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Regulation of aromatase gene expression in Leydig cells and germ cells☆

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Abstract

The ability of the testis to convert irreversibly androgens into estrogens is related to the presence of a microsomal enzymatic complex named aromatase. Although somatic cells and germ cells (GC) have the capacity to produce estrogens the regulation of the *CYP19* gene expression in adult rat testicular cells and specially in freshly purified Leydig cells, pachytene spermatocytes (PS) and round spermatids (RS) is not fully understood. In the present study we have analyzed the putative effects of steroid hormones, transforming growth factor β (TGFβ), cytokine (tumor necrosis factor α, TNFα) and dexamethasone (Dex) on *CYP19* expression in these purified testicular cells from adult rat. In parallel the biological role of seminiferous tubules and Sertoli cells conditioned media on the expression of aromatase was studied. Using a highly specific quantitative competitive RT–PCR we established that testosterone (T) enhances *CYP19* gene expression in Leydig cells and germ cells, and augments the estradiol outputs. The non-aromatizable androgen 5α-DHT induces the same effect as T on P450 aromatase (P450arom) gene expression but was inefficient on the estradiol output. In PS and RS an inhibitory effect on *CYP19* gene transcription was observed with TGFβ (1 ng/ml) alone or in combination with T. Conversely, the addition of TNFα (20 ng/ml) increases the P450arom transcription in PS although an inhibitory effect is observed in RS. Together with T, TNFα decreases the amount of P450arom mRNA in PS and RS. In PS we found that Dex regulates positively *CYP19* expression and negatively in RS. Furthermore in PS a synergistic effect of Dex and TNFα on P450arom mRNA expression was observed whereas an additive one was recorded for RS. Therefore in germ cells TNFα likely enhances expression of aromatase through promoter PI.4 in PS, possibly via an AP1 site upstream the GAS element, while in RS TNFα requires glucocorticoids as a co-stimulator to increase *CYP19* gene expression. Finally in presence of seminiferous tubules or Sertoli cell conditioned media, the amount of aromatase transcripts is increased in both Leydig cells and germ cells therefore suggesting that other locally produced modulators, yet unknown, but from Sertoli cell origin, are concerned in the regulation of the aromatase gene expression in rat testicular cells. In summary, using an in vitro model of mature rat Leydig cells, pachytene spermatocytes and round spermatids, we have shown that several factors direct the expression of the aromatase gene and it is obvious that not only promoter PII but also promoter PI.4 are concerned.

Keywords: Aromatase; Estrogens; Germ cells; Testis; Spermatogenesis; Rodents; Humans

1. Introduction

The mammalian testis is a complex organ characterized by two main functions: synthesis and secretion of steroid hormones and production of spermatozoa. It is well known that normal testicular development and maintenance of spermatogenesis are controlled by gonadotrophins and testosterone whose effects are modulated by locally produced factors and among them estrogens are obviously concerned [1,2]. Even though, the presence of estrogens in the testis is now well documented, unlike androgens the role of estrogens in the physiology of the male reproductive tract has not been fully understood and thus is now extensively studied [3–5]. The cytochrome P450 aromatase (P450arom) involved in the irreversible transformation of androgens into estrogens is a microsomal enzymatic complex composed of two proteins. The first one is a ubiquitous NADPH-cytochrome P450 reductase and the second part, a cytochrome P450 aromatase, which contains the heme and the steroid binding pocket, specific for the estrogen biosynthesis. The P450arom is the product of a single gene called *CYP19*, which belongs to the cytochrome P450 gene family, containing more than 500 members [6]. The human *CYP19* gene stretches on more than 120 kb length with a coding region of nine exons and nine untranslated exons I. The *CYP19* gene expression is regulated by multiple tissue-specific promoters producing alternate 5′-untranslated exons I that are then spliced onto a common 3′-splice acceptor site in the exon II upstream of the translation start site [7,8]. Transcriptional regulation of *CYP19* is the major, although not exclusive, mechanism

