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A Cytochemical Study of the Transcriptional and Translational Regulation of Nuclear Transition Protein 1 (TP1), a Major Chromosomal Protein of Mammalian Spermatids

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Abstract. Immunocytochemical localization and in situ hybridization techniques were used to investigate the presence of spermatid nuclear transition protein 1 (TP1) and its mRNA during the various stages of spermatogenesis in the rat. A specific antiserum to TP1 was raised in a rabbit and used to show that TP1 is immunologically crossreactive among many mammals including humans. During spermatogenesis the protein appears in spermatids as they progress from step 12 to step 13, a period in which nuclear condensation is underway. The protein is lost during step 15. An asymmetric RNA probe generated from a TP1 cDNA clone identified TP1 mRNA in late round spermatids beginning in step 7. The message could no longer be detected in spermatids of step 15 or beyond. Thus, TP1 mRNA first appears well after meiosis in haploid cells but is not translated effectively for the several days required for these cells to progress to the stage of chromatin condensation. Message and then protein disappear as the spermatids enter step 15. In agreement with a companion biochemical study (Heidaran, M. A., and W. S. Kistler. *J. Biol. Chem.* 1987. 262:13309–13315), these results establish that translational control is involved in synthesis of this major spermatid nuclear protein. In addition, they suggest that TP1 plays a role in the completion but not the initiation of chromatin condensation in elongated spermatids.

DURING spermatogenesis, spermatogonia proliferate mitotically to give rise to primary spermatocytes, which undergo meiosis to yield haploid spermatids, which in turn gradually transform into spermatozoa. About midway through spermatid development in mammals and many other organisms, the nucleus undergoes a rather sudden change in shape, and the chromatin condenses. In mammals this change in the chromatin is accompanied by a transition from histones to a class of novel nuclear "transition proteins" (1, 7, 23, 28, 29, 34), which are later replaced by the characteristic arginine and cysteine-rich mammalian protamines (2, 3, 23, 35). Once the chromatin begins to condense, its nucleosomal structure is lost (22, 30), and available evidence indicates that transcription ceases (10, 22). If this is so, then it follows that any messenger RNAs for proteins that will appear at later stages of spermatogenesis must be laid down before the point of chromatin condensation.

It is well established that the mRNAs for the protamines are transcribed early in spermatogenesis and then regulated at the posttranscriptional level until needed. In trout, protamine messages are synthesized before the end of meiosis and are found in a translationally inert ribonucleoprotein particle throughout the early stages of spermatid development (20, 43). In the mouse, a similar situation occurs though in this case the message first appears in round spermatids rather than in spermatocytes (26). Such translational regulation has not yet been established for other types of mRNAs during spermatogenesis.

We were interested to see if translational control also applies to spermatid nuclear transition protein 1 (TP1),[1] one of the first nuclear transition proteins to appear (23, 37). In fact, a biochemical approach to this problem has already shown that TP1 message is detectable during development of young rats several days before synthesis of TP1 can be demonstrated (18). In adult animals, in which TP1 synthesis was occurring, a substantial fraction of its message was associated with polysomes and had variable polyadenylation, as is typical of translationally active mRNAs (11, 41). In contrast, the TP1 mRNA in young animals was found exclusively in a nonpolysomal location and had uniform polyadenylation. Thus, some factor(s) both protects the stored message from nuclease attack and prevents its association with the translational apparatus. While this study provided strong evidence that TP1 message is under translational control, it did not show unequivocally in which testicular cells TP1 message is first made or in which cells TP1 protein first appears. Based on the isolation of specific antisera to TP1, we have now used the techniques of immunocytochemistry and in situ

1. *Abbreviation used in this paper*: TP1, spermatid nuclear transition protein 1.

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hybridization to probe individual spermatogenic cells for each of these molecules.

Materials and Methods

Antibody Preparation

TP1 was purified as described previously (25) incorporating preparative electrophoresis as the final step (24). A female New Zealand White rabbit was immunized by injecting a total of 0.5 mg of TP1 in Freund's complete adjuvant at multiple intradermal sites and by repeating this procedure 10 w later using incomplete adjuvant. Sera were titered by using TP1 labeled with ^{125}I as described by Shima et al. (42) and precipitation of antigen antibody complexes with 44% saturated ammonium sulfate (12).

