

The different inhibitory domains of the Oct-2 transcription factor have distinct functional activities

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Abstract The Oct-2 POU family transcription factor contains three distinct regions whose deletion reduces its ability to inhibit transcription via its octamer binding site. Here we show that only one of these inhibitory domains is capable of also inhibiting the activity of activating molecules bound at adjacent sites upstream of a TATA box-containing promoter whereas the other two regions are inactive in this assay. None of the three regions is able to achieve this effect when located upstream of the same promoter containing an initiator motif. The mechanisms of action of these domains and their role in the functioning of the Oct-2 factor are discussed.

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Key words: Oct-2 transcription factor; Inhibitory domain

1. Introduction

The Oct-2 transcription factor is a member of the POU family of transcription factors which was originally identified as a factor specifically expressed in B lymphocytes [1,2]. In these cells, Oct-2 appears to have a generally stimulatory effect on the expression of specific genes. Thus, the early suggestion that Oct-2 may play a role in activating the immunoglobulin genes which contain its target octamer sequence ATGCAAAT in their promoters and enhancers [3] has now been supplemented by the demonstration that mice in which the gene encoding Oct-2 has been inactivated lack expression of some B cell-specific genes such as CD36 [4] and Crisp3 [5]. Indeed, these knock-out mice lacking functional Oct-2 show defects in B cell maturation which indicates the critical role of Oct-2 in this process [6]. These stimulatory effects on gene expression appear to be mediated by a C-terminal activation domain which is present in the predominant B cell form of Oct-2, Oct-2.1 [7,8].

Although absent in most non-B cell types, Oct-2 has been identified in neuronal cells [9–11]. In these cells, Oct-2 appears to have a predominantly inhibitory effect on gene expression repressing, for example, the expression of the tyrosine hydroxylase gene which also contains an octamer motif in its promoter [12]. Interestingly, alternative splicing of the primary transcript produced by the single gene encoding Oct-2 results in the production of a number of different mRNAs encoding different isoforms of the protein [13]. This effect occurs in a tissue-specific manner so that the predominant forms of Oct-2 in neuronal cells, Oct-2.4 and Oct-2.5, lack the C-terminal activation domain present in the predominant B cell form, Oct-2.1, but are identical at the N-terminus of the protein [14].

These findings have led to a detailed study of the N-terminus of the Oct-2 molecule in order to identify specific regions common to all the forms of Oct-2 which might mediate an inhibitory effect on gene expression in the absence of the C-terminal activation domain present in Oct-2.1 but not in Oct-2.4 or 2.5. In particular, our laboratory defined a 40 amino acid region (amino acids 142–181) whose deletion from intact Oct-2 abolished its ability to inhibit gene expression [15]. When linked to the DNA binding domain of the heterologous Gal4 transcription factor, this domain was able to repress transcription from promoters containing binding sites for GAL4 indicating that it functions as a transferable inhibitory domain [16]. Moreover, when linked in this manner to a heterologous DNA binding domain, this inhibitory domain can also inhibit gene activation by a wide variety of different activation domains delivered to another DNA binding site in the promoter [17]. Thus this inhibitory domain is likely to act downstream of these activation domains presumably by interacting with a component of the basal transcriptional complex [17].

In addition to this domain, other studies have located an N-terminal inhibitory domain between amino acids 42 and 64 of Oct-2.1 [18,19]. This domain was demonstrated to inhibit the activity of the C-terminal activation domain of Oct-2 when fused to it in an artificial construct containing, in addition, the DNA binding domain of Gal4 [18,19]. The effect of this domain on the ability of either the C-terminal activation domain of Oct-2 or other activation domains when delivered to the promoter *in trans* (i.e. via a distinct DNA binding site in the promoter) was not reported, however.

