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The 2009 Nobel Prize in Chemistry: Thomas A. Steitz and the Structure of the Ribosome

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Over the past 200 years, there have been countless groundbreaking discoveries in biology and medicine at Yale University. However, one particularly noteworthy discovery with profoundly important and broad consequences happened here in just the past two decades. In 2009, Thomas Steitz, the Sterling Professor of Molecular Biophysics & Biochemistry, was awarded the Nobel Prize in Chemistry for “studies of the structure and function of the ribosome,” along with Venkatraman Ramakrishnan of the MRC Laboratory of Molecular Biology and Ada E. Yonath of the Weizmann Institute of Science. This article covers the historical context of Steitz’s important discovery, the techniques his laboratory used to study the ribosome, and the impact that this research has had, and will have, on the future of biological and medical research.

INTRODUCTION

One of the fundamental tenets of biology is the relationship between DNA, RNA, and protein. The central dogma states that DNA is transcribed into RNA, and RNA is translated into protein. Transcription and translation occur by very complex mechanisms that continue to be subjects of intense study. It is now a well-known fact that a protein’s structure and its function are closely linked, and the genetic instructions conveyed from DNA to RNA provide specific instructions on how to synthesize proteins with unique functions in-

side and outside the cell. However, none of this was known in the 1930s, when scientists first attempted to deduce the molecular structures of proteins [1]. In fact, it was not until the 1950s that scientists were first able to determine the three-dimensional structure of a protein, myoglobin, on the molecular level using a technique known as X-ray crystallography [2].

X-RAY CRYSTALLOGRAPHY AND PROTEIN STRUCTURE

Everyday objects can be “seen” when light waves are reflected into a detector. For

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†Abbreviations: mRNA, messenger RNA; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*.

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example, an apple is visible because red light is reflected by the skin of the apple into the human retina. The brain then interprets the information from the retina as the color red. However, as we try to visualize objects that are progressively smaller and smaller with only visible light, we reach a physical limit at about 1,000 nanometers, or 0.001 millimeters. This size is approximately the wavelength of red visible light. Thus, even if we had an infinitely powerful magnifying glass, a red object 1,000 nm in size could not be seen because the object is physically smaller than the light waves, making it impossible for them to reflect off the object.

Proteins are even smaller — most range from 4 to 6 nm in diameter, or 200 times smaller than the wavelength of red light. At these sizes, even the short wavelengths used in electron microscopy are insufficient. Furthermore, much of the structural information about proteins is derived from understanding the molecular interactions of individual amino acids.

X-ray crystallography describes a set of techniques that allows the visualization of these extremely small objects, including molecules and atoms, using wavelengths of light on the scale of 1 angstrom, or 0.1 nm. The form of electromagnetic light produced at these wavelengths is called an X-ray. Unfortunately, X-rays cannot be focused in the same way visible light can be by a magnifying glass. Instead, researchers grow crystals of the protein being studied using methods that slowly increase the concentration of precipitant in a protein solution until the protein becomes insoluble, at which point crystals begin forming [3]. Like salt or sugar crystals, protein crystals are arranged as lattices containing many repeating units organized and oriented in a regular fashion. In the case of protein crystals, each crystal contains perhaps millions of identical protein molecules in locked arrangement. Successfully grown protein crystals are soaked in cryoprotectant and flash frozen in liquid nitrogen and then brought to an X-ray beam facility such as a synchrotron [4].

When monochromatic X-ray beams are fired at the protein crystals, the molecular

electron clouds scatter the X-rays, which combine constructively and destructively to form a detectable diffraction pattern. The crystal is rotated through 180 degrees to collect a full set of 180 diffraction patterns, or reflections [5]. This set of reflections is unique to different proteins. One hurdle that arises from the use of X-ray crystallography is the phase problem. Each reflection has an intensity and a phase angle, but only the intensities of the reflections can be measured, not their phases. In order to solve the structure of the protein, the lost phase information must be recovered by another method, such as molecular replacement or multiple heavy atom isomorphous replacement.

