Human surfactant protein B (SPB) is a 79-amino-acid hydrophobic protein that enhances the surface active properties of pulmonary surfactant. SPB is expressed in nonciliated bronchiolar and alveolar type II cells of the respiratory epithelium, and its expression increases markedly late in gestation. In the present study, a human pulmonary adenocarcinoma cell line, H441, was used in both functional and biochemical assays to identify DNA sequences controlling lung cell-specific expression of the SPB gene. DNase I hypersensitive studies demonstrated two distinct regions of lung cell-specific hypersensitivity located proximal to the SPB promoter and within the eighth intron of the gene. To functionally define these DNA sequences, a series of plasmid vectors were constructed in which segments of the human SPB gene and 5′-flanking sequence were linked to a CAT reporter gene and assayed for expression in lung and nonlung cell lines. Whereas far upstream and intronic sequences did not contain enhancer-like elements, a 258-bp-base pair DNA segment (base pair −218 to +41) was sufficient to support lung cell-specific expression. DNase I footprinting demonstrated that this pulmonary epithelial cell-specific promoter fragment contained five nuclear protein-binding sites, two of which bound lung cell-specific nuclear proteins. These results suggest that the pulmonary epithelial cell-specific expression of SPB is determined, in part, by both ubiquitous and cell type-specific protein-DNA interactions within the proximal promoter region.

Surfactant is a complex mixture of phospholipids and proteins secreted by the distal respiratory epithelium of the lung. Surfactant reduces surface tension at the alveolar surface of the lung and is essential for normal respiration. Four surfactant proteins designated surfactant protein A (SPA), surfactant protein B (SPB), surfactant protein C (SPC), and surfactant protein D (SPD) have been identified as distinct functional components of pulmonary surfactant (for reviews, see Refs. 1 and 2). The surfactant protein genes are expressed selectively in epithelial cells of the lung. Recently, molecular studies have provided much insight into the spatial, temporal, and hormonal regulation of surfactant protein synthesis during lung development (3–6). However, little is known about the cis-acting sequences and trans-acting factors that regulate surfactant protein gene expression. In particular, it is unclear whether gene expression in distinct cell types of the lung is regulated by common, overlapping, or unique sets of cis-active elements and trans-acting factors.

SPB, a low molecular weight hydrophobic protein released by proteolytic processing of a preproprotein (7, 8), interacts strongly with surfactant phospholipids to enhance the surface active properties of surfactant mixtures (9, 10). SPB is expressed selectively in epithelial cells of the lung and has been detected in both nonciliated bronchiolar cells and alveolar type II cells in human, mouse, and rat lung tissue (11–14). SPB gene expression increases with advancing gestation and is influenced by a variety of hormonal and cellular factors (for review, see Ref. 1). SPB synthesis is enhanced by glucocorticoids in human fetal lung explant tissue, fetal rat lung in vitro, and in two distinct human adenocarcinoma cell lines (14–17). Other effector molecules, such as phorbol esters and tumor necrosis factor α, inhibit SPB synthesis (18, 19). Thus, precise molecular mechanisms have evolved to regulate SPB gene expression.

In previous studies from this laboratory, we described a human pulmonary adenocarcinoma cell line, H441, that expressed SPB mRNA and preproprotein (20, 21). In this report, transient expression assays as well as DNase I hypersensitivity and DNase I footprinting were used to define cis-active sequences that regulate SPB gene expression in H441 cells. These studies demonstrate that SPB gene transcription in H441 cells is regulated by both ubiquitous and cell type-specific DNA-binding proteins.

