A Rat RuvB-like Protein, TIP49a, Is a Germ Cell-enriched Novel DNA Helicase*

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We have isolated a novel nuclear protein with a molecular mass of 49 kDa (TIP49a) from rat liver. The rat TIP49a showed structural resemblance to several bacterial RuvBs and also displayed Walker A and B motifs. We overproduced the recombinant TIP49a in Escherichia coli and purified it to near homogeneity. Biochemical investigations demonstrated that TIP49a possessed ATPase activity that was stimulated by single-stranded DNA but neither by double-stranded DNA nor by any forms of RNA polymers tested. Moreover, a UV cross-linking assay indicated TIP49a specifically interacted with ATP. Interestingly, we found that DNA duplex was unwound by the recombinant TIP49a in the presence of ATP or dATP. Optimal concentrations of ATP and Mg2+ for the helicase activity were 1−2 mM and 0.25−1 mM, respectively. Displacement of the DNA strand occurred in the 3′ to 5′ direction with respect to the single-stranded DNA flanking the duplex. Western blot analysis revealed that TIP49a was abundantly expressed in testes and moderately in spleen, thymus, and lung. In mouse seminiferous tubules, the protein was restrictively observed in germ lineages from late pachytene spermatocytes to round spermatids. From these observations, we propose that TIP49a is a novel DNA helicase and may play a role in nuclear processes such as recombination and transcription.

The unwinding of parent DNA strands is a prerequisite to basic genetic processes including DNA replication, DNA repair, recombination, and transcription (1, 2). In each of these processes, unwinding of duplex DNA is catalyzed by a DNA helicase, which functions to destabilize hydrogen bonds between complementary base pairs in duplex DNA. The energy necessary for this reaction is provided by hydrolysis of nucleosides and deoxynucleoside 5′-triphosphates, meaning that DNA helicase generally possesses an intrinsic ATPase activity (2).

DNA helicases are found ubiquitously from prokaryotes to eukaryotes. Biochemical studies and computer analysis have revealed that helicases have several characteristic motifs and that some of them are functionally important. Two sequence signatures, so-called Walker A and B motifs, have been identified in all helicases examined and in a wide variety of other NTP-utilizing enzymes (3, 4). On the basis of the primary sequence analysis of the Saccharomyces cerevisiae genome, there are at least 41 helicase genes in the genome (5). However, most of our biochemical knowledge about DNA helicases comes from analyses of bacterial and phage enzymes (1, 2), because little is known about the biochemical properties of most of the eukaryotic DNA helicases. However, recent studies demonstrated that six established or putative helicases are mutated in the human diseases such as xeroderma pigmentosum, Bloom’s syndrome, Cockayne’s syndrome, trichothiodystrophy, Werner’s syndrome, and α-thalassemia (5). Thus, identification of a novel helicase is medically important.

We originally identified a 49-kDa TATA-binding protein (TBP1-interacting protein (TIP49a; termed TIP49 in previous reports) from rat liver nuclear extracts (6, 12). TBP is one of the general transcription factors needed for all classes of RNA polymerases (7). Although we found a protein complex including both TIP49a and TBP in nuclear extracts (6), it is still unclear how TIP49a is involved in transcription and why it forms a complex with TBP. Interestingly, a computer search revealed a high similarity between TIP49a and a prokaryotic DNA helicase, RuvB, which is involved in branch migration of the Holliday junction (8, 9). It is noteworthy that TIP49a contains both the Walker A and B motifs in the RuvB homologous regions (6), suggesting that TIP49a is a putative DNA helicase. Recently, we found highly conserved tip49a-related genes in a variety of organisms from humans to an archaean by a data base search, implying that TIP49a plays a fundamental and essential role throughout the archaean and eukaryotes (10). Moreover, it is generally surprising that a mammalian protein would exhibit such extensive homology to a bacterial one. The eukaryotic counterpart of the bacterial ruvB has not been identified so far. Demonstration and characterization of a new helicase would be expected to help in the elucidation of eukaryotic nuclear processes.

