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Structural and Functional Specificity of FGF Receptors

Juha Partanen, Satu Vainikka and Kari Alitalo

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Structural and functional specificity of FGF receptors

JUHA PARTANEN, SATU VAINIKKA AND KARI ALITALO

Molecular Cancer Biology Laboratory, Department of Pathology, University of Helsinki, Haartmaninkatu 3, 00290 Helsinki, Finland

SUMMARY

Fibroblast growth factors (FGFs) represent a group of polypeptide mitogens eliciting a wide variety of responses depending on the target cell type. The knowledge of the cell surface receptors mediating the effects of FGFs has recently expanded remarkably. Perhaps not surprisingly, the complexity of the FGF family and FGF induced responses is reflected in the diversity and redundancy of the FGF receptors. The molecular cloning of the signal transducing receptors for fibroblast growth factors has revealed a tyrosine kinase gene family with at least four members. Differential splicing and polyadenylation creates further diversity in the FGF receptor system. These numerous receptor forms have both distinct and redundant properties. We are only now beginning to understand how the different receptors are activated by the various FGFs and how they are expressed by various cells and tissues. FGF binding to the tyrosine kinase receptors needs the assistance of heparan sulphate side chains of proteoglycans present at the cell surface and in the extracellular matrix. As several other growth factors share the heparin binding property of FGFs, the dual receptor system for FGFs might be an example of a more widely used principle.

1. EFFECTS OF THE FGFS ON VARIOUS CELL TYPES

Eight factors belonging to the fibroblast growth factor family are known. These are acidic FGF (aFGF), basic FGF (bFGF), *int*-2, Kaposi sarcoma FGF (K-FGF), also known as the product of *hst*-1 oncogene, FGF5, FGF6, keratinocyte growth factor (KGF) (Burgess & Maciag 1989) and androgen induced growth factor (AIGF) (Tanaka *et al.* 1992). These polypeptides are 35–55% identical in their amino acid sequence and the analysed genes have similar exon-intron structures. In contrast to the other members of the family, aFGF and bFGF lack a signal sequence, and the mechanism of their secretion is not yet fully understood.

The most widely studied FGFs, aFGF and bFGF, appear to elicit very similar biological responses in most target cell types. These two factors have effects *in vitro* on a wide variety of cells of mesodermal, neutroectodermal as well as endodermal origin (Gospodarowicz 1990). They support the survival of neural cells and stimulate proliferation of many cell types including fibroblasts, endothelial cells, smooth muscle cells, hepatocytes and skeletal myoblasts. aFGF and bFGF can also affect cellular differentiation: both factors stimulate neurite outgrowth by PC12 rat pheochromocytoma cells and are capable of blocking skeletal myoblast differentiation. In addition, bFGF has been claimed to enhance the cloning efficiency of hematopoietic progenitor cells (Gabbianelli *et al.* 1990). Other FGFs may have more specific functions. For example, the mitogenic activity of KGF seems to be restricted to epithelial cells types.

In vivo, bFGF has been implicated in mesoderm induction in *Xenopus* embryos. Disruption of the FGF signalling by expression of a dominant negative mutant of the *Xenopus* FGF receptor has recently been shown to inhibit the formation of mesodermal tissues (Amaya *et al.* 1991). Other members of the FGF family also seem to function in developmental processes. For example, antisense oligonucleotides complementary to the *int*-2 mRNA, and antibodies against *int*-2 protein were recently shown to block the development of the chick otic vesicle (Represa *et al.* 1991).

In addition to inducing proliferation and migration of endothelial cells in culture, bFGF and aFGF also induce neovascular structures *in vivo* (Folkman & Klagsbrun 1987). It was recently claimed that the progression and neovascularization of fibrosarcomas of transgenic mice carrying the bovine papilloma virus genome is correlated with enhanced secretion of bFGF (Kandel *et al.* 1991).

