Oct-1 interacts with conserved motifs in the human thyroid transcription factor 1 gene minimal promoter

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The homeodomain containing thyroid transcription factor 1 (TTF-1) is a lung- and thyroid-enriched protein implicated in the regulation of a number of pulmonary specific genes. Within the lung TTF-1 is expressed within the epithelial cells. Although the molecular mechanisms that govern this tight cell-type-specific distribution are unclear, transient transfection studies have suggested that tissue specificity is conferred in part by regions of the proximal promoter. Further studies have shown that two functionally important regions (BS1 and BS2) are sites for activation of the TTF-1 gene by the homeodomain protein HoxB3, raising the possibility that Hox proteins might function in the regulation of TTF-1 *in vivo*. The different cellular distributions of the two proteins within the lung suggest, however, that proteins distinct from HoxB3 might be the mediators of expression through these sites. In the present study we have used gel-mobility-shift experiments to show that in a pulmonary adenocarcinoma cell line (NCI-H441) that expresses TTF-1, the same single protein binds to both of these sites. The binding of this protein is competed for specifically by the addition of oligonucleotides containing a range of octamer-binding sites but not by a variety of non-related binding sites. Using specific antiserum we have identified this protein as being the ubiquitously expressed POU-domain protein Oct-1. Reverse transcriptase–PCR performed with degenerated primers suggests that Oct-1 is the major POU-domain-containing protein expressed in H441 cells. These results suggest that BS1 and BS2 are functional octamer sites and might therefore be implicated in the basal rather than the tissue-restricted expression of the TTF-1 gene.

INTRODUCTION

Thyroid transcription factor 1 (TTF-1) is a highly conserved homeobox-containing transcription factor of the Nkx2 family of proteins [1,2]. TTF-1 was originally identified as a regulator of thyroid-specific gene expression, being implicated in the regulation of thyroglobulin and thyroperoxidase gene transcription [1,3]. Northern blotting studies suggest that TTF-1 is also expressed within the lung and brain [1]. Hybridization studies *in situ* have shown that TTF-1 is localized to the pulmonary epithelium, and subsequent immunohistochemistry has confirmed that TTF-1 is found in both the bronchiolar and alveolar epithelium [4,5]. Recent studies have begun to identify the molecular targets of TTF-1 within the pulmonary epithelium. Functional binding sites have been identified within the regulatory sequences of the surfactant apoprotein A (SP-A) and B (SP-B) genes [6–8] as well as in the Clara cell secretory protein (CCSP) gene [9]. As well as playing a role in the regulation of gene expression within the mature lung and thyroid, the early onset of TTF-1 gene expression in these organs, occurring as it does 5 days before the first expression of its known target genes, suggests that TTF-1 might play a role in tissue morphogenesis. This is supported by the observation that specific anti-sense TTF-1 oligonucleotides inhibit lung branching morphogenesis *in vitro* [10]. Furthermore recent gene-targeting studies have confirmed that TTF-1 gene expression is essential for organogenesis of the lung, thyroid, ventral forebrain and pituitary [11]. It is therefore clear that TTF-1 is an important regulator of pulmonary restricted genes and is itself a tissue-restricted gene product.

The genomic elements that restrict expression of the TTF-1 gene to the thyroid and lung have not been identified. Transient transfection studies of rat and human TTF-1 gene chimaeric reporter constructs have suggested that the highly conserved proximal promoter region is implicated in this tissue restriction [5,12]. In addition, transgenic experiments have shown that 2 kb of the mouse gene are sufficient to direct the appropriate expression of a reporter gene to the lung [13]. Studies with the first 142 bp of the rat TTF-1 gene proximal promoter fused to a reporter gene suggest that this region contains elements responsible, at least in part, for the tissue restriction of the TTF-1 gene [12]. Further studies identified two regions (BS1 and BS2) as being sites through which the homeodomain transcription factor HoxB3 was able to activate the same reporter construct specifically, whereas mutation of these sites decreased the activation seen in co-transfection assays [12]. Although these studies clearly suggest that HoxB3 is able to activate TTF-1 gene expression *in vitro*, a role for HoxB3 in the regulation of TTF-1 gene expression *in vivo* seems unlikely in view of the different cellular localization of these two proteins within the lung [14]. The suggestion that further unidentified proteins might play a role in the regulation of TTF-1 gene expression through these sites *in vivo* is supported by the following observations. First, reduced levels of promoter activity were seen in transfection assays performed with the BS1/BS2 mutant constructs in the absence of HoxB3, suggesting that these regions have a functional role in regulating TTF-1 expression; and secondly, gel-mobility-shift reactions performed with the two binding sites as probes suggested that proteins other than HoxB3 were capable of interacting with these sites [12].

