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Short sequence-paper

Cloning, sequencing and expression in MEL cells of a cDNA encoding the mouse ribosomal protein S5 [1]

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Abstract

We describe the isolation and characterization of a cDNA encoding the mouse S5 ribosomal protein. It was isolated from a MEL (murine erythroleukemia) cell cDNA library by differential hybridization as a down regulated sequence during HMBA-induced differentiation. Northern series analysis showed that S5 mRNA expression is reduced 5-fold throughout the differentiation process. The mouse S5 mRNA is 760 bp long and encodes for a 204 amino acid protein with 94% homology with the human and rat S5.

Keywords: S5; Ribosomal protein; Murine erythroleukemia cell; Hexamethylene-bis-acetamide; Differential hybridization; (Mouse)

Murine erytrholeukemia (MEL) cells provide a useful model for studying differentiation in the erythroid lineage. When exposed to a number of chemical agents such as hexamethylene-bis-acetamide (HMBA), MEL cells are induced to enter a differentiation program that resembles the final stages of erythropoiesis [1]. Changes in the expression of several genes, including protooncogenes and histone-variants, have been described as early events that may influence commitment of MEL cell differentiation [2–4]. In an effort to isolate other genes whose expression changes in the first hours of induction, we differentially screened cDNA libraries constructed from HMBA-treated MEL cells. Cell culture, construction of cDNA libraries and differential screening were conducted as previously described [3] with only some minor changes. A Lambda Zap cDNA library was made with polyA$^+$ RNA extracted from MEL-DS 19 cells treated for 8 h with 5 mM HMBA. Duplicate filters were hybridized with ^{32}P-labeled cDNA probes synthesized from uninduced and 8-h induced mRNAs. Positive clones were excised into the pBluescript phagemid using the ExAssist/SOLR System (Stratagene) and subcloned into the *Kpn* I–*Eco* RI sites of pUC18. The cDNA inserts were sequenced from both strands in an ABI-377 automatic sequencer using FS *Taq* polymerase and dye terminators by the dideoxy chain-termination method [5]. Data base searches were performed with the Wisconsin Genetics Computer Group (GCG) software running on a VAX DEC3500/S AXP.

Among the clones that showed a differential hybridization signal after a first screening, one, designated MEL-Pf17, was initially isolated as a down-

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[1] The nucleotide sequence reported in this paper has been submitted to the GenBank/EMBL Data Bank under the accession number U78085.

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regulated gene. The size of the insert was approximately 760 bp. Sequence analysis comparison with the EMBL and Genbank data bases revealed an homology at the nucleotide level of 93 and 88% with the rat and human ribosomal protein S5, respectively [6,7]. The predicted amino acid sequence was 97% homologous to the rat and human protein S5. We concluded we had cloned the sequence encoding for the mouse S5 ribosomal protein. The nucleotide sequence reported in this paper has been submitted to the GenBank/EMBL with accession number U78085.

Fig. 1 shows the complete nucleotide sequence of the cDNA insert and its deduced amino acid sequence. Mouse ribosomal protein S5 has a coding region of 612 bp. The open reading frame begins at an ATG codon which occurs in the context AGGATGA, and ends with a termination codon TGA at position 613–615. A canonical polyadenylation signal AATAAA was found at nucleotides 633–638 (underlined in Fig. 1), 22 nucleotides upstream of a poly(A) tail. The encoded protein is 204 amino acids long and differs from the human and rat S5 by 4 amino acids replacements (Fig. 2). In both human and rat, a threonine at position 6 is replaced by an alanine, and an asparagine at position 59 is replaced by a lysine in the mouse protein. In addition, there are two substitutions in the human S5 at positions 8 and 60 resulting in an alanine residue instead of threonine and alanine instead of arginine. Compared to rat, a glycine at position 60 is replaced by an arginine, and an arginine at position 181 by an alanine in the mouse protein.

