Clustered Regularly-Interspaced Short-Palindromic Repeats (CRISPR) in bioarchaeology - Review

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Abstract

Convergence between social and experimental sciences has been accomplished for bioarchaeology. That has been possible since archaeological remains can be analyzed with molecular-biology methodologies, like Polymerase Chain-Reaction (PCR), as well as nucleic-acid and peptide (protein) sequencing. A revolutionary consequence is the possibility to bring to life or restore extinct species. That has been a scientific dream for some years, and now is much more feasible, due to a recent technological breakthrough. That is known as Clustered Regularly-Interspaced Short-Palindromic Repeats (CRISPR). In short, it allows to edit genomes, including modern and ancient ones. Among other examples, current elephant and extinct mammoth genomes could be compared, to edit the former and to make it to resemble the latter. Thus, CRISPR technology is becoming certainly exciting, holding a great potential not only in medicine and plant and animal breeding, but also in bioarchaeology.

Keywords: CRISPR/Cas9, cisgenic, de-extinction, passenger pigeon, archaeobiology.

Resumen

La convergencia entre las ciencias sociales y experimentales se ha llevado a cabo para la bioarqueología. Ello ha sido posible porque los restos arqueológicos pueden ser analizados con metodologías de biología molecular, como la reacción en cadena de la polimerasa (PCR), así como la secuenciación de ácidos nucleicos y péptidos (proteínas). Una consecuencia revolucionaria es la posibilidad de traer a la vida o restaurar especies previamente extintas. Ello ha representado un sueño científico desde hace algunos años, siendo ahora mucho más factible, gracias a un significativo avance tecnológico. Es conocido como repeticiones palindrómicas cortas, agrupadas y regularmente interespaciadas (CRISPR). En resumen, permite editar genomas, incluyendo los modernos y antiguos. Entre otros ejemplos, los genomas del elefante actual y mamut extinto pueden ser comparados, permitiendo editar el primero para asemejarlo al segundo. Así, la tecnología CRISPR se está convirtiendo en algo realmente excitante, con gran potencial no solo en medicina y mejora de plantas y animales, sino también en bioarqueología.

Palabras clave: CRISPR/Cas9, cisgénico, desextinción, paloma migratoria, arqueobiología.

Introduction

Interestingly, curiosity has driven human advancements. Indeed, it is thought that Neanderthals (*Homo sapiens neanderthalensis*) and other human subspecies like Denisovans (*Homo sapiens denisova*) became extinct (Dorado et al, 2010) because they had less inquisitiveness than modern humans (*Homo sapiens sapiens*). This way, the now extinct subspecies lived in fewer locations when compared to the latter, which extended all over the world. And even then, we were near extinction at least three times in our biological evolution: i) 1.2 million years ago (*Homo sapiens*, *Homo ergaster*, and *Homo erectus* had a worldwide population of ~18,000 people); ii) 150,000 years ago (glacial stage, with only ~600 human survivors); and iii) 70,000 years ago (Toba explosion at Sumatra, leaving between 1,000 to 10,000 people).

Archaeology is a fascinating research area. Thus, it allows to uncover ancient events that otherwise would remain unknown. Until recently, such knowledge subject was considered and classified within social sciences (including arts and humanities), but now it is also considered within experimental sciences. That is the case, for instance, of the Journal of Archaeological Science <http://www.journals.elsevier.com/journal-of-archaeological-science>, which is indexed in both areas of the Journal Citation Reports (JCR) of the Web of Science (WoS) - Web of Knowledge (WoK) of Clarivate Analytics (formerly, Thomson Reuters) <http://apps.webofknowledge.com>. Such integration has taken place since it is now possible to analyze archaeological remains using molecular biology methodologies. That has been accomplished with techniques like Polymerase Chain-Reaction (PCR), as well as nucleic-acid and peptide sequencing. Such methodologies have been further potentiated with substantial advancements in computing, including both hardware (eg., many-core microprocessors) and software algorithms (bioinformatics). All that brings bioarchaeology to a new and exciting territory, close to the study of living entities, like viroids, virusoids viruses and cellular-based ones (prokaryotic and eukaryotic).

