Molecular Biology Through Discovery
Companion to Crick et al (1961) *General Nature of the Genetic Code…*
Nature 192:1227-1232

I. Introduction

By 1960, seven years after publication of the structure of DNA, efforts to determine the nature of the genetic code were at an impasse. Sydney Brenner had demonstrated that any overlapping triplet code would impose restrictions on amino acid order that are not observed in actual proteins.[1] There seemed little more that theory could contribute, and it was not at all clear what kind of experiment could lead to the next insight. This might seem strange to you – why not just compare the sequences of genes and proteins and figure out how to get from one to the other? The problem with this idea was that the sequencing of proteins was tedious and the practical sequencing of nucleic acids was 17 years in the future. However, as you will recall, Seymour Benzer had developed phage genetics to the point where it was capable of distinguishing mutation sites at nearly adjacent nucleotides. Perhaps genetics could be used to obtain a DNA sequence. So thought Brenner.[2]

Sydney Brenner came from South Africa to Oxford University as a graduate student. After completing a PhD thesis on phage mutagenesis and a short stint back home, he was brought to the MRC. There he shared an office with Francis Crick (**Fig. 1**), the same office Watson and Crick had thrashed out details of DNA structure a few years earlier. In both cases, the inhabitants shared not only the office but an inclination to work out their ideas in conversation through intense, daily discussions. One idea on Brenner's mind was to mutagenize phage T4 with base analogues, chemicals that looked like and would substitute for one of the four natural bases of DNA, in the hopes that they would generate mutants with predictable changes (e.g. only G's would be mutated). Then by mapping these mutations in a specific gene (e.g. rII), one could deduce the DNA sequence of that gene. The range and properties of the mutants proved to be very confusing, and Brenner had the idea (drawn from his days in South Africa working in cytology) of using a different sort of mutagen, the DNA-binding dye, proflavine (an acridine).

While Brenner was playing with phage mutagenesis, Crick and Leslie Orgel were toying with a new kind of genetic code, hoping to address the problem of marking what portion of an RNA encoded a protein. The idea was stimulated by the finding that single-stranded RNA could fold back on itself, leading to partially double-stranded regions.[3] Perhaps the folding itself was important in the information carried by the RNA. This had the important implication shown in **Fig. 2**: a mutation that disrupted a base-paired region (and by hypothesis prevented translation of the RNA) might be counteracted by a second mutation – a suppressor mutation – that restored the base-pairing. Suppressor mutations had already been described in phage T4, but this loop theory made the surprising prediction that the order of mutations would run opposite to the order of their suppressors (a*-b*-c*-d*-e*… e'*-d'*-c'*-d'*-a'*). Said Crick a decade later "*I got so excited about this that I thought 'I'll do the experimental work myself*'".[4] This was his only sustained effort in experimental molecular biology, and he made it count.

Figure 1: Sydney Brenner and Francis Crick. Courtesy of MRC Lab of Molecular Biol Archives.

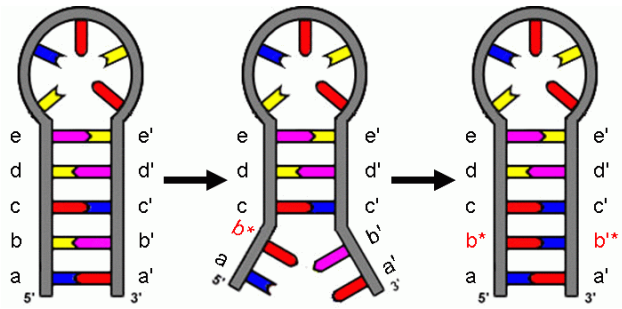

Figure 2: Suppressor mutation rescues hairpin structure. RNA hairpin is stabilized by five base pairs: a-a' – e-e'. A mutation at one of the sites disrupts the hairpin. A second mutation restores the hairpin.

…but not in the way he expected. Crick could not find intelligible suppressor mutations using mutants generated by base-analogues and so he turned to mutants generated by his office-mate's preferred mutagen, acridines.

Acridine-generated mutations turned out to be quite different from others. Most mutagens generated a spectrum of *rII* mutants. Some were tight – showing no plaques at all when the mutant T4 was plated on *E. coli* K12 – but many were leaky – showing small plaques. Mutations generated by most mutagens were revertible, that is at some low frequency they spontaneously became able to infect K12. Acridine-generated mutants, however, were invariably tight and almost never reverted. Many mutagens used at the time became incorporated into the mutated DNA. Acridines do not become an integral part of the DNA.[5]

Why were acridines so different? At the time, mutagenesis – whether spontaneous or chemically induced – was considered to result in a change in the DNA sequence, a substitution of a wrong nucleotide for a correct one. Brenner had the insight that the characteristics of acridine-induced mutants could be explained if the mutagen worked by a different mechanism, producing a gain or loss of a nucleotide.[6]

We now know that he was right. Acridines act by intercalating within the stack of bases of the double helix. If the DNA is in the act of replicating, then if it intercalates into the template strand, then an insertion results, and if it intercalates into the new strand, a deletion results (**Fig. 3**; the actual mechanism is considerably more complex than shown[7]). You may know of another compound that intercalates into DNA, ethidium bromide, a fluorescent compound that binds to DNA and permits its visualization after electrophoresis.

