JOURNAL OF VIROLOGY, June 1974, p. 1346-1355

Vol. 13, No. 6
Printed in U.S.A.

Coiled Rings of DNA Released from Cells Infected with Bacteriophages T7 or T4 or from Uninfected *Escherichia coli*

CAROL BERNSTEIN AND HARRIS BERNSTEIN

Molecular Biology Program, Department of Microbiology, University of Arizona College of Medicine, Tucson, Arizona 85724

Received for publication 1 February 1974

The replicating intracellular DNA of phage T7 was labeled at high specific activity with tritiated thymidine. The DNA of uninfected *Escherichia coli* was similarly labeled. Portions of cells which contained replicating phage T7 or *E. coli* DNA were lysed by a lysozyme, freeze-thaw, sodium lauryl sulfate procedure, and the DNA was spread on Millipore membranes for visualization by autoradiography. The DNA of phage T7 appeared to be highly concatenated reaching lengths of up to 721 μm. Much of the DNA of phage T7 and *E. coli* was retained in compact globular structures. In addition, orderly coiled rings of varying diameter up to about 43 μm were regularly observed. Similar coiled ring structures were also observed in autoradiographs of replicating phage T4 DNA which had been prepared in previous experiments. Worcel and Burgi (27) have presented evidence that *E. coli* chromosomes, when gently extracted from cells, are in a multilooped and superhelically twisted configuration. The coiled rings which we have observed may correspond to the relaxed, multilooped configurations which they find when the superhelical twists have been relieved by one or more nicks in each loop.

The replicating DNA produced by phage T7 during the latter half of its infectious cycle has previously been studied by centrifugation techniques and by electron microscopy. This DNA was observed to be concatenated (9, 22, 25). Most of it occurs in condensed structures which sediment at up to 1,500*S* in a neutral sucrose gradient (9). The replicating DNA of phage T4 similarly has been shown by centrifugation, electron microscopy and autoradiography, to be concatenated and largely in condensed structures which sediment at about 200*S* in a neutral sucrose gradient (6, 11, 13).

The *Escherichia coli* chromosome has been observed to be a circular molecule about 1 mm long (4, 7, 20). It too has been shown to have a folded structure that sediments between 1,300*S* and 2,200*S* when gently extracted from the cell at 25 C (27). A folded, membrane-attached form that sediments at 3,000 to 4,000*S* has also been obtained when cells are extracted at temperatures below 10 C (10, 24, 28). The 1,300 to 2,200*S* structures were shown to contain DNA arranged in 12 to 80 loops associated with an RNA-containing core (27).

We report here on an autoradiographic study of the condensed form of phage T7, phage T4, and *E. coli* replicating DNA. A significant proportion of the condensed DNA from all three organisms is arranged in the form of coiled rings of various dimensions. Our evidence suggests that these rings reflect the internal organization of the DNA within the cell and may correspond to the relaxed folded structures described by Worcel and Burgi (27) for *E. coli* DNA.

MATERIALS AND METHODS

Autoradiographs from former experiments. All of the autoradiographs of intracellular replicating phage T4 DNA were from the experiments described by Bernstein and Bernstein (3). The autoradiographs of *E. coli* DNA prepared by the one-step sodium lauryl sulfate extraction procedure, and the autoradiographs of mature phage chromosomes were from the experiments described by Bernstein (2).

Media and strains. The liquid medium used for growth of phage T7, phage T4, and *E. coli* was M9E. This consisted of M9 medium (1) supplemented with $FeCl_3 \cdot 6H_2O$ (2.7 μg/ml) and Difco vitamin-free Casamino Acids (2.5 mg/ml). When labeling of *E. coli* or phage T7 DNA was required, *E. coli* B3, a thymine-requiring strain was used. *E. coli* B3, when being stored, was kept in liquid medium with 50 μg of thymidine per ml. The wild-type stock of phage T7 used in these experiments was obtained from W. Summers. For titering stocks of phage T7, *E. coli* BB was used as host. Growth of phage on agar plates was performed at 30 C by the agar overlay method (1) using Hershey broth (23).

Labeling of the DNA. For labeling *E. coli* DNA, *E.*

coli B3 was first grown in M9E supplemented with thymidine at 5 μg/ml at 30 C until a cell density of 2.5 × 10^7/ml was achieved. The cells were centrifuged and resuspended in M9E medium containing tritiated thymidine at 4 μg/ml and at a specific activity of about 50 Ci/mM (New England Nuclear Corp.). The labeled thymidine was at an adequate concentration to support growth of *E. coli* B3. Growth was continued for about four generations and then a portion was removed for lysis and autoradiographic analysis of the cell DNA. Under these conditions of labeling the cells had begun growing with a snake-like morphology. This snake-like morphology is characteristic of our strain of *E. coli* B3 when it is grown in media containing [^{3}H]thymidine. The remaining labeled *E. coli* cells were infected with phage T7 at a multiplicity of infection of about 14 phage per snake cell (or 3.5 per normal cell equivalent). After infection the host DNA breaks down and the products of the breakdown are utilized to build up a pool of phage T7 DNA (12, 14, 21). Roughly 90% of the phage T7 DNA is synthesized from host product, whereas about 10% is from extracellular sources (15, 16, 18). Infected cells were removed late in the infectious cycle (at the times indicated in Fig. 1) and their intracellular DNA was released. Uninfected cells make up less than 0.0001% of this population of cells.

