REVIEW

Hormonal regulation of mRNA stability and RNA–protein interactions in the pituitary

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ABSTRACT

Regulating gene expression from DNA to protein is a complex multistage process with multiple control mechanisms. Transcriptional regulation has been considered the major control point of protein production in eukaryotic cells; however, there is growing evidence of pivotal posttranscriptional regulation for many genes. This has prompted extensive investigations to elucidate the mechanisms controlling RNA processing, mRNA nuclear export and localization, mRNA stability and turnover, in addition to translational rates and posttranslational events. The regulation of mRNA stability has emerged as a critical control step in determining the cellular mRNA level, with individual mRNAs displaying a wide range of stability that has been

linked to discrete sequence elements and specific RNA–protein interactions. This review will focus on current knowledge of the determinants of mRNA stability and RNA–protein interactions in the pituitary. This field is rapidly expanding with the identification of regulated *cis*-acting stability-modifying elements within many mRNAs, and the cloning and characterization of *trans*-acting proteins that specifically bind to their cognate *cis* elements. We will present evidence for regulation of multiple pituitary genes at the level of mRNA stability and some examples of the emerging data characterizing RNA–protein interactions.

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INTRODUCTION

Though small, the pituitary, which is situated centrally in the sella turcica, is a major gland that synthesizes and secretes at least six hormones essential for maintaining body metabolism and homeostasis, in addition to mental function. These hormones regulate such diverse functions as growth (growth hormone, GH) and development and function of the thyroid gland (thyroid stimulating hormone, TSH), adrenal cortex (corticotropin, ACTH), male and female gonads (follicle stimulating hormone, FSH; leuteinising hormone, LH) and breasts (prolactin, PRL). The posterior pituitary contains the termini of neurons originating from the hypothalamic nuclei responsible for synthesis of oxytocin and arginine vasopressin (AVP), hormones

involved in suckling and body water homeostasis, respectively. Lesions in the pituitary often produce tumors and symptoms referable to under- or overexpression of these hormones.

There has been great interest in understanding the regulation of expression of each of the pituitary hormones ever since 1886, when Pierre Marie described the association of pituitary enlargement and acromegaly. How these hormones regulate the expression of other genes is also of great interest. Each pituitary hormone has been purified, characterized, cloned and its functional roles clearly delineated. Complex feedback loops have been described for many of the hormones; however, we are only just beginning to understand the complexity of the regulation of expression of the genes coding for each of these hormones. This is also true

FIGURE 1. Factors affecting mRNA stability: locations of mRNA sequence elements involved in regulating mRNA decay. (1) Cap structure, (2) 5'UTR sequences, (3) premature termination codons, (4) open reading frame sequences, (5) 3'UTR sequences including stem-loops and (6) AU-rich elements, and (7) the poly(A) tail. The mRNA may be protected from degradation by the binding of RNA-binding proteins (octopuses) and cap-binding protein (starfish), which protect it from exonucleases (fish), or non-specific endonucleases (crab). Specific sites may be targeted by a specific endonuclease (e.g., swordfish). Adapted from Laird-Offringa (1991).

in the study of regulation of their cognate receptors and other target genes.

Regulation of gene expression occurs at multiple levels, including transcription, RNA processing, mRNA nuclear export and localization, mRNA decay, translation and posttranslational events. However, the focus of this review will be on the current knowledge of posttranscriptional regulatory mechanisms at the level of mRNA stability. A large number of hormones have been shown to influence the mRNA stability of both non-pituitary and pituitary genes. There will be a noticeable bias towards the anterior pituitary, as this reflects the current research status, though a few examples do exist for posttranscriptional gene regulation in the posterior pituitary. Before embarking on a detailed discussion of the pituitary, in the following sections we provide an overview of the mechanisms involved in the regulation of mRNA stability.

mRNA STRUCTURE AND FUNCTION AND RNA-PROTEIN INTERACTIONS

After transcription, pre-mRNAs undergo major processing in the nucleus, whereby introns are removed and exons ligated (mRNA) within a RNA-protein complex known as the spliceosome. The coding sequence of eukaryotic mRNAs is flanked by their 5' and 3' untranslated regions (UTRs) (Fig. 1). The 5' and 3' UTRs of mRNAs may contain *cis*-acting elements that control the translation, degradation and localization of transcripts (Wilson & Brewer 1999). In addition, the 5' and 3' terminal nuclear modifications of eukaryotic mRNAs, the cap ($^7\text{mGpppN}$) and poly(A) tail respectively, play critical roles in mRNA translation and stability (Wickens & Stephenson 1984, Strickland *et al.* 1988, Gallie 1991, Keller & Minvielle-Sebastia 1997, Wickens *et al.* 1997, Deo *et al.* 1999) (Fig. 1). RNA transport from the

nucleus takes place via localizing *cis* elements and nuclear transport *trans*-acting proteins (for further details see Izzaualde & Mattaj 1995, Izzaualde *et al.* 1995, Michael & Dreyfuss 1995, Meyer *et al.* 1996, Visa *et al.* 1996).

After mRNAs have been released from the nuclear export machinery, they are bound by a cytoplasmic cap-binding complex, eIF4F, that is composed of three sub-units: eIF4E (which directly contacts the 7mG cap), the RNA helicase, eIF4A, and eIF4 G (Dreyfuss *et al.* 1996, Sonenberg 1996, Waskiewicz *et al.* 1999, Pyronnet *et al.* 1999) which are bound in the polyribosome complex (Mangus & Jacobson 1999). eIF4E is believed to represent a rate-limiting factor in translation, and it is therefore a potential target for translational control (Marcotrigiano *et al.* 1999). Work from many laboratories has shown recently that the binding of the heterotrimeric complex, eIF4F, to the cap structure is required to initiate the assembly of a translation-competent ribosome on the cap-proximal region of most cellular mRNAs (Sachs *et al.* 1997, Waskiewicz *et al.* 1999, Pyronnet *et al.* 1999). Other proteins that bind with high affinity to this cap-proximal region block the entry of the small ribosomal subunit and repress translation (e.g., iron-responsive elements (IREs) and iron-regulatory proteins (IRPs); Muckenthaler *et al.* 1998, see below).

