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Activation and Repression of Gene Expression by POU Family Transcription Factors

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Activation and repression of gene expression by POU family transcription factors

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SUMMARY

The POU family transcription factors Oct-2 and Brn-3 utilize different mechanisms to produce a variety of effects on gene expression particularly in the nervous system. In the case of Oct-2, a single gene produces a primary RNA transcript. This transcript then undergoes alternative splicing to yield a variety of different mRNAs encoding Oct-2 isoforms which either activate or repress gene expression. In contrast, three distinct genes encode the closely related Brn-3 factors, Brn-3a, Brn-3b and Brn-3c. Although the proteins encoded by the Brn-3a and Brn-3c genes activate their target genes Brn-3b represses these genes and can also interfere with activation by Brn-3a or c. These findings indicate that diverse mechanisms are used to generate activating or repressing forms of POU family transcription factors.

The POU family of transcription factors was originally defined on the basis of a conserved region of approximately 150 to 160 amino acids which was identified in the Pit-1, Oct-1, Oct-2 and Unc-86 regulatory proteins (for review see Verrijzer & van der Vliet 1993; Wegner *et al.* 1993). This central POU domain constitutes the DNA binding domain of these proteins and consists of a POU-specific domain and a POU-homeodomain related to that found in the homeobox proteins.

Each of these original POU proteins plays a critical role in regulating gene expression in specific cell types. Thus, for example, the Pit-1 protein plays an essential role in gene expression in the pituitary gland and its absence results in a failure of pituitary gland development in both mice and humans (for review see Voss & Rosenfeld 1992). Similarly the *unc*-86 mutation in the nematode results in a failure to form specific neuronal cell types specifically sensory neurons (Desai *et al.* 1988; Finney *et al.* 1988). Interestingly, this association of the POU family factors with gene expression in neuronal cell types was strengthened by the observations of He *et al.* (1989) who used a degenerate polymerase chain reaction (PCR) approach to isolate novel POU domain transcription factors. All the factors which they isolated were shown to be expressed in specific neuronal cells suggesting that they are involved in regulating gene expression in such cells. Moreover, the Oct-2 factor, which had originally been suggested to be specifically expressed only in B lymphocytes (Singh *et al.* 1986; Staudt *et al.* 1986) was shown by He *et al.* (1989) to also be expressed in specific neuronal cells although it was absent in most other cell types.

This identification of Oct-2 in neuronal cells has been confirmed subsequently by a number of other laboratories including our own (Scholer *et al.* 1989; Kemp *et al.* 1990; Lillycrop *et al.* 1991; Stoykova *et al.* 1992). Interestingly however, the single RNA transcript produced from the Oct-2 gene is subject to alternative splicing to produce distinct forms of the mRNA in B lymphocytes compared to neuronal cells (Wirth *et al.* 1991; Lillycrop & Latchman 1992) (figure 1). Moreover, the different forms produced in B cells and neuronal cells have distinct effects on gene expression. Thus the predominant B cell form Oct-2.1 has a generally stimulatory effect on gene expression whereas the predominant neuronal forms Oct-2.4 and Oct-2.5 have a generally inhibitory effect on gene expression repressing promoters such as those of the herpes simplex virus immediate early genes (Lillycrop *et al.* 1991) and the cellular tyrosine hydroxylase gene (Dawson *et al.* 1994) These findings explain the contradiction between earlier observations suggesting that an Oct-2 binding octamer motif stimulated gene expression in B cells (Wirth *et al.* 1987) but inhibited gene expression in neuronal cells (Dent *et al.* 1991; Lillycrop *et al.* 1991).

The difference in activity between Oct-2.1 and Oct-2.4 or Oct-2.5 arises from the fact that the alternative splicing event results in Oct-2.4 and Oct-2.5 lacking the strong C-terminal activation domain which is present in Oct-2.1. However all the three forms contain a transcriptional inhibitory domain which is located at the N-terminus of the molecule (Lillycrop *et al.* 1994). In the case of Oct-2.1 the strong C-terminal activation domain overcomes the effects of the N-terminal inhibitory domain resulting in activation of transcription. In contrast, in Oct-2.4 and Oct-2.5 where the C-terminal activation domain is absent, the N-terminal domain is able to repress transcription.

