*Proc. Natl. Acad. Sci. USA*
Vol. 76, No. 8, pp. 3779–3783, August 1979
Biochemistry

# Nicking-closing enzyme assembles nucleosome-like structures *in vitro*

(chromatin/supercoiling of DNA/micrococcal nuclease/histone–DNA interaction/minichromosomes)

JACQUES-EDOUARD GERMOND*†, JOSETTE ROUVIÈRE-YANIV‡, MOSHE YANIV‡, AND DOUGLAS BRUTLAG*§

*Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305; and ‡Département de Biologie Moléculaire, Institut Pasteur, 25 rue du Dr. Roux, Paris 75015, France

*Communicated by James F. Bonner, May 18, 1979*

**ABSTRACT** **The four core histones (H2A, H2B, H3, and H4) and DNA were assembled into nucleosome-like particles at physiological ionic strengths either by an extract of chromatin rich in nicking-closing activity or by the purified nicking-closing enzyme itself. When histone–DNA complexes were assembled *in vitro* from relaxed circular DNA, nearly physiological numbers of superhelical turns were induced in the DNA molecule. Electron microscopy of the complexes assembled by the chromatin extract revealed a beaded structure and a reduction of the contour length compared to free DNA. Micrococcal nuclease digestion of the histone–DNA complexes yielded 145-base-pair DNA fragments typical of nucleosome core particles and shorter subnucleosomal DNA fragments of discrete length.**

The reconstitution of chromatin-like structures from histones and DNA *in vitro* has required, until recently, the use of high ionic strengths. These nonphysiological conditions were needed to neutralize the strong electrostatic interaction between histones and DNA. Chromatin reconstituted by either dialysis or dilution from high ionic strengths to physiological levels contains nucleosomes as determined by a beaded structure shown in the electron microscope (1), a repeating pattern along the DNA revealed by micrococcal nuclease digestion (2), discrete subnucleosomal DNA fragments produced by digestion with several nucleases (3), and the induction of superhelical turns in circular DNA molecules revealed after relaxation of the chromatin by a nicking-closing activity (4).

The assembly of chromatin *in vitro* under physiological conditions of ionic strength and temperature was demonstrated by Laskey *et al.* (5). They showed that circular DNA of simian virus 40 (SV40) was assembled into a chromatin-like structure when incubated in an extract of *Xenopus* eggs that was known to contain a pool of free histones. We felt that chromatin itself might be a source of the factors or enzymes essential for chromatin assembly. Many enzymes that are bound to chromatin can be released by extraction at various ionic strengths. For example, the nicking-closing enzyme, which can relax the supercoiling induced by nucleosome formation, is readily released from chromatin by 150 mM sodium phosphate (4). In this paper we show that such a chromatin extract, rich in nicking-closing activity, and the highly purified nicking-closing enzyme itself are able to mediate the assembly of histones and DNA into nucleosome-like structures under physiological conditions.

## MATERIALS AND METHODS

**Preparation of DNAs, Chromatin Extract, and Histones.** Supercoiled dimer of mini ColE1 DNA (FI) was extracted and purified from MC100 *capR*$^+$ *Escherichia coli* (6). After lysis of the bacteria by lysozyme, supercoiled DNA was purified and circular relaxed DNA was prepared as described (4, 7). The chromatin extract was prepared from mouse L cells (8) or *Drosophila melanogaster* $K_c$ cells (9) as described (4). Highly purified nicking-closing enzyme from rat liver was kindly provided by J. Champoux (10). The four core histones H2A, H2B, H3, and H4 were purified from *D. melanogaster* $K_c$ cells from the low-salt chromatin used to prepare the chromatin extract as described (11). Triton DF-16/polyacrylamide gel electrophoresis (12) indicated the histones were intact, not highly modified, and free of histone H1.

**Gel Electrophoresis.** Agarose was purchased from MCB and acrylamide from Bio-Rad. Two gel electrophoresis systems were used. One percent agarose slab gels in 36 mM Tris/30 mM $NaH_2PO_4$/1 mM EDTA, pH 7.7, were run horizontally at 4–5 V/cm for 5 hr to resolve the superhelical structures of covalently closed DNA. Mixed 5.5% acrylamide, 0.5% agarose gels were prepared as described (13) and used to resolve DNA fragments produced by micrococcal nuclease digestion. As little as 0.16 μg of DNA is detectable on an agarose gel, whereas 0.3–0.4 μg of digested DNA must be loaded on a polyacrylamide/agarose gel. Gels were stained with ethidium bromide and photographed with short wavelength UV light.

