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REVIEW ARTICLE

Advances in the study of protein–DNA interaction

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Abstract Protein–DNA interaction plays an important role in many biological processes. The classical methods and the novel technologies advanced have been developed for the interaction of protein–DNA. Recent developments of these methods and research achievements have been reviewed in this paper.

Keywords Protein–DNA interaction · Biotechnology · EMSA · SELEX

Introduction

Protein–nucleic acid interactions are vital for all living organisms. Many important biological processes such as the transport and translation of RNA, packaging of DNA, genetic recombination, replication, and DNA repair are controlled by interactions of these two kinds of bio-macromolecules. In addition, the manipulation of protein–DNA interactions through biotechnology can regulate the expression of some virulence genes, which may be conducive to treating disease. Therefore, the study of protein–DNA interactions is important for exploring mechanisms as well as for regulating the growth, development, differentiation, and evolution of living beings. Various methods have been developed to explore the DNA–protein interaction space. These include structural methods and computational prediction tools, such as chromatin immunoprecipitation analysis (ChIP)-based methods (ChIP, ChIP-chip, and ChIP-Seq), SELEX, Electrophoretic mobility shift assays (EMSA), DNA footprinting and so on. This review focuses on the classical methods for the study of protein–DNA interaction and their recent advances, to understand this field systemically and promote the development of this field deeply.

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The classical methods

Electrophoretic mobility shift assay

Electrophoretic mobility shift assays, also known as bandshift assays, have become the standard protocol for determining the potential of a DNA sequence to bind a DNA-binding protein (Hellman and Fried 2007; Lane et al. 1992). This technique originated from the early work on rRNA–protein interaction (Dahlberg et al. 1969; Schaup et al. 1970) and its widespread use dates from its development for studies on transcriptional regulation (TF) in bacteria. EMSA is a simple, quick, and extremely sensitive in vitro technology for testing the protein–DNA interaction, which can realize the analysis of the purpose protein qualitatively and quantitatively. It is based on the observation that protein bound to DNA–RNA fragments usually leads to a reduction in the electrophoretic mobility of the fragment in no denaturing polyacrylamide or agarose gels. The nucleic acid probe is usually labeled by radioisotope. EMSAs are especially useful to analyze the dynamic subunit composition of nucleic acid-binding protein complexes under a variety of conditions especially when they are combined with protein identification techniques.

Based on the conventional EMSA protocol, some kinds of combined method have been developed. Smith and

Humphries (2009) developed multiplexed competitor electrophoretic mobility shift assay (MC-EMSA) to identify a previously unreported hepatocyte nuclear factor-3 site, which provides the tools that aid in the low-cost but high-throughput identification of uncertain DNA-binding proteins using arrays of unlabeled DNA competitors. Stenger et al. (2004) reported a method to identify RNA-binding proteins from a dried EMSA gel, which resulted in improved protein identification sensitivity and RNA–protein complex isolation accuracy that can be useful for the large-scale characterization of RNA- or DNA–protein complexes.

DNase I footprinting

Deoxyribonuclease I footprint is a valuable technique for locating the specific binding sites of proteins on DNA. Bound protein protecting the phosphodiester backbone of DNA from DNase I-catalyzed hydrolysis is the basis of this assay. Binding sites are visualized by autoradiography of the DNA fragments that result from hydrolysis, following separation by electrophoresis on denaturing DNA sequencing gels (Brenowitz et al. 1986).

Footprinting has been developed further as a quantitative technique to determine separate binding curves for each individual protein-binding site on the DNA. Brenowitz et al. (2001) described the use of footprinting to identify DNA-binding proteins in crude extracts qualitatively. Quantitative DNase footprint titration is one of the few techniques capable of resolving the microscopic binding affinities containing multiple-binding sites with which DNA-binding proteins interact cooperatively (Connaghan-Jones et al. 2008).

Yeast one-hybrid system

The yeast one-hybrid (Y1H) system is a powerful tool for the identification of proteins that can bind to DNA elements of interest, including cDNA of transcription factors (Li and Herskowitz 1993), methylated DNA (Feng et al. 2010), origin of DNA replication (Lehming et al. 1994), and telomeres (Kim et al. 2003). The Y1H system is conceptually similar to the yeast two-hybrid system (Y2H) which was first devised by Field and Song (1989) on the basis that transcription activator proteins such as yeast Gal4 have a modular structure comprising a DNA-binding domain and a transcription activation domain.Y1H system was used to identify an essential protein that interacts in vivo with the yeast origin of DNA replication.

