Intramembrane proteolysis and post-targeting functions of signal peptides

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Abstract

Signal sequences are the addresses of proteins destined for secretion. In eukaryotic cells, they mediate targeting to the endoplasmic reticulum membrane and insertion into the translocon. Thereafter, signal sequences are cleaved from the pre-protein and liberated into the endoplasmic reticulum membrane. We have recently reported that some liberated signal peptides are further processed by the intramembrane-cleaving aspartic protease signal peptide peptidase. Cleavage in the membrane-spanning portion of the signal peptide promotes the release of signal peptide fragments from the lipid bilayer. Typical processes that include intramembrane proteolysis is the regulatory or signalling function of cleavage products. Likewise, signal peptide fragments liberated upon intramembrane cleavage may promote such post-targeting functions in the cell.

Introduction

Extensive studies during the past three decades have unravelled the prime function of signal sequences; that is, addressing proteins for the secretory pathway. Usually located at the N-terminal end of a precursor protein, they mediate the targeting to the ER (endoplasmic reticulum) membrane [1], which is the entry site into the secretory pathway in eukaryotes, and the insertion into the translocon for transport through the lipid bilayer [2]. Thereafter, signal sequences are cleaved off from the pre-protein by the signal peptidase [3], which cuts the membrane-spanning peptide in the ER lumen close to the exoplasmic surface of the lipid bilayer. The resulting signal peptide is liberated into the ER membrane, whereas the residual protein is further translocated into the ER lumen or integrated into the membrane.

To mediate protein targeting and induce translocation, signal sequences have a characteristic tripartite structure, but otherwise share no particular sequence identity [4]. Most critical is a central h-region of 7–15 hydrophobic residues that is required for targeting and insertion into the translocon, and a subsequent short stretch of 2–9 usually small and polar residues, the c-region, which comprises a rudimentary consensus motif for cleavage by signal peptidase. Besides the relatively high sequence variation in the h- and c-regions, the highest diversity is found in the N-terminal extension of signal sequences [5]. Not only does the amino acid sequence show great variation here, but also the length of the extension, which can be up to 200 residues long, varies greatly.

Many of the features of the N-terminal extensions are not necessarily important for the function of the respective signal sequence in protein targeting and translocation. This leads to the hypothesis that N-terminal extensions may carry features that are relevant for functions beyond protein targeting, e.g. after cleavage by signal peptidase and liberation of the signal peptide from the pre-protein [5]. Research in the past few years has indeed revealed that some signal peptides are required for processes beyond cleavage by signal peptidase, and has brought to light pathways by which signal peptides can fulfill such functions in the cell.

Signal peptide cleavage and processing

In eukaryotic cells, signal sequences mediate the targeting of proteins to the translocation machinery, the so-called translocon or protein-conducting channel, at the ER membrane [2]. Upon insertion into the translocon, a signal sequence adopts a type II transmembrane orientation, that is with the N-terminus in the cytosol and the C-terminal portion in the ER lumen (Figure 1A). When translocation continues, the signal sequence’s c-region appears in the ER lumen and becomes accessible to signal peptidase, which cleaves the signal sequence from the pre-protein. The latter is further translocated into the ER lumen, while the liberated signal peptide is usually not detected in assay systems, implying rapid degradation. In a cell-free in vitro system with ER membranes from canine pancreas, we reported several years ago that after cleavage by signal peptidase, the liberated signal peptide is transiently embedded in the lipid bilayer and undergoes further processing to be released from the membrane [6]. The underlying SPP (signal peptide peptidase) activity was not known at the time, but it was speculated that signal peptidase might also promote this processing step.

The mammalian SPC (signal peptidase complex) is hetero-oligomeric, being composed of five different membrane proteins of 12, 18, 21, 22/23 and 25 kDa [3]. Two of them,
Signal peptide cleavage and processing

(A) A newly synthesized protein containing an N-terminal signal sequence is targeted to the ER membrane and co-translationally translocated into the ER lumen. During this event, the signal sequence is cleaved off by signal peptidase. The free signal peptide, which spans the ER membrane with the hydrophobic region (barrel), is processed by SPP in the centre of the transmembrane region, and cleavage products are released towards the cytosol and ER lumen, respectively. (B) Predicted topology of SPP. The multi-spanning membrane protein contains motifs, YD and LGLGD, of an aspartic protease. They are located in the centre of adjacent transmembrane regions, consistent with the intramembrane cleaving activity. The protein is N-glycosylated twice close to the N-terminus, and contains an ER-retrieval signal, KXXX, at the C-terminus, which faces the cytosol. A third motif of unknown function, QPALLYhhP (where h represents a hydrophobic amino acid), is highly conserved in SPP and similar candidate intramembrane-cleaving proteases.

