International Journal of Computer and Communication Technology

Volume 1 | Issue 3 Article 7

July 2010

Prediction of Sub-cellular Localization of Scramblase Protein Family

Babita Sahoo
Institute of Technical Education and Research, 'SOA' Univ, Bhubaneswar,, babitababy83@gmail.com

Santosh Kumar Sahu
Weill Cornell Medical College, Cornell Uni, NewYork, scramblase@gmail.com

Tripti Swarnkar
Deptt. Of CA, SOA University Bhubaneswar, tripti_sarap@yahoo.com

Follow this and additional works at: https://www.interscience.in/ijcct

Recommended Citation

Sahoo, Babita; Sahu, Santosh Kumar; and Swarnkar, Tripti (2010) "Prediction of Sub-cellular Localization of Scramblase Protein Family," *International Journal of Computer and Communication Technology*: Vol. 1 : Iss. 3 , Article 7.
DOI: 10.47893/IJCCT.2010.1043
Available at: https://www.interscience.in/ijcct/vol1/iss3/7

This Article is brought to you for free and open access by the Interscience Journals at Interscience Research Network. It has been accepted for inclusion in International Journal of Computer and Communication Technology by an authorized editor of Interscience Research Network. For more information, please contact sritampatnaik@gmail.com.

Prediction of Sub-cellular Localization of Scramblase Protein Family

Babita Sahoo
Institute of Technical Education and Research, 'SOA' Univ, Bhubaneswar,
babitababy83@gmail.com

Santosh Kumar Sahu
Weill Cornell Medical College, Cornell Uni, NewYork
scramblase@gmail.com

Tripti Swarnkar
Institute of Technical Education and Research, 'SOA' Univ, Bhubaneswar,
tripti_sarap@yahoo.com

Abstract: ***In the present work, we discuss an anaysis about the localization of different members of scramblase protein family. Different scramblase sequences were picked up from organisms of all eukaryotic phyla and their localization were predicted using the P-SORT programme. Our analysis showed that the scramblase protein family shows multiple sub-cellular localization. Most proteins were found to be localized to the cytoplasm, where as others were found to be present in the nucleus or mitochondria. Interestingly, we found that in yeast, all putative scramblases were localized in the nucleus with a reliability of more than 95%. Our analysis shows that scramblases are a family of protein having diversed cellular localization and hence hypothesized to be performing multiple cellular functions in various organisms.***

Key words: scramblase; apoptosis; localization; nuclear localization signal; plasma membrane

I. INTRODUCTION

Phospholipid scramblases (PLSCR) belong to structurally and functionally unique class of proteins that are the product of a tetrad of genes conserved from *Caenorhabditis elegans* to humans [1, 2]. They are named as PLSCR1-4. Each protein of this family consists of multiple functional domains that are highly conserved across different phyla and are closely related evolutionarily [3, 4]. The best characterized member of this family, phospholipid scramblase 1 (PLSCR1) (318 aa) is a calcium binding, multiple palmitoylated endofacial type II plasma membrane (PM) protein. PLSCR1 was discovered by Sims and co-workers as a protein involved in phospholipid (PL) translocation across plasma membrane [4]. It has been reported that an elevation in intracellular calcium levels initiated rapid redistribution of PL between the inner and outer membrane in order to destroy the normal PM asymmetry thereby exposing phosphatidylserine (PS) to the cell surface [4]. The exposure of PS on the cell surface plays a crucial role in the clearance of injured and apoptotic cells by a mechanism named phagocytosis. Hence, Ca^{2+} induced remodeling of plasma membrane phospholipids is central to both vascular haemostatis and cellular clearance mechanisms [5, 6]. However, recent studies indicate that the protein PLSCR1 has a wide spectrum of functions as opposed to its purported role as a lipid 'scrambler'. These include the potential roles of PLSCR1 in cell differentiation, proliferation and apoptosis [7, 8]. PLSCR1 has been shown to interact with several protein kinases including c-Abl and protein kinase C delta (PKC δ) [9]. Expression of PLSCR1 has been shown to be induced by cytokines such as interferons, epidermal growth factors and leukemic cell differentiation inducing agents such as all-trans retinoic acid (ATRA) [10, 11, 12, 13]. Also, reports such as those which implicate PLSCR1 in the suppression of ovarian carcinoma lead us to believe that PLSCR1 may have a potential role in the pathogenesis of several cancers [6, 14, 15]. Similarly, hPLSCR3 is known to involve in lipid synthesis and metabolism [16, 17]. Though previous work done on scramblases shows an increasing possibility of PLSCR1 serving as an anti-leukemic and a tumor suppressor gene, several questions remain to be answered in this regard. Also as discussed earlier, PLSCR1 belongs to a family of closely related proteins with a number of functional domains in various species. Though existing reports detail the presence of these

