CHAPTER 15

CHAPEVILLE: PROVING THE tRNA HYPOTHESIS

In 1963, F. Chapeville and a number of collaborators used labeled amino acids to demonstrate that the specificity of tRNA molecules was not *determined by the amino acids to which they were attached.*

HOW DOES PROTEIN TRANSLATION OCCUR?

Genetic information is encoded in DNA as a sequence of three-base codons, and nucleotide information is transcribed onto messenger RNA and carried to the cytoplasmic ribosomes, where it is translated into a corresponding sequence of amino acids in a protein. This sequence of "information transfer" steps (what Watson calls "the central dogma") describes in a general way how genes are expressed in a cell. However, it leaves the key question unanswered: how is the translation achieved? There is no chemical correspondence between the structure of an amino acid and that of a nucleotide base. Worse, the code utilized a sequence of *three* bases to specify an amino acid. What did sequence have to do with it?

Crick again had a reasonable suggestion: perhaps there existed a class of molecules that could bind both mRNA *and* amino acids. Such a hypothetical *adapter molecule* would have to recognize an mRNA three-base sequence specifically, and at the same time specifically bind a particular amino acid. The rest of protein synthesis could then proceed in a then-yet unknown, but in principle, straightforward manner. It was the adapter molecule, under this hypothesis, that read the code and delivered the appropriate amino acid to where it belonged, like a postal carrier reading a house number.

ZAMECNIK'S EXPERIMENT

What sort of a molecule might the proposed adapter be? A good candidate was soon found. Paul Zamecnik, attempting to develop a cell-free system to carry out *in vitro* RNA synthesis in 1957, discovered that ^{14}C ATP precursors indeed produced the expected newly-synthesized radioactive RNA (containing ^{14}C adenine). To ensure that the new RNA was not in some manner mated with protein (it could be that the ^{14}C ATP is broken down and metabolized, and the ^{14}C carbons used in amino acid and subsequent protein synthesis), Zamecnik ran ^{14}C leucine as a control. If the new synthesis carried out by his *in vitro* system had indeed been RNA, then a labeled amino acid should not be incorporated. It was. And try as he would, Zamecnik could not separate the ^{14}C RNA from ^{14}C amino acid; it was as if the amino acids were covalently bound to the RNA. HE was able to show that this was exactly what was happening by digesting the complex with ribonuclease (which destroys RNA but not protein). ^{14}C amino acids were then released.

The RNA that was binding the amino acids in this fashion proved to be of a special sort. When ribosomes (and thus ribosomal RNA and any associated mRNA) are spun down into a pellet by centrifuging at 100,000 g's, this RNA is left behind in the supernatant. Evidently very small (about 80 bases), this RNA was called "soluble" RNA, or *sRNA*.

IT'S tRNA!

Many of the characteristics of Crick's hypothetical adapter molecule could be recognized in the molecule Zamecnik isolated. It was possible to separate and purify different sRNA molecules, each specific for different amino acids. The binding of sRNA to amino acid was specific. The key question, of course, was whether the binding of an sRNA-amino acid complex to mRNA was codon specific. Was the code really

being "read" by the amino acid-carrying sRNA molecule? This was shown to be the case in an experiment in which a specific sRNA was allowed to "pick out" its appropriate amino acid, and then that amino acid was experimentally changed into a *different* amino acid while still bound to the sRNA; the sRNA couldn't tell the difference. It placed the new amino acid into protein in an *in vitro* protein synthesizing system just as if it were the unmodified original amino acid. Therefore, once the amino acid was bound to its appropriate RNA carrier, the specificity of binding to mRNA clearly derived from the RNA molecule, not the amino acid. This experiment unambiguously established that the adapter hypothesis was correct, and that a class of small soluble RNA molecules bound specific amino acids and transported them to appropriate positions in mRNA translation. These small soluble RNA molecules are now called *transfer RNA*, or *tRNA*.

THE tRNA HYPOTHESIS

Transfer RNA is thought of as a bifunctional molecule: one end carries a specific amino acid (added with the aid of an activating enzyme) and the other end carries a corresponding anticodon that permits appropriate tRNA-mRNA pairing. If this hypothesis is true, the chemical nature of the amino acid carried by the tRNA should not make any difference. Like a letter, the amino acid would be delivered according to the address, not the contents.

CHAPEVILLE'S EXPERIMENT

This key concept in the tRNA adapter hypothesis was subject to a direct test. In 1962, Chapeville and his colleagues, under the auspices of Seymour Benzer, switched the contents of such a tRNA "letter" to see if it made any difference in where it was delivered (figure 15.1). What they did was charge the UGU anticodon tRNA that normally carries cysteine (tRNA^{cys}) with radioactive amino acid, using the appropriate activating enzyme, to obtain ^{14}C-cysteinyl-tRNA^{cys}. They then chemically modified the attached amino acid without removing it from the tRNA, and looked to see how the new tRNA performed in protein synthesis.

To modify the ^{14}C-cysteinyl-tRNA^{cys}, they reacted it with a special metal catalyst, *Raney nickel*, which removed the –SH sulfur (thiol) group from cysteine, replacing it with a simple hydrogen atom. The resulting molecule was alanine! This treatment thus produced ^{14}C-alaninyl-tRNA^{cys}, a tRNA molecule with the CYS anticodon carrying the amino acid alanine.

