HHS Public Access

Author manuscript

Curr Osteoporos Rep. Author manuscript; available in PMC 2021 June 23.

Published in final edited form as:

Curr Osteoporos Rep. 2019 June ; 17(3): 138–146. doi:10.1007/s11914-019-00512-2.

Nuclear Fibroblast Growth Factor Receptor Signaling in Skeletal Development and Disease

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Abstract

Purpose of Review—Fibroblast growth factor receptor (FGFR) signaling regulates proliferation and differentiation during development and homeostasis. While membrane-bound FGFRs play a central role in these processes, the function of nuclear FGFRs is also critical. Here, we highlight mechanisms for nuclear FGFR translocation and the effects of nuclear FGFRs on skeletal development and disease.

Recent Findings—Full-length FGFRs, internalized by endocytosis, enter the nucleus through β-importin-dependent mechanisms that recognize the nuclear localization signal within FGFs. Alternatively, soluble FGFR intracellular fragments undergo nuclear translocation following their proteolytic release from the membrane. FGFRs enter the nucleus during the cellular transition between proliferation and differentiation. Once nuclear, FGFRs interact with chromatin remodelers to alter the epigenetic state and transcription of their target genes. Dysregulation of nuclear FGFR is linked to the etiology of congenital skeletal disorders and neoplastic transformation.

Summary

Revealing the activities of nuclear FGFR will advance our understanding of 20 congenital skeletal disorders caused by FGFR mutations, as well as FGFR-related cancers.

Keywords

FGFR2; FGFR1; FGF2; Skeletal development; Nuclear RTK, cancer

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Compliance with Ethical Standards

Human and Animal Right and Informed Consent This article does not present any primary studies with human or animal subjects.

Conflict of Interest The authors declare that they have no conflict of interest.

Introduction

The fibroblast growth factor (FGF) signaling pathway, comprised of 22 ligands and 4 high-affinity tyrosine kinase FGF receptors (FGFRs), regulates complex cellular behaviors including migration, proliferation, self-renewal, lineage commitment, senescence, and survival in vertebrates [1, 2]. FGF-mediated regulation of these cellular behaviors in embryonic development drives morphogenetic movements, patterning, and growth from gastrulation through organogenesis. One of the most notable roles for FGF signaling during organogenesis is in the development and growth of the skeleton, as dominant missense mutations in FGFR1, FGFR2, and FGFR3 cause at least 20 congenital conditions with skeletal abnormalities.

During skeletal development, FGFR1, 2, and 3 are expressed in unique and overlapping patterns to regulate sequential steps in bone formation. In intramembranous bone formation, expression of FGFR1 and FGFR2 in condensing osteogenic mesenchyme stimulates osteoprogenitor cell proliferation and differentiation [3, 4]. Later, expression of FGFR1 and FGFR2, along with low levels of FGFR3 are restricted to osteoblasts where they promote terminal differentiation and matrix mineralization [3, 5]. During endochondral ossification, FGFR1 and FGFR2 expression in condensing chondrogenic mesenchyme regulates its proliferation and transition into the cartilage anlagen [6, 7]. As endochondral ossification proceeds, FGFR2 expression becomes restricted to the perichondrium and periosteum surrounding the growing bones and is essential for osteoblast proliferation [8]. In the cartilage anlagen and growth plate, FGFR3 expression in chondrocytes inhibits proliferation, while FGFR1 expression in hypertrophic chondrocytes promotes terminal maturation [5, 9, 10].

A number of FGF ligands are expressed in and around intramembranous and endochondral skeletal elements, including FGF1, 2, 6–10, 17, 18, and 21–23 [3, 11]. The context-dependent response of FGFR signaling is driven in part by the mode of FGF ligand action, which can be classified as paracrine/autocrine or endocrine. Paracrine/autocrine FGFs (FGF 1–10, 16–18, 20, and 22), known as the canonical FGFs, are secreted by the cell and remain tightly bound in the extracellular matrix with their cofactor heparan sulfate proteoglycans (HSPGs). HSPGs limit FGF ligand diffusion and regulate the specificity of FGFR binding [12]. Binding of canonical FGFs to FGFRs triggers receptor dimerization and transphosphorylation of tyrosine residues in the receptor’s activation loop. Phosphotyrosine residues in FGFRs subsequently serve as intracellular docking sites for activators of multiple downstream signal transduction cascades [1, 13, 14]. Like the canonical FGFs, the endocrine FGFs (FGF 15/19, 21, and 23) are secreted and mediate their biological response through an FGFR-dependent manner. However, endocrine FGFs act as circulating hormones that function over long distances and require the Klotho family of transmembrane proteins, rather than HSPGs, as cofactors to bind FGFR within its target tissues [15].

