Journal of Medical Genetics and Genomics Vol. 2(4), pp. 44-56, November 2010
Available online http://www.academicjournals.org/jmgg
ISSN 2141-2278 ©2010 Academic Journals

Full Length Research Paper

Immunodetection of Ribin-like proteins in neuron-based cellular models

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Accepted 14 July, 2010

Extensive bibliographical analysis has demonstrated changes in *ribin genes* expression during several types of stress, especially in neurological tissues or cells during stress inducing neurological disorders. These analyses suggest that Ribin studies could be useful for neurological investigation. The present study was undertaken to detect and localize Ribin in various neuron-based cellular models mimicking neurodegenerative pathologies such as Huntington's disease, amyotropic lateral sclerosis and spinal muscular atrophy, as well as pathologies. Using confocal microscopy immunofluorescence methods, the presence of Ribin was detected in all neuronal models. Change, in the protein level was found at least in one neuronal model, suggesting that this protein could play a physiological role in diseases. Moreover, in contrast to previous experiments, we showed that Ribin-like proteins had principally a cytoplasmic localization. Indeed, BLAST analysis of muridae and human DNA-databases provides evidence that most of the sequence homologies are found within the Ribin COOH-part involved in the loss of the nuclear localization signals, which suggests the synthesis of cytoplasmic Ribin-like proteins.

Key words: Ribin, neuron-based cellular models, amyotropic lateral sclerosis, Huntington's disease, spinal muscular atrophy.

INTRODUCTION

A previous study had shown that a region homologous to the complementary strand of the region homologous to *28S rRNA genes* could encode a protein named Ribin (Kermekchiev and Ivanova, 2001). This protein bound to the rRNA promoter and stimulated its activity. Moreover, green fluorescent protein-ribin fusion proteins shown that Ribin was localized in the nucleus which is congruent with the presence of two predicted nuclear localization sequence elements in Ribin. In addition, transfected cell lines overexpressing Ribin exhibited enhanced rRNA transcription and faster growth. Using a polyclonal antibody raised against the cloned protein it has been shown that in murine cells (mouse N2a neuroblastoma cells, hamster kidney BHK-21 cells and rat hepatoma N1S1 cells) the expressed ribin comigrated with the endogenous one. As bibliographical analysis provides evidence that, on the one hand, stress can induce change of *ribin*-like *gene* expression and that, on the other hand, these changes are preferentially found in neuronal tissues or cells. The Ribin detection was investigated in neuron-based cellular models using immunohistochemical techniques.

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MATERIALS AND METHODS

Blast searches

The nucleotide and predicted protein sequences were analyzed online using NCBI-BLAST (http://blast.ncbi.nlm.nih.gov) and ENSEMBL-BLAST (http://Ensembl.org) servers. Multiple alignments were performed with CLUSTAL X (1.83).

Neuron-based cellular models of neurodegenerative diseases

To mimic Huntington's disease, primary striatal neurons were prepared from E17 rats and electroporated with cDNA plasmids encoding the 480 N-terminal amino acids of human huntingtin containing an expanded (68) CAG repeat (Htt480.68) and a plasmid encoding green fluorescent protein (GFP) as previously described (Valenza et al., 2005). Motor neurons were prepared from E14 rat spinal cord and deprived of trophic factors for 3 days as previously described (Bordet et al., 2007). Finally, cortical neurons were cultured from cortices of E17 rats and treated with 10 µM camptothecin for 16 h after 6 days *in vitro* as previously described (Morris and Geller, 1996).

Immunochemical localization of Ribin

For immunodetection of Ribin, a polyclonal antibody raised in a rabbit against bacterially expressed and gel-purified Ribin protein (Kermekchiev and Ivanova, 2001) was used. To minimize any non-specific antibody binding, cells were first incubated in 10% normal goat serum in PBS (0.1 M), with 0.6% Triton X-100 and 2% bovine serum albumin (PBS buffer), for 1 h at room temperature, before being incubated overnight at 4°C in the same PBS buffer containing a mixture of primary antibodies against Ribin (diluted 1/200, gift from M. Kermekchiev), and S6 ribosomal protein (mouse monoclonal, diluted 1/200; Cell Signaling technology). After being rinsed three times, cells were incubated for 1 h with the appropriate secondary antibodies diluted 1:500 (for striatal cells, TRITC-coupled anti-rabbit IgG (Sigma) was used; for motoneurons and cortical cells, a mixture of Alexa 488-coupled anti-rabbit IgG (Molecular Probes) and TRITC-coupled anti-mouse IgG (Sigma) was used). Finally, all cells were stained with 10 µM DRAQ5™ (cell permeable DNA-interactive agent, Biostatus Limited) in PBS for 10 min at room temperature, rinsed in PBS and mounted in a medium containing antifading (Gel/MountR, Bibmeda, Foster City, CA, USA). Control experiments using preimmune serum diluted 1/200 in the PBS buffer remained negative.

Confocal microscopy

Neuronal cells were examined under a confocal scanning microscope (Leica TCS SP2) using argon (488 nm), helion-neon green (543 nm) and helion-neon red (633 nm) lasers, and scanned sequentially. For quantitative analysis of immunolabellings, Z series of 5 - 7 optical sections (1024 x 1024 pixels) were processed. For each cell type studied, stressed and non-stressed, identical confocal acquisition parameters were applied. Adobe Photoshop (Adobe Systems, San Jose, CA) was used to crop, adjust brightness and contrast, organize layouts, and apply text on the images of optical sections.

