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Chimeric Guides Probe and Enhance Cas9 Biochemical Activity

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Supporting Information

ABSTRACT: DNA substitutions in RNA can probe the importance of A-form structure, 2′-hydroxyl contacts, and conformational constraints within RNA-guided enzymes. Using this approach, we found that Cas9 biochemical activity tolerated significant substitution with DNA nucleotides in the clustered regularly interspaced short palindromic repeat RNA (crRNA). Only minimal RNA content was needed in or near the seed region. Simultaneous substitution at all positions with predicted crRNA−Cas9 2′-hydroxyl contacts had no effect on enzyme activity. The *trans*-activating crRNA (tracrRNA) also tolerated >50% substitution with DNA. DNA substitutions in the tracrRNA-pairing region of crRNA consistently enhanced cleavage activity while maintaining or improving target specificity. Together, results point to a prominent role for guide:target A-form-like helical structure and a possible regulatory role for the crRNA−tracrRNA pairing motif. A model chimeric crRNA with high activity did not significantly alter RNP assembly or target binding but did reduce Cas9 ribonucleoprotein stability, suggesting effects through conformation or dynamics. Cas9 directed by chimeric RNA−DNA guides may represent a cost-effective synthetic or molecular biology tool for robust and specific DNA cleavage.

RNA-guided enzymes are evolutionarily ancient ribonucleoproteins (RNPs) that act upon nucleic acid substrates using associated RNA guides for specificity.[1,2] Understanding how an RNA guide and its associated protein cooperate is important for characterizing enzyme mechanisms and engineering RNA-guided enzymes. One step toward understanding the functional marriage between an RNA and protein is probing with chemically modified residues.[3−5] DNA nucleotides, simply lacking a 2′-hydroxyl group, are effective probes.[6] They are affordable to synthesize and have well-characterized properties. DNA typically forms a B-form double helix when paired to another DNA strand but can readily undergo a transition to an A-form helix depending on the local environment.[7] Chimeric RNA−DNA oligonucleotides prefer to form A-form-like helices due to RNA dominance.[8−10] This is attributed to the relative flexibility of the 2′-deoxyribose sugar when incorporated into nucleic acids, as opposed to more sterically constrained ribonucleotides.[11] Inspiration for incorporating DNA nucleotides also arises from naturally occurring DNA-guided enzymes like Argonaute.[12,13]

Clustered regularly interspaced short palindromic repeat (CRISPR) RNAs (crRNAs) and the factors that associate with them, Cas proteins, are recently discovered RNA-guided proteins.[1,14] Enzymatic CRISPR−Cas complexes can recognize and degrade foreign DNA in bacteria and Archaea.[14] Enzymes like Cas9 from *Streptococcus pyogenes* have been heavily co-opted for genome editing and synthetic biology. They are straightforward to program and can elicit sequence-specific DNA cleavage.[15−17] Their continued development will depend on characterization of mechanism and their tolerance to modifications and unnatural conditions.

We chose the CRISPR−Cas9 complex from *S. pyogenes* (*Sp*Cas9) as a model system to investigate. Because *Sp*Cas9 evolved to use two separate guide RNAs,[14,16] the crRNA and *trans*-activating crRNA (tracrRNA), and to simplify chemical synthesis of RNA−DNA chimeras, we chose to use the more natural dual RNA-guided CRISPR−Cas9 complex (Figure 1A). This is in contrast to the more commonly used artificial single-guide RNA (sgRNA) that fuses a crRNA and tracrRNA together.[16] We assembled *Sp*Cas9 RNP complexes and assessed *in vitro* DNA cleavage activity with purified components (Figure S1A). Using two substrates (Figures S1B and S2), a linearized EGFP plasmid and a 60 bp fluorescently labeled DNA duplex (FAM duplex), we systematically tested DNA substitution of the crRNA. The FAM duplex targets facilitated later biochemical experiments and extended our general conclusions. We found that cleavage activity trends were quite similar for both types of target DNA substrate.

