Choreographed movement – unlocking the inner workings of the ribosome in protein synthesis

Ribosomes, tiny organelles within our cells, conduct the highly choreographed movements of protein synthesis. They ensure that the information encoded in DNA is successfully translated into proteins essential for nearly all chemical processes in our body, with an error rate of just 1 in 5000. **Dr Karissa Sanbonmatsu** and her team at Los Alamos National Laboratory, New Mexico, use cutting edge computational and imaging tools to investigate exactly how these extraordinary structures work. Specifically, they focus on translocation, one of the most complex and little understood processes in protein synthesis.

Ribosomes are only 25 nanometers (billionths of a meter) in diameter. Despite this, they are one of the most essential cellular organelles involved in protein synthesis. They provide the scaffold upon which the decoding of the cell's genetic material is realised, taking a string of RNA (think of it as a recipe book or instruction manual) and building a protein from amino acids (the ingredients or building blocks) in the order specified. In other words, ribosomes convert genetic language into the language of proteins.

But how does the ribosome decode the cell's genetic information? Proteins are made up of small amino acids and how many amino acids are used, and in which order, is specific to each protein. Each amino acid is coded for in DNA by a 'codon' of three base letters e.g. AUG or CCA or UGA. A DNA sequence for a particular protein will list the codons in the order in which the respective amino acids must be linked to one another. However, a ribosome cannot process the string of codons while it is contained within DNA. The DNA must be unwound and copied onto a single strand called mRNA (messenger RNA).

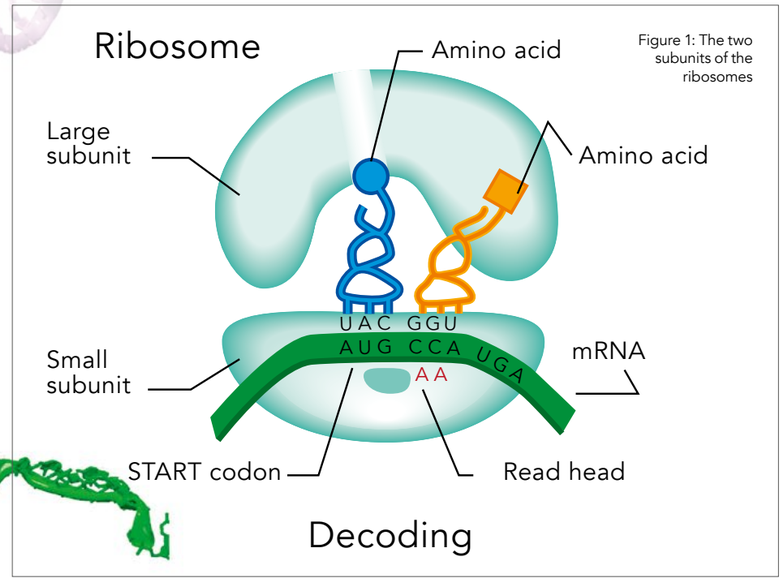


Figure 1: The two subunits of the ribosomes

Ribosomes are made up of two subunits (see Figure 1): a small subunit where the mRNA is attached for decoding of the genetic instructions and a large subunit where the respective amino acids are added to form the growing protein.

The mRNA strand attaches to the small subunit of the ribosome which reads through each codon one at a time. Another molecule, transfer RNA (tRNA), acts as the crucial intermediary. It has a single sequence of three base letters, an 'anticodon', and carries the corresponding amino acid that is encoded by the complementary codon on the mRNA. Think of a lock and a key, where the lock is the mRNA codon and the key is the tRNA anticodon. As soon as the tRNA approaches the small subunit of the ribosome, it tries to fit on to the codon that is currently exposed on the mRNA strand. If the anticodon on the tRNA matches the codon on the mRNA, then the fit is strong and the tRNA will be accepted into the ribosome (accommodation). The amino acid carried by the tRNA will bind to the previous one in the sequence and the growth of the protein – on the large subunit – commences.

