Available online at www.sciencedirect.com
ScienceDirect
Journal of Steroid Biochemistry & Molecular Biology 106 (2007) 62–70
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Mechanisms in the regulation of aromatase in developing ovary and placenta

Carole R. Mendelson [a,b,*], Amrita Kamat [c]

[a] *Department of Biochemistry, The University of Texas Southwestern Medical Center at Dallas, TX, United States*
[b] *Department of Obstetrics & Gynecology, The University of Texas Southwestern Medical Center at Dallas, TX, United States*
[c] *Department of Medicine-Geriatrics and Gerontology, The University of Texas Health Science Center at San Antonio, TX, United States*

Abstract

During human gestation, the placental syncytiotrophoblast develops the capacity to synthesize large amounts of estrogen from C_{19}-steroids secreted by the fetal adrenals. The conversion of C_{19}-steroids to estrogens is catalyzed by aromatase P450 (P450arom), product of the *CYP19* gene. The placenta-specific promoter of the *hCYP19* gene lies ~100,000 bp upstream of the translation initiation site in *exon II*. In studies using transgenic mice and transfected human trophoblast cells we have defined a 246-bp region upstream of placenta-specific *exon I.1* that mediates placental cell-specific expression. Using transgenic mice, we also observed that as little as 278 bp of DNA flanking the 5′-end of ovary-specific *hCYP19 exon IIa* was sufficient to target ovary-specific expression. This ovary-specific promoter contains response elements that bind cAMP-response element-binding protein (CREB) and the orphan nuclear receptors SF-1 and LRH-1, which are required for cAMP-mediated stimulation of *CYP19* expression in granulosa and luteal cells during the estrous cycle and pregnancy. In this article, we review our studies to define genomic regions and response elements that mediate placenta-specific expression of the *hCYP19* gene. The temporal and spatial expression of LRH-1 *versus* SF-1 in the developing gonad during mouse embryogenesis and in the postnatal ovary also will be considered.

Keywords: Estrogen biosynthesis; Human *CYP19* gene; Ovary; Placenta; Tissue-specific expression; Liver receptor homologue-1 (LRH-1); Steroidogenic factor-1 (SF-1)

1. Introduction

In most vertebrates, expression of the aromatase P450 (P450arom/*CYP19*) gene is restricted to the gonads and brain; however, in humans, aromatase is expressed in specific cell populations of a variety of estrogen-producing tissues, including the syncytiotrophoblast layer of the placenta, granulosa and luteal cells of the ovary, Leydig, Sertoli and germ cells of the testis, stromal cells of adipose tissue, bone, discrete nuclei within the brain and in fetal liver [1,2]. *hCYP19* gene expression in various estrogen-producing tissues appears to be driven by tissue-specific promoters upstream of alternative first exons, which encode the tissue-specific 5′-untranslated regions of P450arom mRNA transcripts. These unique promoters not only control tissue-specific expression of these P450arom mRNA transcripts, but also mediate their differential regulation by hormones and factors. The alternative first exons, which are located from ~110 to ~100,000 bp upstream of the *hCYP19* translation initiation site in *exon II*, are alternatively spliced onto a common site just upstream of the translation start site in *exon II*, so that the protein encoded in each of these tissues is identical (Fig. 1). In placenta, the majority of the P450arom mRNA transcripts contain sequences encoded by

* Corresponding author at: Department of Biochemistry University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9038, United States. Tel.: +1 214 648 2944; fax: +1 214 648 3214.
E-mail address: carole.mendelson@utsouthwestern.edu (C.R. Mendelson).

