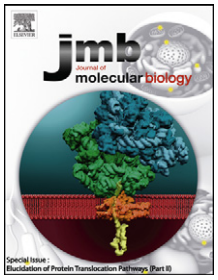
Review



Unconventional Secretion of Fibroblast Growth Factor 2—A Novel Type of Protein Translocation across Membranes?

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Abstract

N-terminal signal peptides are a hallmark of the vast majority of soluble secretory proteins that are transported along the endoplasmic reticulum/Golgi-dependent pathway. They are recognized by signal recognition particle, a process that initiates membrane translocation into the lumen of the endoplasmic reticulum followed by vesicular transport to the cell surface and release into the extracellular space. Beyond this well-established mechanism of protein secretion from eukaryotic cells, a number of extracellular proteins with critical physiological functions in immune surveillance and tissue organization are known to be secreted in a manner independent of signal recognition particle. Such processes have collectively been termed "unconventional protein secretion" and, while known for more than two decades, their underlying mechanisms are only beginning to emerge. Different types of unconventional secretory mechanisms have been described with the best-characterized example being based on direct translocation of cytoplasmic proteins across plasma membranes. The aim of this review is to critically assess our current knowledge of this type of unconventional secretion focusing on fibroblast growth factor 2 (FGF2) as the most established example.
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Mechanisms of Eukaryotic Protein Secretion

The vast majority of soluble secretory proteins is making use of the endoplasmic reticulum (ER)/Golgi-dependent pathway to get access to the extracellular space (Refs. [1] and [2] and Fig. 1). Entering this route of secretion requires the presence of N-terminal signal peptides that are recognized by signal recognition particle [3]. Following a slowdown of translation, ribosomes carrying nascent chains with N-terminal signal peptides are directed to the cytoplasmic surface of the ER coupling protein synthesis to translocation into the ER lumen mediated by Sec61 [4]. Translocation of newly synthesized chains proceeds in a largely unfolded form followed by folding of the translocation substrate into the native state within the ER lumen [5]. Once secretory proteins pass ER quality control [6,7], they are delivered to the cell surface based on vesicular transport and, upon fusion of post-Golgi carriers with the plasma membrane, are released into the extracellular space (Ref. [8] and Fig. 1).

It was long believed that the presence of a signal peptide concomitant with a protein's ability to penetrate the lumen of the ER is an essential requirement for protein secretion from eukaryotic cells. This view was challenged by the identification of signal-peptide-lacking cytokines that mediate defined extracellular functions of fundamental physiological importance [9,10] along with reports presenting direct evidence that they can be secreted in an ER/Golgi-independent manner [11–14]. The most classical examples are fibroblast growth factor 2 (FGF2), a proangiogenic mitogen [15], as well as interleukin 1β (IL1β), a key cytokine in inflammation [16,17]. For both molecules, the cell surface receptors on target cells have been identified and crystal structures of the corresponding ligand–receptor complexes are available [18–21]. Based on this, alternative pathways of protein secretion that operate independently of the ER/Golgi system were proposed to exist in eukaryotic cells [9].

As illustrated in Fig. 1, two principal types of unconventional secretion that are exemplified by FGF2 and IL1β as cargo proteins appear to exist. While FGF2 is not only excluded from the lumen of the