DNA nucleotides were initially incorporated at the 5′ end of the guide. Activity against a single sequence (E2 target site) steadily decreased as the DNA composition expanded beyond nine residues and into the seed region of the guide (Figure 1B and Figure S1C). The seed sequence is the portion of the guide region that nucleates target hybridization.[16] These results suggested a need for RNA in the seed sequence. To test this hypothesis, we substituted eight contiguous seed residues with DNA (crE2-I) and surprisingly observed robust activity. We suspected that the guide may instead require a minimum amount of RNA to maintain A-form-like helical structure upon hybridization to target DNA. Upon complete substitution with

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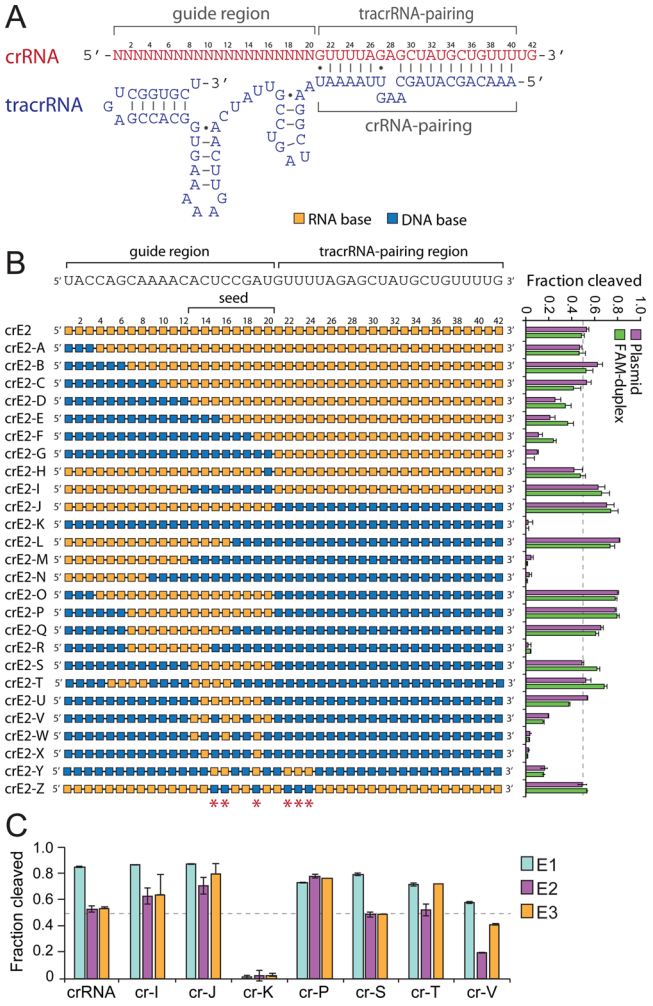

Figure 1. Chimeric RNA–DNA crRNAs are tolerated and can enhance the cleavage activity of *Sp*Cas9. (A) Sequence and secondary structure of a dual-RNA guide for *Sp*Cas9. (B) DNA substitution patterns for crRNA and subsequent *in vitro* cleavage activity for *Sp*Cas9. The DNA (blue) and RNA (orange) composition is schematically shown with the corresponding catalytic activity to the right. Cleavage of the plasmid (purple) or short FAM duplex DNA (green) is shown. Critical contacts between Cas9 and 2′-hydroxyls of crRNA are denoted with red asterisks. Error bars represent the standard error of the mean (SEM). (C) Cleavage of plasmid by *Sp*Cas9 RNPs assembled with chimeric crRNAs targeting different sequences. E2 cleavage data are taken from panel B for comparison. crRNA naming follows the substitution scheme and convention in panel B. Error bars are SEM.

