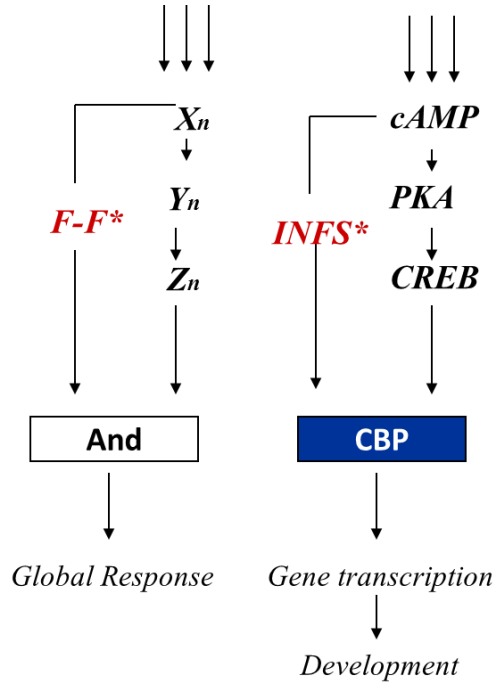
Evidence based theory for integrated genome regulation in ontogeny

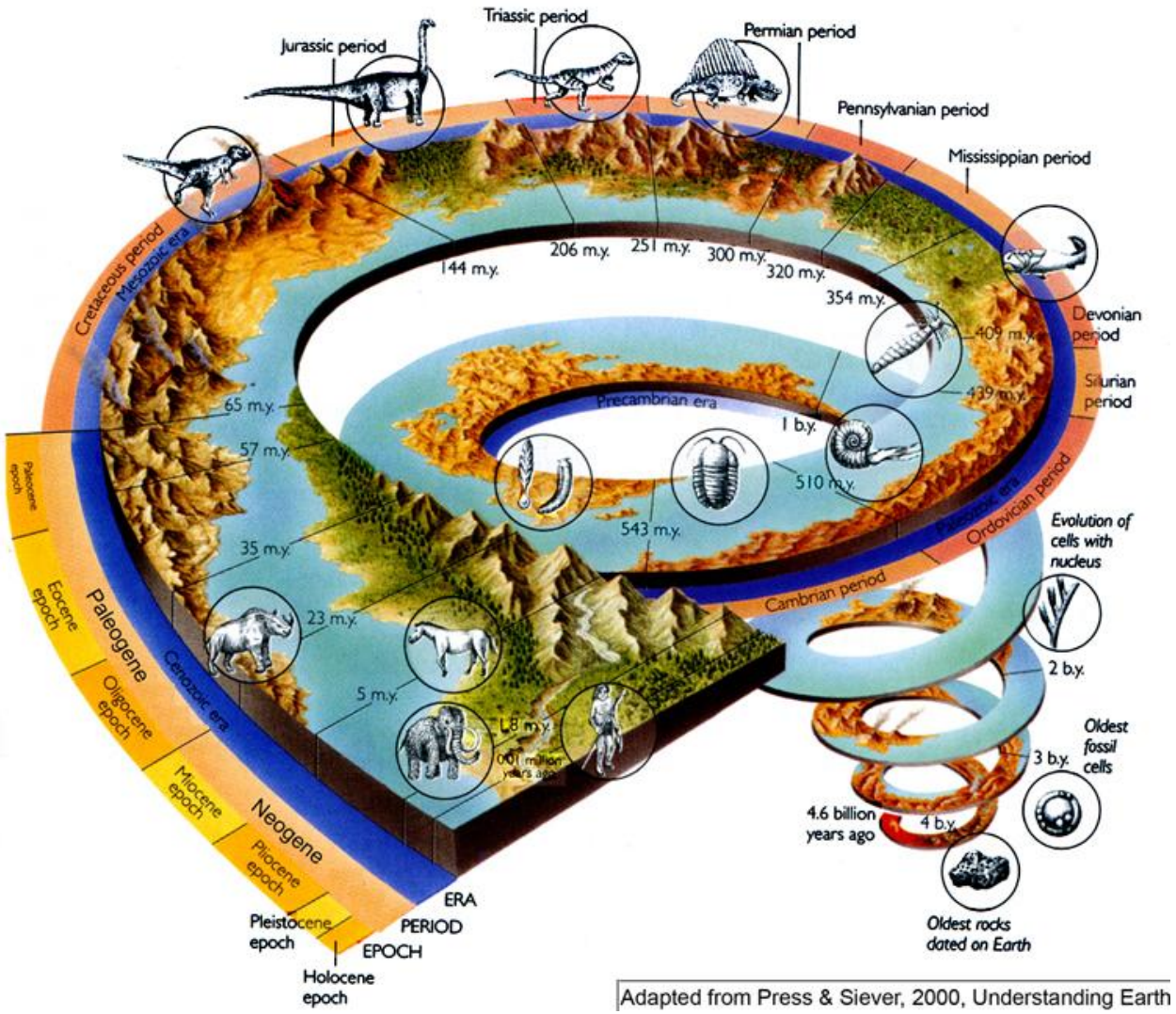
- potential therapeutic utility

Michal K. Stachowiak
Ewa K. Stachowiak
Molecular and Structural Neurobiology and Gene Therapy Program



State University of New York At Buffalo

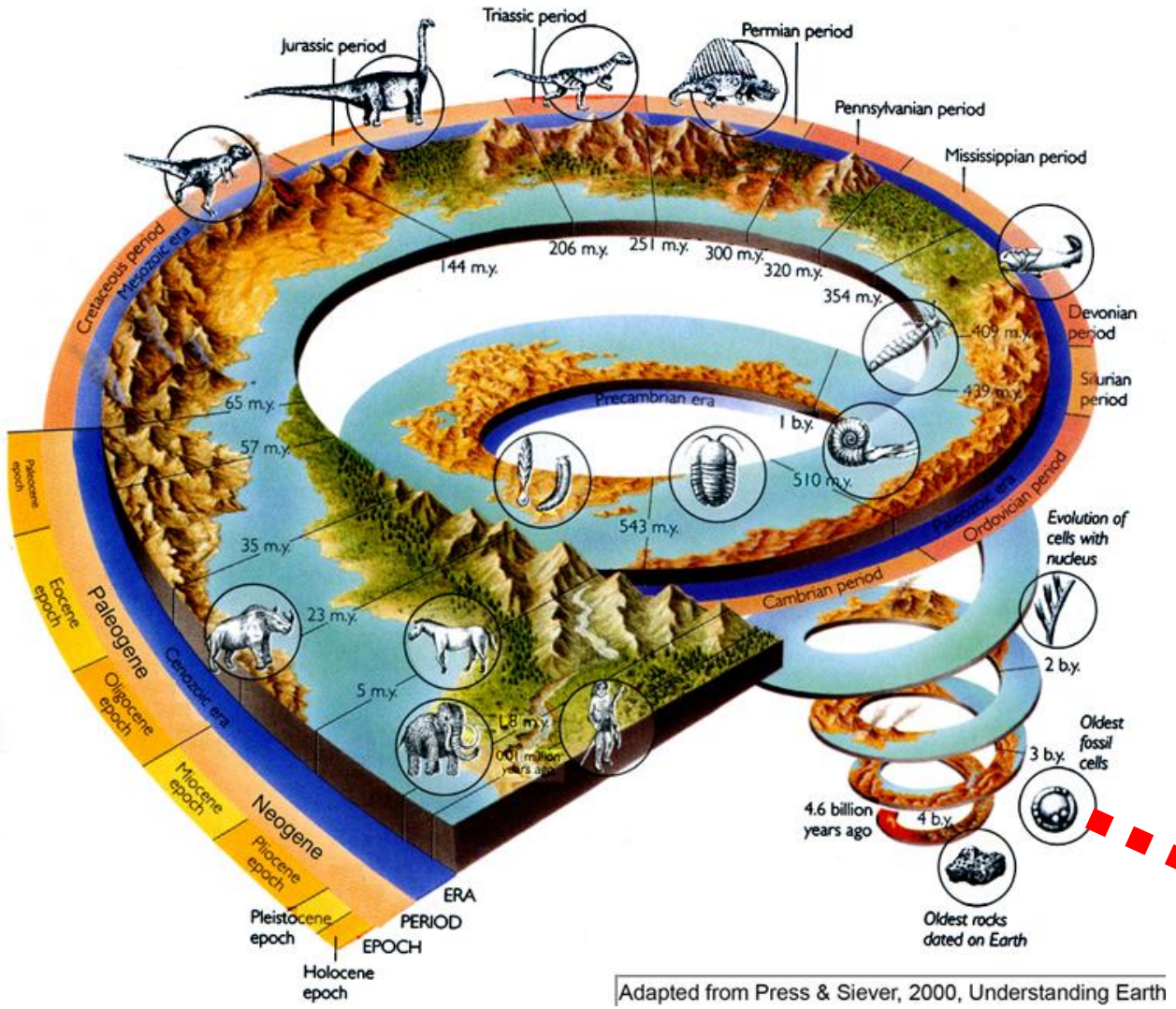
WNYSTEM NYSTEM



Adapted from Press & Siever, 2000, Understanding Earth

Paul Davies: *"Scientists and clinicians who study cancer …….are caught up in the frantic and expensive search for an elusive "cure" but rarely ask why these diseases exist at all and what their place is in the grand story of life on Earth"*

Physics of Cancer: New tools and fresh perspectives, Physics World Vol. 26 No. 7, 2013, 37---40.



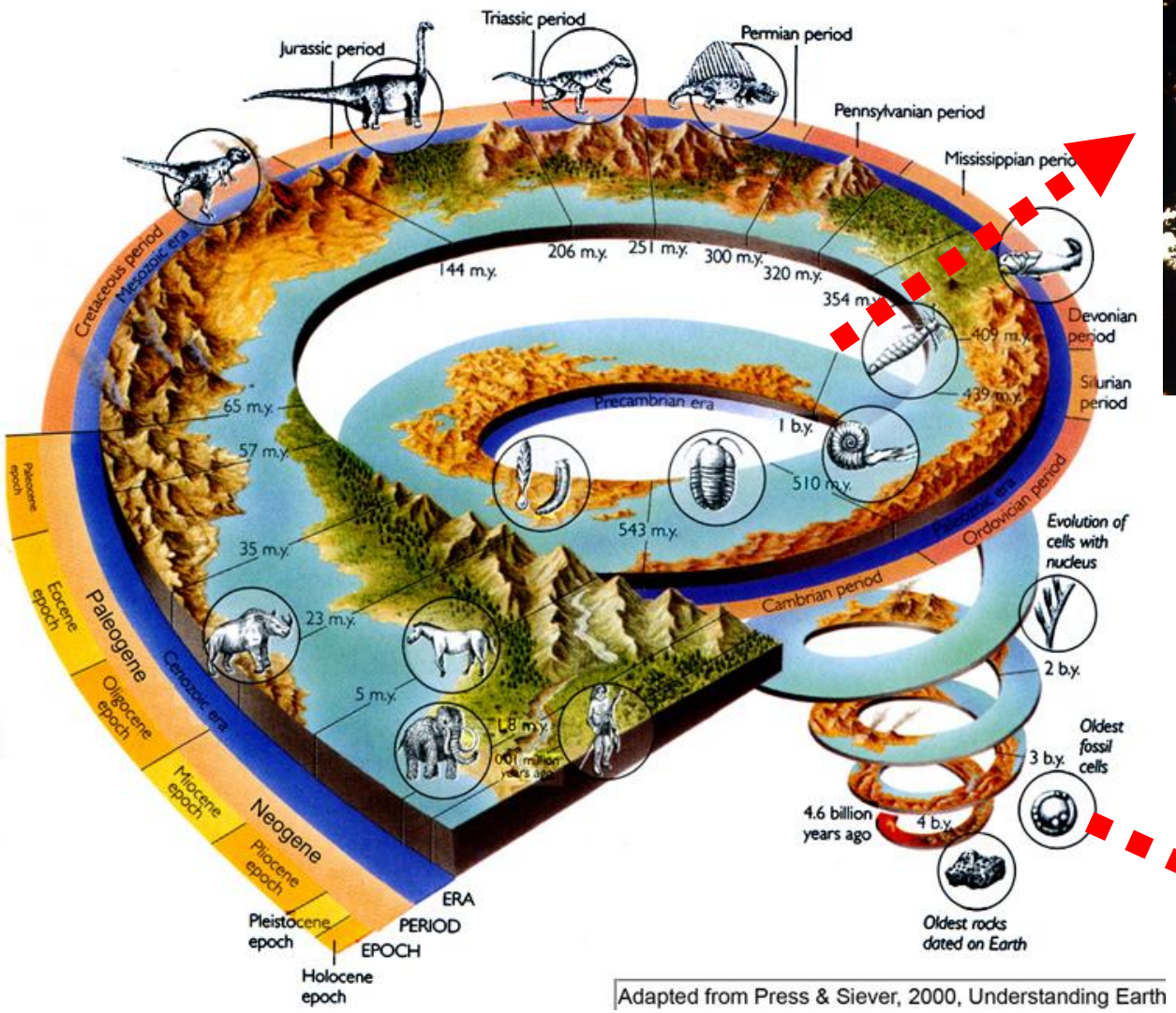
Adapted from Press & Siever, 2000, Understanding Earth

