A Single Amino Acid Change Converts an Inhibitory Transcription Factor into an Activator*

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The closely related POU family transcription factors Brn-3a and Brn-3b differ in their functional activity with Brn-3a activating several target promoters, which are repressed by Brn-3b. Brn-3b also prevents promoter activation by Brn-3a. Here we have altered a single isoleucine residue in the POU homeodomain of Brn-3b to the valine residue found at the equivalent position in Brn-3a. This change not only abolishes the ability of Brn-3b to repress basal and Brn-3a-stimulated promoter activity but also converts it to an activator of similar potency to Brn-3a. Hence a single amino acid difference determines the difference between an activator and a repressor in the Brn-3 family.

The POU (Pit-Oct-Unc) family of transcription factors (for review see Refs. 1 and 2) was originally defined on the basis of a common DNA binding domain in the mammalian transcription factors Pit-1, Oct-1, and Oct-2 and in the regulatory protein Unc-86, whose mutation results in the failure to form specific neuronal cells in the nematode (3, 4). Although many other POU factors are known (see for example Ref. 5) the mammalian POU factors that show the highest homology to Unc-86 are the Brn-3 factors, and these factors together with the Drosophila POU factors I-POU and ti-POU constitute a separate POU IV subfamily within the POU family (2).

Three distinct Brn-3 factors have been identified, which show close homology in the DNA binding POU domain but are much less homologous outside it and are encoded by three different genes (6, 7). These factors are Brn-3a (also known as Brn-3 or Brn-3.0) (5, 8, 9)), Brn-3b (also known as Brn-3.2 (9, 10)), and Brn-3c (also known as Brn-3.1 (8, 11)). Each of these factors is expressed in distinct but overlapping sets of neurons in the developing and adult nervous systems (5, 8, 10–12), suggesting that like Unc-86 they may play a key role in regulating gene expression in neuronal cells.

To investigate this role, we have previously tested the effect of co-transfecting fibroblasts lacking exogenous Brn-3s with plasmids containing the Brn-3a or Brn-3b promoter linked to reporter genes. The results with both promoters show that activation of the test promoter occurred only when the chimera contained the POU domain of Brn-3a and not when it contained that of Brn-3b (15, 16). As the isolated POU domain of both Brn-3a and Brn-3b binds to DNA (6, 10, 19) we were able to test the effect on the test promoter of expressing only the POU domain of Brn-3a or Brn-3b. Indeed that promoter was activated by the POU domain of Brn-3a but not by that of Brn-3b (20), indicating that one or more of the seven amino acid differences in the POU domains of Brn-3a and Brn-3b (6, 9) must be responsible for this difference in their functional activities.

The POU domain consists of two separate subdomains, the POU-specific domain and the POU homeodomain separated by a short flexible linker region (1, 2). Brn-3a and Brn-3b exhibit six differences in this linker region but are identical in the POU-specific domain and have only a single difference in the POU homeodomain (6, 9). As the linker region is very variable between different POU factors and is believed to serve only to allow the two major domains to move relative to one another (1, 2), we concentrated our attention on the single difference in the POU homeodomain at position 22 in the first a-helix. Here we report the effect of altering the isoleucine at this position in Brn-3b to the valine found in Brn-3a.

MATERIALS AND METHODS

Mutagenesis—Brn-3b cDNA (6) was subcloned into the pALTER-1 vector (Promega), which contains a mutated, inactive ampicillin resistance gene. The resulting plasmid was annealed with 100 pmol of an ampicillin repair oligonucleotide (Promega) and the mutagenic oligonucleotide 5'-GGAAGCCTACTTCGCCGTCCAGCCAAGGCCCTC-3', which will convert the isoleucine codon in Brn-3b to a valine codon. The annealed oligonucleotides were used to prime the synthesis of mutated plasmid using T4 DNA polynucleotide (10 units) and T4 DNA ligase (1 unit) in 10 mM Tris-HCl, pH 7.5, 0.5 mM dNTPs, 1 mM ATP, 2 mM dithiothreitol at 37 °C for 1 h and were then incubated for a further 30 min at 25 °C with an additional unit of T4 ligase. Mutated plasmids were isolated by selecting ampicillin-resistant clones following transfection of Escherichia coli BMH repair minus cells, and the presence of the Brn-3b mutation was confirmed by DNA sequence analysis.