Using both amplitude and phase information, electron density maps can be calculated and are used to solve the three-dimensional molecular structure of the protein. With a high-quality protein crystal, today's structure resolutions can be higher than 1 Å, giving information about protein structure on the level of the side chains and torsion angles of individual amino acids. This molecular information about proteins allows researchers to understand the physical mechanisms by which proteins perform their functions. Furthermore, researchers can solve the structures of protein molecules bound to substrates or inhibitors, providing novel insight into how enzymes catalyze reactions with their substrates, or how drugs bind proteins to change their function.

SOLVING THE STRUCTURE OF THE RIBOSOME

Thomas Steitz was introduced to protein crystallography in the 1960s as a graduate student at Harvard University in the laboratory of William Lipscomb. At the time, Max Perutz had just won the 1962 Nobel Prize for solving the crystal structure of human hemoglobin [6]. Steitz attended a series of lectures by Perutz on the structure of myoglobin and became fascinated, realizing that this new way of understanding proteins would certainly be the future [7]. His first project was working on purifying and obtaining the structure of bovine carboxypeptidase A, a digestive enzyme. By

1967, the laboratory group had solved the structure of carboxypeptidase A to a resolution of 2 Å, fine enough to reveal all the molecular and physical details of the molecule. Steitz then spent three years working at the MRC Laboratory of Molecular Biology in Cambridge under David Blow, during which time he helped solve the structure of chymotrypsin, another digestive enzyme [7]. In the fall of 1970, Steitz moved to Yale. Between 1970 and 1995, his laboratory determined the structures of many more proteins and their complexes, including yeast hexokinase, DNA polymerase I, and HIV reverse transcriptase [8,9,10].

Starting in the mid-1990s, Steitz's laboratory decided to tackle the structure and function of the ribosome. Unlike proteins such as carboxypeptidase A and hexokinase, which are composed of hundreds of amino acids, the ribosome is a massive complex consisting of dozens of smaller proteins coupled with thousands of nucleotides of RNA. Analyzing this astounding degree of complexity proved a difficult task. Indeed, it required exponentially better computational power and laboratory technology than was available for most of the 20th century. However, in the 40 years between the discovery of the structure of hemoglobin and that of ribosome, computational power for analyzing diffraction patterns had increased 8,000-fold and the rate of X-ray data collection had increased 10^5 fold, setting the stage for Steitz's laboratory to tackle the last piece of the central dogma that was not yet structurally understood.

The ribosome is a massive protein and RNA complex that carries out the translation of the nucleotide code on messenger RNA (mRNA†) into functional protein. Different mRNAs code for different proteins necessary to life. Eukaryotic organisms, which include humans, have two ribosomal subunits, the large 60S and small 40S, which combine to form the functional 80S complex. In contrast, prokaryotes such as bacteria have similar, but smaller subunits — a large 50S and small 30S, which combine to form a 70S complex. Because bacterial cells are easier to grow and can be used to produce the relatively large

amounts of protein needed to grow crystals, Steitz's team of researchers, led by Nenad Ban, chose to focus first on solving the structure of the prokaryotic 50S subunit, with the goal of obtaining a crystal structure with a high resolution of around 2 Å.

The Steitz laboratory's first breakthrough was in 1998, when they first solved a 9 Å resolution structure of the 50S subunit of the prokaryotic ribosome using crystals of protein purified from the extremophile archaeon *Haloarcula marismortui* [11]. The 9 Å map was a significant improvement over an older 20 Å map, which had been created by cryo-electron microscopy. To solve the phase problem, the researchers had to use a method called multiple isomorphous replacement. They created heavy-atom derivatives of the ribosome using a heavy atom cluster compound containing as many as 18 tungsten atoms and fired X-rays into the derivative crystals [11]. The derivative crystal data sets were then used to solve the phase problem. The most important discovery made with the 9 Å map was that it revealed numerous features on the ribosome consistent with double-helical RNA, providing evidence agreeing with previous studies by the scientific community showing that ribosomes were actually 60 percent RNA by weight [11].

