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Specific domains in anterior pharynx-defective 1 determine its intramembrane interactions with nicastrin and presenilin

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Abstract

γ-Secretase, a multisubunit transmembrane protease comprised of presenilin, nicastrin, presenilin enhancer 2, and anterior pharynx-defective one, participates in the regulated intramembrane proteolysis of Type I membrane proteins including the amyloid precursor protein (APP). Although Aph-1 is thought to play a structural role in the assembly of γ-secretase complex and several transmembrane domains (TMDs) of Aph-1 have been shown to be critical for its function, the importance of the other domains of Aph-1 remains elusive. We screened a series of Aph-1 mutants and focused on nine mutations distributed in six different TMDs of human APH-1aS, assessing their ability to complement mouse embryonic fibroblasts lacking Aph-1. We showed that mutations in TMD4 (G126) and TMD5 (H171) of Aph-1aS prevented the formation of the Nct/Aph-1 subcomplex. Importantly, although mutations in TMD3 (Q83/E84/R85) and TMD6 (H197) of APH-1aS did not affect Nct/Aph-1 subcomplex formation, both mutations prevented further association/endoproteolysis of PS1. We propose a model that identifies critical TMDs of Aph-1 for associations with Nct and PS for the stepwise assembly of γ-secretase components.
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Keywords: Aph-1; Mutagenesis; Nct; PS; Transmembrane domain; γ-secretase

γ-secretase is an aspartyl-type protease membrane protein complex that catalyzes the regulated intramembrane proteolysis of numerous Type I membrane proteins, including Notch and APP (Francis et al., 2002). The requirement for γ-secretase processing of APP to generate amyloid-β, a neurotoxic peptide involved in the pathogenesis of Alzheimer's disease (AD) has lead to intense studies to understand the biology of γ-secretase and towards the development of rationally designed drugs targeting this enzyme for prevention or treatment of AD (Panza et al., 2009).

The γ-secretase is comprised of PS (Takasugi et al., 2003), the catalytic component of this enzyme complex (Haass and Steiner, 2002), and three other essential subunits (Edbauer et al., 2003): Nct (Hu et al., 2002), Pen-2 (Francis et al., 2002), and Aph-1 (Francis et al., 2002). Nct is thought to be the receptor that initially recognizes the processed substrates; (Dries et al., 2009; Shah et al., 2005), although some studies challenged this view (Chavez-Gutierrez et al., 2008). Pen-2 is believed to control the endoproteolysis of PS to form a stable heterodimer composed of N- and C-terminal fragments (PS-NTF and PS-CTF) (Luo et al., 2003; Prokop et al., 2004). Current studies support the view that the formation of mature, active γ-secretase requires the initial formation of Nct/Aph-1 subcomplex (LaVoie et al., 2003), and the subsequent sequential assembly of PS and Pen-2, which assembles with the Nct/ Aph-1/PS ternary complex to initiate endoproteolysis of PS (Hu et al., 2002).

In humans there are two APH-1 homologues, APH-1a and APH-1b, and APH-1a has two C-terminal splicing variants: APH-1aL (long variant) and APH-1aS (short variant). There is an additional homologue, Aph-1c in mice. Al-

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though the homologues are differentially expressed in various tissues (Serneels et al., 2005), Aph-1aL, APH-1aS, and Aph-1b are functionally redundant in terms of their ability to form active γ-secretase complexes with the other three subunits (Shirotani et al., 2004b). Aph-1 has 7 TMDs with its N-terminus in endoplasmic reticulum (ER)/extracellular space and its C-terminus in the cytosol (Fortna et al., 2004). Direct interaction between Aph-1 and Nct for the initial subcomplex has been shown (Shirotani et al., 2004a), and crosslinking experiments have also demonstrated close proximity between Aph-1 and PS and between Aph-1 and Nct (Steiner et al., 2008). The GXXXG motif (Kleiger et al., 2002) in TMD4 of Aph-1 has been identified to be essential for the initial assembly and later maturation of γ-secretase (Lee et al., 2004), recently H171 and H197 in TMD5 and TMD6 were also found to be critical for the function/maturation of γ-secretase (Pardossi-Piquard et al., 2009).

