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CELL SCIENCE AT A GLANCE

FGF2 and IL-1β – explorers of unconventional secretory pathways at a glance

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ABSTRACT

Fibroblast growth factor 2 (FGF2) and interleukin 1β (IL-1β) were among the earliest examples of a subclass of proteins with extracellular functions that were found to lack N-terminal secretory signal peptides and were shown to be secreted in an ER- and Golgi-independent manner. Many years later, a number of alternative secretory pathways have been discovered, processes collectively termed unconventional protein secretion (UPS). In the course of these studies, unconventional secretion of FGF2 and IL-1β were found to be based upon distinct pathways, mechanisms and molecular machineries. Following a concise introduction into various pathways mediating unconventional secretion and transcellular spreading of proteins, this Cell Science at a Glance poster article aims at a focused analysis of recent key discoveries providing unprecedented detail about the molecular mechanisms and machineries driving FGF2 and IL-1β secretion. These findings are also highly relevant for other unconventionally secreted cargoes that, like FGF2 and IL1β, exert fundamental biological functions in biomedically relevant processes, such as tumor-induced angiogenesis and inflammation.

KEY WORDS: FGF2, Fibroblast growth factor 2, IL-1β, Interleukin 1β, Unconventional protein secretion

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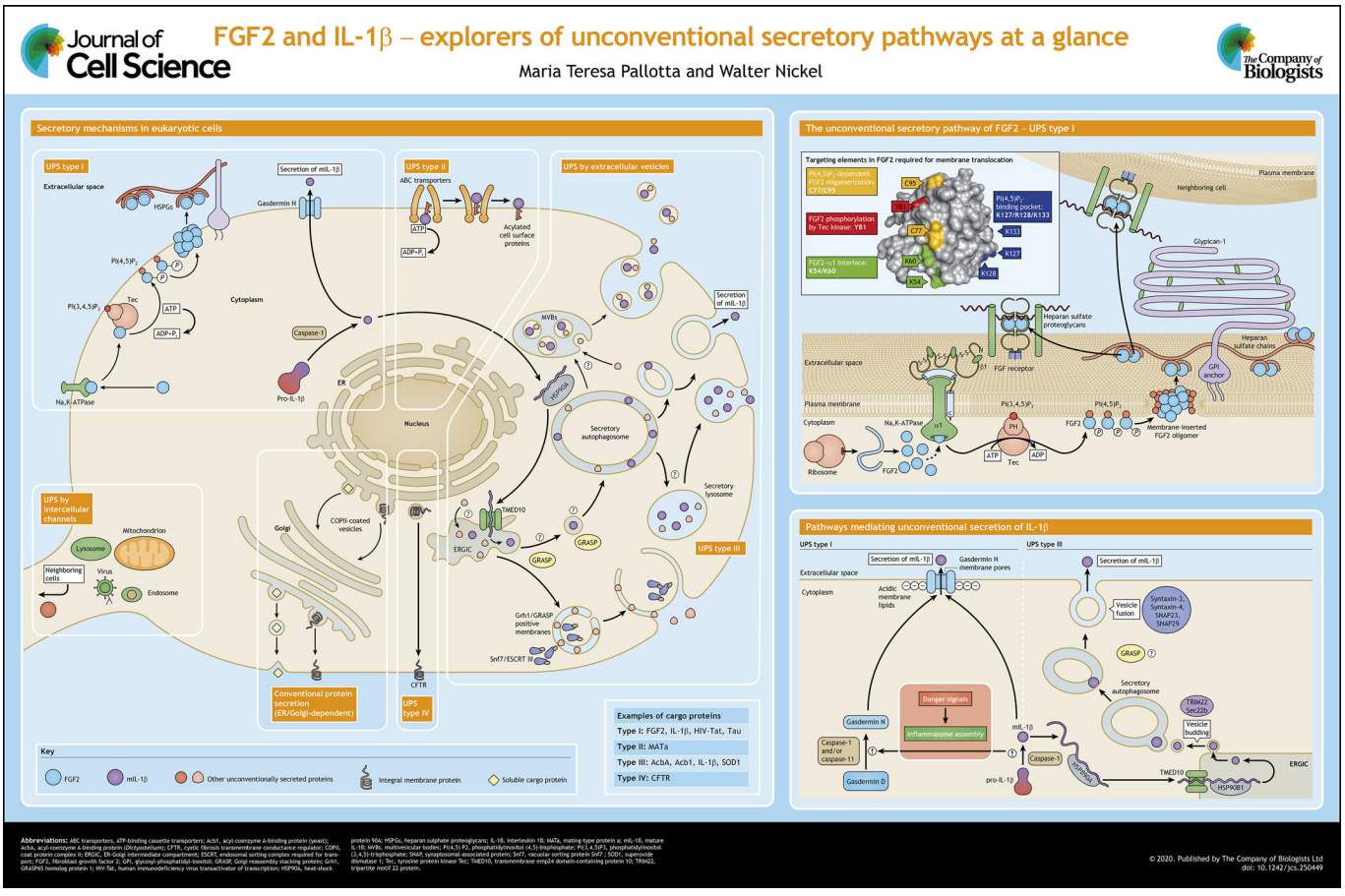
Introduction