**MATERIALS AND METHODS**

DNase I Hypersensitivity—H441 and RAJI cells were disrupted by Dounce homogenization in polyamine buffers modified from that of Hewish and Burgoine (22). The use of the polyamine buffer was critical in that DNA purified from nuclei that contained calcium exhibited substantial cleavage at the typical hypersensitive sites even in the absence of added DNase I. The polyamine buffer contained 0.34 M sucrose, 53 mM KCl, 13 mM NaCl, 2 mM EDTA, 0.5 mM EGTA, 0.13 mM spermine, 0.5 mM spermidine, 14 mM freshly prepared 2-mercaptoethanol, 0.1% Triton X-100, 13 mM Tris-HCl, pH 7.4, 3 mM MgCl₂, and 1 mM freshly prepared phenylmethylsulfonyl fluoride. Nuclei were prepared from the homogenates and centrifuged at 2400 × g for 30 min over a cushion of 1.2 M sucrose in polyamine buffer. The nuclear pellet was washed twice in polyamine buffer without sucrose and detergent and resuspended in a DNase I digestion buffer that contained 60 mM KCl, 5 mM MgCl₂, 0.1 mM EGTA, 0.5 mM dithiothreitol, 5% glycerol, and 15 mM Tris-HCl, pH 7.5. Nuclei were resuspended at a concentration of 1.25 × 10⁶ to 3.5 × 10⁶ nuclei/ml, and gentle DNase I digestions were carried out in a volume of 0.2

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**Cis-active Elements Controlling Lung Cell-specific Expression of Human Pulmonary Surfactant Protein B Gene***

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Dynamics PhosphorImager. To ensure linearity of the assay, data was maintained in RPMI medium containing 10% fetal bovine serum. Cells were changed to RPMI with 10% fetal bovine serum. Cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cells were maintained as described (27). All cell lines were cultured at 37 °C, the samples were set at room temperature and after 5 min digested with DNase I (Promega) for 2 min. The reactions were stopped by the addition of 350 μl of stop buffer containing 230 mM NaCl, 17 mM EDTA, 1.14 mM SDS, 11.4 mM Tris, pH 7.8, and 250 μg/ml proteinase K. DNA was purified by phenol extraction and ethanol precipitation. DNA samples were fractionated on 6% polyacrylamide, 7 M urea sequencing gels.

**RESULTS**

**Identification of DNase I hypersensitive Sites Flanking the SPB Promoter**—Because many enhancer-like elements and other functional regions are associated with perturbations of chromatin structure, DNase I hypersensitivity (DH) assays were used to evaluate the SPB gene and 5'-flanking DNA. A 12.1-kb HindIII fragment was used to map DH sites. This fragment contained over 5 kb of 5' flanking sequence and over 8 kb of intragenic sequence extending to a HindIII site in intron 10. Autoradiograms of the indirectly end-labeled fragments that were generated by DNase I treatment of nuclei are shown in Fig. 1. A and B. Nuclei were analyzed from a human lung adenocarcinoma cell line (H441), nonlung cell lines (RAJI and GM4429B), and human thymus. A total of four hypersensitive sites were identified in H441 cells (Roman numerals, Fig. 1A). These sites, designated DNase I hypersensitive sites I to IV (DHL–DHIIV), were located proximal to the SPB promoter and within intron eight of the gene. Each site was mapped in two separate experiments by comparison of the DNase I liberated fragments to known molecular weight standards. The locations of these sites are summarized in Fig. 1C. An identical procedure detected no DH sites in preparations of RAJI cell nuclei (a nonlung human B-lymphoid cell line), GM4429B cell nuclei (an SV40-transformed human skin fibroblast cell line), or human thymic nuclei (Fig. 1B and data not shown). These data suggest that chromatin in H441 cell nuclei, but not nonlung cell nuclei, exists in a unique structure which is sensitive to DNase I and indicates that important regulatory regions may lie in close proximity to the promoter or within the gene.

**Sequences Flanking the SPB Promoter Direct Lung Cell-specific Expression**—To determine if sequences encompassing DNase I hypersensitive sites I and II were associated with functional transcriptional regulatory domains, 2.7 kb of sequence (−2244 to +436) was linked to a CAT reporter gene.
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FIG. 1. Cell type-specific DNase I hypersensitivity in the SPB gene and promoter region. A and B, nuclei were isolated from lung (H441) and non-lung (RAJI) cell lines and exposed to DNase I for 0, 1, 2.5, 5, 10, and 15 min (indicated 1-6 for each cell line). The sizes of the parental fragment and the fragments liberated by DNase I digestion are indicated in kilobases within parentheses. The sizes of each fragment were determined by comparison to known standards in each gel. Each distinct DNase I-liberated fragment is indicated by a Roman numeral (I-IV). In preparations of H441 cell nuclei, it was not possible to completely eliminate endogenous DNase I activity, and daughter fragments were detected even in the absence of added DNase I (lane 1). C, a schematic of the SPB gene and flanking sequence is shown. Filled boxes represent SPB exons 1-11. The probe used to indirectly end label the parent HindIII and DNase I-liberated fragments is labeled probe. A second HindIII site was mapped approximately 5 kb upstream of the transcription start site (not shown). Arrowheads and Roman numerals indicate the locations of hypersensitive sites. The lower heavy line shows the extent of sequence used to construct p2244/436-CAT.

