

P031 Assembly of a clamp loading, AAA+ motor: the γ -complex controls the δ wrench

Ah Young Park¹, Brandon Rutolo¹, Daniel Barsky^{1,2}, Slobodan Jergic³, Mike O'Donnell⁴, Nick E. Dixon³, Carol V. Robinson¹

¹Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, CB2 1EW, United Kingdom; ²Lawrence Livermore National Laboratory, L-372, Livermore, CA 94550, USA; ³School of Chemistry, University of Wollongong, New South Wales 2522, Australia ; and ⁴Rockefeller University, Howard Hughes Medical Institute, 1230 York Avenue, New York, New York 10021, USA.

DNA replicases from all forms of life contain a processivity factor called a DNA sliding clamp, which wraps around the DNA strands. The clamp must be loaded by a clamp loader, an ATP-dependent AAA+ motor, which in *Escherichia coli* is an asymmetric pentameric ring called the γ -complex, with stoichiometry $\tau_2\gamma\delta\delta'$. The δ -subunit of the γ -complex alone can unload the DNA sliding clamp, a counterproductive, ATP-independent process; to avoid termination of the replicase, the δ “wrench” must somehow be sequestered. We present evidence here that the sequestering component is not a single protein, but in fact the full γ -complex. This role was previously obscured by heterogeneity of the γ -complex, which may contain any combination of the motor proteins γ and τ as three subunits of its pentamer. The γ -subunit is a translational frame-shift product of the τ gene lacking two C-terminal domains that interact with the helicase and polymerase core. Here we report the identification by mass spectrometry of every subspecies formed during the assembly of the clamp loader. Our results show that γ and τ exist as homo and hetero tetramers. The δ' -subunit first induces formation of a $(\tau/\gamma)_3\delta'$ sub-complex, which then binds the δ wrench. Since γ and τ can accomplish large conformational changes, we postulate that the stator δ' induces the “resting” conformation of the γ -complex that then binds δ .