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controlling the amount of aromatase. Expression of P450arom in human placenta is controlled primarily by a promoter that lies at least 40 kb upstream from the start site of translation (promoter I.1) [9]. A promoter proximal to the translation start site, called promoter PII, regulates P450arom expression in ovary and testis [5,7,10]. Promoter PII contains elements such as cAMP responsive element (CRE), AP-1, transforming growth factor β (TGFβ) and SP-1 [11]. In both ovary and testis, FSH and LH act through increasing concentrations of intracellular cyclic AMP to induce expression of P450arom. Promoter PII activity is regulated by cAMP and requires the transcription factors cAMP response element binding protein (CREB), cAMP response element modulator (CREM) and steroidogenic factor-1 (SF-1) [12]. In human adipose tissue, as well as in fetal liver, the primary promoter lies about 15 kb upstream of the start site of translation (promoter I.4 [13]) and is a TATA-less promoter driven by glucocorticoids and class I cytokines such as IL-6 and tumor necrosis factor α (TNFα) [12]. The localization of aromatase within the testis has been a subject of considerable interest for a number of years, however despite much work focused on aromatase specially in male gonad the regulation of P450arom gene expression has not been extensively studied. Using a highly specific quantitative competitive RT–PCR method developed in our lab [14,15] we have analyzed the effects of various hormonal treatments on the amount of specific aromatase transcripts in purified Leydig cells and germ cells (GC) and in parallel, we have measured the estradiol output. We therefore seek to identify which promoter(s) is (are) used in the expression of P450arom transcripts in various testicular cells of rat.

2. Brief overview on the localization of aromatase in mammalian testicular cells and epididymal cells

Past efforts to localize the source of testicular estrogens have been a considerable subject of interest during the last decades and led to the assertion that Leydig cells synthesize estrogen in adults, whereas Sertoli cells are the major source in immature animals (for review see [2]). However, striking species differences exist, since in mouse [16], rat [14,17], bank vole [18] and brown bear [19] the aromatase

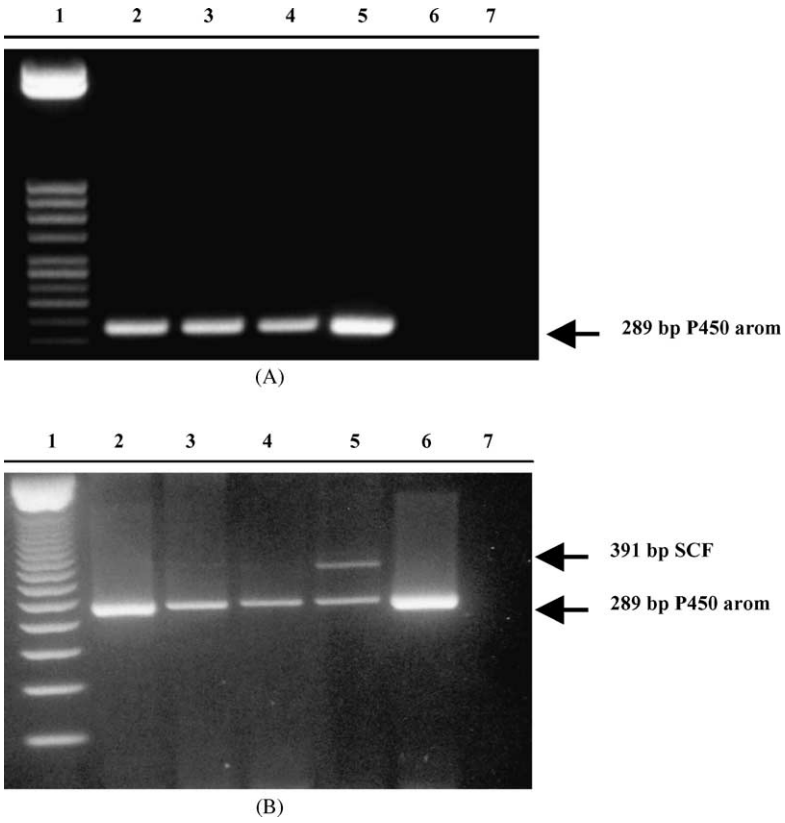

Fig. 1. (A) Detection of P450 aromatase mRNA in purified adult rat germ cells by RT–PCR. Pachytene spermatocytes RNA (lane 2), round spermatides RNA (lane 3) and seminiferous tubules RNA (lane 4) generated RT–PCR products of the predicted size of 289 bp (using P450arom primers 5′-ARO and 3′-ARO). Ovarian RNA (lane 5) was used as positive control and also generated RT–PCR product of the predicted size (289 bp); whereas rat muscle RNA (lane 6) and no RNA (lane 7, water was used instead of RNA) were used as negative controls. Lane 1 corresponds to DNA ladder. (B) Co-amplification of P450arom mRNA and SCF mRNA. Mixed germ cells before purification (lane 2), PS (lane 3), RS (lane 4), Sertoli cells (20 days, lane 5), ovary (lane 6) and no RNA (lane 7, water was used instead of RNA).