The Y1H system has been mainly used with multiple copies of short DNA elements (up to 30 bp) as DNA baits and offers maximal sensitivity because detection of the DNA–protein interaction occurs while proteins are in their native configurations. Deplancke et al. (2004) developed a Gateway-compatible Y1H system to facilitate the high-throughput, unbiased identification of protein–DNA interactions. Yan and Burgess (2012) developed a yeast inverse one-hybrid system which allows for rapid and genomic-wide identification of transcriptional binding targets. The Y1H system is relatively direct, quick, and sensitive; the protein screened has the active function in vivo compared with natural protein. The result gained from this method can reflect the regulation of the eukaryotic cell gene expression more truthfully than the other methods without the complex purification work of proteins.

ChIP

Chromatin Immunoprecipitation (Orlando 2000) is a commonly used technique to detect interactions between proteins and DNA, which is based on the enrichment of DNA associated with a protein of interest. This method has been used to describe physiological interactions between DNA sequences, transcriptional regulators (TFs), and the modification status of associated chromatin (Won and Kim 2006; Fullwood and Ruan 2009). In ChIP assays, living cells are treated with chemical cross-linkers to covalently bind proteins to each other and then to their DNA targets. Once cross-linked to associated proteins, chromatin is extracted and fragmented by sonication, and protein–DNA complexes are isolated using specific antibodies against a target protein. The cross-links that bind proteins to DNA are then reversed, and purified DNA fragments are analyzed by RT-PCR to determine if a specific sequence is present.

ChIP-chip, combining ChIP, and microarray technology (chip) (Horak and Snyder 2002), is based on a tiling-array platform in which genomic DNA oligomers from gene promoters are pre-fixed, enabling scientists to survey genome-wide DNA-binding sites for a given protein. The ChIP-chip technique has been used to identify TFs-binding sites, explore epigenomic information, and investigate factors in DNA replicate/repairs (Tong and Falk 2009; Reimer and Turck 2010). Chromatin immunoprecipitation on tiled arrays (ChIPOTle) is a useful tool for the analysis of ChIP-chip data (Buck et al. 2005). But this method can detect only targets included on the array.

With the advance of next-generation sequencing technologies, ChIP-Seq has been developed, which offers whole-genome coverage and greater sensitivity than the traditional ChIP-chip array (Robertson et al. 2007). Wu et al. (2010) also developed an algorithm, ChIP-PaM, which is based on peak count modeling and pattern matching of a specific tag count distribution of forward and reverse strands generated by protein–DNA binding, followed by de novo motif finding and searching within the

potential binding regions. A public ChIP-Seq dataset (GSE12782) was analyzed to illustrate this algorithm can greatly reduce false-positive findings while maintaining or improving accuracy and statistical power for binding site discovery.

Recently, Re-ChIP methods were developed to allow for the identification of multiple proteins on a single DNA sequence. This assay has enabled the analysis of multiple, simultaneous, posttranslational modifications to histones to determine the combinatorial pattern of modifications associated with transcriptional status of a gene (Truax and Greer 2012).

Advance of the technologies

SELEX

SELEX is an in vitro and combinatorial chemistry method that allows rapid selection from a library consisting of randomly generated oligonucleotides, which could consist of a mixture of RNA and single-stranded (ss) or double-stranded (ds) DNA, and the oligos have appropriate binding affinity to a given molecular target (Brown et al. 1997).

The scheme of the SELEX experiments is typically performed as follows: one prepares a library of oligonucleotides based on the interaction between protein and DNA so the library is incubated with a target. Then the oligonucleotides that are bound by the target from those that are not bound are separated. Selected oligonucleotides are then amplified by PCR in the case of DNA or by RT-PCR followed by in vitro transcription in the case of RNA. One cycle of target binding, selection, and amplification is called a SELEX round. The SELEX rounds are repeated several times, and some of the oligonucleotides selected in the final round of the experiment, which are called aptamers, are sequenced.

