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Nuclear trafficking of the epidermal growth factor receptor family membrane proteins

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Abstract

Multiple membrane-bound receptor tyrosine kinases (RTKs), such as the epidermal growth factor receptor (EGFR) and ErbB-2, have been reported to be localized in the nucleus, where emerging evidence suggests that they are involved in transcriptional regulation, cell proliferation, DNA repair and chemo- and radio-resistance. Recent studies have shown that endocytosis and endosomal sorting are involved in the nuclear transport of cell surface RTKs. However, the detailed mechanism by which the full-length receptors embedded in the endosomal membrane travel all the way from the cell surface to the early endosomes and pass through the nuclear pore complexes is unknown. This important area has been overlooked for decades, which has hindered progress in our understanding of nuclear RTKs' functions. Here, we discuss the putative mechanisms by which EGFR family RTKs are shuttled into the nucleus. Understanding the trafficking mechanisms as to how RTKs are transported from the cell surface to the nucleus will significantly contribute to understanding the functions of the nuclear RTKs.

Keywords

EGFR family receptors; nuclear trafficking; integral membrane proteins

Introduction

Epidermal growth factor receptor (EGFR) family proteins, including EGFR (ErbB-1/HER-1), ErbB-2 (HER-2/neu), ErbB-3 (HER-3) and ErbB-4 (HER-4), have been recognized as oncogenic proteins that are involved in cancer initiation, tumor growth/progression, metastasis and poor patient outcome. Among the EGFR family members, overexpression or constitutive activation of EGFR, ErbB-2 and ErbB-3 is frequently found in many human cancers (Yarden and Sliwkowski, 2001; Irmer *et al.*, 2007; Gazdar, 2009; Hynes and MacDonald, 2009; Linardou *et al.*, 2009). In contrast, ErbB-4 appears to be associated with growth suppression and improved patient prognosis in breast cancer (Jones, 2008; Muraoka-Cook *et al.*, 2008).

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Conflict of interest
The authors declare no conflict of interest.

EGFR family proteins are cell surface receptors that associate with tyrosine kinase activity, except ErbB-3. Activation of these EGFR family receptors by ligand binding results in receptor homo- or hetero-dimerization and subsequent tyrosine kinase activation. However, there is no known ligand that binds to homo-dimer of ErbB-2 with high affinity. Activated EGFR family receptors recruit their substrates and phosphorylate them, leading to the activation of downstream cascades of signaling molecules, such as phosphatidylinositol-3 kinase, mitogen-activated protein kinase, signal transducer and activator of transcription (STAT) and phospholipase C. These signaling pathways downstream of activated EGFR have a critical role in aggressive tumor behaviors, such as increased cell proliferation, metastasis and chemotherapeutic resistance (Yarden, 2001; Citri and Yarden, 2006; Lo *et al.*, 2006b; Huang *et al*., 2009). Therefore, EGFR family receptors have been intensely considered as therapeutic targets, and two major classes of anti-ErbB therapies, ectodomain-binding antibodies and small-molecule tyrosine kinase inhibitors, have been developed, with promising clinical outcomes (Hynes and Lane, 2005; Ciardiello and Tortora, 2008; Sequist and Lynch, 2008; Di Cosimo and Baselga, 2010; Esteva *et al.*, 2010).

Membrane receptors in the nucleus

In addition to the traditional cytoplasmic EGFR signaling pathways, evidence from several groups indicates that EGFR family receptors can be shuttled from the cell surface to the nucleus, where they transduce signals (Lin *et al*., 2001; Ni *et al*., 2001; Offterdinger *et al*., 2002; Wells and Marti, 2002; Giri *et al*., 2005; Dittmann *et al*., 2005a; Edwards *et al*., 2006; Lo and Hung, 2006; Massie and Mills, 2006; Wang *et al.*, 2006; Chen and Nirodi, 2007; Das *et al.*, 2007; Kim *et al.*, 2007; Mosesson *et al.*, 2008; Wanner *et al.*, 2008; de la Iglesia *et al.*, 2008; Carpenter and Liao, 2009; Li *et al.*, 2009; Wang and Hung, 2009).

Nuclear localization of EGFR

Nuclear EGFR was first observed in hepatocytes during liver regeneration (Marti *et al.*, 1991; Marti and Hug, 1995; Marti and Wells, 2000). In line with these studies, EGFR ligands, including EGF and protransforming growth factor-α, were also found in the nucleus of proliferating hepatocytes (Raper *et al.*, 1987; Grasl-Kraupp *et al*., 2002). In a ligand-independent pathway, DNA damage events, such as ultraviolet irradiation, ionizing radiation and cisplatin treatment, also result in the nuclear translocation of EGFR (Dittmann *et al.*, 2005a; Xu *et al.*, 2009). Furthermore, it has been reported that the full-length form of nuclear EGFR is involved in transcriptional regulation, cell proliferation, DNA replication, DNA repair and chemo- and radio-resistance (Lin *et al.*, 2001; Dittmann *et al.*, 2005a; Wang *et al*., 2006; Das *et al.*, 2007; Kim *et al.*, 2007; Wanner *et al*., 2008; Hsu *et al.*, 2009).

