CHAPTER 14

Point-of-care electrochemical biosensors using CRISPR/Cas for RNA analysis

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14.1 Introduction

Pathogenic infections are a major concern in the healthcare sector, accounting for roughly a quarter of human deaths globally and when these findings are normalized to developing countries, the proportion grows exponentially [1].

Pandemics, are epidemics having worldwide diaspora; causing an excessive number of sicknesses, deaths in the world cause disruption in the socioeconomic situation affected countries. Globalization, lifestyle changes, and improvements on the social and economic fronts have caused these emerging infections and accelerated occurrence and circulation of new microbial agents. Globalization has also facilitated sharing information and experiences on diseases transmission and treatment [2].

Over the past 40 years, there are recurrent large-scale epidemics from emerging RNA viruses and pandemics of influenza (Spanish flu, swine flu, bird flu), outbreaks of Ebola and Zika virus, deadly and wide-spread epidemics of MERS and SARS, and the ongoing pandemic of COVID-19, originating in China in 2019, have swept across continents and emerged as the most widespread infections reported during this century.

These biomolecular agents (nucleic acids) are spread by an animal-to-human transmission event, with either clinically apparent or occult spread into vulnerable human populations. Each time, a scarcity of rapid, accessible and accurate molecular diagnostic testing has hindered the general public health response to the emerging viral threat [3].

According to the World Health Organization (WHO), the ideal approach for detecting foreign pathogens should be rapid, specific, sensitive, instrument-free and cost-effective [4,5]. Setting up motions capable of rapid and sensitive

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diagnosis is the need of hour. Although current diagnostics are both specific and sensitive, they happen to be time consuming and expenive; thus, they are not available in resource-limited areas, for the purposes of large-scale screenings and in the case of outbreaks and epidemics (Fig. 14.1).

Focus has shifted in research towards the development of molecular POC devices adhering to the ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users), whose performances should be comparable to traditional molecular techniques [6].

The rapid and accurate detection of nucleic acids with high sensitivity plays a crucial role in different fields such as biotechnology, environmental monitoring, life science, and especially in the medical field. An early diagnosis of pathogens can be achieved by nucleic acid detection, particularly RNA, which is beneficial for better treatment of life-threatening diseases [7].

Small non-coding RNAs contain 18−25 nucleotides which are also termed microRNAs (miRNAs). The mature form of miRNAs bind with specific messenger RNAs which help in gene expression regulations in many biological processes [8,9]. The mRNA plays a key role between genome and

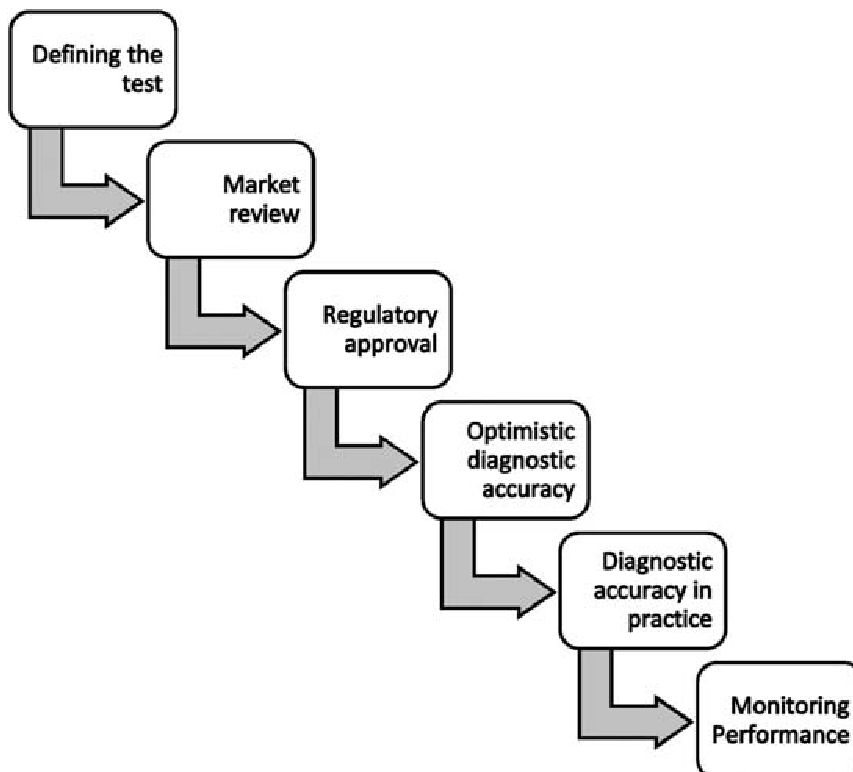

Figure 14.1 Ideal six steps that must be addressed before selecting a diagnostic test.

