tRNA from a genomic perspective

Professor Tao Pan, from the University of Chicago, focuses on investigating the diversity of transfer RNA (tRNA) and its role in both translation and extra-translational processes. In order to achieve these goals, Professor Pan and his team have developed high throughput sequencing methods that quantify the abundance, modification and charging of tRNA. Furthermore, the team are also exploring how mistranslation can be adaptive under stressful environmental conditions.

Sequencing the human genome was one of the most revolutionary biological studies conducted in the twenty-first century. The first draft human genome sequence, published in 2001, revealed areas where our knowledge was limited, for example in the field of ribonucleic acid (RNA). This inspired Professor Pan to focus on the field of RNA biology and in particular transfer RNA (tRNA). By developing new methods to examine many aspects of tRNA, the team was able to conduct in-depth studies which investigate the diversity and function of tRNA from a genomic perspective.

THE ROLE OF tRNA IN GENE EXPRESSION

Gene expression, whereby information provided by the genetic code is used to synthesise proteins, is the foundation of life. Messenger RNA (mRNA) replicates a specific DNA segment which encodes a protein. However, RNA and proteins, which consist of amino acids, have different chemical structures. Therefore, an adaptor molecule is required to interpret the genetic information encoded by the mRNA. These translational tools, cloverleaf in secondary structure consisting of three hairpin loops, and L-shaped in three-dimensional structure, are called transfer RNA (tRNA). One of the hairpin loops contains an ‘anticodon’, a specific three-nucleotide sequence that binds to a complementary mRNA 'codon'. The codon-anticodon pair encodes a particular amino acid which is covalently attached to the tRNA. Eventually an amino acid chain is formed which is then further processed to form functional proteins.

ABUNDANCE, MODIFICATION AND CHARGING

Professor Pan and his colleagues performed functional genomic studies on tRNA. Functional genomics is a field of molecular biology that explores all molecules of the same type (i.e. tRNA) in cells simultaneously. The team explored the three processes that occur during mRNA decoding: abundance, modification and charging.

'Abundance' refers to the quantity of each tRNA type per cell. The human genome has around 600 tRNA genes distributed among around 300 different species. Interestingly, Professor Pan and his team discovered that humans have a high level of tRNA genetic diversity termed 'isodecoders' – tRNAs which have the same anticodon, but have different body sequences. Preventive and identity elements, found in the body sequence, ensure that the correct amino acid is covalently attached to the tRNA. However, altering the body sequence to produce a new isodecoder may remove the vital preventive element, meaning that an incorrect amino acid could be attached to the tRNA, increasing the risk of mistranslation. Outwardly, it appears that isodecoders are harmful. However, studies have shown that isodecoders can actually

Professor Pan and his colleagues have revolutionised the way in which we view tRNA from a genomic perspective

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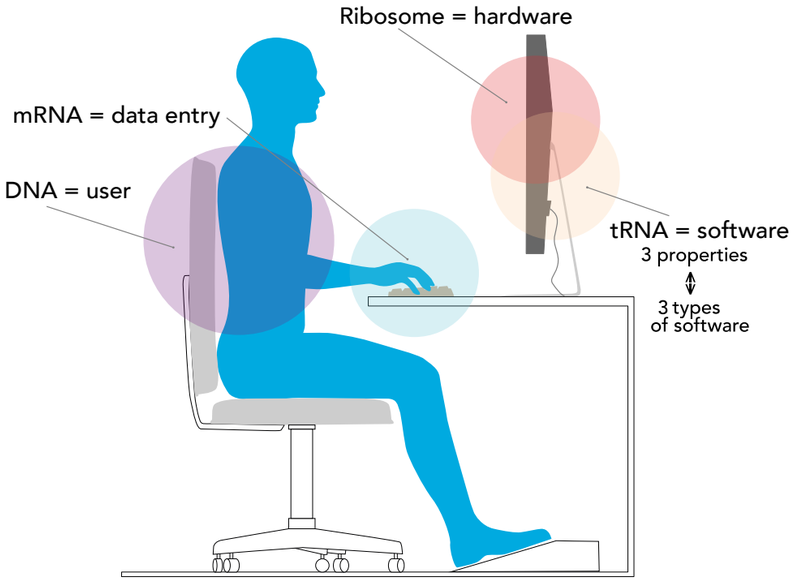
fine-tune translation by enabling cell-specific expression. For example, the isodecoder Arg-UCU is expressed in the mouse central nervous system and reduced abundance of this tRNA results in neurodegeneration.

'Modification' occurs when tRNA is chemically altered. In fact, tRNA is the most highly modified RNA, with an average of 13 modifications per molecule. However, individual tRNAs are modified unevenly, for example, a tRNA from the placenta has as many as 17 modifications, whereas another tRNA from HeLa cells has only three modifications. Changes can occur both within and outside of anticodon region. For example, methylation within the wobble anticodon can enhance codon-anticodon interaction. Methylation outside the anticodon loop can alter the structural integrity and chemical property of tRNA, vital for tRNA stability or effective ribosome interaction. Lastly, 'charging' refers to the frequency of tRNA molecules with the same sequence, carrying the same amino acid.

Interestingly, these three distinct characteristics (abundance, modification and charging) coordinate to affect translation depending on cell type and environmental conditions. For example, the team showed that starvation of a certain amino acid results in differential charging of different tRNA species carrying the specific amino acid. Furthermore, Professor Pan showed that alterations in these tRNA properties are linked to disease. Tumour cells overexpress tRNA at greater quantities compared to healthy cells.

