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CRISPR/Cas system: A powerful tool for de-extinction

Yucong Shen

Guangdong experimental high school, Guangdong, China

20150235602@mail.sdufe.edu.cn

Abstract. The rapid advances in CRISPR technology have opened up new avenues of research and provided hope for a future where extinct species can be brought back to life. De-extinction, the process of resurrecting extinct species by using genetic engineering techniques, is one potential application of CRISPR technology that has gained increasing attention in recent years. The idea is to recover the genetic information of extinct species from preserved tissue samples and recreate them using modern genetic engineering techniques. George Church, a geneticist at Harvard University, is one of the leading scientists exploring the potential of CRISPR for de-extinction. He has spearheaded efforts to resurrect the woolly mammoth, which went extinct over 4,000 years ago, by inserting mammoth DNA into the genome of the Asian elephant. While this project is still in its early stages, it has sparked renewed interest in using CRISPR to bring back other extinct species, such as the Pyrenean ibex, the passenger pigeon, and the Tasmanian tiger. This review paper aims to explore the potential of CRISP technology for de-extinction, including its technical and ethical challenges, and the progress that has been made in various de-extinction projects involving CRISPR technology.

Keywords: De-extinction, CRISPR/Cas system, genetic engineering.

1. Introduction

The northern circumpolar permafrost region, which is one of the most affected areas by climate change, stores and cycles a globally relevant pool of carbon (C) in its ecosystems, with much of the ecosystem C being stored as soil organic carbon (SOC) vulnerable to remobilization under projected permafrost degradation. The widespread release of greenhouse gases resulting from this process would have a positive feedback in a warming climate. The K-Pg mass extinction is the most recent of five mass extinctions known to have occurred on Earth. According to paleontologists, a "mass extinction" is when more than 75% of the species in a geologically brief period — typically little more than 2 million years — disappear [1]. Numerous studies have demonstrated that the primary cause of species extinction, local population decline, extreme weather, drought, fire, etc. is ambient temperature surpassing the physiological limit temperature of species tolerance. In addition to habitat destruction, excessive hunting, toxic pollution, alien species invasion, and other reasons, climate change is one of the paths to species extinction. About one-third of terrestrial vertebrate species, many of which are common species, have declined in recent decades, according to a new study that analyzed data from 27,500 terrestrial vertebrate species from the International Union for Conservation of Nature (IUCN). Over 3,000 species were examined by a team of experts in 2014, and they discovered that since 1970, about half of all animal species had vanished from the earth.

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DISCOVER has suggested that the revival of mammoth populations in tundra areas could have a significant impact on the Earth's carbon emissions. The mammoths, as cold-resistant grazers, have the potential to enhance primary productivity as they move through the terrain. This could lead to the absorption of carbon into phytomass carbon (PC). Additionally, their trampling activity has the potential to wipe out spindly trees that hinder growth and expose healthy grasses that serve as carbon traps. These actions help to reinstate the tundra's crucial function as a climate buffer and maintainer of greenhouse gases, thus highlighting the essential role that mammoths can play in mitigating the effects of climate change. The de-extinction of mammoths could provide valuable insights into the genetics, behavior, and physiology of these animals. It could also help us understand the process of de-extinction itself and its potential for other extinct species. The de-extinction of mammoths could be used as a tool to educate the public about the importance of conservation and the impacts of human activities on the environment.