Since two groups performed the first SELEX experiments initially in 1990 (Brown et al. 1997; Ellington and Szostak 1990), many variations have resulted from it. Most of published SELEX experiments involve ssDNA (Surina et al. 2009; Muller et al. 2009), due to that single-stranded oligos have important diagnostic and therapeutic applications, and can be identified for a large variety of molecular targets (Strong et al. 2011; Feng et al. 2011). With the rapid development of the SELEX technology, a variety of improved technologies have appeared. Stormo and Fields (1998) described an automated microfluidic SELEX prototype that consisted of LabView-controlled actuatable valves and a PCR machine, which achieved simpler, smaller and more rapid SELEX applications. Zhao et al. (2009) introduced a high-throughput SELEX (HT-SELEX) procedure which gives good fits to the selected site distributions, much better than standard motif identification algorithms.

Problems in determining protein–DNA interaction from standard SELEX procedure is that weight matrix elements, which are parameters that could accurately quantify binding specificity of the protein–DNA interaction (Stormo and Fields 1998), determined provide a low level of both specificity and sensitivity. Little is known beforehand about the binding properties of a target molecule in many cases, so one most often starts with a large library of random oligonucleotides. Factors regulating multiple genes often have binding motifs low in information content, making the task of prediction harder (Sengupta et al. 2002).

The experiment by Matos et al. (2010) introduced important modification, which was to combine the SELEX procedure with the serial analysis of gene expression (SAGE) protocol. Nagaraj et al. (2008) used the SELEX-SAGE protocol to analyze low stringency SELEX data for *E. coli* catabolic activator protein (CAP), and the sequences obtained are more accurate.

SELEX has provided a novel research method for the interaction of protein–DNA. Different methodologies based on SELEX have a large potential to reliably infer nucleic acid-binding specificity of proteins and other molecular targets. This potential will likely result in the discovery of a number of novel biological regulatory loops in the future.

Scanning probe microscope (SPM)

Scanning probe microscopy (Jahanmir et al. 1992) is a branch of microscopy that forms images of surfaces using a physical probe that scans the sample. SPM includes atomic force microscopy (AFM; Binnig et al. 1986), scanning tunneling microscopy (STM; Binnig et al. 1982), near-field scanning optical microscopy (NSOM; Betzig et al. 1991) and so on. Basic idea of scanned probe techniques is that probe tip is scanned relative to the sample or sometimes the sample is scanned, the tip measures some property of the surface, and then a feedback mechanism is used to maintain the tip at a constant height above the sample.

The advantage of SPM is obvious. The resolution of the microscopes is not limited by diffraction, but only by the size of the probe-sample interaction volume, which can be as small as a few picometres. Hence the ability to measure small local differences in object height is unparalleled. Laterally, the probe-sample interaction extends only across the tip atom or atoms involved in the interaction. The interaction can be used to modify the sample to create small structures. Unlike electron microscope methods, samples do not require a partial vacuum but can be observed in air at standard temperature and pressure or while submerged in a liquid reaction vessel.

As a novel method on the interaction of protein–DNA, SPM plays an important role. But there are also drawbacks need to be solve, such as the scanning techniques are generally slower in acquiring images, due to the scanning process, scanning probe microscopy is often not useful for examining buried solid–by–solid or liquid–by–liquid interfaces. Scientists are working on the improvement of SPM for years and got some advances. Zhang and Zou (2012) presented an inversion-based iterative feedforward–feedback (II-FF/FB) approach to achieve high-speed force load in force measurement of soft materials in liquid using SPM. Okorafor and Clayton (2011) exploited the SPM's probe–surface interaction measurement capabilities to determine the SPM's lateral positioning dynamics.

Surface plasmon resonance technology (SPR)

Surface plasmon resonance technology is an efficient method for measuring biomolecular interactions that are broadly used in basic research and drug development. SPR is a more sensitive and quantitative biophysical approach that can measure binding affinity and kinetics simultaneously (Hoa et al. 2007). Real-time and label-free assay is another advantage of the SPR technique. The basic experimental protocol can be summarized as immobilizing or capturing one of the binding partners, injecting the second binding partner, and recording a real-time interaction curve; this step is performed using a series of concentrations of the second binding partner, choosing an appropriate kinetic model, and then fitting raw data to extract rate-constant estimates (Szabo et al. 1995).

Recently, spectroscopic SPR and imaging SPR have been further adapted as affinity detection techniques in the proteomic and genomic fields, especially in a protein conformation study (Despeyroux et al. 2000), biomarker profiling, aptamer selections (Murphy et al. 2003), and antibody selections (Wilson and Howell 2002), which have produced high-affinity ligands that specifically recognize protein targets.

