



## Review

## Translational control of gene expression in the gonadotrope



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## ABSTRACT

The study of gene expression in gonadotropes has largely focused on the variety of mechanisms regulating transcription of the gonadotropin genes and ancillary factors that contribute to the overall phenotype and function of these cells in reproduction. However, there are aspects of the response to GNRH signaling that are not readily explained by changes at the level of transcription. As our understanding of regulation at the level of mRNA translation has increased, it has become evident that GNRH receptor signaling engages multiple aspects of translational regulation. This includes activation of cap-dependent translation initiation, translational pausing caused by the unfolded protein response and RNA binding protein interaction. Gonadotropin mRNAs and the mRNAs of other factors that control the transcriptional and signaling responses to GNRH have been identified as targets of regulation at the level of translation. In this review we examine the impact of translational control of the expression of gonadotropin genes and other genes relevant to GNRH-mediated control of gonadotrope function.

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## 1. Introduction

The development and regulation of reproductive tissues is a complex task that operates through a variety of regulatory

mechanisms. In mammals, the reproductive endocrine axis, consisting of the hypothalamus, pituitary, and gonad, (H–P–G axis) is controlled by a number of feed-forward and feedback signals that impact each level. The regulatory signals range from the synaptic and peptidergic control of the hypothalamic neurons producing the primary releasing factor, gonadotropin-releasing hormone (GNRH), interaction of GNRH and other factors such as activin and insulin modulating pituitary gonadotropin output, and finally

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the impact of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) on the cognate cells of the testis and ovary that dictate germ cell maturation and feedback factor production such as sex steroids, activin, and inhibin. There is increasing evidence that there are regulatory signals originating outside the Hypothalamus–Pituitary–Gonad axis that impact gonadotropin production by the pituitary. Inflammatory stress and obesity are both associated with decreased gonadotropins. The interaction of these cues ultimately defines gonadotropin output and there is an emerging appreciation for the role of the pituitary in interpreting multiple signals. Much of the study of gonadotropin gene expression has been focused on the primary regulation of gonadotropin gene transcription. However, gonadotropin production is not easily explained solely on the basis of transcriptional control and a more complete description of gonadotropin gene expression must incorporate other modes of gene regulation, including the regulation of protein synthesis. A number of studies have implicated translational control in the regulation of gonadotropes and gonadotropin production. Further, the increasing appreciation for the role of stress responses and maintenance of endoplasmic reticulum (ER) homeostasis in secretory cells of all types through the unfolded protein response (UPR) suggests that these processes, which largely operate through translational control, may also play a role in gonadotrope biology as well. This review will discuss our current understanding of translational control of gene expression in the gonadotrope.

## 2. Translational control in human disease

As our understanding of the mechanisms of translational control has increased, it has become clear that a number of diseases involve some component of the regulated translation or the unfolded protein response (Scheper et al., 2007; Walter and Ron, 2011). A variety of conditions lead to disrupted or alternatively regulated translation in mammalian cells. Lytic viral infection results in accumulation of protein in the ER. Some viruses, such as Hepatitis B, manipulate the activation of UPR signaling proteins to elicit an ER proliferative response to aid in viral replication and assembly (Li et al., 2007). Other viruses disrupt normal translational initiation by cleavage of factors necessary for translation initiation such as eukaryotic translation initiation factor 4G (EIF4G) and polyA Binding Protein (PABP) (Lloyd, 2006). Other than compromise by infectious agents, dysregulated protein synthesis is identified in a number of diseases in which tissues are exposed to chronic stress, taxing the ability of the cell to maintain homeostasis. One common example of this is the degenerative disease retinitis pigmentosa, which is caused by accumulation of misfolded rhodopsin in retinal photoreceptor cells. Retinitis pigmentosa is associated with a number of other pathologies including mis-sense mutations of numerous RNA processing enzymes and membrane proteins, suggesting an overall sensitivity of photoreceptors to accumulated mis-translated or unfolded protein (Lin and Lavail, 2010). Neurodegenerative diseases also appear to have a significant relationship to disorders of protein translation (Chang et al., 2007). Most similar to the secretory cells of the reproductive endocrine axis, the impact of increased demand on pancreatic beta cells in Type II diabetes mellitus leads to chronic activation of the UPR that eventually causes loss of some members of the population, increasing demand on the remaining cells, thus establishing a recurring and elevating cycle of increased demand and cell loss that ultimately causes a near or complete loss of insulin production (Fonseca et al., 2011). Overall the wide range of disease types suggests different tissues exhibit different levels of sensitivity to disruption of translation and ER homeostasis, resulting in a variety of consequences.

## 3. Evidence for translational control in the hypothalamus–pituitary–gonad axis

Although disorders of pituitary function or tumors of pituitary origin are well studied, their origins have not been directly attributed to dysregulated protein synthesis or disruption via the UPR, nor has this perspective been examined carefully. There is suggestion in a number of studies that gonadotropin secretion is reduced under conditions of high BMI, stress, or hyperinsulinemia (Arroyo et al., 1997; Pagan et al., 2006; Jain et al., 2007) and the post-translational modification of secreted gonadotropins is altered under these conditions (Srouji et al., 2007). It is also documented that inflammation can reduce gonadotropin output. Although it is yet to be conclusively demonstrated, the known impact of inflammatory cytokines and lipopolysaccharides on UPR activation presents the possibility that some aspect of reduced pituitary gonadotropin release under conditions of stress may be due to the translational impact of UPR activation.