DNA at the 3′ end that pairs with the tracrRNA, we observed significantly enhanced cleavage activity (crE2-J) (Table S1). Chimeras with complete DNA substitution in this region, including crE2-L, crE2-O, and crE2-P, also exhibited enhanced cleavage. Cleavage activity was, however, compromised when too many DNA residues were substituted into the guide region, in particular the seed region (i.e., crE2-M, crE2-N, and crE2-R). Minimal RNA content was achieved with as few as six RNA residues in the seed (crE2-U and crE2-V), although activity was reduced. These results suggest that a minimum number of RNA residues might be required in the guide, preferably in or near the seed region, for catalysis. The six critical 2′-hydroxyl contacts predicted between crRNA and Cas9,[18] three of which are in the seed region, are lost to varying degrees in several of these chimeras. To directly test the role of specific 2′-hydroxyl contacts, we generated an all-DNA crRNA with all six positions converted to RNA (crE2-Y). This resulted in activity similar to that of another crRNA with only six RNA residues, crE2-V. In contrast, substituting an all-RNA crRNA with DNA at these same six residues (crE2-Z), thereby eliminating all predicted critical 2′-hydroxyls between crRNA and Cas9, did not significantly alter cleavage activity (Table S1). These results demonstrate that predicted 2′-hydroxyl contacts are dispensable for biochemical activity[19] and indicate that RNA content is important for maintaining an A-form conformation of the crRNA.

To test whether enhanced cleavage was independent of guide region sequence, we targeted two additional sites of the EGFP plasmid, E1 and E3 (Figure 1C and Figure S1D,E). The tested crRNA substitution patterns gave similar results for most targets. Although the E1 target was more efficiently cleaved overall, configurations like cr-J and cr-P consistently conferred high activity.

Other CRISPR–Cas systems may also tolerate DNA substitution. To evaluate this possibility, we assembled dual RNA-guided *Staphylococcus aureus* Cas9 (*Sa*Cas9) RNPs with DNA-substituted crRNAs against the E2 target site. DNA substitution patterns showed high activity for some configurations (crE2-I, crE2-J, and crE2-P) (Figure S3A). However, others that had previously induced efficient cleavage for *Sp*Cas9 provided little activity. These results suggest that chimeric RNA–DNA guides will generally be compatible with other CRISPR–Cas systems, although substitution patterns tolerated in the guide region may vary in an enzyme-specific manner.

We reasoned that higher activity might be the result of nonspecific cleavage. To evaluate this possibility, we resolved radiolabeled short duplex cleavage products on a denaturing polyacrylamide sequencing gel (Figure 2A). We found that

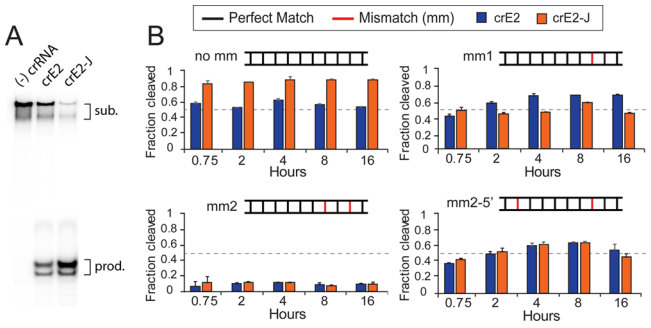

Figure 2. Chimeric crRNA maintains Cas9 specificity. (A) Resolution of radiolabeled *Sp*Cas9 cleavage products on a sequencing gel. Two radiolabeled products that differ by a single nucleotide can be resolved. (B) Cleavage activity using mismatched FAM duplex targets. *Sp*Cas9 RNPs were incubated with the targets indicated. Cleavage activity was measured after the time specified. Mismatch target sequences are listed in Table S2. Error bars are SEM.

*Sp*Cas9 guided by native crE2 and a model chimeric crE2-J catalyzed cleavage of the same phosphodiester bonds, a major and minor product as expected from previous reports.[15,16] Therefore, cleavage remained site-specific. We also tested a FAM duplex target with an NTT instead of an NGG protospacer adjacent motif (PAM) sequence. The PAM sequence is an additional sequence requirement for each Cas protein, with NGG being specific for *Sp*Cas9.[16,20,21] Little or no

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cleavage was observed for crE2 or crE2-J with an NTT PAM (Figure S3B).

