Commentary

Post-transcriptional regulation of gene expression by androgens: recent observations from the epidermal growth factor gene

J C Pascall

Steroid hormones generally mediate their effects by interacting with specific receptors which then bind to defined DNA sequences in the regulatory regions of target genes to activate expression (see Gronmeyer (1992) and references therein). However, the post-transcriptional regulation of gene expression by steroid hormones is also well documented (see Nielsen & Shapiro 1990). Indeed steroid hormones were amongst the first agents to be demonstrated to play a role in mRNA stabilization (Palmiter & Carey 1974). For example, glucocorticoid hormones have been shown to enhance the stability of growth hormone mRNA (Paek & Axel 1987), testosterone has been reported to induce changes in the poly(A) tail length of the mRNA encoding cystatin-related protein (Vercaeren *et al*. 1992) and testosterone and/or oestrogen induce changes in the poly(A) tail length of the vasopressin mRNA (Carter & Murphy 1993) associated with changes in mRNA accumulation. However, it is still unclear how steroids mediate these specific actions. This commentary will discuss recent results from studies on the androgenic regulation of epidermal growth factor (EGF) gene expression which give some clues as to how these mechanisms may operate.

EGF is a 53-amino acid polypeptide, first purified in the early 1960s from its most abundant known source, the male mouse submaxillary gland (SMG) (Cohen 1962). In the SMG, EGF mRNA and protein accumulation can be regulated by several hormones including androgens, thyroid hormones, dexamethasone, progesterone and oestrogen (see e.g. Gresik *et al*. 1981, Gubits *et al*. 1986, Kurachi & Oka 1986, Pascall *et al*. 1989, Maruyama *et al*. 1993). Probably the best studied of these hormonal controls is that mediated by androgens. As early as 1972, a paper was published demonstrating that EGF protein levels were higher in the SMG from male than female animals and that androgen administration to females increased EGF protein in the SMG, whilst castration of males reduced EGF protein levels (Bynny *et al*. 1972). These results were extended in the 1980s to show similar effects on EGF mRNA levels (see e.g. Gubits *et al*. 1986, Pascall *et al*. 1989). However, it is only recently that any information about the mechanisms involved in this androgenic regulation of EGF gene expression have begun to appear.

The first question that needs to be addressed is whether the regulation of EGF gene expression by androgens is a transcriptional or post-transcriptional event. Surprisingly, to my knowledge, there are no reports of the measurement of transcription rates from the EGF gene in nuclei isolated from mouse SMG after androgen administration. Work in my laboratory has shown that a 5·5 kb fragment of the EGF promoter drives expression of a reporter gene to the granular convoluted tubule cells of the SMG in transgenic mice and that levels of expression of the reporter gene are higher in male than in female animals (Pascall *et al*. 1994). This suggests that the 5·5 kb EGF promoter fragment is directly hormonally responsive to androgens. However, these data are difficult to interpret, as the EGF-producing granular convoluted tubule cells are present in higher numbers and are more highly developed in the SMG from male animals than in the SMG of females (Gresik & MacRae 1975). Thus any mRNA or protein specifically targeted to those cells would be present at higher levels in the whole gland of males compared with females. Therefore further work is required to address the issue of whether the EGF gene is transcriptionally responsive to androgens.

In contrast with the lack of good evidence for a transcriptional regulatory mechanism, two recent

