ONCOLOGY REPORTS 24: 1133-1139, 2010

SPANDIDOS PUBLICATIONS

Characterization of an alternative transcript of the human *CREB3L2* gene

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Received May 7, 2010; Accepted July 2, 2010

DOI: 10.3892/or_00000964

Abstract. *CREB3L2*, a member of the CREB3 family of transcription factors, spans >120 kbp and is composed of 12 exons. We characterized a widely expressed transcript of *CREB3L2* generated by an intronic polyadenylation site in intron 4 of the gene. It could be translated to a CREB3L2 variant which is localized both in the nucleus and the endoplasmatic reticulum. The protein retains the N-terminal transactivation domain but lacks the DNA-binding domain, the transmembrane domain and the C-terminal part. Experiments using a GAL4 DNA-binding domain fusion model showed that the transcript is a transactivator but it cannot exert its function through the CRE and ATF6 binding sites and has little effect on the *GRP78* promoter. Whether this transcript has a cellular function or is targeted for degradation by nonsense-mediated RNA decay system of RNA surveillance is currently unknown.

Introduction

The *CREB3L2* gene was first identified as the partner of *FUS* in a chimeric gene found in low grade fibromyxoid sarcoma (LGFMS) (1-3). *CREB3L2* spans >120 kbp, is composed of 12 exons and is expressed in most human tissues (3). The CREB3L2 protein is a member of the CREB3 family of transcription factors and contains an N-terminal transactivation part, a basic DNA-binding domain, a leucine zipper region, an α-helical transmembrane hydrophobic region and a luminal domain (4,5). The full length CREB3L2 protein is an endoplasmatic reticulum (ER)-resident transmembrane protein and is cleaved at the membrane in response to ER stress. In the luminal segment, CREB3L2 contains the sequence RNLL, which fits the RxxL consensus for S1P, a membrane-anchored serine protease in the Golgi lumen, suggesting that CREB3L2 is cleaved by regulated intramembrane proteolysis (RIP), similarly to the other members of the family (4). The cleaved fragment of the protein, containing the N-terminal transactivation domain, the basic DNA-binding domain and the leucine zipper region translocate into the nucleus. There, it binds directly to the CRE site and activates the transcription of CREB3L2-target genes (4). CREB3L2 was shown to be induced at the translational level during ER stress, suggesting that it might contribute to only the late phase of unfolded protein response signalling (4).

The information related to the physiological role of *CREB3L2* is limited. *CREB3L2* was down-regulated in human lymphocytes after *in vitro* irradiation with low doses of gamma rays (6), the homologue *Creb3l2* gene was activated in the notochord of *Xenopus laevis* embryos (7) and chronic ethanol consumption altered the expression of *Creb3l2* in the brains of adult male rats (8). Recently, Saito *et al* (9) showed that *Creb3l2* (-/-) mice had severe chondrodysplasia and died by suffocation shortly after birth because of an immature chest cavity. The *Sec23a* gene, which encodes a coat protein complex II component responsible for protein transport from the ER to the Golgi, was found as a target of *Creb3l2*.

In LGFMS, the N-terminal part of CREB3L2 is replaced by the N-terminal part of FUS, which displays transactivating and oncogenic properties (3). Thus, FUS/CREB3L2 may act as an abnormal transcription factor that undergoes intramembranous proteolysis allowing the N-terminal FUS domain with the B-ZIP domain of CREB3L2 to enter the nucleus and to activate/deregulate a number of target genes. In follicular thyroid carcinoma, however, the N-terminal transactivation part of CREB3L2 (5) is fused to all functional domains of the PPARG nuclear receptor (10) and the receptor comes under the control of the *CREB3L2* promoter.

In the present study, we report the characterization of an alternative transcript from the *CREB3L2* gene generated by an intronic polyadenylation site in intron 4 of *CREB3L2*.

Materials and methods

Vectors. The cDNA clone IMAGE:4185677 (accession no. BC063666) was purchased from Invitrogen (Carlsbad, CA) and was used as template for subsequent cloning experiments. PCR amplifications, ligations and additional auxiliary methods have been described in detail previously (5).

