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SMART MAGAZINE AUTHOR: Evelyn Yi EDITOR: Amanda

Breaking the Limiting Mindset: How the Brain Learns Control

Keywords: Learned Helplessness, Dorsal raphe nucleus, Ventromedial Prefrontal Cortex, Depression

Introduction:

Why would someone choose not to escape a painful electrical shock when given the chance? In 1967, psychologist Martin Seligman conducted a famous experiment on dogs–he strapped the dogs to a harness and administered electrical shocks to them. One group of dogs could terminate the shocks by pressing a lever nearby. The second group received the same shocks, but, pressing the lever would not affect them.

The next day, all dogs were tested in a different environment. They were placed in a “shuttlebox”, a laboratory apparatus separated into two compartments by a low barrier. This time, the dogs were unrestrained; all they had to do to escape the painful shocks was jump over the low barrier.

Surprisingly, the dogs behaved significantly differently depending on whether they had control over the shocks the previous day. 90% of the escapable shock group quickly learned to jump over the barrier and escape the shocks, yet ⅔ of the inescapable shock group did not even attempt to escape. Instead, they lay down passively and endured the shocks, even though escape was possible.

Seligman’s experiment gave rise to the influential psychological concept of “learned helplessness.” Learned helplessness illustrates that when individuals are exposed to repeated or prolonged aversive events, they learn that their actions do not impact have any outcomes. They will integrate this lesson into their beliefs and generalize it to other situations even when they are not considered objectively helpless, or when they do have control over the outcomes.

Discoveries in Human Experiments

Consolidated by later studies, learned helplessness is also observed in human subjects. Studies on college students by delivering escapable vs. inescapable loud noises or asking participants to solve solvable vs. unsolvable anagrams yielded similar patterns to the dog experiment. Those in the inescapable group consistently failed to escape or solve the anagrams. For instance, one report indicates that people from the inescapable group commented, “Nothing worked, so why try?”

Furthermore, researchers discovered that for humans, explanations or excuses people give for their failure to escape can effectively predict the duration and extent of their perceived helplessness. Subjects who attribute their failure to permanent causes (e.g., "I am always bad at solving problems") predict prolonged helplessness. In comparison, subjects attributing make attributions to temporary causes (e.g., "This was a bad day") predict short-lived helplessness. Attributions to pervasive causes (e.g., “most problems are unsolvable”) lead to helplessness across multiple situations. In contrast, attributions to specific causes (e.g., "This particular task is too hard") limit helplessness to the original context.

Lastly, studies on human subjects establish learned helplessness as a laboratory model for understanding the mechanisms underlying depressive disorders. Defined in the DSM-III (1980) and DSM-IV (1994), major depressive disorder is diagnosed when the individual exhibits at least five of the following nine symptoms:

1. Sad mood.
2. Loss of interest or pleasure in activities.
3. Significant weight loss or gain.
4. Sleep disturbances (insomnia or hypersomnia).
5. Psychomotor agitation or retardation.
6. Fatigue or loss of energy.
7. Feelings of worthlessness or excessive guilt.
8. Difficulty concentrating or making decisions.
9. Recurrent thoughts of death or suicide.

The symptoms seen in learned helplessness closely mirror those of clinical depression. Laboratory experiments with animals and humans replicated eight out of nine symptoms of depression. The only symptom not reproducible in the laboratory was suicide or suicidal thoughts, due to the ethical limitations of laboratory experiments. Conversely, depressed individuals who were not exposed to inescapable events in the experiments showed more passivity and more often failed to escape or complete cognitive tasks.

Neuroscience Revises the Theory

New neuroscience investigations revealed that although psychologists were right about the associations between exposure to inescapable negative stimuli and passivity, they got the “learning” part backwards. Passivity and anxiety, it turns out, are not learned; they are the brain’s default, instinctive reactions to harmful or traumatic experiences. Instead, it is the perception of control that must be actively learned.

The original theory of learned helplessness, as articulated by Seligman and colleagues, proposed the following two primary mechanisms:

1. Detect: Animals (or humans) detect whether the consequences of events are controlled by their behaviours.
2. Expect: After detecting uncontrollability, animals form an expectation that future events will also be uncontrollable.

The original model assumed that 'detecting uncontrollability' was the active ingredient–learned helplessness arises when an individual recognises uncontrollability. As more recent neuroscience discoveries emerge, the case proves that proves detecting control (not uncontrollability) is the critical factor for preventing passivity–learned helplessness is inhibited when an individual detects controllability.

1. Passivity/Anxiety

Aversive stimuli activate the serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN). The DRN sends serotonin (5-HT) to several brain regions, such as the periaqueductal grey, striatum, and extended amygdala. In the periaqueductal grey and striatum, 5-HT inhibits active escape behaviours. In the amygdala, 5-HT enhances fear and anxiety responses. Prolonged activation of the DRN leads to sensitisation, which persists for days, causing increased passivity and exaggerated fear and anxiety. Unlike what the detection-expectation theory assumed, this response is triggered by the intensity and duration of the aversive experience per se, not by the organism's perception of uncontrollability. In other words, regardless of whether an individual perceives the situation as uncontrollable, passivity and anxiety responses are automatically activated when intense or prolonged shocks are experienced.

2. Detection and Action (DETECT and ACT)
When control is possible (e.g., escapable shock), the prelimbic ventromedial prefrontal cortex (PL) communicates with the DRN to recognise control. Then, after detecting control, another PL pathway suppresses the DRN, preventing the activation of 5-HT neurons, and thus reducing the response of passivity and fear. This demonstrates that it is the detection of control by the prelimbic medial prefrontal circuits, not its absence that represses the instinctive DRN responses.
3. Expectation of Control (EXPECT):
After experiencing control, the PL-DRN pathway undergoes plastic changes, forming a circuit that "remembers" control. In future stress scenarios, even if the shock is inescapable, this circuit activates as if control were present. For example, an inescapable shock would not be detected as inescapable but would be experienced as if the individual had control over it. Importantly, this proves that EXPECTATION is not a conscious process but rather a neural bias shaped by prior experience of control.

Conclusion

The discovery of the prelimbic cortex and its critical role in detecting and acting upon control reframes helplessness—not as an inevitability of failure, but as a failure to detect power. When the brain learns control, it rewires itself, embedding an expectation of agency that can reinterpret even inescapable trials as valuable opportunities. These insights hint at a promising prospect where individuals can learn to be resilient and gain agency by rewiring their minds to recognise control. By taking on leadership roles or seeking positions that demand initiative, even those prone to passivity can cultivate the ability to assert control and change their previously “hardwired” responses to challenging situations.

References

Baratta, M. V., Seligman, M. E. P., & Maier, S. F. (2023). From helplessness to controllability: Toward a neuroscience of resilience. Frontiers in Psychiatry, 14, 1170417. https://doi.org/10.3389/fpsyt.2023.1170417
Maier, S. F., & Seligman, M. E. (2016). Learned helplessness at fifty: Insights from neuroscience. Psychological Review, 123(4), 349–367. https://doi.org/10.1037/rev0000033
Song, X., & Vilares, I. (2021). Assessing the relationship between the human learned helplessness depression model and anhedonia. PLOS ONE, 16(3), e0249056. https://doi.org/10.1371/journal.pone.0249056

SMART MAGAZINE AUTHOR Evelyn Yi EDITOR: Amanda

Unraveling the Brain: The Mystery of REM and Non-REM Dreams

Keywords: dreaming; sleep stages; REM sleep; NREM sleep; concept of self;

Introduction:

Some dreams we can easily recall. They are vivid, rich in details, and have almost movie-like plot developments. Others are much less memorable—more akin to GIFs or TikTok shorts rather than intense, story-packed experiences. What accounts for these differences in our dreaming experiences? The answer may lie in the distinct phases of sleep during which dreams occur.

When 8-year-old Armond Aserinsky went to bed one night in 1952, researchers first recorded periodic, rolling eye movements that accompanied periods of erratic brain activity. They also found that when awakened during this rapid eye movement period (REM), people more frequently reported having dreamed, that is, 71% of those awakened in REM sleep compared with only 17% in non-REM (NREM) sleep. Since this discovery, the term ‘REM sleep’ has become synonymous with dreaming and conscious experience, giving the impression that dreaming only occurs during REM. Yet such an assumption is incorrect. For instance, pharmacologically produced suppression of REM sleep does not eliminate dreaming nor does dream-suppressing lesions affect REM sleep. Therefore, dreaming is not exclusive to REM sleep but occurs in NREM, or n on-rapid eye movement, sleep.

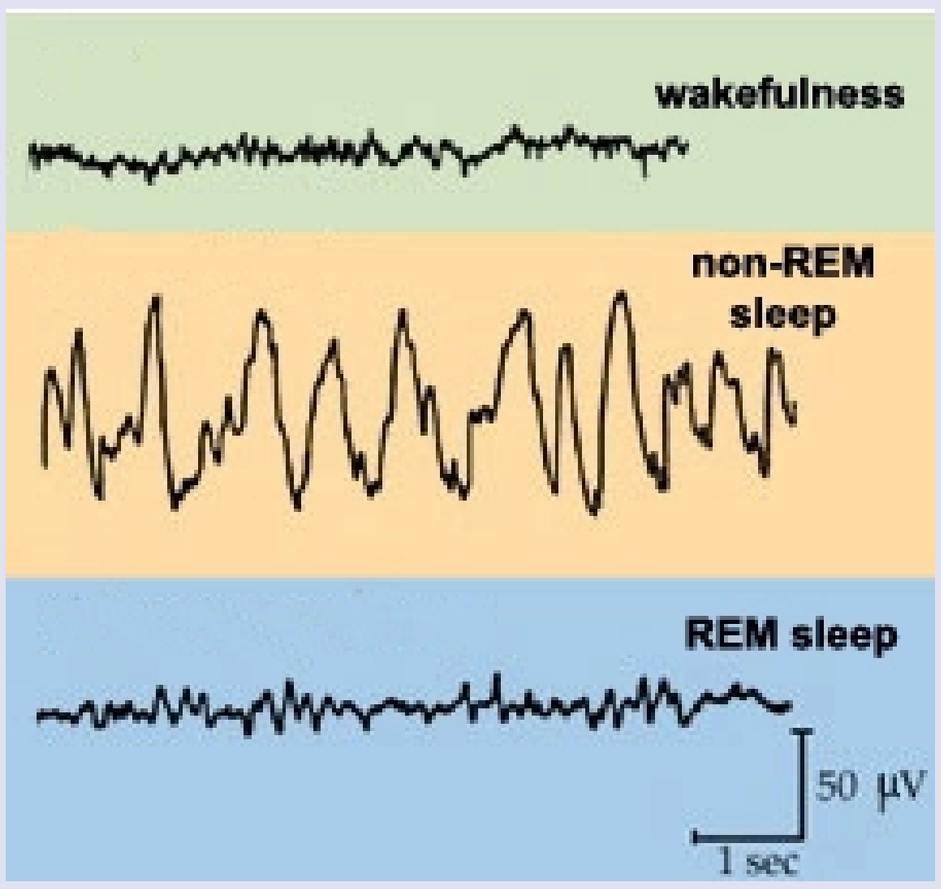


Figure 1: REM sleep and non-REM sleep

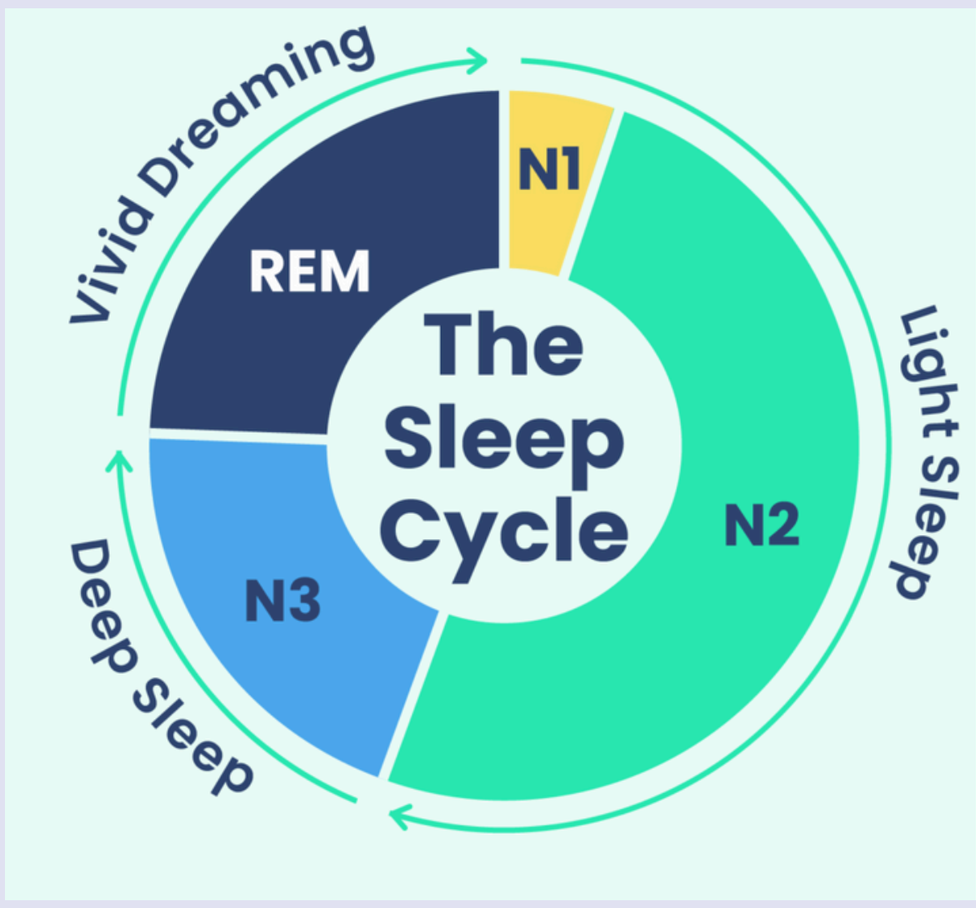


Figure 2:different sleep stages

Sleep Stages:

To understand what accounts for the differences in REM and NREM dream experiences, a closer look at the nightly cycle of sleep stages might help. People experience a night’s sleep in a cycle of 4 stages: N1, N2, N3, and REM. N1, N2, and N3 are considered NREM sleep, and about 75%-80% of our sleep is spent in NREM. Each night, people alternate between REM and NREM, with the time spent on REM gradually increasing for each cycle. A complete sleep cycle typically lasts between 90 to 110 minutes and people usually go through 4-6 cycles per night.

● N1: A person enters N1 when they first fall asleep and they can easily wake up during this stage. During this stage, muscle activities and breathing slow down and hallucinations such as the sensation of sudden falling or floating can happen. N1 lasts about 1-7 minutes.
● N2: We spend the majority of our sleep in N2, which lasts around 10-25 minutes. Individuals are clearly asleep, although they can still be awakened easily. In N2, people's brain waves can show periodic bursts of rapid activity, or sleep spindles.
● N3 (and N4): N3, usually combined with N4, is considered deep sleep. The brain emits slow delta waves and people in this stage find it hard to awaken. N3 is where night terrors and sleepwalking generally occur. People spend 20% of the time in N3 sleep, most of which occurs during the early half of the night and decreases as the night progresses.
● REM: In REM sleep, an individual's heart rate rises, breathing becomes fast and irregular, eyes dart around behind eyelids, and brain activities resemble a waking brain. Because of this, REM dreams are marked by their vividness and emotional intensity. The brain's motor cortex is active during REM but the brainstem blocks motor signals, leaving the person in muscle paralysis. This paralysis can linger as the person awakens, causing people to experience sleep paralysis–when the person is awake but unable to control their body movement. REM sleep is also called paradoxical sleep, as the person appears externally calm and asleep but is internally active.

REM Dreams v. NREM Dreams

Dream Structure

Aside from recall rates, the differences between REM and NREM dreams can also be highlighted regarding linguistic structure. A study solicited and analyzed dream reports from participants by waking them up in REM sleep and the N2 stage of NREM sleep. During N2 awakening participants were more likely to report having not dreamt (19.40% vs. 7.41%) or to have had a white dream (15.67% vs. 1.85%), which is the feeling of having dreamt but could not recall the content. More importantly, their study concludes that, in addition to greater report length, REM dream reports also demonstrate higher structural connectedness than N2 reports, suggesting that REM dreams often feature longer reports with greater structural coherence, resembling ongoing narratives.

In contrast, NREM dream reports are shorter and more fragmented, often reflecting simple, thought-like impressions (Martin et al.). This aligns with findings that NREM dreams lack the hallucinatory and story-like qualities typical of REM dreams. These differences highlight how varying brain activity during REM and NREM shapes the architecture of dreams.

Representation of the "Self"

Another distinction between REM and NREM dream characteristics can be found in their divergent representation of the Self. The properties that compose our awareness of "self"–the "I" in dreams–are notably altered in dreaming compared to how we experience the "self" in waking times. For instance, one might dream of attending a high school class when they graduated years ago or dream of seeing a dead relative. This indicates limited access to autobiographical memory and abnormal emotional reactions, and that the dream self lacks the ability to integrate past and present experiences.

Additionally, dreaming also tends to showcase impaired self-monitoring, as we may dream of nonsensical and completely bizarre happenings that do not appear out of place to the senses of our dream self. Lastly, dreams seem to display an ego-centered perspective, with the dream's narrative revolving around the dreamer's actions, emotions, desires, and experiences. In this sense, the dream self is lacks temporal coherence and an integrative sense of "self".

Due to the differing physiological responses and levels of consciousness observed in REM and NREM sleep, examining the differences in self-representation in REM and NREM dreams may tell us something intriguing about the nature of Self.

One study investigated the differences in the representation of the Self in REM and NREM dreams by systematically analyzing dream reports from a large database. Interestingly, the study found that the self in REM dreams more frequently engaged in aggressive interactions–a strikingly 52%–while the self in NREM dreams rarely acted as the aggressor. Instead, the self is represented as a more friendly image in NREM dreams. Although social encounters in NREM are mostly unpleasant, the same as other dream states, the percent of being a befriender in NREM dreams approaches 90% compared to 54% for REM dreams. Moreover, the REM dream self is more likely to appear with other people who have similar intentions, hence the frequent use of “we” in dream reports, whereas NREM dreams are more often articulated in terms of “I” (McNamara et al., 2009).

Physiological Explanations

REM sleep is characterized by the selective activation of limbic and paralimbic regions, including the lateral hypothalamus, amygdala, parahippocampal cortex, and medial and orbitofrontal cortices. These areas are deeply involved in emotional processing, memory consolidation, and motivational states, which explains why REM dreams tend to be vivid and emotionally charged.

Compared to the waking-like brain activity in REM, NREM generally indicates a global decrease in cerebral energy metabolism in positron emission tomographic (PET) studies and more localized cortical activity in electroencephalographic (EEG) studies. For example, during NREM sleep, although the thalamocortical system remains active, the brain shows reduced cortical connectivity, or diminished capability to interact with other brain regions to produce complex, integrated responses, when stimulated under experimental conditions. This disconnection between regions leads to fragmented, abstract dream experiences in NREM sleep.

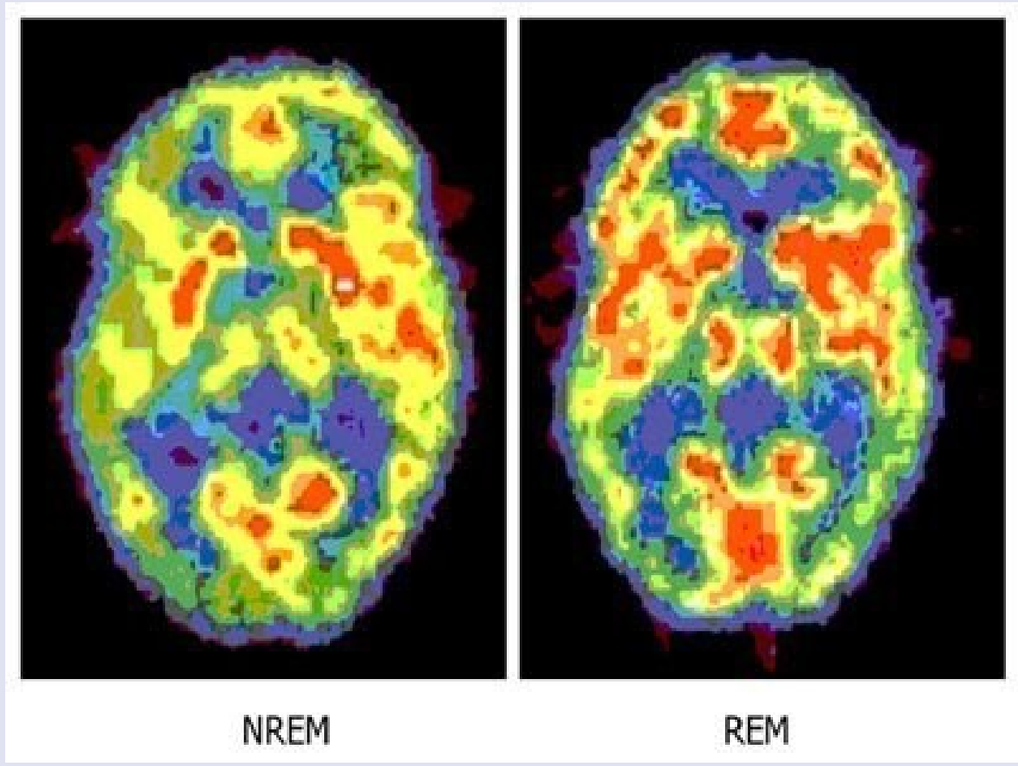


Figure 3: brain activity in NREM and REM

REM sleep, on the other hand, tends to retain most of the connectivity with other brain regions that a waking brain has (Massimini et al., 2011). This widespread communication between brain regions allows the brain to create more coherent and complex experiences, reflected in the vivid, narrative-like dreams of REM sleep.

Conclusion

Dreaming during REM typically occurs when the brain is highly active, driven by intense activity in brain regions associated with emotions and sensory experiences. NREM dreams, however, emerge when the brain is in a quieter, oscillatory state, reflecting the lower level of brain activity during this sleep stage.

Even though the current studies on physiological differences between REM and NREM can justify certain distinctive features of REM dreams such as vividity and emotional richness, they, however, are not sufficient to explain the striking dissociation seen in the roles of the Self in REM versus NREM dreams.

This leaves a host of intriguing questions concerning the nature of the Self unanswered: Why is the NREM Self constrained to engage solely in friendly interactions, while the REM Self is predominantly involved in aggressive encounters? What mechanisms in the brain dictate these divergent roles of the Self in different dream states?

By exploring such questions, we can glean deeper insights into how the brain constructs the sense of self and processes social and emotional experiences.

References

Aserinsky, E., & Kleitman, N. (1953). Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science, 118(3062), 273–274. https://doi.org/10.1126/science.118.3062.273

Martin, J. M., Andriano, D. W., Mota, N. B., Mota-Rolim, S. A., Araújo, J. F., Solms, M., & Ribeiro, S. (2020). Structural differences between REM and non-REM dream reports assessed by graph analysis. PLOS ONE, 15(7), e0228903. https://doi.org/10.1371/journal.pone.0228903

Massimini, M., Ferrarelli, F., Murphy, M., Huber, R., Riedner, B., Casarotto, S., & Tononi, G. (2010). Cortical reactivity and effective connectivity during REM sleep in humans. Cognitive Neuroscience, 1(3), 176–183. https://doi.org/10.1080/17588921003731578

McNamara, P., McLaren, D., & Durso, K. (2007). Representation of the self in REM and NREM dreams. Dreaming, 17(2), 113–126. https://doi.org/10.1037/1053-0797.17.2.113

SMART MAGAZINE AUTHOR: Nox EDITOR: Hecate

The Importance of Autophagy in the Integumentary Tapetum for Embryo Pattern

Introduction:

In May 2024, Professor Zhao Peng's team from Wuhan University published a research paper in Nature Communications titled "Autophagy-mediated degradation of integumentary tapetum is critical for embryo pattern formation" [1]. This paper primarily elaborates on the regulatory significance of autophagy in the integumentary tapetum concerning programmed cell death (PCD) and plant lipid metabolism.

Research Background

The integumentary tapetum (IT) is a specialized layer of cells located on the outer surface of the embryo sac in the ovule of angiosperms, originating from the innermost layer of the integument. Its function is similar to that of the anther tapetum, providing nutrients and signaling molecules (such as gibberellins) to support early embryo and endosperm development. Although the integumentary tapetum itself does not develop into a seed, it regulates normal embryonic development through intercellular communication, and its programmed cell death (PCD) is essential for embryo pattern formation.As the counterpart of the integumentary tapetum in the male reproductive system of plants, the anther tapetum has been extensively studied for its role in promoting male reproduction through PCD. Although the integumentary tapetum also facilitates embryo development via PCD, its underlying mechanism remains unclear. Therefore, the authors conducted this study to explore the mechanism involved.

Autophagy can be categorized into three major types: macroautophagy, microautophagy, and chaperone-mediated autophagy. This study focuses on macroautophagy. Previous research has identified ATG (AUTOPHAGY-RELATED) genes as key regulators of macroautophagy, exhibiting relatively high expression levels and dynamic temporal changes during tobacco seed development. This finding suggests that autophagy plays a crucial role in seed development [2].

Research Content and Results

By utilizing GFP gene fusion targeting ATG genes and immunofluorescence techniques, the researchers found that ATG5, ATG7, and autophagosomes were abundantly present in the integumentary tapetum (IT), confirming the existence of active autophagy in IT. Additionally, electron microscopy revealed the detailed ultrastructure of autophagosomes and autolysosomes within IT cells.

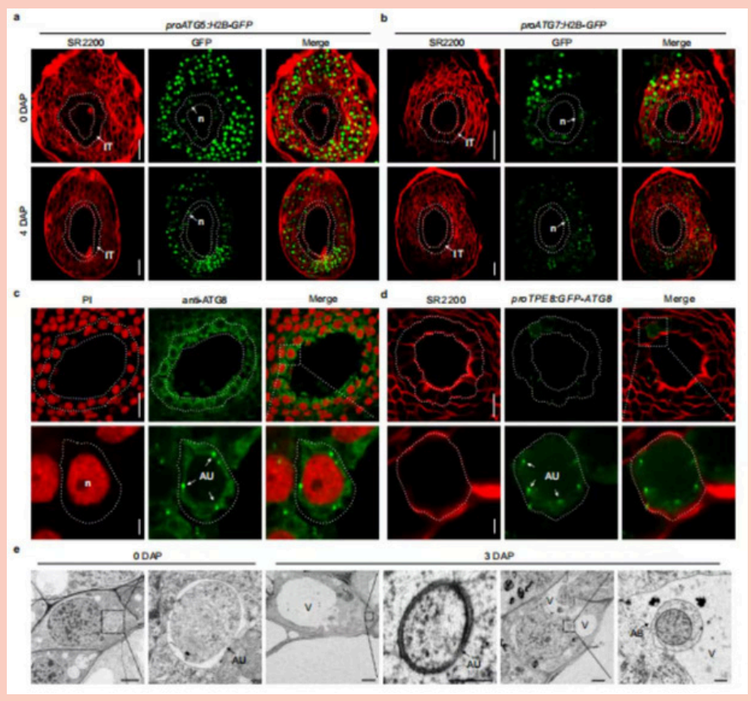


Figure 1. Active autophagy in IT during seed development(a, b, d: Red represents the cell wall, green represents ATG; c: Red represents the nucleus, green represents ATG; e: Transmission electron microscopy (TEM) images, showing typical autophagosomes and autolysosomes in the integumentary tapetum.)

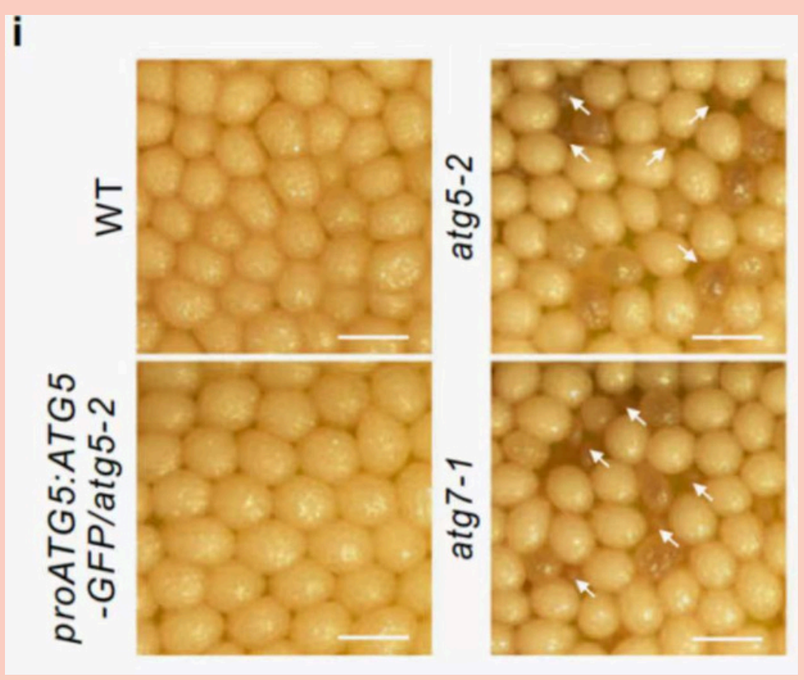


Figure 2. Seed development（mutans exhibit varying degrees of abortion）

To further investigate the impact of IT autophagy on seed development, the research team constructed atg5 and atg7 mutants and found that autophagic activity was significantly reduced in these mutants, leading to a substantial increase in seed abortion rates. However, when ATG5 was reintroduced into the mutants, their phenotype was restored.

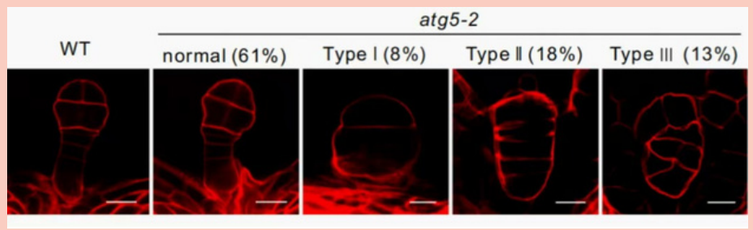
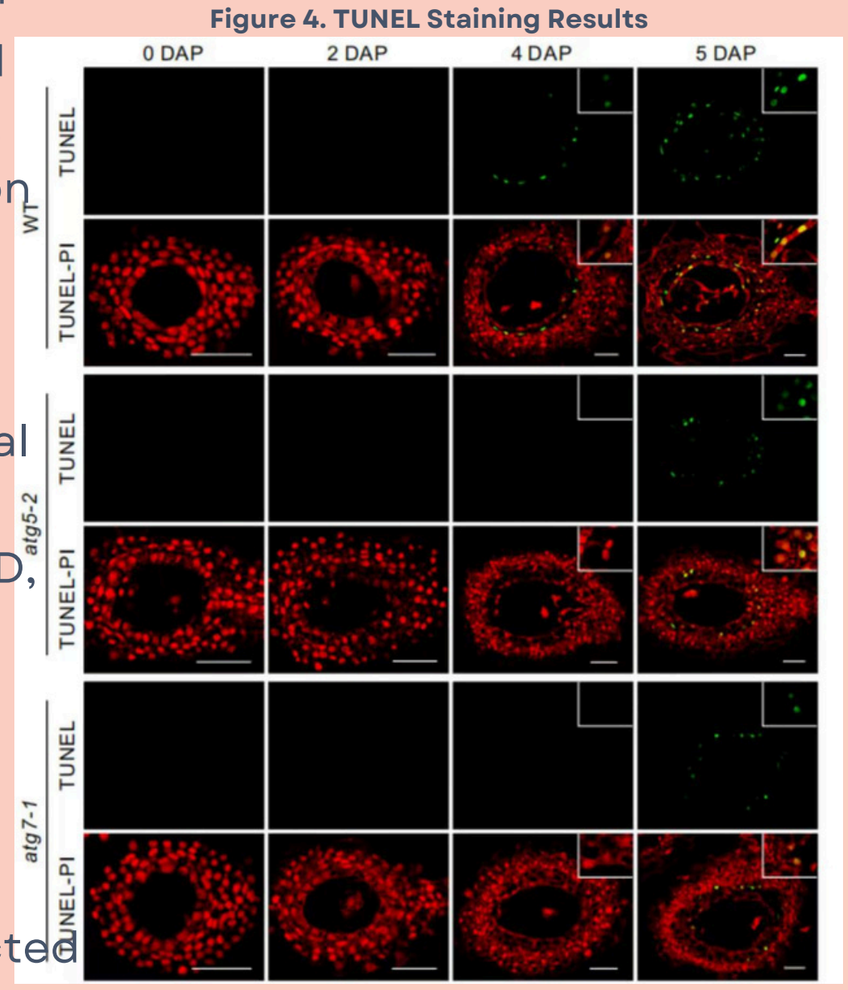


Figure 3. Enbryo development patterns

Additionally, the researchers observed that before fertilization and within the first four days post-fertilization, there was no significant difference between the mutants and the wild type. However, after four days post-fertilization, the loss of autophagic activity in the mutants prevented IT degradation, unlike in the wild type, resulting in a gradual thickening of IT. Moreover, the mutants exhibited three distinct abnormal embryonic development patterns, where zygote division deviated from the normal process. This finding suggests that autophagy plays a crucial role in embryo development.

Figure 4. TUNEL Staining Results



To investigate the relationship between autophagy and programmed cell death (PCD), the researchers performed TUNEL staining to detect DNA fragmentation (a hallmark of PCD) and used transmission electron microscopy (TEM) to observe nuclear membrane integrity.The results showed that four days after fertilization, the wild-type IT exhibited abundant DNA fragmentation, nuclear membrane rupture, and cytoplasmic vacuolization. In contrast, the mutant IT displayed minimal DNA fragmentation, and the nuclear membrane remained intact.This experiment demonstrated that ATG deficiency leads to the loss of PCD, further suggesting that autophagy-related genes play a crucial role in initiating PCD.

How does autophagy influence embryo development through PCD? To investigate the molecular connection between IT and embryo pattern formation, the researchers isolated embryos from wild-type and mutant plants and performed RNA sequencing. They then conducted principal component analysis (PCA) and hierarchical clustering analysis on the sequencing results.The results showed a significant decrease in the number of differentially expressed genes (DGEs) in the mutants, suggesting the regulatory role of ATG in embryo differentiation. Subsequent GO analysis also identified several pathways related to embryo pattern formation, including pattern specification and auxin-related pathways, all of which were regulated by autophagy in IT.This finding further confirmed the non-cell-autonomous role of autophagy in the process of embryo development.

In addition to elucidating how IT autophagy regulates embryo development through PCD, this study also highlights its dual role in lipid metabolism during embryo development. By analyzing the differences in lipid metabolism between wild-type and mutant plants in egg cells and seeds, the researchers found that before fertilization, ATG inhibited triacylglycerol (TG) accumulation, whereas after fertilization, ATG promoted TG accumulation. This indicates that autophagy is involved in both the degradation and biosynthesis of TG in plant lipid metabolism.

Discussion

This study innovatively demonstrates that autophagy in the integumentary tapetum influences plant embryo pattern formation through both PCD and lipid metabolism. While PCD is an important regulatory mechanism of cell life, the role of autophagy in this process remains unclear. The regulation of PCD by autophagy is cell type-specific. For example, autophagy is essential for PCD in proximal root cap cells but not necessary for PCD in distal lateral root cap cells [3]. The regulatory patterns of PCD in different cell types and their physiological significance remain important topics for future research.

References

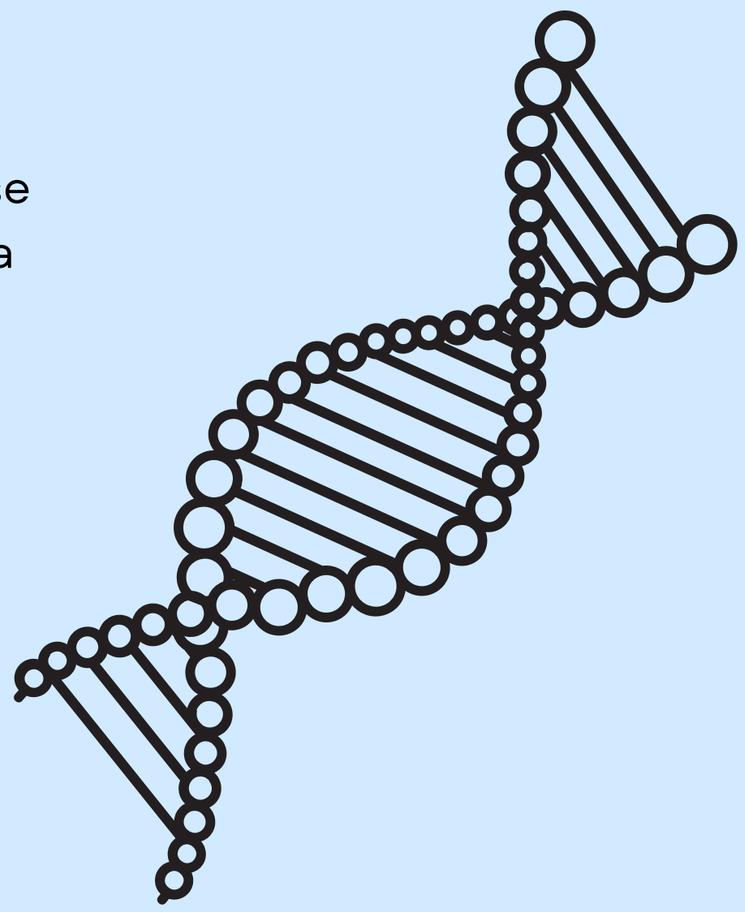
• Zhao, L. L., Chen, R., Bai, Z., Liu, J., Zhang, Y., Zhong, Y., ... & Zhao, P. (2024). Autophagy-mediated degradation of integumentary tapetum is critical for embryo pattern formation. Nature Communications, 15(1), 2676.
• Zhou, X. M., Zhao, P., Wang, W., Zou, J., Cheng, T. H., Peng, X. B., & Sun, M. X. (2015). A comprehensive, genome-wide analysis of autophagy-related genes identified in tobacco suggests a central role of autophagy in plant response to various environmental cues. DNA Research, 22(4), 245-257.
• Feng, Q., De Rycke, R., Dagdas, Y., & Nowack, M. K. (2022). Autophagy promotes programmed cell death and corpse clearance in specific cell types of the Arabidopsis root cap. Current Biology, 32(9), 2110-2119.

SMART MAGAZINE AUTHOR: Ariana EDITOR: Hecate

From Off-Target to On-Demand: How Chinese Innovations in Base Editing Are Revolutionizing Genetic Medicine

Introduction

In recent years, significant breakthroughs have been made in the research of single-base editing technology. The core function of base editing genes is to use a dead Cas9 protein to bring a deaminase to a specific location, where it catalyzes deamination, triggering a cell repair response, ultimately achieving a single-base conversion. As gene editing has advanced, its application in rare diseases has been both the most promising and the first to be practically applied. Base editing is an important gene-editing technology, but there are still some issues that need to be addressed, with hopes for gradual solutions in the future.



Existing Problems in Base Editing

The first issue is the limited types of editing. Professor Liu Ruqian was the first to develop cytosine base editors (CBE) and adenine base editors (ABE). In theory, base editing allows for 12 types of conversions, but only four have been successfully achieved, with eight remaining unachieved. Future work is expected to make these other conversions possible.

Additionally, a serious issue with single-base editors is “bystander editing,” where the editor inadvertently modifies bases adjacent to the target base. Researchers are hoping for more precise editing, as nearly all rare diseases and genetic conditions are caused by single nucleotide changes.

Another issue is the low efficiency of base editing. Currently, to treat a disease, it is necessary to first conduct experiments in cell and animal models or organoid models before proceeding to therapeutic applications. This aspect of base editing lags behind in practical applications.

First-Generation Base Editors

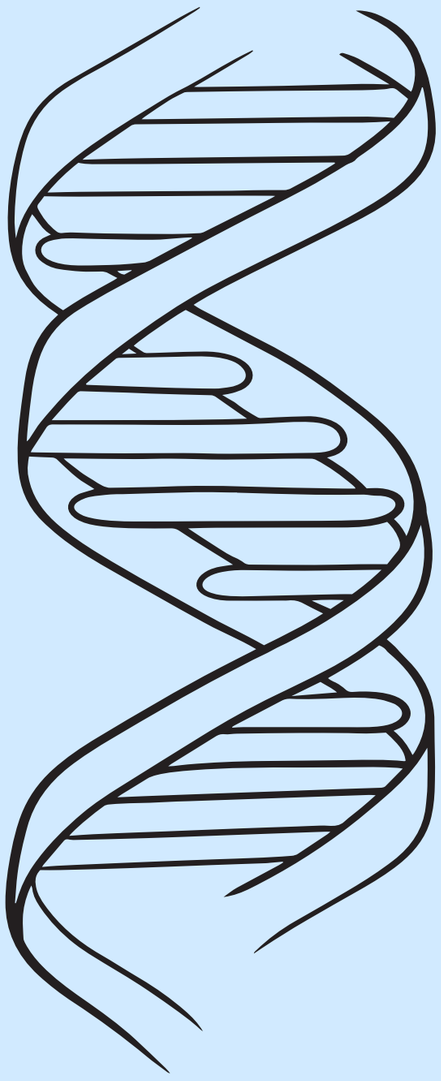
In recent years, China developed the GBE (Gene Base Editor), a base editor for both base conversions and base exchanges. This editor builds on Professor Liu Ruqian’s CBE technology, where a cytosine deaminase is brought to a specific location to deaminate cytosine, converting it into uracil (U). Uracil is recognized as thymine (T) in base pairing, which, during replication, triggers repair, ultimately resulting in a C-to-T conversion. However, if uracil is created by deaminating cytosine, a glycosylase can be used to excise the base from the DNA backbone, leaving an abasic (AP) site. This induces a new DNA repair mechanism and leads to a C-to-G conversion with the GBE base editor.

On this basis, Chinese scientists developed the second-generation GBE base editor, using a more potent and active uracil glycosylase and optimizing the linker structure between the two proteins. Internationally, David Liu and J. Keith Joung's research groups simultaneously developed the second-generation CGBE, with relatively advanced performance.

It is well-known that, in both gene editing and base editing, the crucial aspect is designing the gRNA, as the choice of target site for the gRNA is very important. To address the challenges in selecting gRNA target sites, Chinese scientists have developed a deep learning model for the GBE, collecting high-throughput editing data to train the AI model. After learning, the AI can generate weight maps to indicate which nucleotide compositions at the target site are favorable for improving efficiency. Additionally, scientists have built machine learning models that predict the efficiency of any given gRNA, allowing for high-efficiency gRNA to be identified before formal experiments.

Currently, base editing research in China is relatively limited. Whether in base editing or first-generation CRISPR/Cas9 editing, all editing results are indirect, as they trigger mammalian cell repair systems. Therefore, base editing research is typically carried out in eukaryotic Saccharomyces cerevisiae (baker's yeast) cells, which are highly homologous to mammalian cells, and extensive gene knockout experiments in yeast cells can help identify enzymes critical to the repair process.

Second-Generation Base Editors



This year, Chinese scientists developed a glycosylase-based base editor with high efficiency, but it suffers from serious off-target issues, especially with cytosine. This editor does not depend on Cas9. There are two primary causes for off-target effects: one is the inherent tolerance of Cas9 protein to mismatched sequences, meaning even when the gRNA is not perfectly matched, it may still perform editing, resulting in Cas9-dependent off-target effects. However, the current deaminase is highly active, and after evolution, cytosine deaminase can cause unpredictable, random mutations across the entire genome, which is a significant risk. Therefore, scientists aim to avoid off-target effects at the whole-genome level by directly removing cytosine or other bases from the DNA single strand without relying on deamination.

Researchers are also trying to develop enzymes for cytosine and thymine. Unfortunately, these glycosylases do not exist in nature, and currently, there is no base editor capable of targeting thymine. In Escherichia coli, a mutation screening system has been designed. By constructing a low-activity glycosylase editor and using this system to restore a designed tryptophan mutant to a functional tryptophan synthase, E. coli can synthesize tryptophan and survive in simple media.

