The Ability of the Inhibitory Domain of the POU Family Transcription Factor Oct-2 to Interfere with Promoter Activation by Different Classes of Activation Domains Is Dependent upon the Nature of the Basal Promoter Elements*

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The Oct-2 transcription factor contains an inhibitory domain which is able to repress transcription following DNA binding. Here we show that within the neurally expressed Oct-2.5 form, the inhibitory domain can strongly inhibit activation by transcription factor activation domains which are either composed predominantly of acidic residues or contain the HOB motif, whereas it has a weaker effect or no effect on proline-rich activation domains and on a glutamine-rich domain. In contrast, the isolated inhibitory domain of Oct-2 can efficiently repress all types of activation domains. This effect is observed however, only on TATA box-containing promoters and not on promoters containing an initiator motif. This widespread inhibition of different activation domains and its dependence on the nature of the basal promoter elements indicate that the inhibitory domain is likely to act by contacting a common downstream target of activation domains within the basal transcriptional complex bound at the TATA box rather than quenching specific activation domains by direct interaction. These effects are discussed in terms of the functional role of the inhibitory domain within Oct-2.5 and the mechanism by which it acts.

The Oct-2 transcription factor was originally identified in B lymphocytes where it is involved in the activation of gene expression (1, 2). Thus, for example, the immunoglobulin heavy chain gene enhancer contains the octamer binding site (ATGCATAAT) for Oct-2 (3) and its activity is greatly reduced in B cells derived from mice in which the Oct-2 gene has been inactivated (4). Similarly, Oct-2 has recently been shown to activate the CD36 gene in B cells (5).

Although Oct-2 is absent in most other cell types (1, 2), it has been detected in neuronal cells (6–9). Interestingly, in these cells, Oct-2 acts predominantly as a repressor of gene expression inhibiting the activity of artificial promotors containing its binding site (9, 10) as well as the tyrosine hydroxylase gene promoter (11) and the promotors of the herpes simplex virus (HSV)1 immediate-early (IE) genes, which contain the octamer-related TAATGARAT sequence (12, 13). Hence, Oct-2 can function as an activator or repressor of transcription depending upon the cell type.

This effect occurs because the primary RNA transcript derived from the single gene encoding Oct-2 (1, 14, 15) is alternatively spliced to produce mRNAs encoding different isoforms (16) with the Oct-2.1, -2.2, -2.3, and -2.6 forms containing a strong C-terminal activation domain, which is absent in Oct-2.4 and -2.5. This alternative splicing is regulated in a tissue-specific manner so that the Oct-2.1 form, which contains the C-terminal activation domain predominates in B lymphocytes, whereas the Oct-2.4 and -2.5 forms, which lack this activation domain, predominate in neuronal cells (17). In contrast, several groups including our own have identified an N-terminal region of Oct-2, which has an inhibitory effect on transcription (18–20). In particular, we have shown that the inhibitory effect of Oct-2 is dependent upon a 40-amino acid region near the N terminus of the protein (amino acids 142–181) which is common to all forms of Oct-2 (18). In Oct-2.1, -2.2, -2.3, and -2.6, the strong C-terminal activation domain overcomes the effect of the inhibitory domain so that these forms activate gene expression (17, 18). In contrast when the C-terminal activation domain is absent, as in Oct-2.4 and -2.5, the inhibitory domain is able to act to repress transcription (17, 18).

To investigate the mechanism by which this inhibitory domain acts, we previously linked it to the DNA-binding domain of the yeast transcription factor Gal4 (17). This construct was able to repress different promoters containing Gal4 DNA-binding sites (17, 21). This indicates that the inhibitory domain does not function by binding to DNA and blocking the binding of an activator since no activator proteins binding to Gal4 sites exist in mammalian cells. Similarly, since the isolated Oct-2 inhibitory domain in the absence of any DNA-binding domain does not repress transcription (17, 21), the inhibitory effect does not depend upon the formation of a non-DNA-binding protein-protein complex between the inhibitory domain and an activating molecule.

Apart from these two mechanisms, two other means by which inhibitory domains can repress transcription have been defined (for reviews see Refs. 22 and 23). These are quenching of a DNA-bound activator preventing it from activating transcription or direct interaction with the basal transcriptional complex to reduce its activity. To distinguish these possibili-

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ties, we have investigated the ability of Oct-2 to interfere with transcription when bound at different positions relative to the transcriptional start site and to inhibit activation of different basal promoter elements by a variety of different activation domains.

