Nucleic Acids Research, 1996, Vol. 24, No. 11 **1981–1986**

The transcription factors Sp1 and Oct-1 interact physically to regulate human U2 snRNA gene expression

Anne-Christine Ström, Maud Forsberg, Peter Lillhager and Gunnar Westin*

Department of Medical Genetics, Uppsala University, Biomedical Centre, Uppsala, Sweden

Received March 28, 1996; Accepted April 12, 1996

ABSTRACT

The expression of human small nuclear U2 RNA genes is controlled by the proximal sequence element (PSE), which determines the start site of transcription, and a distal sequence element (DSE). The DSE contains an octamer element and three Sp1 binding sites. The octamer, like the PSE, is essential for U2 transcription. The Sp1 sites contribute to full promoter activity by distance-dependent cooperative interactions with the transcription factors Sp1 and Oct-1. Here we show that purified recombinant Sp1 and Oct-1 bind cooperatively to the DSE and that they physically interact *in vitro*. Furthermore, we show that Sp1 and Oct-1 interact *in vivo* using a yeast two-hybrid system. The domain of Sp1 which interacts with Oct-1 is confined to the region necessary for transcriptional stimulation of U2 RNA transcription. This region contains the glutamine-rich activation domain B and a serine/threonine-rich part. The results demonstrate that Sp1, in addition to binding to a number of other factors, also interacts directly with transcription factor Oct-1.

INTRODUCTION

Transcription of small nuclear RNA (snRNA) genes by RNA polymerase II is dependent on the proximal sequence element (PSE) centred around position –55 and the distal sequence element (DSE) approximately at position –220 (for reviews see 1–4). The PSE determines the start site of transcription (1–4) and may be required for 3′-end formation (5–8). A multi-subunit complex (e.g. PBP, PTF and $SNAP_C$) needed for transcription of both RNA polymerase II- and polymerase III-transcribed snRNA genes binds to PSE *in vitro* (9–13). The DSE regulates the level of transcription and, like all DSEs of U snRNA genes, the DSE of human U2 snRNA genes contains an essential octamer element, which binds transcription factor Oct-1 and other members of the POU family of homeodomain proteins (14–17). In addition to Oct-1, transcription factor Sp1 binds to the DSE of human U2 genes *in vitro* and deletion of these sites leads to an 80% reduction in U2 transcription (14,16,17). Experiments in *Xenopus* oocytes suggested that DSE binding factors could function by stabilizing the formation of transcription complexes at the U2 promoter (18), and in agreement with this it has been shown that Oct-1 potentiates binding of PTF to the PSE *in vitro* (10). A combination of transient expression analysis and *in vitro* binding studies using crude nuclear extracts have revealed distance-dependent cooperative interactions with the Sp1 and Oct-1 factors at the DSE of human U2 genes (19). Analysis of chimeric proteins has revealed U2 promoter-specific activation domains of Oct-1 (20) and we have recently shown that cooperative stimulation of human U2 snRNA transcription requires a region of Sp1 that includes a serine/threonine-rich part in addition to the glutamine-rich activation domain B (21).

Here we show that purified recombinant Sp1 and Oct-1 factors bind DNA cooperatively and that they interact physically *in vitro*. We also show that Sp1 and Oct-1 interact *in vivo* in a yeast two-hybrid assay. Furthermore, the domain of Sp1 that interacts with Oct-1 was mapped to a region necessary for transcriptional stimulation, strongly suggesting that the interaction between Sp1 and Oct-1 is important for U2 snRNA gene transcription.

MATERIALS AND METHODS

Protein preparations

The Oct-1 cDNA, PCR amplified from plasmid pBS/Oct-1 (22), was cloned C-terminally of the histidine-tag in pET-19b (Novagen; 23,24). His/Oct-1 was expressed in *Escherichia coli* BL21 pLysS at 20°C for 3 h and the extract from 1 l of culture was passed twice over a Ni^{2+}–NTA (Qiagen) resin column essentially as described (25). His/Oct-1 eluted with 150 mM imidazole (2.5 ml) was dialyzed into a buffer (20 mM HEPES–KOH, pH 7.9, 20% glycerol, 0.1 M KCl, 2 mM $MgCl_2$) and used directly in the experiments. A control extract was prepared from untransformed bacteria in the same way. The GST/Sp1 expression vector, which contains the complete Sp1 cDNA (kindly provided by Dr E.Wintersberger, Vienna), was transformed into *E.coli* DH5α. Cells were grown, extract prepared and GST/Sp1 proteins purified on glutathione–Sepharose (Pharmacia) as described (26). GST (glutathione S-transferase) was prepared in the same way. The His/Oct-1 and GST/Sp1 preparations were analyzed on SDS–PAGE and major His/Oct-1 and GST/Sp1 polypeptides of the correct sizes were detected by Coomassie

* To whom correspondence should be addressed: Institutionen för Kirurgi, Akademiska Sjukhuset, Forskningsavdelning 2, Ingång 70, Plan 3, Lab 9, S-751 85 Uppsala, Sweden

brilliant blue staining, together with minor degradation products, and by Western blotting with anti-Oct-1 and anti-Sp1 antisera (Santa Cruz Biotechnology Inc.).

