October 4, 1977

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Dear Aaron,

This is the letter I promised you about Bak and Zeuthen. I need not discuss their personalities as you have already met them. Bak has a research student (medically qualified) also called Bak, but no relation. He is the one who actually makes all measurements -- he said he sees unit fibre in his dreams!

Talking with them and looking at their slides soon revealed a number of things which my previous extensive correspondance had not uncovered. In the first place, although the yield of chromosomes is high, they are a mixture of very different stages of condensation. This is because the colcemid is applied for a very long time (19 hours?), so that some of the mitotic chromosomes have seen it only for a short time and others for a long time, depending on when that cell entered mitosis. Thus then the chromosomes tend to stay together in groups, presumably because parts of the spindle are still there. However the really bad feature is the extremely low yield of unit fibres (perhaps 1%); the rest of the material being in irregular clumps. These are produced by the fixative (methanol-acetic acid). It looks to me as if there is a race between unfolding (due to pH?) and fixation.

I told them that it was essential that their yields should be higher; at least 50% and hopefully 90% or greater. I told them to try acid buffers of different pH. I also suggested that they attempt to disperse the chromosomes somewhat, before fixation, by a cold treatment in the hope it might depolymerize the microtubules. Also I suggest that they try (there are tricks to do this) to get the mitotic chromosomes more uniform before they start and to vary conditions to see if the less condensed chromosomes unfolded more, or less, easily than the more condensed ones.

If they found that a pH of, say, 5-0 would unfold the chromosomes to give unit fibres then they might try other fixatives. Also they could try the effects of various salts, etc.

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I told them it was useless to pursue e/m work (except to try out methods) until they had a pure preparation of fibre? For this they should certainly get the yields up and even then might have to try fractionation (to remove unfolded or clumped chromosomes) perhaps using a sucrose gradient.

In short, my message was to stop measuring and instead to try lots of things. Also to work with smaller samples so that they could do many quick experiments. Also to work briefly to explore conditions and only do careful measurements when the conditions looked promising. The younger Bak liked this idea because I think he was sick of measuring! Zeuthen got the various points and I think will act on them, though my impression was that the elder Bak didn't see what all the fuss was about.

Anyway, it was a lesson to me not to write papers with people you've never met! It never occurred to me to ask what their yields were. The point is so elementary.

Incidentally the "doubleness" in the microscope means very little if anything. As you go out of focus the image broadens as it appears double. I suspect almost anything cylindrical will do this. In spite of all the above, I think it's worth their while to try to clear it all up. A major question is whether a unit fibre comes from one chromatid or a pair of chromatids (a metaphase chromosome consists of a pair of chromatids joined only at the centromere). One way to clear this up is to measure the mass/length or DNA/length, but these measurements are very difficult to do accurately. I was not impressed by Zeuthen's preliminary measurements. A better way is to watch the formation of the unit fibre under the microscope and, eventually, make a video tape of the process.

I am trying to arrange for Dan Lindsley, of UCSD, to send them a Drosophila strain with a translocation to make the three major chromosomes of very different lengths. This should produce unit fibres of three clearly distinct size classes. Of course if they really can produce unit fibres in good yield, there is no reason why they shouldn't try a lot of other interesting species, to say nothing of doing good e/m work.

I've just spoken to Michael Levitt. He seems cheerful but time-shifted. My only other news is ~~nice~~ nice: I've been on a diet -- I've lost 12 lbs! Apparently I need only lose another 4-5 lbs. Then I can buy some new trousers.

F. H. C. Crick
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