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THE INTERACTIONS OF SERTOLI CELL GLYCOPROTEINS WITH GERMINAL CELLS IN THE TESTIS

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INTRODUCTION

Because of their unique morphological arrangement in the testis, the Sertoli cells have traditionally been regarded as "nurse" cells whose function is to provide an appropriate biochemical milieu in which germinal cell development can proceed (1). Even though it is clear that Sertoli cells secrete several glycoproteins and metabolites, evidence for the direct in vivo interaction between these products and germinal cells has been lacking. It has been and remains the goal of our research to identify, characterize and localize in the testis the major glycoprotein secretion products of Sertoli cells. In addition, we have examined the synthesis of these glycoproteins in an effort to determine the regulation of Sertoli cell functions both in cell culture and in vivo.

METHODS AND MATERIALS

The methods used in these studies have been described in detail elsewhere.

Cell culture - Sertoli cells from 20 day old rats were prepared and maintained in culture in Ham's F-12 medium (2).

Immunofluorescence - Paraffin embedded sections of Bouin's fixed adult rat testes were deparaffinized and rehydrated. Sections were preincubated in buffer containing 1% calf serum for 10 min prior to staining. Rabbit antisera to rat transferrin or to rat DAG-protein were diluted 50 to 100 fold and incubated on the tissue overnight. The slides were washed with buffer, and incubated with 1:100 dilution of biotinylated goat anti-rabbit IgG for 2 hr. The slides were further washed and incubated with FITC-avidin, re-washed and viewed with a Nikon Model-Ke microscope equipped for epifluorescence illumination and photomicroscopy (3).

Results and Discussion - When Sertoli cells from 20 day old rats are placed in cell culture, they continue to synthesize and secrete glycoproteins (4). If these secretion products are labeled with ^{35}S-methionine and analyzed by 2-dimensional polyacrylamide gel electrophoresis and fluorography several major polypeptides are detected (Fig. 1). Many of these polypeptides exhibit charge heterogeneity characteristic of sialated glycoproteins. Several of the major polypeptides on this electrophoretogram have been purified and identified. Polypeptides numbered 1 and 2 have been shown to be testicular

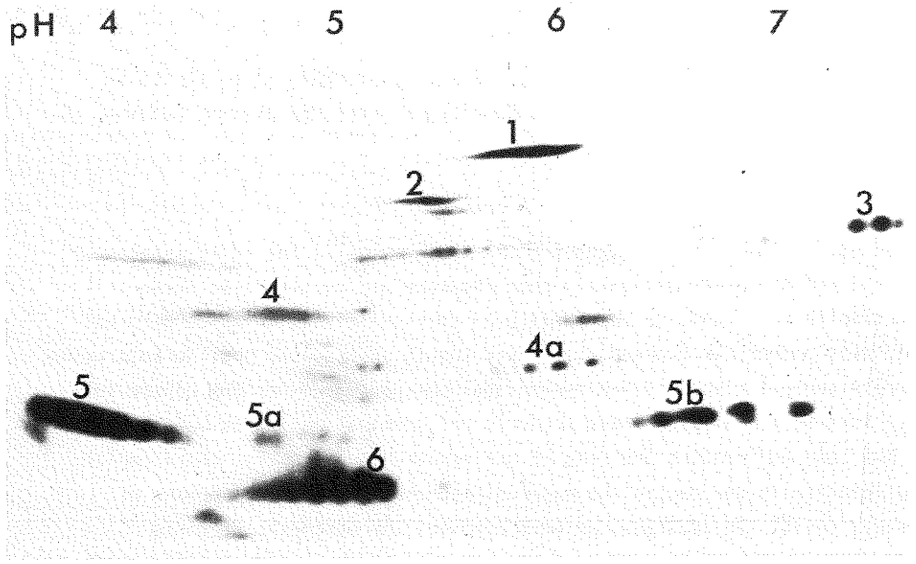

Fig. 1. Fluorogram of 2-dimensional polyacrylamide gel electrophoresis analysis of ^{35}S-methionine labeled proteins secreted by cultured Sertoli cells. The molecular weight of the polypeptides are 1-130 Kdal; 2-115 Kdal; 3-75 Kdal; 5-41 Kdal; 6-29 Kdal.

ceruloplasmin and a proteolytic fragment of testicular ceruloplasmin, respectively (5). Number 4a has been shown to be secreted by peritubular myoid cells which are present in low numbers in Sertoli cell cultures (4). Number 3 has been identified as testicular transferrin (6) and numbers 5 and 6 are monomers of a disulfide linked acidic glycoprotein (DAG-protein) (4). By a number of different analytical procedures, it was estimated that transferrin accounts for 7-15% and DAG-protein for 30-50% of the total mass of the secreted proteins. Most of our further studies have focused on the biochemistry and localization of transferrin and the DAG-protein.

