TWINKLE Has 5′ → 3′ DNA Helicase Activity and Is Specifically Stimulated by Mitochondrial Single-stranded DNA-binding Protein

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Mutations in TWINKLE cause autosomal dominant progressive external ophthalmoplegia, a human disorder associated with multiple deletions in the mitochondrial DNA. TWINKLE displays primary sequence similarity to the phage T7 gene 4 primase-helicase, but no specific enzyme activity has been assigned to the protein. We have purified recombinant TWINKLE to near homogeneity and demonstrate here that TWINKLE is a DNA helicase with 5′ to 3′ directionality and distinct substrate requirements. The protein needs a stretch of 10 nucleotides of single-stranded DNA on the 5′-side of the duplex to unwind duplex DNA. In addition, helicase activity is not observed unless a short single-stranded 3′-tail is present. The helicase activity has an absolute requirement for hydrolysis of a nucleoside 5′-triphosphate, with UTP being the optimal substrate. DNA unwinding by TWINKLE is specifically stimulated by the mitochondrial single-stranded DNA-binding protein. Our enzymatic characterization strongly supports the notion that TWINKLE is the helicase at the mitochondrial DNA replication fork and provides evidence for a close relationship of the DNA replication machinery in bacteriophages and mammalian mitochondria.

The molecular mechanisms by which mtDNA is replicated in mammalian cells are of fundamental biological interest. Saccharomyces cerevisiae has served as a model system for studies of mammalian mtDNA replication, but there are significant differences between yeast and mammalian cells (1). Replication of the S. cerevisiae mtDNA is initiated from multiple sites of the ~86-kb genome, and the mtDNA molecules frequently undergo recombination. In contrast, the smaller mammalian mtDNAs (~16 kb) initiate DNA replication from two specific origins of replication, oriH and oriL, and recombination is a rare or possibly even non-existent phenomenon (2).

Mammalian mtDNA contains two major promoters, the light and heavy strand promoters, which produce near genomic length transcripts that, after RNA processing, release individual mRNAs, tRNAs, and rRNAs. A separate transcription unit for the rRNA genes in mammalian mitochondria has also been reported (3). Transcription from light strand promoters is not only necessary for gene expression but also produces the RNA primers required for initiation of mtDNA replication at oriH (1, 4). DNA synthesis from oriH is unidirectional and proceeds to displace the parental heavy strand. The nascent H strands frequently terminate 700 bp downstream of oriH, giving rise to 7 S DNA (D-loop strand). This termination event produces a characteristic triple-stranded structure, called the D-loop (5). The function of the D-loops is unknown, but they presumably play a role in regulating mtDNA replication.

The mitochondrial DNA polymerase γ is a heterodimer comprising catalytic (A) and accessory (B) subunits of 140 and 54 kDa, respectively. The accessory subunit, polymerase γ B, which is not present in yeast, has been characterized as a processivity factor for the polymerase (6, 7). Polymerase γ B increases the affinity of the polymerase for DNA and promotes tighter nucleotide binding, increasing the polymerization rate (7, 8). The processivity of DNA polymerase γ is specifically stimulated by the mitochondrial single-stranded DNA-binding protein, mtSSB (9, 10).

The TWINKLE gene was originally identified in a search for mutations associated with chromosome 10q24-linked autosomal dominant progressive external ophthalmoplegia (adPEO) (11), which is a human disorder with exercise intolerance, muscle weakness, peripheral neuropathy, deafness, ataxia, cataracts, and hypogonadism. Homology searches revealed a striking sequence similarity between TWINKLE and the bacteriophage T7 gene 4 protein, which contains both the DNA helicase and the primase activities needed at the bacteriophage replication fork. Primary sequence analysis revealed that TWINKLE contains sequence motives typically found in DNA helicases, whereas no obvious similarities could be identified with known primases.

Interestingly, adPEO is characterized by the presence of multiple mtDNA deletions, and the disorder has also been linked to mutations in DNA polymerase γ (12, 13). Taken together, these data demonstrate a functional relationship between TWINKLE and the DNA polymerase and suggest that TWINKLE may be a DNA helicase active in mammalian mitochondrial DNA replication. A molecular characterization of TWINKLE would therefore not only be of interest for a molecular understanding of adPEO but would also reveal important insights into the molecular mechanisms of mtDNA replication.

