Review

CellPress

Understanding intramembrane proteolysis: from protein dynamics to reaction kinetics

D. Langosch[1], C. Scharnagl[2], H. Steiner[3], and M.K. Lemberg[4]

[1] Technische Universität München, Lehrstuhl Chemie der Biopolymere, Weihenstephaner Berg 3, 85354 Freising, and Munich Center for Integrated Protein Science (CIMPS[M]), Germany
[2] Fakultät für Physik E14, Technische Universität München, Maximus-von-Imhof-Forum 4, 85354 Freising, Germany
[3] Ludwig-Maximilians-University Munich, Metabolic Biochemistry and DZNE (German Center for Neurodegenerative Diseases), Munich, Germany
[4] Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Deutsches Krebsforschungszentrum (DKFZ)–ZMBH Allianz, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany

Intramembrane proteolysis – cleavage of proteins within the plane of a membrane – is a widespread phenomenon that can contribute to the functional activation of substrates and is involved in several diseases. Although different families of intramembrane proteases have been discovered and characterized, we currently do not know how these enzymes discriminate between substrates and non-substrates, how site-specific cleavage is achieved, or which factors determine the rate of proteolysis. Focusing on γ-secretase and rhomboid proteases, we argue that answers to these questions may emerge from connecting experimental readouts, such as reaction kinetics and the determination of cleavage sites, to the structures and the conformational dynamics of substrates and enzymes.

Intramembrane proteases are relevant for many biological processes

Intramembrane proteolysis has been first observed in the 1990s. The past two decades have seen a wealth of studies that identified major families of substrates and intramembrane proteases (IMPs, see Glossary) in all kingdoms of life, as well as uncovering the nature and function of some cleavage products (reviewed in [1–4]). Most substrates contain a single transmembrane domain (TMD) harboring the scissile peptide bond. Recently, trimming of lipidated peripheral membrane proteins and cleavage within ectodomains and luminal loops of polytopic substrates have also been described [5–7]. The functional consequences of intramembrane proteolysis include activation of membrane-tethered transcription factors and transcriptional activators, secretion of growth factors, maturation of a bacterial translocation channel, and regulated protein degradation. The malfunction of IMPs can lead to devastating degenerative disorders such as Alzheimer's or Parkinson's disease [1,8].

The structures of some IMPs have been solved [5,9–13] and their mode of action is beginning to unfold. By analogy to soluble proteases, intramembrane proteolysis requires substrate recognition, which is followed by substrate processing. Substrate processing requires exposure of the scissile bond to the catalytic residues, followed by formation of a tetrahedral intermediate structure leading to hydrolysis. Finally, the product is released [1–3]. Unlike most soluble proteases, IMPs tend to be rather slow enzymes [14–16]. It is currently unclear which of the above step(s) limits the kinetics of proteolysis, and how the primary structures and/or the conformational dynamics of substrates and enzymes determine the rate-limiting

Corresponding author: Langosch, D. (langosch@tum.de).
Keywords: intramembrane proteolysis/protease; transmembrane helix; presenilin; γ-secretase; rhomboid; conformational dynamics.