Western Blotting

Protein was extracted from fresh or frozen tissues by direct homogenization in cold 4% (wt/vol) trichloroacetic acid, centrifugation (10,000 × *g* for 10 min) to remove insoluble material, and precipitation of soluble proteins with 20% trichloracetic acid (23). Duplicate samples of material recovered from ~200 mg of tissue were separated electrophoretically in a 15% polyacrylamide gel containing 0.9 M acetic acid and 2.5 M urea (36). Proteins were then transferred electrophoretically to a sheet of nitrocellulose (Schleicher & Schuell, Keene, NH) (45) in a Bio-Rad (Richmond, CA) Trans-Blot cell using 0.7% (vol/vol) acetic acid as electrolyte and a potential of 80 V for 4 h with constant cooling. The nitrocellulose sheet was cut in half. One set of lanes was stained with naphthol blue black, and the other was treated for immunological detection with a Bio-Rad Immuno-Blot Assay Kit. Briefly, the sheet was incubated with 3% (wt/vol) gelatin, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.5, for 1 h and then incubated for 12 h at room temperature in the same solution containing 1% gelatin and a 1:10 dilution of antisera to TP1. The sheet was then washed twice for 20 min in the incubation buffer lacking gelatin but containing 0.05% (vol/vol) Tween-20, and then incubated for 1 h in buffer containing 1% gelatin and a 1:3,000 dilution of goat anti-rabbit IgG conjugated with horseradish peroxidase. The sheet was washed as before followed by one wash without detergent. It was then developed in buffer containing 0.015% (vol/vol) H_2O_2, 0.05% (wt/vol) 4-chloro-1-naphthol and 16% (vol/vol) methanol for 10 min.

Immunocytochemistry

Immunohistological localization was adapted from Rajaniemi et al. (39). Portions of adult testis were fixed in Carnoy's solution (ethanol:acetic acid:chloroform 6:3:1) for 16 h at 4°C, embedded in paraffin, and cut to give 7-μm sections. After removal of paraffin and step-wise hydration, slides were treated with 3 mM dithiotreitol (DTT) in 50 mM Tris-HCl, pH 8.5, for 1 h at room temp. They were then washed in 0.15 M NaCl, 10 mM sodium phosphate, pH 7.5 (PBS), and treated with 0.2% BSA in PBS for 15 min. Next, slides were incubated with a 1:10 dilution of TP1 antiserum in PBS containing 0.3% (vol/vol) Triton X-100 for 1 h at 37°C. They were then washed four times for 5 min in PBS containing Triton and incubated 1 h at 37°C with the same solution containing a 1:1,000 dilution of goat anti-rabbit IgG conjugated with horseradish peroxidase. Sections were washed four times as before and twice in PBS without detergent and then developed for 5 min at room temp with PBS containing the same reagent concentrations used for Western blots. Sections were washed briefly in distilled water, and coverslips were mounted with glycerol. Some sections were stained for 5 min in 0.1% Mayer's hematoxylin and developed in 0.1% (vol/vol) ammonium hydroxide for 5 min. If coverslips were mounted in standard resin (Permount, Fisher Scientific, Pittsburgh, PA), the color from the immunological reagent disappeared immediately.

In Situ Hybridization

A single stranded RNA probe for TP1 was prepared using a 90 bp PstI to Sau3AI fragment from the 3' end of a cloned TP1 cDNA (pMHI) (17). This fragment was inserted in the multicloning site of plasmid pSP64 (Promega Biotec, Madison, WI) next to the promoter for phage SP6 RNA polymerase. Plasmid DNA was purified as described through a single CsCl density gradient (21) and cleaved with Bam HI at the far end of the cDNA insert. The reaction mixture contained 40 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 2 mM spermidine, 10 mM dithiothreitol, 0.5 mM each ATP, CTP, and GTP, 100 μCi [^{3}H]UTP (35 Ci/mmol; ICN Radiochemicals, Irvine, CA) 30 U of RNasin (Pharmacia Fine Chemicals, Piscataway, NJ) and 10 U SP6 RNA polymerase (Boehringer Mannheim, Indianapolis, IN) in a final volume of 25 μl. After incubation at 42°C for 1 h, the nucleic acids were precipitated twice from 2 M ammonium acetate with 2 vol of ethanol and once from 0.2 M sodium acetate, washed with absolute ethanol, and dissolved in sterile water. The specific activity of the product was estimated as 10^7 CPM/μg.