proteome processes. Sometimes miRNA can affect the translation and stability of mRNA by altering its structure and interaction with proteins [10]. Dysregulation of miRNAs can modify human body fluids such as saliva, plasma, serum, seminal, urine cerebrospinal fluid, and amniotic pleural effusion [11]. Some of the body fluids were enriched with specific circulating miRNAs. Different types of miRNAs are present in body fluids and their quantity varies according to the type of disease. In cancer diagnosis, the quality and quantity of circulating extracellular miRNAs are associated with stages of cancer [12]. Therefore such miRNAs are considered as potential diagnostic biomarkers. Different types of circulating miRNAs have been found in body fluids of cancer patients which indicates that these circulating miRNAs are linked with cancer initiation and progression [13]. Therefore, circulating miRNA have been widely used as noninvasive biomarkers for cancer diagnosis.

The ideal biomarker should be inexpensive, noninvasive, specific to the disease, and should be accurate in early detection [11]. The biomarker should give a response before clinical symptoms appear in the patient. For accurate on-site miRNA detection, highly sensitive electrochemical microfluidic biosensors are necessary. Lab-on-chip (LOC) devices are used for on-site detection, which contains multiple immobilization areas in a single channel. Nowadays, novel biosensors used for RNA detection include nucleic acid-based, aptamer-based, optical biosensors, electrochemical biosensors using silver/gold nanoparticles, and Surface Plasmon Resonance [14]. Health professionals have used different types of electrochemical biosensors for early diagnosis of diseases such as enzymatic electrochemical biosensors, bioaffinity-based electrochemical biosensors, and microfluidic LOC methods [15]. In most of the cases, the sample obtained for detection contains minute amounts of miRNAs so traditionally, different methods were used such as polymerase chain reaction (PCR), microarrays, and RNA sequencing methods. These techniques are tedious and also require expensive instruments with well-equipped laboratories and skilled personnel. An advantageous, affordable, and alternative technique for genomic diagnosis is CRISPR/Cas (clustered regularly interspaced short palindromic repeats and associated nuclease proteins) system [16]. Many of the miRNAs require hundreds of transcriptional processes, therefore, it is very difficult to diagnose, but CRISPR-based biosensors fulfill the demand for ultrasensitive nucleotide detection [17]. CRISPR/Cas system is considered a powerful tool for transcription regulation, genome editing, and molecular diagnostics [18,19].

CRISPR was first discovered in 1987 [20]. CRISPR is a natural process found in bacteria and archaea as an adaptive immune defense system protecting against viral invasion [16]. It is mainly composed of two components. First, are short DNA repeats called Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR), and second is CRISPR-associated (Cas) systems [21]. CRISPR repeaters and spacers are attached with CRISPR-associated (Cas) genes and previously, viral or bacterial plasmids could present in between the spacer segments. The CRISPR system gives protection to bacteria from repeated viral attacks. When a virus infects bacteria for the first time then the viral genome gets incorporated as a new spacer DNA amongst existing spacers. These spacers formed from the viral genome act as a genetic memory in the bacterial CRISPR genome. The CRISPR sequence is transcribed to form crRNA molecules which are associated with bacterial molecular machinery (Cas complex) [22,23]. Transactivating CRISPR RNA also called TracrRNA combines with crRNA to form guide RNA (gRNA). When a virus attacks bacteria again, then gRNA guides the bacterial Cas complex to invade the viral genome and when the match is found, then the Cas complex triggered the degradation of invading phage genome. Some CRISPR types require Protospacer Adjacent Motifs (PAM) sequences which are very short sequences present in target nucleic acids. These PAM sequences help guide RNA to find the target sequence and then Cas enzymes cut the target sequence. In this way, CRISPR protects bacteria from viral attacks. This CRISPR-Cas system plays a crucial role in a variety of technologies like genetics and biomedical research [24].

Electrochemical detection of RNAs is an inexpensive and quite easy method to fabricate biosensor chips [16]. Thus early, rapid, and ultrasensitive RNA-based diagnosis involves the use of electrochemical CRISPR biosensors.

14.2 CRISPR/Cas classification

The most recent classification of CRISPR/Cas is based on Cas effector enzymes. These effector Cas proteins provide immunity by cleaving foreign genetic material [25]. CRISPR-Cas is divided into two classes, class 1 and class 2, which are further divided into six types and several subtypes. The effector molecule of class 1 is characterized by a multi-subunit effector complex while the effector molecule of class 2 consists of one single multi-domain effector protein.

