specifically bound and would migrate in complex B1, rather than at the top of the gel.

In lanes 7 to 9, a competition binding experiment determined whether complexes B1 and B2 represent specific protein-DNA complexes. Lane 7 is a standard binding reaction which contains probe, protein and poly(dI-dC)-poly(dI-dC). Lane 8 is the same as lane 7, except that a 100-fold molar excess of an unlabeled DNA fragment identical to the probe (i.e., a specific competitor) was added prior to the addition of protein. Lane 9 is the same as lane 8, except that an unlabeled DNA competitor fragment containing sequences different from the probe (i.e., a nonspecific competitor) was added.

Formation of complex B1 is competed by the specific unlabeled DNA fragment, but not by the nonspecific DNA fragment. Therefore, complex B1 results from the specific binding of a molecule in the extract to the DNA probe. Formation of complex B2 is not competed by either the specific competitor or the nonspecific competitor. Complex B2 results from the nonspecific binding of a molecule in the extract to the DNA probe. This is typical behavior for a nonspecific DNA-protein interaction.

COMMENTARY

Several methods exist for detecting the sequence-specific binding of proteins to DNA including nitrocellulose filter binding, DNase I footprinting, methylation protection, methylation interference, and mobility shift gel electrophoresis. The DNA-binding assay using mobility shift PAGE is based on the observation that DNA-protein complexes migrate through low-ionic-strength polyacrylamide gels more slowly than unbound DNA fragments. This assay is generally simpler, faster, and more sensitive than other methods. Thus, it is an ideal assay for monitoring the purification of DNA-binding proteins. More importantly, the sensitivity of this assay enables femptomole quantities of DNA-binding proteins to be detected routinely. In addition to providing quantitative information on the amount of DNA bound by the protein, the use of mobility shift PAGE provides additional information on the number and type of proteins bound. Also, each distinct species of protein bound to the probe generates a complex of distinct mobility and specificity so that interactions of several proteins binding to a single DNA fragment can be observed. Moreover, even if multiple proteins recognize overlapping sites on the DNA fragment, the complexes formed by each can be resolved and characterized.

Methylation Interference Assay for Analysis of DNA-Protein Interactions

Methylation interference identifies guanines and adenines in the DNA binding site that, when methylated, interfere with binding of the protein. The protocol described below uses a single end-labeled DNA probe that is methylated at an average of one site per molecule of probe. The labeled probe is a substrate for a protein-binding reaction. DNA-protein complexes are separated from the free probe by the mobility shift DNA-binding assay (UNIT 12.2). A DNA probe that is methylated at a position which interferes with binding will not be retarded in this assay. Therefore, the specific DNA-protein complex is depleted for DNA that contains methyl groups on purines important for binding. After gel purification, DNA is cleaved with piperidine. Finally, these fragments are electrophoresed on polyacrylamide sequencing gels and autoradiographed. Guanines and adenines that interfere with binding are revealed by their absence in the retarded complex relative to a lane containing piperidine-cleaved free probe. This procedure offers a rapid and highly analytical means of characterizing DNA-protein interactions.

COMMENTARY

Methylation interference was used initially by Siebenlist and Gilbert (1980) to study the interaction of RNA polymerase and the T7 promoter. This technique utilized the chemistry developed for the G reaction in DNA sequencing (Maxam and Gilbert, 1980). Recently methylation interference has been coupled with the mobility shift DNA-binding assay (UNIT 12.2), resulting in relatively rapid analyses of the contact sites for DNA-binding proteins.

The two procedures typically used to map the region of DNA-protein interaction are methylation interference and DNase I protection. Methylation interference has several advantages over DNase I protection. First, methylation interference analyses are not hindered by incomplete binding because all of the probe in a retarded complex is bound by protein. In contrast, DNase I protection experiments require protein titrations to fully saturate probe DNA. Another disadvantage of DNase I digestions is that protein exchange from the binding site during the digestion can lead to cutting within the binding site. Finally, methylation interference allows the determination of specific nucleotides that are in relatively close contact with the DNA-binding protein. As a result, much more information is obtained about the binding site than from the comparatively large protected region produced by a DNase I footprint. For example, methylation interference has determined single nucleotide differences in binding site specificities that could not be determined by DNase I protection (Baldwin and Sharp, 1988).

Although methylation interference does not measure direct protein contacts with nucleotides, it permits detection of nucleotides that are closely apposed to the proteins. DMS methylates guanine residues at the N-7 position that protrudes into the major groove, and also at the N-3 position of adenines that protrudes into the minor groove. Adenines can be detected in this assay but are much weaker than the G reactions (Maxam and Gilbert, 1980).

A logical extension of methylation interference has been described recently (Brunelle and Schleif, 1987). In this procedure, depurinated and depyrimidated DNA probes are substrates in the binding reaction. Bound and free probes are separated by native gel electrophoresis and cleaved by piperidine, allowing detection of proteins closely apposed to T and C, as well as G and A, residues.

DNase I Footprint Analysis of Protein-DNA Binding

Deoxyribonuclease I (DNase I) protection mapping, or footprinting, is a valuable technique for locating the specific binding sites of proteins on DNA. The basis of this assay is that bound protein protects the phosphodiester backbone of DNA from DNase I--catalyzed hydrolysis. Binding sites are visualized by autoradiography of the DNA fragments that result from hydrolysis, following separation by electrophoresis on denaturing DNA sequencing gels. Footprinting has been developed further as a quantitative technique to determine separate binding curves for each individual protein-binding site on the DNA. For each binding site, the total energy of binding is determined directly from that site's binding curve. For sites that interact cooperatively, simultaneous numerical analysis of all the binding curves can be used to resolve both the intrinsic binding and cooperative components of these energies.