CAAS 2012级博士班

JEV感染与抗病毒先天性免疫及p53下游靶基因的鉴定

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报告内容

一 研究背景

1. p53下游靶基因的鉴定

p53作为转录因子和应激感受器（stress sensor），调控许多下游靶基因的转录，而目前通过试验鉴定出的靶基因只有50多个，还有很多亟需挖掘，这对阐明p53作用网络具有重要的意义。

2. p53与抗病毒先天性免疫

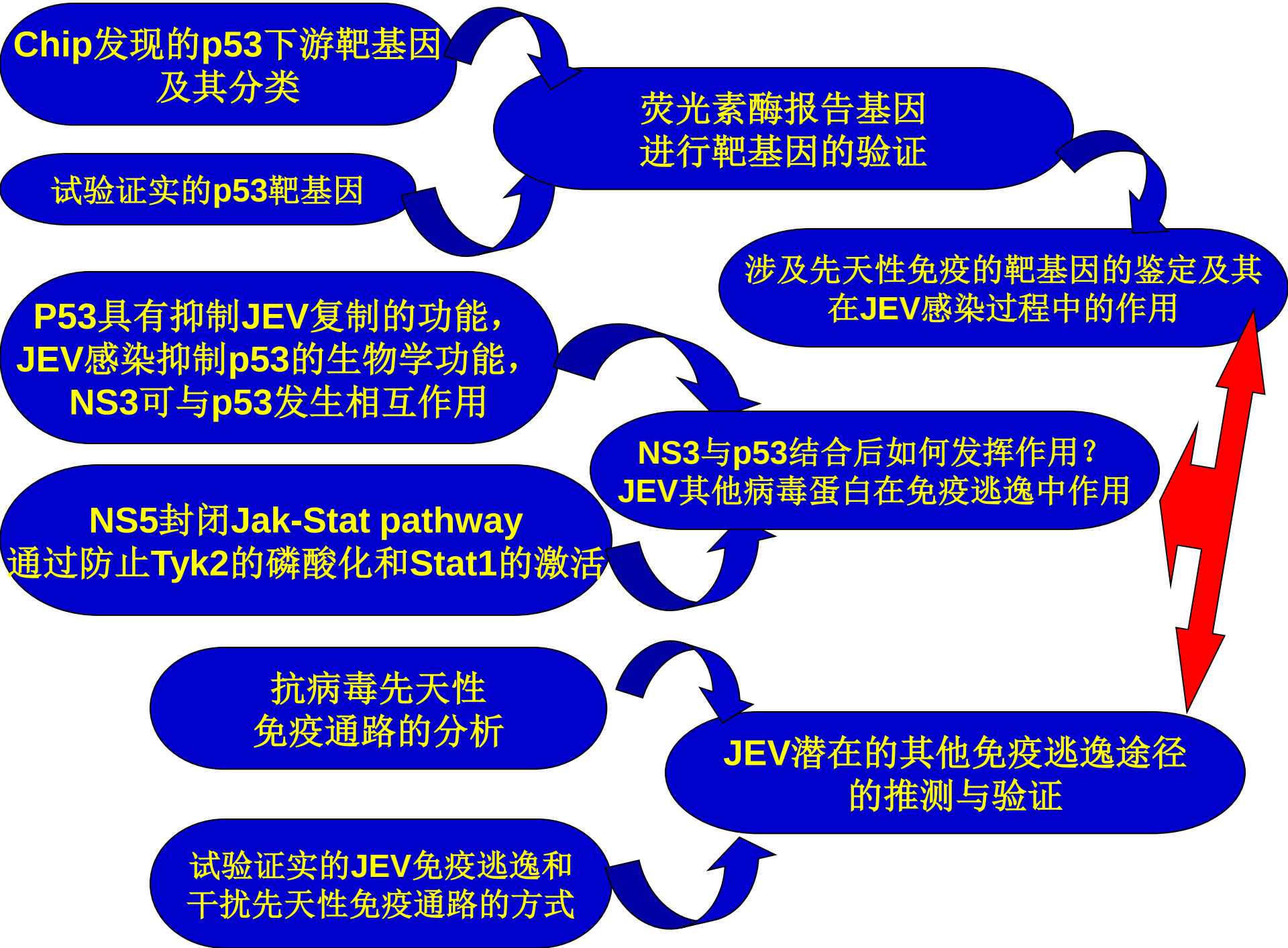
p53是I型IFN的直接靶基因，病毒感染后诱导产生的IFN激活p53的转录表达，有助于病毒感染细胞的凋亡，从而抑制许多不同种类病毒的复制和扩散。有趣的是，IFN诱导基因，例如IRF9、IRF5、IFN刺激基因15（ISG15）和TLR3是p53的直接转录靶基因。这些发现表明p53可能通过病毒感染后诱导细胞凋亡和增强I型IFN反应而发挥抗病毒先天性免疫的作用。

