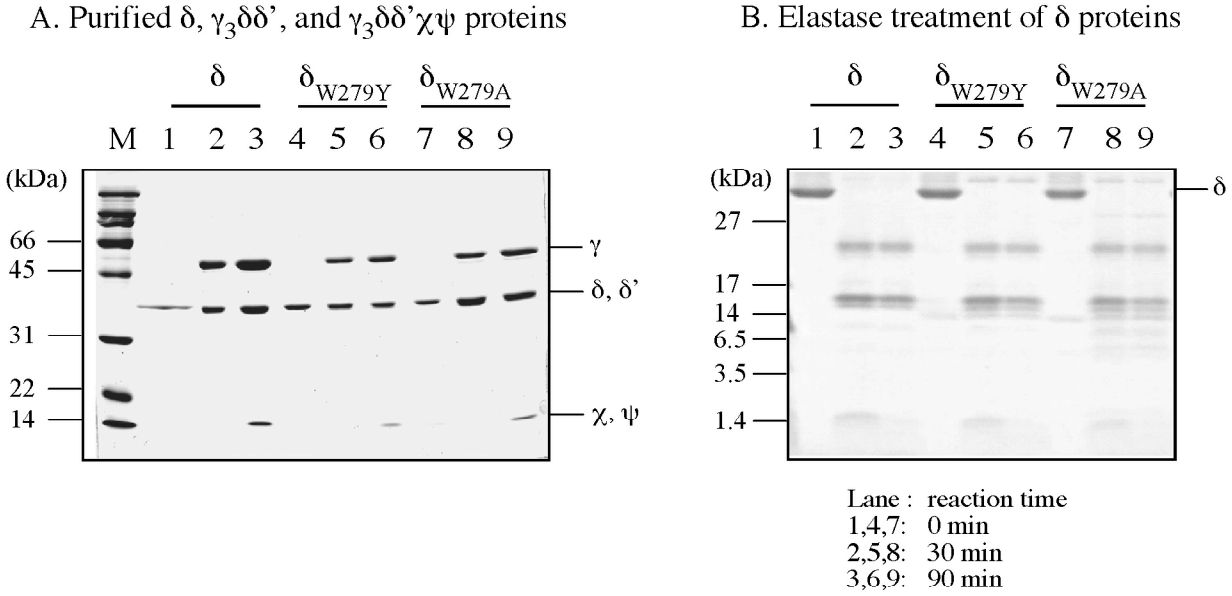
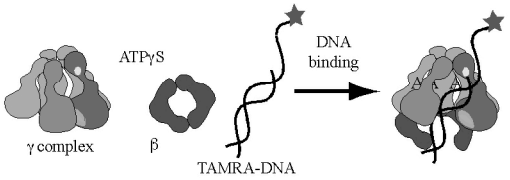
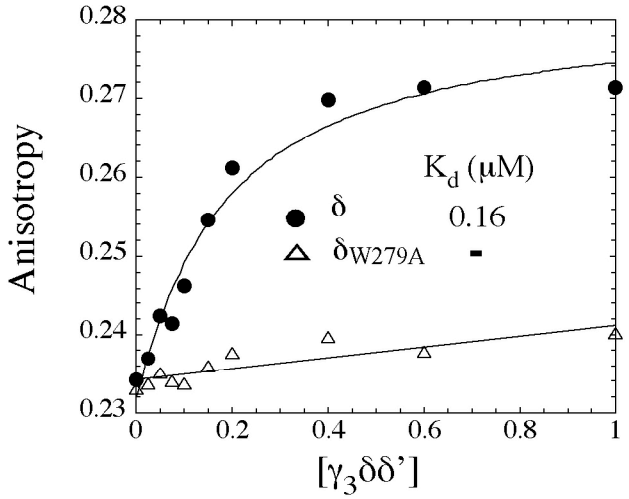

A. Purified δ, γ3δδ', and γ3δδ'χψ proteins
δ
δW279Y
δW279A
M 1 2 3 4 5 6 7 8 9
(kDa)
66
45
31
22
14
γ
δ, δ'
χ, ψ
B. Elastase treatment of δ proteins
δ
δW279Y
δW279A
1 2 3 4 5 6 7 8 9
(kDa)
27
17
14
6.5
3.5
1.4
δ
Lane : reaction time
1,4,7: 0 min
2,5,8: 30 min
3,6,9: 90 min

