We identified a novel gene encoding protein-tyrosine phosphatase using a polymerase chain reaction-based method. Northern blot hybridization of RNAs from various tissues with the polymerase chain reaction-amplified DNA fragment showed that this gene was expressed exclusively in the testis. Complementary DNAs for this gene, termed typ (testis-specific tyrosine phosphatase), were obtained from a mouse testis cDNA library. Nucleotide sequencing of the cDNAs revealed an open reading frame that encoded 426 amino acids. The predicted Typ protein contained a single catalytic domain at the carboxyl-terminal half. No hydrophilic stretch for a possible transmembrane sequence or signal sequence was found, suggesting that Typ is a cytoplasmic protein-tyrosine phosphatase. The amino-terminal half of Typ did not share significant homologies with the other known proteins but contained a region rich in PEST residues. Indirect immunofluorescence studies and in situ hybridization analysis showed that Typ was specifically expressed in testicular germ cells that underwent meiosis. Developmentally, Typ was detected between 2 and 3 weeks after birth, in parallel with the onset of meiosis. Thus, Typ is a new member of the cytoplasmic protein-tyrosine phosphatases that may play an important role(s) in spermatogenesis and/or meiosis.

Protein tyrosine phosphorylation is one of the important regulatory events in cell growth, activation, and differentiation (1). The levels of tyrosine phosphorylation of cellular proteins are regulated by the opposing actions of protein-tyrosine kinases and protein-tyrosine phosphatases (PTPs).1 In contrast to the extensive information available on the protein-tyrosine kinases, that on the biological roles of PTPs is limited. PTPs are classified into two subfamilies, receptor-type PTPs (R-PTP) (14), dual-specific phosphatase Twine (15), and a serine/threonine phosphatase (OST-PTP) (16), a serine/threonine phosphatase (cALPase) (17), and a dual specific phosphatase (Twine) (17). Because of the putative regulatory function of the protein kinases and phosphatases, these proteins are thought to contribute to germ cell differentiation. Indeed, c-Kit is directly involved in the mitotic cell cycle of spermatogonia (9), and the dual-specific phosphatase Twine is expressed specifically in germ cells and is suggested to be important in male meiosis in Drosophila (17).

Here we report identification of the typ gene that encodes a novel cytoplasmic PTP. The typ gene is expressed specifically in spermatocytes that are under meiosis, suggesting an important role of the Typ protein in spermatogenesis.

**EXPERIMENTAL PROCEDURES**

**Amplification of PTP cDNAs—**For the cDNA synthesis and PCR, oligonucleotide primers were designed from conserved regions within the PTP domain. A primer for first-strand cDNA synthesis corresponded to the amino acid sequence GT/E/D/T (primer 1, 5'-CATGAATTC(A/G/T) (A/G)/C/T/G/G/G/G/G/C/T/C/G/T/G (primer 2, sense), 5'-ATGAGGCTTTGAC/T/T/A/C/T/G/G/G/G/C/T/G/C/G/T/G-3').

1 The abbreviations used are: PTP, protein-tyrosine phosphatase; RT, reverse transcription; PCR, polymerase chain reaction; GST, glutathione S-transferase; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; pNPP, p-nitrophenyl phosphate; AS, antisense.

© 1997 by The American Society for Biochemistry and Molecular Biology, Inc.
were incubated at 72 °C for 5 min. PCR products of the expected size (~420 base pairs) were purified through 6% polyacrylamide gel electrophoresis and cloned into the pUC119 vector for sequence analysis.

Colony Hybridization—The pUC119 vector NIH DNAs were ligated with the RT-PCR products amplified using RNAs from MDA-MB453 cells, and transformants were selected on LB plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and ampicillin. These colonies were transferred onto nitrocellulose filters (Schleicher & Schuell), and colony hybridization of the filters was performed using a mixture of 32P-labeled PCR fragments corresponding to B-PTP2 (19), PTP-MEG (20), DEP-1 (21), and LAR (22) as probes. Hybridization was performed in a stringent condition as described (23).

