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DNA Detection Technology Using Zinc Finger Protein

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Abstract

With recent advances in nanotechnology and sequencing technology, DNA diagnostic technology is becoming practical and is advancing at a fast pace. In these detection systems, mainly PCR (particularly real-time PCR) and DNA probe hybridization techniques are used. We suggest that detecting PCR products using double-stranded DNA (dsDNA) is more convenient and powerful compared with DNA probe hybridization technique. Zinc finger protein is the major DNA binding protein in nature and it recognizes dsDNA with sequence specific manner. Additionally, by changing its amino acids sequence, we can design it to recognize the desired DNA sequence to some extent. Using zinc finger protein for DNA detection element, simple, accurate and sensitive DNA detection can be achieved. In this review, dsDNA detection using zinc finger protein is described and compared with recent advanced technology.

Keywords: DNA sequences; Metastatic cancer; *Legionella pneumophila;* DNA methylation

Introduction

Detection of specific DNA sequences has become an important technology in health care, bioterrorism prevention, and genotyping. Some systems are commercially available, such as for diagnosis of single-nucleotide polymorphisms (SNPs) [1], metastatic cancer [2], or epigenetic modification [3]. These systems reveal present, congenital, or future disease problems. SNPs are a genotyping method that reveals differences in one individual's genome from another's and can also provide insights into the most effective drug regimen for a particular patient or reveal susceptibility to specific diseases. Given that DNA methylation [3] and histone modification [4,5] play important roles in gene regulation, epigenetics has become the focus of attention for many researchers and clinicians [6].

In clinical diagnosis, genetic information should be obtained not only by determining the presence, absence, or abundance of particular genetic information but also with simplicity, rapidity, and accuracy [7]. Many DNA detection methods have been described, and some are already commercially available. There are few methods that do not require initial treatments, such as PCR or extraction and purification from biological samples using immune chromatography, often used in clinics [8]. These methods are simple and rapid and may be useful in several fields but often give false-positive or false-negative results because of the presence of unrelated DNA or inhibitors or quenchers [9]. Because false results lead to wrong treatment of patients, these methods may cause unintended health hazards or critical illnesses [10]. Given that in the near future, DNA diagnostic systems will be routinely applied to many patients, their diagnostic accuracy will be critically important.

With recent advances in technology, the efficiency and rapidity of DNA amplification methods, such as PCR, are increasing [11]. Chrissy [12] has reported a diagnostic assay for ocular *Chlamydia trachomatis* infections using digital PCR assay. Microfluidic droplet generator chips are used to partition PCR 1-nl volume micelles that are stable at high temperatures. The droplet PCR is performed in a normal thermal cycler within a few minutes. This technology enables us to perform simple and rapid testing for target DNA amplification.

PCR products must be detected by rapid, simple, and highly sensitive detection methods and with its signal transducer. Electrophoresis is the DNA detection method most routinely used by researchers and is now becoming a more useful technology [13]. Despite its convenience, it detects only the length of DNA and may give false-positive test result. Most currently used detection methods involve DNA probe hybridization, which uses the sequence-specific recognition of single-stranded DNA (ssDNA) [14]. It requires only denaturation of PCR products and annealing with complementary ssDNA to make rapid detection possible. With respect to its sequence specificity, it detects target PCR products accurately. In the last 10 years, several technologies have been proposed and have become commercially available. Nakamura et al. developed a multi-sample detection system employing a tag insertion primer and an electrochemical DNA chip [15]. It is mixed and injected into a cassette that contains the DNA chip with immobilized probes and automatically performs hybridization, washing, and signaling. Sawata has reported a DNA probe hybridization technology using PNA probe technology [16]. PNA is an artificial oligoamide that is capable of forming highly stable complexes with oligonucleotides complementary to DNA sequences because of its neutral electric charge. Real-time PCR is also a useful technology for detecting specific DNA [17]. It detects target DNA by amplification. A fluorescent dye and a quencher-modified DNA probe (e.g., Taqman®probe) hybridize to the template DNA during PCR, and DNA polymerase dissociates the DNA probe. The fluorescent dye and the quencher draw apart from each other, generating a fluorescent signal. The fluorescent signal increases relative to DNA amplification, indicating the rapidity and reliability of real-time PCR. Besides this technology, in recent years, a novel technology that uses a sequence-specific direct double-stranded DNA (dsDNA) recognition element has been developed. It does not need denaturation and hybridization, increasing its speed. In the process of DNA probe hybridization, most DNA re-hybridizes to the original ssDNA, reducing signal intensity.

