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Hyperthermophilic Topoisomerase I from Thermotoga maritima

A VERY EFFICIENT ENZYME THAT FUNCTIONS INDEPENDENTLY OF ZINC BINDING*

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Topoisomerases, by controlling DNA supercoiling state, are key enzymes for adaptation to high temperatures in thermophilic organisms. We focus here on the topoisomerase I from the hyperthermophilic bacterium Thermotoga maritima (optimal growth temperature, 80 °C). To determine the properties of the enzyme compared with those of its mesophilic homologs, we overexpressed T. maritima topoisomerase I in Escherichia coli and purified it to near homogeneity. We show that T. maritima topoisomerase I exhibits a very high DNA relaxing activity. Mapping of the cleavage sites on a variety of single-stranded oligonucleotides indicates a strong preference for a cytosine at position -4 of the cleavage, a property shared by E. coli topoisomerase I and archaeal reverse gyrases. As expected, the mutation of the putative active site Tyr 288 to Phe led to a totally inactive protein. To investigate the role of the unique zinc motif (Cys-X-Cys-X₁₆-Cys-X-Cys) present in T. maritima topoisomerase I, experiments have been performed with the protein mutated on the tetracysteine motif. Strikingly, the results show that zinc binding is not required for DNA relaxation activity, contrary to the E. coli enzyme. Furthermore, neither thermostability nor cleavage specificity is altered in this mutant. This finding opens the question of the role of the zinc-binding motif in T. maritima topoisomerase I and suggests that this hyperthermophilic topoisomerase possesses a different mechanism from its mesophilic homolog.

Hyperthermophilic organisms have evolved a variety of strategies to adapt their physiology to extreme environments. In particular, molecular machineries in charge of genetic information have to function at temperatures near the boiling point of water.

Topoisomerases, a class of enzymes specialized in manipulating DNA, are involved in the maintenance and control of DNA supercoiling. In mesophilic bacteria (*i.e. Escherichia coli*), the DNA supercoiling control is achieved mainly through the balance between negative supercoiling impulsed by gyrases and DNA relaxation by topoisomerase I (1, 2). In hyperthermophilic organisms, this control appears particularly important for life at high temperatures. Indeed, at such temperatures, the opening of the double helix is favored, and the control of supercoiling is crucial to maintaining the stability of the DNA duplex while ensuring initiation of essential processes such as replication, transcription, and recombination/repair.

Little information about the DNA supercoiling control in hyperthermophilic bacteria is available. To get a better view of this process, we used *Thermotoga maritima* as a model organism. In this bacterium, which grows at 80 °C, we have already described three topoisomerases: a gyrase (3) and a topoisomerase I (4), as found in all other bacteria, but also a reverse gyrase (5) as found in hyperthermophilic archaea (6). This third topoisomerase is able to introduce positive supercoils into DNA. Surprisingly, in *T. maritima*, episomal DNA (3, 7) is negatively supercoiled as in *E. coli*, contrary to the hyperthermophilic archaea in which episomal DNA is positively supercoiled (8). Thus, in *T. maritima*, gyrases and topoisomerase I probably control the global level of DNA supercoiling as in mesophilic bacteria.

In the present work, we have focused on the study of the *T*. *maritima* topoisomerase I overexpressed in *E. coli* and used mutational analysis to explore some of its biochemical properties. The protein is composed of 633 amino acids. Sequence analysis (4, 9) has shown previously that this protein is clearly related to the topoisomerase IA subfamily, the representative of which is topoisomerase I from *E. coli*. Similarities are located particularly within the first 540 amino acids of the protein. In this region, all the conserved sequence motifs defined for the other members of the family are found in the *T. maritima* enzyme, and Tyr²⁸⁸ was proposed to be the active site tyrosine (4).

Here, we show that *T. maritima* topoisomerase I relaxes DNA at 75 °C with an unusually high specific activity compared with the enzyme from *E. coli* and is also able to decatenate nicked kinetoplast DNA networks. Analysis of the DNA cleavage reaction, which constitutes the first step of the topoisomerization cycle, shows a strong preference for cytosine at position -4 of the cleavage point as is the case for *E. coli* topoisomerase I (10) and archaeal reverse gyrases (11, 12). We have also confirmed that Tyr²⁸⁸ is the active site residue involved in this reaction. Interestingly, although relaxation is highly sensitive to temperature, the cleavage step by *T. maritima* topoisomerase I is very efficient at 24 °C.

Examining the C-terminal part of the protein including the last 93 amino acids, no similarity with the other topoisomerases of the family was found (4), except a putative zinc-binding motif with the structure Cys-X-Cys-X₁₆-Cys-X-Cys (Fig. 1). This type of tetracysteine motif is present in the C-terminal part of most bacterial topoisomerases I sequences with a variable number of copies of up to four. Interestingly, all of these motifs share a significant similarity (Fig. 1). In *E. coli* topo-

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isomerase I, it has been shown that zinc binding to the three tetracysteine motifs present in the protein is required for DNA relaxation activity (13, 14). Here we show that, contrary to the case of *E. coli*, the unique zinc-binding motif of *T. maritima* topoisomerase I is not required for the topoisomerization reaction. Indeed, the enzyme mutated on the tetracysteine motif loses the ability to bind zinc but retains wild type activities. The function of the zinc motif in *T. maritima* topoisomerase I remains unknown, but our results support the idea that the mechanism of the hyperthermophilic topoisomerase I may be somewhat different from that of its *E. coli* mesophilic homolog.

EXPERIMENTAL PROCEDURES

Cloning and Protein Expression—A DNA fragment encoding T. maritima topoisomerase I was prepared by polymerase chain reaction using genomic DNA as template, *Pfu* DNA polymerase (Expand High Fidelity kit, Roche Molecular Biochemicals) and the following primers: 5'-AGAGGTGGAAGA<u>GGATCC</u>GAGTAAGAAAGTGAAGAAATATAT-CGT-3' as forward primer containing a *Bam*HI restriction site and 5'-TCATACCCGAGT<u>GTCGAC</u>TCAAGAGCCTTTTTTACCCTTTCTC-CGCTT-3' as reverse primer containing a *Sal*I restriction site. Polymerase chain reaction products were inserted into the pET29b expression vector (Novagen) at the *Bam*HI/SalI sites, and constructs were checked by DNA sequencing.

The resulting expression plasmid was transformed into *E. coli* BL21(DE3). A colony of transformed cells was picked and grown at 37 °C in LB medium with 30 µg/ml kanamycin until A_{600} reached 0.5–0.6. Induction was carried out with 1 mM isopropyl-1-thio- β -D-galactopyranoside for 3 h at 37 °C (A_{600} , 1.0–1.2), and the cells were harvested and stocked at -80 °C until required. About 15 g of cells were obtained from 4 liters of culture. The same procedure was followed to express the mutant enzymes.

Protein Purification-Frozen cells were resuspended in 10 volumes of buffer A (50 mM Tris-HCl, pH 7.5, 0.1 M NaCl), lysed by sonication, and centrifuged at 40,000 $\times\,g$ for 15 min at 4 °C. The crude extract was then heated for 15 min at 75 °C and immediately centrifuged at $40,000 \times g$ for 20 min at 4 °C. To remove DNA, polymin P was added to the supernatant to a final concentration of 0.36%. After stirring for 1 h, the solution was centrifuged for 1 h at 90,000 \times g. Ammonium sulfate (70% saturation) was added to the supernatant, and the solution was stirred overnight at 4 °C before centrifugation (24,000 \times g for 30 min). The pellet was dissolved in 7 ml of buffer A and dialyzed extensively against this buffer at 4 °C. The solution was clarified by centrifugation at $24,000 \times g$ for 20 min and applied to a heparin-Sepharose column (BioSepra) equilibrated with buffer A. Elution was performed by applying a gradient of 0.1-1 M NaCl in buffer A. Active fractions eluted around 0.5–0.6 M NaCl. They were pooled and concentrated in Amicon concentrator (Centricon 30) before loading onto a gel filtration column (Superdex S200, Amersham Pharmacia Biotech) equilibrated with 50 mM Tris-HCl pH 7.5, 0.6 M NaCl. Glycerol was added to a final concentration of 15% prior to storage of the active fractions at -20 °C. Protein concentrations were determined by the method of Schaffner and Weissmann (15), with BSA¹ as a standard, or spectrophotometrically using the theoretical molar extinction coefficient of the topoisomerase I $(\epsilon_{\rm 278}$ $= 56254 \text{ M}^{-1} \text{ cm}^{-1}$). A very good correlation was found between these two methods.

Mutagenesis—Site-specific mutagenesis within the *T. maritima* topoisomerase I gene, replacing Tyr²⁸⁸ (TAT codon) by a Phe (TTT codon), was performed using the QuickChange site-directed mutagenesis kit (Stratagene) and the following primers: 5'-GGGCATATCGCGTTCATAACCTTTATGAGAACAGATTCAACGCG-3' and 5'-CGCGTTGAATC-TGTTCTCATAAAGGTTATGAACGCGATATGCCC-3' (forward and reverse primers, respectively). The replacement of Cys⁵⁵⁹ and Cys⁵⁶¹ (the two first cysteines of the tetracysteine motif) by alanines was performed with the ChameleonTM double-stranded site-directed mutagenesis kit (Stratagene). The sequence of the mutagenic oligonucleotide was: 5'-CCGACGAACCAGAAAGCTTCGGCTGGAAAGGAAATGAGG-3' (mutated positions are underlined), resulting in a change of the two cysteine codons TGT into two alanine codons, GCT. Mutants were identified by DNA sequencing of the plasmid DNA.

Zinc Content Measurements—The zinc content of wild type and mutant proteins was determined both by a colorimetric method and flame atomic absorption spectroscopy. The colorimetric assay was conducted as follows. A solution of 2.5 mM *p*-hydroxy(mercuri)benzenesulfonate (PMPS) was added by increments of 1 μ l to a 500- μ l sample of 10 μ M of enzyme (in 10 mM Tris-HCl, pH 7.5, 200 mM NaCl, 5% glycerol) containing 0.1 mM 4-(2-pyridylazo)resorcinol (PAR). Zinc release from the enzyme was monitored by absorbance at 500 nm of the PAR₂:Zn(II) complex for which molar absorptivity is 6.6 10⁴ M⁻¹ cm⁻¹ (16). The zinc content determination using flame atomic absorption was performed using a Varian AA775 spectrometer. The protein samples (49 μ M) were dialyzed against a buffer containing 10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, and 150 mM KCl. The instrument was calibrated using a solution of ZnCl₂. Measurements were performed with three dilutions of the enzyme and using dialysis buffer as a blank.

Fluorescence Measurements—Intrinsic tryptophan fluorescence was measured at 20 °C in a Bio-Tek SFM25 spectrofluorimeter. The excitation wavelength was 295 nm. Fluorescence measurements were carried out in a buffer composed of 50 mM Tris-HCl, pH 7.5, 0.1 mM DTT, and 150 mM KCl with a protein concentration of 0.5 μ M.

DNA Relaxation Assay—The reaction mixture (10 μ l) contained 50 mM Tris, pH 8.0, 0.5 mM DTT, 30 μ g/ml BSA, 10 mM MgCl₂, 120 mM NaCl, and 200 ng of negatively supercoiled plasmid DNA (pTZ 18). The reaction was initiated by adding purified topoisomerase I, which was serially diluted in a buffer containing 50 mM Tris-HCl, pH 8.0, 1 mM DTT, and 100 μ g/ml BSA. After 30 min of incubation at 75 or 50 °C (or as otherwise indicated), the reaction was stopped by adding 2 μ l of stop solution containing 50 mM EDTA, 2.5% SDS, 25% glycerol, and 0.2% bromphenol blue. Samples were electrophoresed through 1.2% agarose gel in TEP buffer (90 mM Tris-phosphate, pH 8.0, 2 mM EDTA) for 2.5 h at 4 V/cm. Gels were stained with 1 μ g/ml ethidium bromide and photographed under UV light.

Decatenation of Catenated Kinetoplast Minicircular DNA (kDNA)— The reaction mixture was identical to that used in the DNA relaxation assay except that negatively supercoiled pTZ 18 was replaced by 200 ng of kinetoplast DNA (TopoGEN). The activity was measured using serial dilutions of enzymes. Incubations were performed for 30 min at 75 °C as described in the paragraph above. The incubation products were analyzed by treatment with 0.5 units of *XhoI* (New England Biolabs) for 30 min at 37 °C. For the control of kDNA decatenation with human topois somerase II (TopoGEN), 2 units of enzyme and 1 mM ATP were added to the reaction mixture, and the incubation was performed at 37 °C for 30 min. After the addition of the stop solution, samples were electrophoresed and stained as described above. When required, 1 μ g/ml ethidium bromide was added in gel and electrophoresis buffer.

Oligonucleotides Radiolabeling—Oligonucleotides were synthesized and purified by MWG Biotec. 10 pmol were 5'-end-labeled by 5 units of T4 polynucleotide kinase (New England Biolabs) in the presence of 20 μ Ci of [γ -³²P]ATP in 10 μ l of the buffer recommended by the manufacturer. After 30 min of incubation at 37 °C, the reaction was stopped by heating the samples at 75 °C for 15 min. The oligonucleotide size markers (Amersham Pharmacia Biotech) were labeled following the manufacturer's protocol.

Oligonucleotide Cleavage Assay—0.1 pmol of 5'-end-labeled oligonucleotide was incubated with 1 pmol of *T. maritima* topoisomerase in a buffer containing 50 mM Tris-HCl, pH 8, 0.5 mM DTT, 30 μ g/ml BSA, and 60 mM NaCl for 15 min at 75 °C (unless otherwise indicated) in a final volume of 10 μ l. The reaction was stopped by the addition of 1% SDS and 10 μ l of loading buffer (97.5% formamide, 10 mM EDTA, 0.3% bromphenol blue, 0.3% xylene cyanol). The samples were denatured by heating at 95 °C for 3 min and loaded onto a 18% polyacrylamide gel (19:1) containing 50% (w/v) urea in TBE buffer (90 mM Tris, 90 mM boric acid, 1 mM EDTA). The gel was electrophoresed at 1300 V for 2 h and autoradiographed. Quantification was performed with the ImageQuant v1.2 software.

RESULTS

Overexpression and Purification of T. maritima Topoisomerase I and Its Mutants—After amplification of the T. maritima topoisomerase I gene by polymerase chain reaction from genomic DNA and subcloning into the pET29b expression vector, the recombinant protein was expressed in E. coli. Expression and purification steps are summarized in Fig. 2. Induction with isopropyl-1-thio- β -D-galactopyranoside at 37 °C led to the production of an additional protein migrating to the expected molecular mass of the recombinant T. maritima topoisomerase

¹ The abbreviations used are: BSA, bovine serum albumin; PMPS, *p*-hydroxy(mercuri)benzenesulfonate; PAR, 4-(2-pyridylazo)resorcinol; DTT, dithiothreitol; kDNA, kinetoplast minicircular DNA.

