TIP49b, a New RuvB-like DNA Helicase, Is Included in a Complex Together with Another RuvB-like DNA Helicase, TIP49a*

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Masato Kanemaki‡, Yumiko Kurokawa‡, Toru Matsu-ura‡, Yasutaka Makino‡, Abdull Masani‡, Katsu-ichiro Okazaki§, Takashi Morishita†, and Taka-aki Tamura‡‡

From the ‡Department of Biology, Faculty of Science, Chiba University, CREST Japan Science and Technology Corp., 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, §Department of Life Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, and †Department of Experimental Chemotherapy, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, Japan

We previously reported that TIP49a is a novel mammalian DNA helicase showing structural similarity with the bacterial recombination factor RuvB. In this study, we isolated a new TIP49a-related gene, termed TIP49b, from human and yeast cells. TIP49b also resembled RuvB, thus suggesting that TIP49a and TIP49b are included in a gene family. Like TIP49a, TIP49b was abundantly expressed in the testis and thymus. Enzyme assays revealed that TIP49b was an single-stranded DNA-stimulated ATPase and ATP-dependent DNA helicase. Most of the enzymatic properties of TIP49b were the same as those of TIP49a, whereas the polarity of TIP49b DNA helicase activity (5’ to 3’) was the opposite to that of TIP49a. TIP49b and TIP49a bound to each other and were included in the same complex of ~700 kDa in a cell. We found that TIP49b was an essential gene for the growth of Saccharomyces cerevisiae, as is the TIP49a gene, suggesting that TIP49b does not complement the TIP49a function and vice versa. From these observations, we suggest that TIP49b plays an essential role in the cellular processes involved in DNA metabolism.

Because most organisms use DNA as their genetic substance, DNA-related nuclear dynamics such as replication, repair, and transcription are critical for most organisms. In these processes, duplex DNA must, as a prerequisite, be unwound. The enzymes that catalyze unwinding double-stranded DNA (dsDNA)1 to single-stranded DNA (ssDNA) in an ATP-dependent manner are DNA helicases (1–3). DNA helicases are widely found in organisms from prokaryotes to eukaryotes and their viruses. Most organisms contain multiple DNA helicases. At least 12 different helicases have been identified in Escherichia coli, whose enzyme activities were confirmed in vitro (3). So far, 10 and at least 15 helicases have been identified in Saccharomyces cerevisiae (4–7) and mammalian cells (8–13), respectively. Recently, genome analysis has identified a number of putative DNA helicases. For example, the S. cerevisiae genome encodes more than 41 putative DNA helicases (14). Thus, there must be many other eukaryotic DNA helicases that have not yet been identified.

Recently, we reported the isolation of TIP49a (TATA-binding protein [TBP]-interacting protein 49a) from rats and humans (15, 16). Interestingly, among eukaryotic proteins, TIP49a has the highest similarity to bacterial RuvB proteins. RuvB is a bacterial DNA helicase whose direction of the helicase reaction is 5’ to 3’ (17). RuvB is involved in homologous recombination and double-strand break repair in bacteria. When the double-strand break happens in DNA by x-ray irradiation or nuclease, the DNA ends would be processed by RecBCD and introduced into homologous sequences in a heterologous duplex by RecA (18). This mechanism forms a homologous recombination-directed intermediate having a 4-way junction, namely the Holliday structure. In the late stage of homologous recombination, RuvB binds to the Holliday structure, and a branch point migrates dependent on the DNA helicase activity of RuvB and its co-factor RuvA functions. Then, RuvC, a Holliday structure-specific endonuclease, resolves the junction (19). Although the above process is also thought to exist in eukaryotes (20), an eukaryotic RuvB homolog has not been identified.

TIP49a was originally identified as a protein that formed a complex with TBP in rat liver (15). This protein had ssDNA-stimulated ATPase activity and ATP-dependent DNA helicase activity (23). TIP49a was also identified as RUVBL1 (RuvB-like protein 1) (21), NMP238 (nuclear matrix protein 238) (22), and Pontin 52 (46) by other groups. Although the physiological role of TIP49a is still unclear, the previous reports imply that TIP49a is involved in essential cellular processes. TIP49a is highly conserved in various kinds of eukaryotes (24). Moreover, the archaeal genome also encodes a TIP49-related protein (25). We noticed the existence of a DNA sequence for another TIP49a-related gene in the S. cerevisiae genome. We tentatively designated it as TIP49b (24). Furthermore, we found a TIP49b-related sequence in the mammalians EST data bases (24). In this paper, we describe the isolation of cDNA for TIP49b from human and S. cerevisiae. We demonstrate ssDNA-stimulated ATPase and ATP-dependent DNA helicase activities in human TIP49b. TIP49b had a characteristic helicase activity, bound to TIP49a, and formed a large complex in a cell.