Although both these inhibitory domains are present in all the different isoforms of Oct-2 [13], an additional inhibitory domain has also been defined which is unique to the Oct-2.3 isoform [20]. This isoform resembles Oct-2.1 at the C-terminus but differs from it in containing an additional 22 amino acids at the N-terminus between positions 72/73 of Oct-2.1. The presence of this domain was associated with a much weaker activation of transcription by Oct-2.3 compared to Oct-2.1 [13]. Subsequently, however, this domain was shown to be unable to activate the C-terminal activation domain when linked to it *in cis* in a construct containing the DNA binding domain of GAL4 [20]. Its effect on other activation domains or on the Oct-2 C-terminal domain when delivered to the promoter *in trans* was not assessed in these experiments however.

In order to directly compare the activity of the two inhibitory domains defined by other laboratories with that observed for the domain we previously defined, we have tested these two other domains for their ability to inhibit activation by a wide variety of activators when delivered to the promoter *in trans* utilising the same system which we previously used to

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characterise the inhibitory domain defined by our laboratory [17].

2. Materials and methods

2.1. Repressor domain cloning

The inhibitory domain defined by our laboratory (designated I1) was linked to the DNA binding domain of the tetracycline repressor as previously described [17]. In order to clone the inhibitory domain located between amino acids 42 and 64 of Oct-2.1 (designated I2) and the inhibitory domain which is unique to Oct-2.3 and is located between amino acids 72/73 of Oct-2.3 (designated I3), PCR primers were used to amplify the region containing amino acids 42–99 (numbered for Oct-2.1) from either Oct-2.1 or Oct-2.3. The primers used were 5'-GAATTCCATCAGAACCCCAAGTAAAG-3' and 5'-GAATCCTAGCTGGCTGCCGTCAGC-3'. When these primers were used to amplify a plasmid encoding Oct-2.1, we obtained the expected 183 bp insert. Similarly, when amplification was carried out using a plasmid encoding Oct-2.3 a 249 bp insert containing the additional 66 bp sequence found in this isoform as well as the region encoding amino acids 42–99 of Oct 2.1 was obtained. Each of these fragments was then cloned into the pTET^r plasmid containing the tetracycline repressor DNA binding domain and the clones obtained verified by DNA sequence analysis.

2.2. Transfections

BHK-21 fibroblast cells [21] and ND7 neuronal cells [22] were co-transfected by the method of Gorman [23]. In each case 5 µg of each repressor domain construct was co-transfected with 5 µg of plasmid encoding a specific activator domain linked to the DNA binding domain of GAL4 and 5 µg of the reporter construct. Activator and reporter constructs have been previously described [17,24]. In particular, each reporter contains seven binding sites for the TET repressor DNA binding domain upstream of five binding sites for GAL4 which in turn is located upstream of either a TATA box or an initiator motif. In all cases chloramphenicol acetyltransferase (CAT) activity was determined by the method of Gorman [23] with all samples being equalised on the basis of DNA uptake as assayed by dot blotting transfected cell extracts with an ampicillin-resistant gene probe derived from the plasmid vector [25]. In CAT assays an amount of extract which would give approximately 15–30% conversion (as assayed in preliminary experiments) was used for the sample lacking any inhibitor domain and the activity compared to an equivalent amount of the other samples. This allows repression to be detected whilst remaining in the linear range of the CAT assay.

3. Results and discussion

In our previous experiments [17] we used a reporter construct containing seven binding sites for the tetracycline repressor DNA binding domain located upstream of five binding sites for the GAL4 DNA binding domain which in turn were located upstream of a TATA box and CAT reporter gene. It was therefore possible to assess the effect of our inhibitory domain on transcription by a variety of activators by linking this domain to the TET repressor DNA binding domain and co-transfecting the reporter with this construct and a variety of activation domains linked to the GAL4 DNA binding domain. We therefore prepared similar constructs containing the Oct-2.1 inhibitory domain defined by Friedl and Matthias between amino acids 42 and 64 of Oct-2.1 [18,19] which we designated I2 and the same region together with the inhibitory domain unique to Oct-2.3 [20] which we designated I3. The inhibitory domain construct previously prepared by our laboratory was designated I1.

