**Molecular mechanism of Cas1-Cas2-mediated**

**memory formation in CRISPR-Cas immunity**

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CRISPR-Cas systems are diverse, RNA-guided adaptive immune systems that defend a broad range of prokaryotes against invasions by viruses and plasmids. A crucial initial step, called ‘‘adaptation’’ or ‘‘spacer acquisition’’ is the updating process of genetic memory (spacer) by integrating foreign DNA fragments (protospacers) in between repeats in host CRISPR arrays for inheritable immunity against future invaders. So far, the molecular mechanism of new spacer acquisition has been relatively less understood. Although heterohexameric Cas1(4)-Cas2(2) complex sufficiently sustain new spacer acquisition promiscuously in *E. coli* type I-E system, it remains unclear how Cas1-Cas2 complex can process loaded protospacer DNAs into the optimal size to be integrated in CRISPR array at the leader-proximal repeat, as well as how the Cas1-Cas2 complex can specifically recognize and load protospacer DNA fragments containing protospacer-adjacent motif (PAM). Most importantly, it has not been revealed how orientation of spacer integration is achieved correctly. To characterize, we developed an assay based on single-molecule total internal fluorescence (smTIRF) microscopy and found underlying mechanism of protospacer loading process with structural and kinetics modeling. Furthermore, we identified DnaQ or DnaQ-like 3'-5' exonucleases as single-stranded overhang trimmers, and their intriguing features of trimming process. Collectively, we provide a model that delayed PAM trimming can drive the correct orientation of spacer integration in type I-E system, which was validated the model by *in vitro* biochemical and smTIRF assay. Further works might be needed in different types to generalize this notion to shed light on the integrated understanding of memory formation in CRISPR-Cas adaptive immunity.