Sp3 regulates Fas expression in lung epithelial cells

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By transducing an apoptotic signal in immune effector cells, Fas has been directly implicated in the control of immunological activity. Expression and functional results, however, have also suggested a role for Fas in regulating cell turnover in specific epithelial populations. To characterize factors responsible for Fas expression in epithelial cells, approximately 3 kb of the 5' flanking region of the mouse Fas gene was isolated. By rapid amplification of cDNA ends and primer extension, transcriptional start sites were identified within 50 bp upstream of the translation start site. Transient transfection of promoter–luciferase constructs in a mouse lung epithelial cell line, MLE-15,

localized promoter activity to the first 77 bp of upstream sequence. By using a 60 bp DNA probe (–18 to –77) in electrophoretic mobility-shift assays, three shifted complexes were found. Incubation with excess cold Sp1 oligonucleotide or an anti-Sp3 antibody inhibited complex formation. Site-directed mutagenesis of the Sp1 site resulted in 60–70 % loss of promoter activity. In *Drosophila* SL-2 cells, promoter activity was markedly increased by co-transfection of an Sp3 expression construct. These results show that the Sp3 protein is involved in regulating Fas gene expression in lung epithelial cells.

INTRODUCTION

Fas is a 45 kDa cell surface receptor that transduces an apoptotic signal after engagement with the Fas ligand [1]. Originally isolated from immune cells, the Fas cDNA encodes for a transcript of approx. 2.1 kb that is highly expressed in T cells [1,2], B cells [1,2] and macrophages [1,2]. By the induction of apoptosis in immune effector cells, the predominant role for Fas has been postulated to be the control of immune cell function in the thymus and at peripheral sites of immune activity [1]. In this regard it is notable that the *lpr* mouse, which contains a loss of function mutation in the Fas gene, develops a lymphoproliferative disorder with auto-immune features [1].

Fas mRNA, however, is also expressed in liver [3], skin [4], ovary [5], small intestine [6] and lung [2,7]. In these organs Fas is localized to specific epithelial progenitor subtypes, including keratinocytes [4], hepatocytes [3], Paneth cells [6] and pulmonary alveolar type II cells [7]. Expression in these cell types suggests the possibility that Fas might be involved in regulating epithelial turnover. Consistent with this, variations in Fas expression accompany epidermal [4] and hepatic inflammation [3], and involution of the ovarian follicle [5]. Moreover, Fas-deficient knockout mice display evidence of hepatocyte hyperplasia [8].

The basis for the expression of Fas mRNA in specific epithelial cell populations is not yet known. In the lung, a tissue characterized by numerous epithelial cell types, the localized expression of Fas mRNA in the alveolar type II cell suggests that specific *cis*-acting and *trans*-acting factors serve to restrict Fas gene expression to this particular lung cell. To examine this further, we isolated the mouse Fas gene promoter and examined its regulation in a mouse lung epithelial type II cell line (MLE-1) [7,9], which we previously found to express Fas mRNA [7]. Here we demonstrate a critical role for the transcription factor Sp3 in controlling Fas expression in lung epithelial cells.

MATERIALS AND METHODS

Cloning of the upstream region of the mouse Fas gene

Anti-sense primers [(+60 to +26) and (+47 to +19)] specific to the first exon were designed to walk upstream from the 5' end of the mouse Fas cDNA (PromoterFinder Walking Kit Clontech). This method generated a 71 bp fragment. Subsequently, an 118 bp DNA fragment containing the 71 bp of PCR-generated 5' Fas gene sequence and 47 bp of contiguous mouse Fas cDNA sequence [2] was used as a probe to screen a mouse λ genomic library (Clontech). Two identical clones were identified by Southern analysis. DNA was purified from the positive clones, digested by *Pst*I, and an insert containing approx. 3 kb of 5' flanking region along with 47 bp of cDNA sequence was cloned into pBluescript (Stratagene). DNA sequence analysis was performed by automatic (Model 373; ABI) and by manual sequencing.