Northern Hybridization—Expression plasmid, pME-Typ, the human Multiple Tissue Northern Blot, Human Multiple Tissue Northern Blot II, and Human Fetal Multiple Tissue Northern blot (CLONTECH) were hybridized to the 32P-labeled human type PCR fragment using the hybridization and washing conditions recommended by the supplier. Samples of total RNAs isolated from adult mice tissues were loaded on each lane (20 μg/ lane) of 1.0% formaldehyde-agarose gels, electrophoresed, and blotted overnight on Hybond-N membrane filters (Amersham). The filters were hybridized to the 32P-labeled human type PCR fragment or the cDNA insert of clone 5 (see Fig. 2A) using the previously described hybridization and washing procedures (24).

DNA Sequencing Analysis and Computer Analysis—DNA sequencing was carried out by the dyeoxy chain termination method (46) using the BcaBEST dideoxy sequencing kit (Takara). The nucleotide sequence coded by the insert were translated in the LAMDA ZAP II cloning vector (Stratagene) in the sense orientation as described (23). Complementary DNA inserts of positive clones were excised and partially sequenced using an antisense oligonucleotide primer corresponding to amino acid sequence WPHTD (5’-AGTGCCTAGGTCGCGCA-3’), a highly conserved sequence in the PTP domain.

Production and Purification of Bacterially Expressed Protein—Amino acid sequences of residues 51–164, 165–285, and 168–427 of the predicted Typ protein were expressed in E. coli as glutathione S-transferase (GST) fusion proteins (GST-TypN1, GST-TypC1, and GST-TypPTP, respectively). For construction of plasmids, the PCR amplification and restriction enzyme digestion. The amplified fragments were subcloned into the plasmid vector (pBluescript II sk–) vector. The resulting plasmids were linearized with appropriate enzymes and transcribed in vitro with T7 RNA polymerase (Stratagene). The transcription products fragment 1 (nucleotide position 21–709 of typ), fragment 2 (nucleotide position 710–1071) were used as the antisense probes (antisense (AS)-probe 1, AS-probe 2, and AS-probe 3, respectively). Fragment 1 was also subcloned into plasmid vector to generate a sense RNA probe. The RNA probes were labeled using the digoxigenin RNA Labeling Kit (Boehringer Mannheim) following manufacturer’s protocol.

Testis samples of 8-week-old mice were prepared in OCT compound (Tissue-Tek, Miles) and frozen. The frozen sections (5–8 μm) were fixed for 20 min with 4% paraformaldehyde freshly prepared in 0.1 M sodium phosphate buffer, pH 7.4. They were then treated with 0.2% HCl and acetylated with 0.1 M triethanolamine containing 0.25% acetic anhydride. After washes in PBS, sections were dehydrated in a series of ethanol washes. The hybridization solution contained 50% formamide, 10 μM Tris, pH 7.6, 1 × Denhardt’s solution, 10% dextran sulfate, 600 mM NaCl, 0.25% SDS, 1 mM EDTA, pH 8.0, 200 μg/ml tRNA, and 5 μg/ml digoxigenin-labeled RNA probes. Hybridization solution (80 μl) was placed on each section and incubated at 50 °C in a moist chamber. After a 16 h incubation, sections were washed in a solution containing 50% formamide and 2 × SSC (20 × SSC = 3 M NaCl, 0.3 M sodium citrate) at 50 °C for 30 min, and washed again in 2 × SSC and in 0.2 × SSC. Sections were stained with 0.1% DAPI and 0.1% PPS4, 50 μM at 50 °C for 60 min. The hybridization signals were visualized with alkaline phosphatase-conjugated anti-digoxigenin antibody (Boehringer Mannheim) by adding the substrate for alkaline phosphatase TNBT (Sigma).

Immunofluorescence Microscopy—Testis sections were cut and fixed as described above and permeabilized by incubation at room temperature with 0.2% (v/v) Triton X-100 in PBS for 10 min. The permeabilized sections were treated with PBS containing 1% (v/v) bovine serum albumin (fraction V from Sigma) for 30 min at room temperature. Then the sections were incubated for 2.5 h with the anti-TypN1 and anti-TypC1 antibodies in PBS containing 0.02% (v/v) Triton X-100 at room temperature. As a control staining, anti-TypN1 antibody in PBS containing 0.02% (v/v) Triton X-100 were pre-incubated with GST-TypN1 (see Fig. 7B). After being washed three times with PBS for 15 min, the sections were incubated for 1 h with the second immune reagent, FITC-labeled goat anti-rabbit IgG. For localization of chromosomes, DNAs were stained with propidium iodide. Samples were observed using laser scanning microscopes (Zeiss and Bio-Rad).