Electrophoretic mobility shift analysis

For the binding reaction 4 ng Sp1 (Promega) and 4 µl His/Oct-1 extract were preincubated with 4 µg poly(dI·dC) (Pharmacia) for 10 min at room temperature in a buffer containing 10 mM HEPES–KOH, pH 7.9, 25% glycerol, 50 mM KCl, 5 mM $MgCl_2$, 0.6 mM DTT and 0.25% non-fat dried milk. An aliquot of 2 fmol of the ^{32}P-labelled probe (Sp1–10–Low Octa), containing a Sp1 binding site separated by 10 bp from a low affinity octamer site (19), was thereafter added and the incubation was continued for another 20 min at room temperature. The total reaction volume was 20 µl. In the competition experiments 1.5 pmol specific competitor DNA, containing an octamer element or two Sp1 binding sites, was added. The reaction mixtures were separated on a 4% polyacrylamide (29:1) gel in 0.25× TEB buffer at 10 V/cm for 75 min (27). The saturating Sp1 binding study was done in the same way except that the probe was incubated with or without saturating amounts of Sp1 (30 ng) and with increasing amounts of His/Oct-1 extract. The amounts of probe and shifted complexes were quantified by PhosphorImager analysis (Molecular Dynamics).

Protein–protein interactions

The purified His/Oct-1, GST/Sp1 and GST proteins and the control extract were coupled to tosyl-activated magnetic beads (Dynabeads; Dynal) essentially as described by the manufacturer. Thereafter, the beads were incubated with bovine serum albumin to saturation. The coupled beads were stored in PBS, 0.1% BSA at 4°C. For one interaction assay 5 µl coated beads were washed twice in 100 µl binding buffer (25 mM Tris–HCl, pH 7.9, 10% glycerol, 0.2 M KCl, 0.1% NP-40 and 1 mM DTT). The beads were then incubated with the various proteins in 50 µl binding buffer for 90 min at room temperature with occasional gentle shaking. After extensive washing in the binding buffer (6× 0.5 ml) protein loading buffer was added to the beads, the samples were boiled and proteins were separated on SDS–PAGE. The *in vitro* transcribed/translated ^{35}S-labeled proteins were detected by autoradiography. Equimolar amounts of *in vitro* translated Sp1 and GAL/Sp1 fusions and of Oct-1 and the Oct-1 POU domain proteins were used in the binding reactions. Aliquots of 30 ng HeLa cell Sp1 (Promega) were used in the binding reactions with the His/Oct-1 beads and with the control beads. Western blotting analysis was performed by standard methods using anti-Sp1 antisera (Santa Cruz Biotechnology Inc.) and the ECL detection kit (Amersham). Ethidium bromide (100 µg/ml) was used (28) to show that binding of ^{35}S-labelled C482 to His/Oct-1 beads was a DNA-independent protein association (data not shown). To produce the ^{35}S-labelled proteins a Sp1 expression vector (amino acids 83–778) and the various GAL/Sp1 constructs (21), which all contain a T7 RNA polymerase promoter, were *in vitro* transcribed/translated using the TNT coupled reticulocyte lysate system (Promega). Two major translation products were obtained from the fusion plasmids. Since both bands were detected by Western blotting with anti-GAL4 antisera (data not shown), the shorter one is the result of abortive translation. ^{35}S-Labelled Oct-1 and Oct-1 POU domain (amino acids 296–455 of Oct-1) were produced from plasmids pBGO-Oct-1 and pBGO-ATG-POU1, respectively (29).

