1 **Novel Amplification Mechanism of Prions through Disrupting Sortilin-Mediated**

2 **Trafficking**

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12 Short title: Prion accumulation via sortilin dysfunction

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ABSTRACT.

Conformational conversion of the cellular prion protein, PrP^C, into the abnormally folded isoform of prion protein, PrP^{Sc}, which leads to marked accumulation of PrP^{Sc} in brains, is a key pathogenic event in prion diseases, a group of fatal neurodegenerative disorders caused by prions. However, the exact mechanism of PrP^{Sc} accumulation in prion-infected neurons remains unknown. We recently reported a novel cellular mechanism to support PrP^{Sc} accumulation in prion-infected neurons, in which PrP^{Sc} itself promotes its accumulation by evading the cellular inhibitory mechanism, which is newly identified in our recent study. We showed that the VPS10P sorting receptor sortilin negatively regulates PrP^{Sc} accumulation in prion-infected neurons, by interacting with PrP^C and PrP^{Sc} and trafficking them to lysosomes for degradation. However, PrP^{Sc} stimulated lysosomal degradation of sortilin, disrupting the sortilin-mediated degradation of PrP^C and PrP^{Sc} and eventually evoking further accumulation of PrP^{Sc} in prion-infected neurons. These findings suggest a positive feedback amplification mechanism for PrP^{Sc} accumulation in prion-infected neurons.

KEYWORDS. Prions, prion protein, sortilin, sorting, VPS10P sorting receptor, protein degradation, lysosome.

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Introduction

Prions are causative agents of prion diseases, a group of fatal neurodegenerative disorders including Creutzfeldt-Jakob disease in humans and bovine spongiform encephalopathy and scrapie in animals.[1] They are widely believed to consist of the abnormally folded, amyloidogenic isoform of prion protein, designated PrP^{Sc}.[1] PrP^{Sc} is produced through conformational conversion of the cellular prion protein, PrP^{C}, by unknown mechanisms.[1] PrP^{C} is a glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein expressed most abundantly in brains, particularly by neurons.[2] The constitutive conversion of PrP^{C} into PrP^{Sc} leads to accumulation of PrP^{Sc} in brains. We and others have shown that the conversion of PrP^{C} into PrP^{Sc} is a key pathogenic event in prion disease, by demonstrating that mice devoid of PrP^{C} neither developed the disease nor propagated prions or accumulated PrP^{Sc} in their brains after intracerebral inoculation with prions.[3-6] Most pathogens usually evade host defense mechanisms to propagate themselves in their hosts. However, the host defense mechanism against prions to suppress prion propagation, or PrP^{Sc} accumulation, remains unknown.

The vacuolar protein sorting-10 protein (VPS10P)-domain receptors, including sortilin, SorLA, SorCS1, SorCS2 and SorCS3, are multi-ligand type I transmembrane proteins abundantly expressed in brains and involved in neuronal function and viability.[7, 8] They function as a cargo receptor to deliver a number of cargo proteins to their subcellular compartments through the VPS10P domain in the extracellular luminal N-terminus.[7, 8] Accumulating lines of evidence indicate that altered VPS10P receptor-mediated trafficking

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could be involved in the pathogenesis of neurodegenerative disorders, including Alzheimer's disease[9-12] and frontotemporal lobar degeneration.[13] Sortilin mediates intracellular trafficking of the amyloid precursor protein (APP)-cleaving enzyme BACE1[14] and the neurotrophic factor receptors Trks.[15] SorLA and SorCS1 are involved in APP transport.[9, 11]

We recently reported that sortilin negatively regulates PrP^{Sc} accumulation by sorting PrP^{C} and PrP^{Sc} to lysosomes for degradation, and that PrP^{Sc} accumulation itself impairs the sotrtilin-mediated degradation of PrP^{C} and PrP^{Sc} by stimulating lysosomal degradation of sortilin, thereby evoking further accumulation of PrP^{Sc} in prion-infected cells.[16] These findings suggest that the sortilin-mediated lysosomal degradation of PrP^{C} and PrP^{Sc} could be a host defense mechanism against prions, and that prions, or PrP^{Sc}, could propagate in infected neurons by evading the sortilin-mediated defense mechanism by inducing lysosomal degradation of sortilin.

