

Nucleotide and derived amino-acid sequences of the CRE-binding proteins from rat C6 glioma and HeLa cells

Marc L.Short, Chitra F.Manohar¹, Manohar R.Furtado², Ghanashyam D.Ghadge¹, Steven M.Wolinsky², Bayar Thimmapaya¹ and Richard A.Jungmann*

Departments of CMS Biology, ¹Microbiology/Immunology and ²Medicine/Infectious Disease Section, Northwestern University Medical School and Cancer Center, Chicago, IL 60611, USA

Submitted June 28, 1991

EMBL accession nos X60002, X60003

The second messenger, cyclic AMP, regulates the expression of a number of mammalian genes through the interaction of DNA-binding proteins with a conserved promoter sequence, the cyclic AMP-responsive element 5'-TGA CGT CA-3' (CRE) (1). Cloning studies of the cDNAs encoding the DNA-binding proteins have identified a complex family of transcriptional *trans*-regulatory proteins exhibiting marked sequence similarities.

We have isolated and sequenced two cDNAs coding for CRE-binding proteins from rat C6 glioma cells and HeLa cells. Both CRE-binding proteins were isolated by polymerase chain reaction (PCR) from rat C6 glioma cell and HeLa cell poly(A)⁺RNA respectively, using primers derived from the base sequence of PC12 cell CREB cDNA (2) which contained an additional EcoRI site at the 5' and a HindIII site at the 3' end. The resulting PCR product was ligated into M13mp18 and M13mp19 (3) and single-stranded DNA was sequenced by the dideoxy chain termination method using Sequenase™ (USB Inc.), or with the Applied Biosystems 370A DNA Sequencer.

The rat C6 glioma cell cDNA (EMBL accession no. X60002) as well as the HeLa cell cDNA (EMBL accession no. X60003) contain an open reading frame of 981 nucleotides having a capacity to encode for a 327-amino acid polypeptide with a calculated molecular size of 35 kDa. This differs from the relative molecular size of 47 kDa experimentally determined for both peptides by Southwestern blotting analysis. Posttranslational modifications may account for the discrepancy between the calculated and experimentally determined values. The rat C6 glioma and HeLa cell CRE-binding proteins exhibit a 94% sequence similarity at the nucleotide and 97% sequence similarity at the amino acid level. The carboxy-terminal regions of the predicted polypeptides contain a heptad repeat of 4 leucines, spaced seven residues apart, characteristic of the leucine zipper motif (4). Adjacent to the leucine zipper motif on the amino-terminal side, a region of approximately 30 amino acids is identified containing a number of basic amino acids. This structural feature is characteristic of the leucine zipper class of proteins and represents a potential DNA-binding domain (5).

Several putative sites of phosphorylative modification by cAMP-dependent protein kinase (ser 119), protein kinase C (ser 107), and casein kinase (ser 142) can be recognized.

Comparison of PC12 cell CREB cDNA (2) and human placental CREB cDNA (6) with rat C6 glioma and HeLa cell CRE-binding protein cDNA reveal extensive sequence similarities. The corresponding polypeptides are highly conserved in their leucine zipper motif, the basic region and putative phosphorylation sites. The majority of sequence changes reside in the amino-terminal region of the peptides. Additionally, rat C6 glioma, HeLa cell as well as human placenta (6) CRE-binding proteins lack a 14 amino acid sequence, termed α CREB transactivating sequence (7), which is present in the amino terminal domain of PC12 cell CREB (2). On the basis of the differences in cDNA sequence and apparent differences in molecular size, we conclude that rat C6 glioma and HeLa cell 47-kDa CRE-binding proteins are part of a larger family of CRE-binding transcriptional modulators.

ACKNOWLEDGEMENTS

Supported by NIH grants GM23895 (to R.A.J.), A120156 (to B.T.) and A1-32535 (to S.M.W.).

REFERENCES

1. Habener, J.F. (1990) *Molecular Endocrinol.* **4**, 1087-1094.
2. Gonzales, G.A., Yamamoto, K.K., Fischer, W.H., Karr, D., Menzel, P., Biggs, W., III, Vale, W.W. and Montminy, M.R. (1989) *Nature* **337**, 749-752.
3. Messing, J., Gronenborn, B., Muller-Hill, B. and Hofschneider, P.H. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3646-3652.
4. Landschulz, W.H., Johnson, P.F. and McKnight, S.L. (1989) *Science* **243**, 1681-1688.
5. Turner, R. and Tjian, R. (1989) *Science* **243**, 1688-1694.
6. Hoeffler, J.P., Meyer, T.E., Yun, Y., Jameson, J.L. and Habener, J.F. (1988) *Science* **242**, 1430-1433.
7. Yamamoto, K.K., Gonzales, G.A., Menzel, P., Rivier, J. and Montminy, M.R. (1990) *Cell* **60**, 611-617.

* To whom correspondence should be addressed