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Common Factor 1 Is a Transcriptional Activator Which Binds in the c-*myc* Promoter, the Skeletal α-Actin Promoter, and the Immunoglobulin Heavy-Chain Enhancer

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Ubiquitously expressed transcription factors play an integral role in establishing and regulating patterns of gene transcription. Common factor 1 (CF1) is a ubiquitously expressed DNA-binding protein previously identified in our laboratory. We show here that CF1 recognizes sites in several diverse transcription elements, and we demonstrate the ability of the c-*myc* CF1 site to activate transcription of a basal promoter in both B cells and fibroblasts.

Analysis of many mammalian transcriptional promoters and enhancers has revealed that only a few of the proteins which bind to them are either tissue specific (4, 12, 21, 41, 43) or gene specific; most transcription factors are more ubiquitously expressed (7, 8, 30, 32, 36). This fact has led to models in which the regulatory properties of particular promoters or enhancers are achieved through a unique combination of binding sites for subsets of generally available transcription factors, which may then interact with or be modified by tissue-specific or inducible proteins to achieve tissue-specific or inducible activity (6, 9, 26). It is important, therefore, to understand the target genes and mechanisms of action of ubiquitously expressed transcription factors. In this paper, we describe studies on one such ubiquitously expressed transcription factor, common factor 1 (CF1).

CF1 was first identified by electrophoretic mobility shift assays (EMSAs) and footprinting as a protein which binds upstream of the murine c-*myc* P1 transcription start site at −260 bp (21); CF1-binding activity was found in all cultured cells and normal tissues tested (22). We also detected a tissue-specific protein, expressed only in plasmacytoma cells, which bound just 5′ of CF1 in the c-*myc* promoter (21). This protein was shown to repress c-*myc* transcription and was named *myc*-plasmacytoma repressor factor (*myc*-PRF). Our studies further suggested that *myc*-PRF interacted with CF1. Because of its interaction with *myc*-PRF and because of its ubiquitous expression, we proposed that CF1 might be a transcription-activating protein (22).

CF1-binding sites in the regulatory elements of other genes have been identified. In order to further characterize CF1, we have searched the sequences of many known transcriptional regulatory regions for sequences homologous to the CF1 site in the c-*myc* gene and have tested the binding ability of these sites by EMSA with CF1 and unrelated oligonucleotides as competitors. The oligonucleotides used as competitors were annealed, concatenated, and ligated prior to use, and 100 ng of oligonucleotide (approximately a 1,000-fold molar excess over probe) was added to the reactions as indicated in Fig. 1. The oligonucleotide containing the −260 c-*myc* site comprises the sequence 5′-GCGCGCGAGAAGAGAAAATG GTCGGGC-3′. The oligonucleotide containing the μE1 site of the IgHE comprises the sequence 5′-GGAGTCAAGAT GGCCGATCAGAACC-3′. The oligonucleotide containing the μE3-binding site from the IgHE comprises the sequence 5′-CCTTGCCACATGACCTGCTTCCT-3′. Nuclear extracts from the pre-B-cell line 1881 were prepared essentially as previously described (21), with the addition of 1 mM iodoacetic acid as a protease inhibitor, and these extracts were used in all experiments. Figure 1A shows that there is a second binding site for CF1 in the c-*myc* promoter. When a c-*myc* promoter probe from −424 to −313 bp was used in an EMSA, a complex with mobility similar to that of the known CF1 complex was observed (Fig. 1A, lane 1), which was specifically sensitive to competition with a CF1 oligonucleotide (lane 2) but not with an unrelated oligonucleotide (lane 4). OP/Cu footprinting was used to map this binding site to 390 bp upstream of the P1 start site of transcription (Fig. 2A). No CF1 binding could be detected with a probe in which the site-directed changes indicated in Fig. 2B had been made.

Figure 1C demonstrates that the protein binding to the previously identified μE1 (13) or B (36) site in the murine immunoglobulin heavy-chain intronic enhancer (IgHE) also appears to be CF1. A μE1 oligonucleotide competitor competes effectively for CF1 binding to both the −260 and the −390 sites in the c-*myc* promoter (Fig. 1A, lane 3, and Fig. 1B, lane 7). When a fragment from the IgHE [*Dra*I(320)-*Dde*I(518)] was used as probe, a complex with mobility similar to that of CF1 was specifically sensitive to competition with the −260 CF1 oligonucleotide (Fig. 1C, lane 10). This site was originally shown to be occupied in vivo (13), and subsequent mutational analyses showed that it was a positive element in the enhancer (24, 33). We have previously partially purified and characterized a protein, which we called IgEBP-B (35), which binds to this site. The elution profiles of IgEBP-B and CF1 are identical on Mono Q anion-exchange columns (data not shown), and both proteins have an off rate under 30 s (22, 35), consistent with the possibility that the same protein binds both sites.