2. CRISPR/Cas system

CRISPR-Cas9 is a remarkable technology that hires RNA-guided nucleases to “cut” foreign genetic elements, though it was first discovered serving as part of an adaptive immune system. Among the three types of CRISPR systems, Type II is the most extensively studied. The system includes the Cas9 enzyme, a cluster of crRNA that encodes guide RNAs, and an accompanying tracrRNA that assists in breaking down the crRNA cluster into distinct units [2]. By expressing human designed Cas9 enzyme and the necessary RNA components, CRISPR-Cas reconstitutes the RNA-guided nuclease function in mammalian cells. By merging crRNA and tracrRNA, a singular, chimeric RNA (sgRNA) can be formed. This sgRNA can manipulate the 20-nt guide sequence to steer Cas9 towards any desired target. With its convenient application and multiplexing capability, Cas9 has facilitated the production of modified eukaryotic cells containing targeted mutations by means of both NHEJ and HDR techniques [3]. By introducing Cas9 mRNA and sgRNA into embryos, it has become possible to quickly produce transgenic mice carrying multiple modified alleles. Cas9 nucleases use the conserved HNH and RuvC nuclease domains to perform strand-specific cleavage, which can be exploited for additional functions through mutation. The Cas9 nickase mutant (Cas9n) can nick DNA to yield single-stranded breaks (DSB) instead of cleaving it [4]. The process of preferential repair through HDR is made possible using appropriately offset sgRNA pairs. This technique has the potential to decrease the occurrence of unwanted indel mutations caused by off-target DSBs. These applications can be used for reporting or modulating gene function. Compared to TALEN, a genome editing technology that uses a fusion of a TAL effector DNA-binding domain with a DNA cleavage domain to cut specific DNA sequences, CRISPR-Cas9 offers distinct advantages in terms of customization. Retargeting to new DNA sequences with CRISPR requires less hands-on time than TALEN. A pair of oligos encoding the 20-nt guide sequence is enough with CRISPR, whereas TALEN necessitates constructing two new TALEN genes, which is substantially more time-consuming.

3. Application

3.1. Woolly mammoths

Dr. Church and his team's work on using CRISPR to create hybrid species of elephants is a fascinating example of the potential for gene editing to address complex problems. The situation is it is nearly impossible to find complete DNA of the mammoths as they died out thousands of years ago, mostly leaving their bodies deep in frozen land. However, by inserting genes related to cold tolerance and long hair growth from woolly mammoths into the genome of Asian elephants, the researchers hope to create a new species that can thrive in the Arctic tundra. Many professional teams are now working with it. For example, Dr. Church and his team is a promising step forward in the field of gene editing [5].

The first step of processing the programme after finding the target genes is to design certain guide RNA in order to match to certain base pairs on Asian elephant’s DNA. Church and his team faced a lot of difficulties in designing CRISPR-Cas9 guide RNAs (gRNAs) with high on-target efficiency. Accurate gRNA design is crucial for efficient and precise genome editing, but it requires high-quality gRNA

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activity data as well as well as efficient modeling. To address this challenge, a novel high-throughput method to measure gRNA activity in cells is created by Dr church and his team, and was proved to be accurate in determining gRNA activity [6]. The scientists used deep sequencing to investigate the prevalence of indel mutations at 16 surrogate sites and their corresponding endogenous genomic regions in HEK293T cells to confirm the validity of this method. With a Spearman's R value of 0.72 and a p-value of 0.0016, the findings showed a significant connection between the surrogate and endogenous sites in terms of indel profiles and frequency. The statistical measure Spearman's R indicates the strength and direction of the association between two variables. In this case, the connection between the indel profiles and frequency of the surrogate and endogenous sites was evaluated. A score of 1 represents the strongest possible correlation, a score of -1 the strongest possible correlation, and a score of 0 the absence of any association. Therefore, the positive association between the surrogate and endogenous sites is indicated by the Spearman's R score of 0.72. The p-value measures the probability that the observed association between the endogenous and surrogate sites is a result of chance. A p-value of 0.0016 suggests that there is a very slim chance (less than 1%) that the observed correlation is due to chance, providing further backing for the methodology's accuracy in gauging gRNA behavior. A model used to prediction was developed by the team. measuring the thermodynamic properties of the PAM and neighboring sequences, 30 nt DNA input sequence constituted of the protospacer, the brand new model could be used to predict certain activity of the gRNA. It is obvious that when a certain chemical reaction happens, the combination of gRNA and target DNA, for example, will directly lead to a change in the gibbes free energy. So an observation of a change in delta G is a key fact of predicting the activity and certain characteristic of a gRNA. It turned out that the first model developed by church and his team, which is called pre- CRISPRon_v0, showed similar ability of prediction as the models already have now, including DEEPHF, DEEPSPCAS9. And of course, the pre- CRISPRon_v0 was well developed and replaced soon, as pre-CRISPRon_v1 and CRISPRon_v1. According to the team, the final CRISPRon_v1 was proved to be better at predicting than all the models mentioned before, reaching an improvement in spearman’s R roughly about 20 percent or more. While testing some specific data it reached a surprising spearmen’s R up to 0.800. Since doctor church and his team is still working with the de-extinction project, soon, more and more advanced model will be developed and the living mammoths are coming [7].

