論文の内容の要旨

応用生命工学専攻
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論文題目　Experimental analysis on the gene duplication in prokaryotic gene evolution
（原核生物の遺伝子進化における遺伝子倍化に関する実験的考察）

Gene duplication toward posttranscriptional advantage

Gene duplications occur as an evolutionary response in bacteria exposed to different selection pressures. Duplicated genes could persist through either the innovation of a novel beneficial function of one copy (neofunctionalization) or the accumulation of mutations reducing the functional capacity of individuals (subfunctionalization). However, gene duplications frequently encounter the reversible fate. One copy could be rapidly lost with the removal of the selective pressures. A variety of knowledge on gene duplications has been provided through significant in silico analysis, with which experimental approaches could lead to explanations on the important evolutional events.

Biological nitrogen fixation has evolved in the Early Archean world and the ability to fix atmospheric nitrogen is distributed across the bacterial and archaeal domains. Nitrogen fixation is conducted by a nitrogenase complex consisting of dinitrogenase and dinitrogenase reductase. Dinitrogenase is a heterotetramer of *nifD* and *nifK* gene products, while dinitrogenase reductase is a homodimer of the *nifH* gene product. The *nif* genes encoding the nitrogenase complex organize a conserved transcriptional unit, *nifHDK*. It is proposed that the distribution of the conserved

cluster among bacterial and archeal domains would be performed through horizontal gene transfer from the last common ancestor. Subsequent intragenomic evolutions under specific selective pressures could rearrange the conserved gene cluster.

Azorhizobium caulinodans is a nitrogen fixing *Rhizobium* that induces symbiotic nodules on a tropical legume *Sesbania rostrata*. The *nifH* gene was reiterated in the chromosome. One copy constitutes a conserved *nifHDK* transcriptional unit, while the other positions upstream of *nifQ* gene. The phylogenetic analysis provided that the gene duplication would have occurred in the common ancestor of some symbiotic *Rhizobia* including *A. caulinodans* and *Bradyrhizobium* species.

Our experimental approach was initiated from the comparative analysis on the duplicated *nifH* genes. The analysis using *nifH* in-frame disruptants resulted in the possibility of the functional differentiation between the *nifH1* and *nifH2*, which could suggest the persistence of the duplication (T. Iki et al., 2007a). However, the approach included the unexpected transcriptional activation on *nifA* gene encoding the transcriptional activator NifA, which might have disturbed the accurate comparison. Further analysis using *nifH* deletion mutants obtained the conclusion that the *nifH2* gene, the additional copy, functioned primarily on the nitrogen fixation.

The parental *nifH1* gene showed higher activities than the daughterly *nifH2* copy in both the transcriptional and translational analysis using *lacZ* gene. Furthermore, the point mutation approach focusing on the 3' terminus nonsynonymous substitution showed that the NifH1 protein functions more efficiently than the NifH2 protein under the same expression level. These results contradicted the significance of the *nifH2* gene on the nitrogen fixation. The contradiction motivated us to focus on the posttranscriptional regulation of the *nif* gene expression. Northern blot analysis showed that the monocistronic mRNA of *nifH2* was significantly accumulated, while that of *nifH1* was not, with the alternative accumulation the bicistronic mRNA of *nifH1D*. The significant accumulation of the monocistronic *nifH2* mRNA was consistent with the severe decrease of nitrogen fixation activity caused by the deletion of *nifH2* gene. It was supposed how the *nifH2* gene could accumulate the monocistron in spite of the rather weak transcriptional activity compared with the *nifH1* gene.

Bacterial polycistronic genomes contain a number of small extragenic palindromic sequences that may affect the expression of flanking genes. It is reported that the stem loops formed by the palindromic sequences stabilize the proximal mRNA against the 3' to 5' exonucleolytic degradation, while affect the distal gene expression either by the transcriptional termination or through the posttranscriptional processing on mRNA. The *nifH1DK* and *nifH2Q* polycistrons of *A. caulinodans* also contain small extragenic palindromic sequences, putatively form stem loops in mRNA, but there is no experimental evidence showing the actual effect on gene expression.

It was shown that the palindromic sequence between *nifH1* and *nifD* attenuated the

expression of distal genes as well as accelerated that of proximal genes. In consideration with the insufficient accumulation of monocistronic *nifH1* mRNA, it was supposed that the dilemmatic functions of the palindromic sequence might be somehow inactivated within the native gene cluster in order to prevent the hazardous attenuation on the expression of *nifDK* genes on nitrogen fixation. In the subsequent analysis, it was found that the cloning of the palindromic sequence with the 5' coding sequence of the *nifD* gene suppressed the function of the palindromic sequence. This result supports the inactivation of the potentially active palindromic sequence within the *nifHDK* mRNA.