The class 1 system of CRISPR includes type I, type III, and type IV systems and is further subdivided into twelve subtypes. Most types belong to the class 1 CRISPR system and are found in various bacteria and archaea. The class 1 system is composed of 4−7 Cas protein subunits [26]. In the class 1 system, type I and type III are most common because they are responsible for various activities such as crRNA, site binding, target cleavage, and spacer regulation. Type I system uses Cas3 enzymes which recognize double-stranded DNA with the help of PAM sequences and introduce a single strand break. Cas3 enzymes are signature enzymes of the CRISPR class 1 system. Type III system is slightly different in its mechanism than type I. The type III system uses Cas10 to introduce single-stranded breaks in DNA and RNA which are specifically involved in the transcriptional process. The type IV system is new and functionally uncharacterized. Type IV system lacks genetic material of Cas nucleases, integrases, and other important enzymes which are commonly observed in other CRISPR systems, therefore it is difficult to predict the mechanism and function of a type IV system.

The class 2 system of CRISPR consists of a single multi-domain effector protein and it includes type II, type V and type VI systems. The class 2 system of CRISPR is identified in various bacteria but is not yet found in archaea. Type II CRISPR system uses Cas9 enzymes that require both crRNA and tracrRNA which combine to form gRNA. This gRNA helps Cas9 to target and produce a blunt end in target double-stranded DNA with the help of PAM sequences. The type V CRISPR system uses Cas12 enzymes which mainly depend on its subtypes. Cas12a requires PAM sequences to create staggered double-stranded breaks in DNA. The difference between Cas9 and Cas12a is that Cas9 recognizes the G-rich PAM sequence while Cas12 recognizes the T-rich PAM sequence [27]. Cas12b requires crRNA and tracrRNA for its activity. The type VI system of CRISPR uses C2c2 enzyme which is also termed as Cas13 enzymes. Cas13 is completely different from the rest of the CRISPR/Cas enzymes because it targets RNA. CRISPR/Cas13 recognizes a Protospacer Flanking Sequence (PFS) in target single-stranded RNA and then the crRNA-Cas13 complex introduces single-stranded breaks in target RNA sequences [26].

CRISPR/Cas13 targets viral RNA sequences as well as RNAs found in plants. Cas13 can detect foreign genomes without PAM sequences. Most viruses and pathogens contain RNA as genetic material. For cancer diagnosis, miRNAs play a key role. Therefore CRISPR/Cas13 can be used for the early diagnosis of cancer and different diseases [28].

14.3 Electrochemical biosensor

Sensors are device operations that convert physical inputs into a readable output. Chemical sensors are gaining a leading position among the presently commercially available ones with a wide array of clinical, industrial, environmental, and agricultural applications. Biosensors have gone viral as analytical and diagnostic tools for widespread use as they outperform any other device presently in use. Thanks to their operational simplicity, low cost, and no skills requirements, they have become the standard man's tools of everyday use. These advantages have won them increasingly wide applications in such varied fields as diabetic and cardiac self-monitoring, forensic investigations, drug discovery, agricultural and environmental detection systems, the food industry, and in biodefense. No doubt, further commercialization of biosensors relies on such improved features as enhanced selectivity, sensitivity, stability, reproducibility, and portability, all at lower costs [29]. Electrochemical biosensors, especially with reference to advancing point-of-care (POC) medical devices, includes low fabrication costs, minimal power requirements, basic designs, facile user interfaces, simple miniaturization, robust measurements, low detection limits and small operating volumes. However, they also reduce turbidity-based biases which are common in conventional optical systems [30] (Fig. 14.2).

