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Review

Engineering nucleic acid chemistry for precise and controllable CRISPR/Cas9 genome editing

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ABSTRACT

The clustered regularly interspaced short palindromic repeats (CRISPR)/associated protein 9 (CRISPR/Cas9) genome editing technology is revolutionizing our approach and capability to precisely manipulate the genetic flow of mammalians. The facile programmability of Cas9 protein and guide RNA (gRNA) sequence has recently expanded biomedical application of CRISPR/Cas9 technology from editing mammalian genome to various genetic manipulations. The therapeutic and clinical translation potential of CRISPR/Cas9 genome editing, however, are challenged by its off-target effect and low genome editing efficiency. In this regard, developing new Cas9 variants and conditional control of Cas9/gRNA activity are of great potential for improving genome editing accuracy and on-target efficiency. In this review, we summarize chemical strategies that have been developed recently to engineer the nucleic acid chemistry of gRNA to enhance CRISPR/Cas9 genome editing efficacy, specificity and controllability. This review aims to highlight the endeavor that has been made to solve bottleneck problems in the field of CRISPR/Cas9 and inspire innovative researches to fulfill the gap between bench and bed.

1. Introduction

Over past decades, the rapid development of gene editing toolsets has invigorated gene therapy, among which CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeat and CRISPR-associated protein 9) is the top hit [1,2]. CRISPR/Cas9 genome editing is adapted from an immune defense system presents in many bacteria and archaea to protect them from the invasion of bacteriophages. It is composed of Cas9 protein, an endonuclease, and guide RNA (gRNA) [3] that anchoring Cas9 to the targeting gene loci flanked by a protospacer-adjacent motif (PAM) (Fig. 1a). In the presence of gRNA, Cas9 protein creates double strand breaks (DSB) which are subsequently repaired via two different pathways (Fig. 1b), non-homologous end joining (NHEJ) or homology-directed repair (HDR) inside cells, enabling a precise manipulation of genetic information of mammalians [4].

Unlike traditional genome editing systems, such as ZFN (zinc-finger nucleases) or TALEN (transcription activator-like effector nucleases) relying on engineering arrays of DNA-binding domains to recognize and target genome sites via protein-DNA interaction, CRISPR/Cas9 simply requires control of a short sequence (usually~20 nt) of gRNA to target gene loci. Based on its modularity and flexibility, orthogonal and multiplexed gene editing can be easily achieved by simply customizing gRNA sequences [5]. In recent years, increasing number of nucleases in CRISPR family are discovered [6] and characterized [7,8], imparting researchers with diverse tools to better elucidate and manipulate gene function. The easy-to-handle and programable properties of CRISPR/Cas9 have led to generation of extensive biomedical applications [9], including gene therapy [10], nucleic acid detection [11] and genome imaging [12]. Catalytically inactive Cas9 (dCas9) and Nickase are also instrumental recently in transcriptional regulation [13,14], base editing [15], epigenetic editing [16], etc.

As a promising therapeutic agent candidate, safety of CRISPR/Cas9 genome editing is of most concern. Chances are CRISPR/Cas9 induces imprecise genomic alteration or edits at unintended loci because mismatch of gRNA is tolerated to a certain extent [17]. Due to the dynamic inferior of off-target activity to on-target activity, off-target activity can be repressed by restricting Cas9 protein to a brief window [18] or mutating it into a high-fidelity variant at an expense of on-target editing activity [19,20]. Paired Cas9 Nickases have been utilized to increase their targeting specificity [21,22], but they incur extra potential off-target sites and make *in vivo* delivery [23] even more troublesome.

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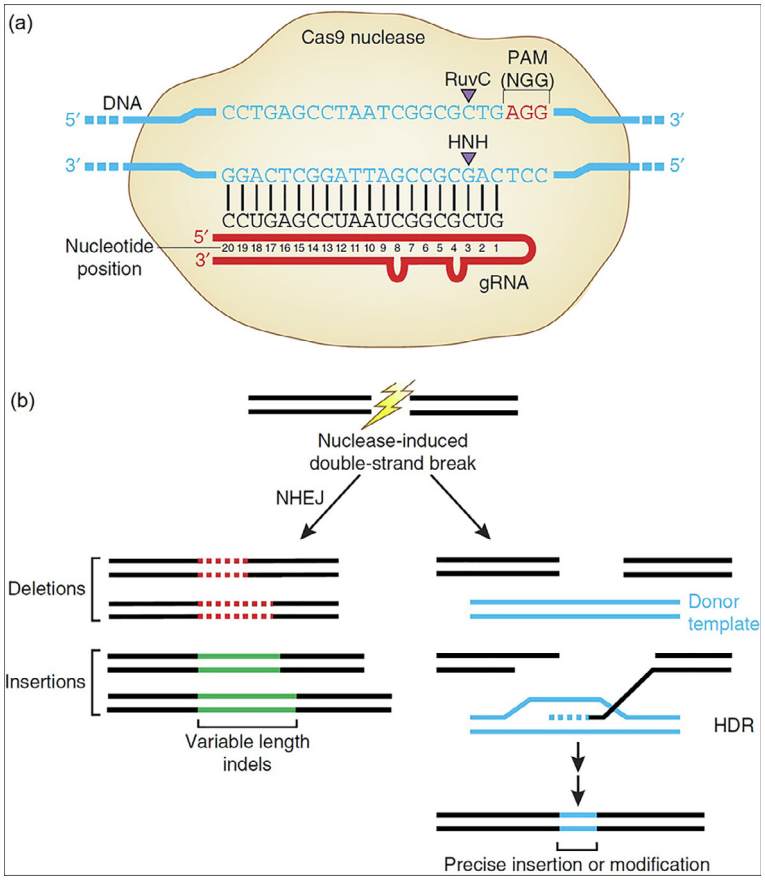

