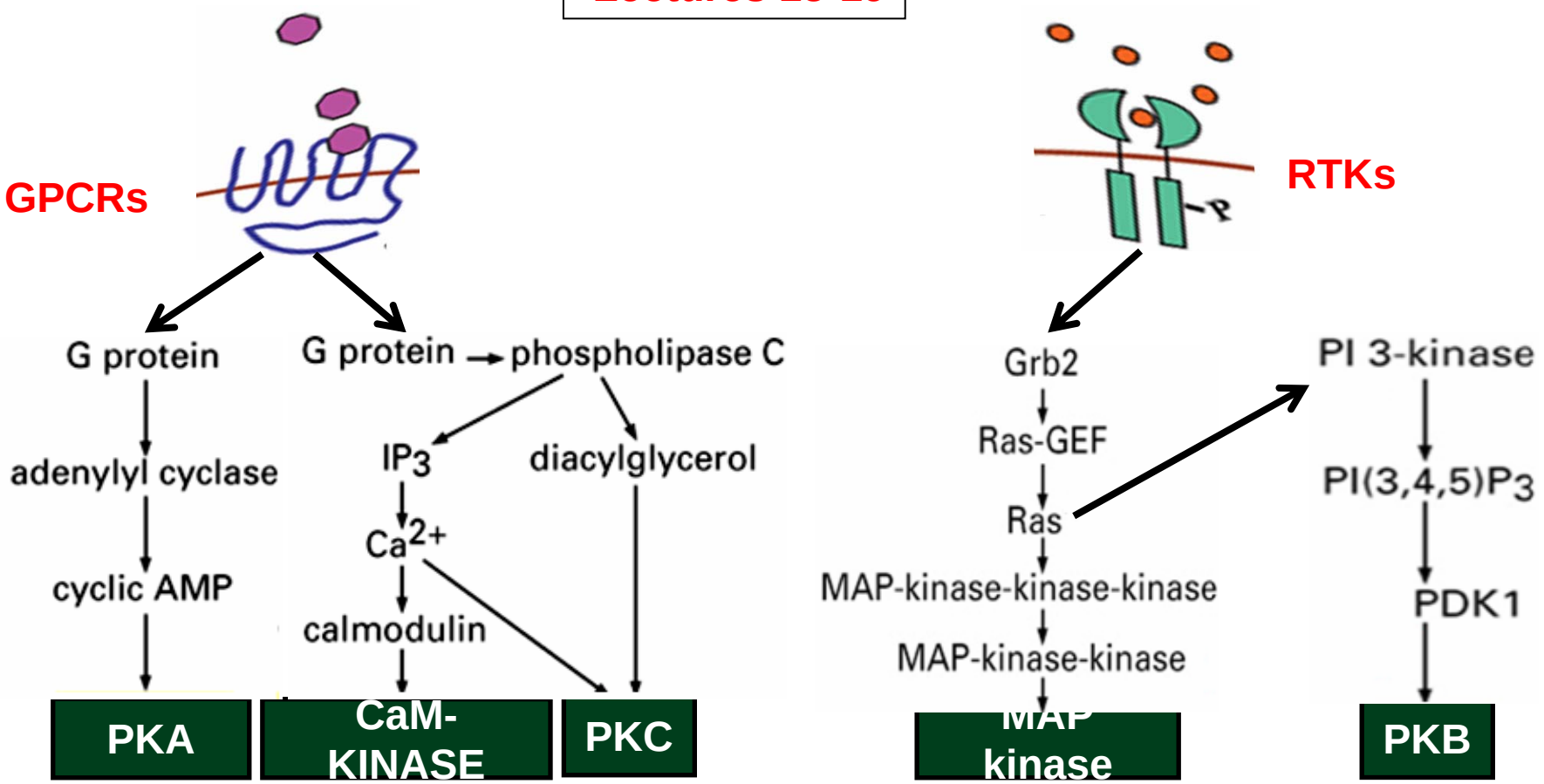
Eukaryotic Gene Expression: Basics & Benefits

P N RANGARAJAN

Lecture 20

Regulation of gene expression by cytokines

Lectures 15-19



Phosphorylation of target proteins

Ex. metabolic enzymes

ion channel proteins

TRANSCRIPTION FACTORS

REGULATION OF GENE EXPRESSION

How cytokines regulate gene expression?

What are cytokines?

Cytokines are proteins produced by diverse cell types and they primarily interact with cells of the immune system in order to regulate the body's response to disease and infection.

Cytokines also regulate several normal cellular processes in the body.

Types of Cytokines

Cytokines are a diverse group of molecules:

- colony stimulating factors (stimulate production of blood cells)
- growth and differentiation factors (function primarily in development)
- immunoregulatory and proinflammatory cytokines (interferons, interleukins and TNF-α that function in the immune system)

How Cytokines Work

Cytokines are released by various cell types cells into the circulation or directly into tissue.

The cytokines locate target immune cells and bind to specific cell surface receptors on the target immune cells.

The cytokine-cytokine receptor interaction activates specific signal transduction pathways leading to the modulation of activity of a number of proteins including transcription factors ultimately leading to distinct physiological responses.

Cytokines and their functions

IL-1 activates Antigen Presenting Cell and CD4+ lymphocytes; affect the differentiation of the B-Cells and T-Cells and other immunocompetent cells and takes part in the regulation of productions of other cytokines and GMCSF (Granulocyte-Macrophage Colony-Stimulating Factor)

IL-2 stimulates the proliferation and activation of B-Cells and T-Cells. IL-4 plays a role in the differentiation of TH2 (T Helper Type-2), in allergic responses, and in the switching of antibody types.

IL-5 stimulates the production and maturation of eosinophils during inflammation.

IL-8 acts as a chemotactic factor that attracts neutrophils, basophils and T-Cells to sites of inflammation.

IL-12 is a critical linker between the innate immunity and adaptive immunity, capable of TH1 (T Helper Type-1) differentiation and IFN-Gamma release by T-Cells and NK cells.

Cytokines and their functions

IL-10 acts to repress secretion of pro-inflammatory cytokines.

IL-3 is a poly potent activator of the hemopoietic cells. It stimulates NK-Cells and acts as a synergist with IL-4 during the induction of CD4+ lymphocyte activation process.

IL-7 is known as the growth factor of the immature B-Cells and T-Cells. It induces apoptosis of tumor cells and causes differentiation of cells from a subgroup of acute myeloblastic leukemia.

IL-9 stimulates the excretion of IL-2, IL-4, IL-6, IL-11, and takes part in a stimulation of cytotoxicity of T-killers and NK-Cells, inducing apoptosis.

IL-11 is a pro-inflammative factor, which regulates the functions of B-Cells and T-Cells. It also takes part in the induction of various killer cells' activities and acts as an autocrine factor for the proliferation of megacaryocytes.

Cytokines and their functions

The secretion of **TNF-Alpha and TNF-Beta** by TH1 cells activates macrophages, inhibits apoptosis of neutrophils and eosinophils, and induces vascular endothelial cells at the sites of infection to change the adhesion molecules they express so phagocytes circulating in the blood can bind to them

IFN-Alpha, IFN-Beta and IFN-Gamma are produced in the area of infection during the early phase of immune response.