- 3 billion years



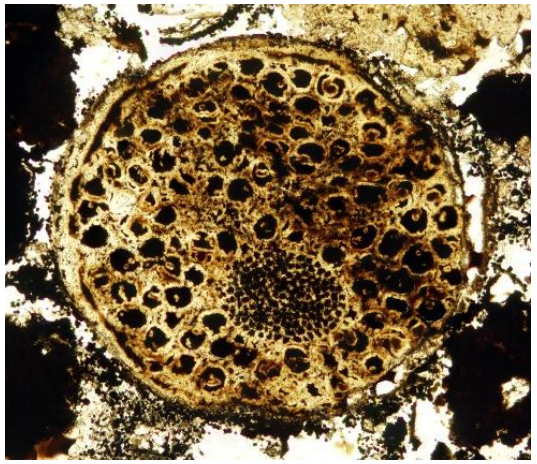
Unicellular

Oncogenes:
P53, cdk, klf4,
Ddk, cyclins,
…



Adapted from Press & Siever, 2000, Understanding Earth

- 0.6 bilion years



Morphogenes:
Hox, Wnt, Notch,
Pax, Rest, mesp2,
…

{ *Oncogenes:*
P53, cdk, klf4,
Ddk, cyclins,
…. }

Multicellular

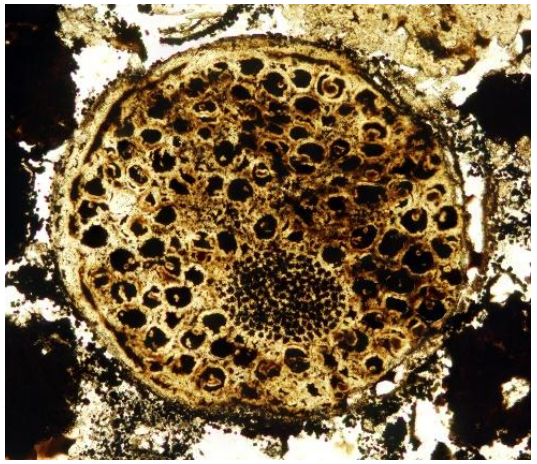
- 3 bilion years



Oncogenes:
P53, cdk, klf4,
Ddk, cyclins,
…

Unicellular

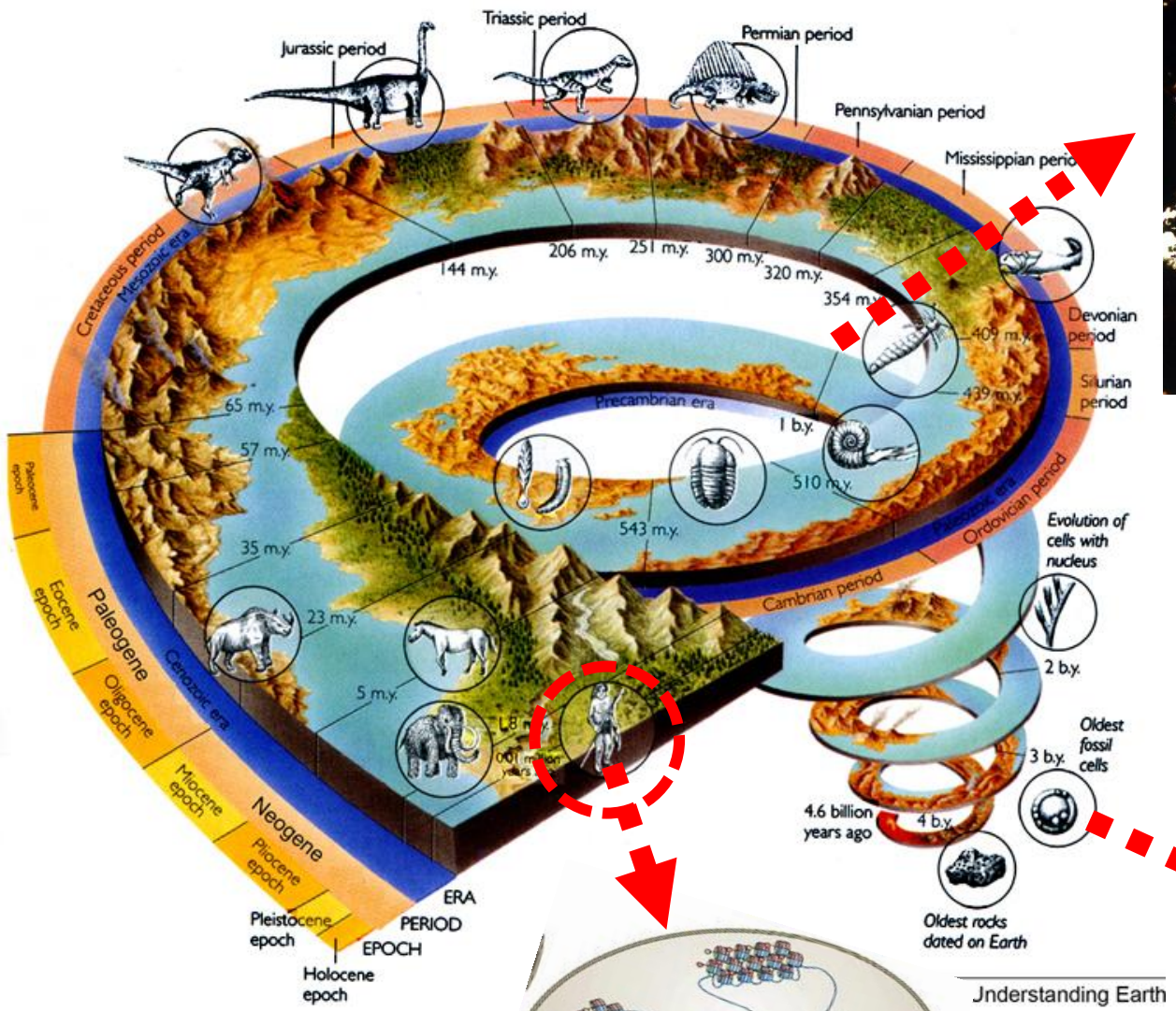
- 0.6 bilion years



Morphogenes:
Hox, Wnt, Notch,
Pax, Rest, mesp2,
…

{ *Oncogenes:*
P53, cdk, klf4,
Ddk, cyclins,
…. }

Multicellular



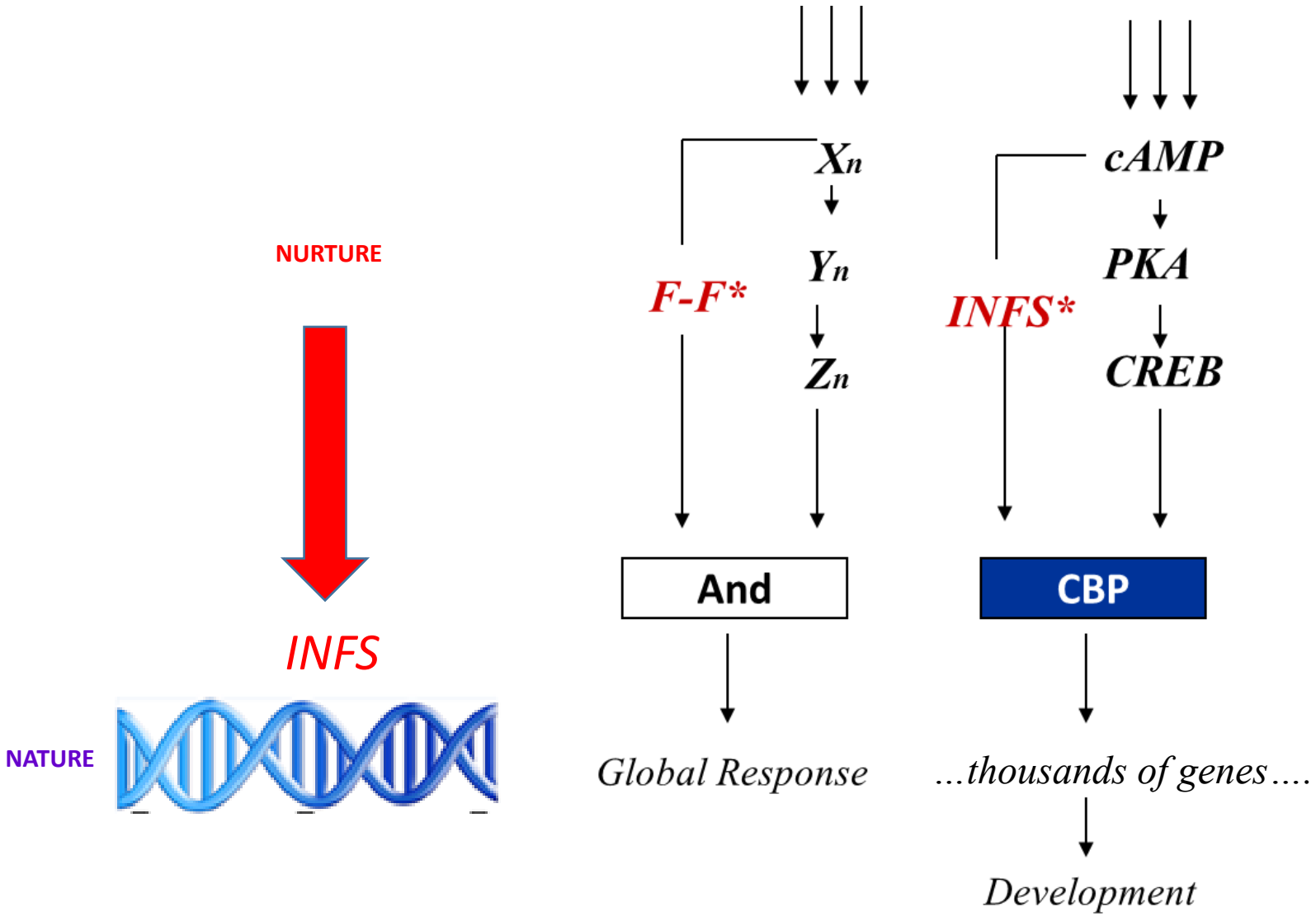
- 3 bilion years



Oncogenes:
P53, cdk, klf4,
Ddk, cyclins,
…

Unicellular

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).

Evolution of FGFs & FGF Receptors as nuclear signaling proteins

FGF general structure:

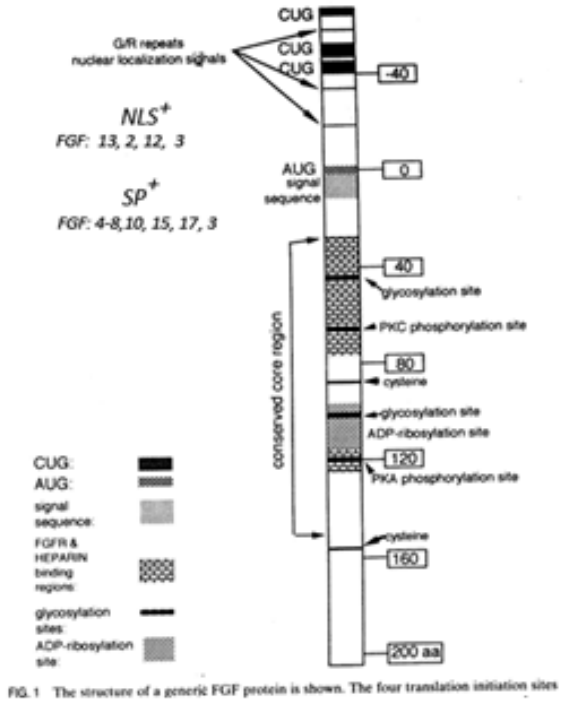


FIG. 1 The structure of a generic FGF protein is shown. The four translation initiation sites

FGF cellular action:

(Nuclear, Extracellular)

C. Elegans:

LET756

(500 mln years ago)



Mammals:

FGF13-like (iFGF)



FGF13, FGF14

FGF4-like

FGF4-like

FGF15-like

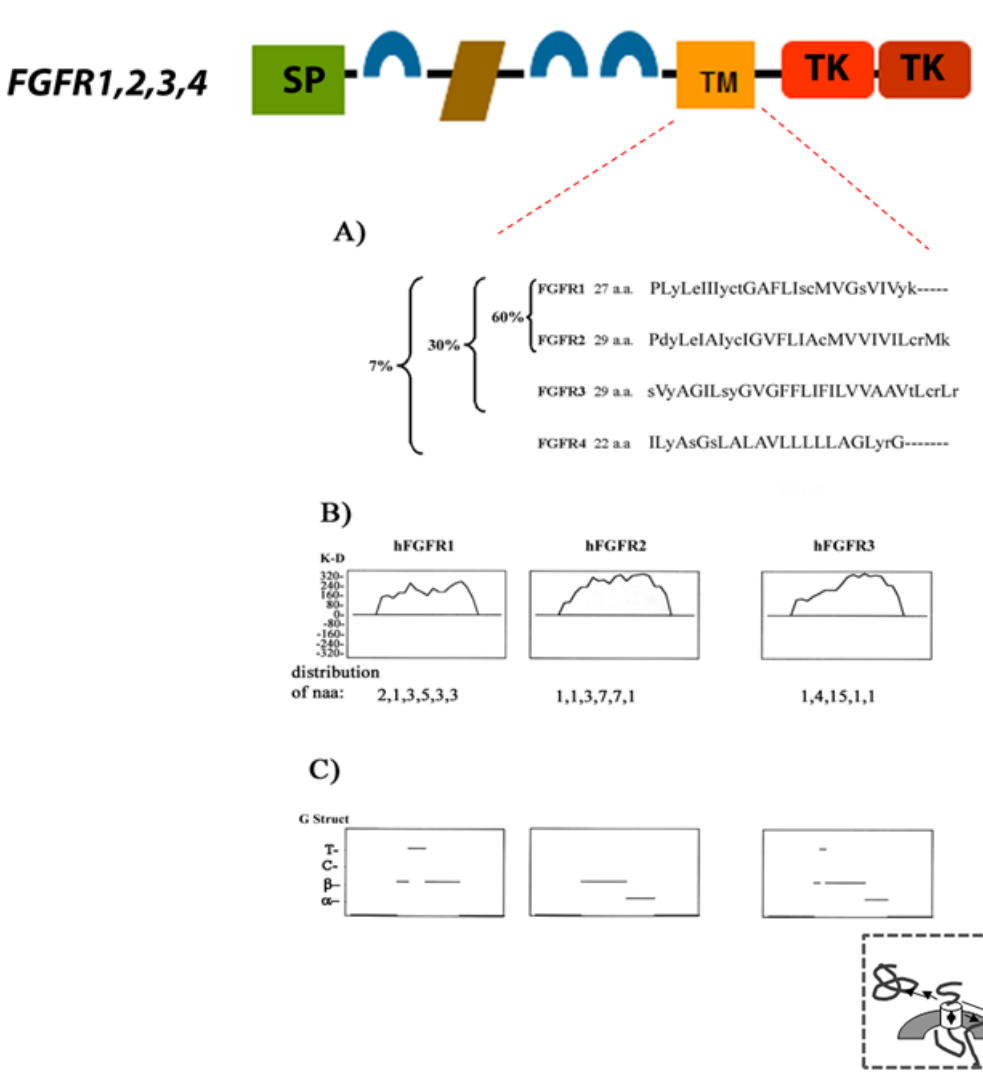
FGF1, FGF2

FGF1,FGF2(?)

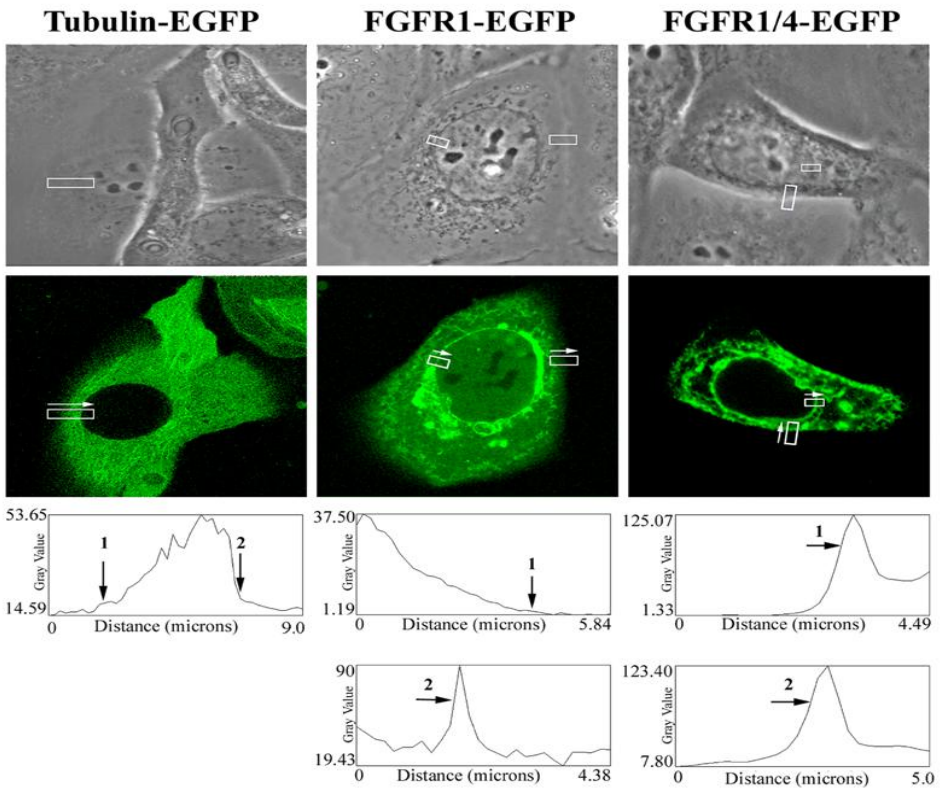
FGF15,FGF21,FGF23

Stachowiak et all, 2015

Unusual properties of FGFR1 transmembrane domain

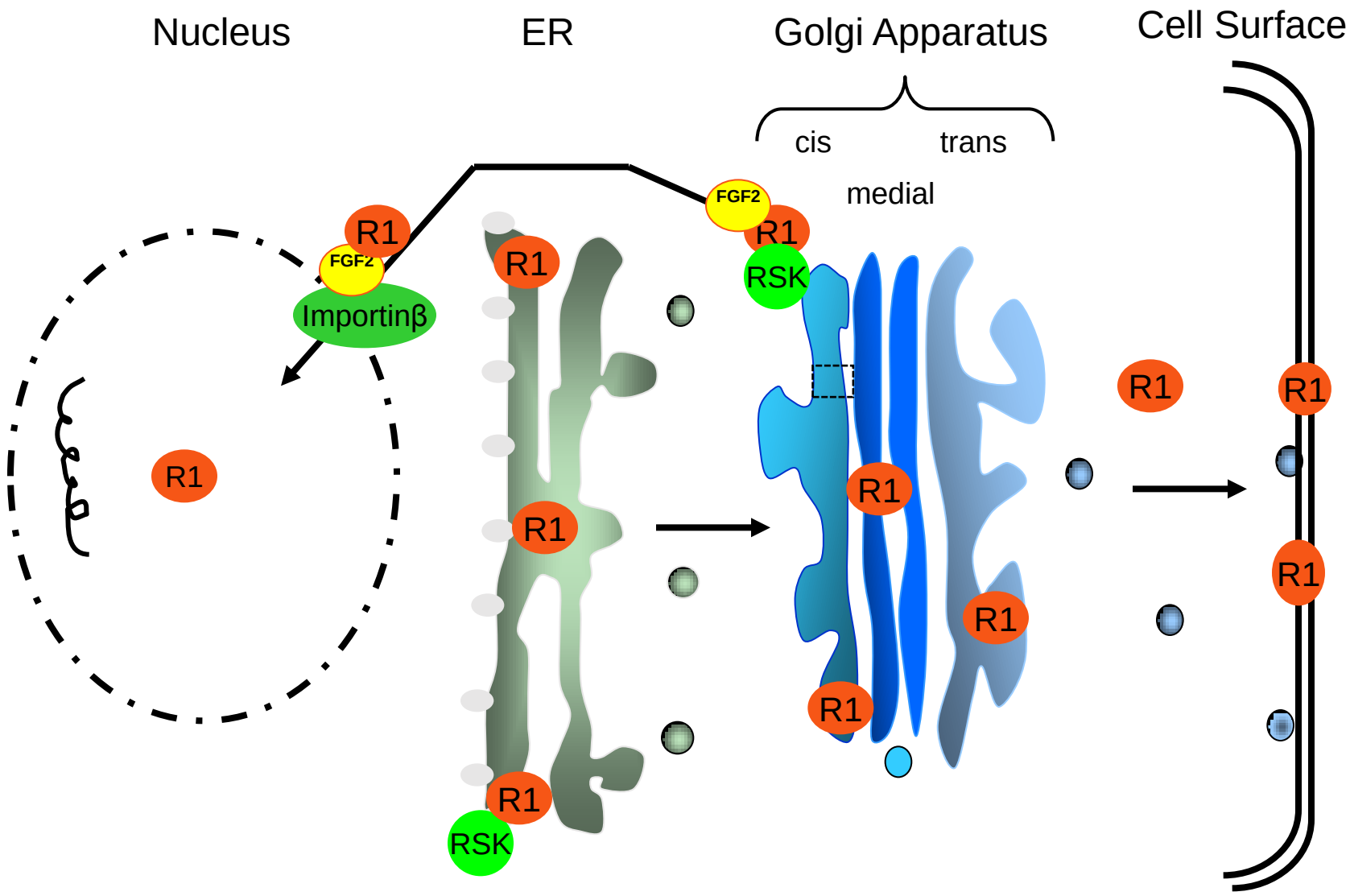






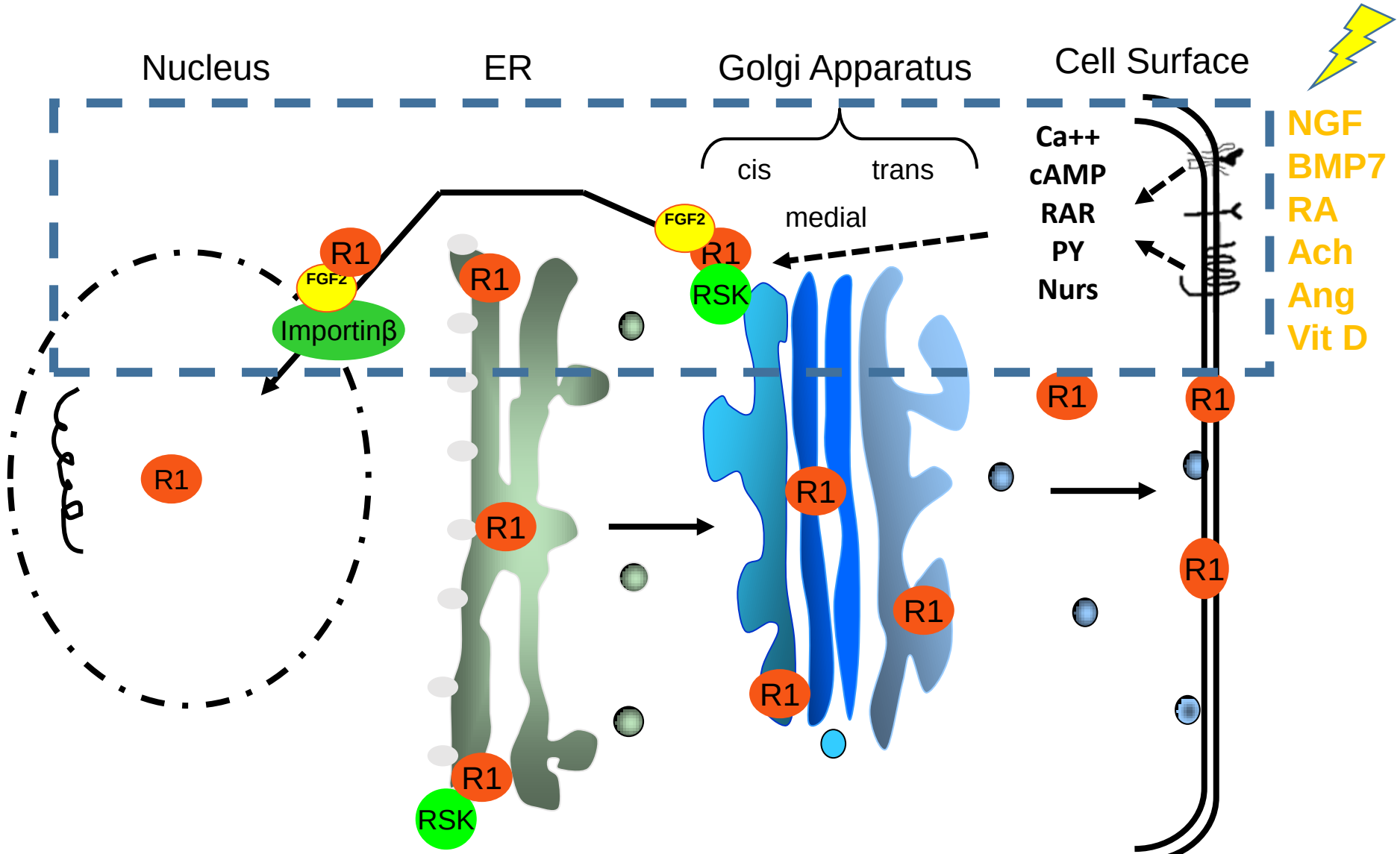
Chou-Fasman and Garnier algorithms predict the TM of FGFR4 to be a typical a-helix. In contrast FGFR1 showed a b-sheet-Turn-b-sheet structure with no predicted a-helix. Both FGFR2 and FGFR3 demonstrated b-sheets followed by shorter a-helices. (α= α-helics; β= β-sheet; T= turn, C=random coil). (Myers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

Nuclear FGFR1 (R1) trafficking by RSK1, FGF & Importin



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.

