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# Endocytosis of Androgen-binding Protein (ABP) by Spermatogenic Cells

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**To test whether Sertoli cell-secreted ABP could serve as steroid carrier to the germ cell (GC) lineage, radiolabeled ABP and SHBG and gold SHBG were used for binding studies and for internalization studies based on transmission electron microscope analyses and autoradiography of the radiolabeled samples. The data clearly showed that: (1) rat and human germ cells possess a single class of binding sites for rat ABP and human SHBG respectively ($K_d$ of 0.78 and 0.56 nM); (2) $1.7 \times 10^{10}$ and $2.7 \times 10^{10}$ sites/mg protein was found in the corresponding plasma membrane preparations; (3) the receptor peak was eluted in the same position as dextran blue: 2000 kDa ($M_r = 2 \times 106$) for labeled rat ABP; (4) in the whole GC lineage, the labeled ligand was internalized through an endocytic pathway involving clathrin coated structures and the distribution was similar throughout the maturation step, however striking differences in the internalization rate were revealed with regard to the maturation step; and (5) this internalization occurred even in ligated seminiferous tubules, via the Sertoli cells cytoplasm. When isolated rat GC were incubated in the presence of ABP, a dose dependent increase in labeled secreted protein was observed for spermatocytes (50–250%) whereas ABP had no effect on spermatids. Addition of steroids and ABP caused a 200 and 50% increase in labeled secreted proteins for spermatocytes and spermatids respectively. 2-D SDS–PAGE analysis revealed that ABP alone increased the secretion of specific spermatocyte proteins whereas steroids in the presence of ABP resulted in the synthesis of new spermatocyte secreted proteins. Taken together these results strongly suggest that ABP may be required for spermatogenesis either as a steroid transmembrane carrier or on its own.**

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## INTRODUCTION

In many mammalian species, for example, in the rat and in man, androgen-binding protein (ABP) is secreted by Sertoli cells [1, 2]. Its synthesis is positively regulated by FSH and testosterone [3–5] and its secretion is considered as a good marker for the Sertoli cell function [6]. The presence of germ cells also regulates the secretion of Sertoli ABP [7].

Human sex hormone-binding globulin (SHBG) or sex steroid-binding protein (SBP) is secreted by the liver and released into the blood. ABP and SHBG are coded for by the same gene [8, 9] and several testicular messengers for SHBG/ABP have been identified in the rat and human [10]. There is roughly 70% homology between the amino acid sequences of the rat ABP and the human SHBG. Immunoreactive SHBG has been found in monkey and human testis [11, 12] and cannot be distinguished from ABP produced by the Sertoli cells.

In the body, ABP is mainly present in the initial part of the male genital tract, namely in the testicular fluids that bathe the spermatogenic cells and in the luminal fluid of the epididymis head.

Some years ago, we demonstrated in the epididymis head that ABP interacts with the principal cells through membrane receptors leading to its internalization [13, 14]. [$^3$H]testosterone uptake by epididymal cells is facilitated by testicular ABP [15]. In the testis, the pioneer work of Steinberger in 1984 [6] suggested the presence of receptors for ABP on spermatocytes. It was therefore relevant to test if ABP could act on germ cell lineage as in the epididymal model, i.e. (1) internalization following membrane-binding; and (2) facilitation of androgen uptake.

It is recognized that spermatogenesis is completely dependent upon testosterone, namely at stages VII and VIII of the spermatogenic cycle which contain specific

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steps of developing germ cells [17]. Testosterone regulates both the overall level of protein secretion as well as the secretion of several specific protein from seminiferous tubules in the rat during the stages VI to VIII of the spermatogenic cycle [18]. However, germ cells are known to be devoid of androgen receptors [19, 20] and the direct action of androgens on these cells has never been observed. Thus, how testosterone acts on germ cells is yet a matter of debate and the demonstration of such binding and internalization should help us understand this question.

Uptake and binding studies using labeled ABP/SHBG and/or steroids have been performed in adult rat, monkey and human germ cells, and the effect of ABP on germ cell protein synthesis was investigated *in vitro* using the rat.

## DEMONSTRATION OF ABP AND SHBG INTERNALIZATION WITHIN MALE GERM CELLS

To study internalization, we have developed uptake protocols using pure rat testicular ABP prepared as described by Gueant [21] or human SHBG prepared by Egloff [22] from late pregnancy serum. After labeling with a tracer molecule we have exposed different species of germ cells to the exogenous labeled complex and examined the intracellular fate at the ultrastructural level.

ABP or SHBG were labeled with tritiated 6-ene-testosterone by a photoaffinity covalent coupling as described by Fremont [23] and Gerard [24]. Radioactive compounds in tissue sections can be detected after radioautographic treatment leading to dense silver grains overlying the radioactive sources.

The use of colloidal gold as a label, in providing unequivoque ultrastructural detection, allows a more accurate localization within small organelles but requires higher magnification [24].

Homo- and heterospecific uptake studies have been performed by incubating isolated germ cells from different species in culture medium containing either radiolabeled rat ABP or human SHBG or gold labeled SHBG for different time periods.

Following incubation in the presence of radiolabeled SHBG or ABP, autoradiography revealed intracellular labeling in all germ cell types from the difference species tested. This labeling is significantly inhibited by preincubation with an excess of unlabeled ABP/SHBG prior to incubation in the presence of radiolabeled SHBG or ABP.

