Molecular Biology Through Discovery

Companion to Brenner et al (1965)

Genetic Code: The 'nonsense' triplets for chain termination and their suppression

Brenner S, Stretton AOW, Kaplan S (1965). Nature 206:994-998

I. Introduction

Jones et al (1962) and related work pointed the way towards how the genetic code could be broken, and by 1965 the task had largely been accomplished. Note that the method we might think of now was not available to those faced with the problem. It was difficult to sequence proteins and virtually impossible to sequence DNA, so the simple solution of comparing gene sequences with their protein products was off the table. And as for gene termination, we can now examine the ends of genes directly (as you did in What is a Gene?), but in 1965 the problem had to be solved by less direct but ingenious methods.

Enter Sydney Brenner. You may remember from Problem Set 5 his clever proof that overlapping triplet codes are impossible. In a week you'll encounter his clever method to reveal the existence of mRNA. The article we are considering here, however, might be the tour-de-force. In it, Brenner et al determined the sequence of two of the three stop codons, using no sequencing technology, none of Marshall Nirenberg's biochemical tricks, none of Gobind Khorana's organic chemistry prowess. The job was done with pure genetic techniques plus logic (plus a bit of prior results).

The keys to the experiments were suppressible mutations. These mutations had been recognized for a few years, particularly in genes of bacteriophages. They were known as strong mutations (the function of the encoded protein was totally lost) but conditional. By this I mean that in some strains of *E. coli*, phage genes containing a certain class of suppressible mutations behave as if wild-type, while in other strains, the same genes behave as if mutant. Suppressible mutations were long suspected of having something to do with termination of protein synthesis (explaining the total loss of function of gene bearing these mutations), but the exact connection remained for Brenner et al to elucidate.

Now would therefore be a good time for you to get the article if you have not done so already. The reference is at the top of this page.

2. First glance at Brenner et al (1965)

I suggest skimming the article so you can see what you're up against. The task is made more difficult because articles in the journal *Nature* (and *Science*) are often without obvious form. You won't see headers, such as **Abstract**, **Introduction**, etc. The form is still there, however, if the authors know how to write an article. We just have to look for it. You can do this by browsing the paragraphs, perhaps just the first sentences, and fitting their contents into the categories we expect.

SQ1. Where does the Introduction end? Where do Brenner et al stop talking about context and prior work and start talking about their own work?

SQ2. Where does the Result section begin and end? Where do Brenner et al stop talking about the observations they made from their experiments and start talking about the conclusions they can draw from those observations?

SQ3. How many experiments do Brenner et al present? Where does the presentation of each experiment begin and end?

SQ4. Where is the Methods section?

You can see where the style imposed by *Nature* can be restricting. Brenner et al can write an article that for the most part is segmented in the usual way (but without the usual labels) and flows along as you might expect, but they cannot insert a section devoted to methods. That would stop the narrative in its tracks unless it is demarcated as a special methods section – and that *Nature* does not allow. The solution is to put methods here and there, sometimes in the text mixed in with the results, sometimes in figure legends or footnotes in tables. We have to live with that.

3. Introduction to the experimental system

In order to do the experiments Brenner et al will describe, they needed an experimental system in which it was easy to identify new mutations (therefore, under some conditions the mutations must not be lethal) but also easy to select strongly for the loss of mutations (therefore, under some conditions the mutations must be lethal). This system, based on the *rII* genes of bacteriophage T4, was developed a decade earlier by Seymour Benzer and used by him to establish the linear nature of the gene.[1,2] It was also used by Crick et al (1961) to work out that the genetic code was very likely triplet and nonoverlapping.[3,4]

In this article, Brenner et al presumed that the rII system was common knowledge – doubtful then, assuredly not true now. I'll introduce the system myself (**Fig. B1**). Phage T4 infects *E. coli*, multiplies within its host, and then lyses (breaks open) the cells to release progeny phage, which go on to infect new *E. coli* cells. If the infection takes place on a Petri plate, you can see dense growth of *E. coli* (represented by gray in **Fig. B1**), except in small regions where a phage and its progeny have lysed the cells in spreading circles of death called "plaques". Wild-type T4 makes small fuzzy plaques (Panel A). Phages mutant in the rII region ("r" stands for "rapid lysis") make large plaques with sharp boundaries on *E. coli*, strain B (Panel B). The variant E. coli strain K12 is also lysed by wild-type T4 and shows the same fuzzy plaques (Panel C) but the strain is immune to rII mutants of the phage (Panel D). E. coli B can therefore be used to grow up large quantities of mutant phage, and E. coli K12 can be used to select for rare revertants of mutant phage that have regained the ability to grow on the strain.