## RESULTS

**Introduction of Superhelical Turns into Circular Relaxed DNA.** The association of the four core histones with circular covalently closed DNA was studied under physiological conditions of salt and temperature in the presence of a protein fraction extracted from chromatin. The extent of the histone–DNA association was measured by the number of superhelical turns introduced into an initially relaxed circular DNA. When DNA wraps around histones to form a nucleosome, a topological constraint equivalent to the unwinding of one turn of the double helix is induced into the DNA duplex (4). These constraints produce a twisted histone–DNA complex, which can then be relaxed by the action of a nicking-closing enzyme. Such a relaxed complex contains a DNA molecule that, after removal of the histones, possesses superhelical turns. The number of nucleosomes is directly related to the number of superhelical turns, which can be estimated by gel electrophoresis of the deproteinized DNA. The results of nucleosome assembly using histones isolated by either salt or acid extraction were similar. The chromatin extract contained an active nicking-closing enzyme and negligible amounts of histones and was free of $Mg^{2+}$-dependent double-strand-specific endonucleases (4).

When the four core histones and circular relaxed DNA (FIr)

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: SV40, simian virus 40; FI DNA, supercoiled circular DNA (form I); FIr DNA, circular relaxed DNA.

† Present address: Institut de Zoologie, Université de Neuchâtel, rue Emile Argand 11, CH-2000 Neuchâtel 7, Switzerland.
§ To whom reprint requests should be addressed.

were incubated in the presence of this chromatin extract, the DNA acquired superhelical turns as demonstrated after deproteinization and gel electrophoresis (Fig. 1). The optimal ionic strength for the induction of superhelical turns was 150 mM NaCl. Divalent metal ions were not essential for this reaction, and $Mg^{2+}$ was not inhibitory (data not shown). Under optimal ionic conditions, the reaction was complete in 1 hr. The maximum number of superhelical turns generated was the same as the physiological number found in SV40 minichromosomes as determined by electrophoresis in the presence of chloroquine (14) (data not shown).

The optimal ratio of histones to DNA by weight was approximately 0.9, which is close to the proportion found in native chromatin. Smaller ratios of histones to DNA resulted in DNA molecules containing intermediate numbers of superhelical turns. When histones were present in excess of DNA, the induction of superhelical turns was markedly reduced, indicating that either chromatin assembly or the nicking-closing enzyme itself was inhibited. Control experiments showed that the induction of superhelical turns depended on both the histones and the chromatin extract. Other basic proteins or polyamines could not replace histones. For example, neither lysozyme nor cytochrome *c* in a 1:1 weight ratio induced superhelical turns in the presence of the chromatin extract. Similar negative results were obtained with putrescine or spermidine in the range 1–5 mM (data not shown). Besides histones, the only protein tested that induced formation of superhelical turns was the histone-like protein HU, isolated from *E. coli* (15, 16). This protein required incubation at lower ionic strength (50 mM NaCl), because its interaction with DNA is weak.

**Formation of a Beaded Nucleoprotein Structure.** Electron microscopy of histone–DNA complexes assembled in the presence of the chromatin extract revealed a beaded structure (Fig. 2 *b* and *c*) comparable to that of the SV40 minichromosome extracted from infected cells (Fig. 2*e*; ref. 19). The average number of beads per molecule was 15.6 ± 7.8 (±SD) for the entire population and 21 ± 1.2 when only the 50% of the complexes having the maximum number of beads was considered. This distribution of beads is in agreement with the distribution of the number of superhelical turns observed by gel electrophoresis (Fig. 1). In this case, too, a fraction of the DNA became fully supercoiled while the rest had intermediate numbers of superhelical turns. The average contour length of the complexes from the population having 21 ± 1.2 beads was 2.4 times shorter than naked DNA (Fig. 2*a*). This contraction ratio is similar to that found for SV40 minichromosomes extracted from infected cells and spread in the same conditions (ratio = 2.6; ref. 17). The diameter of the beads was 120 ± 14 Å, similar to that of nucleosomes from SV40 minichromosomes. Each bead contained an average of 184 ± 14 base pairs of DNA as calculated from the regression line of the total length of the nucleoprotein complexes as a function of the bead number.

