

Cloning of rat Mbnl2 cDNA

Our interest in Mbnl2 was stimulated by results of cDNA microarray analysis studies indicating that the expression of mouse Mbnl2 mRNA increased at night in the pineal gland (Blackshaw *et al.*, unpublished data). As we are interested in the rat pineal gland, we cloned rat Mbnl2 cDNA based on mouse Mbnl2 mRNA sequence. Rat Mbnl2 cDNA was cloned from a rat pineal cDNA using PCR with primers based on rat genome corresponding to mouse Mbnl2 sequence (GeneBank Accession no: XM_193773). Rat primers used in PCR cloning are as follows: 5'-GTGCCATCGTATTGAAGTCAC-3' and 5'-ATCTCCTGGCAAAGCGAACC-3', which are corresponding to those of mouse Mbnl2 sequences (GeneBank accession no: XM_193773); 5'-GTGCCATCGTATTAAAGCCAC-3' and 5'-ATCTCCTGGCAAAGCGAACC-3', respectively. This yielded a 1941 bp sequence that was 95% and 88% identical to the corresponding mouse and human Mbnl2 cDNA sequences, respectively. The deduced amino acid sequence defined 361 amino acids that are 99% and 93% identical to those of mouse and human Mbnl2, respectively.

Expression of Mbnl2 protein:

For expression of Mbnl2 protein in HEK293 cells, the coding region of Mbnl2 cDNA was cloned to pcDNA3.1 (+) expression vector (Invitrogen), which places the cDNA under the control of the cytomegalovirus promoter. PCR for the cloning of Mbnl2 cDNA coding region was done using high fidelity Pfu DNA polymerase (Stratagene, La Jolla, CA). Mbnl2 primers used are as follows: 5'-AAAGAATTCATGGCCTTGAACGTTGCC-3', 5'-AAACTCGAGTTAGCATGCAG TTTGTGGCAAT-3'. Cloned pcDNA3.1(+)-Mbnl2 DNA was confirmed with sequencing and was transfected to HEK293 cells using Lopofectamine 2000 (Invitrogen) according to manufacture's instructions. Cells were transfected overnight in Opi-MEM I and 10% fetal bovine serum. The next day, the media was replaced by complete DMEM media. After further 48 hr incubation, transfected cells were collected for the western blot assay.