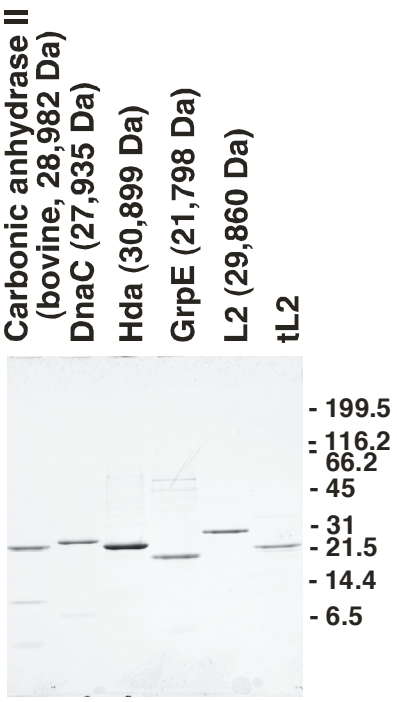
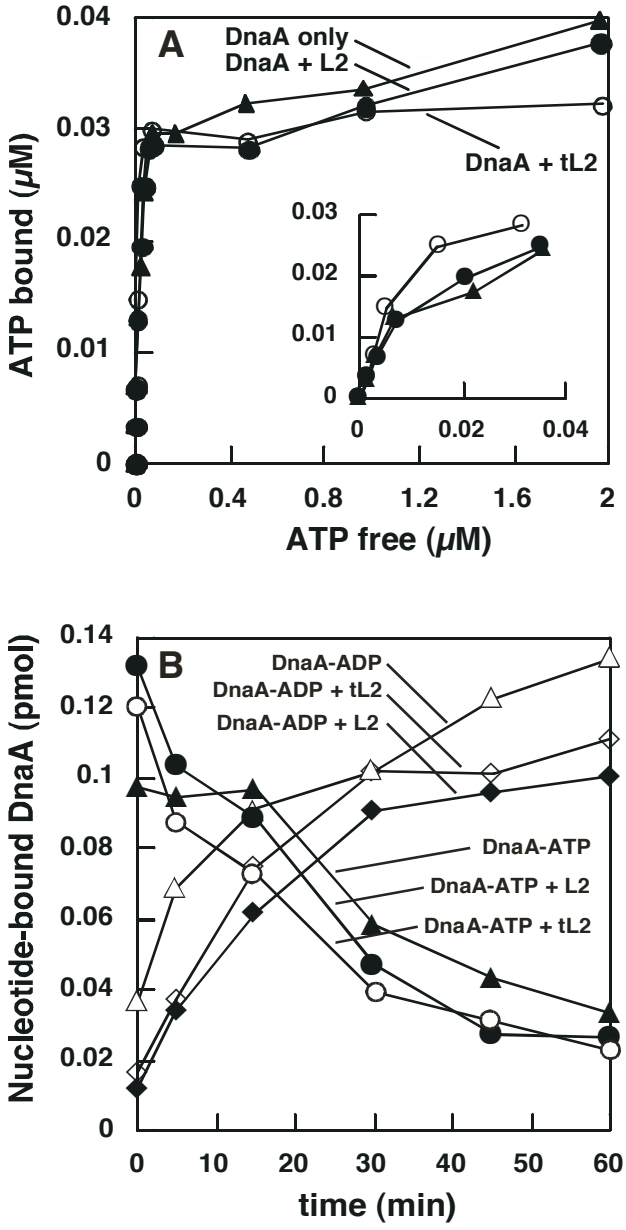
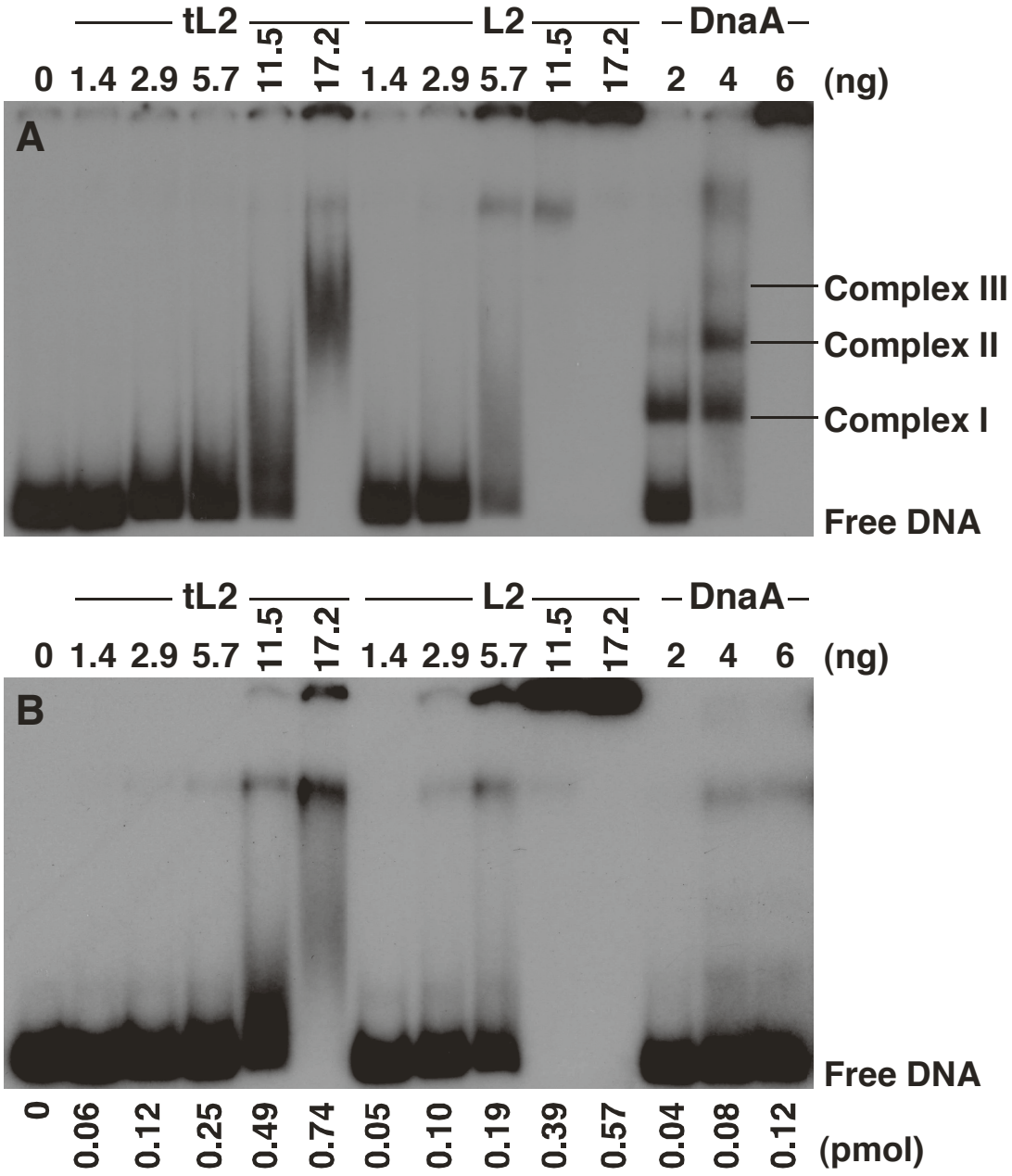
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 1. Bovine carbonic anhydrase II (0.4 μg), DnaC (0.4 μg), Hda (0.78 μg) joined at its N-terminus to the amino acid sequence MGHHHHHHHHHHSSGHIQGRH, GrpE (0.3 μg), L2 (0.38 μg), and the protein isolated in Figure 1 (0.57 μg) were visualized by staining with Coomassie blue after electrophoresis in a 15% SDS-polyacrylamide gel. Except for Hda, the predicted mass of the respective proteins assumes that the N-terminal methionine has not been removed. The molecular weight markers (Bio-Rad prestained SDS-PAGE Standards, Broad Range) shown at the right border are myosin (199.5 kDa), β-galactosidase (116.2 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), and aprotinin (6.5 kDa).

Supplementary Figure 2. *Neither form of L2 substantially affects ATP binding or ATP hydrolysis by DnaA*. In (**A**), reactions (25 μl) were assembled essentially as described (69), and contained DnaA (2 pmol; 100 ng), [α^{32}P]-ATP (1.5 x 10^5 cpm/pmol) at the indicated concentrations, and tL2 or L2 (each at 170 ng) as indicated in buffer composed of 50 mM Tris-HCl pH 8.0, 15% (v/v) glycerol, 0.5 mM magnesium acetate, and 0.1% Triton X-100. After incubation on ice for 20 min, the reactions were filtered through nitrocellulose filters (Millipore HAWP), which were then washed with 300 μl of the above buffer. The amount of radioactivity retained on the filters was measured by liquid scintillation spectrometry. Dissociation constants and estimated stoichiometries for DnaA (K_D of about 0.01 μM with or without tL2 or L2, and n=0.4 with DnaA only) were calculated