Most recently, Qiu et al. (11) reported the identification and characterization of a eukaryotic RuvB-like protein (RUVBL1), which is highly homologous to bacterial RuvB. The amino acid sequence of RUVBL1 is identical to that of human TIP49a (12) and to that of rat TIP49a (6) with one exception; i.e. Val291 in rat TIP49a is replaced by Ile in RUVBL1. However, they reported that ATPase and helicase activities could not be detected in their RUVBL1 preparation. In this study, we purified the recombinant rat TIP49a protein from Escherichia coli to

* This work was supported by grants-in-aid for scientific research on priority areas from the Japanese Ministry of Education, Science, Sports, and Culture. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence reported in this paper has been submitted to the DDBJ/GenBank™/EBI Data Bank with accession number AB002406.

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† The abbreviations used are: TBP, TATA-binding protein; TIP, TBP-interacting protein; RUVBL1, RuvB-like protein 1; PAGE, polyacrylamide gel electrophoresis; ssDNA, single-stranded DNA; ATPγS, adenosine 5′-O-(thiotriphosphate).
near homogeneity and showed that this protein is a DNA-stimulated ATPase and ATP-dependent DNA helicase. We further found that TIP49a was enriched in testes as compared with all somatic tissues examined. Within the testes, TIP49a was localized in a subset of the germ cells from late pachytene spermatocytes to round spermatids. Herein, the biological significance of TIP49a will be discussed.

**EXPERIMENTAL PROCEDURES**

**Plasmids for Expression of Rat TIP49a Protein**—An NdeI site was created at translation initiation site of the TIP49a cDNA isolated from rat liver cDNA library in Agt11(6), and a fragment from NdeI to BamHI (in pBluescript vector downstream from the TIP49a cDNA) including an entire TIP49a coding region was inserted into pET-3a vector (13). TIP49a carrying FLAG tag and histidine tag was constructed by inserting oligonucleotides for amino acid stretch, MDYKDDDDKHHHHHH, at the N-terminal methionine of the rat TIP49a.

**Expression and Purification of Recombinant TIP49a**—The rat TIP49a was overexpressed in *E. coli* BL21(DE3)pLysS strain (13) by isopropyl-1-thio-β-D-galactopyranoside induction. The *E. coli* cells were harvested and suspended in a lysis buffer containing 20 mM Tris/HCl (pH 7.9), 100 mM KCl, 0.1% Nonidet P-40, 1 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, and 10% glycerol. The cells were disrupted by sonication and centrifuged, and the supernatants were then applied onto a nickel-agarose column equilibrated with the lysis buffer. The column was washed with BC100 buffer (20 mM Tris/HCl (pH 7.9), 100 mM KCl, 1 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 10% glycerol) supplemented with 20 mM imidazole/HCl (pH 7.9). TIP49a protein was eluted with BC100 buffer containing 300 mM imidazole/HCl (pH 7.9), and the peak fractions were dialyzed against F buffer containing 20 mM Tris/HCl (pH 7.9), 50 mM MgCl₂, 0.1% Nonidet P-40, 500 mM KCl, and 10% glycerol. Dialyzed materials were incubated with 5 ml of M2 anti-FLAG agarose (Eastman Kodak Co.) for 4 h with agitation. After the column had been washed with F buffer, the bound protein was incubated with the M2-agarose by incubation for 60 min with 5 ml of the FLAG peptide (Kodak) in F buffer (0.5 mg/ml). The eluate was dialyzed against BC40 buffer. The resulting sample was loaded onto a MonoQ (Amersham Pharmacia Biotech) column and eluted with a linear gradient of KCl from 40 to 400 mM using BC buffer. The protein concentration was estimated by use of BCA protein assay reagent (Pierce) with bovine serum albumin as a standard.

**Assay for ATP Hydrolysis—ATPase activity was assayed by use of activated charcoal (Sigma) as described by Armon et al. (14). The reaction (20 μl) contained 0.3 μg of the purified TIP49a, unless specified otherwise.** The TIP49a was incubated at 37° C for 30 min in A buffer (20 mM Tris/HCl (pH 7.5), 70 mM KCl, 2.5 mM MgCl₂, 1.5 mM dithiothreitol, 0.1 mM ATP, and 1.25 μCi of [γ-32P]ATP). One microgram of M13 single-stranded DNA (ssDNA), double-stranded pBluescript DNA, RNA homopolymer d(CCCUUU), or cellular total RNA was added to as indicated. Radioactivity was determined as Cerenkov radiation. Control reactions without TIP49a were carried out in parallel tubes, and the control value (radioactivity) was subtracted from each experimental one. Each assay was done in duplicate, and the results were presented as a simple arithmetic average.