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Because sequence-specific dsDNA detection detects PCR products directly, it permits the rapid and sensitive detection of target PCR products. Osawa et al. have reported a PCR product detection method from *Legionella pneumophila* using zinc finger protein (ZFP), which is the most common DNA-binding motif [18]. ZFP recognizes DNA in a sequence-specific manner with high affinity (*Kd*: nM – pM). Moreover, its binding mode is simple, and it is relatively easy to construct an artificial ZFP that binds to a specific DNA sequence [19,20].

Several ways to detect specific DNA sequences have been described above (Figure 1). In this review, we focus on useful DNA diagnostic technology including recent advanced techniques such as a direct dsDNA detection method using sequence-specific DNA recognition elements. Along with these techniques, we will discuss their application and the possibility of their use in DNA diagnostic technology in the future.

dsDNA recognition technology: Sequence-specific DNA-binding protein

There are many DNA-binding motifs in nature, including leucine zipper and helix-turn-helix [21]. Jarzy described a simple method for isolating specific DNA sequences directly from genomic DNA [22]. They used a fusion protein, the sequence-specific binding protein DnaA fused with glutathione *S*-transferase (GST). DnaA recognizes the *oriC* coding region, the key region for the initiation of chromosomal DNA replication. Takeuchi et al. have reported the detection of PCR products from the genus Salmonella using DnaA IV, the DNA recognition domain of DnaA protein [23].

Given that naturally occurring DNA-binding proteins recognize specific sequences or repeat sequences like those of *Eco*RI, it is difficult to distinguish many DNA sequences. Ideally, for DNA diagnosis as described above, any DNA sequence should be distinguishable by its high affinity and specificity to a DNA-binding protein. One candidate is the zinc finger motif, the largest functional group encoded in the eukaryotic genome. TheCys2–His2 type of zinc finger motif is among the most common types of DNA-binding motif, and its individual zinc finger consists of approximately 30 amino acids in a conserved bba configuration. Several amino acids on the surface of the helix typically contact 3–4 bp in the major groove of DNA, with varying levels of selectivity. The modular structure of ZFPs has made them an attractive framework for the design of custom DNA-binding proteins [20,24,25]. Key to the application of ZFPs for specific DNA recognition is the development of unnatural arrays that contain three to six tandem zinc finger domains that recognize 9–19 bp [26]. Cliff has described a DNA detection method using ZFP, fusing a half fragment of asignal-producing protein in a process named sequence-enabled reassembly (SEER) detection [27,28]. In recognizing a specific DNA sequence, half of the fragments of a protein reassemble while emitting a corresponding signal. GFP and -lactamase has reported fusing one half of the signal protein with a natural zinc finger (zif268) and other half of the signal protein with an artificial reported protein (PBSII). Given its enzymatic activity and low background in colorimetric assays, the b-lactamase system could detect 20 nM of purified target DNA within 5 min. Applying this system to a DNA microarray with immobilized artificial ZFP on poly (ethylene glycol) hydrogel permitted the detection of several DNA samples at the same time [29]. Although SEER is rapid and simple, its sensitivity is not sufficient for DNA detection. The enzymatic activity of a separated protein is decreased at least 10 times compared with that of a full-length enzyme. Kazutoshi et al. have described a method for detection of dsDNA by FRET using GFP and CFP with ZFP [30]. They fused a dimerization peptide to its N terminus to enhance the dimerization of two distinct ZFPs, achieving a sensitivity of 10–30 nM that considerably exceeded that of SEER-GFP or SEER-LAC.

In the application of DNA detection technology with zinc finger, rapidity and simplicity are important. We attempted to develop a dsDNA detection method using ZFP and not relying on protein reassembly or fluorescence detection.