Using a growth-coupled screening system, large numbers of mutants can be accurately screened. After several rounds of screening, mutants with higher activity can be obtained. After nearly 1.5 years of screening, the scientists successfully developed a high-activity cytosine glycosylase and a glycosylase capable of directly excising thymine. After obtaining the glycosylases, they aimed to bring these editors from E. coli to mammalian cells. After codon optimization and structural adjustments, the first-generation glycosylase-based editor was obtained, with a C-to-G editing efficiency of about 40%, comparable to the performance of popular CBE and ABE editors. The T-to-G base editor has a narrower editing window, making it more suitable for applications in genetic and rare diseases.

After further optimizing the first-generation glycosylase-based base editor, a second generation was developed. This second-generation editor outperforms the first in terms of efficiency and specificity, though the editing window has changed. Notably, no RNA off-target effects were observed, and DNA off-target effects remained below the noise level, indicating that the second-generation glycosylase-based base editor is safer than the first generation. Currently, the efficiency of domestic base editors is still slightly lower than the highest-performing international editors, and scientists are continuing optimization efforts to achieve even better glycosylase-based base editors.

Best gRNA Database for Immediate Use

In single-window editors, off-target effects often occur when the gRNA is not perfectly matched, and this effect increases over time. Researchers believe this is due to Cas9's high tolerance for mismatched gRNAs, resulting in off-target effects similar to mismatches. Therefore, scientists introduced non-100% matching sites in the gRNA, greatly reducing off-target rates while increasing single-base editing efficiency. However, the best-performing gRNA is still to be determined, requiring high-throughput testing of different gRNAs.

The ClinVar database now includes over 37,000 known SNV loci for genetic diseases, with more being added daily. To address SNVs corrected by ABE, scientists have cloned all relevant loci and integrated them into cells using lentivirus. By performing high-throughput editing on the cell library and collecting data, they can determine the best gRNA for each disease locus. Most gRNAs have been chemically modified, and using mechanisms like intron splicing or viral cleavage can greatly improve stability, thereby enhancing editing efficiency.

An automated system has been built in microbial cell factories for full automation of cell editing. Within two weeks, several thousand mammalian cells can be edited, provided that the transformation system is sufficiently efficient. The steps for full automation include: 1) automated construction of primer pools, 2) automated generation of editing instructions, 3) automated cell transformation, and 4) fully automated collection of edited cells and measurement of editing efficiency. Although manual intervention is still needed for subsequent operations, such as single-clone isolation from 384-well plates, the editing process itself is fully automated. This system enables high-throughput determination of editing efficiency for thousands of loci, and AI models can be used to predict the relationship between gRNA and editing efficiency. The results of these predictions have been relatively accurate. The impact of chromatin accessibility on editing efficiency is approximately 1:5 for CBE, meaning chromatin accessibility contributes, on average, one-sixth to editing efficiency.

Finally, scientists have found that many cell factors significantly affect base editing efficiency. They have screened for these factors and discovered a class of “pioneer factors” that can greatly enhance editing efficiency before replication and transcription. By fusing these cell factors with the editor, they have seen improvements in efficiency, especially with the PE editing systems (PE3, PE5). This recruitment mechanism can be applied to non-template RNAs or regular RNAs without PEG (polyethylene glycol), showing a significant increase in efficiency compared to the original PE3/PE5 systems.

Applications for Treating Genetic Diseases

Retinitis pigmentosa is a rare genetic disease caused by gene mutations. In the normal sequence, GCG codes for arginine, but in affected individuals, it is mutated to GCA, leading to the replacement of arginine with cysteine. This mutation disrupts the activity of the PD6B enzyme, which plays a crucial role in transmitting retinal light and chemical signals. When PD6B is damaged, toxic intermediates accumulate, damaging retinal neurons and causing blindness.

The principle of base editing to treat this disease is straightforward: researchers use the ABE base editor to correct the GCA mutation to GCG, restoring the normal gene sequence. The key is optimizing the editor’s efficiency using commonly used 293-mode cells. Through gRNA selection and editor optimization, the highest editing efficiency achieved so far is approximately 60%.

After optimizing the base editor, due to the limited capacity of AAV (adeno-associated virus), it must be split into two parts and placed into two separate AAV vectors, which are then delivered to the lens. The researchers injected the vectors into the retina of mice on approximately day 14 for functional validation.

Upon collecting the mice for functional validation, the genome editing efficiency was about 20%, but the editing efficiency of cDNA that actually exerted its function could reach 40%. However, whether this can truly restore visual function in the mice requires further pathological, behavioral, and optoelectronic analysis. In the control group, the outer nuclear layer was severely suppressed, showing almost no growth. However, in the injected areas, the outer nuclear layer grew well, and the response to light was significantly restored. The water maze experiment also demonstrated that, compared to the control group, the treatment group exhibited significant behavioral recovery.

This represents an important application of base editing. Genetic eye diseases are among the most treatable hereditary conditions and present a promising avenue for commercialization, offering hope for application to other diseases in the future.

Citation

Dong, X., Yang, C., Ma, Z., Chen, M., Zhang, X., & Bi, C. (2022). Enhancing glycosylase base-editor activity by fusion to transactivation modules. Cell Reports, 40(3), 111090. https://doi.org/10.1016/j.celrep.2022.111090

Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A., & Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature, 533(7603), 420–424. https://doi.org/10.1038/nature17946

Zuo, E., Sun, Y., Wei, W., Yuan, T., Ying, W., Sun, H., . . . Yang, H. (2019). Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. Science, 364(6437), 289–292. https://doi.org/10.1126/science.aav9973

Kim, H. K., Yu, G., Park, J., Min, S., Lee, S., Yoon, S., & Kim, H. H. (2020). Predicting the efficiency of prime editing guide RNAs in human cells. Nature Biotechnology, 39(2), 198–206. https://doi.org/10.1038/s41587-020-0677-y

Esvelt, K. M., Carlson, J. C., & Liu, D. R. (2011). A system for the continuous directed evolution of biomolecules. Nature, 472(7344), 499–503. https://doi.org/10.1038/nature09929

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., . . . Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature, 576(7785), 149–157. https://doi.org/10.1038/s41586-019-1711-4

Maeder, M. L., Stefanidakis, M., Wilson, C. J., Baral, R., Barrera, L. A., Bounoutas, G. S., . . . Jiang, H. (2019). Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nature Medicine, 25(2), 229–233. https://doi.org/10.1038/s41591-018-0327-9

Landrum, M. J., Lee, J. M., Benson, M., Brown, G. R., Chao, C., Chitipiralla, S., . . . Maglott, D. R. (2017). ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Research, 46(D1), D1062–D1067. https://doi.org/10.1093/nar/gkx1153

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The Intricacies of tRNA and the CCR4-NOT Complex

Keywords: tRNA modifications, tRNA processing, proteomics, CCR4-NOT complex, de-adenylation, tRNA translation mechanisms

1. The Cellular Energy Carrier – tRNA

In the past decade, the mRNA industry has made rapid progress and achieved tremendous success, such as with mRNA vaccines and modification technologies. mRNA-based therapies have shown enormous medical and market potential. The achievements of the mRNA industry also reflect the vast therapeutic potential of RNA modifications. Increasing evidence suggests that tRNA modifications play a crucial role in human diseases, including cancer and neurological disorders. The following focuses on the physiological functions and pathological effects of tRNA modifications.

tRNA Biosynthesis:

tRNA molecules are approximately 70-80 nucleotides long and fold into a secondary "cloverleaf" and tertiary "L-shaped" structure. The cloverleaf structure consists of five parts: the acceptor stem, the 5' and 3' ends containing the tRNA, the D-loop, the anticodon loop, the variable loop, and the T-loop. The process begins with the transcription of tRNA genes by RNA polymerase III (Pol III) and the transcription factors TFIIIB and TFIIIC in the nucleus, generating a precursor tRNA (pre-tRNA) of approximately 100 nucleotides. This precursor tRNA undergoes initial trimming, where RNase P and RNase Z remove the 5' leader sequence and 3' trailer sequence, respectively. The precursor tRNA is then processed by the tRNA splicing endonuclease (TSEN) complex and hematopoietic stem cell 117 (HSPC117) splicing. Additionally, the CCA-adding enzyme synthesizes the 3'-CCA end of the pre-tRNA using ATP and CTP. Before being exported to the cytoplasm via the export protein t (Xpo-t), the pre-tRNA undergoes further modifications and is ultimately processed into a mature tRNA of 70-80 nucleotides in length.

Finally, aminoacyl-tRNA synthetase acetylates the mature tRNA and attaches it to the 3' end in the cytoplasm or nucleus, after which it is catalyzed by elongation factor Tu and GTP complexes (EF-Tu-GTP) to bind to the ribosome's A-site for translation.

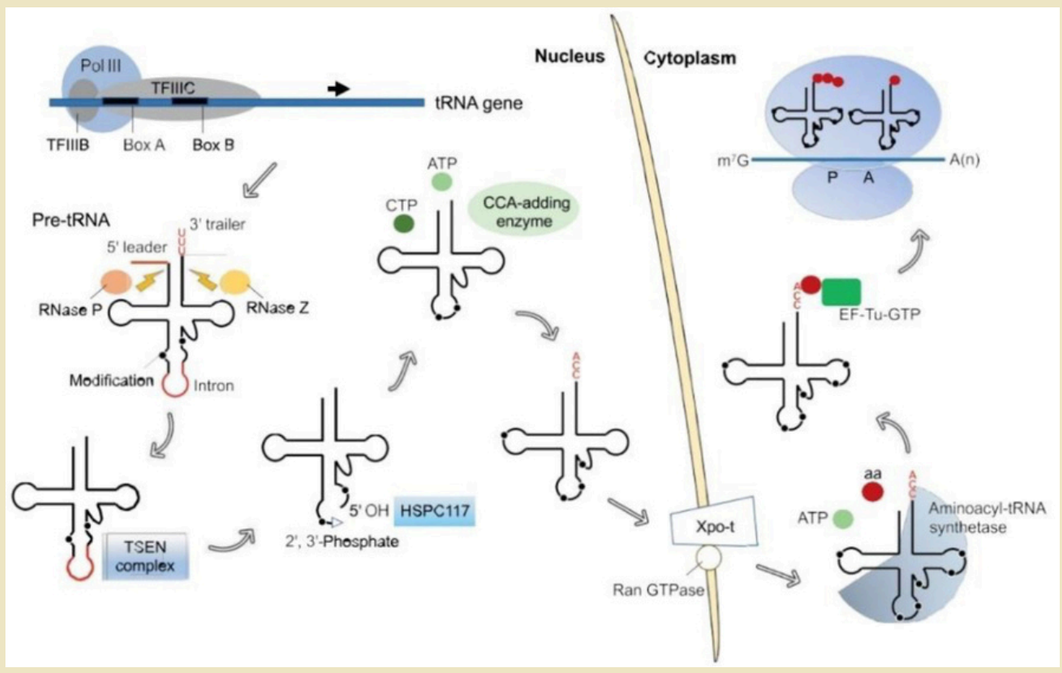

Figure1. The synthesis of tRNA

tRNA Biochemical Modifications:

tRNA modification refers to various chemical modifications made to its nucleotides, which can alter the structure and function of tRNA, thereby impacting protein synthesis and cellular metabolic processes. Representative steps of tRNA modification typically include the processing of the 5' leader and 3' tail, removal of introns, and addition of CCA. There are at least 70 known modifications on tRNA, and each modification affects different regions of the cloverleaf structure (various loops), which leads to different functional outcomes. These modifications can be categorized into the following main types:

1. Expanding or restricting base pairing ability.
2. Enhancing the stability of codon-anticodon binding.
3. Affecting translation initiation and maintaining the reading frame.
4. Regulating intracellular metabolism.

Methods for Analyzing tRNA Expression and Modifications:

The advancement of tRNA modification sequencing technologies is crucial for analyzing and studying the functions of tRNA modifications. Due to the widespread modification and stable three-dimensional structure of tRNA, converting tRNA to complementary DNA (cDNA) can lead to premature termination or mismatches in base pairing. Thin-layer chromatography (TLC), liquid chromatography, and mass spectrometry (LC/MS) can access all modification types in purified tRNA without requiring reverse transcription (RT). Another traditional measurement method, primer extension analysis, can identify tRNA modification sites by detecting RT termination or mismatched base insertions, but it has a high false-positive rate and cannot identify modification types. Currently, sequencing methods such as hydrazine cleavage sequencing (HAC-seq) and m7G reduction and cleavage sequencing (TRAC-seq) are used to analyze specific tRNA modifications more efficiently and accurately through unique chemical treatments. These analytical techniques have significantly enhanced our understanding of tRNA expression and modifications.

Methods for Analyzing tRNA Expression and Modifications:

The CCR4-NOT complex is a major mRNA deadenylase. The core CCR4-NOT complex consists of nine subunits and plays a role in post-transcriptional regulation of mRNA stability by shortening the poly(A) tail or removing adenylation, as well as in other gene regulation functions.

Structure of the CCR4-NOT Complex:

In yeast, the complex is centered around the scaffold protein Not1p, with the other components including CCR4, Caf1, Caf40, Caf130, and Not1-4. Homologs of these subunits are also found in other eukaryotes.

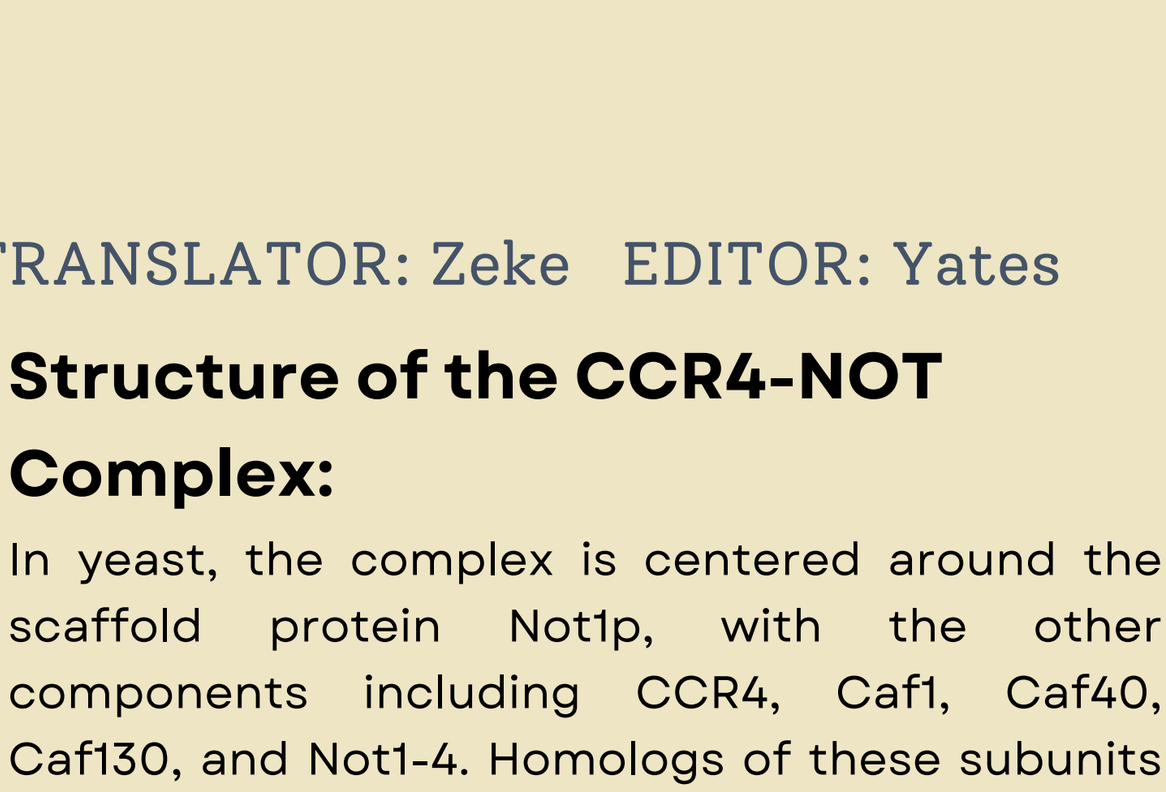

Figure 2. Schematic of the CCR4-NOT Complex

- Not1: The large Not1 protein links the other subunits of the CCR4-NOT complex, serving as the scaffold for the entire complex. In yeast, Not1 is the only protein in this complex that plays a crucial role in yeast fertility.
- Not2: The Not2 protein contains two functional domains. The C-terminal region participates in the function of the CCR4-NOT complex, while the N-terminal domain interacts with the Ada2 protein. The Not2 protein in yeast is essential for the stability of the CCR4-NOT complex and is crucial for yeast survival. In mammals, the loss of Not2 also leads to programmed cell death.
- Not3/Not5: The CCR4-NOT complex in yeast contains the subunits Not3 and Not5, which are linked to each other. Due to their sequence similarity, their functions are redundant. Both Not3 and Not5 contain coiled-coil domains and a presumed HR1 domain. The HR1 domain is believed to bind small Rho GTPases and play a role in signal transduction.
- Not4: In vitro experiments confirm that Not4 is an unstable, conserved subunit of the CCR4-NOT complex and primarily acts as a functional ubiquitin ligase. This protein contains a ring finger domain, a coiled-coil domain, and an RRM domain.
-

•Ccr4: Ccr4 is one of the two catalytic subunits in the CCR4-NOT complex. In vitro experiments have confirmed that yeast Ccr4 has a Mg^{2+}-dependent, poly(A)-specific 3' exonuclease activity. In addition to the nuclease domain, Ccr4 also contains a leucine-rich repeat (LRR) region, which provides a platform for interaction with Caf1.

• Caf1: Caf1 is the second catalytic subunit of the CCR4-NOT complex. The N-terminal domain of Caf1 is rich in glutamine, and no homologous counterpart has been found in mammals. This domain is not essential for function. It is speculated that this protein contains another glutamine-rich domain, which has been experimentally shown to exhibit nuclease activity.

• Caf40: Knockout of the CAF40 gene in Drosophila does not significantly affect mRNA deadenylation. Similarly, in yeast caf40 mutants, no impact on mRNA stability has been observed.

• Caf130: Caf130 contains a presumed transmembrane domain.

Deadenylases and Their Diversity:

Deadenylases are typically defined as magnesium-dependent exonucleases that recognize the poly(A) tail as the primary substrate and hydrolyze RNA in the 3'-5' direction, leading to the release of 5'-AMP. This deadenylation process occurs in both the nucleus and cytoplasm. In the nucleus, deadenylation limits the newly added poly(A) tail of mRNA to an appropriate length, while extensive deadenylation in the cytoplasm triggers degradation or translation repression. Deadenylation is often considered a rate-limiting step in mRNA decay and translation silencing. Currently, based on their nuclease domains, deadenylases are divided into two groups:

1. DEDD-type Exonucleases: Named for the conserved catalytic Asp and Glu residues in the three nucleic acid exonuclease motifs. Major family members of DEDD-type deadenylases include POP2, CAF1Z, Poly(A) specific ribonuclease (PARN), and PAN2.
2. Exonuclease-Endonuclease-Phosphatase (EEP) Family: Includes CCR4, nocturnin, ANGEL, and 2' phosphodiesterase (2'PDE).

CCR4-NOT Deadenylases:

The Saccharomyces cerevisiae CCR4-NOT complex contains two deadenylases, Ccr4p and Pop2p (Caf1p), both of which participate in mRNA degradation, although Pop2p is not necessary for the deadenylase activity of Ccr2p. The two human orthologs of yeast Ccr4p are CNOT6 (hCcr4a) and CNOT6L (hCcr4b), while the two orthologs of Pop2p are CNOT7 (hCAF1) and CNOT8 (hPOP2/CALIF).

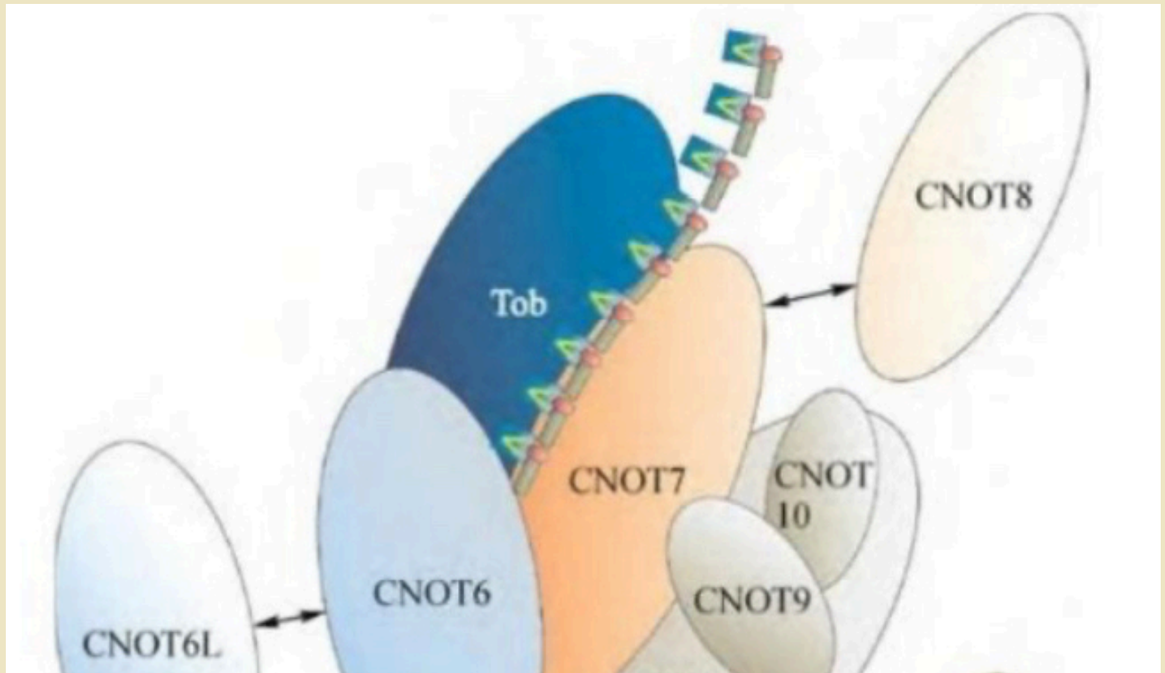

Figure 3. Structural Model of the Human CCR4-NOT Complex

CNOT7 and CNOT8 are critical for cell proliferation and share high amino acid sequence similarity with overlapping functions. CNOT6 and CNOT6L have distinct functions. CNOT7 and CNOT8 have been identified as members of the DEDD-type exonuclease family, while CNOT6 and CNOT6L belong to the EEP superfamily. Furthermore, mammalian cell growth can be reduced by overexpression of CNOT7 or reduction in CNOT6L expression.

Structure and Activity of Pop2 Deadenylase:

The crystal structure of Pop2p was first determined in 2003 from Saccharomyces cerevisiae and later in 2009 from Schizosaccharomyces pombe. The overall structure of Saccharomyces cerevisiae Pop2p is kidney-shaped, containing 13 α-helices and 6 β-strands.

Structural and functional studies of homologous proteins from Schizosaccharomyces pombe and other yeasts have revealed more information about the activity and selectivity of deadenylases, which differ from Saccharomyces cerevisiae Pop2p. The corresponding protein from Schizosaccharomyces pombe indeed contains a complete DEDD motif. From a structural perspective, two divalent metal ions (A and B) located at the active site are critical for its activity.

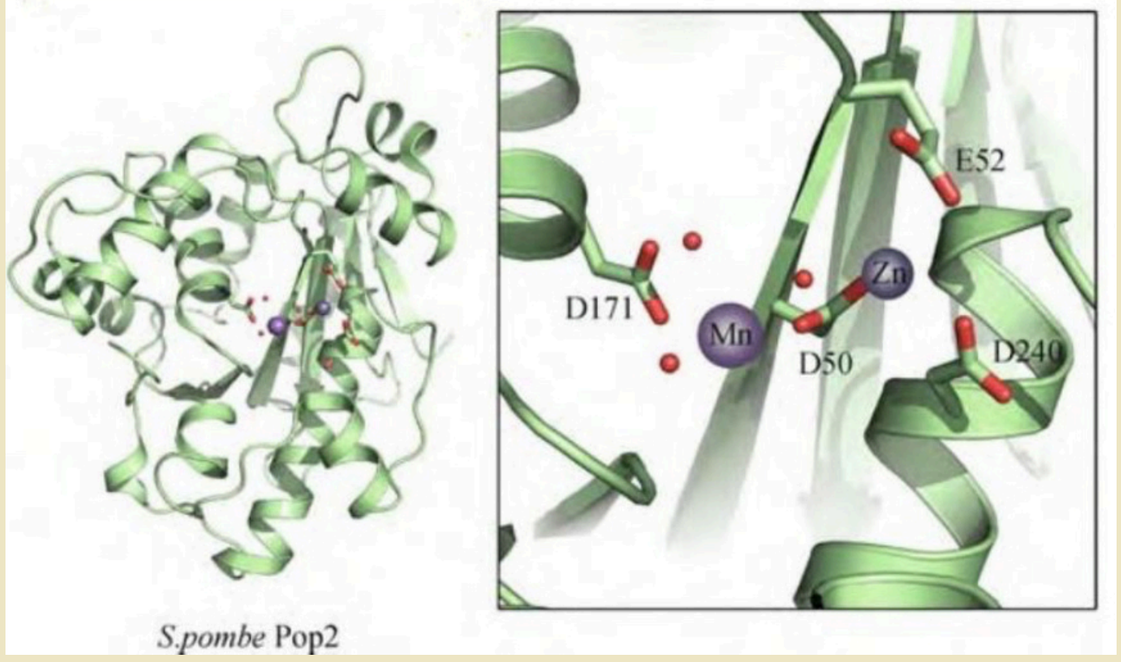

Figure 4. Crystal Structure of S. pombe Pop2 and Magnified View of the Active Site of S. pombe Pop2
(The residues forming the conserved DEDD motif are shown as sticks, Zn^{2+} and Mn^{2+} ions are represented as spheres, and water is shown as red small spheres.)

In the presence of Mg^{2+}, Mn^{2+}, and Zn^{2+} ions, the deadenylation reaction is slow and non-specific. However, in the absence of Zn^{2+}, Pop2p can rapidly and specifically degrade the entire poly(A) tail of RNA substrates, suggesting that changes in cellular Zn^{2+} levels may provide a mechanism to regulate the overall turnover rate of mRNA.

Structure of the Yeast Homolog of Human CNOT7:

Similar to the case of S. pombe Pop2p, the structure of human CNOT7 was studied in the presence of metal ions such as Mg^{2+}, Ca^{2+}, Mn^{2+}, and Co^{2+} to examine the nuclease activity of CNOT7. No activity was observed in the presence of Ca^{2+}. CNOT7 showed significantly higher activity toward RNA substrates than DNA substrates. The highest RNase activity was observed in the presence of Mn^{2+}, indicating that Mg^{2+} is essential for the full activity of CNOT7.

CCR4 Deadenylase:

yCcr4p is the main cytoplasmic deadenylase in yeast and acts as the primary catalytic component. Biochemical studies have classified yeast Ccr4p into the EEP family, and it has been shown to contain three main functional domains.

The N-terminal region is rich in glutamine and asparagine, while the central region contains several tandem copies of a leucine-rich repeat (LRR) domain, which is thought to link yCcr4p with the rest of the complex and other ligands. The LRR domain distinguishes all Ccr4p homologs from other EEP family members and CCR4-like proteins. The C-terminal region contains the deadenylase domain characteristic of the EEP superfamily, with conserved catalytic Asp and His residues in the activation domain. The human homologs, CNOT6 and CNOT6L, share the LRR domain and nuclease domain with yCcr4p but lack the N-terminal region rich in Glu/Asp.

TOB/BTG Anti-Proliferative Proteins:

Tob/BTG proteins acquire anti-proliferative activity by binding to target proteins in the cell. Both Tob and BTG2 have been shown to interact with CNOT7 via the CCR4-NOT complex. BTG2 acts as a co-activator of ERα-mediated transcription via the CCR4-like complex. Tob, BTG2, and TIS21 have similar structures, consisting of five α-helices and four β-strands, forming two anti-parallel β-sheets. The N-terminal region immediately forms a bundle of three α-helices, followed by four β-strands, with two small α-helices inserted between β1 and β2 strands.

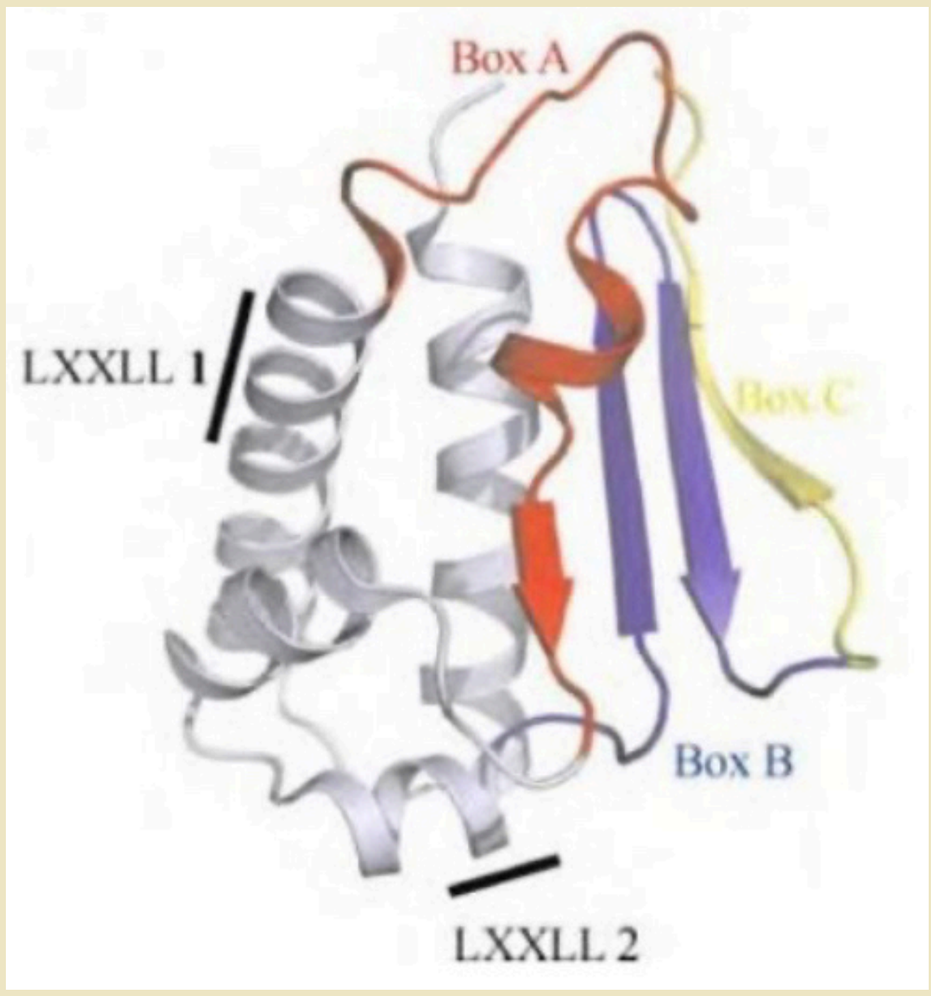

Figure 5. Crystal Structure of Human BTG2
(The conserved BoxA region is shown in red; BoxB is in blue; and the conserved BoxC region is colored yellow. Two conserved LXXLL motifs are indicated.)

The structure of human BTG2, which shares 40% sequence identity with Tob, displays three highly conserved domains in the Tob/BTG2 family. BoxA, also known as the GR (growth regulation) box, consists of β1 strand, a short α3 helix, part of the α2 helix, and the connecting loop between them. Two anti-parallel β-strands (β2 and β3) form BoxB, which is crucial for binding to various molecular targets, including CNOT7. BoxC is formed by the β4 strand and an extended C-terminal loop.

The structure of BTG2 suggests that the relevant interfaces are located on different surfaces of the protein, which may not interfere with each other. This increases the likelihood that BTG2 can simultaneously bind to two or more molecular targets to fulfill different regulatory requirements. Two LXXLL motifs, also known as nuclear receptor boxes (NR boxes), are located on α2 and α5 helices, respectively.

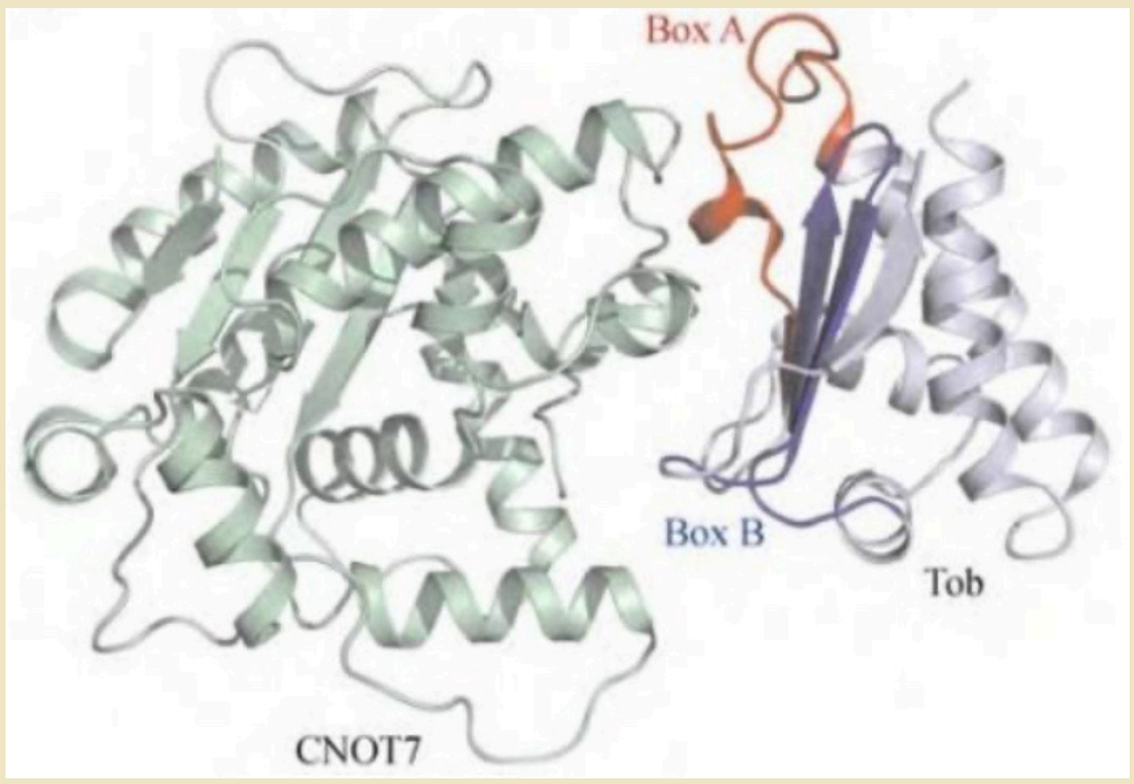

Figure 6. Crystal Structure of Human Tob-CNOT7 Complex

These two motifs are located on the opposite face of BTG2, providing a hydrophilic surface that may facilitate interaction with nuclear receptors, while hydrophobic residues are buried in the core of the protein.

As mentioned earlier, accumulating evidence supports the notion that the Tob/BTG protein family is a common binding partner of CNOT7 in the CCR4-NOT complex. In 2009, Horiuchi and colleagues reported the crystal structure of the Tob-CNOT7 complex, thereby elucidating the interaction pattern between the two proteins and suggesting the mechanism behind the anti-proliferative activity of the complex. The interaction between Tob and CNOT7 is largely hydrophobic and is mediated by the conserved Box A and Box B regions. The presence of Tob does not significantly affect the activity of CNOT7.

Analysis of human BTG2 has shown that BTG2 inhibits the deadenylase activity of CNOT7 in vitro by directly interacting with it. However, structural analysis clearly indicates that the binding interface between BTG2 and CNOT7 is not close to the CNOT7 active site, suggesting that the binding of BTG2 may induce local conformational changes that affect CNOT7 activity, or even distort the active site. BTG2 has recently been shown to be a general activator of mRNA degradation, involving the deadenylase activity of both CNOT7 and CCR4.

1. Specific tRNA Promotes mRNA Decay by Recruiting the CCR4-NOT Complex to Translating Ribosomes

The CCR4-NOT complex is a highly conserved multi-subunit assembly that acts as the major cytoplasmic deadenylase. mRNA deadenylation rates and half-lives vary by several orders of magnitude and are highly influenced by the mechanisms recruiting the CCR4-NOT complex to specific transcripts. Many RNA-binding proteins (RBPs) interact with the CCR4-NOT complex, accelerating the deadenylation of the specific mRNAs they bind. Similarly, microRNAs (miRNAs) target mRNAs by directly recruiting the CCR4-NOT complex–TNRC6 interacts with CNOT9, which is a core component of both the miRNA-induced silencing complex and the CCR4-NOT complex.

The CCR4-NOT complex can also be directly recruited to translating ribosomes to trigger the accelerated degradation of specific transcriptomes. When the ribosome encounters a suboptimal codon, the E-site tRNA may be released before the A-site codon is decoded, resulting in a conformation with empty A- and E-sites. This allows the N-terminal helix bundle of Not5 (the human homolog of CNOT3) to enter the empty E-site. In this way, the CCR4-NOT complex can monitor decoding efficiency and accelerate the degradation of mRNAs rich in suboptimal codons. It has been demonstrated that ribosome stalling on highly suboptimal codons in mammalian cells also leads to the recruitment of CNOT3 to the empty E-site.

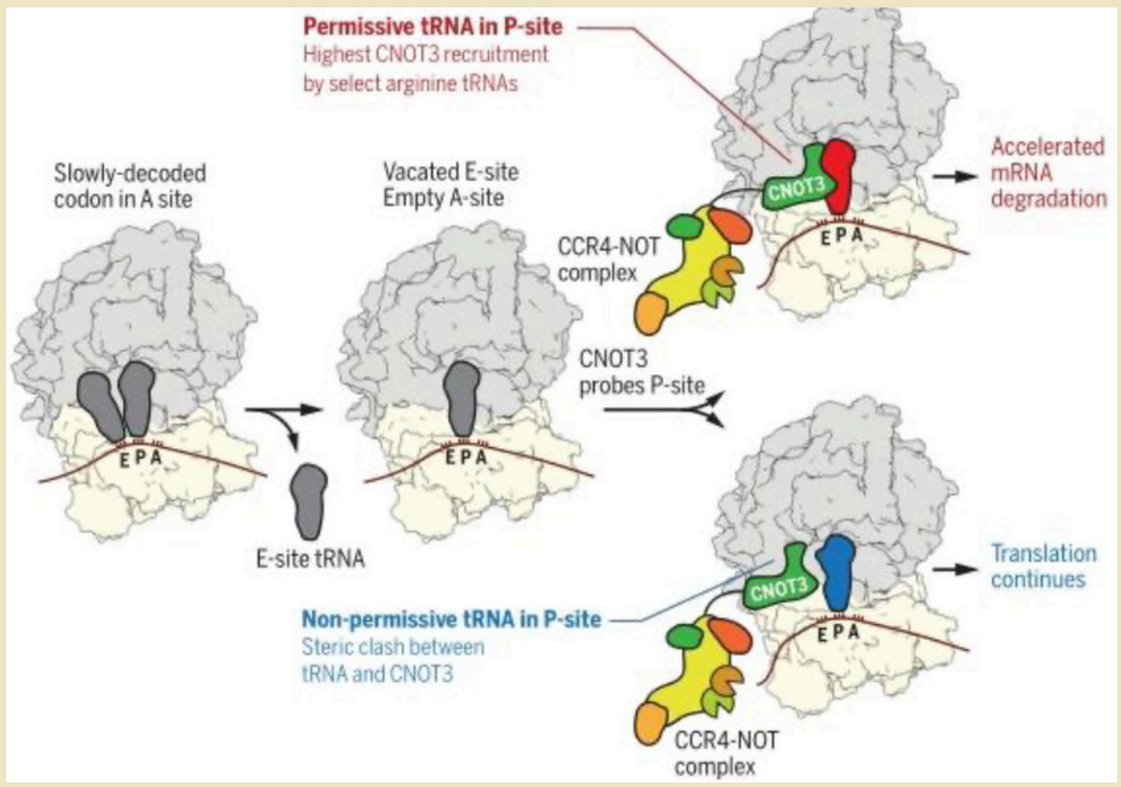

Figure 7. P-Site tRNA Controls Recruitment of the CCR4-NOT Complex to Translating Ribosomes

By using selective ribosome profiling to determine the translation characteristics of mRNAs that lead to CNOT3 recruitment to the ribosome, an previously unrecognized P-site tRNA-mediated mRNA decay (PTMD) pathway was revealed.

Ribosomal P-Site Codons Are the Major Determinants for CNOT3 Recruitment :

Selective ribosome analysis was used to identify the characteristics of mRNAs associated with CNOT3 binding to the ribosome, along with immunoprecipitation (IP) of CNOT3. Sequencing of the observed enriched ribosome footprints confirmed the expected triplet periodicity within the open reading frame (ORF). Simultaneously, in Saccharomyces cerevisiae, it was confirmed that non-optimal codons with low tRNA adaptation index (tAI) are highly enriched at the A-site of ribosomes that are not bound by Ccr4. Codon optimality can be quantified using the tRNA adaptation index (tAI). However, no specific A-site codon was strongly enriched in the experimental dataset, with only one codon showing more than a two-fold enrichment.

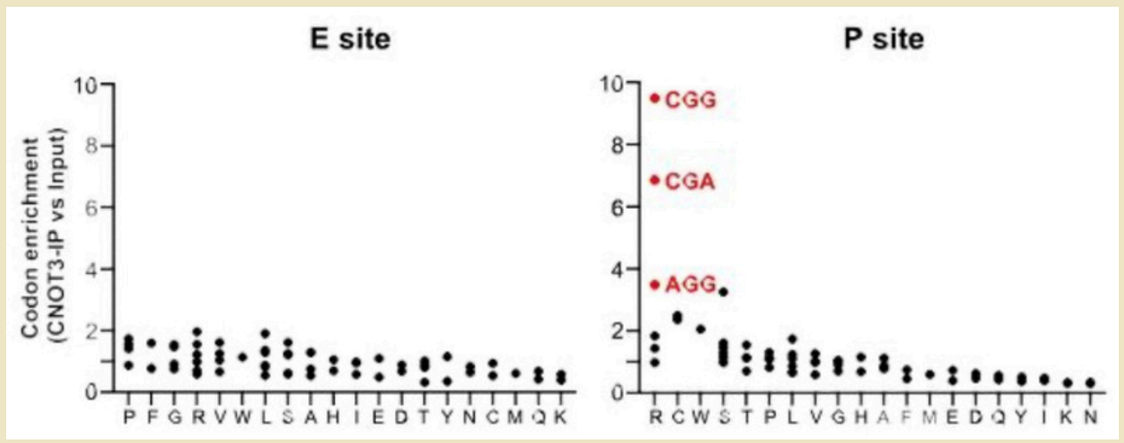

Figure 8. Codon Enrichment at Ribosomal E, P, and A Sites on CNOT3-Bound Ribosomes

This suggests that the slow decoding of A-site codons significantly promotes the recruitment of CNOT3 to translating ribosomes, but it also increases the possibility that other determinants play a more dominant role in co-translational CNOT3 recruitment in mammalian cells.

By studying codon enrichment at selective ribosome P- and E-sites, and analyzing the amino acids encoded by CNOT3-bound ribosome footprints, it was shown that the most enriched codons on ribosomes binding CNOT3 are located at the P-site. The identity of P-site codons is closely related to co-translational CNOT3 recruitment in human cells, with arginine codons at the P-site providing the strongest detectable signal for CNOT3 binding.

CGG, CGA, and AGG Arginine Codons Promote CNOT3-Mediated mRNA Decay :

By calculating the weighted CGG/CGA/AGG scores to stratify mRNAs, it was found that transcripts with high weighted CGG/CGA/AGG scores preferentially stabilize after the loss of CNOT3. Transcripts rich in other arginine codons (CGC, AGA, and CGU) did not exhibit this behavior. Further analysis of mRNA decay rates in CNOT3-depleted Jurkat cells or steady-state mRNA levels in Cnot3 knockout mouse pro-B cells confirmed that transcripts rich in CGG, CGA, or AGG arginine codons, but not CGC, AGA, or CGU-encoded arginine, preferentially stabilized in CNOT3-deficient cells.

