

This Week's Citation Classic®

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Hoagland M B, Stephenson M L, Scott J F, Hecht L I & Zamecnik P C. A soluble ribonucleic acid intermediate in protein synthesis.

J. Biol. Chem. 231:241-57, 1958. [John Collins Warren Labs., Huntington Memorial Hosp., Harvard Univ., Massachusetts General Hosp., Boston, MA]

This paper describes the discovery of, and the early steps in, functional characterization of transfer RNA. [The SCI® indicates that this paper has been cited in over 580 publications since 1958.]

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In 1954 I became one of a group led by Paul Zamecnik at the Massachusetts General Hospital studying protein synthesis. I had just completed a postdoctoral year with Fritz Lipmann in an adjoining lab. Inspired by Lipmann's insights into acyl activation mechanisms and exploiting the Zamecnik group's *in vitro* rat-liver system, I uncovered the mechanism of amino acid activation in 1955. The enzymic activity was concentrated in a "soluble" cellular fraction obtained by adjusting cell sap to pH 5 and redissolving the precipitate. In the presence of ATP and amino acids, the fraction vigorously catalyzed the formation of amino acyl adenylates and inorganic pyrophosphate.¹ Zamecnik's group had already shown that this same fraction was required for incorporation of amino acids into peptide linkages in protein in the presence of microsomes, ATP, and GTP (reviewed in reference 2).

At this time there was no evidence as to how amino acids might be sequenced in protein. Workers in the field assumed that a template must exist on the ribosomes, presumably made of the RNA that was plentiful in them. (The revelation of messenger RNA was still three years in the future.)

Zamecnik's lab shed the first experimental light on coding while attempting to find if certain of the cell fractions that synthe-

sized protein were also making RNA. To test this, he incubated the pH 5 fraction and microsomes separately with radioactive nucleotides and looked for labeling of acid-precipitable material (RNA and protein). By way of a test for "irrelevant"—or nonspecific—labeling, he carried out parallel incubations with radioactive amino acids. To our surprise, there was both labeling of acid-precipitable material with nucleotides (the -CCA end)³ and also considerable labeling by amino acids, particularly in the pH 5 fraction. We found the amino acid label to be in "soluble" mRNA.

The cited studies indicated that each of the amino acids was bound to the new RNA reversibly and at a separate site. The clincher as to its role in protein synthesis was that after it was charged with amino acids and reisolated, its bound amino acids were rapidly and quantitatively transferred to peptide linkages in protein on ribosomes. And that reaction was dependent on GTP.

Preliminary accounts of these findings appeared in 1957.^{3,4} Soluble RNA was soon re-named transfer RNA. The work, more fully set forth in the cited 1958 paper, opened the door to an explosion of research characterizing the role of tRNA molecules as amino acid adaptors and as sequencing vehicles via the pairing of their anti-codons with codons of mRNA on ribosomes.

Another reason for the paper's frequent citation was that tRNA's discovery, which came at a time of heated speculation about the nature of the genetic code, offered the first real hope of cracking the code by assigning nucleotide sequences on tRNA to each of the 20 amino acids. This formidable task was, of course, obviated by the discovery in 1961 that synthetic messenger "RNAs" would promote the synthesis of artificial polypeptides on ribosomes.

My contributions to the understanding of protein synthesis were recognized in 1963 by membership in the American Academy of Arts and Sciences, in 1976 by the award of the Franklin Medal, and in 1984 by membership in the National Academy of Sciences.

1. Hoagland M B, Keller E B & Zamecnik P C. Enzymatic carboxyl activation of amino acids. *J. Biol. Chem.* 218:345-58, 1956. (Cited 530 times.)
2. Zamecnik P C. Historical and current aspects of the problem of protein synthesis. *Harvey Lect.* 54:256-81, 1960. (Cited 125 times.)
3. Zamecnik P C, Stephenson M L, Scott J F & Hoagland M B. Incorporation of C¹⁴-ATP into soluble RNA isolated from 105,000 x g supernatant of rat liver. *Fed. Proc.* 16:275, 1957.
4. Hoagland M B, Zamecnik P C & Stephenson M L. Intermediate reactions in protein synthesis. *Biochim. Biophys. Acta* 24:215-16, 1957. (Cited 335 times.)