Cloning and genomic characterization of sytdep, a new synaptotagmin XIV-related gene

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FOOTNOTES

Abbreviations used: bp, base pair; HRP, horseradish peroxidase; DMSO, dimethyl sulfoxide; ORF, open reading frame; PMN, polymorphonuclear neutrophil; RT-PCR, reverse transcription-polymerase chain reaction; SNARE, soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein (SNAP) receptor; syt, synaptotagmin; sytdep, synaptotagmin XIV-derived protein; SDS, sodium dodecyl sulfate; UTR, untranslated region.

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The nucleotide sequence data for human sytdep (synaptotagmin XIV-derived protein) reported in this work has the GenBank accession no. **AY954513**.

Abstract

We have identified a new human gene coined *sytddep* (synaptotagmin XIV-derived protein) in human neutrophils. *Sytddep* encodes a 188-amino acid sequence with a 21.435 kDa deduced molecular mass, showing 75% identity to human synaptotagmin (syt) XIV. Human neutrophils express *sytddep*, but not *syt XIV*. *Sytddep* was upregulated during HL-60 neutrophil differentiation. *Sytddep* gene is located in human chromosome 4 and contains a unique exon, whereas *syt XIV* gene, located in chromosome 1, comprises 10 exons with 9 introns. Mouse genome did not contain *sytddep*. The N-terminal region of *sytddep* shows no homology with any known protein and, unlike synaptotagmin XIV isoforms, *sytddep* shows a unique C-terminal C2B domain. Polyclonal antibodies against the C2B domain of *syt XIV* recognized *sytddep* as a 27 kDa protein in human neutrophils. Genomic analyses suggest that human *sytddep* could derive from a retrotranslocation of a *syt XIV* transcript into chromosome 4.

Keywords: alternative splicing, genomic characterization, gene expression, C2 domain, synaptotagmin XIV-derived gene, human neutrophils, cloning.

Introduction

Synaptotagmins (syts) are a family of membrane proteins featured by an N-terminal transmembrane domain (type I membrane topology), two C-terminal tandem C2 domains (C2A and C2B), which are responsible for binding calcium and phospholipids, and a short conserved C-terminal sequence, which may be crucial for docking to the plasma membrane and correct folding of the C2B domain [1-4]. Syts and SNARE [soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein (SNAP) receptor] proteins are likely to represent the minimal protein complement for Ca^{2+} -triggered exocytosis [5]. In vertebrates, 16 isoforms of syt have been identified [6], some of which appear to function as Ca^{2+} sensors that couple Ca^{2+} to membrane fusion *in vivo* and *in vitro* [7, 8]. The most abundant isoform, syt I, appears to function as a Ca^{2+} sensor that triggers the rapid exocytosis of synaptic vesicles from neurons, but the functions of the remaining syt isoforms are ill-defined. Syts are known to be evolutionarily and functionally well conserved genes and thought to be derived from common ancestral genes. Distinct variants generated by alternative splicing have been reported, including transmembrane exon skipping and syt VII splice variants that differentially regulate synaptic vesicle recycling [9-12].

The partial sequence of a putative *chr415 syt* gene, so-called by Craxton [13], has been suggested to be a human orthologue of mouse *syt XIV* in the human genome [14]. In the present report, we have completed the sequence of *chr415 syt* gene and found by cloning, sequencing and chromosomal localization that this gene is different from *syt XIV* and encodes a novel protein that shows a high amino acid identity with that of syt XIV. We have coined this new gene as synaptotagmin XIV-derived protein (sytdp) as it is phylogenetically related to syt XIV. In addition, we have found that human polymorphonuclear neutrophils (PMNs) express high levels of *sytdp*, but not *syt XIV*.

Materials and methods

Cell culture and neutrophil isolation. The human acute myeloid leukemia HL-60 cell line was grown in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 24 µg/ml gentamicin at 37 °C in a humidified atmosphere of air/CO₂ (19/1). Neutrophil differentiation of HL-60 cells was induced by adding 1.3 % (v/v) dimethyl sulfoxide (DMSO) as previously described [15]. The human astro-oligodendroglioma (grade II/III) GOS-3 cell line was grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, and 24 µg/ml gentamicin at 37 °C in a humidified atmosphere of air/CO₂ (19/1).

Neutrophils were obtained from fresh human peripheral blood by dextran sedimentation and centrifugation on Ficoll-Hypaque (Pharmacia LKB Biotechnology, Uppsala, Sweden), followed by hypotonic lysis of residual erythrocytes as previously described [16], and the final cell preparation contained more than 99 % neutrophils as assessed by Wright-Giemsa staining. Postnuclear neutrophil extracts were prepared and processed for Western blotting as previously described [17].

