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The structure and function of vertebrate Fibroblast Growth Factor Receptor 1

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ABSTRACT The vertebrate fibroblast growth factor receptor 1 *(FGFR1)* is alternatively spliced generating multiple splice variants that are differentially expressed during embryo development and in the adult body. The restricted expression patterns of FGFR1 isoforms, together with differential expression and binding of specific ligands, leads to activation of common FGFR1 signal transduction pathways, but may result in distinctively different biological responses as a result of differences in cellular context. FGFR1 isoforms are also present in the nucleus in complex with various fibroblast growth factors where they function to regulate transcription of target genes.

KEY WORDS: *fibroblast growth factor receptor, fibroblast growth factor, signal transduction, Notch, vertebrate*

Introduction

The vertebrate fibroblast growth factor receptor (FGFR) gene family consists of four highly related genes, *FGFR1*, *FGFR2*, *FGFR3* and *FGFR4*, encoding polypeptides that are 55% to 72% identical in their amino acid sequence (Johnson and Williams, 1993). Recently a new member of this family (*FGFR5*) was identified in human and mouse (Kim *et al.*, 2001; Sleeman *et al.*, 2001). *FGFR5* is the most distantly related member of the FGFR family, showing approximately 30% amino acid identity to other FGFR proteins. FGF receptors are involved in many biological processes during embryo development and homeostasis of adult body tissues, and disruption of normal *FGFR* functions lead to pathological conditions in humans. For example, mutations in the *FGFR* genes are the cause of several human developmental disorders characterized by skeletal abnormalities such as achondroplasia (i.e. dwarfism) (Muenke and Schell, 1995; Passos-Bueno *et al.*, 1999), and misregulation of *FGFR* mRNA splicing or upregulation of *FGFR* expression may lead to cell transformation and cancer (Yamaguchi *et al.*, 1994; Valve *et al.*, 2001).

Research in recent years has provided a wealth of new information regarding the function of FGF receptors. In particular, the role of *FGFR1* during vertebrate embryo development and in the adult body has been studied in detail. In this review, we will illuminate the biological role of FGFR1 by describing its basic structure and function as a signal transduction molecule regulating numerous cellular processes in vertebrates. We will also focus on the potential role of nuclear FGFR1 as a regulator of transcription.

The General Structure of FGFR1

The general structure of FGFR1 and other FGF receptors is highly conserved during evolution (Johnson and Williams, 1993). A schematic diagram of the longest single-pass transmembrane product encoded by the human *FGFR1* gene is shown in Fig. 1. The extracellular region consists of a signal peptide followed by three immunoglobulin-like domains (Ig domains). Present between immunoglobulin-like domains II and III (IgII and IgIII) is the acidic box domain, including 8 consecutive acidic residues, termed the acidic box. A heparin-binding region important for interactions with extracellular matrix (ECM) components and a cell adhesion molecule (CAM) homology domain (CHD) are located downstream from the acidic box domain (Kan *et al.,* 1991; Doherty and Walsh, 1996). Continuous with the extracellular region is a transmembrane (TM) domain followed by an intracellular region. The latter consists of a juxtamembrane domain next to the TM domain and a tyrosine kinase domain, which is split by a 14 amino acid long non-catalytic interkinase domain and followed by a short C-terminal tail (Johnson and Williams, 1993).

Abbreviations used in this paper: CAM, cell adhesion molecule; CHD, cell adhesion molecule homology domain; ECM, extracellular matrix; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; HSPG, heparan sulphate proteoglycan; Ig domain, immunoglobulin-like domain; MHB, midbrain-hindbrain boundary.

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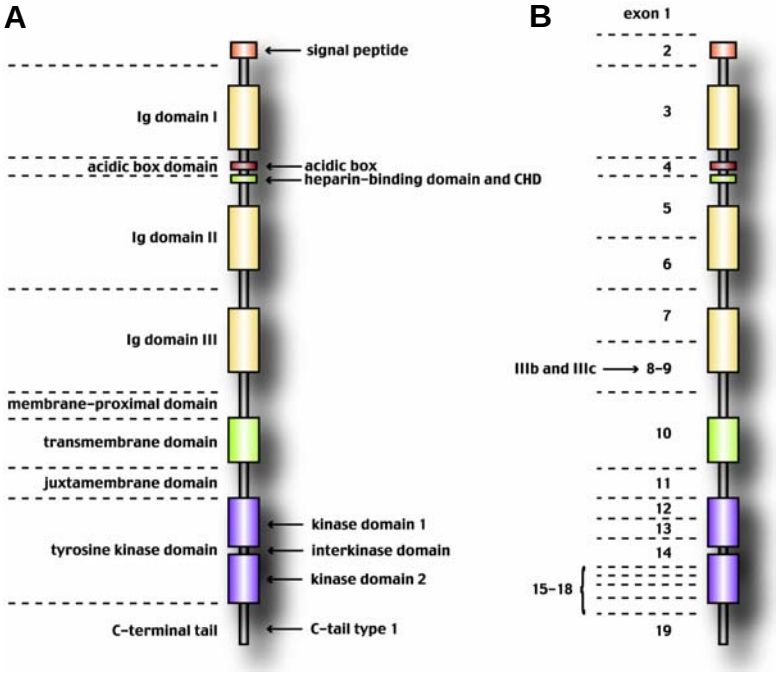