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Key words: *CREB3L2* gene, transcription factor, truncated, transactivator, low grade fibromyxoid sarcoma, intronic polyadenylation

The pBIND vector of the CheckMate Mammalian Two-Hybrid System (Promega, Madison, WI) was used to construct products encoding fusions with the GAL4 DNA-binding domain. This vector expresses the *Renilla reniformis* luciferase, which allows the user to normalize the transfection efficiency. The cDNA fragment was amplified using BBF2H7prim1F (5'-CGGGATCCGTATGGAGGTGCTGGAGAGCG-3') and BBF2bc6-1058R (5'-GTAACGCGTTTTGGAAGCAAAAT TTTTATTTTTTA-3') primers and was inserted in-frame between *Bam*HI and *Mlu*I restriction sites. The pBIND vectors with inserts coding for the full length CREB3L2 protein and the form of CREB3L2 corresponding to amino acids 1-372 (CREB3L2ΔTM) have been described elsewhere (5). All constructs had 7 additional amino acids (PEFPGIR), which were encoded by the vector polylinker, between the GAL4 DNA-binding domain and the coding sequence of the insert. For experiments concerning transcription activation through various DNA-binding sites, the GAL4 DNA-binding domain was removed by double digestion by *Nhe*I and *Bam*HI, after which the plasmids were filled in with T4 DNA polymerase and self-ligated.

The plasmid p5xATF6GL3 was kindly provided by Dr R. Prywers (Columbia University, New York). It contains five repeats of the ATF6 binding sites and the FOS minimal promoter upstream of the luciferase gene (11). The plasmids pGL3-GRP78 and pGL3-CRE have been described elsewhere (5). In the pGL3-GRP78 construct, a 371-bp long fragment from the promoter region of the *GRP78* gene (nt 6-376 of the sequence with accession no. X59969) drives the expression of luciferase activity. In the pGL3-CRE vector, four copies of the sequence AGCCTGACGTCAGAG have been cloned upstream of an SV40 promoter which drives the expression of the luciferase gene.

For experiments concerning subcellular localization, the cDNA fragment was amplified from the clone IMAGE: 4185677 using the primer set BBF2-347F-*Eco*RI (5'-TCGAA TTCTGCAATGGAGGTGCTGGAGAGC -3') and BBF2bc-1058R*Bam*HI (5'-CGGTGGATCCCGTTTGGAAGCAAAA TTTTTATTTTTTA-3') and was subsequently cloned between the *Eco*RI and *Bam*HI sites of a pEGFP-N1 vector (Clontech, Mountain View, CA), in frame with the cDNA coding for enhanced green fluorescence protein (EGFP). The pEGFP-CREB3L2 and pEGFP-CREB3L2ΔTM vectors have been described previously (5). The pDsRed2-ER vector, designed for fluorescent labeling of the ER in living cells, was obtained from Clontech. In this vector the ER targeting sequence of calreticulin is fused in frame to the 5'-end of *Discosoma sp.* red fluorescent protein (DsRed2), and the ER retention sequence KDEL is fused to the 3'-end of DsRed2.

Cell lines and transfection experiments. The human cervix carcinoma cell line HELA (obtained from the German Collection of Microorganisms and Cell Cultures, DSMZ, Braunschweig, Germany, ACC57) was used for transfection experiments. The cells were cultured in RPMI-1640 medium, supplemented with fetal bovine serum (10%), 2 mM L-glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin. For transfections, 5,000 cells (in 100 μl medium) were seeded in 96-well plates, and 24 h later they were transfected using the FuGene HD Transfection Reagent: DNA ratio 3:2 according to the company's recommendations (Roche Diagnostics Scandinavia, Stockholm, Sweden). To study the transcriptional activity 400 ng of pBIND plasmid DNA (empty or with the various inserts) together with 1,600 ng of the pG5*luc* (Promega) vector were used. For experiments concerning transcription activation through various DNA-binding sites 400 ng of pBIND plasmid DNA without the GAL4 DNA binding domain together with 1,600 ng of the p5xATF6GL3, pGL3-GRP78 or pGL3-CRE vector were used.