The transcriptional activity of this construction (p2244/436-CAT) in the indicated cell lines was determined by transient transfection. Increased transcription of the CAT reporter was observed only in H441 cells, where an approximate 10-fold increase in expression relative to promoterless vector pSV0-CAT was observed (Fig. 2A, lanes 1 and 2). Transfection of p2244/436 into A549 cells, a human pulmonary adenocarcinoma cell line that does not express SPB, or HeLa cells did not support CAT transcription above promoterless vector (Fig. 2, B and C, lanes 1 and 2). This result indicated that a human lung adenocarcinoma cell line, H441, was capable of expressing chimeric SPB-CAT genes and that the human SPB gene promoter and flanking sequence contained within -2244 to +436 was transcriptionally active in a cell type-specific manner.

To determine if sequence encompassing hypersensitive sites III and IV contained additional regulatory elements, a genomic subfragment spanning intron eight was subcloned into the BamHI site downstream of the CAT reporter gene and SPB promoter and flanking sequence (-2244 to +436). The transcriptional activity of this construction was similar to p2244/436-CAT (data not shown). This result suggested that DHIII and DHIV were not associated with a typical enhancer element.

Deletion Analysis of Sequence Flanking the SPB Promoter—To better delineate cis-acting sequences that regulate SPB transcription in H441 cells, a series of 5' flanking deletions of SPB sequence were analyzed in transient expression assays. Each 5' deletion mutant had the same 3' end point at +436, containing sequence into SPB exon 2. A summary of the results obtained from transfection of these CAT reporter constructs is shown in Fig. 3. Each construction was assayed for expression in both H441 and HeLa cell lines. CAT activity varied in H441 cells with deletion of 5'-flanking DNA to -218 (pA5'-218), but there was no loss of activity relative to p2244/436-CAT and no construction expressed above the level of pSV0-CAT in HeLa cells. However, deletion of sequence to -80 (pA5'-80) resulted in an 82% reduction in reporter activity compared to p2244/436-CAT, suggesting that a positive cis-active element was located between -218 and -80.

To determine if additional regulatory elements were located downstream of the SPB transcription start site, we constructed a series of 3' intragenic deletion mutants. Each 3' deletion mutant had the same 5' end point at -2244 bp. A summary of the results obtained from transient expression of these CAT reporter constructs in H441 and HeLa cells is shown in Fig. 4. Deletion of 3'-flanking DNA to +41 (pA3'+41) or internal deletion of sequence encompassing most of the first intron (pA1(+112/+318)) did not significantly alter reporter gene activity. Further deletion of 3'-flanking DNA to +7 (pA3'+7) reduced transcriptional activity by 91% compared to p2244/436-CAT. In addition, internal deletion of sequence encompassing nucleotides +8 to +38 (pA(+8/+38)) also reduced transcriptional activity by 91%. This result
plasmid (5 pmol) containing the CAT reporter gene was cotransfected to the SPB promoter-CAT construction, p2244/436-CAT, contains bp vector pSVO-CAT as described under “Materials and Methods.” Activity was measured 48 h later and normalized to β-galactosidase using H441 nuclear extracts on both the coding and noncoding protein-binding sites within the SPB promoter and flanking DNA to -218 and adjacent intragenic DNA to +41 sequence, DNase I footprinting experiments were performed to the corresponding murine sequence revealed uninterrupted conservation of 11 (TGGAGGGGACTCT) and 12 (CAACACTGAGG) nucleotides in the SPB-f1- and SPB-f2-binding sites, respectively. Much less conservation was found in regions protected by both H441 and HeLa cell nuclear extract. Only 4 of 16, 6 of 24, and 15 of 19 nucleotides were conserved in the SPB-f3-, SPB-f4-, and SPB-f5-binding sites, respectively. Within SPB-f4, the murine sequence did not contain an Sp1 motif, however, a 7-bp TATA box element was conserved. Although an AP1-binding site motif was not identified within the murine sequence corresponding to SPB-f5 in exon 1, this motif was identified 7 bp downstream of the murine TATA box. Taken together, these experiments demonstrate that the SPB promoter proximal region contains five nuclear protein-binding sites, two of which bind novel lung cell-specific nuclear protein complexes. In particular, with the exception of the HNF-5 motif in SPB-f2, the sequence of the DNase I footprints specifically protected in H441 cells does not correspond to any known promoter or enhancer binding site motif and was conserved between the human and murine genes, suggesting that these elements represent novel lung cell-specific transcriptional regulatory pathways.