3. JEV感染与抗病毒先天性免疫

细胞本身存在识别外来抗原并启动快速应答并进行免疫防御的机制。其中PRRs中的TLR和RLR识别入侵的病毒，启动免疫应答产生I型IFN，细胞识别干扰素后，启动一系列抗病毒蛋白的表达，抑制病毒的复制与扩散。但病毒也有干扰和阻断先天性免疫通路的机制，发生免疫逃逸。目前已发现JEV可干扰先天性免疫通路，但仍有许多问题未能阐明。

二 课题思路与预期

从p53靶基因鉴定、JEV的先天性免疫逃逸、JEV病毒蛋白如何参与免疫逃逸的途径出发，期望能鉴定出几个p53下游靶基因、阐明JEV免疫逃逸的某一个具体途径


Chip发现的p53下游靶基因
及其分类
试验证实的p53靶基因
荧光素酶报告基因
进行靶基因的验证
涉及先天性免疫的靶基因的鉴定及其
在JEV感染过程中的作用
P53具有抑制JEV复制的功能，
JEV感染抑制p53的生物学功能，
NS3可与p53发生相互作用
NS3与p53结合后如何发挥作用？
JEV其他病毒蛋白在免疫逃逸中作用
NS5封闭Jak-Stat pathway
通过防止Tyk2的磷酸化和Stat1的激活
抗病毒先天性
免疫通路的分析
JEV潜在的其他免疫逃逸途径
的推测与验证
试验证实的JEV免疫逃逸和
干扰先天性免疫通路的方式

三 课题开展前的准备

- p53背景知识的掌握
- p53 与抗病毒先天性免疫
- 抗病毒先天性免疫通路
- JEV与先天性免疫

p53背景知识

1 蛋白与基因名称

Protein name: tumor suppressor p53 (celluar tumor antigen p53)

Gene neme: TP53 (p53)

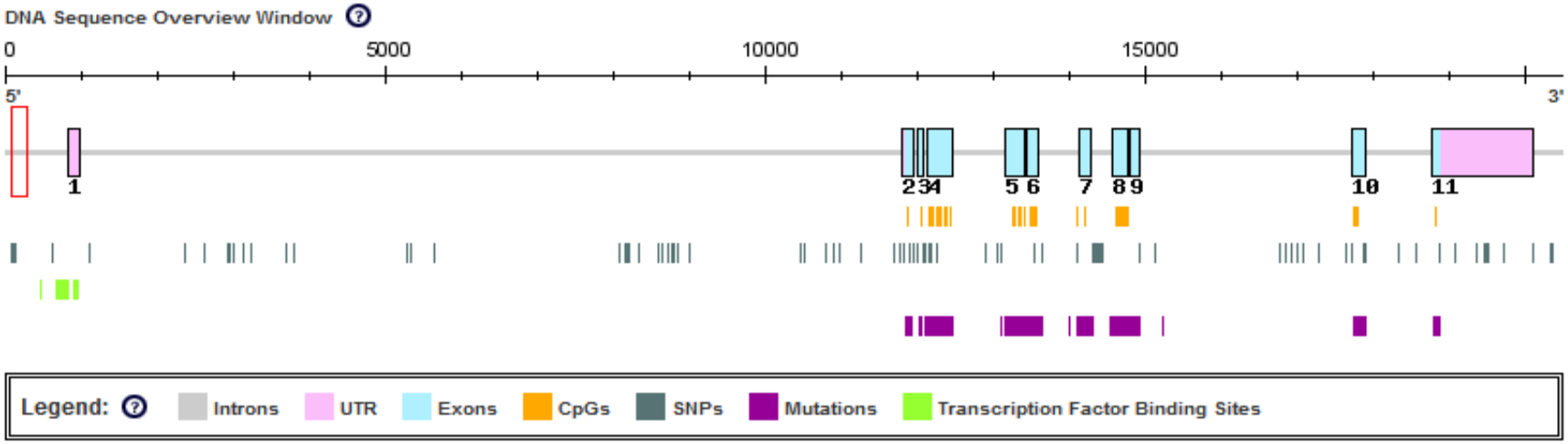
2 蛋白与基因序列

Homo sapiens p53 protein, cellular tumor antigen p53 isoform a, NCBI Reference Sequence: NP_000537.3, 393AA, protein level