3.2. The passenger pigeon

Another example is the de-extinction of the passenger pigeon. The decline of the Passenger Pigeon population was largely due to commercial hunting and habitat loss. In the 19th century, pigeon meat was a popular food source, and pigeon feathers were used for insulation and decoration. This led to widespread hunting, which decimated the population. Additionally, the clearing of forests for agriculture and urbanization destroyed the birds' natural habitat, further contributing to their decline. The extinction of the Passenger Pigeon has had significant ecological consequences. The birds were known to play an important role in dispersing seeds and contributing to forest regeneration. The loss of the Passenger Pigeon has also had cultural and historical impacts, as the birds were once an important part of American folklore and were considered a symbol of abundance and fertility [8].

However, we are taking actions. According to the website “revive and restore”, a programme was started in 2012, The scientific progress made in de-extinction involves a series of five stages, which are in silico, in vitro, in vivo, ex situ, and in situ.

To bring back the Passenger Pigeon, the first step is to detect the genes present in both the extinct Passenger Pigeon and a closely related living species, the Band-tailed Pigeon. Again, the complete gene of the passenger pigeons can hardly be found. So, our goal is to create a hybrid species, and the banded tailed pigeon was selected by the researchers. By comparing the genomes of the two species, fixed genetic differences can be identified that set the Passenger Pigeon apart from the Band-tailed Pigeon. The morphological traits that set the Passenger Pigeon apart from its live counterpart are the result of 37% of the 168,000 fixed genetic differences detected in the protein-coding genes that modify 66% of the protein-coding genes. The genetic code will be converted into live DNA molecules that may be used

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to change the living genome of Band-tailed Pigeons to produce a bird that is genetically similar to the Passenger Pigeon after the fixed genetic differences have been determined [9].

Third, the most efficient method for producing genome-edited birds involves editing genomes in cell cultures, specifically using Primordial Germ Cells (PGCs). Isolated PGCs can be grown in a liquid medium called culture, which provides the necessary nutrients for their survival and growth in the laboratory. By utilizing CRISPR/Cas9 technology, now manipulate the genome of cultured PGCs without the need to hatch new birds every time changes are made. With the "cut and paste" biotechnology, the Cas9 enzyme is directed to a specific site in the genome using the CRISPR portion, where it cuts the DNA. The CRISPR/Cas9 mechanism slices the existing DNA to introduce the Passenger Pigeon's manufactured DNA, which is then incorporated into the genome by homologous recombination, a normal DNA repair process in cells. Then the pgc was injected in to blood of a living pigeon. After the pgc was transferred into sexual organs, it will stay there and sperm and eggs that contains passenger pigeon DNA will be produced. After mating, their offspring will contain these genes.

However, a researcher who is guide “revive and restore” programme, Ben Novak, is managing turning all these into reality. 13 birds are now available in Melbourne, Australia. They don’t look like passenger pigeons, but they are the most special birds ever – their genes contain man-designed CRISPR/Cas9 system, and their offspring are ready for gene editing at any time. Surprisingly, about 25% of the eggs survived and hatched after being injected by cas9 genes according to Novak. These were the effort reached in 2018, and until now, in 2023, good news is still being waited from Melbourne – de-extinction may take a long time, but it should always be paid the patience, and, of course, get prepared to hug it when it becomes true someday.