Nuclear localization of EGFRvIII

Nuclear expression of a constitutively activated EGFR variant (EGFRvIII) was first reported in hormone-refractory prostate cancer (Edwards *et al.*, 2006). Nuclear EGFRvIII is also present in normal glial cells and in primary glioblastomas and associated with transcriptional activities (de la Iglesia *et al.*, 2008). More recently, it has also been showed that nuclear EGFRvIII interacts with STAT3 to activate *COX-2* gene expression (Lo *et al.*, 2010).

Correlation between nuclear EGFR and clinical outcome

Nuclear EGFR has been identified in various tumor tissues, including breast cancer, ovarian cancer, and oropharyngeal and esophageal squamous cell carcinomas, and has been shown to be associated with poor patient outcomes (Psyrri *et al.*, 2005; Lo *et al.*, 2005b; Hoshino *et al.*, 2007; Xia *et al.*, 2009). In addition, the correlation between nuclear EGFRvIII and poor clinical prognoses has also been shown in prostate cancer (Edwards *et al.*, 2006).

Nuclear EGFR and therapeutic response

A recent paper shows that nuclear EGFR is responsible for acquired resistance to cetuximab, an anti-EGFR antibody, treatment (Li *et al.*, 2009), suggesting that nuclear EGFR has a role in therapeutic response to EGFR-targeting drugs. Moreover, cetuximab and gefitinib, an EGFR-tyrosine kinase inhibitors, have been shown to inhibit radiation-induced EGFR nuclear transport and sensitize the cells to radiation (Dittmann *et al.*, 2005b; Bailey *et al.*, 2007). Lapatinib, a dual tyrosine kinase inhibitors of EGFR and HER2, also inhibits the nuclear translocation of EGFR and HER2 and sensitize cancer cells to 5-fluorouracil (Kim *et al.*, 2009). Thus, although the therapeutic role of EGFR-targeting drugs on the nuclear translocation of nuclear EGFR remains unclear, nuclear EGFR may be associated with drug-resistance.

Nuclear localization of other receptors

Furthermore, the rat neu protein (rat version of human ErbB-2) was first identified in the nucleus more than a decade ago as an intact molecule associated with transcriptional activity (Xie and Hung, 1994). In addition, a full-length form of ErbB-3 (Offterdinger *et al.*, 2002) and the C-terminal fragment of ErbB-4 (Ni *et al.*, 2001; Sardi *et al.*, 2006) have been found in the nucleus of cancer cells, although full-length ErbB-4 has also been detected in the nucleus of some normal cells (Bueter *et al.*, 2006; Thompson *et al.*, 2007). Moreover, full-length receptor tyrosine kinases (RTKs) and cell surface receptors other than EGFR family members have been found in the nucleus, including fibroblast growth factor receptor (FGFR), cMet, insulin-like growth factor-1 receptor, vascular endothelial growth factor receptor, TrkA, interleukin receptors, interferon-γ receptor and growth hormone receptors (Lo and Hung, 2006; Carpenter and Liao, 2009; Sehat *et al.*, 2010).

Together, there are ample examples that membrane receptors can be detected in the nucleus. These membrane receptors in the nucleus, membrane receptors in the nucleus (MRIN), may have important functions that have been overlooked (Wang and Hung, 2009).

Biological functions of nuclear EGFR family members

The functions of nuclear EGFR family proteins have been extensively studied and found to involve transcriptional regulation (Wang and Hung, 2009). The carboxyl termini of EGFR, ErbB-2 and ErbB-4 contain intrinsic transactivation activity (Xie and Hung, 1994; Lin *et al.*, 2001; Ni *et al.*, 2001; Wang *et al.*, 2004), and function as transcriptional regulators that activate target genes. In response to EGF stimulation, activated nuclear EGFR binds to an AT-rich response sequence within the promoters of *cyclin D1*, *B-Myb*, *iNOS* and *Aurora-A*, and transactivates them. Although nuclear EGFR alone seems to activate *cyclin D1* promoter (Lin *et al.*, 2001), nuclear EGFR interacts with some transcriptional factors, including STAT3, E2F1 and STAT5, within the *iNOS*, *COX-2*, *B-Myb* and *Aurora-A* promoter regions, respectively (Lo *et al.*, 2005a, 2010; Hanada *et al.*, 2006; Hung *et al.*, 2008). These protein–protein interactions between nuclear EGFR and the transcriptional factors appear to be essential for the transactivation of each target gene. EGFRvIII has also been found to interact with STAT3 in the normal glial cells and contributes to their malignant transformation (de la Iglesia *et al.*, 2008). A recent paper, in which nuclear EGFR target genes are identified using an unbiased approach, further shows that nuclear EGFR and EGFRvIII cooperate with STAT3 to activate *COX-2* gene expression in glioblastoma cells (Lo *et al.*, 2010). Similar to nuclear EGFR, nuclear ErbB-2 transactivates the *COX2* gene through a specific DNA element, the HER2/ErbB-2-associated sequence, which stimulates *COX2* transcription in breast cancer cells (Wang *et al.*, 2004). It should also be mentioned that a truncated form of ErbB-2 (p95HER2) is shown to be in the nucleus, and p95HER2-expressing breast tumors are resistant to anti-HER2-targeting therapies (Scaltriti *et al.*, 2007). In addition, ligand-activated ErbB-4 is cleaved through sequential proteolytic processing by γ-secretase to produce an intracellular domain