Homo sapiens p53 protein mRNA, complete cds, AF307851, 2521 bp; transcript variant 1, NCBI Reference Sequence: NM_000546.5

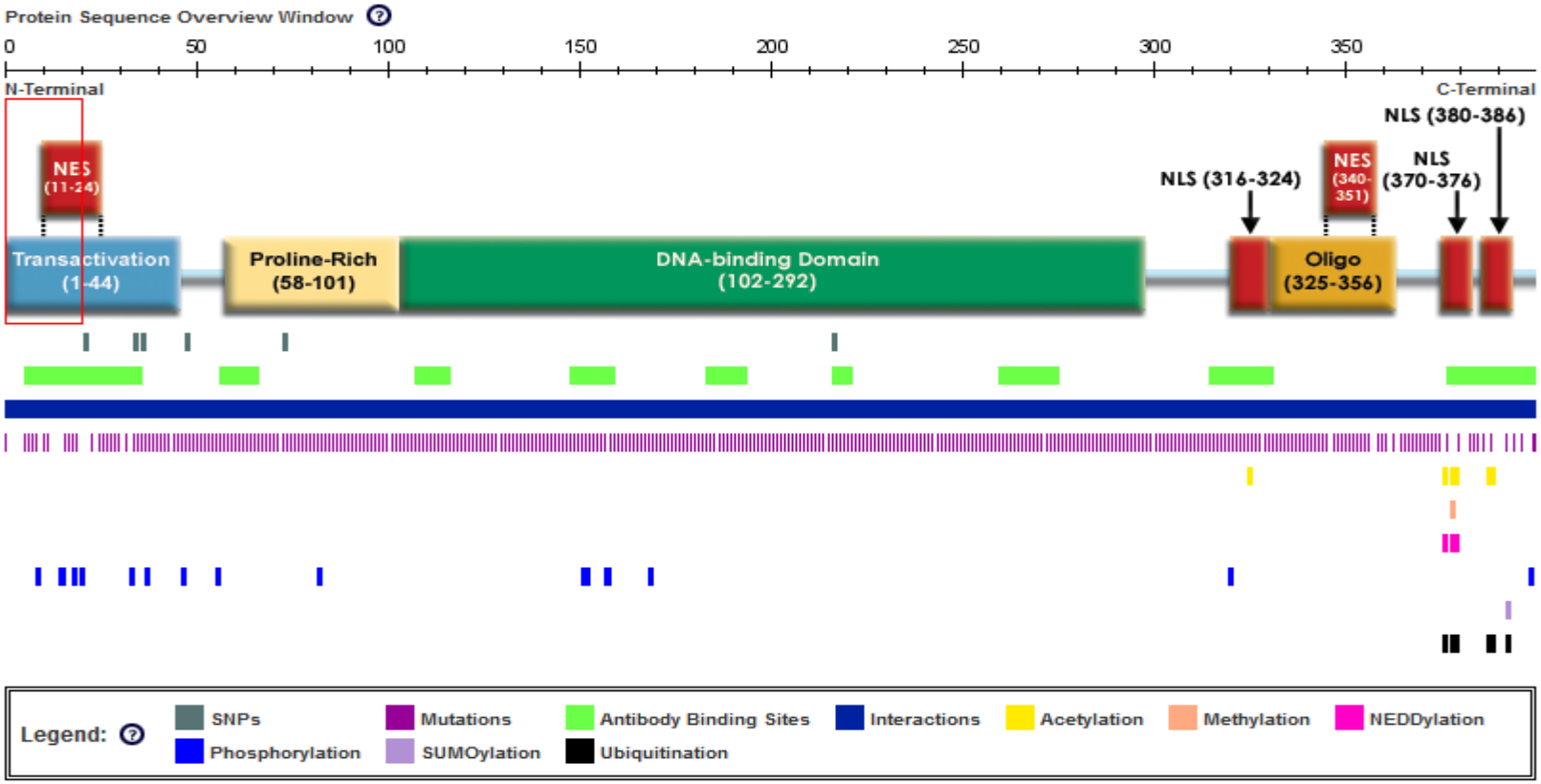
Homo sapiens p53 gene (genomic DNA), GenBank: X54156.1, 20303 bp

