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* Present address: Los Alamos Scientific Laboratory, Los Alamos, N.M. 87544.

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DNA Structure: Evidence from Electron Microscopy

Abstract. *The contour lengths of φX174 DNA duplex and RNA-DNA hybrid molecules were measured by several commonly used electron microscopic techniques. The countour length of the hybrid molecules corresponds to a rise of 2.5 to 2.6 angstroms per base pair, as expected for the A conformation, while the length of φX174 duplex DNA similarly measured corresponds to a 2.9-angstrom rise, very different from 3.4 angstroms of the classic B form. Thus any chromatin structure parameter based on electron microscopy and a rise of 3.4 angstroms must be reappraised. The possibility that DNA in dilute solution also has a rise of 2.9 angstroms and a screw of 10.5 base pairs per turn is discussed.*

The structure of DNA in dilute solution has not been proved to correspond exactly to any of the helical forms observed under conditions necessary for x-ray crystallography. These conditions require that DNA be oriented in very high concentration, and in less than 100 percent relative humidity. The x-ray studies reveal two steriochemical families, A and B (*1*), which differ in the orientation of the base pairs relative to the sugar-phosphate backbone; transitions from the A to B patterns are observed upon changing the ionic conditions and hydration of the DNA fibers. Because the classic Watson-Crick B structure having a rise of 3.4 Å per base pair and a screw of 10.0 base pairs per turn is found at higher relative humidities as compared to the A form, it is generally assumed that this classic B structure is retained when these DNA concentrates are dispersed into dilute solution. Little direct experimental evidence for this assumption exists, however, and recent energy calculations suggest otherwise (*2*). It is therefore important to establish the helical parameters, the rise and screw, of DNA under normal laboratory conditions. Furthermore, because electron microscopy (EM) is commonly used to measure the mass per unit length of DNA, an accurate measurement of the rise of DNA as it is visualized by the common EM techniques is important to many studies.

Now that the exact size of the φX174 genome is known (revised to 5386 base pairs) (*3*) measurement of its length by EM provides a direct means of measuring the average rise of DNA prepared by these techniques. Furthermore, such values might reflect the rise of DNA in dilute solution if changes in length which may occur during the preparation and dehydration of the DNA could be eliminated or understood. One probe into such changes would be a parallel measurement of the length of φX174 RNA-DNA hybrid circles synthesized from single-stranded φX174 DNA and *Escherichia coli* RNA polymerase (*4*). These hybrid molecules are always found in the A helix form in x-ray studies and appear to remain so even in dilute solution (*5*). Their length divided by 5386 base pairs should correspond to the A helix rise of 2.55 Å. A second test would be to determine whether or not open covalently closed DNA circles become supertwisted during preparation for EM. Because of the spiral nature of the DNA double helix, any change in its length should also be accompanied by a change in the rate at which the two strands wrap about each other, that is, the screw. Any such changes, therefore, would be seen as a supertwisting of a formerly covalently closed DNA circle.

This report describes such a study. With the use of the φX174 strain that has been sequenced, several different EM techniques were applied to measure both φX174 DNA duplex and RNA-DNA hybrid circle lenths. Whereas measurement of the φX174 hybrid length yielded a value in agreement with the A helix rise, measurement of the DNA duplex circles yielded a rise of 2.9 Å, very different from the 3.4 Å of the classic B form. Furthermore, covalently closed, open DNA circles remained untwisted during these EM procedures. These results support the suggestion that DNA in dilute solution also has a rise close to 2.9Å. Furthermore, this finding will require reevaluation of any chromatin parameter based on EM and a rise of 3.4 Å.

φX174 (amber 3) double-stranded DNA was prepared for electron microscopy by several different preparative procedures (Fig. 1 and Table 1): (i) direct absorption on carbon supporting films in a physiologic salt mixture followed by slow dehydration with water-ethanol washings and tungsten shadow-casting (*6*), (ii) absorption onto carbon supports followed by brief washing with dilute ammonium acetate buffer, drying in air, and tungsten shadow-casting, and (iii) surface spreading on a cytochrome c film (*7*) followed by picking up on plastic supports and shadow-casting with carbon-platinum.