Fig. 1. (Color online) (a) CRISPR/Cas9 is a RNA guided nuclease adapted for specific gene editing. After positioned by guide RNA (gRNA) in the target DNA sequence downstream of a protospacer-adjacent motif (PAM), HNH domain of Cas9 protein generates a break at target DNA strand and RuvC domain breaks nontarget DNA strand. (b) DNA double-strand breaks (DSB) induced by CRISPR/Cas9 can be repaired via different repair pathways. Non-homologous end joining (NHEJ) is error-prone and creates insertion or deletion mutations (indels) at DSB. In assistance of donor DNA templates, the homology-directed repair (HDR) pathway provides a way to knock in gene fragments. Reprinted with permission from Ref. [4]. Copyright 2014 Springer Nature.

As tight time, space and dose control over CRISPR/Cas9 is highly desirable, scientists have been seeking for approaches to equip CRISPR/Cas9 with molecular safeguards. To date, most repurposed CRISPR/Cas9 systems focus on Cas9 protein engineering. For example, fuse Cas9 protein with other functional domains [24] and incorporate light-responsive unnatural amino acid site specifically [25]. However, inherent limitations have stymied engineering CRISPR/Cas9 through protein regulation. Despite diversity of protein engineering processes [26], they all require sophisticated and burdensome gene manipulation or directed evolution. Furthermore, as Cas9 variants and other CRISPR/Cas9 system keep flourishing, these strategies have to be reinvented from the very beginning.

An alternative way of controllable CRISPR/Cas9 genome editing is to optimize gRNA to modulate its recognition of gene loci. The most widely applied Cas9 is furnished with CRISPR RNAs (crRNAs) and trans-activating crRNA (tracrRNA) or a chimeric version termed single guide RNA (sgRNA) [3]. The crRNA recognizes and hybridizes with matching DNA, forming a R-loop structure which plays a key role in DNA cleavage [27]. The tracrRNA partially complements crRNA and is responsible for maintaining Cas9 protein conformation in active state [28]. Adopting functions of natural dual crRNA-tracrRNA duplex (Fig. 2a), sgRNA mimics their structure and can be easily produced from *in vitro* transcription. The sgRNA (Fig. 2b) is constituted by six conserved modules: the spacer, the lower stem, the upper stem, the bulge, the nexus and the hairpins [29]. The spacer forms RNA:DNA duplex when engaged with genome. While the lower stem and the upper stem are tolerant to mutant, bulge and nexus are conserved and necessary for DNA cleavage. The nexus and the hairpins interact with

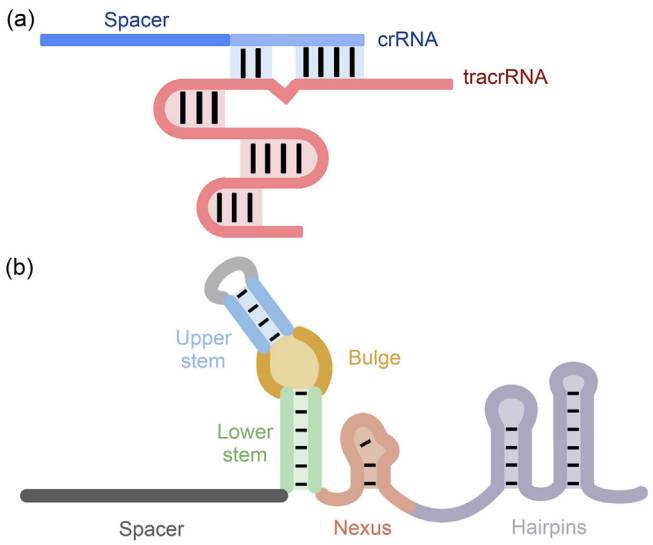

Fig. 2. (Color online) (a) Schematic representation of crRNA (blue) and tracrRNA (red). (b) Cas9 sgRNA includes six functional modules: spacer responsible for DNA targeting (black); the upper stem (blue), bulge (orange), and lower stem (green) formed by the crRNA: tracrRNA duplex and the nexus (red) and hairpins (purple). Reprinted with permission from Ref. [29]. Copyright 2014 Elsevier.

Cas9 protein and define orthogonality of CRISPR/Cas system. Cas9 recognizes a G-rich PAM at 3′ end of protospacer and creates blunt DSB. In contrast to Cas9 protein, gRNA adopts simple structures which can be predicted and depicted by abundant software tools. Deep insight into gRNA interaction [30] and large-scale profile [31,32] permit efficient gRNA design with minimal off-target effect. Straightforward interaction between gRNA and DNA enables various engineering approaches to modulate gRNA structure to improve genome editing. Truncation is believed to decrease excess binding energy of the Cas9-sgRNA:genome complex, rendering lower tolerance of mismatches [33]. In the same vein, gRNA that sterically impede R-loop formation can markedly improve Cas9 specificity [34]. Extension and mutation of gRNA can boost its knockout efficiency [6,12,35–37]. Fractions with special sequence [38] or structure [39–41] that can benefit stability and favor gRNA:protein interaction have been introduced to optimize gRNA. These rational design strategies have general applicability across CRISPR systems.

In this review we discuss several chemical approaches to reengineer CRISPR/Cas9 gRNA in aspect of improving editing efficiency and specificity by optimizing sequence, conformation and chemical property, as well as conditional control of gRNA activity using light, nucleic acids and small molecules.