IFN-Alpha and IFN-Beta induce proliferation of NK-Cells and stimulate innate and adaptive immune responses that are specifically targeted to virus infections.

Upon activation, NK cells release **IFN-Gamma,** which activates macrophages to secrete cytokines that help to activate macrophages to secrete cytokines that help to activate T-Cells and promote the initiation of T-Cell responses.

Cytokines are key regulators of immune functions

They alter gene expression and promote cell growth

They either boost (IL-2) or suppress (i.e., IL-10, and TGFβ1) immunity

Type I IFN and TNFa are mediators of natural/innate immunity

IL-2, IL-4, IL-5, IL-12, IL-1 are regulators of lymphocytic growth, activation and differentiation

IFN g is an activator of inflammatory cells

IL-3, GM-CSF, IL-7 stimulate hematopoiesis

Overproduction of Cytokines

Overproduction or inappropriate production of certain cytokines by the body can lead to diseases.

For example, in case of rheumatoid arthritis, cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha) are produced in excess and these leads to inflammation and tissue destruction.

IL-4 causes allergic diseases

IL-17 causes autoimmune diseases

A number of drugs have been developed which act by inhibiting the action of cytokines

Kineret (anakinra)

Used in the treatment of rheumatoid arthritis.
It works by inhibiting IL-1 binding to its receptor.

Enbrel (etanercept)
Remicade (infliximab)
Humira (adalimumab)

These are TNF-alpha inhibitors (*a.k.a.* TNF blockers)

They bind TNF and prevent TNF from binding to cell surface receptors.

How do these cytokines act?

The first step in Cytokine action

is their binding to cytokine receptors

CYTOKINE RECEPTORS

Cytokine Receptors

- Type I
- Type II
- Soluble

Type I cytokine receptors	Type 1 interleukin receptors Erythropoietin receptor GM-CSF receptor G-CSF receptor Growth hormone receptor prolactin receptor Oncostatin M receptor Leukemia inhibitory factor receptor
Type II cytokine receptors	Type II interleukin receptors Interferon-alpha/beta receptor Interferon-gamma receptor
Immunoglobulin superfamily	Interleukin 1 receptor CSF1 C-kit receptor Interleukin-18 receptor

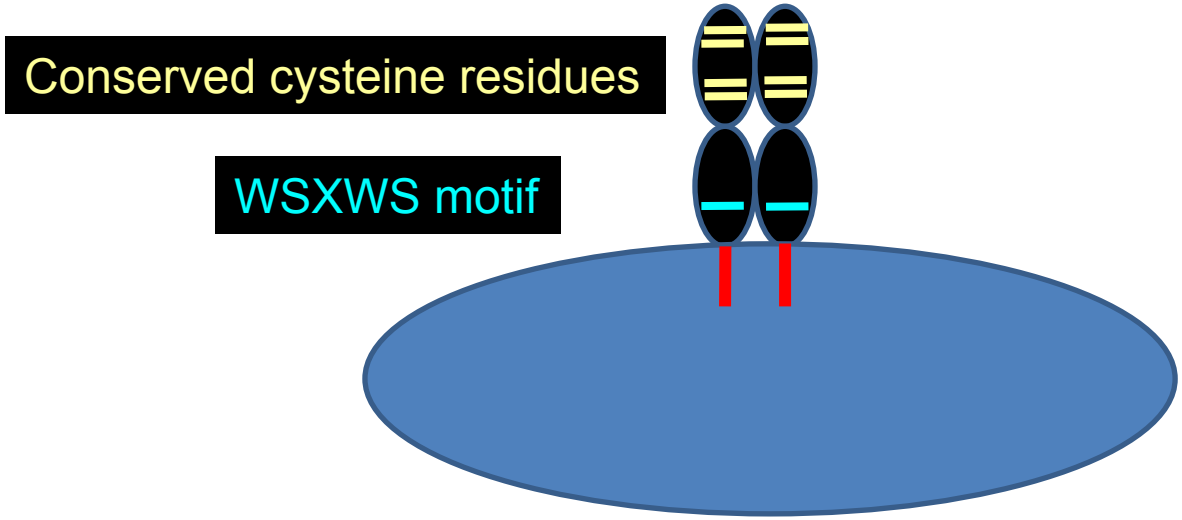
Tumor necrosis factor receptor family	CD27 CD30 CD40 CD120 Lymphotoxin beta receptor
Chemokine receptors	Interleukin-8 receptor CCR1 CXCR4 MCAF receptor NAP-2 receptor
TGF beta receptors	TGF beta receptor 1 TGF beta receptor 2

Type I cytokine receptors

These are transmembrane receptors expressed on the surface of cells that recognize and respond to cytokines with four α-helical strands.

They share a common amino acid motif (WSXWS) in the extracellular portion adjacent to the cell membrane.

Members of the type I cytokine receptor family comprise different chains, some of which are involved in ligand/cytokine interaction and others that are involved in signal transduction.



Type II cytokine receptors

These are transmembrane proteins that are expressed on the surface of certain cells, which bind and respond to a select group of cytokines.

These receptors are similar to type I cytokine receptors **except they do not possess the signature sequence WSXWS that is characteristic of type I receptors.**

The intracellular domain of type II cytokine receptors is typically associated with a tyrosine kinase belonging to the Janus kinase (JAK) family.

Type I cytokine receptors

Interleukin receptors
Interleukin-2 receptor
Interleukin-3 receptor
Interleukin-4 receptor
Interleukin-5 receptor
Interleukin-6 receptor
Interleukin-7 receptor
Interleukin-9 receptor
Interleukin-11 receptor
Interleukin-12 receptor
Interleukin-13 receptor
Interleukin-15 receptor
Interleukin-21 receptor
Interleukin-23 receptor
Interleukin-27 receptor

Colony stimulating factor receptors
Erythropoietin receptor
GM-CSF receptor
G-CSF receptor

Others
growth hormone receptor
prolactin receptor
Oncostatin M receptor
Leukemia inhibitory factor receptor

Type II cytokine receptors

Interferon receptors
interferon-alpha/beta receptor
interferon-gamma receptor

Interleukin receptors
Interleukin-10 receptor
Interleukin-20 receptor
Interleukin-22 receptor
Interleukin-28 receptor

Once the cytokine binds to its receptor

what happens?

