J. Steroid Biochem. Molec. Biol. Vol. 42, No. 3/4, pp. 259–265, 1992
Printed in Great Britain

0960-0760/92 $5.00 + 0.00
Pergamon Press Ltd

THE METHYLTRIENOLONE BINDING PROTEIN OF JEG-3 CELLS AND HUMAN PLACENTA IS LOCALIZED WITHIN THE NUCLEUS AND IS TIGHTLY ASSOCIATED WITH CHROMATIN

J. O. MACAULAY,[1]* G. L. WARNE[1] and Z. S. KROZOWSKI[2]

[1]Department of Endocrinology and Diabetes, Royal Children's Hospital, Flemmington Road, Parkville, Victoria, 3052 and [2]Laboratory of Molecular Hypertension, Baker Institute, P.O. Box 348, Prahran, Victoria, 3181, Australia

(Received 20 July 1991)

Summary—Human placenta contains the methyltrienolone binding protein (MTBP), an androgen binding protein which is distinct from the androgen receptor. This study demonstrates that the human choriocarcinoma cell line (JEG-3) also contains the MTBP and that in both human placenta and JEG-3 cells the MTBP is located exclusively in the nucleus and in particular is associated with DNase 1 resistant chromatin.

INTRODUCTION

Our previous reports of androgen binding in human placenta have demonstrated the presence of the methyltrienolone binding protein (MTBP), an androgen binding protein which is distinct from the androgen receptor [1–3]. The characteristics which distinguish the MTBP from classical androgen receptors and other androgen binding proteins are: it requires the nucleotide NAD^+ for steroid binding activity, unique steroid specificity (high affinity for R1881 but negligible affinity for dihydrotestosterone and mibolerone), a K_d for R1881 of 10 nM, and a molecular weight of 67 kDa.

Early studies of steroid hormone actions proposed that unoccupied steroid receptors were present in cytosol where they bound ligand resulting in the activation or transformation of the receptor into a state with increased affinity for nuclear acceptor sites. Recent studies, however, have shown that both unoccupied and occupied steroid receptors are localized in the nucleus of intact cells [4, 5]. The currently accepted model of steroid hormone action proposes that binding of the hormone to the receptor protein causes the hormone–receptor complex to acquire high affinity for a limited number of nuclear acceptor sites resulting in the modulation of specific gene expression [6, 7]. There are also reports of steroid receptors in other subcellular fractions [8–12], although their function within these structures is unknown. The aim of this study was to determine the intracellular localization of MTBP.

*To whom correspondence should be addressed.

EXPERIMENTAL

Materials

[17-α-methyl-^{3}H]R1881 and radioinert R1881 (17β-hydroxy-17α-methylestra-4,9,11-trien-3-one) were obtained from New England Nuclear Corp. (Boston, MA). Other radioinert steroids were from Sigma Chemical Co. (St Louis, MO). Deoxyribonuclease 1 (DNase 1) was from Sigma. All reagents were of analytical grade.

Tissue samples

Human placentas were obtained immediately after delivery. The tissue was cut into small pieces and either frozen in liquid nitrogen or in dry ice/hexane and stored at −70°C.

Cell structure

Human choriocarcinoma cells, JEG-3 (ATCC HTB 36, American Type Culture Collection, Rockville, MD) were cultured in RPMI medium with 10% fetal calf serum (FCS), 0.29% glutamine, 20 mM HEPES, penicillin and streptomycin at 37°C in a humidified 95% air/5% carbon dioxide atmosphere.

Subcellular fractionation of human placenta

A modification of the method of Dobashi *et al.* [13] was used to isolate nuclei, mitochondria, plasma membranes, microsomes and cytosol. All procedures were performed at 4°C. Human placental tissue was allowed to thaw and was then homogenized using a polytron PT10S homogenizer (Kinematica GmbH Littau, Luzern, Switzerland) at a setting of 4 with two 15 s bursts, allowing 30 s between bursts for cooling. Homogenization was performed at 4°C in 4 vol of Buffer A (0.25 m sucrose, 2 mM Tris–HCl, 1 mM EDTA pH 7.4). The homogenate was filtered through gauze and centrifuged at 1000 *g* for 15 min. The pellet was washed twice in the same buffer. The pooled supernatants were centrifuged at 8000 *g* for 20 min, the pellet washed twice and the pooled supernatants centrifuged at 24,000 *g* for 10 min. The resultant pellet was washed twice in the same buffer and the pooled supernatants centrifuged at 100,000 *g* for 60 min. The final pellet was resuspended in Buffer A.

