Characterization of rat 5 α -reductases type 1 and type 2 expressed in *Saccharomyces cerevisiae*

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Two isoforms of the rat 5 α -reductase (5 α -R), the enzyme that converts testosterone into dihydrotestosterone (DHT), and other Δ^4 -3-keto steroids (e.g. progesterone and corticoids) into their 5 α -reduced metabolites, have been cloned. In this study, a convenient and efficient system was developed to overexpress the two isoenzymes in *Saccharomyces cerevisiae* by using the ubiquitin-fusion expression system. Two yeast expression vectors have been prepared, YEpR1 and YEpR2, which code for 5 α -R type 1 and 5 α -R type 2 respectively; they contain the copper-responsive yeast metallothionein promoter (CUP1) upstream of the ubiquitin coding sequence, and the full-length rat 5 α -R type 1 or 5 α -R type 2 cDNAs in frame to the 3' end of the ubiquitin cDNA. The activity of the two isoenzymes produced in yeast was

determined in cell lysates at the enzyme pH optima (type 1, pH 7.5; type 2, pH 5.5) and a possible differential intracellular distribution was also evaluated. The kinetic parameters were: type 1, K_m 4.6 μ M, V_{max} 100.6 μ g/h per mg of protein; type 2, K_m 68.6 nM, V_{max} 0.84 μ g/h per mg of protein. Yeast cell lysates were fractionated by differential centrifugation and the 5 α -R type 1 activity was maximal in fractions containing nuclei (1000 g and 2500 g), whereas the maximal activity of 5 α -R type 2 was present in subcellular fractions sedimenting at higher speeds (20000 g). The data indicate that yeasts overexpress the two 5 α -R isoenzymes, maintaining their native biochemical properties, and that the two isoforms are probably differentially localized within the yeast cell.

INTRODUCTION

In most androgen-dependent target tissues, testosterone is converted into a more active metabolite, 5 α -androstane-17 β -ol-3-one (dihydrotestosterone, DHT) through the action of the enzyme steroid 5 α -reductase (5 α -R; NADP⁺: Δ^4 -3-oxo-steroid 5 α -oxidoreductase, EC 1.3.99.5) [1]. This enzyme also catalyses the NADPH-dependent reduction of the Δ^4 double bond of several other steroid substrates (e.g. progesterone, androstenedione and corticoids). Two isoforms of the enzyme have been cloned in rat and man; they are encoded by different genes, have a limited degree of homology and possess different structural, biochemical and tissue distribution characteristics [2–5]. The reactions catalysed by the two isoforms of 5 α -R are similar, but some differences exist; for example, finasteride, a specific 4-azasteroid inhibitor used in the treatment of benign prostatic hyperplasia, is about 30-fold more potent against the human type 2 isoenzyme than against the type 1; this difference is much less pronounced in the two rat isoforms. The activation of testosterone in androgen-dependent reproductive tissues by the type 2 isoenzyme has important physiological roles [1] whereas the function(s) of the type 1 isoenzyme is still an open question; this isoform has been found in low amounts in most of the tissues containing the type 2 isoenzyme and in high concentrations in the skin, in the basal epithelial cells of the rat prostate [1], and in epithelial cell lines (DU145 and LNCaP) derived from human prostatic carcinoma [6, P. Negri-Cesi, A. Poletti, P. Magni and M. Motta, unpublished work].

The possibility of using suitable expression vectors for transforming yeasts with DNAs of animal origin is an important approach in studying the structure and function of proteins, in particular those that are membrane-bound and expressed at low levels in tissues. Yeast can be permanently transformed with plasmids that are present in high copy number within the cell and

that overexpress the products of the cloned genes; moreover, yeasts are eukaryotic cells that are much easier to grow and manipulate than animal cells; finally, their cellular biochemistry and regulatory mechanisms are, in several aspects, similar to those of higher eukaryotes [8]. Thus yeasts represent good surrogates for studying the structure and function of eukaryotic gene products. To clarify the role and the cellular distribution of 5 α -R, it was felt of interest to use the yeast *Saccharomyces cerevisiae* to produce high amounts of its two isoforms. In this paper the production of the two rat isoforms is reported by using a ubiquitin-fusion system that has not previously been applied to the study of these enzymes. The amounts of proteins produced by the ubiquitin-fusion technology are significantly higher than those obtained with conventional techniques [9]; the primary products of translation are hybrid ubiquitin–5 α -R proteins, which are rapidly and efficiently cleaved *in vivo* by the endoprotease ubiquitinase [10,11], releasing the authentic proteins. It has been demonstrated with several different types of protein that the hydrolysis of chimaeric proteins is only a function of the presence of the ubiquitin consensus sequence, and that this cleavage is completed a few minutes after the production of the primary translation product [12–14]. The biochemical characterization and the possible different subcellular localizations of the two rat 5 α -R isoenzymes were also investigated.

