**Adaptive Capacity of a DNA Polymerase Clamp Loader**

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Clamp loaders are AAA+ ATPases that load sliding clamps onto DNA to facilitate high-speed DNA replication. Using deep mutagenesis, we mapped the mutational sensitivity of the T4 bacteriophage clamp loader and sliding clamp. Most residues not directly involved in catalysis or binding tolerate mutation well. However, a notable exception is Gln118, located in the AAA+ module at a structurally central but non-catalytic site. Gln118 forms a hydrogen-bonded junction within a helical segment we term the *central coupler*, which links ATPase active sites to DNA and the clamp. Suppressor mutations and molecular dynamics simulations show that this junction maintains structural rigidity in the coupler, a feature likely critical for allosteric signaling. The conservation of this glutamine-mediated junction across diverse AAA+ ATPases suggests that hydrogen-bond networks connecting ATP molecules are a common mechanism for coupling ATP hydrolysis to mechanical work.

We also investigated the consequences of disrupting conserved catalytic residues. In the T4 clamp loaders, ATP hydrolysis depends on interactions between an aspartate in the DEAD-box motif and an interfacial arginine. Mutation of the aspartate to cysteine reduces activity but can be functionally rescued by single-site substitutions at distant sites within the ATPase domain. Cryo-EM analysis reveals an inactive conformation in which DNA binding is blocked and catalytic sites are disassembled. Restorative mutations cluster in regions undergoing conformational changes during activation, suggesting they shift the equilibrium toward the active state by increasing DNA affinity.

To further explore how mutations drive adaptation, we engineered a defective chimeric clamp loader by replacing T4 catalytic domains with those from another phage, reducing activity ~5,000-fold. Directed evolution identified multiple substitutions at a single negatively charged residue, Asp86, that restore function to within ~20-fold of wild type. These mutations eliminate a non-native electrostatic repulsion between Asp86 and the clamp, improving affinity.

Additional compensatory mutations—typically lysine or arginine substitutions at DNA-proximal positions in the clamp loader or clamp—also restore function, highlighting a latent capacity to reinforce weak interactions. Even single-point mutations can reestablish key interfaces disrupted by domain swapping.

Together, these results demonstrate the structural plasticity and evolutionary potential of the clamp-loading machinery. Mutations distant from catalytic sites can fine-tune conformational states and inter-subunit communication, enabling recovery of complex function through diverse, distributed changes. This underscores how molecular machines can evolve new functionality through surprisingly accessible mutational paths.