fragment, which translocates to the nucleus and functions as a transcriptional factor to activate the *β-casein* gene (Ni *et al.*, 2001; Carpenter, 2003; Williams *et al.*, 2004). Furthermore, nuclear ErbB-4 intracellular domain, which directly interacts with estrogen receptor-α, is associated with the improved outcomes for patients on tamoxifen therapy by increasing the sensitivity of the cells to tamoxifen (Naresh *et al.*, 2008). Nuclear ErbB-4 intracellular domain has also been shown to reduce the transcriptional repression mediated by Eto-2, a tumor suppressor candidate in breast cancer (Linggi and Carpenter, 2006). Recently, EGFR has been characterized as a DNA-binding protein using unbiased approaches to analyze the human protein–DNA interactome through the recognition of the predicted DNA motifs (Hu *et al.*, 2009), further supporting the role of EGFR in transcriptional regulation. Besides being transcriptional regulators, nuclear EGFR family proteins have been shown to be directly involved in DNA repair and replication signaling. Nuclear EGFR phosphorylates the chromatin-bound proliferative cell nuclear antigen, resulting in stabilization of active proliferative cell nuclear antigen and stimulation of DNA replication and repair (Wang *et al.*, 2006). Upon DNA damage and oxidative stress, nuclear EGFR interacts with DNA-dependent protein kinase in a ligand-independent pathway, leading to DNA repair and radio-resistance (Dittmann *et al.*, 2005a,b, 2008a,b). Furthermore, the intracellular domain fragment of ErbB-4 phosphorylates and inhibits the nuclear protein mdm2 and, consequently, enhances the levels of p53 and p21 (Arasada and Carpenter, 2005). Together, these observations indicate that nuclear EGFR family proteins are involved in transcriptional regulation and signaling transduction and have an important role in multiple biological functions, including tumor progression, DNA repair, DNA replication and resistance to certain cancer therapy.

Trafficking of the EGFR family proteins

Receptor endocytosis

The transmembrane RTKs serve as mediators of cell signaling from extracellular growth factors, and RTK signaling levels need to be tightly regulated. Receptor endocytosis stimulated by ligand-induced activation is thought to be a key mechanism by which the duration and intensity of RTK signaling is controlled. Upon ligand-induced stimulation, RTKs are immediately internalized, and this process permits the capture of transmembrane RTKs and their extracellular ligands into cytoplasmic vesicles and the sequential removal of RTKs from the cell surface (Conner and Schmid, 2003; Doherty and McMahon, 2009; Sorkin and von Zastrow, 2009). A major pathway of EGFR internalization is clathrin-dependent endocytosis, whereby the activated EGFR is pinched off from the cell surface by clathrin-coated pits and then routed to an early endosomal compartment (Sorkin, 2004). By contrast, EGFR and some other receptors have also been shown to be internalized by different routes, such as clathrin-independent endocytosis or circular dorsal ruffles/waves (Sigismund *et al.*, 2005; Orth *et al.*, 2006; Mayor and Pagano, 2007). After endocytosis, endocytic vesicles carrying EGFR derived from both clathrin-dependent and clathrin-independent endocytosis subsequently fuse with the early endosomes. The fate of the internalized EGFR is decided in the early endosomes; EGFR is either recycled back to the cell surface or degraded by lysosomes (Figure 1). Interestingly, it has been shown that the endocytic process is also involved in the nuclear translocation of EGFR family RTKs (Giri *et al.*, 2005; Massie and Mills, 2006; Mosesson *et al.*, 2008), which we will discuss in detail below (Figure 1).

Recycling and degradation of the EGFR RTKs

After internalization, the endosomal trafficking of cargo proteins is primarily regulated by several small GTPase Rab family proteins, and each Rab protein is involved in a particular stage of the membrane transport machinery (Maxfield and McGraw, 2004; Grant and Donaldson, 2009). For example, the internalized EGFR is first routed to the early endosomes by a Rab5-dependent mechanism. Receptors can be recycled back to the cell surface either

through a Rab4-dependent rapid recycling pathway or sorted to the recycling endosomes containing Rab11 (Ceresa, 2006). In contrast, some RTKs in the early endosomes are sorted into the late endosomes and, subsequently, into lysosomes for degradation. During the transport from the early to the late endosomes of cargo proteins that are destined for degradation, a Rab5-to-Rab7 dynamic conversion is required (Rink *et al.*, 2005). Indeed, Rab7 has been shown to regulate the lysosomal degradation of EGFR (Ceresa and Bahr, 2006).