Yeast interaction assay

The interaction trap assay, a yeast two-hybrid system, was used as described by Gyuris *et al*. (30) and Paroush *et al*. (31). Yeast strain EGY48, with an integrated *LEU2* reporter gene and upstream *LexA* operators, was transformed with pSH18-34. This plasmid contains the *LexA*$_{op}$–*lacZ* reporter. This reporter strain was then transformed with LexA/Sp1(231–485) and/or B42/Oct-1(1–369). The LexA/Sp1(231–485) plasmid was constructed by ligating the *Eco*RI fragment from GAL/Sp1 (231–485) (21) C-terminally of *LexA*(1–202) in pEG202. The 1.2 kbp *Eco*RI fragment from plasmid pBS/Oct-1 (22), encoding amino acids 1–369 of Oct-1, was cloned into the yeast expression vector pJG4-5 (30). This vector allows galactose-dependent expression of Oct-1(1–369) as a fusion protein with N-terminal sequences consisting of a nuclear localization signal, a transcription activation domain (B42) and the haemaglutinin epitope tag. Galactose-dependent LEU$^+$ colonies were picked and grown on glucose X-gal Ura$^-$ His$^-$ Trp$^-$ plates and on galactose X-gal Ura$^-$ His$^-$ Trp$^-$ plates. This selects for the *LexA*$_{op}$–*lacZ* reporter, LexA/Sp1(231–485) and B42/Oct-1(1–369) plasmids, respectively. Blue colonies appeared only on X-gal plates with galactose. Cells were grown in liquid culture with glucose and galactose medium and extracts were prepared using the reporter lysis buffer as described by the manufacturer (Promega). β-Galactosidase activity was determined in a luminometer using a chemiluminescent β-galactosidase system as described by the manufacturer (Clontech).

RESULTS

The purified Sp1 and Oct-1 factors bind DNA cooperatively

Electrophoretic mobility shift analysis was performed to see whether purified recombinant Sp1 and Oct-1 factors bind to the distal sequence element of human U2 snRNA genes in a cooperative way. His-tagged Oct-1 (His/Oct-1) purified from *E.coli* and Sp1 purified from vaccinia virus-infected HeLa cells were incubated with the probe Sp1–10–Low Octa. This probe contains one Sp1 site separated by 10 bp from a low affinity binding site for Oct-1 and has been used to demonstrate cooperative binding in crude nuclear HeLa cell extracts (19). His/Oct-1 bound weakly (complex A) and Sp1 bound strongly (complex B) to the probe, as expected (Fig. 1A, lanes 1 and 2). Addition of both factors resulted in a prominent retarded complex (Fig. 1C, lane 3). This complex was not seen when Sp1- or Oct-1-specific competitor DNAs were added (lanes 4 and 5), showing that complex C consists of templates to which both Oct-1 and Sp1 have bound. These results demonstrate cooperativity in binding to DNA, since the fraction of templates with Oct-1 bound to the low affinity site is increased in the presence of Sp1 (compare lanes 1 and 3). Mobility shift experiments with or without a saturating concentration of Sp1 and small increments of Oct-1 concentrations (Fig. 1B) showed that the fraction of complex C (Sp1 + Oct-1) rapidly increased compared with the fraction of complex A (Oct-1). We have also found that a GAL/Sp1 fusion (C590, see Fig. 5) bound DNA cooperatively together with His/Oct-1 (data not shown). In this experiment the

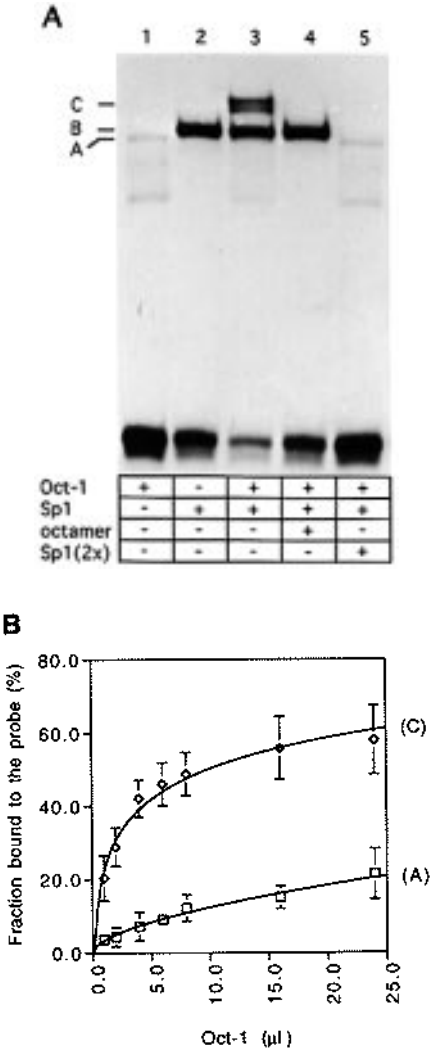

Figure 1. Purified recombinant Sp1 and Oct-1 factors bind DNA cooperatively. (**A**) Electrophoretic mobility shift analysis of affinity-purified *E.coli* His/Oct-1 and HeLa cell Sp1 (Promega). The probe, Sp1–10–Low Octa, contains one Sp1 site separated by 10 bp from a low affinity binding site for Oct-1 (19). Octamer and Sp1(2×) are competitor DNAs for Oct-1 and Sp1, respectively. The faster migrating complex seen in lanes 1, 3 and 5 represents a degraded form of Oct-1. Complex A, Oct-1; complex B, Sp1; complex C, Oct-1 + Sp1. (**B**) Electrophoretic mobility shift analysis with increasing concentrations of Oct-1 with or without a saturating concentration of Sp1. The graph shows fractions of the total amount of probe shifted to complex A (Oct-1) and complex C (Oct-1+Sp1) as a function of the Oct-1 concentration.

probe UAS–10–Low Octa was used (21). This suggests that the DNA binding domain of Sp1 is not involved. We conclude from these experiments that purified Sp1 and Oct-1 factors bind DNA cooperatively.