Silver grains or gold particles were detected in the peripheral cytoplasm within early endosomes after short incubation periods (Fig. 1) and in more deep cytoplasm, in multivesicular bodies (Fig. 2) and Golgi vesicles after longer periods of incubation in the presence of the tritiated testosterone ABP complex. Finally, radioactive sources were found over the nucleus and nuclear envelope.

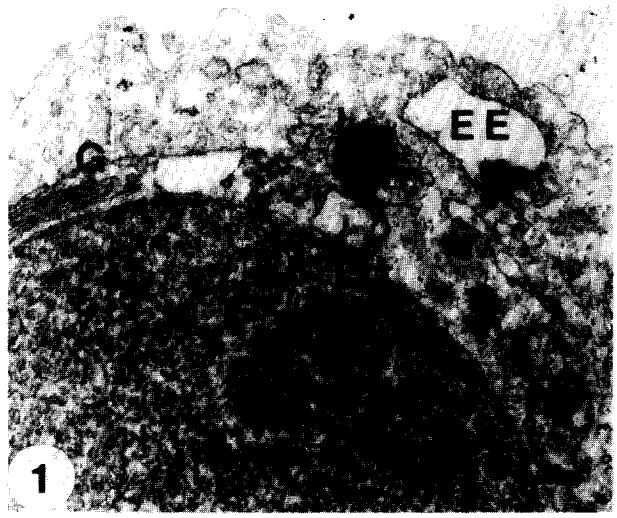


**Fig. 1. Radioactive labeling of a step 2 human round spermatid after incubation in the presence of [$^3$H]6-ene-testosterone-hSHBG for 10 min. Sections were coated with the L4 nuclear emulsion and developed after 3 months exposure. Silver grains appear to be associated with the peripheral membrane of an early endosome (EE) showing that the radiolabeled hSHBG has been internalized within this organelle. N, nucleus; G, Golgi apparatus; L, lipid; bar = 0.2 μm.**

By scoring the grains overlying the cellular compartments, the relative distribution at 15 min clearly showed a significant labeling of the plasma membrane, the cytoplasm, the nucleus and the nuclear envelope (Table 1). Most of the grains were found at the intracellular level indicating a rapid entry of the labeled complexes; in the cytoplasm, taking into account that the endocytic apparatus corresponds to a very small compartment, the large number of grains (% of the

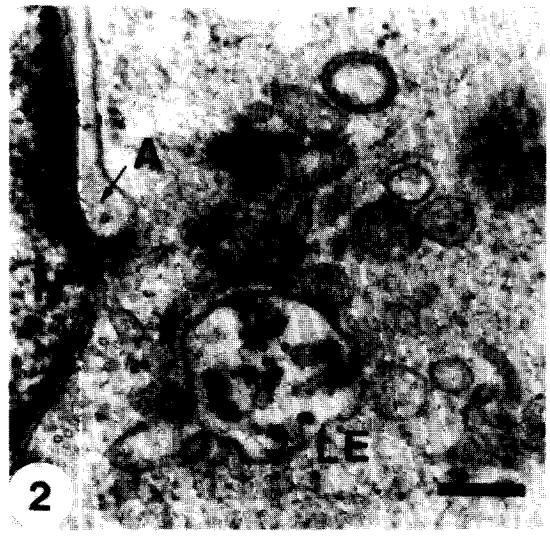


**Fig. 2. Gold labeled step 7 monkey spermatid after the incubation in the presence of gold hSHBG for 30 min. Gold particles are sequestered in a late endosome (LE) which corresponds to a perinuclear multivesicular body. N, nucleus; A, acrosomic vesicle; bar = 0.2 μm.**

*Table 1. Internalization of [$^3$H]6-ene testosterone rat ABP complex within isolated rat germ cells expressed as the silver grain density (grain number per 1000 $\mu m^2$ of cell section)*

| | Cytoplasmic compartment | | | | Nuclear compartment | | |
|---|---|---|---|---|---|---|---|
| | | Grain distribution (%) | | | | Grain distribution (%) | |
| | Grain density/1000 $\mu m^2$ | MB | EndApp | Hyal | Grain density/1000 $\mu m^2$ | NE | N |
| Spermatocytes | 32 | 16 | 50 | 34 | 43 | 26 | 74 |
| Round spermatids | 82 | 13 | 89 | 08 | 25 | 24 | 76 |
| Elongated spermatids | 103 | 08 | 76 | 19 | 1 | | |

The density was calculated using grain counts on autoradiograms where around 500 cells of each type were counted. Moreover the grain distribution is expressed as a percentage of the total grains scored in each cell category. MB, plasma membrane; End App, endocytic apparatus; Hyal, Hyaloplasm; NE, nuclear envelope; N, nucleus. It should be noticed that the grains are mainly located within the cellular compartments rather than the plasma membrane. In addition, the highest level in the nuclear compartment is observed in the early maturation step.

cytoplasmic grains observed) found over it showed that it is highly involved in this process. The density per 1000 $\mu m^2$ calculated in each cell type shows an overall higher density in spermatids than spermatocytes.