2. Split and ligation

While chimeric sgRNA is commonly prepared from *in vitro* transcription, it requests tedious labor of molecular cloning. Chemical synthesis would be a feasible alternative. A solid phase oligonucleotide synthesizer can automatically generate RNAs with different and accurate sequences and is the sole way making site-specific chemical modification of RNA possible. Still it is difficult to synthesize RNA longer than 100 nt with a high yield and purity. Some researchers [42,43] reported a method that synthesizes crRNA and tracrRNA respectively. After annealing into single gRNA, they can effectively mediate gene modification. Not only does synthesis of shorter RNA chains cost less time and money, also the tracrRNA is universal. It is compatible with gRNAs targeting different genes combining with variable crRNA, making the toolbox simple and versatile.

Apart from the base-pair interaction between crRNA and tracrRNA to form gRNA for genome editing, He et al. [44] designed and synthesized 5′ hexyne tracrRNA and 3′-azide crRNA, and ligated via click conjugation (Fig. 3a). In aqueous condition, copper (I) salt can catalyze the ligation reaction to form a triazole-linked gRNA. It has a comparable function to the *in vitro* transcribed dual RNA. Taenaitree et al. [45] adopted a similar split-and-click strategy (Fig. 3b) to generate gRNA for genome editing. Considering the possibility that copper might negatively affect Cas9 activity, they designed two routes to introduce different functional azide to 3′ termini of crRNA, or alkyne to 5′ termini of tracrRNA. Combination two of RNAs and subsequent ligation using copper-catalyzed azide-alkyne cycloaddition (CuAAC) or copper free strain-promoted azide-alkyne cycloaddition (SPAAC) produces four different gRNA. This exemplifies the significance of split-and-click strategy in preparing sgRNA library on a large scale, as it circumvents repetitive and time-consuming construction and polymerase bias that *in vitro* transcription suffers. In order to improve the cleavage activity of clicked sgRNA, they substituted monomer with deoxyribonucleotides and further modified with 2′-OMe modification. The latter gRNA can direct Cas9 gene editing at the similar level to that of *in vitro* transcribed sgRNA. Moreover, the conjugation methodology provides us with positions to incorporate functional structures, for example, moieties for delivery or imaging.

Lee et al. [46] went towards another direction of sgRNA ligation. They investigated the tolerance of gRNA to chemical modifications such as amide, azide, disulfide and DBCO. Introducing modification at 5′ end of Cas9 crRNA will not compromise their activity. So they directly conjugated crRNA with chemically modified donor DNA template and electroporated the complex to BFP-HEK293T or BFP-K562 cells together with Cas9 protein. The edited cells underwent DSB generation and HDR gene insertion with a higher efficiency than traditional methods. By labelling donor DNA with fluorophore Alexa 647, the authors developed an easy approach to sort and enrich gene-edited cells using flow cytometry analysis. The long linker between crRNA and donor DNA also presents the complex with intensive negative charge which boosts electrostatic binding with cationic polymer vectors, resulting in better intracellular delivery. Although some chemical linkers may have a negative effect on activity of sgRNA, giving the diversity of chemical conjugation, more applications of the platform are on their way.

3. Chemical modification

DNA is much more stable than RNA although the most significant difference between them is a single 2′-hydroxyl substitution, which is a notable component in many biological transesterification reactions as an active nucleophile. It also altered C2′-endo to C3′-endo sugar pucker rendering RNA's preference of symmetric A-form helix rather than asymmetric B-form helix. Considering these facts, a lot of RNA chemical modification strategies are related with its 2′-OH (Fig. 4).

2′-O-methyl (2′-O-Me) modification of RNA nucleotide sugar forces RNA to transform into a conformation more favorable for Watson-Crick base pairing and shelters RNA duplex from immune system recognition. Inspired by previous study that 2′-O-Me, 2′-O-methyl-3′-phosphorothioate (MP) or 2′-O-methyl-3′thioPACE (MSP) modified oligonucleotides (Fig. 4) are resistant to serum and nuclease degradation, Hedel et al. [47] synthesized Cas9 sgRNA with these chemical modification at three nucleotides of both termini. To test whether these modifications improve the half-life of sgRNA, they delivered sgRNAs into human primary cells first, followed by Cas9 mRNA transfection 4, 8, 12 or 24 h later. For the native unmodified sgRNA, indel efficiency dropped to undetectable level by the 4-h timepoint. For MSP-modified sgRNA, no decrease of indel efficiency was observed even after a 24-h delay indicating that the chemically modified sgRNAs afford a better intracellular stability. Meanwhile, compared with naked sgRNAs, chemically modified sgRNAs increased genome editing specificity depending on the targeting loci. Ryan et al. [48] also tested MP modification systematically across Cas9 sgRNA spacer and identified modification patterns that boost editing specificity drastically.