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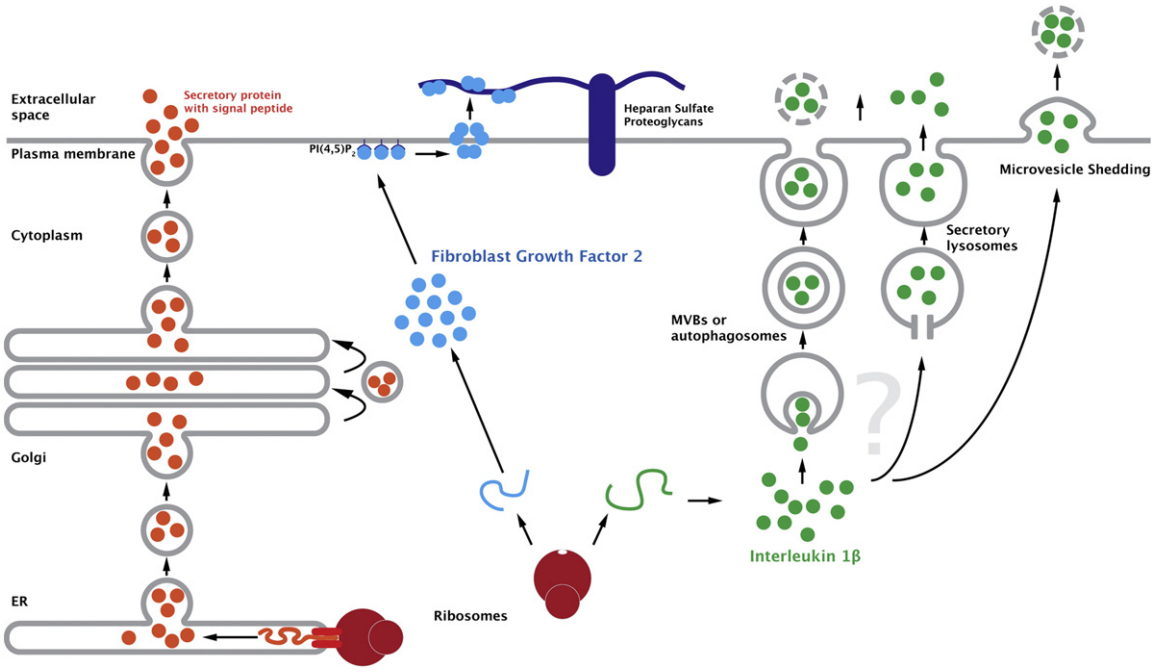

Fig. 1. Principle pathways of protein secretion from eukaryotic cells.

ER, it is also not entering any other type of vesicular intermediates inside cells. Instead, as detailed below, FGF2 has been shown to be capable of translocating across plasma membranes to reach the extracellular space (Fig. 1 and Refs. [22] and [23]). By contrast, as part of its unconventional secretory pathway, IL1β is believed to get incorporated into membrane-bound intermediates of the endocytic membrane system [24–28]. The identity of these intermediates has not unequivocally been clarified; however, a common suggestion has been that they represent multivesicular bodies (MVBs) as indicated in Fig. 1 [28]. In this case, IL1β localized in the cytoplasm would be internalized by endosomes resulting in its localization in intra-endosomal vesicles called exosomes. Alternatively, cytoplasmic IL1β might be initially captured by autophagosomes followed by delivery to endocytic compartments such as MVBs (Fig. 1) [25,26]. This mechanism would be consistent with a proposed pathway of unconventional secretion in lower eukaryotes that has been described for the protein AcbA/Acb1 [25,29–31]. Other suggestions have been a role for secretory lysosomes [13,24,28] or plasma membrane shedding, that is, secretion of IL1β in microvesicles that form at the extracellular surface of cells and incorporate cytoplasmic material (Fig. 1) [28,32]. However, for none of these options of IL1β release from cells, unambiguous evidence has been presented. In particular, IL1β has not been shown to localize inside any of such endocytic intermediates based on biochemical experiments with the identity of such vesicles as secretory intermediates being demonstrated [33]. Another unresolved issue is that, for most of the described options, extracellular IL1β would be at least initially contained inside membrane-bound vesicles released into the extracellular space (Fig. 1). It is, however, unclear how IL1β could escape such vesicles to interact with its receptors in order to exert its biological functions. Finally, the question on how the described pathways are linked to the regulation of IL1β secretion is an unresolved problem. It is well established that processing of the precursor form of IL1β into mature and biologically active IL1β depends on the activation of inflammasomes [34]. This process results in the activation of caspase 1 that, in turn, generates the mature form of IL1β by proteolytic cleavage [28]. However, no conclusive experimental evidence has been reported on where this processing event occurs, that is, in the cytoplasm or inside endocytic intermediates such as secretory lysosomes. It cannot be even excluded that processing of IL1β occurs after secretion, that is, in the extracellular space, as keratinocytes secrete large amounts of the precursor form of IL1β for example [35]. In conclusion, the mechanism by which biologically active IL1β appears in the extracellular space in an inflammasome-dependent manner largely remains an unanswered question with various kinds of endocytic pathways being proposed; however, conclusive evidence that would justify a preference for one of those options is lacking. In our view, it cannot even be excluded that, like FGF2 (see below), IL1β is secreted by a non-vesicular mechanism that is based on direct translocation across plasma membranes as