To summarize: *in vitro*, isolated germ cells rapidly internalized exogenous labeled rat ABP and human SHBG and the labeled complexes were found exclusively associated with intracellular membranes such as endocytic vesicles and Golgi vesicles. The internalization was inhibited by preincubation with unlabeled protein, this indicates the specificity of the internalization mechanism. Moreover, when co-incubating germ cells with horse radish peroxidase (HRP), a fluid phase endocytosis marker, and ABP we exceptionally observed a co-localization of ABP/SHBG and HRP in endocytic vesicles, suggesting a probable receptor mediated internalization mechanism for ABP/SHBG endocytosis. So we then investigated the presence of membrane receptors for ABP/SHBG in germ cells.

## INTERNALIZATION OF ABP/SHBG WITHIN GERM CELLS IS RECEPTOR-MEDIATED

Only a few examples of receptor-mediated endocytosis within germ cells has been reported [25, 26], though, we have previously explored the presence of clathrin, which is well known to be associated with membrane receptor activity, at the cell surface of germ cells.

We have searched for the presence of clathrin on rat germ cell cytopspin preparations using immunocytochemistry. A positive reaction was present for all germ cell types [27]. Moreover, at the ultrastructural level, typical clathrin-like coated pits and vesicles were found in association with the plasma membrane of monkey, rat and human germ cells (Fig. 3). These specialized membrane structures were frequently labeled within the initial steps of uptake protocols, whichever labeled protein was used (Fig. 3). In addition, lowering the incubation temperature to 4°C inhibited the internalization rate and enhanced the membrane binding localization. Gold labeling provided a more accurate localization within the very small organelles (< 100 nm in diameter) than developed silver grains which often mask the underlying structures (Fig. 4).

These results obviously showed that SHBG and ABP enter the germ cell cytoplasm through the clathrin coated pit pathway and taken together with the intracellular strictly membranous location of the internalized protein, it was relevant to look for membrane receptors.

Some years ago, we had already demonstrated that rat ABP was endocytosed in epididymis principal cells [13] and that rat epididymal cells have a specific receptor which binds both rat ABP and human SHBG [14, 27, 28]. Thus, we used photoaffinity-labeled rat ABP as a model with which to investigate the mechanism by which rat ABP interacts with spermatogenic cells. Binding studies were performed in intact rat and

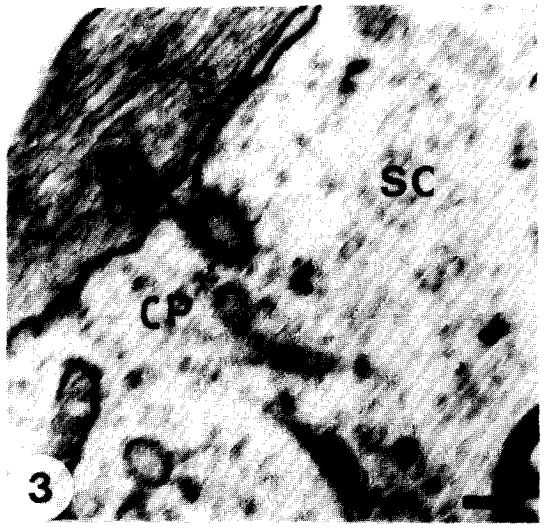


**Fig. 3. Gold labeling of the cell surface of a monkey spermatocyte after incubation in the presence of gold hSHBG for 3 min. Some gold particles (arrows) are seen within a typical coated pit (CP) of 100 nm in diameter open at the spermatocyte (SC) cell surface and facing a Sertoli cell process (S); bar = 0.1 $\mu$m.**

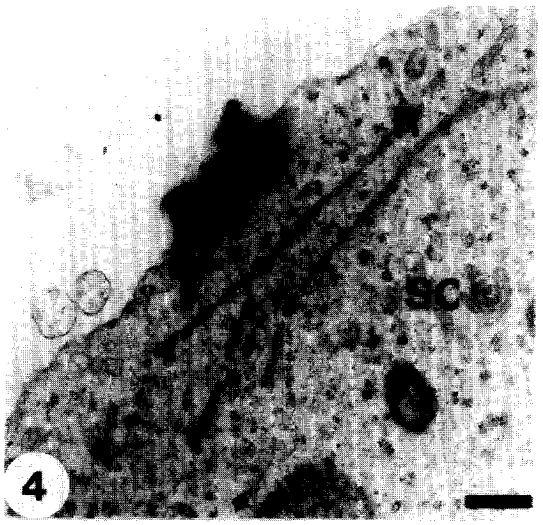


Fig. 4. Radioactive labeling of the plasma membrane of a rat spermatocyte after incubation in the presence of [$^3$H]6-ene-testosterone rat ABP for 2 min. The section was coated with the L4 nuclear emulsion and developed after 3 months exposure. Silver grains which appear demonstrated strongly radioactive sources associated with the plasma membrane but the cluster of filamentous silver grains masks the underlying structures that could be involved in this binding. R, reticulum; bar = 0.5 $\mu$m.

human isolated germ cells or in membrane-enriched preparations [29].

The binding of photoaffinity-labeled ABP to plasma membrane-enriched preparations of germ cells was studied as a function of time. Specific binding reached a maximum after 40 min at 4°C. The displacement of bound labeled ABP by a 100-fold excess of unlabeled ABP demonstrated that the binding was a reversible process (Fig. 5). The binding labeled ABP was pH dependent, it was maximal at pH 6–8.