“Integrative Nuclear FGFR1 Signaling (INFS)”

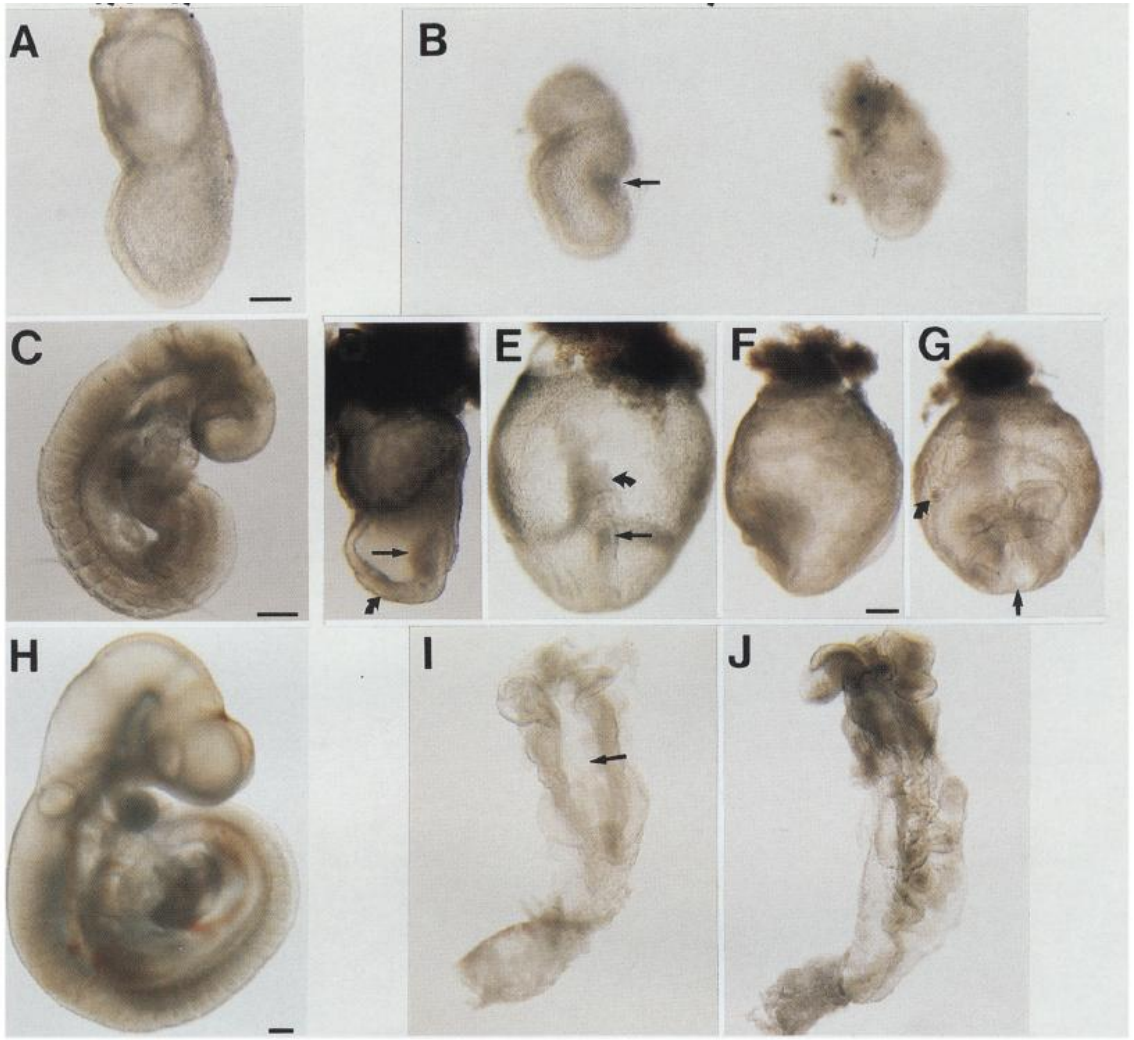


Stachowiak, M.K. Stachowiak E.K. DNA and Cell Biology 2007; Stachowiak,M.K., Stachowiak,E.K., Aletta, J.M. and Tzanakakis,E.S; In: Stem Cells from Mechanisms to Technologies, M.K. Stachowiak, E.S. Tzananakis-editors. World Scientific Publishing, 2011

FGFR1 is indispensable for ontogeny

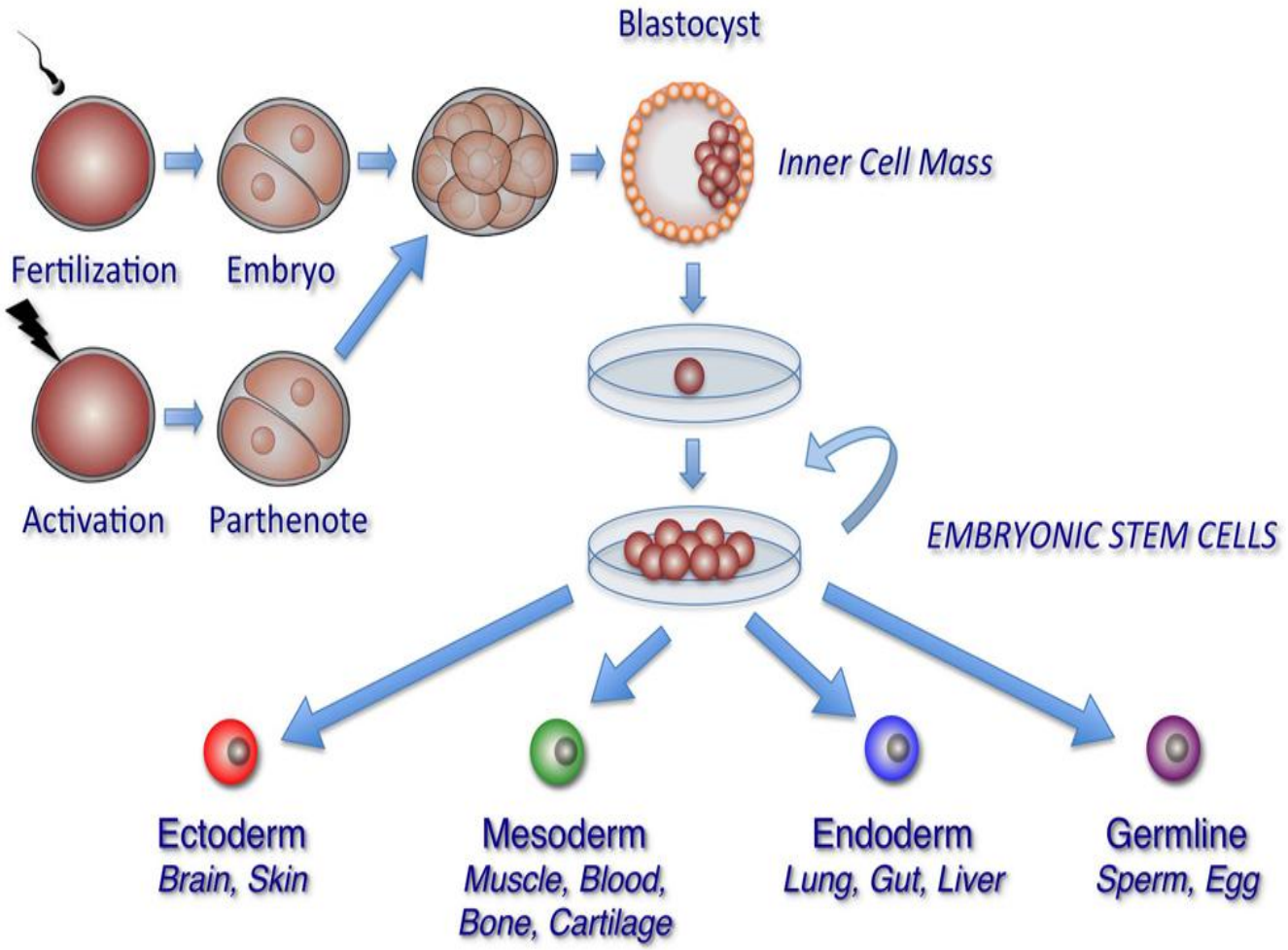
FGFR1 +/+

FGFR1 -/-



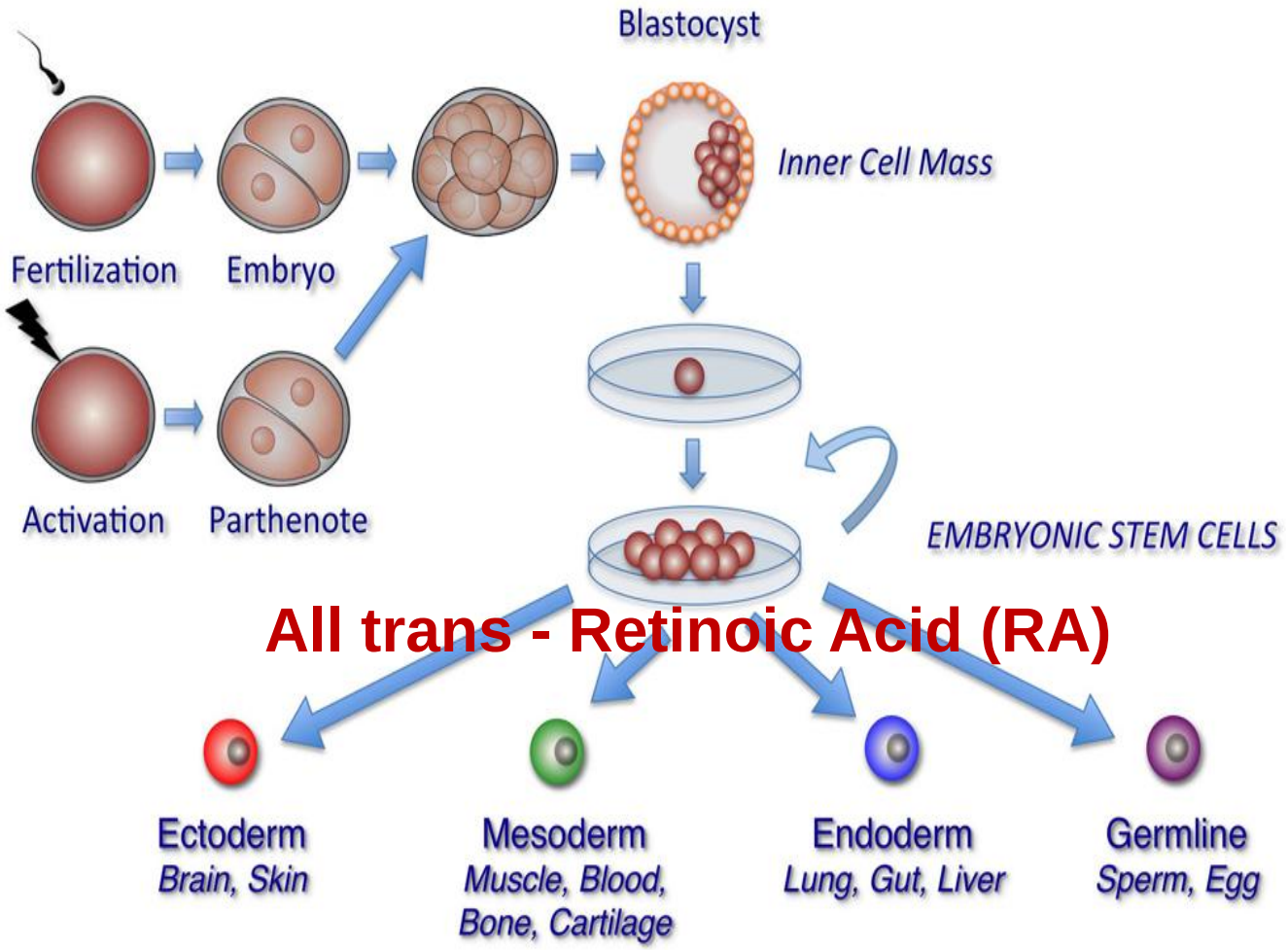
(Gene @ Dev., 8, 3045-57, 1994)

INFS in Pluripotent Embryonic Stem Cells



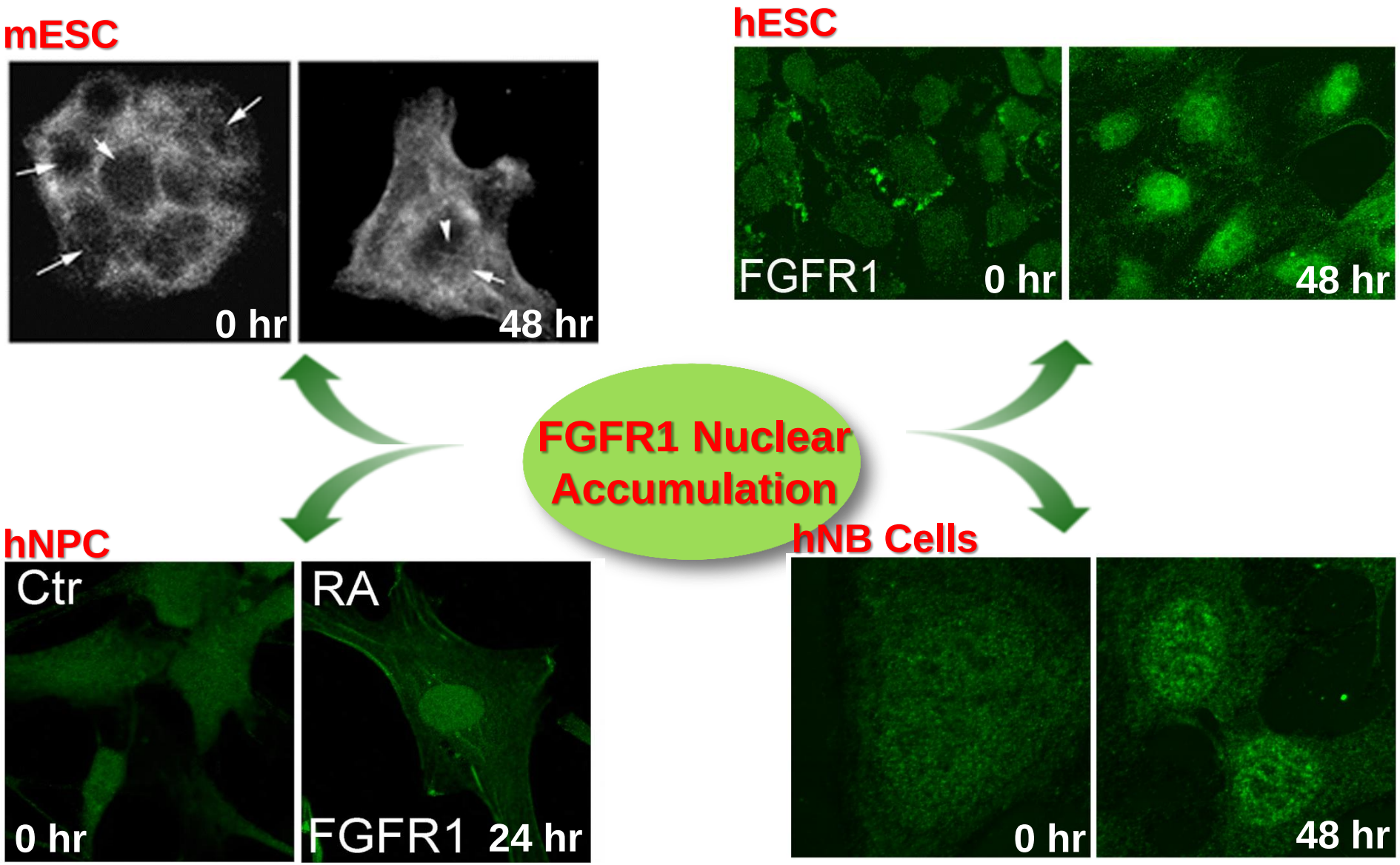
Yabut & Bernstein; Aging Volume 3, No. 5 2005

INFS in Pluripotent Embryonic Stem Cells



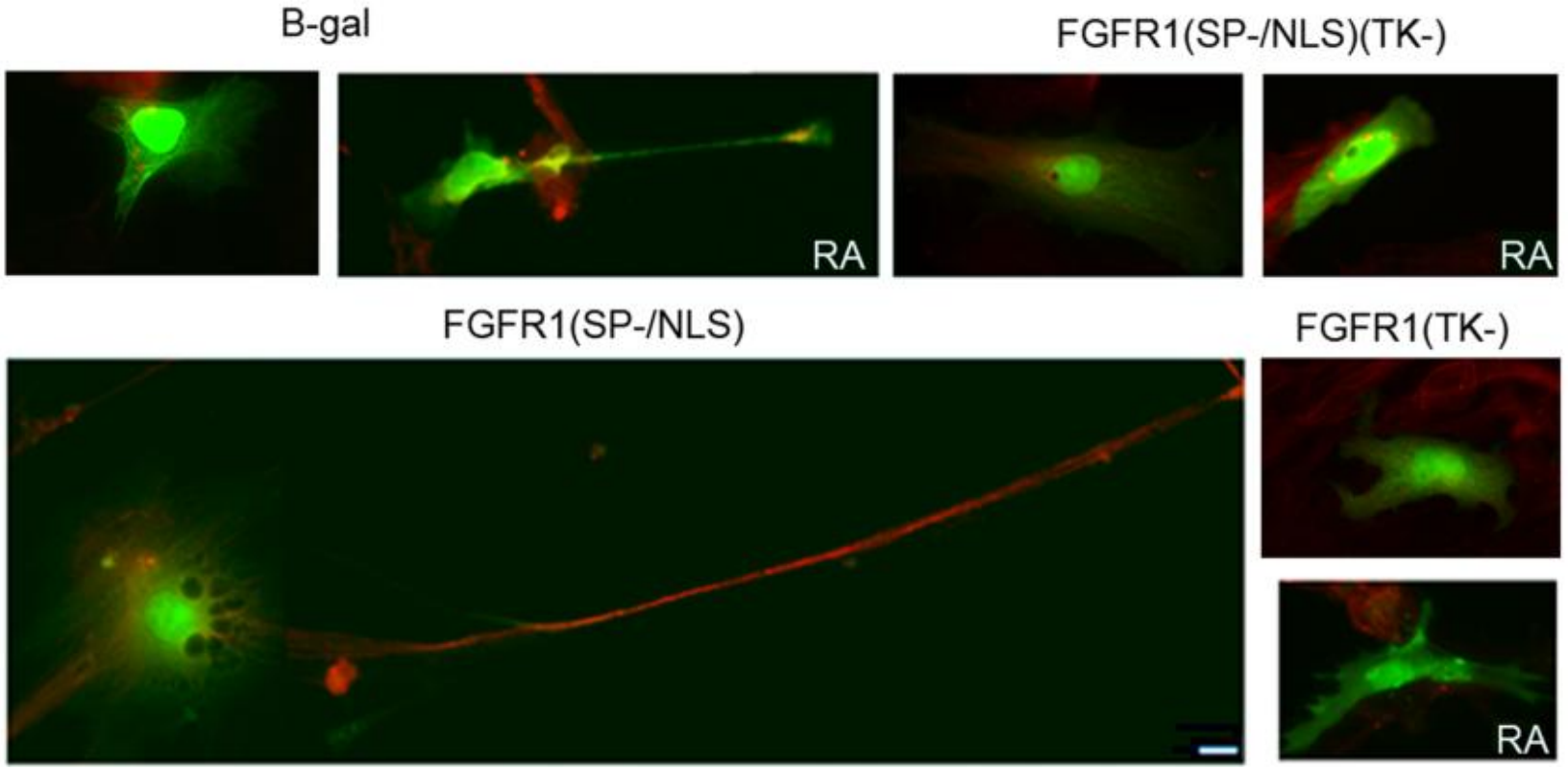
Yabut & Bernstein; Aging Volume 3, No. 5 2005

