Mapping the transactivation domain of the Oct-6 POU transcription factor

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ABSTRACT

The POU transcription factor Oct-6 is expressed in embryonic stem cells, glial progenitor cells and in a restricted set of neurons in the CNS. The protein has been shown to act as a transactivator as well as a repressor. Here we show that the Oct-6 protein activates transcription from three different promoters in HeLa cells. The ability to activate a minimal tk promoter via a multimerized IgH enhancer octamer motif relies on a domain within the aminoterminal third of the protein. Parts of this domain can be deleted without abolishing transactivation, suggesting that there is functional redundancy within this region. The transactivation domain of the Oct-6 protein is different from other described activation domains in that it is highly glycine and alanine rich.

INTRODUCTION

The octamer motif (ATGCAAAT) is a well studied DNA motif present in enhancers and promoters of both ubiquitously and cell type specifically expressed genes (1, 2, 3). This DNA element was shown to be a binding site for a family of nuclear proteins present in different cell types and at different stages of mammalian development (4, 5). A number of these proteins are believed to be involved in determining cell fate through selective regulation of target genes. The cloning of different cDNAs encoding several of these octamer binding factors revealed that they belong to the POU domain gene family (6–13). This family was defined by a region of extensive sequence homology (the POU domain) between three mammalian transcription factors (Oct-1, Oct-2 and Pit-1/GHF-1) and one nematode regulatory protein (Unc-86;(14) and references therein). The POU domain constitutes the DNA binding domain of these proteins and can be subdivided in two regions, separated by a short linker (10, 12, 15, 16). The carboxy-terminal part shows homology with the classical homeobox proteins (the POU_{HD} Domain), while the aminoterminus contains a homology specific for this class of proteins (the POU_{SP} domain).

Oct-6 was originally defined as an embryonic stem cell specific octamer binding factor (4). Differentiation of these cells in vitro leads to a downregulation of the Oct-6 protein. However in undifferentiated P19 EC cells the Oct-6 gene is expressed at very low levels. The gene is transiently upregulated when these cells differentiate into neuronal cell types after aggregation and addition of retinoic acid to the culture medium. Expression increases again after several days of induction, indicating that the protein plays a role in different stages or cell types during neuronal differentiation (7). Cloning and sequencing of cDNAs encoding the Oct-6 protein revealed that the Oct-6 gene is the mouse homologue of the rat SCIP \ Tst-1 gene (17). The SCIP gene is highly expressed during glial cell development in the peripheral and central nervous system. It has been shown that the SCIP protein functions as a repressor of myelin specific genes during a period of rapid cell division that separates a premyelinating from a myelinating phase in Schwann cell development (18). Furthermore,using a PCR based approach, a partial Oct- 6/SCIP was cloned from neonatal rat testis which was named Testes-1 (Tst-1 (19)). Using in-situ hybridization it was shown that the Oct-6/SCIP/Tst-1 gene is also highly expressed in discrete regions of the developing nervous system (13, 19). The Oct-6 protein was shown to function as a positive as well as a negative regulator of transcription depending on the exact promoter architecture (13, 18, 20). Here we show that high level ectopic expression of the Oct-6 protein in HeLa cells can activate transcription from three different promoters. Using a series of Oct-6 deletion mutants we show that the transactivation domain of the protein is located in the first 157 amino acids and is distinct from its DNA binding domain. This domain, which is extremely glycine and alanine rich, can be subdivided in at least two active subdomains.

MATERIALS AND METHODS

Construction of Oct-6 deletion mutants

Deletions of the Oct-6 cDNA were created using naturally occurring restriction sites. The different deletion constructs were cloned in frame into the CMV based expression vectors pEVRF0, pEVRF1, pEVRF2 or pEV3S (26). These vectors allow expression of N terminally deleted proteins as an in frame fusion product with the first four amino acids of the HSV tk protein. Cloning of the full length Oct-6 cDNA in pEVRF0 using the Bal1 restriction site results in a protein that has 7 amino acids inserted between aa 2 and aa 3 as compared to the wildtype

