CELL CYCLE
2016, VOL. 15, NO. 17, 2360–2366
http://dx.doi.org/10.1080/15384101.2016.1203494

REPORT

OPEN ACCESS

Loss of all 3 Extended Synaptotagmins does not affect normal mouse development, viability or fertility

Michel G. Tremblay[a,b] and Tom Moss[a,b]

[a]Laboratory of Growth and Development, St-Patrick Research Group in Basic Oncology, Cancer Division of the Quebec University Hospital Research Center, Quebec, QC, Canada; [b]Department of Molecular Biology, Medical Biochemistry and Pathology, Faculty of Medicine, Laval University, Québec, QC, Canada

ABSTRACT

The extended synaptotagmins, E-Syt1, 2 and 3, are multiple C2 domain membrane proteins that are tethered to the endoplasmic reticulum and interact in a calcium dependent manner with plasma membrane phospholipids to form endoplasmic reticulum - plasma membrane junctions. These junctions have been implicated in the exchange of phospholipids between the 2 organelles. The E-Syts have further been implicated in receptor signaling and endocytosis and can interact directly with fibroblast growth factor and other cell surface receptors. Despite these multiple functions, the search for a requirement *in vivo* has been elusive. Most recently, we found that the genes for E-Syt2 and 3 could be inactivated without effect on mouse development, viability, fertility or morphology. We have now created insertion and deletion mutations in the last of the mouse E-Syt genes. We show that E-Syt1 is specifically expressed throughout the embryonic skeleton during the early stages of chrondrogenesis in a pattern quite distinct from that of E-Syt2 or 3. Despite this, E-Syt1 is also not required for mouse development and propagation. We further show that even the combined inactivation of all 3 E-Syt genes has no effect on mouse viability or fertility in the laboratory. However, this inactivation induces an enhancement in the expression of the genes encoding Orp5/8, Orai1, STIM1 and TMEM110, endoplasmic reticulum - plasma membrane junction proteins that potentially could compensate for E-Syt loss. Given the multiple functions suggested for the E-Syts and their evolutionary conservation, our unexpected findings suggest that they may only provide a survival advantage under specific conditions that have as yet to be identified.

ARTICLE HISTORY
Received 27 April 2016
Revised 9 June 2016
Accepted 13 June 2016

KEYWORDS
Extended-Synaptotagmin; E-Syt1; E-Syt2; E-Syt3; ESyt1; ESyt2; ESyt3; Expression Profiling; Developmental Expression Profiling; Gene Deletion; Viability' Mouse

Introduction

The E-Syts (E-Syt1, 2 and 3) are C2-domain membrane proteins that bind membrane phospholipids in a Ca^{2+} dependent manner.[1] They were initially implicated in glucose transport and fibroblast growth factor (FGF) signaling.[2-5] Subsequently, they were shown to enhance the formation of contact sites between the endoplasmic reticulum (ER) and the plasma membrane (PM) in response to Ca^{2+} signaling,[6,7] see [8],[9] and [10] for reviews. The E-Syts are broadly conserved throughout the animal kingdom and are analogous to the tricalbins (Tcb1, 2 and 3), which perform analogous functions in ER-PM tethering and phospholipid transport in yeast.[11,12] By their ability to form ER-PM junctions, the E-Syts were shown to facilitate the transport of phospholipids to the plasma membrane (PM).[7,13-15] In this way they can act to sustain homeostasis of PM phosopholipids and particularly of phosphatidylinositol (PI) and phosphatidylinositol 4,5 biphosphate (PtdIns(4,5)P_2 or PIP_2). PIP_2 is a major substrate in cell signaling and its hydrolysis by phospholipase C (PLC) activates Ca^{2+} signaling via the release of inositol 1,4,5-trisphosphate (IP_3). Since activation of PLCγ and PIP_2 hydrolysis is a major pathway of FGF signaling, the ability of the E-Syts to replenish plasma membrane PIP_2 would naturally enhance and prolong signaling through this pathway, as was suggested for E-Syt2 during early Xenopus development.[5,9]

The ability of the E-Syts, and particularly E-Syt2, to induce tight ER-PM junctions would logically also promote the bidirectional transfer of phospholipids between the PM and ER via both the ORP5/8 and Nir2/3 exchange proteins, e.g. see.[9] This clearly has broader implications for the maintenance of PM phosphoinositide homeostasis, as shown recently by the demonstration that E-Syt2 dependent ER-PM junctioning enhances the removal of PI(4)P via ORP5/8 and the Sac1 phosphatase.[16] However, targeted inactivation of all 3 E-Syt genes in HeLa cells had no effect on PtdIns(4,5)P_2 dynamics following activation of muscarinic receptor, though it did affect the rate of removal of DAG from the PM.[14] This latter effect is consistent with a reduction in the rate of DAG to PA conversion or more likely PA removal via the Nir2/3 exchange pathway,[10,13] see also.[9]

Given the apparent importance of the E-Syts in the dynamics of membrane lipid composition and in Ca^{2+}, FGF and potentially other signaling pathways, it is essential that we

CONTACT Tom Moss Tom.Moss@crhdq.ulaval.ca Laboratory of Growth and Development, St-Patrick Research Group in Basic Oncology, Cancer Division of the Quebec University Hospital Research Center and Department of Molecular Biology, Medical Biochemistry and Pathology, Faculty of Medicine, Laval University, Edifice St Patrick, 9 rue McMahon, Québec, QC, G1R 3S3, Canada.