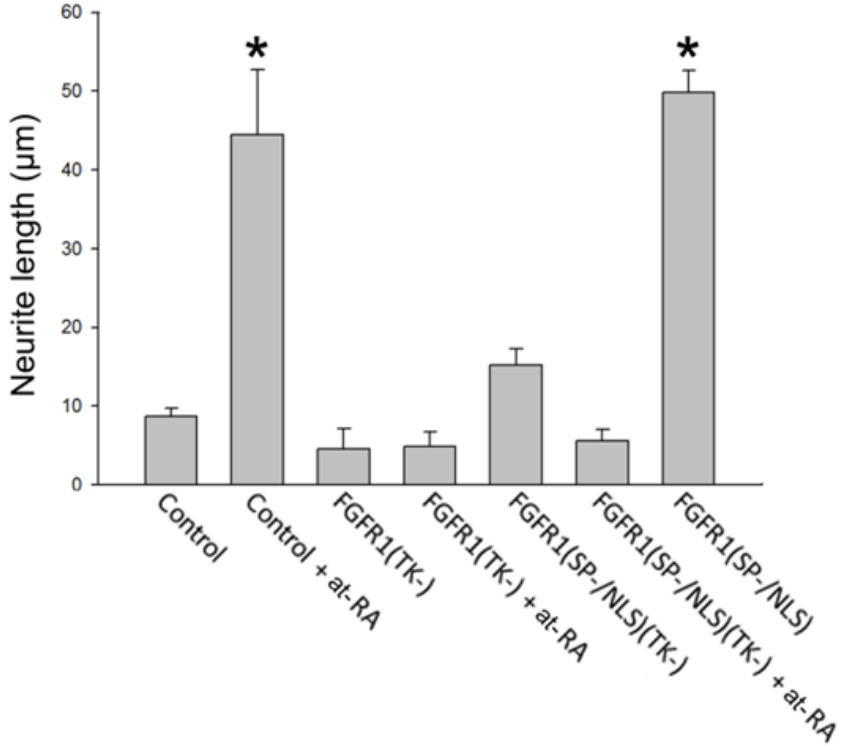
Nuclear Accumulation of FGFR1 is a common response to Retinoic Acid (RA) in Various Cells



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

nFGFR1 mediates RA-induced neuronal programing of pluripotent mESC

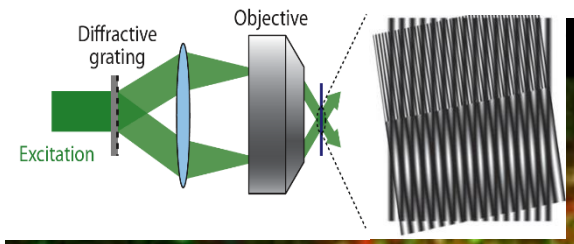




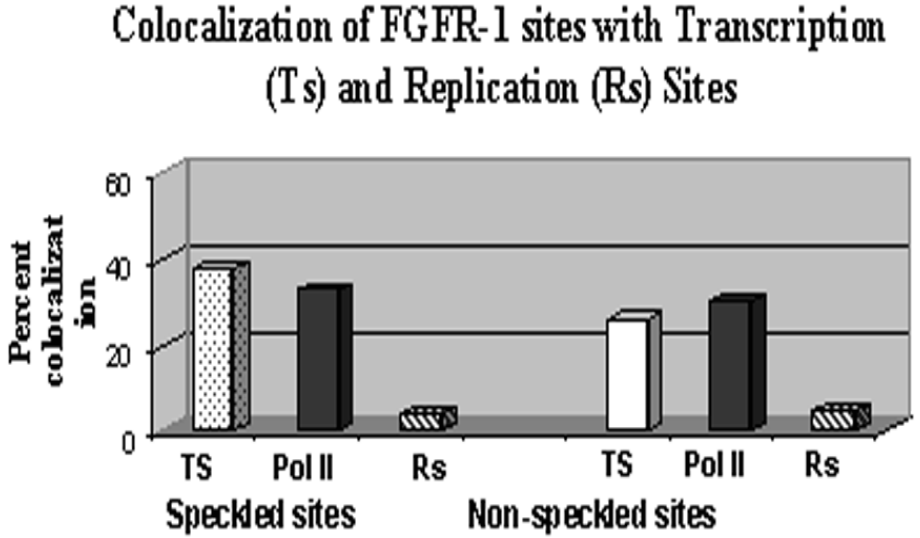
Lee, Y-Wet al., (2012). A novel nuclear FGF Receptor-1 partnership with retinoid and Nur receptors during developmental gene programming of embryonic stem cells J. Cell. Biochem., 113, 2920-2930

FGFR1 localizes within transcription domains

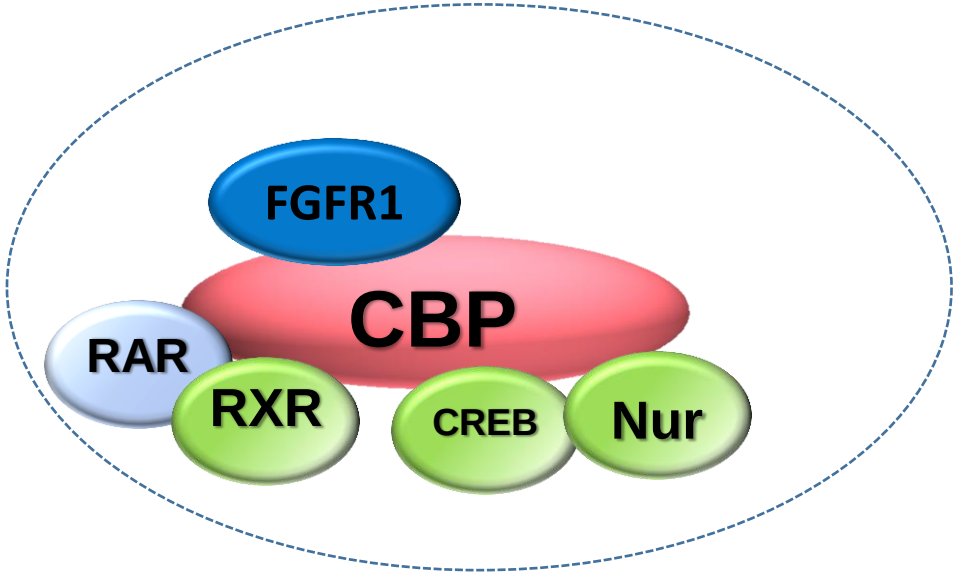
Structured-Illumination Microscopy (SIM)



FGFR1 RNA Polymerase II

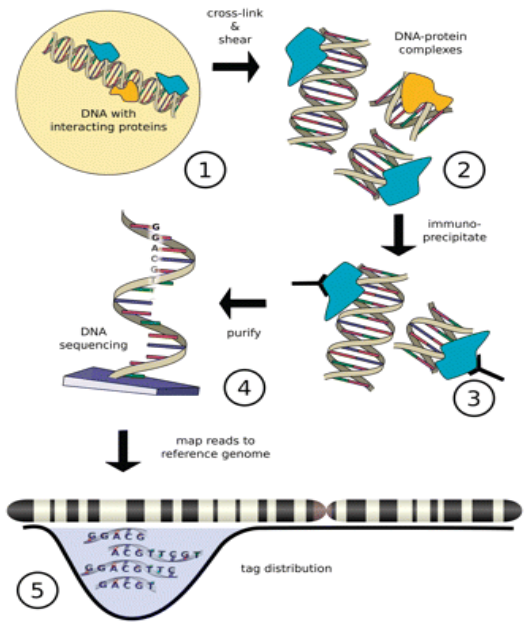


In multiprotein CBP complexes:



Insight into the whole genome !

Cross-linked Chromatin Immunoprecipitation, DNA sequencing (ChIPseq)





Illumina HiSeq 2000

RNA and small RNA sequencing (RNAseq)



cDNA synthesis

sequencing



Illumina HiSeq 2000

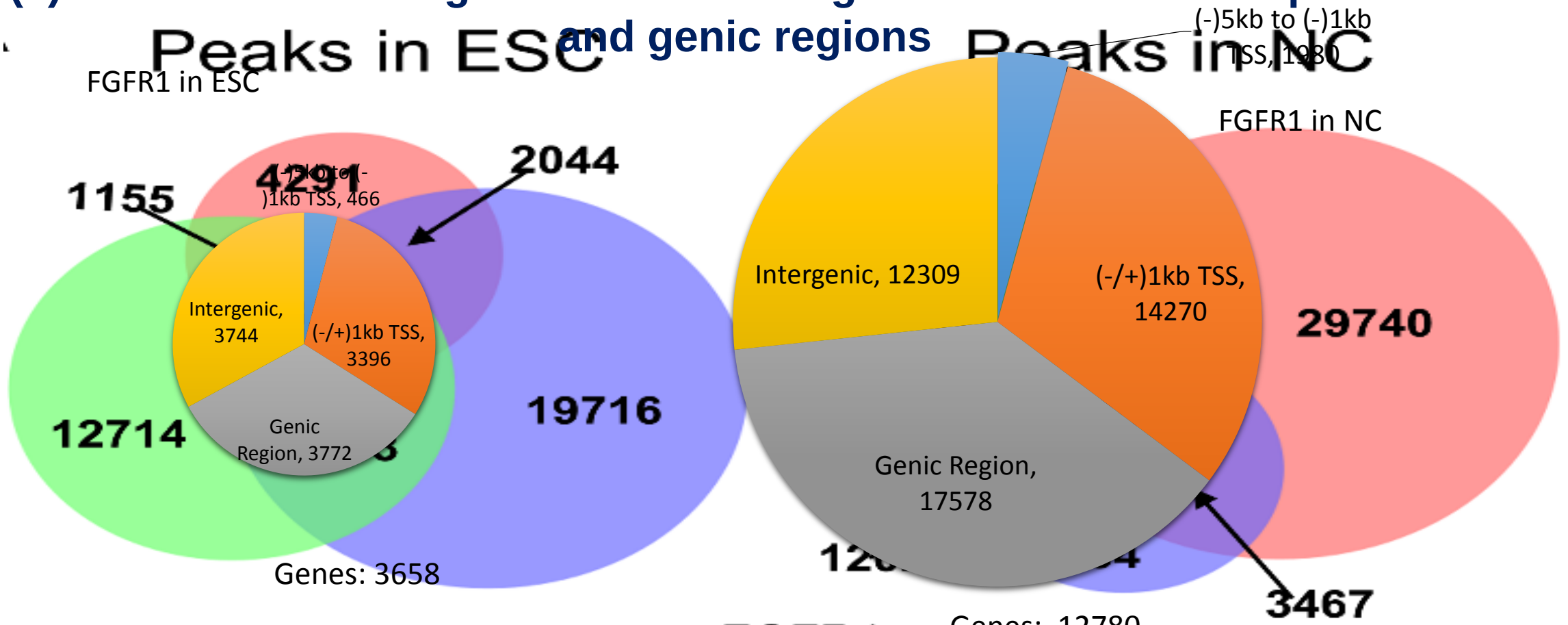
Global developmental gene programing involves a nuclear form of Fibroblast Growth Factor Receptor-1 (FGFR1). 2015, *PLOS One, in press*
*I*ntegrative paradigm for global developmental genome programing in embryonic stem cells by nuclear Fibroblast Growth Factor Receptor-1 (FGFR1) ISSCR, 2015
Utilizing induced Pluripotent Stem Cells (iPSCs) to delineate the neurodevelomental base of schizophrenia, ISSCR **2015.**

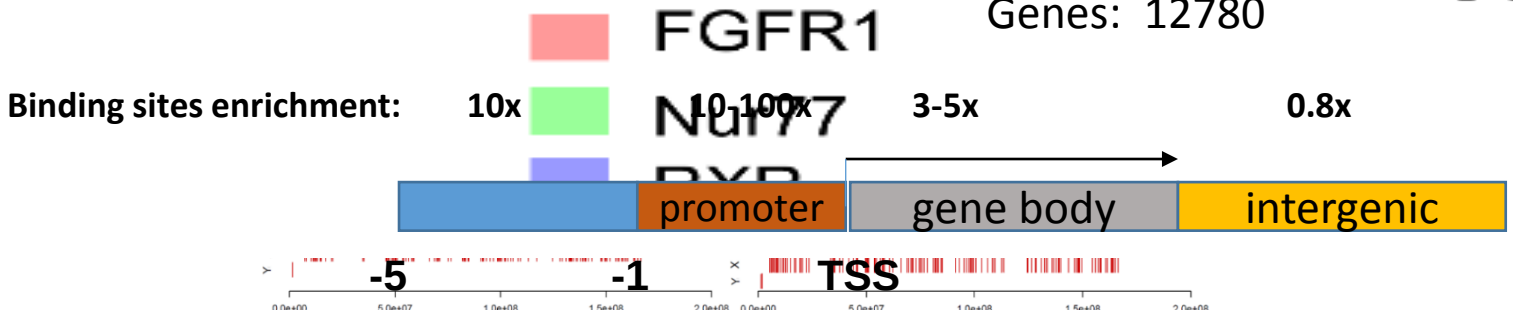
Lessons from ChIPseq:

(1) mESC FGFR1 its partners RXR and Nur77 largely bind [illegible]

(3) FGFR1 targets some but not all RXR and Nur77

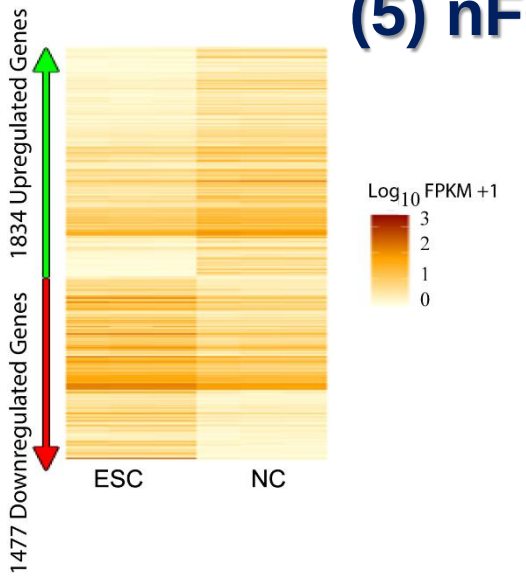
(4) In mESC and NC genomes 2/3 of targeted sites are within promoter and genic regions

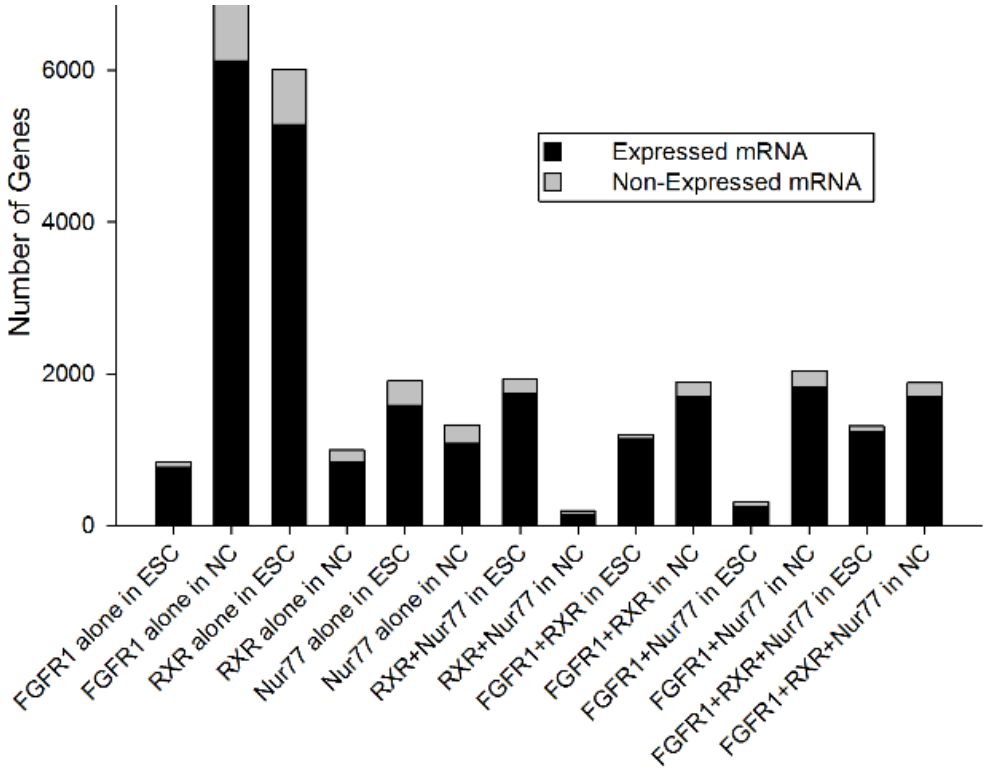


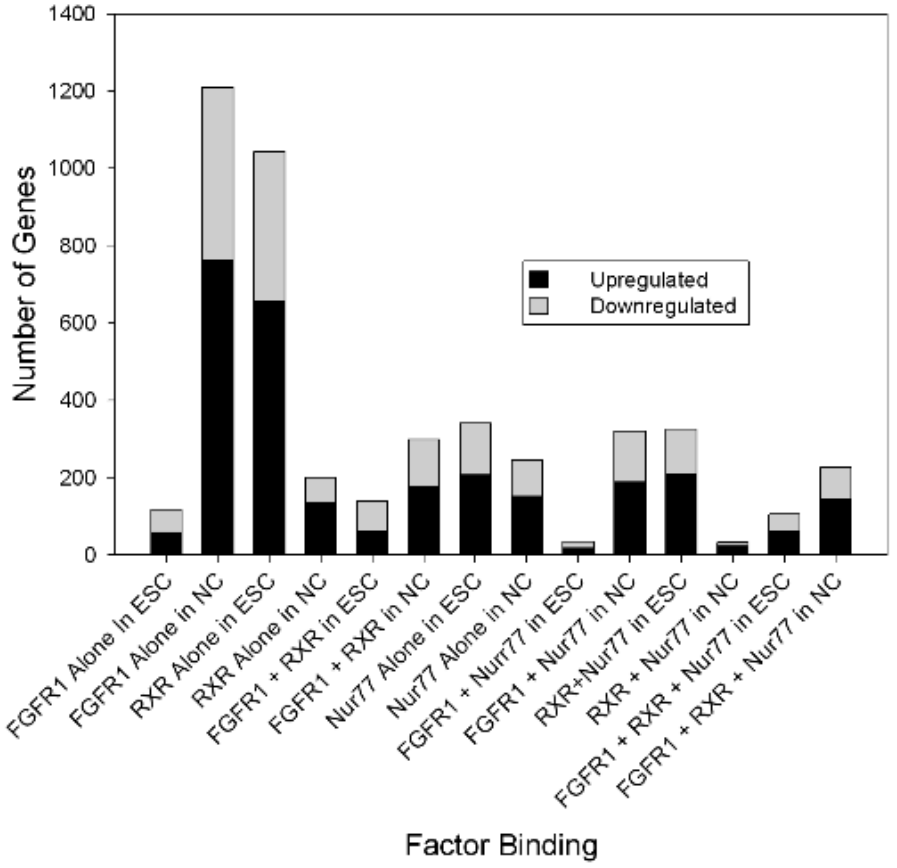


(5) nFGFR1, RXR and Nur77 bind predominantly to proximal promoters of expressed genes

