Serum amyloid A (SAA) is a major acute-phase protein synthesized and secreted mainly by the liver. In response to acute inflammation, its expression may be induced up to 1000-fold, primarily as a result of a 200-fold increase in the rate of SAA gene transcription. We showed previously that cytokine-induced transcription of the SAA3 gene promoter requires a transcriptional enhancer that contains three functional elements: two CCAAT/enhancer-binding protein (C/EBP)-binding sites and a third site that interacts with a constitutively expressed transcription factor, SAA3 enhancer factor (SEF). Each of these binding sites as well as cooperation among their binding factors is necessary for maximum transcription stimulation by inflammatory cytokines. Deletion or site-specific mutations in the SEF-binding site drastically reduced SAA3 promoter activity, strongly suggesting that SEF is important in SAA3 promoter function. To further elucidate its role in the regulation of the SAA3 gene, we purified SEF from HeLa nuclear extracts to near homogeneity by using conventional liquid chromatography and DNA affinity chromatography. Ultraviolet cross-linking and Western experiments indicated that SEF consisted of a single polypeptide with an apparent molecular mass of 65 kDa. Protein sequencing and antibody supershift experiments identified SEF as transcription factor LBP-1c/CP2/LSF. Co-transfection of SEF expression vector with SAA3-luciferase reporter resulted in approximately a 5-fold increase in luciferase activity. Interestingly, interleukin-1 treatment of SEF-transfected cells caused dramatic synergistic activation (31-fold) of the SAA3 promoter. In addition to its role in regulating SAA3 gene expression, we provide evidence that SEF could also bind in a sequence-specific manner to the promoters of the α2-macroglobulin and Aα-fibrinogen genes and to an intrinsic enhancer of the human Wilm’s tumor 1 gene, suggesting a functional role in the regulation of these genes.

The defense processes initiated in most vertebrates after infection or tissue injury are termed the acute-phase response (1). One characteristic of this response is changes in the circulating plasma protein profile, reflecting the synthesis and secretion of proteins involved in immune function and wound repair (2). After tissue injury or infection, macrophages and monocytes near the damaged site detect the infectious agent or damaged cells and respond with a first wave of synthesis of cytokines, mainly of interleukin-1 (IL-1) (3) and tumor necrosis factor. These first-wave cytokines trigger the surrounding cell types, such as fibroblasts and blood vessel endothelial cells, to respond with an amplified second wave of cytokine synthesis, which includes a large amount of IL-6. A significant amount of these cytokines is transported in the blood stream and triggers the acute-phase response in target tissues such as the liver. The liver is one of the major targets for these proinflammatory cytokines because it has the largest number of cells with cytokine receptors as well as a high density of receptors per cell (3–5). The liver responds to the cytokine stimulation by a burst of synthesis of acute-phase plasma proteins. The magnitude of the changes in the relative plasma concentrations of these proteins ranges from less than 2-fold to several hundredfold after acute inflammation.

Elevated expression of acute-phase genes is regulated primarily at the transcriptional level. Analyses of many acute-phase gene promoters have revealed two general types of regulatory cis-acting elements in the transcriptional induction by cytokines: the binding sites for constitutive factors such as C/EBPα, hepatocyte nuclear factor 1, and hepatocyte nuclear factor 3 and the binding sites for inducible transcription factors such as C/EBPβ, C/EBPδ, NFκB, and signal transducer and activator of transcription proteins (STATs). In most cases, full transcriptional activation of these acute-phase gene promoters requires the combined action of a constitutive factor and an inducible transcription factor or factors. For example, induction of β-fibrinogen by IL-6 requires the cooperative interaction of three transcription factors: the constitutively expressed transcription factor hepatocyte nuclear factor 1, the IL-6-inducible C/EBPβ protein, and an unidentified IL-6-responsive factor (6). In the promoter of the C-reactive protein gene, the binding site for members of the C/EBP family and hepatocyte nuclear factor 1 are required for full promoter activity after cytokine induction (7, 8).

The serum amyloid A (SAA) gene family belongs to one of the major acute-phase proteins. In mice, there are four SAA genes (SAA1, SAA2, SAA3, and SAA5) and a pseudogene (9–11). The

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The abbreviations used are: IL-1, interleukin-1; SAA, serum amyloid A; SEF, SAA3 enhancer factor; DRE, distal response element; C/EBP, CCAAT/enhancer-binding protein; EMSA, electrophoretic mobility shift assay; bp, base pair(s); PAGE, polyacrylamide gel electrophoresis; STAT, signal transducer and activator of transcription protein; LC, liquid chromatography; MS, mass spectrometry; CID, collision-induced dissociation; WT1, Wilm’s tumor 1.

