fingerlike specializations that have been described on horizontal cell processes in the cone pedicles during light adaptation [J. P. Raynauld, J. R. Laviolette, H. J. Wagner, *Science* **204**, 1436 (1979)]. It is possible that the release of dopamine by interplexiform cells during darkness and the resultant increase in cyclic AMP in cone horizontal cells may play a role in this phenomenon.

16. It is unclear whether our findings will apply to other animals. Although interplexiform cells appear to occur in most, if not all, retinas, they may not be dopaminergic in many animals. In the cat, for example, only a few interplexiform cells are dopaminergic (C. W. Oyster *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press); most may contain γ-aminobutyric acid [Y. Nakamura, B. A. McGuire, P. Sterling, *ibid.* **77**, 658 (1980)]. It is possible that nondopaminergic interplexiform cells perform the same function as the dopaminergic ones do in fish. On the other hand, dopaminergic amacrine cells are observed in virtually all retinas, and it may be that these neurons alternatively or in addition modulate the center-surround organization of ganglion cells. In the cat retina it has been reported that dopamine reduces the strength of ganglion cell surrounds [P. Thier and V. Alder, *Brain Res.* **292**, 109 (1984)], whereas in the rabbit, dopamine antagonists reduce ganglion cell surround responses [R. J. Jensen and N. W. Daw, *J. Neurosci.* **4**, 2972 (1984)].

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Crystallographic Structure of the Octamer Histone Core of the Nucleosome

Burlingame *et al.* (*1*), have presented the results of their x-ray analysis, nominally at a resolution of 3.3 Å, of crystals of the isolated histone octamer (*2*). Their proposed structure is quite different in shape, size, and internal arrangement from that determined by us from a crystallographic analysis of nucleosome core particles (*3, 4*), which consist of histone octamers associated with their natural complement of DNA. All the same, Burlingame *et al.* argue that their structure for the isolated histone octamer is more relevant to the structure of chromatin than that of the octamer within nucleosome core particles, and go on to propose a different model for the way in which DNA associates with the octamer to form a nucleosome. Their analysis has led to a hydrated spongelike structure and a shape for the octamer which disagrees with the results of x-ray solution scattering on both the octamer and nucleosome core particles (*5–7*), and which cannot be fitted into the lattice of nucleosome core crystals. These large discrepancies suggest that the structure proposed by Burlingame *et al.* is wrong; we attribute this to deficiencies in their x-ray analysis.

First we deal with the relevance of our x-ray work on nucleosome core particles to the structure of intact chromatin which Burlingame *et al.* questioned:

1) Burlingame *et al.* argue that because core particles derive from nucleosomes that have lost histone H1 and the linker DNA, they have an altered structure or are artifacts. [The logic of Burlingame *et al.* (*1*) is baffling, because the isolated histone octamer, which has lost all its DNA, and is only stabilized by the use of high salt concentrations, might, by their argument, be expected to be even less representative of the state of the histones in chromatin.] What is the evidence that nucleosome core particles are present in chromatin? The answer comes from comparisons of the effects of the enzyme deoxyribonuclease I (DNAse I) on intact chromatin and nucleosome cores. Lutter (*8, 9*), using a high resolution gel electrophoresis technique, which can resolve single nucleotide steps in mixed sequence DNA, has shown that the characteristic cutting pattern of DNAse I on the DNA of core particles accounts quantitatively for both the length and frequency distribution of DNA fragments produced from nuclei. Thus the bulk of the chromatin in nuclei contains nucleosome core particles, and it is these that we have crystallized.

2) Could the nucleosome core particles have undergone an extensive structural change on crystallization? This is unlikely, since the crystallization conditions are mild and close to physiological ionic strength (*3*). The shape that emerged from our studies, a disk of diameter 110 Å and height 57 Å, is consistent with the low angle x-ray scattering studies in solution (*5–7*). These spacings are found in x-ray diffraction patterns of both chromatin and nuclei in vivo (*10, 11*) and arise from the packing of the nucleosomal disks in the 300 Å diameter filaments of chromatin (*12, 13*). Furthermore the higher angle x-ray spacings at 37 and 27 Å, which arise from the internal structure of the nucleosomes, are also found in the correct orientation in 300 Å filaments of intact chromatin (*13*), as calculated on the basis of our electron density map (*4*).

