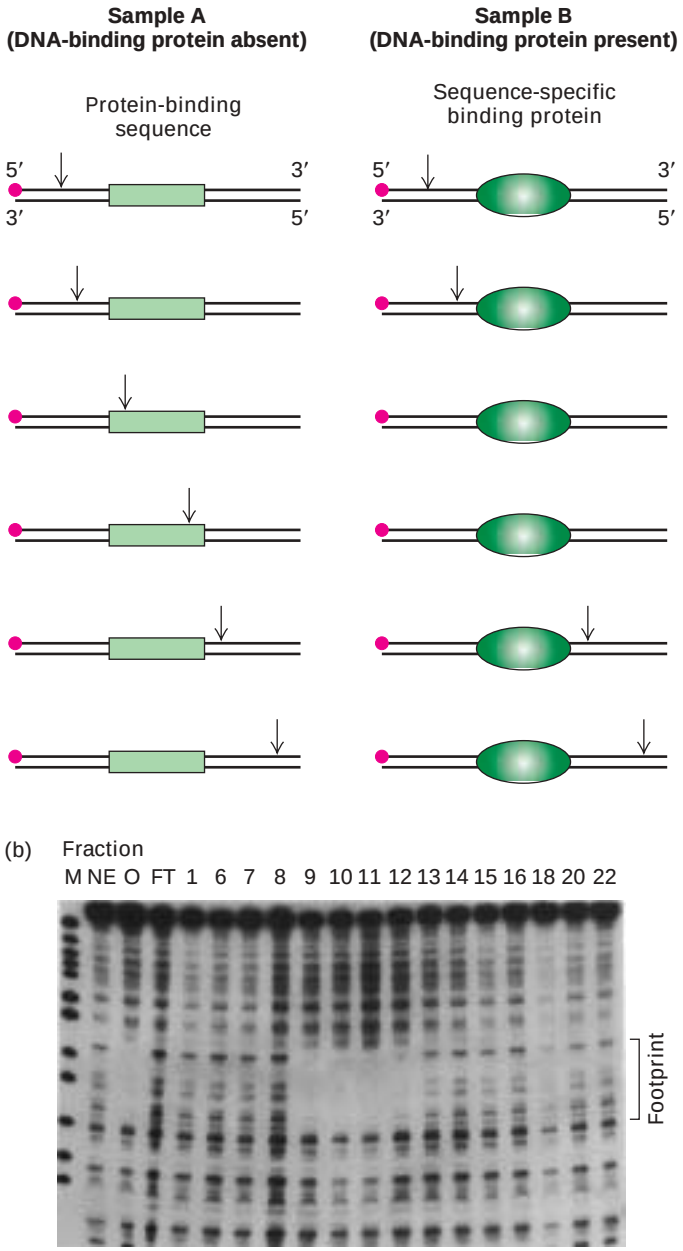

▶ EXPERIMENTAL FIGURE 11-13 **DNase I footprinting reveals control-element sequences and can be used as an assay in transcription factor purification.** (a) DNase I footprinting can identify control element sequences. A DNA fragment known to contain the control-element is labeled at one end with ^{32}P (red dot). Portions of the labeled DNA sample then are digested with DNase I in the presence and absence of protein samples thought to contain a cognate protein. DNase I randomly hydrolyzes the phosphodiester bonds of DNA between the 3′ oxygen on the deoxyribose of one nucleotide and the 5′ phosphate of the next nucleotide. A low concentration of DNase I is used so that on average each DNA molecule is cleaved just once (vertical arrows). If the protein sample does not contain a cognate DNA-binding protein, the DNA fragment is cleaved at multiple positions between the labeled and unlabeled ends of the original fragment, as in sample A on the left. If the protein sample contains a cognate protein, as in sample B on the right, the protein binds to the DNA, thereby protecting a portion of the fragment from digestion. Following DNase treatment, the DNA is separated from protein, denatured to separate the strands, and electrophoresed. Autoradiography of the resulting gel detects only labeled strands and reveals fragments extending from the labeled end to the site of cleavage by DNase I. Cleavage fragments containing the control sequence show up on the gel for sample A, but are missing in sample B because the bound cognate protein blocked cleavages within that sequence and thus production of the corresponding fragments. The missing bands on the gel constitute the footprint. (b) A protein fraction containing a sequence-specific DNA-binding protein can be purified by column chromatography. DNase I footprinting can then identify which of the eluted fractions contain the cognate protein. In the absence of added protein (NE, *no extract*), DNase I cleaves the DNA fragment at multiple sites, producing multiple bands on the gel shown here. A cognate protein present in the nuclear extract applied to the column (O, *o*nput) generated a footprint. This protein was bound to the column, since footprinting activity was not detected in the flow-through protein fraction (FT). After applying a salt gradient to the column, most of the cognate protein eluted in fractions 9–12, as evidenced by the missing bands (footprints). The sequence of the protein-binding region can be determined by comparison with marker DNA fragments of known length analyzed on the same gel (M). [Part (b) from S. Yoshinaga et al., 1989, *J. Biol. Chem.* **264**:10529.]

with a DNA fragment containing a known DNA control element, the appearance of a footprint indicates the presence of a transcription factor that binds that control element in the protein sample being assayed. Footprinting also identifies the specific DNA sequence to which the transcription factor binds.

The electrophoretic mobility shift assay (EMSA), also called the *gel-shift* or *band-shift* assay, is more useful than the footprinting assay for quantitative analysis of DNA-binding proteins. In general, the electrophoretic mobility of a DNA fragment is reduced when it is complexed to protein, causing a shift in the location of the fragment band. This assay can be used to detect a transcription factor in protein fractions incubated with a radiolabeled DNA fragment containing a known control element (Figure 11-14).

In the biochemical isolation of a transcription factor, an extract of cell nuclei commonly is subjected sequentially to several types of column chromatography (Chapter 3). Fractions eluted from the columns are assayed by DNase I footprinting or EMSA using DNA fragments containing an identified regulatory element (see Figures 11-13 and 11-14). Fractions containing protein that binds to the regulatory element in these assays probably contain a putative transcription factor. A powerful technique commonly used for the final step in purifying transcription factors is *sequence-specific DNA affinity chromatography,* a particular type of