Nucleic acid isolation and reverse transcription-polymerase chain reaction (RT-PCR). Both genomic DNA and total RNA were purified from the different cell types used in this work using the DNazol genomic DNA isolation reagent and the TRIZOL reagent (Gibco BRL, Gaithersburg, MD), respectively. RNA preparations were carefully checked by gel electrophoresis and found to be free of DNA contamination. Total RNA (5 µg), primed with oligo-dT, was reverse-transcribed into cDNA at 37°C for 2 h using the first-strand cDNA synthesis kit (Amersham Biosciences, Piscataway, NJ) according to the manufacturer's instructions in a 20-µl volume, and stored at -20°C until use. A 25-µl PCR mixture contained 500 ng of cDNA template or genomic DNA, 20 pmol of

each primer, 0.2 mM dNTPs, 1.5 mM MgCl₂ and 5 units of Taq DNA polymerase (Roche, Basel, Switzerland). PCR reactions were performed in a GeneAmp PCR system model 9600 (Perkin-Elmer, Norwalk, CT). The primers used are listed in Table 1. Amplification of human *β-actin* (GenBank accession no. **NM_001101**) (5'-AATATGGCACCACACCTTCTACA-3' and 5'-CGACGTAGCACAGCTTCTCCTTA-3') was used as an internal and loading control. PCR amplification was as follows: 1 cycle at 95°C for 5 min as an initial denaturation step, then denaturation at 95°C for 30 s, annealing at 53-63°C for 30 s, and extension at 72°C for 90 s (30 cycles), followed by further incubation for 15 min at 72°C (1 cycle). PCR conditions were shown to be at the linear phase of amplification to assess a semiquantitative analysis. PCR products were electrophoresed on 2% agarose gels in 1 X TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) and visualized by ethidium bromide staining.

Cloning and sequencing. PCR products were either extracted from the agarose gel using the ConcertTM kit (Gibco BRL) or cloned into the pCR[®]2.1 vector, using the TA-TOPO cloning kit (Invitrogen, San Diego, CA) following the manufacturer's indications, and sequenced in an ABI PRISM[®] 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). DNA sequencing was performed on both strands from 10 independent cDNA clones. Distinct genomic and cDNA sequences were aligned by ClustalW program [18]. Potential full-length cDNAs were sequenced, analysed, and searched against nucleotide and protein databases by using BLAST programs (BLASTn, tBLASTn, BLASTp).

Protein biotinylation and immunoprecipitation. Cells (20 x 10⁶) were lysed with 50-200 µl of lysis buffer (20 mM HEPES pH 7.5, 10 mM EGTA, 40 mM β-glycerophosphate, 1% NP-40, 2.5 mM MgCl₂, 2 mM orthovanadate, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 20 µg/ml aprotinin, 20 µg/ml leupeptin), and centrifuged at 12000 rpm for 20 min at 4°C. Solubilized proteins (5-30 µg) were biotinylated with 200 µl of 10 mM Sulfo-NHS-Biotin (Sulfo-Succinimidyl-6-

(biotinamido) hexanoate; EZ-LinkTM Sulfo-NHS-LC-Biotin; Pierce Biotechnology, Rockford, IL) for 30 min at room temperature with constant rotation. Then, these biotinylated lysates were precleared with protein A-Sepharose as previously described [19]. Affinity-purified rabbit polyclonal antibody raised against the C2B domain of syt XIV fused to GST [14], and P3X63 myeloma culture supernatant (a kind gift of Dr. F. Sánchez-Madrid, Hospital de La Princesa, Madrid, Spain), used as a negative control, were coupled to protein A-Sepharose beads (Amersham Biosciences) by incubation for 2 h at 4°C with constant rotation. These beads were pelleted, washed twice with lysis buffer, and incubated with the supernatant from the preclearing step for 2 h at 4°C with constant rotation. Sepharose beads were then pelleted and washed 5 times with lysis buffer. Then, 20 µl SDS sample buffer (50 mM Tris-HCl pH 6.8, 100 mM DTT, 2% SDS, 0.1% bromophenol blue, 10% glycerol) were added, and samples were subjected to SDS-15% PAGE under reducing conditions and immunoblotted [20]. Biotynilated proteins were visualized with streptavidin-horseradish peroxidase (HRP) conjugate (diluted 1/1000 in TBST: Tris-buffered saline (TBS) -50 mM Tris-HCl pH 8.0, 150 mM NaCl- containing 0.05% Tween 20), using an ECL detection system (Amersham Biosciences).

