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# Both Oct-1 and Oct-2A contain domains which can activate the ubiquitously expressed U2 snRNA genes

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**The U2 snRNA genes, which are transcribed by RNA polymerase II at high levels in all tissues examined, require both a distal and a proximal sequence element for efficient expression. The distal sequence element which has many properties in common with transcriptional enhancers contains, in addition to Sp1 binding sites, an octamer binding site which mediates activation through interactions with the ubiquitous transcription factor Oct-1. In the present study we have attempted to answer the question whether Oct-1 contains a unique activating domain which is required for activation of snRNA genes or whether ubiquitously expressed and lymphoid specific octamer binding factors both have the capacity to activate snRNA transcription. Our results show that in the presence of Oct-1, overexpression of Oct-2A in HeLa or COS1 cells neither inhibits nor stimulates transcription of U2 constructions which contain octamer binding sites with or without an adjacent Sp1 binding site. Moreover, an Oct-2A–GAL4 fusion protein in which the DNA binding domain of Oct-2A was substituted for by the one of the yeast transcription activator GAL4 activates transcription of a human U2 snRNA gene in which the octamer binding site was replaced by a GAL4 binding site. From the results it is concluded that both Oct-1 and Oct-2A contain domains which can activate the ubiquitously expressed U2 snRNA genes.**

*Key words:* cell-type specific expression/enhancer/Oct-2A/transcription activation/U2 snRNA transcription

## Introduction

The octamer motif (ATGCAAAT) is found in the regulatory regions of a number of lymphoid cell specific and ubiquitously expressed eukaryotic genes. It is present in the promoters of both the heavy chain and light chain immunoglobulin (Ig) genes as well as in the enhancer of the Ig heavy chain gene (Banerji *et al.*, 1983; Falkner and Zachau, 1984; Parslow *et al.*, 1984). This *cis*-acting element has been shown to play an important role in transcription of the Ig genes (Gerster *et al.*, 1987; Scheidereit *et al.*, 1987; Wirth *et al.*, 1987). Several octamer binding proteins have been identified in various tissues and cell lines (Pruijn *et al.*, 1986; Staudt *et al.*, 1986; Gerster *et al.*, 1987; Schreiber *et al.*, 1988; Lenardo *et al.*, 1989; Schöler *et al.*, 1989). Two of these proteins have been well characterized. The ubiquitous protein Oct-1 (also known as OTF-1, OBP100, NFA-1 and NFIII) is present in all tissues tested (Pruijn *et al.*, 1986; Fletcher *et al.*, 1987; Sturm *et al.*, 1988) whereas Oct-2A (also referred to as OTF-2, NFA-2) is expressed only in a few cell types, notably B lymphocytes (Landolfi *et al.*, 1986; Staudt *et al.*, 1986). Based on the correlation between the presence of Oct-2A and transcription of the Ig genes in lymphoid cells it has been concluded that Oct-2A, at least in part, is responsible for the tissue specific expression of the Ig genes.

The most conserved region between Oct-1 and Oct-2A is referred to as the POU domain (Herr *et al.*, 1988). This domain includes a 60 residue homeobox and an adjacent region of 75 residues (POU specific box) which is also found in a number of tissue specific transcription factors, including Pit-1 and unc-86 (Finney *et al.*, 1988; Ingraham *et al.*, 1988; He *et al.*, 1989). The entire POU domain has been shown to be responsible and sufficient for DNA binding of Oct-1 and Oct-2A (Clerc *et al.*, 1988; Sturm and Herr, 1988; Sturm *et al.*, 1988). Two transcription activation domains have been localized in the N-terminal and C-terminal regions of Oct-2A (Gerster *et al.*, 1990; Müller-Immerglück *et al.*, 1990; Tanaka and Herr, 1990). Oct-1 appears to contain only one transcription activation domain in its N-terminal region (Tanaka and Herr, 1990).

