AMP plays a significant role in thyroid gene activation with TSH and Graves' immunoglobulin, thyroid mRNA activation may also be modulated by other cellular messengers.


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Intronic steroid response elements in prostate binding protein genes

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Androgen receptors are assumed to influence gene expression through interaction with a nucleotide response element similar to that recognized by glucocorticoid, mineralocorticoid and progesterone receptors [1]. This assumption arises from the efficiency in heterologous systems by which androgens act through the defined glucocorticoid response elements (GRE) of the mouse mammary tumour virus.

Abbreviations used: GRE, glucocorticoid response element; ARE, androgen response element; MMTV, mouse mammary tumour virus; LTR, long terminal repeat.

Fig. 1. The 450 bp BglII-PvuII (B-P) fragment of the C3(1) gene first intron was (a) 5' (T4 polynucleotide kinase) or (b) 3' (Klenow-labelled with 32P and digested with one of HinfI(H), DraI(D), SacI(Sa), ScaI(Sc) or FokI(F))

Electrophoretically purified, electrophoresed, specific radiolabelled DNA fragments were re-electrophoresed, alone (odd-numbered lanes), or after incubation with androgen-receptor complexes and poly(dI-dC) (even-numbered lanes), in polyacrylamide gels [12]. Coding strand positions and sequences of putative response elements are shown.

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to the FokI cutting site. For further analysis of the Scal–FokI and FokI–PvuII fragments, pairs of complementary oligonucleotides representing these regions were synthesized, respectively, ARE1:

\[5'-CTATAAATATCACAGCTCAGTTAGCCAGTTTGCTCCTTTAACTCT-3']

and ARE2:

\[5'-TATAGGATTTTGAACATAGTCACTGTAAGTGTCT-CAAGATGAGTGAAT-3']

each of which the putative 15-nucleotide hormone response element, comprising the two 'arms' of the imperfect 6-base palindrome and the 3-base spacer [1], is underlined. In DNA cellulose competition assays, both ARE1 (50% competition at 0.05 g equivalents) and ARE2 (73% competition at 0.05 g equivalents) effectively reduced binding of androgen receptor to immobilized calf-thymus DNA. Mutation of the right arm to TTTTCT [10] abolished competitive ability in both cases. Alteration of the left arm of ARE1 to TTGCAA and that of ARE2 to ATTAAG reduced competitive effectiveness at 0.05 g equivalents to 24% and 41%, respectively. Deletion of the three bases immediately 5' to the TGTCTT motif, i.e. those respectively responsible for the critical spacing of dyadic response elements [11], abolished the effectiveness of ARE2 and reduced that of ARE1 to 28% at 0.05 g equivalents. In the latter case, however, removal of the 'spacer' creates TGTIAGCCAAGTCTT, which has greater similarity to ARE2 than does the natural sequence. In terms of binding of androgen receptor, therefore, both putative intronic AREs behave as response elements, with the right arm of the imperfect palindrome placing more stringency on binding than the left arm. ARE2 has the greater similarity to the response elements of the MMTV LTR and, most remarkably, to the most active synthetic element GGTACANNNNGTTCT devised by Ham et al. [3]. This is in line with the relative performances of ARE1 and ARE2 in transfection assays [10], but their role in vivo, and the requirement for the co-operativity of other enhancer factors, including possible ARE elements in the promoter region, remains open to critical appraisal.


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Functional characterization of an androgen response element

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The synthesis of prostatic binding protein (PBP) in rat ventral prostate is strongly dependent on the presence of androgens. This dependency can at least in part be explained by transcriptional regulation of the genes coding for the components C1, C2 and C3 of PBP.

We have screened the complete C1, C2A and C3(1) genes and their flanking sequences for regions displaying affinity in vitro for the androgen receptor in a DNA-cellulose competition assay. Two affinity regions were detected in each gene: one in the promoter region and one in the first intron. These results are in agreement with earlier reports on the 5' part of the C(3)(1) gene [1].

While attempts to delimit androgen response elements (AREs) in the promoter region of C3(1) have been unsuccessful [2], we now have demonstrated that the 450 bp intron fragment of C3(1) confers androgen responsiveness.

Abbreviations used: PBP, prostatic binding protein; ARE, androgen response element; GRE, glucocorticoid response element; PRE, progesterone response element; tk-CAT, thymidine kinase promoter in front of a chloramphenicol acetyltransferase; MMTV LTR, mouse mammary tumour virus long terminal repeat; 5α-DHT, 5α-dihydrotestosterone.

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