Rapid amplification of cDNA ends (RACE)

A mouse Marathon-ready cDNA kit (Clontech) was employed to determine the start site(s) of transcription of the mouse Fas gene. This kit contains a 5' adaptor-ligated full-length double-stranded cDNA library that serves as a template. To determine the start site, two separate nested PCR reactions were performed with adaptor anchor primers as sense primers and two sets of gene-specific anti-sense primers [(+273 to +248) and (+236 to +213); (+136 to +113) and (+90 to +52)]. The PCR products were gel-purified, cloned into a TA vector (InvitroGen) and sequenced.

Primer extension

An oligonucleotide corresponding to bp +44 to +80 of the published mouse cDNA sequence [2] was end-labelled before co-

Abbreviations used: EMSA, electrophoretic mobility-shift assay; MLE, mouse lung epithelial cell; RACE, rapid amplification of cDNA ends.

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precipitation with 50 μ g of total RNA derived from MLE-15 cells (see below). The pellet was resuspended in hybridization buffer [40 mM Pipes (pH 6.4)/1 mM EDTA/4 M NaCl/80 % (v/v) formamide] before reverse transcription. After 1 h the reaction was stopped by the addition of EDTA and the material digested with DNase-free RNase (5 μ g/ml) before analysis on a polyacrylamide/urea sequencing gel.

Luciferase reporter constructs

To measure the promoter activity of the 5' flanking region, pHP-3kb was digested with *SpeI*, blunt-ended by T4 DNA polymerase, and digested with *HindIII*. The isolated 3 kb upstream fragment was then subcloned into the *SmaI* and *HindIII* sites 5' to a luciferase reporter gene in the pGL3B vector (Promega). Progressive unidirectional deletion constructs were engineered by cloning the PCR fragments generated by using a vector forward primer and reverse gene-specific primers. For site-specific mutagenesis, plasmid pGL3B-77M was generated with a reverse primer (−44 to −77) containing the mutated Sp1 site (GGGGCGGGCC to GGTTCGGGCC).

Cell culture, transfection and luciferase assay

MLE-15 cells (kindly provided by Dr. Jeffrey Whitsett, University of Cincinnati, Cincinnati, OH, U.S.A.) were maintained as described previously [9]. The day before transfection, MLE-15 cells were plated into six-well plates at a density of 0.25×10^6 cells per well; 3 hours before transfection the cells were transferred to Dulbecco's modified Eagle's medium containing 10 % FBS. Transfections were performed by the calcium phosphate coprecipitation method without glycerol shock. After 6 h of incubation the medium was removed and replaced by fresh medium. After 24 h the plates were washed three times with ice-cold PBS and the cells were harvested with luciferase lysis buffer (Promega). The supernatant was then immediately assayed for luciferase activity in accordance with the manufacturer's protocol. Each transfection was performed in triplicate. Luciferase units were normalized for transfection efficiency on the basis of β -galactosidase activity from a co-transfected CMV-lacZ construct.

Electrophoretic mobility-shift assay (EMSA)

Nuclear extracts from MLE-15 cells were prepared by a mini-extraction procedure [10] at 4 °C. Briefly, monolayer cells from two confluent 150 mm dishes were washed twice and harvested by scraping into 1 ml of PBS. The cells were then washed once with 1 ml of PBS, pelleted in a Microfuge tube at 700 g for 5 min and resuspended in 400 μ l of buffer A [10 mM Hepes (pH 7.9)/10 mM KCl/0.1 mM EDTA/1.5 mM MgCl₂/1 mM dithiothreitol/0.5 mM PMSF] for 15 min. After the addition of 25 μ l of 10 % (v/v) NP40, the cell pellet was vortex-mixed gently for 5 min and centrifuged at 700 g for 5 min to obtain the nuclear pellet. Nuclear proteins were extracted from the nuclear pellet by a 10 min incubation with intermittent vortex-mixing in one packed nuclear volume of buffer B [20 mM Hepes (pH 7.9)/420 mM NaCl/0.1 mM EDTA/1.5 mM MgCl₂/25 % (v/v) glycerol/1 mM dithiothreitol/0.5 mM PMSF]. After centrifugation at 16000 g for 10 min, the supernatant was recovered and proteins were quantified before storage at −80 °C. EMSAs were performed as described [3]. As a probe, a DNA fragment encompassing −18 to −77 was generated by PCR with Elongase Mix (Gibco BRL), end-labelled with [γ -³²P]dATP with T4 polynucleotide kinase (New England Biolabs), and column-purified. For competition assays, oligonucleotides for Sp1 (5'-

