SUPPLEMENTARY INFORMATION

Dna2 Exhibits a Unique Strand-End Dependent Helicase Function

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Experimental Procedures

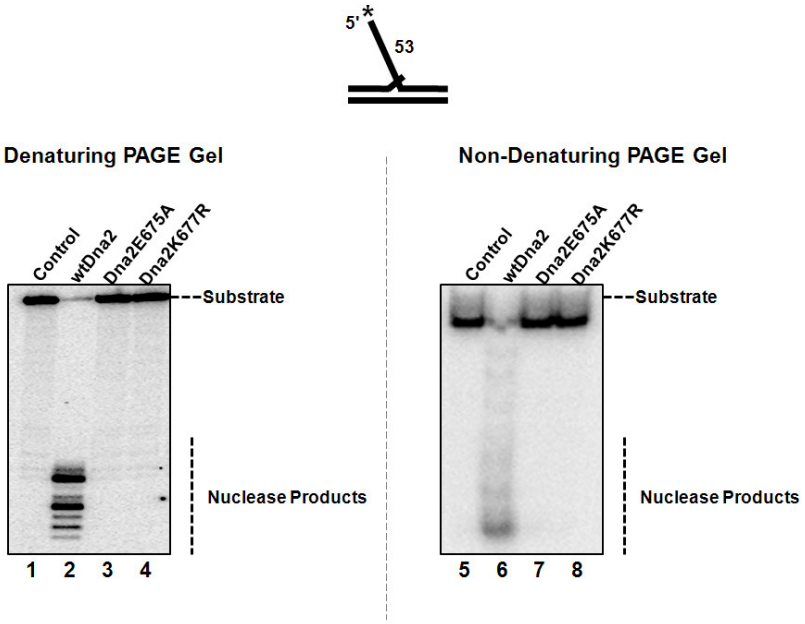
Purified Proteins – Wild-type Dna2 protein from *S. cerevisiae* was overexpressed in baculovirus High Five cells and purified as described (1).

Nuclease assay – Five fmol of substrate (53nt flap substrate) labeled at the 3' end of the downstream primer was incubated with 50 fmol of either wtDna2, Dna2E675A or Dna2K677R in a reaction volume of 20 µl, at 37 °C for 10 min. The reaction buffer consisted of 50 mM Tris-HCl {pH 8.0}, 2 mM dithiothreitol, 30 mM NaCl, 0.1 mg/ml bovine serum albumin, 2 mM $MgCl_2$, and 1 mM ATP. The reactions were terminated using 2X termination dye (90% formamide (v/v), 10 mM EDTA, 0.01% bromphenol blue, and 0.01% xylene cyanole). After termination, samples were heated at 95°C for 5 min and half the reaction volume was loaded onto a denaturing gel (7 M urea) 18% polyacrylamide gel and fractionated by electrophoresis for 1hour 30mins at 3000V. The remaining reactions were loaded onto pre-run 6% polyacrylamide gels in 1 X TBE. Gels were subjected to electrophoresis for 1 hour at constant 250V.

Electrophoretic Mobility Gel Shift Assays (EMSA) – Binding efficiency of Dna2E675A and Dna2K677R to a 53 nt flap substrate was assessed using EMSA. Five fmol of substrate was incubated with 500 fmol of either of Dna2E675A or Dna2K677R and incubated for 10 mins at room temperature in a reaction buffer consisting of 50 mM Tris-HCl (pH 8.0), 2 mM dithiothreitol, 30 mM NaCl, 0.1 mg/ml bovine serum albumin and 5% glycerol. The reactions were loaded onto pre-run 6% polyacrylamide gels in 1 X TBE. Gels were subjected to electrophoresis for 1hour at constant 250V.

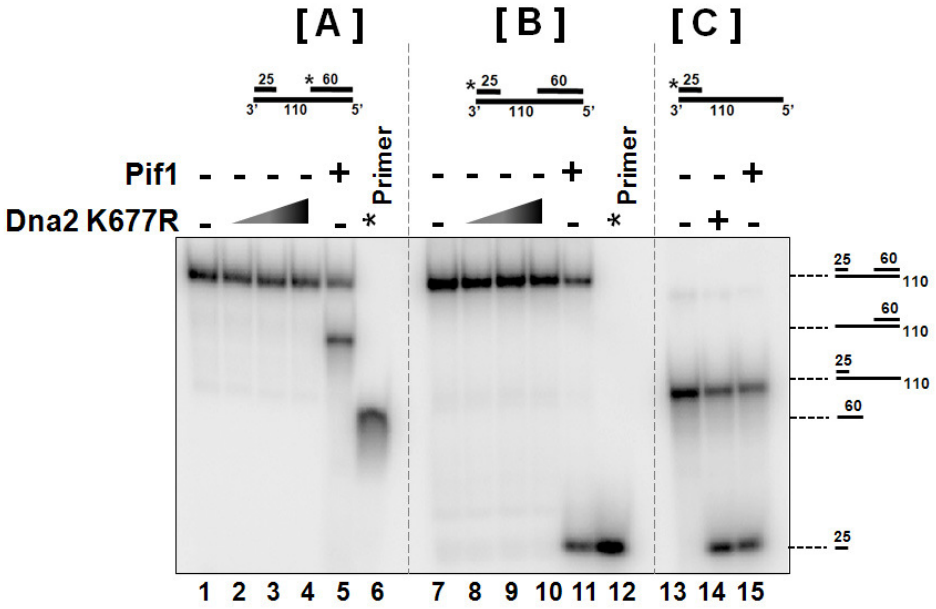
1. Budd, M. E., Choe, W., and Campbell, J. L. (2000) *J Biol Chem* **275**, 16518-16529