Cytokine signal transduction pathways

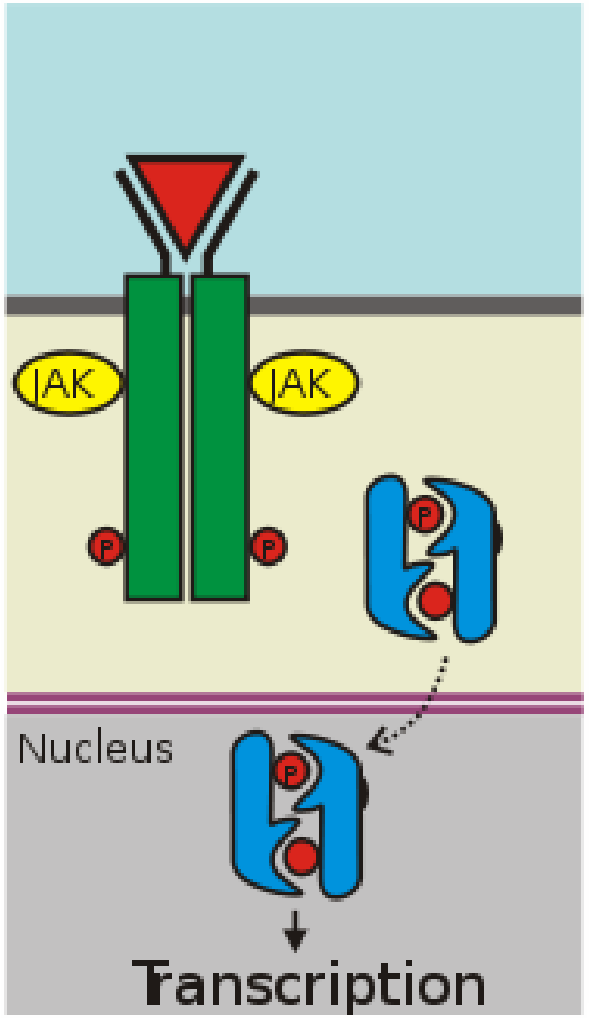
THE JAK-STAT PATHWAY

The cytokine receptors lack catalytic domains (unlike RTKs).

On binding to cytokines, they interact with members of a unique the family of cytoplasmic protein tyrosine kinases known as the **Janus kinases (JAKs)**.

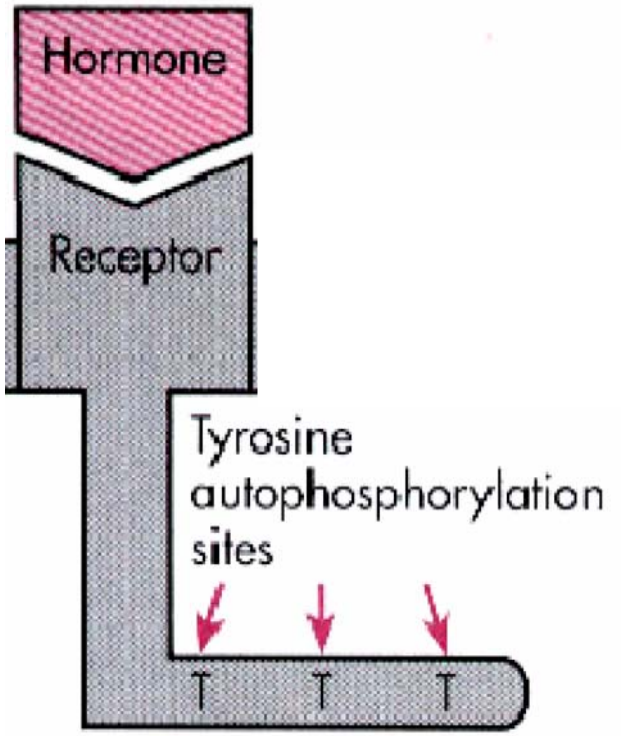
JAKs physically associate with the membrane-proximal region of the ligand-bound receptor, leading to their tyrosine phosphorylation and activation.

The activated JAKs phosphorylate the receptors as well as cytoplasmic proteins belonging to a family of transcription factors called the **signal transducers and activators of transcription (STATs),** providing a novel signaling pathway that is shared by all members of the cytokine receptor superfamily.



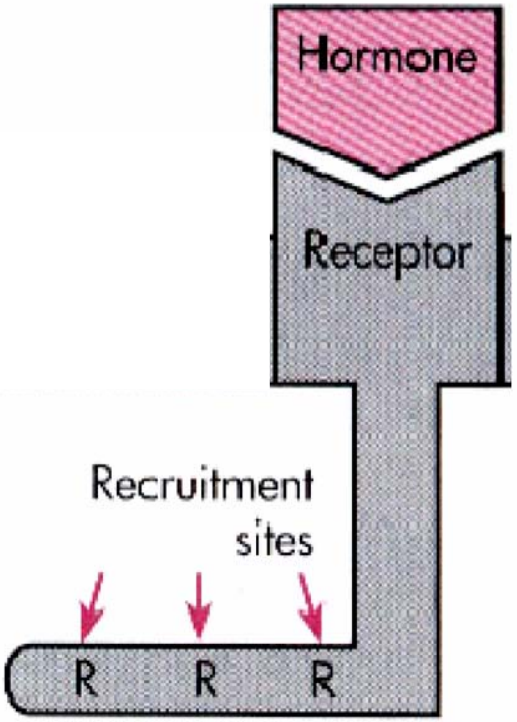
Adapted from: http://en.wikipedia.org/wiki/File:Jakstat_pathway.svg

Growth factor signalling (RTKs)



Receptor becomes tyrosine kinase

Cytokine signalling (JAK-STAT)



Receptor becomes docking site for tyrosine kinases

JANUS KINASES

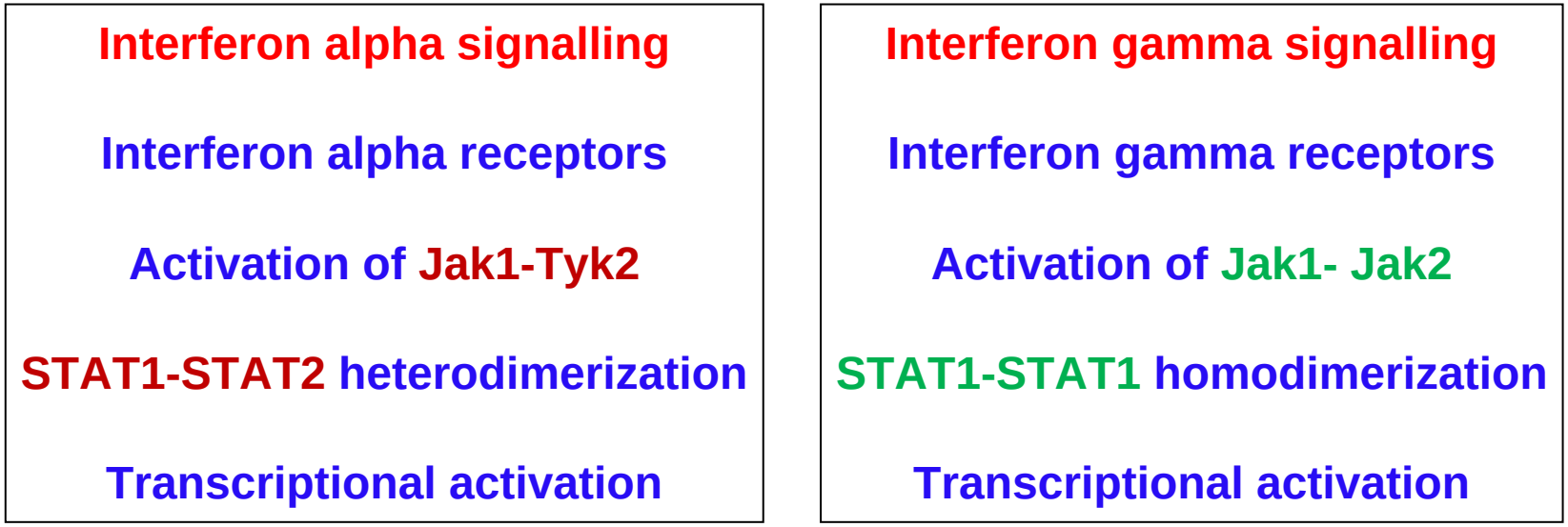
JAK belongs to a family of
non-receptor protein tyrosine kinases
of approximately 130 kDa, comprising of
JAK1, JAK2, JAK3
and
TYK2 (non-receptor Protein Tyrosine Kinase-2).