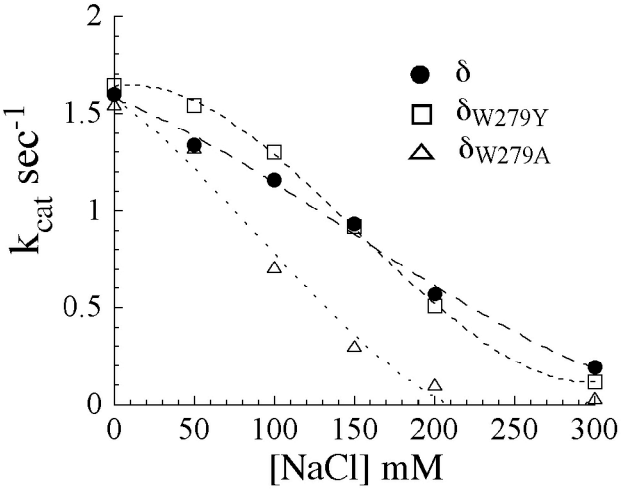
Figure S1

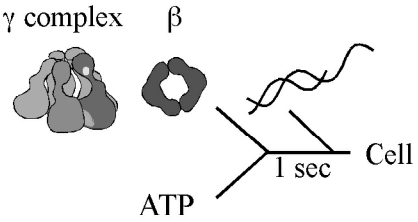


A. $\gamma_3\delta\delta$'-β binding p/tDNA (60 mM NaCl)

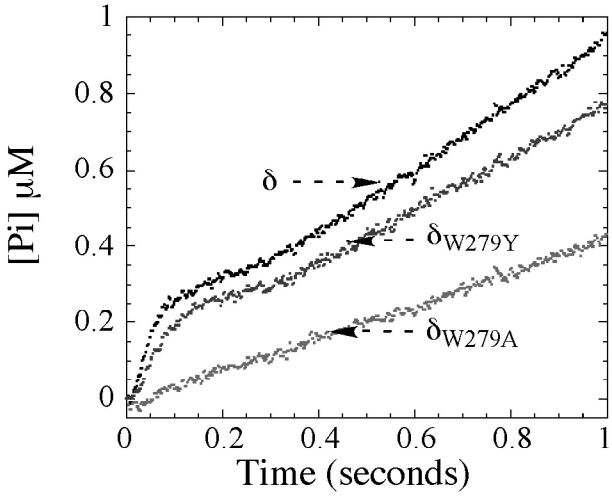


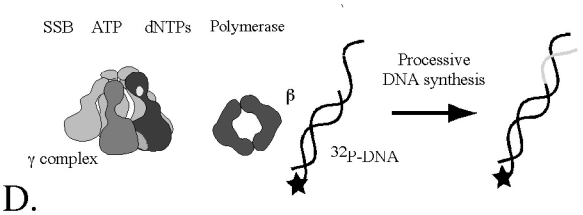
B. $\gamma_3\delta\delta$'-β-p/tDNA ATPase activity





C. $\gamma_3\delta\delta$'-β-p/tDNA ATPase (80 mM NaCl)





D.

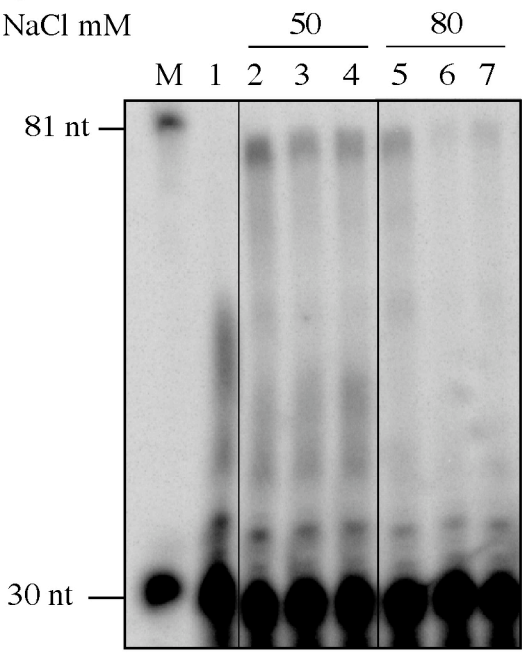
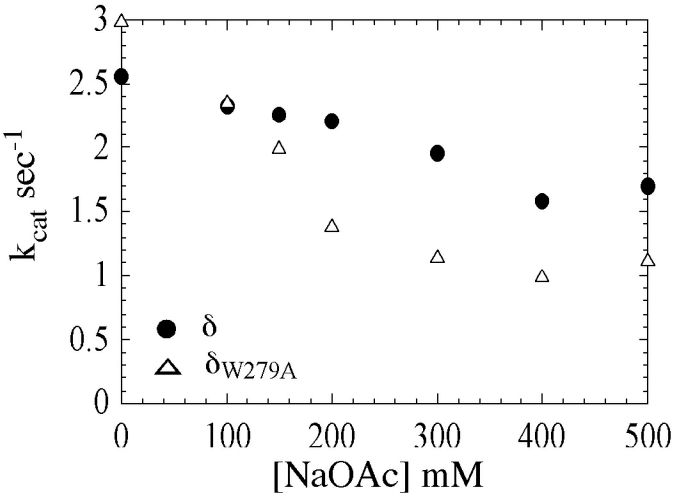
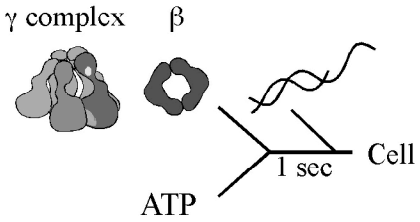


