Communication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 255, No. 20, Issue of October 25, pp. 9523-9525, 1980
Printed in U.S.A.

Sertoli Cells Synthesize and Secrete Transferrin-like Protein*

(Received for publication, July 8, 1980)

Michael K. Skinner and Michael D. Griswold‡

From the Biochemistry/Biophysics Program, Washington State University, Pullman, Washington 99164

One of the major proteins secreted by Sertoli cells (Band 3) has a molecular weight on sodium dodecyl sulfate-polyacrylamide gels which corresponds to rat serum transferrin. The preparation of Sertoli cell-secreted proteins contains an iron-binding protein with electrophoretic properties on native polyacrylamide gels similar to serum transferrin. Antibodies against Sertoli cell-secreted proteins contain a component which will immunoprecipitate serum transferrin. Antibodies to serum transferrin cross-react with and immunoprecipitate a major component (Band 3) of [^{35}S]-methionine-labeled Sertoli cell-secreted proteins. We propose that testicular transferrin is a major secretory product of Sertoli cells in culture. In addition to transferrin, antiserum to rat serum proteins will immunoprecipitate one other polypeptide from preparations of Sertoli cell-secreted proteins.

Sertoli cells have been described as the "nurse cells" of the testis whose primary function is to provide essential growth factors and to create the proper environment for the development of germinal cells (1-3). Part, if not all, of the control of spermatogenesis by hormones (follicle-stimulating hormone and testosterone) seems to be exerted as a result of the action of these hormones on Sertoli cells (2). The response of the Sertoli cells to hormone stimulation could be to interact directly with germinal cells, to communicate with germinal cells by secreted products, or to alter the environment of the lumen of the seminiferous tubule. The unique environment of the tubule lumen is possible because of junctional complexes which form at the basal part of adjacent Sertoli cells and form a functional "blood-testis barrier" (3).

Most of the components of the fluid of the seminiferous tubules are apparently secreted by the Sertoli cells (4). The major polypeptides which are synthesized and secreted by the Sertoli cells in culture have been described by this laboratory (5). Only seven major glycoproteins are secreted and these proteins appear to be uniquely synthesized by the Sertoli cells. The secreted glycoproteins were designated as Bands 1 to 7 based on their mobility in SDS[1]-polyacrylamide gels (5). The functional significance of one protein, androgen binding protein, has been previously investigated (6-8). Androgen binding protein represents only a minor amount (approximately 1%) of the total amount of secreted glycoprotein (5).[2]

* This work was supported by Grants HD 10808 and HD 00253 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom requests for reprints should be sent.

[1] The abbreviations used are: SDS, sodium dodecyl sulfate; SC, Sertoli cell-secreted.

[2] M. K. Skinner and M. D. Griswold, unpublished observations.

We have obtained evidence concerning the structure and function of one of the major secreted glycoproteins which corresponds to Band 3 protein in our previous report (5). The identification of this protein as an iron transport protein (transferrin) has important implications in the study of germinal cell development and Sertoli cell functions. A large percentage of the protein secreted by Sertoli cells is transferrin and this suggests that iron transport is a major function of these cells. The question of how iron is transported across the blood-testis barrier and the importance of iron in spermatogenesis have not been previously considered.

EXPERIMENTAL PROCEDURES

Chemicals—[^{35}S]Methionine (500 Ci/mmol) and ^{59}Fe (2 to 40 Ci/g of iron) were obtained from New England Nuclear. Dibutyryl cAMP and testosterone were obtained from Sigma Chemical Co. Medium for cell culture (F-12) was made from a powdered formulation supplied by GIBCO.