Abbreviations used: CCSP, Clara cell secretory protein; CREB; cAMP response element binding protein; GR, glucocorticoid receptor; HNF-3, hepatocyte nuclear factor 3; HSV, herpes simplex virus; SP, surfactant apoprotein; TTF-1, thyroid transcription factor 1; XRE, xenobiotic response element.

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As an initial step towards elucidating the molecular mechanisms that govern the tissue-restricted expression pattern of TTF-1 we have used a human lung adenocarcinoma cell line, NCI-H441, that expresses TTF-1 [5,9] to study the interaction of nuclear proteins with these binding sites. We provide evidence that a single protein present in these cells interacts with both of these sites and is in all probability the ubiquitously expressed POU-domain protein, Oct-1.

MATERIALS AND METHODS

Tissue culture

The human lung-derived adenocarcinoma cell line, NCI-H441, was obtained from the American Tissue Culture Collection and maintained in RPMI medium containing 10% (v/v) fetal calf serum, 100 units/ml penicillin and 150 μg/ml streptomycin. The human endometrial cell line, Ishikawa, was obtained from Dr. J. D. Croxtal (Biochemical Pharmacology, St. Bartholomew's Hospital) and maintained as for the H441 cells.

Nuclear extract production and gel-mobility-shift assays

Nuclear extracts were prepared from confluent monolayers of tissue culture cell lines (10^7 cells) in the presence of proteinase inhibitors, as described previously [15]. Extracts were sampled and stored at -70 °C. For gel-mobility-shift experiments overlapping complementary oligonucleotides were annealed and labelled by filling in regions with [^{32}P]dNTPs and Klenow polymerase. The oligonucleotides used in this study are listed in Table 1. Additional binding sites for AP-1, AP-2, AP-3, glucocorticoid receptor (GR), cAMP response element binding protein (CREB), Sp1 and NF-1 were obtained from Stratagene. The xenobiotic response element (XRE) site was from the Cyp 1A1 gene [16] and the TTF-1 site was from the rat CCSP gene [9]. The non-specific competitor, an NFκB site oligonucleotide, was as described [17]. Approx. 1 ng of probe (specific radioactivity 10^7 c.p.m./μg) was used in each binding reaction. Binding reactions were performed at room temperature in a 20 μl reaction volume as described [17,18]. After electrophoresis, gels were fixed, dried and exposed to film at -80 °C. In some reactions a 100-fold molar excess of unlabelled competitor oligonucleotides were included in the reactions. For Oct-1 antibody supershift studies the gel shift reactions were prepared as above except that 1 μl of antiserum raised against the N-terminal (amino acid residues 1–370) region of human Oct-1 [19], or 1 μl of preimmune serum, was added to the reaction 10 min before the addition of the labelled probe.

RNA preparation and analysis

Poly(A)$^+$ RNA was isolated directly from cell lines by using the Fast Tract system (Invitrogen). RNA samples were subjected to electrophoresis, transferred to Hybond-N membranes (Amersham) and hybridized at 68 °C in Quick-Hyb (Stratagene) with ^{32}P-labelled random primed cDNA probes with full-length human TTF-1 (S. Gowan and C. D. Bingle, unpublished work) and human SP-A [20].

