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Molecular Cloning of the Rat Tpx-1 Responsible for the Interaction between Spermatogenic and Sertoli Cells

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We previously showed in a primary culture of rat testicular cells that spermatogenic cells specifically bind to somatic Sertoli cells and that this interaction is needed for spermatogenic cells to differentiate *in vitro*. Adopting an expression cloning procedure, we here isolated a cDNA coding for a spermatogenic cell protein whose expression gave a cultured cell line the ability to bind to Sertoli cells. The protein, 243 amino acids with a putative N-terminal signal peptide and a C-terminal Cys-rich region, turned out to be the rat homologue of a testicular protein called Tpx-1 whose function had yet to be determined. A polyclonal antibody raised against bacterially expressed Tpx-1 significantly inhibited the binding of spermatogenic cells to Sertoli cells. The above results indicated that Tpx-1 is a testicular cell adhesion molecule responsible for the specific interaction between spermatogenic and Sertoli cells.

Throughout the mammalian spermatogenic pathway, differentiating spermatogenic cells remain in close contact with somatic Sertoli cells [reviewed in (1, 2)], and this has been considered to be essential for the proliferation, differentiation and survival of spermatogenic cells as well as for the cyclic function of Sertoli cells [reviewed in (3-5)]. It has thus been postulated that many important materials and signals are mutually transferred between the two cell types. However, the molecular basis for such an interaction remains to be clarified. It is likely that the two cell types communicate with each other by direct cell-cell contact [reviewed in (5-7)] and/or via paracrine mechanisms [reviewed in (8-9)]. Although the presence of various cell adhesion molecules on the surface of spermatogenic and Sertoli cells has been reported [reviewed in (5-7)], the question of whether they truly participate in the functional interaction between the two cell types is still a matter of debate. Among such molecules, β1,4 galactosyltransferase (10) and N-cadherin (11) are strong candidates as proteins responsible for Sertoli-spermatogenic cell adhesion, since their antibodies inhibited the binding of the two cell types *in vitro*.

Tres and Kierszenbaum observed that spermatogenic cells specifically bound to Sertoli cells in a primary culture of rat testicular cells, and suggested that this interaction was important for the proliferation and differentiation of spermatogenic cells (12). We have developed a similar primary culture of rat testicular cells, in which spermatogenic cells advance in their differentiation in terms of the occurrence of testis-specific gene expression (13, 14). In this culture, spermatogenic cells are maintained in association with Sertoli cells, and spermatogenic differentiation was abrogated when the two cell types were placed on the opposite side of a permeable membrane (15). This indicated that an association with Sertoli cells is needed for spermatogenic cells to differentiate in culture. We here intended to functionally clone the molecule(s) responsible for the specific interaction between spermatogenic and Sertoli cells, making use of this primary culture system.

MATERIALS AND METHODS

Cell culture. Dispersed testicular cells were prepared from testes dissected from 20-day-old Donryu rats and primary cultured at 32.5 °C in a mixture of F12 and L15 media (1:1) with 10% fetal calf serum (FCS)[2] and norepinephrine (1 μg/ml) as described previously (13, 14). Jurkat Tag cells (provided by G. Crabtree), a human acute lymphocytic leukemia cell line expressing SV40 large T-antigen, were maintained in RPMI1640 medium supplemented with 10% FCS.

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The nucleotide sequence for the rat Tpx-1 cDNA has been deposited in the DDBJ, EMBL and GenBank nucleotide sequence databases under accession number AB009662.

[2] Abbreviations used: AA1, autoantigen 1; AEG, acidic epididymal glycoprotein; CRISP, Cys-rich secretory proteins; FCS, fetal calf serum; GST, glutathione S-transferase.

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Cell adhesion assay. Testicular cells were primary cultured on coverslips (ϕ=3 cm) for 3 days. Cultured spermatogenic cells consisting mostly of spermatocytes (15) were detached from Sertoli cells by pipetting and spun down, and the cells (5×10^5) were added to a monolayer of the Sertoli cells (1.5×10^6). After a three-hour culture, the coverslips were taken out with forceps and washed by shaking them exactly five times in the culture medium. The number of spermatogenic cells remaining attached to the Sertoli cells was determined under a phase-contrast microscope. Ten randomly-chosen microscopic fields were examined, and the cell numbers are presented relative to that of initially loaded spermatogenic cells taken as 100; i. e., the cell adhesion index. To determine the function of cloned cDNA, the binding of Jurkat Tag cells expressing the DNA to Sertoli cells was examined as described above.

