論文内容の要旨

論文題目

Prediction of *miRNA* Gene Promoters and Gene Regulation Involving miR-21.

(*miRNA* 遺伝子のプロモーター予測及び miR-21 に関する遺伝子制御の解析)

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Tissue-specific gene expression in multi-cellular organisms has been previously considered to be principally regulated at the transcriptional level, but it is now known that post-transcriptional regulation also plays key roles in this process. Phylogenetically conserved 20-24 nucleotide RNAs, designated microRNAs (miRNAs) mediate the repression of target mRNAs by suppressing translation or promoting mRNA decay in animal. More than 500 species of miRNAs have now been identified in human, and have now been also predicted to target roughly 30% of the total coding genes in human. Hence, just as transcription factors, *miRNA* genes modulate global patterns of gene expression during differentiation, metabolic activation, stimulus response and also carcinogenesis. Consistent with this, miRNAs exhibit unique expression patterns, and participate in various developmental stages in vertebrates. Moreover, some miRNAs are also thought to be oncogenic or anti-oncogenic due to the observation that they are abundant or scarce, respectively in certain human tumors. However, little is currently known how the *miRNA* gene expression itself is regulated owing to lack of basic information of their gene structure. Global prediction of promoter regions of *miRNA* genes would allow us to explore the mechanisms underlying gene-regulatory

mechanisms involving these miRNAs. To find out evolutionarily conserved regulatory mechanisms that involve vertebrate miRNAs, in this study, a computational approach was first performed to the prediction of promoters of such miRNAs by the use of comparative genomic methods, followed by scrutinizing a specific conserved miRNA system found in the search.

It is speculated that if specific miRNA molecules are involved in evolutionarily conserved regulatory systems in vertebrates, this would entail a high level of conservation of the promoter of *miRNA* gene as well as the miRNA molecule. By our current screening of putative promoter regions of *miRNA* genes (miPPRs) on this base, I identified 59 miPPRs that would direct production of 79 miRNAs (Figure 1). It is found that the position of human miPPRs relative to the corresponding miRNAs are significantly shorter when compared with the 20 sets of background observation miPPRs. Moreover, the miPPRs contains relatively higher CpG dinucleotides than that of the background. These results support that the miPPRs are rich in functional promoter sequences. I have also experimentally assessed the prediction of miPPRs, and show that miR-1-2, miR-133a-1, miR-199a-2 and miR-21 are transcribed from the corresponding miPPRs. The miPPRs for miR-146a and miR-126 are also consistent with previous reports.

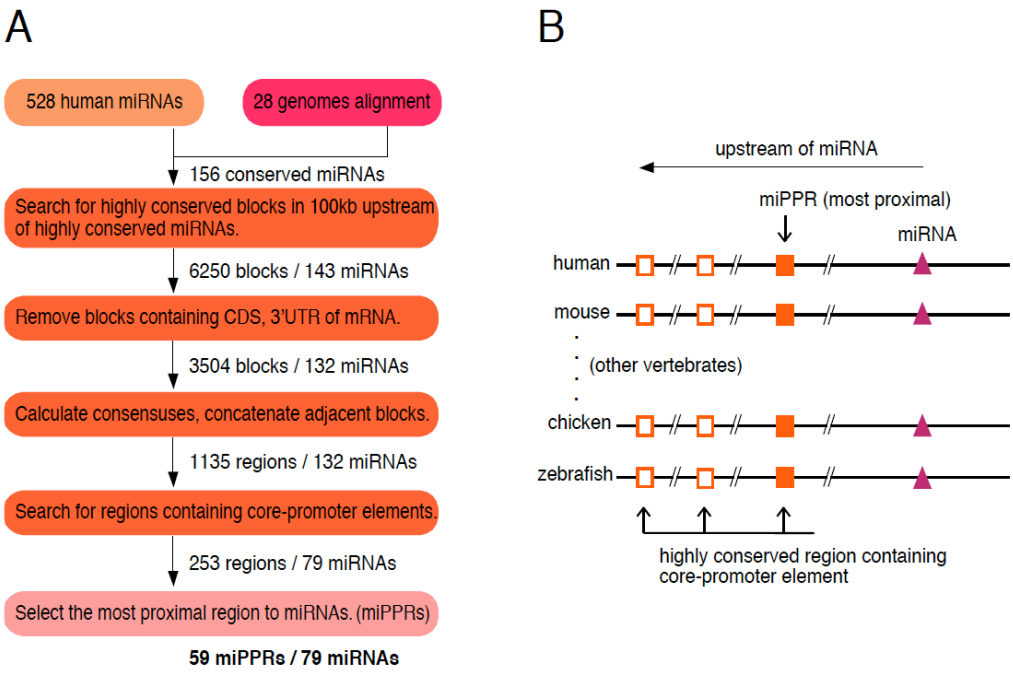

Figure 1. Scheme of the computational prediction of miRNA promoters.