Results and discussion

Identification and molecular characterization of sytdep

A putative syt gene, named *chr415 syt* (GenBank/EMBL accession number, AJ303368), was identified and partially sequenced in a previous study by Craxton [13]. We cloned and sequenced *chr415 syt* in human neutrophils and found its identity to *chr415 syt*, with only one nucleotide difference (Fig. 1A). Recently, this gene was assigned to syt XIV [14] by sequence comparison. However, we have found evidences that questioned this assumption. First, comparison of the partial nucleotide and amino acid sequence of *chr415 syt* with that corresponding to syt XIV showed differences both in DNA and protein sequences (Fig. 1), although amino acid sequence comparison

of chr415 syt with the six isoforms of syt XIV indicated that the protein encoded by this gene showed a strong homology with isoform 2 of syt XIV (Fig. 1B). Secondly, we found that human neutrophils expressed *chr415 syt*, but not *syt XIV* (data not shown), using RT-PCR and a wide array of PCR primers covering the whole sequences of *syt XIV* and *chr415 syt*. On these grounds, we asked whether these two sequences could correspond to two different genes. Using the BLASTn program we analyzed the chromosome location of both genes, aligning all known *syt XIV* isoforms and *chr415 syt* sequence *versus* the full human genome. Surprisingly, two distinct gene loci for *syt XIV* and *chr415 syt* were found in the human chromosomal positions 1q32.2 and 4q13.2, respectively.

In order to characterize the nucleotide sequences of both the genomic locus and transcript corresponding to the gene defined by the partial *chr415 syt* sequence, we carried out a BLASTn analysis of the partial cDNA sequence known of *chr415 syt* in the human chromosome 4 region (positions from 8932396 to 8929693), which led to a prediction of 2584 bp for the full-length *chr415 syt* cDNA (Fig. 2A). By RT-PCR assays we found a cDNA sequence that contained a single ORF encoding a protein of 188 amino acids with a deduced molecular mass of 21.435 kDa (Fig. 2A). These results extended and completed the partial nucleotide sequence of *chr415 syt*. We also found one nucleotide difference at position 479, leading to a Thr-Met change at residue 97 (Fig. 2A). We coined the name sytdep (synaptotagmin XIV-derived protein) for this new gene (GenBank accession no. **AY954513**). The predicted full-length *sytdep* cDNA contained a 189-bp 5'-untranslated region (5'-UTR), a 564-bp ORF, and a 1831-bp 3'-UTR (Fig. 2A). The rather long length of the 3' untranslated sequence might be similar to that highly conserved of rat and human *syt I* gene, arguing for conserved functions in non-coding regions. The *sytdep* cDNA showed high identity with three clones of human chromosome 4 (GenBank accession nos. **AC096727**, **BC041902**, and **AJ617629**).

The amino acid sequence of human sytdep showed 75% identity to synaptotagmin XIV. The primary sequence of sytdep showed a 57% identity to the human syt XIV-related protein (strep14,

a.k.a. syt XVI), and 20-24%, 10-19% and 12% identity to other syt isoforms, syt-like proteins (slps) and slp homologue lacking C2 domain protein (slac2), respectively. On the other hand, the N-terminal region (24 amino acids) of sytdep did not show any significant homology with any known protein domain. A physiological role for the N-terminal region of Syt in neurotransmission has been recently reported [21]. This might suggest a specific role for sytdep that remains to be elucidated.

Unlike most synaptotagmin XIV isoforms (Fig. 1B) that contain two C2 domains, C2A and C2B, sytdep contains a single C2B domain at the C-terminus, and thereby sytdep behaves as a single C2 domain-containing protein. C2 domains are conserved motifs of ~135 amino acids that often serve as Ca^{2+} -binding domains [22]. The Ca^{2+} -binding sites of the C2 domains are formed by five conserved acidic residues present in two flexible loops (loops 1 and 3) [23, 24]. However, sytdep as well as syt XIV [14] lack the above acidic residues, suggesting that sytdep, like syt XIV, is a Ca^{2+} -independent C2 protein. Unlike syts that contain the conserved C-terminus WHXL motif, which may be crucial for docking to the plasma membrane and correct folding of the C2B domain [25], sytdep lacks this motif, being substituted by a WQAL motif (Fig. 1B and 2A) of uncertain function.

