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5'-Terminal Sequence of the mRNA of Mouse Whey Acidic Protein Contains Three Possible Sites of Interaction with 18S rRNA

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SUMMARY - The mRNA sequence of whey acidic protein, a major mouse milk protein, was cloned in full length. The sequence analysis of the cDNA clones revealed that the mRNA contains a 28 nucleotide-long 5'-noncoding region. Three different portions of this region were identified as possible sites of interaction with the 3'-end of 18S rRNA that may facilitate efficient rate of translation of the mRNA.

The major whey protein termed whey acidic protein (WAP) is unique to rodent milks, comprising at least 2.4% of total milk protein and having a molecular weight of 14,000 daltons (1). WAP, like other milk proteins caseins and α-lactalbumin, has been shown to be synthesized in the mammary gland of lactating mouse (1). Several lines of evidence indicate that the regulation of WAP gene expression is different from that of caseins that constitute more than 60% of total milk protein. During the development of the mouse mammary gland that occurs during pregnancy, the mRNA sequence of WAP begins to accumulate only during the second half of pregnancy, whereas the casein mRNA sequences accumulate progressively throughout the period of pregnancy (2). Studies of the mouse mammary gland in organ culture have shown that the combination of insulin, cortisol and prolactin stimulates the synthesis of both WAP and caseins to a level comparable to that of lactating gland, i.e. 2.4% and 55% of total protein, respectively (2). Under the same conditions, however, the amount of WAP mRNA relative to total mRNA is less than 0.1%, whereas the relative amount

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of casein mRNA is about 50%. These findings raised the possibility that the WAP mRNA is translated more efficiently in mammary cells.

To gain some insight into this problem, it was of interest to examine the WAP mRNA structure and its regulatory properties. Although cDNA cloning of the WAP mRNA sequence has been reported for the mouse (3) and the rat (4,5), none of these studies has given the information on the entire 5'-noncoding region that is essential for understanding the regulatory properties of the WAP mRNA. Furthermore, previous studies employed S1 nuclease treatment for the construction of cDNA; this causes loss of 5' sequences and often sequence rearrangement (6-11). We here describe the structure of 5'-noncoding region of the WAP mRNA obtained by sequence analysis of the cDNA clones constructed without S1 nuclease treatment using the method of Land et al. (12). The data show that the 5'-terminal sequence of the WAP mRNA contains three possible sites for interaction with the 3'-end of 18S rRNA.

MATERIALS AND METHODS - [γ-^{32}P]ATP and [α-^{32}P]dCTP were obtained from ICN. Restriction enzymes, nick translation kit were from BRL, and polynucleotide kinase from P-L Biochemicals, Inc.

The plasmid pMG800 containing cDNA insert for mouse WAP mRNA was isolated from the cDNA library of the lactating mouse mammary gland using the ^{32}P-labeled purified mRNA. This was confirmed by the hybrid-selected translation followed by immunoprecipitation using a specific antibody against mouse WAP which was kindly provided by Dr. Piletz (1). Two other plasmids, pMG828 and pMG834 that contained more than 600 base pair inserts, were isolated from the bank using the insert of pMG800 ^{32}P-labeled by nick translation as a specific probe.

Recombinant plasmid DNA was prepared according to the method described (13). The Pst I cDNA inserts isolated by preparative agarose gel electrophoresis were labeled at the 5'-ends with [γ-^{32}P]ATP and polynucleotide kinase using the procedure of Maxam and Gilbert (14). After digestion with Bam Hl, the large fragments were isolated and sequenced using the standard protocols of Maxam and Gilbert (14).

RESULTS AND DISCUSSION - Seven positive cDNA clones for WAP mRNA were obtained by screening about 200 clones in the cDNA library by hybridization with the ^{32}P-labeled insert of pMG800. After size analysis of the inserts, pMG828 and pMG834 were identified as the clones containing more than 600 base pair inserts. The restriction maps of these two clones and pMG800 are shown in Fig. 1.

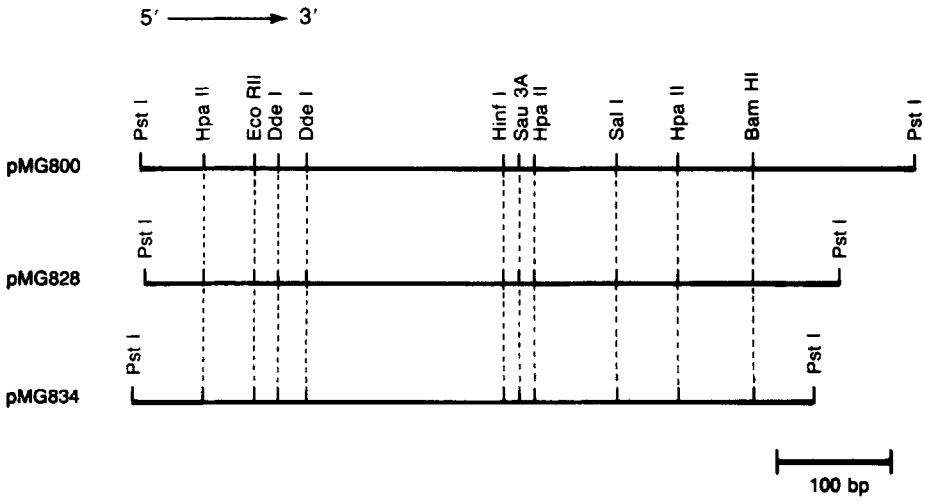

Fig. 1 Restriction maps of the cDNA inserts of pMG800, pMG828 and pMG834. The Pst I sites at both ends were generated during cloning of the double stranded cDNA. The GC-tails are present at both ends.

