Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 19

Regulation of gene expression by Growth factors

Lectures 15 -18

Signal transduction pathways & Regulation of gene expression

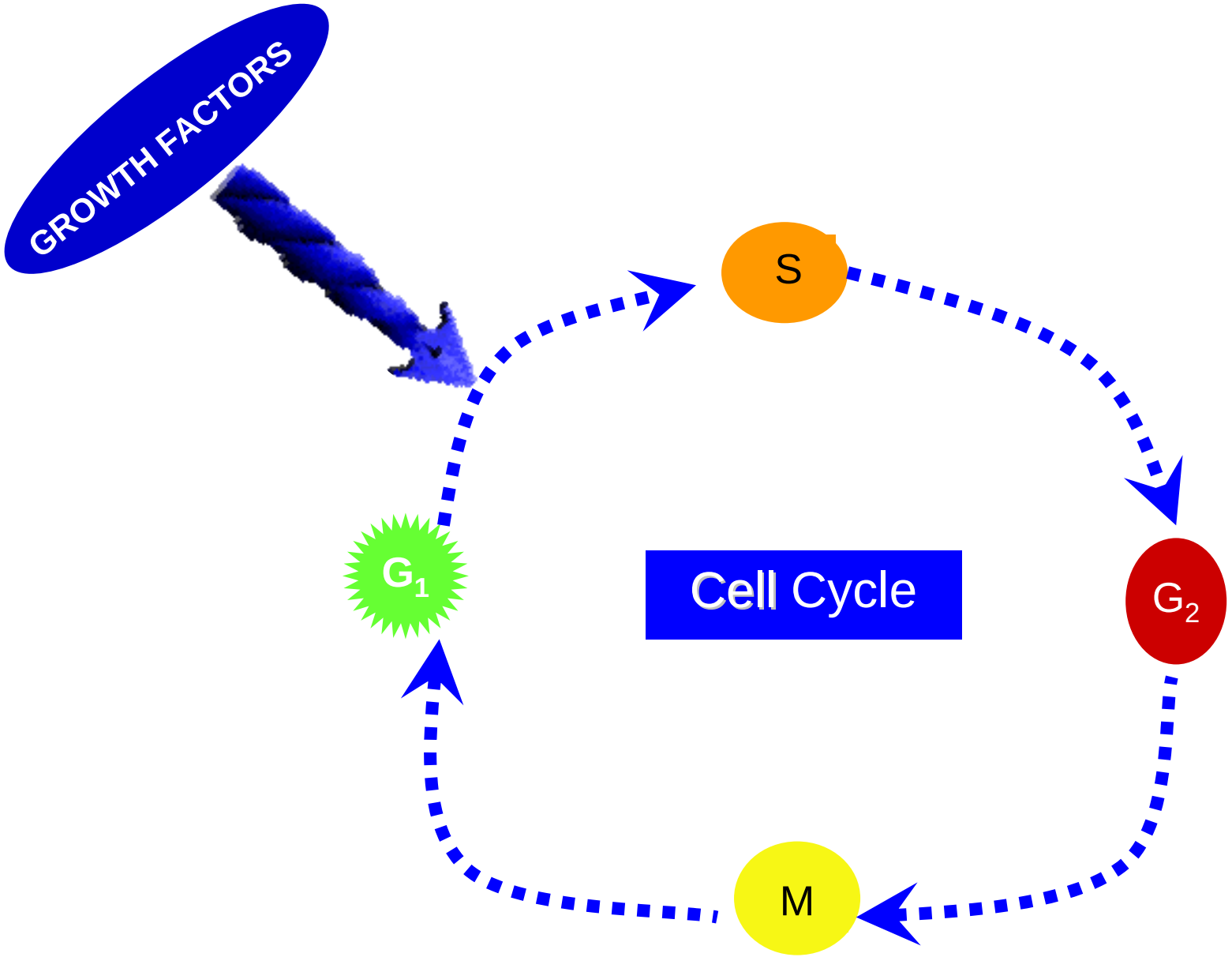
Lipophilic signalling molecules
intracellular receptors

Water soluble signalling molecules
membrane receptors

G protein coupled receptors (GPCRs)
Trimeric G proteins
Gene regulation by second messengers
(cAMP, cGMP, Ca^{2+}, DAG)

This lecture…….

Regulation of gene expression
by
growth factors and growth factor receptors (RTKs)



*Adapted from www.iuphar.org/sections/teaching/docs/**EGF**R_inhibitors.**ppt***

Stanley Cohen

Rita Levi-Montalcini

The Nobel Prize in Physiology or Medicine 1986

The Nobel Prize in Physiology or Medicine 1986 was awarded jointly to Stanley Cohen and Rita Levi-Montalcini *"for their discoveries of growth factors"*

http://nobelprize.org/nobel_prizes/medicine/laureates/1986/

NGF & EGF

In 1952, Rita Levi-Montalcini demonstrated that tumours from mice when transplanted to chick embryos induced potent growth of the chick embryo nervous system, specifically sensory and sympathetic nerves.

Since this outgrowth did not require direct contact between the tumour and the chick embryo, she concluded that the tumour released a nerve growth-promoting factor which had a selective action on certain types of nerves.

In 1958, Stanley Cohen purified NGF from the salivary glands of adult mice and snake venom.

While studying NGF, Cohen observed that the salivary gland extract contained another growth factor apart from NGF.

Cohen termed this substance *epidermal growth factor* (EGF) because it could stimulate the proliferation of epithelial cells in skin and cornea.

How do these growth factors such as EGF and NGF act?

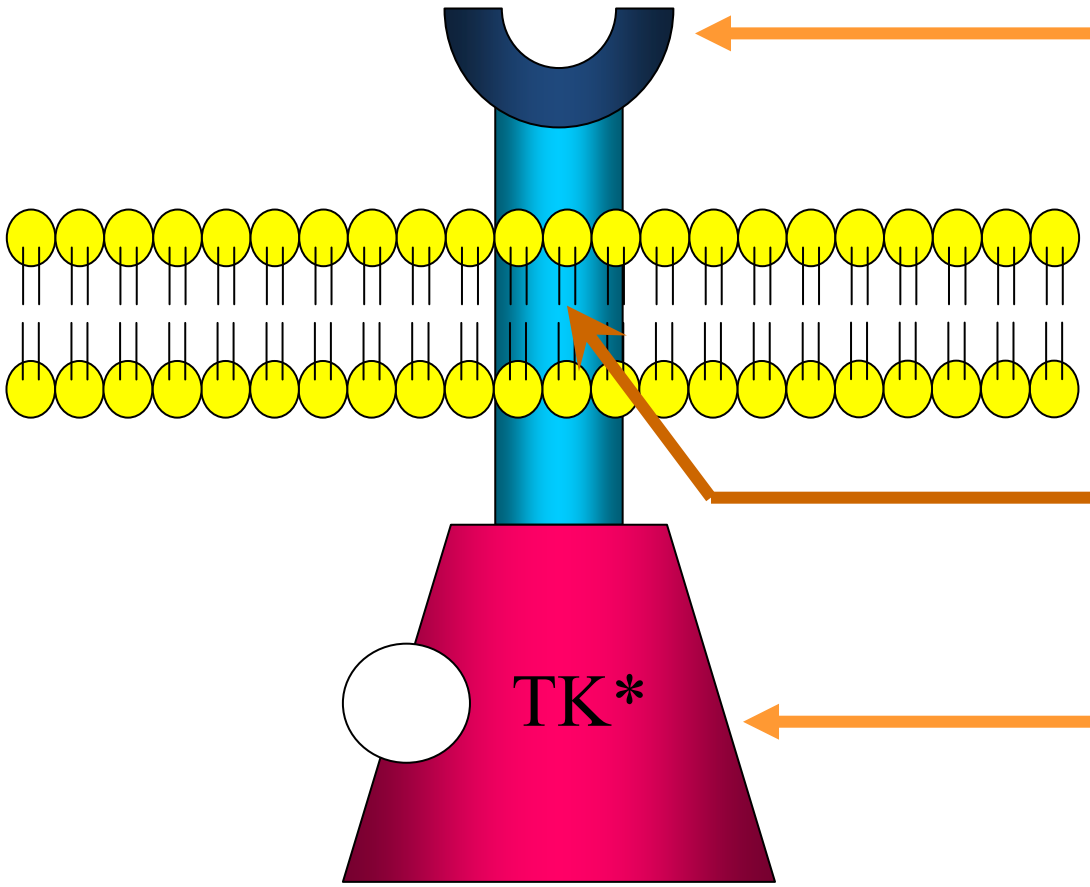
Protein Tyrosine Phosphorylation

Hunter, T. and Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA 77, 1311- 1315*

Stanley Cohen discovered that the receptor for epidermal growth factor was itself a tyrosine kinase whose activity was induced by binding of the ligand.

Ushiro, H. and Cohen, S. (1980)J. *Biol. Chem. 255, 8363-8365.*

EGFR



Extracellular Domain

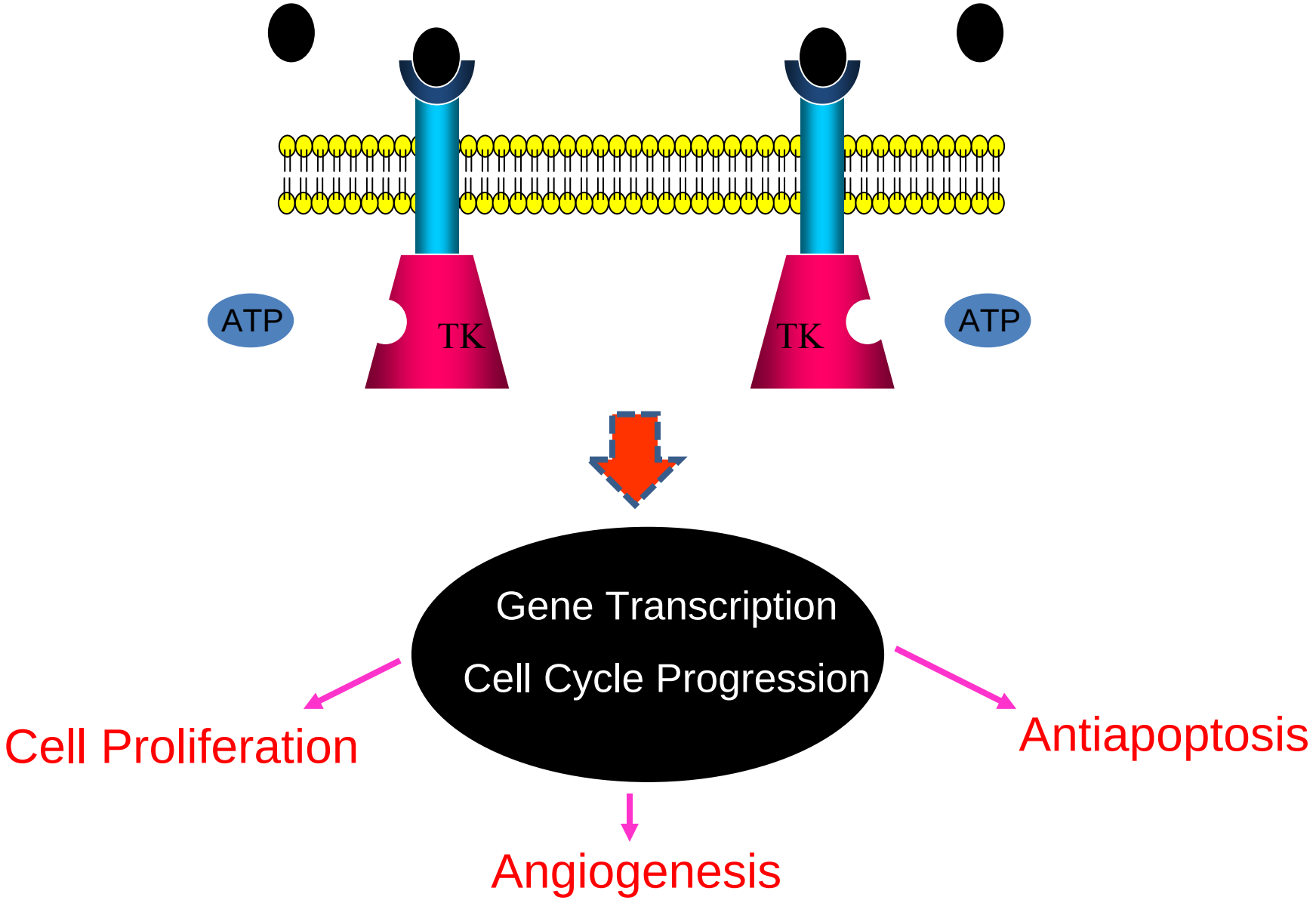
Single pass Transmembrane Domain (unlike GPCRs

Intracellular Domain

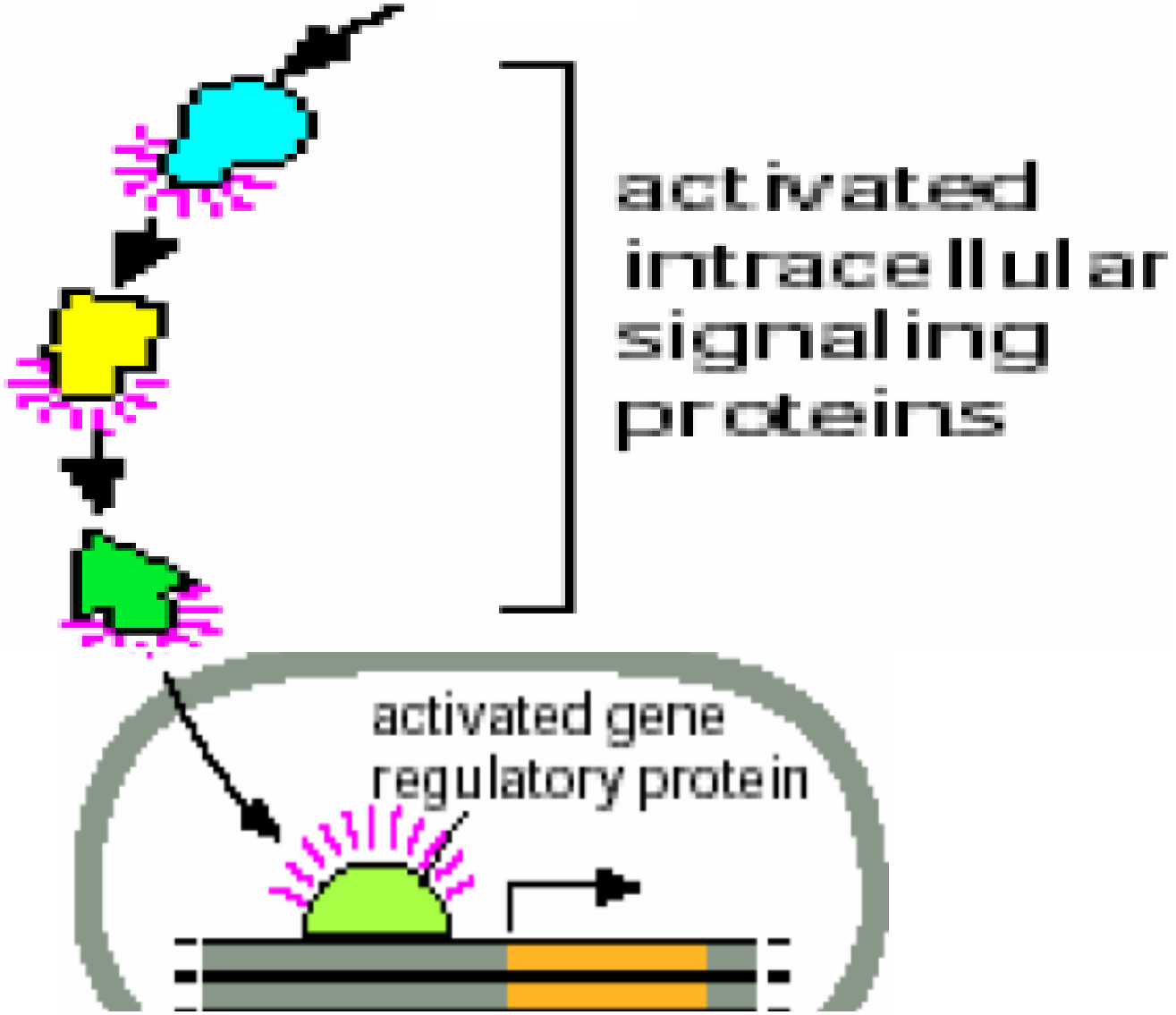
* TK - Tyrosine kinase

*Adapted from www.iuphar.org/sections/teaching/docs/**EGF**R_inhibitors.**ppt***

EGFR Function in Normal Cell



*Adapted from www.iuphar.org/sections/teaching/docs/**EGF**R_inhibitors.**ppt***



MAPK signaling cascades

CELL PROLIFERATION

Growth Factors

Growth Factor	Primary Activity
PDGF	promotes proliferation of connective tissue, glial and smooth muscle cells
EGF	promotes proliferation of mesenchymal, glial and epithelial cells
TGF-α	may be important for normal wound healing
FGF	promotes proliferation of many cells; inhibits some stem cells; induces mesoderm to form in early embryos
NGF	promotes neurite outgrowth and neural cell survival
Erythropoietin	promotes proliferation and differentiation of erythrocytes
TGF-β	anti-inflammatory (suppresses cytokine production and class II MHC expression), promotes wound healing, inhibits macrophage and lymphocyte proliferation
IGF-I	promotes proliferation of many cell types
IGF-II	promotes proliferation of many cell types primarily of fetal origin

