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Intramembrane cleaving proteases (I-CLiPs) as guardians of shuttling proteins

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The concept of proteases cleaving their substrates within the hydrophobic core of their transmembrane domains is very well established. In the past 15 years, various intramembrane cleaving proteases (I-CLiPs) and their respective substrates have been identified.[1] Besides the involvement in specific signaling pathways, for instance regulation of cholesterol metabolism or Notch signaling,[1] a proteasome-like function, removing needless transmembrane segments to prevent clogging of cellular membranes, was attributed to some of those I-CLiPs.[2] Excluding the rhomboid family, a family of serine I-CLiPs, and signal peptide peptidase-like 3 (SPPL3), a member of the aspartyl I-CLiP family, I-CLiPs only accept substrates with short ectodomains.[3] Consequently, intramembrane proteolysis is often regulated by an independent proteolytic cleavage resulting in the truncation of the actual I-CLiP substrate. This 2-step proteolytic cascade is termed regulated intramembrane proteolysis (RIP).[4]

In this issue, Castelli et al.[5] assign an additional biological function to RIP, i.e., controlling the release of a hepatocyte odd protein shuttling (HOPS) isoform from the cellular membrane to allow shuttling between cytosol and nucleus. Using different cell-based model systems and mouse tissues, the authors provide evidence that HOPS exists in 3 isoforms, differing in their molecular weight. The N terminus of the long isoform (lHOPS) comprises an N-terminal hydrophobic signal sequence. In contrast, the short isoform (sHOPS) lacks 54 amino acids at the N terminus, since its translation is initiated at an alternative starting methionine. However, both isoforms are membrane-tethered, since they comprise 2 transmembrane domains within their C termini. Using GFP-tagged reporter constructs of HOPS the authors demonstrate that a presently unknown protease has the capability to release both isoforms from the cellular membrane, allowing their translocation into the cytosol.[5] Yet, only one additional isoform depicting an intermediate molecular weight (iHOPS) was readily detected in cellular lysates and mouse tissue. iHOPS contains neither the N-terminal signal sequence nor any of the 2 C-terminal transmembrane domains. Thus, 2 independent proteolytic cleavages are required to convert lHOPS into the soluble iHOPS. Interestingly, lHOPS and iHOPS are able to bind Nucleophosmin (NPM) in co-immunoprecipitation assays, while sHOPS fails to do so.[5]

The findings reported by Castelli et al.[5] once more corroborate the concept of proteases being the accomplice for their substrates to fulfil various different biological functions. While the membrane-bound isoforms of HOPS probably act in a ubiquitin-like fashion, the soluble iHOPS is able to translocate between cytosol and nucleus, most likely in a complex with NPM, an ubiquitously expressed shuttling protein, that is involved in several cellular functions, for instance ribosome biogenesis and chromatin remodeling, but also in the pathophysiology of acute myeloid leukemia (AML).[6] To exploit this concept therapeutically in the future, it is crucial to identify the particular protease(s) involved in the release of the substrate from the cellular membrane. Although in the case of HOPS it is likely that the C-terminal cleavage occurs within or close to a transmembrane domain, it is not yet clear whether the release, and thus the regulation of HOPS function, is indeed catalyzed by an ICLiP. Moreover, it needs to be clarified whether the N-terminal signal sequence, absent in sHOPS, reflects a classical ER signal sequence. Such signal sequences usually direct the insertion of proteins into the ER-membrane and are released by signal peptidase (SP), an ER-localized enzyme complex with catalytic activity on the ER luminal side.[7] Following this SP cleavage, individual signal peptides are further processed by signal peptide peptidase (SPP), an aspartyl ICLiP.[3] Since the release of those signal peptides by SP occurs co-translationally in the ER, they are usually not detectable in the mature protein. If the N-terminal hydrophobic signal sequence is detectable within endogenous lHOPS, it may function as a regulatory signal for the subsequent C-terminal cleavage and would explain why a soluble HOPS isoform derived from sHOPS is not detectable. This, however, postulates that the release of the N-terminal hydrophobic domain most likely is catalyzed by a protease different from SPP, which, based on current knowledge, exclusively localizes to the ER and depends on a preceding cleavage of SP. To finally identify the respective proteases responsible for the release of HOPS, inhibitor studies as well as the determination of the cleavage sites within HOPS will be required. This will certainly deepen our understanding of how proteolytic cleavage at and close to transmembrane domains controls fundamentally different cellular functions of one protein—an undoubtedly very important aspect in cell biology.

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