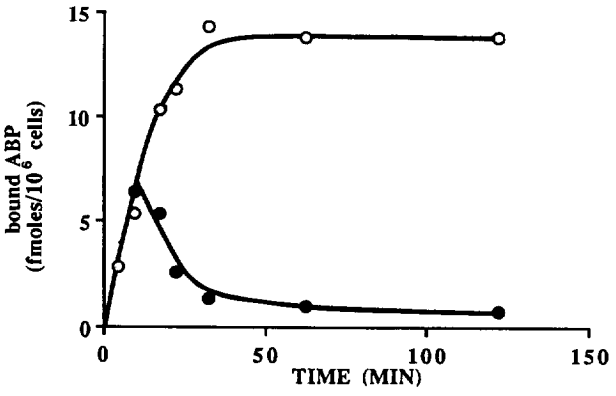


Fig. 5. Time-course of photoaffinity-labeled rat ABP binding to plasma membrane-enriched preparations (0.87 mg/ml). Association (○) was performed at 4°C; 1 pmol labeled ABP was incubated with aliquots of plasma membranes in a final volume of 10 ml. Aliquots (1 ml) of the mixture were collected at incubation times ranging from 2 to 120 min. A second experiment (●) was performed in the same way, except that a 100-fold excess of unlabeled rat ABP was added 15 min after incubating the plasma membranes with the tracer at 4°C. Data are the means of triplicate experiments. Reproduced by permission of the *Journal of Endocrinology*.

Specific binding of ABP with intact germ cells and the corresponding membrane-enriched preparations was analysed by Scatchard plots (Fig. 6). We found a single class of binding sites with apparent dissociation constant $K_d$ values of 0.78 and 0.97 nM in intact germ cells and the corresponding membrane-enriched preparations respectively. The number of binding sites per cell was around 13,000 and $1.7 \times 19^{10}$ sites/mg protein in membrane preparations.

Human SHBG incubated with rat germ cell plasma membranes also showed a single class of binding site with a lower affinity corresponding to a $K_d$ of 1.72 nM. Human SHBG also showed a single class of binding site on human germ cells with a higher affinity corresponding to a $K_d$ of 0.56 nM and $2.7 \times 10^{10}$ sites/mg protein in membrane preparations.

The ABP receptor was eluted from a Superose 6 gel as an aggregate with a molecular weight of 2,000 kDa. It was abolished in the presence of 20 mM EDTA or at pH <4. The receptor complex was also abolished in the presence of a 100-fold excess of either unlabeled rat ABP or unlabeled human SHBG.

In parallel, from quantitative radioautographic analyses, the preincubation of germ cells with either an excess of unlabeled protein or EDTA significantly decreased the labeling density of germ cells indicating an inhibition of the internalization within these cells. Specificity of ABP binding to germ cells was studied by preincubating membrane-enriched preparations with an excess of different unlabeled proteins (lactotransferrin, sertotransferrin, asialofetuin, fetuin, and bovine serum albumin). No inhibition was observed except with unlabeled SHBG which appeared as efficient as unlabeled ABP.

All these results clearly demonstrate that ABP and SHBG are able to bind to membrane receptors present in germ cell plasma membrane and can subsequently be internalized through a receptor-mediated endocytic pathway. The data can be compared with those observed with epididymal epithelial cells [14, 28]. The affinity of rat ABP binding sites of germ cells was lower than that of epididymal epithelial cells ($K_d$ values were 0.97 and 0.27 nM, respectively), but the number of binding sites per cell was similar (13,000 and 12,000 sites/cell) [29].

[$^3$H]6-ene-testosterone-human SHBG was bound almost as efficiently as [$^3$H]6-ene-testosterone-rat ABP ($K_d$ = 1.72 nM). A similar result has been also obtained with epididymal cells and can be explained by the structural homology of the two proteins [8]. However, the $K_d$ values appeared to be somewhat different from the reported values from testis. The reason for the large variation in $K_d$ for the soluble ABP/SHBG receptors noted above could be related to tissue differences in factors that stabilize receptors or technical differences between laboratories. Nevertheless, the SHBG