protein. The first two aa encoded by the tk reading frame are the same as in the Oct-6 reading frame. This expression plasmid is referred to as clone pN2Oct6 or Oct-6 in figure 2. The N stands for a N terminal truncation and the number following the N refers to the number of codons removed from the Oct-6 open reading frame. The C stands for a carboxy terminal truncated protein and the number following the C indicate the number of codons removed from the carboxy terminus of the Oct-6 protein. The translated linker sequence of the truncated clones is as follows: N2, MASWGSGTP; N23, MASWGSGTL; N44, MASWGSGTH; N97, MASWGSGVP; N115, MASWGSGTL; N143, MASWGSGVP; N157, MASWGSGTH; N182, MASWGSGT; N197, MASWGSGVP; N229, MASWGSGY. The chimaeric clone POU/VP16 was constructed by replacing the 3′ part Oct-6 cDNA from the SacII to the XbaI site (encoding the 51 aa carboxy terminus of Oct-6) for the 3′ part of the HSV1 Vmw65 (VP16) gene in clone N229. In this way the Oct-6 POU domain is fused to the 80 aa carboxy terminus of the VP16 protein. This part of the VP16 protein is a strong transactivator (29). Internal deletion mutants were made by excising part of the Oct-6 open reading frame in clone pN2Oct6. The numbers represent the codons at which the Oct-6 ORF is fused. All fusion points were checked by dideoxy sequencing. All deletion constructs were tested by expression in COS cells. Using nuclear extracts of transfected COS cells in a bandshift assay indicated that all mutants were stable, bound to the octamer sequence with high affinity and were located in the nucleus.

Reporter plasmids

The reporter plasmids 6WtkCAT and 6FdtkCAT are described in detail in (22) and were a kind gift of Dr Hans Schöler. The βOCTA promoter was constructed by PCR amplification using a 5′ sense oligo containing an OCTA box or a mutated version thereof and a 3′ anti-sense oligo mapping in front of the rabbit β-globin start codon. A genomic subclone in pBR327 containing the entire rabbit β-globin gene was used as a template. 5′ sense oligo, GCGGATCC<u>ATTTGCATT</u>ACATAGTTCAGGAC-TTGG; 3′ anti-sense oligo, GCGCTCGAGTCTGTTTTGGG-GGATTGC. The amplification products were cloned 5′ of the CAT gene in pBLCAT3 (23). The integrity of the βO⁺ and βO⁻ promoter constructs were checked by dideoxy sequencing. The full sequence of these promoters is as follows; GGATCC-<u>ATTTGCATT</u>ACATAGTTCAGGACTTGGG<u>CATAAAA</u>GG-CAGAGCAGGGCAGCTGCTGCTTACA

The ICP4 CAT (IE175CAT) construct was a kind gift of Dr Peter O'Hare. This construct contains the IE175 (ICP4) promoter from −380 to +30 (25).

Cell transfections

Using the CaPO₄ precipitate method, 0.5×10^6 cells (either HeLa, CHO or MES) were transfected with 5 μg of reporter plasmid and 2.5μg of Oct-6 expression vector (or with 2.5μg of the empty expression vector pEVRF0) and 12.5 μg of pTZ19 plasmid DNA as carrier (27). The DNA/CaPO₄ precipitate was removed after 20 hours. Transfected cells were harvested 48 hrs after removal of the CaPO₄ precipitate. Eighty percent of the cells were used to prepare cell extracts for a CAT assay and the remaining 20% was used to prepare whole cell extracts for monitoring Oct-6 protein expression in a bandshift assay. CAT assays were performed with equal amounts of protein (20 μg). Acetylated and non-acetylated forms of ¹⁴C labelled chloramphenicol were separated using standard thin layer

chromatography. The ratio between acetylated and non-acetylated forms of chloramphenicol were calculated after quantization of the signals using a Molecular Dynamics phosphoimager.

Bandshift experiments and whole cell extracts

Whole cell extracts of transfected HeLa cells (20%; see above) were prepared by resuspending the cells in 40 μl of 20mM Hepes-KOH pH7.9, 400mM KCl, 1mM EDTA, 10% glycerol, 10mM DTT, 1mM PMSF, 5 μg/ml leupeptin, 5μg/ml pepstatin and 10μg/ml chymostatin.Resuspended cells were subject to 4 cycles of freezing in liquid nitrogen and thawing on ice. The cellular debris was removed by centrifugation at 14000g for 5 minutes at 4°C. Equal amounts of cellular extract were used in a bandshift assay using 3 fmol of a ³²P endlabeled double stranded OCTA probe. Probe and protein were incubated on ice for 20 minutes in 20 mM Hepes-KOH ph7.9, 1mM EDTA, 1 mM EGTA, 4% Ficoll in a total volume of 20 μl. Complexed and free probe were separated on a 4% polyacrylamide gel in 0.25×TBE.

