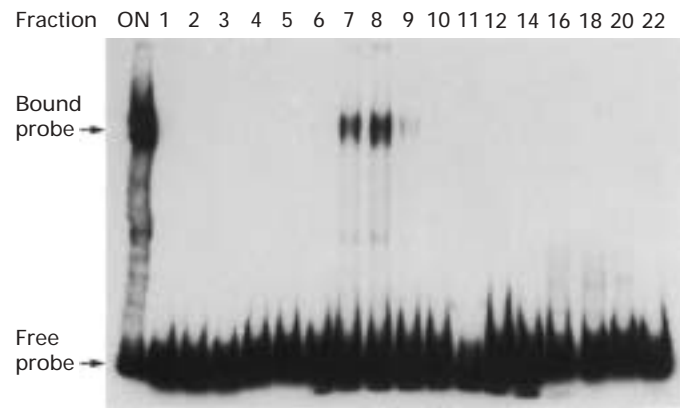
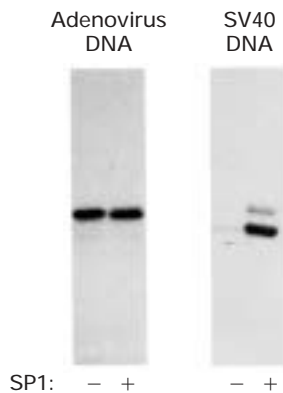


► **EXPERIMENTAL FIGURE 11-14 Electrophoretic mobility shift assay can be used to detect transcription factors during purification.** In this example, protein fractions separated by column chromatography were assayed for their ability to bind to a radiolabeled DNA-fragment probe containing a known regulatory element. After an aliquot of the protein sample loaded onto the column (ON) and successive column fractions (numbers) were incubated with the labeled probe, the samples were electrophoresed under conditions that do not denature proteins. The free probe not bound to protein migrated to the bottom of the gel. A protein in the preparation applied to the column and in fractions 7 and 8 bound to the probe, forming a DNA-protein complex that migrated more slowly than the free probe. These fractions therefore likely contain the regulatory protein being sought. [From S. Yoshinaga et al., 1989, *J. Biol. Chem.* **264**:10529.]

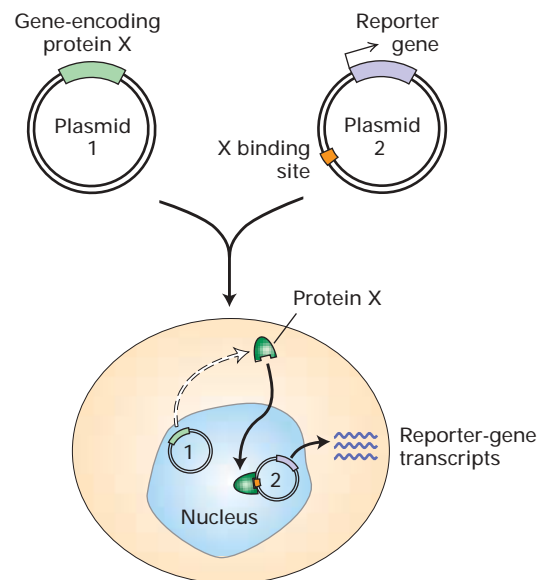


affinity chromatography in which long DNA strands containing multiple copies of the transcription factor-binding site are coupled to a column matrix. As a final test that an isolated protein is in fact a transcription factor, its ability to modulate transcription of a template containing the corresponding protein-binding sites is assayed in an *in vitro* transcription reaction. Figure 11-15 shows the results of such an assay for SP1, a transcription factor that binds to GC-rich sequences, thereby activating transcription from nearby promoters.



▲ **EXPERIMENTAL FIGURE 11-15 Transcription factors can be identified by *in vitro* assay for transcription activity.** SP1 was identified based on its ability to bind to a region of the SV40 genome that contains six copies of a GC-rich promoter-proximal element and was purified by column chromatography. To test the transcription-activating ability of purified SP1, it was incubated *in vitro* with template DNA, a protein fraction containing RNA polymerase II and associated general transcription factors, and labeled ribonucleoside triphosphates. The labeled RNA products were subjected to electrophoresis and autoradiography. Shown here are autoradiograms from assays with adenovirus and SV40 DNA in the absence (–) and presence (+) of SP1. SP1 had no significant effect on transcription from the adenovirus promoter, which contains no SP1-binding sites. In contrast, SP1 stimulated transcription from the SV40 promoter about tenfold. [Adapted from M. R. Briggs et al., 1986, *Science* **234**:47]

Once a transcription factor is isolated and purified, its partial amino acid sequence can be determined and used to clone the gene or cDNA encoding it, as outlined in Chapter 9. The isolated gene can then be used to test the ability of the encoded protein to activate or repress transcription in an *in vivo* transfection assay (Figure 11-16).



▲ **EXPERIMENTAL FIGURE 11-16 *In vivo* transfection assay measures transcription activity to evaluate proteins believed to be transcription factors.** The assay system requires two plasmids. One plasmid contains the gene encoding the putative transcription factor (protein X). The second plasmid contains a reporter gene (e.g., *lacZ*) and one or more binding sites for protein X. Both plasmids are simultaneously introduced into cells that lack the gene encoding protein X. The production of reporter-gene RNA transcripts is measured; alternatively, the activity of the encoded protein can be assayed. If reporter-gene transcription is greater in the presence of the X-encoding plasmid, then the protein is an activator; if transcription is less, then it is a repressor. By use of plasmids encoding a mutated or rearranged transcription factor, important domains of the protein can be identified.