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Synaptotagmin gene content of the sequenced genomes

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Abstract

Background: Synaptotagmins exist as a large gene family in mammals. There is much interest in the function of certain family members which act crucially in the regulated synaptic vesicle exocytosis required for efficient neurotransmission. Knowledge of the functions of other family members is relatively poor and the presence of Synaptotagmin genes in plants indicates a role for the family as a whole which is wider than neurotransmission. Identification of the Synaptotagmin genes within completely sequenced genomes can provide the entire Synaptotagmin gene complement of each sequenced organism. Defining the detailed structures of all the Synaptotagmin genes and their encoded products can provide a useful resource for functional studies and a deeper understanding of the evolution of the gene family. The current rapid increase in the number of sequenced genomes from different branches of the tree of life, together with the public deposition of evolutionarily diverse transcript sequences make such studies worthwhile.

Results: I have compiled a detailed list of the Synaptotagmin genes of *Caenorhabditis*, *Anopheles*, *Drosophila*, *Ciona*, *Danio*, *Fugu*, *Mus*, *Homo*, *Arabidopsis* and *Oryza* by examining genomic and transcript sequences from public sequence databases together with some transcript sequences obtained by cDNA library screening and RT-PCR. I have compared all of the genes and investigated the relationship between plant Synaptotagmins and their non-Synaptotagmin counterparts.

Conclusions: I have identified and compared 98 Synaptotagmin genes from 10 sequenced genomes. Detailed comparison of transcript sequences reveals abundant and complex variation in Synaptotagmin gene expression and indicates the presence of Synaptotagmin genes in all animals and land plants. Amino acid sequence comparisons indicate patterns of conservation and diversity in function. Phylogenetic analysis shows the origin of Synaptotagmins in multicellular eukaryotes and their great diversification in animals. Synaptotagmins occur in land plants and animals in combinations of 4–16 in different species. The detailed delineation of the Synaptotagmin genes presented here, will allow easier identification of Synaptotagmins in future. Since the functional roles of many of these genes are unknown, this gene collection provides a useful resource for future studies.

Background

Synaptotagmin (Syt) 1 was initially found as a protein component of synaptic vesicles [1]. New members of the *Syt* gene family have subsequently been discovered by DNA sequence similarity [2-15]. *Syts* encode proteins which share a common structure: an N-terminal trans-membrane sequence joined to a variable length linker, followed by two tandemly arranged, distinct C2 domains,

C2A and C2B. At present, a great deal more is known about Syt1 than the other Syts because it functions crucially in synaptic vesicle trafficking in the nervous systems of animals [16]. Other Syts are implicated in trafficking events in the nervous system as well as in various other tissues [17,18]. Certain *Syts* are known to express alternatively spliced transcripts [19-21] and RNA editing of *Drosophila Syt1* has been described [22]. Little is known however, about the details of the variations in expression of different Syts.

Public sequence database resources are becoming quite comprehensive, including vast numbers of transcript sequences from a wide variety of organisms as well as a number of relatively complete genome sequences. Systematic identification of *Syts* by database searching makes it possible to begin to address questions such as: what is the evolutionary extent of this gene family? where do these genes appear on the tree of life? and how many of these genes does an organism need?

Building on my previous effort to extract the *Syt* content of the sequenced genomes [13] I have now collected information for 98 *Syts* from organisms with sequenced genomes. Transcript sequences reveal abundant variation in *Syt* expression and indicate the presence of *Syts* in all land plants and all animals.

Results and Discussion

Identification of Syts

Previously [13] I used a 44 amino acid sequence probe, representing the most highly conserved stretch of all the known Syts, and lying within a single exon in the C2B region, to search the sequence databases. This probe detected all the loci within the available genomes which could harbour *Syts*, but in order to confirm that these loci did indeed encode Syts it was necessary to ascertain that all the relevant parts were present (N-terminal transmembrane sequence, variable length linker, C2A and C2B). Whilst some regions (C2A and C2B) are well conserved, there is great variation in the sequences of other regions. It is difficult to predict exons accurately from genomic sequence unless a good degree of sequence similarity is present. Transcript sequences can reveal the true gene structure but few transcripts were available at that time, so although I could locate the already known *Syts* in *Caenorhabditis*, *Drosophila* and *Homo*, it was clear that there were more potential *Syts* in each of these genomes and that *Syt* relatives may even be present in plants, which would indicate a general function for this gene family, not restricted to the operation of nervous systems.

Recently, more genomes have been sequenced and some very good transcript resources have become available. I have also carried out cDNA library screening and RT-PCR to investigate the *Arabidopsis Syts*, the novel *Homo Syts* and the alternative splicing of *Rattus Syt1* (accession numbers aj617615-aj617630). I used tblastn and blastn to search sequences at NCBI [23], EBI [24], Ensembl [25] and JGI [26]. I assembled transcript sequences into gap4 databases [27] and used Spin [27] and Align [28] to compare transcripts with genomic sequence. I have compiled a list of 98 *Syts* from the genomes of *Caenorhabditis*, *Anopheles*, *Drosophila*, *Ciona*, *Danio*, *Fugu*, *Mus*, *Homo*, *Arabidopsis* and *Oryza* ([see Additional File 1] entries 1–98). This list summarizes the results of the database searches and includes genomic locations, amino acid sequences, exon structures and alternative splicing patterns. The identities of all the sequences examined here are summarized in Table 1. Where *Syt* synonyms exist, I have chosen the human gene names used by Ensembl [25] but have also indicated synonyms within parentheses.

Fig. 1 shows the chromosomal locations of *Homo* and *Mus Syts*. *Syt2* and *Syt14*, *Syt6* and *Syt11*, *Syt8* and *Syt9*, *Syt3* and *Syt5*, and *Syt7* and *Syt12*, are linked in both *Homo* and *Mus*. *Syt4*, *Syt15*, and *Syt16* are each solitary in both *Homo* and *Mus*. Linkage of *Syt1*, *Syt10* and *Syt13* is different in *Homo* and *Mus*. Different *Homo* (*Syt9*) and *Mus* (*Syt4*, *Syt12*) *Syts* are associated with overlapping antisense transcripts.

Syt comparisons

I used clustalw at EBI [24] to compare all 98 Syts (fig. 2) and Multalin [29] followed by manual editing to produce mulitple alignments (figs. 3,4,5,6,7,8). Where alternative splicing produces complex sequence variation, I chose one representative sequence. Fig. 2 shows the clustalw cladogram tree of relationships between the Syts. The multiple alignments are arranged in the same way, with N-terminus and linker regions in figs 3,4,5 and C2A to C-terminus regions in figs 6,7,8. Intron positions, alternative splicing and RNA-edited positions are indicated.

Animal *Syts* are distributed over more than 7 main branches of the cladogram tree while plant *Syts* occupy a separate main branch. Groups of orthologues and paralogues appear on closely linked sub-branches. A group of orthologues includes genes from different species for example, all *Syt1* genes. Paralogues are multiple versions of one gene within the same species. The paralogues of *Syt1* in *Mus* and *Homo* are *Syt2*, *Syt5* and *Syt8*. I have used the tree and multiple alignment information to give provisional names to as many *Syts* as possible (Table 1).

