Conformational dynamics of Cas9 governing DNA cleavage revealed by single molecule FRET

Content

Cas9 is a RNA guided, multi-domain DNA endonuclease which has been engineered as a widely-used tool for genomic editing and manipulation. The complementarity between RNA guide sequence and DNA drives Cas9 to recognize its target and to subsequently induce double-strand DNA breaks. However, the off-target binding and cleavage by Cas9 pose as one of the major challenges in its applications. Previous studies have revealed the highly flexible nature of structural conformations of Cas9, but how the conformational dynamics of Cas9 governs its nuclease activity under on- and off-target conditions remains largely unknown. Here, using intra-molecular single molecule fluorescence resonance energy transfer (smFRET) measurements, we revealed that Cas9 in apo, sgRNA-bound, and dsDNA/sgRNA-bound forms all spontaneously transits between three major conformational states, mainly reflecting significant conformational mobility of the catalytic HNH domain as shown by structural studies. We furthermore uncovered a surprising long-range allosteric communication between the HNH domain and RNA/DNA heteroduplex at the PAM-distal end to ensure correct positioning of the HNH domain, which demonstrated a unique proofreading mechanism served as the last checkpoint before DNA cleavage. We showed that several Cas9 residues were likely to mediate the allosteric communication and proofreading step through their interactions with RNA/DNA heteroduplex at the PAM-distal end. Modulating interactions between Cas9 and heteroduplex by engineering mutations on these sites provides an alternative route to improve and optimize the CRISPR/Cas9 toolbox.

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