Absolute molecular lengths were measured with the aid of a ruled grating verified in this laboratory (*8*) to have 54,800 lines per inch (1 inch = 2.54 cm). The grating was used in two ways: (i) consecutive sets of micrographs of the grating and of the sample were taken (*9*) and the DNA lengths were related to the mean grating spacing set-by-set, and (ii) a copper grid supporting the sample and a grid carrying the grating were sandwiched together and photographed simultaneously; the length of each molecule was related to the grating spacing in the same micrograph (Fig. 1A).

The length of φX174 duplex DNA prepared by direct absorption onto carbon supports, slow dehydration, and tungsten shadow-casting yielded values of 2.9 ± 0.05 Å and 3.0 ± 0.10 Å for the average rise by the two methods of measurement. Both DNA directly mounted and blotted dry from dilute ammonium acetate solutions and DNA surface spread with cytochrome c had lengths corresponding to a 2.9 ± 0.10 Å rise.

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ϕX174 RNA-DNA hybrid molecules were constructed by incubating ϕX174 (amber 3) single-stranded DNA with *Escherichia coli* RNA polymerase and ribonucleosidetriphosphates (*4*) (Table 1). Visualization of the total hybrid population revealed three classes: (i) fully single-stranded molecules, (ii) molecules having duplex regions of variable length and single-stranded material forming the remainder of a closed circle (single-stranded DNA or RNA appear tightly clumped and bushlike by the direct mounting and surface spreading methods used), and (iii) smooth contoured circles showing no such discontinuities (Fig. 1C). The third class was found at densities in Cs_2SO_4 equilibrium density gradients corresponding to a DNA to RNA mass ratio of 1:1 (*4*). The length of the fully hybrid molecules (Table 1) corresponds to a rise of 2.5 to 2.6 Å (the value from x-ray studies is 2.55 Å).

These results show that DNA prepared by several common EM procedures has a rise of 2.9 Å. It is of interest to ask whether DNA in dilute solution might also have a similar rise. A recent study (*10*) bears on this question. Covalently closed circular SV40 DNA molecules containing 0, 1, 2, or more supertwists (as judged by agarose gel electrophoresis) were prepared by nicking

Table 1. Length and rise of ϕX174 DNA duplex and DNA-DNA hybrid circles. The direct mounting EM procedures have been reviewed (*6*). ϕX174 form II DNA was diluted to 1 μg/ml in a buffer of 2 m*M* spermidine, 0.12*M* NaCl, 1 m*M* $MgCl_2$, 0.01*M* tris, *p*H 7.5, and adsorbed onto thin, glow-charged carbon films for 5 minutes. The grids were washed in baths of 0, 20, 50, 75, and 100 percent ethanol in water (5 minutes each), dried in air, and shadow-cast with tungsten. For more rapid dehydration, after absorption of the sample, the grids were washed for 10 minutes in 5 m*M* ammonium acetate, *p*H 7.5, blotted dry, and shadow-cast with tungsten. Cytochrome c surface spreading was done with 100 μg of cytochrome c per milliliter from 0.5*M* ammonium acetate, *p*H 7.5, on solutions of 0.25*M* ammonium acetate (*7*). Electron micrographs (take at ×15,000 magnification) were projected onto a Hewlett-Packard 9864A digitizer tablet coupled to a Hewlett-Packard 9825 calculator programmed with a fully smoothed length calculation program. Triplicate measurements were traced for each molecule. ϕX174 RNA-DNA hybrid molecules were constructed essentially as described (*4*). ϕX174 (amber 3) single-stranded DNA (10 μg) was incubated for 5 hours at 37°C with 15 μg of *E. coli* RNA polymerase (containing sigma factor from Boehringer Mannheim) in 0.5 ml of a buffer containing 20 m*M* $MgCl_2$, 1 m*M* $MnCl_2$, 10 m*M* 2-mercaptoethanol, 0.050*M* NaCl, 0.040*M* tris, *p*H 7.5, supplemented with 1 m*M* each of the four ribonucleoside triphosphates. The efficiency of conversion to fully hybrid molecules varied from 5 to 30 percent depending on the incubation time and particular DNA preparation. The reaction was terminated by chilling and the addition of EDTA to 25 m*M*. Fully hybrid molecules were purified by banding in Cs_2SO_4 solutions of average density 1.504 g/ml for 48 hours in a type 40 rotor at 42,000 rev/min.