Protein secretion through the ER- and Golgi-dependent secretory pathway has long been believed to be the exclusive transport mechanism by which eukaryotic cells dispatch proteins into the extracellular space (Palade, 1975; Rothman and Wieland, 1996). This



Journal of Cell Science (2020) 133, jcs250449. doi:10.1242/jcs.250449

view has been challenged through the identification of secreted proteins that lack N-terminal hydrophobic signal peptides for membrane translocation into the ER. A diverse set of distinct pathways has been identified, through which a defined set of soluble proteins can be transported from the cytoplasm into the extracellular space (see poster). The full range of subtypes of unconventional secretory processes has collectively been termed unconventional protein secretion (UPS) (Nickel and Seedorf, 2008; Nickel and Rabouille, 2009; Rabouille et al., 2012; Rabouille, 2017; Dimou and Nickel, 2018) (see Box 1 for a brief overview). In addition to the deposition of such proteins into the extracellular space in a free form (UPS type I, II and III), mechanisms exist that export proteins as part of vesicles that are released into the extracellular space [UPS by extracellular vesicles (EVs)]. One of the major sources of EVs are exosomes that originate from multivesicular bodies (Mathieu et al., 2019). These processes may lead to the intercellular delivery of proteins when EVs fuse with target cells. Another way of intercellular protein delivery is mediated by the formation of channels that physically link cells and are used to exchange proteins and even vesicular structures or viral particles (UPS by intercellular channels) (Korenkova et al., 2020). These structures are functionally equivalent to plasmodesmata in plants (Petit et al., 2020). All pathways of UPS are not only found in mammals, but are also present in plants, flies, fungi and lower eukaryotes, such as yeast (Malhotra, 2013; Pompa et al., 2017).

With many detailed recent reviews (and references therein) available covering the broad range of unconventional secretory processes and cargo molecules as discussed above, this Cell Science at a Glance poster article aims at highlighting our most current knowledge on the UPS pathways taken by FGF2 and IL-1β, by far the best understood examples regarding the molecular mechanisms and machineries involved in ER- and Golgi-independent secretory pathways.