** Experimental Procedures

Cloning of TIP49b—On the basis of two human EST clones (GenBank™ accession numbers R19091 and AA374580), two primers (5’-GAGATCGCTGATGTAAACAGGATGAG and 5’-CTTGTCTCGGAGCCCATAGCGTCG) corresponding to those clones, respectively, were

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‡ A research fellow of The Japan Society For The Promotion Of Science.

** To whom correspondence should be addressed. Tel.: (81)43-290-2823; Fax: (81)43-290-2824; E-mail: btamura@nature.s.chiba-u.ac.jp.

1 The abbreviations used are: dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; TBP, TATA-binding protein; TIP, TBP-interacting protein; PAGE, polyacrylamide gel electrophoresis; h- and r-, human and rat, respectively; GST, glutathione S-transferase; kb, kilobase; ATP-γS, adenosine 5’-O-(thiotriphosphate); AD, activation domain; DB, DNA binding domain.

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designed. These primers were used to amplify DNAs in a human liver cDNA library. The subcloned amplified fragment was used as a probe to screen a cDNA library of human TIP49b (hTIP49b) in a human liver cDNA library. ScTIP49b (YLP235a; GenBank™ accession number S61029) and scTIP49a (YDR190c; GenBank™ accession number S52698) of S. cerevisiae were cloned by polymerase chain reaction based on sequences submitted to the data base.

Expression and Purification of Recombinant Human TIP49b—Recombinant hTIP49b carrying FLAG and oligohistidine tags at the N-terminal region was expressed in E. coli by use of a PET vector system (23). E. coli expressing the recombinant protein were suspended in a lysis buffer (20 m M Tris-HCl, pH 7.9, 100 m M KCl, 0.1% Nonidet P-40, 1 m M 2-mercaptoethanol, 1 m M phenylmethylsulfonyl fluoride, and 10% glycerol) and disrupted by sonication. The soluble fraction was applied on an nickel nitritrocio acid-agarose (Qiagen) column equilibrated with the lysis buffer. The proteins were eluted by 0.1 M KCl buffer (20 m M Tris-HCl, pH 7.9, 0.1 M KCl, 2 m M 2-mercaptoethanol, and 10% glycerol) containing 200 m M imidazole, and the eluates were dialyzed against the P10 buffer (10 mM potassium phosphate, pH 7.2, 50 m M KCl, 5 m M Tris-mercaptoethanol, and 10% glycerol). The sample was loaded to a hydroxypatite column (Bio-Rad) and eluted by linear gradient of the potassium phosphate from 10 to 300 m M in the P10 buffer. The peak fractions were dialyzed against 50 mM KCl buffer same as the above KCl buffer but with 50 m M KCl, loaded to a MonoQ column (Amersham Pharmacia Biotech), and eluted with a linear gradient of 0.1 M KCl from 0.05 to 0.5 M in the same buffer. Finally, the peak fractions were pooled and dialyzed against 20% glycerol-containing 0.1 M KCl buffer. Protein concentration was determined by BCA protein assay reagent (Pierce) with bovine serum albumin as standard.

DNA Helicase Assay—An oligonucleotide with the complementary sequence corresponding to 6291 to 6320 of M13mp18 was synthesized and labeled at the 5'-end with [32P] with T4 polynucleotide kinase. This probe was hybridized with phage ssDNA. The purified hybrid (10 ng) was incubated with 20 m l of solution containing 20 m M Tris-HCl, pH 7.5, 70 m M KCl, 1 m M MgCl2, 1.5 m M dithiothreitol, 0.1 m M ATP, and 1.25 m Ci of [γ-32P]ATP at 37 °C for 30 min. Released phosphates were separated from ATP by activated charcoal method [27] and measured by a liquid scintillation counter. UV cross-linking was performed on ice in 20 m l of ATPase reaction mixture. The sample was irradiated with light for 20 min by UV cross-linker PS1500 (Funko). The samples separated by 10% SDS-PAGE were subjected to Coomasie Brilliant Blue staining and autoradiography.