Sample preparation	Molecules measured (No.)	Length (μm)*	Rise per base pair (Å)
	DNA duplex circles		
Direct mounting	30†	1.62 ± 0.06	3.0 ± 0.10
Slow dehydration	100‡	1.56 ± 0.03	2.9 ± 0.05
Rapid drying	33‡	1.58 ± 0.04	2.9 ± 0.10
Cytochrome c surface spreading	100‡	1.55 ± 0.05	2.9 ± 0.10
	DNA-RNA hybrid circles		
Direct mounting			
Slow dehydration	40‡	1.40 ± 0.08	2.6 ± 0.15
Rapid dehydration	15‡	1.33 ± 0.08	2.5 ± 0.15
Cytochrome c surface spreading	206‡	1.40 ± 0.10	2.6 ± 0.15

*Plus or minus standard deviation. †Sandwich technique used. ‡Consecutive sets of micrographs of the ruled grating and fields of molecules were used.

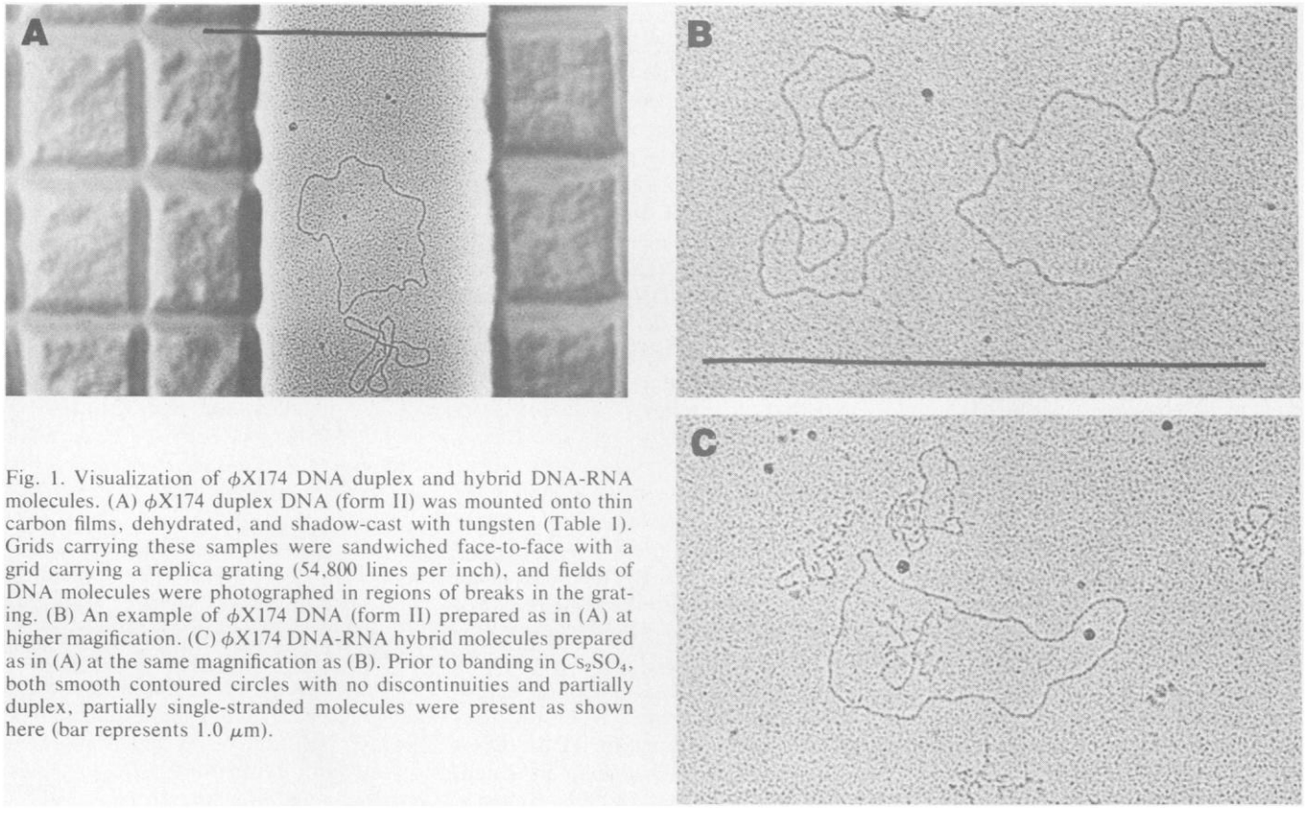

Fig. 1. Visualization of ϕX174 DNA duplex and hybrid DNA-RNA molecules. (A) ϕX174 duplex DNA (form II) was mounted onto thin carbon films, dehydrated, and shadow-cast with tungsten (Table 1). Grids carrying these samples were sandwiched face-to-face with a grid carrying a replica grating (54,800 lines per inch), and fields of DNA molecules were photographed in regions of breaks in the grating. (B) An example of ϕX174 DNA (form II) prepared as in (A) at higher magification. (C) ϕX174 DNA-RNA hybrid molecules prepared as in (A) at the same magnification as (B). Prior to banding in Cs_2SO_4, both smooth contoured circles with no discontinuities and partially duplex, partially single-stranded molecules were present as shown here (bar represents 1.0 μm).

and ligation in dilute buffer, and examined by the direct mounting and cytochrome c surface spreading techniques used here. The same distribution of molecules with 0, 1, 2, or more twists were counted by EM as were observed by gel electrophoresis.