Quantification and statistical analysis

To quantify the Ribin immunoreactivity, an automated quantification approach was adopted using the Biovays ImagePro software program. The validity of this program developed by Biovays SAS, a Contract Research Organization based in France (Marseille, France), has been confirmed on the basis of comparisons between experimental and simulated data (Jaafari et al., 2007). This method of quantification consisted of assessing the rates of colocalization of the pixels corresponding to single fluorescent staining observed in a confocal image. Threshold values were automatically calculated for each monochrome optical section by using an iterative selection method (Ridler and Calvard, 1978) and applied to subtract the non specific staining. Results are expressed as mean ± standard error (SE).

RESULTS

Bibliographical analyses

Several searches have been made on the internet using unique keywords corresponding to Ribin (nucleotide and protein accession numbers, respectively: NM_147136 or U77931 or Q99JC0, NP_671477 or AAK21974; *gene* description: rRNA promoter binding protein; interim symbol: LOC257642). These keywords were also searched in full text of scientific papers in ScienceDirect (www.sciencedirect.com). These analyses show that changes of expression of *genes* homologous to *ribin* are generally found in neuronal tissues or cells during stress simulating neurological disorders (Table 1). Changes of *ribin*-like *gene* expressions have also been found in other tissues or cell types but more rarely; however, these changes are generally observed during viral infection or chemical stress. Activation of *ribin*-like *genes* has also been found in human breast cancer (http://lifesciencedb.jp/cged). The literature shows evidence that, on the one hand, stress can induce change of *ribin*-like *gene* expression and that, on the other hand, these changes are preferentially found in neuronal tissues or cells. Moreover, *ribin*-like *genes* have a widespread evolutionary distribution among organisms including plants suggesting important physiological roles of the corresponding proteins.

Blast analyses

In this work, we screened rat, mouse and human complete genomes from ENSEMBL to find sequences homologous to Ribin. Surprisingly, no region exhibiting a significant homology level with Ribin was found (Figure 1 and Table 2); moreover, no homology was observed between the 5' part of the *ribin* published sequence and these three complete genomes. In addition, all the homologous regions were non-coding with the exception of an rRNA pseudogene found in the human genome. These results are congruent with BLASTs on cDNA and non-coding RNA *genes* of ENSEMBL which did not reveal homology with the entire Ribin.

BLAST searches on NCBI's nucleotide database reveal homologies on the totality of the Ribin sequence for both the rat and mouse. The rat sequence exhibits the

Table 1. Differential expression of *ribin*-like genes in models for the study of neurological disorders.

Animal or plant	Tissue or cell type and conditions	Fold changes with conditions if appropriate	Position versus total number of genes analyzed as compared to the higher or lower value if + or – (if appropriate)	Model for study of …	References
Neurological tissues, cells and/or disorders					
Mouse	Spinal cord anterior of PQBP-1 transgenic mice	+2.4 (aged 12 months) +3.0 (aged 2 months)	3/13	Huntingtin disease	Marubuchi et al., 2005
Mouse	Cerebral cortex of PQBP-1 transgenic mice	-0.53 (aged 12 months) Ratio transgenic mouse/control	1/1	Huntingtin disease	Marubuchi et al., 2005
Rat	Brain regions involved in cocaine addiction	+2.3 (1-week extinction to withdrawal)	2/35	Treating drug addiction	http://www.freepatentsonline.com/y2005/0143295.html
Rat	Hyppocampus after lateral fluid percussion injury	+2.1±0.1 (after 24 h)	252/354	Traumatic brain injury	Li et al., 2004
Rat	Periaqueductal gray of rats displaying high exploratory activity in the elevated plus-maze	+1.47	14/26	Anxiety	Nelovkov et al., 2007
Rat	Spinal cord after recovery from inflammatory hyperalgesia	≈ -1.29 (after 24 hours) ≈ -1.10 (after 28 days)	122/1051 251/1051	Persistent post-injury pain	Yukhananov and Kissin, 2008
Rat	Lumbar part of the spinal cord	-22.57 (24h) and -9.57 (24 days)	519	Genes playing a role in the formation of the long-term hyperalgesia-related imprint in the spinal cord	Yukhananov and Kissin, 2008
Rat	IFN-γ-immature oligodendrocyte	-1.70	2/2	Myelination/remyelination process	Strand, 2006
Rat	Frontal pole of the prenatally stressed adult offspring	-1.74 but not confirmed by real time RT-PCR	20/36	Schizophrenia and bipolar disorders due to stress during pregnancy	Kinnunen et al., 2003
Rat	Adult brain of maternally vitamin D deprived rats	-2.4	30/74	“Imprinting” with low prenatal vitamin D could contribute to the risk of multiple sclerosis and schizophrenia	Eyles et al., 2007
Rat	Primary cortical cell cultures prepared from gestational day 15 fetal rats (neurons represent 70-90% of the culture)	-0,12 (after 1h) (z-ratio)	136/1155 but the transcript is weakly similar to ribin mRNA	Analysis of plasticity-induced late-response genes (response to *N*-methyl-d-aspartate (NMDA))	Hong et al., 2004

Table 1. Contd.