© 2016 Michel G. Tremblay and Tom Moss. Published with license by Taylor & Francis
This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

understand their role *in vivo*. It was, therefore, surprising to find that inactivation of the mouse E-Syt2 and 3 genes had no detectable phenotypic effect, and that $esyt2^{-/-}esyt3^{-/-}$ mice were both viable and fertile. Here we have extended these *in vivo* studies to the last of the E-Syts, E-Syt1. We find that the E-Syt1 gene is discretely expressed in the developing embryonic skeleton, but its functions are apparently also not essential for mouse viability. Indeed, we show that $esyt1^{-/-}$ and even $esyt1^{-/-}esyt2^{-/-}esyt3^{-/-}$ mice are viable and fertile and display no overt functional or morphological anomalies.

Results

Insertional mutation of the E-Syt1 gene

Using a recombinant vector from the EUCOMM consortium, we generated mouse embryonic stem (mES) cells carrying a potentially conditional "flox-neo" insertion in the E-Syt1 gene. Lox recombination sites were placed in intron 2 and intron 7, and a ß-Gal marker gene and a *neo* selection gene flanked by FRT (*Flipper*) sites were inserted within intron 2 (Fig. 1A). Nine independent ES cell clones heterozygous for this $esyt1^{flox\text{-}neo}$ allele were isolated and 2 of these (B11 and D7, Fig. 1B) were used to generate mouse lines carrying this "knock-out first" inactivating mutation (Fig. 1C). The mice were then crossed with FLPo and Sox2-Cre recombinase expressing mice to generate $esyt1^{flox}$, and $esyt1^{\beta Gal}$ insertion and $esyt1^{\Delta}$ ($esyt1^{-}$) deletion alleles (Fig. 1D). Finally, the Cre and Flipper transgenes were removed by backcrossing.

E-Syt1 is strongly expressed in the embryonic skeleton and in adult lung and spleen

Insertion of a β-galactosidase (βGal) gene into the *esyt1* locus permitted the expression pattern of this gene to be determined during early mouse development. E-Syt1 expression was first detected at 11.5 dpc within the developing mouse skeleton and this expression became rapidly stronger and more striking,

