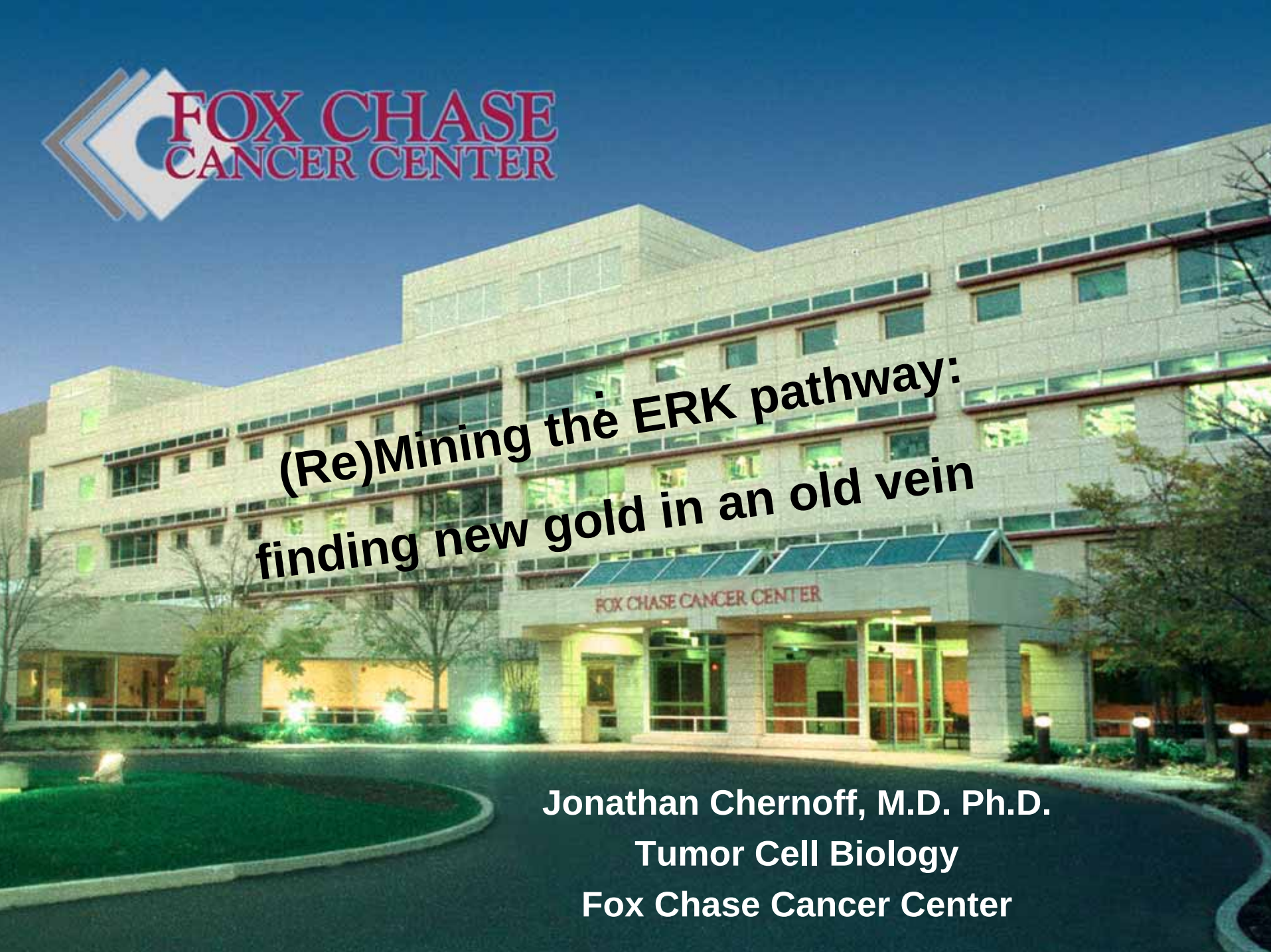
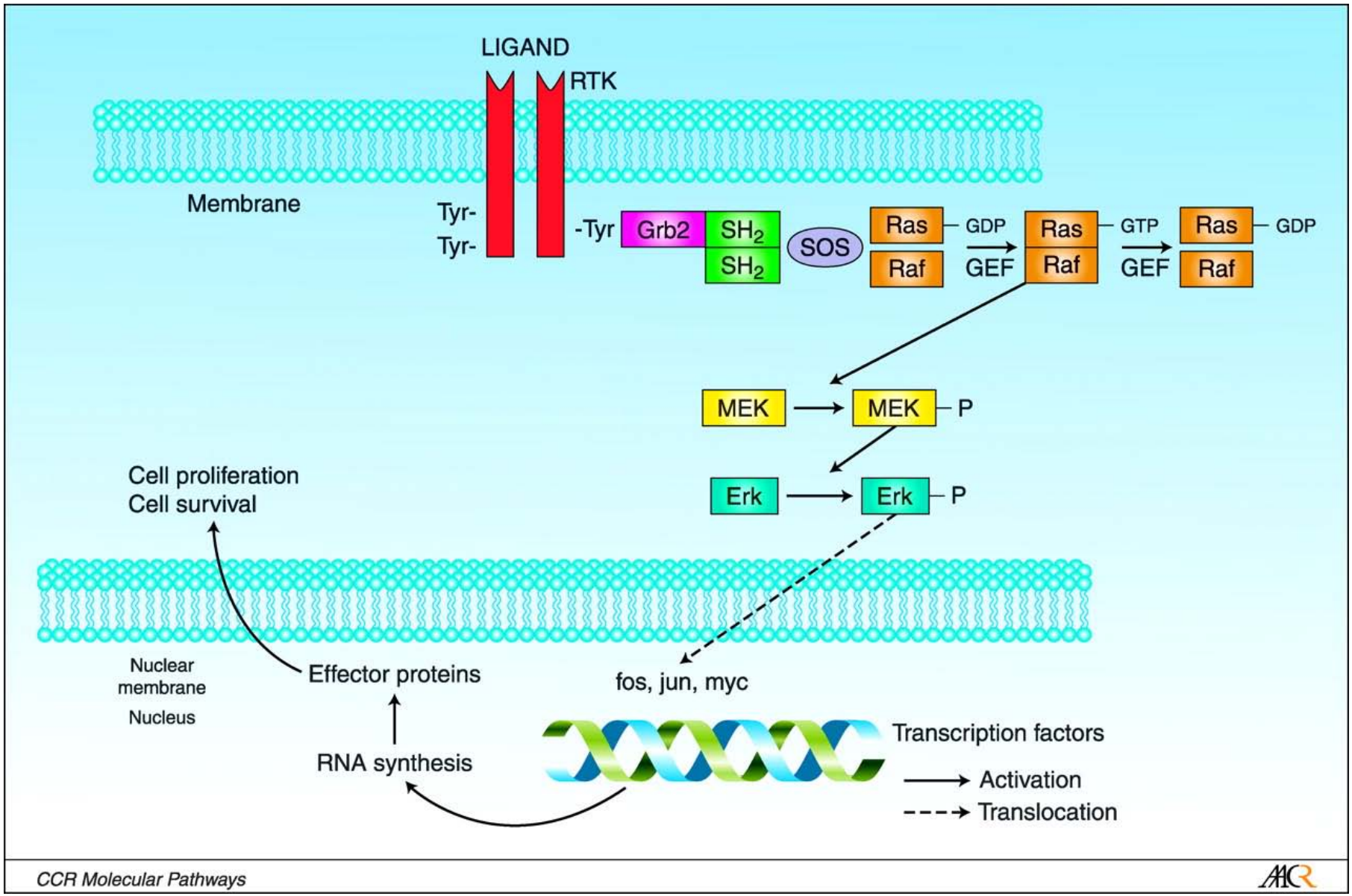

FOX CHASE
CANCER CENTER
(Re)Mining the ERK pathway:
finding new gold in an old vein
FOX CHASE CANCER CENTER
Jonathan Chernoff, M.D. Ph.D.
Tumor Cell Biology
Fox Chase Cancer Center