Had these DNA circles been in the classic B structure in solution but shrunk in length such that their rise was almost exactly halfway between the classic B values (3.4-Å rise, 10.0 base pair screw) and A values (2.55-Å rise, 11 base pair screw), then we would expect this new structure to have a screw of 10.5 base pairs per turn. A change from 10.0 to 10.5 base pairs per turn for a DNA of 5200 base pairs would be accompanied by a gain of about 25 superhelical turns. That no such change was observed even though small changes due to salt effects can be detected is evidence that the rise of these ϕX174 circles in solution was not altered by the EM preparations. This conclusion was further buttressed by the finding that the lengths of the ϕX174 hybrid molecules were exactly that expected for the A helix when prepared by the same EM procedures, and that very different preparative techniques yielded consistent results.

The suggestion that DNA in dilute solution has, on the average, 10.5 base pairs per turn is in agreement with the recent calculations of Levitt (*3*), which yielded the same value. This agreement could be coincidental; we have yet to prove that the ϕX174 DNA retained its length throughout the EM preparations. Indeed, certain EM procedures can induce DNA length changes (*11*). Furthermore, because the A helix is more compact than the classic B helix and the A form is found at lower hydration, it might be more stable than normal duplex DNA. On the contrary, we have observed that the ϕX174 hybrid circles will change length when prepared with low (10 percent) concentrations of the denaturant formamide (*12*). Finally, it is not impossible that DNA would change in length without changing its overall twisting. Until these possible changes can be ruled out absolutely or evaluated, measurement of DNA lengths by EM can be taken as strong evidence for, but no proof of, the contention that the average rise of DNA in dilute solution is closed to 2.9 Å.