0960-0760/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2007.05.001

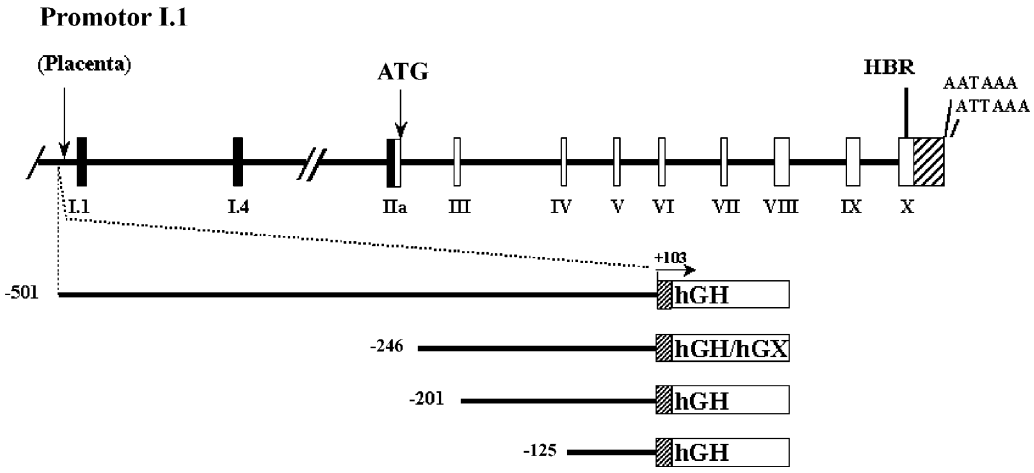

Fig. 1. Schematic representations of the *hCYP19* (aromatase) gene and of h*CYP19I.1:hGH/hGX* fusion genes introduced into transgenic mice. Exons II-X (white boxes), which encode the aromatase protein, and their introns (black lines) comprise a region of ~34 kb in size. The heme binding region (HBR) and two polyadenylation signals in the 3′-untranslated region (striped box) are encoded in exon X. Exons IIa, I.4 and I.1 (black boxes) encode the 5′-UTRs of the aromatase P450 mRNAs in the gonads, adipose tissue and placenta, respectively. The region containing these alternative first exons encompasses ~100 kb. The *hCYP19I.1:hGH/hGX* fusion genes are comprised of *hCYP19* DNA sequences encoding 501, 246, 201 and 125 bp of DNA flanking the 5′-end of *exon I.1* (solid line) and the first 103 bp of *exon I.1* (hatched box) fused to either the wild-type (*hGH*) or mutated, biologically inactive form (*hGX*) of the human growth hormone structural gene, as reporter (white box). The arrow indicates the position of the transcription initiation site and direction of transcription for all fusion gene constructs. Ref. [6], with permission. Copyright 2005, *The Endocrine Society*.

exon I.1, which lies ~100,000 bp upstream of the start site of translation in *exon II*, whereas in ovary, P450arom mRNA transcripts contain 5′-untranslated sequences encoded by *exon IIa* which lies 110 bp upstream of the translation start site [2] (Fig. 1).

2. Use of transgenic mice and transfected cells to define genomic sequences that mediate placenta-specific *hCYP19* expression

In previous studies using primary cultures of human placental cells, we observed that differentiation of cytotrophoblasts to syncytiotrophoblast is oxygen-dependent and associated with a marked induction of aromatase activity and *hCYP19* gene expression [3,4]. Transfection of placental and non-placental cells with reporter gene constructs revealed that placenta-specific *exon I.1* 5′-flanking sequences between −501 and −42 bp mediates trophoblast-specific *hCYP19* gene expression [3]. Studies using transgenic mice also suggested that as little as 501 bp of *exon I.1* 5′-flanking DNA directed reporter gene expression exclusively to the placenta and specifically to the labyrinthine trophoblast layer, which is region of mouse placenta most analogous to the human syncytiotrophoblast [5]. Collectively, these findings suggest that the 5′-flanking DNA within 501 bp of *exon I.1* of the *hCYP19* gene contains *cis*-acting elements that bind placenta-specific transcription factors. Since mouse placenta does not express aromatase, it is likely that placental transcription factors that mediate *hCYP19* gene expression are conserved between mouse and human, while the genetic response elements that bind these factors are not.

More recently, we created transgenic mice carrying fusion genes containing 246, 201 and 125 bp of *exon I.1* 5′-flanking sequence fused either to a mutated (*hGX*) or wild-type (*hGH*) human growth hormone reporter gene (Fig. 1). We found that as little as 246 bp of *hCYP19 exon I.1* 5′-flanking sequence was sufficient to direct placenta-specific expression of *hGX* or *hGH* in transgenic mice (Fig. 2). By contrast, transgenes containing 201 bp or 125 bp of *exon I.1* 5′-flanking DNA were not expressed in mouse placenta [6] (Fig. 2). Furthermore, *hCYP19I.1*$_{-246}$*:hGX* transgene expression was

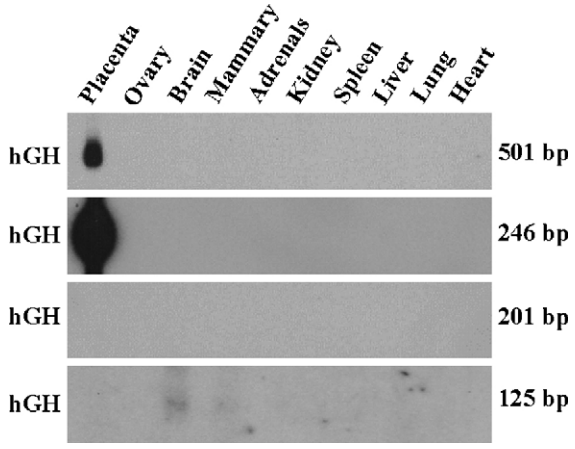

Fig. 2. 246 bp of *hCYP19 exon I.1* 5′-flanking sequence is sufficient to mediate placenta-specific expression in transgenic mice. Aliquots of total RNA (30 μg) isolated from placenta of a E17.5 F1 transgenic mouse or from various tissues of an adult F1 male or female mouse carrying the *hCYP19I.1*$_{-501}$*:hGH*, *hCYP19I.1*$_{-246}$*:hGH*, *hCYP19I.1*$_{-201}$*:hGH* or *hCYP19I.1*$_{-125}$*:hGH* transgenes were analyzed by Northern blotting using a ^{32}P-labeled hGH cDNA probe. Ref. [6], with permission. Copyright 2005, *The Endocrine Society*.