Genome engineering in rodents - status quo and perspectives

Kühn, R.

Laboratory Animals
2022 FEB 01 ; 56(1): 83-87
2021 OCT 21 (first published online)
doi: 10.1177/00236772211051842

Publisher: SAGE Publications

Commentary for the Special issue on Genome Editing

Title

Genome engineering in rodents - Status quo and perspectives

Author information

Ralf Kühn
Max-Delbrück-Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany

Email: ralf.kuehn@mdc-berlin.de

Abstract

The introduction of the CRISPR-Cas9 system in 2013 has revolutionized experimental genetics in mice and rats. This commentary gives an overview on the use of CRISPR either for gene editing in the germline or for editing and beyond editing in somatic cells. Future perspectives are opened by emerging CRISPR technologies that could enable genome engineering at larger scale.

Keywords
CRISPR, Mouse, Rat, Rodents, Genetics, Gene Editing, Genome Editing, Animal Models

Running title
Genome Editing Perspectives

Genome engineering in rodents - Status quo and perspectives

Since the first knockout mouse was described more than three decades ago, engineering of the mouse genome has proven extremely useful for understanding the function of genes *in vivo.* [1] [2] [3] On the time scale of engineering technologies I would like to highlight 1987, 2002 and 2013 as years with key discoveries that opened up new routes for research. The year 1987 witnessed the first gene targeting by homologous recombination (HR) in mouse embryonic stem (ES) cells that established a paradigm for thousands of knockout lines that followed.[4] The technology was later refined further into conditional gene targeting using Cre/loxP recombination.[5] The year 2002 saw the sequencing of the mouse genome, giving us a complete view and access to all genes, greatly enabling their engineering at individual or large scale.[6] The third key development was the application of the CRISPR-Cas9 nuclease system for gene editing in mammalian genomes in 2013, including its use in rodent zygotes.[7] [8] Looking back from here, we can now reflect on eight years of CRISPR-Cas9 applications in mouse and rat zygotes and consider - where do we stand and where do we go?

To answer these questions it is helpful to subdivide the applications of CRISPR-Cas9 into three groups. The first group includes the complete or conditional knockout of genes in the germline as previously achieved through gene targeting in ES cells. However, quantitatively this approach is boosted by CRISPR-Cas9 due to the significantly reduced time and efforts required for gene editing directly in one-cell embryos. Furthermore, the protocols for use of CRISPR-Cas9 in zygotes have evolved in several steps since 2013 and facilitate the production of germline mutants. Firstly, the classical approach of using HR targeting vectors with large homology regions was complemented by the use of synthetic oligonucleotides for introduction of small precise sequence modifications.[8] Secondly knockout alleles can now be easily produced

without targeting vectors using gRNAs that cause frameshift mutations by small deletions or insertions (Indels) or by the deletion of gene segments using pairs of gRNAs.[9] Therefore, more edited lines can be generated in shorter time with less resources, greatly benefitting individual researchers as well as enabling large scale mutagenesis as pursued by the International Mouse Phenotyping Consortium or commercial providers.[10] [11] In addition, the delivery of CRISPR reagents became more convenient and reliable by replacement of *in vitro* transcribed RNAs with recombinant Cas9 protein and synthetic guide-RNAs from commercial vendors.[12] Higher HR frequencies can be obtained by using linear double-stranded DNA donor molecules (HMEJ, TILD) or by using long single-stranded DNA donors (Easi-CRISPR).[13] [14] [15] Another milestone was the establishment of protocols for batch electroporation of zygotes as an easy alternative to serial embryo microinjections, initially restricted for use of Cas9/gRNA complexes and oligonucleotides passing the zona pellucida.[16] [17] [18] The latest advance also enables the delivery of targeting vectors with a size of several kb in combination with zygote electroporation. For this purpose, the donor is packaged into AAV viral particles that can be added to the culture medium in high numbers, pass the zona pellucida, transduce into the zygotes and are beneficial for HR by their single-stranded and end protected genome. Although donor delivery via AAV requires extra efforts for virus preparation it offers the perspective of raising the Knockin frequencies to levels of $\geq$ 50% as reported in several pioneer studies.[19] [20] [21] Under these conditions half or more of the pups born from manipulated zygotes represent the desired founder mutants, requiring only a limited number of embryos to be be handeled within 1 or 2 working days. These high frequencies were previously only reported for the generation of knockout alleles via Indel formation. If the AAV technology can be validated by a wider community across a wider range of target genes, it will make also the production of knockin mouse models more reliable. Another versatile application of CRISPR