The 6 *Arabidopsis Syts* and 8 *Oryza Syts* are each found on three sub-branches. The *Oryza* genome is polyploid so one would expect multiple copies of many genes, and since the genome sequence is incomplete, further *Oryza Syts* may yet be found. Searches of plant transcript sequences,

Table 1: Summary of sequence identities

Number	Organism	Names	Number	Organism	Names	Number	Organism	Names	C2 domains
1	Caenorhabditis		43	Fugu	syt6 Frsyt6	85	Arabidopsis	AtsytB	
2	Caenorhabditis		44	Danio	syt6.1 Drsyt6.1	86	Arabidopsis	AtsytA	
3	Caenorhabditis		45	Danio	syt6.2 Drsyt6.2	87	Arabidopsis	AtsytC	
4	Caenorhabditis	syt1 Cesyt1	46	Fugu	syt12 Frsyt12	88	Oryza		
5	Caenorhabditis		47	Danio	syt13 Drsyt13	89	Oryza		
6	Caenorhabditis	syt7 Cesyt7	48	Fugu	syt16 Frsyt16	90	Oryza		
7	Caenorhabditis	syt4 Cesyt4	49	Danio	syt16 Drsyt16	91	Oryza	OssytB	
8	Anopheles	syt1 Agsyt1	50	Danio	syt14 Drsyt14	92	Oryza	OssytC	
9	Anopheles	syt4 Agsyt4	51	Fugu	syt14.1 Frsyt14.1	93	Arabidopsis	AtsytD	
10	Anopheles		52	Fugu	syt14 Frsyt14	94	Arabidopsis	AtsytE	
11	Anopheles	syt13 Agsyt13	53	Mus	syt1 Mmsyt1	95	Oryza		
12	Anopheles	syt16 Agsyt16	54	Mus	syt2 Mmsyt2	96	Oryza		
13	Drosophila	syt1 Dmsyt1	55	Mus	syt3 Mmsyt3	97	Arabidopsis	AtsytF	
14	Drosophila	syt4 Dmsyt4	56	Mus	syt4 Mmsyt4	98	Oryza	OssytF	
15	Drosophila	syt7 Dmsyt7	57	Mus	syt5 Mmsyt5 (syt9)	99	Ceratopteris		
16	Drosophila		58	Mus	syt6 Mmsyt6	100	Physcomitrella		
17	Drosophila	syt13 Dmsyt13	59	Mus	syt7 Mmsyt7	101	Physcomitrella		
18	Drosophila	syt12 Dmsyt12	60	Mus	syt8 Mmsyt8	102	Pinus		
19	Drosophila	syt16 Dmsyt16	61	Mus	syt9 Mmsyt9 (syt5)	103	Pinus		
20	Ciona	syt1 Cisyt1	62	Mus	syt10 Mmsyt10	104	Pinus		
21	Ciona	syt7 Cisyt7	63	Mus	syt12 Mmsyt12 (syt11)	105	Pinus		
22	Ciona	syt15 Cisyt15	64	Mus	syt11 Mmsyt11 (syt12)	106	Physcomitrella	PpsytF	
23	Ciona	syt16 Cisyt16	65	Mus	syt13 Mmsyt13	107	Ceratopteris	CrsytF	
24	Danio	syt1 Drsyt1	66	Mus	syt14 Mmsyt14	108	Arabidopsis	CaLB	1
25	Fugu	syt1 Frsyt1	67	Mus	syt16 Mmsyt16 (syt14r)	109	Arabidopsis		1
26	Danio	syt5.1 Drsyt5.1	68	Mus	syt15 Mmsyt15	110	Arabidopsis		1
27	Fugu	syt5.1 Frsyt5.1	69	Homo	syt1 Hssyt1	111	Arabidopsis		1
28	Danio	syt5.2 Drsyt5.2	70	Homo	syt2 Hssyt2	112	Arabidopsis		1
29	Danio	syt2 Drsyt2	71	Homo	syt3 Hssyt3	113	Oryza		1
30	Fugu	syt5.2 Frsyt5.2	72	Homo	syt4 Hssyt4	114	Oryza		1
31	Fugu	syt8 Frsyt8	73	Homo	syt5 Hssyt5 (syt9)	115	Oryza		1
32	Danio	syt4 Drsyt4	74	Homo	syt6 Hssyt6	116	Oryza		1
33	Fugu	syt4 Frsyt4	75	Homo	syt7 Hssyt7	117	Oryza		1
34	Danio	syt11.2 Drsyt11.2	76	Homo	syt8 Hssyt8	118	Caenorhabditis	MBC2	3
35	Danio	syt11.1 Drsyt11.1	77	Homo	syt9 Hssyt9 (syt5)	119	Drosophila	CG6643	3
36	Fugu	syt11 Frsyt11	78	Homo	syt10 Hssyt10	120	Homo	KIAA0747	>3
37	Danio	syt9.2 Drsyt9.2	79	Homo	syt12 Hssyt12 (syt11)	121	Homo	CHR3SYT	>2
38	Fugu	syt9.2 Frsyt9.2	80	Homo	syt11 Hssyt11 (syt12)	122	Homo	KIAA1228	3
39	Danio	syt9.1 Drsyt9.1	81	Homo	syt13 Hssyt13	123	Saccharomyces	Tricalbin3	4
40	Fugu	syt9.1 Frsyt9.1	82	Homo	syt14 Hssyt14	124	Saccharomyces	Tricalbin2	3
41	Fugu	syt10 Frsyt10	83	Homo	syt16 Hssyt16 (syt14r)	125	Saccharomyces	Tricalbin1	3
42	Danio	syt10 Drsyt10	84	Homo	syt15 Hssyt15	126	Trypanosoma		1

reveal the presence of *Syts* in all the land plants. Sequences from *Pinus*, *Physcomitrella* and *Ceratopteris* ([see Additional File 1] entries 99–107) demonstrate the presence of plant *Syts* across the whole evolutionary range of land plants.

Animals have a more diverse array: 7 *Syts* in *Caenorhabditis*, 5 or more in *Anopheles* (incomplete genome sequence), 7 in *Drosophila*, 4 or more in *Ciona* (a surprisingly small number perhaps, but an incomplete genome sequence), 13–14 in *Danio* and *Fugu* (incomplete genome sequences) and 16 in *Mus* and *Homo*. Bearing in mind that some of the genome sequences are incomplete, the overall picture appears to reflect both acquisition and loss of different types of *Syt*, with different animals bearing different arrays of *Syts*. I have highlighted a motif (G X X X P E L Y) in the linker region of the *Syt15* orthologues (fig. 4) which