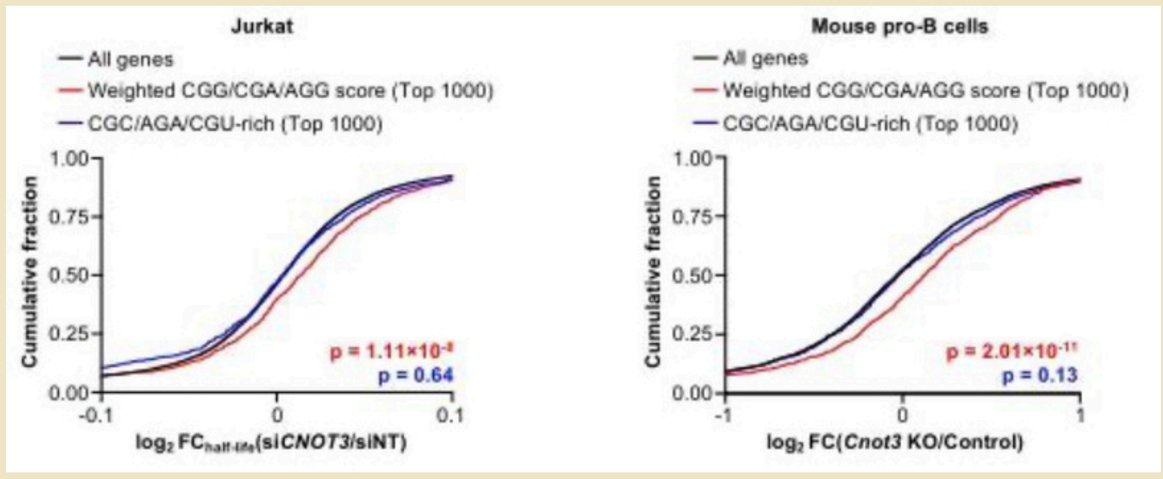

Figure 9. Highest Weighted Scores of mRNAs Rich in CGG/CGA/AGG or CGC/AGA/CGU Arginine Codons

A doxycycline-regulated reporter transcript was constructed, and a control mRNA was designed where each arginine codon was replaced with a codon not enriched at the P-site of CNOT3-bound ribosomes. Experimental results were consistent with whole transcriptome analysis, showing that the decay rate of the arginine-encoding reporter was significantly faster than that of the control reporter,

and it was selectively stabilized after CNOT3 depletion. This indicates that CGG, CGA, and AGG arginine codons recruit CNOT3 to translating ribosomes, leading to accelerated mRNA decay.

Mitochondrial Ribosomal Protein mRNAs Are Rich in CGG/CGA/AGG Codons and Are Regulated by CNOT3 :

By analyzing a gene set of endogenous mRNAs that are most highly regulated by CNOT3 due to the presence of unstable arginine codons, it was found that the gene set encoding mitochondrial ribosomal proteins was the most enriched. These findings suggest that mRNAs encoding mitochondrial ribosomal proteins are regulated by CNOT3. It was also confirmed that CNOT3 depletion in HEK293T or Jurkat cells resulted in a significant increase in the steady-state abundance of these transcripts, with a corresponding increase in mitochondrial mass.

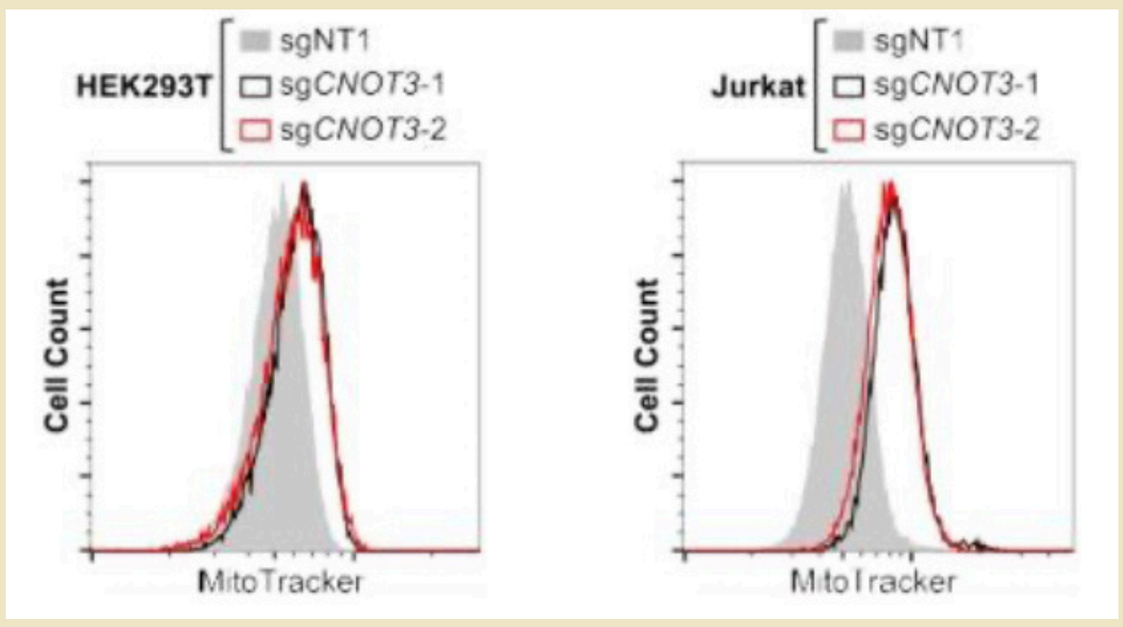

Figure 10. HEK293T Enrichment Analysis

Depletion of the scaffold subunit CNOT1 of the CCR4-NOT complex led to a similar increase in the steady-state abundance of mitochondrial ribosomal protein mRNAs, suggesting that the CCR4-NOT complex regulates mitochondrial ribosomal proteins (encoded by arginine codons CGG, CGA, and AGG) and influences mitochondrial homeostasis in mammalian cells.

Structural Basis of P-Site Arginine Codon Co-Translational Recruitment of CNOT3 to Ribosomes :

A reporter mRNA encoding a sequence of 41 leucine-arginine-aspartic acid repeats was constructed, representing the most abundant tripeptide in CNOT3-bound ribosomes.

Lysine codons (41×LKAAGD) were used to replace the arginine codons as a control. In vitro translation of 41×LRCGGD, rather than 41×LKAAGD, led to significant recruitment of labeled CNOT3 to the polyribosomes, with other CCR4-NOT complex components selectively recruited to the 41×LRCGGD mRNA.

The system was then used to determine the structural basis for CNOT3 recruitment to ribosomes by P-site arginine codons. Through flag immunoprecipitation (IP) enrichment of actively translating 41×LRCGGD mRNA poly-ribosomes, ribosomes bound by CNOT3 were analyzed using cryo-electron microscopy (cryo-EM), revealing linear aggregation of ribosomes, indicating that the polyribosomes remained intact during sample preparation.

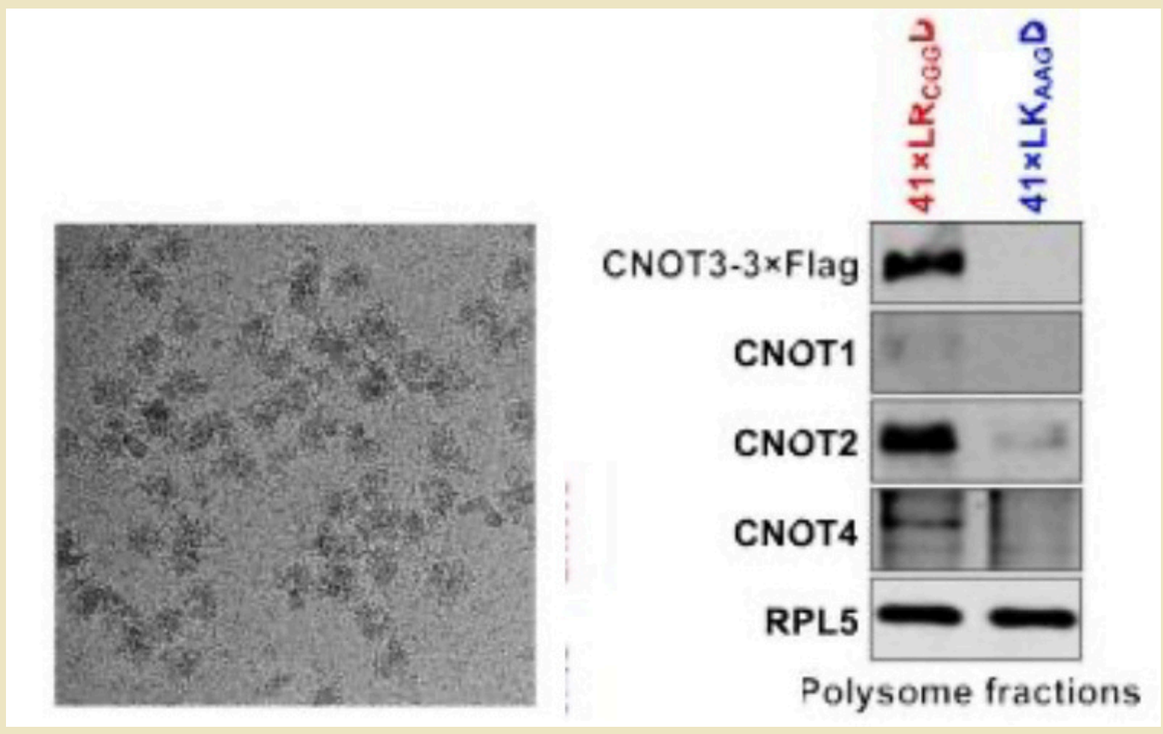

Figure 11. Cryo-EM Electron Microscopy Analysis and In Vitro Control Analysis

By analyzing the structure of individual ribosomes (but not dimers or higher-order polyribosomes), single-particle reconstruction of the ribosome produced a uniform structure with an overall resolution of 2 Å. Density interpretation and model building revealed an empty A-site, tRNA in the P-site, and CNOT3 occupying the E-site.

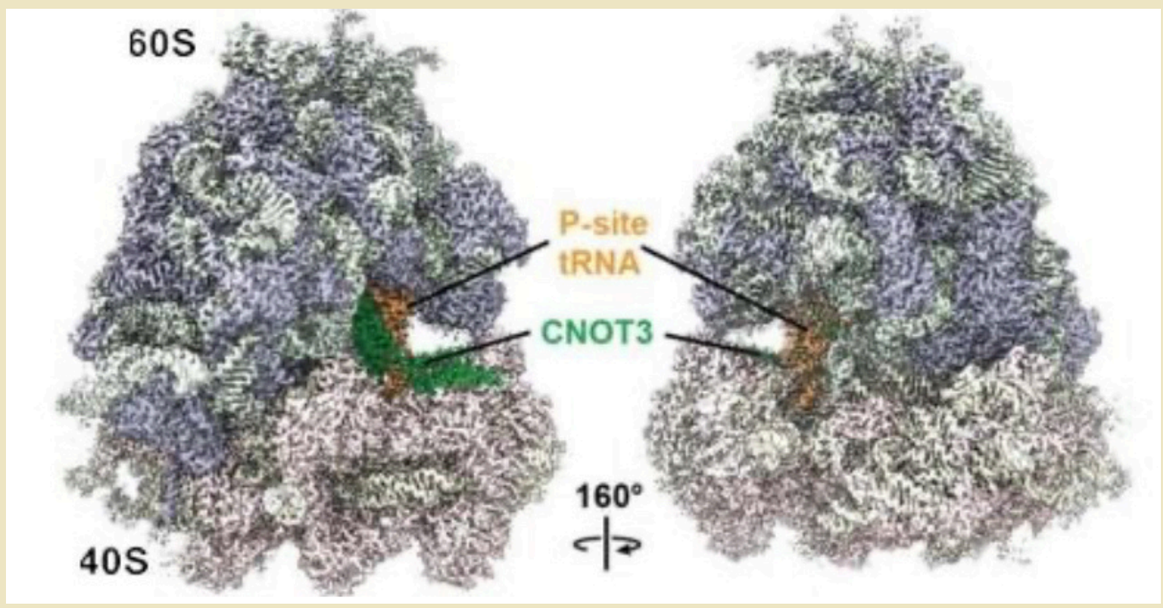

Figure 12. Low-Temperature Electron Density Map

The analysis results are consistent with previous observations, where the N-terminal 3-helix bundle of CNOT3 connects the two ribosomal subunits and interacts with the D-loop, D-stem, and anticodon stem of the P-site tRNA. The arginine encoded by the CGG codon in the P-site was clearly identified, and as predicted by selective ribosome analysis, the CGG codon present in the transcript can be recognized by two tRNA^Arg, CCG, or one of the five tRNA^Arg, UCG, decoders present in HEK293T cells due to wobble base pairing.

P-Site tRNA D-Arm as a Key Determinant for Co-Translational Recruitment of CNOT3:

It has been reported that selective codon pairs in the P- and A-sites of the ribosome distort the structure of the A-site mRNA, leading to decoding impairment, which may cause ribosome stalling and result in CNOT3 recruitment. By simulating the A-site mRNA, the mRNA geometry in the structure was compared to the mRNA structure in the translating ribosome, and tRNA detected the A-site. The conformation of the A-site mRNA and the overall structure of the ribosome bound to CNOT3 are compatible with decoding, indicating that CNOT3 recruitment is not a result of ribosome stalling caused by mRNA distortion.

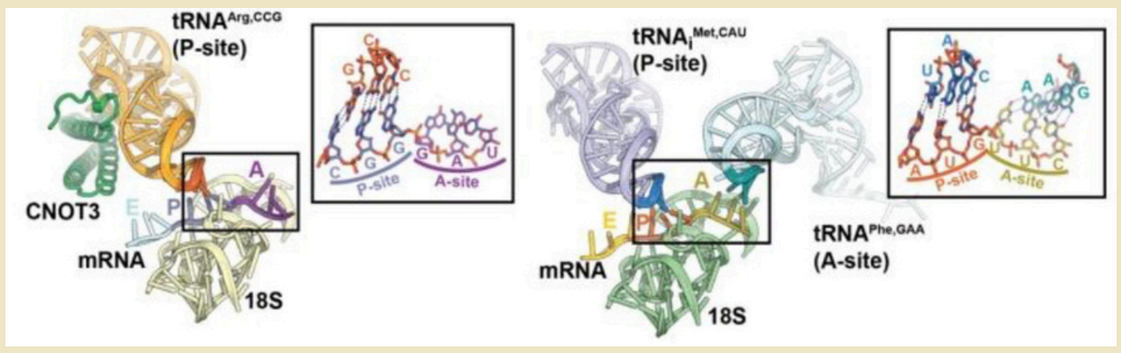

Figure 13. mRNA Configuration in CNOT3 Ribosome Structure

The results also suggest that changes in the D-loop and anticodon stem of the arginine tRNA may alter their affinity for CNOT3 in the context of the translating ribosome, and propose the possibility of nucleotide modifications, particularly the loss of m^7 G at nucleotide 46 in tRNA^Arg, CCG/UCG/CCU involved in the D-stem triplet base interaction, which could affect CNOT3 recruitment. In vitro aminoacylation assays confirmed that no mutations impaired tRNA activity compared to each corresponding parental tRNA. This evidence indicates that the sequence of the P-site tRNA plays a critical role in co-translational CNOT3 recruitment, and this effect does not depend on specific tRNA nucleotide modifications.

By testing different D-stem sequences of tRNA^Arg, ACG and tRNA^Arg, CCG, it was found that adding the D-stem sequence of tRNA^Arg, CCG to tRNA^Arg, ACG (tRNA^Arg, ACG-m5), or introducing a single C13U mutation (tRNA^Arg, ACG-m6) that forms a U13:A22 base pair in tRNA^Arg, ACG, was sufficient for CNOT3 recruitment. Similarly, the inversion of the G12:C23 base pair (tRNA^Arg, ACG-m7) had no effect. In conclusion, these results identify the U13 position in the D-arm of tRNA^Arg, CCG/UCG/CCU, which interacts with A22 and A46 to form a triplet base interaction, as a key feature associated with CNOT3 recruitment to the ribosome. In tRNA^Arg, UCU, the triplet interaction is C13:G22:m7G46, which is a common configuration for these positions in all tRNAs.

Subsequent studies also structurally confirmed that the arginine tRNA, containing the U13:A22:A46 triplet base interaction, enhances the recruitment of CNOT3.

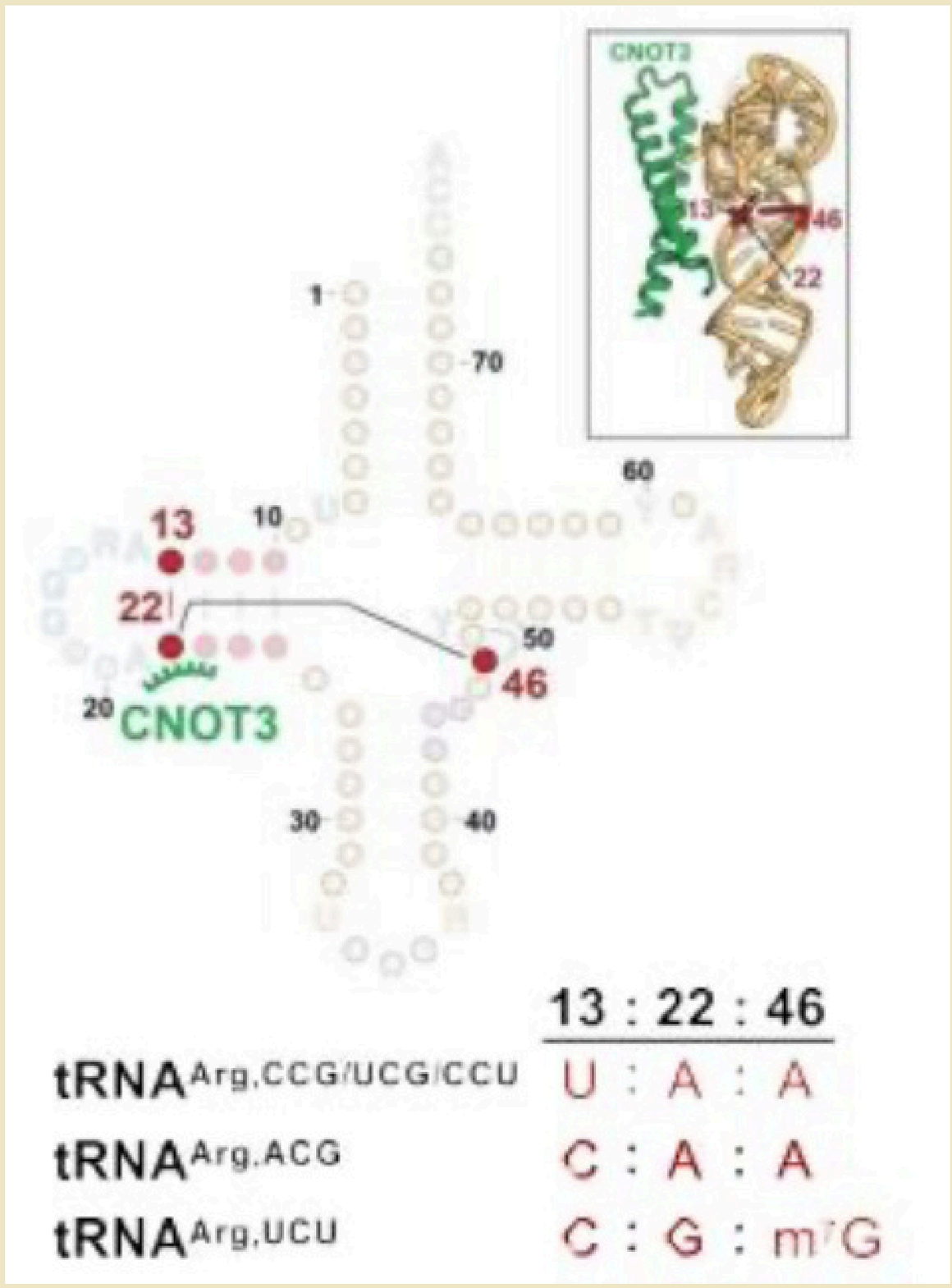

Figure 14. Secondary Structure of tRNA and CNOT3

Impact of P-site tRNA Anticodon Stem Cells on Co-translational CNOT3 Recruitment:

The interaction between CNOT3 and the anticodon stem of the P-site tRNA is mediated by tCM, which forms several direct backbone interactions and water bridge interactions with the G42 and A43 bases of the P-site tRNA.

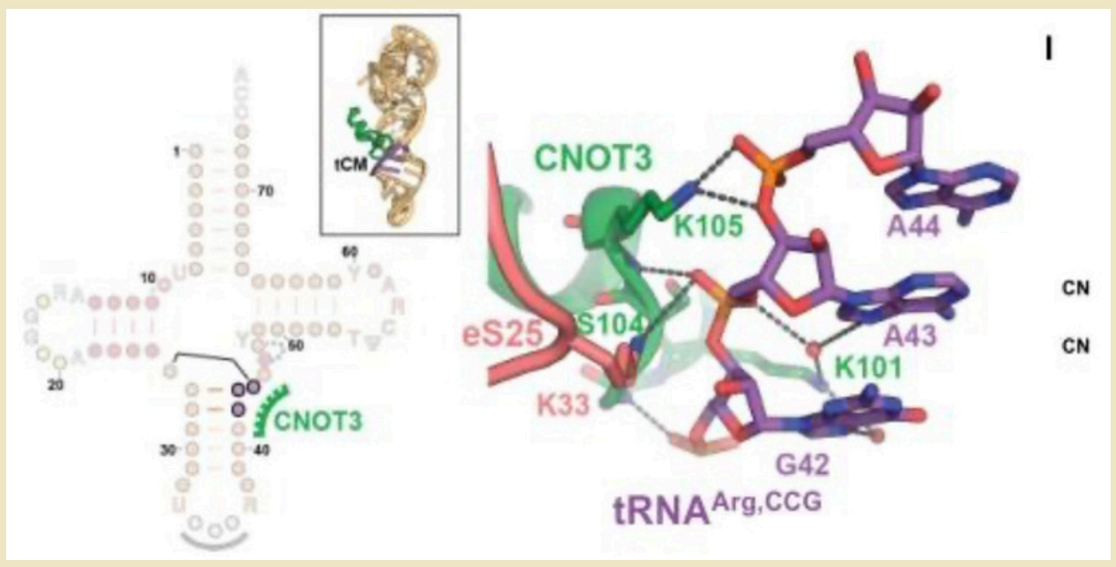

Figure 15. Secondary Structure of CNOT3-tCM Motif and Anticodon Stem Loop

The K105S substitution in tCM abolishes the recruitment of CNOT3 to transcripts rich in CGG/CGA/AGG. Subsequently, tRNAArg, UCU-1 was reprogrammed to recognize CGU arginine codons. Experimental results showed that adding the tRNAArg.CCG U13:A22:A46 triplet, but not adding the tRNAArg.CCG anticodon stem loop, was sufficient to enhance CNOT3 recruitment. Therefore, the presence of the U13:A22:A46 triplet is a key determinant for the recruitment of CNOT3 by arginine tRNA, while the role of the anticodon stem loop is small but measurable.

Additional Nucleotides Upstream of the D-loop GG Motif Prevent CNOT3 Recruitment:

Further tRNA mutagenesis experiments revealed that changes in the D-loop b element do not affect CNOT3 recruitment, as mutations introducing C20A (tRNAMet-m14) or C20U (tRNAMet-m15) had no impact on CNOT3 binding. In conclusion, our results suggest that the D-stem U13:A20:A46 triplet and the short single nucleotide D-loop a element in P-site tRNA are the main determinants for co-translational CNOT3 recruitment, providing a structural basis for explaining the primary pattern of P-site codon enrichment and the depletion of CNOT3-bound ribosomes.

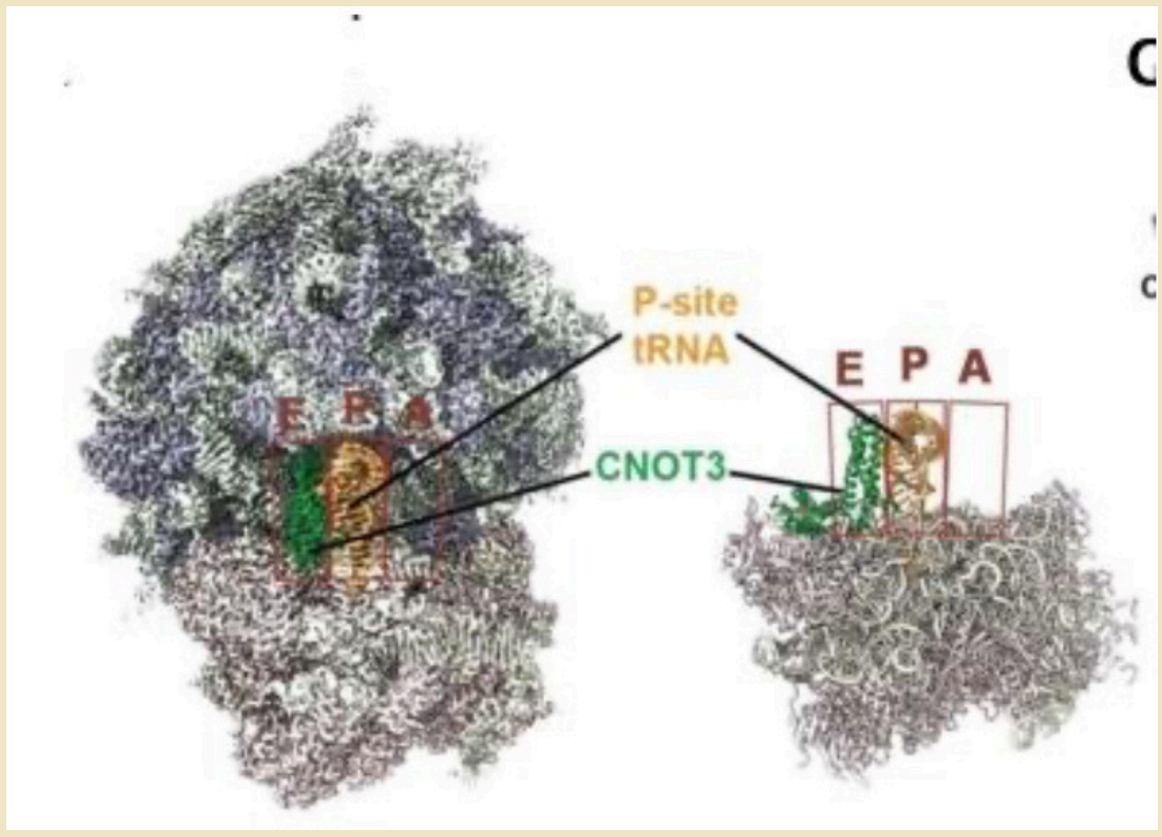

Figure 16. Shear Density Map (left) and Atomic Model (right) Highlighting Ribosome E, P, and A Sites

Finally, a weak but statistically significant correlation was detected between A-site dwell time and CNOT3 recruitment in the selective ribosome analysis data. This suggests that slow decoding can significantly enhance CNOT3 recruitment in human cells. When the P-site is occupied by CGG, CGA, or AGG arginine codons, the correlation between A-site dwell time and CNOT3 recruitment is greatly strengthened. In contrast, when the P-site contains any other codons, no correlation in dwell time is detectable. Slow decoding increases the likelihood of simultaneous vacancies at the ribosome A and E sites, providing an opportunity for CNOT3 to enter the E site. This further demonstrates that the detection of P-site tRNA's D-arm by CNOT3 ultimately determines whether CNOT3 stably binds to the ribosome and initiates mRNA decay.

Reference

[1] Xiaoqiang Zhu, Victor Emmanuel Cruz, He Zhang,(2024), Specific tRNAs promote mRNA decay by recruiting the CCR4-NOT complex to translating ribosomes, https://doi.org/10.1126/science.adq8587, 《 Nature: MOLECULAR BIOLOGY》

[2] Lu Wang, Shuibin Lin,(2023), Emerging functions of tRNA modifications in mRNA translation and diseases, https://doi.org/10.1016/j.jgg.2022.10.002, 《Journal of Genetics and Genomics》

[3] Kentaro Ito, Akinori Takahashi, Masahiro Morita,(2011), The role of the CNOT1 subunit of the CCR4-NOT complex in mRNA deadenylation and cell viability, https://doi.org/10.1007/s13238-011-1092-4,《Protein & Cell》

[4] Mark Bartlam, Tadashi Yamamoto, (2010),The structural basis for deadenylation by the CCR4-NOT complex, https://doi.org/10.1007/s13238-010-0060-8,《Protein & Cell》

[5] 李超 ,(2014)Functional Characterization of Ccr4-NOT complex components in the Rice Blast Fungus, 《 China Agricultural University》

[6] 吴洁，温贵兰，赵静，胡璇,(2023), Ccr4－Not复合物的生物学功能研究进展, 1007－1474(2023) 05－0059－04,《贵州畜牧兽医》

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Genetic Engineering Approaches to Prevent the Outspread of and Cure Prion Diseases: A Focus on Creutzfeldt-Jakob Disease (CJD)

Keywords: Creutzfeldt-Jakob Disease, Machine Learning, Nanobodies, Immunotherapy, Antibody Engineering

Abstract

Creutzfeldt-Jakob Disease (CJD) is a rare and fatal neurodegenerative disorder linked to prion protein misfolding. This study explores genetic engineering and machine learning approaches for CJD diagnosis and treatment. Using Convolutional Neural Networks (CNNs), we compared diagnostic models and found ResNet50 achieved a 97% accuracy rate in classifying MRI images. Additionally, we discuss the therapeutic potential of nanobodies and humanized antibodies, such as PRN100, which offer promising avenues for CJD treatment with reduced side effects. The integration of computational methods and immunotherapy as well as the use of antibody engineering pave the way for advancements in CJD management. In this way, we achieve the detection and treatment of CJD.

Introduction

Prion proteins are self-templating proteins that are in two forms: PrPC and PrPSc (Baraznenock & Kravchuk, 2023; Ribes et al., 2023, p. xx). The non-cytotoxic form of prion protein, PrPC can misfold into the cytotoxic, insoluble and prone to aggregate PrPSc (Zerr et al., 2024, p. xx). Moreover, PrPSc is a shockingly conformationally stable protein (Rouvray, 2005). Irradiation, ultra-violet light exposure, formaldehyde treatment, and caustic soda exposure cannot alter its infectious feature (Rouvray, 2005). Although still not entirely revealed, it was proposed that the aggreagation of insoluble proteins which ultimately damages the neurosystem is caused by the highly crystalised structure of PrPSc, leading to the formation of spongiform cerebra (Rouvray, 2005; Zerr et al., 2024, p. xx). As the cluster of PrPSc meets PrPC, the harmless prion (PrPC) changes its conformation to fit into the cluster (Rouvray, 2005). In another research, it is claimed that PrPSc replicates its structure onto PrPCs', thus carrying out a chain reaction of protein infection, leading to stockage of PrPScs in the brain, and that the process can last up to a decade (Baraznenock & Kravchuk, 2023). However, the definition of prion has broadened, perhaps overused, now including all neurodegenerative diseases that involves protein aggregation, regardless of whether the protein has infectivity (Scheckel & Aguzzi, 2018, p. xx).

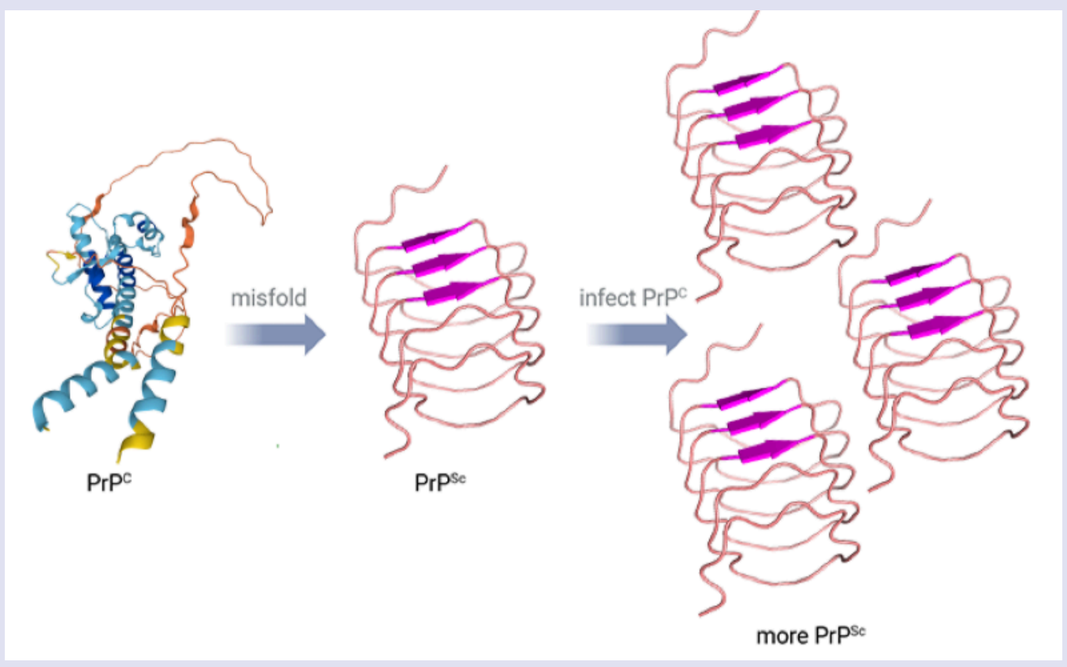


Figure 1 PrPc misfolds and PrPSc aggregates.

Meanwhile, the Brain cellular protein quality control machinery with its central constituents of chaperones and proteases is vital to maintain protein homeostasis under physiological conditions and to protect against acute stress conditions(Rustenhoven, 2021). Imbalances in protein homeostasis also are keys to a plethora of genetic and acquired, often age-related, diseases as well as aging in general.

A public survey was done, and it was found out that only 25.86% of the interviewees claim to have heard of the prion virus (the culprit of CJD), indicating a lack of attention to and public acknowledgement of this disease. Among these interviewers, fortunately, 68.97% of them adopt a serious attitude towards gene screening which can diagnose hereditary CJD. Meanwhile, CJD can also be infected due to contaminated beef (Variant Creutzfeldt-Jakob Disease, vCJD). When asking people's opinions, 30.99%, which is the highest proportion of people, mention that they hope the country can reinforce regulation of food safety. 29.93% of people think that they always go to the place with certificates of food safety for beef. 15.85% and 11.62% of interviewees claim to follow food-safety magazines, channels, official accounts, video makers and websites on a frequent basis, and emphasizing the source of cattle feed on matter as a buyer or producer of beef. However, only 3.52% claim to have experience of work and research in terms of safety of meat products.

Developing Prevention Methods

The level of understanding people has about CJD (Creutzfeldt-Jakob Disease)

In our public survey, it is found that only 25.86% of the interviewees claim to have heard of the prion virus (the culprit of CJD), indicating a lack of attention to and public acknowledgement of this disease. Among these interviewers,

fortunately, 68.97% of them adopt a serious attitude towards gene screening which can diagnose hereditary CJD. Meanwhile, CJD can also be infected due to contaminated beef (Variant Creutzfeldt-Jakob Disease, vCJD). When asking people's opinions, 30.99%, which is the highest proportion of people, mention that they hope the country can reinforce regulation of food safety. 29.93% of people think that they always go to the place with certificates of food safety for beef. 15.85% and 11.62% of interviewees claim to follow food-safety magazines, channels, official accounts, video makers and websites on a frequent basis, and emphasizing the source of cattle feed on matter as a buyer or producer of beef. However, only 3.52% claim to have experience of work and research in terms of safety of meat products.

Significance of Conventional Neural Networks in Medicine

Brilliant achievements have been made by conventional neural networks(CNN), enabling to become one of the most representative neural networks in deep learning. It is computer vision based on conventional neural networks that enables people to accomplish those considered to be impossible in the past few centuries, especially in image recognition (Li, Z. W., et al. 2004). In the year 1959, it was found by Hubel & Wisel that cells in animal visual cortex have the duty to detect light in receptive fields. This discovery inspired Kunihiko Fukushima to propose the neocognition in 1980, which can be considered as the predecessor of CNN. Since then, many models of CNN had been developed. Notably, Krizhevsky et al. proposed a classic CNN architecture and showed significant improvements to previous methods on the image classification task (Gu, J., et al. 2017).

With the progress made in data-driven machine learning, it becomes increasingly important to use machine learning method to analyse medical data. CNNs, as a powerful image processing tool, are increasingly applied across the medical field. Their exceptional performance has been notably demonstrated in various branches such as biomedical image analysis, assistive diagnostics, pathology, radiology, and neuroscience.

Convolutional Neural Networks (CNNs) demonstrate their sophistication not only in their deep analytical capabilities for images but also in their potential contributions to enhancing the quality and efficiency of medical services. By accurately identifying subtle patterns and anomalies in medical images, CNNs can assist doctors in diagnosing diseases at an early stage. Such early detection is crucial for treatment because many diseases are more easily treated and have relatively lower treatment costs when caught early. Most importantly, it has a higher level of accuracy in terms of diagnosis.

Four different models of CNN and comparisons

To decide whether one is infected with CJD, we use the machine to do this diagnosis. We train machines with few shots since CJD is a rare disease and there is very little data about it. Since CJD is a rare disease, it is hard to find the training and testing data needed. There are very few cases of MRI of patients. We spend days and nights visiting medical communities, websites and forums and contacting relevant organizations, hoping to obtain the data we needed, regardless of how slow and difficult this process is. Using the conventional neural network, we can classify the pictures of MRI pictures of patients' brains into with or without CJD. In this way, we can more precisely and reliably diagnose this disease and decide whether to adopt relevant treatment.

Basic CNN

Convolutional Neural Networks (CNNs) are deep learning models specifically designed for processing structured grid data, such as images (LeCun et al., 1998). Their main structures include convolutional layers, pooling layers, and fully connected layers. Convolutional layers extract spatial features from input data through convolution operations, while pooling layers reduce data dimensions through subsampling while retaining important information. Basic CNNs typically consist of four regular layers, each with a kernel size of 3, a stride of 1, and padding of 1. These layers incrementally build an understanding of the image content by extracting and combining features layer by layer.

Results

On the test data, the basic CNN achieved an accuracy rate of 95%.

ResNet34

ResNet34 is a deep residual network designed to address the issues of gradient vanishing and gradient explosion that can occur during the training of deep neural networks (He et al., 2016). The structure of ResNet34 includes multiple residual blocks, each of which bypasses part of the layers through a skip connection to ensure that gradients can be effectively propagated forward. Specifically, ResNet34 repeats 3x3 convolutional layers three, four, six, and three times, respectively, and uses skip connections in each residual block to achieve a deeper network structure without increasing the difficulty of training.

Results

The accuracy rate of ResNet34 on the test data was 94%.

ResNet50

ResNet50 is a deeper version in the ResNet series, which employs more complex residual blocks (He et al., 2016). Each residual block of ResNet50 contains a combination of 1x1, 3x3, and 1x1 convolutional layers. This design not only retains efficient feature extraction capabilities but also reduces computational complexity through the 1x1 convolutional layers. Specifically, ResNet50 repeats these combinations of convolutional layers three, four, six, and three times, respectively, also using skip connections to maintain effective gradient propagation.

Results

ResNet50 achieved an accuracy rate of 97% on the test data.

DenseNet121

DenseNet121 is a densely connected convolutional network designed to maximize information flow and gradient propagation by directly connecting any two layers (Huang et al., 2017). The structure of DenseNet121 includes a 7x7 convolutional layer and a 3x3 max pooling layer, followed by six, twelve, twenty-four, and sixteen repetitions of combinations of 1x1 and 3x3 convolutional layers (referred to as dense blocks). Between each dense block, DenseNet121 transitions through 1x1 convolutional layers and 2x2 average pooling layers, thereby maintaining the compactness and computational efficiency of the network.

Results

The accuracy rate of DenseNet121 on the test data was 96%.

Comparisons

It is found that ResNet50 has the best performance in this picture classifying task. Using ResNet50(accuracy of 97%), we can quickly detect CJD so that they can be cured as soon as possible.

We used Cross Entrophy Loss as the loss function in this model. We can see how loss value in these four processes changes respectively through the following pictures.

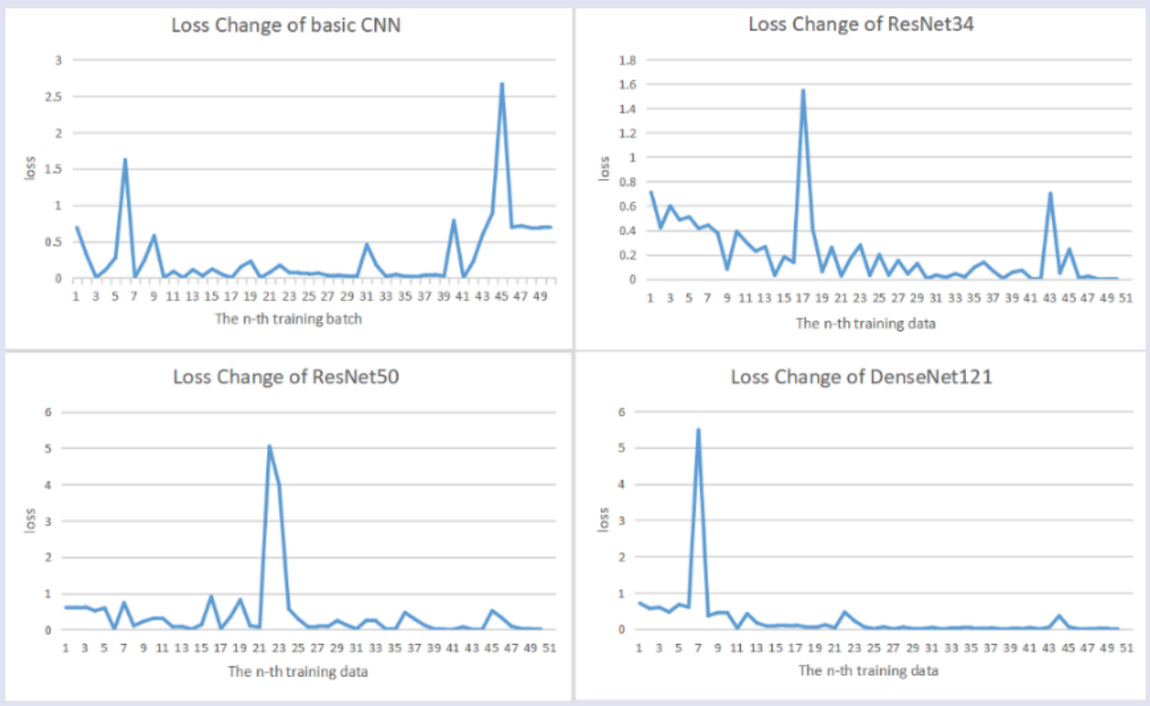


Figure 2 Visualisation of investigation results

Model	Source of Data	Loss	Optimizer	epoches	Validation Set Accuracy	Training Time
Basic CNN	https://www.dxy.cn https://radiopaedia.org/articles/creutzfeldt-jakob-disease Brain Tumor MRI Dataset (kaggle.com)	Cross Entrophy	Adam	10	95%	4m 43.5s
ResNet34	https://www.dxy.cn https://radiopaedia.org/articles/creutzfeldt-jakob-disease Brain Tumor MRI Dataset (kaggle.com)	Cross Entrophy	Adam	10	94%	17m 14.5s
ResNet50	https://www.dxy.cn https://radiopaedia.org/articles/creutzfeldt-jakob-disease Brain Tumor MRI Dataset (kaggle.com)	Cross Entrophy	Adam	10	97%	24m 17.8s
DenseNet121	https://www.dxy.cn https://radiopaedia.org/articles/creutzfeldt-jakob-disease Brain Tumor MRI Dataset (kaggle.com)	Cross Entrophy	Adam	10	96%	14m 47.7s

Figure 3 Comparison between four models

Train and test YOLOv5 classification models to identify whether an MRI image is of a person with CJD

YOLOv5, developed by Ultralytics, is a versatile and powerful model primarily known for object detection. However, it also supports image classification tasks. The classification model in YOLOv5 leverages the same efficient architecture used for detection, making it fast and accurate.

We trained all five sizes of YOLOv5 classification models on an augmented dataset consists of MRI images of people with and without CJD for 100 epochs and recorded the change of train loss, test loss and test accuracy. All models show great accuracy and low train and test loss.

YOLOv5 classification models have five different sizes: n(nano), s(small), m(middle), l(large) and x(ultra). The larger models have more trainable parameters, stronger fit ability but also slower training and prediction speed and bigger risk of overfit.

Results

In our training and testing task, model of all sizes achieved similar loss and accuracy level in the end, but in the early epochs, the fluctuation of accuracy of larger model is more violent.