An exciting consequence of the convergence between such sciences is the possibility to bring to life previously extinct species, which was considered impossible just a few years ago. That is, certainly, a provocative breakthrough. Welcome to de-extinction (sometimes, wrongly named as resurrection, which has a different meaning, related to religion). Such a scientific dream is now much more feasible, thanks to new technological accomplishments, as described below. Again, human curiosity and innovations are driving scientific advancements and practical accomplishments...

Clustered regularly-interspaced short-palindromic repeats

Clustered Regularly-Interspaced Short-Palindromic Repeats (CRISPR) is a revolutionary technology, allowing to edit nucleic-acids in vitro and in vivo (Mojica and Montoliu, 2016). As with some other technological developments, the molecular basis of this process was discovered by chance. At such time, they were considered a mere curiosity (DNA sequences with short and repetitive nucleotide-base stretches). Indeed, the origin and use of interspacing

subsequences were not known at such time. Interestingly, it was later on demonstrated that CRISPR represent the only adaptive immunity system of prokaryotes (Marraffini, 2015; Jackson et al 2017). On the other hand, Cas9 endonuclease is a four-component system that includes two small RNA molecules named CRISPR RNA (crRNA), trans-activating CRISPR RNA (tracrRNA; that binds to crRNA and forms an active complex) and RNase-III (Barrangou, 2015). That makes the CRISPR-Cas9 system.

In short, when bacteria suffer virus infections, sometimes they can survive and store parts of such virus genomes, as a kind of molecular memory. It works this way: i) virus DNA fragments (known as spacers) are stored in CRISPR arrays, within bacterial genome; ii) spacers are transcribed into small RNA guides; iii) such guides bind to proteins like “CRISPR-associated protein 9” (Cas9); y iv) the latter is an RNA-guided DNA endonuclease enzyme, that can recognize and cut DNA hybridizing with the associated RNA template (previously transcribed from virus DNA). Therefore, such an elegant approach can recognize and destroy exogenous DNA to which the bacteria was previously exposed, should a future infection take place (Fig. 1). But, curiously, not all prokaryotes contain CRISPR systems (Grissa et al 2007; Rousseau et al 2009). Thus, they have been identified in 45% of bacteria and 87% of archaea genomes sequenced so far (6,782 and 232, respectively), as shown by “CRISPRs web server” <http://crispr.i2bc.paris-saclay.fr>.