STATs

There are seven STAT proteins:

STAT1 to 6, including STAT5a and STAT5b, which are encoded by distinct genes.

Cytokine receptor	Janus kinases	STATs
IFN-γ	JAK1 and JAK2	Stat1
IFN-α/β	JAK1 and Tyk-2*	Stat2
IL-2	JAK1 and JAK3	Stat5
IL-3	JAK2	Stat5
IL-4	JAK1 and JAK3	Stat6
IL-6	JAK1 (and sometimes others)	Stat3
IL-10	JAK1 and Tyk-2	Stat3
IL-12	JAK2 and Tyk-2	Stat4


Interferon alpha signalling
Interferon alpha receptors
Activation of Jak1-Tyk2
STAT1-STAT2 heterodimerization
Transcriptional activation
Interferon gamma signalling
Interferon gamma receptors
Activation of Jak1- Jak2
STAT1-STAT1 homodimerization
Transcriptional activation

STAT binding sites:

ISRE (STAT1-2)
Interferon Stimulated Response Element
AGTTTNCNTTTCC

GAS (STAT1-5)
Interferon-Gamma Activated Sequence
TTCN3GAA

STAT6

TTCN4GAA

Each of the seven STATs has separate *in vivo* functions, as revealed by knockout experiments.

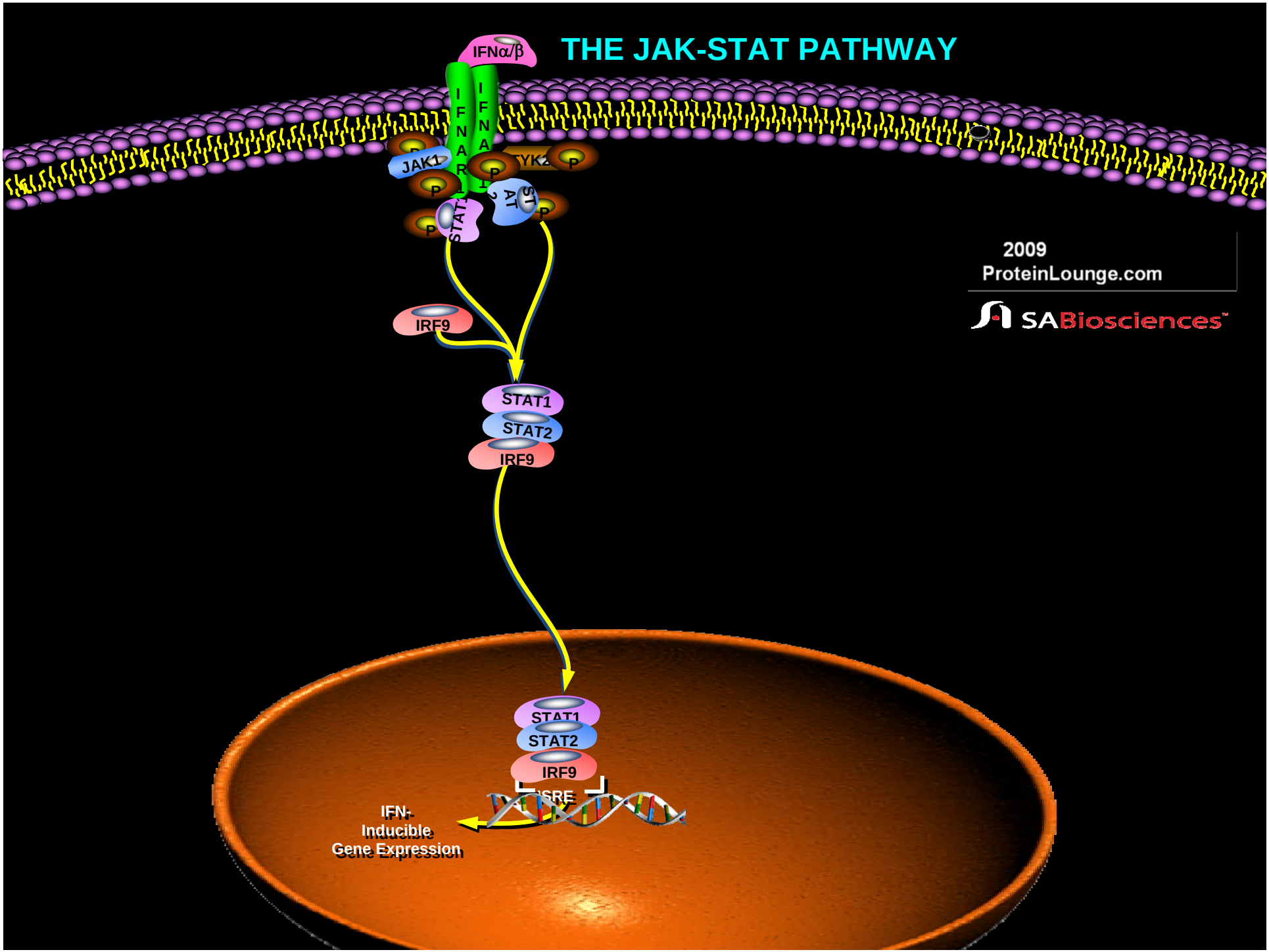
STAT1 knockout mice have an impaired interferon signalling

STAT4 knockouts impaired IL-12 signalling,

STAT5a knockouts impaired prolactin signalling,

STAT5b knockouts impaired growth hormone signalling, and

STAT6 knockout impaired IL-4 and IL-13 signalling.


