学位論文の要約

題目　Chemical biology studies on the structures and biological functions of nucleic acids
（核酸の構造と生物活性についてのケミカルバイオロジー研究）

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序論

In this thesis, we mainly focus on the DNA G-quadruplex structures as well as RNA/DNA triplex formed by long non-coding RNA with duplex DNA and their regulatory functions in the process of transcription, replication, epigenetics and human diseases. Structural characterization in vitro was performed and the biological functions of these structures were also explored.

1. Photoreactivity of the linker region of two consecutive G-quadruplexes formed by human telomeric DNA

Photoreaction method has been developed by our lab to probe the local structure of DNA including A-form DNA, B-form DNA, Z-form DNA, G-quadruplex, and DNA-protein complex. The specificity and efficiency of photoreaction are mainly dependent on the structural features of DNA. In the current study, we report the application of a photoreaction method for probing the two consecutive G-quadruplexes formed by human telomeric DNA. Distinct photoreaction efficiencies were found between two consecutive G-quadruplexes formed by eight TTAGGG repeats in K^+ and Na^+ solutions, suggesting the different higher-order structures formed in K^+ and Na^+ solutions. This method can be applied on the detection of higher-order G-quadruplexes formed by long telomeric repeats.

2. Effect of ATRX and G-quadruplex formation by VNTR sequence on α-globin gene expressions

ATR-X syndrome (α-thalassemia/mental retardation syndrome X-linked) is caused by mutations in chromatin-remodeler ATRX. ATRX mutations and G-quadruplex formation by the VNTR sequence can be linked to α-thalassemia found in ATR-X patients. We investigated the G-quadruplex and i-motif formation of VNTR CGC(GGGGCGGGG)n. The promoter region without the VNTR sequence showed approximately two folds higher luciferase activity than the

promoter region harbouring VNTR sequence, suggesting the potential gene down-regulatory role by the formed G-quadruplex structures. G-quadruplex stabilizers hemin and TMPyP4 reduced the luciferase activity whereas expression of ATRX led to a recovery in reporter activity. Our results demonstrate that stable G-quadruplex formation by the VNTR sequence downregulates the expression of α-globin genes and that ATRX might bind to and resolve the G-quadruplex.

3. G-quadruplex formation in the promoter region of mouse imprinted gene *Xlr3b*

Another server feature of ATR-X syndrome is mental retardation. Mouse model of mutated ATRX have been constructed and the molecular mechanism of mental retardation in mouse have been investigated. Expression of imprinted gene *Xlr3b* has been identified in the mouse with abnormal spine morphology. We found that ATRX mutations are related to the loss of CpGs methylation in the promoter regions of *Xlr3b* gene but no other genes in *Xlr3* family. By comparing the promoter sequences of *Xlr3* gene family, G-quadruplex-forming sequence was found exclusively in the promoter of *Xlr3b* gene. It can form stable parallel G-quadruplex structure *in vitro* and stable G-quadruplex formation can downregulate the gene expression of *Xlr3b* gene. Therefore, we proposed that G-quadruplex-forming sequence and ATRX function are important in the repression of *Xlr3b* imprinted gene. The regulatory functions of G-quadruplex-forming sequence also suggested the probability of countering the abnormal expression of *Xlr3b* gene in ATR-X syndrome by G-quadruplex ligands.

4. RNA/DNA triplexes formation by *Xist* long non-coding RNA

The specific binding of ATRX to the repeat A region of Xist RNA provided an alternative molecular mechanism of mental retardation which is related to the abnormal expression of X-linked genes. *Xist* RNA plays important regulatory roles in the process of XCI (X chromosome inactivation). We proposed the triplex formation between *Xist* RNA and DNA duplex in the X chromosome. Preliminary experiments in vitro suggested that polyadenines sequence appears to form triplex with corresponding DNA. We also proposed that symmetric sequences are quite important I in the formation of triplex between lncRNAs and duplex DNA. In the following steps, we will focus on the repeat A region and investigate its binding affinity towards ATRX and triplex formation. The in vivo studies of triplex formation by mouse *Xist* RNA will also be performed. We anticipate that triplex formation may help explain the spreading, location and regulatory mechanism of *Xist* RNA during XCI.