Deletion of SNAP-23 Results in Pre-Implantation Embryonic Lethality in Mice

Young Ho Suh1,2,3, Aki Yoshimoto-Furusawa5, Karis A. Weih1, Lino Tessarollo4, Katherine W. Roche2, Susan Mackem5, Paul A. Roche1*

1 Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, United States of America, 2 Receptor Biology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, United States of America, 3 Neuroscience Graduate Program, Department of Pharmacology, Ajou University School of Medicine, Suwon, South Korea, 4 Mouse Cancer Genetics Program, Center for Cancer Research (CCR), NCI-Frederick, National Institutes of Health, Frederick, Maryland, United States of America, 5 Cancer and Developmental Biology Laboratory, Center for Cancer Research (CCR), NCI-Frederick, National Institutes of Health, Frederick, Maryland, United States of America

Abstract

SNARE-mediated membrane fusion is a pivotal event for a wide-variety of biological processes. SNAP-25, a neuron-specific SNARE protein, has been well-characterized and mouse embryos lacking Snap25 are viable. However, the phenotype of mice lacking SNAP-23, the ubiquitously expressed SNAP-25 homolog, remains unknown. To reveal the importance of SNAP-23 function in mouse development, we generated Snap23-null mice by homologous recombination. We were unable to obtain newborn SNAP-23-deficient mice, and analysis of pre-implantation embryos from Snap23+/− matings revealed that Snap23-null blastocysts were dying prior to implantation at embryonic day E3.5. Thus these data reveal a critical role for SNAP-23 during embryogenesis.

Citation: Suh YH, Yoshimoto-Furusawa A, Weih KA, Tessarollo L, Roche KW, et al. (2011) Deletion of SNAP-23 Results in Pre-Implantation Embryonic Lethality in Mice. PLoS ONE 6(3): e18444. doi:10.1371/journal.pone.0018444

Editor: Robert Oshima, Sanford-Burnham Medical Research Institute, United States of America

Received January 13, 2011; Accepted February 28, 2011; Published March 29, 2011

This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Funding: This research was supported by the NCI Intramural Research Program (Y.H.S., A.Y.-F., LT, S.M. and P.A.R.), the NINDS Intramural Research Program (Y.H.S., and K.W.R.), and the Integrative Neural Immune Program (Y.H.S. fellowship). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: paul.roche@nih.gov

Introduction

Vesicle-mediated intracellular protein trafficking is essential for a wide-variety of cellular processes including both constitutive protein transport and regulated exocytosis. The protein machinery regulating vesicle trafficking is conserved in organisms ranging from yeast to human, and among them the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) complex has emerged as specialized machinery in mediating vesicle-target membrane fusion [1]. Although there are many factors that interact with and modify the SNARE complex, the trimeric core complex of syntaxin, VAMP/synaptobrevin, and SNAP-25 are the prototypical components of the SNARE complex and together play a key role in membrane fusion process [2].

Since membrane-membrane fusion events are critical for all cell types and are important for maintaining the orderly movement of cargo proteins from one intracellular compartment to another, it is not surprising that there are a wide variety of distinct SNARE isoforms that reside on distinct intracellular compartments, thereby ensuring appropriate homotypic and heterotypic membrane fusion events. For example, there are a wide variety of syntaxin and VAMP isoforms in eukaryotic cells that are expressed on particular organelles in essentially all cell types. By contrast, SNAP-25 is only expressed in neuronal/neuroendocrine cells and the role of SNAP-25 in the SNARE complex in non-neuronal tissues is taken-over by the related protein SNAP-23 [3]. SNAP-23 is ubiquitously expressed and has been shown to play a role in diverse protein trafficking events including GLUT4 transport in adipocytes [4], mast cell degranulation [5–7], dense core granule release in platelets [8], cholecystokinin-regulated exocytosis in pancreatic acinar cells [9], and surface expression/recycling of transferrin receptors [10], the glutamate transporter EAAC1 [11], and NMDA receptors [12,13].