Cells were lysed 24 h after transfection in 40 μl of 1X Passive lysis buffer (Promega) and assayed for the firefly and *Renilla* luciferase activities using the Dual Luciferase Assay System (Promega), according to the manufacturer's instructions. The results were normalized against the *Renilla* luciferase activities. Measurements were performed with the Veritas 96-microplate luminometer (Turner Biosystems, Sunnyvale, CA) using 20 μl cell lysate and 96-well medium binding Lumitrac 200 plates (Greiner bio-one, Kremsmuenster, Austria). Two independent transfection experiments were performed with eight replicas for each construct. The results are presented as the median together with the 25th and 75th percentiles. The Mann-Whitney 2-tailed test was used for the statistical analysis using the statistiXL software (http://www.statistixl.com).

Subcellular localization. For subcellular localization, 400,000 HELA cells were plated in 2 ml medium in a 35-mm culture dish and 24 h later they were transfected with 100 μl transfection complex containing 3 μl FuGene HD Transfection Reagent, 1 μg pEGFP-BC063666 plasmid DNA (or pEGFP-CREB3L2 or pEGFP-CREB3L2ΔTM) and 1 μg pDsRed2-ER vector. The cells were harvested 24 h after transfection, fixed in 4% formaldehyde solution, washed in 1X PBS and spread on a glass slide. The spreads were counterstained with 0.5 mg/ml DAPI (Sigma-Aldrich, St. Louis, MO) and were visualized under an Axioplan 2 imaging microscope (Zeiss, Jena, Germany). Detailed information on the entire protocol is given elsewhere (5).

Expression analysis. Expression analyses were performed using cDNA multiple tissue (Clontech) panels I (brain, heart, kidney, liver, lung, pancreas, placenta and skeletal muscle), II (colon, ovary, peripheral blood leukocytes, prostate, small intestine, spleen, testis and thymus), and a fetal panel (brain, heart, kidney, liver, lung, skeletal muscle, spleen and thymus). In addition, the following cell lines were also used: K562, 697 (DSMZ, ACC42), KARPAS-299 (DSMZ, ACC31), RD-ES (ATCC, HTB166), TTC466 (kindly provided by Dr P. Sorenson, Vancouver), DTC1 (kindly provided by Dr K.-L. Schäfer, Düsseldorf), SJCRH30 (ATCC, CRL2061), RMZ-RCZ (kindly provided by Dr P.L. Lollini, Padova), HEK293, and HELA (DSMZ, ACC57). Total RNA (2.5 μg) from the above mentioned cell lines were reverse-transcribed using 25 μM random hexamers, and 200 units M-MLV Reverse Transcriptase (Invitrogen) as described previously (3). cDNA (3 μl) were used as template for real-time PCR. This volume corresponds to approximately 3 ng cDNA for the samples in the two cDNA panels or 375 ng total RNA of the cell lines.

For the full length CREB3L2 transcript two assays were performed which were supplied from Applied Biosystems (Foster City, CA). Assay Hs01592194_m1 was used for the

The retrieved ESTs using the Blast software and the between nucleotides 900 to 1100 of the BC063666 transcipt.

Accession	Source
CK300074.1	Eye
CD368934.1	Alveolar macrophages
BQ187009.1	Eye
BM477826.1	Leiomyosarcoma
BG236289.1	Lymphocytes
AW136624.1	Colon
AI401161.1	B-cell, chronic lymphocytic leukemia
AI769562.1	Two pooled tumors (clear cell type)
AA861496.1	Testis
BQ014161.1	Chondrosarcoma
AI262707.1	Placenta
N24079.1	Melanocytes
BQ014940.1	Chondrosarcoma
AI765087.1	Colon tumor, RER+"
BI224039.1	Cervical carcinoma cell line
AA367707.1	Placenta
BX330082.2	Placenta
BE732684.1	Choriocarcinoma
BE893857.1	Choriocarcinoma
EL734901.1	Mixed
BI253025.1	Hepatocellular carcinoma cell line

5' end of the transcript and was specific for the exon 4/5 boundary (NM_194071). Assay Hs00811200_m1 was used for the 3' end of the transcript and was specific for the exon 8/9 boundary. For the transcript *BC063666* an assay was developed in which the forward primer CREB3L2-808F (GAACCTCCTCTGGAAATGAACACT), reverse primer CREB3L2bc-934R (GGGCAGACAGACCTTCTTTAGG) and the TaqMan MGB Probe CREB3L2-881 CTCATGAAG TGGATCAGTT were designed.