TOPI Tm 550: VUD. FPTN.QKGSG.KENLSF.GKYGFUKE.EGKTREVKNDE.:551 TOPI Hi 3706: LKG.YDGPVVEDUK.G.G.ADMELKI.GRF.G.LKLHEPNCKTNKALKSDV.654 TOPI VC 707: VKG.YEGPVVEDUK.G.G.SUMVLKN.GRF.G.K.KMGTNDCDMTRKILKNG:752 TOPI VC 3701: LKG.YEGPSELEDK.G.SEMULKN.GRF.G.K.KMGTNDCMTRKLIKNG:748 TOPI EC 3701: LKG.YEGPSELEDK.G.SEMULKN.GRF.G.K.KMGTNDCKMTRKLIKNG:748 TOPI Ba 696: .KSPIVSKIC.EK.Y.V.NNKLKI.GFF.G.K.K.FFGTNFCKMTRKLIKNG:748 TOPI Ba 696: .KSPIVSKIC.EK.Y.V.NNKLKI.GFF.G.K.KFFGTNFCKMTRKLIKNG:748 TOPI TP 1 581: GVPD.KGTNG.K.KS.SENUSK.G.G.G.KMTRT. TOPI TP 1 606: NND.SKCELEKG.GENVYKK.GRF.G. AFLANNY.P.D.CKTRKSKNYPF. TOPI TP 1 591: GVPD.SENTOVY SQ.G.G.TMYKKL.GEF.G. AFLANNY.P.D.CKKRYKTINLEPT. TOPI TP 1 591: RND.JEKCYCWF.G.K.G.SENTOVY SQ.G.G.TMYKKL.GEF.G.W.FCGSGXLPP.KERKTTINLFGE.646 TOPI TP 1 591: RNQ.PTLTNIP TRE.G.G.CMPMIRT.AST.G.G.VVEGSGXLPP.KERKTTINLTGLEE.646 TOPI TP 1 595: RNQ.PTLTNP TRE.G.G.MMKRT.ATT.G.WVEGSGXSLPP.KERKTTINLGGEGEGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG		(ALANDER) SNOLDED CHARMEN BOLDER	
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TODI Pa 703: I.KSPILLEDK. G.S.F.(C.S.F) C.S.F.(C.S.F) C.S.F.(C.S.F) C.S.F.(C.S.F) C.S.F.(C.S.F) C.S.F.(C.S.F) C.S.F.(C.S.F) C.S.F.(C.S.F) D.S.F.F.C.S.G.G.F.F.V.K.S.F.G.S.F.F.C.S.G.G.G.F.V.K.S.F.S.R.S.G.G.G.S.F.V.G.K.F.S.R.G.S.F.F.C.S.G.G.G.F.V.K.F.S.R.G.S.F.G.S.G.G.G.G.S.G.F.F.S.R.G.S.F.G.S.F.G.S.G.G.G.F.V.F.C.S.G.G.G.F.F.C.S.G.G.G.F.V.F.C.S.G.G.G.F.F.K.S.F.G.S.F.F.G.S.G.G.G.G.S.G.G.G.S.G.G.S.F.G.S.G.G.G.S.G.G.G.S.G.G.S.F.G.S.G.G.G.S.G.G.G.S.F.G.S.G.G.G.S.G.G.G.S.F.G.S.G.G.G.S.G.G.G.S.G.G.G.S.F.G.S.G.G.G.G		707: .VKGIEGPTVECDK	G. SDMVLKN GRF G KIMGGTND ACKNTRKILKNG. : 752
TOPI Ba 696: .KSPIYLSKTIQLEKCY.NNMKLKIGPFGKFFKISNRD.L.TIGNTRKLLPNG.: 742 TOPI Bh 559: EIEPSYAGEDELGS.SPMYVKMGRYGKFFKISNRP.D.RNTKFIVKGI.: 615 TOPI Bh 559: EIEPSYAGEDELGS.SPMYVKMGRYGKFFKISNRP.D.RNTKFIVKGI.: 615 TOPI TP 561: GYDSKNGRYGKFFKISNRP.D.RNTKFIVKGI.: 615 TOPI TP 561: GYDSKNGRYGKFFKISNRP.D.RNTKFIVKGI.: 615 TOPI TP 561: GYDKCGTWKKIGRFGFFLGGER.P.E.RNTYTVKGI.: 613 TOPI VC 1.596: PNNIVMTNILDT.C.GG.RKWGITASTGVFLGGSGYGLPP.KERKTTINLGDEE.: 646 TOPI Pa 1.591: RANQPTITNIPREG.G.RMMAITASTGVFLGGSGYSLPP.KERKKTTNILTPEN.: 638 TOPI Da 2.661: INTITSLNCPIC.C.KK001KTAITG.VFLGCGVSNPP.R.ERKTTINLTMILTIN.: 638 TOPI Ma 2.663: INTITSLNCPIC.C.KK001KRRKFNRKN.K.R.TFIGWFLGCSVSNPP.NRFTKKNNAS.: 711 TOPI Mage2 657: INTITSLNCPIC.C.KK001KRTKFNPNK.TFIGCSNP.P.NRFTKKNAS.: 711 TOPI Mage2 663: COKERVER.SKK.N.K.N.K.N.K.R.TFIG.SNP.P.NRFTKKNNAS.: 711 TOPI Mage2 664: COKERVER.SKK.N.K.N.K.N.K.R.TFIG.SNP.P.NRFTKKNNAS.: 711 TOPI Mage2 616: COKERVER.SKK.N.K.N.K.N.K.TKF.NDPNRFTKCGSN		701: .IKGYDGPIVECEK	G.SEMHLKMGRFGKYMACTNEECKNTRKILRNG.:746
TOPI BS 1 569: EIRDEYAGED EL CS. SPMVYEMGRYGKFLACSNEPD.CRNTKFIVKQI.:615 TOPI BJ 561: EIRDEYTGEMEK.G. G.HEVVEKL.GRFGKFLACSNEPD.CRNTKFIVKQI.:615 TOPI BJ 606: NANDEXTOYV SQCG.DTMVKKL.GRFGFLACKF.PD.CRNTKFIVKQI.:615 TOPI EC 590: NOM.NVLTSID PTCG.RKMGIRT.ASTGVFLGCSGXLPP.KER.KTTINLGDEE. TOPI VC 596: NNNDLXTNTNIL PTCG.RKMGIRT.ASTGVFLGCSGXLPP.KER.KTTINLGDEE. TOPI HI 595: PNS.LVETDIK PTCG.RNMAI.T.ASTGVFLGCTGXALPP.KER.KTTINLIGDEE. TOPI TA 591: ANNVETDIK PTCG.RNMAI.T.ASTGVFLGCTGXALPP.KER.KTVINLIPOD. TOPI TA 366: LNNTVPTSLN PICC.KKMGIKT.AITGVFLGCTGXALPP.KER.KTVINLIPOD. TOPI M2 663: NLTLEKTINE PICC.KKMGIKT.AITGVFLGCTGY.PKER.KTVINLIPOD. TOPI M2 663: NLTLEKL.EEL PE. N.SQLVKRRTKF.NNKK.TVGCSNF.PRCRIKKNNLKKKTINLITIN. TOPI M2 661:GVK PK CKKWRR.KKK.K.K.K.K.K.K.K.VFCGSNF.PRCRIKKNNAS.:711 TOPI M3 616:GVK PK CKKEVKR.K.KK.K.K.K.K.K.YFCGSNF.PRCRIKKNDNAS.:715 TOPI M3 653: NAKETIE.GVK PE. CG.CGUVERK.SKK.K.K.K.K.K.YFCGSNF.PRNTKKDNAS.:715 TOPI M3 653: NAKETIE.GVK PE. CG.CGUVERK.SKK.K.K.K.K.K.YFCGSNF.PRNTKKDNAS.:715 TOPI M3 653: NAKETIE.GVK PE.	TopI Pa 3	703: .IKGYEGPSLECDK	CG.SEMQLKTGRFGKFFGCTNPTCKNTRKLLKNG.:748
TOPI BS 1 569: EIRDEYAGED EL CS. SPMVYEMGRYGKFLACSNEPDCRNTKFIVKQI.:615 TOPI BJ 563: EIRDEPTGEMEK.C.G.HEMVYEMGRYGKFLACSNED.CRNTKFIVKQI.:615 TOPI JD 606: NANDEXTOYV SQCG.DTMVKKL.GRFGFLACKFPDCRNTKFIVKQI.:615 TOPI JC 1 581: GVFDEKTOVV SQCG.DTMVKKL.GRFGFLACKNT.PDCRNTKFIVKBLKNTP.:652 TOPI VC 1 590: NUMMVLTSID PTCG.RKMGIRT.ASTGVFLGCSGXALPP.KERKKTTINLGDEE.646 TOPI HI 1 595: PNS.LVETDIKPT.CG.RNMAI.T.ASTGVFLGCTGXALPP.KERKKTTINLGDEE.646 TOPI HA 1 595: PNS.LVETDIKPT.CG.RNMAI.T.ASTGVFLGCTGXALPP.KERKKTVNLIPED.6643 TOPI BA 1 586: LNN.TVPTSLNPFI.CC.KKMGIKT.AITGSLGCTNY.P. CCHTVNLFKGK.6844 TOPI BA 1 586: LNN.TVPTSLNPFI.CG.KKMKR.KR.GKF.GSEGCTNP.P.C.NTKKDNAS.:711 TOPI MD2 663: .NLTLEKL.EEL PE.CN.SQLVKRR.TKF.NNAKK.TFVGCSNP.PR.CNTKKDNAS.:716 TOPI MD2 661:GVK PK CGRSUKRR.KKK.K.K.K.N.VFGGSNP.PRCRTKKKDNAS.:716 TOPI MD2 616:GVK PK CGRSUKRR.KKK.K.K.K.N.VFGGSNP.PRCNTKKDNAS.:711 TOPI MD3 616:GVK PK CGRSUKRR.KKK.K.K.R.VFGGSNP.PRCNTKKDNAS.:711 TOPI MD3 616:GVK PK CGRSUKRR.KKK.K.K.R.VFGGSNP.PRCNTKKDNAS.:71657 <td>TODI Ba 3</td> <td>696: KSPIYLSKTIOCEK</td> <td>Y.NNMKLKIGPFGKFFTCINKICKNTRKILPNG.:742</td>	TODI Ba 3	696: KSPIYLSKTIOCEK	Y.NNMKLKIGPFGKFFTCINKICKNTRKILPNG.:742
TOPI Bh 1 569: EIKDEPTCEMEEK. CG. HERVYTKMGRYGKTMACSNFPDCRNTKFITUKEI.:615 TOPI TP 581: GVFDEKTTOVY SQ. GG.DTYVKKIGRYGAFLACNKYPECNTKSILNTF.:624 TOPI TP 2 606: NANDEAXQELEK. CG.GENVQKFSRNGAFLACNKYPECNTKSILNTF.:651 TOPI KI 1 596: NANVUTTSID CFG.G.RNGHTASTGVFLGCSGVALPP.KERCKTTINLTPEN::661 TOPI HI 591: RANOPTLTNIP REG.RNMAIT.T.ASTGVFLGCSGVALPP.KERCKTTINLIPGD::643 TOPI BI 586: NADTUPTINT PTG.C.KKNGUKRTASTGVFLGCSGVALPP.KERCKATVNLIPGD::633 TOPI BI 2 663: NLTLEKL.ELE PEN.SQUKRR.TRFNSK.TEGCSNFPNCTHTKNDAKS.:711 TOPI MG2 657: KLTLETL.DEL PEN.SQUKRR.TRF.NNNK.TETGCSNFPNCTHTKDNASE::701 TOPI BI 2 616 GVK PSGEGENVERK.SKK.R.R.K.N.K.YETGCSNFPNCTHTKDNASE::706 TOPI BI 2 616 GVK PSGEGENVERK.SKK.R.R.G.K.FYCGSNFPNCNFTKNDASE::701 TOPI CI 2 616 GVK PSGEGENVERK.SKK.R.R.G.K.FYCGSNFPNCNFTKNDASE::701 TOPI DI 2 616 GVK PSGEGENVERK.SKK.R.R.R.VFYCGDAY.PLCNFTKNDASE::701 TOPI DI 3 653:NAKAMGT.GIT PSCXGEVVERK.SKK.R.R.R.VFYCGDAY.PLCNFTKNDASE::702 <tr< td=""><td></td><td>569: ETEP EYAGEDCEL</td><td>S. SPMVYKM. GRYG KFLACSNEPDCRNTKPIVKOI. :615</td></tr<>		569: ETEP EYAGEDCEL	S. SPMVYKM. GRYG KFLACSNEPDCRNTKPIVKOI. :615
TopI Tp 1 581: GVFDEKTDVVCSQC.DTVVKLIGRFGFFLACGKFP.L.CRNTGLVPT: 624 TopI Hp 2 606: NANDEAKQELCEKC.G.GEVVQKFSRNGVFLGCSQXALPP.KERCKTTINVPF.: 652 TopI Ec 1 590: NQMMVLTSIDEPTC.G.RKMGIRTASTGVFLGCSQXALPP.KERCKTTINVPF.: 6531 TopI Vc 1 595: PNNLVETDIK PTS.RPKIRTASTGVFLGCSQXALPP.KERCKTTINLIPEAE: 6467 TopI Ba 1 595: PNNLVETDIK PTC.G.RNMIRTASTGVFLGCSQXLPP.KERCKTTINLIPEAE: 647 TopI Ba 1 586: LNNIVETDIK PTC.C.KKMGIKTAITGVFLGCSQXLPP.KERCKTTILIPEAE: 647 TopI Ba 1 586: SADDTFWDAPCAL.C.G.GQMKVRH.GKF.GSFLGCENN.P.K.KATVNLIPCD.: 643 TopI Mp 2 663: NLTLELL.EELCPL.N.SQLVKRTKF.MPNK.TFVGCSNF.PRC.RIVKKNNAS.: 711 TopI Mg 2 657: KLTLET.DLCPL.N.N.SQLVKR.TKF.MKK.N.K.TFVGCSNF.PRC.RIKKNNAAE.: 706 TopI Mg 2 616:		569 . ETKD EPTGEMOEK	CG HEMUYEM GRY G KEMACSNE P. DCRNTKPTVKET :615
TOPI FD FD GO FANDAL.ERKCELER. G. GREVOKF.SRN.G. AFLACNNY. P. CONTRELLER. CONTENDATION TOPI 1596: NOM.MUTTSIDET. G. REMGIRT.AST.G. VFLGE SCALPP.KERCINTINUEDE.: 646 TOPI 11 5951: PNS.LUNTDIK PT.C.G.REMAIT.AST.G. VFLGE SCALPP.KERCINTINUEDE.: 646 TOPI PA1 591: RANOPTLTNIP REG.REMAIT.AST.GVFLGE SCASLPP.KERCINTINUEDE.: 643 TOPI PA1 586: LANUPTSINUEDE.C.C.KKNGITT.AIT.GVFLGE CCANNTENKREKARTUNUED.: 643 TOPI G.ST.LINN.LUPTSINUEDE.C.C.KKNGITT.AIT.GVFLGE CCANNTENKREKARTUNUEFKG.: 684 TOPI G657: KLTLELELPE.N.SOLVKRE.NEF.NEVCSINP.PRQCHYIKNNAS.: 716 TOPI G257: KLTLELELPE.N.SOLVKRE.NEK.NEK.K.R.VFVGEDR.PEQCHYIKNNAS.: 716 TOPI B2 616: GVKCPS. GEGENVERK.SKK.R.R.VFVGEDR.PEQCHYIKNNAS.: 657 TOPI B2 616: GVKCPS. GEGENVERK.SKK.R.R.VFYGEDR.PK. GVKCPS. 657 TOPI G13 631 KAKANOT.GITC 25. GGGDIALKR.SK.K.R.R.VFYGEDR.PK. <td></td> <td>591. CUED EKTONOCO</td> <td>C DTMUTET OFF C FFT ACEF D FORMODUD :524</td>		591. CUED EKTONOCO	C DTMUTET OFF C FFT ACEF D FORMODUD :524
TopI EC 1 590: NOM. MVLTSIDCTT. G.RRMGIRT. AST. GVFLCGSGALPP. KER KTTINUGDE: :646 TopI Hi 1 595: PNS. IVUTDIKCTT. G.RNMAIT. AST. GVFLCGSGALPP. KER KTTINLIGDE: :646 TopI Ha 1 595: PNS. IVUTDIKCTT. G.RNMAIT. AST. GVFLCGSGALPP. KER KATTINLIGDE: :646 TopI Ba 1 586: LNN. IVUTDIKCT. G.RNMAIT. AIT. GVFLGSGAVLPP. KER KATVILIPED. :643 TopI Cn 2 368: YAD. DTPWDAPCAL. G.GONKVRH. GKF. GSFLGENY. P CHYIKDNAK: KKRINLIPEKG. :684 TopI Mg 2 663: .NLTLEKL.EELCPE. N.NGLVKR. TKF. NPNK. TFVG SNF. PR CRYIKKDNAS. :711 TopI Mg 2 663: .NLTLEKL.EELCPE. N.NGLVKR. TKF. NPNK. TFVG SNF. PR CRYIKKDNAS. :711 TopI Mg 2 661:GVKCPS. GEGNIVERK. SKK. N. K. YFIG TGY. PE CRYIKKDNAS. :710 TopI Mg 2 616:GVKCPS. GEGNIVERK. SKK. R. K. VFIG DRY. PR CRYIKKDNAS. :701 TopI GJ 2 619:GVKCPS. GEGNIVERK. SKK. R. R. C. VFIG DRY. PR ONFISHKPIN. :657 TopI GJ 2 619:GVKCPS. GEGNIVERK. SKK. R. R. C. VFIG DRY. PR ONFISHKPIN. :699 TopI GJ 3 632:DT GVQCPQGKGEVEFF. SK. R. G. KFYG SAY. PRNFISKYRPSE. :666 TopI Mg 3 632:DT GVQCPQGKGEVEKK. SRY. G. K. LFYS STY. PDONFISHKPIN. :799 TopI Mg 1 614:		SOL. GVFDEKIDVVCSQ	
<pre>TopI Vc 1 596: PNH. TVMTNILCTT. G.S.RPMGIRT. AST. GVFLGCSGVGLPP.KERCKTTILLGDEE.:646 TopI Pa 1 591: RANO. PTLTNIPDEE. G.G.RPMMIRT. AST. GVFLGCSGVALPP.KERCKTTILTPEAE:647 TopI Pa 1 586: LNN. TVPTSLNOPIL. C.KKNGIRT. AST. GVFLGCSGVALPP.KERCKTTILTPEAE:647 TopI Pa 2 368: YAD. DTPWDAPCAL. G.GOMKVRH. GKF. GVFLSCLGVNNTDNKKKCKTLITLTA. 638 TopI Cm 2 368: YAD. DTPWDAPCAL. G.GOMKVRH. GKF. GVFLSCLGVNNTDNKKKCKTTLTLTA. 638 TopI Mg 2 663: .NLTLEKL.ELCPE. N.SOLVKR. TKF. NNRK. TFVG SNF. PR RYIKKDRAS. 1711 TopI Mg 2 657: .KLTETL DELCPE. N.SOLVKR. TKF. NARKK. TFIG.SNF. PR RYIKKDRAS. 1716 TopI U 2 627:IL NELCPN. S.KSLVKR. NKK. N. K.YFIG TGY. PE NFIKKLNASE. 1706 TopI B 2 616:GVKCPS. GEGENIVERK. SKK. K.R. R. VFYGO TGY. PE OFFWORDKPIE. 657 TopI C 2 619: .KAKANGT GITCPS. QKGETVERK. SKK. R. R. VFYGO NGY. PE DFVSWDKPIE. 657 TopI B 2 616:GVKCPS. GEGENIVERK. SKK. R.R. VFYGO NGY. PE DFVSWDKPIE. 657 TopI C 353: .NAKETIE.GVKCPE. G.GDIALKR. SK. K.G. SFYGONY. PK NFISKIKPSE. 1666 TopI Mp 3 653: .NAKETIE.GVCCPE. G.GDIALKR. SK. K.G. SFYGONY. PK NFISKIKPSE. 1666 TopI Mp 3 653: .NAKETIE.GVKCPE. G.GDIALKR. SK. K.G. SFYGONY. PKNYTSUNHVPIN. 1725 TopI Cm 1 585: .FIPRIVT.WENDCR. HKGKLVKKW. SKN. R. YFFG SEY. PTDYNTSEERLT. 632 TopI Mg 608: .KKEKELV.ERDCPS. CA.SPLIYPY. TKR.GNE. K. FVGG SNF. PK. KYFESPNKP. 1652 TopI Mg 61 609: .KKEKELV.ERDCPS. CA.SPLIYPY. TKR.GNE. K. FVGG SNF. PK. KYFESPNKP. 1655 TopI Mg 61 609: .KKEKELV.ERDCPS. CA.SPLIYPY. TKR.GNE. K. FVGG SNF. PK. KYFESERST. 657 TopI Mg 61 609: .KKEKELV.ERDCPS. CA.SPLIYPY. TKR.GNE. K. FVGG SNF. PK. KYFESERST. 657 TopI Mg 61 609: .KKEKELV.ERDCPS. CA.SPLIYPY. TKR.GNE. K. FVG SNF. PK. KYFESERST. 657 TOPI Mg 61 609: .KKEKELV.ERDCPS. CA.GELVKKN.SR. YG B. P. K. KYFESERST. 657 TOPI Mg 61 609: .KKEKELV.ERDCPS. CA.GELVKKN.SR. YG B. P. FVG SNF. PK. KYFESERST. 657 TOPI Mg 63: .EUVYES.SPLIYPY. TKR.GNE. K. FVG SNF. PK. KYFESERST. 657 TOPI Mg 63: .EUVYES.SPLIYPY. TKR.GNE. K. FVG SNF. PK. KYYFESERST. 657 TOPI Mg 63: .EUVYES.SPLIYPY. C.G.GELVKKN.SR. YG</pre>		606: NANDEAKQELCEK	G.GEMVQKFSKNGAFLACNNYPECKNTKSLKNTP.:052
<pre>TopI Hi 1 595: PNS.LVETDIKOFT. G.RNMAIT. AST. GVFLG GYALPP KER KTYTNLIPEAE:643 TopI Ba 1 586: LNN. IVPTSLNOPI. C.KKMGIKT. AIT. GVFLG GYSLPP KER KATVNLIPEA. 643 TopI Mg 2 663: NLTLEKL ELG G.GOMKVRH. GKF. GVFLG SYSLPP KER KATVNLIPEA. 643 TopI Mg 2 663: NLTLEKL ELG E. N.SCIVKR. TKF. NPNK. FVG SNT. P CHYIKNAS. 711 TopI Mg 2 657:KITLETL DEL PE. N.NKIVKR. TKF. NPNK. TFVG SNT. PR RYIKKNAS. 711 TopI Mg 2 663:GVKCPS. GEGNIVERK. SKK. K. N. K.YFIG TGY. PE. C.FYSWDKPIA. 670 TopI B 2 616:GVKCPS. GEGNIVERK.SKK. R. R. VFYG DY. PD CFYSWDKPIA. 657 TopI B 2 616:GVKCPS. GEGNIVERK.SKK. R. R. VFYG NY. PR NFISHKKPS. 657 TopI C 2 619:GVKCPS. GEGNIVERK.SKK. R. R. VFYG NY. PR NFISKYRPSE. 656 TopI G 3 633: NAKETIE GYKCPS. GEGNIVERK.SKK. R. R. VFYG SY P NFISKYRPSE. 656 TopI G 463:DT GYQCPQ. KKGNIVERK.SKK. R. R. VFYG SY PD NFISKYRPSE. 656 TopI Mg 682:DT GYQCPQ. KKGNIVERK.SKK. R. RFYG SY PLNFISKYRPSE. 666 TopI Mg 682:DT GYQCPQ. KKGNIVERK.SKY. R. R. VFYG SY PLNFISKYRPSE. 666 TopI Mg 682:DT GYQCPQ. KKGNIVERK.SKY. R. R. VFYG SY PLNFISKYRPSE. 666 TopI Mg 682:DT GYQCPQ. KKGNIVERK.SKY. R. R. VFYG SY PLNFISKYRPSE. 662 TopI Mg 682:DT GYQCPQ. KKGNIVERK.SKY. R. R. VFYG SY PLNFISKYRPSE. 662 TopI Mg 682:DT GYQCPQ. KKGNIVERK.SKY. R. R. VFYG SY PLNFISKYRPSE. 662 TopI Mg 682:DT GYQCPQ. KKGNIVERK.SKY. CFYG SY PLNFISKYRPSE. 662 TopI Mg 6160:KKEKELV ERD FK. NDFLVKYN.SKN R. R. YFFC SY PL</pre>		590: .NQMMVLTSIDCPT	CG.RKMGIRTASTGVFLGCSGYALPP.KERCKTTINVPEN :631
TODI Pa 1591:RANQ.PTLTNIP RE. G.RPMMIRT.AST.GVELG GCYSLPF.KER KATVNLIPGD.f643TODI Ba 1586:LINN.IVPTSLNPI.C.KKMGIKT.ATT.GVELG CGVNLPKRKCKTNILTLATA.638TODI Mp 2663:LINLTLEKL ELL PE.G.GOMKVRH.GKF.GSEGCENY.POHYIVNLFKKG.f848TODI Mg 2663:NLTLEKL ELL PE.N.SQLVKRR.FKF.NPNK.TFVGCSNF.PR.CRYIKKDNAS.111TODI Mg 2627:LINEL PL.NEL PE.N.NKLVKRR.FKF.NAKK.TFVGCSNF.PR.CRYIKKDNAS.1711TODI Du 2627:LINEL PN.CS.KSLVKR.NKK.N.K.N.FFIGCTGY.PE.CNFIKKDNAAE.657TODI BL 2616:GVK OFS.CGEGNIVERK.SKK.R.R.N.FFIGCTGY.PE.CPFVSNDKPIE.657TODI BL 2616:GVK OFS.CGEGNIVERK.SKK.R.R.R.VFYGCNOY.PE.CPFVSNDKPIE.666TODI Hg 3653:.NAKETIE.GVK OFS.CGEGUEKF.SK.R.K.G.R.FYGCSY.PE.CNFISKYKPSE.6666TODI Hg 3653:.NAKETIE.GVK OFS.CG.G.GDIALKR.SKK.R.R.R.VFYGCNY.PE.CDFVSNDKPIE.662TODI Mg 653:.NAKETIE.GVK OFS.CA.SPLLYRY.VRR.GNE.KFYGCSNF.PN.CNFISNHKPIN.659TODI Mg 654:DT.GVC OPC.CKKGNLVERK.SKY.R.R.R.VFYGCSNF.PT.CDVKTSEEELT.652TODI Mg 655:FIPRIVT.EMD PR.CARALVERK.SKY.R.R.VFYGCSNF.PT.CVKTSEEFSORKF.662TODI Mg 600:.KKVEYIE.GVK OPS.CA.SPLLYRY.VRR.GNE.KFVGCSNF.PK.CKYEKSQKP.6657TODI Mg 600:.KKVEYIE.GVK EL.CH.AKLIKNV.AKKGN.RDNTFLGCSNF.PK.CKYEKSEQKF.6557TODI Mg 1563:EIVYEE.GVK EL.CH.AKLIKNV.SK.S.R.YG.E.FIACNY.PK.CKYIKSEQKF.6617TODI Mg 2563:EIVYEE.GVK EL.CH.AKLIKNV.SK.S.R.YG.PC.K.TKSLEPF.K.CKYEKSEQKF.6557TODI Mg 3669:KKVEYIE.GKN EP.C.GEVKNN.S.R.YG.EFIACNY.PK.CKYIKSLEPFK.6617TODI Mg 4564:FIVE.GVK EF.C.G.CHLIKK.GK.GR.G.C.P.FVGCSNF.PK.CKYIKSLEPFK.6617TODI Mg 565:FIVYEE.GVK EL.CH.CKK.GR.GR.YG.G.P.C.	TopI Vc 1		