is present not only in Leydig cells and Sertoli cells but also in germ cells (Fig. 1). In the rat, related to the stage of germ cell maturation, the amount of P450arom mRNA transcripts decreases: it is twice as high in pachytene spermatocytes (PS) than in round spermatids (RS) which have levels 20-fold higher than in spermatozoa [14]. Moreover using polyclonal antibodies against human placental aromatase we have shown in rat that not only Leydig cells but also elongated spermatids are strongly positive [14] as also reported for spermatozoa within the epididymis [17]. Conversely the aromatase activity, likely located in the cytoplasmic droplet of spermatozoa [17] is four to five-fold higher in testicular spermatozoa than in either pachytene spermatocytes or spermatids. All together in the adult rat, the aromatase activity measured in microsomes from purified germ cells accounts for more than 60% of the total testicular enzyme activity [14]. Our observations were therefore in agreement with the data of Nitta et al. [16] who first reported in mouse germ cells the presence of aromatase. In addition, it has been shown that rat epididymal epithelial cells contained specific aromatase transcripts which were likely coding for a biologically active protein since in vitro androgens were transformed into estrogens [20].

In the bank vole, a seasonal breeder, we have shown that the P450arom was much more expressed (in terms of transcripts, activity and immunolocalization) specially in germ cells (pachytene spermatocytes and spermatids) of animals bred in long day light cycle than during the winter resting season [21].

Recent observations made in our group showed that in seminiferous tubules of the stallion there was a positive immunoreactive signal for aromatase not only in Leydig cells but also in cytoplasm surrounding germ cells therefore suggesting strongly the presence of aromatase in Sertoli cells [22]. In the Rhesus monkey, it has been shown that testis and to a lesser extent epididymidis contained P450arom transcripts [23] and moreover, in the epididymal regions a discrepancy was observed between the amount of transcripts and the aromatase activity which was found more active in caput than in cauda although the P450arom mRNA levels were reversed.

Very recently we have examined the ability of human ejaculated spermatozoa to convert androgens into estrogens. When sperm RNA was used as template in RT–PCR we have shown the presence of P450arom transcripts; the sequences alignment from these PCR products and granulosa cells with published human P450arom gene were identical except for some bases unidentified. Using Western blots and a specific monoclonal antibody against aromatase [24] we have evidence of the presence of aromatase in these sperm cells which was obviously more abundant in spermatozoa containing cytoplasmic droplets as well as in motile spermatozoa (data not shown). These observations are consistent with our data on the amount of P450arom transcripts which are 30% lower in immotile than in motile spermatozoa [25].

3. Regulation of aromatase gene expression in testicular somatic cells and germ cells

3.1. Leydig cell purification

The testes from mature Sprague–Dawley rats (>80 days) were decapsulated then submitted to an enzymatic treatment with collagenase–dispase (0.05%), soybean trypsin inhibitor (0.005%) and deoxyribonuclease (0.001%) in Ham's F-12/DME medium (1/1, v/v) for 10 min at 32 °C, and the Leydig cells were purified on discontinuous Percoll gradients [26]. The enriched cell fractions collected after Percoll sedimentation were characterized for the presence of Leydig cells by histochemical staining for the 3β-hydroxysteroid dehydrogenase (HSD); colored cells were identified as Leydig cells (3β-HSD positive).

3.2. Germ cell preparations

Testicular mixed germ cell preparation was obtained by the trypsin-DNase treatment. Pachytene spermatocytes and round spermatids enriched fractions were obtained after purification on a Sta–Put apparatus [14]. Purity of fractionated germ cells was routinely monitored under a phase-contrast microscope. To estimate the Sertoli cells contamination of the germ cell preparations a histochemical staining with Oil Red O [27] was developed. Moreover, RT–PCR method was applied to detect whether stem cell factor (SCF, a specific marker of Sertoli cells [28]) was present in our germ cell preparations; in addition, the 3β-HSD histochemical staining had been used to check for the presence of Leydig cells.

The viability of Leydig cells and germ cells before and after incubation was evaluated using the Trypan blue exclusion test; the stained cells were considered to be dead (Table 1).

