Human M1 subunit of ribonucleotide reductase: cDNA sequence and expression in stimulated lymphocytes

Nigel J.Parker[1], C.Glenn Begley[1, 2] and Richard M.Fox[1]
[1]Department of Haematology and Medical Oncology and [2]Walter and Eliza Hall Institute, Royal Melbourne Hospital, Parkville 3050, Australia

Submitted May 29, 1991 EMBL accession no. X59543

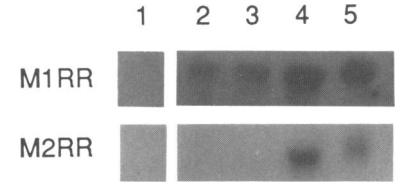
Ribonucleotide reductase (RR) is essential for de novo synthesis of DNA precursors by catalysing the reduction of ribonucleotides. Mammalian RR requires two nonidentical subunits for activity; M1 and M2. The M1 subunit (M1RR) is responsible for allosteric regulation of RR through nucleotide binding sites (1). M1RR protein levels do not vary during the cell cycle in cycling cells (2, 3), although it is absent from quiescent cells, and appears after stimulation (3). A murine (Mu) M1RR cDNA has been previously described (4). Using a 1375 bp BamHI fragment of this cDNA, a human (Hu) bone marrow cDNA library (5) was screened. Two clones were obtained, each was 1.1 kb. They were sequenced in both directions, found to overlap and encode a total of 1.4 kb. A Hu 335 bp BglII fragment was used to screen a Hu hepatoma cDNA library (HepG2 cells, Stratagene). Eight independent clones were obtained and sequenced, these encoded 3.0 kb. In order to define the 5′ end of the mRNA, we employed a polymerase chain reaction (PCR)-based technique (6). The method was followed as described using 10 μg of total RNA from CCRF-CEM cells. Eight clones were obtained and the degree of 5′ extension was found to vary between clones. The composite cDNA sequence is 3075 bp, the first 19 nucleotides (nt) were obtained from a single PCR clone. The first in-frame ATG is at 188 bp and the open reading frame continues for a further 2376 bp. A polymorphism was found at nt 1037, a C or A, both resultant codons encode an arginine. Comparison of the Hu and Mu nt sequences revealed a very high degree of homology, especially in the predicted coding region (90%). The predicted Hu protein is 792 aa and shares 97% homology with the Mu protein. The requirement for this gene for growth is supported by the high degree of homology between the Hu and Mu proteins. To examine Hu M1RR and M2RR mRNA expression, peripheral blood lymphocytes, from normal individuals, were stimulated with 5 μg/ml phytohaemagglutinin (PHA). Aliquots of cells were taken and total RNA isolated, DNA flow cytometry was also performed to determine cell cycle position. The figure shows typical results from experiments performed on the blood of several individuals. It can be seen that (i) the Hu M1RR cDNA detects an RNA species which is proliferation-dependently expressed and (ii) expression of M1RR and M2RR mRNAs is non-coordinate. The latter result contrasts the observations of Björklund *et al.* (7), in that the two mRNA species were similarly expressed in serum deprived Mu cells. Different regulation under these conditions may explain the different expression of Hu and Mu M1RR mRNA.

ACKNOWLEDGEMENTS

Mu RR cDNA fragments were obtained from L.Thelander, University of Umeå, Sweden. This work is supported by grants from the National Health and Medical Research Council (Australia) and the Anti-Cancer Council of Victoria.

REFERENCES

1. Thelander,L., Eriksson,S. and Åkerman,M. (1980) *J. Biol. Chem.* **255**, 7426–7432.
2. Engström,Y., Eriksson,S., Jildevik,I., Skog,S., Thelander,L. and Tribukait,B. (1985) *J. Biol. Chem.* **260**, 9114–9116.
3. Mann,G.J., Musgrove,E.A., Fox,R.M. and Thelander,L. (1988) *Cancer Res.* **48**, 5151–5156.
4. Caras,I.W., Levinson,B.B., Fabry,M., Williams,S.R. and Martin,D.W.,Jr. (1985) *J. Biol. Chem.* **260**, 7015–7022.
5. Begley,C.G., Aplan,P.D., Denning,S.M., HaynesB.F., Waldmann,T.A. and Kirsch,I.R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 10128–10132.
6. Frohman,M.A., Dush,M.K. and Martin,G.R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8998–9002.
7. Björklund,S., Skog,S., Tribukait,B. and Thelander,L. (1990) *Biochemistry* **29**, 5452–5458.



Northern analysis of PHA-stimulated lymphocytes. Lane 1, 0 hrs after stimulation; lane 2, 12 hrs; lane 3, 24 hrs; lane 4, 48 hrs; lane 5, 72 hrs. Ten μg of total RNA was loaded per lane, the filter was hybridized with a 2 kb BamHI fragment of Hu M1RR cDNA, and with Mu M2RR cDNA. The M2RR band shown is 3.2 kb, the 1.8 kb mRNA is similarly regulated. S-phase cells were detectable 48 hrs after stimulation, and by 72 hrs, 40% of cells were in S-phase.