GROWTH FACTORS

Tyrosine kinases

Phospholipase C

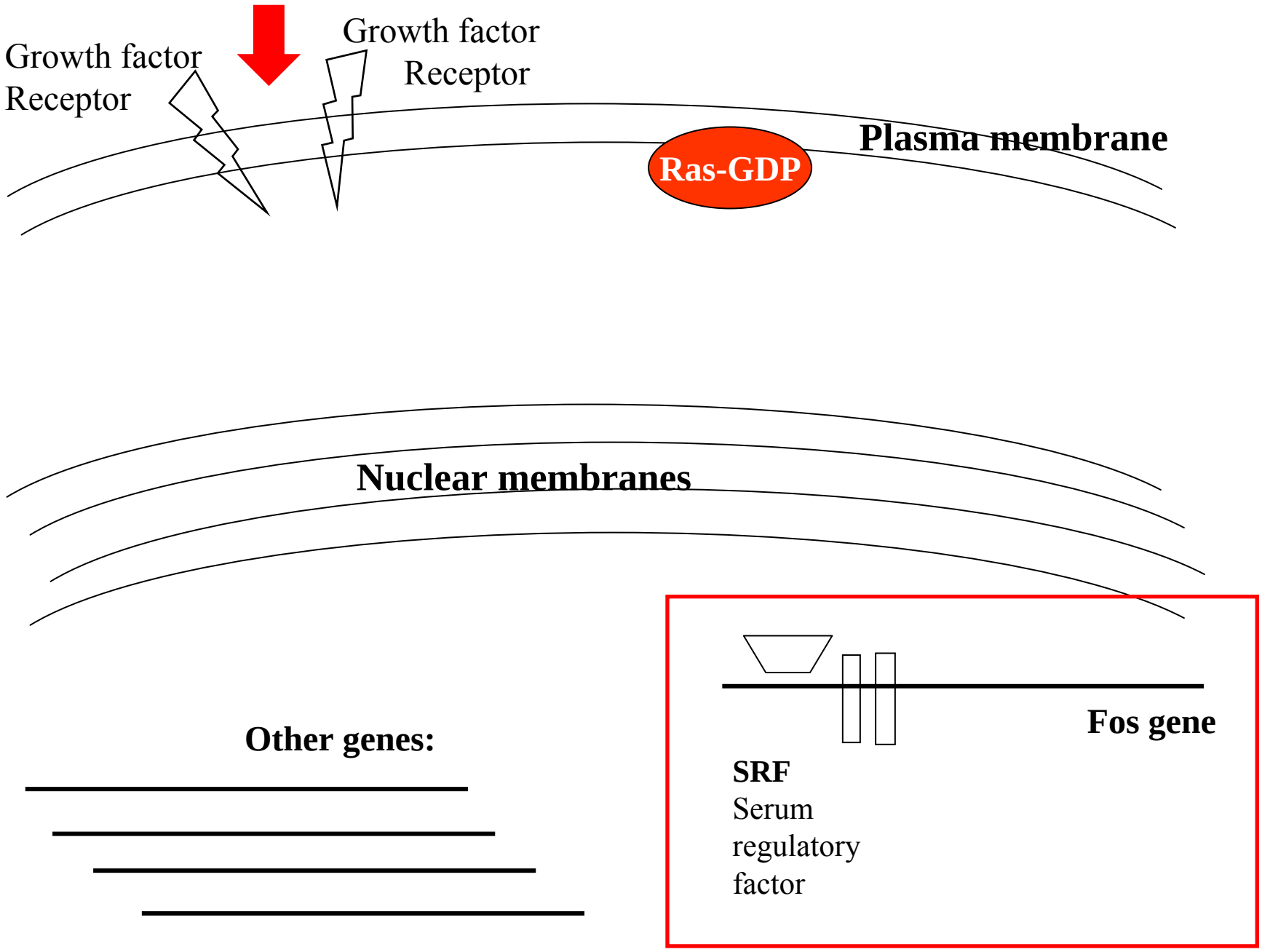
Ras/GAP

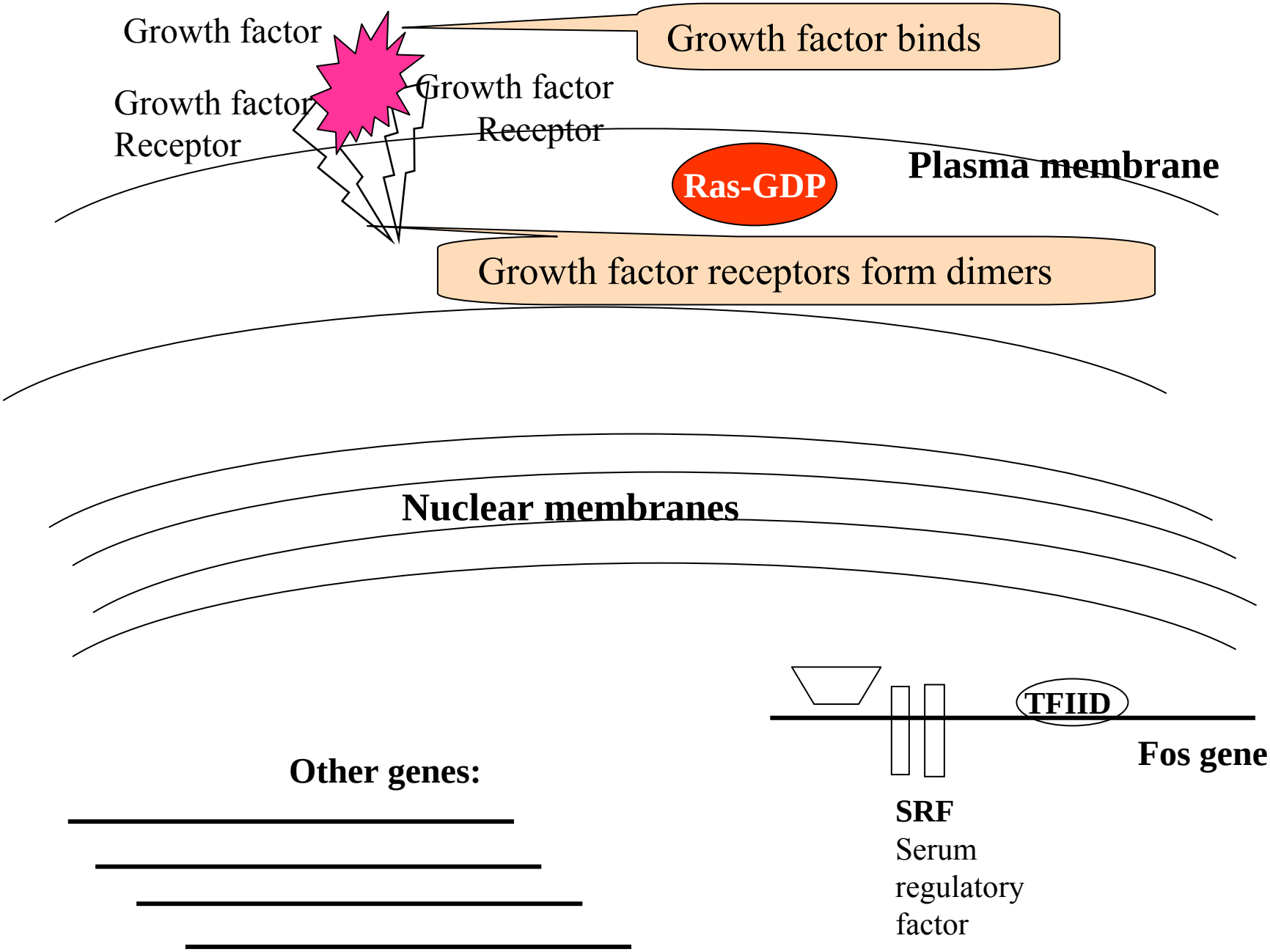
PI3 kinase

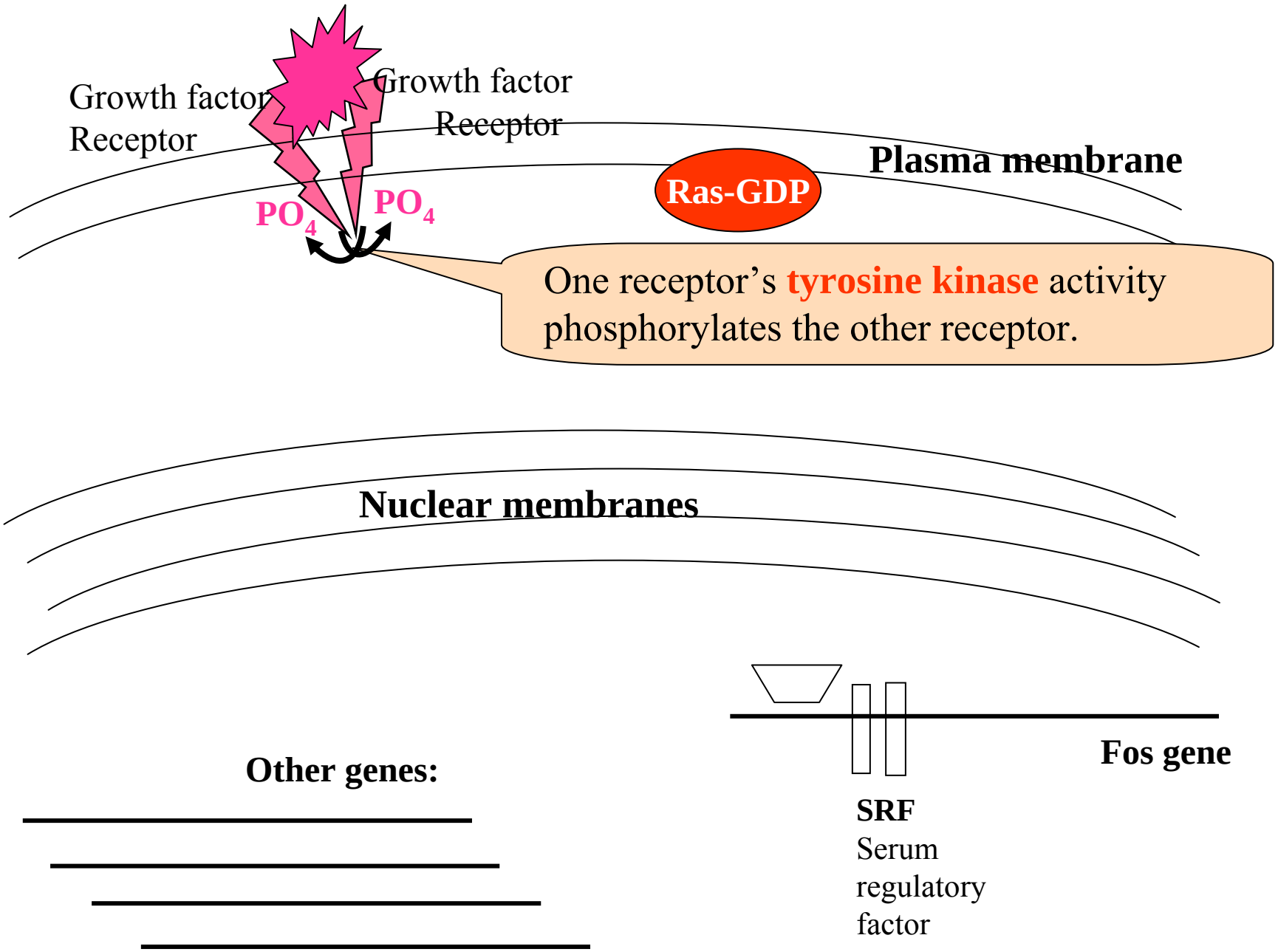
Activation of MAP kinase cascade (Ser/Thr kinases)