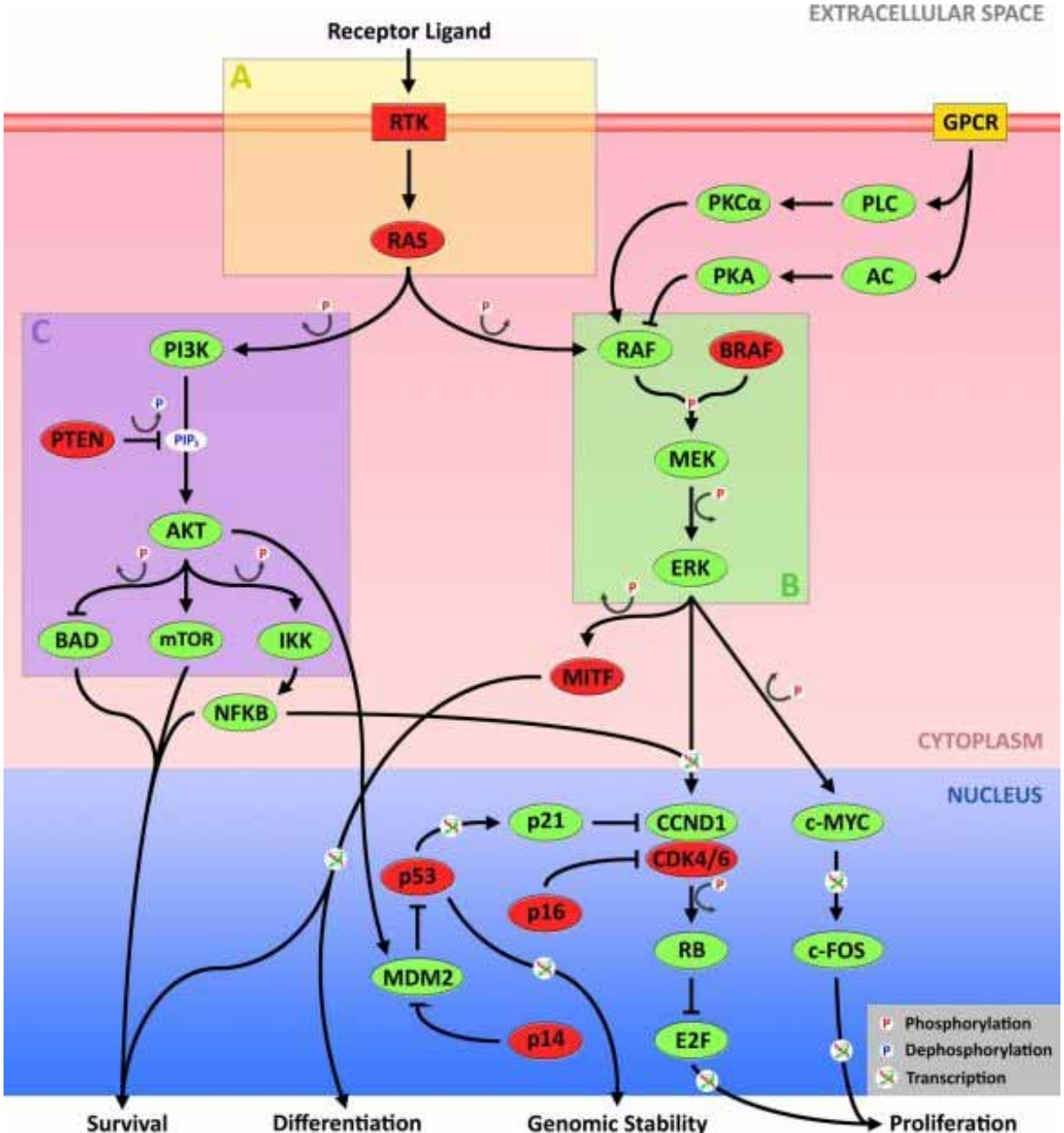
Agenda

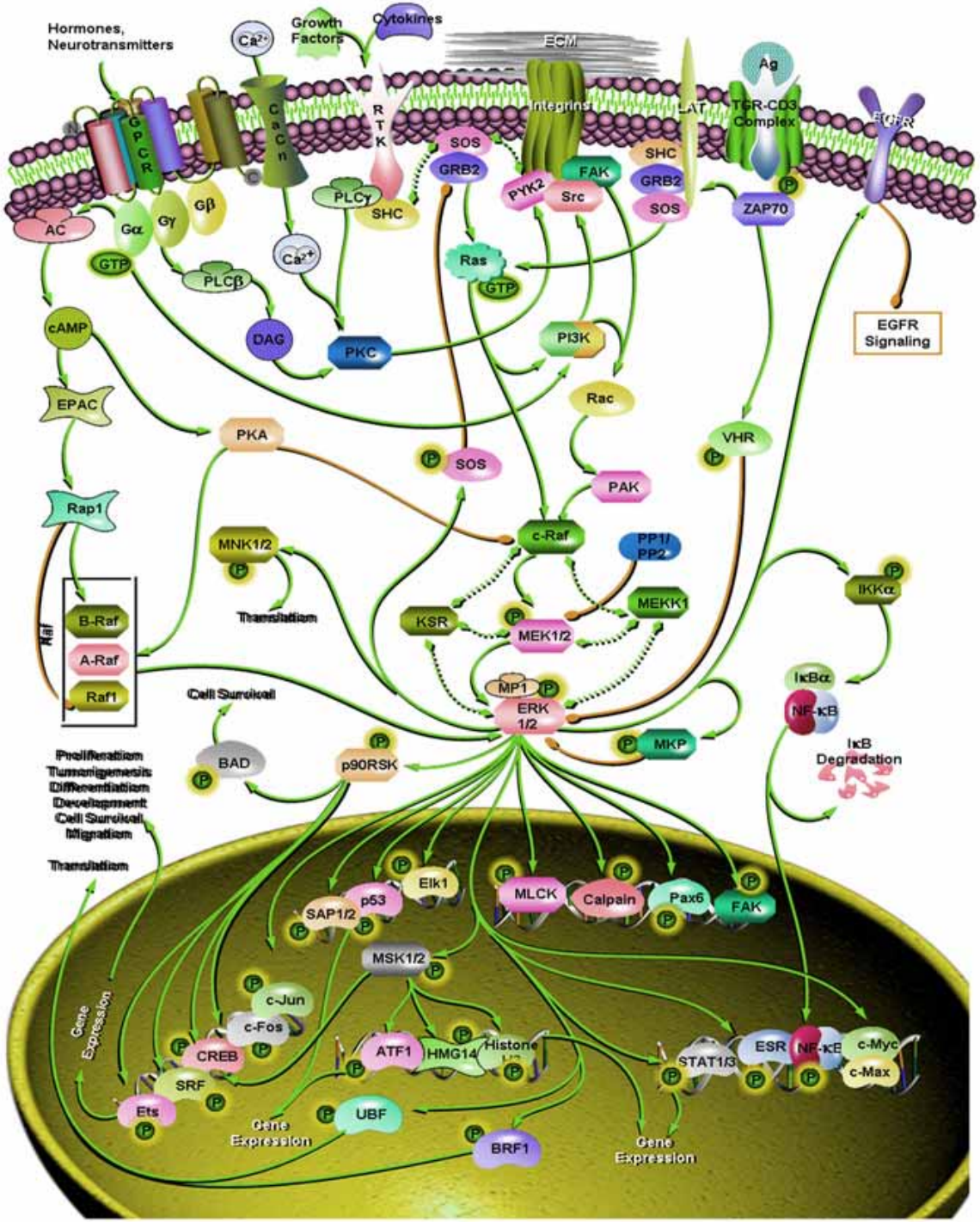
- Introduction to p21-activated kinases (Paks)
- Role of Paks in ERK pathway
- Role of Paks in inflammation and cancer