<pre>TopI Pa 1 591:RANQ.PTLTNIP RE. G.RPMMIRT.AST.GVELGCGCYSLPE.KERCKATVNLIPGD.f643 TopI Em 1 586:LINN.IVPTSLNPE.C.KKMGIKT.ATT.GVELGCGVNFDENKRCKKTNLITLI.F38 TopI Cm 2 366:LINN.DTFWDAPAL.G.G.GOMKVRH.GKF.G.SELGCENY.P.C.OHYIVNLFKKG.f884 TopI Mg 2 663.NLTLEKLEEL PE.N.SQUVKR.TKF.PT.G.S.SELGCENY.P.C.OHYIVNLFKKG.f884 TopI Mg 2 657:.KLTLET.DEL PE.N.NKLVKR.TKF.NRK.TFIGSNF.PR.RYIKKDNAAE.706 TopI Uu 2 627:INEL PN.CS.KSLVKR.NKK.N.K.PFIGCTGY.PE.CNFIKKDNAAE.706 TopI B1 2 616:GVKOPS.GEGNIVERK.KKK.K.N.K.PFIGCTGY.PE.CNFIKKDNAAE.706 TopI B1 2 616:GVKOPS.GEGNIVERK.KKK.R.R.VFYGCNOY.PE.CPFVSWDKPIE.667 TopI B1 2 616:GVKOPS.GEGNIVERK.SKK.R.R.N.K.PFIGCTGY.PE.CDFVSWDKPIE.6657 TopI Cj 2 619:.KAKANGTGITOPS.QKGEIVERF.SK.R.R.G.FYGGNY.PE.CDFVSWDKPIE.666 TopI Hg 3 653:.NAKETIE.GVKOPE.G.G.GDIALKR.SK.K.R.R.LEYSGNY.PL.CNFISNHKPIN.659 TopI Mg 653:.NAKETIE.GVKOPE.G.G.GDIALKR.SK.K.R.R.VFYGCNY.PL.CNFISNHKPIN.659 TopI Mg 653:.NAKETIE.GVKOPE.S.ASPLVRY.VKR.GNE.KFYGGSPY.PD.CVYAWNEPYA.725 TopI Mg 614.KKEKEFV.GRD PS. OASPLVRY.VKR.GNE.K.FYGGSPF.PK.CKYFESQKF.662 TopI Mg 6169.KKVEYIE GND PS. OASPLVRY.VKR.GNE.K.FYGGSPF.PK.CKYFESQKF.662 TopI Mg 61609.KKVEYIE GND PS. OASPLVRY.VKR.GNE.K.FYGGSPF.PK.CKYFESQKF.6657 TopI Mg 61609.KKVEYIE GND PS. G.SUVRE.GRY.G.P.PVGGSPF.PK.CKYFESDKF.662 TopI Mg 2 656EIVYEE.GVE EL.CH.AKLIRRV.AKKGRN.KDNTFLGSNF.PK.CKYFKSEQKF.6557 TOPI Mg 2 656EIVYEE.GVE EL.CH.AKLIRRV.AKKGRN.KDNTFLGSNF.PK.CKYFKSEQKF.6557 TOPI Mg 2 656EIVYEE.GVE EL.CH.AKLIRKV.AKKGRN.KDNTFLGSNF.PK.CKYFKSEQKF.661 TOPI Mg 2 566EIVYEN.FX.GESPD.CG.GELVKKN.SR.YG.F.PIACNN.PK.CKYKSLEPEK.709 TOPI U 1 576.KVPDEKK.NRL FI CALLIRK.SM.K.R.R.G.PIACNN.PK.CKYKSERSK.661 TOPI Mg 2 656EIVYEN.FX.GESPD.CG.GELVKKN.SR.YG.F.F.FXALNP.PK.CKYFKSEQKF.662 TOPI Mg 2 656EIVYEN.FX.GESPD.CG.GELVKKN.SR.YG.F.PIACNN.PK.CKYKSEPEK.709 TOPI U 1 576.KVPDEKK.NRL FI CALLIRK.SMN.FX.FT.GS.K.F.FIGCNY.PK.CKYFKSEEFF.704 TOPI MG 2 656EIVYEN.FX.FC.GRUVKY.SRT.GS.N.FD.FTFECKNY.PK.CKYFKSEEFF.704 TOPI MG 2 656EIVYEN.FX.FC.GFLOFF.GK.G.G.PVYEN.FT.FFC.FYALNP.FYE.CKYFFFFFF.FYE.FYEFFFEFF.FYE.F</pre>	TODI Hi 1	595: .PNSLVETDIKCPT	G.RNMAI.T.ASTGVFLGCTGYALPP.KERCKTTINLIPEAE:647
TODI Ba1586:INT. IVPTSLN PI.C. KKMGIKT. ATT. GVELSCIGYNNTDNKKR KKTINLITLN. :638TODI Cm3663:YAD. DTPWDAP AL. G. GOMKVH. GKF. GSELGCNY P OCHIVUNLFKKG. :684TODI Mge2663:.NLTLEKL EEL PE. N. SQLVKRR. TKF. NPNK. TFVGCSNF. PR CHIKKDNAS. :711TODI Mge2657:.KLTLETL.DEL PE. N. SKLVKRK. NRK. NAKK. TFVGCSNF. PR CHIKKDNAS. :711TODI U627:ILNELPN. CS. KSLVKRK. NRK. NRK. N. FFIGCSNF. PN. CRIKKDNAS. :716TODI B2616:GVKCPS GEGNIVERK. SKK. NR. K. N. FFIGCSNF. PD. CFFVSWDKPIE. :657TODI G1619:.KAKANGT.GITCPS. QKGETVERF. SK. R. R FFIGCSNY. PD. CFFVSWDKPIE. :657TODI M3682:DT.GVQCPQ. CKKGNLVERK. SKK. R. R. G. KFYGCSAY. PK. CNFISNHKPIN. :699TODI M3682:DT.GVQCPQ. CKKGNLVERK. SKNR		591 : RANO PTLTNTPCRE	G. RPMMTRT. AST
TODI Cm 2368: YAD. DTPWDAPCAL.CG.GQMKVRH. GKFGSFLCCENTPOCHYIVNLFKKG.:684TODI Mg 2663: .NLTLEKL.ELL PE.N.SOLVKRR. TKF. NPK. TFVGCSNFPN.CFLVKKDNAAE.:701TODI Mg 2657: .KLTLETL.DEL PE.N.NKTVKRR. TKF. NAKK.TFIGCSNFPN.CFLKKDNAAE.:706TODI Du 2627:L.NELCPNCS.KSLVKRK.NKK.N.K.K.K.TFIGCSNFPN.CFLKKDNAAE.:706TODI Bb 2616:GVKCPS.GECNIVERK.SKK.NK.N.KVFTGCDIXPE.NFIRNLDESNN:670TODI Cj 2619: .KAKANGTGTCPS.CKGETVERK.SKK.K.R.N.K.YFTGCDIXPE.DFVSWDKPIA.:657TODI Cj 2653: .NAKETTE.GVKCPE.G.GDIALKR.SKK.K.R.R.K.G.SFYCCNNYPKOFLSNKKPSE.:666TODI Mj 3682:DT.GVQ PQ.CKKGNLVERK.SKY.G.K.LEYS STY.PD.ONYATWNPPVA.:725TODI Mj 3682:DT.GVQ PQ.CKKGNLVERK.SKY.G.K.LEYS STY.PD.ONYATWNPPVA.:725TODI Mj 1614: .KKEKEFV.GRDCPS.A.SPLLYN.TKR.GNE.K.FVGCSDF.PRCYSEFSDNFY.:656TODI Mg 1614: .KKEKEFV.GRDCPS.A.SPLLYN.TKR.GNE.K.FVGCSDF.PRCYSEFSDNFY.:657TODI Mg 2658:EIVYEE.GVEEL.CH.AKLTKV.SKN.SNY.G.P.FVCCSDF.PRCYSEFSDNFY.:657TODI Mg 3609: .KKVEYIE.GKNCPN.CS.SQLVRRE.GRY.G.P.FVCCSDF.PRCYSEFSDNFY.:657TODI Mg 4609: .KKVEYIE.GKNCPN.CS.SQLVRRE.GRY.G.P.FVCCSNF.PR.CKYESESNF.:657TODI Mg 4562:EIVYEE.GVEEL.CH.AKLTK.SKN.SNY.T.R.KUENTF.EXT.SNF.PT.CYTKSLEPEEL.:662TODI Mg 561:EKVEYES.SPNC.G.GELVKN.SR.VG.G.F.FVACLNF.PT.CKYESESNFE.:627TODI Mg 1562:EVYEE.GVEEL.CH.AKLTK.GKN.SNY.T.G.E.FVACLNF.PT.CKYESESNEFE.:627TODI Mg 1562:EVYEE.GVEEL.CH.AKLTK.GKN.SNY.T.G.E.FVACLNF.PT.CKYYESLEKFE.:627 <tr< td=""><td></td><td>586 . TANK TUPTSTNOPT</td><td>C KENGLET ATT G VELSELGYNNTONKERCKETTNLTTLN .638</td></tr<>		586 . TANK TUPTSTNOPT	C KENGLET ATT G VELSELGYNNTONKERCKETTNLTTLN .638
TOPI Mp 2663:NLTLEKL.EELCPE.N.SOLVKRR. TKF.NPNK. TFVG SNF.PR.RYIKKDNAS.711TOPI U2627:NLTLETL.DELCPE.N.NKLVKRR. TKF.NAKK.TFIG SNF.PN.RFIKKDNAAE.706TOPI U2616:GVKCPS.CGEGNTVERK.SKK.K. K.R.VFG TGY.PD.CEFVSWDKPIE.657TOPI B2616:GVKCPS.CGEGNTVERK.SKK.K.R.R.K.VFG NGY.PD.CEFVSWDKPIE.657TOPI G12619:.KAKANGT.GITCPS.CCKGETVERF.SKK.R.R.R.VFGCSAY.PK.NNFLSNHKPIE.657TOPI Nm 3682:DT.GVQ.PQ.CKGDIALKR.SKK.G.SFYGCSAY.PK.NNFLSNHKPIN.699TOPI Nm 3682:DT.GVQ.PQ.CKGNUVEK.SRY.G.K.R.K.LFYGCSTY.PD.NYATWNPFVA.725TOPI Nm 3682:DT.GVQ.PQ.CKGNUVEK.SRY.G.K.R.K.LFYGCSTY.PD.NYATWNPFVA.725TOPI Nm 3682:DT.GVQ.PQ.CKGNUVEK.SRY.G.K.R.Y.FGCSTY.PT.CNYATWNPFVA.725TOPI Nm 3682:DT.GVQ.PQ.CKGNUVEK.SRY.G.K.R.Y.FGCSTY.PT.CNYATWNPFVA.725TOPI Mge1608:.KKEKELV.ERDCPK.CN.QUPUYRY.TKR.GNE.K.FYGCSDF.PN.CKYNEFSENFF.662TOPI Mge1608:.KKEKELV.ERDCPK.CN.QUPUYRY.TKR.GNE.K.FYGCSDF.PN.CKYNEFSENFF.657TOPI Mge1608:.KKEKELV.ERDCPK.CG.GELVKK.SRY.GNE.K.FTACSDF.PK.CKYNEFSENFF.657TOPI Mge265		260. VAD DUDADOAT	
TODI MGe2657:.KLTLETL DELCER.N.NKLVKRR.NKK.NAKK.TFIG SNF.PN.CRFIKKDNAAE.706TODI Uu627:			
TODI UU 2627:		663: NLTLEKL.EELCPE	CN. SQLVKRR. TKFNPNK. TFVGCSNFPRCRYIKKDNAS:711
TODI BS 2616:		657:KLTLETL.DELCPE	CN.NKLVKRRTKFNAKKTFIGCSNFPNCRFIKKDNAAE.:706
TODI Bh 2616:GVKCPK.CEKGETVERK.SKK.R.R.R.VEYGCNOY.PEODVSWDRPIA.:657TODI Cj 2619:.KAKANGT.GITCPSQKGETVERF.SK.R.G.KEYGCSAY.PK.NFISKYKPSE.:666TODI Hp 3653:.NAKETIE.GVKCPEG.G.DLAKR.SKY.G.K.LEYSCSTY.PD.NFISKYKPSE.:666TODI TM 3682:DT.GVQCPQ.CKKGNLVERK.SKY.G.K.LEYSCSTY.PD.DYXTWNPPVA.:725TODI TM 15851.FIPRIVT.BMDCPR.CHKGKLVKIW.SKN.R.Y.G.K.LEYSCSTY.PD.DYXTWNPPVA.:725TODI Mg 1614:.KKEKEFV.GRDCPS.CA.SPLLYRY.TKR.GNE.K.FVGCSNF.PN.CXYNEFSQNKP.:662TODI Mge1608:.KKEKELV.ERDCPK.CN.QPLVYRY.TKR.GNE.K.FVGCSNF.PK.CXYNEFSQNKP.:665TODI Mga1609:.KKVEYIE.GKNCPN.CS.SQLVRE.GRY.G.P.FVGCSNF.PK.CKYIEKSEQKP::657TODI Mga2658:.EIVYEE.GVECEL.H.AKLIRV.AKKKGRN.KDNTFLGCSNF.PK.CKYIEKSEQKP::657TODI Hp 1562:.QKVHEKT.GQSCPK.G.GELAIRK.GR.FG.E.FIACNNYPK.CKYIKQTENAN.:608TODI TD 3667:FAVCPQ.C.G.GELAIRK.GR.FG.E.FVACINF.PK.CKYIKQTENAN.:608TODI TD 3667:FAVCPQ.C.G.CFLVEKS.NRR.VG.T.YTACVN.PE.CKYIKYENEKSEK:610TODI TD 3667:FAVCPQ.C.G.CFLVEKS.NRR.VG.T.YTACVN.PE.SYTPPDEGK:599TODI Xf 1552:.TLQVSATPHPQC.C.G.KPLRIP.GRM.G.H.FWGCSGF.KEG.CKATYEDKKKK:660TODI Xf 2603:.KKPVEVS.NFAVK.G.G.NPLKHLVKKGRG.G.YD.FWGCSGF.KEG.CKATYEDKKKK:660TODI Xf 2603:.KKPVEVS.NFAVK.G.G.NPLKHLVKKGRG.G.YD.FWGCSGF.KEG.CKATYEDKKKK:660TODI Xf 2603:.KKPVEVS.NFAVK.G.G.NPLKHLVKKGRG.G.YD.FWGCSGF.KEG.CKATYEDKKGK:660TODI Xf 2658:RKPK.G.G.TAMDSY	TopI Uu 2	627:IL.NELCPN	CS.KSLVKRKNRKN.K.YFIGCTGYPECNFIRNLDESNN:670
TODI Bh 2616:GVKCPK.CEKGETVERK.SK.R.R.G.KFYGCNQY.PEODFVSWDRPIA.:657TODI Cj 2619:.KAKANGT.GITCPSQKGETVERF.SK.R.G.KFYGCSAY.PKCNFISKYKPSE.:666TODI Hp 3653:.NAKETIE.GVKCPEG.GUGLAKR.SK.R.G.K.LFYGCSAY.PKCNFISNHKPIN.:699TODI TM 3682:DT.GVQCPQ.CKKGNLVERK.SKY.G.K.LFYGCSTY.PDCNATWNPPVA.:725TODI Mp 1614:.KKEKEFV.GRDCPS.CA SPLLYRY.TKR.GNE.K.LFYGCSNF.PNCXYNEFSQNKP.:662TODI Mge1608:.KKEKELV.ERDCPK.CN.QFLYRY.TKR.GNE.K.FYGCSNF.PNCXYNEFSQNKP.:656TODI Mge1608:.KKEKELV.ERDCPK.CN.QFLYRY.TKR.GNE.K.FYGCSNF.PK.CKYENEFSNPKP.:656TODI Mge1608:.KKEKELV.ERDCPK.CN.QFLYRY.TKR.GNE.K.FYGCSNF.PK.CKYESENPKP.:657TODI Mge1658:.EIVYEE.GVECEL.CH.AKLIRY.AKKKGRN.KDNTFLGCSNF.PK.CKYIKSLEPEK.:709TODI Mge2658:.EIVYEE.GVECEL.CH.AKLIRY.AKKKGRN.KDNTFLGCSNF.PK.CKYIKSLEPEK.:709TODI Hp 1562:.QKVHEKT.GQSCPK.CG.GELAIRK.GR.FG.E.FIACNNYTODI Mg 2632:.AEQAELD.GRECPK.CG.GELAIRK.GR.FG.E.FVACLNF.PK.CKYIKQTENAN.:608TODI TD 3667:FAVCPQ.CR.CFLVEKS.NRR.VG.T.YTACVN.PK.CKYIKQTENAN.:608TODI TD 3667:FAVCPQ.CR.CFLVEKS.NRR.VG.T.YTACVN.PK.CKYIKQTENAN.:608TODI Xf 1552:.TLQVSATPHPQC.CG.CFLVEKS.NRR.VG.T.YTACVN.PK.CKYISPTERGK.:599TODI Xf 2603:.KKPVEVS.NFAVK.CG.NRRLYC.GR.GR.R.FVG.CSGF.KEG.CKATYEDKKGK.:660TODI Xf 2603:.KKPVEVS.NFAVK.GG.NPLKHLVKKGRG.G.YD.FWGCSGF.KEG.CYTPTDEGGK.:599TODI Xf 2658:FYTQELPCC.GG.KELRIP.GRM.G.R.FYTACMN.PL.CYTACMN.PK.CKYEEPEK.:691TODI S3 <td< td=""><td>TODI Bs 2</td><td>616:GVKCPS</td><td>GEGNIVERK., SKKK. R. VFYGCDRYPDCEFVSWDKPIE.: 657</td></td<>	TODI Bs 2	616:GVKCPS	GEGNIVERK., SKKK. R. VFYGCDRYPDCEFVSWDKPIE.: 657
TopI Cj 2619:KAKANGT.GITCPS.COKGETVERF.SK.R.G.SFYGCSAY.PK.CNFISKYKPSE.:666TopI Hp 3653:DT.GVCPC.CKKGRLVERK.SKK.G.SFYGCNY.PK.CNFISNHKPIN.:699TopI Cm 1585:DT.GVCPC.CKKGRLVERK.SKY.G.K.LFYSCSTY.PD.CNYATVNPPVA.:725TopI Cm 1585:DT.GVCPC.CKKGRLVERK.SKY.G.K.LFYSCSTY.PD.CNYATVNPPVA.:725TopI Mp 1614:.KKEKEFV.GRDCPS.CA.SPLYRY.TKR.GNE.K.FYGCSFY.PT.CDYKTSEEELT::632TopI Mge1609:.KKEKELV.ERDCPR.CN.OPLVYRY.TKR.GNE.K.FYGCSDF.PK.CKYSEFSDNPF::666TopI Mga1609:.KKVEYIE.GKNCPN.CS.SQLVRE.GRY.G.P.FYGCSNF.PK.CKYSEFSDNPF::656TopI Mga1609:.KKVEVIE.GVCEL.CH.AKLIRV.AKKKGRN.NDNTFLGCSNF.PK.CKYTKSLEPEK::709TopI Uu 1578:.EVYDEKI.NRLCPI.CA.HDLLIKR.SMN.T.R.FIACSNF.PT.CRFTESLEKPE::627TopI Hp 1562:.QKVHEKT.GQSCPR.CG.GELVKKN.SR.YG.E.FIACNNY.PK.CKYIKSURPKNESK::610TopI TopI Mm 2632:.AEQAELD.GRECPK.CG.GELVKKN.SR.YG.E.FIACNNY.PK.CKYIKNLKSESK::610TopI Tp 3667:FAVCPQ.CC.GC.KFLRRIP.GKN.GR.FG.E.FVACINF.PK.CKYSRNLKSESK::610TopI Tp 3667:FAVCPQ.CC.GC.KFLRRIP.GKN.G.G.YD.FWGCSCY.PD.CSVTFPDEGGK::599TopI Xf 2603:.KKPVEVS.NFACVK.CG.NRERLP.GKN.G.G.YD.FWGCSCY.PD.CSVTFPDEGK::622TopI S3658:RCPK.CG.SVILVEKK.LKK.GC.G.YD.FWGCSCY.PD.CSVTFPDEGGK::599TopI M1565:.KFTQELDETPK.CG.GENVKEKS.INR.Q.UQVQCSSTopI S3658:RCPK.CG.SVILVEKK.LKK.GC.G.TTLELK.LVCGNN.PT.CDYTEDKGK.:691TopI S3658:RCPK.CG.SVILVEKK.LKK.GC.TTLUCGNN.PT.CDYTEDKGKK.:691 <t< td=""><td></td><td>616: GVKCPK</td><td>CENCETVERK SKK B B VEYCONOY PE ODEVSWOKPTA :657</td></t<>		616: GVKCPK	CENCETVERK SKK B B VEYCONOY PE ODEVSWOKPTA :657
TopI Hp653:NAKETIE.GVKCPE.G.GDIALKR.SK.K.G.SFYGCNNY.PK.NNFLSNHKPIN.:699TopI Nm682:DT.GVCPC.CKKGRLVERK.SRY.G.K.LFYSCSTY.PD.CNYATMNPPVA.:725TopI Mp614:KKEKEFV.GRDCPS.CA.SPLLYRY.TKR.GNE.K.FYGCSNF.PN.CNYATSEELT:632TopI Mge1608:KKEKELV.ERDCPK.CN.QPLVYRY.TKR.GNE.K.FYGCSNF.PN.CKYNEFSQNKP::662TopI Mge1609:KKEKELV.ERDCPK.CN.QPLVYRY.TKR.GNE.K.FVGCSNF.PN.CKYSEFSNPKP::656TopI Mge1608:KKEKELV.ERDCPK.CN.QPLVYRY.TKR.GNE.K.FVGCSNF.PK.CKYSEFSNPKP::656TopI Mge1608:KKEKELV.ERDCPK.CN.QPLVYRY.TKR.GNE.K.FVGCSNF.PK.CKYSEFSNPKP::656TopI Mge1658:EIVYEE.GKCELLH.AKLIRV.AKKGRN.KDNTFLGCSNF.PK.CKYSEFSNPKP::657TopI Mga2658:EIVYEE.GVECEL.H.AKLIRV.AKKGRN.KDNTFLGCSNF.PK.CKYTKSLEPEK::709TopI D1578:KVPDEKI.NRLCPI.CA.HDLLIKR.SNN.T.R.FIACSNF.PT.CRFTESLEKPE::627CDITopI N1563:QKTVTKL.GSSCPD.CG.GELAIRK.SN.YG.E.FIACNNY.PK.CKYIKQTENAN::608TopI N2632:AEQAELD.GRECPK.CG.GELVKKN.SR.YG.F.FG.E.FVACLNF.PK.CKYIKQTENAN::668TopI N2632:AEQAELD.GRECPK.CG.GRLVKS.SNR.VG.T.YTACN.PE.CRYVSPTRKNL::706TopI Xf552:TLQVSATPKHPQE.CG.GRLVKS.SNR.G.K.G.F.G.G.K.FUGCSGF.KEG.CKATYEDKGK::599TopI Xf563:.KKTYQELDETCPK.CG.EHLQIKF.GKM.GG.G.YD.FWGSGF.KEG.CKATYEDKKGK::660TopI Xf563:.KKPVEVS.NFACVK.CG.CHLVEKK.LKK.GG.IQVQCVE.CDYKEEEQK.:667:.KCPK.CG.TKK.QUVCVE.TopI B3658:.RKCPK.CG.CHLVEKKEK.LKK.GG.TELELK.CCN.PE.CKIKKEKC100			
TODI Nm 3682:DT. GVQ.PQ.CKKGNLUVERK.SRYG.K.LFYSCSTYPD.CNYATUNPPVA: 725TODI Cm 1585:FIPRIVT.EMD.PR.CHKGKLVKIW.SKNR.YFGCSEYPT.DYKTSEEELT: 632TODI Mg 1614:KKEKEEV.GRD.PSCN.QFLVYRY.TKR.GNE.K.FVGCSNF.PN.CKYNEFSQNKP: 662TODI Mg 1608:KKEKELV.ERD.CPK.CN.QFLVYRY.TKR.GNE.K.FVGCSNF.PK.CKYNEFSQNKP: 656TODI Mg 1609:KKEKELV.ERD.CPK.CN.QFLVYRY.TKR.GNE.K.FVGCSNF.PK.CKYNEFSQNKP: 656TODI Mg 2658:ELVYEE.GVECEL.CH.AKLIRRV.AKKGRN.KDNTFLGCSNF.PK.CKYTKSLEPEK: 709TODI U 1578:KVPDEKI.NRLCFI.CA.HDLIKR.SNN.T.R.FIACSNF.PT.CRFTESLEKPE: 627TODI Hg 1562:QKVHENT.GQS.PK.CG.GELVKKN.SR.YG.E.FIACNNY.PK.CKYTKQTENAN: 668TODI Cj 1563:QKTVTKL.GESCPD.CG.GELAIRK.GR.FG.E.FVACINF.PK.CKYTKQTENAN:TODI Nm 2632:AEQAELD.GRECPK.CG.GRLVYKY.SRT.GS.K.FIG.VNY.PK.CKYTKQLEPKK.1681TODI Xf 1552:TLQVSATPKHPQE.CG.KFLVEKS.NRR.VG.T.YTACINF.PK.CKYSPTKKNL:706TODI Xf 1552:TLQVSATPKHPQE.CG.KPLVEKS.NRR.UG.G.F.UDV.PKC.SGY.PD.CSVTFPDEGGK:599TODI Xf 1552:TLQVSATPKHPQE.CG.KPLVEKS.NRG.G.R.VD.FWGCSGY.PD.CSVTFVDEGGK:599TODI Xf 2638:RKCPK.CG.NFLKK.UCKKG.G.G.YD.FWGCSGY.PD.CSVTFVDEGGK:599TODI M1568:RKCPK.CG.KFLVKKGG.G.YD.FWGCSGY.PD.CSVTFVDEGGK:599TODI M3658:RKCPK.CG.KFLVKKGG.G.G.YD.FWGCSGY.PD.CSVTFVDEKGK:601TODI B3658:RKCPK.CG.KFLVKKGG.G.G.YD.FWGCSGY.PD.CSVTFVDEKGK:691TODI B4658:CFTCRCFK.CG.TKK.QUVVEKKGK.LKKK.GG.TFLECK.CKKKKD.700TODI VC 2658:FTAALAKKCPI.CG.TA		CE2. NAVANGI.GIICES.	CONSTRUCTION AND A CONSTRUCTION
TopI Cm 1585:.FIPRIVT.EMDCPR.CHKGKUVKIW.SKNR.YFGCSKYPT.CDYKTSEELT.:632TopI Mp 1614:.KKEKELV.GRDCPS.CA.SPLLYRY.TKR.GNE.K.FVGCSNFPN.CKYNEFSONKP::652TopI Mge1608:.KKEKELV.ERDCPK.CN.OPLVYRY.TKR.GNE.K.FVGCSDF.PK.CKYNEFSONKP::656TopI Mga1609:.KKVEYIE.GKNCPN.CS.SOLVRE.GRY.G.P.FVGCSDF.PK.CKYSEFSDNKP::657TopI Mga2658:.EIVYEE.GVCEL.CH.AKLIRRV.AKKGRN.KDNTFLGCSNF.PK.CKYIKSLEPEK::657TopI U1578:.KVPDEKI.NRLPI.CA.HDLLIKR.SNN.T.R.FIACSNF.PK.CKYIKSLEPEK::627TopI U1562:.QKVHEKT.GQSCPK.CG.GELVKKN.SR.YG.E.FIACSNF.PK.CKYIKOLEKEFE::627TopI TopI U1562:.QKVHEKT.GQSCPK.CG.GELVKKN.SR.YG.E.FIACSNF.PK.CKYIKOLEKEFE::627TopI TopI 3663:.QKTVTKI.GESCPD.CG.GELAIRK.GR.FG.E.FVACINF.PK.CKYSRNIKSESK::610TopI Tp3667:FAVCPQ.CG.GRLVKY.SRT.GS.K.FIGCVMYPK.CKYSRNIKSESK::610TopI Tp3667:FAVCPQ.CG.GKERIRIP.GKN.NGH.FWGCSOF.KEG.CKATYEDKKKK::620TopI Xf1552:.TLQVSATPKHPQCC.GG.KERRIP.GKM.GG.R.FVACAOYPE.CSYTRVNETA::622TopI S3658:RSCPK.CG.NKLQIKF.GKM.GC.R.FVACAOYPE.CSYTRNNETA::660TopI K1565:.KFTQELDETCPK.CG.KGC.KKLGIKF.GKM.GC.R.FVACAOYPE.CVYKEPCK.:691TopI S3658:RSCPK.CG.SYTVEKK.LKK.GC.TLQVCVECKKKEMKD.:700TopI S4658:RSCPK.CG.TAMDSYLIDPKR.K.LHVCGNN.PN.CEGFIVEEQEF:704TopI S2GENTALRAK.RCPK.CG.TAMDSYLI	TOPI HP 3	053: NAKETIE. GVKCPE	G. GDIALKR. SK KG. SFIGENNI PK ENFLSNAKPIN. :099
