Transgenic Res
DOI 10.1007/s11248-017-0038-x

MEETING REPORT

Advances in transgenic animal models and techniques

Séverine Ménoret · Laurent Tesson · Séverine Remy · Claire Usal · Laure-Hélène Ouisse · Lucas Brusselle · Vanessa Chenouard · Ignacio Anegon

Received: 26 July 2017 / Accepted: 31 July 2017

Abstract On May 11th and 12th 2017 was held in Nantes, France, the international meeting "Advances in transgenic animal models and techniques" (http://www.trm.univ-nantes.fr/). This biennial meeting is the fifth one of its kind to be organized by the Transgenic Rats ImmunoPhenomic (TRIP) Nantes facility (http://www.tgr.nantes.inserm.fr/). The meeting was supported by private companies (SONIDEL, Scionics computer innovation, New England Biolabs, MERCK, genOway, Journal Disease Models and Mechanisms) and by public institutions (International Society for Transgenic Technology, University of Nantes, INSERM UMR 1064, SFR François Bonamy, CNRS, Région Pays de la Loire, Biogenouest, TEFOR infrastructure, ITUN, IHU-CESTI and DHU-Oncogeffe and Labex IGO). Around 100 participants, from France but also from different European countries, Japan and USA, attended the meeting.

Keywords Transgenic · Genome editing · CRISPR · TALEN · ZFN · Animal models · Zygote · iPS cells · ES cells · Microinjection · Electroporation · Gene drive

S. Ménoret · L. Tesson · S. Remy · C. Usal · L.-H. Ouisse · L. Brusselle · V. Chenouard · I. Anegon (✉)
Centre de Recherche en Transplantation et Immunologie UMR1064, INSERM, Université de Nantes, Nantes, France
e-mail: ianegon@nantes.inserm.fr

S. Ménoret · L. Tesson · S. Remy · C. Usal · L.-H. Ouisse · L. Brusselle · V. Chenouard · I. Anegon
Institut de Transplantation Urologie Néphrologie (ITUN), CHU Nantes, Nantes, France

S. Ménoret · L. Tesson · S. Remy · C. Usal · L.-H. Ouisse · L. Brusselle · V. Chenouard · I. Anegon
Platform Rat Transgenesis ImmunoPhenomic, SFR François Bonamy, CNRS UMS3556, Nantes, France

The meeting included oral invited presentations (below) as well as poster presentations.

V. Siksnys (Institute of Biotechnology, Vilnius University, Lithuania) gave an update on the Cas9 and CRISPR diversity for genome engineering applications. The Cas9 protein and guide RNA of Type II CRISPR-Cas bacterial adaptive immune system have been adopted as a robust and facile genome editing tool. The Cas9 derived from *Streptococcus pyogenes* has been utilized most largely. However, naturally occurring Cas9 orthologues are abundant in bacteria. To tap into this largely unexplored diversity for genome editing applications, a phylogeny-guided bioinformatics approach and a rapid biochemical screen for the rapid identification and characterization of the protospacer adjacent motif (PAM) and guide RNA requirement of new Cas9 proteins were developed (Karvelis et al. 2015, 2017). This approach delivered Cas9s with novel PAM recognition that

Published online: 05 August 2017

expand the genome editing capacity by increasing the sequence space targetable by Cas9.

I. Fonfara (Max Planck Institute for Infection Biology, Berlin, Germany) presented the results concerning the CRISPR-associated DNA-cleaving enzyme Cpf1 (*cas* gene of *Pasteurella, Francisella*) recently classified as a Type V-A CRISPR-Cas system. The absence of tracrRNA and homologues of Cas6 or Cas5d in the vicinity of the type V-A locus suggests novel mechanisms for crRNA biogenesis and DNA interference in this system. In this study, they described the biochemical properties of Cpf1 from *Francisella novicida* U112. After recognition of a 5′-YTN-3′ PAM on the non-target DNA strand, crRNA-guided Cpf1 introduces double-stranded breaks in the target DNA to generate a 5′ overhang (Zetsche et al. 2015; Fonfara et al. 2016; Dong et al. 2016). She demonstrated that Cpf1 is a dual specificity nuclease also responsible for pre-crRNA processing. By specifically recognizing the hairpin structure formed by the repeat of crRNA and a four-nucleotide recognition sequence upstream of the hairpin, Cpf1 cleaves pre-crRNA 19nt from the 3′ end of the repeat. Cpf1 is the first enzyme shown to cleave both RNA and DNA in a sequence-specific manner. The Type V-A of *F. novicida* therefore constitutes a minimalistic CRISPR-Cas system, utilizing the same protein for pre-crRNA processing and RNA-guided DNA targeting. Cpf1 complements the existing CRISPR-Cas genome engineering toolbox by enormously facilitating multiplex targeting of genomic loci (Zetsche et al. 2017).

