Regulated Intramembrane Proteolysis of the Frontotemporal Lobar Degeneration Risk Factor, TMEM106B, by Signal Peptide Peptidase-like 2a (SPPL2a)*

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Background: TMEM106B polymorphisms are associated with some forms of dementia.

Results: A pathway for the sequential processing of TMEM106B on the lysosome membrane has been identified.

Conclusion: TMEM106B undergoes processing via removal of its luminal domain, followed by intramembrane cleavage by the protease SPPL2a.

Significance: This may represent a mechanism for regulation of TMEM106B levels.

The sequential processing of single pass transmembrane proteins via ectodomain shedding followed by intramembrane proteolysis is involved in a wide variety of signaling processes, as well as maintenance of membrane protein homeostasis. Here we report that the recently identified frontotemporal lobar degeneration risk factor TMEM106B undergoes regulated intramembrane proteolysis. We demonstrate that TMEM106B is readily processed to an N-terminal fragment containing the transmembrane and intracellular domains, and this processing is dependent on the activities of lysosomal proteases. The N-terminal fragment is further processed into a small, rapidly degraded dent on the activities of lysosomal proteases. The N-terminal fragment; ADAM, a disintegrin and metalloproteinase; MMP, matrix metalloproteinase; SPPL, signal peptide peptidase-like; iCLiP, intramembrane cleaving protease; SPPL, SPPL-like; 3-MA, 3-methyladenine; PMA, phorbol myristate acetate; FTLD, frontotemporal lobar degeneration; NTF, N-terminal fragment; ADAM, a disintegrin and metalloproteinase; MMP, matrix metalloproteinase; Bri2, British dementia protein-2; ITMB2; ER, endoplasmic reticulum.

* This work was supported, in whole or in part, by National Institutes of Health Grant R21 NS081557-01 (to F. H.). This work was also supported by funding from the Weill Institute for Cell and Molecular Biology and from the Association of Frontotemporal Dementia, Alzheimer’s Association (to F. H.). To whom correspondence should be addressed: 345 Weill Hall, Ithaca, NY 14853. Tel.: 607-255-0667; Fax: 607-255-5961; E-mail: fh87@cornell.edu.

¹ The abbreviations used are: RIP, regulated intramembrane proteolysis; ICD, intracellular domain; SPP, signal peptide peptidase; iCLiP, intramembrane cleaving protease; SPPL, SPPL-like; 3-MA, 3-methyladenine; PMA, phorbol myristate acetate; FTLD, frontotemporal lobar degeneration; NTF, N-terminal fragment; ADAM, a disintegrin and metalloproteinase; MMP, matrix metalloproteinase; Bri2, British dementia protein-2; ITMB2; ER, endoplasmic reticulum.
been shown to play a role in the pathogenesis of FTLD-TDP caused by C9ORF72 repeat expansions (20, 21) but not in other classes of FTLD, such as those caused by microtubule-associated protein tau, MAPT mutations. Finally, the TMEM106B risk allele is associated with cognitive impairment in amyotrophic lateral sclerosis and is associated with the pathological presentation of Alzheimer’s disease (22–24).

**EXPERIMENTAL PROCEDURES**

**Pharmacological Reagents and Antibodies**—The following reagents were used in this study: mouse anti-FLAG (M2) from Sigma, mouse anti-HA (HA.11) from Covance, mouse anti-GAPDH from Proteintech Group, mouse anti-v5 from Invitrogen, and rat anti-mouse LAMP1 (1D4B) from BD Biosciences. Rabbit anti–TMEM106B was generated against the ICD as described (18). Rabbit anti–TMEM106A antibodies were generated by Pocono Rabbit Farm & Laboratory using the recombinant Gst–TMEM106A ICD region (amino acids 1–66) purified from bacteria as the antigen. 3-Methyladenine (3-MA), phorbol 12-myristate 13-acetate (PMA), leupeptin, and ammonium chloride were purchased from Sigma. TAPI-2, GM6001, BACE IV inhibitor, and (ZLL)2-ketone were from EMD Millipore.

**Expression Constructs**—Human TMEM106B cDNAs in the pCMV-Sport6 and pDONR223 vectors were obtained from Open Biosystems. SPPL2b, SPPL2c, TMEM106A, and TMEM106C were obtained from ORFeome Collection (kind gifts from Dr. Haiyuan Yu). SPPL2a was obtained from the DNASU Plasmid Repository (Arizona State University). All FLAG-tagged TMEM106 constructs were generated by cloning TMEM106 family cDNAs into the p3XFLAG-CMV7.1 vector (Sigma-Aldrich). All V5 tagged SPPL2 constructs were generated by cloning cDNAs into the p3XFLAG-CMV7.1 vector (Sigma-Aldrich). All V5 tagged SPPL2 constructs were generated by cloning cDNAs into pCDNA3.1(+)-V5/HisA (Invitrogen). The SPPL2a D412A mutant and the TMEM106B Y132D, C105A, G110A, and P118A mutants were generated by cloning cDNAs into pCDNA3.1(+) vector (Stratagene) was kindly provided by Dr. Hening Lin. TMEM106B-V5/HisA (Invitrogen) or IRDye 800 (LI-COR Biosciences) for 1 h at room temperature. Membranes were washed three more times with PBS followed by permeabilization and blocking in blocking buffer (0.05% saponin, 3% BSA in PBS) for 1 h. Primary antibodies were incubated in blocking buffer overnight at 4 °C. The cells were washed and incubated in secondary antibodies conjugated to CF488A, CF568, or CF660C (Biotum). Cells were washed three more times, and coverslips were mounted onto slides with Fluoromount G (SouthernBiotech). Images were acquired on a CSU-X spinning disc confocal microscope (Intelligent Imaging Innovations) with an HQ2 CCD camera (Photometrics) using a 100× objective.