As the I2 but not the Oct-2.3 inhibitory domain had previously been shown to inhibit the activity of the C-terminal activation domain of Oct-2, when linked to it *in cis* via the Gal4 DNA binding domain we tested the effect of all three

inhibitory domains upon this activation domain when delivered to the DNA *in trans*. Thus the TATA box reporter plasmid was co-transfected with a construct containing one of the inhibitory domains linked to the TET repressor DNA binding domain and a construct encoding the Oct-2 C-terminal activation domain linked to the Gal4 DNA binding domain. In this experiment (Fig. 1A) the inhibitory domain which we previously characterised (I1) was able to repress activation by the Gal4 activation domain when delivered to the DNA *in trans* by transfection of ND7 neuronal cells whereas neither the inhibitory domain I2 nor the same domain linked to the Oct-2.3 domain (I3) was able to achieve this effect.

We have previously demonstrated that the Oct-2 I1 domain is cell type-specific in its activity having a particularly strong effect in neuronal cells [16]. It was therefore possible that the lack of inhibitory effect observed with the other domains was due to their similarly have a cell type-specific effect, although in this case being inactive in cells of neuronal origin. Similarly, it was possible that their inhibitory effect was in some way masked by the presence of endogenous Oct-2 in ND7 cells [11] which might be, for example, binding any co-factors required for the inhibitory effect. We therefore carried out similar experiments in BHK fibroblast cells which lack endogenous Oct-

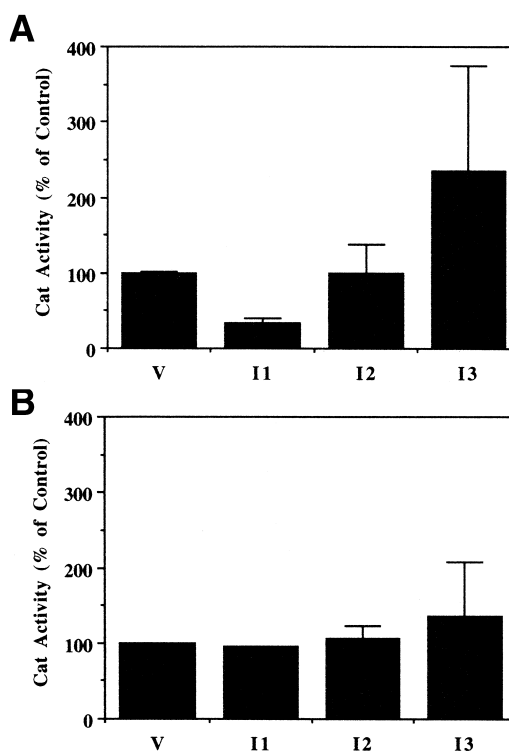


Fig. 1. Chloramphenicol acetyl transferase (CAT) assay following transfection of ND7 neuronal cells (A) and BHK fibroblast cells (B) with the T7 G5-TATA-CAT reporter construct together with a construct containing the C-terminal activation domain of Oct-2 linked to the DNA binding domain of GAL4 and either vector expressing the isolated DNA binding domain of the tetracycline repressor (V) or the same vector expressing the DNA binding domain linked to each of the three distinct inhibitory domains previously defined within the Oct-2 molecule. In each case the degree of activation obtained by co-transfection of the activator is compared to that observed by co-transfection of the activator with the reporter and the vector construct lacking any insert (set at 100%). Values are the average of three independent experiments whose standard error is indicated by the bars.