Like other syts, sytdep protein lacks a signal peptide, and it lacks apparently a transmembrane domain. Sytdep contains two hydrophobic regions of approximately 8 and 16 amino acids (Fig. 2, A and B), which could be sufficient for a putative transmembrane domain, but the location of these regions in the C2B domain make this option particularly implausible as C2B domains do not act as transmembrane domains. Three potential N-glycosylation sites (Asn-Xaa-Ser/Thr; 60-62, 141-143, and 158-160), one potential O-glycosylation (Thr-Xaa-Ser, 39-41) site, and a number of potential Ser, Thr and Tyr phosphorylation sites (12/2/2) were found in the deduced amino acid sequence of sytdep (Fig. 2A). No potential N-myristoylation or prenylation sites were detected, but three Cys residues at the N-terminal domain might be fatty-acylated as it happens in some syts [26].

Genomic structures of human syt XIV and sytdep

The gene structures of human *syt XIV* and *sytdep* were determined by database searching and PCR approaches. The human *syt XIV* and *sytdep* genes were mapped to chromosome 1 and 4, spanning 226,054 and 2,584 bp, respectively. *Syt XIV* gene consisted of 10 exons with 9 introns of various lengths, whereas *sytdep* gene showed a unique exon (Fig. 3). The *Syt XIV* exon 4 encoded the transmembrane domain, and the C2A and C2B domains were encoded by exon 7 and exons 8-10, respectively. *Syt XIV* exon 3 is not translated as it occurs with other human *syt* genes containing 5' non-coding exons [13]. Six different *syt XIV* transcripts have been sequenced, namely: *syt XIV*, *syt XIV-1*, *syt XIV-2*, *syt XIV-3*, *syt XIV-4*, *syt XIV-5* (GenBank accession nos. **NM 153262**, **AJ617623**, **AJ617624**, **AJ617625**, **AJ617626**, **AJ617627**), with ORFs encoding sequences of 555, 457, 517, 265, 503 and 416 amino acids, respectively (Figs. 1B and 3). As shown in Fig. 3, we found that these *syt XIV* isoforms result from alternative splicing. In *syt XIV* and *syt XIV-1*, exon 1 encodes the initial 4 amino acids and the first nucleotide of the 5th amino acid; exons 2 and 4 encode 15 and 56 amino acids, respectively. In *syt XIV-2* and *-3*, exon 4 encodes the initial 37 amino acids, and the putative transmembrane domain is incomplete due to the fact that this exon is likely skipped by alternative splicing at the mRNA level. In all these *syt XIV* isoforms, exons 5, 6, 7 and 8 encode 72, 92, 149 (or 64 in *syt XIV-3*) and 63 amino acids, respectively. So far, the initial amino acids of both *syt XIV-4* and *-5* isoforms remain unidentified, and thereby exons 5 and 6 encode the first known 69 and 64 amino acids, respectively. The so-called *syt XIV* homology domain [14] is encoded by exon 5. Finally, exons 9 and 10 encode 27 and 103 or 102 (due to an amino acid deletion in *syt XIV-4* and *-5*) amino acid residues, respectively. Thus, alternative splicing of the upstream sequence of transmembrane region (exons 1 and 2) and the C2A and C2B domains (exons 7 and 9, respectively) give rise to the distinct isoforms. Table 2 shows the distinct exon and intron compositions as well as the exon-intron junctions of the human *syt XIV* gene, containing the highly conserved sequences for RNA splicing in higher eukaryotes, namely GT at

the 5' splice site (donor site) and AG at the 3' splice site (acceptor site) [27], except exons 7 and 9 that show in addition GC and TG as intron 5'- and 3'-splice donor and acceptor sites.

Using BLAST search from the human genome database and DNA sequencing we mapped *sytddep* locus in chromosome 4 containing the exons 1, 2, 3, 4, 8 and 10 of *syt XIV* with no introns (Fig. 3). Due to the high similarity between *sytddep* and *syt XIV*, it could be envisaged that the human *sytddep* gene might originate from a *syt XIV* transcript derived of chromosome 1, which was retrotranslocated to a genomic region of chromosome 4. Thus, as shown in Fig. 3, the *sytddep* genomic locus could result from a fusion of exons 1, 2, 3, 4, 8 and 10 of *syt XIV* gene.

Interestingly, the mouse genome did not contain *sytddep* by database searching. In addition, we were unable to identify *sytddep* in mouse brain cDNA by RT-PCR (data not shown). Thus, the above putative retrotranslocation leading to *sytddep* apparently occurred in human, but not in mouse.

