Regulated Intramembrane Proteolysis: Signaling Pathways and Biological Functions

Intramembrane cleavage of transmembrane proteins is a fundamental cellular process. Several enzymes capable of releasing domains of integral membrane proteins have been described. Transmembrane protein proteolytic cleavage is regulated and involved not only in degrading membrane spanning segments but also in generating messengers that elicit biological responses. This review examines the role of the released functional protein domain in signaling mechanisms regulating an array of cellular and physiological processes.

Cells are dynamic functional units that maintain an intact plasma membrane to ensure proper cellular homeostasis. Within cells, the membrane-bound organelles represent sets of compartments isolated from the surrounding environment that are capable of autonomous functionality. Although these lipid barriers serve to isolate their enclosed volumes from the surrounding aqueous milieu, they are also engineered to facilitate the selective communication and transmission of information between compartments. Receptor-dependent cell signaling, i.e., the transfer and propagation of external cues across the lipid bilayer by signaling-competent proteins embedded in the lipid bilayer, occurs by a transduction mechanism that utilizes specific messenger molecules that act as conduits to relay information to defined subcellular destinations. A relatively recently identified cell signaling strategy employed by an ever increasing number of recognized membrane proteins occurs via a process known as regulated intramembrane proteolysis (RIP), a highly regulated proteolytic processing event in which an integral membrane protein is ultimately cleaved within its transmembrane domain to yield a soluble protein fragment. This liberated protein domain (LPD) is often a very labile moiety and for many instances of RIP is designed to act as a signaling messenger at discrete locations both within and beyond the parent cell (7). Here, we summarize the prominent features and mechanisms regulating such intramembrane cleavages and provide examples supporting the central involvement of the LPD as a signaling messenger controlling numerous biological processes including apoptosis, cell differentiation and proliferation, and neurogenesis. We have limited our review to RIP processes relevant to animal physiology, but this signaling phenomena is shared across all kingdoms of life, down to unicellular organisms (for a review, see Ref. 61).

Regulated Intramembrane Proteolysis: Properties and Classification

Proteases are a group of catalytic enzymes whose ability to carry out the hydrolytic cleavage of peptide bonds is often tied to the inactivation of their protein substrates. Importantly, however, proteolysis cannot only degrade functional signaling components, but can also elicit the release and activation of dormant signaling molecules (FIGURE 1). With the discovery of RIP, a new perspective on protease action has emerged, and the principle that all proteases are water-soluble enzymes has had to undergo a radical change. The family of proteases that catalyze RIP have been dubbed iCLiPs (intramembrane cleaving proteases) (68), and they are currently represented by three distinct and evolutionarily conserved iCLiP families: the aspartyl protease-like (including presenilin-dependent H9253-secretase, signal peptide peptidase, and signal peptide peptidase-like), the zinc metalloproteinase site-2 protease, and the serine protease family of rhomboids (rhomboid and PARL). These iCLiPs are all polytopic integral membrane proteins that harbor their predicted catalytic residues within the hydrophobic environment of the membrane. Accordingly, catalysis of the peptide bond of a substrate protein by an iCLiP is understood to occur within the plane of the membrane (30).

Insight into the mechanistic details by which iCLiPs are able to functionally cleave their substrate protein within a transmembrane region has been elegantly revealed by the successful crystallization of GlpG, a rhomboid protease isolated from the bacterium Escherichia coli (4, 64, 69). According
to the atomic structure, the rhomboid catalytic site comprised of a His-Ser dyad actually occurs buried ~10 Å below the predicted interface between the aqueous environment and external leaflet of the lipid bilayer in a water-filled cavity formed by the surrounding six transmembrane helices. With respect to the structural and molecular determinants controlling substrate accessibility to the catalytic site, the data suggest that substrate enters between TM2 and TM5 and that TM5 may act as a gating helix to modulate substrate accessibility to the catalytic site (2, 30). Based on similarities in the topological construction of rhomboid and the other iCLiPs, it is not unlikely that the RIP strategy utilized by GlpG for cleaving proteins within their transmembrane domains represents a more general principle shared among the different iCLiP family members.

