Intramembrane proteases hydrolyze peptide bonds within the cell membrane as the decision-making step of various signaling pathways or during general proteostasis. Initially thought to be rare, fourteen proteases from four superfamilies are now known to be distributed among nearly every membrane compartment of a human cell. Each protease is endowed with specific enzymatic properties that determine both substrate choice and outcome.

The Enzymes

Around the turn of the millennium, an extraordinary class of enzymes that cut proteins inside the cell membrane was discovered (Brown et al., 2000). These intramembrane proteases originated independently on at least four occasions during evolution and are now widely distributed across all life. Atomic-resolution structures of prototypic members confirmed that each superfamily adopts a unique fold and uses distinct chemistry to catalyze proteolysis (Sun et al., 2016). The human genome encodes at least 14 active intramembrane proteases, and tissue-specific expression patterns shuffle the complement in different cell types.

Asparyl intramembrane proteases are the most numerous human intramembrane proteases. They contain GxGxD, YD, and PAL active site motifs within their nine transmembrane segments and come in two groups; γ-secretases and signal peptide peptidases (SPPs). Although four proteins are required for γ-secretase activity, presenilin 1 or presenilin 2 provides the catalytic center (Selkoe and Wolfe, 2007). Conversely, SPP and SPP-like enzymes SPPL2a, SPPL2b, SPPL2c, and SPPL3 are presenilin homologs but function without requiring partners (Voss et al., 2013).

Rhomboid proteases evolved a serine-histidine catalytic dyad for peptide bond attack. The human genome encodes five of these serine proteases, called RHBDL4-1 (rhomboid-like) (Lastun et al., 2016) and PARL (PINK1/PGAMS-associated rhomboid-like) (Spinazzi and De Strooper, 2016). Rhomboid proteases have either six or seven transmembrane segments and are active alone.

Site-2 protease (S2P) is a HEXxH-type zinc metalloenzyme with a complex transmembrane topology (Brown et al., 2000). Ras-converting enzyme 1 (Rce1) is a glutamyl protease comprised of three active-site motifs housed within eight transmembrane segments (Manolaridis et al., 2013).

Subcellular Deployment

Subcellular compartmentalization regulates intramembrane proteolysis by segregating substrate from protease until proteolysis is warranted, enhances cleavage efficiency by concentrating enzyme/substrate, and/or selects only subsets of substrate for cleavage. The endoplasmic reticulum (ER) houses four intramembrane proteases that function broadly in protein maturation and/or quality control. SPP cleaves signal peptides after they are released from nascent membrane proteins by signal peptidase, and certain tail-anchored ER proteins—while a fraction of SPP and RHBDL4 (probably within larger assemblies)—cleave aberrant proteins, as they are retrotranslocated for posttranslational degradation. Rce1 matures most prenylated CAAX proteins by trimming off the C-terminal AAX tripeptide. SPPL2c has no described function.

Three intramembrane proteases are stationed in the Golgi apparatus. S2P ultimately triggers cholesterol/fatty-acid biosynthesis or proteostatic stress responses through release of SREBP or ATF6 transcription factors, respectively (Brown et al., 2000). SPPL3 is an aspartyl protease that abrogates membrane association of glycosyltransferases/glycosidases to downregulate protein glycosylation in the cell. The function of RHBDL1 remains unknown, although Golgi-resident rhomboid proteases in other organisms frequently trigger signaling, including EGF ligand processing in Drosophila.

Four intramembrane proteases within the plasma membrane play decisive roles in cell signaling. Although >50 proteins are turned over by γ-secretase/presenilin 1, processing of two substrates dominates its role in the cell. Intramembrane cleavage of ligand-activated Notch receptors liberates their transcription-factor domains for signaling, while ~140 familial Alzheimer’s disease mutations in presenilin 1 alter processing of amyloid-β precursor protein (APP). Although the cell surface is its major site of action, only a minor fraction of γ-secretase resides there and likely within “lipid rafts” or protein super-assemblies. SPPL2b is expressed highly in the brain, where it processes type III neuroregulins and the British dementia protein Bri2. The physiological functions of RHBDL2 and RHBDL3 are uncertain, but processing substrates by the former, including B-type ephrins and thrombomodulin, may facilitate wound healing.

Endosome trafficking provides the predominant residence of γ-secretase/presenilin 2 and where APP cleavage generates an intracellular pool of amyloid β (Sannerud et al., 2016). Mutations in presenilin 1 cause some mislocalization to endosomes/lysosomes and elevate intracellular amyloid β with disease implications. RHBDL3 may also reside in or cycle through endosomes. SPPL2a is targeted to the lysosome, where it processes proteins, including TNFα and Fasl, that are involved in guiding the adaptive immune system. Although both SPPL2a and SPPL2b are able to cleave CD4 with indistinguishable efficiency, only lysosomal processing by SPPL2a is important for B-cell function, emphasizing the contribution of subcellular localization to biological outcome.

PARL is a rhomboid protease whose function reflects the health of mitochondria. In healthy mitochondria, PARL cleaves PINK1 constitutively. When mitochondrial health falters, PARL cleavage stops and accumulated PINK1 recruits/phosphorylates Parkin, leading to mitophagy. This surveillance is compromised in Parkinson’s disease. PARL can also cleave PGAMS in unhealthy mitochondria, which may lead to apoptosis.

Distinguishing Enzymatic Features

Three enzymatic properties shape the features of an intramembrane proteolysis event. Since additional substrates are expected even for the best-characterized intramembrane proteases, these characteristics can help pinpoint which enzyme is responsible for a newly discovered intramembrane cut.

The biological role of intramembrane proteolysis is largely to liberate an effector domain from the membrane (as shown with arrows on the image). Accordingly, the protease active site is positioned in the membrane to facilitate the direction of release. Rhomboid proteases and SPPL3 cleave close to the extracellular/lumenal side of the membrane and release domains to the outside of the cell. Conversely, S2P and most aspartyl intramembrane proteases liberate transcription factor domains and typically cleave close to the cytosolic face of the membrane.

Intramembrane proteases that release proteins into the cytosol require “priming”—cleavage by a different protease at a distinct site to remove a substrate ectodomain (lipidation) in an analogous “priming” requirement for Rce1). Conversely, SPPL3 and all rhomboid proteases cleave full-length, unmodified proteins.

Intramembrane proteases have only loose sequence requirements in substrates, although residues flanking the scissile bond are often restricted because they must fit precisely into the active site. Rhomboid proteases generally cleave only between small residues, whereas many other intramembrane proteases cleave between bulkier residues. More generally, substrate helices are unstable to facilitate dynamics/unwinding prior to cleavage. Polarity of the peptide bond in the active site, as imposed by membrane topology of the substrate, dictates the most rigid rule for proteolysis. For example, relative to γ-secretases, which process only type I proteins (N terminus out), SPPs are flipped in the membrane and cleave only type II proteins (N terminus cytosolic).

REFERENCES