

4th November 1966.

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Gobind,
Dear ~~Khorana~~,

We were delighted to receive your manuscript. I have read it fairly carefully but I have not yet had time to ponder over it. However there are a few points that I should mention to you. I quite agree that the slight incorporation you get of, for example serine and valine with Poly r-UAC is likely to be due to the formation of some Poly r-GUA as you suggest. However, I think it would be much more satisfactory if you could show this in a positive way. Do you think that a much shorter incorporation, say of ten minutes, might remove this spurious effect. It is, for example, a little peculiar that the ratio of valine to serine that you get with Poly r-GUA is about 80:1, whereas the ratio using Poly r-UAC appears to be about 5:1 or even smaller.

The second point that attracted my attention was the very unequal amount you get of the three different amino acids from some of your Repeating Trinucleotide Sequences. Did you notice the fact that the triplets which predominate are those beginning with an A or a G? For example, for Poly r-UAC the incorporation of threonine (ACU) is much greater than that of leucine (CUA) or tyrosine (UAC). The same remarks apply to Poly r-AUC. The most plausible explanation for this is the one you give at the bottom of page 7, namely that the initial base is not random. As I am sure you know natural messenger is supposed to begin mainly with A or G. What you may be observing, therefore, is the selective action of the RNA polymerase. I think it would be very interesting to study this and well worth while to study the end groups of the RNA you were making. As you will see ~~the amber~~ may be important when you come to consider the effects of Poly r-GAU. The fact that you appear to get no amino acid corresponding to UAG might therefore be explained by postulating that all your RNA molecules begin with A or G and never with U. As you will see from what I say below I do not actually think this is the reason but

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I think you ought to check the end group to make sure.

I am not quite sure I understand the point of looking for poly-aspartic acid in the way you do. Is this because polyaspartic acid cannot be assayed by acid insolubility? You seem to imply this on page 10. The reason I ask is that you claim in your footnote that Poly r-AAG, which stimulates the binding of aspartyl-tRNA, does not incorporate aspartic acid during polypeptide synthesis. Was this checked using the chromatographic characterization.

My final point concerns your remarks on page 11 about assignment of AGA for arginine. I do not think the argument as you give it is logically correct. On the evidence that you quote AGA could just as well be glutamic acid. As far as I can see it is not at the moment possible to allocate the single codon using your results alone, although one can make certain deductions, for example AGA cannot be lysine.

I notice you quote the results for your two Repeating Tetra Nucleotide Sequences. Have you yet been able to find the exact amino acid in either of these cases?

Ugurena

Now I must tell you about some rather dramatic developments which have taken place in the last two weeks concerning the third nonsense triplet which we had uncovered by our genetic work on the rII region. You may recall that we had four barriers and one mutant (X655) which were neither ochre nor amber. We thought they were not UGA because they did not appear to revert to ochre. While we were assembling the data in the final stages of preparing our paper we noticed that this had not actually been checked for X655. When Leslie Barnett did the experiment we were astonished and delighted to find that it did indeed revert to ochre. We therefore went back and checked more carefully the reversion of the four barriers. We have now shown quite unmistakably that three of these revert to ochre with 2-aminopurine. The fourth barrier is at the extreme right of our region and it may be that our suppressors do not put in an acceptable amino acid here. Preliminary work with hydroxylamine suggests that these mutants which we call opal ^{9.} give two ochre in such a way that the base change must be G to A, but the ~~triplets~~ ^{triplets} are very poor and we cannot yet be certain of these. All this makes us confident that opal mutants are UGA and not AUA or UUA as we suspected earlier. We are now very actively engaged in trying to decide if UGA chain terminates. The arguments are rather complicated and I think I had better wait to tell you about them until the experiments have been done. At the moment the most likely hypothesis is that UGA is a triplet for which there is no tRNA. We are also looking at the β -galactosidase gene to see if we can find opal mutants there too. In

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the light of your results on Poly UGA I certainly expect that it will turn out to be nonsense in the uninfected cell as well as in the infected cell. I will let you know as soon as we have anything definite. In the mean time I think it would not be a good idea for you to bother about codons for T4 phage infected cells.

I am very sorry that we have misled you about opal mutants in this way. As you know ochre suppression is very poor and this is basically the reason why the reversion of opals to ochres was missed when the experiments were first done. Even now we can only be sure that we have got an ochre when we have taken the further step of converting it into an amber.

It is still possible that UGA chain terminates. If we could show that it does not do this we shall have to try and think of some other function for it. It cannot be anything to do with the starting or stopping of messenger RNA because it is only nonsense if it is read in phase. We are trying to think of some reason why one triplet has to be left without any meaning, but at the moment we have failed to come up with a good idea.

Sydney is in the States at the moment and told me that he planned to ring you up some time during his visit. Unfortunately he did not say when. It may be that you will have heard all this already direct from him. He had a number of ideas he wanted to suggest to you but I will leave these for him to talk to you about. Of course it would make life much easier if we could find a suppressor for UGA. We are trying to do this but of course it may be that any suppressor would be lethal.

John Smith asked me to tell you that he had not forgotten about the tRNA from the suppressor strain which he was going to send you. It is not quite ready but he will send it as soon as it is. We will also send you a copy of our genetic manuscript but it will be posted separately as we shall have to get another one xeroxed.

I have been spending part of my time thinking about the origin of the genetic code. I feel I understand the problem a little better now but have not come up with anything world shaking yet.

With best wishes,

F.H.C. Crick.