An important consequence of iCLiP action common to most of the bona fide examples of RIP is the controlled generation of novel protein fragments that reveal their own latent signaling properties when released from the parental transmembrane protein. Collectively, the RIP processes described to date share a number of common features that may be used to categorize the group as a whole, although not each property has been definitively illustrated for all putative RIP events (Table 1). For many of the iCLiPs (except rhomboids), RIP requires the initial priming of the substrate protein by ectodomain shedding, a cleavage event that occurs at a scissile peptide bond located close to the transmembrane interface (FIGURE 1). This ectodomain shedding is carried out by proteases of the disintegrin and metallopeptase (ADAM) family, matrix metalloproteases, and the aspartyl proteases β-site APP-cleaving enzymes (BACE) and results in the release of a peptide that often possesses its own biological properties. Additional RIP features include the coupled translocation and spatial segregation of the substrate with iCLiP, generation of a liberated protein domain that possesses an essential cell signaling capacity, as well as ligand-dependent control of RIP through physiologically relevant stimuli. For many of the described putative RIP examples, definitive evidence of the molecular, cellular, and physiological sequelae are not complete (Table 2). The ensuing discussion provides a synopsis of the most recent advances in the field that support the emerging role of RIP and LPD-dependent cell signaling in the control of a wide variety of biological functions. Notably, the LPDs of the various substrates processed by RIP may signal either within the nucleus, the cytosol, or the extracellular space, depending on the inherent characteristic properties of each of the cleaved functional proteins (FIGURE 2).

### Table 1. RIP-common principles

<table>
<thead>
<tr>
<th>RIP-Common Principles</th>
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<tr>
<td>Substrate is a transmembrane protein</td>
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<tr>
<td>Proteolysis is mediated by an iCLiP</td>
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<td>Hydrolysis occurs within/close to the membrane</td>
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<td>Regulation by biological stimuli</td>
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<td>LPD possesses a signaling function</td>
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<td>RIP results in a defined biological response</td>
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Table 2. List of selected RIP-mediated LPD signaling events

<table>
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<th>RIP Stimulus</th>
<th>LPD Signaling Function</th>
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<td>CD74</td>
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<td>?</td>
<td>Nuclear signaling via NF-kB activation</td>
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<td>Mechano sensing</td>
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<td></td>
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<tr>
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<td>10</td>
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<tr>
<td>?</td>
<td>Polycystin-1</td>
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<td>Nuclear signaling, JNK, Wnt, STAT6 signaling</td>
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</tbody>
</table>

APP, Alzheimer precursor protein; bFGF, basic fibroblast growth factor; CSF-1, colony-stimulating factor; EGF, epidermal growth factor; iCLiP, intramembrane-cleaving protease; HCV, hepatitis C virus; HLA-E, human lymphocyte antigen E; IFNaR2, type I interferon a receptor 2; II-1R2; interleukin-1 receptor II; LPD, liberated protein domain; LRP, low-density lipoprotein-related protein; MAG, myelin-associated glycoprotein; MHC, major histocompatibility complex; NTR, neurotrophin receptor; PARL, presenilin-associated rhomboid-like; PMA, phorbol 12-myristate 13-acetate; RPTP, receptor protein tyrosine phosphatase; S2P, site-2 protease; SPP, signal-peptide peptidase; SPPL, SPP-like; SREBP, sterol regulatory element-binding protein; STAT, signal transducers and activators of transcription; TLR, Toll-like receptors.
RIP-Dependent Signaling: Functional Roles of the Liberated Protein Domain