RESULTS

Cloning of cDNA for Human TIP49b—To isolate a cDNA for human TIP49b, two human EST clones (GenBank™ accession numbers R19091 and AA374580) were used to partially encode hTIP49b. We amplified related fragments and finally obtained a 1.5-kb candidate cDNA (Fig. 1A). DNA sequencing of this clone revealed an open reading frame with 463 amino acids, and the calculated molecular mass was 51 kDa (Fig. 1A). Although an in-frame stop codon upstream of the putative first methionine was not found, the sequence around the presumed first methionine fit the Kozak rule (34). Anti-TIP49b antibody detected a single protein in the nuclear extract whose size was the same as that of the recombinant hTIP49b (Fig. 1B). The antibody did not cross-react with TIP49a (data partly presented in Fig. 4A). From these data, it was concluded that we had obtained a hTIP49b cDNA. It was found that hTIP49b shows high similarity to other TIP49 family proteins (Fig. 1C). Interestingly, archaeal (Archaeoglobus fulgidus) TIP49 was more similar to TIP49b than several TIP49a s. As expected, hTIP49b also exhibited high identity to the bacterial RuvB. TIP49b had two RuvB-homologous regions that corresponded to critical Walker A and B motif-containing regions (Fig. 1C).

ATPase Activity of hTIP49b—We have determined that rTIP49a is an ssDNA-dependent ATPase and DNA helicase (23). From the marked structural similarity with TIP49a, we assumed that hTIP49b can also have the same activities. We expressed recombinant hTIP49b carrying FLAG and oligohistidine tags in E. coli and purified it to over 95% pure (Fig. 2A, lane 5). A faint 53-kDa band just above the major band was suggested to be a read-through or incorrect translation product of hTIP49b (data not shown). An aliquot of the MonoQ fractions was used for the ATP hydrolysis assay. We found that the fractions exhibited only faint ATPase activity (Fig. 2B, column 1). However, hTIP49b-directed ATPase activity was remarkably stimulated by ssDNA (Fig. 2B, column 2). These results indicated that hTIP49b is a ssDNA-dependent ATPase, as is TIP49a. Four kinds of oligonucleotides did not affect the ATPase activity, and dsDNA only slightly stimulated the ATPase activity (Fig. 2B, columns 3 to 7). To detect ATP binding activity of hTIP49b, a UV cross-linking assay was performed. We found that hTIP49b was effectively cross-linked with ATP (Fig. 2C, lanes 1 and 2). Because no other ATP cross-linked protein was observed, we concluded that the ATPase
reaction was governed by hTIP49b itself. A competition experiment for ATP binding was performed by adding cold NTP, and we found that cold ATP was the strongest competitor (Fig. 5 C, lanes 3 and 4 to 6). These results indicate that hTIP49b selectively binds to ATP. We found that UTP and CTP could only partially compete the ATP binding (Fig. 5 C, lanes 5 and 6).

DNA Helicase Activity of hTIP49b—Because another family protein, TIP49a, is a DNA helicase (23), we investigated whether hTIP49b also has DNA helicase activity. For this purpose, we used an assay by which dissociation of an oligonucleotide probe from a circular ssDNA template is detected by gel electrophoresis. We found that the probe was displaced from the template at amounts that were dependent on the hTIP49b concentration (Fig. 3 A). Another hTIP49b preparation purified by an anti-FLAG antibody column exhibited an equivalent DNA helicase activity (data not shown). From these data, we concluded that hTIP49b is a DNA helicase. The reaction absolutely required ATP and MgCl₂ ion. ADP and a nonhydrolyzable ATP analog, ATP-γ-S, were not able to be substituted by ATP (Table I). The optimal concentrations of ATP and MgCl₂ were 1 and 0.5–1 mM, respectively (data not shown). These enzyme parameters were almost the same as those of TIP49a (23).

The polarity of strand displacement by hTIP49b was determined using two kinds of linear DNA substrates in which a short oligonucleotide had been hybridized with the template at either end (Fig. 3 B). The results indicated that hTIP49b preferentially unwound the 24-base probe (Fig. 3 B, lanes 3 and 7). These observations indicate that the polarity of hTIP49b DNA helicase activity was 3' to 5'. On the other hand, the strand displacement polarity of rTIP49a was opposite (5' to 3') (23) (Fig. 3 B, lanes 4 and 8). It was concluded that TIP49a and TIP49b are enzymatically unequivalent.