RESULTS

Oct-6 transactivates a variety of promoters when ectopically expressed in HeLa cells

To study the transactivation potential of the Oct-6 protein we used three different promoter constructs. A simplified promoter in which the octamer is located 20 bp upstream of the rabbit β-globin TATA box (pβO⁺Cat in figure 1A) driving a CAT
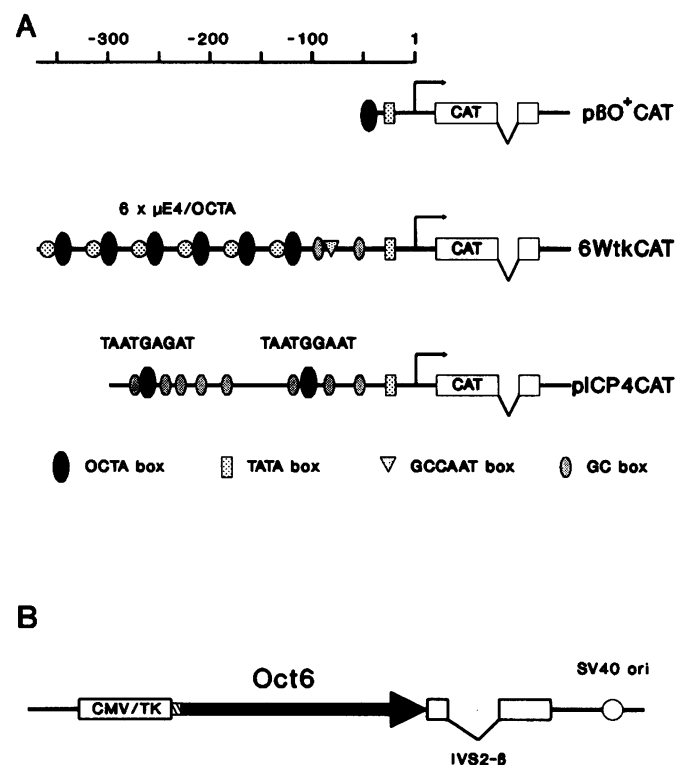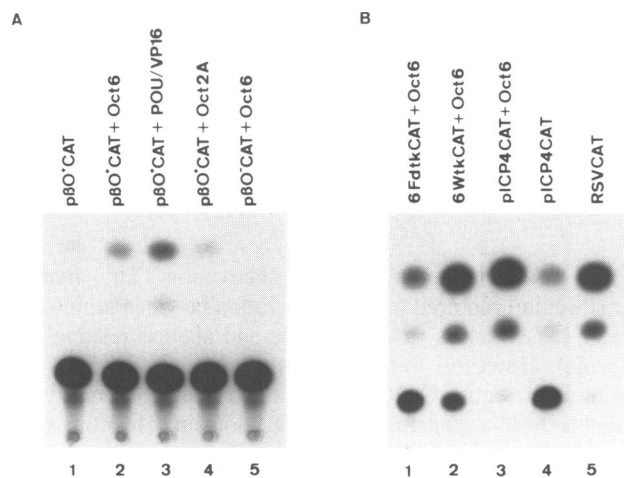

Figure 1. A. Schematic representation of the reporter plasmids used in this study. The reporter gene is the bacterial Chloramphenicol Acetyl Transferase gene (CAT). The pβO⁺CAT, 6WtkCAT and the ICP4CAT constructs do not contain additional enhancers downstream of the CAT gene. The scale bar above the drawing indicates the position (in basepares) of the cis-acting elements relative to the transcriptional start site (arrow). B. Structure of the Oct-6 expression vector.