Hybridization to histological sections was carried out essentially according to Cox et al. (6). Samples of adult testis were fixed in 2.5% glutaraldehyde, 0.2 M sodium phosphate, pH 7.5, 0.15 M NaCl for 16 h at 4°C, embedded in paraffin, sectioned at 7 μm, mounted on glass slides, and hydrated. Sections were digested with 1 μg/ml proteinase K (E. Merck) in 0.1 M Tris-HCl, pH 8.0, 50 mM EDTA for 45 min at 37°C, treated with 0.25% (vol/vol) acetic anhydride (16), and dehydrated to absolute ethanol. The probe (5–10 × 10^4 CPM/section) was heated at 80°C for 3 min in 50% (vol/vol) formamide, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.02% (wt/vol) each of BSA, Ficoll, and Polyvinylpyrollidone, 500 μg/ml yeast tRNA (Sigma Chemical Co., St. Louis, MO), 10% (wt/vol) Dextran Sulfate, and 500 μg/ml poly(A) RNA (Pharmacia Fine Chemicals). The mixture was chilled and applied to each section (5 μl/cm^2) and then covered with a siliconized cover slip. Slides were immersed in mineral oil and incubated for 16 h at 45°C. Slides were washed twice in chloroform to remove oil and then in 0.6 M NaCl, 0.06 M trisodium citrate as the coverslips were carefully removed. They were then treated in 0.5 M NaCl, 10 mM Tris-HCl, pH 8, 1 mM EDTA, containing 20 μg/ml RNase A at 37°C for 20 min, and given two washes in 0.3 M NaCl, 0.03 M trisodium citrate for 20 min at room temperature and a final wash in 0.1 M NaCl, 0.01 M trisodium citrate at 50°C for 30 min.

After dehydration in ethanol solutions containing 300 mM ammonium acetate, slides were dipped in Kodak (Rochester, NY) NTB-2 nuclear liquid emulsion diluted 1:1 with 600 mM ammonium acetate. Slides were air dried for 30 min, incubated in a moist chamber for 2 h at room temperature, and exposed at 4°C for varying periods. Slides were developed in Kodak D-19 developer, fixed 2.5 min in Kodak fixer, and washed for 15 min in distilled water. Sections were stained briefly with hematoxylin and eosin, and coverslips were mounted with Permount. Photomicrographs were made on Kodak Panatomic X film using a Zeiss Photomicroscope III.

Results

Antisera to TP1

To investigate the timing of appearance of TP1 mRNA and TP1 protein, we used specific antiserum for identification of spermatids that contain the protein and a cDNA-derived RNA probe to identify cells that contain TP1 mRNA. An antiserum of suitable specificity was raised in rabbits. As a check of its specificity and also as a demonstration of the conservation of immunological determinants on TP1 found in various species, Western blots of a series of extracts of 4% trichloroacetic acid–soluble testicular proteins were used. After electrophoretic separation in an acetic acid/2.5 M urea gel and transfer to nitrocellulose, a strong reaction was observed for the TP1 band from rat, bull, rabbit, human, boar, and hamster (Fig. 1, lanes *b–g*). No signal was obtained from a control extract of rat liver (Fig. 1, lane *a*).

Immunohistological Identification of TP1

Using a peroxidase-conjugated second antibody procedure for detection of anti-TP1 immune complexes, we tested a variety of fixation and preparative procedures. Carnoy's fixative worked well, but fixation in formaldehyde or glutaraldehyde gave no immunologically positive results. While some reaction was observed with sections stained without thiol treatment, reduction with DTT intensified the signal substantially as was reported for immunocytochemical detection of mouse protamine (40). Since the peroxidase substrate yields a dark blue product, and since the staining is nuclear, counterstaining with hematoxylin had to be done lightly to see the immunological reaction product. With relatively condensed

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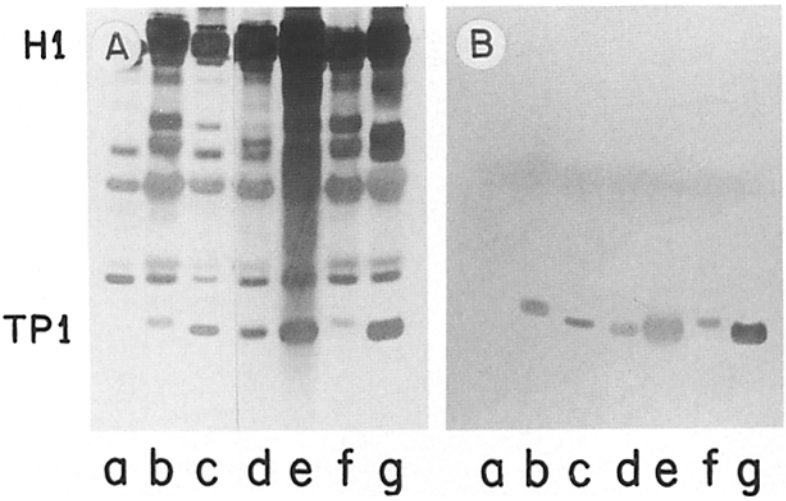