Genetic ablation of various syntaxin and VAMP isoforms does not significantly impair embryonic development, revealing the importance of genetic redundancy of SNARE function in development. Surprisingly, deletion of SNAP-25 does not affect embryo viability, although Snap25-null mice die at birth due to neuromuscular abnormalities [14]. By contrast, the importance of SNAP-23 in mouse development and embryonic viability remains unknown. We now report that deletion of Snap23 results in pre-implantation embryonic lethality, highlighting the importance of this ubiquitous SNARE in mouse development.

Results and Discussion

Generation of SNAP-23-deficient mice

We generated Snap23-deficient mice using a conventional gene replacement targeting method through homologous recombination [15]. A targeting vector was designed to delete Snap23 exon 2, which is the first coding exon of the mouse Snap23 gene [16], by Cre-mediated excision (Figure 1A) and PCR Primer sets were designed to screen for wild-type and targeted Snap23 alleles (Figure 1B). A neomycin-resistance gene under the control of the
PGK promoter (PGK-Neo) was placed in front of exon 2 and PGK-Neo, as well as Snap23 exon 2, were flanked by loxP sequences. The linearized targeting vector was introduced into the CJ7 ES cells and G418/FIAU-resistant clones were screened for homologous recombination at the Snap23 locus. Southern blotting of EcoRI-digested genomic ES cell DNA with a 5' probe revealed a 3.5 kb fragment from the wild-type Snap23 gene as well as a 2.5 kb fragment that was the product of homologous recombination at the 5' end of exon 2 (Figure 2A). Similarly, when ClaI/KpnI-digested DNA was hybridized with a 3' probe we observed a 9.4 kb wild-type fragment as well as a 6.0 kb fragment in targeted ES cells (Figure 2B). Of 85 neomycin-resistant/ganciclovir-
sensitive ES cell clones analyzed by Southern blotting, seven clones had undergone homologous recombination in both the short-arm (5') and long arm (3') sequences of our targeting construct. This Southern blotting result was confirmed using a PCR-based genotyping assay (Figure 1B, Figure 2C, Table 1). Four of these targeted ES cell clones were then used to generate chimeric Snap23^floxed allele. Genomic PCR from this mouse revealed only two PCR fragments corresponding the Snap23^floxed allele and Snap23^allele, indicating that this was a Snap23 floxed exon 2 heterozygous mouse. doi:10.1371/journal.pone.0018444.g002

To generate Snap23 exon 2 floxed mice as well as mice in which Snap23 exon 2 was deleted, Snap23^Neo^/WT mice were mated with transgenic mice expressing Cre under the control of the EIIa promoter. The adenovirus EIIa early promoter is known to be transcriptionally active in mouse oocytes and early embryos prior to implantation in the uterus [17]. Of note, when male EIIa-Cre transgenic mice are mated with female mice harboring multipleloxP sites, partial Cre-mediated excision can occur among differentloxP sites, leading to the production of mice with a mosaic genotype [18]. This mosaic (F2) genotype can be observed by PCR analysis of genomic DNA (Figure 2D) and can be segregated into the discrete recombinant alleles in the next (F3) generation by mating with wild-type mice [18]. This breeding gave rise to Snap23^and Snap23^Neo^/WT mice (Figure 1A, Figure 2D). Genomic PCR from F3 mouse tail DNA identified a mouse (number 182234) that contained only the Snap23^/WT mice (Figure 1A, Figure 2D). These data are consistent with the idea that the EIIa-Cre transgenic mice are marked with female mice harboring multipleloxP sites, partial Cre-mediated excision can occur among differentloxP sites, leading to the production of mice with a mosaic genotype [18]. This mosaic (F2) genotype can be observed by PCR analysis of genomic DNA (Figure 2D) and can be segregated into the discrete recombinant alleles in the next (F3) generation by mating with wild-type mice [18]. This breeding gave rise to Snap23^/WT and Snap23^Neo^/WT mice (Figure 1A, Figure 2D). Genomic PCR from F3 mouse tail DNA identified a mouse (number 182234) that contained only the Snap23^ and Snap23^alleles (Figure 2D), demonstrating that the mosaic alleles were segregated individually in this mouse.