Sytddep expression in human neutrophils

Because *sytddep* and synaptotagmin XIV isoforms showed a high degree of homology, we next asked whether these genes could be differentially expressed. We designed specific primers for the distinct *syt XIV* isoforms and *sytddep*, and carried out RT-PCR experiments with different pairs of primers (Table 1 and Fig. 4), in peripheral blood human neutrophils, human leukemia HL-60 cells, neutrophil-differentiating-HL60 cells, and human astro-oligodendroglioma GOS-3 cell line. The HL-60 cell line has been widely used as a cell culture model to study different molecular aspects of human neutrophils, as it acquires many biochemical and functional characteristics of mature neutrophils following treatment with DMSO for 4 days [15]. As shown in Fig. 4, *sytddep* was highly expressed in mature peripheral blood neutrophils, and resulted upregulated during neutrophil differentiation of HL-60 cells. However, no *syt XIV* expression was detected. GOS-3 cells expressed both *sytddep* and *syt XIV* isoform. Cloning and sequencing of the *syt XIV* amplified band indicated that this amplicon corresponded to *syt XIV* isoform 2.

We next investigated whether the *sytdep* transcript was translated into protein in human neutrophils where we found a high *sytdep* mRNA expression. Due to the high identity (84%) between the C2B domains of syt XIV and sytdep, we assumed that a polyclonal antibody against the C2B domain of syt XIV could detect the C2B-containing sytdep protein. By immunoprecipitating biotinylated cell protein extracts with the above polyclonal antibody, we observed an immunoreactive band of ~27 kDa in the neutrophil extract (Fig. 5A), which could well correspond to the expected size of sytdep protein. The use of protein biotinylation avoided the use of secondary antibodies and thereby the putative interference with the 55-kDa heavy and 25-kDa light immunoglobulin chains. In addition, the above anti-C2B antibody recognized a band of ~27 kDa in the postnuclear extract of human neutrophils by immunoblotting when high amounts of extract protein were used (Fig. 5B). This molecular mass is higher than the predicted molecular mass (21.435 kDa) based on the ORF of the cloned *sytdep* cDNA, and could be in part due to posttranslational modifications.

Because we have found that SNARE proteins are involved in human neutrophil exocytosis [17, 19, 28], and syts have been reported to interact with SNARE proteins [5, 29-31], it is tempting to suggest a putative role for sytdep in the secretory response of human neutrophils where *sytdep* mRNA is particularly highly expressed. The unique major functional domain present in sytdep is the C2B domain, which has been involved in binding to other C2B domains, accessory proteins independent of divalent cations and phospholipids, as well as in exocytosis [32-34]. The polybasic Lys-rich region of C2B domain has been implicated in the binding to phosphatidylinositol (4,5)-biphosphate in the absence of Ca^{2+} [35, 36]. On these grounds, it could be envisaged that the C2B domain of the new gene sytdep might be involved in membrane trafficking in human neutrophils via binding to phosphoinositides. The expression of sytdep in human neutrophils might increase the number of exocytosis-related genes, including SNARE proteins [17, 19, 28], expressed in human neutrophils.

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FIGURE LEGENDS

FIG. 1. *Chr415 syt* and *syt XIV* encode different proteins. (A) The nucleotide sequence of human polymorphonuclear neutrophil (PMN) *chr415 syt* cDNA is compared with the nucleotide sequences of *chr415 syt* and *syt XIV* cDNAs. Human neutrophil *chr415 syt* cDNA was obtained by RT-PCR using distinct pairs of primers shown in Table 1. Dashes have been introduced to maximize sequence identity. Asterisks show positions in the alignment that are perfectly conserved. (B) The partial amino acid sequence of *chr415 syt* gene is compared with the complete amino acid sequences of the six *syt XIV* isoforms. The putative transmembrane region (TM) (in bold), *syt XIV* homology domain (in bold), and the C2A and C2B domains (in black boxes) are indicated. Asterisks indicate the Cys residues that can be fatty-acylated. ▲ indicates the Lys residues (polybasic region) that mediate binding to phosphoinositides. The WHXL motif (in gray boxes) is replaced by the WQAL motif in *chr415 syt*. Arrowheads above the alignment indicate the positions of the alternative splicing sites. Dashes have been introduced to maximize sequence identity. Total number of amino acids is indicated at the end of each sequence.