To further address specificity, we synthesized three FAM duplex targets containing nucleobase changes that would result in mismatches with the crRNA. These substrates can test the substrate specificity of Cas9 assembled with chimeric crRNA. A single seed mismatch at position 16 (mm1) should only moderately impact activity, while two seed mismatches at positions 16 and 12 (mm2) should abrogate cleavage. In contrast, combining a mismatch outside of the seed (position 6) with a position 16 mismatch in the seed (mm2-5′) should be tolerated like a single mismatch in the seed.[22−24] Activity of crE2 was compared to that of substituted crE2-J for all targets (Figure 2B, Figure S3C,D, and Table S2). The level of cleavage of the mm1 target by crE2 increased from ∼40% to almost 70% over time, while the level of cleavage by crE2-J stayed nearly constant at ∼50%. This represents a significant discrimination against the mm1 target at all time points by crE2-J compared to crE2 (Table S1). Cleavage of mm2 and mm2-5′ was similar for both crE2 and crE2-J. These targets represent a small fraction of possible mismatches. Nonetheless, these results together demonstrate that chimeric crE2-J can maintain or improve Cas9 specificity.

Having established tolerance for DNA in the crRNA, we also probed the tracrRNA with DNA substitution (Figure 3).

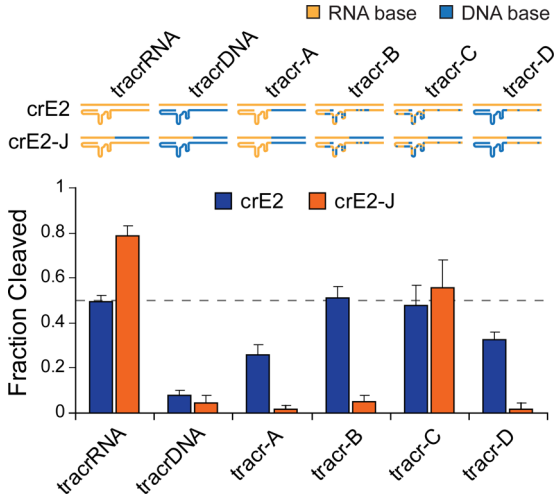

Figure 3. DNA substitutions in tracrRNA are tolerated. Cleavage activity of *Sp*Cas9 RNP assembled with crE2 and crE2-J and chimeric tracrRNA. Substitution illustrations are shown above the graph. Full substitution patterns are listed in Table S1. Error bars are SEM.

Complete conversion of tracrRNA to DNA (tracrDNA) supported little or no activity. Substitution of DNA in the crRNA-pairing region of the tracrRNA (tracr-A) supported approximately half of the usual cleavage activity for crE2 but none for crE2-J. More selective replacement of RNA with DNA in certain parts of the tracrRNA, such as stem structures (tracr-B), improved crE2 activity to that of unsubstituted tracrRNA but did not yield activity for crE2-J. Therefore, we used a crystal structure of an *Sp*Cas9 ternary complex to guide additional substitutions and potentially conserve critical Cas9 contacts or RNA structure.[18] We substituted 34 of 66 (∼50%) RNA nucleotides with DNA (tracr-C) and observed restored activity for both crE2 and crE2-J that was similar to that of an unsubstituted tracrRNA. As an additional control, we synthesized a tracrRNA containing DNA everywhere except for 16 bases in the crRNA-pairing region (tracr-D). Surprisingly, this design (∼75% DNA) supported approximately two-thirds of the activity of a normal tracrRNA for crE2. These results demonstrate that the entire 3′ portion of tracrRNA that anchors Cas9 binding can be completely substituted with DNA and still provide moderate enzyme activity. Thus, binding of tracrRNA to Cas9 may involve an induced fit of the tracrRNA. Combined with substitutions in the crRNA that enhanced activity, these results indicate that elements for regulating Cas9 activity may reside in the crRNA−tracrRNA pairing motif.