The histone octamer in the nucleosome core particles has the shape of a disk about 70 Å in diameter and 57 Å in height (*3, 4*). There is no way in which our electron density map could give the shape proposed by Burlingame *et al.* (*1*), namely a prolate ellipoid of diameter 70 Å and a length of 110 Å. The overall protein density in the nucleosome core particle is limited to 70 Å in two dimensions by the two-turn superhelix of DNA (which is clearly visible) and to less than 60 Å in the third dimension by the DNA in neighboring layers of core particles. The octamer in our crystals is so confined by this adjacent DNA that no significant density could extend beyond the disk.

The structure presented by Burlingame *et al.* also has curious physical chemical properties. The proposed ellipsoid has a volume of 280,000 $Å^3$, three times that of the dry volume of 82,000 $Å^3$ of the histone octamer in solution [as calculated from the molecular weight (108,000) and the partial specific volume of 0.77 at appropriately high salt concentrations (*14*)]. Being penetrated by numerous holes and channels like a sponge, the proposed octamer has an abnormally high water content. A simple calculation shows that even on the most favorable assumptions (*15*), the proposed ellipsoid would have a sedimentation constant of 3.7*S* compared with the experimental value of 4.8*S* (*14*). An octamer of the shape and volume found in the nucleosome core crystals leads to a value of 4.2*S*, more consistent with the observed value.

Despite these disagreements, it could be argued that the structure proposed by Burlingame *et al.* could be correct for the octamer in high salt (the nominal ionic strength of the crystallization buffer is of the order of 7*M*), even if it does not reflect the structure of the octamer when combined with DNA. This, too, must be discounted, since the shape and size of the histone octamer, deduced by image reconstruction from electron micrographs of helical aggregates of octamers prepared at similarly high salt concentrations (*16*), agrees with that present in nucleosome core particles, as determined by neutron diffraction contrast variation at low resolution (*17*) or x-ray analysis (*3, 4*).

We are thus led to the view that either Burlingame *et al.* (*1*) have misinterpreted their map, or that the map contains errors that have led to a structure of the histone octamer at variance with other, firm data. Despite their demonstration of two α-helical rods of density (in which amino acid side chains are not visible), we believe that their map is unreliable. First, it is surprising that the polypeptide chain has not been traced since this should be easily discernible at the resolution of 3.3 Å, but there are grave deficiencies in the crystallographic analysis. Only a single heavy atom derivative has been used, and this is reported to be located at a rather special position with fractional coordinates very close to (1/3,

1/3, 0). This means that two-thirds of the reflections are only weakly phased, by isomorphous replacement or anomalous scattering. It is therefore highly doubtful whether the solvent-flattening procedure used to resolve the phase ambiguity could have produced reliable phases for these reflections. Even in more favorable circumstances, a procedure which relies on a single isomorphous replacement (SIR) map for recognition of the solvent boundaries has its obvious dangers.

The shape of the particle deduced from the octamer crystals may therefore be misleading for the following reason. In electron density maps, the general distribution of density in the unit cell and hence the boundaries of the protein molecules are defined, initially, by the intensities and phases of the low order reflections. The intensities of two-thirds of these reflections would have remained almost unchanged in the single heavy atom derivative because of the closeness of the heavy atom to the special position, so that these reflections would hardly have been phased at all. If this had led to a wrong choice of envelope, it could not have been rectified by flattening the calculated SIR density outside it, although various isolated high resolution features common to the correct and chosen boundaries, such as a piece of right-handed helix, might still show through in weakened form. However, the larger scale distribution of density in the final map would be unreliable. Hence, it is not surprising that an attempt to interpret it has led to a structure which conflicts with all other firm results in the field.