### 3.1. Evidence in animal models

The study of gonadotropin subunit mRNA synthesis in rats provides a strong suggestion that processes other than transcriptional regulation contribute to gonadotropin production. Early examination of the changes in gonadotropin mRNA levels in hemipituitaries subjected to tonic or pulsatile GNRH stimulation showed measurable changes of up to fourfold in LH transcription rate (Shupnik, 1990). Though significant, these rates were less than the typical changes in LH secretion seen during the LH surge or under exogenous GNRH stimulation (Blake et al., 1972; Arimura et al., 1974; Legan and Karsch, 1975). In GNRH-stimulated male rats, LH beta (*Lhb*) steady-state mRNA levels were found to be increased approximately 40% after stimulation with GNRH, although LH secretion was found to be increased by approximately 100 fold (Burger et al., 2001, 2002). The nonlinear increase in protein release by the pituitary indicates that increased gonadotropin production cannot be explained by strict correspondence to increased mRNA. The relatively low level of steady state mRNA response is corroborated by a number of microarray studies that failed to show an increase in *Lhb* mRNA after GNRH stimulation that exceeded the typical 2-fold cutoff for declaring significance (Blake et al., 1972; Legan and Karsch, 1975; Kakar et al., 2003; Zhang et al., 2006; Lawson et al., 2007). Overall, these accumulating observations provide the foundation for the hypothesis that GNRH engages post-transcriptional regulatory processes including the protein synthetic machinery to increase gonadotropin production and release.

### 3.2. Evidence in cell model systems

The regulation of post-transcriptional processes is not well-established in the gonadotrope despite the potential to be a major means of gene regulation. GNRH impacts both mRNA synthesis rates and half-life of *Lhb* mRNA (Shupnik, 1990; Bouamoud et al., 1992; Weiss et al., 1992). The glycoprotein hormone subunit alpha gene (*Cga*) mRNA was shown to be stabilized by GNRH treatment of the immature gonadotrope cell line  $\alpha$ T3-1 (Weiss et al., 1992; Chedrese et al., 1994). Although reports focused on understanding gonadotropin synthesis in the context of transcriptional regulation, evidence emerged shortly thereafter that post-transcriptional control may also contribute to gonadotropin synthesis. Early studies of GNRH receptor (GNRHR) expression showed regulation of receptor synthesis activity despite no change in mRNA content after GNRH stimulation (Tsutsumi et al., 1993, 1995). GNRHR synthesis increased in GNRH-stimulated cells and in *Xenopus* oocytes injected with RNA isolated from these cells, indicating an RNA-based

increase in efficiency of the template (Tsutsumi et al., 1993). A decrease in GNRHR mRNA association with polyribosomes was also found in GNRH-desensitized cells (Tsutsumi et al., 1995). A second indication of the role of translational control was found in the observation of a marked, transient increase in luciferase activity directed by a *Cga* promoter reporter gene (Chedrese et al., 1994). Luciferase values peaked within 4 h of GNRH stimulation, whereas endogenous *Cga* mRNA levels did not reach maximal levels until 24 h after stimulation, at which time luciferase values also stabilized at a lower level. One interpretation of this observation is that luciferase activity was elevated through an increase in protein synthesis that preceded increased mRNA levels. Taken together with the observations in vivo and primary pituitary, a circumstantial case for the contribution of translational control of gonadotropin synthesis can be made.

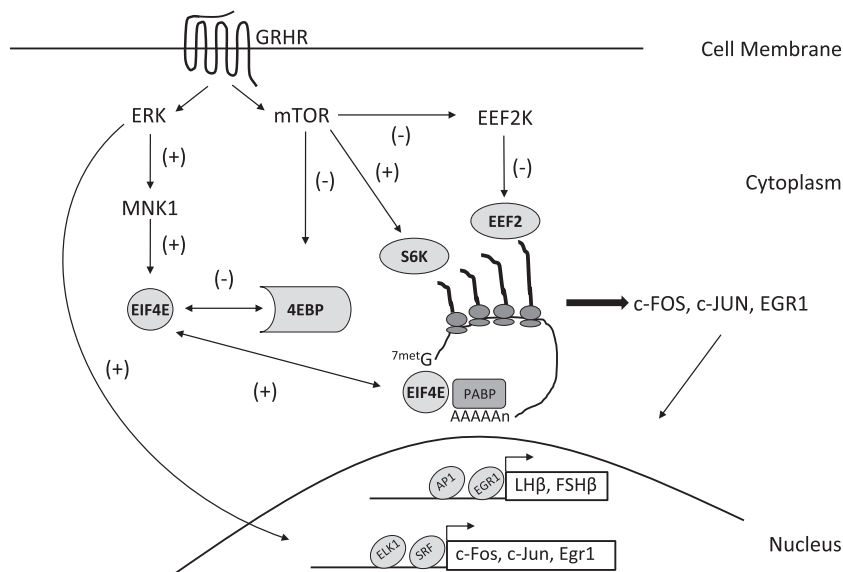
#### 4. Control of mRNA translation

Eukaryotic translation is a complex process that employs many factors which coordinate the interaction of mRNA and aminoacyl-tRNA with the 40s and 60s ribosomal subunits. The translation process can be distinguished by its three main stages of initiation, elongation, and termination. The initiation stage involves the coordinated assembly of the ribosomal subunits at the capped 5' end of the mRNA and identification of the AUG start codon by the 40S ribosomal subunit. Assembly of the initiation complex involves a number of factors known collectively as the eukaryotic translation initiation factors. This process is generally recognized as the rate-limiting step in translation (Rau et al., 1996). The elongation stage is the progressive addition of amino acids to the nascent polypeptide chain from start codon to stop codon and involves the translocation of the ribosome on the template and delivery of charged tRNAs. At the termination stage, the nascent peptide, ribosomes and mRNA binding factors are released from the mRNA or recycled for a subsequent round of translation (reviewed in (Scheper et al., 2007)). Translating polyribosomes are remarkably stable and translation complexes may contain one or more mRNA molecules

in a circular head-to-tail or anti-parallel conformation that efficiently recycles terminating ribosomes (Kopeina et al., 2008). Of particular importance for secretory cells, protein synthesis is compartmentalized based on the nature of the protein being synthesized. Soluble proteins can be synthesized on free ribosomes in the cytosol and secretory proteins, integral membrane proteins, and ER resident proteins are translated on ER-associated ribosomes. For membrane-bound or exported proteins, this allows for the growing polypeptide to be co-translationally exported to the ER lumen, folded, modified, and transported to the final target or shunted to the secretory pathway and packaged into secretory vesicles. It is these key differences in how individual mRNAs are translated that provide some basis of differential regulation of translation by ER-bound regulatory factors.