EXPERIMENTAL

Materials

Restriction enzymes, T4 DNA ligase and NADPH were purchased from Boehringer Mannheim; casamino acids, yeast nitrogen base without amino acids were from Difco (Detroit, MI, U.S.A.). Gel electrophoresis supplies and reagents were

Abbreviations used: CUP1, copper-responsive yeast metallothionein promoter; DHT, dihydrotestosterone; 3 α -diol, 5 α -androstane-3 α ,17 β -diol; 5 α -R, 5 α -reductase; YEpA, yeast episomal plasmid expressing chicken progesterone receptor form A; YEpR1, yeast episomal plasmid expressing rat 5 α -R type 1; YEpR2, yeast episomal plasmid expressing rat 5 α -R type 2.

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Figure 1 Construction of yeast vector (YEpR2 and YEpR1) and expression of the two isoenzymes of 5α-R

The linker realizing the fusion of ubiquitin to 5α-R type 2 was inserted into the full-length cDNA of 5α-R type 2, and the resulting cDNA was cloned in a vector suitable for protein expression in yeast; the same strategy was used for the type 1 isoform. The plasmids encoding 5α-R isoforms were introduced in yeast by using the lithium acetate protocol and selected by tryptophan prototrophy. The recombinant RNAs were induced by the addition of Cu^{2+} to the culture medium and were translated to yield ubiquitin–5α-R fusion proteins; after folding, the chimaeric protein is rapidly processed by a cellular ubiquitinase at the N-terminal part of the Arg-Gly-Gly consensus sequence to release authentic enzymes. CUP1 represents the promoter of the yeast metallothionein genes; CYC1 term is the transcriptional termination signal for the yeast CYC1 gene; TRP1 is the tryptophan-selective marker; 2micron is the replicating DNA of yeast derived from the yeast 2 μm plasmid; AmpR pBR322 is the selectable marker encoding ampicillin resistance.

from Bio-Rad (Richmond, CA, U.S.A.). Testosterone, DHT, 5α-androstan-3α,17β-diol (3α-diol), glucose, adenine, uracil, lyticase and T4 polynucleotide kinase were obtained from Sigma. [^{14}C]Testosterone (specific activity approx. 56.9 mCi/mmol), [^{14}C]DHT (sp. radioactivity approx. 56.9 mCi/mmol), [^3H]DHT and [^3H]3α-diol were purchased from Amersham. The *S. cerevisiae* strain used was BJ3505 (MATα, pep4::HIS3 PR61-Δ1.6R his3 lys2-208 trp1-Δ101 ura3-52 gal2) obtained from the Yeast Genetic Stock Center (Berkeley, CA, U.S.A.).

Construction of the expression vectors

YEpR1 and YEpR2, two high-copy-number yeast expression vectors derived from YEp52GSA [15], produce ubiquitin-fusion proteins cleaved *in vivo* by the enzyme ubiquitinase, to release

authentic proteins [10]. All recombinant DNA procedures were performed by using standard protocols [16]. The two expression plasmids were constructed as follows.

YEpR2

As shown in Figure 1, plasmid pSP72 (Promega, Madison, WI, U.S.A.) was digested with *Hind*III and *Kpn*I and religated with a linker (phosphorylated at the 5' end) that encodes the C-terminal 14 residues of ubiquitin fused, at the ATG_{MET} level, in frame to the coding sequence of the N-terminus of 5α-R type 2, obtaining pSPoli2. The linker possesses cohesive ends of *Hind*III and *Kpn*I. Two complementary oligonucleotides were synthesized to produce the linker, namely AGCTTGTCGACCTTACATC-TTGTCTTAAGACTAAGAGGTGGTATGCAGATTGTTG-