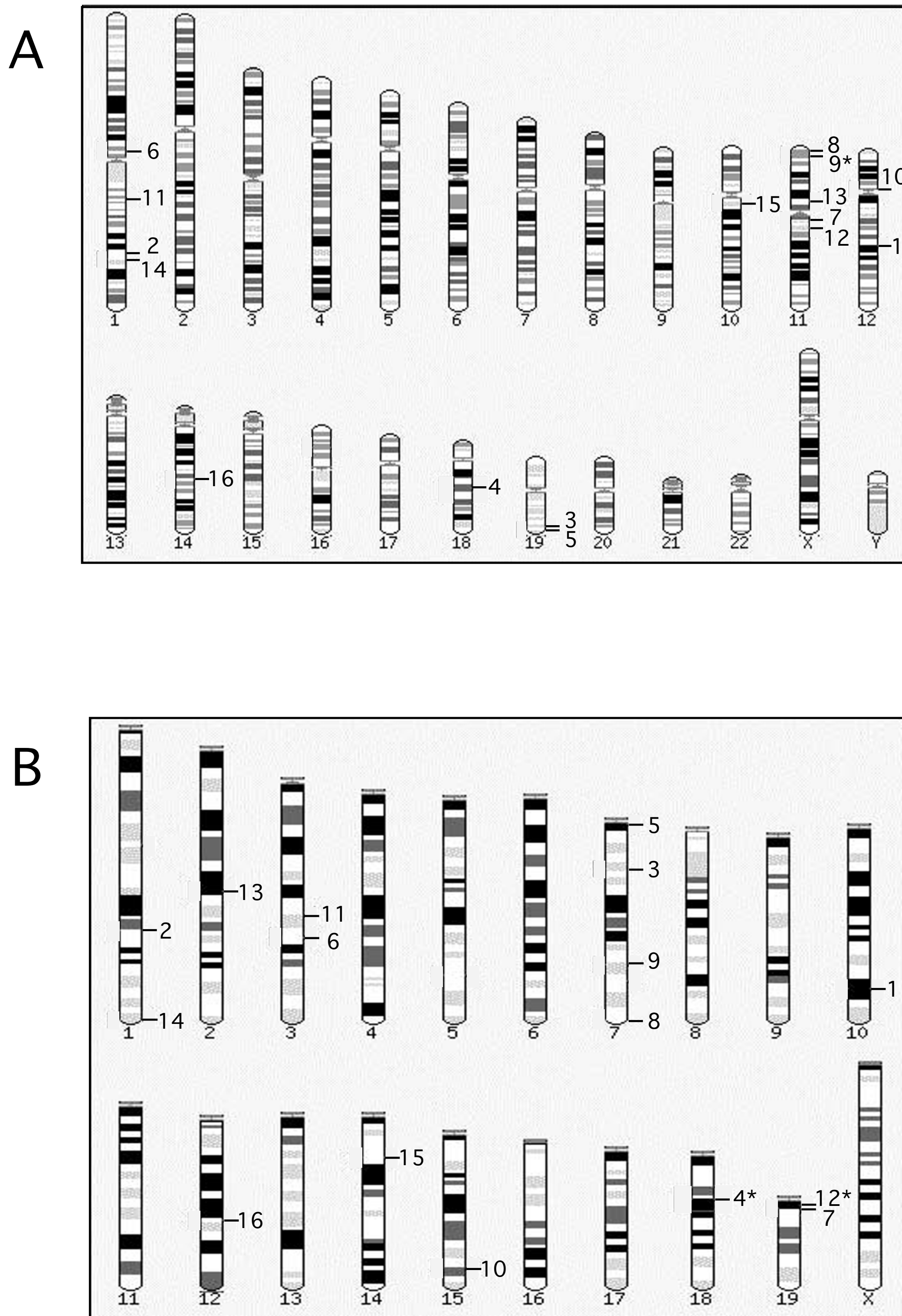

Figure 1
Chromosomal locations of *Homo* and *Mus Syts* I have labelled ideograms produced from blast search results at Ensembl [25] with the locations of *Syt1-Syt16*. (A) *Homo*. (B) *Mus*. Asterisks indicate loci with overlapping antisense transcription.

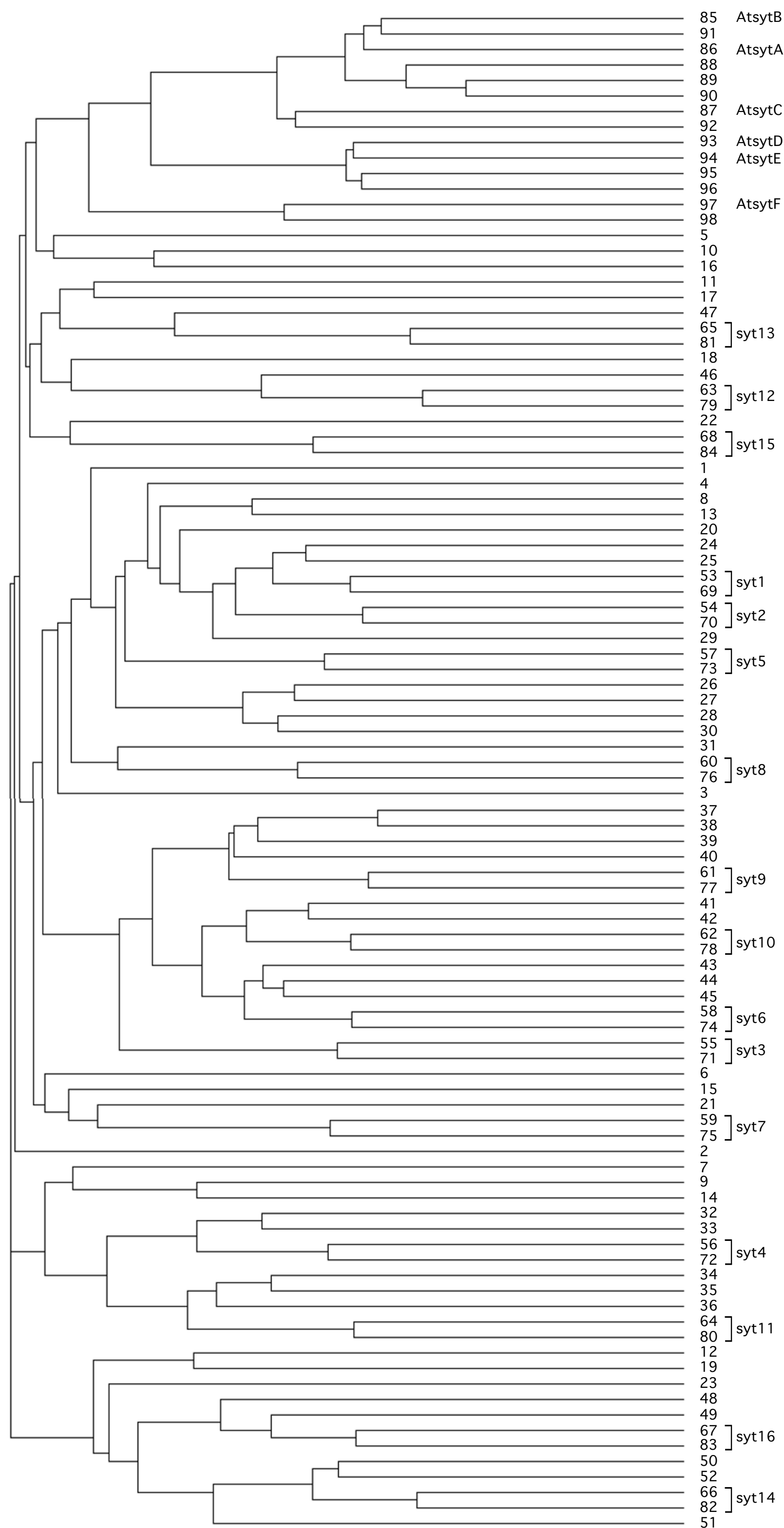

Figure 2

Cladogram tree of Syts Syts are identified on the right. *Mus* and *Homo* Syts are identified with names and are bracketed. *Arabidopsis* Syts are identified with names, following the nomenclature of Fukuda [14].