More well-established chemical modification methods were progressively introduced to enhance the metabolic half-life and hybridization affinity of gRNA. Yin et al. [49] identified phosphorothioate (PS) backbone modifications and 2′-fluoro(2′-F) are all useful ways to optimize crRNA (Fig. 4). Further, they also studied the length effect of the chemically modified crRNA and found that 29-mer crRNA will not compromise its activity. The authors reasoned that modification sites should be broaden to tracrRNA [49]. Certain modification incorporation at a large scale may silence sgRNA activity. Therefore, detailed modification pattern should be carefully designed under the guidance of Cas9-sgRNA structure [50]. For example, modification should be avoided at seed region or nucleotides interacting with Cas9 protein. Optimal chemically modified sgRNA was identified for robust *in vivo* gene editing. Mir et al. [51] skillfully summed up these experiences and developed fully modified sgRNA. They additionally explored sgRNA compatibility with Cy3 fluorophores, N-acetylgalactosamine

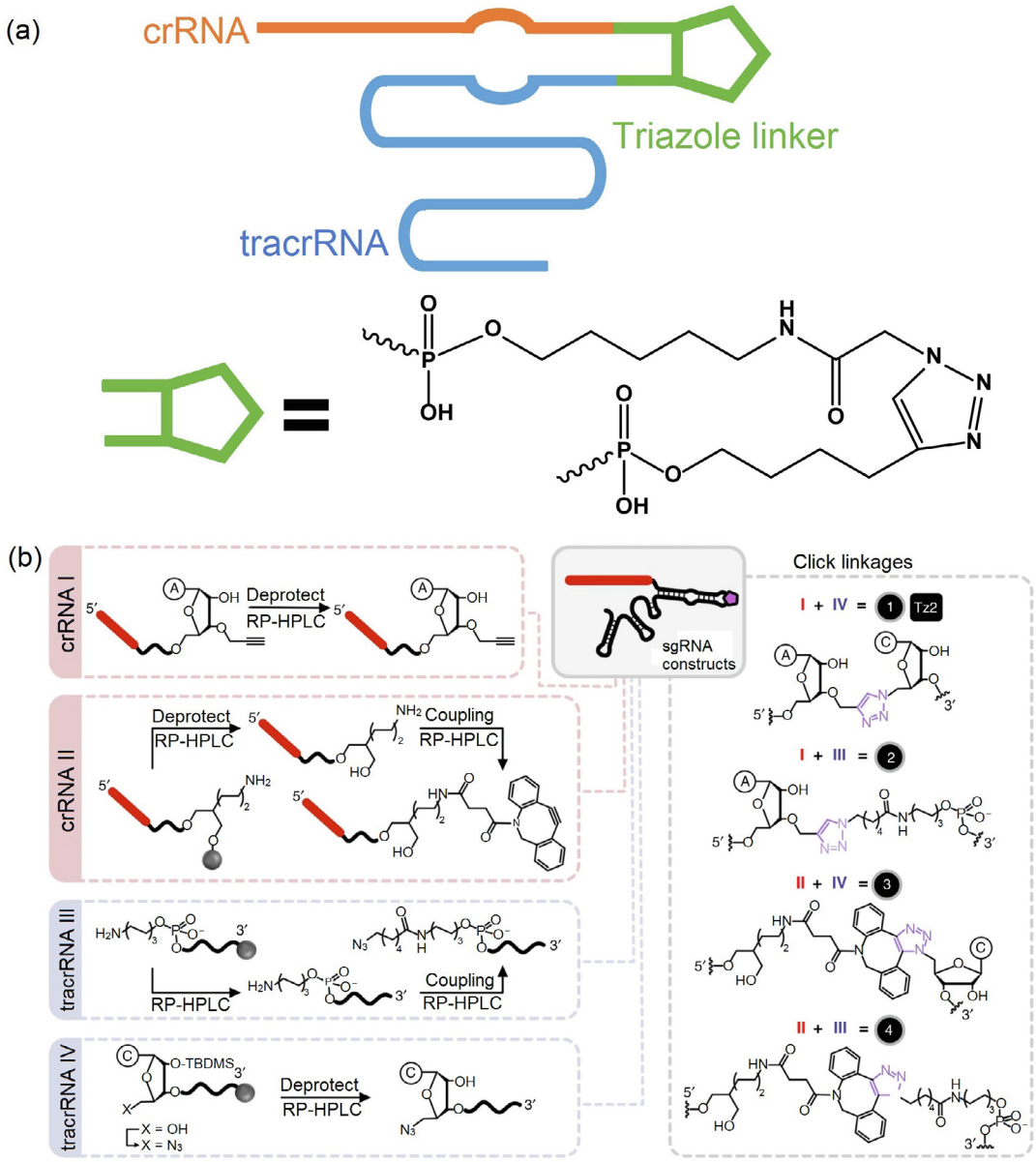

Fig. 3. (Color online) (a) Illustration of conjugated sgRNA with triazole linker. Reprinted with permission from Ref. [44]. Copyright 2016 John Wiley and Sons. (b) Guide RNA precursors containing artificial linkages 1–4 and construction of clicked crRNA-tracrRNA library. Reprinted with permission from Ref. [45]. Copyright 2019 Springer Nature.

(GalNAc), or cholesteroltriethylene glycol (TegChol), providing with handy ways to improve RNA trafficking and track uptake.

In line with Cas9-sgRNA crystal structure, Rueda et al. [52] substituted crRNA with DNA except for locations where Cas9 protein interacts with crRNA. This hybridized crXNA is sufficient in directing specific nuclease cleavage with a lower off-target effect. On the other hand, thymine has an extra methyl compared with uracil, so they replaced uracil with thymine. It turned out the methyl groups don't affect case stacking or Cas9:sgRNA interaction so they barely impact on cleavage activity. Yin et al. [53] adopted a consecutive replacement strategy. They substituted serial RNAs with DNAs at sgRNA tails which are amenable to modification. The chimera is also functional and easier to synthesize in comparison with native sgRNA.

Locked nucleic acids (LNA), S-constrained ethyl ((S)-cEt) [49] for instance, connects 2′ oxygen with 4′ carbon with a covalent bond (Fig. 4). It is an unprecedent way to tune up RNA base stacking, resulting in high binding affinity, nuclease resistance and sensitivity to mismatch. While LNA can enhance RNA thermal stability, its steric bulk could make RNA chain too rigid to form high dimension structure. It tends to aggregate when consecutively modified on RNA and may have profound hepatoxicity. Cromwell et al. [54] utilized a new generation bridged nucleic acid (BNA) analogue, 2′,4′-BNA^{NC}[N-Me], to optimize Cas9 sgRNA. It is believed to be more conformationally flexible and less toxic.

