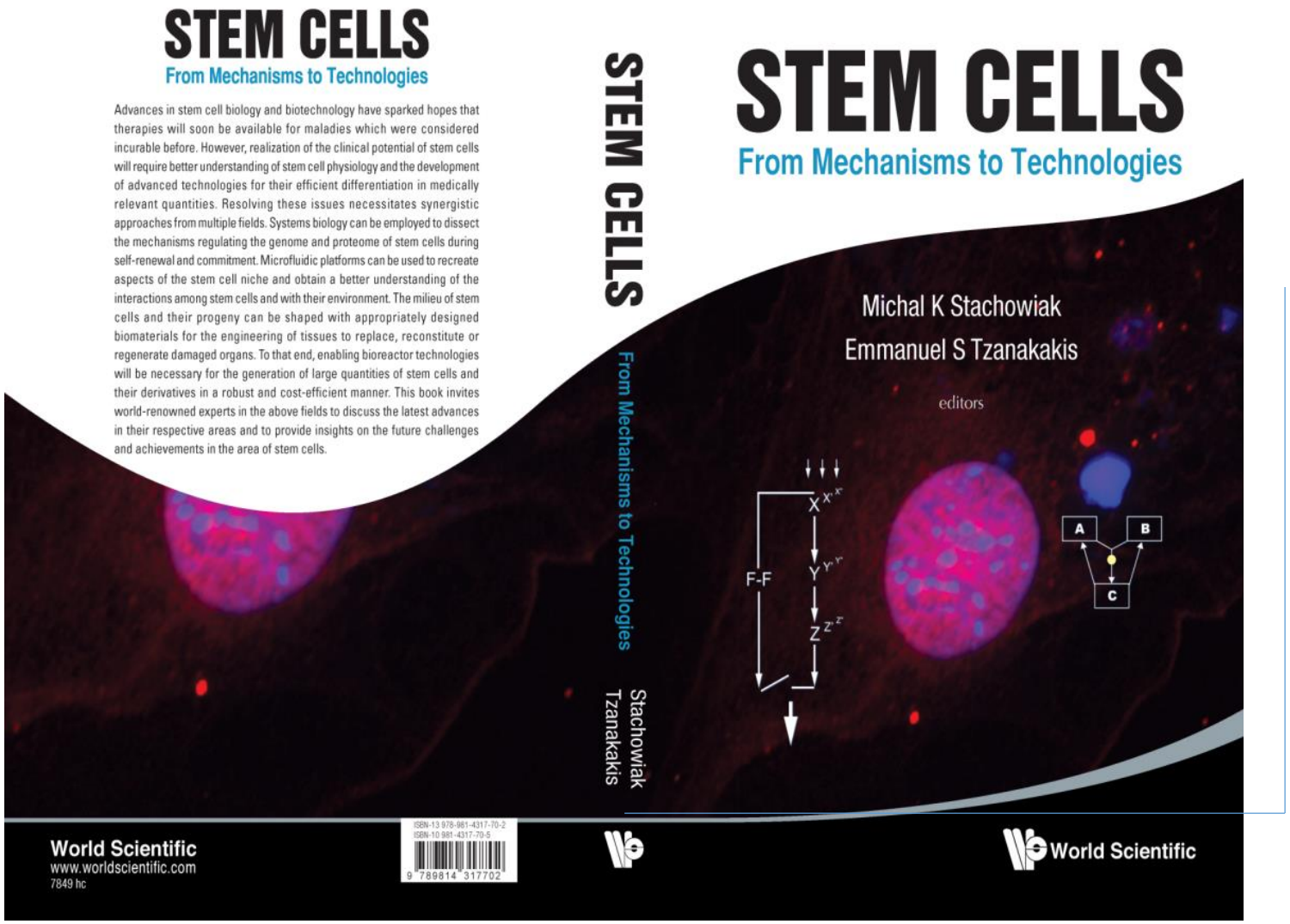
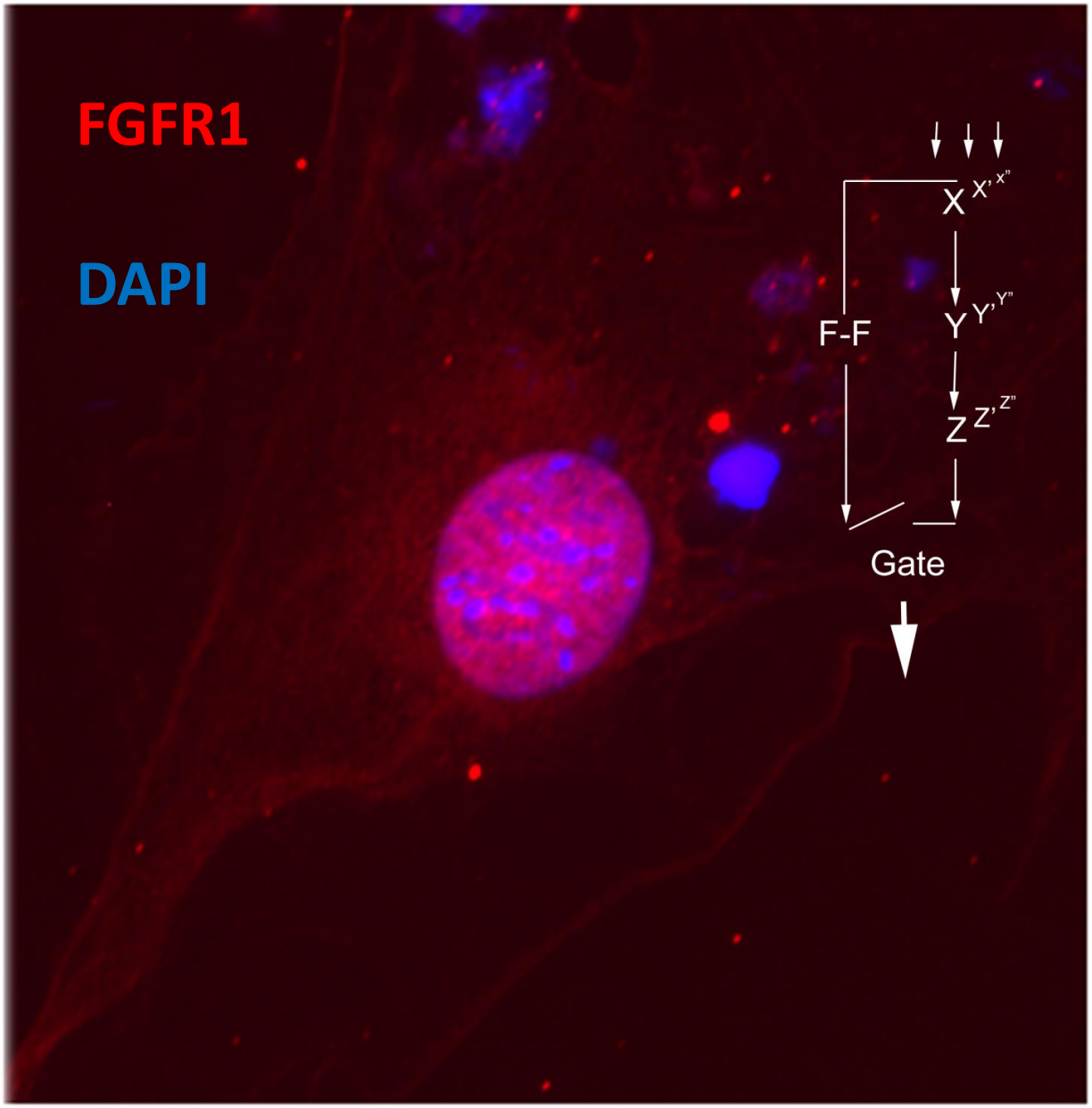
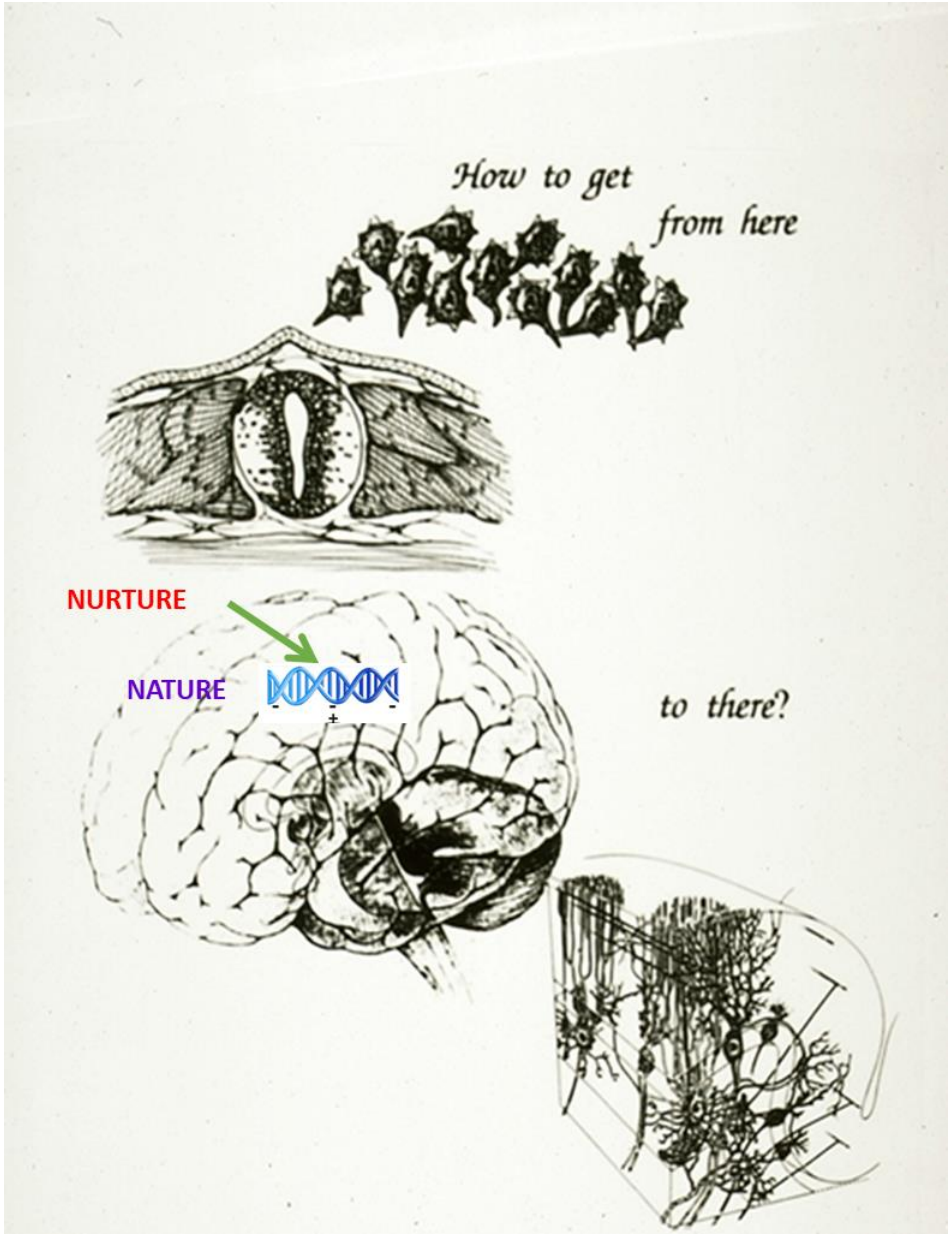
Lecture 7 “Novel Integrative Nuclear Signaling Complements Pluripotency and Cell Cycle Modules in Development”

part1

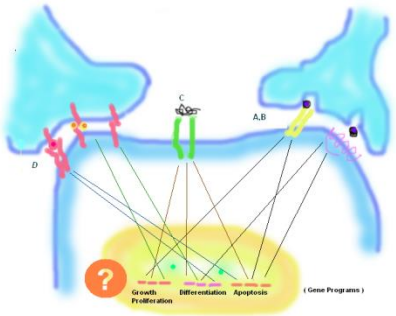


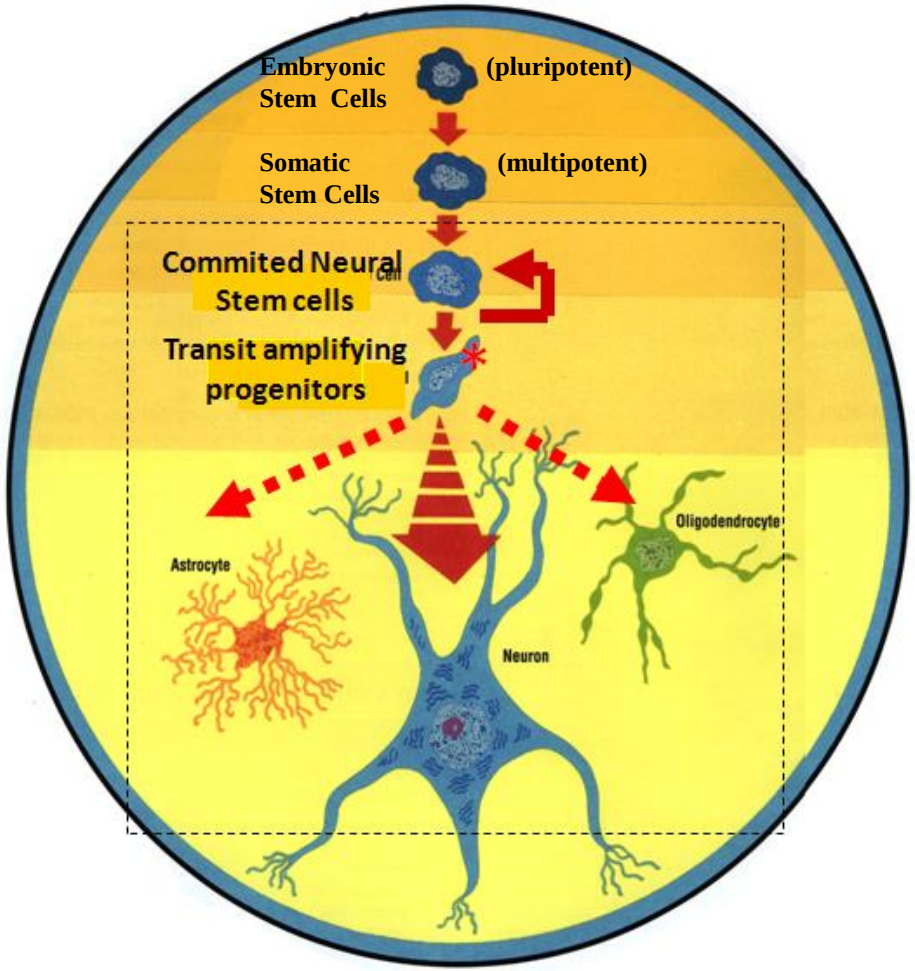

FGFR1
DAPI
X
X', X"
Y
Y', Y"
Z
Z', Z"
F-F
Gate

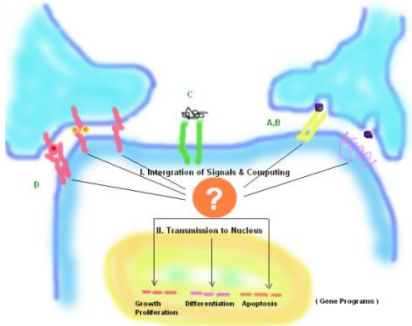
Nervous System
- product of GENOMIC BLUEPRINT and ENVIRONMENTAL FACTORS



Unfolding Developmental Plan – *a reductionist view*

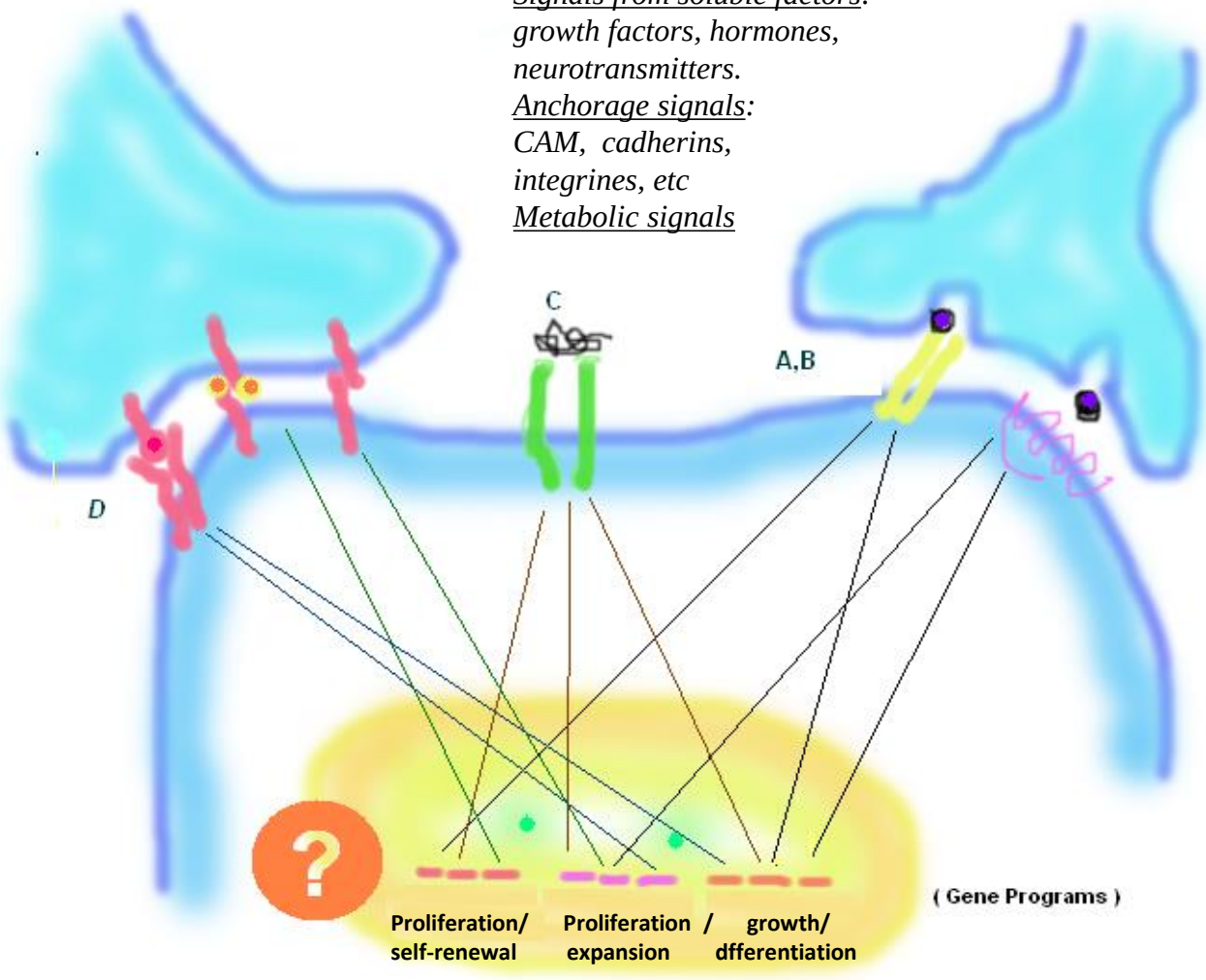






Decisions, decisions …

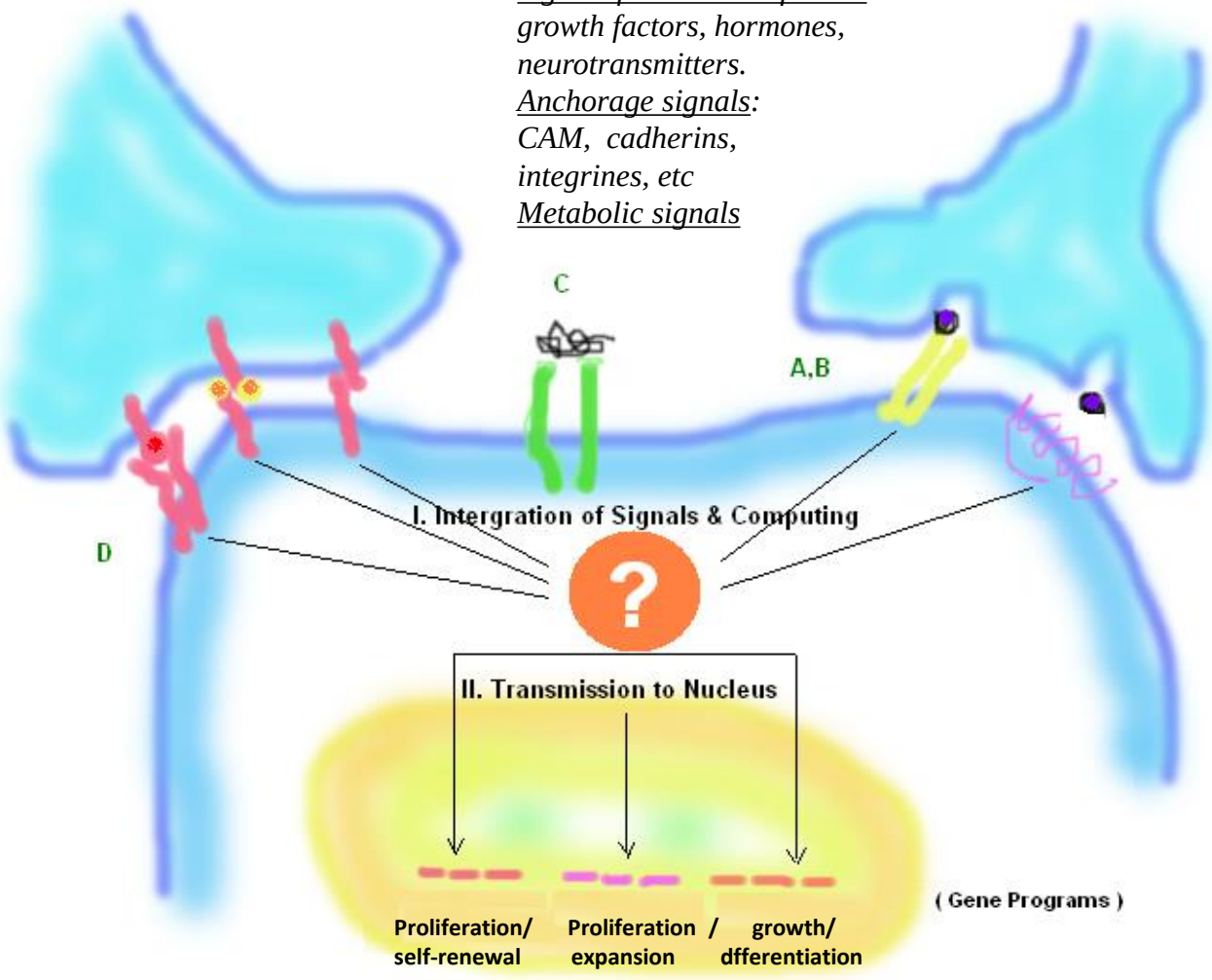
<u>Signals from soluble factors</u>:
growth factors, hormones, neurotransmitters.
<u>Anchorage signals</u>:
CAM, cadherins, integrines, etc
<u>Metabolic signals</u>

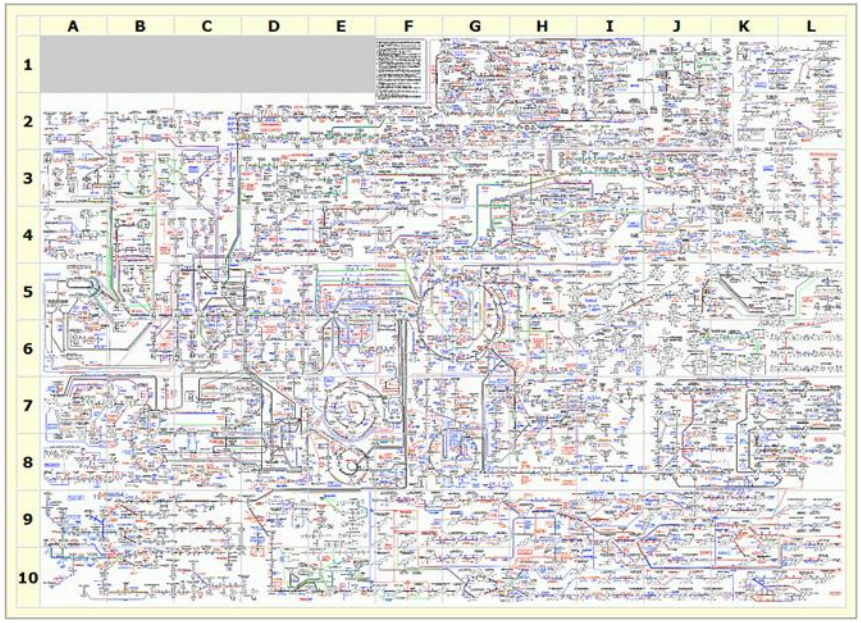


Systems Biology Computational Modules

Digital switches that quantify & integrate epigenetic signals

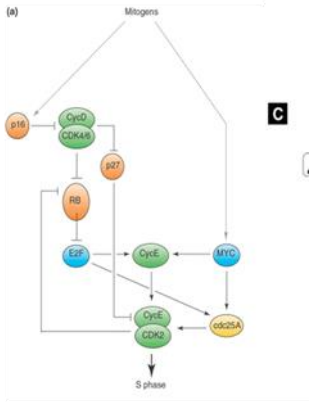
Signals from soluble factors:
growth factors, hormones,
neurotransmitters.
Anchorage signals:
CAM, cadherins,
integrines, etc
Metabolic signals





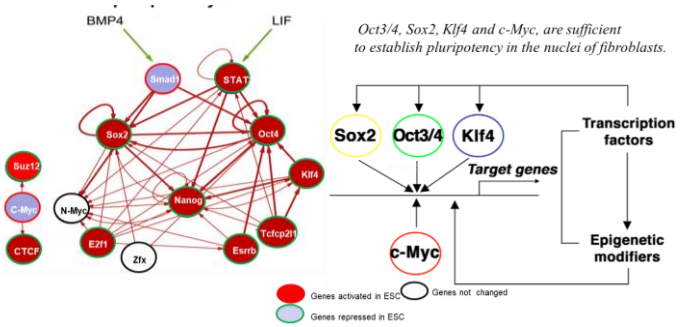
- *Metabolic Pathways*

Mitotic Network - Cyclins and CDKs

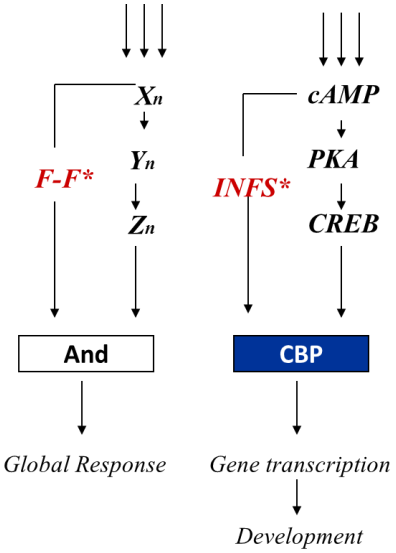


Several signals and complex pathways control accumulation of cyclin E/CDK2.