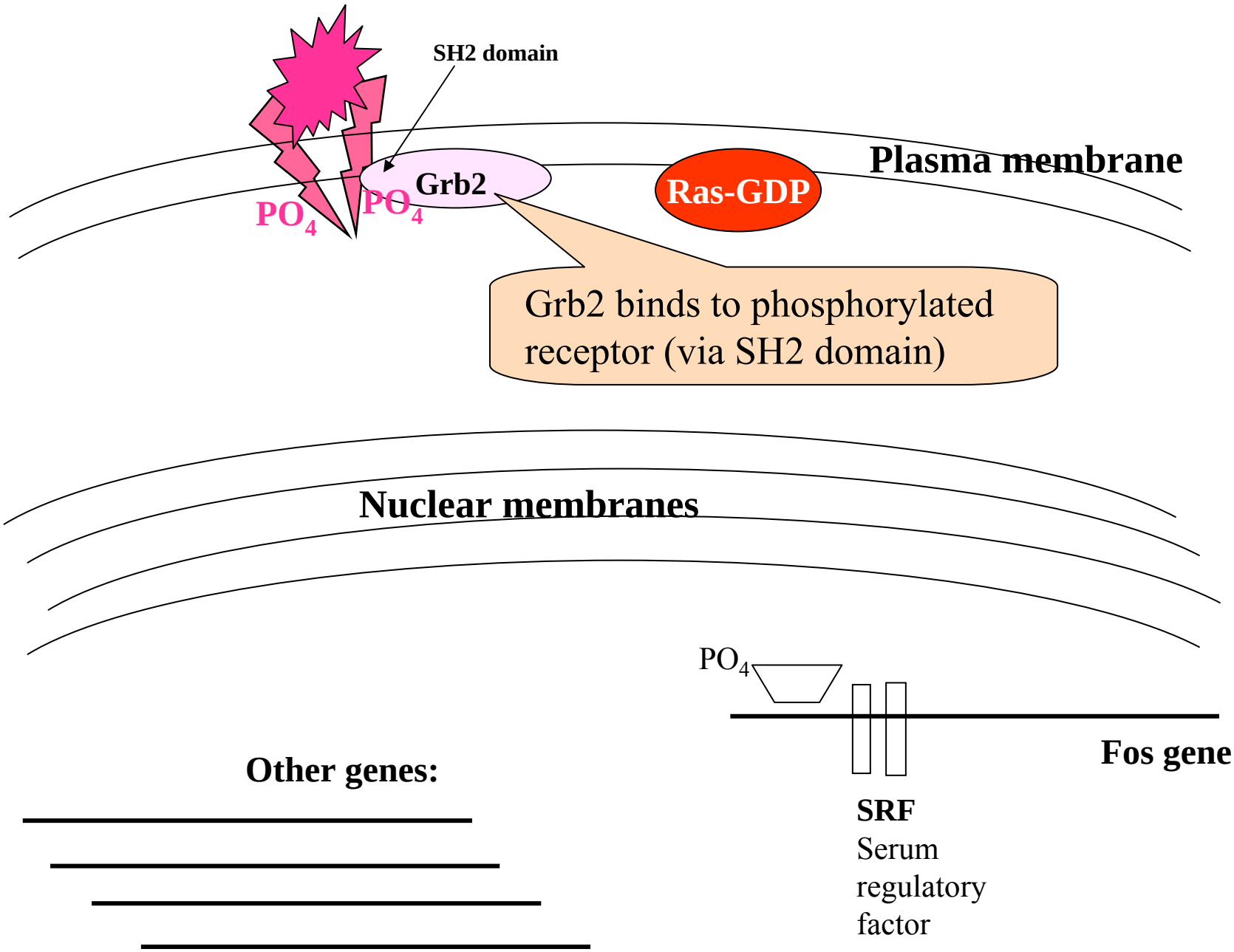
Activation of Transcription factors

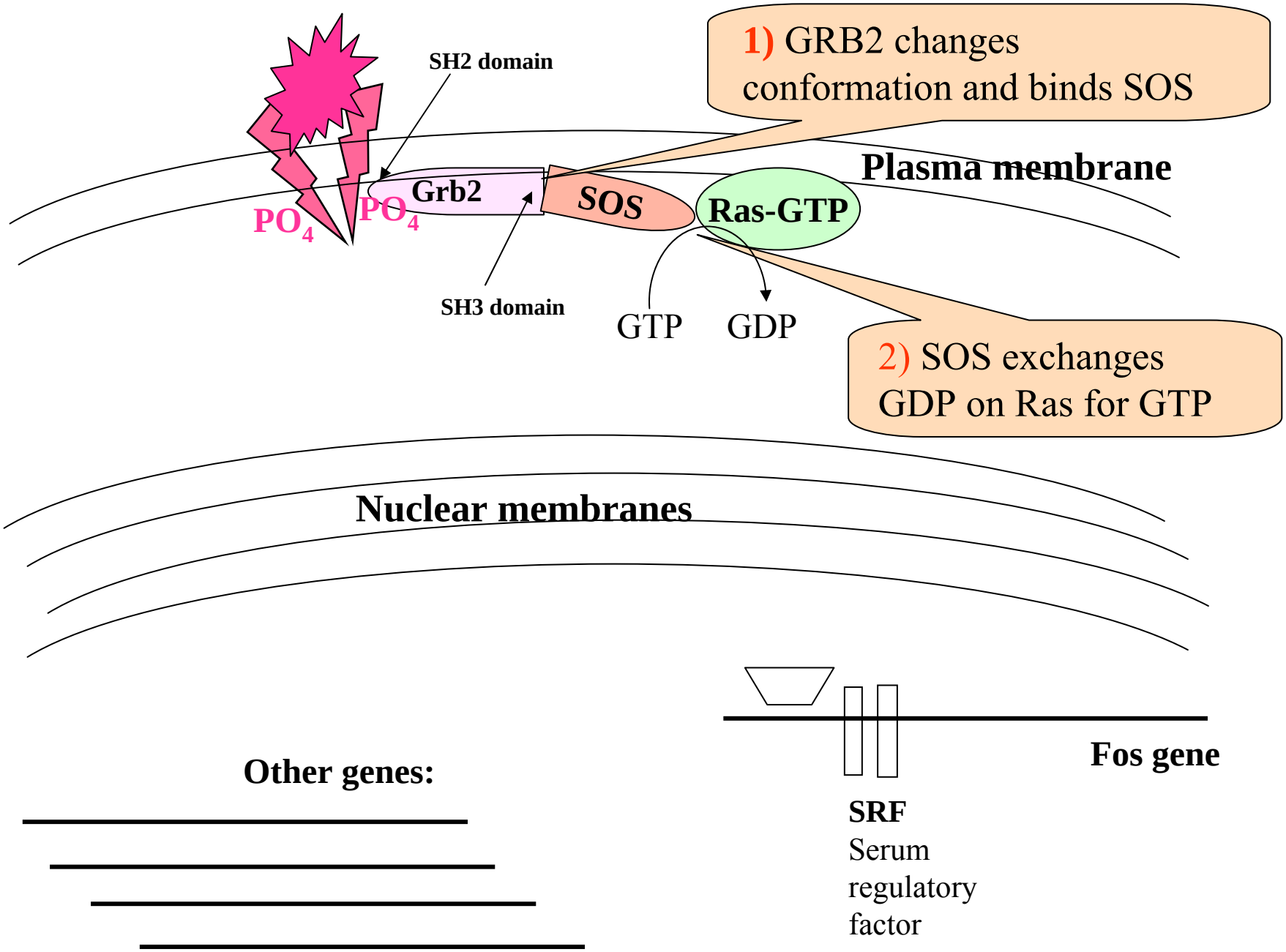
Growth Response Genes


Growth factor
Receptor
Growth factor
Receptor
Plasma membrane
Ras-GDP
Nuclear membranes
Other genes:
Fos gene
SRF
Serum
regulatory
factor

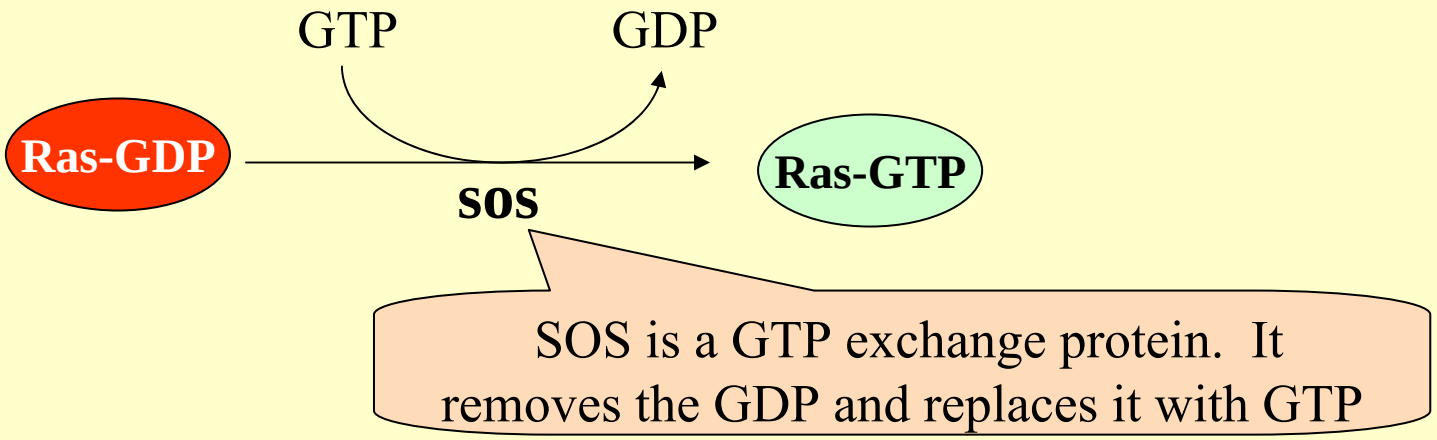

Growth factor
Growth factor binds
Growth factor Receptor
Growth factor Receptor
Plasma membrane
Ras-GDP
Growth factor receptors form dimers
Nuclear membranes
TFIID
Fos gene
Other genes:
SRF
Serum regulatory factor


Growth factor
Receptor
Growth factor
Receptor
PO4
PO4
Plasma membrane
Ras-GDP
One receptor's tyrosine kinase activity phosphorylates the other receptor.
Nuclear membranes
Fos gene
SRF
Serum
regulatory
factor
Other genes:

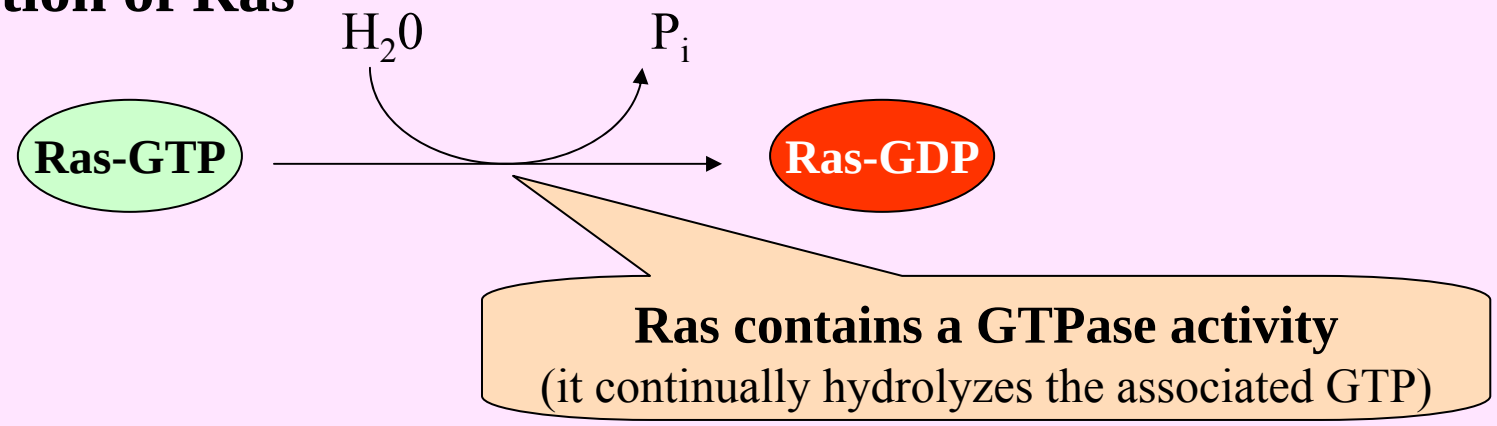

SH2 domain
Plasma membrane
Grb2
Ras-GDP
PO4
PO4
Grb2 binds to phosphorylated receptor (via SH2 domain)
Nuclear membranes
PO4
Fos gene
Other genes:
SRF
Serum regulatory factor

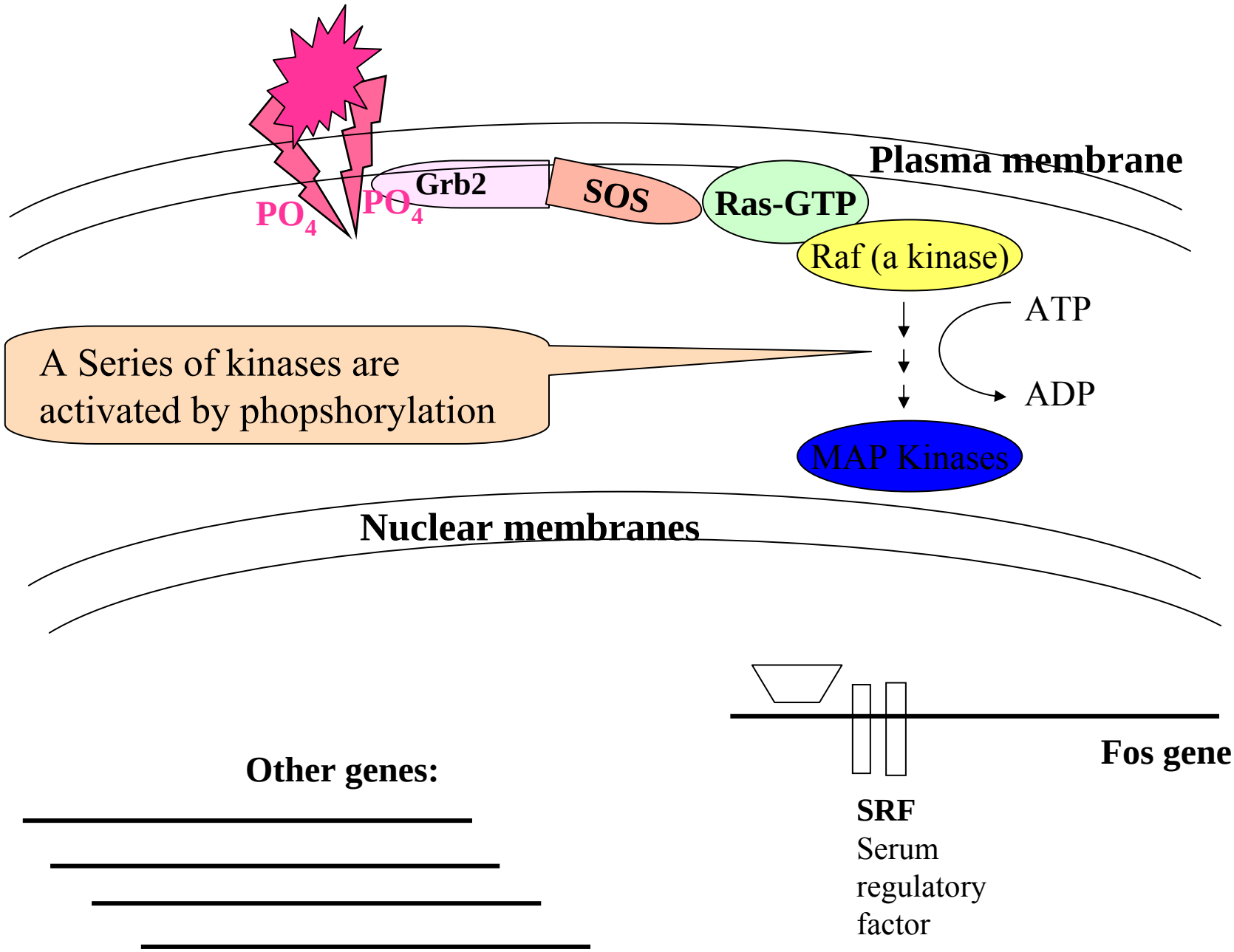

1) GRB2 changes conformation and binds SOS
SH2 domain
Plasma membrane
Grb2
SOS
Ras-GTP
PO4
PO4
SH3 domain
GTP
GDP
2) SOS exchanges GDP on Ras for GTP
Nuclear membranes
Fos gene
Other genes:
SRF
Serum regulatory factor