SREBP RIP

The first RIP-dependent signaling mechanism to be described defined the proteolytic processing of the sterol regulatory element binding proteins (SREBPs) and their involvement in the proper homeostatic control of cellular cholesterol levels (6). Many excellent reviews of SREBP RIP are available, but a brief description is warranted here to bring into focus several of the characteristic features that are often considered requisite for the establishment of other proteolytic events as bona fide RIP processes. SREBP is a transmembrane protein that spans the membrane twice, with its NH₂ and COOH termini facing the cytosol. When cells are replete with sterols and lipids, SREBP resides in the ER membrane. In cells subjected to sterol deprivation, however, SREBP translocates to the Golgi apparatus where it becomes a substrate for sequential cleavages by the site-1 and site-2 proteases (S1P and S2P, respectively) (70). S1P acts first and catalyzes the luminal cleavage of SREBP to yield a single transmembrane segment that has been primed for further processing. This new substrate, with a much shortened luminal peptide domain, is then susceptible to cleavage by S2P just inside the cytosolic face of the protein, within the transmembrane domain, that results in the release of a soluble NH₂-terminal intracellular fragment to the cytosol. This cleavage product, which harbors a nuclear localization signal (NLS), a DNA binding domain, as well as a transcriptional activation domain, then traffics to the nucleus, where it reveals its latent propensity to initiate a genetic program controlling cholesterol production. The sequential proteolytic processing of SREBP and release of its intracellular domain (ICD) is a paragon of RIP signaling and provides the template on which to evaluate more recently reported cases of intramembrane proteolytic events as either cell-specific, biologically relevant signaling mechanisms or as mechanisms designed for the removal and degradation of transmembrane protein segments.

CREBH RIP

CREBH and its structurally similar homologs (ATF6, Luman/CREB3, CREB4, and OASIS) are members of the type 2 transmembrane protein class of endoplasmic reticulum (ER) stress response transducers whose cytosol-facing NH₂ termini possess a latent transcription activation
domain and a basic leucine zipper region (24, 32, 50, 72). ATF6, the founding member of the group, resides in the ER membrane under normal conditions, but on ER stress-specific accumulation of unfolded or misfolded proteins, traffics to the Golgi compartment where it is proteolytically processed by the sequential action of S1P and S2P (70). ATF6 processing therefore occurs in a manner analogous to that of SREBP, wherein RIP of the substrate protein is dependent on the regulated trafficking of the substrate to the site of enzyme action in the Golgi.

RIP-dependent processing of CREBH represents an example for which the relevant stimulus, mechanism of cleavage, and biological role of the released peptide fragment has been thoroughly examined and verified, both in vitro and in vivo. In a recent study, Zhang et al. show that ER stress also induces the cleavage and release of the NH\textsubscript{2}-terminal domain of CREBH (72). This intracellular domain localizes to the nucleus where it modulates the transcriptional induction of not only ER stress response genes but also those coding for the acute phase response (APR). Deficiency of either S1P or S2P prevented ER stress from inducing the accumulation of the nuclear NH\textsubscript{2}-terminal cleavage product. The function of the released ICD was subsequently confirmed using a CREBH-knockdown mouse generated using CREBH-specific siRNA silencing in single-cell mouse embryos. Microarray analysis of the genes affected by CREBH-knockdown revealed reduced expression of a select group of genes involved in the APR. Both proinflammatory cytokine-dependent as well as ER-stress-mediated regulation of APR genes were abrogated. Most notable in the animal model was the ability to detect the cleaved form of CREBH in the livers of wild-type mice but its absence in CREBH-knockdown mice on APR activation with proinflammatory cytokines and LPS. Not only were Zhang et al. able to show that the nuclear form of CREBH could significantly activate gene expression utilizing the SAP and CRP promoters but also that the RIP- liberated nuclear fragments of CREBH and ATF6 physically interact with one another and cooperate synergistically to activate SAR and CRP gene expression by binding to a conserved promoter element in the specific APR genes. This observation provides the most direct evidence for a role of ATF6 in the physiological response to ER stress and indicates that ATF6 can act as a potent co-activator of the APR. Thorough investigation of the RIP of other ATF6 family members and how they are involved in the ER stress response will undoubtedly add further complexity to this cellular activity.