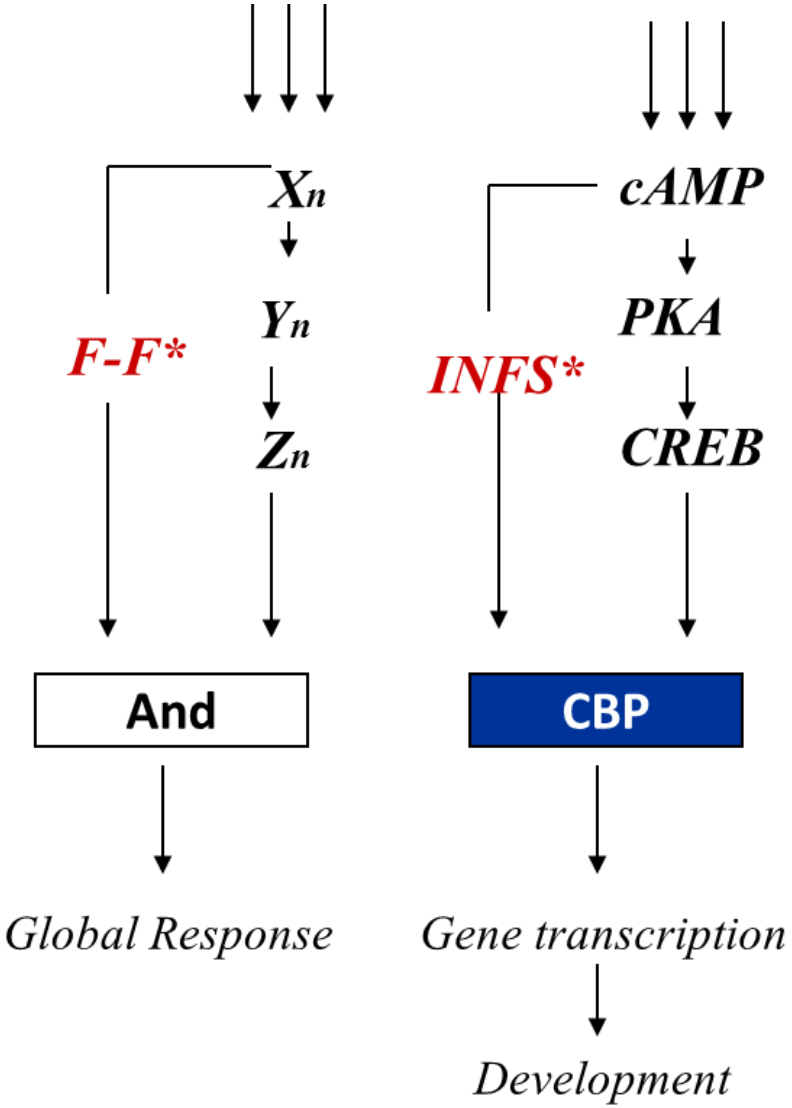
Pluripotency



Differentiation



Integrative Nuclear FGF-2 + FGFR1 (F-F) Signaling - INFS



Stachowiak, M.K., X. Fang, J.M. Myers, S.M. Dunham, R. Berezney, P.A. Maher, and E.K. Stachowiak. 2003b. Integrative nuclear FGFR1 signaling (INFS) as a part of a universal "feed-forward-and-gate" signaling module that controls cell growth and differentiation. J Cell Biochem. 90:662-691; DNA & Cell Biology, 2007).

Integrative Nuclear FGFR1 Signaling (INFS) Overview

- **FGF-2 and FGFR1 as nuclear proteins**
- Integrative Nuclear FGFR1 Signaling (INFS) in cell development
- Targeting nuclear FGFR1 signaling to control neuronogenesis.
- Reconstitution of INFS in Cancer cells
- Nuclear FGFR1 as an essential gene activator
- INFS genome programing

FGFs (n=21) - multifunctional signaling factors expressed in all tissues in multicellular organisms – Metazoa (Stachowiak eta.al., ,Integrative Nuclear Signaling in Cell Development-A Role for FGF Receptor-1. *DNA Cell Biol* **2007,** 26, (12), 811-26.)

Nomenclature of FGF and Some Features of F

Expressed in all tissues.

Name	Alternative names	Genes cloned from[b]
FGF-1	Acidic FGF (aFGF)	Human, hamster, bovine, rat, pig, chick, mouse
FGF-2	Basic FGF (bFGF)	Human, opossum, bovine, rat, chick, mouse, she *Xenopus*, newt
FGF-3	INT-2	Human, chick, fish, mouse, *Xenopus*
FGF-4	HST-1, k-FGF (Kaposi)	Human, chick, bovine, mouse, *Xenopus*
FGF-5		Human, mouse, rat
FGF-6	HST-2	Human, mouse
FGF-7	KGF (keratinocyte GF)	Human, mouse, rat, sheep, dog
FGF-8	AIGF (androgen induce	Human, mouse, chick, *Xenopus*
FGF-9	GGF (glial)	Human, rat, mouse, *Xenopus*
FGF-10		Human, rat, chick, mouse
FGF-11	FHF-3	Human, mouse
FGF-12	FHF-1	Human, mouse, chick
FGF-13	FHF-2	Human, mouse, chick
FGF-14	FHF-4	Mouse
FGF-15		Mouse
EGL-17		*C. elegans*
BNL	Branchless	*Drosophila*

[a] Based on Emoto *et al.* (1997).
[b] Based on searches of Genbank through September 1997.

FGF-2 and other FGFs are produced at some point of development by all tissues and play roles in:

Gastrulation

Neurulation

Anteroposterior specification

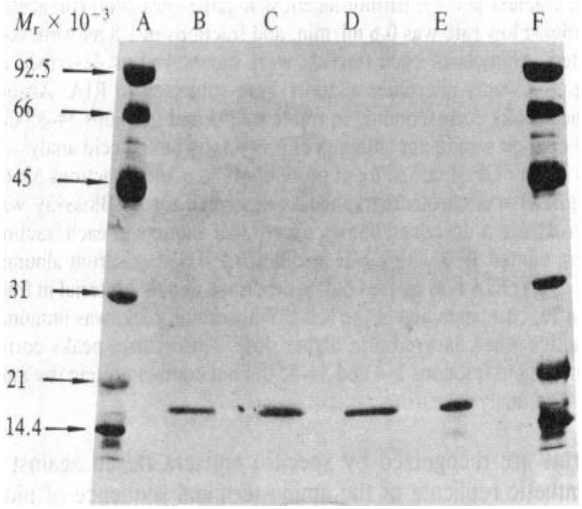
Organ morphogenesis

Axonal growth and guidance

Neuronal survival

M.K. Stachowiak and E.K. Stachowiak,
"Evidence based theory for integrated genome regulation of ontogeny - an unprecedented role of Nuclear FGFR1 signaling".
„J. Cell. Physiol. 231: 1199–1218, 2016.

Fibroblast Growth Factors (FGFs) and FGF Receptors (FGFR)



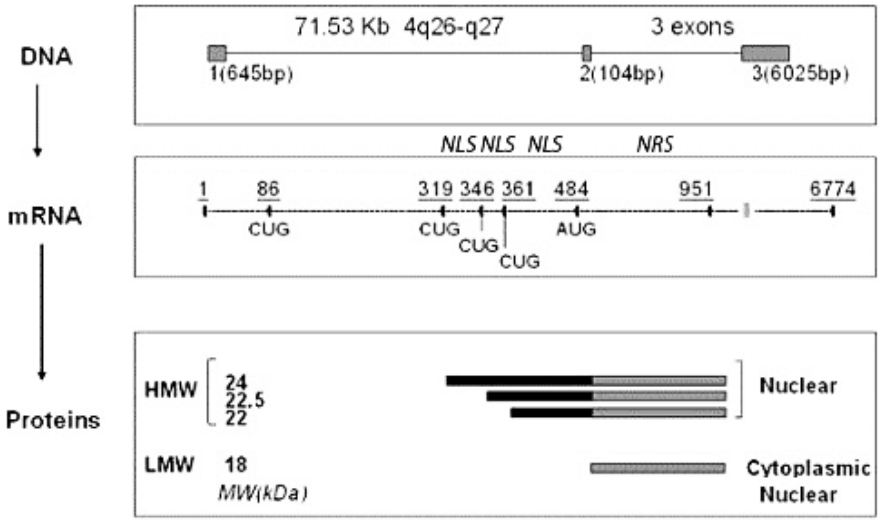
Basic FGF (FGF-2) mitogenic factor

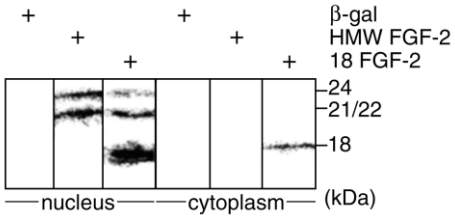
1. **Denis Gospodarowicz, G.Lui, and J. Cheng** ***1982***
Purification in High Yield of Brain Fibroblast Growth Factor by Preparative Isoelectric Focusing at p H 9.6
J. BIOL CHEM 257., pp. 12266-12276,.

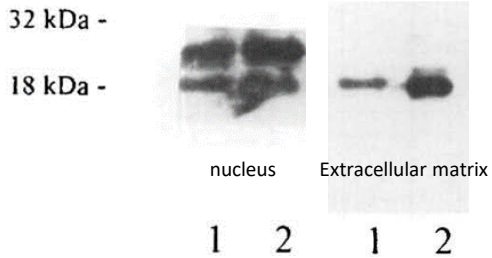
Evolution of FGFs & FGF Receptors as nuclear signaling proteins

1. Denis Gospodarowicz, G.Lui, and J. Cheng ***1982*** *Purification in High Yield of Brain Fibroblast Growth Factor by Preparative Isoelectric Focusing at p H 9.6* ***J. BIOL CHEM 257., pp. 12266-12276,.***

2. Florkiewicz, R. Z., and A. Sommer. 1989. *Human basic fibroblast growth factor gene encodes four polypeptides: three initiate translation from non-AUG codons. Proc. Natl. Acad. Sci. USA. 86:3978-3981.*
Powell PP, Klagsbrun M. Three forms of rat basic fibroblast growth factor are made from a single mRNA and localize to the nucleus. J Cell Physiol. 1991 148:202-10.







Stachowiak M.K., Moffett J., Joy A, Puchacz E., Florkiewicz R., Stachowiak, E.K., 1994, **J. Cell Biol.** 127, 203-223.
Moffett J., Kratz E., Florkiewicz R, Stachowiak M.K.,. **Proc. Natl. Acad. Sci. USA,** 1996, 93: 2470-2475.

Xin Gu1 and EunDuck P. Kay.1998 Investigative Ophthalmology & Visual Science, 39, 2252-58

Evolution of FGFs & FGF Receptors as nuclear signaling proteins

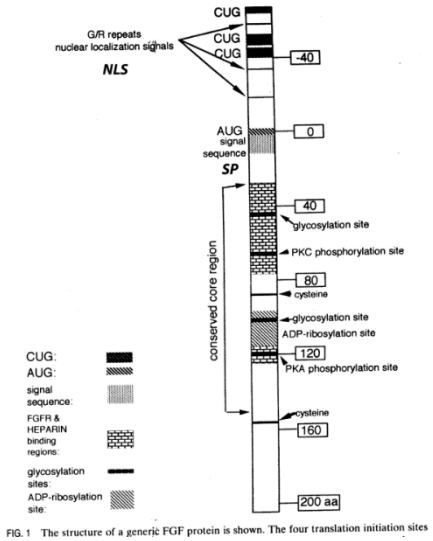


FIG. 1 The structure of a generic FGF protein is shown. The four translation initiation sites

(Nuclear, Extracellular)

C. Elegans:

LET756

(500 mln years ago)

Mammals:

FGF13-like (iFGF)

FGF13, FGF14

FGF4-like

FGF4-like

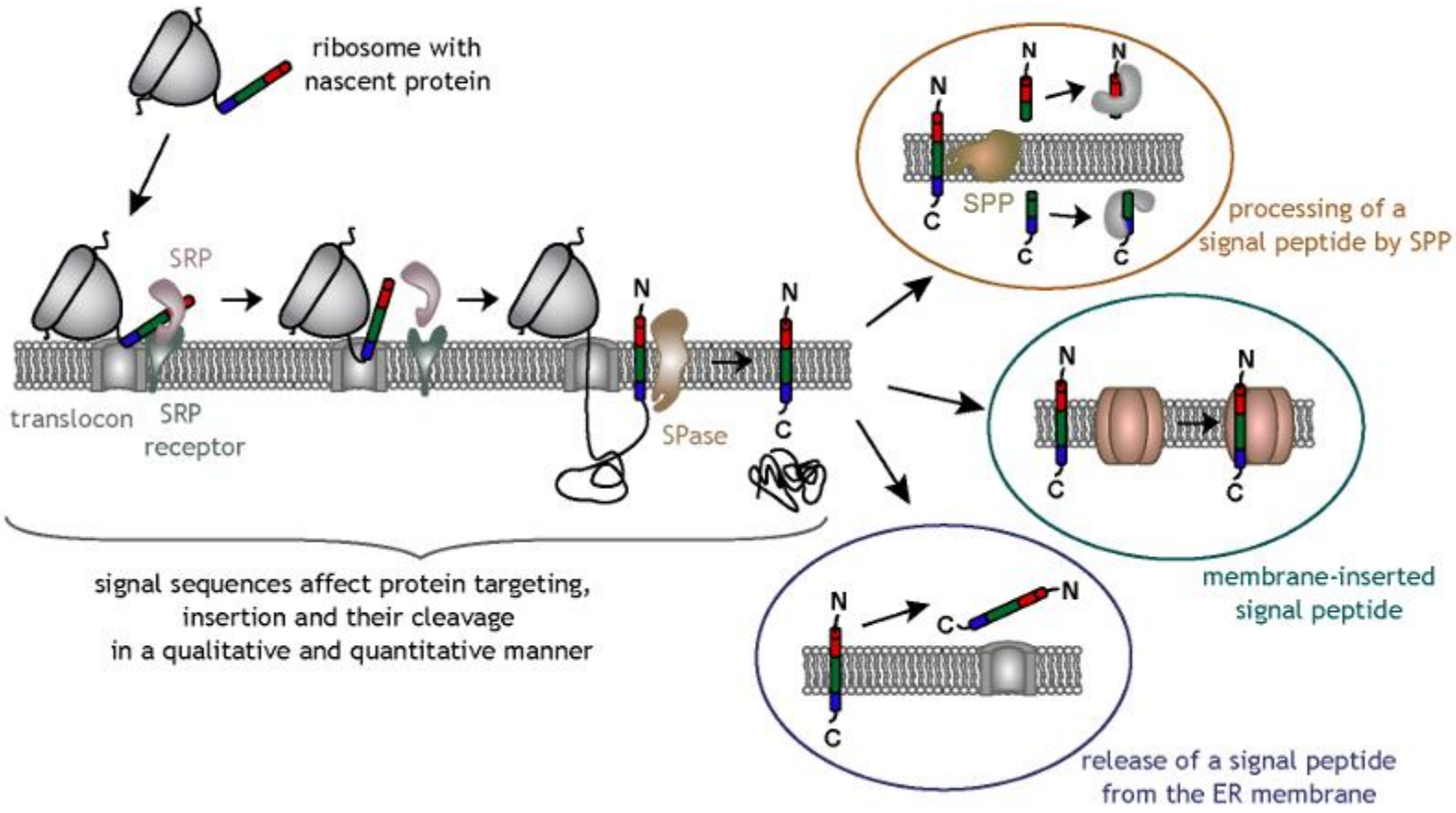
FGF15-like

FGF1, FGF2

FGF15,FGF21,FGF23

FGF1,FGF2(?)

Ornitz and Itoh, 2001


ribosome with
nascent protein
SRP
translocon
SRP
receptor
SPase
N
C
SPP
processing of a
signal peptide by SPP
membrane-inserted
signal peptide
release of a signal peptide
from the ER membrane
signal sequences affect protein targeting,
insertion and their cleavage
in a qualitative and quantitative manner

Curious evolution of FGFs - extracellular and intracellular factors nuclear proteins

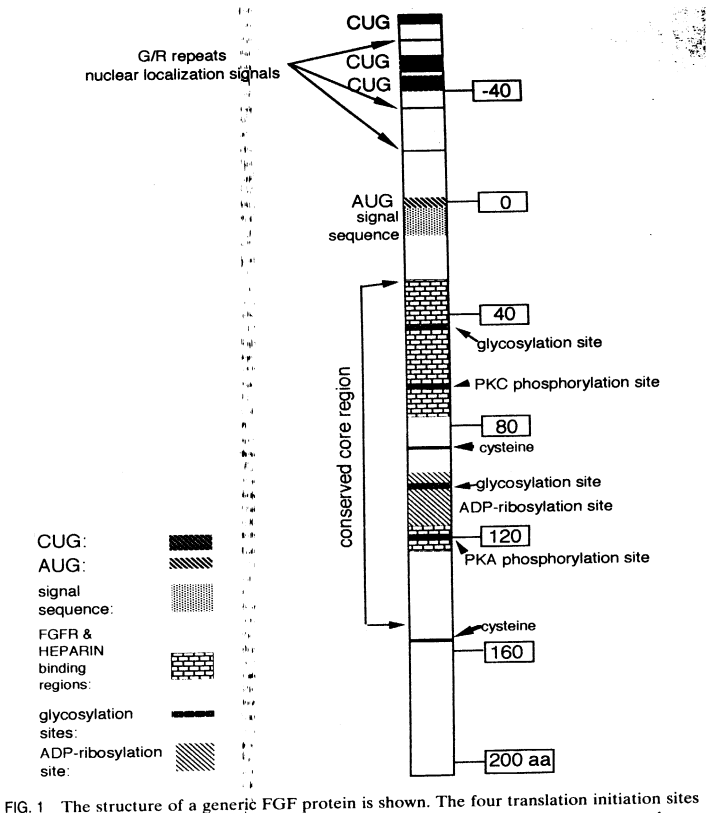
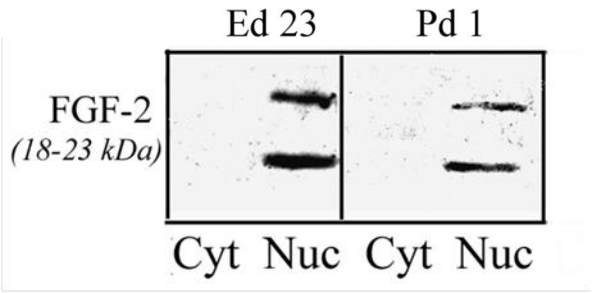


FIG. 1 The structure of a generic FGF protein is shown. The four translation initiation sites

	FGFs:	NLS	Signal peptide:
II	**4,5,6,7,8, 10,15,17**	-	+
IV	**3**	+	+
IIIa	**9,16,20**	(+)	-
IIIb	**1,2,12,13**	+	-
I	**11,18**	-	-

FGF-2 in developing mouse brain



Stachowiak et al. , (2003) J.. Neurochem 84, 1296-1312.

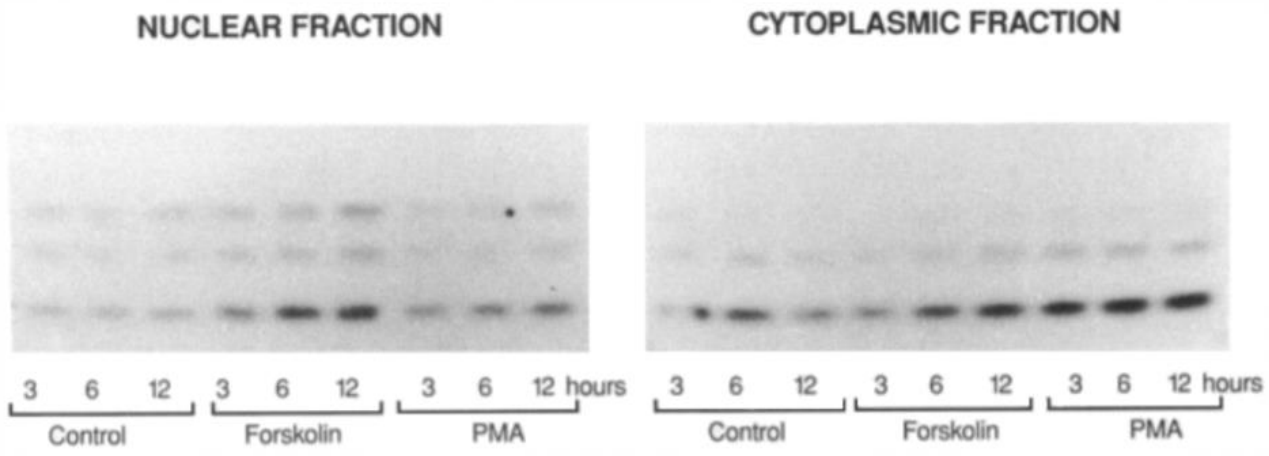


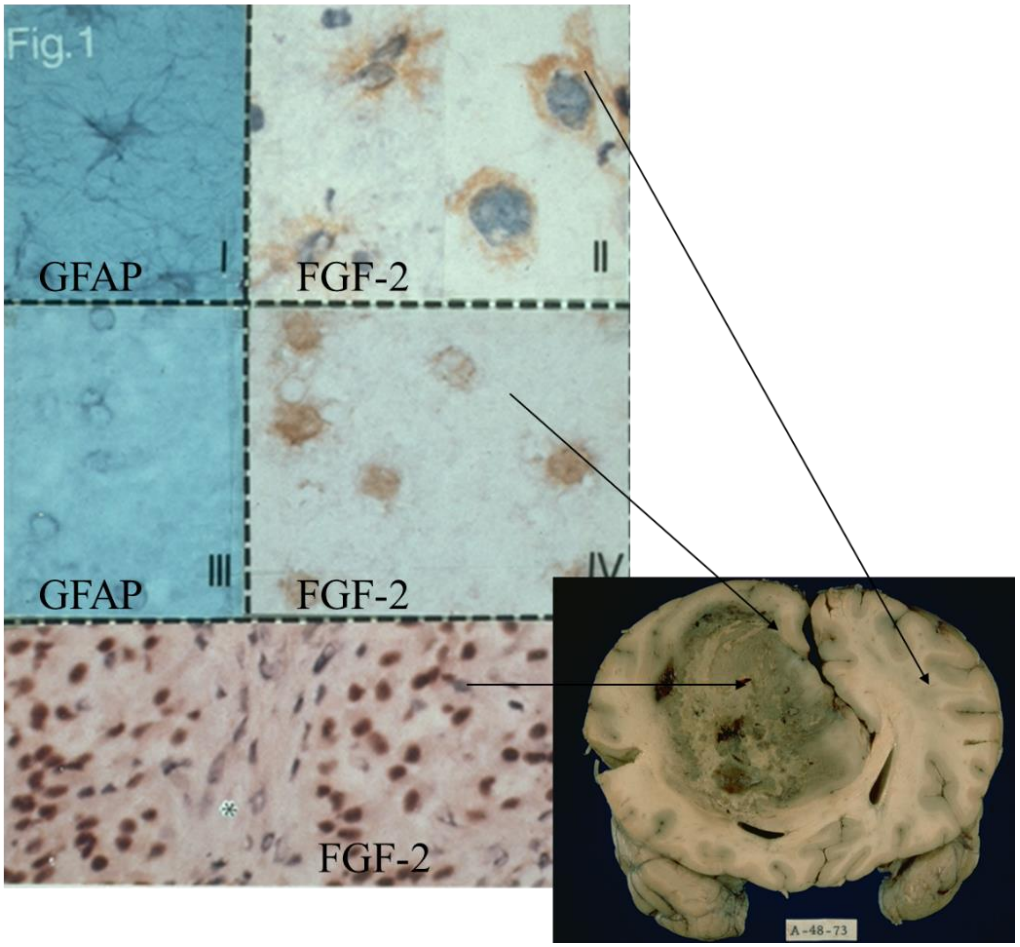
Figure 6. Effect of forskolin and PMA on subcellular distribution of bFGF isoforms in BAMC. Cells were incubated with 5 μM forskolin, 0.2 μM PMA, or 0.007% DMSO for indicated periods of time. Cellular fractions were isolated and bFGF was analyzed as on Fig. 5.