Fig. 1. Human FGFR1 protein and genomic structure. (A) *The domain structure of a 3 Ig domain FGFR1 protein is shown (modified from Johnson and Williams, 1993). A type 1 C-tail is shown, which is found in isoforms that have kinase activity. Truncated, kinase-deficient isoforms have been isolated with a different "type 2" C-tail. For further details on domain structure see the text.* **(B)** *The first exon encodes a non-translated promoter region and exons 8 and 9 encode the alternatively spliced C-terminal half of IgIII. Domain sizes shown are not to scale.*

FGFR1 Splice Variants

The genomic organization of *FGFR1* is still not fully characterised. However, based on genomic comparison to other FGF receptor genes it is likely that the human *FGFR1* gene is comprised of 19 exons (Fig. 1; Johnson *et al.*, 1991; Givol and Yayon, 1992; Cote *et al.*, 1997). Alternative splicing of *FGFR1* transcripts generates a diversity of isoforms, which are differentially expressed in cells and tissues (Johnson and Williams, 1993). Figure 2 shows diagrams of some of the reported FGFR1 splice variants. These are mainly of human or murine origin, but isoforms isolated from frog and fish are also represented. The protein structure of each isoform is deduced from analysis of the cDNA sequence. Alternative sequence usage can generate mRNA isoforms encoding receptors truncated in the extracellular or intracellular region, lacking Ig-like domains, or derived from different exons encoding variants of particular Ig-like domains. For example, secreted receptor isoforms lacking the kinase domain have been shown to result from alternative splicing involving distinct sequences (Johnson *et al.*, 1990; Eisemann *et al.*, 1991, Werner *et al.*, 1992). One such variant is generated by alternative splicing of mRNA from exon 3, which encodes IgI, to normally intronic sequences from upstream of exon 4 (Fig. 2: isoform 1). This mRNA encodes IgI followed by 32 unique amino acids and a stop codon (Eisemann *et al.*, 1991). Alternative splicing of exon 7 sequences to three different downstream sequences generates isoforms with a variable C-terminal half of IgIII (termed FGFR1IIIa, FGFR1IIIb and FGFR1IIIc). The latter two isoforms, produced by splicing exon 7 sequences to those of either exons 8 or 9, have been shown to generate transmembrane proteins with different ligand binding specificities (Fig. 2: isoforms 5-12; and see below; Dionne *et al.*, 1990; Johnson *et al.*, 1990; Reid *et al.*, 1990; Eisemann *et al.*, 1991; Hou *et al.*, 1991; Johnson and Williams, 1993; Gillespie *et al.*, 1995; Wang *et al.*, 1996; Beer *et al.*, 2000; Lopez and Korch, 2000; C. Groth and M. Lardelli, unpublished results). Splicing of mRNA from exon 7 to normally intronic sequences upstream of exons 8 and 9 generates another truncated and secreted FGFR1 product (FGFR1IIIa), which ends downstream of IgIII (Fig. 2: isoforms 2,3; Johnson *et al.*, 1990; Werner *et al.*, 1992).

Little is known about the biological functions of truncated soluble FGFR1 isoforms. The FGFR1IIIa isoform is expressed in postnatal and adult mouse tissues, and in human cell lines (Duan *et al.*, 1992; Werner *et al.*, 1992) and is present in the circulation (Hanneken, 2001). In the adult murine retina the truncated FGFR1IIIa is differentially expressed compared to full-length FGFR1IIIc, suggesting a specific role for this isoform in the retina (Guillonneau *et al.*, 1998). Soluble fibroblast growth factor receptors have also been isolated that lack the transmembrane region (Givol and Yayon, 1992). In our laboratory we have isolated from zebrafish embryos a cDNA representing a FGFR1 variant that lacks the second half of IgIII as well as the transmembrane domain, thus representing a putative soluble isoform (Fig. 2: isoform4; C. Groth and M. Lardelli, unpublished results). However, soluble FGFR1 receptors can also be generated by proteolytic cleavage, resulting in ectodomain shedding. The ectodomains from isoforms of FGFR1IIIb and FGFR1IIIc with either two or three Ig domains have been found in the extracellular matrix and in blood where they may function to regulate the biological activities of the FGFs during cell proliferation and development (Hanneken, 2001; and see below). Several groups have isolated cDNAs representing FGFR1 isoforms with either 2 or 3 Ig domains, termed FGFR1β and FGFR1α, respectively (Fig. 2: isoforms 2,3, 5-8; Dionne *et al.*, 1990; Johnson *et al.*, 1990; Reid *et al.*, 1990; Eisemann *et al.*, 1991; Werner *et al.*, 1992; Johnson and Williams, 1993; Beer *et al.*, 2000). The physiological significance of inclusion or exclusion of IgI is still largely unknown. However, it has been shown that a switch from FGFRα to FGFRβ isoforms correlates with astrocyte malignancy (Yamaguchi *et al.*, 1994). This may be the result of changes in ligand affinity between these two forms of FGFR1 receptor, as it has been shown that FGFR1β isoforms exhibit a 10-fold higher affinity for FGF1 and FGF2 than FGFR1α isoforms. This may lead to a growth advantage for tumourigenic cells, resulting in the development of a brain tumour (Shi *et al.*, 1993; Wang *et al.*, 1995). FGFR1 is not only located at the cell surface but is also present in the nucleus (Kilkenny and Hill, 1996). However, only the FGFRα form has been found in the nucleus, suggesting that IgI is important for nuclear targeting of this receptor (Prudovsky *et al.*, 1996).