Box 1. Brief overview of UPS

UPS pathways have been classified into six general categories, from UPS type I to IV, UPS by EVs and UPS by intercellular channels, based upon the mechanisms and types of cargoes being involved (Rabouille et al., 2012; Rabouille, 2017; Dimou and Nickel, 2018). Type I and II are based upon direct protein translocation across the plasma membrane (see poster). Well-studied example proteins that undergo UPS type I pathway are FGF2 (Steringer and Nickel, 2018) and HIV-Tat (Schatz et al., 2018), whose secretion occurs in a constitutive manner and is regulated by expression levels. Type II UPS pathways are mediated by ABC transporters and translocate lipidated cargoes onto the surface of cells (Dimou and Nickel, 2018). As opposed to type I and II UPS mechanisms, the type III pathway involves intracellular vesicle intermediates (see poster). Most examples of cargoes utilizing this pathway have been seen when triggered by different kinds of cellular stresses, such as nutrient starvation, protein misfolding or acute inflammation. Examples are acyl-CoA binding protein (AcbA/Acb1) in lower eukaryotes (Cruz-Garcia et al., 2018), as well as misfolded cytoplasmic proteins (Ye, 2018) and IL-1β in mammals (Sitia and Rubartelli, 2018). Interestingly, depending on the physiological context, certain cargoes may be capable of entering different types of UPS pathways. Examples are proteins that form toxic aggregates in neurodegenerative diseases, such as Tau, with evidence reported pointing to it undertaking both type I UPS and UPS by EVs (Wegmann et al., 2016; Wang et al., 2017; Katsinelos et al., 2018; Merezhko et al., 2018). Another example is IL-1β, which has been shown to reach the extracellular space through both type I and type III mechanisms (Zhang and Schekman, 2013; Sitia and Rubartelli, 2018). So far, three types of targeting signals have been identified in unconventionally secreted proteins: (i) the PI(4,5)P_2-binding pocket in FGF2 for the type I UPS pathway (see below) (Steringer and Nickel, 2018), (ii) a diacidic motif in AcbA/Acb1 for the type III UPS pathway (Cruz-Garcia et al., 2018), and (iii) a direct interaction of IL-1β with the cytoplasmic domain of TMED10, which initiates membrane translocation of IL-1β as part of the type III UPS pathway (Liu et al., 2020). A fourth type of UPS has been defined for integral membrane proteins that, while traveling from the ER to the plasma membrane, bypass the Golgi (UPS Type IV; see poster) (Gee et al., 2018; Witzgall, 2018). Interestingly, such processes are also frequently induced by cellular stresses and, similar to the type III UPS pathway, depend on GRASP, a potential common denominator of stress-related pathways of unconventional protein secretion (Giuliani et al., 2011; Gee et al., 2018).

The unconventional secretory pathway of FGF2

FGF2 is an extracellular mitogen secreted from a wide range of cell types during development (Beenken and Mohammadi, 2009). Beyond functions of FGF2 in processes, such as the biogenesis of the vascular system, FGF2 also plays critical roles under pathophysiological conditions that promote tumorigenesis, with primary cancer cells expressing and secreting large quantities of FGF2 (Akl et al., 2016). In this context, the extracellular population of FGF2 secreted from tumor cells not only affects target cells through paracrine signaling (e.g. endothelial cells to trigger tumor-induced angiogenesis), but also mediates autocrine signal transmission by which cancer cells protect themselves from undergoing apoptosis (Pardo et al., 2006; Noh et al., 2014). Such mechanisms are thought to contribute to resistance development when cancer cells are challenged by chemotherapies or other interventions (Beenken and Mohammadi, 2009; Akl et al., 2016).