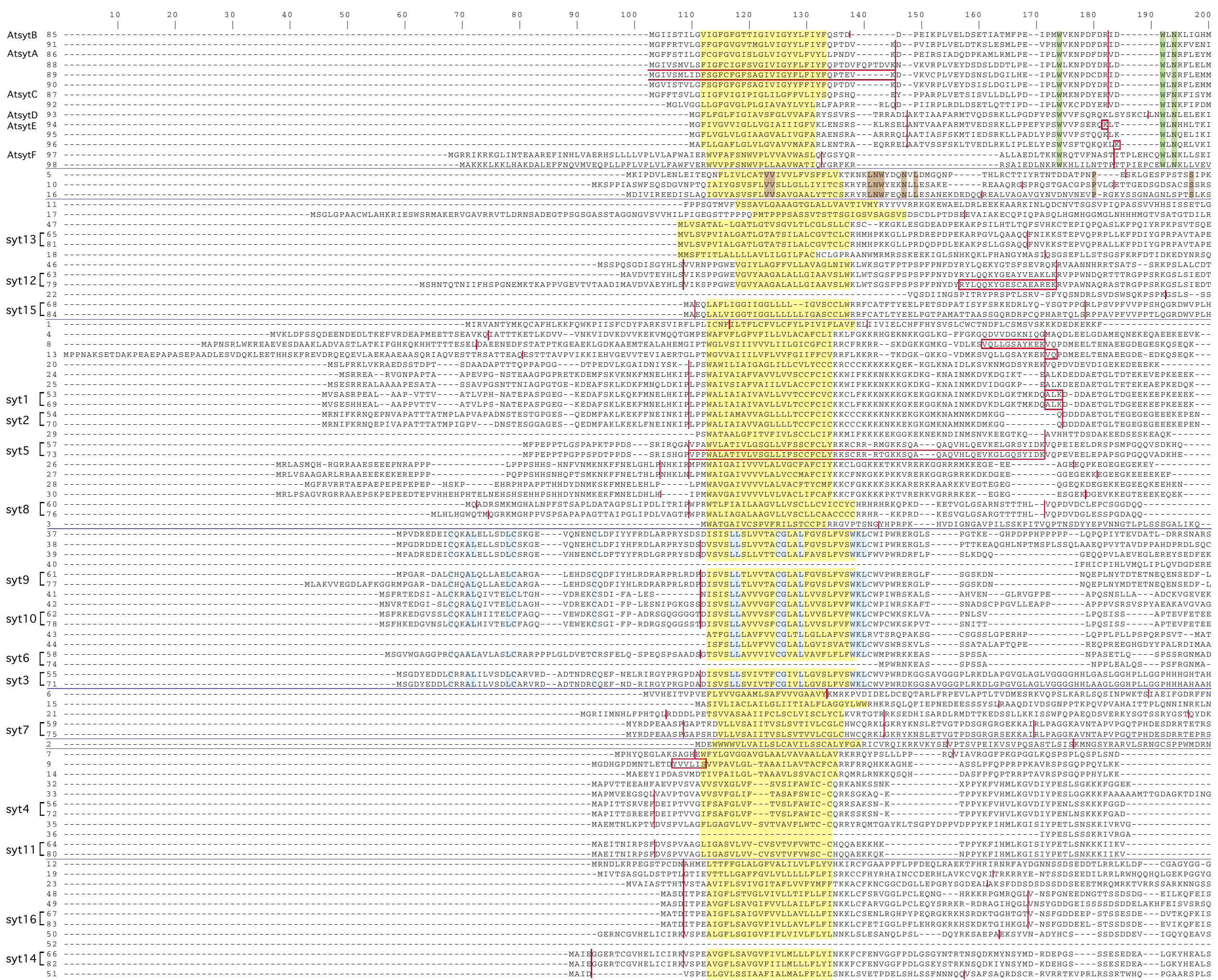

Figure 3
N-terminal regions of Syts Syts are identified on the left. *Mus* and *Homo* Syts are are bracketed and named. *Arabidopsis* Syts are named following the nomenclature of Fukuda [14]. Amino acid sequence length is indicated on top. Putative transmembrane regions are indicated with a yellow background. Intron positions are indicated by red vertical lines. Regions of alternative splicing or RNA editing are enclosed by red boxes. The main branches of Syts are separated by horizontal blue lines. Similarity between members of a main branch is indicated with a coloured background. A motif common to Syt 15, Syt9, Syt10, Syt6 and Syt3 is indicated in blue in fig. 4.

is shared with the otherwise unrelated, vertebrate specific branch of *Syts* which includes *Syt9*, *Syt10*, *Syt6* and *Syt3*. Such a conserved motif probably indicates the specification of a common function.

Expression of variant Syts

Alternative splicing (see Additional File 1) adds a further level of diversity to *Syts*. The large numbers of *Mus* and *Homo* transcripts in particular, show abundant alternative splicing which involves coding regions as well as both upstream and downstream regions. There are common patterns of alternative splicing in *Mus* and *Homo* as well as species specific patterns. For example, both *Mus* and *Homo* *Syt11* transcripts, use atypical GC intron donors in the final intron, rather than the typical GT donors which are present, to specify a change in the second calcium coordinating position in the C2B region (fig. 7). In fish, the same sequence is encoded via typical intron donors. Another such example is *Syt16* where both *Mus* and *Homo* use atypical GC intron donors in the final intron preceding the

Figure 4

N-terminal regions of Syts Syts are identified on the left. *Mus* and *Homo* Syts are are bracketed and named. *Arabidopsis* Syts are named following the nomenclature of Fukuda [14]. Amino acid sequence length is indicated on top. Putative transmembrane regions are indicated with a yellow background. Intron positions are indicated by red vertical lines. Regions of alternative splicing or RNA editing are enclosed by red boxes. The main branches of Syts are separated by horizontal blue lines. Similarity between members of a main branch is indicated with a coloured background. A motif common to Syt 15, Syt9, Syt10, Syt6 and Syt3 is indicated in blue in fig. 4.

C2A region, but *Fugu Syt16* does not. There are numerous examples of differences in the patterns of alternative splicing between *Mus* and *Homo*. Certain regions of the coding sequences are altered in specific *Syts* but overall, these regions range from N-terminus to C-terminus indicating a sophisticated control of many functions.