2. BIOCHEMICAL CHARACTERIZATION OF CELL SURFACE RECEPTORS FOR FGFS

Two classes of FGF binding sites have been characterized on cell surfaces. The low affinity (K_d 2–20 nM), high capacity receptors are cell surface proteoglycans containing heparan sulfate side chains. Of the proteoglycans, FGF binding to heparan sulfate is specific: other closely related glycosaminoglycans such as chondroitin sulfate do not bind FGFs. The high affinity, low capacity receptors bind FGFs with a K_d of 20–600 pM in the presence of heparin. These receptors are

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Table 1. *Basic characteristics of the tyrosine kinase FGF receptors*

	other names	location in human genome	mRNA length	apparent molecular mass	FGF binding
FGFR1	*flg*, *cek*1, bFGFR	8p12	4.2, 4.3 kb	150 kDa	aFGF, bFGF > K-FGF (type IIIc) aFGF > bFGF (type IIIb)
FGFR2	*bek*, KGFR, *cek*3 K-sam, TK14	10q26	4.4 kb	135 kDa	aFGF, bFGF, K-FGF > KGF (type IIIc) aFGF, KGF > bFGF (type IIIb)
FGFR3	*flg*-2, *cek*2	4p16.3	4.5, 7.5 kb	135 (125,97) kDa	aFGF > bFGF
FGFR4		5q35	3.0 kb	110 (95) kDa	aFGF > K-FGF > bFGF

transmembrane glycoproteins containing an intrinsic tyrosine kinase activity and are encoded by a gene family (see below). Co-operation between the proteoglycan and tyrosine kinase receptors is required for FGF binding and appears to be crucial for signal transduction as discussed below. Other, as yet less well characterized receptors have also been reported. Recently, a 150 kDa proteoglycan of rat parathyroid cells was described, which binds acidic FGF with a very high affinity (Sakaguchi *et al.* 1991). Whether the polypeptide of this proteoglycan is related to the high-affinity tyrosine kinase receptors is not known. A high-affinity receptor lacking a tyrosine kinase domain has also been isolated from chicken (Olwin *et al.* 1991). This receptor binds FGFs only in the absence of heparin. In addition to FGFs, many other growth factors, such as granulocyte-macrophage colony stimulating factor (GM-CSF), amphiregulin, interleukin-3 (IL-3), heparin-binding growth associated molecule (HB-GAM) and heparin-binding-epidermal growth factor (HB-EGF) interact with heparin (Ruoslahti & Yamaguchi 1991).

3. MOLECULAR CLONING OF FGF RECEPTORS

A receptor binding bFGF with high-affinity in the presence of heparin was originally affinity purified and its cDNA cloned from chicken (Lee *et al.* 1989). This protein was found to be highly homologous to the *flg* receptor tyrosine kinase cloned from human endothelial cells (Ruta *et al.* 1988). Subsequently, the human and mouse *flg* (FGFR1) cDNAs were shown to encode receptors for bFGF, aFGF and K-FGF (Dionne *et al.* 1990; Mansukhani *et al.* 1990; Wennström *et al.* 1991). The first partial clone for a second FGF receptor (FGFR2) called *bek*, emerged from screening a mouse liver expression library with anti-phosphotyrosine antibodies (Kornbluth *et al.* 1988). The molecular cloning and expression of full-length *bek* cDNAs showed that they encode another receptor for both aFGF and bFGF (Dionne *et al.* 1990; Houssaint *et al.* 1990). One form of FGFR2 cDNA was isolated as a keratinocyte growth factor receptor using an expression cloning approach (Miki *et al.* 1991). cDNAs for two additional fibroblast growth factor receptors, FGFR3 and FGFR4, were isolated from human leukemia cell lines and shown to encode functional FGF receptors (Keegan *et al.* 1991; Partanen *et al.* 1991). Some of the characteristics of these receptors and their genes are listed in table 1.

All four receptor sequences are highly homologous (amino acid sequence identity 60–70%) and are predicted to possess a similar structure. The apparent evolutionary relationships of these genes are shown in figure 1*b*. Three immunoglobulin-like loops can be identified in the extracellular region of the FGFRs and the cytoplasmic tyrosine kinase domain is interrupted by a short (14 amino acids) kinase insert. The second and third immunoglobulin-like loops (which have been implicated in FGF binding) and the tyrosine kinase domains, are highly conserved between the different receptors. Different forms of FGFR1, FGFR2 and FGFR3 proteins generated by differential splicing have been identified (see below).