In addition to the Rab family proteins, ubiquitination of EGFR proteins has a key role in controlling their intracellular trafficking after internalization, including lysosomal targeting and degradation (Sorkin and von Zastrow, 2009). Ubiquitinated receptors are sorted into intraluminal vesicles within the multivesicular bodies, which are the late endosomal intermediates, and then fused with lysosomes. The multivesicular body pathway is regulated by the endosomal sorting complex required for the transport (ESCRT) machinery comprising four complexes, ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III, which have been identified as important regulators of intraluminal vesicle formation and delivery of ubiquitinated EGFR into the intraluminal vesicles (Gruenberg and Stenmark, 2004; Williams and Urbe, 2007; Saftig and Klumperman, 2009; Sorkin and Goh, 2009; Woodman, 2009). In addition to their role in EGFR degradation, the ESCRT family proteins are shown to be involved in the EGFR recycling pathway (Baldys and Raymond, 2009). Although endocytosis is essential for the nuclear translocation of EGFR, the involvement of the Rab family proteins or EGFR ubiquitination on nuclear EGFR is unknown. Therefore, it is an unexplored area whether specific Rab proteins or post-translational modifications of EGFR are involved in EGFR nuclear trafficking.

Nuclear translocation of the EGFR RTKs

Accumulating evidence suggests the existence of a novel pathway by which internalized cell surface RTKs within the early endosomes are transported to the nucleus rather than to either the cell surface or lysosomes (Figure 1). Several studies have shown that inhibition of receptor endocytosis using a dominant-negative dynamin mutant, which abrogates the formation of clathrin-coated pits from the cell surface, blocks the nuclear transport of EGFR, ErbB-2 and FGFR (Bryant and Stow, 2005;Giri *et al.*, 2005;Lo *et al.*, 2006a). Furthermore, EGFR and ErbB-2 have been shown to be associated with the early endosomal marker, early endosome antigen 1, in the nucleus (Giri *et al.*, 2005;Lo *et al.*, 2006a), suggesting that the nuclear entry of EGFR/ErbB-2 is mediated by mechanisms involving endocytosis and early endosomal sorting. During nuclear-cytoplasmic transport, nuclear localization signal (NLS)-containing proteins are transported into the nucleus by forming a complex with either importin α/β or importin-β alone (Jans *et al.*, 2000;Weis, 2003). Importin-β is responsible for nuclear translocation by directly associating with the nucleoporins, which are the constituents of the nuclear pore complexes (NPCs) (Harel and Forbes, 2004;Cook *et al.*, 2007). Many cell surface RTKs, including EGFR, ErbB-2 and FGFR1, translocate to the nucleus by importin β-dependent mechanisms (Reilly and Maher, 2001;Giri *et al.*, 2005;Lo *et al.*, 2006a). Furthermore, the putative NLS of EGFR and ErbB-2 has been identified (Wang *et al.*, 2004;Lo *et al.*, 2005a) and importin-β has been shown to interact with it (Giri *et al.*, 2005;Lo *et al.*, 2006a). The tripartite NLS of EGFR, which contains three clusters of basic amino acids (RRRHIVRKRTLRR; amino acids 645–657) and is conserved among the other EGFR family membranes, is located at the intracellular carboxyl terminus of EGFR (Hsu and Hung, 2007). The NLS has been also found in EGFRvIII (Lo *et al.*, 2010). Interestingly, importin-β colocalizes with ErbB-2 in the endosomes, and importin-β, ErbB-2 and Nup358, one of nucleoporins located at the cytoplasmic filaments of the NPC, form a tricomplex and colocalize near the nuclear envelope (NE) (Giri *et al.*, 2005). These studies suggest that the endocytic vesicles/endosomes may function as vehicles for EGFR/ErbB-2, using importin-β as a driver to carry cargo proteins through the NPC for nuclear translocation. However, the exact mechanisms by which the full-length RTKs embedded in the endosomal membrane travel all

the way from the cell surface to the early endosomes and pass through the NPC are still largely unknown. In addition to nuclear import of EGFR RTKs, the exportin CRM1 may be involved in the nuclear export of EGFR, ErbB-2 and ErbB-3 (Offterdinger *et al.*, 2002;Giri *et al.*, 2005;Lo *et al.*, 2006a); however, nuclear export sequences within these cell surface RTKs have not yet been identified.