Subtle variations of structure have been found in the FGFR1α and β isoforms. These are inclusions or exclusions of a RM dipeptide downstream of the acidic box (Fig. 2: isoforms 2,3,7,8) or of a VT dipeptide within the juxtamembrane domain (Fig. 2: isoform 9). These isoforms are generated by alternative use of slightly different splice donor sites at the exon/intron boundaries of the exons encoding the acidic box and juxtamembrane regions

(Eisemann *et al.*, 1991; Gillespie *et al.*, 1995). The VT dipeptide is part of a protein kinase C (PKC) phosphorylation consensus sequence within FGFR1 (Kennelly and Krebs, 1991). PKC-induced FGFR1 activity is strictly regulated during embryo development (Gillespie *et al.*, 1995), and the relative expression levels of VT-containing and VT-lacking FGFR1 isoforms have been found to regulate mesoderm induction in *Xenopus* (Paterno *et al.*, 2000).

Several kinase-deficient FGFR1 variants have been described. For example, Wang *et al.* (1995) have isolated a cDNA which lacks codons for 37 amino acids in the kinase 2 domain (Fig. 2: isoform 11). Hou *et al.* (1991) have characterized a transcript that, due to the use of an alternative splice donor site in exon 14 of the *FGFR1* gene, leads to a shift in the reading frame that truncates the second kinase domain to 24 amino acid residues followed by 44 unique C-terminal residues (C-tail type 2) (Fig. 2: isoform 12). The presence of kinase-deficient FGFR1 isoforms in cells that also express FGFR1 active forms is thought to be a mechanism for regulating the level of FGFR1 activity since kinase-deficient and active forms can heterodimerize leading to non-functional receptor dimers and down-regulation of ligand-induced signal transduction (Shi *et al.*, 1993). Finally, an FGFR1 splice variant was isolated from human placental tissue that is derived by skipping exons 6 and 7 (encoding the C-terminal half of IgII and N-terminal half of IgIII) and directly splicing exons 5 and 9 together. This results in the generation of a product that can activate mitogenic signalling without phosphorylating FRS2α (FGF receptor substrate 2α) or phospholipase C_γ, key components of two mitogenic signalling pathways (Fig 2: 10; and see below).

The large number of different splice variants of FGFR1 isolated from various vertebrate tissues strongly suggests that FGFR1 isoforms have specific functions during development and in adult homeostasis. However, the number of different FGFR1 isoforms, may to a certain degree, be exaggerated for two reasons. First, many of the FGFR1 variants have been identified as cDNAs isolated from immortalised cell cultures. Thus, although they may well be important for processes in pathological cell states they may not be relevant for our understanding of *FGFR1* biology in the context of normal, healthy tissues. Secondly, RT-PCR, the central technique used to identify *FGFR1* isoforms, is highly sensitive and may amplify cDNAs that are mere by-products of normal *FGFR1* RNA splicing and are present at concentrations too low to have any physiological effect.

FGFR1 Ligands

The generation of multiple FGFR1 isoforms by alternative transcript splicing provides the basis for differential binding of ligands to the receptor resulting in specific cellular responses. FGFR1 has been found to interact with several different types of ligand, including fibroblast growth factors (FGFs), heparan sulphate proteoglycans (HSPGs) and neural cell adhesion molecules (CAMs). FGFR1 was originally identified as a high-affinity receptor for fibroblast growth factors (FGF). In human and mouse, 22 *FGF* genes have been identified (Ornitz and Itoh, 2001) with diverse functions during embryo development and adult tissue homeostasis, including the regulation of cell proliferation, differentiation, wound healing, angiogenesis and malignant transformation as well as migration and neurite outgrowth (for reviews see Szebenyi and Fallon, 1999; Powers *et al.*, 2000). *In vitro* assays with BaF3 cells show that FGFR1 isoforms have distinct FGF-binding affinities and only bind FGF1 and FGF2 with high affinity (Ornitz *et al.*, 1996; Xu *et al.*, 2000). The alternatively spliced C-terminal half of the IgIII domain has been shown to play an essential