We have purified TWINKLE in recombinant form and studied its enzymatic functions. We demonstrate here that TWINKLE is a 5′ → 3′ DNA helicase, which is specifically stimulated by mtSSB. Our findings strongly support the notion that TWINKLE is involved in mammalian mtDNA replication and further demonstrate the remarkable structural and functional similarities between the DNA replication machinery in bacteriophages and mammalian mitochondria.
TWINKLE Is a 5′ → 3′ DNA Helicase

MATERIALS AND METHODS

Recombinant Proteins—Spodoptera frugiperda (Sf9) cells were maintained and propagated in suspension in SFM 900 medium (Invitrogen), contaminated with fetal calf serum, at 27 °C. DNA fragments encoding TWINKLE and mtSSB were PCR-amplified from human cDNAs and cloned into the pBacPAK9 vector (Clontech). The TWINKLE expression construct encoded the mitochondrial form of the protein lacking the import signal (1–42 amino acids). A His6 tag was introduced at the N terminus. The construct for mtSSB also encoded the mitochondrial form of the protein lacking the import signal (1–16 amino acids) but contained no affinity tag. Autographa californica nuclear polyhedrosis viruses recombinant for the individual expression constructs were prepared as described in the BacpakTM manual (Clontech). Protein expression was performed by growing 400 ml of Sf9 cells to a density of 2 × 10^6 cells/ml in suspension. The cells were infected with 10 plaque-forming units/ml of recombinant baculovirus and harvested 72 h after infection. Infected cells were frozen in liquid nitrogen and thawed at 4 °C in 20 ml of lysis buffer containing 25 mM Tris-HCl, pH 8.0, 10 mM β-mercaptoethanol, and 1 × protease inhibitors (for all purifications, a 100 mM phenylmethanesulfonyl fluoride, 200 mM protease inhibitors A, 60 mM leupeptin, and 200 mM benzamidine in 100% ethanol). The cells were incubated on ice for 20 min, transferred to a Dounce homogenizer, and disrupted using 20 strokes of a tight-fitting pestle. Next, NaCl was added to a final concentration of 1.0 M, and the homogenate was swirled gently for 45 min at 4 °C. The extract was cleared by centrifugation at 36,000 rpm for 30 min at 4 °C using a Beckman TLA 100.3 rotor.

Protein Purification—TWINKLE was purified by diluting the protein extracts with an equal volume of buffer A (50 mM Tris-HCl, pH 8.0, 0.6 M NaCl, 20% glycerol, 10 mM β-mercaptoethanol, and 1 × protease inhibitors) containing 20 mM imidazole. The extract was then added to 1 ml of Ni2+-agarose Superflow beads (Qiagen) and incubated for 1 h at +4 °C. Ni2+-agarose beads were collected by centrifugation (JA-17, 2,500 rpm, 10 min, +4 °C), washed once with 15 ml of buffer A containing 40 mM imidazole and centrifuged, and finally loaded into a column. The column was washed with 10 column volumes of buffer A containing 40 mM imidazole and eluted with 15 column volumes of buffer A containing 250 mM imidazole. The recombinant protein was identified by SDS-PAGE and Coomassie Brilliant Blue staining (Fig. 1A). Recombinant TWINKLE migrated as a doublet with an apparent molecular mass of about 70 kDa during SDS-PAGE. The weaker, lower band is a predicted molecular mass of 72 kDa. The purity of the TWINKLE protein was >95% as judged by SDS-PAGE and Coomassie Brilliant Blue staining (see Fig. 5A).