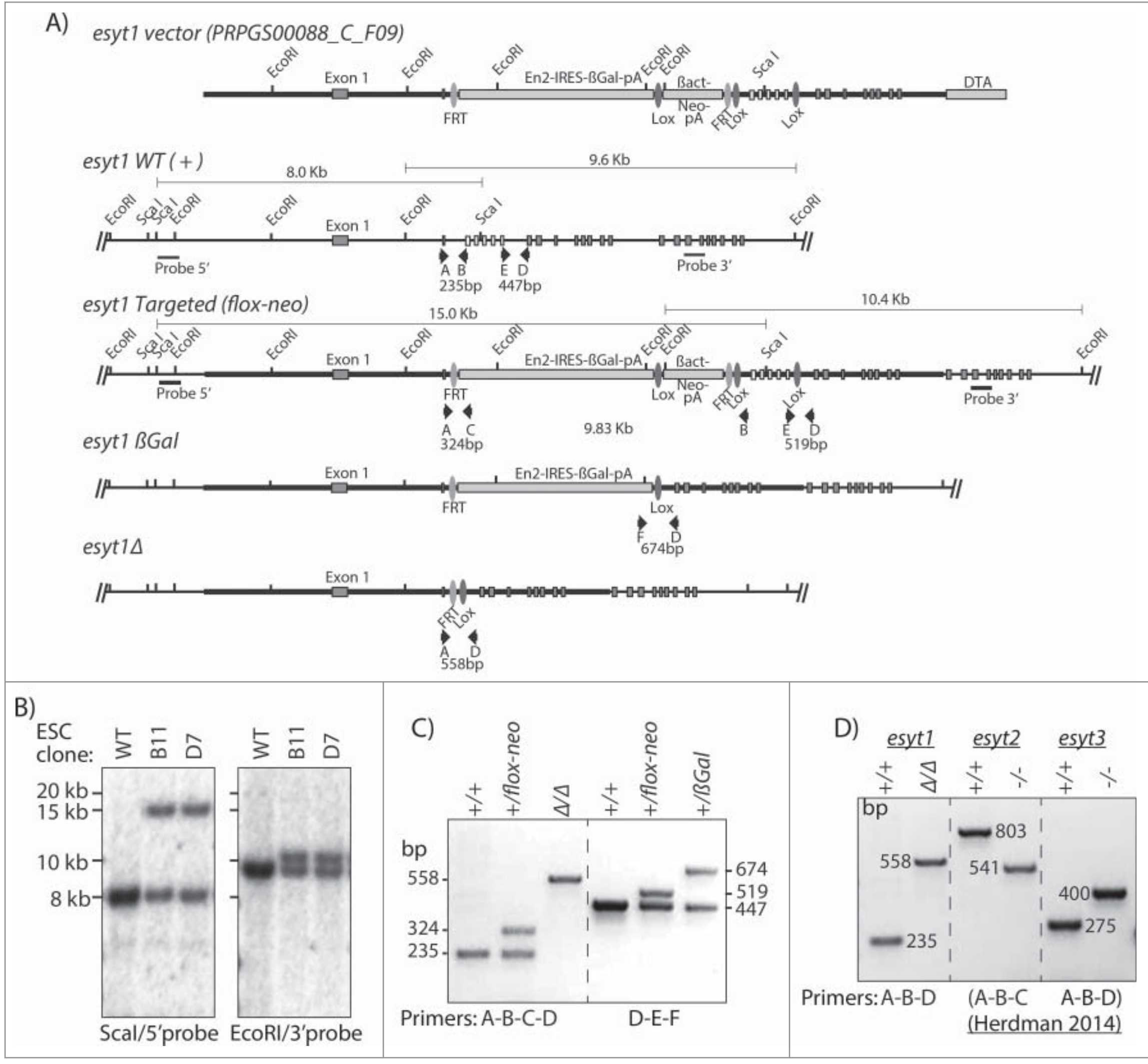

Figure 1. Targeted disruption of the *esyt1* gene in mouse. A) Maps of the EUCOMM *esyt1* Knockout First vector PRPGS00088_C_F09, the wild type (WT, +) mouse *esyt1* gene locus, the initial targeted (flox-neo) gene insertion and the subsequent ßGal insertion and exon 3 to 7 deletion (Δ) products generated by recombination respectively of the inserted FRT and Lox sites. Positions of relevant restriction sites, genotyping PCR primers (thick arrows) and hybridization probes are indicated. B) Southern analysis of the insertion mutant ESC clones used to generate mouse lines. C) Typical example of PCR genotyping of mouse lines carrying the insertion (*flox-neo, ßGal*) and deletion (Δ) mutant *esyt1* alleles in comparison with the WT allele. D) Typical example of PCR genotyping of homozygous *esyt1, 2 and 3* wild type and *esyt1, 2 and 3* null mouse lines. In (C) and (D), the PCR primers used for *esyt1* were those shown in (A), and those for *esyt2* and *esyt3* were as previously described [18].

such that by 13.5 dpc it was observed in a very distinct pattern throughout the developing thoracic skeleton, the cervical, thoracic and lumbar vertebrae, and within the limb buds (Fig. 2). The detail of staining within the lateral mesodermal plate at 13.5 dpc suggested that E-Syt1 was initially expressed at the outer edges of the mesenchymal condensations that presage chondrogenesis (see enlargements in Fig. 2). In fact, its pattern of expression at this stage somewhat resembled that of the transcription factor Runx2, a master regulator of osteoblast differentiation implicated in the process of mesenchymal condensation.[17]

Our previous data showed that E-Syt1 gene was strongly expressed in the lung and spleen of adult mice.[18] Given that this pattern was quite distinct from that previously published for adult human tissues,[1] we repeated the analysis of E-Syt1 expression, and in parallel E-Syt2 and 3, in wild type adult mouse tissues (Fig. 3A). The results concurred with our previous data for all 3 E-Syts,[18] showing strong E-Syt1 expression only in lung and spleen. To further confirm these data, we repeated the extraction of total RNA from kidney, lung, spleen, stomach, testes and heart tissues of wild type mice and determined E-Syt expression levels by Real-Time qRT-PCR (Fig. 3B). Again here, E-Syt1 expression was found to be strong in lung and spleen, weak in stomach and insignificant in kidney, testes and heart, while the data for E-Syt2 and 3 expression closely followed that obtained in Figure 3A and previously.[18]

The E-Syt1 gene is not essential in mouse

Given the very striking skeletal pattern of E-Syt1 expression, we were very surprised to find that mice homozygous for the $esyt1^{flox\text{-}neo}$ "knockout first" insertion mutation developed normally and were viable and fertile, suggesting that the E-Syt1 gene was not essential (Table 1A). Similarly, mice homozygous

