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The hamster androgen receptor promoter: A molecular analysis

Bruno Varriale *, Teresa Esposito

Dipartimento di Medicina Sperimentale, Laboratorio di Biologia Molecolare, Sez. "F. Bottazzi", II Università di Napoli, Via Costantinopoli, 16, 80138 Naples, Italy

Abstract

The steroid/thyroid hormone receptors are members of a very large family of nuclear-activated transcription factors. These receptors play a crucial role in most biological function, including regulation of development, metabolism, behaviour and reproduction. Among androgen receptor (AR), we have recently demonstrated that its expression in the Harderian gland (HG) of the male hamster is under a well-co-ordinated cross-talk between various steroid hormone receptors. Here, are presented data on the sequence of hamster AR promoter region (5′UTR) and the molecular tools of its regulation. The 5′UTR is 1585 bp. The promoter region shows various responsive elements. Two putative CREM elements are present at −71 and −1576 bp. A putative retinoic acid responsive element is present at −1476 bp. An androgen/glucocorticoid responsive element is present at −473 bp. A putative thyroid hormone-responsive element at −381 bp and an estrogen responsive element at −230 bp. Also, a homopurinic stretch is evident between −1199 and −1118. Furthermore, Sp1 sites are also spread along the sequence. As well as for human, mouse, rat and pig, the hamster lacks the canonical promoter TATA and CCAAT boxes. Gel retardation experiments confirm the presence of active responsive elements for AR, estrogen receptor, glucocorticoid receptor and thyroid hormone receptor. Previous data on the regulation of expression of AR by other members of steroid/thyroid hormone receptors well correlate with sequence analysis and gel retardation experiments. Thus, androgens, thyroid hormone, stimulate the AR transcription, while synthetic glucocorticoid (Dex) and estrogen are potent inhibitors of AR expression. The comparison of hamster AR promoter sequence with other AR promoter shows an 89, 82, 84 and 84% identity with human, rat, mouse and pig AR promoter, respectively.

These results, in the light of the extreme plasticity of hamster HG, suggest that the comparative study of expression and regulation of AR gene in the HG of the hamster offers a useful tool to approach the normal and pathological phenotype in human.

Keywords: Androgen receptor mRNA; Promoter; Hamster; Steroid regulation

1. Introduction

The Harderian gland (HG) is a tubulo-alveolar gland present in the medial corner of the orbital cavity, in the majority of land vertebrate. Main products of the HG are lipids and porphyrins, although other products have been described. In rodents, the gland occupies a large portion of the cavity and particularly in the golden hamster, the gland displays sexual dimorphism in both morphological and biochemical parameters [1,2].

Sexual dimorphism is a phenomenon in which male and female individuals from the same species develop different phenotypes [3] both in reproductive and non-reproductive organs [4]. It is commonly accepted that androgens are the mastermind in directing this phenomenon, through their action on target genes [4,5]. In this respect, it has been found that some of the genes involved in the setting and maintenance of the sexual dimorphism in hamster HG are controlled directly or indirectly by androgens [6] and this control involves the transcription of an androgen receptor (AR).

The AR is a transcription-regulating protein that plays a mastermind role in setting male sexual differentiation, development and sexual dimorphism, and mutation or transcriptional defect of AR are involved in aberrant male sexual development [7].

Structurally and functionally, AR belongs to the superfamily of ligand-responsive transcription factors which include the steroid receptors and receptors for thyroid hormone, retinoids and Vitamin D, as well as an even larger group of proteins named orphan receptors whose ligand and/or function are still unknown [8–11]. These receptors

* Corresponding author. Tel.: +39 081 5667621; fax: +39 081 5667536.
E-mail address: bruno.varriale@unina2.it (B. Varriale).

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play a crucial role as transcriptional activators and as repressor in most biological function, including regulation of development, metabolism, behaviour and reproduction. The structural regions that these receptors have in common are a C-terminal ligand-binding domain, an internal DNA-binding domain, consisting of two Cys–Cys zinc finger motifs and an N-terminal regulatory domain, these regions enable them, through binding to hormonal stimulus, to regulate gene transcription through recognition and binding to hormone-responsive elements (HREs) located in the control regions of target genes.

The expression of AR mRNA has been largely investigated in different animal and human models and several papers report the analysis of AR promoter region from diverse species [12] This 5′UTR sequence is one of the main regulatory region involved in ARmRNA expression [13–14].

The aim of this paper is to characterize the 5′UTR of the hamster AR, since in previous paper it has been shown that in primary cultures of hamster HG cells the AR is under a highly coordinated cross-talk between different steroidal compounds [15].