Helicase Substrates—A 60-nt oligonucleotide (MG1, 5′-ACATGAT-AAGATACATGGAATTTGTTGAAACACCAAGTTAAAACGAC-GGCCAGTGCC-3′) was labeled with 32P at its 5′-terminus with T4 polynucleotide kinase (Stratagene) and annealed to M13mp18 single-stranded DNA (Amersham Biosciences) to generate a 20-bp single-stranded region with a 40-nucleotide 5′-tail. The annealed DNA was purified from unannealed oligonucleotide by Centricon 100 (Amicon) using a buffer containing 20 mM Tris-HCl (pH 7.6), 100 mM NaCl, and 0.1 mM EDTA. The helicase substrate used to decide the tail length necessary for unwinding was made by annealing a 60-nt-long oligonucleotide (MG2, 5′-GCGTAACTACAAATGTATTACCTTCCCCCTTTT-3′) or a 20-nt-long one (MG3, 5′-CGGTACGATCTAGAGGACCCC-3′), a 10-nt 3′-tail (MG4, 5′-CGGTCATGCTAGAAGGACCCC-3′), or a 15-nt 3′-tail (MG5, 5′-CGGTACGATCTAGAAGGACCTC3′) (see Fig. 3B).

The helicase substrates used in the directionality assays were made by annealing the radiolabeled MG2 oligonucleotide to either of two different 60-nt-long oligonucleotides (MG6, 5′-CGGTCATGCTTAGAGGACCCCCTGAGAAGGAGGAGT-3′, or MG7, 5′-CTGCGACGAG-AGGACCCAAGGCAGTCAGCCGAGTACCGTGA-TCAGGCC-3′). The MG1/MG6 and the MG1/MG7 annealing reactions also included an additional 40-nt oligonucleotide (MG8, 5′-CTGACTGCGCCGTTCCTGACTCCGAAAATCGTCTTCGAC-3′) to generate the two different fork substrates used in the directionality assays (see Fig. 4A and B). DNA helicase substrates were purified by gel electrophoresis as described (14).

RESULTS

The yield of mtSSB was ~20 mg from a 400-ml starting culture, and the purity was at least 95% as judged by SDS-PAGE and Coomassie Brilliant Blue staining (see Fig. 5A).

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Helicase Assay—The reaction mixture (15 µl) contained 15 fmol of DNA substrate (DNA concentrations in this report are expressed in moles of molecules), 20 mM Tris-HCl (pH 7.6), 10% glycerol, 10 mM dithiothreitol, 4.5 mM MgCl2, 3 mM ATP, 100 µM bsa DNA polymerase, and the indicated amounts of TWINKLE, mtSSB, and Escherichia coli SSB. The reactions were incubated for 20 min at 37 °C for the times indicated and stopped by the addition of 2 µl of stop solution (90 mM EDTA (pH 8.0), 6% SDS, 30% glycerol, 0.25% bromophenol blue, 0.25% xylene cyanol). We did not observe any significant levels of spontaneous reannealing of unwind DNA at the assay conditions used. The products were separated by electrophoresis through a 15% nondenaturing polyacrylamide gel, which was dried onto DE11 (Whatman) and autoradiographed overnight at ~80 °C with an intensifying screen. Intensities of the bands were quantified by densitometry using the program NIH Image (rsb.info.nih.gov/nih-image).

The finding that TWINKLE mutations are associated with mtDNA deletions suggested that the protein might be involved in mtDNA replication, and we therefore characterized the enzymatic activities of the TWINKLE in vitro. We generated a recombinant baculovirus encoding the human TWINKLE gene to obtain sufficient quantities of the protein for studies of its associated biochemical activities. TWINKLE was expressed in insect cells and purified over Ni2+-agarose, hydroxyapatite, SP-Sepharose, and heparin-Sepharose near homogeneity (Fig. 1A). Recombinant TWINKLE migrated as a doublet with an apparent molecular mass of about 72 kDa. The weaker, lower band is a
shorter form of the protein, lacking 15 amino acids at the very C terminus as demonstrated by mass analysis with MALDI-TOF mass spectrometry (data not shown). The truncation is probably due to translational pausing since the relative levels of the two forms are unaffected by protease inhibitors. A strong contaminating exonuclease activity co-purified with TWINKLE over the first two columns, but it was lost at the SP-Sepharose step. Heparin column-purified TWINKLE co-migrated with a strong ATPase activity (Fig. 1B), supporting primary sequence analysis predictions that TWINKLE is a Walker-type ATPase.

