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Decoding how receptor tyrosine kinases (RTKs) mediate nuclear calcium signaling

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Abstract

Calcium (Ca^{2+}) is a highly versatile intracellular messenger that regulates several cellular processes. Although it is unclear how a single-second messenger coordinates various effects within a cell, there is growing evidence that spatial patterns of Ca^{2+} signals play an essential role in determining their specificity. Ca^{2+} signaling patterns can differ in various cell regions, and Ca^{2+} signals in the nuclear and cytoplasmic compartments have been observed to occur independently. The initiation and function of Ca^{2+} signaling within the nucleus are not yet fully understood. Receptor tyrosine kinases (RTKs) induce Ca^{2+} signaling resulting from phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis and inositol 1,4,5-trisphosphate (InsP3) formation within the nucleus. This signaling mechanism may be responsible for the effects of specific growth factors on cell proliferation and gene transcription. This review highlights the recent advances in RTK trafficking to the nucleus and explains how these receptors initiate nuclear calcium signaling.

Graphical Abstract

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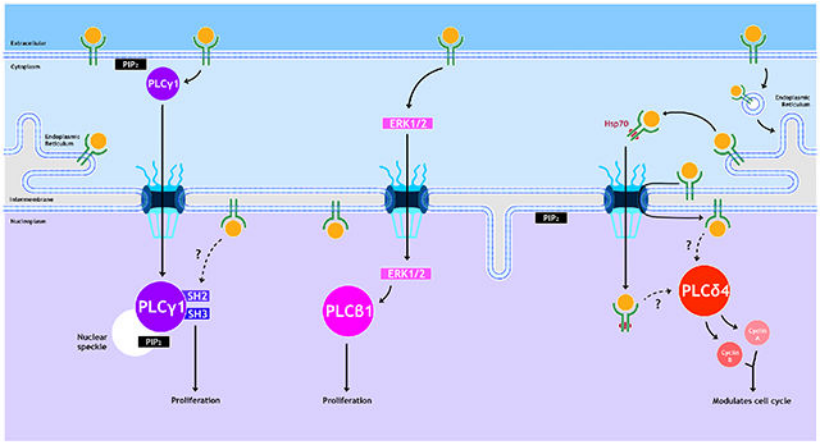

Keywords

Nucleus; calcium signaling; phospholipase C; receptor tyrosine kinases; Nucleoplasmic reticulum; nuclear calcium

1. Introduction

Intracellular Ca^{2+} signaling can regulate several cellular processes such as gene transcription, cell death, and proliferation (Dhaouadi et al., 2023; Lu et al., 2023; Thiel et al., 2021). A wide range of Ca^{2+} signaling systems transmit precise spatial and temporal signals that are essential for the regulation of specific functions within a cell (Berridge, 2007). Ca^{2+} signaling in the nuclear and cytoplasmic compartments may be independently regulated (Bootman et al., 2009). The precise mechanisms that lead to localized increases in free calcium levels within the nucleus are yet to be fully elucidated.

Studies have reported that receptor tyrosine kinases (RTKs) translocate to the nucleus in a ligand-dependent manner (Chen et al., 2020; Faria et al., 2016). These receptors can activate phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP2) to generate two intracellular products: diacylglycerol, an activator of protein kinase C, and inositol 1,4,5-trisphosphate (InsP3), a universal Ca^{2+}-mobilizing second messenger (de Miranda et al., 2019; Irvine, 2016; Michell, 1995). The interior of the nucleus contains all the Ca^{2+} signaling molecules required to generate nuclear Ca^{2+} signaling (Gomes et al., 2021). The translocation of RTK to the nucleus indicates a mechanism to increase Ca^{2+} in the nucleus and a new paradigm to explain the pathways that promote nuclear-specific Ca^{2+} signals. A better understanding of how nuclear Ca^{2+} signals originate and whether they can be separated from cytosolic increases is demonstrated by the fact that nuclear Ca^{2+} and cytoplasmic Ca^{2+} can have distinct functions, such as controlling gene transcription and cell proliferation (Li et al., 2014; Rodrigues et al., 2007). This review offers a concise overview of recent advances in the route of RTK to the nucleus and elucidates how these receptors can initiate nuclear calcium signaling.