technology in zygotes is the introduction of mutant alleles into other genetic backgrounds than the commonly used C57BL/6 strain, such as NSG or NOD mice.[22] [23] Chromosome engineering for the deletion, inversion or duplication of megabase segments, initially performed using CRISPR-Cas9 in ES cells, can also be achieved directly in zygotes.[24] [25] Beyond its application in mouse zygotes, CRISPR-Cas9 had an even greater impact for rat genetics since, in the absence of reliable ES cells in the pre-nuclease era, nuclease technologies, first shown with zinc-finder nucleases and TALEN, enabled targeted manipulation of the rat genome.[26] [27] Enabling the same types of genetic models to be created in rats as have been built in mice.[28] Seen from a perspective before 2013, these developments alone represent a revolution, but the utility of CRISPR is even broader because of its additional application in somatic cells and for genome-wide engineering as discussed in the following sections.

The second category includes CRISPR applications that go beyond mouse germline mutagenesis to enable gene manipulation directly in somatic cells *in vivo* or *ex vivo*, something intractable before 2013. Gene editing for the therapy of monogenetic diseases in somatic cells is an exciting new opportunity. Either Cas9 or Base editors have been successfully used for therapeutic gene editing in the liver, muscle, or the whole body of newborn or adult mice.[29] [30] [31] Presently CRISPR reagents are mostly delivered to somatic cells by AAV vectors, but many in the field are seeking to replace AAV with nonviral delivery of components through various sorts of nanoparticles.[32] [33] [34] In the US such developments are systematically supported by the Somatic Cell Genome Editing (SCGE) funding program of the NIH, including rodent testing centres for development of reporter systems and somatic editing tools.[35] The other application area of CRISPR in somatic cells does not aim for gene repair but for knockout or precise mutagenesis of target genes for cancer modelling or functional CRISPR

screens in primary cells either *in vivo* or *ex vivo*.[36] The establishment of Cas9 transgenic mice was instrumental for this research because it is difficult to deliver Cas9 using a single AAV vector that offers, besides the large Cas9 coding region, only very limited space for promoter and polyA regions. In contrast, the use of Cas9 transgenic mice or cells requires only the delivery of single AAV vectors with a small gRNA expression unit. Furthermore, mice with doxycycline inducible Cas9 transgenes can be employed to generate Indel based sequence barcodes in somatic cells for the tracing and reconstruction of cell lineages.[37] Beyond gene editing, Cas9 variants without nuclease activity, that should not create double-strand breaks, can be used to bind the nuclease-dead dCas9 protein with specific gRNAs to selected genomic target regions. Fusion proteins of dCas9 with regulatory protein domains then allow programmable activation, silencing or the epigenetic repression of a target gene.[38] [39] [40] [41] [42] [43]

The third class of CRISPR applications is just emerging or not yet realised but theoretically feasible with future potential, in particular if editing technologies would be further streamlined. A potential future application could be efficient multiplex gene editing e.g. for studying polygenic traits. However, CRISPR-Cas9 can be efficient for targeting single loci but targeting of two or more loci in a single step in a reasonable fraction of zygotes is presently not routine. Nevertheless, in an ideal world of CRISPR technology Cas9 could process multiple target loci at high efficiency. Further research will be required to clarify whether nuclease efficacy can be further boosted or the enzyme may reach its limit on mammalian genomes that exceed the size of its natural host genome by ~1000-fold. Other elusive CRISPR applications are methods for replacement of large (10 - 1000 kb) exogenous DNA segments that are presently lacking protocols for assembly and site-specific transfer into the genome. Such

methods, once available, would allow the easy humanisation of mouse genes by replacement of e.g. a 50 kb segment by its human counterpart in a single step in zygotes. Finally, for the potential global engineering of an entire mouse genome, as exemplified by the yeast 2.0 project [44], it would be necessary to efficiently modify an inbred strain at many locations simultaneously. One could for example imagine of redesigning a strain with new traits such as resistance to viral pathogens, or to repair genetic defects of lab strains that were fixed homozygously by inbreeding. In the C57BL/6 mouse genome hundred of genes are compromised by missense mutations in protein coding regions or the intronic integration of endogenous retroviral elements.[45 46] In a first endeavour into this direction we are running a pilot study for the repair of 46 loci in the C57BL/6N genome. To this end CRISPR technology could liberate mouse geneticists from the historic origin of inbred strains, an endeavour that was intractable before the CRISPR era.

