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Title: UNDERSTANDING GUIDE-RNA DESIGN RULES FOR EFFICIENT AND SPECIFIC CAS13 RNA-TARGETING

Abstract

Molecular tools to dissect RNA function are valuable for studying the diverse roles RNA plays in cellular function, and as potential novel therapeutics, given thousands of dysregulated RNAs have been observed across a range of diseases. Recently described programmable nucleases, such as the prokaryotic CRISPR-associated nuclease Cas13, offer the potential to develop precise and flexible RNA-targeting technology. Cas13 has been successfully employed for potent and specific RNA-knockdown in eukaryotic cells, and other exciting applications described to date include directing additional effector protein domains to an RNA of interest by fusing to a nuclease-dead Cas13 for use in RNA imaging, RNA-splicing, RNA-detection or RNA-editing applications, for example. Despite growing use, the principles of guide-RNA selection for efficient and specific Cas13:gRNA RNA targeting remain elusive. With all previous evidence considered, we hypothesize that stable Cas13:gRNA binding to a target-RNA requires less base-pair complementarity than subsequent Cas13 RNA nuclease activation, which would lead to a more-relaxed binding specificity vs. cleavage specificity. By using various methods including transcriptome-wide measurements of Cas13 RNA binding specificity in human cells, binding, cleavage and kinetic in vitro studies, we report differential contributions of gRNA:target regions to binding and activity, and have shown that indeed these two activities are decoupled. Additionally, we have performed molecular dynamic simulations which have revealed how target RNA binding allosterically gates nuclease activation, and has allowed structure-guided engineering to generate Cas13 versions with robust on-target cleavage and reduced off-target effects.