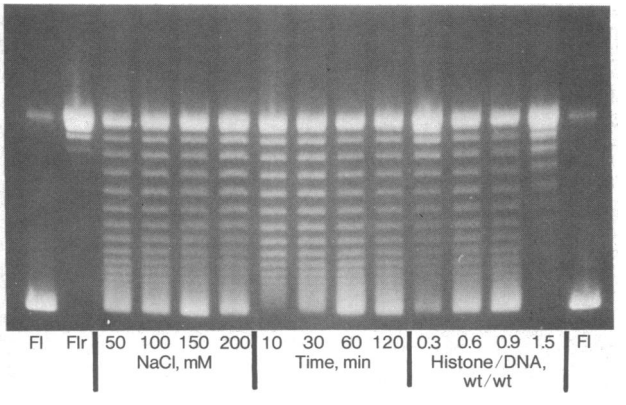


FIG. 1. Assembly of the four core histones and circular relaxed DNA in the presence of a chromatin extract. Incubations were normally performed at 37°C for 90 min in conical Eppendorf tubes, with relaxed DNA at 8 μg/ml and histones at 8 μg/ml in 10 mM Tris·HCl, pH 8.0/1 mM EDTA/150 mM NaCl, in the presence of 12 μl of chromatin extract (≈0.1 mg/ml final concentration of protein) or 400 ng of pure nicking-closing enzyme per μg of relaxed DNA, except that the concentration of NaCl, the time of incubation, and the amount of histones were varied as indicated. The depth of the incubation solution could not exceed the diameter of its surface and the histones had to be added last. After incubation, the samples were made 10% sucrose/0.3% sodium dodecyl sulfate/0.05% bromophenol blue and heated at 60°C for 10 min for direct analysis by agarose gel electrophoresis. The markers are supercoiled DNA (FI) and circular relaxed covalently closed DNA (FIr).

The formation of nucleosome-like structures is dependent on the presence of both histones and the chromatin extract. When histone–DNA complexes were formed from supercoiled DNA in the absence of the chromatin extract, most of the complexes appeared twisted and only a few beaded structures were observed (one to five beads, Fig. 2*d*), but no highly beaded complexes such as those shown in Fig. 2 *b* and *c* were detected.

**Micrococcal Nuclease Digestion of Histone–DNA Complexes Yields Discrete Sized DNA Fragments.** When the histone–DNA complexes assembled in the presence of the chromatin extract were digested to various extents with micrococcal nuclease, a pattern of discrete DNA fragments was produced (Fig. 3 *c* and *d*). These fragments were similar in length to the subnucleosomal DNA fragments from native chromatin (Fig. 3*b*; refs. 13 and 20). The largest discrete DNA fragment was 145 base pairs long, which is similar in length to the DNA in a nucleosome core particle (21, 22). Quantitation of the microdensitometer scans of Fig. 3 indicated that at least 50% of the input DNA had been packaged into a nucleosome-like structure. Micrococcal nuclease digestion of the histone–DNA complex assembled *in vitro* did not generate the 200-base-pair periodicity chracteristic of the digestion of chromatin in nuclei (21, 23, 24). Instead, the DNA fragments longer than 145 base pairs were more heterogeneous and smaller in size than the fragments obtained by digestion of chromatin in nuclei (Fig. 3*a*). This result suggests that the nucleosome core particles assembled *in vitro* were less regularly and more closely spaced along the chromatin fiber than in native chromatin. The absence of histone H1 or specific nonhistone proteins in the assembly reaction may account for this difference. When chromatin is assembled in more complex extracts, such as from *Xenopus* eggs or *Drosophila* embryos, the 200-base-pair repeating pattern can be obtained by micrococcal nuclease digestion (ref. 5; unpublished results).

The formation of DNA resistant to micrococcal nuclease is dependent upon the presence of the chromatin extract during assembly. When the four core histones and circular relaxed DNA were incubated in the absence of extract, no discrete micrococcal nuclease-resistant DNA fragments were generated (Fig. 3 *j* and *k*). Instead, a broad distribution of DNA fragments extended throughout the gel. The conditions of nuclease digestion used were similar to those used in Fig. 3*c* except that the amount of micrococcal nuclease ½ to ⅛. Because the formation of nucleosomes induces superhelical turns in a DNA molecule, we felt that the use of a supercoiled DNA might favor a spontaneous assembly reaction. When the four core histones were incubated with supercoiled DNA in the absence of the chromatin extract, again no discrete DNA fragments were formed upon micrococcal nuclease digestion (Fig. 3 *g*, *h*, and *i*).