homologues as well as the conservation of these functional domains in a few organisms, they leave a few questions open for investigation. One such important question among them is: is there any pattern in the divergence of scramblase family across various species during the course of evolution and their divergence in sub cellular distribution. How do scramblases perform multiple cellular functions ? In the present study, we intend to perform an analysis of the sub cellular localization of different scramblase homologs from different organisms belong to a wide verities of phyla to deduce their probable location in the cell. This analysis gives novel insights into the sub-cellular distribution of various members of the phospholipid scramblase family.

II. MATERIALS AND METHODS

A subcellular localization of various members of phospholipid scramblase family was performed. For this purpose, all the different homologues of scramblase identified by the Pfam HMM domains were chosen [18]. Over and above this a Hidden Markov Model (HMM) search was done with the four scramblases of human mouse and rat species and the generated profile was referred to the non redundant database to select any homologues of scramblase missed out by Pfam. This was done using the FasthHMMER server at the MPI Bioinformatics Toolkit [19]. A multiple sequence alignment was performed on the obtained sequence set using the MUSCLE package [20]. The non repetitive unique scramblase sequences were selected for the localization studies (table 1). The prediction of localization was performed using the PSORT bioinformatics tool kit [21]. The yeast sequences were further analyzed for their mitochondrial localization using the MITOPRED free bioinformatics tool [22]. A sequence analysis of yeast scramblases were performed using CLUSTALW2 software [23]

III. RESULTS AND DISCUSSION

A. *Identification of Scramblase Homologs*

The scramblase sequences were picked up for the localization analysis from the Pfam HMM database. Out of the 103 entries given in Pfam the duplicates and the fragments were weeded out and this list was augmented by a FastHMMER search done with human scramblases as seed sequences. This search gave some five to six new proteins which were not picked up in the Pfam HMM. A list of twenty eight sequences taken for localization analysis (table 1). There were six scramblase homologues in *Caenorhabditis elegans* , four from yeast that included *Schizosachharomyces*, *Sachharomyce*s and *Cryptococcus* species., four from *Drosophila melanogaster*. Among the amphibians homologues *Xenopus laevis* as well as *Xenopus tropicalis* were identified. from *Danio rerio,* In birds a homologues was found in *Gallus gallus.* Among the mammals, apart from the ones in humans, Shimpanzee and mouse were selected.

B. *Multiple Sequence Alignment of Scramblases*

A multiple sequence alignment was performed on the collected set of 76 sequences of scramblase homologues using the MUSCLE software (Figure not shown). From the alignment, it can be observed that the major functional domains outlined earlier are conserved to a good extent. Different domains that were found to be conserved among all the organisms were DNA binding domain [M^{86}-E^{118} in hPLSCR1], Cysteine palmitoylation motif [^{184}CCCPCC^{189} in hPLSCR1], nuclear localization signal [NLS] [^{257}GKISKHWTGI^{266} in hPLSCR1], calcium binding motif [^{273}DADNFGIQFPLD^{284} in hPLSCR1] and transmembrane domain [^{288}KMKAVMIGACFLIDFMFFE^{306} in hPLSCR1]. An interesting observation worth noting is the fact that while in the mammals and higher organisms, the different motifs are conserved to a greater extent, these motifs in the lower organisms like *C. elegans* and yeast are conserved to a lesser extent with many non-specific insertions and deletions. There is more similarity among the different homologues of the species than the cross–species homology. Among the five different motifs, the calcium binding motif and the transmembrane domain are the ones which have the greatest extent of conservation when compared to any other domain. This probably suggests that the higher organisms developed the other functional domains related to the signaling functions of scramblases