3 基因的结构示意图

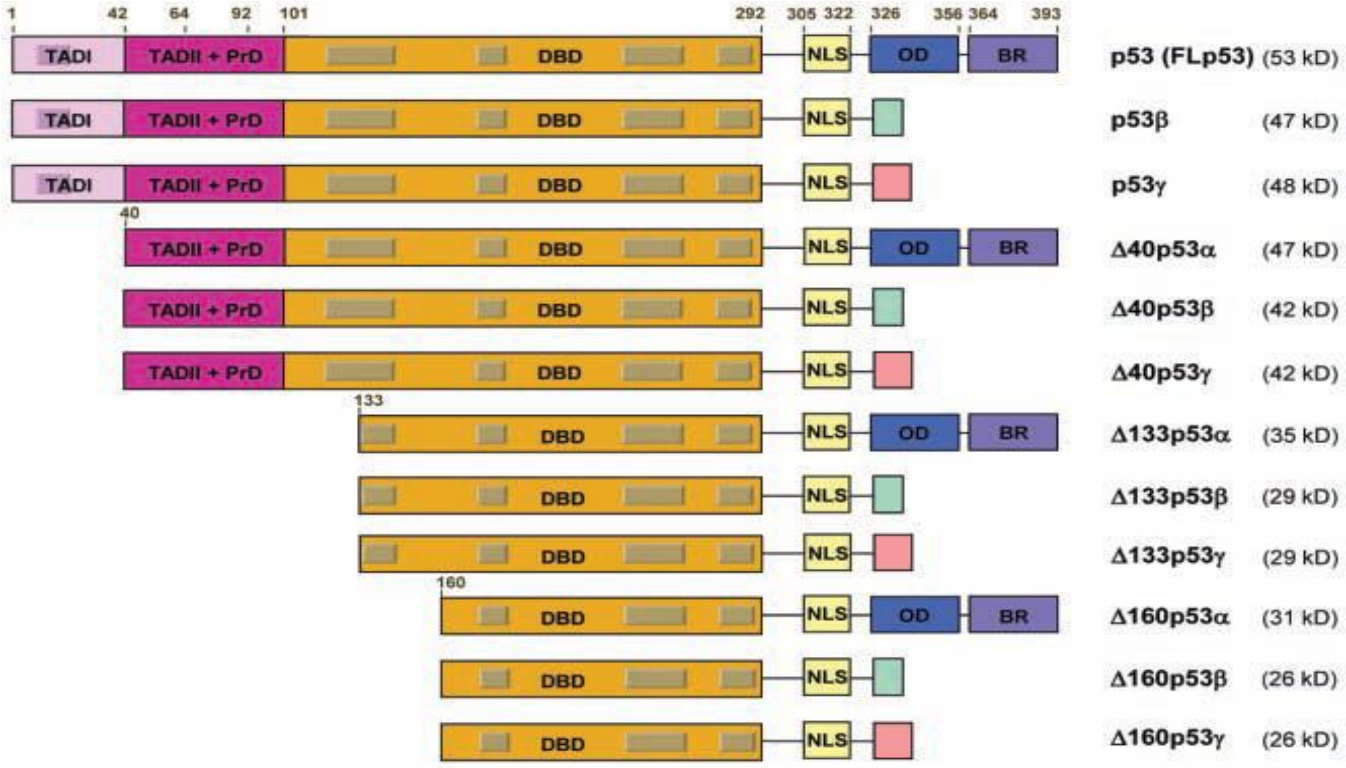


4 p53结构域和功能域

N-terminus TAD（AD1 1-42;AD2 43-63）; proline rich domain 64-92；DBD，102-292；3个核定位信号（NLS）；homo-oligomerisation domain（OD）；C-terminus；核输出信号（NES）