Figure 1. Reaction between TP1 and its antiserum by Western blotting. Samples of proteins extracted from tissues by 4% trichloroacetic acid were separated in an acetic acid/urea gel, transferred to nitrocellulose, and reacted with anti-TP1 serum. (*A*) A set of lanes transferred to nitrocellulose and stained by naphthol blue black. (*B*) A duplicate set of lanes transferred to nitrocellulose and reacted with anti-TP1 serum followed by a chromogenic detection protocol based on a peroxidase-conjugated second antibody. In most cases the protein sample corresponded to the material extracted from 200 mg of tissue. (Lane *a*) Control extract from rat liver; (lane *b*) bull testis; (lane *c*) rat testis; (lane *d*) rabbit testis; (lane *e*) human testis; (lane *f*) boar testis; (lane *g*) hamster testis. The broad TP1 band seen with human (lane *f*) and hamster (lane *g*) results from loading a greater quantity of protein in these lanes. The locations of H1 histones as well as TP1 are indicated. In control blots, no reaction was observed with the TP1 band in the absence of the first antiserum or when the anti-TP1 serum was replaced with a heterologous serum raised to the testis-specific H1 histone variant H1t.

spermatids, it was sometimes difficult to see an immunological reaction following hematoxylin counterstaining. Therefore, serial sections of the paraffin block were used with alternate sections stained for immunological identification and for nuclear morphology with hematoxylin.

In the rat, haploid cell (spermatid) development is divided into 19 steps based primarily on acrosome and nuclear morphology (4, 38). Since a new generation of spermatids results from the completion of meiosis every time an existing spermatid cohort completes step 14, only the first 14 steps are necessary to define the 14 stages of the seminiferous epithelium. In each of these stages a characteristic developmental relationship is observed among the several generations of germinal cells present in a tubular cross section. While some of the steps of spermatid development can be identified only based on acrosome morphology, many can be recognized confidently based on the pattern of cells present in the tubule as a whole, that is, by the stage of the tubule. For the problem at hand, it is particularly useful to note that the point at which the spermatid nucleus begins to change shape (step 8) coincides with the release of the most advanced generation of spermatids from the tubule lumen. The period in which the elongating cells progress through steps 9, 10, and 11 is the only one in which no cells are present with the highly elongated and condensed nuclei of advanced spermatids. In step 12, the elongated nucleus begins to condense, a process that is nearly complete by the end of step 13. As the spermatids pass through step 14, the spermatocytes developing behind them in the same tubule divide twice to give rise to a new crop of round spermatids. Thus, beginning at step 15 the condensed spermatids are followed by step 1 spermatids rather than by spermatocytes.

With these reference points in mind, it was possible to fix the point of appearance and disappearance of TP1 immunoreactivity with considerable confidence. The results were a dramatic confirmation of earlier assignments of TP1 to the nuclei of condensing spermatids by other means (15, 23, 37). TP1 was not detectable in any round spermatid, for example in the tubule at Stage VII shown in Fig. 2, *a* and *b*. Further, it was not present in early elongated spermatids such as those in the Stage XII tubule of Fig. 2, *c* and *d*. Immunological reactivity appeared suddenly in tubules containing late condensing (step 13) spermatids (Fig. 2, *e* and *f*). In these and other tubules, strong immunological reactivity was seen only when nuclei had been cut in cross section. Immunoreactivity was retained through step 14 (Fig. 2, *g* and *h*) and into step 15 (Fig. 2, *i* and *j*), but was then lost rapidly and was not seen in step 16–17 cells (Fig. 2, *k* and *l*). While loss of immunoreactivity could reflect epitope masking, disappearance of TP1 at this point agrees well with results of a variety of previous studies (14, 32, 33, 37). In summary, TP1 immunoreactivity appeared during the transition of spermatids from step 12 to 13 and was retained until some point in step 15. In one testis cross section, 66:295 (22%) of tubules were immunologically reactive, which is in good agreement with the expected distribution based on the durations of the various spermatogenic stages (4).

In Situ Hybridization to TP1 mRNA

Localization of the spermatids containing TP1 mRNA was done by the technique of in situ hybridization as used by Cox et al. (6). In adult testis sections, about half the tubules were unlabeled by the tritiated RNA probe (Fig. 3). Of those that did react with the probe, the pattern varied from a light dusting of silver grains scattered about the outer half of the tubule to examples in which a high concentration of silver grains clustered toward the tubular lumen. Closer examination of these tubules indicated that this labeling pattern reflected the progression of round spermatids through the elongating and condensing phases. Fig. 4 highlights examples of these: in 4 *A*, a tubule with step 7 round spermatids; in 4 *B*, a tubule