during the course of evolution as indicated by their DNA binding domains, Cysteine palmitoylation motif and nuclear localization signal. Probably the scramblases in the primitive organisms were more of a dedicated lipid translocator that was shown primarily by their highly conserved transmembrane domain as well as Ca^{2+} binding domain. It can be hypothesized that due to evolutionary divergence, higher organisms possessed scramblases with a repertoire of functions.

Although the transmembrane domain, nuclear localization signal and cysteine palmitoylation motifs of various members of scramblase superfamily shows a very high degree of conservation among various species, the nuclear localization of human phospholipid scramblase is known. Sofar, there is no reports of nuclear localization of other known members of scramblase homologs. Similarly, although the mitochondrial localization of human phospholipid scramblase 3 is known, there is no other report of the existence of other members of the scramblase family in mitochondria. To get further insight into the localization of various members of scramblase proteins across differerent phyla, we performed a prediction of sub-cellular locations of there proteins using the P-SORT program.

C. Sub-cellular localization analysis of the scramblases

The sequence alignment obtained through the MUSCLE program showed a marked conservation of different domains of scramblase homologs [figure not shown]. This shows that all the domains that are essential for the sub-cellular distribution of scramblase homologs are conserved among different species. However, there exists very little information about the sub-cellular distribution of scramblase family members. We performed the localization analysis of all twenty eight scramblase proteins using PSORT programme [21] that showed a great variation in the localization of scramblase family members. This variation not only existed across different species, but also for different homologs of scramblases of the same organisms. In human, it is known that the hPLSCR1 resides in the plasma membrane and performs the function of scrambling of phospholipids. However, recent findings suggests that the human phospholipid scramblase1 also get translocated into the nucleus during its rapid synthesis in response to growth factors and interferons. Our analysis confirms the previous findings showing that it localizes to nucleus. hPLSCR2, hPLSCR3 and hPLSCR4 were found to be localized to the endoplasmic reticulum, cytoplasm and nucleus respectively. The localization of hPLSCR2 and hPLSCR4 to endoplasmic reticulum and nucleus may throw light on the further experimental analysis of these proteins as the sub-cellular localization of these proteins are not yet found out.

For, shimpanzee, the sub-cellular localization of the scramblases were not clear form the analysis. The proteins show a distribution across various organelles. Similarly, mouse PLSCR1 did not show any confined localization in the cell. However, PLSCR2 and PLSCR3 of mouse showed cytoplasmic localization. Interestingly, all scramblase homologs of *Caenorhabditis elegans* showed high (more than or close to 60%) cytoplasmic distribution of all four known scramblase homologs. However, other two homologs showed mixed sub-cellular distribution (Fig. 1). The analysis of sub-cellular distribution of yeast scramblases showed more than 95% of probability that these proteins reside in mitochondria. Fig. 1 shows the representative protein YP_PLSCR1. To get further insight into the mitochondrial localization of yeast scramblases, all known predicted scramblase homologs were collected from PUBMED data base and a multiple sequence alignment was performed using CLUSTAL W software. The sequences of three different yeast species show moderate conservation of their primary structure. However, the N-terminal domain of all scramblases from yeast showed a cleavable mitochondrial targeting segment (fig. 2). The results show that the scramblase homologs found in yeast reside in the mitochondria.