A PCR-product detection system for pathogenic bacteria using ZFP

L. pneumophila is a major pathogenic bacterium that is difficult to detect rapidly using the culture method, because the bacteria grow slowly. We have developed a rapid and specific detection method using

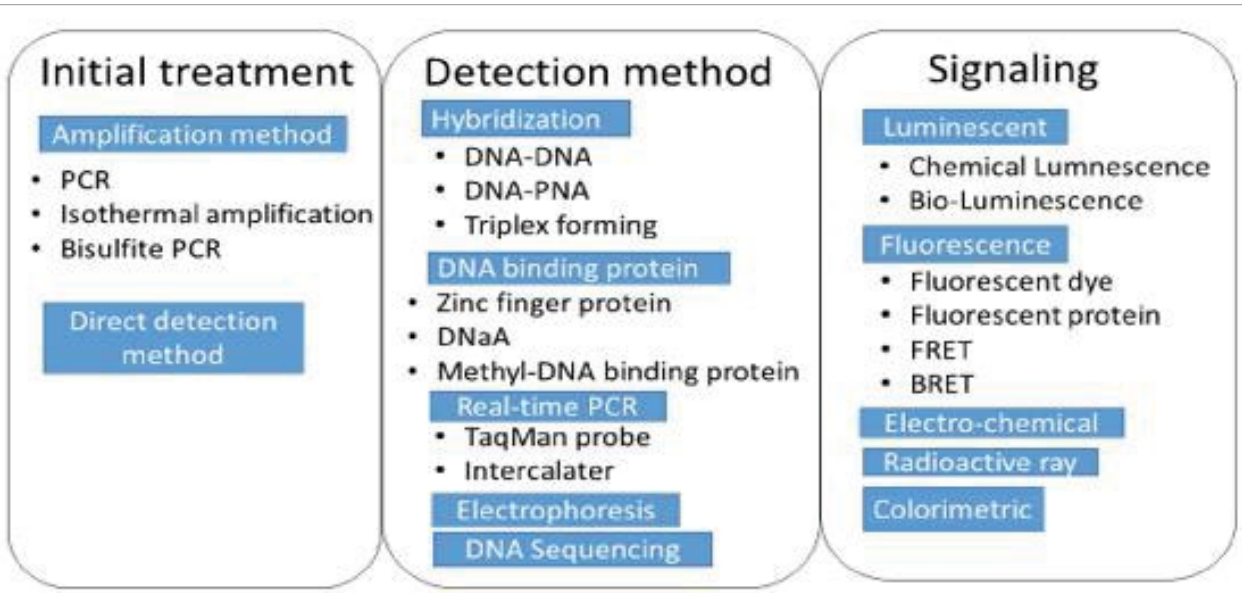


Figure 1: Classification for the strategy for detecting DNA.

PCR products amplified from a *L. pneumophila* genome DNA using a ZFP (Figure 2) [18]. The PCR target region was a 49-bp sequence of a *L. pneumophila* specific region containing a 9-bp Sp1-binding site and two 20-bp primer regions at both ends and is located in the *flh*A gene in the *L. pneumophila* genome. The PCR product was detected by ELISA using horseradish peroxidase-conjugated anti-GST antibody. Only 100 copies of *L. pneumophila* genome were detectable by this detection system. Moreover, using fluorescence depolarization measurement, we were able to detect the PCR products from the *L. pneumophila* genome within only 1 min. Applying this technology to other pathogenic bacteria, *Salmonella* spp. and the influenza A virus using Zif268 and Sp1, respectively, which is the most researched protein in ZFP [31].

