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Vernon, Ellen; Gaston, K

### Nucleic Acids Research

DOI:  
[10.1093/nar/25.18.3705](https://doi.org/10.1093/nar/25.18.3705)

Published: 01/10/1997

Publisher's PDF, also known as Version of record

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*Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):*  
Vernon, E., & Gaston, K. (1997). A functional YY1 binding site is necessary and sufficient to activate Surf-1 promoter activity in response to serum growth factors. *Nucleic Acids Research*, 25(18), 3705-11. <https://doi.org/10.1093/nar/25.18.3705>

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# A functional YY1 binding site is necessary and sufficient to activate Surf-1 promoter activity in response to serum growth factors

Ellen G. Cole and Kevin Gaston\*

Department of Biochemistry, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK

Received April 30, 1997; Revised and Accepted July 23, 1997

## ABSTRACT

The human Surf-1 and Surf-2 housekeeping genes are divergently transcribed and share a bi-directional, TATA-less promoter. Housekeeping promoters typically contain complex arrays of transcription factor binding sites and several studies have suggested that many of these sites might be functionally redundant. The Surf-1/Surf-2 promoter region contains four factor binding sites; members of the ETS family of transcription factors bind to two of these sites whilst YY1 binds to a third site immediately downstream of the major Surf-1 transcription start point. Here we show that Sp1 binds to the fourth transcription factor binding site. Although YY1 and Sp1 have previously been shown to interact both *in vitro* and *in vivo*, these proteins function independently at the Surf-1/Surf-2 promoter. The binding of Sp1 alone is sufficient to bring about full promoter activity in the Surf-2 direction. In contrast, both Sp1 and ETS proteins are required to bring about full promoter activity in the Surf-1 direction. The YY1 binding site is not required for basal transcription in either direction. The YY1 binding site is, however, both necessary and sufficient to confer growth factor inducibility on transcription in the Surf-1 direction. Our data suggest that functionally redundant transcription factor binding sites might not be a general feature of housekeeping promoters.

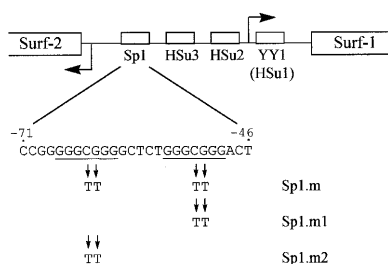
## INTRODUCTION

The promoters recognized by RNA polymerase II fall into two broad classes: the TATA-containing promoters and the TATA-less promoters (1). Tissue-specific genes typically contain a TATA box sequence located ~30 bp upstream of a single transcription start site. Tissue-specific transcription factors generally bind upstream of the TATA box and either activate or repress promoter activity. The promoters of housekeeping genes do not generally contain TATA box sequences and usually display multiple transcription start sites (2). Housekeeping promoters are active in most cell types and often contain binding sites for ubiquitous transcription factors such as Sp1 and YY1 (3 and references therein).

Although housekeeping promoters are probably the most common class of promoters in mammalian DNA, relatively few promoters of this type have been characterized in detail (4). The housekeeping promoters that have been analysed appear to contain complex arrays of transcription factor binding sites. However, mutation or deletion of these sites has revealed extensive functional redundancy. Large deletions that remove several factor binding sites from the mouse HTF9 promoter, for example, have relatively little effect on promoter activity (5). Similar experiments revealed an apparent surplus of regulatory elements within the mouse DHFR promoter (6), the mouse hprt promoter (2) and several promoters of ribosomal protein genes (7,8). Two related models could explain the high level of functional redundancy seen at housekeeping promoters. The first model proposes that different sets of transcription factors might be present in different cell types. Multiple regulatory elements might therefore be required to ensure gene expression in each cellular background. The second model proposes that the activity of housekeeping genes is subject to subtle transcriptional controls and that multiple regulatory elements are required to respond to manifold changes in the cell environment.

The human Surf-1 and Surf-2 genes lie in a cluster of at least six housekeeping genes (Surf-1–Surf-6) that are unrelated by sequence homology (9–11). The Surf-1/Surf-2 intergenic region (shown schematically in Fig. 1) does not contain TATA box sequences and shows multiple transcription start sites in both the Surf-1 and Surf-2 directions (12,13). DNase I footprinting revealed four transcription factor binding sites within this promoter region (13). Three of these factor binding sites (HSu1, HSu2 and HSu3) are also present within the mouse Surf-1/Surf-2 promoter (14). The HSu1 site lies immediately downstream of the major transcription start site of the Surf-1 gene (13). Gel retardation assays, methylation interference studies and specific antibodies have shown that the HSu1 binding factor corresponds to the initiator protein YY1 (15). YY1 binds to sites close to or overlapping the transcription start point at a number of genes and activates transcription initiation (16,17). Over-expression of YY1 activates transcription in the Surf-1 direction and, to a lesser degree, the Surf-2 direction (15). The HSu2 and HSu3 binding factors have not yet been identified, but have characteristics which suggest that they are members of the ETS family of DNA binding proteins (18). The fourth factor binding site within the human Surf-1/Surf-2 promoter shows sequence similarity to Sp1

