Aminoacylation of Disease-Related Mutants of Mitochondrial tRNA^{Leu}(UUR) and (CUN)

Wang En Duo

State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, the Chinese Academy of Sciences

The human mitochondrial LeuRS (hmLeuRS) with high activity was obtained from *E. coli* transformants. Aminoacylation of disease-related mutants of mitochondrial tRNA^{Leu}(UUR) and (CUN) was studied. Five single nucleotide substitutions within the human mitochondrial tRNA^{Leu}(UUR) gene were reported to be associated with MELAS. We provided *in vitro* evidence that the aminoacylation levels of these five tRNA^{Leu}(UUR) transcripts decreased differently. Especially, the T3291C mutant can inhibit aminoacylation of the wild type tRNA^{Leu}(UUR), indicating that it may play the role of an inhibitor in the mitochondrial heteroplasmic environment. Among the 22 human mitochondrial tRNAs, hmtRNA^{Leu}(CUN) corresponds to the most frequently used codon. Even a slight impairment of the function of hmtRNA^{Leu}(CUN) can lead to significant deficiencies in mitochondrial protein synthesis. Our *in vitro* studies reveal that the U48C substitution in hmtRNA^{Leu}(CUN), which corresponds to the pathological T12311C gene mutation, improved the aminoacylation efficiency of hmtRNA^{Leu}(CUN), the flexibility of hmtRNA^{Leu}(CUN) facilitates a T-stem slip resulting in 2 potential tertiary structures. The results indicated that the T-stem slip governs tRNA accepting activity. These results suggest a novel, self-regulation mechanism of tRNA structure and function.

A Novel DNA Phosphorothiolate Modification and its Biological & Biotechnological Implications

Deng Zi Xin

Laboratory of Microbial Metabolism and School of Life Science & Biotechnology, Shanghai Jiaotong University

Representative strains of *Streptomyces and E. coli* were discovered to incorporate sulphur into the DNA backbone as a sequence-selective, stereo-specific phosphorothioate modification[1,2]. The phosphorothioate was found to be of the R_p configuration and to occur with high frequency at G-A or G-G sequence motifs in *E. coli* and *S. lividans*, respectively[1]. Such modification was found to be widespread in *Bacillus, Salmonella, Klebsiella, Enterobacter, Mycobacterium, Vibrio, Pseudomonas, Pseudoalteromonas, Hahella, Oceanobacter, Geobacter, Pelagibacter, Roseobacter, Mesorhizobium, Serratia, Acinetobacter, Clostridium* etc., and in many unidentified marine microorganisms, and seemed to be closely associated with DNA degradation during electrophoresis and to be governed by a *dnd* locus involving a cluster of five genes[3,4]. The *dnd* counterpart gene clusters were all mapped to genomic islands in bacterial chromosomes and a plasmid in taxonomically unrelated bacterial species from various geographic niches[3,4]. Analysis by a combined genetic, biochemical and bio-informatic approaches enabled proposal of a hypothetic pathway leading to DNA phosphorothioation[3-5]. The physiological roles in possible relation to, e.g. DNA replication, transcription, translation, DNA damage/repair, cell defense, stress responses etc., and exact mechanism for the sulfur incorporation remained obscure, but the stimulating research directions of this fascinating new field with biological and biotechnological implications will be extensively discussed.

References:

1. L. Wang, S. Chen, T. Xu, K. Taghizadeh, J. S. Wishnok, X. Zhou, D. You, Z. Deng, Peter C. Dedon. Phosphorothioation of DNA in bacteria by dnd genes, Nature Chemical Biololgy， (2007). 3: 709-710.

2. J. Liang, Z. Wang, X. He, J. Li, X. Zhou, and Z. Deng. DNA modification by sulfur: analysis of the sequence recognition specificity surrounding the modification sites, Nucleic Acids Resrarch, (2007). 35:2944-2954.

3. X. Zhou, X. He, J. Liang., T. Xu, A. Li, T. Kieser, J. D. Helmann and Z. Deng. A novel DNA modification by sulphur, Molecular Microbiology, (2005). 57:1428-1438.

4. X. He, H.Y. Ou, Q. Yu, X. Zhou, J. Wu, J. Liang, W. Zhang, K. Rajakumar and Z. Deng. Analysis of a genomic island housing genes for DNA S-modification system in *Streptomyces lividans* 66 and its counterparts in other distantly related bacteria, Molecular Microbiology. (2007). 65: 1034-1048.

5. D. You, L. Wang, F. Yao, X. Zhou, and Z. Deng. A novel DNA modification by sulfur: DndA is a NifS-like cysteine desulfurase capable of assembling DndC as an iron-sulfur cluster protein in *Streptomyces lividans*, Biochemistry, (2007). 46:6126-6133.

Mutagenesis in Zebrafish by Tol2 Transposon-Mediated Gene Trapping Approach

Meng An Ming

State-Key Laboratory of Biomembrane and Membrane Bioengineering, Institute of Zoology, the Chinese Academy of Science; Protein Science Laboratory of Ministry of Education, Tsinghua University

Recently developed Tol2 transposon technology has been successfully applied to make transgenic lines, and to trap tissue-specific enhancers or developmentally regulated genes in zebrafish. We generated several Tol2-based gene trap vectors using zebrafish-derived splicing signals (SA), each of which contains a GFP reporter downstream of the SA. Following injection with vector DNA and transposase mRNA at the one-cell stage, adult fish (T_0) were mated to wildtype fish, and their progeny (T_1) with GFP expression were isolated and grown up. We found that the majority (>80%) of T_1 lines (and their offspring T_2) express GFP maternally and zygotically, suggesting that Tol2 transposon is preferentially inserted into maternally expressing loci. By intra-crossing T_2 fish, dozens of mutant lines have been identified and several mutant lines have been characterized in details. For example, the *T2EGE*$^{Z8/Z8}$ homozygous mutants show embryonic heartbeat failure due to fewer, shortened myofibrillar filaments in ventricular myocytes. We demonstrate that in this mutant both the hear-specific isoform and vasculature-specific isoform of tropomyosin4 are interrupted. Another example is *T2BGS*^{Z055} mutant line, in which *centromere protein H-like* (*cenphl*) locus is interrupted. The *T2BGS*^{Z055} zygotic mutant embryos appear to develop normally for the first day postfertilization and thereafter show excess cell death in the brain. Our experience tells that Tol2-based gene trap mutagenesis works in zebrafish albeit its efficiency is low.