To improve the rapidness and sensitivity of the assay, we constructed a fusion protein of a signal-producing enzyme and ZFP [32]. Of many types of signaling enzyme, we focused:* on firefly luciferase. Luciferase produces a luminescence signal by catalyzing the oxidation of luciferin in the presence of ATP, Mg^{2+}, and molecular oxygen. The excited state of oxyluciferin then returns to the ground state, accompanied by the emission of light with a high quantum yield (0.88 ± 025). Thus, a highly luminescent signal is observed. Using its substrate specificity against luciferin, it is possible to construct an assay with low background noise [33-35]. Compared with a colorimetric detection or a fluorescent detection assay, a luminescent molecule emits a light signal by its original reaction, leading to a low background signal. These features of luciferase, high signal and low noise (leading to a high S/N ratio), are key characters of biosensors. The use of a fusion protein of luciferase and ZFP (LUC-ZF) permitted the detection of 100 fmol of synthetic dsDNA. In comparison with the ELISA described above, the detection limit was improved more than 100 times. We found that 10 copies of the *L. pneumophila* genome was detectable using PCR and LUC-ZF, but theoretically 35 cycles of PCR from 1 copy of the genome generate approximately 100 fmol (235/6×1023) of dsDNA, so that one copy of the bacterial genome may be detectable. Electrochemical detection is also a useful detection method, owing to its high sensitivity and amenability to miniaturization, for example, in glucose sensing [36]. We focused on glucose dehydrogenase (GDH) in place of luciferase as a signal-producing enzyme [37]. For combining an electrochemical detection system with PCR, it is rather easy to construct an on-chip total analysis system to detect pathogenic bacteria. Ten copies of *Escherichia coli* genome were detectable in this system, the same result as achieved by LUC-ZF.

Detection of DNA and histone modification using ZFP

Methylation of DNA at cytosine bases is an important mechanism widely used to regulate gene expression and transposable elements in eukaryotic organisms [38]. Aberrant methylation is detected in the promoter regions of tumor suppressor genes in tumors, and this aberrant methylation pattern is dependent on the type of tumor [39]. Thus, aberrant DNA methylation is regarded as a biomarker in the early diagnosis of cancer. The sodium-bisulfite sequencing assay has been widely used in DNA methylation analysis but requires several steps to produce results and is accordingly time consuming. We have developed a rapid and sensitive detection method for methylated DNA using a fusion protein of a methyl CpG-binding domain (MBD) with ZFP and LUC-ZF, w named methylated DNA precipitation combined luciferase-fused

ZFP assay (Figure 3) [40]. In the first step, methylated DNA is captured by MBD from a sonicated human genome and immobilized on glutathione-coated magnetic beads via GST. Next, the eluted DNAs from beds and the target region, which contains a zinc finger recognition site, are amplified by PCR using biotinylated primers. Finally, PCR products are immobilized on streptavidin-coated magnetic beads via biotin and detected by LUC-ZF.

Similarly to DNA methylation, histone modifications in specific target genes are regarded as biomarkers [41]. DNA methylation is used for long-term gene silencing, whereas an immediate gene expression state is determined by histone modifications. Detection of histone modifications in specific target regions is important not only for diagnosis but also for evaluation of drug treatment. We have reported a detection system for histone modifications at target genomic regions by chromatin immune-precipitation combinedLUC-ZF-based bioluminescence resonance energy transfer (ChIP-ZF-BRET) assay (Figure 4) [42,43]. At the first step, ChIP was performed to collect the modified histone. Next, PCR was performed against target genomic region, which includes a zinc finger recognition site. At last, homogeneous detection of the PCR product by BRET was performed by LUC-ZF and fluorescent DNA intercalating dye that is excited at luciferase luminescence. To develop a high-sensitivity detection system, we performed a solid-phase detection assay with a bound/free separation procedure using LUC-ZF. In the histone modification detection assay, we demonstrated the BRET assay, which is performed homogeneously. Compared with a solid-phase detection assay, a homogeneous detection assay is more rapidly and conveniently