THE JAK-STAT PATHWAY
IFNα/β
IFNAR1
IFNAR2
JAK1
TYK2
P
STAT1
STAT2
IRF9
2009
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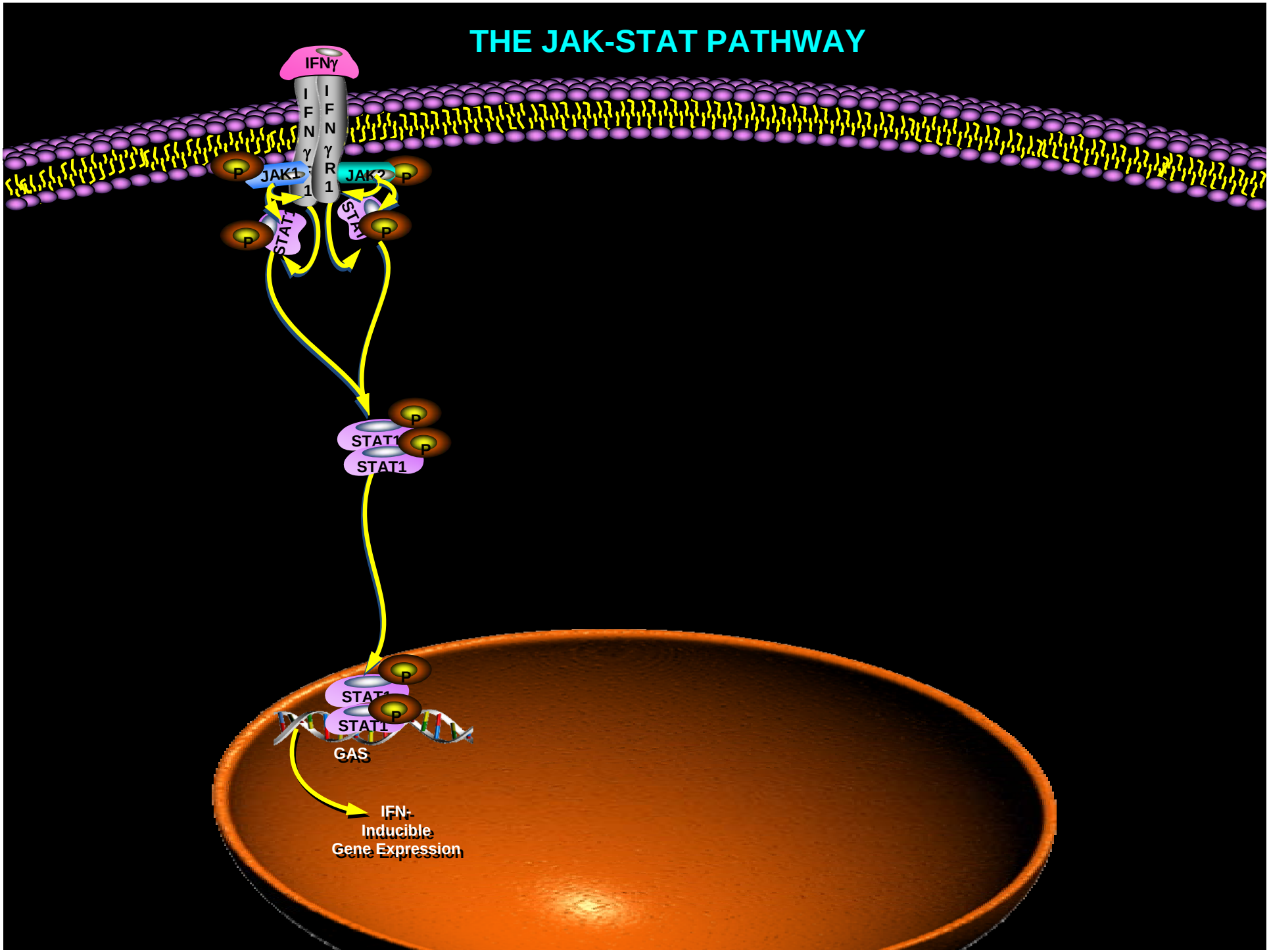