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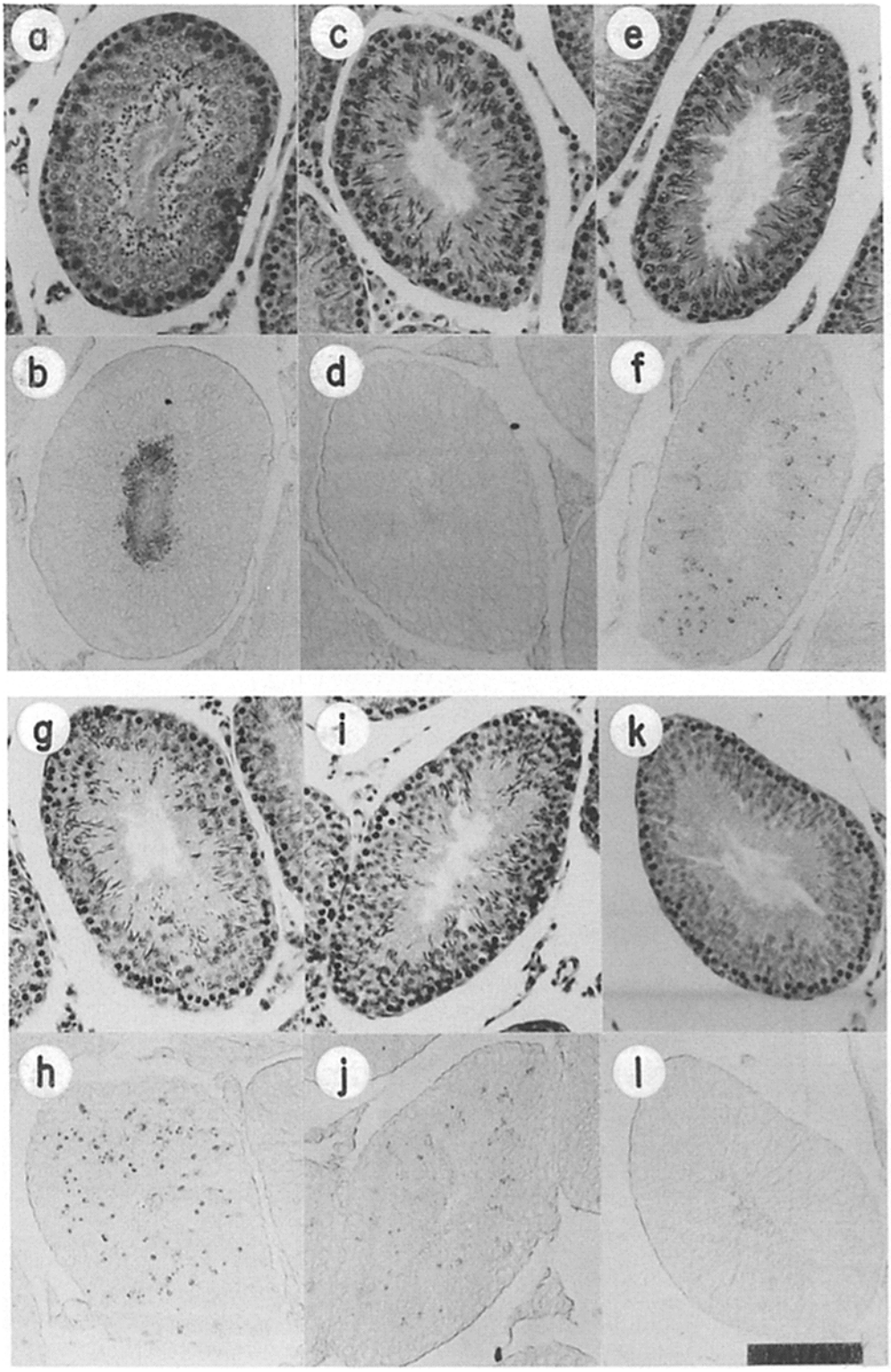

Figure 2. Immunocytochemical localization of TP1 in rat testis. Successive sections were stained immunologically for TP1 with the same general procedure used for Western blots, (*lower panels*) or with hematoxylin (*upper panels*). (*a* and *b*) Stage VII tubule with many step 7 spermatids sandwiched between step 18 spermatids near the lumen and spermatocytes near the periphery (note that the clusters of spermatid tails in the center of stage VII tubules appear dark due to optical effects and not as a result of immunological staining); (*c* and *d*) Tubule of about stage XII with early (step 12) condensing spermatids; (*e* and *f*) tubule of stage XIII, with step 13 condensing spermatids; (*g* and *h*) stage XIV tubule with step 14 spermatids (note meiotic figures in upper left quadrant); (*i* and *j*) stage I tubule with step 15 spermatids; (*k* and *l*) stage II–IV tubule with step 16–17 spermatids. In control reactions, no positive staining was seen when the first antiserum was omitted or when a heterologous antiserum raised against histone H1t was used (not shown). Bar, 100 μm.