4. Conclusions

North America's Eastern forests require disturbance as a critical element in their regeneration cycle, similar to other forest ecosystems. So, the existence of passenger pigeon is like some kinds of stimulus to help the tree’s growth. That means, regenerating forest habitats are more bio-productive, resulting in higher rates of carbon sequestration and photosynthesis.

Critics argue that reintroducing mammoths could disrupt the ecological balance of the northern hemisphere, where they were primarily found. The reintroduction of mammoths could lead to changes in the populations of other species such as red deer, wolves, lynx, and wild boar, which may not be able to compete with the reintroduced mammoths for limited resources. This could have a ripple effect throughout the ecosystem, causing further disruptions to the delicate balance of the ecosystem. Therefore, some have expressed concerns about the potential consequences of the research on de-extinction of mammoths by the church [10].

Critics also have their own thoughts about the restore of the passenger pigeons. They claim that money and researchers should be placed in where they could save the animals that are dying out today.it is estimated that 75 kinds of species vanishes from the earth every day. What’s more, when the passenger pigeon are existing in this world again, what will happen if they reach a population of more than 1 billion individuals, just like what they were in the 1700s? nobody knows how hard the crash is to the north American ecology system, or even to the world’s ecology. The food chain will be affected most strongly, which means the de-extinction of the passenger pigeons may lead to extinction to other species, which should not be accepted.

To make a conclusion, CRISPR offers an efficient way to edit genomes, making it a promising tool for bringing back extinct species. The de-extinction process involves inserting edited DNA into the genome of a closely related living species to create a hybrid that can reproduce and potentially revive the extinct species. Bringing back extinct species using CRISPR could have various benefits, such as restoring lost ecological functions and promoting biodiversity conservation. However, there are also ethical, legal, and environmental concerns that need to be addressed, such as the impact on existing ecosystems, and the opportunity cost of focusing resources on de-extinction instead of conservation efforts.

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References

[1] Siewert M B, Hanisch J, Weiss N, et al. Comparing carbon storage of Siberian tundra and taiga permafrost ecosystems at very high spatial resolution[J]. Journal of Geophysical Research: Biogeosciences, 2015, 120(10): 1973-1994.

[2] Ying YAN,Roswitha A. AUMANN,Irina H?CKER,Marc F. SCHETELIG.CRISPR-based genetic control strategies for insect pests[J].Journal of Integrative Agriculture,2023,22(03):651-668.

[3] XUE Shan,WANG Shuya,LIU Li,ZHONG Qiaofang,CHENG Zaiquan,XIAO Suqin. Research progress of precision gene editing technology based on CRISPR/Cas9[J/OL]. Chinese Journal of Biotechnology:1-13[2023-04-16].

[4] Huang Meiyan, Liu Binghuan, Jie Yonghong, Zhao Hong, Shang Chenyang. Off-target effects and countermeasures of CRISPR/Cas9 system [J/OL]. Molecular Plant Breeding: 1-19 [2023-04-16].

[5] Ryu S M, Hur J W, Kim K. Evolution of CRISPR towards accurate and efficient mammal genome engineering[J]. BMB reports, 2019, 52(8): 475.

[6] Xiang X, Corsi G I, Anthon C, et al. Enhancing CRISPR-Cas9 gRNA efficiency prediction by data integration and deep learning[J]. Nature communications, 2021, 12(1): 3238.

[7] DeFrancesco L. Church to de-extinct woolly mammoths[J]. Nat Biotechnol, 2021, 39(10): 1171.

[8] Greely H. Of science, CRISPR-Cas9, and Asilomar[J]. Law and Biosciences Blog, 2015, 4.

[9] Shapiro B. Mammoth 2.0: will genome engineering resurrect extinct species? [J]. Genome Biology, 2015, 16(1): 1-3.

[10] Application, controversy and future of CRISPR gene editing technology [J]. Science Chinese,2018(10):78-80.