Brains from isolated from Snap23^/WT mice expressed only half as much SNAP-23 protein as Snap23^floxed allele spleen cells, demonstrating that expression of SNAP-23 from the floxed allele was defective (Figure 3A). Identical results were observed when analyzing SNAP-23 expression in mast cells and spleen from Snap23^/WT mice. These data are consistent with the idea that theloxP sites adjacent to the Snap23 exon 2 splice donor/acceptor sites interfered with SNAP-23 expression, a situation observed also in other mouse models ([15] and L.T., unpublished observations). Unlike their male counterparts, female EIIa-Cre transgenic mice completely excise sequences betweenloxP sites [18,19], and

---

**Table 1. Oligonucleotide primers used in this study and estimated size of PCR products.**

| Name of primers | Sequences (5’ to 3’) |
|-----------------|---------------------|
| genoE2 SS       | TGCCCATAGGTTGTCAGACT |
| genoNEO SS      | TCACCTTAAATTGCGAAGTGG |
| genoE2 AS       | ATGTGCTAACCAGACCCTTGA |
| genoE2 rev      | GAGAGACCTCAGTGTTGGAG |
| CreSS           | CCGGCGTGCCACGACACA |
| CreAS           | GGCGCGGACAAACCATTTT |
| SA-4400Spel     | ACTAGTGTTGCTACCTCTCACAAGTTTC |
| SA-6400Sall     | TCGAATCTTCTCCGAGTCACCTCAGTG |
| E2-4400Spel     | ACTAGTGATCAGAAGCCTAGTTAGG |
| E2-3400Kpnl     | GGTACCCCGAAGTCCGAGGTAAACT |
| E2-5probeSS     | TGGCCAGAAGACTGGTAAAGC |
| E2-7300Kpnl     | TGCGTCTGAACTCAATCCTG |
| E2-3probeSS     | GGTTAGACGGATGTTATG |
| E2-3probeAS     | AGAATGCAGCTCTGTAG |

The oligonucleotide sequences used in this study are listed in the upper table. The oligonucleotide primers were used to genotype the mice, to obtain genomic fragments of Snap23, and to generate probes for Southern blot analysis. The estimated sizes of PCR products obtained during genotyping the mice are indicated in the lower table. doi:10.1371/journal.pone.0018444.t001
breeding these mice with male Snap23<sup>fl/wt</sup> mice resulted in the generation of Snap23<sup>fl/fl</sup> mice. As expected, expression of SNAP-23 from the exon 2-deleted allele (Snap23<sup>fl/fl</sup>) was also defective in the brains of Snap23<sup>fl/fl</sup> mouse pups and densitometry confirmed that SNAP-23 expression was only half that observed in Snap23<sup>wt/wt</sup> littermates (Figure 3B). Expression of other SNARE proteins, including syntaxin 1a, syntaxin 3, syntaxin 13, SNAP-25, and VAMP2 was not altered in Snap23<sup>fl/fl</sup> heterozygous mice.

**Deletion of Snap23 leads to early embryonic lethality**

To generate homozygous Snap23<sup>fl/fl</sup> mice, Snap23<sup>fl/fl</sup> mice were mated with each other. Genotyping of tail DNA from nearly 100 live pups failed to reveal any homozygous Snap23<sup>fl/fl</sup> mice, strongly suggesting that the Snap23<sup>fl</sup> allele was not expressed and that deletion of Snap23 resulted in lethality. Instead of characterizing Snap23<sup>fl/fl</sup> mice further, we set out to investigate the effects of Snap23 deletion using exon 2-deleted Snap23<sup>fl/fl</sup> mice. To generate Snap23-deficient mice, Snap23<sup>fl/wt</sup> mice were mated with each other. As expected (based on our analysis of Snap23<sup>fl/fl</sup> mice), Snap23<sup>fl/wt</sup> pups were never obtained from adult heterozygous matings after more than 50 live births (data not shown). Genotyping confirmed that approximately 2/3 of these offspring were Snap23<sup>fl/wt</sup> and 1/3 were Snap23<sup>wt/wt</sup>, demonstrating that deletion of Snap23 leads to embryonic lethality.

