

NEWS RELEASE

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Cracking the Genomic Code: Gene Decoding Revealed at Atomic Level

FOR IMMEDIATE RELEASE

A recent finding by a North Carolina State University biochemist advances the fundamental biology of how genetic information, encoded in DNA, is decoded for the production of proteins.

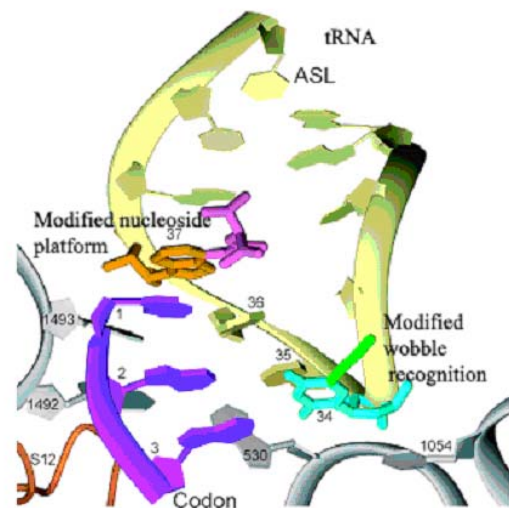
Dr. Paul F. Agris, professor of biochemistry at NC State, and academic colleagues from England and Poland show concrete evidence in favor of the 1966 “Wobble Hypothesis” offered by Francis Crick, the co-founder of the DNA molecule and its double-helix structure, and Agris’ own “Modified Wobble Hypothesis” posed in 1991.

The scientists used x-ray crystallography of the cell’s protein-manufacturing unit, the ribosome, to provide a visual snapshot of the decoding process.

The research is published in the December 2004 edition of *Nature Structural and Molecular Biology*.

The Wobble Hypothesis was Crick’s attempt to make sense of how the cell decodes the genetic information of DNA – the molecule that constitutes all the genetic information in a cell – and then, from that information, makes biologically active proteins, Agris said.

DNA has 61 three-letter codes that are translated by transfer RNA (tRNA) into amino acids; proteins are made of amino acids. But there are only 20 natural amino acids. Squaring the disparity between the number of codes and the number of amino acids – there are three times as



The critical decoding structure produced when modified nucleosides enable tRNA to decode by wobble recognition. Only the decoding region of a 50,000+ atom structure of the ribosome (small subunit) is shown. The modified nucleoside platform (orange) that stabilizes the codon-anticodon interaction, and the modified nucleoside that wobbles (green) are shown. The structure was determined at the atomic resolution of -3 angstroms (3×10^{-10} meters).

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many codes as there are amino acids – became a hurdle for Crick and other early geneticists, Agris explained.

Crick attempted to clear this hurdle with the Wobble Hypothesis. He based this theory on the first report of a tRNA molecule's chemical structure discovered by Robert Holley in 1963.

Normally, RNA molecules are composed of four nucleosides: adenosine, guanosine, cytosine and uridine (A,G,C,U). But the tRNA molecule Holley studied included a modified nucleoside called inosine (I), Agris says. Seeing this inosine in an important area of the tRNA molecule – an area that read the three-letter DNA codes when the cell synthesizes proteins – led Crick to believe that a single tRNA used inosine to read more than one code, and that therefore the 61 codes were decoded by fewer than 61 tRNAs.

As an example, Agris used the amino acid alanine, which has four codes. Crick's hypothesis would allow that only two tRNA molecules could be capable to decode all four alanine codes. Using the modified nucleoside I in place of A, G, C or U, one tRNA may be able to read three codes, effectively “wobbling” the reading.

Twenty-five years after the Wobble Hypothesis, Agris proposed his Modified Wobble Hypothesis. It stated that modified nucleosides other than inosine would in some cases expand tRNAs ability to translate codes by wobbling to greater numbers of three-letter codes, whereas other modified nucleosides would restrict wobble to only one or two codes.

Now, in the recent paper, Agris and colleagues prove Agris' alteration to Crick's hypothesis was correct: Cellular modification of tRNA alters chemistry and structure in a manner critical for tRNA to decode more than one three-letter code.

Using atomic-level resolution – in which researchers can distinguish atom from atom – and working with a tRNA specific for the amino acid lysine, Agris and his colleagues show modified nucleosides enabling tRNA to decode genomic information on the ribosome, the cell's protein synthesis machinery.

Specifically, it shows modifications enabling the decoding of two codes. One modification acts like a platform on which decoding takes place, and the other allows a novel chemical and physical interaction to occur between tRNA and the code, Agris said.

“This is the first visualization that modifications are critical for decoding the genome through wobble,” he said.

Agris says that 15 to 20 percent of tRNAs in all organisms require modified chemistries in order for codes to be properly read and protein synthesis to be successful.

“An understanding of how modified nucleosides enable and improve wobble recognition of the three-letter codes for protein synthesis opens the possibility of using

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modified nucleosides to expand the cells' use of tRNA to make new proteins, or in new ways to target the protein synthesis machinery in pathogens," Agris said.

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Note to editors: An abstract of the paper follows.

“The Role of Modification in Codon Discrimination: tRNA^{Lys}_{UUU}”

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Abstract: The natural modification of specific nucleosides in many tRNAs is essential during decoding of mRNA by the ribosome. For example, tRNA^{Lys}_{UUU} requires the modification N-6-threonylcarbomoyladenine at position 37 (t⁶A₃₇), 3'-adjacent to the anticodon, to bind AAA in the A site of the ribosomal 30S subunit. Moreover, it can only bind both AAA and AAG lysine codons when doubly modified with t⁶A₃₇ and either 5-methylaminomethyluridine or 2-thiouridine at the wobble position (mm⁵U₃₄ or s²U₃₄). Here we report crystal structures of modified tRNA anticodon stem-loops (ASLs) bound to the 30S ribosomal subunit with lysine codons in the A site. These structures allow the rationalization of how modifications in the anticodon loop enable decoding of both lysine codons AAA and AAG.