**RESULTS**

**TMEM106B Is Proteolytically Processed**—When lysates from HEK293T cells overexpressing TMEM106B are subjected to Western blotting with a homemade antibody against the N-terminal intracellular/cytosolic domain of TMEM106B, we observed a strong immunoreactive band at ~14 kDa along with a much fainter band at ~12 kDa in addition to the full-length 43-kDa band (Fig. 1A). We hypothesized that the 14-kDa band was the N-terminal transmembrane stump (the N-terminal fragment (NTF)) after cleavage of the TMEM106B luminal domain and that the fainter 12-kDa band was a short-lived intracellular domain (ICD). Indeed, when C-terminal deletion constructs of TMEM106B were run next to the cleaved fragments of TMEM106B, the NTF ran similarly to the 1–106 fragments, which corresponds to the predicted size of NTF with the cytosolic and transmembrane region. The ICD fragment ran similarly to the 1–106 fragments, which could be the predicted size of the ICD resulting from intramembrane cleavage. Thus, it is very likely that TMEM106B is subject to RIP-mediated processing. Consistent with this hypothesis and the fact that ectodomain shedding is a prerequisite for most known instances of RIP (11-13), we found that the 1–127 fragment can be further cleaved to a smaller fragment with the same apparent size as the ICD generated by full-length TMEM106B. The 1–132 NTF fragment, on the other hand, was very poorly processed, despite similar expression levels. This suggests that the length of the remaining luminal portion of the NTF is critical for recognition by the protease required for conversion to the ICD (Fig. 1A). The FLAG-tagged TMEM106B has significantly decreased
NTF and ICD generated compared with untagged TMEM106B when overexpressed in HEK293T cells (data not shown), indicating that the N terminus of TMEM106B could affect TMEM106B processing.

Next we sought to determine whether TMEM106B processing could be detected in other cell lines without massive overexpression. Stable lines with different levels of TMEM106B expression were generated in the NSC-34 motor neuron-like cell line. In these stable lines, the appearance of NTFs is readily apparent, and the amount of NTF generated appears directly proportional to the level of TMEM106B expressed, with a clone expressing near endogenous levels of TMEM106B showing minimal NTF generation and clones expressing higher levels of full-length TMEM106B exhibiting greater levels of NTF generation.

**FIGURE 1.** TMEM106B undergoes sequential proteolysis. **A**, HEK293T cells transfected with TMEM106B were treated with vehicle control or (ZLL)₂-ketone for 14 h as indicated. Whole cell lysates were subject to anti-ICD Western blot, which revealed a 43-kDa monomeric TMEM106B, a 14-kDa membrane-retained NTF stub, and a 12-kDa ICD. Size standards corresponding to the first 132, 127, 106, and 101 residues of TMEM106B were run next to lysates from full-length (FL) TMEM106B expressing cells as indicated. The NTF runs closest to the 1–127 fragments, whereas the ICD runs closest to the 1–106 fragments. The asterisk indicates a nonspecific band running right below NTF. **B**, (ZLL)₂-ketone inhibits the formation of the ICD, resulting in a relative increase in the NTF levels and a decrease in ICD. The ICD generated was quantitated by densitometry and calculated as a ratio relative to full-length and NTF TMEM106B. (n = 3 ± S.E.; ***, p < 0.001, Student’s t test). **C**, whole cell lysates from a control NSC-34 cell expressing endogenous TMEM106B and a stable line expressing high levels of TMEM106B were blotted with anti-ICD antibodies. Treatment with 5 mM 3-MA for 16 h increases the levels of full-length TMEM106B and ICDs. **D**, NTFs and ICDs of TMEM106B can be detected at endogenous levels in N2a cells. N2a cells were transfected with control siRNA or siRNA against TMEM106B to determine whether these smaller fragments are derived from TMEM106B. Lysates from transfected HEK293T cells were run side by side as a control. The asterisk indicates a nonspecific band running right above NTF in N2a cells. The low molecular weight part of membrane was blotted separately with high concentration of anti-ICD antibodies to allow the detection of low levels of NTF and ICD in N2a cells. **E**, TMEM106B NTFs composed of the amino acids 1–127 retains its lysosomal compartment localization. **F**, the predicted TMEM106B ICD composed of the first 106 residues partially localizes to lysosomes, although less than full-length TMEM106B or NTF. Scale bars, 10 μm in main panels and 2 μm in insets.
markedly less than the NTF and especially full-length level of lysosomal localization of the TMEM106B ICD was which may facilitate insertion into the lipid bilayer. Still, the to be left after intramembrane cleavage are highly hydrophobic, corresponding to the partial transmembrane region predicted for TMEM106B. Second, the last 10 amino acids and the string of basic residues N-terminal to the transmembrane region of TMEM106B. The TMEM106B ICD(1–106) also appeared partially localized to lysosomes, suggesting that the liberated ICD can remain associated with the lysosome membrane after RIP (Fig. 1F). Several features of the ICD may explain the partial lysosomal localization observed. First, electrostatic interactions may mediate contact between negatively charged phospholipid head groups and the string of basic residues N-terminal to the transmembrane region of TMEM106B. Second, the last 10 amino acids corresponding to the partial transmembrane region predicted to be left after intramembrane cleavage are highly hydrophobic, which may facilitate insertion into the lipid bilayer. Still, the level of lysosomal localization of the TMEM106B ICD was markedly less than the NTF and especially full-length TMEM106B, indicating that the ICD is likely capable of being liberated from the membrane after cleavage from the NTF.