A. Activation of Ras

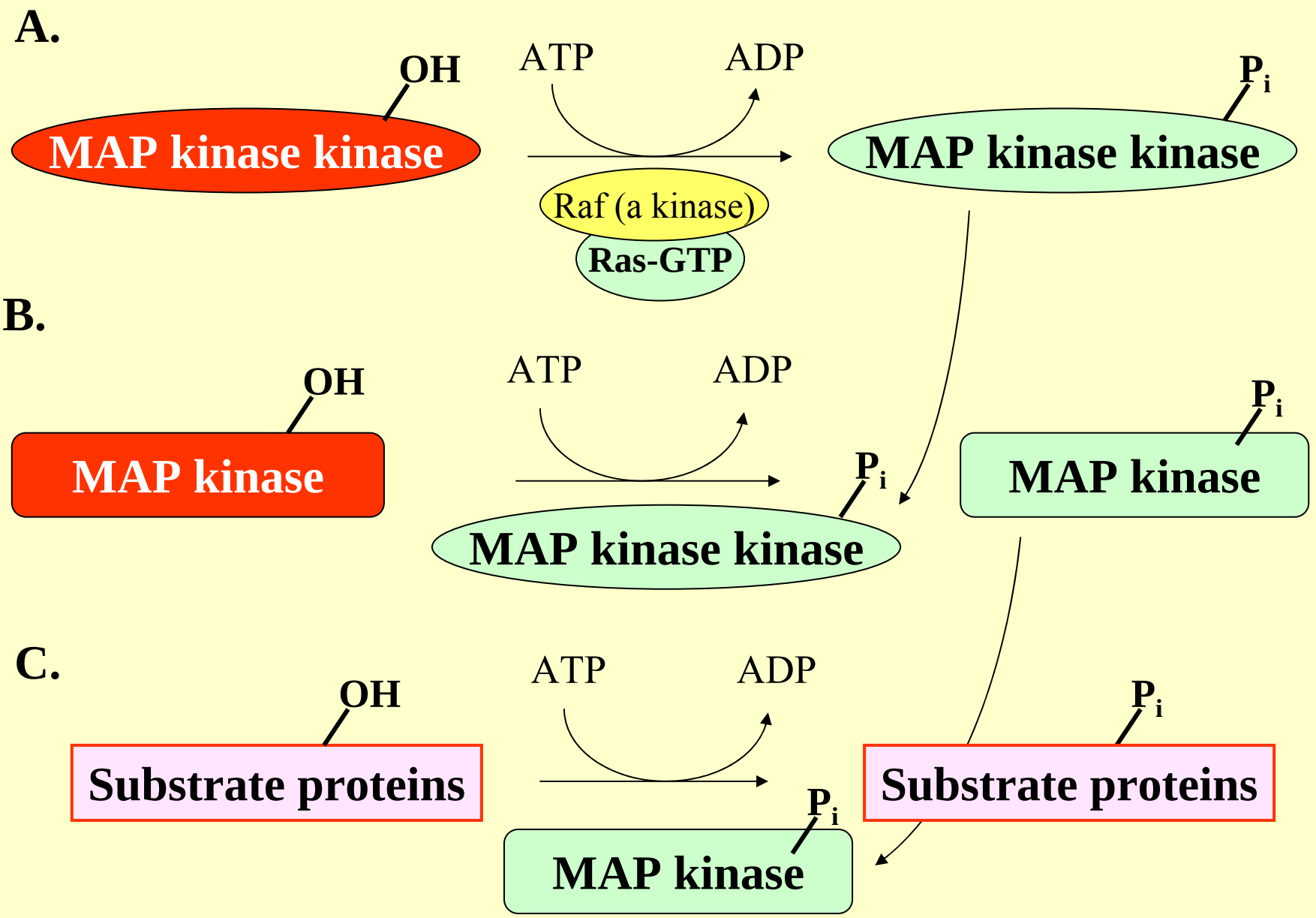


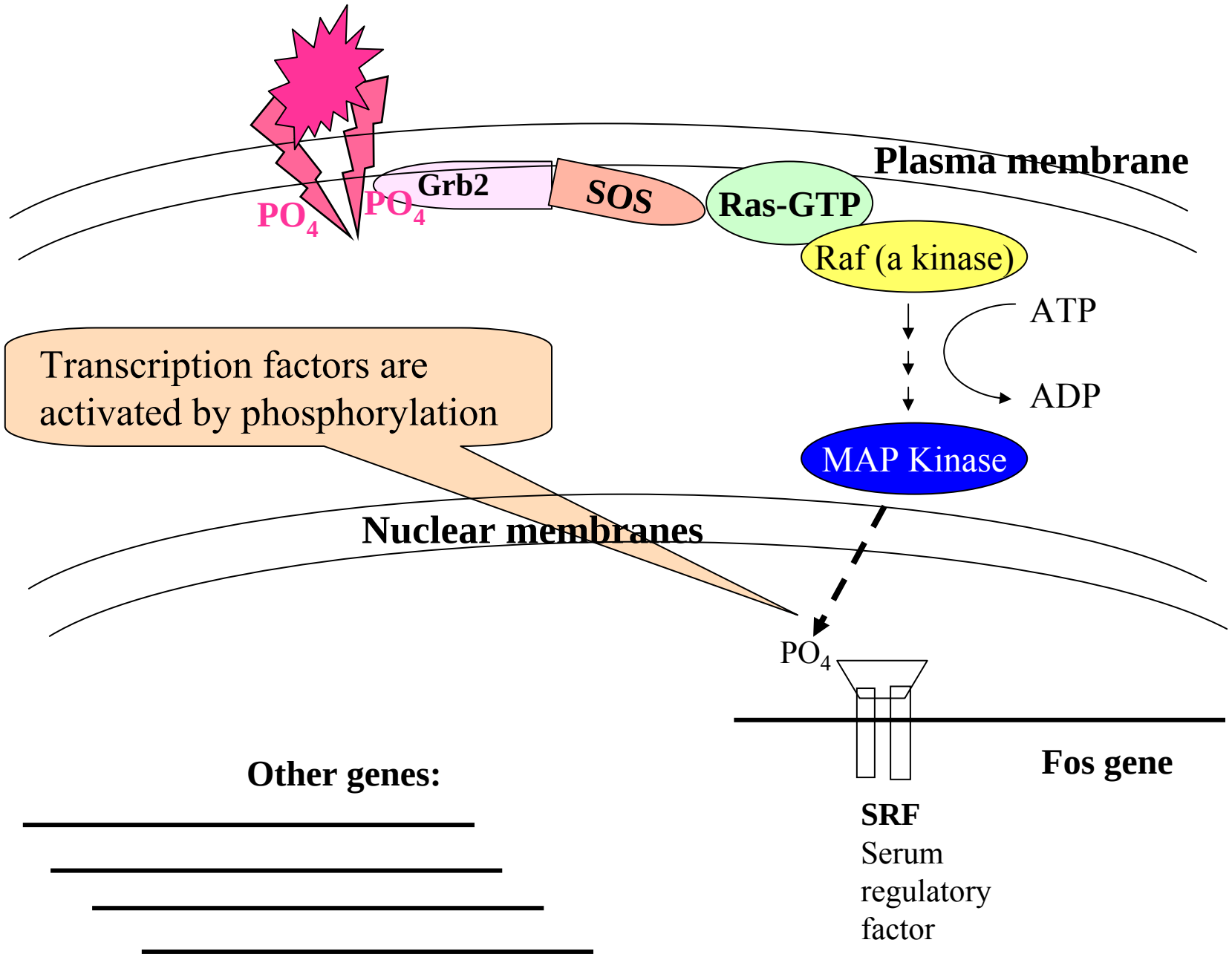
B. Inactivation of Ras

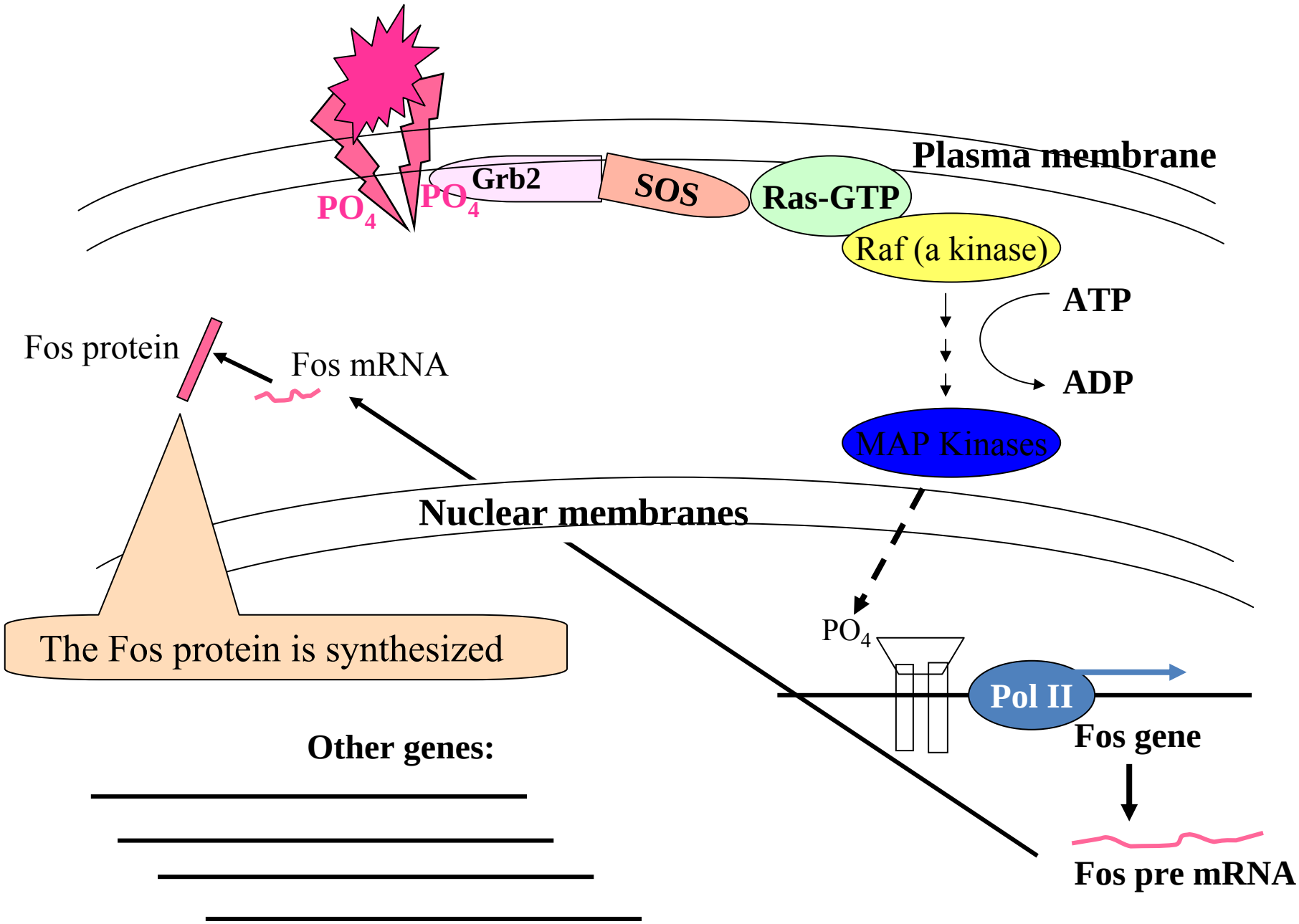


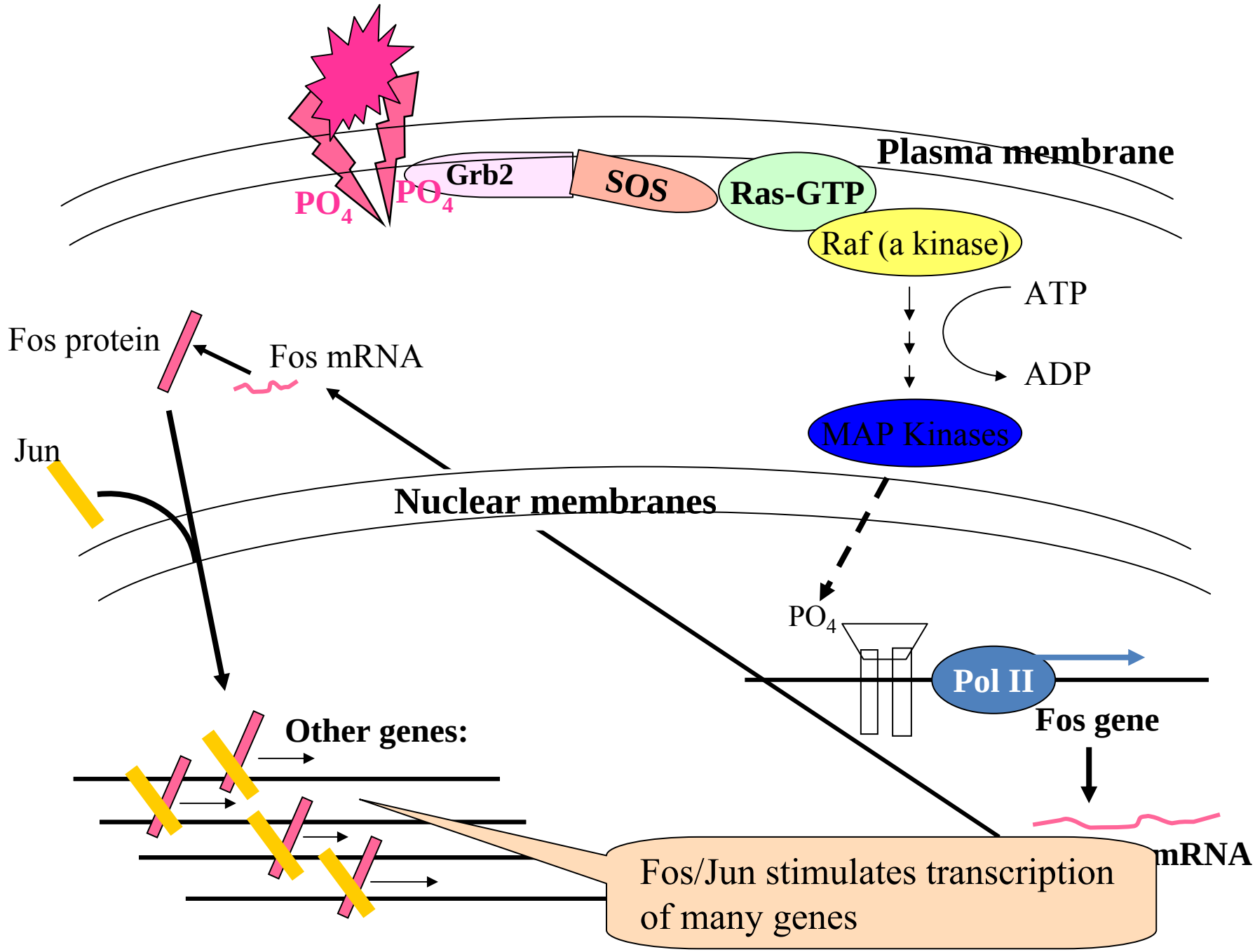

Plasma membrane
PO4
PO4
Grb2
SOS
Ras-GTP
Raf (a kinase)
ATP
ADP
A Series of kinases are activated by phopshorylation
MAP Kinases
Nuclear membranes
Fos gene
Other genes:
SRF
Serum regulatory factor

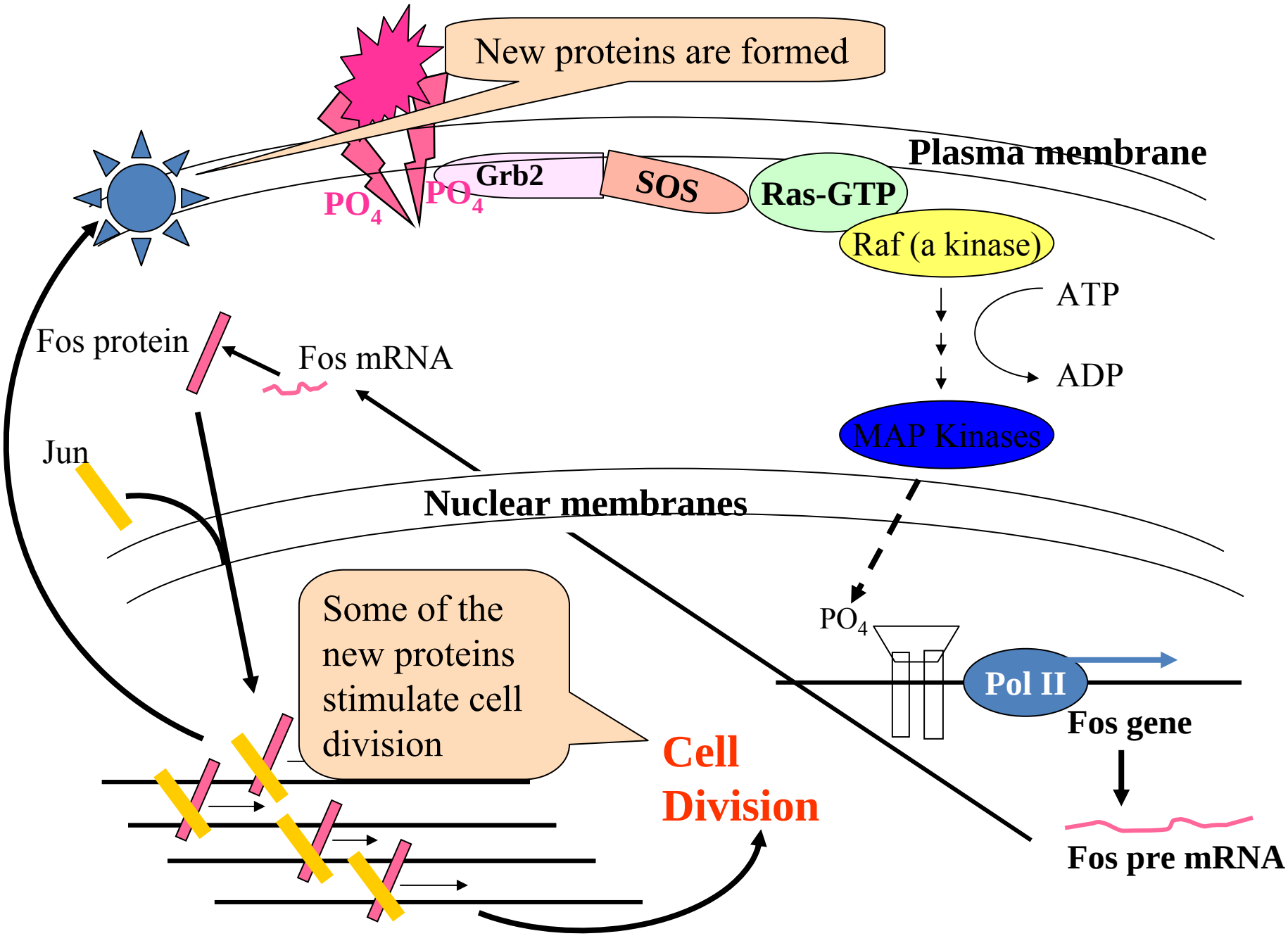
Activating a chain of MAP kinases
(mitogen activated protien kinases)