Isolation of nuclei

The method of Flint and Burton [14], was used to isolate nuclei from human placental tissue. Human placenta which had been frozen on dry ice/hexane was thawed and minced in 1 vol of Buffer B (50 mM Tris–HCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4) and homogenized by hand in a Teflon–glass homogenizer at 4°C. The homogenate was centrifuged for 15 min at 600 *g* and the pellet washed twice in Buffer C (Buffer B containing 0.25 M sucrose). The final pellet was resuspended in Buffer C. An aliquot of the nuclei preparation was resuspended in phosphate buffered saline (PBS) and the nuclei smeared onto glass slides and fixed in methanol (5 min). The slide was then stained with haematoxylin and eosin and the purity of the nuclei preparation checked by phase microscopy. This method yielded intact nuclei with very little cytosolic contamination.

Nuclei were isolated from JEG-3 cells by first releasing the cells from 75 cm^2 flasks with trypsin followed by washing with PBS. The cells were resuspended in Buffer B and snap frozen on dry ice. The cell suspension was then thawed at room temperature and the freeze/thaw cycle repeated 4 more times. The cell suspension was then centrifuged for 10 min at 10,000 *g* and the pellet washed three times in Buffer B. The final pellet was resuspended in Buffer C. The nuclei were fixed and stained as described above.

Extraction of chromatin

Chromatin was extracted by the method of Spelsberg *et al.* [15]. Nuclei prepared as described above, from both placental tissue and JEG-3 cells, were pelleted and resuspended in 50 vol of Buffer D (80 mM NaCl, 20 mM EDTA, pH 6.3) and homogenized by hand in a Teflon–glass homogenizer. The homogenate was then centrifuged at 10,000 *g* for 10 min. The pellet was rehomogenized and centrifuged twice in the same buffer and the supernatants pooled. The pellet was rehomogenized and centrifuged in 5 vol of 0.35 M NaCl followed by 5 vol 0.1 mM EDTA, 2 mM Tris–HCl pH 7.5 to yield the final pellet (chromatin) which was resuspended in 0.1 mM EDTA, 2 mM Tris–HCl.

Binding assays

[^{3}H]R1881 binding was measured in subcellular fractions with 10 nM [^{3}H]R1881 $\pm$ 2 μM R1881 and 200 μM NAD^+ for 1 h at 4°C. Separation of bound from free steroid (in particulate fractions) was performed by centrifugation at 10,000 *g* followed by washing of the pellet and extraction of steroids into 100% ethanol. The DCC method [1] was used to separate bound from free steroid in soluble fractions.

JEG-3 cells were grown in monolayers and the binding assay performed using a modification of the method used in our laboratory to measure androgen receptors in human fibroblasts [16]. JEG-3 cells were grown to confluency in 2 cm^2 24 well plates then washed twice with serum-free medium and incubated with 10 nM [^{3}H]R1881 $\pm$2 μM unlabeled R1881 in serum-free medium for 60 min at 37°C. Cells were than washed 5 times with cold PBS, 0.2 ml trypsin/versene solution added and monolayers incubated at 37°C until cells were released from the plates. 0.3 ml PBS was added and the cell suspension transferred to tubes, sonicated for 30 s and 0.2 ml counted for radioactivity. 0.2 ml aliquots were taken for DNA determination.

Protein and DNA determination

Protein was determined by the method of Bradford [17]. DNA was determined by the method of Cesarone *et al.* [18].

Enzyme assays

5′-Nucleotidase activity was determined as described by Aronson and Touster [19] and glucose-6-phosphatase activity was determined by a modification [20] of the method of Hers [21].

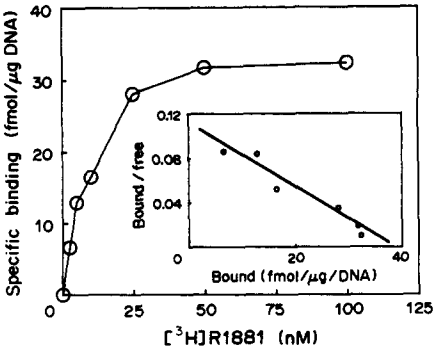

Fig. 1. Saturation curve of and Scatchard analysis of [^{3}H]R1881 binding to JEG-3 cells. JEG-3 cells (2 cm^2 wells) were incubated with 2.5–100 nM [^{3}H]R1881 ± 200-fold excess unlabeled R1881 in serum-free medium for 1 h at 37°C. Free steroid was separated from bound as described in Experimental. Specific binding is plotted. Inset is the Scatchard plot of the data.

