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Kinetic basis for DNA target specificity of CRISPR-Cas12a

Rick Russell
University of Texas at Austin, USA

Class II CRISPR-Cas nucleases are programmable via a guide RNA, enabling genome editing applications in nearly all organisms. However, DNA cleavage at off-target sites that resemble the target sequence is a pervasive problem that remains poorly understood mechanistically. Here, we use quantitative kinetics to dissect the reaction steps of DNA targeting by *Acidaminococcus* sp Cas12a (known previously as Cpf1). We show that Cas12a binds DNA tightly in two kinetically-separable steps. Protospacer-adjacent motif (PAM) recognition is followed by rate-limiting R-loop propagation, leading to inevitable DNA cleavage of both strands. Despite the functionally irreversible binding, Cas12a discriminates strongly against mismatches between the DNA target sequence and the crRNA along most of the target, implying substantial reversibility during R-loop formation and a transition state that is much later than for formation of an isolated helix in solution. Dissociation of Cas12a occurs on the timescale of hours, which together with the binding rate constant indicates an overall binding equilibrium constant of 50 FM for a fully matched DNA target. Mismatches throughout the R-loop exert variable effects on the equilibrium, ranging from 10- to 2000-fold weaker binding, with no discernable trend along the target sequence. The ability of Cas12a to discriminate against mismatches throughout the target sequence and the large energetic effects of PAM-distal mismatches defy common descriptions of a 'seed' sequence for Cas12a. Further, they raise the possibility of a kinetic origin, rather than a thermodynamic one, for observations of seed sequences in other CRISPR-Cas nucleases including the well-studied Cas9 nuclease. Together, our results provide a quantitative physical underpinning for the DNA cleavage patterns of Cas12a measured *in vivo* and observations of greater target specificity of Cas12a than Cas9.

Biography

Rick Russell has expertise in quantitative biochemical and biophysical approaches to dissect the activities of enzymes that manipulate RNA and DNA structures. Work from his lab has focused on RNA helicase proteins that function as chaperones in the complex tertiary folding processes of structured RNAs, and additional research has increased our understanding of the folding mechanisms of these RNAs. Recent work from the lab has also included a focus on DHX36, a human helicase protein that efficiently disrupts G-quadruplex structures in DNA and RNA.

rick_russell@cm.utexas.edu

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