Review Article

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Handshake of DNA and Protein: A Perspective

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Abstract

DNA is a passive molecule and it can not exist on its own in cellular environment. It exist alongwith proteins and their interaction play a pivotal role in various biological processes such as replication, transcription, recombination, and repair etc. and via their interaction they regulate and modulate gene expression. Association of DNA-protein has an utmost importance in biological processes. There are plethora of biochemical and biophysical techniques to study the interaction of DNA-protein. Present article is an effort to give a glimpse of DNA-Protein interaction.

Keywords: DNA-Binding Proteins; Reporter Assay; DNA Foot Printing; CD; Fluorescence Spectroscopy.

Introduction

DNA, a fundamental unit of life, is polymorphic and adopts a variety of unique secondary and tertiary structures that may play functional role in gene regulation. It has been one of the most important sources not only for the understanding of the fundamental basis of human life but also for the development of a novel group of therapeutics modeled on its endogenous structure. The DNA has the potential to adopt numerous conformations depending on the primary structures, hydration, ions, proteins, drugs and spherical stress. Its primary structure dependence is far from being fully understood but in addition to the most common forms of DNA namely the duplex several other forms such as hairpins, cruciform three stranded structures such as triplexes, four stranded structures such as G-quadruplexes and C- quadruplexes (i-motif) and even polyads have also been discovered.

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Evidences shows the flexibility in the DNA topology and this distinctive flexibility are detectable for instance in the growing number of unusual DNA structure and conformations as a result of the intricate interplay between the various factors [1]. Although enormous progress have been made in elucidating the DNA dynamic behavior but still our knowledge is limited to explore DNA characteristics. The structural and conformational variability shown by DNA have been found biologically very important [2,3]. Therefore, it is intriguing to explore DNA and its interaction with various ligands; proteins etc.

It is widely known that various intra/inter molecular interactions perform crucial roles in cellular processes in particular ways. Proteins- DNA interactions are a common feature in cellular processes such as mismatched DNA repair [4]and gene regulation [5] etc. The main function of nucleic acids is the storage and faultless transmission of genetic information to progeny and transmission and coding of this information into proteins whereas proteins are concerned with the execution of biological processes. The function of nucleic acids is controlled and made possible by their interactions with specific proteins thus the interactions between the proteins and nucleic acids play a very

fundamental role in living cells, by controlling every step of the entire mechanism of information, translation, repressor mechanisms and presumably differentiation. For instances, proteins involved in (a) repair and replication is-ligases and repair proteins, nucleases and excision enzymes, gyrases, DNA polymerases. (b) transcription-RNA polymerase, cyclic AMP receptor protein, repressors, rho factors which terminate transcription (c) nucleases-restriction endonucleases, exo- and endonucleases (d) recombination rec A protein and (e) package- histones, protamines, virus condensation proteins.

Specificity in DNA-Protein interactions comes from protein recognition of the linear order of base pairs through hydrogen bonds and salt bridge contacts through the major and minor grooves. Proteins can also recognize particular regions of DNA through an indirect readout mechanism in which contacts are not made with the bases in the major and minor grooves, but with phosphate groups and sugar residues. Proteins involved in package and repair are less specific to the base sequence. The coulombic interactions between the phosphate groups and the basic amino acids residues of the proteins are assumed to be the major source of binding in these cases. In contrast, proteins which facilitate and regulate the transcription are very specific to the nucleotide sequence. Great advances have been made in the detection of protein-DNA interactions which would have a wide applicability in the rapidly developing biological research field. However, we find that today our knowledge of interactions between proteins and nucleic acid is still rather limited. The main problem is that these systems are all rather complex and require simultaneous observation of two associated macromolecules. In order to circumvent the difficulties inherent in these systems, model compounds involving nucleic acid and protein constituents have been studied.Many diseases arises owe to the miscommunication of these two regulatory elements i. e. DNA and protein. Overexpression of transcription factors are usually observed in oncogenes [6]. For this reason extensive studies were done on transcription factors till date. DNA being polymorphic also exists in various canonical and non-canonical structures and various proteins bind specifically to DNA structures. DNA bases are planar and aromatic moieties and aromatic amino acids specifically interact with DNA via base stacking to DNA bases and intercalation mode of binding. DNA contains negatively charged sugar-phosphate backbone so proteins containing positively charged amino acids such as lysine, arginine interact via binding to the DNA backbone. So, basically DNA-protein interacts via specific as well as non-specific binding.The latter interactions can also be specific due to sequence dependent formation of DNA structure. Protein might produce drastic conformational changes in DNA which is essential and responsible for their efficient function. Thus, in order to understand the DNA-protein complex function clearly, various techniques are required and used to investigate binding between DNA-protein. For better understanding we should not rely on a single technique, so we tried to summarize a few of the techniques in this review.

Characterization of DNA-Binding Protein

There are various biochemical and biophysical methods which are currently used for investigation of DNA-protein interaction and these are summarized in Figure 1. Here, in present article a few techniques are discussed.

Nitrocellulose Filter Binding Assay

Nitrocellulose method is now obsolete but earlier it was used very frequently to investigate DNA-protein interaction. This method is very rapid and simple to perform. This process is based on the principle that any protein can bind to the nitrocellulose without losing their capacity to bind DNA. In this method labeled DNA with protein are set to incubate and allow enough time to attain equilibrium [7,8]. Mixture is made to filter through a filter disk composed of nitrocellulose. Protein can bind effectively to nitrocellulose while DNA is unable to bind. If any DNA bind to protein that can be retained on the filter due to complex formation. Now dry the filter and count it. The demerit of this method is that the exact location of binding site can not be recognized as well as if mixture of protein used for incubation it was difficult to explain about the protein binding affinity of different proteins. One more demerit is also worth to discuss here that sometimes single-stranded nucleic acids are also retained at nitrocellulose filters under various conditions which put a question mark on the authentication of this technique.

Foot Printing Assay

Foot printing assay is specifically used in order to investigate about the binding of protein to DNA and precise binding of a protein to a particular sequence of DNA. The DNA region which is subjected to study is end-labelled and allowed to interact with a protein either crude extract or in pure form. Further, the DNA-

protein complex is treated with enzymatic or chemical agent who specifically cleaves DNA, to see the effect of these on protein bound DNA. These reagents do not affect protein bound DNA because protein protect DNA from cleavage. These complexes are then run on denaturating PAGE and subjected to autoradiography. DNA ladder is used for comparing the protein bound DNA and DNA alone (Figure 2). The protected region is observed as gap in the continuous bands of the digestion products. The DNase I was used as the first reagent for DNA foot printing experiment [9,10]. Due to its large size DNA is readily prevented by the attack of DNase owe to the steric hindrance. DNase I reagent is used under mild conditions so that it does not perturb the DNA-protein complex at larger extent [11]. With advantages there are some disadvantages of DNase I as foot printing agent as well. Due to its size it is difficult to cutting it immediately in vicinity of protein bound DNA complex.Along with this a large amount of protein is required to ensure that DNA-binding site is saturated. So, this technique is not suitable where small amount of protein is available. The cleavage of DNA by DNase I is variable i. e. some sequences cleaved very rapidly whereas some sequences are not digested even after long time of incubation. Thus, an uneven ladder of digestion product is observed in electrophoresis.

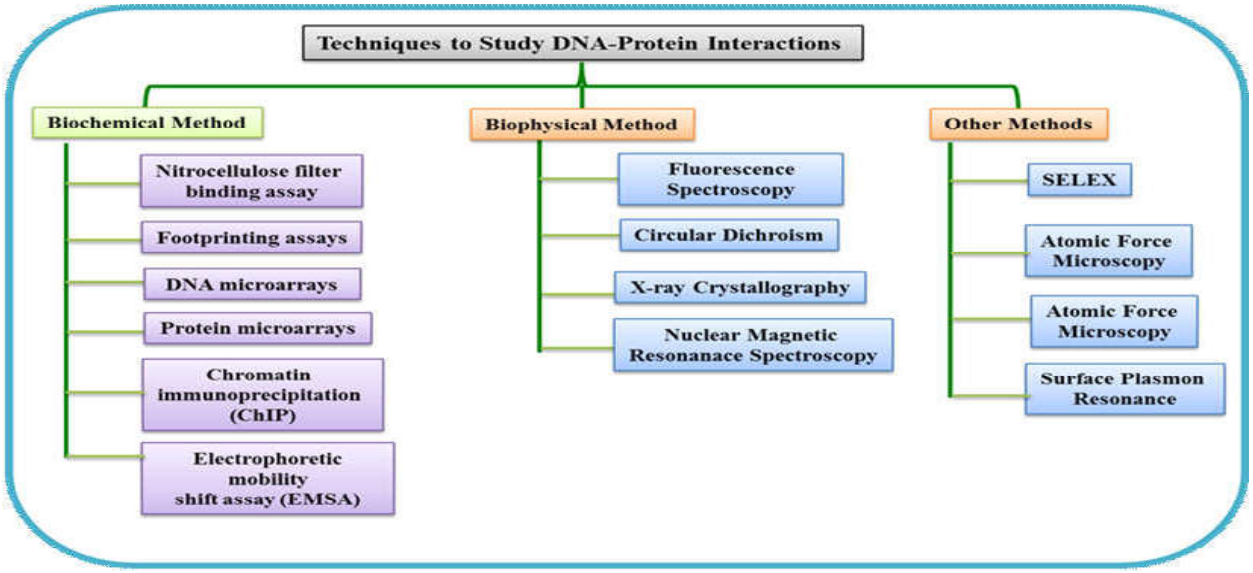

Fig. 1: Schematic representation of techniques used to study DNA-protein interaction.