Cellular Pattern of the Cyanobacteria: Formation and Application

Zhao Jin Dong

Institute of Hydrobiology, the Chinese Academy of Sciences

Cyanobacteria are a group of prokaryotes that carry out oxygenic photosynthesis. Some filamentous cyanobacteria can fix nitrogen in the presence of atmospheric oxygen. The site of the nitrogen fixation by these cyanobacteria is heterocyst, which is derived from a vegetative cell when combined nitrogen is lacking. The distribution of heterocysts on a filament is often regular or semi-regular so that there is a pattern of heterocysts. In recent years, we have been studying the ergulation of cell differentiation and the mechanism of the pattern formation. We found that HetR, the master switch of heterocyst differentiation, is a DNA-binding protein. Inhibition of its DNA-binding leads to an inability to form heterocysts. CcbP, a calcium binding protein from heterocystous cyanobacteria, negatively regulates heterocyst formation. Evidence shows that calcium plays an important role in heterocyst formation. PatS, which is critical to pattern formation, inbibits HetR from binding its target DNA. The interaction of HetR and PatS and their control of cellular pattern formation could be explained by Turing model. A hetR mutant was found to differentiate heterocyst in the presence of combined nitrogen and it raises a possibility to use this mutant to generate hydrogen using solar energy as the only energy supply.

From Molecular Mechanism of High K^+ Efficiency in *Arabidopsis* to Genetic Improvement of Crop K^+ Utilization Efficiency

Wu Wei Hua

State Key Laboratory of Plant Physiology and Biochemistry, China Agricultural University; National Plant Gene Research Centre

Potassium is an essential mineral element for plant growth and development. Normal crop growth and development require millimolar K^+ in the soil or growth medium. However, typical K^+ concentration at the interface of roots and soils is within micromolar range. Under K^+-deficient conditions, most crops show K^+-deficient symptom, typically leaf chlorosis and subsequent inhibition of plant growth and development. The K^+-deficiency has been one of limiting factors for crop production in China for decades.

Our previous study has revealed that a protein kinase AtCIPK23 significantly regulates K^+-uptake, particularly under low-K^+ conditions in *Arabidopsis*. Lesion of *LKS1* significantly reduced K^+-uptake and caused leaf chlorosis and growth inhibition, whereas overexpression of *LKS1* significantly enhanced K^+-uptake and low-K^+ tolerance. It was further demonstrated that CIPK23 positively regulates K^+ transporter AKT1 and two calcineurin B-like proteins CBL1 and CBL9 are upstream positive regulators of CIPK23. It was further hypothesized that this K^+-uptake regulatory pathway may also exist in other plants (crops). During the last two years, we have tried to improve K^+ utilization efficiency in several crop species by genetically modifying transcriptional expression of the components in this pathway. The preliminary results showed that overexpression of AtCIPK23 significantly increased K^+ accumulation and low-K^+ tolerance in transformed tobacco plants, and that overexpression of AtCIPK23 or OsCIPK23, AtCBL1/9 or OsCBL1/9 as well as AtAKT1 significantly increased low-K^+ tolerance in transformed rice plants. Similarly, overexpression of these genes in corn, Bt cotton and soybean also improved K^+-utilization efficiency in the transgenic plants. The detailed preliminary results and potential application of these regulatory components in improvement of crop K^+ utilization efficiency will be discussed.

Towards Molecular Design of Rice Plant Architecture

Li Jia Yang, Wang Yong Hong

Institute of Genetics and Developmental Biology, the Chinese Academy of Sciences

Rice is an ideal system for studying plant architecture of cereal crops. Rice plant architecture, a collection of the important agronomic traits that determine its grain production, is mainly affected by factors including tillering (tiller number and tiller angle), plant height, and panicle morphology. To elucidate molecular mechanisms that control rice plant architecture, we have identified several related mutants and isolated their corresponding genes. Among them, the *MONOCULM1* (*MOC1*) gene has been characterized as a master switch to initiate the formation of rice tiller meristems; the *DWARF27* (*D27*) gene controls rice tiller number by participating in the biosynthesis of strigolactones, a new class of plant hormones; the *LA1* gene plays an important role as a negative regulator of polar auxin transport (PAT) and loss-of-function of *LA1* enhances PAT greatly and thus alters the endogenous IAA distribution in shoots, leading to the reduced gravitropism and therefore the tiller-spreading phenotype of rice plants; the *SHORT PANICLE1* (*SP1*) gene, which encodes a putative PTR transporter, determines the panicle size by regulating the elongation of panicle branches. Our studies indicate that the endogenous expression levels of the above genes are correlated to plant architecture, implying their potential applications in molecular breeding. To further elucidate the regulatory mechanisms of these genes, we are now working on identification of interaction proteins of MOC1 and LA1 and on screening of suppressors of *d27* and *sp1*. Our studies will provide a molecular basis for developing elite rice varieties in the future.