DNase 1 digestion of chromatin

Isolated chromatin was digested with DNase 1 by resuspending the chromatin in 10 mM Tris–HCl, 5 mM $MgCl_2$, pH 7.4 containing protease inhibitors (10 mM *N*-ethylmalamide, 1 mM benzamidine HCl, 0.1 mM phenylmethyl sulfonylfluoride) and incubating with 50 μg/ml DNase 1 at 37°C. Aliquots were taken at 5, 10, 15 and 30 min put on ice and adjusted to 0.01 M EDTA. Samples were then centrifuged at 10,000 *g* for 10 min and the pellet washed and resuspended in 2 mM Tris–HCl, 0.1 mM EDTA pH 7.4.

RESULTS

A human placental cell line, JEG-3, was examined as a possible source of the MTBP. Initially the JEG-3 cells were grown in monolayers and assayed for androgen binding using a modification of the method used in this laboratory to measure androgen receptors in human fibroblasts [16]. Saturation analysis was performed using [^{3}H]R1881 over a range of 0–100 nM. Figure 1 is a representative binding curve derived Scatchard plot. JEG-3 cells have a high affinity R1881 binding site [K_d 8.3 ± 2.4 nM (n = 3)] which is saturable and of limited capacity (55.6 ± 11.3 fmol/μg DNA). These cells therefore contain an androgen binding protein with an affinity for R1881 similar to that of the human placental MTBP.

To determine if the androgen binding was indeed to MTBP and not androgen receptors or other androgen binding proteins, specificity studies were performed with [^{3}H]R1881 in the absence and presence of a 200-fold excess of various unlabeled steroids (Table 1). [^{3}H]R1881 binding was competed for strongly by R1881 (29% remaining), with androstenedione and testosterone the next strongest competitors (46 and 54% remaining, respectively). The other androgens, dihydrotestosterone (DHT) and mibolerone, displaced slightly at this concentration but to no greater extent than the other classes of steroids tested. The steroid specificity of these cells was then examined further by testing the ability of these steroids to displace [^{3}H]R1881 over a range of concentrations (10–1000 nM) (Fig. 2). The results obtained were similar to those obtained in human placental cytosol [2] and strongly suggests that the

Table 1. Steroid specificity of [^{3}H]R1881 binding to JEG-3 cells: percentage of binding remaining in the presence of 200-fold excess of unlabeled steroid

Steroid	% of Total binding remaining
—	100
R1881	28.7 ± 3.3
Androstenedione	46.0 ± 1.3
Testosterone	53.7 ± 6.9
Mibolerone	73.3 ± 4.5
Dehydroepiandrosterone	73.3 ± 2.4
Estrone	76.7 ± 6.8
DHT	78.0 ± 2.6
Progesterone	78.7 ± 3.5
Estradiol	80.7 ± 2.6
Estriol	81.0 ± 3.6
Cortisol	88.9 ± 2.7
Dehydroepiandrosterone-sulfate	91.3 ± 2.0
Dexamethasone	92.0 ± 4.6
Triamcinolone acetonide	95.0 ± 4.5

JEG-3 cells (2 cm^2 wells) were incubated with 10 nM [^{3}H]R1881 ± 2 μM unlabeled competing steroid in serum-free medium for 1 h at 37°C. Free steroid was separated from bound as described in Experimental. Data are the means ± SEM of three separate determinations.

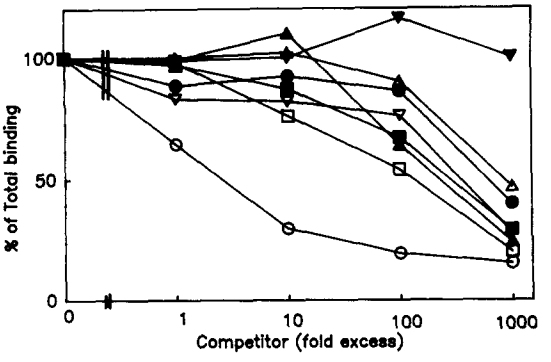

Fig. 2. Steroid specificity of [^{3}H]R1881 binding to JEG-3 cells. JEG-3 cells (2 cm^2 wells) were incubated in the presence of 10 nM [^{3}H]R1881 with or without the addition of 1-, 10-, 100- and 1000-fold excess of nonradioactive competing steroid for 1 h at 37°C. Competing steroids were: R1881 (○), mibolerone (●), DHT (△), testosterone (▲), androstenedione (□), R5020 (■), progesterone (▽) and triamcinolone acetonide (▼). Free steroid was separated from bound as described in Experimental. Binding in the presence of 10 nM [^{3}H]R1881 alone was set at 100%. Data are the means of three separate determinations. SEM were < 10% and are not shown.