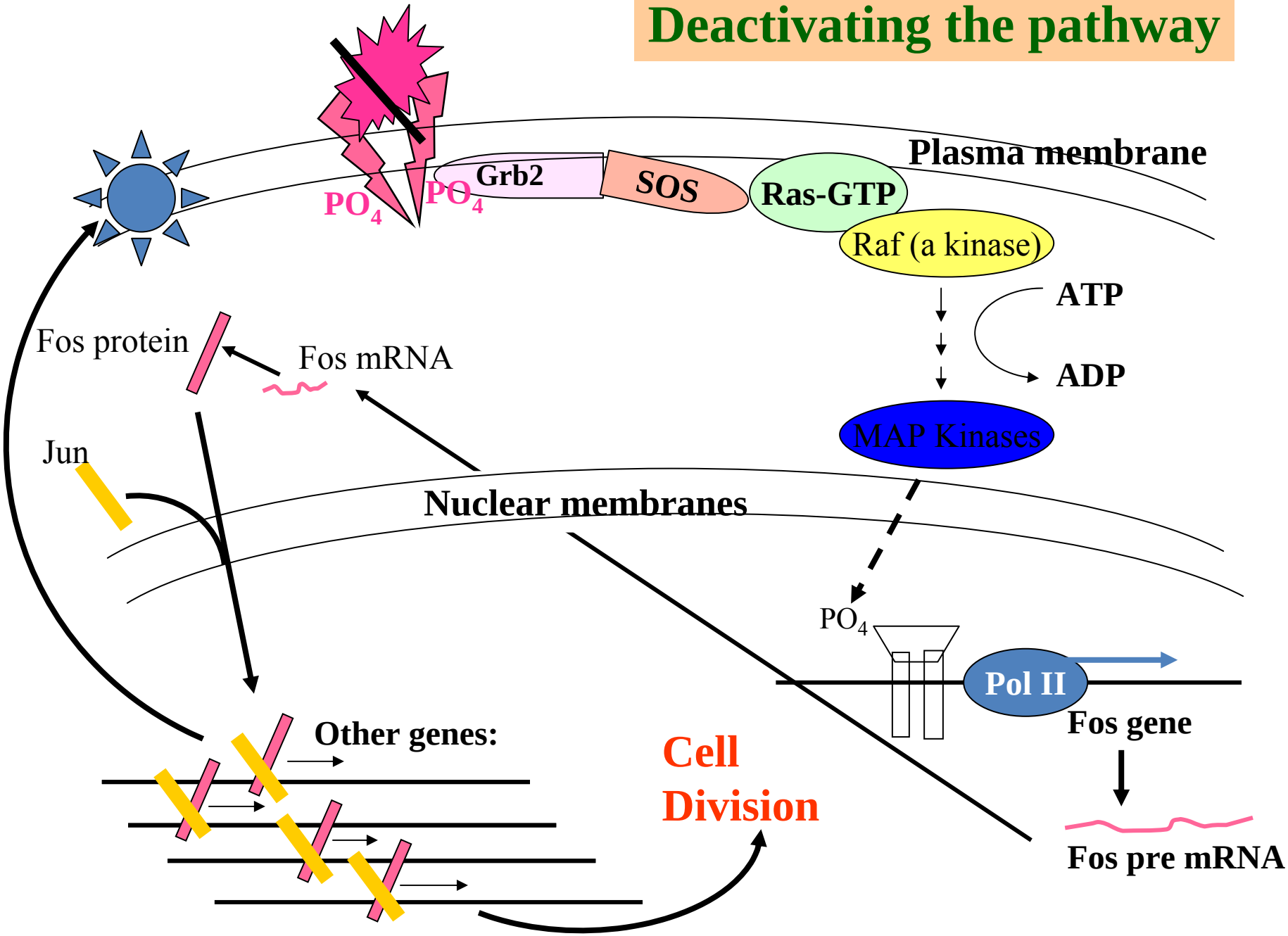

Plasma membrane
PO4
PO4
Grb2
SOS
Ras-GTP
Raf (a kinase)
ATP
ADP
MAP Kinase
Transcription factors are activated by phosphorylation
Nuclear membranes
PO4
Fos gene
Other genes:
SRF
Serum regulatory factor


Plasma membrane
Grb2
SOS
Ras-GTP
Raf (a kinase)
PO4
PO4
ATP
ADP
MAP Kinases
Fos protein
Fos mRNA
Nuclear membranes
The Fos protein is synthesized
PO4
Pol II
Fos gene
Fos pre mRNA
Other genes:

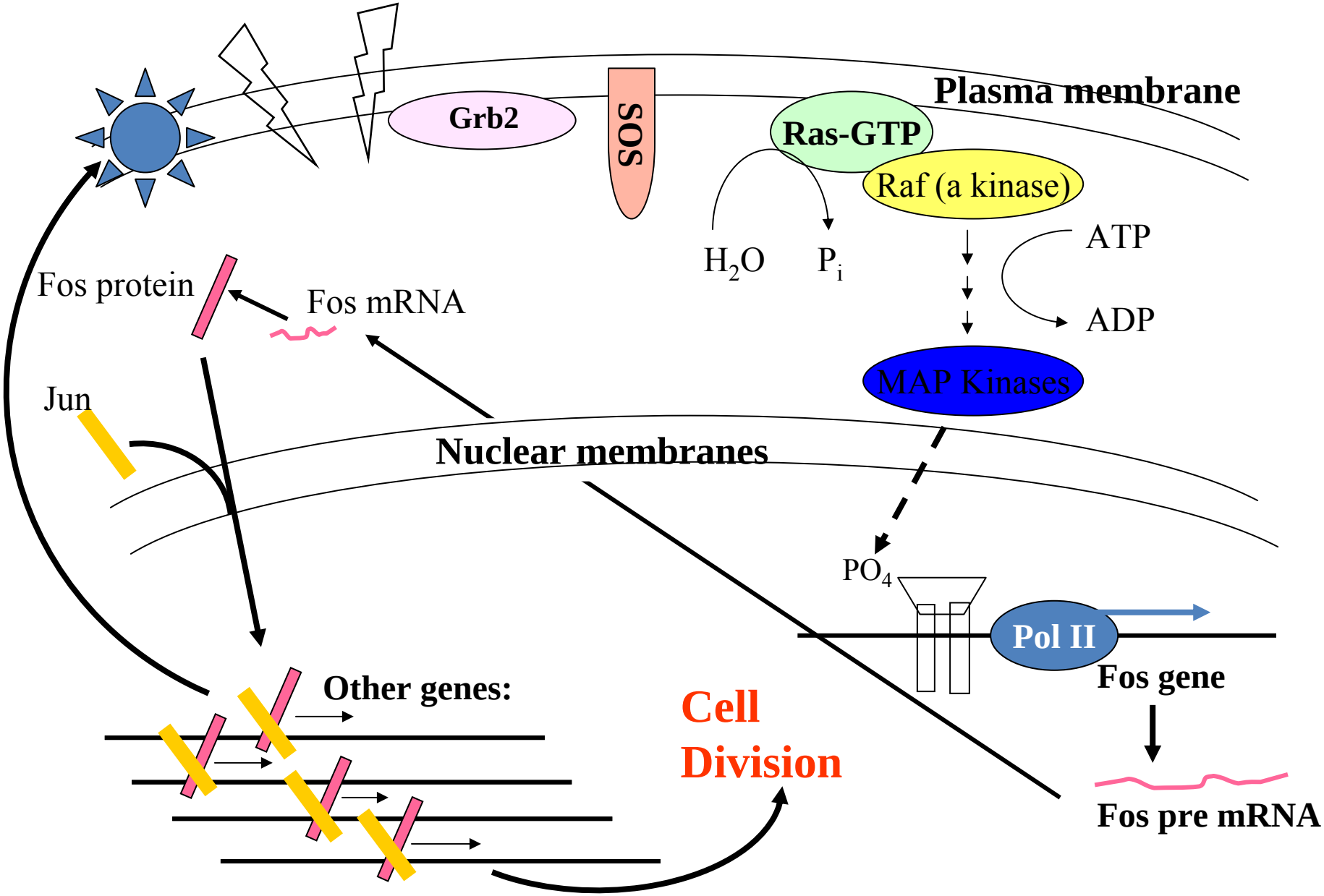

Plasma membrane
PO4
PO4
Grb2
SOS
Ras-GTP
Raf (a kinase)
ATP
ADP
MAP Kinases
Fos protein
Fos mRNA
Jun
Nuclear membranes
PO4
Pol II
Fos gene
mRNA
Other genes:
Fos/Jun stimulates transcription of many genes


New proteins are formed
Plasma membrane
Grb2
SOS
Ras-GTP
Raf (a kinase)
PO4
PO4
ATP
ADP
MAP Kinases
Fos protein
Fos mRNA
Jun
Nuclear membranes
Some of the new proteins stimulate cell division
Cell Division
PO4
Pol II
Fos gene
Fos pre mRNA