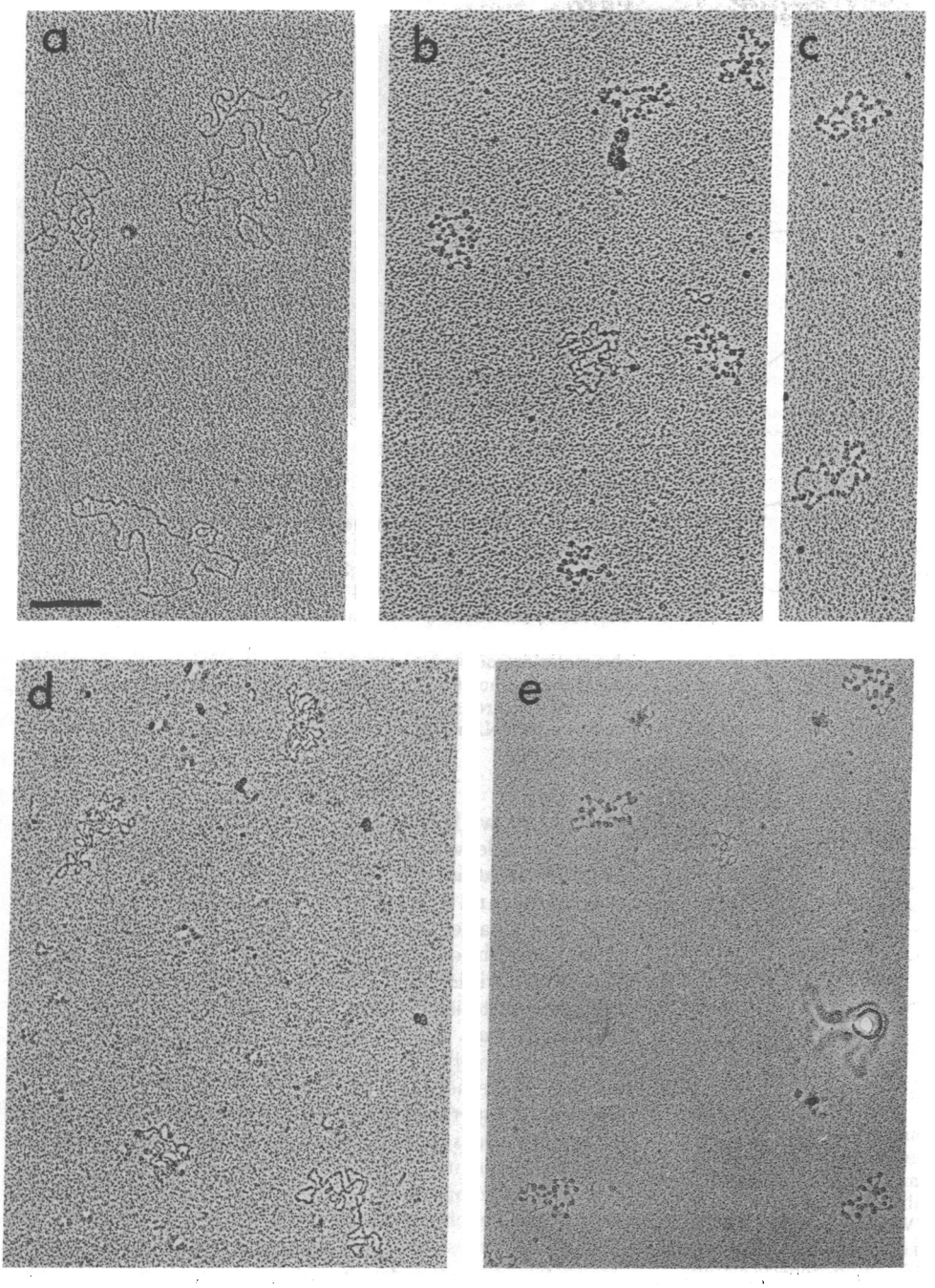


FIG. 2. Electron microscopy of (*a*) SV40 FIr DNA alone; (*b* and *c*) SV40 FIr DNA and calf thymus histones assembled in the presence of a chromatin extract; (*d*) complexes of FI DNA and histones formed in the absence of chromatin extracts; (*e*) SV40 minichromosomes extracted from infected cells (17). The bar corresponds to 200 nm. Aliquots of an incubation mixture prepared as described for Fig. 1 were diluted 1:50 with 10 mM Tris·HCl, pH 7.5/1 mM EDTA and applied to carbon-coated grids activated by glow discharge in amylamine vapor (18). The samples were stained with 2% aqueous uranyl acetate and the grids were rotary shadowed with platinum/palladium (80:20) at an angle of 8°. Photographs were taken with Siemens Elmiskop 101 electron microscope at ×16,000. Length and size measurements were made on ×10 photographic enlargements. The relative increase in diameter by metal deposition was deduced from the size measurements of naked DNA.

**Purified Nicking-Closing Enzyme Mediates Chromatin Assembly.** We thought that the chromatin extract might contain several factors involved in chromatin assembly. In attempts to fractionate this activity, we found that a purified nicking-closing enzyme (10) by itself induced superhelical turns in a relaxed DNA in the presence of histones. Fig. 4 shows that this homogeneous enzyme induced superhelical turns in a relaxed DNA under the conditions found optimal for the chromatin extract. An assembly reaction with the