the findings suggesting a role for endocytic compartments or microvesicles are solely based on indirect evidence.

Hallmarks of FGF2 Membrane Translocation during Unconventional Secretion

As illustrated in Fig. 1, our current view on how FGF2 is secreted from cells is based on a mechanism characterized by direct translocation across plasma membranes; that is, FGF2 is not getting incorporated into intracellular membrane-bound compartments as intermediates in this alternative pathway of secretion [22,23]. Instead, FGF2 is capable of interacting with phosphoinositides at the inner leaflet of plasma membranes, a key step that initiates membrane translocation [36,37]. This process, however, does not appear to rely on a protein-conducting channel but rather on the ability of membrane-recruited FGF2 to oligomerize concomitant with the formation of a membrane pore [38,39]. This structure has been proposed to have a toroidal architecture with the FGF2 oligomer in the center and the lipid binding sites being localized in the periphery. In a way, this structure might share structural similarities with toroidal membrane pores formed by components of the apoptotic machinery involved in the disintegration of the outer mitochondrial membrane [40,41]. Once FGF2 oligomers have formed membrane pores, membrane-proximal heparan sulfate chains of cell surface localized proteoglycans can act as an extracellular trap for FGF2 molecules resulting in their exposure on cell surfaces [42,43]. Thus, the biochemical and biophysical properties of FGF2 allow for a mechanism of membrane translocation with the cargo protein (FGF2 monomer) forming its own translocation machinery (membrane-inserted FGF2 oligomer) triggered by phosphoinositide-induced multimerization. This model is supported by conclusive evidence for membrane translocation of FGF2 in a fully folded state [44,45] requiring the formation of defined oligomers during membrane pore formation. Further support for this model comes from the observation that Tec kinase, a non-receptor tyrosine kinase that has previously been described in the context of T cell development and activation [46], is a regulatory factor of FGF2 secretion. The role of Tec kinase in FGF2 secretion was initially recognized through a genome-wide RNAi screen designed to identify factors involved in this process [47,48]. Tec kinase was further shown to phosphorylate FGF2, a process that facilitates the formation of membrane pores by FGF2 oligomers [38,39]. Since Tec kinase contains a PH domain that mediates both phosphoinositide-dependent membrane recruitment and enzymatic activation [49], FGF2 phosphorylation is likely to occur at the inner leaflet of the plasma membrane [38]. Since FGF2 is a key signaling molecule in the context of many cancers, Tec-kinase-regulated secretion of FGF2 represents an interesting link to the up-regulation of PI3 kinases in many tumor cells [50]. PI3 kinases catalyze the formation of PI(3–5)P_3 and high cellular levels of this phosphoinositide are likely to support efficient secretion of FGF2 that, in turn, promotes tumor cell proliferation.

The Molecular Mechanism of FGF2 Membrane Translocation and Its Path of Discovery

Our current knowledge about the mechanism by which FGF2 physically traverses the plasma membrane to reach the extracellular space explaining the sequence of events as they occur in a cell has previously been described [38,48,51–53]. Here, we would like to recount this path of discovery in chronological order.

