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.
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 multiple 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,
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

Table 1: Summary of sequence identities

| Number | Organism | Names | Number | Organism | Names | Number | Organism | Names | C2 domains |
|--------|----------|-------|--------|----------|-------|--------|----------|-------|-----------|
| 1      | Caenorhabditis | syt1   | 2      | Caenorhabditis | syt1   | 3      | Caenorhabditis | syt1   | 4         |
| 2      | Danio     | syt6   | 3      | Caenorhabditis | syt6   | 4      | Caenorhabditis | syt6   | 5         |
| 3      | Danio     | syt7   | 4      | Caenorhabditis | syt7   | 5      | Caenorhabditis | syt7   | 6         |
| 4      | Anopheles | syt1   | 5      | Anopheles   | syt1   | 6      | Anopheles   | syt1   | 7         |
| 5      | Anopheles | syt4   | 6      | Anopheles   | syt4   | 7      | Anopheles   | syt4   | 8         |
| 6      | Danio     | syt5   | 7      | Danio       | syt5   | 8      | Danio       | syt5   | 9         |
| 7      | Danio     | syt9   | 8      | Danio       | syt9   | 9      | Danio       | syt9   | 10        |
| 8      | Anopheles | syt1   | 9      | Anopheles   | syt1   | 10     | Anopheles   | syt1   | 11        |
| 9      | Anopheles | syt4   | 10     | Anopheles   | syt4   | 11     | Anopheles   | syt4   | 12        |
| 10     | Anopheles | syt7   | 11     | Anopheles   | syt7   | 12     | Anopheles   | syt7   | 13        |
| 11     | Anopheles | syt13  | 12     | Anopheles   | syt13  | 13     | Anopheles   | syt13  | 14        |
| 12     | Drosophila| syt1   | 13     | Drosophila  | syt1   | 14     | Drosophila  | syt1   | 15        |
| 13     | Drosophila| syt4   | 14     | Drosophila  | syt4   | 15     | Drosophila  | syt4   | 16        |
| 14     | Drosophila| syt7   | 15     | Drosophila  | syt7   | 16     | Drosophila  | syt7   | 17        |
| 15     | Drosophila| syt13  | 16     | Drosophila  | syt13  | 17     | Drosophila  | syt13  | 18        |
| 16     | Drosophila| syt12  | 17     | Drosophila  | syt12  | 18     | Drosophila  | syt12  | 19        |
| 17     | Drosophila| syt16  | 18     | Drosophila  | syt16  | 19     | Drosophila  | syt16  | 20        |
| 18     | Drosophila| syt14  | 19     | Drosophila  | syt14  | 20     | Drosophila  | syt14  | 21        |
| 19     | Drosophila| syt16  | 20     | Drosophila  | syt16  | 21     | Drosophila  | syt16  | 22        |
| 20     | Ciona     | syt1   | 21     | Ciona       | syt1   | 22     | Ciona       | syt1   | 23        |
| 21     | Ciona     | syt7   | 22     | Ciona       | syt7   | 23     | Ciona       | syt7   | 24        |
| 22     | Ciona     | syt15  | 23     | Ciona       | syt15  | 24     | Ciona       | syt15  | 25        |
| 23     | Danio     | syt1   | 24     | Danio       | syt1   | 25     | Danio       | syt1   | 26        |
| 24     | Danio     | syt5.1 | 25     | Danio       | syt5.1 | 26     | Danio       | syt5.1 | 27        |
| 25     | Fugu      | syt1   | 26     | Fugu        | syt1   | 27     | Fugu        | syt1   | 28        |
| 26     | Fugu      | syt5.1 | 27     | Fugu        | syt5.1 | 28     | Fugu        | syt5.1 | 29        |
| 27     | Fugu      | syt5.2 | 28     | Fugu        | syt5.2 | 29     | Fugu        | syt5.2 | 30        |
| 28     | Fugu      | syt8   | 29     | Fugu        | syt8   | 30     | Fugu        | syt8   | 31        |
| 29     | Fugu      | syt4   | 30     | Fugu        | syt4   | 31     | Fugu        | syt4   | 32        |
| 30     | Fugu      | syt5   | 31     | Fugu        | syt5   | 32     | Fugu        | syt5   | 33        |
| 31     | Fugu      | syt9   | 32     | Fugu        | syt9   | 33     | Fugu        | syt9   | 34        |
| 32     | Danio     | syt11  | 33     | Danio       | syt11  | 34     | Danio       | syt11  | 35        |
| 33     | Danio     | syt1    | 34     | Danio       | syt1   | 35     | Danio       | syt1   | 36        |
| 34     | Danio     | syt9.2 | 35     | Danio       | syt9.2 | 36     | Danio       | syt9.2 | 37        |
| 35     | Danio     | syt9.1 | 36     | Danio       | syt9.1 | 37     | Danio       | syt9.1 | 38        |
| 36     | Danio     | syt10  | 37     | Danio       | syt10  | 38     | Danio       | syt10  | 39        |
| 37     | Danio     | syt9.1 | 38     | Danio       | syt9.1 | 39     | Danio       | syt9.1 | 40        |
| 38     | Danio     | syt9.2 | 39     | Danio       | syt9.2 | 40     | Danio       | syt9.2 | 41        |
| 39     | Danio     | syt9.1 | 40     | Danio       | syt9.1 | 41     | Danio       | syt9.1 | 42        |
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. Introns 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 colour 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
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 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.
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 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. Introns 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 Syt15, Syt9, Syt10, Syt6 and Syt3 is indicated in blue in fig. 4.
Additional File 1 and accession numbers a617615-a617619 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.
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
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 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.
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 (e.g. *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 Pfu-turbo 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 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.
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.

**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.
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