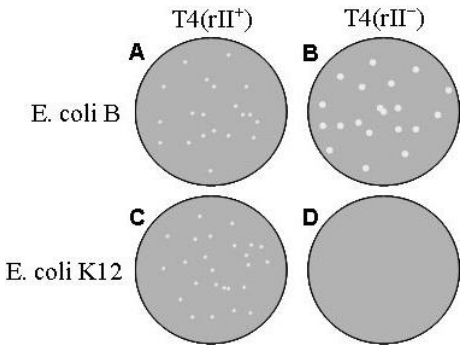


Figure B1. Plaque formation by phage T4 wild-type and rII mutant on two strains of E. coli.

SQ5. Suppose you mixed T4(rII$^+$) and T4(rII$^-$) in a ratio of 1:1 and then plated it on a lawn of *E. coli* B. What plaques would you observe the next day? What if you did the same experiment plating instead on *E. coli* K12?

With that in mind, consider the fourth paragraph of the article, where Brenner et al introduce suppressible mutants. That may not be readily apparent at first, because they use several different names: "ambivalent mutants", "suppressible mutants", "*hd* or *sus* mutants", "*amber* mutants"… they all refer to the same kind of mutant. In this article, they're called "nonsense mutants", divided into two subclasses: "*amber*" and "*ochre*". They differ as to which *E. coli* strains may suppress them, collectively called *su*$^+$ strains (suppressor strains), those able to somehow ignore the

mutations found in *amber* or *ochre* mutants. The su^+ strains may also be divided into two classes: *Amber* suppressor strains suppress only *amber* mutants, while *Ochre* suppressor strains may suppress both *amber* and *ochre* mutants. These relationships are summarized in **Table B1**. For now, let it remain mysterious as to why some mutation/strain combinations lead to suppression while others don't. By the end of the article, it should be more clear.

Table B1: Suppression of suppressible mutations in different strains*

	Strain		
Mutation	su^-	su^+ (*amber*)	su^+ (*ochre*)
(none)	+	+	+
amber	-	+	+
ochre	-	-	+

*A "+" indicates that a mutation may be suppressed. There are many mutation/strain combinations where suppression still does not occur.

SQ6. Interpret Table 1 from Brenner et al in light of Table B1.

It isn't necessary to dwell on the evidence put forth to support the hypothesis that suppressible mutations are caused by nonsense mutations, resulting in premature chain termination during translation of the mRNA in which they reside (Brenner et al, paragraph 5). The first argument is the easiest to understand.

SQ7. How does the finding of Garen and Siddiq bear on the question of whether an *amber* mutation is related to premature chain termination? Draw a crude picture of the gene encoding alkaline phosphatase and imagine the position of the *amber* mutation. Draw a picture illustrating how the wild-type gene is expressed as a protein (alkaline phosphatase) and who the gene with the *amber* mutation fares.

By the end of the (implied) Introduction section, Brenner et al have finished their argument that suppressible mutations are caused by nonsense codons, which lead to premature termination of protein synthesis. At this point, it would be nice to have a clear statement of the question their experiments will address. They don't provide this, but from Paragraph 3, the question is clear.

SQ8. What question is experimentally addressed in this article?