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References and Notes

1. R. W. Burlingame, W. E. Love, B.-C. Wang, R. Hamlin, N.-H. Xuong, E. N. Moudrianakis, *Science* **228**, 546 (1985).
2. R. W. Burlingame, W. E. Love, E. N. Moudrianakis, *ibid.* **223**, 413 (1984).
3. J. T. Finch, L. C. Lutter, D. Rhodes, R. S. Brown, B. Rushton, M. Levitt, A. Klug, *Nature (London)* **269**, 29 (1977).
4. T. J. Richmond, J. T. Finch, B. Rushton, D. Rhodes, A. Klug, *ibid.* **311**, 532 (1984).
5. J. F. Pardon, P. L. Worcester, J. C. Wooley, R. I. Cotter, P. M. J. Lilley, B. M. Richards, *Nucleic Acids Res.* **4**, 3199 (1977).
6. P. Suau, G. G. Kneale, G. W. Braddock, J. P. Baldwin, E. M. Bradbury, *ibid.*, p. 3769.
7. G. W. Braddock, J. P. Baldwin, E. M. Bradbury, *Biopolymers* **20**, 327 (1981).
8. L. C. Lutter, *J. Mol. Biol.* **124**, 391 (1978).
9. ———, *Nucleic Acids Res.* **6**, 41 (1979).
10. J. P. Langmore and J. R. Paulson, *J. Cell Biol.* **96**, 1120 (1983).
11. J. R. Paulson and J. P. Langmore, *ibid.*, p. 1132.
12. L. Sperling and A. Klug, *J. Mol. Biol.* **112**, 253 (1977).
13. J. Widom and A. Klug, in preparation.
14. J. O. Thomas and P. J. G. Butler, *J. Mol. Biol.* **116**, 769 (1977).
15. For a prolate ellipsoid of dimensions 110 × 70 Å, the radius of the equivalent sphere is 40.7 Å, giving $f_0 = 7.74 \times 10^{-8}$ g sec. From standard tables, f/f_0 is 1.03, for zero hydration, whence $f = 7.97 \times 10^{-8}$. The sedimentation constant $s = M(I - \bar{V}\rho)/Nf$, which gives, using the experimentally measured value of 0.167 for $\delta\rho/\delta c = I - \bar{V}\rho$ (14), $s = 2.98 \times 10^{-20}/f$. Hence, for zero hydration, $s = 3.7$; any allowance for hydration will increase f, and hence reduce s.
16. A. Klug, D. Rhodes, J. Smith, J. T. Finch, J. O. Thomas, *Nature (London)* **287**, 509 (1980).
17. G. A. Bentley, J. T. Finch, A. Lewit-Bentley, *J. Mol. Biol.* **145**, 771 (1981).

10 June 1985; accepted 20 June 1985

We agree with two of the major points in the letter of Klug *et al.* (*1*): the structure of the histone octamer determined by us (*2*) is very different from the structure determined earlier by them; and both structures cannot correctly represent the histone octamer even if a small DNA-dependent increase in its compaction is assumed. We differ on all their remaining points, which represent prevailing views and interpretations rather than "firm data." We can show that the properties of our structure are not inconsistent with the reported data.

1) We never made a value judgment as to whether the "isolated histone octamer is more relevant to the structure of chromatin than that of the octamer within nucleosome core particles." The histone octamer is a physiological entity that exists as such within cells (*3*) before it becomes associated with DNA and not after it "has lost all its DNA." It deserves to be described for its own sake. However, nucleosome core particles are not naturally "present in chromatin," but result from the deliberate enzymatic digestion of chromatin. We questioned not the significance of the removal of H1 or the putative "linker DNA" but instead the consequences of eliminating the torsional information present in the continuum of the native chromatin domains. This is a legitimate and logical question since the work of the Mirzabekov group (*4*) suggests that some internal rearrangements do take place. We believe however, that the structures of both the histone octamer and the nucleosome need to be accurately determined.

2) We did not challenge the crystallization procedures of the MRC group. To do so would have been presumptious on our part since their exact crystallization conditions [references 3 and 4, in (*1*)] such as *p*H, temperature, cofactors, and others, remain unpublished and thus beyond analysis. Likewise, we have not challenged their data handling procedures, such as the heavy atom parameters in each of the approximately 40 (our assumption) data sets and their scaling, the specifics of which have not been published [reference 4 in (*1*)].