The role of the cap in mRNA stabilization has been demonstrated clearly in *Saccharomyces cerevisiae*, in which decapping of the 5'-terminus represents part of a common degradation pathway for stable and unstable mRNAs (Caponigro & Parker 1995, Tuite 1996, Deo *et al.* 1999, see below). Decapping is preceded by deadenylation (removal of the poly(A) tail at the 3'-terminus), which reflects the role of the poly(A) tail in translation regulation and mRNA turnover and indicates an important communication between the two termini (Strickland *et al.* 1988, Jacobson & Peltz 1996, Tanguay & Gallie 1996, Deo *et al.* 1999).

Inherently unstable mRNAs can approach a new steady-state level more rapidly than stable mRNAs after changes in transcriptional activity (Hargrove & Schmidt 1989, Beelman & Parker 1995, Wilson & Brewer 1999). This property of unstable mRNAs allows their cytoplasmic concentrations, and hence their potential for translation, to be altered quickly in response to a change in the transcription rate. A number of mRNAs display altered stability in response to hormonal, developmental and environmental factors (Wilson & Brewer 1999, Williams *et al.* 1993). These inducible effects on mRNA stability have the potential to change cytoplasmic

mRNA concentrations rapidly without alteration in transcriptional activity, or to augment the magnitude of mRNA induction by coordinating changes in transcription and mRNA stability.

In summary, specific fundamental RNA-protein interactions occur within both the nucleus (mRNA processing and localization) and the cytoplasm (localization, translation, mRNA stability) of eukaryotic cells, and it is these interactions that govern the subsequent rate of decay of a particular mRNA. This review will focus predominantly on cytoplasmic mRNA decay in the pituitary.

CYTOPLASMIC mRNA DECAY: SEQUENCE DETERMINANTS

mRNA decay is not a random, indiscriminate nuclease degradation process, but a precise process dependent on a variety of specific *cis*-acting stability-modifying mRNA sequences and *trans*-acting protein factors (Figs 1, 2). Nucleases, mRNA sequence elements and regulated RNA-binding proteins required for the promotion or inhibition of mRNA decay have been identified, and the molecular mechanisms involved are being elucidated. Generically, mRNA decay is triggered by at least three types of initiating event: poly(A) tail shortening, arrest of translation at a premature nonsense codon, and endonucleolytic cleavage (Figs 1, 2) (Stevens 1993). Steps subsequent to poly(A) tail shortening or premature translational termination converge in a pathway that progresses from removal of the 5' cap to exonucleolytic digestion of the body of the mRNA (Jacobson & Peltz 1996, Wilson & Brewer 1999). Fragments generated by endonucleolytic cleavage are most probably removed by exonucleolytic decay also (Fig. 2). Recent studies have indicated that decay of mRNA commences while the mRNA is still being translated and is still attached to the polyribosomal complex (Mangus & Jacobson 1999). However, translation initiation is required to induce mRNA decay (Posch *et al.* 1999). The role of different mRNA sequences and structures (*cis* elements) and RNA-binding proteins (*trans* elements) in the determination of mRNA decay will be discussed below.

Exonuclease mRNA decay: poly(A) tail-dependent degradation

Role of 5' cap and poly(A) tail

The mRNA 5' cap structure generally remains unchanged after mRNA export into the cytoplasm. The 5'-5' phosphodiester bond of the cap makes it inherently resistant to general ribonucleases

DEADENYLATION-DEPENDENT DECAY

DEADENYLATION-INDEPENDENT DECAY

FIGURE 2. mRNA decay pathways in eukaryotes. The top pathway depicts deadenylation-dependent decay, followed by decapping and 5'–3' decay, or 3'–5' decay by exonucleases and/or endonucleases. The vast majority of eukaryotic mRNAs may undergo decay in this way unless they are specifically targeted for rapid deadenylation-independent decapping (bottom) by a nonsense codon, or for endonucleolytic cleavage, by the presence of a cleavage site. After deadenylation-independent decapping (bottom pathway), mRNA is degraded in a 5'–3' manner. Endonucleolytic cleavage of mRNA may serve as a one-step deadenylation that leads to decapping and 5'–3' decay. Adapted from Beelman & Parker (1995) with permission.

(Furuichi *et al.* 1977, Shimotohno *et al.* 1977), though binding of the eIF4 G complex may also be responsible for protecting this region from nuclease attack (Stevens 1993, Dreyfuss *et al.* 1996).

Most eukaryotic mRNAs have a 3' poly(A) tract; though contentious, this is not regarded as a determinant of mRNA stability, even though it does influence mRNA decay (Strickland *et al.* 1988). Poly(A) tracts are gradually shortened in the cytoplasm in an mRNA- and organism-specific manner, and may be completely removed in some cases (Wilson & Brewer 1999, Imataka *et al.* 1998, Jacobson & Peltz 1996). In some instances, shortening of the tail to an oligo(A) form is the rate-determining event that triggers turnover of the body of the transcript (Jacobsen & Peltz 1996). In other cases, deadenylation occurs and may be an obligate event in the decay pathway, but it is not the rate-determining step (Decker & Parker 1993, Muhlrads *et al.* 1995).

Shortening of the poly(A) tail by 3'–5' exonucleases is followed by decapping at the 5' end, which may or may not be a rapid process and is mRNA-specific (Decker & Parker 1993, Muhlrads *et al.* 1995, Imataka *et al.* 1998). Removal of the 5' cap allows the subsequent entry of exoribonucleases that digest the mRNA in a 5' to 3' direction. This has been shown to occur in all eukaryotic organisms studied; examples include mammalian c-fos (Shyu *et al.* 1991), yeast MFA2 (Muhlrads *et al.* 1994) and oat phytochrome A (Higgs & Colbert 1994).