CTCATGAAGTGGATCAGTT were designed using the Primer Express Software for real-time PCR version 3.0 (Applied Biosystems). Human *ACTB* was used as endogenous control for relative gene expression quantification [Human ACTB (ß-actin) Endogenous Control FAM/MGB Probe, Non-Primer Limited, Applied Biosystems]. Three replicates of each sample and endogenous control were performed. For the commercial assays the 20 μl reaction volume contained 1X TaqMan Universal Mix, 1X of the 20X TaqMan Gene Expression mix and 3 μl cDNA. For the *BC063666* assay, the reaction contained 1X TaqMan Universal mix, 0.9 μM of each of CREB3L2-808F and CREB3L2bc-934R, 0.25 μM of the TaqMan MGB Probe CREB3L2-881, and 3 μl cDNA. The PCR was run on a 7500 real-time PCR System (Applied Biosystems) using the standard 7500 run mode. The thermal cycling included an initial step at 50˚C for 2 min, followed by 10 min at 95˚C and 50 cycles of 15 sec at 95˚C, and 1 min at 60˚C. Data were analyzed using the SDS software 1.3.1 (Applied Biosystems). Additionally, the efficiency of the BC063666 assay was measured using the Ct slope method. With this method, the expected slope for a 10-fold dilution series of template DNA is -3.32, when the efficiency of target amplification (E_X) is 1.0. Dilution series of both the pEGFP-BC063666 plasmid and the cDNA clone IMAGE:4185677 were applied. Using the pEGFP- BC063666 as template the measured efficiency of the assay was: y=-3.3473x + 11.8966, R2=0.9997 and the calculated efficiency for the assay was 98.95%. Using the clone IMAGE: 4185677 as template the measured efficiency of the assay was: y=-3.4741x + 12.3426, R2=0.9965 and the calculated efficiency for the assay was 94.02%. For the relative quantification of *BC063666* and *CREB3L2*, RNA expression was calculated using the comparative threshold cycle (Ct) method (12): the value of the target, normalized to an endogenous control *(ACTB)* and relative to a calibrator, was expressed as $2^{-\Delta\Delta Ct}$ (fold difference), where ΔCt = the Ct of the target gene - the Ct of the endogenous control gene, and $\Delta\Delta$Ct = the ΔCt of the target gene samples - the ΔCt of the target gene calibrator (12). The Peripheral blood leukocytes, fetal spleen and the cell line 697 were the calibrators for the cDNA panels I and II, the fetal panel and the cell lines, respectively.

Results

Using the Blast software and the sequence between nucleotides 900 to 1100 of *BC063666*, 21 ESTs were retrieved (Table I). This finding prompted us to investigate further the expression of *BC063666* and compare it with the expression of the full length *CREB3L2* transcript in various tissues. Quantitative real-time PCR showed that the *BC063666* transcript is widely expressed in adult and fetal tissues as well as in various cell lines (Fig. 1). Brain, heart, placenta and skeletal muscle had strong *BC06366* expression while, intestine and spleen weak (Fig. 1A). In the fetal tissues, heart and thymus had the highest and lowest expression, respectively (Fig. 1B). Of the cell lines, HEK293 and HELA had the highest expression of *BC06366*, while KARPAS-299 the lowest (Fig. 1C). Moreover, the *BC063666* transcript was more strongly expressed than the full length *CREB3L2* in both adult and fetal brain, and in adult skeletal muscle, colon and lung. To determine whether BC063666 is a transcription activator or not, a plasmid expressing the GAL4-BC063666 fusion protein was co-transfected with a luciferase reporter plasmid containing the GAL4 DNA-binding element (pG5*luc*) into HELA cells. The transfected *BC063666* cDNA fused to GAL4 activated the reporter expression (Fig. 2A), indicating that it encodes a peptide which contains an activation domain. The activity was higher than that of the CREB3L2ΔTM ($P<0.0001$) but less than that of the full-length CREB3L2 ($P<0.0001$). To assess whether BC063666 can activate transcription through the ATF6 and CRE binding sites and to examine its effect on the expression of a reporter gene carrying the *GRP78* promoter, we co-transfected BC06366-expressing plasmid and luciferase reporter constructs containing the CRE and ATF6 elements or the promoter of *GRP78* into HELA cells. Two plasmids expressing CREB3L2, CREBL2ΔTM were also used as positive controls (5). Relative to control plasmid pBIND (empty vector), BC06366 did not activate