RESULTS

Isolation of a Novel PTP cDNA Fragments—To identify novel PTPs, we employed the RT-PCR-based cloning procedure. Complementary DNAs were synthesized by RT of mRNAs prepared from MDA-MB453 cells and TIG1 cells. The RT reactions were primed with a degenerate primer (primer 1) designed from amino acid sequence QRE/A/E/D/Q, which was conserved among PTPs. The PCRs were performed using the cDNA and degenerate primers corresponding to other conserved regions in PTPs (primers 2 and 3 corresponding to DYINA and HCSA, respectively). The PCR products were subcloned into the human 293T kidney cells by the calcium phosphate method.

Western Blotting—293T cells were lysed in a lysis buffer containing 50 mM Tris-HCl (pH 8.0), 1% Nonidet P-40, 0.1% SDS, 20 μg EDTA, 150 mM NaCl, 50 mM NaF, 0.1 mM Na3Vo4, and aprotinine (50 units/ml) after 4 h of transfection. Cell lysates of the testis, ovary, and brain were prepared by using the same lysis buffer. Samples of 25 μg of proteins from the lystate of 293T cells and 100 μg of proteins from the lystate of mouse tissues were fractionated by 10% SDS-PAGE. The filters were subjected to immunoblot analysis as described (24).

In Vitro Translation—The sense strand of the insert of clone 5 was transcribed using T7 RNA polymerase (Stratagene). The proteins encoded by this insert were translated in vitro in the presence of [35S]Metionine by using the wheat germ cell extract (Promega).

Phosphatase Assay—The catalytic activity of GST-TypPTP was assayed using p-nitrophenyl phosphate (pNPP) as a substrate (24, 28). The glutathione beads capturing 0.25–3 μg of GST-TypPTP or GST were subjected to the reaction.

In Situ Hybridization—Three DNA fragments (fragments 1–3, see below) were prepared from the cDNA insert of clone 5 (see Fig. 2A) by PCR amplification and restriction enzyme digestion. The amplified fragments were subcloned into the plasmid vector (pBluescript II sk–) vector. The resulting plasmids were linearized with appropriate enzymes and transcribed in vitro with T7 RNA polymerase (Stratagene). The transcripts were hybridized to the filters representing 1×106 plasmids. The hybridization solution contained 50% formamide, 10 μM Tris, pH 7.6, 1 × Denhardt’s solution, 10% dextran sulfate, 600 mM NaCl, 0.25% SDS, 1 mM EDTA, pH 8.0, 200 μg/ml tRNA, and 5 μg/ml digoxigenin-labeled RNA probes. Hybridization solution (80 μl) was placed on each section and incubated at 50 °C in a moist chamber. After a 16 h incubation, sections were washed in a solution containing 50% formamide and 2 × SSC (20 × SSC = 3 M NaCl, 0.3 M sodium citrate) at 50 °C for 30 min, and washed again in 2 × SSC and in 0.2 × SSC. Sections were stained with 0.1% DAPI and 0.1% PPS4, 50 μM at 50 °C for 60 min. The hybridization signals were visualized with alkaline phosphatase-conjugated anti-digoxigenin antibody (Boehringer Mannheim) by adding the substrate for alkaline phosphatase TNBT (Sigma).

Immunofluorescence Microscopy—Testis sections were cut and fixed as described above and permeabilized by incubation at room temperature with 0.2% (v/v) Triton X-100 in PBS for 10 min. The permeabilized sections were treated with PBS containing 1% (v/v) bovine serum albumin (fraction V from Sigma) for 30 min at room temperature. Then the sections were incubated for 2.5 h with the anti-TypN1 and anti-TypC1 antibodies in PBS containing 0.02% (v/v) Triton X-100 at room temperature. As a control staining, anti-TypN1 antibody in PBS containing 0.02% (v/v) Triton X-100 were pre-incubated with GST-TypN1 (see Fig. 7B). After being washed three times with PBS for 15 min, the sections were incubated for 1 h with the second immune reagent, FITC-labeled goat anti-rabbit IgG. For localization of chromosomes, DNAs were stained with propidium iodide. Samples were observed using laser scanning microscopes (Zeiss and Bio-Rad).
Typ, a Novel Tyrosine Phosphatase Expressed in the Testis