**DNA Helicase Assay**—A complementary oligonucleotide corresponding to nucleotide positions 6291–6520 in M13mp18 ssDNA was synthesized and labeled at the 5'-end by T4 polynucleotide kinase and [γ-32P]ATP. The labeled oligonucleotide was annealed with the phage ssDNA by incubation at 95° C for 10 min and 60 min at 37° C. The product was purified by Suprec02 (Takara Shuzo) to remove the unannealed oligonucleotide. In the case of the experiment to determine the direction of DNA helicase, another substrate was constructed as described previously (15). A complementary oligonucleotide (54-mer) including the SmaI site, corresponding to nucleotide positions 6226–6279 in M13mp18 ssDNA, was synthesized and hybridized with the phage ssDNA. The oligonucleotide was labeled with T4 DNA kinase for 5'-end labeling or with terminal deoxynucleotidyl transferase and [γ-32P]dATP for 3'-end labeling. After SmaI digestion, this partial duplex DNA was used as a substrate.

For the DNA helicase assay, the reaction mixture (20 μl) contained 20 mM Tris/HCl (pH 7.5), 2 mM dithiothreitol, 50 mg/ml BSA, 0.5 mM MgCl₂, 80 mM KCl, 1 mM ATP, and 10 μCi of [γ-32P]ATP. It was irradiated by a UV cross-linker, LS1500 (Funakoshi), from a distance of 2 cm at 4° C for 20 min. The samples were stained with Coomassie Brilliant Blue and autoradiographed.

**Western Blotting and Immunocytochemistry**—The purified TIP49a was incubated with anti-FLAG antibody and anti-antibody (kindly provided by Kanemaki *et al.* (6). The TIP49a antibody was affinity-purified by use of TIP49a-immobilized Hitrap NHS-activated beads (Amersham Pharmacia Biotech). Rat tissues were homogenized in lysis buffer (20 mM Tris/HCl (pH 7.4), 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml phosphatase A, 0.5 μg/ml leupeptin, and 1 mM benzamidine) and then centrifuged. The protein content of the supernatant was determined using the BCA protein assay kit. Sample lysates (10 μg) were mixed with SDS sample buffer and then boiled for 5 min. The protein sample was subjected to 10% SDS-PAGE and then electrophoretically transferred to polyvinylidene difluoride membranes (Millipore Corp.). The proteins were visualized by the ECL protocol (Amersham Pharmacia Biotech).

**Immunohistochemical Analysis of TIP49a in Mouse Testes**—Sections were processed as described previously (18, 19). Localization of TIP49a was examined with anti-TIP49a antibody. Deparaffinized and rehydrated sections were immersed in 0.3% H₂O₂ in methanol and then preincubated with 0.5 mg/ml normal goat IgG in 1% bovine serum albumin/phosphate-buffered saline for 1 h. The sections were next reacted with rabbit anti-TIP49a antisera (1:600) diluted with 1% bovine serum albumin in phosphate-buffered saline for 3 h. After having been washed with 0.075% Brij 35/phosphate-buffered saline, each section was incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:100) for 1 h and washed with 0.075% Brij 35/phosphate-buffered saline. Negative control sections were incubated with normal rabbit serum at 1:800 dilution.

**RESULTS**

**TIP49a Is Structurally Similar to the Bacterial RuvB**—We found earlier that rat TIP49a showed a significant homology with RuvB, which is a bacterial recombination factor and possesses ATPase/DNA helicase activities (8, 9). There were mainly two conserved regions between TIP49a and the bacterial RuvBs, and in these regions the sequence identity and similarity were 38 and 25% and 55 and 46%, respectively (12). The RuvB-homologous regions in the TIP49a protein described above included Walker A and B motifs, which are responsible for ATP binding and ATP hydrolysis, and are characteristic of RNA and DNA helicases (Fig. 1). Therefore, we assumed that TIP49a is a putative ATPase and also possibly a DNA or RNA helicase. A database search indicated the presence of TIP49a-related proteins in various organisms such as humans and *S. cerevisiae*, and also in archaea (*Archaeoglobus fulgidus*) (10). Amino acid identities of the human, yeast, and archaean proteins to the rat TIP49a protein were 99.8% (with only one amino acid substitution), 70%, and 46–48%, respectively (Fig. 1). It is remarkable that the tip49a-related genes are so highly conserved among bacteria, eukaryotes, and archaea, suggesting that TIP49a must play a fundamental role in biological processes.