MATERIALS AND METHODS

Plasmid DNAs—The plasmids containing Gal4 sites at different positions and orientations relative to the SV40 promoter have been described previously (24). The reporter plasmid was constructed by cloning three octamer motifs (ATGCTAATGAGAT; Ref. 25) into the vector pG5B Cat (26, 27) (a kind gift of Dr. U. M. Hansen and Professor M. Ptashne), which contains five Gal4 DNA-binding sites upstream of the adenovirus E1B TATA box. The constructs containing the Gal4 DNA-binding domain linked to different activation domains were kind gifts of Dr. U. M. Hansen and Professor M. Ptashne (full-length Gal4, Gal4/Spi; Ref. 27), Professor W. Schaffner and Dr. P. Douville (Gal4/CTF, Gal4/AP2; Ref. 28), Dr. T. Kouzarides (Gal4/Fos; Ref. 29), Dr. M. R. Green (GAL4/EIA-30); and Dr. P. Baeuerle (Gal4/NFκB; Ref. 31). The target plasmid and Gal4 construct were co-transfected with ether the cDNA done of one of the different alternatively spliced forms of Oct-2 under the control of the constitutive IE promoter of cytomegalovirus (16) or a series of Oct-2 mutants lacking either the entire N- or C-terminal region (32) or containing different N- or C-terminal deletions (32, 33) or the isolated POU domain (18). The target plasmids containing the initiator sequence have been described previously (34) and were co-transfected with Gal4-linked activators and the Oct-2 inhibitory domain linked to the tetraarginine repressor DNA-binding region.

DNA Transfection—BHK-21 cells (clone 13; Ref. 35), which lack endogenous Oct-2 (13, 36), and ND7 cells (37), which express Oct-2 (12, 17), were transfected by the method of Gorman et al. (38). Transfections were carried out with 2 × 10⁶ cells on a 90-mm diameter plate transfected with 10 μg of reporter plasmids, 10 μg of the Oct-2 expression vectors, and 10 μg of the Gal4 plasmids, except in the case of Gal4/Fos or Gal4/VP16, where only 5 μg was used. These amounts of the Gal4/activation domain plasmids were shown to direct equivalent levels of protein production in transfected as assayed by DNA mobility shift on a Gal4 DNA-binding site (data not shown).

CAT Assays—Assays of CAT activity in the transfected cells were carried out as described by Gorman et al. (38), extracts having been previously equalized for protein content as described by Bradford (39). The percentage conversion of chloramphenicol to the acetylated form was determined by analysis on a PhosphorImager (Bio-Rad). In all cases, the values obtained were equalized for differences in plasmid uptake between samples based on the results of dot blot hybridization of an aliquot of the transfected cell extract with a DNA probe for the ampicillin resistance gene (40).

RESULTS

As a first step in distinguishing whether the inhibitory domain acted via quenching or as a direct repressor, we investigated the ability of a Gal4-inhibitory domain construct to repress a set of promoters in which the Gal4 sites were located in different orientations or different positions. Thus in many cases repressors that quench a specific activator have to bind to a site adjacent to the activator in order to exert their effect (see, for example, Ref. 41). In previous experiments (21) we had shown that the inhibitory domain could function when bound at different positions relative to the promoter, however, the constructs used in that study were prepared by cloning a large DNA fragment containing the Gal4 sites at different positions (42) and therefore maintained the relationship of the Gal4 sites with any adjacent activator binding site. We therefore repeated these experiments with constructs containing isolated Gal4 binding sites at different positions (Fig. 1a).

In these experiments (Fig. 1b), the Gal4-inhibitory domain construct was able to repress all three SV40 promoter constructs, which contained the Gal4 binding sites in different orientations and at different positions relative to the transcriptional start site. These experiments are thus not consistent with a model in which the inhibitory domain acts by quenching an adjacent activator, although they do not distinguish whether it acts by quenching a distant activator or by interact-
activity is not affected by Oct-2 (Table I). Thus, any effects of Oct-2 that were observed in the presence of activators would represent effects on activator-mediated transcription. This construct was co-transfected into BHK-21 cells (35), which lack endogenous Oct-2 (36) with the empty expression vector plj 7 (43) and individual constructs containing different activation domains linked to the DNA-binding domain of Gal4 (see Fig. 2 for details of the reporter and activator plasmids). The level of Cat activity was then compared to that observed when the reporter was co-transfected with empty expression vector and a construct encoding only the DNA-binding domain of Gal4. In these experiments (Table I), all the activation domains clearly activated the reporter gene with the strongest effect being observed with the acidic activation domain of the HSV virion protein VP16. These effects were specific to the reporter plasmid-containing Gal4 sites with no activation or nonspecific protein VP16. These effects were specific to the reporter plasmid lacking such sites (data not shown).