A low-affinity receptor for bFGF was isolated from a hamster kidney cell line by a cell panning method (Kiefer *et al.* 1990). This protein is a transmembrane proteoglycan core protein known as syndecan (Saunders *et al.* 1989), which contains heparan sulphate side chains capable of FGF binding. Basic FGF binding to the syndecan type receptor can be blocked by heparan sulphate and heparan, but not by other glycosaminoglycans such as keratan sulphate, chondroitin sulfate or dermatan sulphate (Kiefer *et al.* 1990).

4. MULTIPLE FORMS OF TYROSINE KINASE FGFRS

The published cDNA sequences for both FGFR1 and FGFR2 differ in their extracellular domains: FGFR1 and FGFR2 forms, with two or three immunoglobulin-like loops, are generated by differential splicing

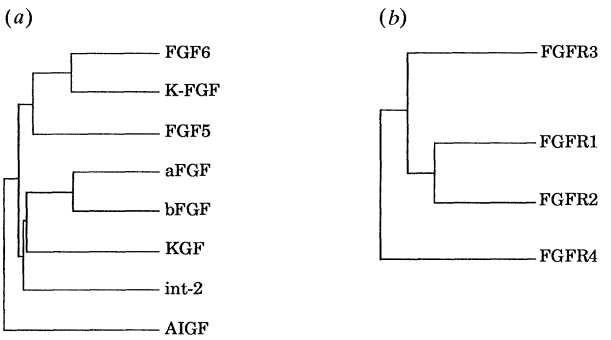

Figure 1. Deduced evolutionary relationships of (*a*) the FGFs and (*b*) the FGFRs. The trees were constructed using the Pileup program of the software package of the Genetics Computer Group of Wisconsin University (Devereux *et al.* 1984).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

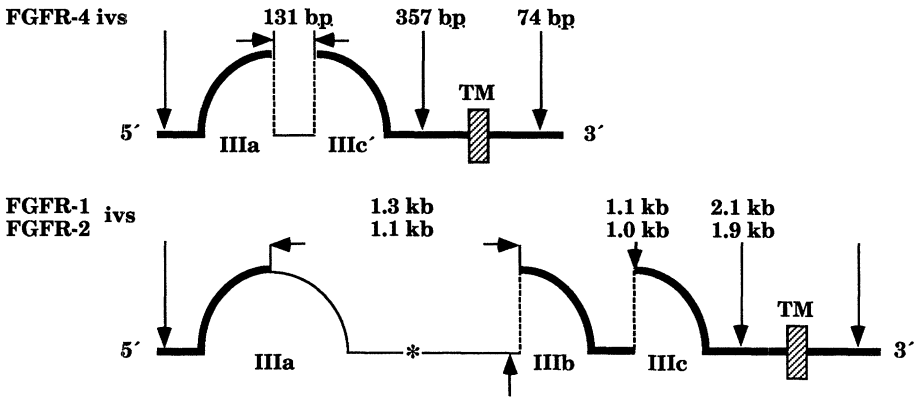

Figure 2. Comparison of the genomic structures of FGFR-4, FGFR-1 and FGFR-2 in the region encoding the III Ig loop. The schematic drawing in the upper pannel is based on analysis of the FGFR-4 genomic sequence (submitted to the EMBL database; accession number X68559) and comparison with data published by Johnson *et al.* (1991). The sites of the introns (ivs) have been marked with arrows along the cDNA (thick line) and their sizes are given. Thus, the III Ig-loop of FGFR-4 is encoded in exons IIIa and IIIc′, separated by a 131 b.p. ivs (thin line). In the FGFR-1 and FGFR-2 the III Ig-loop is encoded by exon IIIa and either exon IIIb or IIIc, depending on the splicing of the transcripts. Asterisk: stop codon, AATAA: polyadenylation site, TM: transmembrane region.