CCATCAGGTGCCGGTGTCTGGCAGGTAGCGCCACAT-TGGCCACTATGGGGACCCGCATGCGGTAC-3' and CG-CATGCGGGTCCCCATAGTGGCCAATGTGGCGCTA-CCTGCCAGCACCGGCACCTGATGGCAGACAATCTGC-ATACCACCTCTTAGTCTTAAGACAAGATGTAAGGTC-GACA-3'. pSPoli2 was sequenced [17] and partly digested with *Bgl*II, and to completion with *Sph*I. The 2500 bp fragment was ligated to the 870 bp *Bgl*II-*Sph*I fragment of pT801 (a vector containing the full-length cDNA of rat 5 α -R type 2, kindly provided by Dr. David W. Russell, UT Southwestern Medical Center, Dallas, TX, U.S.A.). The resulting plasmid, pSP5 α R2, was digested with *Sal*I/*Kpn*I and the 900 bp fragment, containing the 3' end of the ubiquitin sequence fused in frame to the complete open reading frame of the 5 α -R type 2, was inserted into the cognate sites of YEpA [12] giving rise to YEpR2; the plasmid produces rat 5 α -R type 2 under the control of the copper-responsive yeast metallothionein promoter (CUP1) [13].

YEpR1

As shown in Figure 1, plasmid pSPoli2 was digested to completion with *Afl*II and *Kpn*I and religated with a linker (previously phosphorylated at the 5' end with T4 polynucleotide kinase), which encodes the C-terminal six amino acids of the ubiquitin fused in frame to the coding sequence of the N-terminus of the 5 α -R type 1, to obtain pSPoli1; the two complementary synthetic oligonucleotides were TTAAGACTAAGAGGTGGCATGGA-GTTGGATGAGCTGTGCCTGCTCGGCATGCGGTAC-3' and CGCATGCCGAGCAGGCACAGCTCATCCAACTCC-ATGCCACCTCTTAGTC-3'. pSPoli1 was amplified, sequenced, digested to completion with *Sph*I and ligated to the 900 bp *Nsp*I fragment (*Nsp*I produces cohesive ends complementary to those of *Sph*I) of pB5 α RED1 (containing the full-length cDNA of rat 5 α -R type 1, kindly provided by Dr. David W. Russell), giving rise to pSP5 α R1. This plasmid contains a mutation (A $\rightarrow$ G) at the *Sph*I site, which was removed by digestion with *Sph*I and *Bgl*II (partial), and ligation in the presence of two oligonucleotides (a, 5'-CGACATG; b, 5'-TCGAGC) reproducing the sequence of the 5 α -R type 1 cDNA at this level (not shown in Figure 1); the correct sequence was confirmed by DNA sequencing. After digestion with *Afl*II and *Kpn*I (partial) the 960 bp fragment (containing the ubiquitin 3'-end fused in frame to the complete open reading frame of the 5 α -R type 1) was inserted into the cognate sites of YEpA [12], giving rise to YEpR1; this plasmid produces rat 5 α -R type 1 under the control of CUP1 [13].

Transformation of *S. cerevisiae* strain BJ3505

YEpR1 and YEpR2, which contain TRP1 as a selectable tryptophan-selective marker, were used to transform the protease-deficient *S. cerevisiae* strain BJ3505 by using the lithium acetate protocol [18]. Transformants were selected by tryptophan prototrophy. BJ3505[YEpR1] produces the type 1 and BJ3505[YEpR2] produces the type 2 5 α -R enzyme.

Preparation of yeast lysate

Transformed cells were grown overnight at 30 °C in CAAU medium [2 % (w/v) glucose, 0.1 % casamino acids, 0.67 % yeast nitrogen base, 0.001 % adenine, 0.002 % uracil]; 2×10^5 cells per ml were inoculated into fresh CAAU containing 100 μ M CuSO₄ (to induce CUP1 promoter) (Figure 1) and grown until D_{600} reached 1.0 (early mid-exponential phase). Spheroplasts were prepared as previously described [19]. Cells were lysed by osmotic shock (30 min at 4 °C) in low-salt buffer (PBS; 10 mM

potassium phosphate buffer, 150 mM KCl, 1 mM EDTA, pH 7.5 for 5 α -R type 1, pH 5.5 for 5 α -R type 2) and sonicated for 5 s at the lowest power.

Yeast cell fractionation

All steps were performed at 4 °C in PBS buffer. Yeast cell lysates were centrifuged at 1000 *g* for 10 min. The pellets were resuspended (2 ml) and centrifuged three times. The combined supernatants were centrifuged successively at 2500 *g* for 10 min, 20000 *g* for 15 min and 110000 *g* for 30 min. The pellets formed after each centrifugation were retained and resuspended. Protein concentrations were determined by the method of Bradford [20].