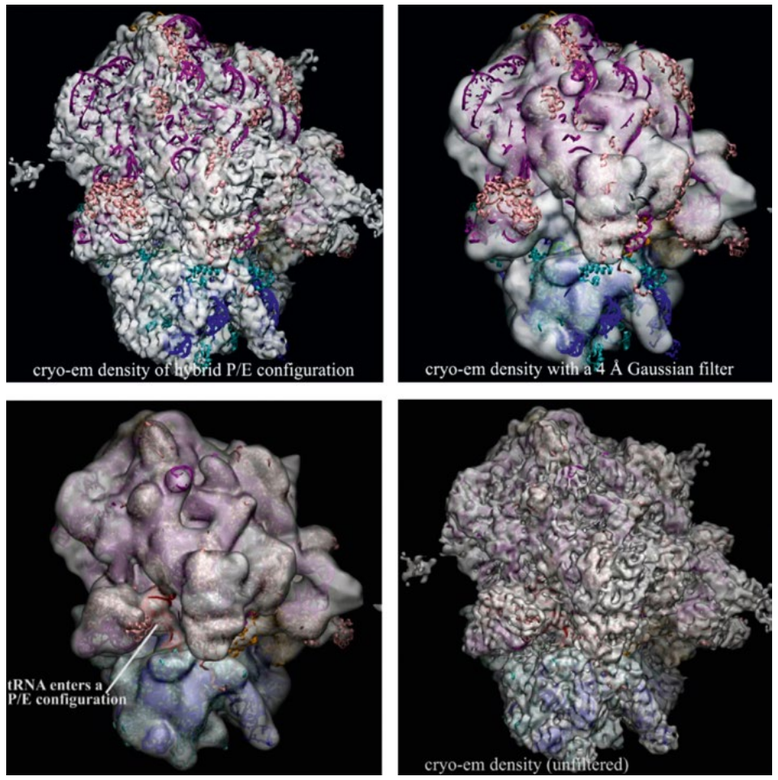
A MODEL EXPLANATION

It sounds relatively simple, but for the past 40 years researchers have focused on the movement of tRNA through the ribosome without offering any adequate or logical explanation for the underlying molecular mechanism that dictates this process. In fact, there are many cases where the tRNA fit is weak, indicating that it is carrying the wrong amino acid sequence. When this happens, the tRNA is physically rejected by ▸

Large-scale computer simulation of the ribosome using supercomputers at Los Alamos National Laboratory. Newton's equations of motion are solved several million times for each atom in the ribosome and each atom in the surrounding solution environment (total of 2.6 million atoms). White, large subunit RNA; magenta, small subunit RNA; light green, large subunit proteins; pink, small subunit proteins; yellow, aminoacyl tRNA; cyan, peptidyl tRNA; green ribbon, messenger RNA; green spheres and red spheres, amino acids.

the ribosome. This is where the work of Dr Karissa Sanbonmatsu demonstrates its profound significance and brilliance. Dr Sanbonmatsu is a principal investigator at Los Alamos National Laboratory, New Mexico – one of the most famous national institutions in the US. She has dedicated her research to using computational and experimental approaches to understand the mechanism of a diverse array of non-coding RNA systems, including ribosomes, riboswitches and long non-coding RNAs.

Her current focus aims to take advantage of powerful supercomputers together with innovative imaging modalities to address the molecular mechanisms behind the accommodation or rejection of tRNA by mRNA, right down to the atomic level. Once the correct tRNA is accepted by the ribosome, the tRNA must navigate through the complicated crevices inside the ribosome so it can escape and make room for the next incoming tRNA. This process, called translocation, is one of the most complicated in protein synthesis and remains an enigma at the molecular level. The realisation of this objective will offer a greater insight regarding the origin of the genetic code and, ultimately, of life itself. More specifically, these molecular simulations focus on the ribosome head swivel, the rate-limiting step of tRNA translocation through the ribosome. In this key movement, the 'head' of the small subunit pivots around its neck, over the body of the small subunit, moving the mRNA and tRNA by exactly one codon. The next codon is then exposed, ready to bind with the next tRNA. The purpose of these simulations is to create efficient predictions of the respective energy landscape of the head swivel, thus identifying regions of the ribosome that place constraints on the maximal displacements of the head.



High resolution cryogenic electron microscopy (cryo-EM) is an emerging field, revolutionising structural biology. Grey surfaces are based on cryo-EM images of the ribosome from Christian Spahn, a leader in cryo-EM ribosome science. Colored ribbons beneath grey surfaces, are atomic models from the Sanbonmatsu group. Atomic models are produced from computer simulations of the ribosome, integrating the cryo-EM information. The result is an atomistic view of the cryo-EM study, enabling detailed insights into the mechanism of the ribosome (e.g., head swivel). Upper panels show the ribosome before head swivel; lower panels, after head swivel. Lower right, final atomistic model is consistent with cryo-EM grey surface. Magenta, large subunit RNA; pink, large subunit proteins; blue, small subunit RNA; cyan, small subunit proteins.