\*To whom correspondence should be addressed. Tel: +44 117 928 7439; Fax: +44 117 928 8274, E-mail: gaston@bsa.bristol.ac.uk



**Figure 1.** The human Surf-1/Surf-2 promoter. The top line shows the organization of the human Surf-1/Surf-2 intergenic region. The bent arrows indicate the major transcription start points in each direction (for clarity the minor transcription start points are not shown). The open boxes represent the transcription factor binding sites revealed by DNase I footprinting. The second line shows an enlarged view of the potential Sp1 binding sites and includes the top strand sequence from -71 to -46 relative to the Surf-1 major transcription start site. The sequences similar to other Sp1 binding sites have been underlined. The small arrows indicate the positions at which mutations were introduced.

binding sites. YY1 and Sp1 have been shown to interact physically *in vitro* and *in vivo* and many TATA-less promoters contain binding sites for both of these proteins (19,20).

Here we show that Sp1 binds to the Surf-1/Surf-2 promoter and activates transcription of Surf-1 and Surf-2. However, Sp1 and YY1 appear to function independently and do not synergistically activate Surf-1 promoter activity. We also show that although the YY1 binding site makes little contribution to basal promoter activity, it is essential for activation of transcription in the Surf-1 direction in response to serum growth factors. Our data suggest that the factor binding sites within the Surf-1/Surf-2 promoter perform specific regulatory roles.

## MATERIALS AND METHODS

### Plasmids used in this study

The reporter plasmids used in this study are derivatives of pGL2-basic (Promega) and contain the human Surf-1/Surf-2 bi-directional promoter cloned upstream of the luciferase gene in either the Surf-1 (pGL-HS1) or Surf-2 (pGL-HS2) orientation. Construction and use of these reporters and a promoter construct containing a mutation in the YY1 binding site have been previously described, although the names have been abbreviated here for clarity (15). Promoter constructs containing mutations in the other transcription factor binding sites were made using PCR-directed mutagenesis. In each case the mutations introduced have previously been shown to block binding of the relevant transcription factor *in vitro* (13,15,18). The mutagenic primers are shown below; the underlined bases mismatch the template and produce the desired mutation:

Sp1<sub>forward</sub> 5'-GGAGCCGGGGTTTGGGGCTCTGGTTTGGGACT-3';  
 Sp1<sub>reverse</sub> 5'-AGTCCCAACCAGAGCCCCAACCCCCGGCTCC-3';  
 HSu2<sub>forward</sub> 5'-GCAGATGCCTCCTGCGTCCCGAAGCGCCCGC-3';  
 HSu2<sub>reverse</sub> 5'-GCGGGCGCTTAAGGGACGCAGGAGGCATCTGC-3';  
 HSu3<sub>forward</sub> 5'-GCGGCGCCCCTTATGCAGATGCCTCCTGCGTC-3';  
 HSu3<sub>reverse</sub> 5'-GACGCAGGAGGCATCTGCATAAGGGGGCGCCGC-3'.

Each forward primer was used in combination with the pGL2-basic-specific primer GL1 (Promega) to amplify half of the promoter sequence. Each reverse primer was used in combination with the pGL2-basic-specific primer GL2 (Promega) to amplify the opposite half of the promoter sequence. Corresponding promoter

fragments were then mixed and full-length promoter constructs amplified using the pGL2-basic-specific primers. Combinations of mutations were obtained using mutated promoter constructs as template for further rounds of PCR-directed mutagenesis. Each mutant promoter was cloned into pGL2-basic in either the Surf-1 or the Surf-2 orientation and sequenced using a Sequenase kit according to the supplier's instructions (USB).

### Gel retardation assays

The single-stranded oligonucleotide shown in Figure 1 (100 ng) was 5'-end-labelled with [ $\gamma$ -<sup>32</sup>P]ATP using T4 polynucleotide kinase. After annealing to a complementary oligonucleotide free label was removed using a NucTrap column (Stratagene). Labelled oligonucleotides (20 000 c.p.m.) were incubated with 10  $\mu$ g HeLa cell nuclear extract and 4  $\mu$ g poly(dI-dC):poly(dC-dI) in buffer containing 12 mM HEPES, pH 7.9, 5 mM MgCl<sub>2</sub>, 60 mM KCl, 1 mM DTT, 50  $\mu$ g/ml BSA, 0.5 mM EDTA, 0.05% NP40 and 10% glycerol. After 30 min at 20°C the complexes were resolved on 5% non-denaturing polyacrylamide gels run in 0.5 $\times$  TBE and visualized by autoradiography. Competitor oligonucleotides and Sp1-specific antibodies (Santa Cruz Biotechnology Inc.) were added at the beginning of incubation.