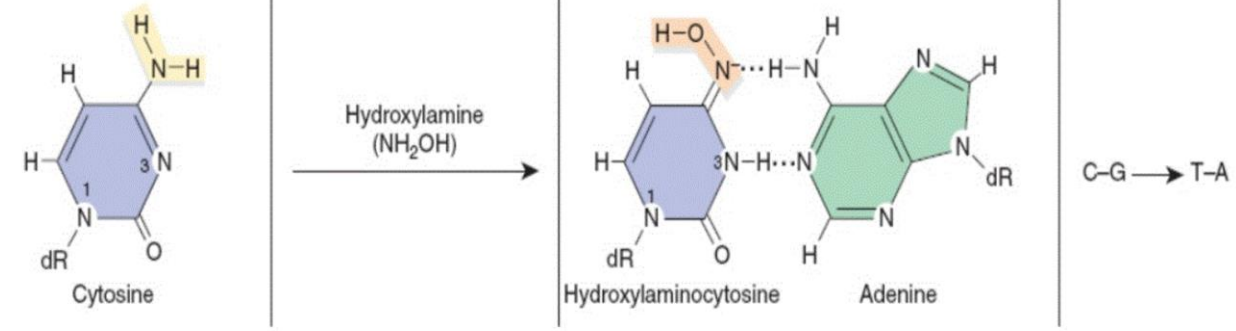
4. Results: Experiment #1

In the interest of time, I'm ignoring this experiment, except to make note of one finding: a site with an *amber* mutation can be mutated by hydroxylamine to produce an *ochre* mutation. This implies (for one thing) that the *amber* codon is one nucleotide different from the *ochre* codon.

5. Results: Experiment #2

Beginning at the top of p.997, Brenner et al describe how they mutated the T4 rII region using mutagens with specific properties, under conditions that selected for mutations on one strand or the other, and observed how many *amber* and *ochre* mutations resulted. Look over the first 25 lines of the first full paragraph on p.997, particularly lines 17-25. Don't be discouraged by terms or procedures you don't understand.

SQ9. Diagram the two experimental conditions described (SetB and Set K). Make sure your diagram contains all seemingly relevant procedures, making note of aspects you don't understand. What is the significance of the treatment of wild-type phages? The two different *E. coli* hosts (*E. coli* B and *E. coli* K12 (λ) su^-)? What essential procedure is implied but not stated for the Set K condition?



B

generation 0

ATGTACAATATTAAATGCCTGACCAAAAACGAACAAGCTG
TACATGTTATAATTTACGGACTGGTTTTTGCTTGTTCGAC

Treat with hydroxylamine

mutation

ATGTACAATATTAAATGCCTGACCAAAAACGAAUAAGCTG
TACATGTTATAATTTACGGACTGGTTTTTGCTTGTTCGAC

DNA replication

ATGTACAATATTAAATGCCTGACCAAAAACGAAUAAGCTG
TACATGTTATAATTTACGGACTGGTTTTTGCTTATTCGAC

+

generation 1

ATGTACAATATTAAATGCCTGACCAAAAACGAACAAGCTG
TACATGTTATAATTTACGGACTGGTTTTTGCTTGTTCGAC

Figure B2: Action of hydroxylamine. (**A**) Hydroxylamine alters cytosine to a form, functionally similar to uracil, that basepairs with adenine. Taken from Peter J. Russell, *iGenetics*, 3rd Edition (2010), Pearson Education, Inc. (**B**) DNA originally (***generation 0***) has normal, complementary strands. When exposed to hydroxylamine (***mutation***), a random cytosine residue is modified to hydroxylaminocytosine, similar to uracil. When the DNA is replicated, the modified cytosine pairs with A, not G.

There is no doubt that hydroxylamine is at the core of this article, and we will need to understand what it is about. **Fig. B2.A** shows how hydroxylamine adds on to the free amino group of cytosine, changing it from a proton donor in hydrogen bonding to a proton acceptor. In this it is like thymine, so hydroxylaminocytosine basepairs with adenine, not guanine. Mutagenesis by hydroxylamine therefore has two separate but related effects. First, when the strand bearing the mutated nucleotide is used as a template for transcription, the resulting mRNA has an A rather than a G at the mutated position. Second, when the strand is replicated, what used to be a C-G basepair becomes a T-A basepair (partially shown in **Fig. B2.B**).

SQ10. Take replication shown in Fig. B2 to one more generation. What would be the resulting double-stranded DNA? What about after many generations?

