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# Characterisation of a new splice variant of *MASK-BP3*$^{ARF}$ and *MASK* human genes, and their expression patterns during haematopoietic cell differentiation

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**Abstract**

In this study we report the characterisation of a new splice variant, here denominated splice variant 4 (accession number AF258557) of the human Multiple Ankyrin repeats Single KH domain (hMASK) (accession number AF521882) and the hMASK-4E-Binding Protein 3 Alternative Reading Frame (hMASK-BP3$^{ARF}$) (accession number AF521883), containing a number of ANK-repeat motifs. Ankyrin (ANK) repeat-containing proteins carry out a wide variety of biological activities and are involved in processes, such as cell differentiation and transcriptional regulation. The present study reports the computer analysis of these splice variant cDNAs and their broad mRNA expression in different normal human tissues and cancer cell lines. An upregulation of the splice variant mRNAs expression was observed after HL-60 and erythroblast differentiation. The upregulation of *splice variant 4* mRNA was considerably higher than those of the other variants, during erythroid differentiation.


*Keywords:* Ankyrin repeat domain; Alternative splicing; Haematopoietic cell differentiation

## 1. Introduction

MASK protein (Multiple Ankyrin repeats Single KH domain) was first described in *Drosophila melanogaster* and was shown to be crucial for photoreceptor differentiation, cell survival and proliferation (Smith et al., 2002). The Ankyrin (ANK)-repeat is one of the most common protein sequence motifs, which leads to its own variation in overall domain size by simple sequence duplication or deletion. Thus, ANK-repeat proteins do not bind selectively to a single class of protein target. Rather, the diversity of biological roles of ANK-repeat proteins is paralleled by the diversity of unrelated proteins with which they interact (Sedgwick and Smerdon, 1999). Functions of these proteins vary widely and include, for example, proteins that control cell differentiation and transcriptional factors (Michaely and Bennett, 1993). The K homology (KH) domain, which is present in hMASK and hMASK-BP3$^{ARF}$, was first identified in the human heterogeneous nuclear ribonucleoprotein K. This domain possesses approximately 70 amino acids and is present in a wide variety of fairly diverse nucleic acid-binding proteins (Gibson et al., 1993; Siomi et al., 1993). The function of the KH domain in hMASK-BP3$^{ARF}$ and hMASK is still uncertain.

Herein, we report the characterisation of a new splice variant, denominated splice variant 4 (accession number AF258557) of

*Abbreviations:* ANK, ankyrin; KH, K homology domain; ATRA, all-*trans*-retinoic acid; EST, expressed sequence tag; NCBI, National Center for Biotechnology Information; BLAST, basic local alignment search tool; hMASK, human Multiple Ankyrin repeats Single KH domain; hMASK-BP3$^{ARF}$, human Multiple Ankyrin repeats Single KH domain-4E-BP3 Alternative Reading Frame; EIF4EBP3, Eukaryotic Initiation Factor 4E-Binding-Protein 3; 4E-BP3, 4E-Binding Protein 3; RACE, Rapid amplification of cDNA ends; RTK, receptor tyrosine kinase; MAPK, Mitogen-Activated Protein Kinase.

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hMASK (accession number AF521882) and hMASK-BP3$^{ARF}$ (accession number AF521883) (Poulin et al., 2003), containing a number of ANK-repeat motifs. The present study reports the computer analysis of these splice variant cDNAs and their expression in different normal human tissues and cancer cell lines. Furthermore, we report the mRNA expression of these splice variants during haematopoietic cell differentiation.

## 2. Materials and methods

### 2.1. Computational analysis

In order to identify new potential cytoskeletal proteins as a part of our functional genomics project, we searched the ORESTES database (available at http://www.ludwig.com.br) using keyword search against all expressed sequence tags (ESTs) classified as potential paralogs. Among other ESTs, *RC3-CT0255-200100-024-c05* (GenBank Accession No. AW854359) was selected and further analysed through similarity searches against different databases available at the National Center for Biotechnology Information (NCBI), using the BLAST search algorithm (available at http://www.ncbi.nlm.nih.gov/BLAST). Prosite database searches were performed at http://www.expasy.cbr.nrc.ca/tools, using the InterProScan search tool. We also used the sim4 software (downloaded from http://globin.cse.psu.edu) to compare all splice variant cDNAs with their genomic DNA regions to obtain their exon–intron structures. Internal donor sites were predicted by NetGene2 Server (www.cbs.dtu.dk/services/NetGene2). Polyadenylation signals were predicted by the software program *Polyadq* (Tabaska and Zhang, 1999) (http://rulai.cshl.org/tools/polyadq/polyadq_form.html) and *Erpin* (Legendre and Gautheret, 2003) (http://tagc.univ-mrs.fr/erpin/).