FIG. 2. Nucleotide and deduced amino acid sequences of human *sytdep*. (A) Nucleotides are numbered in the 5' to 3' direction and the predicted amino acid sequence is shown in single-letter code below the nucleotide sequence. Nucleotides and amino acids are numbered on the right. In-frame termination codons are marked by $\frac{||}{\perp}$. The C2B domain includes from C-35 to R-188. Two hydrophobic regions are boxed. # indicates the Cys residues that may be fatty-acylated. A number of potential Ser (*), Thr (¶) and Tyr (Δ) phosphorylation sites are labeled. Lys residues (▲, polybasic region) that can mediate binding to phosphoinositides are also indicated. The WHXL motif present in *syt XIV* is replaced by the underlined WQAL motif. Putative polyadenylation signal (ATTAAA motif) are also underlined. The nucleotide and predicted amino acid sequences of human *sytdep* are available from GenBank accession no. [AY954513](#). (B) Hydrophobicity profile of human *sytdep*. This profile was obtained according to [37] and shows two hydrophobic regions.

FIG. 3. Genomic organization and transcription of the human *syt XIV* and *sytdep* genes. Exons and introns are represented by boxes and solid bars. Exons are numbered (1 to 10) on top of each box. Exons encoding the transmembrane domain (TM), the C2 domains, and the non-coding (untranslated) regions are represented by black, hatched, and dotted boxes, respectively. The number of amino acids encoded by each transcript is shown. The exon/intron boundaries are

indicated by arrows, and the numbers above the arrows describe position in the codon at which the coding sequence is separated by an intron (0 = at the codon junction; 1 and 2 = after the first codon and the second codon position, respectively). Broken lines indicate the corresponding exons of the *syt XIV* and *sytdep* genes. The numbers on top of the unique exon of *sytdep* indicate the exons of *syt XIV* gene locus which lead to this monoexonic gene. Chromosome location of *syt XIV* and *sytdep* genes are indicated in parenthesis. Letters below the exons stand for primers used in gene cloning from Table 1.

FIG. 4. Differential expression of *syt XIV* and *sytdep* in human cells. Total RNA was purified from human peripheral blood neutrophils, GOS-3 cells as well as HL-60 cells and neutrophil-differentiated HL-60 cells following a 4-day DMSO incubation [HL-60(DMSO)]. The expression of *sytdep* and *syt XIV* was assessed by RT-PCR. Primers used, corresponding to those listed in Table 1, are shown in parenthesis. Amplification of β -actin was used as an internal control. The PCR products were electrophoresed and stained with ethidium bromide. The size of each amplified PCR fragment is shown. Data shown are representative of three separate experiments.

FIG. 5. Sytdep protein in human neutrophils. (A) Biotinylated neutrophil extracts were immunoprecipitated with rabbit polyclonal antibody against the C2B domain of syt XIV or P3X63 myeloma supernatant, as a negative control, and immunoprecipitates were subjected to SDS-PAGE and immunoblotted with streptavidin-HRP. (B) Different amounts of postnuclear extract proteins from resting human neutrophils were run on SDS-PAGE and analyzed by immunoblotting using the above anti-C2B domain antibody. The molecular mass of the immunoreactive band is indicated on the right. Data shown are representative of three separate experiments.

Table 1*Primers used for sytdep and syt XIV cloning*

Primer location in the corresponding GenBank sequences of human origin are indicated.

Type of primer	Primer name	Sequence	Position	GenBank accession no.	Gene name
Forward	A	CAGTTGGTGCGGTCCATG	121342-121359	AC096727	Clone chr4
"	"	"	6-23	AJ617623	Syt XIV-1
"	B	CCTGATGCTGCTTCTTTTCTC	121199-121220	AC096727	Clone chr4
"	"	"	125-146	NM_153262	Syt XIV
"	"	"	181-206	AJ617623	Syt XIV-1
"	"	"	288-309	AJ617624	Syt XIV-2
"	"	"	321-342	AJ617625	Syt XIV-3
"	C	CAGGATGACAGTGGTTCTCC	519-538	NM_153262	Syt XIV
"	"	"	539-558	AJ617623	Syt XIV-1
"	"	"	682-701	AJ617624	Syt XIV-2
"	"	"	715-734	AJ617625	Syt XIV-3
"	"	"	272-291	AJ617626	Syt XIV-4
"	"	"	31-50	AJ617627	Syt XIV-5
"	D	CCAGAAATTCT(C/T)ATT(A/G)GCCTGC	120999-121020	AC096727	Clone chr4
"	"	"	15-36	AJ303368	chr415 syt
"	"	"	1263-1284	NM_153262	Syt XIV
"	"	"	1283-1304	AJ617623	Syt XIV-1
"	"	"	1483-1304	AJ617624	Syt XIV-2
"	"	"	1188-1209	AJ617625	Syt XIV-3
"	"	"	1016-1037	AJ617626	Syt XIV-4
"	"	"	775-796	AJ617627	Syt XIV-5
Reverse	E	TCATCGTGACTCTAGCAACG	120604-120624	AC096727	Clone chr4
"	"	"	1666-1685	NM_153262	Syt XIV
"	"	"	1825-1848	AJ617624	Syt XIV-2
"	"	"	1587-1610	AJ617625	Syt XIV-3
"	"	"	1493-1516	AJ617626	Syt XIV-4
"	"	"	1231-1254	AJ617627	Syt XIV-5
"	F	GGAGAACCACTGTCATCCTG	539-558	NM_153262	Syt XIV
"	"	"	682-701	AJ617624	Syt XIV-2
"	"	"	715-734	AJ617625	Syt XIV-3
"	"	"	272-291	AJ617626	Syt XIV-4
"	"	"	31-50	AJ617627	Syt XIV-5
"	G	TTCATCGTGACTCTAGCAACG	120603-120623	AC096727	Clone chr4
"	"	"	412-432	AJ303368	chr415 syt
"	"	"	1652-1668	NM_153262	Syt XIV
"	"	"	1666-1686	AJ617623	Syt XIV-1
"	"	"	1829-1849	AJ617624	Syt XIV-2
"	"	"	1591-1611	AJ617625	Syt XIV-3
"	"	"	1497-1517	AJ617626	Syt XIV-4
"	"	"	1235-1255	AJ617627	Syt XIV-5