**Fig. 2. Cell-specific function of the SPB promoter region.** The SPB promoter-CAT construction, p2244/436-CAT, contains bp -2244 to +436 and is described under “Materials and Methods.” Each plasmid (5 pmol) containing the CAT reporter gene was cotransfected along with 2.5 pmol of pCMV-βgal into the indicated cell lines. CAT activity was measured 48 h later and normalized to β-galactosidase activity. The activity in each cell line is compared to the promotorless vector pSVO-CAT as described under “Materials and Methods.” pRSV-CAT is an external positive control for CAT activity. The results shown are representative of at least three independent transfections.

**Identification and Cellular Specificity of Nuclear Protein-binding Sites within the SPB Promoter**—To identify nuclear protein-binding sites within the SPB promoter and flanking sequence, DNase I footprinting experiments were performed using extract prepared from lung (H441) and nonlung (HeLa) cell lines. Five nuclear protein-binding sites were identified using H441 nuclear extracts on both the coding and noncoding DNA strands (single and double lines, Fig. 5, A and B). In addition, multiple DNase I hypersensitive sites, reflected as more intense bands of digestion, were observed between and within some of the footprinted regions (arrowheads, Fig. 5, A and B). This type of DNase I footprint has been described previously for complex promoters and enhancers containing multiple closely spaced cis-active elements and may reflect the bending of DNA adjacent to these sites (32, 33). Two footprinted regions, designated SPB factor 1 (SPB-f1; bp -107 to -93) and SPB factor 2 (SPB-f2; bp -90 to -73), were protected only with H441 cell nuclear extract (double lines, Fig. 5, A and B). The 5′-most binding site, SPB-f1, did not contain any previously identified enhancer or promoter motif. SPB-f2 contained a sequence motif for hepatocyte nuclear factor 5 (HNF-5; TGGTTTGT), a transcription factor previously described in liver (34, 35). Three additional nuclear protein-binding sites were identified in both H441 and HeLa cell nuclear extracts (single lines, Fig. 5, A and B) and designated SPB factor 3 to 5 (SPB-f3 to SPB-f5). SPB-f3 contained a six of nine match to the consensus CAAT box. Notably, SPB-f5 was located entirely within the protein coding region of the gene and encompassed a consensus AP1-binding site motif (5′-TGAGTCA). The locations of protected sequences and binding site motifs are summarized in Fig. 6.

Comparison of the human SPB promoter proximal region to the corresponding murine sequence revealed uninterrupted conservation of 11 (TGGAGGGGACTCT) and 12 (CAACACTGAGG) nucleotides in the SPB-f1- and SPB-f2-binding sites, respectively. Much less conservation was found in regions protected by both H441 and HeLa cell nuclear extract. Only 4 of 16, 6 of 24, and 15 of 19 nucleotides were conserved in the SPB-f3-, SPB-f4-, and SPB-f5-binding sites, respectively. Within SPB-f4, the murine sequence did not contain an Sp1 motif, however, a 7-bp TATA box element was conserved. Although an AP1-binding site motif was not identified within the murine sequence corresponding to SPB-f5 in exon 1, this motif was identified 7 bp downstream of the murine TATA box. Taken together, these experiments demonstrate that the SPB promoter proximal region contains five nuclear protein-binding sites, two of which bind novel lung cell-specific nuclear protein complexes. In particular, with the exception of the HNF-5 motif in SPB-f2, the sequence of the DNase I footprints specifically protected in H441 cells does not correspond to any known promoter or enhancer binding site motif and was conserved between the human and murine genes, suggesting that these elements represent novel lung cell-specific transcriptional regulatory pathways.