A. Rezza (GenOway, Lyon, France) presented the pros and cons of nucleases-based technologies for the generation of genetically-modified mouse models. She presented different CRISPR/Cas9 optimization techniques, either by using multiple online tools for improved sgRNA design, or by studying cleavage efficiency/specificity. These approaches made possible with a Cas9 nickase to improve specificity and reduce off-target, activity. Another optimization was the use of Cas9 protein limiting the time of Cas9 cleavage activity compared to mRNA. Several factors that can increase the efficiency of KI have been evaluated including the use of small molecules that enhance HDR by inhibiting NHEJ, such as SCR7 (a DNA ligase IV) inhibitor, but that did not increase efficiency. A. Rezza also clarified that genotype screening and validation is the most time consuming step that this can also be very rewarding with different test methods and RNA concentrations for KI.

A. Geurts (Genome Editing Rat Resource Center, Medical College of Wisconsin, Milwaukee) gave an update on the Dahl salt sensitive rat as a rat model which shares many features with salt-sensitive human hypertensive patients. He also highlighted specific projects and described the resources and gene knockout models that have been created for exploring a variety of diseases in this model, and the initial development of driver strains for Cre/loxP conditional mutagenesis—focused on the cardiovascular system and kidney (Endres et al. 2014; Geurts et al. 2015).

A. Tedgui (PARCC-Inserm U970, Paris, France) A. Ignatowski described in 1908 that experimental atherosclerosis could be induced in rabbits by feeding them a diet of milk and egg yolk. The lipid theory of atherosclerosis that predominated for most of the twentieth century. A significant leap came in 1979 when Brown and Goldstein showed that the LDL receptor is not involved in macrophage foam-cell formation, and proposed that a macrophage receptor that recognized acetylated LDL plays a key role in this process (Goldstein et al. 1979). The role of oxidized LDL (oxLDL) in the pathogenesis of atherosclerosis was described (for a review, Steinberg et al. 2010), and a number of scavenger receptors mediating their uptake by macrophages were identified. The model of the Watanabe heritable hyperlipidemic rabbit, introduced in 1980 was particularly useful in establishing the role of oxLDL in atherogenesis. A second revolution occurred at the beginning of the 1990s when mice deficient for apolipoprotein E (apoE)- and LDL receptor (LDLr) were generated (Ishibashi et al. 1993; Plump et al. 1992; Zhang et al. 1992). In contrast to the previous models, mice lacking functional apoE or LDLr genes were shown to develop widely distributed arterial lesions that progress from foam cell-rich fatty streaks to fibro-proliferative plaques with lipid/necrotic cores, typical of the spectrum of human lesions. The possibility of abolishing the expression of a single gene of interest, or of overexpressing it, opened a new era of atherosclerosis research at a mechanistic level (for a review, Getz et al. 2016).

F. Stewart (Center for Molecular and Cellular Bioengineering, Technische Universität Dresden, Germany) presented results on complex genome engineering with Cas9 and recombineering. Designer

nucleases like CRISPR/Cas9 enable fluent site directed damage or small mutations in many genomes. Strategies for their use to achieve more complex tasks like regional exchanges for gene humanization or the establishment of conditional alleles are still emerging. To optimize Cas9-assisted targeting, he measured the relationship between targeting frequency and homology length in targeting constructs using an HPRT assay in mouse ES cells. Targeting frequency with supercoiled plasmids improved steeply up to 2 kb total homology and continued to increase with even longer homology arms, thereby implying that Cas9-assisted targeting efficiencies can be improved using homology arms of 1 kb or greater. To humanize the Kmt2d gene, he built a hybrid mouse/human targeting construct in a BAC by recombineering. To simplify the possible outcomes, he employed a single Cas9 cleavage strategy and best achieved the intended 42 kb regional exchange with a targeting construct including a very long homology arm to recombine ~42 kb away from the cleavage site. He recommend the use of long homology arm targeting constructs for accurate and efficient complex genome engineering.