#### 4.1. Cap-dependent and elongation translational control

Translation initiation in eukaryotes is a complex process that provides opportunity for regulation through targeting of the activity and availability of cap-binding initiation factors. Eukaryotic translation initiation factor 4E (EIF4E) binds the 7-methylguanylate cap that occurs on most eukaryotic mRNAs derived from the nucleus. The association of EIF4E with capped mRNA is the rate-limiting step in translation initiation (Fig. 1). Subsequent to cap binding, EIF4E promotes the formation of the cap-dependent initiation complex that incorporates both 5' and 3' untranslated region (UTR) binding factors such as PABP, EIF4G mediating circularization of the initiating mRNA and the association of the 40S ribosomal subunit. The availability of EIF4E is regulated through phosphorylation of its binding protein and negative regulator, 4E-binding protein1 (4EBP1) and its related proteins (Fig. 1). 4EBP1 is a substrate of the mammalian target of rapamycin (mTOR) kinase and bears multiple serine phosphorylation target sites. 4EBP1 competes with the scaffolding protein EIF4G for EIF4E binding and its phosphorylation facilitates EIF4E release, allowing eIF4E to form a complex with EIF4G and establish a functional initiation complex. (Gray and Wickens, 1998; Kleijn et al., 1998).



**Fig. 1.** Cap-dependent and elongation translational control by GNRH. Activated GNRH receptor leads to a rapid activation of the mTOR and ERK signaling cascades. mTOR reduces 4E binding protein (4EBP) activity by inhibitory phosphorylation, releasing the 5' cap-binding protein EIF4E. EIF4E is also positively regulated by phosphorylation through the ERK/MNK1 pathway. Binding of EIF4E to the 5' cap structure stimulates formation of the initiation complex and promotes ribosome binding and translation initiation. Translation elongation is also affected by mTOR through activating phosphorylation of the p70 ribosomal subunit S6 kinase (S6K) and by inhibitory phosphorylation of the Eukaryotic Elongation Factor 2 Kinase (EEF2K), a suppressor of Elongation Factor 2 (EEF2). Thus GNRH receptor promotes translation initiation and elongation through a combination of activating and inhibitory phosphorylation events that modulate exposure of mRNA to translational machinery through initiation and that modulate translation efficiency through regulation of elongation.

Similarly, EIF4E may also be controlled by the fragile X mental retardation protein FMR1 through cytoplasmic FMR1 interacting protein (CYF1P1), which also binds EIF4E (Napoli et al., 2008; Iacoangeli and Tiedge, 2013).

In the  $\alpha$ T3-1 cell line GNRH rapidly stimulates hyperphosphorylation of 4EBP1, suggesting an increase in cap-dependent translational activity (Sosnowski et al., 2000). Evidence for cap-dependent translation was examined using a bicistronic reporter gene directing synthesis of a single mRNA bearing a cap-dependent open reading frame encoding luciferase followed by the murine encephalomyocarditis virus internal ribosomal entry site (IRES) mediating cap-independent translation of a second reading frame encoding  $\beta$ -galactosidase. Under GNRH stimulation luciferase reporter activity derived from the cap-dependent reading frame was increased relative to IRES-mediated  $\beta$ -galactosidase activity, demonstrating an increase in cap-dependent gene expression. The impact of increased translational activity on gonadotropin synthesis was demonstrated by the observation that increased LH synthesis in response to GNRH stimulation could occur independently of increased mRNA synthesis (Nguyen et al., 2004). Pre-treatment of the gonadotropin-expressing cell line L $\beta$ T2 with the transcriptional inhibitor actinomycin D did not block increased LH synthesis after GNRH stimulation. The increase in LH occurred within 4 h of stimulation, prior to the known peak of mRNA synthesis and similar to the increase in reporter gene expression reported above, indicating that the acute LH synthesis response can be explained by increased protein synthesis in the absence of increased mRNA synthesis (Nguyen et al., 2004).

Modulation of protein synthesis through regulation of the cap-binding factors operates through a combination of positive and negative regulatory signaling events that increases activity of some factors and inhibits the activity of constitutive repressors. Regulation occurs mainly through the Extracellular Signal Regulated Kinase (ERK, or MAPK 1/3) and the mammalian Target of Rapamycin (mTOR) (Fig. 1). Signaling through mTOR increases translation through phosphorylation of 4EBP1, reducing binding to EIF4E and promoting formation of the capped initiation complex. Signaling by mTOR also increases translation through phosphorylation of ribosomal protein S6 kinase (p70<sup>S6k</sup>), stimulating mRNAs containing 5' terminal oligopyrimidines. A third effector, eukaryotic elongation factor 2 kinase (EEF2 Kinase) is also targeted by inhibitory mTOR (Fig. 1). The EEF2 Kinase inhibits ribosomal translocation by phosphorylation of eukaryotic Elongation Factor 2. Thus inactivation of EEF2 Kinase relieves repression of EEF2 activity, thereby stimulating elongation (Kleijn et al., 1998; Dennis et al., 1999; Proud, 2006). Activation of mTOR signaling (Raught et al., 2000) and 4EBP1 may be additionally targeted by ERK activation (Kleijn et al., 1998), thus providing potential cross-talk between other receptors and signaling cascades. EIF4E activity may also be regulated through phosphorylation by the Map Kinase Interacting Kinase1 (MNK1) (Kleijn et al., 1998). There are conflicting data suggesting the phosphorylated form of EIF4E has higher affinity for the 5' cap and also evidence that interaction with EIF4G may stabilize cap interaction (Rhoads, 1993; Scheper and Proud, 2002; Slepnev et al., 2008). Inhibition of MAP kinase activity reduces GNRH-induced phosphorylation of EIF4E and EIF4G. The MAP kinase interacting kinase MNK targets EIF4E (Ueda et al., 2004; Walsh and Mohr, 2004) and it was shown to be sensitive to blockade or ERK activation by GNRH (Nguyen et al., 2004). Furthermore, the mTOR inhibitor rapamycin reduces the translation activity induced by GNRH in both  $\alpha$ T3-1 and L $\beta$ T2 cells (Sosnowski et al., 2000; Nguyen et al., 2004).