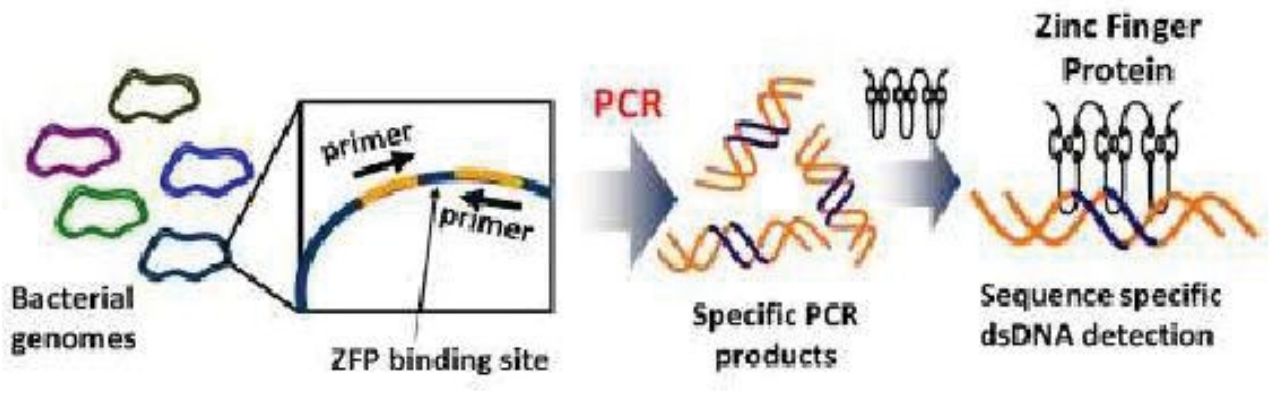
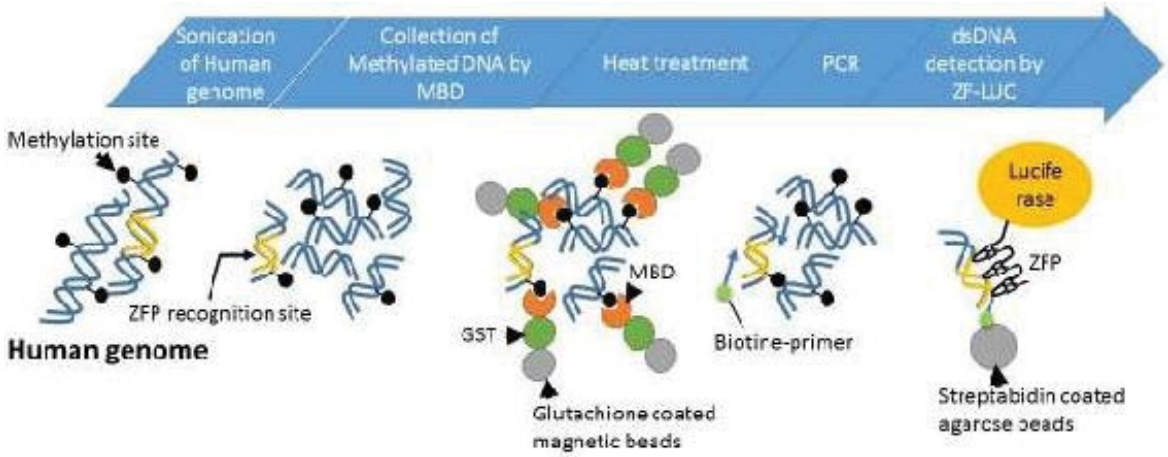
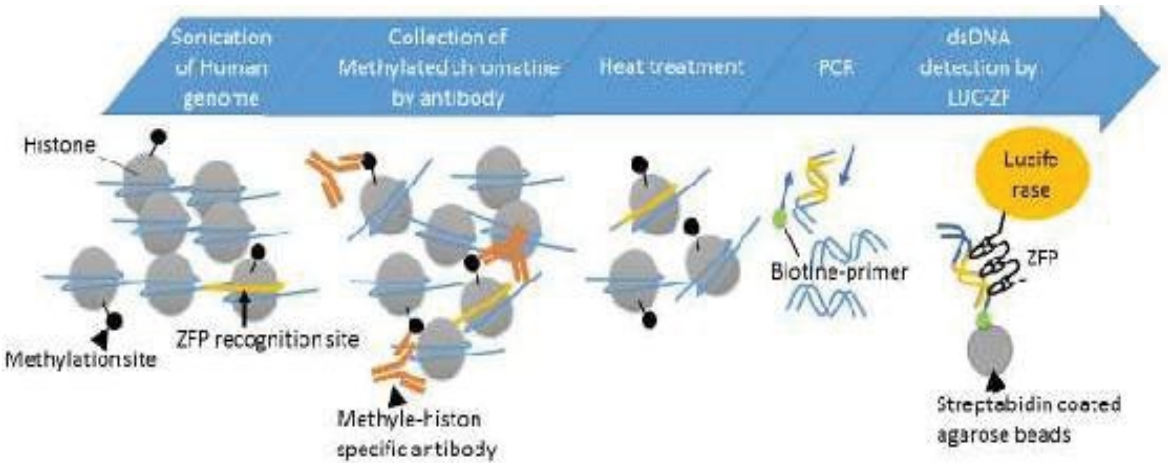


Figure 2: Bacterial genomic DNA detection method using zinc finger protein. A specific region of bacterial genomic DNA that contains the zinc finger protein recognition region was amplified by PCR. The PCR product was detected by zinc finger protein.