Even though FGF2 lacks a signal peptide for transport along the ER- and Golgi-dependent secretory pathway, it is capable of getting access to the extracellular space to exert the functions described above. Having been a mystery for a long time, substantial insight into the molecular mechanism of FGF2 secretion has been obtained in recent years. Unconventional secretion of FGF2 from cells is now known to be mediated by a type I UPS pathway that is based upon direct translocation of FGF2 across the plasma membrane (Schäfer et al., 2004; Steringer et al., 2017; Dimou and Nickel, 2018; Steringer and Nickel, 2018; Dimou et al., 2019) (see poster). All molecular components known to date to play a role in unconventional secretion of FGF2 are physically associated with the plasma membrane. These factors include the Na,K-ATPase (Zacherl et al., 2015), the kinase Tec, which is recruited to the inner leaflet through binding to the phosphoinositide phosphatidylinositol (3,4,5)-trisphosphate [PI(3,4,5)P_3] (Ebert et al., 2010; Steringer et al., 2012; La Venuta et al., 2016), and phosphatidylinositol (4,5)-bisphosphate [PI(4,5)P_2], the most abundant phosphoinositide at the inner leaflet of the plasma membrane (Temmerman et al., 2008; Temmerman and Nickel, 2009; Nickel, 2011). FGF2 has been demonstrated to engage in direct physical interactions with all three of these components. Through interactions with PI(4,5)P_2 mediated by a cluster of basic amino acids on the molecular surface of FGF2 (K127, R128 and K133; see poster) (Temmerman et al., 2008; Müller et al., 2015; Steringer et al., 2017), the core mechanism of FGF2 membrane translocation is triggered. This process involves PI(4,5)P_2-dependent FGF2 oligomerization, which drives the formation of a lipidic membrane pore in which membrane-spanning FGF2 oligomers are accommodated (Steringer et al., 2012, 2017; Steringer and Nickel, 2018). Two cysteine residues (C77 and C95) have been identified in FGF2, and these form intermolecular disulfide bridges during oligomerization (Müller et al., 2015; Steringer et al., 2017; Dimou and Nickel, 2018). Lipidic membrane pores induced by FGF2 oligomerization have been proposed to be characterized by a toroidal architecture (Steringer

Journal of Cell Science (2020) 133, jcs250449. doi:10.1242/jcs.250449

et al., 2012; Müller et al., 2015; Steringer and Nickel, 2018). This view is supported by several independent observations, including simultaneous membrane passage of fluorescent tracers and transbilayer diffusion of membrane lipids that is triggered by PI(4,5)P_2-dependent FGF2 oligomerization and membrane insertion (Steringer et al., 2012; Steringer and Nickel, 2018). In further support of this, diacylglycerol, a cone-shaped lipid that antagonizes PI(4,5)P_2-induced membrane curvature, was found to inhibit membrane insertion of FGF2 oligomers (Steringer et al., 2012; Steringer and Nickel, 2018). Furthermore, when FGF2 variant forms were used, such as an FGF2 fusion protein with GFP, the pore cut-off was observed to increase, a typical phenomenon for toroidal membrane pores (Gilbert et al., 2014). Based upon these findings, the role of PI(4,5)P_2 in unconventional secretion of FGF2 has been proposed to be three-fold with it (i) mediating recruitment of FGF2 to the plasma membrane, (ii) orienting FGF2 molecules at the inner leaflet to drive oligomerization and (iii) stabilizing local curvature to allow for a toroidal membrane structure surrounding membrane-inserted FGF2 oligomers that are accommodated within a hydrophilic environment (Dimou and Nickel, 2018; Steringer and Nickel, 2018).