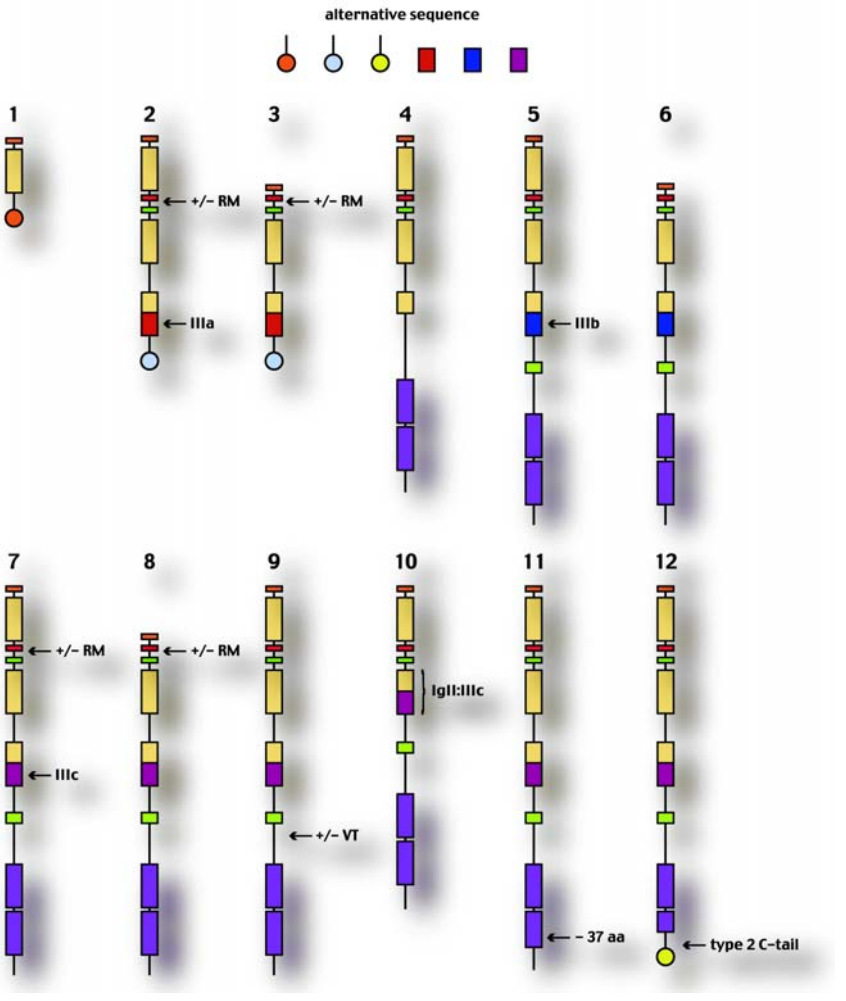

Fig. 2. FGFR1 isoform structures. *Isoforms 1-4 are soluble receptors. 5-12 are transmembrane variants. Isoforms 11 and 12 are transmembrane proteins but are truncated in the kinase domain and therefore represent kinase-deficient isoforms. Alternative sequences are indicated by the colour code above the figures. The various isoforms were compiled from the following sources: (1) Eisemann* et al., *1991; (2) Werner* et al., *1992; (3) Johnson* et al., *1990; Werner* et al., *1992; (4) C. Groth and M. Lardelli, unpublished result; (5) Johnson and Williams, 1993; (6) Beer* et al., *2000; (7) Dionne* et al., *1990; Reid* et al., *1990; Eisemann* et al., *1991; (8) Johnson* et al., *1990; Reid* et al., *1990; Eisemann* et al., *1991; (9) Johnson* et al., *1990; Reid* et al., *1990; Eisemann* et al., *1991; Gillespie* et al., *1995; (10) Lopez and Korc, 2000; (11) Wang* et al., *1996; (12) Hou* et al., *1991.*

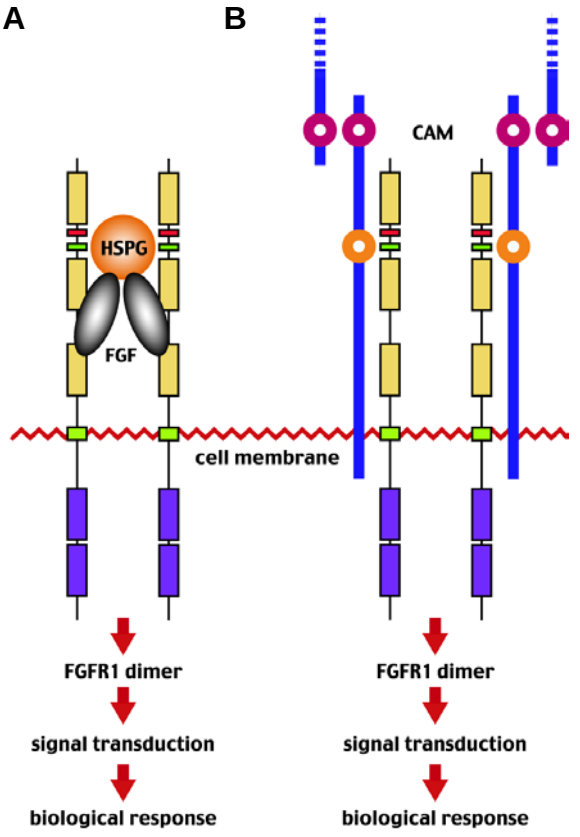

Fig. 3. Putative FGF- and CAM-mediated FGFR1 activation complexes. **(A)** *HSPG facilitated binding of FGFs to FGFR1 leads to receptor activation.* **(B)** *The receptor may also be activated through interactions with CAM molecules.*