In 2002, the setup of a cell-based assay to quantify FGF2 secretion was the starting point of these studies as this experimental system provided convincing evidence that FGF2 is secreted by a controlled mechanism [11]. It also confirmed observations by other laboratories demonstrating that FGF2 secretion does not result in the appearance of soluble FGF2 in cellular supernatants [14]. Rather, following membrane translocation, we found that FGF2 retained on cell surfaces bound to heparan sulfate proteoglycans. Further observations in the initial phase of these studies indicated that the mechanism of FGF2 secretion from cells is unusual in at least two ways. On the one hand, we found that FGF2 has the ability to directly translocate across plasma membranes that had been affinity purified as so-called inside-out vesicles [23]. This finding suggested that FGF2 not only is incapable of penetrating the lumen of the ER/Golgi system but also does not need to make use of other membrane-bound intermediates such as endocytic compartments to reach the extracellular space. These results were initially interpreted as a possibility for a plasma membrane resident protein-conducting channel being responsible for membrane translocation of FGF2. This hypothesis appeared plausible also because ABC transporters were known in the plasma membrane mediating the externalization of peptides [54,55]. However, a second key finding at the time was that FGF2 secretion from cells does not involve an unfolded form of FGF2 as an obligatory intermediate [44]. Since both peptide and protein-conducting channels from various membranes including the ER and mitochondria were known to form narrow channels through which largely unfolded translocation substrates are threaded, it appeared unlikely that a plasma membrane resident protein-conducting channel is behind the principle of FGF2 membrane translocation. This view was supported by additional findings

demonstrating that membrane-proximal heparan sulfate proteoglycans on cell surfaces are required to complete FGF2 membrane translocation into the extracellular space [43]. Since FGF2 binding to heparin depends on FGF2 being folded properly [45], folding of FGF2 is required for translocation to cell surfaces. The same is true for FGF2 binding to the phosphoinositide $PI(4,5)P_2$ that is both required for FGF2 recruitment at the inner leaflet and essential for FGF2 secretion from cells [36,37]. Again, binding to $PI(4,5)P_2$ was found to require FGF2 in a folded form demonstrating that FGF2 needs to be folded to undergo membrane translocation [45]. Finally, it was shown that FGF2 is capable of transporting other proteins out of cells based on engineered non-covalent interactions that depend on proper folding [45]. These experiments confirmed earlier findings mentioned above and unequivocally demonstrated that unfolded forms are not obligatory intermediates in FGF2 membrane translocation and secretion from cells. In conclusion, as illustrated in Fig. 1, it was clear that unconventional secretion of FGF2 occurs by direct translocation across plasma membranes, involves sequential interactions with the phosphoinositide $PI(4,5)P_2$ at the inner as well as heparan sulfates at the outer leaflet, and is based on a mechanism that not only is likely to be compatible but also rather requires FGF2 in a properly folded form. The latter aspect was interpreted as a potential quality control mechanism ensuring that only folded and, therefore, biologically active forms of FGF2 reach the extracellular space [45]. Thus, in 2009, the search for a novel type of protein translocation across membranes that is independent of protein-conducting channels began.

$PI(4,5)P_2$-Induced FGF2 Oligomerization and Membrane Pore Formation: A Transient Intermediate in FGF2 Membrane Translocation

As a starting point to obtain insight into the mechanism of FGF2 membrane translocation, we sought to conduct a global analysis of gene products involved in unconventional secretion of FGF2. In 2010, a genome-wide RNAi screen identifying a large set of candidate proteins potentially involved in FGF2 membrane translocation was completed. The bioinformatic analysis, validation and functional analysis of these proteins are still ongoing; however, we were lucky to identify and validate one gene product already during pilot experiments that turned out to play a key role, Tec kinase [47]. Both RNAi-mediated down-regulation and pharmacological inhibition of Tec kinase impaired FGF2 secretion. Moreover, biochemical experiments revealed a direct interaction and phosphorylation of FGF2 by Tec kinase at tyrosine residue 81 [47]. FGF2 variant forms lacking this tyrosine were found to be impaired in secretion and, most importantly, a phosphomimetic substitution of tyrosine 81 rendered FGF2 secretion from cells independent of Tec kinase providing conclusive evidence for its role in FGF2 secretion. The identification of Tec kinase was particularly intriguing as this protein contains a PH domain that mediates phosphoinositide-dependent recruitment to the inner leaflet of plasma membranes [49]. Tec kinase has a binding preference for $PI(3–5)P_3$ and this interaction causes activation of its enzymatic activity [49]. With this finding, principal components required for FGF2 membrane translocation were discovered: the phosphoinositides $PI(4,5)P_2$ and $PI(3–5)P_3$, the heparan sulfate chains of cell surface proteoglycans and Tec kinase. All of these factors are physically associated with the plasma membrane, the proposed site of FGF2 membrane translocation.