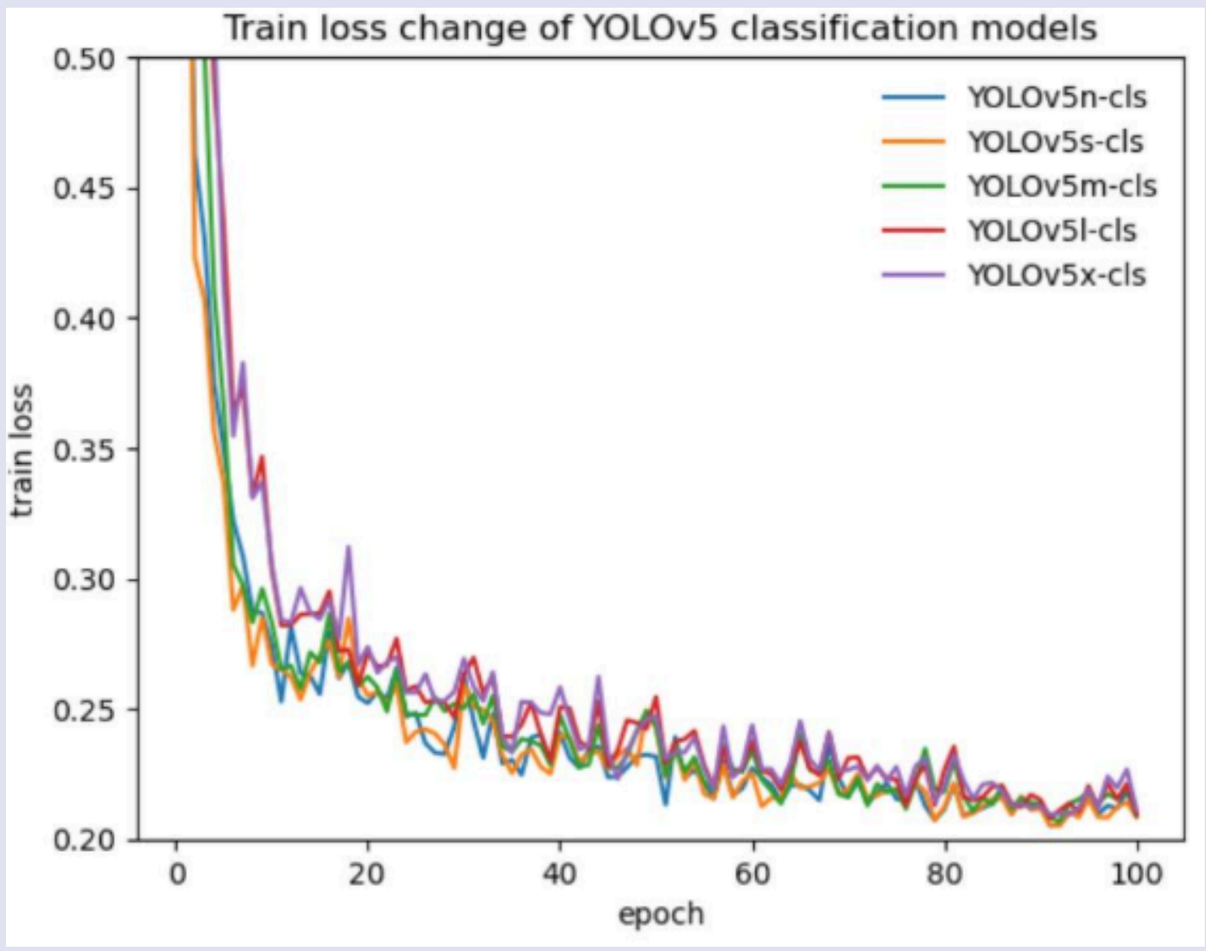


Figure 4 Train loss change of YOLOv5 classification models. all models achieved similar train loss level in the end

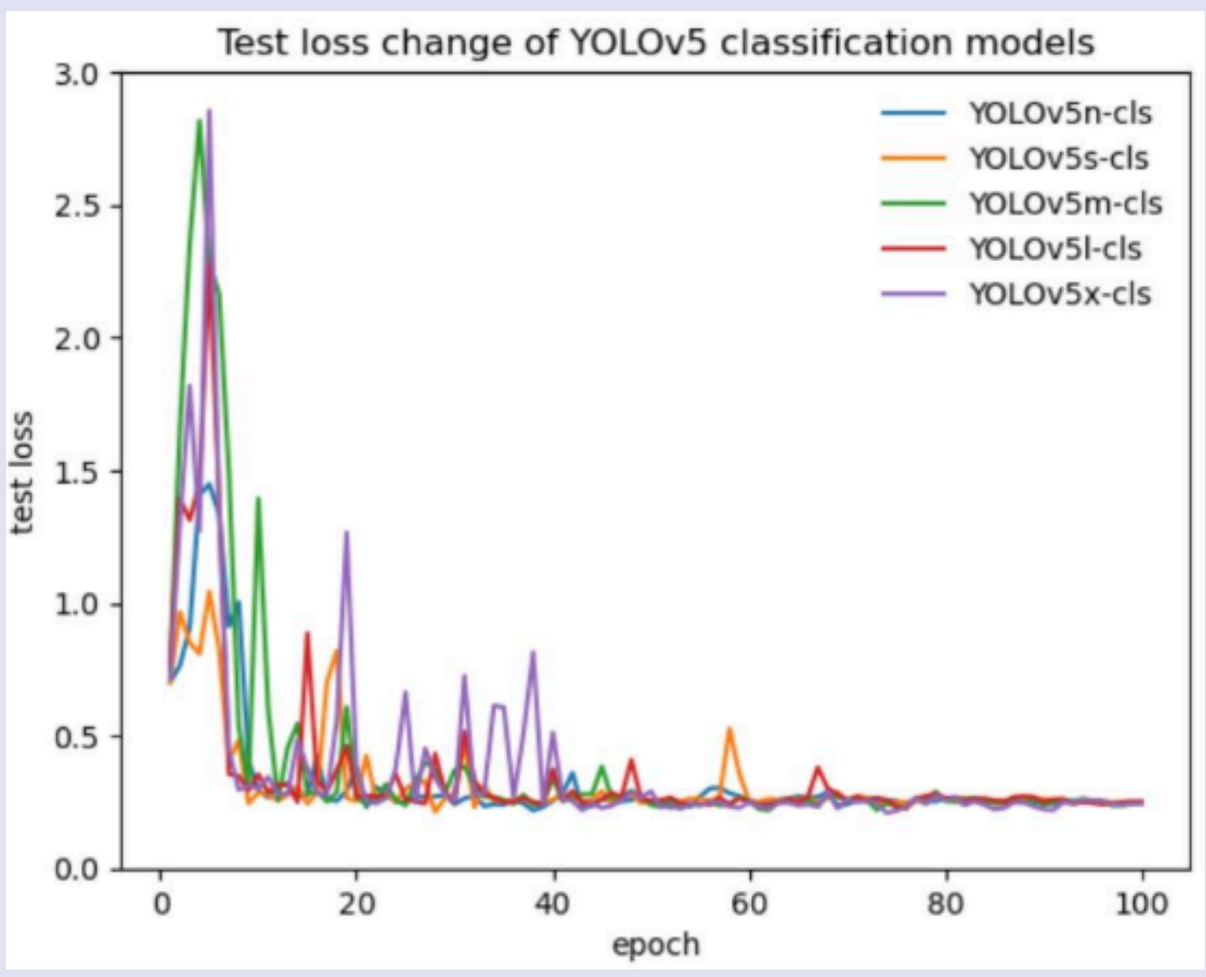


Figure 5 Test loss change of YOLOv5 classification models. There is no overfit in all models.

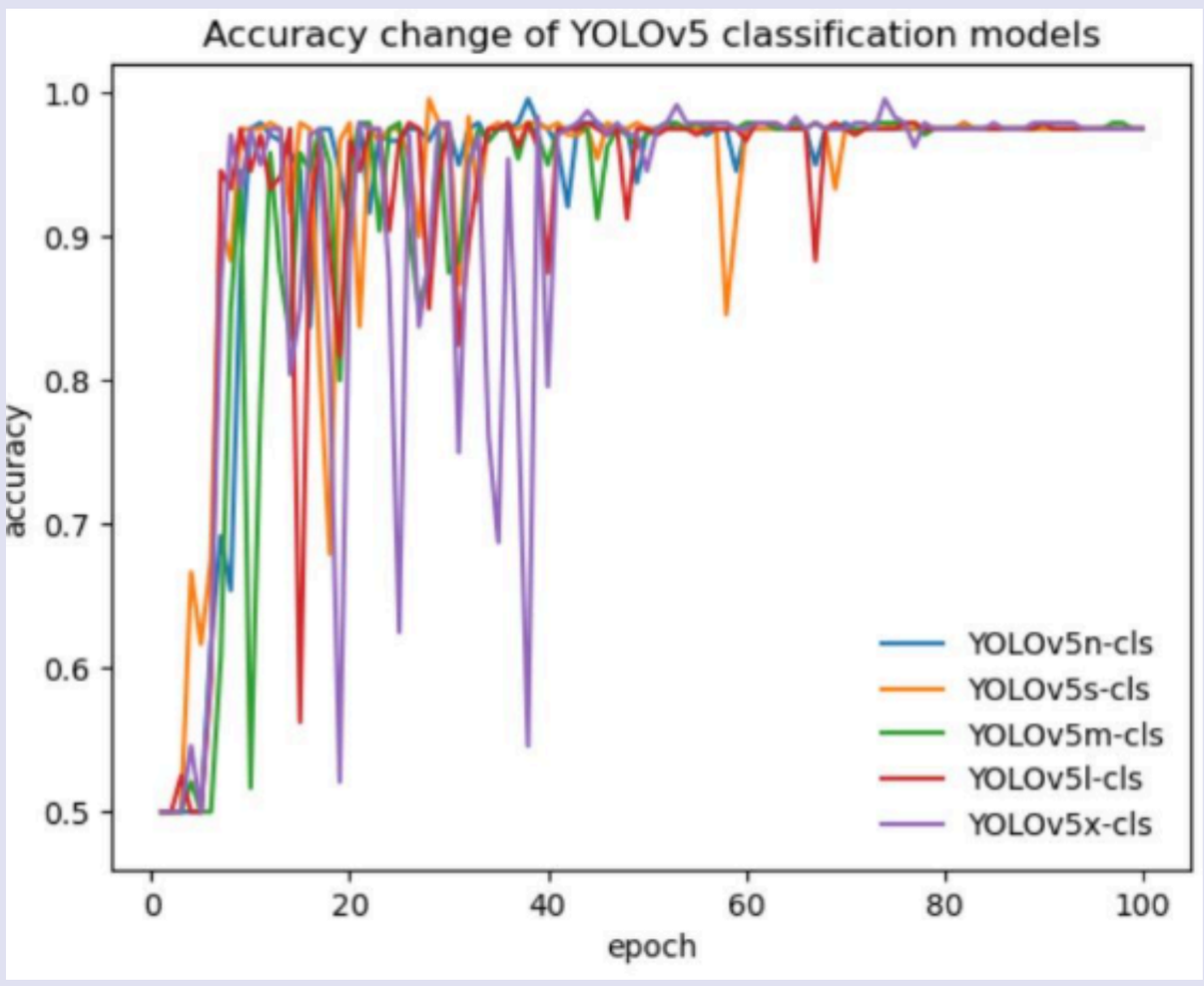


Figure 6 Accuracy change of YOLOv5 classification models. Model of all sizes achieved similar accuracy level in the end, but in the early epochs, the fluctuation of accuracy of larger model is more violent.

Reflections

We use too little data when training the model. Although that is because CJD is very rare so there are only a few pieces of data about it. Only 100 pictures of CJD MRI brain are collected and 200 pictures of non-CJD MRI brain are collected. Therefore, we use data augmentation and resizing the pictures. However, there is a problem with data augmentation. Only rotation, linear transformation, flip and mirror are applied to data. This may cause data leakage. Moreover, due to the change in shape to fit the MRI pictures into the model, some are over-stretched, making shape a factor in determining whether one has CJD, which is obviously not a factor. Hence, in the future, we will, and we advise everyone to make the following improvements. First, establish a community for the collection of cases of rare dieseases. Second, use part of the MRI instead of the whole picture of it to compare the detailed difference. Third, group several MRI pictures of one brain together into a tensor.

Limitations

There might be false negative and false positive problems. Human doctors are more likely to make false negative mistakes. Meanwhile, machines are considered to have the tendency to make false positive mistakes since they are sometimes 'too careful'. Therefore, it is hard to decide whether to believe in a machine. This field concerns Philosophy and Ethics and needs further research.

Designing Antibodies

Immunotherapy, known for its strong specificity and few side effects, has huge potential in curing prion disease.
Immunotherapy has made progress in Alzheimer disease through the discovery of Aducanumab, a human antibody targeting Aβ aggregates. And since different prion diseases have minor structural differences between the sequence of amino acids. And to be effective, these molecules would need to target a region that is crucial for the seeding activity. Thus, a small molecule that fits the seeding region is likely to be a good inhibitor for a specific prion strain, but the probability for such a small molecule to have a universal anti-prion molecule is not going to be high. Thus, antibodies treatment is seen to be more effective, since it can usually tolerate the structure differences and have more stable efficacy.
Antibodies treatment have made progress in curing CWD (Chronic Wasting Disease), a prion disease that was popular in deer, cows, and other mammals and could affect humans.
In the sick animals, the antibodies induced by vaccination will at least reduce PrPSc in the peripheral tissues and prevent the shedding of CWD prions, which is believed to be a main reason for the efficient lateral transmission of CWD (Chames, 2009).
Scientists focus more on using monoclonal antibodies to do immunotherapy, for example, Professor Simon's team have generated the Prion protein monoclonal antibody (PRN100) therapy for Creutzfeldt–Jakob disease. In 2022 April, the first-in-human treatment programme was finished. However, here we want to mention another strategy that is relatively rare, nanobodies.
Nanobodies are naturally found in the Camelidae family, including the dromedaries, alpacas and llamas (Sun, 2021). They are famous for better efficiency and less side effects compared to the regular antigens. In addition, they can also be produced by bacteria due its smaller size, strong hydrophilicity, and single-domain properties. Nanobodies can be rapidly produced in Gram-negative bacteria (such as Escherichia coli), Gram-positive bacteria (such as Bacillus brevis Little Rock), certain lactic acid bacteria, and bifidobacteria. The advantage of bacterial systems is that they are relatively easy to change and more economically viable as production systems (Bhavar, 2022). Nanobodies provide the incredible specificity of antibodies within a single immunoglobulin VHH domain that includes more extended and mixed CDRs, meaning that it has higher efficiency of binding to specific antigens. Complementarity-determining regions (CDRs) is a part of the variable chains in antibodies (generated by B-cells) and T cell receptor (generated by T-cell) (Nanomedicine, 2021). Due to its better resistance to the environment, nanobodies can express in bacteria, yeast and mammalian hosts. Nanobodies also have resistance to protease degradation and PH variation. (My BioSource, 2023) That gives a higher possibility of binding. The smaller size (around 30kda) allows them to target antigens and bind to receptors that are not available for full size conventional antibodies (Wesdorp, 2023).

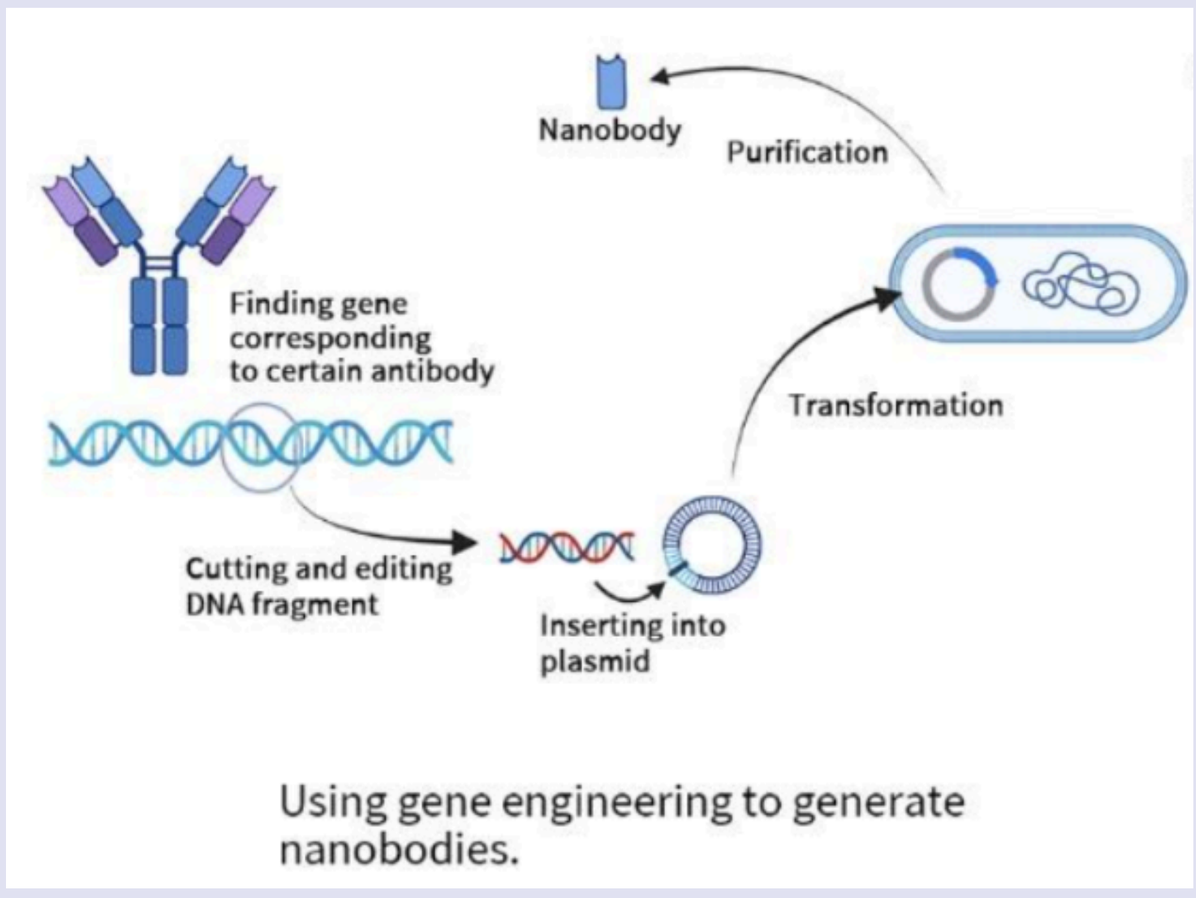


Figure 7 Gene engineering generating nanobodies.

Also, nanobodies have fewer side effects. Patients used to suffer from side effects caused by monoclonal antibodies. They are called infusion reactions, which are most common while the drug is first being given. Infusion reactions may lead to these symptoms: fever, chills, weakness, headache, nausea, vomiting, ddiarrhoea, low blood pressure, and rashes (ACS, 2022).
Despite that passive immunotherapy is rather convenient in treating prion diseases, by ensuring the antibodies are readily prepared before being injected into the patient and saves the time for patients to develop antibodies on their own, it has a fatal flaw, which is the possibility of causing neurodegeneration (Frontzek & Aguzzi, 2020, p. 169). Furthermore, some antibodies that are used in passive immunity treatments often are obtained from infected mice, or other nonhuman organisms, thus making humanization of the antibodies crucial (Wang et al., 2021, p.45-46). Through antibody humanization, the safety risks could be minimised, and the efficacy could be improved (Hummer & Deane, 2023, p. xx).
To elaborate, the conventional method of humanization is complementarity-determining regions (CDR) grafting, in which a nonhuman antibody is transferred and added onto a human antibody framework (Hummer & Deane, 2023, p. xx). Where the sequence of the antibody defines its efficacy, developability and humanness, despite a balance between them is needed (Hummer

& Deane, 2023, p. xx). Currently, there has been interdisciplinary crosses between computer science and immunology. This collaboration between different subject areas greatly improved the work efficiency and opens new possibilities for technical evolution. For example, the use of computational grafting and energy-based ranking method to humanise antibodies has greater efficiency (more than 2000 human acceptors can become attached to non-human CDRs) than the one-to-one result from the conventional experimental grafting (Hummer & Deane, 2023, p. xx; as shown in figure 8).

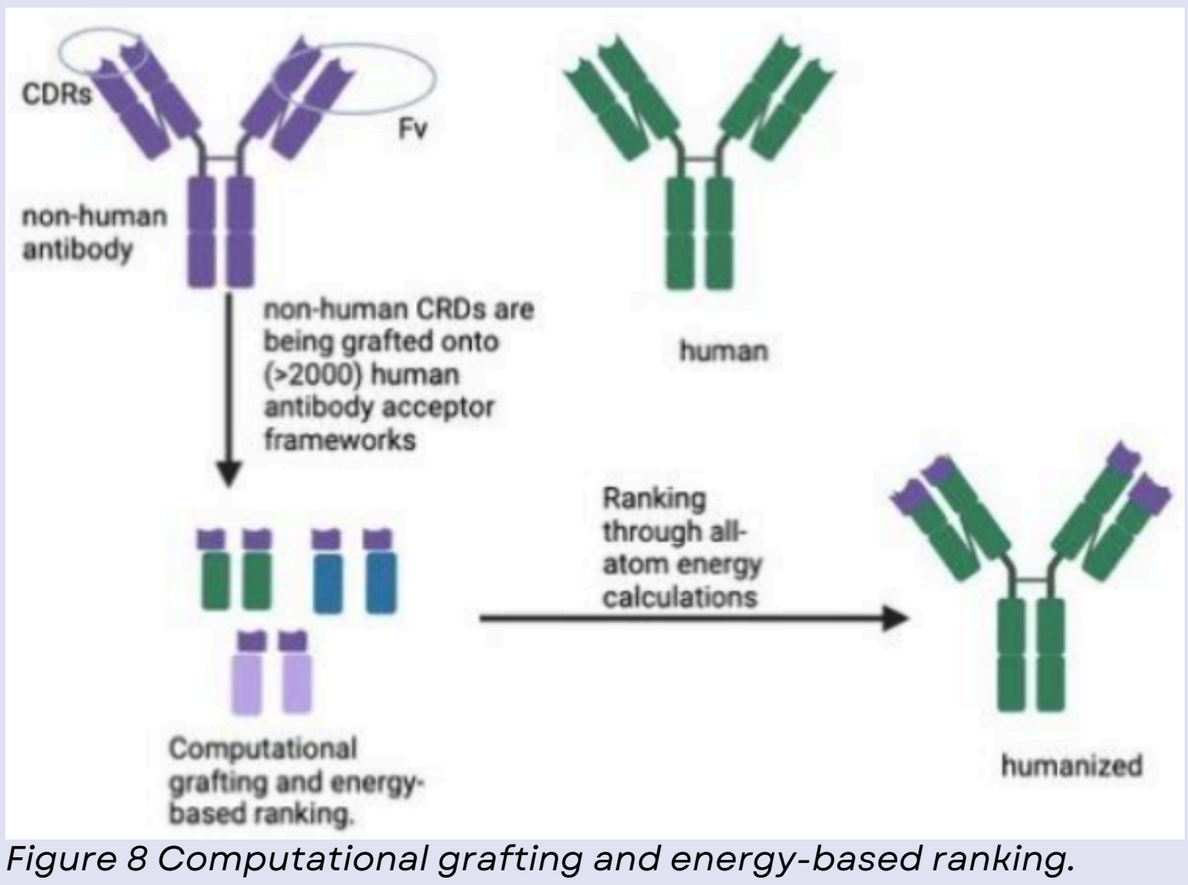


Figure 8 Computational grafting and energy-based ranking.

Discussion and Limitations

The current research presents an in-depth exploration of genetic engineering approaches in the context of prion diseases, specifically focusing on Creutzfeldt-Jakob Disease (CJD). Our study underscores the complexity and challenges associated with the diagnosis and treatment of CJD, given the rarity of the disease and the unique properties of prion proteins.

One of the pivotal findings of our research is the effectiveness of Convolutional Neural Networks (CNNs) in diagnosing CJD through the analysis of MRI images. The ResNet50 model demonstrated the highest accuracy rate of 97%, indicating its potential as a reliable diagnostic tool. However, the limitations of data availability for training these models cannot be overlooked. The rarity of CJD cases has necessitated the use of data augmentation techniques, which, while helpful, may introduce biases or inaccuracies in model training. The potential for data leakage and the distortion of MRI images due to resizing are concerns that warrant further investigation and refinement of our methodology.

Moreover, the discussion on the development of antibodies for immunotherapy against prion diseases highlights the promise and challenges of this therapeutic approach. The use of nanobodies, derived from the Camelidae family, presents a novel avenue for treatment due to their high specificity, reduced immunogenicity, and ability to target antigens not accessible to traditional antibodies. The progress made in the humanization of antibodies, as illustrated by the PRN100 therapy, is a significant step towards minimizing adverse effects and enhancing the efficacy of immunotherapy.

However, our research also reveals the limitations and ethical considerations of using machine learning and immunotherapy. The potential for false positives and false negatives in machine learning models, as well as the risk of neurodegeneration associated with passive immunotherapy, are issues that require careful consideration. The balance between the benefits of early and accurate diagnosis and the risks of overdiagnosis and overtreatment is a delicate one that must be navigated with caution.

Furthermore, the public survey conducted as part of this research reveals a concerning lack of awareness about CJD and prion diseases among the general population. This finding emphasizes the need for increased public education and awareness campaigns to ensure that individuals are informed about the risks, symptoms, and preventive measures associated with CJD.

In conclusion, while our research presents significant advancements in the diagnosis and treatment of CJD, it also highlights the need for further research, improved methodologies, and increased public awareness. The integration of machine learning and immunotherapy holds great promise, but it must be pursued with a thorough understanding of the limitations and ethical implications. Future work should focus on expanding the dataset for training diagnostic models, refining the techniques for data augmentation, and conducting more extensive clinical trials for immunotherapies to ensure their safety and effectiveness.

Supplementary Information

The Results from the survey

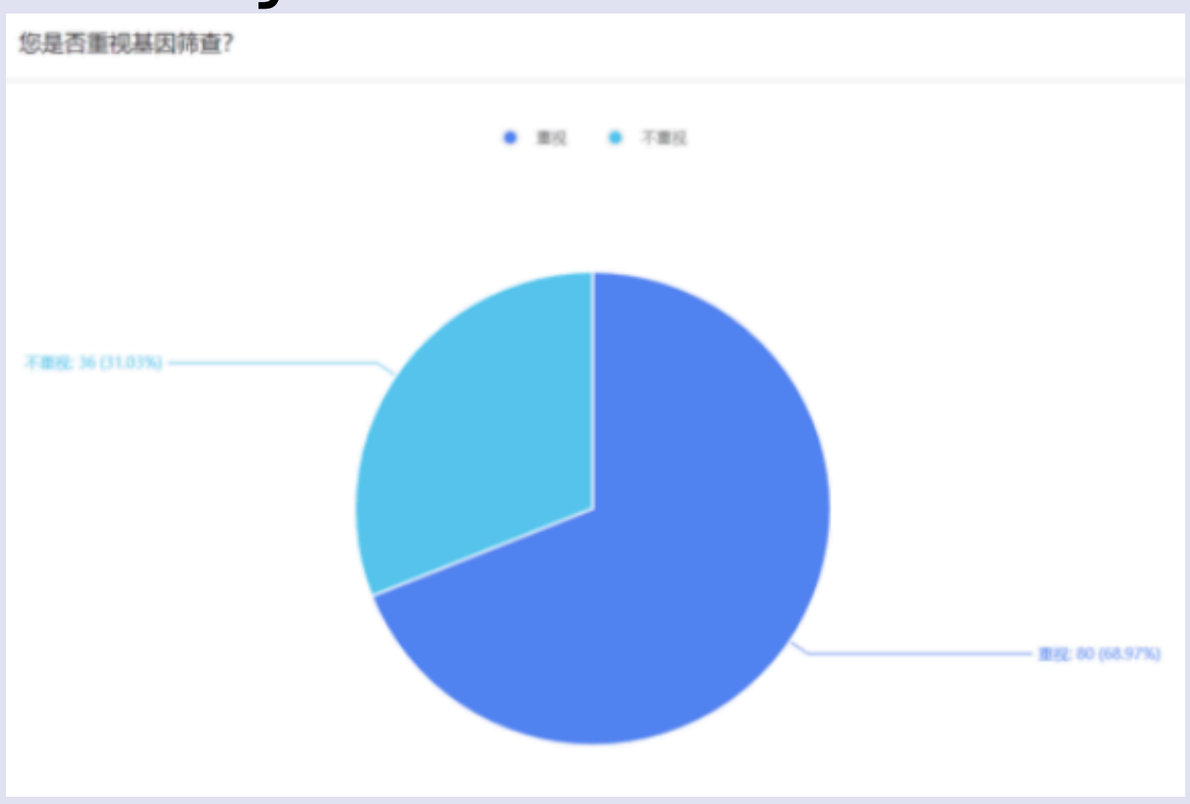


Figure 9 Question: Do you value genetic screening/testing? Darker Blue-Yes； Lighter Blue-No

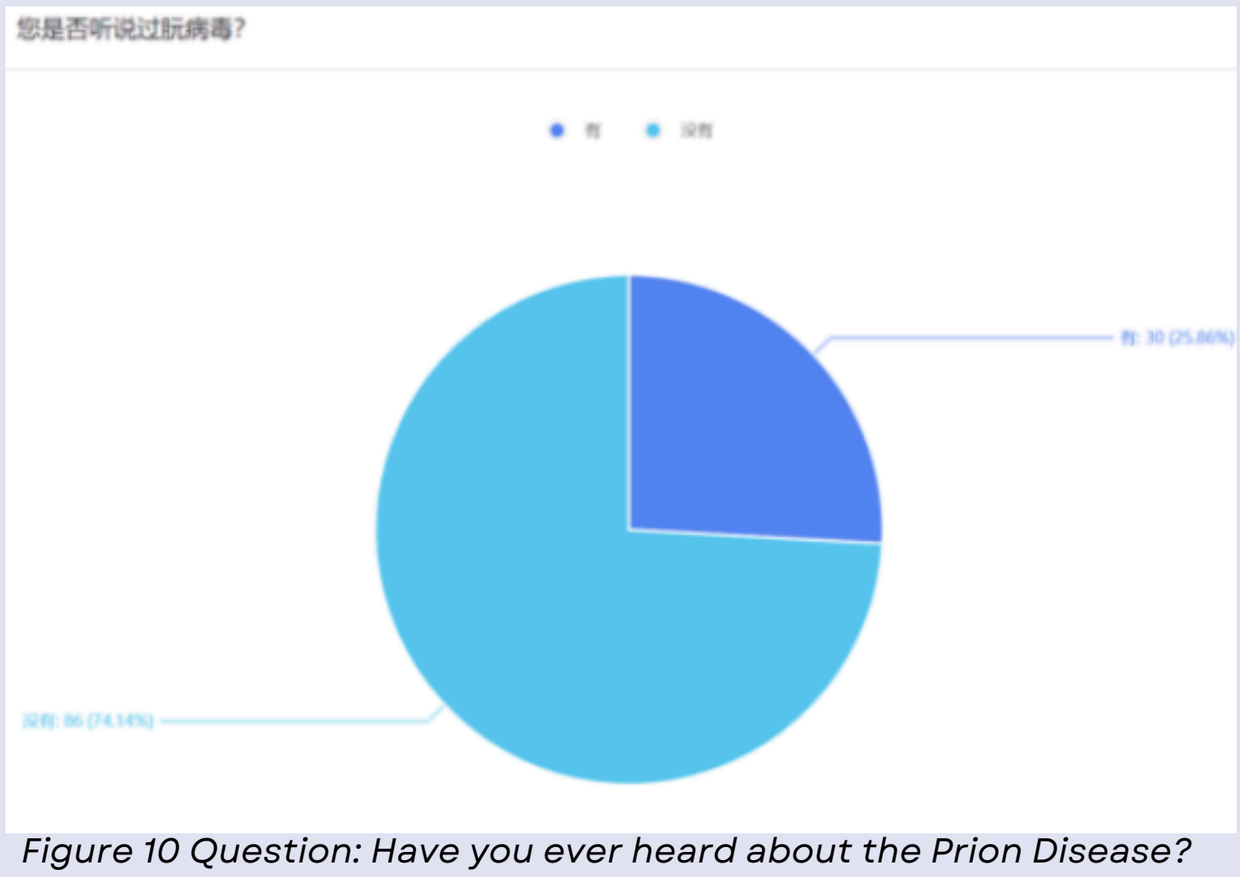


Figure 10 Question: Have you ever heard about the Prion Disease? Dark Blue- Yes； Light Blue-No

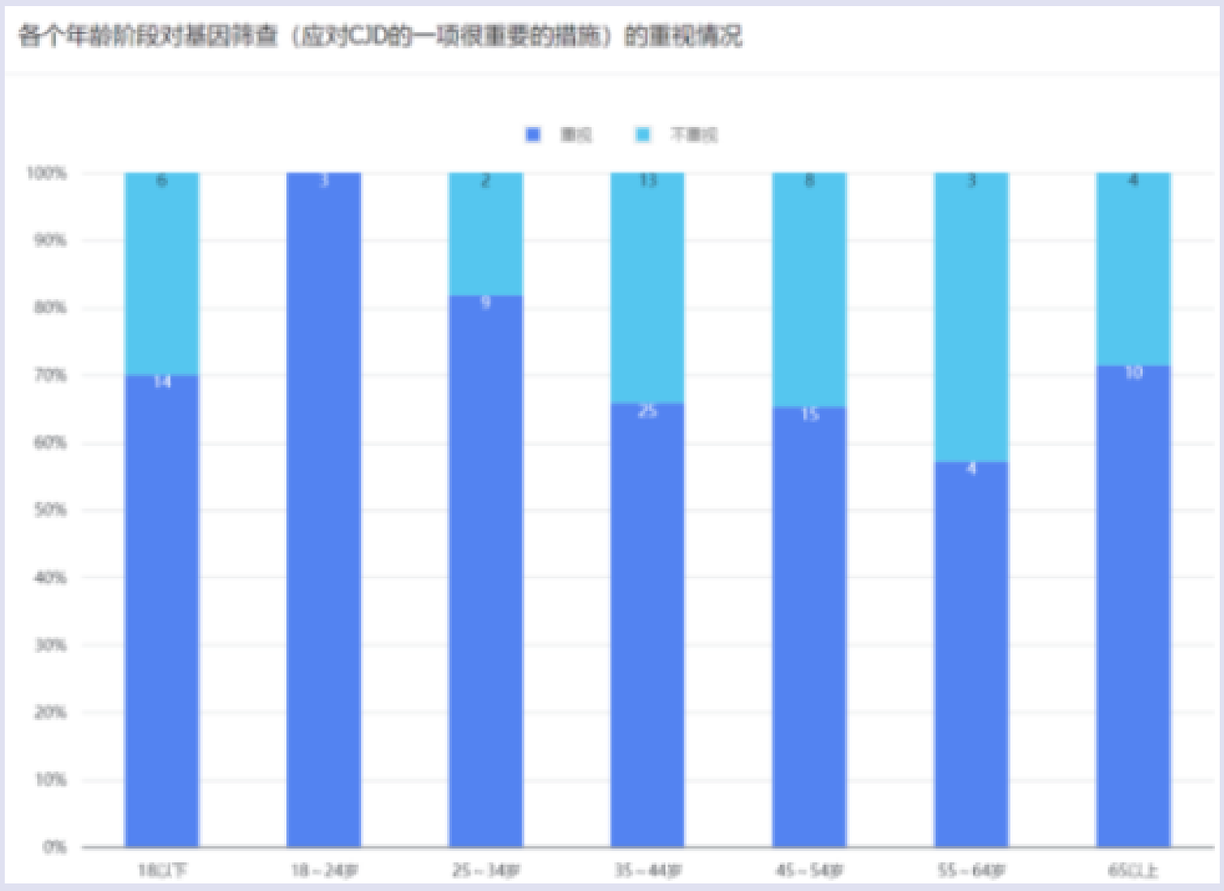


Figure 11 Results of an Investigation on the attitude towards genetic screening across different age groups

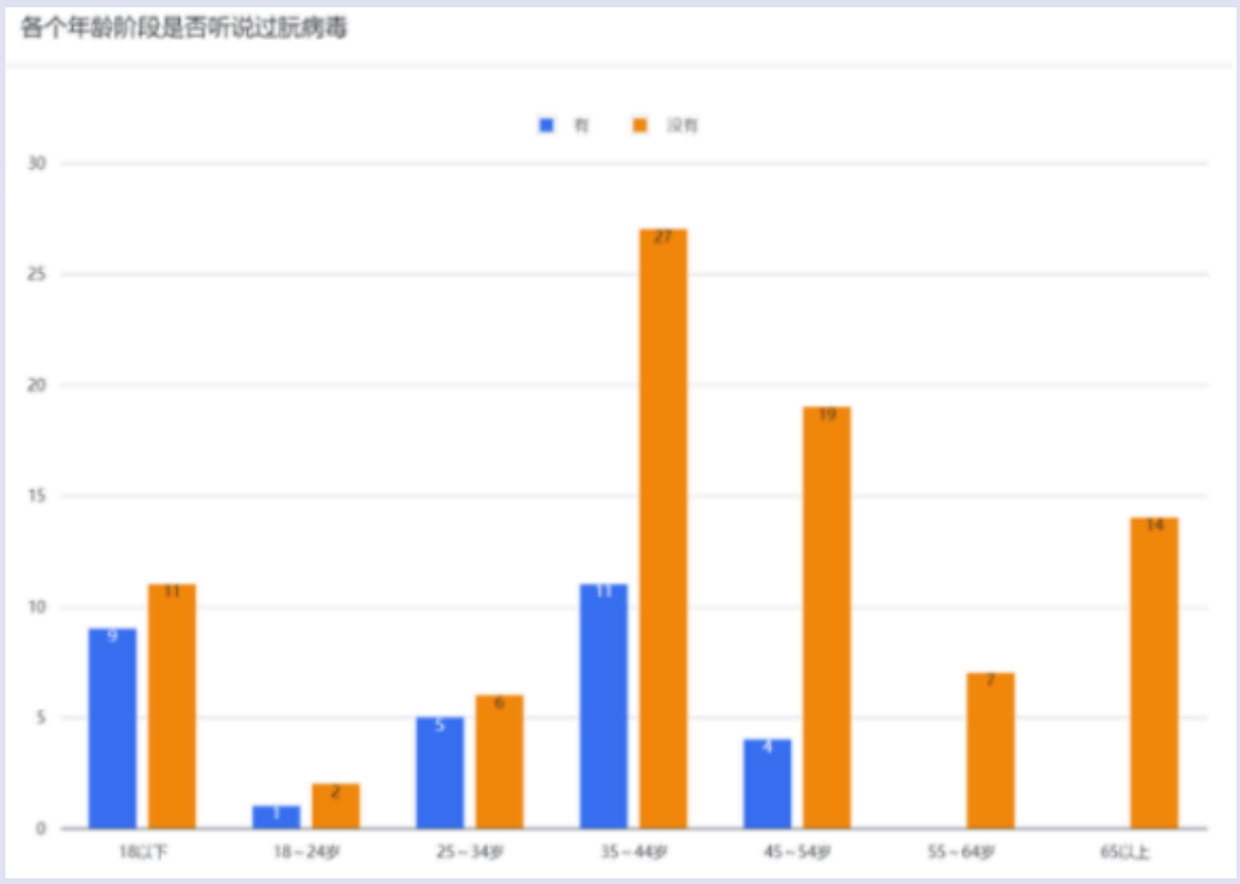


Figure 12 The results for an investigation on the acknowledgement of prion diseases across different age groups

Reference

Aguzzi, A., & Frontzek, K. (2020). Recent developments in antibody therapeutics against prion disease. Emerging Topics in Life Sciences, 4 (2), 169-173. https://doi.org/10.1042/etls20200002

American Cancer Society. (2022). Monoclonal Antibody Side Effects. Www.cancer.org. https://www.cancer.org/cancer/managing-cancer/treatment-types/immunotherapy/monoclonal-antibodies.html

Baraznenock, E., & Kravchuk, S. (2023, February 8). Prions: How can proteins be infectious? And what that tells us about other neurodegenerative diseases. Grey Matters. https://greymattersjournal.org/prions-how-can-proteins-be-infectious-and-what-tells-us-about-other-neurodegenerative-diseases/

Chames, P., Van Regenmortel, M., Weiss, E., & Baty, D. (2009). Therapeutic antibodies: successes, limitations and hopes for the future. British Journal of Pharmacology, 157(2), 220–233. https://doi.org/10.1111/j.1476-5381.2009.00190.x

Gu, J., Wang, Z., Kuen, J., Ma, L., Shahroudy, A., Shuai, B., ... & Chen, T. (2017). Recent advances in convolutional neural networks. Pattern Recognition, 77, 354-377. https://doi.org/10.1016/j.patcog.2017.10.013

He, K., Zhang, X., Ren, S., & Sun, J. (2016). Deep residual learning for image recognition. In Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition (pp. 770-778). https://doi.org/10.1109/CVPR.2016.90

Hummer, A. M., & Deane, C. M. (2023). Designing stable humanized antibodies. Nature Biomedical Engineering, 8 (1), 3-4. https://doi.org/10.1038/s41551-023-01168-1

Hubel, D. H., & Wiesel, T. N. (1959). Receptive fields of single neurones in the cat's striate cortex. The Journal of Physiology, 148 (3), 574-591. https://doi.org/10.1113/jphysiol.1959.sp006308

Huang, G., Liu, Z., Van Der Maaten, L., & Weinberger, K. Q. (2017). Densely connected convolutional networks. In Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition (pp. 4700-4708). https://doi.org/10.1109/CVPR.2017.243

Krizhevsky, A., Sutskever, I., & Hinton, G. E. (2012). ImageNet classification with deep convolutional neural networks. In Advances in Neural Information Processing Systems (pp. 1097-1105).