By screening $1 \times 10^6$ independent clones, nine positive clones were obtained. Nucleotide sequencing revealed that the inserts of eight clones were overlapping, suggesting that they were derived from the same mRNA species (Fig. 2A). One remaining clone had a sequence for a putative mouse homologue of PTP-MEG (23). This clone might have been selected by cross-hybridization because the screening was carried out under the low stringent condition. A composite sequence of 3,090 nucleotides constructed from the inserts of four clones, clones 2, 5, 6, and 8, contained the largest open reading frame of 426 amino acid residues. The coding frame began with a consensus-initiating methionine codon at position 218 that was preceded by five in-frame stop codons (Fig. 2B). We tentatively concluded that the open reading frame encoded the mouse Typ protein. Nucleotide sequence analysis also revealed that the cDNA insert of clone 2 contained internal 441-base pair deletions in the coding region compared with the insert of the other clones. This resulted in the precise deletion of 147 amino acid residues (amino acids 168–314) in the predicted sequence. Therefore, the downstream coding frame was not affected. We then performed RT-PCR analysis of total RNAs extracted from mouse testis. Two pairs of oligonucleotide primers that flanked the deletion were utilized. Both sets of primers failed to amplify the fragment corresponding to the insert of clone 2 but was able to amplify the DNAs corresponding to the other cDNA clones. This suggested that the deletion was an artifact that occurred during the library construction. However, the possibility that the clone 2 cDNA was derived from an alternatively spliced mRNA species, which was expressed in very low abundance, cannot be excluded.

The nucleotide sequence of the mouse typ cDNA and the deduced amino acid sequence showed 74.8 and 84.1% identity, respectively, with the corresponding sequences of the human typ. The similarities between human and mouse were relatively low when compared with those of the other PTPs. For example, predicted amino acid sequences of mouse SHP2 (29) and the first PTP domain of mouse PTP5 (30) are 99.6 and 96.3% identical with the corresponding sequences of their human homologues, respectively (31–35). The human and mouse Mos proteins, protein kinases involved in meiosis (12, 36), are less homologous to each other (~78% identity) even in their catalytic domains (37, 38). We then examined the expression pattern of mouse typ and compared it with that of human typ. RNAs were extracted from adult mouse tissues and analyzed by Northern blot hybridization under stringent conditions. Using the $^{32}$P-labeled cDNA insert of clone 5 as a probe, typ mRNA of 3.2 kilobases, the same size as that detected in human testis, was detected only in testis among seven tissues examined (Fig. 1B). The same result was obtained when the blot was probed with the human typ PCR fragment under a relaxed condition (data not shown). These data substantiated that we have cloned cDNAs for the mouse typ gene.

Characterization of the Typ Protein—The deduced amino acid sequence of the mouse typ protein revealed the presence of the PTP domain at the carboxyl-terminal half. The Typ protein had neither a signal peptide nor a membrane-spanning segment, indicating that Typ is a cytoplasmic PTP. The putative PTP domain contained the consensus sequences such as the catalytic core sequence (V)H/CXGXXR(S/T)G. Comparison of amino acid sequences between Typ and other PTPs at the catalytic domain revealed that the identity was about 45% at most. A computer-assisted search of the GenBank™ data base revealed no significant homologies of the amino-terminal half of the Typ protein to the other known proteins. However, there was a region rich in proline, glutamate, serine, and threonine residues that was flanked by positively charged amino acids.