Nuclear localization of FGF-2 in reactive and neoplastic astrocytes

Control brain

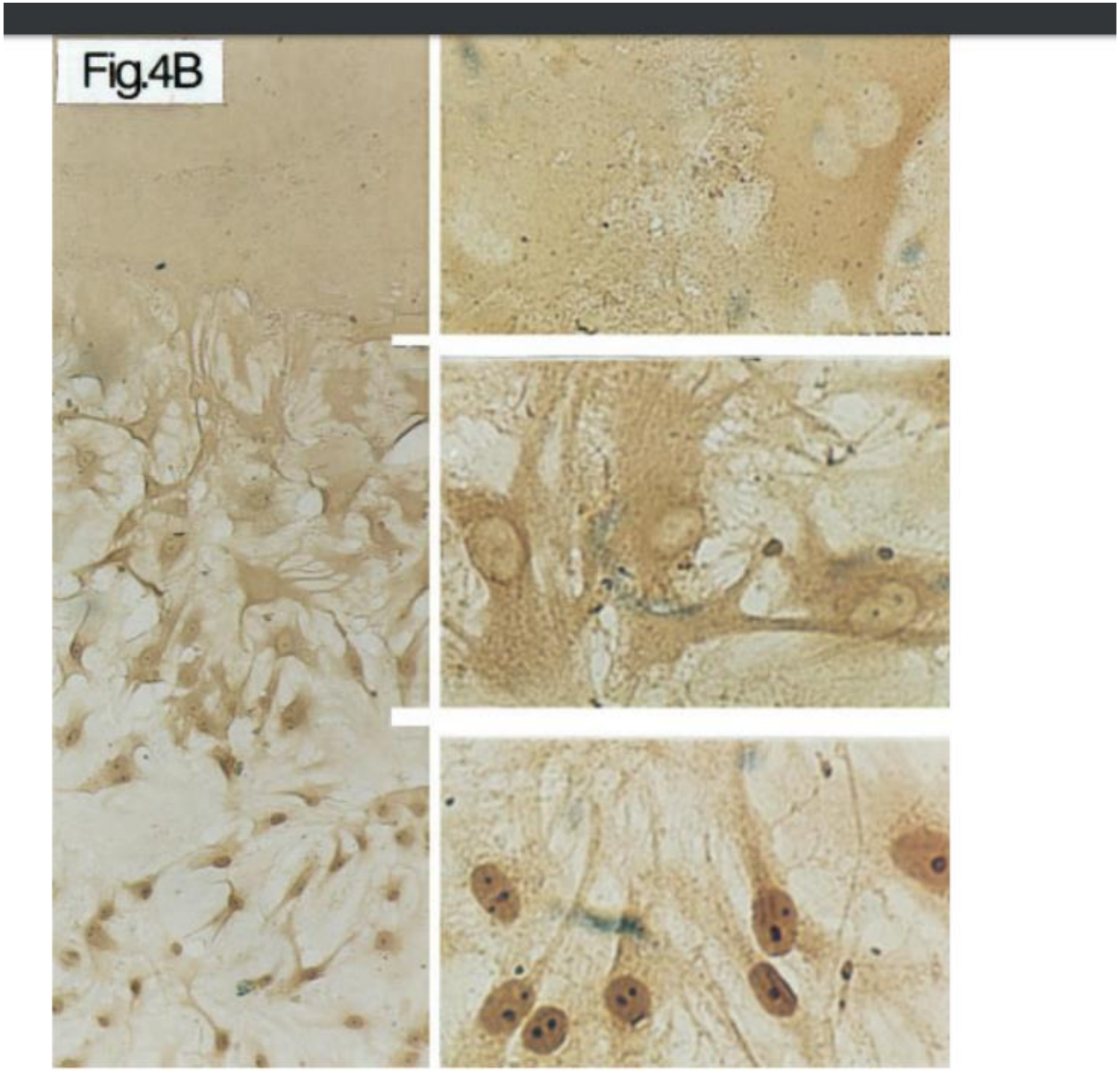
Reactive astrocytes

Glioblastoma tumor



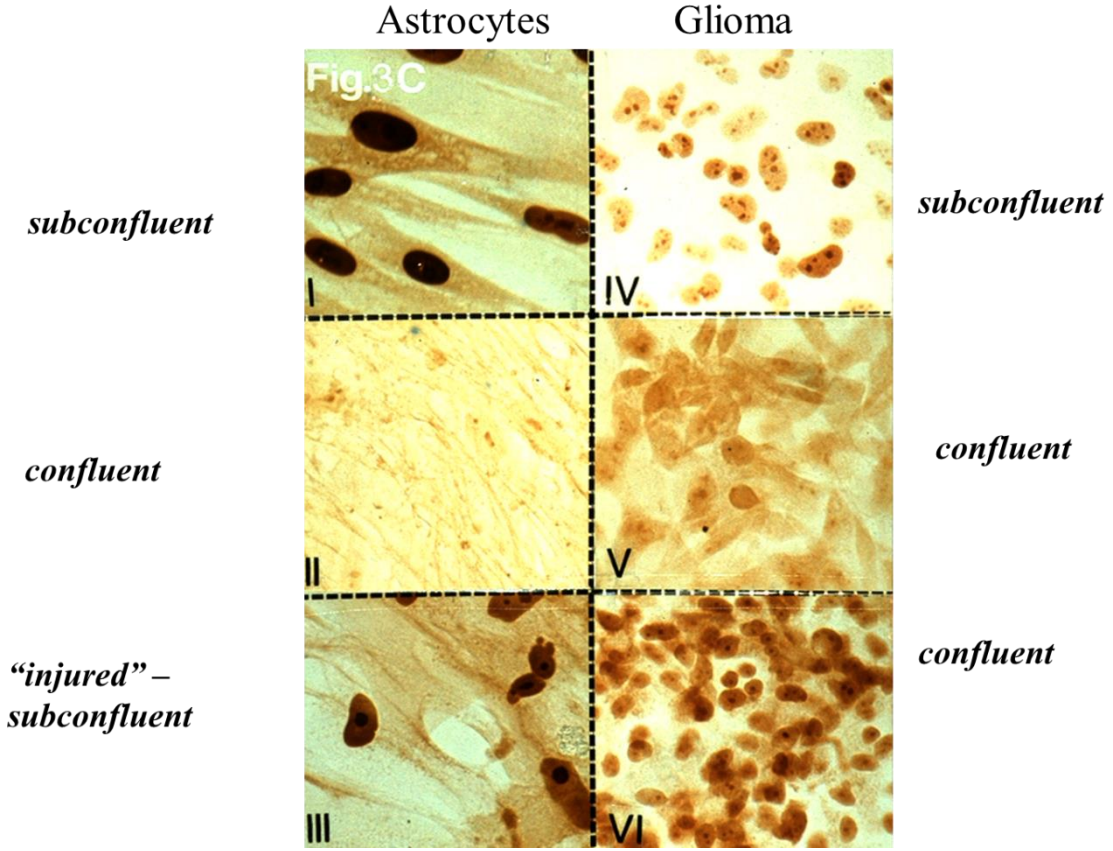
Stachowiak EK, Maher PA, Tucholski J, Mordechai E, Joy A, et al. **Nuclear accumulation of fibroblast growth factor receptors in human glial cells--association with cell proliferation**, Oncogene. 1997; 14(18):2201-11.

Human Astrocytes ibn 2D cultures



Stachowiak EK, Maher PA, Tucholski J, Mordechai E, Joy A, et al. **Nuclear accumulation of fibroblast growth factor receptors in human glial cells--association with cell proliferation,** Oncogene. 1997; 14(18):2201-11.

Cell-density dependent nuclear expression of FGF-2



Moffett J, Kratz E, Florkiewicz R, Stachowiak MK., Promoter regions involved in density-dependent regulation of basic fibroblast growth factor gene expression in human astrocytic cells Proceedings of the National Academy of Sciences of the United States of America. 1996; 93(6):2470-5.

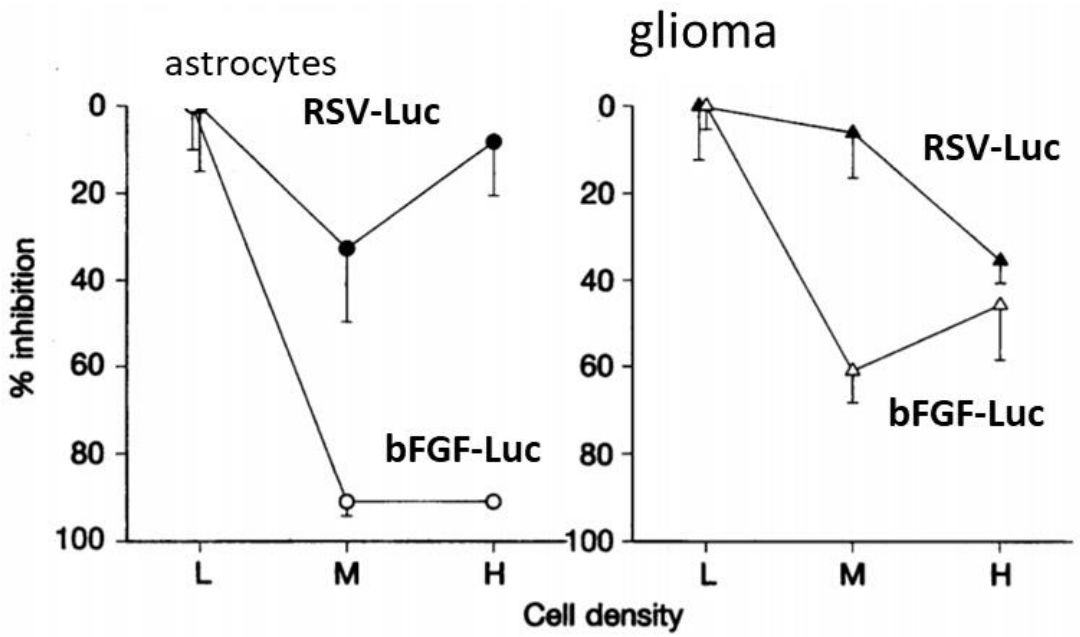


FIG. 4. Cell-density-dependent expression of the (−1800/+314)bFGFLuc reporter gene in cultures of human astrocytes and glioma cells. Approximately 1×10^7 astrocytes (QG strain) or U251MG glioma cells were electroporated with 150 μg of (−1800/+314)bFGFLuc or RSVLuc. Cells were seeded into 12-well dishes at the indicated densities yielding 100%, 70%, and 35% confluent cultures (see Fig. 3). Cells transfected with bFGFLuc expressed luciferase activity above background. Each point is the mean ± SEM of 4–10 samples. The results of statistical analysis are discussed in text. Astrocytes: ○, (−1800/+314)bFGFLuc; △, RSVLuc. U251MG cells: ●, (−1800/+314)bFGFLuc; ▲, RSVLuc.

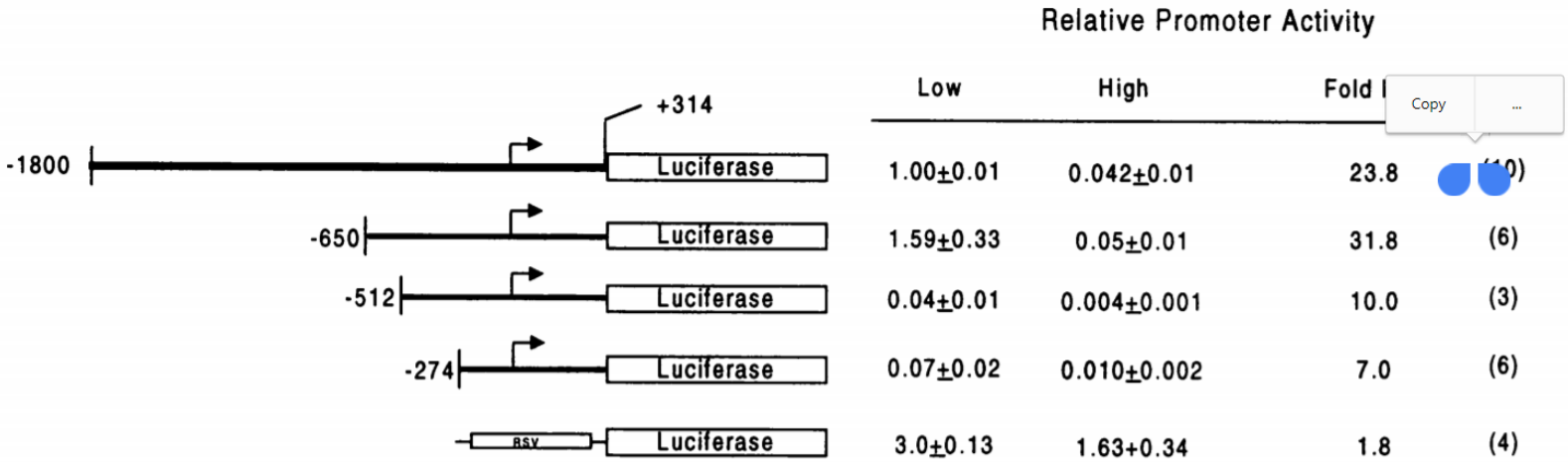


FIG. 5. Deletion analysis of bFGF promoter in human astrocytes. Astrocytes (QG) were transfected. The numbers given for DNA constructs indicate the included region of the bFGF gene. Luciferase activity was measured and normalized to the amount of transfected plasmid DNA. Luciferase activity is shown relative to the levels of (−1800/+314)bFGFLuc activity under low-density condition. The terms low and high refer to the density of the cultures at the time of harvest for luciferase assays. Fold Inh, the fold inhibition, was calculated by dividing the luciferase activity at low density by the value obtained at high density for each individual plasmid. The overall effects of cell density, promoter deletions, and the interaction between these two variables were statistically significant ($P < 0.00001$; 2-way ANOVA). Effects of individual deletions on basal promoter activity were assessed by Neuman–Kuels post hoc test (see text). To compare the effects of cell density between individual plasmids, the results for each plasmid were normalized to luciferase activity in high-density culture. The inhibition of (−512/+314)bFGFLuc or (−274/+314)bFGFLuc in high-density cultures was reduced compared to (−1800/+314)bFGFLuc ($P = 0.056$ and $P < 0.05$, respectively; 1 way ANOVA). The inhibition of (−1800/+314)bFGFLuc, (−650/+314)bFGFLuc, or (−520/+314)bFGFLuc was significantly greater than that of the RSVLuc ($P < 0.01$, $P < 0.05$, and $P < 0.001$, respectively).

FGF-2 gene promoter EMSA - Astrocytes

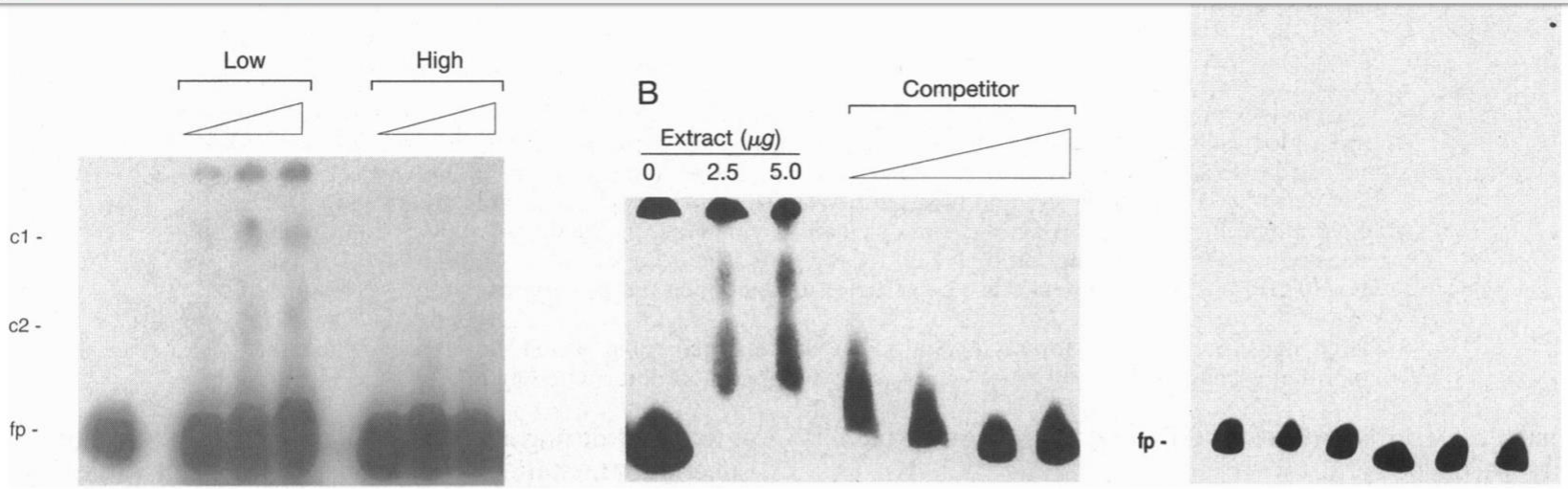


FIG. 6. Nuclear extracts from astrocytes bind to the region of the bFGF promoter from bp −650 to −453 in a cell-density-dependent manner. (*A*) Extracts from astrocytes (1, 2.5, and 5.0 μg) from low- and high-density cultures were incubated with ^{32}P-labeled fragment for 10 min. The two protein–DNA complexes formed are labeled c1 and c2 (fp, free probe). (*B Left*) Increasing amounts of extract from low-density astrocytes were added to ^{32}P-labeled probe (bp −650 to −453). (*B Right*) Five micrograms of nuclear extract was incubated with 25, 50, 100, or 250 ng of unlabeled competitor promoter fragment from bp −650 to −453. (*C*) Labeled probe (bp −512 to −453) was incubated with 1, 2.5, and 5.0 μg of protein from nuclear extracts isolated from low- and high-density cultures.

FGF-2 gene promoter EMSA - Astrocytes

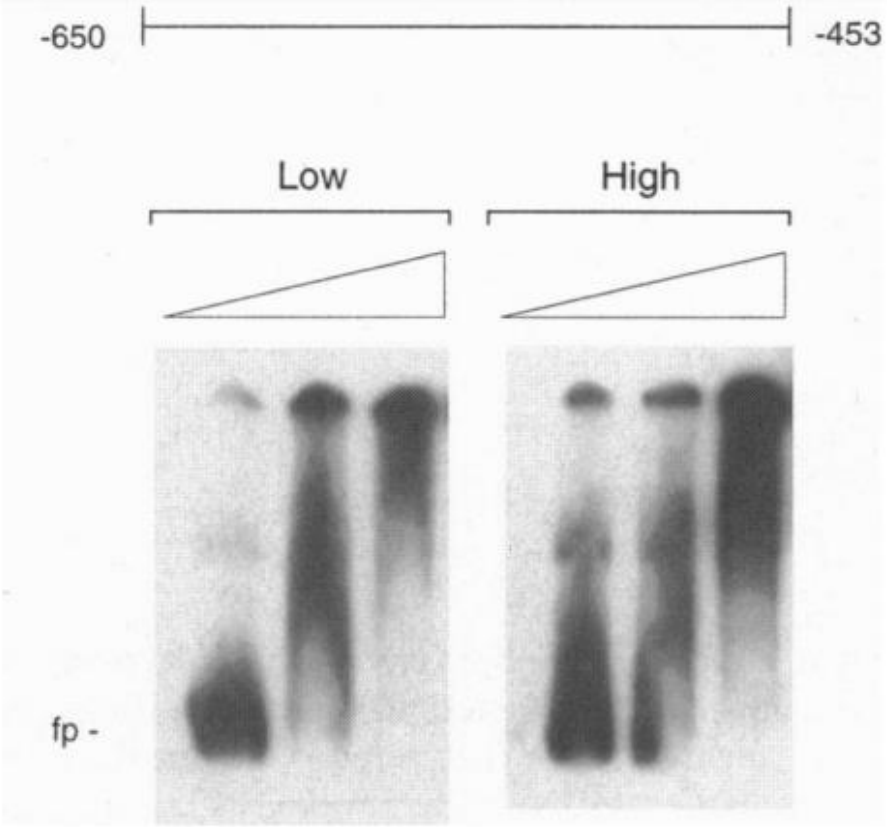


FIG. 7. Nuclear extracts from glioma U251MG bind to the bFGF gene promoter in a density-independent manner. Labeled promoter fragment (bp −650 to −453) was incubated with 1, 2.5, or 5.0 μg of nuclear proteins extracted from U251MG cells grown at low or high density.

Evolution of FGFs & FGF Receptors as nuclear signaling proteins

FGFR1-4 share common structure

The common pathway employed by **FGF2** is the mitogen-activated protein kinase (**MAPK**) pathway. The process involves the lipid-anchored docking protein Fibroblast growth factor receptor substrate 2 (**FRS2**) that constitutively binds **FGFR1** . **FGFR1** can phosphorylate **FRS2** and Src homology 2 domain containing transforming protein (**Shc**) **.** Phosphorylated **FRS2** bind s the adapter protein Growth factor receptor bound 2 (**GRB2**) and the Protein tyrosine phosphatase, non-receptor type 11 (**SHP-2**).
In **FGFR1**/ **FRS2** signaling pathway, **SHP-2** acts as adapter protein. **Shc** and **GRB2**form a complex with the Guanine nucleotide exchange factor Son of sevenless proteins (**SOS**).

