CRISPR/Cas9 mediated gene drive in the fight against malaria

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written by Michael Skoda & Valentin Schmidt

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1. Preface

Through a method of genetic engineering called «gene drive», scientists are in the process of creating a completely new and, if successful, extremely potent weapon in the fight against malaria, one of the deadliest infectious diseases worldwide, taking up to half a million lives every year (cf. World 2019). With this technique, the spread of selected genes in populations could be accelerated drastically, as gene drive significantly increases the expected inheritance of a desired gene over successive generations. Even though the first known descriptions of malaria were made in ancient Italy (cf. Planet Wissen 2020), only through modern science the development of such a method and with it the possibility of defeating the disease completely has become a reality, which is what caught our attention about this topic and caused a fascination for the scientific development, leading us to choose it for our term paper without hesitation. A notable aspect is perhaps the strong controversy that has been evoked by the scientific interest in gene drives due to their promising feasibility and efficiency on the one hand and environmental risks they could pose, as well as their potential to be used as biogenetic weapons, on the other hand.

In this paper we hope to answer the following questions:

- How do gene drives function?
- When could gene drives be applied in nature?
- What are the advantages of the technique compared to currently used methods of malaria prevention?
- What are the risks and dangers of the technique?
- What are the ethical problems of gene drives?

2. Introduction

Because malaria is responsible for such an immense number of deaths every year, additionally causing enormous economic losses in the most affected countries, most of which are located in Africa and other tropical and subtropical regions (cf. Wikipedia Malaria), finding a way of effectively combating the disease has been a great scientific task for a long time. Before genetic engineering was made possible on a large scale with the development of the CRISPR/Cas9 method (cf. Wikipedia CRISPR), the most promising branches of research for achieving control over malaria were vaccines, drugs, diagnostics and vector management tools like insecticides, environmental modification and bed nets, all of which have not shown complete or permanent success in their application to this day. This can be explained mainly by the complexity of the unicellular parasite Plasmodium, which is the organism that causes the disease by entering the human body via the saliva of mosquitoes when they bite (see figure 1) and the emergence of insecticide-resistant mosquitoes and drug-resistant parasites (cf. National Institute 2011).

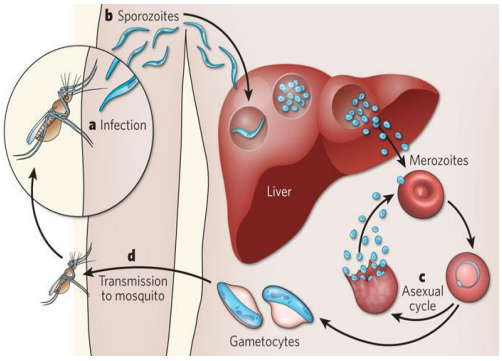

Figure 1. Life cycle of plasmodia (drawing from Chegg Prep, enlarged version in the figure bibliography).

2.1 Recent scientific history

The idea of a mechanism like gene drive was first introduced in 2003 by Austin Burt, who worked as an evolutionary geneticist at the Imperial College London at that time (cf. Wikipedia Gene drive). His thesis was based on observations about selfish genetic elements, which have been a matter of biological discussion since 1964 (cf. Rostami). He proposed the use of gene drives to suppress the ability of mosquitos to transmit malaria or to completely eliminate the mosquito species that can carry malaria (cf. Wikipedia Gene drive). The research about such genes continued in the following years, but only through the discovery of the CRISPR/Cas9 gene editing technique by Jennifer Doudna, Emmanuelle Charpentier, George Church, and Feng Zhang in 2012, which highly increased the efficiency and speed of genetic engineering (cf. Bitesize Bio 2020), research on gene drive at a larger scale became possible and desirable. Just one year later, Kevin M. Esvelt at MIT developed a plan to apply CRISPR in a gene drive system with the aim of altering the genome of wild populations of organisms (cf. Esvelt). In 2015 M. Gantz and Ethan Bier completed a study in which they showcased the applicability of the CRISPR technology to generate gene drives in *Drosophila melanogaster*. This study was followed up in 2016 by Hammond A et al., who generated gene drive by targeting female reproduction in the Anopheles gambiae complex, which contains one of the main vectors of malaria (cf. Hillary 2020; Wikipedia Anopheles). There were even some experiments conducted on mice in 2018, which resulted in a successful change of coat colour in the females of the population (cf. Collins 2018).