Tissue Distribution of TIP49b—Although TIP49a is expressed ubiquitously in rat tissues, it is highly expressed in the mammalian testis and thymus (23). We assayed TIP49b in rat tissues using specific antibodies. We found that the antibodies were subtype-specific, because anti-TIP49b antibody did not detect rat TIP49a, even at a 10-fold-higher amount than that detected by anti-TIP49a antibody, and vice versa (Fig. 4 A). Western blotting indicated that TIP49b was highly expressed in the testis and thymus (Fig. 4 A, upper panel), which was similar to the findings for TIP49a (Fig. 4 A, lower panel). This tissue distribution pattern was similar to that of Rad51, the most established eukaryotic recombination factor. Northern blot analysis also detected abundant TIP49b RNAs in the human testis (data not shown).

TIP49b Binds to TIP49a—It is known that some DNA helicases form a homo- or hetero-oligomer, i.e., typically dimer or hexamer (2). We decided to investigate whether hTIP49b could interact with another TIP49 protein homologously and heterologously. We examined in vitro binding of TIP49-family pro-
teins using GST fusion constructs. Glutathione beads conjugated with either TIP49 protein were mixed with FLAG-tagged and oligo(histidine)-tagged hTIP49b or rTIP49a proteins, and proteins associated with GST fusion protein were analyzed using the anti-FLAG antibody. RPB6, a subunit of the RNA polymerase II, was used as a negative control protein. GST alone bound to neither hTIP49b nor rTIP49a (Fig. 5A, lane 2). It was clearly demonstrated that GST-rTIP49a bound to hTIP49b (Fig. 5A, lane 3) and that GST-hTIP49b bound to rTIP49a, reciprocally (Fig. 5A, lane 4). Nevertheless, no homologous interactions were observed for each protein (Fig. 5A, lanes 3 and 4). RPB6 with FLAG and oligo(histidine) tags did not bind to GST fusion constructs of TIP49 proteins (Fig. 5A, lane 2).

**Fig. 2. ATPase and ATP binding activities of TIP49b.** A, expression and purification of the recombinant hTIP49b. hTIP49b carrying FLAG and oligo(histidine) tags was expressed in E. coli and purified as described “Experimental Procedures.” Two μl of the sample was analyzed by SDS-PAGE and Coomassie Brilliant Blue staining. Lane 1, crude E. coli lysate (-). Lane 2, crude isopropyl-1-thio-β-D-galactopyranoside-treated E. coli lysates (+). Lane 3, nickel-agarose column eluates (Ni). Lane 4, hydroxyapatite column eluates (HAP). Lane 5, MonoQ column eluates (MonoQ). M, molecular weight marker. B, ATPase activity of hTIP49b with (0.5 μg) (columns 2 to 7) or without (column 1) nucleic acid was demonstrated. Column 2, M13mp18 ssDNA; column 3, pBluescript dsDNA; column 4, poly(U); column 5, poly(A); column 6, poly(G); column 7, poly(C). C, UV cross-linking assay of hTIP49b. hTIP49b was labeled with [α-32P]ATP under UV irradiation (lanes 2 to 6) or no irradiation (lane 1). For competition to hot ATP, 0.3 mM cold ATP (A, lane 3), GTP (G, lane 4), UTP (U, lane 5), or CTP (C, lane 6) was added to the reaction. Upper and lower panels display autoradiography and Coomassie Brilliant Blue staining, respectively. The arrowhead indicates the position of labeled hTIP49b.

**Fig. 3. DNA helicase activity of TIP49b.** Structures of substrate hybrids and probes are schematically illustrated. Asterisks show 32P-labeled positions. A, DNA helicase activity of MonoQ fraction. Ten ng of the substrate hybrid made of M13mp18 ssDNA and hybridized oligonucleotide probe was used. One μl of an aliquot of MonoQ fractions (lanes 3 to 9) was added to the reaction mixture. Lane 1, control reaction without protein. Lane 2, heated (at 98 °C for 3 min) substrate. Positions of the intact hybrid and displaced probe are indicated by arrowheads. The upper panel indicates Coomassie Brilliant Blue staining of the MonoQ fraction. B, polarity of strand displacement by hTIP49b. Substrates (schematically indicated below the panel) were prepared as described under “Experimental Procedures.” One μg hTIP49b (lanes 3 and 7) and rTIP49a (lanes 4 and 8) were used for one reaction. Lanes 1 and 5, heated substrate; lanes 2 and 3, without protein. Arrowheads indicate positions of the displaced 30-base and 24-base probes. Arrows indicate the direction of the enzyme reaction.
hTIP49b efficiently binds to rTIP49a in vivo and that the binding affinity of each homologous association drastically decreases. These in vivo results agreed well with those obtained in vitro (Fig. 5A).

