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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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

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