The NTF Is Generated by Lysosomal Proteases—Because TMEM106B is a late endosomal/lysosomal protein (16–18), and ectodomain shedding generally refers to the extracellular release of a soluble protein domain, we henceforth refer to this analogous shedding event to generate TMEM106B NTFs as lumenal domain shedding. To narrow down the possible enzymes required for the initial lumenal domain shedding event, we screened a number of compounds that are capable of processing of the full-length protein to the NTF (Fig. 2, C and D). Thus, Tyr132 may play an important role in the recognition or cleavage of the TMEM106B lumenal domain by resident proteases. To rule out that this is not due to mislocalization of the Y132D mutant, the localization of TMEM106B Y132D mutant was examined in N2a cells. TMEM106B Y132D strongly localized to lysosomes much like wild type TMEM106B, thus confirming that the defects in NTF formation are due to decreased proteolysis in lysosomes and not reduced substrate accessibility (Fig. 2E).
The Y125D mutant, as well as the Y125D/Y132D double mutant also showed almost completely abolished NTF formation and also increased levels of full-length TMEM106B (data not shown); however, these mutants were severely mislocalized and were retained primarily in the ER (unpublished observations). Nonetheless, these results support a model in which resident lysosomal proteases are responsible for the lumenal cleavage of TMEM106B to generate the NTF.

The TMEM106B NTF Is a Substrate for Intramembrane Proteolysis by SPPL2a and SPPL2b—The SPP family of iCLiPs has been shown to cleave a number of type II transmembrane proteins at various subcellular locations (10, 33–36). Treatment of TMEM106B-overexpressing HEK293T cells with the specific SPP class protease inhibitor (ZLL)\(_2\)-ketone led to an increase in the relative amount of TMEM106B NTF generated and a concomitant decrease in the ICD, suggesting that an endogenous SPP class protease plays a role in cleaving the TMEM106B NTF in HEK293T cells (Fig. 1, A and B). Among the SPP family members, SPPL2a and SPPL2b have recently been implicated in the intramembrane proteolysis of several type II membrane proteins. To date, five known substrates have been identified: TNF\(\alpha\), Fas Ligand, British dementia protein-2/ITMB2 (Bri2), Transferrin Receptor 1, and CD74 (33–41). In some cases, SPPL2a and SPPL2b appear to share a common substrate such as TNF\(\alpha\) and Bri2. To test whether TMEM106B could be a substrate of either of these enzymes, we...
co-transfected TMEM106B into HEK293T cells with vector control, or expression constructs for SPPL2a, SPPL2b, and their paralog, SPPL2c. We found that both SPPL2a and SPPL2b, but not SPPL2c, were capable of cleaving the TMEM106B NTF to generate smaller ICD fragments, and the activity of SPPL2a, but not SPPL2b, was inhibited by treatment with (ZLL)$_2$-ketone (Fig. 3, A and B). Additionally, D412A, a catalytically inactive mutant of SPPL2a (D412A) also fails to induce NTF conversion to ICD. SPPL2b promotes conversion of NTF to ICD and is somewhat resistant to (ZLL)$_2$-ketone inhibition under the conditions tested. SPPL2c fails to promote NTF to ICD conversion. Size standards of 132, 127, 106, and 101 amino acids were included as reference to highlight the relative sizes of the NTF and ICD fragments. B, the ratio of ICD to NTF and full-length (FL) TMEM106B was quantitated from the data presented in A. (n = 4–5 ± S.E.; * p < 0.05; **, p < 0.01, Student’s t test). C, processing of the TMEM106B C105A, G110A, and P118A mutants expressed in HEK293T cells. The asterisk indicates a nonspecific band running right below NTF.