Acute increases in translational activity in response to GnRH can contribute to the rapid synthesis of immediate early genes such as c-FOS, c-JUN, and EGR1, which contribute to gonadotropin mRNA synthesis (Fig. 1). Maximal activation of cap-binding protein

by GNRH occurs within 30 min of stimulation, providing a rapid mechanism to increase gene expression in conjunction with immediate early gene transcription. However, generalized stimulation of cap-dependent translational activity by GNRH does not explain gene-specific regulation. Although evidence of differential utilization of mRNA based on 5'UTR structure has been presented (Ding et al., 2012; Rao et al., 2013) and this may contribute to sensitivity to cap-dependent initiation, targeting of specific mRNAs may require additional regulatory schemes that affect other aspects of the length and structure of the 5'UTR and mRNA exposure to the translational machinery (Wegrzyn et al., 2008).

Internal ribosome entry sites (IRES) are highly structured nucleotide sequences located in the 5'UTR of mRNAs that promote translation in a cap-independent manner. Activity of IRES have been demonstrated in the 5' noncoding region of picornavirus RNAs (Jang et al., 1988). Other IRES's are also found in the mRNAs of *Hspa5*, or BiP (Macejak and Sarnow, 1991) and now many examples of eukaryotic mRNAs are found to contain IRES sequences (Lopez-Lastra et al., 2005; Mokrejš et al., 2006). Another related mechanism has also been identified that involves internal ribosome binding to a polyadenylate tract upstream of the initiator AUG and subsequent ATP-dependent scanning for the initiation sequence (Shirokikh and Spirin, 2008). To date it is not clear if IRES-containing or internally initiated mRNAs are targeted by GNRH signaling in gonadotropes.

#### 4.2. The unfolded protein response and translational control

The ER is an oxidative environment where protein folding and post-translational modification of proteins that are secreted or targeted to the plasma membrane occurs (Wickner and Schekman, 2005). Because of the compartmentalization of translation of these proteins in the ER, the integrity of the ER is important to maintain the fidelity of translation. The UPR is a quality control pathway that maintains this integrity by monitoring changes in the ER lumen that perturb protein folding capacity. Disruption of the ER lumen is a consequence of pathological conditions such as hypoxia, viral infection and starvation, or of normal physiological processes such as secretion or increased protein synthetic demand. ER stress can also be induced experimentally by the overexpression of misfolded proteins or by pharmacological insult that targets glycosylation, calcium, or oxidative balance. The UPR seeks to re-establish balance by decreasing the burden through attenuating translation and degrading misfolded proteins, as well as increasing synthetic capacity by increasing the size of the ER and the capacity of the protein-folding machinery. If balance is not reached, the UPR induces apoptosis. The UPR is a multifaceted regulatory process that modulates translational activity and posttranslational processing of proteins, a process has been reviewed in detail (Walter and Ron, 2011). The major ER-resident signaling proteins mediating the UPR are eukaryotic translation initiation factor 2- $\alpha$  kinase 3 (EIF2AK3 or PERK) (Merrick, 2004), endoplasmic reticulum to nucleus signaling 1 (ERN1 or IRE1), and activating transcription factor-6 (ATF6). Each of these sensors plays a distinct role in the modulation of translation and transcription in response to stress, low nutrition, or high protein synthesis and secretion demands. The folding and transport of exported or membrane-bound protein occurs within the endoplasmic reticulum. Over production of protein or loss of the oxidative environment in the ER lumen caused by displacement of calcium during a secretory event may lead to accumulation of misfolded proteins, which induces ER stress via the UPR (Fig. 2). Both EIF2AK3 and ERN1 exist as monomers in the ER membrane and share the common characteristics of a luminal domain that acts as a sensor of ER homeostasis paired with a cytosolic domain with kinase and/or RNase activity. The sensing mechanism is not fully understood but involves either association

with the HSP70 chaperone or calcium ion to maintain monomeric protein. Upon loss of this interaction caused by increased unfolded protein or loss of redox tone, EIF2AK3 and ERN1 independently form homodimers (Fig. 2) or larger complexes in the membrane and trans activate via their homologous kinase domains (Korennykh et al., 2009; Li et al., 2010). Subsequently EIF2AK3 and ERN1 initiate both translational and transcriptional responses via their respective kinase and RNase activities. Alternatively, ERN1 is also capable of interaction with unfolded or highly hydrophobic proteins and this may serve as a method of direct activation (Gardner and Walter, 2011; Kawaguchi and Ng, 2011). The UPR is crucial for the function of secretory cells, including  $\beta$  cells, hepatocytes, and osteoblasts (Gass et al., 2004; Wu and Kaufman, 2006; Volchuk and Ron, 2010), all of which have heavy protein synthesis demands and thus rely on the proper function of the ER in order to maintain secretory output. It has also been recently described in the Leydig cell where UPR induction causes a decrease in steroid output through activation of the ATF6 pathway and ER stress-mediated apoptosis (Park et al., 2013).