**DISCUSSION**

There is currently very little information on the DNA regulatory elements or transcription factors that direct lung-specific gene expression. The distinct regulated expression of SPB in both nonciliated bronchiolar and alveolar type II cells of the respiratory epithelium suggested that studies of the transcriptional regulation of this gene may provide important insights into lung-specific gene expression. This report demonstrates that the lung cell-specific transcription of the SPB gene is dependent on a 259-bp promoter fragment. This region is associated with a prominent domain of lung cell-specific DNase I hypersensitivity and interacts with both ubiquitous and lung cell-specific nuclear DNA-binding proteins.

To identify putative distal regulatory elements, we have exploited the DNase I hypersensitivity assay (36, 37). This method has provided consistent correlation between the location of DNA regulatory elements, such as enhancers or silencers, and the occurrence of DNase I hypersensitive sites (36, 37). The most striking finding in examining the DNase I hypersensitivity pattern of the SPB gene and 5′-flanking region was the cellular specificity of DH sites found close to or within the SPB promoter region and the lack of additional hypersensitivity within 5 kb of additional upstream sequence. Because those enhancers which have been examined are associated with DH sites (36, 37), this result suggested that sequence far upstream of DH and DHH did not contain characteristic enhancer domains. In agreement with this finding, deletion of sequence between -2241 and -218 did not significantly alter the maximal transcriptional activity of the SPB promoter in transient expression assays. Taken together,
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These data demonstrate that sequences sufficient to direct lung cell-specific expression of SPB reside within the proximal promoter region.

The failure of DHIII and DHIV to alter CAT activity in transient expression experiments indicates that these sites do not correspond to typical enhancer elements. Although it has been emphasized that not all DH sites denote transcriptionally functional domains (36, 37), the function of these sites may not be discernible outside of their genomic context or by transient expression analysis. For example, Darnell et al. (38) have identified (within hypersensitive regions upstream of the transthyretin gene) in vivo footprints which are tissue specific but have little or no discernible effect on the level of transient expression in hepatocytes. These same DNA sequences though, are within stretches of DNA required for tissue-specific expression in transgenic mice (39). This requirement for stable integration is also true for the hemoglobin locus control region and the recently identified distal regulatory region of the mouse MyoD gene (40, 41). In none of these cases is there an understanding of the role integration plays in regulating the activity of these regions. To determine if higher order structure influences the regulatory properties of the SPB gene promoter and flanking sequence, we are now examining the spatial and temporal expression conferred by these sequences in transgenic mice and stable transfection experiments. Preliminary results indicate that the SPB promoter proximal region (bp -218 to +41) is sufficient to direct lung-specific expression of a CAT reporter gene in transgenic mice; however, the level of expression from this transgene is low and may reflect a species-specific difference or, alternatively, a lack of appropriate regulatory elements. If additional elements exist, they may contain integration-dependent enhancer elements similar to the locus control region and the distal regulatory region or elements like the A element of the lysozyme gene (42) and DHIII of the adenosine deaminase gene (43) which give copy number-dependent expression but lack enhancer activity. Alternatively, additional regulatory regions may be located either far upstream or downstream of the SPB gene locus; a muscle-specific enhancer is located more than 24 kb downstream of the myosin light-chain 1/3 gene locus (44).

DNase I footprint analysis of the human SPB promoter revealed five nuclear protein-binding sites between bp -102 and +32. The two 5'-most binding sites, SPB-f1 and SPB-f2, interacted with nuclear proteins present only in H441 cells, and deletion of these sites resulted in significant reduction in the transcriptional activity of the SPB promoter. With the exception of an HNF5 motif identified in SPB-f2, the se-

3 R. J. Bohinski, unpublished observations.
before partial digestion with DNase I. Standard Maxam and Gilbert purine bp fragment were end labeled and incubated in the absence containing the SPB lung cell-specific promoter was subjected to specific promoter region.