However, what is the function of Tec-kinase-mediated tyrosine phosphorylation in FGF2 membrane translocation? To tackle this problem, we decided to generate a recombinant form of FGF2 in which tyrosine 81 was substituted by an unnatural amino acid, pCMF (*p-c*arboxy*m*ethyl*p*henylalanine). This residue is a close structural mimetic of a phosphotyrosine; thus, we analyzed the biochemical and biophysical properties of this variant form of FGF2 in biochemical reconstitution experiments. Though occurring independently of the phosphomimetic residue, a key observation in these experiments was that binding to $PI(4,5)P_2$-containing membranes causes FGF2 to oligomerize [39]. Based on small fluorescent tracers and various kinds of artificial membranes including large and giant unilamellar vesicles, it became clear that FGF2 oligomers form membrane pores allowing for the passage of small molecules. In addition, transbilayer diffusion of membrane lipids was observed during FGF2-induced membrane pore formation suggesting a toroidal architecture [39]. Most intriguingly, a phosphomimetic substitution in FGF2 (FGF2-Y81pCMF) strongly increased the rate of membrane pore formation providing direct evidence that Tec-kinase-mediated phosphorylation of FGF2 facilitates membrane insertion of FGF2 oligomers. This, in turn, suggests that inhibition of FGF2 secretion in the absence of Tec kinase activity (e.g., following RNAi-mediated down-regulation) is due to inefficient membrane insertion of FGF2 oligomers as membrane translocation intermediates.

However, how are membrane-inserted FGF2 oligomers removed from the plasma membrane in order to appear on cell surfaces? The proposed mechanism of $PI(4,5)P_2$-induced membrane pore formation actually provides a simple explanation for our early findings establishing a role for cell surface heparan sulfates in the final step of FGF2 membrane translocation [42,43]. Since basic residues in FGF2 that are required for heparin binding are also needed

for binding to $PI(4,5)P_2$ (e.g., lysine 133; Ref. [36]), it is possible that binding of FGF2 to heparan sulfates *versus* $PI(4,5)P_2$ is mutually exclusive. At the same time, the affinity of FGF2 for heparan sulfates is about 100 times higher ($K_D \approx 10$ nM; Ref. [56]) compared to that of $PI(4,5)P_2$ ($K_D \approx 1$ μM; Ref. [37]). This suggests that, once FGF2 molecules become accessible to extracellular heparan sulfates as part of oligomers within a lipidic membrane pore, they can compete against $PI(4,5)P_2$ for binding to FGF2. We propose that this is the underlying mechanism by which cell surface heparan sulfates mediate the final step of FGF2 membrane translocation resulting in the exposure of FGF2 on cell surfaces. This model also provides a basis for directionality of FGF2 membrane translocation that is defined through sequential interactions of FGF2 with $PI(4,51)P_2$ at the inner leaflet and heparan sulfates at the outer leaflet. It also emphasizes that $PI(4,5)P_2$ is required not only for membrane recruitment of FGF2 but also to support oligomerization in the context of plasma membranes. Finally, due to its cone-shaped structure, $PI(4,5)P_2$ is likely to be important for the toroidal architecture of lipidic membrane pores induced by FGF2 oligomerization. Direct experimental evidence for this view comes from the fact that diacylglycerol, a membrane lipid with the opposite cone-shaped structure of $PI(4,5)P_2$, inhibits transbilayer diffusion of membrane lipids during membrane pore formation [39]. Thus, $PI(4,5)P_2$ is likely to be required to stabilize the high membrane curvature that is generated during the formation of a toroidal membrane pore (Fig. 2).