2. InsP3 mediates nuclear calcium signaling via the nucleoplasmic reticulum.

The nuclear envelope (NE) is a structure comprising the outer nuclear membrane (ONM) and inner nuclear membrane (INM), which are both lipid bilayers. Nuclear envelope invaginations have been observed in various cell types since the 1960s (Bourgeois et al., 1979) and have been classified into two main types (Malhas et al., 2011). Type I invaginations involve invaginations of only the INM into the nucleoplasm, whereas type II invaginations implicate both the NE membranes (Figure 1). These invaginations are observed under both normal and pathological cellular conditions (Bourgeois et al., 1979; Malhas et al., 2011; Schoen et al., 2017). The NE lumen is contiguous with the endoplasmic reticulum (ER). Therefore, Ca^{2+} can freely diffuse from the ER lumen to the lumen of the nuclear envelope. The demonstration that these two organelles are interconnected calcium stores can be observed using low-affinity fluorescent Ca^{2+} probes (Gerasimenko et al., 1995; Petersen et al., 1998). In the context of nuclear calcium signaling, NE invaginations are referred to as nucleoplasmic reticulum (NR). Notably, the NR is not a distinct organelle but rather a term that emphasizes the role of NE in calcium release. This term has also been used to highlight the processes by which NE invaginations are formed (Drozdz et al., 2017).

The most extensively studied mechanism of nuclear Ca^{2+} release involves activation of inositol 1,4,5-trisphosphate receptors (ITPRs). Vertebrate genomes encode three distinct isoforms of ITPRs (Prole and Taylor, 2016). All ITPR isoforms are present in both NE membranes of many cell types (Echevarría et al., 2003; Huh and Yoo, 2003; Yoo et al., 2005). The role of NR in InsP3-mediated Ca^{2+} signaling was characterized in experiments performed in the SKHep1 cell line by Echevarria et al. (2003), who demonstrated that local photo-release of caged-InsP3 in the nucleus resulted in a small increase in Ca^{2+} in the nucleoplasm. This observation suggests that the nucleus contains ITPRs but raises the question of whether endogenous nuclear PIP2 hydrolysis occurs in a ligand/receptor-dependent manner. Robin Irvine and colleagues demonstrated that insulin-like growth factor I (IGF-I) treatment induces a rapid reduction in PIP2 mass within Swiss 3T3 cell nuclei (Divecha et al., 1991; Michell, 1992). Subsequently, studies showed that hepatocyte growth factor, insulin and epidermal growth factor (EGF) can induce nuclear PIP2 hydrolysis and InsP3 generation (de Miranda et al., 2019; Gomes et al., 2008; Rodrigues et al., 2008).

Now, which nuclear PLCs can mediate nuclear PIP2 hydrolysis? In mammals, the PLC family is comprised of 13 isozymes: PLCβ (1, 2, 3, and 4), PLCγ (1 and 2), PLCδ (1, 3, and 4), PLCε, PLCη (1 and 2), and PLCζ (Kanemaru and Nakamura, 2023). Interestingly, certain PLC isoforms such as PLCβ1 (Cocco et al., 1999; Manzoli et al., 1997; Martelli et al., 1992), PLCγ1 (Bertagnolo et al., 1995; Ferguson et al., 2007), PLCδ1 (Yagisawa, 2006) and PLCδ4 (de Miranda et al., 2019; Kunrath-Lima et al., 2018) are localized within the cell nucleus. After calcium is released from the ER or NR, the restoration of calcium homeostasis is crucial. Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA) plays a pivotal role in the active transport of Ca^{2+} within the ER. These Ca^{2+} pumps are also present in NE (Collado-Hilly et al., 2010; Lanini et al., 1992).

Remarkably, all signaling cascade components that lead to Ca^{2+} mobilization are present in the nucleus (Gomes et al., 2021). This enables autonomous regulation of nuclear Ca^{2+}. Nonetheless, a significant point of contention in the field of nuclear Ca^{2+} signaling revolves around whether Ca^{2+} transients originate independently within the nucleoplasm and whether they can be differentiated from cytosolic Ca^{2+} fluctuations. The presence of RTKs within the nucleus is a crucial factor to investigate when examining whether nuclear calcium can be autonomously generated. Recent studies have highlighted that RTKs can be transported from the plasma membrane to the nucleus to initiate calcium signaling (de Miranda et al., 2019; Faria et al., 2016; Gomes et al., 2008; Rodrigues et al., 2008). In this review, we summarize the routes of RTK transport to the nucleus and its role in nuclear calcium signaling.