Proline is the most helix destabilizing amino acid, and Pro$_{118}$ immediately C-terminal to the transmembrane region was also mutated to alanine (44). The G110A and P118A mutations had no effect on the ratio of ICD to NTF and full-length (FL) TMEM106B was quantitated from the data presented in A. (n = 4–5 ± S.E.; * p < 0.05; **, p < 0.01, Student’s t test). C, processing of the TMEM106B C105A, G110A, and P118A mutants expressed in HEK293T cells. The asterisk indicates a nonspecific band running right below NTF.
ialization of these proteases in TMEM106B expressing cells, we analyzed the localization of the v5-tagged SPPL2 proteins in N2a cells along with endogenous TMEM106B. TMEM106B appears primarily distributed across the limiting membranes of LAMP1-positive vesicles. SPPL2a as well as the D412A mutant could be detected on vesicles containing both LAMP1 and TMEM106B, indicating that SPPL2a could likely access lysosomal TMEM106B as a substrate (Fig. 4, A and B). SPPL2b localizes very poorly to these TMEM106B-positive vesicles (Fig. 4 C). Instead, SPPL2b accumulates in large perinuclear stacks, possibly Golgi (Fig. 4 C). SPPL2b might process TMEM106B in the Golgi because a small population of TMEM106B is also observed in the Golgi at the steady state. SPPL2c shows a reticulated pattern of localization, indicative of a possible ER localization consistent with a previous report (Fig. 4D) (33). In agreement with this, SPPL2C has no activity toward TMEM106B (Fig. 3, A and B).

The TMEM106B Paralogs TMEM106A and TMEM106C Are Not Subject to Intramembrane Proteolysis—To test the specificity of TMEM106B as a substrate for SPPL2a-mediated intramembrane proteolysis, we decided to explore the localization and cleavage patterns of two paralogs of TMEM106B: TMEM106A and TMEM106C. N-terminally FLAG-tagged TMEM106A and TMEM106C constructs were used to determine intracellular localization. Like TMEM106B, TMEM106A is primarily localized to lysosomes in N2a cells (Fig. 5A). TMEM106C does not localize to lysosomes but rather shows a reticulated pattern indicative of an ER localization (Fig. 5B). To determine whether TMEM106A and TMEM106C can be cleaved by SPPL2a, untagged TMEM106A construct and FLAG-TMEM106C constructs were co-transfected with SPPL2a in HEK293T cells. No specific degradation products consistent with a size expected of a homologous NTF or ICD were detected for TMEM106A with SPPL2a overexpression (Fig. 5C). TMEM106C exhibits a series of closely spaced bands at ~37 kDa, possibly because of different levels of glycosylation. Additionally, specific bands of ~21 and ~18 kDa were detected. These bands could be degradation products and could conceivably correlate with an NTF and ICD product. However, SPPL2a had no effect on TMEM106C processing (Fig. 5D). SPPL2b and SPPL2c also had no effect on TMEM106A or TMEM106C NTF processing (data not shown). These results suggest that SPPL2a cleavage of TMEM106B is relatively specific, because even a close homolog, TMEM106A, expressed at similar levels and localized to the same subcellular compartment, fails to be cleaved by SPPL2a under our experimental conditions. Although iCLiPs can have a large array of potential substrates, they do appear to display some selectivity even for potentially very similar substrates. This modality is supported by experiments by Martin et al. (12) in which they demonstrate that SPPL2b efficiently cleaves the Bri2 protein, but not the highly homologous Bri3, even after it is artificially truncated to mimic the NTF of Bri2.

Model of TMEM106B Processing and RIP—A cartoon schematic of the predicted major proteolytic events in TMEM106B
processing is proposed in Fig. 6 (A and B). An initial series of shedding events occurs in the lysosomal lumen by resident proteases to generate a stable TMEM106B NTF of ~127 amino acids. This NTF is subsequently cleaved by SPPL2a on lysosome membranes, yielding a short-lived and highly unstable ICD product.

Close examination of the ICDs generated by TMEM106B reveals the appearance of two or more extremely closely spaced bands, suggesting that the ICDs could actually result from at least two different cleavage events close to amino acid 106 (Figs. 1A, 2A, and 3A). This may suggest some degree of degeneracy in the cleavage site by SPPL2a, which has been reported for the
other SPPL2a substrate TNFα (37). It has been argued that these multiple cleavage sites are required for the liberation of the TNFα ICD into the cytosol (7). This lack of absolute specificity of the intramembrane cleavage site may be a more general feature of the GxGD family of iCLiPs with certain substrates, because similar patterns of intramembrane cleavage are seen by proteases such as presenilin (46).

TMEM106B is a highly conserved protein, detected throughout the vertebrate lineage. Sequence alignment shows that the transmembrane region and membrane-proximal luminal domain region is absolutely conserved in most mammals and is largely identical even in Danio rerio and Xenopus laevis (Fig. 6C). Similarly, intramembrane proteolysis is a ubiquitous mechanism across all domains of life. This points to the prospect that RIP of TMEM106B may also be an evolutionarily conserved event.

DISCUSSION

In this study we demonstrate the selective processing of the lysosomal membrane protein TMEM106B via the sequential actions of luminal domain shedding and RIP. This TMEM106B processing bears a striking resemblance to the processing of CD74 on the late endosomes/lysosomes of B cells, in which CD74 is sequentially cleaved by luminal cathepsin S followed by intramembrane cleavage by SPPL2a. Knock-out of SPPL2a causes an accumulation of intermediate sized fragments of CD74 between that of the full-length protein and NTF, suggesting multiple processing steps occur in the acidified lumen (35, 40).