Sonicated digested methylated DNA was collected by Methyl binding domain.
Collected methylated DNA was amplified by PCR and detected by ZF-LUC.

Figure 3: Detection scheme of MELZA.



Chromatin immunoprecipitation was performed using target histone modification antibodies. The target region containing a zinc finger recognition site was amplified by PCR. The PCR product was detected by BRET using ZF−luciferase and a fluorescent DNA intercalating dye that is excited by luciferase luminescence.

Figure 4: ChIP-ZF-BRET assay.

performed. A mixture of LUC-ZF and the fluorescent molecule BOBO-3 was added to the PCR product from chromatin DNA that contains a zinc finger recognition site, and 550 and 602 nm emission spectra were measured after the addition of the luciferase substrate. Emission at 602 nm was not increased in the presence of non-target dsDNA.

Comparison with real-time PCR technology

Real-time PCR produces a signal in proportion to the amplification of specific DNA. Use of TaqMan® probe DNA is a powerful tool for analyzing sequences of PCR products. However, real-time PCR systems using the TaqMan® probe can be affected by sample impurities, and the inhibitory activity of impurities against DNA polymerase depends on the type of DNA polymerase [44-48]. This observation suggests that suitable DNA polymerases may not always be used in real-time PCR because the system requires DNA polymerases that exhibit exonuclease activity. Thus, a sequence-specific post-PCR detection system is required for detection of pathogens in real samples.

Another aspect of real-time PCR is that it depends on detecting the amplification of DNA using a fluorescent signal. Although fluorescence detection is a useful method, it requires a special device for signal detection and is thus not preferred to use as an on-site detection tool. Shunsuke et al. have developed a rapid and sensitive method for detection of bacteria using a microchip with real-time PCR [48]. To develop a portable detection system, they detected PCR products by endpoint detection of fluorescent signal. By contrast, the standard PCR method is not limited to fluorescence detection but can detect by luminescence or electrochemically, as described above. In particular, electrochemical detection methods are suitable for compact PCR detection systems [38].

A new candidate for a dsDNA-binding molecule

Transcription activator-like effectors (TALEs) are transcriptional factors encoded by pathogenic bacteria of the genus Xanthomonas to alter transcription of genes in host plant cells. TALEs are injected into host plant cells via the type III secretion system and bind to genomic

DNA to alter transcription in these cells, thereby facilitating pathogenic bacterial colonization [49,50]. DNA binding by TALE is mediated by highly conserved 33- to 35-amino acid repeat arrays. Each TALE repeat in an array specifies a single base of DNA determined by amino acids at positions 12 and 13 of a domain called repeat variable di-residues (RVDs). Crystal structures of TALE DNA-binding domains bound to their cognate sites reveal that individual repeats comprise a two-helix V-shaped structure and that the amino acids at positions 12 and 13 are positioned in the DNA major groove. By engineering those residues, a simple and useful TALE recognition code was constructed [51]. The authors provided experimental evidence for the TALE repeat code by constructing the first examples of engineered TALE repeat arrays with novel specificities [52]. The TALE repeat code was also confirmed by another group via computational analysis of the binding specificities of the TALE [53]. Subsequent reports provided additional evidence that engineered TALE repeats with desired specificities can be created using the code. At present, nearly all engineered TALE repeat arrays use four domains with the residues NN, NI, HD, and NG, for the recognition of G, A, C, and T, respectively. There are several interactions involved in recognition of DNA by TALE, including basic amino acid direct hydrogen bonds, weaker van der Waals interaction, and hydrophobic interactions such as by steric exclusion of interactions with alternative nucleotides. Interestingly, using HD and NG in RVD, unmethylated and methylated cytosines may be discriminated, because NG recognizes a methylated cytosine, whereas HD does not. Moreover, a well-conserved thymine is present at the 0 position of most natural TALE target sites [52,54]. T0 recognition probably serves as a binding anchor from which the protein wraps around a DNA helix and probes a nucleotide sequence [55,56]. Too many strong RVDs in the N-terminal part of the TALE DNA interaction domain may recognize a non-target sequence. Thus, owing to its simple recognition of DNA, an artificial TALE is rather easy to construct and may have particular specificity to the target site, but its recognition is asymmetric across the protein–DNA interface, and it binds preferentially to DNA. This observation suggests that an engineered TALE does not always recognize the target DNA with high affinity and specificity.