By screening $1 \times 10^6$ independent clones, nine positive clones were obtained. Nucleotide sequencing revealed that the inserts of eight clones were overlapping, suggesting that they were derived from the same mRNA species (Fig. 2A). One remaining clone had a sequence for a putative mouse homologue of PTP-MEG (23). This clone might have been selected by cross-hybridization because the screening was carried out under the low stringent condition. A composite sequence of 3,090 nucleotides constructed from the inserts of four clones, clones 2, 5, 6, and 8, contained the largest open reading frame of 426 amino acid residues. The coding frame began with a consensus-initiating methionine codon at position 218 that was preceded by five in-frame stop codons (Fig. 2B). We tentatively concluded that the open reading frame encoded the mouse Typ protein. Nucleotide sequence analysis also revealed that the cDNA insert of clone 2 contained internal 441-base pair deletions in the coding region compared with the insert of the other clones. This resulted in the precise deletion of 147 amino acid residues (amino acids 168–314) in the predicted sequence. Therefore, the downstream coding frame was not affected. We then performed RT-PCR analysis of total RNAs extracted from mouse testis. Two pairs of oligonucleotide primers that flanked the deletion were utilized. Both sets of primers failed to amplify the fragment corresponding to the insert of clone 2 but was able to amplify the DNAs corresponding to the other cDNA clones. This suggested that the deletion was an artifact that occurred during the library construction. However, the possibility that the clone 2 cDNA was derived from an alternatively spliced mRNA species, which was expressed in very low abundance, cannot be excluded.

The nucleotide sequence of the mouse typ cDNA and the deduced amino acid sequence showed 74.8 and 84.1% identity, respectively, with the corresponding sequences of the human typ. The similarities between human and mouse were relatively low when compared with those of the other PTPs. For example, predicted amino acid sequences of mouse SHP2 (29) and the first PTP domain of mouse PTP5 (30) are 99.6 and 96.3% identical with the corresponding sequences of their human homologues, respectively (31–35). The human and mouse Mos proteins, protein kinases involved in meiosis (12, 36), are less homologous to each other (~78% identity) even in their catalytic domains (37, 38). We then examined the expression pattern of mouse typ and compared it with that of human typ. RNAs were extracted from adult mouse tissues and analyzed by Northern blot hybridization under stringent conditions. Using the $^{32}$P-labeled cDNA insert of clone 5 as a probe, typ mRNA of 3.2 kilobases, the same size as that detected in human testis, was detected only in testis among seven tissues examined (Fig. 1B). The same result was obtained when the blot was probed with the human typ PCR fragment under a relaxed condition (data not shown). These data substantiated that we have cloned cDNAs for the mouse typ gene.

Characterization of the Typ Protein—The deduced amino acid sequence of the mouse typ protein revealed the presence of the PTP domain at the carboxyl-terminal half. The Typ protein had neither a signal peptide nor a membrane-spanning segment, indicating that Typ is a cytoplasmic PTP. The putative PTP domain contained the consensus sequences such as the catalytic core sequence (V)H/CXGXXR(S/T)G. Comparison of amino acid sequences between Typ and other PTPs at the catalytic domain revealed that the identity was about 45% at most. A computer-assisted search of the GenBank™ data base revealed no significant homologies of the amino-terminal half of the Typ protein to the other known proteins. However, there was a region rich in proline, glutamate, serine, and threonine residues that was flanked by positively charged amino acids.
known as PEST sequences (39) (positions 66–87 in the predicted protein sequence). In addition, two potential phosphorylation sites for the p34\(^{\text{cdc2}}\) kinase (S/TP\(^X\)K) (40) were present at amino acid positions 3 and 39 within the amino-terminal proximal region.

To detect the protein product of the \(\text{typ} \) gene, antibodies were raised against the two different portions of the predicted \(\text{Typ} \) protein: residues 51–164 and 165–285. The resulting antibod-
The ATG-218 codon was translated containing the entire open reading frame that initiates from be utilized for an initiation methionine. Then the 45-kDa protein. Alternatively, a downstream ATG codon might rise to only the 40-kDa form of Typ upon transfection into 293T cells (Fig. 3). The 45-kDa Typ protein was initiated from the ATG-218 and that the Typ protein was expressed in vitro in the presence of radioactive amino acid. As shown in Fig. 3A, the 45- and 40-kDa proteins were synthesized (lanes 1–3) and 293T cells (lanes 2–10) were fractionated by 10% SDS-PAGE and analyzed by immunoblotting with anti-TypN1 antibodies. Taken together, it was suggested that in addition to the first methionine encoded by the ATG-218 codon, an internal translation initiation site was used. These results indicated that the 45-kDa Typ protein was expressed in vitro and in vivo as a translation initiation site. Developmentally Regulated typ Gene Expression in Testicular Germ Cells—To understand the role of Typ, we analyzed its expression during spermatogenesis. We first examined the developmental expression of the Typ products in mouse testis by Western blotting. As shown in Fig. 5, the 45-kDa Typ protein was detected in the lysate of a 3-week-old mouse testis, whereas the 40-kDa Typ product was not detected until 4 weeks after birth (Fig. 3A). This indicates that the Typ protein becomes detectable between 2 and 3 weeks after birth, in parallel with the appearance of the pachytene spermatocyte (41). Then we examined typ mRNA expression by in situ hybridization.
Typ, a Novel Tyrosine Phosphatase Expressed in the Testis

