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COMMENTARY

Structure of nicastrin unveils secrets of γ-secretase

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Despite afflicting tens of millions worldwide, Alzheimer's disease (AD) remains a devastating brain-destroying malady with no effective treatment or cure. A central hallmark of AD is the deposition of amyloid plaques in the brain. These plaques are primarily composed of the amyloid β-peptide (Aβ), a byproduct of proteolytic processing of the amyloid precursor protein (APP) within its transmembrane domain by γ-secretase. Mutations in both APP and presenilin, the catalytic component of γ-secretase, are associated with dominant early-onset AD, highlighting their central importance in pathogenesis (1). Gaining a detailed mechanistic understanding of how γ-secretase processes APP—and its other substrates, such as Notch receptors—is critically important for biology and medicine (2). Despite a wealth of biochemical studies, key details of how γ-secretase functions have remained elusive because of a lack of high-resolution structural data. In PNAS, Xie et al. present a crystal structure of nicastrin, the first atomic-resolution structure of a component of a γ-secretase complex (3).

The membrane-embedded γ-secretase complex is comprised of four proteins that are necessary and sufficient for its activity: Presenilin, Pen-2, Aph-1, and nicastrin (4). Presenilin, an aspartyl protease, contains catalytic aspartates on transmembrane domains (TMDs) 6 and 7 of its nine TMD helices. Upon assembly of the four components within the endoplasmic reticulum, presenilin undergoes autoproteolysis between TMDs 6 and 7 to form catalytically active γ-secretase. Pen2 has two TMDs and is thought to be important for triggering presenilin self-cleavage (5, 6). Little is known about the biochemical function of the seven-TMD Aph-1 other than that it is required for complex formation and full maturation of γ-secretase, although a recent study suggests a role in determining the length of Aβ peptide proteolytic products (7). The fourth component of the complex, nicastrin, is a single-TMD protein with a large ectodomain.

γ-Secretase is a member of a broader family of intramembrane-cleaving proteases, which include the site 2 protease (S2P) family of metalloproteases and the rhomboid family of serine proteases (8). Unlike S2P and rhomboid, whose high-resolution structures were solved some years ago, detailed structural information of γ-secretase has been slow to develop. Until recently, the only structural information known about γ-secretase has been gleaned from low-resolution (∼12 Å at best) electron microscopy (EM) studies. With the advent of advanced cryo-EM techniques and equipment, a much more detailed structure of the complex was recently obtained (9). Although the resolution of this structure is still too poor to see atomic details, the overall architecture of the complex was visualized for the first time. Individual TMDs could be seen, although not assigned with certainty to each component of the complex. The most well-resolved portion of the complex was the ectodomain of nicastrin, although all of the glycosylation sites and several long segments of amino acids were not observed, presumably because of their intrinsic flexibility.

At 709 amino acids, nicastrin is the largest component of γ-secretase, with the majority of its mass located to its large, heavily glycosylated ectodomain. Although a controversial hypothesis (10), the nicastrin ectodomain has been proposed to bind to the free N terminus of ectodomain-shed substrates of γ-secretase, thereby acting as substrate receptor for the enzyme (11, 12). The mechanism by which this may occur has remained obscure, in part because of a lack of structural information.

Xie et al. (3) solved the crystal structure of nicastrin from the amoeboid eukaryote *Dictyostelium purpureum*, an organism with a complete and functional γ-secretase complex (13). The 1.95 Å-resolution structure (Fig. 1*A*) reveals a bilobed protein with one large and one small lobe. Five glycosylation sites and six stabilizing disulfide bridges were seen that were not observed in the recent cryo-EM structure of the complex. The two lobes are joined by a collection of hydrophobic interactions at the center of a half circle of hydrogen bonds.