Membrane-spanning FGF2 oligomers have been proposed to act as key intermediates in FGF2 membrane translocation based on an assembly–disassembly mechanism that drives the directional transport of FGF2 across the plasma membrane (see poster) (Dimou and Nickel, 2018; Steringer and Nickel, 2018). This process depends on membrane-proximal heparan sulfate proteoglycans on cell surfaces, which form an extracellular trap required for FGF2 translocation into the extracellular space (Zehe et al., 2006; Nickel, 2007; Nickel and Seedorf, 2008; Nickel and Rabouille, 2009). Thus, cell surface heparan sulfates mediate the final step of FGF2 membrane translocation and subsequently lead to the retention of FGF2 on cell surfaces without release into cellular supernatants (see poster). From there, FGF2 undergoes intercellular spreading through direct cell–cell contacts, probably mediated by direct exchange between heparan sulfate chains that are physically associated with opposing cell surfaces (Zehe et al., 2006). Thus, during the lifetime of an FGF2 molecule, the role of heparan sulfate proteoglycans is threefold with them (i) mediating the final step of FGF2 secretion (Zehe et al., 2006; Nickel, 2007), (ii) protecting FGF2 on cell surfaces against degradation (Nugent and Iozzo, 2000) and (iii) mediating FGF2 signaling in the recipient cell as part of a ternary complex comprising FGF2, heparan sulfate chains and high-affinity FGF receptors (Presta et al., 2005; Ribatti et al., 2007; Belov and Mohammadi, 2013). The sequential interactions of FGF2 with PI(4,5)P_2 at the inner leaflet of the plasma membrane and, following the formation of membrane-spanning FGF2 oligomers, with heparan sulfates on cell surfaces, thus offers a molecular basis for directional FGF2 transport into the extracellular space. This model is consistent with previous studies demonstrating that FGF2 membrane translocation occurs with FGF2 in a fully folded state (Backhaus et al., 2004; Torrado et al., 2009; Nickel, 2011), requiring the formation of membrane-spanning oligomers. Since FGF2 membrane translocation occurs at the level of the plasma membrane, these findings suggest an intrinsic quality control mechanism that limits unconventional secretion to fully folded and, therefore, functional forms of FGF2 (Torrado et al., 2009; Nickel, 2011). Recently, by employing biochemical reconstitution experiments using an inside-out membrane model system based on giant unilammelar vesicles (Steringer et al., 2017), the minimal machinery required for FGF2 membrane translocation was established to consist of the phosphoinositide PI(4,5)P_2 and heparan sulfate chains (mimicked by synthetic heparin molecules) on opposing sides of the membrane along with the ability of FGF2 to oligomerize in a PI(4,5)P_2-dependent manner (Steringer et al., 2017). This work also demonstrated that the interactions between FGF2 and either PI(4,5)P_2 or high-affinity heparin chains are mutually exclusive, providing a compelling explanation for directional FGF2 transport across the plasma membrane (Steringer et al., 2017).

More recent key discoveries have provided substantial new insights into the spatio-temporal coordination of FGF2 secretion in living cells. These studies utilized a real-time total internal reflection fluorescence (TIRF) microscopy setup to detect single events of FGF2 membrane translocation from the cytoplasm to the cell surface with single-molecule sensitivity (Dimou et al., 2019). This experimental system allowed the researchers to determine the entire time interval required for FGF2 membrane translocation, including recruitment of a single FGF2 molecule at the inner plasma membrane leaflet, its oligomerization concomitant with membrane insertion and eventually the heparan-sulfate-dependent translocation of FGF2 molecules at this particular site on the cell surface. With an average time interval of ∼200 ms, this process was found to be surprisingly fast and, by mathematical modeling, could be dissected into two non-overlapping steps with a slow and a fast component, which were attributed to FGF2 oligomerization and membrane insertion (relatively slow) and heparan sulfate-mediated translocation to the cell surface (fast) (Dimou et al., 2019). Thus, both biochemical reconstitution experiments (Steringer et al., 2017) and studies in living cells establish that the UPS of FGF2 is based upon a type I UPS mechanism (Dimou et al., 2019).