Sortilin is a negative regulator for PrP^{Sc} accumulation

We found that PrP^{C} directly interacts with sortilin, but not with other VPS10P molecules, on the plasma membrane in PrP^{C}-overexpressing neuroblastoma N2a cells, designated N2aC24 cells.[16] The interaction of both molecules was also confirmed in mouse brain homogenates.[16] SiRNA-mediated knockdown of sortilin increased PrP^{Sc} in prion-infected N2aC24L1-3 cells, which are N2aC24 cells persistently infected with 22L scrapie prions.[16] In contrast, overexpression of sortilin in N2aC24L1-3 cells decreased PrP^{Sc}.[16] We also

76 showed that sortilin-knockout mice had accelerated prion disease caused by early

77 accumulation of PrP^{Sc} in their brains after infection with RML scrapie prions.[16] These

78 results indicate that sortilin could negatively regulate PrP^{Sc} accumulation in prion-infected

79 cells and mice.

80

81 **Sortilin traffics PrP^{C} to non-raft domains and to late endosomes/lysosomes**

82 PrP^{C} is synthesized in the endoplasmic reticulum (ER) and trafficked to the plasma

83 membrane through the Golgi apparatus.[17] PrP^{C} undergoes several posttranslational

84 modifications during its biosynthesis, including cleavage of the N-terminal signal peptide,

85 removal of the C-terminal peptide for attachment of a GPI anchor at the C-terminus and

86 formation of a disulfide bond at the C-terminal domain in the ER, and addition of two core

87 N-linked oligosaccharides at the C-terminal domain in the ER that are further modified in

88 the ER and then in the Golgi apparatus.[17] Like other GPI-anchored proteins, PrP^{C} is

89 predominantly located at raft domains and, to a lesser extent, at non-raft domains.[16, 17] After

90 internalization, some PrP^{C} molecules are delivered back to the plasma membrane directly or

91 indirectly via the recycling endosome compartments and others are transported to

92 lysosomes for degradation.[18] Copper and zinc stimulate endocytosis of PrP^{C} by binding to

93 histidine residues in the octapeptide repeat (OR) region located in the N-terminal

94 domain.[19-21] It has been postulated that PrP^{C} interacts with an as yet unidentified raft

95 molecule via the N-terminal domain including the OR region, thereby being retained at raft

96 domains.[20] The binding of copper or zinc to the OR region causes structural changes in the

97   N-terminal interacting region of PrP^C, thereby PrP^C leaves raft domains to non-raft domains

98   to be endocytosed via the clathrin-dependent pathway.[20] Low-density lipoprotein

99   receptor-related protein 1 has been reported to be involved in the clathrin-dependent

100   endocytosis of PrP^C.[22] The clathrin-independent pathways including caveolae, which is

101   considered to be formed by clustering raft domains, or caveolae-like domains have been

102   also reported to mediate the endocytosis of PrP^C.[18]

103   We found that sortilin was predominantly located at non-raft domains in

104   prion-uninfected N2aC24 cells.[16] Sortilin knockout caused marked shift in localization of

105   PrP^C from non-raft domains to raft domains in N2aC24 cells and mouse brains.[16] These

106   findings suggest that sortilin could function to recruit PrP^C from raft domains to non-raft

107   domains. We also found that, after internalization, PrP^C was transported to both late

108   endosomes and recycling endosomes in N2aC24 cells.[16] However, PrP^C was preferentially

109   transported to recycling endosomes with reduced localization at late endosomes/lysosomes

110   in sortilin-knockdown and -knockout N2aC24 cells,[16] indicating that sortilin also could

111   function as an endocytic receptor for PrP^C at non-raft domains to be sent to lysosomes for

112   degradation (Fig. 1A). Consistent with this, sortilin-deficient N2aC24 cells showed higher

113   PrP^C on their plasma membranes than control N2aC24 cells.[16] Sortilin-knockout mice also

114   showed higher PrP^C in their brains compared to WT mice.[16] Moreover, inhibition of

115   lysosomal enzymes by NH$_4$Cl increased PrP^C markedly in N2aC24 cells, but only slightly

116   in sortilin-knockout N2aC24 cells.[16]

117   The plasma membrane or raft domains are considered to be major sites for the

118 conversion of PrP^C into PrP^{Sc},[23] although the exact site of PrP^{Sc} production remains

119 controversial. It is thus likely that sortilin could negatively regulate PrP^{Sc} accumulation by

120 reducing PrP^C on the plasma membrane, particularly at raft domains through recruiting

121 PrP^C to non-raft domains from raft domains and sorting it to the late endosome/lysosome

122 protein degradation pathway.