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SAA plasma concentration rises from 0.5 μg/ml to more than 1000 μg/ml 24 h after injection of bacterial lipopolysaccharide (12). SAA circulates as an apolipoprotein of high density lipoprotein particles, and at the peak of inflammation, it constitutes up to 20% of the total protein in the high density lipoprotein particles (12). SAA has been suggested to play a role in reverse cholesterol transport of high density lipoprotein by affecting the activity of the enzyme lecithin-cholesterol acyltransferase, which converts cholesterol to cholesteryl esters (13). However, continuous overproduction of SAA associated with chronic inflammation often results in secondary amyloidosis, an incurable and frequently fatal disorder (14).

The large increase in the hepatic synthesis of SAA is primarily a consequence of dramatically increased transcription of SAA genes (10, 15). Thus, transcriptional induction of SAA genes is an excellent model system for studying differential gene expression in response to a specific stimulus. To dissect the molecular mechanisms of SAA gene regulation, we have studied the promoters of the rat SAA1 (16, 17) and mouse SAA3 genes (18–20). Our studies of the rat SAA1 promoter have shown the functional importance and cooperative interaction between NFκB and C/EBP proteins in cytokine-induced expression. Our studies of the mouse SAA3 promoter demonstrated that a 350-bp promoter fragment was necessary and sufficient to confer cytokine responsiveness. Two elements were identified in this 350-bp promoter fragment: a proximal response element, which contains two adjacent C/EBP binding sequences that enhance SAA3 gene expression in liver-derived cells, and a distal response element (DRE), which confers responsiveness to cytokine induction and has properties of an inducible transcription enhancer (19). We demonstrated that DRE consists of three functionally distinct elements: the A element, a weak binding site for C/EBP family proteins; the B element, which also interacts with C/EBP family proteins but with a much higher affinity; and the C element that interacts with a constitutive nuclear factor, which was named SAA3 enhancer factor (SEF). Deletions and site-specific mutation studies revealed that all three elements are required for maximum promoter activity. Deletions and mutations of the C element drastically reduce both basal and inducible activities of SAA3 promoter. Furthermore, although the C element does not interact with C/EBP directly and mutation of this element does not alter C/EBP binding to elements A and B, mutation of the C element nevertheless dramatically reduces the transactivation of the SAA3 promoter by C/EBP (20). Taken together, these functional studies clearly demonstrated that SEF is a critical component in the regulation of SAA3 promoter activity. To further our understanding of SAA3 gene regulation, we purified and characterized SEF from HeLa nuclear extracts and provide some evidence that SEF may play a broad role in regulating other gene promoters.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Preparation of Nuclear Extracts**—HeLa cells were grown in suspension in Spinner’s minimum essential medium supplemented with 5% (v/v) bovine calf serum (HyClone). The cells were maintained by daily dilution with fresh complete medium to 4.5 × 10^5 cells/ml and were grown to a density of 9 × 10^6 cells/ml before harvesting. Nuclear extracts were prepared as described previously (21). The cell pellet from 12 liters of cells was resuspended with 5 volumes of hypotonic buffer (10 mM HEPES, pH 7.6, 1.5 mM MgCl2, 10 mM KCl, 1 mM benzamidine, and freshly added 0.2 mM phenylmethylsulfonyl fluoride and 14 mM β-mercaptoethanol) and applied to a 160-ml DEAE-Sepharose column at a flow rate of 3 ml/min. After loading, the column was washed extensively with Buffer A, and SEF activity was subsequently eluted with 0.2 M NaCl in Buffer A. The DEAE eluates were loaded directly onto a 50-ml heparin-agarose column at a flow rate of 1 ml/min. After washing with 0.2 M NaCl in Buffer A, the bound SEF activity was eluted with 0.5 M NaCl in Buffer A. The eluate from the heparin-agarose column was then diluted to 0.25 M NaCl with Buffer A before being applied to a phenyl-Sepharose column (2.5 × 10 cm). The phenyl-Sepharose column was washed sequentially with Buffer A containing 0.25 M NaCl and Buffer A containing 0.25 M NaCl and 30% ethylene glycol before the SEF activity was eluted with Buffer A containing 65% ethylene glycol. The eluate from phenyl-Sepharose column was first dialyzed in TEG buffer (20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 10% glycerol, 0.05% Nonidet P-40, 1 mM benzamidine, 0.2 mM phenylmethylsulfonyl fluoride, and 14 mM β-mercaptoethanol) and then dialyzed in TEG buffer containing 0.1 mM NaCl for an additional 2 h. The dialyzed sample was mixed directly with the DNA affinity beads. The amount of beads and poly(dI-dC) used in the incubation depended on the amount of protein in the phenyl-Sepharose eluate. In general, approximately 100 μg of protein was incubated with 1.5 mg of DNA affinity beads and 50 μg of poly(dI-dC). This mixture was incubated in a roller at 4 °C for 30 min before being subjected to magnetic separation. After the magnetic separation, the SEF-bound magnetic beads were washed twice by resuspension in TEG buffer containing 0.1 M NaCl. SEF binding activity was then eluted from the DNA affinity beads with 0.4 M NaCl in TEG buffer. Unless otherwise stated, all purification procedures were performed at 4 °C. Protein concentrations were measured by the Bradford assay (23), and SEF activities were determined by electrophoretic mobility shift assays (EMSA).