Transient Transfection and Chloramphenicol Acetyltransferase Assay—Transfection of DNA was carried out according to the method of...

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RESULTS AND DISCUSSION

The mutant Brn-3b containing valine at position 22 in the homeodomain was co-transfected into both BHK-21 cells (22), which do not express any form of Brn-3, and into ND7 neuronal cells (17), which express Brn-3a and Brn-3b (9, 15), together with the target promoter containing a Brn-3 octamer binding site cloned upstream of the tk promoter (15, 16). In both cell types, the mutant Brn-3b activated the promoter to a similar extent to Brn-3a, whereas wild type Brn-3b had no stimulatory effect and actually inhibited activity of the promoter (Fig. 1).

Hence mutation of isoleucine to valine can convert full-length Brn-3b from a repressor to an activator of similar potency to Brn-3a. In our previous experiments (20) we showed that activation of the test promoter could also be achieved by the POU domain of Brn-3a, whereas the POU domain of Brn-3b had little or no effect. We therefore prepared a construct expressing the POU domain of Brn-3b with the valine mutation. This construct was indeed able to activate gene expression exactly as occurs for the POU domain of Brn-3a (Fig. 1).

Full-length Brn-3b has also been shown to inhibit transactivation by Brn-3a (15, 16). We therefore tested the effect of co-transfecting combinations of Brn-3a, Brn-3b (I), and the mutant Brn-3b (V). As shown in Fig. 2, Brn-3b (V) had no inhibitory effect on gene activation by Brn-3a, and this combination produced slightly stronger activation than either factor alone. In contrast gene activation by either Brn-3a or Brn-3b (V) was effectively inhibited by co-transfecting the wild type Brn-3b (I). Similar inhibition of gene activation by the isolated Brn-3a POU domain was also observed upon co-transfection of Brn-3b (I) but not with Brn-3b (V).

Thus the single mutation of isoleucine at position 22 of the homeodomain of Brn-3b to the valine found in Brn-3a abolishes the inhibitory effect of Brn-3b on basal transcription and gene activation by Brn-3a and also allows Brn-3b to act as an activator. As the POU domain of unmutated Brn-3b can bind to DNA (6, 10, 19), this effect does not appear to involve the acquisition of DNA binding ability due to the mutation. Indeed position 22 of the homeodomain is located at the C terminus of the first a helix and is thus not in contact with the DNA according to crystallographic analysis of the POU domain of the related Oct-1 protein (25).

Rather this amino acid appears to be located on the surface of the POU domain away from the DNA representing a site of potential protein-protein interaction. Such a role for the amino acid at this position is supported by the finding that substitution of the alanine at this position in Oct-2 with the glutamic acid found at the equivalent position in Oct-1 allows the mutant Oct-2 to interact with the herpes simplex virus virion protein Vmw65, which is normally a property of Oct-1 only (26, 27). This effect appears to depend on the length of the side chain of the amino acid concerned rather than its charge. Thus
Oct-1 can still interact with Vmw65 when this glutamic acid is replaced by glutamine but not when it is replaced by aspartic acid or alanine (27). Thus, in the case of Brn-3a and Brn-3b it is possible that the shorter branched side chain present in valine allows a closer packing of an interacting protein than the longer side chain of isoleucine. This might allow Brn-3a and Brn-3b (V) to stably interact with and thereby recruit an activating molecule to the promoter whereas Brn-3b could not do so.

Further studies will be required to confirm this possibility and identify the recruited activator. However, it is already clear that a single amino acid difference can produce a difference in functional activity in the Brn-3 family and that alteration of this amino acid can convert a repressor into an activator.

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