123

124 Sortilin is involved in degradation of PrP^{Sc}

125 We also found that sortilin could function to direct PrP^{Sc} for degradation.[16] Sortilin

126 interacted with PrP^{Sc} in prion-infected N2aC24L1-3 cells.[16] Sortilin-knockout significantly

127 slowed down the degradation of PrP^{Sc} in N2aC24 cells infected with RML or 22L prions.[16]

128 PrP^{Sc} is found at various intracellular compartments, including the plasma membrane,

129 various endosomal compartments such as early and late endosomes, recycling endosomes,

130 and lysosomes, and the Golgi apparatus.[18] Enzymatic release of PrP^C from the plasma

131 membrane by phosphoinositide-specific phospholipase C was shown to reduce PrP^{Sc} in

132 infected cells,[24] and formation of PrP^{Sc} was inhibited by lowered temperature,[25] which

133 blocks the endocytosis and internalization of PrP^C. These suggest that the conversion of

134 PrP^C into PrP^{Sc} might occur at the plasma membrane, where exogenous PrP^{Sc} is likely to

135 first contact endogenous PrP^C, or after its internalization in the endosomal compartment.

136 Internalized PrP^{Sc} could also undergo retrograde transport to the Golgi apparatus and/or to

137 the ER,[26, 27] where the transported PrP^{Sc} might trigger the conversion of PrP^C into PrP^{Sc}.

138 PrP^{Sc} molecules on the plasma membrane are trafficked to lysosomes for degradation via

139   the endolysosomal pathway.[28, 29] The PrP[Sc] retrogradely transported to the Golgi apparatus

140   are subjected to Golgi quality control and trafficked to lysosomes for degradation.[27] Sortilin

141   localizes in para-nuclear vesicles, in the trans-Golgi network, and on the plasma

142   membrane.[30, 31] It is thus possible that sortilin could be involved in both degradation

143   trafficking pathways of PrP[Sc]. However, sortilin and PrP[Sc] molecules differed in their

144   microdomain localization on the plasma membrane in N2aC24L1-3 cells. Sortilin was

145   predominantly detected in non-raft fractions while PrP[Sc] was exclusively located in raft

146   fractions (Fig. 1B).[16] Therefore, the sortilin-mediated lysosomal degradation of PrP[Sc]

147   located on the plasma membrane might be a minor event.

148

149   **PrP[Sc] stimulates degradation of sortilin in lysosomes**

150   Interestingly, we found that sortilin was markedly reduced in both prion-infected cells and

151   mouse brains, and that the reduced sortilin levels in prion-infected cells were recovered by

152   treatment with lysosomal inhibitors but not with proteasomal inhibitor.[16] These findings

153   suggest that sortilin is increasingly degraded in lysosomes in prion-infected cells. We also

154   found that PrP[Sc] accumulation preceded the reduction of sortilin in N2aC24 cells freshly

155   infected with RML prions.[16] Immunofluorescent staining showed that sortilin was barely

156   detectable in PrP[Sc]-positive cells but still abundantly observed in PrP[Sc]-negative cells.[16] It is

157   thus likely that PrP[Sc] produced after prion infection could stimulate sortilin degradation in

158   lysosomes in a cell-autonomous fashion, and that the negative role of sortilin in PrP[Sc]

159   accumulation could be impaired in prion-infected cells, therefore PrP[Sc] progressively

8

160   accumulates in prion-infected neurons.

161

162   **Conclusions**

163   We presented a novel accumulation mechanism of PrP^{Sc} through degradation of sortilin.

164   Sortilin could form the host defense mechanism against prions, by functioning to sort PrP^{C}

165   and PrP^{Sc} to the late endosomal/lysosomal compartments for degradation (Fig. 1A, B).

166   Conversely, PrP^{Sc} itself stimulates degradation of sortilin in lysosomes, reducing sortilin

167   levels and impairing its defense function against prions. As a result, PrP^{C} is increasingly

168   converted to PrP^{Sc}, and PrP^{Sc} degradation is delayed, and eventually PrP^{Sc} progressively

169   accumulates in prion-infected cells (Fig. 1B). Accelerating the sortilin-mediated lysosomal

170   degradation of PrP^{C} and PrP^{Sc} might be beneficial for treatment of prion diseases.

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172   **DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

173   The authors declare no competing interests.

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180   contributions.