TopI Mp 1614:.KKEKEEV.GRDCPS.CA.SPLLYRY.TKR.GNE.K.FVGCSNF.NN.CKYNEFSONKP::662TopI Mge1608:.KKEKELV.ERDCPK.CN.OPLVYRY.TKR.GNE.K.FVGCSDF.PK.CKYSEFSONKP::656TopI Mga1609:.KKVEYIE.GKNCPN.CS.SOLVRE.GRY.G.P.FVGCSNF.PK.CKYIEKSEQKP::657TopI Mga2658:.EIVYRE.GVECEL.CH.AKLIRKV.AKKKGRN.KDNTFLGCSNF.PK.CKYIEKSLEPEK::709TopI Ju 1578:.KVPDEKI.NRLCPI.CA.HDLLIKR.SNN.T.R.FIACSNF.PT.CRFTESLEKPE::627TopI D1562:.QKVHEKT.GQSCPK.CG.GELVKKN.SR.YG.E.FIACNNY.PK.CKYIKQTENAN::608TopI D1563:.QKTVTKL.GESCPD.CG.GELAIRK.GR.FG.E.FVACINF.PK.CKYIKQTENAN::608TopI Nm 2632:.AEQAELD.GRECPK.CG.GELVKKN.SR.YG.S.K.FIGCVNY.PK.CKYISNILKSESK::610TopI Nm 2632:.AEQAELD.GRECPK.CG.GRLVKY.SRT.GS.K.FIGCVNY.PK.CKYISPTEKKNL::706TopI Xf 1552:.TLQVSATPKHPQE.CR.CFLVEKS.NRR.VG.T.YTACVN.PE.CRYVSPTKKNL::706TopI Xf 2603:.KKPVEVS.NFACVK.CG.NPLKHLVKKGKG.G.YD.FWGCSGF.KEGTopI S3658:.RKCPK.CG.GKHLVEKK.LKK.GG.IQVQCVE.TopI S3658:.RKCPK.CG.CKHLVEKK.LKK.GG.IQVQCVE.TopI S3658:.RKCPK.CG.CG.TMDSYLIDFKR.KLUKY.GG.TFLELK.TopI S3658:.RSCPK.CG.TMDSYLIDFKR.KLUKY.GG.TFLELK.TopI S3658:.RCPK.CG.TAMDSYLIDFKR.KLUKY.GG.TFLELK.TopI S4657:.ETAALRAKRCPT.CG.TAMDSYLIDFKR.K.TopI C3667:.ECAALRAKRCPT.CG.TAMDSYLIDFKR.K.TopI C4658:.ETAALRAKRCPT.CG.TAMDSYLIDFKR.K.TopI F42654:.ESAVLRGKHRCTK.CG.TAMDSYLIDFKR.K.TopI F42654:.ESAVLRGKHRCTK.CG.TAMDSYLIDFKR.K.<		682:DT.GVQCPQ	CKKGNLVERKSRYGKLFYSCSTYPDCNYATWNPPVA.:725
TopI Mge1608:.KKEKELV.ERDCPK.CN.QPLVYRY.TKR.GNE.K.FVGCSDF.PK.CKYSEFSNPKP.:656TopI Mga1609:.KKVEYIE.GKNCPN.CS.SQLVRRE.GRY.GP.FVGCSNF.PK.CKYSEFSNPKP.:657TopI Mga2658:.EIVYES.GVECEL.CH.AKIIRRV.AKKKGRN.KDNTFLGCSNF.PK.CKYTKSLEPEK:709TopI Uu 1578:.KVPDERI.NRLCPI.CA.HDLLIKR.SNN.T.R.FIACSNF.PT.CKYTKSLEPEK:667TopI Hp 1562:.QKVHEKT.GQSCPK.CG.GELVKKN.SR.YG.E.FIACNNY.PK.CKYIKOTENAN.608TopI TopI Nm 2632:.AEQAELD.GRECPK.CG.GRLVKKY.SRT.GS.K.FIGCVNY.PK.CKYSRNLKSESK:610TopI Tp 3667:		585:FIPRIVT.EMDCPR	CHKGKLVKIWSKNRYFFGCSEYPTCDYKTSEEELT.:632
TODIMce1608:.KKEKELV.ERDCPKCN.QPLVYRYTKR.GNE.KFVGCSDFPKCKYSEFSNPKP.:656TODIMga1609:.KKVEYIE.GKNCPN.CS.SQLVRRE.GRY.GP.FVGCSNFPKCKYSEFSNPKP.:656TODIMga2658:.EIVYEE.GVECEL.CH.AKITRRV.AKKKGRN.KDNTFLGCSNF.PKCKYTKSLEPEK:1657TODI Uu578:.KVPDEKI.NRLCPI.CA.HDLLIKR.SNN.T.R.FIACSNF.PT.CRFTESLEKPE:627TODI Hp562:.QKVHEKT.GQSCPK.CG.GELVKKN.SR.YG.E.FIACNNY.PK.CKYIKQTENAN.1608TODI Cj563:.QKTVTKL.GESCPD.CG.GELAIRK.GR.FG.E.FVALNF.PK.CKYIKQTENAN.1608TODI Nm632:.AEQAELD.GRECPK.CG.GRLVYKY.SRT.GS.K.FIGCVNY.PK.CKYSRNLKSESK:610TODI Xf552:.TLQVSATPKHPQ.C.CC.GLVEKS.NRR.VG.T.YTACVN.PE.CRYVSPTKKNL:706TODI Xf552:.TLQVSATPKHPQ.C.CC.GKPLRRIP.GKN.G.H.FWGCSOY.PD.CSVTFPDEGGK:599TODI Xf603:.KKPVEVS.NFACVK.CG.NPLKHLVKKGKG.G.G.VD.FWGCSGF.KEG.CKATYEDKKGK:660TODI S55:.KFTTGELDETCPK.CGEHKLQIKF.GKN.GC.R.FVAACYPE.CSYTRNVHETA:622TODI Bs658:.RKCPK.CG.KMLVEKK.LKK.GC.IGVVQQCSSCDYKEEPQK.691TODI S0658:.RKCPK.CG.TAMDSYLIDPKR.K.LHVCGNN.PT.CDGYELEEGEF:704TODI C2652:.ETNALRAKRCPI.CE.TAMDAYLIDDKR.K.LHVCGNN.PT.CDGYELEEGEF:704TODI G2658:.ETAALRAKRCPI.CE.TAMDAYLIDDKR.K.LHVCGNN.PT.CDGYELEEGEF:704TODI G3664:.KKISLQLUCKDL.CN.MYMDSYFINEKL.KHICGNN.PD.CPGYELEEGEF:704TODI G2658:.ETAALRAKRCPI.CE.TAMDAYLIDDKR.K.LHVCGNN.PD.CDGYLLEEGEF:704TODI G3664:.KKISLQLUCKCR.STAMDSYVIDAR.K.LHVCGNN.PD.CDGYLLEEGEF:704TODI G3664:.KKISLQLUCKDI.CN.MYMDSYFINEKL.	TODI Mp 1	614:KKEKEFV.GRDCPS	CA.SPLLYRYTKR.GNEKFVGCSNFPNCKYNEFSONKP.:662
TopI Mga1609:.KKVEYIE.GKNCPNCS.SQLVRRE.GRY.GPFVGCSNFPKCKFIEKSEQKP.:657TopI Mga2658:.EIVYEE.GVECELCH.AKLIRRV.AKKGRN.KDNTFLGCSNFPKCKFIEKSEQKP.:657TopI U11578:.KVPDEKI.NRLCPICA.HDLLIKR.SNNT.R.FIACSNFPKCKFIEKSEQKP.:627TopI Hp1562:.QKVHERT.GQSCPKCG.GELVKKN.SR.YG.E.FIACSNFPKCKYIKQTENAN.:608TopI TopI Hp1562:.QKVHERT.GESCPL.CG.GELVKKN.SR.YG.E.FIACSNFPKCKYSRNLKSESK:610TopI Tp632:.AEQAELD.GRECPKCG.GRLVYKY.SRT.GS.K.FIGCVNY.PKCKYSRNLKSESK:6610TopI Tp3667:FAVCPQ.CR.CFLVEKS.NRR.VG.T.YTACVN.PE.TopI Tp3667:FAVCPQ.CR.CFLVEKS.NRR.VG.T.YTACVN.PE.TopI Xf1552:.TLQVSATPKHPQCE.CG.KPERIP.GKN.GG.H.FWGCSCF.DE.CSVTFPDEGK.:599TopI Xf2603:.KKPVEVS.NFAV.CG.NRLVKKGKG.G.G.YD.FWGCSCF.KEG.CKATYEDKKGK.:660TopI Nm1565:.KFTQELDETCPK.CG.KMLVKKKKGG.G.G.YD.FWGCSCF.KEG.CKATYEDKKGK.:661TopI S3658:RSCPK.CG.SYLVEKK.LKK.GC.IQVQCVE.CDYKEEPQK.:691TopI S4658:RSCPK.CG.SYLVEKK.LKK.GC.TLUVCVE.CDYKEEPQK.:691TopI S2652:.ETNALRAK.RCPK.CG.TAMDSYLIDPKR.K.LHVCGNN.PT.CDGYLEEGEF:699TopI VC2658:.ETAALRAKKRCPI.CE.TAMDSYLIDPKR.K.LHVCGNN.PT.CGFTVEEGEF:704TopI Ba654:.ESTVLRGKHRCPI.CG.TAMDSYLIDPKR.K.LHVCGNN.PN.CEGFTVEEGEF:704TopI Ba648:.KKISLQLIQKDIN.MYMDSYFINEKL.KHICGNN.PD.CGFYLEEGQY:701TopI Ba648:.KKISLQLIQKDIS.TAMDSYLIDPKR.KHICGNN.PD.CGFYLEEGQY:701TopI Ba648:		608: KKEKELV, ERDCPK	CN. OPLVYRY. TKR. GNE. K FVGCSDF PK CKYSEFSNPKP. : 656
TODI MG22658:EIVYEE.GVECELAKLIRRV.AKKKGRN.KDNTFLGCSNFPK.CKYTKSLEPEK.:709TODI Uu578:KVPDEKI.NRLCPI.CA.HDLLIKK.SMN.T.R.FIACSNFPK.CKYTKSLEPEK.:709TODI Uu576:KVPDEKI.NRLCPI.CA.HDLLIKK.SMN.T.R.FIACSNFPT.CRFTESLEKPE.:627TODI Du1562:KVVDEKI.NRLCPICG.GELVKKN.SR.YG.E.FIACNNY.PK.CKYIKQTENAN.:608TODI Cj1563:KVVEVLGSPDCG.GELAIRK.GR.FG.E.FVALNF.PK.CKYIKQTENAN.:608TODI Nm2632:.AEQAELD.GRECPKCG.GELVKKN.SRT.GS.K.FIGCVNY.PK.CKYUSPTKKNL:706TODI Nm2632:FAVCPQ.CR.CFLVEKS.NRR.VG.T.YTACVN.PE.CRYVSPTKKNL:706TODI Xf1552:KVYEVSNFACVK.CG.NPLKHLVKKGKG.G.YD.FWGCSGF.KEG.CKATYEDKKGK:660TODI Xf2603:.KKPVEVS.NFACVK.CG.CHLVEKS.NRR.UG.GFVGCSGF.KEG.CKATYEDKKGK:660TODI Nm1565:KFTYQELDET PR.CGEHKLQIKF.GKM.GG.AG.IQVQCVE.CJYKEEPQK.:691TODI Bs3658:RKCFK.CG.CKMLVEKK.LKK.GG.IQVQCVE.CJYKEEPQK.:691TODI Bs3658:RKCFK.CG.TAMDSYLIDFKR.K.LHVCGNN.PT.CDGYELEEGEF:704TODI Cj3667:EKCEE.CG.TAMDSYLIDFKR.K.LHVCGNN.PT.CDGYLIEEGEF:704TODI TQ2658:ETAALRAKKRPI.CE.TAMDASYLIDFKR.K.LHICGNN.PN.CEGFIVEEGEF:704TODI Hi2654:ESRVLRGKHRCTK.CG.TAMDSYLIDFKR.K.LHICGNN.PN.CEGFIVEEGEF:704TODI Fa2654:ESRVLRGKHRCTK.CG.TAMDSYLIDFKR.K.LHICGNN.PN.CEGFIVEEGEF:704TODI TD2654:ESRVLRGKHRCTK.CG.TAMDSYLIDFKR.K.LHICG		609: KKVEYTE GKNCPN	CS SOLVERE GRY G P. FYGCSNE PK. CKETEKSEOKP : 657
TODI U1578:KVPDEKI.NRL PICA.HDLLIKR.SNN.T.R.FIACSNF.PT.CRFTESLEKPE.:627TODI Hp562:OKVHEKT.GQSCPK.CG.GELVKKN.SR.YG.E.FIACNNY.PK.CKYIKQTENAN.:608TODI C]563:OKTVTKL.GESCPD.CG.GELAIRK.GR.FG.E.FVALNF.PK.CKYSRNLKSESK:610TODI TD632:AEQAELD.GRECPK.CG.GRLVYKY.SRT.GS.K.FIGCVNY.PK.CKYSRNLKSESK:610TODI TD3632:AEQAELD.GRECPK.CG.GRLVYKY.SRT.GS.K.FIGCVNY.PK.CKYSRNLKSESK:610TODI TD3667:FAVCPQ.CR.CFLVEKS.NRR.VG.T.YTACVN.PE.CRYVSPTKKNL:706TODI Xf552:TLQVSATPKHPCQE.CG.KPLRRIP.GKN.G.H.FWGCSGY.PD.CSVTFPDEGGK::599TODI Xf603:.KKPVEVS.NFACVK.CG.NFLKHLVKKGKG.G.G.YD.FWGCSGF.KEG.CKATYEDKKGK::660TODI Xf655:.KFTTQELDETCPK.CGEHKLQIKF.GKM.G.R.FVAAAGY.PE.CSVTFPDEGGK::691TODI Bs658:.RKCPK.CG.KMLVEKK.LKK.G. IQVQCVEDYKEEPQK.:691TODI Bh658:.RKCPK.CG.SIVLEKR.TKK.Q.VQVQCSS.CDYKEEANCJKKEMKD.:700TODI C2652:.ETAALRAKRCPI.CG.TAMDSYLIDPKR.K.LHVCGNN.PT.CDGYEIEEGEF:704TODI Bc2658:.ETAALRAKRCPI.CG.TAMDSYLIDPKR.K.LHVCGNN.PT.CDGYLIEEGEF:704TODI Ba644:.KKISLOLIQKDK.CG.TAMDSYLIDPKR.K.LHUCGNN.PD.CDGYLIEEGEF:704TODI Ba644:.KKISLOLIQKDK.ST.MDAYLIDDKR.K.LHUCGNN.PD.CDGYLIEEGEF:704TODI C3667:.ETKALMDRKCTK.GG.TAMDSYLIDPKR.K.LHUCGNN.PD.CDGYLIEEGEF:704TODI Ba644:.KKISLOLIQKDK.CG.TAMDSYLIDPKR.K.LHUCGNN.PD.CDGYLIEEGEF:704TODI Ba644:.KKISLOLIQKDK.ST.MDAYLIDDKR.K.LHUCGNN.PD.CDGYLIEEGEF:704TODI C3665:.ETKALMDRKCTK.GG.TAMDSYLIDPKR.K.LHUCGNN.PD.CDGYLIEEGEF:704 <td></td> <td>659. PTUVER CUPOPI</td> <td>CHANT TODU AVYVCON KONTEL COOME DE CEVERCI. FOFE .700</td>		659. PTUVER CUPOPI	CHANT TODU AVYVCON KONTEL COOME DE CEVERCI. FOFE .700
TopI Hp562:QKVHEKT.GQSCPKGG.GELVKKN.SR.YG.E.FIACNNY.PK.KYIKQTENAN.:608TopI Cj563:.QKTVTKL.GESCPD.GG.GELAIRK.GR.FG.E.FVACLNF.PK.CKYSRNLKSESK:610TopI m2632:.AEQAELD.GRCPK.G.G.RLVYKY.SRT.GS.K.FIG.CVNY.PK.CKHVEPLEKPK.:681TopI Tp3667:FAVCPQ.CR.CFLVEKS.NRR.VG.T.YTACVN.PE.CKHVEPLEKPK.:610TopI Tp3667:FAVCPQ.CR.CFLVEKS.NRR.VG.T.YTACVN.PE.CKHVEPLEKPK.:610TopI Xf1552:.TLQVSATPKHPQE.CG.KPLARLF.GKN.G.H.FWGCSGY.PD.SVTFPDEGGK.:599TopI Xf2603:.KKPVEYS.NFACVK.G.GNEKHLVKKGG.G.YD.FWGCSGF.KEG.KATYEDKKGK.:600TopI Nm1565:.KFTTQELDETCPK.CGEHKLQIKF.GKM.G.R.FVACAGY.PE.CSYTRNVNETA.:622TopI Bs3658:RKCPK.CG.KMLVEKK.LKK.G. IQVQCVE.CDYKEEPQK:691TopI Cj3667:KCPK.CG.TAMDSYLIDPKR.K.LVK.G. TFLECLK.CKIKKEMKD700TopI Cj3667:KCPK.CG.TAMDSYLIDPKR.K.LHV.GNN.PT.CDGYELEEGEF.:699TopI Hi2654:.ETAALRAKRCPT.CE.TAMDAYLIDDKR.K.HICGNN.PN.CDGYLIEEGEF.:704TopI Fa2654:.ETAALRAKRCPT.CS.TMDAYLIDDKR.K.HICGNN.PD.CPGYLIEEGEF.:704TopI Fa2654:.ESRVLRGKHRCPT.S.TAMDAYLIDDKR.K.HICGNN.PD.CPGYLIEEGSF.:704TopI Fa2654:.ESRVLRGKHRCPT.CS.TAMDAYLIDEKL.K.HICGNN.PD.CPGYLIEEGGF.:704TopI Cm3686:.AGSEPEE.IVSCPAEGCT.GHLVKRR.SRFN.K.MFYSCSEY.PA.SVIGNSVDAV.735TopI Tp22625:.LAKCPRPACD.GNIVGKKRGRK.E.FFYGCTRF.P.VCDFVTHKPT.:666TopI Tp2625:.LAKCPRPACD.GNIVGKKRGRK.E.FYGCTRF.P.VCDFVTHKPT.:665TopI Tp2625:		E70. VUDDENT NOT OT	A UNIT TYP CAN B D TTACATE DE DEPERTING
TopI C] 1563:QKTVTKL.GESCPDCG.GELAIRK.GRFG.EFVACLMFPKCKYSRNLKSESK:610TopI Nm 2632: .AEQAELD.GRECPKCG.GRLVYKY.SRT.GS.K.FIGCVNY.PKCKHVEPLEKPK.:681TopI Tp 3667:FAVCPQCR.CFLVEKS.NRR.VG.T.YTACVN.PECKHVEPLEKPK.:681TopI Xf 1552: .TLQVSATPKHPQE.CG.KFLVEKS.NRR.VG.T.YTACVN.PECYVSPTRKNL:706TopI Xf 2603: .KKPVEVS.NFACVK.CG.KPLRRIP.GKN.G.H.FWGCSGY.PD.CSVTFPDEGGK.:599TopI Xf 1565: .KFTYQELDETCPK.CGEHKLQIKF.GKM.G.G.YD.FWGCSGF.KEG.CKATYEDKKGK.:660TopI Bs 3658:RKCPK.CG.KMLVEKK.LKK.GG.IQVQCVECDYKEEPQK.:691TopI Bs 3658:RKCPK.CG.KMLVEKK.LKK.G. IQVQCVECDYKEEPQK.:691TopI Cj 3667:EKCEC.CG.TAMDSYLIDFKR.K.LKK.G. TFLELKCKIKKEMKD.:700TopI YC 2652:ETNALRAK.RCPK.CG.TAMDSYLIDFKR.K.LHVCGNN.PT.CDGYEIEEGEF:704TopI Hi 2657:ETKALMDRKRCTK.CG.TAMDSYLIDFKR.K.LHICGNN.PN.CEGFIVEEGEF:704TopI Fa 2654:ESRVLRGKHRCPI.CS.TAMDSYLIDFKR.K.LHICGNN.PD.CPGYEIEEGEF:704TopI Fa 2654:ESRVLRGKHRCPI.CS.TAMDSYLIDEKR.K.LHICGNN.PD.CPGYEIEEGEF:704TopI Fa 2654:ESRVLRGKHRCPI.CS.TAMDSYLIDEKR.K.LHICGNN.PD.CPGYEIEEGEF:704TopI Fa 2654:ESRVLRGKHRCPI.CS.TAMDSYLIDEKR.K.LHICGNN.PD.CPGYEIEEGEF:704TopI Fa 2654:ESRVLRGKHRCPI.CS.TAMDSYLIDEKR.K.LHICGNN.PD.CPGYEIEEGEF:704TopI Cm 3686: .AGSEPEE.IVSCPAEGCT.GHLVKRR.SRFN.K.MFYSCSEY.PASVIGNSVDAV:735TopI Tp 2625:LAKCPRPACD.GNIVGKKRGKR.E.F.FYGCTFF.P.VCDFVTHFKPT.:665TopI Tm 4726:CHAVCHTIKT.TRK.E.FYR.WG.VEKVPCK.E.C.GVEPAE.765		576: KVPDEKI. NRLEPI	CA. HOLLINK. SMN
TopI Nm 2632:.AEQAELD.GRECPKCG.GRLVYKY.SRT.GS.K.FIGCVNY.PKCKHVEPLEKPK.:681TopI Tp 3667:FAVCPQ.CR.CFLVEKS.NRR.VG.T.YTACVN.PE.CRVSPTRKNL:706TopI Xf 1552:.TLQVSATPKHPQE.CG.KPLRRIP.GKN.G.H.FWGCSGY.PD.CSVTFPDEGGK.:599TopI Xf 2603:.KKPVEVS.NFACVK.CG.NPLKHLVKKGKG.G.G.YD.FWGCSGF.KEG.CKATYEDKKGK.:660TopI M1565:.KFTTQELDETCPK.CG.KHLQIKF.GKM.G.R.FVAAGY.PD.CSVTFPDEGGK.:691TopI Bs 3658:.RKCPK.CG.KHLVEKK.LKK.G.IQVQCVE.CDYKEEPQK.:691TopI Bh 3658:.RKCPK.CG.SYLVEKR.TKK.Q.VQVQCSS.CDYKEEPQK.:691TopI Cj 3667:.KKCEC.GC.TVIKELDKKR.TKK.Q.VQVQCSS.CDYKEEPQK.:691TopI C 2652:.ETAALRAK.RCPK.CG.TAMDSYLIDPKR.K.LHVCGNN.PT.CDYKEEPQK.:691TopI C 2652:.ETAALRAK.RCPI.CE.TAMDAYLIDDKR.K.LHVCGNN.PT.CDGYEIEEGEF:704TopI Pa 2654:.ESTVLRGKHRCPI.CE.TAMDSYVIDAHR.K.LHVCGNN.PN.CEGFIVEEGEF:704TopI Ba 2648:.KKISLQLIQKCDI.CN.MYDSYFINEKI.K.TopI Ba 2648:.KKISLQLIQKCDI.CN.MYDSYFINEKI.K.TopI Top 2625:CKISPACT.G.GTHUVKRR.SRFN.K.TopI Top 3686:.AGSEPEE.IVSCPAEGCT.GHLVKRR.SRFN.K.TopI Top 3686:.AGSEPEE.IVSCPAEGCT.GHLVKRR.SRFN.K.TopI Top 3686:.AGSEPEE.IVSCPAEGCT.GHLVKRR.SRFN.K.TopI Top 3625:		562:QKVHEKT.GQSCPK	G.GELVKKN.SRYG.EFIACNNYPKCKYIKQTENAN.:608
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TopI Xf 2603:KKPVEVS.NFACVKCG.NPLKHLVKKGKGG.YD.FWGCSGFKEG.CKATYEDKKGK.:660TopI Nm 1565:KFTTQELDETCPKCGEHKLQIKF.GKM.G.R.FVACAGY.PECSYTRNVNETA::622TopI Bs 3658:RCPK.CG.SYLVEKR.IKK.G.UQVQCYECDYKEEEQK.:691TopI Ds 3658:RCPK.CS.SYLVEKR.TKK.Q.VQVQCSSCDYKEEEQK.:690TopI Cj 3667:EKCEE.CG.ETLVIKE.LKK.GCKIKKEMKD.:700TopI Ec 2652:.ETNALRAK.RCPK.CG.TAMDSYLIDPKR.KLNVCGNN.PT.TopI Vc 2658:EKCEF.CG.TAMDSYLIDPKR.KLNVCGNN.PN.CopI Hi 2657:.ETAALRAKKRCPI.CE.TAMDAYLIDDKR.KLHVCGNN.PN.CopI Hi 2654:.ESRVLRGKHRCPI.CS.TAMDAYLIDDKR.KLHICGNN.PN.CopI Pa 2654:.ESRVLRGKHRCPI.CS.TAMDAYLIDEKH.KLHICGNN.PD.CopI Cm 3686:.AGSEPEE.IVSCPAEGCT.GHLVKRR.SRFN.KHICANN.PS.CSGYKFEKGYF.:695TopI Tp 2625:LAKCPRPACD.GNIVGKKRRGRK.E.F.FGCTF.P.VCDFVHFKPT.:TopI Tp 2625:LAKCPRPACD.GNIVGKKRGRK.E.F.FGCTF.P.VCDFVHFKPT.:TopI Tp 2625:LAKCPRPACD.GNIVGKKRGRK.E.F.FCGTF.P.VCDFVHFKPT.:TopI Tp 2625:LAKCPRPACD.GNIVGKKRGRK.E.F.FCGTFF.P.VCDFVHFKPT.:TopI Tp 2625:LAKCPRPACD.GNIVGKKRGRK.E.F.FCGTFF.P.VCDFFFKFT.TopI Tp 2625:LAKCPRPACD.GNIVGKKTGRK.E.F.FCGTFF.P.VCDFFFFF.FFFTopI Tm 4765CHWFFTKTGKT.TRR.WG.VEKVF0KC.E.CGFFFF.FFF	TODI Xf 1		
TODI Nm 1565:KFTTQELDETCPKCGEHKLQIKFGKMGRFVACACYPECSYTRNVNETA.:622TODI Bs 3658:RKCPKCG.KMLVEKK.LKKGIQVQCSSCDYKEEPQK691TODI Bh 3658:RSCPKCS.SYLVEKR.TKK.QVQVQCSSCDYKEEPQK691TODI Cj 3667:	TODT Xf 2		
TopI Bs 3658:RKCPK. CG.KMLVEKK.LKK.G.IQVCVE.CDYKEEPQK691TopI Bh 3658:RSCPK.CS.SYLVEKR.TKK.Q.VQVQSS.CDYKEEAN690TopI G3667:EKCE.G.G.TLVIKE.LKK.G.TFLELKCKIKKEMKD700TopI C2652:ETNALRAK.RCPK.CG.TAMDSYLIDPKR.K.LHVCGNN.PT.CDYLEEGEF:658:ETAALRAKKCPI.CG.TAMDSYLIDPKR.K.LHVCGNN.PT.CDYLEEGEF:658:ETAALRAKKCPI.CG.TAMDSYLIDPKR.K.LHVCGNN.PT.CDJI VC 2658:ETAALRAKKCPI.CG.TAMDSYLIDPKR.K.LHVCGNN.PT.CDJI Pa 2654:ESRVLRGKHRCPI.CG.TAMDSYLIDPKR.K.LHUCGNN.PN.CDJI Ba 2654:ESRVLRGKHRCPI.CG.TAMDSYLIDPKR.K.LHICGNN.PD.TopI Ba 2654:.ESRVLRGKHRCPI.CG.TAMDSYLIDPKR.K.LHICGNN.PD.CDJI Ba 2648:.KKISLOLIQKCDI.CN.MYMDSYFINEKL.K.HILGNN.PD.CDJ CM 3686:.AGSEPEE.IVSCPAEGT.GHLVKRR.SFN.K.MFYSCSEY.PA.CSVIGNSVDAV:735TopI Tp 2625:.LAKCPRPACD.GNIVGKKTRGKR.EFYGCTRF.P.VCDFVTHKPT.:666TopI Ma 726:	TopI Nm 1		
TopI Bh 3 658: RSCPK.CS.SYLVEKR.TKK.Q. VQVQCSS. CDYKEEAN:690 TopI Cj 3 667: EKCEE.CG.ETLVIKE.LKK.G. TFLECLK. CKIKKEMKD.:700 TopI C2 652: ETNALRAK.RCPK.CG.TAMDSYLIDFKR.K. LHVCGNN.PT.CDGYEIEEGEF::699 TopI VC 2 658: ETAALRAKKCPI.CE.TAMDSYLIDFKR.K. LHVCGNN.PN.CEGFIVEEGEF::704 TopI Hi 2 657: ETKALMDRKCTK.CG.TAMDSYLIDFKR.K. LHVCGNN.PN.CEGFIVEEGEF::704 TopI Pa 2 654: ESRVLRGKHRCPI.CS.TAMDAYLIDDKR.K. LHICGNN.PN.CDGYLIEEGSF::704 TopI Pa 2 654: CS.TAMDAYLIDEKH.K. LHICGNN.PN.CDGYLIEEGSF::704 TopI Pa 2 654: CS.VLRGKHRCPI.CS.TAMDAYLIDEKH.K. LHICGNN.PN.CDGYLIEEGSF::704 TopI Ba 2 648: .KKISLQLIQK.DI.CN.MYMDSYFINELL.K. HICANN.PS.CSGYKFEKGVF::695 TopI Cm 3 686: AGSEPEE.IVSCPAEGCT.GHLVKRR.SRFN.K. MFYSCSEY.PA.CSVIGNSVDAV::735 TopI Tp 2 625: LAKCPRPACD.GNIVGKKTRGRK.E.F.FGCTF.P.VCDFVTHFKPT.:666 TopI Mm 4 726: .CHWPUTKTKT.TKR.WG.VEKVPOK.E.CGWKE0IEPPA::765			
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TOPI HP 4 700:KREEK. CH.YLMSERIYRKKKAHECIQCKERVFLEEDNG:736		726:EECLN	CHWPVLTIKTTKRWGVEKVCPQKECGWKEQIEPPA.:765
	TopI Hp 4	700:KRCEK	CH.YLMSERIYRKKKAHECIQCKERVFLEEDNG:736