Rat	Primary cortical cell cultures prepared from gestational day 15 fetal rats (neurons represent 70-90% of the culture)	0,55 (after 72h) (z-ratio)	136/1155 but the transcript is weakly similar to ribin mRNA	Analysis of plasticity-induced late-response genes (response to Maximal Electroconvulsive Seizures (MECSs))	Hong et al., 2004
Rat	Penumbra cortex transcriptome after postischemic brain (transient middle cerebral artery occlusion (MCAO))	8.8 to 10.1 (12h after sham-operation)	(24 to 38) 6072	Biological processes relevant for cell death and survival in the brain following stroke	Rickhag et al., 2006
Rat	Microglial cells cultures harvested from the neonatal rat brain	+5.38/microglia under standart culture conditions		Genes expressed in all microglia cultures	Duke et al., 2004
Viral infection					
Rat	Expression in the primary rat embryonic fibroblast (REF) cells versus human foreskin fibroblasts (HFF) cells	3.75	47/~80 (Genes induced by PRV infection in REF cells but not HFF cells)	Transcriptional response to infection by Pseudorabies virus (PRV)	Ray and Enquist, 2004
Rat	Expression in the primary rat embryonic fibroblast (REF) cells versus human foreskin fibroblasts (HFF) cells	4.7	107/~180 (Genes induced by HSV infection in REF cells but not HFF cells)	Transcriptional response to infection by herpes simplex virus type 1 (HSV-1)	Ray and Enquist, 2004
Rat	pancreatic beta cells	-3.3	~73/87	Study of viral infections IFN-γ induced beta-cell dysfunction and death	Rasschaert et al., 2003
Rabbit	Latent trigeminal ganglia	-1.49 (Mean log2 difference)	~/300	Infection by herpes simplex virus (HSV)	Clement et al., 2008
chicken	chick embryo fibroblasts (CEFs)	Upregulation	~180	Infection with Herpesvirus of turkeys (HVT)	http://www.chickest.udel.edu/
Chicken	DT40 bursal lymphoma cell line	-1.84	721	Retrovirus-mediated lymphomagenesis in the bursa of Fabricius provides an experimental model system for molecular analysis of neoplastic change in a developmental B-cell lineage	Neiman et al., 2006
Other types of stress					
Human	WI-38 fibroblasts	>31		Responses to microgravity stress	Liu and Wang, 2008

Table 1. Contd.

Rat	Angiotensin II rapid response in primary adrenal glomerulosa cells	1.4	826/~15,200	Hormonal response	Nogueira et al., 2007
Rat	Extraocular muscles	+2.69/internal control	238/257	Muscles affected in mitochondrial myopathies	Fisher et al., 2002
Rat	Alveolar macrophage cell line (CRL-2192)	+2.8	~14/60	Gene Expression Changes Induced By Bismuth In A Macrophage Cell Line	Magnusson et al., 2005
Rat	Rat renal proximal tubule (PT) cells	7.53±1.05 (above background)	7,502	Cell-specific AQP2 gene expression in renal collecting duct revoir	Yu et al., 2009
Rat	Impregnated female rats	3.09	~3/65	Toxic effects of ethanol during pregnancy (on development)	Shankar et al., 2006
Rat	Liver	Upregulation	24/24	toxic effects of a ternary mixture containing (benzene, trichloroethylene and methyl mercury)	Hendriksen et al., 2007
Rat	Kidney (metanephric mesenchyme)	+2.1/ureteric bud	19/35	kidney development	Stuart et al., 2003
Rat	Pregnant rats	2.1 (Preterm) - 1.5 (Term) - 2.9 (Postnatal)	4/39 (Preterm) - 14/39 (Term) – 29/39 (Postnatal)	consequence of maternal vitamine A	Yokoyama et al., 2007
plant: *Sesbania drummondii*	Plant during germination	Upregulation		consequence of lead-treatment	Srivastava et al., 2007

greatest homology level in the complementary strand of a *28S rRNA gene*. However, it contains numerous frameshifts (at least 10). In the mouse, two sequences that exhibit more than 95% of identical amino acids with Ribin were found; one corresponds to the complementary strand of a *28S rRNA gene* and the other to a cadherin–related neuronal receptor *gene*. In the human, two deduced sequences from *28S rRNA genes* exhibit relatively strong homologies with the COOH-part of Ribin.

BLAST analyses on mRNA sequences show that most of the sequences homologous to *ribin genes* correspond to the complementary strand of these last *genes* and so encodes a protein that is different from Ribin. With the exception of three mouse sequences, only homologies with the Ribin COOH-part were found, suggesting production of Ribin-like proteins. Several sequence homologies have been found in EST libraries. EST sequences in rat and mouse (≈ 15,960), and human (≈ 540) have more than 85% of identical amino acid residues, in a contiguous region of at least 100 residues without stop codon and/or frameshift. These homologies are found almost exclusively in the corresponding Ribin COOH-part.

Neuron-based cellular models

Using immunofluorescence, Ribin detection was investigated in three neuron-based cellular models. In all the cellular models, co-immunolabellings of Ribin with the S6 Ribosomal protein and the DRAQ5, a highly cell permeable DNA-interactive agent, demonstrated the localization of Ribin protein in cytoplasm and neuronal branchings (Figures 2, 3 and 4). Whereas, this protein was more