LeCun, Y., Bottou, L., Bengio, Y., & Haffner, P. (1998). Gradient-based learning applied to document recognition. Proceedings of the IEEE, 86 (11), 2278-2324. https://doi.org/10.1109/5.726791

Li, Z., Yang, W., Peng, S., & Liu, F. (2004). A survey of convolutional neural networks: Analysis, applications, and prospects. Journal of Artificial Intelligence Research, 55, 345-377. https://doi.org/10.1613/jair.4327

Mead, S., Khalili-Shirazi, A., Potter, C., Mok, T., Nihat, A., Hyare, H., Canning, S., Schmidt, C., Campbell, T., Darwent, L., Muirhead, N., Ebsworth, N., Hextall, P., Wakeling, M., Linehan, J., Libri, V., Williams, B., Jaunmuktane, Z., Brandner, S., & Rudge, P. (2022). Prion protein monoclonal antibody (PRN100) therapy for Creutzfeldt–Jakob disease: evaluation of a first-in-human treatment programme. The Lancet Neurology, 21 (4), 342–354. https://doi.org/10.1016/s1474-4422(22)00082-5

Ribes, J. M., Patel, M. P., Halim, H. A., Berretta, A., Tooze, S. A., & Klöhn, P. (2023). Prion protein conversion at two distinct cellular sites precedes fibrillisation. Nature Communications, 14 (1). https://doi.org/10.1038/s41467-023-43961-1

Rouvray, D. (2005, September 28). Battle with the prions. Chemistry World. https://www.chemistryworld.com/features/battle-with-the-prions/3004499.article

Rustenhoven, J. (2021). A privileged brain. Science, 374 (6567), 548–548. https://doi.org/10.1126/science.abl7122

Scheckel, C., & Aguzzi, A. (2018). Prions, prionoids and protein misfolding disorders. Nature Reviews Genetics, 19 (7), 405-418. https://doi.org/10.1038/s41576-018-0011-4

Sun, S., Ding, Z., Yang, X., Zhao, X., Zhao, M., Gao, L., Chen, Q., Xie, S., Liu, A., Yin, S., Xu, Z., & Lu, X. (2021). Nanobody: A Small Antibody with Big Implications for Tumor Therapeutic Strategy. International Journal of Nanomedicine, 16, 2337–2356. https://doi.org/10.2147/IJN.S297631

Wang, B., Gallolu Kankanamalage, S., Dong, J., & Liu, Y. (2021). Optimization of therapeutic antibodies. Antibody Therapeutics, 4 (1), 45-54. https://doi.org/10.1093/abt/tbab003

Zerr, I., Ladogana, A., Mead, S., Hermann, P., Forlorni, G., & Appleby, B. S. (2024). Creutzfeldt–jakob disease and other prion diseases. Nature Reviews Disease Primers, 10 (1). https://doi.org/10.1038/s41572-024-00506-0

The

CHINESE VERSION

English Version in the Front

SMART MAGAZINE AUTHOR: Evelyn Yi EDITOR: Amanda

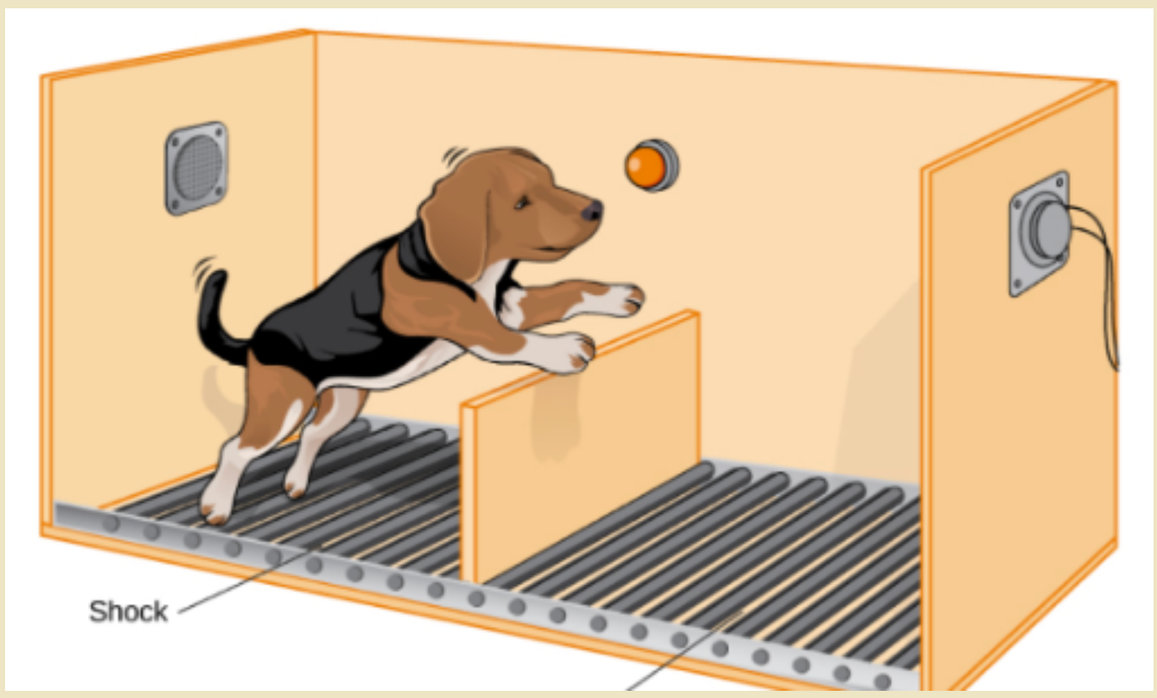
打破局限性思维：大脑如何学会掌控生活?

关键词：习得性无助、背侧剑突核、腹内侧前额叶皮层、抑郁症

导言

在有机会时, 为什么有些人在有机会时，却选择不去逃避痛苦的电击? 1967年，心理学家马丁·塞利格曼（Martin Seligman）进行了一项著名的实验。他将狗固定在安全带上，并对它们施加电击。其中一组狗可以通过按下附近的杠杆来终止电击，而另一组狗则即使按下杠杆也无法改变电击的结果。

第二天，所有的狗都放置在一个新的环境中——“穿梭箱”。穿梭箱是一个实验装置，中间由一个低矮的障碍物将其分隔成两个隔间。这一次，狗没有被束缚，它们只需跳过低矮的障碍物，就能避开痛苦的电击。



令人意外的是，根据狗在前一天是否能够控制电击的经历，它们的反应表现出了显著的差异。90% 的“可逃避电击组”狗迅速学会通过跳过障碍物来避免电极电击，而“不可逃避电击组”中有三分之二的狗甚至没有尝试逃避电击。相反，它们只是被动地躺下，忍受电击，尽管实际上是可以逃脱的。

塞利格曼的实验引入了一个极具影响力的心理学概念——“习得性无助”。“这一概念表明，当个体长期或反复暴露于无法控制的负面事件中时，会逐渐形成一种信念，认为自己的行为无法改变结果。即使在客观上并非无助·，或在实际上可以掌控结果时，他们仍会将这种无助感延续并扩展到其他情境中。

人体实验的发现

通过后续研究的验证，习得性无助的现象在人体实验中得到了相同的观察。在对大学生的研究中，研究人员通过设置可躲避和不可躲避的巨响，或让参与者解答可解和不可解的谜题，得出了与狗实验类似的结果。那些属于“无法逃脱组”的参与者始终无法逃脱或成功解决谜题。例如，有一份报告提到，无法逃脱组的参与者曾评论道：“什么方法都试过了，为什么还要尝试呢？”

此外，研究人员还发现，人类对于未能逃脱的原因所做的解释或归因，可以有效预测他们感知到的无助感的持续时间和严重程度。将失败归因于永久性原因的受试者（如“我总是不擅长解决问题”）往往会表现出较长时间的无助感。相比之下，将失败归因于暂时性原因的受试者（例如“今天状态不好”）通常只会产生短暂的无助感。若将失败归因于广泛的原因（如“大多数问题根本无法解决”），受试者在多个情境中都会感到无助；而如果归因于具体的原因（如“这个任务太难了”），无助感则会被限制在特定的情境中。

最后，以人为对象的研究进一步确立了“习得性无助”作为理解抑郁障碍内在机制的一种实验室模型。根据《精神障碍诊断与统计手册》（DSM-III，1980年；DSM-IV，1994年）的定义，当患者在以下九种症状中出现至少五种时，即可被诊断为重度抑郁症：

1. 悲伤的情绪
2. 对活动失去兴趣或乐趣
3. 体重显著减轻或增加
4. 睡眠障碍（失眠或嗜睡）
5. 精神运动性激动或迟缓
6. 疲劳或失去活力
7. 感到无价值或过度内疚
8. 注意力难以集中或决策困难
9. 反复出现死亡或自杀的念头

习得性无助的症状与临床抑郁症的症状非常相似。在实验室中，研究人员通过动物和人类的实验成功复制了抑郁症九种症状中的八种。唯一无法在实验室条件下复制的症状是自杀或自杀念头，这主要由伦理限制所致。相比之下，那些没有经历无法逃避事件的抑郁症患者在实验中表现出更多的被动性，并且更频繁地未能逃脱或完成认知任务。

神经科学修正了这一理论

新的神经科学研究表明，尽管心理学家关于暴露于无法逃避的负面刺激与被动性之间联系的观点是正确的，但他们对‘学习’部分的理解存在误差。事实上，被动性和焦虑并非学来的，而是大脑对有害或创伤性经历的本能反应。相反，感知控制感才是需要积极学习的内容。

塞利格曼及其同事提出的“习得性无助”原始理论包含了以下两种主要机制：

- 检测：动物（或人类）会检测事件的结果是否能被自己的行为所控制。
- 预期：在检测到无法控制后，动物会形成一种预期，认为未来的事件也将是不可控的。

最初的模型认为，“检测到不可控性”是产生习得性无助的关键成分——当个体意识到事件无法控制时，便会产生习得性无助感。然而，随着最新神经科学发现的出现，事实证明，检测到“控制感”（而非不可控性）实际上才是防止被动性的关键。当个体意识到自己可以控制某种情况时，习得的无助感便会减弱或消失。

1. 被动性/焦虑 （PASSIVITY/ANXIETY）

厌恶性刺激会激活背侧网状结构（DRN）中的血清素能（5-HT）神经元。DRN的长期激活会导致敏感化，这种敏感化可以持续数天，进而加剧被动性，并且恐惧和焦虑反应也会随之增强。与“检测-预期”理论假设的不同，这种反应是由厌恶体验本身的强度和持续时间所引发的，而不是由个体对不可控性的感知所引起的。换句话说，不论个体是否认为情况无法控制，当遭遇强烈或长时间的冲击时，被动性和焦虑反应都会自动激活。

2. 发觉与行动（DETECT 和 ACT）

当情况是可控的（例如，能够逃避电击时），前边缘腹侧前额叶皮层（PL）会与 DRN 通信，识别出控制感。检测到控制后，PL 的另一条通路会抑制 DRN，从而阻止 5-HT 神经元的激活，减少被动性和恐惧反应。这表明，正是前边缘内侧前额叶回路在检测到控制，而不是缺乏控制抑制了 DRN 的本能反应。

3. 预期（EXPECT）

在经历过控制的情境后，PL-DRN 回路会发生可塑性变化，形成一个“记住”控制的回路。在未来的压力情境中，即使电击是无法逃避的，这一回路也会激活，就像个体仍然拥有控制感一样。

例如，无法逃避的电击不会被感知为无法逃避，而是会被体验为个体可以控制的情况。重要的是，这证明了“预期”并非一个有意识的过程，而是一种由先前控制经验形成的神经偏差。

结论

前边缘皮层的发现及其在检测控制并根据控制采取行动方面的关键作用，重新定义了无助感——它不是失败的必然结果，而是检测能力的缺失。当大脑学会控制时，它会发生重塑，形成一种对自主性的期望，这种期望甚至能够将无法逃避的考验重新诠释为宝贵的机会。这些新的理解揭示了一个充满希望的前景：个人可以通过重新塑造大脑来识别控制感，从而学会并获得能动性。通过担任领导职务或寻找需要主动性的位置，即使是那些容易感到被动的人，也能够培养出掌控局面的能力，并改变他们以前对挑战性情境的“硬性反应”。

参考文献

Baratta, M. V., Seligman, M. E. P., & Maier, S. F. (2023). From helplessness to controllability: Toward a neuroscience of resilience. Frontiers in Psychiatry, 14, 1170417. https://doi.org/10.3389/fpsyt.2023.1170417

Maier, S. F., & Seligman, M. E. (2016). Learned helplessness at fifty: Insights from neuroscience. Psychological Review, 123(4), 349–367. https://doi.org/10.1037/rev0000033

Song, X., & Vilares, I. (2021). Assessing the relationship between the human learned helplessness depression model and anhedonia. PLOS ONE, 16(3), e0249056. https://doi.org/10.1371/journal.pone.0249056

SMART MAGAZINE AUTHOR Evelyn Yi EDITOR: Amanda

揭秘快速眼动期与非快速眼动期梦境的奥秘

简介：

有些梦境我们能够轻松回忆起来。它们生动、细节丰富，情节发展几乎像电影一样。而另一些梦则不那么令人印象深刻——它们更像是GIF或抖音短视频，而非充满戏剧性的、有故事情节的体验。是什么导致了这些梦境体验的差异呢？答案可能与梦境发生的不同睡眠阶段有关。

1952年，8岁的阿蒙德·阿瑟林斯基（Armond Aserinsky）在某个晚上入睡时，研究人员首次记录到伴随不规则大脑活动的周期性眼球运动（REM）。他们还发现，当人在快速眼动期（REM）睡眠中被唤醒时，更常报告自己做了梦——也就是说，在快速眼动期（REM）被唤醒的人中，71%报告做了梦，而在非快速眼动期（NREM）睡眠中，只有17%的人做梦。自此之后，“REM睡眠”成为了做梦和意识体验的代名词，给人留下了只有在快速眼动期才能做梦的印象。然而，这种假设并不准确。例如，通过药物抑制快速眼动期睡眠并不会完全消除做梦。因此，做梦并非快速眼动睡眠的专利，它同样发生在非快速眼动睡眠阶段。

关键词：做梦；睡眠阶段；快速眼动睡眠（REM）；非快速眼动睡眠（NREM）；自我概念

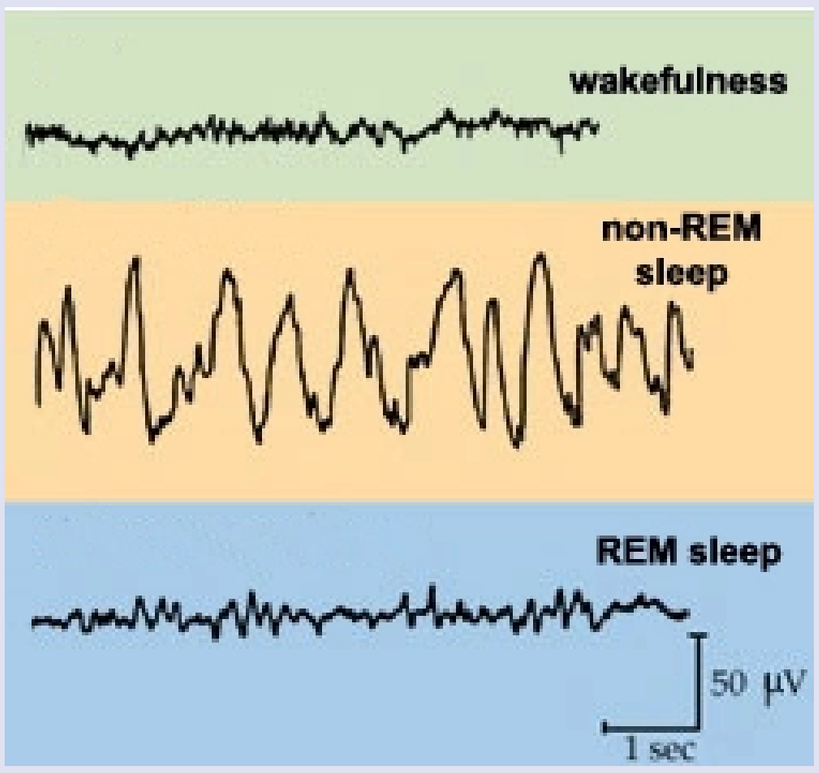


图1: 快速眼动期 vs. 非快速眼动期

睡眠阶段

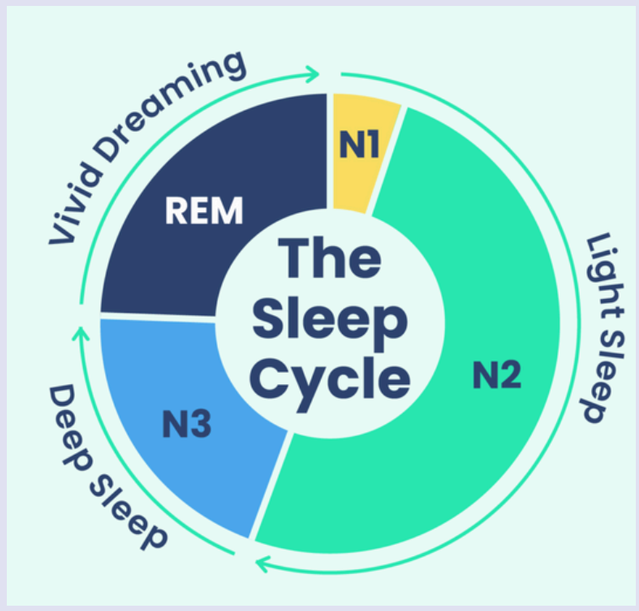


图2: 人类的睡眠周期

要了解快速动眼期（REM）和非快速动眼期（NREM）梦境体验差异的原因，深入研究每晚的睡眠阶段周期可能会有所帮助。人的睡眠周期分为四个阶段：N1、N2、N3 和 REM。N1、N2 和 N3 被归类为非快速眼动睡眠，而我们大约 75%-80% 的睡眠是在非快速眼动睡眠中度过的。每晚，人们在快速动眼期和非快速动眼期之间交替睡眠，在每个周期中，花在快速动眼期的时间会逐渐增加。一个完整的睡眠周期通常持续 90 到 110 分钟，人们每晚通常要经历 4 到 6 个睡眠周期。

N1 阶段：

人在刚入睡时会进入 N1 阶段，此时很容易被唤醒。在这一阶段，肌肉活动和呼吸会减慢，并可能出现幻觉，例如突然坠落或漂浮的感觉。N1 阶段通常持续 1 到 7 分钟。

N2 阶段：

我们大部分的睡眠时间都在 N2 阶段度过，大约持续 10 到 25 分钟。虽然仍然可以被唤醒，但不会像 N1 阶段那样容易。此时，人已明显处于睡眠状态。在 N2 阶段，人们的脑电波会呈现周期性的快速活动，称为睡眠纺锤波。

N3 阶段（与 *N4* 阶段）：

N3 通常与 *N4* 阶段合并，统称为深度睡眠。这一阶段，大脑会发出缓慢的 *delta* 波，处于深度睡眠状态的人很难被唤醒。夜惊和梦游通常发生在 *N3* 阶段。人有 *20%* 的时间处于 *N3* 阶段睡眠，其中大部分发生在前半夜，随着夜晚的推移而逐渐减少。

快速动眼期（*REM*）：

在快速动眼期睡眠中，心率会加快，呼吸变得急促且不规律，眼睛在眼睑后快速转动，大脑活动与清醒时相似。因此，快速动眼期的梦境往往更加生动且富有情感。这一阶段，尽管大脑的运动皮层非常活跃，但脑干会阻断运动信号，使人处于肌肉麻痹状态。这种麻痹有时会持续到人醒来，导致所谓的睡眠麻痹——即醒来后无法控制身体运动。快速动眼期睡眠也被称为“矛盾睡眠”，因为从外表看，人在这一阶段显得平静且熟睡，但内心却非常活跃。

SMART MAGAZINE AUTHOR Evelyn Yi EDITOR: Amanda

REM 梦与 NREM 梦：

梦的结构

除了梦的回忆率外，快速动眼期（REM）和非快速动眼期（NREM）梦境在语言结构方面也存在显著差异。一项研究通过唤醒处于 REM 和 NREM N2 阶段的参与者，收集并分析了他们的梦境报告。

在 N2 阶段唤醒时，参与者更可能报告没有梦境（19.40% 对 7.41%）或经历了“白梦”(15.67% 对 1.85%)，即意识到自己曾做梦但无法回忆内容的感觉。此外，研究发现，REM 梦境报告不仅长度更长，还表现出更高的结构连贯性。这表明，REM 梦境报告通常较长，并且类似于具有清晰叙述的连贯故事。相比之下，NREM 的梦境报告通常较短且零散，反映出更多简单的、类似思维的印象。这一现象与 NREM 梦缺乏 REM 梦典型的幻觉性和故事性的这一研究结果一致（马丁等人）。

这些差异反映了 REM 和 NREM 大脑活动的不同如何在梦境结构中体现：REM 梦境更具叙述性和生动性，而 NREM 梦境则更片段化、简单化。这进一步强调了大脑在不同睡眠阶段对梦境体验的塑造作用。

“自我 "的表征

REM 梦与 NREM 梦的另一个显著区别在于对“自我”的表述方式。梦中的“自我”--即梦中的“我”--在属性上与清醒状态下对“自我”的体验相比，发生了显著变化。例如，一个已经毕业多年的人可能会梦见自己在上高中，又或者是梦到与已故的亲人见面。这种现象表明，梦中的自我在获取自传体记忆方面存在局限，情绪反应异常，缺乏将过去和现在经历整合在一起的能力。

此外，梦中的自我监控能力也常常受损，这使得我们可能梦见一些无厘头或完全离奇的事情，而在梦中的自我视角下却完全不觉得不妥。梦境还往往以一种以自我为中心的方式呈现，梦的叙述通常围绕做梦者的行为、情感、欲望和经历展开。从这个角度来看，梦中的自我缺乏时间上的连贯性以及完整的“自我”感。

由于 REM 和 NREM 睡眠中的生理反应和意识水平存在差异，研究这两种睡眠阶段梦境中“自我”表述的不同可能有助于揭示“自我”的本质特征。

一项研究通过系统分析大型数据库中的梦境报告，调查了 REM 梦与 NREM 梦中“自我”表征的差异。研究发现，在 REM 梦中，“自我”更常参与攻击性互动，比例高达 52%；而在 NREM 梦中，“自我”几乎不会扮演攻击者的角色。在 NREM 梦中，“自我”的表现更为友好。尽管 NREM 梦中的社交接触大多是不愉快的（其它睡眠阶段也是同样的），其中近 90% 的梦境涉及建立友好的关系，而 REM 梦中这一比例仅为 54%。

此外，REM 梦中的“自我”更倾向于与其他意图相似的角色一同出现，因此梦境报告中更频繁地使用“我们”来描述互动。而在 NREM 梦中，更多时候是以“我”为中心进行叙述（McNamara 等人，2009）。

这些发现表明，REM 和 NREM 梦境中的“自我”表征有着显著不同，这种差异可能为我们探索“自我”的本质提供新的视角。

生理学解释：

REM 睡眠的特点是特定脑区的选择性激活，主要集中在边缘系统和旁边缘区域，包括下丘脑外侧、杏仁核、海马旁皮质、内侧和眶额皮质。这些区域深度参与情绪处理、记忆巩固和动机状态，这也解释了为什么 REM 梦境通常更加生动且充满情感。

在正电子发射断层扫描（PET）研究中，与清醒状态相比，REM 睡眠显示出大脑能量代谢的全面下降。此外，脑电图（EEG）研究则揭示了 REM 睡眠期间大脑皮层活动的更强局部化特征。在 NREM 睡眠期间，尽管丘脑-皮层系统仍保持活跃，但大脑皮层的区域连通性有所降低。换句话说，在实验条件下受到刺激时，大脑皮层与其他脑区相互作用以产生复杂、综合反应的能力会减弱。这种区域间的脱节导致 NREM 睡眠中的梦境更为零碎、抽象。

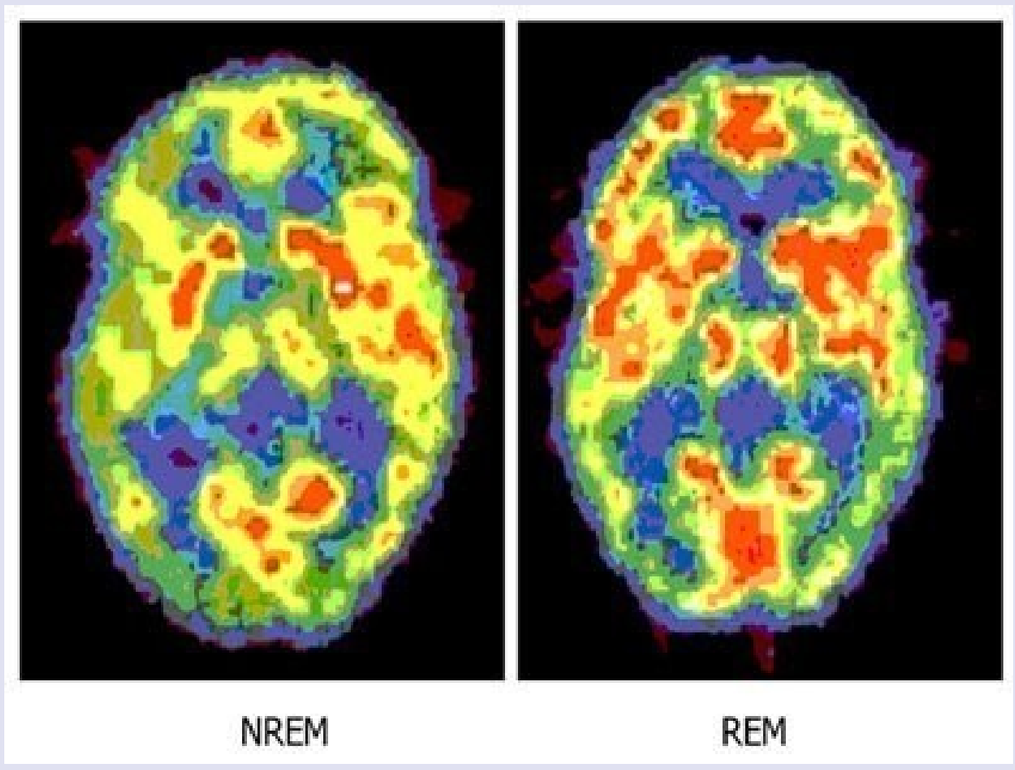


图3: REM和NREM的大脑皮层活动

相比之下，REM 睡眠则保留了清醒状态下大脑各区域之间的大部分连通性（Massimini 等人，2011）。这种区域间的广泛交流使得 REM 睡眠可以创造出更加连贯和复杂的体验，表现为 REM 梦境通常具有生动、叙事性强的特征。

这种大脑活动的差异说明了 REM 和 NREM 睡眠在梦境体验上的显著不同，并揭示了大脑如何通过不同的连接模式塑造梦境的内容和结构。

结论：

REM 梦通常发生在大脑高度活跃的状态下，由与情绪和感官体验相关的脑区强烈活动所驱动；而 NREM 梦则是在大脑较安静的振荡状态中出现，反映了这一睡眠阶段中大脑较低的活动水平。尽管现有关于 REM 和 NREM 生理差异的研究能够解释 REM 梦境的一些显著特点，如生动性和情感丰富性，但这些研究尚不足以完全揭示“自我”在 REM 和 NREM 梦境中所扮演角色的显著差异。

这引发了一些关于“自我”本质的深刻问题：为什么 NREM 梦境中的“自我”主要参与友好互动，而 REM 梦境中的“自我”则倾向于更多攻击性接触？哪些大脑机制决定了“自我”在不同梦境中扮演的角色？

通过探索这些问题，我们可以进一步理解大脑如何构建自我意识并处理社会与情感体验。这不仅有助于阐明梦境的本质，还可能为我们揭示意识、记忆和社会认知的神经机制提供重要线索。

引用：

Aserinsky, E., & Kleitman, N. (1953). Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. Science, 118(3062), 273–274. https://doi.org/10.1126/science.118.3062.273

Martin, J. M., Andriano, D. W., Mota, N. B., Mota-Rolim, S. A., Araújo, J. F., Solms, M., & Ribeiro, S. (2020). Structural differences between REM and non-REM dream reports assessed by graph analysis. PLOS ONE, 15(7), e0228903. https://doi.org/10.1371/journal.pone.0228903

Massimini, M., Ferrarelli, F., Murphy, M., Huber, R., Riedner, B., Casarotto, S., & Tononi, G. (2010). Cortical reactivity and effective connectivity during REM sleep in humans. Cognitive Neuroscience, 1(3), 176–183. https://doi.org/10.1080/17588921003731578

McNamara, P., McLaren, D., & Durso, K. (2007). Representation of the self in REM and NREM dreams. Dreaming, 17(2), 113–126. https://doi.org/10.1037/1053-0797.17.2.113

SMART MAGAZINE AUTHOR: Nox EDITOR: Hecate

珠被绒毡层自噬对胚胎模式建成的重要性

引言

2024年5月，武汉大学赵鹏教授团队于Nature Communications 上发表了一篇名为“Autophagy-mediated degradation of integumentary tapetum is critical for embryo pattern Formation”的研究论文[1]，该文章主要阐述了了珠被绒毡层中的自噬对程序性死亡(programmed cell death, PCD)和植物脂代谢的调控意义。

研究背景

珠被绒毡层（integumentary tapetum, IT）是被子植物胚珠中位于胚囊外围的一层特殊细胞，来源于珠被的最内层。其功能类似于花药绒毡层，通过提供营养物质和信号分子（如赤霉素）支持胚胎和胚乳的早期发育。珠被绒毡层本身并不会发育成种子，但它能通过细胞间通信调控胚种的正常发育，且其程序性死亡（PCD）对胚胎模式形成至关重要。作为珠被绒毡层在植物雄性生殖系统中的对应，花药绒毡层通过PCD促进雄性繁殖的机制已有详细的研究阐述，虽然珠被绒毡层也通过PCD促进胚胎发育，但目前机制尚不清楚，所以作者针对该机制进行了研究。

自噬分为大自噬、微自噬和分子伴侣介导自噬三大类，本文探讨的是大自噬。先前研究发现ATG（AUTOPHAGY-RELATED）基因作为介导大自噬调控的重要基因，在烟草种子发育过程中表现出相对较高的表达水平并随时间动态变化，这暗示了自噬在种子发育过程中发挥了重要作用[2]。

研究内容与结果

研究者利用针对ATG的GFP基因融合和免疫荧光技术，发现ATG5、ATG7、自噬体在IT中含量丰富，证实了IT中存在活跃的自噬现象。并且通过电镜观察到了IT中自噬体和自噬小体的细微状态。

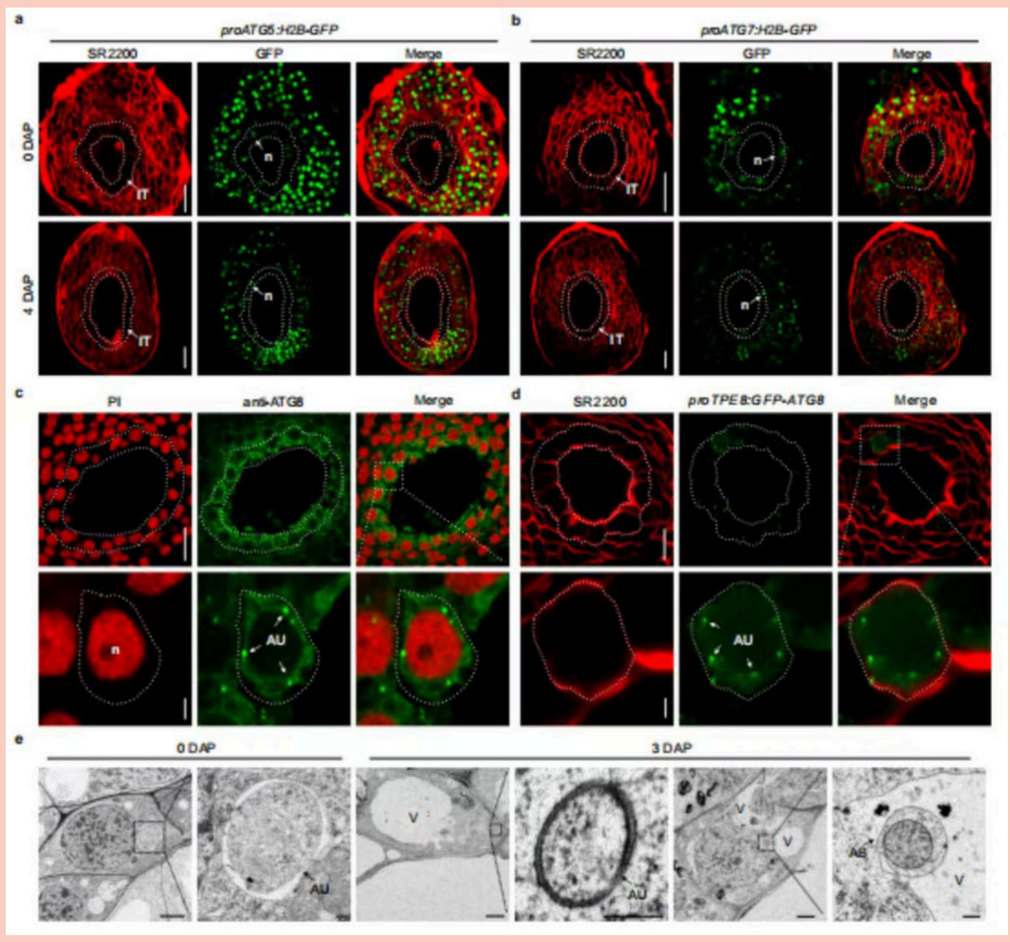


图1. 种子发育过程中IT自噬活跃（a、b、d.红色为细胞壁，绿色为ATG；c.红色为核，绿色为ATG；e.透射电镜观察图，透射电镜观察显示，在皮肤绒毡层中有典型的自噬小体和自噬小体）

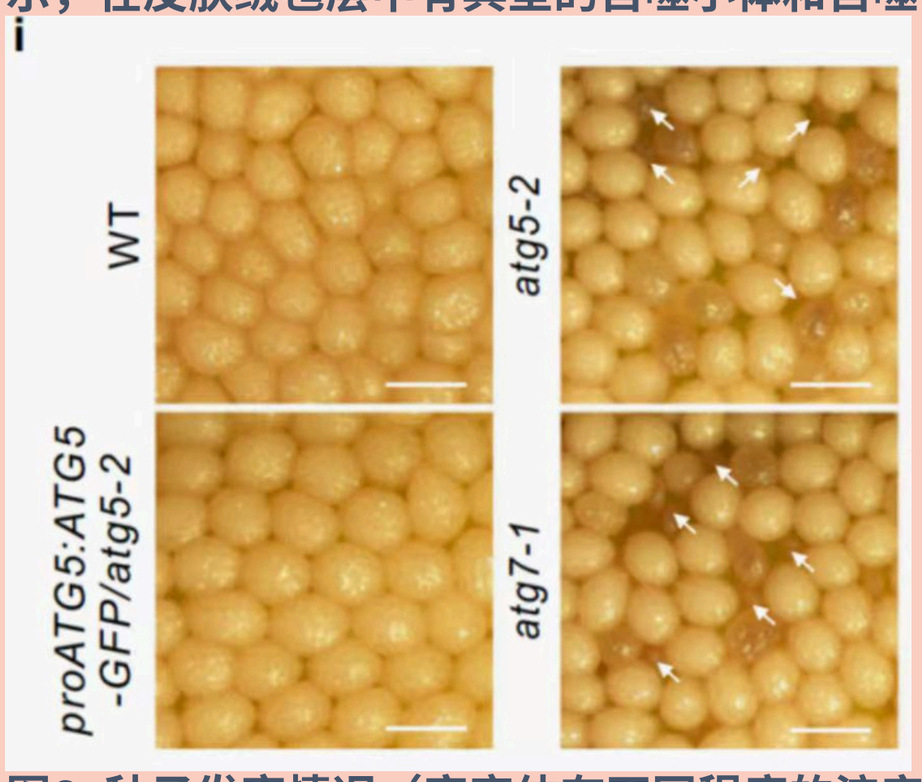


图2. 种子发育情况（突变体有不同程度的流产）

为了进一步探究IT自噬对种子发育的影响，研究团队构建了atg5、atg7的突变体，并发现突变体中自噬活动大幅下降，种子流产率大幅提高。而在向突变体重新引入ATG 5 后表型恢复。研究者还发现，在受精前及受精后4天以内，突变体与野生型并无明显区别。然而在受精4天后，突变体由于自噬活动的丧失导致IT无法像野生型一样降解，导致IT逐渐增厚。不仅如此，突变体的还出现了三种异常的胚胎发育模式，即合子分裂形式与正常状态下不同。这暗示了自噬对胚胎发育的重要意义。

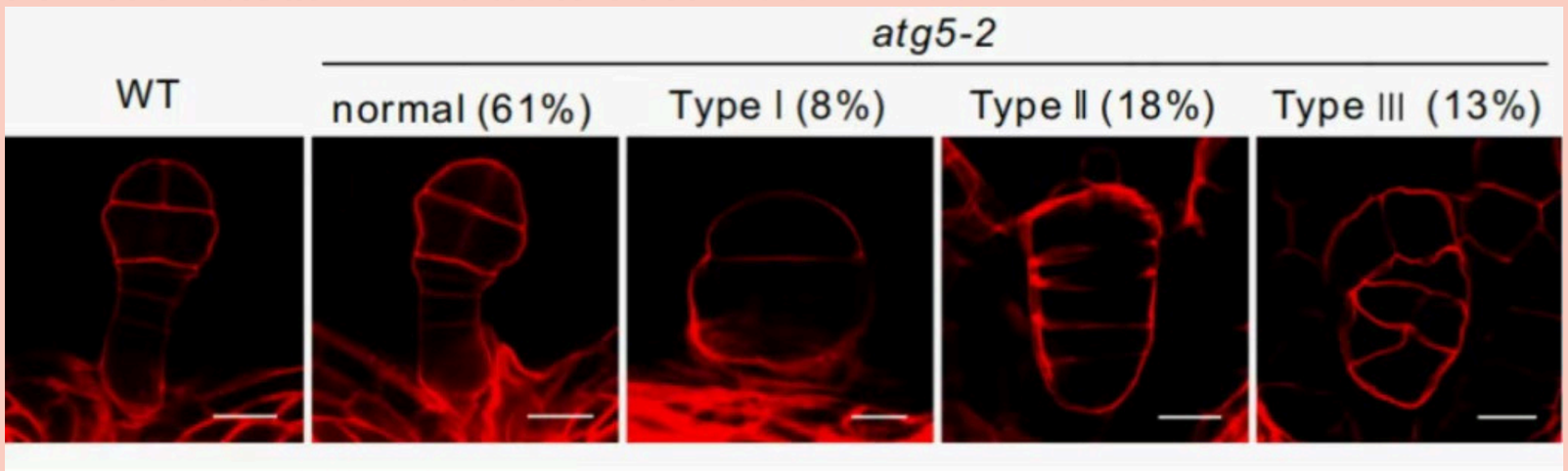


图3. 胚胎发育模式

为探究自噬与PCD的关系，研究人员利用TUNEL染色DNA碎片（PCD会产生大量DNA碎片）和透射电镜（TEM）观察核膜状态。结果显示受精4天后，野生型个体IT中存在大量DNA碎片、核膜破裂、胞质液泡话，而突变体DNA碎片量很少，且核膜保持完整。该实验说明ATG缺失会导致PCD丧失，进一步暗示了自噬相关基因对PCD的开启具有重要意义。

那么自噬又是如何通过PCD影响胚胎发育的呢，研究人员为了探究IT与胚胎模式形成间的分子联系，将野生型和突变体的胚胎分离后进行了RNA测序，并对测序结果进行主要成分分析（principal component analysis, PCA）和全连锁聚类分析。结果显示突变体中分化表达基因（differentially expressed genes, DGEs）含量大幅下降，暗示了ATG对胚胎分化的调控意义。之后进行的GO分析还确定了几个与胚胎模式相关的模式形成的途径，包括模式规范和生长素相关途径，这些途径都受IT的自噬调控。这一发现进一步证实了自噬在胚胎发育过程中的非细胞自主作用。

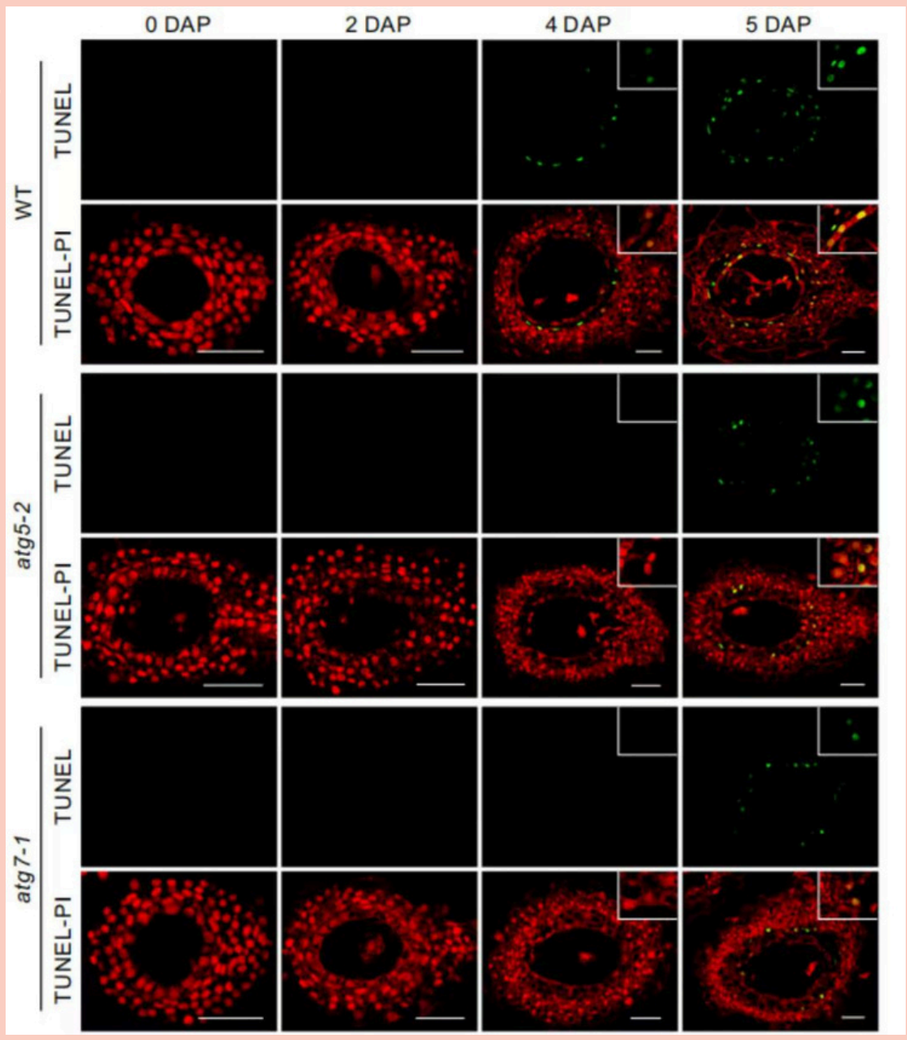


图4. TUNEL染色结果

该研究除了阐明IT自噬通过PCD调控胚胎发育外，还指出其对胚胎发育中脂代谢的双重作用。通过分析野生型和突变体在卵子及种子脂质代谢中的差异，发现受精前ATG抑制三酰甘油（TG，种子发育中含量最高的脂类）积累，而受精后ATG侧促进TG积累。这表明了自噬在植物脂质代谢中既参与TG降解，也参与其生物合成。

讨论

本文创新地指出了珠被绒毡层中的自噬活动通过PCD和脂代谢两个方面影响植物胚胎模式建成。PCD作为一个重要的细胞生命调控形式，自噬在其中的作用仍然不太清楚。因为自噬对PCD的调控具有细胞类型特异性，比如自噬对于近端根帽细胞中PCD是必要的，但对于远端侧根帽细胞的PCD是非必要的[3]。各种细胞的PCD调控模式及其产生差异的生理意义在后续研究中仍有很大研究意义。不仅如此，自噬对脂代谢的调控机制也有待后续研究的阐明。

引用

• Zhao, L. L., Chen, R., Bai, Z., Liu, J., Zhang, Y., Zhong, Y., ... & Zhao, P. (2024). Autophagy-mediated degradation of integumentary tapetum is critical for embryo pattern formation. Nature Communications, 15(1), 2676.

• Zhou, X. M., Zhao, P., Wang, W., Zou, J., Cheng, T. H., Peng, X. B., & Sun, M. X. (2015). A comprehensive, genome-wide analysis of autophagy-related genes identified in tobacco suggests a central role of autophagy in plant response to various environmental cues. DNA Research, 22(4), 245-257.

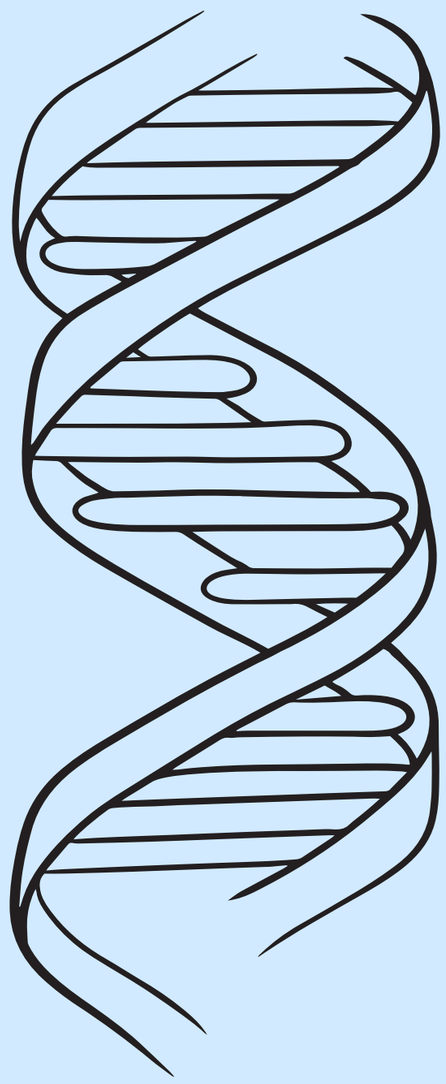
• Feng, Q., De Rycke, R., Dagdas, Y., & Nowack, M. K. (2022). Autophagy promotes programmed cell death and corpse clearance in specific cell types of the Arabidopsis root cap. Current Biology, 32(9), 2110-2119.