The Sp1 and Oct-1 factors interact physically

Cooperativity in binding to DNA can be achieved through a direct interaction between the two proteins or could be a result of structural changes in one of the binding sites, induced by binding of one factor, leading to increased binding of the second factor. To test whether the Sp1 and Oct-1 factors interact without DNA, His/Oct-1 was coupled to magnetic beads and incubated with Sp1 from HeLa cells or with ^{35}S-labelled Sp1 synthesized by *in vitro* translation (Fig. 2). Both protein preparations bound to the Oct-1 beads. ^{35}S-Labelled Sp1 did not bind to the control beads but Sp1 from HeLa cells showed some binding (Fig. 2A and B). The reverse experiment was also performed (Fig. 2C). A GST/Sp1 protein preparation was coupled to magnetic beads and incubated with ^{35}S-labelled Oct-1 or with the Oct-1 POU domain. Oct-1, but not the Oct-1 POU domain, bound to the GST/Sp1 beads. Neither protein preparation bound to control beads coupled with GST protein (Fig. 2C). GST/Sp1 could bind DNA cooperatively together with His/Oct-1, using the mobility shift assay described above (data not shown). We conclude from these experiments that the Sp1 and Oct-1 factors interact directly and that this interaction contributes to the cooperativity in binding to DNA.

An Oct-1 interaction domain is located between amino acids 304 and 485 of Sp1

Equimolar amounts of ^{35}S-labelled GAL/Sp1 fusion proteins were tested for their ability to interact *in vitro* with His/Oct-1 beads and with control beads as described above (Fig. 3). Fusions C590, C482, C406, N231, 231–485 and 304–485 bound to the Oct-1 beads and not to control beads (see Fig. 5 for a map of the GAL/Sp1 fusions and a summary of the results). The GAL4 DNA binding domain and fusions C350, C262, 263–437, 368–485 showed no binding in this assay (Fig. 3). The binding of fusion C482 to Oct-1 was found to be resistant to 100 µg/ml ethidium bromide (28), indicating that contaminating DNA was not involved (data not shown). Fusion C482 bound most efficiently to Oct-1, C406 bound weakly and C350 not at all. This suggests that efficient binding to Oct-1 requires a region between amino acids 350 and 482 of Sp1. Since fusions 231–485 and 304–485, but not 368–485, bound to Oct-1, we conclude that the shortest region of Sp1 able to bind directly to Oct-1 contains amino acids 304–485.

Sp1 and Oct-1 interact *in vivo*

We have used the interaction trap (30,31), a yeast two-hybrid system (32), to study the interaction between Sp1 and Oct-1. In these experiments, physical association between Sp1(231–485) fused to LexA and the N-terminal part of Oct-1 (1–369) fused to a transcriptional activation domain (B42) was analyzed (Fig.4A). Transcription of the two reporter genes, *LEU2* and *lacZ*, that contain upstream LexA binding sites was measured and only yeast transformed with both the LexA/Sp1(231–485) and B42/Oct-1(1–369) plasmids were found to be galactose-dependent LEU$^+$ and galactose-dependent blue on X-gal indicator plates (Fig. 4B). We conclude from these results that Sp1 and Oct-1 interact *in vivo*. The part of Oct-1 that interacts with Sp1 contains the POU-specific domain and glutamine-rich regions (22). Interaction between Sp1(231–485) and a B42/Oct-1 fusion containing the complete open reading frame of Oct-1 was not detected (data not shown).

Correlation between transcriptional stimulation and Sp1/Oct-1 interaction

We have previously analyzed the ability of GAL/Sp1 fusion proteins to cooperatively stimulate U2 snRNA transcription together with Oct-1, using a transient expression assay in COS7 cells (21). These experiments showed that a region of Sp1