ATTCGATCGGGGCGGGGCGAGC-3') and Ap2 (5'-GATCGAAGTACCGCCCGCGGCCCGT-3') were utilized (Promega). To analyse DNA-protein interactions, 1 μ l of nuclear extract (approx. 10 μ g/ μ l) was incubated in a 20 μ l reaction mixture [12 mM Hepes (pH 7.9)/4 mM Tris/HCl (pH 7.9)/25 mM KCl/5 mM MgCl₂/1 mM EDTA/1 mM dithiothreitol/0.2 mM fresh PMSF/1 μ g of poly(dI-dC)] at room temperature for 20 min. The probe (approx. 100000 d.p.m.) was added and incubated at 30 °C for an additional 30 min. As indicated in antibody supershift assays, 2 μ l of anti-Sp1, anti-Sp3, anti-Sp4 or anti-Ap2 (Santa Cruz Biotechnology) was added to the mixture at room temperature for 20 min before the addition of the probe. Bound and free probes were separated on 5 % (w/v) non-denaturing LongRanger Gels (FMC) with 2 % (v/v) glycerol and detected by autoradiography.

Transfection of *Drosophila* cells (SL-2)

SL-2 cells were maintained in Schneider medium (Gibco BRL) supplemented with 10 % (v/v) FBS at 25 °C. The cells were plated in six-well plates at a density of 10^6 per well 1 day before transfection. Each well received 3 μ g of a Fas promoter construct or a promoterless construct (pGL3B), with or without 1 μ g of the Sp3 expression vector pPacSp3 kindly provided by J. M. Horowitz (Duke University, Durham, NC, U.S.A.). After 48 h the cells were washed with PBS, lysed and luciferase assays were performed.

RESULTS

Cloning and nucleotide sequence analysis of the 5' region of the mouse Fas gene

Employing as templates a series of adaptor-ligated genomic libraries, a PCR-based method was utilized to walk upstream from the 5' end of the published mouse Fas cDNA sequence [2]. By using this method a 71 bp fragment of immediate upstream sequence of the Fas gene was generated. This 71 bp fragment was confirmed to be contiguous with the 5' end of the Fas cDNA by sequence analysis of a PCR product derived from amplification of mouse genomic DNA (results not shown). Because this was the longest fragment obtained by this method, a 118 bp Fas probe containing the 71 bp of PCR-derived sequence along with 47 bp of contiguous cDNA was used to screen a mouse λ genomic library. Two positive clones were isolated and characterized further. These two clones were found to be identical by Southern analysis. Sequence analysis of the first 1 kb revealed the lack of a TATA box; this is in agreement with the structure of the human Fas gene promoter [11]. Computer analysis [12] identified putative transcription factor-binding sites for CEBP/ β , NF- κ B, HNF3 β , c-Myb, Ap1, Ap2, and Sp1 (Figure 1).

Transcriptional start sites of the mouse Fas gene

To identify the transcriptional start site, adaptor-ligated double-stranded cDNA was used as a template during RACE analyses (see the Materials and methods section). PCR products were amplified in nested reactions with adaptor upstream primers and two sets of downstream gene-specific primers located in exons 1, 2 and 3. Twelve independent clones containing amplified fragments of the most 5' end region of the cDNA were sequenced. By this method, several start sites were identified within a 50 bp fragment upstream from the translation start site (Figure 1, filled arrowheads). The location of these sites is consistent with the previously published sequence of the putative full-length mouse Fas cDNA and with the approximate size of mouse Fas mRNA as noted by Northern analysis [2].

Figure 1 Nucleotide sequence of the 5' flanking region of the mouse Fas gene

The start sites of transcription, as determined by RACE, are indicated by open arrowheads. The most distal start site of transcription is designated +1. The start sites as determined by primer extension are indicated by the closed arrowheads. The cDNA sequence is in bold type. Potential binding sites of transcriptional factors are indicated.