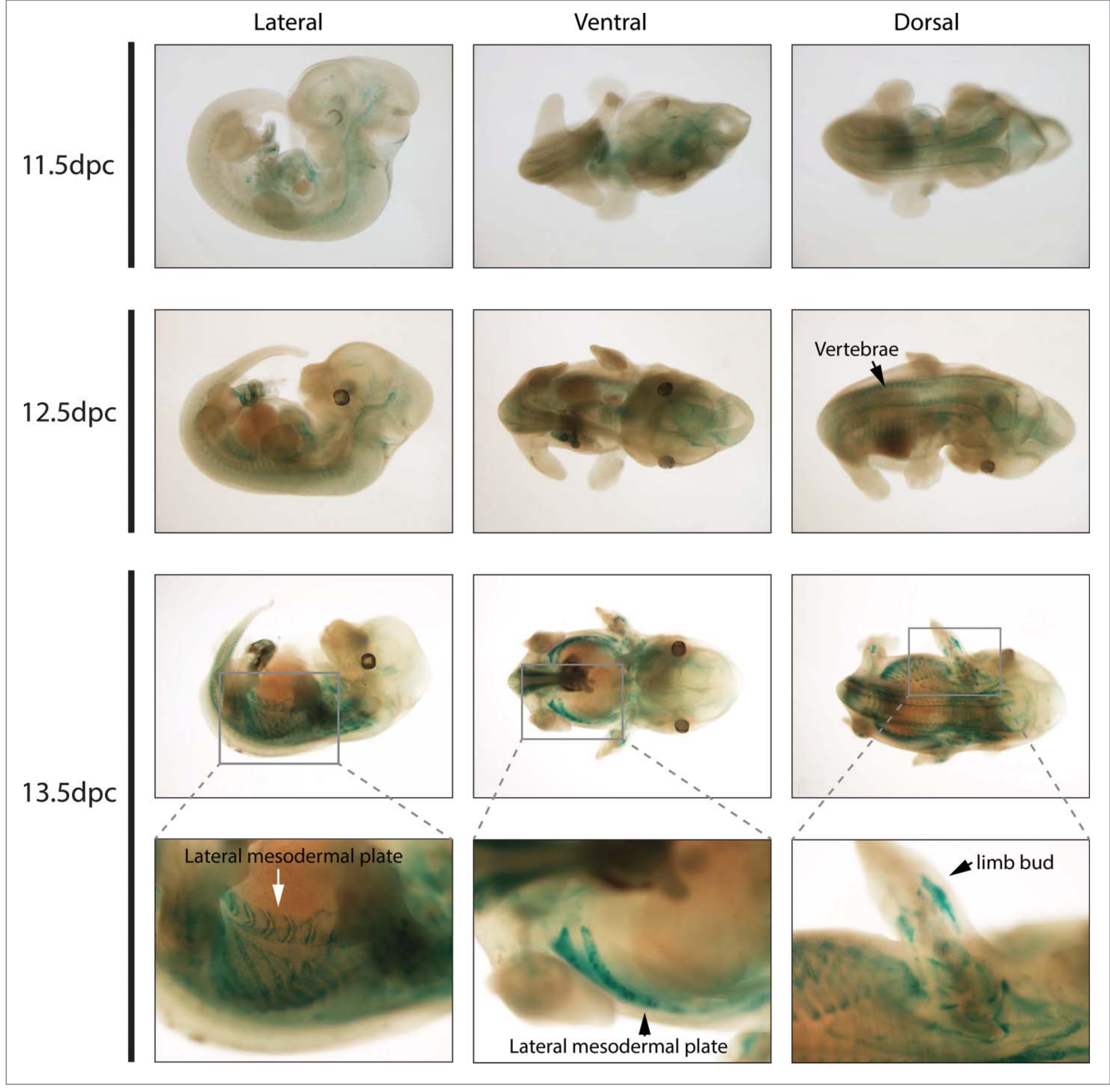

Figure 2. Expression pattern of the *esyt1* gene in early mouse embryos carrying the β-galactosidase (βGal) allele (Fig. 1A). Expression was determined at stages 11.5, 12.5 and 13.5 dpc by conversion of X-Gal (blue-green), see Materials and Methods. Enlarged panels below the whole mount views of the 13.5 dpc embryos show detail of the lateral mesodermal plate and anterior limb-bud. The images are of embryos homozygous for the βGal insertion allele which lack a functional *esyt1* gene. The pattern staining in these embryos was indistinguishable from that of $esyt1^{+/\beta Gal}$ mice but was somewhat stronger consistent with a gene dosage effect.

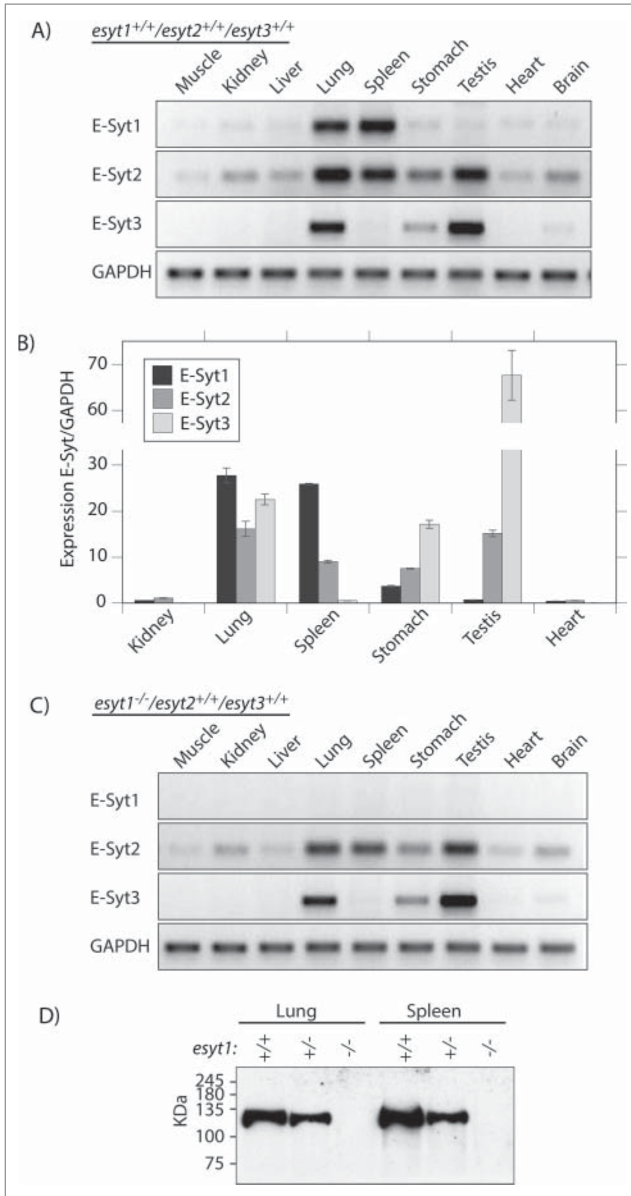

Figure 3. A) RT-PCR analysis of E-Syt1, −2 and −3 mRNA levels in wild type adult mouse tissues. B) Real-Time qRT-PCR analysis to verify E-Syt1, −2 and −3 mRNA levels from a selected subset of wild type adult mouse tissues. C) RT-PCR analysis of E-Syt1, −2 and −3 mRNA levels in E-Syt1-null (*esyt1*$^{-/-}$/*esyt2*$^{+/+}$/*esyt3*$^{+/+}$) adult mouse tissues. D) E-Syt1 protein levels in lung and spleen of mice carrying homozygous or heterozygous deletions (−/− and +/−) of E-Syt1 as compared to the tissues from wild type (+/+) mice. See Materials and Methods for primers.