While paracrine/autocrine and endocrine FGF signaling ascribe a central role to FGFR complexes at the plasma membrane, a growing body of evidence suggests that nuclear localization of FGFRs lend an additional layer of regulatory complexity. For over two decades, it has been recognized that FGFRs, along with canonical FGF ligands, enter the

nucleus of multiple cell and tissue types. More recent studies have shown that once nuclear, FGFRs exert effects on proliferation, lineage commitment, and gene expression. In this review, we will discuss our current knowledge of nuclear FGFR signaling, emphasizing the mechanisms driving nuclear translocation and the biological function of nuclear FGFR activities in the context of skeletal development and disease.

Nuclear Translocation of FGFRs

Nuclear localization of FGF ligands and FGFRs has been documented in multiple tissue types in vivo, as well as in many cell types in vitro. Despite these observations, our understanding of the precise mechanism for how these membrane-integrated receptor-ligand complexes translocate into the nucleus is incomplete. Thus far, nuclear FGFRs have been detected using plasmid-based overexpression of epitope-tagged FGFRs or anti-FGFR antibodies. However, the indirect nature of these techniques has provoked questions about the reliability of these unexpected observations. To develop a direct means of labeling FGFR2 in live cells, we used CRISPR-Cas9 to knock-in an Emerald fluorescent tag into the FGFR2 locus of the mouse calvarial preosteoblast cell line MC3T3-E1. Live confocal analysis of FGFR2-Emerald distribution showed that the endogenous receptor localized to both the plasma membrane and nucleus (Fig. 1). Live tracking of FGFR2-Emerald is consistent with previous reports of nuclear FGFR2 and also provides a valuable resource to address mechanistic questions related to nuclear translocation of endogenous FGFR2.

Structurally, FGFRs contain an external ligand binding domain, a monotopic transmembrane domain (TMD), and an intracellular tyrosine kinase domain [13, 16••]. The hydrophobicity of the TMD appears to be a rate-limiting step in nuclear trafficking, at least in the case of FGFR1 [17]. Mutations that reduce the hydrophobicity of the TMD in FGFR1 affect its subcellular distribution [17]. Given the high homology between FGFR1 and FGFR2 and that both receptors have only modestly hydrophobic TMDs to anchor within the membrane's lipid bilayer, it is not surprising that mutations in the TMD of FGFR2 alter its subcellular localization [17]. For example, FGFR2 mutations ($FGFR2^{M391R}$ and $FGFR2^{Y381D}$) that cause the skeletal disorder bent bone dysplasia syndrome (BBDS) are located in the TMD, reduce plasma membrane levels of FGFR2, and amplify its nuclear and nucleolar targeting [18]. Post-translational modifications, such as glycosylation, that normally direct the subcellular targeting of FGFRs also contribute to nuclear localization. For example, the FGFR2 mutation $FGFR2^{C278F}$ that causes the skeletal disorder Crouzon syndrome leads to incomplete FGFR2 glycosylation, blocks membrane localization, and induces the receptor's perinuclear accumulation [19].

In addition to the structural elements of the receptor, nuclear localization of full-length FGFRs occurs through a ligand-dependent mechanism (Fig. 2). Stimulation of cells with labeled FGF2 revealed that a significant portion of nuclearlocalized FGFR1 was derived from the cell surface [20, 21]. Like FGF2, FGF1 and FGF10 localize to the nucleus with FGFR1 [22, 23]. Each of these ligands contains a nuclear localization signal (NLS) to facilitate nuclear import [24•, 25, 26]. FGF2 has five isoforms, four of which are high molecular weight isoforms corresponding to 22, 22.5, 24, and 34 KDa in humans that are transcribed from nonconventional CUG codons upstream of a low molecular weight (LMW)

18 KDa isoform that has a traditional AUG start codon [27, 28]. While all of the FGF2 isoforms contain nuclear localization signals, only the LMW FGF2 isoform is secreted and acts as an autocrine/paracrine growth factor [26, 29]. Interestingly, FGF2 and FGF10 have also been found to localize to the nucleolus, a subnuclear domain critical for ribosome biogenesis [24•, 30].