This experiment was then repeated substituting an Oct-2.5 expression vector for the empty expression vector. As illustrated in Table I, the presence of Oct-2.5 had virtually no effect on the basal activity of the promoter observed in the absence of any activation. However, Oct-2.5 reduced the degree of trans-activation produced by all the activation domains with the exception of the glutamine-rich activation domain of Sp1 (44). The degree of inhibition observed in the different cases did not appear to be related to the magnitude of the trans-activation observed in each case.

However, when the percentage inhibition of trans-activation produced by Oct-2.5 in each case was determined (by comparing the fold transactivation observed in the presence or absence of Oct-2.5), an interesting pattern emerged (Fig. 3a). Thus, Oct-2.5 was particularly effective in inhibiting trans-activation by the acidic activation domains of the HSV virion protein VP16, the adenovirus EIA protein, the cellular NFkB protein, and of Gal4 itself and also strongly inhibited trans-activation by the HOB motif activation domain contained in amino acids 1–81 of the c-Fos oncoprotein (29). In contrast, a weaker effect was observed on the proline-rich activation domains derived from CTF/NF1, AP-2 (28), and the C terminus of Oct-2 itself (32, 33), whereas no inhibition was observed on the glutamine-rich activation domain of Sp1 (44). In no case did we observe any effect of the activation domain plasmids on the level of Oct-2.5 in the transfected cells as assayed by DNA mobility shift assay (data not shown). Hence, the effects we observe are dependent upon the ability of Oct-2 to interfere with gene activation by the different activators.

To investigate these effects further, we carried out similar experiments in the ND7 cell line, which is derived from dorsal root ganglion neurons (37) and expresses endogenous Oct-2 (12). In these experiments (Fig. 3b) inhibition of both the acidic activation domains and the HOB domain was observed as in BHK-21 cells indicating that overexpression of Oct-2.5 can achieve this effect even in a cell line expressing endogenous Oct-2. Similarly, inhibition of proline-rich activation domains was also observed in the ND7 cells. Interestingly, however, some inhibition was also observed in the case of the glutamine-rich activation domain of Sp1, in contrast to the result in BHK-21 cells.

These results suggest therefore that, when tested within Oct-2.5, the inhibitory domain can strongly inhibit acidic activation domains or the HOB domain and has a weaker effect on proline and glutamine-rich domains. The cell type-specific differences observed in different cell types may relate to our

### Table I

| Activation domain fused to Gal4 DNA-binding domain | Type      | Fold transactivation |
|-----------------------------------------------------|-----------|----------------------|
| None                                                |          | 1 ± 0                |
| C-terminal domain of VP16                            | Acidic   | 70 ± 15              |
| C-terminal domain of Gal4                            | Acidic   | 11 ± 3               |
| N-terminal domain of EIA                             | Acidic   | 33 ± 2               |
| C-terminal domain of NFkB                            | Acidic   | 52 ± 6               |
| C-terminal domain of Sp1                             | Glutamine-rich | 8 ± 1               |
| N-terminal domain of AP2                             | Proline-rich | 16.5 ± 0             |
| C-terminal domain of CTF/NF1                         | Proline-rich | 15 ± 0               |
| N-terminal domain of c-fos                           | HOB motif | 71 ± 1               |
| C-terminal domain of Oct2                            | Proline-rich | 25 ± 5               |

### FIG. 2

Structure of the reporter plasmid, activator-encoding plasmids, and inhibitor-encoding plasmids used in these experiments. Note that since different activators had been linked to either amino acids 1–147 or 1–93 containing the DNA-binding domain of Gal4, a plasmid containing the appropriate region of Gal4 alone was used as a control in each case.
previous finding that the inhibitory domain is more active in ND7 cells compared to BHK-21 cells (21). This greater activity might thus allow it to overcome any activation effects of other regions of Oct-2.5, which might otherwise mask its inherent ability to repress a glutamine-rich activation domain.