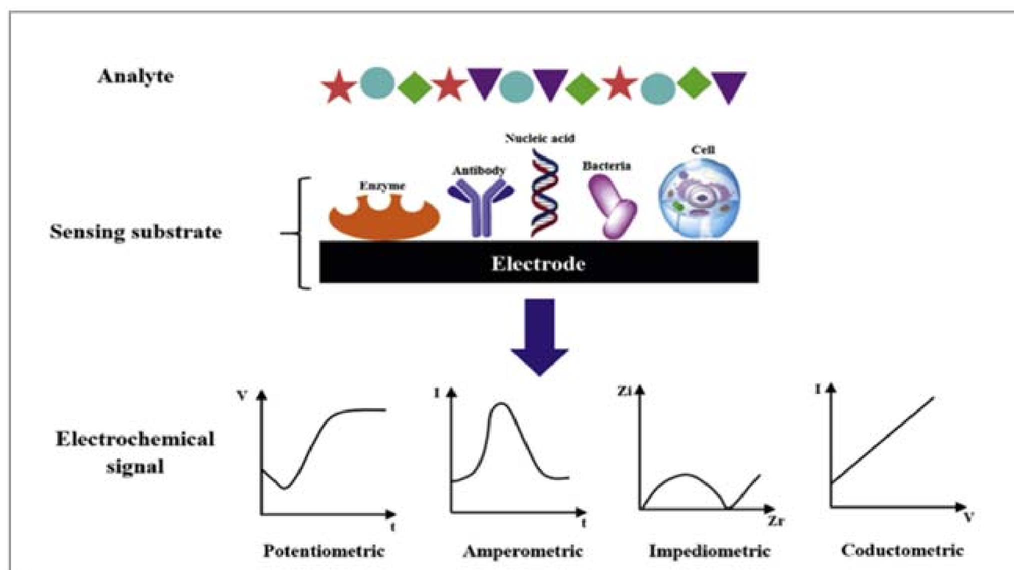

Figure 14.2 Schematic representation of an electrochemical biosensor [29].

14.4 CRISPR/Cas

CRISPR is an inherent bacterial defense scrutiny that assists in the identification of unknown genetic signatures, such as bacteriophages. Using CRISPR-associated proteins (Cas), the bacteria fragmentizes foreign nucleic acid and thus has rendered protection from the bioparticle.

CRISPR-associated protein is an emerging technology for gene editing and diagnostics development. CRISPR/Cas system has been differentiated into types and subclasses on the basis of its loci and signatures of protein. Although employed for genome altering operations, the CRISPR/Cas effectors have entered the biosensing forefront.

14.5 Crispr/Cas biosensing systems

It is an established fact that electrochemical biosensing of viruses is not an easy climb and is therefore considered ambitious. However, electrochemistry provides several well-known advances for biosensor design, such as innate high sensitivity, relatively low-cost sensors and equipment, user-friendly manipulation, fast analysis, and suitability for miniaturization, hence creating POC devices [1,31].

Nucleic acids are important targets for molecular diagnostic techniques, detecting infectious diseases, antimicrobial resistance genes, mutations associated with cancer or genetic diseases (e.g., Huntington, CF and Duchenne muscular dystrophy), single nucleotide polymorphism (SNPs) and biomarkers (miRNA) [32]. Several efficient and effective nucleic acid diagnostic kits have been formulated and authenticated using Cas9, Cas12, and Cas13 proteins [33].

CRISPR/Cas systems have displayed remarkable potential in developing novel biosensing applications for macromolecule detection due to the collateral cleavage activity of Cas effector proteins (Cas12, Cas13, etc.).

Owing to their sequence programmability, much research has focused on the use of CRISPR-Cas technologies for improved accuracy in the development of disease treatment and diagnostic platforms. Despite the potential risk of nonspecific RNA targeting effects, it is believed that the CRISPR-Cas13 system presents a better level of specificity over the available RNA-targeting approaches. Continued development of Cas variants with increased specificities and activities is critical for the application of these technologies to viral infection control [34]. Diagnostics that require processing of samples in a well-equipped centralized laboratory in resource-limited settings, where a

lack of infrastructure and skilled medical personnel preclude effective and fast diagnosis. Hence, attempts for engineering of reliable and rapid diagnostic tests that can be conducted outside the clinical laboratory is vital for effective disease treatment and management. Development of rapid initial screening tests that can be used at the POC will minimize the delay and expense of testing in centralized laboratories [1] (Fig. 14.3).

The latest finding of the collateral RNA cleavage property of the Cas13a effector has ignited an unparalleled interest in developing novel biosensing technologies for nucleic acid detection, promising significant advances in CRISPR diagnostics. Now, alongside the invention of Cas12 collateral cleavage activities on single-stranded DNA (ssDNA), several CRISPR/Cas systems have been established for detecting various targets, including bacteria, viruses, cancer mutations, etc. Based on the key Cas effectors, we offer an in-depth classification of CRISPR/Cas biosensing systems and propose their future utility. As the field continues to mature, CRISPR/Cas systems have the potential to become promising candidates for next-generation diagnostic biosensing platforms [35].