To investigate the distribution of the mouse S5 message through MEL cell differentiation we carried out Northern blot analysis from HMBA-treated cultures. Induction of cell differentiation was monitored by the benzidine staining reaction and by the presence of mRNA globin at later stages [3]. Total RNA was isolated by the Ultraspec RNA isolation system (Biotecx) following the manufacturer instructions. 20

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                                -60                             -30
                               TCGGCACGAGGACGGCGCGTGGTCCACGCCGAGCGACTGAGAAGCCCA
                    +1
GTCTGCGCCCTCAGG ATG ACT GAG TGG GAA GCA GCC ACA CCA GCG GTG GCA GAG ACC CCT  45
                Met Thr Glu Trp Glu Ala Ala Thr Pro Ala Val Ala Glu Thr Pro  15

GAC ATC AAG CTC TTT GGG AAA TGG AGC ACT GAT GAC GTG CAG ATC AAC GAT ATT TCT  102
Asp Ile Lys Leu Phe Gly Lys Trp Ser Thr Asp Asp Val Gln Ile Asn Asp Ile Ser  34

CTG CAG GAT TAC ATT GCT GTG AAG GAG AAG TAT GCC AAG TAC CTG CCC CAC AGT GCC  159
Leu Gln Asp Tyr Ile Ala Val Lys Glu Lys Tyr Ala Lys Tyr Leu Pro His Ser Ala  53

GGA CGG TAT GCT GCC AAG CGC TTC CGC AAA GCA CAA TGT CCC ATC GTG GAG CGC CTT  216
Gly Arg Tyr Ala Ala Lys Arg Phe Arg Lys Ala Gln Cys Pro Ile Val Glu Arg Leu  72

ACT AAC TCC ATG ATG ATG CAT GGT CGT AAC AAC GGC AAG AAG CTC ATG ACT GTG CGA  273
Thr Asn Ser Met Met Met His Gly Arg Asn Asn Gly Lys Lys Leu Met Thr Val Arg  91

ATT GTC AAG CAT GCC TTT GAG ATC ATC CAC CTG CTC ACT GGT GAG AAC CCT CTG CAG  330
Ile Val Lys His Ala Phe Glu Ile Ile His Leu Leu Thr Gly Glu Asn Pro Leu Gln  110

GTC CTG GTG AAT GCT ATC ATC AAC AGT GGC CCC CGA GAA GAC TCA ACA CGC ATT GGG  387
Val Leu Val Asn Ala Ile Ile Asn Ser Gly Pro Arg Glu Asp Ser Thr Arg Ile Gly  129

CGG GCC GGT ACA GTG AGA CGA CAG GCT GTG GAT GTG TCC CCA CTG CGT CGA GTG AAT  444
Arg Ala Gly Thr Val Arg Arg Gln Ala Val Asp Val Ser Pro Leu Arg Arg Val Asn  148

CAG GCC ATC TGG CTG CTG TGC ACA GGG GCT CGT GAG GCT GCT TTC CGG AAC ATC AAG  501
Gln Ala Ile Trp Leu Leu Cys Thr Gly Ala Arg Glu Ala Ala Phe Arg Asn Ile Lys  167

ACC ATC GCC GAG TGC CTT GCA GAT GAG CTC ATT AAT GCT GCC AAG GGC TCC TCC AAT  558
Thr Ile Ala Glu Cys Leu Ala Asp Glu Leu Ile Asn Ala Ala Lys Gly Ser Ser Asn  186

TCC TAT GCC ATC AAG AAG AAA GAT GAA CTG GAG CGT GTG GCC AAG TCT AAC CGC TGA  615
Ser Tyr Ala Ile Lys Lys Lys Asp Glu Leu Glu Arg Val Ala Lys Ser Asn Arg ***  205

TTTCCCAGCTGCTGCCTAATAAACTGTGTCCTTTGGAACAACTATAAAAAAAAAAAAAAAAAAACTCGAGGGGGGG

CCCGGTAC
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Fig. 1. Nucleotide and deduced amino acid sequence of mouse S5 ribosomal protein. The coding region begins at position + 1. The stop codon for translation is designated by asterisks (*) and the polyadenylation signal AATAAA at position 633–638 is underlined. The sequence data has been deposited in the GenBank/EMBL under accession number U78085.