for the *esyt1* deletion allele (*esyt1Δ*) displayed no evident phenotype or any significant effects on viability, fertility or indeed longevity (of the 75 *esyt1*$^{-/-}$ mice used for breeding, 47 were sacrificed at > 5 months, 27 at > 6 months, 14 at > 7 months and 7 > 8 months, none died of natural causes or suffered from any obvious disorder). Further, the *esyt1*$^{-/-}$ allele displayed Mendelian inheritance (Table 1A). To verify that the E-Syt1 gene had in fact been inactivated, we analyzed a broad range of tissues from *esyt1*$^{-/-}$ and wild type mouse siblings for E-Syt1, 2 and 3 mRNA levels, and both spleen and lung for E-Syt1 protein (Fig. 3C and D). The *esyt1*$^{-/-}$ mice expressed no functional E-Syt1 mRNA, and no E-Syt1 protein could be detected in either tissue. Thus, *esyt1*$^{-}$ represented a true null allele. These data further showed that loss of E-Syt1 mRNA was not compensated by an enhancement in the level of E-Syt2 or 3 mRNAs (Figure A and C). It was therefore concluded that E-Syt1 is not essential for mouse viability, fertility or longevity.

Combined deletion of all 3 E-Syt genes has no effect on viability and fertility

Given the surprising finding that the E-Syt1 gene was not essential, we asked if its function was redundant in the presence of the other 2 E-Syt genes. We had previously generated mice lacking functional E-Syt2 and E-Syt3 genes and showed them also to be viable and phenotypically normal.[18] Thus, we attempted to generate mice in which all 3 E-Syt genes were inactivated. Unexpectedly, we found that mice lacking all 3 genes were viable and the null alleles displayed Mendelian inheritance. For example, crosses of *esyt1*$^{-/-}$/*esyt2*$^{+/-}$/*esyt3*$^{-/-}$ mice showed Mendelian inheritance of the *esyt2*$^{-}$ allele (Table 1B). Further, mice lacking all 3 E-Syt genes (*esyt1*$^{-/-}$/*esyt2*$^{-/-}$/*esyt3*$^{-/-}$) displayed no overt phenotype, displayed no premature mortality and were fertile. Indeed, when E-Syt-null (*esyt1*$^{-/-}$/*esyt2*$^{-/-}$/*esyt3*$^{-/-}$) mice were crossed they generated typical sized litters and offspring displayed no evident phenotype. Thus, not one of the 3 E-Syt genes is essential for normal mouse survival or fertility and mice lacking all 3 genes are phenotypically normal.

Loss of the E-Syts may be partially compensated by upregulation of other ER-PM tethering proteins

The data from yeast suggest that loss of the E-Syt analogs Tcb1 to 3 is in greater part compensated by the actions of other the

Table 1. Genotype analysis of the progeny born A) from *esyt1*^{target}/+ and *esyt1*$^{+/-}$ crosses, and B) from *esyt1*$^{-/-}$/*esyt2*$^{+/-}$/*esyt3*$^{-/-}$ crosses. "n" indicates the number of offspring.

A)	*esyt2*$^{+/+}$/*esyt3*$^{+/+}$/		
Cross	*esyt1*$^{+}$	*esyt1*$^{+/-}$	*esyt1*$^{-/-}$
esyt1$^{target\ /+}$ x esyt1$^{target\ /+}$	23%(n = 17)	50%(n = 37)	27%(n = 20)
esyt1$^{+/-}$ x esyt1$^{+/-}$	42%(n = 8)	37%(n = 7)	21%(n = 4)
Total	27%(n = 25)	47%(n = 44)	26%(n = 24)

B)	*esyt1*$^{-/-}$/*esyt3*$^{-/-}$/		
Cross	*esyt2*$^{+}$	*esyt2*$^{+/-}$	*esyt2*$^{-/-}$
esyt1$^{-/-}$/esyt2$^{+/-}$/esyt3$^{-/-}$x esyt1$^{-/-}$/esyt2$^{+/-}$/esyt3$^{-/-}$	28%(n = 17)	48%(n = 29)	23%(n = 14)

ER-PM tethering proteins.[12] Hence we asked if loss of the mouse E-Syts increased expression of other tethering proteins. The Osh homologs Orp5/8, like the E-Syts, are ER membrane proteins that bind PM phospholipids and induce ER-PM tethering.[8-10] The PM Ca^{2+} channel protein Orai interacts with the ER membrane protein STIM1 during Store Operated Calcium Entry (SOCE) and also tethers ER to PM, an action stimulated by TMEM110. The genes for Orp5, Orai1 and TMEM110 were all strongly expressed in lung, coinciding with strong expression of E-Syts 1 to 3 (Fig. 3A and B and Fig. 4). Expression of Orp5, Orai1 and TMEM110 was found to be 1.5 to 2 times higher in lung from E-Syt-null mice as compared with wild type mice of the same background. E-Syt 1 and 2 expression in spleen corresponded with the expression of Orp8, Orai1, STIM1 and TMEM110, and in each case expression was also significantly increased in E-Syt-null mice. Thus, enhanced expression of a range of tethering proteins may at least in part have compensated for the loss of the E-Syts, providing a potential explanation for the lack of an E-Syt-null phenotype.