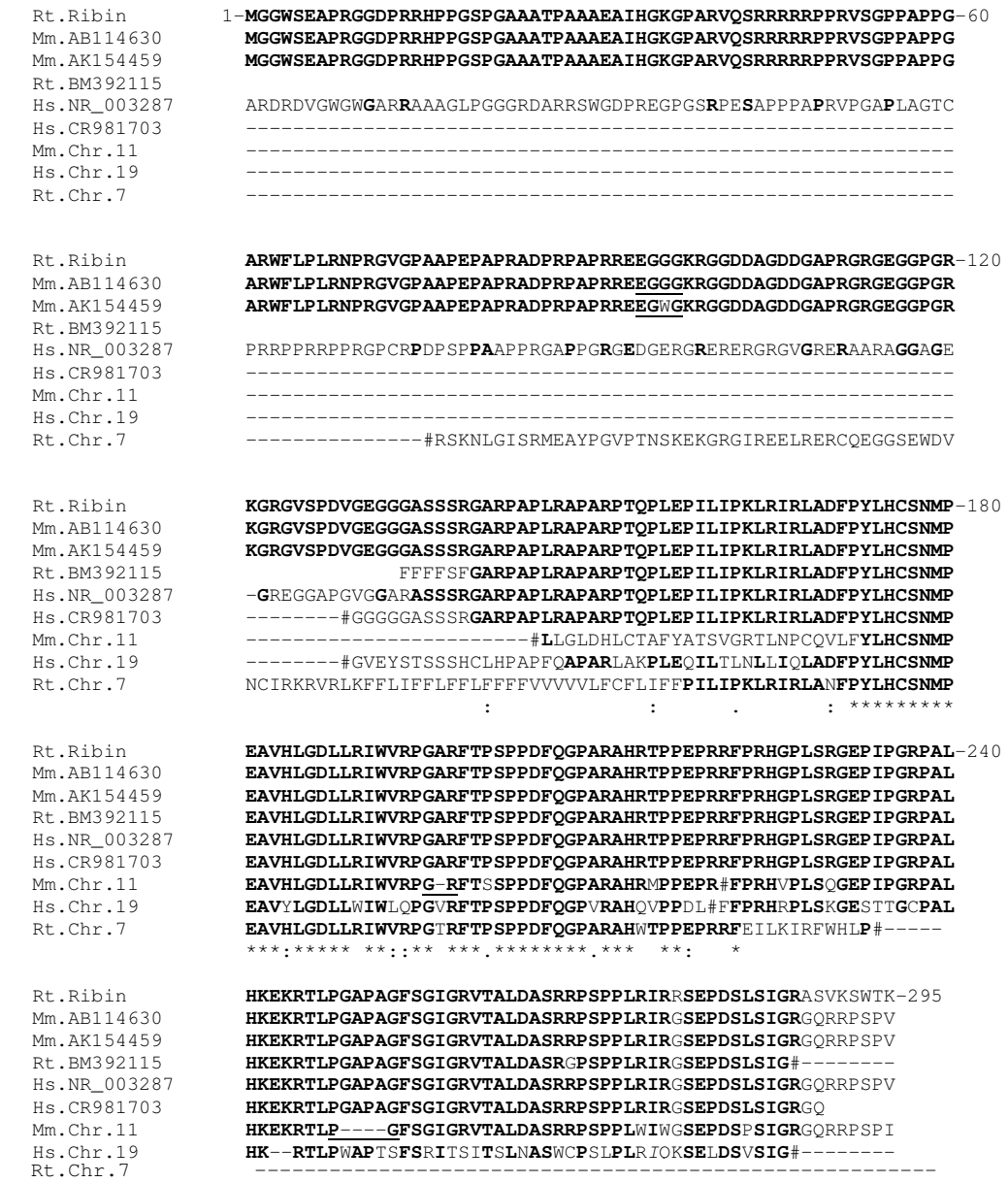

Figure 1. Alignment of deduced amino acid sequences of rat, mouse and human regions which are homologous to Ribin. The characteristics of the sequences are in Table 1; for each species, the sequence closest to Ribin deduced from the complete genome and to the mRNA databank are shown; in each case, the chromosome number or the accession number is given. Amino acids are in standard single letter code. Amino acid residues which are identical to those of *Rattus* Ribin (Rt.Ribin, Acc. number: NP_671477) are in bold letters. A star indicates identity between all the sequences. Where no sequence homology was found before a stop codon, the amino acid residues are symbolized by a dash. When a sequence is shorter, dashes are not inserted. Frameshift regions are underlined and # are stop codons. Abbreviations: Rt, Mm and Hs are respectively *Rattus norvegicus*, *Mus musculus* and *Homo sapiens*.

Table 2. List of rat, mouse and human amino acid deduced sequences which show homologies with Ribin sequence.

Species	Accession number	Molecule type	*Gene* name if known	Orientation / published sequence	Position / published ORF	FS*	Stop codon *	Cell or tissue type, if known	% amino acid identity / Ribin corresponding lenght	Miscellaneous
Rattus norvegicus	Chr.7 (Ensembl.org)	genomic DNA	non coding region	+		0	0		95.4%/65aa	region analyzed: 99314533-99315627
Rattus norvegicus	Chr.4 (Ensembl.org)	genomic DNA	non coding region	-		0	0		88.9%/45aa	region analyzed: 221569580-221570680
Rattus norvegicus	Chr.17 (Ensembl.org)	genomic DNA	non coding region	-		4	2		41.3%/227aa	region analyzed: 62933516-62934478
Rattus norvegicus	V01270	genomic DNA	28S rRNA	-		10	0		82.0%/295aa	
Rattus norvegicus	AY539885	mRNA	unknown protein	+	in another frame	0	0	liver	99.2%/124aa	liver regeneration-related
Rattus norvegicus	AY325217	mRNA	Aa1-330	+	in another frame	0	0	liver	99.1%/106aa	liver regeneration-related
Rattus norvegicus	BM392115	mRNA (EST)		+		0	0		94.9%/156aa	adult
Rattus norvegicus	CB724455	mRNA (EST)		-		0	0	cortical neurons	92.9%/154aa #	
Rattus norvegicus	AW918520	mRNA (EST)		+		0	0	mixed tissues including brain	95.6%/115aa	
Rattus norvegicus	BF558017	mRNA (EST)		-		0	0		99.1%/108aa #	embryo
Mus musculus	Chr.11 (Ensembl.org)	genomic DNA	Non coding region	+		2	1		83.9%/124aa	region analyzed : 108872399-108873499
Mus musculus	Chr.17 (Ensembl.org)	genomic DNA	Non coding region	-		2	1		81.7%/113aa	region analyzed : 23014071-23012890
Mus musculus	AB114630	genomic DNA	cadherin-related neuronal receptor	-	3'UTR	1	0	brain	96.9%/295aa	possible chimeric gene as it has a reverse-transcription origin
Mus musculus	BK000964	genomic DNA	28S rRNA	-		3	0		95.9%/295aa	
Mus musculus	AK154459	mRNA	protein tyrosine phosphatase, receptor type Z, polypeptide 1	-	5'UTR	1	0	dendritic cells	96.6%/295aa	numerous stop codons found in the gene

Table 2. Contd.