with early condensing spermatids of late step 8; in 4 *C*, a tubule with nearly condensed spermatids of about step 13; in 4 *D*, a tubule with round spermatids earlier than step 7 and elongated spermatids later than step 14 (e.g., a tubule of stage I to V). These later tubules were uniformly negative. Thus, TP1 message was definitely detectable in step 7 round spermatids, several steps younger than the step 13 cells that first displayed the protein. As these cells develop and move toward the lumen of the tubule, TP1 mRNA accumulates, but then disappears as the spermatids reach step 15 and begin to associate in clusters around the tubule periphery. In one testis cross section, 160:320 (50%) of tubules were labeled above background by the hybridization probe. This accords well with the expected distribution of tubules having spermatids from step 7 through 14 (4).

A summary of the cellular location of TP1 and its mRNA

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is diagrammed in Fig. 5, which focuses on just the haploid portion of spermatogenesis. Based on the duration of the various steps of spermatid development (4), we can estimate that TP1 mRNA is present for ~5 d before it is translated to a significant extent. The protein is then retained in the nucleus for only ~2 d.

Discussion

The results presented here confirm and extend our earlier conclusion (18), based on biochemical techniques, that TP1 mRNA accumulates only in haploid cells and that it is subject to translational regulation. In that work we could not identify directly the cells in which either mRNA or protein appeared. Here we have exploited histological techniques and found that TP1 mRNA was first detectable in late round spermatids around the beginning of step 7 of development. Immunologically reactive protein was first seen in spermatids that had elongated but which still had incompletely condensed chromatin (late step 12 or early step 13). Both protein and its mRNA were retained during step 14 but were lost rapidly thereafter, though protein was still detected in some step 15 cells.

Certain possible reservations to our conclusions are worth considering. For example, one can imagine certain factors that might mask the presence of a specific RNA in a given cell. One would be the occurrence of an antisense RNA (13) covering the region of homology to the probe. Perhaps other such mechanisms exist as well. We see no reason to suspect such masking of the message in this case as the appearance of TP1 mRNA in step 7 spermatids is exactly what was predicted from our developmental analysis, when RNA was extracted and assayed for hybridization to a TP1 probe via Northern blotting (18). As with our previous study (18), the technique of in situ hybridization does not address the question of whether transcription is occurring but only whether transcription has led to the accumulation of stable products. Thus it remains a formal possibility that transcription occurs in cells prior to step 7 spermatids but does not lead to accumulation of mRNA. Concerns could also be raised with the possible masking of the antigenicity of TP1. The only suspected modification of TP1 is phosphorylation (our unpublished observations), but its extent must be slight since the protein has never been observed to give multiple bands on acetic acid/urea gels under conditions in which rat protamine is readily resolved into several phosphorylated components (31; Kistler, W. S., unpublished observations). Furthermore, newly synthesized TP1 has the same electrophoretic mobility as the mature protein (9, 18), which further argues against extensive side chain modifications. Recent analysis of the TP1 gene (Heidaran, M., and W. S. Kistler, manuscript submitted for publication) shows that the message can not encode a larger precursor form of the protein. A more difficult issue to address is whether remaining histones might mask TP1 immunoreactivity until they were removed or modified. In the absence of any data relative to this last point, we will assume that the cytochemical data are a faithful reflection of the occurrence of both TP1 and its mRNA.

TP1 was first suggested to be in elongating spermatids based on its appearance during sexual development (24). Later it was localized more accurately by analysis of the nuclear proteins of separated classes of spermatids (14, 37), and