chromatin extract is also shown for comparison. The relative amounts of nicking-closing activity necessary for nucleosome assembly were much greater than the amount necessary to relax the same amount of supercoiled DNA. Indeed, the relative ratios of the assembly and relaxing activities were the same for both the chromatin extract and the homogeneous enzyme. Fig. 4 shows that 20 times more chromatin extract was needed for assembly of histones and relaxed DNA than for relaxation of the same amount of supercoiled DNA. Nucleosome assembly with the homogeneous nicking-closing enzyme also required 20-fold more enzyme than the relaxation reactions. On a molar basis about one molecule of nicking-closing enzyme was present per nucleosome formed. In the absence of histones, even this large amount of nicking-closing enzyme did not affect the relaxed state of the DNA, indicating that the supercoiling reaction is dependent upon histone–DNA association.

Micrococcal nuclease digestion of the histone–DNA complexes assembled in the presence of the purified nicking-closing enzyme resulted in DNA fragments of discrete size (Fig. 3 *e* and *f*). These fragments were similar in length to those obtained by digestion of either native chromatin (Fig. 3*b*) or DNA–histone complexes assembled in the presence of the chromatin extract (Fig. 3 *c* and *d*). These results indicate that the homogeneous nicking-closing enzyme itself was sufficient to assemble histones and DNA into a nucleosome-like structure. This conclusion is