ERK Signaling in the Good Old Days

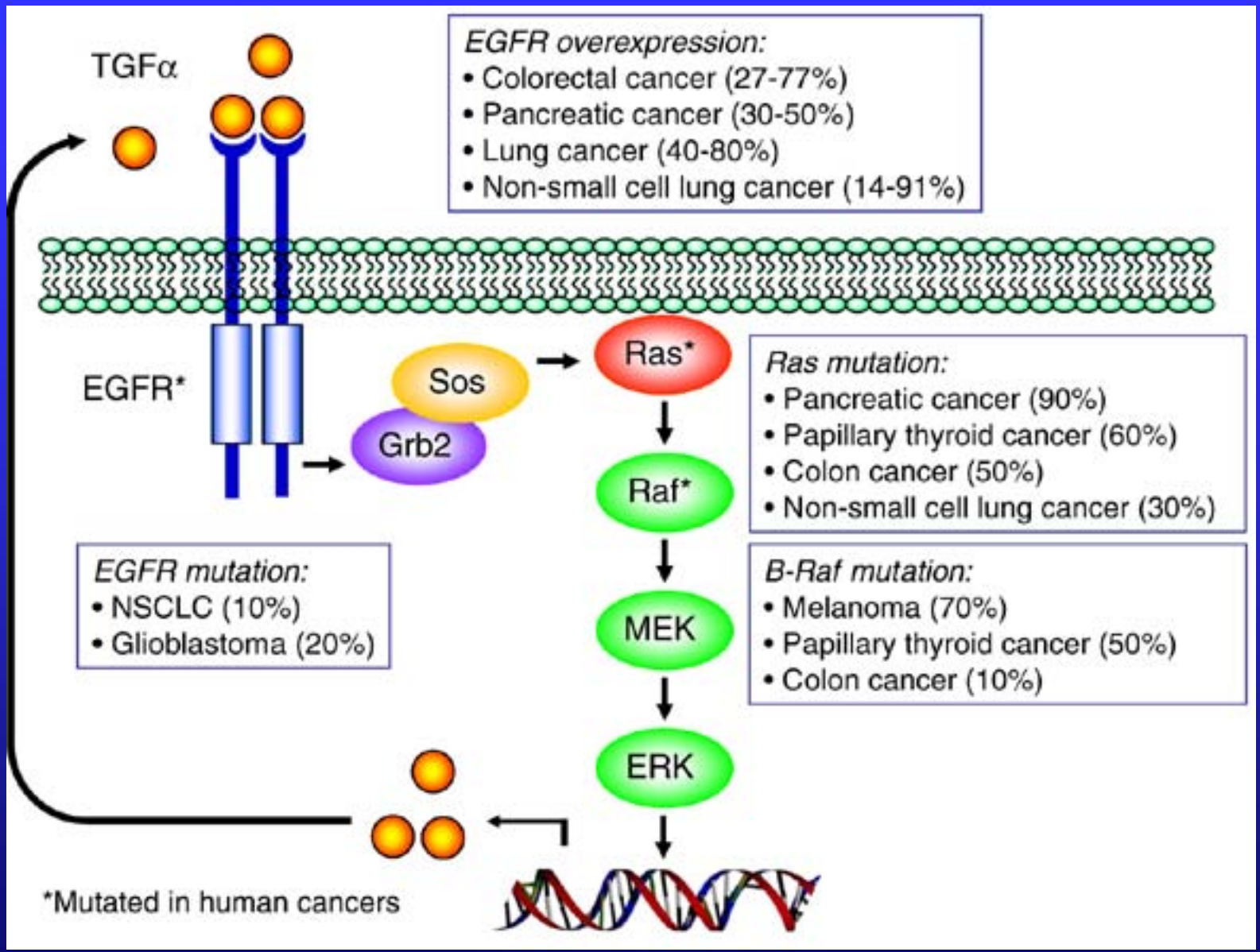


ERK Signaling, circa 2000



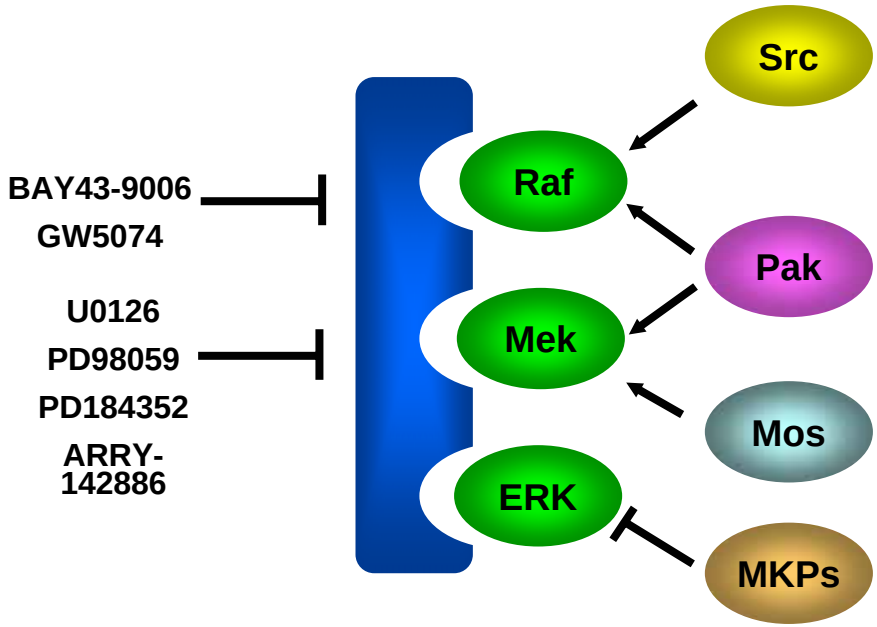

Hormones, Neurotransmitters
Ca2+
Growth Factors
Cytokines
ECM
Integrins
LAT
Ag
TCR-CD3 Complex
EGFR
GPCR
CaCn
RTK
SOS
GRB2
SHC
PLCγ
PYK2
FAK
Src
ZAP70
AC
Gα
Gγ
Gβ
GTP
PLCβ
Ca2+
Ras
GTP
cAMP
DAG
PKC
PI3K
EGFR Signaling
EPAC
PKA
SOS
Rac
PAK
VHR
Rap1
MNK1/2
Translation
c-Raf
PP1/ PP2
IKKα
Raf
B-Raf
A-Raf
Raf1
KSR
MEK1/2
MEKK1
IκBα
NF-κB
MP1
ERK 1/2
MKP
Cell Survival
BAD
p90RSK
IκB Degradation
Proliferation
Tumorigenesis
Differentiation
Development
Cell Survival
Migration
Translation
Elk1
p53
SAP1/2
MLCK
Calpain
Pax6
FAK
MSK1/2
c-Jun
c-Fos
CREB
ATF1
HMG14
Histone
STAT1/3
ESR
NF-κB
c-Myc
c-Max
SRF
Ets
Gene Expression
UBF
BRF1
Gene Expression
Gene Expression