The octamer site is also important for transcription of the snRNA genes although their expression is not restricted to lymphoid cells (Mattaj *et al.*, 1985; Ares *et al.*, 1987; Ciliberto *et al.*, 1987; Janson *et al.*, 1987, 1989; Dahlberg and Schenborn, 1988; Tanaka *et al.*, 1988). Transcription of human U2 genes is controlled by a proximal sequence element (PSE) and a distal sequence element (DSE) which is also called the U2 enhancer (Westin *et al.*, 1984; Ares *et al.*, 1985; Mangin *et al.*, 1986; for review see Dahlberg and Lund, 1987). The basic enhancer unit of the human U2 snRNA gene contains Sp1 binding sites combined with an octamer sequence (Janson *et al.*, 1987, 1989; Ares *et al.*, 1987). Transient expression experiments in HeLa cells have shown that there is an absolute requirement of the octamer motif for U2 transcription (Ares *et al.*, 1987; Janson *et al.*, 1989). Although an Sp1 binding site alone is unable to stimulate transcription, its deletion results in a 5-fold reduction in transcription (Janson *et al.*, 1989). Recently, it has been demonstrated that Sp1 and Oct-1 bind to the U2 enhancer in a co-operative fashion suggesting that these two factors interact physically (Janson and Pettersson, 1990).

The involvement of octamer motifs in the activation of genes which are expressed in a tissue specific and ubiquitous manner presents an enigma since it appears from previous studies that the ubiquitous and the lymphoid specific octamer binding factors bind with identical affinity to any given octamer motif (Scheidereit *et al.*, 1987). Thus an important question is how genes which are activated by the ubiquitous

octamer binding factor interact with other octamer proteins in lymphoid cells. Do the snRNA genes have a specific requirement for Oct-1 or are the octamer binding factors able to substitute for each other? In this study we have attempted to answer this question by asking whether the lymphoid specific Oct-2A factor is able to activate snRNA genes: Our results show that an Oct-2A−GAL4 fusion protein can indeed activate transcription of the human U2 snRNA gene, suggesting that both Oct-1 and Oct-2A contain domains which are capable of transactivating the ubiquitously expressed snRNA genes.

## Results

### *Expression of Oct-2A in HeLa cells or COS1 cells neither stimulates nor inhibits U2 transcription*

Two possible models can be considered regarding the roles of the different Oct-factors in activation of the ubiquitously expressed U2 snRNA genes. (i) The U2 snRNA promoter functions in combination with the activating domains present in both Oct-1 and Oct-2A. (ii) The unique promoter element of U2 snRNA genes requires a unique activating domain which is present only in Oct-1. If model (ii) were correct a mechanism would be required for selection of Oct-1 in cells which express more than one octamer binding factor. Transcription factor Sp1 which is bound adjacently to the octamer motif could be involved in the selection of Oct-1 through protein−protein interactions or through steric hindrance.

In order to distinguish between the two possibilities, plasmids carrying U2 maxigene constructs U2M:Sp1,Octa or U2M:Octa (Figure 1A), which have been described previously (Janson *et al.*, 1989), were transfected together with an expression vector encoding Oct-2A (Müller *et al.*, 1988) into HeLa cells. As a positive control another reporter gene, OCTA(2), which carries a lymphoid specific promoter (Müller *et al.*, 1988) was also co-transfected with or without the Oct-2A expression vector into HeLa cells. If model (ii) is correct, expression of Oct-2A will result in a decrease of U2 transcription from the template U2M:Octa due to competition for the octamer binding site between active Oct-1 and inactive Oct-2A. Following transient expression, correctly initiated U2 transcripts were quantitated by S1 nuclease analysis using an 86 bases long oligonucleotide probe. Figure 1B shows that the transcription activities of the two U2 constructs did not change significantly when increasing amounts of Oct-2A expression vector were introduced into the cell (Figure 1B, lanes 1−4 and lanes 6−9). The control experiment showed that the expression of Oct-2A in HeLa cells did induce transcription from a lymphoid specific promoter (Figure 1C).

It could, however, be argued that the level of Oct-2A expression from the plasmid was insufficient to allow competition with the pre-existing Oct-1 in the cell. To examine whether U2 transcription could be affected at higher concentrations of Oct-2A, the Oct-2A expression vector was co-transfected with constructs U2M:Sp1,Octa or U2M:Octa into COS1 cells. These cells allow replication of the Oct-2A expression vector from a SV40 origin of replication and this results in high levels of Oct-2A protein (Müller-Immerglück *et al.*, 1990). The results showed no significant effect of the Oct-2A expression on U2 transcription from either of the two templates (data not shown). The co-transfection experiments were also carried out in two steps to rule out the possibility that the octamer binding sites of the U2 templates were already saturated with Oct-1 at the time when maximal Oct-2A expression was achieved. In these experiments the expression vector was first introduced into the COS1 cells and the U2 constructs were transfected separately into the same culture 12 h later. Figure 1D shows that the expression of Oct-2A neither stimulated nor inhibited U2 transcription under these experimental conditions.