181

182   **FUNDING**

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189   **REFERENCES**

190   [1]   Prusiner SB. Prions. Proc Natl Acad Sci U S A 1998; 95:13363-83.

191   [2]   Stahl N, Borchelt DR, Hsiao K, Prusiner SB. Scrapie prion protein contains a

192   phosphatidylinositol glycolipid. Cell 1987; 51:229-40.

193   [3]   Bueler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M, et al. Mice devoid

194   of PrP are resistant to scrapie. Cell 1993; 73:1339-47.

195   [4]   Prusiner SB, Groth D, Serban A, Koehler R, Foster D, Torchia M, et al. Ablation of

196   the prion protein (PrP) gene in mice prevents scrapie and facilitates production of

197   anti-PrP antibodies. Proc Natl Acad Sci U S A 1993; 90:10608-12.

198   [5]   Manson JC, Clarke AR, McBride PA, McConnell I, Hope J. PrP gene dosage

199   determines the timing but not the final intensity or distribution of lesions in scrapie

200   pathology. Neurodegeneration 1994; 3:331-40.

201 [6]   Sakaguchi S, Katamine S, Shigematsu K, Nakatani A, Moriuchi R, Nishida N, et al.

202   Accumulation of proteinase K-resistant prion protein (PrP) is restricted by the

203   expression level of normal PrP in mice inoculated with a mouse-adapted strain of

204   the Creutzfeldt-Jakob disease agent. J Virol 1995; 69:7586-92.

205 [7]   Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, et al. Sortilin is

206   essential for proNGF-induced neuronal cell death. Nature 2004; 427:843-8.

207 [8]   Nykjaer A, Willnow TE. Sortilin: a receptor to regulate neuronal viability and

208   function. Trends in neurosciences 2012; 35:261-70.

209 [9]   Rogaeva E, Meng Y, Lee JH, Gu Y, Kawarai T, Zou F, et al. The neuronal

210   sortilin-related receptor SORL1 is genetically associated with Alzheimer disease.

211   Nature genetics 2007; 39:168-77.

212 [10] Caglayan S, Takagi-Niidome S, Liao F, Carlo AS, Schmidt V, Burgert T, et al.

213   Lysosomal sorting of amyloid-beta by the SORLA receptor is impaired by a familial

214   Alzheimer's disease mutation. Science translational medicine 2014; 6:223ra20.

215 [11] Reitz C, Tosto G, Vardarajan B, Rogaeva E, Ghani M, Rogers RS, et al. Independent

216   and epistatic effects of variants in VPS10-d receptors on Alzheimer disease risk and

217   processing of the amyloid precursor protein (APP). Translational psychiatry 2013;

218   3:e256.

219 [12] Reitz C, Cheng R, Rogaeva E, Lee JH, Tokuhiro S, Zou F, et al. Meta-analysis of

220   the association between variants in SORL1 and Alzheimer disease. Arch Neurol

221   2011; 68:99-106.

222   [13]   Hu F, Padukkavidana T, Vaegter CB, Brady OA, Zheng Y, Mackenzie IR, et al.

223   Sortilin-mediated endocytosis determines levels of the frontotemporal dementia

224   protein, progranulin. Neuron 2010; 68:654-67.

225   [14]   Finan GM, Okada H, Kim TW. BACE1 retrograde trafficking is uniquely regulated

226   by the cytoplasmic domain of sortilin. J Biol Chem 2011; 286:12602-16.

227   [15]   Vaegter CB, Jansen P, Fjorback AW, Glerup S, Skeldal S, Kjolby M, et al. Sortilin

228   associates with Trk receptors to enhance anterograde transport and neurotrophin

229   signaling. Nature neuroscience 2011; 14:54-61.

230   [16]   Uchiyama K, Tomita M, Yano M, Chida J, Hara H, Das NR, et al. Prions amplify

231   through degradation of the VPS10P sorting receptor sortilin. PLoS Pathog 2017;

232   13:e1006470.

233   [17]   Harris DA. Trafficking, turnover and membrane topology of PrP. Br Med Bull 2003;

234   66:71-85.

235   [18]   Campana V, Sarnataro D, Zurzolo C. The highways and byways of prion protein

236   trafficking. Trends Cell Biol 2005; 15:102-11.

237   [19]   Pauly PC, Harris DA. Copper stimulates endocytosis of the prion protein. J Biol

238   Chem 1998; 273:33107-10.

239   [20]   Taylor DR, Watt NT, Perera WS, Hooper NM. Assigning functions to distinct

240   regions of the N-terminus of the prion protein that are involved in its

241   copper-stimulated, clathrin-dependent endocytosis. J Cell Sci 2005; 118:5141-53.