reporter gene. An almost identical promoter construct was shown to constitute a B-cell specific promoter (8, 21). This promoter could be readily activated by ectopically expressed Oct-2A, Oct-4 and Oct-6 proteins (8, 13, 22). Transcription from this promoter was shown to be dependent on an intact octamer motif. A second promoter construct consists of a minimal HSV-1 TK gene promoter flanked by a six times repeated IgH enhancer octamer/μE4 motif (6WtkCat; (23, 24)). This type of enhancer was shown to be at least 1000 fold more active than its mutated counterpart in EC cells (6FdtkCAT in which only the octamer box was mutated)(24). In this particular arrangement the closest octamer motif is 147 bp separated from the tk TATA box. A third promoter used in this study is the Herpes Simplex Virus 1 ICP4(IE175K) gene promoter (25). This promoter contains one TAATGARAT motif at position -260 and a second related motif (TAATGGAAT) at position -110. The distal TAATGARAT motif of the ICP4 promoter has been shown to be a strong binding site for Oct-6 whereas it is a weak binding site for Oct-1 (7). The reporter constructs are schematically depicted in figure 1A. In order to express the Oct-6 protein in HeLa cells the Oct-6 cDNA was cloned in a CMV promoter/enhancer based expression vector ((26); see M&M for details). The Oct-6 expression vector and the different CAT reporters were co-transfected in different combinations in HeLa cells. Reporter gene expression was measured by CAT assays (27). Expression of the Oct-6 protein was monitored by a bandshift assay (not shown). As can be seen in figure 2A, Oct-6 activates the simple βO^+ promoter confirming earlier reports (13). Furthermore this activation is dependant on an intact octamer motif (compare lane 2 with lane 5). The same promoter could be activated by Oct-2A (lane 4 and (8)) and a chimaeric protein POU/VP16. This chimaeric protein
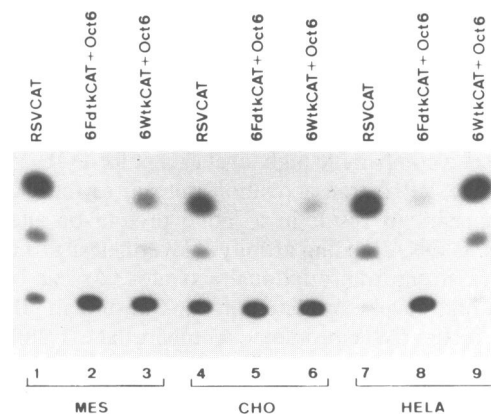
consists of the Oct-6 DNA binding domain (POU domain) coupled to the VP16 transactivation domain ((28, 29);see M&M) and is a strong transcriptional activator (see also figure 4, lane 15). Although this promoter construct is clearly responsive, the absolute levels of expression are rather low. In a second set of transactivation experiments we used the 6WtkCAT reporter and its mutated counterpart 6FdtkCAT. Clearly Oct-6 is able to transcativate the tk promoter via the IgH octamer motif (fig 2B, lane 1 and 2) This result contrasts with an earlier report by Suzuki *et al.* (13) who reported that the Oct-6 protein is not able to activate this reporter (see discussion). Furthermore the HSV1 ICP4 promoter is also strongly induced by Oct-6. Thus Oct-6 is able to activate transcription via octamer and octamer related sequences in different promoter settings.

Transactivation by ectopically expressed Oct-6 is not restricted to HeLa cells

In an earlier report by Suzuki *et al.* it was shown that Oct-6 is not able to activate transcription from a distal position (with respect to the transcription initiation site) when expressed in HeLa cells (13). Similar findings were reported for the B cell specific Oct-2A and the stem cell specific Oct-4 proteins (22, 30). One explanation for the ability of Oct-6 to activate transcription from a more distal position in the experiments presented here is that our HeLa cell line contains factors that are missing in their particular line or that our cell line misses factors that otherwise would prevent transactivation. To test this possibility we performed transactivation experiments using the 6WtkCAT reporter in cell lines of different origin. The MES cell line is stable derivative of the P19 EC cell line and CHO is a Chinese Hamster Ovarian cell line. As can be seen in figure 3 Oct-6 activates the 6WtkCAT reporter gene in all three cell lines tested, indicating that there are no qualitative differences between the cell lines with respect to supporting transactivation. However there are clear quantative differences in the level of activation in the different cellular backgrounds. Transfection efficiency and levels of activation are highest in HeLa cells. Therefore this cell line was chosen for mapping of the transactivation domain of the Oct-6 protein.

Figure 2. A. Oct-6 activates transcription from a simple octamer/TATA promoter. The expression vectors pOct-6,pPOU/VP16 and Oct-2A and reporter plasmids were cotransfected into HeLa cells as indicated. The structure of the Oct-6 expression vector is outlined in figure 1. The structure of the POU/VP16 vector is described in Materials and Methods. The Oct-2A vector is described in (8). When no expression vector is indicated the reporter plasmid is cotransfected with an equal amount of the empty expression vector pEVRF0. B. Oct-6 activates also more complex promoters. The Oct-6 expression vector (see figure1) was cotransfected with CAT reporter plasmids as indicated. Reporter constructs are as outlined in figure 1. The 6Fdtk promoter construct is the mutated counterpart of the 6Wtk promoter (22). The RSVCAT construct serves as a positive control, in which the CAT gene is driven by the strong Rous Sarcoma Virus promoter.
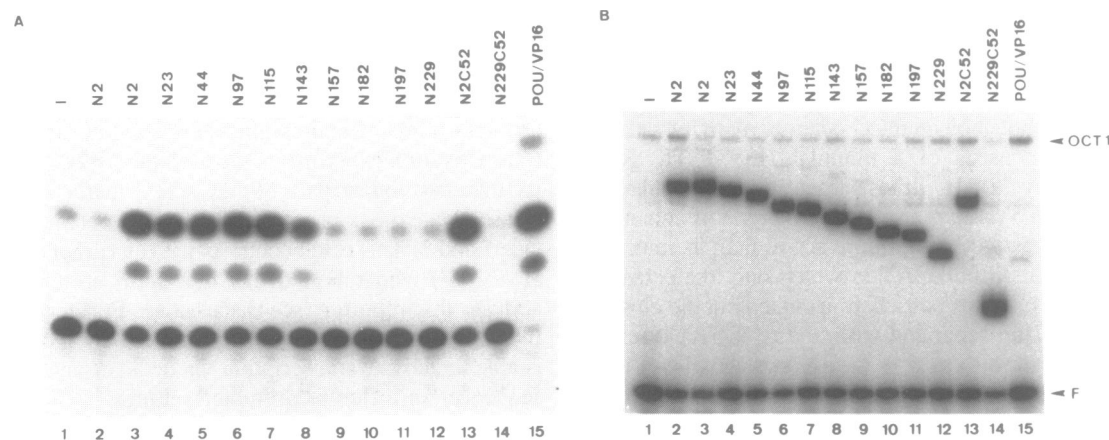