3. RTKs trafficking to the nucleus.

Nuclear trafficking of EGF receptor (EGFR) family members has been the most extensively studied. In this context, we highlight EGFR nuclear transport as the primary illustration of RTK trafficking. We focused on the nuclear transport mechanisms of full-length receptors that contain a transmembrane domain.

Tyrosine kinase receptors are predominantly activated on the cell surface (Carpenter and Liao, 2009). These receptors undergo conformational changes upon ligand binding, leading to dimerization and autophosphorylation of their cytoplasmic tyrosine kinase domains (Schlessinger, 2014). Autophosphorylation significantly enhances the kinase activity of RTKs and the endocytic process begins. The internalization of ligand-activated RTKs depends on either clathrin-dependent or - independent endocytosis.

Nuclear EGFR accumulation is attenuated by the downregulation of caveolin and clathrin (Bazzani et al., 2018; De Angelis Campos et al., 2011; Lo et al., 2006). Several studies have reported that nuclear EGFR accumulation is abrogated in cells that experience inhibition of dynamin, a key protein involved in membrane fission during clathrin-coated pit formation (Bazzani et al., 2018; De Angelis Campos et al., 2011). However, exceptions to the caveolin- and clathrin-mediated endocytosis pathways have been reported (Dunham-Ems et al., 2006; Myers et al., 2003; Porębska et al., 2018).

Internalized EGFR is transported to the Golgi apparatus by syntaxin 6 (Du et al., 2014; Zhang et al., 2019), a protein that mediates retrograde endosome-to-trans-Golgi transport (Laufman et al., 2011). Moreover, disturbances in the Golgi apparatus caused by brefeldin A reduce nuclear transport of EGFR (Wang et al., 2010; Zhang et al., 2019). Subsequently, EGFR destined for the nucleus is transported from the Golgi to the ER. Mutation of the Golgi-to-ER retrograde transport coat protein I (COP-I) complex disrupts the nuclear translocation of EGFR (Wang et al., 2010), suggesting that EGFR remains in membranous vesicles during transport to the ER.

Two main mechanisms have been reported to contribute to the nuclear trafficking of EGFR family receptors from the ER to the nucleus (Carpenter and Liao, 2009; Chen et al., 2020). First, EGFR localized in the ER is extracted from the lipid layers into the cytoplasm via the ER-associated protein degradation (ERAD) pathway (Carpenter and Liao, 2009). While

there is currently no well-established RTK trafficking mechanism that directly removes transmembrane receptors from their lipid bilayer, a protein translocon and Hsp70 are involved in facilitating this process. In particular, the Sec61 translocon, situated within the ER, is recognized for its role in orchestrating the transport of specific extracellular toxins from the cell surface to the cytoplasm as an integral component of the ERAD pathway (Carpenter and Liao, 2013). The knockdown of a Sec61 abrogates nuclear localization of EGFR. Furthermore, Hsp70 is necessary for the retro-translocation process by interacting with the receptor transmembrane domain, thereby maintaining the receptor in a soluble state following its extraction from the ER membrane. Subsequently, cytoplasmic EGFR is transported to the nucleus via the nuclear pore complex (Figure 2) (Carpenter and Liao, 2013). Second, the nuclear transport of EGFR can occur via a mechanism called integral trafficking from the ER to NE (INTERNET), which refers to the INM-targeting process for the ER-to-NE transport of integral membrane proteins (Chen et al., 2020). This pathway may be an important transport mechanism utilized by full-length RTKs to reach NR. The mechanism governing the localization of integral membrane proteins to the INM depends on several factors, including the dimensions of the extraluminal domains, the presence of nuclear localization signals (NLSs), and the binding affinity of NLSs for importin nuclear transport factors (Lusk et al., 2007).