Mus musculus	AK133917	mRNA	protein tyrosine phosphatase, receptor type Z, polypeptide 1	-	partially in the ORF	1	0		96.3%/295aa	embryo - numerous stop codons found in the gene
Mus musculus	AJ428208	mRNA	phosphacan	+	3'UTR	3	0	brain	80.0%/295aa	isoform due to alternative splicing
Mus musculus	AK155692	mRNA	unnamed protein (136 aa)	-	partially in the ORF	0	0	dendritic cells	94.3%/141aa #	
Mus musculus	AK172585/AK172469	mRNA	unclassifiable product	-	partially in the ORF	0	0	Activated spleen	92.8%/111aa	numerous stop codons found in the gene
Mus musculus	CF581302	mRNA (EST)		-		0	0	pancreas	92.5%/280aa #	
Mus musculus	CF581002	mRNA (EST)		-		0	0	pancreas	75.1%/273aa #	
Mus musculus	CV674890	mRNA (EST)		-		0	0	pancreas	98.5%/200aa #	
Homo sapiens	Chr.19 (Ensembl.org)	Genomic DNA	Non coding region	+		1	0		64.3%/157aa	region analyzed: 23974840-23975892
Homo sapiens	Chr.2 (Ensembl.org)	Genomic DNA	Non coding region	+		1	0		69.3%/153aa	region analyzed: 132753987-132755055
Homo sapiens	Chr.1 (Ensembl.org)	Genomic DNA	Non coding region	+		2	0		78.4%/104aa	region analyzed: 107913852-107914973
Homo sapiens	Chr.5 (Ensembl.org)	Genomic DNA	rRNA pseudogene	-		0	0		74.5%/94aa	region analyzed: 71183498-71182348
Homo sapiens	Chr.3 (Ensembl.org)	Genomic DNA	Non coding region	-		1	1		47.9%/119aa	region analyzed: 109733831-109732650
Homo sapiens	Chr.1 (Ensembl.org)	Genomic DNA	Non coding region	+		2	0		78.4%/104aa	region analyzed: 107913852-107914973
Homo sapiens	M11167	Genomic DNA	28SrRNA	-		0	0		99.3%/153aa	
Homo sapiens	NR_003287	Genomic DNA	28SrRNA	-		0	0		98.7%/155aa	
Homo sapiens	AK129843	mRNA	6-phosphofructokinase pseudogene	-	5'-part of the ORF	0	0	heart	98.4%/128aa	
Homo sapiens	BC050745	mRNA	NADH dehydrogenase 1 pseudogene	-	Middle of the ORF	0	0	Lung carcinoma	99.0%/101aa	
Homo sapiens	DQ779565	mRNA	immunoglobulin heavy chain variable region	-	in the ORF	0	0	neuronal cell line	95.5%/90aa #	Antibody found in Sydenham's Chorea which is is a CNS disorder

Table 2. Contd.

Homo sapiens	AK092819	mRNA	immunoglobulin heavy constant alpha 1	-	3'-end of the ORF	0	0	small intestine	98.6%/71aa
Homo sapiens	CR981703	mRNA (EST)		-		0	0	T-Lymphocytes	99.4%/157aa # adult
Homo sapiens	CT002021	mRNA (EST)		-		0	0	T-Lymphocytes	99.3%/155aa # adult

These sequences were found using BLAST against the public databases Ensembl and GenBank. For each species and each molecule type, only the closest sequences to Ribin are shown. The accession numbers are given for all the sequences, except sequences that had BLAST hits to ENSEMBL (Ensembl.org); for these sequences, the chromosome number and the positions of the region analyzed are indicated. The orientation is noted + and – for the same and opposite orientation respectively. Symbols: *, frameshift or stop codon in the Ribin-like region; #, amino acid deduced sequence is shorter than those of Ribin. Abbreviations: FS, frameshift; aa, amino acid residue(s).

slightly found in nucleus with the exception of striatal cells (Figures 2, 3, 4 and Table 3). Moreover, no very significant difference in the level of Ribin, were observed between stressed and non-stressed cells (Table 4). However, in motoneurons a weak increase (at least x 1.3) has been detected.

DISCUSSION

Bibliographical analysis has suggested that changes of *ribin*-like *genes* expression are mainly observed in neuronal tissues or cells during stress simulating neurological diseases. Moreover, previous *in situ* hybridizations have shown a strong expression of *ribin*-like *genes* in the nervous system of a marine invertebtrate (chaetognath) (Barthélémy et al., 2010). In this organism, the cerebral and ventral ganglia, which are the two main nervous centres, were particularly labelled. BLAST analyses gave evidence that complete genomes of rat, mouse and human sequenced to date do not contain a region strongly homologous to the entire *ribin* published sequence. With the exception of some mouse sequences, sequence homologies are only found with the deduced Ribin COOH-part. Similarly, several transcripts contain regions homologous to the 3'-part of the *ribin* ORF. Taken together and added to the high level of amino acid identity found in the COOH-part of the deduced protein (Figure 1), these results suggest that Ribin-like proteins corresponding to the COOH-part of the published Ribin sequence are synthesized both in muridae and in humans.