FLEXIBILITY, ELONGATION AND FLUCTUATION

Ribosomes are made up of ribosomal RNA (rRNA) and proteins. A great part of Dr Sanbonmatsu's research focuses on the flexibility with which the rRNA can be chemically modified – a process that has long been used in order to probe ribosome structure and function. The prospect of creating well-defined datasets of rRNA base flexibilities is key to understanding the dynamics of the ribosome. Excitingly, the current work performed by the Sanbonmatsu group in collaboration with Jonathan Dinman (University of Maryland) has already revealed the existence of fluctuation hotspots. Such fluctuation hotspots have the capacity to allow large scale conformation changes that are essential for the translocation of tRNA through ribosomes. Translocation is catalysed by an elongation factor that dictates the precise movement of both the two tRNAs and the mRNA within the ribosome. If we can better understand how this process works then we open the door to understanding both how our own ribosomes work and how we may be able to target ribosomal activity in pathogens.

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Conclusively, Dr Sanbonmatsu's pioneering research has already provided the scientific community with a greater insight into the underlying mechanisms of a diverse array of non-coding RNA systems. Her special interest in ribosomes and her promising findings set the stage for joint computational and experimental studies that could lead to breakthroughs in the development of bio-inspired nanoscale computers but also allow for a greater insight regarding the origin of life itself.

Q&A

Can you briefly explain to us the principles of the computer model you have developed for understanding the molecular mechanism behind tRNA translocation?

Our model focuses on data from single molecule fluorescence experiments and cryo electron microscopy (cryo-EM). We simulate the ribosome atom for atom as well as the surrounding environment, including several million atoms. The ribosome is constantly in motion due to collisions with water molecules and other factors. When the time is right, it undergoes large changes in its structure, similar to a fashion model striking different poses, or a martial artist adopting different stances. For the ribosome, the succession of different stances helps shuttle the cargo (transfer RNA) through the ribosome. By solving Newton's equations for each atom several million times, we are able to simulate this process. We work closely with Scott Blanchard (Cornell) to compare with his single molecule fluorescence experiments of the ribosome and with Christian Spahn (Charite) to ensure consistency with his cryo-EM structures. We are working with Jonathan Dinman (Maryland) to calibrate our computer simulations with biochemical studies of local motions of the ribosome.

Provided that your research successfully explains the molecular mechanism behind tRNA translocation, what will the implications be in both research and industry?

Scott Blanchard (Cornell) is identifying a potential emerging class of antibiotics that target ribosome dynamics. While over 50% of antibiotics used in the US target the ribosome, most block tRNA binding or clog up the ribosome tunnel, preventing the nascent protein from escaping. Blanchard is using single molecule studies to identify antibiotics that stop the ribosome from shifting from stance to stance. If you think of the ribosome as an automobile engine, the antibiotic would be a monkey wrench, grinding the ribosome to a halt. While the single molecule experiments show the specific step that the antibiotic blocks, our simulations reveal the atomistic details of how this goes down.

Ribosomes provide the scaffold upon which the decoding of the cell's genetic material is realised

How would you explain, in layman's terms, the concept of 'bio-inspired nanoscale computers'?

The ribosome is one of the only molecular machines in the cell that actually processes information (it's certainly the only one that processes info in a complex way). Specifically, it performs a 'look-up table' operation, an operation widespread in computer programming. Given a three-letter RNA codon on the messenger RNA, the ribosome selects the amino acid corresponding to this codon. In this manner, the ribosome converts the four-letter language of nucleic acids (DNA and RNA) to the 20-letter language of proteins using a look-up table of the ensemble of tRNAs (~40 tRNAs in bacteria). The size of the ribosome is ~20 nm. In this sense, the ribosome is a nanoscale computer. One could imagine rigging the ribosome to perform other calculations.

Which factors do you think are the most important in order to develop comprehensive datasets of rRNA base flexibilities?

Recently, Illumina sequencing has enabled high throughput SHAPE probing on the genome-wide scale, especially in the field of long non-coding RNA in epigenetics and development. Performing SHAPE-seq on ribosomes in many species would help provide interesting data. Jonathan Dinman has made great strides in this direction.

What are your hopes for the future of your research?

We hope to uncover the quantitative energy landscape of translocation.

Detail

RESEARCH OBJECTIVES

Dr Sanbonmatsu's research is focused on using molecular simulations to understand tRNA movement through the ribosome and interpret cryo-EM data. She is also using chemical probing to study structures of long non-coding RNAs in her wet lab.

FUNDING

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COLLABORATORS

- Scott Blanchard, Weill Cornell Medical College
- Wataru Nishima, New Mexico Consortium
- Kara Fischer, New Mexico Consortium
- Christian Spahn, Charite Universitätsmedizin Berlin
- Jonathan Dinman, University of Maryland

BIO

Dr Sanbonmatsu has been a principal investigator at Los Alamos National Laboratory since 2001. She received her BA in Physics from Columbia College at Columbia University and PhD in Astrophysical, Planetary and Atmospheric Sciences from the University of Colorado at Boulder. In 2012, she was elected fellow to the American Physical Society for 'pioneering the computer simulation of molecular machines and biomolecular complexes'.

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