Potential pathways of retrograde transport from the endosomes

In addition to endosomal sorting to the recycling endosomes or the lysosomes, endosomal trafficking after endocytosis to the biosynthetic/secretory compartments, such as the endoplasmic reticulum (ER) and the Golgi apparatus, known as retrograde transport, is also important for diverse cellular functions (Johannes and Popoff, 2008). Several mammalian cargo proteins and exogenous viruses/toxins are routed from the early endosomes to the Golgi apparatus and the ER, respectively, by retrograde transport (Sandvig and van Deurs, 2002; Spooner *et al.*, 2006; Johannes and Popoff, 2008). Earlier we discussed that endocytosis is required for the nuclear translocation of EGFR proteins. However, after endocytosis, it is unclear which pathway routes EGFR proteins to the nucleus. It is possible that the nuclear translocation of EGFR family RTKs destined in the early endosomes may involve retrograde transport through membrane compartments such as the Golgi apparatus and the ER (Figure 1). A potential route for EGFR nuclear translocation through the ER-associated degradation pathway has been proposed, whereby membrane-bound EGFR is released from the ER to the cytoplasm, where EGFR as a cytoplasmic protein interacts with importin-β and then enters the nucleus through the NPC (Liao and Carpenter, 2007). However, this model requires EGFR to remain as a soluble protein after its translocation from the ER to the cytoplasm. Thus, one critical question about the nuclear trafficking of EGFR is still unsolved: because EGFR has a hydrophobic transmembrane domain, it is still unclear as to how cytoplasmic EGFR could be soluble in its membrane-bound structure and sequentially overcome the energy barrier to pass through the aqueous channels of the NPC. One possibility yet to be further confirmed is that it might use a mechanism similar to the nuclear translocation of FGFR possessing an atypical transmembrane domain, which is discussed in the next section.

Nuclear trafficking mechanisms of membrane proteins other than the EGFR family proteins

In addition to the EGFR family RTKs discussed above, we will review the nuclear trafficking mechanisms for different groups of membrane proteins and their associated factors in the following section (Table 1). Understanding the mechanisms of nuclear trafficking of other MRIN proteins may help to understand the mechanisms of EGFR RTKs nuclear transport.

FGFR1 RTKs

Nuclear FGFR1 activates transcription in cooperation with a transcriptional co-activator, cyclic adenosoine monophosphate responsive element-binding protein, by stimulating the recruitment of RNA polymerase II and chromatin remodeling in a kinase activity-independent manner (Fang *et al.*, 2005). Nuclear translocation of FGFR1 through the integrative nuclear FGFR1 signaling pathway is dependent on a clathrin-independent endocytosis pathway that does not involve uptake by either clathrin or caveolae (Bryant and Stow, 2005). The transmembrane FGFR1 RTK also serves as a soluble protein present in the cytosol and nucleus (Stachowiak *et al.*, 2007). After ligand-activated internalization, FGFR1 possessing an atypical transmembrane domain is released from the endosomes into the cytosol, which is facilitated by its association with pp90 ribosomal S6 kinase-1. Cytosolic FGFR1 is then transported into the nucleus by an indirect interaction with importin-β (Myers *et al.*, 2003). One proposed model suggests that FGFR1, which does not have a NLS itself, is chaperoned to the nucleus by a

higher-molecular-weight isoform of FGF-2 ligand harboring NLS in an importin β-dependent manner (Stachowiak *et al.*, 1996; Reilly and Maher, 2001; Peng *et al.*, 2002).

Inner nuclear membrane proteins

The lipid bilayer NE comprises the outer nuclear membrane (ONM) and the inner nuclear membrane (INM). The ONM is contiguous to the ER membrane, whereas the INM has a protein composition different from that of the ONM and is associated with the underlying chromatin and lamina. The INM and ONM are joined at the NPC, which form aqueous channels embedded in the NE (Stewart *et al.*, 2007). Nuclear translocation of INM proteins is a related example for studying the nuclear trafficking of integral membrane proteins. Multiple mechanisms of nuclear transport, which depend on the unique characteristics of the INM proteins, have been reported, including simple or gated lateral diffusion, vesicle fusion events and classical NPC-mediated nuclear import (Zuleger *et al.*, 2008). The rules governing the targeting of integral membrane proteins to the INM depend on several characteristics, such as the size of the extralumenal domains, the involvement of the NLSs and the affinity of the NLSs for the karyopherin/importin nuclear transport factors (Lusk *et al.*, 2007). Integral INM proteins are initially inserted into the ER membrane, in which the NLSs present in the extralumenal domains bind to karyopherins/importins, and the proteins are then targeted to the INM of the NE through the ONM and NPC. We propose the use of the term INTERNET, standing for the integral trafficking from the ER to the NE transport, to refer to the INM targeting process for the ER-to-NE transport of integral membrane proteins. For example, the yeast INM proteins Heh1 and Heh2, harboring NLSs, travel into the INM through the INTERNET pathway, which is mediated by the interaction between the NLSs of the INM proteins and karyopherins/importins (King *et al.*, 2006). Furthermore, an INM-sorting motif sequence, a hydrophobic transmembrane sequence of 18–20 amino acids that follows positively charged residues within 4–8 residues of the end of the sequence, can be recognized by an isoform of importin-α to target an insect INM protein to the INM (Saksena *et al.*, 2006). Thus, as EGFR appears to be detected in the INM or nuclear matrix (Wang *et al.*, 1998; Klein *et al.*, 2004), it is worthwhile to test whether this INTERNET mechanism is involved in the nuclear translocation of other integral membrane proteins such as cell surface RTKs (Figure 2).