We showed that inhibition of lysosomal hydrolases with either ammonium chloride or leupeptin reduces luminal domain shedding of TMEM106B. The tyrosine at position 132 appears to play a role in TMEM106B luminal shedding. Mutation of this residue results in impaired luminal shedding, although not a complete block. It is conceivable that the TMEM106B luminal shedding event may be mediated by one or more soluble lysosomal proteases such as cathepsins present within the lumen. Alternatively, this event may be mediated by an as yet unidentified sheddase present within the lysosome membrane. Lysosomal proteases such as the cathepsins have highly redundant substrate specificities, and it is likely that multiple residues are implicated in substrate recognition. Further experiments with a more systematic series of mutations and more specific cathepsin inhibitors or in specific cathepsin knock-out backgrounds may help clarify the exact role of different proteases involved in luminal domain shedding. It will be also interesting to understand whether this shedding happens constitutively in response to elevated levels of TMEM106B or whether it is regulated by other factors.

We show that the GxGD proteases SPPL2a and SPPL2b are capable of cleaving TMEM106B when overexpressed; however, SPPL2a appears to be more specifically inhibited by the SPP family inhibitor (ZLL)₂-ketone, and it co-localizes much better with TMEM106B on the lysosomes, in agreement with previous reports showing that SPPL2a is predominantly trafficked to endosomes and lysosomes (33, 45). SPPL2b localizes to the cell surface and is also observed to accumulate intracellularly when expressed in N2a cells. We speculate that this overexpression may have saturated the machinery normally required for SPPL2b trafficking to the cell surface, causing it to accumulate in the secretory pathway where it may mediate the constitutive cleavage of newly synthesized TMEM106B. Microarray studies performed by Friedmann et al. (9) show that SPPL2b is expressed at low levels in most tissues except the adrenal cortex and mammary glands; SPPL2a, on the other hand, is expressed at high levels in a large number of tissues, with the highest levels detected in the brain. Expressed sequence tag profiles of TMEM106B also show TMEM106B expression in a large number of tissues, including the brain (47). Because of these considerations, we predict SPPL2a, and not SPPL2b, to be the major physiologically relevant iCLiP responsible for processing the TMEM106B NTF in vivo.

RIP generation of soluble ICDs has been proposed to mediate a large variety of signaling events both in the cytosol and in the nucleus to regulate transcription (48–50). However, because of the extremely short-lived nature of the vast majority of ICDs generated by RIP, many have yet to be detected under endogenous conditions. We see no evidence of the TMEM106B ICD fragments in the nucleus when overexpressed. Instead, the TMEM106B ICD seems somewhat lysosomally localized (Fig. 1F). A recent study reported that lysosomal TMEM106B may act as a brake against retrograde dendritic trafficking through its interaction with the microtubule-associated protein MAP6 (51). It will be interesting to see whether the cleavage of TMEM106B by RIP could serve as a mechanism to further fine tune lysosomal trafficking. Although we cannot rule out that processing of TMEM106B by RIP has a signaling function, another possibility is that RIP processing of TMEM106B serves as a membrane protein quality control mechanism allowing the efficient removal of excess TMEM106B and controlling TMEM106B levels, which might be essential for proper lysosomal function (17, 18). Given the accumulating evidence linking elevated TMEM106B levels to FTLD risk, identifying pathways that regulate TMEM106B levels may represent an important avenue in developing strategies for therapeutic intervention.

Acknowledgments—We thank Dr. Haiyuan Yu and Dr. Hening Lin for kind gifts of cDNAs and plasmids and Xiaochun Wu for technical assistance.

REFERENCES

1. Lemberg, M. K. (2011) Intramembrane proteolysis in regulated protein trafficking. Traffic 12, 1109–1118
2. Cao, X., and Südhof, T. C. (2001) A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60. Science 293, 115–120
3. Mumm, J. S., Schroeter, E. H., Saxena, M. T., Griesemer, A., Tian, X., Pan, D. J., Ray, W. L., and Kopan, R. (2000) A ligand-induced extracellular cleavage regulates γ-secretase-like proteolytic activation of Notch1. Mol Cell 5, 197–206
4. Lemberg, M. K., Bland, F. A., Weihofen, A., Braud, V. M., and Martoglio, B. (2001) Intramembrane proteolysis of signal peptides: an essential step in the generation of HLA-E epitopes. J. Immunol. 167, 6441–6446
5. Kopan, R., and Ilagan, M. X. (2004) γ-Secretase: proteasome of the membrane? Nat. Rev. Mol. Cell Biol. 5, 499–504
6. Lichtenhaler, S. F., Haass, C., and Steiner, H. (2011) Regulated intramembrane proteolysis: lessons from amyloid precursor protein processing.
7. Flulhrer, R., Steiner, H., and Haass, C. (2009) Intramembrane proteolysis by signal peptide peptidases: a comparative discussion of GXXGD-type aspartyl proteases. J. Biol. Chem. 284, 13975–13979

8. Weihoien, A., Binns, K., Lemberg, M. K., Ashman, K., and Martoglio, B. (2002) Identification of signal peptide peptidase, a presenilin-type aspartic protease. Science 296, 2215–2218

9. Friedmann, E., Lemberg, M. K., Weihoien, A., Dev, K. K., Dengler, U., Rovelli, G., and Martoglio, B. (2004) Consensus analysis of signal peptide peptidase and homologous human aspartic proteases reveals opposite topology of catalytic domains compared with presenilins. J. Biol. Chem. 279, 50790–50798