To define further the transcriptional start sites, we performed a primer extension analysis on RNA derived from MLE-15 cells. By this method, two start sites slightly upstream from the most 5' site identified by RACE were localized (Figure 1, open arrowheads). Importantly, the region encompassing these two start sites has sequence similarity to a consensus initiator element [13], the putative site of transcriptional initiation in TATA-less genes [13]. The possibility of multiple start sites in close proximity, as determined by RACE and primer extension, is in agreement with observations in other genes that contain GC-rich proximal TATA-less promoters [14]. These findings are also consistent with the possibility of tissue-specific transcription initiation sites in the Fas gene.

Transcriptional activity of the 5' region of the Fas gene in MLE-15 cells

To localize the *cis*-acting element(s) responsible for promoter activity in MLE-15 cells, a series of 5' deletion constructs were generated by PCR. The resulting constructs contained 5' flanking regions from +47 bp to -3, -1.1, -0.5, -0.23, -0.19 and -0.077 kb (pGL3B-77). There were no statistically significant differences in the level of luciferase activity between each of the six constructs (Figure 2). In contrast, we found that a construct containing -23 bp of upstream promoter sequence had similar luciferase activity as a promoterless construct (results not shown).

To determine whether other Fas-expressing cells displayed a similar pattern of promoter activity, we performed transfection studies in NIH3T3 cells; these cells express Fas mRNA, as determined by reverse transcriptase-mediated PCR (results not

Figure 2 Deletion analysis of the 5' flanking region of the mouse Fas gene promoter during transient transfection of MLE-15 or NIH3T3 cells

Luciferase expression plasmids were constructed by inserting various lengths of the 5' flanking sequence into the promoterless expression vector pGL3B. Luciferase activity was normalized to β -galactosidase activity and is expressed as the fold increase in activity relative to a promoterless construct. Results are means $\pm$ S.E.M. for three independent experiments performed in triplicate. Abbreviation: ND, not determined.

shown). In contrast with Fas promoter regulation in MLE cells, we found that Fas promoter activation in NIH 3T3 cells involves additional distal elements located 5' to -77 (Figure 2).

Identification of protein binding to an Sp1 site within -77 bp of upstream sequence by EMSAs

To identify potential DNA–protein-binding sites involved in Fas promoter activity, a 60 bp (–18 to –77) DNA fragment was utilized in EMSAs. By employing nuclear extracts isolated from MLE-15 cells, three major DNA–protein-binding complexes were resolved (Figure 3, left panel). The use of a longer fragment (+47 to –77) resulted in a similar pattern of complex formation (results not shown). The formation of each of these complexes was abolished by a 200-fold molar excess of a non-radiolabelled 60 bp fragment. Because a consensus Sp1 site (–48 to –55) and a less stringently matched Ap2 site (–23 to –32) were identified (Figure 1), we further examined the potential role of these putative binding sites. Notably, all three complexes were completely inhibited by incubation with a 200-fold molar excess of a purified consensus Sp1 oligonucleotide but not by a purified Ap2 oligonucleotide (Figure 3, left panel).

To identify the proteins present in these three complexes, antibodies against Ap2, Sp1 and Sp4 were utilized. Interestingly, we found that the formation and migration of all three complexes associated with the 60 bp probe (−18 to −77) were not affected by the preincubation of nuclear lysates with these antibodies (Figure 3). The presence of Sp1 protein in MLE-15 nuclear lysates was confirmed by the generation of a supershifted complex in EMSAs performed with a purified consensus Sp1 oligonucleotide (results not shown).

To examine the role of the Sp3 protein in complex formation, EMSA studies of the 60 bp fragment were performed after the incubation of lysates with an anti-Sp3 antibody. Importantly, we found that the formation of all three complexes was markedly attenuated by the anti-Sp3 antibody (Figure 3, right panel). Taken together, these studies suggest that this proximal Sp1 site, through interactions with an Sp3-containing protein complex, mediates Fas promoter activity in MLE-15 cells.