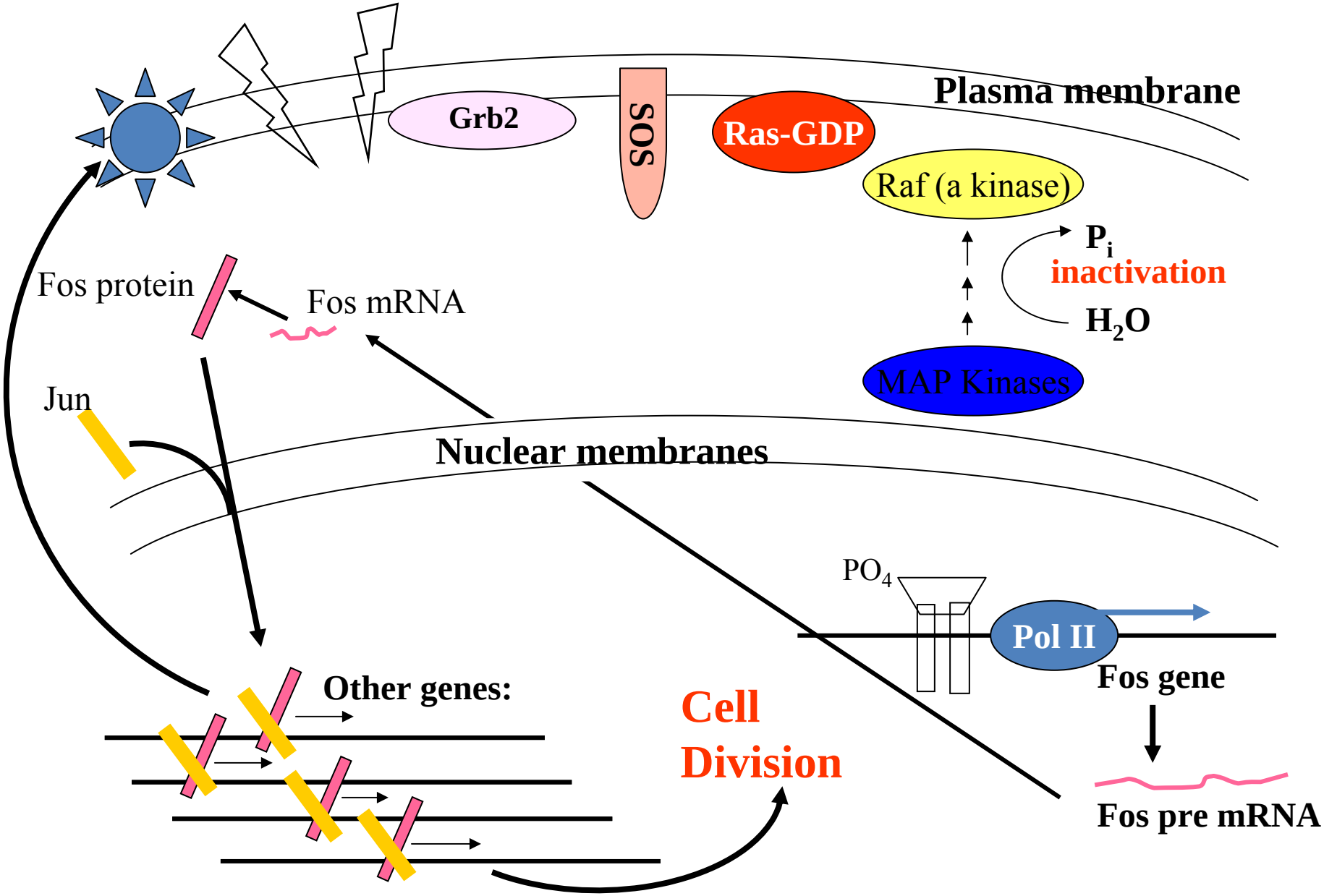
Deactivating the pathway



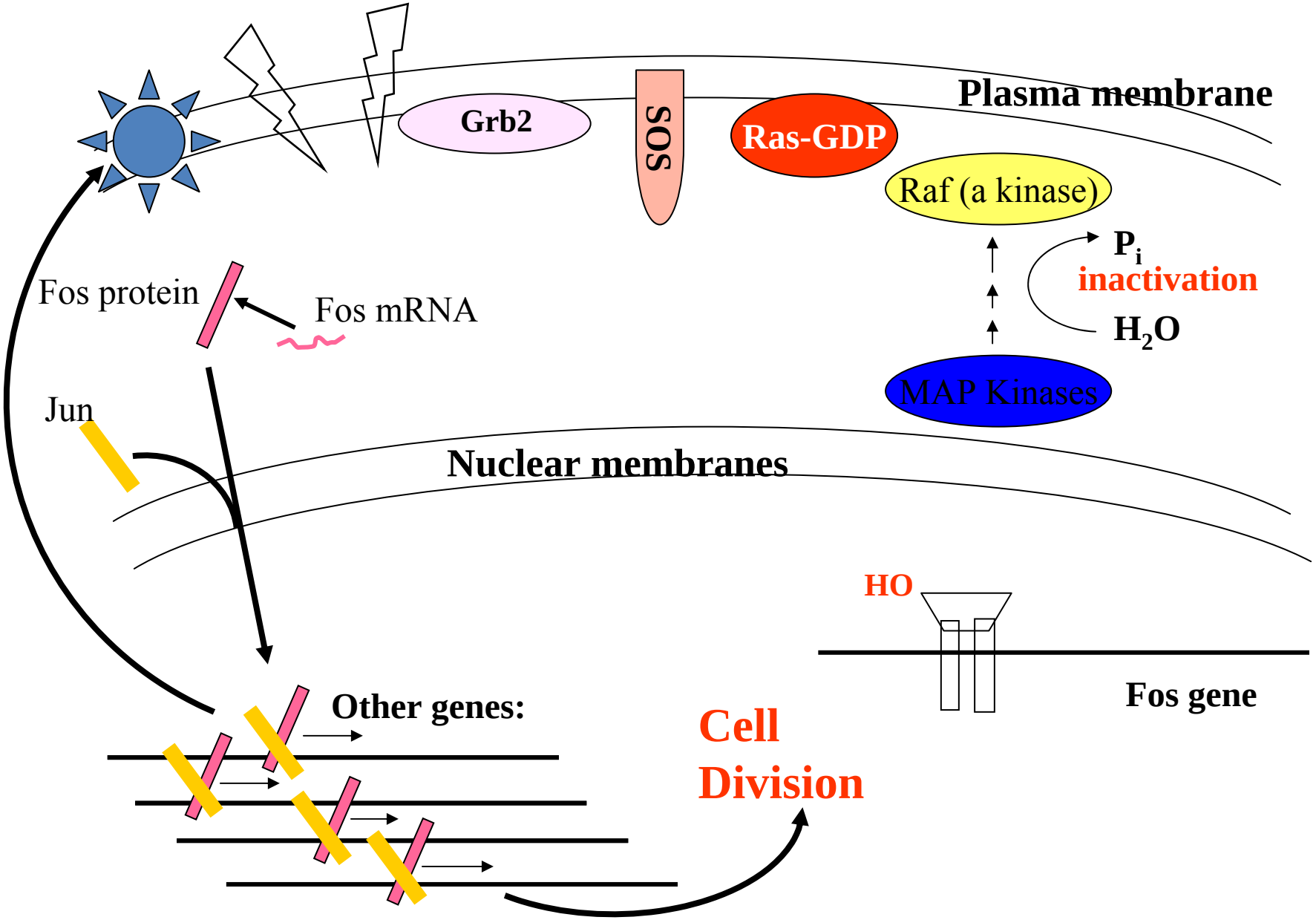
Deactivating the pathway



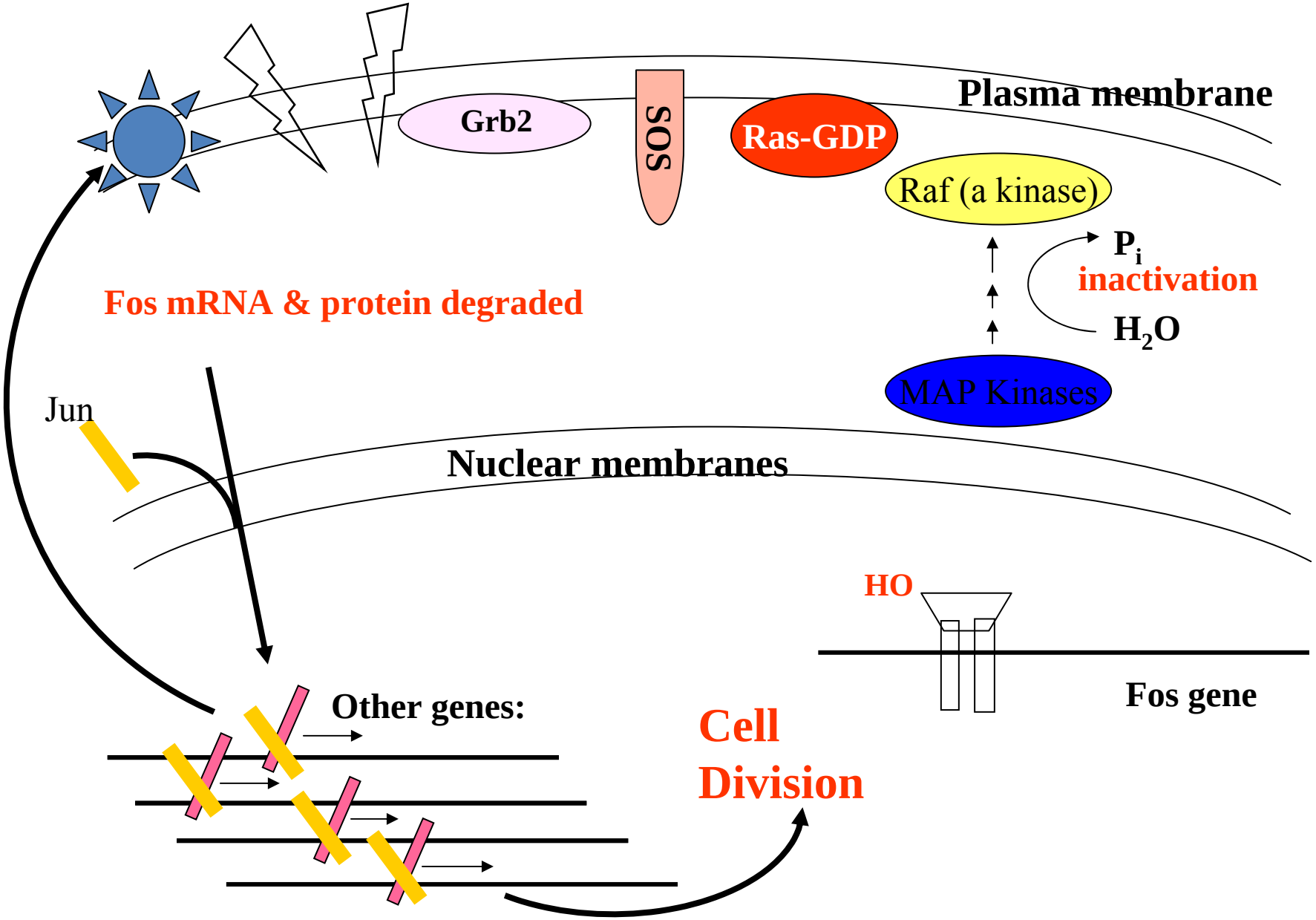
Deactivating the pathway



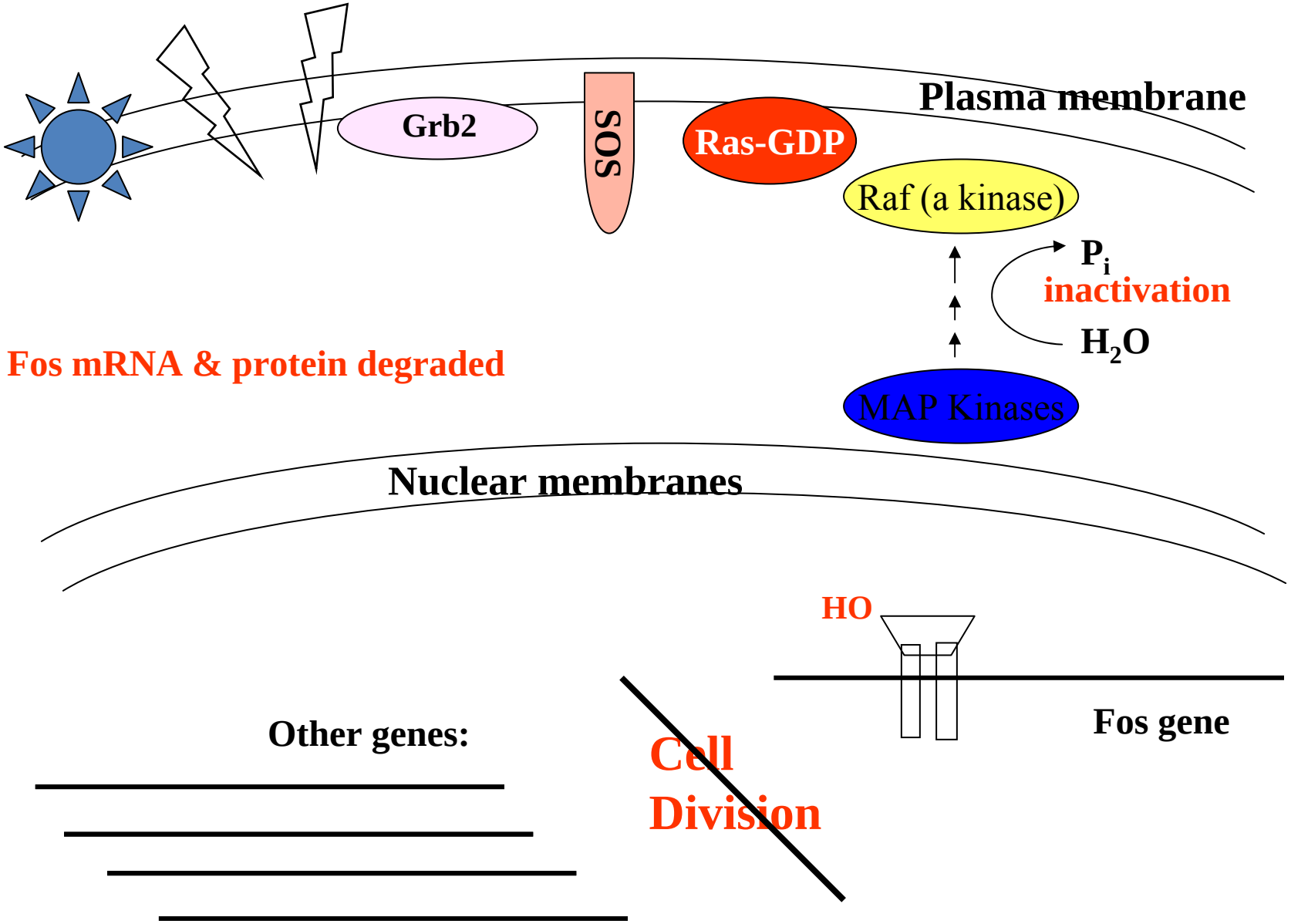
Deactivating the pathway



Deactivating the pathway



Deactivating the pathway



Human Epidermal Growth Factor Receptor Family

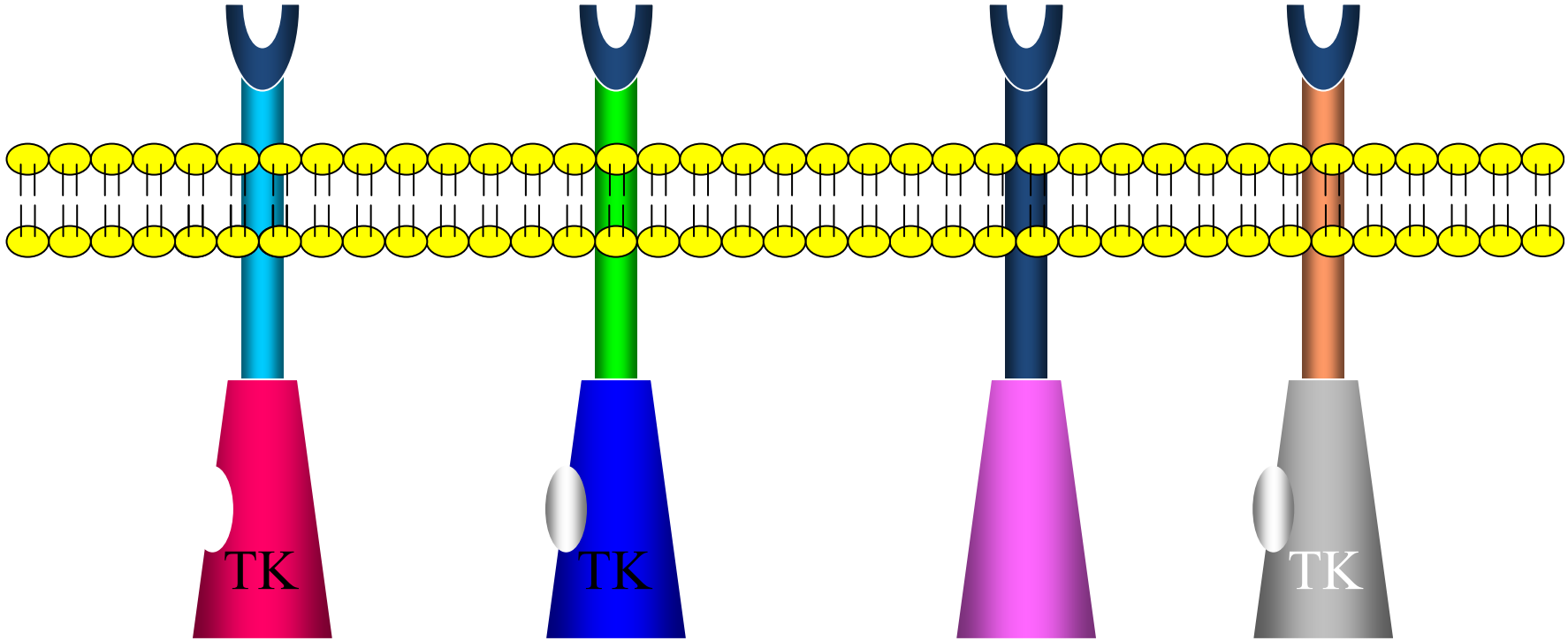
EGF, TGFα , β Cellulin

Amphiregulin, HB-EGF

No specific ligands - often acts as dimer partner

Heregulins

NRG2
NRG3
Heregulins
β-cellulin



erbB1
HER1
EGFR

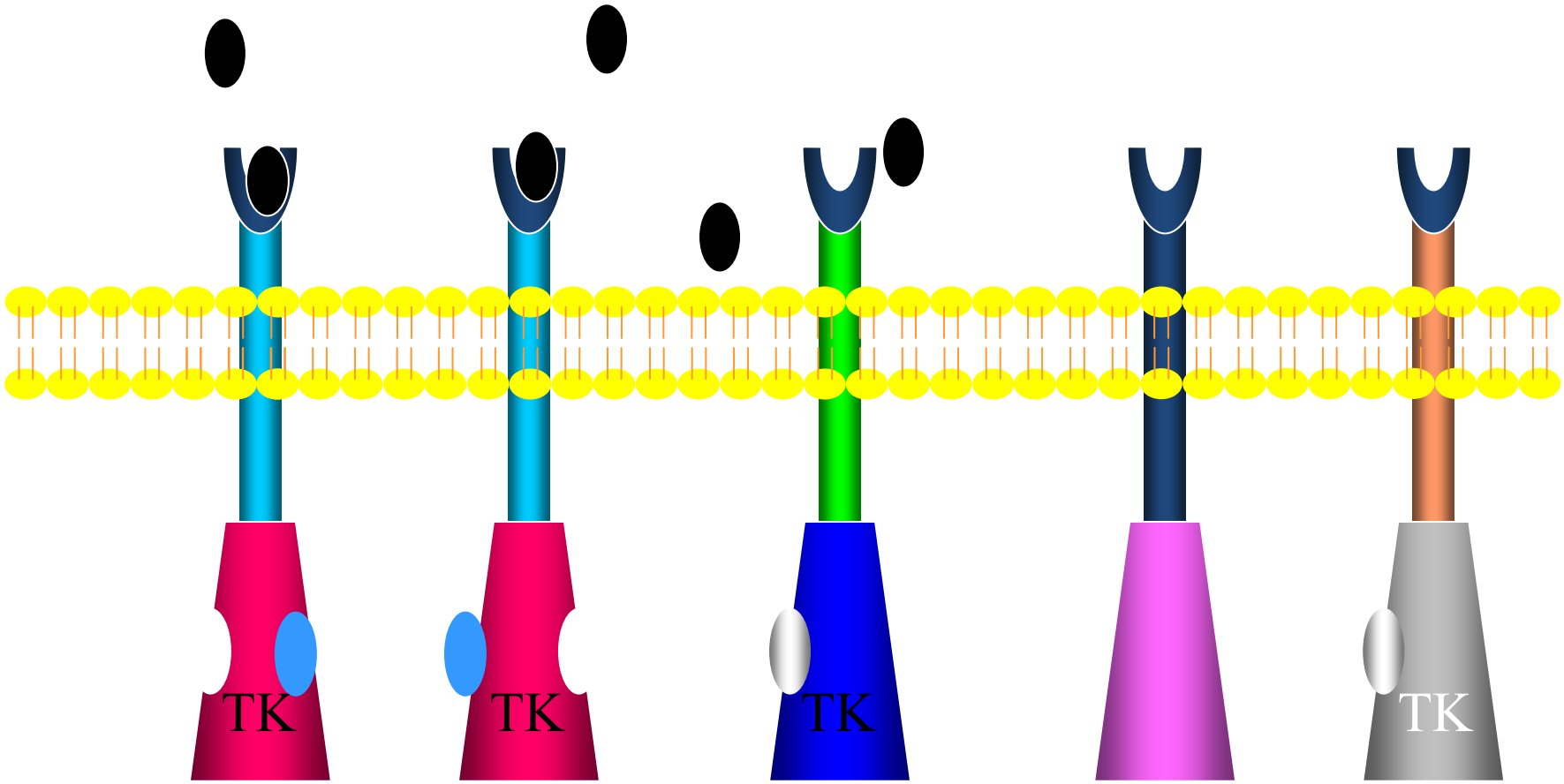
erbB2
HER2
neu

erbB3
HER3

erbB4
HER4

*Adapted from www.iuphar.org/sections/teaching/docs/**EGF**R_inhibitors.**ppt***

EGFR Stimulation & dimerisation



EGFR Homo Dimerisation

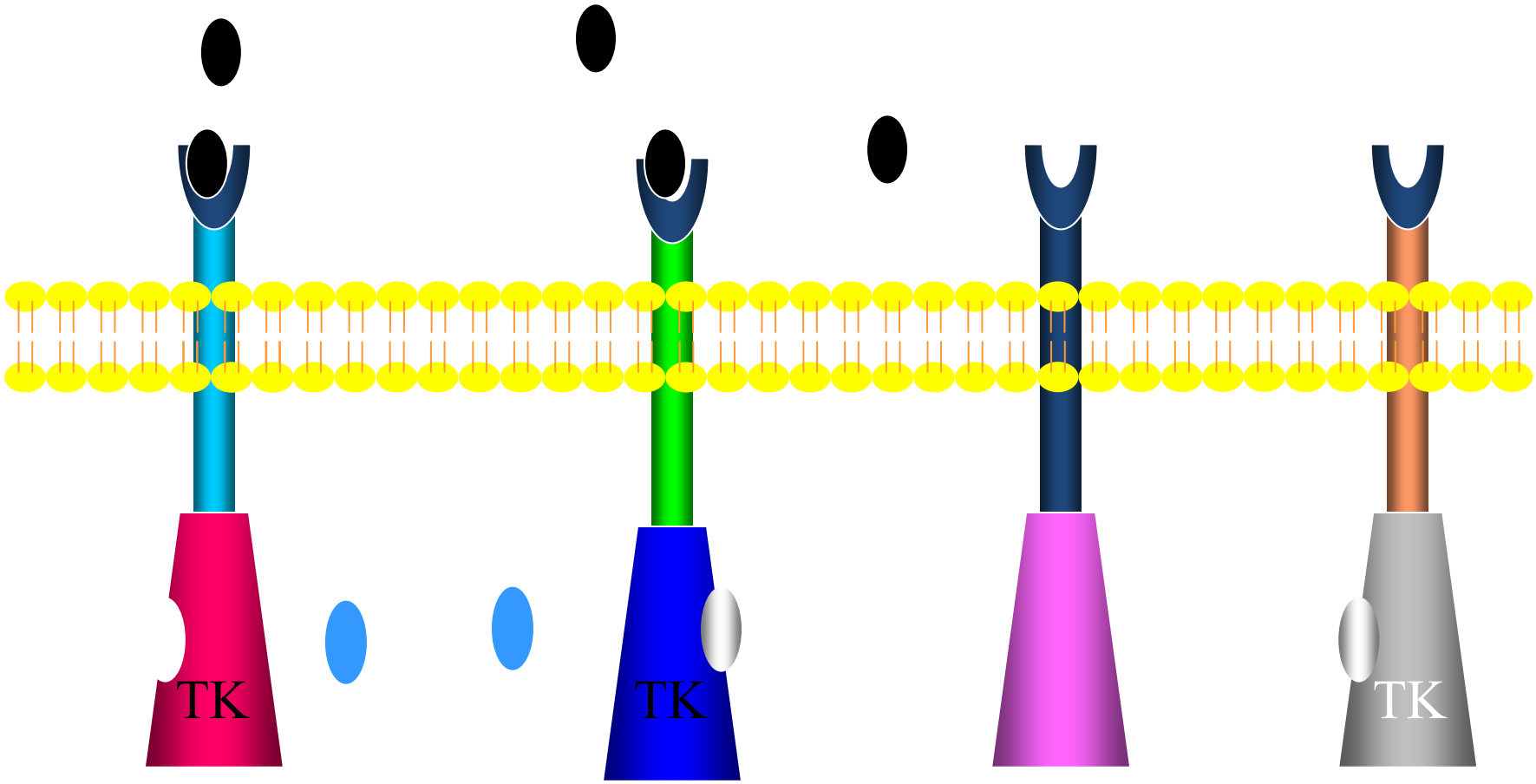
erbB1
HER1
EGFR

erbB2
HER2
neu

erbB3
HER3

erbB4
HER4

*Adapted from www.iuphar.org/sections/teaching/docs/**EGF**R_inhibitors.**ppt***



Hetero Dimerisation

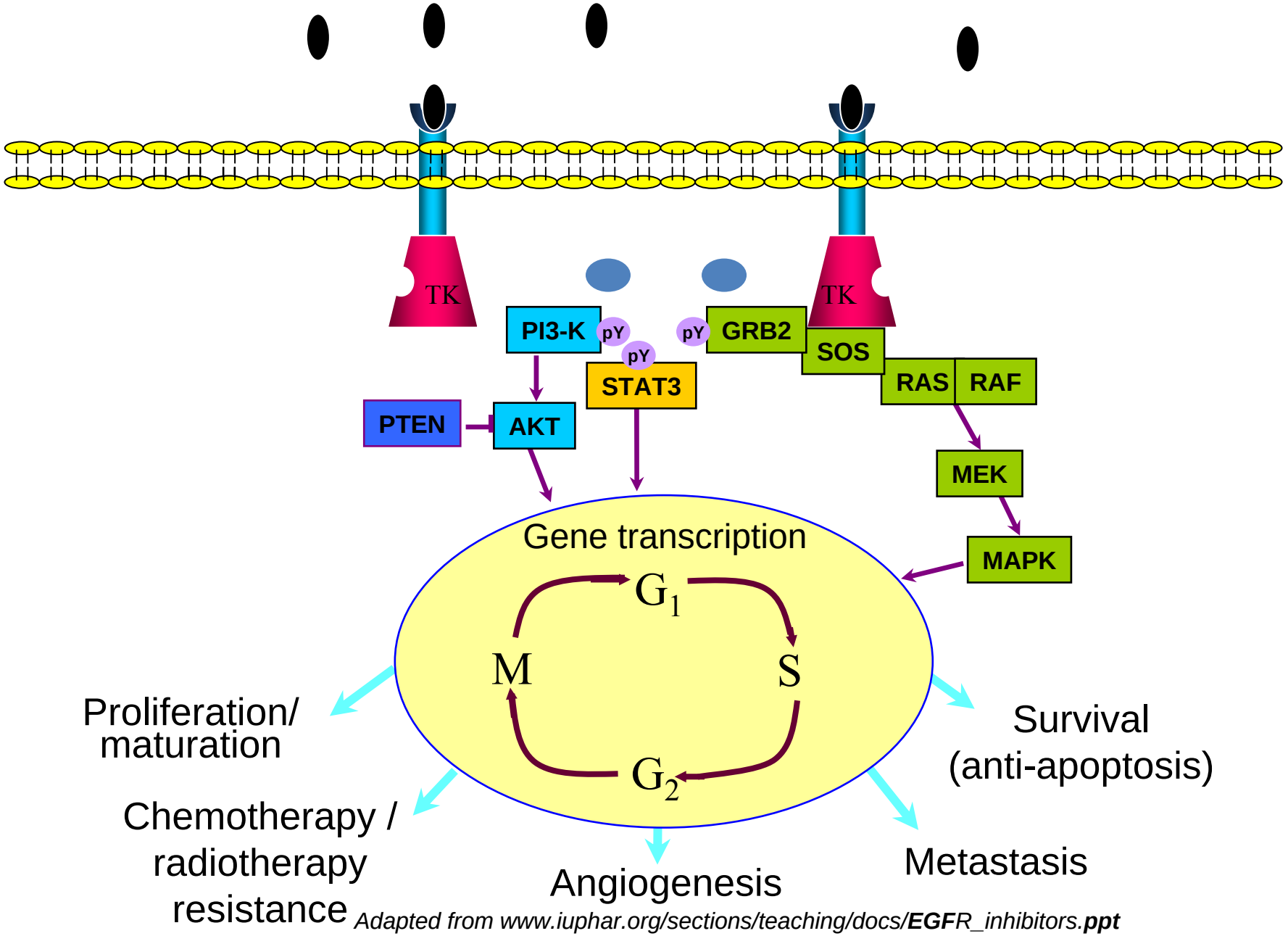
erbB1 **erbB2** **erbB3** **erbB4**

HER1 **HER2** **HER3** **HER4**