Lysis procedure. Cells to be lysed were diluted into ice-cold lysis medium containing 0.1 M KCN and 0.05 M EDTA at pH 8.0. As described by Bernstein (2), 0.5-ml portions of cells at 10^6/ml were pipeted into dialysis cups covered with 50-nm membranes (Millipore Corp.). The membranes were fixed to the open end of the cups with their shiny sides facing inward. The cells were lysed in the cups by using the simplified three-step lysozyme-EDTA, freeze-thaw, and sodium lauryl sulfate treatment of Broker and Lehman (5), except that the sodium lauryl sulfate was used at room temperature. The lysates were dialyzed against a solution of 0.05 M EDTA and 0.01 M NaCl at pH 8.0 for 15 h at room temperature. The dialysis solution was changed every 2 h. After dialysis the cups were drained by putting them on their sides, making tiny holes in the top and bottom of the membranes with a needle, and draining out the liquid with tissues. Much of the DNA remained on the Millipore filter when the cups were drained. This was the DNA used for autoradiography.

Autoradiography. Kodak AR-10 film was used as previously described (2) except that exposure was at 4 C for 44 to 46 days and the developer used was Kodak D19. For each different type of infected or uninfected cells the labeled DNA obtained from 10 dialysis cups on 10 individual Millipore filters was autoradiographed.

Photography. The stripping film was removed from the Millipore membrane immediately after photographic processing and, while still wet, was allowed to adhere to gelatin-coated slides prepared as recommended by Kodak for AR10 film. After each piece of film dried on its slide, the slide was dipped in xylene. Permount mounting medium was put on the film, and the film was covered with a number 1.5 cover slip. Each piece of film was completely scanned at ×125. For photomicroscopy at high magnification a Zeiss microscope with a Planapochromat oil immersion ×63 bright field Zeiss objective, a ×12.5 eyepiece, and a 35-mm camera back with a ×2 focusing telescope was used. For photographing the long structures or larger areas of Fig. 3 a ×40 objective was used. The film used for photomicroscopy was Kodak Panatomic X. It was developed in Microdol X diluted 1:3. Contact prints were made from the negatives on Kodabromide F3 paper.

RESULTS

To estimate the best times for extracting late replicating phage T7 DNA, the time course of phage progeny production under our growth conditions, but with unlabeled thymidine, was determined. Figure 1 shows the single-step growth curve obtained. At the times indicated by the arrows little or no long *E. coli* DNA would be expected to be remaining, and large replicating phage T7 DNA structures should be present (12, 14, 21).

In a parallel culture, *E. coli* B3 was grown in [^{3}H]thymidine-labeled media and infected with phage T7 as described in Materials and Methods. Labeled phage T7 DNA was released from portions taken at 13, 17, and 20 min after infection, the three times chosen on the basis of the growth curve in Fig. 1. Labeled *E. coli* DNA

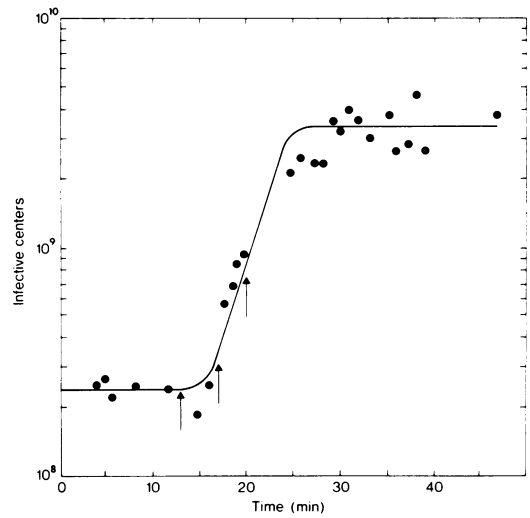

FIG. 1. *One-step growth curve of phage T7. A log-phase culture of E. coli B3 growing in M9E supplemented with nonradioactive thymidine at 5.0 μg/ml was infected with wild-type phage T7. Samples were assayed for infective centers at various times. On the basis of the results obtained the times 13, 17, and 20 min (as indicated by the arrows) were selected for sampling late replicating phage T7 DNA in a parallel T7-infected culture. The burst size in this experiment was 51 phage per bacterium. Most of the infective centers measured prior to the rise period are due to unadsorbed phage.*

was also released from a portion of cells taken prior to infection. An additional portion of labeled phage T7-infected cells was allowed to complete the infectious cycle. The burst size was 26 phage per cell. In our experience, relatively low burst sizes of viable phage are obtained when cells are grown in high specific activity [^{3}H]thymidine-labeled media. A suicide curve was determined with these labeled phage (Fig. 2). It can be seen that there was approximately 90% killing in 90 h with the remaining survivors resistant to further inactivation. The approximately 10% unlabeled phage may be explained by the fairly large proportion (about 30 to 50%) of unlabeled parental phage DNA which is conserved and transmitted to progeny during phage T7 infection (8).

Although much of the phage T7 DNA was tangled and difficult to characterize, there appeared to be three main distinguishable configurations. First, compact globular structures, such as shown in Fig. 3f were very common. Second, long, linear strands of phage T7 DNA, as shown in Fig. 3a to d, were also very frequent. The structures shown in Fig. 3a to d are 527 to 721 μm in length. Since a mature phage T7 chromosome is about 11 to 15 μm in length when visualized in the electron microscope (14, 19), the structures shown in Fig. 3a to d are interpreted as concatenated DNA which is between 35 to 66 times the length of a mature phage T7 chromosome. The linear DNA seen was almost always longer than a mature chromosome. A third regularly observed configuration of the phage T7 DNA was a coiled ring structure, examples of which are shown in Fig. 4a to o and Fig. 5a to o. These rings have considerably more DNA per unit length around their circumference than a free strand of DNA. They appear to be composed of multiple loops of DNA which are adhering to one another. The rings varied from about 43 μm in diameter down to the smallest sizes resolvable by autoradiography. Examples of larger rings are shown in Fig. 4 and smaller ones in Fig. 5. Sometimes these rings were relatively smooth in appearance, such as in Fig. 4g, h, and i or Fig. 5f, g, and h, and sometimes they had quite a few strands emerging, such as in Fig. 4b, d, and l or Fig. 5a, c, and i. Often the emerging strands came mostly from one are a, forming a tail as in Fig. 5b or a blob as in Fig. 4i, m, and n. However, though the appendages varied, the ring structure itself generally had rather rounded and regular contours.