A study of the sort VI CRISPR/Cas system demonstrated the collateral cleavage enzymatic activity of Cas13a after its target-specific binding. This is evident in the study of Bruch et al. [18] to develop an electrochemical macromolecule (miRNA) biosensor. Collateral cleavage-based CRISPR/Cas technology has the potential to significantly affect the sector of biosensors by offering a more rapid and precise method for ultrasensitive macromolecule detection. Talk of creating CRISPR/Cas-based deployable POC paper devices are in the works. Crispr /Cas has gauged

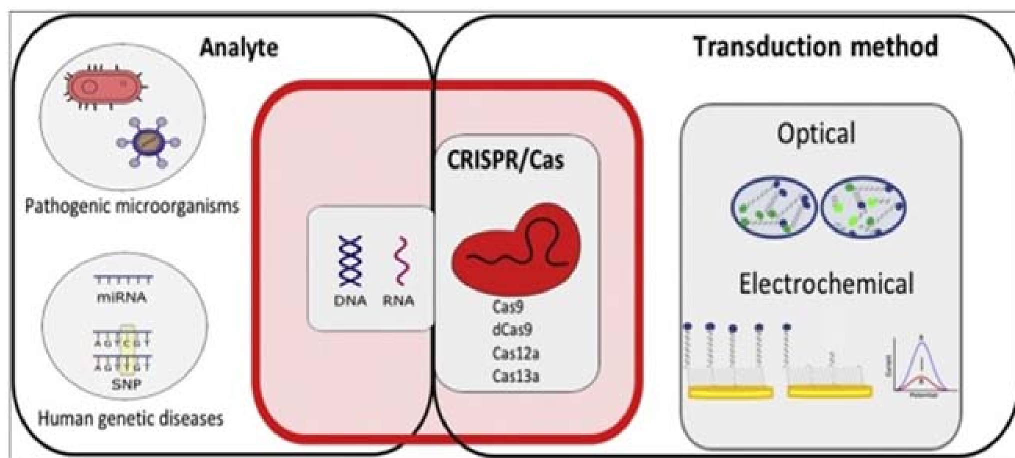

Figure 14.3 Electrochemical biosensor of CRISPR/Cas [32]. *CRISPR/Cas*, Clustered regularly interspaced short palindromic repeats and associated nuclease proteins.

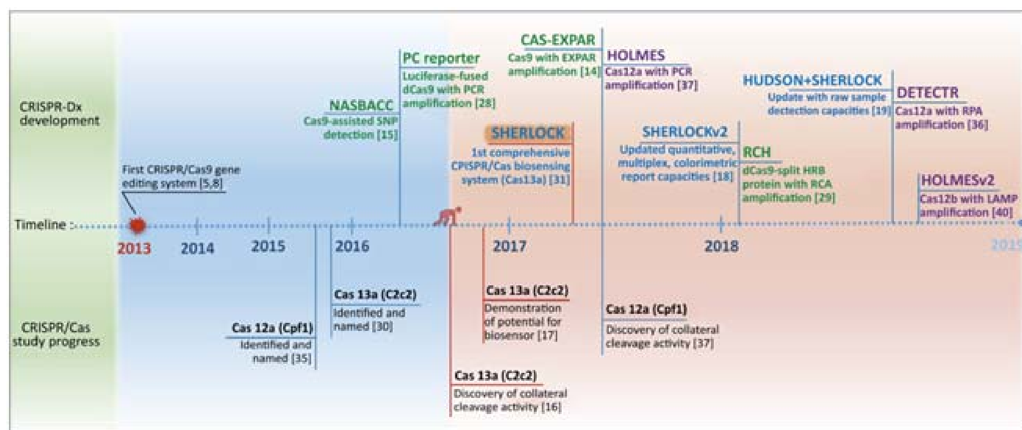

Figure 14.4 The development of CRISPR/Cas biosensing technology [35]. *CRISPR/Cas*, Clustered regularly interspaced short palindromic repeats and associated nuclease proteins.

the interest of many scientists from all walks of life implicated through its compressive publication in the recent years [31] (Fig. 14.4).