Cell Culture—Sertoli cells from 20-day-old rats were prepared and cultured essentially as previously described (5, 9). However, calf serum was not used in the media at all. This alteration of the procedure was necessary to ensure that bovine serum proteins could not contaminate the preparation of Sertoli cell-secreted proteins (SC protein). The medium contained 0.1 mM dibutyryl cAMP and 0.1 M testosterone throughout the culture period. The medium was collected every 4 days for a total of 16 days. On Day 3 to 4 of incubation, the cells were incubated for 24 h with F-12 which contained [^{35}S]-methionine (1 Ci/ml of F-12). The medium from all of the collections was pooled and the proteins were concentrated by ultrafiltration as previously described (5). Each liter of medium was concentrated to 25 ml and was then passed over a column of Bio-Rad P-6 gel which had been previously equilibrated with a 10 mM Tris-HCl buffer (pH 7.4).

Gel Electrophoresis—The concentrated, desalted, secreted proteins were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, on 5 to 15% acrylamide gradients. All samples for electrophoresis contained 1% mercaptoethanol. Fluorography of the gels was done as previously described (5, 10, 11).

Antibodies and Immunoprecipitation—To obtain antibodies to total Sertoli cell-secreted (SC) proteins, 2 mg of secreted proteins and 1 ml of Freund's complete adjuvant were injected intraperitoneally into adult female rabbits. Three injections were made at biweekly intervals and serum was prepared from the blood. Rabbit anti-rat transferrin was obtained from Cappell Laboratories, Inc., and goat anti-rat serum was obtained from both Cappell Laboratories and from Bio-Rad Laboratories. Agar gel double diffusion analysis of antibody-antigen interactions was done according to the method of Ouchterlony (12). Immunoprecipitation was done by adding sufficient antibody to completely precipitate the antigens. The immunoprecipitated proteins were washed by repeated centrifugation through a Tris/saline solution (0.9% NaCl solution) which contained 0.25 M sucrose.

RESULTS

The Sertoli cells from 20-day-old rats were plated in serum-free F-12 medium which contained dibutyryl cAMP (0.1 mM) and testosterone (0.1 M). Sertoli cell-secreted proteins were labeled with [^{35}S]methionine, collected, and concentrated. Rabbit antiserum directed against the total Sertoli cell-secreted proteins (SC proteins) was shown by double immunodiffusion to contain antibodies to several of the secreted proteins. In addition, the antiserum to SC protein contained antibodies to purified rat transferrin (Fig. 1). When antiserum to purified transferrin was analyzed by double diffusion, it was shown that SC protein and purified transferrin contain a homologous antigen (Fig. 1).

It has previously been shown that one of the major secreted

polypeptides (designated Band 3) from Sertoli cells had a molecular weight of 71,000 (5). In Fig. 2, the electrophoresis in SDS-polyacrylamide gels of SC proteins and rat transferrin is compared. Both the Coomassie blue-stained gels and the fluorographs of the ^{35}S-labeled proteins show that Band 3 protein has the same apparent molecular weight as transferrin. It was previously reported that Band 3 consisted of two bands designated Bands 3 and 3a. However, Band 3a has been shown to be the result of the incomplete reduction of the disulfide bonds of a dimer formed by Bands 5 and 6 (data not shown).

The SC proteins and rat transferrin were incubated for 2 h with ^{59}Fe and the samples were analyzed by gel electrophoresis and autoradiography (13). The transferrin and SC protein samples both contained an iron-binding component of similar electrophoretic mobility (Fig. 3).