The concept originated from observations of selfish genetic elements in nature, which enhanced their own inheritance through copying themselves onto their homologous chromosome before meiosis (cf. Rostami). This caused conversion of the gene variant from heterozygote to homozygote state. If applied in germ cells or embryos, super-Mendelian inheritance will result, as shown in Figure 2.

Gene drives could enable scientists to alter a whole population of a species by releasing a few genetically modified individuals into the wild. Without gene drives, increasing the frequency of the modified trait in a population would be impossible, unless the trait provided a competitive advantage to its carriers (cf. Rostami).

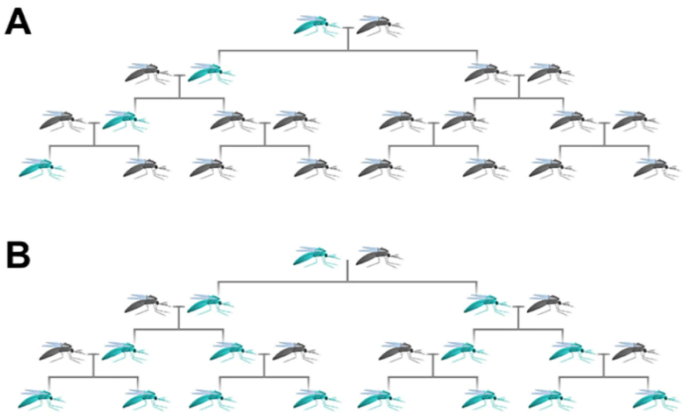

Figure 2. A) Mendelian inheritance of an autosomal dominant trait. Approximately 50% of offspring are expected to inherit the gene variant (marked in blue-green color). B) Gene drive increases the percentage of offspring that inherits the variant trait though conversion of the gene variant from heterozygosity to homozygosity in the male germ cells (drawing from Wyss Institute).

3. Explanation of the applied technique

Over the last decade, several pre-existing or newly modified nucleases capable of introducing targeted mutations in the genome have been tested for their capacity to generate gene drive in populations of organisms in the laboratory. At first, scientists looked for a way to hijack naturally occurring selfish genetic elements. I-Scel, a member of the homing endonuclease genes family, was used in the first engineered gene drive in 2011(cf. Windbichler). This first engineered gene drive reached an efficacy of over 60% in Anopheles gambiae, which surpassed the minimum requirements to be used for vector control. Zinc Finger Nucleases (ZFNs) and TALENs (transcription activator-like effector nucleases) were studied as well, but these “easy-to-engineer” endonuclease were less suited for gene drive (cf. Hammond, 2017). The discovery of the CRISPR/Cas9 technology created new hope to generate a gene drive system.

3.1 CRISPR

CRISPR, an acronym for “clustered regularly interspaced short palindromic repeats “is a locus found on the chromosomes of many Bacteria, for which it acts as an anti-viral defense system (cf. iBiology Science Stories). It consists of multiple short palindromic repeats, meaning sequences of 30-40 bases that read the same from left or right, interspaced with spacer DNA, containing sequences that are frequently found in viruses. In addition, the CRISPR locus contains so-called Cas genes (crispr associated genes), which encode Cas proteins. These Cas proteins are homologues of helicases and nucleases. The spacers and Cas genes together construct the defense system. If a virus, previously encountered by the bacteria, attaches onto the bacteria and releases its virus DNA, the bacteria will express Cas protein and transcribe the spacer DNA into what is called crRNA (crisprRNA) that matches the virus DNA. This crRNA then helps the Cas protein to recognize the viral DNA that is then destroyed by CRISPR/Cas protein-RNA complex. If the bacteria have not been exposed to the invading virus before, one of the Cas proteins cuts the DNA and makes a copy of the sequence, which it then introduces between the repeats. Thus, the spacers are essentially the memory of the defense system, where the bacteria store previously encountered virus sequences. This defense system can be compared to the immune system of humans on a smaller and simpler scale (cf. Bozeman Science).