Typ, a Novel Tyrosine Phosphatase Expressed in the Testis

Cryostat sections of the 8-week-old mouse testis were hybridized with digoxigenin-labeled typ antisense RNA probe, which was complementary to nucleotides 21–709 (AS-probe 1). Expression of the typ mRNA was confined to rings of cells adjacent to the spermatogonial layer of the tubule circumference (Fig. 6A). Both the expression pattern and the morphology of typ-expressing cells strongly suggested that typ was expressed in primary spermatocytes. Under lower magnification, typ mRNA was not detectable in all seminiferous tubules (Fig. 6C). The process of spermatogenesis in mature testis proceeds asynchronously and can be divided into 12 steps according to the composition and distribution of spermatocytes, spermatids, and sperm (42). The failure to detect the typ mRNA in some tubules indicated that expression of the typ gene is restricted to particular stages of the spermatogenetic cycle. The other two antisense probes (AS-probe 2 and 3, see “Experimental Procedures”), gave essentially identical results (data not shown), and the sense control probe did not give any specific signals (Fig. 5).

The distribution of Typ proteins during spermatogenesis was also determined immunocytochemically by employing an indirect immunofluorescence technique. Consistent with the result of the in situ hybridization analysis, strong signals were detected in the primary spermatocytes by both anti-TypN1 and anti-TypC1 antibodies. No significant signals were detected in spermatogonia, early round spermatids, sperm, or in testicular somatic cells such as Sertoli cells (Fig. 7, panels D, E, G, and H). Signals were blocked by preincubation with the immunizing antigen (Fig. 7B). Together these results strongly suggest that expression of the typ gene is temporarily regulated during spermatogenesis and is restricted to germ cells at a particular meiotic stage, possibly at the pachytene stage.

DISCUSSION

In this study to identify novel PTPs, a modified RT-PCR-based method was employed for identifying mRNAs expressed in low abundance within the cell. To eliminate the previously known and/or abundantly amplified sequences from the PCR products, we first generated a plasmid library of amplified PCR products. Then the library was subjected to colony hybridization with probes of a mixture of the 32P-labeled PCR amplified products. Using the negative selection screening method, we cloned a DNA fragment encoding a novel PTP named Typ. Northern blot analysis revealed that the typ gene is expressed exclusively in the testis of both human and mouse. The transcript of the typ gene was not detected in any human cell lines examined, including MDA-MB453, from which the template cDNA for PCR amplification was prepared. Thus, the negative selection screening method allowed identification of the typ mRNA present in low abundance in MDA-MB453 cells.

The predicted Typ protein does not contain a signal peptide or a membrane-spanning region but has a single catalytic domain located in the carboxyl terminus-proximal half. The PTP domain of Typ shows an overall homology with that of other PTPs, although the shared identity is about 45% at most. This indicates that Typ is a new member of the cytoplasmic PTPs. The amino terminus-proximal nonenzymatic region of Typ does not share significant homologies with the other known proteins. The noncatalytic domain contains PEST-like sequences, characteristic of proteins that display very short half-lives (39). Therefore, Typ might be an unstable protein.