role in determining specificities for FGF binding (Duan *et al.*, 1992; Werner *et al.,* 1992; Ornitz *et al.*, 1996; Beer *et al.*, 2000), however a number of other sequence segments in the IgI and IgII domains and upstream of IgI may also significantly modulate ligand specificity (Chelliah *et al.,* 1999). The actual FGF ligand-binding region of FGFR1 has been located within IgII and IgIII (Zimmer *et al.*, 1993; Chellaiah *et al.*, 1999).

It is becoming increasingly evident that the *in vivo* binding of FGFs to FGFR1 is facilitated by HSPGs, which not only function as co-receptors, but may play a prominent role in determining the specificity of binding of FGFs to FGFR1. Thus, the results from *in vitro* binding assays may not reflect the binding affinity of FGFs to FGFR1 *in vivo*. This is illustrated by the developmental process of patterning of the brain where *FGF8* expression is highly restricted at the midbrain-hindbrain boundary (MHB) and certain other regions (Mason *et al.*, 2000). However, *FGFR1*, which is the only FGF receptor expressed at the MHB and so is the most likely candidate for a cognate FGF8 receptor, does not bind FGF8 in *in vitro* assays (MacArthur *et al.*, 1995; Ornitz *et al.*, 1996; Blunt *et al.*, 1997), indicating that endogenous HSPGs may alter the binding affinity of FGF8 for FGFR1, leading to signal transduction *in vivo*.

HSPGs can be divided into cell surface and extracellular matrix (ECM) proteins (for reviews see Iozzo, 1998; Bernfield *et al.*, 1999). HSPG chains are linear polysaccharides made up of repeating disaccharide unit backbones, which are synthesised and attached to the HSPG core protein in the Golgi-complex. They are then modified further in several steps, including the addition of sulphate groups at various positions. This provides the basis for generation of structurally different HSPGs, contributing to the generation of differential binding affinities of FGFR1 for FGFs (Perrimon and Bernfield, 2000). For example, it has been shown that in the neural precursor cells of the developing murine nervous system, the expression of specific HSPGs is highly regulated, leading to a shift in affinity from FGF2 to FGF1. This may have a significant effect on FGFR signalling at a critical phase during murine brain development where the differentiation of hundreds of different subclasses of neurons and glia is initiated (Nurcombe *et al.*, 1993). HSPGs have been shown to be involved in a variety of biological processes during embryo development and in the adult organism, including cell proliferation, differentiation, wound healing, angiogenesis, regulation of blood coagulation, cell adhesion and malignant transformation (for review see Tumova *et al.*, 2000).

It is now well established that specific FGFs facilitated by HSPGs activate FGFR1 by inducing dimerization. Based on this, a general model has been proposed where two FGF molecules interact with one HSPG to facilitate and stabilise the binding of the FGFs to two FGFR1 molecules, leading to activation of the tyrosine kinase domain (Fig. 3; Plotnikov *et al.*, 1999).

FGFR1 has also been implicated in interactions with the neural cell adhesion molecules (CAMs) such as L1, NCAM and N-cadherin that play an important role during the development of the nervous system by mediating cell-cell interactions necessary for cell migration, neurite outgrowth and other physiological processes. CAMs have also been shown to be involved in the regeneration and synaptic plasticity of the neurons in the adult nervous system (Doherty and Walsh, 1996; Benson *et al.*, 2000). Although no direct evidence has been established for the binding of FGFR1 to CAMs, several studies, using inhibitors against components of the PLCγ cascade and FGFR1 have demonstrated, that in the presence of these inhibitors both CAM- and FGF-stimulated neurite outgrowth could be blocked. This suggests that FGFs and CAMs utilize a common PLCγ pathway via FGFR1 to initiate the neurite outgrowth response (Doherty and Walsh, 1996). Furthermore, a putative evolutionarily conserved CAM binding sequence (CAM homology domain, CHD) has been identified in FGFR1 and thought to interact directly with a CHD binding motif in the CAMs. Sharing of common binding motifs may lead to *cis* and *trans* homophilic/heterophilic binding between CAMs and FGFR1 and subsequent clustering of CAM/FGFR1 complexes followed by activation of FGFR1 and signal transduction (Fig. 3; Doherty and Walsh, 1996). Co-clustering of N-cadherin and FGFR1 has recently been demonstrated, lending support for this model (Utton *et al.*, 2001). It is worth pointing out, that even if both CAMs and FGFs interact with FGFR1, the cellular responses may be different, due to possible differences in the way the ligands interact and mediate dimerization and activation of FGFR1 (Doherty and Walsh, 1996).