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S5human MTEWETAAPAVAETPDIKLFGKWSTDDVQINDISLQDYIAVKEKYAKYLP  50
  S5rat MTEWETATPAVAETPDIKLFGKWSTDDVQINDISLQDYIAVKEKYAKYLP  50
S5mouse MTEWEAATPAVAETPDIKLFGKWSTDDVQINDISLQDYIAVKEKYAKYLP  50

S5human HSAGRYAANAFRKAQCPIVERLTNSMMMHGRNNGKKLMTVRIVKHAFEII 100
  S5rat HSAGRYAANGFRKAQCPIVERLTNSMMMHGRNNGKKLMTVRIVKHAFEII 100
S5mouse HSAGRYAAKRFRKAQCPIVERLTNSMMMHGRNNGKKLMTVRIVKHAFEII 100

S5human HLLTGENPLQVLVNAIINSGPREDSTRIGRAGTVRRQAVDVSPLRRVNQA 150
  S5rat HLLTGENPLQVLVNAIINSGPREDSTRIGRAGTVRRQAVDVSPLRRVNQA 150
S5mouse HLLTGENPLQVLVNAIINSGPREDSTRIGRAGTVRRQAVDVSPLRRVNQA 150

S5human IWLLCTGAREAAFRNIKTIAECLADELINAAKGSSNSYAIKKKDELERVA 200
  S5rat IWLLCTGAREAAFRNIKTIAECLADELINARKGSSNSYAIKKKDELERVA 200
S5mouse IWLLCTGAREAAFRNIKTIAECLADELINAAKGSSNSYAIKKKDELERVA 200

S5human KSNR- 204
  S5rat KSNR- 204
S5mouse KSNR- 204
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Fig. 2. Alignment of the S5 ribosomal protein with the human (U14970) and rat (X58465) sequences. The alignment was created using GCG's pileup command (gap creation penalty = 30, gap extension penalty = 0.1) and displayed using the prettyplot command. The mouse protein is 97% identical to the human and rat sequences with only 4 amino acid changes (see text).

μg of total RNA from each time-point was electrophoretically separated on 1.2% agarose-formaldehyde gels and transferred to nylon membranes (Zetaprobe, BioRad). Hybridization was performed as previously described [3] using the ^{32}P labeled cDNA insert as a probe. Figs. 3 and 4 show that S5 mRNA is reduced 5-fold throughout differentiation. This reduction occurs gradually along the process with slightly pronounced drops between 2 and 8 h, and after 48 h of treatment. During induced MEL differentiation, as well as in normal erythropoiesis, cells gradually cease proliferating [12]. The down regulation of S5 mouse mRNA is probably a consequence

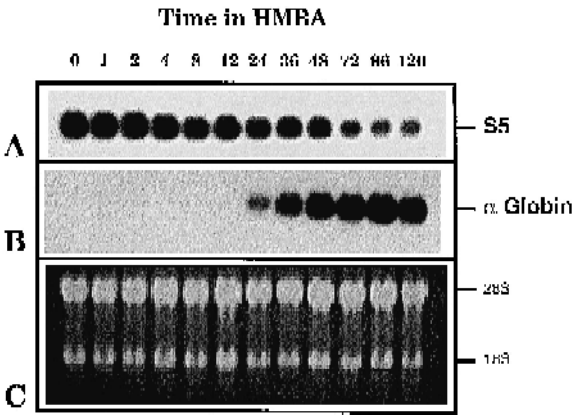

Fig. 3. Northern blot analysis of mouse S5 mRNA expression in differentiating MEL cells. The cells were exposed to 5 mM HMBA for the times indicated (in hours) above each lane. The percentage of benzidine-positive cells after 120 hr in these experiments was 92%. Twenty μg of total RNA from each time point was extracted by the Ultraspec RNA isolation system (Biotecx), separated onto a 1.2% agarose-formaldehyde gel and transferred to nylon membranes (Zetaprobe, BioRad). Northern filters were hybridized with ^{32}P-labeled probes from: (A) The full-length cDNA sequence of the S5 ribosomal protein, (B) A 0.7 kb cDNA fragment of the mouse α-globin [3]. (C) Ethidium bromide stained agarose gel (used in A) showing the relative loading of RNA samples. A similar loading was used for B (data not shown).