Table 1 Sequence of oligonucleotides used for gel-shift studies

TTF-1 BS2 (wild-type)	CATGTAAGCTAATTATCTCGGGCAAGA
TTF-1 BS2 (mutant)	CATGTAAGCTAGCGATCTCGGGCAAGA
TTF-1 BS1	CACTCAAGCCAATTAGGAGGA
Oct consensus	CATCGAATGCAAATCACTAGCT
3′ rat CCSP HNF-3	AGATGACTAAGTAAATAGTGCAATTTCTT
Oct B HSV(IE4/5)	TCGAGCGGTAATGAGATCTAGAAGATC
Oct C HSV(IE1)	TCGAATGCTAATGATATATCTAGAAGATC

Amplification of POU domains by reverse transcriptase–PCR (RT–PCR)

RT–PCR was performed with H441 cell poly(A)$^+$ RNA and the following degenerate POU-domain primers: POU forward (deg), 5′-TT(CT)AA(A/G)AC(A/G)(A/G)G(C/A)(A/C)G(A/G)A-TIAA(/G)(C/T)I(C/T)GGA-3′; POU reverse (deg), 5′-CTG-(C/G)C(T/G)I(C/T)G(G/A)TT(G/A)CA(T/G)AACCAIAC-ICG-3′. These primers were designed to amplify all potential POU domains, and inosine was substituted in positions of complete degeneracy. Poly(A)$^+$ RNA (200 ng) was reverse-transcribed with an oligo(dT) primer with the cDNA Cycle Kit (Invitrogen) in accordance with the manufacturer's instructions. A 2 μl aliquot of the resulting reaction was used as a template for PCR under the following conditions: 94 °C for 1 min, 45 °C for 2 min, and 72 °C for 3 min (30 cycles). A 1 μl aliquot of the initial reaction was reamplified under the same conditions and the resulting 350–450 bp band was ligated directly into pCRII (Invitrogen). Mini-prep DNA was subjected to digestion with *Eco*RI to produce a diagnostic pattern. The Oct-1 POU domain contains an internal *Eco*RI site not shared with other known POU domains. Sequencing of representative POU domains was performed with the fmole system (Promega).

RESULTS

H441 cells are an appropriate line in which to study TTF-1 gene regulation

The H441 cell line has been used extensively for studies on the regulation of pulmonary-specific gene expression [6–9,17,18]. Recently it has been shown that this cell line expresses TTF-1 [5,9]. However, it has been observed that the expression of pulmonary-specific markers within this cell line, particularly of SP-A, is highly variable depending on culture conditions [21]. Thus we used Northern blotting to ensure that the pulmonary phenotype of the cell line was maintained in our hands. A specific TTF-1 transcript was clearly seen in the H441 cell RNA (Figure 1, lane 1) but not in the endometrium-derived cell line Ishikawa (Figure 1, lane 2). The Ishikawa cell line has been shown to support expression of CCSP gene reporter constructs in transient transfection assays [22], although TTF-1-binding activity is undetectable in these cells (C. D. Bingle and S. Gowan, unpublished work). When we rehybridized the blot with a probe for human SP-A, a single transcript was observed in the H441 cells (Figure 1, lane 1) but not in the Ishikawa cells (Figure 1, lane 2). The presence of undegraded RNA in the Ishikawa cell sample was confirmed by rehybridizing the blot with a probe to β-actin [23] (Figure 1, lanes 1 and 2). Additional RT–PCR experiments performed with primers specific for human SP-A also failed to demonstrate SP-A in these cells (results not shown). These results confirm that the H441 cells are an appropriate line in which to study the regulation of the TTF-1 gene and additionally suggest that the Ishikawa cells, as cells not expressing TTF-1, are also useful as a negative control for these studies.

A single protein in H441 cell nuclear extract binds to the BS2 site

The two binding sites identified as being HoxB3 sites in the rat TTF-1 gene are completely conserved in the human, rat and

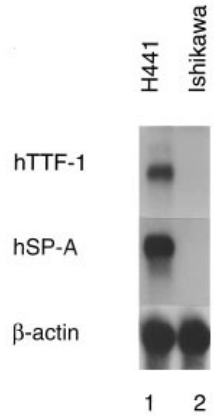

Figure 1 Co-expression of TTF-1 and SP-A in H441 cells suggests that these cells are an appropriate line in which to study TTF-1 gene expression

A Northern blot containing 2 μg of poly(A)$^+$ RNA from H441 cells (lane 1) or Ishikawa cells (lane 2) was hybridized with a ^{32}P-labelled random primed cDNA probe for human TTF-1. The blot was sequentially stripped and rehybridized with ^{32}P-labelled random primed probes to full-length human surfactant protein A and β-actin.