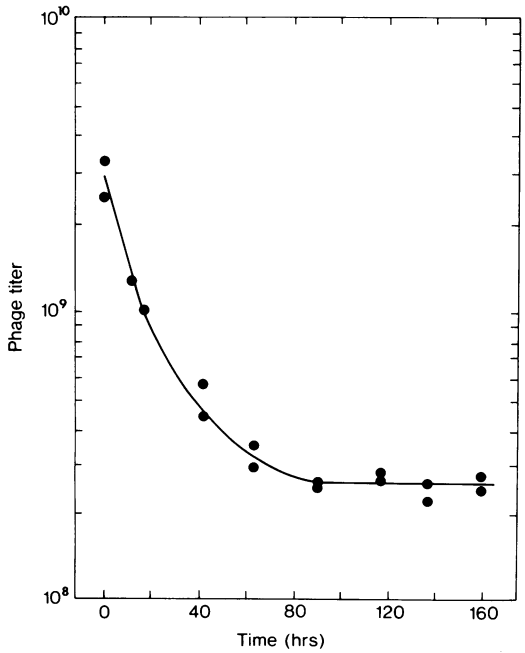

FIG. 2. *Inactivation curve of progeny phage T7 obtained from the E. coli B3 cell culture grown and infected in [^{3}H]thymidine-labeled medium. Samples of infected cells removed from this culture prior to lysis were used for extracting the phage T7-replicating DNA examined in the autoradiographs.*

Often structures were observed which appeared to be partial or interrupted rings (Fig. 6a to o). Sometimes the DNA made an almost complete ring, as in Fig. 6a, and in other cases a large segment was void of DNA, as in Fig. 6l.

The rings and interrupted rings were quite prevalent, and on the order of hundreds of such structures were seen per Millipore membrane. Ring structures with their associated strands represented perhaps a few percent of the DNA, although the percentage varied from one membrane to another.

No striking differences were noted in the characteristics of the multistranded rings extracted at 13, 17, and 20 min after infection. At 20 min there appeared many small dense DNA condensates which are presumably mature intact phage. These condensates can be seen in the backgrounds of the autoradiographs of 20-min samples.

The *E. coli* DNA extracted by the three-step lysis procedure looked very much like the phage T7 DNA. It also occurred in three main distinguishable configurations: a compact globular form, a linear form, and a coiled ring form. Examples of the coiled ring form of *E. coli* DNA are shown in Fig. 3u to y and Fig. 4u to y, and examples of broken rings of *E. coli* DNA are shown in Fig. 6u to y. Although we did not obtain examples of completely unfolded replicating chromosomes, such as those of Cairns (7), Bleecken et al. (4), and Rodriguez et al.