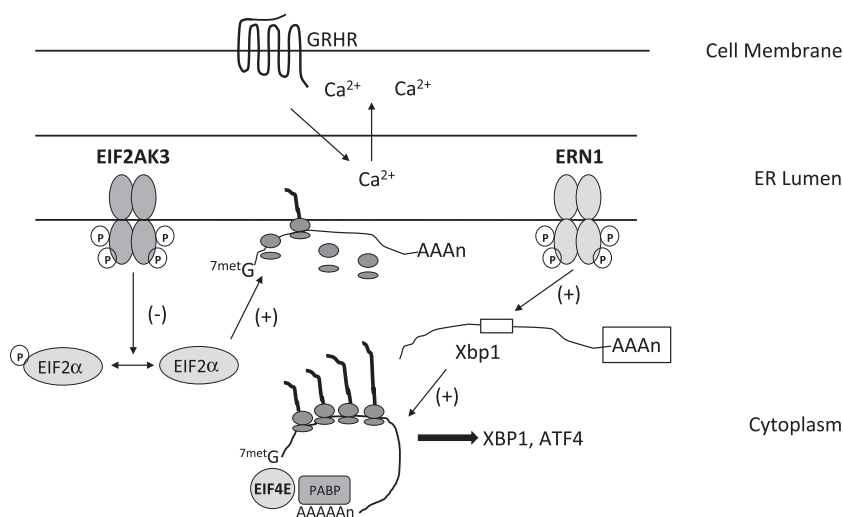
#### 4.2.1. EIF2AK3 and translation initiation

The activation of EIF2AK3 leads to an immediate attenuation of general translation through phosphorylation of translation initiation factor eukaryotic translation initiation factor 2A (EIF2A) (Shi et al., 1998; Rouschop et al., 2013). EIF2A forms part of the tripartite initiation complex EIF2, itself a component of the ternary complex that also contains the initiator methionyl-tRNA and GTP. EIF2A phosphorylation inhibits the formation of this complex and blocks further initiation of translation, thus reducing the flux of new protein into the ER lumen by translational pausing (Fig. 2). Pausing can be resolved by dephosphorylation by the GADD34/PP1 holophosphatase complex (Novoa et al., 2001, 2003; Brush et al., 2003). Mice that lack functional EIF2AK3 in insulin-secreting pancreatic  $\beta$  cells have elevated serum glucose levels compared to wild-type littermates and eventually experience  $\beta$  cell apoptosis and diabetes (Harding et al., 2001; Zhang et al., 2002a). Transgenic mice harboring a mutation in EIF2A (Ser51Ala), which eliminates phosphorylation, show impaired insulin production and loss of

insulin-positive  $\beta$  cells. Most transgenic neonates die within 18 h of birth (Scheuner et al., 2001). Loss of ER sensing by EIF2AK3 or the inability to constrain protein export both contribute to cell death indicating that the appropriate regulation of protein synthesis in is essential to  $\beta$  cell development or differentiated function. In somatotropes, aberrant growth hormone synthesis due to inappropriate mRNA splicing leads to cell death, supporting the essential role for proper control of protein quality and translation in secretory cells of the anterior pituitary (Ariyasu et al., 2013).

Not all mRNAs are affected by translational pausing caused by EIF2A phosphorylation. The mRNA encoding ATF4 is efficiently translated in these conditions (Fig. 2). ATF4 is a bZIP transcription factor which stimulates genes that further the UPR program, is translationally activated by an alternative reinitiation mechanism that is increased by translational pausing due to EIF2A phosphorylation (Vattem and Wek, 2004; Dey et al., 2010). ATF4 target genes are involved in amino acid transport and synthesis, metabolism, and the antioxidant response (Shan et al., 2009; Han et al., 2013). Microarray studies have shown that GNRH treatment of gonadotrope cells increases mRNA levels of *Atf3* (Kakar et al., 2003; Zhang et al., 2006; Lawson et al., 2007), a transcription factor targeted by the EIF2AK3/ATF4 arm of the UPR (Jiang et al., 2004).

EIF2AK3 phosphorylation is induced by GNRH in  $L\beta T2$  cells, but is unique in that the overall level of activation is moderate in comparison to the typical pharmacological insult used in most studies (Do et al., 2009). Similarly, EIF2A phosphorylation is moderate as well. Accordingly, both *Atf4* mRNA and its target gene *Ddit3* (also called CHOP) are increased in response to GNRH stimulation, and have a peak activation level under tonic rather than pulsatile stimulation, indicating an elevated stress response under tonic stimulation (Lawson et al., 2007). Interestingly, GNRH induction of the UPR results in an overall remodeling of the polyribosome profile in  $L\beta T2$  cells that is also modest in comparison to pharmacological induction of the UPR by dithiothreitol (Do et al., 2009). Both *Cga* and *Lhb* mRNAs are targeted by this inhibition, as would be expected of secretory proteins passing through the ER. It is also significant that GNRH induction of the UPR is transient, and both the remodeling of the polyribosomal profile and pausing of *Lhb* and *Cga*



**Fig. 2.** GNRH engages the UPR effectors EIF2AK3 and ERN1 to regulate translation. GNRH receptor activation elicits the UPR possibly through loss of calcium from the ER. As part of the secretion response to GNRH stimulation, GNRH receptor activates of the IP<sub>3</sub> signaling cascade resulting in displacement of calcium ion from the ER lumen and potentially altering redox homeostasis. Consequently, accumulated unfolded protein or loss of chaperone interaction initiates formation of EIF2AK3 homodimers and phosphorylation-induced transactivation. Subsequently, EIF2AK3 inactivates EIF2 $\alpha$  by inhibitory phosphorylation. As part of the EIF2 component of the translation initiation ternary complex delivering the initiating methionyl tRNA to the ribosome, EIF2 $\alpha$  inhibition results in a blockade of translation initiation. Similarly, ERN1 forms homodimers in response to GNRH receptor activation and initiates cytoplasmic processing of *Xbp1* mRNA which, in conjunction with the alternative translation of *Atf4* mRNA and the UPR-induced processing and release of ATF6, initiates a program of ER adaptation and proliferation. Unresolved activation of the ERN1 signaling arm of the UPR can lead to apoptosis (not shown).

mRNA is transient, resolving within 60 min after GNRH treatment. The transient nature of activation suggests the presence of efficient negative feedback control that is not well described, and a physiological integration of the UPR with the normal GNRH response.

