Mutational analyses of U13 small nucleolar RNA : a guide RNA involved in the acetylation of 18S ribosomal RNA.

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U13 small nucleolar RNA (U13 snoRNA) is essential for 4-acetylcytidine modification in the 3' -end region of 18S ribosomal RNA (Fig. 1). According to oligo RNA-ligation and RT-PCR on U13 snoRNA in human, mouse and chicken cells, we demonstrated that there are only two nucleotides between box D and the 3' -end of U13 snoRNA. This suggests that the terminal stem of the k-turn structure in U13 snoRNA is truncated (Fig. 2). The k-turn in snoRNAs is known as the target motif for binding of 15.5kd protein, one of the snoRNA core proteins. A recombinant 15.5kd protein bound to the truncated k-turn *in vitro* and its binding was disrupted when the box D sequence was mutated. A single base substitution in box D also decreased the expression of U13 snoRNA *in vivo*. These observation s suggest that the box C/D sequence of U13 snoRNA, a truncated k-turn, make a functional binding site for core proteins and is important for the assembly of U13 snoRNP complex.

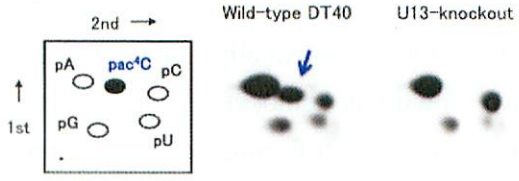

Figure 1

U13 RNA has two regions complementary to 3' -end of 18S rRNA. To identify sequences essential for recognition of the acetylation-target site, we introduced base-substitution mutations in the rRNA-complementary region on U13 RNA gene. There are some primary sequences essential for expression of the RNA, however, we could not find any mutation that lost target-recognition. These observation suggest that U13 snoRNA recognizes the target site not by a short and rigid complementarity but by a long and loose complementarity.

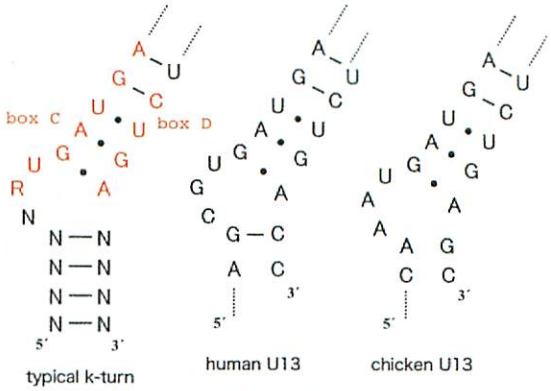

Figure 2

Expression of human or mouse U13 snoRNA in a U13-knockout DT40 cell partially recover the acetylation activity. This implies that the protein machineries recognize common structures on these U13 RNAs and "acetylation-guidance" by U13 snoRNA is conserved, therefore, important function in higher eukaryotes.