Several applications using TALE for DNA-binding domains have been reported in the past 5 years. Properties of the TALE DBD offer great potential for research, biotechnology, and gene therapy, such as fusing activation, silence domain [57], or nuclease, which is often called TALEN [58]. TALEN is a fusion protein consisting of a TALE DNA-binding domain with a *Fok*I nuclease domain that is often used in zinc finger nuclease (ZFN) technology [59]. ZFN technology has yielded substantial achievements in a variety of model organisms and cell types [51] that were previously inaccessible by classical gene targeting methods. The technical knowhow that was established in ZFN technology was subsequently adapted to TALEN. TALEN has been successfully used for targeted genome editing in yeasts, plants, and eukaryotes [60-63].

Engineering method for ZFP

Almost 20 years have passed since an artificial ZFP was reported. To date, many artificial ZFP selection methods have been developed, but at present three construction technologies are mainly used: modular assembly [64], selection-based methods in bacteria [65,66], and a proprietary technology owned by Sangamo BioSciences [67].

The modular assembly generates candidate ZFPs for target sequence against 3 bp by phage display [68] or derived from natural ZFPs [69] and links them into finger modules targeted to a specific sequence. Modular assembled ZFNs are functional in several organisms [70,71] and human cell lines [72] with relatively low toxicity. Against this simplicity, many studies have shown that the success rate for isolating ZFP with high specificity is low. The reason may be the recognition complexity of the zinc finger–DNA interaction. However, Ramirez et al reported that modular assembly had unexpectedly high failure rates, with only 6% of zinc finger module pairs predicted to produce an active ZFN. Zinc finger recognizes target 3 base by -1, 2, 3, 6 positions in a-helix making hydrogen bond contact but each individual finger interact with each other and constructing the structure stability. For example, an aspartate in position 2 of a-helix recognizes mostly C and sometimes A in the opposite strand of the neighboring DNA triplet [73], thus requiring a 5' -GNN or 5' -TNN as an adjacent triplet in the target site [74].

Context-dependent selection of zinc finger arrays has been established *in vitro* [75] and also in bacteria [65,76]. Among them, the most successful selection system is named OPEN and is based on the bacterial two-hybrid assay. The method involves two distinct steps. First, low restricted parallel selections are performed for binding of randomized fingers to each triple finger in the targeted sequence. Next, fingers from these pools are linked by a combinatorial method and the products are selected at high restricted environment for binding to the final target. ZFPs derived from the OPEN system show high activity with low toxicity and have been applied to a few model organisms [65] and human cell lines. Success rates of 25% have been achieved with ZFN [77,78]. However, OPEN is complicated and laborious, with 5–8 weeks needed for identifying a ZFP with high *Kd* and specificity to specified DNA. Also, the library is limited to arrays that recognize all 16 GNN (e.g., GAG, GCT) and a few TNN triplets. An advanced method reported by Sander et al. is Context-Dependent Assembly (CoDA), which is the rapid assembly of parts of OPEN arrays. CoDA ZFNs were shown to successfully cleave 50% of their chromosomal targets. However, the range of targetable sequences was a subset of OPEN and insufficient to target.

Sangamo BioSciences has developed a proprietary platform based on two specialized fingers of three finger domains with the 9-aa linker –LRQKDGERP– [67]. Applying this approach, they have reported 103-fold greater affinity than three fingers of a two-finger domain. Researchers may purchase customized ZFNs made by the Sangamo approach through the Sigma-Aldrich CompoZr service, but the cost of these proteins limits the scale and scope of projects that can be performed.

Underlying such a study of ZFP, several prediction algorithms for contact between zinc finger and DNA have been developed. These design tools enable us to save labor and time in selecting specific ZFPs. SVM Model [79]; ZIFIBI [80], ZiFiT [81], ZiF-BASE [82], ZiF-Pridict [83], zinc finger tools [84], ZiFDB [85], enoLOGOS [86], and predicting DNA recognition [87] have been developed and are available on Web sites [88].