Table 2. Characterization of [^{3}H]R1881 binding to nuclei from JEG-3 cells

	Supernatant	Nuclei
Binding activity (% of total)	1.6 ± 1.3	98.4 ± 1.3
Glucose-6-phosphatase activity (% of total)	100	—
5′-Nucleotidase activity (% of total)	100	—

JEG-3 cells were lysed and [^{3}H]R1881 binding measured with 10 nM [^{3}H]R1881 ± 2 μM R1881 and 200 μM NAD$^+$ for 1 h at 4°C. For nuclei binding free steroid was separated from bound by centrifugation at 10,000 *g* followed by washing and extraction of steroids in 100% ethanol. Binding in supernatant was measured with the DCC method. Enzyme activity was determined as in Experimental. Data are the means ± SEM of three separate determinations.

androgen binding species in the JEG-3 cells is the MTBP.

When JEG-3 cells were lysed and nuclei isolated 98% of [^{3}H]R1881 binding was found to be associated with the nuclei (Table 2). Marker enzymes were assayed as a criterion of purity of the nuclei. 5′-Nucleotidase, a marker for plasma membranes, and glucose-6-phosphatase, a marker for endoplasmic reticulum, were not associated with the nuclear fraction (Table 2), confirming that the nuclei were not contaminated with these fractions. Haematoxylin and eosin staining showed intact nuclei with very little cytosolic contamination (data not shown). These data demonstrate that in JEG-3 cells R1881 binding activity is associated predominantly with nuclei.

To determine if the MTBP in human placenta was also associated with nuclei a crude subcellular fractionation of human placental tissue was performed. The data in Table 3 shows that 62% of the [^{3}H]R1881 binding activity was associated with the 1000 *g* nuclear pellet. The remaining [^{3}H]R1881 binding activity was primarily in the cytosol where 20% of the binding activity was found. Low [^{3}H]R1881 binding was found in the other fractions. Marker enzymes were again assayed as a criterion of purity of the subcellular fractions. 5′-Nucleotidase was predominantly in the nuclear pellet as well as in the 24,000 *g* pellet and the cytosolic fraction. Glucose-6-phosphatase was found predominantly in the 24,000 *g* pellet. These data suggest that the R1881 binding activity is associated mainly with the nuclear fraction.

Table 3. Binding of [^{3}H]R1881 in subcellular fractions of human placenta

Fraction	Specific R1881 binding (% of total)	Enzyme activity: 5′-Nucleotidase (% of total)	Enzyme activity: G-6-Pase (% of total)
1000 *g* pellet (nuclei)	62.2 ± 7.2	49.0 ± 1.8	32.1 ± 2.1
8000 *g* pellet (mitochondria)	3.1 ± 0.4	5.8 ± 0.1	2.2 ± 0.1
24,000 *g* pellet (plasma membranes)	8.6 ± 0.4	23.2 ± 2.2	58.8 ± 1.9
100,000 *g* pellet (microsomes)	5.8 ± 0.3	7.4 ± 0.8	6.8 ± 1.1
Cytosol	20.1 ± 2.5	14.5 ± 1.8	—

Subcellular fractionation of human placenta was performed as described in Experimental. [^{3}H]R1881 binding was measured with 10 nM [^{3}H]R1881 ± 2 μM R1881 and 200 μM NAD$^+$ for 1 h at 4°C. For particulate fractions separation of bound from free steroid was by centrifugation at 10,000 *g* followed by repeated washing of the pellets and extraction of steroids in 100% ethanol. The DCC method was used to separate bound from free steroid in the cytosol. Enzyme activity was determined as described in Experimental. Data are the means ± SEM of three separate determinations and are expressed as the percentages of total activity recovered.

To confirm that the MTBP was indeed associated with the nuclear fraction placental tissue was fractionated using a protocol for the isolation of purified nuclei. The purity of the nuclear preparation was assessed by haematoxylin and eosin staining and measurement of marker enzyme activity. The nuclei prepared by this method had very little cytoplasmic contamination (data not shown) and there was very little contamination of nuclei with either plasma membranes or endoplasmic reticulum (Table 4). When [^{3}H]R1881 binding activity was measured in the nuclei and low speed supernatant 92% of the R1881 binding activity was found in the nuclei. These data confirm that human placenta, like JEG-3 cells contain R1881 binding activity associated predominantly with nuclei.