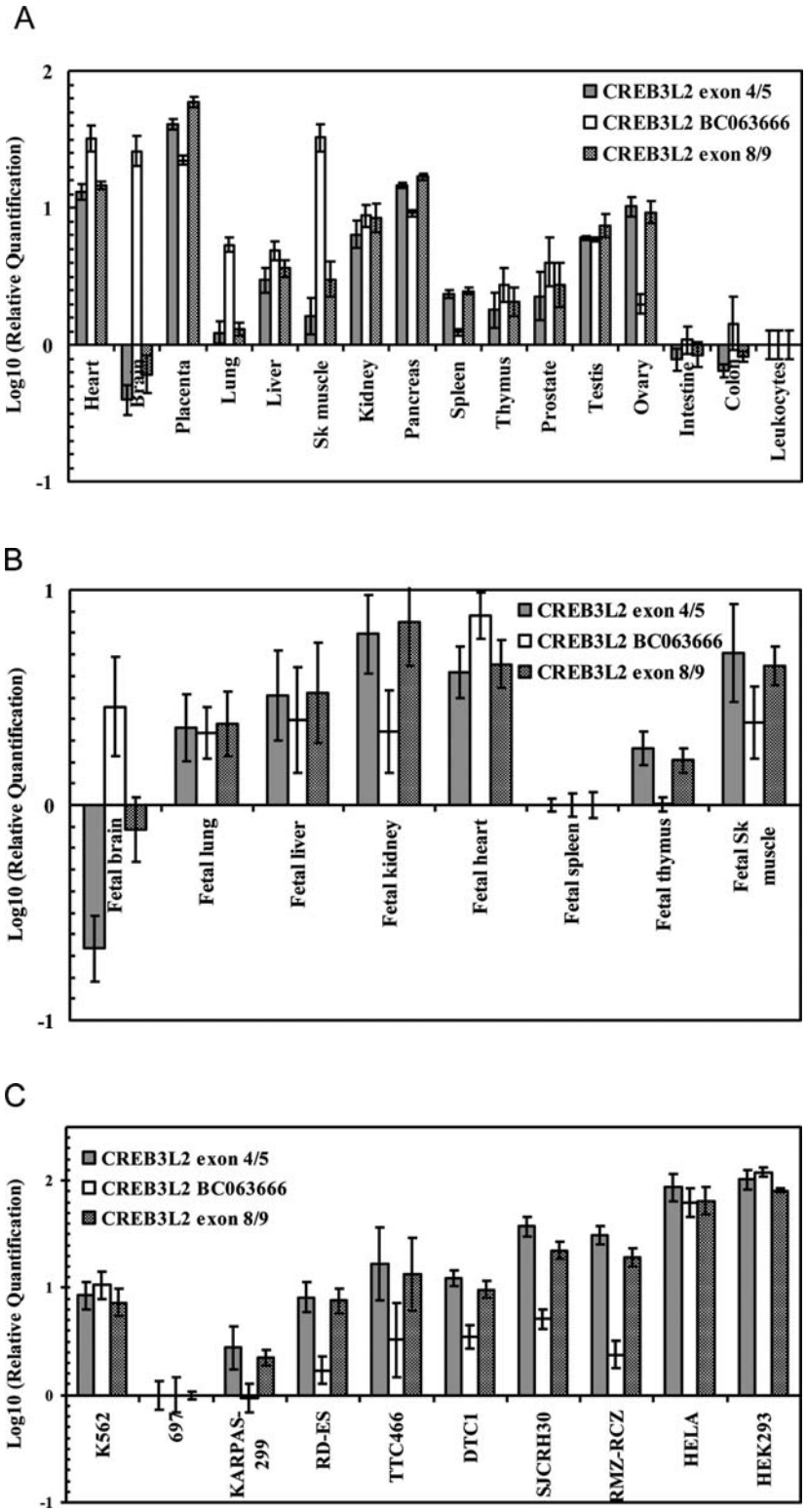

Figure 1. Expression analysis of *BC063666* and *CREB3L2* in various tissues. For the full length *CREB3L2* transcript (NM_194071) two assays were performed. One was used for the 5' end of the transcript and was specific for the exon 4/5 boundary. The other was used for the 3' end of the transcript and was specific for the exon 8/9 boundary. (A) Adult tissues, (B) fetal tissues, (C) various cell lines.

the reporter expression and had little effect on the *GRP78* promoter (Fig. 2B-D).