Proteins containing NLS are transported into the nucleus by forming complexes with either importin α/β or importin β (Jans et al., 2000; Weis, 2003). EGFR translocates to the nucleus via importin β-dependent mechanisms (Giri et al., 2005; Lo et al., 2005; Reilly and Maher, 2001). It has been shown that EGFR has a tripartite NLS (Hsu and Hung, 2007) and that importin-β interacts with it (Giri et al., 2005; Lo et al., 2005). Furthermore, the colocalization of importin-β and EGFR in INM has been demonstrated using electron microscopy (Lo et al., 2005; Wang et al., 2010). In addition, EGFR interacts with the translocon Sec61-β at the INM (Carpenter and Liao, 2009; Wang et al., 2010). It is important to note that full-length EGFR colocalizes with Sec61-β (Carpenter and Liao, 2009). The transport of EGFR from the ER to the INM remains membrane-bound and then is released by translocon Sec61 into the nucleoplasm (Figure 2) (Wang et al., 2010).

4. RTKs can activate PLCs.

Receptor tyrosine kinases play an important role in the activation of nuclear PLCs. The mechanism by which these receptors stimulate this reaction involves tyrosine-phosphorylation-dependent activation (Schlessinger, 2014). RTKs can trigger nuclear PLCs through two distinct mechanisms: indirectly or directly. Regarding these processes, four isoforms of PLC are localized within the nucleus: PLCβ1 (Cocco et al., 1999; Manzoli et al., 1997; Martelli et al., 1992), PLCγ1 (Bertagnolo et al., 1995; Ferguson et al., 2007), PLCδ1 (Yagisawa, 2006) and PLCδ4 (de Miranda et al., 2019; Kunrath-Lima et al., 2018).

PLCβ1, PLCγ1 and PLCδ4 are reported to be activated by RTKs, but only PLCγ is directly phosphorylated by various RTKs such as the vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and EGFR (Mandal et al., 2021). Two mammalian isoforms of PLCγ have been identified: PLCγ1, which is ubiquitously expressed, and PLCγ2, primarily expressed in hematopoietic cells (Kanemaru

and Nakamura, 2023). Similar to other PLC isoforms, PLCγ1 is a Ca^{2+}-dependent phosphodiesterase with a core structure consisting of an N-terminal PH domain, an array of EF-hands, an N-terminal PH domain, a catalytic TIM domain divided into X- and Y-boxes, and a C-terminal C2 domain (Mandal et al., 2021). The uniqueness of the PLCγ isoform lies in the fact that this enzyme is characterized by the insertion of a motif consisting of a split PH domain flanked by two tandem SH2 and SH3 domains between the X and Y halves of the catalytic TIM domain (Kanemaru and Nakamura, 2023). Upon activation by external stimuli, RTKs undergo dimerization and autophosphorylation. This process allows RTKs to specifically interact with the SH2 domain of PLCγ (Mandal et al., 2021).

PLCγ1 has been observed in the nuclei of rat liver cells and various other cell lines, including REF52 (rat embryo fibroblasts), HCT116 (human colon carcinoma cells), SaOS-2 (human osteosarcoma) and PC12 (rat adrenal pheochromocytoma cells) (Bertagnolo et al., 1995; Ferguson et al., 2007; Mazzoni et al., 1992; McBride et al., 1991; Santi et al., 1994; Ye et al., 2002). Despite the lack of a recognizable nuclear localization signal, the PLCγ1 isoform primarily resides in the cytoplasm but dynamically shuttles to the nucleus upon receiving mitogenic stimuli (Ferguson et al., 2007). PLCγ1 was detected in isolated nuclei of rat liver cells by western blotting and immunoprecipitation. Moreover, a phosphoinositidase activity assay demonstrated that PLCγ1 was active in rat liver nuclei (Bertagnolo et al., 1995).

Confocal microscopy and immunocytochemical analysis revealed an increase in PLCγ1 nuclear content in both tissue sections and isolated nuclei after partial hepatectomy of the rat liver. These alterations have been associated with InsP3 synthesis, resulting from phosphodiesterase activity and PIP2 restoration under conditions that enhance PIP kinase activity (Neri et al., 1997). In rat liver nuclei, PLCγ1 is tightly associated with the nuclear matrix and lamina (Bertagnolo et al., 1995). The tight association of this PLC isoform with the nuclear matrix is consistent with the evidence that this enzyme can bind cytoskeletal proteins. (Neri et al., 1997). Researchers have demonstrated that the SH3 domain is essential for PLCγ1 to function as a nucleotide exchange factor (GEF) for nuclear phosphatidylinositol-3-OH kinase enhancer (PIKE). This mechanism enables PIKE to activate nuclear phosphatidylinositol-3-OH kinase (PI3K), thereby promoting cell proliferation (Ye et al., 2002). Nuclear PIK3 localizes to both the nuclear envelope and internal nuclear matrix, which fits with the localization of its substrate PIP2 (Ye, 2006). Interestingly, the mitogenic activity of PLCγ1 is not dependent on its phospholipase activity but requires its SH3 domain (Huang et al., 1995; Smith et al., 1994; Ye et al., 2002).