Klug *et al.* (*1*) questioned the "curious physical chemical properties" of our structure which were not measured by them, but presumed. They, as we did at the beginning, must have assumed that on first approximation, the volume of our rugby-ball-shaped octamer is equivalent to that of a smooth-surfaced prolate ellipsoid 110 Å long and 70Å diameter. However, our photograph [figures 3 and 6, a and b, in (*2*)] demonstrate that the overall circumference of the model at planes perpendicular to its long axis is definitely not circular but indeed deeply concave at several places. We have now directly measured the volume of our model. The model was placed inside a thin, unsealed, water-tight bag and was submerged in water which caused the bag to conform to the surface topography of the model. Extending the Archimedes principle, we determined that the volume for the octamer is 184,000 Å^3, which is equivalent to a sphere of 35.3 Å radius. Using the formulas and the partial specific volume cited by Klug *et al.*, we calculate that the sedimentation coefficient (*S*) of our octamer is 4.4, not 3.7. If instead, the more accurately determined value of 0.753 (*5*) for partial specific volume is used in the calculation, the *S* value of our octamer is 4.98. It appears that the hydrodynamic properties of our structure are just fine within the scope of the criticism and the treatment of the data outlined by Klug *et al.* However, we have reservations concerning some assumptions employed in this treatment.

Klug *et al.* cite solution x-ray studies [references 5, 6, and 7 in (*1*)], which incidently are neutron diffraction studies, as providing firm data against our structure. However, Braddock *et al.* found that the pitch of the DNA superhelix in the nucleosome was 37Å. Furthermore they measured the radius of gyration and found it to be "substantially greater than that expected" [reference 7 in (*1*)] for a "flat disc or wedge" shaped model. To resolve this discrepancy, they assigned 25 percent of the histone mass to flexible "tails" and the remaining 75 percent of the octamer mass to the volume of an equivalent cylinder of dimensions 40 by 70 Å, that is, 153,000 Å^3. If we assume standard protein density for the remaining mass [reference 16 in (*1*)], the volume of the whole octamer would be 188,000 Å^3. Although this number compares well with ours, we do not rely on it as support for our structure, in line with earlier arguments of Finch *et al.* that "scattering in solution which yields spherical averages of intensities, can never prove a model" [reference 3 in (*1*)]. Nevertheless, we predict that when

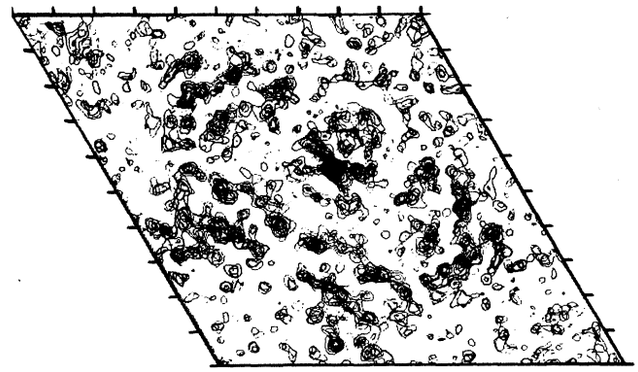

Fig. 1. The unfiltered map. This electron density map shows the range from −0.25 to +0.75 in x, from −0.25 to +0.75 in y, and from −0.048 to +0.048 in z axes. It was calculated from the phases determined by isomorphous and anomalous scattering differences only, with the use of programs from a crystallographic package of G. A. Petsko (Massachusetts Institute of Technology). No filtration of any kind was applied to it. The crystallographic statistics in Table 1 were derived from the same data used to make this map. Comparing this map to that in [figure 2a in (*2*)] it is evident that the filtration process did not alter the overall features of the protein, but eliminated noise in the solvent region, and consequently reduced the well-known artifactual electron density at the heavy atom site while sharpening the density of the protein.

the features of our octamer structure are used to calculate neutron-scatter functions, the resulting curves will correlate more closely with the observed scatter curves than any heretofore calculated.

The statement of Klug *et al.*, "These large discrepancies suggest that the structure proposed by Burlingame *et al.* is wrong; we attribute this to deficiencies in their x-ray analysis," is undocumented and does not serve to resolve the issue. Klug *et al.* have neither analyzed our diffraction data nor have they seen our electron density map in its entirety. The specific criticisms of Klug *et al.* are summarized in the following four points. (i) "[O]nly a single heavy atom (derivative) was used." (ii) ". . . [T]wo-thirds of the reflections are only weakly phased." (iii) Given the above, solvent flattening introduced artifacts. (iv) "Burlingame *et al.* have misinterpreted their map."