Further experiments were performed to determine if the R1881 binding associated with the nuclei from both the placental tissue and the JEG-3 cells was to the MTBP and if any androgen receptors were present. The MTBP has a clearly distinct pattern of steroid binding which is readily distinguishable from androgen receptors [1, 2]. Nuclei from human placenta and JEG-3 cells were incubated with [^{3}H]R1881 in the presence of a 200-fold excess of competing steroid. R1881 competed strongly while neither

Table 4. Binding of [^{3}H]R1881 in nuclei from human placenta

	Supernatant	Nuclei
Binding activity (% of total)	7.7 ± 1.6	87.8 ± 3.5
Glucose-6-phosphatase activity (% of total)	91.0 ± 7.3	8.1 ± 1.2
5′-Nucleotidase activity (% of total)	87.8 ± 3.5	12.1 ± 2.0

Nuclei were prepared as described in Experimental. [^{3}H]R1881 binding measured with 10 nM [^{3}H]R1881 ± 2 μM R1881 and 200 μM NAD$^+$ for 1 h at 4°C. For nuclei free steroid was separated from bound by centrifugation at 10,000 *g* followed by washing and extraction of steroids in 100% ethanol and the DCC method was used for the supernatant. Enzyme activity was determined as in Experimental. Data are the means ± SEM of three separate determinations.

Table 5. Steroid specificity of [^{3}H]R1881 binding to nuclei from human placenta and JEG-3 cells: percentage of binding remaining in the presence of 200-fold excess of competing steroid

Competing steroid	% of Total binding remaining	
	Placenta	JEG-3 cells
—	100	100
R1881	33.4 ± 5.3	39.1 ± 1.9
Mibolerone	92.6 ± 3.2	96.3 ± 1.3
DHT	89.8 ± 2.3	91.1 ± 1.0

Nuclei were incubated with 10 nM [^{3}H]R1881 ± 200-fold excess of unlabeled competing steroid and 200 μM NAD$^+$ for 1 h at 4°C. Separation of bound from free was as described in Experimental. Data are the means ± SEM of three separate determinations.

mibolerone nor DHT competed to any significant extent (Table 5). These data confirm that the R1881 binding measured in nuclei from both placental tissue and the JEG-3 cells is to the MTBP.

Nuclear steroid hormone receptors have previously been reported to be associated with chromatin [22–25]. Therefore chromatin was isolated from both the nuclei of placental tissue and JEG-3 cells to determine if the MTBP is also associated with this nuclear fraction. Nuclei were isolated, then homogenized and extracted in a series of buffers as described in Experimental. R1881 binding activity was assayed in supernatant fractions as well as the final chromatin pellet. The data in Table 6 shows that most of the MTBP R1881 binding activity was found to be associated with the chromatin pellet with negligible R1881 binding activity found in the other fractions. Therefore the MTBP in both placental tissue and the human choriocarcinoma cell line JEG-3 is tightly associated with chromatin.

Since chromatin acceptor sites for steroid receptors have been reported to be resistant to DNase 1 activity [25–27] we tested the chromatin

Table 6. Isolation and binding of [^{3}H]R1881 to chromatin from placental and JEG-3 cell nuclei

Fraction	% of Total binding	
	Placenta	JEG-3 cells
Nuclei	100	100
S1	6.6 ± 0.3	16.2 ± 1.5
S2	1.3 ± 0.2	1.2 ± 0.2
S3	0.3 ± 0.1	—
Chromatin pellet	92.0 ± 1.2	82.5 ± 2.5

Nuclei were prepared as described in the Experimental and chromatin extracted by homogenizing in 50 vol of 80 mM NaCl, 20 mM EDTA pH 6.3 followed by centrifugation at 10,000 *g* for 10 min. The pellet was then rehomogenized and centrifuged twice in the same buffer and the supernatants pooled (S1). The pellet was rehomogenized and centrifuged in 0.35 M NaCl (S2) and 0.1 mM EDTA, 2 mM Tris–HCl pH 7.5 (S3) to yield the final pellet (chromatin). All fractions were incubated with 10 nM [^{3}H]R1881 ± 2 μM R1881 and 200 μM NAD$^+$ for 1 h at 4°C. Bound from free steroid was separated by the DCC method for S1, S2 and S3 and in nuclei and the chromatin pellet was separated by centrifugation at 10,000 *g* followed by washing and extraction of the steroids in 100% ethanol. Data are the means ± SEM of three separate determinations.