Extracts. The coding did not reveal elements with significant homology to SPB-fl genes (45). A search of the 5'-flanking regions of genes that homology to more than 150 functional elements for vertebrate and or SPB-f2. However, it is possible that once important bases for binding are identified and/or transcriptional proteins are isolated or cloned, binding sites in these or other lung genes.

did not reveal elements with significant homology to SPB-fl or SPB-f2. The coding (left) and noncoding (right) strands of the 300-bp fragment were end labeled and incubated in the absence (control, lane 2) or presence of H441 (lane 3) or HeLa (lane 4) nuclear extracts before partial digestion with DNase I. Standard Maxam and Gilbert purine (A + G) sequencing reactions of the same fragments were run in parallel (lane 1). Protected sequences identified with only H441 nuclear extract are indicated with double lines and labeled SPB-fl and SPB-f2. Sequences protected by both H441 and HeLa cell nuclear extracts are indicated with single lines and labeled SPB-fl, SPB-f4, and SPB-f5. Arrowheads denote sites hypersensitive to DNase I.

Fig. 5. DNase I footprint analysis of the SPB lung cell-specific promoter region. A 300-bp fragment (bp -220 to +80) containing the SPB lung cell-specific promoter was subjected to DNase I footprint analysis using H441 lung cell and HeLa cell nuclear extracts. The coding (left) and noncoding (right) strands of the 300-bp fragment were end labeled and incubated in the absence (control, lane 2) or presence of H441 (lane 3) or HeLa (lane 4) nuclear extracts before partial digestion with DNase I. Standard Maxam and Gilbert purine (A + G) sequencing reactions of the same fragments were run in parallel (lane 1). Protected sequences identified with only H441 nuclear extract are indicated with double lines and labeled SPB-fl and SPB-f2. Sequences protected by both H441 and HeLa cell nuclear extracts are indicated with single lines and labeled SPB-fl, SPB-f4, and SPB-f5. Arrowheads denote sites hypersensitive to DNase I.

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Sequences of SPB-fl and SPB-f2 did not contain significant homology to more than 150 functional elements for vertebrate genes (45). A search of the 5'-flanking regions of genes that are expressed in the lung, including human and murine surfactant proteins A and C, and rat Clara cell secretory protein, did not reveal elements with significant homology to SPB-fl or SPB-f2. However, it is possible that once important bases for binding are identified and/or transcriptional proteins are isolated or cloned, binding sites in these or other lung genes will become evident. Comparison of the human and murine SPB 5'-flanking sequence demonstrated that SPB-fl and SPB-f2 were evolutionarily conserved in spite of sequence divergence outside of this region. The final indication that SPB-fl and SPB-f2 are important to the lung cell specificity of SPB gene regulation was the low promoter activity in HeLa cells which lacked SPB-fl and SPB-f2 binding activity but contained SPB-f3 to SPB-f5 binding activity.

The HNF5 motif in SPB-f2 is noteworthy for several reasons. First, the interaction of HNF5 with its binding site within the tyrosine aminotransferase (TAT) 5'-flanking DNA results in an in vitro DNase I footprint characterized by the presence on each strand of a DNase I-hypersensitive site that is situated between the fifth and sixth base of the motif (34, 35). This unique pattern of protection and hypersensitivity is similar to the footprint associated with the HNF5 motif within SPB-f2. Second, HNF5 is involved in a complex mechanism of TAT gene activation involving the concerted action of HNF5 and a glucocorticoid receptor (34, 35). Interestingly, SPB gene expression has been shown to respond dramatically to corticosteroids in both fetal lung explants and two distinct pulmonary adenocarcinoma cell lines (14–17), and part of this response has been attributed to modulation of SPB transcription (16). Third, several hepatocyte-enriched transcription factors have also been identified in the lung, including hepatocyte nuclear factor 3 and CCAAT/enhancer-binding protein family members (46). Recently, Sawaya et al.4 have described a functional hepatocyte nuclear factor 3-binding site within the rat Clara cell secretory protein promoter, a lung cell-specific gene with expression that overlaps but is distinct from SPB (30). Although the tissue distribution of HNF5 and the precise cellular distribution and transcriptional role of hepatocyte nuclear factor 3 and CCAAT/enhancer-binding protein family members within the lung is not currently known, it is possible that transcription factors specifying hepatocyte-specific gene expression have also been adapted to direct gene expression in the lung.