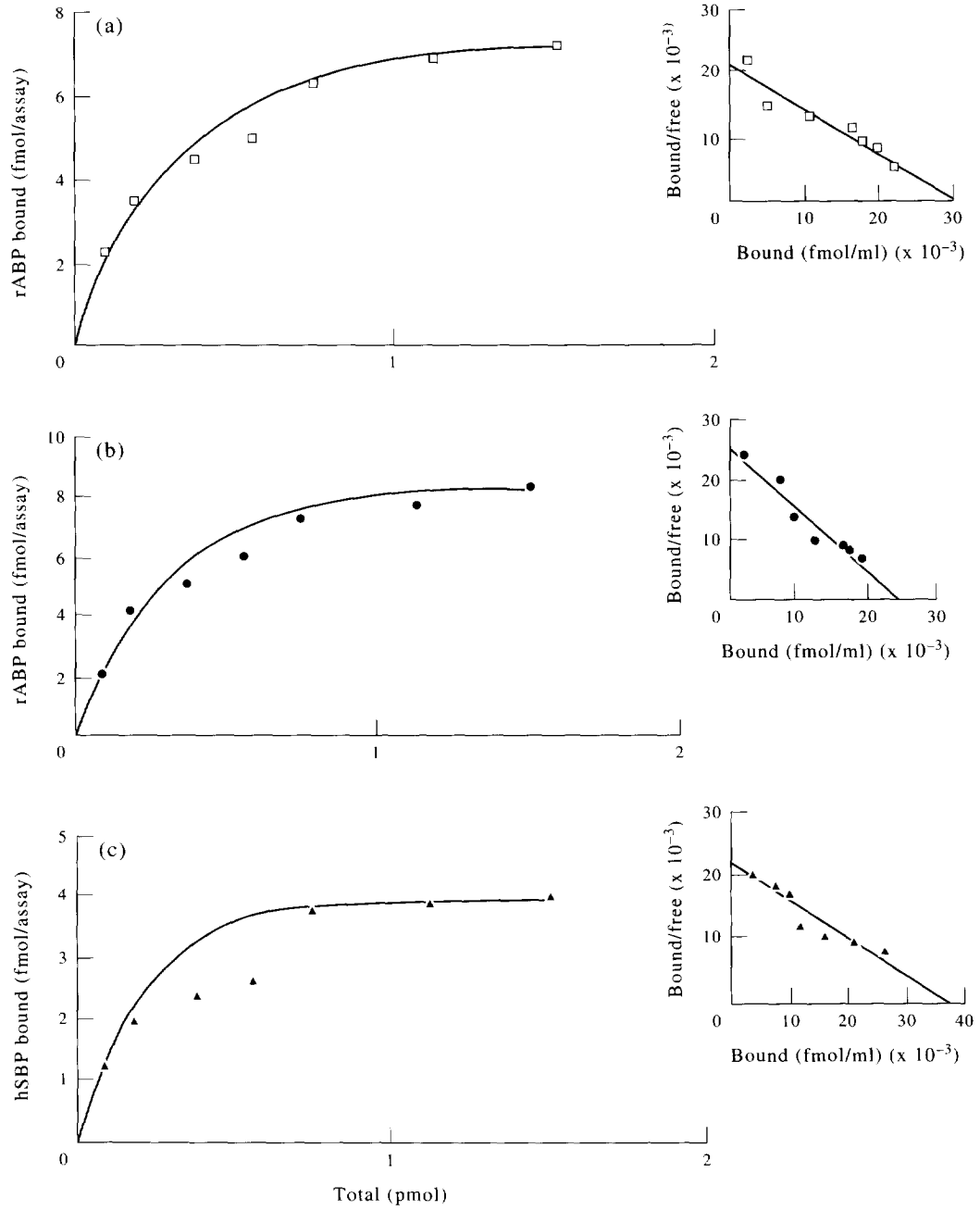


**Fig. 6. Saturation curves and Scatchard plots (inset) of the interactions of intact germ cells with photoaffinity-labeled rat ABP (a) and of plasma membranes of germ cells with photoaffinity-labeled rat ABP (b) and (c) photoaffinity-labeled human SHBG (SBP). Binding assays were performed at 4°C. Reproduced by permission of the *Journal of Endocrinology*.**

receptor in testes had an affinity 10 times larger than that of the receptor from MCF-7 cells using the same methods [30].

It has been reported that only tissues recognized as being strictly dependent on androgens or estrogens possess the membrane receptor for SHBG (human tissues like lymphocytes and colon mucosae do not bind SHBG) [31]. Since germ cells are also dependent on androgens, it would seem likely that they too have a receptor for ABP and SHBG. It remains to be established whether the internalization within germ cells controls a down regulation of receptor activity or plays a role at the intracellular level related to a specific maturation step.

## INTERNALIZATION OF ABP BY *IN SITU* RAT GERM CELLS

In order to verify if germ cells within seminiferous tubules (*in vivo* situation), were capable of internalizing ABP from the Sertoli cells cytoplasm instead of directly

from the culture medium, two other uptake protocols were designed:

—The first protocol: small fragments of isolated seminiferous tubules were ligated at both ends in order to not expose luminal germ cells to the exogenous labeled ABP. In fact, the labeled protein was delivered outside of the blood–testis barrier and must cross this barrier to reach the luminal germ cells via the Sertoli cell cytoplasm.

—The second protocol: small fragments of isolated seminiferous tubules were opened in order to expose luminal germ cell to the exogenous labeled ABP. In this case, as in isolated cell protocols, all luminal germ cells were exposed to the labeled protein however in the presence of Sertoli cells. In the ligated tubule experiment, after short times of incubation, radioactive sources were found outside of the seminiferous tubules and over the basal plasma membrane of Sertoli cells and also over basal germ cells. After longer periods of incubation, the labeled protein was found inside the seminiferous wall, over the finger-like processes of Sertoli cells which are in close contact with germ cells. After prolonged incubation, silver grains were observed within luminal germ cell and Sertoli cell cytoplasms. Quantitative analysis revealed a positive correlation between the labeling density and the time of incubation, however, a clear delay was seen, with no detectable labeling being observed up to 20 min, when isolated cells were heavily labeled. In opened tubule experiments, the labeling density of germ cells followed a similar time-dependent pattern observed in ligated tubule experiments, however, the labeling started earlier and reached a higher level than in ligated tubules. After 45 and 60 min, the total labeling of Sertoli cells did not seem to be different between ligated and opened tubule experiments.