ERK today

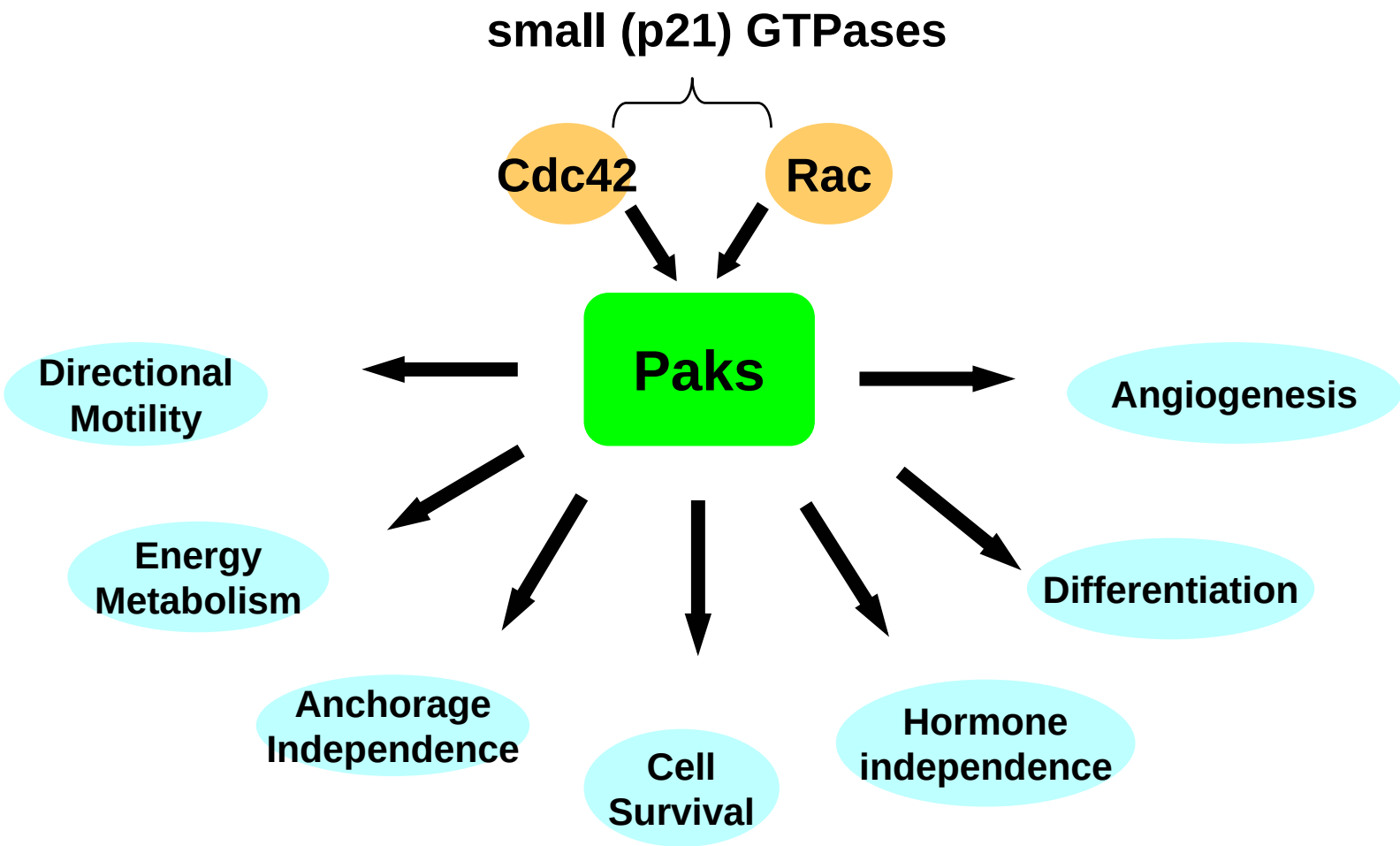


Roberts and Der, Oncogene, 2007, 26:3291

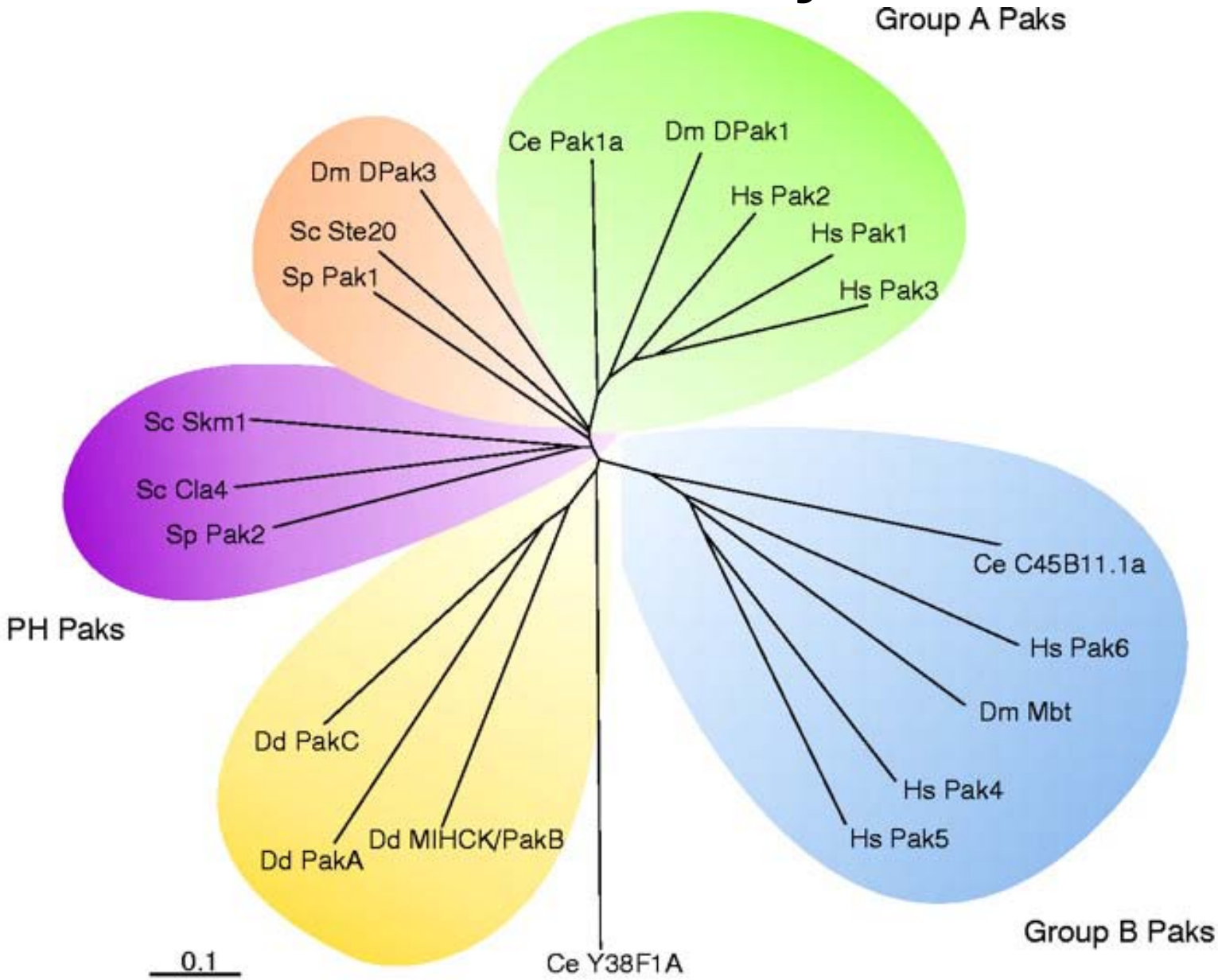
New opportunities to inhibit ERK



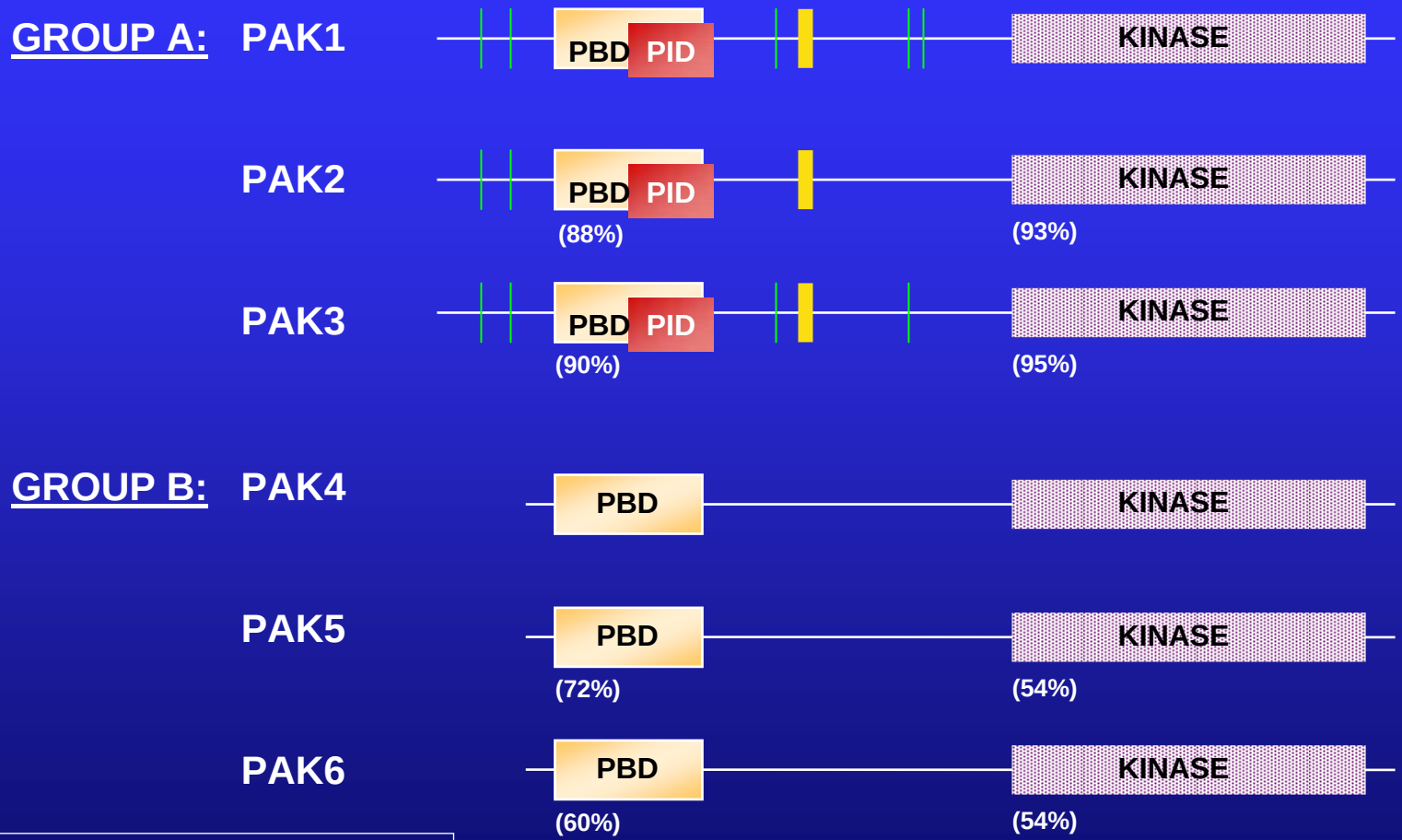
p21-activated kinases (Paks): cancer relevant pathways