3.3. Preparation of seminiferous tubules and Sertoli cell conditionned media

Isolated segments of 10–20 mm (total length 200 mm) of seminiferous tubules were dissected by transillumination [29]. After 20 h of incubation, the seminiferous tubules conditionned media (STMs) were charcoal-treated to remove endogenous steroids then added to purified Leydig cells or germ cells. Sertoli cells from adult rat were prepared as described elsewhere and conditioned medium collected after day 5 of culture [30].

3.4. Incubation procedure

Purified Leydig cells (3×10^5 cells/ml) were incubated at 32 °C incubated under an humidified atmosphere of 5% CO_2 and 95% air in Ham's F-12/DME medium with or without steroid hormones, STM, oLH or cyclic AMP analogue

Table 1
Influence of incubation time on cell viability and on P450 aromatase gene expression in purified adult rat Leydig cells and germ cells (PS–RS)

Time (h)	Cell viability	Quantity of P450arom mRNA
Leydig cells		
0	100	100 ± 3
6	100	108 ± 3 NS
24	98	110 ± 2 NS
PS		
0	100	100 ± 16
6	100	86 ± 3 NS
12	99	130 ± 14 NS
18	99	179 ± 31*
RS		
0	100	100 ± 14
6	100	145 ± 5 NS
12	99	177 ± 5*
18	99	244 ± 33**

Values are expressed as percentages ± S.E.M. of three different experiments. NS, not significant; when compared to time 0.
* $P < 0.05$.
** $P < 0.01$.

(cAMP). Germ cells (2.5×10^6 PS/ml and 4.5×10^6 RS/ml) were incubated under the same conditions in medium supplemented with antibiotics, 10 mM pyruvate and 6 mM glucose and with various hormonal treatments including steroid hormones, cAMP, dexamethasone (Dex), TGFβ and TNFα.

3.5. RT–PCR and quantitative RT–PCR

The cDNAs obtained were further amplified by PCR using selected oligonucleotides. The P450arom primers have been selected to amplify a highly conserved sequence including helical and aromatic regions of the P450arom gene (Table 2). Using a highly specific quantitative RT–PCR method [14] the amounts of P450arom mRNA present in the total RNAs were determined [15]. To estimate the Sertoli cell contamination of the germ cell preparations, stem cell factor primers were synthesized (Table 2).

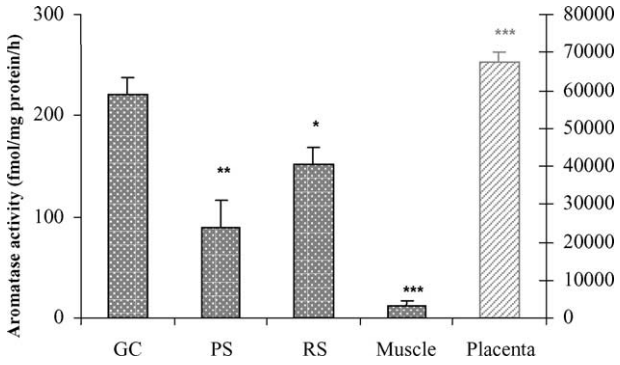

Fig. 2. P450 aromatase activity in adult rat germ cells: mixed germ cells (GC), pachytene spermatocytes (PS), round spermatids (RS). Muscle and human placenta were used as negative and positive controls, respectively. Values are mean ± S.E.M. ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, when compared to mixed germ cells.

3.6. Aromatase activity (Fig. 2) and estradiol determinations

Microsomes were isolated from homogenates of purified germ cells by differential centrifugation and aromatase activity was assayed by measuring the rate of incorporation of [^{3}H] from [^{3}H] androstenedione to [^{3}H] water [14]. In Leydig cell and germ cell culture media, 17β-estradiol was measured by RIA according to a previously reported method [30].