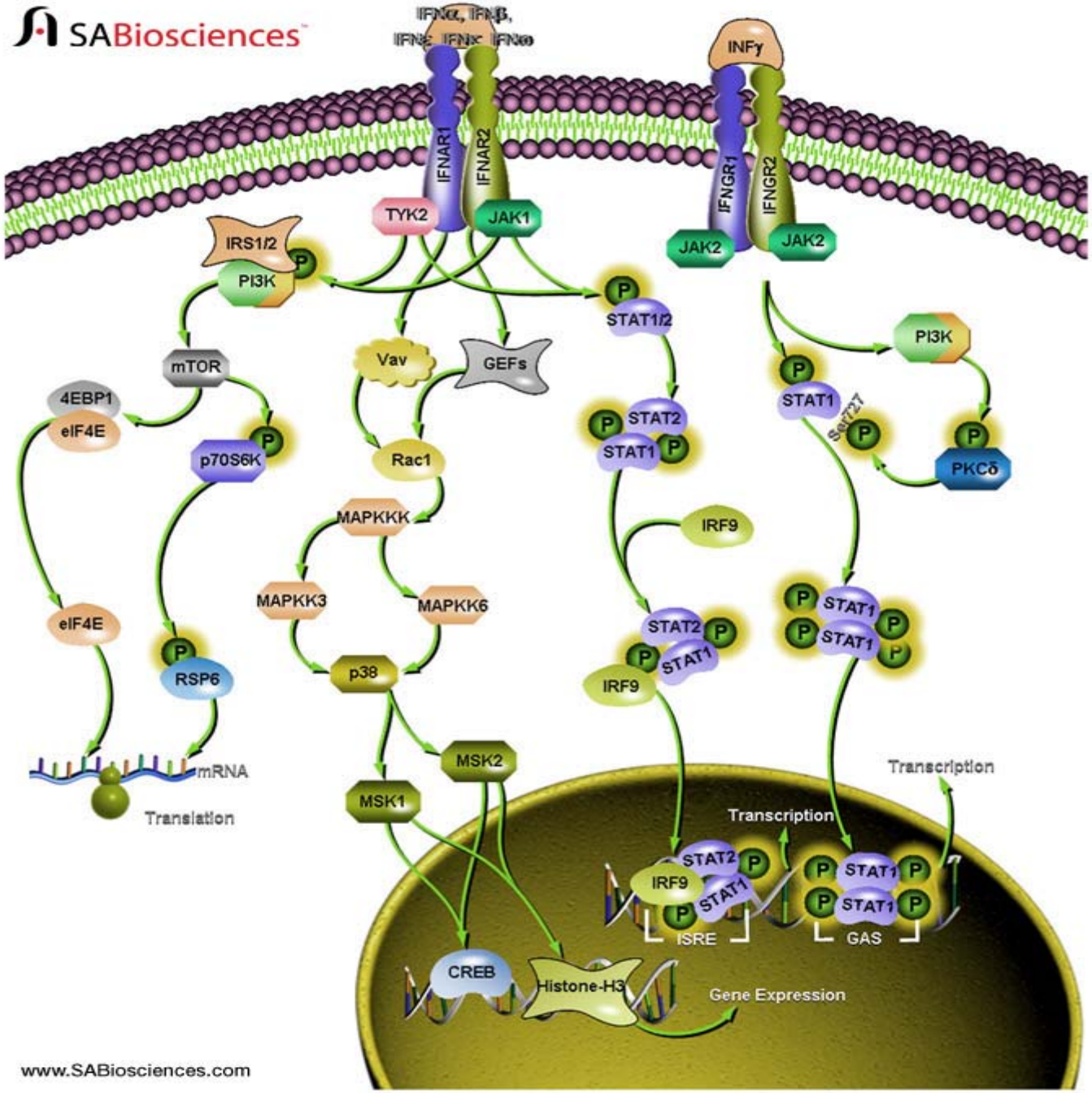
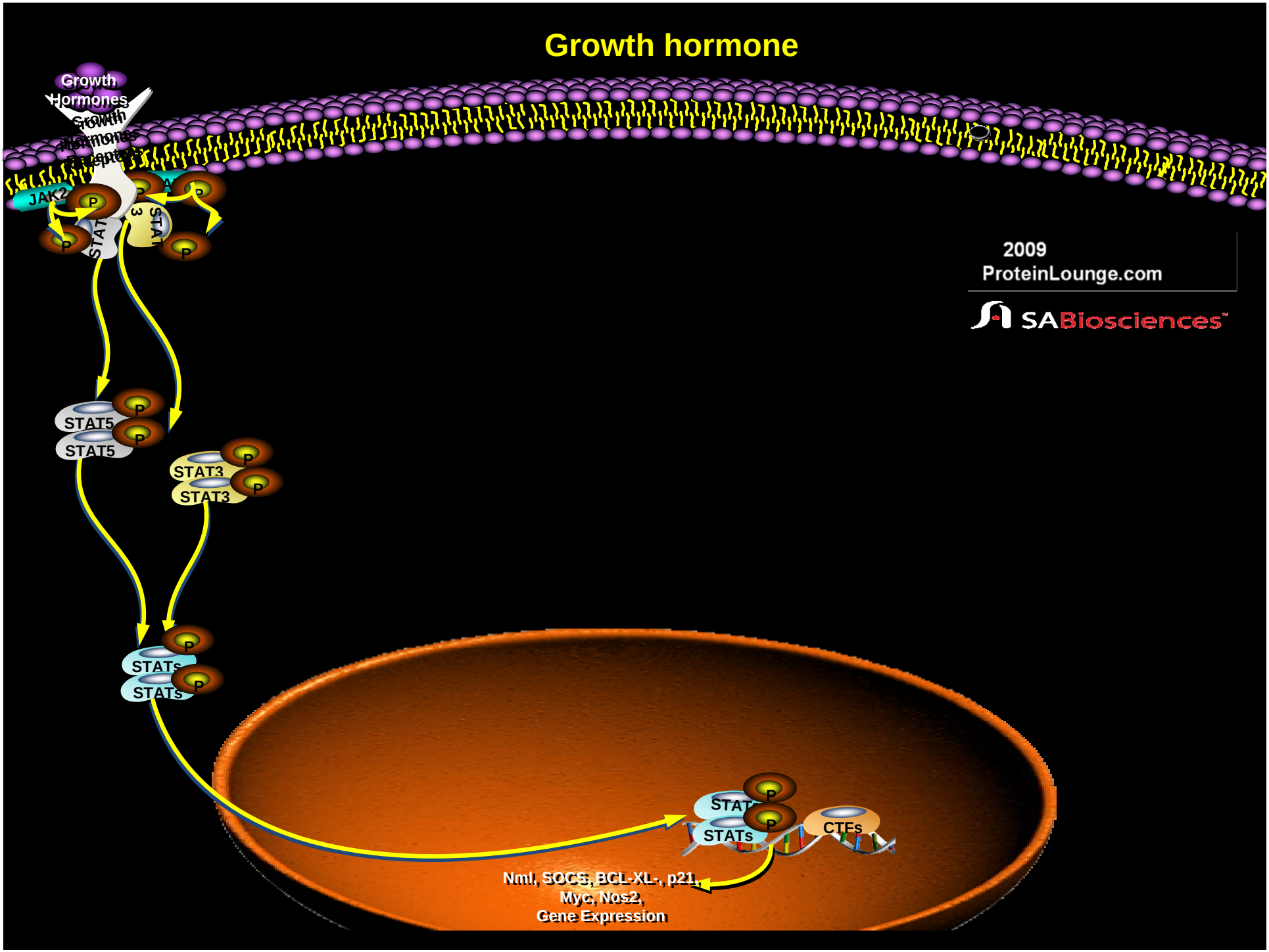
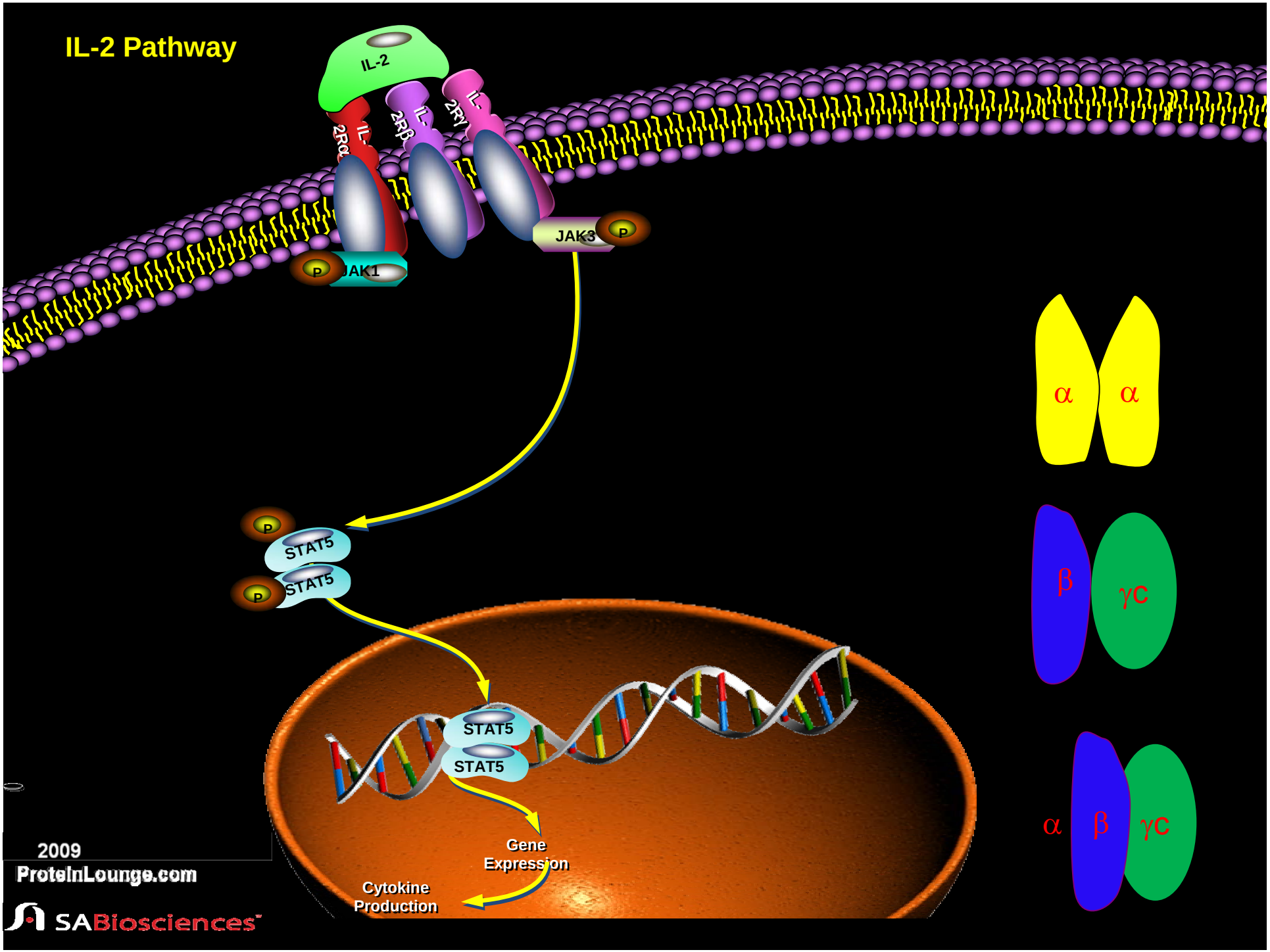
SABiosciences
STAT1
STAT2
IRF9
ISRE
IFN-
Inducible
Gene Expression