By deletion analysis (Lillycrop *et al.* 1994) we have shown that the inhibitory domain comprises the 40 amino acids from 142 to 181 of the Oct-2 molecule. This region constitutes a separable inhibitory domain which, when linked to the DNA binding domain of the yeast GAL4 transcription factor, can repress promoters

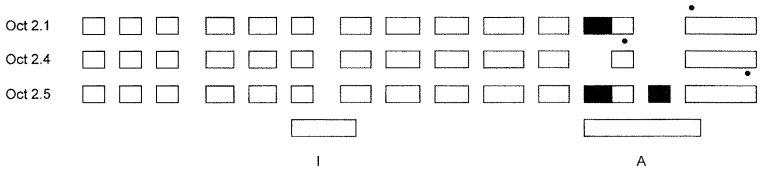

Figure 1. Structure of the different Oct-2 isoforms used in this study. Exons are indicated by boxes with the alternatively spliced exons shaded. Dots indicate the positions of in frame translational stop condons. The hatched boxes labelled I and A indicate the positions of the N-terminal inhibitory domain and the C-terminal activation domain.

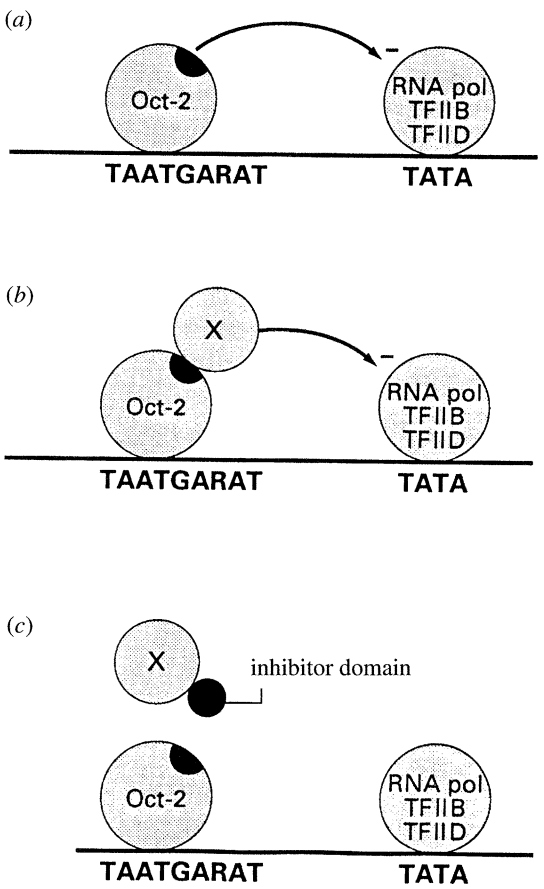

Figure 2. Inhibition of the basal transcriptional complex by Oct-2 could occur either: (*a*) directly; or (*b*) indirectly by recruitment of a second inhibitory protein (X). (*c*) In the latter case repression would be relieved if protein X is removed from the promoter by excess inhibitory domain expressed without a DNA binding domain.

containing GAL4 binding sites. This effect is dependant on the presence of GAL4 binding sites in the gene promoter but can occur regardless of whether such sites are positioned at increasing distances upstream or downstream from the gene promoter (Lillycrop & Latchman 1995).

It is unlikely therefore that Oct-2 represses gene expression simply by passively blocking gene activation by positively acting factors. Thus no such positive acting factors are known which bind to GAL4 sites when such sites are introduced into mammalian cells. Similarly the repression is dependant on DNA binding to the GAL4 site indicating that Oct-2 cannot act simply by removing positively acting factors from the DNA. It is probable therefore, that the Oct-2 inhibitory domain acts by interacting with the basal transcriptional complex to inhibit its activity (figure 2*a*). Of course such interaction may occur directly (figure 2*a*) or indirectly by Oct-2 recruiting a second inhibitory factor (figure 2*b*). This later possibility is supported by the finding that repression by the Oct-2 inhibitory domain is cell type specific being observed in a number of cell types but not, for example, in 3T3 fibroblasts (Lillycrop & Latchman 1995). Moreover the repressive effects can be relieved by cotransfecting cells with a construct expressing only the isolated inhibitory domain without any DNA binding domain. Thus this isolated domain may relieve the repression by competing for the binding of the second directly inhibitory factor (figure 2*c*). By screening a cDNA expression library with labelled inhibitory domain protein we have isolated several candidate cDNA clones which encode proteins capable of interacting with the inhibitory domain. Further studies are in progress to characterize these clones and their interactions with the wild type inhbitory domain as well as mutant forms of this domain which do not inhibit gene expression.