One year later, in 1999, further progress was made toward a 2 Å resolution structure. Steitz's team reported in the journal *Nature* that they had definitively placed protein and RNA structures into a 5 Å resolution map. The new structure revealed the positions of major structural motifs on the ribosome, including the polypeptide exit tunnel, the binding sites for elongation factors G and Tu, and the sarcin-ricin loop [12]. These three motifs are critical components of the functional ribosome. The polypeptide exit tunnel is the exit route for proteins being synthesized in the ribosome, and obstruction of the tunnel can stop protein synthesis. Elongation factors G and Tu are required for polypeptide synthesis and are secured to the ribosome by the binding sites. Finally, the sarcin-ricin loop is the target of the castor bean protein ricin, and its modification by

ricin inactivates the entire ribosome [13]. In the future, a compound could be developed to block ricin's activity and prevent ricin toxicity.

In 2000, Steitz's laboratory finally reached the goal of obtaining a high-resolution structure, publishing a 2.4 Å resolution map in the journal *Science*. At this resolution, the researchers were able to definitively place nearly all of the 50S subunit's 3,045 nucleotides and 31 proteins. This high-resolution structure revealed the stabilizing role of the ribosomal proteins in the ribosomal complex and allowed researchers to begin looking at the molecular interactions between different side chains [14]. It also opened the way for discovering the molecular mechanisms of many antibiotics. By solving the crystal structure of the 50S subunit in complex with antibiotics that inhibit ribosomal translation, researchers can better understand the molecular interactions, including hydrophobic interactions, hydrogen bonds, and covalent bonds that form upon antibiotic binding, and how those binding interactions affect ribosomal activity.

Steitz continues to study the molecular mechanisms of antibiotic actions to this day. For example, in 2002, the laboratory described how four different macrolide antibiotics, including azithromycin and erythromycin, bind the 50S subunit. Macrolide antibiotics were found to exert their effects by binding in the polypeptide exit tunnel of the 50S subunit, inhibiting translation [15]. In 2005, Steitz's group described a single-nucleotide mutation in *H. marismortui* that conferred macrolide antibiotic resistance [16]. And in 2008, a publication in the *Journal of Molecular Biology* described eleven mutations in in *H. marismortui* ribosome that could render it resistant to the antibiotic anisomycin [17].

Such structural studies reveal the mechanisms by which antibiotic resistance can develop. They also provide the groundwork for rational drug design — the synthesis of novel antibiotics that may fit in structurally important grooves and rides on the protein. In 2001, Steitz and others founded Rib-X Pharmaceuticals, a company devoted to de-

veloping novel broad-spectrum antibiotics. By incorporating a structural understanding of how current antibiotics interact with ribosomes, Rib-X Pharmaceuticals hopes to develop antibiotics that can overcome known mechanisms of bacterial resistance. Two such drugs currently in development are delafloxacin, a fluoroquinolone that is effective against Methicillin-resistant *Staphylococcus aureus* (MRSA), and radezolid, a novel oxazolidinone compound with broad activity against Gram-positive bacteria such as vancomycin-resistant *Enterococcus* (VRE) [18]. This work is critical to the battle against constantly evolving pathogens, many of which can develop resistance that makes previous-generation antibiotics no longer effective.

CONCLUSION

Since the 1950s, the structures of thousands of proteins have been solved by X-ray crystallography, but Thomas Steitz's work on the structure and function of the ribosome allowed the scientific community to understand a fundamental component of translation. His work continues to have broad consequences in biology and medicine, especially in the battle against antibiotic-resistant bacteria such as MRSA and VRE.

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