Determination of 5 α -R

The activity of 5 α -R was measured in PBS buffer (250 μ l) in the presence of 1 mM NADPH and [¹⁴C]testosterone [21]. The enzyme pH optima were analysed at pH values ranging from 3.5 to 8.0. The following conditions were also adopted: type 1 activity, [¹⁴C]testosterone 3–5 μ M, total protein 0.005 mg; type 2 activity, [¹⁴C]testosterone 0.3–0.5 μ M, total protein 0.02 mg. Yeast lysates of cells transformed with the plasmid YEpA (used to prepare both YEpR1 and YEpR2) [12] were used to determine basal activities of 5 α -R and 3 α -hydroxysteroid dehydrogenase. Kinetic studies were performed at the pH optima using [¹⁴C]testosterone concentrations ranging from 0.35 to 12 μ M for type 1, and from 0.01 to 0.36 μ M for type 2. K_i values were calculated by using 20 and 40 nM of the 5 α -R inhibitor finasteride. All incubations were performed for 30 min at 37 °C under a stream of O₂/CO₂ (98:2). The detection of metabolites was performed by TLC as previously described [21].

RESULTS

Figure 1 schematically shows the construction of the plasmids YEpR2 and YEpR1 expressing, respectively, the rat type 2 and the type 1 5 α -R isoenzymes, and the production of type 1 or type 2 5 α -R isoenzymes after transformation of the yeast strain BJ3505. Yeast cells bearing the plasmids were grown in the presence of 100 μ M CuSO₄ to activate the CUP1 promoter, which controls the expression of the ubiquitin-5 α -R cDNA. The chimaeric protein produced, ubiquitin-5 α -R isoenzymes, are cleaved *in vivo* by the endogenous yeast endoprotease ubiquitinase, immediately after the C-terminal sequence of ubiquitin [10,11].

Preliminary titration studies were performed on transformed yeast cell lysates to determine the optimum condition of incubation (time and amount of protein to be incubated) for kinetic studies. In control experiments, 5 α -R activity was also measured at different pH values in cell lysates obtained from the yeast strain BJ3505 transformed with the plasmid YEpA, which does not contain any 5 α -R coding sequences; in these experiments very small amounts of [¹⁴C]testosterone metabolites were found. The amount of DHT formed by BJ3505[YEpA] cell lysates, assayed at pH 7.5, was only 0.26 % of that observed for BJ3505[YEpR1]; DHT formation in BJ3505[YEpA] cell lysates, assayed at pH 5.5, was only 0.86 % of that observed for BJ3505[YEpR2]. These results indicate that the yeast strain BJ3505 does not possess intrinsic 5 α -R activity. In these preliminary studies it was also observed that strain BJ3505 possesses a very low 3 α -hydroxysteroid dehydrogenase activity, which may be disregarded in kinetic studies.

The variation of the enzymic activities of the two isoenzymes produced in yeast as a function of pH is summarized in Figure

Figure 2 Variation of enzymic activity as a function of pH

Cell lysates obtained from yeast transformed with either YEpR1 or YEpR2 were incubated for 30 min at 37 °C in PBS at pH values ranging from 3.5 and 8, in the presence of [14 C]testosterone as substrate and NADPH as cofactor; [14 C]DHT formation was determined as described in the Experimental section. The enzymic activity is expressed as ng of DHT formed per mg of protein. Each point represents a mean $\pm$ S.E.M. for experiments done in triplicate. Type 1, enzymic activity determined for yeast cell producing 5 α -R type 1; type 2, enzymic activity determined for yeast cell producing 5 α -R type 2.

Figure 3 Saturation curve and Lineweaver-Burk plot for [14 C]testosterone using yeast cell-expressed 5 α -R type 1

Aliquots of 5 μ g of protein from yeast cell lysates containing 5 α -R type 1 were assayed at pH 7.5 for 5 α -R type 1 activity by using various concentrations of [14 C]testosterone in the presence of 1 mM NADPH, as described in the Experimental section. The data are also shown (inset) in a reciprocal plot of $1/V$ against $1/[S]$ to determine kinetic parameters.

2. Yeast cell lysates of the strain BJ3505[YEpR1] activated by CuSO_4 convert testosterone into DHT; this isoform possesses a wide pH optimum, ranging from 6.0 to 7.5 (Figure 2, upper panel). In contrast, yeast cell lysates containing the type 2 isoform show a narrow pH optimum of about 5.5 (Figure 2,

Figure 4 Saturation curve and Lineweaver-Burk plot for [14 C]testosterone using yeast cell-expressed 5 α -R type 2

Aliquots of 20 μ g of protein from yeast cell lysates containing 5 α -R type 2 were assayed at pH 5.5 for 5 α -R type 2 activity by using various concentrations of [14 C]testosterone in the presence of 1 mM NADPH, as described in the Experimental section. The data are also shown (inset) in a reciprocal plot of $1/V$ against $1/[S]$ to determine kinetic parameters.

lower panel); very low conversion rates were observed at pH values higher than 6.5 or lower than 4.5.