(Johnson *et al.* 1990; Miki *et al.* 1992; Reid *et al.* 1990). Differential splicing and differential polyadenylation can also lead to a structural variation in the second half of the third immunoglobulin-like loop. The genomic sequence of this region reveals two exons (IIIb and IIIc) encoding alternative second halves of the third immunoglobulin-like loop (Johnson *et al.* 1991; Miki *et al.* 1992). These exons are used to create different FGFR1 and FGFR2 transmembrane proteins, whereas use of differential polyadenylation sites preceding these alternative exons results in FGFR1 and FGFR2 proteins lacking transmembrane and cytoplasmic sequences (type IIIa) (Johnson *et al.* 1991; Werner *et al.* 1992). Numerous additional FGFR1 and FGFR2 variants that differ in their extracellular and cytoplasmic domains have recently been observed. Both FGFR1 and FGFR2 cDNAs have been identified which encode receptors lacking a potential threonine phosphorylation site in the juxtamembrane domain or differing in their carboxyl terminal tails (Champion-Arnaud *et al.* 1991; Hou *et al.* 1991). Other, potentially secreted, forms of FGFR1 and FGFR2 have also been identified (Crumley *et al.* 1991; Eisemann *et al.* 1991; Katoh *et al.* 1992). Recently, a variant encoding a potential truncated intracellular receptor has been reported to have transforming activity (Yan *et al.* 1992). The published FGFR3 and FGFR4 sequences are more related to the type IIIc variant than to type IIIb. Interestingly, the genomic structure of FGFR4 does not contain alternative exons for the third immunoglobulin-like loop (Vainikka *et al.* 1992). The comparison of the genomic structure of FGFR-1, FGFR-2 and FGFR-4 in the region encoding the third Ig-loop is shown in figure 2. Two abundant mRNA species can be detected with FGFR3 probes. The larger of these mRNAs seems to correspond to cDNA clones with some retained introns and may encode a secreted form of the receptor (M. Hayman, personal communication).

5. LIGAND BINDING CHARACTERISTICS OF THE TYROSINE KINASE FGFRS

The various FGF receptors bind different FGFs in a partly overlapping and complex manner. When expressed in mammalian cells the FGF receptor cDNAs encode cell surface glycoproteins (95–150 kDa) binding FGFs with a high affinity (K_d 20–600 pM). Both human FGFR1 and FGFR2 containing type IIIc third immunoglobulin-like loops have been shown to bind aFGF and bFGF with high affinity (Dionne *et al.* 1990). In addition, the murine type IIIc FGFR1 lacking the first immunoglobulin-like loop binds, and is activated by, bFGF and K-FGF (Mansukhani *et al.* 1990). K-FGF binds to FGFR1 with 20-fold lower affinity than bFGF, whereas these two factors bind to FGFR2 with similar affinities (Dionne *et al.* 1991; Mansukhani *et al.* 1992; Mansukhani *et al.* 1990). The mouse FGFR3 has been reported to bind aFGF and K-FGF, and less efficiently, bFGF (Orniz & Leder 1992), although the human FGFR3 expressed in *Xenopus* oocytes can be activated with both aFGF and bFGF (Keegan *et al.* 1991). Similarly, human FGFR4 binds aFGF with high affinity and K-FGF and bFGF more weakly (Partanen *et al.* 1991; Vainikka *et al.* 1992).

The differential splicing discussed above further regulates the FGF binding characteristics of the FGF receptors. The first immunoglobulin-like loop seems to be dispensable for FGF binding. FGFR1 forms lacking the first loop can be activated by aFGF and bFGF equally well (Hou *et al.* 1991; Johnson *et al.* 1991). Similarly, KGF binding to the type IIIb variant of FGFR2 is not affected by the absence of the first immunoglobulin-like loop (Miki *et al.* 1992). In contrast, the ligand specificity of FGFR1 and FGFR2 is affected by which exon is used to encode the third immunoglobulin-like loop. A variant of mouse and human FGFR2 containing the type IIIb third immunoglobulin-like loop has been isolated as a high-affinity receptor for KGF (Miki *et al.* 1992; Miki *et al.*

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

1991). This kind of FGFR2 also binds aFGF, but bFGF only with a 50-fold lower affinity. In contrast, the type IIIc FGFR2 binds both aFGF and bFGF with high affinity but does not bind KGF (Dionne *et al.* 1990; Miki *et al.* 1992; Yayon *et al.* 1992). Similarly, murine FGFR1 containing the type IIIb third immunoglobulin loop binds aFGF with about 50-fold higher affinity than bFGF, and might be another receptor for KGF (Werner *et al.* 1992). The truncated, soluble FGFR1 form is also capable of FGF binding and preferentially binds bFGF (Duan *et al.* 1992).