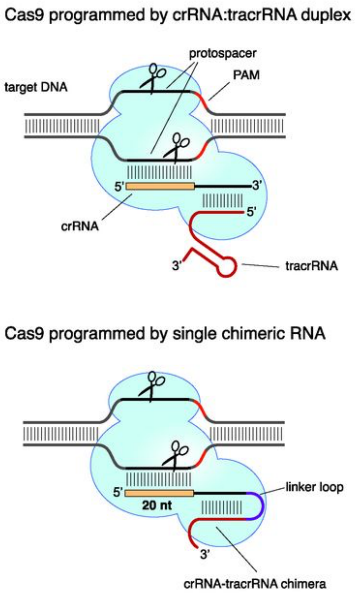

Figure 3. Top) Naturally occurring CRISPR/Cas9 RNA protein complex.
Bottom) Genetically modified CRISPR/Cas9 complex, where crRNA and tracrRNA have been connected (drawing from Jinek, enlarged version in the figure bibliography).

The technology usually referred to as CRISPR uses Cas9, one of the Cas-nuclease proteins. Naturally, Cas9 is made up of the protein, a crRNA as well as a tracrRNA that holds the crRNA in place. The main purpose of Cas9 is cutting virus double strand DNA. Scientists from the labs of Jennifer Doudna and Emmanuelle Charpentier genetically modified this protein to generate a simple and easily accessible gene editing technology. The changes they implemented were to simplify the system and make it programmable. They connected the crRNA with the tracrRNA to form the

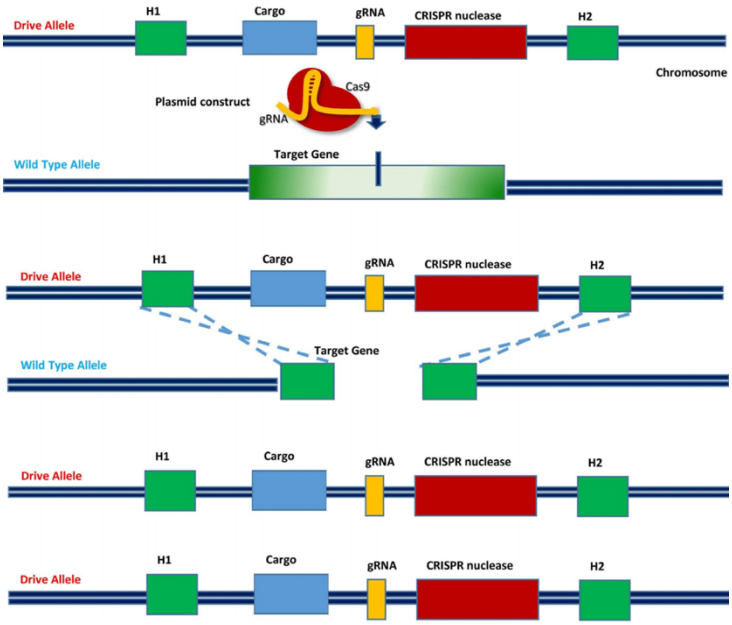

Figure 4. Gene drive system with CRISPR copying itself onto the homologue chromosome (drawing from Rostami, enlarged version in the figure bibliography).

"tracrRNA-crRNA Chimera", which is often called guideRNA (gRNA), as seen in Figure 3. This gRNA is easily edited in the lab, thus making it possible to program Cas9 to cut in a specific location in the genome by synthesizing gRNA with a sequence complementary to the DNA sequence of the desired locus. This is also possible with other gene editing tools. However, what sets CRISPR apart from other endonuclease systems is its efficiency, versatility and, most importantly, its simplicity and accessibility (cf. iBiology Science Stories).

Scientists have engineered a gene drive system using CRISPR/Cas9 as its nuclease. As shown in Figure 4, the gene drive system contains CRISPR, gRNA, the cargo gene and homologue sequences 1 and 2 (H1& H2). The gRNA guides the Cas9 complex to the target gene. There, the Cas9 complex cuts the DNA. Homologue-directed repair is triggered because of H1 and H2, which are homologue to the ends of the cut target gene, and the whole gene drive system is copied onto the homologue chromosome, completing the gene drive (cf. Rostami).