0968-0004/
 http://dx.doi.org/10.1016/j.tibs.2015.04.001

Glossary

Amyloid precursor protein (APP): an N_{out} single-span membrane protein that may have a role in cell–cell adhesion. It serves as substrate for shedding by α- or β-secretase. Shedding by β-secretase produces the C-terminal C99 fragment whose TMD helix is cleaved by γ-secretase. Cleavage yields an intracellular fragment plus a series of Aβ peptides that form cell toxic oligomers and amyloid fibrils that are believed to cause Alzheimer's disease.
Deuterium/hydrogen exchange kinetics: kinetics of the reaction by which deuterons bound to helix backbone amides exchange for solvent protons. The reaction is determined by the open/closed equilibria of intrahelical amide hydrogen bonds.
Familial Alzheimer's disease (FAD): hereditary amino acid mutations located within presenilin, the catalytic subunit of γ-secretase, or APP, that lead to altered APP processing which causes early-onset Alzheimer's disease (http://www.alzforum.org).
Michaelis–Menten kinetics: analyzing and interpreting enzyme kinetics according to the Michaelis–Menten formalism yields K_M, V_{max}, and k_{cat} values. V_{max} describes the initial catalytic rate at saturating substrate concentration. V_{max} thus represents the theoretical maximal velocity of catalysis that occurs when all substrate-binding sites are occupied. K_M denotes the substrate concentration required to induce a half-maximal catalytic rate and approximates to the dissociation constant K_D, in other words the affinity, provided that the rate of the dissociation of uncleaved substrate from the enzyme vastly exceeds k_{cat}. The catalytic turnover number k_{cat} is calculated by dividing V_{max} by the concentration of the active enzyme, and thus represents the catalytic turnover per enzyme molecule.
Transmembrane domain (TMD) topology: location of the N and C termini of membrane-traversing protein domains, usually α-helices, with respect to both surfaces of a membrane; N_{out} refers to exposure of the N terminus of a protein towards the extracellular side.

step(s). In addition, how disease-related point mutations affect these properties and how substrates are discriminated from non-substrates remain open, unanswered questions (Box 1). We review concepts that connect experimental findings to the structure and dynamics of substrates and proteases to illuminate the mechanism of intramembrane proteolysis. We focus on those types of IMPs whose functions and structures have been characterized most intensively in recent years; that is, the γ-secretase and rhomboid proteases.

Box 1. Outstanding questions

- How do intramembrane proteases distinguish substrates from non-substrates, and how do they recognize the sites to be cleaved?
- Which mechanistic steps limit the rate by which an intramembrane protease processes its substrates?
- How does the conformational dynamics of a substrate, and in particular its transmembrane helix, influence its recognition and its cleavage?
- How does the membrane environment affect substrate recognition and the conformational transitions leading to cleavage?

Intramembrane proteases come in different structural families

IMPs are distinguished by their transmembrane topologies and by the nature of their membrane-embedded active site residues (Figure 1). Aspartate IMPs contain two catalytic aspartate residues and comprise presenilins, signal peptide peptidase (SPP), and SPP-like (SPPL) proteases [1,3]. Because the C-terminal aspartate is part of a Gly-X-Gly-Asp active site motif, these proteases are also known as GXGD-type proteases [17]. The aspartate IMP presenilin, the catalytic subunit of γ-secretase, has an N_{in} topology and is associated in a 1:1:1:1 stoichiometry with the nicastrin, PEN-2 (presenilin enhancer-2), and APH-1 (anterior pharynx-defective 1) subunits, thus forming the γ-secretase complex [1,18]. SPP and SPPL proteases exhibit an inverted (N_{out}) membrane topology relative to presenilin and do not need invariant cofactors for their activity [19]. Serine IMPs are also known as rhomboid proteases and comprise a characteristic Ser-His catalytic dyad [2,4]. Rhomboid proteases are defined by a six-TMD core, which is extended by an additional TMD plus cytosolic domains in some cases [20]. Rhomboid proteases in the eukaryotic secretory pathway and the bacterial plasma membrane expose their active sites towards the extracytoplasmatic space. The mitochondrial rhomboid protease PARL (presenilin-associated rhomboid-like protein) has an inverted topology: an extra TMD is appended to the N terminus and its active site faces the