On the whole, most chemical modifications offer gRNA with resistance to hydrolysis and enzymatic degradation and alter its binding affinity with genomic DNA and Cas9 protein, leading to higher mismatch discrimination. Chemically modified RNA may also prevent innate immune response and tends to be freely uptake by cells in culture [55]. As work about chemical modification continues apace, it may reach a plateau of direct gRNA optimization. For instance, heavy RNA modification may not be able to protect it from degradation in serum as efficiently as lipid nanoparticle. More diverse chemical modifications remain to be found.

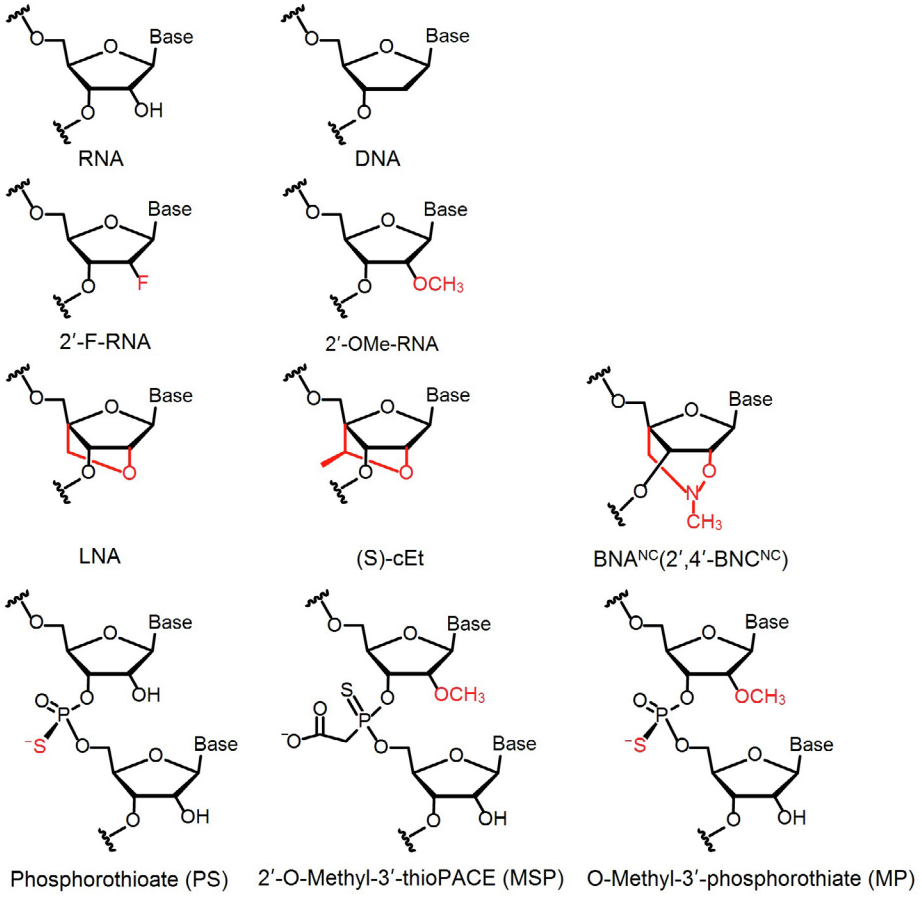

Fig. 4. (Color online) Structures of chemical modifications of oligonucleotide backbone and sugar mentioned in this review.

4. Light-responsive sgRNA for optical control of genome editing

Light is a widely adopted inducer of protein and nucleic acid chemistry and functionality with an extraordinary spatiotemporal resolution. As a non-invasive approach, optogenetic regulation alleviates the requirement for transfection or injection. Previous light-controllable CRISPR/Cas9 systems are burdened with laborious fusion of light responsive protein with Cas9 protein. Alternatively, it has been reported sequestering sgRNA is a convenient way to abrogate CRISPR/Cas9 function temporarily. Jain et al. [56] designed light-inducible blocking single-strand DNA (ssDNA) and developed a light-responsive system, called CRISPR-plus (CRISPR-precise light-mediated unveiling of sgRNA) (Fig. 5). They linked every 6-nt DNA oligonucleotides with a photoactive group and formed a protecting chain complementary to the spacer of sgRNA. The protector:sgRNA complex has a high melting temperature, thus is very stable. The protector can eliminate the function of sgRNA for Cas9 cleavage activity to a large extent. Upon light exposure, the linkers undergo photolysis and the protector is partite and unstably binds with spacer due to lower melting temperature, sgRNA is then released and restores the ability to pair with target sequence. The authors utilized CRISPR-plus to edit different genes, activating CRIPSR/Cas activity precisely and indirectly with light. The photoactive linker can be effortlessly synthesized and substituted with other functional groups. Moreover, this strategy can be easily applied to various targeting genes or CRISPR toolkits. One disadvantage is that on-off ratio of CRISPR-plus is rather low even after modulating the length of protector and number of photoactive linkers carefully.

5. Molecule responsive

Nucleic acid strand displacement reaction is one of the widely-used ways to regulate nucleic acids chain because of its sequence programmability and structure predictability. Based on strand displacement reaction, antisense RNA (asRNA) has emerged as one of powerful RNA regulators. Initially, it was designed to bind cognate mRNA and regulate gene expression at the post-transcription level. Lee and co-workers [57] combined asRNA with CRISPR/dCas9 to target the promotor of a reporter gene, in which antisense RNA was allowed to bind with the spacer of sgRNA, prohibiting it from the targeting genomic DNA.