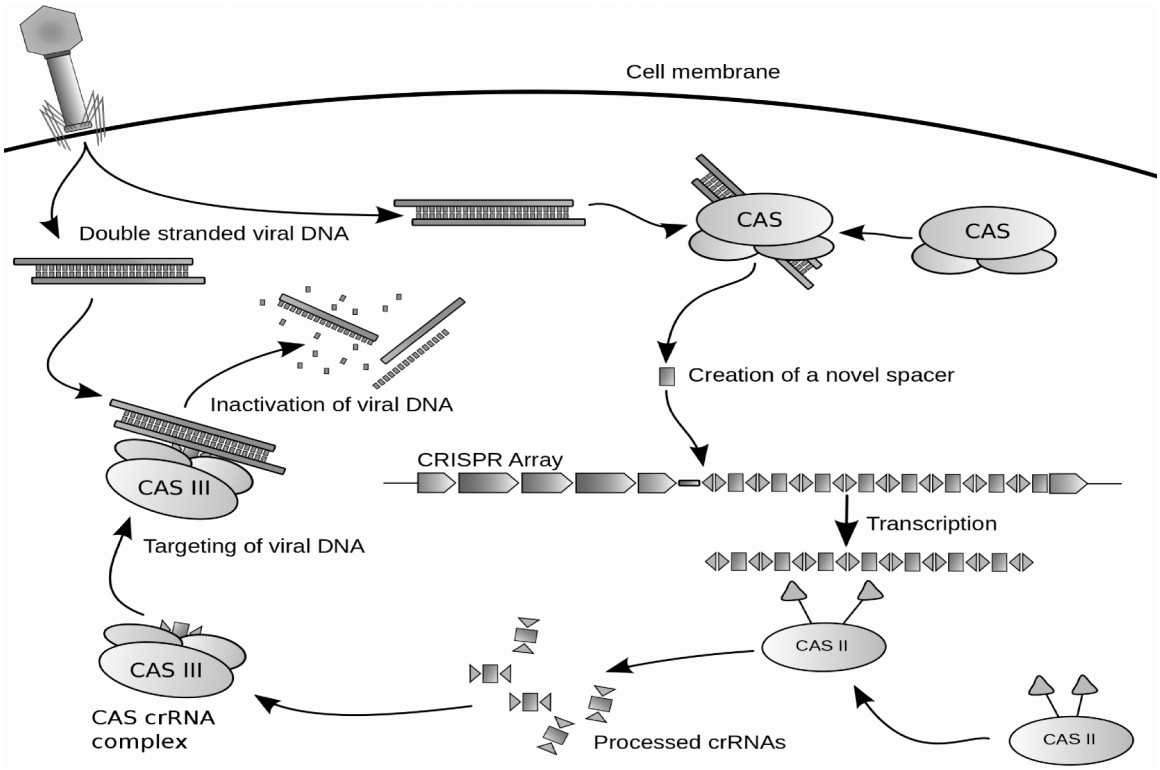

Figure 1. CRISPR/Cas9 bacterial defence system. Cas proteins bind to foreign DNA, allowing to generate CRISPR arrays containing such sequences, which are further transcribed. Such RNA is processed by Cas II proteins to generate fragments (crRNA) that bind to Cas III proteins. This way, the latter RNA can hybridize to exogenous DNA and destroy it, should a further infection occur. © 2017 Danandmike, Wikimedia Commons <http://commons.wikimedia.org> and Creative Commons <http://creativecommons.org>.

Interestingly, this bacterial immunological system can be engineered and exploited as a powerful and flexible genome-editing tool (Gasiunas et al 2012; Jinek et al 2012; Singh et al 2016; Yi and Li, 2016; Kan et al 2017). This way, synthetic guide RNA (gRNA) can be used to direct it towards any genomic site. Thus, genomic sequences (genes of interest, etc) can be removed or added as desired. Therefore, CRISPR can be applied to edit genomes without using nucleic-acids from other species. That way, the resulting constructs would be cisgenics, instead of transgenics (containing genetic material from other species).

CRISPR in bioarchaeology

CRISPR could be exploited to edit current genomes, so that they harbor desired genes from extinct species. That can be accomplished comparing modern and ancient DNA (aDNA) from such species. Characteristics of interest may include productivity and resistance to abiotic (flooding, drought, salinity, high- or low-temperatures, etc) and biotic stresses (diseases and pests). That is particularly relevant to fight the current trend of global warming and climate change. Curiously, it is known that Neanderthals and Denisovans had stronger immune systems than modern humans. Indeed, they inbred with modern humans, which harbor some of such genes (Dannemann et al 2016; Deschamps et al 2016). Information about ancient and stronger immune systems could be exploited, for instance, to make cattle less prone to diseases.

On the other hand, several projects have been proposed to restore extinct species (Dorado et al 2013). Interestingly, CRISPR has increased the possibilities of accomplishing such a scientific dream of bringing extinct species back to life. One of them involves woolly mammoth (*Mammuthus primigenius*), which was extinct about 4,000 years ago, probably due to climate change and human overhunting. Some researchers are trying to use archaeological nuclei to clone the species, using modern elephants as surrogate gestating females. Additionally, CRISPR technology could be used to edit current elephant genomes, to make them cold-resistant and hairy. One approach is using immature cells that differentiate into sperm or eggs [known as Primordial Germ Cells (PGC)], to edit modern genomes to resemble extinct counterparts. This way, a reserve park for them could be created in cold areas, like Siberia. Another de-extinction project currently being considered involves passenger pigeon (*Ectopistes migratorius*). Milliards of such birds were extinct by excessive human hunting in the late nineteenth century in North America (Reardon, 2016).