Thus in the case of the Oct-2 factor, alternative splicing is used to generate different forms of the protein with diverse effects on gene expression. However, in the case of the Brn-3 POU family factors a different mechanism is used. Thus the original Brn-3 factor was isolated as a novel POU protein by He *et al.* (1989) using the degenerate PCR approach described above. Subsequently, however, it became clear that there are in fact three distinct Brn-3 factors which are encoded by distinct but related genes (Theil *et al.* 1993, 1994). These factors are known as Brn-3a (also known as Brn-3.0: Lillycrop *et al.* 1992; Gerrero *et al.* 1993), Brn-3b (also known as Brn-3.2: Lillycrop *et al.* 1992; Turner *et al.* 1994) and Brn-3c (also known as Brn-3.1: Gerrero *et al.* 1993; Ninkina *et al.* 1993). Although these factors are each encoded by distinct genes they are closely related to one another and exhibit the strongest homology of any mammalian factor to the nematode regulatory factor *unc*-86.

All three factors are expressed in specific subsets of neuronal cells but not by other cell types (Gerrero *et al.* 1993; Turner *et al.* 1994) suggesting that like *unc*-86 they play a critical role in regulating neuronal specific gene regulation. Interestingly, when the immortalized ND7 neuronal cell line (Wood *et al.* 1990) is induced to cease dividing and differentiate to a neuronal-like phenotype bearing neuritic processes, the levels of Brn-3a expression increase greatly whereas Brn-3b falls and Brn-3c remains unchanged. This opposite expression pattern of Brn-3a and Brn-3b is paralleled by their

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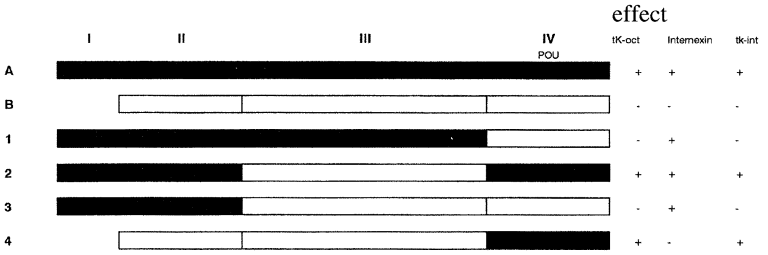

Figure 3. Summary of gene trans-activation data obtained using either the tk promoter carrying a synthetic octamer motif (tk-Oct) (data from Morris *et al.* 1994) or carrying the response element from the α-internexin promoter (tk-int) or with the intact α-internexin promoter (Budraham-Mahadeo *et al.* 1995) together with Brn-3a or -b or with constructs encoding chimeric proteins with different regions derived from Brn-3a or Brn-3b. The division of Brn-3a and -3b subdomains I,II,III and IV is as follows. For Brn-3a: domain I, amino acids 1–40; domain II, amino acids 41–108; domain III, amino acids 109–267; domain IV (POU domain), amino acids 268-end. For Brn-3b: subdomain II, amino acids 1–92; subdomain III, amino acids 93–169; subdomain IV (POU domain), amino acids 170–end. Region IV contains the POU domain.

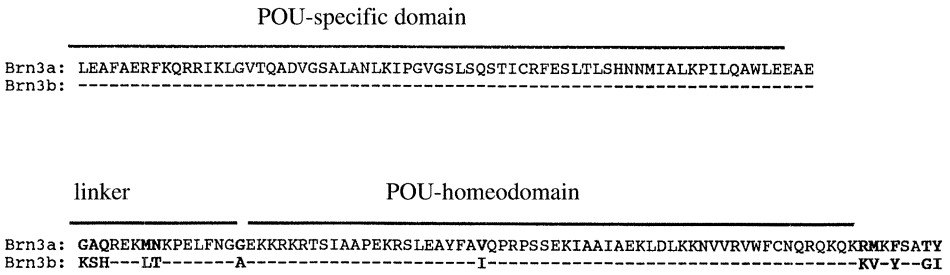

Figure 4. Comparison of the C-terminal subdomain IV in the Brn-3a and Brn-3b proteins. The positions of the POU-specific domain, linker, and POU-homeodomain components of the POU domain are indicated. The proteins contain a short region of eight amino acids C-terminal to the POU domain which is followed by the translational stop codon.

