REFERENCES

1. Arnott, S., R. Chandrasekaran, A. K. Banerjee, R. He, and J. K. Walker. 1983. New wrinkles on polynucleotide duplexes. *J. Biomol. Str. Dyn.* 1:437–452.
2. Hall, K., P. Cruz, I. Tinoco, Jr., T. M. Jovin, and J. H. van de Sande. 1984. 'Z-DNA' — a left-handed RNA double helix. *Nature (Lond.)*. 311:584–586.
3. Milman, G., R. Langridge, and M. J. Chamberlin. 1967. The structure of a DNA-RNA hybrid. *Proc. Natl. Acad. Sci. USA.* 57:1804–1810.
4. O'Brien, E. J., and A. W. MacEwan. 1970. Molecular and crystal structure of the polynucleotide complex: polyinosinic acid plus polydeoxycytidylic acid. *J. Mol. Biol.* 48:243–261.
5. Wang, A. H. J., S. Fujii, J. H. van Boom, G. A. van der Marel, S. A. A. van Boeckel, and A. Rich. 1982. *Nature (Lond.)*. 299:601–604.
6. Arnott, S., R. Chandrasekaran, I. H. Hall, and L. C. Puigjaner. 1983. Heteronomous DNA. *Nucl. Acids Res.* 11:4141–4155.
7. Zimmerman, S. B., and B. H. Pheiffer. 1981. A RNA-DNA hybrid that can adopt two conformations: an x-ray diffraction study of poly (rA)·poly (dT) in concentrated solution or in fibers. *Proc. Natl. Acad. Sci. USA.* 78:78–82.

STRUCTURE AND DYNAMICS OF RECA PROTEIN-DNA COMPLEXES AS DETERMINED BY IMAGE ANALYSIS OF ELECTRON MICROGRAPHS

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The recA protein of *Escherichia coli* mediates genetic recombination; regulates its own synthesis; controls the expression of other genes; acts as a specific protease; forms a helical polymer; and has an ATPase activity, among other observed properties (1–3). The binding of recA to DNA has been shown to alter dramatically the conformation of DNA (4). A key question in understanding DNA recombination, protein-nucleic acid interactions, and protein function in general is how a 38k mol wt molecule can perform all of these activities. Many of the activities of the recA protein are dependent on an ATPase, and previous electron microscopic work has shown that the recA polymer can be seen in different conformations as a function of the presence or absence of ATP and an ATP analogue (5–7). We have used computed image analysis of electron micrographs of recA-DNA complexes to gain more understanding of this fascinating system.

METHODS

Several different complexes have been examined in the electron microscope: pure recA polymers formed with a nonhydrolyzable ATP analogue, ATP-γ-S; recA-double stranded DNA (dsDNA) complexes formed with ATP-γ-S (8); recA-single stranded DNA (ssDNA) complexes formed with ATP (9); and recA-ssDNA complexes formed in the absence of nucleotide cofactors. Negatively stained specimens were used for three-dimensional reconstruction, while platinum-shadowed specimens were used for the determination of the hand of the helices.

The greatest difficulty in processing images of the polymer is the large disorder within the filaments. Large numbers of filaments were examined to find a few which gave rise to useful transforms. In the ATP and ATP-γ-S state, the most striking feature of the transforms is that the pitch of the nominally 95 Å helix can vary by over 10%. Further, the recA polymer is very flexible, and most filaments examined had to be corrected for curvature.

RESULTS

At the lowest resolution, the recA polymer seems to exist in one of two states: a very "open" structure, characterized by a 95 Å-pitch helix, and a compact "closed" filament, characterized by a 64 Å-pitch helix. Both helices are right handed. The 95 Å-pitch helix is formed in the presence of ATP and ATP-γ-S, and the 64 Å-pitch structure is formed in the absence of nucleotide cofactors. Fig. 1 shows the striking differences between these two states as seen in the helically averaged densities. Because of the large disorder, layer lines extracted from individual filaments did not generally average together well. However, the layer lines arising only from the right-handed one-start helix (of either 64 or 95 Å nominal pitch) did average together better, and the resulting views that are shown are equivalent to averaging the detailed density of the polymer along the one-start helix. The effect of removing the nucleotide cofactor is to dramatically compress the deep grooves of the filament.