1) In 1970, the structure of rubredoxin (*6*), and in 1972 the structure of flavodoxin (*7*) were determined by means of single isomorphous replacement (SIR) and anomalous scattering (AS). More recently, the structures of dihydrofolate reductase (*8*) and troponin C (*9*) were solved with SIR and AS, while the Eco RI–DNA complex (*10*) and two Bence Jones proteins (*11, 12*) have been determined from a single derivative without AS data. Thus we believe that the validity of this technique has been established.

2) We, too, were concerned that the heavy atom might have insufficient phasing power for a significant fraction of the reflections. However, we have justified (*2*, p. 547) why "its position caused no significant problems." As documented in Table 1, the heavy atom contributes with high statistical significance to *all* the reflections, a testament to the superb precision of modern data collection methods.

3) Contrary to the assumption of Klug *et al.*, in the iterated single isomorphous replacement (ISIR) procedure (*13*), neither high nor low order reflections dominate the process. In a test (*14*) of the relative influence of high and low order reflections on the boundary of Bence Jones protein Rhe (*15*), the molecular envelope calculated with the 5 to 3 Å reflections is nearly identical to the envelope calculated with the infinity to 3 Å reflections. The ISIR procedure objectively locates the molecular envelope and properly, on the basis of probability, combines the phases of the back transform of the solvent flattened map with the experimentally determined phases.

Figure 1 shows a 3.3 Å resolution map calculated with phases derived solely

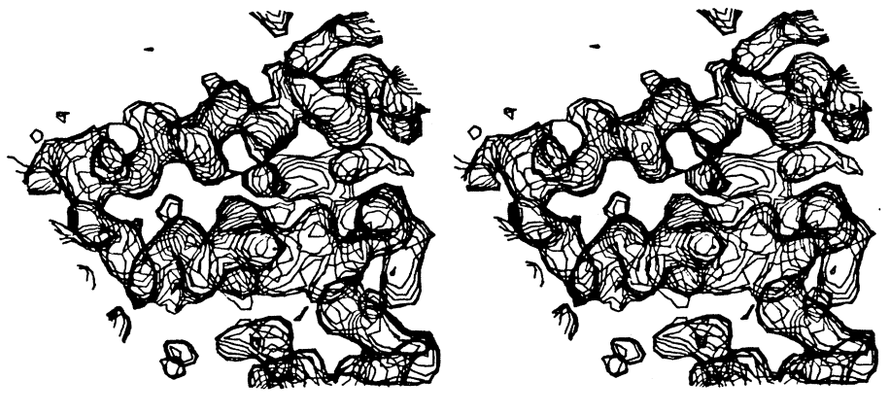

Fig. 2. Helices with visible side chains. Two helices from H2A are contoured at a lower level than shown in [figure 1 in (*2*)] and a thicker slab of electron density is shown. Density above and below the helix can be seen, including side chains. This map is contoured at only the lowest contour level, for clarity.

Table 1. Statistics for the SIR and AS data, without any filtering. For all 12,942 reflections, the phasing power is 1.77 and the figure of merit is 0.57. The phasing power represents the root-mean-square calculated heavy atom contribution to the structure factor divided by the rms lack of closure error. The figure of merits were calculated by the method of Blow and Crick (*17*), and used in obtaining Fig. 1. The maps in (*2*) were generated with phases refined by the ISIR procedure of B. C. Wang (*13*), which calculates the figure of merit by the method of Hendrickson and Lattman (*18*), and yields different numbers. Before noise filtering, the figure of merit calculated by the method of Hendrickson and Lattman was lower than that reported here, while after the ISIR procedure, it was higher. The ISIR procedure substantially improved the phases of the higher order reflections. FOM, figure of merit.