Site-specific mutagenesis of the Sp1 element

To determine the functional role of the proximal Sp1 site at -48 to -55 , a two-base mutation (GG $\rightarrow$ TT) was performed at

Figure 3 Sp3 binds to a proximal Sp1 site (–46 to –55) in the mouse Fas promoter

Left panel: DNA–protein complex formation in EMSA of MLE-15-derived nuclear lysates with a 32 P-labelled 60 bp DNA promoter fragment (–18 to –77). The effects of no additions (lane labelled 0) or incubation with an excess of unlabelled wild-type 60 bp DNA promoter fragment (Wt), consensus Sp1-binding oligonucleotide (Sp1) or a consensus Ap2-binding oligonucleotide (Ap2) are shown. The effects of preincubating nuclear lysates with a polyclonal antibody directed against Sp1 (Anti-Sp1) and Ap2 (Anti-Ap2) is also shown. Three shifted DNA–protein complexes are indicated by arrowheads. Right panel: Anti-Sp3 antibody preincubation abolishes DNA–protein complex formation. EMSAs with the 32 P-labelled 60 bp DNA fragment (–18 to –77) were performed as in the left panel. The effects of preincubating nuclear lysates with no polyclonal antibody (lane labelled 0) or with polyclonal antibodies directed against Sp1 (Anti-Sp1), Sp3 (Anti-Sp3) or Sp4 (Anti-Sp4) is shown. Three shifted DNA–protein complexes are indicated by arrowheads.

Table 1 Promoter activity of wild-type and mutated constructs in MLE cells

Wild-type (pGL3B-77) or mutated (pGL3B-77M) constructs containing 77 bp of upstream sequence were transfected into MLE cells. To generate pGL3B-77M, a 2 bp substitution (GG → TT, positions –52 and –53) was inserted within the consensus Sp1 site. Luciferase and β -galactosidase assays were performed as in Figure 2. The results are the fold increase in activity over a promoterless construct (means $\pm$ S.E.M. for triplicates in two independent experiments).

Vector	Relative luciferase activity
pGL3B-77	7.9 ± 0.18
pGL3B-77M	3.0 ± 0.11

positions –52 and –53 in pGL3B-77. We found that the promoter activity of the mutated construct (pGL3B-77M) was diminished by approx. 60–70% in comparison to wild-type pGL3B-77 (Table 1). Consistent with this finding, a DNA fragment (from –18 to –77) containing these two mutated sites at 200-fold molar excess had no effect on the formation of the three DNA–protein complexes in EMSAs (Figure 4). In contrast,

Figure 4 A 60 bp DNA fragment (–18 to –77) with an Sp1 site mutation (GG → TT at –52 and –53) loses the ability to abolish DNA–protein complex formation

By using the wild-type 32 P-labelled 60 bp DNA fragment (–18 to –77) as a probe during EMSAs, competition assays were performed by the addition of no competitor (lane labelled 0) or 2–200-fold molar excesses of unlabelled wild-type (Wt) or mutated fragments (mutant). The three DNA–protein-binding complexes are indicated by arrowheads.

Table 2 Effect of Sp3 on Fas promoter activity in *Drosophila* SL-2 cells

pGL3B-77 or a promoterless construct (pGL3B) were co-transfected without or with an Sp3 expression vector (pPacSp3) into *Drosophila* SL-2 cells. After 48 h luciferase assays were performed. Results are means $\pm$ S.E.M. for triplicates in two independent experiments.

Vector	$10^{-7} \times$ Activity (c.p.m.)	
	Without pPacSp3	With pPacSp3
pGL3B	0.13 ± 0.01	0.15 ± 0.03
pGL3B-77	0.15 ± 0.008	5.16 ± 0.26

complex formation was diminished by a 2–20-fold molar excess of the wild-type fragment and was completely abolished by a 200-fold molar excess.

Transcriptional activity of pGL3B-77 during co-transfection with an Sp3 expression vector (pPacSp3) in SL-2 cells

To examine further the role of Sp3 in mouse Fas gene regulation, transient co-transfection assays were performed with pGL3B-77 and an Sp3 expression vector (pPacSp3) in *Drosophila* SL-2 cells; these cells do not express any endogenous Sp proteins [15]. As shown in Table 2, minimal activity was detectable in SL-2 cells transfected with pGL3B-77 alone, and in cells transfected with a promoterless construct (pGL3B) along with pPacSp3. However, a marked increase in promoter activity was induced by co-transfection of pGL3B-77 with pPacSp3.