Risk for cancer

*Adapted from www.iuphar.org/sections/teaching/docs/**EGF**R_inhibitors.**ppt***

EGFR signal transduction in tumour cells



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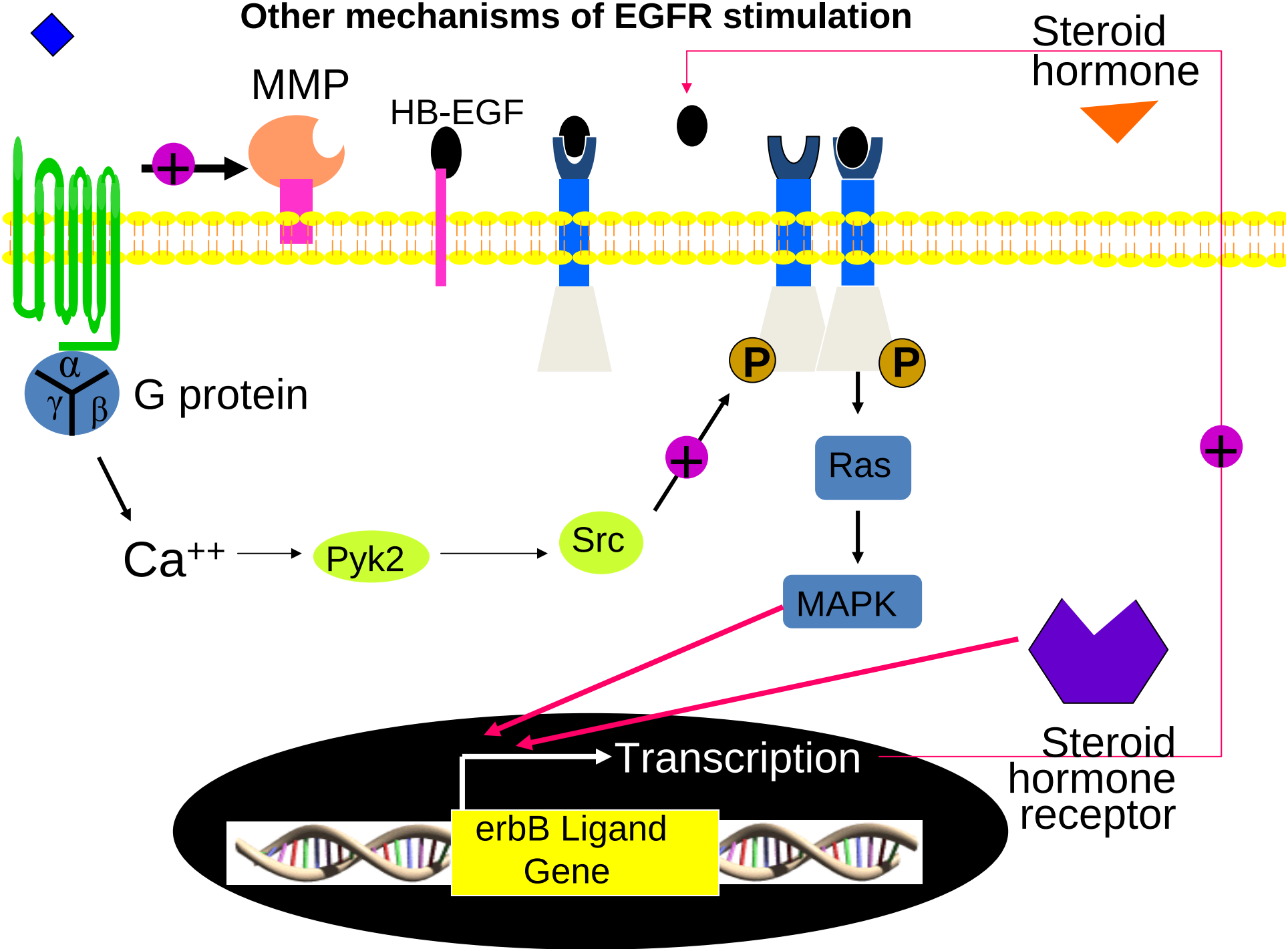
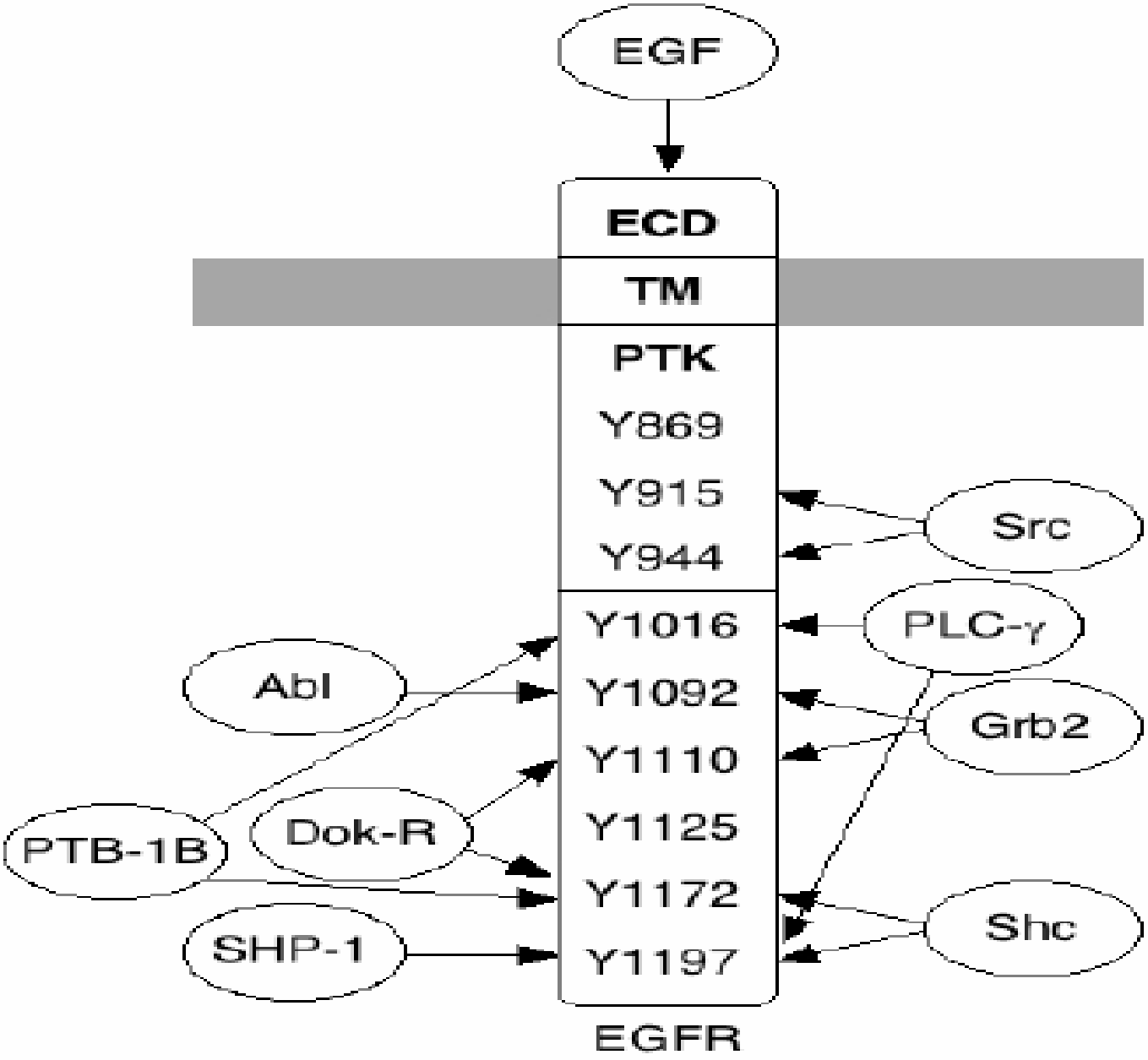

Other mechanisms of EGFR stimulation
Steroid hormone
MMP
HB-EGF
G protein
α
γ
β
P
P
Ras
Ca++
Pyk2
Src
MAPK
Transcription
erbB Ligand Gene
Steroid hormone receptor

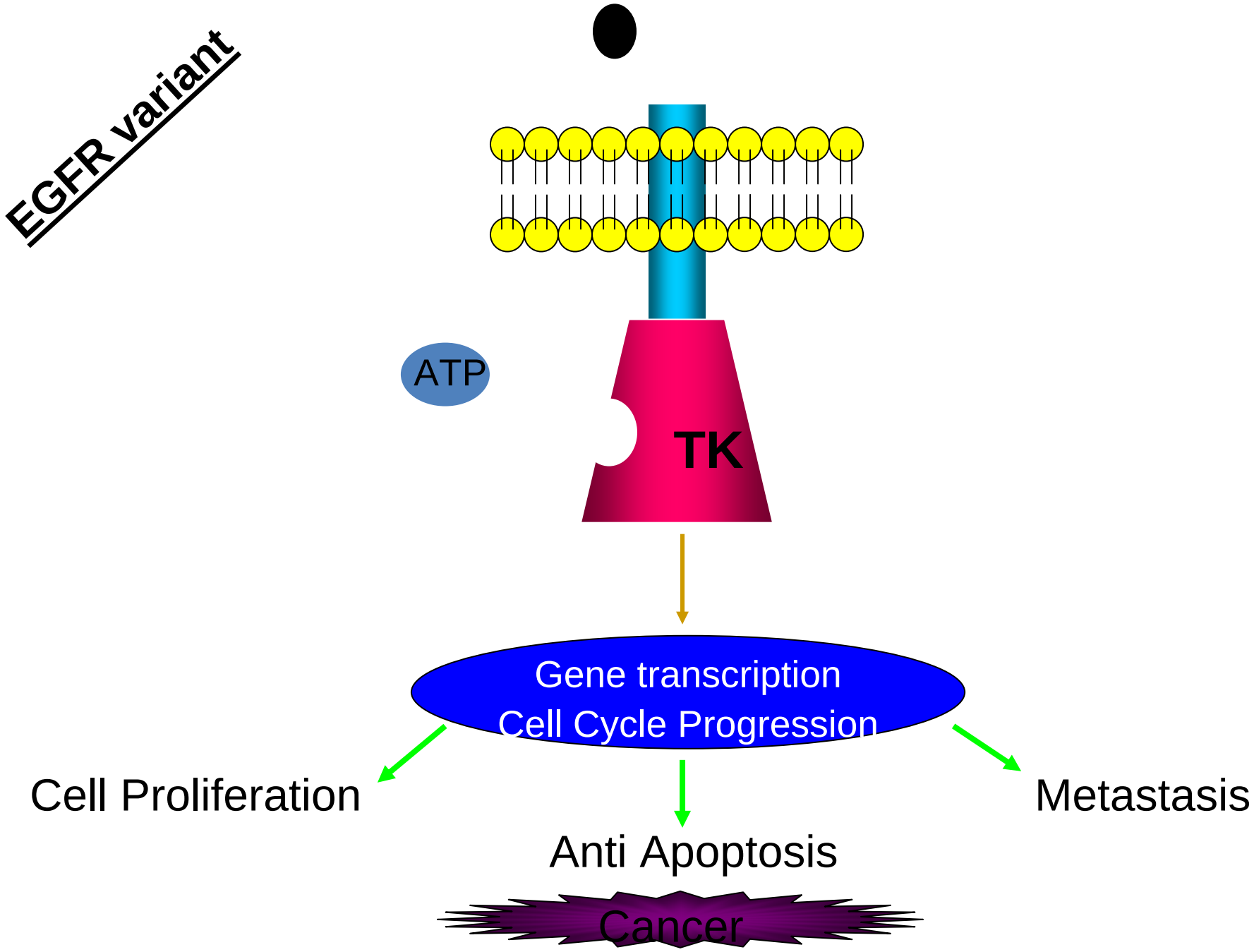
- The effects of activation of GPCRs and RTKs is more complicated than a simple step-by-step cascade
- Stimulation of either GPCRs or RTKs often leads to production of multiple second messengers, and both types of receptors promote or inhibit production of many of the same second messengers
- in addition, RTKs can promote a signal transduction cascade that eventually acts on the same target as the GPCR
- **therefore the same cellular response may be induced by multiple signaling pathways by distinct mechanisms**
- Interaction of different signaling pathways permits fine-tuning of cellular activities