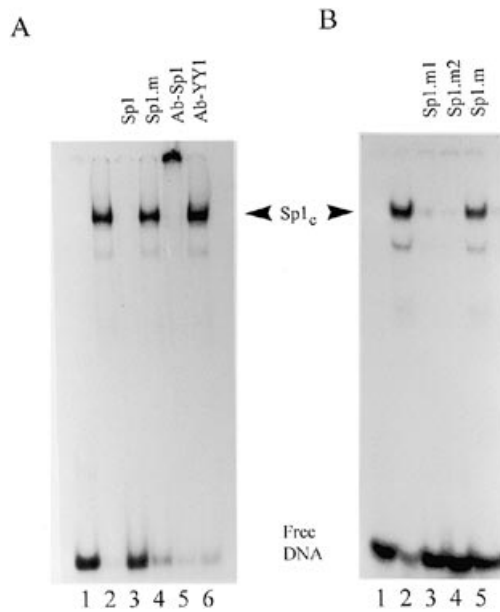
### Transient transfection assays

HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum (FCS) to a density of  $\sim 2 \times 10^7$  cells/15 cm Petri dish. The cells were transiently transfected with a total of 15  $\mu$ g plasmid DNA by electroporation (270 V, 950  $\mu$ F). After 24 h the cells were washed twice with phosphate-buffered saline (PBS) and luciferase activity determined using the Luciferase Assay System (Promega) according to the manufacturer's instructions. In serum stimulation experiments the transfected cells were left to recover for 18 h in medium containing 10% foetal calf serum before being serum starved for 24 h. The cells were then harvested at time points after the addition of 10% FCS to the medium. The plasmid pRSV- $\beta$ gal (5  $\mu$ g) was included in each experiment as an internal control for transfection efficiency.

## RESULTS

### Sp1 activates transcription of Surf-1 and Surf-2

DNase I footprinting revealed four transcription factor binding sites within the human Surf-1/Surf-2 promoter (see Fig. 1). The factor binding site immediately upstream of the Surf-2 gene contains two DNA sequences that closely resemble binding sites for Sp1 (underlined in Fig. 1, line 2). To determine whether protection of these sequences from DNase I was caused by binding of Sp1 we used gel retardation experiments and Sp1-specific antibodies. Figure 2A shows the results of a gel retardation experiment in which an oligonucleotide carrying the potential Sp1 binding sites was incubated with HeLa cell nuclear extract. The addition of nuclear extract resulted in formation of a protein-DNA complex (indicated by the arrowhead in Fig. 2A, lane 2). This complex was competed away by addition of an excess of unlabelled oligonucleotide but was not competed away by an oligonucleotide containing mutations in both potential Sp1 binding sites (Fig. 2A, lanes 3 and 4 respectively). The addition of Sp1-specific antibodies resulted in a supershift of this complex,

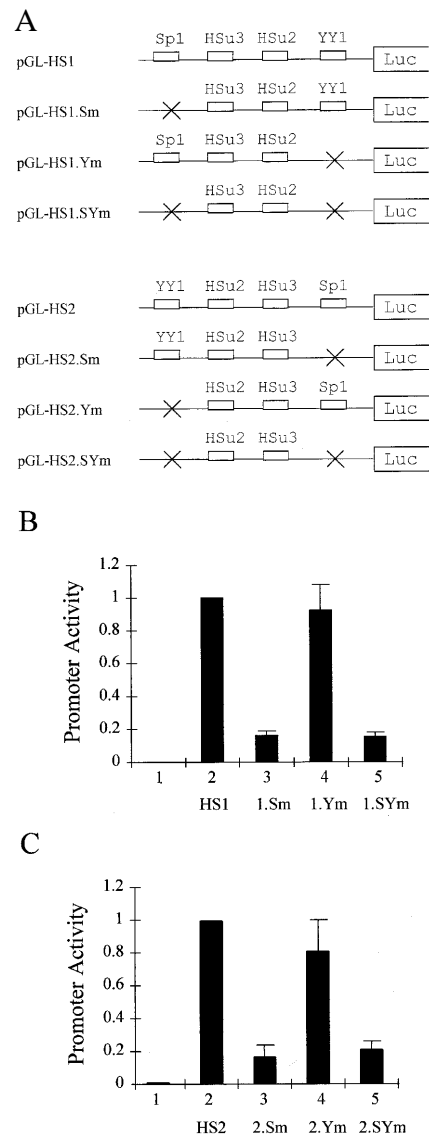


**Figure 2.** Sp1 binds to the human Surf-1/Surf-2 promoter. (A) A labelled oligonucleotide carrying the potential Sp1 binding sites (shown in Fig. 1) was incubated with HeLa cell nuclear extract under the conditions described in the text. Free and bound DNA were resolved on a 5% polyacrylamide gel and visualized by autoradiography. The addition of nuclear extract resulted in formation of a retarded complex (Sp1<sub>c</sub>), indicated by the arrowhead (lane 2). This complex was competed away by addition of a 200-fold excess of unlabelled oligonucleotide but not by addition of an oligonucleotide carrying mutations in both potential Sp1 binding sites (lanes 3 and 4 respectively). The addition of Sp1-specific antibodies resulted in a supershift of the complex (lane 5). The addition of YY1-specific antibodies had no effect (lane 6). (B) The Sp1-DNA complex (lane 2) was competed away by an oligonucleotide carrying a mutation in the Surf-1 proximal or the Surf-1 distal Sp1 binding site (lanes 3 and 4 respectively).

whereas antibodies raised against a different protein (YY1) had no effect (Fig. 2A, lanes 5 and 6 respectively).