10. Krawitz, P., Haffner, C., Flulhrer, R., Steiner, H., Schmid, B., and Haass, C. (2005) Differential localization and identification of a critical aspartase suggest non-redundant proteolytic functions of the presenilin homologues SPPL2b and SPPL3. J. Biol. Chem. 280, 39515–39523

11. Struhl, G., and Adachi, A. (2000) Requirements for presenilin-dependent cleavage of notch and other transmembrane proteins. Mol Cell 6, 625–636

12. Martin, L., Flulhrer, R., and Haass, C. (2009) Substrate requirements for SPPL2b-dependent regulated intramembrane proteolysis. J. Biol. Chem. 284, 5662–5670

13. Shah, S., Lee, S. F., Tabuchi, K., Hao, Y. H., Yu, C., LaPlant, Q., Ball, H., Dann, C. E., 3rd, Sdhof, T., and Yu, G. (2005) Nicastrin functions as a γ-secretase-substrate receptor. Cell 122, 435–447

14. Van Deerlin, V. M., Sleiman, P. M., Martinez-Lage, M., Chen-Plotkin, A., Wang, L. S., Graff-Radford, N. R., Dickson, D. W., Rademakers, R., Boeke, B. F., Grossman, M., Arnold, S. E., Mann, D. D., Pickering-Brown, S. M., Seelaar, H., Heutink, P., van Swieten, J. C., Murrell, J. R., Gheb, S., Spina, S., Graffman, J., Hodges, J., Spillanenti, M. G., Gilman, S., Lieberman, A. P., Kaye, J. A., Woltjer, R. L., Bigio, E. H., Mesulam, M.-H., Van Swieten, J., Troake, G., Rosenberg, N. C., White, C. L., 3rd, Ferrington, L., Lladó, A., Neumann, A., Kretzschmar, H. A., Hulette, C. M., Welsh-Bohmer, K. A., Miller, B. L., Alzualde, A., Lopez de Munain, A., Al-Sarraj, S., Gelpi, E., Ballestero, R. P., and Martoglio, B. (2004) Consensus analysis of signal peptide peptidase, a presenilin-type aspartic protease. Hum. Mol. Genet. 13, 1187–1196

15. Van Deerlin, V. M., Sleiman, P. M., Martinez-Lage, M., Chen-Plotkin, A., van der Zee, J., Van Langenhove, T., Kleinberger, G., Sleegers, K., Engelborghs, S., Van Broeckhoven, C., Seilhean, D., Galasko, D., Gaskell, S. P., Gajdusek, D., Elman, L., McCluskey, L., Lee, V. M., Van Deerlin, V., and Chen-Plotkin, A. S. (2014) TMEM106B is a genetic modifier of frontotemporal lobar degeneration with C9orf72 hexanucleotide repeat expansions. Acta Neuropathol. 127, 407–418

16. Van Blitterswijk, M., Mullen, B., Nicholson, A. M., Biemiek, K. F., Heckman, M. G., Baker, M. C., DeJesus-Hernandez, M., Finch, N. A., Brown, P. H., Murray, M. E., Hsiung, G. Y., Stewart, H., Karydas, A. M., Finger, E., Kretzschmar, H. A., Bigio, E. H., Untraub, S., Mesulam, M., Hatana, K., I. White, C. L., 3rd, Strong, M. J., Beach, G. T., Wszolek, Z. K., Lippa, C. A., Castelli, R., Petruccelli, L., Josephs, K. A., Parisi, J. E., Knopman, D. S., Peters, R. C., Mackenzie, I. R., Seeley, W. W., Grinberg, L. T., Miller, B. L., Boylan, K. B., Graff-Radford, N. R., Boeke, B. F., Dickson, D. W., and Rademakers, R. (2014) TMEM106B protects C9ORF72 expansion carriers against frontotemporal dementia. Acta Neuropathol. 127, 397–406

17. Lu, R. C., Wang, H., Tan, M. S., Yu, J. T., and Tan, L. (2014) TMEM106B and APOE polymorphisms interact to confer risk for late-onset Alzheimer’s disease in Han Chinese. J. Neural Transm. 121, 283–287

18. Rutherford, N. J., Carrasquillo, M. M., Li, M., Biscoglio, G., Menke, J., Josephs, K. A., Parisi, J. E., Petersen, R. C., Graff-Radford, N. R., Younkin, S. G., Dickson, D. W., and Rademakers, R. (2012) TMEM106B risk variant is implicated in the pathologic presentation of Alzheimer disease. Neurology 79, 717–718

19. Vass, R., Ashbridge, E., Guer, F., Hu, W. T., Grossman, M., Clay-Falcone, D., Elman, L., Mccluskey, L., Lee, V. M., Van Deerlin, V. M., Trojanowski, J. Q., and Chen-Plotkin, A. S. (2011) Risk genotypes at TMEM106B are associated with cognitive impairment in amyotrophic lateral sclerosis. Acta Neuropathol. 121, 373–380

20. Vampa, A. R., Vinardoraj, S., Parra, K. V., Jasti, M., Gonzalez-Garcia, M., and Ballestrero, R. P. (2004) Use of polyethyleneimine polymer in cell culture as attachment factor and lipofection enhancer. BMC Biotechnol. 4, 23