EGF
ECD
TM
PTK
Y869
Y915
Y944
Y1016
Y1092
Y1110
Y1125
Y1172
Y1197
EGFR
Src
PLC-γ
Grb2
Shc
Abl
PTB-1B
Dok-R
SHP-1

Tumour	EGFR Expression Rate
Breast	14 % - 91 %
Colon	25 % - 77 %
Lung Cancer (Non small cell)	40 % - 80 %
Head & Neck	80 % - 95 %
Ovarian	35 % - 70 %
Pancreatic	30 % - 50 %

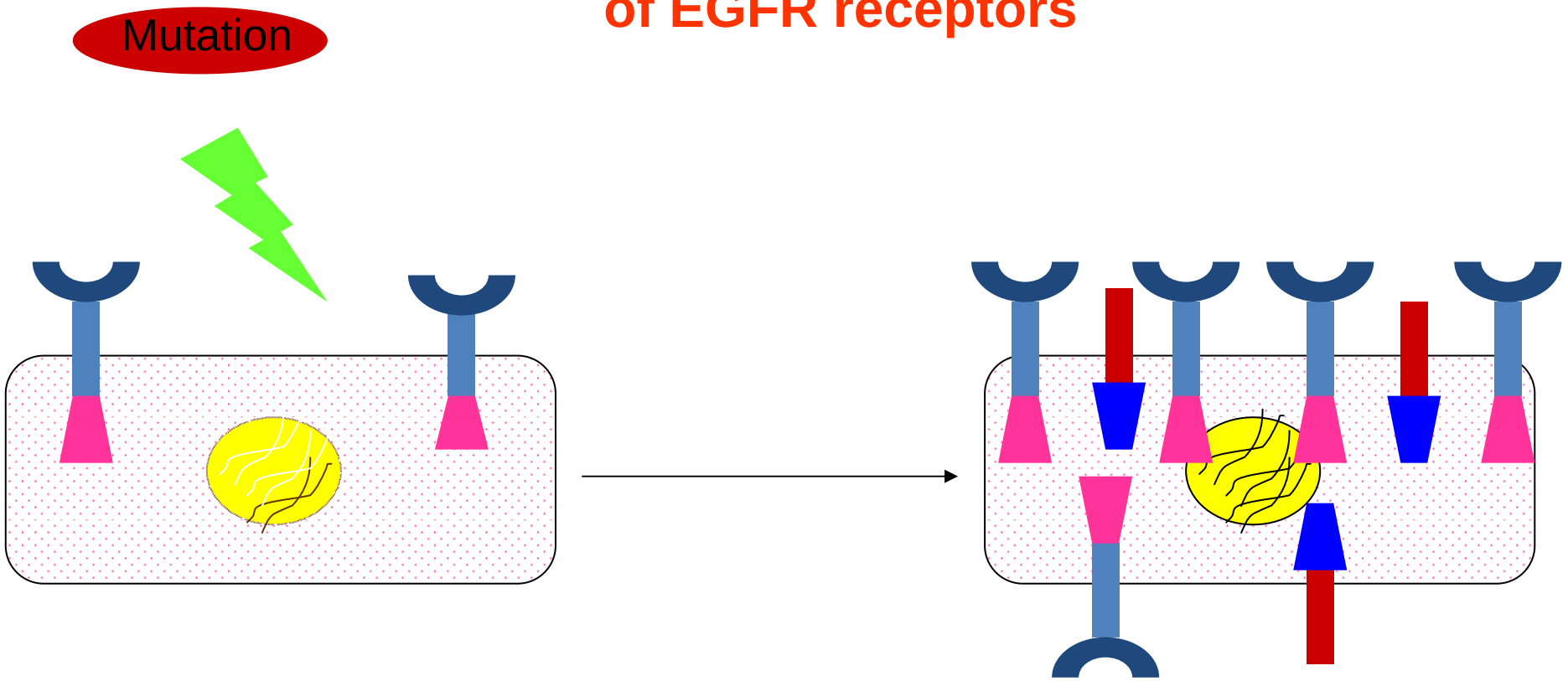
EGFR variants and cancer

EGFR - Variant III	EGFR – Wild Type
No extracellular domain	Present
Ligand cannot bind	Can bind
TK constitutively active	TK activated by ligand binding
Cannot dimerise	Can dimerise
Not found in normal cells	Found normally
More propensity for cancer	Up regulation leads to cancer



*Adapted from www.iuphar.org/sections/teaching/docs/**EGF**R_inhibitors.**ppt***

Consequence of proliferation of EGFR receptors



*Adapted from www.iuphar.org/sections/teaching/docs/**EGF**R_inhibitors.**ppt***

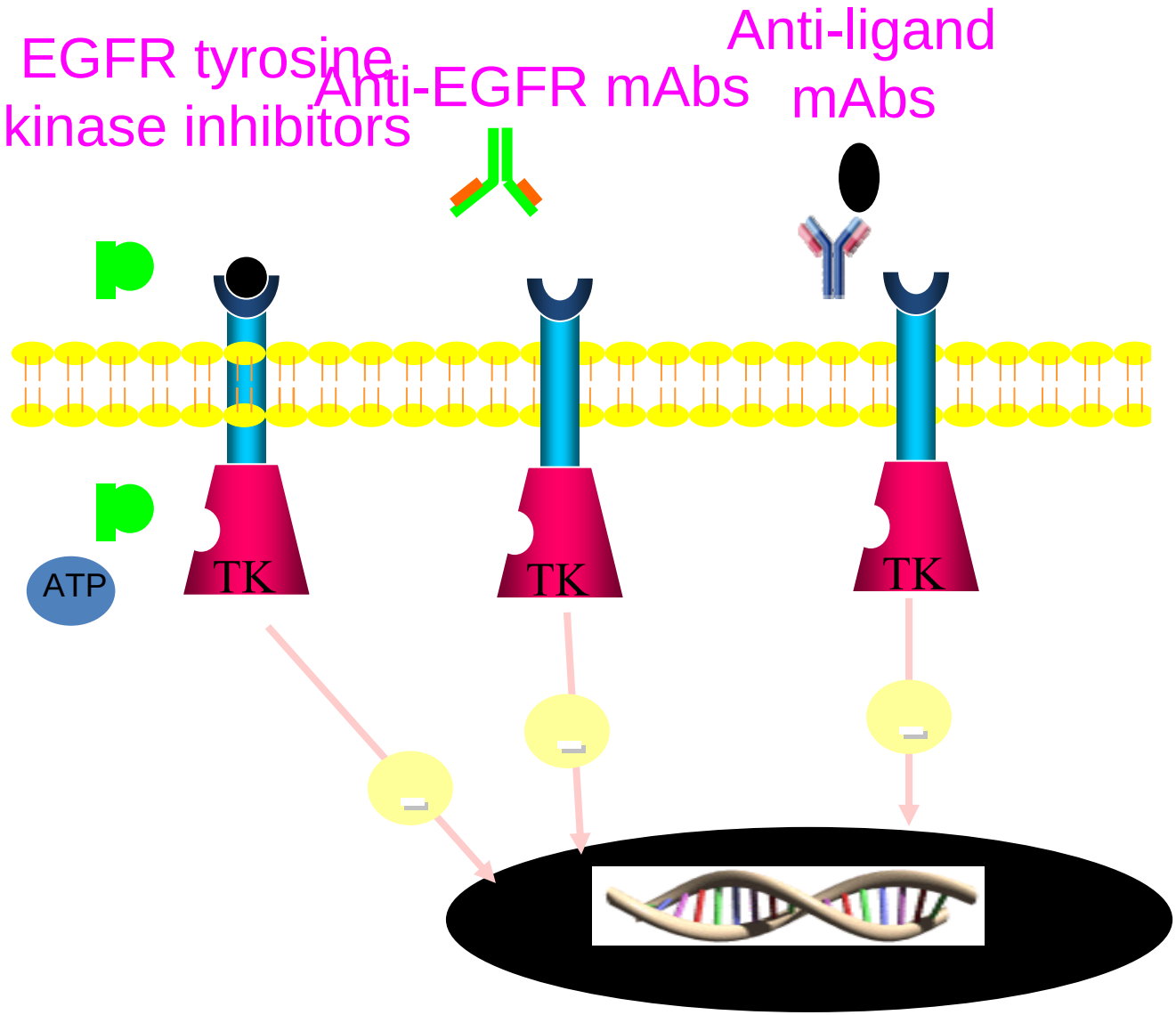
EGFR – a good target for non small cell lung carcinoma

- High level of receptor expression compared with healthy tissue.
- EGFR - Key role in tumour cell growth & function.
- EGFR inhibition can inhibit downstream activity.
- EGFR inhibitors have no severe toxicity.

Rationale for EGFR Inhibitors in Head & Neck cancer

- EGFR expressed in > 90% of head & neck cancers.
- EGFR over expression associated with decreased survival.
- Increased EGFR expression is an early event in carcinogenesis & even present in premalignant lesions.
- Inhibition of EGFR – TK slows the growth of xenograft tumour models of head & neck.

Strategies to inhibit EGFR signaling



*Adapted from www.iuphar.org/sections/teaching/docs/**EGF**R_inhibitors.**ppt***

Drugs Available

- Gefitinib
- Erlotinib

Highly selective, potent & reversible EGFR Tyrosine Kinase Inhibitor

- Cetuximab – Monoclonal Anti EGFR antibody

- H 447
- MDX 210

Bispecific Anti EGFR antibody linked to Anti CD 64

Growth factors with Oncogenic Potential

PDGF, originally shown to regulate proliferation, was also shown to have homology to **v-sis**, the simian sarcoma virus.

Other viral oncogenes encoded protein products that were growth factors that often **overexpressed** in cancer such as TGF-a.

PDGF family
- A chain
- B chain (c-sis)

FGF Family
- acidic FGF
- basic FGF

EGF Family
- EGF
- TGF-a

Neurotrophins
- NGF
- BDNF
- NT3

Cytokines (Hematopoietic)
- IL-2
- IL-3
- M-CSF
- GM-CSF

GF Receptors with Oncogenic Potential

Many oncogenes have been shown to encode for GFRs.

EGFR family

erbB1 (*c-erbB*)

erbB2 (*neu*)

FGF Family

FGFR-1(*fig)*

FGFR-2(K-*sam)*

PDGFR Family

CSF-1R (c-*fms*)

SLF R (c-*kit*)

Insulin Receptor family

IGF-1 (c-*ros*)

Neurotrophins

NGFR (*trk*)

BDNFR (*trk*-B)

NT3 R (*trk*-C)

On-Line Resources

Mechanisms of Signal Transduction
http://www-isu.indstate.edu/thcme/mwking/signal-transduction.html
Clear, illustrated summaries of the various mechanisms of signal transduction

Pathways
http://www.biocarta.com/genes/PathwayGeneSearch.asp?geneValue=g
Comprehensive illustrations of signaling pathways

Extracellular Signal Molecules
http://www.grt.kyushu-u.ac.jp/spad/menu.html
Signals and the pathways stimulated by each

Mammalian MAPK signalling pathways
http://kinase.oci.utoronto.ca/signallingmap.html
MAPK signaling pathway, with information on each component

Small Molecule Platform
http://www.onyx-pharm.com/onyxtech/small_molecule_platform.html
The development of anti-cancer drugs that act on the *ras* signaling pathway

Signal Transduction
http://www.kumc.edu/biochemistry/bioc800/siglofra.htm
Signal transduction from a medical viewpoint

Viruses and Cancer
http://www.geocities.com/tumorbio/vir/vir.htm
History and current summary of viruses and human cancer

Science Maagazine Signal Tansduction Knowledge Environment-Pathways
http://stke.sciencemag.org/cm/index.dtl

Review Articles

1.Soler R.P. HER1/ EGFR Targeting :Refining the strategy. Oncologist 2004 ; 9 : 58 – 67.

2. Herbst R.S, Fukuoka M, Baselga J. Gefitinib – a novel targeted approach to treating canver. Nature rev cancer 2004 ; 4 : 956 – 65.

3. Strausberg R.L, Simpson A.J.G, Old L.J, Riggins G.J. Oncogenomics and the development of new cancer therapies. Nature 2004 ; 429 : 469 – 74.

4. Noble M.E.M, Endicott J.A, Johnson L.N. Protein kinase inhibitors : Insights into drug design from structure. Science 2004 ; 303 : 1800 – 05.

5.Glover K.Y, Soler R.P, Papadimitradopoulou V.A. A review of small molecule Epidermal Growth Factor Receptor specific tyrosine kinase inhibitors in development for non small cell lung cancer. Sem. Oncol. 2004 ; 31 suppl : 83 – 92.

6. Janmaat M.L, Giaccone G. Small molecule Epidermal Growth Factor Receptor tyrosine kinase inhibitors. Oncologist 2003 ; 8 : 576 – 86.

Review Articles

7. Yano S, Nishioka Y, Goto H, Sone S. Molecular mechanism of angiogenesis in non small cell lung cancer and therapeutics trageting related molecules. Cancer sci. 2003 ; 94 : 479 – 85.

8. Vlahovic G, Crawford J. Activation of tyrosine kinases in cancer. Oncologist 2003 ; 8 : 531 – 8.

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Mini Review

1. Levin E.R. Bidirectional signalling between the estrogen receptor and the epidermal growth factor receptor. Mol. Endocrinol. 2003 ; 17 : 309 – 17.

Original Articles

1. Kelly K, Averbuch S. Gefitinib : Phase II and III results in advanced non small cell lung cancer. Sem. Oncol. 2004 ; 31 suppl1 : 93 – 9.
2. Pao W, Wang T, Riley G.J, Miller V.A, Pan Q, Varmus H.E *et al .* KRAS mutations and primary resistance of lung adenocarcinoma to Gefitinib or Erlotinib. PLOS Medicine 2005 ; 2 : e17.

Review Articles by discoverers of EGF and NGF

Stanley Cohen
Origins of Growth factors
Journal of Biological Chemistry August 12, 2008

Rita Levi-Montalcini & Pietro Calissano
The Nerve-Growth Factor. Scientific American 1979, **240**, pp. 44-53.

Landmark papers

1962

Stanley Cohen discovered epidermal growth factor (EGF) in mice

Cohen S. J Biol Chem 1962;237:1555–62

1980

Isolation of human EGF receptor (EGFR) by Stanley Cohen

Cohen S, et al. J Biol Chem 1980;255:4834–42

1984

Human EGFR gene cloned and sequenced

Ullrich A, et al. Nature 1984;309:418–25

On-Line Resources

Mechanisms of Signal Transduction
http://www-isu.indstate.edu/thcme/mwking/signal-transduction.html
Clear, illustrated summaries of the various mechanisms of signal transduction

Pathways
http://www.biocarta.com/genes/PathwayGeneSearch.asp?geneValue=g
Comprehensive illustrations of signaling pathways

Extracellular Signal Molecules
http://www.grt.kyushu-u.ac.jp/spad/menu.html
Signals and the pathways stimulated by each

Mammalian MAPK signalling pathways
http://kinase.oci.utoronto.ca/signallingmap.html
MAPK signaling pathway, with information on each component

On-Line Resources

Small Molecule Platform
http://www.onyx-pharm.com/onyxtech/small_molecule_platform.html
The development of anti-cancer drugs that act on the *ras* signaling pathway

Signal Transduction
http://www.kumc.edu/biochemistry/bioc800/siglofra.htm
Signal transduction from a medical viewpoint

Viruses and Cancer
http://www.geocities.com/tumorbio/vir/vir.htm
History and current summary of viruses and human cancer

Science Maagazine Signal Tansduction Knowledge Environment-Pathways
http://stke.sciencemag.org/cm/index.dtl