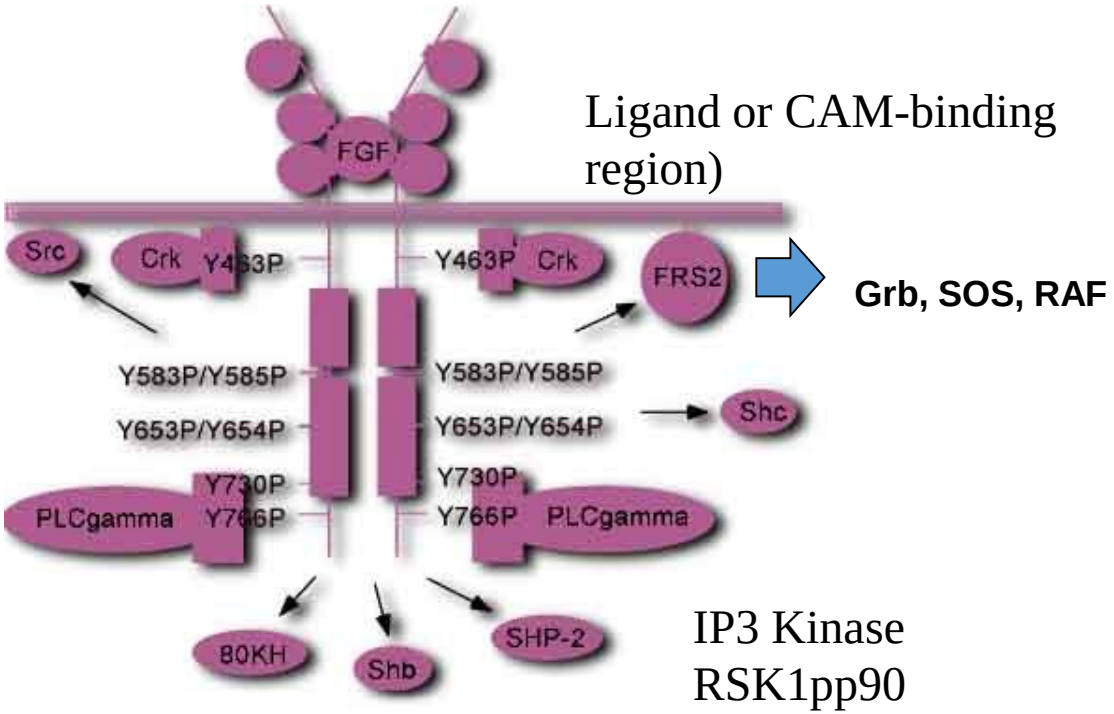
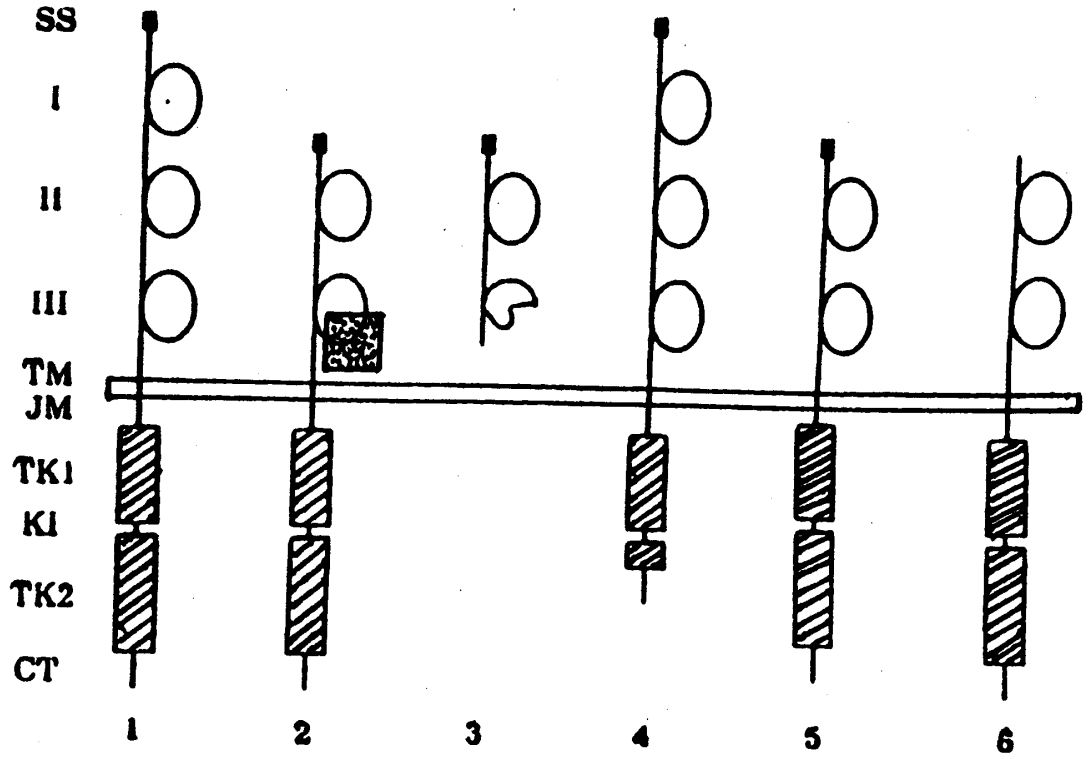
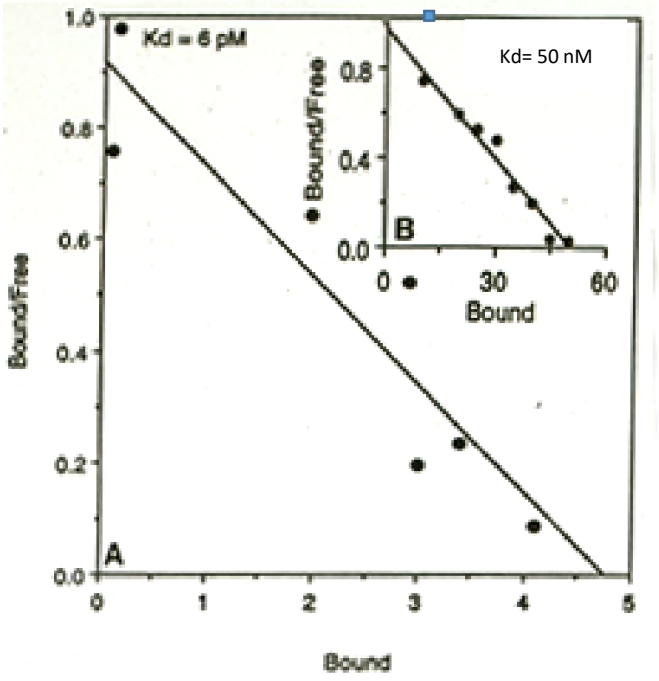
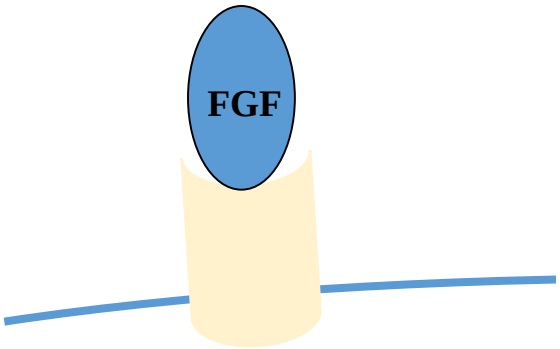


Fig. 3. Exon splicing generates multiple isoforms of FGFRs. Generation of FGFR-1 receptor subtypes by alternative splicing. Six of the 48 different isoforms of FGFR-1 are shown. The region of Ig-loop III encoded by exon 6 is enclosed by a shaded box. Splice forms II and VI differ by just two amino acids in their extracellular domains.

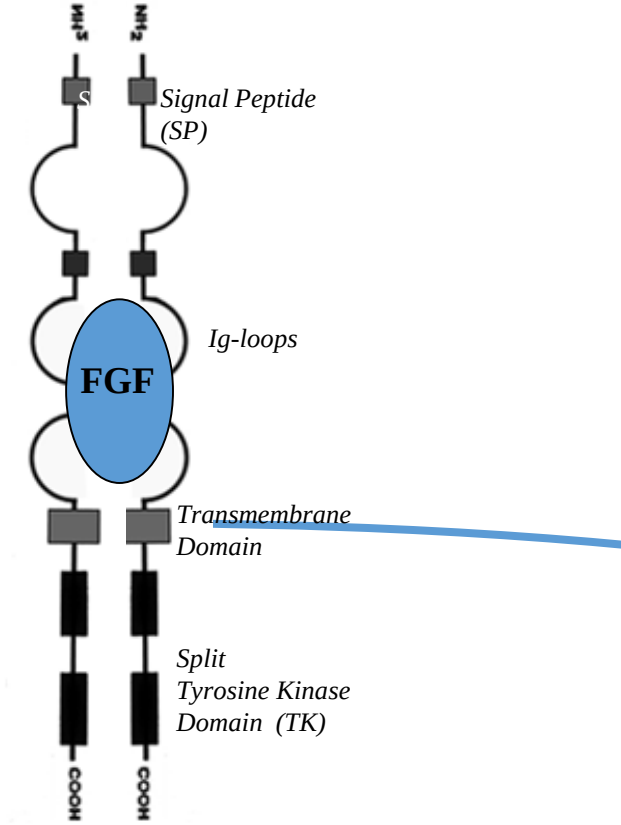


High Affinity FGF Receptors (FGFR1-4) have a general structure of type I membrane proteins.



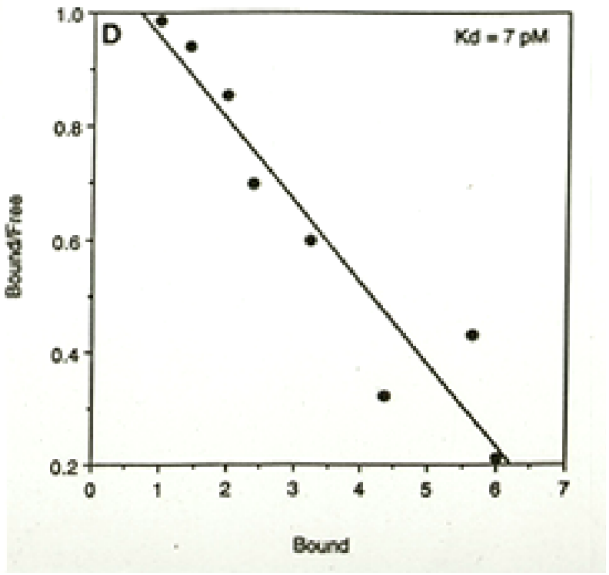


FGFR1-4



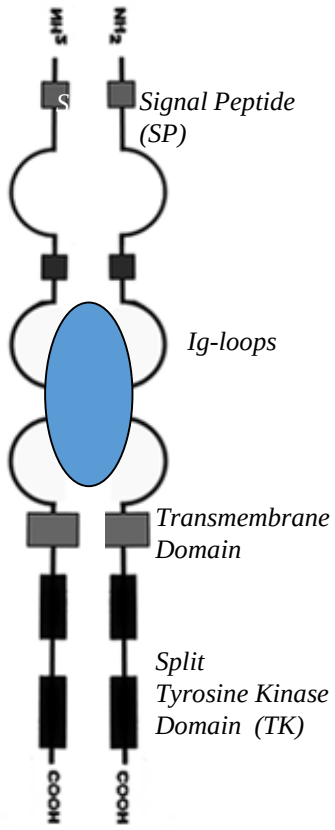
Kd=[nM] *Kd=[pM]*

Nucleus contains High Affinity FGF-2 binding sites Formed by FGFR1



Kd=[pM]

FGFR1-4

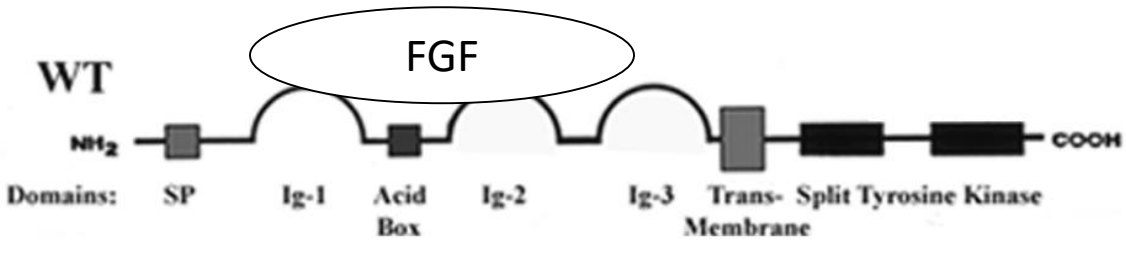


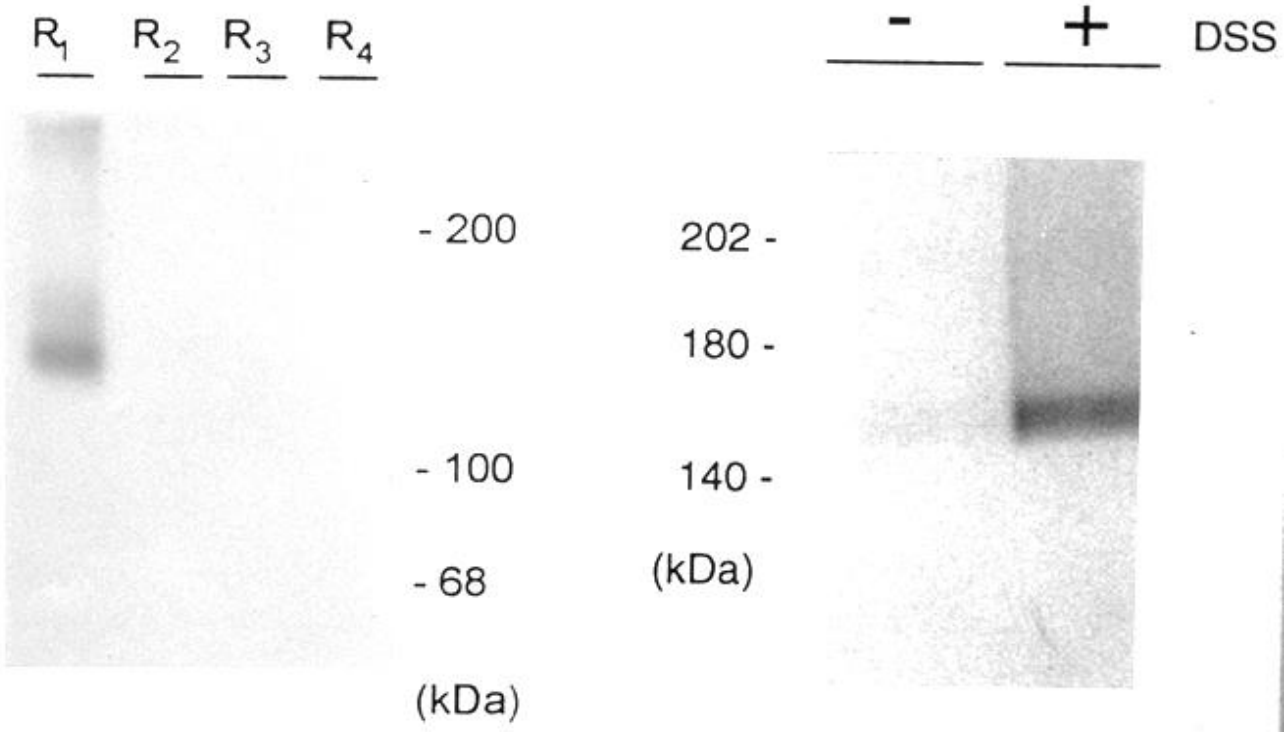
Stachowiak et al., Mol Biol Cell 1996; Moffet et al., PNAS 1996

High affinity FGF-2 binding nuclear sites formed by FGFR1

(Immunoprecipitation of FGFR1-4 crosslinked to ^{125}I FGF-2)

General structure of FGFR1-4:





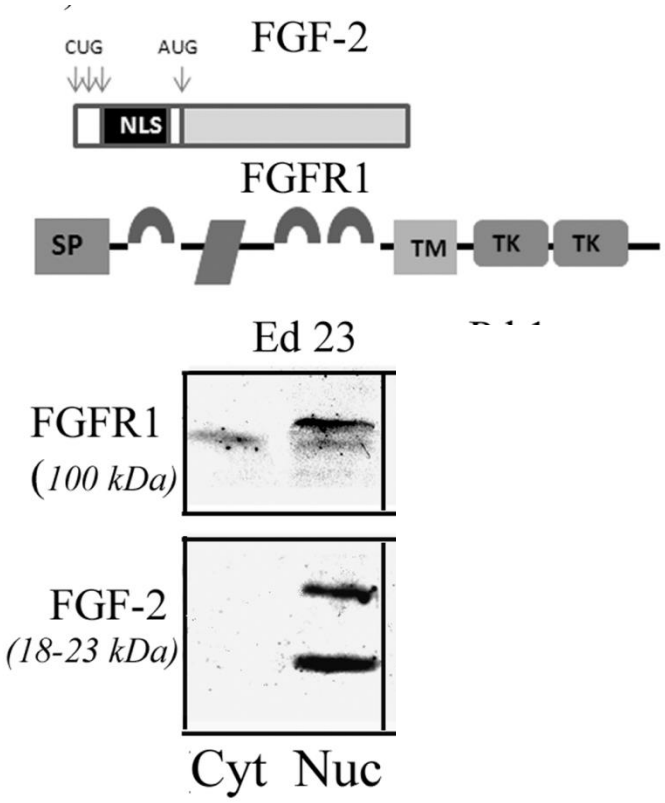
Nuclear **FGFR1**: Baird lab 1995; Stachowiak lab: Mol Biol Cell 1996; Oncogene 1997a,b, PNAS 1996 (FGFR1); Maher lab J. Cell Biol 1996;. (Bryant and Sto,w, 2005; (Clarke et al., 2001; Gonzalez et al., 1995); R. Grose : Nuclear translocation of FGFR1 and FGF2 inpancreatic stellate cells facilitates pancreatic cancer 2014, EMBO J.

Nuclear **FGFR2:**(Marchetti et al., 2006; Sabbieti et al., 2005; Schmahl et al., 2004

Nuclear **FGFR3 (**mutant): (Johnston et al., 1995)

Evolution of FGFs & FGFR as nuclear signaling proteins

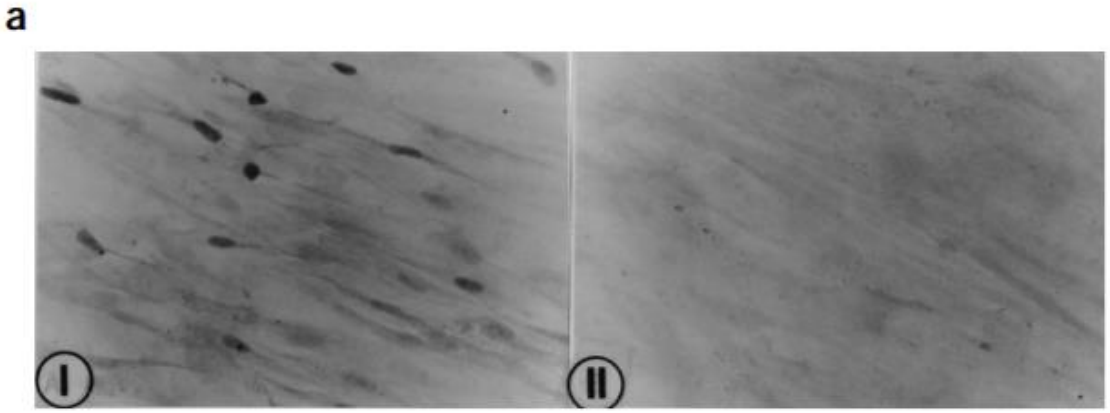
Endogenous proteins in developing brain

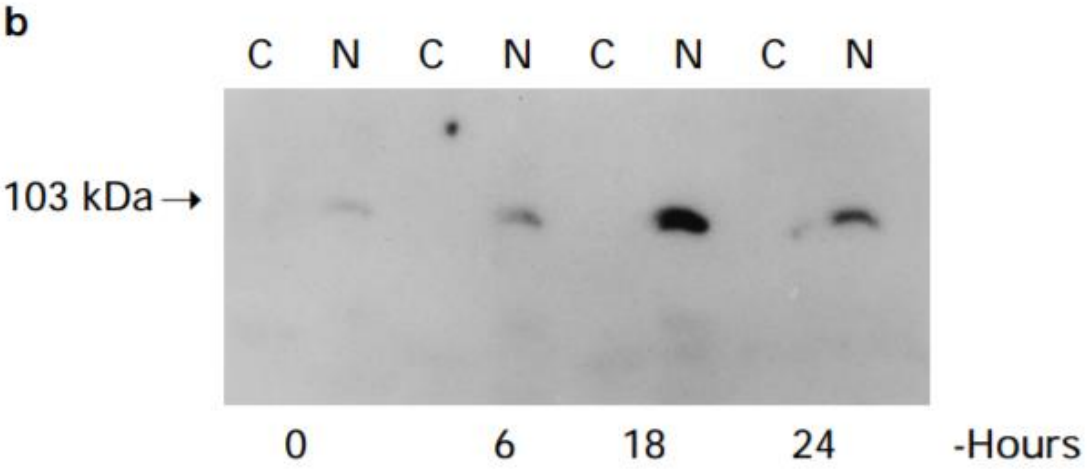


J.M. Myers, G.G. Martins, J.Ostrowski, and M.K. Stachowiak. (2003) Nuclear trafficking of FGFR1: a role for the transmembrane domain. **J. Cell. Biochem.** 88, 1273-1291

Human Astrocytes

Sub-confluent confluent





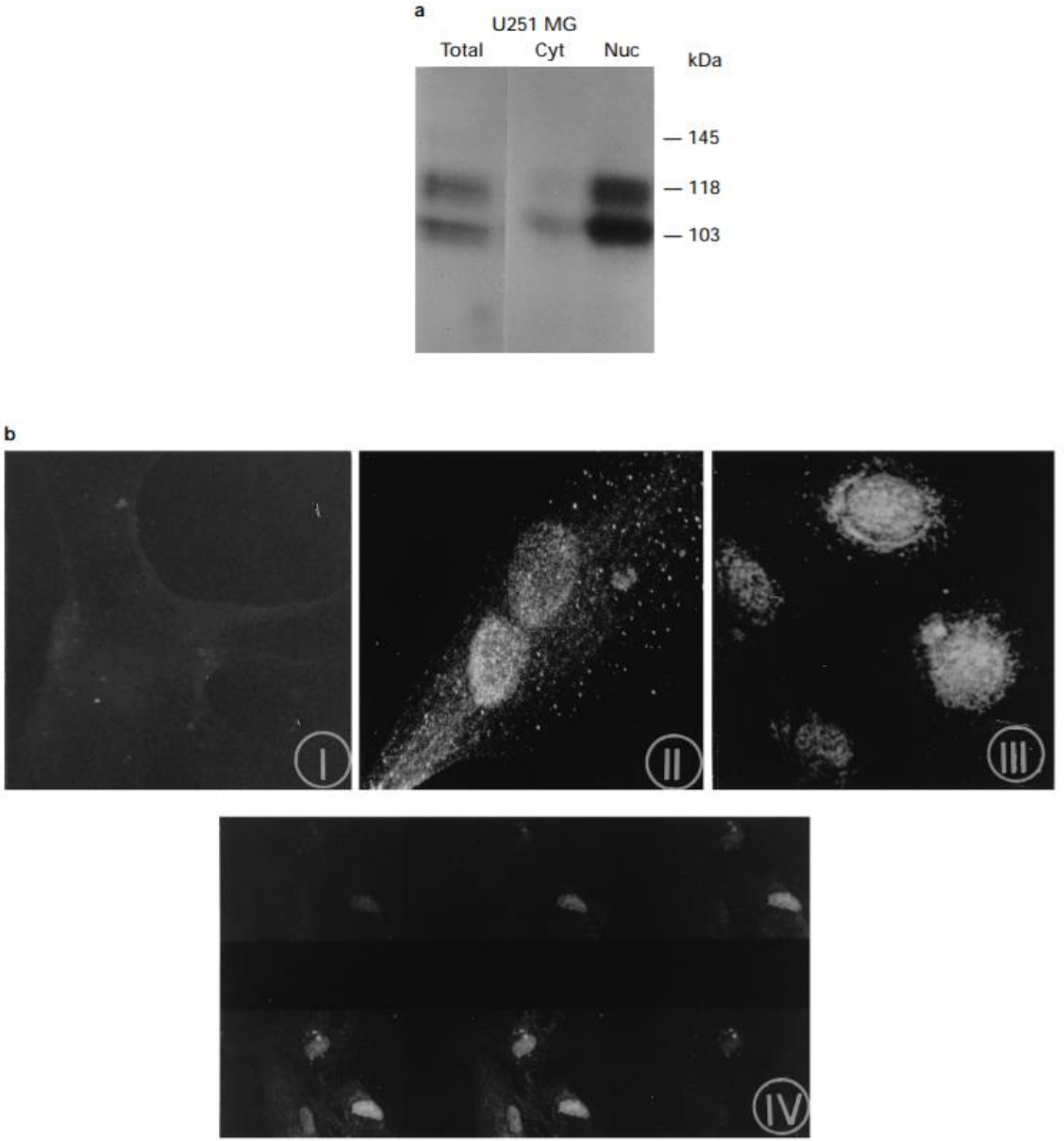
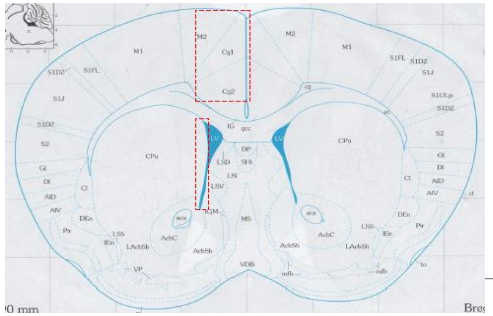
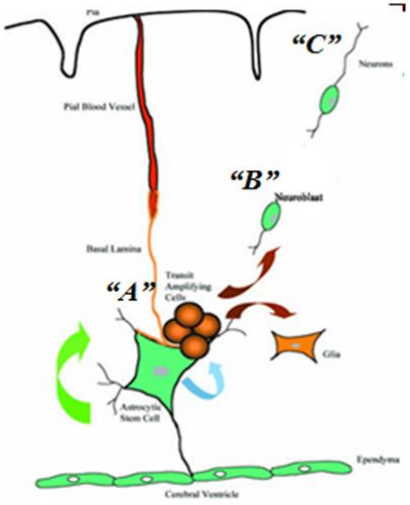
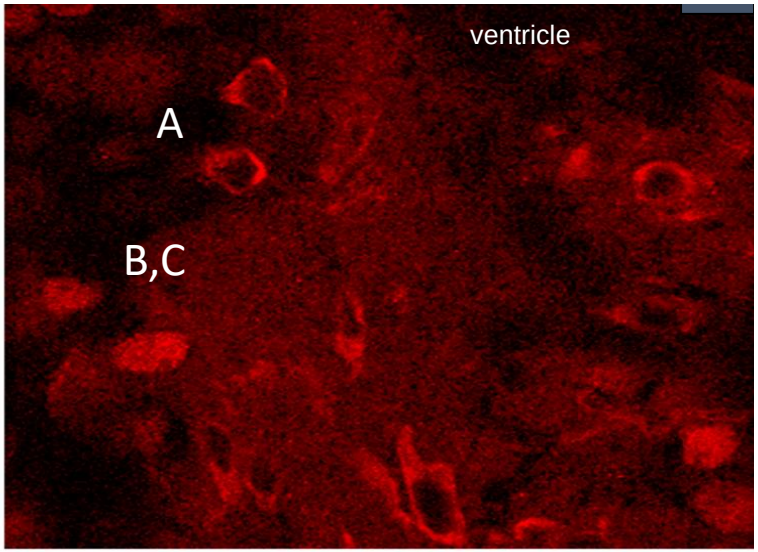


Figure 2 Subcellular distribution of FGFR1 proteins in human glioma cells and astrocytes. (**a**) Analysis of FGFR1 in total cellular lysate and in cytoplasmic (cyt) or nuclear (nuc) fractions of the U251MG glioma cells. Blots were probed with monoclonal McAb6 antibody. Molecular weights of FGFR1 bands are indicated at left. (**b**) Immunocytochemical localization of FGFR1 in human astrocytes (I, II-QG strain, IV-SC strain) and U251MG glioma cells (III). Cells were incubated with C-term FGFR1 Ab and the immune complexes stained with CY^3-conjugated IgG. I-III – photographs represent stacked confocal laser sections taken 1 μm apart. No staining was observed when primary antibody was omitted (I). IV – shows individual consecutive (top to bottom) confocal sections taken 2 μm apart to illustrate intranuclear localization of FGFR1

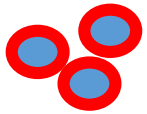
FGFR1-immunoreactivity in the cytoplasm and nuclei of SVZ cells