#### 4.2.2. ERN1 and Xbp1 splicing

Activation of the kinase/endoribonuclease ERN1 initiates the splicing of *Xbp1* mRNA, another bZIP transcription factor central to the UPR transcriptional response program. Spliced *Xbp1* mRNA is also translated under conditions of cellular stress, thus avoiding the generalized decrease in translation caused by EIF2A phosphorylation (Fig. 2). In concert with ATF6 (discussed below) XBP1 targets an extensive network of over 70 genes involved in all aspects of ER homeostasis and protein folding including *Dnaj*, *Hsp5A*, and *Xbp1* itself (Lee et al., 2003; Shoulders et al., 2013). Loss of *Xbp1* in B lymphocytes in mice results in failure to differentiate into immunoglobulin-secreting plasma cells and ultimately failure to mount an immune response to polyoma virus infection (Reimold et al., 2001). ERN1 is also required for proper immunoglobulin production and plasma cell differentiation (Zhang et al., 2005). Both primary pituitary cells and L $\beta$ T2 cells respond to GNRH treatment by inducing *Xbp1* splicing. XBP1 acts as a sensing molecule of ER stress by activating either an adaptive or apoptotic response based on degree and duration of activation. Chronic activation of ERN1 results in sustained XBP1 production that, unresolved, leads to the induction of apoptosis in a number of cell types (Zeng et al., 2009; Allagnat et al., 2010; Jiang et al., 2012; Park et al., 2013).

In gonadotropes, as with EIF2AK3 activation, *Xbp1* splicing is not exhaustive in response to GNRH. This observation is significant in the context of the finding that truncated XBP1 expressed from the pre-mRNA acts as a negative regulator of the fully active form expressed from the mature, spliced mRNA. Thus the modest induction of *Xbp1* splicing may indicate the maintenance of negative feedback in the physiological context of GNRH stimulation (Yoshida et al., 2006). The chronic high level activation of *Xbp1* splicing by extreme insult or stress as is found with pharmacological activation of the UPR with dithiothreitol or peroxide may represent a fully activated stress response mediated by unrestrained XBP1 production, whereas the moderate induction by GNRH may represent a balanced induction capable of rapid resolution rather than conversion to an apoptotic response characteristic of unresolved or chronic stress.

#### 4.2.3. ATF6 and target gene expression

A third arm of the UPR regulating gene expression involves the proteolytic activation of the bZIP transcription factor ATF6. Activation ATF6 does not directly involve translational control and is unique in character. ATF6 exists as an ER membrane-resident transcription factor that is anchored by a luminal domain. Accumulation of unfolded proteins triggers transport of vesicles containing ATF6 to the golgi where the luminal domain is sequentially removed by S1P and S2P proteases normally associated with sterol response element binding protein processing (Ye et al., 2000; Shen and Prywes, 2004). The exact mechanisms of proteolytic processing of ATF6 and the nature of luminal-domain sensing of ER stress are not well defined but it is proposed that the unusual structure of the luminal domain contain redox-sensitive disulfide bridges or chaperone interaction domains that monitor the status of the ER (Walter and Ron, 2011).

ATF6 targets a number of genes independently or in concert with XBP1 and co-regulates the expression of genes that maintain ER homeostasis. These include *xbp1* and the chaperone *Hspa5* but also include a number of genes involved in protein folding (Shoulders et al., 2013), degradation and disulfide bond formation. The cooperative action of ATF6 and XBP1 in target gene activation provides a mechanism of coordinated regulation of adaptation to stress or

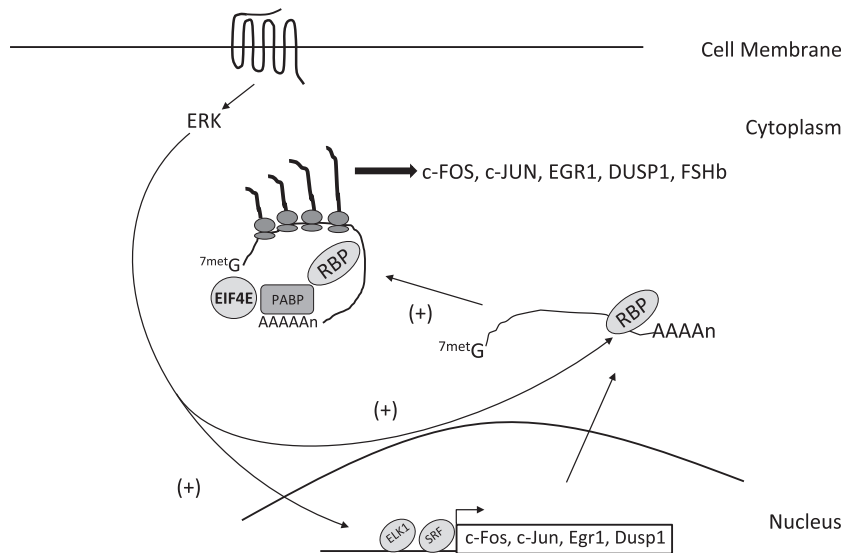
initiation of apoptosis that is not only tied to changes in the ER lumen but also engages the Golgi. Although ATF6 is expressed in L $\beta$ T2 gonadotrope cells and target genes such as *Ddit3*, *Hspa5* are activated in response to GNRH stimulation (Lawson et al., 2007), it has not yet been shown that ATF6 itself is processed in response to GNRH.