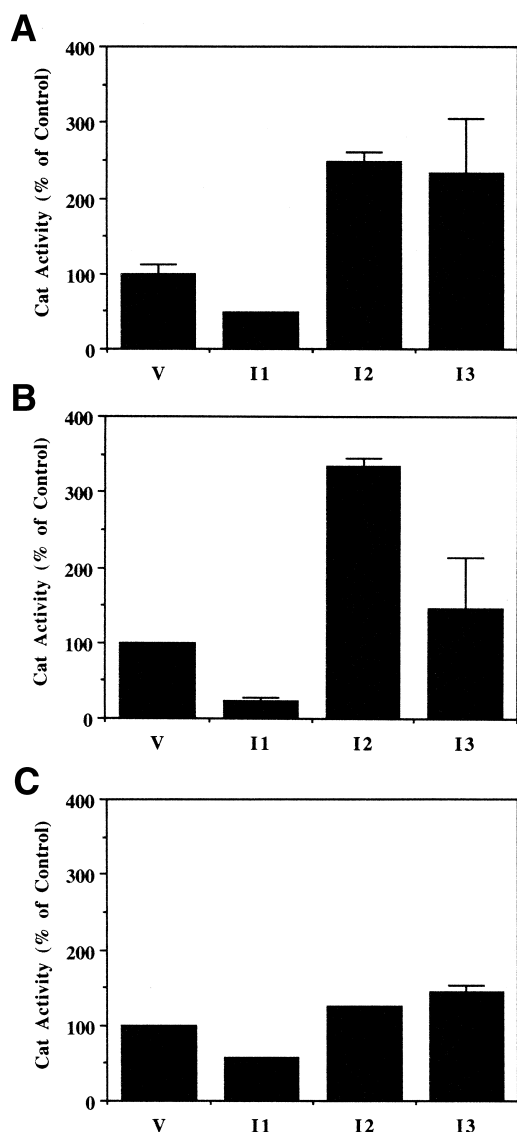


Fig. 2. CAT assay indicating the effect of the various inhibitory domains on activation of the T7 G5-TATA-CAT construct by expression vectors containing the Gal4 DNA binding domain linked to the activation domains of either VP16 (A), NFkB (B) or Sp1 (C). In each case the degree of activation is compared to that observed with that activation domain upon co-transfection of the reporter and TET repressor DNA binding domain construct in the absence of any linked inhibitory domain. Values are the average of three experiments whose standard error is shown by the bars.

2. As illustrated in Fig. 1B, the effect of the I1 domain was very weak in this situation in accordance with our previous experiments and similarly, no effect of the other inhibitory domains was observed. Hence it appears that unlike the I1 inhibitory domain neither of the other inhibitory domains is able to repress gene activation by the Oct-2 C-terminal domain when delivered to the DNA *in trans* in either neuronal or non-neuronal cell types. Indeed the I3 construct appeared to have a weak activating effect on gene expression in this system.

To determine whether this lack of effect of these domains was unique to the Oct-2 C-terminal domain which is rich in proline residues we carried out similar experiments with other activation domains. In these experiments the inhibitory do-

main similarly had no inhibitory effects on activation by the acidic activation domains of VP16 (Fig. 2A) or NFkB (Fig. 2B) or the glutamine-rich activation domain of Sp1 (Fig. 2C).

Indeed the only effect observed was a stimulation of the activation effect which was observed for both I2 and I3 in individual cases. As expected the I1 inhibitory domain repressed the activity of all the activators in accordance with our previous experiments [17]. A similar lack of inhibitory effect on other activators was also observed in similar experiments involving the proline-rich activation domain of CTF, the acidic activation domain of E1A and the Hob motif contained within the N-terminal region of c-Fos (data not shown).

Hence the inhibitory domain I2 and the same domain linked to the inhibitory domain of Oct-2.3 in I3 are unable to repress the activity of a wide variety of activation domains of different classes when delivered to the DNA *in trans* although the inhibitory domain I1 which we previously defined can do so.