Unconventional Secretion—What Is It Good for?

When discussing unconventional protein secretion with colleagues or students, a regular question coming up is: What is it needed for, that is, why do cells need an alternative to the ER/Golgi-dependent secretory pathway? One way of looking into this was to analyze FGF2 variant forms that contain engineered signal peptides at their N-termini. Following expression in mammalian cells, we asked whether they are secreted in an ER/Golgi-dependent manner and whether the secreted population is biologically active [57]. The first question by no means was a trivial one since FGF2 is a lectin that, upon binding to heparan sulfate chains, tends to oligomerize. Thus, premature binding to its ligands in the lumen of the trans-Golgi network might cause aggregation resulting in a block of secretion and potentially even disintegration of Golgi structure. However, FGF2 containing a signal peptide derived from FGF4 was secreted with high efficiency [57], yet a remarkable difference to authentic FGF2 was observed. FGF2 secreted by the ER/Golgi-dependent pathway was no longer retained on cell surfaces but rather accumulated in the supernatant of cells. When analyzed by biochemical means, we realized that the signal-peptide-containing version of FGF2 was modified running as a rather undefined population with an apparent molecular mass of about 80 kDa. A biochemical analysis revealed that, when forced to travel through the lumen of the ER/Golgi system, FGF2 becomes post-translational modified by O-linked chondroitin sulfates. As a consequence, this form of FGF2 failed to interact with heparan sulfates on cell surfaces resulting in its accumulation in cellular supernatants [57]. Since binding of FGF2 to heparan sulfate proteoglycans is essential to form ternary signaling complexes containing FGF high-affinity receptors, FGF2 secreted through the ER/Golgi-dependent pathway is impaired in its biological activity. Based on these findings, it is a plausible hypothesis that unconventional secretion permits export from cells of unmodified forms of FGF2 that retain their biological activity. This concept may actually reflect a general aspect of unconventional secretory processes, the ability of mammalian cells to secrete proteins avoiding modifications that potentially occur in the lumen of the ER/Golgi system such as O-linked glycosylation.

Another interesting aspect emerges when one considers the evolution of the FGF protein family [58]. Four of the more than 20 members have been defined as so-called intracellular FGFs (FGF11–FGF14, termed iFGFs) that are not secreted from cells but are functioning solely inside cells. The iFGFs are believed to be the ancestors of the FGF family [58]. It appears possible that they were followed by FGF1 and FGF2, the two members that are secreted by unconventional means. Only later in evolution, signal-peptide-containing FGFs appeared complementing the FGF family as we know it today. This suggests that FGF1 and FGF2 were the first family members that managed to exit cells in order to execute new functions outside cells. However, how did FGF2 find its way into the extracellular space? Based on the mechanism proposed here (Fig. 2), one may wonder whether the ability of FGF2 to form lipidic membrane pores is related to membrane pores generated by some bacterial toxins [59] or by components of the apoptotic machinery that promote the formation of toroidal membrane pores in the outer membrane of mitochondria to cause programmed cell death [41,60]. In case of FGF2, however, these pores are not formed to destruct the membrane but rather to build transient intermediates of membrane translocation. Unconventional secretion may allow for a double life of proteins with functions in different localizations based on an equilibrium that can be dynamically changed by cells to match physiological needs. By contrast, signal-peptide-containing proteins can be considered professional secretory proteins that are doomed for quantitative secretion with their sole function in the extracellular space.