3.2 Gene drive techniques applied to Malaria

There are two general strategies to apply gene drive for the fight against malaria:

- "Population suppression approach" aims to reduce the size of the malaria transmitting mosquito population by shifting the sex ratio from 50/50 towards predominantly male individuals. Because size of the *Anopheles* mosquito population is primarily dependent on the number of females, male predominance would result in a suppression of the *Anopheles* population to levels where transmission is no longer possible or full extinction (Rostami). This can be achieved by modifying X chromosomes of gamete cells, or mutating genes that can result in female infertility (cf. Galizi; cf. Hammond, 2016).
- "Population modification approach" aims to genetically alter the mosquito to inhibit parasite transmission over subsequent generations by inserting antimalarial effector genes into the mosquito that interfere with the sexual cycle of the malaria parasite Plasmodium. These effector genes get inserted into an expressed locus of the genome through the gene drive (cf. Carballar-Lejarazú).

Both strategies have been successfully applied in laboratory studies, generating immunity to Plasmodium or extinguishing a small cage population within 6 generations. Both strategies are viewed as potential vector control methods, yet they are still a long way from achieving vector control outside of the laboratory setting.

4. Interview

Figure 5: Nikolai Windbichler (image from Imperial College).

For our interview we contacted Dr Nikolai Windbichler (see figure 3), who is currently working in the Faculty of Natural Sciences at the Imperial College of London with a focus on genetic control of the human malaria mosquito (cf. Windbichlerlab; Imperial).

- Which gene drive approach (suppression or alteration) are you doing research on?

We are working on population replacement for the malaria vector Anopheles gambiae and also population suppression to target insect pests of agriculture.

- What gene drive system are you currently using?

We are using classic homing drives, often as split drives or integral gene drives.

- Has it been tested outside laboratory conditions?

No gene drive has so far been tested by anyone outside the laboratory.

- Would it spread as planned if it was released into the wild right now? If not, what are some problems or complications?

Gene drives would spread. However, they may spread slower than expected due to populations that are poorly connected and do not exchange genetic material frequently.

For most gene drives resistance against the drive is the main complication. But even if it arises that does not mean the intervention has necessarily failed. The goal is the break the transmission cycle e.g. Malaria.

- What are the next steps of your research and experimentation?

We are currently testing what molecules can block parasite development in the mosquito and if they can do so with the genetically diverse parasites found in Africa.

- Do you think that this is the most promising method to fight malaria?

All of them together. Vaccines, insecticides, bednets and possibly genetic control as well.

- Do you have any thoughts on the ethical aspects of gene drives? Are they part of your research?

They are, but I consider gene drives, a very species-specific intervention, to have far fewer issues than many other people see in them.

5 Discussion

5.1 Future Steps

Over the last decade, the attempts to use gene drive for malaria vector control have made long strides towards the goal. Thanks to dozens of research teams and the invention of the CRISPR/Cas9 technology, the field has moved from the first engineered gene drive system in 2011 to an array of gene drive malaria transmission controls with efficacies of 90%-100%. While these approaches look very promising, it is still a long way from implementation in the wild.

There are 3 steps to go through for an idea to become an established practice: Discovery, Development and Delivery (cf. Carballar-Lejarazú). Discovery is where scientists conceive an idea and carry out proof-of-principle experiments to demonstrate the potential. This stage has been achieved for gene drive systems, with great promise. The Development step consists of upscaling and testing robustness and efficiency of the system under more physiologic conditions. This comprises assessing potential development of resistance against the gene drive through mating selection or mutations at the target locus on the homologue chromosome, that would make them resistant to cleavage or CRISPR-induced off target mutations that impair the desired gene drive. For this stage, tests have to be carried out in larger test populations, taking factors like temperature changes and daylight cycles into consideration (cf. Hammond, 2016). Techniques also have to be adjusted to different vector species with sometimes fluctuating population sizes and/or different climatic conditions. The practice has to show the same efficacy in populations of Anopheles gambiae in the planes of sub-Saharan Africa as in the swamps of East Asia. An effective method of how to control or reverse the gene drive has to made available, to terminate possible unwanted effects (cf. Carballar-Lejarazú). The last step, Delivery, encompasses producing quality-controlled vectors, fulfilling regulatory requirements and complying with social and ethical principles. Given these still to be fulfilled steps, large scale implementation is not to be expected before 2040.

5.2 Ethical aspects

The most controversial aspect of using genetically modified mosquitoes to stop malaria is using gene drives in nature, as the technique brings many new possibilities, as well as some risks. The ethical discussion about this technology is very complex and has started as soon as the development of gene drives commenced.