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reports have suggested a role for post-transcriptional regulation of EGF gene expression by androgens (Sheflin *et al*. 1996*a*,*b*). The possibility that EGF gene expression may be regulated post-transcriptionally arose from the observation that whereas renin mRNA is increased in mouse SMG within 1 h of testosterone administration (Catanzaro *et al*. 1985), there is a delay of 2 days before an increase in EGF mRNA is seen (Sheflin *et al*. 1996*a*). This result suggests that the effect of testosterone on EGF mRNA levels is mediated not at the transcriptional level, but rather by a post-transcriptional mechanism. One obvious mechanism whereby testosterone could mediate an increase in EGF gene expression would be to increase the mRNA half-life. As the stability of some mRNAs is modified by changes in the length of their poly(A) tails (see for reviews Bernstein & Ross (1989) and Nielsen & Shapiro (1990)), and it has been shown previously in several studies that steroid hormones can modulate the poly(A) tail lengths of specific mRNAs (see e.g. Carter & Murphy 1993), the poly(A) tail lengths of mouse EGF mRNA transcripts isolated from male and female SMGs were investigated, using a PCR-based strategy (Sheflin *et al*. 1996*a*). First, it was demonstrated that the profiles of poly(A) tail length on EGF mRNA molecules varied between the sexes. In females they fell into five distinct size groups of approximately 20, 50, 70, 100 and 200 adenosine residues. In contrast, in males there was a more heterogeneous population with a range of 20-100 adenosine residues, with a mean size of 60 residues. Secondly, when female mice were treated with testosterone, the profile of poly(A) lengths in the EGF mRNA from the SMG was altered to resemble that seen in male animals. In the kidney, where the EGF gene does not appear to be androgen-responsive, no change in polyadenylation profile was observed. The authors comment that the duration of testosterone exposure required to induce the change in polyadenylation pattern (3-7 days) is similar to the time course of the increase in EGF mRNA in the SMG during testosterone administration to female mice. This raises the possibility that the change in poly(A) tail length after androgen administration is associated with increased EGF mRNA stability, which in turn would result in elevated EGF mRNA levels. In addition, as the fold-increase in EGF protein levels after androgen administration is greater than that of EGF mRNA itself (Sheflin *et al*. 1996*a*), it is possible that the change in poly(A) tail length may be associated with increased translatability. Changes in poly(A) tail length have previously been shown to be associated with changes in translatability of several mRNA species (for discussion see Jackson & Standart (1990) and Sachs & Wahle (1993)).

Whilst this work is interesting, there are two major problems. First and most fundamentally, it has not yet been demonstrated that testosterone treatment increases either the half-life of EGF mRNA or its translatability, either in the mouse SMG *in vivo* or in cell culture *in vitro*. The effect of poly(A) tail length on EGF mRNA turnover could be tested by labelling RNA in explants from male and female mouse SMGs using [^{3}H]uridine, and then following the turnover of labelled EGF mRNA either in the presence of excess unlabelled uridine or after actinomycin D treatment to block *de novo* RNA synthesis. To determine whether poly(A) tail length has an effect on EGF mRNA translatability, the poly(A) profile of EGF mRNA species in polysomal RNA could be compared with that of total EGF mRNA. Any differences in the profiles would suggest an effect on translatability. Secondly, whilst this study adds to the body of evidence demonstrating that steroid hormones can effect changes in poly(A) tail lengths, it does not provide any mechanism to explain how the effect may be mediated.

Recent studies from the same group (Sheflin *et al*. 1996*b*) may have at least begun to address the issue of how testosterone treatment could directly impinge on the EGF mRNA species. Initially it was observed that the 3′-untranslated region of EGF mRNA contains several regions of sequence that are conserved between a number of species. In particular, a 23-base sequence including the major polyadenylation sequence was noted that is totally conserved between EGF mRNA sequences of rat, mouse and human. It was demonstrated that a ^{32}P-labelled RNA oligonucleotide of this sequence with a short 3′-terminal poly(A) tail could be UV cross-linked to a 47 kDa protein present in a cytosolic fraction from female SMG tissue. The binding was efficiently competed for by excess unlabelled oligonucleotide (50% reduction by a 50-fold excess of unlabelled oligonucleotide), but only inefficiently by poly(A) alone, suggesting specific binding of this protein to the 23-base RNA sequence. Moreover, binding activity was eightfold higher in cytosolic fractions of SMG from female compared with male mice. Interestingly, 2 days of treatment of female mice with testosterone resulted in an increase in this binding activity in SMG cytosol. However, after 5 days of treatment binding activity had fallen to 64% of the level in untreated animals and by 7 days it was close to that seen in SMG cytosol from male mice.