SPC18 and SPC21, are highly similar to the yeast Sec11, which is an essential component for signal peptide activity and cell viability in *Saccharomyces cerevisiae*. They contain serine, histidine and aspartic residues important for catalysis, and are thus considered proteolytic subunits with active-site domains located in the ER lumen. The function of the other three subunits is not yet clear. In yeast, the counterparts are not essential for signal peptidase activity. They may stabilize the complex, be involved in the retention of the complex in the ER, or be required for the interaction with the translocation machinery, as has been reported for SPC25. They may also be relevant for the fate of the liberated signal peptide.

SPP

Recently, we addressed the SPP activity in the mammalian ER. The corresponding protease was identified by a classical biochemical strategy using an affinity probe to label the candidate protease among the microsomal membrane proteins [7]. The labelled protein was then isolated and sequenced. To demonstrate the proteolytic activity of the discovered protein, we cloned the putative human SPP and reconstituted SPP activity in the yeast *S. cerevisiae*, which does not contain such an enzyme in its genome. Together with active-site mutations, the reconstitution provided strong evidence that the identified protein was the signal-peptide processing activity.

SPP comprises all the features of an intramembrane cleaving protease [8]. It is a multi-spanning membrane glycoprotein of ≈40 kDa, and contains 7–9 putative transmembrane regions and motifs of an aspartic protease (Figure 1B). The transmembrane regions may assemble the protease’s proteolytic domain in the plane of the lipid bilayer such that the active-site motifs YD and LGLGD, which are located in the centre of adjacent transmembrane regions, are positioned in the middle of the membrane. Such an arrangement would be consistent with the observed cleavage of substrate signal peptides in the centre of their membrane-spanning portion. SPP contains also a KXXX ER retrieval signal at the C-terminus, in agreement with its function in the ER membrane.

Are signal peptides candidates for functions beyond protein targeting?

The discovery of SPP and its role in signal peptide processing revealed that some signal peptide fragments are liberated from the ER membrane upon intramembrane proteolysis [8]. By analogy with other intramembrane proteolysis events, this finding supported the hypothesis that distinct signal peptide fragments may be required for processes beyond protein targeting and thus have post-targeting functions. Intramembrane proteolysis is considered to be part of a novel signalling paradigm widely applied in higher eukaryotes [8–10]. Quite a number of membrane-bound signalling molecules seem to be activated in regulated processes that culminate in their liberation upon cleavage in the membrane anchor. This basic principle was first exemplified for the activation of the dormant membrane-bound transcription factor SREBP (sterol regulatory element-binding protein) [11] and the signalling molecule NOTCH-1 [12]. Both factors are activated and released from cellular membranes by an intramembrane-cleaving protease. In the case of SREBP, this cut is catalysed by the metalloprotease S2P [13], whereas in the case of NOTCH-1, intramembrane cleavage is dependent on presenilin [14], an aspartic protease implicated in the cause of Alzheimer’s disease and with a limited relationship to SPP [7].

If the purpose of intramembrane proteolysis is to liberate bioactive proteins or peptides from membranes, could this also be the objective of the SPP-catalysed generation of signal peptide fragments? In other words, do the cleavage products generated by SPP have functions in the cell? Unfortunately, the physiological role of SPP is still a mystery. We do know...
Figure 2 | Generation of signal-peptide-derived HLA-E epitopes

(A) Signal sequences of polymorphic MHC class I molecules contain a highly conserved stretch of nine residues, the HLA-E epitope (white), at the interface between the n- and h-regions. (B) During biosynthesis, the signal sequence of an MHC class I molecule is first cleaved by signal peptidase, then processed by SPP, and the epitope-containing fragment is released towards the cytosol. The fragment is further trimmed at the N- and C-termini, and transported back into the ER lumen via the transporter associated with antigen presentation (TAP). In the ER lumen, the nine-residue epitope is loaded on to a non-polymorphic MHC class I molecule HLA-E, and the complex is eventually transported to the cell surface for presentation to natural killer cells.

Many signal peptides that are substrates for SPP [15]. Some of them appear to have potential properties to be 'bioactive', but it is hard to imagine that they are all involved in post-targeting functions. It appears that SPP is required to clear the ER membrane of signal peptides or initiate degradation of membrane-spanning peptides, rather than to produce and activate bioactive peptides. An argument against such a housekeeping role of SPP is the fact that the protease is found only in the genomes of animals and plants, but not in fungi or bacteria, which also produce many proteins with a signal sequence [7]. It is therefore possible that another, so far undiscovered, basic SPP-independent clearance pathway for signal peptides exists, and hence the prime function of SPP is another, e.g. liberating bioactive peptides.