Our prediction of subcellular loclalization of different homologs of scramblase protein family provides insights into further experimental analysis of these scramblases. Infact, human phospholipid scramblase1 shows dual localization. In the plasma membrane as well as in the nucleus. However, the hPLSCR3 has been shown to localize to the mitochondria and translocates cardiolipin across the mitochondrial inner membrane. Our study shows

that hPLSCR2 and hPLSCR4 although have similar primary structure, show localization in the ER and in the nucleus respectively. In yeast, however, the function of scramblase is not clear. Our study shows that the scramblases in yeast might play an important role in cardiolipin translocation and maintenance of the structure and function of its mitochondria.

ACKNOWLEDGEMENT

The authors acknowledge Indian Institute of Technology, Madras for providing the work facility. Santosh Kumar Sahu acknowledge Council of Scientific and Industrial Research for providing fellowship

TABLE 1. List of the 28 scramblase sequences taken up for localization analysis. The sequences were selected as described in materials and methods.

Protein	Organism
hPLSCR1	Scramblase 1 (*Homo sapiens*)
hPLSCR2	Scramblase 2 (*Homo sapiens*)
hPLSCR3	Scramblase 3 (*Homo sapiens*)
hPLSCR4	Scramblase 4 (*Homo sapiens*)
Ptr PLSCR1	Scramblase 1 (*Pan troglodytus*)
Ptr PLSCR2	Scramblase 2 (*Pan troglodytus*)
MHPLSCR1	Scramblase 1 (*Mus musculus*)
MHPLSCR2	Scramblase 2 (*Mus musculus*)
MHPLSCR3	Scramblase 3 (*Mus musculus*)
MHPLSCR4	Scramblase 4 (*Mus musculus*)
GahPLSCR1	Scramblase 1 (*Gallus gallus*)
GahPLSCR2	Scramblase 2 (*Gallus gallus*)
XehPLSCR1	Scramblase1 (*Xenopus tropicalis)*
XehPLSCR2	Scramblase 2 (*Xenopus tropicalis)*
XehPLSCR3	Scramblase 3 (*Xenopus tropicalis)*
DaPLSCR1	Scramblase 1 (*Danio rerio*)

DaPLSCR2	Scramblase 2 (*Danio rerio*)
YP PLSCR1	Putative scramblase (*Sachharomyces cerevisiae*)
DRPLSCR1A	Scramblase 1 (isoform a) (*Drosophila melanogaster*)
DRPLSCR1B	Scramblase 1 (isoform b) (*Drosophila melanogaster*)
DRPLSCR1C	Scramblase 1 (isoform c) (*Drosophila melanogaster*)
DRPLSCR2	Scramblase 2 (*Drosophila melanogaster*)
CEPLSCR1	Scramblase1 (*Caenorhabditis elegans*)
CEPLSCR2	Scramblase 2 (*Caenorhabditis elegans*)
CEPLSCR3	Scramblase 3 (*Caenorhabditis elegans*)
CEPLSCR4	Scramblase 4 (*Caenorhabditis elegans*)
CEPLSCRL1	Putative cramblase1 (*Caenorhabditis elegans*)
CEPLSCRL2	Putative scramblase2 (*Caenorhabditis elegans*)