FIG. 1. Sequence alignment of tetracysteine motifs in 19 bacterial topoisomerases I. The alignment was performed with Clustal X software. When a topoisomerase sequence contains more than one tetracysteine motif, each of them is numbered (from 1 to 4). The positions of the motifs in the sequences are indicated. Cysteines are *boxed* in *red*, and amino acids that are similar are *boxed* in *blue*. Organism names and their NCBI gene identification numbers are as follows: *Tm*, *Thermotoga maritima*, 1174737; *Fi*, *Fervidobacterium islandicum*, 7531209; *Hi*, *Hemophilus influenzae*, 1174735; *Vc*, *Vibrio cholerae*, 11387201; *Ec*, *Escherichia coli*, 730966; *Ba*, *Buchnera aphidicola*, 11135225; *Pa*, *Pseudomonas aeruginosa*, 14423974; *Bs*, *Bacillus subtilis*, 730965; *Bh*, *Bacillus halodurans*, 11135320; *Tp*, *Treponema pallidum*, 6094493; *Hp*, *Helicobacter pylori* J99, 7531219; *Cm*, *Chlamydia muridarum*, 7190053; *Mge*, *Mycoplasma genitalium*, 1351261; *Mga*, *Mycoplasma galliseptium*, 14423975; *Cj*, *Campylobacter jejuni*, 14423978; *Nm*, *Neisseria meningitidis*, 7225334; *Mp*, *Mycoplasma pneumoniae*, 2501239; *Uu*, *Ureaplasma urealyticum*, 13358155; *Xf*, *Xylella fastidiosa*, 10956714.