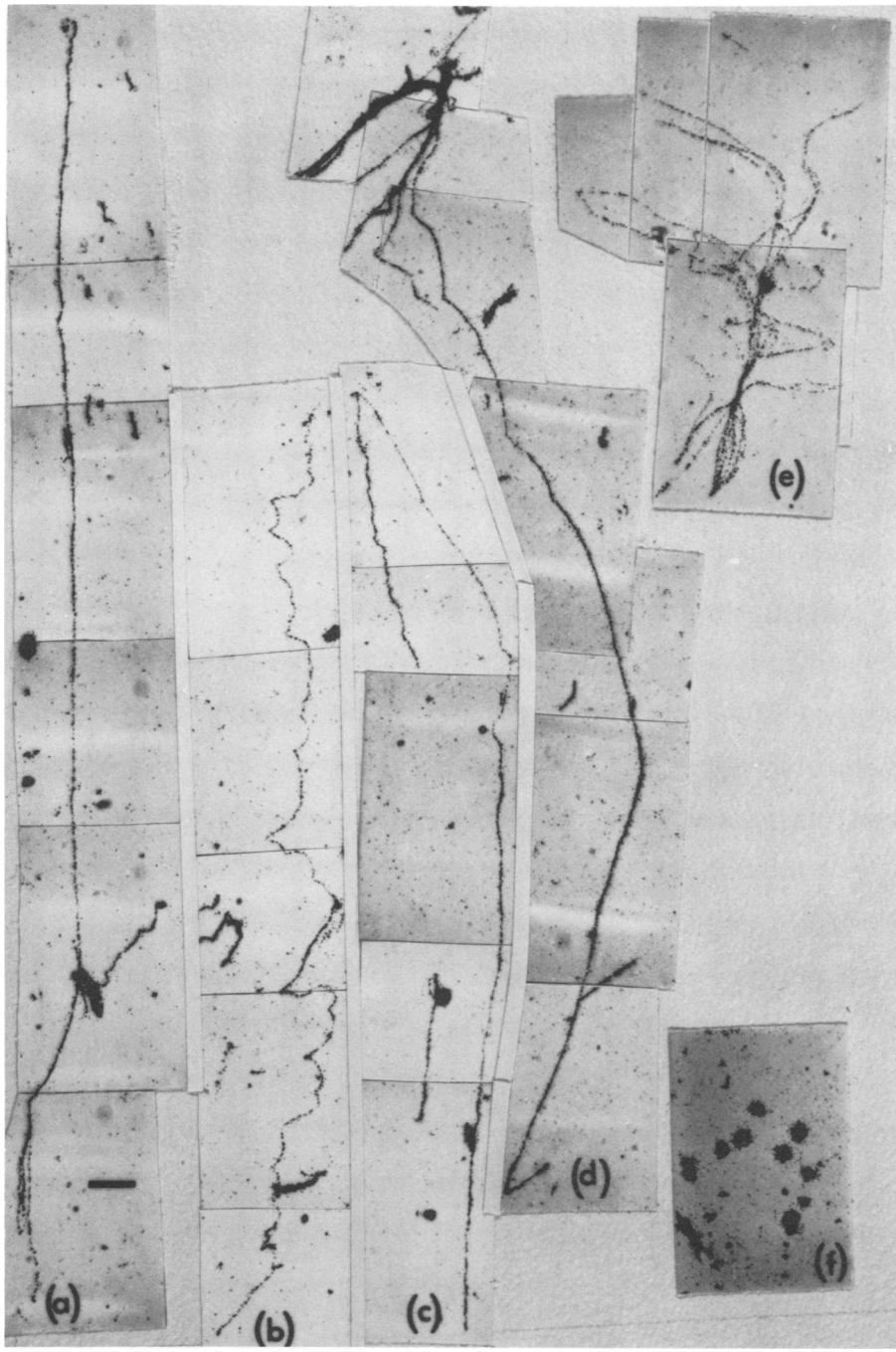

FIG. 3. *(a to d) Concatenated DNA extracted from phage T7-infected cells. The length of the DNA and the time of extraction are indicated for each configuration; (a) 527 μm, 17 min, (b) 626 μm, 17 min, (c) 709 μm, 17 min, (d) 712 μm, 13 min. (e) Partly unfolded DNA complex extracted from phage T4 infected cells. (f) Compact DNA structures from phage T7-infected cells extracted at 17 min. The bar measures 25 μm.*

(20), their lack could be expected since we used sodium lauryl sulfate in our extraction procedure, and this tends to prevent the large replicating circles from being displayed (4, 7).

Autoradiographs of replicating phage T4 DNA, which had been obtained in previous experiments (3) by using the same lysis procedure as that used here, were reexamined. We

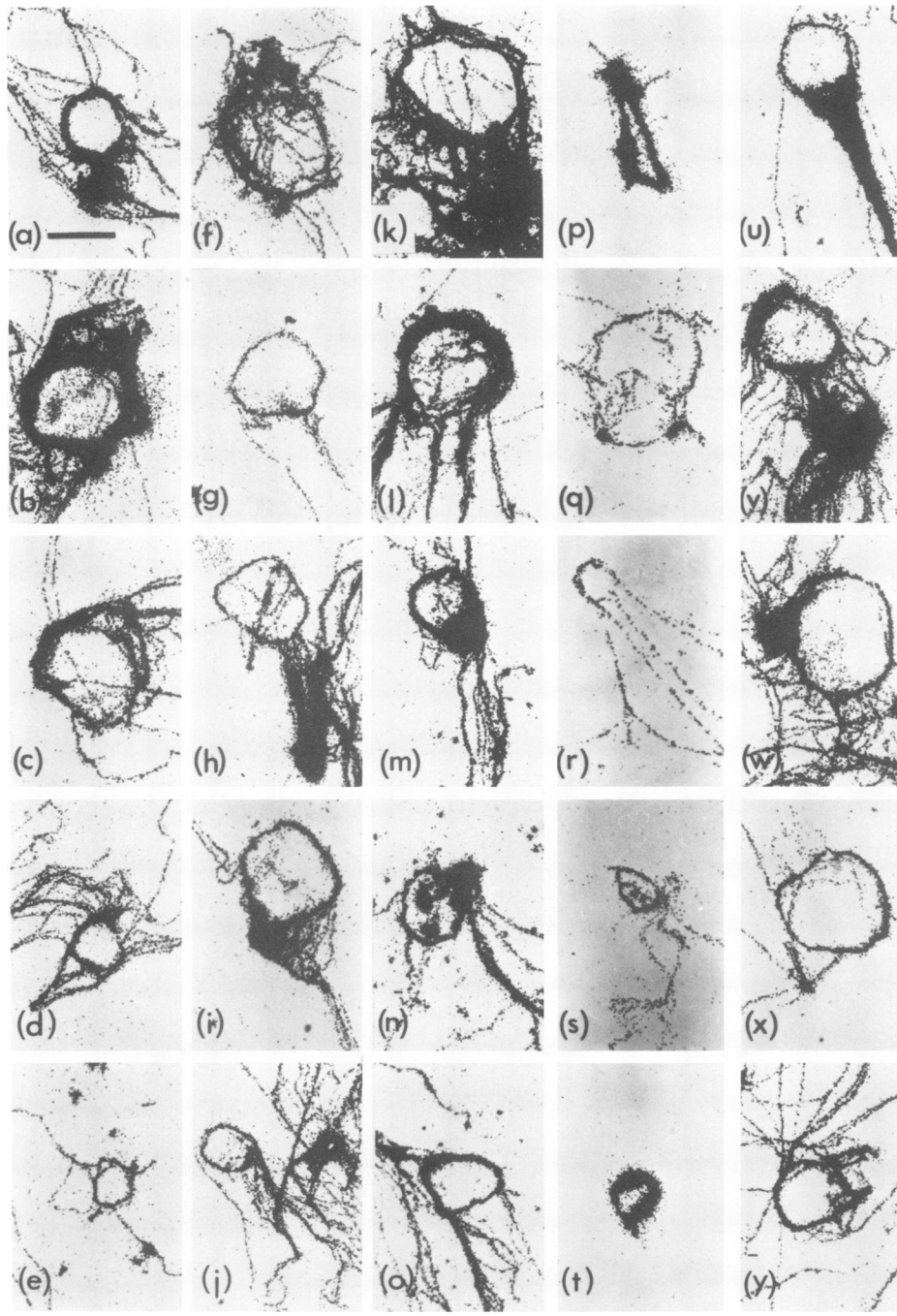

FIG. 4. *Large ring configurations. DNA extracted from phage T7-infected cells at 13 min (a to e); at 17 min (f to j), and at 20 min (k to o). DNA extracted from phage T4-infected cells (p to t), and from uninfected E. coli (u to y). The bar measures 25 μm.*

had previously described the linear and compact configurations of phage T4 DNA and referred to frequent additional "tangled spread out structures". With the prominent ring configurations of phage T7 and *E. coli* DNA in mind, we could identify, among the tangled structures in phage T4 DNA, ring forms similar to those of phage T7 and *E. coli* DNA though at substantially lower frequency. Some phage T4 rings are shown in Fig. 3p to t and Fig. 4p to t.