## 5. Post-transcriptional control of gene expression

Primary transcript RNAs must undergo post-transcriptional processing and transport prior to use as a template for protein production. This process includes splicing, export, localization, turnover and translation initiation. Post-transcriptional regulation of gene expression provides a mechanism for modulation of protein synthesis that acts on template availability and efficiency of utilization, and therefore can have a significant impact on translation. In general, mRNA transport and utilization depends on the action of specific RNA binding proteins (RBPs) binding to the 5' or 3' UTR that targets individual mRNAs to the translation apparatus or alternatively to storage or degradation (Fig. 3). Post-transcriptional regulation may play a significant role in the expression of gonadotropins genes in the pituitary. In studies described above (Burger et al., 2001, 2002), rapid GNRH pulses stimulated *Lhb* primary transcript levels 6 to 9-fold after 8 h of treatment, but mature *Lhb* mRNA only increased 1.5-fold and serum LH increased approximately 100 fold prior to the maximal change in either RNA species. It was not clear if primary transcript levels increased due to elevated synthesis or delay in processing. Activity of the *Lhb* promoter is increased by GNRH stimulation, and *Lhb* mRNA half-life is approximately 2.7 h after GNRH stimulation (Dalkin et al., 2001). This indicates that post-transcriptional control participates in establishment or maintenance of mRNA levels by delaying the maturation of *Lhb* mRNA. Moreover, the GNRH-stimulated increase in translational capacity of  $\alpha$ T3-1 cells occurs concurrently with previously reported changes in *Cga* mRNA stability (Bouamoud et al., 1992). It has been shown that *Cga* mRNA half-life is increased in  $\alpha$ T3-1 cells with GNRH treatment from 1.2 to 8 h (Bouamoud et al., 1992) However, in untreated cultured rat pituitary cells the half-life of mRNA is about 6 h (Bouamoud et al., 1992). It is possible that factors in addition to GNRH contribute to *Cga* mRNA stability. Here, we focus on recent global studies of mRNA stability, and RBPs in the gonadotrope.

### 5.1. Control of mRNA stability

Stabilization of short-life transcripts stimulates high steady-state levels and contributes to dramatic variations in gene expression. In general, there is a positive correlation between the half-lives of mRNAs and proteins, with short-lived mRNAs usually encoding short-lived proteins and vice versa (Hargrove and Schmidt, 1989; Hollams et al., 2002). The stability of mRNA depends on its structure and decay is triggered by at least three types of initiating events; poly(A) tail shortening, arrest of translation at a premature nonsense codon, and endonucleolytic cleavage (Hollams et al., 2002). The regulated turnover of mRNA depends on a variety of protein factors interacting with *cis*-acting sequences motifs that play a role in determining the basal half-life of a particular mRNA species. Most *cis*-elements are located in the 3'UTR and are targets for the binding of RBPs that determine the fate of the mRNA. A number of *cis*-elements have been identified such as AU-rich elements (AREs), the thyrotropin-releasing hormone (TRH)-receptor element, the iron-responsive element (IRE), histone stem loop, poly (A) binding element, and the stem loop destabilizing element (Hollams et al., 2002). AREs have been widely studied and are found in the 3'UTRs of a variety of short half-life of mRNAs



**Fig. 3.** RNA binding proteins modulate mRNA stability and accessibility to translational machinery. The RNA-binding proteins (RBP) HuR, NF90, or CSDA interact with the 3' UTR of immediate-early gene mRNAs encoding *c-Fos*, *c-Jun*, and *Egr1*, the ERK feedback regulator *Dusp1*, and the *Fshb* gonadotropin subunit to control stabilization and/or availability to the translation initiation machinery. RBP's themselves are targets of ERK or other MAP kinase activity that promotes nuclear translocation or interaction with other factors. The precise roles of RBPs in mRNA utilization or degradation is not defined and interaction with some factors may not persist in the translationally-active mRNA-ribosome complex.

such as cytokines, proto-oncogene, and transcription factors (Doller et al., 2008).

### 5.2. Regulation by RNA-binding proteins

After transcription, RNA is associated with one or several RBPs. A number of RBPs regulate the splicing, export of mRNA to the cytoplasm, maintenance of mRNA in the cytoplasm for translation, and finally decay of the mRNA (Fig. 1). RBPs contain one or more RNA-binding domain (Hollams et al., 2002; Glisovic et al., 2008). And affinity for their target *cis*-elements are regulated by varying factors such as hormone signaling, cytokines, UV light, and developmental stage (Glisovic et al., 2008). RBPs known to promote translation of target mRNAs have been examined in the context of gonadotropin gene expression, the cold-shock domain protein A (CSDA) and embryonic lethal abnormal vision (ELAV)-like protein 1/human antigen R (HuR) (Fig. 3).

Cold-shock domain-containing (CSD) proteins contain the evolutionarily conserved nucleic acid binding domain found in eubacteria, archaeobacteria, plants, and animals. The cold-shock domain is capable of binding RNA as well as single or double stranded DNA. The general function of CSD proteins are as participants in mRNA stability and as mRNA chaperones that promote translation. In L $\beta$ T2 cells CSDA is highly expressed relative to  $\alpha$ T3-1 cells and binds to the 3'UTR of *Egr1* mRNA (Chauvin et al., 2012). Differences in CSDA expression correlate to differences in expression of EGR1. GNRH induction of EGR1 protein is pronounced and mRNA half-life is extended from 11 to 24 min in L $\beta$ T2 cells compared to  $\alpha$ T3-1 cells. Additionally, expression of a luciferase reporter gene bearing the *Egr1* CSDA interaction sequence is enhanced and GNRH treatment of L $\beta$ T2 cells results in increased association of *Egr1* mRNA with CSDA. Overall these observations provide strong evidence that translational enhancement of *Egr1* mRNA occurs in the presence of CSDA in gonadotropes.