Notch RIP

Another one of the founding members and most extensively studied pathways of RIP-dependent nuclear signaling involves activation of the Notch receptor. Notch is a type I cell surface membrane spanning receptor that determines cell fate decisions and plays a fundamental role during development. The canonical Notch pathway involves the activation of a signaling cascade that leads to transcriptional regulation of CSL-dependent transcription factors. Regulation of the intramembrane cleavage of Notch begins at the cell surface where ligand (delta, jagged, serrate) binding to Notch elicits a conformational change that facilitates the proteolytic shedding of the Notch ectodomain by a member of the disintegrin and metalloproteinase (ADAM) family. The action of this sheddase results in the priming of Notch for subsequent proteolytic attack by the aspartyl protease, presenilin-dependent \(\gamma\)-secretase complex (11). This iCLiP cleaves Notch in its transmembrane domain and results in the liberation of the Notch COOH-terminal tail into the cytoplasm. The Notch-ICD then translocates to the nucleus where it acts, not directly as a transcription factor, but rather as a transcriptional regulator by virtue of its propensity to activate members of the CSL family of transcription factors.

Amyloid-\(\beta\) precursor protein RIP

The detailed knowledge concerning the regulation of Notch receptor signaling was gleaned largely through initial efforts to understand the biological function of presenilin-1, the catalytic component of the \(\gamma\)-secretase complex whose enzymatic activity causes the release of amyloid \(\beta\)-peptide from membrane-bound amyloid-\(\beta\) precursor protein (APP). Accumulation of extracellular amyloid \(\beta\)-peptide is considered the central cause of neuron degeneration in Alzheimer’s disease (12). Although \(\gamma\)-secretase action has been known for some time to generate, in addition to the amyloid \(\beta\)-peptide product, an amyloid intracellular domain (AICD), a relevant biological regulator of this APP processing event as well as a definitive functional role of the AICD has remained largely enigmatic. A report from Ma et al., however, now provides evidence that AICD formation is indeed a regulated process (36). Their studies of APP processing indicate that, in the neural stem cell niche of the fetal ventricular zone of the developing mouse, AICD production is triggered by the ligand-dependent binding of TAG1 to APP. Moreover, and very importantly, this research group was able to demonstrate that aberrant neurogenesis observed in TAG-1-deficient mice could be reversed solely by exogenous AICD complementation. This study has thereby provided two significant contributions to the classification.
of γ-secretase-dependent APP processing as a legitimate example of RIP, namely, a ligand-mediated mechanism of substrate processing and a biological functionality to the soluble ICD.

Questions remain, however, regarding the nuclear function of the AICD and its complement of target genes. A role for the AICD in gene transcription was first demonstrated by the apparent transactivation potential of the AICD as revealed by its binding to the Fe65 scaffolding protein using gene reporter assays based on a chimeric APP-DNA binding domain fusion protein (8). More recently, however, studies indicate that Fe65 may have a more general role in controlling gene transcription activation and that the AICD is in fact not unique in its ability to stimulate Fe65-dependent promoter activity (18). A number of genes previously identified as downstream targets of the AICD (as revealed using in vitro expression systems) could not be recapitulated in either relevant cell lines treated with γ-secretase inhibitors or in biological models genetically deficient for AICD generation. Such findings suggest that the γ-secretase-dependent cleavage of APP and consequent generation of the AICD may not be immediately involved in the apparent control of a nuclear signaling pathway. It remains possible that intramembrane proteolysis of APP may also be a mechanism designed for the degradation of the transmembrane segment of the native protein.

**EpCAM RIP**

One of the most recent additions to the family of RIP-specific signaling events involves EpCAM, a transmembrane glycoprotein that frequently shows elevated expression levels in various cancers (37). Dual sequential cleavage of EpCAM by ADAM17 and γ-secretase results in the production of a soluble ectodomain fragment as well as a short ICD fragment comprised of a predicted 26 amino acids. Formation of the EpCAM ICD can be ligand stimulated by the soluble EpCAM ectodomain in a manner that likely involves homotypic interactions with the unprocessed transmembrane protein. Credence to the unique biological significance and requirement of EpCAM processing and EpCAM ICD liberation in cell signaling is provided by a number of convincing observations, among them, the requirement of the isolated EpCAM ICD to stimulate cell proliferation in vitro and the propensity of EpCAM ICD expressing cells to cause tumor formation in xenografted mice (37). The oncogenic potential of the EpCAM ICD, which appears to largely recapitulate the oncogenic nature of EpCAM itself, may be related to its molecular association with β-catenin, Lef-1, and FHL2 in a nuclear signaling complex (37).