Most of the phage T4 multistranded rings were smaller and less smoothly organized than the ones found with phage T7 and *E. coli* DNA, and were like the examples shown in Fig. 5r, s, and t. The small ring shown in Fig. 4t is also presented at lower magnification in Fig. 3e as part of a larger partially unfolded structure to illustrate the overall relationship of a ring to the DNA which has apparently become unraveled from it.

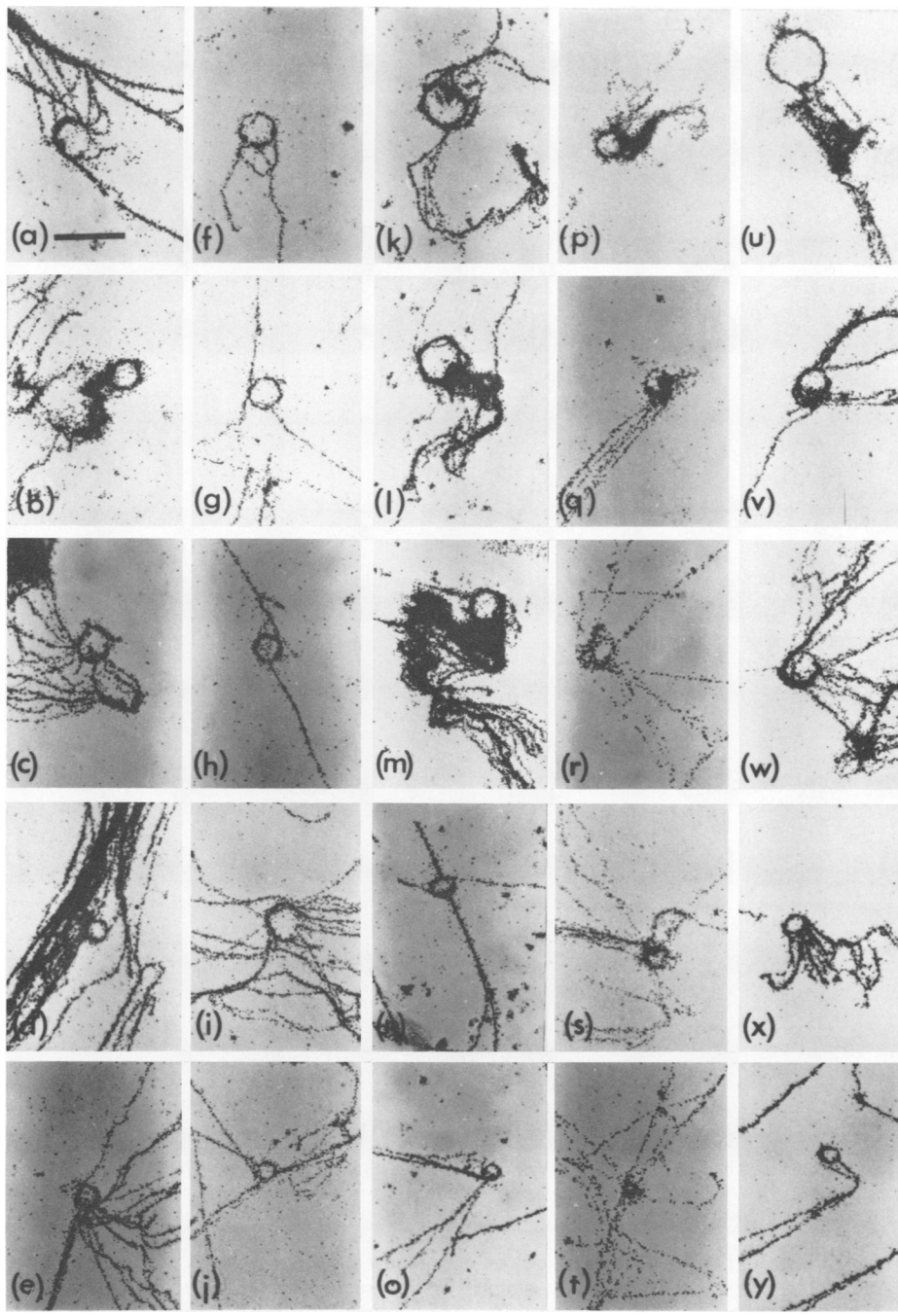

FIG. 5. *Small ring configurations. DNA extracted from phage T7-infected cells at 13 min (a to e); at 17 min (f to j); and at 20 min (k to o). DNA extracted from phage T4-infected cells (p to t), and from ininfected. E. coli (u to y). The bar measures 25 μm.*