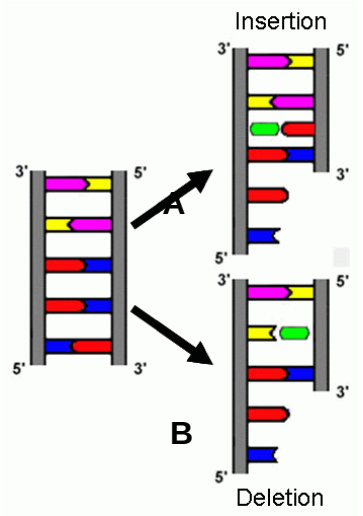

Figure 3. Deletion and insertion induced by an acridine. (**A**) During DNA replication, acridine (green) lodges itself between bases of the template strand. The growing strand encountering an acridine as the template base inserts an arbitrary base across from it. (**B**) Alternatively, if the acridine intercalates into the growing strand, opposite a base in the template, the complement of that base does not appear in the new DNA.

SQ1. Fig. 2 seems to imply that a suppressor of a mutation at site b could occur only at one site. Why couldn't it occur elsewhere?

SQ2. In what sense might the order of sites of mutations and sites of suppressor mutations be considered a palindrome?

SQ3. Why does mutagenesis by acridine not occur in the absence of DNA replication?

SQ4. Using your current knowledge of molecular biology, why would acridine-generated mutations be expected always to be tight?

SQ5. Acridine-generated mutants almost never revert, but apparent reversion is common when such a mutant is exposed again to acridine. What does this imply?

So Crick looked for repressors of acridine-induced mutations in the *rII* gene of phage T4. If the loop-dependent genetic code (**Fig. 2**) were true, he might expect to find one suppressor mutation. Instead he found many. The loop code was immediately forgotten, because Crick and Brenner saw in the result and the proposed explanation for it an opportunity to test for the first time what is the length of codons.

This is where Crick et al (1961),[8] the primary focus of this companion, ***begins*** – not at loops and at palindromic repressor sites but a consideration of triplet codons. Nor will you find any discussion of loop codes in the article Crick wrote to explain the experiment to the general public.[9] That's the way it often is, particularly with research articles. They do not purport to describe the tortuous history that led to the new

insight but rather to present a new picture of nature and what evidence bears on that question. The rest is for the historians. So don't be fooled by research articles. They're not about the process of science. In fact, as often as not, you come to the dance with one idea and leave with another, and if that doesn't happen, you're probably working in a boring area where surprises never happen, or you're blinding yourself to the surprises nature throws at you in favor of the fixed concepts of your own mind.

Time to go to the article.

II. The article

Crick et al (1961)[8] is a typical Crick article, rich in big ideas artfully put together but poor in experimental detail. You may gain from it the logic leading to their conclusions, but you'll have to go elsewhere to find insight into the nuts and bolts of how the experiment was done. One place to go is Crick (1962).[9] There you'll find a good description of the principle behind the experiment. Another place to look is Benzer (1962),[10] where you'll get an overview (or perhaps review) of the T4 system. The companion[11] to Benzer's 1959 article is another possible resource. Perhaps the best way of understanding what experiment was done is to do the experiment yourself. You can, through the simulation[12] provided along with this companion.

II.A. Introduction

The introduction lists four conclusions Crick et al draw regarding the genetic code. Three of these are addressed by experiments described in the article. The fourth, the nonoverlapping nature of the code, relies on prior work.

SQ6. Why are the results of Whittmann and Tsugita and Fraenkel-Conrat described in the introduction incompatible with an overlapping genetic code?

II.B. Experimental Results

SQ7. Are you able to explain how the T4 *rII* system can be used to find new mutants defective in *rII* and to find new mutants or recombinants that have regained the wild-type phenotype? (if not, then see resources above)

SQ8. What is the "B1 segement" introduced in the fourth paragraph of the results? If you don't know, consult Crick (1962).[9]

SQ9. Use the simulation[12] to do the experiment described by Crick et al to obtain suppressor mutants of FC0 and to separate the suppressor mutations from the FC0 mutation.

SQ10. What simple cross can you perform to determine whether the separated mutation is a new suppressor mutation or the old mutation from FC0? Do that test.