Another PLC isoform activated by RTKs is PLCγ1. This PLC isoform exists as two isoenzymes: 150 kDa (PLCγ1a) and 140 kDa (PLCγ1b), which differ only in a short region of their carboxyl-termini (Bahk et al., 1994). The NLS of this enzyme is common to both isoforms and is determined by a cluster of lysine residues (between positions 1055 and 1072) (Kim et al., 1996). PLCγ1b exists almost entirely in the nucleus, whereas PLCγ1a localizes to the nucleus and plasma membrane (Bahk et al., 1998). Activation of PLCγ1 by IGF-1-dependent stimulation in Swiss 3T3 cells occurs via nuclear translocation of extracellular regulated kinase (ERK) 1/2, which phosphorylates PLCβ1 at serine 982 (Xu

et al., 2001). PLCβ1 knockdown abrogated IGF-I mitogenic response in Swiss 3T3 cells, indicating a role for this enzyme in cell proliferation (Manzoli et al., 1997).

New evidence suggests that phosphorylated receptors, such as the hepatocyte growth factor receptor (HGFR/c-Met), insulin receptor and EGFR, can translocate from the cytoplasm to the nucleus to activate InsP3-mediated calcium signals (de Miranda et al., 2019; Gomes et al., 2008; Rodrigues et al., 2008). It has been demonstrated that HGFR can hydrolyze PIP2 and generate InsP3 within the nucleus, which in turn can release calcium in the nucleoplasm (Gomes et al., 2008). This mechanism differs from Ca^{2+} signaling induced by G protein-coupled receptors (GPCRs). Specifically, Ca^{2+} signaling triggered by HGFR reached its peak after approximately 200 s, whereas GPCRs achieved this response in less than a second. The peak of HGFR nuclear translocation also occurred after 200 seconds. Furthermore, biotinylated HGFR on the cell surface before stimulation with HGF is recovered from the nucleus after stimulation. These findings suggested that HGFR translocates from the plasma membrane to the nucleus and may play a role in direct nuclear InsP3 formation (Gomes et al., 2008). Live cell studies conducted on SKHep1 cells revealed that EGFR reached its peak within the nucleus after 10 min of exposure to EGF (De Angelis Campos et al., 2011).

Confocal microscopy was employed to validate the nuclear localization of the EGF/EGFR complexes within these cells. Subsequently, this technique was used to monitor the trajectory of labeled EGF. The acquired images revealed that the ligand/receptor complex was present in both the cytoplasm and nucleus. These observations demonstrate that both total and activated EGFR translocate to the nucleus upon EGF stimulation, suggesting that EGF can migrate with its receptor to the nucleus (De Angelis Campos et al., 2011). It was later demonstrated that EGF-induced Ca^{2+} signaling was inhibited when EGFR translocation was compromised. Additionally, nuclear Ca^{2+} signals were attenuated by selective buffering of InsP3 within the nucleus. Notably, EGF triggered the hydrolysis of nuclear PIP2, a process facilitated by intranuclear PLCδ4 rather than PLCγ1. Furthermore, protein kinase C, a downstream target of EGF, exhibits activity in the nuclei of stimulated cells. PLCδ4 and InsP3 also modulate cell cycle progression by regulating the expression of cyclins A and B1 (de Miranda et al., 2019). These findings support the notion that EGF-induced nuclear signaling is mediated by nuclear PLCδ4.