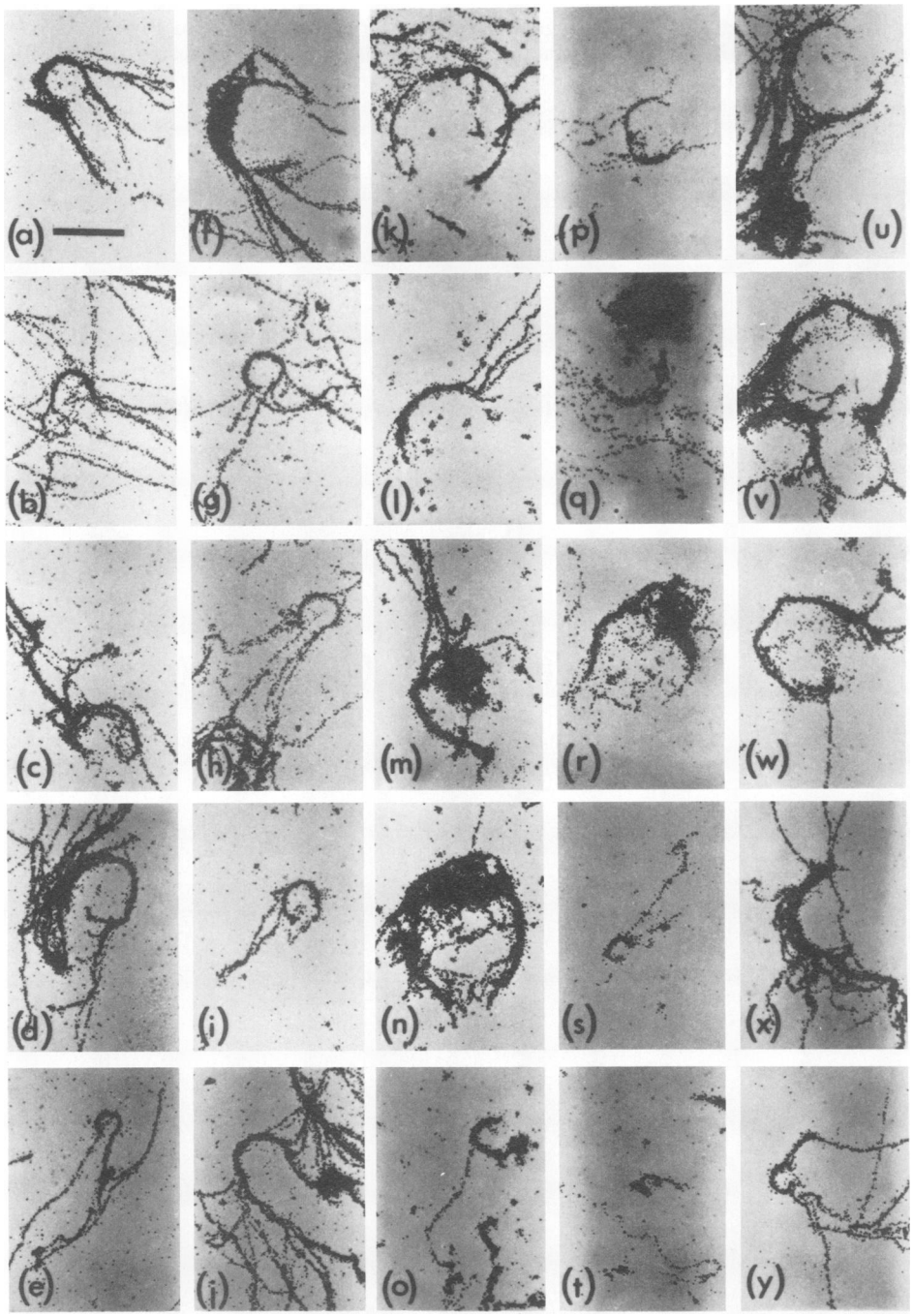

FIG. 6. *Interrupted ring configurations. DNA extracted from phage T7-infected cells at 13 min (a to e); at 17 min (f to j); and at 20 min (k to o). DNA extracted from phage T4-infected cells (p to t), and from uninfected E. coli (u to y). The bar measures 25 μm.*

Examples of *E. coli* DNA complexes in the form of large coiled rings or discontinuous rings which were extracted by the one-step sodium lauryl sulfate procedure, rather than the three-step procedure are shown in Fig. 7. DNA extracted by this simple procedure was not as well spread as that extracted by the three-step procedure, and multistranded rings were not as frequently seen. The sodium lauryl sulfate extracted rings were also not very regular.

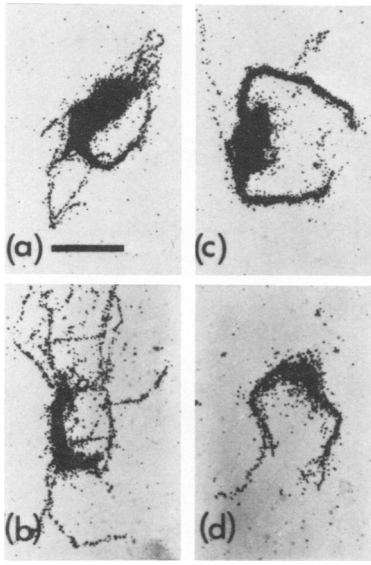

FIG. 7. *DNA configurations obtained from uninfected E. coli lysed with sodium lauryl sulfate. The bar measures 25 μm.*

When T4 DNA is released from mature phage by osmotic shock or 5 M $NaClO_4$ (2) at DNA concentrations similar to the ones used here for *E. coli*, phage T7 and phage T4 replicating DNA, and then subjected to our standard dialysis and autoradiography, only linear molecules were observed. Thus, our conditions of dialysis and autoradiography do not seem to promote spontaneous ring formation in free DNA.

DISCUSSION

We consider that the coiled DNA rings which occur in extracts of wild-type phage T7 and wild-type T4-infected cells, as well as in extracts of uninfected *E. coli* may be equivalent to the multiple loop structure postulated by Worcel and Burgi (27). According to their model, the folded *E. coli* chromosome has 12 to 80 loops, and these loops are restrained in one region by an RNA-containing core. The loops were shown to have superhelical turns causing the DNA to have a compact form and allowing it to fit within the *E. coli* cell. A single-strand nick with DNase was shown to allow free rotation of the DNA chains within a loop, eliminating the superhelical turns of that particular loop without affecting the superhelical form of the rest of the chromosome.