Table 2*Exon-intron junctions in the human syt XIV gene*

Exon and intron sequences are in upper and lower case letters, respectively. Start and stop codons are labeled in bold. The gene contains the typical gt and ag at the 5' splice site (donor site) and 3' splice site (acceptor site) for RNA splicing, required for intron removal. The variant gc and tg dinucleotides for 5'- and 3'-splice donor and acceptor sites are underlined. Sizes in bp of introns and exons are indicated.

Exon	Exon size (bp)	Exon-intron junctions	Intron	Intron size (bp)
1	45	ATCAT TGGTTGAAGgtaagt	1	14431
2	47	aatagGTGGAG.....GGAAAGgtaatt	2	297
3	205	tcagAATCTG.....GGAAAGgtaatt	3	60374
4	169	ctctagTATC....TTGAT GCTTTGTAAgtatcg	4	7236
5	215	aaatagATAATT.....ATGAAGgtaaga	5	73067
6	272	tatcagGAAACT.....CCTGAAgtaagt	6	5418
7	450	tcttagGATATG.....CATTCTgtgagt	7	55258
7'	179	tcttagGATATG.....CTCATG <u>gca</u> agt	7'	55531
8	190	ttacagGGCTGT.....CACCCAgtgagt	8	3509
			8'	3488
9	57	tgtcagATGGAC.....TCCGAGgtgagt	9	1451
9'	78	ttact <u>g</u> CTTTCC.....TCCGAGgtgagt		
10	>314	ctttagATACAT....GAGTCAT GA ...		

Figure 1A
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PMN Chr415 syt -----CCAGAAATTCATTAGCCTGCTTTATAATGCCACAAATGGGAGACTATCAGCAGAAGTGATAAAAGGCAGC 70
Chr415 syt -----AAGACGGGTCAGTTCCAGAAATTCATTAGCCTGCTTTATAATGCCACAAATGGGAGACTATCAGCAGAAGTGATAAAAGGCAGC 86
Syt XIV TGTAGTGAAAGTACATCCTCATGTGTCAGTCTCTTGAACATGGCTCAGTTCCAGAAATTCATTAGCCTGCTTTATAATGCCACAAATGGGAGACTATCAGCAGAAGTGATAAAAGGCAGC 1320
          ***** *** *****

PMN Chr415 syt CACTTAAAAAATTGGGCAGCAAACAGACCACCCAATACATATGTTAAGTTAACTCTACGAAATCCATGGATCAAGAGATGTCCAAATGCAAGATATCCATCCGCAGAGGGCGGCCAAAT 190
Chr415 syt CACTTAAAAAATTGGGCAGCAAACAGACCACCCAATACATATGTTAAGTTAACTCTACGAAATCCACGGATCAAGAGATGTCCAAATGCAAGATATCCATCCGCAGAGGGCGGCCAAAT 206
Syt XIV CACTTCAAAAATTTGGCAGCAAACAGACCACCCAATACATATGTTAAGTTAACTCTACTGAATTCATGGGTCAAGAGATGTCCAAATGCAAGACATCCATCCGCAGAGGGCAGCCAAAT 1440
          ***** *****