Supplementary Figure 1



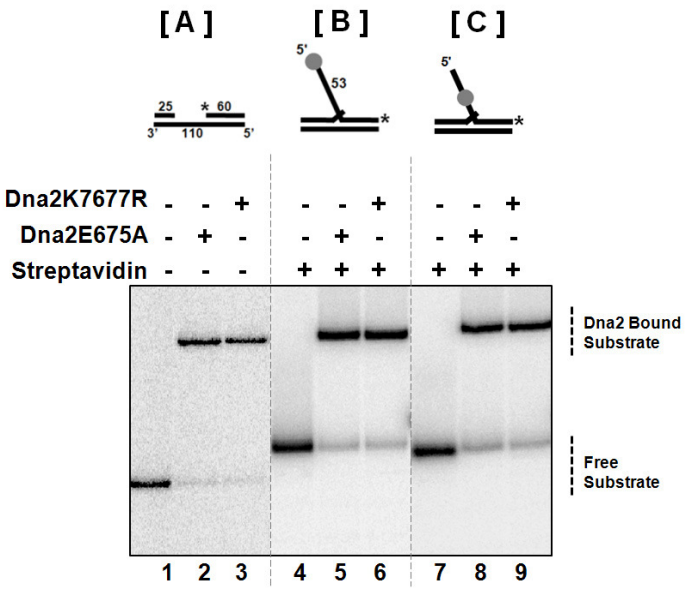
A 53nt flap substrate labeled at the 5' end of the downstream primer was used to measure the 5'-3' endonuclease activity of scDna2. Reactions containing 5 fmol of substrate and 500 fmol of either of wtDna2, Dna2E675A or Dna2K677R were incubated for 10 mins at 37ºC. Reactions were spilt and loaded on either an 18% denaturing PAGE gel or a 6% non-denaturing PAGE gel. The labeled substrate is depicted above the gel with the *asterisk* indicating the site of the ^{32}P label. The 5'- 3' Dna2 cleavage products are indicated beside the gel.

Supplementary Figure 2



Helicase activity was assayed as described in the Methods section. Experimental substrates used were: (A) A substrate consisting of a 110 nt template with a 5' labeled 60 nt primer annealed at the template 5' end and a 25 nt primer annealed at the template 3' end (U1:T1:D1). (B) The substrate had the same structure as in A, but the 5' label was on the 25 nt primer. (C) The substrate was the same as in B but with no 60 nt primer (U1:T1). When two primers were present the gap between them was 25 nt. Reactions containing 5 fmol of each substrate were incubated with either Dna2K677R or Pif1 helicase for 15 mins at 37°C. Lanes 1, 7, 13 are the substrate alone controls and lanes 6, 12 are the labeled primer alone controls. Dna2K677R was titrated into the reactions at a concentration of 100 fmol (lanes 2, 8), 250 fmol (lanes 3, 9) and 500 fmol (lanes 4, 10, 14). Lanes 5, 11, 15 contained 500 fmol of Pif 1. The *asterisk* on the substrates depicted above the figures denotes the position of the radiolabel. Positions of the substrate and helicase products are indicated in the figure.

Supplementary Figure 3



Substrate binding efficiency of Dna2E675A and Dna2K677R were assessed using electromobility gel shift assay (EMSA). The experimental substrates used were (A) a substrate consisting of a 110 nt template with a 60 nt primer annealed at the template 5' end and a 5' labeled 25 nt primer annealed at the template 3' end (U1:T1:D1); (B) a streptavidin blocked 53 nt flap substrate (U4:T3:D6); (C) a 53nt flap substrate with streptavidin conjugated to the internal biotin (U4:T3:D7). Five fmol of substrate was incubated with 500 fmol of either Dna2E675A or Dna2K677R and incubated for 10 mins at room temperature and separated on a 6% polyacrylamide gel. The labeled substrates are depicted above the gel with the *asterisk* indicating the site of the ^{32}P label. The substrate alone and complexes containing Dna2 bound substrate are indicated beside the gel at the right.