I. Anegon (INSERM UMR 1064-CRTI and Transgenic Rats ImmunoPhenomic platform, Nantes, France) presented results obtained on the use of gene-specific nucleases for the generation of genetically engineered rats for the analysis of immune responses. Since 2009, ZFNs have been used to readily generate several KO and a few KI rats (Cui et al. 2011), including for heavy and light chain antibody genes (Geurts et al. 2009; Menoret et al. 2010). These rats were then crossed with transgenic rats in which human immunoglobulin genes introduced by BAC and YAC microinjection (Osborn et al. 2013). These rats showed human V gene diversity comparable to the one of human antibodies and monoclonal antibodies of high affinity were generated from these rats using hybridoma technology. These rats were also used to generate human monoclonal antibodies using a new technique based on single B cell antigen-specific sorting followed by expression cloning of the immunoglobulin genes of these B cells (Ouisse et al. 2016). I-CreI-engineered meganucleases were used to generate rats KO for *Rag1* with a SCID phenotype (Menoret et al. 2013). TALENs were used to generate rats KO for the gamma chain of the IL2 receptor (*Il2rg*) (Ménoret et al. manuscript in preparation). *Rag1* and *Il2rg* KO rats were crossed to obtain double KO rats (RRG) with a more pronounced T, B and NK immunodeficient phenotype that each single KO. To inhibit phagocytosis of human cells by rat macrophages, human *SIRPalpha* transgenic rats were generated using piggyBac-mediated BAC transgenesis (Jung et al. 2016) that are being crossed with RRG animals. TALENs were also used to generate dystrophin KO rats to reproduce Düchenne disease (Larcher et al. 2014) and immune/inflammatory mechanisms exacerbating the disease are being characterized (Ouisse et al. manuscript in preparation). The availability of gene-specific nucleases provide an extremely useful tool to generate KO and KI rats for the analysis of gene function, generate disease models and biotechnological applications in the area of immunology.

C. Giovannangeli (INSERM U1154, CNRS UMR7196, Sorbonne Universités, Museum National d'Histoire Naturelle; Paris, France) presented recent results on the introduction of precise mutations introduced through homology-directed repair but efficiency is usually lower than for gene knock-out (KO), and optimized and robust strategies still remain to be defined. She illustrated different approaches she has developed to enhance genome editing efficiency exploiting different double-strand break DNA repair pathways (Renaud et al. 2016). First, efficient generation of predictable KO mutants can be obtained based on the induction of inter-microhomology deletions likely due to microhomology mediated end-joining pathway. Second, chemically modified short ssODN can be used to improve precise sequence modification and in this case genome editing takes place by 2 steps of single-strand annealing and not by homologous recombination (HR). Finally strategies are also developed to improve gene targeting with a double-strand DNA donor by favoring HR pathway.

V. Brault (IGBMC, Illkirch, France) presented an update on rodent models for the analysis of cognitive disorders. Intellectual disability (ID) affects 2–3% of the population. Chromosomal disorders such as William syndrome, Down syndrome (DS), Cri du Chat syndrome and the 16p11.2 deletion, are among the leading causes of ID. The etiology of such complex genetic syndromes involving many genes is still poorly understood. Mouse models have been an invaluable model to study such complex genetic disorders since they are amenable to chromosomal manipulation reproducing the genetic anomaly

observed in humans and allow to address the phenotype-genotype relationship. DS is a complex genetic disease involving about 300 genes. Mouse models for DS have been developed using genetic engineering. The combination of the site-specific Cre/loxP recombinase strategy coupled to homologous recombination in mouse embryonic stem cells has enabled to generate large (megabases) tandem duplications of specific predetermined mouse chromosomal regions syntenic to Hsa21 (Brault et al. 2007; Hérault et al. 2012). Mouse behavioural phenotyping has enable to identify the *DYRK1A* gene, coding for a serine/threonine kinase, as a major gene involved in DS cognitive deficits (for review, Duchon et al. 2016).