Despite significant progress in recent years, the prevailing CRISPR/Cas-based biosensing platforms have several limitations, including dependence on proper amplification methods, overpriced fluorescence detection equipment, or lateral flow biosensor (LFBMolecular methods for detecting nucleic acids based on CRISPR-Cas systems appear to be highly sensitive, specific, and capable of one-step detection of both RNA and DNA) [4]. CRISPR-Cas, a CRISPR-associated nuclease that can promiscuously cleaves RNAs, enables a rapid and inexpensive test for single-molecule detection and single-base discrimination of nucleic acids. This technique can also detect bacteria, microRNAs and cancer mutations, in a simple and easily scalable manner, by merely changing target-specific crRNA/sgRNA. The CRISPR−Cas12-based lateral flow assay technique is easy to implement and is an accurate and good replacement for real-time diagnosis by reverse transcription PCR (RT-PCR) [36,37]. More importantly, they have been successfully integrated in POC devices with performances like routine techniques [32]. The CRISPR-Cas system has recently shown promise in identifying nucleic acids during a POC testing format, which might remove the necessity for expensive laboratory equipment and would offer rapid and cost-effective detection with high sensitivity and specificity in various RNA and DNA samples. Fig. 14.5 summarizes various CRISPR-Cas diagnostic biosensing systems and their properties of such including SHERLOCK, SHERLOCKv2, HOLMES, HOLMESv2, DETECTR, CAS-EXPAR, NASBACC, STOPCovid, ctPCR, and AIOD-CRISPR, are developed [38,39].

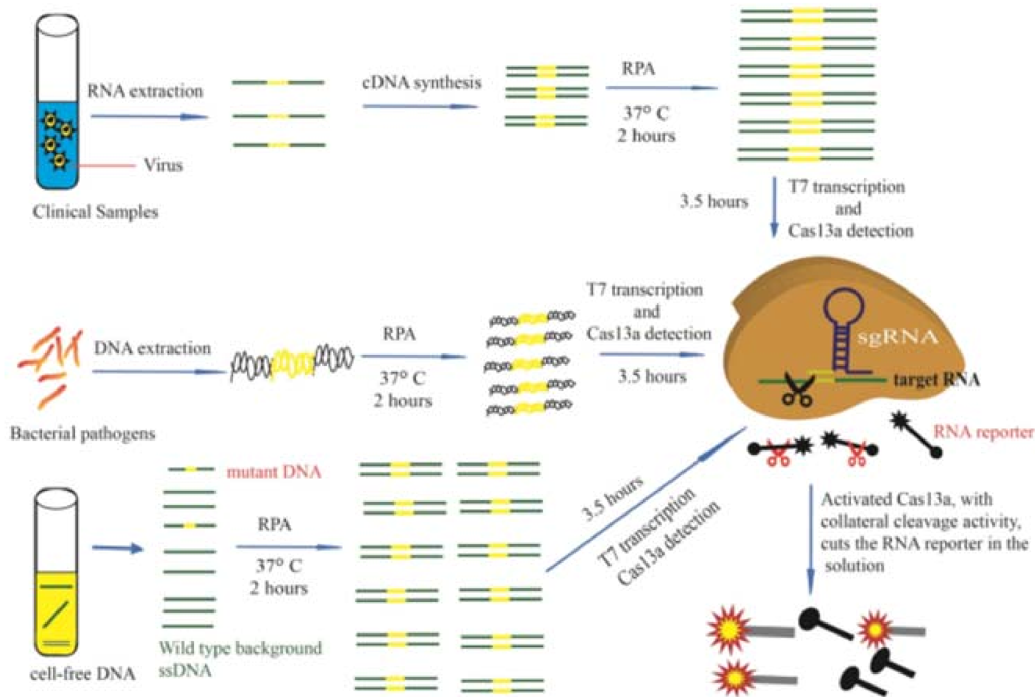

Figure 14.5 Schematic for SHERLOCK system. Target sequence detection involves the following steps: (1) isothermal amplification of target sequence with RPA or RT-RPA, (2) transcription of amplified DNA to RNA with T7 RNA polymerase, (3) activation of Cas13a by identifying and cutting target sequence, and (4) activated Cas13a, with collateral cleavage activity cuts the RNA reporter in the solution of nucleic acid biosensing systems developed using CRISPR/Cas [38].

14.6 Mechanism of RNA targeting by CRISPR/Cas13

CRISPR/Cas class II type VI system targets RNA elements with help of gRNA and is also termed as Cas13. Cas13 enzymes protect prokaryotes from RNA elements like RNA phages. The size of Cas13 family enzymes is between 900–1300 amino acids [40]. Cas13 family is furthered divided into 4 types such as Cas13a (C2c2), Cas13b (C2c6), Cas13c (C2c7), and Cas13d. CRISPR/Cas13 targets and cleaves particularly ssRNA.