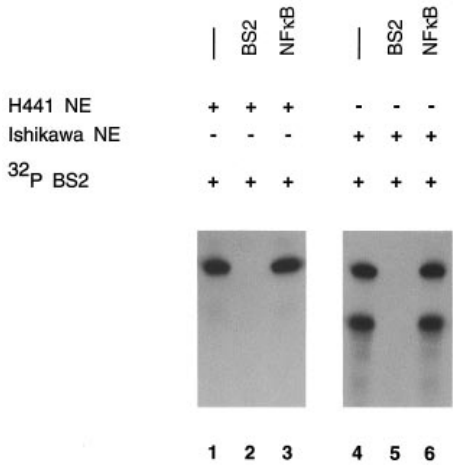

Figure 2 The BS2 oligonucleotide binds to a single protein in H441 cell nuclear extracts

Gel mobility shift assays were performed as outlined in the Materials and methods section by using 1 μl of H441 (lanes 1–3) or Ishikawa (lanes 4–6) cell nuclear extracts. A ^{32}P-labelled BS2 oligonucleotide was used as a probe in the absence (lanes 1 and 4) or presence of a 100-fold molar excess of unlabelled double-stranded BS2 (lanes 2 and 5) or NFκB (lanes 3 and 6) oligonucleotides.

mouse genes [5,12,24]. Therefore we attempted to identify this and other proteins capable of interacting with these sites in both TTF-1-expressing (H441) and non-expressing (Ishikawa) cells. When the labelled BS2 probe was used in gel-mobility-shift assays with nuclear extracts from the H441 cells, a single, specific, retarded complex was observed (Figure 2, lane 1). This was competed by a 100-fold molar excess of the unlabelled specific oligonucleotide (Figure 2, lane 2) but not by an excess of the non-specific oligonucleotide (Figure 2, lane 3). When nuclear extracts from the non-expressing cell line, Ishikawa, were used in an identical reaction, two retarded complexes were observed (Figure 2, lane 4). The slowest migrating band had the same apparent mobility as that seen in the H441 cell extracts. Both

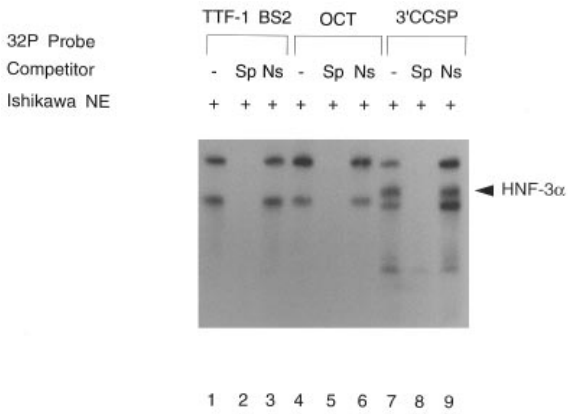

Figure 3 Octamer-containing binding sites bind proteins of similar mobility to the BS2 site

Gel-mobility-shift assays were performed as outlined in the Materials and methods section, with 1 μl of Ishikawa cell nuclear extracts. ^{32}P-labelled BS2 (lanes 1–3), octamer consensus (lanes 4–6) or 3′ CCSP HNF-3 (lanes 7–9) oligonucleotides were used as probes in the absence (lanes 1, 4 and 7) or presence of a 100-fold molar excess of specific (lanes 2, 5 and 8) or non-specific (NFκB; Ns) (lanes 3, 6 and 9) double-stranded binding-site oligonucleotides. The position of the HNF-3α retarded complex is indicated by the arrow.

bands were shown to be specific by competition with an excess of unlabelled probe (Figure 2, lane 5) but not by the non-specific oligonucleotide (Figure 2, lane 6). Using BS1 as a probe in identical reactions gave essentially similar results (results not shown).