Future prospects and concluding remarks

It is now clear that CRISPR represents a revolution for sciences dealing with living entities, such as medicine (for instance, to cure diseases like diabetes), as well as plant and animal breeding. But this powerful technology also holds a great potential for sciences working with entities or parts of them that were once alive, like bioarchaeology. In this context, and taking into account studies of aDNA and cultures of embryos of archaeological maize (Vásquez et al, 2011), genomes of these and modern ones could be compared,

to edit the latter to resemble the former, in order to fight abiotic and biotic stress, increase productivity, etc. In fact, the number of publications about CRISPR is growing exponentially, as can be seen just by considering the reviews about it published in 2017, at the time of writing this report (Biagioni et al 2017; Carroll and Zhou, 2017; Chin et al 2017; Chira et al 2017; Chuai et al 2017; De La Fuente-Núñez and Lu, 2017; Doerflinger et al 2017; Doetschman and Georgieva, 2017; Fellmann et al 2017; Gee et al 2017; Gerace et al 2017; Gibson and Yang, 2017; Goren et al 2017; Jiang and Doudna, 2017; Komor et al 2017; Li et al 2017; Lu et al 2017; Mao et al 2017; Mout et al 2017; Murovec et al 2017; Puchta, 2017; Puschnik et al 2017; Sayin and Papagiannakopoulos, 2017; Shen et al 2017; Shmakov et al 2017; Stout et al 2017; VanDiemen and Lebbink, 2017; Zischewski et al 2017). Such exponential growth of publications resembles other recent revolutions in life sciences, ignited by the development of breakthrough technologies like PCR, as well as Second-Generation Sequencing (SGS) and Third-Generation Sequencing (TGS), sometimes known by the ambiguous name of "Next"-Generation Sequencing (NGS). Interestingly, they have also had –and have now– revolutionary impacts on bioarchaeology (Dorado et al 2007-2009, 2011, 2013-2016).

As an example of partial, albeit illustrative examples, CRISPR potential includes generating desired offspring, avoiding male or female culling (indeed, eggXYt company <https://www.eggxyt.com> has just announced it for chickens). Likewise, ensuring that cattle are polled, lacking dangerous long-horns. Other interesting potential applications are the generation of domestic animals with CRISPR integrated (CRISPi) into their genomes (not to be confused with CRISPI, which is a CRISPR Interactive database, as published by Rousseau et al, 2009). That should allow easier genome editing and production of pharmaceutical drugs, as proposed for CRISPi chickens. This technology could also be applied to generate genetically modified mosquitos, to fight and even eradicate devastating diseases, like dengue or the one caused by Zika virus. That can be effectively accomplished using gene drives, which are synthetic selfish-genetic-elements, ensuring that almost all offspring inherit two copies of the desired gene. Thus, the selected gene effectively spreads and takes over the population in future generations. For instance, gene drives have been developed to fight and even eradicate malaria, also known as paludism (Alphey, 2016), either making mosquitoes resistant to malaria parasite (Gantz et al 2015) or generating sterile females (Hammond et al 2016). The first approach would render *Anopheles stephensi* less prone to transmit malaria, whereas the second has the potential to exterminate *Anopheles gambiae*, effectively wiping out their populations (Reardon, 2016). Indeed, the latter strategy (albeit, sterilizing males with X rays) has been successful in eradicating horrible myiasis from some areas, such as the one caused by flesh-eating fly known as screwworm (*Cochliomyia hominivorax*) (Kouba, 2004).

Yet another application of CRISPR is the generation of small plants that can be easily grown as hedges, facilitating mechanical harvesting. Likewise, for small animals, like micropigs that grow to about 15 kg, for research and as pets. This technology can also be applied to change the size, color and patterns of fishes like koi carp. Also, to cats and dogs, including police, guide and herding ones. The use of CRISPR to design pets has been criticized as

being frivolous and maybe harming animal well-being. Yet, that is equivalent to classical Mendelian breeding, and, in fact, CRISPR could be used to remove undesirable characteristics, as happens with many dog breeds. On the other hand. rodents, ferrets, marmosets and other monkeys are used as disease models in biomedical research, and are being modified by CRISPR to better fit such purposes. A further remarkable example to apply CRISPR for neuroscience research is the Etruscan shrew (*Suncus etruscus*), which is the smallest mammal known, with about 1.8 grams and a tiny brain (Reardon, 2016).

