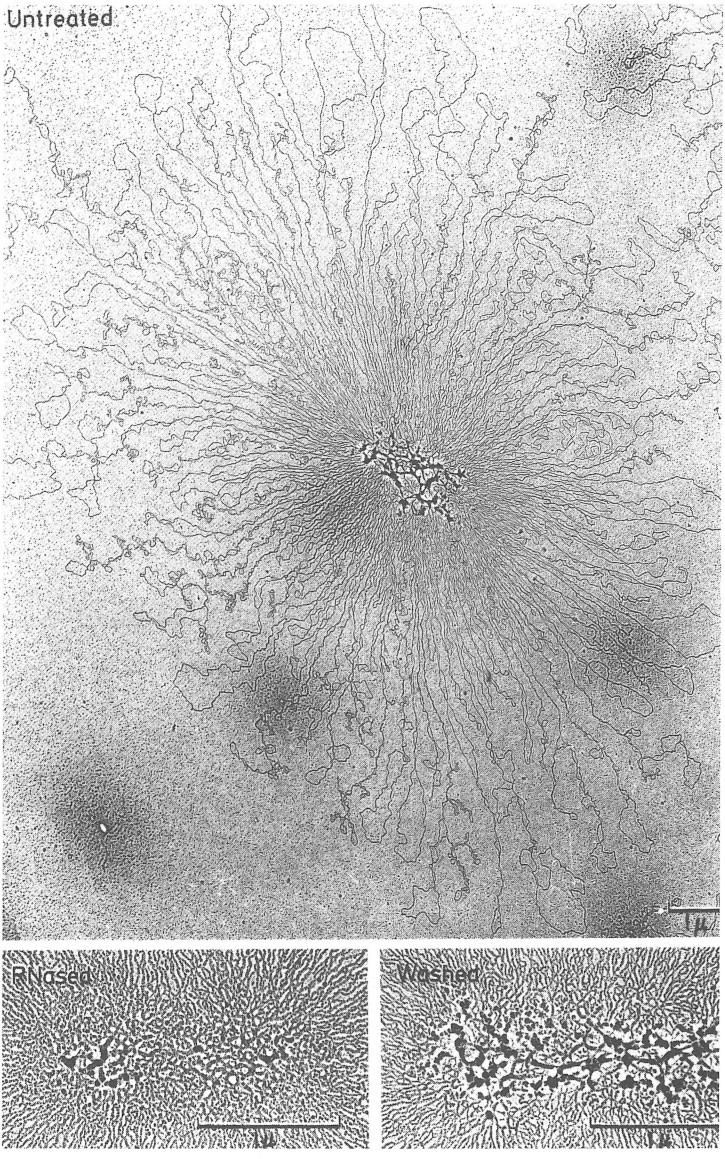


Figure 4. Electron micrograph of a folded chromosome spread out on 0.4 M salt (see text). Insets below show central regions of chromosomes spread on 0.4 M salt and then washed with either buffer alone or buffer containing RNase (from Kavenoff and Bowen 1977).