EGFR family ligands

In addition to EGFR family receptors, ligands for the EGFR family members, such as EGF and protransforming growth factor-α, are also found to be translocated to the nucleus (Raper *et al.*, 1987; Grasl-Kraupp *et al.*, 2002). Another EGFR ligand, Schwannoma-derived growth factor, is capable of translocating to the nucleus and binding to A + T-rich DNA sequences (Kimura, 1993) that are similar to the promoter-binding sequences for nuclear EGFR (Lin *et al.*, 2001). In addition, the intracellular domain of neuregulin-1, which is a ligand for ErbB-3/ErbB-4 receptors, enters the nucleus to prevent neuronal apoptosis (Bao *et al.*, 2003). A membrane-anchored precursor of an EGFR family ligand, heparin-binding EGF-like growth factor (proHB-EGF), has also been detected in the nucleus (Adam *et al.*, 2003). However, the mechanism for the nuclear translocation of EGFR family ligands has not been shown convincingly. A study shows that both the transmembrane forms of unshed proHB-EGF and the carboxy-terminal fragment (HB-EGF-CTF) generated by proHB-EGF shedding are targeted to the INM of the NE (Hieda *et al.*, 2008; Higashiyama *et al.*, 2008). Unlike the EGFR family RTKs containing NLS sequences (Hsu and Hung, 2007), no interaction has been detected between HB-EGF and importin proteins, likely because HB-EGF does not have an NLS (Hieda *et al.*, 2008). It is unclear if the membrane-anchored HB-EGF translocates to the INM by active transport, as larger molecules (>40 kDa) do, or by passive diffusion, as smaller molecules (≤40 kDa) do (Hoelz and Blobel, 2004; Stewart, 2007; Terry *et al.*, 2007). Further systematic study is required to determine whether the cognate receptor EGFR serves as an

active transporter and forms a ligand–receptor complex to translocate the ligand into the nucleus (Figure 2).

Adaptor membrane proteins

Certain adaptor membrane proteins that are associated with the early endosomes are also found in the nucleus. For example, in response to EGF stimuli, a Rab5-binding effector, APPL (adaptor protein containing a pleckstrin homology domain, a phosphotyrosine binding domain and a leucine zipper motif), translocates from the early endosomes to the nucleus, leading to cell proliferation by its association with components of the nucleosome remodeling and histone deacetylase complex (Miaczynska *et al.*, 2004). Another example of an endosomal membrane-associated adaptor protein is huntingtin-interacting protein-1. After androgen treatment, huntingtin-interacting protein-1 has been shown to be translocated to the nucleus and function as a transcriptional co-activator to modulate the transcriptional activity of nuclear androgen receptors (Mills *et al.*, 2005). However, given that potential NLSs have been identified in both adaptor membrane proteins, the trafficking mechanism that results in the nuclear translocation of these membrane-associated adaptor proteins is still largely unexplored. In addition, several endocytic adaptors, including Eps15, Eps15R, Epsin1 and the clathrin assembly protein lymphoid myeloid, have also been found in the nucleus (Pilecka *et al.*, 2007). Interestingly, the membrane-bound early endosomal marker EEA1 is colocalized with EGFR and ErbB-2, not only in the nucleus but also in the vicinity of the NE, as assessed by immuno-electron microscopy (Giri *et al.*, 2005; Lo *et al.*, 2006a). It will be interesting to study further whether the nuclear transport of EGFR/ErbB-2 RTKs and other MRINs involves membrane-bound trafficking from the cell surface all the way to the nucleus, as nuclear EGFR/ErbB-2 RTKs are still associated with components of the early secretory machinery from the early endosomal membranes.

Conclusion

Multiple integral membrane proteins have been reported to be translocated to the nucleus through several potential mechanisms, and their nuclear functions are gradually being discovered (Table 1). Regarding the nuclear trafficking of EGFR family proteins, endocytosis and endosomal sorting are required for the nuclear transport of full-length EGFR/ErbB-2 RTKs. However, the detailed mechanism by which the internalized EGFR proteins are routed to the nucleus through the NPC is still unclear. It has been proposed that EGFR localized in the ER is extracted from lipid layers to the cytoplasm through the ER-associated degradation pathway, where cytoplasmic EGFR is transported to the nucleus through the NPC by association with importin-β (Liao and Carpenter, 2007). In addition to the ER-associated degradation pathway, other mechanisms may also exist to explain how EGFR harboring a hydrophobic transmembrane domain can overcome the energy barrier and move through the hydrophilic channels of the NPC. The INTERNET model provides a logical route for the nuclear translocation of INM proteins and may be a general mechanism for the nuclear transport of membrane-bound ligands, RTKs, or other cell surface receptors. This model may describe a new direction for understanding how membrane-bound EGFR family RTKs are trafficked from the cell surface to the nucleus (Figure 2). As the function of nuclear RTKs is gradually being discovered, understanding the trafficking mechanism for nuclear RTKs will be critical to move this important MRIN research ahead that has been overlooked in the past.