Binding of proteins to BS2 is competed for by octamer-protein-binding sites

To begin to identify the proteins interacting with BS2 we performed an identical series of reactions in which the specific competitors were replaced with known double-stranded binding-site oligonucleotides. The binding sites utilized were for AP-1, AP-2, AP-3, HNF-3, NFκB, Sp1, Oct-1, CREB, GR, NF-1 and XRE proteins. With this series of competitors we were able to show that binding sites for HNF-3 and Oct-1 acted as competitors for binding of the specific protein complexes in both H441 and Ishikawa cell nuclear extracts (results not shown). The results suggested that the BS2-binding proteins were capable of interacting with octamer- and HNF-3-containing binding sites. We therefore compared the binding profile of these probes directly with proteins from H441 and Ishikawa cell nuclear extracts. These experiments clearly demonstrate that all three probes bound to proteins of the same apparent mobility in the Ishikawa cell nuclear extract (Figure 3). In the gel shifts performed with the probe for the HNF-3 site from the rat CCSP gene [17] a further retarded complex of intermediate mobility was observed (Figure 3, lanes 7–9). This corresponds to HNF-3α. This binding site has been shown previously to bind to both Oct-1 and HNF-3 proteins [17,25]. A similar situation was also seen when the H441 cell nuclear extracts were used with the same three probes (results not shown), except that only the slowest migrating band was seen with the BS2 and Oct probes and the HNF-3α complex with the HNF-3 probe was much greater, obscuring the specific BS2-binding band. This reflects the fact that HNF-3α is expressed at much higher levels in the H441 cells than in the Ishikawa cells [26].

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TTF-1 BS2                CATGTAAGCTAATTATCTCGGGCAAGA
                           |*|| |||||
TTF-1 BS1                CACTCAAGCCAATTAGGAGGA
                           | || |||||
Oct                      CATCGAATGCAAATCACTAGCT
                           ||||||||||
3' rat CCSP HNF-3     AGATGACTAAGTAAATAGTGCAATTTCTT
                           | | |||||
Oct B HSV(IE4/5)         TCGAGCGGTAATGAGATCTAGAAGATC
                           |  |||||
Oct C HSV(IE1)           TCGAATGCTAATGATATATCTAGAAGATC
                           |||| |||||
Oct consensus               ATGCAAATNA
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Figure 4 Alignment of BS2 and BS1 sites with octamer-binding sites

The BS2 and BS1 oligonucleotides are aligned with the octamer consensus sequence and the octamer binding sites used in the gel mobility shift studies. The vertical line underneath a nucleotide indicates conservation with the corresponding position in the octamer consensus sequence, shown at the bottom. The asterisk indicates a nucleotide that is conserved between BS2 and BS1 but is not found in the octamer consensus.

The ubiquitously expressed Oct-1 protein binds to both BS2 and BS1

The above results are consistent with the possibility that the proteins interacting with the TTF-1 BS2 site might represent octamer-binding proteins. Alignment of BS2 and BS1 with known octamer-binding sites clearly supports this contention (Figure 4). BS2 and BS1 contain a region of sequence that is identical in nine out of ten bases. Alignment of the octamer-binding site and the 3′ rat CCSP oligonucleotides used in the experiments in Figure 3 clearly shows the high degree of conservation, as do two additional octamer-binding sites, B and C, from herpes simplex virus (HSV) genes [27] and a derived octamer consensus motif. Both BS2 and BS1 are identical in eight out of ten bases compared with the consensus and the positions of divergence are both bases at which the other known octamer-containing sites differ from the consensus. To confirm directly that BS2 and BS1 contain functional octamer binding sites we performed a further series of gel shifts with octamer-binding sites and antiserum to human Oct-1. The complex formed with the H441 cell nuclear extract was effectively competed for by the addition of an excess of the BS2 and BS1 oligonucleotides (Figure 5, upper panel, lanes 2–4) as well as oligo B (Figure 5, upper panel, lane 4) and oligo C (Figure 5, upper panel, lane 5). The addition of 1 μl of pre-immune serum to the reaction produced no change in the mobility of the retarded complex (Figure 5, upper panel, lane 7) whereas the addition of 1 μl of anti-(human Oct-1) antiserum caused the complex to be supershifted (Figure 5, upper panel, lane 8). Addition of specific competitor oligonucleotides to the supershift reactions resulted in abolition of the supershifted band (results not shown), again confirming the specificity of the reaction. Repeating the same series of reactions with the Ishikawa cell nuclear extract produced identical results (Figure 5, lower panel) except that the addition of the Oct-1 antiserum resulted only in the supershifting of the upper complex (Figure 5, lower panel, lane 8). A further series of gel-mobility shifts performed with the labelled BS1 probe also gave consistent results (results not shown). These results strongly suggest that the complex formed on the BS2 and BS1 probes in H441 cell nuclear extracts is formed by Oct-1.

