

11 March 1977

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Dear Francis,

Thank you for your letters of 17 January and 23 February. I am sorry I have not written earlier, but I am only now beginning to surface after my visit to the States and the meetings in London.

Firstly, about your note on Len's work. I have discussed the various points you raised in detail with Len and he is going to do the controls you mention. He is away for two weeks and will be doing them immediately on his return. In the meantime, we do not understand your statement that the removal of nicks by the enzyme might fuzz his result. In any case he plans to repeat the experiment in which the nicks have been earlier sealed with ligase. As for the point about a single band moving down in toto, he cannot of course detect a small level of upward movement but he is pretty confident that almost all does move down.

I have already written about your paper with Bak and Zeuthen. Bak described the matter quite clearly at the Royal Society, but did put forward their picture of a double helix for the final level of folding. Callan answered him firmly but politely that he could not believe that the telomere turns back and tucks into the centromere. Zeuthen spoke at the EMBO meeting where there was much more time and he was more cautious in his approach on this point. Indeed I found Bak rather fixed in his ideas and Zeuthen altogether more open to argument, and I think he is the brighter man. I had raised the question in public as to whether the opening up in 1 M hexyleneglycol could be an artefact, but only later, in private conversation, did Bak say that they had observed the phenomenon in other buffers. On the whole the response to the supersolenoid was one of caution and I think that if your name hadn't been attached to it, it would probably have been disbelieved. Someone at the back at the Royal Society meeting raised the relationship of Bak and Zeuthen's work to that of Stubblefield and Wray, presumably the same point about which I wrote to you on 28 January. Bak's answer wasn't at all clear.

The meetings themselves went quite well. At the Royal Society Discussion there must have been 400-500 people present on both days, with younger people sitting in the aisles, on the platform and perched all around. Zachau had nothing new, but Chambon presented some

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results on reconstitution with H3 and H4 only. This leads to particles of about 75-80 Å diameter (compared with 95 Å for semisomes) and they seem able to organise 120-130 base pairs on their own, indeed rather like the results claimed by Felsenfeld in another type of experiment. I saw Felsenfeld briefly in Boston who told me they had yet another paper in press on this subject. Varshavsky turned out to be very impressive, although in his talk he showed so many gel patterns, mostly unlabelled, that it became very difficult to follow. However, he came to the Lab. the following week with Engelhardt and gave a much clearer seminar. He spoke mostly about three different types of monomers and the sub-nucleosomal fractions. There is a wealth of detail and, rather than summarise it all, I am sending you a copy of his manuscript. I must say I don't know what conclusions one can draw from it but there are some rather interesting associations of non-histone proteins with some of the fractions. One generalisation is that the smaller fragments of DNA seem to be associated with about one histone each. Varshavsky speaks very good English and was altogether very impressive. I am glad we invited him but I rather fear that he may run into trouble in the future since he is rather outspoken about conditions there and his own situation (Having blurted quite a lot out over lunch here as though he were impelled to do so, He then asked us not to repeat anything in case the word got back.) He thinks he may not be able to come to the Cold Spring Harbor meeting because both Georgiev and Mirzabekov will be going. I am writing a letter of thanks to Engelhardt in which I will end by saying I hope to see Varshavsky at CSH.

Roger gave an excellent talk. What he showed was there was no phasing, but the most striking result came out as a by-product. He uses a combination of exonuclease 3 (which only removes one strand of double stranded DNA) plus S1 (which only acts on single stranded DNA) to trim monomers and dimers down to a limit in separate experiments. The monomer band sharpens up beautifully and this is the control. The dimer does not, so the conclusion is that there is a variable length of DNA between the two <sup>adjacent</sup> nucleosomes of the dimer. I regard this result as conclusive, and Roger pointed out that this explained why phasing would in any case be impossible, although in the discussion people raised the question as to whether the phasing could be restored in the next higher level of structure, say at the dodecamer level.

I suppose one could invent other explanations such as the two free ends of a DNA on the dimer coming close together and therefore inhibiting trimming, but I prefer Roger's simple explanation. The rest of the meeting went very well I thought. Many of the people had already been to two earlier meetings on the subject of DNA sequences (the Harden Conference last September and one in Switzerland in January) but, to most of the audience, a lot of the results were new and very well presented. Hogness' work in particular was spectacular but I assume you know all about that. There was a lot of discussion as to whether beads are present on the DNA during transcription and Franke was adamant on the basis of e.m. evidence that they are not. Laird was guarded. The situation wasn't made any clearer by Joel Gottesfeld's paper. He spoke very clearly and I must say I really can't see what is wrong with the experiments now that he

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experiment seems to be that he can convert the 14S particle to the 11S particle in the same tube by treatment with RNase. He is writing this up for Nucleic Acids Research and I will make sure that you get a copy so that, even if it all turns out to be wrong, it won't be obvious at this stage.

I think the hit of the meeting was Ashburner who substituted for Brian Clarke who was ill. Ashburner went into a very detailed analysis of the zeste white region of drosophila and pointed out that there are strong reasons to dispute the one gene-one band hypothesis. He points out that tests for saturation would be difficult or invalid (since the phenomenon didn't obey poisson statistics), that George Lefebvre had shown that many mutations were not without macroscopic phenotype, that there was at least one band which produced three enzymes and also (adding that he could practically hear you calling from California) that the largest bands were probably too large for a single gene and that they were unlikely to contain special bands. I must say that I hope there isn't a straw man involved here. He gave the impression that the one gene-one band hypothesis was so sloppy as not to be worthwhile, but surely one can modify it to one band-one set of genes operating together et sim. However I must say that looking at some of Hogness' results there doesn't seem to be a lot of spare DNA in the genes he has looked at, which are now 18S + 28S RNA, the histone genes and heat shock genes. *FHCC; space between histone genes about twice as long as coding sequences*

Worcel gave a good talk and essentially described his model which you had already alerted me to. It is rather clever but I am beginning to think that clever conjectures by bright people, without any supporting evidence, are rapidly becoming the ban~~e~~ of the chromatin field. What Worcel didn't talk about was the parallel between bacteria and drosophila but I tried to make this clear in some remarks I made.

At the EMBO Workshop, most of the stuff was structural, and the only new thing was a model by Pardon and Richards who presented a model to fit the neutron and scattering data in solution. This was a flat disk of about 50 Å thick by 110 Å in diameter with the DNA right on the outside, two turns, about 30 Å apart. It may not be such nonsense as some of the audience thought because people are fixed on the idea of a spherical bead. The Workshop itself was not a great success because there were so many wanting to speak that I simply decided to allow them 10 to 15 minutes each. There was a good thing by Keller (I enclose a copy of his abstract). Bruce Ponder also described his work. This was the first time I had followed it in detail. I found it amazing that he got the same pattern of eco R1 bands whichever cut of the original monomer material he took, i.e. the 140-170 base pair region or 170-200. Since one gets discrete bands in the eco R1 digestion, one seems forced to conclude that there is a correlation between the alternative positions of the eco R1 site and the alternative lengths of the micrococcal nuclease digestion products.

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That's all for now. Sydney and Max have told me about your plans and although we haven't talked about it the word seems to have got around.

I will write soon about progress with the crystals. We now have some evidence that the packing I described to you in my letters of September and October is correct but we don't know what the units are that are being packed, either nucleosomes or semi-nucleosomes. I hope to prepare an internal memo on the subject and will send you a copy. In the meantime, we are trying to grow bigger crystals to measure the density, of which we only have limits.

Yours ever,

A. Klug

Encs.