At this level of resolution the structure can illuminate some of the observed mechanical properties of the filaments. The spring-like conformation of the ATP-γ-S state, with no apparent groove-spanning contacts, should readily permit the structure as a whole to compress and extend axially, as it is observed to do. This structure can also explain the enormous flexibility of the polymer, since the grooves can be easily compressed and extended during bending. For purposes of comparison, recA polymers in the ATP-γ-S state are ~10 times more flexible than actin, even though they have a greater mass/unit length.

A few filaments in the ATP-γ-S state generated additional layer lines arising from the modulation of continuous

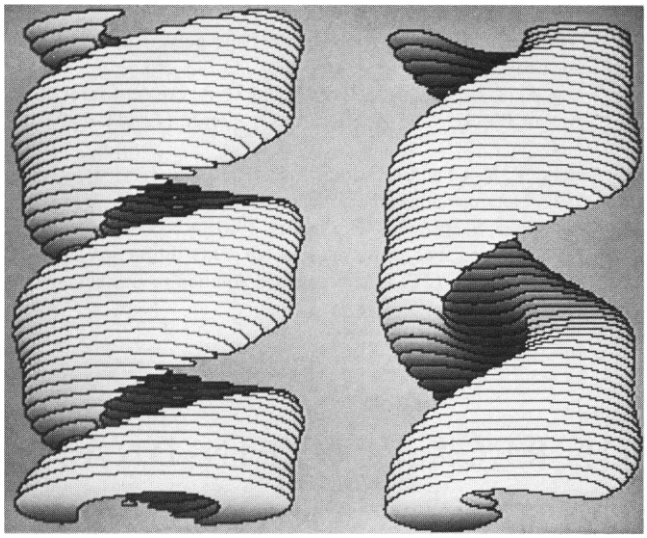

FIGURE 1 The surfaces of the averaged density distributions along the right-handed one-start helix are shown for two states of the complex. On the *left* is recA protein on ssDNA, in the absence of nucleotide cofactors. On the *right* is recA protein on dsDNA, in the presence of ATP-γ-S. The major conformational change is believed to arise from the protein-nucleotide interaction, and not depend on the DNA.

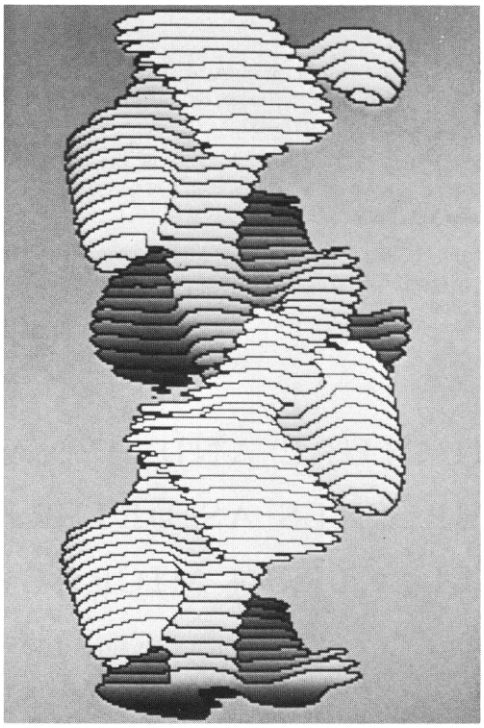

FIGURE 2 The surface of a more detailed reconstruction from recA on dsDNA in the presence of ATP-γ-S. This section has ~5.1 subunits per turn of a 100 Å-pitch right-handed helix. The DNA is believed to bind stain positively in these preparations, and therefore does not appear as part of the stain exclusion volume shown. The best estimate for the location of the DNA is in the deep groove near the filament axis.