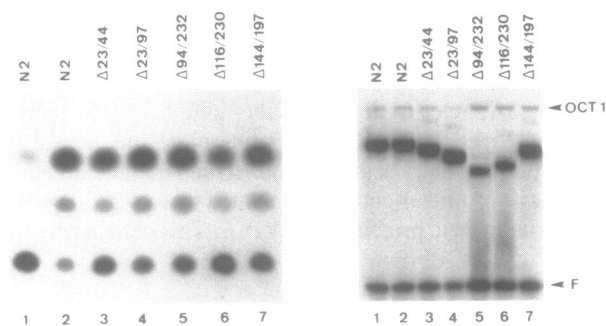**Figure 3.** Oct-6 transactivates the 6Wtk promoter in different cellular backgrounds. MES, CHO and HeLa cells were transfected with the expression and reporter plasmids as indicated. CaPO₄/DNA precipitates were prepared as described and split into three portions and applied to the different cells. The background activities of the 6FdtkCAT and the 6WtkCAT without Oct-6 were low and similar for all three cell lines tested.

Figure 4. A. Transactivation of the 6Wtk promoter by truncated Oct-6 proteins. HeLa cells were transfected with the 6WtkCAT reporter plasmid and the different expression vectors as indicated except for lane 2 where the reporter plasmid is the 6FdtkCAT construct. Lane1 shows the basal level of expression of the 6WtkCAT construct (cotransfected with the empty expression vector pEVRF0). The structure and the relative transactivation potentials of the different Oct-6 mutant proteins is outlined in figure 6. B. Bandshift assays with whole cell extracts of a fraction of HeLa cells transfected with the different truncated Oct-6 proteins.

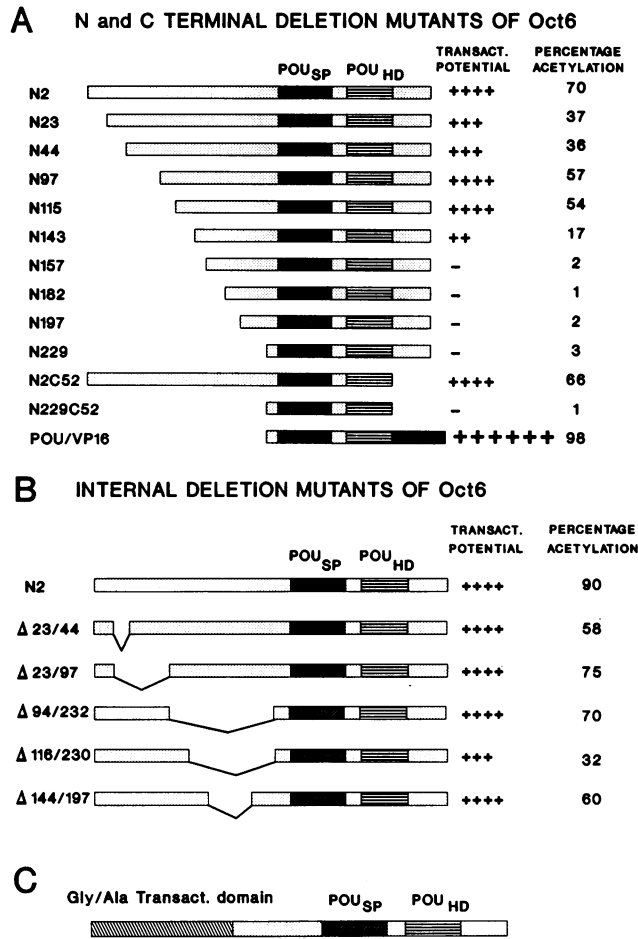## Deletion mapping of the Oct-6 transactivation domain

