29th April, 1965.

Dr. H. Gobind Khorana,
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Dear Gobind,

Very pleased to get your letter and to have the details, especially about poly AG. I am however very puzzled by your results with aspartic. I think it is most important to see whether any aspartic is incorporated. My general impression, both from your results and from Nirenberg's, is that binding studies by themselves, can be very misleading. The only safe way is to test each trinucleotide against all 20 amino acids. It is often found that a triplet binds one amino acid fairly well, but others less well; the latter are usually artefacts. These errors are increased by high Mg and low temperature. Moreover, there are triplets which we believe to be authentic, which bind very poorly. Thus in your table I shall disregard all assignments based on binding alone.

After all, there is no reason why the binding test using trinucleotides should work at all, since it does not exactly correspond to what occurs in protein synthesis. For this reason I attach much more importance to incorporation. However, I am not clear why binding to the polymer should be misleading, and very puzzled that arginine S-RNA binds so poorly, and why aspartic S-RNA binds at all, to poly AAG. This is why I think it important to be sure no aspartic is incorporated.

I am still very pussled about the poly AAG results. If it were not for your results with poly AG I would not accept AGA for arginine. Incidentally are you certain that poly AG incorporates no aspartic?

It's always worth remembering that you may have a strain with a suppressor in it, and that eventually the result should be checked using a different strain. If you had a "suppressor" which was misreading in the third place it would explain your results. However such a strain might incorporate some aspN with poly A. Could you check this? It might also incorporate some ser using poly AAG.

On other matters. I have heard from Jacques Fresco. His results are more or less what one would expect; only a small amount of tryp is incorporated and this may well be because the repeating sequence is not exact. His results suggest that AUA is Ileu. However this could be a mistaken sequence, such as AUU. It could be checked by taking a rigorously repeating poly AU and reacting it (when melted) to turn some A into I, not 90% as Fresco did, but only, say, 30% - just enough to destroy the secondary structure. Then if Ileu were incorporated we should know that AUA was Ileu. Could you try this?

Now as to tri- and tetra-sequences, especially in relation to ochres and ambers. We should very much like to have

poly UAA

and poly UAG (or poly UAI)

(N.B. of course poly UAA is near enough the same as poly AAU or poly AUA - I won't repeat this remark in what follows)

because we could use them to test the mechanism of suppression. I have written out all possible repeating tri- and tetra-sequences, and enclose copies of my notes (please check for slips). The best tetra's would appear to be:

poly UAAA

poly UAAC

poly UAAG

poly UAGA

poly UAGU

However I have grave doubts that you will be able to make accurate repeating tetra's by your present methods, and suggest you try poly UAA and poly UAG first.

There have been no further developments here about suppressors, except that they have shown that Yanofsky's strain of E. coli has an amber suppressor in it which puts in Tyr.

About the Gordon Conference. Many thanks for the promise of the money. I enclose a copy of a letter to Jim and Marshall, which explains itself. I do hope that Yanofsky can come to the Gordon Conference. In addition you should know that Streisinger and his colleagues have the following results for phage lysozyme.

Wild type:

Double mutant:

Using the latest version of the code (copy enclosed - it incorporates their results) you will easily be able to show that there is only one solution, and that reading is from 5' to 3', as Ochoa claims. Their result also gives us 5 new codons. Do you think that Streisinger, or one of his people, could be squeezed into the Gordon Conference?

Finally I should mention that Zachau now has for the serine S-RNA (from yeast) the sequence ... pUpUpIpGpApUp ... which is the anti-codon I predicted for the pair of codons UCU and UCC. I thus believe that the anti-codon for phe is IAA (or GAA) - not AAA - and for tyr it is IUA (or GUA)

F. H. C. Crick