he nucleotide sequence and the gene structure of chicken U17 small nucleolar RNA (snoRNA)

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Two major classes of small nucleolar RNAs (snoRNAs), involved in pre-rRNA processing and modification, have recently been characterized. Members of one group show short conserved sequence motifs, denominated box H (5'-ANANNA-3') and box ACA. Box H/ACA snoRNAs generally function as guide RNAs in site-specific pseudouridylation of pre-rRNA. U17 snoRNA is one of the most abundant H/ACA snoRNAs in the animal cells. However, in spite of the presence of H and ACA boxes, its length is larger than the typical one of H/ACA snoRNAs and it shows a more complex secondary structure. Moreover, its secondary structure does not show any evident pseudouridylation pocket. From these characteristic features, U17 snoRNA may play new role in rRNA biogenesis. To clarify the function of U17 snoRNA, we tried to disrupt the U17 snoRNA gene in a chicken B cell line DT40 by targeted integration.

First of all, we purified U17 snoRNA from total RNA of DT40 cells by the using of gel filtration and polyacrylamide gel electrophoresis. The nucleotide sequence of purified RNA was determined by direct sequencing method using various RNases. The 5'- and the 3'- terminal sequences were determined by oligo-RNA ligation method. Chicken U17 snoRNA consists of 206 nucleotides and 73% identity with human U17 snoRNA.

Then we cloned the U17 snoRNA gene locus using U17 RNA sequence as a probe and sequenced around the RNA gene. U17 snoRNA has been found to be encoded in two (the 1st and the 2nd) introns of the human gene for RCC1 and in each of the six introns of the Xenopus and Fugu genes for ribosomal protein S7 (rpS7). Like human case, chicken U17 snoRNA was encoded in the RCC1 gene, although only one copy was found in the 2nd intron. However, U17 snoRNA coding sequence was not observed in the introns of the chicken rpS7 gene.

To disrupt the U17 snoRNA gene in DT40 cells, the targeting vectors containing blasticidin and puromycin resistance genes were constructed and then transfected to DT40 cells. Although introduction of blasticidin containing vector gave U17 snoRNA gene +/- clones, U17 snoRNA gene -/- clone was never obtained by successive transfection of puromycin containing vector. Probably U17 snoRNA is essential for maintenance of cells. Further experiments using conditional expression mutant cells for U17 snoRNA might be necessary for determination of the U17 snoRNA function in the cells.