helical density by discrete subunits. These layer lines yield information about both the number of subunits per turn of the helix and the shape of the subunit. The values for the number of subunits/turn of the 95 Å helix ranged from 5.1 to 6.8, in good agreement with the average value found by Di Capua et al. of 6.2 (10). Because the number of DNA base pairs/turn of the 95 Å helix under these conditions is 18.6, the stoichiometry in the presence of ATP-γ-S is three base pairs/recA monomer. A reconstruction from the best ATP-γ-S recA-dsDNA complex is shown in Fig. 2. The average pitch of the filament section used in the reconstruction is ~100 Å, so this filament corresponds to one extremum in the range of extension and compression. Individual subunits appear to be elongated, with their long axis nearly parallel to the filament axis.

Because the image analysis was based on negatively stained specimens, the reconstructions shown are of the stain-excluding volume. The dsDNA in the ATP-γ-S complexes is most probably binding stain positively, and therefore is not appearing in the reconstruction as positive density. Rather, the best estimate for the location of the DNA in these complexes is within the deep groove near the filament axis (11).

Several lines of evidence suggest that the large conformational change that the recA polymer undergoes as a result of ATP hydrolysis is relevant to the mechanism of DNA recombination. These include the fact that only the recA polymer, and not the recA monomer, have recombinatory activity; that an ATPase is necessary for strand exchange; and that recA acts in some stage of the recombination process to change the conformation of bound DNA greatly. The image analysis of the recA polymer provides a starting point from which to model the mechanism of genetic recombination. The extension of the present results to more states with higher resolution will greatly aid this effort.

Received for publication 10 May 1985.

REFERENCES

1. Radding, C. M. 1981. Recombination activities of *E. coli* recA protein. *Cell.* 25:3–4.
2. Dressler, D., and H. Potter. 1982. Molecular mechanisms in genetic recombination. *Annu. Rev. Biochem.* 51:727–761.
3. Howard-Flanders, P., S. C. West, and A. Stasiak. 1984. Role of recA protein spiral filaments in genetic recombination. *Nature (Lond.).* 309:215–220.
4. Stasiak, A., E. DiCapua, and T. Koller. 1983. Unwinding of duplex DNA in complexes with recA protein. *Cold Spring Harbor Symp. Quant. Biol.* 47:811–820.
5. Flory, J., and C. M. Radding. 1982. Visualization of recA protein and its association with DNA: a priming effect of single-strand-binding protein. *Cell.* 28:747–756.
6. Dunn, K., S. Chrysogelos, and J. Griffith. 1982. Electron microscopic visualization of recA-DNA filaments: evidence for a cyclic extension of duplex DNA. *Cell.* 28:757–765.
7. Koller, T., E. DiCapua, and A. Stasiak. 1983. Complexes of recA protein with single stranded DNA. *In* Mechanisms of DNA Replication and Recombination. Alan R. Liss, Inc., New York. 723–719.
8. West, S. C., E. Cassuto, and P. Howard-Flanders. 1981. Reca

protein promotes homologous pairing and strand exchange reactions between duplex DNA molecules. *Proc. Natl. Acad. Sci. USA.* 78:6149–6153.
9. Flory, J., S. S. Tsang, and K. Muniyappa. 1984. Isolation and visualization of active presynaptic filaments of recA protein and single-stranded DNA. *Proc. Natl. Acad. Sci. USA.* 81:7026–7030.
10. Di Capua, E., A. Engel, A. Stasiak, and T. Koller. 1982. Characterization of complexes between recA protein and duplex DNA by electron microscopy. *J. Mol. Biol.* 157:87–103.
11. Howard-Flanders, P., S. C. West, J. R. Rusche, and E. H. Egelman, 1984. Molecular mechanisms of general genetic recombination: the DNA binding sites of recA protein. *Cold Spring Harbor Symp. Quant. Biol.* 49:571–580.