In order to map the domain(s) of Oct-6 involved in transactivation, a set of progressive aminoterminal and carboxyterminal deletions were constructed, using natural restriction sites in the Oct-6 gene (7). These truncated cDNAs were cloned in frame into CMV/enhancer based expression vectors (26). All mutant Oct-6 proteins have an intact POU domain. To test whether the different deletion constructs encode stable proteins that bind to the octamer motif and are localized in the nucleus, plasmids were transfected into COS-1 cells. The expressed mutant Oct-6 proteins were assayed in a bandshift experiment using nuclear extracts from the transfected COS-1 cells and a radiolabelled OCTA probe. All constructs express Oct-6 mutant proteins of the expected size at high levels. These proteins appear to be stable and are located in the nucleus (data not shown). The transcriptional activation potential of the mutant Oct-6 proteins was tested in cotransfection experiments with the 6WtkCAT reporter plasmid in HeLa cells and quantitative CAT assays (figure 4, see also figure 6). To exclude the possibility that differences in CAT activity are due to different levels of Oct-6 mutant proteins, the amount of protein was estimated in a bandshift assay using whole cell extracts of transfected HeLa cells. As can be seen in figure 4B all mutant proteins are expressed at a comparable high level except for POU/VP16. The fusion of the VP16 transactivation domain to the Oct-6 POU domain appears to result in a labile protein or alternatively influences its DNA binding affinity. Nevertheless this chimaeric protein is a strong transcriptional activator. As can be seen in figure 4A high level expression of Oct-6 results in 30–50 fold induction of the 6Wtk promoter (compare lane 1 and 3). This activation is dependent on an intact Octa motif within the 6W enhancer as this stimulation of CAT expression is not observed with the mutant version of this promoter (lane 2). Deleting the first 115 amino acids does not reduce the ability to activate the 6Wtk promoter. Although expression of mutants N23 and N44 resulted in a slightly lower transcactivation level, the mutants N97 and N115 showed wild type activity. Further deleting the Oct-6 protein to amino acid 143 resulted in a drop of activity to approximately 30% of the wild type level. Deleting beyond

Figure 5. A. Transactivation of the 6Wtk promoter by Oct-6 internal deletion mutant proteins. HeLa cells were cotransfected with the 6WtkCAT reporter plasmid and the different expression plasmids as indicated except for lane 1 where the reporter is the 6FdtkCAT plasmid. The structure and the relative transactivation potential of the different Oct-6 proteins is depicted in figure 6. B. Bandshift assays with whole cell extracts of HeLa cells transfected with the different Oct-6 mutant proteins.

a.a. 157 completely abolished transactivation. This maps a minimal region required for transactivation between amino acid 115 and 157 which is rich in glycine and alanine residues (19 gly + ala). However, this feature is not characteristic for this domain, as the entire Oct-6 protein is extremely rich in glycine and alanine residues, apart from its POU domain. Therefore other features than just a high gly and ala content must be involved in mediating transactivation.

The Oct-6 protein domain carboxy terminal of the POU domain is rich in proline residues (13 out of 52). Proline rich transactivation domains have been described for a number of transcription factors. However no such function can be ascribed to this part of the Oct-6 protein as the aminoterminal deletion mutants N157 to N229 all contain this domain but fail to transactivate. Furthermore the N2C52 deletion mutant in which the entire carboxy terminal domain is removed shows a wild type level of transactivation. The POU domain by itself (N229C52) even seems to repress basal level of expression (compare lane 14 with lane1).

A N and C TERMINAL DELETION MUTANTS OF Oct6

	POU$_{SP}$ POU$_{HD}$	TRANSACT. POTENTIAL	PERCENTAGE ACETYLATION
N2		++++	70
N23		+++	37
N44		+++	36
N97		++++	57
N115		++++	54
N143		++	17
N157		-	2
N182		-	1
N197		-	2
N229		-	3
N2C52		++++	66
N229C52		-	1
POU/VP16		++++++	98

B INTERNAL DELETION MUTANTS OF Oct6

	POU$_{SP}$ POU$_{HD}$	TRANSACT. POTENTIAL	PERCENTAGE ACETYLATION
N2		++++	90
Δ 23/44		++++	58
Δ 23/97		++++	75
Δ 94/232		++++	70
Δ 116/230		+++	32
Δ 144/197		++++	60