Protein	ID	N	M	C	V	E	EC	VA	G	PM	C/N	Pre	R
Hplscr1	NP_066928.1	21.7	4.3	21.7	17.4	8.7	4.3	4.3	4.3	--	--	N	55.5
Hplscr2	NP_065092.1	11.1	33.3	--	--	55.6	--	--	--	--	C	E	89
HPLSCR3	NP_065093.2	8.7	13.0	73.9	4.3	--	--	--	--	--	C	C	89
HPLSCR4	NP_065086.2	65.2	--	13.0	8.7	--	--	--	4.3	4.3	N	N	70.6
Ptr Hplscr2	XP_001135229.1	13.0	13.0	26.1	--	8.7	--	4.3	4.3	8.7	C	C	55.5
Ptr Hplscr1	XP_001135163.1	13.0	13.0	26.1	17.4	--	--	4.3	4.3	--	C	C	70.6
MHPLSCR1	NP_035766.2	8.7	13.0	21.7	21.7	8.7	--	8.7	8.7	8.7	C	C	55.5
MHPLSCR2	NP_032906.2	17.4	--	43.5	4.3	--	--	4.3	--	--	C	C	70.6
MHPLSCR3	NP_076053.1	13	8.7	69.6	4.3	--	--	--	4.3	--	C	C	89
MHPLSCR4	NP_848826.1	52.5	--	26.1	4.3	---	--	--	4.3	4.3	N	N	70.6
GahPLSCR1	XP_001231237.1	8.7	13	21.7	21.7	8.7	--	4.3	4.3	13	C	C	55.5
GahPLSCR2	XP_422696.2	8.7	--	21.7	21.7	8.7	--	4.3	4.3	4.3	C	C	55.5
XehPLSCR1	NP_001089425.1	22.2	11.1	11.1	11.1	22.2	--	--	11.1	11.1	N	N	55.5
XehPLSCR2	NP_001090508.1	17.4	4.3	17.4	21.7	8.7	4.3	4.3	8.7	8.7	N	N	70.6
XehPLSCR3	AAH88016.1	21.7	4.3	26.1	13	13	4.3	--	8.7	4.3	N	N	76.7
DaPLSCR1	NP_998031.1	60.9	--	17.4	4.3	--	--	--	8.7	4.3	N	N	55.5
DaPLSCR1	NP_001121867.1	13	13	26.1	21.7	4.3	--	4.3	4.3	8.7	C	C	94.1
YP PLSCR1	ABN66823.2	4.3	95.7	--	--	--	--	--	--	--	C	M	89
DRPLSCR1A	AAF50165.3	21.7	13.0	52.2	--	8.7	--	--	--	--	C	C	70.6
DRPLSCR1B	AAN11924.1	21.7	13.0	52.2	--	8.7	--	--	--	--	C	C	70.6
DRPLSCR1C	AAN11923.1	43.5	8.7	30.4	4.3	--	--	--	8.7	--	N	N	55.5
DRPLSCR2	AAF47705.1	22.2		22.2	33.3	11.1	--	--	11.1	--	C	C	76.7
CEPLSCR1	ABQ01576.1	17.4	17.4	56.7	4.3	--	--	4.3	--	--	C	C	94.1
CEPLSCR2	ABQ01577.1	13	13	69.6	4.3	--	--	--	--	--	C	C	94.1
CEPLSCR3	ABQ01578.1	17.4	13	60.9	--	--	--	--	4.3	--	C	C	89
CEPLSCR4	ABQ01579.1	8.7	--	65.1	4.3	--	--	4.3	4.3	--	C	C	94.1
CEPLSCRL1	AAA98029.1	17.4	13	26.1	17.4	4.3	--	4.3	4.3	8.7	C	C	70.6
CEPLSCRL2	AAA98028.1	13	13	26.1	17.4	8.7	--	4.7	4.7	8.7	C	C	76.7