Another recent advance has shed light onto the role of the Na,K-ATPase in FGF2 secretion (Legrand et al., 2020). In that study, a subdomain in the cytoplasmic part of the α1 subunit of the Na,K-ATPase was identified that was both necessary and sufficient to directly bind to FGF2. This, in turn, allowed for structural investigations, including nuclear magnetic resonance (NMR) spectroscopy and molecular dynamics simulations of the α1–FGF2 interface. Residues involved in physical interactions on both sides of the interface could be identified, paving the way for functional studies in intact cells. Using single-molecule TIRF microscopy to quantify FGF2 recruitment under various conditions, these studies revealed that the Na,K-ATPase acts upstream from PI(4,5)P_2, thus being the first contact of FGF2 with the inner plasma membrane leaflet. Furthermore, the interaction of FGF2 with the α1 subunit of the Na,K-ATPase has been demonstrated to be a prerequisite for the subsequent interactions of FGF2 with PI(4,5)P_2, with the latter triggering FGF2 membrane translocation to the cell surface (Legrand et al., 2020). These findings have important implications for future research directions as they suggest a potential regulation of the membrane-potential-generating activity of the Na,K-ATPase by FGF2. In this context, an interesting hypothesis would be that FGF2-induced upregulation of the Na,K-ATPase at sites of FGF2 membrane translocation triggers a process that involves the formation of lipidic membrane pores that may compromise plasma membrane integrity in a transient manner. Finally, this work also addressed the mechanism by which ouabain, an inhibitor of the Na,K-ATPase, blocks unconventional secretion of FGF2 (Florkiewicz et al., 1998; Dahl et al., 2000; Engling et al., 2002). Using an in-cell proximity assay, the authors showed that ouabain reduces interactions between FGF2 and the α1 subunit of the Na,K-ATPase in the vicinity of the plasma membrane, which are required for efficient FGF2 secretion (Legrand et al., 2020).

Journal of Cell Science (2020) 133, jcs250449. doi:10.1242/jcs.250449

Intriguingly, the mechanism of FGF2 secretion also appears to be relevant for other extracellular proteins secreted by unconventional means. In particular, secretion of HIV-Tat and Tau from various kinds of cell types has been shown to occur by direct translocation across the plasma membrane, a process that also in these cases depends on $PI(4,5)P_2$ at the inner leaflet and heparan sulfates at the outer leaflet of the plasma membrane (Rayne et al., 2010; Debaisieux et al., 2012; Zeitler et al., 2015; Katsinelos et al., 2018; Merezhko et al., 2018). In addition, similar to FGF2, unconventional secretion of HIV-Tat has been reported to involve the Na,K-ATPase (Agostini et al., 2017). Furthermore, as discussed in detail in the following section, a number of aspects of the UPS pathway for FGF2 may also be relevant to IL-1β secretion. Under certain physiological conditions, this process involves IL-1β targeting to the plasma membrane in a manner that is mediated by $PI(4,5)P_2$ and based on the formation of membrane pores; these are triggered by phosphoinositide-dependent oligomerization of an N-terminal fragment of Gasdermin D in inflammasome-containing immune cells (He et al., 2015; Martin-Sanchez et al., 2016; Brough et al., 2017; Evavold et al., 2017; Monteleone et al., 2018).

Pathways implicated in the secretion of IL-1β under various physiological conditions

IL-1β is a cytokine that is essential for acute inflammatory responses to pathogen products or factors released from damaged tissues (Garlanda et al., 2013). Under these conditions, in a complex interplay of various types of signals, inflammasomes assemble, become activated and coordinate downstream processes that lead to the activation of caspase-1 (see poster). This, in turn, triggers proteolytic processing of the IL-1β precursor (pro-IL1β), a process that produces the mature form of IL-1β (mIL-1β), which is secreted and exerts biological activity in the extracellular space (Sitia and Rubartelli, 2018).

For decades, the molecular mechanism by which immune cells secrete IL-1β remained elusive. Various kinds of experimental observations led to a diverse set of models, including pathways that either involve intracellular vesicle intermediates (UPS type III), or mechanisms that are based upon direct translocation of IL-1β across plasma membranes (UPS type I). The different pathways for IL-1β secretion have been proposed to depend on different levels and combinations of immune stimulation, the time scale by which sustained danger signals are present and the different types of immune cells that are involved (Sitia and Rubartelli, 2018).