Overexpression of Oct-2A thus seems to have no influence on U2 transcription. Moreover, identical results were obtained with constructions containing an octamer motif with or without an adjacent Sp1 binding site thus showing that the binding of Sp1 to the template does not provide a mechanism to select for Oct-1. Our interpretation of the results is that Oct-1 is already present at a saturating level and that Oct-1 and Oct-2A are able to stimulate U2 RNA transcription with comparable efficiencies.

### *Oct-2A−GAL4 fusion protein can stimulate U2 transcription in HeLa cells*

In order to test directly the possible involvement of Oct-2A in U2 transcription without the contribution of endogenous Oct-1, we used an Oct-2A−GAL4 fusion expression vector (pOct-2A−GAL4), in which the DNA binding domain of Oct-2A (the POU homeobox) is replaced by the DNA binding domain of the yeast transcription activator GAL4 (Figure 2A). Reporter plasmids U2M:1×Gal and U2M:2×Gal were constructed by insertion of oligonucleotides bearing single or double GAL4 binding sites into the upstream region of an enhancerless U2 maxigene (Figures 1A and 2B). As a negative control, an oligonucleotide which contained mutated GAL4 binding sites was also inserted in the same position (U2M:2×MGal, Figure 2B). These reporter plasmids were transfected individually into HeLa cells with or without the pOct-2A−GAL4 expression vector. The transcription activities were measured by S1 nuclease analysis. Figure 2C shows that transcription from template U2M:1×Gal was stimulated after co-transfection with pOct-2A−GAL4 (Figure 2C, lane 4) and that the level of transcription obtained was similar to that obtained from the original U2M:Octa template interacting with endogenous Oct-1 (Figure 2C, lane 8). No detectable U2 transcripts were observed after transfection with the reporter plasmids alone (Figure 2C, lanes 1 and 3) and the reporter plasmid U2M:2×MGal, which contained mutated GAL4 binding sites, also failed to produce any detectable U2 transcripts regardless of the presence of the expression vector pOct-2A−GAL4 (Figure 2C, lanes 5 and 6). As expected, the enhancerless U2 construct (U2M:-Enh) was also transcriptionally inactive in the presence of pOct-2A−GAL4 (Figure 2C, lane 7). The construct U2M:2×Gal which contains two tandemly arranged GAL4 binding sites was stimulated to a very high level in the presence of pOct-2A−GAL4 (Figure 2C, lane 2). Similarly, U2 RNA transcription is stimulated to a very high level by enhancers which contain two tandem Oct-1 binding sites (Figure 2D, lane 1). To exclude the possibility that the GAL4 DNA binding domain by itself had a stimulatory effect on U2 transcription, plasmid pGAL4/DBD (Figure 2A) which expresses the DNA binding domain of GAL4 was co-transfected with U2M:1×Gal or U2M:2×Gal into HeLa cells. No activation on these U2 constructs was observed (Figure 2D, lanes 3 and 4).

## Discussion

In this study we have addressed the question of whether the lymphoid specific factor Oct-2A is capable of activating transcription of the human U2 snRNA gene. Our experiments show that overexpression of Oct-2A in HeLa or COS1 cells neither inhibits nor stimulates transcription of U2 constructs which contain octamer binding sites with or without an adjacent Sp1 binding site. Our explanation for the failure of Oct-2A to influence U2 transcription is that the endogenous Oct-1 is present at a saturating level in HeLa cells and that both Oct-1 and Oct-2A have the capacity to stimulate U2 transcription. This interpretation is supported by our demonstration that the Oct-2A−GAL4 fusion protein can activate U2 transcription in HeLa cells, favouring a model in which both Oct-1 and Oct-2A are involved in stimulation of transcription of the U2 gene. Our results, however, do not exclude the unlikely possibility that only an Oct-2A protein, whose homeodomain is replaced by the GAL4 DNA binding domain, but not a genuine Oct-2A protein, is able to perform the activation *in vivo*.