I (76 kDa) on SDS-polyacrylamide gel (*lane 2*). A 15-min heating step at 75 °C led to the elimination of most of the *E. coli* proteins, which precipitate at this temperature, whereas the thermophilic topoisomerase remained soluble (*lane 4*). This fraction was further purified on a heparin-Sepharose column, and topoisomerase I eluted from the column at around 0.6 M NaCl. The eluate contained nearly pure topoisomerase (*lane 5*). To eliminate traces of a remaining nuclease contaminant, we finally performed an exclusion gel chromatography (Superdex S200). The resulting purified protein is shown in *lane 6*. We obtained about 2–3 mg of *T. maritima* topoisomerase I from 1 liter of culture.

To determine the functional relevance of the predicted zincbinding domain described in Fig. 1, the two first cysteines of the tetracysteine motif were replaced by two alanines (C559A/ C561A mutant) using site-directed mutagenesis, thus preventing a potential coordination of a zinc atom by the cysteine residues. We also performed a mutagenesis on Tyr²⁸⁸ of the active site (Y288F mutant) to produce an inactive form of the enzyme used as a control. The two mutant proteins were purified to near homogeneity (Fig. 2, *lanes* 7 and 8) by the same procedure used to purify the wild type enzyme.

T. maritima Topoisomerase I Binds One Zinc Atom—To determine whether one zinc atom was indeed bound to the unique putative zinc binding site of *T. maritima* topoisomerase I, the zinc content was titrated by using the sulfhydryl reagent