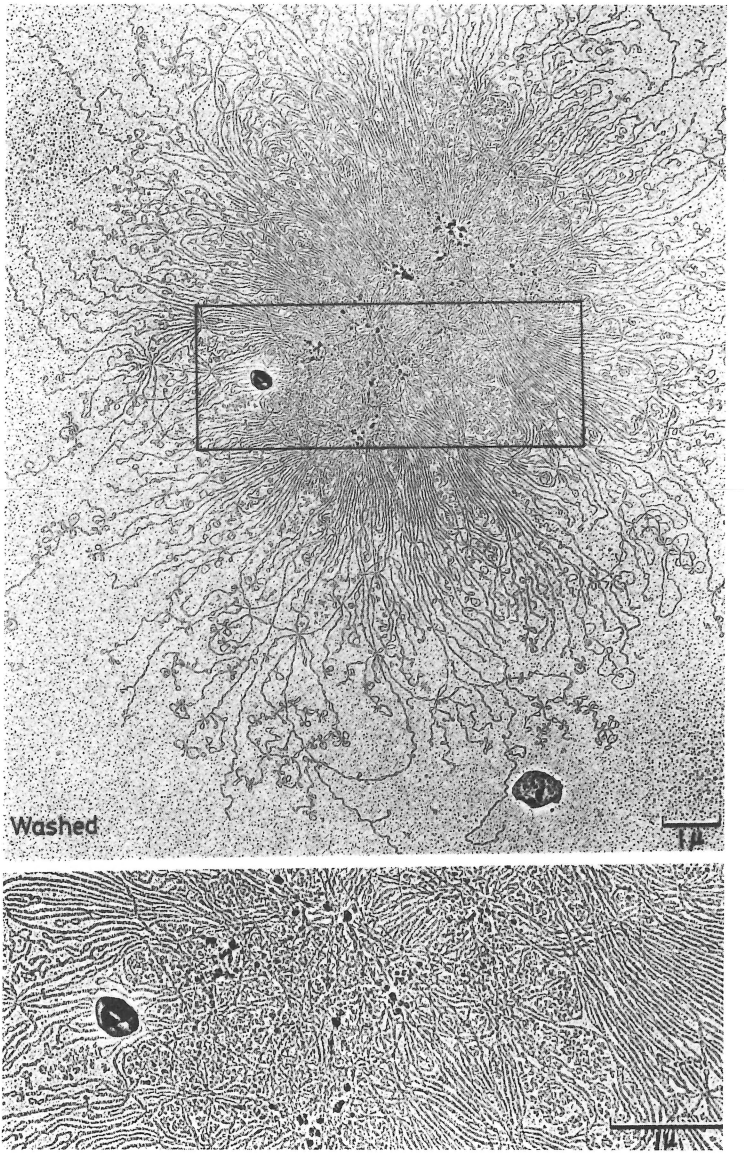
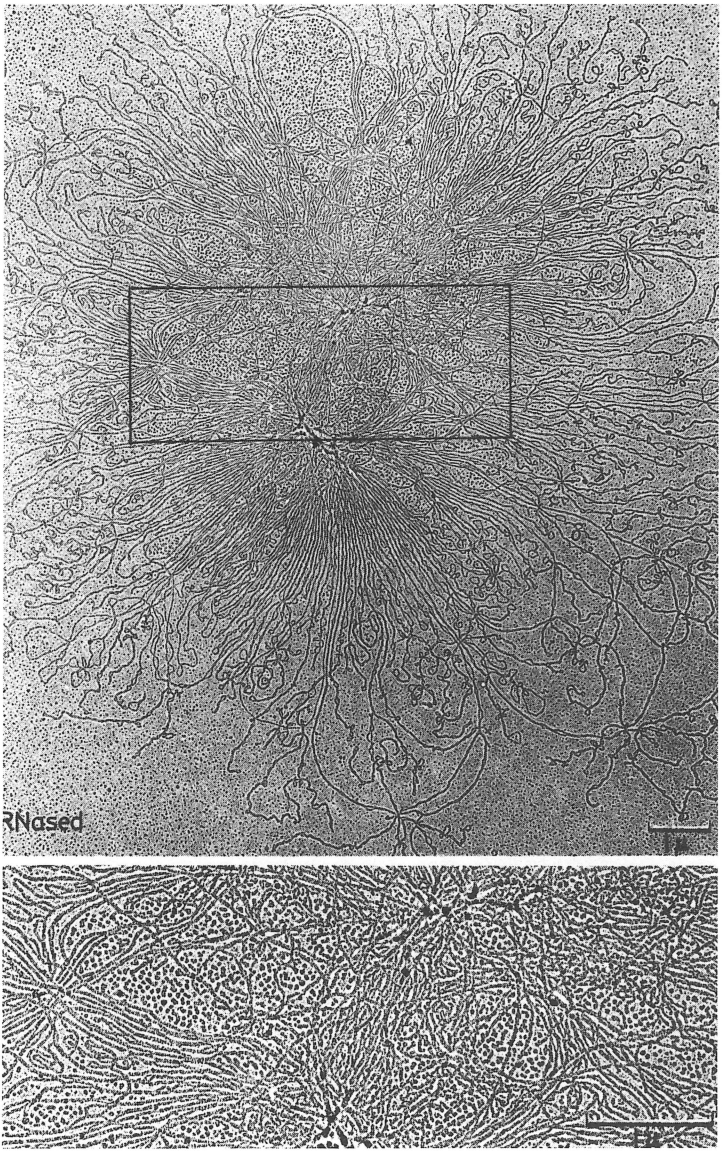


Figure 5. Electron micrographs of folded chromosomes spread on 0.15 M salt (see text) and then washed with either



buffer alone (Washed, left) or buffer containing RNase (RNased, right) (from Kavenoff and Bowen 1977).