Gupta et al. (2012) developed SPR immunosensor using 4-mercaptobenzoic acid (4-MBA) modified gold SPR chip for the detection of flagellin-specific antibodies of *Salmonella typhi* (*S. typhi*) for the first time. Liu et al. (2010) reported a method to enhance detection sensitivity in SPR spectroscopy by coupling a polymerization initiator to a biospecific interaction and inducing inline atom transfer radical polymerization (ATRP) for amplifying SPR response. Xia et al. (2009) carried out the interaction of tumor suppressor p53 with apo-metallothionein (apo-MT), that MT was tethered onto the carboxymethylated dextran film, using a flow injection-surface plasmon resonance (FI-SPR) instrument. Pollet et al. (2009) first reported fiber optic SPR using DNA aptamers as bioreceptors, and detected hlgE in the low nanomolar range (2 nM), while quantification was possible in the concentration range from 6 to 100 nM with low cost. Hayano et al. (2008) developed an automated system to analyze protein complexes by integrating a SPR biosensor with highly sensitive nanoflow liquid chromatography–tandem mass spectrometry (LC–MS/MS), which was validated using either FK506-binding protein or ribosomal protein S19.

One of the major challenges for SPR assay is how to immobilize ligand without affecting its biological activity (Ling et al. 2008); Khan et al. (2012) developed a novel label-free method to immobilize basic proteins onto the C1 chip for SPR assay at physiological pH, which is simple, efficient, and reversible as compared with current ligand immobilization methods.

Compared with EMSA analysis, which needs intact complexes to be highly stabled to be detected by electrophoresis, SPR can observe and measure the association and dissociation of the complex, and Gibbs free energy difference. The SPR analysis is more reliable and sensible to compare between a wild-type protein and its mutants. Moreover, it is a much faster technique and less laborious making it possible to extract more information from the data obtained (Matos et al. 2010; Henriksson-Peltola et al. 2007).

Other methods

Except those methods discussed above, many other technology also can be used to research the interaction of protein–DNA. Ligation-mediated polymerase chain reaction (LM-PCR) provides adequate sensitivity for nucleotide-level analysis of single-copy genes (Mueller and Wold 1989); it can function with DNA modification and cutting reagents, such as DNase I, DMS, and potassium permanganate, for the protein–DNA interaction. Fluorescence technology has been widely used in the interaction between biomolecules. Capillary electrophoresis with laser-induced fluorescence (CE-LIF) is one of high sensitive detection method for biological, chemical, and medical research (Zhang et al. 2004). Methylation interference assay (and methylation protection) also offers a high-resolution method for studying the interaction of proteins with major-groove guanines; a ^{32}P-end-labeled DNA containing a recognition site is methylated by DMS and then protein is allowed to bind (Carey and Smale 2007).

Conclusion

Since the 1990s, a lot of novel methods for studying the interaction of protein–DNA have been developed, such as molecular simulation, electrochemical technology, nanotechnology,

and protein microarray technology. These techniques have made lots of achievements in this field, but also a number of problems need to be solved. Considering that such technologies have their own limitations and the emphasis on the study of protein–DNA interaction is different, kinds of methods should be cross-used to solve the bottleneck of identification of the protein–DNA interaction. For example, EMSA and DNase I footprinting assays were combined to indicate that SabR-modulated nikkomycin biosynthesis is an enhancer via interaction with the promoter region of sanG (Pan et al. 2011).

At present, most of the new technologies on the applications of the protein and nucleic acids interaction are still in the stage of exploration and development. The rapid development of bioinformatics, such as the analysis of gene network and the construction of the protein–DNA recognition database, provides a great convenience for life-science research. But limited to the current situation of the development of computer and algorithm field, the results obtained from bioinformatics analysis and practical experiments still have a certain deviation. Further experimental work is needed for verification.

In recent years, the development and improvement of the research of the protein and nucleic acid interaction provide a powerful tool for clarifying further the complex life phenomenon such as gene expression and regulation, pathogenesis, growth, and cell proliferation. This will have positive and profound effects on the research of functional genomics and proteomics. The analysis methods mentioned in this review are all representative technologies for the research of protein–DNA interaction. With the development of science and technology and strong cooperation between the various disciplines, research strategy with innovative thinking and novel methods will continue to emerge. It can be predicted that research on the interaction law of the protein and nucleic acid will make considerable progress in the near further.

Conflict of interest We declare that we have no conflict of interest about this manuscript.

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