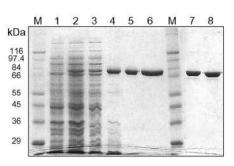


FIG. 2. Purification of recombinant *T. maritima* topoisomerase I wild type and mutant enzymes. Aliquots of the different steps of the purification of the wild type enzyme were analyzed by 9% SDSpolyacrylamide gel electrophoresis (*lanes 1–6*). *Lane 1*, uninduced cells; *lane 2*, induced cells; *lane 3*, crude extract; *lane 4*, extract after heating; *lane 5*, heparin-Sepharose eluate; *lane 6*, purified Superdex S200 fraction; *lane 7*, purified fraction of C559A/561A mutant enzyme; *lane 8*, purified fraction of Y288F mutant enzyme. *Lane M*, molecular mass markers.

PMPS. This compound is known to release zinc(II) from zincbinding proteins by modification of cysteine residues (17). The addition of the metallochrome indicator PAR allowed us to monitor the amount of Zn(II) released due to the formation of the PAR·Zn(II) complex, which absorbs at 500 nm (Fig. 3). Using the molecular absorptivity of $6.6.10^4$ M⁻¹ cm⁻¹ at this

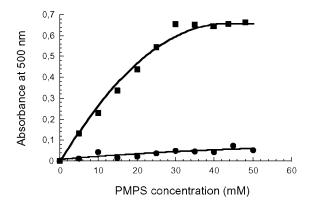


FIG. 3. Titration of the zinc content in wild type *T. maritima* topoisomerase I and C559A/C561A mutant enzyme. Increasing amounts of PMPS were added to 10 μ M wild type protein (\blacksquare — \blacksquare) or C559A/C561A mutant protein (\blacksquare — \bullet) in the conditions described under "Experimental Procedures." The control without enzyme was subtracted from each point of absorbance.

wavelength and a maximal absorbance of 0.658 for the wild type enzyme, the calculated stoichiometry was around one zinc atom (0.98)/molecule of topoisomerase I. Similar results were obtained by using the atomic absorption spectroscopy (results not shown).

By contrast, the zinc content analysis of the C559A/C561A mutant revealed no detectable zinc bound to the enzyme. From this analysis, we conclude that T. maritima topoisomerase I is indeed able to coordinate one zinc atom and that alteration of the zinc-binding domain totally abolishes the ability of the protein to bind zinc.

Conformational Changes in the Double Cysteine Mutant Compared with Wild Type Enzyme—To investigate the potential conformational changes that could affect the mutant proteins, fluorescence emission spectra upon excitation at 295 nm of wild type and mutant enzymes were compared (Fig. 4). At this wavelength, the fluorescence essentially monitors the local environment of tryptophan residues. Four tryptophans are present in *T. maritima* topoisomerase I at positions 94, 156, 365, and 410 (4).

The wild type enzyme fluorescence spectrum is characterized by a maximum emission wavelength at 335 nm (Fig. 4, *curve a*). Whereas no significant change was observed in the emission fluorescence spectrum of the active site Y288F mutant (*curve* b), the spectral properties of the C559A/C561A mutant were significantly affected. A red shift from 335 to 344 nm in the fluorescence emission maximum occurred concomitantly with an increase in fluorescence intensity (*curve c*). These data suggest that the microenvironment around one or several tryptophan residues changes in the protein that has lost the ability to bind zinc. The same differences in spectra were observed between wild type and C559A/C561A mutant when experiments were performed at 60 instead of 20 °C (results not shown).

T. maritima Topoisomerase I Has a High DNA Relaxing Activity, Which Is Not Dependent on Zinc Binding—We first measured the relaxation of a negatively supercoiled DNA by serial dilutions of the wild type enzyme in a buffer containing 10 mM MgCl₂. Fig. 5A shows that as little as 0.1 fmol of *T.* maritima topoisomerase I is sufficient to completely transform 100 fmol of the plasmid DNA after 30 min of incubation at 75 °C. This very high activity level is at least 100-fold greater than that of *E. coli* topoisomerase I (18–20). The optimal temperature of incubation for the assay was around 75 °C (Fig. 5*B*), although a significant activity was still observed at 37 °C when 1 pmol of topoisomerase I was added to the reaction mixture (results not shown).

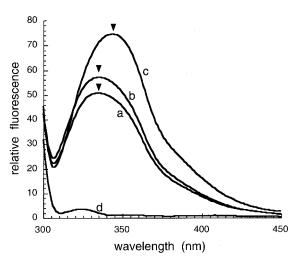


FIG. 4. Fluorescence spectra of wild type and mutants of *T.* maritima topoisomerase I. Fluorescence spectra of wild type enzyme (*curve a*), Y288F mutant enzyme (*curve b*), and C559A/C561A mutant enzyme (*curve c*). *Curve d* is the emission fluorescence spectrum of the buffer alone. The excitation wavelength was 295 nm. Enzyme concentration was 0.5 μ M. The arrowheads indicate the wavelengths of maximal intensities of fluorescence emission.

An examination of the effect of mutations on the relaxation activity showed that the Y288F mutant is totally inactive (Fig. 5A) even with an amount of protein 10,000-fold higher than that used to observe activity of the wild type enzyme. This result indicates that Tyr^{288} , defined as the active site tyrosine from sequence analysis, is probably the amino acid involved in the DNA cleavage step of the topoisomerase reaction.

An unexpected result was obtained with the C559A/C561A mutant, which exhibited an activity similar to that of the wild type enzyme (Fig. 5A) with the same optimal temperature for activity (Fig. 5B). This result contrasts with E. coli topoisomerase I for which the apoenzyme without zinc is totally inactive in DNA relaxation (13, 14). Moreover, the thermostability of the C559A/C561A mutant is comparable with that of the wild type enzyme (Fig. 6). In these assays, enzymes were incubated at 80 °C for 30 min prior to measurement of the remaining DNA relaxation activity. To increase the accuracy of measurements, the test temperature was lowered to 50 °C allowing an increase in the amount of enzyme (*i.e.* reduction of the dilution factors). The results show that both wild type and C559A/C561A mutant enzymes remained fully active after 30 min at 80 °C, suggesting that zinc binding is not involved in the thermostability of T. maritima topoisomerase I.

T. maritima Topoisomerase I Decatenates Nicked Kinetoplast DNA-In regard to the relaxation results, we were interested in analyzing other enzymatic properties of the T. maritima topoisomerase I and comparing them with those of the mutant enzymes. Besides relaxation, another test for type I topoisomerases is their ability to decatenate nicked DNA. In E. coli, topoisomerase I is able to decatenate double-stranded DNA intertwined dimers provided that one of the circular DNA molecules contains a nick (21). In addition, another type I topoisomerase present in this organism, topoisomerase III, was identified as a potent decatenase (22) efficient in the resolution of plasmid replication intermediates in vitro (23). In the case of T. maritima, an analysis of the whole genome sequence (24) did not reveal the presence of a topoisomerase III equivalent. We therefore investigated the ability of T. maritima topoisomerase I and its mutant forms to decatenate kinetoplast DNA (Fig. 7). a substrate usually dedicated to topoisomerase II assays and never tested with a topoisomerase I. This particular DNA forms a network of interlinked minicircles in which a signifi-

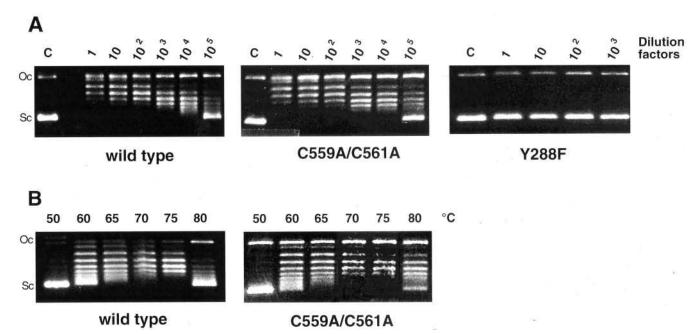
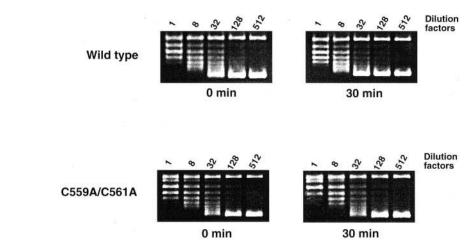


FIG. 5. Relaxation activity of wild type and mutants of *T. maritima* topoisomerase I. *A*, effect of mutations on the activity. 200 ng of pTZ 18 were incubated at 75 °C with serial dilutions of proteins. *Lane C*, control DNA incubated without protein. Dilution factors of 1, 10, 10^2 , 10^3 , 10^4 , and 10^5 , indicated *above* each *panel*, correspond to 1, 0.1, and 0.01 pmol and 1, 0.1, and 0.01 fmol of protein, respectively. The products were analyzed by agarose gel electrophoresis. *B*, effect of temperature on relaxation activity. 0.1 fmol of protein was used in the assay. The incubation temperature is indicated *above* each *panel*. *Sc*, negatively supercoiled DNA; *Oc*, open circular DNA.

FIG. 6. Thermostability of wild type *T. maritima* topoisomerase I compared with C559A/C561A mutant enzyme. Proteins $(1 \ \mu\text{M})$ were preincubated in their storage buffer (50 mM Tris-HCl, pH 7.5, 0.6 M NaCl, 15% glycerol) at 80 °C, as indicated, prior to being tested in a DNA relaxation assay at 50 °C. Dilution factors of 1, 8, 32, 128, and 512 correspond, respectively, to 500, 125, 32, 8, and 2 fmol of protein in the assay.



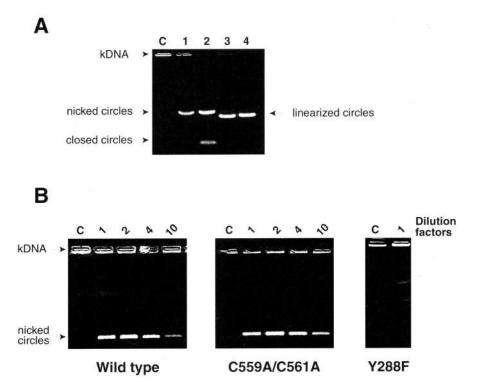
cant fraction can be nicked. When kDNA was incubated with *T*. maritima topoisomerase I at 75 °C under the conditions of the DNA relaxation assay, a product appeared that migrated as nicked circles in an agarose gel containing ethidium bromide (Fig. 7A, lane 1). A fraction of kDNA was not transformed (material remaining in the well). As a control and to confirm the nature of the decatenation product observed, we performed a decatenation assay with human topoisomerase II, which is known to decatenate interlinked minicircles (25). The electrophoretic pattern (Fig. 7A, lane 2) showed that the totality of the kDNA was transformed and yielded two products, one of them migrating as nicked circles and the other exhibiting a higher electrophoretic mobility presumably corresponding to closed circular monomers. This assay confirmed the presence of both intact and nicked minicircles in the kDNA. The products of incubation of kDNA with T. maritima topoisomerase I were further treated by XhoI endonuclease, which cuts once by minicircle; linear monomers were obtained that migrated as the digestion product of kDNA by XhoI (Fig. 7A, lanes 3 and 4). From these results, we conclude that T. maritima topoisomerase I is able to decatenate interlinked DNA if a nick is present

on the molecules to be decatenated.