Although we have not addressed this question experimentally by comparing Oct-2A−GAL4 and Oct-1−GAL4 chimeras it appears that the transactivating domains of Oct-1

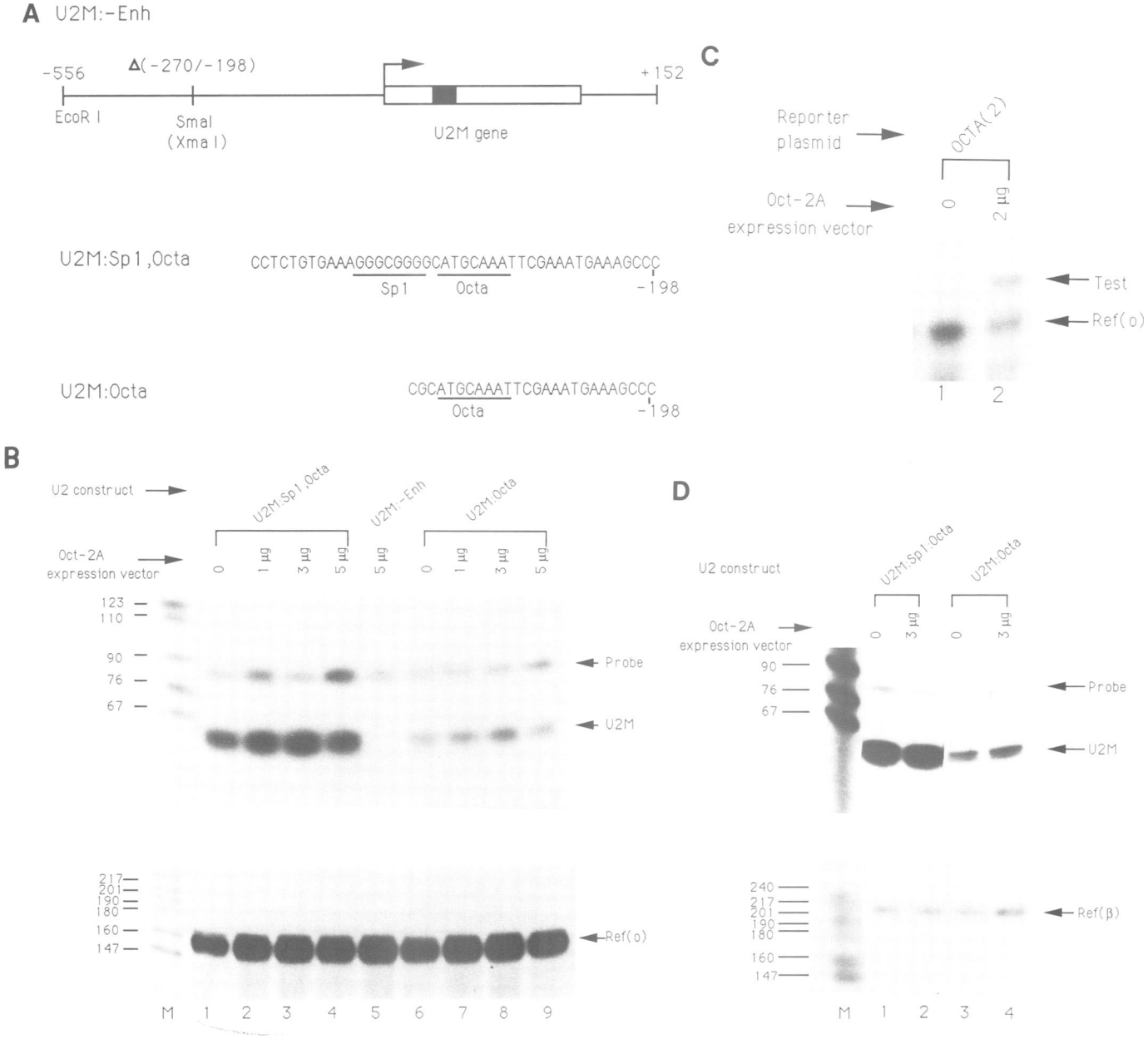


**Fig. 1.** Transient expression of U2M constructs in HeLa and COS1 cells. (**A**) Map of the different U2M constructs. The U2M maxigene contains a 20 bp fragment carrying the pM13+ polylinker at position 26 of the U2 snRNA gene (black box). U2M:-Enh contains sequences up to position −556, but the *SmaI* fragment between positions −198 and −270 is deleted. Sequences upstream of position −198 are shown for U2M:Sp1,Octa and U2M:Octa (Janson *et al.*, 1989). (**B**) Transcription of the U2M constructs in HeLa cells in the absence or presence of increasing amounts of the Oct-2A expression vector. (**C**) Activation of the OCTA(2) promoter construct (Müller *et al.*, 1988) in HeLa cells by Oct-2A protein. (**D**) Transcription of U2M constructs in COS1 cells in the absence or presence of the Oct-2A expression vector. Probe, undigested S1 probe; U2M, correctly initiated U2M transcripts; Ref(o), reference gene transcripts from the Ovec-ref. plasmid; Ref(β), reference gene transcripts from the pSXβ+ plasmid; Test, β-globin gene transcripts from the reporter plasmid OCTA(2); M, size marker (pBR322 digested with *HpaII*).