5.2.1 Advantages

Gene drives could not only be used to eradicate malaria completely, they provide the ability to alter wild populations of any rapidly spreading organism, like controlling or completely eliminating invasive or parasitic species, giving them an immensely large range of possible applications (cf. Wikipedia Gene drive). With regard to malaria, gene drives are therefore, in theory, the most feasible and effective method of control, as there are currently no reliable vaccines, drugs or external tools, such as insecticides, environmental modification or bed nets (cf. National Institute 2011). Researchers at the Imperial College of London also believe an

ethical advantage of gene drives to be the ability to target just the species of mosquitoes responsible for the transmission of malaria, *Anopheles gambiae*, *Anopheles coluzzii* and *Anopheles arabiensis,* instead of causing harm to innocuous species by using insecticides (cf. Albert 2020). The technique thus raises great hope for a permanent solution to the malaria crisis, as well as many other infectious diseases, would it not be for the great risks and ethical dilemmas it brings with it.

5.2.2 Disadvantages

The main concern about gene drives that is often expressed by opponents of their application is certainly the unpredictable, possibly irreversible interference with nature that could lead to environmental catastrophes if control over its spread is lost. Because gene drives are constructed to spread rapidly and exponentially if set free, even minor mistakes or unexpected negative traits of the altered species, such as mutations or undiscovered diseases, could theoretically cause new plagues or other biological threats with the potential of posing a risk to entire ecosystems and humanity itself. However, the technique would only pose a risk of such magnitude if there were no limiting factors to the spread at all, which is certainly not the case. According to the interview we conducted with Dr Nikolai Windbichler, he does not consider gene drives to be the boundlessly powerful biological tool they are often portrayed as, referring to them as a «very species-specific intervention» with limitations due to poor connections between populations and resistance against it. But despite the notorious accuracy of the CRISPR/Cas9 method and the possibility of conducting gene drives in a specific and controlled manner, the ability to make such significant changes in nature they could provide is bound to evoke complex legal and political issues about the extent of their application (cf. Reynolds 2020), which makes an imminent introduction of this technology into nature unlikely (cf. SCNAT 2018). A further significant problem seems to be the probability of the targeted organisms building up a resistance against gene drives, which is one hundred percent, says Dr Windbichler in another Interview. The development of a solution to this issue is therefore of great importance in the process of completing the gene drive technique, one idea being the insertion of multiple gene drives into the genome so the organism is not able to react to all of them (cf. SCNAT 2018).

6 Summary

CRISPR/Cas9 mediated gene drives are a powerful tool to change the genetic traits of rapidly spreading populations, providing great new scientific possibilities, but also bringing complex ethical and political complications with them. The gene drive technique increases the expected inheritance of a desired gene over successive generations and thus causes an accelerated spread of selected genes in populations. As there are currently no reliable strategies to prevent or heal malaria, gene drives could play an important role in the development of a permanent solution to the crisis caused by this disease. But despite the numerous successful applications in laboratory conditions, there are several substantial obstacles that prevent them from being ready for application in the foreseeable future.

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Figure 5 Imperial College: Last accessed February 5th, 2021, < https://www.imperial.ac.uk/people/n.windbichler>