Comparison with ZFP and TALE

Owing to its simple recognition mode, TALE is easy to design against new DNA targets [89]. In contrast, it is rather difficult to construct a designated ZFP that recognizes a specific sequence. Indeed, in application to the therapeutic field-like ZFN, it prefers high sequence specificity and selectability of DNA sequence because of off-target effects derived from nonspecific binding or frame shifts. However, for use of these proteins in particular fields, ZFP may function more effectively than TALE. The main reason is the large MW of TALE. The single finger of the zinc finger recognizes three base pairs, in contrast

to one base pair for each TALE module, meaning that ZFP is more compact than TALE. Focusing on this property, there are three main advantages. First, for constructing a fusion protein with functional protein (e.g., a nuclease, transcriptionally activated protein or labeled enzyme (luciferase, GDH)), several functional protein properties decrease. We have compared firefly luciferase enzymatic activity by fusing only one unit of ZFP with that using two units of zinc finger and GST. Enzyme activity decreased 1/5–1/10 in the first fusion protein and1/10–1/100 in the second fusion protein (data not shown). Luciferase consists of two domains, the active center lies between two domains, and when it reacts with luciferine and ATP, two subunits interact with each other and promote the oxy-luciferil reaction. Thus, the mobilities of both proteins are important for reaction, so that fusing a large protein or several units to the luciferase decreases its activity. Second, the expression and purification efficiency of luciferase decrease in proportion to its MW. Third, background signal derived from nonspecific interaction increases with MW in the DNA detection step, because

sometimes the number of amino acids participates in nonspecific interaction with base material. At the present stage, zinc finger prefers to bind GNN triplets, and the GNNGNNGNN recognition site has an occurrence probability of 1.56%. It is difficult to insert specific DNA at specific sites in genome manipulation but in pathogenic bacterial detection method is not so restricted by narrow recognition field. Using bioinformatics tools like BLAST, it is almost possible to confirm whether a target bacterium is detectable. Additionally, recently designed ZFPs are becoming easier to construct with high DNA sequence specificity. Cong et al. has described the selection of two-finger modules recognizing GRNNYG and selecting the non-GNN recognition finger [90]. An estimation strategy of artificial zinc finger that contains a non-GNN triplet has been developed [91-93]. Sangamo Biosciences and Sigma-Aldrichhave started a construction service of customized ZFPs made by the Sangamo approach through the CompoZr service, but the cost approaches $25,000 and limits the scale and scope of projects that can be performed [94]. The zinc finger consortium has offered several ZFPs constructed by Joung and Voytas using the OPEN system or CoDA. These zincfinger-coded plasmids are available in Addgene. Also, ZiFiT, the zinc finger consortium, offers online software as an effort to provide a simple and easy tool for ZFP design [80]. It is a popular Web tool that provides an integrated modular design approach by incorporating three different datasets enumerating zinc finger-binding patterns for independent modules developed by Barbas, Sangamo, and ToolGen [95-99]. The user may enter a query DNA sequence and choose one or more of these sets. Scores are given with predictions of their chances of success, as measured by a bacterial two-hybrid assay. Indeed, the range of recognition sequences and the design strategy of zinc finger are expanding.

Conclusion

With recent advances in DNA diagnostic systems, anyone can read individual genomes or SNPs to understand his or her health status. In parallel, simple, rapid, and accurate DNA detection systems are also becoming more important. DNA–DNA hybridization including real-time PCR is the standard method. DsDNA detection using a DNA-binding protein does not need steps such as denaturation of dsDNA and recognizes in a sequence-specific manner, so that it is a simple, rapid, and accurate detection method. Engineered ZFPs have been studied for almost 20 years and are still expanding in utility. A nucleotide recognition module named TALE is becoming a bioengineering tool at an explosive pace and is gradually taking the place of ZFP. In gene manipulation applications, TALE may be more useful, but its large MW may limit its advancement.

As an alternative to TALE, ZFP is one of the most compact DNA recognition motifs in nature. Advances in technology will determine the better approach for dsDNA detection.

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