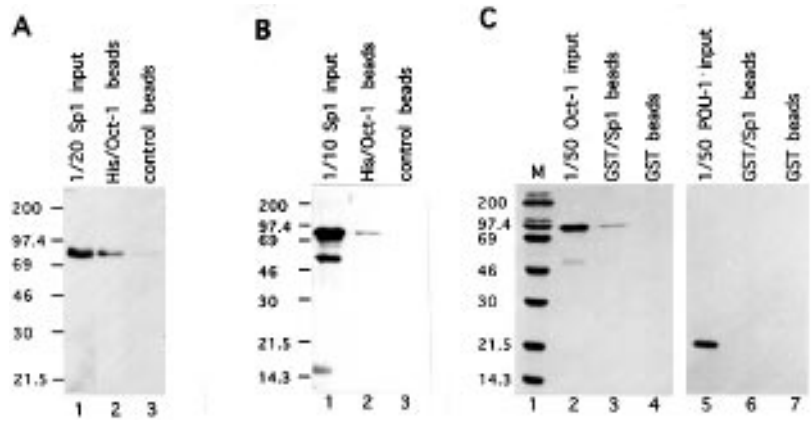

Figure 2. The Oct-1 and Sp1 factors interact physically. (**A**) His/Oct-1 or control extract coupled to tosyl-activated magnetic beads were incubated with HeLa cell Sp1. After extensive washing in binding buffer the bound Sp1 was analyzed by SDS–PAGE and Western blotting with anti-Sp1 antisera. (**B**) Binding of *in vitro* transcribed/translated ^{35}S-labelled Sp1 to His/Oct-1 or to control beads. (**C**) Binding of ^{35}S-labelled Oct-1 and Oct-1 POU domain to magnetic beads coupled with GST/Sp1 or with GST.

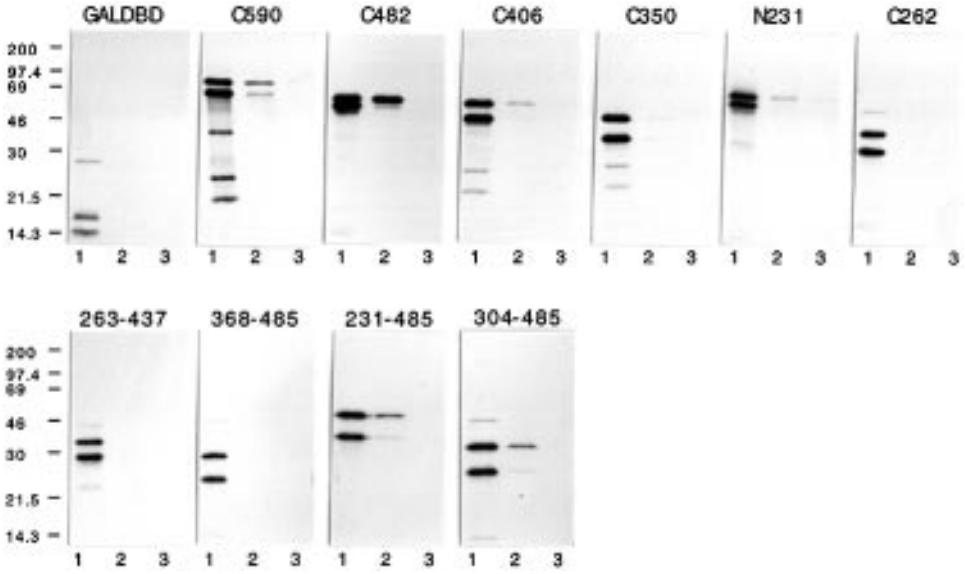

Figure 3. An Oct-1 interaction domain of Sp1. Binding of equimolar amounts of *in vitro* transcribed/translated ^{35}S-labelled GALDBD and GAL/Sp1 fusion proteins to His/Oct-1 beads or to control beads. Two major translation products are obtained from the fusion plasmids, where the shorter one is the result of abortive translation (data not shown). A map of the GAL/Sp1 fusions are shown in Figure 5. Lane 1, 1/10 of protein input; lane 2, binding to His/Oct-1 beads; lane 3, binding to control beads.

containing the glutamine-rich activation domain B together with an N-terminally located serine/threonine-rich part (amino acids 231–485) was sufficient for stimulation of U2 gene transcription. In addition, we found that a GAL/Sp1 fusion with an N-terminal truncation of this region (304–485) also stimulated transcription. Figure 5 summerizes the activation data (21) and the results presented in Figure 3. The results show a correlation between transcriptional stimulatory activity *in vivo* and binding of Oct-1 and GAL/Sp1 fusions *in vitro*. Thus, the part of Sp1 that interacts with Oct-1 is located in a region necessary for transcriptional stimulation of U2 RNA transcription. Moreover, the demonstration that Sp1 and Oct-1 also interact in yeast strengthens the conclusion that this interaction is functionally relevant.

DISCUSSION

The RNA polymerase II-dependent human U2 snRNA genes are tandemly repeated, ubiquitously expressed and have a promoter that activates transcription to very high rates, with a minimum of one transcript/gene/2–4 s (33,34). The DSE contains an octamer element and three Sp1 binding sites. The octamer element, like the PSE, is essential for U2 transcription and the Sp1 sites contribute to full promoter activity, by distance-dependent cooperative interactions with the Sp1 and Oct-1 factors (19).