as described (69). The inset shows results obtained at the lower ATP concentrations. In (**B**), ATPase assays were performed essentially as described (26,44). Reactions (90 μl) contained 50 mM Tris-HCl pH 8.0, 15% (v/v) glycerol, 2.5 mM magnesium acetate, 0.1% Triton X-100, 7 mM DTT, 5 μM [α^{32}P]-ATP (1.5 x 10^5 cpm/pmol), and DnaA (10 pmol; 0.5 μg). After incubation on ice for 15 min, M13*oriC*2LB5 supercoiled DNA (230 fmol; 1 μg), and tL2 or L2 (each at 1 μg) were added as indicated, followed by incubation at 37°C. At the indicated times, 5 μl aliquots were removed and filtered through nitrocellulose filters as described above. The radioactivity on each filter extracted with 1 M formic acid (100 μl) in a 10 μl aliquot was quantified by liquid scintillation spectrometry. ATP and ADP in another 10 μl aliquot were separated by thin layer chromatography in 1 M formic acid and 0.5 M LiCl to determine the relative amount of ATP hydrolyzed. The plot represents the amount of ATP or ADP extracted from each filter.

Supplementary Figure 3. *tL2 and L2 bind to DNA nonspecifically*. Gel mobility shift assays were performed as described (45) with a 337 base-pair EcoR1 DNA fragment (25 fmol) carrying *E. coli oriC* from M13*oriC*2LB5 DNA (**A**), or with a 400 base-pair EcoR1-Eco0119I DNA fragment (25 fmol) encoding part of the β-lactamase gene of pUC19 (**B**) in 20 mM HEPES-KOH pH 8, 1 mM EDTA, 5 mM magnesium acetate, 0.5 μM ATP, 4 mM DTT, 0.2% Triton X-100, 0.1 mg/ml BSA, and the indicated amounts of DnaA, tL2 or L2. Both DNAs were 3' end-labeled with [α^{32}P]-dATP and the large fragment of DNA polymerase I. After electrophoresis in a 4.5% polyacrylamide gel, detection was by autoradiography of the dried gels.