3.7. Effects of incubation time on the expression of P450arom gene in Leydig cells and germ cells

According to the duration of incubation, the Leydig cell and germ cell viabilities were not altered during 18 or 24 h. The magnitude of P450arom mRNA was significantly different in Leydig cells after 24 h of incubation when compared to time zero. An increase of P450arom mRNA level was also observed in PS as well as in RS after 18 h of incubation (Table 1). Therefore according to the quality of Leydig cells and germ cells on the one hand, and to the significant effects observed on aromatase gene expression on the other

Table 2
Primers used for RT–PCR

Sequence		Orientation
Primer		
5′-ARO	GCTTCTCATCGCAGAGTATCCGG	Sense (1555–1577)*
3′-ARO	CAAGGGTAAATTCATTGGGCTTGG	Antisense (1821–1844)*
5′-STD	TAATAGCACTCACTATAGGGAGAGCTTCTCATCGCAGAGT ATCCGGCACACTGTTGTTGGTGACAGAGACATA	Sense
3′-STD	TTTTTTTTTTTTTTTTTCAAGGGTAAATTCATTGGGCTTGG	Antisense
5′-SCF	ACACCACTGTTTGTGCTGGA	Sense (146–166)[a]
3′-SCF	TTCTTCCATACATGCCACGA	Antisense (535–575)[a]

* Numbered as described by Hickey et al. [52] (GenBank accession no. M33986).
[a] Numbered as described by Martin et al. [53] (GenBank accession no. M59966).

hand, a 18 and 24 h incubation periods have been chosen, respectively, for germ cells and Leydig cells in all further experiments unless otherwise indicated.

4. Effects of gonadotrophins (cyclic AMP) and steroid hormones (T, DHT, estradiol)

It is noteworthy that in rat Leydig cells, whatever the age, the level of P450arom mRNA was almost identical. By contrast in mature rat Sertoli cells the amount of these transcripts was 10-fold lower than that in Sertoli cells from 20-day-old animals [31]. In mature rat Leydig cells, the addition of either LH (or dbcAMP) or testosterone induced a dose-related increase of P450arom mRNA levels and estradiol productions [15].

In Sertoli cells whatever the age of the rat, FSH (or dbcAMP) or testosterone were shown to improve the P450arom expression; in addition, together gonadotrophins plus testosterone exerted an additive effect. In both PS and RS testosterone stimulated the expression of P450arom gene expression and the aromatase activity. In order to analyze the androgenic effect we have used 5α-DHT (a non-aromatizable androgen) and observed the same effect as for testosterone on P450arom gene expression both in Leydig cells and germ cells but without any increase of estradiol output as expected [32] Cyclic AMP enhanced the aromatase gene expression in PS whereas an opposite effect (although not statistically significant) was recorded in RS (Figs. 3 and 4).

Estradiol down-regulated the aromatase transcription in germ cells: a 70% decrease was found in RS whereas in PS the diminution was 40%; conversely a positive effect (20% increase) has been noted in Leydig cells (data not shown). The half-life of P450arom mRNAs was studied in Leydig cells and for that purpose the cells were incubated with actinomycin D (5 μg/ml) for various periods (0–12 h) in the presence or absence of either oLH, or testosterone or LH plus testosterone. We have observed a 50% decrease of the amount of P450arom mRNA after 5.8 h of treatment in basal conditions. The addition of oLH or testosterone did not change the results but conversely all together, LH plus testosterone, induced a two-fold increase of the half-life of *CYP19* transcripts [15].

5. Effects of growth factors and cytokines (±dexamethasone)

In germ cells TGFβ induced a dose and time-related decrease of the amount of P450arom transcripts and aromatase activity; the maximal effect on the *CYP19* transcription was recorded with 1 ng/ml of TGFβ after 18 h of treatment (52 and 70% decreases, respectively, in PS and RS), and for the aromatase activity the decreases were of 42 and 47%, respectively (data not shown). Conversely TNFα enhanced