Resolution range	$-h + k + l \neq 3n$			$-h + k + l = 3n$		
	Reflections (No.)	Phasing power	FOM	Reflections (No.)	Phasing power	FOM
120.00–6.30	1290	1.71	0.73	655	4.69	0.97
6.30–5.00	1251	1.89	0.70	631	3.91	0.90
5.00–4.37	1232	1.52	0.58	620	2.13	0.77
4.37–3.97	1217	1.54	0.48	620	1.94	0.55
3.97–3.68	1238	1.32	0.45	615	1.56	0.48
3.68–3.47	1177	1.28	0.43	570	1.04	0.42
3.47–3.29	1209	1.16	0.40	617	0.80	0.34
120.00–3.29*	8614	1.49	0.54	4328	2.34	0.64

*Entire range.

from SIR and AS information and this should be compared with figure 2a in (*2*).These maps are "before" and "after" solvent flattening respectively, and clearly demonstrate that our noise filtering procedure did not introduce artifacts into the map.

4) Klug *et al.*, seem to have overlooked the information presented (*2*, p. 547) and concluded that we have incorrectly chosen the protein boundaries. On the contrary, 95 percent of the boundaries of the octamer are unambiguously delineated by large solvent regions between molecules [Fig. 1 and figure 2 in (*2*)] and the constraints imposed by the crystallographic symmetry elements (*2*, p. 547). Five helices, not two, were mentioned in our paper (*2*), and these plus several smaller helices make up about 50 percent of the protein mass, consistent with circular dichroism and Raman spectroscopy (*16*). Furthermore, we have reported that the chains of H2A and H2B have been traced nearly from end to end, and sufficient segments of characteristic amino acid sequences have been identified in the map to allow the assignment of the polypeptides. The quality of the map itself proves the validity of our procedures. The accompanying stereo pair (Fig. 2) is a replot of the alpha-helical region we showed before [figure 1 in (*2*)], contoured at a lower level and thus illustrating some side chains.

We believe that the resolution of the causes for the differences between the two structures will come about through further experimentation rather than rhetoric and argumentation. The results of our ongoing efforts in fitting the amino acids to the electron density map should shed some light on the resolution of the differences between the two structures.

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References and Notes

1. A. Klug, J. T. Finch, T. J. Richmond, *Science* **229**, 1109 (1985).
2. R. W. Burlingame, W. E. Love, B-C. Wang, R. Hamlin, N-H. Xuong, E. N. Moudrianakis, *ibid.* **228**, 546 (1985).
3. W. C. Earnshaw, O. P. Rekvig, K. Hannestad, *J. Cell Biol.* **92**, 871 (1982).
4. A. V. Belyavsky, S. G. Bavykin, E. G. Goguadze, A. D. Mirzabekov, *J. Mol. Biol.* **139**, 519 (1980).
5. T. H. Eickbush and E. N. Moudrianakis, *Biochemistry* **17**, 4955 (1978).
6. J. R. Herriott, L. C. Sieker, L. H. Jensen, W. Lovenberg, *J. Mol. Biol.* **50**, 391 (1970).
7. K. D. Watenpaugh, L. C. Sieker, L. H. Jensen, J. Legall, M. Dubourdieu, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3185 (1972).
8. D. A. Mathews *et al.*, *J. Biol. Chem.* **253**, 6946 (1978).
9. M. Sundaralingam *et al.*, *Science* **227**, 945 (1985).
10. C. A. Frederick *et al.*, *Nature (London)* **309**, 327 (1984).
11. C. H. Chang *et al.*, *Biochemistry*, in press.
12. J. Rose *et al.*, in preparation.
13. B. C. Wang, *Acta Cryst.* **A40**, C12 (1984); *Methods Enzymol.*, in press.
14. ———, unpublished results.
15. ———, C. S. Yoo, M. Sax, *J. Mol. Biol.* **129**, 657 (1979); W. Furey, Jr., B. C. Wang, C. S. Yoo, M. Sax, *ibid.* **167**, 661 (1983).
16. N. V. Beaudette, A. W. Fulmer, H. Okabayashi, G. D. Fasman, *Biochemistry* **20**, 6526 (1981); G. J. Thomas, Jr., B. Prescott, D. E. Olins, *Science* **197**, 385 (1977).
17. D. M. Blow and F. H. C. Crick, *Acta Cryst.* **12**, 794 (1959).
18. W. A. Hendrickson and E. E. Lattman, *ibid.* **B26**, 136 (1970).