It has been shown that FGFR-FGF complexes are endocytosed from the membrane and undergo retrograde trafficking prior to nuclear import (Fig. 2). A comparison between FGFR1–3 highlights both similarities and differences in the mechanisms that mediate receptor internalization (Table 1). FGFR1 and FGFR2, but not FGFR3, can be endocytosed through a clathrin-dependent mechanism [31–33]. Conversely, both FGFR1 and FGFR3 can be endocytosed through a clathrin-independent mechanism, but it is not yet clear if FGFR2 shares this ability [34]. However, following internalization and during sorting, FGFR1, 2, and 3 associated with markers of both early and late endosomes. For nuclear transport, FGFR1 and FGFR2 have been shown to require β-importin, suggesting an active nuclear pore-mediated import mechanism [22, 40]. Interestingly, there also appears to be a ligand-independent mechanism for nuclear FGFR1 import that is cell cycle-dependent [35].

In addition to the nuclear translocation of full-length receptors, cleaved FGFR1 and FGFR3 have also been found in the nucleus [23, 41]. Proteolytic cleavage of FGFR1 by granzyme B leads to nuclear accumulation of the C-terminal portion of the receptor in invading cancer cells [23]. Additionally, intramembrane proteolysis of FGFR3, mediated in part by a γ-secretase, produces an intracellular domain (ICD) that moves into the nucleus in multiple cells lines [41]. While ligand binding triggers the proteolytic event, nuclear translocation occurs in a ligand-independent manner. Still, the mechanism for nuclear localization of cleaved ICDs of FGFR1 and FGFR3 is unclear, although other studies show that cleaved ICDs is derived from other RTKs associated with transcription factors or chaperones for stabilization during translocation through the nuclear pore complex [42, 43].

Nuclear Activities of FGFRs

Once in the nucleus, FGFRs promote gene expression by influencing the epigenetic state of target genes through their interactions with histone remodeling factors. Nuclear FGFR1 occupies the proximal promoters of genes and interacts with CREB-binding protein (CBP), a histone acetyltransferase, to enhance RNA polymerase II recruitment to transcriptionally active genes in multiple cell types [44•, 45]. In embryonic stem cells (ESCs) and neuronal cells, FGFR1 occupancy corresponds with active transcription of pluripotency genes, Wnt/β-catenin signaling components, and p53 [44•]. Nuclear FGFR2 also promotes activation of gene transcription through epigenetic mechanisms. In preosteoblasts, FGFR2 and FGF2 localize to the nucleolus where they recruit histone remodeling factors, such as the CBP homolog p300, to ribosomal DNA (rDNA) and activate RNA polymerase I-mediated transcription [30, 40, 46••]. Since the transcription of rDNA is the rate-limiting step in building ribosomes, an FGFR2-mediated activation of this process increases ribosome biogenesis and subsequently, protein synthesis [46••, 47].

FGFR1 and FGFR2 act as signaling nodes between proliferation and differentiation in multiple cell types. Nevertheless, how FGF signaling elicits a primary response in gene

expression is not clearly delineated because the transcription factors that are known targets of the pathway are ubiquitously expressed and employed by multiple signaling pathways [48]. Nuclear FGFR-mediated regulation of transcription suggests a mechanism through which FGF signaling can directly induce specific and rapid changes in gene expression following pathway activation. Gene expression changes induced by nuclear FGFRs correlate with the binary choice of cells to proliferate or differentiate. In osteoprogenitor cells, nuclear FGFR2-mediated regulation of rDNA transcription promotes self-renewal over terminal osteoblast differentiation [40, 46••, 47]. FGFR2 mutations in BBDS, which enhance nuclear and nucleolar accumulation of the receptor, lead to an increase in the number of transcriptionally active rDNA genes, promote osteoprogenitor cell proliferation, and delay osteoblast differentiation. Nuclear FGFR1, like nuclear FGFR2, acts as a switch between proliferation and differentiation in neural progenitor cells. Nuclear accumulation of FGFR1 in neural progenitor cells is induced upon differentiation signals and is sufficient for differentiation [49, 50]. Nuclear FGFR1 promotes neural differentiation in ESCs by repressing select pluripotency and mesodermal genes, while activating neurodevelopment genes [44•].