Toehold switch has been proved to be another powerful gene regulator for nucleic acid interaction due to its sequence flexibility and orthogonality [58]. Siu and Chen [59] inserted a toehold into sgRNA to constitute toehold-gated gRNA, termed thgRNA (Fig. 6a). Initially, the spacer is occluded by the hairpin structure. Then an anti-toehold strand is added to displace toehold fragment and triggers conformation change of thgRNA to uncage spacer. They adapted the thgRNAs for both endogenous and artificial sequence, and successfully activated thgRNAs selectively and orthogonally. This “plug and play” system has the potential to be a multi-input logic programmable operation. Likewise, Li et al. [60] embedded a toehold sequence into sgRNA and developed a mRNA detection biosensor (Fig. 6b). The toehold sequence deprives sgRNA of its unique secondary structure, so it’s unavailable for Cas9 protein binding. Function of sgRNA is initiated only in presence of targeting mRNA. Repurposed sgRNA is smartly designed to cut the target sequence between the T7 promotor and the

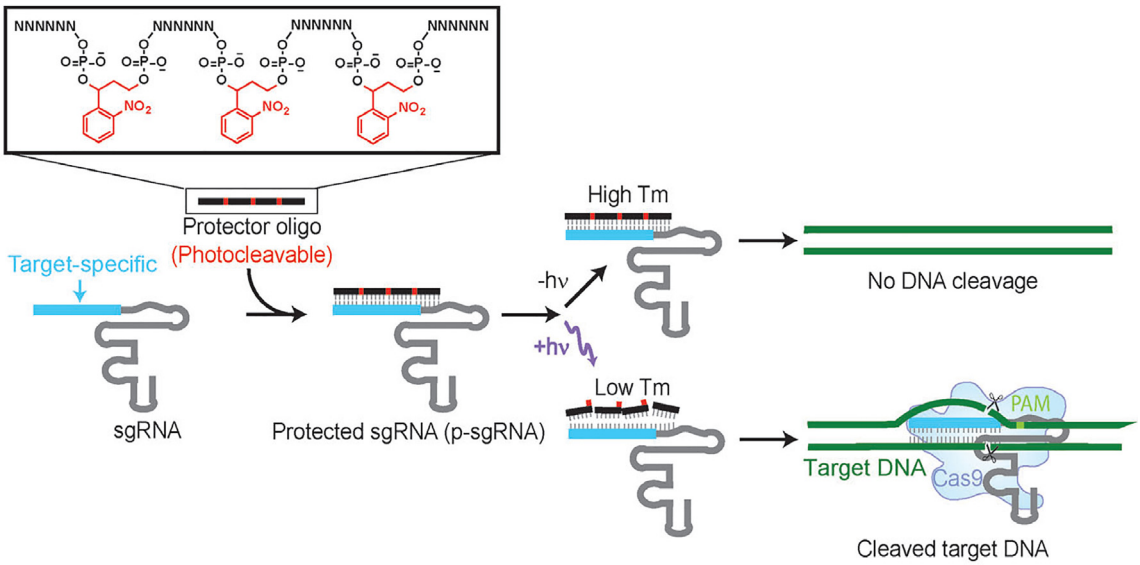

Fig. 5. (Color online) CRISPR-plus achieves photoactivatable Cas9-mediated gene editing by introducing complementary ssDNA oligonucleotide protectors that contains light-cleavable linkers. Reprinted with permission from Ref. [56]. Copyright 2016 John Wiley and Sons.