**ErbB4 RIP**

Activation of the receptor tyrosine kinase ErbB4, a member of the EGF receptor family, is classically viewed in terms of its canonical signaling pathway involving receptor dimerization, tyrosine kinase activation, autophosphorylation, adapter protein recruitment, and subsequent signal cascade activation. In addition to this established mechanism of action, it is now clear that ErbB4 also undergoes ligand-activated RIP, wherein the released ErbB4 receptor fragment acts to repress transcription of genes involved in astrocyte differentiation (FIGURE 2) (55). ErbB4 was previously shown to undergo regulated ectodomain shedding via neuregulin-mediated activation of ADAM17 (53) with the remaining membrane-tethered ErbB4 fragment being an available substrate for a γ-secretase-mediated cleavage in the transmembrane domain that results in the release of the cytosolic domain of ErbB4 (E4ICD) (47). Although this fragment possesses an NLS and was shown to traffic to the nucleus (as similarly observed for Notch processing), a definitive role for this fragment in nuclear signaling was not confirmed since the E4ICD displayed little transcriptional activity when measured in a Gal4-dependent transcriptional activation assay. Whereas the cleavage of ErbB4 and release of E4ICD was shown to regulate the induction of neuregulin-mediated cell death and maturation of oligodendrocytes, the extent to which the soluble E4ICD, per se, contributed to this biological response was not clear. A subsequent study published by Sardi et al. (55) has now conclusively demonstrated that the presenilin-dependent cleavage of ErbB4 results in the formation of an E4ICD ternary complex together with TAB2 and N-CoR that undergoes nuclear translocation and acts to repress transcription of several genes required for neuronal precursor cell differentiation into astrocytes. Based on studies employing transgenic mice null for ErbB4 (other than in the heart), these authors were able to demonstrate by in utero electroporation that the phenotypic increase in astrocytic markers observed in mice deficient in ErbB4 could be rescued by the reintroduction of E4ICD.

Notably, ErbB4 is not the only member of the receptor tyrosine kinase family that signals via a γ-secretase-mediated cleavage event. Ryk, a receptor for Wnt ligands necessary for neuronal differentiation, also undergoes dual proteolytic cleavage processing. In the case of mouse cortical neurogenesis, it is the intracellular cleavage product Ryk ICD that functions as the signaling molecule that transduces Wnt signals from the extracellular compartment to the nucleus and that thereby promotes neuronal differentiation (35). Although the
protease responsible for ectodomain cleavage of Ryk remains unknown, formation of Ryk ICD appears to be constitutive, with its nuclear accumulation dependent on the presence of Wnt ligand.

As with many substrates of the \(\gamma\)-secretase complex, it is often difficult to assign a conclusive functional role to the ICD that is completely independent of the effects of substrate processing itself. A plethora of additional \(\gamma\)-secretase substrates have been reported (see Table 2), but whether they all represent bona fide RIP members as defined by the archetypical SREBP and Notch pathways of proteolytic processing still awaits further testing. It is nonetheless of interest to consider a few putative examples for which a signaling function of the ICD has been described.

For other \(\gamma\)-secretase substrates, such as the closely related SorLA/LR11 (5), low-density lipoprotein-related protein (LRP) members (33, 42, 44), and megalin (73), the study of RIP nuclear signaling is still in its infancy. Although \(\gamma\)-secretase-dependent cleavage of these substrates occurs in what appears to be a manner very similar to that described for Notch and APP, the triggers and biologically relevant signaling-dependent effects remain largely unknown. Much of the evidence for RIP of these proteins has not been tested in animal models, and the consequences of the liberation of their respective ICDs for cellular function are not completely known. With respect to SorLA, the ICD could be detected in the nucleus of cells heterologously overexpressing SorLA-ICD, but the transcriptional potential appears to be weak (5). LRP1B has recently been shown to undergo intramembrane proteolysis in a manner that give rises to an LRP1B-ICD that accumulates in the nucleus and that alone possesses the capacity to suppress tumor cell anchorage-independent growth (33). Megalin, a scavenger receptor with a central role in renal protein absorption, colocalizes with and is processed by presenilin-1/\(\gamma\)-secretase in the brush border of the kidney proximal tubules (73). Although the detection and function of a megalin-ICD remains ambiguous, ectodomain shedding appears to be regulated in a ligand-dependent manner, and megalin processing may be involved in the regulation of specific gene expression (31).