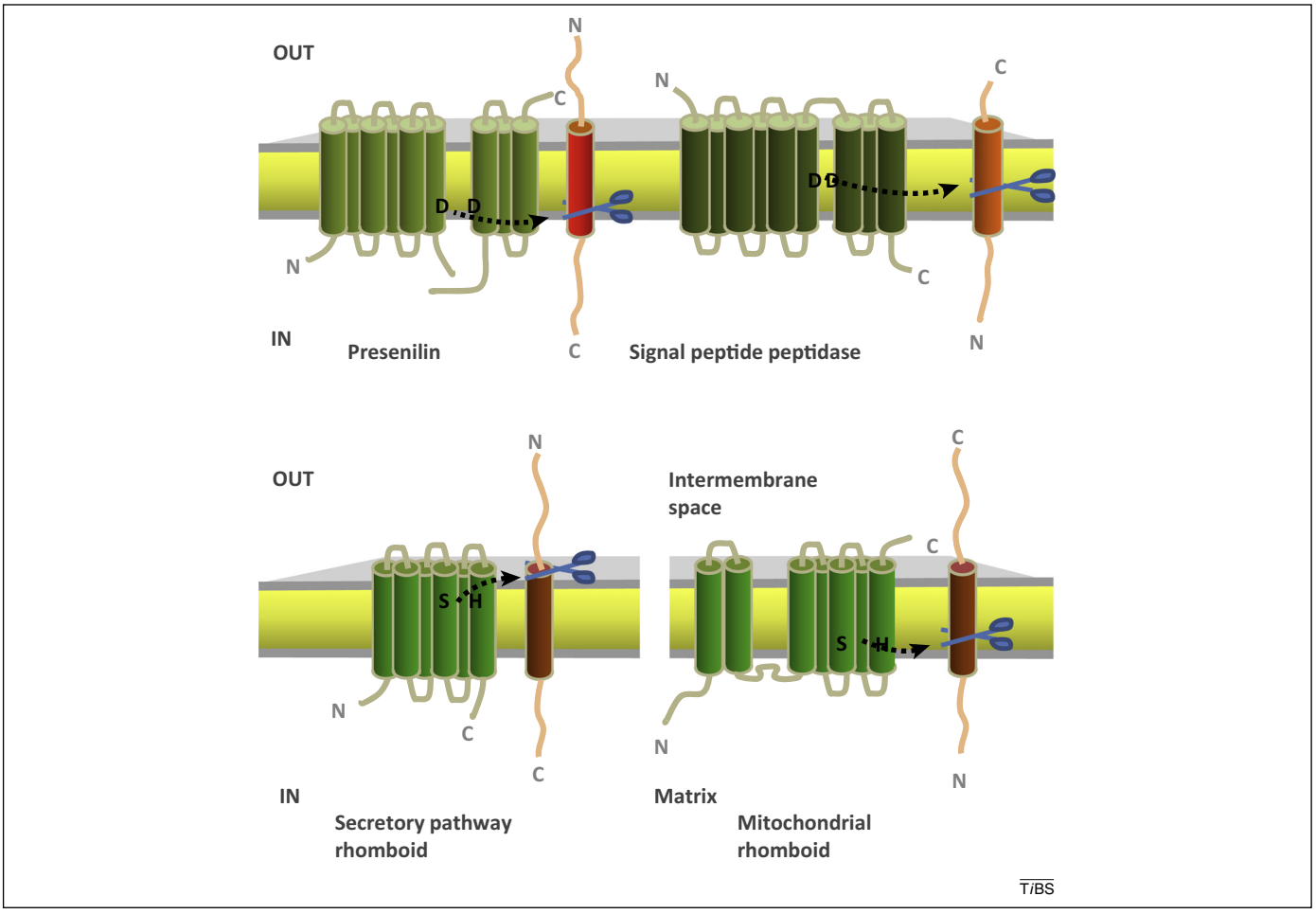

Figure 1. Transmembrane topologies of intramembrane proteases and their substrates. Uppercase letters within transmembrane domains (TMDs) represent catalytic residues, and scissors symbolize cleavage sites. Note that many rhomboid proteases possess a seventh TMD appended to the C terminus of the secretary pathway rhomboid protease scaffold [20]. IN and OUT refer to the cytoplasm and extracytoplasmic space, respectively. Enzymes are in green while substrates are in red or brown.