5. Conclusions

Receptor Tyrosine Kinases play a crucial role in the regulation of various cellular processes including metabolism, motility, differentiation and proliferation (Schlessinger, 2014). Dysregulation of RTK signaling is associated with human diseases, notably cancer (Chen et al., 2020). The mechanism by which RTKs activate nuclear PLC involves direct or indirect modulation (Gomes et al., 2021). Among the 13 PLC isoforms, PLCγ1 is the only one that can be directly activated by RTKs (Kim et al., 2000). However, this isoform is primarily localized in the cytoplasm. After activation, PLCγ1 translocates to the nucleus (Ferguson et al., 2007). The best-known mechanism for direct nuclear PLC activation is via PLCβ1, which undergoes phosphorylation due to nuclear translocation of ERK following stimulation by IGF-1 (Cocco et al., 2002). Recent evidence suggests that phosphorylated

receptors, such as insulin, epidermal growth factor, and hepatocyte growth factor receptors, can also translocate to the nucleus (de Miranda et al., 2019; Gomes et al., 2008; Rodrigues et al., 2008). Therefore, InsP3-mediated nuclear calcium signaling is activated. The presence of RTKs within the nucleus implies that these receptors may directly mediate nuclear PLC activation. Although evidence, such as the kinetics of c-Met and EGFR translocation coinciding with the peak of calcium levels, suggests that these receptors could play a role in nuclear PLC activation, direct activation of nuclear PLCs by RTKs remains to be conclusively demonstrated (de Miranda et al., 2019; Gomes et al., 2008).

The cytoplasm and nucleus exhibit similarities in InsP3-mediated calcium signaling, which stems from the molecules involved. Research has demonstrated that insulin receptors and EGFR are initially homogeneously distributed on the cell surface and these receptors can aggregate into patches on the cell membrane (Schechter et al., 1979; Schlessinger et al., 1978b, 1978a). Notably, EGFR and its ligand also aggregate within the nucleus (Faraco et al., 2018). The aggregation and internalization of hormone-receptor complexes have been associated with specific aspects of hormone action (Schlessinger et al., 1978b), which may be crucial for the nuclear actions of RTKs. To gain deeper insights into the calcium signaling process in the nucleus, it is essential to scrutinize each molecule involved in this pathway from a novel perspective. For instance, nuclear EGFR can leave the inner nuclear envelope membrane and function as a transcription factor (Chen et al., 2020). Interestingly, PIP2, which is also involved in gene expression, is present in nuclear compartments that are not bound to membranes (Boronenkov et al., 1998). Therefore, further investigations are necessary to determine whether nuclear RTKs directly participate in nuclear PIP2 hydrolysis.

In summary, our understanding of how RTKs govern nuclear PLCs has grown substantially. As we explored how RTKs affect nuclear PLC regulation, intriguing questions emerged. For example, how does RTK-mediated InsP3 signaling influence chromatin organization within the nucleus? Understanding this interplay may reveal the novel mechanisms of gene regulation. Additionally, how does the spatial distribution of PLCs within the nucleus affect their function? Is there preferential localization near certain genomic regions? As we continued our scientific journey, these questions motivated us toward exciting breakthroughs in understanding PLC-driven nuclear processes. Unraveling the specific role of RTKs in nuclear PLC activation remains a captivating challenge for future research.

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- Ca^{2+} is a highly versatile intracellular messenger that orchestrates several cellular processes.
- Ca^{2+} signaling patterns vary across different cell regions, and signals in the nuclear and cytoplasmic compartments can occur independently.
- Receptor tyrosine kinases (RTKs) induce Ca^{2+} signaling by triggering phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis and inositol 1,4,5-trisphosphate (InsP3) formation within the nucleus.
- This signaling mechanism likely mediates the effects of specific growth factors on cell proliferation and gene transcription.
- This review highlights recent breakthroughs in understanding how RTKs traffic to the nucleus to initiate nuclear calcium signaling, shedding light on this critical cellular process.

