CRISPR-Based Genetic Switches and Other Complex Circuits: Research and Application

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Abstract: CRISPR-based enzymes have offered a unique capability to the design of genetic switches, with advantages in designability, modularity and orthogonality. CRISPR-based genetic switches operate on multiple levels of life, including transcription and translation. In both prokaryotic and eukaryotic cells, deactivated CRISPR endonuclease and endoribonuclease have served in genetic switches for activating or repressing gene expression, at both transcriptional and translational levels. With these genetic switches, more complex circuits have been assembled to achieve sophisticated functions including inducible switches, non-linear response and logical biocomputation. As more CRISPR enzymes continue to be excavated, CRISPR-based genetic switches will be used in a much wider range of applications.

Keywords: CRISPR; genetic switch; transcription; translation; genetic circuit

1. Introduction

As a fast-growing multidisciplinary field, synthetic biology has aimed to revolutionize biological research with the concept of engineering since its earliest days. In two decades, numerous synthetic parts with increasing complexity have emerged with the capability of mimicking the function of a number of basic electric circuits, including biological bistable switches [1], oscillators [2], spatial pattern formation [3,4], logic gates [5–7], memory devices [8,9], intercellular communication [10–13] and so on. Built upon these parts, synthetic biological circuits have been introduced into a wide range of applications including autonomous metabolic engineering [14–17], cell-based therapies [18], antibiotic-free pathogen control [19,20] and so on. These circuits operate on all levels of life including transcription, translation and post-translation levels. Nevertheless, at their core, the essence of synthetic circuits is to exert precise spatial and temporal control of gene expression as output signals in response to input signals.

Compared to the traditional gene regulation elements such as inducible promoters, synthetic circuits are far superior because of their complexity that enables a sensitive or adaptive response, multiple inputs/outputs and logical signal computation. However, attempts to further increase the complexity of genetic circuits have been hindered by the inherent properties of basic synthetic parts. For example, transcription factors (TFs), the most widely adopted gene expression regulator in circuits, are known to be challenging from an engineering perspective. Specifically, the TFs offer important characteristics such as a high dynamic range and ultrasensitive response to the regulation of gene expression, but engineering a TF with specific parameters is a difficult task because of (1) poor designability,
(2) a limited choice of TFs and (3) a physiological burden to host. These unsolved problems urge the development of modular, orthogonal and easily programmable synthetic parts to enable the building of more sophisticated genetic circuits.

In recent years, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems have shed light on these problems by offering a unique category of synthetic parts, which are particularly useful for building complex genetic circuits. The CRISPR systems are natural defense mechanisms in bacteria and archaea for inactivating foreign nucleic acids [21]. Basically, CRISPR enzymes identify exogenous nucleic acids and record short fragments of them in the CRISPR array of the host genome as “immune memory”. Then, the short fragments (often referred to as “spacers”), along with repeat elements separating them, are transcribed as pre-crRNA, which is subsequently processed by specific endoribonucleases into “guide” RNAs (gRNA) for directing CRISPR endonucleases to the invading nucleic acids. With such “memory”, subsequent invasion by recognizable foreign DNA (or RNA) will be detected by the “guide” and inactivated by endonucleases.

CRISPR endonucleases are particularly suitable to be engineered as genetic switches because of their abundance, orthogonality and programmability. First, CRISPR systems are widely discovered in bacteria and archaea, which bestows an enormous pool of enzymes with diverse functions capable of targeting both DNA and RNA [22]. Compared to the protein-DNA binding of TFs, the fact that CRISPR endonuclease utilize a highly programmable oligonucleotide to recognize DNA makes it much more specific, reliable and friendly to standardize in in silico design. The nearly infinite number of possible oligonucleotide sequences also offers unrivaled potential for orthogonality. More importantly, conventional genetic switches, such as the TF-based switches, operate only on a transcription level, but the diverse nature of CRISPR systems allows the development of genetic switches that control gene expression through both transcription and translation, which greatly expands the territory of gene regulation. Therefore, by harnessing these unique features, a large number of highly modular and orthogonal CRISPR-based genetic switches have been developed in recent years (Table 1).