TWINKLE Is a DNA Helicase
To investigate whether TWINKLE is an active DNA helicase in vitro, we annealed a 32P-labeled 60-nt oligonucleotide to the complementary region of M13mp18 single-stranded DNA to form a helicase substrate with a 20-bp double-stranded region and a 40-nt 5' single-stranded tail. Examination of the purified protein showed that

![Figure 1. ATPase and DNA helicase activities follow the TWINKLE protein peak from the heparin column.](image1)

**A**

![ATPase activity](image2)

**B**

![Helicase activity](image3)

**C**

![Time course of DNA unwinding](image4)

**Figure 1.** ATPase and DNA helicase activities follow the TWINKLE protein peak from the heparin column. In A, TWINKLE peak fractions (10 μl) from the heparin column were separated by SDS-PAGE (15%) and revealed with Coomassie Brilliant Blue staining. The protein concentration in the peak (fraction 26) was 0.25 mg/ml. M, molecular size marker. In B, the ATPase activity of the protein fractions (1 μl) in panel A was analyzed in the presence of activated calf thymus DNA. C, a helicase assay over the peak fraction from the heparin column. One μl from each fraction was added to the reaction mixture as described under “Materials and Methods” and incubated for 30 min. Lane 1, substrate heated to 100 °C before loading; Lane 2, untreated substrate; S, double-stranded substrate; P, single-stranded product.

**Figure 2.** Helicase activity of TWINKLE. Assays were performed as described under “Materials and Methods” in the presence of the indicated amounts of TWINKLE. A, increasing amounts of TWINKLE incubated for 30 min. In B, helicase reactions were performed as in panel A and then analyzed with phosphorimaging, and the fractional amounts of base-paired substrate and single-stranded DNA product were determined. Each data point is the average of at least three experiments. Error bars represent the S.D. C, time course of DNA unwinding reactions. A reaction mixture (150 μl) was prepared in the presence of 30 nM TWINKLE. At the times indicated, 15-μl aliquots were removed and analyzed by 15% non-denaturing polyacrylamide gel electrophoresis. The gel was scanned and quantified as in panel B.
TWINKLE Is a 5’ → 3’ DNA Helicase

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It indeed possessed a strong DNA unwinding activity, which coincided perfectly with the peak of TWINKLE protein eluting from heparin-Sepharose (Fig. 1C). The helicase activity was not found when mock-injected insect cell extracts were purified in a similar way (data not shown). We next analyzed DNA unwinding as a function of TWINKLE protein concentration and found that addition of increasing concentrations of protein to our helicase substrate revealed a linear increase of displaced oligonucleotide (Fig. 2, A and B). Extensive DNA unwinding (>25%) was observed at protein concentrations above 30 nM of the protein, demonstrating that TWINKLE is a potent helicase in vitro. A time course experiment (Fig. 2C) demonstrated that TWINKLE initiates unwinding of the substrate without any apparent lag phase. The unwinding reaction is dependent on NTP hydrolysis and was inhibited at low concentrations of the non-hydrolyzable ATP analogue, ATPγS (Fig. 3A), TWINKLE was next incubated with a variety of different nucleoside 5’-triphosphates to analyze their ability to act as co-effectors for the helicase activity (Fig. 3B). UTP efficiently supported DNA unwinding, and to a lesser extent, so did ATP, GTP, and dTTP. CTP was a very poor co-effector.

FIG. 3. Co-factor and substrate requirements for the TWINKLE helicase. In A, helicase assays were performed as described under “Materials and Methods” in the presence of 15 fmol of substrate and 30 nm TWINKLE protein. The ATP concentration was kept constant at 5 mM, and increasing amounts of ATPγS were added. Lane 1, substrate heated to 100 °C before loading; lane 2, untreated substrate; lane 3, no ATP; lane 4, 50 μM ATPγS; lane 5, 100 μM ATPγS; lane 6, 500 μM ATPγS. S, double-stranded substrate; P, single-stranded product. In B, helicase assays were performed as described under “Materials and Methods” except that the ATP was changed to indicated nucleoside 5’-triphosphates (3 mM). Reactions were incubated for 40 min in the presence of 15 fmol of substrate and 20 nm TWINKLE protein. C, helicase assays with varying lengths of the 3’-tail. An increasing amount of TWINKLE protein was added to 20 fmol of template and incubated for 30 min. A schematic representation of the templates used is included under the figure. Lanes 1, 6, and 11, untreated substrates; lanes 2, 7, and 12, substrates heated to 100 °C before loading.