The coiled rings shown in Fig. 4 and 5 could be the structure postulated by Worcel and Burgi under conditions in which the superhelical turns have been relaxed due to nicking during extraction or dialysis. (Prolonged standing, as in our conditions of dialysis, was shown to cause nicks [27]). Our experiments suggest that in any particular ring the DNA loops are of equal size and that when relaxed by nicking, and under our conditions of extraction, the loops adhere to each other to form a parallel coiled ring arrangement.

That the rings are apparently composed of loops of approximately equal size may reflect the way in which they were formed. Perhaps, after the first superhelically coiled loop is made within the cell, successively synthesized loops are laid down adjacent to their predecessors along the same route. Thus, in any coiled ring complex the loops would all be of the same size, but different ring complexes in individual cells would have different sizes depending on the route of the original loop. This could explain the variation in ring size which we have observed (Fig. 4 and 5). Alternatively, the variation in ring size may be explained if cells contain an organized chromosome structure which can dissociate on extraction into rings of different sizes.

Worcel and Burgi (27) estimated that in *E. coli* chromosomes of 1,000 μm there are from 12 to 80 loops per chromosome, implying an average loop diameter from 4 to 27 μm. By comparison the *E. coli* rings shown in Fig 4u to y and Fig. 5u to y were between 5.5 and 40 μm.

Figure 6 shows examples of semicircular multistranded structures which we interpret as remnants of disrupted rings. The integrity of these semicircular forms may reflect adhesion forces in the ring. Perhaps when the DNA chains are combined to form the ring the structure acquires a stability that is partially retained even after the ring is disrupted. On the other hand, these semicircular forms could be the result of partial adhesion of a ring to the Millipore membrane, with subsequent breaking away of a nonadhering portion. Adhesion of DNA to membranes was indicated in the work of Bleecken et al. (4).

When applied to uninfected *E. coli*, the simple sodium lauryl sulfate lysis procedure, compared to the three-step lysis procedure, yielded a much lower frequency of ring structures, and the ones found were less well displayed (Fig. 7). Thus, the display of the regular ring structure probably depends on some element of our three-step lysis procedure. No other investigators, to our knowledge, have used this procedure with *E. coli*, phage T7 or replicating phage T4 DNA, and this is probably why these ring structures have not been reported before.

That the phage T4 rings are not as frequent or well displayed as the phage T7 or *E. coli* rings suggests that the three-step lysis procedure is inefficient in releasing phage T4 DNA from its

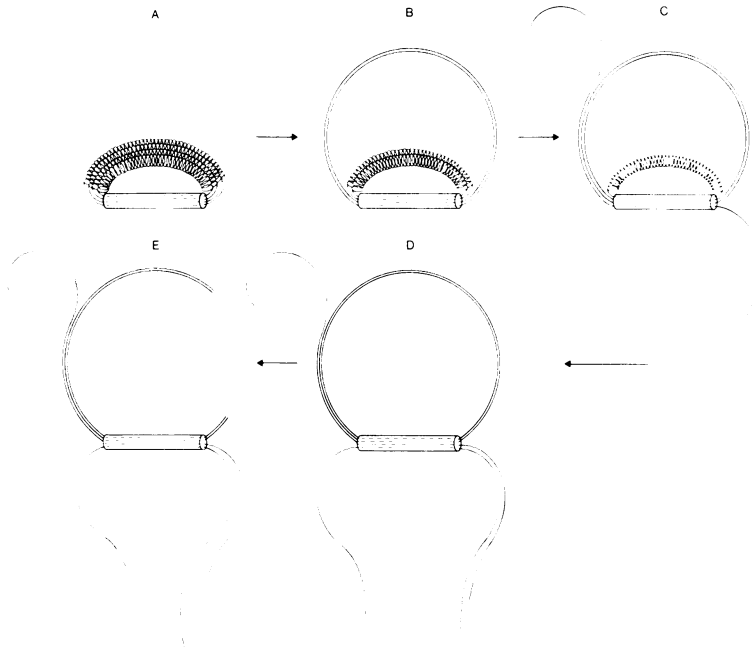

FIG. 8. *A simplified version of the condensed DNA as adapted from Worcel and Burgi (27) is shown in (A). Four superhelically coiled DNA loops are indicated in association with a non-DNA core. When a loop acquires one or more nicks it loses its superhelical turns and takes on a relaxed loop form. The relaxation of superhelical turns in the nicked loop is not transmitted to other loops due to the restraint imposed by the core. As the number of nicks increases multilooped configurations appear (B). These loops are of the same size and adhere to one another in the form of a ring. When one of the loops acquires a complete break in the DNA chain, the DNA may be released from the ring structure (C and D). However, even when all the DNA chains are broken in one portion of the ring, a remnant of the ring may still retain a semicircular form (E).*

compact form. Thus, packaging forces appear to be present in phage T4 DNA complexes which are more difficult to overcome than those in *E. coli* and phage T7 DNA complexes.

Figure 8 is an adaptation of the model of Worcel and Burgi (27) to illustrate the sequence of events that may occur as an increasing number of nicks are introduced into the condensed DNA of *E. coli*, phage T7 or phage T4. The illustration is intended to show diagrammatically a possible way in which ring structures with condensed regions and associated DNA strands as observed in the autoradiographs may have been formed.

The organized form of intracellular DNA indicated by our results may reflect a need for an orderly arrangement of DNA during replication, segregation and gene expression. That the DNA of *E. coli* and two different *E. coli* phages are organized in a way that yields coiled rings upon release suggests that this kind of arrangement may represent a fundamentally important type of DNA packaging.

We have interpreted the multistranded rings as reflecting the natural organization of the DNA within the *E. coli* cell. The alternative possibility, that these structures are formed subsequent to lysis, seemed unlikely because of the frequency, regularity, and organized appearance of the rings. Furthermore, when DNA was extracted from a lysate of mature phage T4 and subjected to dialysis and autoradiography, the unravelled DNA was entirely in the form of linear pieces. No tendency to form rings or other ordered structures was noted. This suggests that our dialysis and autoradiographic procedures do not in themselves promote ring formation subsequent to lysis.