Figure 1: [a]Different abbreviations used in the figure are as follows. ID represents the genpept identification number of the protein from PUBMED. N: nuclear, M: mitochondia, C: cytoplasm, V: vesicular trafficking network, E: endoplasmic reticulum, EC: extracellular, VA: vacuole, G: golgi, PM: plasma membrane, C/N: ratio of cytoplasmic to nuclear distribution as determined by PSORT programme. P: prediction of the localization of the protein as determined by PSORT programme. R represents the reliability of the prediction.

```
PS_PLSCR      ------MSLLRTTLRGVHLRSPAAFSSSIFIRSFGTSSLLKFPRKTRITQEISAEEVKRY 54
YJR_PLSCR     -----------------MHRTAIFLTYRSCMRNFSTLSKTLTVSSGKVIRNGPFRRVIR- 42
SS_PLSCR      --------------------MLEILWANITPIQTFVSSNHLTMLYGLKRFGCRLYHHSKST 41
CN_PLSCR      MLPRSITASALHVGTSRSMLARSIATAVPLLRQQERLPRGHVRPTRRPIRQIPFRTGTGT 60

PS_PLSCR      EEQQHQQGNNAKDFYYRTNPSSDAGEYRNEPSSFHTIFNIPPNENGLITPEDGIYD-ILK 113
YJR_PLSCR     EKNQITKAPSVKAFKENSN-------------------------SGIIKVHDPIATTILN 77
SS_PLSCR      RYIDATAKVVSQEPAAISS-------------------------TGAIPLNSPAAP-LLS 75
CN_PLSCR      DEHYQSTAAGPQAFHAYEPYDPLSTPIHASG-----PVDIPPDPSGVLG-DSHAAREILG 114

PS_PLSCR      EPTLVIERQIEIANVILGFEQANRYKIMNSTGEQIGYMQEK-DLGILKVIGRQFFRLHRP 172
YJR_PLSCR     EPTVIIERQIEFMNVFLGFEQANRYAIMDVNGNKIASMMER-DFSITKAIMRQFYRLHRP 136
SS_PLSCR      QDVLIVERQLEMMNVFLGYEQANRYVILNQQGQHLGYIAEQGASSILSSLSRQFFHTHRA 135
CN_PLSCR      HESLVIVRQLEMLNVFMGFEQANRYAIHSPDGQLVGFLAEQ-EQGILSTISRQALRTHRP 173

PS_PLSCR      FDIDVFNNYGDLLLTIKRPFSFINSHIKCFLP-----GYDTDNSLIFEK-----IGESIQ 222
YJR_PLSCR     FLVDVFDNWGNVIMTIKRPFSFINSHIKTIIPPSAYVDNGSDSTHYHDGKEGTTVGETIQ 196
SS_PLSCR      FKADVMDSNGQLVLQLNRPFSWINSRLQ---------IHSIDYSKFSST----LVGEVLQ 182
CN_PLSCR      FKSIVMDRHGKPVLWIQRPFAFINSRIF------------VHSSEDRDSR---LVGEAQQ 218

PS_PLSCR      SWHLWRRRYNLFKLEDEVTDDFNQFGAIDAPFLSFDFPVKNQNGDVIASVDRNWVGLGRE 282
YJR_PLSCR     NWHLWRRRYELFQKDGVEGSTFDQFGKIDAPFLSFDFPVTDADGKIMASVDRNWVGLGRE 256
SS_PLSCR      KWHLWRRRYELFLAK---RSMFEQFAKIDERVLSWEFLLRNEQDRILGSVSRNFMGLPRE 239
CN_PLSCR      QWHPWRRRYNLFQSR--ESETFRQFAKVDSGFLAWDFWLKDKDDRLLASINRNFRGIGRE 276

PS_PLSCR      LFTDTGVYIVRMDPASFAGMGELYPTVAGP------------LTLDQRAILLGNAVSIDF 330
YJR_PLSCR     MFTDTGVYVVRFD--SQRCFDNIYPTEMLSSQ---------VLTLDQRAVLLANAVSIDF 305
SS_PLSCR      FFTDTGNYVLRFTSTSAANGSVNENQLLQAAHGIANDVCARDMSLEERAVMLGSAVTIDF 299
CN_PLSCR      LFTDTGQHSPQQYQVCTRDTNR-----------------------ENSALKPLCVVVDF 312

PS_PLSCR      DYFSRHSRGPGGG---FLSFGGGE---------------------------------- 351
YJR_PLSCR     DYFSRHSRQTGG----FLSFGGGYDE------------------------------- 327
SS_PLSCR      DYFSRIHGGPALGLNIPFMFGGSSSNHDYPAEDLSAQEILKNDQETTPSTNDSSSETKSP 359
CN_PLSCR      DYFSRHSGSGGLG-FPFFFWGGGDGGADAQSGGRPSDVQPSPDGGAGAGGVAAGAAAGAA 371
```