6. PROTEOGLYCAN AND TYROSINE KINASE RECEPTORS COLLABORATE IN FGF BINDING

Heparin and heparan sulphate present in the side chains of cell surface proteoglycans have been shown to protect bFGF from proteolytic degradation (Saksela *et al.* 1988) and have been suggested to act as a storage reservoir for FGFs. These low-affinity FGF binding sites have recently been shown to play a more direct role in FGF signal transduction. Digestion of cell surface heparan sulphate or prevention of its sulfation has been shown to repress bFGF, aFGF, and K-FGF binding to tyrosine kinase receptors and thus to inhibit their biological activity (Olwin & Rapraeger 1992; Rapraeger *et al.* 1991). Furthermore, heparan sulphate-deficient Chinese hamster ovary (CHO) cells transfected with FGFR1 are not capable of bFGF binding, but high-affinity binding can be reconstituted by addition of exogenous heparin or heparan sulphate (Yayon *et al.* 1991). Heparin is also required for aFGF and bFGF binding to FGFR1-transfected FDC-P1 cells, and for the substitution of their IL-3 dependence by FGFs (Bernard *et al.* 1991). Similarly, heparin dependence has been shown for bFGF and K-FGF binding to FGFR2 (Mansukhani *et al.* 1992). Recently, the requirement of heparin for bFGF binding to FGFR1 has been demonstrated in a cell free system using soluble FGFR1 and FGFR3 extracellular domains (Kiefer *et al.* 1991; Orniz & Leder 1992; Orniz *et al.* 1992). Heparin was observed to affect bFGF dimerization in a concentration dependent manner and it was proposed that this heparin mediated FGF dimerization is also responsible for FGF receptor dimerization and activation.

7. EXPRESSION OF THE TYROSINE KINASE FGF RECEPTORS

The various FGF receptors appear to have different, although overlapping, expression patterns. During embryonic development FGFR1 and FGFR2 are first seen in the primitive ectoderm of egg cylinder-stage mouse embryos (Orr-Urtreger *et al.* 1991). Later, they are also present more widely in mesoderm- and neuroectoderm-derived tissues (Orr-Urtreger *et al.* 1991; Wanaka *et al.* 1991). In general, FGFR2 transcripts are found in various epithelia of embryonal mouse tissues, whereas FGFR1 is found mainly in the mesenchyme. FGFR1 is expressed in sclerotomal tissue condensating into intervertebral disks, while FGFR2 transcripts are present in the vertebral bodies. In the nervous system of chicken and mouse embryos the FGFR1 gene is expressed in a developmentally regulated manner alternating with nerve growth factor receptor expression (Heuer *et al.* 1990; Wanaka *et al.* 1991). Postnatally, FGFR1 has been observed in specific nerve cell populations, including hippocampal neurons and the trigeminal and dorsal root ganglia (Heuer *et al.* 1990; Wanaka *et al.* 1991). FGFR3 is expressed in neuronal cells in many regions of fetal and adult brain and also in fetal kidney, lung, small intestine and calvarial bone (Thompson *et al.* 1991).

In contrast to other FGF receptors FGFR4 does not seem to be expressed in the embryonal nervous system. Instead, its mRNA has been detected in several tissues of endodermal and mesodermal origin in mouse embryos. (Korhonen *et al.* 1992; Stark *et al.* 1991). These results are consistent with data on FGFR4 expression in the 17–18 week human fetus, where highest levels of mRNA were present in the adrenal gland, lung, striated muscle, intestine, pancreas, kidney and spleen (Partanen *et al.* 1991). FGFR4 mRNA has been detected in the developing skeletal muscles of mouse embryos but not in other muscle types such as cardiac muscle. Mesenchymal tissue associated with the first pharyngeal arch and along the prevertebrae in the myotomal portion of somites which will differentiate into muscle tissue, expresses particularly high amounts of FGFR4 mRNA (Korhonen *et al.* 1992; Stark *et al.* 1991). Moreover, FGFR4 expression follows the myogenic differentiation of P19 embryonal carcinoma cells (Stark *et al.* 1991). FGFR4 may thus be important for the differentiation of skeletal muscle and organs of endodermal origin. In adult mouse tissues, FGFR4 mRNA has been detected in liver, lung and kidney (Korhonen *et al.* 1992; Stark *et al.* 1991). In general, although overlap between the expression of the various FGFs and FGF receptors has been observed, no strict correlation of the expression patterns exist. This is consistent with the view that the FGF receptors interact with several ligands.