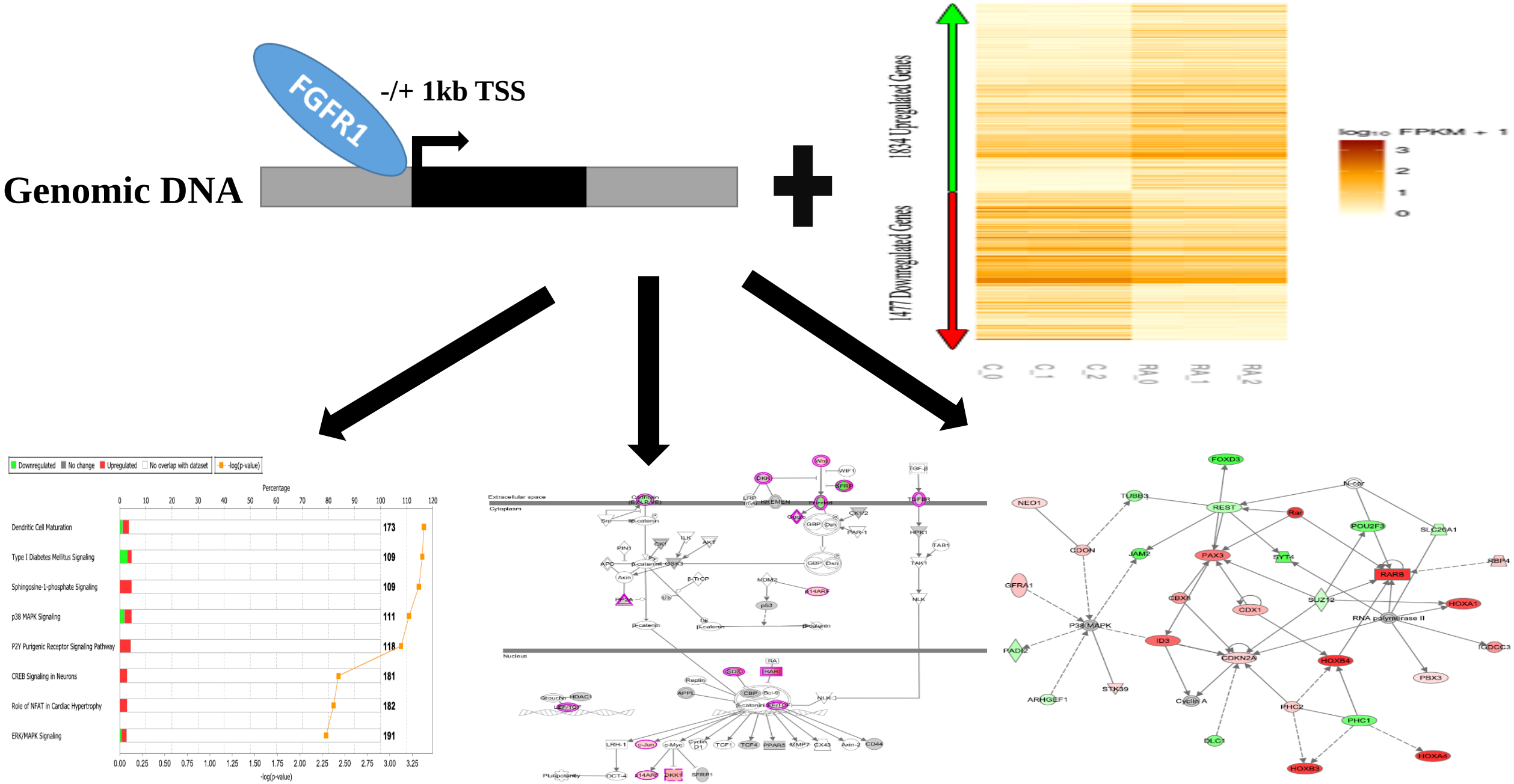
(ChIPseq+RNAseq)



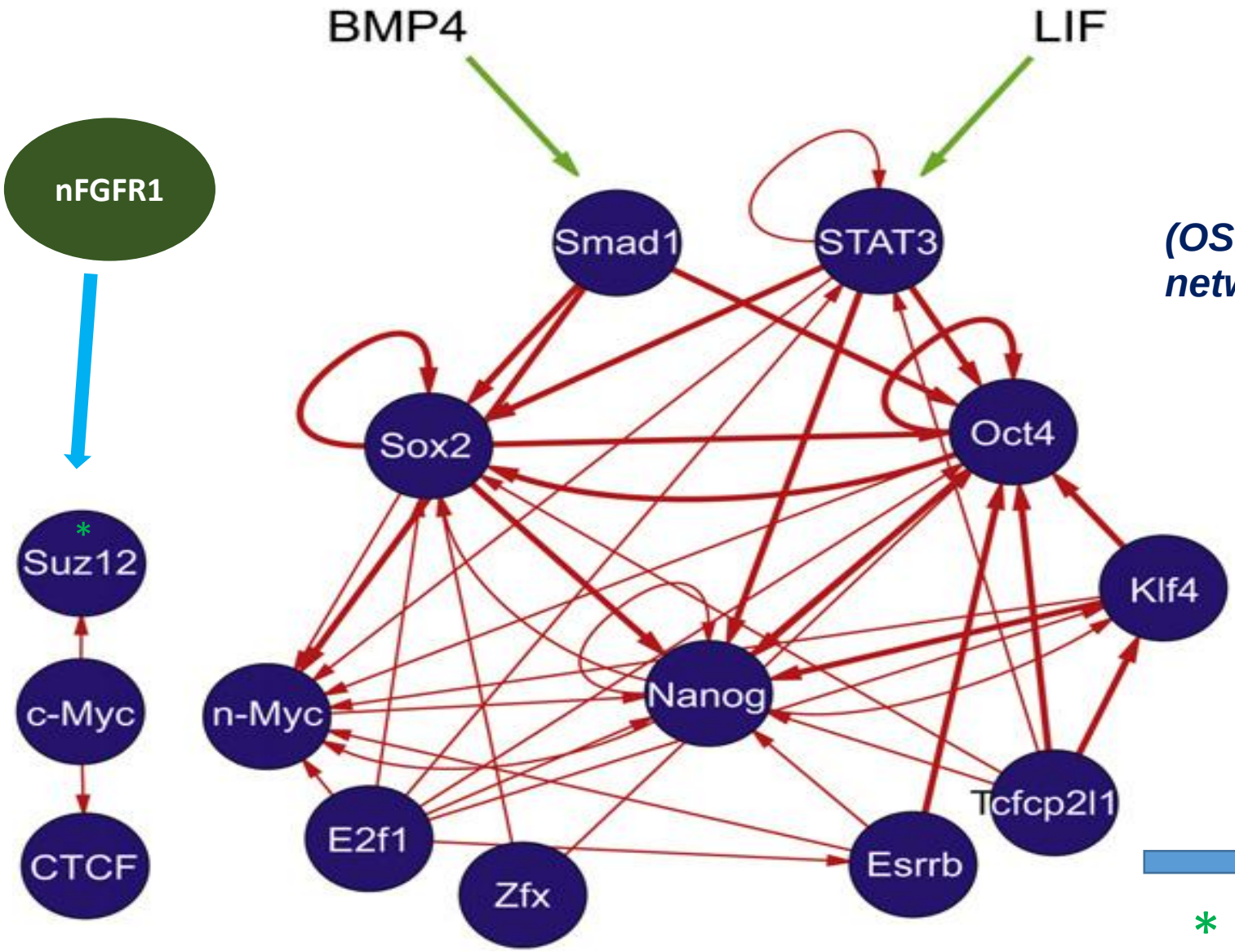




Ingenuity Pathway Analysis (IPA): Combination of ChIP- and RNA-seq

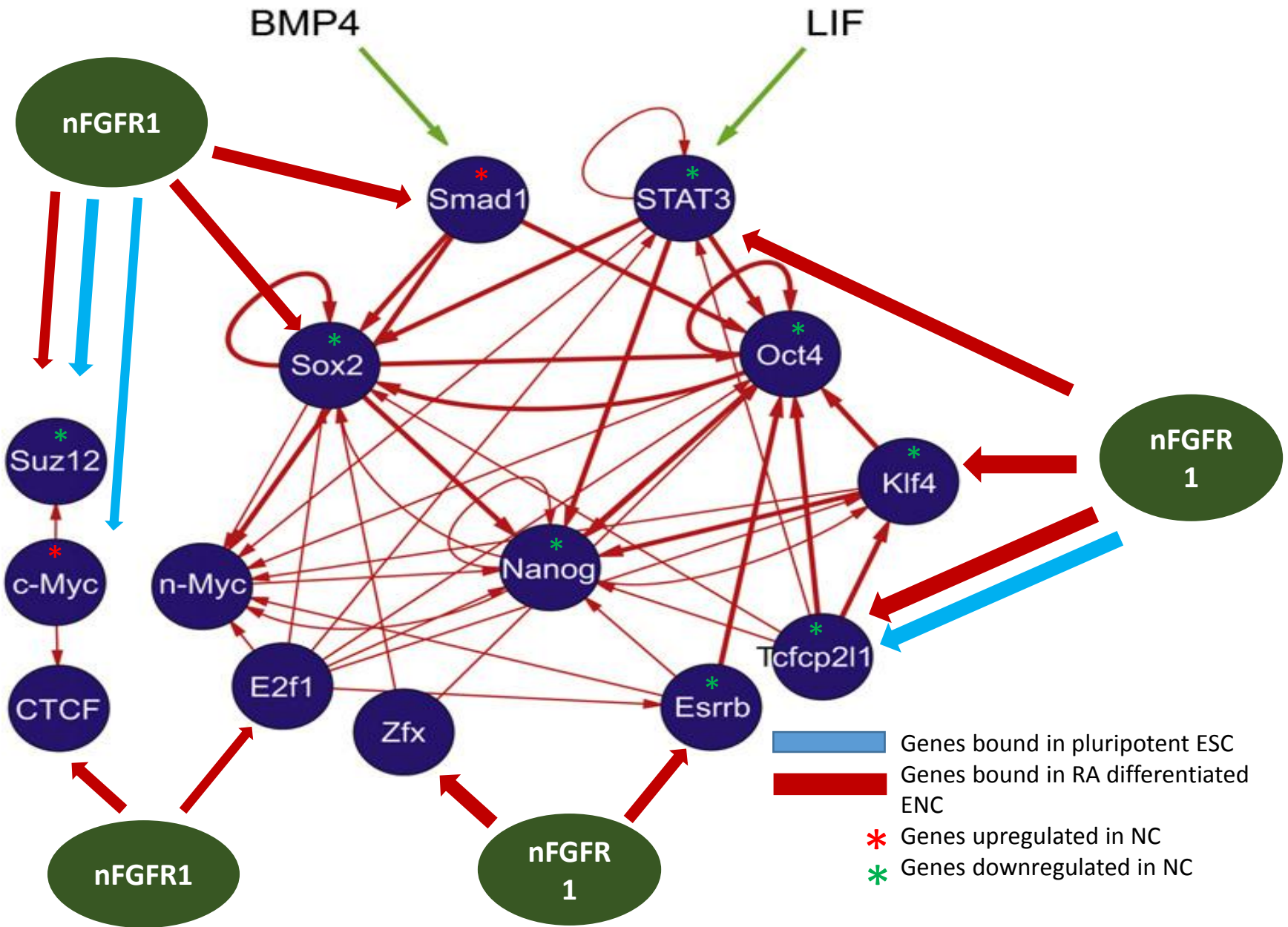


In ESC nFGFR1 controls Pluripotency transcriptional network by targeting Suz12



(OSKM) Oct4, Sox2, Klf4, Myc network

nFGFR1 turns off pluripotency transcriptional network during Retinoic Acid (RA) induced ESC differentiation



nFGFR1 targets genes of the cell proliferation and survival pathways

Top biological functions, networks and diseases:

A

Molecular and Cellular Functions

Name	P-Value	# of Molecules
Cellular Growth and Proliferation	6.20E-05 – 2.15E-02	28
Cell Death and Survival	1.12E-04 – 2.18E-02	29
Cell Cycle	1.12E-04 – 1.18E-02	12
Cellular Development	1.32E-04 – 2.18E-02	33
DNA Replication, Recombination, and Repair	7.66E-04-1.84E-02	8

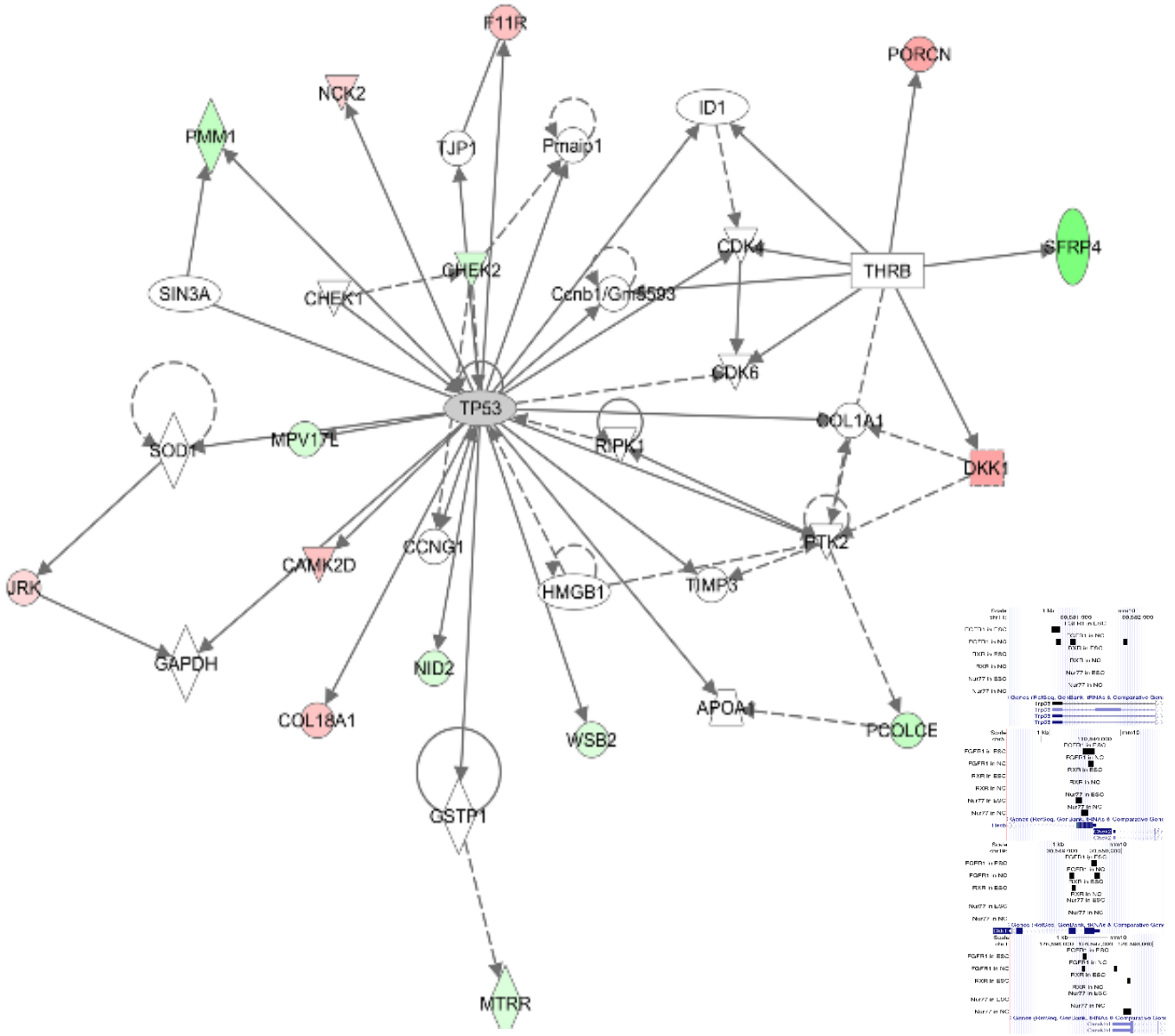
Physiological System Development and Function

Name	P-Value	# of Molecules
Organismal Survival	6.20E-05 – 2.15E-02	32
Tissue Morphology	1.15E-04 – 1.84E-02	29
Embryonic Development	3.21E-04 – 2.18E-02	22
Organismal Development	3.21E-04 – 2.18E-02	25
Nervous System Development and Fusion	5.55E-04 – 1.84E-02	24

Diseases and Disorders

Name	P-Value	# of Molecules
Cancer	9.28E-05 – 2.15E-02	26
Dermatological Diseases and Conditions	1.05E-04 – 1.84E-02	9
Organismal Injury and Abnormalities	1.12E-04 – 1.84E-02	15
Reproductive System Disease	1.12E-04 – 1.84E-02	6
Hematological Disease	5.19E-04 – 1.84E-02	9

Top network controlling cell proliferation and survival:

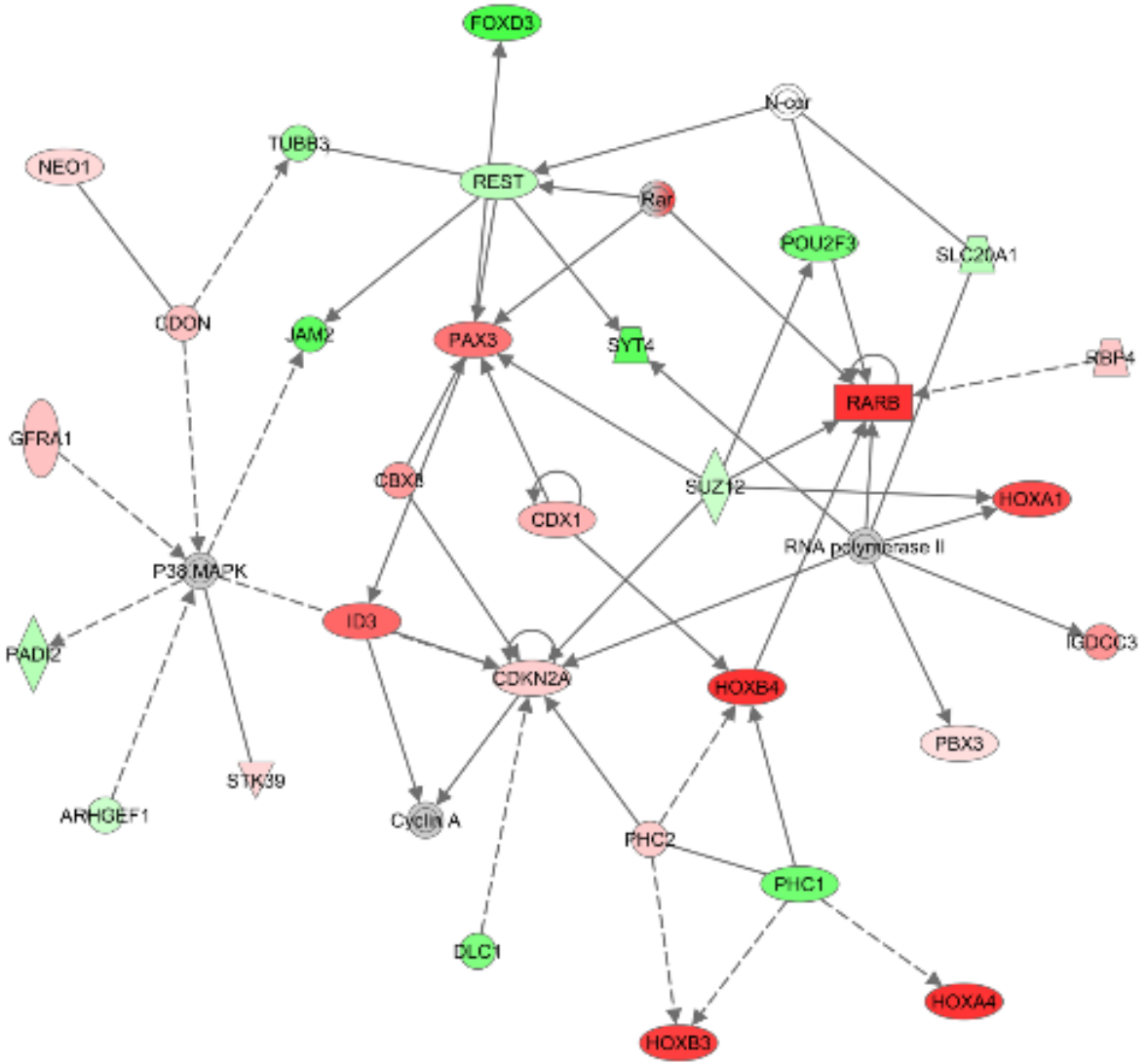


Building body parts and axes:

nFGFR1 mediates RA activation of the Homeobox genes (*Hox* clusters genes)