To determine at what embryonic stage Snap23-deficient mice were dying we obtained embryos isolated from the timed-pregnant Snap23<sup>fl/wt</sup> heterozygous matings. No Snap23<sup>fl/wt</sup> embryos were recovered from 48 embryos obtained from embryonic day 16.5 (E16.5), E12.5, E11.5, E9.5, or E7.5, with 35 heterozygous and 13 wild-type embryos isolated (Table 2). Immunoblot analysis confirmed that SNAP-23 protein expression from heterozygous embryos was reduced by half as compared to wild-type embryos (data not shown). These results suggest that Snap23-null mice are dying at an early, pre-implantation developmental stage.

To determine if the Snap23 null mutation is lethal before uterine implantation, blastocyst stage embryos were recovered by flushing from the uterus of pregnant mice at day E3.5. We noted that 5 among a total of 27 blastocysts isolated using this procedure appeared grossly abnormal and had not expanded properly, suggesting that these blastocysts were dying, and unlike the normal blastocysts, they failed to develop any further after 24 hrs of culture (arrows in Figure 4A). Genomic DNA was isolated from each blastocyst and genomic PCR was performed. Each of five small, degenerating blastocysts showed a strong endogenous Snap23 exon 2-deleted allele (Snap23<sup>fl/fl</sup>) No. of Het embryos (+/−) No. of Homozygote embryos (−/−)

| Age of embryos | No. of dissected embryos | No. of WT embryos (+/+) | No. of Het embryos (+/−) | No. of Homozygote embryos (−/−) |
|----------------|--------------------------|--------------------------|--------------------------|-------------------------------|
| E16.5          | 7                        | 4                        | 3                        | 0                             |
| E12.5          | 8                        | 2                        | 6                        | 0                             |
| E11.5          | 8                        | 2                        | 6                        | 0                             |
| E9.5           | 14                       | 3                        | 11                       | 0                             |
| E7.5           | 11                       | 2                        | 9                        | 0                             |
| E3.5           | 27                       | 11                       | 11                       | 5                             |

Embryo age, number of dissected embryos, and Snap23 genotype results are summarized as indicated.

doi:10.1371/journal.pone.0018444.002
SNAP23 Is Essential for Blastocyst Development

The use and care of animals used in this study followed the guidelines of the NIH Animal Research Advisory Committee. C57BL/6 mice were obtained from NCI-Frederick (Frederick, MD) and E10a-Cre mice were obtained from The Jackson Laboratories (Bar Harbor, ME). All protocols were approved by the National Cancer Institute-Center for Cancer Research Animal Care and Use Committee (protocol numbers EIB-076 and EIB-094). Rabbit SNAP-23 antibody is described in a previous publication [13]. Syntaxin 1a (HPC 1; Wako chemicals), syntaxin 5 (Alomone labs), syntaxin 13 (15G2, Abcam), VAMP-2 (Cl 69.1; Synaptic Systems), α-tubulin (Sigma) antibodies were purchased from commercial sources as indicated.

Generation of Snap23 targeted mice

The Neo(+) Snap23 exon 2 targeting allele was constructed from a BAC clone derived from a 129/SvJ mouse genomic library [16] by flanking exon 2 of the mouse Snap23 gene with loxP sites. The targeting vector was constructed as follows: a 2 kb genomic DNA fragment upstream of Snap23 exon 2 was obtained as a short arm by PCR using primers SA-4400Spel and SA-6400SalI via SpeI/SalI restriction sites. All oligonucleotide primer sequences are provided in Table S1 of the Supplemental Information. In conclusion, we generated Snap23 knockout mice and demonstrated that Snap23 deletion is lethal. Specifically, we showed that Snap23-null embryos die prior to implantation in the uterus. These data indicate that SNAP-23 plays a unique and essential role as a membrane fusion protein that is essential for cell viability.