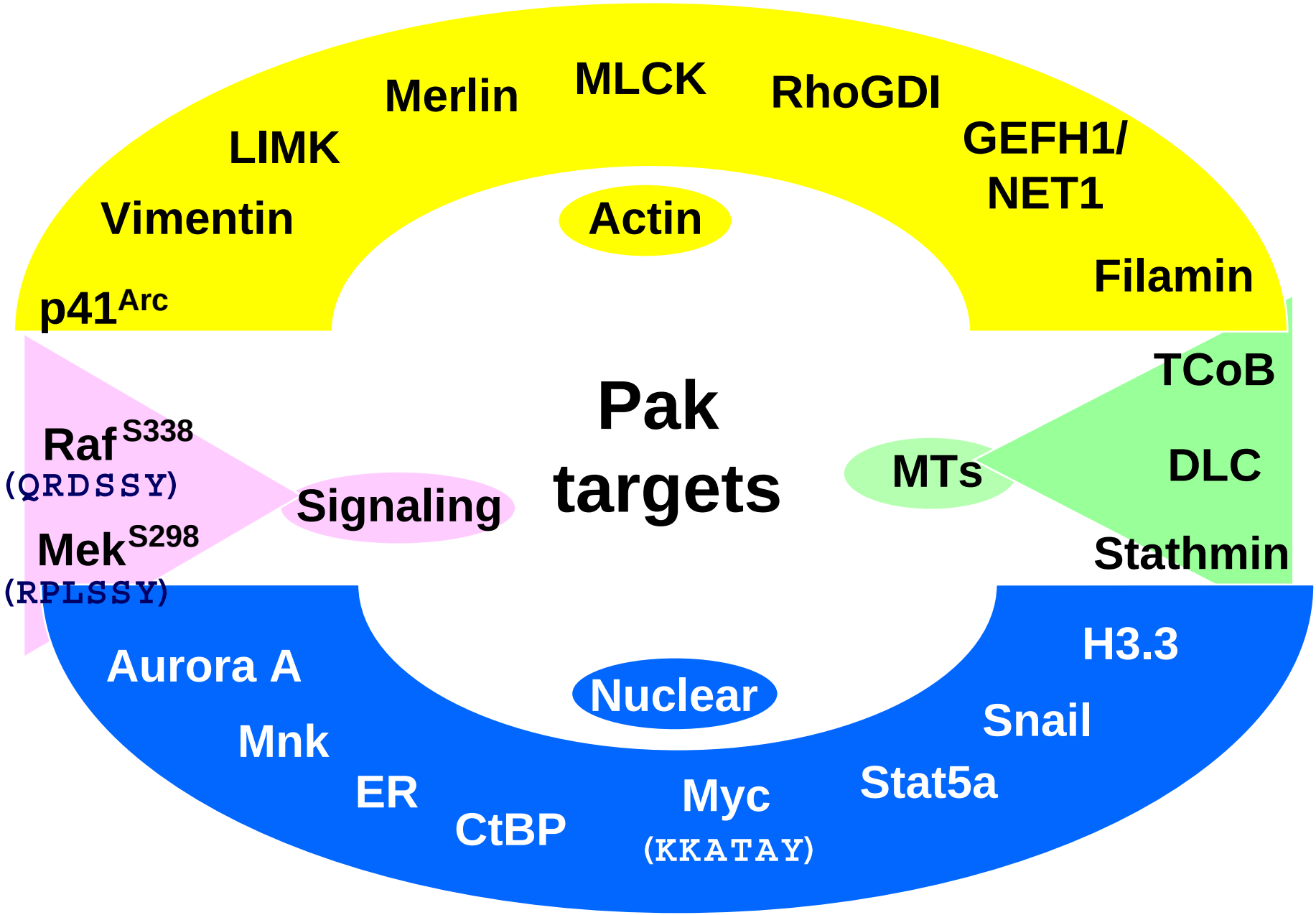


The Pak family

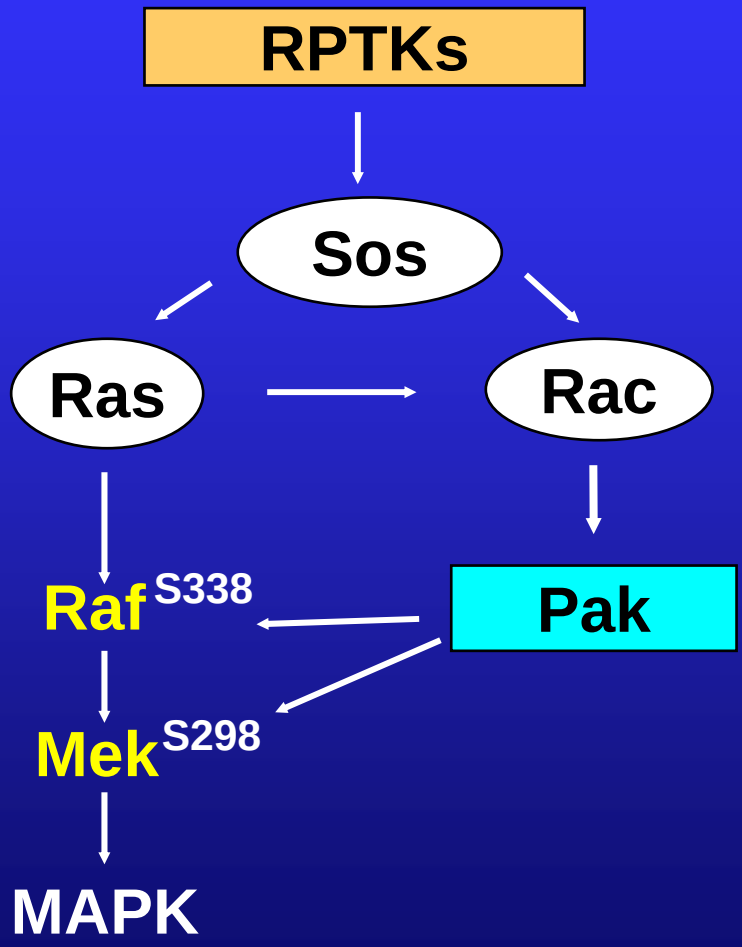


Structural comparison of the Group A and Group B Paks

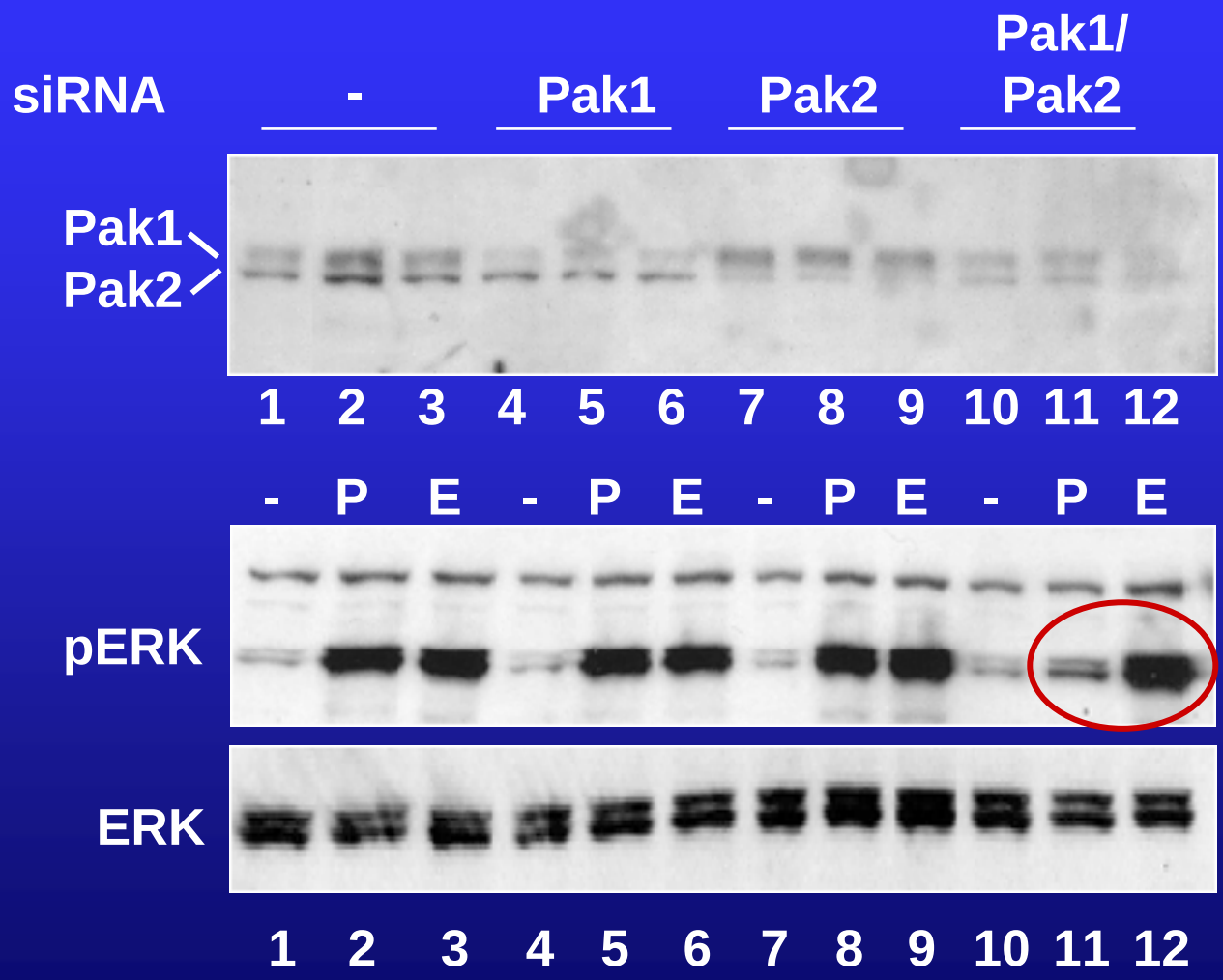



Merlin
MLCK
RhoGDI
LIMK
GEFH1/
NET1
Vimentin
Actin
Filamin
p41Arc
TCoB
Pak
targets
RafS338
(QRDSSY)
MTs
DLC
Signaling
MekS298
(RPLSSY)
Stathmin
H3.3
Aurora A
Nuclear
Snail
Mnk
Stat5a
ER
Myc
CtBP
(KKATAY)

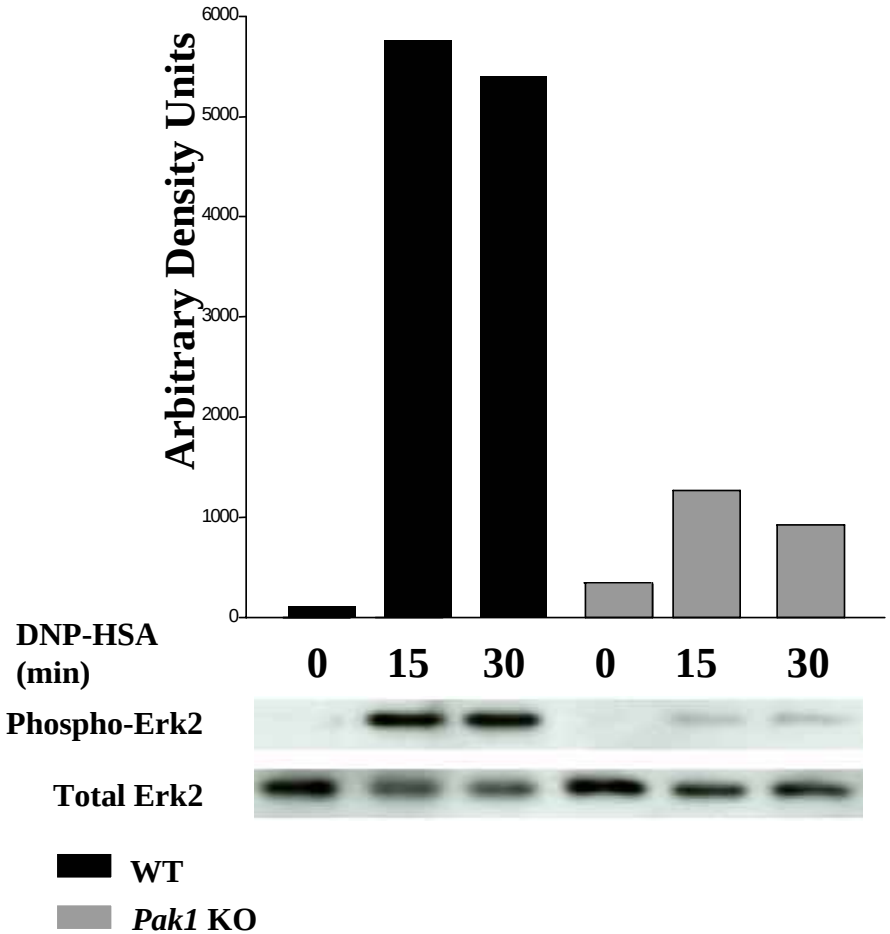
p21-activated kinase (Pak) signaling

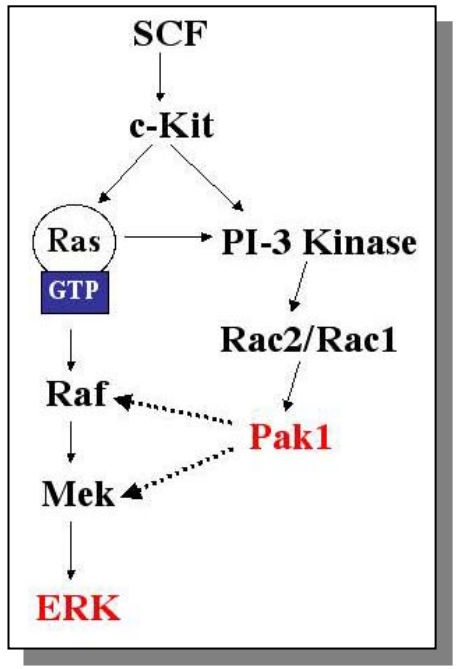


Knock down of Pak1 + Pak2 selectively affects PDGF activation of ERK



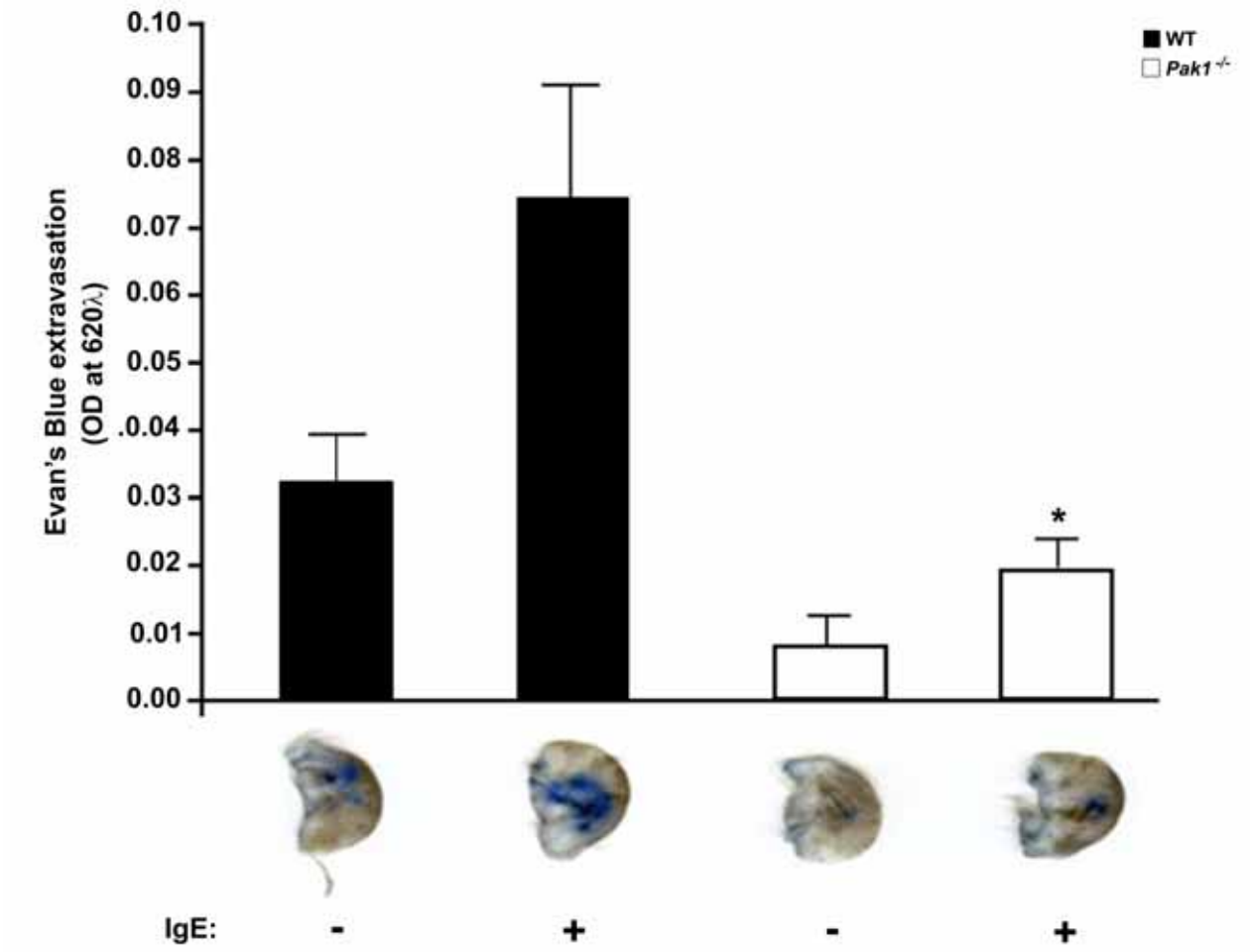
IgE-stimulated phospho-Erk is reduced in *Pak1* KO mast cells



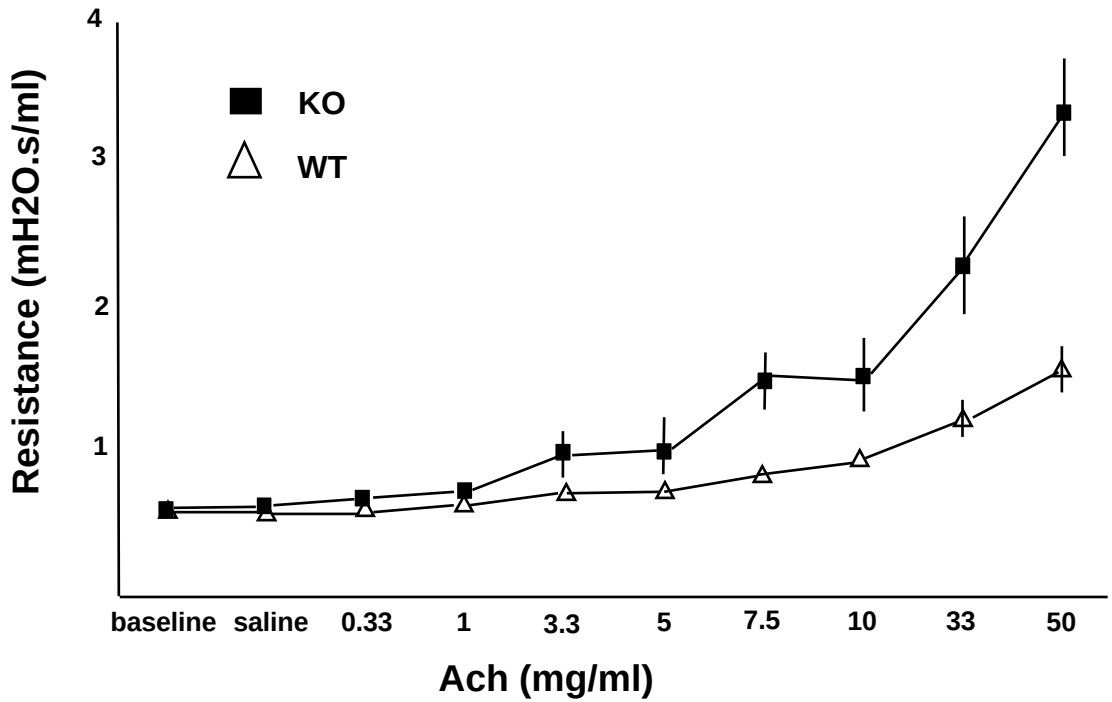


Allen

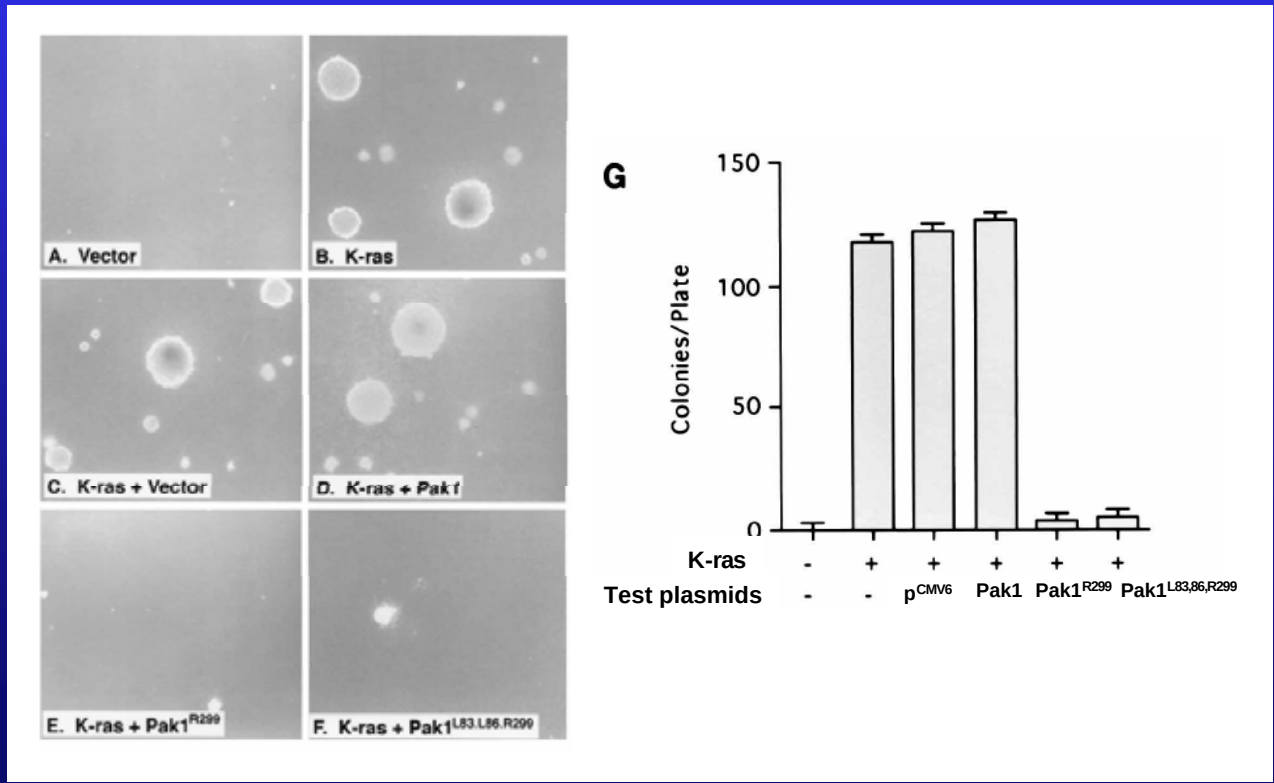
IgE-stimulated degranulation/cutaneous anaphylaxis is reduced in *Pak1* KO mast cells



Pak in asthma: Pak1-null mice don’t wheeze



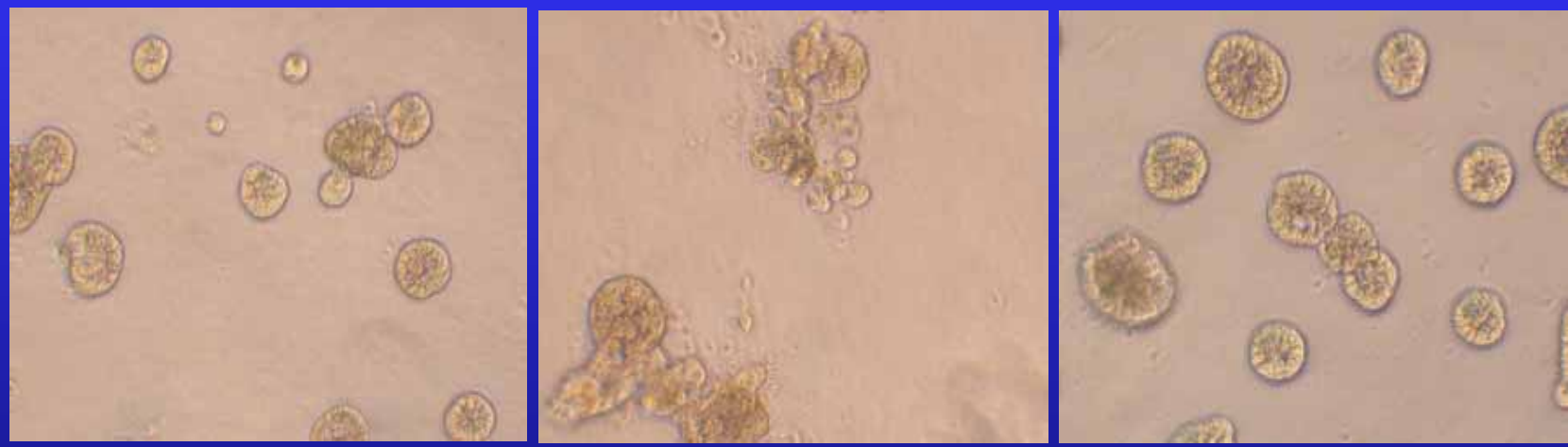
Q: Why would anyone want to inhibit Group A Paks?
A: Because they are required for Ras signaling.