We have previously analyzed Sp1 activating functions at the U2 snRNA promoter and found that the glutamine-rich activation domains A and B, which both strongly stimulate a TATA box

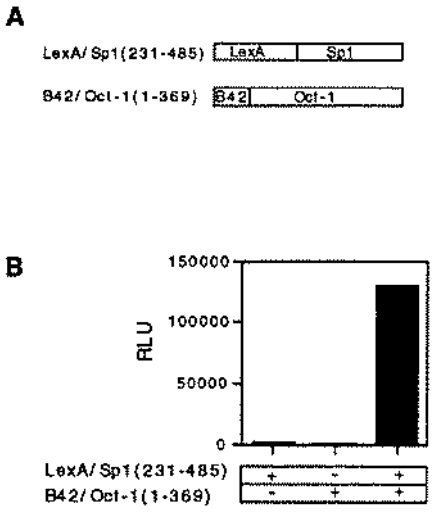

Figure 4. Sp1 and Oct-1 interact in a yeast two-hybrid assay. (**A**) The LexA/Sp1(231–485) and B42/Oct-1(1–369) expression vectors used in the experiments. The plasmids were transformed into yeast strain EGY48 together with a *LexA*$_{op}$–*lacZ* reporter plasmid. This strain has an integrated *LEU2* reporter gene with upstream *LexA* operators (30,31). (**B**) The expression vectors were transformed, alone or in combination, into EGY48. Yeast transformed with both plasmids gave galactose-dependent LEU$^+$ colonies. Cultures were grown in glucose and galactose and extracts were assayed for β-galactosidase activity using a luminometer. Only the result from galactose-induced cultures are shown. The mean relative light units (RLU) from three independent measurements are shown.

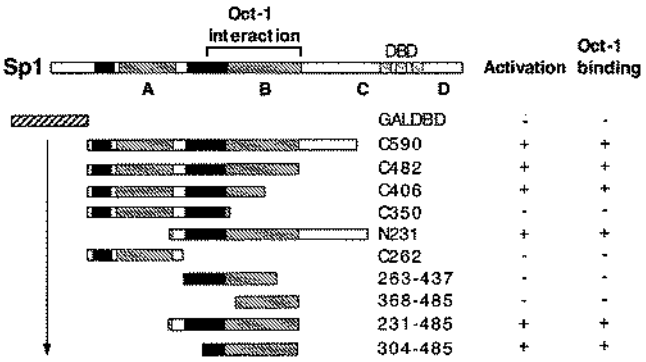

Figure 5. Correlation between transcriptional stimulation and interaction between Sp1 and Oct-1. A summary of the data presented in Figure 3, including activation data published elsewhere (21). The various parts of human transcription factor Sp1 are linked C-terminally to the GAL4 DNA binding domain (amino acids 1–147). GAL/Sp1 fusion C590 contains amino acids 83–590 of Sp1. Functional domains of Sp1 are also shown. A and B, glutamine-rich domains; C and D, transcriptional activation domains. Dark boxes represent serine/threonine-rich parts. DBD, DNA binding domain (43,44). + represents 50–100% transcriptional activation and binding to Oct-1.

promoter, are not sufficient for U2 gene activation. Stimulation of U2 transcription requires a region between amino acid residues 231 and 485 of Sp1, which contains a serine/threonine-rich part in addition to glutamine-rich activation domain B (21). The results described here show that Sp1 contains an Oct-1 interaction domain located in the part of Sp1 required for U2 gene transcription. Thus, in addition to interacting with factors, such as dTAF$_{II}$110, the initiator element binding factor YY1, TBP, E1a and hTAF$_{II}$55 (35–40), Sp1 also binds to the ubiquitously expressed transcription factor Oct-1.