To better understand enhanced activity with chimeric crRNAs, we performed time-course cleavage, RNP assembly, target binding, and RNP stability assays. In time-course experiments, we observed rapid cleavage of the linearized plasmid by *Sp*Cas9 when guided by native crE2, confirming previous reports of fast kinetics[15,16] (Figure 4A). Cleavage was largely complete within 30 s. The crE2-J chimera likewise showed rapid kinetics but a greater overall level of cleavage, supporting our usual end-point assays. These data suggest that crE2-J may force assembly of Cas9 into a conformation that is more poised for cleavage. To determine whether DNA substitutions affected RNP assembly or global conformation, we performed electrophoretic mobility shift assays (EMSAs), or gel shifts. A catalytically inactive "dead" *Sp*Cas9 (dCas9) was titrated with radiolabeled tracrRNA−crRNA and resolved on a native polyacrylamide gel (Figure 4B and Figure S4A−C). Either crE2, crE2-J, or crE2-K as a control was tested for RNP assembly. The gel shift patterns and calculated apparent binding affinities from replicate gels were nearly the same for all three crRNAs irrespective of DNA content (Table S1). Because crE2-K Cas9 RNPs are catalytically inactive, we instead

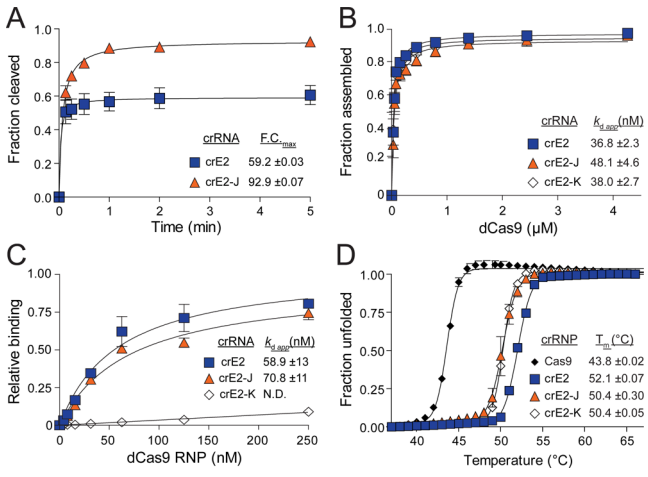

Figure 4. Model chimeric crRNA maintains RNP assembly and target engagement but reduces RNP stability. (A) *Sp*Cas9 RNP complexes were assayed for plasmid cleavage over time. $F.C._{max}$ is the maximum fraction cleaved. Error bars are SEM. (B) Quantification of binding of dCas9 to radiolabeled tracrRNA and indicated crRNAs by an EMSA. Error bars and the error of calculated apparent dissociation constants ($k_{d\ app}$) are reported as SEM. (C) Quantification of preassembled dCas9 RNP binding to radiolabeled duplex DNA as determined by dot-blot filter binding. Error bars and the error of calculated apparent dissociation constants ($k_{d\ app}$) are reported as SEM. (D) Thermal stability of *Sp*Cas9 alone or assembled into RNPs as determined by ultraviolet (UV) melts (280 nm). UV melt profiles are the average of two replicates, and the error is reported as SEM for calculated T_m values.

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tested their ability to engage a double-stranded DNA target. We assembled RNP complexes with dCas9 and titrated them with radiolabeled duplex DNA targets. RNP−target complexes were separated by filter binding. No significant difference in binding between crE2 and crE2-J was observed (Figure 4C and Table S1). In contrast, target binding by crE2-K RNPs resulted in a nearly complete loss of target binding. Finally, to assess RNP stability, we assembled *Sp*Cas9 RNP complexes and monitored their melting at 280 nm (Figure 4D). The *Sp*Cas9 protein alone had a T_m of 43.8 °C. For assembled RNP, we observed a small but significant decrease in the thermal stability with crE2-J and crE2-K (T_m of 50.4 °C each) versus crE2 (T_m of 52.1 °C) (Table S1).