DISCUSSION

In the present study we isolated 3 kb of the 5' flanking region of the mouse Fas gene. In agreement with the previously identified human Fas gene promoter [11], the proximal control region does not contain a TATA box, is GC-rich and contains potential binding sites for several well-characterized transcription factors. We found that, in contrast with transfected NIH 3T3 cells, nearly all promoter activity in transfected MLE-15 cells localized to a perfectly matched proximal Sp1 site (−46 to −55). Mutation of this site resulted in a marked diminution of promoter activity. Our findings are consistent with previous studies showing that the transcription of other TATA-less promoters frequently involves the action of a proximal Sp1 site [16]. Our functional studies, however, suggest that transcriptional initiation of the Fas gene in MLE-15 cells involves a proximal interaction of Sp3 with the RNA polymerase II complex. Notably, Sp3 has the same binding affinity and specificity as Sp1 [17].

By using a proximal promoter DNA fragment we identified three DNA–protein-binding complexes in EMSAs; these complexes were disrupted by an excess of unlabelled Sp1 oligonucleotide or by preincubation of nuclear lysates with an anti-Sp3 antibody. These results indicate that the formation of all three complexes involves the Sp3 protein. In several different studies Sp3 has been reported to function as either a transcriptional activator or a repressor [17,18]. Our results demonstrate that Sp3 functions as an activator during transcription of the Fas gene. Recent work suggests that the dual activities of transcriptional activation and repression are mediated by different regions of the Sp3 protein and are determined by the spacing and context of other Sp3-binding sites [17].

Overall, these results are in agreement with numerous studies showing that tissue-specific expression of a variety of genes involves widely expressed transcription factors. Such examples include the expression of the Clara cell-specific protein CC-10, which involves Ap1 [19], and the expression of the Wilm's tumour product 1, which involves Sp1 [20]. Possibly pertinent to our studies, the action of the Sp3 protein has been implicated in the control of genes that mediate keratinocyte differentiation [18]. It is also noteworthy that a specific protein component (TAFII105) of the proximal-binding TFIID complex has been shown to be selectively expressed during B cell differentiation [21]. It is thought that TAFII105, through interactions with ubiquitously expressed factors, mediates the transcription of B-cell-specific genes [21]. This model suggests the existence of a similar type of proximal-acting cell-specific protein that interacts with Sp3, thereby restricting Fas gene transcription. In support of this we found in preliminary work that, although mouse myoblasts contain Sp3 protein, they do not express Fas mRNA and do not support Fas promoter activity.

In addition to effects mediated through protein–protein interactions, the regulation of tissue specific genes by widely expressed transcription factors might involve direct protein modifications, causing changes in DNA binding and transactivation capability. For example, the reversible oxidative-mediated inactivation of Sp1 binding has been reported [22]. Moreover, expression of the tissue factor gene is correlated with hyperphosphorylation of Sp1 [23]. Lastly, our results do not rule out a role for repressive factors and DNA methylation in cell-specific Fas gene expression.

Examination of the mouse Fas promoter demonstrates the presence of other consensus transcription factor-binding sites

including CEBP/ β , NF- κ B, HNF3 β and Ap2. In the liver, which is characterized by high Fas mRNA levels [2], the CEBP and HNF family of transcription factors are thought to have a key role in hepatocyte differentiation [24]. Though these factors are also involved in regulating distal lung epithelial cell differentiation [25,26], deletion analysis of these binding sites did not affect Fas promoter activity in MLE-15 cells. Additional work will be required to determine whether an Sp3-dependent mechanism or the action of tissue specific differentiation factors controls transcription of Fas in hepatocytes and other epithelial cell populations.

