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Stability of casein mRNA is ensured by structural interactions between the 3′-untranslated region and poly(A) tail via the HuR and poly(A)-binding protein complex

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Abstract

The maintenance of mRNA stability has emerged as a mechanism of post-transcriptional control. We demonstrate that β-casein mRNA stability is dictated by the poly(A) tail and sequences in the 3′-UTR. An in vitro mRNA decay assay revealed that β-casein mRNA with a long poly(A) tail had higher stability than that with a short poly(A) tail. The addition of poly(A) homopolymer and 3′-UTR cRNA as competitor induced rapid degradation of β-casein mRNA. The interaction between full-length β-casein mRNA and poly(A) homopolymer was inhibited by the addition of the 3′-UTR cRNA in the lysates, which indicates that one region of the 3′-UTR associates with the poly(A) tail through an RNA–protein interaction. The putative AU-rich element (ARE) is present at nt 897–905; deletion and mutation analysis showed that the ARE site was required for maintaining the stability of the β-casein 3′-UTR. In the immunoprecipitation analysis, the poly(A)-binding protein (PABP) and the RNA-binding protein HuR were pulled down by 3′-UTR cRNA, and the absence of the ARE site reduced the binding of these proteins. These experiments further revealed that the rapid degradation of β-casein mRNA was induced by incubation with HuR- and PABP-depleted RRLs. Collectively, our results suggest that β-casein mRNA is protected from degradation by virtue of the structural interaction between the 3′-UTR and poly(A) tail via a protein complex of HuR and PABP.

Keywords: Stability; Casein; 3′UTR; Poly(A) tail; PABP; HuR

1. Introduction

The mammary gland is unique in that its tissues exhibit distinct phases of cellular proliferation during early pregnancy, differentiation during late pregnancy, and milk synthesis throughout lactation. The lactating mammary gland secretes milk proteins in response to stimulation by various peptides and steroid hormones, such as insulin, glucocorticoids, and/or prolactin [1–3]. Since the lactating mammary gland synthesizes larger quantities of proteins than any other organ, it needs to switch on and off the expression of mRNAs for milk proteins in response to changing environmental or developmental cues. The steady-state levels of mRNAs depend upon their combined rates of transcription in the nucleus and post-transcriptional processing, which includes mRNA stability in the cytoplasm. Following the addition of prolactin, the β-casein mRNA level is elevated over 70-fold, while the transcription rate is raised only 2- to 4-fold [4], which suggests that β-casein mRNA stability is increased significantly upon the onset of milk synthesis. The poly(A) tail of β-casein mRNA is elongated just before parturition, and an mRNA transcript with a longer poly(A) tail has a longer half-life [5]. Moreover, the length of the poly (A) tail parallels the expression of poly(A) polymerase (PAP) during stimulation with insulin and prolactin [6,7]. Prolactin regulates mammary gland function by binding to its receptor, which is present on the mammary cell membrane. Upon the termination of milk synthesis, the number of mammary prolactin receptors decreases to the minimum level [8]. These observations reveal that the poly(A) tail elongated by stimulation acts as one of the important structures for β-casein mRNA stability.

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Table 1
Oligonucleotide primers used for PCR reactions

Name	Sequence of forward and reverse primer	Product (b)
β-casein		
Full-S	5′–[T7]–ATCATCCTTTCAGCTTCACC–3′	1120
	5′–ATATGCCTAAAGGATTATTT–3′	
CR-S	5′–[T7]–GTCCCACAAAACATCCAGCC–3′	334
	5′–GTGCTACTTGCTGCAGAAAG–3′	
CR-A	5′–[T7]–GTCCCACAAAACATCCAGCC–3′	
	5′–ATATGCCTAAAGGATTATTT–3′	
UTR-S	5′–[T7]–TTGAACTGACTGAAACTGGA–3′	333
	5′–ATATGCCTAAAGGATTATTT–3′	
UTR-A	5′–TTGAACTGACTGAAACTGGA–3′	
	5′–[T7]–ATATGCCTAAAGGATTATTT–3′	
G3PDH	5′–TGAAGGTCGGTGTGAACGGATTTGGC–3′	982
	5′–[T7]–CATGTAGGCCATGAGGTCCACCAC–3′	