**EMSA**—32P-labeled C element DNA containing a SEF-binding site (4 × 10^7 cpm) was incubated with protein samples from different stages of purification to assess SEF activity (20). Approximately 1–2 μg of protein was incubated with the radioactively labeled probe in TEG buffer containing 100 mM NaCl for 30 min at 4 °C. In assays with affinity-purified SEF samples, 5 μg/ml acetylated bovine serum albumin was included in the reaction buffer to minimize nonspecific loss of SEF protein. After incubation, the reaction mixtures were loaded onto a 5% polyacrylamide gel (19.1 cross-linking ratio) in glycerine buffer and subjected to electrophoresis at 200 V for 90 min at 4 °C. The gel was dried before autoradiography. The SEF activity was quantified with a PhosphorImager (Molecular Dynamics). One unit of SEF binding activity was defined as the amount of protein required to retard 10% of the labeled DNA under our standard assay conditions.

**Rabbit polyclonal antibody (anti-LCL)** raised against an N-terminal peptide, LPLADDEVIESGVLQQD, corresponding to amino acid residues 7 to 21 (30) was used in supershift experiments. DNA-affinity-purified SEF was incubated in 2 μl with 32P-labeled C element, and the presence of rabbit anti-LCL antiserum (1:1200 dilution) or preimmune serum for 30 min at 4 °C. The reaction mixtures were then subjected to electrophoresis as above.

**Electrophoresis and Silver Staining**—SDS-PAGE was performed as described by Laemmli (24), and protein sizes were determined by comparison with prestained molecular weight markers (Bio-Rad). Electro-
Purification and Identification of SEF

RESULTS

Purification of SEF—Originally identified in HepG2 and Hep3B cells, SEF activity was subsequently detected at high levels in several other cell types, including HeLa cells (20). As HeLa cells can be easily cultured and grown as cell suspensions to a high cell density, we chose to use HeLa nuclear extracts as our starting material for the purification of SEF. Steps in the purification were carried out as described under “Experimental Procedures.” Protein elutes from each purification step were assayed for SEF binding activities using end-labeled C element containing the SEF-binding site as probe. As shown in Fig. 1, nuclear extracts and eluates from DEAE, heparin, and phenyl-Sepharose columns all showed strong SEF binding activities. Moreover, the binding activity is sequence-specific because the SEF-DNA complex could be completely inhibited by an excess of wild-type C element but not by the mutated C element. Although the DEAE-Sepharose and heparin steps only modestly increased the specific activity of SEF (Table 1), they nevertheless efficiently concentrated the SEF activity and also eliminated some of the major contaminants in the crude nuclear extracts.

Phenyl-Sepharose Chromatography—The steps that achieved the most significant purification were the phenyl-Sepharose and DNA affinity chromatography steps. More than 90% of the protein from the heparin-agarose column either did not bind to the phenyl-Sepharose column or was eluted in the 30% ethylene glycol, 0.25 mM NaCl wash (Fig. 2A). Only about 6% of the protein loaded remained on the column and was eluted with 65% ethylene glycol. Some of the C element binding activity that apparently migrated at the same position as SEF was found in the flow-through fraction. There are two possible explanations for this observation, which are that the column capacity was insufficient for the amount of protein loaded, or the C element binding activity in the flow-through fraction may be not SEF but some interfering protein or proteins. To test the first possibility, we collected the flow-through and reloaded it onto a freshly prepared phenyl-Sepharose column. The binding activity was again recovered in the flow-through fraction; no binding activity was detected in the 30% and 65% ethylene glycol eluates (data not shown). The binding activity in the flow-through fraction was therefore not due to overloading of the column but rather may be due to another binding protein or proteins with properties different from those of SEF. To determine the sequence specificities of this binding activity, compe-
Purification and Identification of SEF