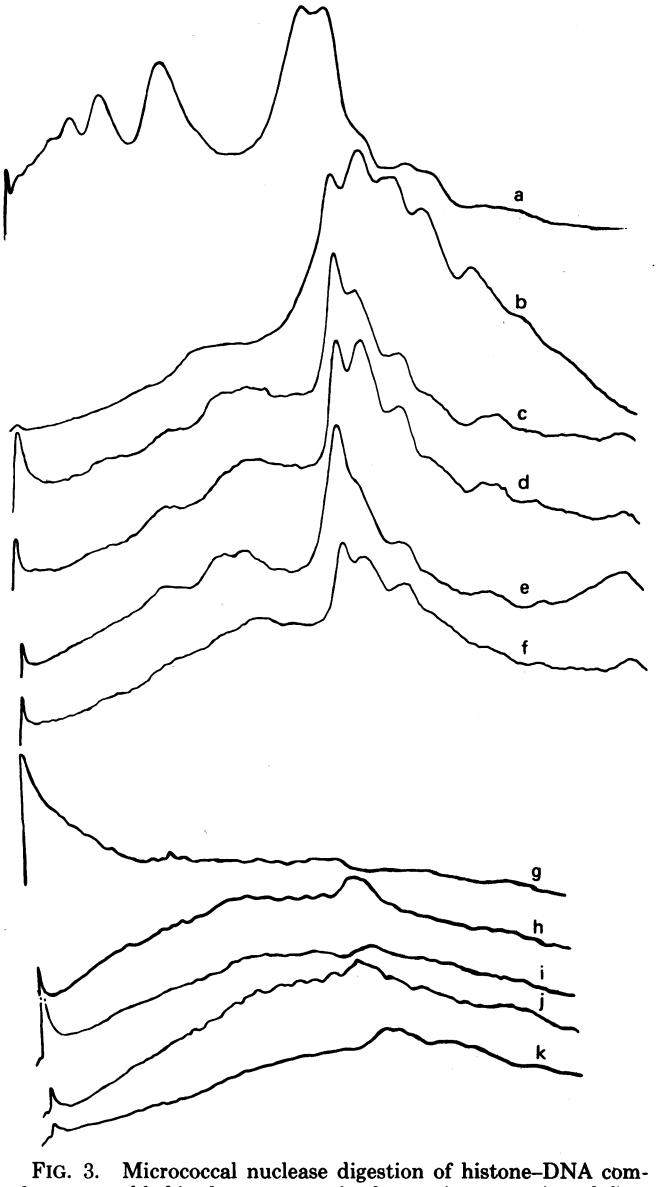


FIG. 3. Micrococcal nuclease digestion of histone–DNA complexes assembled in the presence of a chromatin extract (*c* and *d*) or a homogeneous nicking-closing enzyme (*e* and *f*) as described for Fig. 1. After assembly the complexes were incubated at 23°C in the presence of 50 units of micrococcal nuclease (Worthington) per ml and 1 mM $CaCl_2$ for 5 min (*c* and *e*) and 15 min (*d* and *f*). After digestion, the samples were made 10 mM EDTA/0.3% sodium dodecyl sulfate, and extracted with phenol and then with ether before being precipitated with cold ethanol. DNA was then resuspended in 10 mM Tris·HCl, pH 8.0/1 mM EDTA/10% sucrose/0.05% bromophenol blue for analysis by polyacrylamide/agarose gel electrophoresis. DNA fragments from nuclei digested with micrococcal nuclease to two different extents as described (ref. 13; *a* and *b*). Control experiments, in which supercoiled DNA was incubated with histone in the absence of chromatin extract and then digested for 5 min with 5, 12, or 25 units of micrococcal nuclease (*g*, *h*, and *i*, respectively) per ml. FIr DNA was also mixed with histones in the absence of extract and the resulting complexes were digested with 6 or 25 units of micrococcal nuclease (*j*, *k*) per ml.

reinforced by the fact that the same relative amount of nicking-closing activity was necessary for assembly whether the chromatin extract or the pure nicking-closing enzyme was used. However, the copurification of a highly catalytic assembly activity with the nicking-closing enzyme cannot be excluded.

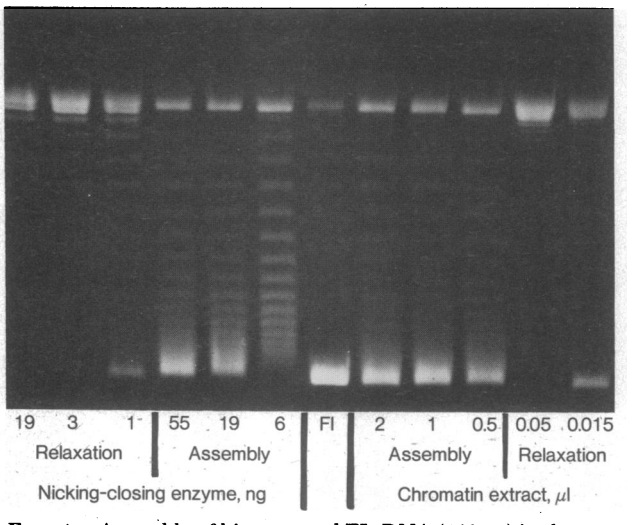


FIG. 4. Assembly of histones and FIr DNA (160 ng) in the presence of indicated amounts of the nicking-closing enzyme or the chromatin extract in the assembly reactions as described for Fig. 1. FI DNA (160 ng) was incubated in the presence of the nicking-closing enzyme or the chromatin extract in the relaxation reactions under ionic conditions identical to those for the assembly reaction. The chromatin extract was ≈1 mg/ml in protein. DNA was then analyzed by gel electrophoresis as described for Fig. 1. The double bands in the two right slots are due to the presence of both negatively and positively supercoiled DNA molecules resolved under the conditions of electrophoresis.