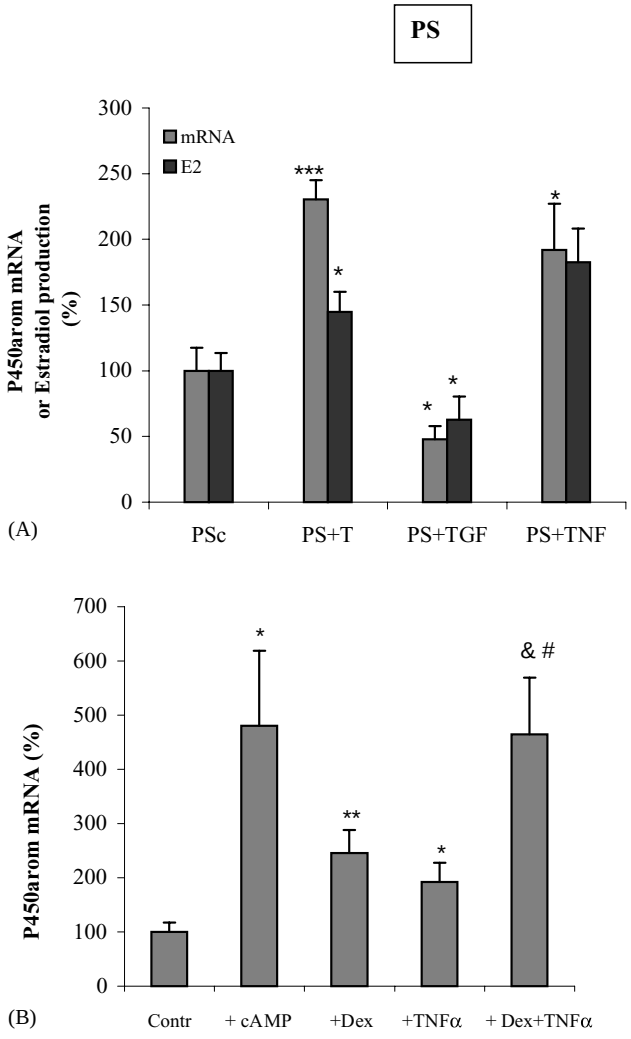

Fig. 3. (A) Effects of TGFβ$_1$, testosterone (T) and TNFα on P450arom mRNA levels and on estradiol production in pachytene spermatocytes (PSc). The data are expressed in percentages; control cells (PSc) are considered to be 100%. Values are mean ± S.E.M. ($n = 3$). $^{*}P < 0.05$, $^{***}P < 0.001$, when compared with control cells. (B) Effect of other treatments: cAMP, dexamethasone (Dex), TNFα and Dex + TNFα on *CYP19* gene expression and aromatase activity in PS. The data are expressed in percentages; values are mean ± S.E.M. ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$, when compared with control cells (Contr), $^{\&}P < 0.05$ compared to TNFα and $^{\#}P < 0.05$ compared to Dex.

in a dose and time related manner the P450arom transcription in PS although an inhibitory effect was observed in RS. The optimal effects were obtained after 18 h of treatment with 20 ng/ml of TNFα in both germ cell fractions (90% increase in PS although a 50% decrease was noted in RS). It is noteworthy that TGFβ reversed dramatically (170 and 190 diminutions in PS and RS) as well as TNFα 120 and 60% decreases in PS and RS) the positive effects induced by testosterone on the aromatase expression (Figs. 3 and 4). When dexamethasone was added to the germ cells incubation media a large improvement (150%) of *CYP19* expression was observed in PS and conversely a 50% decrease was noted in RS [32].

