Functional interaction between different isoforms of the Oct-2 transcription factor expressed in neuronal cells

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The predominant neuronal isoforms of the Oct-2 transcription factor, Oct 2.4 and Oct 2.5, repress the herpes simplex virus immediate-early promoter both in neuronal cells and in fibroblasts normally lacking Oct-2. In contrast, the predominant B lymphocyte form Oct 2.1, which is present at a lower level in neuronal cells, activates the immediate-early promoter in fibroblasts but represses it in neuronal cells. We show here that both

Oct 2.4 and Oct 2.5 can functionally interact with Oct 2.1 and convert it from an activator into a repressor. Hence, the cell type-specific activity of Oct 2.1 results from the presence of Oct 2.4 and 2.5 in neuronal cells and their absence in other cell types. The significance of this effect is discussed in terms of the role of Oct-2 in the regulation of viral and cellular gene expression in neuronal cells.

INTRODUCTION

The octamer-binding proteins activate the expression of a wide variety of cellular genes that contain the octamer motif (ATGCAAT) in the gene promoter (for review, see [1]). In particular, the expression of the octamer-binding protein Oct-2 in B lymphocytes but not in most other cell types [2,3] plays a critical role in the B cell-specific expression of the immunoglobulin genes whose promoter and enhancer elements contain this motif [4,5]. Interestingly, although absent in most cell types other than B cells, Oct-2 has been detected in neuronal cells [6–8]. In neuronal cells, however, Oct-2 appears to act predominantly as a repressor of gene expression, inhibiting the activity both of the herpes simplex virus (HSV) immediate-early (IE) genes, which contain the octamer-related TAATGARAT motif in their promoters [9], and of test promoters which contain an artificially inserted octamer motif [10,11].

The problem of the cell-type specific activity of the Oct-2 factor was partially resolved by the demonstration that the Oct-2 RNA is subjected to tissue-specific alternative splicing, resulting in the production of different isoforms in neuronal cells and B cells [12]. Thus, while B cells contain predominantly the Oct 2.1 form, which has a strong activation domain at its C-terminus, neuronal cells synthesize predominantly the Oct 2.4 and 2.5 forms, which have a truncated or altered C-terminus (Figure 1) [12,13]. Hence, Oct 2.4 and 2.5 can repress the HSV IE promoter when co-transfected into fibroblasts lacking endogenous Oct-2 whereas Oct 2.1 activates this promoter upon co-transfection [12].

However, up to 40 % of the Oct-2 mRNA in neuronal cell lines can encode the activating Oct 2.1 isoform [12] and yet these cell lines exhibit strong repression of the HSV IE promoter [9]. Similarly, when Oct 2.1 is co-transfected into these cell lines, it can repress the IE promoter rather than activate it, as in fibroblasts [9,12]. Hence, the Oct 2.1 factor has a cell type-specific activity, repressing the HSV IE promoter in neuronal cells and activating it in other cell types.

In view of the finding that different octamer-binding proteins can interact with one another [14,15], one possible mechanism for this effect would be for the activity of Oct 2.1 to be altered

from activator to repressor by the endogenous Oct 2.4 and 2.5 factors which are present in neuronal cells but absent in other cell types [12]. We have therefore tested the effect of these forms of Oct-2 on the ability of Oct 2.1 to modulate the activity of the IE promoter.

MATERIALS AND METHODS

Cell culture and isolation of stably transfected cell lines

BHK cells [16] were routinely grown in RPMI medium containing 10 % (v/v) foetal calf serum. To prepare cell lines derived from baby hamster kidney (BHK) fibroblasts that stably expressed either of the predominant neuronal forms Oct 2.4 and Oct 2.5, 2×10^6 BHK cells on a 90 mm plate were transfected with $10 \mu\text{g}$ of plasmids containing full-length cDNA clones for each of the Oct-2 forms expressed under the control of the IE promoter of cytomegalovirus (CMV) [13] and the neomycin resistance gene expressed under the control of the murine sarcoma virus promoter. Transfections were carried out using the calcium phosphate procedure [17]. Stably transfected cells were isolated on the basis of their neomycin resistance and, following cloning, were screened for Oct-2 expression by PCR amplification of the mRNA with Oct-2-specific primers [12]. The resulting PCR products were digested with restriction enzymes that distinguish between human and rodent Oct-2 to confirm that the cells expressed an exogenous human Oct-2 mRNA derived from the transfected plasmid.