10 July 1985; accepted 20 July 1985

We have solved the crystallographic structure of the nucleosome core particle (histone octamer with 146-bp DNA) (*1*). We are having considerable difficulty in rationalizing the interpretation of the histone octamer of Burlingame *et al.* (*2*) with the nucleosome structure we have solved (*1*), with those of Richmond *et al.* (*3*) and Bentley *et al.* (*4*), and with many other biophysical studies. We see no problem with the electron density of the octamer presented by Burlingame *et al.* It appears to be of very high quality. It is the interpretation of the structure that we think should be reexamined. Burlingame *et al.* seem predisposed to assume that the DNA will lie in the grooves of the histone octamer. Our own nucleosome structure, along with those of Richmond *et al.* and Bentley *et al.*, indicated that in many locations rather than lying in the grooves of the histone octamer, the DNA actually rides on the ridges or "high points" of the proteins. The "helical ramp" is therefore somewhat discontinuous and complex. It appears to us, from statements in their article, that Burlingame *et al.* have ignored all but the most obvious (to them) possibilities for placing the model DNA on the octamer.

It seems quite possible to us to place superhelical DNA onto their histone core in a way that is consistent with our own and the other nucleosome structures, and which has a superhelical radius (~43 Å), superhelical pitch (~28 Å), and number of superhelical turns (~1.85 for 146 bp DNA) to be consistent with the bulk of previous experimental evidence about the nucleosome (Fig. 1).

Also, in this orientation, the octamer seems to have a size and extent, and occupy a volume which is not much different from what is seen in the crystallographic studies of nucleosome core particles. Some of our early modeling studies, which tested model nucleosomes with ellipsoidal histone octamers against diffraction data, showed us that the best ellipsoidal representation for the octamer has its long axis *not on the superhelical axis*, but about 45° from it and points in the direction of the DNA ends (Fig. 1). If at 0.15*M* ionic strength, in the presence of the DNA, the H2A region in the octamer of Burlingame *et al.*, (that is, the protein region shaded dark) moves in ~20 Å to fill the apparent solvent channel, the resulting structure (with the DNA bound as we propose) bears a very good resemblance to the crystallographic structures of the whole nucleosome core particle. The maximum thickness of the octamer in the superheli-

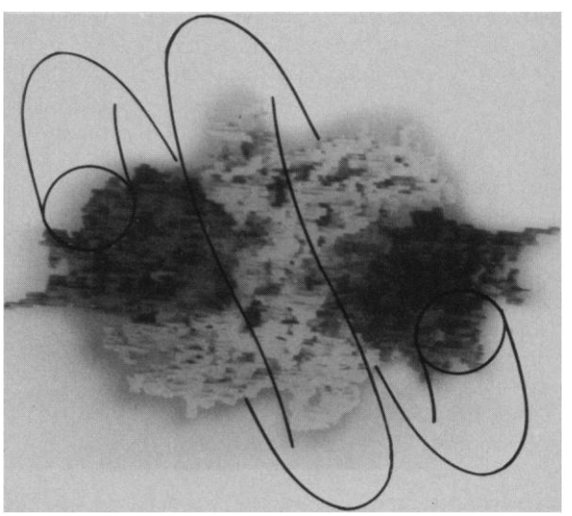

Fig. 1. Diagram of the nucleosome core particle showing proposed placement of superhelical DNA on the histone octamer model of Burlingame *et al.*, which is consistent with the nucleosome crystal structures and other biophysical studies.

cal axis direction would then be approximately 75 Å, which is very close to what is seen in the nucleosome structures. Furthermore, the octamer of Burlingame then forms ramp and groove-like regions precisely where they are seen in our own nucleosome structure. We suggest that Burlingame *et al.* seriously reconsider the model DNA placement on a condensed histone octamer structure.

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References and Notes

1. E. C. Uberbacher and G. J. Bunick, *J. Biomol. Struct. Dynam.* **2**, 1033 (1985).
2. R. W. Burlingame, W. E. Love, B.-C. Wang, R. Hamlin, N.-H. Xuong, E. N. Moudrianakis, *Science* **228**, 546 (1985).
3. T. J. Richmond, J. T. Finch, B. Rushton, D. Rhodes, A. Klug, *Nature (London)* **311**, 532 (1984).
4. G. A. Bentley, A. Lewit-Bentley, J. T. Finch, A. O. Podjarny, M. Roth, *J. Mol. Biol.* **176**, 55 (1984).