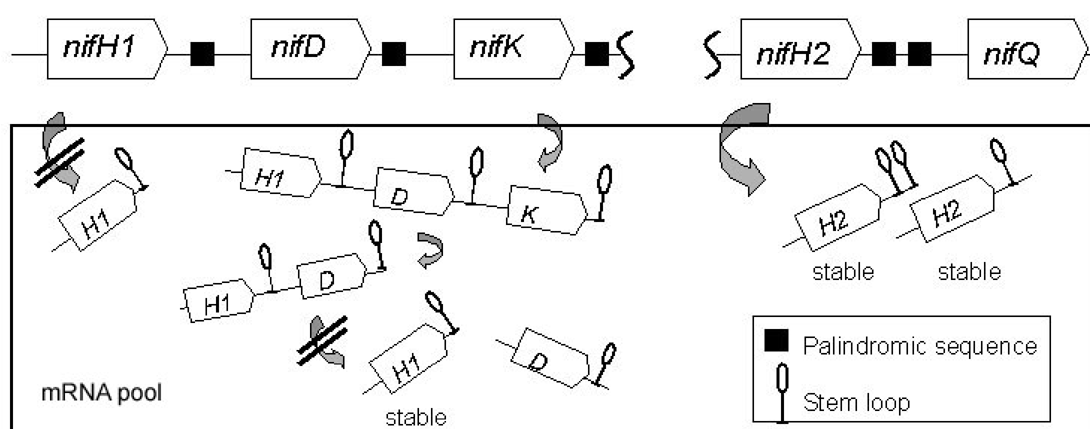

Fig. 1. The schematic mRNA synthesis during the nitrogen fixation by *A. caulinodans.*

The duplication of *nifH* gene would have solved the dilemma, enabling the additional *nifH2* gene to dedicate itself into the synthesis of the stable monocistronic *nifH* mRNA, which is stable and thus required for the efficient nitrogen fixation (Fig. 1). It was concluded that the gene duplication of *nifH* would be advantageous for *A. caulinodans* in the posttranscriptional level of the gene expression (T. Iki et al., 2007b, submitted).

B. japonicum have lost the parental copy which once constituted the conserved *nifHDK* cluster. The primal function of the *nifH2* gene might confer a fatal gene loss of *nifH1* and the consequent translocation of the *nifH* gene in *A. caulinodans*. In this sense, the putative gene translocation in a group of symbiotic *Rhizobia* would not the result of the reversible gene duplication in the transient selective pressures, but the progressive fate under the constant selective pressures purifying more efficient nitrogen fixers.

Characterization of a conserved GTPase, HflX, in bacterial mRNA decay

Through the experimental analysis on the prokaryotic gene duplication, it was recognized that the second structure of mRNA affected the gene expression considerably enough to confer impact on the gene evolution. The mRNA decay has been significantly studied in other bacteria such as *Escherichia coli*, while the current knowledge on the mRNA decay is not sufficient. One of the most important trans acting factors involving to the mRNA decay is the RNA chaperone, Hfq. The pleiotropic functions of the hexameric protein complex have been well documented. On the other hand, there is no report about *hflX* gene, consisting the conserved gene cluster with *hfq* in the prokaryotic genomes. Interestingly, the *hflX* gene is conserved in the eukaryotic and archaeal genomes (Fig. 2). In *A. caulinodans*, the *hfq-hflX* gene cluster positions adjacent to *ntr* genes which involved in the nitrogenous signal transduction, and it has been reported that the *hfq* is required for the expression of *nifA* in the posttranscriptional level. It was assumed that the *hflX* gene might play some significant role on the *nif* gene expression in *A. caulinodans*.

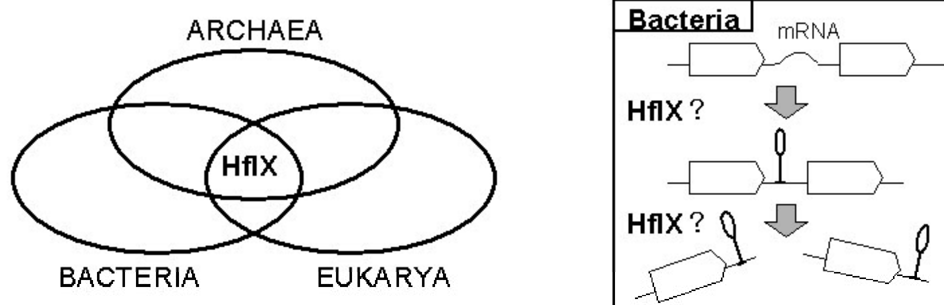

Fig. 2. The distribution of HflX (left), and the possible function in bacteria (right) was shown.

We challenged the venturous study to obtain several stimulating results indicating the involvement of HflX in the mRNA decay. The *hflX* mutant of *A. caulinodans* showed higher transcriptional activity of *nifA*, which was absent in the *hfq* mutant. Furthermore, it was found that the *hflX* mutant inhibited the stabilization of proximal mRNA by the extragenic palindromic sequence between *nifH1* and *nifD* genes (T. Iki et al., preparing). Considering the distribution of *hflX* gene orthologs within the three kingdoms, it is possible that HflX might function in a variety of RNA maturations on both the prokaryotic and eukaryotic cells (Fig. 2). Our study could lead to the first detailed characterization of the highly conserved GTPase, HflX.

References

Iki, T., Aono, T., Oyaizu, H. (2007a) *FEMS Microbiol Lett* **274**: 173-179

Iki, T., Aono, T., Oyaizu, H. (2007b) *FEMS Microbiol Lett* (submitted)