Initial trials to assess the requirements for cleavage by SPP brought to light the fact that the protease does not randomly attack membrane proteins in the ER membrane [15]. The SPP substrates have to be generated first by a preceding cleavage by signal peptidase. Thus SPP acts in a two-step proteolytic process typical of proteases catalysing intramembrane proteolysis. The first cleavage event may be regulated or otherwise controlled. Cleavage by signal peptidase may be delayed or even regulated, as reported for the processing of the HIV-1 gp160 [16], and, in principle, control the induction of the generation of signal-peptide-derived bioactive peptides. Even if signal peptides are produced by signal peptidase, they are not necessarily processed by SPP.

Indeed, not all of the few signal peptides tested so far are processed by SPP [15]. A critical requirement for this cut is one or more helix-breaking or helix-bending residues in the membrane-spanning region, possibly to disclose a peptide bond for proteolysis. Also, residues flanking the membrane-spanning region affect processing, possibly by influencing the perpendicular flexibility of the signal peptide's potentially extended transmembrane helix. Although such requirements are rather ill defined as yet, they are indicative of a control in the action of SPP and are consistent with the typical function of intramembrane-cleaving proteases, that is releasing bioactive peptides from membranes.

**HLA-E (human lymphocyte antigen-E) epitopes and HCV (hepatitis C virus) core protein**

The first process to be discovered that depends on SPP and yields a product with a post-targeting function was the generation of HLA-E epitopes in humans [17]. These epitopes impose a key function in the human immune system. Here, biosynthesis of polymorphic MHC class I molecules, which present antigens at the surface of almost every nucleated cell, is of critical importance and therefore highly controlled. One mode of control comprises the HLA-E epitopes that are derived from the signal sequence of the polymorphic MHC class I molecules (Figure 2A) [18].
During biosynthesis of the latter, their signal sequence is cleaved first by signal peptidase, then by SPP, and the epitope-containing N-terminal cleavage product is released into the cytosol (Figure 2B) [17]. Upon further trimming and transport into the ER lumen, the nine-residue-long epitope is loaded on to the HLA-E molecule and transported to the cell surface for presentation to natural killer cells [19]. Interaction with inhibitory receptors on natural killer cells reports proper biosynthesis of polymorphic MHC class I molecules to the immune system. Conversely, when HLA-E epitope generation is disturbed and no or an insufficient amount of epitopes is presented, which can be the case in tumour cells or virus-infected cells, natural killer cells are not inhibited and attack the impaired cell.

The action of SPP is not restricted to typical N-terminal signal sequences. It is also involved in the processing of proteins directed to the ER with an internal signal sequence. This route is exemplified by the processing of the HCV core protein [20]. In HCV-infected cells, viral components are synthesized as a single ~3000-residue-long polyprotein. For the fragmentation of the most N-terminal subcomponents, the viral core and the membrane glycoproteins E1 and E2, the virus makes use of proteases already present in the host cell (Figure 3). During biosynthesis of the polyprotein, an internal signal sequence in between the core protein and E1 directs the growing polypeptide chain to the ER membrane. Maturation of the core protein, which, in principle, is an N-terminal ~170-residue-long extension of the signal sequence, involves cleavage by signal peptidase and subsequent processing by SPP. Processed core protein is then liberated from the ER membrane and is free for trafficking to lipid droplets in the cytosol [20].

**Perspectives**

Intramembrane-cleaving proteases catalyse peptide bond hydrolysis in the plane of cellular membranes and promote the liberation of bioactive peptides and proteins into the cytosol or exoplasmic space [8]. They are versatile key elements of cell regulation, signalling and protein processing [9,10]. SPP is one of these newly discovered proteases. It catalyses intramembrane cleavage of some free signal peptides and initiates their release from the ER membrane. Some of the cleavage products seem to be required for functions in the cell, as has been revealed for the signal peptide-derived HLA-E epitopes [17] and can be envisaged for signal peptide fragments that bind to calmodulin [21]. These may just be first examples of many more post-targeting functions awaiting discovery. The high degree of sequence and length variation of signal sequences, which has long been a puzzle, may indicate a high complexity and versatility of signal sequence function. We are just beginning to discover the full functional potential of signal sequences. Analysis of the fate of signal peptides and their fragments after cleavage and processing emerges as a new branch of the study of signal sequence functions. There are almost undoubtedly other components that interact with signal peptide fragments in the cytosol or the extracellular space that are yet to be discovered.

**References**


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