Discussion

We previously generated mice lacking the E-Syt2 and 3 genes and found that they were viable, fertile and displayed no morphological abnormalities. This finding was already very surprising given the various studies implicating these proteins in plasma membrane homeostasis and key cell signaling pathways. Here we have shown that inactivation of the E-Syt1 gene, and even the combined inactivation of all 3 E-Syt genes, also has no discernable phenotypic effect, *esyt1*$^{-/-}$/*esyt2*$^{-/-}$/*esyt3*$^{-/-}$ first (F1) and even second generation (F2) mice being viable, fertile and displaying normal survival. However, the present study would not have revealed subtle changes in mouse tissue structure, physiology or behavior. This said, despite the greatly increased complexity of the mouse system, our findings are in fact in line with data on the inactivation of the Tcbs of yeast.[11,12] ER-PM junctions in yeast were only significantly affected when all 6 ER-PM tethering proteins (Ist2, Scs2 and 22, and Tcbs 1, 2 and 3) were inactivated, and even then the growth rate in rich medium remained unaffected.[12] It is, therefore, likely that also in mammals the E-Syts function redundantly with other ER-PM tethering proteins. Indeed we observed a significant enhancement in the expression of the Osh homologs Orp5/8 and the SOCE associated proteins Orai1, STIM1 and TMEM110 known to tether the ER to the PM. As we recently discussed,[9] the paradoxical finding that E-Syt2 knockdown in early Xenopus embryos affects FGF signaling [5] may be related to the size of these embryos, the complex nature of their membranes and the need for extremely rapid lipid transport during cleavage divisions. Thus, it may be necessary to study extreme situations of ER or PM functioning or stress, or other behavioral anomalies, before we can identify an *in vivo* requirement for the mammalian E-Syts. One possible function for the E-Syts, one that could explain their broad evolutionary conservation, is in the regulation viruses-cell interactions such as has been observed for the Arabidopsis synaptotagmin SYTA protein.[19]

Materials and methods

Generation of the E-Syt1 conditional mutation

The *esyt1* gene was mutated using the targeted KO-first, conditional ready, lacZ-tagged mutant vector PRPGS00088_C_F09 vector from the EUCOMM targeting project 69413. The vector

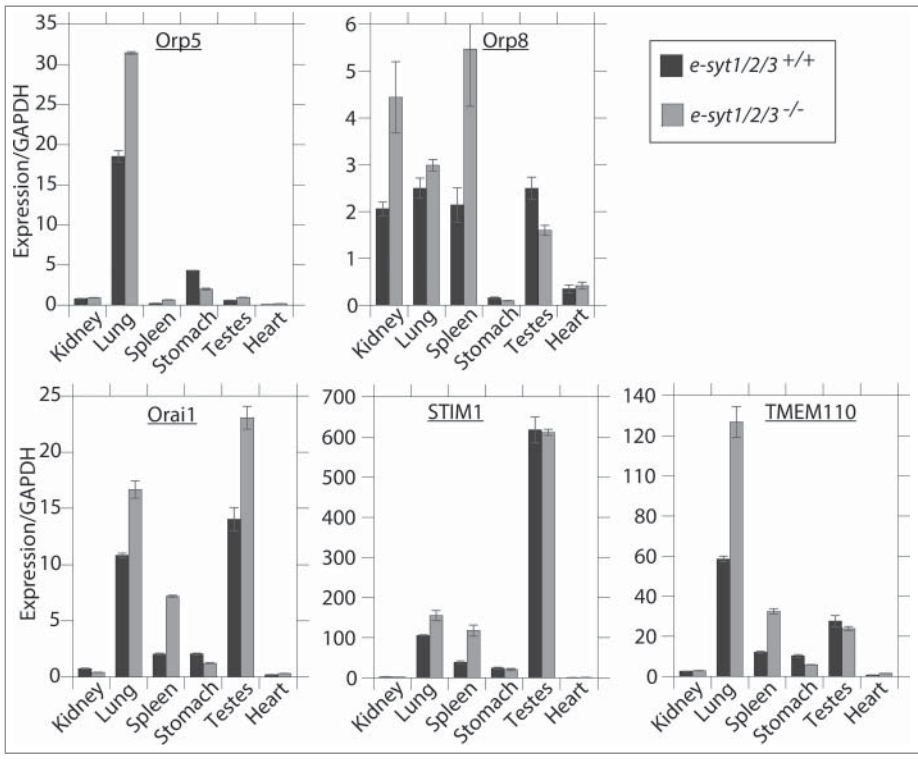

Figure 4. Analysis of Orp5/8, Orai1, STIM1 and TMEM110 tethering protein expression levels in tissues from wild type and E-Syt-null mice. See Materials and Methods for primers.

was linearized and used to electroporate WW6 ES cells, which were then selected with G418. Resistant clones were amplified and analyzed by 5′ and 3′ Southern blotting to identify correctly recombined clones (Fig. 1A and B). These clones were then used to generate 2 independent mouse lines using the services of the McGill Transgenic Core Facility. The subsequent crossing to induce recombination of FRT and Lox sites used the mouse strains codon-optimized FLP recombinase (FLPo) (#012930) and Sox2-Cre (#004783) from the Jackson Laboratory. The mice were housed and manipulated according to the guidelines of the Canadian Council on Animal Care and experiments were approved by the institutional animal care committee.

Genotyping of mice

Genotypes of animals derived from the various crosses were routinely determined by PCR amplification of genomic DNA, (Fig. 1C and D). Primers used for *esyt1* were A (5′-CTCTTTC GATGCCTTTCCAC-3′), B (5′-AGGGTCCCAGAATCATGA AG-3′), C (5′-CCACAACGGGTTCTTCTGTT-3′), D (5′-TCT GGCTGAGCTCGAATTTGT-3′), E (5′-ATCCTGGGCAGAG GTTCAGA-3′) and F (5′-CGGTCGCTACCATTACCAGT-3′)

For *esyt2*, primers were: A (5′-CCAATCAGCAGTCTTAC-CAT), B (5′-CGTCTCAAGGGAAGGAAATAA) and C (5′-CG CCATACAGTCCTCTTCAC). For *esyt3*, primers were A (5′- C TGAAGCCTCCCAGTAGGTG), B (5′-CCATCACCCCTAGT TGTTGC), and D (5′-GAGGCTCCAGGCCTTAGTTT).