C

Gly/Ala Transact. domain   POU$_{SP}$ POU$_{HD}$

Figure 6. Structure and transactivation potential of the Oct-6 deletion mutants used in this study. The naming of the different mutants reflects the number of codons removed from the Oct-6 ORF either from the aminoterminus (N) or the carboxyterminus (C). The construction of the different expression plasmids is detailed in Materials and Methods. The N2 Oct-6 protein is considered to be the wild type protein. The POU-specific and the POU-homeodomain are indicated by shaded boxes. The relative transcriptional activation of the 6Wtk promoter by the different mutant proteins is indicated. The percentage acetylation refers to the percentage of acetylated forms of chloramphenicol in the experiments presented in figure 4 and 5. CAT activity of the 6WtkCAT transfected cells is 2% (lanes 1 in figure 4 and 5). A. Structure of the N- and C-terminal deleted Oct-6 proteins. B. Structure of internal deletion mutant Oct-6 proteins. The portion of the Oct-6 protein deleted is indicated by the number of the codons at which the ORF is fused. C. Structure of the Oct-6 protein, highlighting the domain involved in transcriptional activation and DNA binding (the POU domain; POU$_{SP}$ and POU$_{HD}$).

The experiments described above mapped a minimal transcriptional activation domain between amino acid 115 and 157. To check whether this domain is required and sufficient for transactivation or whether there is some functional redundancy within the aminoterminal one third of the protein, a small set of internal deletion mutants was constructed and tested for its ability to transactivate the 6Wtk promoter. The results of this experiment is shown in figure 5A, while the internal deletion mutants are schematically depicted in figure 6. Surprisingly all internal deletion mutants transactivate the 6Wtk promoter albeit at a lower level than the full length Oct-6 protein. The level of the Δ94/232 and Δ116/230 proteins is lower than that of the other deletion mutants. This was consistently observed in a number

of transfection experiments, which might indicate a lower stability of these mutant proteins (note the smear in lanes 5 and 6 in figure 5, right panel) leading to underestimating the transactivation potential of the mutant proteins. As expected, internal deletions in the first 98 amino acids (Δ23/44; lane 3 and Δ23/97; lane 4) did not abolish transactivation as these proteins retain the domain between a.a. 115 and 157. However, neither deletion of aa 95 to aa 231 (lane 5) nor smaller deletions in this region (Δ116/230; lane 6 and Δ144/197; lane 7) resulted in a complete loss of transactivation. Thus the combined analysis of the N terminal and internal deletion mutants suggest that the entire aminoterminal third of the protein is involved in transactivation and can be split up in functionally redundant subdomains.

Interestingly this aminoterminal region contains a long stretch of ala residues (9) that is polymorphic. The Oct-6 cDNA cloned by Suzuki *et al.* encodes an Oct-6 protein with a stretch of 8 Ala residues and the rat Oct-6 homologue SCIP has 11 ala residues at this position (13, 18). The Alanine stretch is located in a large region that is likely to adopt an α helical structure.

DISCUSSION

The six times repeated IgH enhancer octamer element (6W) was shown to strongly enhance transcription from the linked HSV tk promoter in ES and F9 EC cells. These cells express three octamer binding factors; Oct-1, Oct-4 and Oct-6 (24). The Oct-4 and Oct-6 factors are downregulated upon differentiation. At the same time the 6W enhancer is extinghuised in these differentiating cells, implicating either of the two or both factors in enhancer function. However ectopic expression of Oct-4 in HeLa cells failed to activate the tk promoter via the 6W enhancer (22). In contrast with the results presented here, Suzuki *et al.* reported that also Oct-6 is incapable to activate the 6W enhancer in HeLa cells whereas it activates a simple OCTA/TATA promoter (this promoter is similar to the pβO$^+$ promoter used here; figure2)(13). It is unlikely that this discrepancy is due to intrinsic differences in the cell lines used, as we show that high level expression of Oct-6 results in the activation of the 6Wtk promoter in different cell lines. More likely these differences are due to differences in the levels at which the effector protein is expressed. It is possible that only high levels of Oct-6 effectively compete for binding with the endogenous Oct-1 protein and stimulates transcription in HeLa cells. Thus our results suggest that the Oct-6 protein could play a role in stimulation of transcription of several gene promoters in EC/ES cells.