The authors (Sheflin *et al*. 1996*b*) draw particular attention to the initial increase in binding activity of

the 47 kDa protein after treatment of female mice with androgen, which precedes the increase in EGF mRNA, and speculate that the regulation of the binding of this protein to EGF mRNA could be a step in the pathway whereby testosterone regulates EGF mRNA polyadenylation and/or translatability. However, to postulate a role for the binding protein in this regulation would be more understandable if the binding-protein activity did not transiently increase in the female SMG after androgen treatment, but started to fall immediately towards male levels. In addition, the demonstration of binding *in vitro* does not demonstrate that the binding has any functionality *in vivo* or even whether it occurs. So does this binding protein play any significant role in the post-transcriptional regulation of EGF mRNA level/translatability? A direct test of this hypothesis will require further experiments. For example, modulation by testosterone of the polyadenylation/stability/translatability of synthetic mRNA species, containing the 23-base binding site inserted into the 3′-untranslated region, could be investigated in transgenic mice, using a cell/tissue-specific promoter (such as the EGF promoter itself) to direct expression of the synthetic RNA to the granular convoluted cells of the SMG. In addition, after the 47 kDa protein has been cloned, its ability to alter the polyadenylation/stability/translatability of synthetic RNAs containing the binding site could be investigated in androgen-responsive cell lines.

Despite the fact that the post-transcriptional regulation of gene expression by steroids has been known to occur for more than 20 years, it is still not clear how steroids mediate their post-transcriptional actions on accumulation of individual mRNAs. The demonstration of the modulation of a specific EGF mRNA-binding protein activity by testosterone is therefore of particular interest, as it provides a possible mechanism whereby a steroid hormone could regulate either the stability or translatability of an individual mRNA species within the total RNA population. In several mRNA species, motifs have been identified associated with mRNA instability (see Chen & Shyu 1995). Sheflin and co-workers (1996*b*) draw attention to a 13-base sequence within the 23-base binding site for the 47 kDa protein, of which 10 or 11 bases are conserved within at least four other mRNA species, the lung tumour enzyme PC1/PC5, alkaline phosphatase, the c-myc-associated zinc-finger protein and the TIS11 immediate early gene, some of which are known to be unstable mRNAs. This sequence includes an AUUUA motif which functions as a destabilizing element for mRNAs (see Chen & Shyu 1995). It is possible that the 47 kDa protein could influence mRNA turnover by altering the interaction of additional proteins with these sequences.

An obvious remaining question is whether the binding-protein activity is restricted to an interaction with EGF mRNA or whether it plays a more general role in the post-transcriptional regulation of gene expression by androgens and perhaps by other steroids. The answer to this question will have to await the analysis of proteins that interact with the 3′-untranslated region of other genes believed to be post-transcriptionally regulated by androgens, such as the follicle-stimulating hormone β subunit mRNA (Paul *et al*. 1990), and a detailed analysis of the steroid specificity of the effect. In parallel work, an oestrogen-inducible binding-protein activity has been demonstrated that interacts specifically with a 27-nucleotide region of the 3′-untranslated region of the oestrogen-stabilized *Xenopus laevis* vitellogenin mRNA (Dodson & Shapiro 1994). This binding-protein activity has been suggested to be involved in mediating oestrogen-induced stabilization of this mRNA. Interestingly, the level of binding-protein activity is modulated not only by oestrogen but also by testosterone in *Xenopus* tissues (Dodson *et al*. 1995). Whilst this binding protein has apparent molecular masses very much greater (71 kDa and 141 kDa; Dodson & Shapiro 1994) than that described for the EGF mRNA binding protein (47 kDa), it is tempting to speculate that they mediate their activities by similar mechanisms, and perhaps may be members of a family of proteins involved in hormonally regulated mRNA stabilization. The cloning of these proteins to allow structural and functional analysis should lead to interesting new insights into the post-transcriptional steroidal regulation of gene expression.

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The Babraham Institute,
Babraham Hall,
Cambridge CB2 4AT,
UK