TWINKLE Has 5’ → 3’ Helicase Directionality—We next investigated the efficiency by which TWINKLE could unwind different DNA substrates. To this end, we made a series of constructs with a 60-nt-long oligonucleotide annealed to complementary oligonucleotides to form helicase substrates with a 20-bp double-stranded region, a 40-nt single-stranded 5’-tail, and 3’-tails of varying lengths (0, 10, 15 nt). No unwinding was observed with substrate lacking a 3’-tail, low levels of unwinding were observed with the 10-nt 3’-tail, whereas the 15-nt 3’-tail template was efficiently unwound (Fig. 3C). Similar experiments were also performed with a 40-nt 3’-tail substrate and varying lengths of the 5’-tail (0, 10, 15 nt). We found that unwinding was dependent on a free 5’-tail as well. No unwinding was observed on the template lacking a 5’-tail. TWINKLE displayed a moderate activity on the 10-nt 5’-tail substrate and efficiently unwound the 15-nt 5’-tailed template (data not shown).

TWINKLE needs a fork-like structure with both a 5’- and a 3’-single-stranded stretch of DNA to efficiently initiate DNA unwinding, similar to what had been shown previously for the T7 gp4 helicase (15). We could not use common DNA substrates for a directionality assay, due to this specific substrate requirement. Specific DNA substrates have been developed to circumvent this problem for the T7 gp4 protein. We used a directionality assay with substrates containing a 20-bp double-stranded region with one single-stranded and one double-stranded tail (Fig. 4, A and B) (16). TWINKLE requires a single-stranded stretch of DNA to initiate unwinding, and we could therefore determine the directionality of the enzyme by introducing a single-stranded 5’- or 3’-tail. We found that TWINKLE could only unwind the substrate with a 5’-single-stranded tail, thus demonstrating that TWINKLE unwinds DNA in the 5’ to 3’ direction, as does the T7 gp4 helicase.

The TWINKLE Helicase Is Stimulated by mtSSB—We determined whether human mtSSB had any stimulatory effect on the TWINKLE helicase activity by using the same substrate as in Fig. 1C. We expressed recombinant mtSSB in insect cells and purified the protein to near homogeneity (Fig. 5A). We found that mtSSB had a strong stimulatory effect on the unwinding activity of the TWINKLE protein (Fig. 5B). The stimulation by mtSSB was specific because no such effect was observed with the E. coli SSB.
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T.W. A., W. B., and C. C. (1999) Annu. Rev. Biochem. 66, 409–435
2. E. Walker, A., and A. Walker, C. (2001) J. Mol. Biol. 30, 430–435
3. M. Christiansen, T., Levens, D., Rahimowitz, M., and Attardi, G. (1992) Proc. Natl. Acad. Sci. U. S. A. 79, 7185–7189
4. D. D. and D. H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 351–355
5. K. Dobson, L., and Vinograd, J. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 2252–2257
6. C. E., Libby, M., L., and Copeland, W. C. (1999) J. Biol. Chem. 274, 38197–38203
7. D. H., T., K., and B. C. (1999) Biochemistry 38, 1792–1798
8. M. B., Marsault, J., and Barat-Gueride, M. (1988) Eur. J. Biochem. 174, 479–484
9. C. L., W., Y., and K., L. (1999) J. Biol. Chem. 274, 14779–14785
10. J. N., L. Y., T., V., N., K., Y., P., T., W., M., V., G., G., M., R., B., F., P., J., A., J., H., T., Z., M., and L. (2001) Nat. Genet. 28, 222–231
11. L. A., and K., J. (2001) Am. J. Med. Genet. 106, 53–61