To summarized: (1) spermatids exhibited a higher density than spermatocytes whatever protocol is used. (2) In opened tubules after 15 min, the density in each cell type is 50% lower than isolated cells. In addition, 45–60 min were necessary to obtain a similar level of density as in isolated cells at 15 min. This indicates that the presence of Sertoli cells interferes with the internalization rate. This most likely suggests that the endogenous secreted ABP occupies the germ cell receptors and competes with the labeled exogenous ABP. (3) When the tubules were ligated, the exogenous ABP crosses the blood–testis barrier, through the Sertoli cell cytoplasm and later reaches luminal germ cells indicating that ABP present in the Sertoli cell cytoplasm has been translocated to the adjacent germ cells through a receptor-mediated endocytosis mechanism.

Physiologically, Sertoli cells are responsible for ABP secretion [12]. The above experiments using ligated seminiferous tubules clearly showed that germ cells were capable of internalizing ABP which came from Sertoli cell cytoplasm as must be the case *in vivo*. At the same time our results demonstrated for the first time that ABP, in addition to being synthesized by Sertoli cells, can be captured at the basal pole of the Sertoli cells, transported up to the adluminal part and transferred to the germ cells. Such a process has recently been described for transferrin both synthesized by Sertoli cells and transported across the blood–testis barrier, up to and within the germ cells [26]. These findings are also consistent with the results of Sakiyama [32], who showed that the testosterone-binding globulin–sex steroid complex could cross the blood–testis barrier. The intimate mechanism of this transport is at the moment under study in our laboratory. It is clear, however, that such a steroid-binding protein traffic should be held responsible for the known transport of the sex steroid hormones across the blood–testis barrier, as has been suggested for the functions of the intracytoplasmic Sertoli-ABP [33].

## ENDOCYTOSIS OF RAT ABP AND HUMAN SHBG IS RELATED TO THE GERM CELL MATURATION STEP

Qualitative observations did not reveal any differences in the cellular localization of silver grains within spermatocytes and, round and elongated spermatids. Steroid-binding proteins enter the germ cells through the endocytic pathway from the clathrin coated structures of the plasma membrane up to the nucleus [27]. However, quantitative analysis of the autoradiograms in monkey and rat isolated germ cells following incubation in the presence of [$^3$H]6-ene-testosterone-human SHBG or rat ABP complexes pointed out striking differences in the label density of the intracellular labeled compartments as shown in Table 1. A positive correlation ($r = 0.92$, $P < 0.05$) has been found between the labeling density of the cytoplasm and the maturation step. The labeling was at its peak at the late spermatid step which revealed silver grains sequestered within late endosomes and multivesicular bodies located in the perinuclear cytoplasm. On the contrary, the nuclear compartment labeling was negatively correlated ($r = 0.95$, $P < 0.05$) to the maturation process and was highest in early germ cells. In all testicular fragments incubated in the presence of radiolabeled proteins numerous radioactive sources have been found on spermatogonia nuclei (Fig. 7). In addition, one third of the radioactive sources was related to the nuclear envelope. Furthermore, within the labeled nuclei, the labeling is detected in the less condensed form of chromatin. Further examination of these proteins interacting with the nuclear components is warranted.

The endocytosis pattern appears to be related to the germ cell maturation step by way of a different intracellular traffic of the protein and evidence is beginning to emerge that immunolocalization of ABP within

testicular cells demands more serious consideration taking into account the germ cell maturation steps and the stage of the spermatogenic cycle.

We used rat ABP antibodies prepared with a highly purified protein extracted from rat testes (kindly provided by J. Closset) to localize the protein within rat Sertoli and germ cells. A nuclear location was detected in basal cells spermatogonia and preleptotene spermatocytes (Fig. 8), while only cytoplasmic immunopositive ABP was detectable in spermatids (in press).

Previous immunocytochemical data demonstrated a positive reaction, using rat epididymal ABP antibodies, in the basal part of the adult rat seminiferous tubules most likely within Sertoli cell cytoplasm [34], and faintly in the surrounding germ cells [35]. More recently, in the adult mouse testis, Wang [36] reported a strong positive immunostaining in the luminal part of the seminiferous tubules using a rat ABP antiserum without any information regarding to the spermatogenic cycle and the maturation step of the germ cells. Our immunolocalization data are highly consistent with autoradiographic results and support the notion that the secretion of ABP and the endocytosis in the surrounding germ cells occurs specifically at some stages of the spermatogenic cycle. Whether these differences are related to a specific need of testosterone carried by ABP or to a specific effect of ABP on its own remains to be examined.