In addition to 5'-flanking sequences, an intragenic DNA sequence (bp +8 to +38) was critical to SPB transcriptional activity. DNase I footprinting demonstrated that this site (SPB-f5) interacted with nuclear protein(s) from both H441 and HeLa cell nuclear extracts. Sequence analysis of this region identified a consensus DNA-binding site for the AP1 transcription factor (47). Interestingly, this site was contained entirely within the protein coding region of the gene. A similarly positioned cis-active element has previously been reported within the first translated exon of the glial fibrillary acidic protein and has been shown to function as an initiator element (48). Initiator elements have been proposed to act independently (49) or in conjunction with additional elements (50) to accurately direct the initiation of transcription. This process is distinct from that proposed for elements, including AP1-, Sp1-, and CAAT box-binding protein, that modulate the rate of transcription (51, 52). Although the sequence motifs associated with SPB-f5 are not homologous to any previously described initiator elements, additional studies will be necessary to define the role of this binding site in SPB promoter function.

Finally, these results demonstrate that the positive regulatory influence of SPB-fl and SPB-f2 or SPB-f5 cannot function independently in the context of the SPB promoter. Deletion of either site resulted in a low level of expression, and preliminary data from heterologous gene systems indicate that these elements alone will not support the normal regulatory activity associated with SPB. Rather, it is likely the

4 P. L. Sawaya, B. R. Stripp, J. A. Whitsett, and D. L. Luse, manuscript in preparation.
concerted action of each of these regions that contributes to the expression of this gene. Current studies are investigating whether individual or multiple lung specific and/or ubiquitous trans-factors must interact within this region to produce appropriate expression. The finding that the SPB promoter region contains two evolutionarily conserved and previously undescribed nuclear protein-binding sites and that at least one of these sites is not related to any previously described lung regulatory region or to other consensus sites, strongly suggests the existence of novel lung cell-specific transcription factors. These results should facilitate studies designed to elucidate the mechanisms of cell type-specific gene expression within the lung.