In this study, we used DNA substitutions to probe the structure−function relationship between guide RNA and Cas9 protein. Our results emphasize a requirement for A-form-like structure for target binding and cleavage rather than a conservation of 2′-hydroxyl contacts between Cas9 and crRNA, in contrast to a previous report.[19] Substantial DNA substitution could be tolerated, with as few as six to eight RNA residues in or near the seed region, and removal of all 2′-hydroxyls at predicted Cas9 contacts had no effect on activity. A fully substituted, all-DNA guide was able to assemble stable RNP complexes but unable to efficiently engage target DNA. It is likely that a B-form DNA−DNA guide:target duplex cannot fit into the Cas9 RNP. Structures of the CRISPR−Cas9 complex bound to target DNA show that the guide:target duplex assumes an A-form-like architecture.[18,23,25] It is possible that DNA−DNA hybridization thermodynamics could also reduce the level of Cas9 RNP target binding and therefore reduce activity. However, comparing the activity of other crRNAs, like crE2-M and crE2-S, reveals that DNA placement in the guide is the determining factor rather than the overall DNA content. We also performed a thermal denaturation of crRNA crE2, crE2-J, and crE2-K when annealed to complementary target DNA and found very similar T_m values (Figure S4D). Thus, our results argue for structural constraints when accommodating an R-loop structure and fitting substrate DNA into the Cas9 RNP.[23]

DNA substitutions of tracrRNA showed that the 3′ hairpins and nexus could be completely replaced by DNA and still support moderate cleavage. Cas9 may be able to remodel the DNA and induce it to fit into the RuvC and PI domains.[18,25,26] Many induced fit interactions have been described for RNA−protein complexes and usually involve conformational plasticity of the RNA.[27−29] The flexibility of DNA makes it a useful probe for studying induced fit interactions.[6] Previous studies have noted that high-affinity interactions between Cas9 and truncated sgRNAs can still occur, although they do not support catalysis.[26,30]

Substitution of DNA in the tracrRNA-pairing region of crRNAs consistently enhanced Cas9 biochemical activity. Cleavage activity was site-specific, even showing specificity greater than that of a native crRNA for a single seed mismatch. This same substitution pattern had no impact on RNP assembly but did reduce RNP stability. Effects on enzyme conformation or dynamics are possible explanations for these observations. DNA nucleotides could destabilize inactive conformations or allow more frequent sampling of catalytically competent conformations.[25,30,31]

The duplex between crRNA and tracrRNA may be sensitive to DNA substitutions as it forms a unique bulged motif due to imperfect pairing.[18] This motif is partially buried in the α-helical REC lobe of *Sp*Cas9.[18,32] It makes significant contacts with two short coiled regions of the REC lobe (amino acids 103−126) and the NUC lobe (amino acids 1109−1135) that lack strong α-helical or β structure. These two regions are completely unresolved in the apo *Sp*Cas9 structure,[32] indicating that RNA binding may play a role in stabilizing them through an induced fit of the protein.[27] The paired crRNA−tracrRNA motif might bridge Cas9 domain communication through these two regions, suggesting a possible allosteric role in regulation.[32] For example, large REC lobe movements during guide RNA binding[30] are necessary to maintain an open structure for engaging and processing target DNA.[23,25,30]

Our results indicate a need for conserving A-form-like helical structure in the crRNA of the CRISPR−Cas9 complex and suggest a potential new regulatory role for the crRNA−tracrRNA pairing motif. DNA-substituted crRNAs may find utility in molecular or synthetic biology applications as affordable alternatives to native RNA guides while maintaining predictably robust and specific DNA cleavage. Our results also contribute to the rational design of chemically modified CRISPR systems.