Nuclear FGFR Signaling in Development

Control over the transition from cell proliferation to differentiation is the basis for normal growth and development. Nuclear localization of FGFR signaling has been noted at these discrete times during the development of several organ systems. During gonadal development, FGFR2 is first localized to the plasma membrane of Sertoli progenitor cells where it promotes FGF9-dependent proliferation. During the early stages of Sertoli cell specification and differentiation, FGFR2 is localized to the nucleus with Sry and Sox9, transcription factors critical for Sertoli cell lineage commitment [51]. Temporal deletion of FGFR2 specifically in pre-Sertoli cells blocked their terminal differentiation, which demonstrated that nuclear localization of the receptor has a function distinct from the role of the membrane-localized receptor in progenitor cell proliferation [52]. This role of nuclear FGFR2 in the proliferation-differentiation transition is cell-type specific, as nuclear FGFR2 is not found in the developing female gonad [52].

There are also cases during development in which nuclear FGFR2 is associated with proliferating progenitor cells. During branching morphogenesis of the developing salivary gland, nuclear FGFR2 is specifically located in proliferating epithelial cells at the branch tips in response to FGF10 [53]. Similarly, during branching morphogenesis of the pubertal mammary gland, nuclear FGFR2 is found in proliferating luminal epithelial cells in the terminal end bud at the tips of the primary ducts, as well as in a few basal cells [54]. Once the mammary gland epithelium has matured, FGFR2 becomes localized to the cytoplasm and plasma membrane. While FGFR2 is necessary for both mammary gland and salivary gland development, the specific role of nuclear FGFR2 in these tissues has yet to be determined [54–56].

Nuclear FGFR Signaling in Disease

Biological significance for nuclear FGFR is supported by human congenital conditions caused by mutations in FGFR2. The dominant missense FGFR2 mutations in BBDS

(FGFR2^{M391R} and FGFR2^{Y381D}) decrease the receptor localization to the plasma membrane while enhancing nuclear and nucleolar localization in patient-derived growth plate chondrocytes [18]. BBDS is characterized by coronal craniosynostosis, flattened faces, reduced mineralization of the calvaria, hypoplastic clavicles and pubis, bony nodules on the metacarpals and phalanges, and thickening of the periosteum/perichondrium and bowing of the long bones within the legs [18, 57]. This constellation of clinical findings in BBDS is unique, showing only partial overlap with craniosynostosis disorders caused by FGFR2 gain-of-function mutations. Of the ten skeletal dysplasias caused by FGFR2 mutations, BBDS is the only disorder that presents with bent long bones. Bent long bones are the result of enhanced FGFR2 signaling in the nucleus/nucleolus and is supported in a chick model for BBDS. Targeted expression of FGFR2^{M391R} and FGFR2^{Y381D} in the lateral plate mesenchyme that gives rise to the hindlimb skeleton in the developing chick recapitulates the bent long bone phenotype in BBDS [58]. Moreover, expression of wild-type FGFR2 appended with a nuclear or a nucleolar localization signal also induces the bent long bone phenotype. Together, this study suggests that an increase in the nuclear/nucleolar activities of FGFR2 plays a mechanistic role in the etiology of the BBDS skeletal phenotype. In future studies, it will be important to examine the extent to which the skeletal abnormalities induced by an increased nuclear/nucleolar FGFR2 signaling are the result of altered proliferation and differentiation of skeletal progenitor cells.

Phenotypes common between BBDS and the FGFR2 gain-of-function syndromes suggest a common etiology that includes enhanced nuclear FGFR2 signaling. Premature fusion of the coronal suture, known as coronal craniosynostosis, is found in both BBDS and the FGFR2 gain-of-function disorders: Apert, Pfeiffer, Crouzon, Jackson-Weiss, Beare-Stevenson, and Antley-Bixler syndromes. Among the FGFR2 gain-of-function mutations biochemically tested, enhanced receptor function results from increased ligand affinity, ligand-independent activation, and elevated tyrosine kinase activity [59–61]. While the contribution of these mutations to nuclear translocation and function have not been formally determined, two FGFR2 mutations that cause Crouzon syndrome (FGFR2^{C278F} and FGFR2^{C342F}) result in incomplete receptor glycosylation, formation of disulfide-linked receptor dimers, and intracellular retention of the receptor in preosteoblasts [19]. Conventional FGF signaling models are centered around FGFR activation at the plasma membrane; however, FGFR2^{C278F} and unglycosylated wild-type FGFR2, which do not localize to the plasma membrane, signal intracellularly. These results provide a rationale to examine the consequence of other disease-causing FGFR2 mutations on receptor trafficking and nuclear FGFR2 function.