Table I

| Purification step            | Total protein | Total activity | Yield | Specific activity | Purification |
|------------------------------|---------------|----------------|-------|-------------------|--------------|
| Nuclear extracts             | 140           | 56             | 100   | 4.0 \times 10^2   | 1            |
| DEAE-Sepharose               | 50            | 52             | 95    | 1.0 \times 10^3   | 2.6          |
| Heparin-agarose              | 37            | 51             | 91    | 1.4 \times 10^3   | 3.5          |
| Phenyl-Sepharose             | 2             | 25             | 45    | 1.3 \times 10^4   | 32           |
| DNA affinity                 | 6 \times 10^{-4} | 11           | 20    | 1.8 \times 10^6   | 4500         |

* One unit of SEF activity is defined as the amount of protein required to retard 10% of labeled DNA under standard assay conditions.

**Fig. 2.** Phenyl-Sepharose chromatography. A, elution profile of C element binding activities from phenyl-Sepharose column as detected by EMSA. The reaction mixture contained 4 µl of input sample, 10 µl of flow-through fraction (FT), 10 µl of 30% ethylene glycol eluate (30% EG), and 4 µl of 65% ethylene glycol eluate (65% EG). The numbers denote the fraction numbers from each of the elution steps. The SEF-DNA complex is indicated. NS, nonspecific binding. B, competition analysis of C element binding activities in different fractions from phenyl-Sepharose chromatography. Protein samples (flow-through fraction (FT), 30% ethylene glycol (30% EG), and 65% ethylene glycol (65% EG) eluates) were incubated with labeled C fragment with or without a 100-fold molar excess of wild-type (WT) or mutant (mt) SEF oligos as competitors. The numbers denote the fraction numbers after each elution step. The positions of SEF and nonspecific (NS) complexes are indicated.

Purification of SEF from HeLa cell nuclear extracts

DNA Affinity Chromatography—To facilitate DNA affinity purification, we sought to define some parameters that would minimize protein degradation, preserve the integrity of the DNA affinity beads, and at the same time maintain maximum SEF binding. We examined the effects of various concentrations of EDTA, NaCl, and poly(dI-dC) on the ability of SEF to bind DNA. Our results showed that SEF binding activities were at or near optimal levels under a wide range of concentrations (2 to 18 mM EDTA, 50 to 110 mM NaCl, and 50 to 100 µg of poly(dI-dC)) (data not shown). Therefore, buffers used in DNA affinity chromatography included 10 mM EDTA, 100 mM NaCl, and 50 µg of poly(dI-dC) to maximize specific SEF binding and at the same time limit binding of nonspecific proteins to DNA affinity beads.

Because ethylene glycol severely interfered with SEF binding in our EMSA, it may therefore also affect binding of SEF to the DNA affinity beads and greatly reduce the efficiency of the DNA affinity column. To circumvent this problem, the 65% ethylene glycol eluate was dialyzed at 4 °C sequentially in TEG buffer containing 0.1M NaCl for an additional 2 h before incubation with the DNA affinity beads.

To confirm that the binding activities detected in the 0.4 M NaCl eluate were specific for SEF binding to the C element and to determine the efficiency of the DNA affinity column, we performed EMSA assays. As shown in Fig. 3, the wild-type C element oligonucleotides effectively competed for binding, but the mutant C element did not. Approximately 50% of the input SEF binding activity was recovered from this step (Table I).

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several minor bands were recovered in the DNA-affinity eluate when poly(dI-dC) was not included in the wash (Fig. 4, lane 5).

After an additional wash with poly(dI-dC), only three major protein species, with apparent molecular weights of 140, 105, and 65 kDa, remained (Fig. 4, lane 6). Overall, approximately 20% of the SEF activity was recovered, resulting in a 4500-fold purification (Table I).