To investigate the subcellular localization of BC063666, and compare it with the localization of the full length CREB3L2 and the truncated CREB3L2ΔTM, the pEGFP-N1 vector, containing the cloned transcript fused to the 5' end of the EGFP, was in each assay co-transfected with the pDsRed2-ER vector (RFP). In cells expressing a full-length CREB3L2, the EGFP-signal corresponded to the distribution of the RFP signal outside the nucleus. This finding indicated that protein encoded by full-length *CREB3L2* sequence was located to the reticular structures of the cytoplasm. In cells expressing the

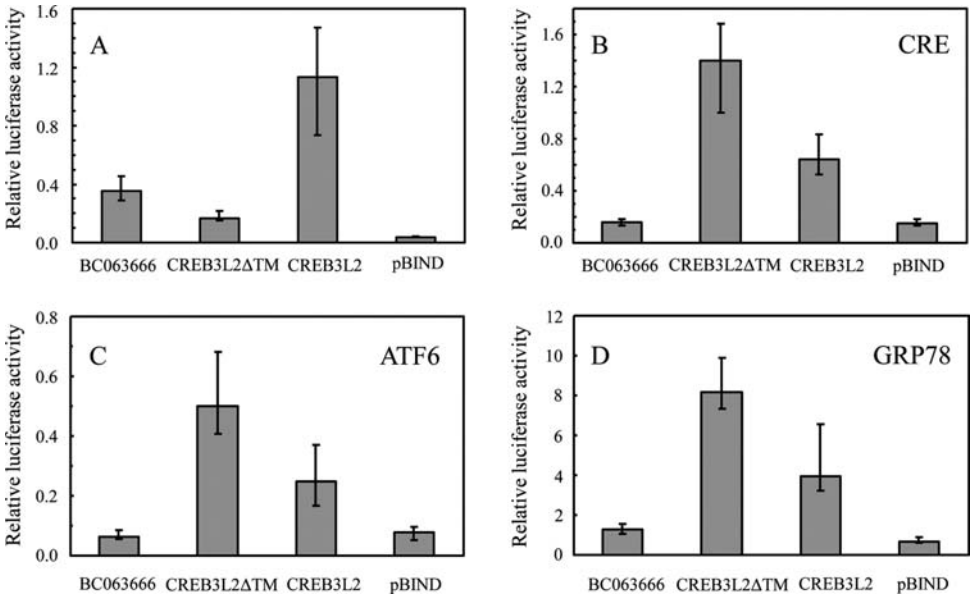

Figure 2. Transcription activation potential of BC063666. (A) cDNA fragments coding for BC063666, CREB3L2 and CREB3L2ΔTM were in-frame inserted into a pBIND vector and co-transfected with the pG5*luc* vector into HELA cells. The encoding fusions with the GAL4 DNA-binding domain activated the luciferase expression. (B-D) BC063666, CREB3L2, CREBL2ΔTM, and empty vector (pBIND) were co-transfected with luciferase reporter constructs containing the CRE (B) and ATF6 binding sites (C) and the GRP78 promoter (D) into HELA cells. Relative to empty vector, the CREB3L2- and CREBL2ΔTM-expressing constructs activated the reporter expression whereas BC063666 had no effect.