E

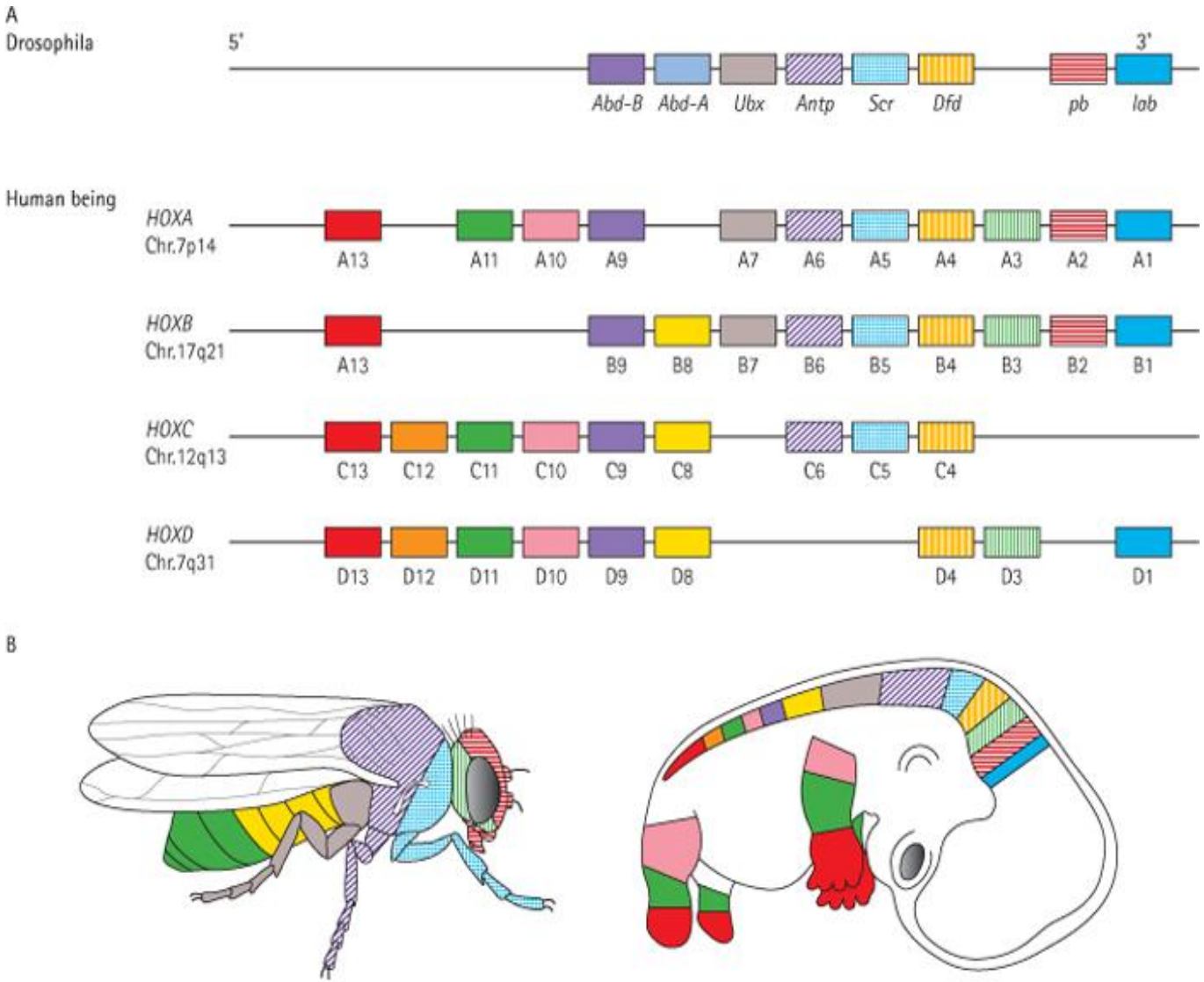
Name	P-Value	# of Molecules
Embryonic Development	2.33E-15 - 3.35E-03	271
Organismal Development	2.33E-15 - 3.35E-03	385
Organismal Survival	8.15E-14 - 6.81E-04	291
Cardiovascular System Development and Function	1.60E-11 - 3.23E-03	167
Nervous System Development and Function	7.10E-10 - 3.29E-03	251



RED: UPREGULATED

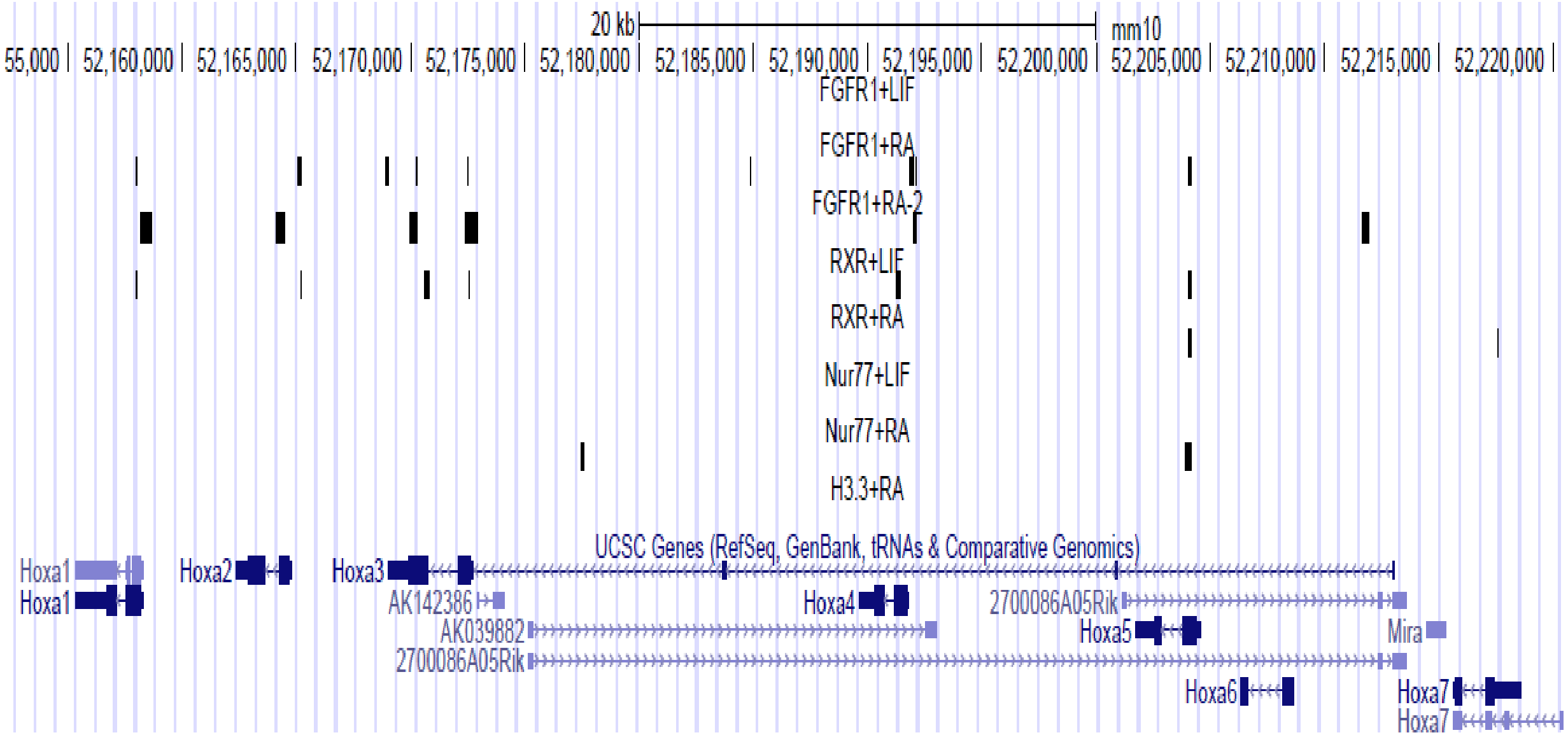
GREEN: DOWNREGULATED

Homeobox (Hox) genes are critical for organism development and patterning - including CNS

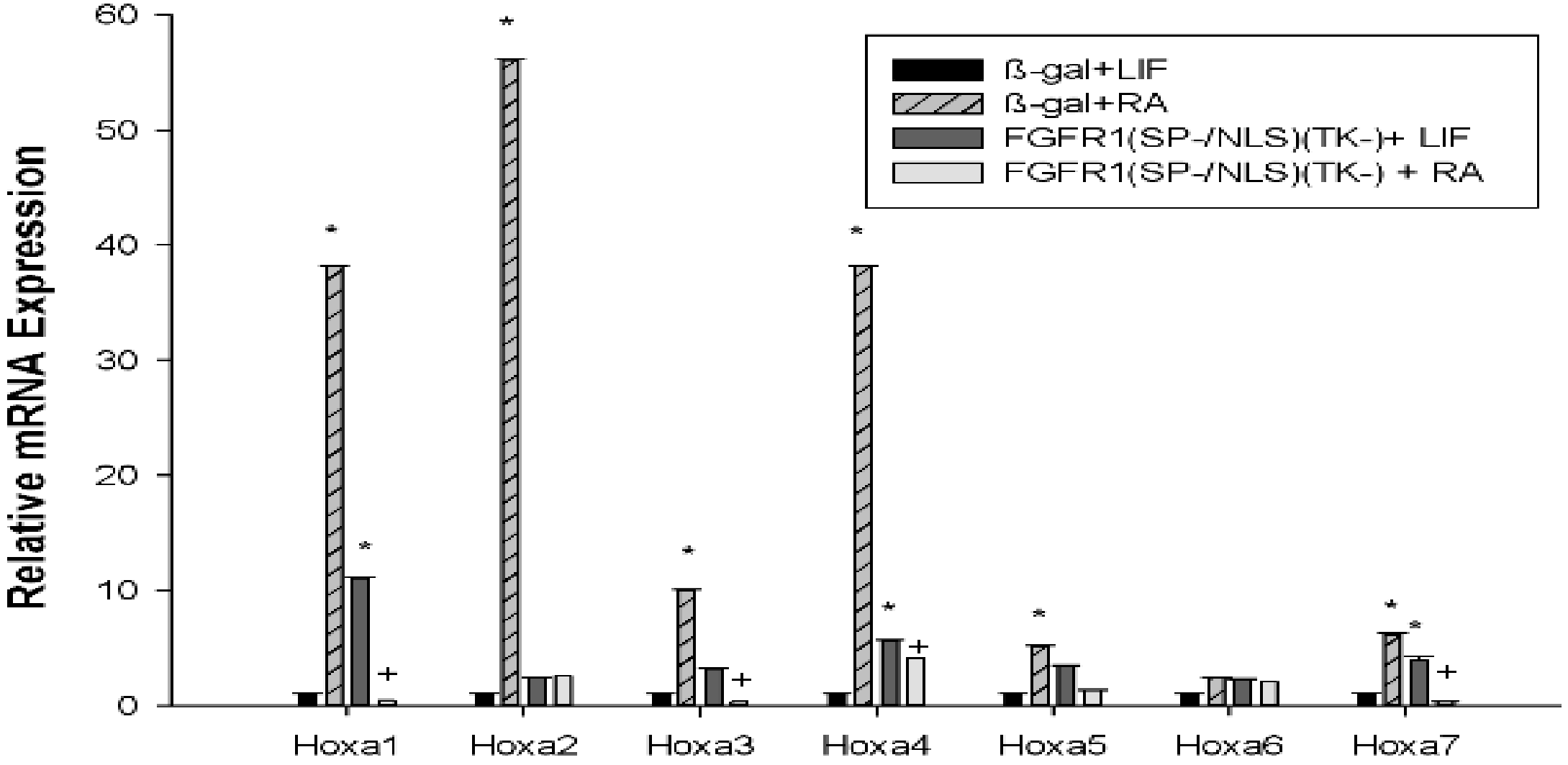


© Elsevier. Turnpenny & Ellard: Emery's Elements of Medical Genetics 13e - www.studentconsult.com

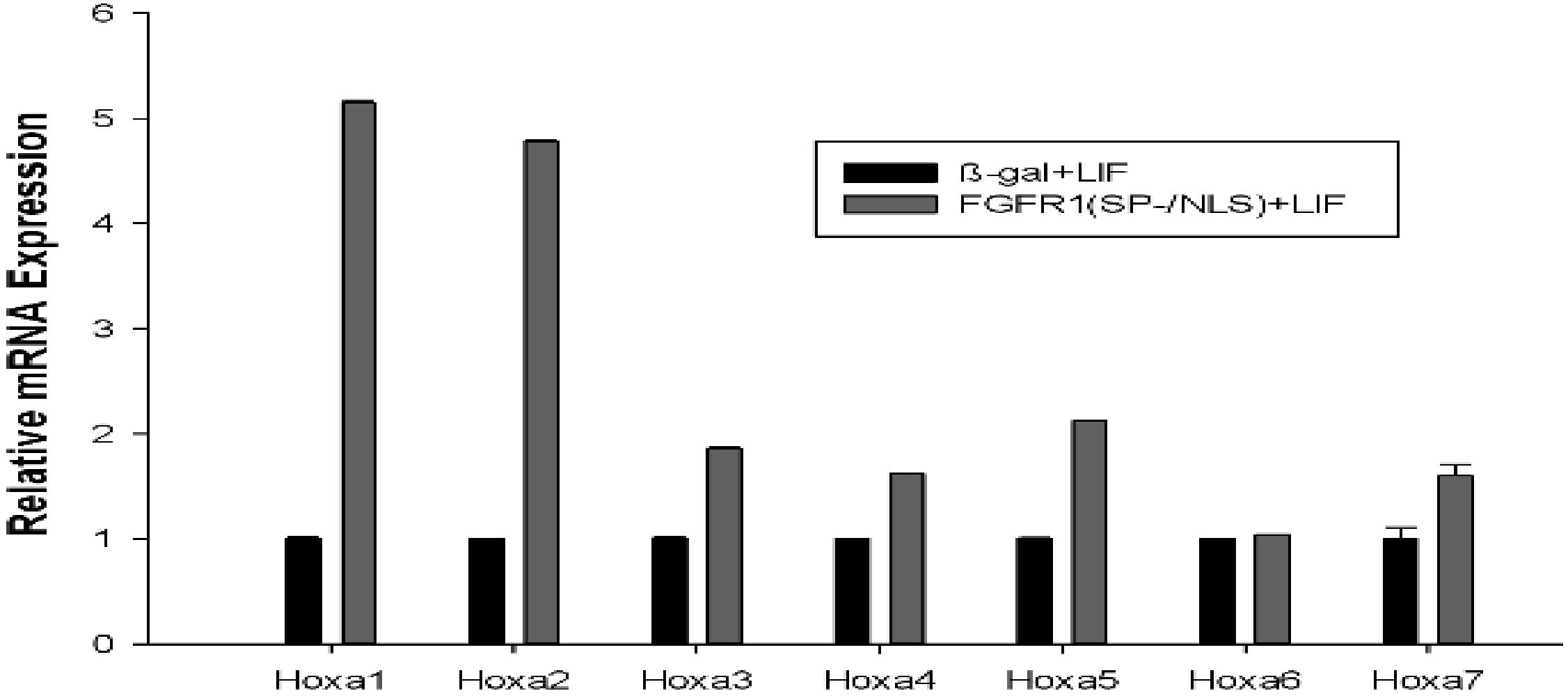
Nuclear FGFR1 binding to 3’ Hoxa gene cluster in the presence of RA



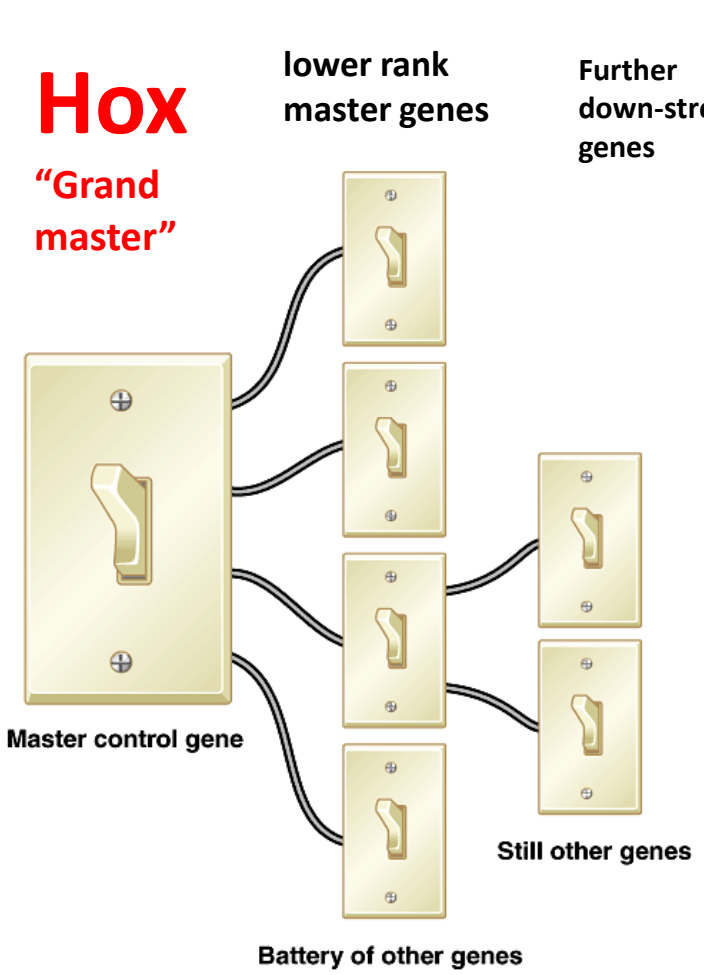
Dominant negative nuclear FGFR1 (NLSTK-) blocks RA upregulation of core 3' Hoxa genes



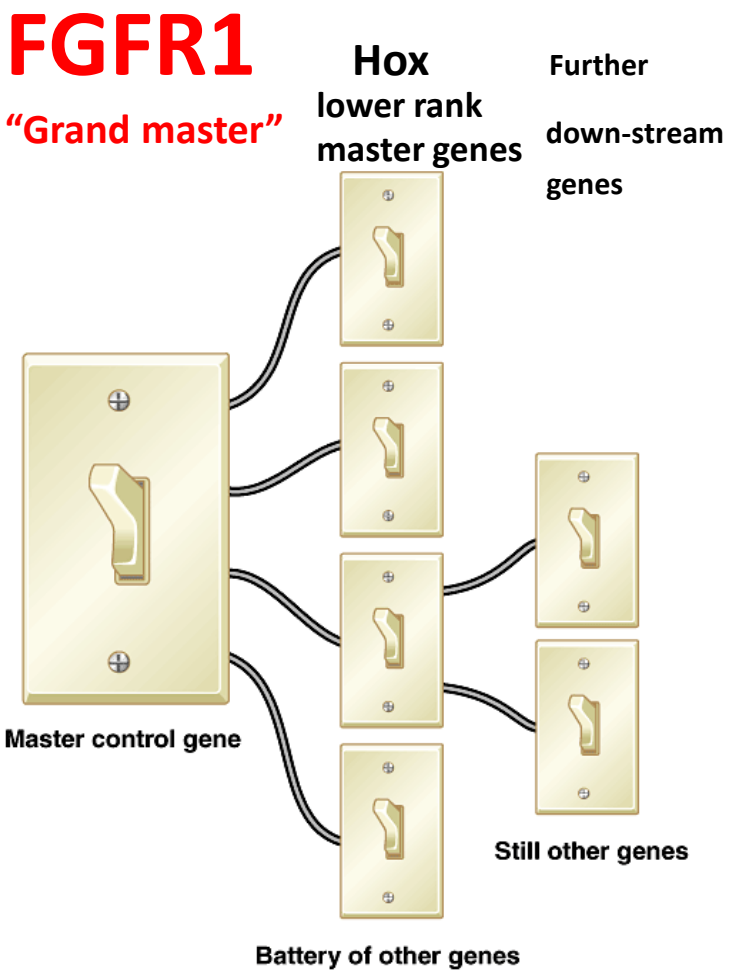
Nuclear active nuclear FGFR1 (NLS) upregulates core 3' Hoxa genes in the absence of RA