The oligonucleotide used in these experiments contains two sequences that resemble binding sites for Sp1. To determine whether Sp1 binds to one or both of these sequences, we used competitor oligonucleotides containing mutations in either the Surf-1 proximal site or the Surf-1 distal site (Sp1.m1 and Sp1.m2 in Fig. 1, lines 4 and 5 respectively). An excess of either the Sp1.m1 oligonucleotide or the Sp1.m2 oligonucleotide competed away the Sp1-DNA complex (Fig. 2B, lanes 3 and 4). These data suggest that Sp1 binds to both of the sequences underlined in Figure 1.

Having shown that Sp1 binds to the Surf-1/Surf-2 promoter *in vitro*, we next set out to determine the role this protein plays in regulation of transcription in intact cells. Site-directed mutagenesis was used to introduce the Sp1.m mutations shown in Figure 1 into the Surf-1/Surf-2 promoter region. The wild-type and mutant promoters were cloned upstream of the luciferase gene in plasmid pGL2-basic. Plasmid pGL-basic contributes no promoter or enhancer elements and, in the absence of a promoter sequence, produces little or no luciferase activity in transfected cells. Transcription in the Surf-1 direction was measured using plasmids pGL-HS1 and pGL-HS1.Sm (shown in Fig. 3A), which contain wild-type and mutated Sp1 sites respectively. Similarly, transcription



**Figure 3.** Sp1 activates transcription in the Surf-1 and Surf-2 directions. (A) The reporter plasmids used in this study contain the human Surf-1/Surf-2 bi-directional promoter cloned upstream of the luciferase gene (Luc). The open boxes represent the transcription factor binding sites present in each construct. Mutated factor binding sites are marked with a cross. Transcription in the Surf-1 direction was measured using the pGL-HS1 series of plasmids (lines 1-4), whereas transcription in the Surf-2 direction was measured using the pGL-HS2 series (lines 5-8). (B) The graph shows the levels of luciferase activity found in HeLa cell extracts 24 h after transfection with: (1) pGL-basic; (2) pGL-HS1; (3) pGL-HS1.Sm, which contains mutations in the Sp1 binding sites; (4) pGL-HS1.Ym, which contains a mutation in the YY1 binding site; (5) pGL-HS1.SYm, which contains mutations in both the Sp1 and YY1 binding sites. Luciferase activity has been normalized using co-transfected pRSV-βgal and is presented as promoter activity relative to the wild-type Surf-1 construct. Values represent the average of at least four independent experiments. (C) The promoter activity of the pGL-HS2 series of constructs was determined exactly as described in (B).

in the Surf-2 direction was measured using plasmids pGL-HS2 and pGL-HS2.Sm. These constructs were transiently transfected into HeLa cells and assayed for promoter activity. The results of this experiment are shown in Figure 3B and C. Mutation of the Sp1 binding sites reduced transcription in the Surf-1 direction to



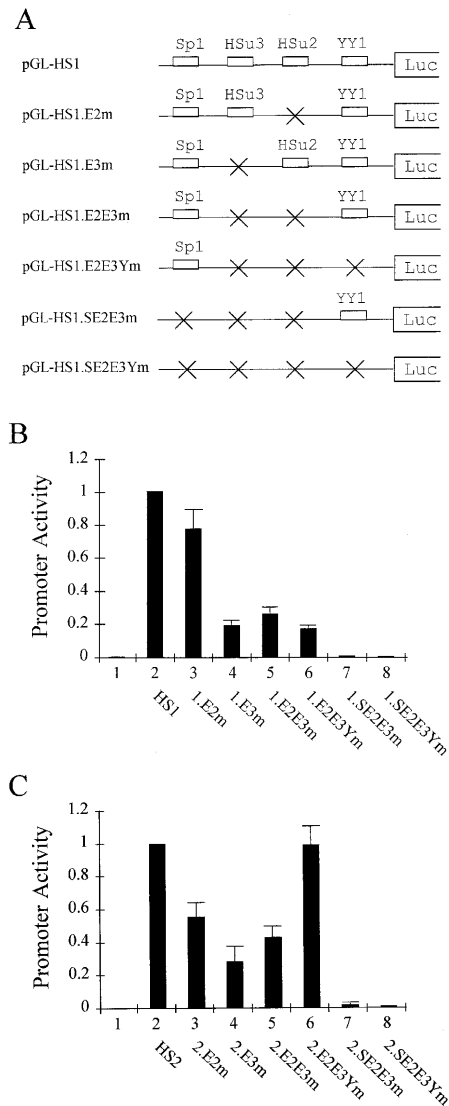
<20% of that seen with the wild-type promoter (compare columns 2 and 3 in Fig. 3B). Mutation of the Sp1 sites had a similar effect on transcription in the Surf-2 direction (columns 2 and 3 in Fig. 3C). Taken together with the results of the gel retardation experiments, these data suggest that Sp1 or a closely related protein binds to the human Surf-1/Surf-2 promoter and activates transcription in the direction of both Surf-1 and Surf-2.