## DISCUSSION

We have shown here that the assembly of histones and DNA to form a nucleosome-like structure can be mediated by either a chromatin extract or a highly purified nicking-closing enzyme isolated from rat liver (10). These agents assemble chromatin at physiological ionic strength (150 mM NaCl), at physiological rates, and with equal amounts of DNA and histone by weight. The characteristics of the complexes formed satisfy most of the important criteria defining chromatin structure at the level of the nucleosome. The circular DNA extracted from the complexes is supercoiled, indicating that the initially relaxed DNA acquired superhelical turns during complex formation. The number of superhelical turns is in good agreement with the number of beads observed by electron microscopy. This relationship of one nucleosome to one superhelical turn is characteristic of chromatin (4). The digestion of the complexes with micrococcal nuclease generates a series of discrete DNA fragments, the largest of which is 145 base pairs in length. DNA fragments of this size are associated with histones in a complex called a nucleosome core particle, which is a major intermediate in the digestion of chromatin from many sources (21, 24). In the electron microscope the complexes appear as a chain of nucleosomes with a diameter of 120 Å, each containing about 180 base pairs of DNA as determined by the length reduction of the DNA molecule. These data for nucleosome-like particles assembled *in vitro* agree with the values established for nucleosomes in native chromatin (21).

The assembly of histones and DNA into a chromatin-like structure can be facilitated by a heat-treated, trypsin-resistant, acidic protein of 29,000 moleular weight isolated from *Xenopus* eggs (25). This protein interacts with the core histones and then the complex attaches to DNA to form a nucleosome. Treatment of our chromatin extract with even limited amounts of trypsin led to the simultaneous inactivation of both assembly and relaxing activities (data not shown).

The nicking-closing enzyme used in this work was purified by J. Champoux and is homogeneous as determined by sodium

dodecyl sulfate gel electrophoresis and by gel filtration analysis (10). This enzyme can relax both positive and negative superhelical turns in a circular DNA molecule by a mechanism involving a single-strand scission of the DNA and a covalent linkage of the polypeptide to the DNA (26, 27). This activity has been shown to be catalytic, because 1 molecule of enzyme is able to relax more than 100 molecules of SV40 DNA (10). Under our conditions for nucleosome assembly one molecule of the nicking-closing enzyme is present per nucleosome formed. We do not know whether the pure enzyme plays a stoichiometric or catalytic role in assembly *in vitro*. Because similar large amounts of nicking-closing activity are required for assembly with the chromatin extract, this enzyme is probably the principal factor present in the extract that mediates assembly.

There are several possible mechanisms whereby nicking-closing enzyme could mediate the assembly of histones and DNA into nucleosomes. The simplest is that histones and DNA associate spontaneously, and the nicking-closing enzyme simply relaxes the nucleoprotein complexes. Results from nuclease digestion and electron microscopy make this mechanism unlikely. Micrococcal nuclease digestion of the complexes formed in the absence of nicking-closing enzyme showed only small amounts of 145-base-pair DNA fragments. This material may represent a small amount of spontaneous assembly. However, even the use of DNA that was already supercoiled did not induce nucleosome formation. In another possible mechanism the nicking-closing enzyme might directly facilitate the proper folding of the DNA around the histone core by acting as a swivel. A swivel might also be needed to allow the DNA to maintain a relaxed conformation throughout chromatin assembly *in vitro*. The large amount of enzyme required suggests that the enzyme might act by coating the DNA and preventing the rapid nonspecific associations that occur when histones and DNA are mixed at physiological ionic strength. The enzyme would then be displaced only when the DNA had properly folded around the histone core. The enzyme might also actively fold the DNA or serve as a nucleation site for histone association. Our current results favor mechanisms in which the nicking-closing enzyme interact first with DNA, because better assembly was observed when these components were preincubated prior to addition of histones.

The formation of nucleosome-like structures *in vitro* can be mediated: by the nicking-closing enzyme, which interacts with nucleic acids as described here; by an acidic protein from *Xenopus* eggs that associates with histones (25); or by nonphysiological ionic strengths (1). All these mechanisms involve a reduction or a competition of the strong electrostatic interaction between histones and DNA. In these reactions, neither histones nor DNA is in a state comparable to that found *in vivo*. Core histones are known to be modified after synthesis, primarily by acetylation, and then deacetylated after association with DNA (28, 29). The use of acetylated histones in these reactions may result in different mechanisms of assembly. The DNA molecules used in these assays were present as long regions of free nucleic acid. *In vivo*, most of the DNA is associated with histones in the form of chromatin or with enzymes involved in DNA metabolism. Perhaps the assembly of chromatin *in vivo* usually involves the displacement of enzymes such as the nicking-closing enzyme, by histones to form nucleosomes. Our data suggest that the nicking-closing enzyme may have two distinct functions in chromosome replication, one relaxing the topological constraints imposed during replication and chromatin assembly and the other facilitating the association of histones and DNA to form nucleosomes.

