EXPERIMENTAL PROCEDURES

Gel shift assays—Double-strand DNA probes containing either the serum response element (SRE) or the cyclic-AMP response element (CRE) were prepared by annealing the following synthetic oligonucleotides,
SRE:
5'-TTCCTCCCCCCTTACACAGGATGTCCATATTAGGACATCTGCGTCAGCAGGTTTCCACGG-3' and
5'-TTCCGTGGAAACCTGCTGACGCAGATGTCCTAATATGGACATCCTGTGTAAGGGGGGAGG-3' for SRE and
5'-TTCGCCACCGTGGTTGAGCCCGTGACGTTTACACTCATTCATAAAACGCT-3' and
5'-TTAGCGTTTTATGAATGAGTGTAAACGTCACGGGCTCAACCACGGTGGCG-3' for CRE.
The probes were labeled with [α^{32}-P]dATP by Klenow fragment (New England Biolabs), and each probe (2~5 x 10^3 cpm) was incubated with transcription factors at 30°C for 30 minutes in the presence of 1 mM DTT and 0.2 mg/ml of poly(dI-dC)-poly(dI-dC). The reaction products were separated directly on a 4% polyacrylamide gel at 4°C. For competition assays, the following oligonucleotides were annealed to prepare wild-type and mutant SRE or CRE. The underlines indicate the mutated nucleotides that abolish the binding of corresponding transcription factor(s). The used oligonucleotides are as follows. SRE:
5'-AATTAGATGCCCATATATGGGCATCT-3' and
5'-AGATGCCCATATATGGGCATCTAATT-3' for wild-type SRE;
5'-AATTAGATGCCGATATATCGGCATCT-3' and
5'-AGATGCCGATATATCGGCATCTAATT-3' for mutant SRE;
5'-TTGAGCCCGTGACGTTTACACTCATT-3', and
5'-AATGAGTGTAAACGTCACGGGCTCAA-3' for wild-type CRE; and
5'-TTGAGCCCGTGCATTTTACACTCATT-3' and
5'-AATGAGTGTAAAATGCACGGGCTCAA-3' for mutant CRE.

DNase I footprint assays—To create the probes, fp1 and fp2, for DNase I footprint assays, the promoter regions (from -393 to -13) or (from -393 to +108) of the c-fos gene spanning the SRE, CRE and TATA box were PCR amplified using the following primers;
5'-GGCCGAATTCGGTACCGCCTCCCCCCGCACTGCACCCTCGGTGTTG-3' and
5'-GGCCAAGCTTGAATTCGCATGCCCGCAGCCACTGCTTTTATAAC-3' for fp1, and
5'-GGCCGAATTCGGTACCGCCTCCCCCCGCACTGCACCCTCGGTGTTG-3' and
5'-GGCCAAGCTTGAATTCGCATGCTTTGGGAAATATAGAAGAAGGA-3' for fp2.
After digestion with *Kpn*I and *Hind*III, the PCR fragments were subcloned between the *Kpn*I and *Hind*III sites of pUC19 to create pUC19-fp1 and pUC19-fp2. After confirmation of their DNA sequences, the plasmids were digested with *Kpn*I and *Eco*RI (non-transcribed strand), or *Eco*RI and *Sph*I (transcribed strand), and the probe fragments were separated and purified from a polyacrylamide gel. The purified probes were labeled with [α-^{32}P]dATP by Klenow fragment and chased with dATP and dTTP. Footprint assays were performed with 20~40 ng of each factor or 5 pmol of each competitor DNAs.

LEGENDS TO SUPPLEMENTAL FIGURES

Supplemental Figure S1. Binding of the activators to their cognate cis-elements. *A*. Gel mobility shift assays using the purified SRF and Elk-1. The radiolabeled DNA probe containing the *c-fos* SRE was incubated with various combinations of the purified recombinant SRF and Elk-1 as indicated. The positions of SRE/SRF and SRE/SRF/Elk-1 are indicated on the right by a single and double asterisk(s), respectively. For competition assays, excess amounts (0.1 and 1.0 pmoles) of unlabeled SRE or mutated SRE (mSRE) probe were added to the reactions. *B*. Gel mobility shift assays using the purified CREB and ATF1. The radiolabeled DNA probe containing the c-fos CRE was incubated with various combinations of the purified recombinant CREB and ATF1 as indicated. The positions of CRE/CREB, CRE/CREB/ATF1 and CRE/ATF1 are indicated on the right by a single, double and triple asterisk(s), respectively. For competition assays, 0.1 and 1.0 pmoles of unlabeled CRE or mutated CRE (mCRE) probe were added to the indicated reactions.

Supplemental Figure S2. Binding of the activators to the *c-fos* promoter. The indicated combinations of recombinant activators were incubated with each DNA fragment containing the c-fos promoter region was incubated with the indicated combinations of recombinant activators, subjected to DNaseI digestion and separated on a denaturing gel. The DNA fragments were labeled on either the non-transcribed strand (*A*) or the transcribed strand (*B*). For competition assays, excess amounts of unlabeled DNA probe containing the c-fos SRE, mutated SRE (mSRE), c-fos CRE or mutated CRE (mCRE) were added. The schema of the c-fos promoter and the positions of SRE, FAP-1 and CRE are indicated on the right side of each panel.

Supplemental Figure S3. Immunoblot analyses of the TFIID subunits, TAF6 and TBP, in the D0.1 and 0.3 fractions. The P11-derived 0.85M fraction was fractionated by DE52 as described in Fig. 4A, and 6 μl each of the D0.1 and D0.3 fractions was analyzed by immunoblotting using anti-TAF6 (upper panel) and anti-TBP antibodies (lower panel), respectively.

Supplemental Figure S4. Effect of hnRNP R on GAL4-VP16-dependent transcription. Transcriptional activity of hnRNP R was tested in the transcription assays using pG5HMC2AT, in which the presence of GAL4-VP16 and PC4 is indicated by +. The amounts of recombinant hnRNP R used for the assays are indicated in the figure.