Examples of common patterns of sequence variation in certain *Syts* include the alternative splicing of the short linker of *Syt1* in *Anopheles*, *Drosophila*, *Mus* and *Homo* (fig. 3). The functional consequences of this alternative splicing have recently been investigated [30]. In *Syt1*, the C2B region undergoes alternative splicing in *Caenorhabditis* and RNA editing in *Anopheles* and *Drosophila*. Alternative splicing equivalent to that of *Caenorhabditis* has just also been described in *Aplysia* [31]. There is no evidence for equivalent alteration of *Ciona*, *Danio*, *Fugu*, *Mus* or *Homo Syt1*. It is intriguing to note that this region in the most

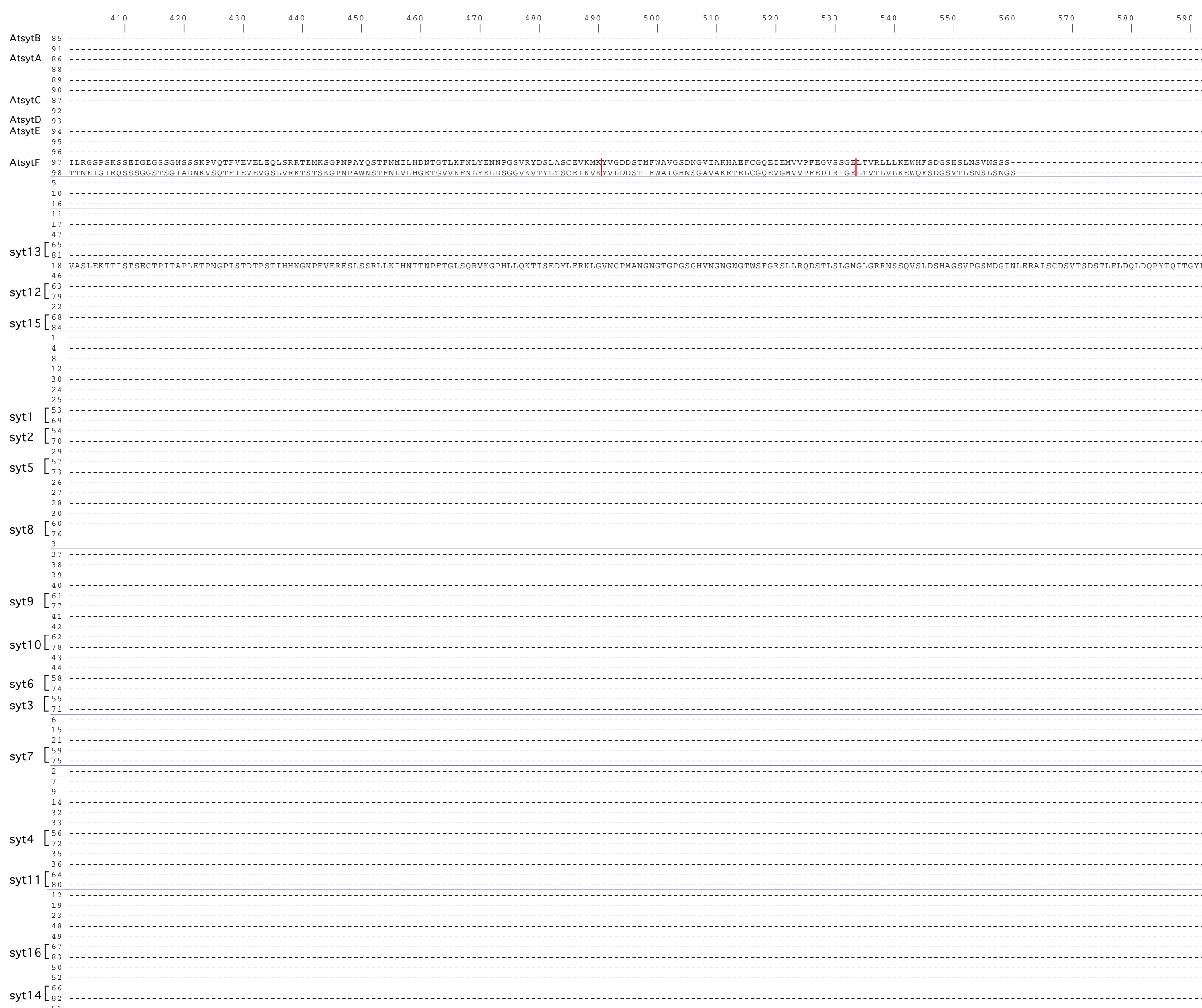

Figure 5

N-terminal regions of Syts Syts are identified on the left. *Mus* and *Homo* Syts are are bracketed and named. *Arabidopsis* Syts are named following the nomenclature of Fukuda [14]. Amino acid sequence length is indicated on top. Putative transmembrane regions are indicated with a yellow background. Intron positions are indicated by red vertical lines. Regions of alternative splicing or RNA editing are enclosed by red boxes. The main branches of Syts are separated by horizontal blue lines. Similarity between members of a main branch is indicated with a coloured background. A motif common to Syt 15, Syt9, Syt10, Syt6 and Syt3 is indicated in blue in fig. 4.

abundantly expressed *Arabidopsis Syt* is also encoded by alternative exons. Alterations of the N-terminal end of *Syt6* and the C-terminal ends of many *Syts* in the same vertebrate specific branch, as well as variable insertions into the linker region of *Mus* and *Homo Syt7* (although nothing similar is found in other *Syt7* orthologues) and insertions into the C2B region of the *Syt14* homologues are further examples of common patterns of sequence variation in certain *Syts*. The true complexity of *Syt* alternative splicing needs to be examined systematically in detail.

It is fortunate that the transcript sequencing projects in *Mus* and *Homo* have generated sequences from many different cell types at different stages of development, as it is likely that the production of variant *Syts* is under cell type and temporal control. Alternative splicing of exons in the 5' untranslated (UTS) region of *Syt1* in mammals (see