21. Vant, A., Ogier-Denis, E., Blommaert, E. F., Meiir, A. J., and Codogno, P. (2000) Distinct classes of phosphatidylinositol 3′-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. J. Biol. Chem. 275, 992–998

22. Edwards, D. R., Handsley, M. M., and Pennington, C. J. (2008) The ADAM family of metalloproteinases. Mol. Aspects Med. 29, 258–289

23. Yu, Q., and Stamenkovic, I. (2000) Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis. Genes Dev. 14, 163–170

24. Nils, D. F., and Courtneidge, S. A. (2003) The ADAMS family of metalloproteinases: mult DOMAIN proteins with multiple functions. Genes Dev. 17, 7–30

25. Vass, R., Ashbridge, E., Guer, F., Hu, W. T., Grossman, M., Clay-Falcone, D., Elman, L., Mccluskey, L., Lee, V. M., Van Deerlin, V. M., Trojanowski, J. Q., and Chen-Plotkin, A. S. (2011) Risk genotypes at TMEM106B are associated with cognitive impairment in amyotrophic lateral sclerosis. Acta Neuropathol. 121, 373–380

26. Vampa, A. R., Vinardoraj, S., Parra, K. V., Jasti, M., Gonzalez-Garcia, M., and Ballestrero, R. P. (2004) Use of polyethyleneimine polymer in cell culture as attachment factor and lipofection enhancer. BMC Biotechnol. 4, 23

27. Vant, A., Ogier-Denis, E., Blommaert, E. F., Meiir, A. J., and Codogno, P. (2000) Distinct classes of phosphatidylinositol 3′-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. J. Biol. Chem. 275, 992–998

28. Edwards, D. R., Handsley, M. M., and Pennington, C. J. (2008) The ADAM metalloproteinases. Mol. Aspects Med. 29, 258–289

29. Yu, Q., and Stamenkovic, I. (2000) Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis. Genes Dev. 14, 163–170

30. Seals, D. F., and Courtneidge, S. A. (2003) The ADAMS family of metalloproteinases: mult Domain proteins with multiple functions. Genes Dev. 17, 7–30

31. Vass, R., Bennett, B. D., Babu-Khan, S., Saha, S., Mondia, E. A., Den, D., Depo, D. B., Ross, S., Amarante, P., Loeloff, R., Luo, Y., Fisher, S., Fuller, J., Edson, S., Lile, J., Jarosinski, M. A., Biere, A. L., Curran, E., Burgess, T., Louis, J. C., Collins, F., Treanor, J., Rogers, G., and Citron, M. (1999) β-Secretase cleavage of Alzheimer’s amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286, 735–741

32. Tanaka, R. D., Li, A. C., Fogelman, A. M., and Edwards, P. A. (1986) Inhibition of lysosomal protein degradation inhibits the basal degradation of
Processing of TMEM106B by SPPL2a