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Instead of using a heavy atom derivative to phase their 15 Å data set, Uberbacher and Bunick (*1*) used molecular replacement and model building to obtain a structure for the nucleosome core particle. In this process they used the structure of Richmond *et al.* (*2*) as their starting point. Their structure is dominated by the features of their starting model, since this is the expected result of the molecular replacement operation. Continuing their model building, they have now drawn lines representing DNA on our photographs (*3*), thus developing an additional model for the structure of the core particle. They appear to have generated this model in order to compact our octamer structure and force it to resemble the model of Richmond *et al.* We do not feel the need to do so and our reasons are presented in our reply to Klug *et al.* (*4*).

The model proposed by Uberbacher and Bunick requires that the length of the histone octamer be condensed to 75 Å. The so-compacted structure, which represents an averaging of the length of the structure of Richmond *et al.* with ours, does not fit parameters imposed by diffraction data. It is well known that the diffraction pattern of chromatin consists of the first- and higher orders from a Bragg spacing of 110 Å, that is, 1/110, 1/55, 1/37, 1/27 (*5*). Our model-built nucleosome is roughly spherical and is 110 Å in diameter. The pitch of the DNA is about 37 Å, and the tripartite protein core is roughly divided into three 37-Å long pieces. Thus the first-order reflections from the DNA superhelix and from the internal arrangement of the protein would superimpose on the third-order from the whole particle. Both our model and that of Richmond *et al.* are consistent with the above criteria, but the model presented by Uberbacher and Bunick would give additional reflections, which are not observed. Averaging two differing structures does not yield the correct one.

In the modeling studies of Uberbacher and Bunick, the value initially assumed for the length of the octamer was 50 Å, and no new value was reported as a result of their procedures [reference 1 in (*1*)]. The dimensions that they cite now for the structure in Fig. 1 (*1*) differ from those that can be obtained by measuring directly the model shown there, when the dimensions of our balsa wood model are used as a scale. The superhelical radius is 48 Å (not 43 Å), the superhelical pitch is 37 Å (not 28 Å), and the length of the particle parallel to the superhelical axis and measured only to the outermost edges of the DNA is 94 Å (not 75 Å). Furthermore, direct inspection of our three-dimensional octamer structure reveals that there is some room for *small-scale* closure of the dimer-tetramer channels at the *front* if the dimer is allowed to pivot about the dimer-tetramer contact point at the back. However, there is no space available to permit the dimer to shift inward along the entire channel, and there is no evidence suggesting that the dimensions of the octamer change drastically when it associates with DNA (*4*).

We have already stated that we have attempted several alternate placements of the DNA around the histone octamer (*3*, p. 551). We have published our preferred orientation in which the DNA "follows the path dictated by these grooves and ridges" (*3*, p. 550), not just the grooves. We found two other interesting orientations. In one (left-tilt), the DNA path is tilted 30 to 45 degrees to the left (similar to theirs) of the path it occupies in our preferred orientation, while in the other (right-tilt) the DNA path is tilted about 45 degrees to the right. In the right-tilt model the DNA rides on the front of the long "propeller" of the H3. The histone octamer remains 110 Å long in all three models, each of which has its own probability of existing in vivo. However, in the absence of direct information on the DNA location, we did not present these models to avoid contributing to excessive speculations.

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References and Notes

1. E. C. Uberbacher and C. J. Bunick, *Science*, **229**, 1112 (1985).
2. T. S. Richmond *et al.*, *Nature (London)* **311**, 532 (1984).
3. R. W. Burlingame, W. E. Love, B.-C. Wang, R. Hamlin, N. H. Xuong, E. N. Moudrianakis, *Science* **228**, 546 (1985).
4. A. Klug, J. T. Finch, T. J. Richmond, *ibid.* **229**, 1109 (1985).
5. J. F. Pardon, M. H. F. Wilkins, B. M. Richards, *Nature (London)* **215**, 508 (1967); B. M. Richards and J. F. Pardon, *Exp. Cell Res.* **62**, 184 (1970).

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