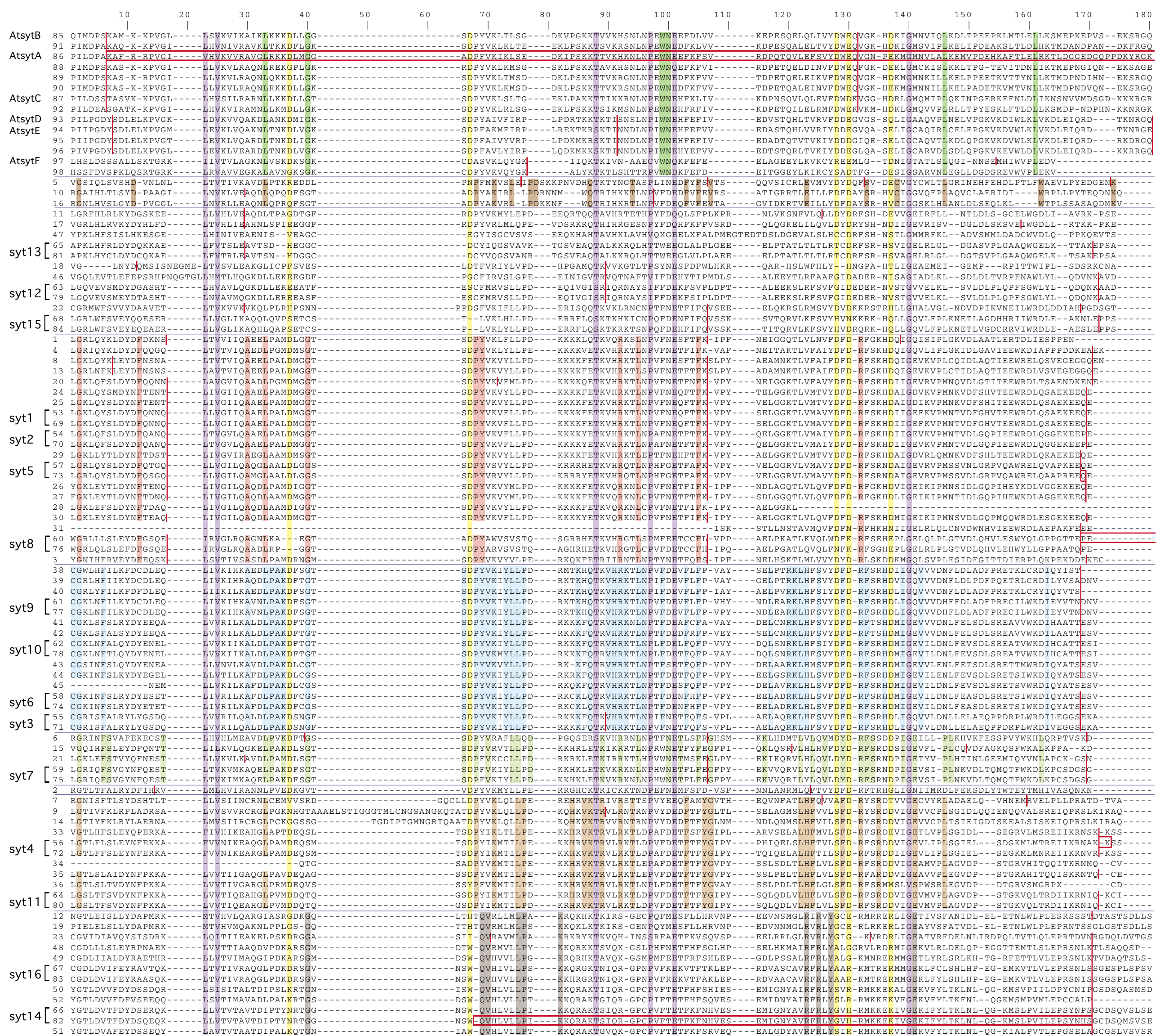

Figure 6

C2A to C-terminal regions of Syts Syts are identified on the left. *Mus* and *Homo* Syts are are bracketed and named. *Arabidopsis* Syts are named following the nomenclature of Fukuda [14]. Amino acid sequence length is indicated on top. Intron positions are indicated by red vertical lines. Regions of alternative splicing or RNA editing are enclosed by red boxes. The main branches of Syts are separated by horizontal blue lines. Similarity between members of a main branch is indicated with a coloured background. The calcium coordinating positions of Syt1 and Syt3 [37,38] are indicated by a yellow background. Positions with greater than 90% conservation are indicated with a purple background.

Additional File 1 and accession numbers aj617615-aj617619 for alternative splicing in *Homo*, *Mus* and *Rattus*) seems to be particularly complex and is the likely explanation for the described variations [32]. This was not seen in the original 5' mapping work [33] but RNase protection (RPA) analysis in *R. norvegicus* and *R. rattus* (fig. 9) confirms the evidence of complex, species specific alternative splicing in this region of *Syt1* in the sequence databases. Alternative splicing of this region is also evident in *Ciona Syt1* and a functional analysis of this region in the related organism *Halocynthia* has recently been carried out [34]. Insufficient transcript evidence is currently available from other organisms to establish the universality of *Syt1* 5'UTS alternative splicing.