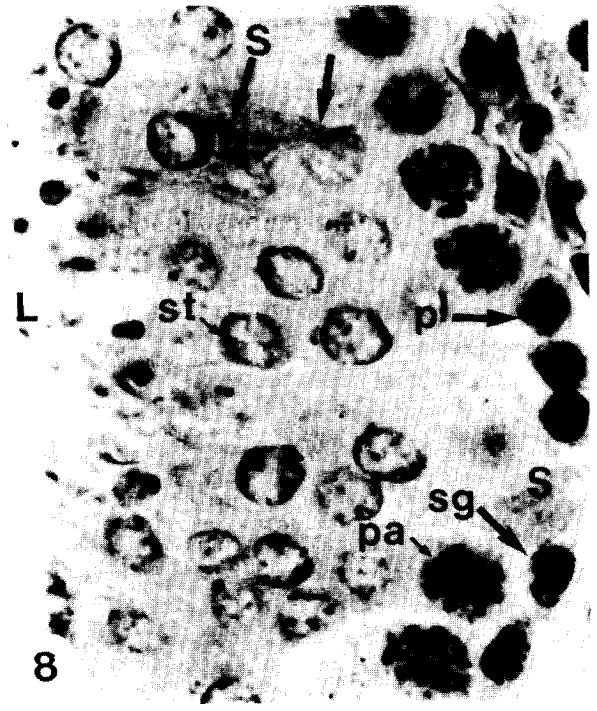


Fig. 8. Immunostaining of a paraffin testis rat section treated with ABP antibodies using the LSAB revelation system (Dako). A peroxidase reaction product is clearly apparent in the nuclei of basal germ cells corresponding to spermatogonia (SG) and preleptotene spermatocytes (Pl). Sertoli cell cytoplasmic processes (S), surrounding luminal round spermatids (ST), also exhibited a positive reaction (arrows). PA, pachytene spermatocytes; L, lumen.


Fig. 7. Radioactive labeling of a monkey spermatogonia following the incubation of a seminiferous tubule fragment in the presence of [$^3$H]6-ene-testosterone hSHBG for 30 min. The section was coated with the L4 nuclear emulsion and developed after 3 months exposure. Silver grains are seen on the nuclear compartment (N) including the nuclear envelope (NE) of the spermatogonia SG. A Golgi apparatus (G) and mitochondria (m) are seen in the cytoplasm. Se, Sertoli cell. bar = 0.5 $\mu$m.

To investigate whether the observed binding of ABP and SHBG to germ cells has any functional significance we set up studies to assess the regulation of germ cell protein synthesis, using an enriched preparation of rat testicular ABP.

## EFFECTS OF RAT ABP ON GERM CELLS PROTEIN SYNTHESIS

Enriched populations of spermatocytes and spermatids were isolated [37] and cultured for 24 h with 60 $\mu$Ci $^{35}$S-labeled methionine, ABP and/or steroids. Initial studies involved culturing spermatocytes and spermatids with increasing concentrations of the enriched ABP extract (equivalent to 30–200 ng/ml ABP as determined by DHT binding) for 24 h. A dose-dependent increase in labeled secreted proteins was observed for spermatocytes (50–250%) (Fig. 9) but there was no significant modification for spermatids with ABP alone.

Addition of either testosterone or 17$\beta$-oestradiol (50 ng/ml) to germ cell cultures containing ABP caused a 200–600% and 50–150% increase in labeled secreted proteins for spermatocytes and spermatids, respectively (Fig. 9). Addition of BSA and/or steroids produced

no effect on the overall germ cell protein secretion, indicating a specific function for the ABP/steroid complex. Analysis of the labeled secreted proteins after 2-D SDS–PAGE performed according to Sharpe [18] using the Phoretix 2-D analysis software (Biometra Ltd) revealed that ABP in the absence of steroid increased the synthesis of specific proteins already produced by spermatocytes. Whereas steroid in the presence of ABP resulted in the synthesis of new spermatocyte secreted proteins (data not shown). The above findings show that ABP/±steroid stimulates the synthesis of spermatocytes and spermatid secreted proteins. Further detailed 2-D SDS–PAGE analyses are required to identify the proteins and to determine their role in the maturation processes.

In conclusion the interactions of ABP and/or the ABP–steroid complex, including binding and internalization, could be related to the functional control of spermatid and spermatocyte protein synthesis. It is important to note that ABP alone appeared to regulate some proteins produced by spermatocytes. These proteins can be further isolated and characterized for the use as endpoints for ABP effects *in vivo*. It also remains to determine how ABP can drive the synthesis machinery.

## GENERAL COMMENTS

Although considerable attention has been focused on steroid-binding proteins, very limited information has been published concerning the physiological role of ABP in male reproductive processes. Among its potential functions is to provide a suitable androgen concentration for the maintenance of spermatogenesis and epididymal function.

We demonstrated here that spermatogenic cells are able to take up ABP produced by Sertoli cells by a very specific mechanism and respond by modifying protein synthesis. In turn, the presence of spermatogenic cells and residual bodies has been demonstrated to control ABP secretion levels by Sertoli cells [7]. Since, in addition, ABP synthesis is stimulated by FSH it ensures that this binding-protein must play a local integrative role and is involved in the "cross-talk" between Sertoli cells and germ cells. Thus, ABP should definitively be considered as a paracrine factor within seminiferous tubules, even if the precise intracellular targets and the intimate mechanisms remain to be