### YY1 and Sp1 act non-synergistically at the Surf-1/Surf-2 promoter

Sp1 and YY1 have been shown to interact both *in vitro* and in intact cells (19,20). Furthermore, in the adeno-associated virus P5 promoter, the presence of multiple Sp1 binding sites upstream of a YY1 binding site has been shown to result in high levels of transcription initiation (16). Our previous work has shown that YY1 binds to the HSu1 site within the human Surf-1/Surf-2 promoter and that over-expression of YY1 activates transcription in the Surf-1 direction ~4-fold (15). Since we have now shown that Sp1 also binds to this promoter, we next looked at the effects of mutations in the Sp1 and YY1 binding sites. The Surf-1/Surf-2 YY1 binding site was mutated either alone or in combination with mutations in the Sp1 binding sites (see Fig. 3A) and the resulting constructs assayed for promoter activity in both the Surf-1 and Surf-2 directions. Mutation of the YY1 binding site had little effect on transcription in either direction (column 4 in Fig. 3B and C). Mutation of both the Sp1 sites and the YY1 site significantly reduced promoter activity in both directions (column 5 in Fig. 3B and C), however, this double mutation had the same effect on transcription as a mutation in the Sp1 sites alone. Furthermore, over-expression of either YY1 alone, Sp1 alone or both Sp1 and YY1 did not result in synergistic activation of promoter activity in either direction (data not shown). Thus, although both Sp1 and YY1 bind to the Surf-1/Surf-2 promoter these proteins activate transcription in the Surf-1 and Surf-2 directions non-synergistically.

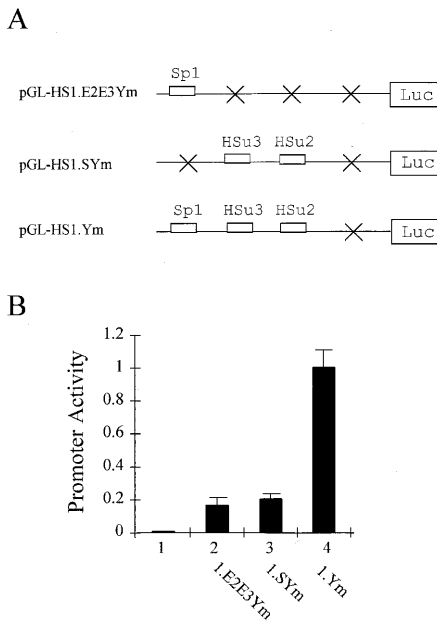
### Sp1 alone produces full transcription in the direction of Surf-2 but not Surf-1

In addition to Sp1 and YY1 binding sites, the human Surf-1/Surf-2 promoter also contains two binding sites for members of the ETS family of transcription factors (sites HSu2 and HSu3 in Fig. 1). At least two members of the ETS family, ETS-2 and PEA3, are capable of binding to the HSu2 and HSu3 sites *in vitro* (18). However, the identity of the factors that bind to these sites *in vivo* is not yet known. In order to determine the contribution these ETS sites make to Surf-1/Surf-2 promoter activity we mutated either HSu2, HSu3 or both HSu2 and HSu3, either alone or in combination with mutations in the Sp1 and/or YY1 binding sites (see Fig. 4A). Mutation of the HSu2 site resulted in a slight reduction in transcription in both the Surf-1 and Surf-2 directions (column 3 in Fig. 4B and C). In contrast, mutation of the HSu3 site reduced transcription in the Surf-1 and Surf-2 directions by ~80% and 70% respectively. Mutation of both the HSu2 and HSu3 sites reduced transcription in both directions to around the same levels seen in the presence of the HSu3 mutation alone (compare columns 4 and 5 in Fig. 4B and C). These data suggest that members of the ETS family of transcription factors positively regulate transcription in the direction of Surf-1 and Surf-2. Interestingly, the contributions of the HSu2 and HSu3 binding factors to promoter activity appear to be non-synergistic.