Western blot experiments were carried out by Kermekchiev and Ivanova (2001) on baby kidney hamster (BHK) cells and mouse N2A neuroblastoma cells. In these experiments, only one type of Ribin protein could be detected suggesting that the other members of the Ribin family are relatively rare even in a neuronal cell type (N2A). Moreover, this has been confirmed by Western or Southwestern techniques that the recombinant Ribin protein comigrates only with endogenous form. Minor proteins have not been detected even in primate cells (African green monkey kidney [VERO] cells). These experiments, together with complete genome analyses and the present immunodetection, strongly suggest that even if several Ribin-like proteins could be produced they are generally produced at a low level or their half-life is very short. In the future, these monoclonal antibodies must be developed in order to identify the members of the Ribin protein family.

Immunochemical analyses of Ribin-like proteins in neuron-based cellular models have failed to show quantitative changes in two onto three stressed cell types and their respective controls (Table 4). However, this is probably due to the fact that changes - if there are any - are too low to be detected with the methods used. Moreover, Table 1 shows that the maximum Ribin-like *gene* expression varies by a factor of three; if there is a strict correlation between transcription and translation, differences in the protein level could not be detected by antibody experiments. A correlation between transcription and translation has already been established in some cases; however, in general, transcriptional activity is notnecessarily closely linked to corresponding mRNA levels and protein abundance, especially for mutant *genes*, chloroplast *genes*, and mitochondrial *genes*, pointing to extensive post-transcriptional and post-translational control of *gene* expression. A weak fluctuation in mRNA half-life or protein half-life could have significant effects on steady-state levels of mRNA or protein (Shu and Hong-Hui, 2004). Surprisingly, in the three models used, the Ribin-like localization is principally cytoplasmic; this is congruent with BLAST analyses suggesting

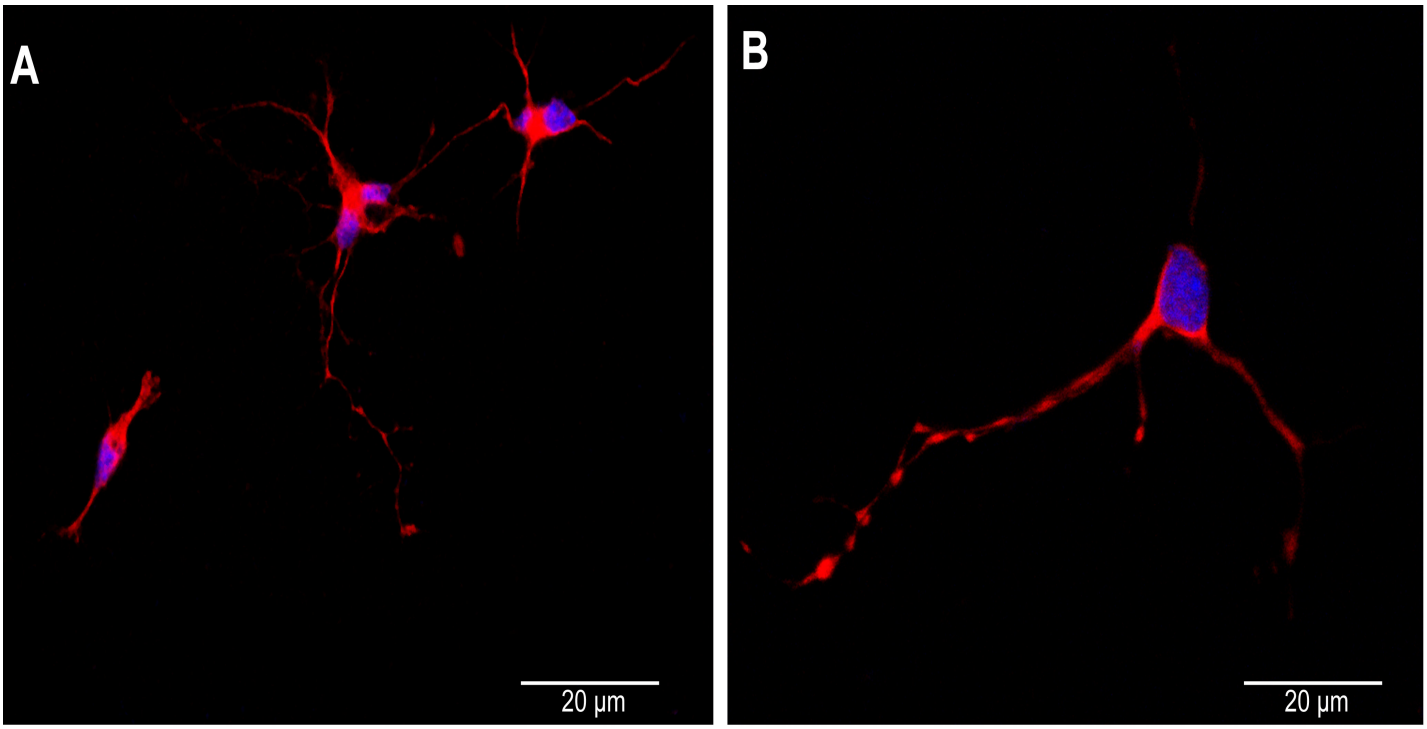

Figure 2. Immunolocalization of Ribin-like protein (red) in striatal cells, electroporated with cDNA plasmids encoding the 480 N-terminal amino acids of human huntingtin, combined with a nuclear marker (DRAQ5, blue); (A) Cells treated with the BDNF neuroprotector factor; (B) Cells grown without BDNF.