and Oct-2A stimulate U2 RNA transcription with similar potencies. This conclusion is based on the observation that similar levels of U2 RNA were transcribed in HeLa cells transfected with a construction containing a single octamer site to those in HeLa cells, transfected with Oct-2A−GAL4 expression vector together with a construction containing a single GAL4 binding site. The HeLa cell appears to contain a level of Oct-1 that saturates the transfected template since transfection of an Oct-1 expression vector fails to increase U2 transcription further (Tanaka and Herr, 1990). Similarly, the Oct-2A−GAL4 fusion protein seems to be present at a saturating level since transfection of increasing amounts of Oct-2A−GAL4 expression vector failed to increase U2 RNA transcription further from the template U2M:1×Gal (data not shown). Thus in cells containing seemingly saturating amounts of the respective octamer factor equivalent levels of U2 RNA transcription were obtained, suggesting that the transactivating domains of Oct-1 and Oct-2A stimulate U2 RNA transcription with similar efficiencies.

Our experiments show moreover that construction U2M:2×Gal carrying double GAL4 binding sites is stimulated at least 10 times more efficiently by Oct-2A−GAL4 than construct U2M:1×Gal which carries a single

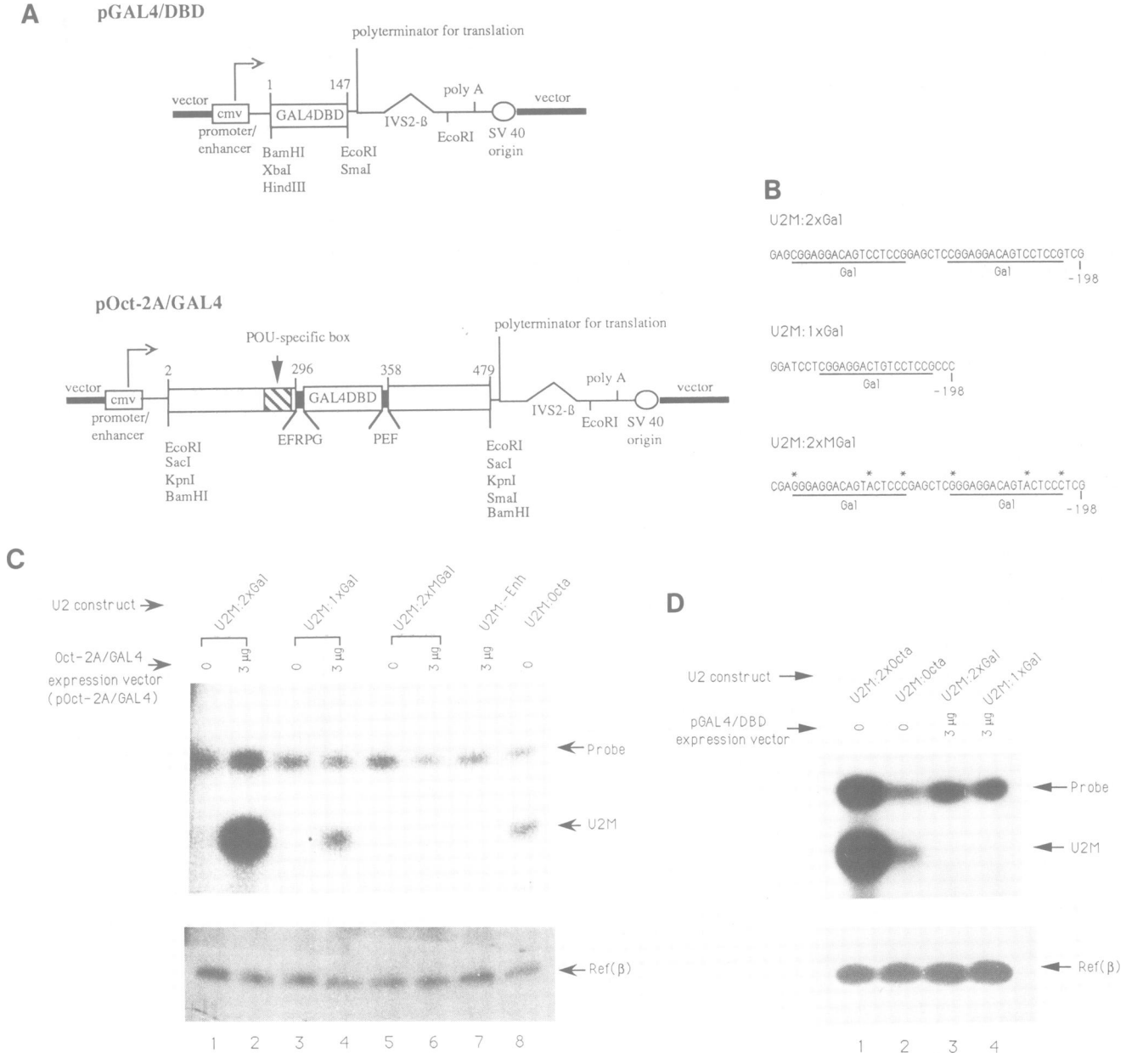


**Fig. 2.** Expression of U2M:Gal constructs.(**A**) Maps of expression vectors pGAL4/DBD and pOct-2A−GAL4. (**B**) Maps of U2M:Gal constructs. Sequences upstream from position −198 of U2M:2×Gal, U2M:1×Gal and U2M:2×MGal are shown. In the constructs U2M:2×Gal and U2M:2×MGal, U2 sequences up to position −556 are present upstream of the GAL4 binding sites. The GAL4 binding sites (Giniger *et al.