FGFR1 Signal Transduction

After binding of the ligand to FGFR1, the signal must be transmitted across the plasma membrane and generate an appropriate biological response in the cell. Initiation of signal transduction is believed to be achieved by conformational changes in the receptor upon ligand binding, leading to dimerization and subse-

quent activation of FGFR1 by autophosphorylation of the intracellular domain (McKeehan *et al.*, 1998). Seven tyrosine residues have been identified as potential substrates for phosphorylation and are important for kinase activity and receptor signalling (Mohammadi *et al.*, 1996). Activation of the receptor allows proteins containing Src homology (SH2) or phosphotyrosine binding (PTB) domains to bind to sequence recognition motifs in FGFR1, resulting in phosphorylation and activation of these proteins (Pawson *et al.*, 1993; Forman-Kay and Pawson, 1999; Dhalluin *et al.,* 2000). For example, phosphorylated FGFR1 tyrosine 766 is a high-affinity binding site for phospholipase C_γ (PLCγ) (Mohammadi *et al.*, 1992). Activated PLCγ can hydrolyse phospatidylinositol-4,5-diphosphate (PIP_2) to inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 induces Ca^{2+} release from intracellular stores, whereas DAG is a PKC activator. Both CAM- and FGF2-stimulated neurite outgrowth has been suggested to rely on the same PLCγ cascade, involving conversion of DAG to arachidonic acid (AA) by DAG lipase and an AA-induced increase in calcium influx into neurons via calcium channels leading to activation of calcium-dependent proteins (Fig. 4; Doherty and Walsh, 1996).

The Ras/mitogen activated protein kinase (MAPK) pathway is another important signalling cascade utilised by FGF-mediated activation of FGFR1. The adaptor protein FRS2α has been shown to link FGFR1 activation to this cascade. The PTB domain of FRS2α interacts with the juxtamembrane region of FGFR1 (Ong *et al.*, 2000), and following tyrosine phosphorylation by FGFR1 it binds the adaptor protein Grb2 (Growth factor receptor-bound protein 2), which can form a complex with Sos (Son of sevenless), a guanine nucleotide exchange factor. The proximity of the Grb2/Sos complex to the cell surface allows Sos to activate the G protein Ras by GDP for GTP exchange, leading to activation of the Ras/MAPK signalling pathway (Fig. 4; Kouhara *et al.*, 1997; Ong *et al.*, 2000; Hadari *et al.*, 2001). Activated MAPK can phosphorylate cellular proteins (e.g. Sos), and also migrate into the nucleus and activate transcription factors by phosphorylation (e.g. JUN and FOS) (for a review see Karin and Hunter, 1995). Several groups have demonstrated the importance of FRS2α in FGFR1-mediated signal transduction in various biological processes such as cell proliferation, migration and embryo development (Ong *et al.*, 2000; Hadari *et al.*, 2001). It is interesting that the FRS2α binding site overlaps with a phosphorylation consensus sequence, which allows for PKC regulation of FGFR1 signalling (see above; Gillespie *et al.*, 1995). An alanine substitution for valine in the VT dipeptide significantly diminishes FRS2α binding to FGFR1 and phosphorylation of FRS2α by FGFR1, leading to a reduction in MAPK activity (Ong *et al.*, 2000). In addition, FRS2α-deficient mice die early during embryo development (Hadari *et al.*, 2001). Taken together these findings suggest that FRS2α and PKC interactions with FGFR1 play an important role during early vertebrate development.

A more detailed understanding of the role of the FGFR1 pathway in certain aspects of brain development is slowly emerging due to recent work by Faux *et al.* (2001) who provide evidence of cross-talk between the FGFR1 signalling pathway and the Notch signalling pathway (another important developmental signalling system). It has long been known that stimulation of FGFR1 signalling by FGF1 or FGF2 inhibits neuronal differentiation (Murphy *et al.*, 1990). Cell-cell interactions mediated by the Notch signalling pathway are essential for regulation of neuron differentiation by maintaining precursor cells in an undifferentiated and proliferative state and, thus, inhibiting neurogenesis (Lewis, 1998). However, the downstream developmental cascade responsible for transduction of the FGF signal that inhibits neurogenesis remained elusive. The neurons and glia, which make up the intricate neuronal network of the nervous system, originate from neuroepithelial precursor (NEP) cells found within the neural tube (Faux *et al.*, 2001). A connection between FGFR1 and Notch signalling was established using murine forebrain NEP cells *in vitro* to demonstrate that FGF1- and FGF2-mediated FGFR1 signalling regulates the expression of Notch and Delta, key components of the Notch pathway, which subsequently leads to inhibition of neurogenesis (Faux *et al.*, 2001).