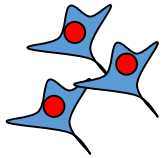




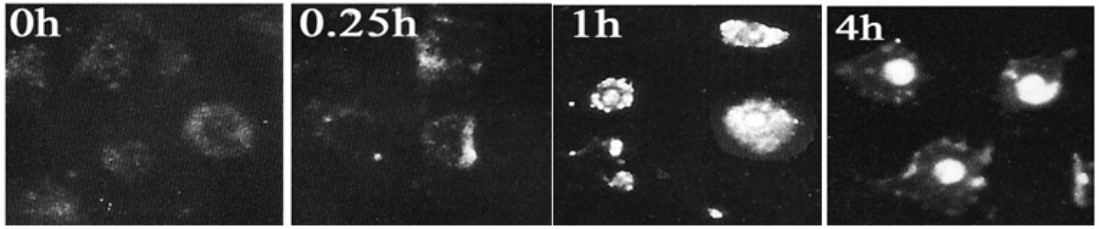
I. **Proliferating Neural Stem/Progenitor Cells**



II. **Differentiating Neural Progenitor Cells**

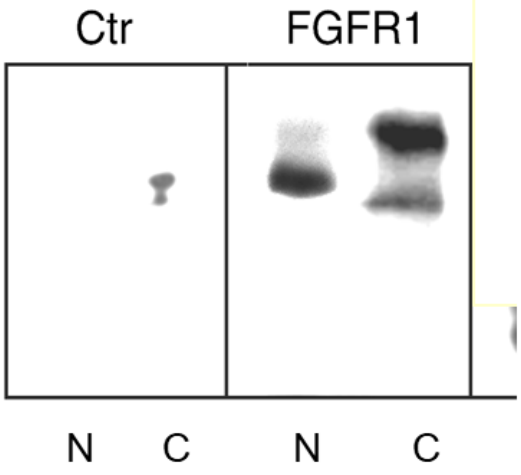


Nuclear FGFR1 is not derived from the cell surface

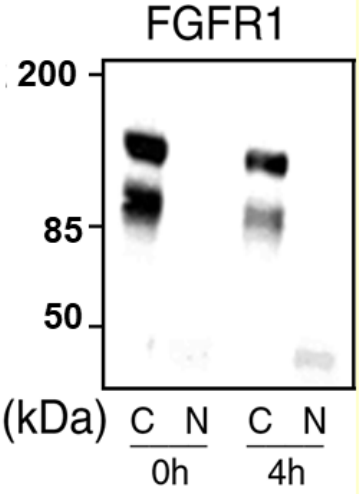


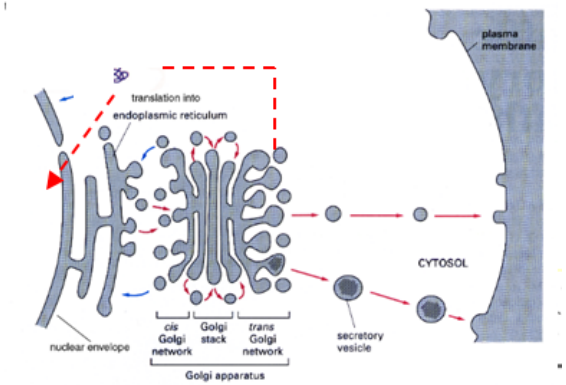
Labeling surface proteins with NHS-sulfobiotin

(Direct western with FGFR1Ab)



(precipitation with avidin-agarose beads, western with FGFR1Ab)

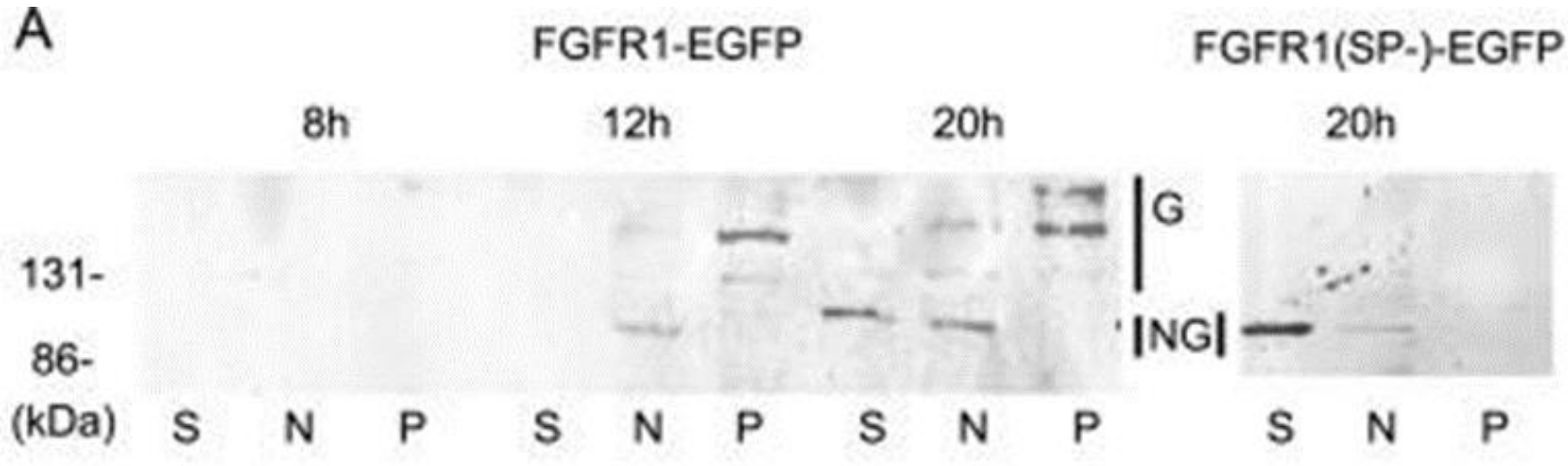




Transfected FGFR1 is detected in the cell nucleus

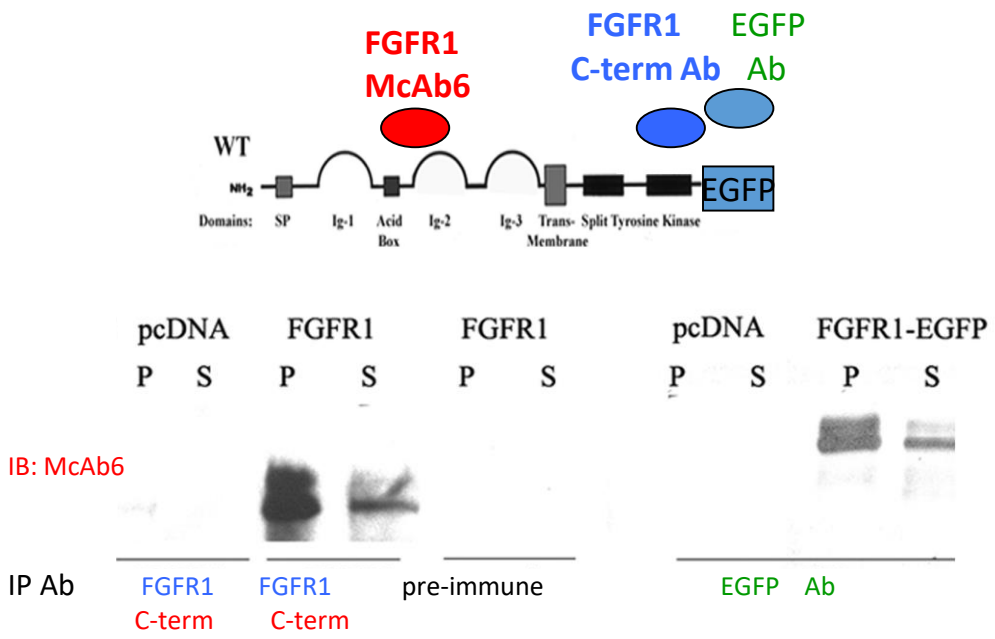
Biotinylated surface FGFR1 does not enter cell nucleus

Transfection of FGFR1-EGFP or FGFR1(SP-)-EGFP

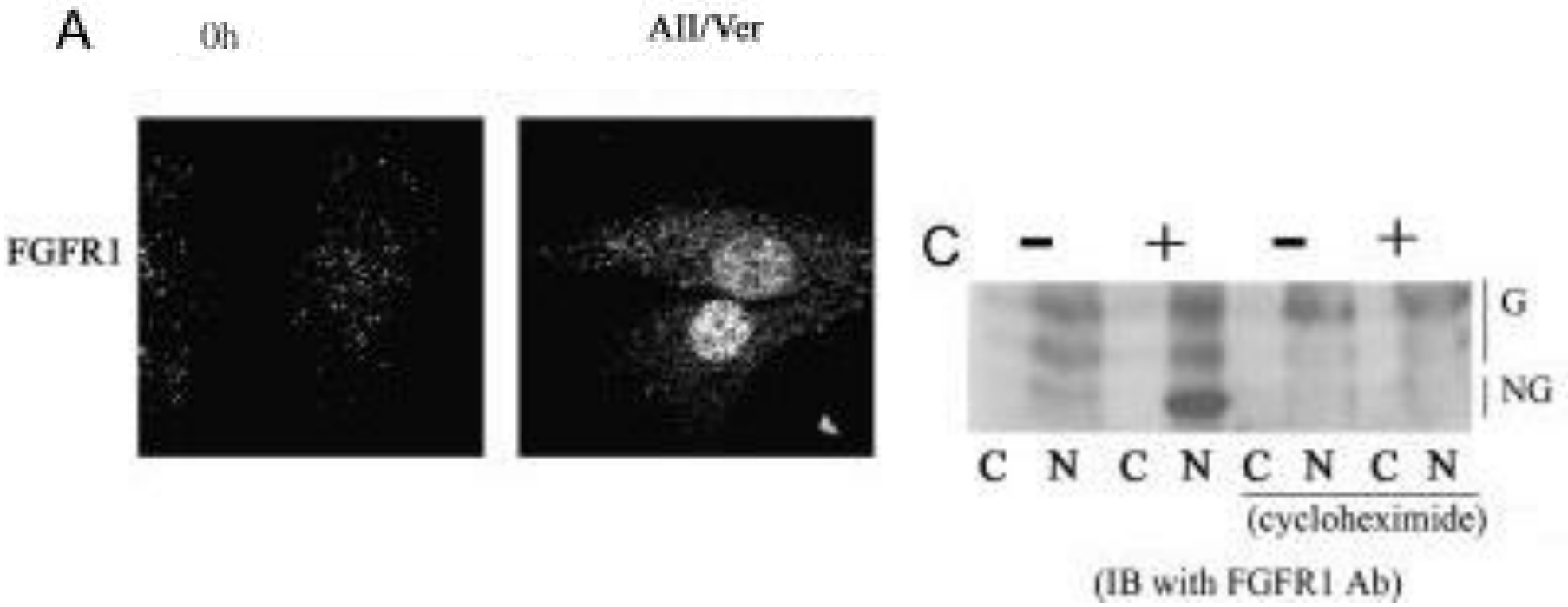


Nuclear FGFR1 is not cleaved

IP with C-term FGFR1 or EGFP Ab, IB with N-term FGFR1 McAb6:

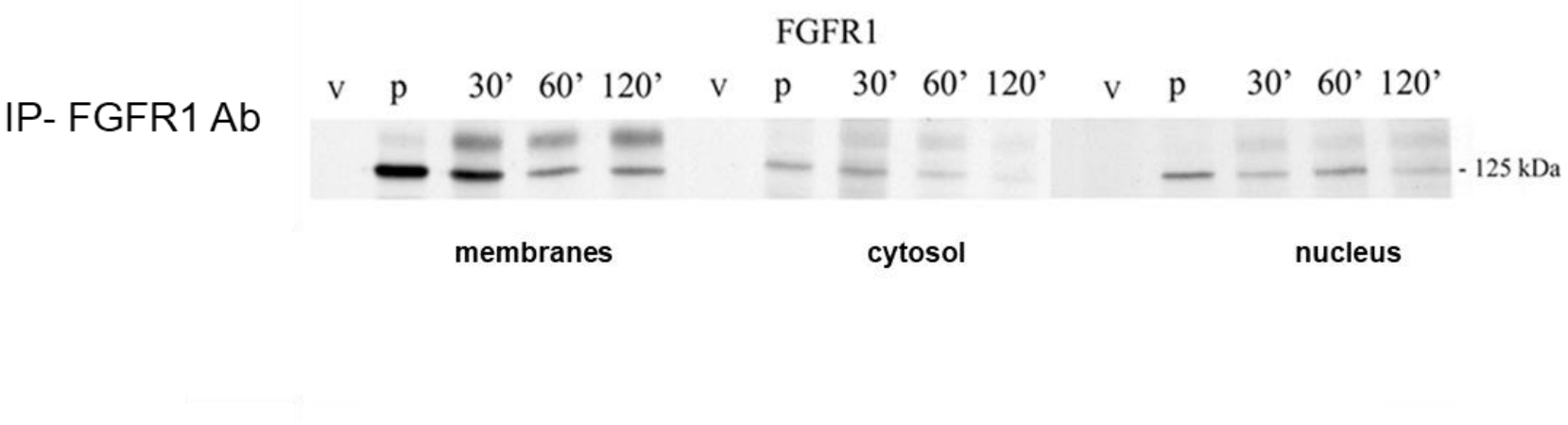


Nuclear (N) accumulation of FGFR1 requires protein synthesis:

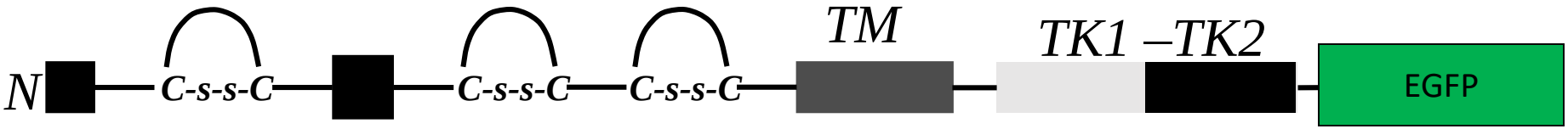


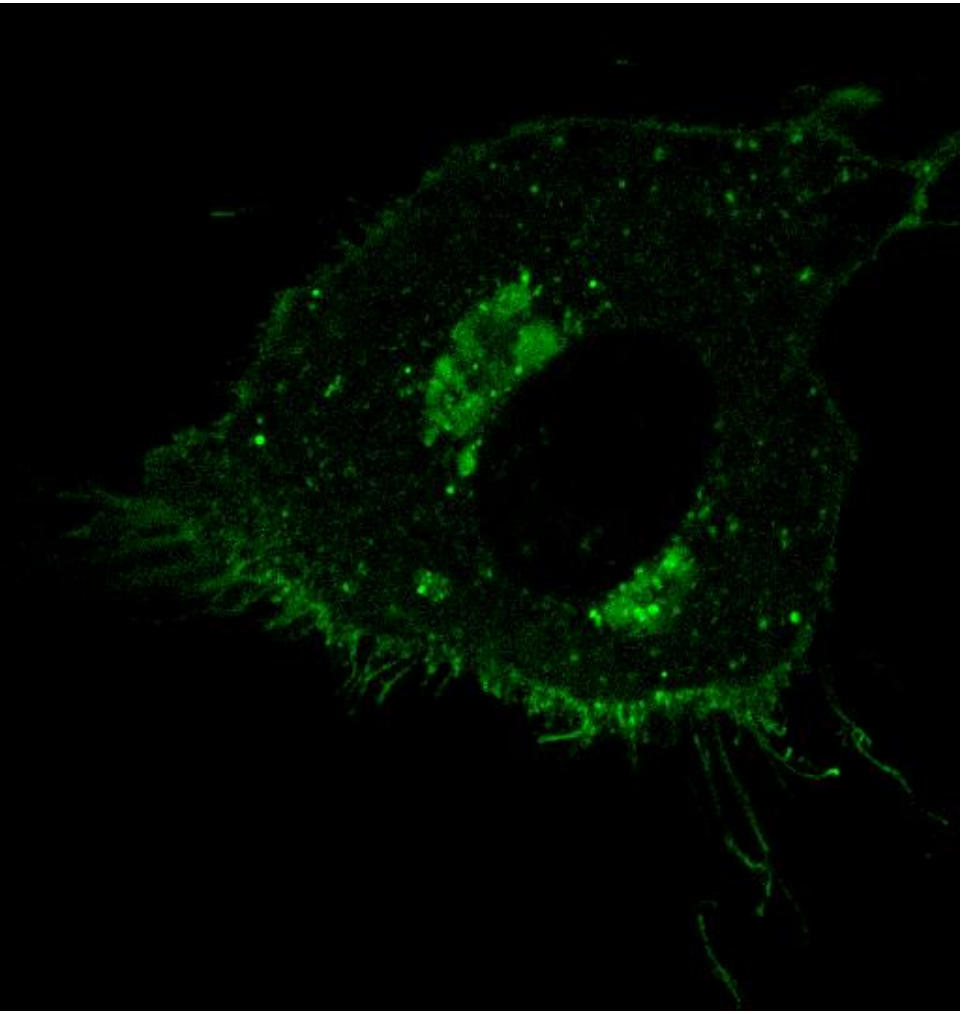
Cytosolic and nuclear FGFR1 molecules represent newly synthesized nonglycosylated receptor

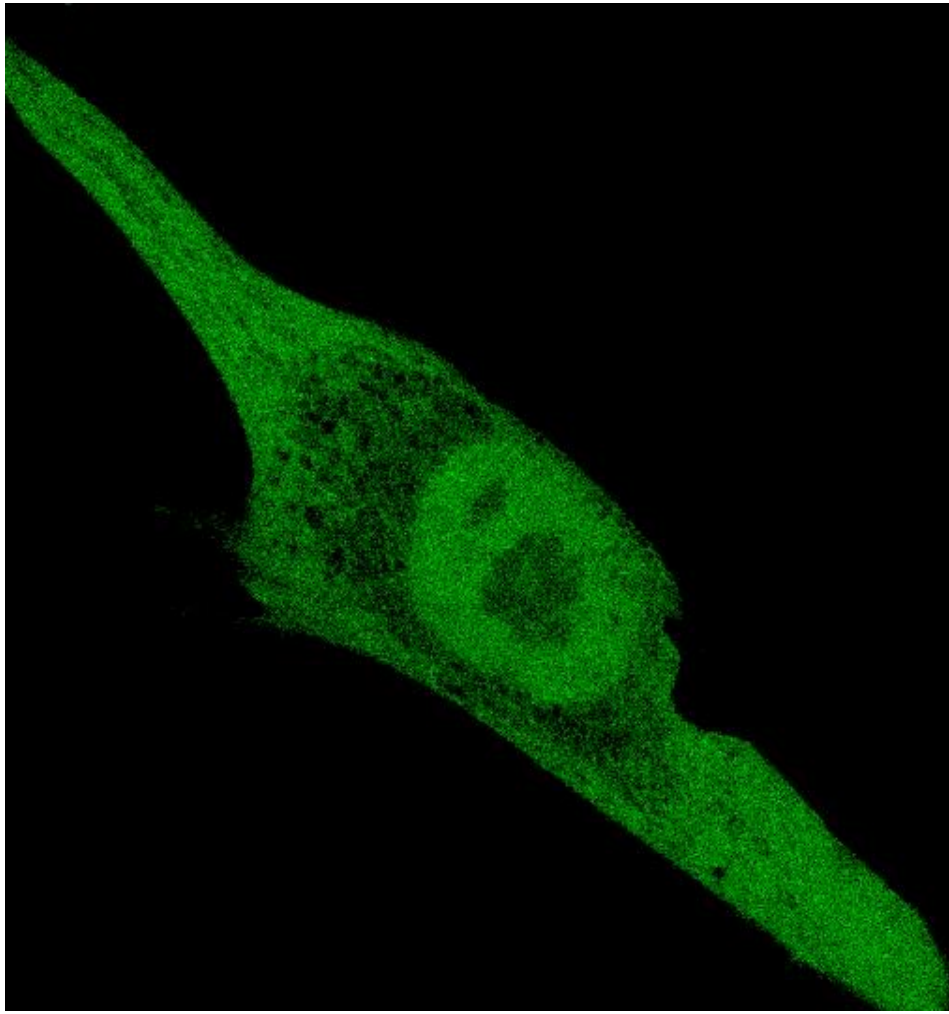
^{35}S-methionine/cysteine pulse-chase:



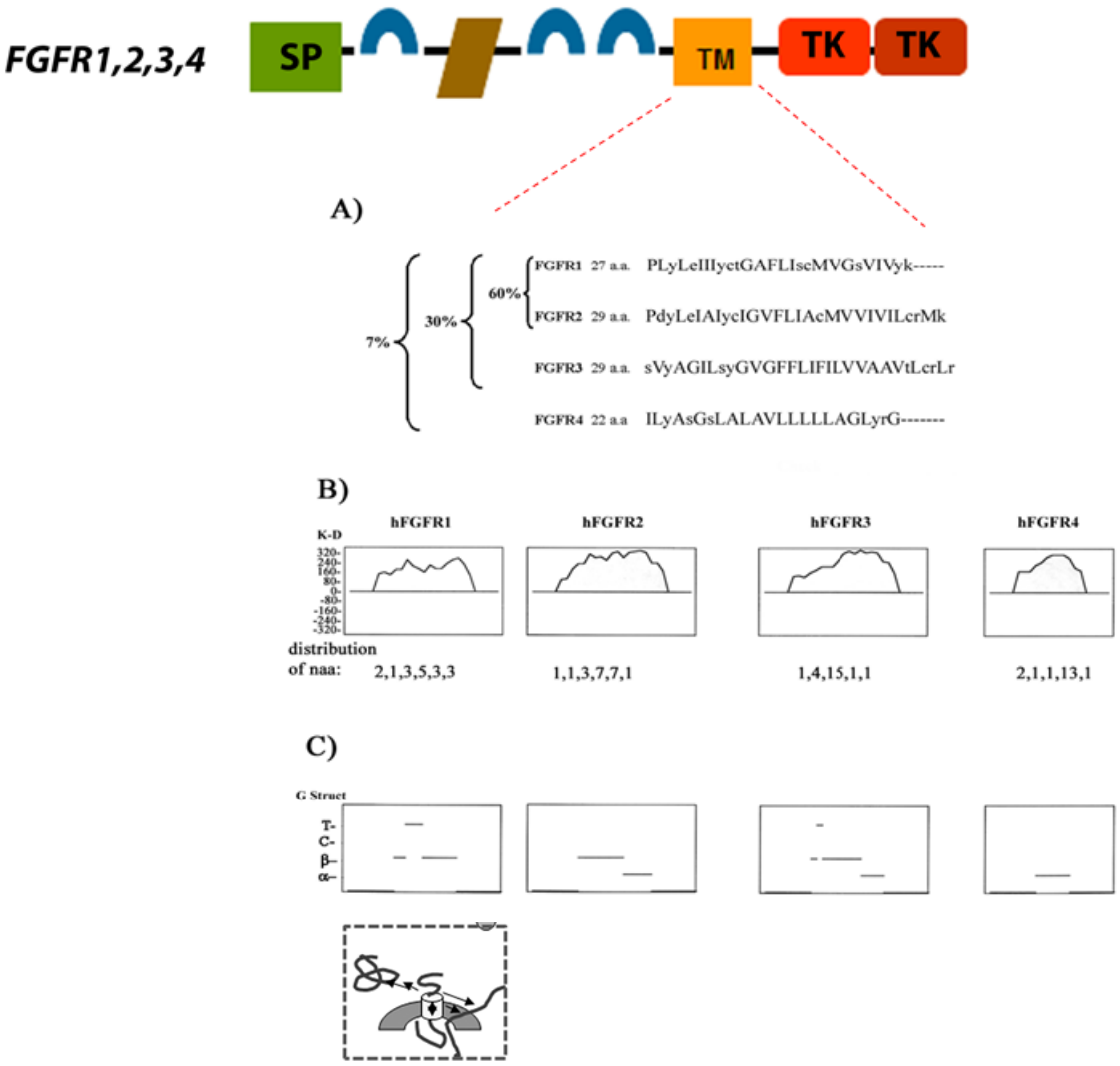
Bimodal processing in live cells of FGFR1-fused to enhanced Green Fluorescent Protein (EGFP)