MTBP binding sites for susceptibility to DNase 1 digestion. Digestion of chromatin from human placenta and JEG-3 cells for 30 min at 30°C with 50 μg DNase 1 resulted in no loss of MTBP [^{3}H]R1881 binding activity, suggesting that placental and JEG-3 chromatin MTBP acceptor sites are resistant to DNase 1 digestion (data not shown).

DISCUSSION

The currently accepted model for the action of androgens is that they enter the cell and then bind to androgen receptors which, both in the presence and absence of ligand, are located in the nucleus [28, 29]. However there are reports of androgen receptors associated with endoplasmic reticulum [8, 9] and of other classes of steroid receptors associated with endoplasmic reticulum and plasma membranes [10–12]. It was therefore important to determine if cytosol, where we had initially identified the MTBP, was the only site of MTBP or if MTBP was also present in other intracellular fractions.

In previous studies we reported the presence of MTBP in human placental cytosol and demonstrated the difference between the MTBP and classical androgen receptors [1–3]. In contrast, the data reported here indicate that the MTBP is found exclusively in the nucleus. In experiments using placental tissue approx. 90% of the MTBP R1881 binding activity was associated with nuclei while in JEG-3 cells 98% of the MTBP R1881 binding was associated with nuclei. These results contrast with those of our previous studies where we observed high levels of MTBP in the cytosolic fraction. This discrepancy is probably due to differences in methodology. In our previous studies the placental tissue had been frozen by immersion in liquid nitrogen and the tissue homogenized with a polytron. These conditions possibly disrupt the nuclei in addition to breaking the cells. In the studies reported here placental tissue was cut into small pieces and frozen in dry ice/hexane, conditions under which cellular structure is maintained intact. The tissue was then gently homogenized at a lower speed for a shorter period of time, conditions which would be less likely to rupture the nuclear membrane.

In a previous report examining androgen binding in the human placenta it was found that placental nuclei did in fact bind androgens but it was concluded that the binding was to androgen receptors [30]. Our previous studies have

shown conclusively that the R1881 binding in human placental cytosol is not to androgen receptors but to the MTBP [1–3]. The steroid specificity data reported here, which shows that both the placental and JEG-3 cell nuclei have a high affinity for R1881, but exhibit negligible binding of DHT or mibolerone, confirms that in both human placental tissue and JEG-3 cells the nuclear R1881 binding species is the MTBP and not androgen receptors. Thus, this cell line is a suitable model for studying the biological actions of MTBP. Murphy and Hyde [37] have reported that certain steroids increase the binding of R1881 in human placental homogenates but in our studies no such effects were observed.

In the present study we also found that the MTBP is tightly bound to chromatin but is not released by DNase 1 digestion. The nuclear binding sites for steroid receptors represent the first nuclear event in the steroid alteration of gene transcription. In order for steroids to regulate gene expression, an interaction between steroid hormone receptors and the nuclear acceptor sites in the genome is required. Acceptor sites for androgen receptors have been reported to be associated with chromatin [22–25, 31] as have acceptor sites for other classes of steroid receptors [32, 33]. Studies of chromatin acceptor sites for androgen and other steroid receptors have found that these sites are resistant to DNase 1 digestion [25–27] and it has been suggested that the receptors are probably bound to adjacent proteins [23]. The resistance to DNase digestion has also been interpreted to suggest that the chromatin acceptor sites may be related to acceptor sites associated with the nuclear matrix [26]. The association of the MTBP with chromatin raises the question of whether it may act as a transcription factor similar to classical steroid receptors.

Our previous data showed that MTBP has many characteristics which differentiate it from classical androgen receptors, in particular its requirement for the nucleotide NAD^+ for steroid binding activity [3]. An NADPH dependent tri-iodo-L-thyronine (T_3) binding protein has previously been described by Hashizume *et al.* [34]. This cytosolic protein is thought to be involved in both the transport of T_3 to the nucleus and to serve as a reservoir of cytoplasmic T_3. It is, however, quite distinct from the nuclear T_3 receptor [35]. One form of the cytosolic T_3 binding protein has been demonstrated to bind to nuclei and the nuclear acceptor sites shown to be resistant to DNase 1 treatment [36]. These authors suggest that the cytosolic T_3 binding protein binds to nuclei and may act as a donor of T_3 to the nuclear receptor present in the nuclear matrix. Although the T_3 binding protein resembles MTBP in its requirement for nucleotide cofactors it is probably quite distinct in function as it has both nuclear and cytosolic forms while the MTBP has been demonstrated to be exclusively localized to the nucleus in both term placenta and cultured human choriocarcinoma cells.