However, 3'–5' exonucleolytic degradation after deadenylation also occurs, as in yeast PGK1 (Muhlrads & Parker 1994, Muhlrads *et al.* 1995), oat phytochrome A (Higgs & Colbert 1994) and mammalian c-myc (Brewer 1999) mRNAs. Ford *et al.* (1997) determined that the presence of a poly(A) tail of at least 30 nucleotides prevented 3'–5' degradation by blocking the assembly, but not the action, of an exonuclease. This effect was shown to be position dependent, as placing a poly(A) sequence internal to the 3' end destabilized the transcript, without altering its interaction with poly(A) binding protein (PABP) (see below). There is growing evidence to support 3'–5' decay. Most significantly, *in vivo* and *in vitro* studies by Brewer (1999) recently demonstrated that the 3'–5' decay pathway contributed to the turnover of c-myc mRNA. Thus, the 3'–5' decay pathway may represent a major turnover pathway in mammalian cells, resulting in a major paradigm shift for those working in the field of mRNA decay.

Deadenylation-independent decapping by decapping protein 1 (Dcp1) occurs in mRNAs that contain nonsense codons (Hagan *et al.* 1995). This process is considered to be a translation-related

event that allows exoribonuclease entry to the mRNA and subsequent digestion of the message (Jacobson & Peltz 1996). This mechanism exists in eukaryotes mainly to ensure that aberrant mRNAs are not translated, but is also related to the degradation process initiated in non-aberrant mRNAs that all contain stop codons.

Role of sequence elements

The differences in decay rates in stable and unstable mRNAs are attributable to the presence or absence of specific sequence elements. With the exception of the stabilizer sequences that appear to be associated with the yeast PGK1 mRNA (Heaton *et al.* 1992, Peltz *et al.* 1993) and the human α -globin mRNA (Weiss & Liebhaber 1995), all identified elements promote mRNA destabilization. These sequences include the well characterized adenosine (A)+uridine (U)-rich elements (AREs) located in the 3'UTRs of mammalian mRNAs, in addition to other sequences in mRNA coding regions, 3'UTRs and 5'UTRs.

AU-rich elements (AREs) In 1986, Shaw & Kamen observed that an ARE in the 3'UTR of granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNA could stimulate the degradation of the normally stable β -globin mRNA. Similarly, the 3'UTR of c-fos, which contains a 69 nt ARE, reduced the stability of β -globin mRNA (Chen & Shyu 1995, Chen *et al.* 1995). Subsequently, AREs that function as RNA destabilizing elements have been found in numerous mRNAs, including c-myc, junB, nur77, β -interferon, interleukin (IL)-1 α and IL-3 mRNAs (Cleveland & Yen 1989, Chen & Shyu 1994, 1995, Gorospe & Baglioni 1994). The sequence consensus for the ARE is loosely defined as a pentamer comprising AUUUA, repeated once or several times within the 3'UTR. It is often found within a U-rich region of the mRNA. However, recent work has suggested that a nonamer, UUAUUUA(U/A)(U/A), is more indicative of rapid destabilization (Lagnado *et al.* 1994, Zubiaga *et al.* 1995). It is now becoming clear that it is the combination of functionally and structurally distinct sequence motifs, such as AU-pentamers, nonamers and U-rich stretches, that determines the ultimate destabilizing ability of each particular ARE.

Other 3'UTR elements associated with poly(A) tail shortening There is a growing number of non-ARE 3'UTR instability elements associated with poly(A) tail shortening. Two well-characterized *cis* elements have been identified within the 3'UTRs of the ribonucleotide reductase R1 and R2 mRNAs (Chen *et al.* 1993, Amara *et al.*

1995, Fan *et al.* 1996); regulation of expression of R2 is especially important in tumorigenesis. R1 and R2 mRNAs contain sequences within their 3'UTRs that are bound by the specific proteins R1 BP and R2 BP, respectively (Chen *et al.* 1993, Amara *et al.* 1995). Increased binding of these RNA-binding proteins leads to accelerated R1/R2 mRNA decay (Amara *et al.* 1995, Fan *et al.* 1996). Interestingly, tumorigenesis was associated with decreased binding of R1 BP and R2 BP to their respective cognate 3'UTR mRNA sequences, which resulted in overexpression of R1 and R2 proteins.

Coding region elements Destabilizing sequences are not limited to the 3'UTR and can be found throughout the mRNA. The open reading frame (ORF) of β -tubulin contains an N-terminal tetrapeptide that provides a signal for rapid decay of the mRNA when tubulin monomer is in excess (Yen *et al.* 1988). Similarly, the c-fos mRNA contains a second destabilizing sequence within the ORF; however, it is only utilized when the message is transiently expressed after growth factor stimulation (Shyu *et al.* 1989). In addition, c-myc has a *cis* element located within the coding region (Shyu *et al.* 1989).

Endonuclease mRNA decay: poly(A) tail-independent degradation

Deadenylation-independent endonuclease degradation of certain mRNAs does occur and cleavage sites have been identified in the 3'UTRs and coding regions of a small number of mRNAs. Examples of transcripts that undergo degradation by this method include mammalian 9E3 (Stoeckle & Hanafusa 1989), insulin-like growth factor-II (Nielson & Christiansen 1992), transferrin receptor (TfR) (Binder *et al.* 1989, 1994) and *Xenopus* Xihbox2B (Brown *et al.* 1993) mRNAs. Sequence-specific endonuclease target sites are likely to be limited to individual mRNAs or classes of mRNAs, and their presence probably allows for specific control of the decay rates of these particular transcripts.

Endonucleolytic cleavage of some of these mRNAs is regulated by RNA-binding proteins that bind in the vicinity of the cleavage site that renders the site inaccessible to nucleolytic attack. One of the most studied examples is the iron-responsive element (IRE) within the TfR mRNA, in which decay is regulated, not only in response to cellular iron concentrations, but to multiple stimuli (Klausner *et al.* 1993, Leedman *et al.* 1996, Posch *et al.* 1999, Thomson *et al.* 1999a). The 3'UTR of

this mRNA contains five distinct stem-loop structures capable of binding the IRE-binding protein (iron-regulatory protein, IRP) (see below). Conditions that favor binding of the IRP lead to stabilization of the mRNA sequence as it protects the functional cleavage site between two of the IREs (Lok & Ponka 1999, Posch *et al.* 1999). Destabilization of the TfR mRNA occurs in conditions that favor a reduction of IRP affinity for the TfR IREs (Fig. 3).