**Purification of Recombinant TIP49a**—For biochemical investigation of TIP49a, we overexpressed rat TIP49a protein in *E. coli*. Codons for two peptide motifs, the FLAG octapeptide, and a stretch of six histidines, were added to the 5'-end of the TIP49a sequence to facilitate the subsequent purification of the recombinant protein. The TIP49a carrying two tags was expressed in *E. coli* (data not shown) as described under “Experimental Procedures.” The TIP49a protein was thoroughly purified by nickel-chelate chromatography, anti-FLAG antibody-immobilized affinity chromatography, and MonoQ column chromatography, as outlined in Fig. 2A. The final MonoQ frac-
tion of TIP49a protein was found to include no detectable contaminating protein by SDS-PAGE and Coomassie Brilliant Blue staining (Fig. 2B, top).

**TIP49a Is a DNA-stimulated ATPase**—The presence of the characteristic Walker motifs in the primary TIP49a sequence predicted that the protein would be an ATPase. Therefore, we examined whether purified TIP49a protein had ATPase activity. The final MonoQ fractions were incubated with [γ-32P]ATP in the presence or absence of DNA, followed by determination of released phosphates as described under "Experimental Procedures." Fig. 2B (bottom) shows that the TIP49a was associated with an ATPase activity that was strongly stimulated by the presence of ssDNA. We also observed a weak ATPase activity in the absence of DNA (Fig. 2B). The level of ATPase activity correlated well with the amount of TIP49a present in each fraction (Fig. 2B). A parallel sample prepared from control *E. coli* had no ATPase activity (data not shown).

To further confirm that the TIP49a is an ATP-utilizing enzyme, the specific interaction between the purified TIP49a and ATP was analyzed by a UV cross-linking assay. This reaction, in which ATP is photolyzed by UV light in the presence of an ATP-binding protein, yields a covalent adduct interacting with ATP and the protein. By use of [α-32P]ATP, specific radioactive proteins were able to be visualized by SDS-PAGE and autoradiography. As shown in Fig. 3A, illumination with UV light resulted in the labeling of TIP49a protein (lane 2), and the labeling was strongly inhibited by cold ATP (lane 3). Incubation of [α-32P]ATP did not cross-link the ATP to the protein without UV illumination (Fig. 3A, lane 1). The addition of cold UTP to the reaction had a little effect on the ATPase activity of TIP49a, whereas cold GTP and CTP moderately reduced the ATPase activity (Fig. 3A, lanes 4–6). This result suggests that TIP49a does not interact nonspecifically with radioactive ATP; i.e., the labeling is attributed to the specific interaction between TIP49a and radioactive ATP. From these experiments, we concluded that TIP49a possesses an intrinsic ATPase activity.

To examine the nucleotide specificity for the TIP49a-catalyzed hydrolysis, we employed unlabeled nucleotides as competitors. The addition of UTP to the reaction had a little effect on the ATPase activity of TIP49a, whereas GTP and CTP moderately reduced the ATPase activity (Fig. 3B). This result suggests that TIP49a is unable to interact efficiently with nucleotides except ATP. Since it is thought that some proteins containing the Walker motif exhibit ATPase activity stimulated by nucleic acids, we next investigated the effects of various nucleic acids on the TIP49a ATPase activity. Fig. 3C shows that neither homoribopolymers nor cellular RNAs affected the enzyme activity of TIP49a. The closed circular form of plasmid DNA (pBluescript) exhibited only a weak stimulating effect, whereas the ATPase activity of TIP49a was remarkably stim-
The ATPase activity was assayed in the presence of M13 ssDNA. Cold nucleoside triphosphates were added to the mixture at a final concentration of 3 mM. Relative ATPase activities are shown. C, effect of nucleic acids on TIP49a ATPase activity. The ATPase activity was assayed in the presence or absence (−) of nucleic acids as indicated. One microgram of M13 ssDNA, pBluescript as a double-stranded DNA, cellular total RNA prepared from rat liver (17), or various RNA homopolymers (Amersham Pharmacia Biotech) was added to the reaction mixture.