The Importance of Nuclear FGFR1

FGFR1 is generally recognized as a protein of the plasma membrane, transmitting signals to the nucleus via the Ras/MAPK pathway (Karin and Hunter, 1995). However, recent findings suggest that FGFR1 may function after internalisation by

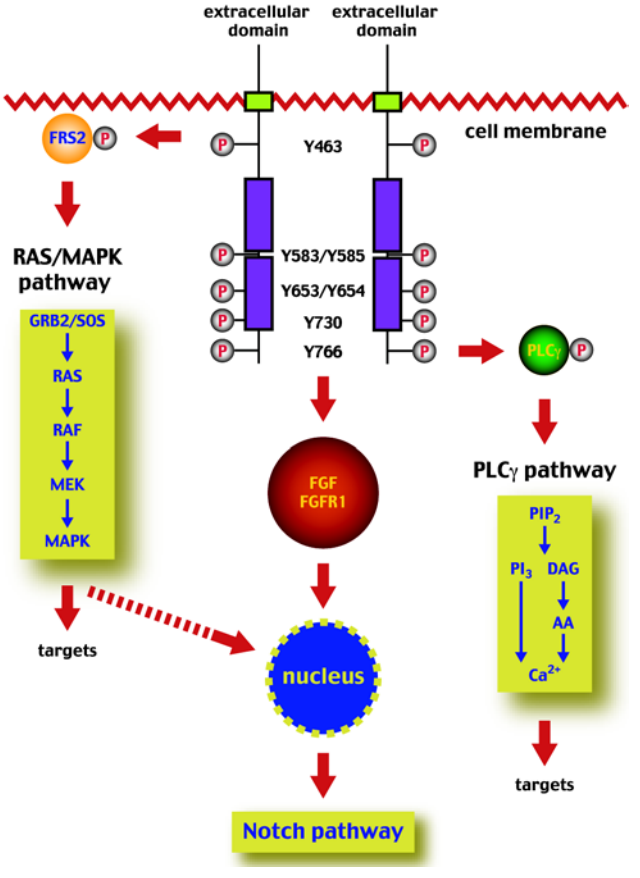

Fig. 4. Intracellular signalling pathways activated by FGFR1. *Two major signal transduction pathways, Ras/MAPK and PLCγ, are shown. The positions of the potential phosphotyrosine sites in human FGFR1 are indicated. FGFR1 signalling may activate the Notch signalling pathway via the Ras/MAPK cascade or possibly, via a direct interaction of nuclear FGF/FGFR1 complexes.*

receptor-mediated endocytosis, which is the principal mechanism for inactivation of the receptor (Sorokin *et al.,* 1994; Reilly and Maher, 2001). A fraction of the internalised FGFR1 is targeted to the nucleus as FGF/FGFR1 complexes where it may be involved in the induction of cell proliferation and differentiation (Karesztes and Boonstra, 1999). Nuclear translocation of FGF2/FGFR1 complexes is mediated through interaction of FGFR1 with importin β, a critical component of multiple nuclear import pathways (Reilly and Maher, 2001). Nuclear FGFR1 shows kinase activity (Maher, 1996) and has been implicated in regulation of the cell cycle by inducing the expression of c-Jun, which subsequently leads to the induction of cyclin D1 expression (the principal G_1-phase cyclin, followed by cell proliferation) (Reilly and Maher, 2001). It is tempting to speculate that the interaction between the FGFR1 and Notch signalling pathways demonstrated by Faux *et al.* (2001) could be accomplished by nuclear FGF/FGFR1 complexes regulating *Notch* and *Delta* gene expression.

The Role of FGFR1 during Development

Vertebrates share a common evolutionary origin, which can be dated back more than 500 million years (Holland and Chen, 2001). Comparative analysis of *FGFR1* expression patterns between vertebrates may help to gain insights into the evolution of vertebrate *FGFR1* function during development. Expression analyses of *FGFR1* in different vertebrates demonstrate a high degree of evolutionary conservation during vertebrate embryo development. For example, *FGFR1* is expressed during the period of mesoderm induction in chick, mouse and frog (*Xenopus*) (Yamaguchi *et al*., 1992; Golub *et al*., 2000; Walshe and Mason, 2000). Embryos from both *FGFR1*-deficient mice and from *Xenopus* overexpressing a dominant negative FGFR1 exhibit defects in mesoderm formation, suggesting an important role for *FGFR1* in this developmental process (Amaya *et al*., 1991; Yamaguchi *et al*., 1994). *FGFR1* is ubiquitously expressed during development of the brain in chick, mouse and *Xenopus*, with high levels of expression reported in the forebrain and midbrain-hindbrain boundary (MHB) in mouse and *Xenopus* (Yamaguchi *et al*., 1992; Golub *et al*., 2000; Walshe and Mason, 2000). Interestingly, chick embryos do not show any distinct high level *FGFR1* expression domains during early neural development while in Medaka fish (*Oryzias latipes*) *FGFR1* expression is excluded from the anterior region of the developing brain (Carl and Wittbrodt, 1999; Walshe and Mason, 2000). We have recently isolated the zebrafish (*Danio rerio*) *fgfr1* gene (C. Groth and M. Lardelli, unpublished results). In contrast to observations in Medaka, the expression pattern of zebrafish *fgfr1* is similar to that of *Xenopus*, showing ubiquitous expression during early neural development with high level expression domains in the forebrain and MHB. The MHB, also termed the isthmus organizer, produces signalling molecules such as FGF8 that induce the specification and patterning of adjacent brain structures (Reifers *et al*., 1998). *FGFR1* is expressed at the MHB in all of the above vertebrates except Medaka. In chick and mouse, *FGFR1* is the only known FGF receptor expressed at the MHB (Wilke *et al*., 1997). Furthermore the elevated FGFR1 expression in this region in zebrafish and *Xenopus* suggests that FGFR1 is the receptor for FGF8-mediated signal transduction at the MHB in these vertebrates. In Medaka neither *FGFR1* nor any of the other three highly related *FGFR* genes (*FGFR2*, *FGFR3* and *FGFR4*) show expression overlapping with that of *FGF8* at the MHB. Thus, FGF8 may transduce its signal at the MHB via a yet unidentified receptor in this species. The expression patterns of *FGFR1* during somite formation share many similar features among amphibians, fish, birds and mammals (Yamaguchi *et al*., 1992; Carl and Wittbrodt, 1999; Golub *et al*., 2000; Walshe and Mason, 2000; C. Groth and M. Lardelli, unpublished observations). *FGFR1* is expressed in the presomitic mesoderm just posterior to newly formed somites and in the first 2-5 somites but is absent from older somites. A more detailed analysis of *FGFR1* expression has been performed in mouse and zebrafish, showing that the expression is restricted to the anterior half of the somites, whereas in Medaka *FGFR1* expression is found in the lateral areas of somites (Yamaguchi *et al*., 1992; Carl and Wittbrodt, 1999; C. Groth and M. Lardelli, unpublished observations).