Finally, as with some breakthrough developments, CRISPR raises bioethical concerns, mostly for germline editing, which should be properly considered. As a security mechanism, and to prevent unexpected consequences of releasing genetically-modified plants or animals into the environment, reverse gene drives have been developed, effectively canceling out the original constructs. Likewise, ensuring that individuals are sterile, so that they cannot reproduce should they escape from where they are confined, like research laboratories, farms, or growing fields (Reardon, 2016). Ultimately, it is obvious that CRISPR has great potential to improve human life. Yet, to avoid irrational rejection from the general public, which may have devastating effects, blocking human technological development and welfare, information about such technology should be fully disclosed. Additionally, scientists and educators should also properly explain it, with appropriate political support. Much as is currently done with transgenic insulin, which is injected into the body, and yet –of course– saves millions of lives worldwide each single day.

Acknowledgements. Supported by “Ministerio de Economía y Competitividad” (MINECO grant BIO2015-64737-R) and “Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria” (MINECO and INIA RF2012-00002-C02-02); “Consejería de Agricultura y Pesca” (041/C/2007, 75/C/2009 and 56/C/2010), “Consejería de Economía, Innovación y Ciencia” (P11-AGR-7322 and P12-AGR-0482) and “Grupo PAI” (AGR-248) of “Junta de Andalucía”; and “Universidad de Córdoba” (“Ayuda a Grupos”), Spain.

References

Alphey L (2016): Can CRISPR-Cas9 gene drives curb malaria? Nat Biotechnol 34:149-150.

Barrangou R (2015): Diversity of CRISPR-Cas immune systems and molecular machines. Genome Biol 16: 247 (pp).

Biagioni A, Chillà A, Andreucci E, Laurenzana A, Margheri F, Peppicelli S, Del Rosso M, Fibbi G (2017): Type II CRISPR/Cas9 approach in the oncological therapy. J Exp Clin Cancer Res 36: 80 (8 pp).

Carroll M, Zhou X (2017): Panacea in progress: CRISPR and the future of its biological research introduction. Microbiol Res 201: 63-74.

Chin WX, Ang SK, Chu JJ (2017): Recent advances in therapeutic recruitment of mammalian RNAi and bacterial CRISPR-Cas DNA interference pathways as emerging antiviral strategies. Drug Discov Today 22: 17-30.

Chira S, Gulei D, Hajitou A, Zimta AA, Cordelier P, Berindan-Neagoe I (2017): CRISPR/Cas9: Transcending the Reality of Genome Editing. Mol Ther Nucleic Acids 7: 211-222.

Chuai GH, Wang QL, Liu Q (2017): In Silico Meets In Vivo: Towards Computational CRISPR-Based sgRNA Design. Trends Biotechnol 35: 12-21.

Dannemann M, Andrés AM, Kelso J (2016): Introgression of Neandertal- and Denisovan-like haplotypes contributes to adaptive variation in human toll-like receptors. Am J Hum Genet 98: 22-33.

DeLaFuente-Núñez C, Lu TK (2017): CRISPR-Cas9 technology: applications in genome engineering, development of sequence-specific antimicrobials, and future prospects. Integr Biol 9: 109-122.

Deschamps M, Laval G, Fagny M, Itan Y, Abel L, Casanova JL, Patin E, Quintana-Murci L (2016): Genomic signatures of selective pressures and introgression from archaic hominins at human innate immunity genes. Am J Hum Genet 98: 5-21.

Doerflinger M, Forsyth W, Ebert G, Pellegrini M, Herold MJ (2017): CRISPR/Cas9-The ultimate weapon to battle infectious diseases? Cell Microbiol 19: e12693 (10 pp).

Doetschman T, Georgieva T (2017): Gene Editing With CRISPR/Cas9 RNA-Directed Nuclease. Circ Res 120: 876-894.