OTHER FUNCTIONS OF tRNA

Professor Pan and his team also show that tRNA interacts with a variety of proteins involved in processes not directly related to protein synthesis. Using a combination of computational and experimental approaches, his team found numerous human proteins that interact with tRNA in cells, even though these proteins were not previously considered to be RNA binding proteins. These tRNA-protein interactions can play a communicative role in adjusting the activity of cellular pathways to that of translation. For example, mitogen-activated protein kinase kinase (MEK) interacts with tRNA which alters its enzymatic activity with the potential to regulate cell cycle progression.



ADAPTIVE MISTRANSLATION

It is vitally important that cells balance translational accuracy with speed. Inevitably, this results in mistranslation with approximately one error per 1,000 to 100,000 amino acids. Error is a fundamental part of all biological systems and typically leads to deleterious effects. However, Professor Pan has showed that, in the case of translation, error can be actively induced and even be beneficial. One of the best examples of beneficial mistranslation, seen in all three domains of life, is methionine (Met) mistranslation, whereby the amino acid methionine is charged to non-methionyl-tRNAs, leading to cells making proteins with non-methionine to Met substitutions. In human cells, it was shown that Met mistranslation can increase up to ten-fold in response to oxidative stress and enhances the oxidative stress response. This is likely due to the antioxidant properties of methionine residues.

Additionally, this stress response is also seen in the archaeon *Aeropyrum pernix*, found in deep sea hydrothermal vents. Under optimum temperature conditions (90°C), there is no evidence of Met mistranslation. However, Met mistranslation is induced when *A. pernix* is grown at 75°C. This is because the hyperthermophilic proteins in *A. pernix* made according to the DNA code function better at higher temperatures. In cooler environments the proteins become more rigid and their activity is reduced. However Met mistranslation can enhance the flexibility of hyperthermophilic proteins, improving their enzymatic activity at cooler temperatures. Inducible Met mistranslation also occurs within the bacterial domain. For example, the protein integrity of *Escherichia coli* is compromised with greater levels of Met mistranslation during antibiotic stress or anaerobic growth. Met mistranslation increases the diversity of the cellular proteins. As a result, the bacteria are much more stress resistant.

Overall, Professor Pan and his colleagues have revolutionised the way in which we view tRNA from a genomic perspective. However, more research is needed to fully appreciate the diversity and variety of functions that tRNA performs. Professor Pan thinks that future research should investigate the role of isodecoders, the importance of tRNA extra-translational processes and the complex and dynamic relationship between abundance, modification and charging in decoding.

tRNA interacts with a variety of proteins involved in processes that are not directly related to protein synthesis

Q&A

What has been your most exciting research finding so far?

There have been quite a few throughout my career. For tRNA, the most exciting discovery about ten years ago was the inducible Met mistranslation in human cells. Follow up studies identified this biological mechanism in stress response and adaptation in eukaryotes, archaea and bacteria. This mechanism expands the cellular protein diversity by reading the genetic code in another way. Recently, we developed high throughput sequencing technologies to measure tRNA abundance, modification and charging. These new technologies are being applied to a wide range of human health and disease investigations including disease biomarkers, diagnostics, and microbiome characterisations.

What is the role of transfer RNA in the process of gene expression?

tRNA reads the genetic code through pairing with mRNA and carries a covalently attached amino acid that is cognisant of the mRNA code. Decoding occurs on a molecular machine called ribosome that produces proteins according to the mRNA code. This flow of genetic information can be compared to a computer: mRNA is the input data, ribosome is the hardware, and tRNA is the software that converts input data to files or products. The three properties of tRNA represent three types of software such as web browser, office suite, and graphics which all together expand the capability of the computer.

How can modification affect the function of tRNA?

Modification increases the chemical diversity of the tRNA, expanding the four letter code of RNA to over 50 in the case of human tRNA. Generally speaking, modifications in the anticodon loop affect the efficiency, selectivity, and accuracy of decoding, whereas modifications outside the anticodon loop affect the folding, stability, localisation, and interaction with the ribosome. Most tRNA modifications are not essential for life, but they are important for cells to respond to stress and to adapt to changing environments. Approximately 2% of all bacterial genes code for tRNA modification enzymes, and they are still being discovered in humans.

What role does tRNA have in extra-translational processes?

By the number of molecules, tRNA is the most abundant RNA molecule in cells. Although the primary role of tRNA is for protein synthesis, we know that tRNA can also interact with other proteins to alter their activities or prevent them from interacting with other cellular components. This is the hypothetic role of tRNA as 'communicators' or cellular sensors for the level of translation activity. High translation activity leaves fewer tRNAs to be available to interact with other proteins, and vice versa. In this way, cells can adjust the speed of pathway progression in response to the level of translation.

Why is mistranslation sometimes induced as a response to stress?

Genetic code specifies only one protein sequence for each gene. Inducible mistranslation instead makes a protein 'library' from the same gene. This library expands the chemical diversity and the activity profile of the original protein encoded in the gene. Stresses come in many flavours caused by different chemicals, heat, presence of oxygen, etc., so that the response may require distinct, optimal activity profile for the stress response protein. By making a protein library through inducible mistranslation, some proteins in the library can function optimally under one stress, and other proteins under another stress condition.

Professor Pan shows that, in the case of translation, error can be actively induced and even beneficial

Detail

RESEARCH OBJECTIVES

Professor Pan's research is focused on functional genomics and the biology of transfer RNA (tRNA) and epitranscriptomics of messenger RNA modifications (also known as RNA epigenetics).

FUNDING

- National Institutes of Health (NIH)
- Congressionally directed Medical Research Programs (CDMRP)

COLLABORATORS (current)

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BIO

Dr Pan is a Professor of Biochemistry and Molecular Biology at the University of Chicago. He received his Ph.D. from Yale University and carried out his postdoctoral research at University of Colorado, Boulder. His current research focuses on the functional genomics of tRNA and the epitranscriptomics of RNA modifications.

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