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Review

Detecting pathogens with Zinc-Finger, TALE and CRISPR- based programmable nucleic acid binding proteins

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ABSTRACT

The recent availability of highly adaptable engineered nucleic acid binding proteins has brought about a revolution in the genome-editing field. Now, several studies have harnessed these Zinc-Finger-based, Transcription Activator-like Effector (TALE)-based, and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based programmable DNA and RNA-binding proteins to develop very specific diagnostic platforms to detect pathogen's nucleic acids, with sensitivities that go down to single molecule resolution. Particularly, the very recent descriptions of novel CRISPR associated enzymes with different target specificities and activities, including Cas13a (C2c2), Cas12a (Cpf1) and Csm6, have subsided the development of methods such as the DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) and the Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK). The reliabilities of these new diagnostic technologies have already been demonstrated for detection of nucleic acids from classical bacterial pathogens and also for emerging viral threats, including Zika virus. Besides, efficient genotyping capabilities have been reported for some technologies, that were shown, for instance, to be able to differentiate between human papillomavirus (HPV) types 16 and 18. Recent advancements that include improvements in multiplexing and quantitative capabilities, and instrument-free detection of results will potentially leverage the introduction of these new technologies to bacterial and viral detection at the point-of-care.

1. Introduction

In recent years, the emergence of "genome-editing" technologies has been accompanied by an increasing number of genetic tools for the efficient design and expression of programmable nucleic acid-binding proteins, based on Zinc-Finger (ZF) protein domains, Transcription Activator-like Effector (TALE) protein domains, and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-derived RNA-guided engineered proteins (such as dCas9, Cas13 and Cas12a). Several therapeutic and biotechnological applications for these engineered DNA-binging proteins have been proposed, including the fusion with nuclease domains for inducing DNA double-strand breaks in specific targets and with transcriptional activator domains for artificial gene expression control (Kim, 2016; Nakade et al., 2017; Murugan et al., 2017).

With the possibility of designing new nucleic acid-binding proteins for virtually any nucleic acid sequence of interest, there is also a great potential for using these engineered proteins in the molecular diagnostics field as novel biosensing tools for pathogen's DNA or RNA. In fact, there is already a growing number of publications demonstrating the reliabilities of such programmable proteins for detection of different bacterial and viral pathogens, including the emerging threat of Zika virus (Zuo et al., 2017). Herein, we review basic aspects about these new molecular diagnostic technologies and provide a technology outlook of future applications for detection of bacterial and viral pathogens at the point of care.

2. Zinc finger(ZF)-domain containing proteins (ZFPs)

Zinc Finger nucleases (ZFNs) were the first engineered proteins to

Abbreviations: ZFP, Zinc-Finger proteins; SEER, SEquence-Enabled Reassembly; TALE, Transcription Activator-like Effector; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; RPA, Recombinase-Polymerase Amplification; Cas, CRISPR-associated protein; DETECTR, DNA Endonuclease-Targeted CRISPR Trans Reporter; SHERLOCK, Specific High-Sensitivity Enzymatic Reporter UnLOCKing; HOLMES, a one-HOur Low-cost Multipurpose highly Efficient System; NASBA, Nucleic acid sequence-based amplification

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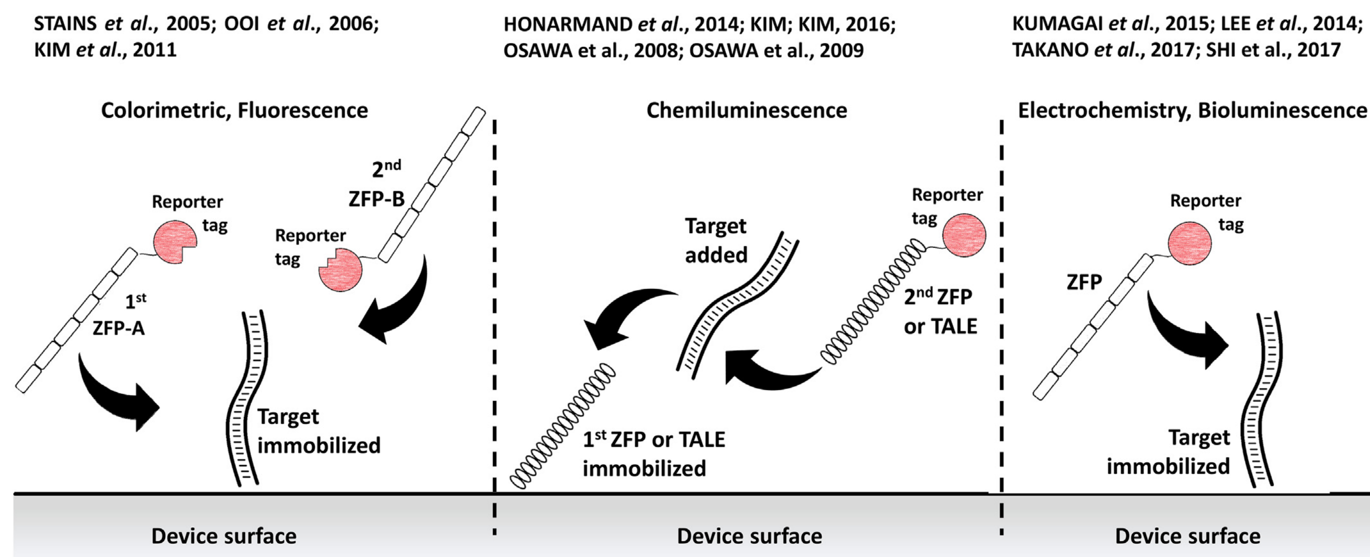