■ ASSOCIATED CONTENT

Supporting Information

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Experimental details and supporting data (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Koonin, E. V. (2017) Evolution of RNA- and DNA-guided antivirus defense systems in prokaryotes and eukaryotes: common ancestry vs convergence. *Biol. Direct 12*, 5.

(2) Huttenhofer, A., and Schattner, P. (2006) The principles of guiding by RNA: chimeric RNA-protein enzymes. *Nat. Rev. Genet. 7*, 475−482.

(3) Kubota, M., Tran, C., and Spitale, R. C. (2015) Progress and challenges for chemical probing of RNA structure inside living cells. *Nat. Chem. Biol. 11*, 933−941.

(4) Nilsen, T. W. (2015) Nucleotide analog interference mapping. *Cold Spring Harbor Protocols 2015*, 604−608.

(5) Peacock, H., Kannan, A., Beal, P. A., and Burrows, C. J. (2011) Chemical modification of siRNA bases to probe and enhance RNA interference. *J. Org. Chem. 76*, 7295−7300.

DOI: 10.1021/acs.biochem.8b00107
Biochemistry 2018, 57, 3027−3031

(6) Ui-Tei, K., Naito, Y., Zenno, S., Nishi, K., Yamato, K., Takahashi, F., Juni, A., and Saigo, K. (2008) Functional dissection of siRNA sequence by systematic DNA substitution: modified siRNA with a DNA seed arm is a powerful tool for mammalian gene silencing with significantly reduced off-target effect. *Nucleic Acids Res. 36*, 2136−2151.
(7) Kulkarni, M., and Mukherjee, A. (2017) Understanding B-DNA to A-DNA transition in the right-handed DNA helix: Perspective from a local to global transition. *Prog. Biophys. Mol. Biol. 128*, 63−73.
(8) Wahl, M. C., and Sundaralingam, M. (2000) B-form to A-form conversion by a 3′-terminal ribose: crystal structure of the chimera d(CCACTAGTG)r(G). *Nucleic Acids Res. 28*, 4356−4363.
(9) Fedoroff, O., Salazar, M., and Reid, B. R. (1993) Structure of a DNA: RNA hybrid duplex. Why RNase H does not cleave pure RNA. *J. Mol. Biol. 233*, 509−523.
(10) Salazar, M., Fedoroff, O. Y., Miller, J. M., Ribeiro, N. S., and Reid, B. R. (1993) The DNA strand in DNA.RNA hybrid duplexes is neither B-form nor A-form in solution. *Biochemistry 32*, 4207−4215.
(11) Saenger, W. (1984) *Principles of Nucleic Acid Structure*, Springer-Verlag Inc., New York.
(12) Swarts, D. C., Jore, M. M., Westra, E. R., Zhu, Y., Janssen, J. H., Snijders, A. P., Wang, Y., Patel, D. J., Berenguer, J., Brouns, S. J., and van der Oost, J. (2014) DNA-guided DNA interference by a prokaryotic Argonaute. *Nature 507*, 258−261.
(13) Yuan, Y. R., Pei, Y., Ma, J. B., Kuryavyi, V., Zhadina, M., Meister, G., Chen, H. Y., Dauter, Z., Tuschl, T., and Patel, D. J. (2005) Crystal structure of A. aeolicus argonaute, a site-specific DNA-guided endoribonuclease, provides insights into RISC-mediated mRNA cleavage. *Mol. Cell 19*, 405−419.
(14) Mojica, F. J., and Rodriguez-Valera, F. (2016) The discovery of CRISPR in archaea and bacteria. *FEBS J. 283*, 3162−3169.
(15) Gasiunas, G., Barrangou, R., Horvath, P., and Siksnys, V. (2012) Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proc. Natl. Acad. Sci. U. S. A. 109*, E2579−2586.
(16) Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., and Charpentier, E. (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science 337*, 816−821.