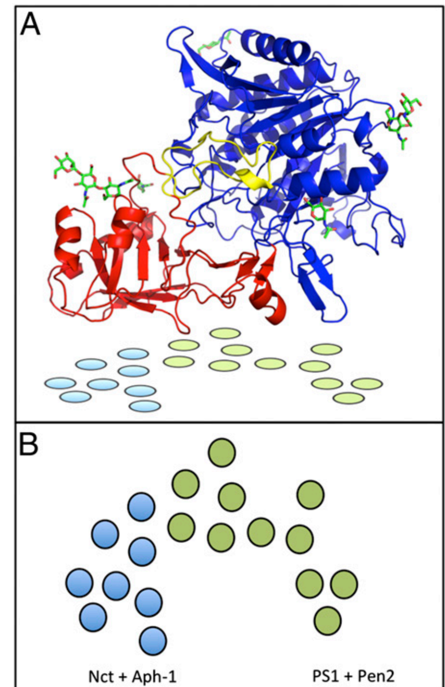

Fig. 1. (*A*) The crystal structure of nicastrin from the organism *D. purpureum* at a resolution of 1.95 Å. The bilobed protein is structurally homologous to a bacterial aminopeptidase, although nicastrin itself lacks proteolytic activity. A loop (yellow) extends from the small lobe (red) to cover the putative substrate-binding pocket on the large lobe (blue). Using this structure to improve the cryo-EM model of the human γ-secretase complex revealed that this pocket is oriented above the opening of the horseshoe-shaped arrangement of γ-secretase TMDs (blue and green ovals). (*B*) New speculative arrangement of the TMDs of γ-secretase components within the lipid bilayer. Nicastrin, Aph-1, and presenilin CTF are located to the thick end of the horseshoe shaped structure determined by cryo-EM. Pen2 and presenilin NTF are located to the thin end in this model.

With this more complete structure, a structural homology search revealed that the ectodomain of nicastrin most closely resembles that of a bacterial aminopeptidase. Nicastrin, however, lacks the key zinc-binding amino

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acids required for a catalytically active metalloprotease. What would be the catalytic pocket in the large lobe of nicastrin still maintains a similar architecture to the active site of the bacterial aminopeptidase. Thus, this pocket could act as a substrate-binding site consistent with previous speculation.

One striking feature not previously seen in the cryo-EM structure is the presence of a loop extending from the small lobe to the large lobe. This loop of roughly 24 amino acids covers the putative substrate-binding pocket on the large lobe. Xie et al. (3) therefore call this loop a "lid" and suggest that it would block substrate entry into the binding pocket. In order for the N terminus of a γ-secretase substrate to gain access to this pocket, the lid would have to move out of the way. This process could occur by rotation of the large lobe relative to the small lobe, or simply by the lid moving in and out of the pocket if it has some intrinsic flexibility. The cryo-EM structure of the whole protease demonstrates that this proposed substrate-binding pocket is positioned directly above the substrate entry site of the complex and at a reasonable distance above the arrangement of TMDs (Fig. 1*A*).

Using this atomic resolution structure of nicastrin from *D. purpureum*, along with the recent cryo-EM structure, Xie et al. (3) were able to generate an improved model of human nicastrin. This model contains roughly 100 additional amino acids and numerous structural features that were not seen by cryo-EM. These features include the aforementioned lid covering the putative substrate-binding pocket, as well as small globular domains present in both small and large lobes.

This new model of human nicastrin also allowed Xie et al. (3) to revisit their speculative assignment of the TMDs from the cryo-EM structure. The recent cryo-EM structure revealed that the TMDs of the four γ-secretase components are organized in a horseshoe shape, with one thick end and one thin end (9). The improved resolution obtained in this study provides a more precise placement of the TMD of nicastrin. Whereas previously the nicastrin TMD was placed in the thin end of the horseshoe, it is now apparent that it most likely resides in the thick end. With this knowledge in hand, the position of presenilin could be assigned to the structure with more certainty using a model of presenilin obtained from the crystal structure of an archaeal presenilin homolog (14). Based on this new model, it is likely that nicastrin, Aph-1, and presenilin CTF (C-terminal fragment, TMDs 7–9) are located to the thick end of the horseshoe shape, whereas Pen2 and presenilin NTF (N-terminal fragment, TMDs 1–6) are located toward the thin end (Fig. 1*B*).

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This new study (3) fuels speculation that nicastrin may indeed serve as a receptor of γ-secretase substrates. Clearly, more rigorous testing will be needed to determine if this is true. The improved structural resolution and model of human nicastrin in this report should provide a good framework for designing such experiments. Although determining the high-resolution structure of nicastrin is a big step forward, much work is still required to elucidate the atomic-level details of the other components of γ-secretase, in particular the TMD of each protein and their relative configuration within the lipid bilayer. Detailed structures of presenilin are of the highest importance, as this component not only contains the active site but also allosteric sites for pharmacological modulators that specifically decrease the production of the most aggregation-prone forms of Aβ (15). Such details should provide further insight into the molecular mechanisms that govern γ-secretase activity, as well as pave the way for a structure-based design of potential AD therapeutics for which there is such a pressing need.

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