Finally, Fig. 1D shows that CF1 also appears to bind to the −90 (downstream CBAR) site in the skeletal α-actin promoter. This site was originally shown to be important for

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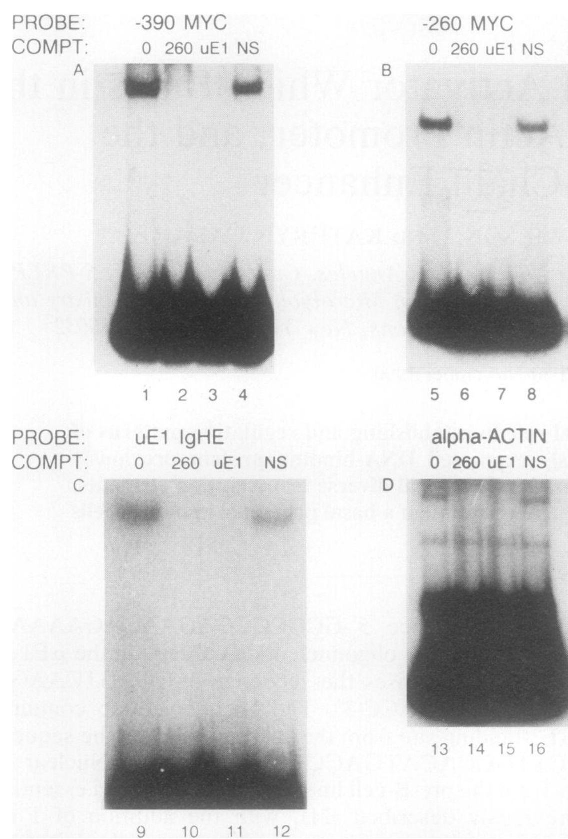

FIG. 1. Cross-competition EMSA of CF1 binding to multiple sites. (A through D) Competition for CF1 bound to the murine c-*myc* promoter fragment *Ava*I(−424)-*Hae*II(−313) (numbering relative to the P1 start site of transcription), the murine c-*myc* promoter fragment *Hae*II(−313)-*Sca*I(−199), the murine IgHE fragment *Dra*I(320)-*Dde*I(518) (IgEBP-B) (numbering as in reference 13), and the murine skeletal α-actin promoter fragment *Xma*I(−152)-*Xma*I(−41) (numbering relative to the start site of transcription), respectively. Oligonucleotides contained the −260 c-*myc* site (lanes 260), the μE1 site of the IgHE (lanes μE1), and the μE3-binding site from the IgHE (lanes NS). COMPT, Competitor.

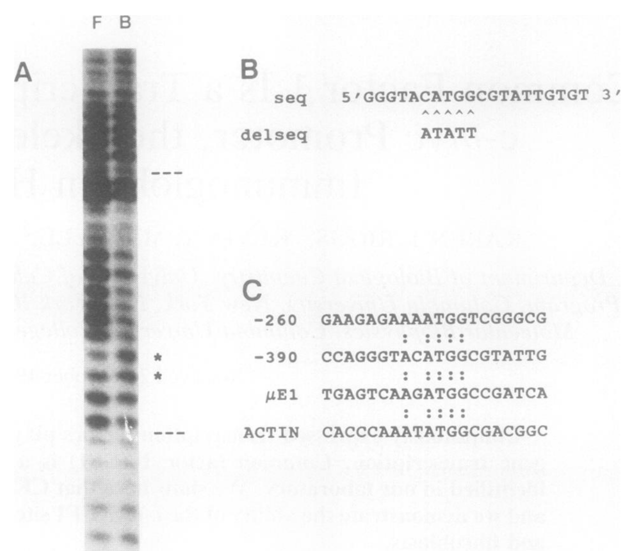

FIG. 2. Binding sites for CF1. (A) OP/Cu footprint of CF1 bound to the −390 c-*myc* fragment *Ava*I(−424)-*Hae*II(−313). F and B, free and bound probe, respectively. Asterisks indicate hypersensitive sites. (B) Sequence. The sequence "seq" indicates the regions of the fragment either protected by CF1 binding or made hypersensitive. Changes indicated by arrows were produced in seq by site-directed mutagenesis to produce the sequence indicated in "delseq." (C) Comparison of the c-*myc* −260, c-*myc* −390, μE1, and skeletal α-actin CF1-binding site sequences. Colons indicate nucleic acids conserved among all four sequences.

function of the chicken skeletal α-actin promoter (10) and is completely conserved in the murine skeletal α-actin promoter (17). A complex migrating with the same mobility as CF1 was detected by EMSA by using a probe containing this site [*Xma*I(−152)-*Xma*I(−41)] (Fig. 1D, lane 13). This complex was sensitive to competition with the *myc* −260 CF1 and the μE1 oligonucleotides but not with a heterologous competitor (Fig. 1D, lanes 14 through 16). Two smaller probes were made such that the only region of overlap between them occurred at the −90 site. Both probes bound a protein with the same mobility as that seen with the larger probe, and the bound proteins were subject to competition with −260 and μE1 oligonucleotides but not with a heterologous competitor (data not shown).