**TIP49b Forms a Large Complex with TIP49a in a Cell**—To investigate the complex formation of TIP49 proteins in vivo, we performed an immunoprecipitation assay with HeLa cell nuclear extracts. For this assay, we prepared a mouse polyclonal antibody against TIP49a to overcome rabbit IgG-derived heavy backgrounds in Western blotting. We detected TIP49b in immunoprecipitates prepared with anti-TIP49a antibody (Fig. 5C, lane 3). Moreover, TIP49a was detected in immunoprecipitates prepared with anti-TIP49b antibody (Fig. 5C, lane 6). These results suggested that TIP49b and TIP49a were included in an identical complex. HeLa cell nuclear extracts were fractionated by gel filtration, and TIP49b and TIP49a were analyzed. TIP49b and TIP49a were roughly coeluted (Fig. 5D). Although both proteins were observed in >2000–500-kDa fractions, some of them were concentrated in 800–600-kDa fractions (Fig. 5D, fractions 36 to 44). We obtained similar results by using glycerol gradient centrifugation (data not shown). We detected several other proteins in the TIP49-containing protein complex. Thus, it is most likely that TIP49b and TIP49a form a large nuclear complex in the nucleus together with other proteins.

**TIP49b Gene Is Essential for Growth of S. cerevisiae**—TIP49a is an essential gene for growth of yeast (*S. cerevisiae*) (21). To investigate the requirement of TIP49b gene in yeast (scTIP49b), we performed gene disruption analysis. The scTIP49b gene was disrupted as described under “Experimental Procedures,” and its requirement for cell growth was examined by tetrad analysis. The scTIP49a gene (YDR190c) was also employed as a control. One allele of scTIP49b or scTIP49a was confirmed by Southern blotting to be disrupted in diploid cells (Fig. 6B). Setip49a/scTIP49b and setip49b/scTIP49a diploids were sporulated and subjected to tetrad analysis. Eight kinds of tetrads yielded only two viable spores (Fig. 6C, lanes 1–8), indicating that scTIP49b was indispensable for the growth of yeast. The requirement of the scTIP49a gene for cell growth, which had been reported by Qiu et al. (21), was reproduced. All viable spores did not contain the maker gene (data not shown). These results indicate that, as is scTIP49a, scTIP49b is an essential gene for the growth of yeast. We found that growth of spores bearing the disrupted setip49b or setip49a gene were aborted and arrested after a few cycles of cell division (data not shown).

**DISCUSSION**

**Identification of a New TIP49 Family Protein**—We previously isolated a RuvB-like DNA helicase TIP49a from rats and humans (15, 16). In this work, we identified a cDNA for TIP49b, another RuvB-like protein, from humans and yeast. The predicted amino acid sequence of TIP49b revealed 40% identity to various TIP49a proteins (Fig. 1C). Thus, TIP49b and TIP49a were judged to belong to a novel protein family, the TIP49 family. We detected proteins equivalent to TIP49b in rats, *Xenopus*, and *Drosophila* (data not shown). A *Caenorhabditis elegans* gene submitted as T22D1.10 was thought to be a nematode TIP49b counterpart (24, 36). Moreover, a gene resembling TIP49b was also found in archaea. These findings suggest that TIP49b homologs are generally conserved from archaea to humans. TIP49b, like TIP49a, showed considerable identity to the bacterial recombination factor RuvB (Fig. 1C).

**TABLE I**

| Reaction requirement of TIP49b DNA helicase | % Unwinding |
|------------------------------------------|-------------|
| Complete | 85 |
| –hTIP49b | <1 |
| –ATP | <1 |
| +dATP | 85 |
| +ATP | <1 |
| +ADP | <1 |
| +AMP | <1 |
| +CPT, GTP, or UTP | <1 |
| +dCTP, dGTP, or dUTP | <1 |
| -MgCl₂ | <1 |
| +MgSO₄ | 90 |
| +Mg(OAc)₂ | 90 |
| +CaCl₂ | 1 |
| +ZnCl₂ | 1 |
| +MnCl₂ | 87 |
| +CuCl₂ | >1 |

* The concentration of nucleotides and divalent cations was 1 mm.
*b One μg of hTIP49b and 10 ng of the substrate were added to the reaction mixture.

not bind to any kind of GST fusion proteins (Fig. 5A, lower panel), suggesting that the tags are not responsible for the association of hTIP49b and rTIP49a.