Endonucleolytic cleavage of some mRNAs is dependent upon current translation, and this may reflect ribosome displacement of bound RNA-binding proteins in the coding region, the melting of secondary structures that leads to exposure of linear sequences, or accumulation of specific endonucleases (Jacobsen & Peltz 1996, Deo *et al.* 1999). Deadenylation or endonucleolytic cleavage may be two independent means of disrupting RNA-protein complexes and creating substrates for exonucleases responsible for the bulk of mRNA degradation.

CYTOPLASMIC mRNA: TRANS-ACTING RNA-BINDING PROTEINS

Realization of the complexity of *cis* elements led to the development of assays to detect RNA-binding proteins targeting these regions. Development of the RNA electrophoretic mobility shift assay (REMSA) and u.v. cross-linking assay (Liebold & Munro 1988, Wilson & Brewer 1999, Thomson *et al.* 1999b), which involve the incubation of a radiolabeled RNA probe with cell protein extract, transformed investigation in this area. With these assays, specific RNA-protein interactions were readily detected, the effects of various treatments easily discerned, and the size of the proteins determined. Identification of specific proteins was made with the addition of specific antibodies that supershift the protein band in a REMSA. Using these approaches, regulated RNA-binding proteins have been characterized in the pituitary that target several different mRNAs, including TSH β and thyrotropin-releasing hormone receptor (TRHR) mRNAs. In the past several years, a vast array of proteins has been described that bind to various mRNA sequences implicated in mRNA destabilization. However, to date, few of these proteins have been shown to play a definite role in modulating mRNA turnover. Identification and cloning of some of these protein factors has provided enormous insight into the mechanisms involved in regulated mRNA turnover.

FIGURE 3. Posttranscriptional regulation of ferritin and TfR mRNAs by interactions of IRPs 1 and 2 with IRE. IRP1 and IRP2, when bound to the 5'UTR IRE of ferritin mRNA, block the binding of the ribosomal initiation complex and inhibit translation. The binding of an uncharacterized RBP to the acute box (AB) stem-loop also plays a role in translational initiation. Both stem-loop structures may be important for steric presentation of the mRNA to the initiation complex. (Interaction of poly(A) tail and associated proteins in translation is not shown in this diagram.) When IRPs 1 and 2 are bound to the five IREs in the TfR mRNA 3'UTR, they block endonuclease attack, which results in an increase in TfR mRNA stability. Thyrotropin-releasing hormone (TRH) and tri-iodothyronine (T₃) can increase or decrease IRP1 and IRP2 binding activity to the ferritin IRE, depending on the cell type. PKCs, protein kinases C; Epo, erythropoietin.

Poly(A) binding protein (PABP)

PABP is a highly conserved 71 kDa protein found in the cytoplasm and nucleus that binds to the poly(A) tail with high affinity and plays an important part in regulating its length (Blobel 1973, Baer & Kornberg 1983, Bernstein *et al.* 1989, Jackson & Standart 1990, Munroe & Jacobson 1990, Görlach *et al.* 1994, Smith *et al.* 1997, Afonina *et al.* 1998, Deo *et al.* 1999). PABP contains four RNA-recognition motifs (RRMs 1–4) and a proline-rich carboxy terminus (Burd *et al.* 1991, Deo *et al.* 1999). The cocrystal structure of human PABP with poly(A) was recently determined (Deo *et al.* 1999). RRM1 and RRM2 are responsible for poly(A) binding (Deo *et al.* 1999), but RRM4 is mainly involved in

non-specific polypyrimidine RNA binding (Smith *et al.* 1997). Recent studies have shown that PABP is also able to bind to AU-rich stretches of mRNA (Afonina *et al.* 1997). Interestingly, RNA binding, but not high-affinity poly(A) binding, is correlated with the ability of PABP to sustain yeast viability (Sachs *et al.* 1987), reinforcing the notion that PABP plays a major role in regulating mRNA stability, independently of its role in poly(A) length regulation (Caponigro & Parker 1995, Deardoff & Sachs 1997, Craig *et al.* 1998, Deo *et al.* 1999).

mRNA stabilization by PABP requires current translation of the mRNA, and PABP preferentially stabilizes mRNAs the decay of which is deadenylation-dependent, with little or no effect on mRNAs that decay independently of deadenylation

(Coller *et al.* 1998). A model has been postulated in which PABP acts to hold both ends of an mRNA in close proximity. PABP does interact *in vitro* with the translation initiation factors eIF4 G, eIF4F and eIF4B in plants and yeast, both in the presence and the absence of poly(A) RNA (Le *et al.* 1997, Tarun & Sachs 1996, Gallie 1998). In humans, eIF4 G has recently been shown to bind to PABP (Imataka *et al.* 1999). This interaction inhibits poly(A)-dependent translation, but has no effect on translation of deadenylated mRNA (Imataka *et al.* 1999). Mammalian PABP also binds to PABP-interacting protein-1 (PAIP-1) (Craig *et al.* 1998). PAIP-1 shares 25% homology with a 470 amino acid region of mammalian eIF4 G. Remarkably, increasing the concentration of PAIP-1 *in vivo* resulted in a modest 2.8-fold increase in translation in COS-7 cells (Craig *et al.* 1998), suggesting an important role for this protein in the regulation of translational efficiency (Deo *et al.* 1999).

AU-rich element binding proteins (AUBPs)

A family of proteins has been characterized by REMSA that bind to AU-rich, and sometimes U-rich RNAs with high affinity. In most cases, binding of these AUBPs (30–45 kDa) increases the turnover of ARE-containing mRNAs (DeMaria & Brewer 1996, Joseph *et al.* 1998).