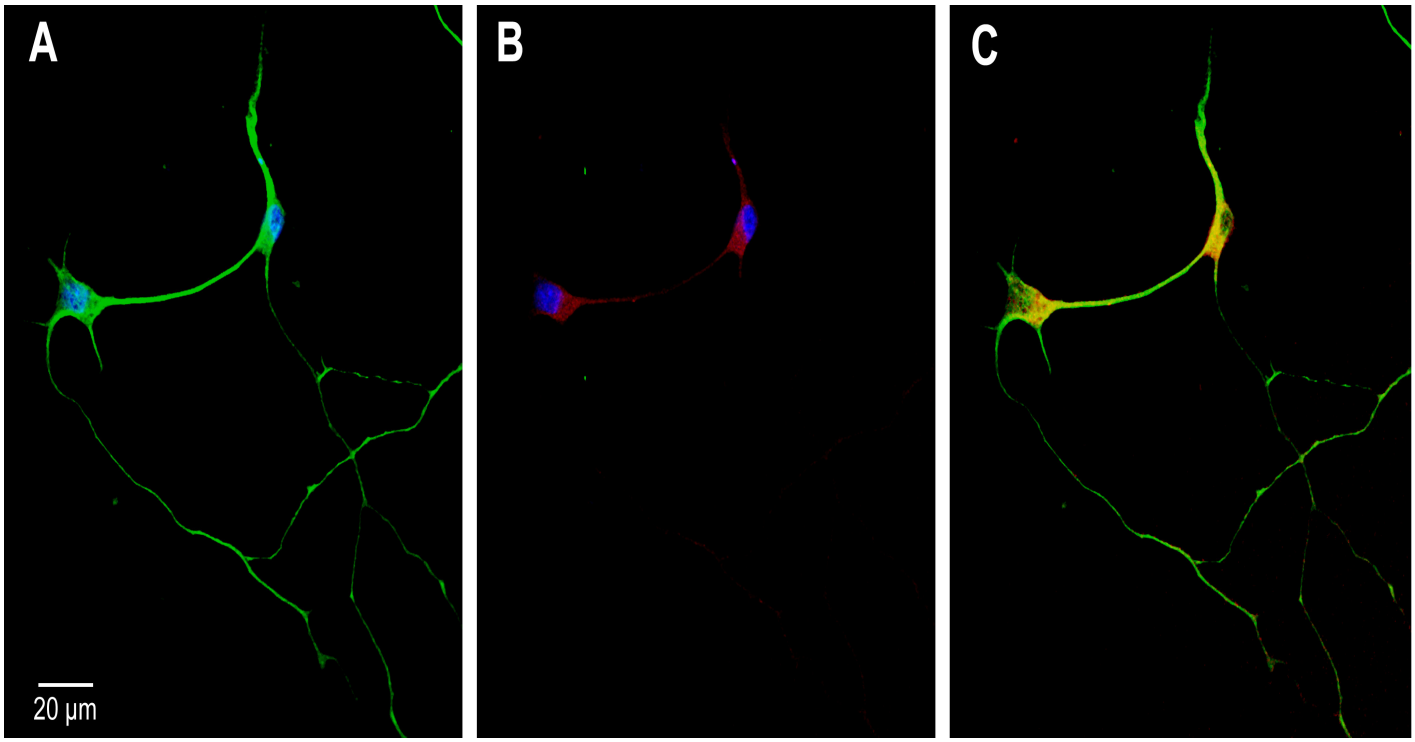

Figure 3. Immunolocalization of Ribin-like protein (green) in motoneurons grown under trophic factor deprivation conditions (A). In (B), localization of DRAQ5, a nuclear marker (blue) and S6 ribosomal protein (red) in the same cells. In (C) colocalization of Ribin-like protein (green) and S6 ribosomal protein (red).