| CRISPR Enzyme | Additional Module | Function | Host | Reference |
|---------------|------------------|----------|------|-----------|
| dCas9         | /                | Transcriptional repression | bacteria, yeast | [23–26] |
| dCas9         | KRAB (F)         | Transcriptional repression | yeast, mammalian cell | [25–30] |
| dCas9         | Mxi1 (F)         | Transcriptional repression | yeast | [26] |
| dCas9         | SRDX (F)         | Transcriptional repression | Arabidopsis | [30] |
| dCas12a       | /                | Transcriptional repression | bacteria, mammalian cell | [31–35] |
| dCas12a       | KRAB (F)         | Transcriptional repression | mammalian cell | [33,36] |
| dCas12a       | Mxi1 (F)         | Transcriptional repression | yeast | [37] |
| dCas12a       | SRDX (F)         | Transcriptional repression | Arabidopsis | [38] |
| Csy4          | /                | Translational repression | E. coli, mammalian cell | [39] |
| Csy4          | dCas9            | Translational repression | mammalian cell | [40] |
| Csy4          | dCas9            | Translational repression | yeast, mammalian cell | [41] |
| Csy4          | dCas9            | Translational repression | yeast | [42] |
| Csy4          | dCas9/dCas12a    | Translational repression | yeast | [43] |
| dCas9         | RNAP Ω subunit (F) | Transcriptional activation | E. coli | [24] |
| dCas9         | AsIA (F)         | Transcriptional activation | E. coli | [44] |
| dCas9         | MCP-SoxS (R)     | Transcriptional activation | E. coli | [45,46] |
| dCas9         | λN22plus-PspFAITH (R) | Transcriptional activation | E. coli | [47] |
| dCas9         | VP64 (F)         | Transcriptional activation | yeast, mammalian cell, Arabidopsis | [25,26,30,48,49] |
| dCas9         | p65 (F)          | Transcriptional activation | mammalian cell | [25] |
| dCas9         | VPR (F)          | Transcriptional activation | yeast, mammalian cell | [50] |
| dCas9         | VTR3 (F)         | Transcriptional activation | mammalian cell | [51] |
| dCas9         | SunTag (F)       | Transcriptional activation | mammalian cell | [27,52] |
| dCas9         | SAM (F)          | Transcriptional activation | E. coli, mammalian cell | [53,54] |
| dCas12a       | VP64 (F)         | Transcriptional activation | mammalian cell | [33] |
| dCas12a       | p65 (F)          | Transcriptional activation | mammalian cell | [55] |
| dCas12a       | VPR (F)          | Transcriptional activation | mammalian cell | [53] |
| Csy4          | /                | Translational activation | yeast, mammalian cell | [41] |

(F) indicate module directly fused to CRISPR enzyme; (R) indicate module recruited by CRISPR enzyme or its associated gRNA.
2. CRISPR-Based Genetic Switches in Transcription Level

The first widely used CRISPR-based genetic switches functioning at transcription level are the CRISPR/dCas9 systems designed based on the Cas9 endonuclease, an RNA-guided DNA endonuclease originating from subtype II of the class 2 CRISPR system [57]. As a CRISPR endonuclease, Cas9 identifies its target through a short PAM (protospacer adjacent motif, NGG in *Streptococcus pyogenes*), binds to the adjacent 20 nt dsDNA sequence through two RNA duplexes and cleaves at a specific location [58]. Such specificity and programmability make Cas9 an ideal candidate as a tool for gene editing and gene expression regulation. For simplicity and convenience, the two RNA duplexes for Cas9 were combined as a single guide RNA (sgRNA) [59]. In addition, although the typical length of dsDNA binding region within a sgRNA is 20 nt, shortening such a length to 17–18 nt could benefit the on-target cleavage efficiency of Cas9 [60].

To engineer endonucleases as a part of genetic switches, the endonuclease activity of these enzymes was deactivated, leaving only the DNA binding activity. Take Cas9 as an example. Two mutations (D10A and H840A) were introduced to the RuvC and HNH endonuclease domain of SpCas9 (Cas9 from *Streptococcus pyogenes*), which abolished its ability to cleave dsDNA. The resulting deactivated Cas9, with the capability to tightly bind to DNA, was named dCas9 and often used as a programmable “roadblock” of transcription in genetic switches [23] (Figure 1A, left). Such a “roadblock” can serve as a transcriptional repressor, which is often referred to as CRISPR interference (CRISPRi). Therefore, genetic switches based on CRISPRi would function as a NOT gate, which generates an output decreased signal as the input signal increases. In recent years, dCas9 has been successfully demonstrated as CRISPRi alone in bacteria [23,24], alone or with fused additional Mxi1 domain in yeast [26], and a fused KRAB (Krüppel associated box) [25–29] or SRDX (EAR-repression) [30] domain in mammalian cells and plants, respectively (Figure 1A, right). On the other hand, dCas9 can also be engineered as a transcriptional activator (CRISPRa), by incorporating an activation domain by either direct fusion, RNA-scaffold recruitment, or the combination of both approaches (Figure 1B). For prokaryotic cells, the activation domains available to be directly fused to dCas9 include the phage activator AsiA and the RNAP ω (omega) subunit [24,44]. Some other activation domains, including SoxS and PspF, can be fused to RNA binding domains and indirectly recruited by dCas9 through binding with the RNA scaffolds connected to sgRNA [45–47]. Interestingly, the output of transcription regulation can be tuned by manipulating the DNA binding region of sgRNA by introducing mismatches [47]. In eukaryotic cells, all the reported dCas9-based transcriptional activators are designed by direct fusion of an activation domain, including multiple copies of Herpes simplex viral protein 16 domains (VP64) [25,26,30,48,49], the p65 domain [25], the combined VPR (VP64-p65-RTA) [50] and VTR3 [51] domains, the multimeric peptide array (SunTag) [27,52] and the synergistic activation mediator (SAM) [53,54] (Figure 1C).

To push the CRISPR technology forward, new CRISPR endonucleases besides Cas9 have been continuously excavated in the past decade. One subtype V endonuclease of the class 2 CRISPR system, Cas12a (previously known as Cpf1), has drawn more attention than others for its unique dual functionality to process its own pre-crRNA before cleaving dsDNA [61]. In the same way as Cas9, Cas12a has also been mutated to serve as transcription regulators. Multiple versions of DNase deactivated Cas12a (dCas12a), some of which have shown great potential for replacing dCas9 in genetic switches [31–33]. For example, dCas12a has been used alone or fused with KRAB or SRDX domains as CRISPRi, and functions as CRISPRa after fusing with VP64, p65 or VTR domain [31–36,38,55].

As with transcriptional factors, the CRISPR-based systems are also not perfect. The CRISPR/Cas9 system has been troubled by (1) the toxicity from off-target effects [62–64], (2) the large size of Cas9/dCas9 and (3) the lack of non-linearity [65,66]. First, the toxicity of Cas9 has severely limited its application potential, particularly in gene therapy where safety is the top concern [67]. Meanwhile, in a complex circuit with multiple dCas9-based switches, the off-target binding of dCas9 could also affect the orthogonality among these genetic switches. Second, the relatively large size of dCas9 makes it easy to cause physiological
burden to the host, which limits its use in instances that require a high expression level of dCas9. For example, the limitation of dCas9 expression level could cap the maximum activation signal in CRISPRa or increase the background signal in CRISPRi, both conditions that pose a negative impact on the performance of dCas9-based genetic switches by reducing their dynamic range. Notably, the search for endonuclease smaller than Cas9 has led to the discovery of more compact endonucleases, including Cas12a [61], Cas12b [68], CasΦ [69] and CasX [70], which greatly promoted the delivery of CRISPR endonuclease in gene therapy.

Third, the lack of non-linear behavior (e.g., ultrasensitivity) of CRISPR-based genetic switches is determined by the binding mechanism of CRISPR endonuclease, which functions by a monomeric endonuclease binding to DNA via a single binding site [65]. Achieving non-linear functions with CRISPR-based systems requires the cooperative activity of multimers or multiple binding sites [71], as in the TF-based genetic switches [72,73]. Therefore, to achieve non-linear functions with CRISPR-based switches, a more complex design is required.

Figure 1. Schematic representation of CRISPR-based genetic switches in transcription level. (A) Schematic of dCas9 or dCas12a function as a transcriptional repressor by blocking RNAP by itself (Left), with a fused repressor such as Mxi1, KRAB or SRDX domain (Right). (B) Schematic of dCas9 or dCas12a function as a transcription activator by fusing with an activation domain such as AsiA and the RNAP ω (omega) subunit, VP64, VPR, etc. (left), or by recruiting an activator through the aptamer fused with sgRNA such as SoxS and PspF (Right). (C) Schematic of dCas9 or dCas12a function as a transcriptional activator in combination with various types of activation domains. Red light indicates repression, green light indicates activation.

3. CRISPR-Based Genetic Switches in Translation Level

Besides DNA endonuclease, CRISPR systems also contain endoribonuclease that recognize specific RNA sequences and structures on the repeats of pre-crRNA and cleaves at a certain location [74]. The genetic switches designed based on these endoribonucleases operate at the level of translation, which can work alongside the switches at the transcription level in genetic circuits [75].
The first widely used CRISPR endoribonuclease in a genetic switch is Csy4, a type I CRISPR endoribonuclease discovered in \textit{Pseudomonas aeruginosa} that recognizes a 28-nt RNA hairpin structure and precisely cleaves between the 20G and 21C [76,77] (Figure 2A). Such precision enables Csy4 to control translation via cleaving of its target RNA hairpin inserted at the 5′ capping or 3′ UTR region of an mRNA, which could severely reduce mRNA stability and, consequently, translation rate [39] (Figure 2B). Furthermore, Csy4 has been demonstrated to control translation in a more sophisticated way for better performance and programmability. For example, a Csy4-based translational activator has been engineered by inserting the Csy4 target RNA hairpin between an RBS (Ribosome Binding Site) and a cis-repressive element [56]. As a result, mRNA translation is inhibited as the cis-repressive element binds to RBS in a complementary manner, and will be restarted after Csy4 cleavage that releases the cis-repressive element from the RBS (Figure 2C). As an endoribonuclease, Csy4 has also been used in many multiplexed gene regulations at translation level in yeast and mammalian cells [40–43]. By simultaneously processing multiple gRNAs with different target sequences for directing dCas9, Csy4, along with other similar endoribonuclease such as Cas5d, Cas6a and Cse3, can build complex logic gates for precisely regulating gene expression [56] (Figure 2D). Moreover, Cas12a, with the dual functionality of cleaving dsDNA and processing its own gRNA, is also valuable in multiplexed gene regulation because it allows simultaneous control of multiple genes using a single enzyme pairing with different gRNAs, which is much simpler than the Csy4/dCas9 systems [34–37,55] (Figure 2E).

**Figure 2.** Schematic representation of CRISPR-based genetic switches in translational level. (A) Csy4 cleavage of the RNA transcript in the processing of gRNA. (B) Csy4 function as a translational repressor by decreasing mRNA stability through cleavage of the 3′ end. (C) Csy4 function as a translational activator. (D) Csy4 and dCas9 in multiplexed gene regulation. Different color of sgRNA indicates different sgRNA that directs dCas9 to the corresponding target genes. (E) Multiplexed gene regulation based on the dual functionality of Cas12a. Different color of sgRNA indicates different sgRNA that directs dCas12a to the corresponding target genes. Red light indicates repression, green light indicates activation.
Besides RNA endoribonuclease, another category of CRISPR enzyme, the RNA-guided RNA endonuclease, have also emerged in recent years with the potential as genetic switches at translational level, namely the Cas13a and Cas7-11 endonuclease [78–80]. These enzymes possess two distinct RNase activities: (1) processing their own pre-crRNA in a way similar to Csy4 [81]; (2) targeting specific RNA sequence for cleavage. Such dual functionality makes them particularly ideal for the knockdown of RNA transcripts in vivo. Specifically, Cas13a, a subtype VI effector in class 2 CRISPR-Cas systems, previously known as C2c2, has been used for gene silencing in *E. coli*, plants and human cells [80,82]. Cas13a possesses a unique “collateral” cleavage effect, which means, upon recognition of its target, Cas13a engages cleavage of nearby non-target RNA [79]. Such a cleavage pattern of Cas13a has been harnessed in a nucleic acid detection method known as SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing), in which the target nucleotide sequence is incorporated in the gRNA, and an RNA probe is designed to be fluorescent after being cleaved via “collateral” cleavage [83]. Cas7-11, on the other hand, is a subtype III effector in the class 1 CRISPR-Cas system recently reported to have a similar function as Cas13a [78]. Currently, although these endonucleases are primarily being employed for gene silencing, it is apparent that they have great potential to be engineered as multiplexed genetic switches similar to Csy4 and Cas12a.

4. Application of CRISPR-Based Switches in Genetic Circuits

With the individual genetic switches, larger circuits can be built to offer more sophisticated functions and provide greater potential for a wider range of applications. Thus far, a number of CRISPR-based genetic circuits have been reported, which primarily incorporate CRISPR-based genetic switches in three different ways: (1) Ligand/Light-inducible genetic switches, (2) genetic switches with certain non-linear behavior or feedback patterns, (3) multi-input biocomputation circuits (Table 2). The first refers to CRISPR-based genetic switches capable of sensing input signals (e.g., chemical ligands) and generates certain output signals; the second mainly includes the circuits that still function as a genetic switch, but offer non-linear functions such as ultrasensitivity, biostability, oscillation or IFFL (incoherent feed-forward loop); the third implies the circuits capable of computing Boolean logics including AND, NOT, NOR and XOR, etc.

Ligand/Light-inducible genetic switches are powerful tools for understanding the spatial and temporal pattern of gene expression because they offer the crucial capability of controlling genetic switches with external or internal input signals, such as chemicals or the light of specific wavelengths (Figure 3A, left and middle). Typically, such inducibility is achieved by endonuclease (mainly dCas9) fused with chemical-induced dimerizing domains (CID) or optogenetically inducible dimerizing domains (OID) that sense chemicals or light signals, respectively. Specifically, the two parts of dimerization domains are separately fused with endonucleases (dCas9 or dCas12a) and the effector domain. Then, dimerization occurs when certain chemical ligands or lights of certain wavelengths are detected, which completes the recruitment of the effector domain to the endonuclease binding sites on DNA. Thus far, the reported CIDs include FKBP-FRB [84,85] and DmrA-DmrC [55] induced by rapamycin, ABI-PYL1 induced by abscisic acid (ABA) [84,86] and GID1-GAI24 induced by gibberellin (GA) [86]. Meanwhile, various OIDs have also been reported, including PhyB-PIF induced by red light [87], and pMag-nMag and CRY2-CIB1 induced by blue light [88–91]. Alternatively, genetic switches can also be constructed based on split dCas9 (Figure 3A, right). In this case, dCas9 is split into two pieces and each piece is fused with part of a heterodimer [53,85,89]. Upon ligand induction, heterodimerization leads to the restoration of dCas9 enzymatic activity and subsequent DNA binding, which allows dCas9 to act as a transcriptional repressor [85]. Similarly, Cas9 can also be split and restored as an inducible genome editor [89].
Table 2. Applications of CRISPR-based genetic switches in circuits.

| CRISPR Enzyme | Dimerization Domain | Effector Domain | Input Signal | Circuit Type | Reference |
|---------------|---------------------|-----------------|--------------|-------------|-----------|
| dCas9         | FKBP-FRB            | VPR             | rapamycin    | ligand-inducible genetic switch | [84]      |
| Split dCas9   | FKBP-FRB            | VPR64           | rapamycin    | ligand-inducible genetic switch | [85]      |
| dCas12a       | DmrA-DmrC           | p65, VPR        | rapamycin    | ligand-inducible genetic switch | [55]      |
| dCas9         | ABI-PYL1            | VPR             | ABA          | ligand-inducible genetic switch | [84,86]   |
| dCas9         | GID1-GAI24          | VPR             | GA           | ligand-inducible genetic switch | [86]      |
| dCas9         | PhyB-PIF            | /               | Red light    | Light-inducible genetic switch | [87]      |
| dCas9         | pMag-nMag           | p65, VPR4       | Blue light   | Light-inducible genetic switch | [88]      |
| Split Cas9    | pMag-nMag           | /               | Blue light   | Light-inducible genome editor  | [89]      |
| dCas9         | CRY2-CIB1           | p65, VPR4       | Blue light   | Light-inducible genetic switch | [88–91]   |
| Cas12a        | /                   | fluorescent DNA probe nucleotide sequence | in vitro diagnostic toolbox  | [92–95]   |
| Cas13         | /                   | fluorescent RNA probe nucleotide sequence | in vitro diagnostic toolbox  | [96–97]   |
| dCas9         | split luciferase    | luciferase      | nucleotide sequence | in vitro diagnostic toolbox  | [98]      |
| dCas9         | /                   | /               | AHL & Ara    | toggle switch | [99]      |
| dCas9         | /                   | /               | Ara          | IFFL        | [99]      |
| dCas9         | /                   | KRAB, VPR       | DNA copy number | IFFL      | [102]     |
| dCas9         | /                   | /               | Anti-CRISPR protein | IFFL    | [103]     |
| dCas9         | /                   | /               | DAPG and Ara | NOR/AND/OR gate   | [65]      |
| dCas9         | /                   | Mxi1            | gRNA         | NOR gate/NOT gate | [104]     |
| dCas9         | GID1-GAI24          | VPR             | GA and ABA   | AND gate    | [86]      |
| dCas9         | /                   | VPR64           | β-estradiol  | AND gate    | [105]     |
| dCas9         | /                   | /               | Ara          | NOT gate    | [99,106]  |
| dCas9         | /                   | VP16            | gRNA         | NOT gate    | [107]     |
| dCas9         | /                   | /               | aTe          | NOT gate    | [108]     |
| dCas9         | /                   | /               | KRAB         | gRNA        | [109]     |

GA: gibberellin; ABA: abscisic acid; DAPG: 2,4-Diacetylphloroglucinol; Ara: Arabinose; AHL: acyl homoserine lactone; aTe: Anhydrotetra-cycline.

Besides chemicals and light signals, oligonucleotide sequences can also be a form of input signal that activates inducible CRISPR-based genetic switches. In other words, CRISPR endonuclease (e.g., dCas9 or dCasd12a) recognizes specific DNA or RNA sequences as input signals, and activates subsequent signals accordingly. Such switches are particularly suitable for nucleotide detection, which makes them quite popular as IVD (in vitro diagnostic) methods. For example, several toolkits for detecting SARS-CoV-2 have been developed based on Cas12a or Cas13a, which functions by detecting specific RNA and cleaving complementary DNA or RNA probes as output signals, respectively [92–97] (Figure 3B, left). Additionally, IVD methods using dCas9 have also been reported, in which the two parts of a split luciferase are fused with two dCas9 molecules. Positive signals are generated when the enzymatic activity of luciferase is restored upon proper placement of the two dCas9 on the target DNA [98] (Figure 3B, right).

As described above, one of the problems of CRISPR-based genetic switches is the lack of non-linear function. However, such a shortcoming can be overcome with a more complex design of CRISPR-based circuits that offers ultrasensitive, bistable and oscillatory signals. For example, toggle switches with ultrasensitive and bistable signals have been built with dCas9 in E. coli that achieved bistable toggle between two states [99] (Figure 4A). Moreover, oscillators based on Cas9 and Cas12a have also emerged recently with a robust oscillatory state in microfluid chambers [99–101]. Additionally, circuits that exhibit spatial-temporal behaviors can also be constructed with CRISPR-based genetic switches. By combining CRISPRi and CRISPRa, IFFL circuits have been constructed in E. coli and mammalian cells that display pulse-generating and stripe-forming patterns [99,102,103].

Biocomputation with logic gates is an important task of genetic circuits for signal integration, processing and logic computation. The foundation of biocomputation is various types of logic gates with robust performance. Many Boolean logic gates, such as AND, NOT and NOR gates, have been constructed with CRISPR-based circuits that functions in E. coli, yeast and human cells. For example, one-input logic gate, such as a NOT gate, can simply be a dCas9-based repressor, which converts “1” to “0” (Figure 4B).
Likewise, more layer of repressors (or activators) can be connected in a consecutive manner, which will further convert the signal back to “1” and then jump back and forth between “0” and “1” [65,66,99,104,106–109]. Thus far, the input signal can be converted up to seven times with NOT gates [104]. On the other hand, the circuit designs to integrate two signals with a single logic gate are more complex and diverse. A straightforward design strategy is to control the expression of two orthogonal sgRNAs and their corresponding endonucleases with two different inducers that serve as input signals [65]. In this case, the type of logic gate is determined by the design of the two endonucleases. Specifically, two transcriptional activators will make the circuit an AND gate [105] (Figure 4C, right), while two transcriptional repressors will result in a NOR gate [65,104] (Figure 4D). Alternatively, an AND gate can be built with other methods such as heterodimerization induced by two different ligands [53,85,86,89] (Figure 4C, left). Notably, a NOR gate is known to be “Boolean-complete”, which means all types of logic gates can be built by combining NOR gates in different ways. For example, converting the output signal of a NOR gate with a NOT gate creates an OR gate [65] (Figure 4E). Similarly, by converting the two input signals of a NOR gate with two NOT gates beforehand, the combination of the three gates (two NOT gates and one NOR gate) equals to an AND gate [65].

Figure 3. Schematic representation of genetic switches inducible by light, chemical ligands and nucleotide sequences. (A) Genetic switches activated by light- (left) or chemical ligand- (middle) induced dimerization that connects CRISPR endonuclease (dCas9 or dCas12) and activation/repression domain, as well as dimerization of split dCas9 induced by chemical inducer (right). Activation or repression depends on the type of the domains recruited after dimerization. Red light indicates repression, green light indicates activation. (B) Genetic switches activated by nucleotide sequences identified by Cas12a (left), Cas13 (middle) and dCas9 (right). Glows indicate fluorescence on probes (left and middle), or luminescence stimulated by luciferase.
is to control the expression of two orthogonal sgRNAs and their corresponding endonucleases with two different inducers that serve as input signals [65]. In this case, the type of logic gate is determined by the design of the two endonucleases. Specifically, two transcriptional activators will make the circuit an AND gate [105] (Figure 4C, right), while two transcriptional repressors will result in a NOR gate [65,104] (Figure 4D). Alternatively, an AND gate can be built with other methods such as heterodimerization induced by two different ligands [53,85,86,89] (Figure 4C, left). Notably, a NOR gate is known to be “Boolean-complete”, which means all types of logic gates can be built by combining NOR gates in different ways. For example, converting the output signal of a NOR gate with a NOT gate creates an OR gate [65] (Figure 4E). Similarly, by converting the two input signals of a NOR gate with two NOT gates beforehand, the combination of the three gates (two NOT gates and one NOR gate) equals to an AND gate [65].

Figure 4. Schematic representation of CRISPR-based non-linear and Boolean logic circuits. (A) Toggle switch built by dCas9 with two different gRNA controlled by two different input signals. (B–E) Boolean logic circuits including NOT gate (B), AND gate (C), NOR gate (D) and OR gate (E).

5. Conclusions

The enzymes discovered from CRISPR systems, with their abundance, diversity and unrivaled programmability, have shown remarkable value and potential in the construction of genetic switches operating in both transcription and translation levels. The prevalence of their existence in nature has also been a great gift that has bestowed Cas9, Cas12a, Cas13, Csy4, and Cas7-11 and much more to come. The advantages of genetic switches constructed with these enzymes have attracted the interest of many circuit designers who have reported a number of circuits with diverse functions in return. It is apparent that CRISPR-based genetic switches will continue to stay in the spotlight of the research of synthetic biology and make their potential into reality in various applications.

Author Contributions: Conceptualization, C.L. and P.D.; resources, P.D., X.Z., X.J. and W.W.; writing—original draft preparation, P.D.; writing—review and editing, P.D., C.L., X.Z. and Q.W. All authors have read and agreed to the published version of the manuscript.
Funding: This research was funded by the National Key Research and Development Program of China grant number [No. 2020YFA0907102]; the Chinese Academy of Sciences grant number [No. QYZDB-SSW-SMC050, No. XDPB1801] and Shenzhen Institute of Synthetic Biology grant number [No. JCHZ20200005].

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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