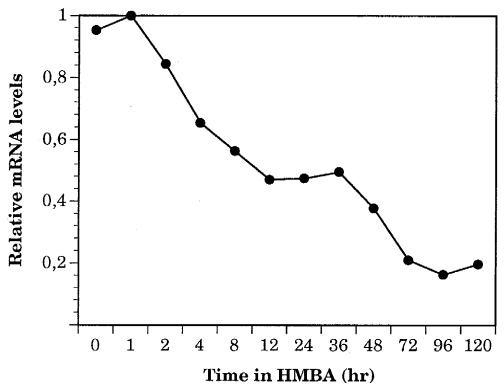

Fig. 4. Relative levels of S5 mRNA in differentiating MEL cells. The relative concentrations of S5 mRNA throughout MEL differentiation was determined by densitometry from the autoradiograms of the Northern (Fig. 3A), standardized to the 18S ribosomal RNA (Fig. 3C). The mRNA/rRNA was calculated for each lane and the values, normalized to 1, were plotted against time.

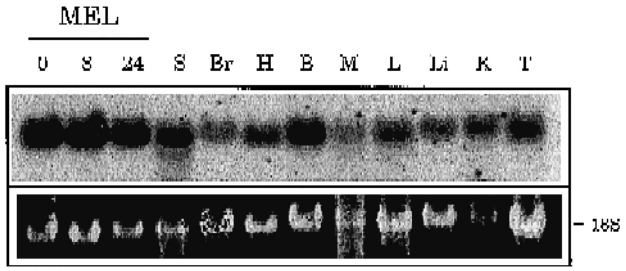

Fig. 5. Northern blot analysis of tissue expression of the mouse S5 ribosomal protein gene. RNA samples and hybridization were performed as described in Fig. 3. Total RNA samples from MEL cells untreated or treated with 5 mM HMBA for 8 and 24 h were included as comparison for mRNA expression and size of the transcript. S = spleen; Br = brain; H = heart; B = bone marrow; M = muscle; L = lung; Li = liver; K = kidney; T = testis.

of the cessation of cell division. Down regulation of some ribosomal proteins has been described also in HL-60 cells induced-differentiation [8], in myoblast and intestinal differentiation [9,10], and during apoptosis of leukaemic cells [11]. It is likely that the reduction observed in the mRNA of ribosomal proteins during these processes also follows the cessation of cell division.

Northern blot analysis of total RNA from various murine tissues showed that S5 cDNA hybridizes to the 760 bp mRNA in all cases (Fig. 5). A high level of expression is particularly observed in kidney.

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References

[1] P. Marks, R. Rifkind, Ann. Rev. Biochem. 47 (1978) 419–448.
[2] G. Cheng, A. Skoultchi, Mol. Cell. Biol. 9 (1989) 2332–2340.
[3] D. Krimer, G. Cheng, A. Skoultchi, Nucleic Acids Res. 21 (1993) 2873–2879.
[4] R. Ramsay, K. Ikeda, R. Rifkind, P. Marks, Proc. Natl. Acad. Sci. USA 83 (1986) 6849–6853.
[5] F. Sanger, S. Nicklen, A. Coulson, Proc. Natl. Acad. Sci. USA 74 (1977) 5463–5467.
[6] Y. Kuwano, J. Olvera, I. Wool, J. Biol. Chem. 267 (1992) 25304–25308.
[7] J.-M. Frigerio, J.-C. Dagorn, J. Iovanna, BBA 1262 (1995) 64–68.
[8] R. Mailhammer, H. Szöts, J. Bönisch, P. Dörmer, Exptl. Cell Res. 200 (1992) 145–148.
[9] Y. Maheshwari, M. Rao, D. Sykes, A. Tyner, M. Weiser, Cell Growth Differ 4 (1993) 745–752.
[10] M. Agrawal, L. Bowman, J Biol Chem 262 (1987) 4868–4875.
[11] S. Goldstone, M. Lavin, BBRC 196 (1993) 619–623.
[12] J. Gusella, R. Geller, B. Clarke, V. Weeks, D. Housman, Cell 9 (1976) 221–229.