We further investigated in vivo interaction of these two proteins by the mammalian two-hybrid system. A positive control experiment in which MyoD and its heterodimer counterpart Id (35) were employed gave high fluorescence intensity (21-fold activation) as expected (Fig. 5B). In this assay, the helicase reaction mixture.

A major objective was to examine the requirements of TIP49 proteins for in vivo DNA unwinding. For this purpose, we analyzed the reaction mixture.

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The helicase reaction was carried out as described under “Experimental Procedures.” Mg(OAc)₂, magnesium acetate.

FIG. 4. Detection of TIP49b in rat tissues. A, specificity of anti-TIP49b and anti-TIP49a antibodies. Five (lanes 1 and 4), 25 (lanes 2 and 5), and 50 (lanes 3 and 6) ng of hTIP49b and rTIP49a, respectively, were separated by SDS-PAGE and subjected to Western blotting with anti-TIP49b (upper panel) and anti-TIP49a (lower panel) antibodies. B, tissue distribution of TIP49b and TIP49a. Whole cell extracts (20 μg each) from various rat tissues were analyzed by Western blotting with α-TIP49b and -TIP49a antibodies.

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**DISCUSSION**

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Perhaps TIP49 family proteins may be eukaryotic RuvB homologs and play a RuvB-like role.

**TIP49b Is a Novel DNA Helicase**—hTIP49b-containing column fractions (Fig. 2A, lane 5) exhibited ssDNA-dependent ATPase and ATP-dependent DNA helicase activities (Fig. 2B and 3A and Table I). A UV cross-linking assay showed that there was no other ATP-binding protein in our preparation except hTIP49b (Fig. 2C). Moreover, as TIP49a and TIP49b exhibited different reaction polarities, the tag moiety of the recombinant TIP49b is thought to have nothing to do with the enzyme activity. As demonstrated previously (23), the other family protein, TIP49a, is a DNA helicase as well as ATPase. hTIP49b utilized ATP and dATP as energy-supplying nucleotides and Mg$^{2+}$ and Mn$^{2+}$ as divalent cations (Table I). The optimal concentration of those co-factors was 0.5–1 m$m$ (data not shown), and those parameters were analogous to those of TIP49a (23).

hTIP49b helicase preferentially moves from the 5’ to 3’ direction for strand displacement (Fig. 3B, lanes 3 and 7). RuvB is enzymatically most similar to hTIP49b among the known bacterial DNA helicases since it has 5’ to 3’ helicase activity (17) and preferentially hydrolyzes ATP and dATP (37). Human DNA helicase IV (28), bovine DNA helicase B, C, and D (38), *Schizosaccharomyces pombe* DNA helicase II (4), human XPD/ERCC2 (11), and its *S. cerevisiae* homolog Rad3 (6) are eukaryotic 5’ to 3’ DNA helicases. However, these helicases had little homology with hTIP49b. Hence, hTIP49b was determined to be a novel 5’ to 3’ DNA helicase.

There are a few known cases of structurally related DNA helicases. UvrD and Rep of *E. coli* have 40% similarity (39). However, they both preferentially move in the 5’ direction. Human RecQ-like family DNA helicases (*i.e.*, WRN (12), and BLM (13)), their yeast homolog Sgs1 (7), and *E. coli* RecQ (40) all move in the 3’ to 5’ direction in their DNA helicase reactions. Our findings provide the first evidence that two closely related DNA helicases move in opposite directions for DNA displacement.

The direction for the helicase reaction was a major difference between TIP49b and TIP49a. Although the amino acid sequence of hTIP49b was similar to that of rTIP49a (41% identity) throughout all regions (Fig. 1A), the C-terminal region of hTIP49b (412–463) was 11 amino acids longer than that of rTIP49a (Fig. 1D). Moreover, there were fewer regions from 146–179 and 196–288 in hTIP49b compared with the corresponding regions in rTIP49a (Fig. 1D). These divergent regions may be responsible for the subtype-specific direction of TIP49 DNA helicases.