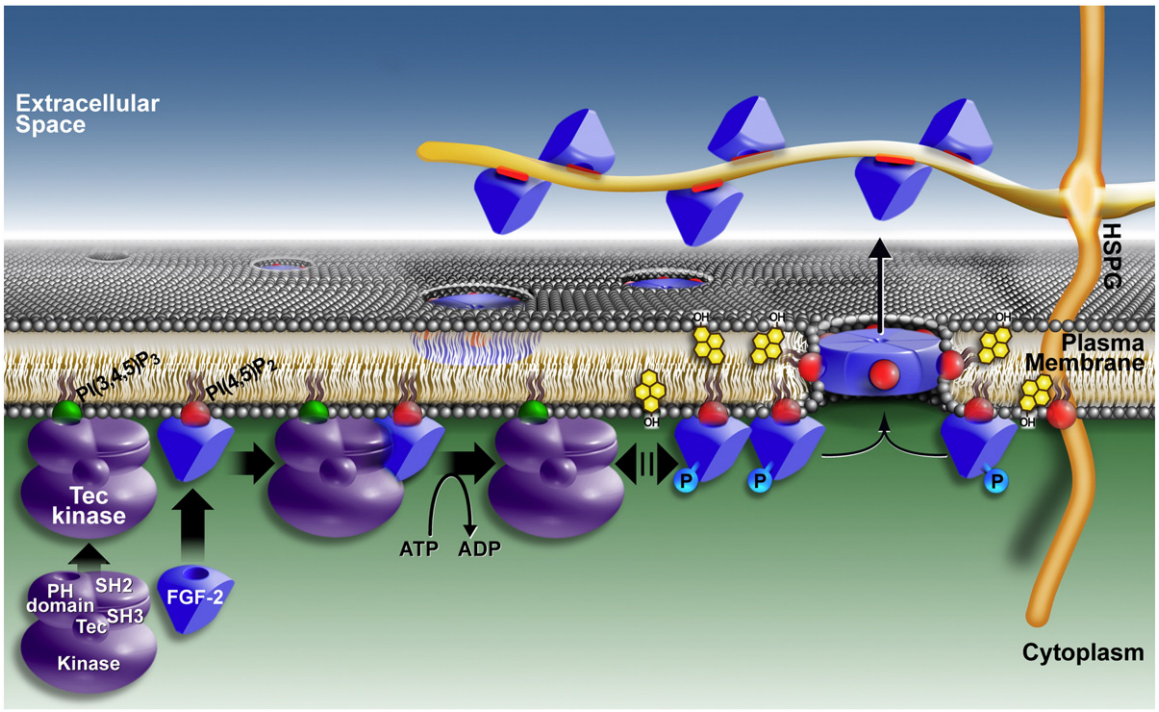

Fig. 2. A current view on the molecular mechanism of FGF2 membrane translocation as part of its unconventional secretory pathway.

What Is Next?

New experimental strategies are required to verify its hallmarks and predictions to challenge the model of FGF2 membrane translocation discussed in this review. A major aim will be to reconstitute a full cycle of FGF2 membrane translocation with chemically defined components. A promising approach will be to use giant unilamellar vesicles with heparin trapped in their lumen to mimic membrane-proximal cell surface heparan sulfates. This will allow us for testing the capability of fluorescently labeled species of phosphomimetic FGF2 to physically traverse the membrane under these conditions, a key prediction of the model shown in Fig. 2. In this context, it is also attractive to address other unconventional secretory proteins such as HIV (*h*uman *i*mmunodeficiency *v*irus)-Tat, a critical transactivator of transcription required for the life cycle of HIV [61]. In addition to its classical role in the nucleus of infected T cells, HIV-Tat was shown to be secreted [62,63] and is present in substantial amounts in the blood of HIV-infected individuals [64]. In cell-based experiments, it has been shown that HIV-Tat is indeed secreted from infected T cells using an ER/Golgi-independent pathway [65]. These studies further suggested that HIV-Tat is capable of translocation across plasma membranes, a process that depends on the ability of HIV-Tat to bind to the phosphoinositide PI(4,5)P_2 [65,66]. Another example is FGF1, the second FGF family member secreted by unconventional means [10]. Its secretion has been suggested to occur at the level of plasma membranes depending on binding to acidic phospholipids and local destabilization of membrane integrity [67]. As discussed above, it is not even clear whether the basic principle behind unconventional secretion of IL1β differs from that of FGF2. This is because the reported evidence for a role of endocytic subcompartments is of indirect nature. A critical shortcoming of experiments supporting a role of MVBs and exosomes for example is that IL1β so far has not been localized to transport vesicles whose nature as intermediates of this pathway has been demonstrated unambiguously [33]. Therefore, one may wonder whether the substantial structural similarity of mature IL1β to FGF2 and the typical disruption of plasma membrane integrity observed during LPS/ATP-triggered secretion of IL1β [68] indicates a core mechanism that resembles the one we propose for FGF2 (Fig. 2).