Fig. 1. Different assay designs for detecting pathogen's DNA using TALE-based and ZF-based DNA-binding proteins. Left panel – ZFP detection by SEER, in which the target DNA is immobilized on the surface of the plate followed by re-assembly of a dimerized reporter protein through binding of two ZFPs; Middle panel – detection assay with ZFPs or TALE-based proteins, in which the first protein is immobilized on the membrane, thus the DNA sample is added followed by the second ZFP/TALE that is bound to a reporter protein; Right panel – the biotinylated target DNA is first immobilized and the detection chimeric ZFP fused to a reporter protein is then added.

be used for gene editing technologies and, subsequently, ZF-domain proteins (ZFPs) were the first ones to be tested for *in vitro* detection of DNA (Perez-Pinera et al., 2012). The ZF DNA-binding domain consists of a ~ 30 amino-acids protein structural motif stabilized by $Zn2+$ ions that specifically recognizes a 3 base-pair stretch on a target DNA (Gaj et al., 2013). A pioneering work by Stains and collaborators (Stains et al., 2005) proposed the application of ZFPs for detection of DNA targets *in vitro* using the so-called SEER (SEquence-Enabled Reassembly) technology. Initially, the method was comprised of a pair of ZFPs (capture molecules) fused to one of the two halves of a split reporter protein (GFP or β-lactamase); upon binging to target DNA (Fig. 1), the reporter proteins would re-assemble then providing a colorimetric output (Ooi et al., 2006; Stains et al., 2005). The oligomerization-dependent protein reassembly system guarantees specificity of the method because signal generation only occurs when the heterodimerization of both moieties of reporter proteins is accomplished. Five years after this proof-of-concept study, the same research team established an improved and simpler method for rapid detection of *Escherichia coli* DNA with high sensitivity using ZFPs (Kim et al., 2011). The now called SEER-LAC system can be executed in 5 min and is capable of detecting as low as 50 fmol of target DNA. A visual result is then obtained when the revealing substrate nitrocefin changes from yellow to red due to hydrolysis of the β-lactam ring by re-assembled β-lactamase (Fig. 1; Table 1).

Osawa and co-workers searched the genomic sequences of *Legionella pneumophila*, *Salmonella* spp. and the Influenza A virus for conserved binding sites of the ZFP proteins Zif268 and Sp1 (Osawa et al., 2009; 2008). The authors fused the ZFPs to the GST tag and used the chimeric proteins to bind biotinylated- PCR products; an ELISA using anti-GST antibody was performed to reveal the results. Alternatively, fluorescein isothiocyanate (FITC)-labeled oligonucleotides were used to measure fluorescence depolarization upon ZFP binding to target DNA. More recently, Lee and colleagues reported on a method for detecting *E. coli* O157 DNA composed of a ZFP domain fused to the enzyme glucose dehydrogenase (GDH) (Fig. 1) (Lee et al., 2014). The use of ZFP-GDH could be exploited for using with electrochemical detection devices, for example, the personal glucometer which measures the activity of the same GDH (Lee et al., 2017). The system was sufficiently sensitive to detect 10 copies of genomic DNA, following target amplification by PCR (Lee et al., 2014). Additionally, the same team designed a ZFP fused to firefly luciferase (Fig. 1) to detect PCR amplicons of *Legionella pneumophila* and *E. coli O157* (Abe et al., 2012) with the same specificities reached by previous systems (Table 1). Both techniques were composed initially of immobilized biotinylated DNA amplicons which were subsequently hybridized with one chimeric ZFP-GDH/luciferase. Later, the same research group manufactured a pipette tip-based automatic system to detect *E. coli* O157 DNA with the same ZFP-luciferase used before (Takano et al., 2017) (Fig. 1).

In a different strategy, Shi and collaborators used a firefly luciferase-ZFP fusion to specifically detect the 16S rDNA of a set of pathogens (*Staphylococcus aureus*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), by including the ZFP-binding site on species-specific primer pairs (Shi et al., 2017). In addition, a multiplex assay using ZFPs was conceived by Kim and Kim (2016) for detecting DNA of *Staphylococcus aureus* and *E. coli* O157, based on an immobilized biotinylated ZFP (Kim and Kim, 2016) (Fig. 1). The chemiluminescent assay was able to distinguish between strains and detected as low as 50 fmol target DNA without the need for DNA amplification (Fig.1; Table 1).

3. Transcription activator-like effectors (TALEs)

TALE (Transcription Activator-Like Effectors)-domain containing proteins are modular proteins that can be engineered to target a specific DNA sequence, similarly to ZFPs. Nevertheless, each TALE monomer binds only one nucleotide whereas each ZFP domain binds three (Kim and Kim, 2014). DNA binding TALE domains can be likewise fused to different protein domains to confer novel functions such as target gene regulation and genome editing. The first use of TALE proteins to detect a pathogen's DNA was reported by Honarmand and colleagues, in 2014 (Honarmand et al., 2014). This assay consisted of a TALE-based sandwich detection similar to the ZFP-based assay described by Kim and Kim (Fig. 1). However, in this case the technique did not depend on heterodimerization of reporter proteins fused to the TALE-domain proteins. Basically, the first TALE-domain containing proteins is immobilized onto a nitrocellulose strip and the target DNA sample is added; then, a second TALE-domain protein fused to β-lactamase is used to reveal the detection. Until now, no other studies have exploited

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