Structural Basis for Cas9-Mediated DNA Interrogation and Editing With 5' Truncated Sgrnas

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The efficiency and accuracy of CRISPR/Cas9 targeting varies considerably across genomic targets and remains a persistent concern for implementing this system in cells and clinical studies. It has been shown that the use of 5' truncated sgRNAs can reduce the rate of unwanted off-target recognition while still maintaining on-target specificity. However, it is not well-understood how reducing target complementarity enhances specificity or how truncation past 15nt prevents full Cas9 activation without compromising on-target binding. To better understand this mechanism, we performed cryo-EM analysis on a Cas9 complex bound to truncated sgRNA. Here, we report two distinct conformations of the sgRNA-DNA duplex as well as reorganization of the RuvC, REC2 and REC3 domains. We find that approximately half of the Cas9 complexes in our sample exhibit a linear sgRNA-DNA duplex, which prevents enzyme activation. In the other predominant state, we surprisingly find that the sgRNA-DNA duplex takes on a kinked conformation and the nuclease lobe undergoes reorganization, resembling the active Cas9 conformation. Importantly, we find that the REC2 domain remains docked onto the duplex and prevents full activation of the HNH and RuvC nuclease domains. Guided by this structural information, we designed Cas9 complexes harboring large domain deletions enabling more efficient cleavage with 5' truncated sgRNAs as well as exhibiting multi-turnover kinetics. Our results provide a structural basis for Cas9 targeting with 5' truncated sgRNAs and support the development of compact, high-fidelity Cas9 complexes in future studies.