The three restriction maps of the cDNA inserts overlapped in the approximately 500 base pair-long central region of the inserts and coincided with the map reported by Henninghausen and Sippel (3). In both boundary regions to the Pst I sites, however, there was some heterogeneity in the length of the Hpa II-Pst I fragments at the 5'-end, and of the Bam Hl-Pst I fragments at the 3'-end. It appeared likely that some of those clones contained the entire 5'-end sequence of the mRNA because all three clones possessed much longer 5'-sequence than pWAP1 (3).

These three plasmids were sequenced in the region of the 5'-end of the mRNA. The results are summarized in Table 1. The three inserts actually

Table 1. Summary of DNA sequences corresponding to 5'-end of WAP mRNA.

Plasmid	Length of the insert (base pair)	Length of the GC-tail at the 5'-end (base pair)	The sequence of cDNA corresponding to the 5'-end of WAP mRNA
pMG800	600	35	tail G/ATCAGTCACTGTACCTAACA
pMG610	610	30	tail G/ATCAGTCACTGTACCTAACA
pMG834	600	40	tail G/ATCAGTCACTGTACCTAACA
pWAP1*	600	not known	tail G/·····CCTGACA

*From reference 5.

contained longer cDNA sequence corresponding to the 5'-end of the mRNA than pWAP1. Although the length of their GC tails was variable, the sequence of cDNA corresponding to the 5'-end of the WAP mRNA was identical.

The present method used for cloning cDNA of the WAP mRNA has been previously shown to give a clone that contains the complete 5'-end without the ^{7m}G cap structure of mRNA sequence. This was shown in studies of chicken lysozyme mRNA (12) and human skeletal muscle α-actin mRNA (16). The present finding that all three clones have identical limits on their 5'-end suggests that they are complete in this region. Although a primer extension method is often used to determine the 5'-end of mRNA, its principle is essentially the same as that of double stranded cDNA synthesis used here. A primer extension experiment, however, may uncover the existence of a minor cap site.

Based on the cDNA sequence of the 5'-noncoding region described here together with the study of the coding and the 3'-noncoding regions of the WAP mRNA (3), we estimate that the mRNA was 561 nucleotides in length plus a poly A tail with an average length of 60 nucleotides. It contained the 5'-noncoding region of 28 nucleotides and the 3'-noncoding region of 131 nucleotides.

Fig 2B illustrates the 5'-noncoding region of the WAP mRNA that shows homology with 18S rRNA. Actually there are three regions in the WAP mRNA that can interact with the 3'-end of conserved non-contiguous sequence in the 18S rRNA, 3'·····UAGGUGG·····5' (see Fig. 2A). We could not identify any sequences that had significant complementarity to a highly conserved contiguous sequence at the 3'-end of eukaryotic 18S rRNA, 3'·····UAGGAAGG-AGU·····5' (17). According to the model recently proposed by Sargan et al. (18), interaction of these sequences with 18S rRNA may provide a means for effecting a high rate of translation of mRNA. Thus, the present findings are in accord with the efficient synthesis of WAP in the mammary gland (2), suggesting that the structure of the 5'-end of mRNA is an important determinant of the rate of protein synthesis.

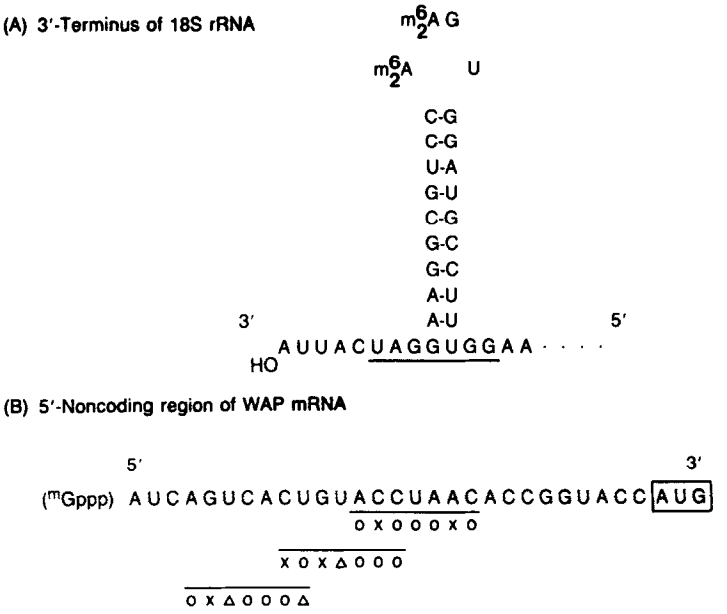

Fig. 2 The sequence of the highly conserved 3'-terminal loop of 18S rRNA (A) and the sequence of the 5'-noncoding region of the WAP mRNA (B). The solid line beneath the 18S rRNA shows the conserved non-contiguous sequence required for interaction with the 5'-end of mRNA, according to the model of Sargan et al. (18). The solid lines beneath the WAP mRNA show the possible three sequences that can interact with the 18S rRNA sequence above, matching at 5 or more of the 7 bases. (o) represents perfect match, (Δ); G-U pairing, (x); non-pairing. A putative cap structure of the WAP mRNA is shown in parentheses.

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