Tang et al, MCB 1997

and HER2 transformation of breast epithelial cells

MCF10A cells



HER2	**-**	**+**	**+**
Pak inhibitor	**+**	**-**	**+**

Chernoff

and group A Paks are frequently activated in breast cancer

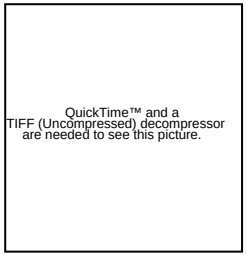
- Many metastatic breast cancer cell lines show constitutive activation of Rac3 and Pak.
- Pak1 is frequently upregulated in human breast tumors, and is associated with tamoxifen resistance.
- In mouse models, transgenic expression of activated Rac1 or activated Pak1 in breast epithelia gives rise to hyperplastic glands with aberrant ductal development that can progress to full-blown malignancy.

and other cancers too

Table 2 | **Human cancers with altered expression of Pak family proteins**

Cancer type	Pak isoform	Type of alteration	References
Breast	PAK1	55% protein overexpression	18
Breast	PAK1	Protein overexpression and tamoxifen resistance	33
Colon	PAK1	Protein overexpression	117
Ovarian	PAK1	30% gene amplification; 85% protein overexpression	118
Bladder	PAK1	Gene amplification	H. Nishiyama and O. Ogawa, personal communication
Pancreas	PAK4	Gene amplification; protein overexpression	12
Brain	PAK1	Protein overexpression	Y. Kondo and K. D. Aldape, personal communication
Neurofibromatosis	PAK1	Protein stabilization	24
T-cell lymphoma	PAK1	Gene amplification	119

Kumar *et al.* Nature Rev Cancer 6:456, 2006



Pak1::K5-kras$^{(D12)}$ Study

K5-tet **X** ***pak1*** **HET** **Tet-kras X** ***pak1*** **HET**

K5-tet::pak1 **HET X** ***tet-kras*****::*****pak1*** **HET**

K5-tet::tet-kras::pak1 **WT***

K5-tet::tet-kras::pak1 **HET***

K5-tet::tet-kras::pak1 **KO***

***induce transgene expression w/doxycycline pellets (2mg/kg)**

K5-*kras(D12)* Induction in WT and *pak1* KO mice

#228 KO female;
2.4 mos;
follicular and
sebacious
hyperplasia;
SCC

#240 KO male;
1.1 mos;
SCC

#237 WT female;
1.1 mos;
papillomas, SCC

K5-*kras(D12)* Induction in WT and *pak1* KO mice

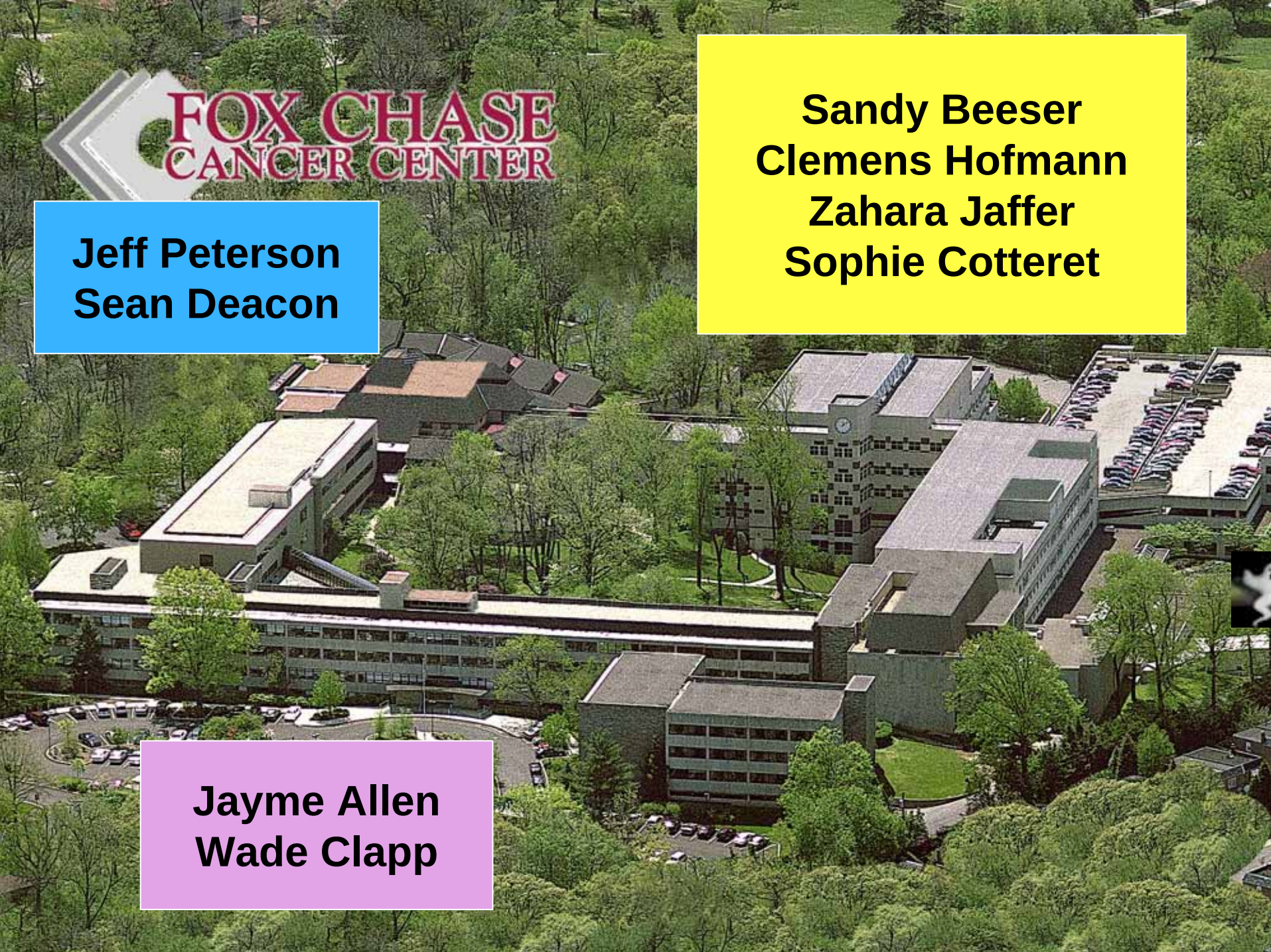
	WT (N = 19)	KO (N = 20)
No disease	0	6
Hyperplasia/papilloma	8	10
SCC	11	4

Summary

- In higher eukaryotes, there are two distinct groups of Paks. These two groups differ in structure, biochemical properties, and function.
- Inhibition of Group A Pak by siRNA or gene deletion results in decreased ERK activity in response to selected growth factors.
- Inhibition of Pak blocks transformation by Ras and HER-2 in vitro.
- *Pak1*$^{-/-}$ mice are healthy, but show defects in mast cell function and resist transformation by Ras.

Pak inhibitor screen

- No current selective Pak inhibitors are available.
- Pak inhibitors could be useful in probing Pak function.
- Such inhibitors might have therapeutic value.


FOX CHASE
CANCER CENTER
Jeff Peterson
Sean Deacon
Sandy Beeser
Clemens Hofmann
Zahara Jaffer
Sophie Cotteret
Jayme Allen
Wade Clapp