C. Huchet (Therassay Platform, UMR INSERM 1089, Université de Nantes, France) presented rodent models of muscular genetic diseases. Neuromuscular disorders are a heterogeneous group of genetic diseases, caused by mutations in genes coding sarcolemmal, sarcomeric or cytosolic proteins leading to progressive loss of locomotor ability. Several animal models manifesting phenotypes observed in neuromuscular diseases have been identified in nature or generated in the laboratory. Mouse models are largely used to analyze physiopathological mechanisms and to perform genetic, cellular and pharmacological therapeutic approaches. These mouse models display physiological muscular and cellular alterations, but not always similar to those observed in human patients. Duchenne Muscular Dystrophy (DMD) is a severe muscle-wasting disorder caused by mutations in the gene encoding dystrophin. For pre-clinical evaluation of therapeutic approaches, large animal models of DMD such as dogs or pigs are available but expensive, difficult to handle and display important clinical heterogeneity. *Mdx* mice is an often used rodent model of DMD they exhibit only limited chronic muscular lesions and muscle weakness. Thus, a new rodent model could represents a strong interest. A line of Dmd mutated-rats (Dmd^{mdx}) was generated using TALENs and muscle function characterized during the first 4 months of life (Larcher et al. 2014). Further analyses has shown that life span of Dmd^{mdx} rats is significantly reduced. Weight, blood biomarkers concentrations, muscle strength and fatigue measured by grip force test, muscle calcium homeostasis and histology in skeletal muscles, diaphragm and heart, are all significantly impaired as soon as the age of 1.5 months and show a clear stepwise evolution along with age. Moreover, echo and electrocardiography approaches highlighted a significant and rapid concentric remodeling associated to an alteration of diastolic function, which progressed with age towards systolic heart failure with rhythm disorders. In conclusion, with systematic and stepwise aggressive phenotypes at both the muscular and the cardiac levels, similar to what occurs in DMD patients, this unique and newly developed Dmd^{mdx} rat model is now one of the best animal models for preclinical evaluations of treatments for DMD. The study of animal models for genetic diseases can provide important clues to the understanding of the pathogenesis of these disorders.

Heiner Niemann (Institute of Farm Animal Genetics, Hannover Medical School, Germany) presented new strategies to produce multi-transgenic pigs for xenotransplantation (Fischer et al. 2016). The generation of pigs with a genetic knockout of the α1.3-galactosyltransferase gene (GGTA1) was a milestone down the road towards clinical application of porcine xenografts since hyper acute rejection can now be reliably prevented and significantly extended survival times after pig-to-baboon xenotransplantation up to a maximum of 83 days for kidneys and more than 2 years for heterotopically transplanted hearts have been reported. For life supportive heart transplants of pig origin into primates the average survival of the recipient is now 30–50 days. Subsequently porcine xenografts are rejected due to inflammatory symptoms and severe perturbation of coagulation. Thus, the acute vascular rejection is mediated by antibodies activating and damaging endothelium followed by thrombotic microangiopathy remain the bottlenecks to clinical xenotransplantation. Non-anti-Gal antibody binding activates the endothelium and results in cellular damage and. The current view is that long-term survival of xenografts after transplantation into primates requires multiple modifications of the porcine genome and a specifically tailored immunosuppressive regimen compliant with current clinical standards. The presentation provided an update on the current status in the production and use of multi-transgenic pigs for xenotransplantation, including not only GGTA1 but also hTM, hHO-1, hA20 and CTLA4Ig.

A. Hammond (Imperial College, London, UK) presented new results concerning engineering gene drive for population suppression of mosquito propagating malaria by reducing their capacity to reproduce. Theory