7.1.1 Figure enlargement

Figure 1

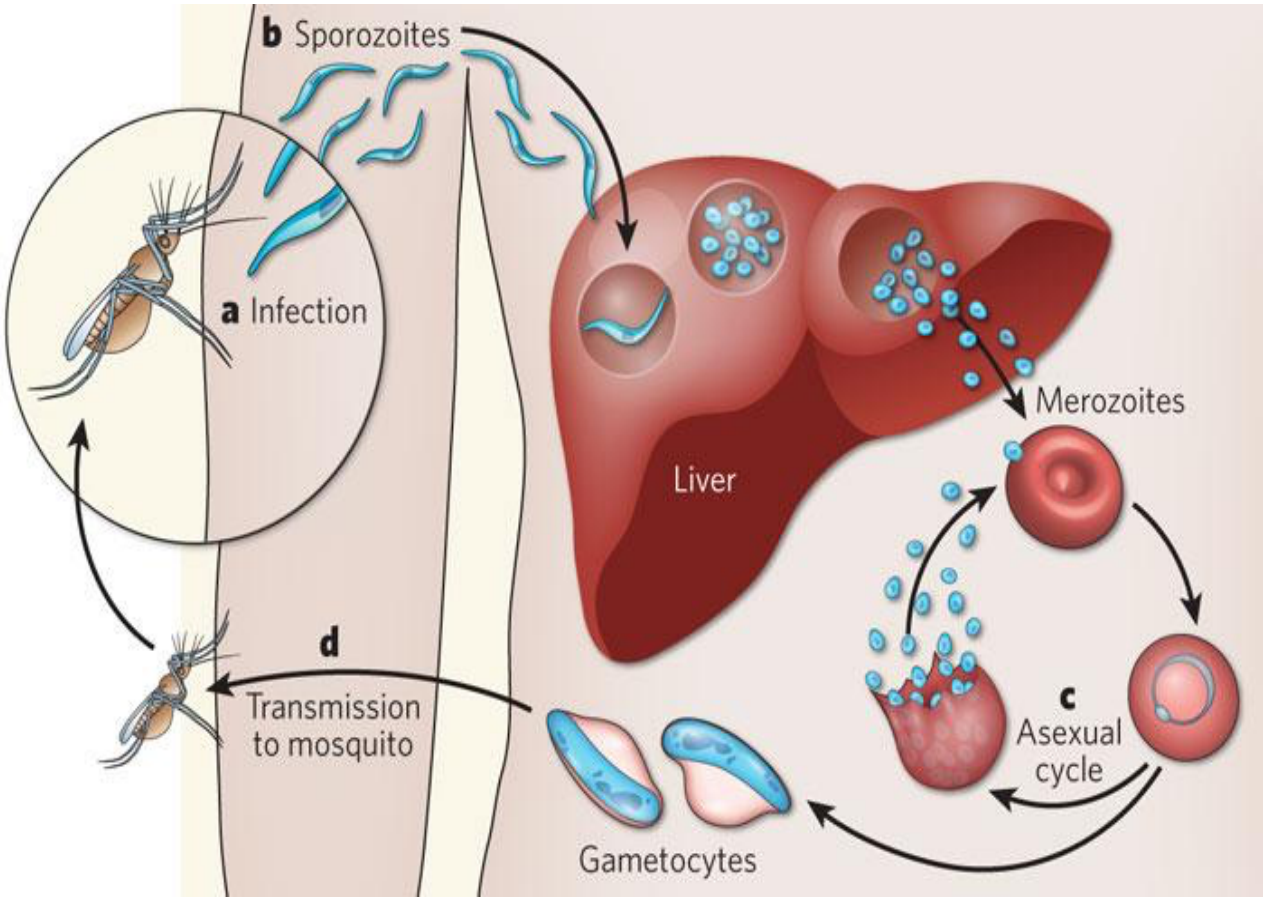

Figure 3

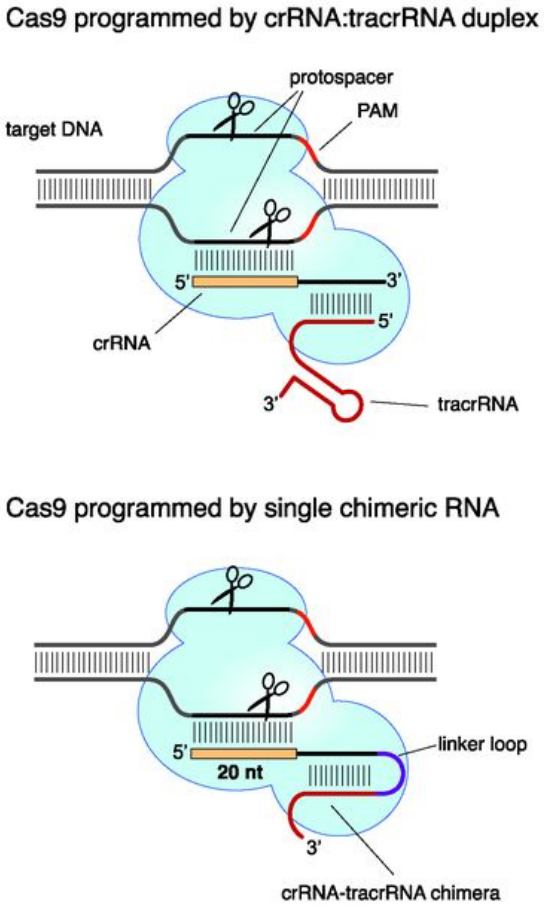

Figure 4