Taken together, the initial phase of CRISPR mediated gene editing in mice and rats already brought us unprecedented freedom and new ways of modifying the genome in somatic and germ cells. In the coming years the universe of CRISPR applications will likely being further expanded and make genetic modeling an even more fascinating scientific endeavour.

References

1. Capecchi MR. Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. *Nat Rev Genet* 2005; 6: 507–512.
2. Brown SDM, Holmes CC, Mallon A-M, et al. High-throughput mouse phenomics for characterizing mammalian gene function. *Nat Rev Genet* 2018; 19: 357–370.
3. Birling M-C, Yoshiki A, Adams DJ, et al. A resource of targeted mutant mouse lines for 5,061 genes. *Nat Genet* 2021; 53: 416–419.
4. Thomas KR, Capecchi MR. Site-directed mutagenesis by gene targeting in mouse embryo-derived stem cells. *Cell* 1987; 51: 503–512.

5. Gu H, Marth JD, Orban PC, et al. Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting. *Science* 1994; 265: 103–106.

6. Mouse Genome Sequencing Consortium, Waterston RH, Lindblad-Toh K, et al. Initial sequencing and comparative analysis of the mouse genome. *Nature* 2002; 420: 520–562.

7. Wang H, Yang H, Shivalila CS, et al. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell* 2013; 153: 910–918.

8. Yang H, Wang H, Shivalila CS, et al. One-step generation of mice carrying reporter and conditional alleles by CRISPR/Cas-mediated genome engineering. *Cell* 2013; 154: 1370–1379.

9. Singh P, Schimenti JC, Bolcun-Filas E. A mouse geneticist's practical guide to CRISPR applications. *Genetics* 2015; 199: 1–15.

10. IMPC, https://www.mousephenotype.org/.

11. GemPharmaTech, http://www.gempharmatech.com/en/.

12. Wefers B, Bashir S, Rossius J, et al. Gene editing in mouse zygotes using the CRISPR/Cas9 system. *Methods* 2017; 121–122: 55–67.

13. Yao X, Wang X, Hu X, et al. Homology-mediated end joining-based targeted integration using CRISPR/Cas9. *Cell Res* 2017; 27: 801–814.

14. Yao X, Zhang M, Wang X, et al. Tild-CRISPR Allows for Efficient and Precise Gene Knockin in Mouse and Human Cells. *Dev Cell* 2018; 45: 526-536.e5.

15. Quadros RM, Miura H, Harms DW, et al. Easi-CRISPR: a robust method for one-step generation of mice carrying conditional and insertion alleles using long ssDNA donors and CRISPR ribonucleoproteins. *Genome Biol* 2017; 18: 92.

16. Chen S, Lee B, Lee AY-F, et al. Highly Efficient Mouse Genome Editing by CRISPR Ribonucleoprotein Electroporation of Zygotes. *J Biol Chem* 2016; 291: 14457–14467.

17. Qin W, Dion SL, Kutny PM, et al. Efficient CRISPR/Cas9-Mediated Genome Editing in Mice by Zygote Electroporation of Nuclease. *Genetics* 2015; 200: 423–430.

18. Hashimoto M, Takemoto T. Electroporation enables the efficient mRNA delivery into the mouse zygotes and facilitates CRISPR/Cas9-based genome editing. *Sci Rep* 2015; 5: 11315.

19. Chen S, Sun S, Moonen D, et al. CRISPR-READI: Efficient Generation of Knockin Mice by CRISPR RNP Electroporation and AAV Donor Infection. *Cell Rep* 2019; 27: 3780-3789.e4.

20. Yoon Y, Wang D, Tai PWL, et al. Streamlined ex vivo and in vivo genome editing in mouse embryos using recombinant adeno-associated viruses. *Nat Commun* 2018; 9: 412.

21. Mizuno N, Mizutani E, Sato H, et al. Intra-embryo Gene Cassette Knockin by CRISPR/Cas9-Mediated Genome Editing with Adeno-Associated Viral Vector. *iScience* 2018; 9: 286–297.

22. Tirado-Gonzalez I, Czlonka E, Nevmerzhitskaya A, et al. CRISPR/Cas9-edited NSG mice as PDX models of human leukemia to address the role of niche-derived SPARC. *Leukemia* 2018; 32: 1049–1052.

23. Lin X, Pelletier S, Gingras S, et al. CRISPR-Cas9-Mediated Modification of the NOD Mouse Genome With Ptpn22R619W Mutation Increases Autoimmune Diabetes. *Diabetes* 2016; 65: 2134–2138.