Again, the decatenase activity of wild type and C559A/C561A mutant enzymes showed no significant difference (Fig. 7B). Nicked DNA circles were still produced by incubation with 0.2 pmol of either protein. As expected, the Y288F mutant was totally inactive (Fig. 7B).

Cleavage by T. maritima Topoisomerase I Shows a High Preference for Cytosine at Position -4 of the Cleavage Site—In their catalytic mechanism, type I topoisomerases transiently cleave one DNA strand to permit strand passage through the break and then reseal the cleaved strand. Although all type IA topoisomerases cleave single-stranded DNA by linking to the 5'-phosphoryl end of cleaved DNA, the specificity of the cleavage sequence is somewhat variable. The derived consensus sequence is CTT \downarrow for *E. coli* topoisomerase III (26), CANNN \downarrow for human topoisomerase III (27), ANN \downarrow for yeast topoisomerase III (28), and CNNN \downarrow for *E. coli* topoisomerase I (10) and archaeal reverse gyrases (11, 12). We therefore investigated the cleavage of a single-stranded oligonucleotide at 75 °C by *T. maritima* topoisomerase I. A 22-base oligonucleotide (22N, sequence shown in Fig. 8*B*), already described as a substrate for

FIG. 7. Decatenation activity of wild type T. maritima topoisomerase I and mutant enzymes. A, analysis of the products obtained in a kDNA decatenation assay. 200 ng of kDNA were incubated for 30 min either with 0.5 pmol of wild type T. maritima topoisomerase I at 75 °C (lane 1) or with 2 units of human topoisomerase II at 37 °C (lane 2) as described under "Experimental Procedures." The reaction product observed in lane 1 was treated further with XhoI (lane 3). As a control of linear monomer, kDNA was digested with XhoI (lane 4). Samples were electrophoresed through agarose gel containing 1 μ g/ml ethidium bromide. Lane C corresponds to the kDNA control without protein. B, a comparison of kDNA decatenation activities of the wild type enzyme, C559A/C561A mutant, and Y288F mutant enzymes. Activities were measured by serial dilutions of enzymes. Dilution factors of 1, 2, 4, and 10 correspond to 2, 1, 0.5, and 0.2 pmol, respectively.



E. coli topoisomerase I and topoisomerase III (29, 30), was labeled at its 5' terminus and used as a substrate for T. maritima topoisomerase I. The results (Fig. 8) showed that the enzyme strongly cleaves two sites leading to the production of two labeled fragments of 11 and 17 nucleotides (Fig. 8A, lane 2). Cleavage positions are indicated by *arrows* on the substrate sequence (Fig. 8B). A minor cleavage site was also observed at position 14. In all cases, a cytosine was present in position -4of the cleavage point, and the efficiency of cleavage appeared highly dependent on its presence. Indeed, when cytosine 8 or 14 was changed to thymine (oligonucleotides 22N C8T and 22N C14T; sequences and cleavage positions shown in Fig. 8B), the corresponding cleavage sites (positions 11 and 17, respectively) totally disappeared (Fig. 8A, lanes 4 and 6). Furthermore, when both cytosines 8 and 14 were mutated (oligonucleotide 22N C8TC14T; sequence and cleavage positions shown in Fig. 8B) only several minor sites were observed (Fig. 8A, lane 8). Finally, we tested an oligonucleotide (21N RG; sequence shown in Fig. 8B) known to contain a strong cleavage site for Sulfolobus shibatae reverse gyrase (12). An 11-nucleotide labeled fragment was predominantly produced (Fig. 8A, lane 10). The corresponding cleavage position (Fig. 8B) was the same as the one found for reverse gyrase (12) and exhibited again a cytosine in position -4. Taken together, these results indicate that T. maritima topoisomerase I cleaves DNA with a strong preference for sites located 4 bases after a cytosine, as observed for *E*. coli topoisomerase I and archaeal reverse gyrase.

Guanine at Position -1 Decreases Cleavage Efficiency—Although cleavage by *T. maritima* topoisomerase I showed a high preference for cytosine in position -4 of the cleavage site, it appeared that all cytosines were not equivalent, since only two sites are cleaved almost exclusively in the 22N, whereas this oligonucleotide contains 5 cytosines. This prompted us to investigate the importance of the bases located at the -1 and +1positions of the cleavage for their specificity. We therefore defined 11 oligonucleotides derived from 22N C8T (exhibiting only one cleavage site), in which the bases adjacent to the cleavage site were changed. The results are summarized in Table I. It appeared that the base in position +1 has very little influence on the cleavage efficiency. On the other hand, the presence of a guanine in position -1 decreased by 2–3-fold the level of cleavage. Similarly, in the case of the *E. coli* enzyme, guanine residues were not found at position -1 in 98% of the cleavage sites studied (10). This result could explain the absence of observable cleavage at position 20 of 22N, but other structural or sequence reasons may be advanced to explain the strong preference for only two sites, while five potential sites are possible. Nevertheless, we can propose the sequence 5'-CNNH \downarrow N (H corresponding to A, C, or T) as a preferential sequence for cleavage by *T. maritima* topoisomerase I.

Cleavage Step of the Hyperthermophilic T. maritima Topoisomerase I Is Not Dependent on Temperature—We have shown that the DNA relaxation activity, which reflects the whole topoisomerization reaction, is highly dependent on temperature with an optimum around 70–75 °C (Fig. 5B). By contrast, when we tested the influence of temperature on the cleavage, which is the first step of the reaction, we observed that the cleavage efficiency was not significantly different in a range of temperature going from 24 °C to 75 °C (Fig. 9). This result suggests that the sensitive temperature step in the topoisomerization is not the trans-esterification reaction but more likely the strand passage event.

Cleavage Specificity Remains Unchanged in the Double Cysteine Mutant—To know whether the loss of the ability to bind zinc changes the specificity of the cleavage, and to confirm that Tyr²⁸⁸ of *T. maritima* topoisomerase I is the residue involved in the cleavage step, the cleavage pattern of the 22N oligonucleotide by the wild type *T. maritima* topoisomerase I was compared with mutant enzymes. The results (Fig. 10) showed that the Y288F mutant is totally inefficient in cleavage, attesting to the fact that Tyr²⁸⁸ is the catalytic amino acid of the active site. On the other hand, cleavage positions obtained with the C559A/C561A mutant were similar to those observed with the wild type enzyme. Preference for cytosine at position -4 was retained, and cleavage intensities were comparable. Alteration of the zinc-binding domain therefore does not affect the efficiency and specificity of the cleavage reaction.

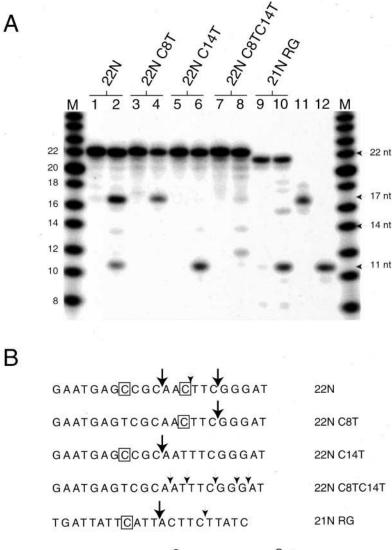


FIG. 8. Analysis of DNA cleavage site specificity of T. maritima topoisomerase I. A, cleavage assay with different 5'-end-labeled oligonucleotides analyzed by urea-polyacrylamide gel electrophoresis. The sequences of the different substrates are indicated in panel B. Lanes 1, 3, 5, 7, and 9 correspond to oligonucleotides without enzyme added. Lanes 2, 4, 6, 8, and 10 contain the reaction products after incubation with the enzyme as described under "Experimental Procedures." Lanes 11 and 12 are 17- and 11-nucleotide oligonucleotide controls, respectively, both derived from 22N. M represents the oligonucleotide size markers. nt, nucleotides. B, map of cleavage sites. The larger arrows correspond to strong cleavage sites and the *smaller arrows* to minor cleavage sites. The cytosines at position -4 of each strong cleavage site are boxed.

TABLE I Efficiency of cleavage by T. maritima topoisomerase I obtained with oligonucleotides derived from 22N C8T

Bases located at positions -1 and +1 of the cleavage site (*underlined positions*) were changed in the sequence of each labeled nucleotide. The arrow represents the cleavage site. The reaction conditions are described in Experimental Procedures. The percentage of cleavage for each substrate was determined by densitometry of 17 nt produced after incubation with *T. maritima* topoisomerase I, compared with the densitometry of the corresponding uncleaved substrate.

Oligonucleotide sequence	% Cleavage
GAATGAGTCGCAACTTC↓GGGAT 22N C8T	26
GAATGAGTCGCAACTT <u>C A</u> GGAT	26
GAATGAGTCGCAACTT <u>C T</u> GGAT	27
GAATGAGTCGCAACTT <u>A G</u> GGAT	20
GAATGAGTCGCAACTTA AGGAT	21
GAATGAGTCGCAACTT <u>T</u> GGAT	23
GAATGAGTCGCAACTT <u>G</u> GGAT	26
GAATGAGTCGCAACTT <u>T</u> GGAT	20
GAATGAGTCGCAACTT <u>T</u> GGAT	28
GAATGAGTCGCAACTT <u>G</u> GGAT	12
GAATGAGTCGCAACTT <u>G</u> AGGAT	8
GAATGAGTCGCAACTT <u>G</u> AGAT	10

DISCUSSION

This work describes the purification and biochemical properties of the hyperthermophilic topoisomerase I from T. maritima and two of its mutants. One was altered in the active site of the enzyme on the putative tyrosine involved in the cleavage step of DNA. The other was mutated on the two first cysteines

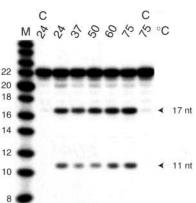


FIG. 9. Cleavage of 5'-end-labeled 22N oligonucleotide by *T.* maritima topoisomerase I at different temperatures. Reactions containing 0.1 pmol of *T. maritima* topoisomerase I and 1 pmol of 22N were incubated at the indicated temperatures and analyzed by ureapolyacrylamide gel electrophoresis. *C* represents the 22N oligonucleotide incubated without enzyme at 24 or at 75 °C. *M*, molecular size markers; *nt*, nucleotides.

of the tetracysteine motif (Cys-X-Cys- X_{16} -Cys-X-Cys) (Fig. 1) located in the C-terminal part of the protein. The thermophilic character of *T. maritima* enzyme and its mutants allowed us to obtain all of the proteins in large amounts and at a high degree of purity by introducing a heating step in the purification procedure.

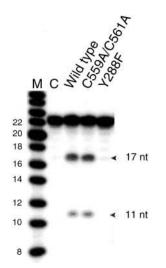


FIG. 10. Comparison of cleavage of 22N oligonucleotide by wild type *T. maritima* topoisomerase I and mutant enzymes. 22N was incubated with proteins at 75 °C as described under "Experimental Procedures" and analyzed by urea-polyacrylamide gel. The amount of protein was 1 pmol for the wild type and the C559A/C561A mutant enzyme and 2 pmol for the Y288F mutant enzyme. *C*, 22N oligonucleotide incubated without enzyme; *M*, molecular size markers; *nt*, nucleotide.

Based on sequence similarities, it was proposed previously that topoisomerase I from *T. maritima* belongs to the *E. coli* topoisomerase I family (4, 9). The results presented here clearly confirm this hypothesis, even though significant differences in their properties are pointed out.

A striking result of our study was the exceptionally high DNA relaxation activity of T. maritima topoisomerase I, with a specific activity at least 100-fold greater than that of the enzyme from E. coli. One molecule of T. maritima topoisomerase I can catalyze about 500-1000 strand-passage events/min as compared with about 5 for E. coli (20). This suggests that a large number of strand passages might occur during a given topoisomerization cycle, as in the case of topoisomerases IB (about 1200 rotations/min for the vaccinia virus topoisomerase I) (31). For this family of topoisomerases, a "controlled rotation" mechanism has been proposed, in which the end of the cleaved strand that is non-covalently attached to the enzyme, rotates around the uncleaved strand in a controlled way (32). Today, insufficient arguments are available to propose such a mechanism for T. maritima topoisomerase I. The reasons for such a high specific activity remain unknown, but it may be required in vivo to locally increase, with great efficiency, the DNA linking number and thus prevent the double-helix opening at high temperature.

Besides its DNA relaxation activity, T. maritima topoisomerase I exhibits an efficient decatenation activity of nicked minicircles present in kinetoplastic DNA. In E. coli, topoisomerase III, rather than topoisomerase I, is efficient to decatenate multiply inter-twisted nicked dimers (22, 33). The lack of a topoisomerase III equivalent in T. maritima (24) suggests that in this organism, topoisomerase I could also be involved in the resolution of replicating daughter DNA molecules at the end of replication.

An examination of oligonucleotide cleavage by the wild type enzyme from *T. maritima* showed the constant presence of a cytosine in position -4 of the cleavage point, as is the case for *E. coli* topoisomerase I (10) and archaebacterial reverse gyrases (11, 12). Although cleavage efficiency was not affected by the identity of the base in position +1, a guanine in position -1clearly reduced cleavage intensity. Interestingly for a thermophilic enzyme, cleavage did not appear to be dependent on temperature, suggesting that this step of the topoisomerization cycle is not rate-limiting. We also show that Tyr²⁸⁸ is the amino acid involved in the trans-esterification reaction, because its mutation to phenylalanine totally abolishes cleavage.

The major differences between *T. maritima* and *E. coli* topoisomerases I are located in the C-terminal region of the enzymes (4) (9). Indeed, the *T. maritima* topoisomerase I C terminus is much shorter than the one from *E. coli* enzyme, and it contains a unique tetracysteine motif instead of three tetracysteines in the *E. coli* topoisomerase I. The present results show that this putative zinc-binding motif is functional because *T. maritima* topoisomerase I does coordinate one Zn(II). Moreover, the double cysteine C559A/C561A mutant was unable to bind zinc, and its fluorescence properties were modified, suggesting a change of conformation in the protein lacking zinc.

Surprisingly, analysis of the enzymatic properties of the C559A/C561A mutant showed that the relaxation as well as decatenation activities are similar to those of the wild type enzyme. Furthermore, the thermal stability and DNA cleavage properties are not affected in this mutant despite the loss of zinc. These results contrast with those found for E. coli topoisomerase I, in which zinc binding to the three tetracysteine motifs is required for DNA relaxation activity (13, 14) and disruption of the second zinc motif deeply affects thermal stability and cleavage specificity (18). Indeed, it was proposed that the zinc binding domain would contribute to the stabilization of the association of the 3'-end of the cleaved DNA with topoisomerase I and would assist the passage of uncleaved strand across the break (14, 34). This model is difficult to imagine with T. maritima topoisomerase I, because we have showed that the unique zinc motif is not involved in the topoisomerase activity at least in vitro. Taken together, our results suggest that the two enzymes may have distinct ways of functioning.

The absence of involvement of the zinc-binding domain of T. maritima topoisomerase I in the topoisomerization reaction stresses the question of the putative role of this domain in other processes. The tetracysteine motif present in T. maritima topoisomerase I is close to those of 19 bacterial topoisomerases I examined (Fig. 1). Strikingly, all of these conserved motifs are located within the highly divergent C-terminal part of these enzymes. The similarity of the tetracysteine motif includes the high frequency of a Gly following Cys², the number of residues between Cys² and Cys³ (15-17 residues), and a hydrophobic region just upstream of Cys³ (Fig. 1). It was recently proposed that these tetracysteine motifs could be members of the zinc ribbon domain superfamily (35), also found in transcription factors TFII B, TFII S, TFII E large subunit (36-38), RNA polymerase II subunit 9 (RPB9) (39), adenovirus E1A transactivator (40), and bacteriophage T7 DNA primase (41). All of these proteins are part of multiprotein-nucleic acid complexes, but the proposed function of the zinc-binding domain appears very variable. Some are involved in interactions with singlestranded DNA, RNA, or other proteins (40-43), whereas in other cases, the role of this motif remains unclear (44).

Thus, the question of the role of the zinc-binding motif in *T. maritima* topoisomerase I is largely open. One hypothesis could be that it interacts with other proteins of the replication or transcription machinery, given the involvement of topoisomerases in these processes. We are presently interested in searching for the potential protein partners of *T. maritima* topoisomerase I.

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