DISCUSSION

The mechanisms of mammalian mitochondrial mtDNA replication have not yet been fully defined (1). We have initiated a project aimed at reconstituting mtDNA replication in vitro, which we hope will generate new insights into this fundamental cellular process. According to the generally accepted model, mammalian mtDNA replication is continuous on both strands and takes place in a strand-asymmetric mode. DNA synthesis is initiated from two different sites, one for each strand (17). Activation of oriL and the heavy strand origin oriH are physically and temporally distinct. DNA synthesis first commences at oriH, which is localized in the non-coding region of mtDNA. After leading strand synthesis has reached two-thirds of the genome, it comes to oriL, which is activated, and the DNA synthesis then initiates in the opposite direction. Recently, however, this strand-asymmetric model for mtDNA replication has been challenged by two-dimensional gel electrophoresis analysis demonstrating the presence of conventional duplex mtDNA replication intermediates, indicative of coupled leading and lagging-strand DNA synthesis (18, 19). A detailed biochemical analysis of these processes in vitro may help to clarify the molecular mechanisms of mtDNA replication.

We demonstrate here that the mammalian TWINKLE protein displays the classical features of a DNA helicase: it catalyzes the ATP-dependent unwinding of a DNA duplex with a 5′ → 3′ polarity. The protein requires specific substrates with a single-stranded 5′-DNA loading site and a short 3′-tail to initiate unwinding. The preferred substrate thus resembles the conformation of a DNA replication fork, a structure with which the TWINKLE protein would be expected to interact. The substrate requirement is also similar to what has been described previously for the T7 gp4 protein (15) and other hexameric helicases, such as DnaB (20). The ability of the TWINKLE protein to utilize various nucleoside 5′-triphosphates as co-effectors for helicase activity is interesting. ATP efficiently supports TWINKLE-mediated DNA unwinding, but UTP is clearly a much more potent cofactor. The physiological relevance of this observation remains to be established.

mtSSB has a stimulatory effect on the rate of DNA unwinding, and this effect is specific as the E. coli SSB cannot substitute for mtSSB. The observed specificity may be due to a direct interaction between mtSSB and the TWINKLE protein. Physical interactions between replicative helicases and their endogenous single-stranded DNA-binding proteins have been demonstrated in other systems, e.g. the herpes simplex virus type 1 helicase-primase complex is specifically stimulated by the viral SSB, ICPS (21, 22). The specific stimulatory effect by mtSSB therefore supports the notion that TWINKLE is the replicative DNA helicase in mammalian mitochondria.

The identification of TWINKLE as a DNA helicase means that only one essential function, a primase, is missing from the mitochondrial replisome. A primase activity is constantly required at the replication fork for coordinated leading and lagging DNA strand synthesis, whereas a strand-asynchronous mode of replication would only need primase activity for initiation of lagging strand DNA synthesis at oriL. The T7 gp4 protein contains a primase activity, which synthesizes primers at the bacteriophage DNA replication fork (23). Primary sequence comparisons suggest that TWINKLE has lost its primase activity, but such an activity cannot be ruled out without careful biochemical analysis (11). Wongs and Clayton (24, 25) previously reported a primase activity specifically acting at oriL in mammalian mitochondria. The oriL region forms a conserved hairpin structure, which may function as an attenuation site for the TWINKLE helicase and the mitochondrial replisome. The paused replisome may then recruit a mitochondrial primase, which can synthesize the primer needed for initiation of light strand DNA synthesis. Alternatively, the hairpin structure could function as a signal to activate an atypical primase activity in the TWINKLE protein itself. Future efforts will be directed toward investigating these intriguing possibilities for additional functions of TWINKLE.