F. Chapeville and his colleagues then tested the hybrid tRNA to see how it behaved in protein synthesis. They added ^{14}C-alaninyl-tRNA^{cys} to an *in vitro* protein-synthesizing system, using the synthetic polynucleotide poly-UG as a messenger RNA. In parallel experiments run as controls, they instead added the normal charged tRNAs, ^{14}C-alaninyl-tRNA^{ALA} and ^{14}C-cysteinyl-tRNA^{cys}. This random polynucleotide could make eight possible triplets:

1st		2nd: U	2nd: G	3rd		U	G	
1st	U	UUU	UGU	U	U	PHE	CYS	U
		UUG	UGG	G		LEU	TRP	G
	G	GUU	GGU	U	G	VAL	GLY	U
		GUG	GGG	G		VAL	GLY	G

Because one of the eight possible triplets was CYS (UGU), a tRNA with the corresponding CYS anticodon should incorporate amino acids into protein in this poly-UG system, while a tRNA with an ALA anticodon should not, as none of the triplets specified alanine.

CONFIRMATION OF THE ADAPTER HYPOTHESIS

The results of these experiments unambiguously confirmed the adapter hypothesis:

1. The poly-UG *in vitro* system, when challenged with the hybrid Raney-nickel tRNA, incorporated ^{14}C-alanine into protein. Subsequent amino acid analysis confirmed that the added amino acid was indeed alanine.
2. The incorporation was not an artifact in the makeup of the poly-UG mRNA, as this system *did* incorporate ^{14}C-cysteine when challenged with normal CYS tRNA. Nor was the incorporation due to sloppy base pairing, as the system would *not* incorporate ^{14}C-alanine when challenged with normal ALA-tRNA.

These results clearly indicated that the specificity of the tRNA molecule is not determined by the amino acids that they carried. The experiment has been repeated employing other mRNA molecules. When hemoglobin mRNA was used, the single peptide of alpha-hemoglobin that normally contains cysteine was converted to one containing ^{14}C-alanine when challenged with Raney-nickel tRNA, while none of the many alanine-containing peptides acquired any ^{14}C-alanine from this hybrid tRNA.

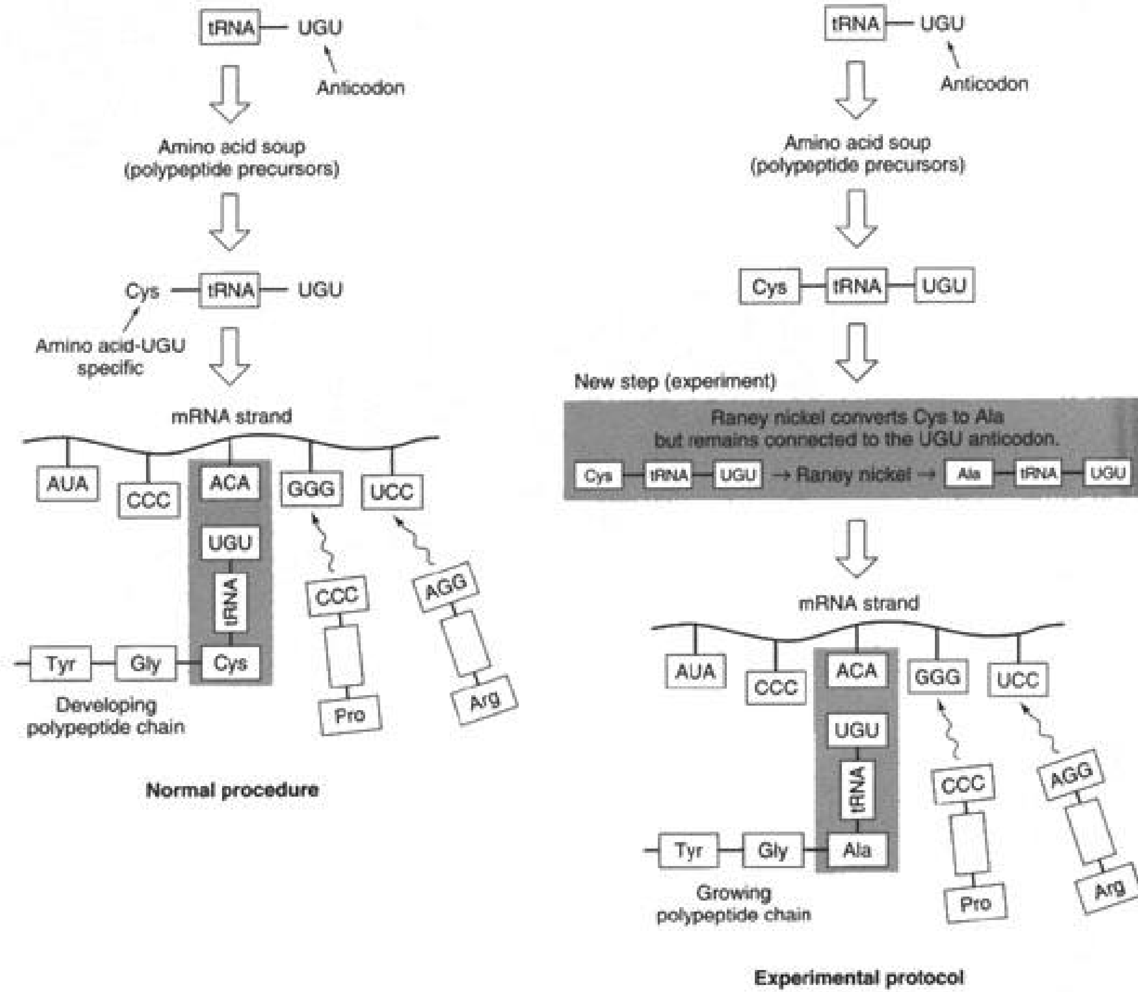

Figure 15.1
Chapeville's experiment.