**N-cadherin and E-cadherin RIP**

The cadherins belong to a major family of intercellular adhesion molecules that mediate cell-cell adhesion via calcium-dependent homophilic interaction of their ectodomains. These type I membrane proteins are also implicated in a wide range of intracellular signaling processes via the interaction of their cytoplasmic domains with a-catenin and \(\beta\)-catenin complexes. Studies indicate that ADAM10, a zinc-dependent metalloproteinase, is able to cleave the ectodomain of both N-cadherin and E-cadherin molecules (40, 52). In the case of E-cadherin, a requirement for ADAM10 proteolytic activity for proper cell adhesion, migration, and proliferation of epithelial cells has been demonstrated (40). Interestingly, however, it has also been noted that the membrane-tethered fragment of E-cadherin can also be further processed by \(\gamma\)-secretase to yield a soluble COOH-terminal fragment (CTF2) of 33 kDa (38). The putative function of this ICD remains to be investigated in detail, and it is currently unclear what portion of the effects of ADAM10 processing of E-cadherin can be solely accredited to ectodomain shedding and which may be contributed to by subsequent \(\gamma\)-secretase-mediated liberation of the cytosolic domain. It has been speculated that the CTF2 of E-cadherin, which does not accumulate in the nucleus, may have a signaling role in the cell as a result of its ability to alter the quantity of \(\beta\)-catenin accessible for nuclear signaling.

In the case of N-cadherin, \(\gamma\)-secretase-dependent proteolytic processing of N-cadherin in the transmembrane domain (most likely following ADAM10-mediated ectodomain shedding) produces an N-cadherin CTF2 for which a biological function has been documented (FIGURE 2) (39, 60). Production of CTF2, which is under agonist-induced activation by NMDA, binds to the CBP transcription factor and promotes its proteasomal degradation (39). The \(\gamma\)-secretase-dependent cleavage product of N-cadherin, therefore, acts as a potent repressor of CRE-dependent transactivation by virtue of its ability to bind and deplete cytosolic CBP.

**P75 RIP**

The p75 neurotrophin receptor, a member of the NGF/TNF receptor superfamily, regulates neurotrophin signaling and neuronal survival and differentiation by serving as a co-receptor in heteromeric association with Trk receptor tyrosine kinases. p75 undergoes shedding of its ectodomain, and, more recently, it has been shown that this ectodomain shedding is a priming event necessary to bring about a further \(\gamma\)-secretase-mediated proteolytic cleavage to yield a p75-ICD of 25 kDa (20). Although this study was unable to assign any specific function to this fragment, it was demonstrated that intramembrane proteolytic cleavage of p75 resulted in a loss of neurotrophin-dependent signaling by preventing p75 from forming a functional signaling complex with Trk. Thus intramembrane proteolysis of p75 may regulate cell signaling by removal of the receptor transmembrane domain rather than through formation of a soluble p75-ICD.
Additional examination of the processing of p75 has confirmed the sequential cleavage of p75 by α- and γ-secretase activity and, more importantly, has provided both an activating ligand as well as a signaling role for the p75-ICD (13). Both α- and γ-secretase-dependent cleavage products could be detected endogenously in primary cerebellar neurons treated with the myelin-associated glycoprotein (MAG) (13). Functionally, the dual cleavage of p75 was required for the ability of MAG to inhibit neurite outgrowth, and heterologous expression of a p75-ICD fragment was on its own sufficient to elicit the same response. At least part of the upstream signaling cascade responsible for the functional effects of p75 intramembrane proteolysis and p75-ICD formation involves PKC-dependent activation of the small GTPase Rho.