The affinities of the natural substrate testosterone for the two isoforms expressed in yeast were calculated. Figure 3 shows that for 5 α -R type 1, saturation is obtained with 7 μ M [14 C]testosterone with a range of concentrations from 0.5 to 12 μ M. The apparent K_m and V_{max} (Figure 3, inset) of 5 α -R type 1 produced in yeast were 4.6 μ M and 100.6 μ g DHT formed/h per mg of protein. The apparent K_i determined for the 5 α -R inhibitor finasteride under the same conditions was of 14.66 nM, with a competitive model (results not shown).

The saturation analysis of 5 α -R type 2 determined in yeast cell lysates with [14 C]testosterone (from 10 to 350 nM) shows (Figure 4) that saturation occurs at very low concentrations of substrate (about 850 nM). A very high affinity for testosterone was observed for the enzyme, with an apparent K_m of 68.6 nM; the V_{max} was 0.84 μ g/h per mg of protein (Figure 4, inset). The apparent K_i value for finasteride was 2.7 nM (results not shown), with a competitive model; this result indicates that, for the rat 5 α -R isoenzymes produced in yeast, finasteride is about 7-fold more active on type 2 than on type 1.

To evaluate a possible different intracellular localization of the two isoforms, fractionation experiments, sequential centrifugations, substantially similar to those used to separate membranes and subcellular fractions from mammalian cells and tissues, were performed. The fractions obtained were analysed by electron microscopy; the results indicated that the 1000 g and 2500 g pellets contained mostly nuclei and cellular debris, whereas the 20000 g and 110000 g pellets were enriched in membranes and ribosomes. As indicated in Table 1, 5 α -R type 1 activity is enriched in the 1000 g and 2500 g pellets, as shown by the fact that the conversion of testosterone to DHT is increased 4–5-fold over the total cellular lysate. The enzymic activities of the 20000 g and 110000 g pellets were respectively 1.5-fold and one-half that of the starting material. No activity was found in the cytosolic fraction. A different pattern of distribution of the enzymic activity was found in the subcellular fractions of samples obtained from yeast cells producing 5 α -R type 2. The differences are evident, even if the conversion capacity of the type 2

Table 1 Cellular fractionation of yeast cell lysates containing 5 α -R1 or 5 α -R2

Transformed yeast cells were centrifuged at different sequential speeds and pellets were saved and incubated with [14 C]testosterone as described in the Experimental section. The formation of [14 C]DHT (ng of steroid formed per 30 min per mg of protein) with equal amounts of protein obtained from the subcellular fractions of yeast cell lysates containing 5 α -R types 1 or 2 is shown. Each point is the mean $\pm$ S.E.M. of experiments done in triplicate.

Subcellular fraction	BJ3505[YEPR1]	BJ3505[YEPR2]
Total cellular lysate	23 263 $\pm$ 2377	931 $\pm$ 18
1000 g pellets	98 274 $\pm$ 7186	1201 $\pm$ 23
2500 g pellets	80 413 $\pm$ 6953	1276 $\pm$ 9
20000 g pellets	30 935 $\pm$ 2407	1801 $\pm$ 22
100000 g pellets	11 291 $\pm$ 304	1259 $\pm$ 104
Cytosolic fractions	183 $\pm$ 13	24 $\pm$ 1

isoenzyme is lower than that of type 1 (see the different scales of Figures 3 and 4). In fact, only a slight increase in the ability to form DHT was observed in the 1000 g, 2500 g and 110000 g pellets. The type 2 5 α -R activity was maximal in the 20000 g pellet, at values double those present in the total cellular lysate. Only negligible amounts of DHT were formed by the cytosolic fraction.

DISCUSSION

Yeast systems have recently been used to express a number of proteins involved in the mechanism of action of hormonal steroids (e.g. receptors for progesterone [12,14,19], oestrogen [22,23], glucocorticoid [24] and vitamin D [9], human placental aromatase [25] and rat 5 α -R type 1 [26]). The data obtained suggest that the post-translational modifications (e.g. glycosylation [27] or phosphorylation [19]) required for the activity of proteins involved in steroid action are conserved in yeast cells.