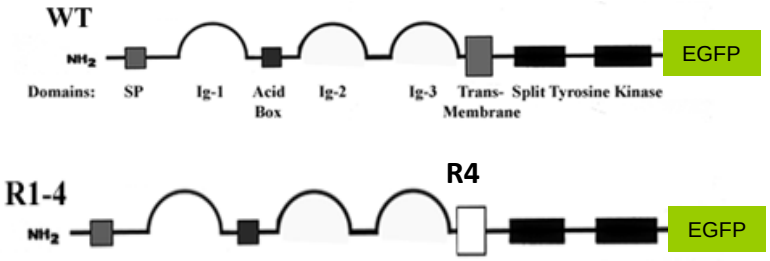


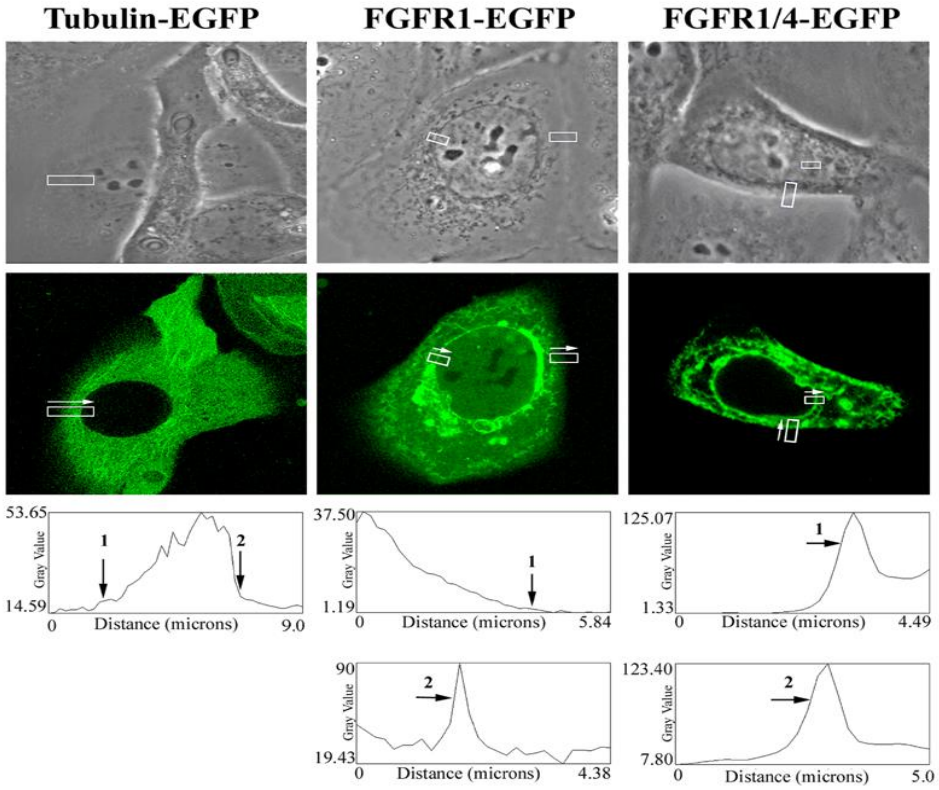
Unusual properties of FGFR1 transmembrane domain

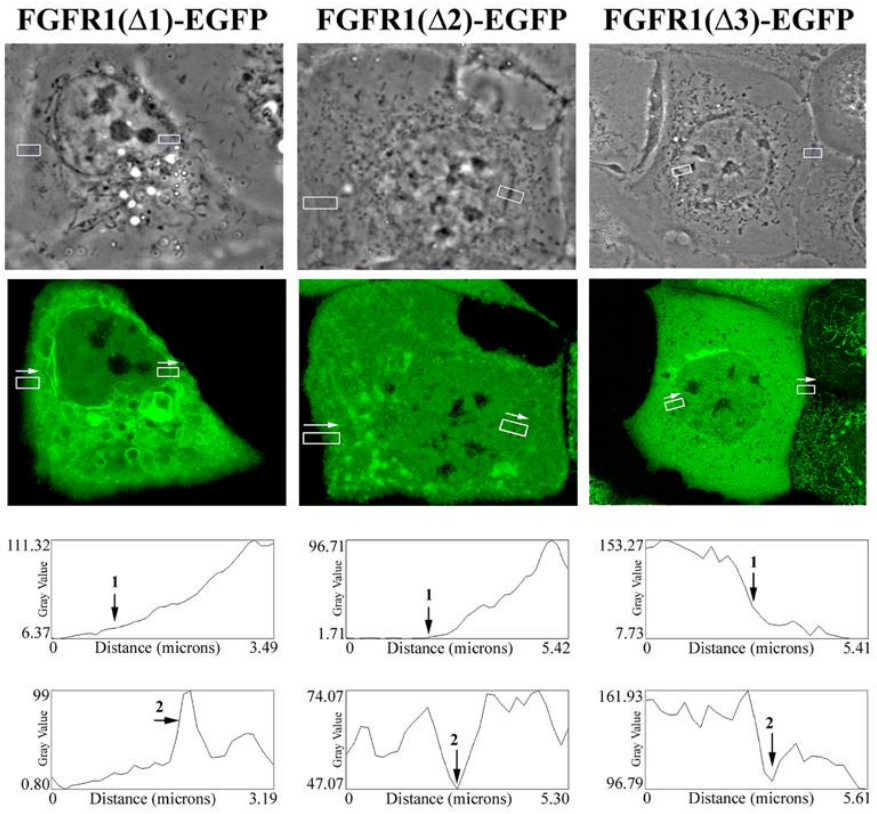


Chou-Fasman and Garnier algorithms predict the TM of FGFR4 to be a typical a-helix. In contrast FGFR1 showed a b-sheet-Turn-b-sheet structure with no predicted a-helix. Both FGFR2 and FGFR3 demonstrated b-sheets followed by shorter a-helices. (α= α-helics; β= β-sheet; T= turn, C=random coil). (Myers et al. 2001)

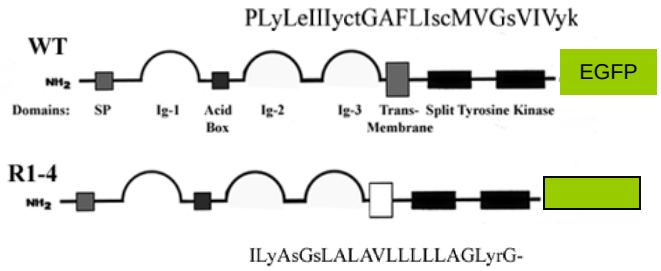
Atypical TRANSMEMBRANE DOMAIN allows cytosolic and nuclear FGFR1 accumulation:

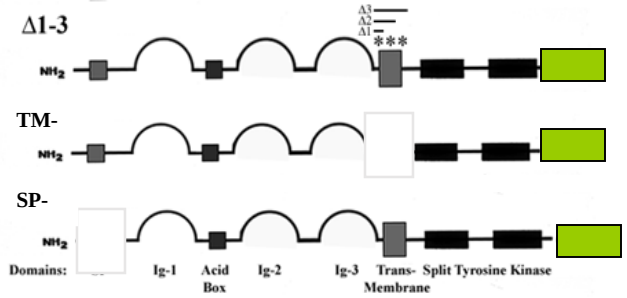


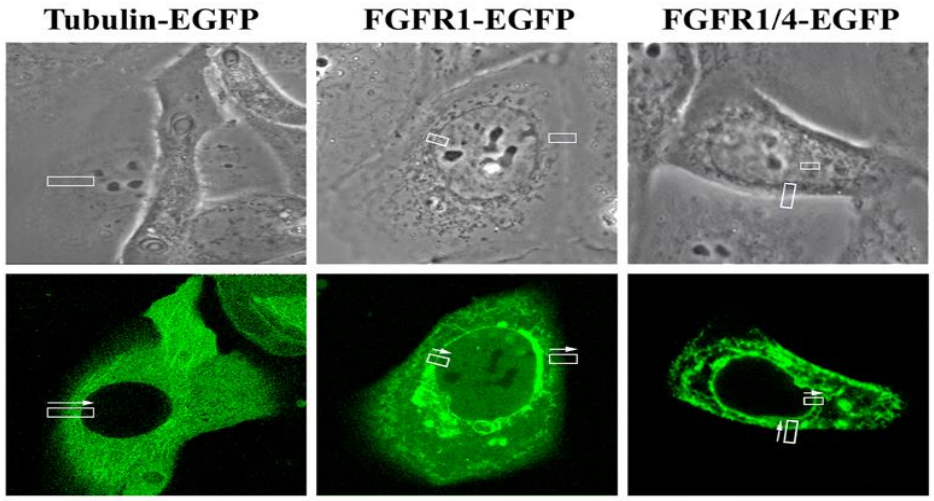


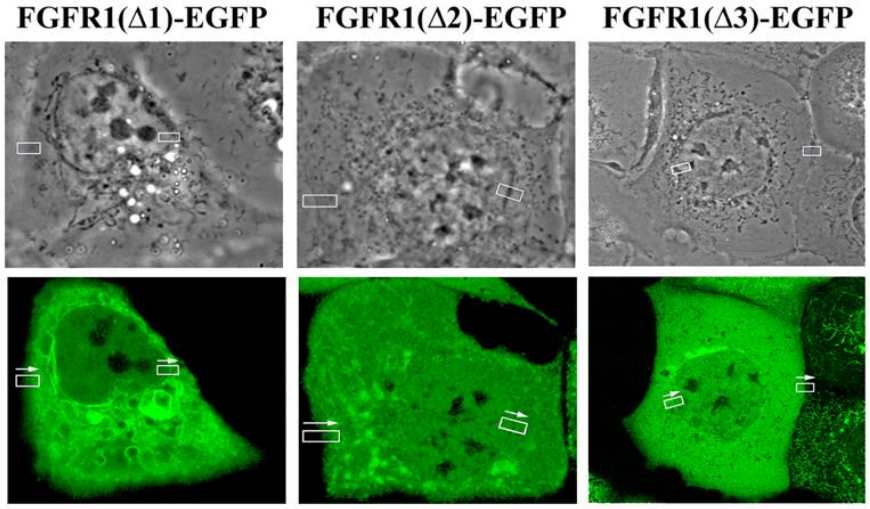


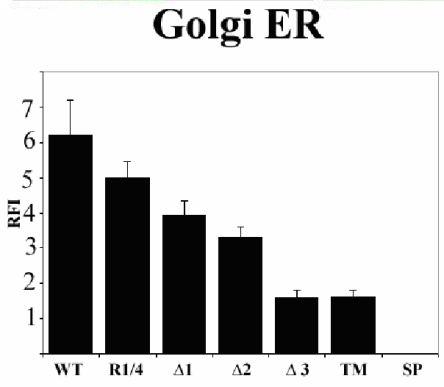
Atypical Transmembrane Domain allows cytosolic and nuclear FGFR1 accumulation:

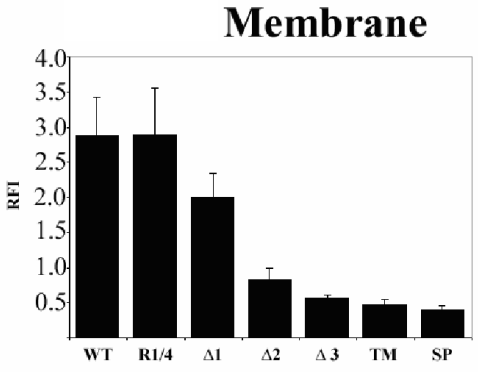


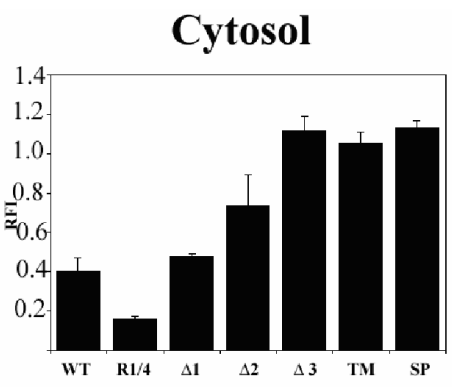


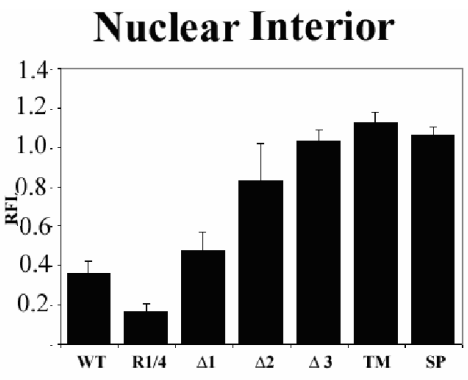




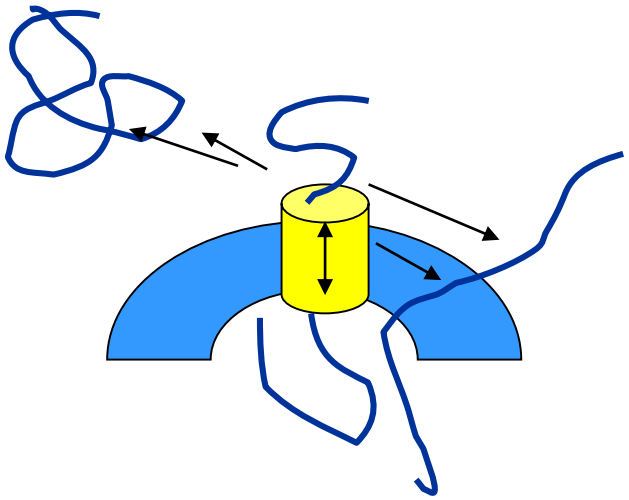






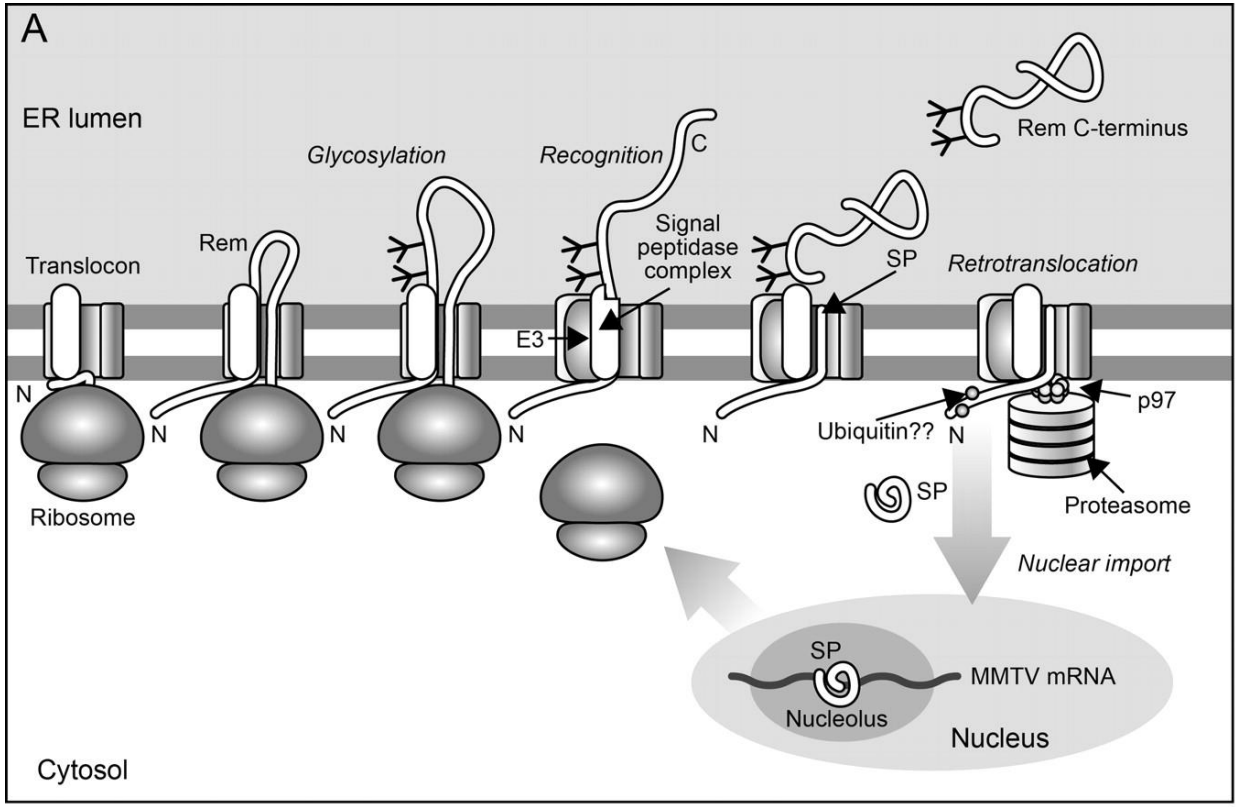


Forward/retrograde movement via SEC61-formed channel and is release from ER membrane.



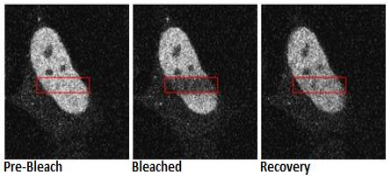
Myers et al., 2002
(J. Cell. Bioch.)

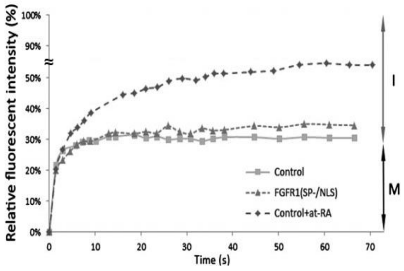
MMTV model for protein release from ER and nuclear translocation



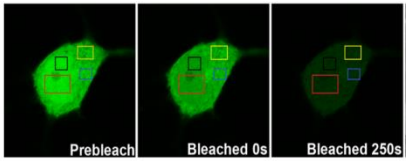
Imaging - Biophotonics reveals Real Time protein movement and interactions - gene expression at work

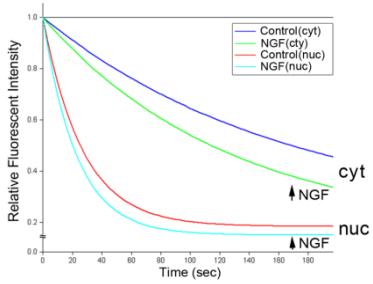
FRAP



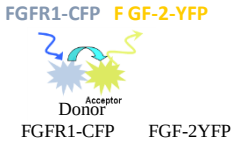


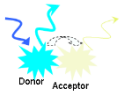
FLIP

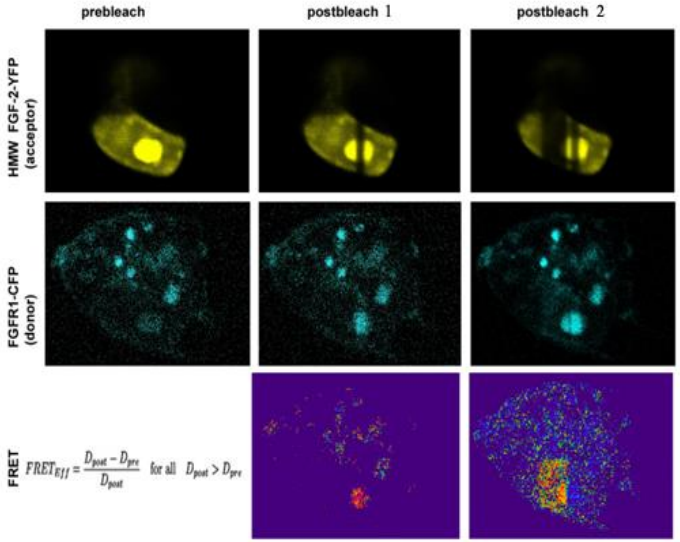




FRET







S.M. Dunham, H. Pudavar, P. Prasad, M.K. Stachowiak (2004) Cellular Signaling and Protein-Protein Interactions Studied Using Fluorescence Recovery after Photobleaching. **J. Physical Chem,** 108,10540-10546.

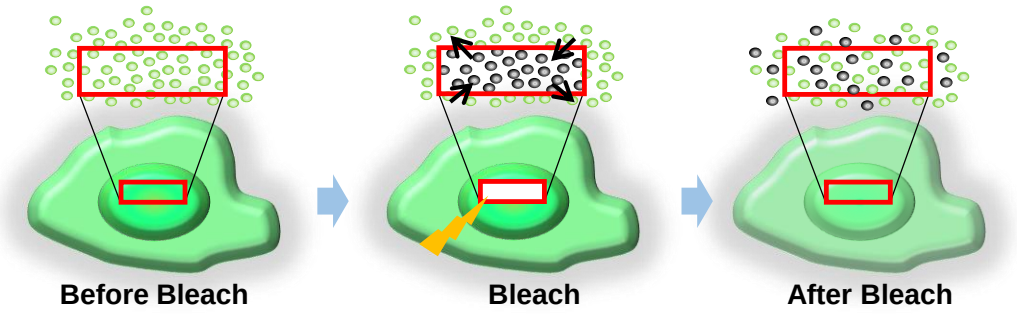
S. M. Dunham-Ems, H. Pudavar, J. Myers, P. Prasad, and M. K. Stachowiak. (2006). Factors controlling Fibroblast Growth Factor Receptor-1's (FGFR1) cytoplasmic trafficking and its regulation revealed by FRAP analysis. **Mol. Biol. Cell** 17, 2223-2235.

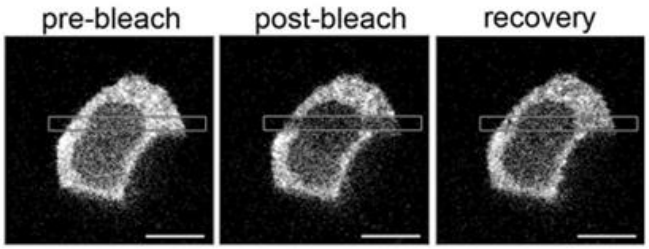
S. M. Dunham-Ems, Yu-Wei Lee, E. K. Stachowiak, H. Pudavar, P. Claus, P.N. Prasad, M. K. Stachowiak. Fibroblast Growth Factor Receptor-1 (FGFR1) nuclear dynamics reveal a novel mechanism in transcription control. **Mol. Biol. Cell,** 2009,

Lee, Y-W., Terranova, C., Birkaya, B., Narla, S., Kehoe, D., Parikh,P., Dong, S., Ratzka, A., Brinkmann, H., Aletta, J., Tzanakakis, E., Stachowiak, E.K., Claus, P., and Stachowiak, M.K. (2012). A novel nuclear FGF Receptor-1 partnership with retinoid and Nur receptors during developmental gene programming of embryonic stem cells **J. Cell. Biochem.,** 113, 2920-2930

O. Baron, B. Foerthmann, Y-W. Lee, C. Terranova, A. Ratzka, E. K. Stachowiak, C. Grothe, P. Claus and M.K. Stachowiak (2012) Cooperation of nuclear FGFR1 and Nurr1 offers a new interactive mechanism in postmitotic development of mesencephalic dopaminergic neurons. **J. Biol. Chem.** 287, 19827-19840

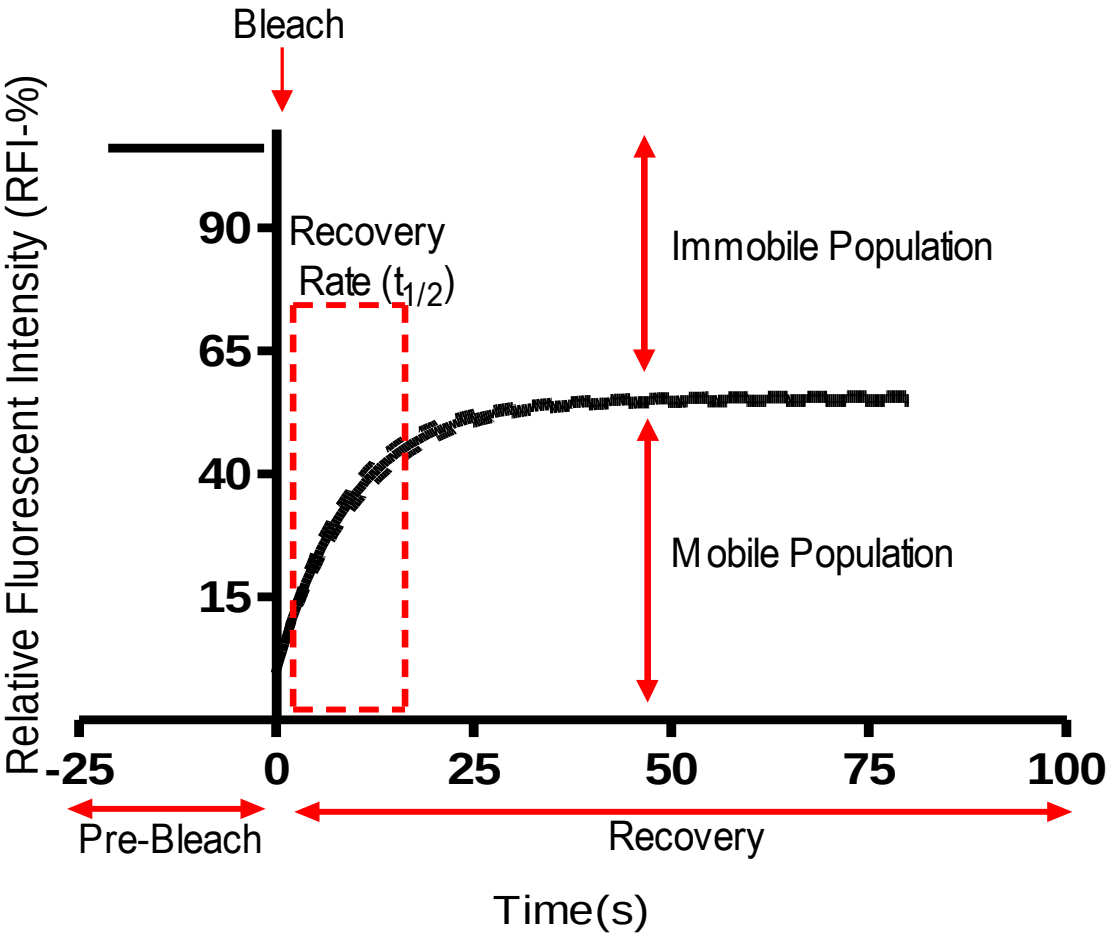
Fluorescence Recovery After Photobleaching (FRAP) of FGFR1-EGFP - kinetic model of gene regulation by nuclear FGFR1



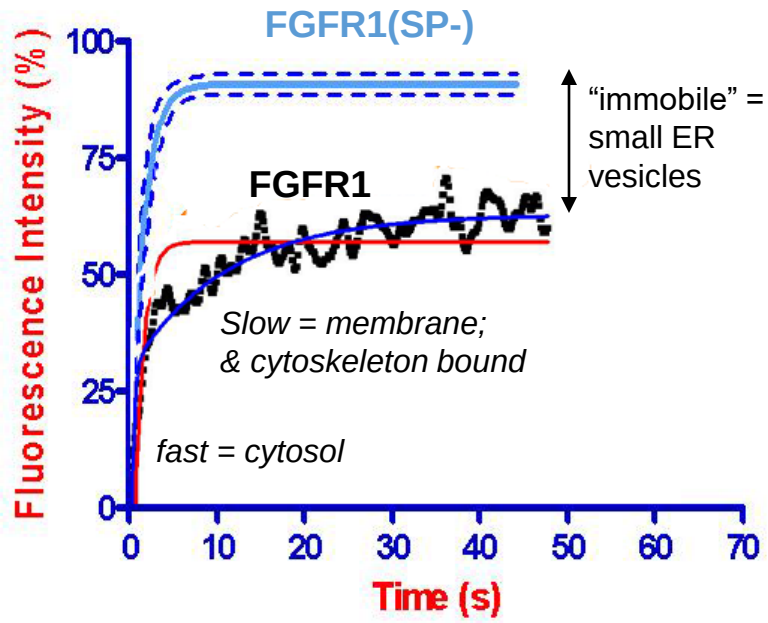


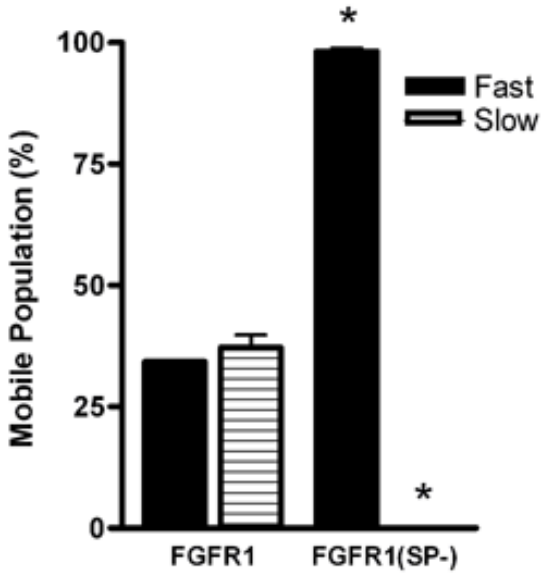
S. M. Dunham-Ems, Yu-Wei Lee, E. K. Stachowiak, H. Pudavar, P. Claus, P.N. Prasad, M. K. Stachowiak. Fibroblast Growth Factor Receptor-1 (FGFR1) nuclear dynamics reveal a novel mechanism in transcription control. Mol. Biol. Cell, 2009, 20, 2401 – 241
2S.M. Dunham, H. Pudavar, P. Prasad, M.K. Stachowiak (2004). **J. Physical Chem,** 108,10540-10546. S. M. Dunham-Ems, H. Pudavar, J. Myers, P. Prasad, and M. K. Stachowiak. (2006). s. **Mol. Biol. Cell** 17, 2223-2235.