Materials and Methods

Animals and antibodies

The use and care of animals used in this study followed the guidelines of the NIH Animal Research Advisory Committee. C57BL/6 mice were obtained from NCI-Frederick (Frederick, MD) and E10a-Cre mice were obtained from The Jackson Laboratories (Bar Harbor, ME). All protocols were approved by the National Cancer Institute-Center for Cancer Research Animal Care and Use Committee (protocol numbers EIB-076 and EIB-094). Rabbit SNAP-23 antibody is described in a previous publication [13]. Syntaxin 1a (HPC 1; Wako chemicals), syntaxin 5 (Alomone labs), syntaxin 13 (15G2, Abcam), VAMP-2 (Cl 69.1; Synaptic Systems), α-tubulin (Sigma) antibodies were purchased from commercial sources as indicated.

Generation of Snap23 targeted mice

The Neo(+) Snap23 exon 2 targeting allele was constructed from a BAC clone derived from a 129/SvJ mouse genomic library [16] by flanking exon 2 of the mouse Snap23 gene with loxP sites. The targeting vector was constructed as follows: a 2 kb genomic DNA fragment upstream of Snap23 exon 2 was obtained as a short arm by PCR using primers SA-4400Spel and SA-6400SalI via SpeI/SalI restriction sites. All oligonucleotide primer sequences are provided in Table S1 of the Supplemental Information. In conclusion, we generated Snap23 knockout mice and demonstrated that Snap23 deletion is lethal. Specifically, we showed that Snap23-null embryos die prior to implantation in the uterus. These data indicate that SNAP-23 plays a unique and essential role as a membrane fusion protein that is essential for cell viability.

Materials and Methods

Animals and antibodies

The use and care of animals used in this study followed the guidelines of the NIH Animal Research Advisory Committee. C57BL/6 mice were obtained from NCI-Frederick (Frederick, MD) and E10a-Cre mice were obtained from The Jackson Laboratories (Bar Harbor, ME). All protocols were approved by the National Cancer Institute-Center for Cancer Research Animal Care and Use Committee (protocol numbers EIB-076 and EIB-094). Rabbit SNAP-23 antibody is described in a previous publication [13]. Syntaxin 1a (HPC 1; Wako chemicals), syntaxin 5 (Alomone labs), syntaxin 13 (15G2, Abcam), VAMP-2 (Cl 69.1; Synaptic Systems), α-tubulin (Sigma) antibodies were purchased from commercial sources as indicated.

Generation of Snap23 targeted mice

The Neo(+) Snap23 exon 2 targeting allele was constructed from a BAC clone derived from a 129/SvJ mouse genomic library [16] by flanking exon 2 of the mouse Snap23 gene with loxP sites. The targeting vector was constructed as follows: a 2 kb genomic DNA fragment upstream of Snap23 exon 2 was obtained as a short arm by PCR using primers SA-4400Spel and SA-6400SalI via SpeI/SalI restriction sites. All oligonucleotide primer sequences are provided in Table S1 of the Supplemental Information. In conclusion, we generated Snap23 knockout mice and demonstrated that Snap23 deletion is lethal. Specifically, we showed that Snap23-null embryos die prior to implantation in the uterus. These data indicate that SNAP-23 plays a unique and essential role as a membrane fusion protein that is essential for cell viability.
arm fragment into Clal/Sall-digested pBS (long arm-HSV-tk) for double selection. Finally, a SpeI linker was generated on the 3' end of a short arm-loxP-Neo-loxP-exon 2-loxP fragment, which was further cloned into a long arm-HSV-tk in pBS by SpeI digestion. The nucleotide sequences of exon 2, loxP, PGR-Neo, and parts of short arm and long arm were verified by DNA sequencing. The targeting vector was linearized by NotI digestion and electroporated into the C57BL/6 mouse strain to generate germ-line chimeric offspring [31]. Ossipring bearing the targeted Snap23 allele were backcrossed more than four generations onto the C57BL/6 background before use to remove potential ES cell mutations not linked to the targeted allele.