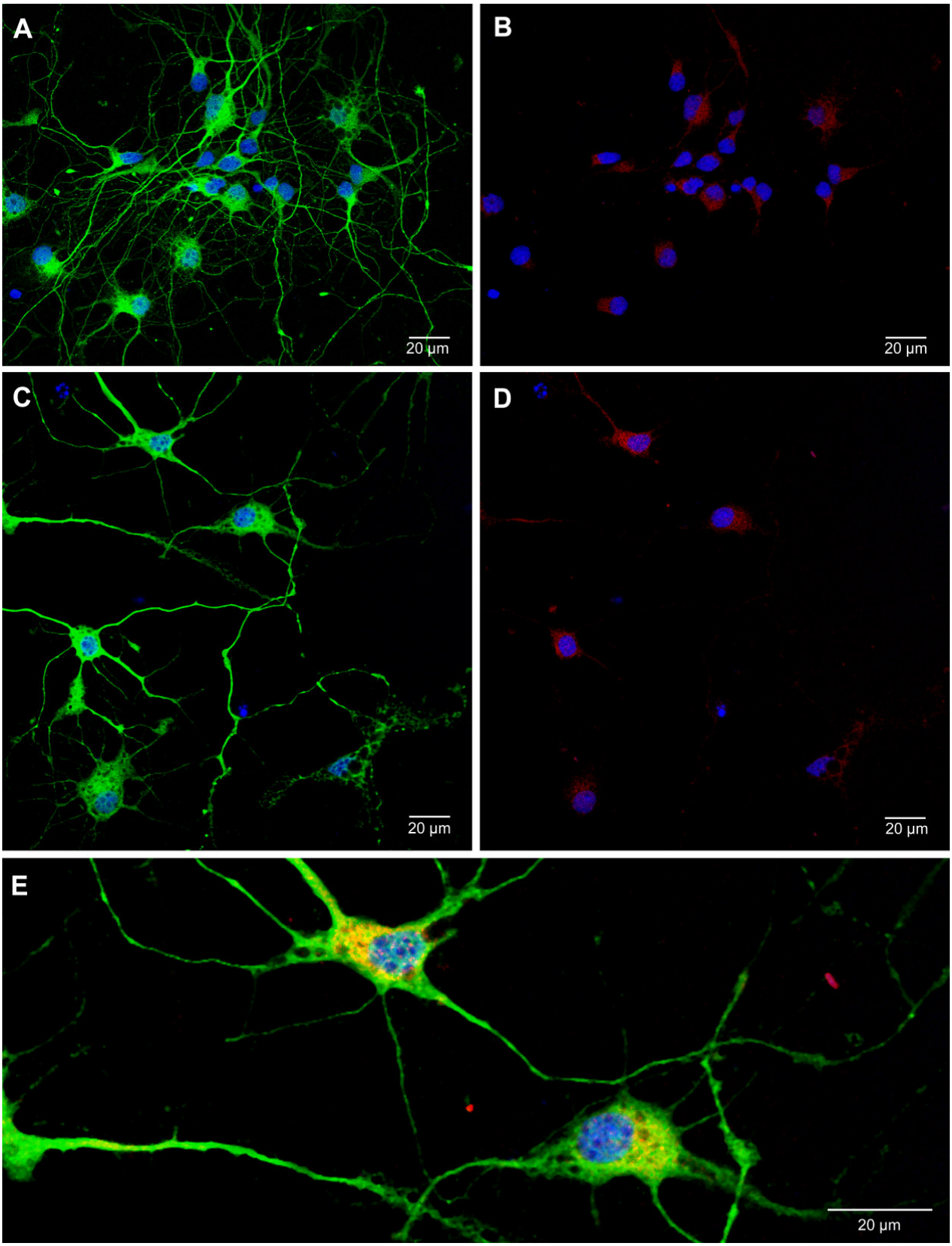

Figure 4. Immunolocalization of Ribin-like proteins (green) in non-stressed cortical cells (A) and in cortical cells treated with 10 µM camptothecin during 16 h (C). In (B and D), immunodetection of the nuclear marker DRAQ5 (blue) and S6 ribosomal protein (red) in the same non-stressed and stressed cortical cells respectively. (E) Colocalization of Ribin-like protein (green), DRAQ5 (blue) and S6 ribosomal protein (red) in cortical cells treated with 10 µM camptothecin for 16 h.

Table 3. Comparison of ribin immunoreactivity (Ribin-IR) in the nucleus and cytoplasm of the three cell types submitted to a specific treatement.

Cell types	Treatment	Ribin-IR (%)	
		Nucleus	Cytoplasm
Striatal cells	Electroporation with cDNA plasmids encoding mHtt	32.6	67.4
Motoneurons	Trophic factor deprivation	7.1	92.9
Cortical cells	10 µM camptothecin / 16 h	14.2	85.7

Table 4. Comparison of ribin immunoreactivity in stressed and non-stressed cells

Cell types	Treatment	Average intensity
Striatal cells	Without	127.24 ± 23.83
	Electroporation with cDNA plasmids encoding mHtt	153.44 ± 33.25
Motoneurons	Without	110.67 ± 02.08
	Trophic factor deprivation	176.25 ± 23.35
Cortical cells	Without	172.33 ± 34.21
	10 µM camptothecin / 16 h	175.18 ± 29.31

that only the COOH-part of the Ribin could be produced inducing a loss of the nuclear localization signals located in the Ribin NH_2-part. This was not the case in the previous study of Kermekchiev and Ivanova (2001) which provided evidence that Ribin has a nuclear localization. Indeed, these authors detected a 31-32 kDa protein signal in Western blot with various cells (which corresponds to the size predicted by the cDNA clone), yet all homologs found/predicted so far presume a shorter protein for the Ribin C-terminal moiety. However, the percentage of Ribin in the nucleus even if it varied according to the cell types (from 7 - 32%) shows evidence that two great types of Ribin-like proteins coexist, at least in rodents.

Conclusion

The physiological role(s) of Ribin-like protein(s) remain unknown; however, evidence of both differential *gene* expression and the relatively conserved regions found in the animal kingdom and even in plants, suggest that these proteins play essential physiological role(s). The results suggest that within the Ribin family, proteins could have at least two functions, a nuclear role as suggested by Kermekchiev and Ivanova (2001) and bibliographical analysis supports that Ribin could also be involved in cellular immune response, brain tissue surveillance, neuronal migration and proliferation. Moreover, experiments using motoneurons could suggest a putative role in neuronal pathogenicity, or at least an overexpression of *ribin*-like *genes* in stressed cells. In the future, the authors will extend this preliminary study; experiments will be carried out to investigate the role of Ribin in neuronal tissues that could further the comprehension of neurological disorders. This requires the development of monoclonal antibodies against the members of the Ribin protein family.

ACKNOWLEDGEMENTS

The authors acknowledge Dr Milko Kermekchiev (DNA Polymerase Technology, Saint Louis, MO 63104, USA, milko@klentaq.com) for the donation of Ribin antibodies and for his helpful comments. They also thank Trophos (Marseille, France, http://www.trophos.com/) for supplying neuronal models, Dr Jean-Claude Gilhodes (Biovays SAS, Marseille, France, http://www.biovays.com) for his help in the quantitative analysis and Dr Thierry Bordet (tbordet@trophos.com) and Pr Jean-paul Casanova (Université de Provence) for their useful comments. The authors thank Carina Richards (communication manager, Biovays, Marseille, France) for helping to improve the English of the text.

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