Deciphering FRAP curves



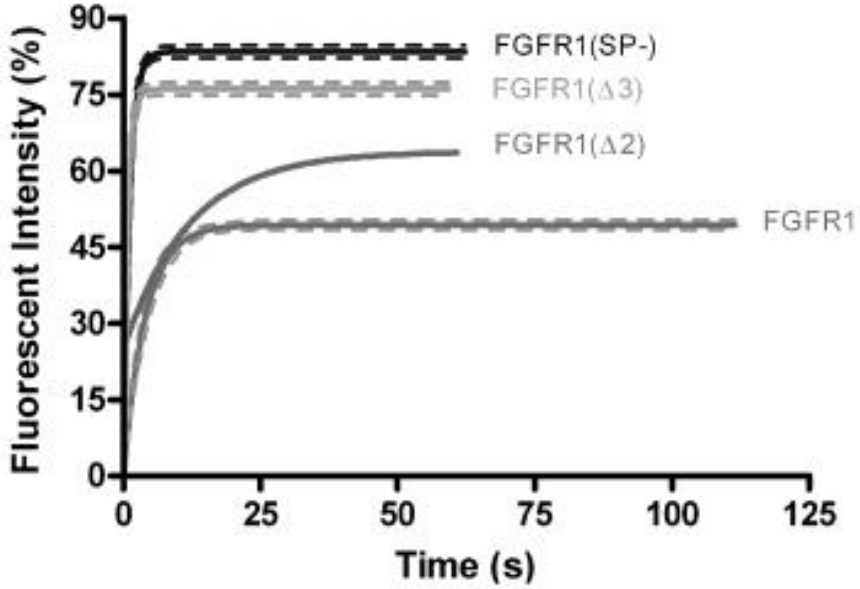
FRAP reveals 3 kinetic pools of FGFR1-EGFP in live cells:



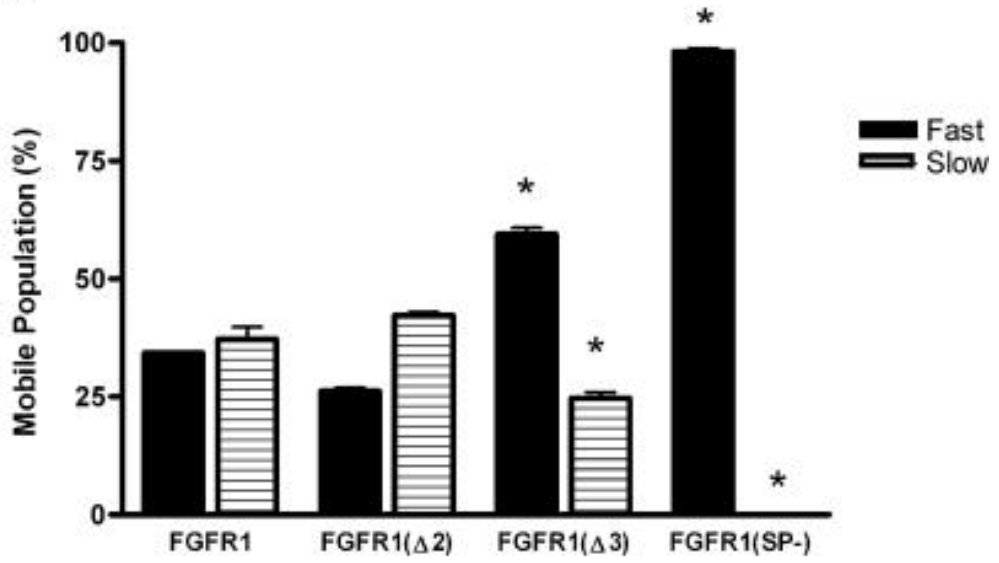


Fast ($t_{1/2}$)	0.224 +/- 0.0236	0.734 +/- 0.412
Slow ($t_{1/2}$)	69.00 +/- 7.378	ND

A



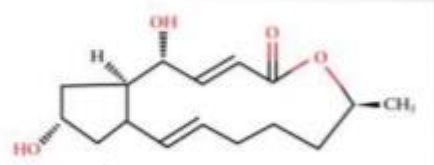
B

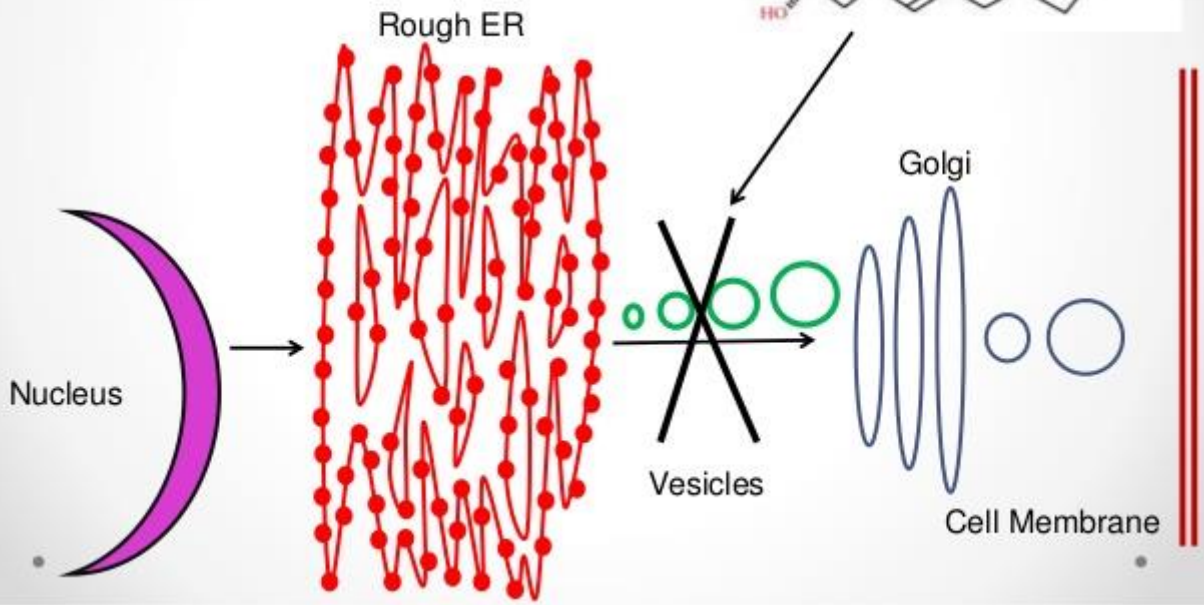


Fast ($t_{1/2}$)	0.224 +/- 0.0236	0.468 +/- 0.06003	0.234 +/- 0.431	0.734 +/- 0.412
Slow ($t_{1/2}$)	69.00 +/- 7.378	14.75 +/- 0.630	6.495 +/- 0.551	ND

What is Brefeldin A?

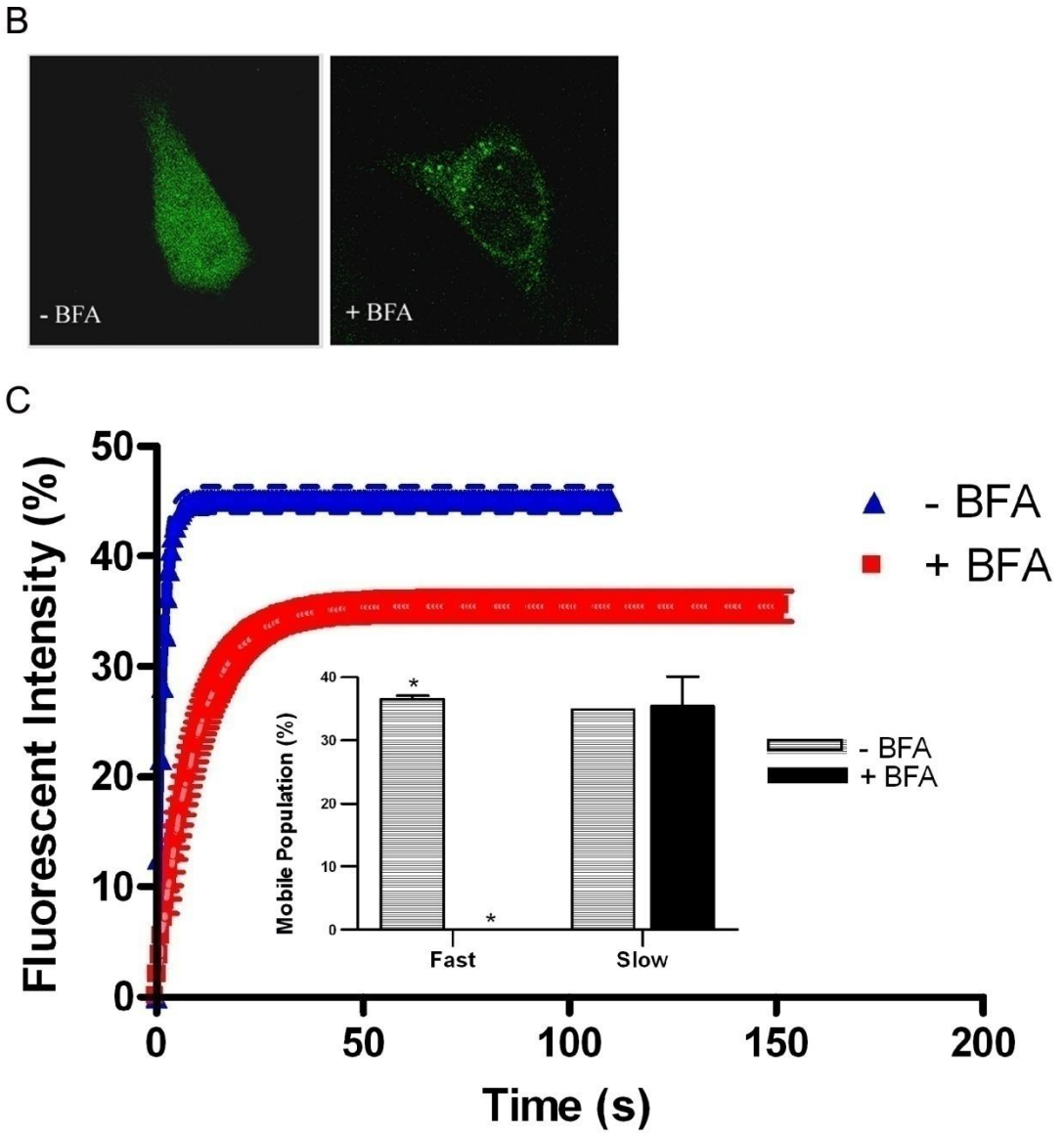
- Fungal metabolite
- Blocks transport from ER → Golgi





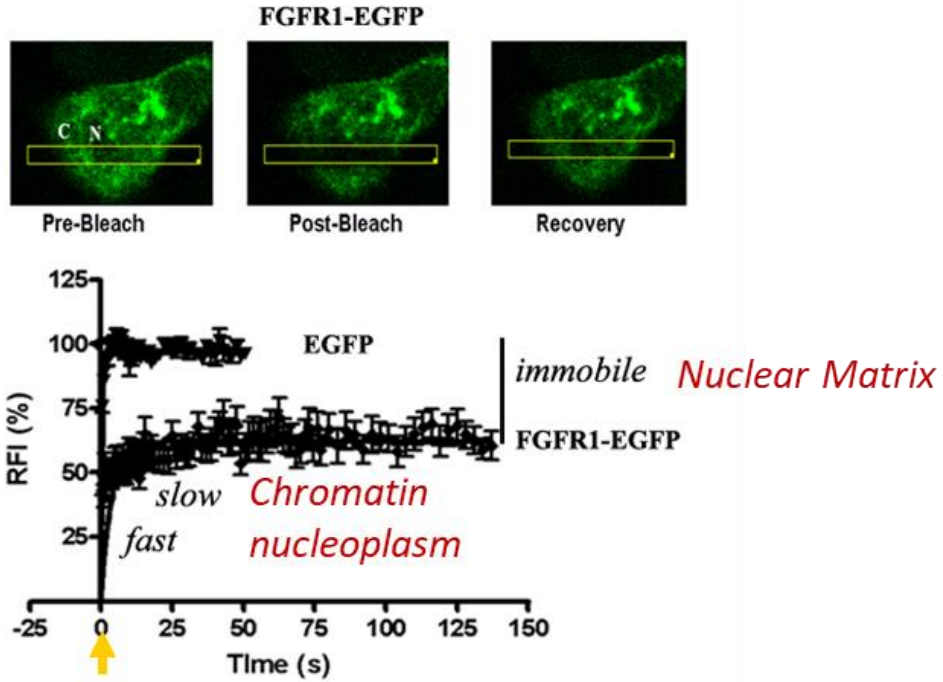
ER Golgi fusion, but not protein degradation determines FGFR1 dynamics.

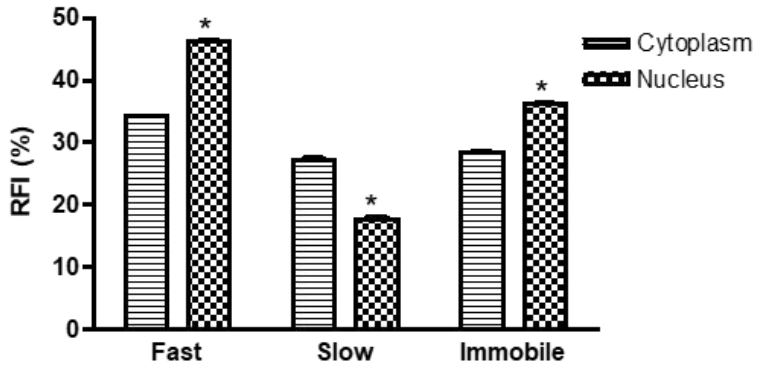
"Fast" cytosolic FGFR1 is released from ER
(Brefeldin A depletes cytosolic and nuclear FGFR1).



Dunham et al., Mol. Biol. Cell 2006

FRAP reveals 3 kinetic populations of FGFR1 in the nucleus



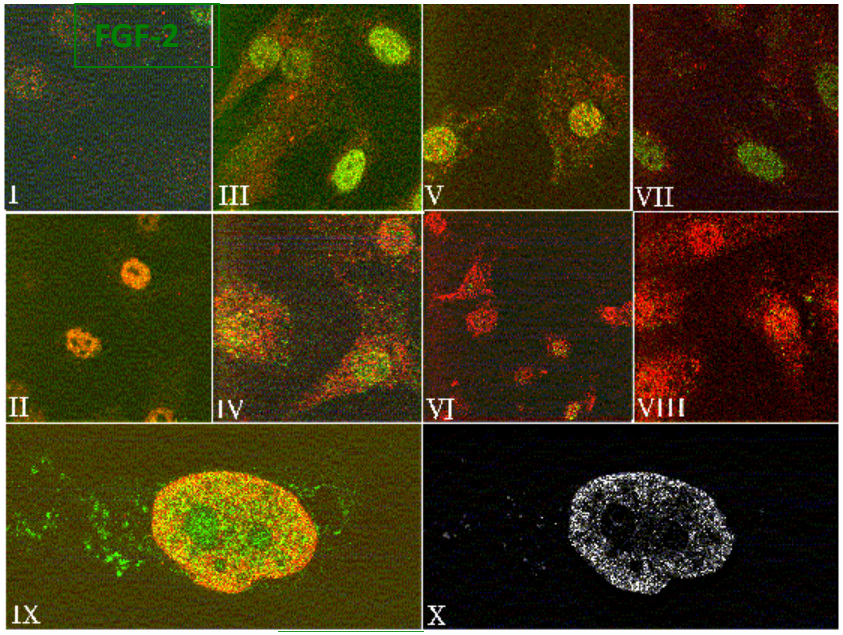


	Nucleus	Cytoplasm
Fast ($t_{1/2}$-s)	0.287 +/- 0.015	0.224 +/- 0.24
Slow ($t_{1/2}$-s)	24.412 +/- 0.221*	69.00 +/- 7.38

Population

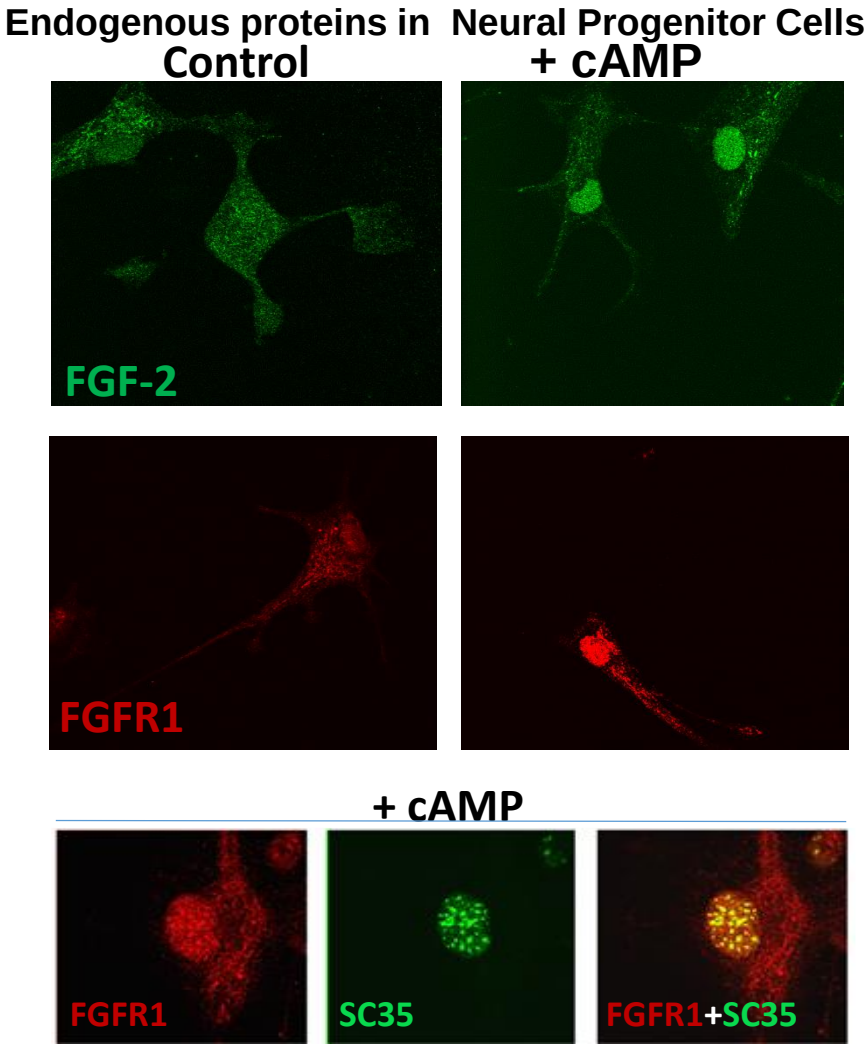
Interaction of nuclear FGF-2 and FGFR1

- Stimuli that increase cellular growth or proliferation, such as angiotensin II, carbachol, forskolin and PMA, activate the FGF-2 and FGFR1 genes and induce an accumulation of FGF-2 and FGFR1 proteins directly in the cell nucleus.



FGFR1

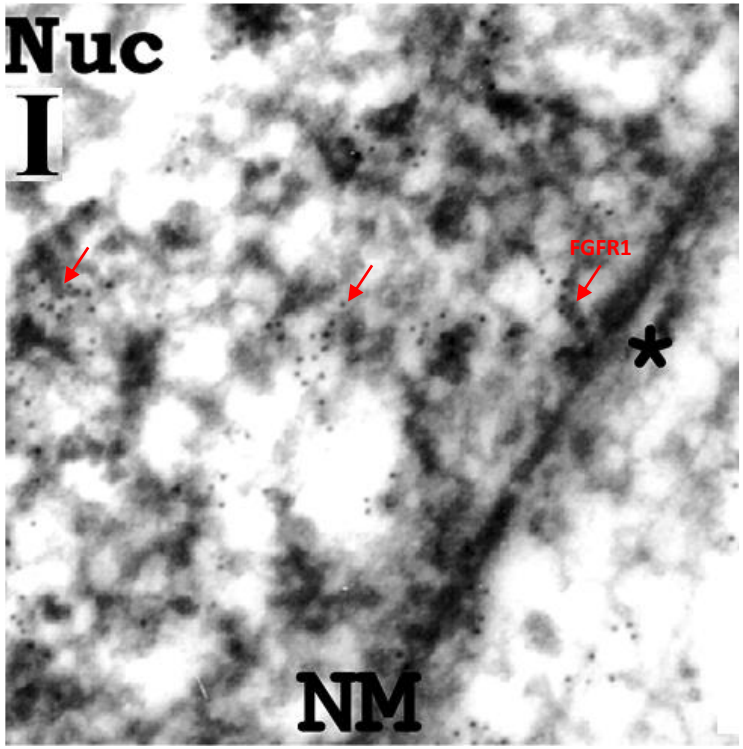
Cytoplasmic - Nuclear co-trafficking of FGF2 and FGFR1

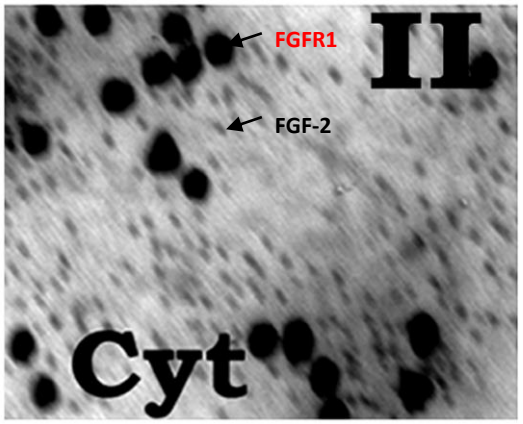


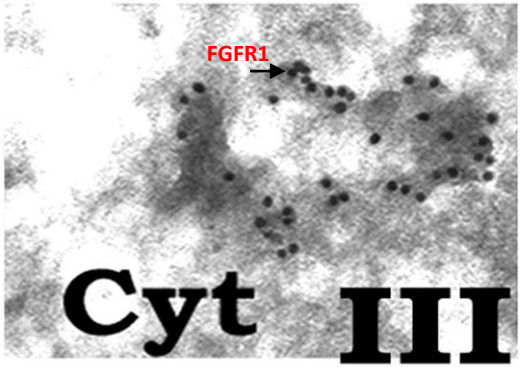
*Stachowiak, et al., (2003). cAMP-induced differentiation of human neuronal progenitor cells is mediated by nuclear FGF receptor-1 (FGFR1**). J. Neurochem.** 84, 1296-13*

*Nuclear **FGFR1**: Baird lab 1995; Stachowiak lab: Mol Biol Cell 1996; Oncogene 1997a,b, PNAS 1996 (FGFR1); Maher lab J. Cell Biol 1996;. (Bryant and Sto,w, 2005; (Clarke et al., 2001; Gonzalez et al., 1995); R. Grose : Nuclear translocation of FGFR1 and FGF2 inpancreatic stellate cells facilitates pancreatic cancer 2014, EMBO J.*
*Nuclear **FGFR2:(**Marchetti et al., 2006; Sabbieti et al., 2005; Schmahl et al., 2004*
*Nuclear **FGFR3 (**mutant): (Johnston et al., 1995)*

Immuno-electron microscopy - FGFR1 in ER (III), cytosol-like (II) and nuclear (I) compartments.