Another key point is that for a given gene, only one of the two strands serves as a template for transcription. If only the template strand is mutated, then mRNA will be affected, but if only the non-template strand is mutated, mRNA will be no different from wild-type.

With that in mind, consider Fig. 2 from Brenner et al.

SQ11. Unfortunately, the terms "sense" and "antisense" are used in opposite ways by different people. Which strand, sense or antisense (as defined in Fig. 2), is the template strand for mRNA synthesis?

SQ12. Which strands are mutated in Set B and Set K conditions?

That's a trick question. Both initially strands are mutated, because hydroxylamine mutagenesis is indiscriminate. I'll try again.

SQ13. rII mutants recovered in the SetB and SetK conditions will be mutated in which strand(s) – template and/or non-template?

There are a few other procedures in this experiment that are important to understand.

SQ14. How are rII mutants recovered? (If you need to, pay a visit to Fig. B1 and SQ5)

SQ15. How can it be determined whether an rII mutant is an *amber* mutant, an *ochre* mutant, or a nonsuppressible mutant?

The critical observation in this experiment is how many *amber* mutations of specific types are recovered under the two conditions. By "specific type" I mean specific nucleotide location. For example, the mutation shown in **Fig. B2.B** is at a specific location and would be given a specific name (e.g. "N24"). How is the location of a mutation determined? To put it another way, how is the mutation ***mapped***? We don't have to go too deeply into this, but **Fig. B3** may illustrate the general idea.

We come finally to the main result, **Table 5B** of Brenner et al, which shows evidence of two kinds of *amber* mutations affecting the rII genes. Read their description of this critical result on p.997, left, lines 26-37 (and a bit beyond.

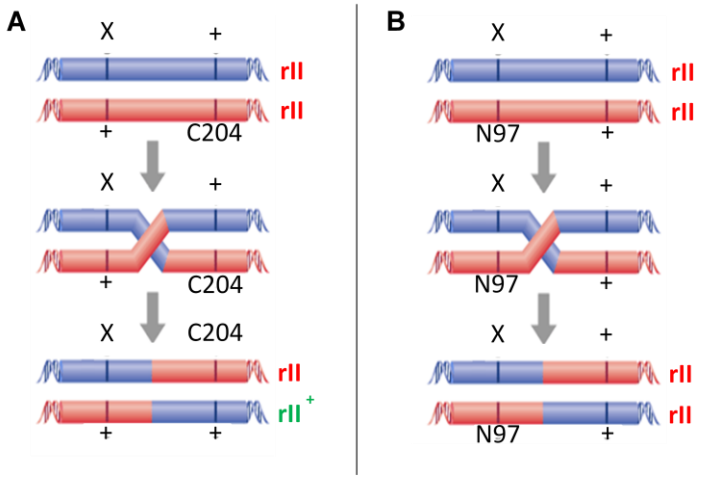


Fig. B3. Principle behind mapping of a mystery mutation (X) by recombination. A phage bearing mutation X in *rII* is coinfected with a second phage carrying a known mutation in *rII*. Initially, the infection is done in *E. coli* B to allow growth and recombination. Then the infection is diluted into *E. coli* K12. Plaques form only if recombination produces wild-type phage. In (**A**), recombination is possible between the two strains. In (**B**), the sites of the mutations are identical, so recombination is not possible. X can thus be identified as N97.

SQ14. Describe the two kinds of *amber* mutants in terms of changes in the sense (template) strand and antisense (non-template) strand. Draw an example of each mutant with made up DNA sequences.

Brenner et al draw the critical conclusion in the last full sentence of the left column of p.997.

SQ15. Using the drawing you made in SQ14 and the results in Table 5B, explain which strand is mutated at site N97 and why. Do the same with site S99.

SQ16. Why do the results imply that amber codons must have at least one A and at least one U on the template strand and the same on the non-template strand?

At this point Brenner et al have identified two of three nucleotides of an amber codon (though not their order). What about the third nucleotide? That also is in reach. Hearken back to the first experiment, which I shrugged off except for one of its conclusions.