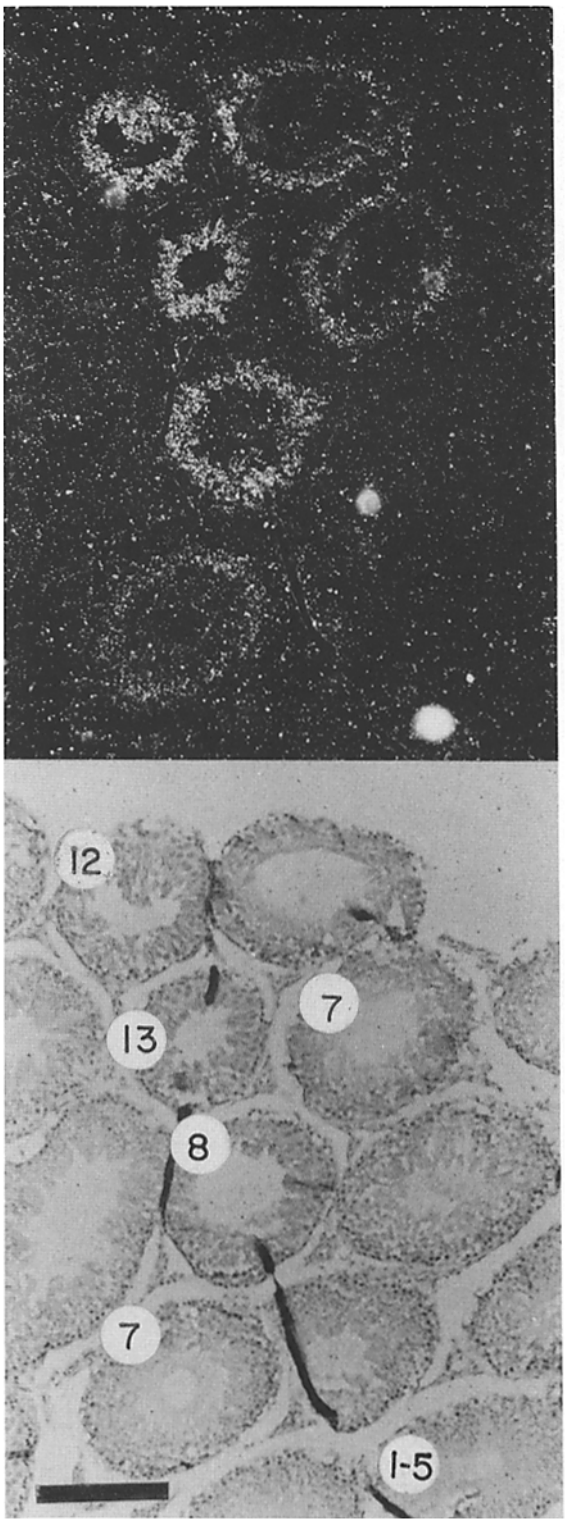

Figure 3. Cytochemical localization of TP1 mRNA by in situ hybridization. The same section is viewed at low magnification by pseudo dark-field optics (*top*) or by bright field illumination (*bottom*). The stage of selected tubules is indicated (which corresponds to the developmental step of the least mature spermatid generation). Exposure time was 7 d. Higher power views of portions of tubules designated 7, 8, 13, and 1–5 are shown in Fig. 4. The specificity of the hybridization was shown by the failure of a heterologous probe (to seminal vesicle secretory protein IV) to react with any testis tubules and by the corresponding failure of the TP1 probe to react with seminal vesicle epithelial cells (not shown). Bar, 200 μm.

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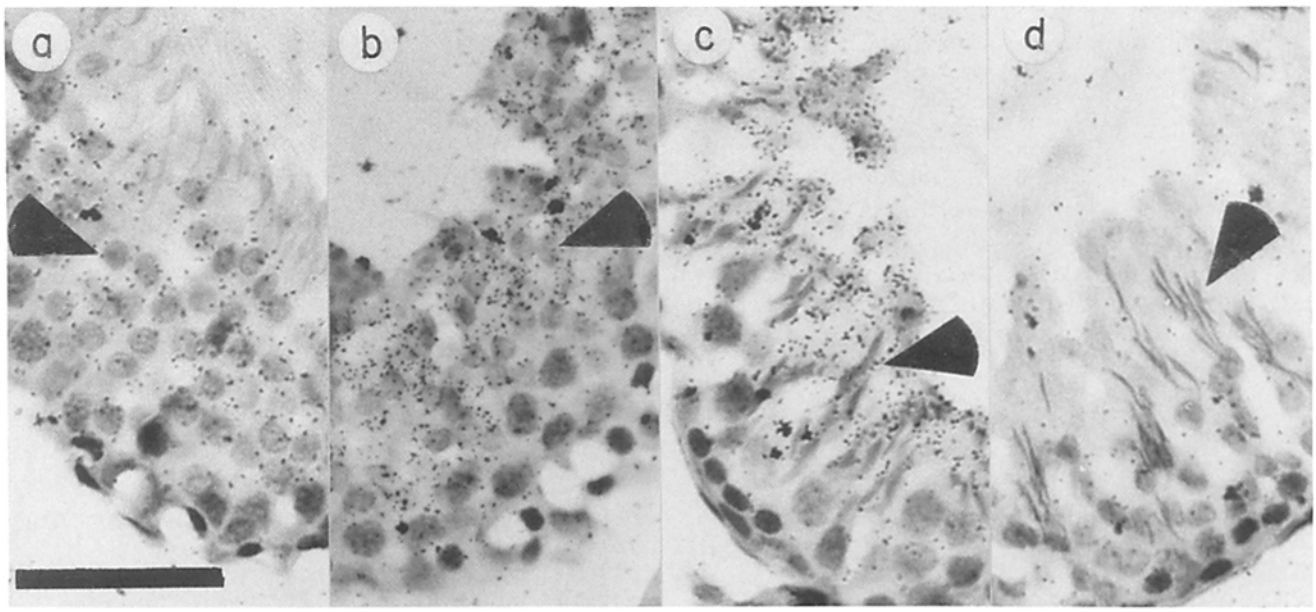