### 2.2. Subjects

Mononuclear cells from peripheral blood and bone marrow of normal controls were obtained by Ficoll–Hypaque gradient separation for RNA extraction. Human tissues were obtained from autopsy. The National Ethical Committee has approved the study.

### 2.3. Cell lines

Human cancer cell lines were obtained from ATCC, Philadelphia, PA.

### 2.4. PCR and sequencing

The in silico inclusion of new sequences to the transcript of splice variant 4 was confirmed experimentally using PCR of K562 cell line cDNA. The cDNA was obtained by reverse transcription of K562 cell line poly(A)+ mRNA using Superscript II (Invitrogen Life technologies). Primers were designed using the GeneRunner software (available at http://www.generunner.com) and their sequences are available upon request. A schematic representation of the location of the primers in relation to the transcript sequence is depicted in Fig. 1A. Amplified products (Fig. 1B) were purified and sequenced in an ABI310 prism automated sequencer. The chromatograms obtained were assembled using the Phred/Phrap/Consed software package (http://www.phrap.org).

### 2.5. Rapid amplification of cDNA ends (RACE)

The 3′ RACE was performed according to the 3′ RACE System (Life Technologies) protocol. In this case reverse transcription was carried out using the 3′ RACE adapter primer

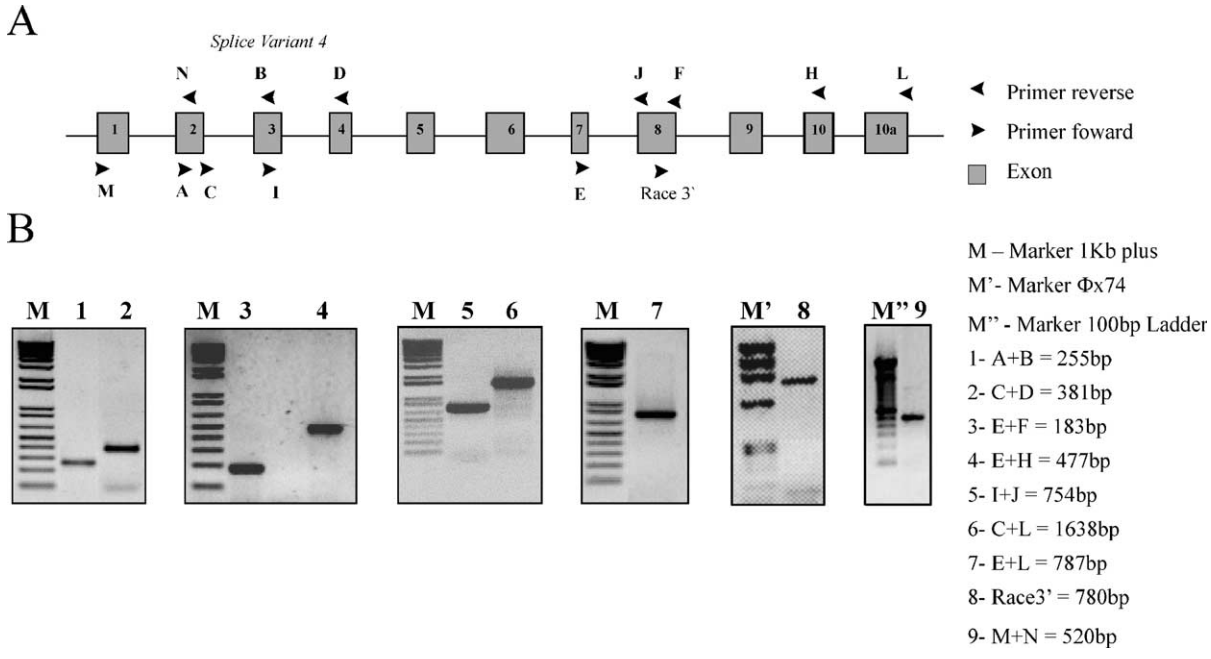


Fig. 1. (A) Schematic representation of the set of primers designed to confirm the splice variant 4 cDNA sequence and to obtain the 3′ end sequences by RACE. Letters were used to designate the primers. (B) Amplified products obtained from the combination of the set of primers described above.