Transient transfection

BHK cells (2×10^6 cells on a 90 mm plate) were transfected with $5 \mu\text{g}$ of the IE-CAT (CAT, chloramphenicol acetyltransferase) plasmid containing the HSV IE3 promoter (base pairs –330 to +33) [18] linked to the CAT gene and the indicated amount of expression vectors (see Figure legends) containing the Oct 2.1, 2.4 or 2.5 forms expressed under the control of the CMV IE promoter [13]. In all cases, the amount of DNA transfected was kept constant by inclusion of the appropriate amount of the pJ7 CMV expression vector plasmid lacking any insert [19].

Following transfection, CAT activity was assayed by the

Abbreviations used: HSV, herpes simplex virus; IE, immediate-early; BHK, baby hamster kidney; CMV, cytomegalovirus; CAT, chloramphenicol acetyltransferase.

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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 codons.

method of Gorman [17] on cell extracts that had been equalized for protein content by the method of Bradford [20]. The values obtained in this way were then equalized for any differences in plasmid uptake between the different samples on the basis of the results obtained by dot blot hybridization of DNA prepared from an aliquot of the transfected cell extract with a CAT DNA probe [21].

RESULTS AND DISCUSSION

In initial experiments, we determined the concentration dependence of the alteration in IE promoter activity obtained by co-transfection with each of the forms of Oct-2 (Figure 1). As expected, co-transfection of increasing amounts of Oct 2.1 resulted in progressively increased activity of the IE promoter

whereas increasing amounts of either Oct 2.4 (Figure 2a) or Oct 2.5 (Figure 2b) resulted in progressively decreased activity.

These results were then used to predict the effect on IE promoter activity of co-transfecting progressively decreasing amounts of Oct 2.1 with increasing amounts of either Oct 2.4 (Figure 3a) or Oct 2.5 (Figure 3b), based on the assumptions that the two forms do not interact and that each form would affect promoter activity identically in the presence and absence of the other form. When this experiment was actually carried out, however, the results obtained were dramatically different from those expected on this basis (Figure 3). Thus, even a small amount of Oct 2.4 or Oct 2.5 was able to inhibit the IE promoter even in the presence of a large amount of Oct 2.1. For example, with the addition of 15 μg of Oct 2.1 and 5 μg of Oct 2.4, one would expect the activity of the IE promoter to be increased. Instead, the promoter was actually repressed below the level observed upon co-transfection of plasmid vector alone.

These data suggest therefore that Oct 2.4 or 2.5 do not act independently of Oct 2.1 when co-transfected with it; rather, they can interact with Oct 2.1 and convert it from an activator to a repressor. This possibility is supported by the finding (Figure 3) that a similar degree of repression of the promoter is observed for 15 μg of Oct 2.4/2.5 mixed with 5 μg of Oct 2.1 as observed for 20 μg of Oct 2.4/2.5 alone. This rules out a model where Oct 2.4 or 2.5 simply compete for binding with Oct 2.1, and strongly suggests that Oct 2.1 is converted from an activator to a repressor by interaction with Oct 2.4 or 2.5.

Figure 2 (a) Concentration-dependence of the effect of the Oct 2.1 (□) or Oct 2.4 (◇) isoforms on the activity of the IE promoter

In each case, the indicated amount of Oct 2.1 or 2.4 was transfected with the IE promoter together with the appropriate amount of pJ7 plasmid vector to keep the DNA amount constant in each case. All values are expressed as the percentage activation of the IE promoter compared to the level of CAT activity observed when the promoter was co-transfected with 20 μg of pJ7 alone. Values are the means of at least three determinations whose range is shown by the bars. (b) As for (a) but showing the concentration dependence of the effect of Oct 2.1 (□) or Oct 2.5 (◇) on the activity of the IE promoter.