SMART MAGAZINE AUTHOR: Ariana EDITOR: Hecate

罕见病的曙光：中国AI赋能碱基编辑技术迈向临床试验

背景

近年来，对于单碱基编辑技术的研究取得了重大突破，碱基编辑基因最核心的功能是用失活的Cas9蛋白把脱氨酶带到一个特定的位置，再脱氨引起细胞修复反应，最终完成单碱基转换。基因编辑发展到现在，作用于罕见疾病是最好的，也是最早能实现真正应用的，碱基编辑技术是重要的基因编辑技术，发展到目前还存在一些问题，希望未来逐步解决。



碱基编辑现存的问题

第一个问题是编辑种类有限，刘如谦教授最先发展出了胞嘧啶编辑器（CBE）和腺嘌呤编辑器（ABE），在碱基编辑完全的情况下有12种转换，现已实现4种转换，还有8种未实现，期待后面的工作能够让它们实现。

其次，单碱基编辑器也有一个严重的问题。它会编辑目标碱基旁边的碱基，即“旁观者编辑”。人们希望它真正能够精确到目标碱基，因为几乎所有的罕见病和疾病产生都是单位点。

此外，碱基编辑效率较低，目前如果想治疗一个疾病，一定要先做细胞再上动物模型或者类器官模型才能成药。这方面的应用相对滞后。

第一代编辑器

近年来我国开发了碱基转换、碱基颠换的编辑器GBE，这是在刘如谦教授CBE的基础上发展起来的，CBE把胞嘧啶脱氨酶带到特定的位置，对胞嘧啶进行脱氨形成尿嘧啶U，U在碱基配对中被认为是T，在复制中引起修复，最终完成C到T的转换。若把胞嘧啶变成尿嘧啶U，再通过糖基化酶从DNA双链上的骨架上切除，就会形成一个没有嘌呤和嘧啶的AP位点。这样诱使了一种新的DNA修复机制，最终开发出能把C特异性转变成G的GBE碱基编辑器。

在此基础上，中国科学家研发了第二代的GBE碱基编辑器，选用了功能更强、活性更高的尿嘧啶糖基化酶，在编辑器两个蛋白中间的Linker的构象做出了优化。国外的David Liu和J.Keith Joung研究组同时做了第二代CGBE，性能相对领先。

众所周知，无论在基因编辑还是在碱基编辑里面，重要的一环是设计gRNA，gRNA选择的靶点非常重要。针对目前gRNA选择靶点的困难，我国科学家对GBE设计了深度学习模型，收集高通量编辑的数据用于AI模型学习，学习后AI就可生成权重图，告诉大家目的gRNA在目标范围内哪些碱基组成有利于效率提高。此外，科学家通过构建机器学习模型，能预测任何gRNA效率，进而在正式实验前获得高效率的gRNA。

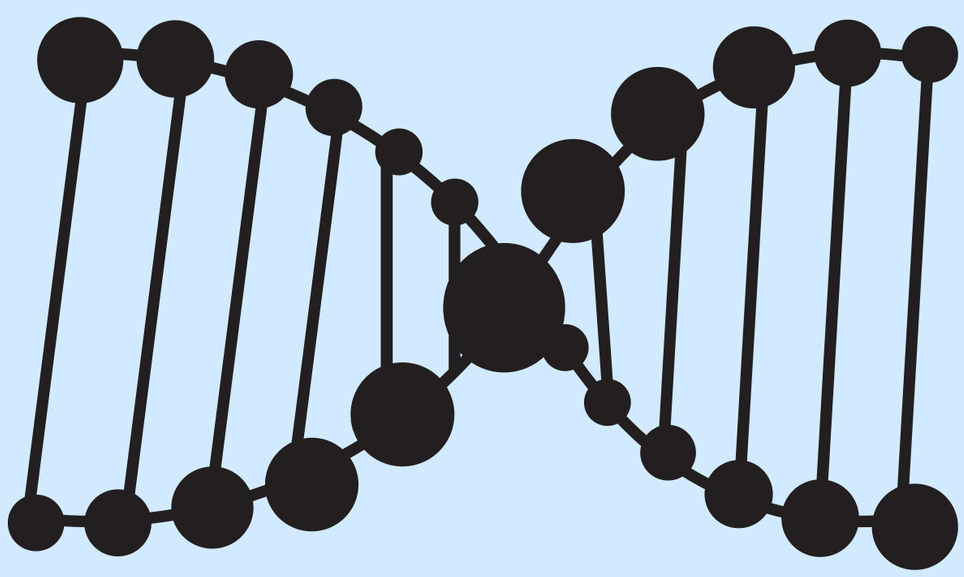
目前国内解析碱基编辑的研究较少，因为无论是碱基编辑还是CRISPR/Cas9第一代的编辑器，作用都是扰动或破坏DNA双链，所有的编辑结果都不是直接的结果，而是通过触发哺乳动物细胞的修复系统，最终修复完成后获得DNA形态的编辑结果。目前解析碱基编辑的工作通过和哺乳动物细胞高度同源的真核酿酒酵母细胞完成，对酿酒酵母细胞进行大量的基因敲除，可获得一些在修复过程中发挥重要作用的酶。

第二代编辑器

我国今年开发的糖基化酶碱基编辑器效率很高但易脱靶，实验中发现胞嘧啶脱靶严重，且不依赖Cas9。目前脱靶主要有两种原因。一种是由于Cas9蛋白本身对不同序列的容忍性，不是100% 匹配gRNA的位置也会进行编辑，这是Cas9依赖性脱靶。但现在使用的脱氨酶活性非常强，尤其是胞嘧啶经过进化后，可以在全基因组中造成很难预测的随机突变，危害比较大。所以科学家们希望避免全基因组的脱靶，从根本来解决这个问题。

他们设想直接用糖基化酶在DNA单链上，直接切除胞嘧啶或其它碱基，过去的方法是先脱氨，然后再把尿嘧啶或者是次黄嘌呤切除，极易脱靶，现在可以在单链上不通过脱氨酶直接去除，避免基因组脱靶。

科学家们希望开发针对胞嘧啶和胸腺嘧啶的酶，非常遗憾的是，这两种糖基化酶自然界中不存在，所以目前没有从胸腺嘧啶T出发的碱基编辑器。他们在大肠杆菌里设计了一个突变筛选系统，构建了活性非常低的糖基化编辑器，它在有活性的情况下，把设计好的色氨酸突变体恢复成正常的色氨酸合成酶，这样大肠杆菌才可以合成色氨酸，在简单培养基中存活。



有了和生长偶联的筛选系统，就可以精确筛选大量突变体，科学家构建了突变体库可以进行多次筛选，一开始筛到的活性非常低，把活性低的再进行突变并进行下一轮筛选，有可能获得活性越来越高的突变体。

中国科学家用了接近一年半的时间，在经过多轮突变筛选后获得了活性较高的胞嘧啶糖基化酶和直接能切除胸腺嘧啶的糖基化酶。获得糖基化酶后，科学家希望把在大肠杆菌中筛到的编辑器带到哺乳动物细胞中，先经过密码子优化，再经过一些结构的调整和编辑器的优化，就获得了对C到G编辑有较强特异性的第一代糖基化酶编辑器，在测试位点基本能达到40%左右的编辑效率，可以和目前比较流行的CBE和ABE相提并论，且T到G碱基编辑器的窗口较窄，更适合应用到遗传疾病、罕见病领域。科学家通过优化第一代糖基化酶碱基编辑器，获得了第二代，它们和第一代不同的是效率和专一性得到进一步的提高，但窗口发生了改变。

令人非常高兴的是，目前没有观察到RNA的脱靶，DNA全基因组脱靶也在信噪水平以下，证明第二代糖基化酶碱基编辑器安全性优于第一代的碱基编辑器。目前国内碱基编辑器效率还是稍微低于国外效率最高的编辑器，现在科学家仍然在继续进行优化，希望未来能带来更满意的糖基化酶碱基编辑器。

可直接使用的最佳gRNA数据库

单窗口编辑器在gRNA100%匹配的情况下，旁观者编辑的情况特别多，且和时间正相关，科学家认为这是因为Cas9对错配 gRNA的容忍性高，引起类似脱靶的效应。因此，科学家在gRNA引入了一个不是100% 匹配的位点，脱靶率大大降低且单碱基效率提高，但目前没有找到效果最好的gRNA，需要先用高通量的方法测试不同gRNA的效果。

在ClinVar的数据库，至少有37000多种遗传疾病的SNV位点已知，且每天都在增加。科学家针对ABE纠正SNV，克隆了所有位点，通过慢病毒整合到细胞，对细胞库进行高通量编辑并收取数据，每一个位点使用了一组20个gRNA，收集完数据就可对所有的疾病位点求出最佳gRNA。目前大多数gRNA经过化学修饰改造，但使用引用内含子的剪切机制或一些病毒的剪切机制，在体外或体内都能大大提高环化后稳定度，进而提高编辑效率。

通过在微生物细胞工厂构建一套全自动系统，可实现全自动的细胞编辑，两星期内可编辑几千个哺乳动物细胞，在转化系统的效率足够高的情况下，可实现以下步骤的全自动化：1、引物池的全自动构建；2、引物池构建后，全自动获得编辑指令的库；3、自动进行细胞的转化；4、细胞转化后完全全自动的获得每个编辑好的细胞并且测出编辑效率。

如果想拿到一个特定编辑的细胞，后续操作如从384孔板上取出细胞进行单克隆的分离仍需人工完成，但整个编辑是全自动完成的。通过这套系统，首次能够获得几千个原位的编辑效率，这样就可以使用AI的模型学习gRNA和编辑效率之间的关系。输入gRNA序列染色体可及性，可预测编辑效率。目前预测结果大概准确。另外，染色体对编辑效率的影响和gRNA序列对编辑效率的影响在碱基编辑器CBE上是1:5的比例，也就是说染色体可及性平均对编辑效率影响是1/6。

最后一点，科学家发现很多细胞因子对碱基编辑效率影响很大，因此他们筛选了很多细胞因子对碱基编辑的影响。发现有一类“先锋因子”，在复制转录前对细胞编辑有很高的促进作用，因此他们构建了融合细胞因子的编辑器，效率得到了提高。这类细胞因子在同时用两个gRNA来进行编辑，两条链都切割的PE编辑系统里发现效率有较大提高，尤其对PE3、PE5。可以把这种招募机制应用在非模板的RNA、非PEG（聚乙二醇）的普通RNA上，效率比原始的PE3、PE5系统又有较大的提高。人眼色素变性是一种由基因突变引起的罕见遗传病，正常人的序列中GCG编码的精氨酸，在患病个体中被突变成GCA，精氨酸突变成半胱氨酸后破坏PD6B酶的活性，PD6B酶在视网膜光信号、化学信号的传导过程中是核心，被破坏后有毒的中间体会积累，损坏视网膜神经造成失明。
碱基编辑治疗这个疾病原理很简单，研究人员把GCA的A用ABE的碱基编辑器纠正成GCG的G，使疾病得到治疗。它的重点首先是在常用的293模式细胞优化编辑器效率，通过gRNA选择优化编辑器，进而优化编辑器蛋白序列，目前最高的编辑效率是60%左右。
获得优化好的编辑器后，由于AAV的容量有限，必须把它拆分成两个，分别放到两个AAV载体里，递送到晶状体，研究人员大概在第14天把它注射到小鼠的视网膜中，最后进行功能的验证。
在收取小鼠进行功能验证时，基因组的编辑效率能达到20%左右，但cDNA真正发挥作用的编辑效率可以达到40%，但是否能真正恢复小鼠的视觉功能，还需要病理学、行为学和光电学的分析。对照组小鼠外核层受到了严重的抑制，几乎没有生长，但在被注射的区域外核层长良好，对光线的响应也得到了极大的恢复。水迷宫实验也说明在对照群落里，治疗组相比对照组，行为学得到了很大的恢复。
这是碱基编辑一个重要的应用，遗传眼病是相对最好治疗遗传病，也是市场化率很好的突破口，未来有希望应用于其他的疾病。

引用

Dong, X., Yang, C., Ma, Z., Chen, M., Zhang, X., & Bi, C. (2022). Enhancing glycosylase base-editor activity by fusion to transactivation modules. Cell Reports, 40(3), 111090. https://doi.org/10.1016/j.celrep.2022.111090

Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A., & Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature, 533(7603), 420–424. https://doi.org/10.1038/nature17946

Zuo, E., Sun, Y., Wei, W., Yuan, T., Ying, W., Sun, H., . . . Yang, H. (2019). Cytosine base editor generates substantial off-target single-nucleotide variants in mouse embryos. Science, 364(6437), 289–292. https://doi.org/10.1126/science.aav9973

Kim, H. K., Yu, G., Park, J., Min, S., Lee, S., Yoon, S., & Kim, H. H. (2020). Predicting the efficiency of prime editing guide RNAs in human cells. Nature Biotechnology, 39(2), 198–206. https://doi.org/10.1038/s41587-020-0677-y

Esvelt, K. M., Carlson, J. C., & Liu, D. R. (2011). A system for the continuous directed evolution of biomolecules. Nature, 472(7344), 499–503. https://doi.org/10.1038/nature09929

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., . . . Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. Nature, 576(7785), 149–157. https://doi.org/10.1038/s41586-019-1711-4

Maeder, M. L., Stefanidakis, M., Wilson, C. J., Baral, R., Barrera, L. A., Bounoutas, G. S., . . . Jiang, H. (2019). Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nature Medicine, 25(2), 229–233. https://doi.org/10.1038/s41591-018-0327-9

Landrum, M. J., Lee, J. M., Benson, M., Brown, G. R., Chao, C., Chitipiralla, S., . . . Maglott, D. R. (2017). ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Research, 46(D1), D1062–D1067. https://doi.org/10.1093/nar/gkx1153

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tRNA与CCR4-NOT复合物的奥妙

关键词: tRNA修饰tRNA处理、蛋白质组学、CCR4-NOT复合物、脱烯化、tRNA翻译机制

一． 细胞中能量的搬运工——tRNA

近十年来，mRNA产业取得了快速发展和巨大成功，如mRNA疫苗和修饰技术。mRNA疗法展现出巨大的医学和市场潜力。mRNA行业的成就也反映了RNA修饰的巨大治疗潜力。越来越多的证据表明，tRNA修饰在癌症和神经系统疾病等人类疾病中起着至关重要的作用。 下面将 重点介绍tRNA修饰的生理功能和病理效应 。

tRNA的生物合成：

tRNA长约70-80个核苷酸，折叠成“三叶草叶”二级和“L形”三级结构。tRNA三叶叶由五个部分组成：受体茎 、 含有tRNA的5'和3'-末端 、 D环、反相干环、可变环和T环 。首先由 RNA聚合酶III（Pol III）和核中的转录因子转录因子IIIB（TFIIIB）和转录因子IIIC（TFIIIC）将 tRNA基因转录成大约100个核苷酸的前体tRNA（前 体 tRNA） 。随后 完整的前tRNA进行初步 剪切， 使用 RNase P和RNase Z对它们进行末端处理，分别去除5'前导序列和3'尾序列tRNA前体由tRNA剪接内切酶（TSEN）复合物和造血干细胞11 7（HSPC117）剪接 。其中 CCA添加酶负责利用ATP和CTP从头合成tRNA前体3'-CCA。这个前 体 tRNA在通过输出蛋白t（Xpo-t）从细胞核输出到细胞质之前会经历进一步的修饰，在那里它们最终被修饰成70-80个核苷酸长的成熟tRNA。最终，氨酰tRNA合成酶负责乙酰化 并且 将成熟的tRNA连接到细胞质或细胞核中的3'-末端 ，接下来由 延伸因子Tu和GTP复合物（EF-Tu-GTP）催化氨酰tRNA结合A位点 核糖体。

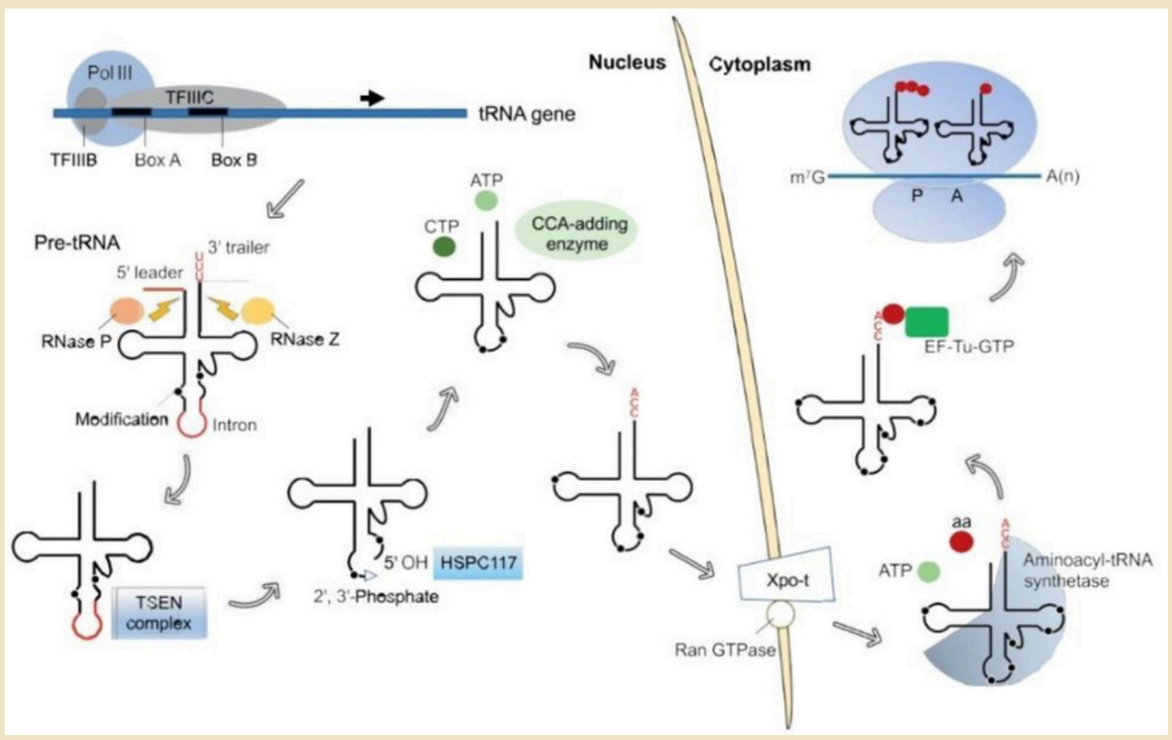

图1. tRna主要合成途径

tRNA的生物修饰：

tRNA的修饰是指在其核苷酸上进行的各种化学修饰，这些修饰可以改变tRNA的结构和功能，从而影响蛋白质的合成和细胞的代谢过程。其中 tRNA 修饰 的代表性步骤通常是5'前导和3'尾部的末端处理 ， 以及 内含子去除和CCA添加 。 tRna上的修饰大概有至少70种，每种修饰因其发生的部位不同（在三叶草结构中的不同环），作用也不同，主要分为以下几类： ① 扩展或限制碱基配对的能力； ② 提高密码子和反密码子结合的稳定性； ③ 影响翻译的起始和维持阅读框； ④ 调控细胞内部代谢。

tRNA的表达和修饰的分析方法：

tRNA修饰测序技术的进步对于tRNA修饰的分析和功能研究至关重要。由于tRNA的普遍修饰和稳定的空间结构，tRNA转化为互补DNA（cDNA）会导致过早终止或碱基对错配。薄层色谱（TLC）或液相色谱和质谱（LC/MS）可以在不进行逆转录（RT）的情况下访问纯化tRNA中的所有修饰类型 ， 另一种传统的测量方法 ——引物延伸分析，可以通过识别RT终止或不匹配的碱基插入来识别tRNA修饰位点 ，但 它的假阳性率很高，无法识别修饰类型 。目前， 肼苯胺裂解测序（HAC-seq ）、m7G还原和裂解测序（TRAC-seq） 等测序方法通过使用独特的化学处理来更有效、更准确地分析特定的tRNA修饰 。 这些 分析 技术显著提高了 人们 对tRNA表达和修饰的理解。

二． CCR4-NOT复合物的结构基础

CCR4-NOT复合物是一个主要的mRNA去腺苷化酶。核心的CCR4-NOT复合物包含九个亚基，并且在基因的转录中，通过缩短poly(A)尾巴或者去除腺苷化来转录后调控mRNA的稳定性，以及在基因调控中的一些其他的功能中发挥作用。

CRR4-NOT复合物的结构：

在酵母中， 该复合物以支架蛋白Not1 p 为中心， 其余部分 由 CCR4 、 Caf1 、 Caf40 、 Caf130 以 及 Not1-4 组成，在其他的真核生物中也存在这些亚基的同源物。

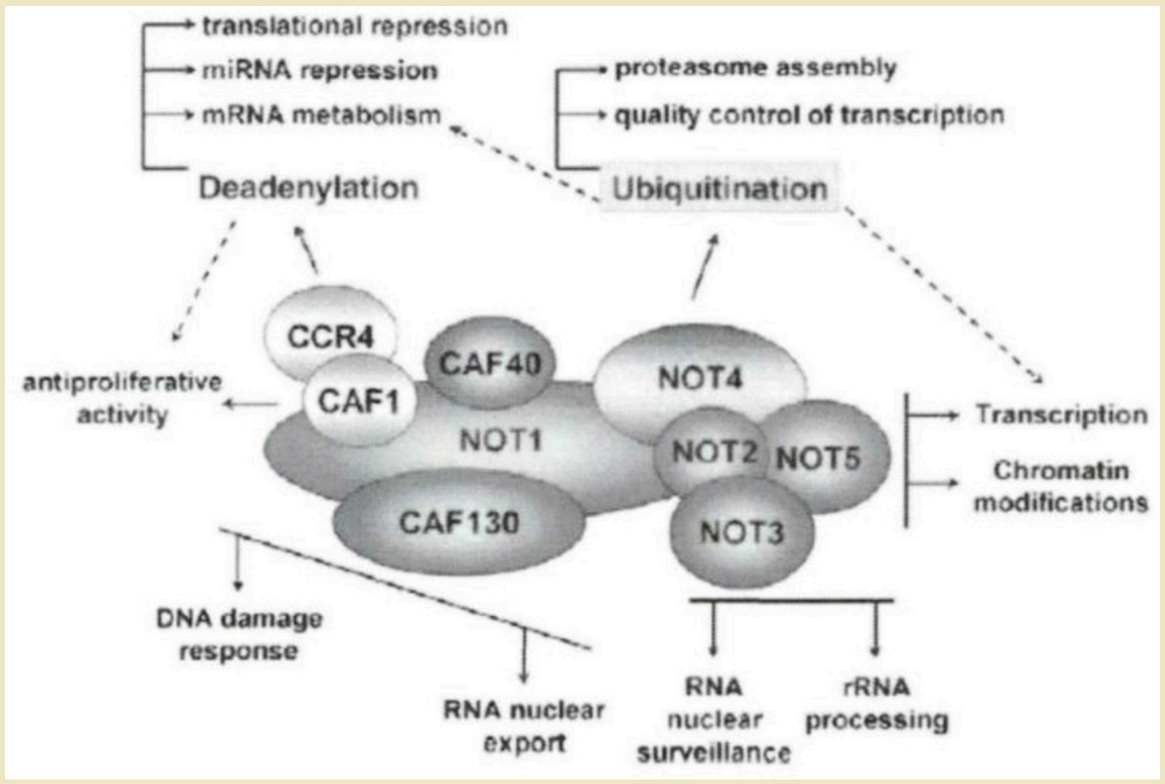

图2. CCR4-NOT复合物的系统图

(1) Not1: 大的Not1蛋白将CRR4-NOT复合体的其他他部分亚基联系起来，作为整个复合体的骨架。在酵母中，Not1蛋白是作为这个复合体中唯一的一个对酵母育性起到重要作用的蛋白。

(2) Not2: Not2蛋白含有两个功能性结构域，其中C端区域参与CCR4-NOT复合物的功能，N端结构域能与Ada2蛋白发生互作。酵母的Not2蛋白对于CCR4-NOT复合物的稳定性很重要，并且对于酵母的生存是很关键的。在哺乳动物中，Not2的缺失还会导致细胞程序性死亡。

(3) Not3/Not5：酵母中的CCR4-NOT复合物包含Not3和Not5这两个彼此联系的亚基，由于Not3和Not5的序列相似性，二者的功能也发生了冗余。在Not3和Not5中含有coiled-coil结构域，以及一个推测的HR1结构域。HR1结构域被认为可能结合小rha G蛋白，并且在信号传导中发挥着一定的作用。

(4) Not4：体外实验证实，Not4是一个不稳定的，CCR4-NOT复合物的保守亚基，起主要作用是一个功能性的泛素连接酶。该蛋白含有一个环指结构域：一个coiled-coil结构域，以及一个RRM结构域。

(5) Ccr 4：Ccr 4是CCR4-NOT复合物中两个催化亚基中的一个。体外实验证实，酵母的Ccr 4具有依赖的，poly(A)-特异性的3’外切核酸酶活性。Ccr 4 除了包含核酸酶结构域，同时也包含一个富含亮氨酸的重复区域(LRR)，这个LRR区域提供了该蛋白与Caf1互作的平台

(6) Caf1：Caf1是CCR4-NOT复合物中的第二个催化亚基。Caf1蛋白的N末端结构域富含谷氨酸，该结构域在哺乳动物中未发现同源类似物，并且该结构域并不是功能上必须的。据推测，该蛋白还含有另一个富含谷氨酸的结构域，并且有实验证实，这个区域表现出核酸酶的活性。

(7) Caf40：果蝇中的CAF40基因被敲除后没有对mRNA的去腺苷化产生明显的影响。在酵母的突变体中，也没有发现mRNA的稳定性受到影响。

(8) Caf130：Caf130有一个假定的跨膜结构。

Deadenylases及其多样性：

Deadenylases通常被定义为镁依赖性外切核糖核酸酶，它将poly(A)尾部识别为主要底物，并在3'-5'方向水解RNA，导致5'-AMP的释放。这种脱腺苷反应可发生在细胞核和细胞质中。在细胞核中，去烯基化将mRNA的新添加的聚 (A) 尾限制在适当的长度，而细胞质中mRNA的广泛去烯化会引发降解或抑制。去烯基化过程通常被认为是mRNA衰变和翻译沉默的限速步骤。目前，根据其核酸酶结构域，脱腺苷酶分为两组：

(1) DEDD型核酸酶：以三个核酸外切酶基序中保守的催化Asp和Glu残基命名。DEDD型deadenylase的主要家族包括POP2、CAF1Z、聚(A) 特异性核糖核酸酶（PARN）和PAN2。

(2)核酸外切酶-内切酶磷酸酶（EEP）家族：包括CCR4、夜曲蛋白、ANGEL和2'磷酸二酯酶（2'PDE）。

CCR4-NOT Deadenylases：

酿酒酵母CCR4-NOT复合物包含两种deadenylase，Ccr4p和Pop2p（Caf1p），这两种酶都参与mRNA降解，尽管Pop2p对于Ccr2p的deadenylases活性来说是不必要的。酵母Ccr4p的两个人类直系同源物是CNOT6（hCcr4a）和CNOT6L（hCcr4b），而Pop2p的两个直系同源物则是CNOT7（hCAF1）和CNOT8（hPOP2/CALIF）。

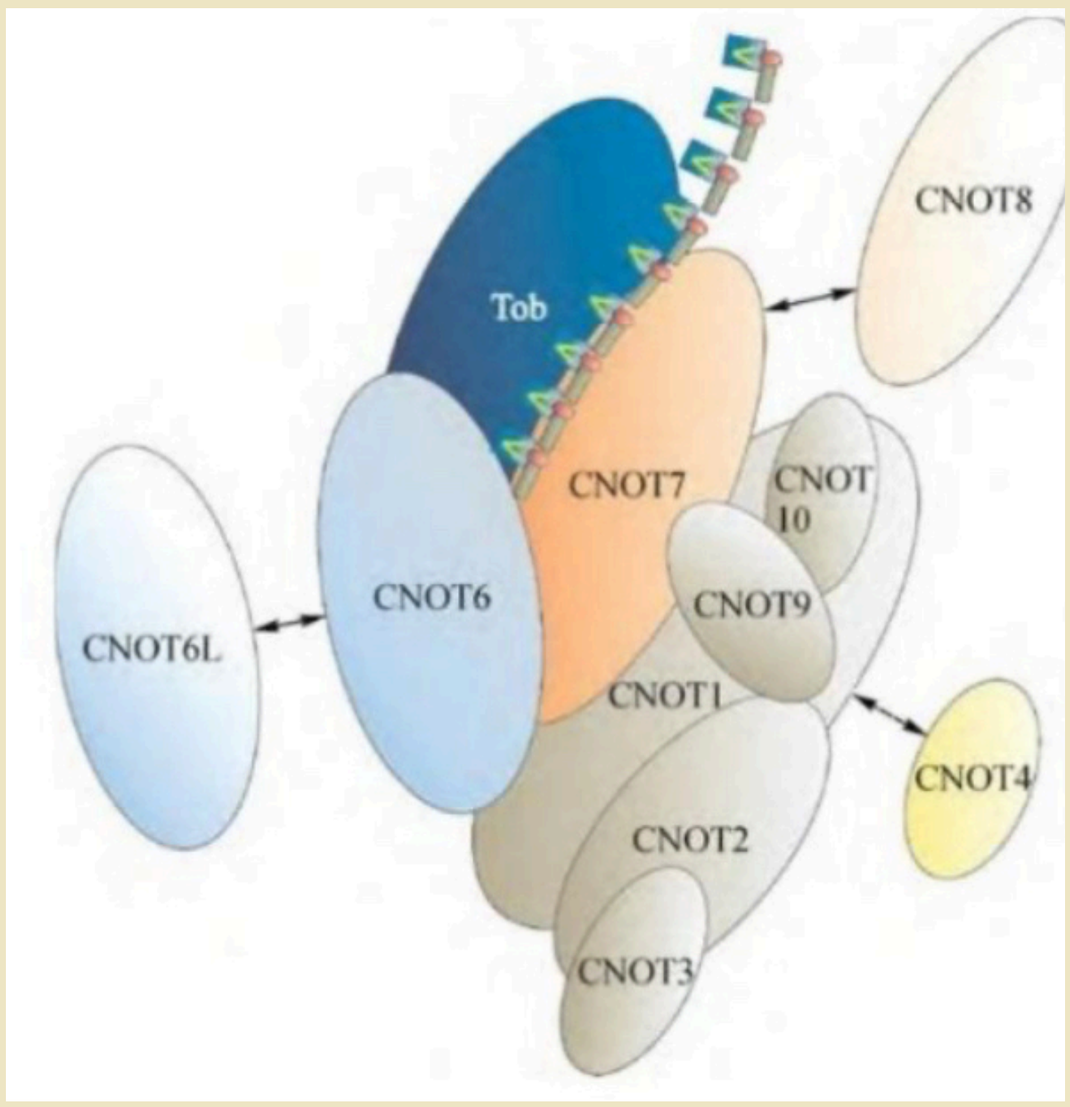

图3. 人类CCR4-NOT复合物的结构模型

其中 CNOT7和CNOT8对细胞增殖很重要， 并且 具有高度的氨基酸序列相似性和部分重叠的功能。CNOT6和CNOT6L具有不同的功能。CNOT7和CNOT8已被鉴定为属于DEDD型核酸酶，而CNOT6和CNOT6L属于EEP超家族 。 此外，哺乳动物的细胞生长可以通过CNOT7的过表达 或CNOT6L表达的减少来减少 。

POP2脱烯酶的结构与活性：

Pop2p的晶体结构于2003年首次从酿酒酵母中确定，后来于2009年从粟酒裂殖酵母中测定 。 酿酒酵母Pop2p的整体结构呈肾形，有13个α-螺旋和6个β-链 。 而 来自粟酒裂殖酵母或分裂酵母的同源蛋白的结构和功能研究揭示了更多关于死烯基酶的活性和选择性与酿酒酵母Pop2p不同，其对应的粟酒酵母确实具有完整的DEDD基序。从结构来看，位于活性位点的两个二价金属离子（A和B）对活性至关重要 。

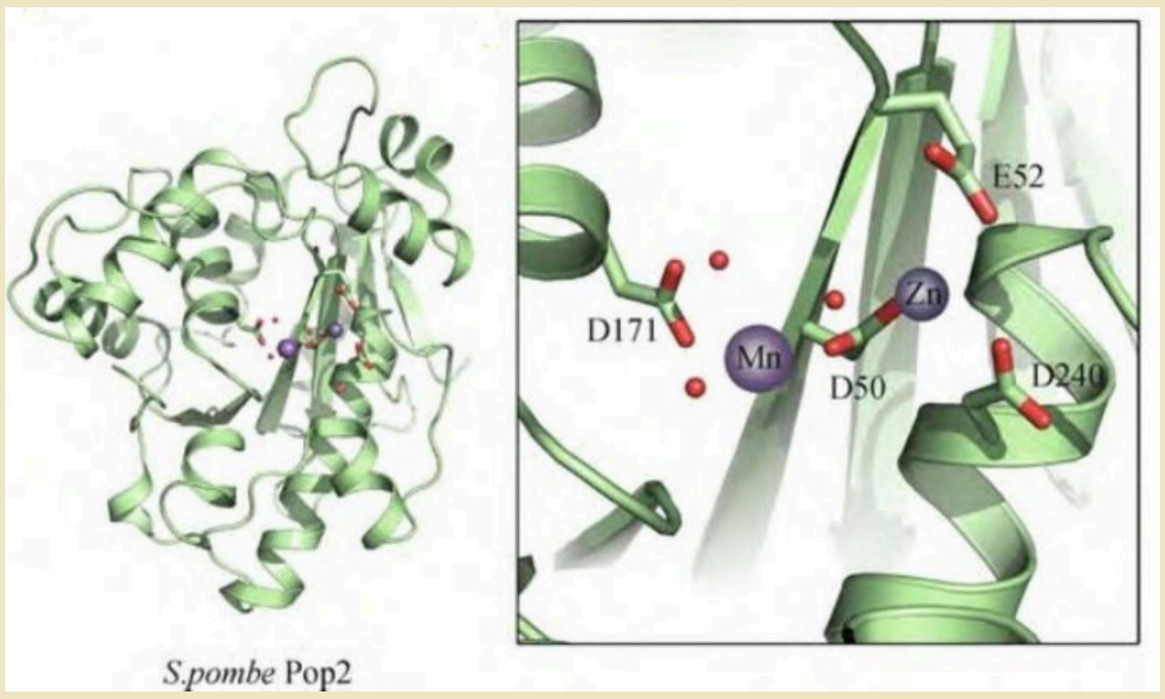

图4. S.pombe Pop2的晶体结构和S.pombe Pop2活性位点的放大图
（其中残基构成保守的DEDD基序，以棒状表示，Zn2+和Mn2+离子显示为球体，水显示为红色小球）

在Mg2+、Mn2+和Zn2+离子的存在下，脱烯反应缓慢且不具特异性。然而，在缺乏Zn2+的情况下，Pop2p能够快速且特异性地降解RNA底物的整个poly（A）尾部，这表明了细胞Zn2+水平的变化可能提供了一种调节mRNA总周转率的方法。

酵母直系同源物人CNOT7的结构：

与S.pombe Pop2p结构的情况一样，人类CNOT7结构在金属离子 、 、 和 的存在下检测了CNOT7的核酸酶活性。在 存在下没有观察到活性，CNOT7对RNA底物的活性明显高于DNA底物。在 存在的情况下观察到最高的RNase活性 ，这一结果表明了了 是CNOT7完全活性所必需的 。

CCR4二烯基酶：

yCcr4p是酵母中主要的细胞质死烯基酶，并作为主要的催化成分 。目前生化研究 已将酵母Ccr4p归入EEP家族，并已证明其含有三个主要功能结构域。N-末端区域富含谷氨酰胺和天冬酰胺 ， 中心区域包含富含亮氨酸重复序列（LRR）结构域的几个串联拷贝 ， 该结构域被认为是连接yCcr4p与复合物的其余部分和其他配体的纽带。LRR结构域将所有Ccr4p直系同源物与其他EEP家族成员和CCR4样蛋白区分开来 。 C末端区域包含EEP超家族特有的死烯基酶结构域，在激活结构域中具有 保 守 的 催 化 Asp 和 His 残 基 。人类直系同源物CNOT6 和CNOT6L与yCcr4p共享LRR结构域和核酸酶结构域，但缺少N端富含Glu/Asp的区域

TOB/BTG抗增殖蛋白：

Tob/BTG蛋白由于与细胞中的靶蛋白结合而获得抗增殖活性 ， Tob和BTG2均已被证明通过CNOT7与CCR4-NOT复合物相互作用 ， BTG2通过CCR4样复合物作为ERα介导的转录的共激活剂 。 Tob、BTG2和TIS21具有相似的结构，由五个α-螺旋和四个β-链组成，形成两个反平行的β-片。N端立即形成一束三个α-螺旋，接着是四条β-链，其中两个小α-螺旋插入在链β1和β2之间 。

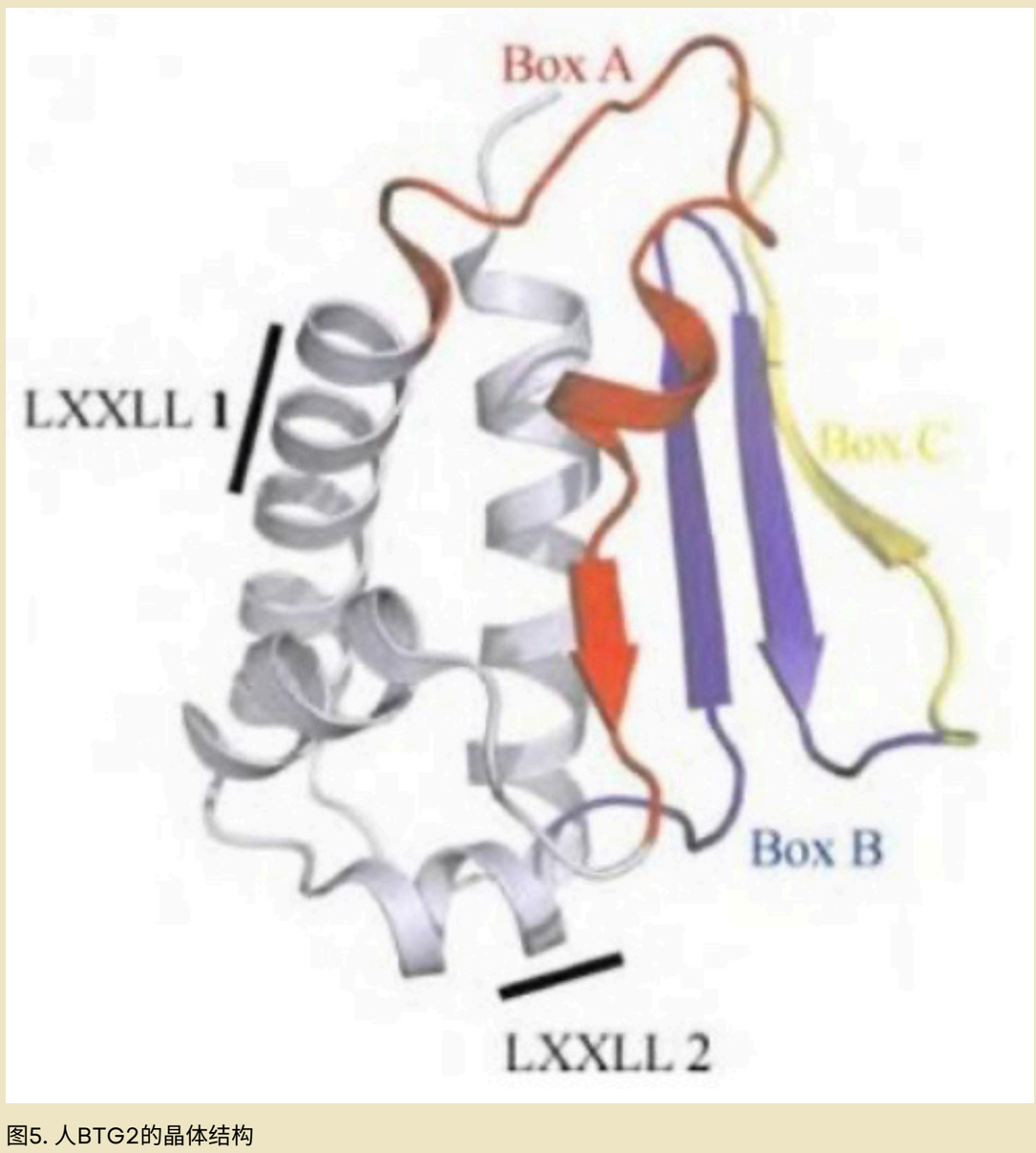

图5. 人BTG2的晶体结构
(保守的BoxA区域呈红色；方框B区域为蓝色；并且保守的Box C区域被着色为黄色。指出了两个保守的LXXLL基序)

人类BTG2的结构，占40%与Tob的序列同一性显示了Tob/BTG2家族中的三个高度保守的结构域。盒A，也称为GR（用于生长调节），由链β1、短螺旋α3、α2螺旋的一部分以及它们之间的连接环组成。两条反平行的β链（β2和β3）形成盒B，这对结合包括CNOT7在内的许多分子靶标很重要。盒子C由链β4和扩展的C端子回路。

BTG2结构表明，相关界面位于BTG2的不同表面上，可能不会相互干扰，这增加了BTG2能够同时结合两个或多个分子靶标以满足不同调控要求的可能性。两个LXXLL基序，也称为核受体盒（NR盒），分别位于螺旋α2和α5上。

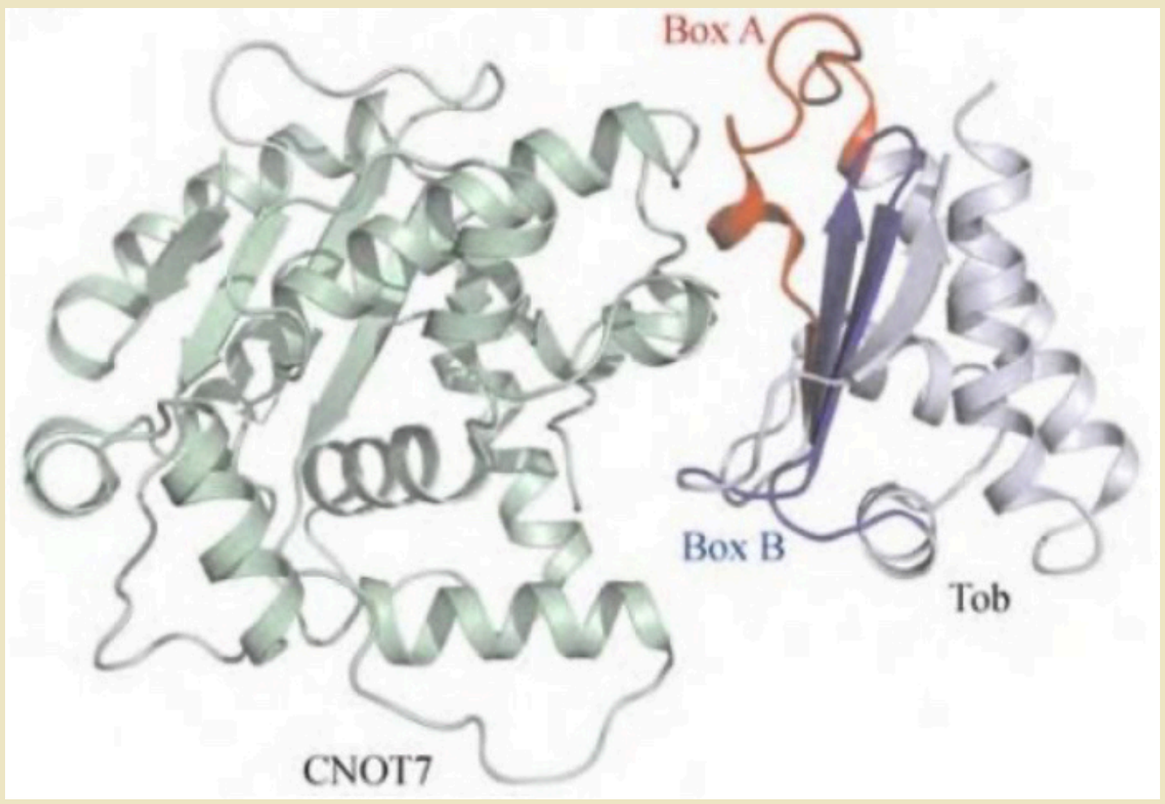

图6. 人类Tob-CNOT7复合物的晶体结构

这两个基序位于BTG2的相对面上，并提供亲水性表面，这可能有助于与核受体接触，而疏水性残基则埋在蛋白质的核心内。

如上所述，越来越多的证据支持Tob/BTG蛋白家族是CCR4-NOT复合物中CNOT7的常见结合伴侣。2009年，Horiuchi及其同事报道了Tob-CNOT7复合物的晶体结构，从而证明了两种蛋白质之间的相互作用模式，并提出了该复合物抗增殖活性的机制。Tob与CNOT7的相互作用在很大程度上是疏水的，并由保守的Box A和Box B区域介导。Tob的存在对CNOT7的活性没有明显影响。