Oct-1 is the major POU-domain protein present in H441 cells

The antibody supershift results presented above suggest that Oct-1 is the major (if not the only) octamer-binding protein

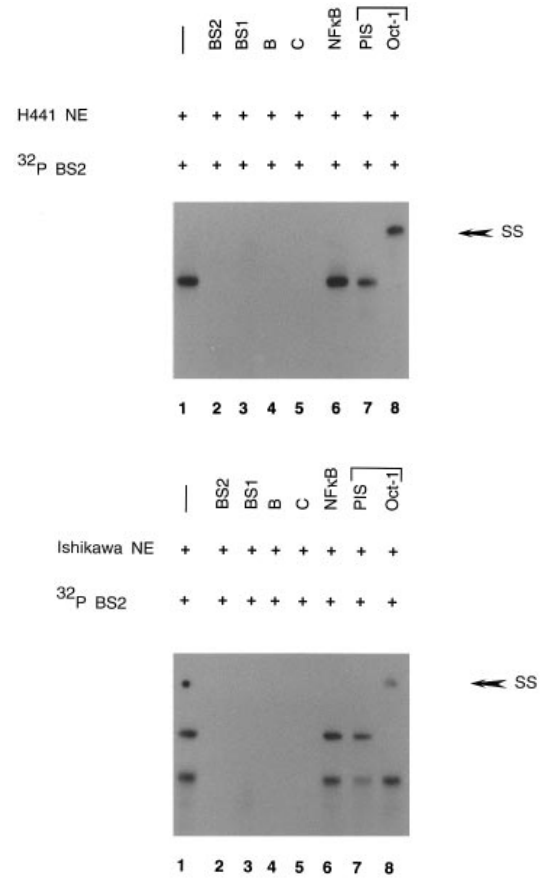

Figure 5 Oct-1 binds to the BS2 site

Gel-mobility-shift assays were performed as outlined in the Materials and methods section with 1 μl of H441 cell nuclear extract (NE) (upper panel) or 1 μl of Ishikawa cell nuclear extract (lower panel), with the ^{32}P-labelled BS2 oligonucleotide as a probe. Reactions were performed in the absence (lane 1) or presence of a 100-fold molar excess of the following unlabelled double-stranded oligonucleotides: BS2 (lane 2), BS1 (lane 3), Oct B HSV (lane 4), Oct C HSV (lane 5) or NFκB (lane 6), or in the presence of 1 μl of pre-immune serum (lane 7) or anti-human Oct-1 antiserum [19] (lane 8). The position of the supershifted complex is indicated by the arrow.

expressed in H441 cells. To investigate whether other POU-domain proteins are expressed in these cells we employed the well established technique of RT–PCR with degenerate POU-domain primers [28]. Such primers have been used extensively to identify a variety of POU proteins. RT–PCR with H441 cell poly(A)$^+$ RNA followed by direct cloning and characterization of over 40 POU-domain-containing recombinants failed to identify any POU domains distinct from Oct-1 (results not shown), again strongly suggesting that Oct-1 is the major POU-domain protein present in the H441 cell line.

DISCUSSION

Previous studies had indicated that the proximal promoter region of both the human and rat TTF-1 gene contains elements that mediate the tissue-specific expression of TTF-1 [5,12]. In the present study we have concentrated on two regions, BS2 and BS1, that have been shown by mutagenesis to play a functional role in regulating TTF-1 gene expression [12]. These regions have also been shown to be binding sites through which the homoeotic gene HoxB3 can bind to and activate TTF-1 gene expression in

transient transfection studies [12]. However, in both the lung and the thyroid, expression of HoxB3 is confined to the mesenchyme [14] whereas expression of TTF-1 is restricted to the epithelium [4,5], suggesting that proteins other than HoxB3 are likely to play a role in regulating the TTF-1 gene through these binding sites *in vivo*.