**Figure 4.** Sp1 and ETS factors are required for promoter activity. (A) The diagram shows the series of reporter plasmids used to assay promoter activity in the Surf-1 direction. Functional transcription factor binding sites are indicated by open boxes, mutated binding sites are marked with a cross. (B) The graph shows the luciferase activity found in HeLa cell extracts 24 h after transfection with: (1) pGL-basic; (2) pGL-HS1; (3) pGL-HS1.E2m, which contains a mutation in the HSu3 binding site; (5) pGL-HS1.E2E3m, which contains mutations in both HSu2 and HSu3; (6) pGL-HS1.E2E3Ym, which contains mutations in HSu2, HSu3 and the YY1 binding site; (7) pGL-HS1.SE2E3m, which contains mutations in HSu2, HSu3 and the Sp1 binding sites; (8) pGL-HS1.SE2E3Ym, which contains mutations in all four factor binding sites. Luciferase activity was normalized using co-transfected pRSV- $\beta$ gal and is presented as promoter activity relative to wild-type pGL-HS1 construct. The values shown are the average of at least four independent experiments. (C) The promoter derivatives described in (A) were cloned in the opposite orientation upstream of the luciferase gene (not shown) and used to assay transcription in the Surf-2 direction exactly as described in (B).

A promoter containing mutations in the YY1 binding site and the ETS binding sites produced the same level of transcription in the Surf-1 direction as a promoter containing mutations in the ETS sites alone (columns 5 and 6 in Fig. 4B). However, mutation of the YY1 binding site and the ETS binding sites resulted in



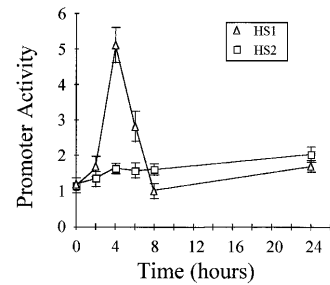
**Figure 5.** Sp1 and ETS factors activate the Surf-1 promoter synergistically. (A) The diagram shows the reporter plasmids used to determine the contribution of the Sp1 and ETS binding sites to promoter activity in the Surf-1 direction. Functional binding sites are indicated by open boxes, mutated sites are marked with a cross. (B) The graph shows luciferase activity found in HeLa cell extracts 24 h after transfection with: (1) pGL-basic; (2) pGL-HS1.E2E3Ym, which contains Sp1 sites alone; (3) pGL-HS1.SYm, which contains ETS sites alone; (4) pGL-HS1.Ym, which contains both Sp1 and ETS binding sites. Luciferase activity was normalized using co-transfected pRSV- $\beta$ gal and is presented as promoter activity relative to pGL-HS1. The values shown are the average of four independent experiments.

wild-type levels of transcription in the Surf-2 direction (column 6 in Fig. 4C). These data suggest that the presence of Sp1 binding sites alone is sufficient to produce transcription in the Surf-2 direction. Promoters containing mutations in both the Sp1 binding sites and the ETS binding sites or mutations in all four factor binding sites produced little or no transcription in either direction (columns 7 and 8 in Fig. 4B and C).

The experiments described above suggest that the ETS binding sites work together with the Sp1 sites to produce full transcription in the Surf-1 direction. To investigate the possibility of cooperative effects we transfected the series of plasmids shown in Figure 5A into HeLa cells and assayed promoter activity. The presence of Sp1 sites alone generated ~20% of wild-type promoter activity in the Surf-1 direction (column 2 in Fig. 5B). Similarly, the presence of ETS sites alone generated ~20% of wild-type promoter activity in this direction (column 3 in Fig. 5B). However, the presence of both Sp1 binding sites and ETS binding sites generated full Surf-1 promoter activity. Thus Sp1 and ETS factors activate transcription in the Surf-1 direction synergistically.

### Serum growth factors stimulate transcription in the Surf-1 direction

The experiments described above show that a mutation in the Surf-1/Surf-2 YY1 binding site has little or no effect on transcription in either the Surf-1 or the Surf-2 directions. This result is surprising given that both the human and the mouse Surf-1/Surf-2 promoters contain YY1 binding sites and that over-



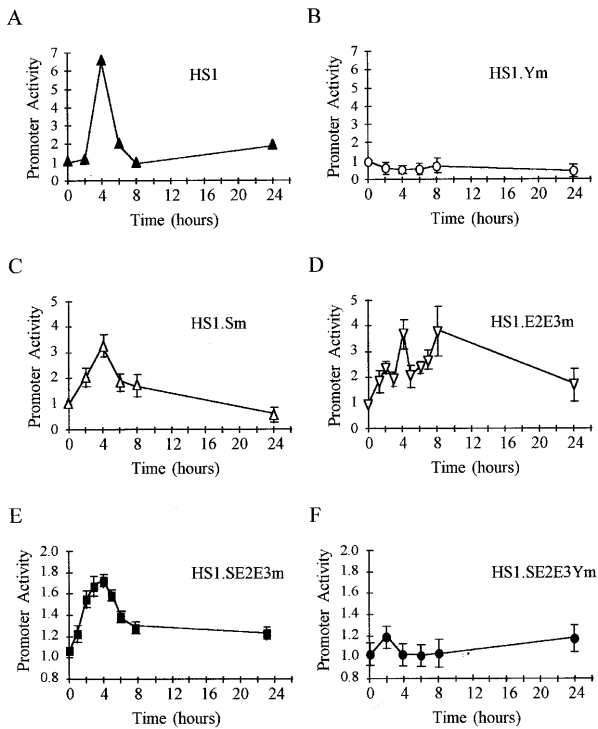
**Figure 6.** Serum growth factors activate transcription in the Surf-1 direction. HeLa cells were transiently transfected with either pGL-HS1 (triangles) or pGL-HS2 (squares). The transfected cells were grown in serum-free medium for 24 h then stimulated by the addition of medium containing 10% foetal calf serum. Cells were harvested at the time points indicated and assayed for luciferase activity exactly as described in Figure 3. Luciferase activity has been normalized using co-transfected pRSV- $\beta$ gal and is presented as promoter activity relative to that seen immediately prior to serum stimulation. The values shown are the average of six independent experiments.