As a first approximation, the distinct types of IL-1β secretion can be classified as type I and type III mechanisms of UPS (see poster) (Rabouille et al., 2012; Rabouille, 2017; Dimou and Nickel, 2018). In the presence of both bacterial lipopolysaccharides and extracellular ATP, as strong and sustained danger signals in macrophages, IL-1β appears to be secreted via a type I mechanism of UPS that involves the formation of pores in the plasma membrane (Martin-Sanchez et al., 2016). Under these conditions, within a few minutes and without any apparent involvement of intracellular vesicle intermediates, the entire cytoplasmic pool of mature IL-1β was found to be released into cellular supernatants by direct translocation across the plasma membrane (Martin-Sanchez et al., 2016). These findings were followed by studies that identified the N-terminal domain of gasdermin D (denoted gasdermin N; this is generated through proteolytic cleavage by caspases following inflammasome assembly) as the structural component that is essential for pore formation in inflammasome-activated macrophages (Evavold et al., 2017). Importantly, it could be demonstrated that macrophages remain viable in this state of hyperactivation and secrete IL-1β through gasdermin N membrane pores without a general loss of other cytoplasmic components (Evavold et al., 2017; Heilig et al., 2017). Another effect of the formation of gasdermin N pores is pyroptosis concomitant with cell death (Liu et al., 2016); this allows for a massive and local inflammatory response at a site of infection. Intriguingly, even when pyroptosis is induced in a gasdermin N-dependent manner, repair mechanisms are in place that are capable of preventing cell death as a result of pyroptosis (Rühl et al., 2018). Taken together, these observations suggest that both hyperactivated and pyroptosis-induced immune cells do not necessarily die and, therefore, may continue to serve the organism by contributing immunomodulatory activities. This, in turn, demonstrates that IL-1β secretion is not merely passively released from dying cells, but rather presents an active and controlled mechanism in intact and viable immune cells.

The formation of IL1β-conducting membrane pores by the N-terminal domain of gasdermin D can also be reconstituted in artificial membranes (Ding et al., 2016; Evavold et al., 2017). Furthermore, the structural basis of this process was elucidated based on high-resolution structures of gasdermin N oligomers obtained by cryo-electron microscopy and X-ray crystallography (Ding et al., 2016; Ruan et al., 2018). Intriguingly, pore formation was found to be linked to the ability of gasdermin N to bind to acidic membrane lipids including phosphoinositides (Ding et al., 2016). Although clearly distinct from the type I UPS pathway described for FGF2 (see above), there are several similarities with gasdermin N-dependent secretion of IL-1β, such as the role of acidic membrane lipids, protein oligomerization and membrane pore formation. This aspect is further emphasized by observations that microdomains containing the phosphoinositide $PI(4,5)P_2$ play a role in targeting mature IL-1β to the inner leaflet of the plasma membrane (Monteleone et al., 2018). However, as opposed to FGF2, IL-1β itself does not bind to $PI(4,5)P_2$ (Martin-Sanchez et al., 2016), suggesting that additional factors are involved to support relocation of mature IL-1β from the cytoplasm to the plasma membrane in preparation for gasdermin N-dependent secretion of IL-1β into the extracellular space.

In addition to gasdermin N-dependent membrane translocation of IL-1β through a UPS type I mechanism, other pathways have been described that involve intracellular vesicle intermediates, namely, type III UPS (see poster). The various secretory routes that IL-1β has been proposed to take may reflect the spectrum of physiological conditions at different levels, such as the type of organism or the type of immune cell being studied, the type, combination, timing and strength of danger signals that trigger IL-1β secretion, and the general state of cells, for example with regard to redox stress (Sitia and Rubartelli, 2018). With regard to the nature of the vesicular intermediates involved, a number of options have been discussed, including secretory lysosomes, multivesicular bodies and secretory autophagosomes (Rabouille, 2017; Dimou and Nickel, 2018; Sitia and Rubartelli, 2018). Although it is possible that all of these organelles have distinct roles in IL-1β secretion under various physiological conditions, an alternative explanation is that the vesicular intermediate that carries IL-1β is not identical to any of these organelles, but rather represents a unique subpopulation that is characterized by components and functions derived from various kinds of organelles. With regard to the biogenesis of the vesicle intermediates involved, a common denominator appears to be GRASP (Golgi reassembly stacking proteins GRASP55 and GRASP65, also known as GORASP1 and GORASP2), a component of the early secretory pathway that has been described by several groups to be important for IL-1β secretion (Dupont et al., 2011; Zhang et al., 2015; Chiritoiu et al., 2019). These findings also