242   [21]   Lee KS, Magalhaes AC, Zanata SM, Brentani RR, Martins VR, Prado MA.

243   Internalization of mammalian fluorescent cellular prion protein and N-terminal

244   deletion mutants in living cells. J Neurochem 2001; 79:79-87.

245 [22] Taylor DR, Hooper NM. The low-density lipoprotein receptor-related protein 1

246   (LRP1) mediates the endocytosis of the cellular prion protein. Biochem J 2007;

247   402:17-23.

248 [23] Taraboulos A, Scott M, Semenov A, Avrahami D, Laszlo L, Prusiner SB.

249   Cholesterol depletion and modification of COOH-terminal targeting sequence of the

250   prion protein inhibit formation of the scrapie isoform. J Cell Biol 1995; 129:121-32.

251 [24] Enari M, Flechsig E, Weissmann C. Scrapie prion protein accumulation by

252   scrapie-infected neuroblastoma cells abrogated by exposure to a prion protein

253   antibody. Proc Natl Acad Sci U S A 2001; 98:9295-9.

254 [25] Borchelt DR, Taraboulos A, Prusiner SB. Evidence for synthesis of scrapie prion

255   proteins in the endocytic pathway. J Biol Chem 1992; 267:16188-99.

256 [26] Beranger F, Mange A, Goud B, Lehmann S. Stimulation of PrP(C) retrograde

257   transport toward the endoplasmic reticulum increases accumulation of PrP(Sc) in

258   prion-infected cells. J Biol Chem 2002; 277:38972-7.

259 [27] Goold R, McKinnon C, Rabbanian S, Collinge J, Schiavo G, Tabrizi SJ. Alternative

260   fates of newly formed PrPSc upon prion conversion on the plasma membrane.

261   Journal of cell science 2013; 126:3552-62.

262 [28] Yamasaki T, Suzuki A, Shimizu T, Watarai M, Hasebe R, Horiuchi M.

263   Characterization of intracellular localization of PrP(Sc) in prion-infected cells using

264   a mAb that recognizes the region consisting of aa 119-127 of mouse PrP. J Gen

265   Virol 2012; 93:668-80.

266   [29]   Veith NM, Plattner H, Stuermer CA, Schulz-Schaeffer WJ, Burkle A.

267   Immunolocalisation of PrPSc in scrapie-infected N2a mouse neuroblastoma cells by

268   light and electron microscopy. European journal of cell biology 2009; 88:45-63.

269   [30]   Petersen CM, Nielsen MS, Nykjaer A, Jacobsen L, Tommerup N, Rasmussen HH, et

270   al. Molecular identification of a novel candidate sorting receptor purified from

271   human brain by receptor-associated protein affinity chromatography. J Biol Chem

272   1997; 272:3599-605.

273   [31]   Nielsen MS, Madsen P, Christensen EI, Nykjaer A, Gliemann J, Kasper D, et al. The