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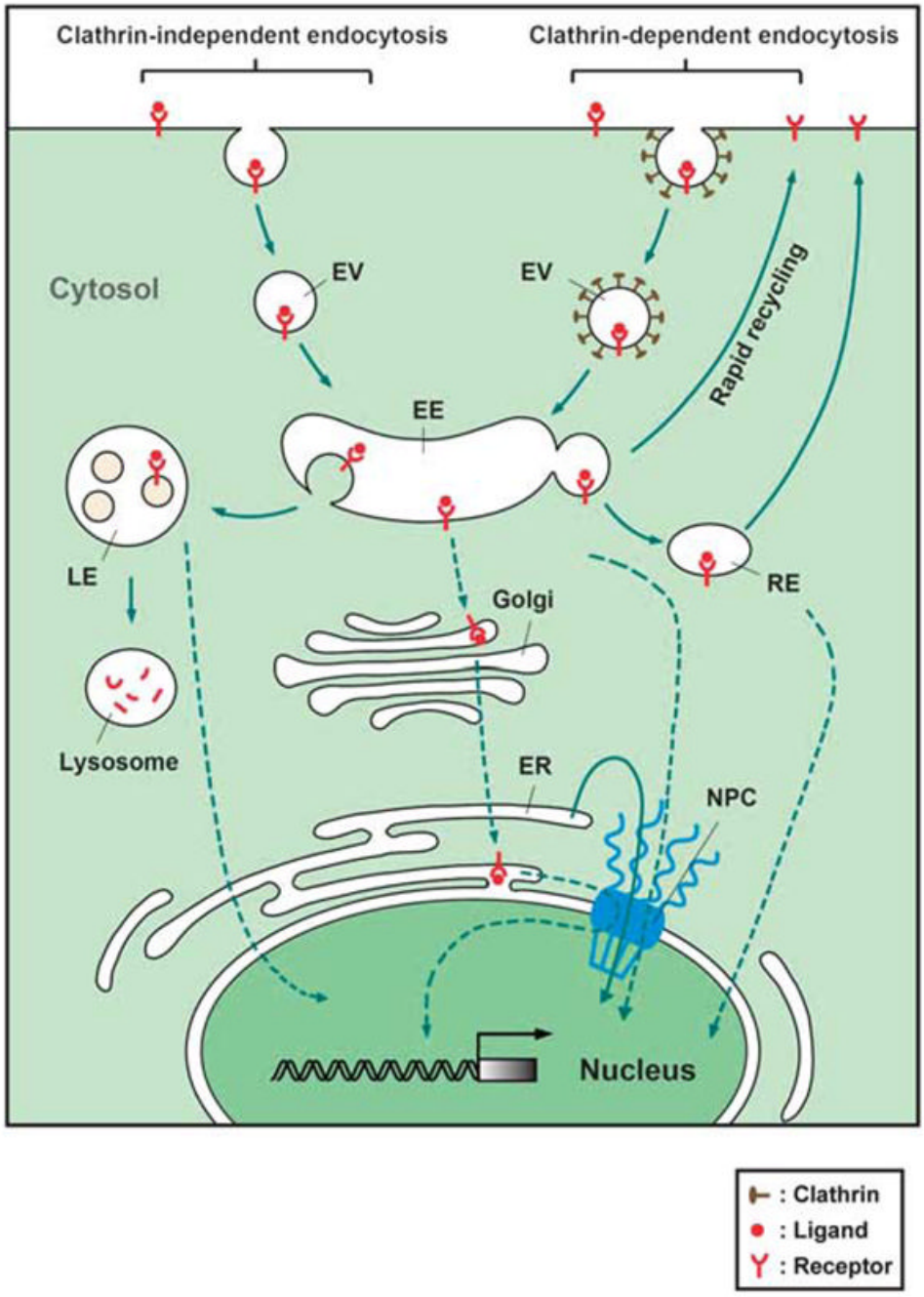

Figure 1.

A diagram of the EGFR family receptors trafficking. The endocytic vesicles carrying EGFR derived from either clathrin-dependent or clathrin-independent endocytosis subsequently fuse with the early endosomes. There are three possibilities of the internalized EGFR decided in the early endosomes. First, EGFR can be recycled back to the cell surface either through a rapid recycling or sorted to the recycling endosomes. Second, EGFR can also be sorted to the late endosomes and then degraded by lysosomes. The third and novel pathway is shown, by which the internalized EGFR embedded within the early endosomes is transported to the nucleus. Several potential mechanisms may be involved in the nuclear trafficking of the EGFR family receptors. For example, EGFR localized in the ER is extracted from lipid layers to the

cytoplasm through the ERAD pathway, and the cytoplasmic EGFR is transported to the nucleus through the NPC. In addition to the ERAD pathway, other mechanisms such as retrograde transport from the early endosomes to the Golgi/ER may also exist. Furthermore, it is unexplored yet whether the nuclear transport of EGFR occurs from the recycling endosomes or the late endosomes. The scale of the diagram does not reflect the relative sizes of different molecules or subcellular structures. EV, endocytic vesicle; EE, early endosomes; LE, late endosomes; RE, recycling endosomes; NPC, nuclear pore complex; ER, endoplasmic reticulum.