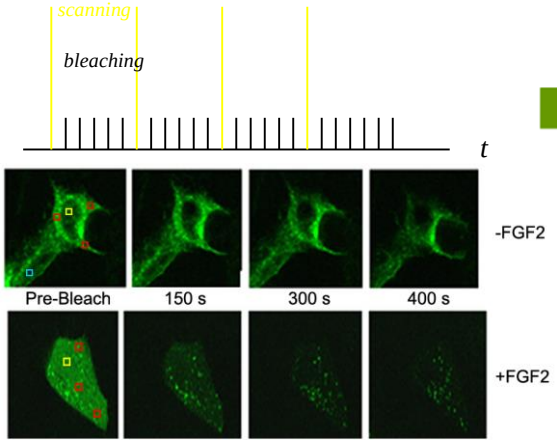


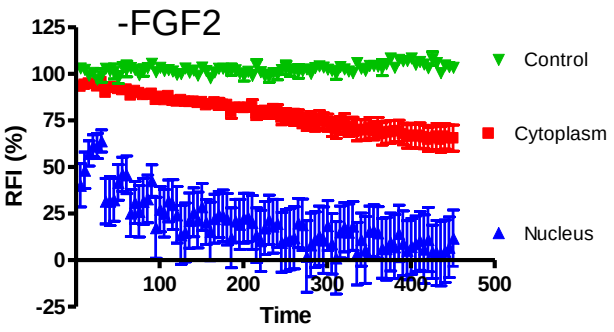
M.K. Stachowiak, P. Maher, E. Mordechai, A. Joy, E.K. Stachowiak. ***Molec. Biol. Cell*** *2006, 7, 1299-1317.*
E.K. Stachowiak, P. Maher, J. Tucholski, E. Mordechai A. Joy,S. Coons, M.K. Stachowiak (1997) ***Oncogene*** *14, 2201-2211*

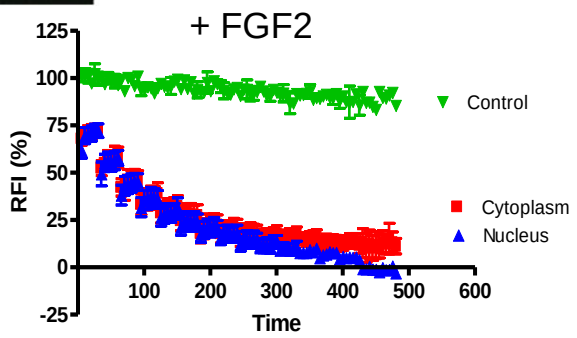
Dynamic equilibrium

FGF-2 increases FGFR1 Cytoplasmic-Nuclear Shuttling.

FLIP - Fluorescent Loss
In Photobleaching



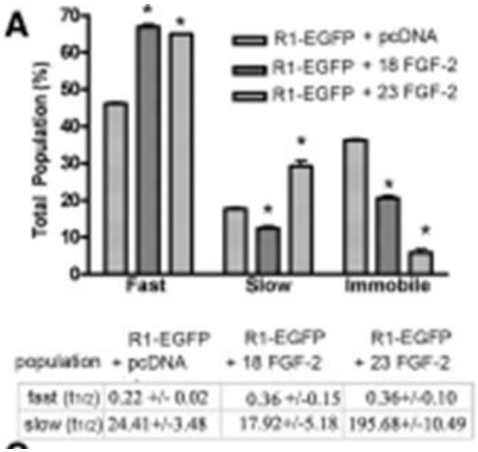




"Stem Cells – From Mechanisms to Technologies" Editors – Michal K. Stachowiak and Emanuel S. Tzanakakis, World Scientific Publishing, 2011
Lee et al. PLOS One, 2013

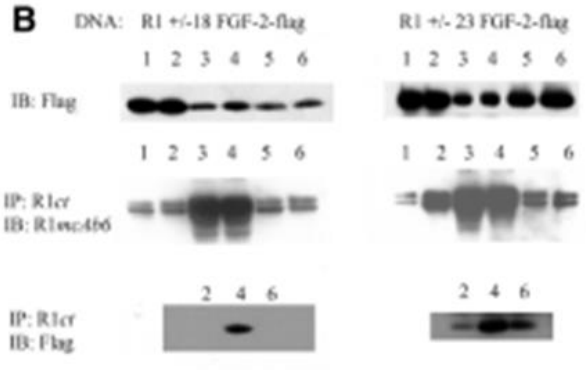
Interaction of nuclear HMW FGF-2 and FGFR1
HMW FGF-2 modulates FGFR1's dynamics and is co-IP with nuclear FGFR1

FRAP



population	R1-EGFP + pcDNA	R1-EGFP + 18 FGF-2	R1-EGFP + 23 FGF-2
fast (t½)	0.22 +/- 0.02	0.36 +/-0.15	0.36+/-0.10
slow (t½)	24.41+/-3.48	17.92+/-5.18	195.68+/-10.49

co-IP



Fractions: microsomal (lanes 1 and 2),
cytosolic (lanes 3 and 4),
nuclear (lanes 5 and 6)

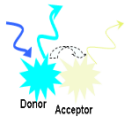
FRET - Fluorescence (Förster) Resonance Energy Transfer

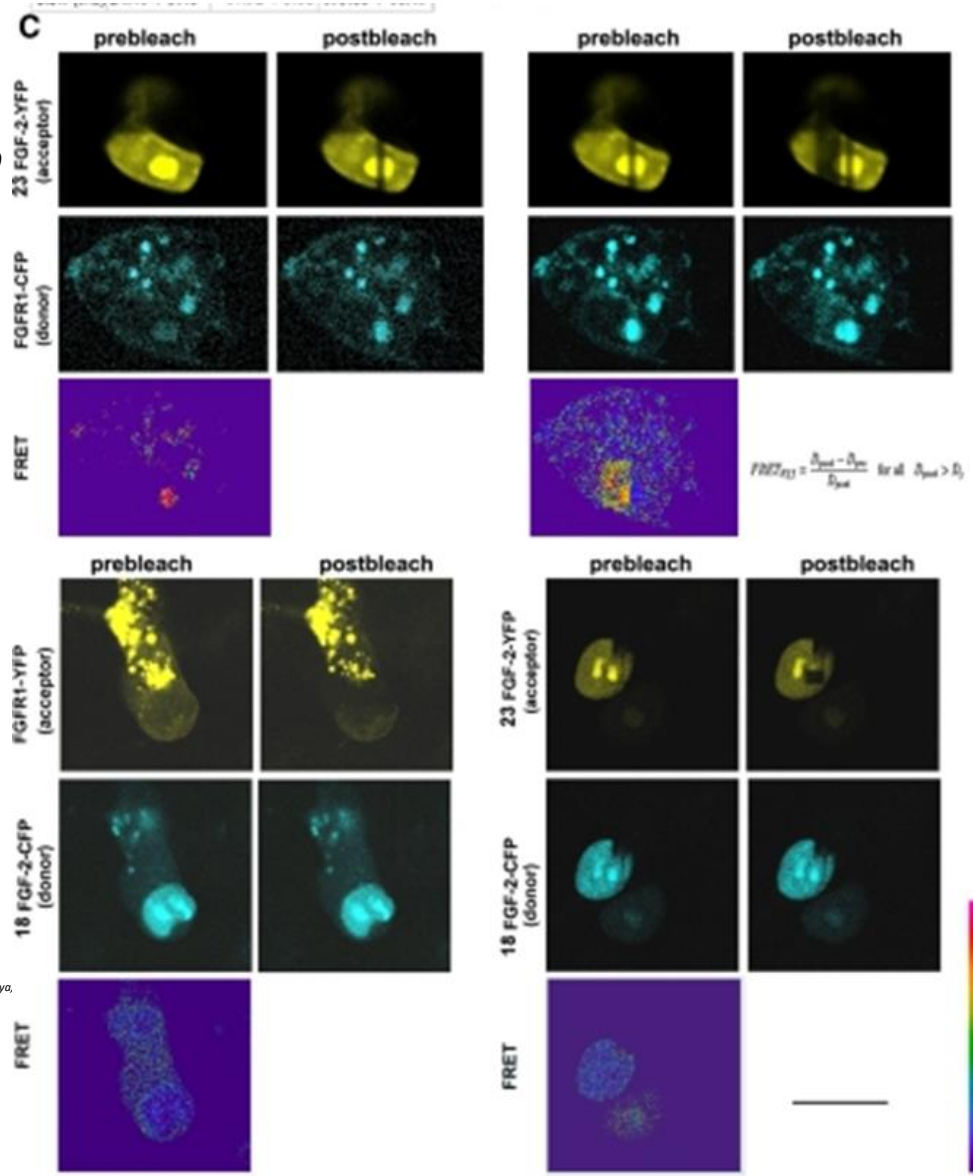
Interaction between nuclear HMW (23 kDa) FGF-2 –YFP and FGFR1-CFP

(Acceptor bleaching technique)

F-2-YFP

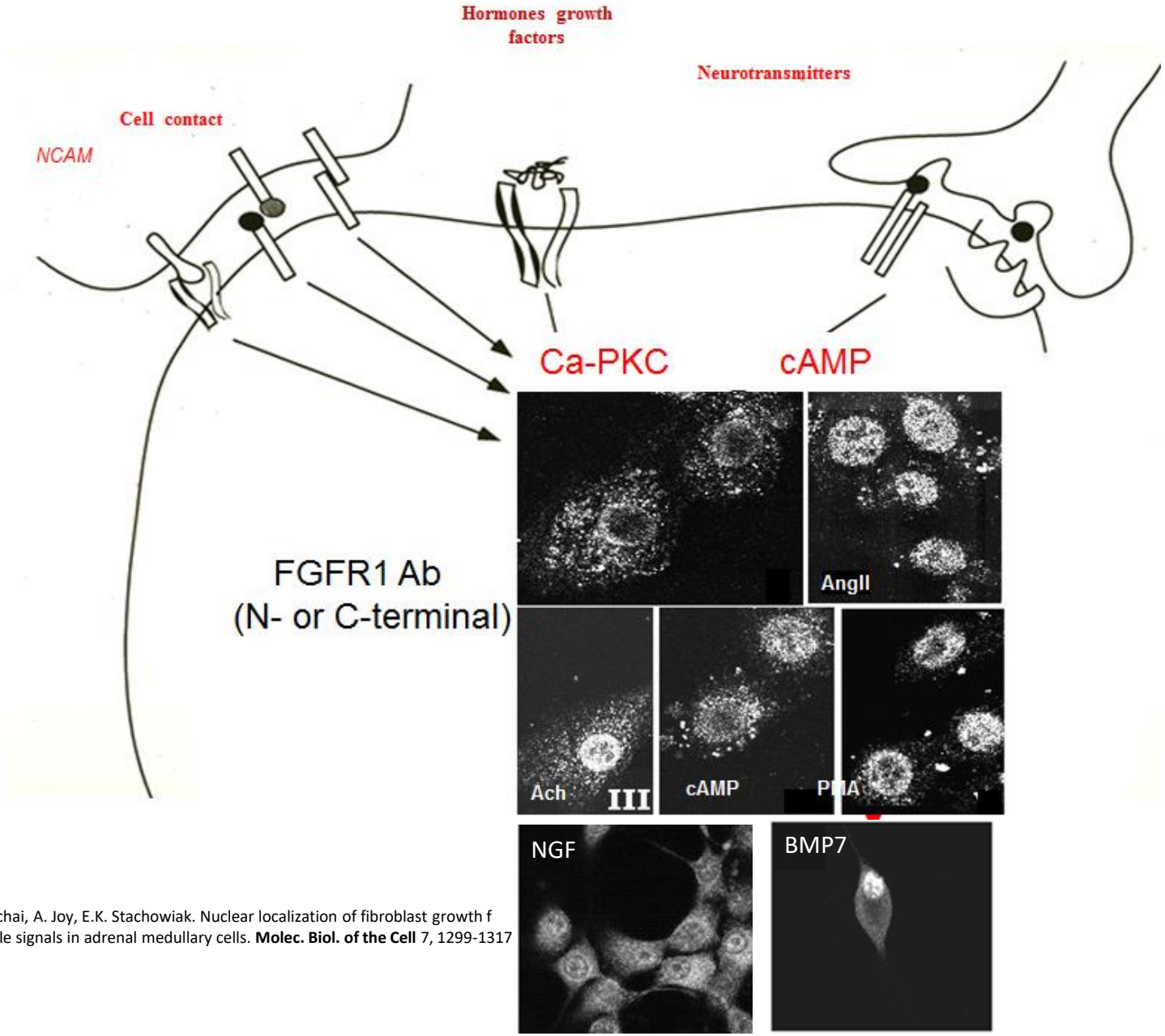
FGFR1-CFP FGF-2YFP





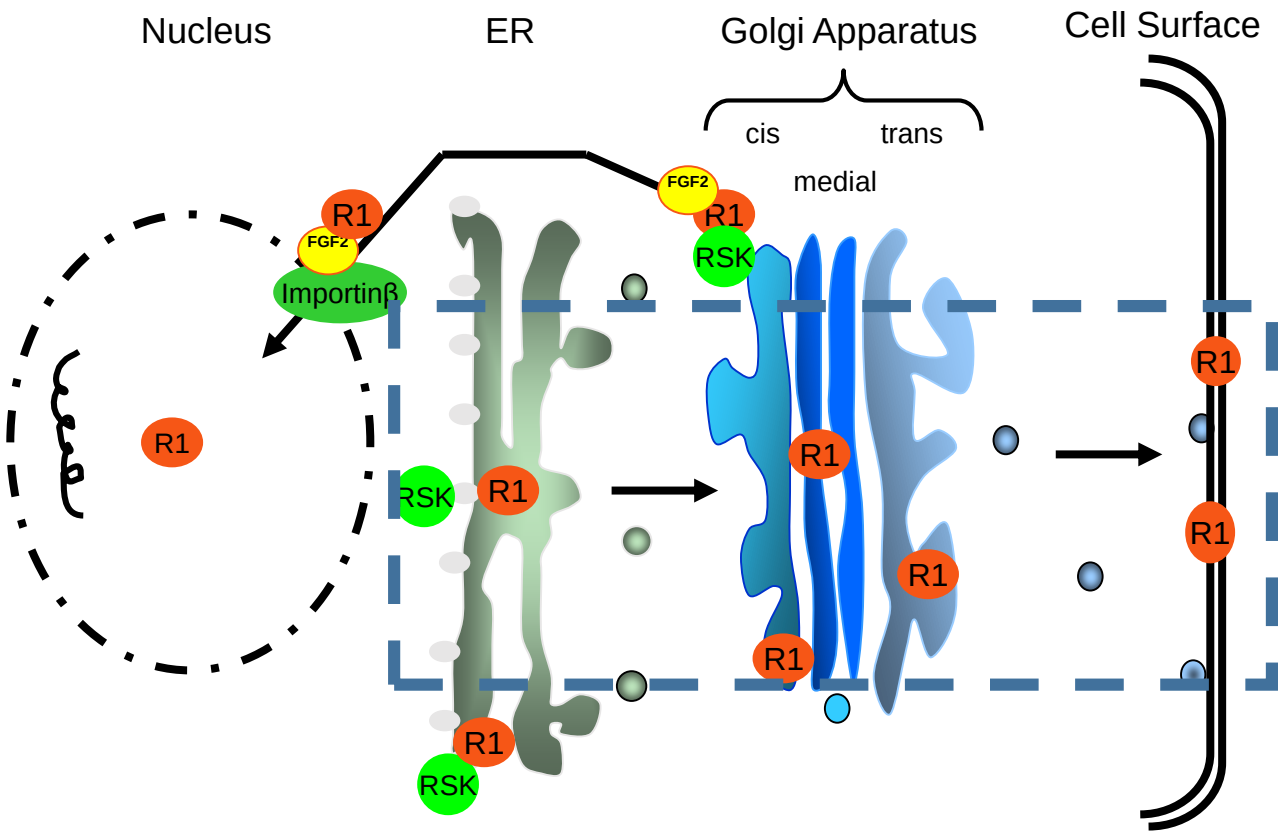
Lee, Y-W., Terranova, C., Birkaya,

“Integrative Nuclear FGFR1 Signaling (INFS)”



M.K. Stachowiak, P. Maher, E. Mordechai, A. Joy, E.K. Stachowiak. Nuclear localization of fibroblast growth f actor receptors is regulated by multiple signals in adrenal medullary cells. **Molec. Biol. of the Cell** 7, 1299-1317

Plasma membrane FGFR1 (R1) trafficking- Constitutive Pathway



Stachowiak, M.K. Stachowiak E.K. DNA and Cell Biology 2007; Dunham, Prasad, Stachowiak, J. Biophys. 2006;
Reilly, J.F., and P.A. Maher. 2001. Importin beta-mediated nuclear import of fibroblast growth factor receptor: role in cell proliferation. *J Cell Biol*. 152:1307-1312
Bryant, D.M., Wylie, F.G., and Stow, J.L. (2005). Mol Biol Cell *16*, 14-23.
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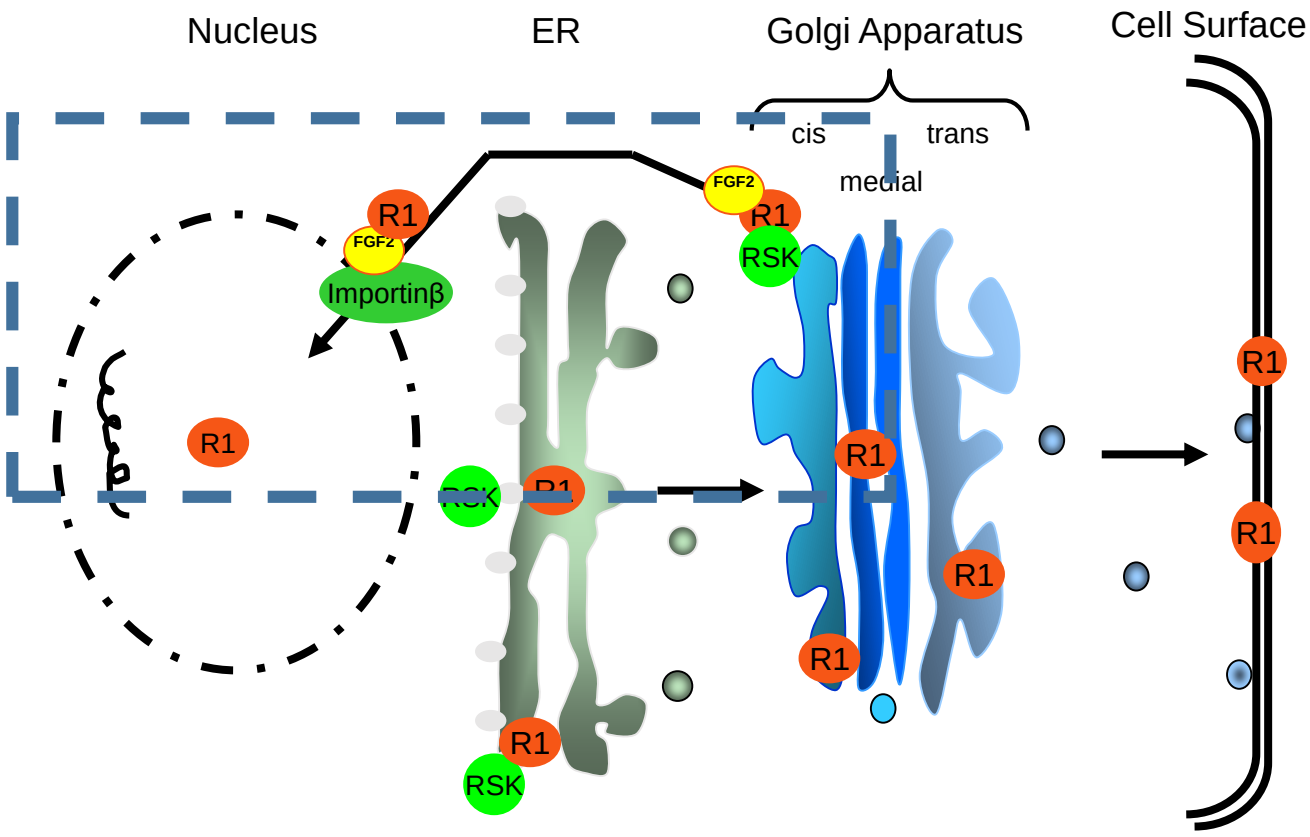

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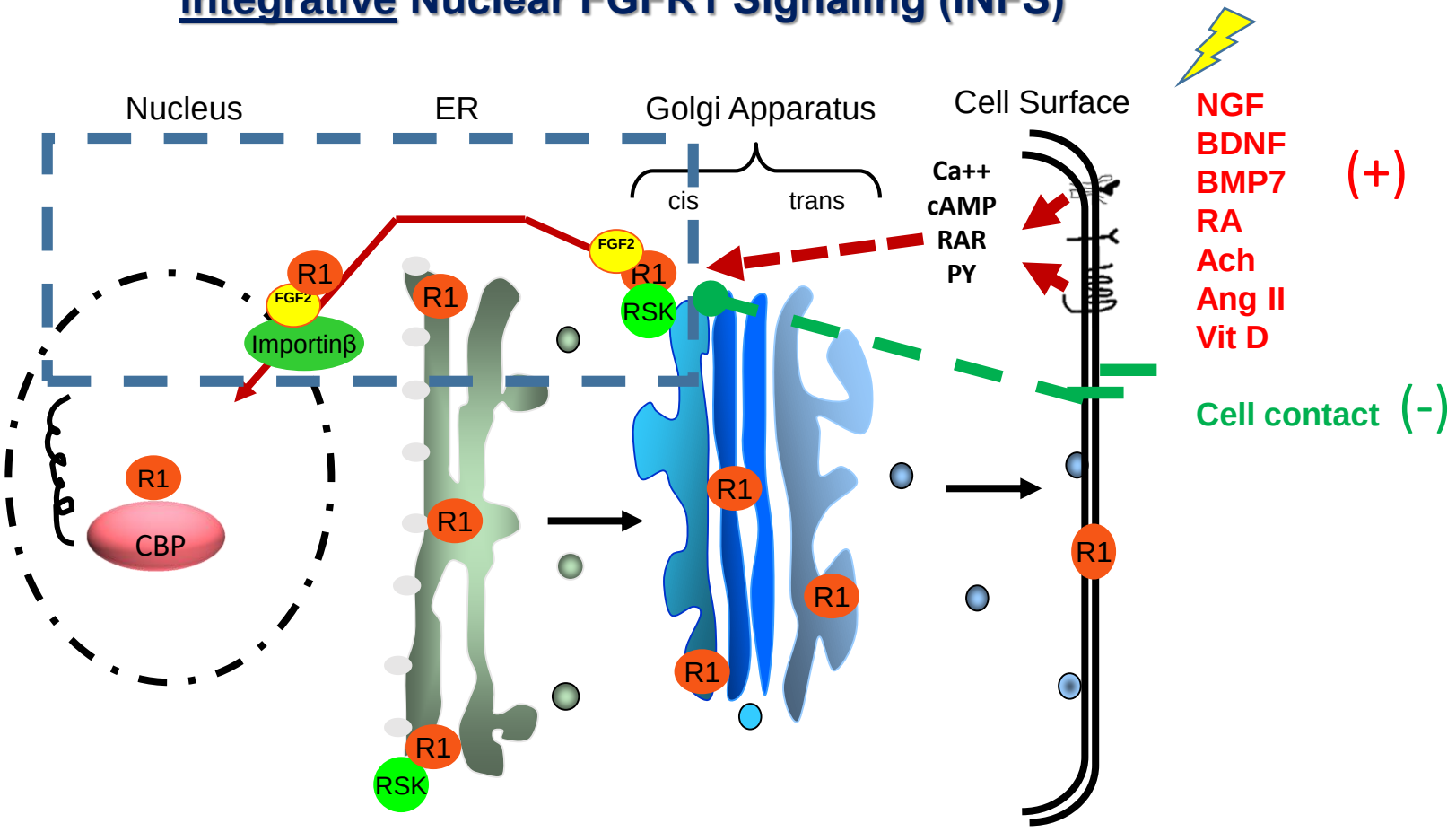

Stachowiak, M.K. Stachowiak E.K. DNA and Cell Biology 2007; Stachowiak,M.K., Stachowiak,E.K., Aletta, J.M. and Tzanakakis,E.S; In: Stem Cells from Mechanisms to Technologies, M.K. Stachowiak, E.S. Tzananakis-editors. World Scientific Publishing, 2011