Figure 4. Cytochemical localization of TP1 mRNA by in situ hybridization. Segments of selected tubules from Fig. 3 are shown at higher magnification to permit identification of the cell types with which silver grains are associated. (*a*) Grains located over round spermatids of about step 7 (*arrow*) (from the lower tubule designated "7" in Fig. 3). (*b*) Grains located over slightly elongated spermatids of late step 8 (*arrow*). (*c*) Grains clustered over elongated and condensing spermatids of early step 13. (*d*) This tubule is representative of those in stages of the cycle after the division of secondary spermatocytes to yield round spermatids but prior to the clustering of the most mature step 18–19 spermatids near the lumen of the tubule. No grains above background are seen above either the condensed spermatids of step 15–17 (*arrow*) or above the early round spermatids only faintly stained in the same tubule. Bar, 50 μm.

our results agree well with those obtained by Meistrich's laboratory. Similarly, in the mouse (32, 33) tritiated lysine was seen to be incorporated readily into the nuclei of step 12 and 13 nuclei but was retained only through step 14. This label almost certainly reflected the combined presence of transition proteins 1 and 2, which are rich in lysine (5, 14, 15, 23). The low lysine content of protamine 1 (2, 3, 46) was apparently not detected by the conditions of the experiment, and protamine 2, which is prominent in mouse, lacks lysine (46).

A pretreatment with DTT markedly enhanced the immunological detection of TP1 in all classes of positive tubules. This suggests that some type of disulfide barrier blocks access to the protein even when it first appears. TP1 itself lacks cysteine in the rat (25). TP2, which is found in the same isolated cell fractions as TP1 contains substantial cysteine (5, 14, 15). Rat protamine, like other mammalian protamines, is also rich in cysteine (2, 3, 23). Since studies with isolated cell fractions indicated that protamine appears after TP1 in spermatid development (14), TP2 may account for the disulfide barrier.

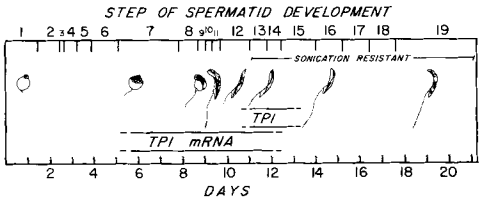

Figure 5. Diagram indicating the presence of TP1 mRNA and immunoreactive protein during the steps of spermatid development. The duration of the steps is taken from data summarized by Clermont (4) and assumes a 12.5-d cycle for the seminiferous epithelium (19), which may vary slightly depending on the strain of rat (44). Note that the first 14 steps of spermatid development define the 14 stages of the rat seminiferous epithelium and thus one complete cycle of the epithelium (4). The period in which the nuclei are resistant to sonication is taken from Grimes et al. (14).

To the extent that this can be established at the level of the light microscope with the detection reagent we have used, it seems that TP1 is present relatively uniformly throughout the interior of the nuclei of condensing spermatids. It was detected poorly if at all on the nuclear surface and reacted strongly with the antiserum only when a nucleus was cut in cross section. Its presence does not correlate with the early morphological changes that lead to an elongated nucleus or to the earliest steps of chromatin condensation that occur during step 12. Clearly this implies that TP1 does not serve to initiate condensation but rather to play a role during the later phase of this process. TP2 remains a possible candidate for participating in the initiation of condensation.

Translational regulation is now well established for protamine synthesis in both trout (20, 43) and mouse (26). TP1 is the first additional spermatid protein shown to share in this form of control. A clear difference between the regulation of protamine synthesis in trout and in mouse is that the message appears before the meiotic divisions in the former and only postmeiotically in the latter. This difference presumably is dictated by a need to avoid the determination of the spermatid message population by the haploid genome and the attendant dominance of otherwise recessive genetic alleles. In mammals developing spermatogenic cells are joined by cytoplasmic bridges that presumably facilitate the transfer of most proteins and perhaps mRNAs between cells (8) so that each

cell is presumably under control of a polyploid nucleus. The mechanism of translational regulation is not worked out but seems to involve a masking effect by salt-extractable proteins rather than any sort of covalent modification of the message itself (43). The isolation of the gene for rat TP1 (Heidaran, M., and W. S. Kistler, manuscript submitted for publication) now makes this system available for the application of modern genetic manipulation as a technique to help define the role of the message sequence in its translational regulation.

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