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Dear Francis,

Thank you for your letter of 17 September, with the enclosed internal memo. The latter is very clear and it is good to have your ideas written down.

I believe I may now have an explanation of the packing in the crystals which relates to other information and ideas on chromatin structure. I would be very glad to have your opinion soon on this as I am considering mentioning these ideas in the paper on crystals (so far not beyond the draft stage!).

The unit cell dimensions are a = 110 Å, b = 193 Å, c = 340 Å, and all odd reflections are missing on the principal reciprocal lattice lines, suggesting a halving in projection onto these lines. My interpretation is as follows. Columns of nucleosomes parallel to the c axis are hexagonally packed to give the pseudo-hexagonal cell of the ab plane. Neighbouring columns are staggered by half a nucleosome to account for the halving of the 00ℓ reflections. There are three nucleosome cores in the repeat distance of 340 Å, successive particles being rotated through 120°.

How does this relate to other ideas? Along a natural nucleofilament, the successive nucleosomes must be related by translation only so that they can form a solenoid in which, as you have pointed out, all nucleosomes must be equivalent, relative to the helical axis. Consider an ideal solenoid which has the minimum amount of DNA, i.e. just sufficient to make two turns of helix, without the additional loop which you postulate as an excrescence looping around the H1 on the outside of the nucleofilament. The number of base pairs to two turns is say 170 - 180 (we need to know the minimum repeat size in different species - physarum is 171). Now what happens in a Zachau reconstitution which gives a repeat of about 140 base pairs? Presumably the nucleosomes pack more tightly into the "screwed down" position and there will now be 140/170 x 2 turns per bead. We know that the DNA does not have to be continuous for nucleosome association, so one assumes that the packing in the columns in the crystals is the same as in Zachau's reconstituted material. If there were 1 2/3 turns of DNA per 140 base pair nucleosome core, and the packing was such as to make the DNA geometrically continuous, then the 120° screw along the 340 Å axis would come out naturally

If this picture is right, then the inter-nucleosome spacing in the screwed down position is 113 Å but, in the natural position in a nucleofilament, this spacing would be increased by a factor of 6/5, i.e. to 130 Å. If one takes the Sperling and Tardieu determination of mass per unit length, indeed one finds this is the spacing of nucleosomes. Moreover, Chambon has always quoted the diameter of a nucleosome (by which I think he means inter-nucleosome spacing) in the SV40 complex as 125 - 135 Å. If this is correct, then the pitch of the DNA helix would be 130/2 = 65 Å, a value not inconsistent with the apparent helical character of the a axis photograph of the crystals (at face value here the pitch would be more like 340/6 = 58 Å, but this is dominated by the sampling). Some of the curves Linda Sperling obtained in Paris show peaks more in the 60 Å region than the 55, the latter will of course be the second order of the spacing between turns of the solenoid or columns of the crystal.

from nucleosomes

This picture is of course over-simplified. From looking at the X-ray photographs, it seems likely that the ideal structure described above is perturbed. Moreover it would be neater if the nucleosome particles contained 150 base pairs, i.e. 1 6/9 turns of a helix kinked à la Sobell. This would also give a three fold character in projection onto the ab plane. The minimum nucleofilament structure containing two turns would then have 180 base pairs. Is this all too neat?

Everybody is now back after holidays and conferences, but we miss Daniela, who will be at home with her baby for three to six months, and Barbara has been ill, so we have not made much progress in finding out just what the nature of the histone degradation is that makes the big crystals. I am pretty convinced that the packing in the micro-crystals (i.e. the hexagonal layers of which we had e.m.s and powder photographs) which are made by intact core particles, is different from that of the large crystals. The simplest explanation is that neighbouring columns pack in a register to give a sheet structure, but the packing isn't as neat so that the crystals don't grow as long.

More news. Uli Laemmli came and, by his new technique, produced some very long 300 Å fibres which John Finch has looked at in e.m. and by X-rays. The X-rays don't show anything new because, I think, the solenoids are twisted and need straightening. One assumes that in the nuclei, solenoids are not perfect, but take up various modifications as the gene is packed. However, the material is certainly a great advance, and we hope to be able to get a good estimate of the mass per unit length (we may find how to straighten it out in solution) and also use it for making oriented specimens for X-ray work. The material is fairly stable because one doesn't need to add magnesium: it seems to contain a fair number of non-histone proteins and probably represents the most intact extracted material anybody has so far obtained. We are not advertising our work on this new material because Uli Laemmli wants a breathing space to study the physical chemistry, and, likewise, we need to get the feel of the material which will take a few months, but I think we do have a way in now to higher order structures in a more natural state.

However the number of things that needs to be done is still enormous. We need to know the mass per unit length of the Zachau reconstituted material, the distance between nucleosomes in this and in natural nucleofilaments and so on, so that even dealing with the ordered structures alone is going to keep our hands full.

Len is going to write up his stuff, but he is trying to obtain some good gel patterns, all taken on the same specimen, for publication.

By the way, what do you think of this idea? It is quite striking that the DNAase I digestion pattern of the degraded nucleosome cores is so similar to that of the particles with intact histones. On the assumption that the amino acids come from the N-terminus, this would mean that the so called tails of the histones are not vital to packing the DNA onto the core - this will be done by other interactions. What then are the tails for? Presumably to enable nucleosomes to pack side by side in the contacts necessary to form solenoids. This would explain why we get a different packing in the crystals when the tails are removed. Clearly to prove the idea will be difficult, but I am thinking of ways of testing this point.

Enjoy sunny California. Here it has been raining almost every day and the grass is green and high.

Yours ever,

Aaron