Olins and Olins (17) have attempted to determine the effect of histones from calf thymus on DNA structure. They were able to demonstrate that phage T7 DNA extracted from mature phage shows a salt-dependent interaction with specific histone fractions to form aggregates which appear in the electron microscope as small donuts, about 0.1 to 0.3 μm in diameter. Such structures would be below the level of resolution of the autoradiographic methods used here. Nevertheless, these results suggest the possibility of a role for histone-like proteins in determining the structures of the much larger rings observed in the present study.

ACKNOWLEDGMENTS

We would like to thank C. Minner for his excellent photography.

This research was supported by National Science Foundation, grant GB8760 and a General Research Support Grant from the National Institutes of Health.

LITERATURE CITED

1. Adams, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. Bernstein, C. 1970. A comparison of the number of nucleotides per unit length in *Escherichia coli* and phage T4 chromosomes. Biophys. J. **10**:1154-1172.
3. Bernstein, H., and C. Bernstein. 1973. Circular and branched circular concatenates as possible intermediates in bacteriophage T4 DNA replication. J. Mol. Biol. **77**:355-361.
4. Bleecken, S., G. Strohbach, and E. Sarfert. 1966. Autoradiography of bacterial chromosomes. Z. Allg. Mikrobiol. **6**:121-123.
5. Broker, T. R., and I. R. Lehman. 1971. Branched DNA molecules: intermediates in T4 recombination. J. Mol. Biol. **60**:131-149.
6. Cairns, J. 1962. The application of autoradiography to the study of DNA viruses. Cold Spring Harbor Symp. Quant. Biol. **27**:311-318.
7. Cairns, J. 1963. The chromosome of *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. **28**:43-45.
8. Carlson, K. 1968. Intracellular fate of deoxyribonucleic acid from T7 bacteriophages. J. Virol. **2**:1230-1233.
9. Center, M. S. 1972. Replicative intermediates of bacteriophage T7 deoxyribonucleic acid. J. Virol. **10**:115-123.
10. Delius, H., and A. Worcel. 1973. Electron microscopic visualization of the folded chromosome of *Escherichia coli*. J. Mol. Biol. **82**:107-110.
11. Frankel, F. R. 1966. Studies on the nature of the replicating DNA in *Escherichia coli* infected with certain amber mutants of phage T4. J. Mol. Biol. **18**:127-143.
12. Hausmann, R., and B. Gomez. 1967. Amber mutants of bacteriophages T3 and T7 defective in phage-directed deoxyribonucleic acid synthesis. J. Virol. **1**:779-792.
13. Huberman, J. A. 1968. Visualization of replicating mammalian and T4 bacteriophage DNA. Cold Spring Harbor Symp. Quant. Biol. **33**:509-524.
14. Kelley, T. J., and C. A. Thomas. 1969. An intermediate in the replication of bacteriophage T7 DNA molecules. J. Mol. Biol. **44**:459-475.
15. Labaw, L. W. 1951. The origin of phosphorus in *Escherichia coli* bacteriophages. J. Bacteriol. **62**:169-173.
16. Labaw, L. W. 1953. The origin of phosphorus in the T1, T5, T6, and T7 bacteriophages of *Escherichia coli*. J. Bacteriol. **66**:429-436.
17. Olins, D. E., and A. L. Olins. 1971. Model nucleohistones: the interaction of F1 F2a1 histones with native T7 DNA. J. Mol. Biol. **57**:437-455.
18. Putnam, F. W., D. Miller, L. Palm, and E. A. Evans. 1952. Biochemical studies of virus reproduction. X. Precursors of bacteriophage T7. J. Biol. Chem. **199**:177-191.
19. Ritchie, D. A., C. A. Thomas, L. A. MacHattie, and P. C. Wensick. 1967. Terminal repetition in non permuted T3 and T7 bacteriophage DNA molecules. J. Mol. Biol. **23**:365-376.
20. Rodriguez, R. L., M. S. Dalbey, and C. I. Davern. 1973. Autoradiographic evidence for bidirectional DNA replication in *Escherichia coli*. J. Mol. Biol. **74**:599-604.
21. Sadowski, P. D, and C. Kerr. 1970. Degradation of *Escherichia coli* B DNA after infection with DNA defective amber mutants of bacteriophage T7. J. Virol. **6**:149-155.
22. Schlegel, R. A., and C. A. Thomas, Jr. 1972. Some special structural features of intracellular bacteriophage T7 concatemers. J. Mol. Biol. **68**:319-345.
23. Steinberg, C. M., and R. S. Edgar. 1962. A critical test of a current theory of recombination in bacteriophage. Genetics **47**:187-208.
24. Stonington, O. G., and D. E. Pettijohn. 1971. The folded genome of *Escherichia coli* isolated in a protein-DNA-RNA complex. Proc. Nat. Acad. Sci. U.S.A. **68**:6-9.
25. Stratling, W., E. Krause, and R. Knippers. 1973. Fast sedimenting deoxyribonucleic acid in bacteriophage T7-infected cells. Virology **51**:109-119.
26. Warner, H. R., and J. E. Barnes. 1966. Deoxyribonucleic acid synthesis in *Escherichia coli* infected with some deoxyribonucleic acid polymeraseless mutants of bacteriophage T4. Virology **28**:100-107.
27. Worcel, A., and E. Burgi. 1972. On the structure of the folded chromosome of *Escherichia coli*. J. Mol. Biol. **71**:127-147.
28. Worcel, A., and E. Burgi. 1973. Properties of a membrane-attached form of the folded chromosome of *Escherichia coli*. J. Mol. Biol. **82**:91-106.