Change in the Hierarchy of Master Genes



Old

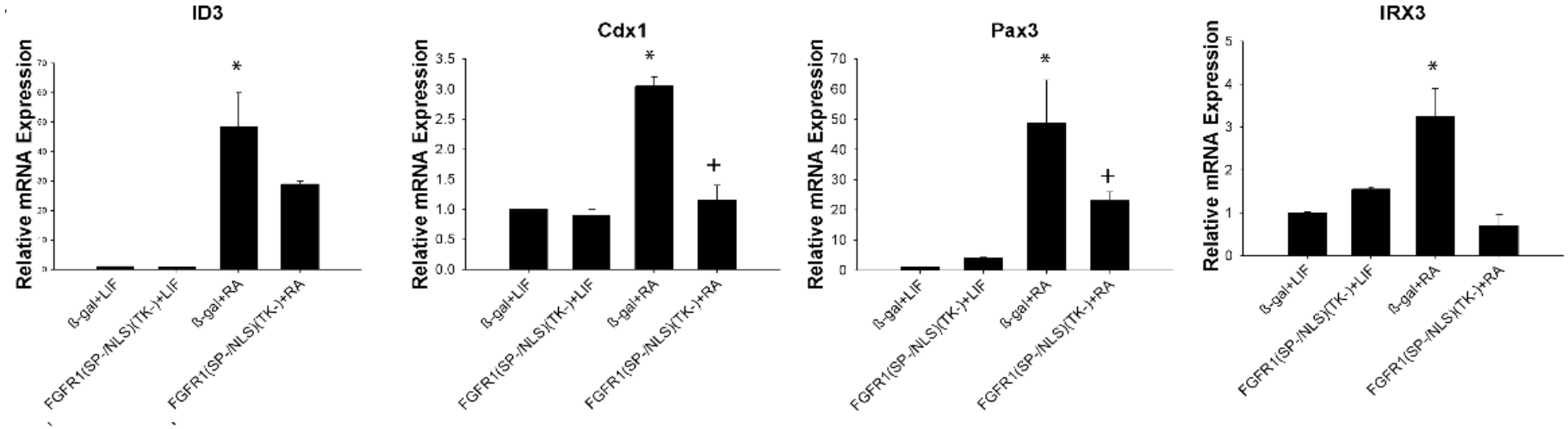


New

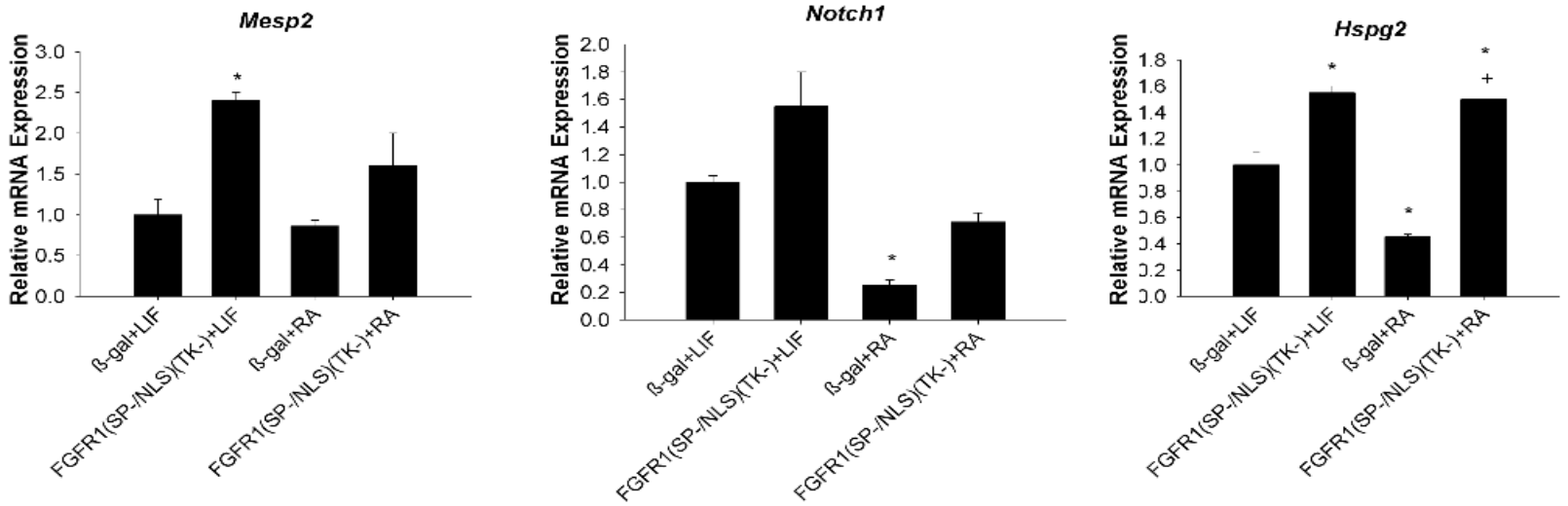
Decisions – brains or muscles?

nFGFR1 upregulates neuronal and downregulates mesodermal genes:

Neuronal genes:

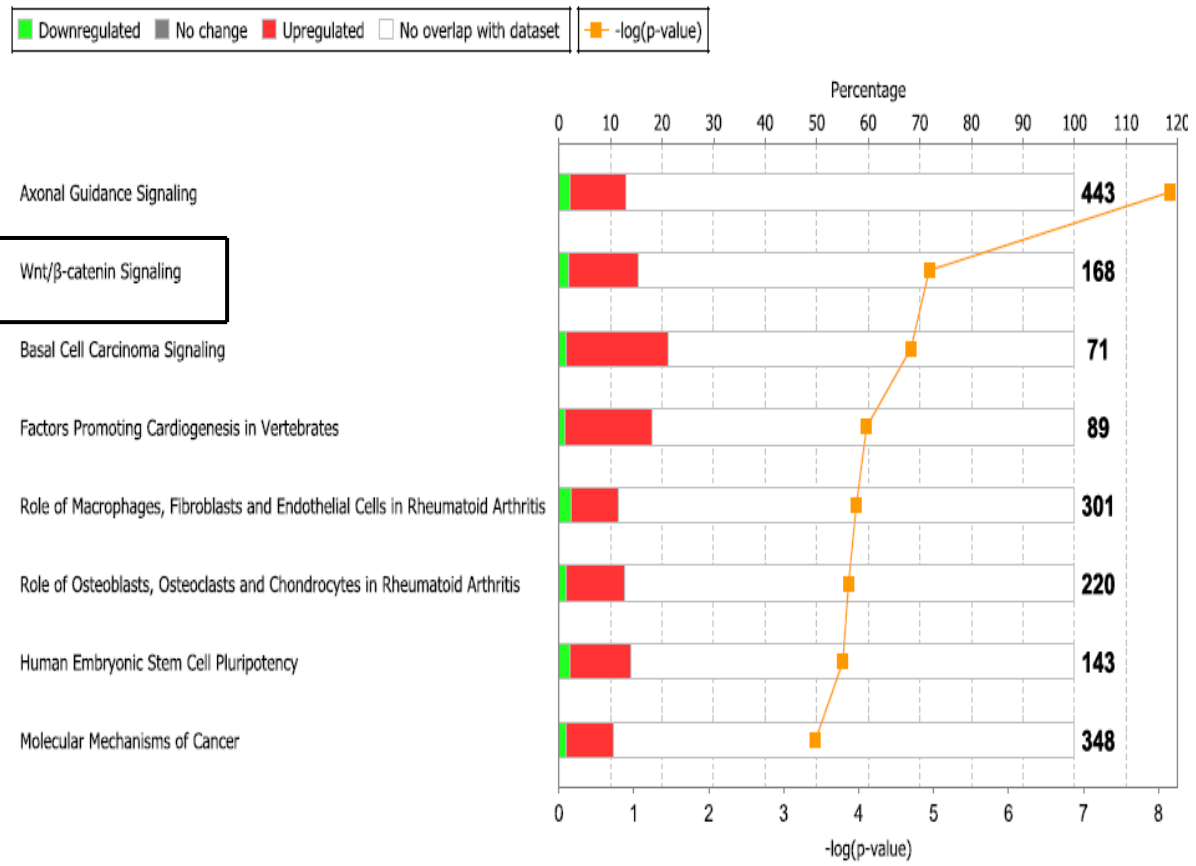


Mesodermal genes:

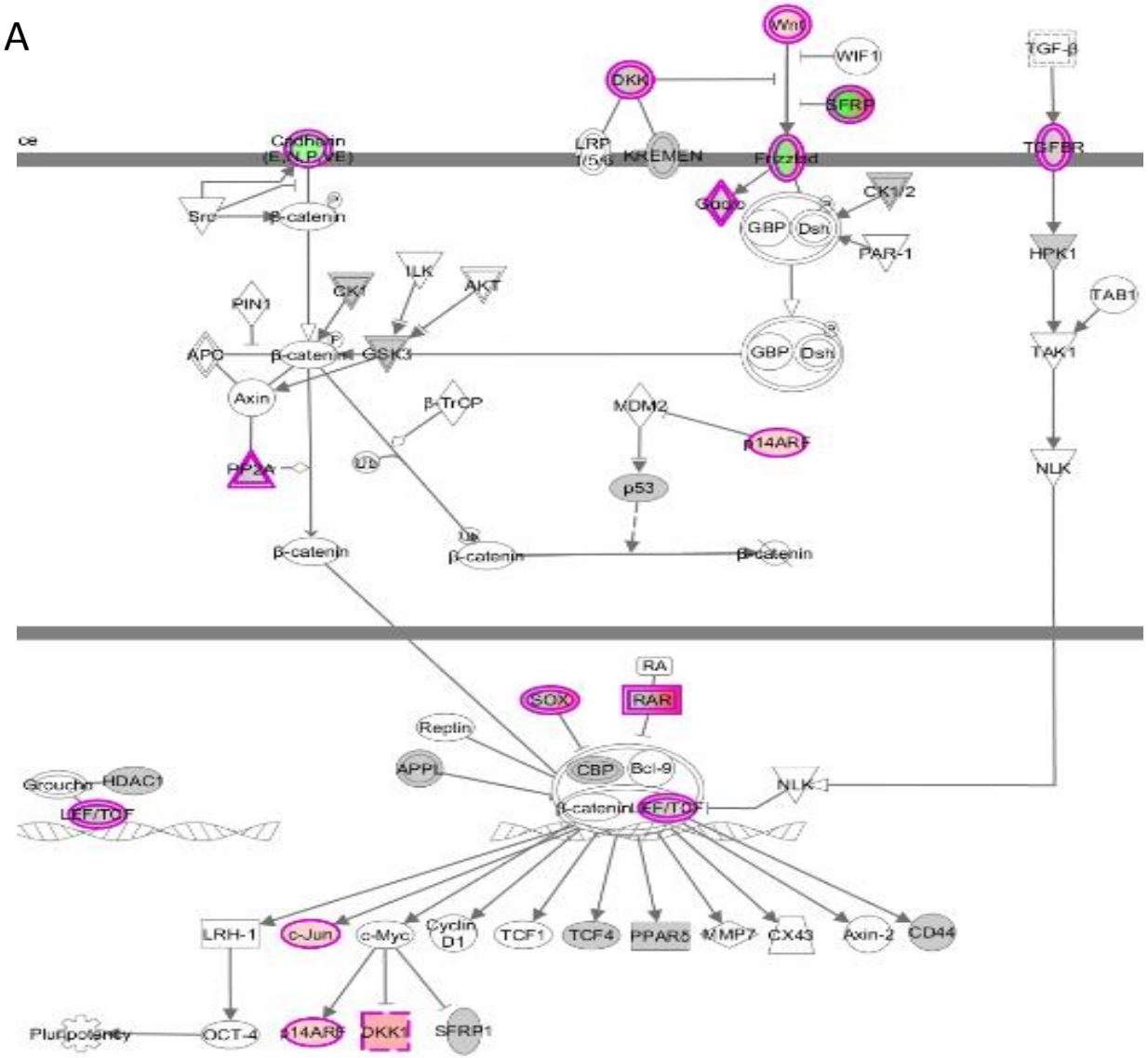


Building Brains - nFGFR1 targets:

Activated Wnt/β-catenin signaling pathways in NCs

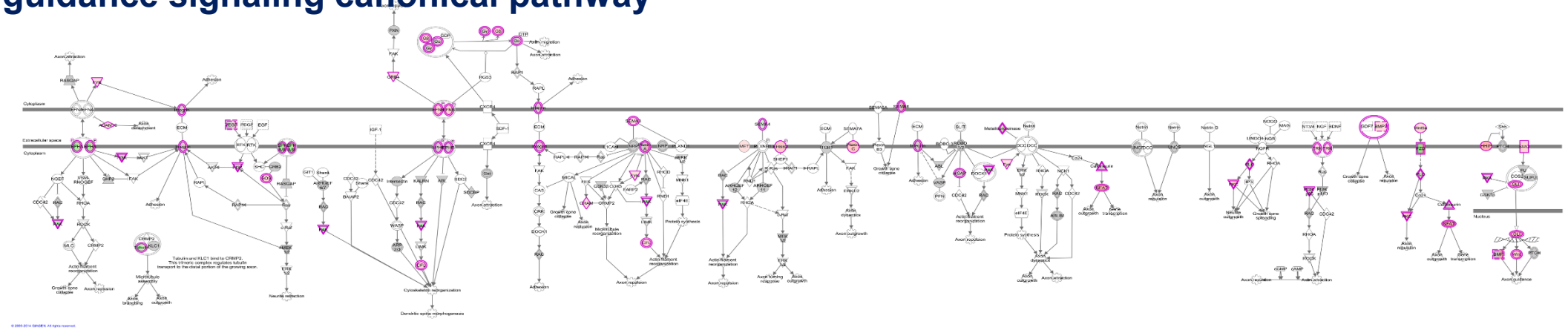


A

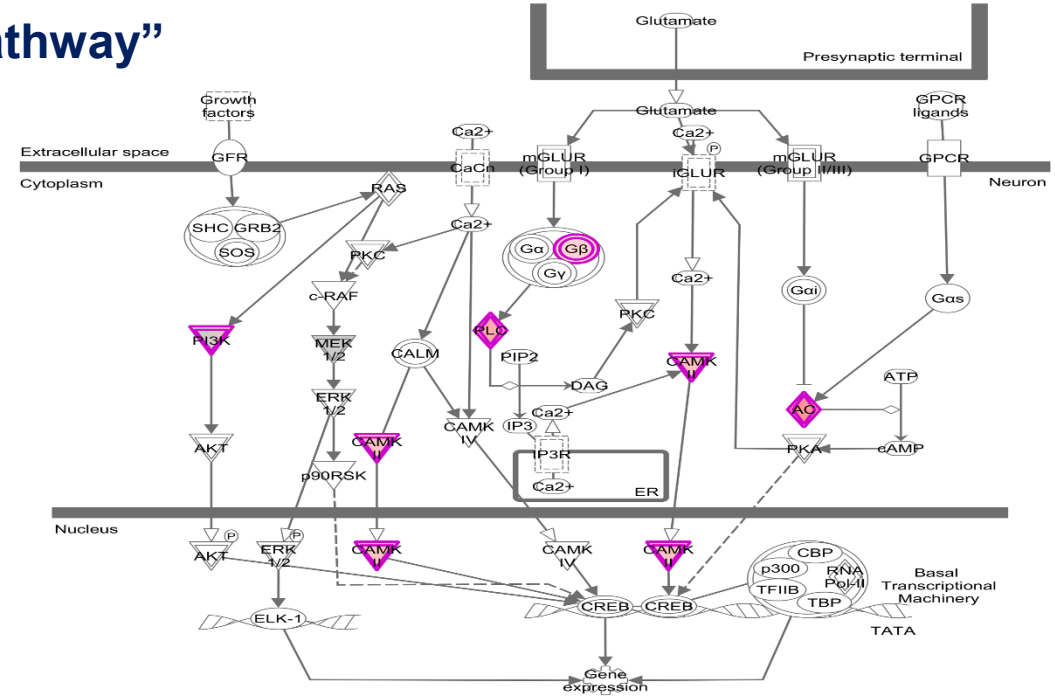


Building Brains - nFGFR1 targets:

“Axonal guidance signaling canonical pathway”



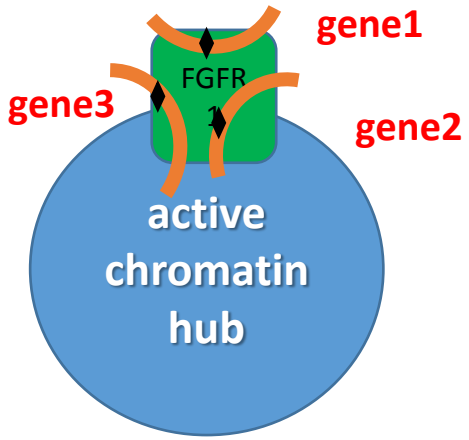
“CREB signaling in neurons pathway”

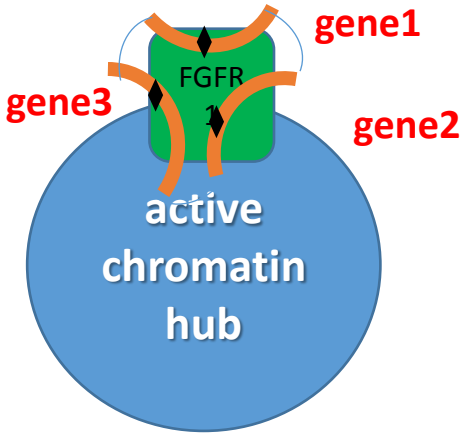


How to coordinate regulation of the thousands of genes?

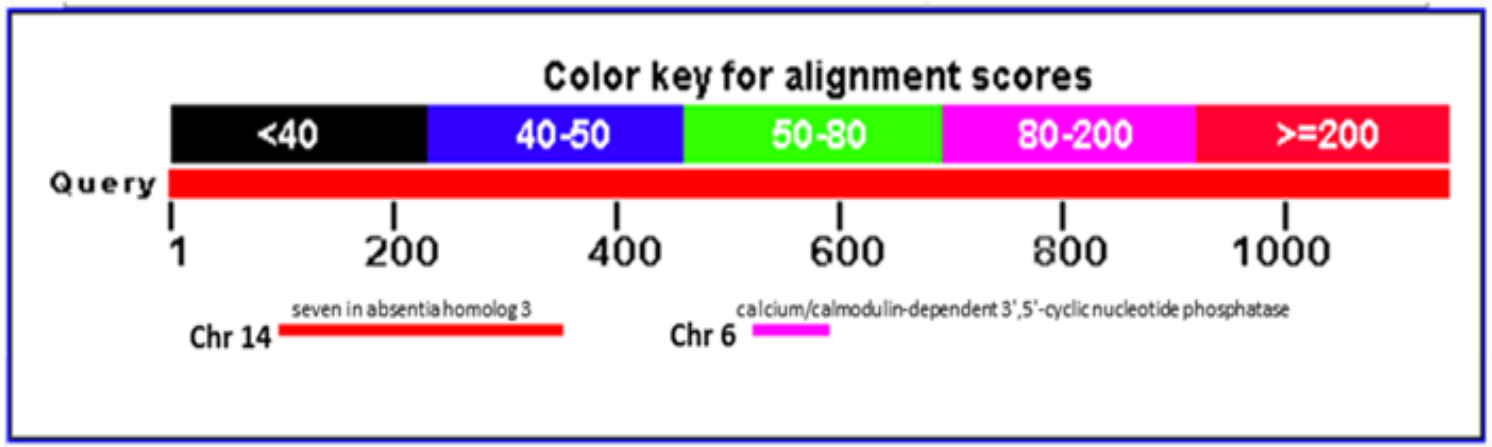
Role of multi-gene chromatin hubs "Transcriptional factories":

