

MRC

Medical Research Council

MRC Laboratory of Molecular Biology
University Postgraduate Medical School
Hills Road, Cambridge, CB2 2QH
England

telephone Cambridge (0223) 48011

telex - 81532

6 April 1977

Dr. F. H. C. Crick, F.R.S.,
The Salk Institute,
Post Office Box 1809,
San Diego,
California 92112, U.S.A.

Dear Francis,

Thank you for your letters of 30 March and 1 April, with the two enclosures. It seems that George P., having been thwarted in getting his ideas into our article, has managed to get them published in Time. I am glad to have Roy Doi's paper on protease. I haven't written about this before, but I have spent a lot of time in the last six months following individual nucleosome preps as they were made, and we now know in which step the protease works and probably can control it in future preps. It has been a long story which I haven't bothered you with, but the general picture became clear to me from a dossier I had drawn up in September, and the only thing was to find out the whereabouts of the criminal and arrest him.

I was glad to have all your comments in the letter of 30 March. I am sending, under separate cover, xeroxes of the papers by Zachau and Joel. Since the organisers of the Discussion have the job of "editing" the manuscripts, I would be glad to have any comments or corrections. I have found in the past that one can get an author to change part of the text by a process which doesn't quite amount to refereeing. I shall send you Kornberg and Chambon's manuscripts when I get them.

In my last letter I forgot to explain the origins of the paper which Sperling and Amos wrote. You may remember the curly fibres ($\sim 50 \text{ \AA}$ diameter, rather regular) of individual histones which were observed in the e.m. here in 1972. Roger persuaded us that they must be artefacts since they didn't dissociate after prolonged dialysis, and also because these were the preparations, which when mixed, 4 histones together, with DNA failed to give the X-ray pattern.

Ruth Sperling in Rehovot has been following this up and finds similar fibres given by mixtures of histones. However in her first paper she reported a periodicity of 150 \AA . This was an error. Linda Amos has "sorted" this all out and I saw the results for the first time last September. My own feeling is that the histones in these fibres are in a not quite native state since Ruth Sperling can't get the Kornberg tetramer prepared directly from chromatin to behave the same way as her mixtures in H3 and H4. However, these aggregates

histones can potentially form (the analogy I have in mind is that of the stacked disk of TMV protein which, though it has a cleaved polypeptide chain, is closely related to the protein helix structure). I therefore take these results fairly seriously, particularly now that the periodicities present are similar to those found along the c axis of our crystals.

I enclose some further memos, but the one on the space group has probably been overtaken by events. The experiments in which one tried to follow crystallization of "degraded" particles in the e.m. have paid off. John has now obtained photographs of material which forms wavy cross-striated columns, about 100 \AA apart. The main transverse periodicity along the columns is about 50 \AA , presumably corresponding to a platysome, but there are finer striations of about 25 \AA . The correspondence with the crystal X-ray photos seems clear. The strongest groups of reflections along the c axis are at 57 \AA and $28\frac{1}{2} \text{ \AA}$, so it seems virtually certain that we are dealing with a two-layer platysome like that sketched in my last memo. I have spent many hours in the last few months scrutinising electron micrographs, but what finally convinced me is seeing a column curved into an arc and noticing that the 25 \AA paired striation stayed together as an entity. (Indeed the parallel now with the stacked disk structure of TMV protein - two rings of protein $26\frac{1}{2} \text{ \AA}$ apart forming a 53 \AA disk - is now quite striking, but I must say it didn't figure in my thinking a few weeks ago.)

One of the enclosed memos deals with the problem of packing these two layer disks, and I wonder whether the possible wedge shape (on which you have been so keen) is really related to that which may occur in the intact 200 base pair particle which forms the nucleofilament and solenoid. What puzzles me is still the question of mass per unit length along the nucleofilament. It could be that the estimates are wrong and that in Chambon's em pictures the flat disks are laying on their faces. It seems to me a disk is a rather odd thing to use to pack into a higher order unless there are little beads between the disk consisting of H1 and the 60 base pairs which provide the necessary articulation for the flexible joint. But if so, how does one then explain the crosslinking experiments at pH 8 which show a continuous column of the four histones without H1. One can, of course, draw models in which fingers reach out from the flat faces of a disk to meet the next disk about 100 \AA away and the empty space is filled up by H1 and the 60 base pairs.