The elav-like proteins

One of the best characterized AUBPs is HuR, a 36 kDa member of the embryonic lethal abnormal vision (elav) family of RRM-containing RNA-binding proteins (Levine 1993, Joseph *et al.* 1998). The elav-like protein family contains four proteins, namely HuC, Hel-N1, HuD and HuR (Myer *et al.* 1997). HuR has been shown to bind with high affinity to ARE sequences (Levine 1993, Joseph *et al.* 1998). Most importantly, HuR has an active role in the stabilization of specific mRNAs containing AREs, such as glucose transporter 1, c-fos, GM-CSF, plasminogen activator inhibitor type 2 and p21^{waf1} (Jain *et al.* 1997, Levy *et al.* 1998, Fan & Steitz 1998, Maurer *et al.* 1999, Joseph *et al.* 1998). The ability of elav/Hu proteins to stabilize ARE-containing mRNAs was confirmed recently using an *in vitro* system (Ford *et al.* 1999). Interestingly, HuR appears to shuttle between the nucleus and the cytoplasm, and it has been postulated that this function is to chaperone AREs out to the cytoplasm (Fan & Steitz 1998). Aberrant shuttling may therefore render the ARE susceptible to decay in the cytoplasm. HuR may also have an effect at the translational level, coupling mRNA

stability and translation, an association studied extensively by Jacobson & Peltz (1996).

AU-binding factor 1 (AUF1)

The proto-oncogene, c-myc, contains ARE sequences in the 3'UTR of its mRNA. These sequences act as destabilizing elements and bind the RNA-binding protein, AUF1 (Brewer 1991, Zhang *et al.* 1993, DeMaria *et al.* 1997, Wilson & Brewer 1999, Wilson *et al.* 1999). AUF1 has been implicated in the regulation of many cytokine and G protein-coupled receptor mRNAs (Wilson & Brewer 1999), and plays a major role in development (Lafon *et al.* 1998). Different splice variants of AUF1 exist and all bind to ARE sequences. The 37 and 40 kDa isoforms are found throughout the cell, whereas the p42 and p45 isoforms reside only in the nucleus (Wilson & Brewer 1999). Interestingly, four 3'UTR splice variants also exist that alter expression of AUF1 (Wilson *et al.* 1999). AUF1 contains two non-identical RRM s that interact with other sequences within the protein structure to facilitate RNA-binding (Wilson & Brewer 1999). AUF1 is also known to bind to eIF4 G and PABP. AU-rich mRNA decay is associated with displacement of eIF4 G from AUF1, ubiquitination of AUF1 and degradation of AUF1 by proteasomes (Laroia *et al.* 1999).

Other ARE binding proteins

An intriguing finding has been the isolation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and enoyl coenzyme A (CoA) hydratase from ARE-binding protein screens (Nagy & Rigby 1995, Wilson & Brewer 1999). These proteins contain ARE-binding motifs, but their significance in RNA turnover has yet to be established.

Non-ARE-binding proteins

Reports characterizing novel non-ARE RNA-binding proteins are increasing, although we shall focus on the most studied of these proteins, the IRPs. Two mammalian isoforms exist, IRP1 and IRP2, IRP1 acting as a cytoplasmic aconitase under conditions favoring low-affinity mRNA binding (Klausner *et al.* 1993, Leedman *et al.* 1996, Thomson *et al.* 1999a).

Posttranslational modification of IRP1 and IRP2 alters their binding affinity for their specific cognate mRNA hairpin structures, the IREs, and controls the expression of genes posttranscriptionally. IREs present in the 5'UTR of ferritin, erythroid 5-aminolevulinate synthase and mammalian mitochondrial aconitase mediate the translational efficiency of these mRNAs (Klausner *et al.* 1993,

TABLE 1. Summary of non-pituitary-derived genes that are known to be regulated by hormones

mRNA affected	Regulation	Reference
Vitellogenin	mRNA stabilized 30-fold by estrogen	Brock & Shapiro (1982)
ApoVLDII	mRNA stabilized and poly(A) tail increased by estrogen	Cochrane & Deely (1988)
Ovalbumin	mRNA stabilized by estrogen or progesterone	Cox (1977)
Conalbumin	mRNA stabilized by estrogen or progesterone	McKnight & Palmiter (1979)
MCH	mRNA stabilized by lithium	Presse <i>et al.</i> (1997)
Proteolipid protein	mRNA stabilized 10–20-fold by retinoic acid	Lopez-Barahona <i>et al.</i> (1993)
HMG-CoA reductase	mRNA stabilized twofold by T ₃	
NADH dehydrogenase subunit 3	mRNA stabilized threefold by T ₃ ; mRNA destabilized five- to sixfold by Dex or hydrocortisone	Simonet & Ness (1989)
Vasopressin	mRNA stabilized 20-fold by ACTH	Raikhinstein & Hanukoglu (1993)
PAM	mRNA stabilized by dehydration through increased poly(A) tail length	Zingg <i>et al.</i> (1988) Robinson <i>et al.</i> (1988)
CRH	mRNA destabilized by estrogen	El Meskini <i>et al.</i> (1997)
	mRNA stabilized 16-fold and poly(A) tail length increased by protein kinase C	Adler <i>et al.</i> (1992)
Inhibin α	mRNA stabilized by cAMP	Najmabadi <i>et al.</i> (1993)
Renin	mRNA stabilized three- to fourfold by cAMP	Chen <i>et al.</i> (1993)
Malic enzyme	mRNA stabilized twofold by T ₃	Song <i>et al.</i> (1988)
FSHR	mRNA stabilized 1.5–2-fold by activin	Tano <i>et al.</i> (1997)
Casein	mRNA stabilized 20-fold by prolactin	Guyette <i>et al.</i> (1979)
PR	mRNA stabilized by LH or FSH	Iwai <i>et al.</i> (1991)
TRHR	mRNA destabilized by TRH	Fujimoto <i>et al.</i> (1992b)
LH/hCGR	mRNA destabilized threefold by LH	Lu <i>et al.</i> (1993)
FSHR	mRNA stabilized sixfold by FSH	Tilly <i>et al.</i> (1992)
AR	mRNA stabilized by DHT	Yeap <i>et al.</i> (1999)
EGFR	mRNA stabilized by EGF	McCulloch <i>et al.</i> (1998)

apoVLDII, Very-low-density lipoprotein II; MCH, melanin concentrating hormone; PAM, peptidyl-glycine α -amidating mono-oxygenase; CRH, corticotropin releasing hormone; PR, progesterone receptor; LH/ hCGR, luteinizing hormone/human chorionic gonadotropin-receptor; AR, androgen receptor; DHT, dihydro-testosterone; Dex, dexamethasone.