Chromatin Conformation Capture – 6C analysis

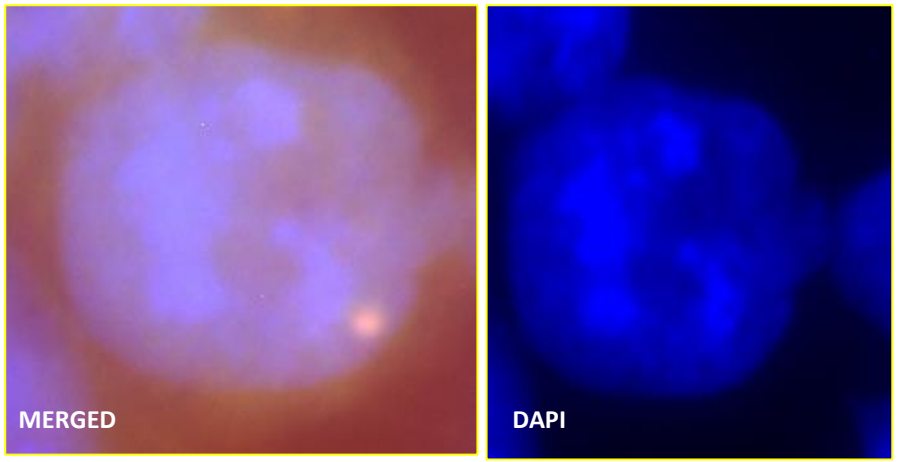


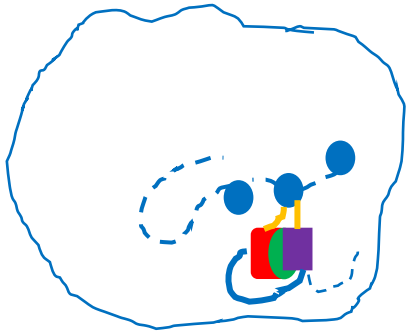


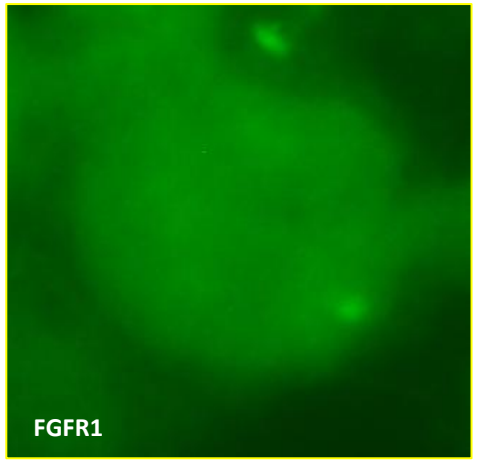
Results 6C: Gene Regions Identified

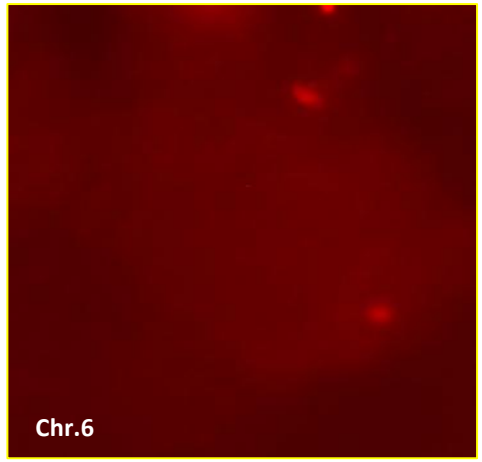


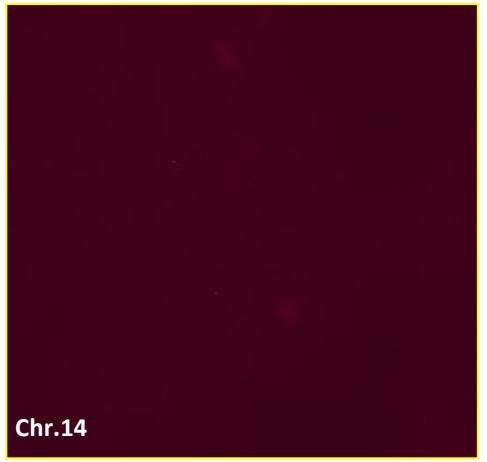
In Situ Hybridization/Immunocytochemistry verifies interchromosomal interactions involving nFGFR1











RA #14

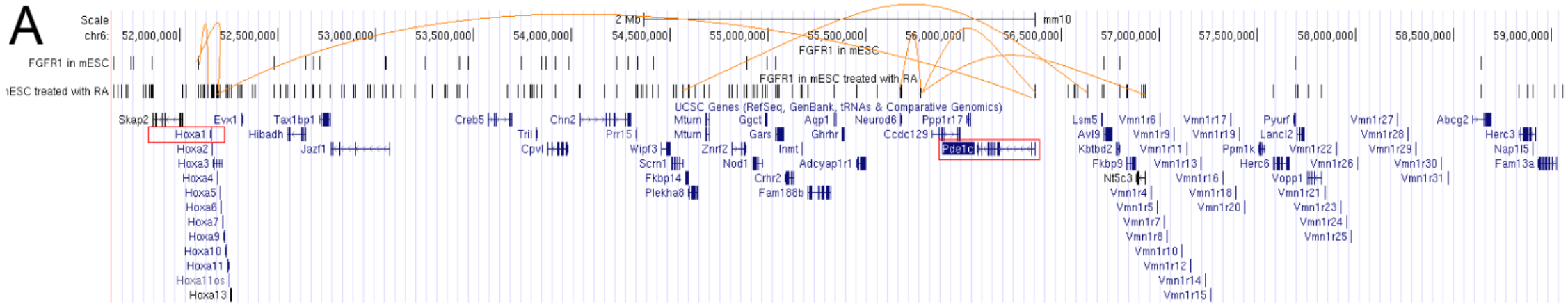
Chromosome 6-- 5' Alexa Fluor 546

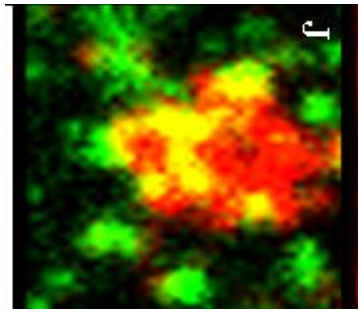
chromosome6-- 14' Alexa Fluor 546

FGFR1 –Alexa 488

Looping interactions within intra-chromosomal (Chr. 6) loci:

Interactions of *pde1c* (red arches) and *hoxA1* (orange arches).

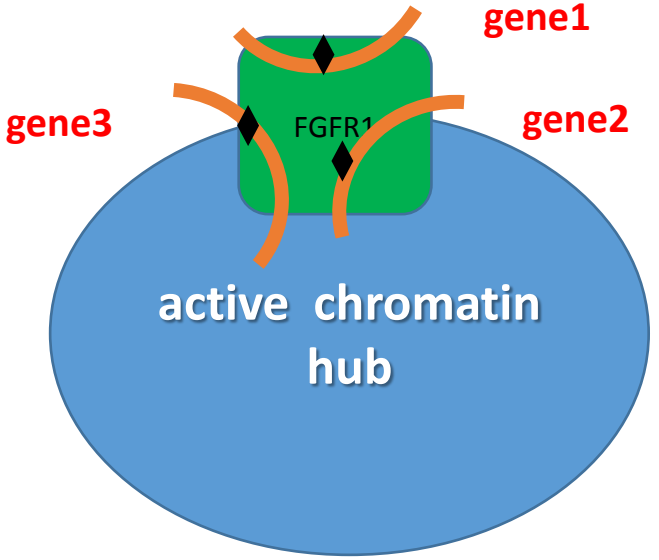




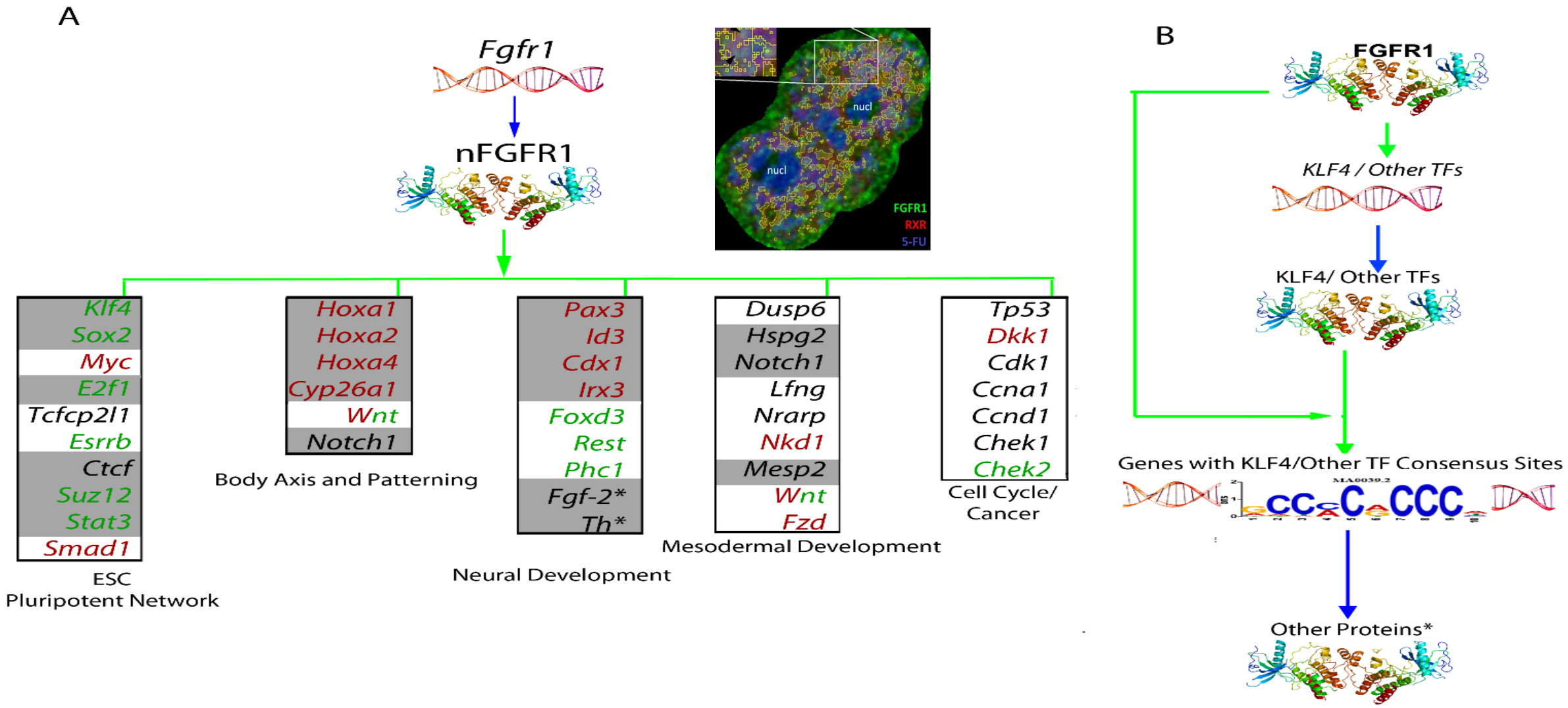
FGFR1 and RNA Polymerase II in shared nuclear domains

0.4 – 2.0 um speckle

nFGFR1 forms multi-gene chromatin hubs "Transcriptional factories":

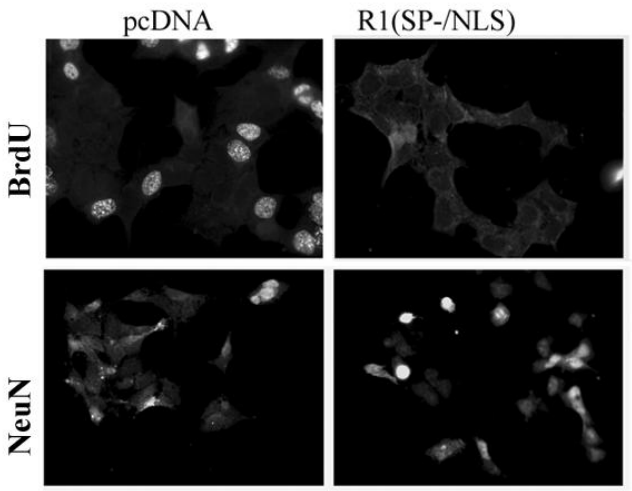


Evidence based theory of ontogeny – Nuclear FGFR1 a new type master gene regulator



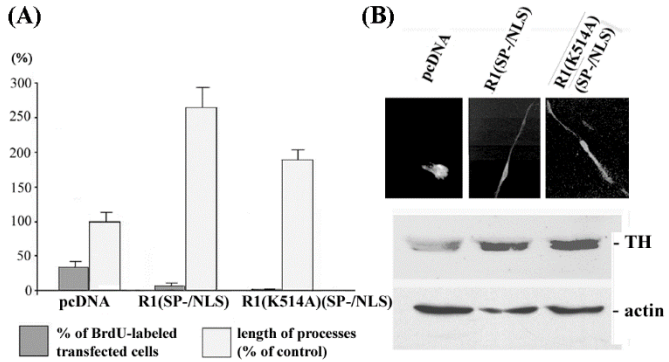
Global developmental gene programing involves a nuclear form of Fibroblast Growth Factor Receptor-1 (FGFR1). 2015, *PLOS One, in press*

Human Neuroblastoma



Mol. Biol. Cell, 2009, 20, 2401 – 2412.

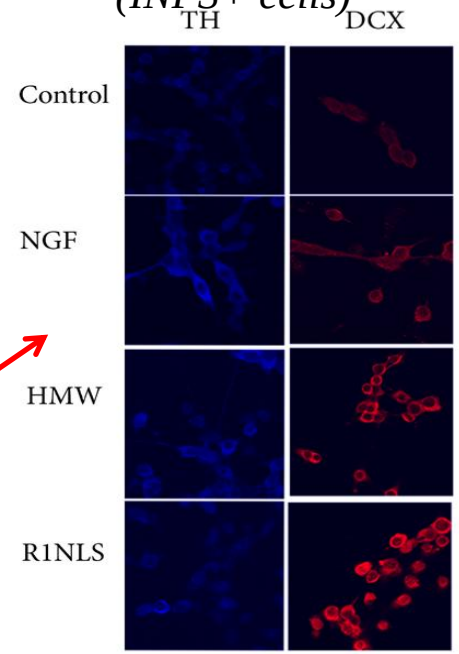
Human Medulloblastoma (TE671)



J. Biol. Chem. – 280(31):28451-62.

Rat pheochromocytoma (PC12)

(INFS+ cells)



PloS one. 2013, 8:e68931

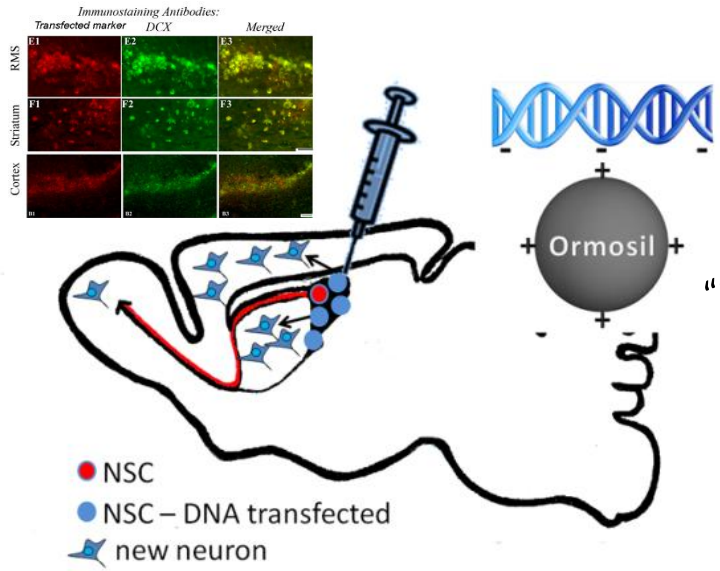
FGFR1(SP-/NLS)

FGFR1(SP-/NLS)

differentiates cancer cells

"Therapeutic" Targeting of INFS

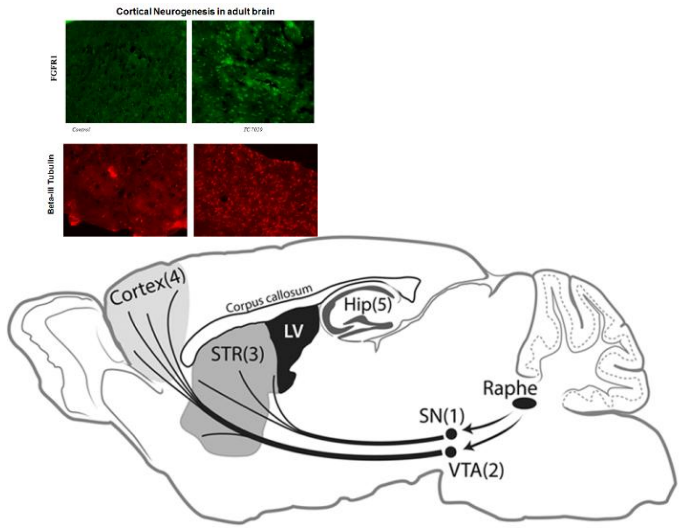
New neurons in adult brain



"Gene therapy"

FGFR1(SP-/NLS)

α7 Nicotinic agonist

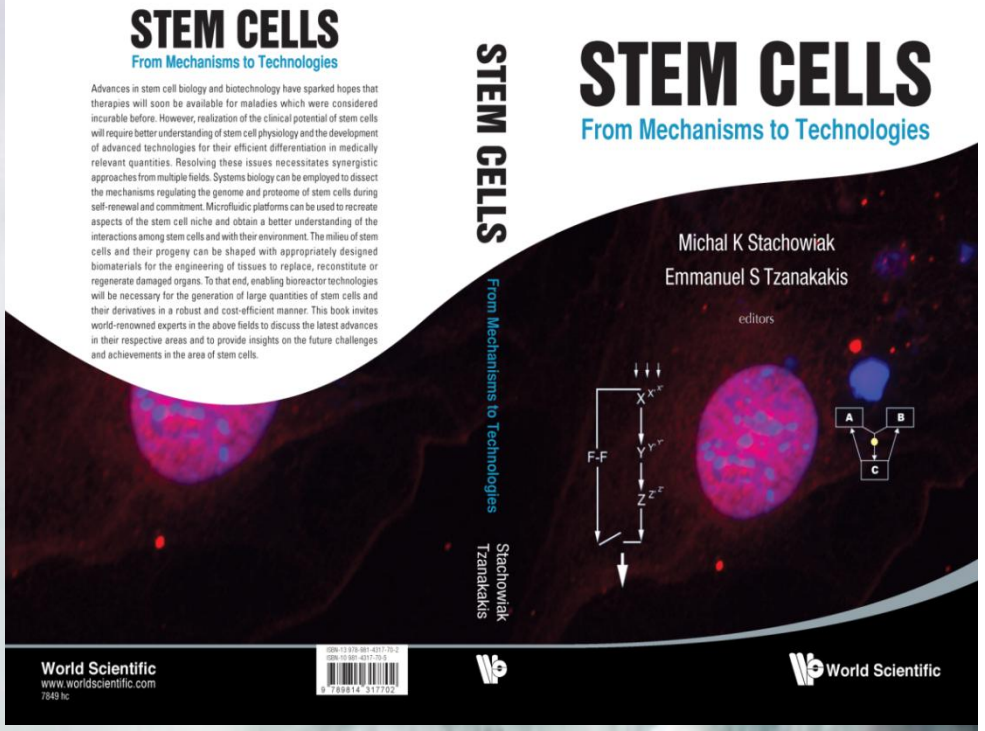


PNAS US A. 2005 02(32):11539-44; Integrative Biology, 2009, 1, 394 – 403.

Stem cells translational medicine 2013, 2:776-788.

MICHAL AND EWA'S LABs:

Barbara Birkaya
Yu-Wei (Eli) Lee
Christopher Terrranova
Sridhar Narla
Nived Nair
David Freedman
Courtney Benson
Seerat Elahi
Brendon Decker



Collaborators:

John Aletta H3 Biosystems
Merouanne Bencherif (Targacept Inc.)
Ronald Berezney (UB)
Dhruba Bharali (UB Dr. Prasad lab)
Michael Buck (UB)
Peter Claus (Hannover Med. Sch., Germany)
Janusz Morys, Ilona Klejbor (Med. Univ. Gdansk, Poland
Hari Shroff (NIH)
Manolis Tzanakakis (Tufts Univ,)

State University of New York
At Buffalo

Support: NIH (3), NSF (3), NYSTEM(2), Patrick Lee Foundation & Esther Trachtman Foundation for Schizophrenia, National Parkinson Foundation, Rett Foundation, Targacept Inc..

The End