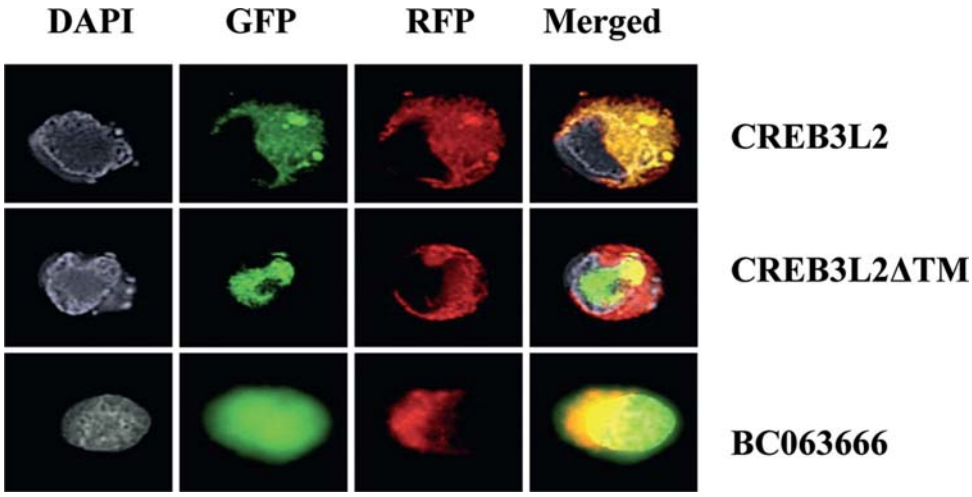

Figure 3. Epifluorescence microscopy images showing the distribution of DAPI fluorescence (cell nucleus), EGFP fluorescence (CREB3L2, CREB3L2ΔTM and BC063666) and RFP fluorescence (pDsRed2-ER vector). Protein encoded by an intact *CREB3L2* sequence localized to the ER, by *CREB3L2ΔTM* sequence localized to the nucleus while protein encoded by *BC063666* was not distinctly localized to particular cell structures, but was rather unspecifically distributed across the nucleus and the ER.

CREB3L2ΔTM protein lacking the transmembrane domain and the carboxyl part of CREB3L2, the EGFP-signal was detected distinctively within the nucleus whereas the RFP-signal was distributed outside the nucleus (Fig. 3). This finding indicated that the truncated protein CREB3L2ΔTM was located in the nucleus and not in the cytoplasm. In cells expressing BC063666, the EGFP-signal was detected inside the nucleus but also in the cytoplasm, co-localizing with the RFP signal. This finding indicated that the BC063666 protein was not distinctly localized to a particular cell structure, as CREB3L2 and CREB3L2ΔTM, but was rather unspecifically distributed across the nucleus and the ER (Fig. 3).

Discussion

In the present study, we characterized a truncated transcript of *CREB3L2* which is composed of exons 1-4 and part of intron 4. It is widely expressed in adult and fetal tissues as well as in cell lines (Fig. 1) and could be translated to a CREB3L2 protein which lacks the DNA-binding domain, the transmembrane domain and the C-terminal part, but it has the N-terminal transactivation domain. Experiments using a GAL4 DNA-binding domain fusion model showed that indeed the transcript has the potential to act as a transactivator (Fig. 2A). However, it cannot exert its function through the CRE and ATF6 binding

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ATGAGGTGGA AAGTGAGAAA TGGTACCTGT CTACAGACTT CCCTTCAACA TCCATCAAGA

CAGAGCCAGT TACAGACGAA CCACCCCCAG GACTCGTTCC GTCTGTCACT CTGACCATCA

                                                   Exon 3 | Exon 4
CAGCCATCTC CACCCCGTTG GAAAAGGAGG AACCTCCTCT GGAAATGAAC ACTGGGGTTG
                                  CREB3L2-808F (→)

ATTCCTCGTG CCAGACCATT ATTCCTAAAA TTAAGCTGGA GCCTCATGAA GTGGATCAGT
                                                CREB3L2-881
                   Exon 4 | Intron 4
TTCTAAACTT CTCTCCTAAA GAAGgtctgt ctgccctccc tgtgtccctt tgggttatgg
                    (←)CREB3L2-934R
atatggtctc tgggtctaca gagagggaat atggcgagag agctgggatg agtttgtacc

acagatgttg tagctggctt tatgaaatag ctctgttctt aaaaaataaa aattttgctt

ccaaataaaa attttgcaag ctaactatta ttttcccata tatgcacgtg attaaatttc

agctaagtgt taccatactt ttacttctaa ccagggtgat atttttcaaa acttttatgg
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Figure 4. Partial sequence of the BC063666 and intron 4 of CREB3L2. The stop codon (taa) is in bold. The three polyadenylation signals are in box. The position (a) where the polyadenylation starts is underlined. The primers CREB3L2-808F, CREB3L2-934R and the probe CREB3L2-881 used for quantification of the transcript are underlined. Arrows indicate the orientation of the primers.

sites and has little effect on the *GRP78* promoter (Fig. 2B-D). The full length CREB3L2 protein is localized to reticular structures of the cytoplasm and is involved in unfolded protein response signalling. Upon ER stress it undergoes intramembrane proteolysis allowing the CREB3LΔTM, i.e., the N-terminal part, the DNA binding and the bZIP domains, to enter the nucleus and activate a number of target genes. The protein encoded by the *BC063666* transcript was localized both in the nucleus and the ER (Fig. 3) indicating that it might not be associated with ER stress; it might have a different response to ER stress, or another cellular role.