3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Lipid Res. 27, 261–273
32. Verspurten, J., Gevaert, K., Declercq, W., and Vandenberghe, P. (2009) Site
33. Friedmann, E., Hauben, E., Maylandt, K., Schleeger, S., Vreugde, S., Licht-
34. Zahn, C., Kaup, M., Fluhrer, R., and Fuchs, H. (2013) The transferrin
35. Schneppenheim, J., Dressel, R., Hüttl, S., Lüllmann-Rauch, R., Engelke, M.,
36. Zörnig, M. (2007) The Fas ligand intracellular domain is released by
37. Fluhrer, R., Grammer, G., Israel, L., Condron, M. M., Haffner, C., Fried-
38. Lemberg, M. K., and Martoglio, B. (2002) Requirements for signal peptide
39. Beisner, D. R., Langerak, P., Parker, A. E., Dahlberg, C., Otero, F. J., Sutton,
40. Bergmann, H., Yabas, M., Short, A., Miosge, L., Barthel, N., Stu-}
41. Vermeulen, J., Gevaert, K., Declercq, W., and Vandenabeele, P. (2009)
42. Friedmann, E., Hauben, E., Maylandt, K., Schleeger, S., Vreugde, S., Licht-
43. Kirkin, V., Cahuzac, N., Guardiola-Serrano, F., Huault, S., Lückerath, K.,
44. Pace, C. N., and Schultz, J. M. (1998) A helix propensity scale based on
45. Behnek, J., Schnepf, J., Koch-Nolte, F., Haag, F., Saftig, P., and Schröder,
46. Steiner, H., Fluhrer, R., and Haass, C. (2008) Intramembrane proteolysis by
47. Sayers, E. W., Barrett, T., Benson, D. A., Bryant, S. H., Canese,
48. Wang, X., Sato, R., Brown, M. S., Hua, X., and Goldstein, J. L. (1994)
49. Ye, J., Rawson, R. B., Komuro, R., Chen, X., Davé, U. P., Prywes, R., Brown,
50. Selkoe, D., and Kopan, R. (2003) Notch and Presenilin: regulated intramem-
51. Schwenk, B. M., Lang, C. M., Hog, S., Tahirovic, S., Orozco, D., Rentzsch, K., Lichtenthaler, S. F., Hoogenraad, C. C., Capell, A., Haass, C., and Ed-
52. Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez,
53. Bertram, E. M., Mackay, F., Rimmer, A. J., Cornall, R. J., Field, M. A., Andrews, T. D., Goodnow, C. C., and Enders, A. (2013) B cell
54. Field, M. A., Andrews, T. D., Goodnow, C. C., and Enders, A. (2013) B cell
55. Schubert, D., and Kerjaschki, D. (2008) Regulated intramembrane proteolysis of Bri2 (Itm2b) by ADAM10 and SPPL2b cleavage in T-cells. Cell
56. Beisser, D. R., Langerak, P., Parker, A. E., Dahlberg, C., Otero, F. J., Sutton, S. E., Poiriot, L., Barnes, W., Young, M. A., Niessen, S., Wiltshire, T., Bodendorf, U., Martoglio, B., Cravatt, B., and Cooke, M. P. (2013) The intramembrane protease SPPL2a is required for B cell and DC development and survival via cleavage of the invariant chain. J. Exp. Med. 210, 23–30
57. Bergmann, H., Yabas, M., Short, A., Miosge, L., Barthel, N., Teh, C. E., Roots, C. M., Bull, K. R., Jeelall, Y., Horikawa, K., Whittle, B., Balakishnan, B., Sjoëlfga, G., Bertram, E. M., Mackay, F., Rimmer, A. J., Cornall, R. J., Field, M. A., Andrews, T. D., Goodnow, C. C., and Enders, A. (2013) B cell survival, surface BCR and BAFFR expression, CD74 metabolism, and CD8+ dendritic cells require the intramembrane endopeptidase SPPL2A. J. Exp. Med. 210, 31–40
58. Schneppenheim, J., Hüttl, S., Mentrup, T., Lüllmann-Rauch, R., Rothaug, M., Engelke, M., Dittmann, K., Dressel, R., Araki, M., Araki, K., Wienands, J., Fluhrer, R., Saftig, P., and Schröder, B. (2014) The intramembrane proteases signal Peptide peptidase-like 2a and 2b have distinct functions in vivo. Mol. Cell. Biol. 34, 1398–1411
59. Lemberg, M. K., and Martoglio, B. (2002) Requirements for signal peptide peptidase-catalyzed intramembrane proteolysis. Mol. Cell 10, 735–744
60. Fluhrer, R., Martin, L., Klier, B., Haug-Krömer, M., Grammer, G., Nuscher, B., and Haass, C. (2012) The α-helical content of the transmembrane domain of the British dementia protein-2 (Br2) determines its processing by signal peptide peptidase-like 2b (SPPL2b). J. Biol. Chem. 287, 5156–5163
61. Pace, C. N., and Schultz, J. M. (1998) A helix propensity scale based on experimental studies of peptides and proteins. Bioophys. J. 75, 422–427
62. Behnek, J., Schnepf, J., Koch-Nolte, F., Haag, F., Saftig, P., and Schröder, B. (2011) Signal-peptide-peptidase-like 2a (SPPL2a) is targeted to lysosomes/late endosomes by a tyrosine motif in its C-terminal tail. FEBS Lett. 585, 2951–2957
63. Steiner, H., Fluhrer, R., and Haass, C. (2008) Intramembrane proteolysis by γ-secretase. J. Biol. Chem. 283, 29627–29631
64. Sayers, E. W., Barrett, T., Benson, D. A., Bolton, E., Bryant, S. H., Canese, K., Chetverinin, V., Church, D. M., Micu, M., Federhen, S., Feolo, M., Fingerman, I. M., Geer, L. Y., Helmberg, W., Kapustin, Y., Krasnov, S., Landsman, D., Lipman, D. J., Lu, Z., Madden, T. L., Madej, T., Maglott, D. R., Marchler-Bauer, A., Miller, V., Karsch-Mizrachi, I., Ostell, J., Panchenko, A., Phan, L., Pruitt, K. D., Schuler, G. D., Sequeira, E., Sherry, S. T., Shumway, M., Sirotkin, K., Slotta, D., Souvorov, A., Starchenko, G., Tus- tosuva, T. A., Wagner, L., Wang, Y., Wilbur, W. J., Yaschenko, E., and Ye, J. (2012) Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 40, D13–D25
65. Wang, X., Sato, R., Brown, M. S., Hua, X., and Goldstein, J. L. (1994) SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. Cell 77, 53–62
66. Ye, J., Rawson, R. B., Komuro, R., Chen, X., Davé, U. P., Prywes, R., Brown, M. S., and Goldstein, J. L. (2000) ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell 6, 1355–1364
67. Selkoe, D., and Kopan, R. (2003) Notch and Presenilin: regulated intramembrane proteolysis links development and degeneration. Annu. Rev. Neurosci. 26, 565–597
68. Schwenk, B. M., Lang, C. M., Hog, S., Tahirovic, S., Orozco, D., Rentzsch, K., Lichtenthaler, S. F., Hoogenraad, C. C., Capell, A., Haass, C., and Ed- bauer, D. (2014) The FTLD risk factor TMEM106B and MAP6 control dendritic trafficking of lysosomes. EMBO J. 33, 450–467
69. Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., Lopez, R., McWilliam, H., Remmert, M., Söding, J., Thompson, J. D., and Higgins, D. G. (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. 7, 539