*, 1985) are underlined. Mutations in the GAL4 binding sites (Giniger and Ptashne, 1988) of U2M:2×MGal are marked with asterisks. (**C**) Activation of U2M:Gal constructs by the Oct-2A−GAL4 fusion protein. (**D**) Transcription of constructs U2M:2×Octa and U2M:Octa in HeLa cells and of constructs U2M:2×Gal and U2M:1×Gal in the presence of expression vector pGAL4/DBD. P, undigested S1 probe; U2M, correctly initiated U2M transcripts; Ref(β), transcripts from the reference plasmid pSXβ+.

GAL4 binding site, indicating that the Oct-2A−GAL4 fusion protein activates U2 transcription synergistically when interacting with adjacent binding sites. A similar level of U2 RNA transcription was observed when a construction with two tandem octamer motifs was transfected into HeLa cells. The functional co-operation between the fusion proteins could be due to interactions between either the GAL4 DNA binding domains or the Oct-2A part of the molecules (Giniger and Ptashne, 1988; Kemler *et al.*, 1989; LeBowitz *et al.*, 1989; Poellinger *et al.*, 1989). It might also be due to co-operation with another component of the transcription apparatus (Pettersson and Schaffner, 1990).

Recently, the functional relationships between Oct-1 and Oct-2A have been studied extensively. Deletion analysis has revealed that Oct-2A carries at least two separate activation domains: an N-terminal proline, glutamine and leucine-rich region and a C-terminal proline-rich region (Gerster *et al.*, 1990; Müller-Immerglück *et al.*, 1990; Tanaka and Herr, 1990). Oct-1 contains only an N-terminal glutamine-rich domain which can functionally replace the N-terminal domain of Oct-2A (Tanaka and Herr, 1990). Accordingly it has been postulated that any difference in the properties of Oct-2A and Oct-1 would primarily be determined by the strength of activating surfaces exposed on the octamer binding factors Schaffner, 1989; Tanaka and Herr, 1990). These differences may be smaller than originally anticipated (Kemler *et al.*, 1991). Our results are in line with the latter data and demonstrate that both Oct-1 and Oct-2A contain domains which can activate the ubiquitously expressed U2 snRNA genes.