Functions of Oct-1 and Sp1 at the human U2 snRNA promoter

The *Xenopus laevis* U2 enhancer contains Oct-1 and Sp1 sites and experiments in oocytes showed that the enhancer promotes the formation of stable transcription complexes (18,41). It is conceivable that Sp1 and Oct-1 also participate in the formation of stable transcription complexes at the U2 enhancer in human cells. Several multicomponent PSE binding activities have been reported, e.g. PBP, PTF and SNAP$_C$ (9–13). Both PTF and SNAP$_C$ contain four polypeptides of similar size and so far one of the subunits has been shown to be identical in the two complexes. However, TBP (TATA box binding protein) was reported to be a part of SNAP$_C$ but only loosely associated with PTF (42). Interestingly, it has been found that Oct-1 potentiates binding of PTF to different PSE elements *in vitro* (10), suggesting a role for Oct-1 in the formation of stable transcription complexes. Potentiation of PTF binding required only the POU domain of Oct-1 (10), a region not sufficient for the interaction with Sp1 (Fig. 2C). Analysis of Oct-1 activation function at U2 snRNA and mRNA promoters showed that Oct-1 contains redundant U2 promoter-specific activation domains. These were found in the N- and C-terminal parts of Oct-1, not including the POU domain (20). Thus, from these experiments it seems that the POU domain is not necessary for activation of U2 transcription, although it is possible that the Pit-1 POU domain, which replaced the Oct-1 POU domain in these experiments, fulfils this function. On the other hand, the *in vitro* potentiation of PTF binding by Oct-1 may not be involved in transcription of all snRNA genes. It is not known yet if the U2 promoter-specific activation domains of Oct-1 are involved in the functional interaction with the Sp1 factor that we describe here, since those experiments were performed using a reporter gene with multiple octamer sites and without Sp1 binding sites (20). Interestingly, we have found that the N-terminal 369 amino acids of Oct-1 interact with Sp1 in a yeast two-hybrid assay. We are currently investigating the role of this part of Oct-1 in the regulation of U2 snRNA gene transcription. It is not clear whether the function of Oct-1 is to stabilize the PTF/SNAP$_C$–DNA complex and/or if Oct-1 has activation domains that contact other components of the basic transcription machinery.

Sp1 and Oct-1 bind DNA cooperatively in crude nuclear extracts (19). This is not, however, dependent on other activities in the nuclear extract, since we have demonstrated that purified recombinant Sp1 and Oct-1 factors also bind DNA cooperatively. Furthermore, we show that the two proteins interact directly, without DNA. The Oct-1 interaction domain is located between amino acids 304 and 485 of Sp1. Fusion C406, with amino acids 83–406 of Sp1, binds Oct-1 weakly, suggesting either that the region between 304 and 406 is sufficient for the interaction or that the N-terminal part of this fusion also contributes to binding. Our results suggest that the binding of Sp1 to Oct-1 is relevant for U2 transcription in the living cell, since we find a correlation between binding and transcriptional activation. The Oct-1 interaction domain of Sp1(304–485) contains the glutamine-rich activation domain B (368–485) and a serine/threonine-rich part (see Fig. 5). This is a different region of Sp1 to that involved in binding to the human YY1 factor, the adenovirus E1a factor and hTAF$_{II}$55,

which all require the C-terminal part of Sp1, including the DNA binding domain (35,38,39). Several functions of transcription factor Sp1 at the U2 snRNA promoter could be envisaged. The Oct-1 interaction domain of Sp1 could stabilize binding of Oct-1 to the DSE and thereby stabilize the formation of transcription complexes at the U2 promoter. It is also possible that parts of Sp1 outside or overlapping with the Oct-1 interaction domain make contacts with the PSE binding complex or with other factors which are involved in transcription initiation of U2 snRNA genes. Since the transcriptional control elements coincide with elements involved in human U2 RNA 3′-end formation (5), there is a possibility that the Sp1 and Oct-1 factors participate in this process as well.

ACKNOWLEDGEMENTS

We are grateful to Roger Brent, Winship Herr, Patrick Matthias and Erhard Wintersberger for the generous gift of plasmid constructs. We thank Cathrine Phillips for linguistic revision and Ulf Pettersson for critical reading of the manuscript. This work was supported by grants from the Swedish Medical Research Council and The Göran Gustafsson Foundation.