expression of YY1 activates transcription in the Surf-1 direction by ~4-fold. YY1 interacts with a number of transcription factors, including Sp1, E1a and Myc (19–22). The nuclear oncoprotein Myc has been implicated in control of many cellular functions, including proliferation, differentiation and apoptosis (23). After mitogenic stimulation, expression of Myc is rapidly and transiently induced, resulting in entry of quiescent cells into the cell cycle (24). To determine whether the YY1–Myc interaction might confer mitogenic regulation on the Surf-1/Surf-2 promoter we assayed promoter activity at a number of time points after stimulation of cells with FCS.

To measure promoter activity in the Surf-1 and Surf-2 directions, HeLa cells were transiently transfected with either pGL-HS1 or pGL-HS2, exactly as described previously. The transfected cells were then serum starved for 24 h prior to addition of 10% FCS. Cells were harvested at various time points after stimulation with FCS and assayed for luciferase activity. Figure 6 summarizes the results of several experiments. Transcription in the Surf-1 direction was transiently activated after addition of FCS, reaching a peak ~4 h after serum stimulation and returning to pre-stimulation levels after ~8 h. In contrast, addition of serum growth factors had no effect on transcription in the Surf-2 direction.

Having shown that Surf-1/Surf-2 promoter activity responds to serum stimulation we next set out to determine whether the YY1 binding site mediates this effect. Promoter constructs containing mutations in either the YY1 site, the Sp1 sites or the ETS binding sites were transiently transfected into HeLa cells and serum stimulated as described above. Mutation of the YY1 binding site totally eliminated the response to serum growth factors (Fig. 7B). In contrast, mutations in the Sp1 binding sites had little effect on the response to serum (Fig. 7C). Mutation of the ETS binding sites did not prevent serum stimulation of transcription in the Surf-1 direction but did alter the duration of the response (Fig. 7D). In the absence of ETS binding sites transcription in the Surf-1 direction increased dramatically after serum stimulation, however, in this case transcription failed to return to pre-stimulation levels after 8 h (compare Fig. 7A and D).

Further evidence to suggest that the YY1 binding site mediates the response of this promoter to serum growth factors was obtained using a promoter derivative containing mutations in both the Sp1 binding sites and the ETS binding sites. The presence of a



**Figure 7.** A functional YY1 binding site mediates the response to serum growth factors. HeLa cells were transiently transfected with: (A) pGL-HS1 (filled triangles); (B) pGL-HS1.Ym (open circles); (C) pGL-HS1.Sm (open triangles); (D) pGL-HS1.E2E3m (inverted triangles); (E) pGL-HS1.SE2E3m (filled squares); (F) pGL-HS1.SE2E3Ym (filled circles). The transfected cells were serum starved for 24 h, stimulated by addition of 10% foetal calf serum and assayed for luciferase activity, exactly as described in Figure 6.

YY1 binding site alone was sufficient to generate a response to serum growth factors, although the level of activation was much less than that seen with the intact promoter (Fig. 7E). A promoter derivative containing mutations in all of the known factor binding sites showed no response to serum growth factors (Fig. 7F), as did a reporter plasmid lacking promoter sequences (data not shown). Taken together, these experiments show that although the YY1 binding site present in the Surf-1/Surf-2 promoter is not required for basal transcription in either direction, this site is necessary and sufficient to bring about mitogen stimulation of transcription in the Surf-1 direction.

## DISCUSSION

The human Surf-1 and Surf-2 genes are divergently transcribed from a single bi-directional promoter (13). At present, the functions of the Surf-1 and Surf-2 gene products are unknown, however, Surf-1 and Surf-2 are expressed in all tissues and cell lines examined to date, suggesting that these genes encode proteins with housekeeping roles. The architecture of the Surf-1/Surf-2 promoter provides further evidence to support this conclusion. The Surf-1/Surf-2 promoter has all the characteristic features of a housekeeping gene promoter: it is located within a CpG island, has multiple transcription start sites, contains several binding sites for ubiquitous transcription factors and does not contain TATA box-like sequences (12–15). We have used

site-directed mutagenesis to investigate the degree of functional redundancy within the Surf-1/Surf-2 promoter region.