A comparison of the nucleic acid sequence of the CF1-binding sites at −390 and −260 in the c-*myc* promoter, the μE1 site in the IgHE, and the −90 site in the skeletal α-actin promoter is shown in Fig. 2C. A conserved core sequence, ANATGG, is shared by the four binding sites. On the basis of the cross-competition by EMSA and sequence similarities of the binding sites, we conclude that the proteins binding these sites have the same binding specificity and may be identical.

To determine more conclusively whether the same or different proteins recognized these sites, we used a proteolytic clipping band shift assay (39) to determine the protease sensitivity of the proteins bound to the c-*myc* and IgHE sites; for technical reasons, the skeletal α-actin site was not tested. The −260 and μE1 oligonucleotides (see the legend to Fig. 1) were cloned into the *Hin*cII site in the pUC19 polylinker and excised with *Eco*RI and *Hin*dIII to produce −260 c-*myc* and μE1 IgHE probes free from additional protein-binding sites. Reactions were allowed to bind for 10 min after the addition of probe, and then buffer or 1, 5, 10, 25, or 50 ng of V8 protease was added. Digestion was allowed to proceed for 10 min, and the reaction was stopped by loading on an EMSA gel. When a panel of five proteases was tested, four proteases (trypsin, chymotrypsin, elastase, and proteinase K) destroyed binding to all three sites (data not shown). When *Staphylococcus aureus* V8 protease (EC 3.4.21.19) was used for proteolytic clipping band shift assay analysis of CF1 bound to the −260 c-*myc* site, a distinctive pattern of digestion showing three subfragments with binding ability was obtained (Fig. 3B, fragments F1, F2, and F3). When the proteins binding to the −390 c-*myc* site and to the μE1 site were subjected to the same digestion, the identical distinctive pattern of proteolytic cleavage was obtained (Fig. 3A and C). This shows that proteins binding to the three sites have similar protease sensitivities and that the V8 fragments formed which still retain binding have the same mobilities. We might not have detected very small amino acid differences, such as alternate splicing of a small exon, or differences in posttranslational modification by this technique, but the results are most consistent with the suggestion that the

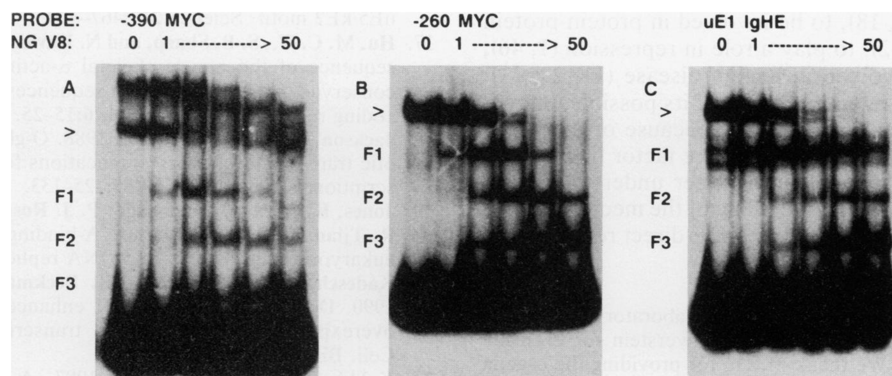

FIG. 3. Limited *S. aureus* V8 protease digestion of CF1 binding to multiple sites. (A through C) V8 digestion of CF1 bound to the −390 c-*myc* promoter fragment *Ava*I(−424)-*Hae*II(−313), the cloned −260 c-*myc* oligonucleotide, and the cloned μE1 oligonucleotide, respectively. Arrows indicate intact CF1. F1, F2, and F3 indicate DNA-binding proteolytic subfragments.

same protein binds to all three sites. Thus, we conclude that the same protein (or extremely similar proteins) binds to sites at −390 and −260 in the c-*myc* promoter and the μE1 site in the IgHE. Henceforth, we call the protein which recognizes these sites CF1.

