DNA-binding site to the binding protein. Irradiation of DNA with UV light produces purine and pyrimidine free radicals. If a protein molecule is in close proximity to the free radical, a covalent bond can be formed, crosslinking the protein to the DNA.

In solution, pyrimidines are approximately 10-fold more sensitive to photochemical alteration than purines. Several amino acids are known to form photoadducts with pyrimidine bases, including cysteine, serine, methionine, lysine, arginine, histidine, trytophan, phenylalanine, and tyrosine. Cells that have incorporated halogenated analogs of thymine--such as bromodeoxyuridine (BrdU)--into their DNA are several times more sensitive to UV-induced crosslinking with protein than normal cells. This is because replacement of the thymidine methyl group with the bromine atom creates a molecule more susceptible to free radical formation in the presence of UV light. Because the bromo group is approximately the same van der Waals radius as a methyl group, several cellular enzymes will use thymidine and BrdU interchangeably. Thus, it is quite simple to generate BrdU-substituted DNA probes.

Another important reason for using BrdU is that the longer wavelength of UV light used to crosslink these probes is less damaging to proteins than a shorter wavelength. In addition, substitution of BrdU into a binding site sometimes increases the affinity of the protein-DNA interaction being studied.

Purification of DNA-Binding Proteins Using Biotin/Streptavidin Affinity Systems

Short fragments of DNA--either natural or formed from oligonucleotides--containing a high-affinity site for a DNA-binding protein provide a powerful tool for purification. The biotin/streptavidin purification system is based on the tight and essentially irreversible complex that biotin forms with streptavidin. The experimental design of this system is illustrated in Figure 12.6.1. First, a DNA fragment is prepared that contains a high-affinity binding site for the protein of interest. A molecule of biotinylated nucleotide is incorporated into one of the ends of the DNA fragment. The protein of interest is allowed to bind to the high-affinity recognition site present in the biotinylated fragment. The tetrameric protein streptavidin is then bound to the biotinylated end of the DNA fragment. Next, the protein/biotinylated fragment/streptavidin ternary complex is efficiently removed by adsorption onto a biotin-containing resin. Since streptavidin is multivalent, it is able to serve as a bridge between the biotinylated DNA fragment and the biotin-containing resin. Proteins remaining in the supernatant are washed away under conditions that maximize the stability of the DNA-protein complex. Finally, the protein of interest is eluted from the resin with a high-salt buffer.

ALTERNATE PROTOCOL: PURIFICATION USING A MICROCOLUMN

Although the batch method in the basic protocol is rapid and well-suited for analytical-scale purification, larger volumes of biotin-cellulose resin can be better handled in a microcolumn. This method is also used to elute the protein in as small a volume (i.e., as high a concentration) as possible.

ALTERNATE PROTOCOL: PURIFICATION USING STREPTAVIDIN-AGAROSE

When high-quality free streptavidin is not available or cellulose is an inappropriate resin, a simple

variation on the basic protocol may be employed. In this protocol, the same biotinylated DNA fragment is used but is removed from solution directly by streptavidin-agarose (see Fig. 12.6.2).

COMMENTARY

The biotin/streptavidin purification method works well because the interaction between biotin and avidin (or avidin-like proteins) is one of the strongest known noncovalent interactions. The dissociation constant for the streptavidin-biotin complex is ~10-15 M. Avidin, from egg white, and streptavidin, from Streptomyces avidinii, are tetrameric proteins containing four high-affinity binding sites for the vitamin biotin. Since streptavidin is multivalent, it is able to serve as a bridge between the biotinylated DNA fragment and the biotin-containing resin. The strong interaction is extremely useful for purification of DNA-binding proteins, because DNA-affinity columns with streptavidin/biotin bridges can be washed under a wide variety of conditions (i.e., 2 M KCl and 1% SDS) without removing either the streptavidin or the biotinylated DNA fragment from the matrix.

Two properties of streptavidin make it more suitable than avidin for use in DNA-affinity purification. The first is that streptavidin, unlike avidin, is not a glycoprotein, and the second is that streptavidin is slightly acidic whereas avidin is basic. Therefore, streptavidin is less likely to bind nonspecifically to cellular glycoproteins and to acidically charged cell components such as nucleic acids.

Several factors make this method simple, rapid, and effective. The same binding conditions and DNA fragment used in the mobility-shift DNA-binding assay to identify a protein (UNIT 12.2) can be used to effect its purification. In addition, the binding of the protein to its DNA recognition site in solution is more efficient than protein-DNA interactions that take place on a column matrix (discussed below). Most importantly, binding in solution allows each reaction parameter to be optimized on an analytical scale by using the gel binding assay.

As an analytical technique, biotin/streptavidin DNA-affinity purification permits the direct identification of a wide variety of sequence-specific DNA-binding proteins. It has already been successfully used to identify hormone receptors, components in mRNA splicing complexes, and RNA polymerase II and III transcription factors (Haeuptle et al., 1983; Grabowski and Sharp, 1986; Chodosh et al., 1986; Kasher et al., 1986).

Another method is commonly used for purifying DNA-binding proteins, whereby DNA-protein binding interactions occur on a column matrix. In this technique, catenated DNA-binding sites are covalently coupled with cyanogen bromide to Sepharose CL-2B (Kadonaga and Tjian, 1986; Rosenfeld and Kelly, 1986). A partially purified protein fraction is combined with competitor DNA and passed through a DNA-Sepharose resin, binding the protein to the surface of the matrix. Generally, multiple passes through the column are used to effect purification, with 25- to 50-fold purification achieved with each column passage. The biotin/streptavidin technique, on the other hand, typically yields 50- to 250-fold purification.

Detection, Purification, and Characterization of cDNA Clones Encoding DNA-Binding Proteins