To determine the polarity of the DNA unwinding by TIP49a, we prepared the following DNA substrates. For substrate preparation, a 5′- or 3′-end-labeled 54-mer oligonucleotide was annealed with M13 ssDNA to construct a partial duplex substrate. This substrate was incubated with the final MonoQ fractions. The reaction products were separated on a polyacrylamide gel and detected by autoradiography. In agreement with the results of the ATPase assay (Fig. 2B), the helicase activity was also correlated with the amount of TIP49a in each fraction (Fig. 4A), demonstrating that TIP49a possesses a DNA helicase activity.

To determine the polarity of the DNA unwinding by TIP49a, we prepared the following DNA substrates. For substrate preparation, a 5′- or 3′-end-labeled 54-mer oligonucleotide was annealed with M13 ssDNA. This partial duplex DNA was digested with SmaI to yield a linear ssDNA with either a labeled complementary 24-mer or a labeled 30-mer bound to its ends. Following incubation of the substrates with the TIP49a protein, no increase in unwound 24-mer oligonucleotide over background was observed (Fig. 4B, lanes 4 and 6). The recombinant TIP49a was found to efficiently displace the 5′-labeled 30-mer (Fig. 4B, lane 3), showing that TIP49a has a 3′ to 5′ unwinding polarity with respect to the ssDNA flanking the duplex.

Characterization of TIP49a DNA Helicase—Next, we determined the optimal conditions for the DNA helicase activity of TIP49a. The optimal temperature for the helicase reaction was 37 °C, and the optimal pH range was 7.5–8 (data not shown). For the maximal helicase activity, TIP49a required 1–2 mM ATP and 0.25–1 mM MgCl₂, and 4 mM ATP and 10 mM MgCl₂ considerably inhibited the enzyme activity (Fig. 5A). TIP49a helicase activity was not detected in the absence of ATP or MgCl₂ (Table I), demonstrating that TIP49a DNA helicase activity is dependent on the presence of both Mg²⁺ and ATP. The helicase reaction required the hydrolysis of a β-γ bond of ATP, since the poorly hydrolyzable analog ATP₇S, ADP, and AMP did not exhibit the cofactor function (Table I). Compared with ATP, other nucleoside 5′-triposphates were inert as a co-factor, with the exception of dATP, which supported more than 90% of the activity of ATP (Table I). We next examined the requirement of various divalent cations for TIP49a helicase activity. Although the presence of Mn²⁺ at a 1 mM concentration resulted in 75% of the activity of Mg²⁺, all other tested cations such as Ca²⁺, Cu²⁺, and Zn²⁺ could not replace Mg²⁺ for the enzyme activity (Table I). MgCl₂ was able to be replaced by either MgSO₄ or Mg(CH₃COO)₂.

A time course of the TIP49a-mediated strand displacement reaction under the optimal assay conditions is shown in Fig. 5B. Percentage of unwound fragment was determined. In the presence of 300 ng of TIP49a protein, we found that >50% of the labeled fragments were displaced in 5 min. The unwinding activity increased linearly up to 5 min and reached a plateau in 20 min.

Expression of TIP49a Protein in Rat Tissues—Finally we examined the expression of TIP49a in various rat tissues by Western blot analysis using affinity-purified anti-TIP49a antibody. TIP49a protein was exclusively detected as a single band of 50 kDa (Fig. 6A). It was moderately expressed in the thymus, spleen, and lung and abundantly in the testis. This distribution pattern coincided well with that of Rad51, an authentic eukaryotic recombination factor known to be involved in mitotic and meiotic recombination (Fig. 6A) (20, 21). We suspect that TIP49a participates in some way in recombination.
The testes contain multiple types of germ and nongerm cells. Moreover, each type of germ cells reflects a particular stage of spermatogenesis. We next performed immunohistochemistry to examine what kinds of testicular cells predominantly express TIP49a proteins. Fig. 6B, a, shows that the epitope reacted with anti-TIP49a antibody is specifically localized in a subset of the germ cells at the stage of pachytene (stages IX–X) to round spermatids. Immunostaining using normal serum resulted in no staining (Fig. 6B, c), demonstrating that the detected epitopes represent TIP49a. In contrast, little signal was detected in pachytene spermatocytes (stages IV–VI; Fig. 6B, b). These results indicated that TIP49a is predominantly expressed in testicular germ cells at stages from late pachytene spermatocytes to round spermatids. No or little signal was observed in somatic Sertoli or Leydig cells (Fig. 6B, a and b). These observations suggest that TIP49a plays a critical role in meiosis.