REFERENCES

1. Shadel, G. S., and Clayton, D. A. (1997) Annu. Rev. Biochem. 66, 409–435
2. Eyre-Walker, A., and Awadalla, P. (2001) J. Mol. Biol. 30, 430–435
3. Montoya, J., Christianson, T., Levens, D., Rahimowitz, M., and Attardi, G. (1992) Proc. Natl. Acad. Sci. U. S. A. 79, 7185–7189
4. Chang, D. D., and Clayton, D. A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 351–355
5. Kasamatsu, H., Robberson, D. L., and Vinograd, J. (1971) Proc. Natl. Acad. Sci. U. S. A. 68, 2252–2257
6. Carrodeguas, J. A., Kobayashi, R., Lin, S. E., Copeland, W. C., and Bogenhagen, D. F. (1999) Mol. Cell. Biol. 19, 4039–4046
7. Lim, S. C., Longley, M. J., and Copeland, W. C. (1999) J. Biol. Chem. 274, 38197–38203
8. Johnson, A. A., Tsai, Y., Graves, S. W., and Johnson, K. A. (2000) Biochemistry 39, 1702–1708
9. Mignotte, B., Marsault, J., and Barat-Gueride, M. (1988) Eur. J. Biochem. 174, 479–484
10. Farr, C. L., Wang, Y., and Karginski, L. S. (1999) J. Biol. Chem. 274, 14779–14785
11. Spellman, J. N., Li, F. Y., Tiranti, V., Nikali, K., Yuan, Q. P., Tarqi, M., Wangrooj, S., Garrido, N., Coma, G., Morandi, L., Santoro, L., Toscano, A., Fabrizi, G. M., Somer, H., Croxon, R., Beeson, D. Poulton, J., Suomalainen, A., Jacobs, H. T., Zeviani, M., and Larsson, C. (2001) Nat. Genet. 28, 222–231
12. Suomalainen, A., and Kaukonen, J. (2001) Am. J. Med. Genet. 106, 53–61

Figure 5: The TWINKLE helicase is specifically stimulated by mtSSB. In A, mtSSB (2 μg) purified over hydroxypatite was separated by SDS-PAGE (14–20%) and revealed with Coomassie Brilliant Blue staining. The faint smear below the protein is an artifact in the SDS-PAGE analysis. The protein migrated as one single peak in mass determination with MALDI-TOF mass spectrometry. In B, DNA helicase assays were performed as described under “Material and Methods” using the MG01/M13mp18 helicase substrate. The reactions contained 60 nM TWINKLE protein when indicated and increasing amounts of mtSSB and E. coli SSB (EcSSB). The reactions were incubated for 30 min. The unwinding reaction was unaffected by EcSSB but stimulated 2.6–2.8 fold (lane 9) with mtSSB. Lane 1, substrate heated to 100 °C before loading; lane 2, untreated substrate; lane 4, 475 nM mtSSB; lane 5, 475 nM E.Coli SSB; lane 7, 47.5 nM mtSSB; lane 8, 153 nM mtSSB; lane 9, 475 nM mtSSB; lane 11, 47.5 nM E.Coli SSB; lane 12, 153 nM E.Coli SSB; lane 13, 475 nM E.Coli SSB. Double-stranded substrate, lane 1, single-stranded product.
13. Ponamarev, M. V., Longley, M. J., Nguyen, D., Kunkel, T. A., and Copeland, W. C. (2002) J. Biol. Chem. 277, 15225–15228
14. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Vol. 1, Section 6.46–6.47, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
15. Matson, S. W., and Richardson, C. C. (1983) J. Biol. Chem. 258, 14009–14016
16. Ahnert, P., and Patel, S. S. (1997) J. Biol. Chem. 272, 32267–32273
17. Clayton, D. A. (1982) Cell 28, 693–703
18. Holt, I. J., Lorimer, H. E., and Jacobs, H. T. (2000) Cell 100, 515–524
19. Yang, M. Y., Bowmaker, M., Reyes, A., Vergani, L., Angeli, P., Gringeri, E., Jacobs, H. T., and Holt, I. J. (2002) Cell 111, 495–505
20. LeBowitz, J. H., and McMacken, R. (1986) J. Biol. Chem. 261, 4738–4748
21. Tanguy Le Gac, N., Villani, G., Hoffmann, J. S., and Boehmer, P. E. (1996) J. Biol. Chem. 271, 21645–21651
22. Falkenberg, M., Bushnell, D. A., Elias, P., and Lehman, I. R. (1997) J. Biol. Chem. 272, 22766–22770
23. Benkovic, S. J., Valentine, A. M., and Salinas, F. (2001) Annu. Rev. Biochem. 70, 181–208
24. Wong, T. W., and Clayton, D. A. (1985) J. Biol. Chem. 260, 11530–11535
25. Wong, T. W., and Clayton, D. A. (1985) Cell 42, 951–958
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