In situ hybridisation studies to localise *FGFR1* expression during embryo development and in the adult body have mainly been performed using probes covering the kinase domain. Thus, these analyses show the cumulative expression of all transcripts not encoding intracellularly-truncated receptors. However, the few studies based on Northern blots, RNase protection assays and immunological staining that have included isoform-specific probes show that the isoforms are differentially expressed (Reid *et al*., 1990; Werner *et al*., 1992; Guillonneau *et al*., 1998; Beer *et al*., 2000). The lack of detailed knowledge about the expression patterns of the various *FGFR1* splice forms is surprising considering the large number of isoforms that have been isolated (Fig. 2), indicating that a driving force in the evolution of *FGFR1* function has been the generation of isoforms to perform specific functional roles during embryo development and adult homeostasis. In the adult body, the FGFR1IIIc isoforms are preferentially expressed compared to FGFR1IIb isoforms (Werner *et al*., 1992; Beer *et al*., 2000). In addition, mice with targeted deletions of exon 8 or 9 (required for isoforms FGFR1IIIb or IIIc, respectively) have been generated. The developmental defects of FGFR1IIIc-isoform deficiency were severe and these mouse embryos died early during development while mice lacking FGFR1IIIb isoforms were viable and fertile with few developmental abnormalities. This indicates that the FGFR1IIIc isoforms are critical for embryo development, whereas splice variants encompassing the FGFR1IIIb specific sequence apparently are not (Partanen *et al*., 1998). Also, targeted disruption in mice of exon 3 that encodes IgI, a domain found exclusively in FGFR1α isoforms, has demonstrated that these splice variants play a pivotal role during mesoderm induction and other early developmental processes, but may not be essential during somitogenesis (Xu *et al*., 1999).

Conclusions

Since the isolation of the first complete cDNA of a vertebrate FGF receptor 1 more than 10 years ago a great variety of FGFR1 splice variants have been isolated. However, only a few of these have received more than initial characterisation. A major challenge in the coming years will be to establish the spatio-temporal expression patterns of the various isoforms during embryo development and to analyse the functional significance of each of the FGFR1 variants in a developmental context as well as in adult tissues homeostasis. Based on recent years studies, a conceptual understanding is slowly emerging of how FGFR1 signalling can result in specific biological responses of particular cell populations. In order to get a restricted and highly local response only the cells intended

to respond to signals in their environment must be able to be transmit these signals across the cell membrane and initiate signal transduction events, whereas other cells in the local environment must remain unresponsive. This is likely to be achieved by the combined differential expression of FGFR1 isoforms, FGFs and HSPGs, leading to highly restricted activation of FGFR1 signalling only in cells with the right combination of ligand and receptor molecules. The biological outcome of signals generated at the cell surface in response to ligand induced FGFR1 activation is strongly dependent on the cellular context. For example, during early embryo development, FGFR1 plays an important role in control of cell migration, a process crucial for mesodermal patterning and gastrulation, while the activation of FGFR1 signalling in fibroblasts promotes cell proliferation (Schlessinger, 2000). This suggests that common intracellular signalling pathways activated by FGFR1 are able to interact with cell-type specific effector proteins and transcription factors, leading to a specific biological response. The characterisation of components of the various FGFR1 activated signalling pathways and their regulation is progressing rapidly. However, the role of nuclear FGFR1 in regulation of transcription is less well studied and deserves more attention in the future.

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