CF1 can activate transcription. The functional importance of the CF1 (formerly μE1 or B) site in the IgHE has been previously established (24, 33, 44). It appears to be a positive activator. CF1 binds adjacent to the IgHE μE5 site, which appears to bind both positive and negative regulators (16, 20, 30, 45). Although extrapolation must be made from chickens to mice, the putative CF1-binding site in the skeletal α-actin promoter also appears to be a positive element essential for promoter function (10). We have previously suggested that the repressor *myc*-PRF either displaces CF1 from the −260 c-*myc* site or engages it in a complex and alters its DNA-binding characteristics (22). We wished to determine directly the functional activity of the CF1 sites in the c-*myc* promoter. When a construct in which 1.14 kb of 5′ flanking sequence and the first exon of c-*myc* control the transcription of the chloramphenicol acetyltransferase (CAT) gene (22), or the same construct from which the block to elongation had been removed by deletion of the region between *Not*I(335) and *Xho*I(516), was used, site-directed mutation of the −260 c-*myc* site or both the −390 and the −260 c-*myc* CF1-binding sites did not produce any decrease in the levels of CAT expressed (data not shown). However, deletions in this construct have previously produced conflicting results (2, 22, 27, 37), and deletion of individual sites from complex transcriptional regulation regions has frequently shown functional redundancy among multiple sites (24, 33, 44). Therefore, we adopted an alternate strategy for assessing the function of CF1. The c-*myc* −260 CF1 site was multimerized, and its ability to activate transcription of a heterologous promoter was tested. Four copies of the −260 CF1 oligonucleotide were cloned into the *Xba*I site upstream of the partial (−109 to +55 bp) herpes simplex virus thymidine kinase promoter present in the construct pBLCAT2 (25), creating a new construct, pBLCAT2(CF1-4). Twenty micrograms of these constructs was transfected into L-cell (fibroblast) and P3X63-Ag8 (plasmacytoma) cell lines by calcium phosphate precipitation (28). Cells were harvested approximately 48 h after transfection; levels of CAT activity after correction for transfection efficiency as determined by cotransfection of 2 μg of the growth hormone construct pXGH5 (40) for L cells or of 50 μg of pCH110 (15) for P3X cells are shown in Table 1. A low level of CAT activity was detected upon transfection of pBLCAT2 into L cells or P3X cells. When pBLCAT2(CF1-4) was transfected into L cells, the amount of CAT produced was sevenfold greater than that produced by pBLCAT2. In P3X cells, expression of CAT by pBLCAT2(CF1-4) was 2.5-fold greater than CAT expression by pBLCAT2 [elimination of pCH110 from the P3X transfections resulted in a substantial increase in the production of CAT from pBLCAT2(CF1-4) and not from pBLCAT2, but these results could not be corrected for transfection efficiency]. These data demonstrate that the c-*myc* −260 CF1 site binds a positive activator which can activate transcription from a heterologous promoter. Although we have not directly shown that the CF1 sites play a positive role in the context of the complete c-*myc* promoter, our data are most consistent with that possibility. A positive role for CF1 in the c-*myc* promoter is also consistent with the previously demonstrated positive activity of the CF1 site in the IgHE and the positive role of the related site in the skeletal α-actin promoter.

In conclusion, our data suggest that CF1 is a transcriptional activator which binds to sites in the c-*myc* promoter, the IgHE, and the skeletal α-actin promoter. Because CF1 is expressed at approximately the same level in all cell types and tissues examined, this makes CF1 a new addition to the growing collection of ubiquitously expressed transcription factors which are known to activate more than one gene (5, 7, 14, 19, 29, 30, 38). Other ubiquitously expressed DNA-binding proteins have been shown to undergo posttransla-

TABLE 1. Transient transfection of and CAT expression in P3X and L cells

Cell line and construct	% CAT activity (mean ± SEM)[a]
P3X	
pBLCAT2	2.8 ± 0.5
pBLCAT2(CF1-4)	6.6 ± 0.5
L	
pBLCAT2	0.5 ± 0.6
pBLCAT2(CF1-4)	3.6 ± 0.8

[a] CAT activity was determined as percent conversion of unacetylated chloramphenicol to acetylated chloramphenicol and adjusted for transfection efficiency. Results are means of at least four independent transfections which were normalized to cotransfected controls.

tional modification (3, 18), to be involved in protein-protein interactions (34, 38, 42), to play a role in repression (1, 46), and perhaps to be involved in human disease (11, 23, 31). CF1 is particularly intriguing because of its possible interaction with the repressor *myc*-PRF and because of the juxtaposition of its binding site and a negative factor binding site in the IgHE. We anticipate that a better understanding of CF1 will lead to a better understanding of the mechanisms by which the c-*myc* promoter and the IgHE direct regulation of gene expression.

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