CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

8. November, 1957

Dear Jim,

A transfer experiment with bacterial DNA has been completed. E.Coli was grown from 10^4 to 10^8 cells per ml in N^{15} M9. The generation time was 45 minutes in this medium and was the same in a parallel N^{14} culture. Upon reaching 10^8, a 20-fold excess of N^{14} was added to the N^{15} culture along with adenosine and uridine. Samples of bacteria were withdrawn just before the change of medium and afterwards for two generation times. The generation time, as measured both by colony formation assays and direct particle counts remained constant at 45 minutes. The bacterial samples were chilled and centrifuged immediately upon their withdrawal. The sedimented cells were resuspended in versene, treated with lysozyme and then duponol and placed in the refrigerator. This treatment yields a clear lysate which is added to CsCl and centrifuged. Nothing is thrown out. DNA bands of three discrete densities were found in the various samples as shown in the following table. Times are given in units of one division time.

	fraction of total DNA of each type		
time	heavy	intermediate	light
0	1	0	0
0.28	0.7	0.3	0
0.71	0.2	0.8	0
1.14	0	0.9	0.1
1.57	0	0.7	0.3
2.00	0	0.5	0.5

Clean as a whistle! Who would have imagined that, with all the other great good luck we've had, the DNA molecules would replicate all at the same rate?

The new photometer which is being airmailed from Britain has not yet arrived and accordingly the films have been judged by eye, not photometered. Our old machine is too much trouble to fire up. The bands all seem to have the same width and the separations are clean looking. The intermediate band seems to be just in the middle.

I was all set to send you a collection of verses the overall mood of which is set by the lines

"Now N^{15} by heavy trickery
Ends the sway of Watson-Crickery..."

But now we have WC with a mighty vengeance...or else a diabolical camouflage. Formally, what the centrifuge says is that the nitrogen of the units which form a band is divided equally*, between two sub units and that upon replication the sub-units

* so the thymines are not highly concentrated in one sub-unit.

separate from each other and become associated with new sub-units built from nitrogen quite recently present in the medium. Sub units are conserved. Note that this is exactly analogous to the formal implication of Taylors experiment.

The units which form a band have a molecular weight of about 8 million and a frictional constant related to their MW as would be expected from centrifuge experiments with phage, calf, and other DNA samples. Also, these units have the same behavior upon denaturation as phage and salmon DNA. Nevertheless this does not prove that they are single DNA duplexes. Until the photometer arrives I can't say whether these units are monodisperse.

Aside from the question of the nature of the units themselves, we are also ignorant of the nature of the sub-units. Even if the units are WC duplexes, the sub-units need not be single strands.

In all of this the temptation is great to believe in pure, simple WC explanations but we'll try to determine more definitely the identity of the units and sub-units. I'll write to you later of how we will attempt this. Please give it some thought and send along any ideas you come up with.

As well as can be told without photometry, we are getting all the DNA of the cells to show up in the bands. Certainly it's unlikely that any more than half is hiding at the top or bottom of the centrifuge cell. This estimate is based on knowledge of the number of Coli cells present in the spinco cell.

If we assume that the centrifuge experiment would have the same outcome if it had been done with the DNA from Vicia Faba, then it is necessary that one sub-unit of the centrifuge unit be associated with one sub-unit of the Taylor unit. This model of the chromosome includes Taylors proposal

as well as others such as

and

In Taylors model there are two sorts of attachment of the DNA to the Taylor unit: a 3' and a 5' attachment. In the right-hand lower model both attachments are the same. Also, in this model, new material might be laid down on each strand always in the same chemical direction, say the 3' to 5' direction. The two-fold rotational symetry of the molecule would then be conserved even during replication. A"disadvantage" might be that, with this kind of symetrical replication, ~~up/up~~ half of the molecule would be single strand ~~//////////////~~ when replication is half completed/ Maybe the strands could nevertheless be kept from collapsing into an undesireable configuration by some accessory structure. The unwinding of the DNA in the symetrical chromosome might be made difficult by the requirement that, if the molecules remain attached to the Taylor units, uncoiling requires simultaneous breakage of all the inter-chain hydrogen bonds. Taylors' own model still allows residue-by-residue breakage. I wonder if the synthesis of new

DNA might not take place without unwinding--some four stranded creature being an intermediate. Then the Taylor units might comprise some sort of machine for unwinding these. We shall try to look at DNA just after DNA doubling in synchronized cultures in hopes of finding interesting intermediates. We will survey the entire density spectrum so as not to miss interesting DNA-RNA-protein complexes. N^{15} of course makes possible transfer experiments for nearly any celular componant which will band. Already we have found some interesting "mystery bands" which seem to be very large but homogeneous nucleoprotein structures. Do you have any ideas for preventing nucleo-protein dissassociation in the CsCl. We will try adding Mg^{++}, keeping the pH low and using low temperatures and fresh samples.

The questions regarding organization of DNA molecules into larger structures perhaps of importance in ordering the genetic determinants and making recombination possible are the ones that interest us most just now. I think a good program in this area, in addition to the above experiments with cell lysates, would be to characterize the DNA of lambda hoping to find more than one piece. Lambda can be banded in CsCl very beautifully. If there is more than one piece of DNA in the virus, then N^{15} transfer experiments with whole phage will tell if these molecules remain associated during their biological career. If they do, we can perhaps find gentle enough preparative methods (injection on bacterial membranes) to obtain these interesting aggregates. We might then find how they are build. If the molecules come apart during their careers, we have the puzzle of keeping order on the genetic map unless each piece or one piece is a complete (known) genome. A result very interesting in itself and which will provide a very good handle to the problems of DNA organization is that wheras lambda gives one sharp band, lambda plus lambda Gal gives two!! I think T1 should be as good a material for asking these questions--I hope you are having good luck with it. Please keep us posted on your results. Have you a nice way of getting out the DNA? We'll try pyrophosphate, duponol, urea, and injection with lambda.

We have taken some very nice pictures of TMV strains provided by UCLA which give symetrical bands. Helgas virus gives a skewed band. When photometer arrives, we'll measure MW's. I reccommend using whole unpurified leaf juice in the CsCl method. There is no great trouble with materials of nearby density.

Paul has offered me an instructorship at Harvard next year as you no doubt know. I'm writing him that I simply don't think I could keep up the momentum in research if there was also teaching to be done. I think I should stay here in Pasadena (alas) another year at least. What do you think?

Give by best to Helga, Paul, and the Litts. I'll be in New York over Christmas with a flute player I met and liked in Aspen last year. Is there a chance you might come down?

As ever,

Matt