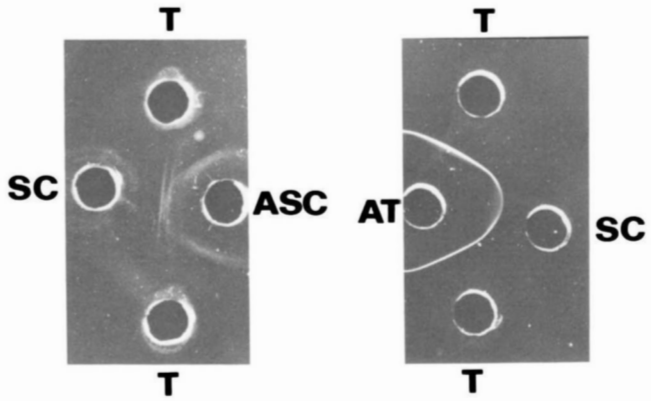

FIG. 1. **Agar gel double diffusion analysis of antisera against Sertoli cell-secreted proteins and against transferrin.** Rabbit serum (anti-Sertoli cell-secreted protein (*ASC*)) was placed in the center well on the left side of the figure. The rabbit anti-rat transferrin serum (*AT*) was placed in the center well on the right side of the figure. The following antigens were put in the outer wells: Sertoli cell-secreted proteins (*SC*), and purified rat transferrin (*T*). The precipitin lines were allowed to develop for 48 h at 22°C.

[^{35}S]Methionine-labeled SC proteins were immunoprecipitated by rabbit anti-SC protein, by rabbit anti-rat transferrin, and by goat anti-rat serum. The immunoprecipitates were washed as described and analyzed by gel electrophoresis and fluorography (Fig. 4). The anti-SC protein precipitated most of the SC polypeptides which have been previously described (5). The anti-rat transferrin immunoprecipitated a single protein which corresponds to ^{35}S-labeled Band 3 protein, and the anti-rat serum immunoprecipitated ^{35}S-labeled Band 3 as well as one other protein. This additional protein corresponds to Band 1 in the previous study and has a molecular weight of 140,000 (5).

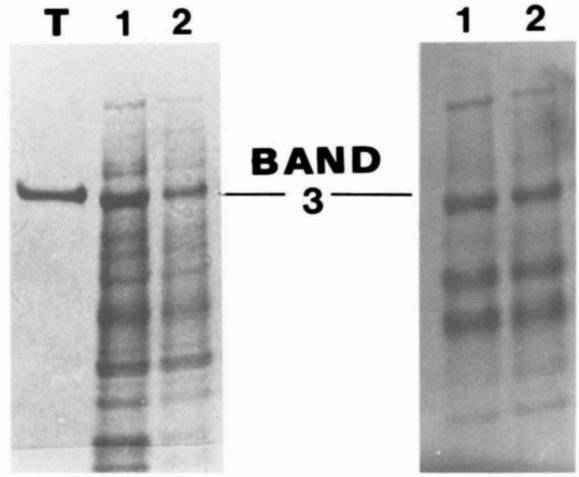

FIG. 2. **Electrophoresis of Sertoli cell-secreted proteins in gradient gels which contain 5 to 15% acrylamide and SDS.** On the left is a gel which has been stained with Coomassie blue and on the right is the fluorograph of the same gel. There was approximately 6000 cpm of ^{35}S-labeled protein in both samples and the fluorograph was exposed for 6 days. Two separate preparations of Sertoli cell protein (No. *1* and *2*) and purified rat transferrin (*T*) are shown for comparison.

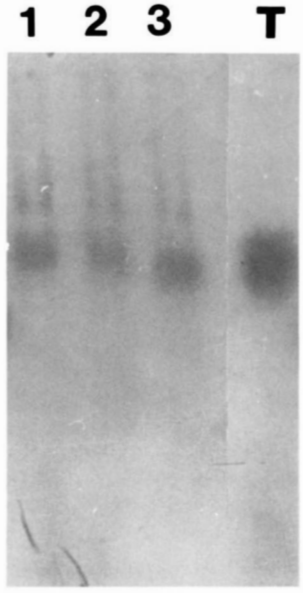

FIG. 3 (*left*). **Autoradiograph of ^{59}Fe-labeled proteins after electrophoresis in acrylamide slab gels.** The Sertoli cell-secreted proteins from three separate preparations (*1* to *3*) and rat transferrin (*T*) were incubated with 20 μCi of ^{59}Fe (New England Nuclear) and then analyzed by electrophoresis as described (5) except that no SDS was added. After electrophoresis, a film was exposed to the gel for 6 h.