predicts that a significant reduction in the population of *Anopheles gambiae* mosquitoes will enable malaria to be eliminated. He identified three genes (*AGAP005958*, *AGAP011377* and *AGAP007280*) that confer a recessive female-sterility phenotype upon disruption, and inserted into each locus CRISPR-Cas9 gene drive constructs, named $CRISPR^h$, designed to target and edit each gene to contain a drive (Hammond et al. 2016). At each of the three loci, they observed a strong bias in transmission of the gene drive to the progeny at rates of 91.4–99.6%. At one locus, *AGAP007280*, these high rates of transmission bias combined with a weaker fertility cost in heterozygosity suggests that the $CRISPR^h$ gene drive meets the minimum requirements to spread infertility throughout an insect population. Gene drives are susceptible to resistance in the form of nucleotide variation at target site that prevents recognition and cleavage by the endonuclease. Moreover, these variant alleles are likely to be generated by the gene drive itself if nuclease activity results in dsDNA breaks that are repaired by end-joining. If these mutants are non-functional, they will not be able to restore female fertility and will be selected out of the population. However mutant alleles that restore fertility will come under strong selection in the face of a suppression gene drive. He saw that the frequency of $CRISPR^h$ continuously decreased after 8 generations, indicating a rise in frequency of nuclease-resistant alleles that retain at least some of the functionality of the original target gene. For that he used a targeted amplicon sequencing approach to interrogate the nature of the target site in mosquitoes before and after the emergence of resistance and observed a strong increase in the frequency of putatively resistant alleles. Finally, he has developed new gene drives that restrict spatiotemporal activity of the Cas9 and have observed almost complete restoration of fertility in heterozygous females (Beaghton et al. 2017). In this way, gene drives will be able to tolerate target site variation at multiple sites without affecting their ability to drive into the chromosome containing the partially resistant allele.

T. Kaneko (Division of Science and Engineering, Iwate University, Japan) presented the electroporation method to generate gene-edited animals. Microinjection of endonucleases into pronuclear-stage embryos is the standard method but it requires specialized equipment and high skill training. Moreover, injecting endonucleases into embryos one by one is time consuming. He recently developed simple and high effective method that can be introduced endonuclease into intact embryos using electroporation instead of microinjection (Kaneko et al. 2014, 2015, 2017). Using this method, knock-out/in mice and rats can be produced by introduction of Cas9 protein and mRNA of ZFN, TALEN and CRISPR into intact embryos.

M. Müller (University of Freiburg, Freiburg, Germany) presented new results concerning *Streptococcus thermophilus* CRISPR-Cas9 systems enable specific editing of the human genome. Cas9 proteins from different species recognize distinct PAM sequences. Although SpCas9-based RGNs are widely used (Mussolino et al. 2013), several studies have reported high frequencies of off-target mutagenesis (Fu et al. 2013) driving researchers to develop novel strategies to increase the fidelity of the SpCas9 system. Since the PAM is a major determinant of CRISPR/Cas9 specificity, he hypothesized that Cas9 recognizing longer PAMs could be more specific. To this end, he focused on two CRISPR/Cas9 systems derived from *Streptococcus thermophilus* (St) and characterized their efficacy and specificity. St1Cas9 and St3Cas9 are encoded by the St CRISPR1 or CRISPR3 loci and bind to NNAGAAW and NGGNG PAMs respectively. He show that these Cas9 proteins are well tolerated in human cells and that the cleavage activities of the StCas9 nucleases are comparable to the established SpCas9-based RGNs. Importantly, as compared to the Sp-derived CRISPR/Cas9 system, the cleavage activities at predicted off-target sites is considerably lower for both St1Cas9 and St3Cas9-based RGNs (Müller et al. 2016). These novel CRISPR/Cas9 systems therefore represent a valid alternative for expanding the targeting range of RGNs in general and for safe human genome editing in particular.

Acknowledgements LabCom SOURIRAT Project (ANR-14-LAB5-0008), "TEFOR" «Investissements d'Avenir» (ANRII-INSB-0014), Labex IGO Project «Investissements d'Avenir» (ANR-11-LABX-0016-01) and IHU-Cesti Project «Investissements d'Avenir» (ANR-10-IBHU-005) as well as Nantes Métropole and Région Pays de la Loire.