Alternative splicing is an important mechanism by which an eukaryotic gene is able to produce several proteins and to regulate the level of RNA processing and alternative polyadenylation is one of the mechanisms in cells that gives rise to a variety of transcripts from a single gene. Tian *et al* (13) studied the polyadenylation in introns and found that 20% of human genes have at least one intronic polyadenylation site that potentially could result in different protein products. Intronic polyadenylation sites can lead to either conversion of an internal exon to a 3'-terminal one or inclusion of an otherwise skipped exon into the transcript. Exons associated to those types are termed composite terminal and skipped terminal exons, respectively (13,14). The *BC063666* transcript is associated with a composite terminal exon with the first four exons to be followed by a short sequence of intron 4. There are two AATAAA polyadenylation signals within 40-bp upstream of the polyadenylation site, and one ATTAAA signal 27 nucleotides downstream of it (Fig. 4). From the 16 known polyadenylation signals, AATAAA and ATTAAA are the two most common ones in human and mouse transcripts (13,14). Comparison of the human intron 4 of CREB3L2 with the corresponding intron 4 of mouse *Creb3l2* (Ensembl gene ID: ENSMUSG00000038648) showed that the two above mentioned polyadenylation signals are not conserved in mouse *Creb3l2*.

Tian *et al* (13) reported that 90% of human intronic polyadenylation sites do not have orthologous sites in mouse or rat genomes. Moreover, it was demonstrated that the intronic polyadenylation activity varies under different cell conditions, that several tissues tend to use polyadenylation sites that are biased towards certain location of a gene and that retina, placenta brain and other tissues are using polyadenylation sites which are not frequently used in other tissues (13,15). The present data from real-time PCR (Fig. 1) indicate that both full-length and the truncated *BC063666* transcripts of *CREB3L2* are expressed but that the expression differs among various tissues and cell lines. Adult brain, colon, skeletal muscle and fetal brain preferentially express the *BC063666* transcript, suggesting a biased usage of the polyadenylation site in intron 4 of *CREB3L2*. Adult spleen and ovary, fetal thymus and the cell lines KARPAS-299, RD-ES, RMZ-RCZ, TT466, SJRH30 and DTC1 much more strongly express the full length *CREB3L2* transcript, suggesting a biased usage of the polyadenylation site at the 3' untranslated region of *CREB3L2*. The remaining samples showed comparable levels of expression for the *BC06366* and the full length transcript of *CREB3L2*, suggesting that both polyadenylation sites at intron 4 and at the 3' untranslated end can be used with the same efficiency.

The elimination of mRNAs that prematurely terminate translation is a very important and well orchestrated process in the cell, since possible resulting proteins might have the potential to be nonfunctional or acquire dominant-negative or gain-of-function activities (16). Such transcripts undergo a rapid degradation via the nonsense-mediated decay (NMD) surveillance mechanism (16). However, there are numerous instances in which intronic polyadenylation signals play an important cellular role (14). For example, the immunoglobulin heavy chain genes have intronic polyadenylation sites and subsequently composite exons can switch between being internal or 3'-terminal (14). Previously, Thomas *et al* (17) showed that in the human *FLT1* locus intronic polyadenylation signals and alternative splicing generate soluble Flt1 variants and regulate the abundance of soluble Flt1 in the placenta. Moreover, Gilat and Shweiki (18) have shown that alternative

ylation can function as a rescue pathway from NMD ice. Whether the *BC06366* transcript of *CREB3L2* mRNA has a cellular function or instead is targeted for degradation by nonsense-mediated RNA decay (NMD) system of RNA surveillance is currently unknown.

Acknowledgements

We thank Margareth Isaksson for her technical assistance. This study was supported by The The Swedish Childhood Cancer Foundation and The Swedish Research Council.

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