CHROMATIN FOLDING INTO HIGHER ORDER STRUCTURE

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An exciting chapter in the study of biological structure is reaching quantitative levels with recently reported advances in nucleosome structure determination. The structure and structural transitions of chromatin, of which the nucleosome is a repeating unit, is presently not amenable to high resolution by x-ray diffraction because of the size of the particles ($\sim 1.2 \times 10^7$ dalton for a chromatin sample comprising 50 nucleosomes with radii of gyration R_g between 30 and 100 nm, depending on experimental conditions). It thus becomes necessary to use lower resolution electron microscopy or solution procedures. Hydrodynamics, sedimentation in the ultracentrifuge and diffusion, and the study of dependence of the intensity of scattered radiation (light, x-rays or neutrons) on the scattering angle, have been methods of choice. In this contribution we would like to demonstrate that conclusions derived from one or two methods only are usually model dependent and do not lead to uniquely defined results. We have therefore combined total intensity and quasielastic light scattering, ultracentrifugation, and small-angle x-ray scattering to characterize chromatin folding with a minimum of model-dependent assumptions. A self-consistent model of chromatin folding into the higher order structure, achieved with increase in either NaCl or $MgCl_2$ concentrations, will be presented. Better characterization of chromatin folding is important in understanding how DNA is packaged in higher organisms into tractable structures, and yet remains easily accessible for biological function.

Chromatin is a complex of double-stranded DNA with the core histones H2A, H2B, H3, and H4 and the linker histones Hl and H5 (in the case of chicken erythrocyte chromatin), organized in chromatosome structure. The repeat length for chicken erythrocyte chromatin, with which we are concerned, is 208 DNA base-pairs, of which 165 are coiled in two superhelical turns in the chromatosome and 43 form the linker connecting the chromatosomes. At low salt concentrations, ~1–2 mM NaCl at neutral pH, chromatin is in a flexible, unfolded so-called "10 nm" filament form. Details of chromatosome arrangement in the structure have been presented (1), though the three-dimensional spatial arrangement is not well understood. With increase in NaCl concentration chromatin folds into a more compact so-called "30 nm" higher order structure. Similar effects can also be achieved by the addition of small quantities of $MgCl_2$. This process has been followed by electron microscopy and x-ray diffraction (2). In solution it has been studied by the determination of frictional coefficients by sedimentation and diffusion (2); shape and size parameters have been studied by light and small-angle x-ray scattering (3, 4) and electrical dichroism (5). Though it is generally believed now that the higher order folded structure is a solenoidal helix, and not an arrangement of superbeads of chromatosomes, its precise nature has not been established. A solenoidal structure with 11 nm helical pitch, ~15 nm radius, and 6–8 chromatosomes per tightly wound helical turn is currently favored.

METHODS

Chromatin from chicken erythrocytes was prepared by digesting nuclei to ~1% acid solubility with micrococcal nuclease in 100 mM KCl, 50 mM Tris-HCl (pH 8.0), 1 mM $CaCl_2$. The reaction was stopped by addition of EDTA to 5 mM and the reaction mixture cooled to ice temperature. Nuclei were lysed and chromatin extracted in various ways (3). Well-characterized chromatin fractions were obtained by sedimentation on a sucrose gradient at 4°C, in 25 mM NaCl, 5 mM Tris-HCl (pH 8.0), 0.1 mM EDTA. The solubility of the chromatin fractions was studied in NaCl, in mixtures of NaCl and $MgCl_2$, and in a variety of other divalent cations (6).

RESULTS

After the NaCl concentration was increased from 1 or 5 mM to 37.5 M NaCl, both the sedimentation coefficient s, determined in the ultracentrifuge, and the diffusion coefficient D, determined by quasielastic light scattering, increased; the radius of gyration R_g, determined by total laser intensity light scattering, decreases correspondingly, indicating a decrease in size of the total structure. At the same time molar masses M, determined by light scattering intensity and by combination of s and D (the Svedberg equation) remain unchanged, in good correspondence with