THE JAK-STAT PATHWAY
IFNγ
IFNγR1
IFNγR1
JAK1
JAK2
P
STAT1
STAT1
STAT1
STAT1
GAS
IFN-Inducible Gene Expression


SABiosciences
IFNα, IFNβ, IFNε, IFNκ, IFNω
INFγ
IFNAR1
IFNAR2
IFNGR1
IFNGR2
TYK2
JAK1
JAK2
JAK2
IRS1/2
PI3K
STAT1/2
PI3K
mTOR
Vav
GEFs
STAT1
Ser727
4EBP1
eIF4E
p70S6K
Rac1
STAT2
STAT1
PKCδ
MAPKKK
IRF9
MAPKK3
MAPKK6
eIF4E
STAT1
STAT1
RSP6
p38
STAT2
STAT1
IRF9
mRNA
Translation
MSK2
MSK1
Transcription
Transcription
STAT2
IRF9
STAT1
STAT1
STAT1
ISRE
GAS
CREB
Histone-H3
Gene Expression
www.SABiosciences.com


Growth hormone
Growth Hormones
Growth Hormones Receptors
JAK2
P
STAT5
STAT5
STAT3
STAT3
STATs
STATs
STATs
CTFs
Nml, SOCS, BCL-XL-, p21,
Myc, Nos2,
Gene Expression
2009
ProteinLounge.com
SABiosciences


IL-2 Pathway
IL-2
IL-2Rα
IL-2Rβ
IL-2Rγ
JAK3
P
JAK1
P
P
STAT5
P
STAT5
STAT5
STAT5
Gene Expression
Cytokine Production
α
α
β
γc
α
β
γc
2009
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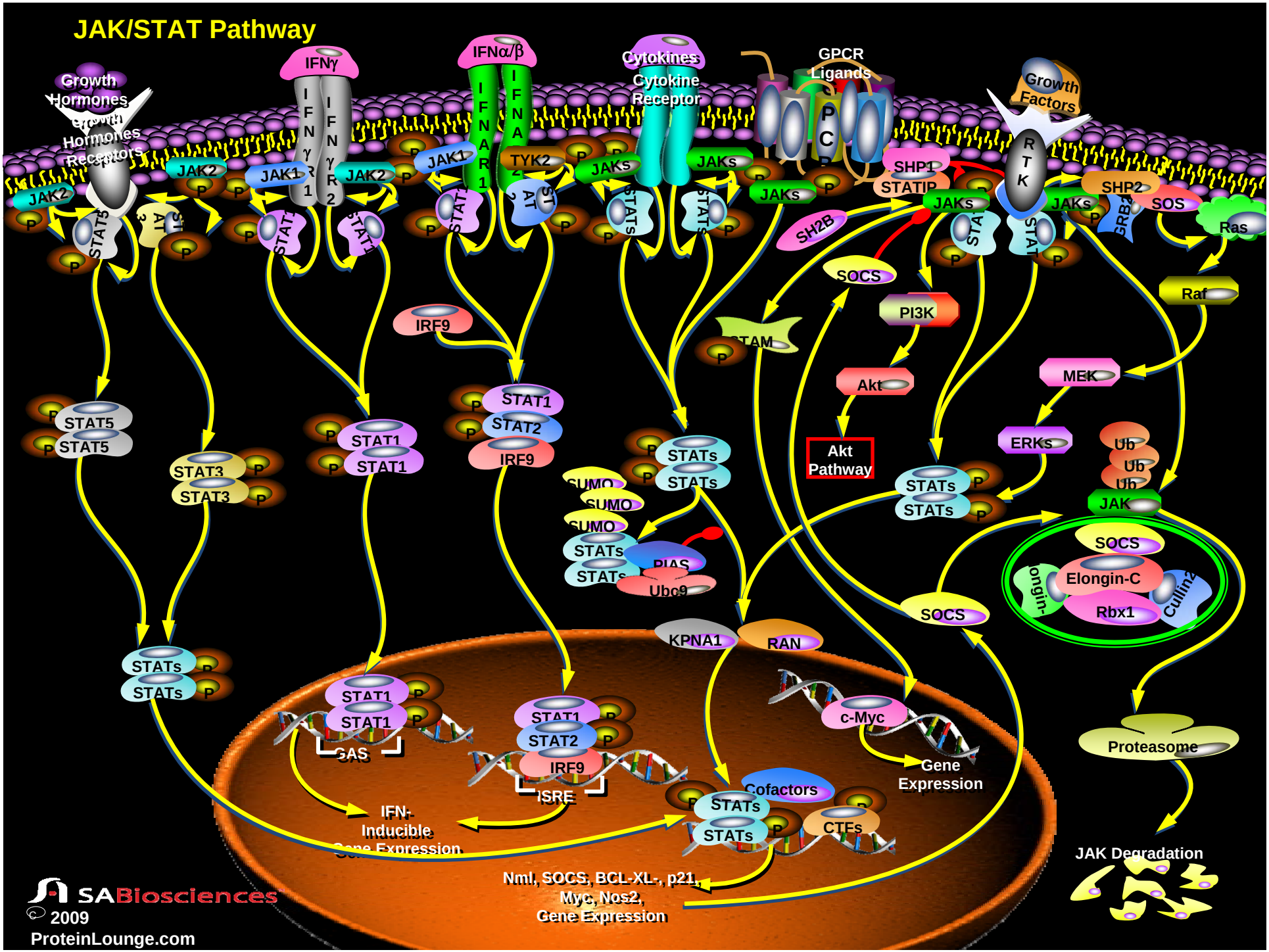
The IL-2 receptor forms homo/heterodimers

Low affinity
$10^{-8,}$
expressed by activated T cells,
No function

Intermediate affinity
$10^{-9,}$ resting T cells.