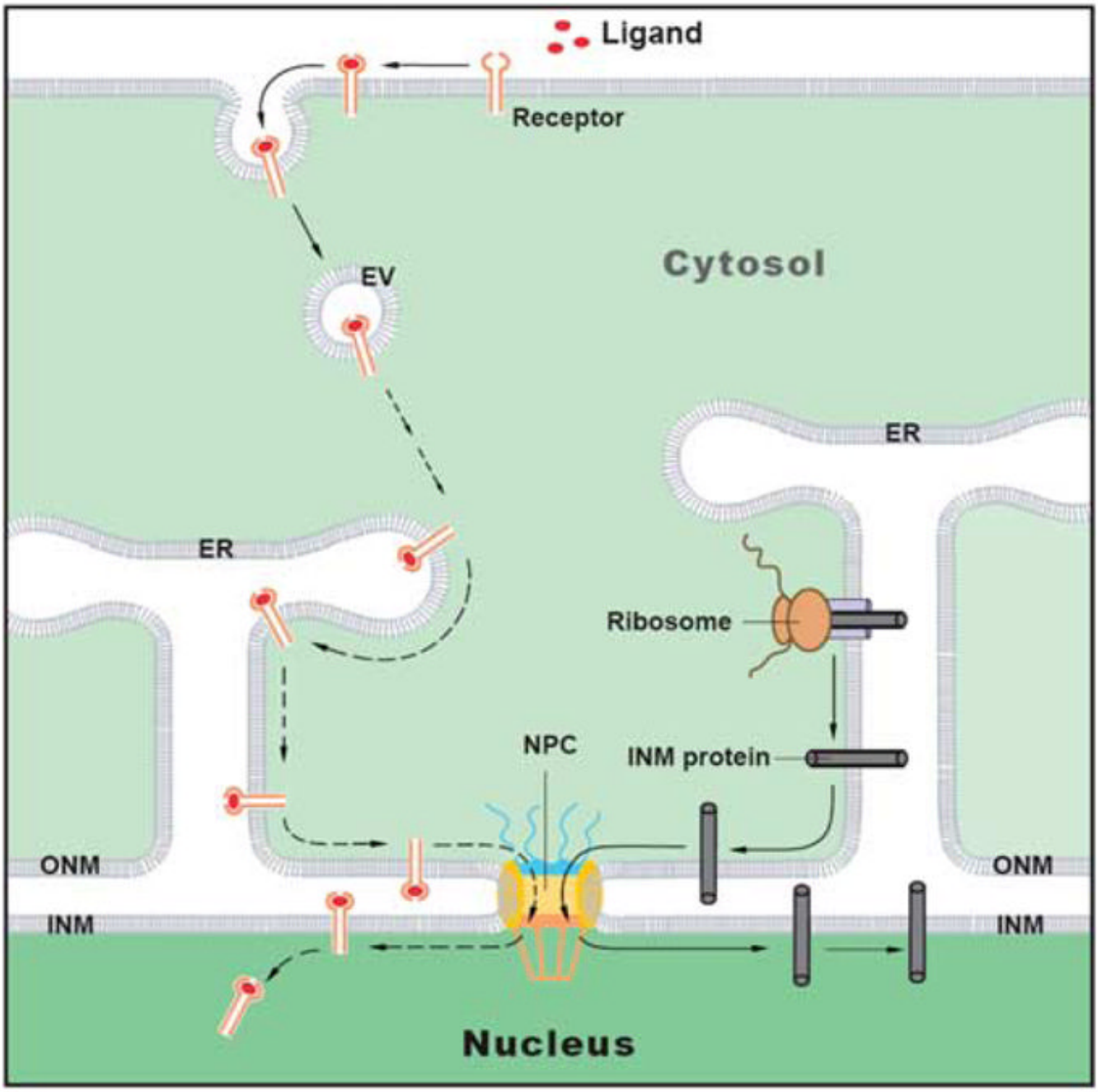

Figure 2.

A model of integral trafficking from the ER to the NE transport (INTERNET). Integral INM proteins initially inserted into the ER membrane are targeted to the INM of the NE through the ONM and NPC. This INTERNET model may be involved in the nuclear transport of other integral membrane proteins such as cell surface EGFR RTKs (see Table 1). The cognate receptor may also serve as an active transporter and form a ligand–receptor complex to transport the ligand into the nucleus. The scale of the diagram does not reflect the relative sizes of different molecules or subcellular structures. ONM, outer nuclear membrane; INM, inner nuclear membrane.

Table 1

Mechanisms of nuclear transport involving membrane-bound trafficking

	MW (kDa)	'INTERNET'	ER localization	Importins involvement	Endocytosis	Reference
RTKs/EGFR/ErB-2	170/185	?	+	+	+	Lo and Hung (2006); Giri *et al.* (2005)
RTKs/FGFR-1	120,145	−		+	+	Reilly and Maher (2001)
INM proteins	>40	+	+	+	−	King *et al.* (2006); Saksena *et al.* (2006)
Ligands/HB-EGF	20–30	+		+		Hieda *et al.* (2008)

Abbreviations: EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; FGFR, fibroblast growth factor receptor; HB-EGF, heparin-binding epidermal growth factor; INM, inner nuclear membrane; INTERNET, integral trafficking from the endoplasmic reticulum to the nuclear envelope transport; MW, molecular weight; RTK, receptor tyrosine kinase.

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