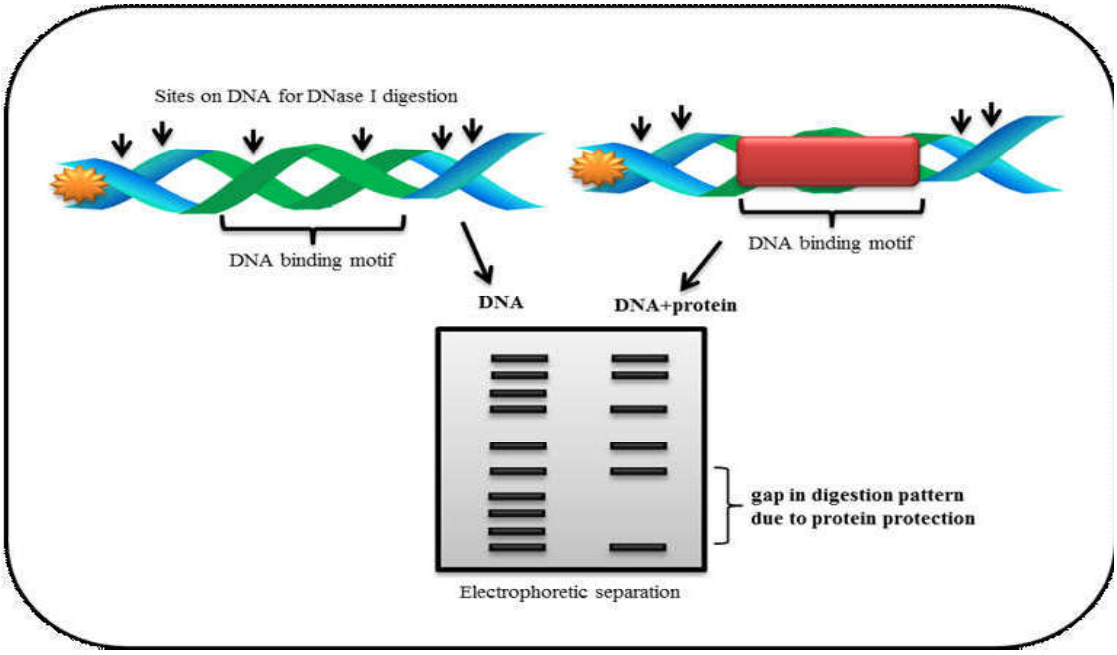

Fig. 2: Schematic representation of DNase I Foot printing

Characterization by Electrophoretic Mobility Shift Assay

EMSA is also known as gel retardation assay in which a radiolabeled duplex DNA or a labelled restriction fragment which consists of one or more binding sites for a specific DNA-binding protein motif. The restriction fragment can be labeled at both ends or at one end and it can give a single band in gel [12].Then incubation is set up for fragment either in absence of protein and increasing concentration of protein. These are allowed to incubate for some time to facilitate binding. Then these samples are loaded in polyacrylamide or agarose gel. The gel buffer consists of low salt concentration for the stabilization of DNA-protein complex. Distribution of radioactivity can be visualized by foot printing method. The DNA-protein complex migratesslowly in comparison to the DNA without any protein. So, this is the reason why we call it gel retardation assay.

Biophysical Techniques

Biophysical techniques include spectroscopic methods to investigate the DNA-protein complex formation. All the biophysical techniques which are used for structure study of DNA and protein alone can also be applied for the binding interaction. The most sensitive and valuable techniques are fluorescence and circular dichroism spectroscopy. Other spectroscopic techniques such as nuclear magnetic resonanceand X-ray techniques require large sample amount as well as more time for data analysis. These techniques need very expensive instruments as well.

- *Fluorescence Spectroscopy*

Fluorescence spectroscopy is a very sensitive techniqueused to elucidate DNA-protein interaction. Protein can produce intrinsic emission spectra due to presence of tryptophan and tyrosine amino acid in its structure. Often quenching of intrinsic emission spectrum and/or shift in wavelength maxima is observed on protein binding to DNA. So, by comparing the change observed in free protein spectra after addition of DNA is considered for calculation of binding constant and other binding parameters [13]. Fluorescence anisotropy is also used for the investigation of DNA-binding curve when molecular size of Protein-DNA complex is sufficiently different from the fluorescing component which is specifically in all cases is protein. Sometimes an extrinsic fluorophore can also be attached to DNA single strand or duplex at 5′ ends to increase the sensitivity and investigation of DNA-protein interaction [14].

- *Circular Dichroism*

CD is also a very informative technique which is used to investigate DNA-Protein interaction. Generally it is not used for calculation of binding parameters and binding constants rather it is applied for analyzing structural changes in DNA-protein interaction [15]. The far UV-CD (170-250nm) is exploited for the secondary structure of proteins and investigation is carried out to see the effect of DNA binding to protein in this region whereas near UV-CD (250-300nm) signal is specifically arise due to DNA component and study is done to reveal conformational changes in the DNA structures when protein binds to it. A large distortion is observed when aromatic amino acids of protein intercalate between the DNA bases [16]. CD is found to be less sensitive technique to study DNA-protein complex.

Conclusion

DNA-protein association and interaction has an utmost importance in the biological system. Several biochemical and biophysical techniques are available to study and interpret the binding interaction of DNA-protein. Every technique is self-efficient in one or other aspect of elucidation of DNA-protein interaction. Many topics have been left here owing to the restriction of space but all the techniques should combined to study these interactions and give insight about the exact mechanism involve in DNA-protein binding.

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