Applications of CRISPR Technologies for Biosensors Using Nucleic Acid Detection

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Introduction

In 2012, CRISPR was discovered to be the mechanism that allows genome editing by creating guide RNA to target specific regions of a genome to alter and modify gene function in the DNA. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are short repetitions of base sequences in a segment of DNA that are found in bacteria and archaea's immune systems. However, CRISPR has been further developed to be applicable to other species, specifically humans, to accomplish gene manipulation and regulation.

When a virus or plasmid injects its DNA into a bacterial cell, a piece of their viral DNA is integrated into the bacterial genome at a site called CRISPR that include a series of repeated sequences that bound each inserted piece of viral DNA. The viral DNA integration creates a permanent genetic record of the past infections that the bacterial cell encountered. Afterwards, the sequences are copied into strands of RNA and chopped into functional units, each containing one viral sequence plus the bounded repeat. The chopping process requires a separate RNA called tracrRNA that together bind to the Cas9 protein, a component of CRISPR immunity, to form a surveillance complex.

Once another part of the DNA has a matching sequence, the Cas9-RNA binds onto the matching DNA sequence, unwinds it, and cuts each strand of the DNA double helix. In bacteria, the broken viral DNA is chopped up and destroyed by other proteins, thereby halting the infection.

As mentioned above, the "other proteins" are Cas, CRISPR-associated proteins that associate with CRISPR RNAs to bind and alter target sequences. Furthermore, within the CRISPR-Cas system, enzymes are produced the CRISPR system, such as Cas9, Cas12, and Cas13. As previously mentioned, Cas9 is the sole enzyme that detects and cuts target stretches of DNA, specifically, a short genetic sequence called protospacer-adjacent motif (PAM), that is embedded in the target DNA sequence (Doudna et al., 2012). In addition, Cas9 is also the protein that guides CRISPR RNA (crRNA) to silence foreign DNA, meaning that Cas9 is a DNA endonuclease that is guided by two RNA molecules.

Similar to Cas9, the Cas12 protein is a DNA endonuclease but additionally does crRNA processing. The Cas13 protein, however, is a RNA endonuclease, but also does crRNA processing like the Cas12 protein. All three of these proteins fall under the CRISPR-Cas Class II system, which is complementary and alternative to what CRISPR-Cas9 can already do (Nidhi et al., 2021).

With the CRISPR-Cas system enzymes being able to detect and cleave specific DNA sequences, CRISPR has been used greatly for genomic editing which opens opportunities for biosensing for infectious diseases and pathogens (Aman et al., 2020). Gene-editing tools have been utilized to help modify genes that deal with infectious diseases, cancers, and pathogens, as well as to create diagnostics for these diseases. The CRISPR-Cas system can be applicable to

humans, animals, water, and plants meaning that the CRISPR-Cas system can be manipulated to guide endonucleases to a specific target gene (Nidhi et al. 2021) that can later be applied to single nucleotide polymorphism (SNP) detection and genotyping, cancer screening, viral infection diagnosis, bacterial and parasitic infection diagnosis, water and sanitation and lastly, antibiotic resistance (Aman et al., 2020).

Equally important, the continued development of the CRISPR-Cas biosensing system grew to include the Cas effectors such as Cas12a and Cas13a, the Class II systems that complement CRISPR-Cas9.

Body

Nucleic acid detection is essential in CRISPR-Cas biosensing applications because as the building blocks of nucelotides that make up our DNA, nucleic acids play a role in every species' heredity, growth and mutations. As a result, looking into the sensing and detection of nucleic acids in our genome can prevent a wide range of diseases for generations to come.

Established CRISPR-Cas-based nucleic acid biosensing systems are differentiated based on the Cas effectors proteins used for the system such as Cas9, Cas12a, and Cas13a. Cas12a and Cas13a are studied extensively for their nucleic acid-sensing and detection with Cas12a being utilized for DNA targeting and Cas13a for RNA targeting. Diagnostic tools for nucleic acid detection were created based on the Cas12a and Cas13a effectors such as DETECTR (Cas12a) and SHERLOCK (Cas13a).

Specific High-Sensitivity Enzymatic Reporter Unlocking (SHERLOCK) and DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) were the first two sensing systems

in molecular diagnostics with different signal readouts such as fluorescent, colorimetric, electrochemical signals and more (Yin et al., 2021). DETECTR is able to detect viruses with the greatest sensitivity, so much that this biosensor is able to detect foreign nucleic acids are the subattomolar level (Aman et al., 2020). Moreover, DETECTR was developed for rapid detection of both target DNA and RNAs (Li et al., 2019) and has been applied to human HIV detection, Ebola and Lassa virus, and the hepatitis B virus. SHERLOCK, however, is based on the Cas13 cleavage and collateral activity that helps detect the presence of the target DNA for cleavage and has proven to detect specific strains of the virus Zika and Dengue (Yin et al., 2021). Furthermore, SHERLOCK was able to differentiate the mutation in HIV and HBV that expressed drug-resistance within specific crRNAs (Yin et al., 2019).

With the help of CRISPR-Cas nucleic acid biosensing systems, the creation of diagnostic tests came to light for pathogen detection, cancer mutation screens, and single nucleotide polymorphism identification, in other words, identification of disease-causing genes in humans (Li et al., 2019). Compared to other current biosensing technologies, CRISPR-Cas systems express rapid and simple results due to their high sensitivity, specificity and cost-effectiveness.

Conclusions and Future Directions

In the end, nucleic acid detection as a whole can prevent diseases and create diagnostic tests for the detection of discrimination within an organism's genome. SHERLOCK and DETECTOR, two Cas effectors biosensors stemmed from Cas12a and Cas13a, proved to detect the foreign nucleic acids and strains that are present in organisms that host them. The applications of biosensing for plants, animals, insects are continue to grow once +++ Mutations within nucleic acids can alter the DNA expression in organisms, leading to genome evolution.

With using the CRISPR-Cas system for biosensing applications, we can hope to continue to develop these biosensors to see what CRISPR can possible cure in terms of human diseases. Although the CRISPR-Cas system is fairly recent from 2012, applications for biosensing today are still being proposed to look into recent diseases such as COVID-19 and its various strains.

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