In conclusion, we have reported that the human choriocarcinoma cell line JEG-3 contains the MTBP and that in both human placenta and JEG-3 cells the MTBP is located exclusively in the nucleus and in particular is associated with DNase 1 resistant chromatin. The presence of the MTBP in nuclei and its tight association with chromatin suggests that MTBP may be acting directly or indirectly to modulate gene transcription. JEG-3 cells provide a potential model for investigating the genomic effects of R1881 mediated by the MTBP.

Acknowledgements—We would like to thank the Royal Women's Hospital for providing the human placental tissue. This work was supported by a National Health and Medical Research Council grant and by the Royal Children's Hospital Research Foundation.

REFERENCES

1. Macaulay J. O., Warne G. L. and Krozowski Z. S.: Human placenta contains a high affinity R1881 binding site that is not the androgen receptor. *J. Steroid Biochem.* **29** (1988) 497–503.
2. Macaulay J. O., Krozowski Z. S. and Warne G. L.: Characterization and covalent labeling of human placental methyltrienolone binding protein. *J. Steroid Biochem.* **32** (1989) 493–498.
3. Macaulay J. O., Warne G. L., Smith A. I. and Krozowski Z. S.: The methyltrienolone binding protein of human placenta requires nicotinamide adenine dinucleotide cofactor(s) for steroid binding. *Endocrinology* **126** (1990) 2506–2513.
4. King M. J. and Greene G. L.: Monoclonal antibodies localize oestrogen receptors in the nucleus of target cells. *Nature* **307** (1984) 745–747.
5. Welshons W. V., Lieberman M. E. and Gorski J.: Nuclear localization of unoccupied oestrogen receptors. *Nature* **303** (1984) 747–749.
6. Brown T. R. and Migeon C. J.: Mechanism of action of androgens: androgen receptors. In *Androgens in Childhood* (Edited by M. G. Forest). *Pediat. Adolesc. Endocr.* Basel, Karger (1989) pp. 24–36.
7. Evans R. M.: The steroid and thyroid receptor superfamily. *Science* **240** (1988) 889–895.
8. Steinsapir J., Bryhan M. and Muldoon T. G.: Relative binding properties of microsomal and cytosolic androgen receptor species of the ventral prostate. *Endocrinology* **125** (1989) 2297–2311.
9. Steinsapir J., Evans A. C., McDonald T. and Muldoon T. G.: Association of androgen binding sites with the endoplasmic reticulum of rat ventral prostate. *Biol. Reprod.* **42** (1990) 337–349.

