

## Involvement of U13 small nucleolar RNA in cytidine modification of 18S rRNA.

Y. Kido and F. Harada

Since 1990's, many small nucleolar RNAs (snoRNAs) are shown to be involved in 2'-O-methylation or pseudouridylation of nucleosides on rRNAs. U13 snoRNA was first identified in 1986 as a trimethylguanosine-capped, box C/D class snoRNA isolated from HeLa cell. According to the complementarity to 3'-end of 18S rRNA, U13 RNA has been hypothesized to be involved in maturation of 3'-end region of 18S rRNA. Cavaille et al. (1996) discussed the involvement of U13 snoRNA in cleavage of pre-rRNA at the 18S rRNA / ITS1 junction. There is, however, no direct evidence supporting the suggested function of U13 snoRNA so far.

In order to elucidate the function of U13 snoRNA we have established U13-knockout DT40 cell lines and have compared all the nucleoside (and its modification) of the 3'-end region of 18S rRNA between wild-type and knockout DT40 cells by 2-dimensional TLC. In all independent U13-knockout cell lines examined so far a modified cytidine (N4-acetylcytidine) on position 1796, which detected in wild-type cell, was not detected. By transfection of U13 RNA gene into a knockout strain, we have detected expression of U13 snoRNA at the same level as that of wild-type. In all independent transfectants examined so far, cytidine acetylation at the position 1796 was restored. These results strongly suggest that U13 snoRNA is involved in the modification of C1796 of 18S rRNA.

U13 snoRNA has a complementarity to the 3'-end of 18S rRNA (see Figure). To test the importance of the complementarity in acetylation activity, we are introducing several nucleotide substitutions on the complementary region of U13 snoRNA gene and are analyzing the modification status of 18S rRNA isolated from the individual transfectants of the mutant U13 snoRNA genes.

To study acetylation of the 3'-end of 18S rRNA biochemically, *in vitro* system of cytidine acetylation in a cell extract is required. So far, however, we have not been able to detect rRNA acetylation *in vitro*.