PMN Chr415 syt CCAGTATACAAGGAACTTTTGTCTTCAAGTGACCCCTATTTTCAGCTTTCTCATGTGACACTCATGCTGTCTGTGTATAACAAAAGCAGCATGAGAAGAAA---GATGATAGGCTGGATT 307
Chr415 syt CCAGTATACAAGGAACTTTTGTCTTCAAGTGACCCCTATTTTCAGCTTTCTCATGTGACACTCATGCTGTCTGTGTATAACAAAAGCAGCATGAGAAGAAA---GATGATAGGCTGGATT 323
Syt XIV CCAGTATATAAGGAACTTTTGTCTTCAAGTGGCCCTATTTTCAGCTTTCTGATGTGACACTCATACTGTCTGTGTATAACAAACGCAGCATGAAAAGAGAAGAGATGATAGGCTGGATT 1560
          ***** *****

PMN Chr415 syt TATTTAGGTCTCAACAGCTCTGGAGAAGAA--CTCAATCACTGGACTGAAATGAAAGAGTCAAAGGACGGCAAGTACGTAGATGGCAGGCGTTGCTAGAGTCACGATGAA 416
Chr415 syt TATTTAGGTCTCAACAGCTCTGGAGAAGAA--CTCAATCACTGGACTGAAATGAAAGAGTCAAAGGACGGCAAGTACGTAGATGGCAGGCGTTGCTAGAGTCACGATGAA 432
Syt XIV TCTTTAGGTCTCAACAGCTCTGGAGAAGAACTCAATCACTGGACTGAAATGAAAGAGTCAAAGGACAGCAAGTATGTAGATGGCATGCGTTGCTAGAGTCATGA---- 1668
          * *****

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Figure 1B
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Figure 2A
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cagttggtgcggtccatggtgagctcatcatggcgattgaaggtggagaga 51
 gaacctgtggagtacatgaacttacctgttttagaaaagtatctccagagg 102
 cagttgggtttttgtcagctgtttgggtgtttattatcctgatgctgcttc 153
 tttttctctatattaataagcagttctgttttgaaaATGTTGGAGGGCTTC 204
 CAGATCTTGGTTCAGAATACAGTACAAGGAACAATTCAAGATAAAATGT 5
 Q I L V Q N T V Q G T I H K I K C 22
 GTCTGTGACTCCCAATGAGCGTGTGAGAAATGTCTTGTAGTGAAAGTACA 306
 V C D S Q M S V S E M S C S E S T 39
 TCCGCATGTGAGTCTCTCGAAGACGGGTGAGTTCCAGAAATTCATTAGC 357
 S A C Q S L E D G S V P E I L I S 56
 CTGCTTTATAATGCCACAAATGGGAGACTATCAGCAGAAGTGATAAAAGGC 408
 L I Y N A T N G R L S A E V I K G 73
 AGCCACTTAAAAAATTTGGGAGCAAAACAGACCCCAATACATATGTTAAG 459
 S H L K N W A A N R P P N T Y V K 90
 TTAAGTCTACGGAATCCATGGATCAAGAGATGTCCAAATGCAAGATATCC 510
 L T L R K S M D Q E M S K C K I S 107
 ATCCGCAGAGGGCGGCCAAATCCAGTATACAAGGAAACTTTTGTTCAAA 561
 I R R G R P N P V Y K E T F V F K 124
 GTGACCCTATTTTCTCATGTGACACTCATGTGTCTGTGTATAAC 612
 V T L F Q L S H V T L M L S V Y N 141
 AAAAGCAGCATGAGAAGAAAGATGATAGGCTGGATTTATTTAGGTCTCAAC 663
 K S S M R R K M I G W I Y L G L N 158
 AGCTCTGGAGAAGAACTCAATCACTGGACTGAAATGAAAGAGTCAAAAGGA 714
 S S G E E L N H W T E M K E S K G 175
 CGGCAAGTACGTAGATGGCAGGCGTTGCTAGAGTCACGATGAatacaataa 765
 R Q V R R W Q A L L E S R 188
 gcaaacagttaccatccaatgcagcatattttctaattacaacattactgtt 816
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 aaaaaagtcactatataattattataaaactagaacttggtattaccataataa 2346
 aaaaagacctgtaatttctgtatttttcaatttttttttttttttttttttg 2397
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 atgaggtttgagaatgtgttttacagggtgcattaaagggaataattcat 2499
 attttattgttacatcaatgagcttttccaggattttttacaataaaacgag 2550
 taggaattagaacctgaatcaaatggcgagagat 2584

C2B

Figure 2B
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Figure 3
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Figure 4
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Figure 5
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