Evolutionary and mechanistic diversity of CRISPR RNA-guided transposases

**ABSTRACT** Conventional CRISPR–Cas systems maintain genomic integrity by leveraging guide RNAs for the nuclease-dependent degradation of mobile genetic elements, including plasmids and viruses. In a remarkable inversion of this paradigm, bacterial transposons have coopted nuclease-deficient CRISPR–Cas systems to catalyze RNA-guided integration of mobile genetic elements into the genome. Here we show that programmable transposition occurs at a fixed distance downstream of target DNA sequences, accommodates variable length genetic payloads, and functions robustly in diverse bacterial species. Deep sequencing experiments reveal highly specific, genome-wide DNA integration, which is enabled by the coordinated and sequential recruitment of transposase factors to target sites specified by Cascade. By exploring a large set of evolutionarily diverse CRISPR-transposon systems, we further define key sequence motifs that establish transposase-transposon specificity during DNA excision and integration. The discovery of a fully programmable, RNA-guided transposase lays the foundation for kilobase-scale genome engineering that obviates the requirements for DNA double-strand breaks and homologous recombination.