Cloning of Tpx-1 cDNA. Poly(A)-containing RNA (5 μg) prepared from cultured spermatogenic cells was used to synthesize cDNA using a commercial kit (Great Lengths cDNA Synthesis Kit; Clontech, Palo Alto, CA), and the resulting cDNA was ligated with the pcDNAI/Amp vector (Invitrogen, NV Leek, Netherlands). The cDNA library consisted of 2×10^6 independent clones with insert sizes ranging from 0.5 to 3 kbp. The library (200 μg DNA) was introduced into Jurkat Tag cells (5×10^7) by electroporation using Gene Pulser (Bio-Rad Laboratories, Hercules, CA) at 0.25 kV with a capacitance of 960 μF. The cells were cultured in RPMI1640 medium with 10% FCS for 48 h and then subjected to a screening in the cell adhesion assay with Sertoli cells. The cells remaining attached to the Sertoli cells were detached by pipetting and selected again in the same adhesion assay. Plasmid DNA was then recovered from the selected cells and amplified in *E. coli* TOP10F′. The DNA was introduced again into Jurkat Tag cells (1×10^7), and the cells were subjected to another round of screening. After four rounds of this screening, plasmid DNA was extracted from the selected cells and about 50 cDNA clones were sequenced. We obtained three clones that contained reasonably long open reading frames. The inserts of these clones were ligated with the pHook-2 vector (Invitrogen), and the resulting DNA (40 μg) was introduced into Jurkat Tag cells (1×10^7) by electroporation. After a 48-h culture, the cells expressing the cDNA (1×10^5) were collected using phOx-coated magnetic beads (Capture-Tec pHook-2 kit; Invitrogen) and subjected to the final cell adhesion assay with Sertoli cells. About 80% of the cells selected by the above procedure expressed an introduced DNA when examined with a control pHook-2-*lacZ* DNA (data not shown). Eventually, one clone (#97) coded for a protein possessing the desired activity.

Preparation of anti-Tpx-1 antibody. The region between nucleotide positions 345 and 807 (amino acids 89-243) of clone #97 was inserted into a pET-15b (Novagen, Madison, WI)-derived His-tag vector, and the resulting DNA was introduced into *E. coli* BL21. Expression of the His-tagged Tpx-1 was induced with 1 mM isopropyl β-D-thiogalactoside. The His-tagged Tpx-1 recovered as inclusion bodies were separated on a 15% polyacrylamide gel containing SDS. A portion of the gel containing the fusion protein was excised and the protein was electroeluted using Maxyield-NP (Atto, Tokyo, Japan). Tpx-1 fused with glutathione S-transferase (GST) was similarly prepared by inserting the region between nucleotide positions 345 and 807 into a vector (pGX-KG; Amersham Pharmacia Biotech, Tokyo, Japan), and *E. coli* BL21 was transformed with the resulting DNA. The GST-Tpx-1 fusion protein (GST-Tpx-1c) was purified from *E. coli*, as was the His-tagged protein. GST was purified using glutathione Sepharose (Amersham Pharmacia Biotech, Stockholm, Sweden) under standard procedures. Rabbits were immunized with about 0.4 mg of the purified His-tagged Tpx-1, and the antibody titer was determined by Western blots of GST-Tpx-1c. The total IgG fraction was obtained from sera by successive fractionation with ammonium sulfate precipitation and DEAE-cellulose chromatography (16). IgG specific to Tpx-1 was further purified through an affinity chromatography with His-tagged Tpx-1-conjugated Ni-NTA agarose (Qiagen, Hilden, Germany).

Western blots. Proteins were separated on a 12% polyacrylamide gel containing SDS and electrophoretically transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The membrane was blocked with 0.2% skim dry milk and incubated with a primary antibody in a buffer consisting of 10 mM Tris-HCl (pH 8), 0.15 M NaCl, and 0.5% Tween 20. The membrane was washed and reacted with a secondary antibody conjugated with alkaline phosphatase. The signals were detected using the Immun-Star system (Bio-Rad Laboratories).

Immunohistochemistry. Spermatogenic cells prepared from 20-day-old rats were fixed with 4% paraformaldehyde and 2% sucrose for 15 min at room temperature, then with methanol for 1 min at room temperature. The fixed cells were treated first with affinity-purified anti-Tpx-1 IgG, then with a FITC-conjugated secondary antibody, and examined under a confocal laser microscope.