References

Beaghton A et al (2017) Requirements for driving anti-pathogen effector genes into populations of disease vectors by homing. Genetics 205(4):1587–1596

Brault V et al (2007) Cre/loxP-mediated chromosome engineering of the mouse genome. Handb Exp Pharmacol 178:29–48

Cui X et al (2011) Targeted integration in rat and mouse embryos with zinc-finger nucleases. Nat Biotechnol 29:64–67

Dong D et al (2016) The crystal structure of Cpf1 in complex with CRISPR RNA. Nature 532:522–526

Duchon A et al (2016) DYRK1A, a dosage-sensitive gene involved in neurodevelopmental disorders, is a target for drug development in down syndrome. Front Behav Neurosci 10:104

Endres BT et al (2014) Mutation of Plekha7 attenuates salt-sensitive hypertension in the rat. Proc Natl Acad Sci USA 111:12817–12822

Fischer K et al (2016) Efficient production of multi-modified pigs for xenotransplantation by 'combineering', gene stacking and gene editing. Sci Rep. 29(6):29081

Fonfara I et al (2016) The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. Nature 532:517–521

Fu Y et al (2013) High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells. Nat Biotechnol 31:822–826

Getz GS et al (2016) ApoE knockout and knockin mice: the history of their contribution to the understanding of atherogenesis. J Lipid Res 57:758–766

Geurts AM et al (2009) Knockout rats via embryo microinjection of zinc-finger nucleases. Science 325:433

Geurts AM et al (2015) Maternal diet during gestation and lactation modifies the severity of salt-induced hypertension and renal injury in Dahl salt-sensitive rats. Hypertension 65:447–455

Goldstein JL et al (1979) Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. Proc Natl Acad Sci USA 76:333–337

Hammond A et al (2016) A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. Nat Biotechnol 34(1):78–83

Hérault et al (2012) The in vivo Down syndrome genomic library in mouse. Prog Brain Res 197:169–197

Ishibashi S et al (1993) Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J Clin Invest 2:883–893

Jung CJ et al (2016) Comparative analysis of piggyBac, CRISPR/Cas9 and TALEN mediated BAC transgenesis in the zygote for the generation of humanized SIRPA rats. Sci Rep 6:31455

Kaneko T (2017) Genome editing in mouse and rat by electroporation. Genome editing animals method and protocols. Methods Mol Biol 1630:81–89

Kaneko T et al (2014) Simple knockout by electroporation of engineered endonucleases into intact rat embryos. Sci Rep 4:6382

Kaneko T et al (2015) Simple genome editing of rodent intact embryos by electroporation. PLoS ONE 10:e0142755

Karvelis T et al (2015) Rapid characterization of CRISPR-Cas9 protospacer adjacent motif sequence elements. Genome Biol 16:253

Karvelis T et al (2017) Methods for decoding Cas9 protospacer adjacent motif (PAM) sequences: a brief overview. Methods 121–122:3–8

Larcher T et al (2014) Characterization of dystrophin deficient rats: a new model for Duchenne muscular dystrophy. PLoS ONE 9:e110371

Menoret S et al (2010) Characterization of immunoglobulin heavy chain knockout rats. Eur J Immunol 40:2932–2941

Menoret S et al (2013) Generation of Rag1-knockout immunodeficient rats and mice using engineered meganucleases. Faseb J 27:703–711

Müller M et al (2016) Streptococcus thermophilus CRISPR-Cas9 systems enable specific editing of the human genome. Mol Ther 24(3):636–644

Mussolino C et al (2013) RNA guides genome engineering. Nat Biotechnol 31:208–209

Osborn et al (2013) High-affinity IgG antibodies develop naturally in Ig-knockout rats carrying germline human IgH/Igkappa/Iglambda loci bearing the rat CH region. J Immunol 190:1481–1490

Ouisse L-H et al (2016) Antigen-specific single B cell sorting and expression-cloning from immunoglobulin humanized rats: a rapid and versatile method for the generation of high affinity and discriminative human monoclonal antibodies. BMC Biotechnol 17(1):3

Plump AS et al (1992) Severe hypercholesterolemia and atherosclerosis in apolipoprotein-E-deficient mice created by homologous recombination in ES cells. Cell 71: 343–353

Renaud JB et al (2016) Improved genome editing efficiency and flexibility using modified oligonucleotides with TALEN and CRISPR-Cas9 nucleases. Cell Rep 14(9):2263–2272

Steinberg D et al (2010) Oxidized low-density lipoprotein and atherosclerosis. Arterioscler Thromb Vasc Biol 30: 2311–2316

Zetsche B et al (2015) Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 163:759–771

Zetsche B et al (2017) Multiplex gene editing by CRISPR-Cpf1 using a single crRNA array. Nat Biotechnol 35:31–34

Zhang SH et al (1992) Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein-E. Science 258:468–471