2. Material and methods

2.1. Animals

Adult male golden hamsters (*Mesocricetus auratus*) were purchased from Nossan (Italy) and housed (3/cage) under a light:dark cycle of 14:10 h at 20 °C. Water and food were supplied ad libitum. Animals were caged and cared at Istituto Zooprofilattico Sperimentale del Mezzogiorno (IZSM).

2.2. Chemicals

All reagents were molecular biology grade and tissue culture tested. [α^{32}P]dCTP (3.000 Ci/mmol) and [α^{32}P]dTTP (3.000 Ci/mmol) were purchased from Amersham International (Amersham, Italy). Testosterone (T), 17β-estradiol (E), dexametasone (Dex), triiodiotyronine (T_3), all-*trans* retinoic acid (RA) were purchased from Sigma (St. Louis, MO).

2.3. RNA extraction

Total cellular RNA was extracted by the procedure of Chomzynski and Sacchi [16], with minor modifications. The yield and the quality of RNA were assessed by the 260/280 nm optical density ratio (1.93 ± 0.05) and by electrophoresis under either non-denaturising and denaturising conditions on 1.2% agarose gels.

2.4. Northern and slot blot analyses

Northern blots and slot blot of RNA were performed as described by Varriale [17]. Hybridisation were performed with following template: a 2.0 kb *Ava*I–*Pvu*II fragment from rat thyroid hormone receptor α [18]; a 2.1 kb *Eco*RI fragment from mouse RARα cDNA (pSG5) [19]; a 1.95 kb *Bam*HI–*Eag*I fragment from mouse RARβ cDNA (pSG5) [19]; a 1.8 kb *Eco*RI fragment from mouse RARγ cDNA (pSG5) [19]; a 2.43 kb *Eco*RI fragment from rat AR cDNA (pGM3Z) [20]; a 1.9 kb *Ava*I fragment from the human GR cDNA (pKCR2) [21]; a 2.4 kb *Eco*RI fragment from the mouse ER cDNA (pKS$^-$) [22]; a 1.9 kb *Bam*HI fragment from the human β-actin cDNA (pHFbA-1) [23]; a 1.6 kb *Bam*HI–*Eco*RI fragment of 18 S rRNA (pBR322) [24]. Probes were labelled with [α^{32}P]dCTP and [α^{32}P]dTTP by random priming to a specific activity of about 5×10^8 cpm/μg [25]. Pre-hybridisations and hybridisations were performed in 50% deionised formamide, 5 × SSC, 0.1% SDS, 50 mM phosphate buffer (pH 6.8), 5 mM EDTA, 5 × Denhardt's solution, 100 μg/ml yeast tRNA. Hybridisations were carried out at 42 °C overnight. Filters were washed twice with 2 × SSC, 0.1% SDS at 65 °C, once with 0.2 × SSC, 0.1% SDS at 65 °C and once with 0.1 × SSC, 0.1% SDS at 68 °C. Dried filters were exposed to X-ray film (Fuji HRH) for a time ranging between 24 and 96 h.

2.5. Amplification of 5′UTR of hamster AR mRNA

Amplification of the 5′UTR of the AR mRNA was completed through the rapid amplification of cDNA ends (RACE) technique (4), using the 5′/3′ RACE cDNA Amplification Kit from Roche, Italy. Shortly, 1 μg of total RNA from male hamster HG was used as source of template to perform the initial reverse transcriptase reaction (20 μl) as indicated by the manufacturer. A specific amplification of the desired 5′cDNA by nested PCR was achieved through two consecutive PCR reactions, according to the kit instructions and including the specific oligonucleotides 5′acagcctgttgaactcttct3′ from 5′UTR ARmRNA of Homo sapiens, 5′ggagctgccctttcctct3′ from 5′UTR ARmRNA of Rattus norvegicus, 5′agctgccctttcctcttcg3′ from 5′UTR ARmRNA of Mus musculus domesticus, 5′agcctgttgaactcttctca3′ from 5′UTR ARmRNA of Sus domesticus as forward primers and 5′catccttgagcttggctgaa3′ from 5′UTR ARmRNA of Homo sapiens, 5′catccttgagcttgtcgtag3′ from 5′UTR ARmRNA of Rattus norvegicus, 5′catcctttagcttgtctctag3′ from 5′UTR ARmRNA of Mus musculus domestucus, 5′catccttgagcttggcagaa3′ 5′UTR ARmRNA of Sus domesticus as reverse primers. The products of the PCR amplification were sequenced from both ends.

2.6. Sequencing of 5′UTR of hamster AR mRNA

The purified cDNA containing the 5′UTR of AR were sequenced from both strands according to the conditions outlined by the Big Dye Terminator Kit (Perkin-Elmer/Applied Biosystem) and runs on ABI 377 Sequencing System (Perkin-Elmer/Applied Biosystem). Sequence data were analysed with DNA-STRIDER and BLAST SEARCH programs.