Dysregulation of FGFR signaling in a subset of cancer types, with either gain-of-function mutations or gene amplifications of FGFR1, FGFR2, and FGFR3, is associated with intracellular FGFRs. At the invasive front of human pancreatic cancer, FGFR1 and FGF2 ligand are localized to the nucleus where they promote proliferation and invasion in the tumor microenvironment [62]. Similarly, FGFR1 undergoes nuclear translocation and activates the transcription of genes critical for cell migration in invading breast cancer cells [23]. Endometrial carcinoma harbor somatic FGFR2 mutations that overlap germline FGFR2 mutations that cause congenital skeletal disorders, including the FGFR2^{M391R} mutation in BBDS. In this case, nuclear localization of the receptor is associated with the

atrophic endometrium that is adjacent to endometrial carcinomas [63, 64]. Another FGFR2 activating mutation in endometrial cancer, $FGFR2^{Y376C}$, has increased perinuclear localization and appears to be either directly or indirectly involved in disrupting cell polarity in metastatic cells [38••]. Expression of $FGFR2^{Y376C}$ in an endometrial cancer cell model blocks polarization of intracellular pools of FGFR2 toward the migrating front of cells, induces Golgi fragmentation, and disrupts directional migration. Intracellular FGFR2 has also been found in as many as half of mammary carcinomas with different hormone dependence [65, 66, 67, 68•]. Although the significance of the finding remains unclear, nuclear and cytoplasmic FGFR2 is correlated to increased tumor size and a much lower overall survival rate [67]. In mucinous carcinoma of the breast, nuclear FGFR2 is commonly found colocalized with STAT-5 and Runx2 [68•]. Altered intracellular localization of FGFR3 has been identified in several tumor types. Nuclear FGFR3 levels in breast, bladder, and pancreatic cancers cells are high compared to that in corresponding nontumor tissues [69, 70, 71•]. In pancreatic cancer, nuclear FGFR3 correlates with metastatic disease and poor overall prognosis [70]. Coordination of differentiation with cell cycle exit is critical for tissue homeostasis, and altogether these studies suggest that nuclear FGFRs play an important role in this process and, when dysregulated, lead to neoplastic transformation.

Conclusion

Beyond its well-established mechanism in transmembrane signaling, FGFRs directly communicate FGF signals between the membrane and nucleus. Nuclear localization of RTKs is not uniqueto the FGF pathway. RTKs for EGF, VEGF, ephrin, and IGF also localize to the nucleus and regulate gene expression [42]. In future studies, it will be important to more clearly define the precise mechanisms for nuclear FGFR translocation and determine the extent to which these mechanisms are shared by other RTKs. Once the components necessary for nuclear translocation are identified, experiments testing the sufficiency and necessity of nuclear FGFRs can be more elegantly designed. While the role of nuclear FGFR signaling in ribosome biogenesis and gene expression have been described in cultured cells, genetic tools are required to test the function of these and other yet undiscovered nuclear FGFR activities in vivo. Recently, we generated a conditional knock-in mouse that harbors the BBDS mutation $FGFR2^{M391R}$. This genetic model, by inducing tissue-specific expression of $FGFR2^{M391R}$ during skeletal development, will provide not only new insights into the disease mechanisms of BBDS, but also a more complete understanding of the biological role for nuclear FGFR2 in the skeleton.

Acknowledgments

The authors thank all the members of the Merrill laboratory for insightful discussions and, in particular, Lauren Bobzin for her critical review of this manuscript.

Funding Information This work was supported by the National Institutes of Health R01DE025222 to A.E.M.