One of the aims of this study was to localize the domain(s) in the Oct-6 protein involved in the transactivation function of this protein. First we tested the responsiveness of three different promoters to high levels of Oct-6. The three promoters are different with respect to the number and the exact sequence of Oct-6 binding sites, the distance of these sites to the TATA box, and the nature, number and position of other cis-acting elements (see figure 1). All three promoters can be activated by coexpression of Oct-6. Thus in our test system Oct-6 can activate transcription from octamer and octamer related sequences in different promoter/enhancer settings. To determine which part of the Oct-6 protein is involved in this function we constructed a series of deletion mutants that were tested for their capacity to stimulate transcription from the 6Wtk promoter. All deletion mutants have an intact POU domain and thus retain the ability to bind the octamer motif. The data from the Oct-6 deletion

analysis indicate that the transactivation function is located in the aminoterminal one third of the protein. The entire activation domain is extremely rich in glycine and alanine residues and is composed of multiple, functionally redundant, subdomains. Protein domains have been determined, involved in the transactivation function of a large number of transcription factors. There are no apparent structural similarities between these domains, although they can be roughly classified as being particularly rich in certain amino acids (see (31) and references therein). As far as data are available, the transcativation domains of the POU proteins are either rich in glutamine (Oct-1) glutamine/leucine/proline (Oct-2), proline (Oct-3/Oct-4) or serine/threonine (Pit-1/GHF-1) rich (9, 16, 30, 32 – 35). The transactivation domain of the Oct-6 protein would form a new class, being glycine and alanine rich. Interestingly the proline rich region carboxy terminal of the POU domain is dispensable for transactivation of the 6Wtk promoter, suggesting that a high concentration of a particular amino acid residue is not enough to make up an activation domain. This is also illustrated by the fact that the region between aa 157 and 229 of the Oct-6 protein is also rich in glycine and alanine residues but does not activate transcription. A direct approach to address this issue for the Oct-2 protein has been reported by Gerster et al.(35). The aminoterminal transactivation domain of the Oct-2 factor is characterised by a high content of glutamine, leucine and proline residues. The analysis of substitution mutants of subdomainI in which the glutamine residues were replaced by asparagine or the leucine by isoleucines showed a drastic reduction in transactivation potential. Thus the particular arrangement and interaction with other residues are critical for the activation function of this domain.

The transactivation domains of the Oct-6 and Oct-4 factors belong to different classes (being gly/ala rich and pro rich respectively). This might indicate that Oct-4 and Oct-6 interact with different components of the basic transcription apparatus or alternatively that they interact with different socalled coactivators associated with the TATA box binding protein (TBP; (36) see also (37)). Thus taking into account the interaction with other DNA binding proteins and the differences in affinity between the two proteins for different octamer binding sites, it is likely that the two octamer factors regulate different sets of genes in ES/EC cells.

The importance of interactions between octamer factors and other transcription factors is illustrated by the observation that the octamer motif can also mediate repression of transcription in F9 EC cells (38). Obviously the sequence context of a cis-acting element is highly critical. Several mechanisms can be envisaged by which transcription factors can function as repressors (see (39)), one of which is competitive binding. For instance, the SV40 B element consist of two directly repeated Sph motifs of which the junction forms an octamer binding site. Both Sph motifs are required for enhancer function in HeLa cells, while high level expression of Oct-1 or Oct-2 represses the activity of the Sph motif via competitive binding (33).

The rat homologue of Oct-6, SCIP, is expressed at high levels in proliferating Schwann cells (17). The expression of SCIP/Oct-6 seems to antagonize the expression of glial specific genes like MBP and P_0 (18). Transfection of a SCIP/Oct-6 expression vector into cultured Schwann cells results in the downregulation of cotransfected MBP, P_0 and NGF-R promoters. These results indicate that the SCIP/Oct-6 protein serves as a negative regulator of Schwann cell specific genes. Significantly, downregulation of

the P_0 promoter by SCIP was also observed when it was cotransfected in the monkey cell line CV-1, indicating that no Schwann cell specific components are needed to downregulate this promoter (20). The P_0 promoter contains five SCIP/Oct-6 binding sites, that are remotely related to the octamer motif (20). One of the weaker binding sites overlaps with the P_0 TATA box. It was suggested that this binding sites serves as a negative element for SCIP/Oct-6 regulation of P_0 gene transcription through a competition mechanism (20). It is not known whether the POU domain of SCIP/Oct-6 suffices for repression of the P_0 promoter or that in addition other domains of the protein are required for this function. It is of interest to note here that a domain capable of mediating repression in HeLa en CV-1 cells has been identified in the Drosophila Krüppel protein. It was shown that a N-terminal alanine rich domain of this protein fused to the DNA binding domain of the lac repressor can repress transcription of target genes containing lac operator sequences (40). Such an alanine rich region can also be found in the Drosophila transcriptional repressors engrailed and evenskipped. Similarly, alanine rich domains are present in the Oct-6 protein interwoven with the transcriptional activation domains as determined in this study. It is possible that these regions in the Oct-6 protein are also involved in mediating repression of glial cell specific genes.

ACKNOWLEDGEMENTS

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