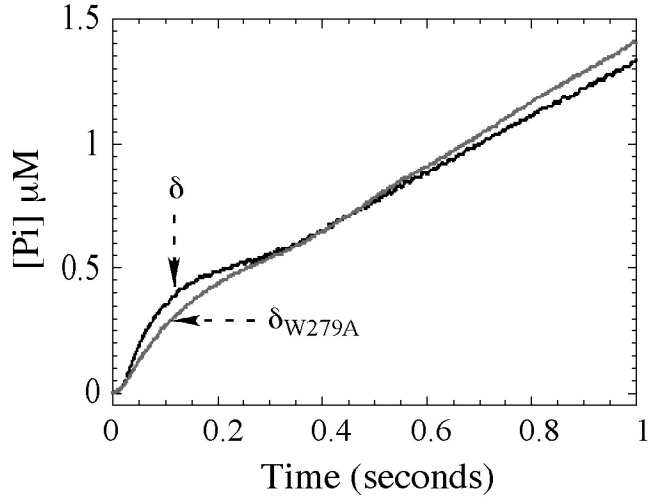
Figure S2

A. ATase activity of γ complexes in NaOAc





B. γ complex-β-p/tDNA ATPase (100 mM NaOAc)



C. γ complex-β-p/tDNA ATPase (200 mM NaOAc)

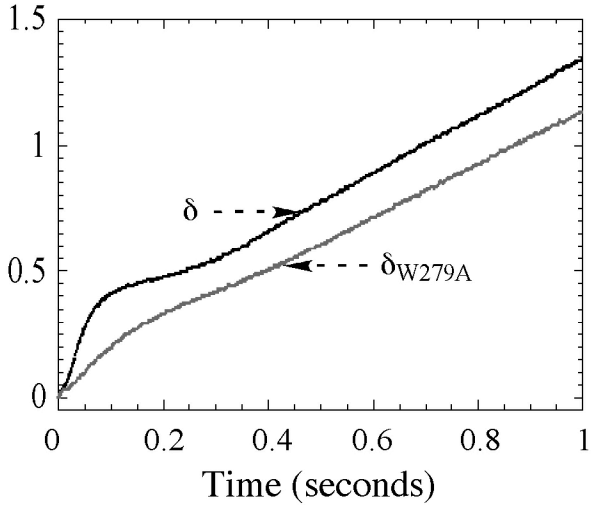
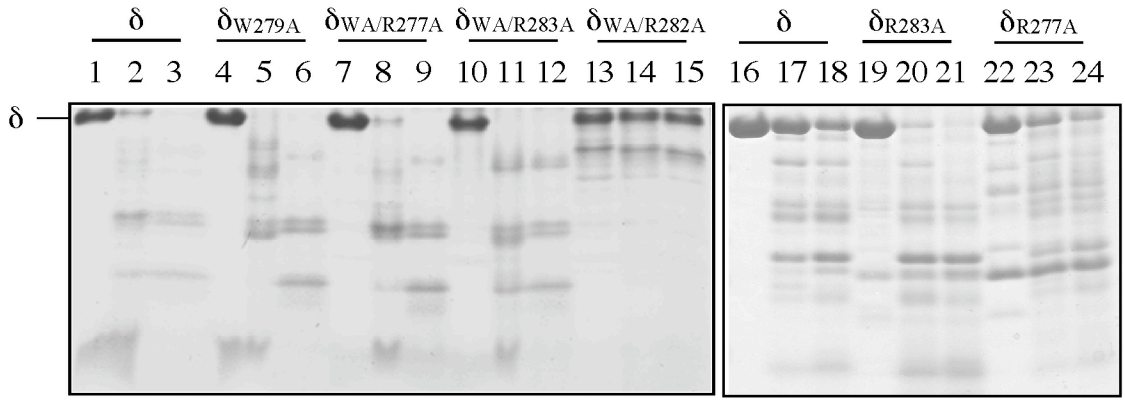