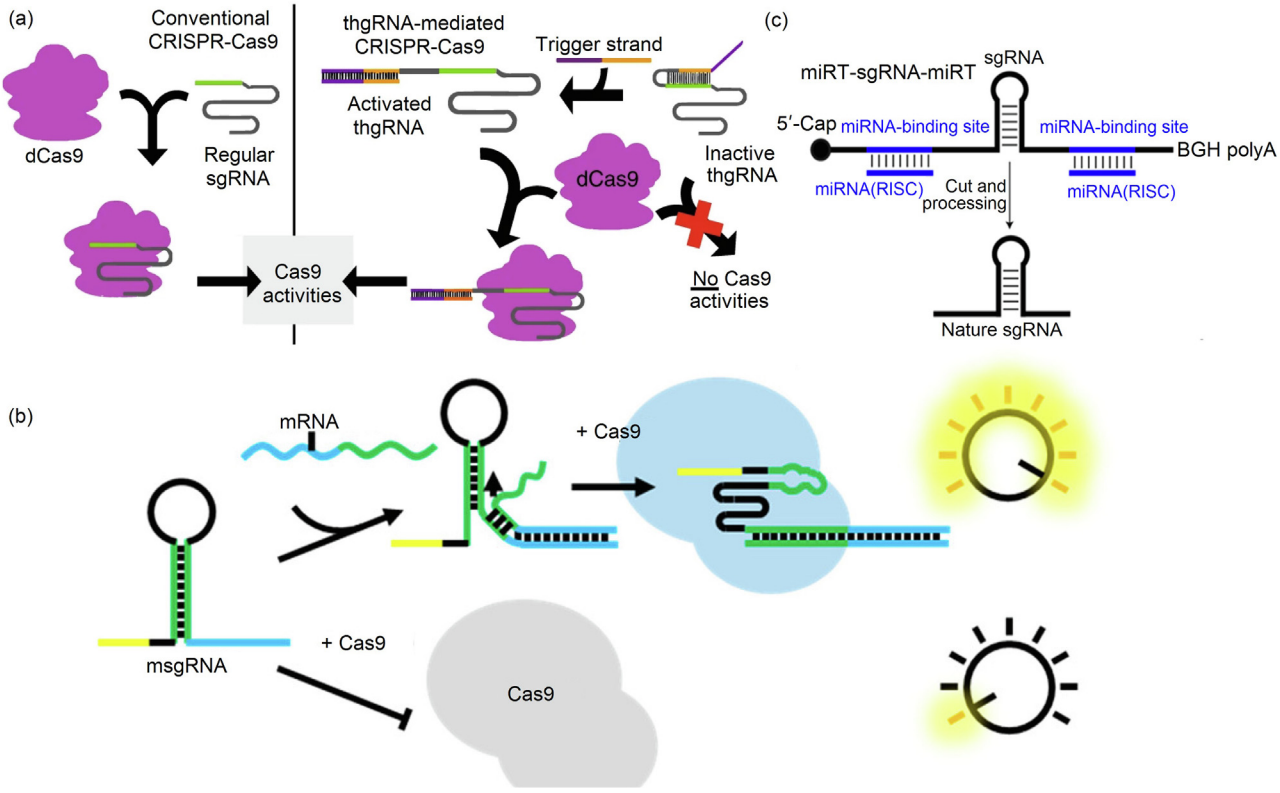

Fig. 6. (Color online) (a) Schematic representation of thgRNA-gated CRISPR-Cas9 system. Reprinted with permission from [59]. Copyright 2019 John Wiley and Sons. (b) Design of mRNA sensing CRISPR/Cas9 system. Activity of sgRNA is forbidden by insertion of mRNA complementary sequence. Reprinted with permission from Ref. [60]. Copyright 2019 American Chemical Society. (c) Pre-matured sgRNA flanked with two miRNA binding sites. It is activated after processing. Reprinted with permission from Ref. [62]. Copyright 2019 Springer Nature.

following sequence encoding spinach RNA aptamer. When the plasmid is intact, spinach RNA aptamer is transcribed and outputs fluorescence signal. When the sgRNA is turned on by targeting mRNA, the signal is turned off. It can be expanded to consist of different targeting sequences and anti-mRNA sequences. To demonstrate the multiplex regulation capability of the system, NOR and NAND logic gate programs were constructed using two sgRNA elements. Both of them exhibit good orthogonality. The main weakness with this biosensor is that it only works in bacteria.

Although the sequence of toehold or asRNA is rather arbitrary, there remains a struggling challenge that its structure should be exquisitely adjusted. For example, elongating duplex stabilizes the structure, inserting bulge on the contrary. Cas9 protein and trigger strand compete to bind with sgRNA, therefore the strand

displacement only occurs when the Gibbs free energy of sgRNA: Cas9 complex is higher than sgRNA:trigger strand complex.

Lessons learned from synthetic biology continues to add diversity to repertoire of inducible CRISPR/Cas9 genome editing. Ferry et al. [61] used a Cas9 sgRNA framework composing of a reflexed 5′-overhang to block spacer. The connecting loop is let to be the cleavage site of Cys4 endoribonuclease or Cas6A, so trigger enzymes can switch sgRNA from off-state to on-state. On the other hand, they sought to make the loop sequence complementary to antisense oligonucleotides (ASOs). Connecting loop of sgRNA hybridizes with ssDNA ASOs and then undergoes degradation mediated by RNase H, blocking sequence released at the same time. Leveraging these regulation modules, branching or orthogonal gene networks were implemented. One drawback is that these enzymes are from prokaryote, so relevant plasmid delivery is required to induce sgRNA activity restoration in cells. More recently, Wang et al. [62] flanked sgRNA with two fragments that can bind with miRNA (Fig. 5c). After formation of RNA-induced silencing complex (RISC), it undergoes AGO2-mediated cleavage and releases functional sgRNA. This CRISPR/Cas9 platform meets the end of cell-type-specific regulation and can be adapted for miRNA detection.

By inserting blocking segment into Cas9 sgRNA tetraloop or extending 5′ end with a complementary sequence, Tang et al. [63] demonstrated that blocking the spacer can abolish CRISPR/Cas9 activity more effectively than blocking the annealing region. A self-cleaving hammerhead ribozyme was integrated between sgRNA and blocking sequence to modulate their binding and blocking interaction. After cleavage, base pair between sgRNA and blocking sequence is transformed from strong intramolecular interaction into weak intermolecular interaction so the blocking sequence is removed. As hammerhead ribozyme catalyzes cleavage spontaneously, the authors additionally interrupted it with theophylline-binding RNA aptamer (Fig. 7a) or guanine-binding RNA aptamer. Upon addition of corresponding ligands, function of inactive sgRNAs recovered and directed endogenous gene editing and base editing with high specificity and generality.

Kundert et al. [64] rationally designed three ways to coordinate sgRNA conformation and activity: (1) grafting aptamers on different stem, (2) splitting sgRNA and recombining with aptamers, (3) inducing different base-pairing pattern. By screening the massive sgRNA library by *in vitro* assay, it is illustrated the last strategy is most successful in controlling CRISPR/Cas9 activity. Theophylline aptamer can deactivate or activate sgRNA depending on its insertion site. Reengineered sgRNA exhibits a linear response to the concentration of theophylline. In order to verify its superiority in independent control of genome editing with different ligands, the authors introduced 3-methylxanthine aptamer. This aptamer is strictly specific to its ligand whereas theophylline aptamer recognizes both. Based on these two regulators, they activated, deactivated and surprisingly, reactivated protein expression with different stimuli.