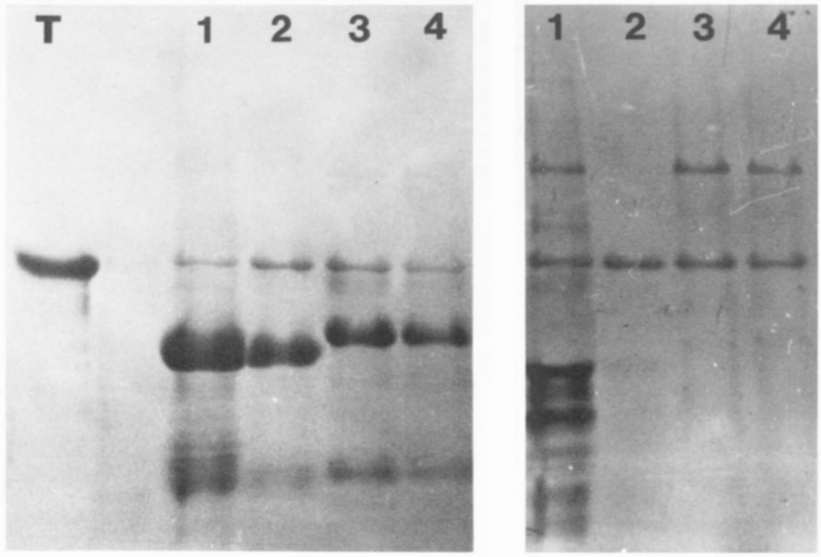

FIG. 4 (*center* and *right*). **Electrophoretic analysis of immunoprecipitated Sertoli cell-secreted proteins.** Electrophoresis was on gradient slab gels of 5 to 15% acrylamide which contained SDS. On the left is the gel after it was stained with Coomassie blue and on the right is the fluorograph of the same gel. *T* is rat transferrin; *1* to *4* are ^{35}S-labeled Sertoli cell-secreted proteins which have been immunoprecipitated by rabbit anti-SC protein (*1*), rabbit anti-rat transferrin (*2*), goat anti-rat serum from Cappell Laboratories (*3*), and goat anti-rat serum from Bio-Rad Laboratories (*4*).

DISCUSSION

Transferrin is a serum protein found in all vertebrates. It is a single glycopeptide of a reported molecular weight of 70,000 to 80,000 and functions as an iron transport protein (for a review, see Ref. 14).

We have concluded that transferrin or a transferrin-like protein is synthesized and secreted by Sertoli cells. We have based this conclusion on the following results: (*a*) antibodies to Sertoli cell-secreted proteins (SC proteins) reacted with rat serum and with purified rat transferrin; (*b*) a Sertoli cell-secreted protein (Band 3) and transferrin are antigenically homologous; (*c*) one of the secreted proteins which bound ^{59}Fe had an identical electrophoretic mobility to transferrin; (*d*) antibodies to rat transferrin selectively precipitated [^{35}S]methionine-labeled Band 3 protein from a preparation of SC protein.

It has been previously reported that, in cultures from 20-day-old rats, 5 to 15% of the total proteins synthesized by the Sertoli cell are secreted glycoproteins (5). We have estimated by densitometry that from 25 to 35% of the [^{35}S]methionine label in the secreted glycoproteins appears in testicular transferrin (data not shown) so that between 1 and 5% of the total Sertoli cell proteins synthesized is transferrin (assuming a uniform distribution of methionine among proteins).

Why do the Sertoli cells synthesize transferrin at all? All germinal cells which are more advanced than pre-leptotene spermatocytes are sequestered from contact with plasma transferrin by Sertoli cell tight junctions which prevent the movement of macromolecules into the lumen of the tubule (3). We propose that, *in vivo*, testicular transferrin is secreted into the lumen of the seminiferous tubule. The transferrin thus serves as a source of iron for the heme proteins or for non-heme metalloproteins in developing germinal cells. The Sertoli cell serves as an intermediary in the transport of iron from serum transferrin to the germinal cells.