通过对人类BTG2的分析表明，BTG2通过直接相互作用在体外抑制CNOT7脱烯酶活性。然而，结构分析清楚地表明，BTG2和CNOT7的结合界面并不靠近CNOT7活性位点，这表明BTG2的结合可能会引起局部构象变化，影响CNOT7活动，甚至扭曲活性位点。BTG2最近被证明是mRNA降解的一般激活剂，其中涉及CNOT7和CCR4的deadenylase活性。

三． 特异性tRNA通过募集CCR4-NOT复合物翻译核糖体来促进mRNA衰变

CCR4-NOT复合物是一种高度保守的多亚基组装，作为主要的细胞质死烯基酶发挥作用。 mRNA脱烯率和半衰期相差几个数量级，并且受到将CCR4-NOT复合物募集到特定转录本的机制的高度影响。许多RNA结合蛋白（RBPs）与CCR4-NOT复合物相互作用，从而加速它们所结合的特定mRNA的去烯基化.同样，microRNAs（miRNAs）通过直接诱导CCR4-NOT来靶向mRNAs-TNRC6与 CNOT9的相互作用 ，CNOT9分别是miRNA诱导沉默复合物和CCR4-NOT复合物的核心成分。

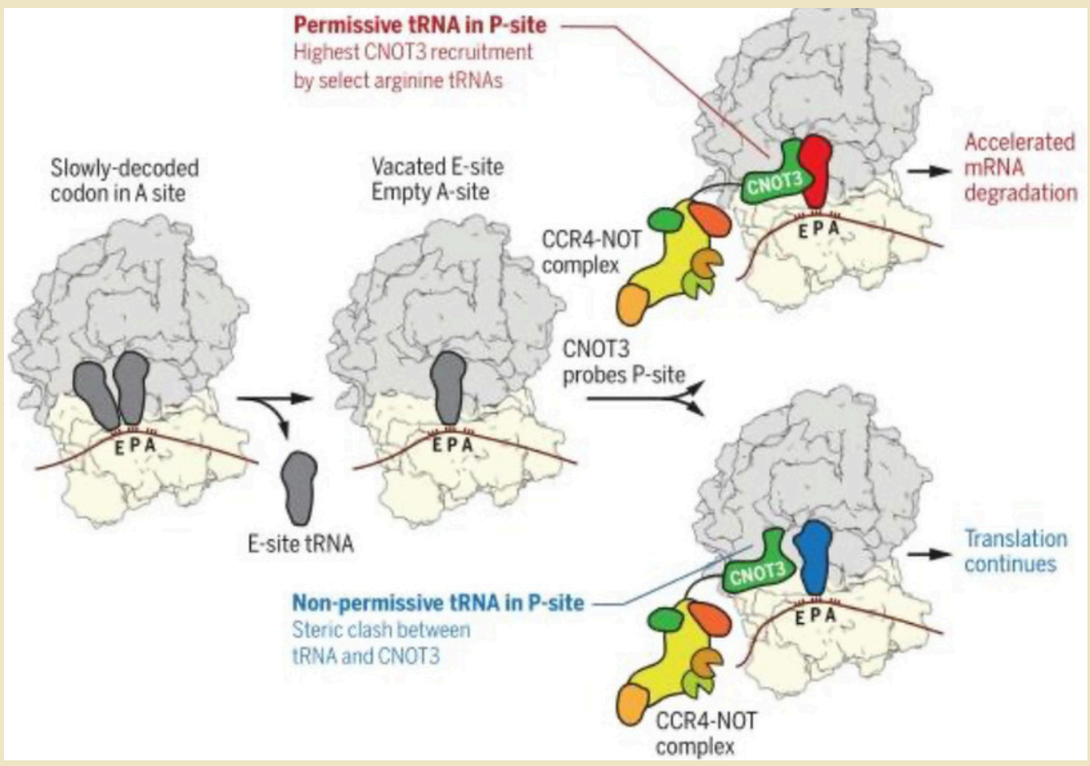

图7. P位点tRNA控制CCR4-NOT复合物向翻译核糖体的募集

通过 使用选择性核糖体谱来确定其翻译导致CNOT3募集到核糖体的mRNA的特征 ， 揭示了一种以前未被认识的P-位点tRNA介导的mRNA衰变（PTMD）途径 。

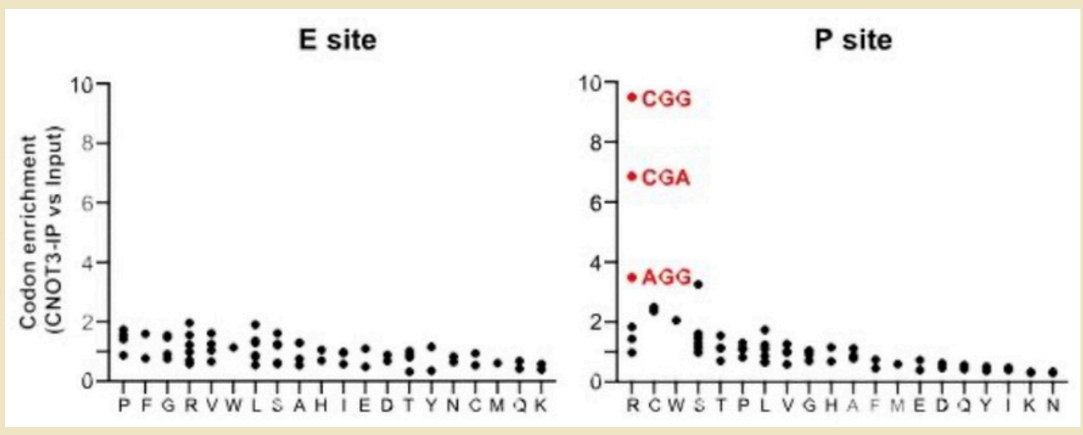

图8. 编码子在CNOT3结合核糖体的核糖体E、P和A位点富集

这表明A位密码子的缓慢解码可显著促进CNOT3向翻译核糖体的募集，但也增加了其他决定因素在哺乳动物细胞共翻译CNOT3募集中发挥更主导作用的可能性。

通过研究选择性核糖体P-和E-位点的密码子富集情况，以及对CNOT3结合核糖体足迹编码的氨基酸的分析，表明CNOT3结合核糖体中最富集的密码子位于P位点，并且P位点密码子的同一性与人类细胞中共翻译的CNOT3募集密切相关，P位点的精氨酸密码子为CNOT3结合提供了最强的可检测信号。

CGG、CGA和AGG精氨酸密码子促进CNOT3介导的mRNA衰变：

通过计算加权CGG/CGA/AGG评分对mRNA进行分层，具有高加权CGG/CGA/AGG评分的转录在CNOT3丢失后优先稳定。富含其他精氨酸密码子（CGC、AGA和CGU）的转录本没有表现出这种行为。CNOT3耗竭的Jurkat细胞mRNA衰变率分析或Cnot3敲除小鼠pro-B细胞中的稳态mRNA水平进一步证实，富含CGG、CGA或AGG精氨酸密码子但不含CGC、AGA或CGU编码的精氨酸的转录本在CNOT3缺陷细胞中优先稳定。

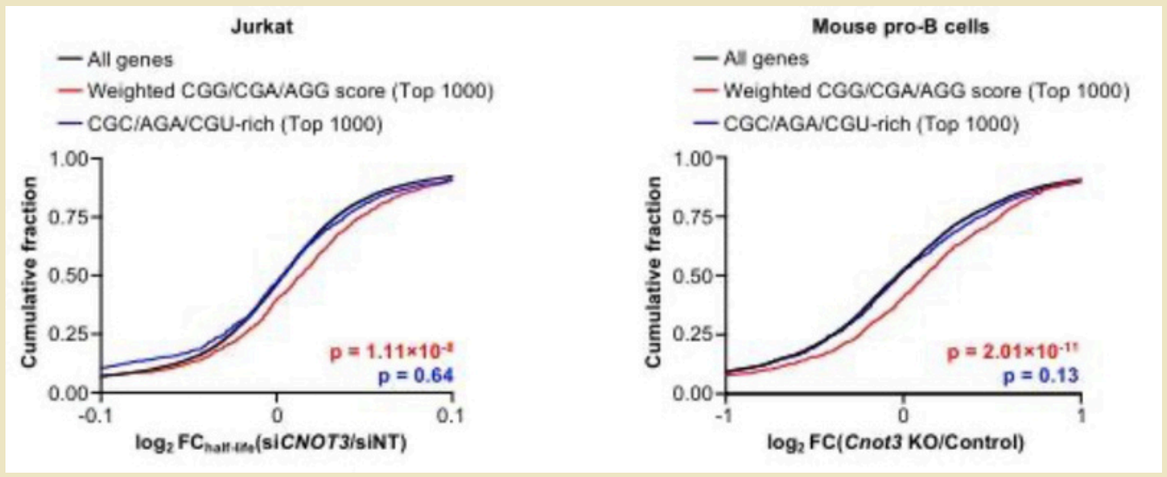

图9. 富含CGG/CGA/AGG的mRNA或富含CGC/AGA/CGU精氨酸密码子的mRNA的最高加权评分

随后， 构建了一个多西环素调节的报告转录物 ，并且设计 了一种对照mRNA，其中每个精氨酸密码子都被一个未在CNOT3结合核糖体的P位点富集的密码子所取代 。实验结果 与全转录组分析一致，精氨酸编码报告子的衰减速度明显快于对照报告子，并在CNOT3耗竭后选择性稳定 。 表明CGG、CGA和AGG精氨酸密码子将CNOT3募集到翻译核糖体中会导致mRNA降解加速 。

线粒体核糖体蛋白mRNAs富含CGG/CGA/AGG密码子，受CNOT3调控：

通过 研究 因为 存在不稳定的精氨酸密码子而受到CNOT3最强调控的内源性mRNA基因集富集分析 ，发现 含有线粒体核糖体蛋白的基因集是最富集的基因集 ， 这些发现表明，编码线粒体核糖体蛋白的mRNAs受CNOT3的调节 。同时证实了 HEK293T或Jurkat细胞中CNOT3的耗竭导致这些转录物的稳态丰度显著增加，线粒体质量相应增加 。

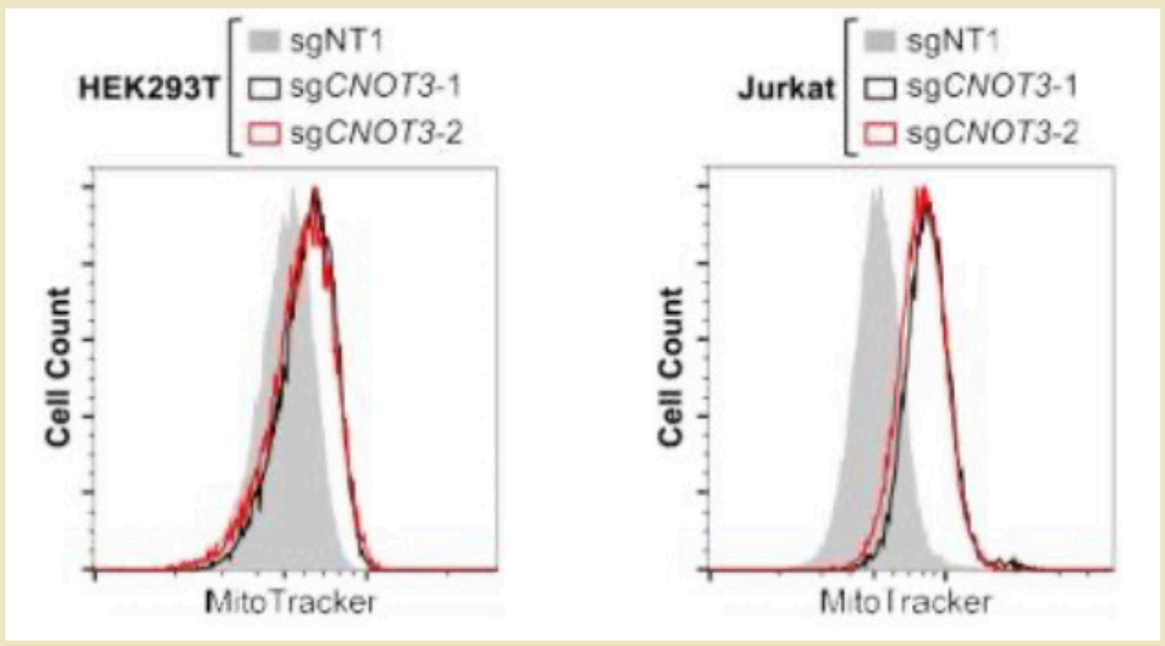

图10. HEK293T富集分析

CCR4-NOT复合物的支架亚基CNOT1的耗竭导致线粒体核糖体蛋白mRNA稳态丰度的类似增加 ，这表明 CCR4-NOT 对 线 粒 体 核 糖 体 蛋 白 （富 含 由CGG、CGA和AGG密码子编码的精氨酸）的调节会影响哺乳动物细胞中的线粒体稳态 。

线粒体核糖体蛋白mRNAs富含CGG/CGA/AGG密码子，受CNOT3调控：

通过构建了一个编码41个亮氨酸-精氨酸-天冬氨酸重复序列的mRNA，是CNOT3结合核糖体中最丰富的三肽。并且使用赖氨酸密码子（41×LKAAGD）替换精氨酸密码符作为对照，体外翻译41×LRCGGD，而不是41×LKAAGD，导致标记的CNOT3被大量招募到多聚体中，其他CCR4-NOT复合成分也被选择性地招募到41×LRCGGD mRNA中。

随后，利用该系统确定CNOT3与P-位精氨酸密码子募集核糖体的结构基础。通过flag IP富集主动翻译41×LRCGGD mRNA 转 录 物 的 多 核 糖 体 中 结 合CNOT3的核糖体，并通过冷冻EM进行分析，显示了线性聚集的核糖体，表明多核糖体在样品制备过程中保持完整。

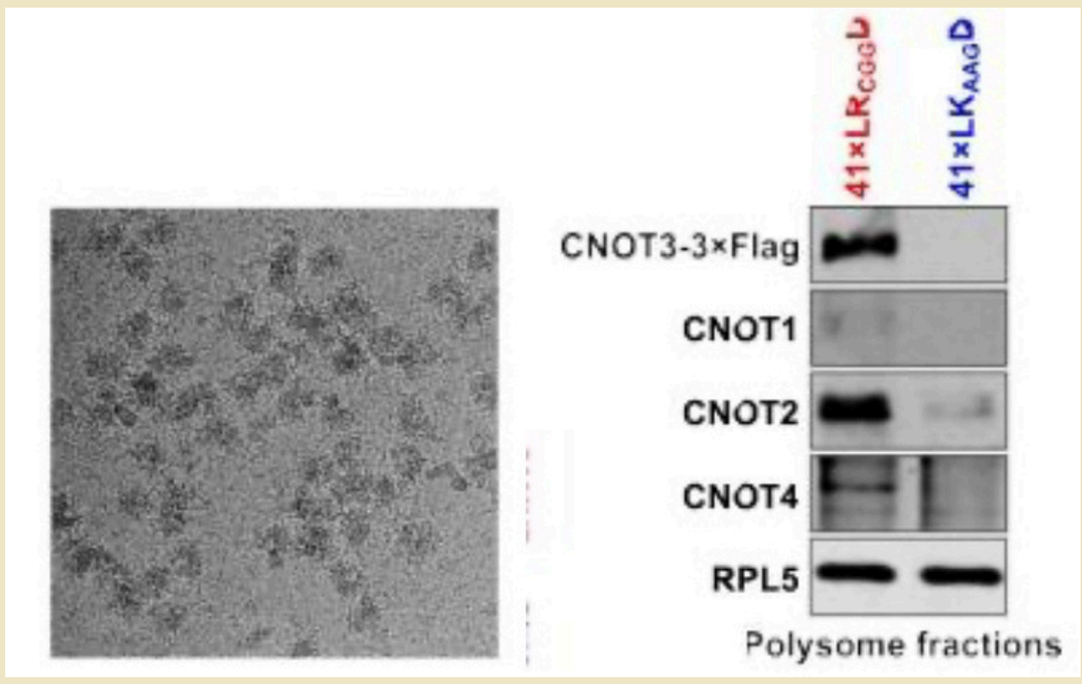

图11. 冷冻EM电镜分析和对照组的体外对照分析

通过 分析单个核糖体（但不是二聚体或高阶多聚体）的结构测定 ， 核糖体的单颗粒重建产生了一个整体分辨率为2Å的均匀结构。密度解释和模型构建揭示了一个空的A位点、P位点中的tRNA和占据E位点的CNOT3 。

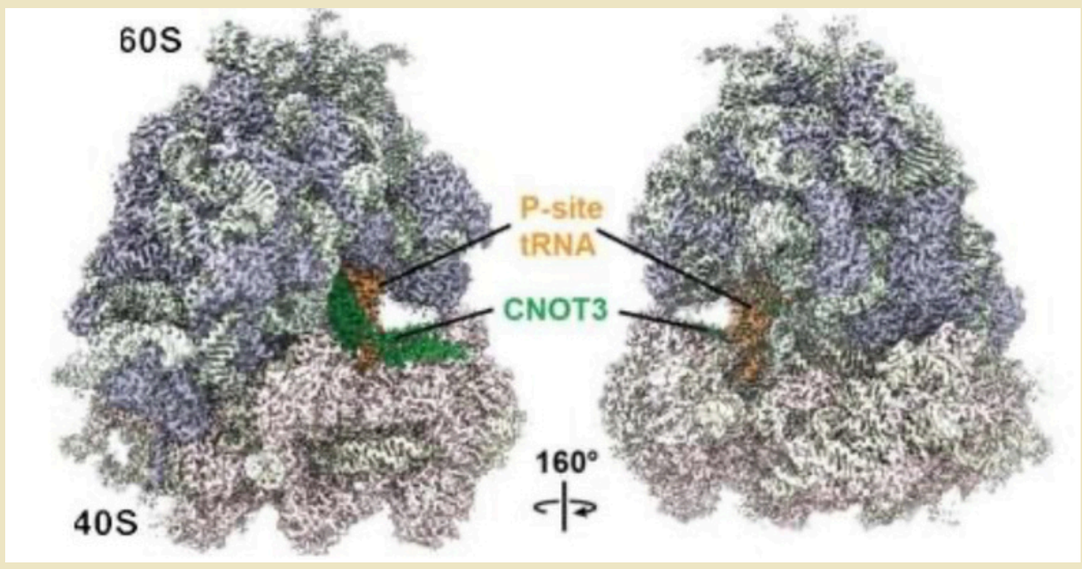

图12. 低温电磁密度图

分析结果与之前观察的结果一致， CNOT3的N端3螺旋束连接两个核糖体亚基 ， 并与P位tRNA的D环、D干和抗密码子干接触 。

明确地鉴定了P-位点中编码CGG密码子的精氨酸，正如选择性核糖体分析数据所预测的 结果一致， 转录本中存在的CGG密码子可以被两个tRNAArg、CCG等解码器识别，或者由于摆动碱基配对，可以被HEK293T细胞中存在的五个tRNAArg、UCG等解码器之一识别 。

P位点tRNA D臂是共翻译CNOT3募集的关键决定因素：

据报道，核糖体P-和A-位点中的选择密码子对会扭曲A-位点mRNA的结构，导致解码受损 ，这 可能会导致核糖体停滞导致CNOT3募集 。通过模拟A位mRNA，将结构中的mRNA几何形状与翻译核糖体中的mRNA结构进行了比较，tRNA探测了A位。A位mRNA的构象以及与CNOT3结合的核糖体的整体结构与解码相容，这表明CNOT3的募集不是由于mRNA畸变导致的核糖体停滞的结果 。

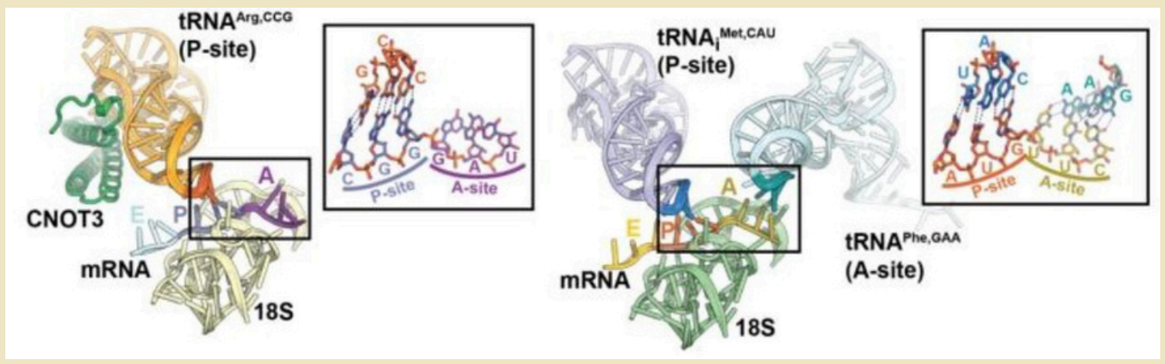

图13. CNOT3的核糖体结构中的mRNA配置

同时表明精氨酸tRNA的D-和抗密码子干的变化可能会在翻译核糖体的背景下改变它们对CNOT3的亲和力，并提出了核苷酸修饰的可能性，特别是参与上述D-茎三联体碱基相互作用的tRNAArg 、CCG/UCG/CCU中核苷酸46处的缺失，可能会影响CNOT3的募集。体外氨酰化试验证实，与每个相应的亲本tRNA相比，没有突变损害tRNA。这些证据表明P位点tRNA的序列在共翻译CNOT3募集中起着关键作用，并表明这种作用不依赖于特定的tRNA核苷酸修饰。

通过测试tRNAArg、ACG和tRNAArg、CCG的不同D-stem序列，发现将 tRNAArg 、 CCG 的 D- 茎 序 列 添 加 到tRNAArg 、 ACG （tRNAArg ， ACG-m5），或在tRNAArg、ACG中形成U13:A22碱基对的单个C13U突变（tRNAArg、ACG-m6），足以实现CNOT3 的 募 集 。 同 样 ， 翻 转 G12:C23 碱 基 对（tRNAArg，ACG-m7）在这种情况下没有效果。总之，这些结果确定了tRNAArg 、CCG/UCG/CCU位于D 臂中的U13位置，该位置与A22和A46形成三重碱基相互作用，这是与CNOT3募集到核糖体相关的关键特征。在tRNAArg ， UCU中 ， 三 重 态 相 互 作 用 是C13:G22:m7G46，这是所有tRNA中这些位置的核苷酸的常见配置。

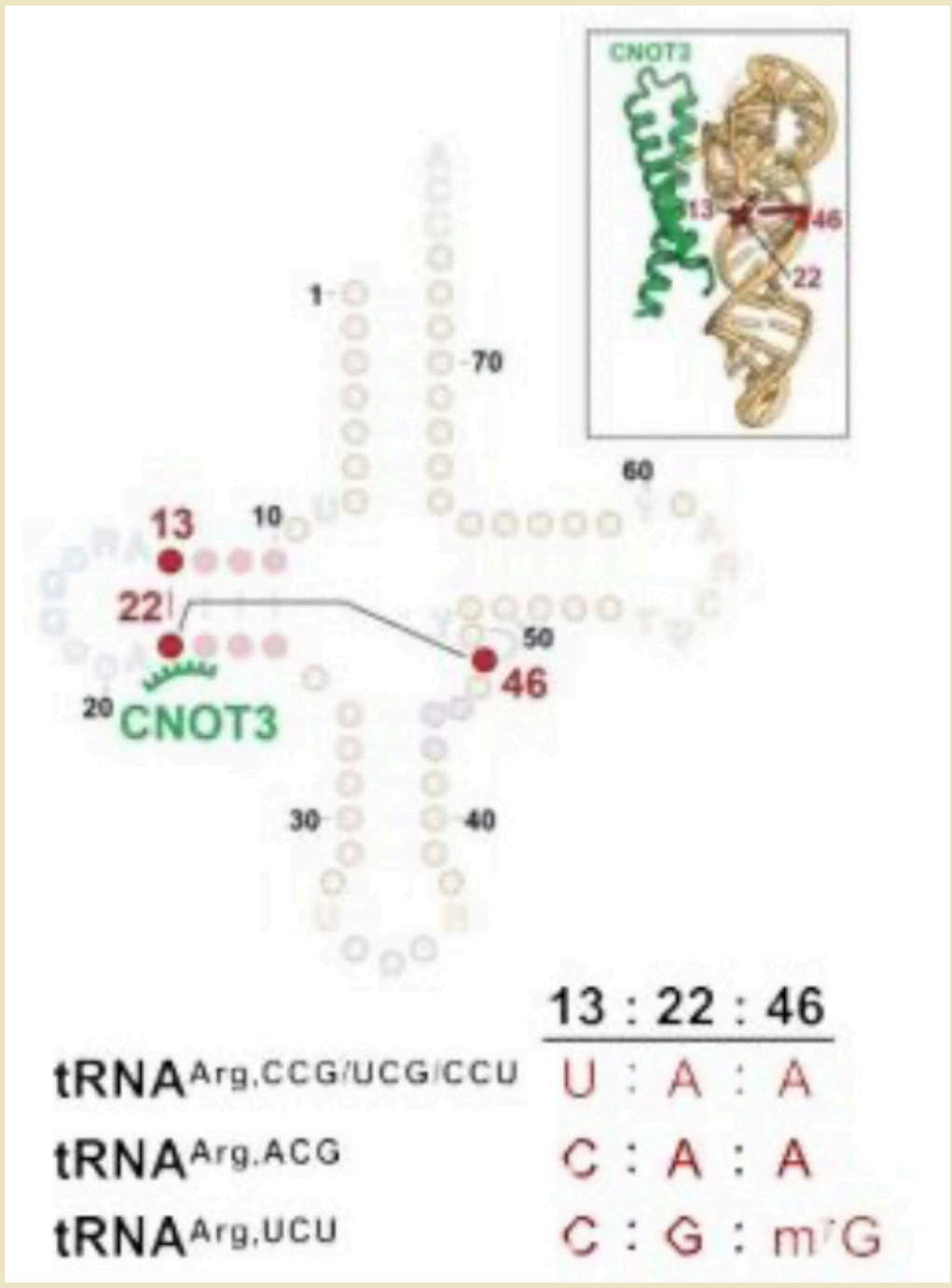

图14. tRNA和CNOT3的二级结构

在后续研究中， 也从结构上 证实了 含有U13:A22:A46三重碱基相互作用的精氨酸tRNA增强CNOT3的募集。

P-位点tRNA抗凝血干细胞对共翻译CNOT3募集的影响：

CNOT3与P-位点tRNA的抗密码子干之间的相互作用由tCM介导，tCM与P-位点-tRNA的碱基G42和A43形成了几种直接的骨架相互作用以及水桥相互作用 。

图15. CNOT3-tCM基序的二级结构和抗凝血干环

其中 tCM 中 的 K105S 替 代 消 除 了 CNOT3 向 富 含CGG/CGA/AGG的转录本的招募 。随后 对tRNAArg、UCU-1进行了重新编程 ， 使其能够识别CGU精氨酸密码子 。实验结果表明， 添加tRNAArg.CCG U13:A22:A46三联体 ， 但不添加tRNAArg.CCG抗密码子干，就足以增加CNOT3的募集。因此，U13:A22:A46三联体的存在是精氨酸tRNA募集CNOT3的主要决定因素，而抗凝血干细胞的作用较小，但可以测量 。

D-环GG基序上游的额外核苷酸阻止CNOT3募集：

通过进行进一步 tRNA诱变实验，发现 D-loop b元件的变化不影响CNOT3的募集，因为引入C20A（tRNAMet-m14）或C20U（tRNAMet m15）突变对CNOT3结合没有影响。 综上所述，我们的结果表明，P-位点tRNA中的D-干U13:A20:A46三联体和短的单核苷酸D-环a元件是CNOT3共翻译募集的主要决定因素，为解释P-位点密码子富集的主要模式提供了结构基础CNOT3结合核糖体的耗竭。

图16. 剪切密度图（左）和原子模型（右）突出显示核糖体E、P和A位点

最后，在 选择性核糖体分析数据中检测到a位点停留时间与CNOT3募集之间存在微弱但具有统计学意义的相关性 表明 缓慢的解码可显著促进人类细胞中CNOT3的募集，并且 当P位点被CGG、CGA或AGG精氨酸密码子占据时，A位点停留时间与CNOT3关联之间的相关性大大增强。相比之下，当P位点存在任何其他密码子时，停留时间相关性无法检测到。其中 缓慢的解码增加了核糖体A和E位点同时空缺 的可能性，为CNOT3进入E位点提供了机会。也就证明了 CNOT3随后探测P位点tRNA的D臂最终决定了CNOT3是否与核糖体稳定结合并启动mRNA衰变。

参考文献

[1] Xiaoqiang Zhu, Victor Emmanuel Cruz, He Zhang,(2024), Specific tRNAs promote mRNA decay by recruiting the CCR4-NOT complex to translating ribosomes, https://doi.org/10.1126/science.adq8587,《Nature: MOLECULAR BIOLOGY》

[2] Lu Wang, Shuibin Lin,(2023), Emerging functions of tRNA modifications in mRNA translation and diseases, https://doi.org/10.1016/j.jgg.2022.10.002,《Journal of Genetics and Genomics》

[3] Kentaro Ito, Akinori Takahashi, Masahiro Morita,(2011), The role of the CNOT1 subunit of the CCR4-NOT complex in mRNA deadenylation and cell viability, https://doi.org/10.1007/s13238-011-1092-4,《Protein & Cell》

[4] Mark Bartlam, Tadashi Yamamoto, (2010),The structural basis for deadenylation by the CCR4-NOT complex, https://doi.org/10.1007/s13238-010-0060-8,《Protein & Cell》

[5] 李超,(2014)Functional Characterization of Ccr4-NOT complex components in the Rice Blast Fungus,《China Agricultural University》

[6] 吴洁，温贵兰，赵静，胡璇,(2023), Ccr4－Not复合物的生物学功能研究进展, 1007－1474(2023) 05－0059－04,《贵州畜牧兽医》

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预防和治愈朊病毒病的基因工程方法：以克雅氏病（CJD）为重点

关键词：克雅氏病、机器学习、纳米抗体、免疫疗法、抗体工程

摘要

克雅氏病 （CJD） 是一种罕见且致命的神经退行性疾病，与朊病毒蛋白错误折叠有关。本研究探讨了 CJD 诊断和治疗的基因工程和机器学习方法。使用卷积神经网络 （CNN），我们比较了诊断模型，发现 ResNet50 在对 MRI 图像进行分类方面达到了 97% 的准确率。此外，我们还讨论了纳米抗体和人源化抗体（如 PRN100）的治疗潜力，它们为 CJD 治疗提供了有前途的途径，同时减少了副作用。计算方法和免疫疗法的整合以及抗体工程的使用为 CJD 管理的进步铺平了道路。通过这种方式，我们实现了 CJD 的检测和治疗。

引入

克雅氏病 （CJD） 是一种罕见且致命的神经退行性疾病，与朊病毒蛋白错误折叠有关。本研究探讨了 CJD 诊断和治疗的基因工程和机器学习方法。使用卷积神经网络 （CNN），我们比较了诊断模型，发现 ResNet50 在对 MRI 图像进行分类方面达到了 97% 的准确率。此外，我们还讨论了纳米抗体和人源化抗体（如 PRN100）的治疗潜力，它们为 CJD 治疗提供了有前途的途径，同时减少了副作用。计算方法和免疫疗法的整合以及抗体工程的使用为 CJD 管理的进步铺平了道路。通过这种方式，我们实现了 CJD 的检测和治疗。

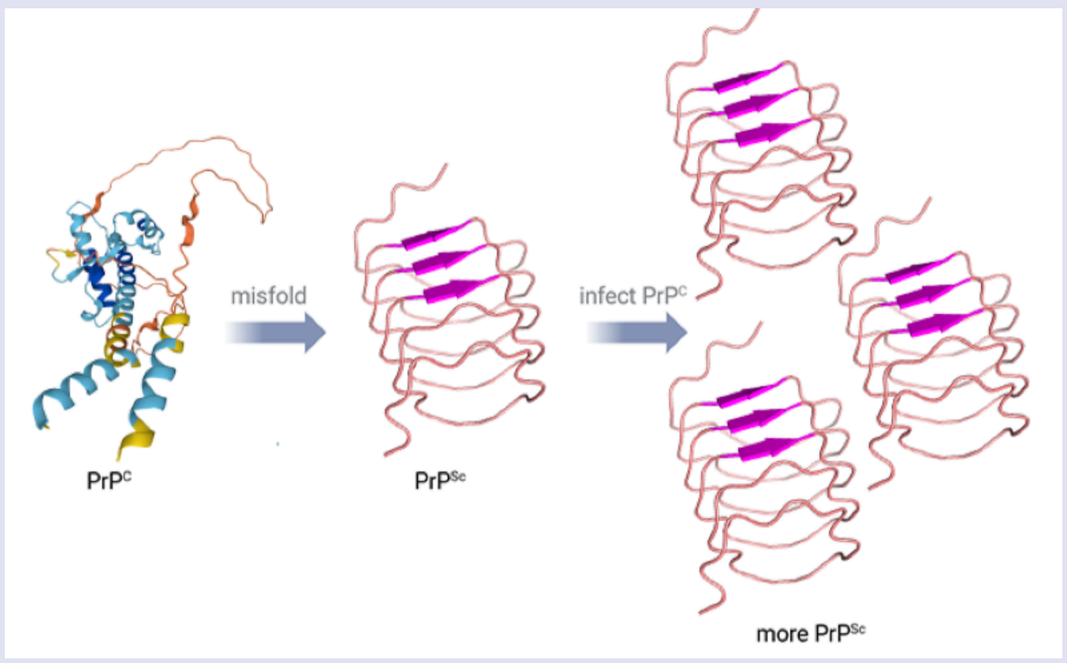

Figure 1 PrPc misfolds and PrPSc aggregates.

与此同时，大脑细胞蛋白质量控制机制（其核心组成成分为伴侣蛋白和蛋白酶）对于维持生理条件下的蛋白稳态以及抵御急性应激条件至关重要（Rustenhoven, 2021）。蛋白稳态失衡也是导致众多遗传性和后天性疾病（通常与年龄相关）以及衰老的关键因素。

一项公众调查显示，仅有25.86%的受访者表示听说过朊病毒（克雅氏病的元凶），这表明公众对该疾病的关注度和认知度不足。在这些受访者中，幸运的是，有68.97%的人对基因筛查持认真态度，因为基因筛查可以诊断遗传性克雅氏病。此外，克雅氏病还可能因食用受污染的牛肉而感染（即变异型克雅氏病）。当被问及意见时，比例最高的人群（30.99%）表示希望国家能够加强对食品安全的监管。29.93%的人认为他们总是去有食品安全认证的地方购买牛肉。15.85%和11.62%的受访者表示，他们经常关注食品安全相关的杂志、频道、公众号、视频制作者和网站，无论是作为牛肉的买家还是生产者，都强调牛饲料来源的重要性。然而，仅有3.52%的人表示自己有过肉类产品安全方面的工作或研究经验。

研究预防方法

公众对克雅氏病（Creutzfeldt-Jakob Disease, CJD）的认知程度

在我们的公众调查中，仅有25.86%的受访者表示听说过朊病毒（CJD的罪魁祸首），这表明公众对该疾病的关注度不高且认识不足。在这些受访者中，幸运的是有68.97%的人对能够诊断遗传性CJD的基因筛查持认真态度。同时，CJD还可能因食用被污染的牛肉而感染（即变异型克雅氏病，vCJD）。在询问人们的意见时，30.99%的人（占比最高）表示希望国家能加强食品安全监管。29.93%的人认为自己总是去有食品安全认证的牛肉销售点购买。15.85%和11.62%的受访者表示经常关注食品安全方面的杂志、频道、公众号、视频制作者和网站，并且在购买或生产牛肉时会强调牛饲料的来源。然而，仅有3.52%的人表示自己有过肉制品安全方面的工作或研究经验。

传统神经网络在医学中的重要性

传统神经网络（CNN）取得了卓越成就，已成为深度学习中最具代表性的神经网络之一。基于传统神经网络的计算机视觉使人们能够完成过去几个世纪被认为不可能的事情，尤其是在图像识别方面（Li, Z. W., 等人，2004）。1959年，Hubel和Wisel发现动物视觉皮层中的细胞负责在感受野中检测光线。这一发现启发了Kunihiko Fukushima在1980年提出新认知机，这可以看作是CNN的前身。自此以后，开发了许多CNN模型。值得注意的是，Krizhevsky等人提出了一种经典的CNN架构，并在图像分类任务上相较于之前的方法取得了显著改进（Gu, J., 等人，2017）。

随着数据驱动的机器学习的进步，使用机器学习方法分析医学数据变得越来越重要。CNN作为一种强大的图像处理工具，在医学领域的应用越来越广泛。其在生物医学图像分析、辅助诊断、病理学、放射学和神经科学等多个分支中均表现出色。

卷积神经网络（CNN）不仅以其对图像的深度分析能力而著称，还因其有可能提高医疗服务的质量和效率而备受瞩目。

CNN能够准确识别医学图像中的细微模式和异常，从而协助医生在疾病早期阶段进行诊断。这种早期诊断对于治疗至关重要，因为许多疾病在早期发现时更容易治疗且治疗成本相对较低。最重要的是，CNN在诊断方面具有更高的准确性。

四种不同的CNN模型及其比较

为了判断一个人是否感染了CJD，我们使用机器进行诊断。由于CJD是一种罕见疾病且相关数据很少，因此我们使用少量样本对机器进行训练。由于CJD的罕见性，很难找到所需的训练和测试数据。患者的MRI案例也非常少。我们夜以继日地访问医学社区、网站和论坛，并联系相关组织，希望能获得所需数据，尽管这个过程既缓慢又困难。使用传统神经网络，我们可以将患者大脑的MRI图像分类为有CJD或无CJD。通过这种方式，我们可以更精确、可靠地诊断该疾病，并决定是否采取相关治疗。

基本CNN

卷积神经网络（CNN）是专门为处理结构化网格数据（如图像）设计的深度学习模型（LeCun等人，1998）。其主要结构包括卷积层、池化层和全连接层。卷积层通过卷积运算从输入数据中提取空间特征，而池化层则通过子采样降低数据维度，同时保留重要信息。基本CNN通常包含四个常规层，每层核大小为3，步长为1，填充为1。这些层通过逐层提取和组合特征来逐步构建对图像内容的理解。

结果

在测试数据上，基本CNN的准确率为95%。

ResNet34

ResNet34是一种深度残差网络，旨在解决深度神经网络训练过程中可能出现的梯度消失和梯度爆炸问题（He等人，2016）。ResNet34的结构包括多个残差块，每个残差块都通过跳跃连接绕过部分层，以确保梯度可以有效向前传播。具体而言，ResNet34分别重复3x3卷积层三次、四次、六次和三次，并在每个残差块中使用跳跃连接，以实现更深的网络结构而不增加训练难度。

结果

ResNet34在测试数据上的准确率为94%。

ResNet50

ResNet50是ResNet系列中更深层次的版本，采用了更复杂的残差块（He等人，2016）。ResNet50的每个残差块都包含1x1、3x3和1x1卷积层的组合。这种设计不仅保留了高效的特征提取能力，还通过1x1卷积层降低了计算复杂度。具体而言，ResNet50分别重复这些卷积层组合三次、四次、六次和三次，也使用跳跃连接来保持有效的梯度传播。

结果

ResNet50在测试数据上的准确率为97%。

DenseNet121

DenseNet121是一种密集连接的卷积网络，旨在通过直接连接任意两层来最大化信息流和梯度传播（Huang等人，2017）。DenseNet121的结构包括一个7x7卷积层和一个3x3最大池化层，后面跟着六个、十二个、二十四个和十六个1x1和3x3卷积层组合（称为密集块）的重复。在每个密集块之间，DenseNet121通过1x1卷积层和2x2平均池化层进行过渡，从而保持网络的紧凑性和计算效率。

结果

DenseNet121在测试数据上的准确率为96%。

比较

我们发现，ResNet50在此图像分类任务中表现最佳。使用ResNet50（准确率为97%），我们可以快速检测CJD，以便患者能够尽快得到治疗。

在这个模型中，我们使用 Cross Entrophy Loss 作为损失函数。我们可以看到损失

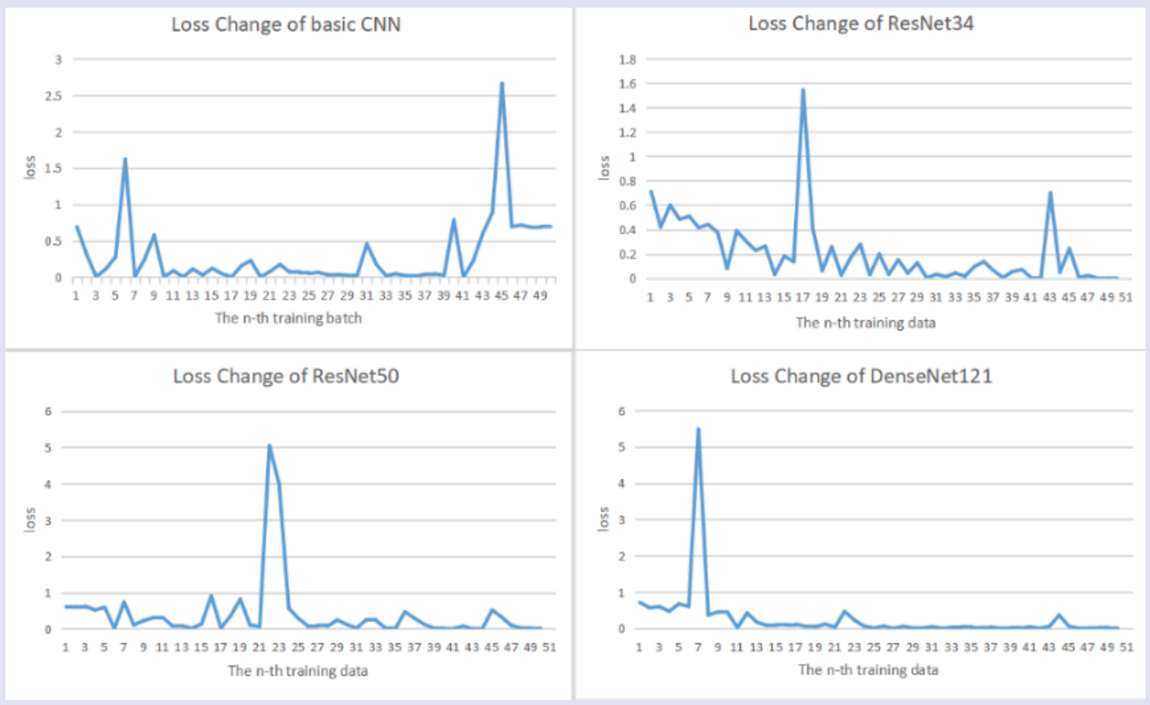

Figure 2 Visualisation of investigation results

Model	Source of Data	Loss	Optimizer	epoches	Validation Set Accuracy	Training Time
Basic CNN	https://www.dxy.cn https://radiopaedia.org/articles/creutzfeldt-jakob-disease Brain Tumor MRI Dataset (kaggle.com)	Cross Entrophy	Adam	10	95%	4m 43.5s
ResNet34	https://www.dxy.cn https://radiopaedia.org/articles/creutzfeldt-jakob-disease Brain Tumor MRI Dataset (kaggle.com)	Cross Entrophy	Adam	10	94%	17m 14.5s
ResNet50	https://www.dxy.cn https://radiopaedia.org/articles/creutzfeldt-jakob-disease Brain Tumor MRI Dataset (kaggle.com)	Cross Entrophy	Adam	10	97%	24m 17.8s
DenseNet121	https://www.dxy.cn https://radiopaedia.org/articles/creutzfeldt-jakob-disease Brain Tumor MRI Dataset (kaggle.com)	Cross Entrophy	Adam	10	96%	14m 47.7s

Figure 3 Comparison between four models

训练和测试 YOLOv5 分类模型，以确定 MRI 图像是否为 CJD 患者

由 Ultralytics 开发的 YOLOv5 是一款多功能且功能强大的模型,主要用于物体检测,但也支持图像分类任务。
YOLOv5 中的分类模型利用了用于检测的相同高效架构,使其快速而准确。