In the present study, cDNAs for rat 5 α -R types 1 and 2 have been cloned into yeast plasmid vectors to produce the corresponding functional enzymes. The availability of consistent amounts of these proteins will permit an easier biochemical and kinetic characterization, mutational studies and an evaluation of the efficacy of specific inhibitors. The strategy of having the yeast synthesizing the two isoforms as ubiquitin-fusion proteins was adopted because previous results had shown that this procedure enhances the integrity by minimizing the N-terminal heterogeneity of the recombinant protein [9]. This integrity is especially important for the two isoforms of 5 α -R because the N-terminal domains of these two proteins have been described as the sites of interaction with their substrates and with specific inhibitors such as finasteride [1]. In this connection it has been demonstrated that a segment of four amino acids located at the very beginning of the N-terminal portion of rat 5 α -R type 1 (from residue 26 to residue 29) confers the sensitivity towards finasteride [28]. Moreover, single amino acid substitutions in the N-terminal region of the human type 2 isoenzyme greatly impair its affinity for testosterone and are responsible for the clinical syndrome of 5 α -R deficiency [1,29].

The expression in yeast of rat 5 α -R type 1 (as deduced from the sequence analysis, but not by kinetic studies) has been described by Ordman et al. [26]. However, those authors used a different promoter, PHO5, and did not use the ubiquitin-fusion protein system. The expression driven by the CUP1 promoter produces 10-fold more enzyme (as shown by the specific activities of 5 α -R type 1) than when the PHO5 promoter is used. Moreover, the

PHO5 promoter necessitates a longer induction time (e.g. 48 h), which is longer than the 12–16 h needed when the CUP1 promoter is used. Finally, the importance of the strategy with the ubiquitin-fusion protein system has been stressed above.

The kinetic studies showed that both the type 1 and type 2 isoenzymes of 5 α -R are highly expressed and fully active in *S. cerevisiae*. The type 2 isoenzyme has a higher affinity for testosterone but a lower capacity for conversion than type 1. It has already been reported that the two rat isoforms have different sensitivities to finasteride [28], and this observation has been confirmed in the present study with the enzymes produced in yeast: the K_i for finasteride was found to be higher for type 1 (14.66 nM) than for type 2 (2.7 nM). Finally, the two isoforms expressed in yeast have the same pH optima as the respective native enzymes, i.e. a wide pH range (from 6 to 7.5) for type 1, and a narrow pH optimum at 5.5 for type 2.

The fractionation studies indicate that the two isoenzymes may have different intracellular localizations. It was found that the highest enzymic activity of type 2 was present in the 20000 g pellet. Even if a complete resolution of the major yeast organelles by subcellular fractionation was not established as firmly as for mammalian cells, the 20000 g fraction seems to contain most of the cellular membranes derived from the endoplasmic reticulum [8]. A preferential localization in the perinuclear ergastoplasm has been described for the type 2 isoenzyme in human androgen-sensitive tissue [30,31]. In the present study the activity of the type 1 isoform was observed in the 1000 g and 2500 g pellets (two fractions known to contain the cell nuclei). The perinuclear or nuclear localization of the type 1 isoenzyme might facilitate the formation of DHT, and its subsequent binding to nuclear androgen receptors [32], before the rapid inactivation of DHT to less potent androgens (such as 3 α -diol by the action of the cytoplasmic enzyme 3 α -hydroxysteroid dehydrogenase) [33]. It is probably premature to extrapolate to mammalian tissues the different localizations of the two 5 α -R isoenzymes found in the yeast cellular fractions; if this occurs also in mammals, different intracellular physiological role(s) of the two enzymes could be postulated. The association of the 5 α -R type 1 with the enriched nuclear fraction in yeast partly conflicts with the results by Ordman et al. [26], who found the highest activity of type 1 isoenzyme produced in yeast cells in the 12000 g fraction. The discrepancy is probably explained by the difference in the homogenization procedure adopted; in fact, homogenization of the yeast spheroplasts with a Teflon homogenizer, reported by Ordman et al., might have damaged the nuclei and altered the sedimentation properties of the organelles. In the present study, homogenization was obtained by slight sonication, which prevents nuclear damage.

The present data on the preferential nuclear association of 5 α -R type 1 find support in studies performed in mammals, even if a final agreement on the cellular compartmentalization of the native 5 α -R isoenzymes in rat and human tissues is not yet available [1,31,34–41].

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