Testicular transferrin was purified from spent Sertoli cell culture medium by a combination of hydrophobic chromatography and immuno-affinity chromatography. The polypeptide portion of rat testicular transferrin is identical to that of rat hepatic transferrin (7). The synthesis of transferrin by cultured Sertoli cells was examined by radioimmunoassay and by hybridization

TABLE I

SECRETION OF TRANSFERRIN AND ACCUMULATION OF TRANSFERRIN mRNA BY CULTURED SERTOLI CELLS

The transferrin was measured by a radioimmunoassay of spent medium from cultured Sertoli cells accumulated over a 2 day period on days 4-6 of cultures. The mRNA was measured in similar cultures on day 4 by hybridization of poly A^+ RNA to nick translated DNA for transferrin.

Hormone treatment	mg Transferrin/ 10^5 cells/48hr (avg. of 9 determinations)	cpm ^{32}P DNA hybridized/ ng cellular DNA (avg. of 12 determinations)
control	25 ± 5	90,960±18,800
FSH, Insulin, Retinol, Testosterone	164 ± 22	209,190±68,000

of mRNA to a nick translated cloned cDNA probe constructed from purified transferrin mRNA. (8, 9) Using these analyses, it was shown that the secretion of transferrin and the presence of transferrin mRNA were maximal and were stimulated when the cells were cultured in the presence of FSH, insulin, retinol and testosterone. (Table I) Antisera to transferrin was used to localize the protein on fixed paraffin embedded sections of testis (3). The transferrin was localized in the interstitium as hepatic or serum transferrin and in the acrosome region of developing spermatids, presumably as testicular transferrin (Fig. 2, left).

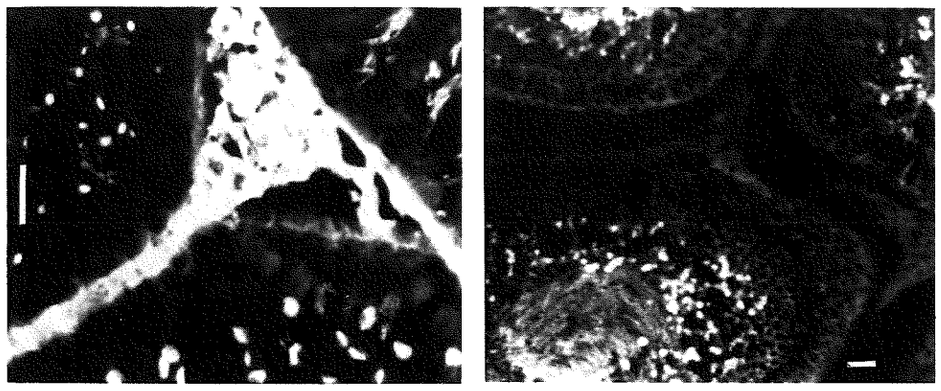

Fig. 2. Localization by immunofluorescence, in paraffin sections of testis, of transferrin (left) and DAG-protein (right). Bar = 25 microns.

DAG-protein was purified by column chromatography and by high performance liquid chromatography. Biochemical studies have shown that DAG-protein is a heavily glycosylated, sulfated, hydrophobic protein (10). Antisera to DAG-protein has been used to localize the protein in tissue sections in a procedure similar to the studies with transferrin. A unique pattern of localization showed DAG-protein present on released testicular spermatozoa, in the tubular milieu, and in the epididymal lumen (Fig. 2, right). Further studies have shown that DAG-protein is actively synthesized and secreted by the caput epididymus. The DAG-protein becomes localized on the sperm membrane over the anterior acrosome, the neck region and the distal tail (10).

Thus, two of the major secretion products of the Sertoli cells can be shown to interact with specific but separate stages of germinal cells during spermiogenesis. The function of the transferrin is presumably to supply Fe^{+3} to developing spermatids - a "nurse" cell activity of the Sertoli cells. The function of the DAG-protein is unknown but apparently is confined to interactions with spermatozoa.

ACKNOWLEDGMENTS

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REFERENCES

1. Fawcett SW (1975) In: Hamilton D W, Grup R D (eds.) Handbook of Physiology, vol. 5, American Physiological Society, Bethesda, MD, pp 21-56
2. Wilson RM, Griswold MD (1979) Exp. Cell Res. 123:127-135
3. Sylvester SR, Griswold MD (1984) Biol. of Reprod. in press
4. Kissinger C, Skinner MK, Griswold MD (1982) Biol. of Reprod. 27:233-240
5. Skinner MK, Griswold MD (1983) Biol. of Reprod. 28:1225-1229
6. Skinner MK, Griswold MD (1980) J. Biol. Chem. 225:1923-1925
7. Skinner MK, Cosand WL, Griswold MD (1984) Biochem. J. 218:313-320
8. Skinner MK, Griswold MD (1982) Biol. of Reprod. 27:211-221
9. Huggenvik J, Sylvester SR, Griswold MD (1984) Annals of N.Y. Acad. Sci. in press
10. Sylvester SR, Skinner MK, Griswold MD (1984) submitted