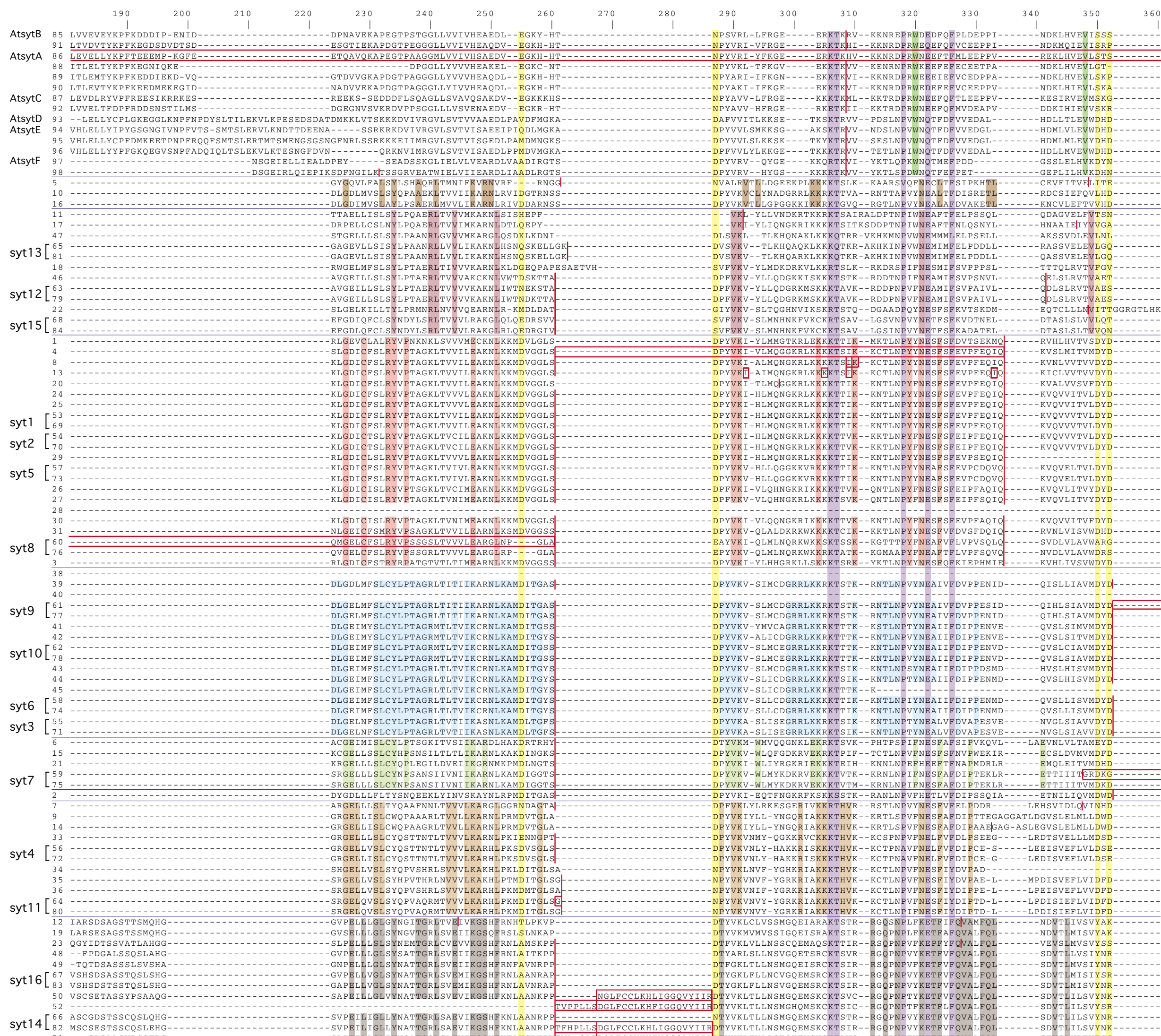

Figure 7

C2A to C-terminal regions of Syts Syts are identified on the left. *Mus* and *Homo* Syts are are bracketed and named. *Arabidopsis* Syts are named following the nomenclature of Fukuda [14]. Amino acid sequence length is indicated on top. Intron positions are indicated by red vertical lines. Regions of alternative splicing or RNA editing are enclosed by red boxes. The main branches of Syts are separated by horizontal blue lines. Similarity between members of a main branch is indicated with a coloured background. The calcium coordinating positions of Syt1 and Syt3 [37,38] are indicated by a yellow background. Positions with greater than 90% conservation are indicated with a purple background.

Conclusions

I have described more than 98 *Syts* from a broad range of animals and plants. Much remains to be done to understand the control of the expression and location of the range of variants produced by each *Syt*. There is no evidence of *Syts* in single cell organisms or those with the most simple forms of multicellularity (algae, fungi, slime moulds) leading one to speculate that these genes may be necessary for communication in more differentiated cell systems. Although C2 domains are present in the simpler eukaryotes, the distinctly conserved C2A-C2B arrangement is unique to *Syts*. All of the plant *Syts* share the transmembrane-linker-C2A region with a family of genes which encode proteins with variable numbers of C2

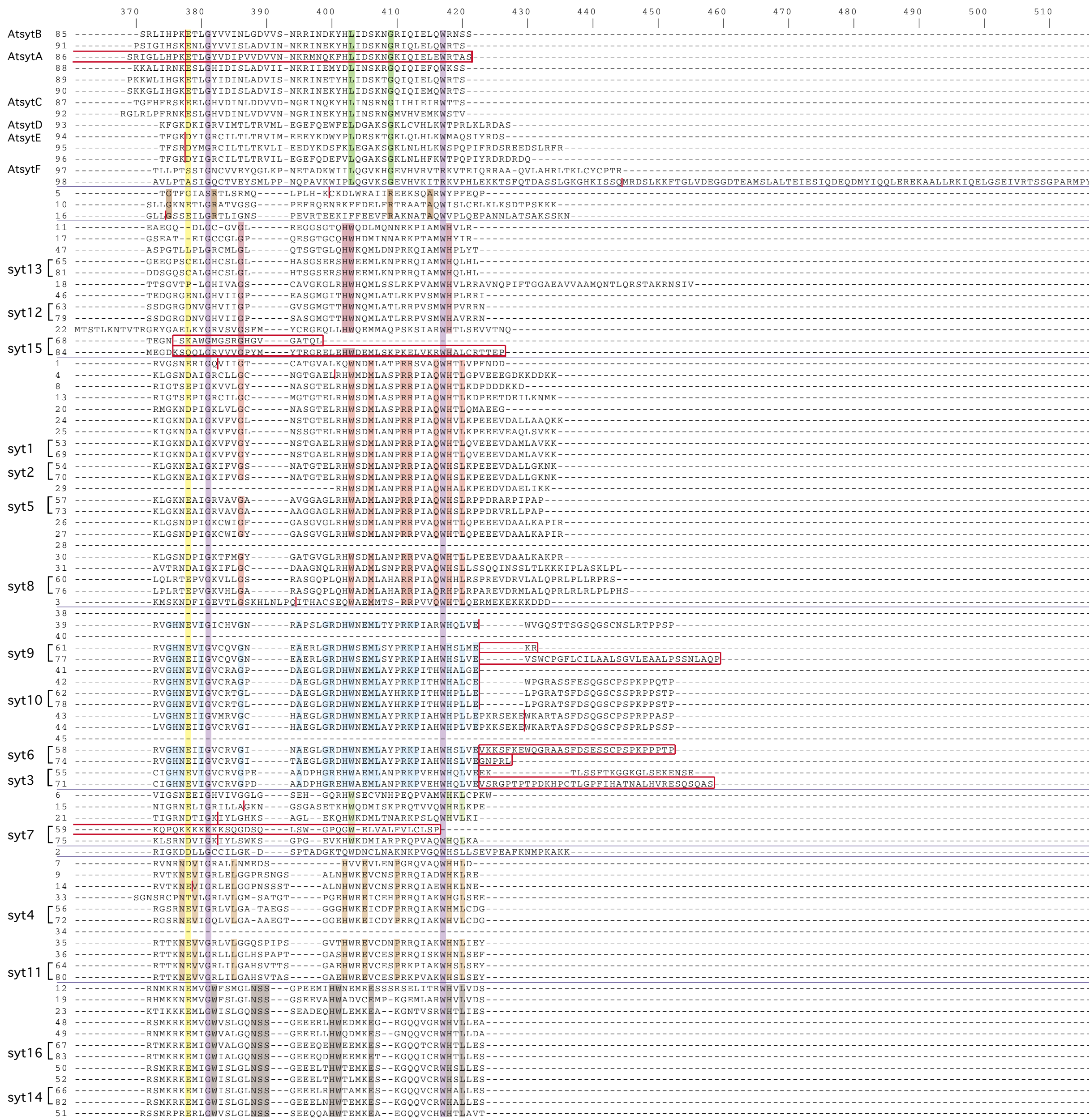

Figure 8
C2A to C-terminal regions of Syts Syts are identified on the left. *Mus* and *Homo* Syts are are bracketed and named. *Arabidopsis* Syts are named following the nomenclature of Fukuda [14]. Amino acid sequence length is indicated on top. Intron positions are indicated by red vertical lines. Regions of alternative splicing or RNA editing are enclosed by red boxes. The main branches of Syts are separated by horizontal blue lines. Similarity between members of a main branch is indicated with a coloured background. The calcium coordinating positions of Syt1 and Syt3 [37,38] are indicated by a yellow background. Positions with greater than 90% conservation are indicated with a purple background.

domains. This family has members in yeast, fungi, metazoa, land plants and trypanosoma, but there is no evidence of family members in other eukaryotes at present. The first functional analysis of the yeast members (tricalbins) has just been published [35] but the family is poorly characterized otherwise. Additional File 1 entries 108–