Hu proteins are vertebrate homologs of the *Drosophila* protein ELAV, which are involved in nervous system development and function. The posttranscriptional regulation of gene expression by Hu proteins has been implicated in a wide range of processes involved in cell growth and differentiation and their function is also

sensitive to regulation via ERK (Yang et al., 2004; Yashiro et al., 2013). There are several ELAV-like (Hu) protein family members including the predominantly cytoplasmic and neuron-specific (HuB/Hel-N1, HuC and HuD) and the nuclear-localized (HuA/HuR) (Hollams et al., 2002). ELAV proteins contain three RNA recognition motifs that are components of their RNA-binding domains. The bovine, mouse, human and rat *Fshb* mRNAs have six HuR-targeted ARE elements (AUUUUUUU) in the 3'UTR (Manjithaya and Dighe, 2004). The 3'UTR of bovine *Fshb* mRNA reduced expression of a reporter gene in both NIH/3T3 and  $\alpha$ T3-1 cells but this was overcome in NIH/3T3 cells by overexpression of HuR (Manjithaya and Dighe, 2004). Interestingly, rescue was not possible in  $\alpha$ T3-1 cells, indicating that factors other than HuR may also interact with the *Fshb* 3'UTR and prevent HuR-mediated stabilization and translation (Fig. 3). The ARE recognized by HuR is also recognized by the mRNA-stability regulator ZFP36 and this and other factors may also contribute to the overall stability and translatability of the mRNA (Zhang et al., 2002b; Sanduja et al., 2011).

A second HuR target is also relevant to translational control and gonadotropin gene expression. The dual specificity protein phosphatase 1 (DUSP1) is an important negative feedback regulator of ERK activity. DUSP1 is constitutively expressed in L $\beta$ T2 cells, and mRNA and protein levels are increased in response to GNRH stimulation. DUSP1 protein modulates ERK activation in L $\beta$ T2 cells and affects *Lhb* promoter activity (Nguyen et al., 2010). The *Dusp1* mRNA contains a 3'UTR ARE which binds HuR, causing improved stabilization and translation (Kuwano et al., 2008). This site is also recognized by the translational repressor NF90 (Kuwano et al., 2010). Other immediate-early response genes induced by GNRH, including *c-Fos*, *c-Jun*, and *Egr1* contain AREs in their 3'UTR (Peng et al., 1998; Mou et al., 2012). Elevation of cytoplasmic HuR level inhibits ARE-mediated decay of *c-Fos* mRNA and HuR protein binding to AREs is associated with reduced deadenylation (Hollams et al., 2002). Therefore, high expression of HuR proteins may regulate stability of *Dusp1* *c-fos*, *c-Jun*, and *Egr1* mRNA in gonadotropes. Finally, HuR is associated with sucrose gradient fractionation of polyribosomes, suggesting that HuR-bound mRNAs are actively engaged in translation (Mou et al., 2012). However, clear evidence has yet to be presented showing that HuR presence in

polyribosome-containing sucrose gradient fractions is due to association with active polyribosomes or due to co-sedimentation with RNA engaged in other ribonucleoprotein complexes.

## 6. Conclusions and future directions

It is clear that translational control is an important component of the overall regulatory regime that determines gene expression in the gonadotrope. As detailed in this review, translation is particularly prominent in gene regulation through GNRH receptor signaling and translational activation provides an acute response to receptor signaling that complements the transcriptional response. Consideration of the role of translational control of gonadotropin gene expression has provided a more complete description of the response of the gonadotrope to GNRH stimulation. A complete model of gene expression in the gonadotrope must incorporate transcriptional control, translational control and the content and activity of the proteome. Translational control is an important link between the nucleus and the proteome; a greater understanding of both will benefit from a thorough understanding of the regulatory process modulating protein production. A number of questions remain to be addressed with respect to the impact of translational control on gene expression in the gonadotrope. Although GNRH receptor signaling rapidly engages the cap-dependent initiation machinery, the specificity of this action is not clear and it remains to be determined if it is a generalized response or targets particular mRNAs, particularly the immediate early response genes that subsequently modulate gonadotropin subunit gene expression. It may be that activation of cap-dependent initiation acts as a counterbalance to the transient inhibition of translation caused by the UPR, facilitating a rapid recovery from an acute stress such as secretion. Further, the magnitude of UPR induction by GNRH is intermediate in character to that induced by pharmacological insult, which is commonly used to study the UPR. The partial response may be due in part to the moderating effect of cap-dependent translation activation. The study of the UPR in gonadotropes was conducted in cells naïve to GNRH stimulation. It is presumed that the UPR will lead to increased ER proliferation and adaptation to GNRH stimulation, but this has yet to be determined. It is possible that UPR induction in cells repeatedly exposed to GNRH in a pulsatile manner may further reduce the extent of UPR induction as they adapt. Examination of UPR induction and resolution under pulsatile GNRH conditions will shed light on the role of the UPR and the adaptive response in gonadotropin production throughout the ovulatory cycle.

Finally, the discovery of the role of the UPR and translational control in gonadotropin production provides a new perspective on disorders of reproduction and how the gonadotrope may be a point of integration of other signals that impact reproductive fitness. The UPR can be invoked by a number of mechanisms including hypoxia, inflammation, lipopolysaccharide signaling, and generation of reactive oxygen species through receptor signaling and fatty acid metabolism. All of these may potentially impact the gonadotrope and compromise gonadotropin production through chronic activation of the UPR. The suppression of gonadotropin output in individuals with high BMI as noted in the studies above may potentially reflect a chronic stress imposed by the endocrine and metabolic signals associated with increased adiposity. The sensitivity of gonadotropin production to stress signaling may participate in the overall determination of reproductive fitness and modulate interpretation of hypothalamic input. Evidence of stress signaling in the Leydig cell by gonadotropin signaling suggests that similar stress sensing occurs in other tissues of the H–P–G axis and in concert, can lead to profound changes in reproductive fitness. The UPR is also known to play an important role in

neuronal function and these studies suggest that GNRH or other hypothalamic neurons may also be subject to restraint through stress signaling. More generally, other endocrine axes may also be subject to similar physiological feedback control through stress signaling and translational control. These exciting topics remain to be addressed in future studies.

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