(17) Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., and Church, G. M. (2013) RNA-guided human genome engineering via Cas9. *Science 339*, 823−826.
(18) Nishimasu, H., Ran, F. A., Hsu, P. D., Konermann, S., Shehata, S. I., Dohmae, N., Ishitani, R., Zhang, F., and Nureki, O. (2014) Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell 156*, 935−949.
(19) Rueda, F. O., Bista, M., Newton, M. D., Goeppert, A. U., Cuomo, M. E., Gordon, E., Kroner, F., Read, J. A., Wrigley, J. D., Rueda, D., and Taylor, B. J. M. (2017) Mapping the sugar dependency for rational generation of a DNA-RNA hybrid-guided Cas9 endonuclease. *Nat. Commun. 8*, 1610.
(20) Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., and Horvath, P. (2007) CRISPR provides acquired resistance against viruses in prokaryotes. *Science 315*, 1709−1712.
(21) Shah, S. A., Erdmann, S., Mojica, F. J., and Garrett, R. A. (2013) Protospacer recognition motifs: mixed identities and functional diversity. *RNA Biol. 10*, 891−899.
(22) Wu, X., Scott, D. A., Kriz, A. J., Chiu, A. C., Hsu, P. D., Dadon, D. B., Cheng, A. W., Trevino, A. E., Konermann, S., Chen, S., Jaenisch, R., Zhang, F., and Sharp, P. A. (2014) Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells. *Nat. Biotechnol. 32*, 670−676.
(23) Jiang, F., Taylor, D. W., Chen, J. S., Kornfeld, J. E., Zhou, K., Thompson, A. J., Nogales, E., and Doudna, J. A. (2016) Structures of a CRISPR-Cas9 R-loop complex primed for DNA cleavage. *Science 351*, 867−871.
(24) Hua Fu, B. X., Hansen, L. L., Artiles, K. L., Nonet, M. L., and Fire, A. Z. (2014) Landscape of target:guide homology effects on Cas9-mediated cleavage. *Nucleic Acids Res. 42*, 13778−13787.
(25) Jiang, F., Zhou, K., Ma, L., Gressel, S., and Doudna, J. A. (2015) A Cas9-guide RNA complex preorganized for target DNA recognition. *Science 348*, 1477−1481.
(26) Wright, A. V., Sternberg, S. H., Taylor, D. W., Staahl, B. T., Bardales, J. A., Kornfeld, J. E., and Doudna, J. A. (2015) Rational design of a split-Cas9 enzyme complex. *Proc. Natl. Acad. Sci. U. S. A. 112*, 2984−2989.
(27) Williamson, J. R. (2000) Induced fit in RNA-protein recognition. *Nat. Struct. Biol. 7*, 834−837.
(28) Turner, B., Melcher, S. E., Wilson, T. J., Norman, D. G., and Lilley, D. M. (2005) Induced fit of RNA on binding the L7Ae protein to the kink-turn motif. *RNA 11*, 1192−1200.
(29) Qin, F., Chen, Y., Wu, M., Li, Y., Zhang, J., and Chen, H. F. (2010) Induced fit or conformational selection for RNA/U1A folding. *RNA 16*, 1053−1061.
(30) Sternberg, S. H., LaFrance, B., Kaplan, M., and Doudna, J. A. (2015) Conformational control of DNA target cleavage by CRISPR-Cas9. *Nature 527*, 110−113.
(31) Sternberg, S. H., Redding, S., Jinek, M., Greene, E. C., and Doudna, J. A. (2014) DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. *Nature 507*, 62−67.
(32) Jinek, M., Jiang, F., Taylor, D. W., Sternberg, S. H., Kaya, E., Ma, E., Anders, C., Hauer, M., Zhou, K., Lin, S., Kaplan, M., Iavarone, A. T., Charpentier, E., Nogales, E., and Doudna, J. A. (2014) Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. *Science 343*, 1247997.

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Biochemistry 2018, 57, 3027−3031