October 5, 1962

Dr. S. E. Luria
Massachusetts Institute of Technology
Department of Biology
Cambridge 39, Massachusetts

Dear Lu and Naomi:

I hope you will pardon the delay but I have been so rushed that I did not get the time really to get the necessary tracings done so that you could get an idea of the data. It turned out that verifax copies of our results as we plot them was too confusing. We, therefore made tracings of the data so that you could see them. I have given you only some of the experiments and representative runs. Figures 1a and 1b illustrate the kind of discrepancy one observes as a result of induction of an ordinary wild type. They represent Hershey columns of mixed RNA from cells treated according to the diagrammatic protocol on the top of each. In all cases the dotted lines represents optical density profile and represents the bulk of the RNA components. The green is always the tritiated preparation and the red is the C^{14}. The gradient is indicated by a dashed pencil line. 1a is a control run in which neither one was induced. You see that there is good concordance between the two profiles. In figure 1b the tritiated preparation came from a cell induced. The C^{14} was uninduced. You will notice then two discordant peaks in the tritium profile. Another control is given in figure 2 in which a lac deletion mutant was induced and not induced. Here induction had no effect.

These results are completely reproducible and have been obtained many times. We have a fairly detailed story on the kinetics of the appearance of these discordancies during the induction of a wild type. The regions of interest in figure 1 were arbitrarily designated and the fractions within each pooled. Figures 2a, b, and c represent cesium chloride gradient runs of hybridizing experiments carried out with a P^1 carrying lac. It is clear the region two contains the most hybridizable material. The amounts of counts put in each were approximately the same. Here, as in all other cases, we are only looking at the RNA resistant counts. 3a and 3b represent two types of controls. Here the same region from a labeled induced deletion negative shows no ability to hybridize with the P1 carrying lac. 3b shows that the same region in the gradient taken from an uninduced wild type inducible shows a faintly detectable possible hybrid. It is clear from comparison with 3a and 3b that induction leads to the onset of transcription. Figures 4a and 4b represent the other types of controls in which region 2 again was mated with the P1 DNA not carrying lac and there is virtually no detectable hybrid. 4b checks the same thing with RNA from a non-induced wild type again from the same region. Again, there is no hybrid.

I might say that we have also looked at other regions of figure 1b for hybridizible RNA with the P^1 carrying lac and the results have been negative. We are now running a few matings with unfractionated RNA as a final check. Also there are a few other repeat controls which are spinning now and if everything checks out I think we can regard the story as established.

With kindest regards,

Sincerely yours,

S. Spiegelman
Professor of Microbiology

SS:rn