Thus, although the Oct-2.5 molecule lacks the strong C-terminal activation domain present in other forms of Oct-2, it does contain an alternative C-terminal sequence, which has some similarity to that of Oct-1 and can trans-activate some promoters such as those encoding the small nuclear RNAs (45). To extend our observations, we therefore used the same experimental protocol to test the inhibitory effect in BHK cells of a construct (derived from Oct-2.2; Ref. 33) encoding only the N-terminal domain of Oct-2 (containing the inhibitory domain) and the central POU domain (allowing binding to the octamer motif) and lacking any C-terminal sequences. For comparison we also included a construct containing only the POU domain of Oct-2 but lacking the inhibitory domain.

In these experiments (Fig. 4), the N-terminal region was able to strongly inhibit trans-activation by the acidic domains of VP16, EIA, NFκB, and Gal4 as well as by the HOB motif of c-Fos paralleling the ability of Oct-2.5 to do this. Interestingly, in the case of the acidic domains the effect of the N terminus was much stronger than that of Oct-2.5, resulting in almost total repression of trans-activation.

This effect also resulted in the N terminus being able to significantly repress trans-activation in BHK-21 cells by the proline-rich activation domains of CTF/NF1 and AP2 as well as by the glutamine-rich activation domain of Sp1. In all cases only very weak or no inhibition of activation was observed with the construct expressing the POU domain alone, indicating that the effects are dependent upon the N terminus of Oct-2. Hence, the N terminus is able to inhibit activation by a variety of activation domains of different classes, although some of these effects are masked within intact Oct-2.5.

Interestingly, however, in these experiments the N-terminal region of Oct-2 was able to only partially inhibit its own proline-rich activation domain when the two regions are delivered to the DNA via separate DNA-binding domains. To investigate this effect further, we used a construct in which the DNA-binding domain of Gal4 had been linked in tandem to the N and C-terminal domains of Oct-2 so that both these domains were located on a single molecule. When this construct was co-transfected with a construct containing five Gal4 sites cloned at position −105 relative to the transcriptional start site of the thymidine kinase promoter (42) strong activation of transcription was observed (Fig. 5). Hence, the C-terminal activation domain can function in the presence of the N-terminal region when both are linked in cis to Gal4. In contrast, much weaker activation was observed using target constructs in which the Gal4 sites had been removed to either −770 or +1000 relative
to the transcriptional start site (42). This is in accordance with the weak stimulation of transcription produced when the Oct-2 C-terminal activation domain is bound at an enhancer position (32) and the ability of the Oct-2 inhibitory domain to inhibit all three of these constructs (21).

Although the intact N-terminal region is thus able to inhibit its own C-terminal activation domain only weakly it is a more effective inhibitor overall than intact Oct-2.5 (compare Figs. 3 and 4). We therefore investigated the effects of the different regions of Oct-2 on the inhibition of activation in more detail. In particular we tested the effects of a series of N- and C-terminal deletions of Oct-2.2 (32) on activation by the Gal4/VP16 fusion protein in BHK cells. All these constructs had previously been shown to direct similar levels of expression of the appropriate form of Oct-2 when transfected into different cell types including BHK-21 cells (18, 32, 33).

As illustrated in Fig. 6, progressive deletion of the N terminus of Oct-2.2 leaving the C terminus intact resulted in a progressive reduction in the ability to inhibit transactivation by the acidic activation domain of VP16 until a construct lacking the first 161 amino acids of Oct-2.2 was unable to inhibit activation. Interestingly, however, progressive deletion of the C terminus of Oct-2.2 removing the activation domain resulted in an increase in the ability to inhibit trans-activation. Thus, constructs containing the entire N terminus but truncated at amino acids 370 or 357 at the C terminus showed a stronger ability to inhibit trans-activation than the intact Oct-2.2 construct. As expected the ability to inhibit was lost upon further C-terminal truncation to amino acid 344, since this truncation disrupts the POU domain and would therefore prevent DNA binding.

These findings suggested that a construct containing the minimal repression domain defined in our previous studies would be likely to inhibit trans-activation in BHK cells in the absence of the C-terminal activation domain. As shown in Fig. 6, this is indeed the case with strong inhibition being produced by a construct encoding amino acids 154–376 and therefore containing only the inhibitory domain and the DNA-binding POU domain.