Dorado G, Jiménez I, Rey I, Sánchez-Cañete FJS, Luque F, Morales A, Gálvez M, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2013): Genomics and proteomics in bioarchaeology - Review. Archaeobios 7: 47-63.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Pérez-Jiménez M, Raya P, Gálvez M, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2015): Second-generation nucleic-acid sequencing and bioarchaeology - Review. Archaeobios 9: 216-230.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Pérez-Jiménez M, Raya P, Sáiz J, Sánchez A, Martín J, Rosales TE, Vásquez VF, Hernández P (2016): Sequencing ancient RNA in bioarchaeology - Review. Archaeobios 10: 103-111.

Dorado G, Rey I, Rosales TE, Sánchez-Cañete FJS, Luque F, Jiménez I, Gálvez M, Sáiz J, Sánchez A, Vásquez VF (2009): Ancient DNA to decipher the domestication of dog (REVIEW). Archaeobios 3: 127-132.

Dorado G, Rey I, Rosales TE, Sánchez-Cañete FJS, Luque F, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Hernández P, Vásquez VF (2010):

Biological mass extinctions on planet Earth (REVIEW). Archaeobios 4: 53-64.

Dorado G, Rosales TE, Luque F, Sánchez-Cañete FJS, Rey I, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Vásquez VF, Hernández P (2011): Ancient nucleic acids from maize - A review. Archaeobios 5: 21-28.

Dorado G, Sánchez-Cañete FJS, Pascual P, Jiménez I, Luque F, Pérez-Jiménez M, Raya P, Gálvez M, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2014): Starch genomics and bioarchaeology - Review. Archaeobios 8: 41-50.

Dorado G, Vásquez V, Rey I, Luque F, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Hernández P (2008): Sequencing ancient and modern genomes (REVIEW). Archaeobios 2: 75-80.

Dorado G, Vásquez V, Rey I, Vega JL (2007): Archaeology meets Molecular Biology (REVIEW). Archaeobios 1: 1-2.

Fellmann C, Gowen BG, Lin PC, Doudna JA, Corn JE (2017): Cornerstones of CRISPR-Cas in drug discovery and therapy. Nat Rev Drug Discov 16: 89-100.

Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E, James AA (2015): Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. Proc Natl Acad Sci USA 112: E6736- E6743.

Gasiunas G, Barrangou R, Horvath P, Siksnys V (2012): Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci USA 109: E2579-86.

Gee P, Xu H, Hotta A (2017): Cellular Reprogramming, Genome Editing, and Alternative CRISPR Cas9 Technologies for Precise Gene Therapy of Duchenne Muscular Dystrophy. Stem Cells Int 2017: 8765154 (11 pp).

Gerace D, Martiniello-Wilks R, Nassif NT, Lal S, Steptoe R, Simpson AM (2017): CRISPR-targeted genome editing of mesenchymal stem cell-derived therapies for type 1 diabetes: a path to clinical success? Stem Cell Res Ther 8: 62 (10 pp).

Gibson GJ, Yang M (2017): What rheumatologists need to know about CRISPR/Cas9. Nat Rev Rheumatol 13: 205-216.

Goren M, Yosef I, Qimron U (2017): Sensitizing pathogens to antibiotics using the CRISPR-Cas system. Drug Resist Updat 30: 1-6.

Grissa I, Vergnaud G, Pourcel C (2007): The CRISPRdb database and tools to display CRISPRs and to generate dictionaries of spacers and repeats. BMC Bioinformatics 23: 8:172.

Hammond A, Galizi R, Kyrou K, Simoni A, Siniscalchi C, Katsanos D, Gribble M, Baker D, Marois E, Russell S, Burt A, Windbichler N, Crisanti A, Nolan T (2016): A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. Nat Biotechnol 34: 78-83.

Jackson SA, McKenzie RE, Fagerlund RD, Kieper SN, Fineran PC, Brouns SJ (2017): CRISPR-Cas: Adapting to change. Science 356: eaal5056 (10 pp).

Jiang F, Doudna JA (2017): CRISPR-Cas9 Structures and Mechanisms. Annu Rev Biophys 46: 505-529.

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012): A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816-821.