link IL-1β secretion to unconventional secretory processes in lower eukaryotes, such as yeast, where they are mediated by a specialized organelle, termed cup-shaped membranes (CUPS), that might be related to autophagosomes (Kinseth et al., 2007; Bruns et al., 2011). In addition, as expected for a vesicular pathway involving membrane fusion events at multiple steps, SNARE proteins have been implicated in unconventional secretion of IL-1β (see poster) (Kimura et al., 2017a,b).

A major open question with regard to GRASP-dependent type III UPS of IL-1β was how IL-1β, which does not have a signal peptide for ER translocation, enters into the lumen of intracellular vesicle intermediates from where it is expelled into the extracellular space upon their fusion with the plasma membrane. Recently, elegantly combining an IL-1β construct whose folding state can be controlled with a small molecule and a biochemical cross-linking approach, TMED10, a member of the p24 family of integral membrane proteins in the early secretory pathway, was identified as a candidate for IL-1β membrane translocation (Zhang et al., 2020). Through silencing or CRISPR knockouts, TMED10 was found to be of critical importance for IL-1β secretion in various cell lines and primary macrophages, as well as *in vivo* in a mouse model. Furthermore, mature IL-1β was demonstrated to interact with the cytoplasmic domain of TMED10 in lipopolysaccharide-stimulated cells, a process that depends on IL-1β unfolding and the cytoplasmic chaperone HSP90A (Zhang et al., 2020). The latter finding is consistent with the presence of a KFERQ motif in IL-1β, which is recognized by HSP90, and has been shown to be important for IL-1β membrane translocation (Zhang et al., 2015). Moreover, silencing of TMED10 not only inhibits IL-1β secretion, but also that of other type III UPS cargoes, such as galectin-1 and galectin-3, whereas known type I UPS cargoes, such as FGF2 and HIV-Tat, were secreted normally when TMED10 levels were downregulated. In addition, by using biochemical reconstitution experiments, TMED10 could be demonstrated to be sufficient for membrane translocation of IL-1β and other unconventionally secreted proteins. Based on an analysis in cells, the site of membrane translocation could be identified as the ER–Golgi intermediate compartment (ERGIC). Finally, membrane translocation of mature IL-1β further involved oligomerization of TMED10 (presumably to form the protein-conducting channel) and another chaperone on the luminal side, HSP90B1 (Zhang et al., 2020). Thus, this study solved a long-standing problem with regard to type III UPS-mediated IL-1β secretion, namely, the mechanism by which IL-1β enters the membrane-bound structures that represent the precursors of vesicle intermediates, through which it is trafficked to the plasma membrane and then delivered into the extracellular space.

Future perspectives

While extracellular proteins lacking signal peptides for ER- and Golgi-dependent secretion, such as FGF2 and IL-1β, were identified more than 30 years ago, a detailed knowledge of the underlying mechanisms and pathways began to emerge only recently. The pathways undertaken by FGF2 and IL-1β exemplify the two major modes by which a defined group of soluble cytoplasmic proteins can reach the extracellular space by UPS. The unprecedented in-depth knowledge available for these two examples will pave the way for the elucidation of the mechanisms and pathways of other cargoes secreted by unconventional means. A detailed understanding of the molecular machineries involved will provide unique opportunities to develop novel classes of drugs with great value in fighting cancer or chronic inflammatory diseases, among others.

Competing interests
The authors declare no competing or financial interests.

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