24. Birling M-C, Schaeffer L, André P, et al. Efficient and rapid generation of large genomic variants in rats and mice using CRISMERE. *Sci Rep* 2017; 7: 43331.

25. Boroviak K, Doe B, Banerjee R, et al. Chromosome engineering in zygotes with CRISPR/Cas9. *Genesis* 2016; 54: 78–85.

26. Li D, Qiu Z, Shao Y, et al. Heritable gene targeting in the mouse and rat using a CRISPR-Cas system. *Nat Biotechnol* 2013; 31: 681–683.

27. Hu X, Chang N, Wang X, et al. Heritable gene-targeting with gRNA/Cas9 in rats. *Cell Res* 2013; 23: 1322–1325.

28. Chenouard V, Remy S, Tesson L, et al. Advances in Genome Editing and Application to the Generation of Genetically Modified Rat Models. *Front Genet* 2021; 12: 615491.

29. Ran FA, Cong L, Yan WX, et al. In vivo genome editing using Staphylococcus aureus Cas9. *Nature* 2015; 520: 186–191.

30. Long C, Amoasii L, Mireault AA, et al. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. *Science* 2016; 351: 400–403.

31. Koblan LW, Erdos MR, Wilson C, et al. In vivo base editing rescues Hutchinson-Gilford progeria syndrome in mice. *Nature* 2021; 589: 608–614.

32. Lee K, Conboy M, Park HM, et al. Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair. *Nat Biomed Eng* 2017; 1: 889–901.

33. Xu Y, Liu R, Dai Z. Key considerations in designing CRISPR/Cas9-carrying nanoparticles for therapeutic genome editing. *Nanoscale* 2020; 12: 21001–21014.

34. Li J, Røise JJ, He M, et al. Non-viral strategies for delivering genome editing enzymes. *Adv Drug Deliv Rev* 2021; 168: 99–117.

35. Saha K, Sontheimer EJ, Brooks PJ, et al. The NIH Somatic Cell Genome Editing program. *Nature* 2021; 592: 195–204.

36. Platt RJ, Chen S, Zhou Y, et al. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. *Cell* 2014; 159: 440–455.

37. Bowling S, Sritharan D, Osorio FG, et al. An Engineered CRISPR-Cas9 Mouse Line for Simultaneous Readout of Lineage Histories and Gene Expression Profiles in Single Cells. *Cell* 2020; 181: 1693–1694.

38. Hunt C, Hartford SA, White D, et al. Tissue-specific activation of gene expression by the Synergistic Activation Mediator (SAM) CRISPRa system in mice. *Nat Commun* 2021; 12: 2770.

39. Zhang X, Lv S, Luo Z, et al. MiniCAFE, a CRISPR/Cas9-based compact and potent transcriptional activator, elicits gene expression in vivo. *Nucleic Acids Res* 2021; 49: 4171–4185.

40. van der Weyden L, Harle V, Turner G, et al. CRISPR activation screen in mice identifies novel membrane proteins enhancing pulmonary metastatic colonisation. *Commun Biol* 2021; 4: 395.

41. Attenello FJ, Tsung K, Bishara I, et al. In vivo CRISPR screening for novel noncoding RNA functional targets in glioblastoma models. *J Neurosci Res*. Epub ahead of print 10 May 2021. DOI: 10.1002/jnr.24850.

42. Deutsch M, Günther A, Lerchundi R, et al. AAV-Mediated CRISPRi and RNAi Based Gene Silencing in Mouse Hippocampal Neurons. *Cells*; 10. Epub ahead of print 4 February 2021. DOI: 10.3390/cells10020324.

43. Himeda CL, Jones TI, Jones PL. Targeted epigenetic repression by CRISPR/dSaCas9 suppresses pathogenic DUX4-fl expression in FSHD. *Mol Ther Methods Clin Dev* 2021; 20: 298–311.

44. Pretorius IS, Boeke JD. Yeast 2.0-connecting the dots in the construction of the world's first functional synthetic eukaryotic genome. *FEMS Yeast Res*; 18. Epub ahead of print 1 June 2018. DOI: 10.1093/femsyr/foy032.

45. Timmermans S, Libert C. Overview of inactivating mutations in the protein-coding genome of the mouse reference strain C57BL/6J. *JCI Insight*; 3. Epub ahead of print 12 July 2018. DOI: 10.1172/jci.insight.121758.

46. Qin C, Wang Z, Shang J, et al. Intracisternal A particle genes: Distribution in the mouse genome, active subtypes, and potential roles as species-specific mediators of susceptibility to cancer. *Mol Carcinog* 2010; 49: 54–67.