274   sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS

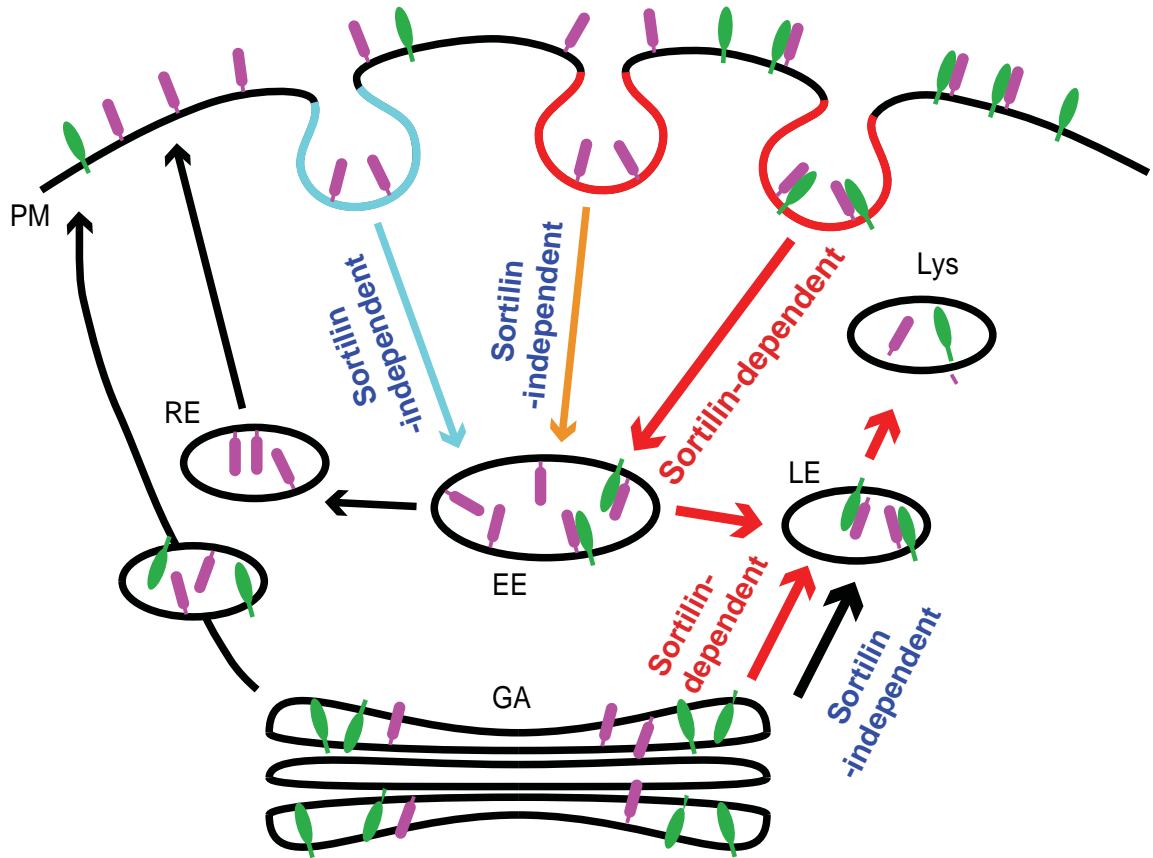275   domain of the GGA2 sorting protein. Embo J 2001; 20:2180-90.

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Figure Legends

279 **Figure 1. A model of the sortilin-mediated intracellular trafficking of PrP^C and PrP^{Sc}**

280 **in prion-uninfected and infected neurons.** (A) Sortilin-dependent and -independent

281 endocytosis of PrP^C in uninfected neurons. Sortilin mediates endocytosis of PrP^C on the

282 plasma membrane (PM), particularly at non-raft domains, via the clathrin-dependent

283 pathway to early endosomes (EE) and then traffics it to late endosomes/lysosomes (LE/Lys)

284 for degradation. Other PrP^C molecules are trafficked either to LE/Lys for degradation or to

285 the recycling endosome (RE) pathway in a sortilin-independent way. There also might be

286 sortilin-dependent and -independent trafficking pathways from the Golgi apparatus (GA) to

287 LE/Lys for degradation. (B) Intracellular trafficking of PrP^C and PrP^{Sc} in prion-infected

288 neurons. Prion infection stimulates lysosomal degradation of sortilin via an unknown

289 mechanism, thereby impairing the sortilin-mediated trafficking of PrP^C and PrP^{Sc} to LE/Lys

290 for degradation. As a result, PrP^C and PrP^{Sc} are increased at raft domains and endocytosed

291 via the sortilin-independent pathway to RE, causing accumulation of PrP^{Sc} and increasing

292 conversion of PrP^C into PrP^{Sc} in prion-infected neurons. PrP^{Sc} could undergo retrograde

293 transport to the GA. However, sortilin might also be functionally impaired in the GA,

294 thereby being unable to traffic PrP^{Sc} in the GA to LE/Lys for degradation. The decreased

295 degradation of PrP^{Sc} in LE/Lys and the increased conversion of PrP^C into PrP^{Sc} in raft

296 domains or RE could both contribute to the constitutive production of PrP^{Sc} in

297 prion-infected neurons. Dashed arrows indicate restricted trafficking.

A

PM

RE

EE

Sortilin-independent

Sortilin-independent

Sortilin-dependent

Lys

LE

Sortilin-dependent

Sortilin-independent

GA

B

PM

RE

EE

Sortilin-independent

Sortilin-independent

Sortilin-dependent

Lys

LE

Sortilin-dependent

Sortilin-independent

GA

⬮ : Sortilin   ⬮ : PrP^C   ✦ : PrP^Sc   ⊂ : raft domain   ⊂ : non-raft domain