## Materials and methods

### *Construction of U2M templates and expression vectors*

The construction of U2M:-Enh, U2M:Sp1,Octa and U2M:Octa has been described previously (Janson *et al.*, 1989). Constructs U2M:2×Gal and U2M:2×MGal were generated by insertion of double stranded oligonucleotides, shown in Figure 2B, into the *Sma*I site of U2M:-Enh (Figure 1A). U2M:1×Gal was constructed by insertion of a double stranded oligonucleotide (Figure 2B) into the *Eco*RI and *Xma*I sites of U2M:-Enh. pGAL4/DBD was made in the following way. A fragment from plasmid pMA424 (Ma and Ptashne, 1987) containing the GAL4 DNA binding domain (amino acids 1−147) was cloned into an expression vector similar to pSTC-X556 (Severne *et al.*, 1988). The expression vector, that was kindly provided by Dr S.Rusconi, contains the human CMV promoter/enhancer and 5′ untranslated leader, rabbit β-globin gene splicing and polyadenylation signals, and the SV40 origin of replication. pOct-2A−GAL4 was constructed as follows: a continuous ORF encoding the first 147 amino acids of GAL4 flanked by two *Eco*RI sites was excised from the plasmid R147R (P.Broad and M.Ptashne, unpublished results) and cloned into the *Eco*RI site of a *Sfi*I−*Eco*RI−*Pst*I polylinker. The GAL4 ORF was subsequently excised as a *Sfi*I−*Pst*I fragment and used to replace the POU homeobox (which is immediately flanked by an *Sfi*I site 5′ and a *Pst*I site 3′) of the Oct-2A cDNA in the full length expression vector pO/2-479 (Müller-Immerglück *et al.*, 1990). A map and the amino acids within the junctions are shown in Figure 2A. The pOct-2A−GAL4 expression vector stimulated transcription from an Ig promoter containing a GAL4 binding site (M.M.Müller-Immerglück, K.Seipel and W.Schaffner, unpublished data).

### *Transfections*

HeLa or COS1 cells were transfected by the calcium phosphate co-precipitation method (Graham and van der Eb, 1973). A total of 14 μg DNA was used per 6 cm plate. 3 μg of the different U2M constructs and various amounts of expression vectors, as described in the figures, were used in each transfection. 3 μg of either plasmid pSXβ+ (Banerji *et al.*, 1981) or plasmid Ovec-ref. (Westin *et al.*, 1987) was used as reference in the transfection experiments. Sonicated calf thymus DNA was used as carrier.

### *S1 nuclease analysis and RNase protection mapping*

The U2M maxigene transcripts were analysed by S1 nuclease mapping using a single stranded oligodeoxyribonucleotide (5′-CAGATACTACACTTG-ATCTCGACTCTAGCTAGAGGATCTTAGCCAAAAGGCCGAGAA-GCGATGCGCTCGCCTTCGCGCCCGCCGTC-3′) as a 5′ end labelled probe. This 86 base probe hybridizes to the first 62 bases of U2M transcripts. The rabbit β-globin transcripts produced by pSXβ+ were measured by S1 nuclease mapping as described by Janson *et al.* (1989). The transcripts produced by plasmids OCTA(2) and Ovec-ref. were measured by RNase protection mapping as described by Müller *et al.* (1988).