In view of this fact that the strong inhibition produced by this minimal construct was much greater than that produced by intact Oct-2.2 or 2.5, we tested its effect upon activity by the other activation domains. As shown in Fig. 7, the minimal inhibitory domain was able to significantly repress all the activation domains tested including the glutamine rich (Q) and proline-rich (P) activation domains are indicated together with the leucine-zipper (Leu-Z) and the repressor domain (RD). Panel b shows the results of three replicate experiments whose range is shown by the bars. All values are expressed relative to the degree of trans-activation produced by the construct in the presence of pJ7 vector lacking any insert. Constructs labeled N begin at the indicated amino acid and end at the C terminus of the protein (amino acid 479); constructs labeled C begin at the N terminus of the protein (amino acid 2) and end at the indicated amino acid. Construct labeled N154/C376 encodes amino acids 154–376.

In view of this fact that the strong inhibition produced by this minimal construct was much greater than that produced by intact Oct-2.2 or 2.5, we tested its effect upon activity by the other activation domains. As shown in Fig. 7, the minimal inhibitory domain was able to significantly repress all the activation domains tested including the glutamine rich activation domain of Sp1 and even had an effect on the C-terminal activation domain of Oct-2 itself.

The target promoters used in all these experiments contained a TATA box downstream of the Oct-2 and activator binding sites. We therefore wished to test the ability of the Oct-2 inhibitory domain to repress a promoter which lacks a TATA box and in which the basal transcriptional complex is
assembled at an initiator element (Inr), which overlaps the transcriptional start site (for review see Ref. 46).

To do this we used a construct (Ref. 34; see also Fig. 8a) which contains an initiator motif as well as upstream binding sites for Gal4 and the tetracycline repressor. This construct was transfected with four different Gal4 linked activators representing each of the four classes, i.e. acidic, proline-rich, glutamine-rich, and the HOB motif) and the Oct-2 inhibitory domain linked to the DNA-binding domain of the tetracycline repressor.

As illustrated in Fig. 8b, the inhibitory domain was unable to inhibit activation of initiator-driven transcription by the VP16, CTF, or Fos activation domains, although it did have an effect on activation by the Sp1 motif. Moreover, the same lack of effect on activation by VP16, CTF, or Fos was observed on a similar construct containing both an initiator and the E1B TATA box used in all the other target promoters (Fig. 8c), indicating that the lack of effect on initiator driven transcription predominates when both motifs are present. As expected, the Oct-2 inhibitory domain linked to the tetracycline repressor DNA-binding domain was able to repress the action of activators using the equivalent reporter construct containing only the TATA box and no initiator element (data not shown). Hence,
the inhibitory domain in this construct is functionally active but is unable to act when an initiator motif is present either alone or in the presence of the TATA box.

DISCUSSION

Following the identification of activation domains in a number of different transcription factors, three major types of domain have been identified, which are, respectively, rich in acidic, glutamine, or proline residues (for reviews, see Refs. 23 and 47). Here we show that the N-terminal inhibitory domain of the Oct-2 transcription factor can inhibit activation by members of all three these classes as well as by the HOB activation domain of c-Fos (29) when delivered to DNA via the Oct-2 POU domain, in the absence of other Oct-2 sequences. Interestingly, one of the activation domains that was mostly weakly inhibited by the inhibitory domain was the C-terminal activation domain of Oct-2 itself. This is in accord with our previous findings that, in the Oct-2 isoforms that contain this domain (Oct-2.1, -2.2, -2.3, and -2.6), it can overcome the inhibitory effect of the N-terminal domain (18). This results in Oct-2 activating transcription in B lymphocytes where these forms predominate (17).

We have shown previously, however, that in the neuronal forms Oct-2.4 and 2.5, which lack this C-terminal activation domain (16), the inhibitory domain predominates resulting in these forms inhibiting gene expression (18). Nonetheless, at least in the case of Oct-2.5, the alternative C-terminal sequences that are present modulate the activity of the inhibitory domain so that it is more selective in its trans inhibition of activation domains. Thus, within Oct-2.5 the inhibitory domain can strongly repress acidic activation domains and the HOX domain. In contrast, however, it has a weaker effect on several proline-rich domains and on a glutamine-rich domain.