Kan Y, Ruis B, Takasugi T, Hendrickson EA (2017): Mechanisms of precise genome editing using oligonucleotide donors. Genome Res 27: 1099-1111.

Komor AC, Badran AH, Liu DR (2017): CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes. Cell 168: 20-36.

Kouba V (2004): History of the screwworm (Cochliomyia hominivorax) eradication in the Eastern Hemisphere. Hist Med Vet 29: 43-53.

Li Y, Song YH, Liu B, Yu XY (2017): The potential application and challenge of powerful CRISPR/Cas9 system in cardiovascular research. Int J Cardiol 227: 191-193.

Lu Q, Livi GP, Modha S, Yusa K, Macarrón R, Dow DJ (2017): Applications of CRISPR genome editing technology in drug target identification and validation. Expert Opin Drug Discov 12: 541-552.

Mao Y, Botella JR, Zhu JK (2017): Heritability of targeted gene modifications induced by plant-optimized CRISPR systems. Cell Mol Life Sci 74: 1075-1093.

Marraffini LA (2015): CRISPR-Cas immunity in prokaryotes. Nature 526: 55-61.

Mojica FJ, Montoliu L (2016): On the Origin of CRISPR-Cas Technology: From Prokaryotes to Mammals. Trends Microbiol 24: 811-820.

Mout R, Ray M, Lee YW, Scaletti F, Rotello VM (2017): In Vivo Delivery of CRISPR/Cas9 for Therapeutic Gene Editing: Progress and Challenges. Bioconjug Chem 28: 880-884.

Murovec J, Pirc Z, Yang B (2017): New variants of CRISPR RNA-guided genome editing enzymes. Plant Biotechnol J 15: 917-926.

Puchta H (2017): Applying CRISPR/Cas for genome engineering in plants: the best is yet to come. Curr Opin Plant Biol 36: 1-8.

Puschnik AS, Majzoub K, Ooi YS, Carette JE (2017): A CRISPR toolbox to study virus-host interactions. Nat Rev Microbiol 15: 351-364.

Reardon S (2016): The CRISPR zoo. Nature 531: 160-163.

Rousseau C, Gonnet M, LeRomancer M, Nicolas J (2009): CRISPI: a CRISPR interactive database. Bioinformatics 25: 3317-3318.

Sayin VI, Papagiannakopoulos T (2017): Application of CRISPR-mediated genome engineering in cancer research. Cancer Lett 387: 10-17.

Shen S, Loh TJ, Shen H, Zheng X, Shen H (2017): CRISPR as a strong gene editing tool. BMB Rep 50: 20–24.

Shmakov S, Smargon A, Scott D, Cox D, Pyzocha N, Yan W, Abudayyeh OO, Gootenberg JS, Makarova KS, Wolf YI, Severinov K, Zhang F, Koonin EV (2017): Diversity and evolution of class 2 CRISPR-Cas systems. Nat Rev Microbiol 15: 169-182.

Singh A, Chakraborty D, Maiti S (2016): CRISPR/Cas9: a historical and chemical biology perspective of targeted genome engineering. Chem Soc Rev 45: 6666-6684.

Stout E, Klaenhammer T, Barrangou R (2017): CRISPR-Cas Technologies and Applications in Food Bacteria. Annu Rev Food Sci Technol 8: 413-437.

VanDiemen FR, Lebbink RJ (2017): CRISPR/Cas9, a powerful tool to target human herpesviruses. Cell Microbiol 19: e12694 (9 pp).

Vásquez VF, Rosales TE, Dorado G (2011): Estudio de la respuesta al cultivo de semillas y embriones de maíz (Zea mays) antiguo de la época Chimú (1420 años d.C.). Archaeobios 5: 4-15.

Yi L, Li J (2016): CRISPR-Cas9 therapeutics in cancer: promising strategies and present challenges. Biochim Biophys Acta 1866: 197-207.

Zischewski J, Fischer R, Bortesi L (2017): Detection of on-target and off-target mutations generated by CRISPR/Cas9 and other sequence-specific nucleases. Biotechnol Adv 35: 95-104.