Anti-Typ antibodies recognized 45- and 40-kDa proteins in testicular lysates. In vitro translation of the typ open reading frame resulted in the production of the same sizes of proteins. The results demonstrate that the two proteins represent the typ gene products and arise from a single unprocessed typ mRNA species. As shown in Fig. 3C, even when the 45-kDa protein was not synthesized due to the mutation in the ATG-218 codon, the 40-kDa Typ protein was detected. Therefore, that 40-kDa Typ protein was not produced by the proteolytic cleavage of the 45-kDa Typ protein. In addition, mutations in the other three internal ATG codons and two CTG codons, which might be putative translation initiator, did not affect the production of the two proteins. These results suggest that the 40-kDa form of Typ is produced by utilizing non-ATG nor CTG codon as a translation initiator. Since pME-Flag-4M directs the synthesis of a protein slightly smaller than 40 kDa, the translation initiation site for this protein is confined within a se
cells cease to proliferate and enter meiosis about 13.0 days postcoitum. All oocytes are in the diplotene stage of the prophase of the first meiotic division by 5 days after birth. Thus, oocytes in adult ovary are in a phase later than the equivalent stage of the testicular germ cells in which typ is expressed. Further analysis of typ expression in the ovaries of female mouse embryo will demonstrate whether the expression of the typ gene is restricted to testicular germ cells or commonly found in both male and female germ cells.

In conclusion, we have reported the isolation and characterization of a novel cytoplasmic PTP Typ. Typ expresses exclusively in the testis. The highest level of the typ mRNA and Typ proteins were detected in the primary spermatocyte of the adult mouse testis. Accumulating evidence suggests that protein tyrosine phosphorylation is a key reaction in various biological systems. Protein-tyrosine kinases such as c-Kit (9) and Fer-T (45) are implicated in spermatogenesis. However, the molecular mechanisms of spermatogenesis that involve protein tyrosine phosphorylation remain to be clarified. Less understood is the relevance of tyrosine phosphatases in spermatogenesis, though receptor-type PTPs termed OST-PTP (osteotesticular protein-tyrosine phosphatase) is reported to be expressed within the seminiferous tubule as well as in bone (15). Further investigation of the function of Typ will provide an important insight into the signal transduction pathway that regulates spermatogenesis or the maturation of mammalian germ cells.

Acknowledgments—We thank M. Shiota and Y. Morishita for advice on the in situ hybridization study and Human Genome Center (Institute of Medical Science, University of Tokyo) for the sequence analysis package of the Genetics Computer Group (GCC).