The differentially spliced variants of FGFR1 and FGFR2 are also expressed in a tissue specific fashion. In the mouse FGFR1 type IIIb is expressed mainly in skin whereas FGFR1 type IIIc expression is more widespread (Werner *et al.* 1992). Similarly, the expression of FGFR2 type IIIb is restricted to epithelial cells, whereas FGFR2 type IIIc is expressed by many other cell types (Miki *et al.* 1992). This is consistent with the observation that FGFR2 type IIIb is a receptor for KGF, an epithelial cell specific mitogen (see above).

8. SIGNAL TRANSDUCTION BY THE FGF RECEPTORS

As with other receptor tyrosine kinases, the first step of signal transduction via FGF receptors seems to involve receptor dimerization and transphosphorylation (autophosphorylation). It has been suggested (Orniz *et al.* 1992) that heparin could play a critical role in FGF receptor dimerization by dimerizing the ligand.

The distinct high-affinity receptors also interact. It

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

has been demonstrated that heterologous transphosphorylation occurs between FGFR1 and FGFR2 (Bellot *et al.* 1991). In *Xenopus* oocytes the expression of truncated FGFR1 containing no tyrosine kinase catalytic domain inhibits signal transduction by wild-type FGFR1, FGFR2 and FGFR3 (Ueno *et al.* 1992), demonstrating that ligand induced dimerization of the extracellular domains of different FGFRs can occur. Tyrosine residue 766 at the carboxy terminus of the FGFR1 is one of the tyrosine autophosphorylation sites (Mohammadi *et al.* 1991). Phospholipase C-gamma-1 (150 kDa) has been identified as a major target of aFGF induced tyrosine phosphorylation. It has been shown to bind to the FGFR1 protein in the region containing the phosphotyrosyl 766 through SH2 domains (Mohammadi *et al.* 1991). Interestingly, a tyrosine residue corresponding to residue 766 of FGFR1 is found in all other members of the FGFR family. A point mutation at this site of FGFR1 abolishes FGF-induced binding and phosphorylation of PLC-gamma-1, phosphatidylinositol turnover and calcium flux. Nevertheless, this mutation does not affect receptor autophosphorylation, phosphorylation of other substrate proteins or growth signal transduction FGFR1 (Mohammadi *et al.* 1992; Peters *et al.* 1992). In addition to PLC-gamma-1, FGF stimulation has been reported to activate the *raf*-1 kinase. However, FGFR1 does not seem to bind either the *ras* specific GTPase activating protein (*ras*GAP) or phosphatidyl inositol-3-kinase (PI-3K; L. T. Williams, personal communication). Interestingly, different FGFRs appear to have distinct sets of substrate proteins. In addition, PLC-gamma-1 does not appear to be phosphorylated by FGFR4 to the same degree as by FGFR1 (Vainikka *et al.* 1992).

9. CHROMOSOMAL LOCALIZATION OF FGF RECEPTOR GENES AND THEIR POSSIBLE INVOLVEMENT IN HUMAN DISEASE

The various FGF receptor genes are located on different human chromosomes (see table 1; Adnane *et al.* 1991; Armstrong *et al.* 1991; Dionne *et al.* 1992; Thompson *et al.* 1991). One form of the FGFR2 cDNA was isolated as a product of an amplified and overexpressed gene in a gastric carcinoma cell line (Hattori *et al.* 1990). FGFR1 and FGFR2 genes have also been found to be amplified in about 10% of human breast cancers (Adnane *et al.* 1991). However, current evidence does not favour the view that FGFR1 or FGFR2 is the target gene driving these amplicons (D. Birnbaum, personal communication). It has also been suggested that the FGF receptors on the cell surface aid viral penetration to cells. Contradictory reports exist in the literature concerning the role of proteoglycan and tyrosine kinase FGF receptors in Herpes simplex virus type 1 infection (Kaner *et al.* 1990; Mirda *et al.* 1992; Muggeridge *et al.* 1992).

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES