CRISPR/Cas: a potential gene-editing tool in the nervous system

Yanxia Gao, Kexin Gao and Hui Yang *

Abstract

The rapidly developmental RNA-guided CRISPR/Cas system is a powerful tool for RNA and DNA editing in a variety of cells from different species and makes a great contribution to gene function research, disease model generation and gene therapy development in the past few years. The ease of use, low cost and high efficiency of CRISPR/Cas make it commonly used in various conditions. In this review, we introduce the CRISPR/Cas system and its diverse applications in nervous system briefly, which provides a better understanding for its potential application values.

Background

The CRISPR/Cas system is gaining more and more popularity in gene editing and therapy since first discovered in 1987. Up to now, on one hand, different types of the CRISPR/Cas system were discovered to improve its size, editing efficiency and PAM limitations; on the other hand, by fusing different factors to the mutant Cas protein which inactivates its nuclease activity but retains its ability to bind a specific DNA target site by a guide RNA, different types of engineered CRISPR/Cas9 tools were developed to perform modification of a specific gene, like DNA methylation or demethylation, histone acetylation or deacetylation and so on. Here, we briefly introduce these tools and their applications in the nervous system.

Main Text

The CRISPR/Cas system

The Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated system (Cas) was first discovered in 1987 as a type of RNA-mediated adaptively immunity to defend foreign nucleotides in bacteria and archaea (Ishino et al. 1987; Wiedenheft et al. 2012) and later Charpentier, et al. revealed its potential of RNA-programmable genome editing in 2012 because of its ability of making site-specific DNA double-stranded breaks (DSBs) in vitro (Jinek et al. 2012). In the following year, researchers successfully used the CRISPR/Cas system to edit genome in mammalian cells, which indicated its strong application value in genome editing (Cho et al. 2013; Cong et al. 2013; Mali et al. 2013). Since then, the CRISPR/Cas system becomes more and more widely used in genomic editing because of its high efficiency, easy operation, low cost and diverse applications. Usually, CRISPR/Cas systems are divided into 2 classes, including 6 types (Makarova et al. 2015; Shmakov et al. 2017; Koonin et al. 2017; Pickar-Oliver and Gersbach 2019a), some of which are widely used as editing tools.

The class 2 type II CRISPR/Cas9 is one of the most popular editing tools, and simply composed of the CRISPR RNA (crRNA), the trans-activating crRNA (tracrRNA) and the Cas9 protein. In the engineered CRISPR/Cas system, it is usually simplified by transforming tracrRNA and crRNA into a chimeric single guide RNA (sgRNA) (Jinek et al. 2012), which can also direct the Cas9 to the target DNA sequence and cleave the DNA by recognizing the protospacer adjacent motif (PAM) based on Watson-Crick base pairing rules to edit genomic DNA through non-homologous end joining.© The Author(s). 2021, corrected publication 2021. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

* Correspondence: huiyang@ion.ac.cn
Institute of Neuroscience, State Key Laboratory of Neuroscience, Key Laboratory of Primate Neurobiology, CAS Center for Excellence in Brain Science and Intelligence Technology, Shanghai Research Center for Brain Science and Brain-Inspired Intelligence, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

* Correspondence: huiyang@ion.ac.cn
Institute of Neuroscience, State Key Laboratory of Neuroscience, Key Laboratory of Primate Neurobiology, CAS Center for Excellence in Brain Science and Intelligence Technology, Shanghai Research Center for Brain Science and Brain-Inspired Intelligence, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

© The Author(s). 2021, corrected publication 2021. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Lachnospiraceae bacteria have two Cas12a orthologues, Cas9 from Staphylococcus aureus (SpCas9, 1368 amino acids) and Streptococcus thermophilus (StCas9, 1121 amino acids) respectively recognize the PAM of 5′-NGG (N represents A, T, C or G) PAM (Jinek et al. 2012), however, its recognition limits the availability of SpCas9 targeting specific sites in the genome editing. To improve its availability, more Cas9 proteins from other species are found and engineered with various PAMs. For instance, Staphylococcus aureus Cas9 (SaCas9, 1053 amino acids), Neisseria meningitidis Cas9 (NmCas9, 1082 amino acids), Streptococcus thermophilus Cas9 (StCas9, 1121 amino acids) and Campylobacter jejuni Cas9 (CjCas9, 984 amino acids) respectively recognize the PAM of 5′-NGGRRT (R represents A or G), 5′-NNNNNGATT, 5′-NNAGA (W represents A or T), and 5′-NNNVRYM (V represents A, C or G; Y represents C or T) (Cong et al. 2013; Esvelt et al. 2013; Zhang et al. 2013; Hou et al. 2013; Ran et al. 2015; Friedland et al. 2015; Yamada et al. 2017). The identification and improvement of Cas9 proteins that recognize different PAMs provides us more target sites for genome editing. In addition, RCas9 (O’Connell et al. 2014), SaCas9 (Strutt et al. 2018), CjCas9 (Dugar et al. 2018) and Francisella novicida Cas9 (FnCas9) (Sampson et al. 2013; Price et al. 2015) can also edit RNA at the same time.

In contrast with the CRISPR/Cas9 system, the Cas12a (known as Cpf1), which belongs to the Class 2 type V CRISPR/Cas system, is guided by the crRNA to the target site without the tracrRNA, and cleaves DNA in a staggered way by recognizing a T-rich PAM of 5′-T/ TTN (Jinek et al. 2012; Garneau et al. 2010; Deltcheva et al. 2011; Chylinski et al. 2013; Zetsche et al. 2015). There are two Cas12a orthologues, Acidaminococcus sp. Cas12a (AsCas12a, 1307 amino acids) and Lachnospiraceae bacterium Cas12a (LbCas12a, 1228 amino acids), which have efficiency activity of genome editing in mammalian cells. The Cas12a can process its own crRNAs that makes it easier to target multiple sites (Zetsche et al. 2017), and has less off-target effects, compared to Cas9 (Kim et al. 2016). In conclusion, the CRISPR/Cas12a offers a choice of precise genomic modifications in the different applicational condition.

The recently discovered Cas13a is an RNA-targeting nuclease and belongs to class 2 type VI CRISPR/Cas13 system. The Cas13a contains two higher conservative eukaryotes and prokaryotes nucleotide-binding (HEPN) domains, which is guided by a crRNA to the target single-stranded RNA (ssRNA) and recognized by a H (H represents A, U or C) protospacer flanking sequence (PFS) in the 3′ end of the target sequence (Abudayeh et al. 2016). Moreover, the Cas13b targets RNA to accomplish RNA cleavage by recognizing the double-sided PFS (Smargon et al. 2017). The Cas13d provides a higher efficiency of RNA targeting in various cells and organisms at the optimal cleavage temperature 24～41°C (Konermann et al. 2018). In addition, the Cas13 (Cas13d-NLS from Ruminococcus flavefaciens strain XPD3002) with a smaller size can be easily packaged into adeno-associated virus (AAV) and delivered to cells and organisms (Konermann et al. 2018), which expands the genome editing toolbox beyond DNA to RNA and plays a critical role in nucleic acid engineering, transcriptome-related study and therapy development.

**Mechanisms and applications of the CRISPR/Cas system in the nervous system**

In the engineered CRISPR/Cas system, the Cas protein combining with sgRNA can target the specific gene loci and cut the DNA double-strand (DSB). Afterwards these DSBs are predominantly repaired by the error-prone non-homologous end joining (NHEJ) in eukaryotes (Wyman and Kanaar 2006; Pickar-Oliver and Gersbach 2019b), which bring about insertion or deletion (indels) in the target loci, in turn, result in gene inactivation. In addition, these DSBs are also repaired by homologous-directed recombination (HDR) and microhomology-mediated end joining (MMEJ) (Wyman and Kanaar 2006; Pickar-Oliver and Gersbach 2019b). If homologous donor sequence including our interesting sequence like gene markers tags or fluorescence protein are given during repair process, these markers possibly insert to sgRNA-targeted sites, which achieved site-specific insertion (Pickar-Oliver and Gersbach 2019b). As a result, as a new gene-editing tool, the CRISPR/Cas system has been being focused by more and more researchers including neuroscientists, since biological characterizations of the Cas protein were first found enabled to edit genes in 2012 (Jinek et al. 2012; Gasiunas et al. 2012).

The nervous system, as the most complex system in animals and humans, still has many mysteries in biology (Salles et al. 2019). For example, how does the nervous system especially the brain develop at embryo and which genes play essential roles in this process (Salles et al. 2019)? In addition, it is known that this system uses electrical and chemical means to help all parts of the body communicate with each other and performs many functions like sleep and wakefulness, mood, learning and memory, cognition and so on (Salles et al. 2019); however, many questions still need to be further explored, such as how neurons and genes in the brain achieve these functions. Besides, neuron typing also is a hard question because the number of neurons is very large, about 100 billion neurons in the humans brain (Herculano-Houzel 2009). When the CRISPR/Cas system is identified as an efficient gene-editing tools, researchers...
can hardly wait to explore its application in the nervous system and try their best to uncover these mysterious masks. In the following, we mainly review uses of the CRISPR/Cas system in the nervous system.

**Knock-out and knock-in**

Conventional methods of gene knock-out (KO) and knock-in (KI) like homologous recombination and LoxP/Cre-mediated conditional insertion and deletion are money and time consuming as they need to generate first chimeras and later KO or KI individuals (Doyle et al. 2012). Later, RNA interference enriches methods and saves time of gene knockout, although it still has some limitations, such as it cannot totally eliminate effects of a gene and knockdown gene expression level (Hannon 2002). However, the Cas-mediated tool shows competitive advantages in gene KO and KI due to its efficiency, complete deletion and time saving (Heidenreich and Zhang 2016).

Gene knock-out mediated by CRISPR/Cas in the nervous system are being reported. For example, CRISPR/sgRNA-mediated knockout successfully was achieved in induced pluripotent stem cells (iPSC)-derived neurons (Liu et al. 2016; Ortiz-Virumbras et al. 2017), in brain slice neurons in vitro (Incontro et al. 2014) and in neurons in vivo (Shen et al. 2014; Sweiech et al. 2015; Kalebic et al. 2016; Heman-Ackah et al. 2016; Park et al. 2019). Additionally, without limitations in the number of the gene loci, knockout of multiple genes, multi-copy genes and noncoding RNAs are achieved more easily than before. Typically, Amin, et al. identified functions of the multi-copy microRNA miR-218 in motor neurons by complete deletion with CRISPR/Cas (Amin et al. 2015). Moreover, it get easier access to knockout model for function identity of the gene in the nervous system, for example, Cdk5 roles in cortex folding and Mettl3 in neuronal differentiation were uncovered by its knockout with the help of CRISPR/Cas (Batista et al. 2014; Shinmyo et al. 2017). Moreover, CRISPR/Cas-mediated gene knockout dramatically saves time and money in construction of primate knockout model so that Prrt2-knockout and Bmal1-knockout monkeys were soon born in the Institute of Neuroscience (ION) from Chinese Academy of Sciences (Zuo et al. 2017; Qiu et al. 2019).

Specific insertion is becoming more convenient under the help of CRISPR/Cas. In the next year that CRISPR/Cas were used to edit genes, mice model carrying a fluorescent marker in the endogenous OCT4, NANOG, and SOX2 genes was one-step generated by injecting Cas9 mRNA, different sgRNAs and donor DNA vectors into zygotes (Yang et al. 2013), and mCherry knock-in monkey also was constructed (Yao et al. 2018). Another, Huntingtin knock-in pig model were also generated by CRISPR/Cas, which enables us to mimic the feature of Huntingtin neurodegeneration which is unavailable in the mice model (Yan et al. 2018a).

CRISPR/Cas are providing new strategies for study and therapy of neurological diseases, especially genetic disorders like Huntington’s disease (HD) and spinal muscular atrophy (SMA). HD is characterized by early striatal atrophy, which result from HTT level decreases in the brain due to CAG repeat expansion in huntingtin (HTT) gene (Jimenez-Sanchez et al. 2017). Earlier studies generated genetical HD mice models to simulate HD-like phenotypes (Mangiariini et al. 1996; Schilling et al. 1999; Ehrnhoefe et al. 2009), but there is still no efficient treatment for HD. However, researchers recently did not only generate HD pig model (Yan et al. 2018a), but also eliminated mutant HTT protein and relieved neuropathology by CRISPR/Cas-mediated inactivation of mutant HTT gene in vitro model (Shin et al. 2016; Kolli et al. 2017) and in mice model (Monteys et al. 2017; Yang et al. 2017), which provides promise for its cure. Moreover, SMA, along with general weakness and atrophy of spinal cord motor neurons and skeletal muscles, is also a severe autosomal recessive disease caused mainly by nucleotide mutations of the survival motor neuron 1 (SMN1) gene (Bergin et al. 1997; Schrank et al. 1997; Hamilton and Gillingwater 2013). However, its symptoms can be alleviated when its duplicate gene SMN2 is edited to increase SMN protein level by delivering CRISPR/Cas-sgRNA to SMA mice zygotes (Li et al. 2019), which give some cues for SMA treatment by CRISPR technology. Besides, researchers are exploring new strategies for other neurological disease like Parkinson’s disease by using the CRISPR system (Ortiz-Virumbras et al. 2017; Park et al. 2019).

**Base editing**

Cytidine deaminase and adenine deaminase can convert cytosine into thymine (C to T) and adenine into guanine (A to G) separately. When they are fused to mutant Cas protein which can bind DNA but not cleave DNA double-strand, they can change base in the activity window of Cas protein, at the upstream of the protoscaler adjacent motif (PAM) (Komor et al. 2016; Gaudelli et al. 2017; Molla and Yang 2019). Base editing is based on this principle. Considering PAM limitation to editing sites, besides discovering different Cas proteins in nature (Ma et al. 2019), numerous engineered Cas versions are also developed to extend scope of base editing, for example, the PAM of different SpCas9 variants SpCas9, VQR- SpCas9, VRER-SpCas9 and EQR-SpCas9 are separately 5′-NGG, 5′-NGAN or 5′-NGNG, 5′-NGCG and 5′-NGAG (Pickar-Oliver and Gersbach 2019b; Molla and Yang 2019; Kleinstiver et al. 2015). In addition, base editing activity also depends on deaminase which has different activities on a genomic site (Cheng...
et al. 2019). As a result, proper composition of Cas protein and deaminase is important for accurate and efficient base editing of different genes.

Noticeably, David Liu, et al. got up to 70% editing efficiency by separately fusing improved cytidine deaminase enzyme APOBEC1 and adenine deaminase TadA to mutant SpCas9 (also called dead Cas9, dCas9) (Fig. 1a), where they successfully converted an amino acid of APOE4, a gene related to Alzheimer’s disease in vitro (Komor et al. 2016; Gaudelli et al. 2017). Given no DSBs and accurate editing, engineered Cas-mediated point mutation shows potential advantages in correcting genetic diseases including neurological diseases. In addition, base editing provides a new strategy for inactivation of gene function by converting a protein-coding sequence into a stop codon (Zhang et al. 2018a).

**Epigenomic modification**

Epigenomic modifications like DNA methylation/demethylation, histone acetylation/deacetylation and nucleosome remodeling/positioning play important roles in neuronal differentiation and diseases (Feng et al. 2007; Zhou et al. 2018a). However, it is difficult to uncover how these epigenomic modifications of the specific gene loci affect gene functions in organisms with the conventional gene KO tool like the LoxP/Cre system because enzymes for epigenetic modification cannot target specific loci of genes, promoters or enhancers. CRISPR/
Cas9 has recently emerged as an unparalleled tool for the interrogation of epigenome at individual loci, which is fused to different factors to complete different modification (Vora et al. 2016).

The dCas9-p300 system fusing dCas9 to histone acetyltransferase P300 developed by Hilton et al. efficiently activates specific gRNA-targeted promotors and enhancers (Fig. 1b) (Hilton et al. 2015). In addition, dCas9 fusing to the catalytic domain of ten-eleven translocation methylcytosine dioxygenase 1 (Tet1) (Fig. 1b) or DNA methyltransferase DNMT3A efficiently methylate specific loci in vivo and in vitro (Valton et al. 2012; Vojta et al. 2016; McDonald et al. 2016), while dCas9 fusing to lysine-specific histone demethylase 1 (LSD1) (Fig. 1b) repressed expression of genes Oct4 and Tbx1 by targeting their enhancers, in turn to affect the cellular state of embryonic stem cells (Kearns et al. 2015). In conclusion, these systems have many potential applications in exploring epigenomic editing and regulation in neuronal development without causing global alterations (Zentner and Henikoff 2015; Pulecio et al. 2017).

Another epigenomic modification, RNA editing, is also enriched because of the discovery of the RNA-targeting Cas system such as the Cas13 protein. The Cas13 family can cut RNA strand by its nuclease domain, following targeting the specific loci mediated by the sgRNA (Cox et al. 2017; Yan et al. 2018b; Zhang et al. 2018b). Because of its small size, researchers show much interests in mRNA editing by the modified Cas13d protein. Cheng et al. fused the splicing factor Fox1 element to dCas13d in order to inhibit Exon 7 skipping of SMN2 (Jillette and Cheng 2018), which gives new insights in SMA cures.

Regulation of gene expression
The dCas9 protein is also further fused to different transcription activation or inactivation domains, which constructs different CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) systems to regulate gene expression (Chavez et al. 2016). The most used CRISPRa system fused the dCas9 protein to three transcriptional activators VP64, p65 and Rta at the same time, which is proved successful to activate multigene expression to induce neuronal differentiation of human iPSCs (Fig. 1c) (Chavez et al. 2015). Moreover, sgRNAs also are modified by adding two RNA hairpin aptamers. They bind to dimers of the bacteriophage MS2 coat proteins which are fused to additional activators such as p65 and the human heat shock factor 1 (HSF1) to recruit more activation molecules for each dCas9 molecule and higher efficiently amplify multigene expression (Fig. 1c) (La Russa and Qi 2015). Zhou et al. successfully achieved transcriptional activation of multiple genes in the mammalian developmental brain and efficiently converted astrocytes into functional neurons in vivo by improved CRISPRa systems (Zhou et al. 2018b). In the contrary, fusing the Krüppel-associated box of the transcriptional repressor Kox1 (Krab) to dCas9 can effective repress gene expression in cells (Fig. 1c) (Gilbert et al. 2013; Gilbert et al. 2014). Zheng et al. used the CRISPRi tool to conditionally repress synaptotagmin I (Syt1) and found that the dentate gyrus of the hippocampus has distinct regulatory roles in learning and affective processes in mice (Zheng et al. 2018).

Inducible regulation
Fusing promotors like doxycycline- or light-dependent promotors to dCas9 achieves inducible regulation of Cas9 expression, in turn to regulate turn-on and turn-off of the Cas-based editing system. Doxycycline-inducible dCas9-based system has been used in reversible disease modelling in iPSC-derived cardiomyocytes, while the light-inducible dCas9-based system induced neuronal differentiation successfully (Mandegar et al. 2016; Nihongaki et al. 2017; Shao et al. 2018). This suggests that the inducible system achieves more precise regulation in specific gene expression in cell fate, neuronal differentiation or neuronal diseases.

High-throughput screen
The modified Cas9 system can efficiently change expression levels of large-scale genes through targeting them by sgRNA libraries, which makes a breakthrough to annotate functional characteristics of genetic elements in neuronal differentiation, normal neurobiological processes and diseases. Liu et al. identified transcription factors that efficiently promote neuronal fate of ESCs by high-throughput CRISPRa screening with serial pooled sgRNA librararies, while Tian et al. revealed neuron-specific roles of genes for survival, transcriptomics states, and morphology by CRISPRi-based knockdown with a pooled sgRNA libarary (Liu et al. 2018; Tian et al. 2019).

Future directions
CRISPR/Cas-based gene editing has been widely used in the nervous system because of its high efficiency, easy operation, low cost and diverse application, which help us uncover mysterious masks of various neurons and neuronal diseases. However, some potential risks still cannot be ignored. Firstly, DNA cleavage with the Cas endonuclease facilitates small insertions or deletions of nucleotides in unwanted sites (Zhang et al. 2015) and the cytosine/adenine deaminase may target non-specific RNA (Grunewald et al. 2019; Zhou et al. 2019; Zuo et al. 2019). These off-target editing may confound experiment results if it existed in a CRISPR-based animal model and could permanently disrupt normal gene function and lead to unpredictable complications if it is
located in a patient’s DNA during CRISPR-based treatment. Secondly, considering the size of the Cas gene, it is difficult to enter cells directly. At present, Cas-sgRNA is addressed into cells mainly by liposome, virus vectors, ribonucleoprotein and so on, which have some drawbacks like limitation in size of the cargo, delivery efficiency and safety (Lino et al. 2018). In the future, more and more focus will be put on exploring hard neurobiological problems, correcting genetic diseases and performing cell therapies in the nervous system with CRISPR/Cas-based methods by overcoming its risks.

Acknowledgements

We thank the members of the Yang Laboratory for their valuable advice.

Authors’ contributions

Gao Y., Gao K. and Yang H. wrote the paper and Yang H. edited and revised the paper. The author(s) read and approved the final manuscript.

Funding

This work is supported by China National Funds for Distinguished Young Scientists (31925016) and Major Research Plan (91957122) from the National Natural Science Foundation of China.

Competing interests

The authors have declared that no competing interests exist.

Received: 1 March 2020 Accepted: 1 April 2020

Published online: 06 August 2020

References

Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaxmeyer IM, Cox DB, Shimkhov S, Makarova KS, Semenova E, Minakhin L, Severinov K, Regan A, Lander ES, Kooein EV, Zhang F. C2C2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science. 2016;353(6299):aaaf5573. https://doi.org/10.1126/science.aaf5573.
Amin ND, Bai G, Kugl JR, Bonanomi D, Pankratz MT, Gifford WD, Hinckley CA. Authors. We thank the members of the Yang Laboratory for their valuable advice.

Bergin A, Kim G, Price DL, Sisodia SS, Lee MK, Rabin BA. Identification and characterization of a mouse homologue of the spinal muscular atrophy–neuronal function. Pediatr Res. 2007;61(5 Pt 2):58R–63R.

Bhatia PJ, Mollinie B, Wang J, Qu K, Zhang J, Li L, Bouley DM, Lujan E, Haddad B, Daneshvar K, Carter AC, Flynn RA, Zhou C, Lim KS, Dedon P, Wernig M, Mullenn AC, Xing Y, Gallouzarakis CC, Chang HY. m (6) A RNA modification controls cell fate transition in mammalian embryonic stem cells. Cell Stem Cell. 2014;15(6):707–19. https://doi.org/10.1016/j.stem.2014.09.019.

Bergin A, Ginn G, Price DL, Sisodia SS, Lee MK, Rabin BA. Identification and characterization of a mouse homologue of the spinal muscular atrophy–determining gene, survival motor neuron. Gene. 1997;204(1-2):47–53. https://doi.org/10.1016/S0378-1119(97)00510-6.

Chavez A, Scheiman J, Vora S, Prutt BK, Tuttle M, PRI E, Lin S, Kiern S, Guzman CD, Wiegeon DJ, Ter-Ovanesyan D, Braff JL, Davidsohn N, Housden BE, Perrimon N, Collins JJ, Church G. Comparison of Cas9 activators in multiple species. Nat Methods. 2015;12(4):326–8. https://doi.org/10.1038/nmeth.3312.

Chavez A, Tuttle M, Prutt BK, Ewen-Campen B, Chari R, Ter-Ovanesyan D, Haque SJ, Cecchi RI, Kowal EK, Buchthal J, Housden BE, Perrimon N, Collins JJ, Church G. Programmable base editing of A.T to G.C in genomic DNA without DNA cleavage. Nature. 2017;551(7681):464. https://doi.org/10.1038/nature24644.

Gilbert LA, Horbeck MA, Adamson B, Willats JE, Chen Y, Whitehead EH, Guimaraes C, Panning P, Ploegh HL, Basisk MC, Qi LS, Kampmann M, Weissman JS. Genome-scale CRISPR-mediated control of gene repression and activation. Cell. 2014;159(3):647–60. https://doi.org/10.1016/j.cell.2014.09.029.

Gillett KA, Larson MH, Monstut L, Liu Z, Brar GA, Torres SE, Stern-Ginossar N, Brandman O, Whitehead EH, Doudna JA, Lim WA, Weissman JS, Qi LS. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell. 2013;154(2):442–51. https://doi.org/10.1016/j.cell.2013.06.044.

Grunewald J, Zhou R, Garcia SP, Iyer S, Lareae CA, Ayyee MJ, Joung JK. CRISPR–Cas9 as a programmable RNA-binding protein for any RNA target. Nature. 2013;499(7458):437–40. https://doi.org/10.1038/nature12384.

Hilton IB, D’Impolito AM, Vockley CM, Thakore PI, Crawford GE, Reddy TE, Gerbich CA. Epigenome editing by a CRISPR-Cas9-based acetyltransferase
responsible brain research for the benefit of society. Neuron. 2019;101(3):380–4. https://doi.org/10.1016/j.neuron.2019.01.005.

Sampson TR, Saroj SD, Llewellyn AC, Tzeng YL, Weiss DS. A CRISPR/Cas system mediates bacterial innate immune evasion and virulence. Nature. 2013; 497(7448):254–7. https://doi.org/10.1038/nature12048.

Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, Kozak JA, Slunt HH, Ratovitski T, Cooper JK, Jenkins NA, Copeland NG, Price DL, Ross CA, Borchelt DR. Intracellular nucleosomes and neuritogen aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. Hum Mol Genet. 1999;8(3):397–407. https://doi.org/10.1093/hmg/8.3.397.

Schnirch B, Gotz R, Gunnersen JM, Ure JM, Toyka KV, Smith AG, Sendtner M. Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc Natl Acad Sci U S A. 1997;94(18):9920–5. https://doi.org/10.1073/pnas.94.18.9920.

Shao J, Wang MY, Gu Z, Zhu S, Heng BC, Wu J, Ye H. Synthetic far-red light-regulated by accessory proteins Csx27 and Csx28. Mol Cell. 2017;65(4):618–28. https://doi.org/10.1016/j.molcel.2016.12.023.

Shmakov S, Abudayyeh OA, Essletzbichler P, Shmakov S, Makarova KS, Koonin EV, Zhang F. Diversity and evolution of class 2 CRISPR-Cas systems. Nat Rev Microbiol. 2015;13(11):691–704. https://doi.org/10.1038/nrmicro.2015.37.

Stahl EA, Heintz N, Zhang X, Xiao S, Wang H, Hao Z, Li Z, Shao J, Sun Y, Guo Y, Wang J, Hu Y, Yang J, Chen Y, Ye M, Zhang Y. Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. Proc Natl Acad Sci U S A. 2018;115(29):E6722–30. https://doi.org/10.1073/pnas.1802448115.

Tian R, Gachechiladze MA, Ludwig CH, Laurie MT, Hong JY, Nathaniel D, Prabhu V, Vojta A, Dobrinic P, Tadic V, Bockor L, Korac P, Julg B, Klasic M, Zoldos V. CRISPR interference-based platform for multimodal genetic screens in human iPSC-cells. Cell. 2015;163(3):759–71. https://doi.org/10.1016/j.cell.2015.09.038.

Tschöke B, Heidenreich M, Mahapatra P, Fedorova I, Kneppers J, DeGennaro EM, Winokur N, Choudhury SR, Abudayyeh QQ, Gootenberg JS, Wu WY, Scott DA, Severinov K, van der Oost J, Zhang F. Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. Nat Biotechnol. 2017;35(3):113–41. https://doi.org/10.1038/nbt.3973.

Zhang L, Konermann S, Biermeier NJ, Lodt F, Wu XB, Novick SJ, Strutzenberg T, Griffin PR, Hsu PD, Lyumkis D. Structural basis for the RNA-guided RNase-homology activity of CRISPR-Cas13d. Cell. 2018;171(5):212. https://doi.org/10.1016/j.cell.2018.09.001.

Zhang H, Pan H, Zhou CY, Wei Y, Ying WQ, Li ST, Wang QG, Li C, Ren YF, Li G, Ding X, Sun YD, Li GL, Song L, Li XY, Yang H, Liu ZY. Simultaneous zygotic inactivation of multiple genes in mice through CRISPR/Cas9-mediated base editing. Development. 2018;145(20):dev168906. https://doi.org/10.1242/dev.168906.

Zhang YH, Tee LY, Wang XG, Huang QS, Yang SH. Offset-target effects in CRISPR/Cas9-distributed gene engineering. Mol Ther Nucleic Acids. 2015;4:e264. https://doi.org/10.1038/mtna.2015.37.

Zhang Y, Heidrich N, Ampatuj BJ, Gunderson CW, Seifert HS, Schoen C, Vogel J, Sontheimer EJ. Processing-independent CRISPR RNAs limit natural transformation in Neisseria meningitidis. Mol Cell. 2015;59(4):486–503. https://doi.org/10.1016/j.molcel.2015.03.001.

Zeng C, Konermann S, Biermeier NJ, Lodt F, Wu XB, Novick SJ, Strutzenberg T, Griffin PR, Hsu PD, Lyumkis D. Structural basis for the RNA-guided RNase-homology activity of CRISPR-Cas13d. Cell. 2018;171(5):212. https://doi.org/10.1016/j.cell.2018.09.001.

Zhou HB, Liu JL, Zhou CY, Yao J, Liu Y, Li H, Hu XD, Li CL, Yao X, Shen XW, Sun YD, Wei Y, Liu F, Yang WQ, Zhang JM, Gage FH, Hu X, Yang H. In vivo simultaneous transcriptional activation of multiple genes in the brain. Nat Neurosci. 2018;21(3):447–54. https://doi.org/10.1038/s41593-017-0060-6.

Zhou CY, Ying WQ, Wang QF, Chen RC, Shen Q, Xu GL, Sun Q, Xiong ZQ, Yang H. One-step generation of complete gene knockout mice and monkeys by CRISPR/Cas9-mediated gene editing with multiple sgRNAs. Cell Res. 2017;27(7):219–24. https://doi.org/10.1038/cr.2017.81.