We are indebted to Jim Champoux for his generous gifts of the highly purified nicking-closing enzyme, to David Clayton for supplying us with mouse L-cells, and to D. Cany for the help in the preparation of the electron microscope photographs. We thank Jim Whitlock for critical reading of this manuscript and Roger Kornberg and Tao Hsieh for helpful discussions. This work was supported by grants from the National Institute of General Medical Sciences and by the French Centre National de la Recherche Scientifique. J.E.G. is an International Fogarty Fellow of the National Institutes of Health sponsored by the Fonds National Suisse pour la Recherche Scientifique.

1. Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Cell* **4**, 281–300.
2. Thomas, J. O. & Butler, P. J. G. (1977) *Cold Spring Harbor Symp. Quant. Biol.* **42**, 119–125.
3. Sollner-Webb, B., Camerini-Otero, R. B. & Felsenfeld, G. (1976) *Cell* **9**, 179–193.
4. Germond, J. E., Hirt, B., Oudet, P., Gross-Bellard, M. & Chambon, P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1843–1847.
5. Laskey, R. A., Mills, A. D. & Morris, N. R. (1977) *Cell* **10**, 237–243.
6. Avni, H., Berg, P. E. & Markovitz, A. (1977) *J. Bacteriol.* **129**, 358–366.
7. Germond, J. E., Vogt, V. M. & Hirt, B. (1974) *Eur. J. Biochem.* **43**, 591–600.
8. Stanner, C. P. & Till, J. E. (1960) *Biochim. Biophys. Acta* **37**, 406–419.
9. Eschalier, G. & Ohanessian, A. (1970) *In Vitro* **6**, 162–172.
10. Champoux, J. J. & McConaughy, B. L. (1976) *Biochemistry* **15**, 4638–4642.
11. Germond, J. E., Bellard, M., Oudet, P. & Chambon, P. (1976) *Nucleic Acids Res.* **3**, 3173–3192.
12. Alfageme, C. R., Zweidler, A., Mahowald, A. & Cohen, L. J. (1974) *J. Biol. Chem.* **249**, 3729–3736.
13. Bellard, M., Oudet, P., Germond, J. E. & Chambon, P. (1976) *Eur. J. Biochem.* **70**, 543–553.
14. Shure, M., Pulleyblank, D. E. & Vinograd, J. (1977) *Nucleic Acids Res.* **4**, 1183–1204.
15. Rouvière-Yaniv, J. & Gros, F. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3428–3432.
16. Rouvière-Yaniv, J., Yaniv, M. & Germond, J. E. (1979) *Cell* **17**, 265–274.
17. Cremisi, C., Pignatti, P. F., Croissant, O. & Yaniv, M. (1976) *J. Virol.* **17**, 204–211.
18. Dubochet, J., Ducommun, M., Zollinger, M. & Kellenberger, E. (1971) *J. Ultrastruct. Res.* **35**, 147–167.
19. Griffith, J. D. (1975) *Science* **187**, 1202–1203.
20. Camerini-Otero, R. D., Sollner-Webb, B. & Felsenfeld, G. (1976) *Cell* **8**, 333–347.
21. Kornberg, R. D. (1977) *Annu. Rev. Biochem.* **46**, 931–954.
22. Sollner-Webb, B. & Felsenfeld, G. (1975) *Biochemistry* **14**, 2915–2920.
23. Noll, M. (1974) *Nature (London)* **251**, 249–251.
24. Lohr, D., Corden, J., Tatchell, K., Kovacic, R. T. & Van Holde, K. E. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 79–83.
25. Laskey, R. A., Honda, B. M., Mills, A. D. & Finch, J. T. (1978) *Nature (London)* **275**, 416–420.
26. Champoux, J. J. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 3488–3491.
27. Champoux, J. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3800–3804.
28. Ruiz-Carrillo, A., Wangh, L. J. & Allfrey, V. G. (1975) *Science* **190**, 117–128.
29. Jackson, V., Shires, A., Tanphaichitr, N. & Chalkley, R. (1976) *J. Mol. Biol.* **104**, 471–483.