At all events I am pretty confident of the two layer disk now, and I have decided to write it all up, but please don't break silence until this has all been done and submitted.

I should say that in the electron micrograph the " $28\frac{1}{2} \text{ \AA}$ " striations go all the way to the edge of a column and it would seem that the DNA is wound in two rings, perhaps connected by a short* longitudinal

join rather than wound helically. (The parallelism of the striations is more pronounced than may be expected from a helix formed to give the kind of splay you have mentioned earlier. So although one can't rule out a rather flat two turn helix of both histones and DNA, the simplest view is to make them ring like.)

(Of course we can't be quite certain that the DNA on the rim of a histone layer is like a bicycle tyre, it is just possible that the DNA lies between the layers, like a sandwich, but appears blocked in the electron micrographs because it is being "positively stained" by the negative stain. However this would require one large turn of DNA and I don't think this model is likely.)

I now realise that this type of model probably can explain some of the earlier photographs of chromatin. You may remember that in Pardon & Richards, Experimental Research (1970) paper which showed an orientation of the 110 Å reflection, the 55 Å reflection went all the way round in an arc, so that the meridional part and indeed equatorial part looked as if it had a different origin from merely being a second order of the 110. It was indeed this sort of feature that led me towards the solenoid as an interpretation. This would require the disks to be organised with their planes approximately parallel to the axis of the solenoid, which again raises the question of the mass per unit length. The other point is that the origin of the solo 27 Å reflection obtained on drying is not (in necessarily) pulled-out DNA, but in the fact that the only feature of the structure which survives is the two layers. The DNA in the two layers, if it does run round like a bicycle tyre, would presumably remain about 27 Å apart (an attraction of the model). In the more recent X-ray photographs of native chromatin, described in the Sperling & Klug paper, you may remember that the X-ray pattern of dry material shows both the "55 Å and 27 Å" bands (photo enclosed). If one does a radial distribution of the intensities in the crystals then one also finds two peaks at 55 Å and 27 Å, and Steve Harrison has pointed out that the density in a 12 nucleosome unit cell crystal would be about 58% which is close to that in the chromatin specimens.

A further thought about the two layer model is that there would be no problem in explaining Simpson's results that the ends of the DNA can crosslink equally to H3 and H4. In fact the major difficulty is that pointed out in my memo of reconciling a model of this type with Felsenfeld's results on reconstitutes containing only H3 and H4. He claimed this could be done by a single tetramer, but on looking up notes of what Chambon said at the Royal Society meeting, it seems that it takes four H3-H4 dimers to get the maximum yield of the 90 Å particles he sees on reconstitution in the e.m. and which contain about 120-130 base pairs. Here we are getting on to the question of what might be the organisation of the four histones in a layer. Based on the crosslinking results, one can draw a number of models. However, we shall now go on to try to find the shape of the octamer by e.m. of aggregates and so on.

In summary, just as has happened before in other large systems we have dealt with, it is the combination of e.m. and low angle X-ray which gives us the general picture of the structure. The e.m. on its

6 April 1977

own would be pretty useless, because it is only when one can get e.m. pictures "which agree with the X-ray patterns and spacings" one can trust them. Equally when one has e.m.'s of distorted or not quite regular aggregates, one has all the more got to relate these spacings to e.m.'s of regular aggregates and these in turn to the X-rays. You may not know that it took about a year for us to solve the packing of the TMV protein disks in the crystals and this was also done by a combination of X-rays and e.m. This again was a cell with an asymmetric unit of 600,000!

Yours ever,

Aaron

A. Klug

Encs.