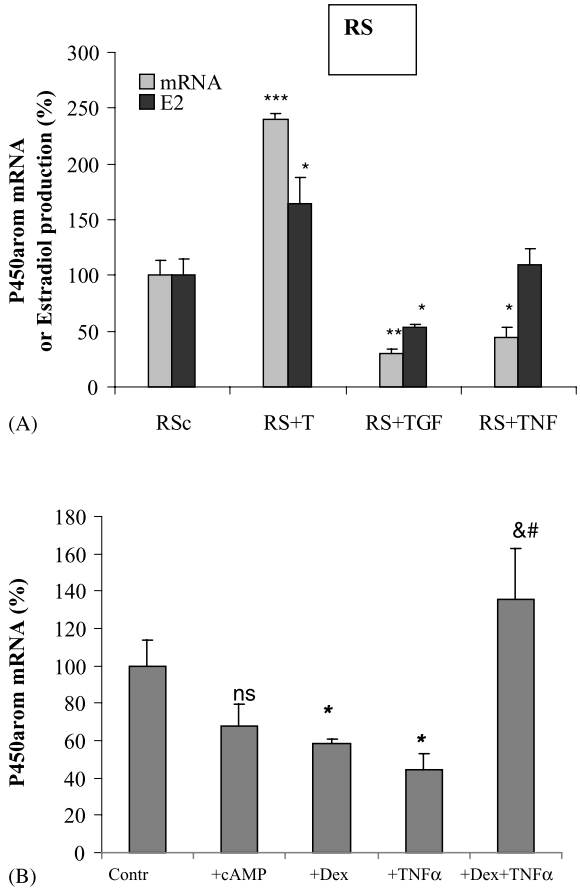

Fig. 4. (A) Effects of TGFβ$_1$, testosterone (T) and TNFα on P450arom mRNA levels and on estradiol production in round spermatocytes (RSc). The data are expressed in percentages; control cells (RSc) are considered to be 100%. Values are mean ± S.E.M. ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, when compared with control cells. (B) Effect of other treatments: cAMP, dexamethasone (Dex), TNFα and Dex + TNFα on *CYP19* gene expression and aromatase activity in RS. The data are expressed in percentages; values are mean ± S.E.M. ($n = 3$). $^{*}P < 0.05$, when compared with control cells (Contr); $^{\&}P < 0.05$ compared to TNFα and $^{\#}P < 0.05$ compared to Dex.

6. Effects of other factors presents in STM (or SCM) on *CYP19* gene expression

When purified Leydig cells were incubated with increasing concentrations of STM, a dose dependent enhancement of the P450arom mRNA was observed, the maximal effect (42%) was achieved in presence of 40% (v/v) STM without modification of the half-life of the aromatase transcript. In both PS and RS, the Sertoli cell conditioned medium (40%, v/v) induced an increase in P450arom mRNA levels (data not shown).

7. Other modulators

In order to analyze the putative role of some other factors such as nuclear transcription factor (SF-1 or the liver receptor homologue LRH-1 [33]) on the aromatase gene expression RT–PCR were realized using various purified testicular cells from mature rat. Our preliminary observations showed that mRNA of SF-1 but not LRH-1 was present in somatic cells (Leydig and Sertoli cells) whereas in germ cells the signals for both SF-1 and LRH-1 were detectable (unpublished observations). In addition, using the RT–PCR method and various primers located in the 3′-end of the P450arom coding region we have reported the existence of alternative splicing events of P450arom mRNA in rat somatic cells, PS and RS. At least two isoforms of P450arom were expressed (the exon 9 truncated transcripts represented 35% of the total amplified products in pachytene spermatocytes, 15% in spermatids and only 4% in Leydig cells. These aromatase transcripts were also demonstrated to be unable to encode biologically active protein [34].

8. Discussion

From these in vitro studies performed with various purified testicular cells from adult rat we have demonstrated that the aromatase gene expression is constitutively expressed and controlled by various factors. Among them gonadotropins, cyclic AMP, steroid hormones TGFβ and TNFα together with other paracrine factors likely from Sertoli cell and germ cell originsmodulate the *CYP19* expression in Leydig cells and germ cells. Our data are in agreement with our previous experiments developed by RACE–PCR establishing that the promoter located immediately upstream of the translation start site (promoter II) directs the expression of aromatase not only in Sertoli cells and Leydig cells, but also in germ cells of the rat [10]. Indeed, whatever the somatic cells studied gonadotropins (or cAMP) provoke a dose-related increase of the P450arom mRNA levels but the nadirs are different: 3–6 h in Sertoli cells [35], whereas the maximum is achieved at 24 h in Leydig cells. All together these observations are in keeping with the presence of CRE motifs localized in the promoter PII of the aromatase gene [7]. In germ cells specially in PS the positive control of *CYP19* by cAMP is in agreement with the presence of both CREM and CREB transcription factors which bind to CREs in these cells [36]. In addition, whatever the testicular cells studied we have shown that androgens are strong modulators of aromatase gene expression and activity. Similar observations have been reported in the brain of the rat [37] and of the male Rhesus monkey [38]. However as far as germ cells are concerned our data are thus in favor for the presence of androgen receptors since an antiandrogen (nilutamide) abolishes the positive effect induced by testosterone (data not shown); nevertheless the existence of androgen receptors in germ cells is currently a subject of debates. The role of estrogens in Leydig cells has been reported as negative [39] but that is not the case in mature rat Leydig cells as far as the *CYP19* expression is concerned [15]. In germ cells estradiol down-regulates