Testicular transferrin is immunologically and electrophoretically similar to serum transferrin. We have previously reported that Band 3 protein contains fucose (5). Serum transferrin has been reported to lack fucose, so the possibility remains that the two transferrins might be glycosylated with different sugar moieties.

When SC proteins were immunoprecipitated with goat anti-rat serum antibodies, testicular transferrin and one other protein (Band 1) were immunoprecipitated. Ceruloplasmin can function as a ferroxidase and a copper transport protein. The ferroxidase activity of ceruloplasmin has led to suggestions that its function and presence are coupled to the function and presence of transferrin (15). The molecular weight of Band 1 (140,000), the presence of Band 1 protein in serum, and the association with transferrin suggest that Band 1 may be ceruloplasmin (15). However, the identification of Band 1 protein will require additional studies.

The presence of serum proteins in the tubular fluid has been reported previously but it was possible to explain the presence of these proteins by the contamination of the collected fluid by serum (16, 17). It was reported by Thorbecke *et al.* (18), who surveyed several tissues with antibodies, that transferrin is synthesized by both the testis and the ovary. The significance of testicular transferrin can only be postulated, and further studies of iron metabolism during spermatogenesis are needed.

Acknowledgments—We extend our appreciation to Alice Karl for her technical help and to Barbara Comstock for her help in the preparation of this manuscript.

REFERENCES

1. Means, A. R., Fakunding, J. L., Haskins, C., Tindall, D. J., and Vitale, R. (1976) *Recent Prog. Horm. Res.* **32,** 477-527
2. Fritz, I. B. (1973) *Curr. Top. Cell Regul.* **7,** 129-174
3. Fawcett, D. W. (1975) in *Handbook of Physiology: Male Reproductive System* (Hamilton, D. W., and Grup, R. D., eds) Vol. 5, pp. 21-56, American Physiological Society, Washington, D. C.
4. Waites, G. M. H. (1977) in *The Testis* (Johnson, A. D., and Gomes, R., eds) Vol. 4, pp. 91-117, Academic Press, New York
5. Wilson, R. M., and Griswold, M. D. (1979) *Exp. Cell Res.* **123,** 127-135
6. Fritz, I. B., Kopec, B., Lam, K., and Vernon, R. G. (1974) *Curr. Top. Mol. Endocrinol.* **1,** 311-327
7. Tindall, D. J., Cunningham, G. R., and Means, A. R. (1978) *J. Biol. Chem.* **253,** 166-169
8. Hansson, V., Trygstad, O., French, F. S., McLean, W. S., Smith, A. A., Tindall, D. J., Waddington, S. C., Petrusz, P., Nayfeh, S. N., and Ritzen, E. M. (1974) *Nature* **250,** 387-391
9. Dorrington, J. H., and Fritz, I. B. (1975) *Endocrinology* **96,** 879-889
10. Bonner, W. M., and Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 83-88
11. Laskey, R. A., and Mills, A. D. (1975) *Eur. J. Biochem.* **56,** 335-341
12. Ouchterlony, O. (1949) *Acta Pathol. Microbiol. Scand.* **26,** 507-511
13. Giblett, E. R., Hickkman, C. G., and Smithies, O. (1959) *Nature (Lond.)* **183,** 1589-1590
14. Putnam, F. W. (1975) in *The Plasma Proteins* (Putnam, F. W., ed) Vol. I, pp. 265-316, Academic Press, New York
15. Poulik, M. D., and Weiss, M. L. (1975) in *The Plasma Proteins* (Putnam, F. W., ed) Vol. II, pp. 51-108, Academic Press, New York
16. Koskimies, A. I., and Kormano, M. (1973) *J. Reprod. Fertil.* **34,** 433-444
17. Kormano, M., Koskimies, A. I., and Hunter, R. L. (1971) *Experientia (Basel)* **27,** 1461-1462
18. Thorbecke, C. J., Liem, H. H., Knight, S., Cox, K., and Muller-Eberlard, U. (1973) *J. Clin. Invest.* **52,** 725-731