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REFERENCES

- 1 Itoh, N. S., Yonehara, S., Ishii, A., Yonehara, N., Mizushima, S., Sameshima, M., Hae, A., Seto, Y. and Nagata, S. (1991) *Cell* **66**, 233–243
- 2 Watanabe-Fukunga, R., Brannan, C. I., Itoh, N., Yonehara, S., Copeland, N. G., Jenkins, N. A. and Nagata, S. (1992) *J. Immunol.* **148**, 1274–1279
- 3 Hiramatsu, N., Hayashi, N., Katayama, K., Mochizuki, K., Kawanshi, Y., Kasahara, A., Fusamoto, H. and Kamada, T. (1994) *Hepatology* **19**, 1354–1359
- 4 Sayama, K., Yonehara, S., Watanabe, Y. and Miki, Y. (1994) *J. Invest. Dermatol.* **103**, 330–334
- 5 Guo, M., Mori, E., Xu, J. P. and Mori, T. (1994) *Biochem. Biophys. Res. Commun.* **203**, 1438–1446
- 6 Moller, P., Walczak, H., Reidl, S., Strater, J. and Krammer, P. H. (1996) *Am. J. Pathol.* **149**, 9–13
- 7 Fine, A., Anderson, N. L., Rothstein, T., Williams, M. C. and Gochuico, B. R. (1997) *Am. J. Physiol.* **273** (Lung Cell. Mol. Physiol.), L64–L71
- 8 Adachi, M., Suematsu, S., Kondo, T., Ogasawara, J., Yoshida, N. and Nagata, S. (1995) *Nature Genet.* **11**, 293–300
- 9 Ikeda, K., Clark, J. C., Bachurski, C. J., Wikenheiser, K. A., Cuppoletti, J., Mohanti, S., Morris, R. E. and Whitsett, J. A. (1994) *Am. J. Physiol.* **267** (Lung Cell. Mol. Physiol.), L309–L317
- 10 Bohinski, R. J., Di Lauro, R. and Whitsett, J. A. (1994) *Mol. Cell. Biol.* **14**, 5671–5681
- 11 Cheng, J., Liu, C., Koopman, W. J. and Mountz, J. D. (1995) *J. Immunol.* **154**, 1239–1245
- 12 Quandt, K., Frech, K., Karas, H., Wingender, E. and Werner, T. (1995) *Nucleic Acids Res.* **23**, 4878–4884
- 13 Javahery, R., Khachi, A., Lo, K., Zenzie-Gregory, B. and Smale, S. T. (1994) *Mol. Cell. Biol.* **14**, 116–127
- 14 Dynan, W. S. and Tijan, R. (1983) *Cell* **35**, 79–87
- 15 Vindevoghel, L., Chung, K. Y., Davis, A., Kouba, D., Kivirkko, S., Alder, H., Uito, J. and Mauviel, A. (1997) *J. Biol. Chem.* **272**, 10196–10204
- 16 Azizkhan, J. C., Jensen, D. E., Pierce, A. H. and Wade, M. (1993) *Crit. Rev. Eukaryotic Gene Expr.* **3**, 229–254
- 17 Majello, B., DeLuca, P. and Lania, L. (1997) *J. Biol. Chem.* **272**, 4021–4026
- 18 Prowse, D. M., Bolgan, L., Molnár, A. and Paolo Dotto, G. (1997) *J. Biol. Chem.* **272**, 1308–1314
- 19 Sawaya, P. L., Stripp, B. R., Whitsett, J. A. and Luse, D. S. (1993) *Mol. Cell. Biol.* **13**, 3860–3871
- 20 Cohen, H. T., Bossone, S. A., Zhu, G., McDonald, G. A. and Sukhatme, V. P. (1997) *J. Biol. Chem.* **272**, 2901–2913
- 21 Dikstein, R., Zhou, S. and Tijan, R. (1996) *Cell* **87**, 137–146
- 22 Hamm-Künzelmann, B., Schäfer, D., Weigert, C. and Brand, K. (1997) *FEBS Lett.* **403**, 87–90
- 23 Lin, M. C., Almus-Jacobs, F., Chen, H. H., Parry, G. C. N., Mackman, N., Shyy, J. Y. J. and Chien, S. (1997) *J. Clin. Invest.* **99**, 737–744
- 24 Clevidence, D. E., Overdier, D. G., Tao, W., Qian, X., Pani, L., Lai, E. and Costa, R. H. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3948–3952
- 25 Hackett, B. P., Brody, S. L., Liang, M., Zietz, I. D., Bruns, L. A. and Gitlin, J. D. (1995) *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4249–4253
- 26 Li, F., Rosenberg, E., Smith, C. I., Notarfrancesco, K., Reisher, S. R., Shuman, H. and Feinstein, S. I. (1995) *Am. J. Physiol.* **269** (Lung Cell. Mol. Physiol.), L241–L247