High affinity
$10^{-11,}$ activated T cells


JAK/STAT Pathway
Growth Hormones
Growth Hormones Receptors
IFNγ
IFNγR1
IFNγR2
IFNα/β
IFNAR1
IFNAR2
Cytokines
Cytokine Receptor
GPCR Ligands
PC
Growth Factors
RTK
JAK1
JAK2
TYK2
JAKs
SHP1
STATIP
SHP2
SOS
Ras
Raf
MEK
ERKs
SH2B
SOCS
PI3K
Akt
Akt Pathway
IRF9
STAT1
STAT2
STAT3
STAT5
STATs
SUMO
PIAS
Ubc9
Ub
JAK
Elongin-C
Cullin2
Rbx1
KPNA1
RAN
c-Myc
Gene Expression
Proteasome
JAK Degradation
GAS
ISRE
IFN-Inducible Gene Expression
Cofactors
CTFs
Nml, SOCS, BCL-XL-, p21, Myc, Nos2, Gene Expression
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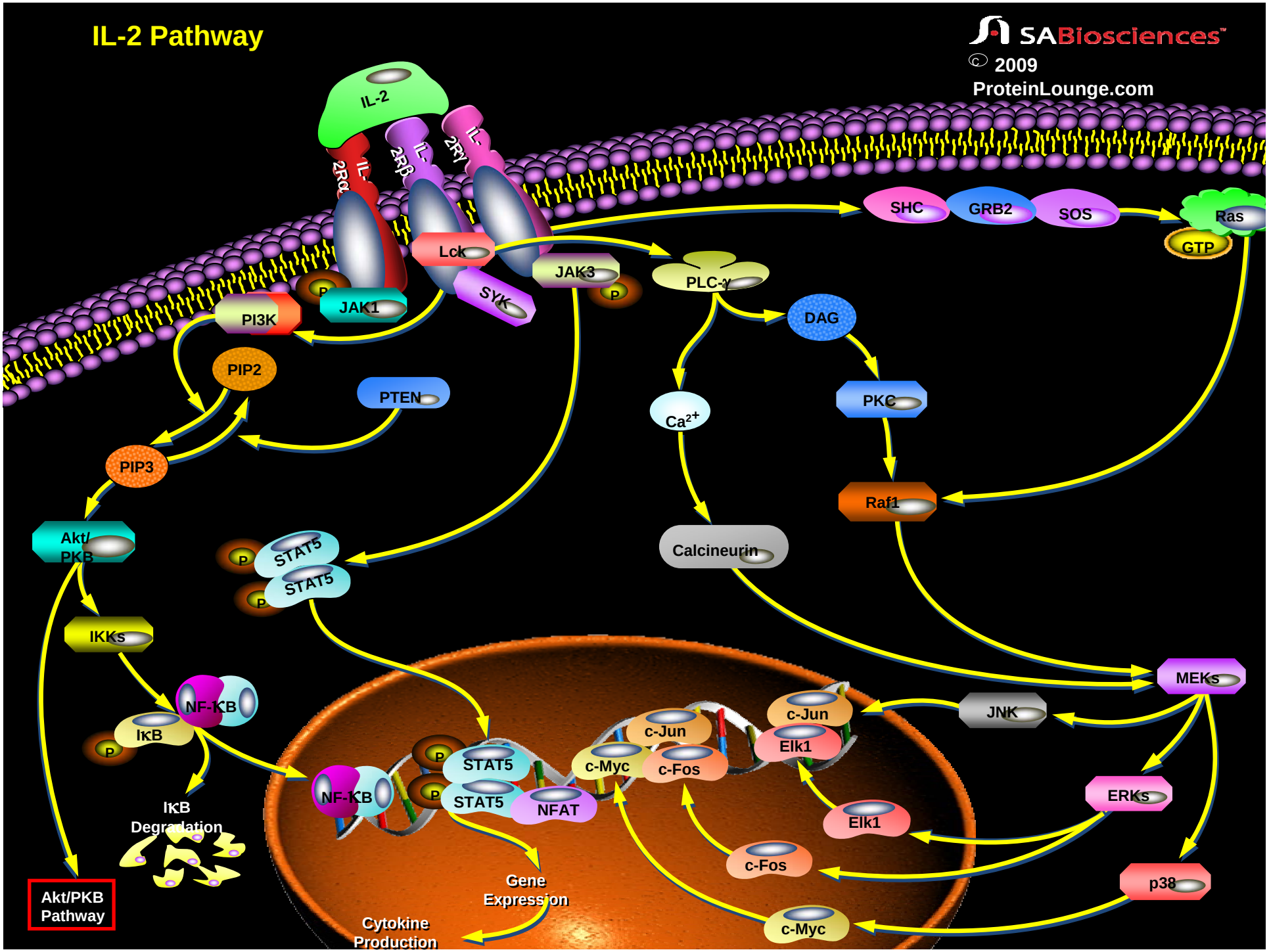
IL-2 Pathway
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IL-2
IL-2Rα
IL-2Rβ
IL-2Rγ
Lck
JAK3
JAK1
SYK
PI3K
PIP2
PIP3
PTEN
SHC
GRB2
SOS
Ras
GTP
PLC-γ
DAG
Ca2+
PKC
Raf1
Calcineurin
Akt/PKB
STAT5
STAT5
IKKs
NF-κB
IκB
IκB Degradation
Akt/PKB Pathway
MEKs
JNK
c-Jun
Elk1
c-Myc
c-Fos
ERKs
p38
NFAT
Gene Expression
Cytokine Production

Termination of Jak/STAT signaling

1. Phosphotase

Receptor-Shp-1 (HCP, SH-PTP1, and PTP1c),
Shp-2 (Syp and PTP1D), and SHIP

Nuclear phosphotase

2. Suppressors of Cytokine Signaling

(SOCS). SOCS1-7.

3. Degradation of Stats through

ubiquitination/proteasome pathway.
The PIAS family

Alexander et. al. Annu. Rev. Immunol. 2004. 22:503

DRUGS

ENBREL	MAb AGAINST TNFa RECEPTOR	RHEUMATOID ARTHRITIS, CROHN'S DISEASE
INTRON A	INTERFERON A-2B	HEPATITIS C MELANOMA
EPOGEN	ERYTHROPOIETIN	STIMULATES RBC PRODUCTION

DRUGS

ACTIMMUNE	INTERFERON γ 1β	CHROMINC GRANULOMATOUS DISEASE OSTEROPOROSIS
NEUPOGEN	G-CSF	STIMULATES PRODUCTION OF NEUTROPHILS REDUCTION OF INFECTION IN CANCER PATIENTS UNDERGOING CHEMOTHERAPY

DRUGS

LEUKINE	GM-CSF	STIMULATES PRODCUTION OF MYELOID CELLS AFTER BONE MARROW TRANSPLANTATION
NEUMAGA / NEULASTA	INTERLEUKIN 11	STIMULATES PRODUCTION OF PLATELETS

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ACKNOWLEDGEMENT

SA BIOSCIENCES
Pathway Central

http://www.sabiosciences.com/pathway.php?sn=JAK_STAT_Pathway