我们对包含有CJD（克雅氏病）和无CJD人群MRI图像的增强数据集进行了100轮训练，训练了五种不同大小的YOLOv5分类模型，并记录下了训练损失、测试损失和测试准确率的变化。所有模型均表现出极高的准确率，且训练损失和测试损失均较低。
YOLOv5分类模型分为五种不同大小：nano（微型）、small（小型）、middle（中型）、large（大型）和ultra（超大型）。模型越大，可训练参数越多，拟合能力越强，但训练和预测速度越慢，过拟合的风险也越大。
结果
在我们的训练和测试任务中，尽管所有尺寸的模型最终都达到了相似的损失和准确率水平，但在训练初期，较大模型的准确率波动更为剧烈。

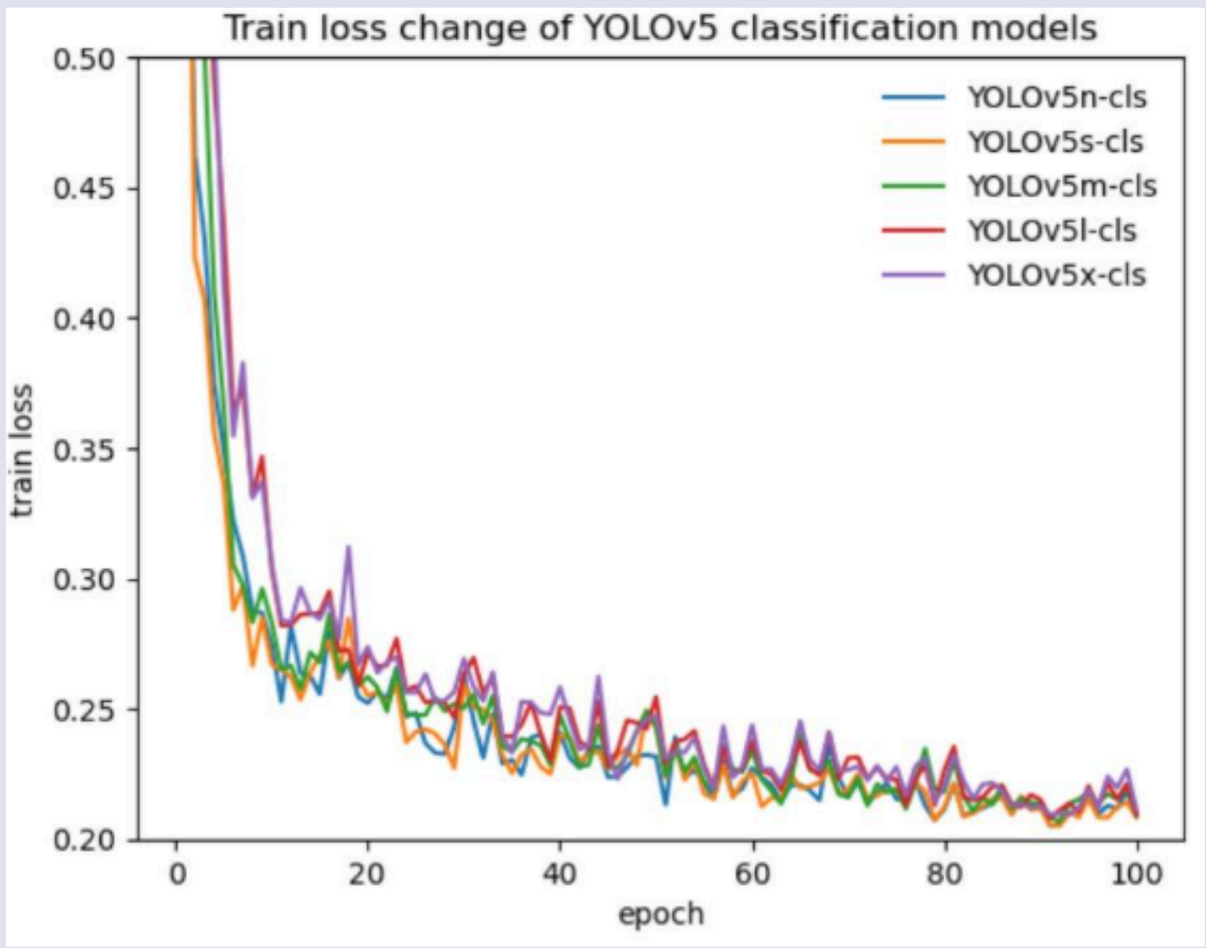

Figure 4 Train loss change of YOLOv5 classification models. all models achieved similar train loss level in the end

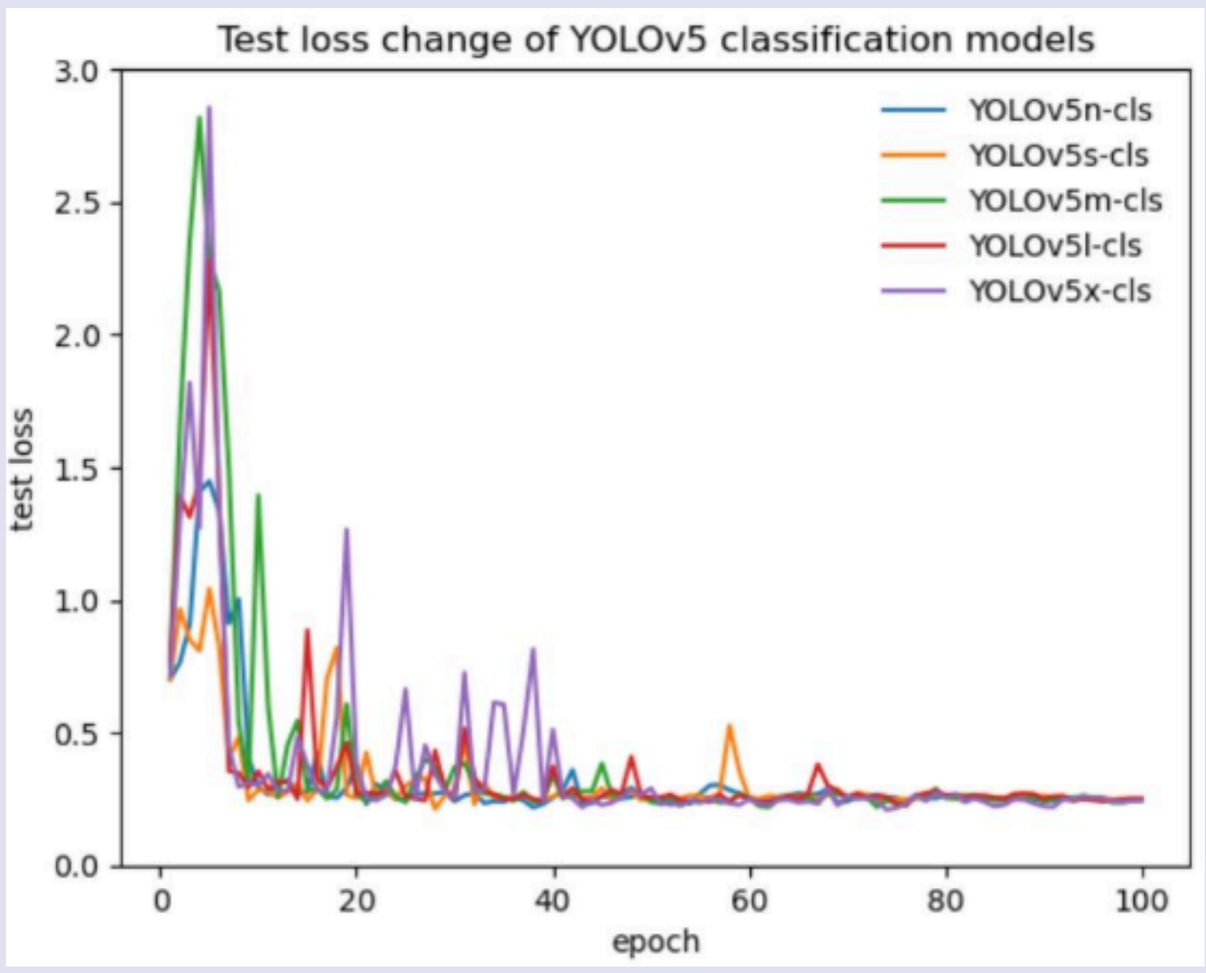

Figure 5 Test loss change of YOLOv5 classification models. There is no overfit in all models.

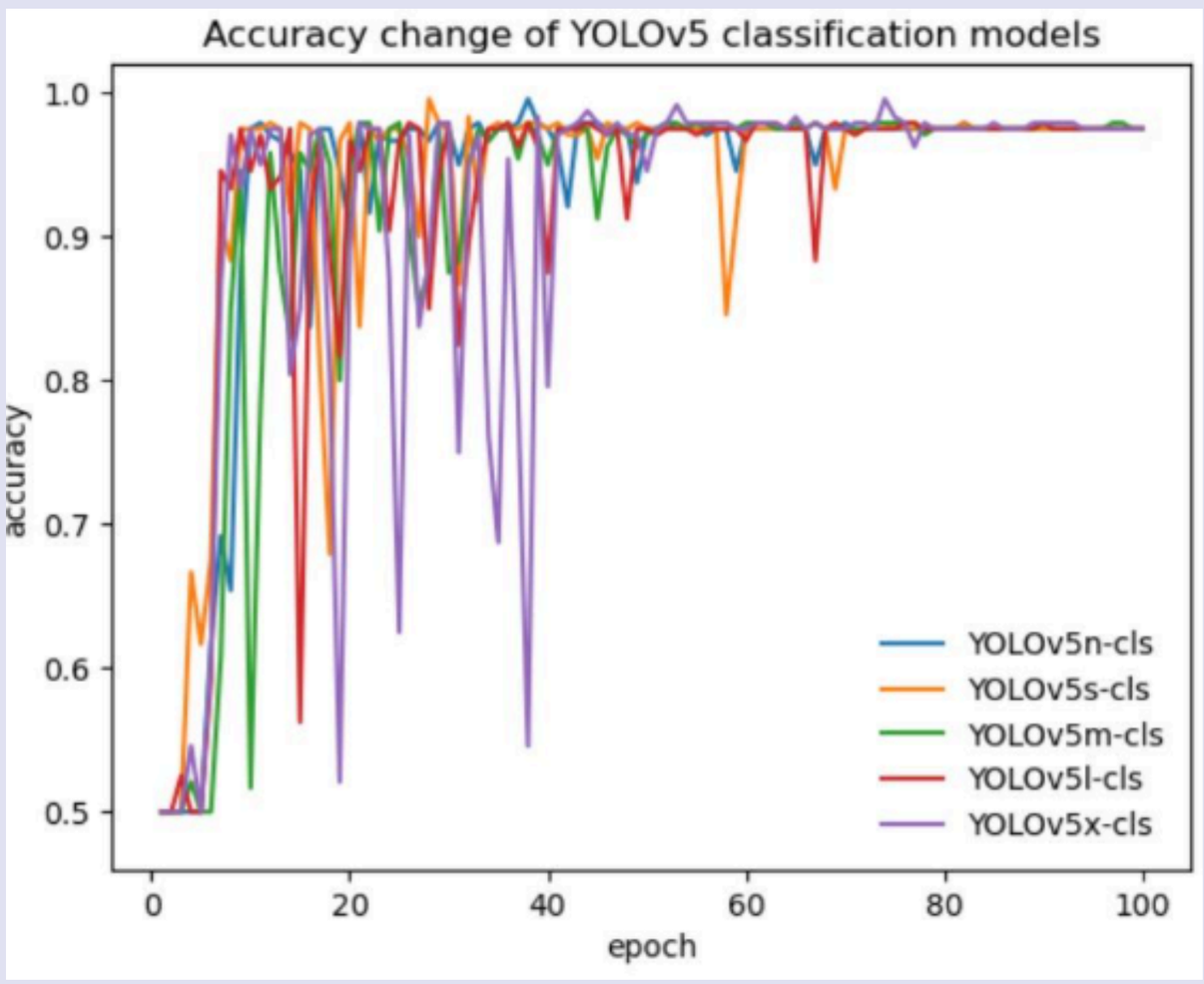

Figure 6 Accuracy change of YOLOv5 classification models. Model of all sizes achieved similar accuracy level in the end, but in the early epochs, the fluctuation of accuracy of larger model is more violent.

反思

在训练模型时，我们使用的数据量太少。这主要是因为克雅氏病（CJD）非常罕见，因此相关数据很少。我们仅收集了100张克雅氏病患者的脑部MRI图片和200张非克雅氏病患者的脑部MRI图片。因此，我们采用了数据增强技术并调整了图片大小。然而，数据增强技术存在一个问题。我们只对数据进行了旋转、线性变换、翻转和镜像处理。这可能会导致数据泄露。此外，为了适应MRI图片的形状以符合模型要求，一些图片被过度拉伸，导致形状成为判断一个人是否患有克雅氏病的一个因素，这显然是不合理的。因此，在未来，我们将采取以下改进措施，并建议所有人都这样做。首先，建立一个罕见病例收集社区。其次，使用MRI的部分图片而不是整张图片来比较细节上的差异。第三，将同一个大脑的多个MRI图片组合成一个张量。

限制

可能会存在假阴性和假阳性问题。人类医生更容易犯假阴性错误，即误诊为阴性（即未患病但实际上患病）。同时，机器则因为有时“过于谨慎”而倾向于犯假阳性错误，即误诊为阳性（即患病但实际上未患病）。因此，很难决定是否应该相信机器的诊断。这个领域涉及哲学和伦理问题，需要进一步研究。

设计抗体

免疫治疗以其高度的特异性和较少的副作用而闻名，在治疗朊粒病方面具有巨大潜力。通过发现针对Aβ聚集物的人源抗体Aducanumab，免疫治疗在阿尔茨海默病方面取得了进展。由于不同的朊粒病在氨基酸序列上存在细微的结构差异，为了有效治疗，这些分子需要针对对播种活性至关重要的区域。因此，一个与播种区域匹配的小分子可能是特定朊粒株的良好抑制剂，但这种小分子成为通用抗朊粒分子的可能性并不高。相比之下，抗体治疗被认为更有效，因为它通常能够容忍结构差异并具有更稳定的疗效。

在治疗慢性消耗性疾病（CWD）这一鹿、牛和其他哺乳动物中流行的朊粒病（可能感染人类）方面，抗体治疗已取得进展。在患病动物中，接种疫苗诱导的抗体至少可以减少外周组织中的PrPSc（朊粒蛋白），并防止CWD朊粒的脱落，这被认为是CWD高效横向传播的主要原因（Chames, 2009）。

科学家更多地关注使用单克隆抗体进行免疫治疗。例如，西蒙教授的团队已经为克雅氏病开发出了朊粒蛋白单克隆抗体（PRN100）疗法。2022年4月，该疗法的首次人体治疗计划已完成。然而，本文还想提及另一种相对罕见的策略，即纳米抗体。

纳米抗体天然存在于骆驼科动物中，包括单峰驼、羊驼和骆马（Sun, 2021）。与普通抗原相比，纳米抗体以更高的效率和更少的副作用而闻名。此外，由于其体积小、亲水性强和单域特性，纳米抗体也可由细菌生产。纳米抗体可以在革兰氏阴性菌（如大肠杆菌）、革兰氏阳性菌（如短芽孢杆菌Little Rock）、某些乳酸杆菌和双歧杆菌中快速生产。细菌系统的优点是易于改变且作为生产系统更具经济可行性（Bhavar, 2022）。

纳米抗体在单个免疫球蛋白VHH域内提供了抗体的不可思议的特异性，该域包含更长且混合的互补决定区（CDRs），这意味着它具有与特定抗原结合的更高效率。CDRs是抗体（由B细胞产生）和T细胞受体（由T细胞产生）中可变链的一部分（Nanomedicine, 2021）。由于纳米抗体对环境具有更好的抵抗力，它们可以在细菌、酵母和哺乳动物宿主中表达。纳米抗体还具有抗蛋白酶降解和酸碱度变化的能力（My BioSource, 2023），这提高了结合的可能性。其较小的体积（约30kDa）使它们能够靶向传统全尺寸抗体无法结合的抗原和受体（Wesdorp, 2023）。

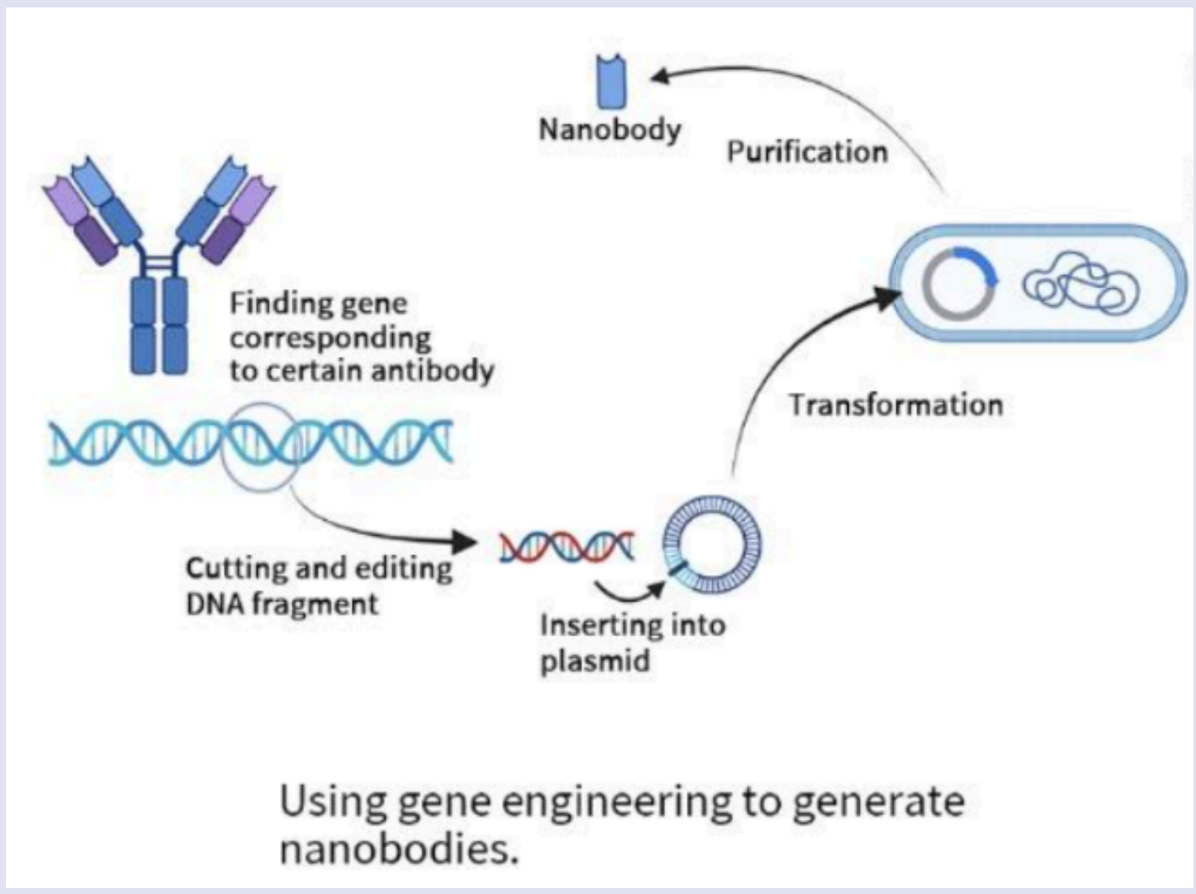

Figure 7 Gene engineering generating nanobodies.

此外，纳米抗体产生的副作用较少。患者过去常常因单克隆抗体而产生副作用，这些被称为输注反应，通常在首次给药时最为常见。输注反应可能导致以下症状：发热、寒战、虚弱、头痛、恶心、呕吐、腹泻、低血压和皮疹（ACS，2022）。

尽管被动免疫治疗在治疗朊病毒疾病方面相当方便，因为它能确保抗体在注射给患者前已准备好，从而节省了患者自身产生抗体的时间，但它有一个致命缺陷，即可能导致神经退行性病变（Frontzek & Aguzzi，2020，第169页）。此外，被动免疫治疗中使用的一些抗体通常是从感染的小鼠或其他非人类生物体中获得的，因此使抗体人源化变得至关重要（Wang等，2021，第45-46页）。通过抗体人源化，可以最大限度地降低安全风险，并提高疗效（Hummer & Deane，2023，第xx页）。

具体来说，传统的人源化方法是互补决定区（CDR）移植，即将非人类抗体的CDR移植并添加到人类抗体的框架上（Hummer & Deane，2023，第xx页）。抗体的序列决定了其疗效、可开发性和人源性，尽管这三者之间需要保持平衡（Hummer & Deane，2023，第xx页）。目前，计算机科学和免疫学之间已经出现了跨学科交叉。不同学科之间的这种合作大大提高了工作效率，并为技术进化开辟了新的可能性。例如，使用计算移植和基于能量的排序方法来人源化抗体的效率（超过2000个人类受体可以与非人类CDR结合）高于传统实验移植的一对一结果（Hummer & Deane，2023，第xx页；如图8所示）。

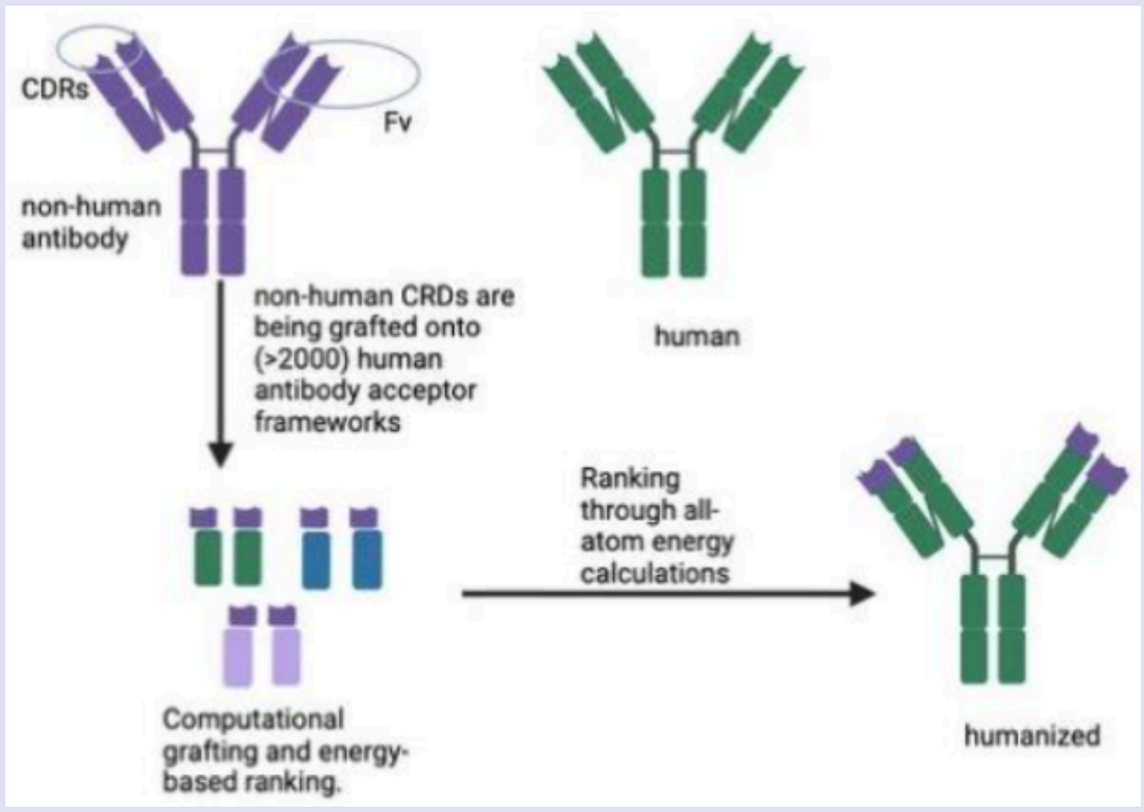

Figure 8 Computational grafting and energy-based ranking.

讨论和限制

当前研究深入探讨了基因工程方法在朊病毒病，特别是克雅氏病（CJD）中的应用。鉴于该病的罕见性以及朊蛋白的独特性质，我们的研究强调了CJD诊断和治疗的复杂性和挑战。

研究的一个关键发现是，卷积神经网络（CNN）在分析MRI图像诊断CJD方面具有有效性。其中，ResNet50模型展示了高达97%的准确率，表明其有望成为可靠的诊断工具。然而，训练这些模型所需数据的稀缺性不容忽视。由于CJD病例罕见，我们不得不使用数据增强技术，尽管这项技术有所帮助，但可能会在模型训练中引入偏差或不准确性。数据泄露的潜在风险以及MRI图像因调整大小而产生的失真问题，需要我们对方法进行进一步的调查和改进。

此外，关于开发针对朊病毒病的免疫疗法抗体的讨论，突显了这一治疗方法的潜力和挑战。源自骆驼科的纳米抗体因其高特异性、低免疫原性和能够靶向传统抗体无法触及的抗原，为治疗开辟了一条新途径。以PRN100疗法为例，抗体人源化方面的进展是减少不良反应、增强免疫疗法疗效的重要一步。

然而，我们的研究也揭示了使用机器学习和免疫治疗的局限性和伦理考量。机器学习模型中存在假阳性和假阴性的可能性，以及被动免疫治疗相关的神经退行性病变风险，都是需要仔细考虑的问题。在早期准确诊断的好处与过度诊断和过度治疗的风险之间找到平衡，是一项需要谨慎处理的任务。

此外，作为本研究一部分开展的公众调查显示，普通人群对CJD和朊病毒病的认识严重不足。这一发现强调了加强公众教育和宣传活动的重要性，以确保人们了解CJD的风险、症状和预防措施。

综上所述，虽然我们的研究在CJD的诊断和治疗方面取得了显著进展，但也强调了需要进一步研究、改进方法和提高公众意识。机器学习与免疫治疗的结合前景广阔，但必须在对局限性和伦理影响有充分了解的基础上进行。未来的工作应侧重于扩大诊断模型训练的数据集、完善数据增强技术，并进行更广泛的免疫治疗临床试验，以确保其安全性和有效性。

补充信息

调查结果

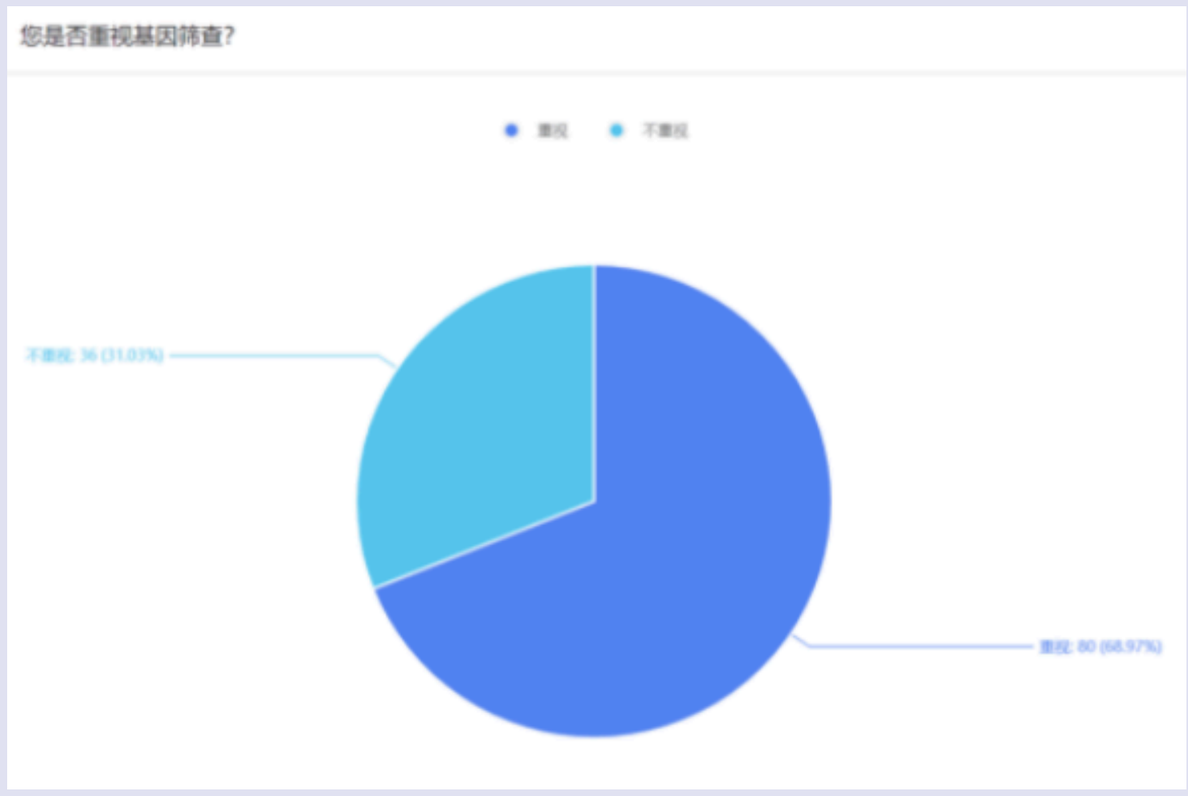

Figure 9 Question: Do you value genetic screening/testing? Darker Blue-Yes； Lighter Blue-No

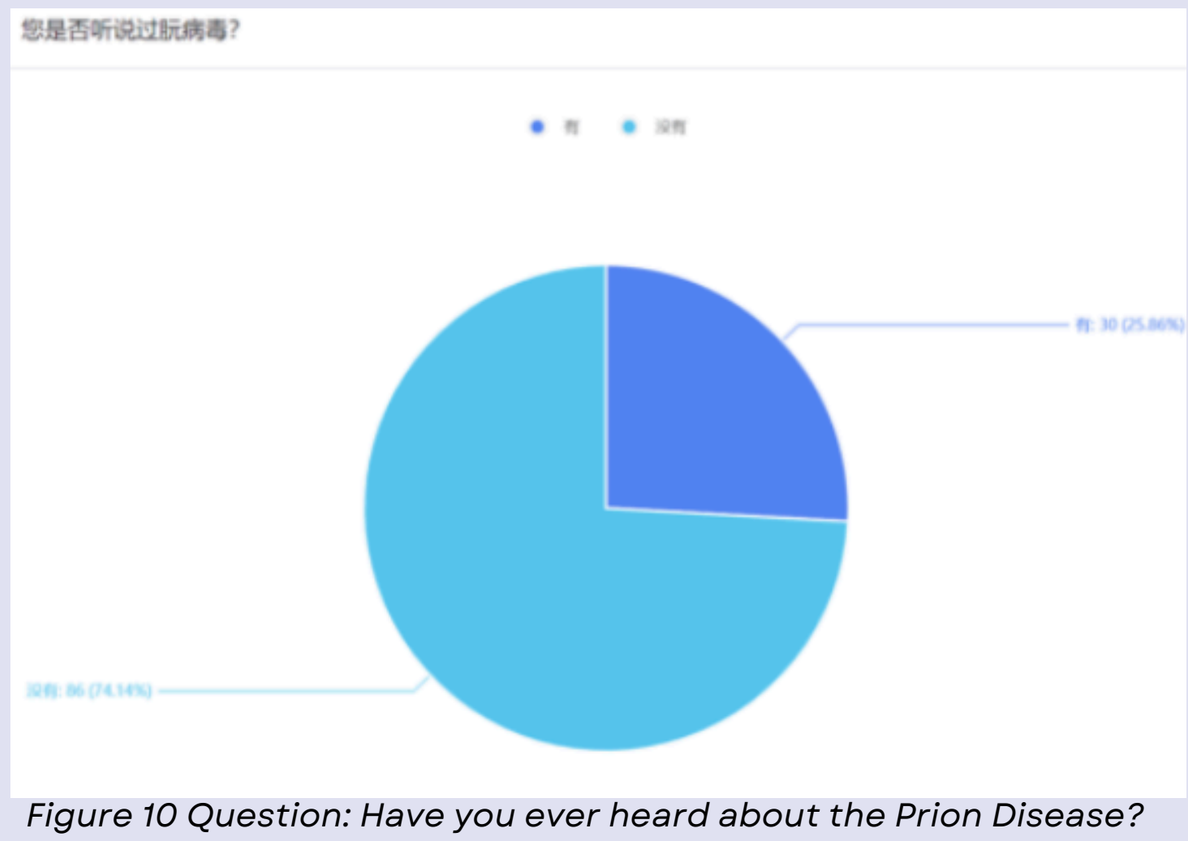

Figure 10 Question: Have you ever heard about the Prion Disease? Dark Blue- Yes； Light Blue-No

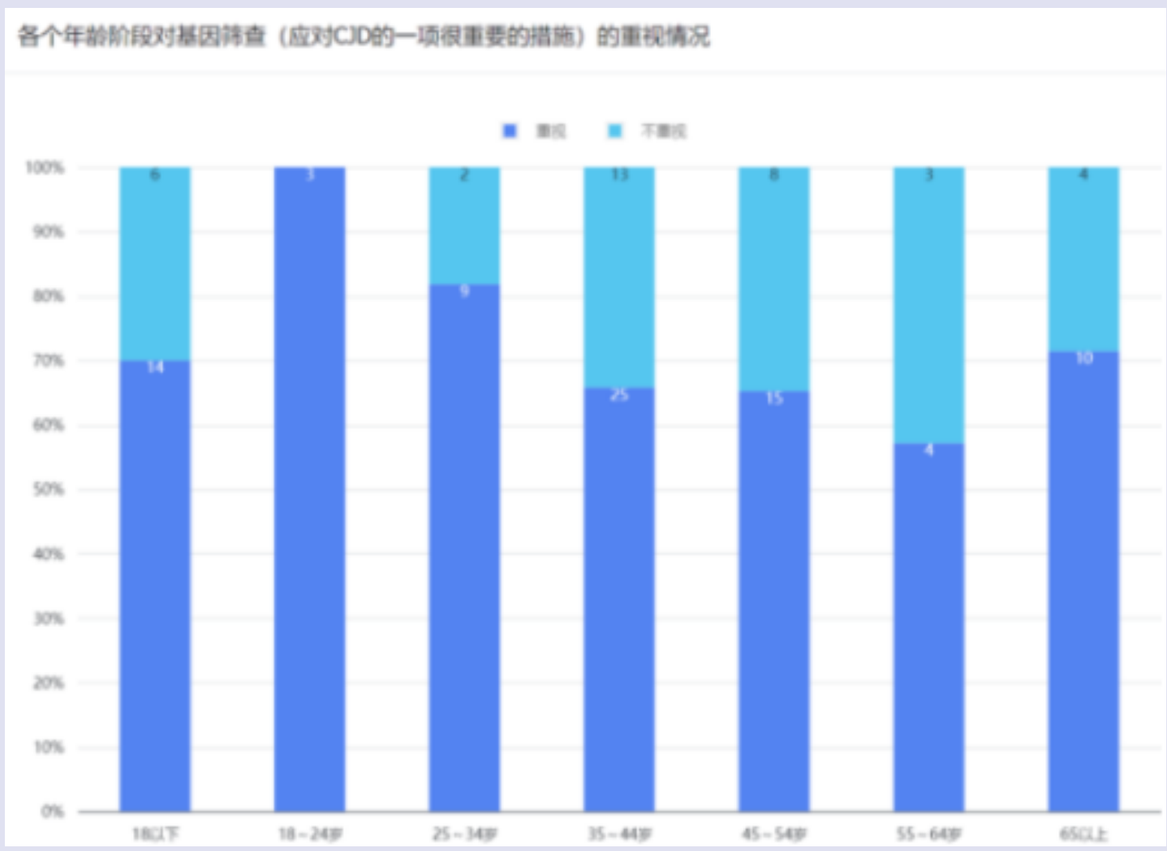

Figure 11 Results of an Investigation on the attitude towards genetic screening across different age groups

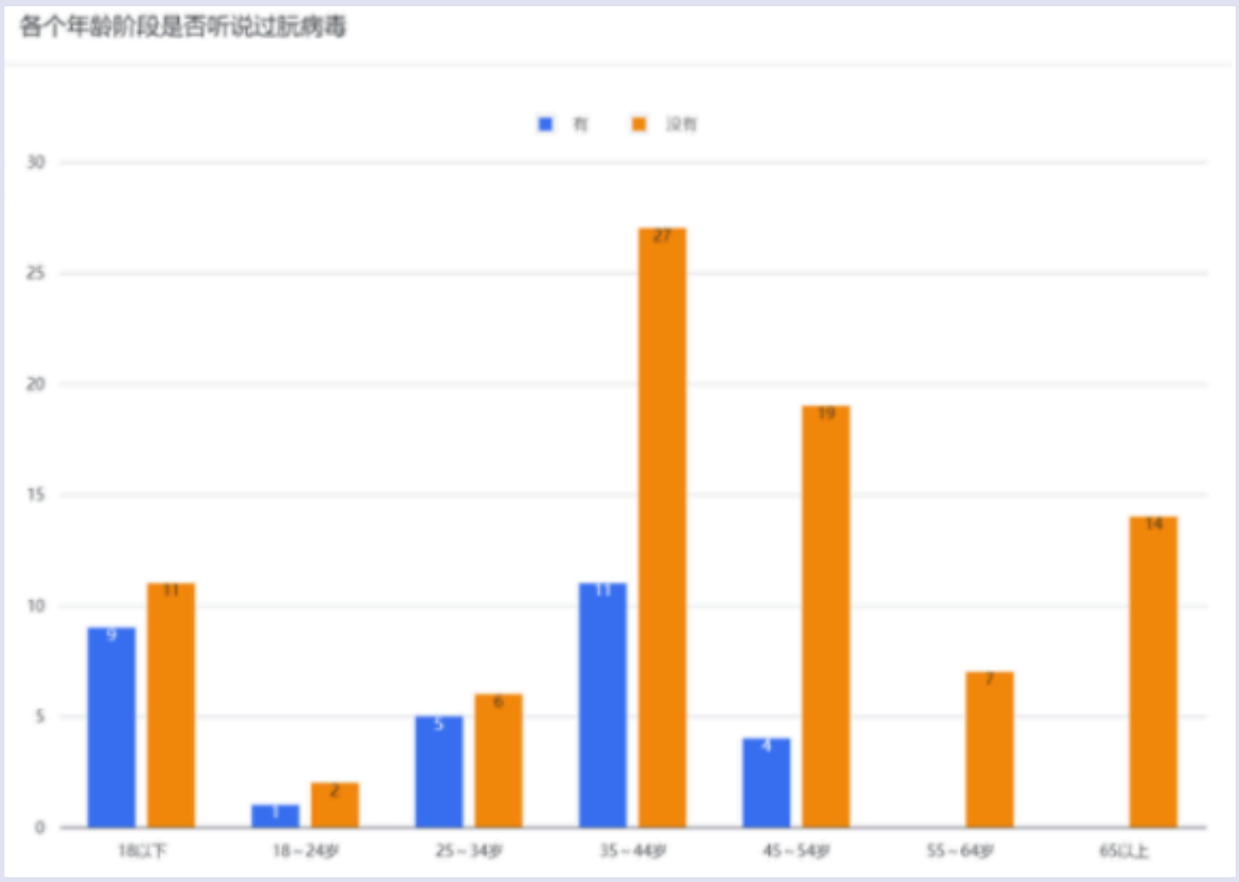

Figure 12 The results for an investigation on the acknowledgement of prion diseases across different age groups

引用

Aguzzi, A., & Frontzek, K. (2020). Recent developments in antibody therapeutics against prion disease. Emerging Topics in Life Sciences, 4 (2), 169-173. https://doi.org/10.1042/etls20200002

American Cancer Society. (2022). Monoclonal Antibody Side Effects. Www.cancer.org. https://www.cancer.org/cancer/managing-cancer/treatment-types/immunotherapy/monoclonal-antibodies.html

Baraznenock, E., & Kravchuk, S. (2023, February 8). Prions: How can proteins be infectious? And what that tells us about other neurodegenerative diseases. Grey Matters. https://greymattersjournal.org/prions-how-can-proteins-be-infectious-and-what-tells-us-about-other-neurodegenerative-diseases/

Chames, P., Van Regenmortel, M., Weiss, E., & Baty, D. (2009). Therapeutic antibodies: successes, limitations and hopes for the future. British Journal of Pharmacology, 157(2), 220–233. https://doi.org/10.1111/j.1476-5381.2009.00190.x

Gu, J., Wang, Z., Kuen, J., Ma, L., Shahroudy, A., Shuai, B., ... & Chen, T. (2017). Recent advances in convolutional neural networks. Pattern Recognition, 77, 354-377. https://doi.org/10.1016/j.patcog.2017.10.013

He, K., Zhang, X., Ren, S., & Sun, J. (2016). Deep residual learning for image recognition. In Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition (pp. 770-778). https://doi.org/10.1109/CVPR.2016.90

Hummer, A. M., & Deane, C. M. (2023). Designing stable humanized antibodies. Nature Biomedical Engineering, 8 (1), 3-4. https://doi.org/10.1038/s41551-023-01168-1

Hubel, D. H., & Wiesel, T. N. (1959). Receptive fields of single neurones in the cat's striate cortex. The Journal of Physiology, 148 (3), 574-591. https://doi.org/10.1113/jphysiol.1959.sp006308

Huang, G., Liu, Z., Van Der Maaten, L., & Weinberger, K. Q. (2017). Densely connected convolutional networks. In Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition (pp. 4700-4708). https://doi.org/10.1109/CVPR.2017.243

Krizhevsky, A., Sutskever, I., & Hinton, G. E. (2012). ImageNet classification with deep convolutional neural networks. In Advances in Neural Information Processing Systems (pp. 1097-1105).

LeCun, Y., Bottou, L., Bengio, Y., & Haffner, P. (1998). Gradient-based learning applied to document recognition. Proceedings of the IEEE, 86 (11), 2278-2324. https://doi.org/10.1109/5.726791

Li, Z., Yang, W., Peng, S., & Liu, F. (2004). A survey of convolutional neural networks: Analysis, applications, and prospects. Journal of Artificial Intelligence Research, 55, 345-377. https://doi.org/10.1613/jair.4327

Mead, S., Khalili-Shirazi, A., Potter, C., Mok, T., Nihat, A., Hyare, H., Canning, S., Schmidt, C., Campbell, T., Darwent, L., Muirhead, N., Ebsworth, N., Hextall, P., Wakeling, M., Linehan, J., Libri, V., Williams, B., Jaunmuktane, Z., Brandner, S., & Rudge, P. (2022). Prion protein monoclonal antibody (PRN100) therapy for Creutzfeldt–Jakob disease: evaluation of a first-in-human treatment programme. The Lancet Neurology, 21 (4), 342–354. https://doi.org/10.1016/s1474-4422(22)00082-5

Ribes, J. M., Patel, M. P., Halim, H. A., Berretta, A., Tooze, S. A., & Klöhn, P. (2023). Prion protein conversion at two distinct cellular sites precedes fibrillisation. Nature Communications, 14 (1). https://doi.org/10.1038/s41467-023-43961-1

Rouvray, D. (2005, September 28). Battle with the prions. Chemistry World. https://www.chemistryworld.com/features/battle-with-the-prions/3004499.article

Rustenhoven, J. (2021). A privileged brain. Science, 374 (6567), 548–548. https://doi.org/10.1126/science.abl7122

Scheckel, C., & Aguzzi, A. (2018). Prions, prionoids and protein misfolding disorders. Nature Reviews Genetics, 19 (7), 405-418. https://doi.org/10.1038/s41576-018-0011-4

Sun, S., Ding, Z., Yang, X., Zhao, X., Zhao, M., Gao, L., Chen, Q., Xie, S., Liu, A., Yin, S., Xu, Z., & Lu, X. (2021). Nanobody: A Small Antibody with Big Implications for Tumor Therapeutic Strategy. International Journal of Nanomedicine, 16, 2337–2356. https://doi.org/10.2147/IJN.S297631

Wang, B., Gallolu Kankanamalage, S., Dong, J., & Liu, Y. (2021). Optimization of therapeutic antibodies. Antibody Therapeutics, 4 (1), 45-54. https://doi.org/10.1093/abt/tbab003

Zerr, I., Ladogana, A., Mead, S., Hermann, P., Forlorni, G., & Appleby, B. S. (2024). Creutzfeldt–jakob disease and other prion diseases. Nature Reviews Disease Primers, 10 (1). https://doi.org/10.1038/s41572-024-00506-0