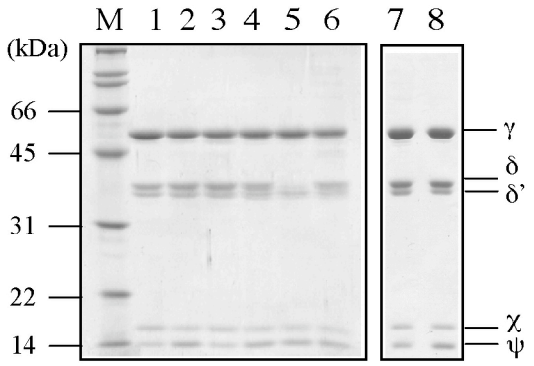
Figure S3

A. Elastase treatment of δ proteins



Lane : reaction time
1,4,7,10,13,16,19,22: 0 min
2,5,8,11,14,17,20,23: 30 min
3,6,9,12,15,18,21,24: 90 min

B. Purified $\gamma_3\delta\delta'\chi\psi$ complexes



Lane: protein
1: δ
2: δ_{W279Y}
3: δ_{W279A}
4: $\delta_{W279A/R277A}$
5: $\delta_{W279A/R282A}$
6: $\delta_{W279A/R283A}$
7: δ_{R277A}
8: δ_{R283A}

Figure S4

Supplementary material

Figure S1. Preparation and initial characterization of δ W279-γ complex mutants. (A) SDS-PAGE analysis of purified δ, δ_{W279Y} and δ_{W279A} proteins, and corresponding γδδ'χψ (γ complex), and γδδ' clamp loader complexes; both mutants assemble into γ complexes as expected for wild type δ. (B) Elastase treatment performed to assess the structural integrity of δ mutants shows that wild type and mutant δ proteins have similar proteolysis patterns. Partial proteolysis of δ mutants was performed by incubating 6 μM δ in buffer (30 mM HEPES-NaOH pH 7.5, 0.1 mM EDTA pH 8.0, 2 mM DTT, 25 mM NaCl, 7% glycerol) with 0.5 units Elastase (Sigma Aldrich) at 37 ºC in a final volume of 40 μl. Next, 20 μl aliquots of the reaction were quenched at different times (30 and 90 minutes) with 10 μl SDS dye solution (1.5 M Tris base, 3% SDS, 35% glycerol, 150 mM DTT, bromophenol blue). The samples were heated at 90 ºC for 2 minutes and analyzed by tricine gel electrophoresis on a 16.5% acrylamide gel at 4 ºC (Schagger, H., and von Jagow, G. (1987) *Anal Biochem* **166**, 368-379), followed by staining with GelCode Blue Stain Reagent (Pierce).

Figure S2. Analysis of minimal clamp loader, γδδ', activities. (A) Interaction between γδδ' and p/t DNA is highly sensitive to salt concentration, with K_D = 156 ± 50 nM for wild type γδδ' and barely detectable binding to δ_{W279A}-γδδ' even at 60 mM NaCl. (B) Steady state ATPase activity of the δ_{W279A}-γδδ' complex (△) is more sensitive to NaCl than wild type γδδ' (●), and (C) δ_{W279A}-γδδ' does not catalyze a burst of ATP hydrolysis even at 60 – 80 mM NaCl. (D) DNA replication reactions containing p/t DNA, SSB, ATP, β, dNTPs, and core polymerase, in the absence of clamp loader (lane 1) or with γδδ' complex (lanes 2, 5), δ_{W279A}-γδδ' (lanes 3, 6), and δ_{W279Y}-γδδ' (lanes 4, 7) reveal loss of processive DNA replication with δ_{W279A}-γδδ' at 80 mM NaCl. The markers are ^{32}P-labeled 30 and 81 nt DNAs.

Figure S3. Effect of sodium acetate on clamp loader function mimics that of sodium chloride. (A) Steady state ATPase activity of the δ_{W279A}-γ complex (△) is more sensitive

to NaOAc than wild type γ complex (●). (B, C) Increasing NaOAc concentration lowers the rate and amplitude of the burst ATP hydrolysis phase for δ_{W279A}-γ complex (●) compared with wild type γ complex (●), indicating that the impact of δ-W279A mutation on clamp loader function is independent of the type of anion present in the reaction.

Figure S4. Preparation and initial characterization of δ mutants. (A) Elastase treatment performed, as described above for Figure S1, to assess the structural integrity of δ proteins shows that the mutants have similar proteolysis patterns as wild type (0, 30, 90 minutes), except for the one harboring the δ-R282A mutation, which indicates distinct structural changes in this protein (impurities in the δ_{R277A} preparation are separated during purification of δ_{R277A}-γ complex). (B) SDS-PAGE analysis of purified γ complexes containing various δ proteins; all mutants, except for $\delta_{W279A/R282A}$, assemble into γ complexes as expected for wild type δ.