DNA analysis

ES cells, tissues, or mouse tails were lysed in 500 μl of DNA lysis buffer containing 40 mM Tris-HCl, pH 7.6, 200 mM NaCl, 20 mM EDTA, 0.5% sodium dodecyl sulfate (SDS), and 60 μg/ml proteinase K (Sigma-Aldrich) at 56°C overnight. Genomic DNA was extracted using phenol-chloroform followed by ethanol precipitation. The purified DNA was digested with EcoRI or Clal/KpnI and analyzed by standard Southern blotting. All restriction enzymes were purchased from New England Biolabs. DNA templates for probe were amplified from a BAC DNA by PCR using primers E2-5probeSS and E2-5probeAS for 5’ DNA templates for probe were amplified from a BAC DNA by ClaI/KpnI and analyzed by standard Southern blotting. All was extracted using phenol-chloroform followed by ethanol 20 mM EDTA, 0.5% sodium dodecyl sulfate (SDS), and 60 buffer containing 40 mM Tris-HCl, pH 7.6, 200 mM NaCl, 2 mM EDTA, 1% NP40, 0.1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate) containing EDTA-free complete protease inhibitor (Roche). The lysates were incubated for 30 min on ice and centrifuged at 20,000 g for 20 min. The supernatants were collected, resolved by SDS-PAGE, transferred to PVDF membranes, and analyzed by immunoblotting with the relevant antibodies as indicated. Bound antibodies were revealed using Western Lightening Chemiluminescence Reactagen Plus (Perkin Elmer LifeSciences, Inc., Boston, MA).

Isolation of blastocyst stage embryos

Four week-old Snap23<sup>−/−</sup> females were induced to superovulate with sequential injections of FSH and HCG and bred with Snap23<sup>−/−</sup> males using standard protocols [32]. The plug was examined the following day (noon considered as E0.5) and blastocysts were recovered at E3.5 by flushing the uterus. Collected blastocysts were maintained in culture for one day in M16 medium to reduce contamination by maternal tissues, and then lysed to purify genomic DNA for analysis.

Acknowledgments

We thank Eileen Southon and Susan Reid for technical assistance in the generation of the Snap23 mutant mice.

Author Contributions

Conceived and designed the experiments: YHS AY-F KAW LT KWR SM PAR. Performed the experiments: YHS AY-F KAW LT SM PAR. Analyzed the data: YHS AY-F KAW LT KWR SM PAR. Wrote the paper: YHS AY-F KAW LT SM PAR.