REFERENCES

1 Dahlberg,J.E. and Lund,E. (1988) In Birnstiel,M. (ed.), *Small Nuclear RNAs*. Springer Verlag, Heidelberg, Germany, pp. 38–70.
2 Hernandez,N. (1992) In McKnight,S. and Yamamoto,K.R. (eds), *Transcriptional Regulation.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 281–313.
3 Parry,H.D., Scherly,D. and Mattaj,I.W. (1989) *Trends Biochem. Sci.*, **14**, 15–18.
4 Reddy,R. and Singh,R. (1991) *Prog. Mol. Subcell. Biol.*, **12**, 1.
5 Hernandez,N. and Lucito,R. (1988) *EMBO J.*, **7**, 3125–3134.
6 Neuman de Vegvar,H.E., Lund,E. and Dahlberg,J.E. (1986) *Cell*, **47**, 259–266.
7 Neuman de Vegvar,H.E. and Dahlberg,J.E. (1989) *Nucleic Acids Res.*, **17**, 9305–9318.
8 Parry,H.D., Tebb,G. and Mattaj,I.W. (1989) *Nucleic Acids Res.*, **17**, 3633–3644.
9 Henry,R.W., Sadowski,C.L., Kobayashi,R. and Hernandez,N. (1995) *Nature*, **374**, 653–656.
10 Murphy,S., Yoon,J.B., Gerster,T. and Roeder,R.G. (1992) *Mol. Cell. Biol.*, **12**, 3247–3261.
11 Sadowski,C.L., Henry,R,W., Lobo,S.M. and Hernandez,N. (1993) *Genes Dev.*, **7**, 1535–1548.
12 Wanandi,I., Waldschmidt,R. and Seifart,K.H. (1993) *J. Biol. Chem.*, **268**, 6629–6640.
13 Yoon,J.-B., Murphy,S., Zhengxin Wang,L.B. and Roeder,R. (1995) *Mol. Cell. Biol.*, **15**, 2019–2027.
14 Ares,M.,Jr, Chung,J.-S., Giglio,L. and Weiner,A.M. (1987) *Genes Dev.*, **1**, 808–817.
15 Herr,W., Sturm,R.A., Clerc,R.G., Corcoran,L.M., Baltimore,D., Sharp,P.A., Ingraham,H.A., Rosenfeld,M.G., Finney,M., Ruvkun,G. and Horvitz,H.R. (1988) *Genes Dev.*, **2**, 1513–1516.
16 Janson,L., Bark,C. and Pettersson,U. (1987) *Nucleic Acids Res.*, **15**, 4997–5016.
17 Janson,L., Weller,P. and Pettersson,U. (1989) *J. Mol. Biol.*, **205**, 387–396.
18 Mattaj,I.W., Lienhard,S., Jiricny,J. and DeRobertis,E.M. (1985) *Nature*, **316**, 163–167.
19 Janson,L. and Pettersson,U. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 4732–4736.
20 Tanaka,M., Lai,J.S. and Herr,W. (1992) *Cell*, **68**, 755–767.
21 Forsberg,M., Ström,A.-C., Lillhager,P. and Westin,G. (1995) *Hoppe-Seyler Z. Biol. Chem.*, **376**, 661–669.
22 Sturm,R.A., Das,G. and Herr,W. (1988) *Genes Dev.*, **2**, 1582–1599.
23 Hoffmann,A. and Roeder,R.G. (1991) *Nucleic Acids Res.*, **19**, 6337–6338.
24 Studier,F.W., Rosenberg,A.H., Dunn,J.J. and Dubendorff,J.W. (1990) *Methods Enzymol.*, **185**, 60–89.
25 Tan,S.-H., Leong,L.E.-C., Walker,P.A. and Bernard,H.-U. (1994) *J. Virol.*, **68**, 6411–6420.
26 Ausubel,F., Brent,R., Kingston,R.E., Moore,D.D., Seidman,J.G., Smith,J.A. and Struhl,K. (1994) *Current Protocols in Molecular Biology,* Suppl. 28, 16.7. Greene Publishing, New York, NY.
27 Westin,G. and Schaffner,W. (1988) *EMBO J.*, **7**, 3763–3770.
28 Lai,J.S. and Herr,W. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 6958–6962.
29 Strubin,M., Newell,J.W. and Matthias,P. (1995) *Cell*, **80**, 497–506.
30 Gyuris,J., Golemis,E., Chertkov,H. and Brent,R. (1993) *Cell*, **75**, 791–803.
31 Paroush,Z., Finley, Jr.,R.L., Kidd,T., Wainwright,S.M., Ingham,P.W., Brent,R. and Ish-Horowicz,D. (1994) *Cell*, **79**, 805–815.
32 Fields,S. and Song,O. (1989) *Nature*, **340**, 245–246.
33 Ares Jr.,M., Mangin,M. and Weiner,A.M. (1985) *Mol. Cell. Biol.*, **5**, 1560–1570.
34 Westin,G., Zabielski,J., Hammarström,K., Monstein,H.J., Bark,C. and Pettersson,U. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 3811–3815.
35 Chiang,C.-M. and Roeder,R.G. (1995) *Science*, **267**, 531–536.
36 Emili,A., Greenblatt,J. and Ingles,C.J. (1994) *Mol. Cell. Biol.*, **14**, 1582–1593.
37 Hoey,T., Weinzierl,R.O.J., Gill,G., Chen,J.-L., Dynlacht,B.D. and Tjian,R. (1993) *Cell*, **72**, 247–260.
38 Lee,J.S., Galvin,K.M. and Shi,Y. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 6145–6149.
39 Liu,F. and Green,M.R. (1994) *Nature*, **368**, 520–525.
40 Seto,E., Lewis,B. and Shenk,T. (1993) *Nature*, **365**, 462–464.
41 Bohmann,D., Keller,W., Dale,T., Schöler,H.R., Tebb,G. and Mattaj,I.W. (1987) *Nature*, **325**, 268–272.
42 Yoon,J.-B. and Roeder,R.G. (1996) *Mol. Cell. Biol.*, **16**, 1–9.
43 Courey,A.J. and Tjian,R. (1988) *Cell*, **55**, 887–898.
44 Kadonaga,J.T., Carner,K.R., Masiarz,F.R. and Tjian,R. (1987) *Cell*, **51**, 1079–1090.