10. Perrot-Applanat M., Groyer-Picard M-T., Logeat F. and Milgrom E.: Ultrastructural localization of the progesterone receptor by an immunogold method: effect of hormone administration. *J. Cell Biol.* **102** (1986) 1191–1199.
11. Trueba M., Rodriguez P., Vallejo A. I., Marino A., Sancho M. J. and Macarulla J. M.: Binding of progesterone to specific sites in isolated hepatic cells and purified plasma membrane fraction. *Exp. Clin. Endocr.* **95** (1990) 169–180.
12. Yunghans W. N., Day R. N., Schiari S. C., Desmond T. J., Morre D. J. and Bruner M. W.: Steroid interactions with plasma membrane of decidual endometrium of the rat. *Biochem. Int.* **21** (1990) 387–395.
13. Dobashi K., Ajika K., Kambegawa A. and Arai K.: Localization and distribution of unconjugated steroid hormones in normal placenta at term. *Placenta* **6** (1985) 445–454.
14. Flint A. P. F. and Burton R. D.: Properties and ontogeny of the glucocorticoid receptor in the placenta and fetal lung of sheep. *J. Endocr.* **103** (1984) 31–42.
15. Spelsberg T. C., Knowler J. T. and Moses H. L.: Specific methods for the isolation of nuclei from chick oviduct. *Methods Enzym.* **31** (1974) 263–279.
16. Morrow A. F., Gyorki S., Warne G. L., Burger H. G., Banagh M. L., Outch K. H., Mirovics A. and Baker H. W. G.: Variable androgen receptor levels in infertile men. *J. Clin. Endocr. Metab.* **64** (1987) 1115–1121.
17. Bradford M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Analyt. Biochem.* **72** (1976) 248–254.
18. Cesarone C. F., Bolognesi C. and Santi L.: Improved microfluorometric DNA determination in biological material using 33258 Hoechst. *Analyt. Biochem.* **100** (1979) 188–197.
19. Aronson N. N. and Touster O.: Isolation of rat liver plasma membrane fragments in isotonic sucrose. *Methods Enzym.* **31** (1974) 90–102.
20. Nordlie R. C. and Arion W. J.: Glucose-6-phosphatase. *Methods Enzym.* **IX** (1966) 619–625.
21. Hers H. G.: Glycogen storage disease. In *Advances in Metabolic Disorders* (Edited by R. Levine and K. Luft). Academic Press, New York (1964) p. 36.
22. Davies P. and Thomas P.: Interaction of androgen receptors with chromatin and DNA. *J. Steroid Biochem.* **20** (1984) 57–65.
23. Foekens J. A., Rennie P. S., Cheng H. and Bruchovsky N.: *In situ* cross-linking of androgen receptors to nuclear acceptor sites of rat prostate with formaldehyde. *J. Biol. Chem.* **260** (1985) 10093–10098.
24. Xu Y. H., Luo R. S. and Wang T. Y.: Preferential binding of androgen to transcriptionally active chromatin in rat prostate. *J. Steroid Biochem.* **26** (1987) 647–652.
25. Spelsberg T. C., Ruh T., Ruh M., Goldberger A., Horton M., Hora J. and Singh R.: Nuclear acceptor sites for steroid receptors; comparisons of steroids and antisteroids. *J. Steroid Biochem.* **31** (1988) 579–592.
26. Hora J., Horton M. J., Toft D. O. and Spelsberg T. C.: Nuclease resistance and the enrichment of native nuclear acceptor sites for the avian progesterone receptor. *Proc. Natn. Acad. Sci. U.S.A.* **83** (1986) 8839–8843.
27. Spelsberg T. C., Goldberger A., Hortom M. and Hora J.: Nuclear acceptor sites for sex steroid hormone receptors in chromatin. *J. Steroid Biochem.* **27** (1987) 133–147.
28. Chang C., Chodak G., Sarac E., Takeda H. and Liao S.: Prostate androgen receptor: immunohistoligical localization and mRNA characterization. *J. Steroid Biochem.* **34** (1989) 311–313.
29. Husmann D. A., Wilson C. M., McPhaul M. J., Tilley W. D. and Wilson J. D.: Antipeptide antibodies to two distinct regions of the androgen receptor localize the receptor protein to the nuclei of target cells in the rat and human prostate. *Endocrinology* **126** (1990) 2359–2368.
30. McCormick P. D., Razel A. J., Spelsberg T. C. and Coulam C. B.: Evidence for an androgen receptor in the human placenta. *J. Obstet. Gynec.* **140** (1981) 8–13.
31. Rennie P. S., Bowden J., Bruchovsky N., Frenette P., Foekens J. A. and Cheng H.: DNA and protein components of nuclear acceptor sites for androgen receptors in rat prostate. *J. Steroid Biochem.* **27** (1987) 513–520.
32. Singh R. K., Ruh M. F., Butler W. B. and Ruh T. S.: Acceptor sites on chromatin for receptor bound estrogen versus antiestrogen in anti-estrogen sensitive and resistant MCF-7 cells. *Endocrinology* **118** (1986) 1087–1095.
33. Wrenn C. K. and Katzenellenbogen B. S.: Cross-linking of estrogen receptor to chromatin in intact MCF-7 human breast cancer cells; Optimization and effect of ligand. *Molec. Endocr.* **4** (1990) 1647–1654.
34. Hashizume K., Kobayashi M. and Miyamoto T.: Active and inactive forms of 3,5,3′-triiodo-L-thyronine (T3)-binding protein in rat kidney cytosol: possible role of nicotinamide adenine dinucleotide phosphate in activation of T3 binding. *Endocrinology* **119** (1986) 710–719.
35. Hashizume K., Miyamoto T., Ichikawa K., Yamauchi K., Kobayshi M., Sakurai A., Ohtsuka H., Nishii Y. and Yamada T.: Purification and characterization of NADPH-dependent cytosolic 3,5,3′-triiodo-L-thyronine binding protein in rat kidney. *J. Biol. Chem.* **264** (1989) 4857–4863.
36. Hashizume K., Miyamoto T., Ichikawa K., Yamauchi K., Sakurai A., Ohtsuka H., Kobayshi M., Nishii Y. and Yamada T.: Evidence for the presence of two active forms of cytosolic 3,5,3′-triiodo-L-thyronine (T_3) binding protein (CTBP) in rat kidney. *J. Biol. Chem.* **264** (1989) 4864–4871.
37. Murphy B. E. P. and Hyde M. J.: The influence of various steroids on the binding of methyltrienolone (R1881) to human placenta: evidence for a second binding site which stabilizes the R1881 binding site. *J. Steroid Biochem.* **33** (1989) 923–926