Cas13 possesses RNA-guided RNase activity with the help of two Higher Eukaryotic and Prokaryotic Nucleotide-binding (HEPN) domains. Cas13 family contains HEPN domains that process pre-CRISPR RNA into mature crRNAs. The crRNA-Cas13 complex searches Protospacer by scanning the ssRNA. These Protospacer Flanking Sequences (PFS) help the Cas13 complex to find and cleave the target ssRNA. When the crRNA-Cas13 complex binds with ssRNA then the HEPN1 and HEPN2 domains of the Cas13 complex undergo conformational changes which bring both HEPN domains close to each other. This conformational change causes

activation of the HEPN nuclease site. HEPN domains combine to form an RNA-guided RNA cleavage complex. This Cas13 complex cleaves target RNA under cis-cleavage activity and nonspecific RNA under trans-cleavage activity also called collateral cleavage activity. Cas13 cleaves specifically at the Uridine (U) and Adenosine (A) rich regions. Sometimes Cas13 cleaves host RNAs. Collateral cleavage activity helps to develop specific and sensitive pathogen detection methods [28]. In this catalytic cleavage, RNA which are complementary to the target sequence is cleaved by Cas13 complex and also triggers the catalytic cleavage of nonspecific RNA molecules in trans-cleavage [41]. The cis-cleavage mechanism of target RNA is still unclear.

The activation of the Cas13 complex after binding with target RNA sequence causes a collateral cleavage mechanism which is a promising characteristic of Cas 13 enzymes. With the help of this mechanism, the Cas13 complex can be used for the diagnosis of miRNA. A cas13 complex is a tool used for the optical detection of viral RNAs. The CRISPR/Cas13 technology can be successfully used to create electrochemical biosensors which help in measuring miRNA levels in serum samples of patients.

14.7 Electrochemical biosensor for RNA detection

For rapid and early diagnosis of pathogenic diseases, different types of samples are used. Detection of pathogens based on genetic material such as DNA/RNA plays a crucial role in the diagnosis and accurate treatment of diseases. Scientists created different types of biosensors for the detection of nucleic acids which involves various detection techniques such as fluorescent detection, colorimetric detection, and electrochemical detection. Some electrochemical biosensors use the CRISPR/Cas13 mechanism for miRNA detection.

An electrochemical biosensor for miRNA-21 was developed by Cui et al. [12] combining CRISPR/Cas13 system and catalytic hairpin assembly (CHA) [12]. CHA reaction used two different DNA strands for the preparation of stable hairpin DNA structures. Target miRNA hybridizes with the spacer region of Cas13-crRNA complex. This hybridization activates trans-cleavage activity of CRISPR-Cas13 complex and cleaves hairpin DNA which produces amplified electrochemical signals with the help of a CHA reaction [12]. This electrochemical biosensor shows a good linear relationship of miRNAs with logarithmic concentrations from 10 fM to 1 nM with a detection limit of 2.6 fM. This ultrasensitive biosensor shows better results than previously reported analytical methods [28]. Cui et al. used this CRISPR-Cas13 and CHA cascade signal amplification

method to develop an ultrasensitive electrochemical biosensor for miRNA-21 detection [12].

Bruch et al. (2019) were the first scientists to successfully develop a microfluidic electrochemical biosensor for the detection of miRNA with the help of CRISPR-Cas13. This CRISPR-Cas 13 based microfluidic electrochemical biosensor chip can be used for on-site miRNAs detection. In this method, streptavidin (SA) molecules are applied on-chip to functionalize it and a vacuum is used to remove unbound molecules from the chip while Bovine Serum Albumin (BSA) is used to block remaining surface channels to prevent adsorption of unnecessary biomolecules. For CRISPR-Cas13, a complex mechanism and collateral cleavage of reporter RNA (reRNA), CRISPR-Cas 13 effector molecule with target-specific crRNA, 6-FAM-biotin labeled reRNA, and sample with target miRNA are mixed separately. Then, this mixture is applied on a microfluidic chip. If the sample does not contain target miRNAs then the CRISPR-Cas13 complex fails to cleave 6-FAM-biotin-labeled reRNA and bound to SA molecules on the surface channels. Then GOx (glucose oxidase)—conjugated anti-6-FAM-antibody binds with 6-FAM-biotin labeled reRNA which triggers an enzymatic reaction in which glucose is converted into hydrogen peroxide (H_2O_2). This H_2O_2 is amperometric and is detected with the help of an electrochemical cell. The amount of amperometric signals produced by the enzymatic reaction is directly proportional to the amount of GOx-conjugated anti-6-FAM-antibody bound to uncleaved 6-FAM-biotin-labeled reRNA and hence inversely proportional to the number of target miRNAs present in the sample. If the sample contains target miRNAs, then CRISPR-Cas13 complex triggers the cleavage of 6-FAM-biotin-labeled reRNA due to which 6-FAM-antibody will not bind and no enzymatic reaction will occur. This method helps in the detection of miRNAs levels in brain tumor patients.