**EphrinB RIP**

The concerted catalytic activity of a matrix metalloproteinase and γ-secretase has been elegantly shown to mediate reverse signaling in EphrinB expressing cells following binding of EphrinB ligands to EphrinB receptors (EphB) (16). The capacity for EphB to stimulate EphrinB2 RIP and EphrinB2-ICD formation controls a signaling cascade responsible for regulating the sprouting of endothelial cells. More specifically, the EphrinB2-ICD was demonstrated to physically interact with tyrosine kinase Src in such a manner that it displaces the COOH-terminal Src inhibitory kinase and thus allows Src autophosphorylation and catalytic activity. Two downstream signaling effects of Src activation are noteworthy. One is that Src is able to phosphorylate EphrinB2 and inhibit its processing by γ-secretase, thereby establishing a potential negative feedback mechanism for control of signal transduction. Second, the recruitment of SH2 domain containing adaptor proteins such as Grb4 to EphrinB2 requires γ-secretase activity.

**OPA1 RIP**

The rhomboid family of serine-protease iCLiPs is responsible for activation and regulation of the EGF signaling pathway in *Drosophila* by virtue of its intramembrane proteolytic processing of type I transmembrane protein EGF receptor ligands (FIGURE 2) (28, 62). A new member of the rhomboid substrate family, Star, a type II transmembrane protein that also functions in the EGF signaling cascade, has recently been identified (59). Despite the evolutionary conservation of rhomboids across all kingdoms of life, the signaling role of rhomboids in mammals is still unclear. Work from the laboratory of De Strooper and Scoranno recently defined a role for the mitochondrial rhomboid, PARL, in the control of apoptosis in a manner that may be dependent on PARL-mediated RIP of OPA1, a dynamin-related protein that localizes to the inner membrane of mitochondria. PARL appears to have an anti-apoptotic function, since it protects cells against activation of the intrinsic mitochondrial pathway of apoptosis by controlling cytochrome C release (10). This protective action of PARL may be dependent on its ability to generate a short form of OPA1 in the inner membrane space region of mitochondria (IMA-OPA1). Generation of the IMA-OPA1 is necessary to form functional hetero-oligomers with integral OPA1 to regulate the structural integrity of mitochondrial cristae and to control cytochrome C release as well as apoptosis. Although PARL is an iCLiP and a central role of its action appears to be anti-apoptotic, what factors control PARL activity is not clear.

As with other rhomboid-mediated RIP events, ectodomain shedding may not be required.

**FIGURE 3. Regulated intramembrane proteolysis of polycystin-1 regulates intracellular signaling**

Polycystin-1, an integral membrane protein localized to the lipid membrane of the plasmalemma, endoplasmic reticulum, and primary cilia, is subject to at least two proteolytic cleavages that take place in or near its COOH-terminal tail and that result in the release of protein fragments of ~14 kDa and ~34 kDa. Formation of the ~34-kDa fragment is consistent with proteolysis occurring within the last transmembrane spanning domain of polycystin-1. Both the ~14-kDa and the ~34-kDa fragments localize to the nuclear compartment where they bind signaling proteins and regulate gene transcription. The ~34-kDa fragment enters the nucleus by virtue of a nuclear localization signal embedded in its primary amino acid sequence. Polycystin-1 also undergoes proteolytic processing at its NH2-terminal GPS. Mechanosensation by and ligand binding to polycystin-1 may constitute possible regulatory stimuli for polycystin-1 intramembrane proteolysis. RIP of polycystin-1 and release of the COOH-terminal tail may also lead to inactivation of intracellular signaling events normally regulated by the COOH-terminal tail of full-length polycystin-1.
**TNF-α RIP**