Figure 2. A sequence alignment of all yeast scramblases that showed above 95% localization in the mitochondria. The abbreviations are as follows: PS_PLSCR: *Pichia pastoris* scramblase, YJR_PLSCR: YJR like protein *of Sachharomyces cereviseae*, SS_PLSCR: *Sachharomyces* hypothetical protein homologous to scramblase and CN_PLSCR represents the *Cryptococcus* PLSCR. The N-terminal shaded peptide shows the portion of the protein that is cleaved during the translocation of the protein into the mitochondria. The sequence
analysis was performed by using CLUSTAL W and the N-terminal clevable sequences were predicted using the programme MITOPRED [22]

REFERENCES

[1] T. Wiedmer, Q. Zhou, D. Kwoh, and P. J. Sims, "Identification of three new members of the phospholipid scramblase gene family", Biochimica et Biophysica Acta (BBA)- Biomembranes, vol. 1467, pp. 244-253, July 2000.

[2] P. J. Sims and T. Wiedmer, "Unraveling the mysteries of phospholipid scrambling", Thromb Haemost, vol. 86, pp 266-275, July 2001.

[3] F. Basse, J. G. Stout, P. J. Sims and T. Wiedmer, "Isolation of an Erythrocyte Membrane Protein that Mediates Ca^{2+}-dependent Transbilayer Movement of Phospholipid", J. Biol. Chem., vol. 271, pp 17205-17210, July 1996.

[4] Q. Zhou, J. Zhao, J. G. Stout, R. A. Luhm, T. Wiedmer and P. J. Sims, "Molecular Cloning of Human Plasma Membrane Phospholipid Scramblase. a protein mediating transbilayer movement of plasma membrane phospholipids", J. Biol. Chem., vol. 272, pp 18240-18244, July 1997.

[5] S. K. Sahu, S. N. Gummadi, N. Manoj and A. Gopalakrishna, "Phospholipid scramblases: An overview", Arch. Biochim.Biophys., vol. 46, pp 103-114, June 2007.

[6] Y. Huang, Q. Zhao, and G. Q. Chen, "Phospholipid scramblase 1", Acta Physiologica Sinica, vol. 6, pp 501-510, September 2006a.

[7] K. W. Zhao, D. Li, Q. Zhao, Y. Huang, R. H. Silverman, P. J. Sims and G. Q. Chen, "Interferon-{alpha}-induced Expression of Phospholipid Scramblase 1 through STAT1 Requires the Sequential Activation of Protein Kinase C{delta} and JNK", J. Biol. Chem., vol. 280, pp 42707-42714, December 2005.

[8] Q. Zhou, J. Zhao, T. Wiedmer, and P. J. Sims, ''Normal hemostasis but defective hematopoietic response to growth factors in mice deficient in phospholipid scramblase 1'', Blood, vol. 99, pp 4030-4038, June 2002.

[9] Y. Huang, Q. Zhao, C. X. Zhou, Z. M. Gu, D. Li, H. Z. Xu, P. J. Sims, K. W. Zhao and G. Q. Chen, "Antileukemic roles of human phospholipid scramblase 1 gene, evidence from inducible PLSCR1-expressing leukemic cells", Oncogene, vol. 25, pp 6618-6627, October 2006b.

[10] B. Dong, Q. Zhou, J. Zhao, A. Zhou, R. N. Harty, S. Bose, A. Banerjee, R. Slee, J. Guenther, B. R. G. Williams, T. Wiedmer, P. J. Sims and R. H. Silverman, "Phospholipid Scramblase 1 Potentiates the Antiviral Activity of Interferon", J. Virol, vol. 78, pp 8983-8993, April 2004.