Thomson *et al.* 1999a). IREs present in the 3'UTR of the TfR mediate mRNA stability (Posch *et al.* 1999). This coordinate but divergent posttranscriptional regulation of IRP/IRE RNA–protein interactions governs iron homeostasis by modulating target gene expression posttranscriptionally. Interestingly, the binding affinity of IRP1 and IRP2 is modulated by various hormones, including thyroid hormone (tri-iodothyronine, T₃) (Leedman *et al.* 1996), TRH (Thomson *et al.* 1999a), and erythropoietin (Weiss *et al.* 1997).

HORMONE-REGULATED mRNA TURNOVER

As outlined below, the targets for the hormones that influence pituitary and non-pituitary mRNA stability are distributed widely and involve endocrine and non-endocrine organs (Table 1). For example, several hormones influence the mRNA stability of their own receptor in a feedback regulation mechanism (Table 1). In many cases, hormones regulate expression at transcriptional, posttranscriptional and posttranslational levels of their target genes.

Hormonal regulation of non-pituitary gene mRNA stability

Steroid hormones were one of the earliest agents shown to control the degradation of specific mRNAs and to regulate the stability of a substantial number of mRNAs. The best characterized is the stabilization of vitellogenin mRNA by estrogen (Nielsen & Shapiro 1990a). Vitellogenin mRNA levels increase >10 000-fold upon estrogen stimulation in *Xenopus* liver (Brock & Shapiro 1982, 1983). The estrogen-mediated increase in vitellogenin mRNA is effected by an increase in both transcription and mRNA stability (Brock & Shapiro 1982, 1983). The mRNA half-life of hepatic vitellogenin increases from 16 h to 500 h after the administration of estrogen (Brock & Shapiro 1983). Further analysis revealed that an estrogen-regulated *cis*-acting element was situated in the 3'UTR of vitellogenin mRNA (Nielsen & Shapiro 1990b), and required association of the mRNA with ribosomes for stabilization (Blume & Shapiro 1989).

More recently, a polysome-associated protein of molecular mass 150–155 kDa was shown to bind

specifically within a 27 nt region of a 94 nt segment (required for maximal binding) of the 3'UTR of vitellogenin mRNA in an estrogen-inducible manner (Dodson & Shapiro 1994, 1997, Dodson *et al.* 1995, Kanamori *et al.* 1998). This protein has subsequently been identified as vigilin. It is ubiquitously expressed and contains 14 human ribonucleo protein K (hnRNPK) homology (KH) domains and had previously been shown to bind tRNA (Kruse *et al.* 1996). The 14 KH domains in vigilin allow its interaction with multiple mRNAs that contain specific, mainly single stranded, regions that constitute long RNA binding sites (~75 nt, (A)_nCU and UC(A)_n motifs) (Kanamori *et al.* 1998); its association with other proteins may also define binding specificity or cytoplasmic targeting. Vigilin is present in both the cytoplasm and the nucleus of cells (Kugler *et al.* 1996), and may be involved in shuttling of mRNAs from nucleus to cytoplasm. A clear functional role of RNA binding of vigilin has not been established (Kanamori *et al.* 1998), but its estrogen-regulated binding to vitellogenin mRNA and associated increase in vitellogenin mRNA stability emphasize its importance in regulating mRNA metabolism.

Other hormones known to influence posttranscriptional events include T₃, which increases the stability of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase mRNA approximately threefold (Simonet & Ness 1989). This stabilization was blocked by hydrocortisone and dexamethasone, each of which reduced the stability of HMG-CoA reductase mRNA five- to sixfold (Simonet & Ness 1989). More recently, T₃ was shown to increase the stability of proteolipid (the major component of myelin) mRNA approximately twofold. Interestingly, retinoic acid induced a ~10-fold increase in proteolipid mRNA stability in C6 glioma cells (Lopez-Barachona *et al.* 1993). Other effects of T₃ include the stabilization of malic enzyme mRNA in rat liver (Back *et al.* 1986, Song *et al.* 1988).

The ovary and adrenals are also sites at which hormonal regulation of mRNA stability has been observed. Treatment of ovarian granulosa cells with LH or FSH for 3 h increased progesterone receptor mRNA eight- to ninefold (Iwai *et al.* 1991). Incubation of adrenal cortex cells with ACTH stabilized mitochondrial mRNA (Raikhinstein & Hanukoglu 1993) and also NADH dehydrogenase subunit 3 mRNA, increasing the latter mRNA approximately 20-fold after 6 h of exposure.

An increasing number of hormones have been shown to regulate expression of their own receptor at the posttranscriptional level. For example, the stability of the epidermal growth factor receptor (EGFR) mRNA was increased more than threefold

by EGF and by transforming growth factor α (TGF α) in epidermoid cancer cells (McCulloch *et al.* 1998), and approximately twofold by EGF and TGF α in prostate cancer cells (Seth *et al.* 1999). Recent studies utilizing prostate cancer LNCaP cells showed that androgens (dihydro-testosterone) increased the stability of the androgen receptor mRNA approximately twofold (Yeap *et al.* 1999). Finally, the TRHR mRNA is destabilized by TRH in pituitary cells (Narayan *et al.* 1992).