Several homologs of the SPP family of iCLiPs have recently been identified, and accumulating evidence now indicates that SPP-like (SPPL) enzymes are also iCLiPs that catalyze intramembrane proteolysis. It has been known for some time that ectodomain shedding of TNF-α (to release the soluble and biologically active TNF-α receptor ligand) also gives rise to a residual membrane-anchored TNF-α-NH₂-terminal fragment (NTF). It has now been shown that the TNF-α-NTF is a substrate for additional cleavage by SPPL2a and SPPL2b enzymes localized to the plasma membrane and ER and that proteolysis results in the release of a detectable TNF-α-ICD (14–15). The cleavage sites within the TNF-α transmembrane domain have been determined and indicate a multiple cleavage pattern similar to that commonly observed for γ-secretase-mediated substrate cleavages (14). To evaluate the cellular function of SPPL2a- and SPPL2b-mediated TNF-α processing, Friedmann et al. studied the production of the proinflammatory cytokine IL-12 in LPS-activated dendritic cells using a series of pharmacological and genetic strategies (15). They found that both SPPL2a and SPPL2b are required for IL-12 expression in mature dendritic cells and that expression of the TNF-α-ICD could recapitulate the same biological response as the SPPL2a- and SPPL2b-mediated processing of TNF-α. The biological activity of the TNF-α-ICD was independent of the formation and action of soluble TNF-α and thus suggests a dedicated physiological role for the membrane-anchored TNF-α-NTF. The molecular mechanism by which the TNF-α-ICD mediates IL-12 production remains to be examined.

**Polycystin-1 RIP**

Work from our laboratory as well as others has led to the suggestion that polycystin-1, the protein involved in the majority of all cases of autosomal dominant polycystic kidney disease (ADPKD), may also undergo RIP (9, 34) (FIGURE 3). Two distinct intracellular cleavage products have been identified, one of ~34 kDa and one of ~14 kDa. A fragment of the polycystin-1 COOH-terminal tail has been found localized to the nucleus in cells heterologously expressing polycystin-1 as well as in cyst lining epithelial cells derived from both animal and human subjects with ADPKD. It has been demonstrated that the larger, soluble polycystin-1 ICD, formed by what is predicted to be an intramembrane cleavage event occurring within the last transmembrane domain of polycystin-1, inhibits the canonical Wnt signaling pathway by virtue of an autonomous nuclear localization signal that also serves as a molecular motif that facilitates direct or indirect binding to β-catenin (26). The smaller COOH-terminal fragment of the polycystin-1 tail interacts with STAT6 and P100 and undergoes nuclear translocation as a consequence of these interactions (34). It is worth noting that heterologous expression of a membrane-tethered polycystin-1 cytosolic tail fused to a transmembrane anchor (which is unlikely to undergo RIP) in various mammalian cell lines demonstrates numerous intracellular signal transduction properties (for a review, see Ref. 48). It seems likely, therefore, that intramembrane cleavage of polycystin-1 may function as a switch that determines the repertoire of intracellular signaling cascades that can be modulated through the influence of full-length polycystin-1.

It is also of note that polycystin-1 undergoes ectodomain shedding (as similarly observed for many other iCLiP substrates) (49) and that the inability to proteolytically cleave the ectodomain of polycystin-1 is sufficient to cause cystogenesis (71). Whether this cleavage event is a prerequisite for the liberation of the ICD remains unclear. Additional studies are required to establish whether there is a true functional link between the two cleavage events as well as to determine the specific iCLiP involved in releasing the ICD.

**Summary**

Regulated intramembrane proteolysis of integral membrane proteins represents a fascinating strategy for cellular signaling that, as outlined above, is required for such diverse biological processes as apoptosis, inflammation, lipid metabolism, and neurogenesis. To be classified unambiguously as RIP, a cleavage event requires, among other criteria, conclusive evidence of regulated cleavage within or close to the transmembrane domain by a dedicated iCLiP. Additionally, identification of a signaling role for the nascent cleavage products is also an important aspect in defining the biological outcome of RIP. The lability of the intracellular protein fragments generated in many cases of RIP can make it difficult to detect them in vivo, and this has hampered efforts to assess the putative biological relevance of the cleaved peptide fragments. New experimental models and strategies have nonetheless led to the identification of a growing number of RIP substrates as well as to the signaling roles of the liberated protein domains in many cases. Developments in this field of research over the coming years should doubtless reveal novel RIP-dependent mechanisms of signal transduction that should continue to expand the repertoire of this intriguing posttranslational modification.
References