Antibodies used in this study. Mouse anti-GST monoclonal antibody (B-14; Santa Cruz Biotechnology, Santa Cruz, CA); alkaline phosphatase-labeled anti-rabbit IgG antibody (Bio-Rad Laboratories); alkaline phosphatase-labeled goat anti-mouse IgG antibody (Bio-Rad Laboratories), fluorescein-conjugated goat anti-rabbit IgG antibody (Immunotech, Marseilles, France).

RESULTS

Expression cloning of a cell adhesion molecule of spermatogenic cells. When rat testicular cells were primary cultured, spermatogenic cells were maintained in association with Sertoli cells that grew as a monolayer (Fig. 1A). In order to identify the molecule(s) responsible for the specific interaction between the two cell types, we first established a quantitative cell adhesion assay. Cultured spermatogenic cells were isolated and loaded on the Sertoli cell culture, and the mixture was left for 3 h. The culture was then washed carefully and cell adhesion indices were determined as described in the Materials and Methods section. In this assay, spermatogenic cells were shown to bind to Sertoli cells much more efficiently than did a cultured T-cell line, Jurkat Tag (Fig. 1B).

We then aimed at cloning the molecule(s) responsible for this interaction, using an expression cloning procedure which was adopted for cloning a cell adhesion protein presumably involved in embryo implantation (18). We chose the human T-cell line Jurkat Tag as parent cells for the following reasons; the cells grow in suspension, do not bind to Sertoli cells (see Fig. 1B), and express SV40 large T-antigen that allows a plasmid containing the SV40 replication origin to replicate extrachromosomally. A cDNA library prepared from the mRNA of cultured spermatogenic cells was introduced into Jurkat Tag cells, and the cells that acquired the ability to bind to Sertoli cells were selected as described in the Materials and Methods section. After four rounds of screening, plasmids containing the cDNA were recovered from the selected cells and sequenced. We obtained three cDNA clones that contained the complete coding sequences for reasonably large peptides. To determine whether these cDNA coded for proteins with the desired activity, the final cell adhesion assay was conducted. Two of the clones

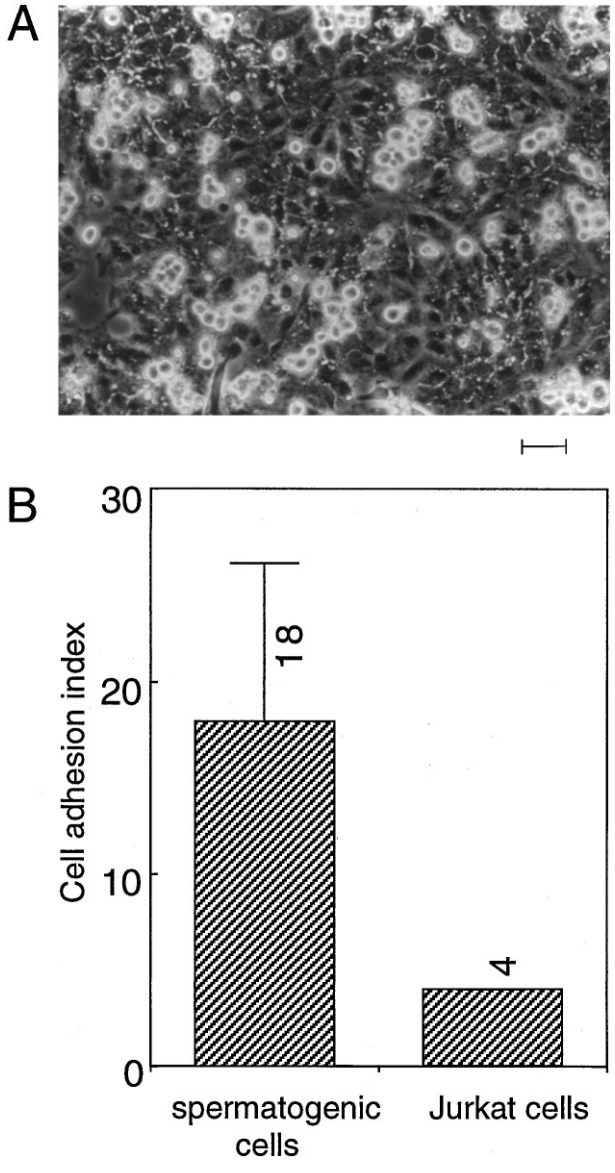

FIG. 1. Specific binding of spermatogenic cells to Sertoli cells *in vitro*. (A) A phase-contrast microscopic view of primary cultured testicular cells of 20-day-old rats. Spermatogenic cells are maintained in association with Sertoli cells that grow attached to dishes. Scale bar = 50 μm. (B) Adhesion of spermatogenic cells and Jurkat Tag cells to Sertoli cells. Shown are the mean and standard deviations from one experiment of three with similar results.

(#78 and #97) were inserted into the pHook-2 vector, and the resultant DNA was introduced into Jurkat Tag cells. We were unable to ligate the insert of the third clone with pHook-2 for an unknown reason. Cells expressing the cDNA were selected using magnetic beads, and their ability to bind to Sertoli cells was examined. The cells with clone #97 bound to Sertoli cells as efficiently as did spermatogenic cells, but those expressing clone #78 or the vector alone did not (Fig. 2). These results showed that clone #97 is the one that coded for a protein with the desired activity.

Structure of the cell adhesion protein. The cDNA clone encoded a protein with 243 amino acids including an N-terminal hydrophobic sequence and a Cys-rich region at the C-terminal half (Fig. 3A). Characteristically, the Cys residues repeated five times with an interval of eight amino acids near the C-terminus (Fig. 3A). The entire amino acid sequence of this protein showed a significant similarity to that of a testicular protein called Tpx-1 or autoantigen 1 (AA1); 85, 65, and 68% identity with mouse, guinea pig, and human proteins, respectively (Fig. 3B). We thus concluded that the protein is the rat homologue of Tpx-1. Tpx-1 is a member of the CRISP (standing for Cys-rich secretory proteins) family of proteins, which contain the N-terminal signal peptide and are rich in Cys residues at the C-terminal half (18, 19).

Role of Tpx-1 in spermatogenic and Sertoli cell adhesion. In order to assess the role of Tpx-1 in the specific adhesion of spermatogenic cells to Sertoli cells, a polyclonal antibody was raised against a bacterially-expressed His-tagged Tpx-1. The specificity of the antibody was first examined by Western blots. *E. coli* proteins containing a GST-Tpx-1 fusion protein were analyzed with anti-Tpx-1 and anti-GST antibodies in the presence and absence of corresponding antigen proteins. As seen in Fig. 4A, GST-Tpx-1c of about 44 kDa was detectable with either antibody. When the reaction with primary antibodies was conducted in the presence of GST-Tpx-1c or GST, the signal with anti-Tpx-1 disappeared only in the presence of GST-Tpx-1c (lanes 1-3), while that with anti-GST was abolished by the addition of either protein (lanes 4-6). These results showed that the antibody raised against bacterially-expressed Tpx-1 specifically recognizes the Tpx-1 protein in Western blots. The same antibody was then examined to determine whether it reacts with native

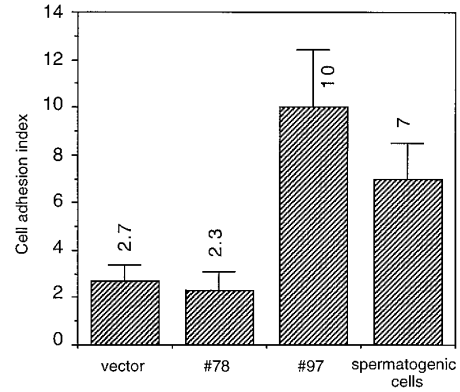

FIG. 2. Cell adhesion assay of the cloned cDNA. Jurkat Tag cells expressing the pHook-2 vector alone, the cDNA clone #78, the cDNA clone #97, and the control spermatogenic cells were analyzed for their ability to bind to Sertoli cells. Cell adhesion indices with the mean and standard deviations from one experiment of three with similar results are shown.

A

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   1  CTC CTG ATC TTC AAA CAT CAG AAG AAA GGA CAA GAT AAG GCA GAT ATT TCA ACT   54
  55  GTC AAA TCA ACA CTT CCA GCC ATG GCT TGG TTC CAG GTG ATG CTG TTT GTC TTT  108
   1                              M   A   W   F   Q   V   M   L   F   V   F   11
 109  GCT GTG CTG CTA CCA TTG CCA CCC ACA GAA GGA AAG GAT CCA GAC TTC GCT ACT  162
  12   A   V   L   L   P   L   P   P   T   E   G   K   D   P   D   F   A   T   29
 163  TTG ACA ACC AAC CAA ATA CAA GTT CAA AGA GAG ATC ATA GCT AAA CAC AAT GAA  216
  30   L   T   T   N   Q   I   Q   V   Q   R   E   I   I   A   K   H   N   E   47
 217  CTG AGG AGA CAA GTT AGC CCC CCT GGC AGC AAC ATA CTA AAA ATG GAA TGG AAC  270
  48   L   R   R   Q   V   S   P   P   G   S   N   I   L   K   M   E   W   N   65
 272  GTA CAA GCA GCA GCA AAT GCT CAA AAG TGG GCT AAT AAC TGT ATT TTA GAA CAC  324
  66   V   Q   A   A   A   N   A   Q   K   W   A   N   N   C   I   L   E   H   83
 325  AGT AGT ACA GAA GAC CGG AAA ATC AAT ATA AAA TGT GGC GAG AAT CTC TAT ATG  378
  84   S   S   T   E   D   R   K   I   N   I   K   C   G   E   N   L   Y   M  101
 379  TCG ACT GAC CCT ACA TCC TGG AGA ACC GTA ATT CAA AGC TGG TAT GAA GAA AAT  432
 102   S   T   D   P   T   S   W   R   T   V   I   Q   S   W   Y   E   E   N  119
 433  GAA AAC TTC GTT TTC GGC GTA GGA GCT AAA CCC AAT TCC GCT GTC GGA CAC TAC  486
 120   E   N   F   V   F   G   V   G   A   K   P   N   S   A   V   G   H   Y  137
 487  ACT CAG CTT GTT TGG TAT TCA TCT TTC AAA GTT GGA TGT GGA GTT GCT TAC TGT  543
 138   T   Q   L   V   W   Y   S   S   F   K   V   G   C   G   V   A   Y   C  155
 544  CCC AAT CAA GAT ACC CTG AAA TAC TTC TAT GTT TGC CAT TAC TGT CCT ATG GGT  594
 156   P   N   Q   D   T   L   K   Y   F   Y   V   C   H   Y   C   P   M   G  173
 595  AAC AAC GTG ATG AAA AAG AGT ACC CCA TAT CAT CAA GGG ACA CCT TGT GCT AGT  648
 174   N   N   V   M   K   K   S   T   P   Y   H   Q   G   T   P   C   A   S  191
 649  TGT CCC AAT AAC TGT GAT AAT GGA TTG TGC ACC AAT AGC TGT GAT TTT GAA GAT  702
 192   C   P   N   N   C   D   N   G   L   C   T   N   S   C   D   F   E   D  209
 703  TTA CTT AGT AAC TGT GAA TCC TTG AAG AGT TCA GCA GGC TGT AAA CAT GAG TTG  756
 210   L   L   S   N   C   E   S   L   K   S   S   A   G   C   K   H   E   L  227
 755  CTC AAG GCA AAG TGT GAG GCT ACT TGC CTA TGT GAA GAC AAA ATT CAT TAA CAT  810
 228   L   K   A   K   C   E   A   T   C   L   C   E   D   K   I   H   *      243
 811  GCC CAG CGT GCA GCA TGA CAG ACT ACA TGA GAA GGG GTA CAG ACT TAG TTG AGA  864
 865  CAT GAC AGG GAA AAC CTA TAG GAG AGT AGT GAA ACA GTG CAT CCC AAA TGA CAA  918
 919  GGC TTC TTT CCT TCC TGG ATT TAT ATA GAA ATG TCT TTC ATA CAG CCA TTA AGA  972
 973  AAG GTG TCA TTT AGG ATA ACA ACT CTG GAT TTT GAC CAA CTT TGC TGC TTC AAA 1026
1027  TGT AGT GAA GCG AAT CAA GTG GAG AAT TTT GAA AGT TGT ACC ATA ACT GGT CAT 1080
1081  TCA CCT CTA GAA CTT TGA AAA GGA GAG AAC TGT TTG TGT CCT AAA CCA ACC TGC 1134
1135  AAT GGA AGA ATG GGC TGT AGT TAC ATC ACC ATC AAC CTA CTT CAT AGT GCC TAC 1188
1189  CAG GAT GAA TCT TGA CAT CTA GAT TTG TCT TAT GTC TTC TTA CTT TAA CAC AAA 1242
1243  TGA TCA TCT TTT CCA ATA AAG AAT TCA AGC TAC CAC AAA AAA AAA AAA AAA AAA 1296
1297  AA
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B

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                    **   *  *     **          **** *     *  * *** **  *** **
# 97 (rat)           1 MAWFQVMLFVFAVLLP/LP/P/TEGKDPDFAT/LTTNQIQVQREIIAKHNELRR  50
Tpx-1 (mouse)       1 ------------L--RS///-L--------TSL--/---------VN--------  50
AA-1 (guinea pig)   1 --LLP-VV-LITM---CVL///-N----A-TALI--/-S---N---N---Q--K  50
TPX-1 (human)       1 --LLP-/--LVT-----S---///A------A-TAL----/-L------VN------K  49

                      * *  *  *****       *** *** * * **   ***    ******** *
# 97 (rat)          51 QVSPPGSNILKMEWNVQAAANAQKWANNCILEHSSTEDRKINIKCGENLYMSTD 104
Tpx-1 (mouse)       51 S-N-T--D------SI--TT-------K-------KD------R---------- 104
AA-1 (guinea pig)   51 S-T--A--M-----SRE--V-------R-T-V--NPD---TST---------S- 104
TPX-1 (human)       50 A----A--M-----SREVTT---R---K-T-Q--DP----TSTR--------S- 103

                    *  *   ****  *   *  *** *  ** ***********    *** *****
# 97 (rat)          105 PTSWRTVIQSWYEENENFVFGVGAK/PNSAVGHYTQLVWYSSFKVGCGVAYCPN 157
Tpx-1 (mouse)      105 --L-S-------N---D--Y-----/-------------------I---I----- 157
AA-1 (guinea pig)  105 -S--SDA----FD-SQD-T----P-SH-AV-----------YL----I------ 158
TPX-1 (human)      104 ----SSA-----D-ILD--Y---P-S--AV----------TYQ----I----- 157

                   ** *** *** *** ***   * *** ** *** **  *  *******   ***
# 97 (rat)          158 QDTLKYFYVCHYCPMGNNVMKKSTPYHQGTPCASCPNNCDNGLCTNSCDFEDLL 211
Tpx-1 (mouse)      158 --N-----------------------Q-----------E-------------- 211
AA-1 (guinea pig)  159 --S---Y---Q---A----YT-N---K--I------GH-E--------EY---- 212
TPX-1 (human)      158 --S---Y---Q---A---MNR-N---Q------G--DD--K-------QYQ--- 211

                      *** ***  *** * **   ** *** ** **
# 97 (rat)          212 SNCESLKSSAGCKHELLKAKCEATCLCEDKIH                    243
Tpx-1 (mouse)      212 -------T----------T--Q----------                    243
AA-1 (guinea pig)  213 -------NT---E-Q--VE--K---R-----Y                    244
TPX-1 (human)      212 ---D---NT---E------E--K------N--Y                    243
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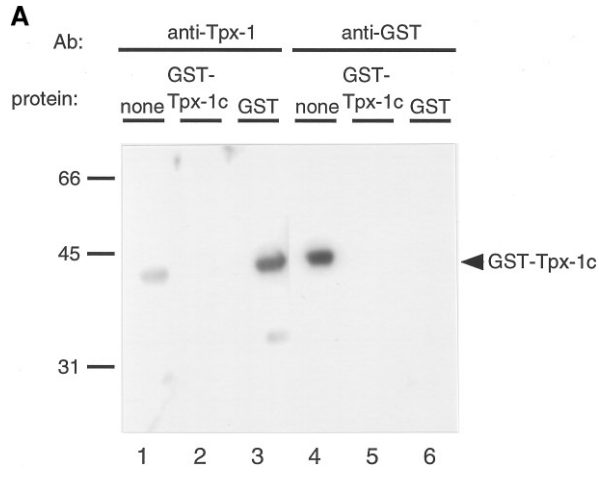
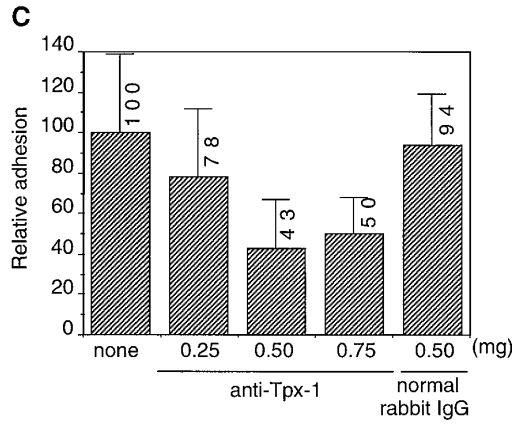
FIG. 3. Structure of the protein encoded by clone #97. (A) Nucleotide and deduced amino acid sequences of the protein encoded by the cDNA clone #97. The N-terminal hydrophobic sequence and a presumed polyadenylation signal are underlined, and the five Cys residues appearing every nine amino acids near the C-terminus are circled. The nucleotides and amino acid residues are numbered relative to the 5′-end of the cDNA and the translation start codon, respectively. (B) Similarity in the amino acid sequence of the protein encoded by clone #97 to mouse, guinea pig and human Tpx-1. Amino acid residues are aligned to give a maximum similarity; gaps are indicated by slashes. Amino acid residues identical to those of the rat Tpx-1 are shown by horizontal bars, and those conserved among the four proteins are asterisked.





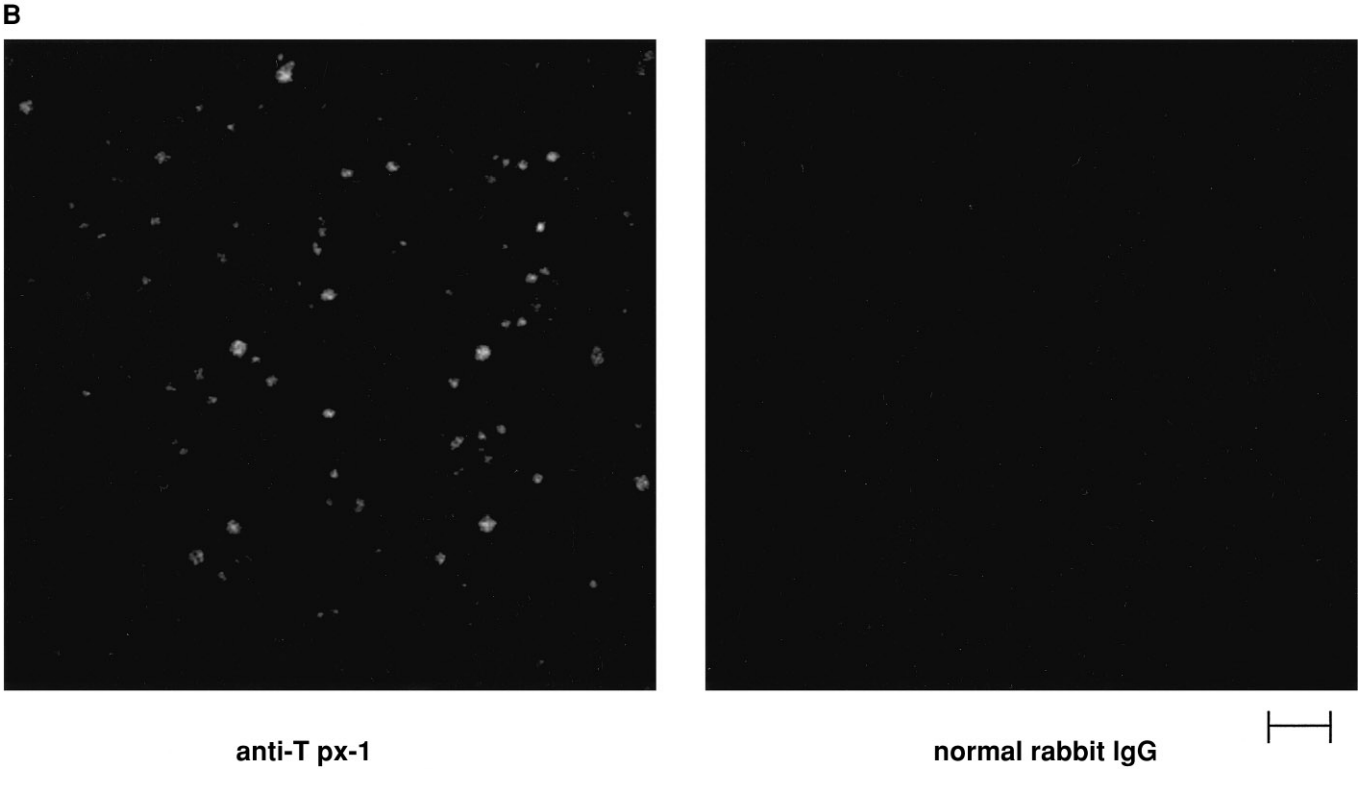


FIG. 4. Role of Tpx-1 in spermatogenic-Sertoli adhesion. (A) Western blots of a GST-Tpx-1 fusion protein. *Escherichia coli* proteins containing GST-Tpx-1 were analyzed with a polyclonal antibody raised against bacterially-expressed His-tagged Tpx-1 or an anti-GST antibody. The reaction with primary antibodies was conducted in the presence (lanes 2, 3, 5, and 6) and absence (lanes 1 and 4) of the corresponding antigen proteins. (B) Immunohistochemical detection of Tpx-1 in spermatogenic cells. Spermatogenic cells of 20-day-old rats were subjected to an immunofluorescence analysis with an affinity-purified anti-Tpx-1 antibody (left) and control normal rabbit IgG (right) and examined under a confocal laser microscope. Scale bar = 100 μm. (C) Effect of anti-Tpx-1 on adhesion between spermatogenic and Sertoli cells. Cell adhesion assays were conducted in the presence of an anti-Tpx-1 antibody or normal rabbit IgG. The extent of cell adhesion is shown relative to that with no added antibody. The mean and standard deviations from one experiment of three with similar results are shown.

Tpx-1. When spermatogenic cells of 20-day-old rats were immunohistochemically analyzed with the affinity-purified anti-Tpx-1 antibody, most of the cells showed signals (Fig. 4B). We then assessed the effect of anti-Tpx-1 on the adhesion between spermatogenic and Sertoli cells. The adhesion was inhibited in a manner responding to the amount of added antibody, and the maximum inhibition was about 50% (Fig. 4C). The control normal rabbit IgG showed little effect. These results indicated that Tpx-1 is responsible, at least in

part, for the adhesion between spermatogenic and Sertoli cells.

DISCUSSION

A number of cell adhesion proteins such as cadherins, Ca^{2+}-independent cell adhesion molecules and integrins exist in the testis, seemingly on the surface of both spermatogenic and Sertoli cells (5-7). Although such proteins are likely candidates responsible for the specific contact between these two cell types, no direct evidence of this has been presented. We attempted in this study to isolate the cDNA for the molecule(s) that defines the binding of rat spermatogenic cells to Sertoli cells, employing an expression cloning method. One such cDNA clone, obtained after repeated screening in a cell adhesion assay with Sertoli cells, coded for a protein distinct from authentic cell adhesion molecules. The protein turned out to be the rat homologue of a known testicular protein called Tpx-1 (20, 21) or AA1 (18), which is abundantly present in the acrosome. The synthesis of the mRNA and protein of AA1 (guinea pig Tpx-1) becomes detectable at the pachytene spermatocyte stage, reaching a peak in round spermatids, and it is thereafter stably present in spermatogenic cells (18). Since the spermatogenic cells used in this study consisted mostly of spermatocytes, it seemed reasonable that a cDNA for acrosomal Tpx-1 was isolated from a library made with the RNA of cultured spermatogenic cells. Tpx-1 may not be solely responsible for the adhesion of spermatogenic cells to Sertoli cells, since an anti-Tpx-1 polyclonal antibody inhibited it by only 50%. Other testicular proteins, e.g., β1,4 galactosyltransferase (10) and N-cadherin (11), are likely to also be involved in the specific interaction between the two cell types.

Tpx-1 was first identified as a protein encoded by one of the randomly-cloned genomic fragments from mouse chromosome 17 (22). It was eventually revealed to be the mouse homologue of a guinea pig protein named AA1, a component of the acrosome (18). Tpx-1 consists of about 240 amino acids with a presumed N-terminal signal peptide and a Cys-rich C-terminal half, with no sequences identified that are considered to be transmembrane regions or N-linked glycosylation sites. Within the Cys-rich region, five Cys residues appear every nine amino acids near the C-terminus. These characteristics of Tpx-1 indicate that it is a member of the CRISP family of proteins (18, 19). Another member of this family is an epididymal protein called acidic epididymal glycoprotein (AEG) that is synthesized in the epididymal epithelium and exists attached to the plasma membrane covering the sperm head (23). AEG has been suggested to be involved in sperm-egg fusion (24). Since more than 55% of the amino acid sequence of Tpx-1 is identical to that of AEG, these two proteins could possess similar biological functions. We here showed that Tpx-1 functions as a cell adhesion protein not for the sperm-egg interaction but for the association between spermatogenic and Sertoli cells.

Sertoli cells appear to functionally interact with spermatogenic cells in a fashion different from that focused on in this study; that is, they play a central role in the exclusion of degenerating spermatogenic cells (15, 25), and they presumably induce the apoptosis of spermatogenic cells via the Fas/Fas-ligand system (26). It is necessary to clarify the molecular basis underlying such interactions for a deeper understanding of the mammalian spermatogenic pathway.

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