**TIP49b and TIP49a Bind to Each Other**—We showed that hTIP49b and rTIP49a bound to each other both in vitro and in vivo (Fig. 5). Moreover, reciprocal immunoprecipitation-immunoblotting revealed that they were included in the same complex in a cell (Fig. 5C). Bacterial RuvB forms a hexameric ring...
surrounding the DNA stretch (41). UvrD, a bacterial DNA helicase involved in DNA replication, forms a homodimer and heterodimer with Rep (39), even though the relevance of the multimerization has not been elucidated. The present study provided evidence that two related DNA helicases associate with each other. The tag moiety of the recombinant proteins is thought not to be responsible for the protein-protein interaction. Although stimulation of each helicase activity was not observed when hTIP49b and rTIP49a were included in the reaction mixture, the native enzyme activities of both enzymes were restored even when they were mixed (data not shown). This implies that these two enzymes can work even if they exist in a complex.

Role of TIP49b and a TIP49a-TIP49b Complex—We showed that scTIP49b and scTIP49a were indispensable for the growth of yeast (Fig. 6). This fact indicated that these two similar genes are involved in fundamental and nonredundant cellular processes. Moreover, it was thought that each gene was not complemented by the other. Different polarities in TIP49a and TIP49b functions with TIP49a in a large complex. Since Qiu et al. (21) reported that TIP49a is included in the RNA polymerase II holoenzyme together with BRCA1 and CREB-binding protein, TIP49b might also be included in the RNA polymerase II holoenzyme. TIP49a was included in a TBP-containing complex (15). We found that TIP49b was also included in a TBP-containing complex. As TIP49 family proteins were DNA helicases, TIP49a and TIP49b were thought to be involved in DNA-related nucleic acid metabolism, especially in transcriptional regulation. An analogous situation is seen in TFIIH. TFIIH is one of the general transcription factors that contains two DNA helicases (XPD/ERCC2 and XPB/ERCC3) (11, 42) and is included in the RNA polymerase II holoenzyme (43). TIP49b and TIP49a may play an analogous role to that of TFIIH in transcriptional regulation.

Alternatively, TIP49b may be involved in homologous recombination and/or recombination repair, since it is structurally related to a bacterial recombination factor, RuvB (Fig. 1C), and it has the same direction as RuvB DNA helicase (17) (Fig. 3B). TIP49b might be a eukaryotic RuvB homolog, although we have not yet been able to detect its branch migration ability. The fact that TIP49b is enriched in the testis and thymus (Fig. 4B) implies that TIP49b participates in recombination. However, since scTIP49b is an essential gene, it is unlikely that TIP49b participates in recombination alone. XPD/ERCC2 and XPB/ERCC3 in TFIIH (see above) participate in nucleotide excision repair as well as transcriptional regulation. Hence, TIP49b may have multiple roles in the nuclear events.

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REFERENCES
1. Gorbalenya, A. E., and Koonin, E. V. (1993) Curr. Opin. Struct. Biol. 3, 419–429
2. Lohman, T. M., and Bjornson, K. P. (1996) Annu. Rev. Biochem. 65, 169–214
3. Matson, S. W., Bean, D. W., and George, J. W. (1994) Bioessays 16, 13–22
4. Lee, C., and Seo, Y. S. (1998) Biochem. J. 334, 377–386
5. Li, X., Yoder, B. L., and Burgers, P. M. (1992) Chromosoma (Berl.) 102, 93–99
6. Sung, P., Prakash, L., Matson, S. W., and Prakash, S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8951–8955
7. Bennett, R. J., Sharp, J. A., and Wang, J. C. (1998) J. Biol. Chem. 273, 9644–9650
8. Tuteja, N., Tuteja, R., Rahman, K., Kang, L. Y., and Falaschi, A. (1990) Nucleic Acids Res. 18, 6785–6792
9. Sung, P., Bailly, V., Weber, C., Thompson, L. H., Prakash, L., and Prakash, S. (1993) Nature 365, 852–855
10. Shen, J. C., Gray, M. D., Oshima, J., and Leeb, L. A. (1998) Nucleic Acids Res. 26, 2879–2885
11. Karrow, J. K., Chakraverty, R. K., and Hickson, I. D. (1997) J. Biol. Chem. 272, 30611–30614
12. Ellis, N. A. (1997) Curr. Opin. Genet. Dev. 7, 354–363
13. Kanemaki, M., Makino, Y., Yoshida, T., Kishimoto, T., Koga, A., Yamamoto, K., Yamamoto, M., Moncollin, V., Egly, J.-M., Muramatsu, M., and Tamura, T. (1997) Biochem. Biophys. Res. Commun. 235, 64–68
14. Makino, Y., Miomeri, T., Kuzke, C., Kanemaki, M., Kurokawa, Y., Inoue, S., Kishimoto, T., and Tamura, T. (1998) Biochem. Biophys. Res. Commun. 245, 819–823
15. Tsaneva, I. R., Muller, B., and West, S. C. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 1315–1319
16. Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K., Launder, S. D., and Rehrauer, W. M. (1994) Microbiol. Rev. 58, 401–465
17. Shinagawa, H., and Iwasaki, H. (1996) Trends Biochem. Sci. 21, 107–111
A New Eukaryotic RuvB-like DNA Helicase TIP49b

