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

27 January 1977

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

Dear Francis,

Thank you for your letter enclosing the preprint of the paper with Bak and Zeuthen. It reads very well and I think the way you have dealt with the final level of folding can't lead to any objections. Bak and Zeuthen are both coming to the EMBO Workshop and one of them will also speak as a discussant in the Royal Society meeting. It certainly makes a nice story but I still have my doubts as to whether the original organisation of the metaphase chromosome hasn't been drastically changed by the extraction procedure. 1M hexyleneglycol is rather severe, the times are long and the calcium concentration high. I think the paper should have included a short discussion of this possibility and referred to work on e.m. sections which don't show a lumen.

Thanks also for the news about Worcel. Since you left, one doesn't have the fresh flow of news which may stop one from getting into a rut in one's thinking. I think I understand the general idea, but I can't see where the original postulate comes from that the 190 base pair repeat leads to a straight filament.

I am going to the USA next Monday, 31 January, and will be visiting Rochester all that week. Henry Sobell asked me to give some lectures but I also hope to try to build systematic models of different types of kink, beginning by seeing what the combination of our kink and the Sobell kink lead to. In the following week I shall be at Haverford College for a few days visiting Chris Goff, two days in Boston and return to England on the evening of Friday 11 February, just in time to get ready for the Royal Society meeting.

I should say that my confidence in kinks has been diminished since Len Lutter showed me some patterns of DNAase I digests run on extended gels where the bands in the 10, 20, 30 region were so expanded that one could see clear sub-bands corresponding to changes of one base pair. The point is that the bands 10N ± 1, 10N ± 2 aren't all that much weaker than the 10N band, suggesting that the cuts can be made fairly easily on either side of the 10N bases, albeit with reduced efficiency. This would be explained naturally by continuously bent DNA and an enzyme which acts most efficiently at the two-fold position, but has a gradient of activity as the distortions from the dyad increase. Len will no doubt tell you about this next

spread might reflect "fraying" at the ends of the DNA during the original micrococcal digestion. In answer to this, Len showed me gels where long chromatin had been digested and which showed the same effect. On close examination, one of the patterns showed the 10N band less intense than the 10N ± 1 bands. So what might be happening is that these latter bands are produced by subsequent action of the DNAase after one of the 10, 20 or 30 long pieces has fallen off.

We still haven't solved the packing of the crystals and we have been much limited by supplies. We are still trying to find conditions for reproducible production of the crystals. We had some excitement when an apparently new form was produced, but it turned out to be the same crystal structure, but with a quite different habit. I was, however, encouraged to see that, although the crystals were even smaller than before, the pattern went out somewhat further so that the disorder beyond 20 Å may only be due to the small size of the crystals. What is new is that we have calculated the 3D Patterson to a resolution of 25 Å and the section at Z = ½, i.e. along the 340 Å axis, doesn't show many peaks compared with other sections. It thus seems, as I have suspected for some time, that there isn't a true screw axis parallel to Z and that the halvings in that direction are low order packing effects.

John Finch has been taking electron micrographs of crystals, crystal fragments, etc. to try to see if we can correlate them with the X-ray patterns. Although he has some very nice pictures, the end effect is to leave one more bewildered than ever. The only thing one can be reasonably sure of is that the nucleosome has a strong division into two parts. However it is certainly not 'semi-somes" that we have crystalised because the DNAase I digestion gives a standard pattern.

We have tried to measure the density several times and the best answer we get is somewhere between 8 and 14! I am aware of the point you passed on from Lubert Stryer that one should look not for equilibrium in a flotation method but for a pause in the sinking curve (indeed we have done this before).

Other news. Vaughn Jackson continues what he began. He gave a talk the other day at one of our chromatin meetings (which are much rarer nowadays - I am so busy that I don't always call them) and he won't move off formaldehyde cross-linking. Ron Laskey has been continuing the search for the assembly intermediate (Barry Honda is now helping him) but recently seems to have come across a different phenomenon. He tried to isolate the relaxing activity on sucrose gradients and found a beautiful sharp 11S peak. This turns out not to require histones at all and he believes it may have something to do with unwinding plus nicking and cutting activity. He therefore may have stumbled on something to do with replication. However there is a broader peak which may contain a partially assembled histone intermediate and he is now following that up.

Jean Thomas has at last got some reconstitution experiments to work and certainly the filaments made without H1 have a roughly 140 base pair repeat, thus confirming Zachau, but they don't look any straighter than natural nucleofilaments. The experiments with reconstitution including H1 are being done now.

Thats all for now. Perhaps we can speak on the phone when I am in Rochester.

Yours ever,

Aaron

A. Klug