The 65-kDa Protein Band Possesses SEF Binding Activity—Because three major protein species remained in the DNA affinity eluate, we performed UV cross-linking and Southwest-ern experiments to identify the protein that possesses the SEF binding activity. Results from the UV cross-linking experiment revealed one major DNA-protein complex on polyacrylamide gels with the adjusted protein molecular mass of approximately 65 kDa (Fig. 5A). Formation of this protein-DNA complex could be specifically competed by oligonucleotide containing the wild-type SEF-binding site but not by the mutant oligonucleotide. Similar results were obtained with in-gel UV cross-linking (data not shown). To confirm these findings, we analyzed the SEF-binding activity in the DNA-affinity purified samples by Southwestern analysis. Consistent with our UV cross-linking results, the polypeptide that bound to the radiolabeled, oligomerized wild-type C element (Fig. 5B, lane 1) but not the mutant probe (Fig. 5B, lane 2) was estimated to be 65 kDa. Taken together, our results indicate that the 65-kDa protein purified by the DNA affinity chromatography indeed possesses SEF binding activity.

Identification of SEF as LBP-1c/CP2/LSF—To determine the identity of this 65-kDa SEF protein, two peptides, Peptide-1 and Peptide-2, from the trypsin digestion were sequenced by mass spectrometry. The amino acid sequences obtained from both peptides were found to match exactly with two regions from the transcription factor LBP-1c/CP2/LSF (30–32). Peptide-1, with the amino acid sequence KLGELPEINGK, corresponds to amino acids 103 to 115 in LBP-1c, and Peptide-2, with the amino acid sequence AETNDSYHIILK, corresponds to residues 491 to 502 (30). In addition, the molecular mass of SEF and its ubiquitous tissue distribution characteristics are also consistent with it being LBP-1c/CP2/LSF. To further determine whether SEF and LBP-1c/CP2/LSF are indeed identical and are antigenically related, specific rabbit polyclonal antibodies against the N-terminal peptide of LBP-1c/CP2/LSF were used in supershift experiments with purified SEF protein. As shown in Fig. 6, purified SEF formed a strong SEF-DNA complex with 32P-labeled C element. The addition of anti-LCL antibodies, but not preimmune serum, completely supershifted the SEF-DNA complex. Taken together, our results demonstrated that SEF is identical to LBP-1c/CP2/LSF.

Transactivation of SAA3 Promoter by SEF—To investigate the function of SEF in the regulation of SAA3 promoter, we cotransfected HepG2 cells with wild-type pSAA3(-306)Luc reporter gene along with a SEF expression plasmid. As shown in Fig. 7, cotransfection of SEF increased the luciferase activity.

**Fig. 3.** DNA-binding properties of affinity-purified SEF. Protein samples from 65% ethylene glycol eluate from the phenyl-Sepharose column (PS), unbound fraction (Unbound), and 0.4 M NaCl eluate from the DNA affinity beads (DNA Affinity) were incubated with labeled C element with or without a 100-fold molar excess of wild-type (WT) or mutant (mt) SEF-binding site digonucleotides.

**Fig. 4.** SDS-PAGE analysis of SEF at different stages of purification. Protein samples at different stages of purification were subjected to SDS-PAGE and visualized by silver staining. NE, nuclear extracts; DEAE, DEAE-Sephacel; PS, phenyl-Sepharose; DNA Affinity, 0.4 M NaCl eluate from DNA affinity beads; and DNA Affinity*, 0.4 M NaCl eluate from DNA affinity beads after washing with poly(dI-dC).

**Fig. 5.** Identification of the protein band that possesses SEF binding activity. **A**, UV-induced cross-linking of protein-DNA complexes. Affinity-purified SEF was incubated with uniformly labeled and 5-bromodeoxyuridine-substituted C element and irradiated with UV light to covalently cross-link the polypeptide to the DNA probe. The DNA-protein adduct was resolved by SDS-PAGE and visualized by autoradiography. **B**, Southwestern analysis. The affinity-purified proteins were separated on a 7.5% SDS-PAGE, transferred to a nitrocellulose membrane, and incubated with 32P-labeled, multimerized oligonucleotides containing wild-type (WT) or mutated (mt) SEF-binding sites. The specific protein that bound the radioactive probe was visualized by autoradiography.

**Purification and Identification of SEF**

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was 32P-labeled and incubated with purified SEF in EMSA assays. The activity of the control and noncotransfected cells, to which a value of 1.0 (control) or with IL-1 (50 ng/ml). The results were normalized to the expression plasmids. Transfected cells were treated with medium alone because of saturation of a limited number of binding sites by an

by nearly 5-fold. Interestingly, although IL-1 alone induced the reporter gene activity by approximately 10-fold, stimulation of SEF-transfected cells with IL-1 resulted in dramatic synergistic activation of the SAA3 promoter with more than a 31-fold increase in luciferase activity. Consistent with its important functional role, mutations in the SEF binding site greatly reduced SAA3 promoter activity (20). Taken together, our data indicated that SEF is an important regulatory component at the SAA3 gene promoter and appears to cooperate with other IL-1-inducible factor(s) to confer the dramatic up-regulation in SAA3 gene expression.