Guide RNAs containing various aptamers as riboswitches were developed into signal conductors in response to a series of internal or external molecule signals [65]. Transcription regulators were recruited by CRISPR/Cas9 to be the linker between signal concentrations (input) and gene-expression events (output). For example, tetracycline aptamer was incorporated into the 3′ end of sgRNA targeting VEGF gene to temporarily block its activity and dCas9-VP64, a transcriptional activator was co-expressed. Tetracycline can induce restoration of sgRNA activity thus increase level of VEGF mRNA and protein in a dose-dependent way. Apart from transcription regulation, they are also competent to construct Boolean logic gates, outputting luciferase signal and rewire signaling pathways.

In addition to repurposing responsive CRISPR/Cas9, combination of gRNA with aptamers that can recruit various protein has greatly broadened its possible applications [66], for example, simplified gene activation [67,68], enhanced gene knock-in [69] and orthogonal gene manipulation [70].

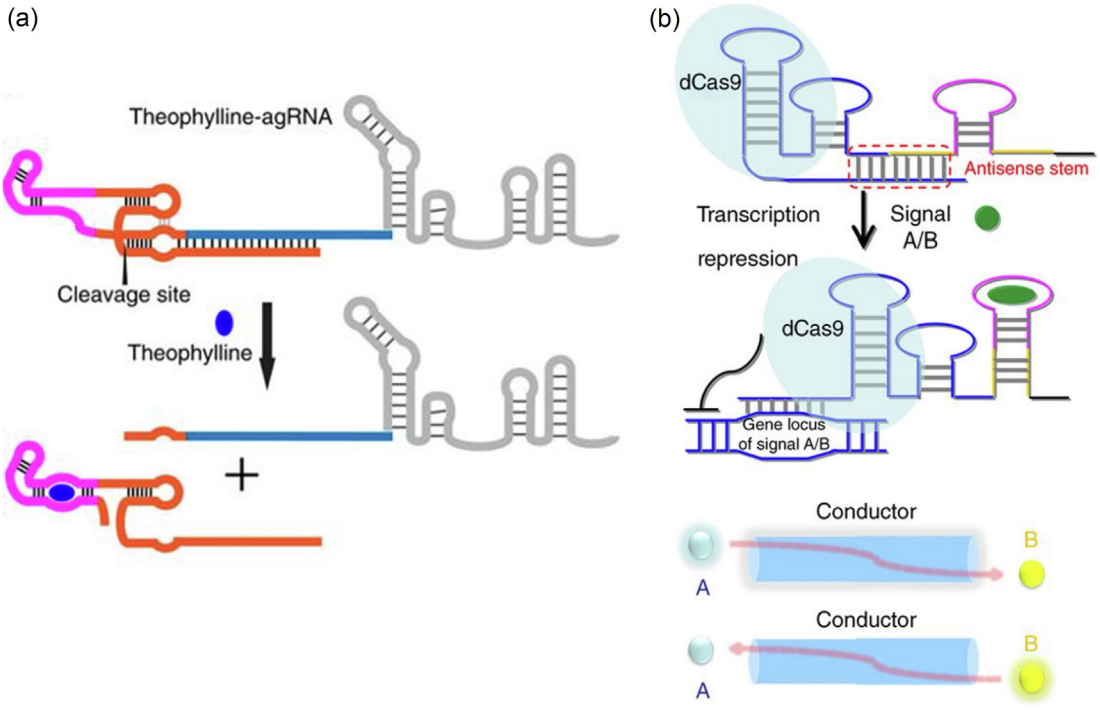

Fig. 7. (Color online) (a) Restoration activity of guide RNA with a hammerhead ribozyme interrupted by Theophylline aptamer. Reprinted with permission from Ref. [63]. Copyright 2017 Springer Nature. (b) Small molecule inducible CRISPR/Cas9 signal conductor. Reprinted with permission from Ref. [65]. Copyright 2016 Springer Nature.

The promising aptamer-reprogrammed sgRNA has a potential to take the merit of numerous aptamers but also suffers from aptamers' background activity without binding ligands and limited dynamic range. Besides, bulky aptamer imposes steric hinderance on sgRNA, making inhibition not ideal. There requires more effort to search for better aptamers and optimize aptamer-sgRNA structure.

6. Summary

With unprecedented simplicity, versatility and flexibility, CRISPR/Cas9 is a revolutionary gene editing tool. However, concerns for safety and efficacy persist. Therefore, it is highly desirable to develop new chemical tools to control Cas9 and sgRNA activity for precise and controllable genome editing. To this end, chemical modified gRNA has various advantages such as enhanced stability, selectivity, editing efficiency and can be produced on a large scale. For instance, 2′-O-Me modification of RNA improves binding affinity and nuclease resistance. LNA modification reduces flexibility of nucleic acid and increases its binding affinity. 2′-F modification changes conformation of nucleic acid strands. Phosphorothioate backbone modification stabilizes RNA. In the future, a larger concern may be that endowing gRNA with new controllable functional moieties, such as in responsive to endogenous or exogenous stimuli, including but not limited to light, targeted ligand and independent of blocking strand.

As more details of mechanisms of CRISPR/Cas9 being unraveled, the development of new chemical approaches to modulate the activity of gRNA by harnessing nucleic acid chemistry and molecular biology tools would further optimize CRISPR/Cas9 genome editing, providing opportunities for precise gene therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contributions

Both authors equally contributed to researching data for the article, the discussion of content, writing of the manuscript and its editing before submission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scib.2019.07.035.

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