The stabilization of mRNA has emerged as a key step in the regulation of mammalian gene expression. Mammalian mRNAs have an m7G cap at the 5′-terminus and a poly(A) tail at the 3′-end. These two elements, along with the cap-binding proteins and poly(A)-binding protein (PABP), provide a basal level of mRNA stability by preventing ribonucleolytic degradation [9–11]. In addition to the provision by the mRNA structural components of a basal level of mRNA stability, elements inherent to the mRNA are also important, since each mRNA has an intrinsic half-life, ranging from several minutes to several days, which contributes to its expression level. The differential mRNA stabilities are determined by interactions between cis-acting sequences that lie in the 3′-UTR and the trans-acting proteins that bind to them [12,13]. To date, the cis and trans activities of the β-casein 3′-UTR have not been characterised extensively.

AU-rich elements (AREs), which were originally identified in the 3′-UTRs of several cytokine genes, function as potent destabilizing elements that cause rapid degradation of the respective mRNAs. Some classes of AREs have slightly different sequence determinants, almost all of which include the core pentamer AUUUA [14,15]. The β-casein mRNA contains a putative ARE with one AUUUA in the 3′-UTR. ARE-mediated mRNA turnover is influenced by many exogenous factors, which is consistent with the possibilities that ARE plays a critical role in the regulation of gene expression during cell proliferation and differentiation [16–20]. Numerous proteins have been identified as trans-acting factors that bind ARE and/or affect the stability of ARE-containing transcripts. These include AUF1, TTP, KSRP, BRF1, and HuR [21–25]. However, the functional and physiological significance of these RNA–protein interactions and their exact roles in ARE-directed mRNA degradation remain enigmatic.

HuR, which is a ubiquitously expressed member of the Hu/ELAV family, is involved in the stabilization of several ARE-containing mRNAs [26–28]. Stabilization by HuR is thought to be due to competition with AUF1 and other degradation-promoting proteins for binding to the same ARE on target mRNAs [20,21,29]. A recent study indicates that HuR and AUF1 can bind jointly to common target mRNAs [30]. Furthermore, other investigations have suggested that AREs consist of functionally distinct domains, which may be targeted by different populations of ARE-binding proteins [31,32]. It has also been reported that the trans-acting factor α-CP, which is a protein binding to a C-rich element (CRE) in the α-globin 3′-UTR, acts together with PABP to prevent ribonucleolytic degradation [33–35]. Taken together, these observations lead us to postulate that HuR binding to ARE and PABP, along with the poly(A) tail, play an important role in maintaining high-level mRNA stability.

The specific aims of the present investigation were to define the molecular mechanisms that determine the fate of β-casein mRNA, and to asses the potential roles in mRNA stability of the long poly(A) tail and 3′-UTR including the putative ARE, as well as the structural interactions between these elements. First, we investigated the influences on β-casein mRNA stability of the addition of poly(A) homopolymer and 3′-UTR complementary RNA (cRNA) in the in vitro RNA decay assay. Second, we confirmed the function of ARE or subdomain thereof in the β-casein 3′-UTR for the maintenance of mRNA stability by deletion and mutation analysis. Finally, we ascertained how interactions between the 3′-UTR and poly (A) tail *via* HuR and PABP complex contribute to β-casein mRNA stability.

2. Materials and methods

2.1. Animals

ICR mice were purchased from SLC (Shizuoka, Japan), maintained at 23 ± 1 °C under a 14-h lighting schedule (lights on 05:00 to 19:00 h), and food and tap water were given ad libitum. The day of parturition was designated as day 0 of lactation, and the litter size was adjusted to 10 pups per dam on day 0. The pups were separated from their mothers on day 8 (W0) and weaned for 24 h (W24); the mammary glands were collected at these time-points (W0 and W24).

2.2. RNA extraction and in vitro synthesis of RNA transcripts

Whole mammary glands were collected immediately after cervical dislocation. Total RNA was isolated using Isogen (Nippon Gene, Tokyo,