These differences may be of biological significance. Thus, in neuronal cells, Oct-2 is involved in repressing the HSV IE promoters (13, 17, 18) resulting in a failure of lytic infection and leading to asymptomatic latent infections (for reviews, see Refs. 48 and 49). This is achieved by Oct-2 binding to the TAATGARAT (R = purine) motifs in the HSV IE promoters and both directly inhibiting transcription and preventing the binding of the complex of Oct-1 and the HSV virion protein VP16, which is essential for trans-activation of the IE genes during lytic infection (50, 51). Interestingly, however, each IE promoter contains several TAATGARAT motifs (52, 53). The ability of Oct-2.5 to inhibit acidic activation domains in general and that of VP16 in particular would ensure that IE gene expression was inhibited, even when one of the TAATGARAT motifs was bound by an activating Oct-1/VP16 complex provided at least one other TAATGARAT had bound Oct-2.

Subsequently, however, the reactivation of latent HSV must be achieved by activating IE gene expression in the absence of virion proteins such as VP16. This is likely to be achieved by the activation of specific cellular transcription factors that bind to other sites in the IE promoters and activate transcription. Thus, for example, treatment of neuronal cells with cyclic AMP can reactivate latent virus (54), and this is associated with the activation of the CREB transcription factor (55), which binds to the HSV IE1 gene promoter and activates it (56). In this situation, therefore, the relatively weak inhibition of non-acidic activation domains by Oct-2.5 could allow cellular factors containing such domains to stimulate IE gene transcription and reactivation even in the presence of DNA-bound Oct-2.5.

Whatever the precise significance of the differential effect of Oct-2.5 on different activation domains, it is clear that the Oct-2 inhibitory domain itself can inhibit a wide range of different activation domains. This finding renders it unlikely that the inhibitory domain acts by quenching the activity of activation domains. Thus, in cases of this type of repression, the inhibitory factor interacts with the activation domain; hence, such factors normally inhibit only a single type of activation domain (see for example Refs. 41, 57, and 58). This conclusion is in accordance with our finding that the inhibitory domain linked to Gal4 can function when the Gal4 sites are located at various positions relative to the transcriptional start site (19) since quenching factors normally function only when their DNA-binding site is located adjacent to the binding site of the activator (41). It is likely, therefore, that the Oct-2 inhibitory domain acts as a direct inhibitor of transcription interacting with the basal transcriptional complex to reduce its assembly and/or activity. This would allow it to interfere with gene activation by different classes of activation domains, which all act to stimulate this complex. This possibility is also supported by our previous finding that Oct-2 can repress a simple promoter containing only an octamer motif and TATA box with no binding sites for upstream activating molecules (17).

Interestingly, however, the ability of the Oct-2 inhibitory domain to repress transcription is dependent upon the TATA box, since it was unable to prevent activation by several different activation domains when transcription was directed by an initiator motif rather than by a TATA box. This effect parallels the inability of the KRAB (Kruppel-associated box) inhibitory domain to block the activation of initiator mediated transcription, although, as in the case of the Oct-2 inhibitory domain, it did have some effect on activation of the initiator by the SP1 activation domain (34). However, the KRAB inhibitor domain was able to prevent activation of a promoter containing both an initiator motif and a TATA box. In contrast Oct-2 did not repress such a construct, although it repressed constructs containing the same E1B TATA box without the initiator when delivered via either the Gal4 or Tet repressor DNA-binding domains. It is therefore possible that, in the case of Oct-2, the initiator motif can actually prevent repression of TATA box-mediated transcription as well as being itself refractory to such repression.

Whatever the reason for this difference between the Oct-2 and KRAB domains, the ability of the Oct-2 inhibitory domain to repress many different classes of activator and its dependence on basal promoter elements indicates that it is likely to act by interacting directly with the basal transcriptional complex bound at the TATA box. The target for such repression may be a specific component of such complexes, which is absent in complexes bound at the initiator. Such factors would include the TATA-binding protein-associated factors, which differ in the TFIID, complexes formed at the initiator and the TATA box (59). Alternatively, Oct-2 could interact with a component common to both types of complex such as the TATA-binding protein component of TFIID (60) if such a component were present in the TATA box complex in a different configuration, which allows it to interact with Oct-2. We are currently investigating the ability of the Oct-2 inhibitory domain to interact with the components of the TATA box, basal transcriptional complex (61) to determine whether one of these factors is the target which is inhibited by Oct-2. It is already clear, however, that this factor must directly inhibit a central component of the transcriptional complex bound at the TATA box, downstream of the target factors stimulated by different classes of activation domain.

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