Interestingly, however, we previously showed [17] that the ability of the I1 inhibitory domain to achieve this effect is abolished when the TATA box in the test promoter is replaced by an initiator element [17]. We therefore tested, whether in contrast to I1, either of the other domains could mediate repression of gene activation by an activator on a promoter using an initiator element. Similar transfections were therefore carried out using a target promoter in which the TATA box used in our previous experiments had been replaced by an initiator element [17,24]. In these experiments (Fig. 3) neither of the constructs was able to repress activation of this initiator-containing promoter by either the acidic activation domain of VP16 or the glutamine-rich domain of Sp1. Thus these domains are incapable of repressing activation regardless of whether the promoter tested contained a TATA box or an initiator motif.

These findings therefore establish distinct functional differences between the different inhibitory domains that have been defined within the Oct-2 molecule and indicate that they are likely to have distinctly different roles in the functioning of this factor. Thus the I2 inhibitory domain defined by Friedl and Matthias between amino acids 42 and 64 of Oct-2.1 [18,19] appears to be able to repress the C-terminal activation

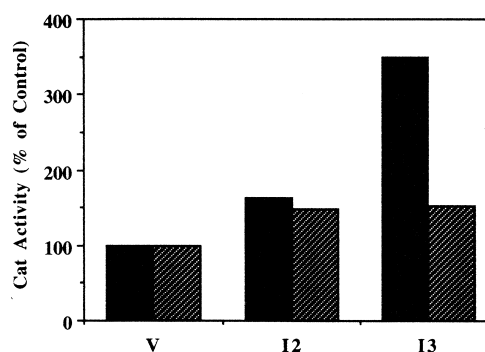


Fig. 3. CAT assay indicating the effect of the Oct-2 repressor domains on activation of a T7 G5-Initiator-CAT construct by Gal4-Sp1 (solid bars) or Gal4-Vp16 (hatched bars). In each case the degree of activation observed is compared to that observed with the reporter construct, the appropriate activator and the construct expressing the DNA binding domain of the tetracycline repressor alone (set at 100%).

domain only when linked to it *in cis* either naturally or with a heterologous DNA binding domain. It is incapable, however, of repressing this activation domain or other activation domains *in trans*. This domain is therefore likely only to act to reduce the activation ability of Oct-2 in particular situations when it is bound to its binding site on specific gene promoters.

The activity of the inhibitory domain unique to Oct-2.3 as defined by Annweiler et al. [20] is even more restricted since it only acts within the context of intact Oct-2 and is inactive even against its own C-terminal domain when linked to it via a heterologous DNA binding domain [20]. Similarly, we have demonstrated that when linked to the I2 domain, the construct containing this domain is still inactive in inhibiting activation by the C-terminal activation domain of Oct-2 or other activation domains when delivered to the DNA *in trans*. The activity of the Oct-2.3 domain is therefore likely to be confined to specifically reducing the activation ability of Oct-2.3 and perhaps as suggested by Annweiler et al. [20], it may compete for binding with other more potent activating forms of Oct-2 thereby reducing the activation mediated by an Oct-2 binding site when this factor is present. Hence Oct-2.3 may act as a passive repressor which is able to reduce the rate of gene expression by blocking the binding of more potent activators to its binding site.

In contrast, the I1 inhibitory domain which we previously defined [15,17] is not only capable of producing this effect but can also allow Oct-2 to act as a direct repressor which reduces the activity of a promoter compared to that which would be observed in the absence of its specific DNA binding site. Hence this factor is able to interfere with gene activation by other positively acting factors bound at adjacent sites and is therefore likely to be responsible for the ability of Oct-2 to dramatically reduce the rate of transcription of some promoters such as that encoding tyrosine hydroxylase [12].

The ability of this domain to block the activity of a wide variety of activators in this way is likely to be dependent upon its ability to interact with the basal transcriptional complex and reduce its activity thereby acting downstream of the different classes of activators. Although further studies will be required to define the target of the I1 inhibitory domain in the basal transcriptional complex it is already clear that this domain is far more potent than the other inhibitory domains defined within the Oct-2 molecule and is likely to be responsible for the direct inhibitory effect of the Oct-2 factor transcription in neuronal cells.

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