To extend those findings, here we tested the functional role of TMDs of Aph-1 in the assembly of components of the γ-secretase complex using site-directed mutagenesis to generate 12 single, double, or triple mutations of the conserved amino acid residues within TMD1 to TMD6 of human APH-1aS (hAPH-1aS) and assessed their ability to complement Aph-1 null MEFs (Aph-1 ab −/−; Aph-1c shRNA-suppressed). Our results are consistent with the previous findings that GXXXG, H171, and H197 are critical for structure/function of γ-secretase complex. In addition to an extra critical region identified, Q83/E84/Q87 in TMD3, we found those disrupting mutants differentially affected 2 steps in γ-secretase maturation: mutation in TMD4 or TMD5 of Aph-1 disrupted formation of the initial Aph-1/Nct subcomplex whereas those in TMD3 and TMD6 decreased association/endoproteolysis of PS after the formation of the Nct/Aph-1 subcomplex.

1. Methods

1.1. Mutagenesis

Mutations were introduced into human APH-1aS cDNA and cloned into pcDNA3.1-V5/His (Invitrogen) using QuickChange site-directed mutagenesis as described by the manufacturer (Stratagene). Human APP695 expressing plasmid was transfected as the substrate to assess functional competence of γ-secretase; thus, the accumulation of its C-terminus (APP-CTF) would indicate the impaired γ-secretase function. LacZ expression vector served as the mock transfection control.

Nine amino acids were chosen for mutagenesis (Fig. 1A) as potential functionally critical residues using the following rationale: E84, R87, H171, or H197 are potentially charged; W42, S-45, and Q83 are polar; P16 distorts the helix; G126 is known to disrupt assembly and activity of γ-secretase (Lee et al., 2004). The targeted amino acids span TMD1–6 of the hAPH-1 peptide sequence. The targeted amino acids were replaced by residues with hydrophobic side chain that is compatible with preserved α-helix formation and intramembrane location.

For most of the TMDs, there was only one site targeted for mutagenesis. However, TMD2 had 2 (W42V and S45A) and TMD3 had 3 (Q83L, E84V, R87L) sites targeted. Because multiresidue interaction could mediate TMD interactions, double or triple mutations were generated to enhance the sensitivity of complementation for TMDs with more than one targeted mutation. Thus, 3 hAPH-1aS compound-

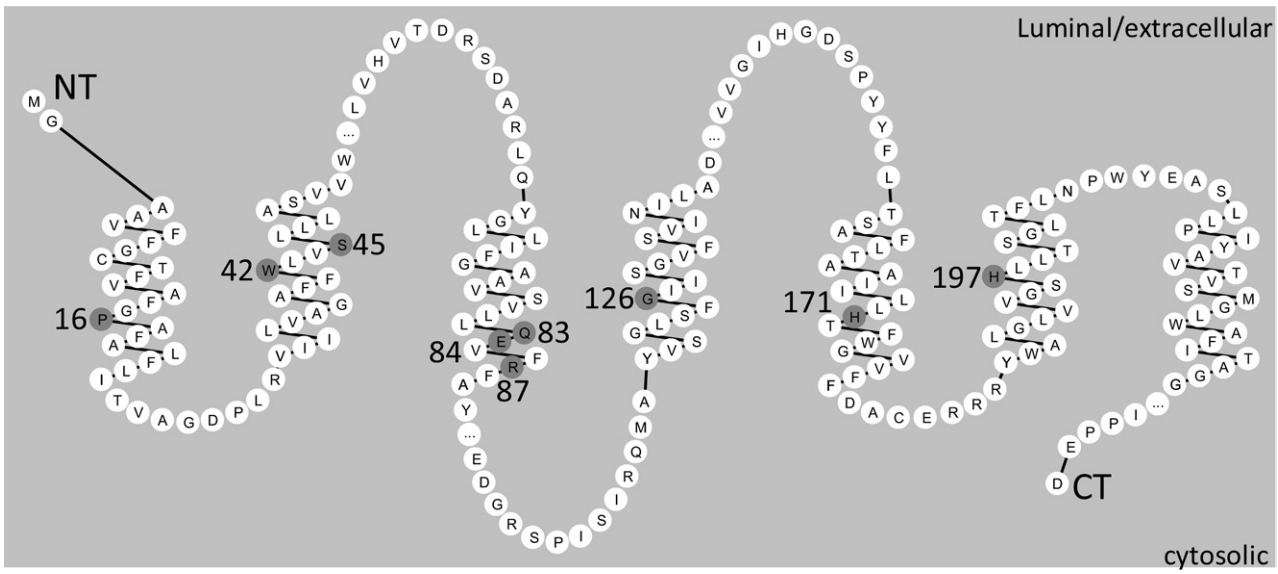

Fig. 1. Topology of Aph-1 depicted by Residue-based Diagram Editor (Campagne and Weinstein, 1999; Konvicka, et al., 2000; Skrabanek, et al., 2003). The residues in TMDs targeted for mutation highlighted in gray.

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