References

1. Jahn R, Scheller RH (2006) SNAPReceptors—engines for membrane fusion. Nat Rev Mol Cell Biol 7(6): 631–643.
2. Brugger AT (2005) Structure and function of SNAPRE and SNAPRe-interacting proteins. Q Rev Biophys 38(3): 1–47.
3. Ravichandran VK, Chawla A, Roche PA (1996) Identification of a novel syntaxin- and synaptobrevin/VAMP-binding protein, SNAP-23, expressed in non-neuronal tissues. J Biol Chem 271: 18794–18792.
4. Rea S, Martin LB, McIntosh S, Macaulay SL, Ramsdale T, Baldini G, James DE (1990) Syndet, an adipocyte target SNARE involved in the insulin-induced translocation of GLUT4 to the cell surface. J Biol Chem 275: 18794–18792.
5. Guo Z, Turner C, Castle D (1996) Retlocation of the tSNARE SNAP-23 from lamellipodia-like cell surface projections regulates compound exocytosis in mast cells. Cell 94: 537–548.
6. Vaidyanathan VV, Puri N, Roche PA (2001) The last exon of SNAP-23 regulates granule exocytosis from mast cells. J Biol Chem 276: 25101–25106.
7. Hepp R, Puri N, Hohenstein AC, Crawford GL, Whiteheart SW, Roche PA (2005) Phosphorylation of SNAP-23 regulates exocytosis from mast cells. J Biol Chem 280(30): 45927–45936.
8. Chen D, Bernstein AM, Lemons PP, Whiteheart SW (2000) Molecular mechanisms of platelet exocytosis: role of SNAP-23 and syntaxin 2 in dense core granule release. Blood 95(3): 921–929.
9. Huang X, Shen L, Tamori Y, Trimble WS, Gaiasno HY (2001) Cholesterol-kainate-regulated exocytosis in rat pancreatic acinar cells is inhibited by a C-terminus truncated mutant of SNAP-23. Pancreas 23(2): 125–133.
10. Leung SM, Chen D, DasGupta BR, Whiteheart SW, Apodaca G (1998) SNAP-23 Requirement for Transferin Recycling in Streptolysin-O-permeabilized Madin-Darby Canine Kidney Cells. J Biol Chem 273(34): 21733–21741.
11. Fournier KM, Robinson MB (2006) A dominant-negative variant of SNAP-23 decreases the cell surface expression of the neuronal glutamate transporter EAAC1 by slowing constitutive delivery. Neurochem Int 48(6-7): 596–603.
22. Fujiwara T, Mishima T, Kofuji T, Chiba T, Tanaka K, Yamamoto A, Akagawa K (2006) Analysis of knock-out mice to determine the role of HPC-1/syntaxin 1A in expressing synaptic plasticity. J Neurosci 26(21): 5767–5776.

23. Schoch S, Deak F, Konigstorfer A, Moshayeva M, Sara Y, Sudhof TC, Kavalali ET (2001) SNARE function analyzed in synaptobrevin/VAMP knockout mice. Science 294(5544): 1117–1122.

24. Verhage M, Maia AS, Plomp JJ, Brussaard AB, Heeroma JH, Vermeer H, Tooten RF, Hammer RE, van den Berg TK, Misler M, Greue HJ, Sudhof TC (2000) Synaptic assembly of the brain in the absence of neurotransmitter secretion. Science 287(5454): 864–869.

25. Geppert M, Goda Y, Hammer RE, Li C, Rosahl TW, Stevens CF, Sudhof TC (1994) Synaptotagmin I, a major Ca2+ sensor for transmitter release at a central synapse. Cell 79(4): 717–727.

26. Puri N, Roche PA (2008) Mast cells possess distinct secretory granule subsets whose exocytosis is regulated by different SNARE isoforms. Proc Natl Acad Sci U S A.

27. Wang CC, Ng CP, Lu L, Atlashkin V, Zhang W, Sert LF, Hong W (2004) A role of VAMP8/endobrevin in regulated exocytosis of pancreatic acinar cells. Dev Cell 7(3): 359–371.

28. Bresnahan P, Kearns B, Champion K, Kerman S, Bankaitis V, Novick P (1994) Sec9 is a SNAP-25-like component of a yeast SNARE complex that may be the effector of Sec1 function in exocytosis. Cell 79: 245–258.

29. Yang C, Goker KJ, Kim JK, Mora S, Thurmond HG, Davis AC, Yang B, Williamson RA, Shulman GI, Pesin JE (2001) Syntaxin 4 heterozygous knockout mice develop muscle insulin resistance. J Clin Invest 107(10): 1311–1318.

30. Southon E, Tessarollo L (2009) Manipulating mouse embryonic stem cells. Methods Mol Biol 530: 165–195.

31. Reid SW, Tessarollo L (2009) Isolation, microinjection and transfer of mouse blastocysts. Methods Mol Biol 530: 269–285.

32. Nagy A, Gertsenstein M, Vintersten K, Behringer R (2002) Manipulating the Mouse Embryo: A Laboratory Manual (Cold Spring Harbor Laboratory Press) 3rd Ed p 890.