SQ17. Revisit that conclusion in light of your new understanding of how hydroxylamine works. What can you say about the missing nucleotide of amber codons?

Brenner et al now knew all three nucleotides on the template and non-template strands of an *amber* codon, though they did not know which strand was which, and they didn't know the order of the nucleotides. Don't be fooled by their conclusion at the top right of p.997. "(UAC)" means "order unknown".

Now to resolve the final mysteries. To do this, they drew on many results from others, but in retrospect, one of them would have sufficed, Near the bottom right of p.997, they referred to a recent result from Nirenberg's group that CAG (order known) is a codon for glutamine (the same work also established CAA as a synonymous codon).[5] Combine this with the result shown in Fig. 1 from Brenner et al (taken from another work[6]) that the *amber* codon can be derived from a glutamine codon in a single mutation, one that can be induced by hydroxylamine.[*]

SQ17. Which of the two glutamine codons can (in one hydroxylamine-induced mutational event) produce the three established nucleotides of an *amber* codon? At which of the three positions of the codon must the mutation take place?

SQ18. What therefore must be the identity of the *amber* codon? Revisit and outline the argument that led to this conclusion.

There's much more in this article, but the elucidation of the *amber* codon is for me a fitting climax.

6. Discussion

I can't resist one more observation. Go to the last paragraph of the article, which provides three bottom-line claims. The first – the identity of two nonsense codons – has taken root in every pertinent textbook for the past 50 years. The second – the functional role of nonsense codons – made sense of a collection of otherwise bewildering observations. It continues to be molecular dogma (though predictably, reality is more complicated than theory[7]). What about the last claim?

SQ19. Are there special tRNA's (modern term for sRNA's) that specifically recognize stop codons and mediate chain termination? How did that idea hold up?

[*] The *amber* mutant H36 shown in Fig. 1 was reported by Sarabhai et al (1964) as induced by either hydroxylamine or one of two other mutagens, 2-aminopurine and 5-bromouracil, both of which cause transition mutations, as does hydroxylamine. The conclusions would be unchanged regardless of which mutagen was actually used.

REFERENCES

1. Benzer S (1959). On the topology of the genetic fine structure. Proc Natl Acad Sci USA 45:1607-1620.
2. Benzer S (1962). The fine structure of the gene. Sci Am 206:70-84 (January, 1962).
3. Crick FHC, Barnett L, Brenner S, Watts-Tobin RJ (1961). General nature of the genetic code for proteins. Nature 192:1227-1232.
4. Crick FHC (1962). The genetic code. Sci Amer 207:66-77 (October, 1962).
5. Nirenberg M, Leder P, Bernfield M, Brimacombe R, Trupin J, Rottman F, O'Neal C (1965). RNA codewords and protein synthesis, VII. On the general nature of the RNA code. Proc Natl Acad Sci USA 53:1161–1168.
6. Stretton AOW, Brenner S (1965). Molecular consequences of the amber mutation and its suppression. J Mol Biol 12:456-465.
7. Schueren F, Thoms S (2016). Functional Translational Readthrough: A Systems Biology Perspective. PLoS Genet 12:e1006196.

[1] Benzer S (1959). On the topology of the genetic fine structure. Proc Natl Acad Sci USA 45:1607-1620.

[2] Benzer S (1962). The fine structure of the gene. Sci Am 206:70-84 (January, 1962).

[3] Crick FHC, Barnett L, Brenner S, Watts-Tobin RJ (1961). General nature of the genetic code for proteins. Nature 192:1227-1232.

[4] Crick FHC (1962). The genetic code. Sci Amer 207:66-77 (October, 1962).

[5] Nirenberg M, Leder P, Bernfield M, Brimacombe R, Trupin J, Rottman F, O'Neal C (1965). RNA codewords and protein synthesis, VII. On the general nature of the RNA code. Proc Natl Acad Sci USA 53:1161–1168.

[6] Stretton AOW, Brenner S (1965). Molecular consequences of the amber mutation and its suppression. J Mol Biol 12:456-465.

[7] Schueren F, Thoms S (2016). Functional Translational Readthrough: A Systems Biology Perspective. PLoS Genet 12:e1006196.