Figure 3 Interactions between Oct 2.1 and Oct 2.4 (a) and between Oct 2.1 and Oct 2.5 (b)

(a) The observed effect of mixing the indicated amounts of Oct 2.1 and 2.4 and co-transfecting them with the IE promoter ($\triangle$) is compared with the predicted values ($\square$) based on the data in Figure 2 and assuming that Oct 2.1 and 2.4 act independently on the IE promoter and do not interact with one another. The data are expressed as a percentage of the CAT activity observed when the IE promoter was co-transfected with 20 μ g of pJ7 plasmid vector alone. Observed values are the means of at least three determinations whose range is shown by the bars. (b) The data are as described in panel (a) except that Oct 2.5 was used instead of Oct 2.4.

Table 1 Modulation of the HSV IE promoter by Oct 2.1 in BHK cells stably expressing Oct 2.4 or Oct 2.5

The activity of the IE promoter when co-transfected into each of the cell lines with Oct 2.1 is expressed as a percentage of its activity when co-transfected with the vector control. All values are the means of two experiments whose range is indicated.

Cell line	Stably expressed protein	Activity (%)
Parental BHK	—	203 $\pm$ 18
2.4/1	Oct 2.4	82 $\pm$ 21
2.4/2	Oct 2.4	60 $\pm$ 3
2.5/1	Oct 2.5	120 $\pm$ 8
2.5/2	Oct 2.5	83 $\pm$ 0

We confirmed this possibility by using a different system in which we constructed BHK cells stably expressing either Oct 2.4 or 2.5. Two cell lines of each type were selected on the basis that they expressed high levels of Oct 2.4 or 2.5 that were comparable to that expressed naturally by neuronal cell lines (results not shown). These cells were then co-transfected with Oct 2.1 and the IE-CAT plasmid, and the effect on IE promoter activity compared with that observed when it was co-transfected into each cell type with the plasmid expression vector alone.

In these experiments (Table 1), Oct 2.1 activated the IE

promoter upon co-transfection into the parental BHK cells, as expected. In contrast, no significant activation of the IE promoter by Oct 2.1 was observed in two independently isolated BHK cell clones stably expressing Oct 2.4 or in two similar independently isolated clones expressing Oct 2.5. In both the Oct 2.4-expressing clones and one of the Oct 2.5-expressing clones, the activity of the IE promoter when co-transfected with Oct 2.1 was actually below that observed when it was co-transfected into the same clone with the pJ7 expression vector alone. Hence, in these cells, Oct 2.1 can repress the IE promoter, exactly as in neuronal cells naturally expressing Oct 2.4 and 2.5. The ability of Oct 2.1 to repress the IE promoter when co-transfected into neuronal cells [9,12] can thus be reproduced by expressing either of the neuronal isoforms of Oct 2 (Oct 2.4 or Oct 2.5) in non-expressing fibroblast cells.

Our data using both transiently and stably transfected cells indicate therefore that Oct 2.4 and/or Oct 2.5 can interact with Oct 2.1 and convert it from an activator of the IE promoter into a repressor. Thus, despite the relatively high level of Oct 2.1 in neuronal cell lines [12], the IE promoter is strongly repressed in these cells [9] both because of the direct inhibitory activity of Oct 2.4 and 2.5 and because of their ability to functionally interact with Oct 2.1.

Although different octamer-binding proteins can interact with one another to form dimers both in solution and when two adjacent binding sites are present in the target DNA, they bind

to individual octamer binding sites as monomers [14]. The functional interaction that we have observed is therefore likely to be dependent on the presence of two octamer-binding TAATGARAT sequences in the HSV IE3 promoter [22], with interaction occurring when an Oct 2.1 molecule is bound at one site and Oct 2.4 or 2.5 is bound at another site. If this is the case, it would provide a new aspect to the modulation of gene expression by Oct-2 in neuronal cells, with promoters containing multiple octamer-binding sites being repressed by Oct 2.1 as well as Oct 2.4/5, and those with single binding sites being modulated by the balance between the Oct 2.1 activator and the Oct 2.4/5 repressors. Our recent finding that Oct 2.4 and Oct 2.5 can repress the tyrosine hydroxylase promoter, which contains two adjacent octamer-binding sites (S. Dawson and D. S. Latchman, unpublished work), suggests that the significance of these effects may not be confined to the regulation of viral gene expression in neuronal cells.

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