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REFERENCES

1. Weaver, T. E., and Whislet, J. A. (1991) Biochem. J. 273, 249-264
2. Hagwood, S., and Shiffer, K. (1991) Annu. Rev. Physiol. 53, 375-394
3. Maudeloun, C. R., and Boggaram, V. (1991) Annu. Rev. Physiol. 53, 415-440
4. Wert, S. E., Glasser, S. W., Korfhagen, T. R., and Whislet, J. A. (1993) Dev. Biol., in press
5. Glasser, S. W., Korfhagen, T. R., Bruno, M. D., Dey, C., and Whislet, J. A. (1990) J. Biol. Chem. 265, 21996-21991
6. Wert, S. E., Glasser, S. W., Wert, S. E., Bruno, M. D., Daugherty, C. C., McNeish, J. D., Stock, J. L., Potter, S., and Whislet, J. A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 6122-6126
7. Weaver, T. E., and Whislet, J. A. (1989) Am. J. Med. 95, L100-L108
8. O'Reilly, M. A., Weaver, T. E., Pilot-Matias, T. J., Sarin, V. K., Gazdar, A. F., and (1986) Biochem. Biophys. Acta 1011, 146-148
9. Cochrane, C. G., and Revak, S. D. (1991) Science 254, 566
10. Baizs, J. E., Ellidge, B., and Whislet, J. A. (1990) Biochemistry 29, 6714-6729
11. Stahlman, M. T., Gray, M. E., and Whislet, J. A. (1992) J. Histochem. Cytochem. 40, 1471-1480
12. Phelps, D. S., and Flores, J. (1988) Am. Rev. Respir. Dis. 137, 939-942
13. D'Amore-Bruno, M. A., Wikenheiser, K. A., Carter, J. E., Clark, J. C., and Whislet, J. A. (1992) Am. J. Pathol. 140, 1-17
14. Phelps, D. S., and Flores, J. (1991) Am. J. Pathol. 140, L146-L152
15. Phelps, D. S., and Flores, J. (1990) Am. J. Pathol. 146, L146-L152
16. Whislet, J. A., Weaver, T. E., Clark, J. C., Sawtell, N., Glasser, S. W., Korfahgen, T. R., and Hull, W. M. (1987) J. Biol. Chem. 262, 15018-15023
17. O'Reilly, M. A., Clark, J. C., Whitsett, J. A., and Phuyher, G. S. (1992) Am. J. Physiol. 262, L688-L693
18. O'Reilly, M. A., Gazdar, A. F., Morris, R. E., and Whislet, J. A. (1988) Biochem. Biophys. Acta 970, 194-204
19. Gazdar, A. F., Linnola, R. I., Kurita, Y., Oin, H. K., Muihine, J. L., Clark, A. J., and Whislet, J. A. (1990) Cancer Res. 50, 5467-5467
20. Hewis, D. R., and Burgoyne, L. A. (1973) Biochem. Biophys. Res. Commun. 52, 504-510
21. Phillips, T. J., Kitzer, S. E., Fox, J. L., Kropk, K., Glasser, S. W., and Whislet, J. A. (1989) DNA 8, 75-86
22. Gorman, C. M., Mofat, L. F., and Howard, B. H. (1982) Mol. Cell. Biol. 2, 1044-1051
23. Miller, J. H. (1972) in Experiments in Molecular Genetics, pp. 352-355, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Alam, J., Yu, S., Irias, S., Cook, J. L., and Vig, E. (1991) BioTechniques 10, 423-425
25. Annov, B., Lattier, D., Silberman, D., Dusen, S., Hutton, J., Jones, G., Stock, J., McNeish, J., Potter, S., Witte, D., and Wigniton, D. (1989) BioTechniques 10, 2253-2254
26. Shapiro, D. J., Sharp, P. A., Walth, W. W., and Keller, M. J. (1988) DNA (N. Y.) 7, 47-55
27. Stripp, R. B., Sawaya, P. L., Luse, D. L., Wikenheiser, K. A., Wert, S. E., Huffman, J. A., Latter, D. L., Singh, G., Katyal, S. L., and Whislet, J. A. (1992) J. Biol. Chem. 267, 14703-14712
28. McNeil, J. P., and Sambrook, J. (1989) Molecular Cloning: a Laboratory Manual, 2nd ed., pp. 11.31-11.32, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
29. Whitsett, J. A. (1990) Mol. Cell. Biol. 10, 5486-5495
30. Ho, I.-C., Yang, L.-H., Morle, J., and Leiden, J. M. (1989) Proc. Natl. Acad. Sci. U. S. A. 66, 6714-6718
31. Rigaud, G., Roux, J., Pictet, R., and Grunge, T. (1991) Cell 67, 97-56
32. Gross, D. S., and Garett, W. T. (1988) Annu. Rev. Biochem. 57, 197-1977
33. Eisenberg, J. C., Cartwright, I. L., Thomas, G. H., and Elgin, S. C. R. (1985) Annu. Rev. Genet. 19, 486-506
34. Gross, D. S., and Garett, W. T. (1986) Nucleic Acids Res. 14, 8995-8999
35. Moore, A. M., and Ley, J. J. (1991) Histochemistry 100, 2272-2284
36. Samac, A., and Ley, J. J. (1991) Blood 77, 2272-2284
37. Tapparel, S., Lasser, A. B., and Weintraub, H. (1992) Mol. Cell. Biol. 12, 4904-5003
38. Bonffer, C., Hecht, A., Sauvageau, H., Winter, D. M., and Sippel, A. E. (1991) J. Cell. Biochem. 47, 89-108
39. Aronow, B. J., Silberman, N., Dusen, S., Stock, J. L., Yaeger, K., Potter, S. S., Hutton, J. J., and Wigniton, D. (1992) Mol. Cell. Biol. 12, 4170-4175
40. Donoghue, D., Ernt, H., Wentworth, B., Nadal-Ginard, B., and Rosenthal, N. (1990) Biochemistry 29, 4077-4079
41. Faisst, S., and Meyer, S. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 1161-1164
42. Faisst, S., and Meyer, S. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 4253-4254
43. Kakatani, Y., Brenner, M., and Freese, E. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4253-4254
44. Xanthopoulos, K. G., Preitzios, V. R., Chen, W. S., Sladek, F. M., Cortese, R., and Darnell, J. E. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3807-3811
45. Risso, G., Jooe, K., Neuberg, M., Bruller, H. J., and Muller, R. (1989) EMBO J. 8, 2635-2641
46. Kakatani, Y., Brenner, M., and Freese, E. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4388-4393
47. Smale, S. T., and Baltimore, D. (1989) Cell 57, 103-113
48. Carone, J., Buckhinder, L., and Reinberg, D. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8052-8056
49. Manistas, T., GoodBourn, S., and Fischer, J. A. (1987) Science 236, 1237-1244
50. Levin, B. (1990) Cell 61, 1161-1164