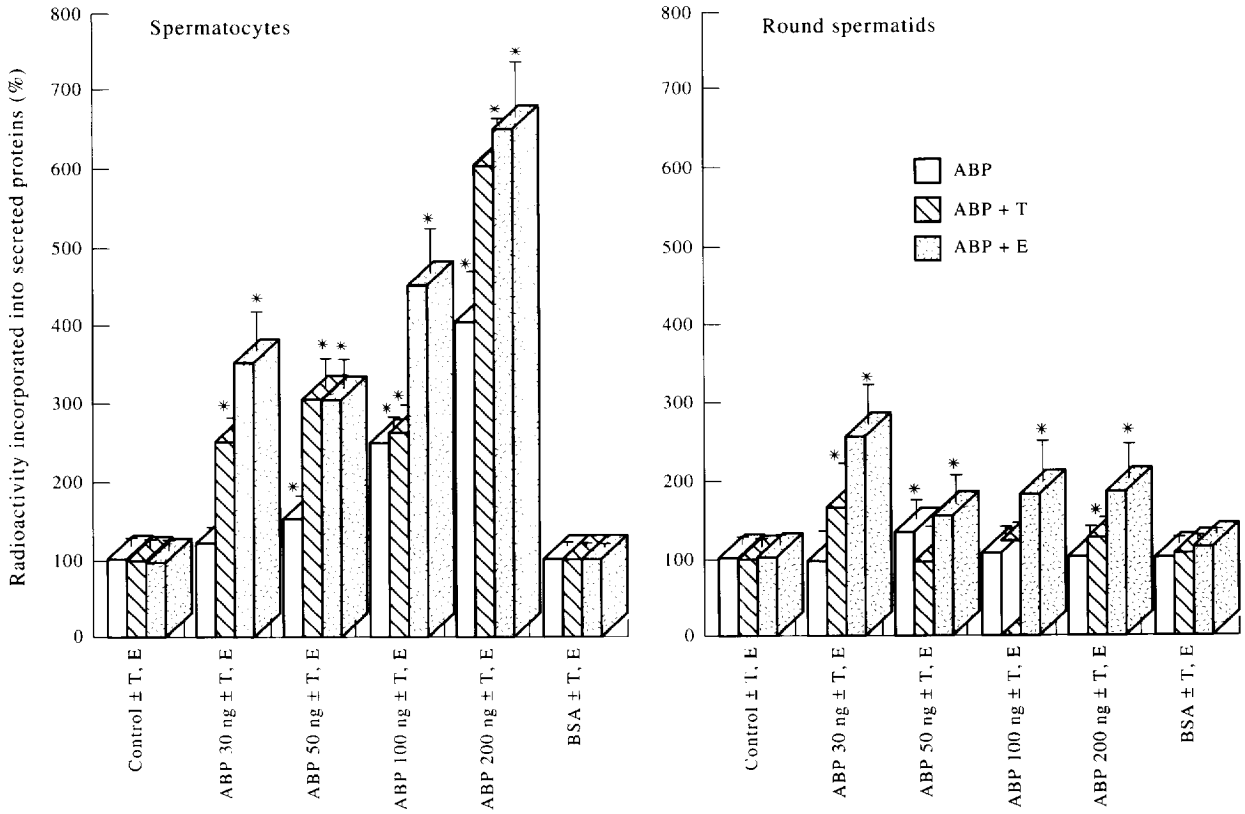


**Fig. 9. Effect of the enriched ABP fraction and testosterone (T) or 17β oestradiol (E) on the secretion of radiolabeled proteins by isolated spermatocytes or round spermatids. Cells were plated at $1.5 \times 10^6$/500 μl/well incubated for 24 h with 60 μCi $^{35}$S-labeled methionine. BSA (50 ng/ml) was used as control. Testosterone or oestradiol were added at a concentration of 50 ng/ml. The amount of radioactivity incorporated into newly synthesized proteins was expressed as a percentage of the radioactivity incorporated in controls. Values are mean ± SEM ($n = 4$). The values were compared using Student's $t$-test. *Significant increase compared to the control, $P < 0.01$.**

determined, especially since there is a possibility that it plays a role by itself independent of the steroid transport function.

Concerning its intratesticular function, a noteworthy point is that germ cell protein synthesis and secretion are modulated by ABP. In the same way, it is of great relevance that transgenic mice expressing highly abnormal levels of rat ABP mRNA [40] exhibited striking spermatogenesis defects associated with reduced fertility (manuscript in preparation).

Until now, however, we have not been able to get the firm proof that internalization was an obligatory step in the functional effect of ABP on germ cells and we cannot exclude that binding to membrane receptors could initiate the stimulation of secondary messengers. If such a mechanism exists, it should be consistent with the functional model proposed by Rosner [41]. However, it must be pointed out that germ cell maturation steps involved in the stimulation of secreted proteins under the control of ABP are the same as those which exhibited a large nuclear labeling after incubation with the labeled ABP. Since the interactions of steroid-binding proteins with nuclear components have already been observed by others, we can speculate on the necessity of an intranuclear step in ABP action: one hypothesis is that some of the known effects of androgen on germ cell maturation could be mediated by the translocation of the ABP–steroid complex to the nuclear compartment and by the following activation of target genes, thus explaining the fact that testosterone has an effect on germ cell maturation, although these cells are not known to have testosterone receptors [19, 20]. This exciting question is, at present, under scrutiny in our laboratory.

Consequently, if such a nuclear step does exist, the biological effect of ABP on germ cell metabolism depends more on the mechanisms for controlling its intracellular traffic than on the number and activity of binding sites present on the plasma membrane. All in all, ABP should no longer be considered as only a regulator of free testosterone concentration within the testis as originally proposed.

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