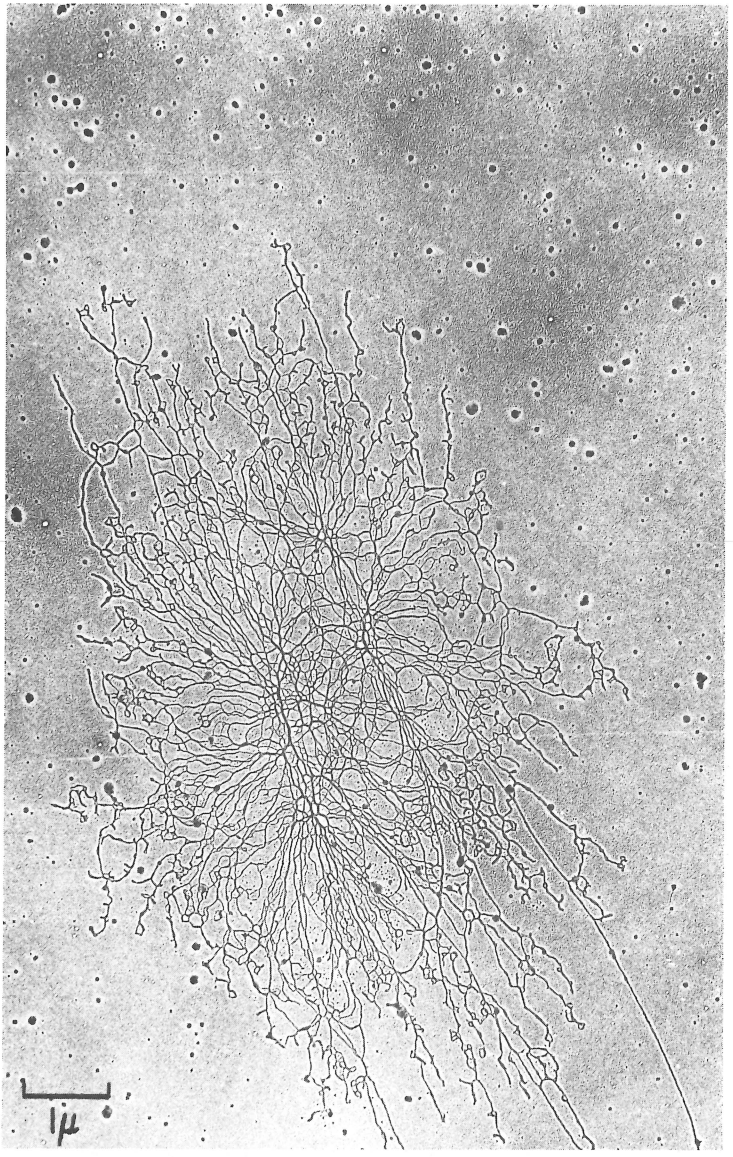


Figure 6. Electron micrograph of a typical folded chromosome spread out on 0.15 M salt (see text) and washed first for five seconds with 0.1 M ammonium acetate, then washed for two seconds with 0.1 M ammonium acetate containing 10 μg/ml Pronase (calbiochem, nuclease-free) and then washed for five seconds with 0.1 M ammonium acetate containing 40 μg/ml RNase (as described in Kavenoff and Bowen 1977).

that is, the bushes are converted to large dots. (These large dots are further degraded if the spread chromosomes are treated with pronase before RNase (Fig. 6). So there is protein associated with the RNA, but we cannot say whether the protein is cytochrome c which was mixed with the chromosomes before spreading or some other, more interesting protein.)

When Delius and Worcel spread folded chromosomes with much lower salt concentrations and formamide, they did not see such well-organized supercoiled loops or RNA cores (Delius and Worcel 1973, 1974). They observed fairly extended RNA chains. RNA chains were particularly concentrated in the central region, and sometimes RNA chains seemed to hold DNA strands together.

Thus all of the electron microscopy shows a large fraction of the RNA in the central region--this is a surprising result. This should be tested more quantitatively by autoradiography. In the meantime, a reasonable hypothesis is that the loops of DNA are stabilized by an RNA core resulting from the aggregation of the nascent RNA chains when the cells are lysed with 1 M salt. This hypothesis is consistent with reports that folded chromosomes from *E. coli* treated with rifampicin (which inhibits the initiation of RNA synthesis) are very unstable in 1 M salt concentrations (Dworsky 1975b) and with the well-known tendency of RNA to aggregate in the presence of high salt concentrations (e.g., Boedtker 1968). This raises the question as to whether there is any association of different nascent RNA's *in vivo* as might be mediated by hybridization of homologous "leader sequences" at the 5' ends of nascent RNA's.

QUESTIONS FOR FUTURE STUDY

Many basic questions remain concerning the organization of folded chromosomes from bacteria. One question is whether RNA is the only substance stabilizing the DNA loops. Another question is how the DNA in loops is further compacted (for example, compare Fig. 1 with Fig. 3). A fundamental question is the exact relation between the organization of isolated folded chromosomes and the organization of the chromosomes (including nascent RNA!) *in vivo*.

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