antagonistic effects on promoter activity. Thus Brn-3a strongly activates both an artifical promoter containing an appropriate binding site (Budhram-Mahadeo *et al.* 1994; Morris *et al.* 1994) and the promoter of the gene encoding the neuronal intermediate fillament protein α-internexin (Budhram-Mahadeo *et al.* 1995). In contrast Brn-3b can both inhibit the basal activity of these promoters and can abolish their stimulation by Brn-3a, although, it can activate the promoter of the neuronal nicotinic acetylcholine α2 receptor gene (Milton *et al.* 1995). As Brn-3c has only a weak stimulatory effect on promoter activity the net effect of ND7 cell differentiation is therefore to decrease the level of the inhibitory Brn-3b and increase the level of the activating Brn-3a. As expected from this, the activity of both the test promoters rises when transfected ND7 cells are induced to differentiate and extend neuritic processes confirming that the observed changes in the endogenous levels of different forms of Brn-3 can regulate target gene activity (Budhram Mahadeo *et al.* 1994).

Given the distinct activities of Brn-3a and Brn-3b on target promoters, it is possible to use chimaeric constructs encoding different regions of Brn-3a and Brn-3b (figure 3) to map the regions of Brn-3a which are required for activation of the different target promoters. This approach has allowed us to define two activation domains within the Brn-3a molecule. Thus an N-terminal activation domain is required for activation of the α-internexin promoter (Budhram-Mahadeo *et al.* 1995) whereas the DNA binding POU domain is required for activation of the test promoter containing a Brn-3 binding site (Morris *et al.* 1994). Insertion of the Brn-3 binding site from the α-internexin promoter into the same context in the test promoter results in its activation becoming dependant upon the POU region activation domain. Thus it appears that the context of the Brn-3 binding site in different promoters rather than its precise sequence controls the activity of each activation domain.

Interestingly, promoters which are responsive to the POU domain in the chimaeras are also responsive to the POU domain of Brn-3a when it is expressed in isolation. Thus the isolated POU domain of Brn-3a can activate a test promoter whereas the corresponding POU domain of Brn-3b has no effect (Budhram-Mahadeo *et al.* 1995). This effect must be dependant on one of the seven amino acid differences in Brn-3a compared to Brn-3b within the POU domain (see figure 4). Interestingly although six of these differences are located in the flexible linker region between the POU specific domain and POU homeodomain, one of them is found within the POU homeodomain. This difference is located at a position within the POU homeodomain which has been shown to be critical for its interaction with other proteins. Thus alteration of the amino acid at this position within the Oct-2 transcription factor to that normally found within the Oct-1 factor, confers upon Oct-2 the ability to interact with the herpes simplex virus protein VP16 which is

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normally a property of Oct-1 alone (Lai *et al.* 1992). It is likely therefore that this amino acid difference may allow Brn-3a to recruit a second activating molecule to the promoter whereas Brn-3b cannot do this and binds to DNA preventing activation by Brn-3a.

As well as analysing the molecular mechanisms of gene activation/repression by the Brn-3 factors, we have also attempted to characterize the function of these factors. Thus, using an antisense approach, we have inhibited the increased Brn-3a expression which occurs during the differentiation of the ND7 neuronal cell line. This treatment has no effect on the normal growth arrest of ND7 cells but prevents them extending neurite processes, suggesting that Brn-3a may play a critical role in this process (Lakin *et al.* 1995). In agreement with this idea, we have shown that Brn-3a is capable of transactivating the promoter of the gene encoding the SNAP25 protein which is essential for neurite outgrowth by a number of different types of neuronal cell (Osen-Sand *et al.* 1993). Thus the rise in Brn-3a expression upon ND7 differentiation may play a critical role in stimulating the expression of genes whose protein products are required for this process. Conversely Brn-3b may act to restrain neurite outgrowth ensuring that it does not occur inappropriately. Thus the different expression patterns and functional activities of the Brn-3a and Brn3b factors may play a critical role in controlling neuronal phenotype.

It is clear therefore that the POU proteins of the Oct-2 and Brn-3 families play a critical role in gene expression in neuronal cells. The diverse activities of these factors are facilitated by the generation of different but related forms by splicing of the Oct-2 gene primary transcript and by the existence of three distinct but related genes encoding the different forms of Brn-3.

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