The human Surf-1/Surf-2 promoter region contains four transcription factor binding sites. YY1 binds to the HSu1 site and over-expression of YY1 activates transcription in the Surf-1 direction (15). Members of the ETS family of transcription factors bind to the HSu2 and HSu3 sites *in vitro*, although the factors that bind to these sites *in vivo* have not yet been identified (18). Here we have shown that Sp1 binds to the fourth factor binding site within the Surf-1/Surf-2 promoter region. Mutation of the Sp1 binding sites significantly reduces transcription in the direction of both Surf-1 and Surf-2. Mutation of the HSu2 and HSu3 binding sites also reduces transcription in both directions. Although binding of Sp1 alone is sufficient to bring about full promoter activity in the Surf-2 direction, both Sp1 and ETS proteins are required to bring about full promoter activity in the Surf-1 direction. The important role played by Sp1 in transcriptional regulation of the human Surf-1 and Surf-2 genes is somewhat surprising given that although the HSu1, HSu2 and HSu3 factor binding sites are present in both the human and the mouse Surf-1/Surf-2 promoter regions, the mouse promoter does not contain any sequences that resemble Sp1 binding sites (14). Hence, our data add weight to the view that the conserved transcription factor binding sites present within the promoter regions of the same gene from different species are not necessarily the only binding sites important for gene regulation.

A mutation in the HSu1 site that completely prevents binding of YY1 has little or no effect on basal transcription in either the Surf-1 or Surf-2 directions, suggesting that the YY1 binding site might be functionally redundant. However, here we have shown that transcription in the Surf-1 direction is activated ~6-fold following stimulation of HeLa cells with serum growth factors and that a mutation in the YY1 binding site completely blocks this activation. YY1 has also been shown to play at least two roles in regulation of *c-fos* promoter activity in response to growth factors: first, YY1 facilitates binding of the serum response factor to the *c-fos* serum response element (25); second, YY1 blocks activation of *c-fos* by the cAMP response element binding protein (26). Thus regulation of the *c-fos* promoter by YY1 appears to require additional transcription factor binding sites. In contrast, we have shown that a functional YY1 binding site is sufficient to mediate growth factor stimulation of Surf-1 promoter activity. YY1 interacts with several cellular transcription factors, including Myc (22). The addition of serum growth factors to serum-starved cells results in a rapid and transient induction of *c-myc* gene expression (24). Further experiments will be required to determine whether the YY1–Myc interaction is required for activation of Surf-1 promoter activity in response to serum growth factors.

Relatively few housekeeping promoters have been characterized in detail. However, in several of those housekeeping promoters which have been examined mutations that remove or inactivate transcription factor binding sites have been shown to have little or no apparent effect on promoter activity. In the hamster HMG-CoA promoter, for example, mutations in two factor binding sites were found to have no effect on transcription initiation *in vitro* (27). Similarly, extensive deletions within the mouse HTF9 promoter produced little change in promoter activity in intact cells (5). These and other similar studies suggest that housekeeping promoters contain compensatory or redundant transcription factor binding sites (5–8). However, our data suggest that each factor binding site within the Surf-1/Surf-2



promoter region has a role in generation of full promoter activity. The absence of functionally redundant elements within the Surf-1/Surf-2 promoter implies that the 'redundant' factor binding site within these other promoters might in fact play important roles in transcription initiation. One possibility is that these sites could mediate the response of housekeeping genes to as yet unidentified stimuli. Alternatively, these sites might be involved in maintenance of CpG methylation patterns. Most housekeeping promoters are located within unmethylated CpG islands (28). Methylation of CpG sequences within these islands often leads to repression of promoter activity (29). One function of the 'redundant' factor binding sites might be to maintain the methylation-free status of these promoters. Another possibility is that these sites might be important in the formation of nucleosome-free regions. Active promoters are typically associated with nuclease hypersensitive sites in which nucleosomes are either absent or partially disrupted (30). The formation of a nucleosome-free region within the chicken  $\beta^A/\epsilon$  globin gene enhancer has recently been shown to be an all-or-nothing event that requires binding of several tissue-specific transcription factors (31). The seemingly redundant transcription factor binding sites within housekeeping promoters might act collectively to prevent transcriptional silencing mediated by nucleosomes.

In conclusion, we have shown that the Surf-1/Surf-2 housekeeping promoter contains four transcription factor binding sites, each of which is required for full promoter function. Our data suggest that functionally redundant transcription factor binding sites are not a general feature of housekeeping promoters. Further experiments will be required to determine whether the apparently redundant sites present within other housekeeping promoters are also required for full promoter activity.

## ACKNOWLEDGEMENTS

We are grateful to Dr Padma-Sheela Jayaraman for comments on the manuscript. This work was supported by a Wellcome Trust Project Grant and a BBSRC PhD Studentship.

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