Gene expression analysis by RT-PCR and real-time RT-PCR

Total RNA was extracted from mouse tissues using Trizol (Invitrogen) and analyzed by RT-PCR in the linear amplification range (qRT-PCR) as previously described,[18] or by Real-Time qRT-PCR. For Real-Time qRT-PCR, the RNA was reverse transcribed as for RT-PCR, but amplification was carried out in triplicate in 20 μl reactions containing 10 μl QuantiFast SYBR Green and 1μM of each primer. 35 reaction cycles of 10 s at 95°C and 30 s at 58°C were carried out on a Multiplex 3005 Plus (Stratagene/Agilent). The primers used for the E-Syts were: mE-Syt1.FOR (5′-TGGGATCCTGGTATCTCAGC), mE-Syt1.REV (5′-CTGGGAGATCACGTCCATTT), mE-Syt2.FOR (5′- CGAATCACCGTTCCTCTTGT), mE-Syt2.REV (5′-GCTCTGGAAGATTTGGTTGC), mE-Syt3.FOR (5′- CAAGC CCTTCATAGGAGCTG), mE-Syt3.REV (5′- AGCAAATG-GACTCGGATCAC), mGAPDH.FOR (5′- AACTTTGGCATT GTGGAAGG), mGAPDH.REV (5′ ACACATTGGGGGTAG-GAACA). Amplicons were of the expected sizes of 296 bp for E-Syt1, 192 bp for E-Syt2, 246 bp for E-Syt3 and 223 bp for GAPDH. Products were sub-cloned and sequenced to confirm their specificity. Primers for other tethering proteins were taken from Primer Bank (https://pga.mgh.harvard.edu/primerbank/) [20]; Orai1.FOR (5′-GATCGGCCAGAGTTACTCCG), Orai1.REV (5′-TGGGTAGTCATGGTCTGTGTC), Orp8.FOR (5′-A TGGAGGCAGCCTTAGCAGA), Orp8.REV (5′-CAAATGCT-GAGGTTCGTCACT) STIM1.FOR (5′-TGAAGAGTCTACC-GAAGCAGA), STIM1.REV (5′-AGGTGCTATGTTTCACTG TTGG), TMEM110.FOR (5′-GCGCTCATGCACAGTTTCG), TMEM110.REV (5′-ACAGTGAACAAGGGTCCTCTT), Orp5.FOR (5′-TTCTGGGCTGCGAAAATGAG), Orp5.REV (5′-GT CAGATCCATTGCATAGCCTG), GAPDH.FOR (5′-AGGTC GGTGTGAACGGATTTG), GAPDH.REV (5′-TGTAGACCA TGTAGTTGAGGTCA)

X-gal Staining

Mouse embryos were isolated at E11.5 to E13.5 and fixed for 30 minutes in 1% Formaldehyde, 0.2% Gluteraldehyde, 0.02% NP-40 in 1 x PBS, washed 3 times 20 min. each in Wash Solution (2 mM MgCl2, 0.02% NP40, 1 x PBS). Embryos were protected from light and incubated overnight at R/T in the Staining buffer solution (5 mM potassium ferricyanide, 5 mM potassium ferrocyanide and 1 mg/ml X-gal in Wash Solution). Embryos were rinsed 3 times, 20 min. each, in 1 x PBS. Clarification was performed with "Sca*l*e" solution as described previously.[21]

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Profs Jean Charron and Lucie Jeannotte for their guidance and advice throughout this study. We also wish to acknowledge the services of the McGill University Transgenic Core Facility and the animal house staff of the Quebec University Hospital Research Center (RC-CHU de Québec).

Funding

This work was supported by operating grants from the Cancer Research Society (CRS/SRC) and the National Science and Engineering Council (NSERC) of Canada. The Research Center of the CHU de Québec is supported by a grant from the Fonds de recherche du Québec-Santé (FRQS).

References

[1] Min SW, Chang WP, Sudhof TC. E-Syts, a family of membranous Ca^{2+}-sensor proteins with multiple C_2 domains. Proc Natl Acad Sci U S A 2007; 104:3823-8; PMID:17360437; http://dx.doi.org/10.1073/pnas.0611725104

[2] Lalioti V, Muruais G, Dinarina A, van Damme J, Vandekerckhove J, Sandoval IV. The atypical kinase Cdk5 is activated by insulin, regulates the association between GLUT4 and E-Syt1, and modulates glucose transport in 3T3-L1 adipocytes. Proc Natl Acad Sci U S A 2009; 106:4249-53; PMID:19255425; http://dx.doi.org/10.1073/pnas.0900218106

[3] Tremblay MG, Herdman C, Guillou F, Mishra PK, Baril J, Bellenfant S, Moss T. Extended Synaptotagmin Interaction with the Fibroblast Growth Factor Receptor Depends on Receptor Conformation, Not Catalytic Activity. J Biol Chem 2015; 290:16142-56; PMID:25922075; http://dx.doi.org/10.1074/jbc.M115.656918

[4] Jean S, Tremblay MG, Herdman C, Guillou F, Moss T. The endocytic adapter E-Syt2 recruits the p21 GTPase activated kinase PAK1 to mediate actin dynamics and FGF signalling. Biol Open 2012; 1:731-8; PMID:23213466; http://dx.doi.org/10.1242/bio.2012968