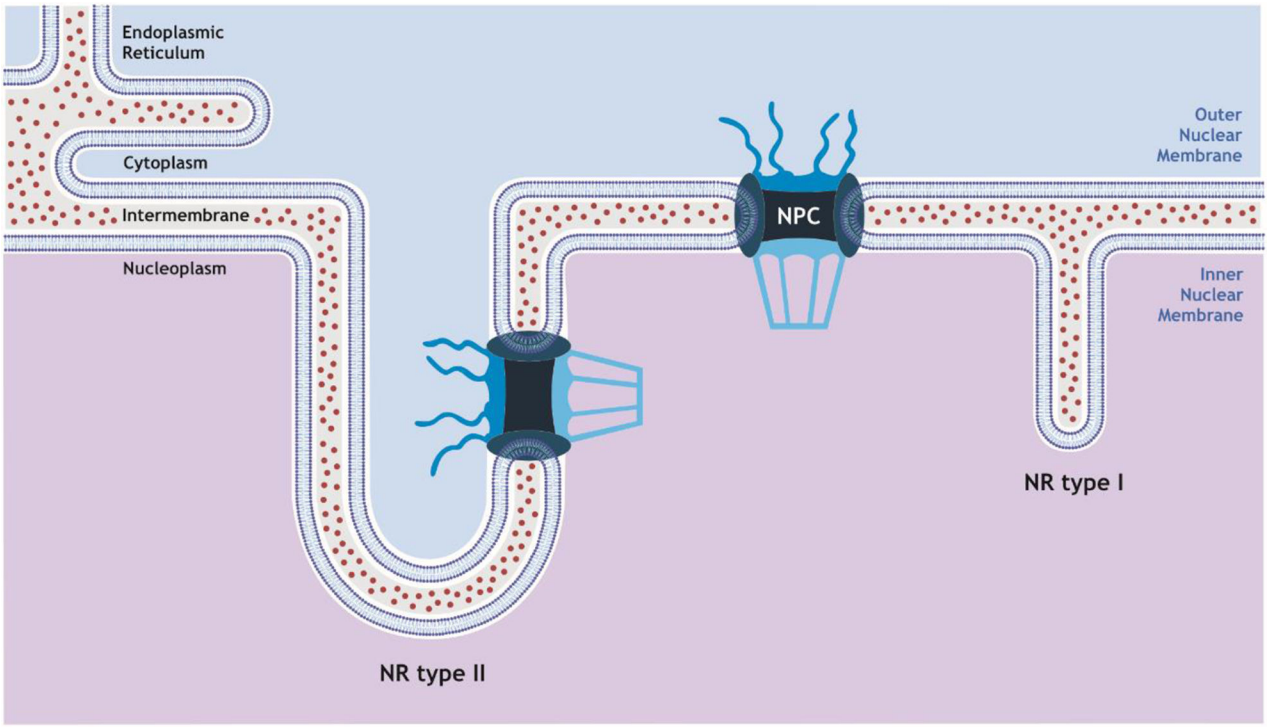

Figure 1. Types of membrane invaginations in the nucleoplasmic reticulum.

The nuclear envelope (NE) intermembrane space is contiguous with the endoplasmic reticulum (ER). Therefore, Ca^{2+} can freely diffuse between ER lumen and nuclear membrane lumen. The nuclear envelope can form invaginations. These invaginations, in the context of nuclear calcium signaling, are termed the nucleoplasmic reticulum (NR) and have been classified into two types: Type I invaginations, in which the inner nuclear membrane (INM) invaginates the nucleoplasm. Type II invaginations occur when both the INM and outer nuclear membranes propagate into the nucleoplasm. Ca^{2+} is represented by red spheres. NPC = Nuclear Pore Complex.