Other key aims in the analysis of the molecular mechanism by which FGF2 is secreted from cells will be to analyze the structure–function relationship of membrane-inserted FGF2 oligomers as translocation intermediates in unconventional secretion and the identification of further components of this pathway. In the long run, we aim at the development of pharmacological inhibitors against each molecular component and step of this process. Furthermore, this knowledge will allow for systematically addressing

the question whether indeed multiple, unrelated pathways of unconventional secretion exist or whether one common principle is behind this phenomenon with variations at the regulatory level depending on cell type and physiological conditions.

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Q&A with Walter Nickel

Q1

DM: *How widespread do you think unconventional protein secretion is, both across eukaryotic kingdom as well as within one particular cell? How helpful do you think systems approaches, like bioinformatics, proteomics and genome-wide protein localization screens, will be in identification of novel substrates that are secreted in an ER/Golgi-independent manner?*

WN: It is currently unclear as to whether unconventional secretion is a broad phenomenon across all kingdoms of life or whether it reflects very specialized properties of a small set of atypical secretory proteins. To date only one example is known in lower eukaryotes such as yeast and Dictyostelium. Beyond well characterized examples such as FGF2 and interleukin 1 beta, about 20 to 30 proteins in mammals and plants have been proposed to get secreted by unconventional means, however, convincing experimental evidence has not been established in all cases. Therefore, systematic and quantitative studies on the extracellular proteome of various cell types along with advanced bioinformatical tools are indeed required to obtain comprehensive insight into this phenomenon.

Q2

DM: Elucidation of the majority of intracellular protein sorting pathways started with analyses of one or at best few substrates to in the end reveal mechanisms used by tens or even hundreds of different proteins. How likely do you think there will be "convention" in unconventional protein secretion?

WN: To date, two principal pathways of unconventional protein secretion appear to exist, one being exemplified by FGF2 and the other by interleukin 1 beta. Most likely, most of the proteins to be discovered by future studies as unconventionally secreted proteins will make use of one of these two pathways, so in that sense, there will be some degree of convention as part of unconventional secretion. However, it cannot be excluded that additional pathways of ER/Golgi-independent protein secretion exist. Unconventional secretion in general appears to be a heterogenous phenomenon.

Q3

DM: *Though ER and mitochondrial membranes clearly contain protein conducting channels that allow trasport of only largely, if not completely, unfolded proteins, translocation of fully folded proteins has been described for translocation across peroxisomal and thylakoid membranes. Interestingly, in both these cases, transient pore models have been suggested, similar to what you have proposed for FGF2 translocation. Do you think these are examples of convergent evolution or can one find some homologies between the systems as well?*

WN: Indeed, protein import into peroxisomes has been shown to involve the formation of membrane pores along with translocation substrates that remain folded during transport across the membrane. However, there is no experimental evidence that these translocation intermediates themselves are capable of forming these pores. Rather, a number of gene products have been identified to build the machinery for perioxisomal protein import. By contrast, as outlined in this review, it is a central aspect of the mechanism of FGF2 membrane translocation that, upon PI(4,5)P2-dependent oligomerization and membrane insertion, FGF2 itself forms membrane pores, i.e. it represents both cargo and machinery. In this sense, protein import into peroxisomes and FGF2 translocation across plasma membrane do not appear to be related mechanistically.

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Abbreviations used:
ER, endoplasmic reticulum; FGF2, fibroblast growth factor 2; IL1β, interleukin 1β; MVB, multi-vesicular body.

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