**DISCUSSION**

**TIP49a Is a Novel Eukaryotic DNA Helicase**—We originally identified a 49-kDa TBP-interacting protein (TIP49a) from rat liver nuclear extracts by use of an in vitro binding assay (6). Immunofluorescence analysis using anti-TIP49a antibody revealed a typical dot-shaped nuclear staining pattern, as previously reported (12), indicating that TIP49a to be a nuclear protein. This finding is consistent with a recent report that TIP49a (or NMP38) was identified as a nuclear matrix protein (22). TIP49a contains Walker A and B motifs, which are characteristic of DNA/RNA helicases. Thus, we suspected it to be a putative DNA/RNA helicase. In this work, the recombinant TIP49a was purified to near homogeneity from *E. coli* and was defined to be an ssDNA-stimulated ATPase and ATP-dependent DNA helicase based on the following biochemical evidence. (i) The ATPase activity was co-purified with the recombinant TIP49a on the final MonoQ column (Fig. 2B, top) and the same result was obtained in the case of the helicase activity (Fig. 4A). (ii) A UV cross-linking assay indicated that TIP49a specifically interacted with ATP (Fig. 3A). Thus, it is unlikely that a contaminating protein was involved in the ATPase/helicase activity. (iii) The ATPase activity was remarkably stimulated by ssDNA (Fig. 3C). (iv) The helicase activity was dependent on the presence of ATP or dATP, but ATPγS could not substitute for ATP (Table I).

Most recently, RUVBL1, human counterpart of rat TIP49a, was isolated from human cells. However, the purified RUVBL1 expressed in insect cells did not possess ATPase and helicase activity, although RUVBL1 and TIP49a are 99.8% identical (11). We also found that TIP49a preparations purified from insect cells using the baculovirus expression system did not
hydrolyze ATP (data not shown). The failure to detect the enzyme activity in insect cell-expressed TIP49a and RUVBL1 might be due to the modification of the protein and/or the presence of an inhibitory factor in insect cells.

TIP49a exhibits significant sequence similarity to bacterial RuvB, which is a recombination factor having ATP-dependent DNA helicase activity. A functional and structural homologue of bacterial RuvB has not been found in eukaryotes yet (see below). We herein provided evidence that a mammalian RuvB-like TIP49a is an intrinsic DNA-stimulated ATPase and DNA helicase. However, we found some differences between TIP49a and RuvB in terms of biochemical properties. TIP49a required 1–2 mM ATP and 0.25–1 mM MgCl2 for the maximal helicase activity (Fig. 5A), whereas concentrations of ATP of >0.5 mM and MgCl2 at 10–20 mM are optimal for RuvB helicase activity (23). The TIP49a helicase activity was considerably inhibited at 10 mM MgCl2. Furthermore, despite the sequence similarity between RuvB and TIP49a, TIP49a has a 3’ to 5’ polarity, which is the opposite of that of RuvB. If TIP49a possesses RuvB-like activity, this difference may be relevant to the finding that the polarity of strand transfer promoted by Rad51 is also the opposite of that observed in RecA (24).