Binding of SEF to the a2-Macroglobulin and Aa-fibrinogen Promoters and Wilms Tumor 1 Intronic Enhancer—Since SEF binding activities could be detected in nearly all cell lines and tissues examined (20), we sought to identify other potential target genes that may be regulated by SEF. A computer search for sequences homologous to SEF-binding sites identified several genes that contain SEF-like binding sequences. To determine whether SEF binds to these sequences, the oligonucleotides that correspond to the sequences from the a2-macroglobulin (33–35) and Aa-fibrinogen (36) promoters and the WT1 intronic enhancer (37) were end-labeled and used as probes in the EMSA assays. As shown in Fig. 8, when incubated with partially purified SEF, all three probes formed intense protein-DNA complexes that could be specifically competed by wild-type but not by mutated SEF binding oligonucleotides, suggesting that SEF may have a functional role in the regulation of these genes.

**DISCUSSION**

We previously demonstrated that a 350-bp promoter fragment from the mouse SAA3 gene was necessary and sufficient to confer cytokine-induced expression in hepatoma cells (19). Deletion studies identified a DRE that is responsible for the cytokine response and has the properties of an inducible transcriptional enhancer (20). We also demonstrated that the DRE consists of three functionally distinct elements: the A element, a weak binding site for C/EBP family proteins; the B element, which also interacted with C/EBP family proteins but with a much higher binding affinity; and the C element, which interacted with a novel constitutive nuclear factor, SEF (20). Each of these binding sites is required for maximum transcription activation by inflammatory cytokines. Deletion and site-specific mutations of the SEF binding site drastically reduced both the basal and inducible activities of the SAA3 promoter. Therefore, to understand the molecular mechanisms by which SEF regulates the SAA3 promoter, perhaps by cooperating with other transcription factors, we performed studies aimed at determining the identity of the SEF protein. Here, we have described the purification and initial characterization of SEF from HeLa nuclear extracts. By several chromatographic steps, including DNA affinity, we purified SEF to near homogeneity. The purified SEF had the same DNA-binding specificities as the HeLa and HepG2 nuclear extracts; they bound with identical DNA sequence specificity. Although all the purification steps contributed to SEF purification, the most important steps were the phenyl-Sepharose and DNA affinity chromatography. In the phenyl-Sepharose step, the amount of contaminating proteins was greatly reduced after sequential washes of the column. More importantly, two major nonspecific DNA binding activities that were still present after the DEAE-Sepharose and DNA affinity chromatography. In the DNA affinity chromatography column was by far the most efficient step. The basis of DNA affinity chromatography is the differential sensitivity of sequence-specific and nonspecific DNA-protein interactions to increases in the ionic strength of the buffer conditions (38, 39). Ideally, the protein samples would be loaded onto a DNA affinity column at an ionic strength optimal for specific protein binding and minimal for nonspecific interactions. Because the affinity of the SEF-binding site is such that the DNA affinity column must be loaded at relatively low salt concentrations, a potential problem arose because of saturation of a limited number of binding sites by an
excess of nonspecific DNA-binding proteins. We circumvented this problem by enriching the SEF activity using a series of conventional chromatographic separations before DNA affinity chromatography and by the addition of nonspecific competitor DNA to the pool of proteins. This strategy allowed us to achieve a more than 100-fold purification in a single purification step.

Several lines of evidence suggested that the 65-kDa polypeptide purified by DNA chromatography under native conditions is the SEF activity that specifically binds to the C element.

First, the 65-kDa polypeptide was one of the major bands present in an amount sufficient to account for the observed SEF binding activity. Second, the photoactivated protein-DNA cross-linking experiments detected a 65-kDa polypeptide that could be specifically competed by the wild-type oligo containing the SEF-binding site but not by the mutant. Third, a Southwestern assay showed that the 65-kDa polypeptide bound only to the wild type and not to the mutant multimerized probes.

Protein sequencing and antibody supershift experiments identified SEF as the transcription factor LBP-1c/CP2/LSF (30–32). LBP-1, CP2, and LSF were initially identified as cellular, immunological, and developmental processes. Future studies will aim to understand the molecular mechanisms by which SEF regulates the transcription of these and other genes involved in various cellular, immunological, and developmental processes.

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