Hormonal regulation of pituitary gene mRNA stability

A large number of genes expressed in the pituitary are regulated at the level of mRNA stability (Table 2). For example, mRNA stability of the common α -glycoprotein subunit was recently compared with that of LH β and FSH β mRNAs (Bouamoud *et al.* 1992). Interestingly, there was broad variation in the basal half-life for each mRNA, with values of 1.0, 6.5 and 44 h for FSH β , the α -subunit and LH β mRNAs, respectively. Rapid disappearance of these mRNAs was associated with a progressive shortening of the poly(A) tail (Bouamoud *et al.* 1992). In addition, the stability of each of these mRNAs was regulated extrinsically by various ligands. For example, the mRNA stability of the α -subunit was increased six- to sevenfold in the presence of gonadotropin releasing hormone (GnRH) in α T₃ pituitary cells (Chedrese *et al.* 1994). Similarly, GnRH stabilized LH β mRNA, and this stabilization was further augmented by progesterone, or a combination of estrogen and progesterone (Park *et al.* 1995). Interestingly, FSH β subunit mRNA, but not α -subunit or LH β mRNA, was stabilized by testosterone in male rat gonadotropes (Paul *et al.* 1990). In a separate study, estrogen was shown to increase the mRNA stability of another pituitary hormone receptor, TRHR, approximately twofold in rat pituitary primary cultures (Kimura *et al.* 1994).

We and others have shown that TSH β subunit mRNA is regulated posttranscriptionally by T₃, reducing its half-life from 24 h to approximately 8 h in both rat and murine pituitary cells (Krane *et al.* 1991, Staton & Leedman 1998). This reduction in mRNA stability was accompanied in both species with a reduction in the length of the poly(A) tail from 160–180 to ~30 nt. TSH β mRNA was also destabilized by bromocryptine, which reduced the half-life approximately twofold (Levy & Lightman 1990).

The effect of T₃ on the posttranscriptional regulation of GH expression has been studied extensively by several groups. T₃ destabilized and

TABLE 2. Summary of pituitary-derived genes that are known to be regulated by hormones

	Regulation	Reference
mRNA affected		
TRHR	mRNA stabilized twofold by estrogen	Kimura <i>et al.</i> (1994)
TSH β	mRNA destabilized three- to fourfold and poly(A) tail shortened by T ₃ ;	Staton & Leedman (1998)
FSH β	mRNA destabilized twofold by bromocryptine	Levy & Lightman (1990)
GH	mRNA stabilized twofold by testosterone	Paul <i>et al.</i> (1990)
	mRNA stabilized and poly(A) tail length increased by glucocorticoids;	Paek & Axel (1987)
	mRNA stabilized twofold by T ₃	Diamond & Goodman (1985)
LH β	mRNA stabilized 50-fold and poly(A) tail length increased by Dex+T ₃ ;	Jones <i>et al.</i> (1990)
	mRNA stabilized by GnRH; GnRH-induced mRNA stability augmented with progesterone or progesterone+estrogen	Park <i>et al.</i> (1995)
LH/FSH/TSH α -subunit	mRNA stabilized six- to sevenfold by GnRH	Chedrese <i>et al.</i> (1994)

Dex, Dexamethasone.

deadenylated GH mRNA in rat pituitary cells (Murphy *et al.* 1992). However, when T₃ was combined with dexamethasone, a 50-fold increase in GH mRNA was observed, predominantly as a result of stabilization of the mRNA associated with an increase in the length of the poly(A) tail (Diamond & Goodman 1985, Paek & Axel 1987, Jones *et al.* 1990).

There are abundant examples of pituitary ligands regulating the mRNA stability of their own receptor in non-pituitary tissue. LH decreased the stability of LH/hCG-receptor mRNA approximately threefold in rat ovary primary cultures (Lu *et al.* 1993). The stability of FSH- and activin-stabilized FSH receptor (FSHR) mRNA is increased several-fold in ovarian granulosa cells (Tilly *et al.* 1992, Tano *et al.* 1997). Recent studies identified an LH receptor mRNA stability LH-receptor RNA-binding protein (LRBP-1) of molecular mass 50 kDa that bound to a 180 nt region within the ORF of LH receptor mRNA (Kash & Menon 1998). Moreover, the binding of LRBP-1 increased approximately threefold after 12 h of incubation with LH (Kash & Menon 1998).

Very little is known about posttranscriptional regulation of gene expression in the posterior pituitary. In 1988, Zingg *et al.* observed that dehydration caused an increase in the poly(A) tail length of vasopressin (from 210 nt to 330 nt), associated with increased mRNA stability. Robinson *et al.* (1988) investigated this further and determined that the variation in length occurred in the supra-chiasmatic hypothalamic nuclei, the location of an endogenous mammalian circadian pacemaker. These results suggested that the variation in mRNA poly(A) tail length may underlie the circadian

rhythm of vasopressin peptide levels in cerebrospinal fluid (Robinson *et al.* 1988).

In summary, the regulation of expression of hormones secreted by the pituitary has been studied extensively, the vast proportion being regulated, in part, at the level of mRNA stability. However, the mechanisms underlying these changes in mRNA turnover for the majority of these genes remain largely unknown.

Hormonal regulation of RNA-protein interactions and mRNA decay in the pituitary

There are a few examples of regulated mRNA decay in the pituitary for which RNA-protein interactions have been more clearly defined.

Posttranscriptional regulation of TSH β mRNA expression

Several studies have shown that T₃ decreases TSH α - and TSH β -subunit steady-state mRNA levels, in part as a result of reduced transcription (Surks & Litschitz 1977, Ross *et al.* 1983, Shupnik *et al.* 1985). TSH β mRNA is also regulated by T₃ at the posttranscriptional level, accompanied by a shortening of the poly(A) tail (Krane *et al.* 1991, Staton & Leedman 1998). In the presence of actinomycin D, but the absence of T₃, TSH β mRNA was deadenylated without inducing rapid mRNA decay, suggesting that deadenylation alone was not sufficient for the acceleration of TSH β mRNA turnover.

