Supplemental Data

DiaA dynamics are coupled with changes in initial origin complexes leading to helicase loading

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Legends for Supplemental Figures

Supplemental Fig. S1. Activities of DnaA F46A in DiaA/DNA/ATP/ADP binding and RIDA. *A.* Purified wild-type DnaA (WT) and DnaA F46A (500 ng) were stained with Coomassie Brilliant Blue after SDS-10% PAGE. *B.* The indicated amounts of wild-type DnaA (WT) or DnaA F46A were incubated on ice for 15 min in the presence (+) or absence (-) of a biotin-tagged *oriC* fragment (bio-*oriC*; 100 fmol) and/or His-DiaA (2.5 pmol as monomer). Proteins bound to bio-*oriC* were isolated using streptavidin-beads, eluted in 1% SDS, and analyzed by SDS-13% PAGE and silver staining. The bands were quantified by densitometry, and the recovered amounts of DnaA and DiaA were determined using standard curves. *C, D.* Gel-mobility retardation assay for DNA binding. This assay was performed as previously described (1,2). The indicated amounts of wild-type DnaA (WT) or DnaA F46A mutant were incubated on ice for 20 min in buffer (10 μl) containing a 15-mer DNA carrying a single DnaA box (2.5 pmol). The samples were analyzed by 8% PAGE at 4°C and Gel star (Cambrex) staining (*C*). The protein-free DNA (Free DNA) was quantified by densitometric scanning (*D*). *E, F.* The affinities of DnaA for ATP/ADP were determined by a filter-retention assay as described previously (1). DnaA protein (2 pmol) was incubated on ice for 20 min in buffer containing various concentrations of [α-^{32}P] ATP or [^{3}H] ADP. *G.* DnaA-ATP hydrolysis was assessed using a staged RIDA reconstituted system. The [α-^{32}P] ATP-DnaA (0.25 pmol) was incubated at 30°C for 20 min in buffer containing the indicated amounts of Hda protein in the presence, or absence, of the DNA-loaded clamp (10 ng). The ratio of ADP-DnaA to total ATP-/ADP-DnaA is shown as a percentage.

Supplemental References

1. Kawakami, H., Ozaki, S., Suzuki, S., Nakamura, K., Senriuchi, T., Su'etsugu, M., Fujimitsu, K., and Katayama, T. (2006) *Mol. Microbiol.* **62**, 1310-1324

2. Keyamura, K., Fujikawa, N., Ishida, T., Ozaki, S., Su'etsugu, M., Fujimitsu, K., Kagawa, W., Yokoyama, S., Kurumizaka, H., and Katayama, T. (2007) *Genes Dev.* **21**, 2083-2099

Supplemental Figure S1