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[PubMed: 26823787]

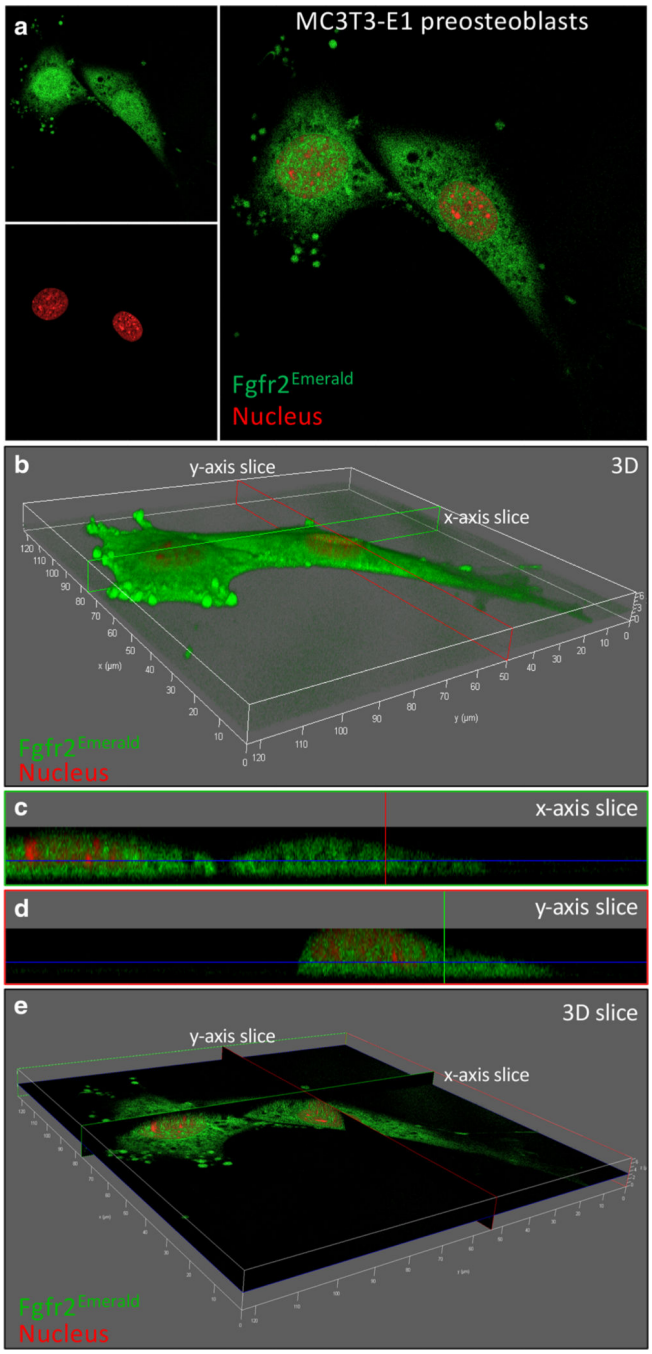

Fig. 1.

Subcellular localization of FGFR2 in live cells. (**a**) Confocal imaging of live MC3T3-E1 calvarial preosteoblasts expressing endogenous FGFR2-Emerald (green) with nuclei counterstained by DRAQ5 (red). CRISPR-Cas9 was used to integrate a floxed Emerald cDNA cassette in frame with endogenous 3′UTR coding region of FGFR2. Cells were subjected to FACS to obtain Emerald-positive single cell isolates. (**b**) 3D reconstruction of confocal stack shows localization of FGFR2-Emerald to the membrane. (**c–d**) x-axis and y-

axis orthogonal slices through the 3D reconstruction at the level of the nuclei, as indicated in panel (**b**), and (**e**), shows that FGFR2-Emerald (green) is localized within the nucleus (red)