REFERENCES

1. Hunter, T. (1995) Cell 80, 225–236
2. Fischer, E. H., Charbonneau, H., and Tonks, N. K. (1991) Science 253, 401–405
3. Walton, K. M., and Dixon, J. E. (1993) Annu. Rev. Biochem. 62, 101–120
4. Stone, R. L., and Dixon, J. E. (1994) J. Biol. Chem. 269, 3123–3132
5. Guan, K. L., and Dixon, J. E. (1991) J. Biol. Chem. 266, 17026–17030
6. Iwaki, Y., Matsuda, H., Mutter, G. L., Watrin, F., and Wolgemuth, D. J. (1993) Exp. Cell Res. 206, 212–219
7. Manova, K., Nocka, K., Besmer, P., and Bachvarova, R. F. (1990) Development 110, 1057–1069
8. Sorrentino, V., Giorgi, M., Geremia, R., Besmer, P., and Rossi, P. (1991) Oncogene 6, 149–151
9. Yoshinaga, K., Nishikawa, S., Ogawa, M., Hayashi, S., Kuniataka, T., Fujimoto, T., and Nishikawa, S. (1991) Development 113, 689–699
10. Keshet, E., Itin, A., Fischman, K., and Nir, U. (1990) Mol. Cell. Biol. 10, 5021–5025
11. Matsushime, H., Jinno, A., Takagi, N., and Shibuya, M. (1990) Mol. Cell. Biol. 10, 2291–2298
12. Mutter, G. L., and Wolgemuth, D. J. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 5301–5305
13. Toshima, J., Ohashi, K., Okano, I., Nonoue, K., Kishioka, M., Kuma, K., Miyata, T., Hirai, M., Baba, T., and Mizuno, K. (1995) J. Biol. Chem. 270, 31331–31337
14. Letwin, K., Mizzen, L., Motto, B., Ben, D. Y., Bernstein, A., and Pawson, T. (1992) EMBO J. 11, 3521–3531
15. Mauro, L. J., Olmsted, E. A., Skrobacz, B. M., Mourey, R. J., Davis, A. R., and Dixon, J. E. (1994) J. Biol. Chem. 269, 30659–30667
16. Muramatsu, T., Giri, P. R., Higuchi, S., and Kincaid, R. L. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 529–533
17. Alphay, L., Jimenez, J., White, C. H., Dawson, I., Nurse, P., and Glover, D. M. (1992) Cell 69, 973–984
18. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
19. Smith, D. B., and Johnson, K. S. (1988) Gene 70, 31–40
20. Guan, K. L., Broyles, S. S., and Dixon, J. E. (1991) Nature 350, 359–362
21. Kuramochi, S., Matsuda, S., Masuda, Y., Satoh, T., Ohashi, M., and Yamamoto, T. (1996) FEBS Lett. 378, 7–14
22. Adachi, M., Sekiya, M., Arimura, Y., Takekawa, M., Itoh, F., Hinoda, Y., Imai, K., and Yachi, T. (1993) Cancer Res. 52, 737–740
23. Semb, K., Kamata, T., Toyoshima, K., and Yamamoto, T. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 6497–6501
24. Ostman, A., Yang, Q., and Tonks, N. K. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 9680–9684
25. Streuli, M., Krueger, N. X., Hall, L. R., Schlossman, S. F., and Saito, H. (1988) J. Exp. Med. 168, 1523–1530
26. Takebe, Y., Seki, M., Fujisawa, J., Hoya, Y., Yokota, K., Arai, K., Yoshida, M., and Arai, N. (1988) Proc. Natl. Acad. Sci. U. S. A. 90, 5404–5408
27. Deng, W. P., and Nickoloff, J. A. (1992) Anal. Biochem. 200, 81–88
28. Gu, M., Warshawsky, I., and Majerus, P. W. (1992) Proc. Natl. Acad. Sci. U. S. A.
Typ, a Novel Tyrosine Phosphatase Expressed in the Testis

Feng, G. S., Hui, C. C., and Pawson, T. (1993) Science 259, 1607–1611

Mizuno, K., Hasegawa, K., Katagiri, T., Ogimoto, M., Ichikawa, T., and Yakura, H. (1993) Mol. Cell. Biol. 13, 5513–5523

Freeman, R. J., Plutzky, J., and Neel, B. G. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11239–11243

Adachi, M., Sekiya, M., Miyachi, T., Matsuno, K., Hinoda, Y., Imai, K., and Yachi, A. (1992) FEBS Lett. 314, 335–339

Ahmad, S., Banville, D., Zhao, Z., Fischer, E. H., and Shen, S. H. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2197–2201

Vogel, W., Lammers, R., Huang, J., and Ullrich, A. (1993) Science 259, 1611–1614

Krueger, N. X., Streuli, M., and Saito, H. (1990) EMBO J. 9, 3241–3252

Mutter, G. L., Grills, G. S., and Wolgemuth, D. J. (1988) EMBO J. 7, 683–689

Sagata, N., Oskarsson, M., Copeland, T., Brumbaugh, J., and Vande, W. G. (1998) Nature 335, 519–525

Watson, R., Oskarsson, M., and Vande, W. G. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 4078–4082

Rogers, S., Wells, R., and Rechsteiner, M. (1986) Science 234, 364–368

Kennelly, P. J., and Krebs, E. G. (1991) J. Biol. Chem. 266, 15555–15558

Bellve, A. R., Caviezel, J. C., Millette, C. F., O’Brien, D. A., Bhatnagar, Y. M., and Dym, M. (1977) J. Cell Biol. 74, 68–85

Oakberg, E. F. (1956) Am. J. Anat. 99, 391–413

Peabody D. S. (1989) J. Biol. Chem. 264, 5031–5035

Noce, T., Fujiwara, Y., Sezaki, M., Fujimoto, H., and Higashinakagawa, T. (1992).Dev. Biol. 153, 356–367

Fischman, K., Edman, J. C., Shackleford, G. M., Turner, J. A., Rutter, W. J., and Nir, U. (1990) Mol. Cell. Biol. 10, 146–153

Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467