[5] Jean S, Mikryukov A, Tremblay MG, Baril J, Guillou F, Bellenfant S, Moss T. Extended-synaptotagmin-2 mediates FGF receptor endocytosis and ERK activation in vivo. Dev Cell 2010; 19:426-39; PMID:20833364; http://dx.doi.org/10.1016/j.devcel.2010.08.007

[6] Giordano F, Saheki Y, Idevall-Hagren O, Colombo SF, Pirruccello M, Milosevic I, Gracheva EO, Bagriantsev SN, Borgese N, De Camilli P. PI(4,5)P_2-dependent and Ca^{2+} -regulated ER-PM interactions mediated by the extended synaptotagmins. Cell 2013; 153:1494-509; PMID:23791178; http://dx.doi.org/10.1016/j.cell.2013.05.026

[7] Chang CL, Hsieh TS, Yang TT, Rothberg KG, Azizoglu DB, Volk E, Liao JC, Liou J. Feedback regulation of receptor-induced Ca^{2+} signaling mediated by E-Syt1 and Nir2 at endoplasmic reticulum-plasma membrane junctions. Cell Rep 2013; 5:813-25; PMID:24183667; http://dx.doi.org/10.1016/j.celrep.2013.09.038

[8] Henne WM, Liou J, Emr SD. Molecular mechanisms of inter-organelle ER-PM contact sites. Curr Opin Cell Biol 2015; 35:123-30; PMID:26025028; http://dx.doi.org/10.1016/j.ceb.2015.05.001

[9] Herdman C, Moss T. Extended-Synaptotagmins (E-Syts); the extended story. Pharmacol Res 2016; 107:48-56; PMID:26926095; http://dx.doi.org/10.1016/j.phrs.2016.01.034

[10] Chang CL, Liou J. Homeostatic regulation of the PI(4,5)P_2-Ca^{2+} signaling system at ER-PM junctions. Biochim Biophys Acta 2016; PMID:AMBIGUOUS

[11] Creutz CE, Snyder SL, Schulz TA. Characterization of the yeast tricalbins: membrane-bound multi-C2-domain proteins that form complexes involved in membrane trafficking. Cell Mol Life Sci 2004; 61:1208-20; PMID:15141306; http://dx.doi.org/10.1007/s00018-004-4029-8

[12] Manford AG, Stefan CJ, Yuan HL, Macgurn JA, Emr SD. ER-to-plasma membrane tethering proteins regulate cell signaling and ER morphology. Dev Cell 2012; 23:1129-40; PMID:23237950; http://dx.doi.org/10.1016/j.devcel.2012.11.004

[13] Chang CL, Liou J. Phosphatidylinositol 4,5-Bisphosphate Homeostasis Regulated by Nir2 and Nir3 Proteins at Endoplasmic Reticulum-Plasma Membrane Junctions. J Biol Chem 2015; 290:14289-301; PMID:25887399; http://dx.doi.org/10.1074/jbc.M114.621375

[14] Saheki Y, Bian X, Schauder CM, Sawaki Y, Surma MA, Klose C, Pincet F, Reinisch KM, De Camilli P. Control of plasma membrane lipid homeostasis by the extended synaptotagmins. Nat Cell Biol 2016; PMID:27065097

[15] Fernandez-Busnadiego R, Saheki Y, De Camilli P. Three-dimensional architecture of extended synaptotagmin-mediated endoplasmic reticulum-plasma membrane contact sites. Proc Natl Acad Sci U S A 2015; PMID:25787254

[16] Dickson EJ, Jensen JB, Vivas O, Kruse M, Traynor-Kaplan AE, Hille B. Dynamic formation of ER-PM junctions presents a lipid phosphatase to regulate phosphoinositides. J Cell Biol 2016; 213:33-48; PMID:27044890; http://dx.doi.org/10.1083/jcb.201508106

[17] Kimura A, Inose H, Yano F, Fujita K, Ikeda T, Sato S, Iwasaki M, Jinno T, Ae K, Fukumoto S, et al. Runx1 and Runx2 cooperate during sternal morphogenesis. Development 2010; 137:1159-67; PMID:20181744; http://dx.doi.org/10.1242/dev.045005

[18] Herdman C, Tremblay MG, Mishra PK, Moss T. Loss of Extended Synaptotagmins ESyt2 and ESyt3 does not affect mouse development or viability, but in vitro cell migration and survival under stress are affected. Cell Cycle 2014; 13:2616-25; PMID:25486202; http://dx.doi.org/10.4161/15384101.2014.943573

[19] Levy A, Zheng JY, Lazarowitz SG. Synaptotagmin SYTA forms ER-plasma membrane junctions that are recruited to plasmodesmata for plant virus movement. Curr Biol 2015; 25:2018-25; PMID:26166780; http://dx.doi.org/10.1016/j.cub.2015.06.015

[20] Wang X, Spandidos A, Wang H, Seed B. PrimerBank: a PCR primer database for quantitative gene expression analysis, 2012 update. Nucleic Acids Res 2012; 40:D1144-D9; PMID:22086960; http://dx.doi.org/10.1093/nar/gkr1013

[21] Hama H, Kurokawa H, Kawano H, Ando R, Shimogori T, Noda H, Fukami K, Sakaue-Sawano A, Miyawaki A. Scale: a chemical approach for fluorescence imaging and reconstruction of transparent mouse brain. Nat Neurosci 2011; 14:1481-8; PMID:21878933; http://dx.doi.org/10.1038/nn.2928