JACK D. GRIFFITH
Cancer Research Center, Departments of Bacteriology and Immunology and Biochemistry and Nutrition, University of North Carolina School of Medicine, Chapel Hill 27514

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8. A diffraction replica grating prepared by Ted Pella Company from a grating ruled to 54,800 lines per inch by Bausch & Lomb Company, was verified in the following manner. The square holes of the copper grid supporting the carbon grating replica, measured by light microscopy, were 38.75 ± 0.05 μm across. The grating lines were parallel to the sides of the square holes and counted by EM; 84 ± 1 lines crossed the 38.75-μm distance. One line, therefore, is 0.461 μm by this measurement and 0.464 μm by calculation from 54,800 lines per inch.
9. After every fifth micrograph taken of the sample, two were taken of the ruled grating, then five of the sample, and so on. Only those sets in which the variation in magnification was 1 percent or less were used. This required that the instrument (Hitachi H500) be left on (and unused) for 8 hours prior to taking the micrographs.
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11. In a recent study applying a detergent film technique [H. J. Vollenweider, A. James, W. Szybalski, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 710 (1978)], a spectrum of rises for DNA including 2.9 Å were observed, depending on the details of dehydration. In the procedures used here, no such effects were observed: the molecules fell into single-sized distributions and exhibited the same length whether the grids were dehydrated with ethanol, or blotted dry after a brief washing with dilute buffer.
12. Both the ϕX174 DNA duplex and RNA-DNA hybrid molecules that were spread from 40 percent formamide solutions onto 10 percent solutions had lengths corresponding to rises of 3.0 to 3.2 Å. This is in agreement with the finding of Chow *et al.* [L. Chow, J. M. Roberts, J. B. Lewis, T. R. Broker, *Cell* **11**, 818 (1977)].
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Histidine Transfer RNA Levels in Friend Leukemia Cells: Stimulation by Histidine Deprivation

Abstract. *Friend leukemia cells incubated with sublethal concentrations of histidinol for 5 to 6 days show up to twofold increases in their relative concentrations of histidine transfer RNA and no change in the relative concentrations of leucine transfer RNA. A similar effect is seen when cells are grown to stationary phase in the presence of 0.2 times the amount of histidine in Eagle's minimum essential medium. These observations support the theory that the concentrations of specific transfer RNA's are regulated by a mechanism that is sensitive to the extent of their aminoacylation.*

Intracellular concentrations of specific transfer RNA's (tRNA's) are closely correlated with the needs of the cell for their cognate amino acids in protein synthesis (*1*, *2*). However, the signals that regulate the synthesis and degradation of specific tRNA's and thereby control their concentrations are not understood. Although rates of degradation and synthesis of different tRNA species differ (*3*, *4*), it is not known how these rates are regulated to produce shifts in the tRNA population in cells undergoing differentiation.

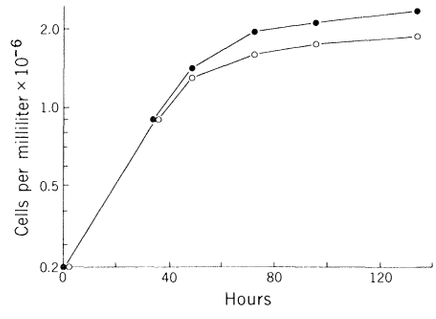

Fig. 1. Growth curves for Friend leukemia cells in Eagle's MEM (closed circles) and in MEM containing less (0.2 times) histidine (open circles). Each point is the average of two determinations on duplicate cultures.

The extent of aminoacylation might play a role in controlling tRNA concentrations. If an amino acid is not being used for protein synthesis, its cognate tRNA's will tend to become 100 percent aminoacylated. If, on the other hand, an amino acid is in great demand, the steady-state level of aminoacylation of its cognate tRNA's will tend to decrease (*5*). We postulated that the synthesis of a particular tRNA species would be stimulated or that its degradation would be inhibited when the extent of deacylation of that particular tRNA species increased. Our experiments indicate that, at least for the case of histidine tRNA ($tRNA^{His}$) in Friend leukemia cells, this postulate is correct.

L-Histidinol, a potent inhibitor of protein synthesis in cultured mammalian cells, acts by competetively inhibiting

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