I have next scrutinize the miR-21 whose promoter was predicted here. miR-21 has been reported to be highly expressed in various cancers and also to be inducible in a human promyelocytic cell line, HL-60, after PMA treatment. To examine molecular mechanisms involved in miR-21 expression, the structure of the *miR-21* gene was analyzed by determining its promoter and primary transcripts by primer extension and northern analysis, respectively. While a promoter of *miR-21* gene was previously reported, I found that it is incorrect and further showed that miPPR-21 (putative promoter of miR-21) is an authentic promoter. A single precursor RNA containing miR-21 was transcribed just downstream from the TATA box in this promoter, which is located in an intron of a coding gene *TMEM49* in the same direction. Importantly, transcription of *TMEM49* is completely PMA-independent and all its transcripts are polyadenylated before reaching the miR-21 hairpin embedding region, indicating that *miRNAs* could have their own promoter even if overlapped with other genes. The mutated reporter analysis of miPPR-21 and the reporter analysis with expression vector containing AP-1 family genes show that miPPR-21 is activated by AP-1 dependent manner. Moreover, performing gel shift assay and ChIP analysis, I show that AP-1 binds the conserved sites in the promoter identified here, and then activates the *miR-21* transcription in conjugation with SWI/SNF complex, after PMA stimulation. The previous findings of enhanced miR-21 expression in several cancers may therefore reflect the elevated AP-1 activity in these carcinomas.

I also found a conserved binding site for NFIB in miPPR-21. The reporter analysis further shows that miPPR-21 activation by PMA was canceled out by NFIB, and the ChIP experiment shows that NFIB protein usually binds the *miR-21* promoter in HL-60 cells as a negative regulator and is swept off from the *miR-21* promoter during PMA-induced macrophage differentiation of HL-60. Importantly, by available algorithms predict that the *NFIB* mRNA is a target of miR-21. The 2'OMe-RNA mediated inhibition of miR-21 enhanced the protein level of NFIB, and the mutation at miR-21 recognition site in *NFIB* 3'UTR reporter rescued the repression of its luciferase activity. Since exogenous miR-21 expression moderately induced endogenous miR-21, an evolutionarily conserved double negative feedback regulation would be operating as a mechanism to sustain miR-21 expression (Figure 2). I also found that *NFIB* gene was also suppressed at transcriptional level after PMA stimulation in HL-60 cells, and also that inhibition of miR-21 only enhanced the protein level, suggesting that the translational repression of *NFIB* mRNA by miR-21 accelerates clearance of NFIB in parallel with the simultaneous miR-21-independent transcriptional repression of *NFIB* after PMA stimulation. This suggests that the coherent feed forward loop including miRNA may be implemented for not only fail-safe mechanism but for rapid shut-off of target genes.

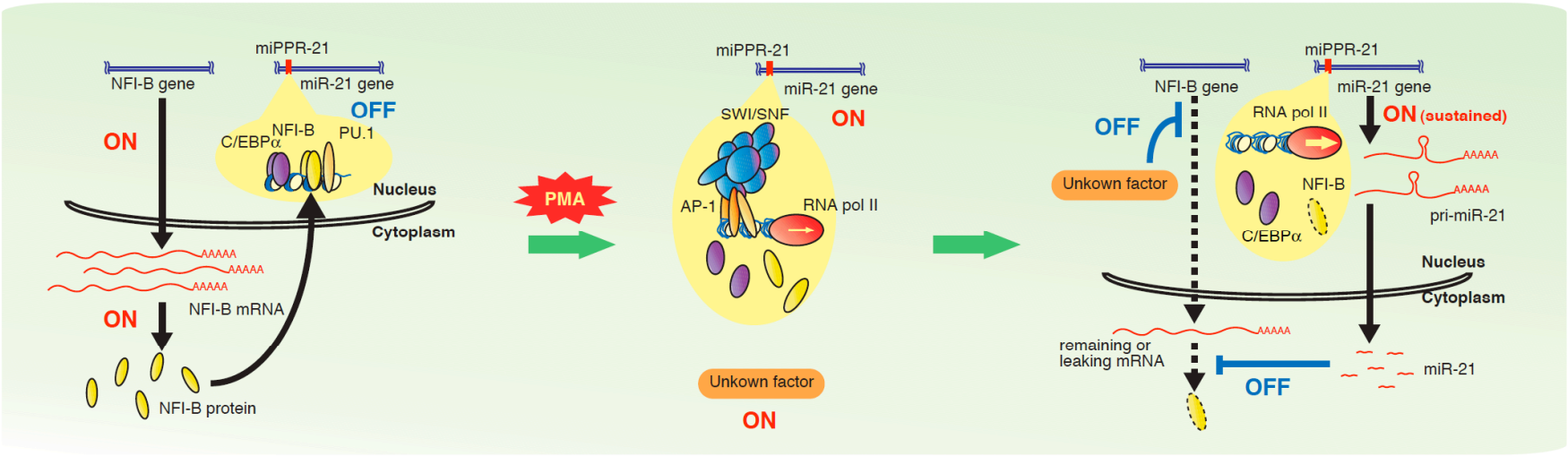

Figure 2. Scheme of the double-negative feedback regulation of *miR-21* gene