These CRISPR/Cas13-based RNA detection technologies provide high sensitivity, are inexpensive, and require less time. CRISPR-Cas13-based electrochemical biosensors were successfully used for onsite miRNA detection which helps in the detection of early-stage cancers and other life-threatening pathogenic diseases.

14.8 Applications

CRISPR/Cas13-based RNA targeting tools show promising results in medical research, biotechnology, and therapeutic industries. These RNA targeting tools are especially used as diagnostic tools.

Electrochemical biosensors based on CRISPR/Cas13 systems are used to detect and target RNA viruses. Bruch et al. (2019) developed a CRISPR/Cas13a-based low-cost electrochemical microfluidic biosensor for the detection of tumor markers such as miR-19b and miR-20 from serum samples of children [42].

Other applications of the CRISPR/Cas13 system show promising results in diagnosis and as a defense mechanism Lu et al. [42] and Cox et al. [43] developed a tool with the help of Cas13b termed REPAIRv2 (RNA Editing for Programmable A to I Replacement version 2). This REPAIRv2 is useful to study G to A disease-relevant mutations and also used in the treatment. RESCUE is an RNA editing tool used for the conversion of cytidine to uridine. Live imaging of RNAs in living cells is beneficial to understand their function. For such live imaging, RNA-guided RNA targeting Cas13 RNases (dCas13) are used by Yang and co-workers (2019). Yang et al. [44] combined dCas13 and Ms2-MCP for dual-color imaging of RNAs in single cells. Wang et al. [45] developed a new tool termed Live-cell Fluorescent in situ hybridization (LiveFISH) by combining Cas9 and Cas13 systems for real-time live imaging of both DNA and RNA in living cells. Mahas et al. studied the CRISPR/Cas13 system to target RNA viruses in plants [46]. They constructed CRISPR/Cas13d (CasRx) which can target one RNA virus at a time or else can target two RNA viruses simultaneously and made this helpful to study plant virology easily. Freije et al. developed Cs13 assisted restriction of viral expression and readout (CARVER) [47]. CARVER is used to detect RNA viruses that can infect humans and cleave ssRNA with the help of collateral activity of Cas13 [47]. Abbott et al. [48] demonstrated a CRISPR/Cas13 based system that helps to degrade RNA from live influenza virus (IAV) and SARS-CoV-2 in epithelial cells of the human lung. This helps to decrease the viral load of IAV from the respiratory epithelial cells of human lungs. Tng et al. discovered *Prevotella* sp. *P5—125* CRISPR/Cas13b system used to target RNA viruses of chikungunya (CHIKV) in mosquitoes [49].

CRISPR/Cas13 system requires a lot of studies so that this mechanism can be used to target various life-threatening RNA viruses. New research and study of CRISPR/Cas13 will help for RNA manipulation, transcriptome engineering, RNA imaging, and nucleic acid detection.

14.9 Conclusion

CRISPR/Cas system is divided into different types and subtypes from which some are used as diagnostic tools. Collateral cleavage activity of

CRISPR/Cas13 specifically cleaves ssRNA transcripts. The electrochemical biosensor based on a combination of CRISPR/Cas13 with enzymatic reaction is used for miRNA detection in patients' body fluids. For such diagnoses, miRNAs serve as biomarkers in the clinical field. The new technologies based on CRISPR/Cas13 electrochemical biosensors can be used for early, on-site, accurate, and sensitive diagnosis to provide effective treatment to patients. Different types of CRISPR/ Cas13 electrochemical biosensors are successfully used for the detection of RNA viruses and tumors with the help of enzymatic reactions such as CHA, Glucose-oxidase (GOx)—H_2O_2 microfluidic system, and catalytic hairpin DNA circuit. Furthermore, the discoveries in CRISPR/Cas technology will transform clinical and biotechnological research. CRISPR is an emerging technology that will undoubtedly help to combat viral outbreaks and antibiotic-resistant pathogenic species in the near future.

In a nutshell, CRISPR/Cas systems provide biosensing ease with its diagnostic biodata. Scientists are hopeful that with time and more research on the CRISPR/Cas systems, many insights are promised and minor shortcomings can be resolved. These trying times of COVID-19 serve as a reminder to the healthcare community for the necessity in maturation of diagnostic setups.

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