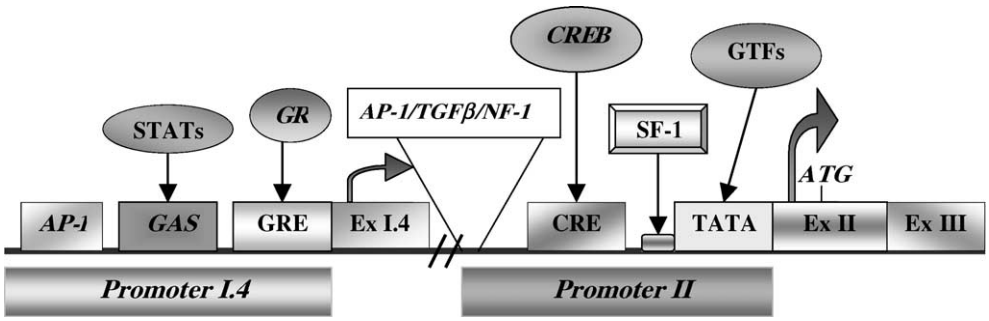

Fig. 5. Schematic representation of parts of the aromatase gene with the promoters concerned in rat testicular cells.

the aromatase gene expression likely via ERβ [40] which suggest that estrogens could have a paracrine and/or autocrine role on germ cell function and maturation therefore in modulating spermatogenesis as proposed in ArKO male mice [5,41].

Germ cells produce TGFβ and contain receptors for this peptide [42,43]; indeed in our purified germ cells, TGFβ inhibits the aromatase gene expression both in absence and in presence of testosterone which positively controls the transcription. The mechanism of action is not yet elucidated but SMADs and or oncogen (*c-fos*) may be involved [44] as well as a TGFβ response element since a conserved sequence is present on the PII promoter [11]. When germ cells were co-incubated with Dex and TGFβ an increase of aromatase transcription was noted (data not shown) suggesting an effect likely through the GRE sequence on promoter PI.4. This is in agreement with the data reported by Shozu et al. [45] showing that TGFβ increases the aromatase activity induced by Dex in osteoblast-like cells.

TNFα is also synthesized by germ cells and has been involved in numerous cellular functions such as an increase aromatase expression in adipose tissue in presence of Dex which thus involves the PI.4 promoter and an AP1 site [46]. In germ cells TNFα exerts a positive role in PS whereas in RS a negative control of aromatase is observed. Consequently, since both TNFα and Dex induce the same effect on aromatase gene expression that will suggest the requirement of PI.4 in the control of the *CYP19* expression. Consequently, TNFα may direct the expression of *CYP19* via promoter PI.4 in germ cells and used glucocorticoïds as a co-stimulator in RS.

Moreover it has been shown that rat [15] and human Leydig cells [47] as well as in germ cells that Sertoli-secreted factors modulate the aromatase expression suggesting the presence of other transcriptional factors either repressor or activator giving rise to either biologically active aromatase or inactive protein [34]. We may postulate that these paracrine factors and the alternative splicing events are necessary to control the aromatase expression and thus, the amount of endogenous estrogens in the rat testis. Finally it is also important to take into account the half-life of the aromatase transcripts from such various origin in rat testicular cells and in our conditions, hormonal treatments increase the stability of the mRNAs in Leydig cells without any changes at the transcriptional level as reported elsewhere [48]; that could be an other mechanism to control the amount of estradiol within the testis. It should be added that other modulators (SF-1 or the LRH-1) are involved in the regulation of *CYP19* expression at least in rat Leydig cells [49]. Finally from a recent work in male mice it appears that three distinct promoters exist to regulate the expression of *CYP19* in brain, ovary and testis [50].

All together in PS aromatase gene expression is in general up-regulated whereas it is down-regulated in more mature germ cells such as RS in which transcription is obviously much lower. Thus our results confirm that promoter PII is largely used in the control of aromatase gene expression but we cannot exclude a contribution of a PI.4 promoter in germ cells (Fig. 5). Age-related studies are on the way in our lab to better understand the precise localization of aromatase in the various testicular cells together with the concerned P450arom promoters.

Now it is clear that not only testicular somatic cells (Leydig and Sertoli cells) but also germ cells are able to express P450arom mRNA which is translated in a biologically active protein able to transform androgens into estrogens in several species of mammals. Germ cells (both meiotic and post-meiotic cells) do not only produce estrogens but contain estrogen receptors. This would explain part of the role (autocrine and/or paracrine) of estrogens in male germ cell development. The mechanisms of action of estrogens in the reproductive organs of the male remain to be clarified as well as the regulation of the aromatase gene expression, especially in germ cells during testicular development. Furthermore, one should keep in mind that not only rodent spermatozoa but ejaculated human spermatozoa contain specific P450arom transcripts together with ER [51] which thus open new considerations about the role of estrogens throughout the male genital tract.

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