What Is the in Vivo Role of TIP49a?—Our findings that TIP49a is a nuclear factor possessing a DNA helicase activity led us to an assumption that TIP49a is involved in DNA-associated nuclear processes such as recombination, transcription, and replication. Considering the high sequence conservation in archaea and yeast as well as in higher eukaryotes (Fig. 1), we suggest that TIP49a may play an important role in nuclear processes. Immunological analysis indicated that Rad51, which is responsible for an early stage in homologous recombination (i.e. a sequential pathway up to the Holliday junction formation), was highly expressed in the thymus, spleen, and testis, as expected (Fig. 6A). Like Rad51, TIP49a was also significantly enriched in tissues where recombination frequently takes place. Moreover, it is interesting that TIP49a protein was highly accumulated in the meiotic cells at stages from late pachytene to round spermatids (Fig. 6B), since formation of the Holliday junction is assumed to occur in germ cells staying at the pachytene stage (28). Chiasmata represent the physical manifestation of crossing over and reciprocal recombination and are cytologically detected when homologues begin to separate at diplotene stage (29). It may be that the TIP49a expression pattern during meiosis reflects the late stage of recombination. Thus, we propose a hypothesis that TIP49a participates in a recombination process like RuvB.

In bacteria, RecA generates the Holliday structure. E. coli RuvB, which is an ATP-dependent DNA helicase, binds to the Holliday junction, and promotes the branch migration together with RuvA during the late stage of recombination (8, 9). Rad51 in eukaryotes and RadA in archaea were established to be RecA functional homologues (30, 31), suggesting that a recombination mechanism is conserved among the organisms. However, in eukaryotes, RuvB-like activity has not been identified. It was a general surprise that rat TIP49a is highly homologous to bacterial RuvBs. Thus, with all evidence taken together, it is conceivable that TIP49a helicase activity is involved in some aspect of RuvB-associated recombination in eukaryotes.

Alternatively, TIP49a may function as a transcription factor, because it was identified in a TBP-carrying complex (6). This hypothesis is supported by a recent finding that RUVBL1, which is a human homologue of TIP49a, is co-purified with the RNA polymerase II transcription complex (11). We cannot exclude a possibility that TIP49a directly participates in the transcriptional regulation. Bauer et al. (37) reported that β-catenin and TBP were binding partners of the human TIP49a (Pontin52). As another possibility, TIP49a may involved in a transcription-recombination coupling reaction. Indeed, several subunits of the TFIIH complex, which is a general transcription factor, are involved in nucleotide excision repair (32). Rad51 and BRCA1 are found to be components of the RNA

### Table I

| Reaction condition* | Unwinding % |
|---------------------|-------------|
| Complete            | 98          |
| Without TIP49a      | <1          |
| Without ATP         | <1          |
| With dATP           | 90          |
| With ATP$_2$S       | <1          |
| With ADP            | <1          |
| With AMP            | <1          |
| With CTP, GTP, or UTP | <1  |
| With dCTP, dGTP, or dTTP | <1  |
| Without MgCl$_2$    | <1          |
| With MgSO$_4$       | 95          |
| With Mg(0Ac)$_2$    | 94          |
| With CaCl$_2$       | <1          |
| With ZnCl$_2$       | <1          |
| With MnCl$_2$       | 75          |
| With CuCl$_2$       | <1          |

*Helicase reaction was carried out as described under “Experimental Procedures.” Concentrations of nucleotides and divalent cations were 1 mM.

![Fig. 6. Expression of TIP49a protein in tissues. A, tissue-specific distribution of TIP49a in various rat tissues. Ten micrograms of total protein of lysates was used for Western blot analysis using anti-TIP49a antibody (top) or anti-Rad51 antibody (bottom). B, immunohistochemical staining of mouse testis sections. Sections were reacted with anti-TIP49a antibody (a and b) or normal rabbit serum (c). Stages of seminiferous tubule sections in a and b are IX–X and IV–VI, respectively. P, pachytene spermatocyte; RS, round spermatid; Sr, Sertoli cell; Ly, Leydig cell.](image)
polymerase II-holoenzyme complex (33, 34), and BRCA1 has a potential to enhance transcription (35). These facts suggest that some DNA recombination-repair proteins may be associated with the transcriptional apparatus. It is possible that recombination-repair may be linked to transcription. If this is the case, TIP49a may play a pleiotropic role in DNA metabolism. Further investigation of TIP49a should provide new insight into the nuclear events that transpire in eukaryotes.

Acknowledgments—We thank Dr. T. Ohta for the generous gift of anti-Rad51 antibody. We also thank Drs. T. Ogawa and H. Shinagawa for valuable discussions.

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