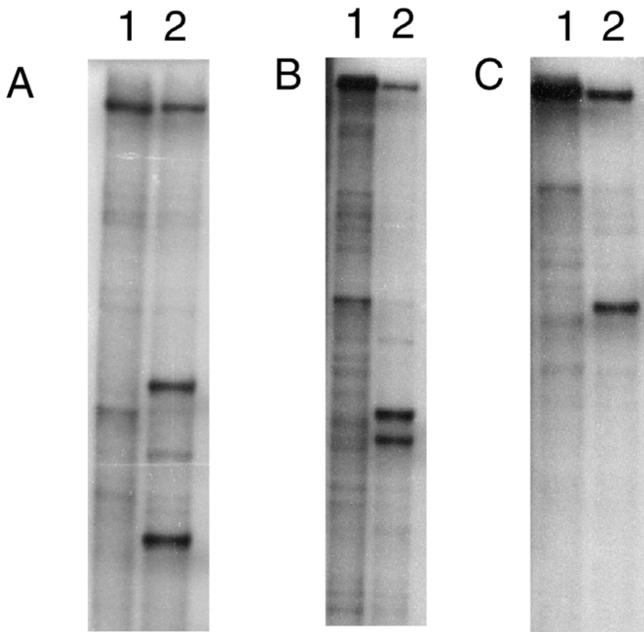

Figure 9
RNase protection analysis of 5'UTS region of *Rattus Syt1* (A) 5'UTS probe aj617620. (B) 5'UTS probe aj617621. (C) 5'UTS probe aj617622. Lane 1: *Rattus norvegicus* brain mRNA. Lane 2: *Rattus rattus* brain mRNA. The uppermost bands are full-length products from mRNA transcripts which match the input probe across its whole length. Shorter products result from partially matching mRNA transcripts.

126 describe the non-*Syt* members of this gene family in *Arabidopsis*, *Oryza*, *Caenorhabditis*, *Drosophila*, *Homo*, *Saccharomyces* and *Trypanosoma*. The analogous situation is not found in animals, where the relation between *Syts* and other gene families is restricted to C2 domain sequence similarity. The clustalw cladogram tree of all the sequences described in this paper is shown in fig. 10.

The advantages of performing an evolutionary analysis of *Syts* and attempting to understand their origins and diversity include the possibility of exhaustively defining the functions of a minimal set in a model organism (eg. *Arabidopsis*, *Ciona*). Comparative analysis of subgroups of *Syts* from a range of evolutionary lineages helps to define exactly which sequences are required to maintain function and which are able to diversify (see [36] for a structural evolutionary analysis of the C2 domains of Syts). The patterns of alternative splicing displayed by certain groups of *Syts* indicate enormous functional diversity that is only beginning to be understood. It will be fascinating to discover what it is about certain animal *Syts* that distinguishes them as essential players in neurotransmission.

Methods

RT-PCR and cDNA library screening

RT-PCR from *Rattus* brain mRNA was carried out with Pfuturbo polymerase. A *Homo* brain cDNA library (Clontech) was screened with probes for the 6 novel human loci identified in [13] (accession numbers aj303363-aj303368). An *Arabidopsis* whole plant cDNA library (Stratagene) was screened with probes for the loci identified in [13]. The probes were produced by PCR from genomic DNA which was a gift from Ian Furner (Cambridge University department of Genetics).

RNase protection analysis

RNase protection analysis (RPA) analysis was carried out as described [20]. *Rattus rattus* brain was a gift from S.Redrobe at Bristol Zoo. Brain mRNA was prepared from *Rattus rattus* and from *Rattus norvegicus* (Sprague-Dawley) by guanidine isothiocyanate followed by polyA selection with oligo-dT cellulose. Regions of the 5' untranslated (5'UTS) portion of sequence accession x52772 (*Rattus rattus Syt1)* were cloned using RT-PCR with *Rattus* brain mRNA. RPA probes were produced using the Maxiscript kit (Ambion) from pBSIIKS- clones containing insert sequences aj617620-aj617622.

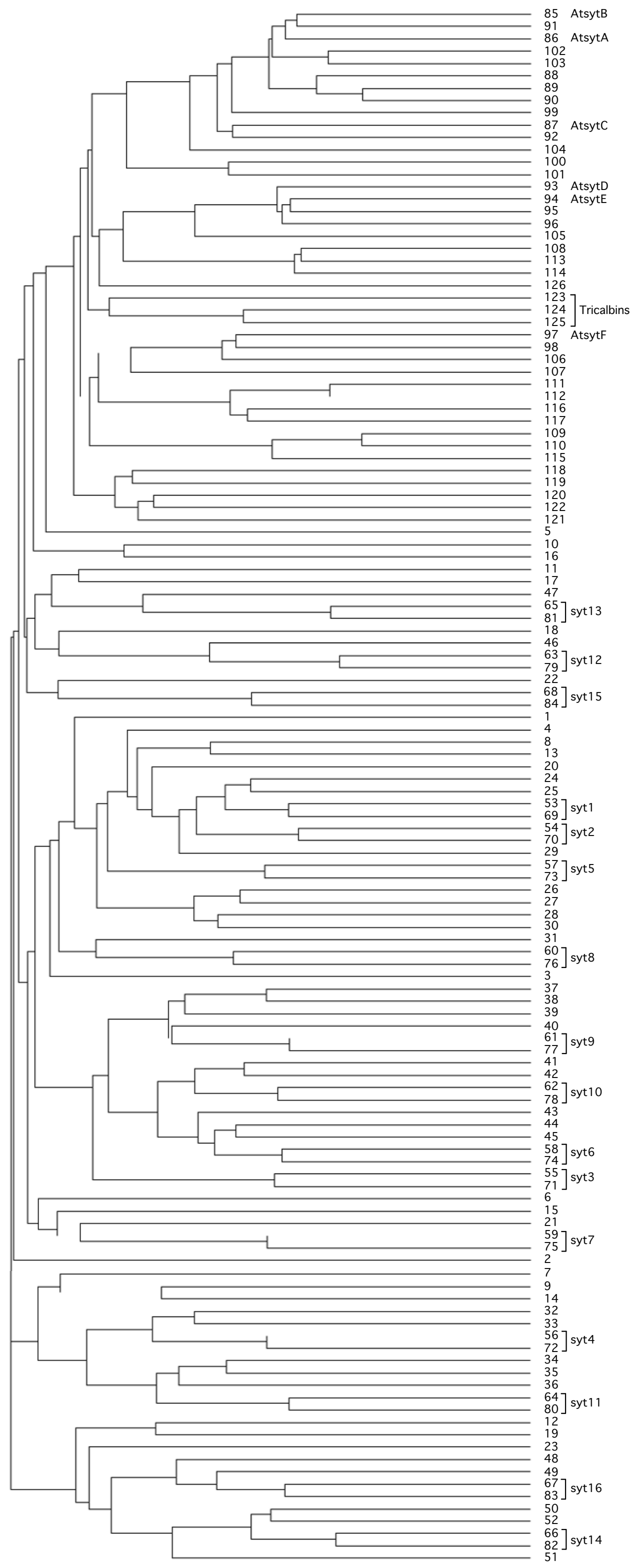

Figure 10
Cladogram tree of all sequences Sequences are identified on the right. *Mus* and *Homo* Syts are named and bracketed. *Arabidopsis* Syts are named following the nomenclature of Fukuda [14]. Yeast tricalbins are named and bracketed.

Additional material

Additional File 1

This text file describes the detailed features of all 98 Syts as well as information about a further 28 related genes mentioned in this paper.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2164-5-43-S1.txt]

Acknowledgements

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