20. Shinohara, A., and Ogawa, T. (1995) Trends Biochem. Sci. 20, 387–391
21. Qiu, X. B., Lin, Y. L., Thome, K. C., Pian, P., Schlegel, B. P., Weremowicz, S., Parvin, J. D., and Dutta, A. (1998) J. Biol. Chem. 273, 27786–27793
22. Holzmann, K., Gerner, C., Korosec, T., Pollt, A., Grimm, R., and Sauermann, G. (1998) Biochem. Biophys. Res. Commun. 252, 39–45
23. Makino, Y., Kanemaki, M., Kurokawa, Y., Koji, T., and Tamura, T. (1999) J. Biol. Chem. 274, 15329–15335
24. Kurokawa, Y., Kanemaki, M., Makino, Y., and Tamura, T. (1999) DNA Sequence 10, 37–42
25. Klenk, H. P., Clayton, R. A., Tomb, J. F., White, O., Nelson, K. E., Ketchum, K. A., Dodson, R. J., Gwinn, M., Hickey, E. K., Peterson, J. D., Richardson, D. L., Kerlavage, A. R., Graham, D. E., Kyrpides, N. C., Fleischmann, R. D., Quackenbush, J., Lee, N. H., Sutton, G. G., Gill, S., Kirkness, E. F., Dougherty, B. A., McKechnie, K., Adams, M. D., Loftus, B., Venter, J. C., et al. (1997) Nature 390, 364–370
26. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
27. Armon, T., Ganoth, D., and Hershko, A. (1990) J. Biol. Chem. 265, 26723–26726
28. Tuteja, N., Rahaman, K., Tuteja, R., and Falaschi, A. (1991) Nucleic Acids Res. 19, 3613–3618
29. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4350–4354
30. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489
31. Simanis, V., and Lane, D. P. (1985) Virology 144, 88–100
32. Rothstein, R. J. (1983) Methods Enzymol. 101, 202–211
33. Sherman, F., and Hicks, J. (1991) in Methods in Enzymology Guide to Yeast Genetics and Molecular Biology (Guthrie, C., and Fink, G. R., eds) Vol. 194, pp. 21–37, Academic Press, San Diego, CA
34. Kozak, M. (1986) Cell 44, 283–292
35. Finkel, T., Duc, J., Fearon, E. R., Dang, C. V., and Tomasetti, G. F. (1993) J. Biol. Chem. 268, 5–8
36. Chervitz, S. A., Aravind, L., Sherlock, G., Ball, C. A., Koonin, E. V., Dwight, S. S., Harris, M. A., Dolinski, K., Mohr, S., Smith, T., Weng, S., Cherry, J. M., and Botstein, D. (1998) Science 282, 2022–2026
37. Iwasaki, H., Shiba, T., Makino, K., Nakata, A., and Shinagawa, H. (1989) J. Bacteriol. 171, 5276–5280
38. Thommes, P., Ferrari, E., Jessberger, R., and Hubscher, U. (1992) J. Biol. Chem. 267, 6063–6073
39. Wong, I., Amaratunga, M., and Lehman, T. M. (1993) J. Biol. Chem. 268, 20386–20391
40. Umezu, K., Nakayama, K., and Nakayama, H. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 5363–5367
41. Stasiak, A., Tsaneva, I. R., West, S. C., Benson, C. J., Yu, X., and Egeland, E. H. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7618–7622
42. Coin, F., Bergmann, E., Tremeau-Bravard, A., and Egly, J-M. (1999) EMBO J. 18, 1357–1366
43. Ossipow, V., Tassan, J. P., Nigg, E. A., and Schibler, U. (1995) Cell 83, 137–146
44. Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) Nucleic Acids Res. 22, 4673–4680
45. Southern, E. M. (1975) J. Mol. Biol. 98, 503–517
46. Bauer, A., Huber, O., and Kemler, R. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 14787–14792