Our studies have shown that in a TTF-1-expressing cell line, a single protein is able to bind to both the BS2 and BS1 sites. BS2 and BS1 are highly conserved in a region of 10 bases (nine out of ten) suggesting that this portion of the sequence is the site that binds to the protein. HoxB3 was shown to interact with ATTA motifs centred on both of these binding sites [12]. Mutation of three of these shared residues in the BS2 site renders the sequence unable to act as a competitor for the binding of proteins to both BS2 and BS1, suggesting that it is this conserved portion of BS2 and BS1 that is involved in the binding of the H441 cell protein. These sites bind to proteins that recognize a series of octamer-containing binding sites with and without ATTA cores.

We have identified the protein responsible for the generation of the retarded complex as being the ubiquitiously expressed octamer binding protein Oct-1. We have based this conclusion on the following observations. First, the ubiquitous nature of Oct-1 expression: Oct-1 has been shown to be expressed in many cell types whereas other POU-domain proteins have a more tissue-restricted expression pattern [28]. Oct-1 has also previously been shown to be expressed in lung cancer cell lines, both small cell carcinoma lines and adenocarcinoma lines [29]. The NCI-H441 line is an adenocarcinoma. Secondly, the antibody super-shift studies: the antiserum we have used was raised against a recombinant N-terminal fragment of human Oct-1, including the POU domain. Therefore we cannot exclude the formal possibility that it might cross-react with epitopes from other POU-domain proteins, particularly Oct-2, the POU domain of which is very similar to Oct-1 [30]. However, combining this with the information known about the expression of Oct-2 makes it unlikely that Oct-2 is present in H441 and Ishikawa cells [28]. Thirdly, the RT–PCR results: we could find no evidence of expression of POU-domain proteins other than Oct-1 with the use of RT–PCR, again suggesting that Oct-1 is the major POU-domain protein found in the H441 cells. Irrespective of the absolute identification of the H441 protein as Oct-1, these results do suggest indirectly that in the NCI-H441 cell line HoxB3 is unlikely to be a regulator of TTF-1 gene expression.

Oct-1 is one of the initial members of the POU-domain family of transcription factors. POU-domain proteins are a group of developmental regulators found in a wide range of organisms, first recognized as a distinct family by sequence similarity between the pituitary restricted Pit-1, the ubiquitous Oct-1, the B-cell enriched Oct-2 and the *Caenorhabditis elegans* protein Unc-86 [30]. Oct-1 is the most widely distributed member of the POU-domain family [28] and therefore it would seem to be an unlikely candidate to play a major role in governing the tissue-specificity of TTF-1 gene expression.

Octamer-binding motifs are frequently found close to the TATA box or initiator elements of both cell-type-specific and ubiquitously expressed genes. It is possible to envisage three roles for the octamer-binding sites in the TTF-1 gene. First, Oct-1 sites might play no role in determining the cell-type specificity of the TTF-1 gene, functioning only in the basal regulation of the TTF-1 gene. Secondly, Oct-1 binding to the BS1/BS2 sites might be a target for cell-specific or promoter-context-specific co-activator protein binding, which might help to mediate the specificity of the TTF-1 gene. Oct-1 and Oct-2 co-activator proteins have recently been identified: though lacking direct transactivation potential of their own, they specifically stimulate transcription through Oct-proteins binding to octamer motifs [31,32]. Thirdly, evidence has recently been provided that suggests that octamer binding proteins might play a direct role in the recruitment of the basal pre-initiation complex to TATA-less promoters in a manner similar to that proposed for Sp1 sites in TATA-less promoters [19]. The TTF-1 gene has no canonical TATA box, possessing instead two TAAAA motifs just 5′ to the transcription start site [5,12,24]. The TTF-1 gene also contains a CG-rich region 5′ to these TAAAA motifs, which is a specific binding site for members of the Sp1 family of zinc finger-containing transcription factors [33,34] (C. D. Bingle and S. Gowan, unpublished work). This site might also serve, either alone or with the octamer sites, to mediate the binding of the pre-initiation complex to the TTF-1 gene promoter. Site-directed mutations of these binding sites need to be constructed to characterize the functional role that they play in the regulation of the TTF-1 gene.

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