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## References

Ares,M., Jr, Mangin,M. and Weiner,A.M. (1985) *Mol. Cell. Biol.*, **5**, 1560−1570.
Ares,M., Jr, Chung,J.-S., Giglio,L. and Weiner,A.M. (1987) *Genes Dev.*, **1**, 808−817.
Banerji,J., Rusconi,S. and Schaffner,W. (1981) *Cell*, **27**, 299−308.
Banerji,J., Olson,L. and Schaffner,W. (1983) *Cell*, **33**, 729−739.
Ciliberto,G., Palla,F., Tebb,G., Mattaj,I.W. and Philipson,L. (1987) *Nucleic Acids Res.*, **15**, 2403−2416.
Clerc,R.G., Corcoran,L.M., LeBowitz,J.H., Baltimore,D. and Sharp,P.A. (1988) *Genes Dev.*, **2**, 1570−1581.
Dahlberg,J.E. and Lund,E. (1987) In Birnstiel,M. (ed.), *Small Nuclear RNAs*. Springer Verlag, Heidelberg, pp. 38−70.
Dahlberg,J.E. and Schenborn,E.T. (1988) *Nucleic Acids Res.*, **16**, 5827−5840.
Falkner,F.G. and Zachau,H.Z. (1984) *Nature*, **310**, 71−74.
Finney,M., Ruvkun,G. and Horvitz,H.R. (1988) *Cell*, **55**, 757−769.
Fletcher,C., Heintz,N. and Roeder,R.G. (1987) *Cell*, **51**, 773−781.
Gerster,T., Matthias,P., Thali,M., Jiricny,J. and Schaffner,W. (1987) *EMBO J.*, **6**, 1323−1330.
Gerster,T., Balmaceda,C.-G. and Roeder,R.G. (1990) *EMBO J.*, **9**, 1635−1643.
Giniger,E. and Ptashne,M. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 382−386.
Giniger,E., Varnum,S.M. and Ptashne,M. (1985) *Cell*, **40**, 767−774.
Graham,F.L. and van der Eb,A.J. (1973) *Virology*, **52**, 456−467.
He,X., Treacy,M.N., Simmons,D.M., Ingraham,H.A., Swanson,L.W. and Rosenfeld,M.G. (1989) *Nature*, **340**, 35−42.
Herr,W., Sturm,R.A., Clerc,R.G., Concoran,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.
Ingraham,H.A., Chen,R., Mangalam,H.J., Elsholtz,H.P., Flynn,S.E., Lin,C.R., Simmons,D.M., Swanson,L. and Rosenfeld,M.G. (1988) *Cell*, **55**, 519−529.
Janson,L. and Pettersson,U. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 4732−4736.
Janson,L., Bark,C. and Pettersson,U. (1987) *Nucleic Acids Res.*, **15**, 4997−5016.
Janson,L., Weller,P. and Pettersson,U. (1989) *J. Mol. Biol.*, **205**, 387−396.
Kemler,I., Schreiber,E., Müller,M.M., Matthias,P. and Schaffner,W. (1989) *EMBO J.*, **8**, 2001−2008.
Kemler,I., Bucher,E., Seipel,K., Müller-Immerglück,M.M. and Schaffner,W. (1991) *Nucleic Acids Res.*, **19**, 237−242.
Landolfi,N.F., Capra,J.D. and Tucker,P.W. (1986) *Nature*, **323**, 548−551.
LeBowitz,J.H., Clerc,R.G., Brenowitz,M. and Sharp,P.A. (1989) *Genes Dev.*, **3**, 1625−1638.
Lenardo,M.J., Staudt,L., Robbins,P., Kuang,A., Mulligan,R.C. and Baltimore,D. (1989) *Science*, **243**, 544−546.
Ma,J. and Ptashne,M. (1987) *Cell*, **51**, 113−119.
Mangin,M., Ares,M., Jr and Weiner,A.M. (1986) *EMBO J.*, **5**, 987−995.
Mattaj,I.W., Lienhard,S., Jiricny,J. and De Robertis,E.M. (1985) *Nature*, **316**, 163−167.
Müller,M.M., Ruppert,S., Schaffner,W. and Matthias,P. (1988) *Nature*, **336**, 544−551.
Müller-Immerglück,M.M., Schaffner,W. and Matthias,P. (1990) *EMBO J.*, **9**, 1625−1634.

Parslow,T.G., Blair,D.L., Murphy,W.J., Granner,D.K. and Yamamoto, K.R. (1984) *Proc. Natl Acad. Sci. USA*, **81**, 2650–2654.
Pettersson,U. and Schaffner,W. (1990) *J. Mol. Biol.*, **214**, 373–380.
Poellinger,L., Yoza,B.K. and Roeder,R.G. (1989) *Nature*, **337**, 573–576.
Pruijn,G.J.M., van Driel,W. and van der Vliet,P.C. (1986) *Nature*, **322**, 656–659.
Schaffner,W. (1989) *Trends Genet.*, **5**, 37–39.
Scheidereit,C., Heguy,A. and Roeder,R.G. (1987) *Cell*, **51**, 783–793.
Schreiber,E., Matthias,P., Müller,M.M. and Schaffner,W. (1988) *EMBO J.*, **7**, 4221–4229.
Schöler,H.R., Hatzopoulos,A.K., Balling,R., Suzuki,N. and Gruss,P. (1989) *EMBO J.*, **8**, 2543–2550.
Severne,Y., Wieland,S., Schaffner,W. and Rusconi,S. (1988) *EMBO J.*, **7**, 2503–2508.
Staudt,L.M., Singh,H., Sen,R., Wirth,T., Sharp,P.A. and Baltimore,D. (1986) *Nature*, **323**, 640–643.
Sturm,R.A. and Herr,W. (1988) *Nature*, **336**, 601–604.
Sturm,R.A., Das,G. and Herr,W. (1988) *Genes Dev.*, **2**, 1582–1599.
Tanaka,M. and Herr,W. (1990) *Cell*, **60**, 375–386.
Tanaka,M., Grossniklaus,U., Herr,W. and Hernandez,N. (1988) *Genes Dev.*, **2**, 1764–1778.
Westin,G., Lund,E., Murphy,J.T., Pettersson,U. and Dahlberg,J.E. (1984) *EMBO J.*, **3**, 3295–3301.
Westin,G., Gerster,T., Müller,M.M., Schaffner,G. and Schaffner,W. (1987) *Nucleic Acids Res.*, **15**, 6787–6798.
Wirth,T., Staudt,L. and Baltimore,D. (1987) *Nature*, **329**, 174–178.

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