Figure 2 shows a genetic map of the beginning of phage T4 *rIIB*. The authors don't explain how they obtained this map – not good! Mapping was accomplished in the same fashion as Thomas Morgan and his group mapped fruit fly genes 50 years earlier, by quantitative recombination. If two mutants are crossed, recombination between them occurs at some frequency, and half of the recombinants will be wild-type, hence able to form plaques on E.coli K12. The number of plaques relative to the number of applied phage is a measure of how frequently recombination occurred, and that frequency is

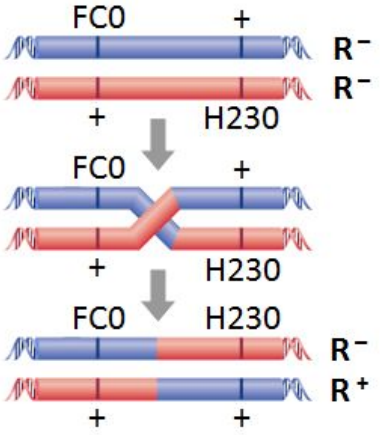

Figure 4: Basis of quantitative recombination. Crossing mutants carrying two different *rII* mutations will result in some number of recombinants that are wild type. The frequency is proportional to the distance between the mutations. (Thanks to Richard Twyman, U Warwick)

proportional to how many nucleotides separate the two mutations. Therefore, it is possible to count wild-type phage resulting from a cross between two mutants to determine the distance between the two mutations.

SQ10. Which mutants shown in Figure 2 of Crick et al are suppressors of FC0?

SQ11. Why are all of those suppressors clustered as compared to the more dispersed mutants shown on the other lines?

SQ12. Use the simulation[12] to map the suppressor mutations you obtained in response to SQ9.

SQ13. Choose one of the suppressors of FC0 that you obtained in SQ9 (I mean the suppressor after having been separated from the FC0 mutation) and obtain suppressors of it. As before, separate several of them to make strains with individual mutations.

Crick et al had no idea whether FC0 was an insertion or a deletion (and so far as I'm aware, the strain is not available, and so we'll never know[*]). They arbitrarily labeled it (+) and suppressors of FC0 (-).

SQ14. Suppose that FC0 indeed is an insertion. Then why should suppressors of FC0 be deletions?

SQ15. How would you label the suppressors of the suppressors of FC0?

II.C. Double Mutants

SQ16. Crick et al state in the second paragraph that their theory predicts that combining one + mutation with another + mutation should yield a strain with a mutant r (or R^-) phenotype. What theory? Why a mutant phenotype?

SQ17. How did they obtain double mutants?

Good luck on that last one! I don't see that they gave any description at all. Evidently the matter was considered too obvious (perhaps after close examination of Benzer's articles) to warrant any discussion. Suppose you crossed two + mutants hoping to get a + + mutant after recombination (**Fig. 4** might provide a model of the experiment).

SQ18. What is the phenotype expected of the + + mutant? What strain of E. coli should you plate the cross on in order to see plaques from this mutant?

Understand that recombination between sites within a gene is quite uncommon. In T4, you shouldn't expect more then 8% recombination between the most distant sites within a large gene. And you're looking for recombination between two sites that are ***not*** the most distant sites.

SQ19. How far apart are FC0 and another + mutant (your choice). Express your answer as x% of the *rIIB* gene.

SQ20. So, if the + + mutant will occur far less often than any of the parents (i.e. FC0 and some other + mutant), and all three have the R^-, then how can you distinguish what you're looking for from the vast majority of plaques that are single mutants? Hint: Consider your answer to SQ10.

This is a lot of work… but do-able.

SQ21. Do it. Use the simulation to construct at least two double (+ +) mutants. Choose FC0 to be one of the single (+) mutants and choose the other (+) mutant to have a mutation as far from that of FC0's as possible.

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[*] Actually, combining the evidence provided by the article with the now known sequence of the *rIIB* gene, you can be certain whether FC0 is really + or -.

II.C. Triple Mutants and the Coding Ratio

SQ22. How would you go about making a triple mutant? You might think you just cross a third single (+) mutant with one of the double (+ +) mutants you created in SQ21. If you go that route, but you increase your chances by using the trick shown in Fig. 5. If you cross two double mutants with the central mutation in common, then recombination anywhere between the two outer markers will give you what you want. Try it out.

SQ23. You can cross and screen, as you did in SQ21, but there's a second time-saving trick here. If the theory of Crick et al is correct, then what would you expect if you plated the cross on E.coli K12? How does this differ from the cross of SQ21?

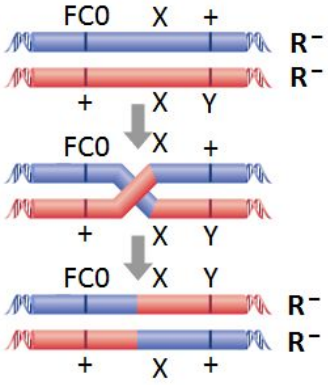

Figure 5: Recombination of two double mutants to produce a triple mutant.

SQ24. What results would you expect if the genetic code were duplet? quadruplet?

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