[11] T. Nakamaki, J. Okabe-Kado, Y. Yamamoto-Yamaguchi, K. Hino, S. Tomoyasu, Y. Honma and T. Kasukabe, "Role of MmTRA1b/phospholipid scramblase1 gene expression in the induction of differentiation of human myeloid leukemia cells into granulocytes", Exp. Hematol., vol. 30, pp 421-429, May 2002.

[12] R. H. Silverman, A. Halloum, A. Zhou, B. Dong, F. Al-Zoghaibi, D. Kushner, Q. Zhou, J. Zhao, T. Wiedmer and P. J. Sims, "Suppression of Ovarian Carcinoma Cell Growth in Vivo by the Interferon-inducible Plasma Membrane Protein, Phospholipid Scramblase 1", Cancer Res., vol. 62, pp 397-402, January 2002.

[13] A. Rami, P. J. Sims, G. Boetz and J. Winckler, "Spatial resolution of phospholipid scramblase 1 (PLSCR1), caspase-3 activation and DNA-fragmentation in the human hippocampus after cerebral ischemia", Neurochem. Int., vol. 43, pp 79-87, July 2003.

[14] Y. Li, K. Rogulski, Q. Zhou, P. J. Sims and E. V. Prochownik, "The Negative c-Myc Target Onzin Affects Proliferation and Apoptosis via Its Obligate Interaction with Phospholipid Scramblase I", Mol. Cell. Biol., vol. 26, pp 3401-3413, May 2006.

[15] T. Kasukabe, J. Okabe-Kado, and Y. Honma, "TRA1, a Novel mRNA Highly Expressed in Leukemogenic Mouse Monocytic Sublines but not in nonleukemogenic sublines'', Blood, vol. 89, pp 2975-2985, April 1997.

[16] T. Wiedmer, J. Zhao, L. Li, Q. Zhou, A. Hevener, J. M. Olefsky, L. K. Curtiss, and P. J. Sims, "Adiposity, dyslipidemia, and insulin resistance in mice with targeted deletion of phospholipid scramblase 3 (PLSCR3)", Proc. Natl. Acad. Sci. U.S.A., vol. 101, pp 13296-13301, August 2004.

[17] A. Yu, C. R. McMaster, D. M. Byers, N. D. Ridgway, and H. W. Cook, "Stimulation of Phosphatidylserine Biosynthesis and Facilitation of UV-induced Apoptosis in Chinese Hamster Ovary Cells Overexpressing Phospholipid Scramblase", J. Biol. Chem., vol. 278, pp 9706-9714, March 2003.

[18] R. D. Finn, J. Mistry, B. Schuster-Bockler, S. Griffiths-Jones, V. Hollich, T. Lassmann, S. Moxon, M. Marshall, A. Khanna, R. Durbin, S. R. Eddy, E. L. L. Sonnhammer, and A. Bateman, "Pfam: clans, web tools and services", Nucl. Acids Res., vol. 34, pp D247-D251, October 2006.

[19] A. Biegert, C. Mayer, M. Remmert, J. Soding, A. N. Lupas, ''The MPI Bioinformatics Toolkit for protein sequence analysis", Nucl. Acids Res., vol. 34, pp W335-W339, March 2006.

[20] R. Edgar, "MUSCLE: a multiple sequence alignment method with reduced time and space complexity", BMC Bioinformatics, vol. 5, pp 113, August 2004.

[21] Y. Yu. Nancy, R. Wagner; R. M., Laird, G. Melli, S. Rey, R. Lo, P. Dao, S. C. Sahinalp, M. Ester, L. J. Foster, F. S. L. Brinkman, "PSORTb 3.0: Improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes", Bioinformatics, doi: 10.1093, May 13 2010.

[22] M. Kumar, R. Verma, and G.P.S. Raghava, "Prediction of Mitochondrial Proteins Using Support Vector Machine and Hidden Markov Model", J. Biol. Chem., vol. 281, pp 5357-5363, March 2005.

[23] J. D. Thompson, D. G. Higgins, and T.J. Gibson, "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice", Nucleic Acids Res., vol. 22, pp 4673-4680, November 1994.