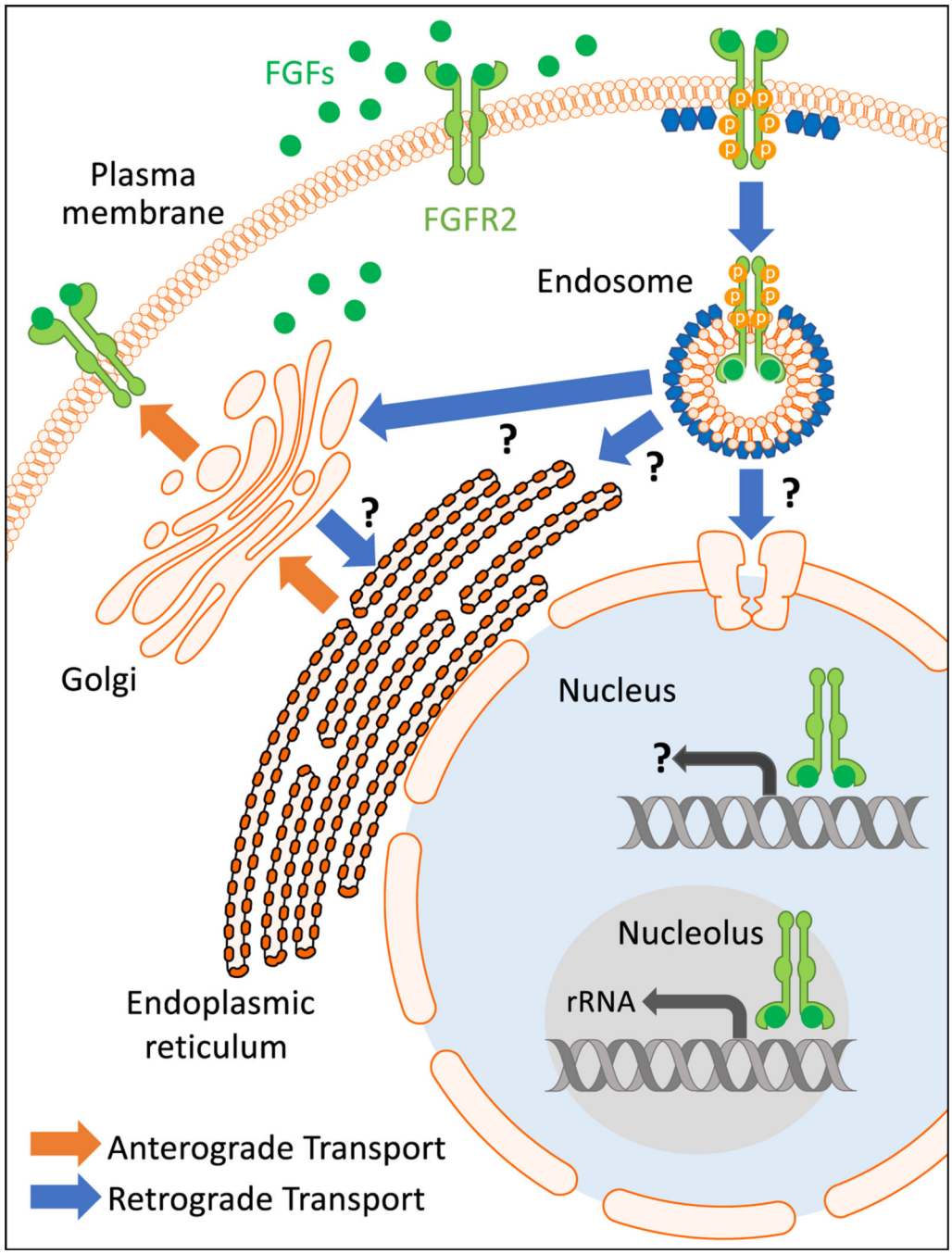

Fig. 2.

Transport of FGFR2. During anterograde transport (orange arrows), newly synthesized FGFR2 is integrated into the membrane of the endoplasmic reticulum and then trafficked to the Golgi where it is glycosylated before being transported to the plasma membrane. Upon binding to extracellular FGF2 ligand and the cofactor heparin sulfate, FGFR2 dimerizes and auto-phosphorylates tyrosine residues in the kinase domain to undergo activation. During retrograde transport (blue arrows), FGFR2 is endocytosed by a clathrin-mediated process. The mechanism(s) for nuclear transport and whether this requires retrograde transport

through the ER or Golgi remains unclear. However, β-importin has been shown to associate with FGFR2, suggesting a nuclear pore-mediated import mechanism

Table 1

Comparison of FGFR1–3 association with cellular compartments

Cell compartment	FGFR1	FGFR2	FGFR3	References
Endocytosis				
Clathrin-dependent	+	+	–	[31–34]
Dynamin	+	+		[31–33]
Arf6	+			[31]
α-Adaptin		+		[33]
Clathrin-independent	+		+	[34, 35]
Endosome				
EEA1 (early)	+	+	+	[31, 33, 34]
Rab5 (early)	+	+		[36, 37]
LAMP-1 (late)	+	+	+	[34, 37]
Rab7 (late)	+	+		[37, 38••, 39]
Nuclear pore/membrane				
Importin-β	+	+		[22, 40]