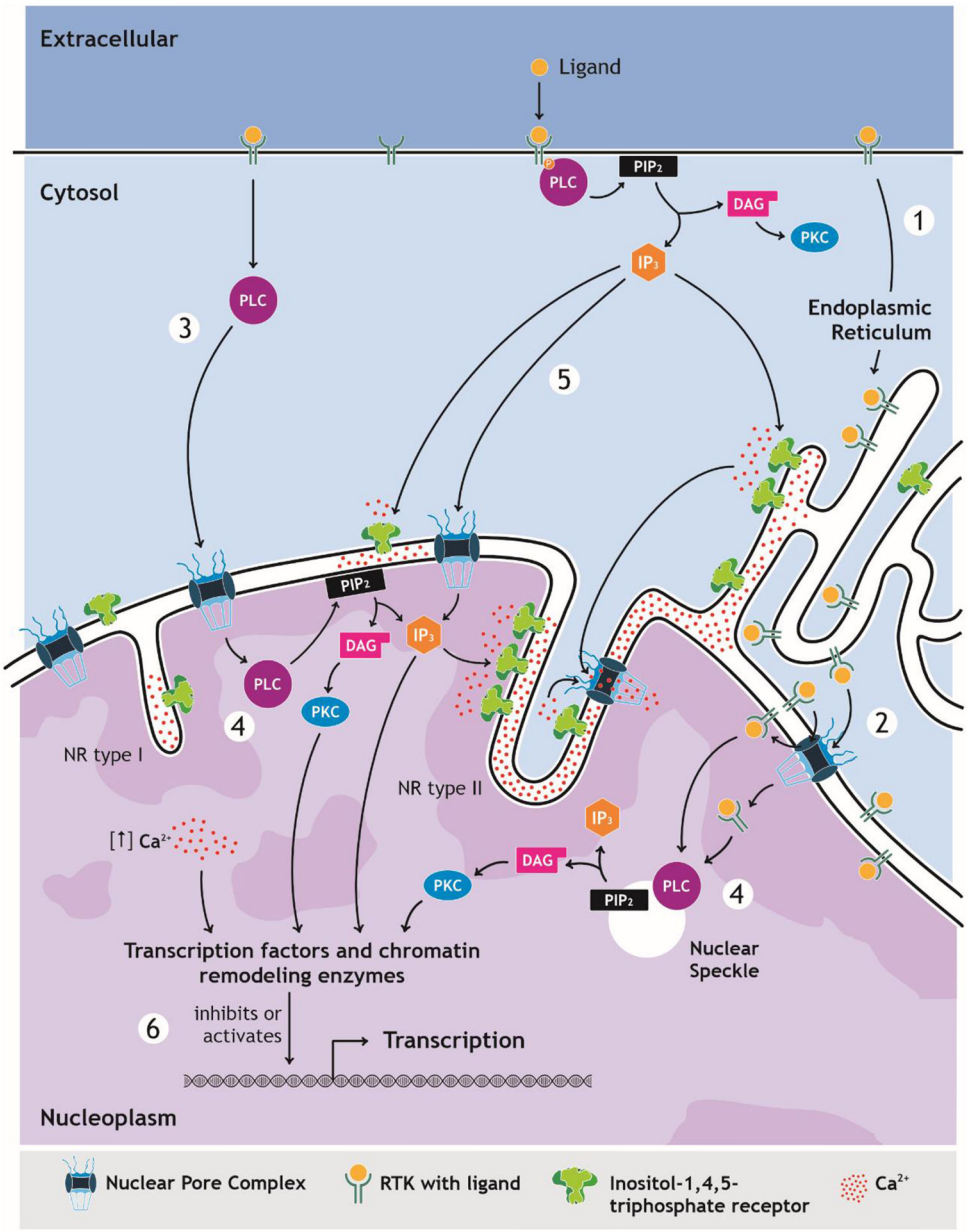

Figure 2. Pathways regulating nuclear RTK translocation and PLC Activation.

Receptor tyrosine kinases (RTKs) translocate to the nucleus. Two main mechanisms have been reported to contribute to the nuclear trafficking of EGFR. (1) First, the EGFR localized in the ER is extracted from the lipid layers into the cytoplasm via the ER-associated protein degradation (ERAD) pathway. Sec61 translocon and Hsp70 are involved in this process to transport receptors from the ER membrane to the cytoplasm and to stabilize the transmembrane domain of the receptors, respectively. Subsequently, cytoplasmic EGFR is transported to the nucleus via the nuclear pore complex (NPC). (2) Second, nuclear trafficking of EGFR can occur via a mechanism called integral trafficking from the ER to the NE (INTERNET). The transport of EGFR from the ER to INM remains membrane-bound and the receptors are released by Sec61 into the nucleoplasm. (3) PLCs can translocate to

the nucleus to generate InsP3 or nuclear PLCs could be directly activated. For example, ERK can translocate to the nucleus to phosphorylate PLCβ1 (4) Nuclear PLCs can hydrolyze PIP2 to generate InsP3 to release calcium from NR. InsP3 receptors (ITPRs) are found in both the outer nuclear membrane (ONM) and inner nuclear membrane (INM). (5) Nuclear calcium levels can also be mediated by InsP3 diffusion through NPC, which in turn activates nuclear ITPRs. (6) Nuclear calcium mediates transcription and cell proliferation.