We recently isolated a *cis*-acting element located within the 70 nt of the 3'UTR of TSH β mRNA (unpublished observations). This sequence does not

FIGURE 4. Schematic model of T_3 -mediated decay of TSH β mRNA in pituitary cells. In the absence of T_3 , TSH β mRNA is polyadenylated and stable; in the presence of T_3 , it is deadenylated and undergoes decay by exonucleases and/or endonucleases. T_3 regulates binding of 3'UTR mRNA-binding proteins, PABP and TSH β BP2, which play a role in facilitating TSH β mRNA decay.

contain any AREs or other well characterized *cis*-acting destabilizing elements (Leedman *et al.* 1995). Interestingly, it was the target for T_3 -regulated cytoplasmic RNA-binding proteins derived from murine thyrotrope cells. U.v. cross-linking assays identified two major TSH β mRNA specific RNA-binding complexes (TSH β BP1 and BP2) of molecular mass ~ 60 and ~ 72 kDa (unpublished observations). Remarkably, we demonstrated that the 72 kDa complex contained PABP. A proposed model in which T_3 coordinately regulates the shortening of the poly(A) tail and the activity of RNA-binding proteins binding specifically to the *cis*-acting element within the 3'UTR of TSH β mRNA is illustrated in Fig. 4. The role of PABP in this process is still being elucidated. However, our findings suggest a novel dual role for PABP in the deadenylation and accelerated decay of TSH β mRNA, by its ability to bind poly(A) and non-poly(A) sequences, respectively. Cloning and characterization of the other TSH β mRNA RNA-binding protein(s) will facilitate careful functional analysis of the role of each protein in regulated TSH β mRNA decay, and provide considerable insight into the mechanisms of action of T_3 at the posttranscriptional level.

Posttranscriptional regulation of TRHR mRNA

Posttranscriptional regulatory mechanisms have been shown to have a critical role in the regulation of TRHR gene expression (Fujimoto *et al.* 1992a, b). Several actinomycin D chase experiments have shown that TRH regulates TRHR gene expression at the level of mRNA turnover. Endogenous TRHR mRNA in rat pituitary cells was decreased by TRH, and increased by estradiol (E_2) (Kimura *et al.* 1994). In rat pituitary cells stably transfected with murine TRHR, TRH destabilized TRHR mRNA (Fujimoto *et al.* 1992a). A cell free mRNA decay assay showed that TRH regulated RNase activity in rat pituitary GH3 cells, governed by specific sequences within the 3'UTR of TRHR mRNA (Narayan *et al.* 1992). In deletion studies, a truncated form of the TRHR missing the entire 3'UTR (~ 2 kb) was more stable than the full-length receptor. Interestingly, deletion of the last 143 nt from the 3'UTR, which formed a stable stem-loop structure, prevented TRH from enhancing TRHR mRNA decay (Narayan *et al.* 1992). Thus the TRHR 3'UTR appears to contain two distinct regions. One, the stem-loop at the 3' end of the 3'UTR, is necessary for conferring TRH-regulated TRHR mRNA degradation. The other,

which is less well defined, comprises a *cis*-acting element responsible for TRH-mediated decay. Within this region, there are several AU-rich putative mRNA stability modifying *cis*-acting regions that are conserved across species. There are four AUUUA pentamers, including one interesting double pentamer, AUUUAUUUA, motif just 5' to the stem-loop in mouse and rat. We presume that one or more of these AU-rich regions comprise the *cis* element. Interestingly, the human TRHR 3'UTR (~0.9 kb) contains many AU-rich regions and a structurally conserved stem-loop at the 5' end of the 3'UTR.

We recently identified RNA-binding proteins unique for each of the aforementioned TRHR AU-rich regions and the stem-loop in cytoplasmic extracts from thyrotrope and lactotrope pituitary cells (unpublished observations). Binding activity of these RNA-protein complexes was rapidly upregulated approximately fivefold in both cell types after TRH and phorbol-12 13-myristate acetate stimulation. In marked contrast, however, T₃ down-regulated binding activity of these RNA-protein complexes. These data provide the first evidence for divergent regulation by TRH and T₃ of the binding of these novel RNA-binding proteins to the stem-loop and AU-rich regions of TRHR mRNA. This divergent hormone-regulated change in binding is consistent with the opposing physiologic actions of T₃ and TRH expression in these cell types. Furthermore, these data suggest that a complex system of hormonally regulated RNA-protein interactions is involved in the control of TRHR mRNA decay in the pituitary.

Posttranscriptional regulation of ferritin gene expression in the pituitary

As alluded to above, control of mammalian intracellular iron homeostasis is largely due to posttranscriptional regulation of binding by IRPs to IREs within ferritin and TfR mRNAs. IRP binding is tightly controlled such that it responds to changes in intracellular iron requirements in a coordinate manner, differentially regulating ferritin mRNA translational efficiency and TfR mRNA stability. Most interestingly, we have found recently that T₃ and TRH are capable of modulating the binding of two pituitary IRPs, IRP1 and IRP2, to a ferritin IRE (Thomson *et al.* 1999a). TRH was found to regulate ferritin protein concentrations similarly in thyrotrope and lactotrope cells but, unexpectedly, differentially modulated the binding of IRP1 and IRP2 to a ferritin IRE. However, T₃ downregulated IRP binding in both cell types (unpublished observations). TRH induced an increase in IRP

binding in thyrotropes, associated with IRP phosphorylation and protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) activation. However, in lactotropes, TRH decreased IRP binding despite PKC and MAPK activation. These data demonstrate a divergent and cell-specific regulation of IRP binding by TRH and T₃ in the pituitary. They also represent a further example of a specific RNA-protein interaction in the pituitary that was divergently regulated by TRH and T₃.

These data suggest that multiple RNA-protein interactions may be subject to hormonally divergent regulation in a cell-specific manner. Clearly, we are only just beginning to unravel some of the complexities involved in the fine-tuning of basic cellular functions, such as the control of iron homeostasis. However, this work illustrates the central role that hormones have in the pituitary as regulators of RNA-binding protein binding-activity and intracellular iron concentrations.

CONCLUDING REMARKS

The main thrust of molecular research in understanding hormone action in the pituitary has, in the past, concentrated on transcriptional regulation and nuclear hormone receptor action. However, it is now evident that mRNA stability and hormonal regulation of RNA-protein interactions within pituitary cells also play a crucial role in regulating pituitary cellular function. Investigations in this field are adding to the global knowledge of RNA-binding proteins, RNA-stability sequences, the role of translation in mRNA stability, mechanisms of RNA degradation, and understanding of the molecular effects of hormone action in the pituitary. The cloning and characterization of novel pituitary RNA-binding proteins will allow, for the first time, a detailed analysis of the impact of these interactions on the vast secretory function of the pituitary gland.

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