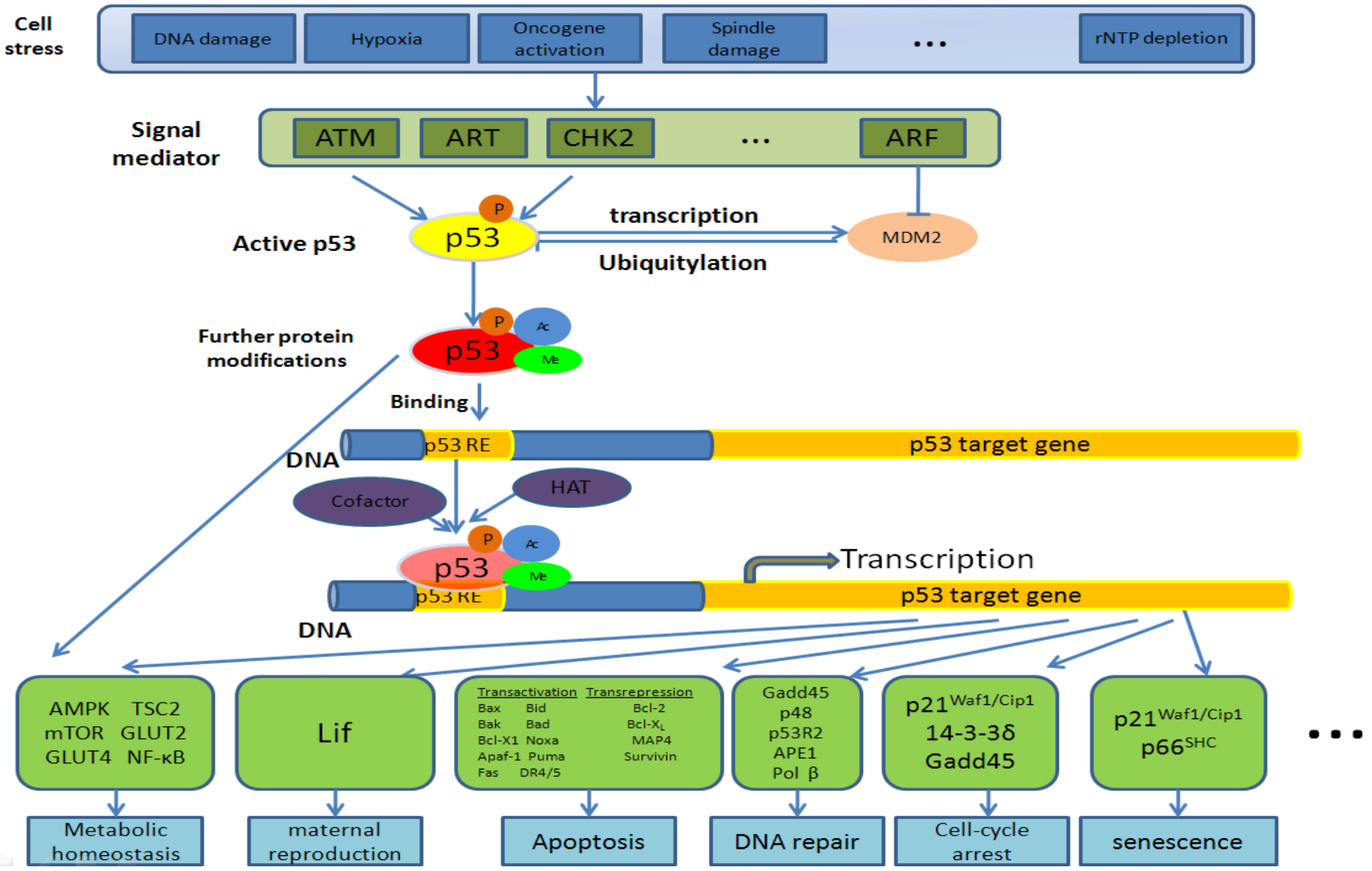
5 p53的可变剪接



6 p53家族（p53 p63 p73）

p63和p73在皮肤、神经系统、雌性繁殖等方面的发育起到必不可少的作用，在某些情况下扮演肿瘤抑制剂的作用。奇怪的是p53、p63和p73有非常相近的DNA结合区，结合相似的DNA序列并诱导一些相同基因的转录，也能诱导特定细胞类型中非常不同基因的转录。

7 p53的功能与调节

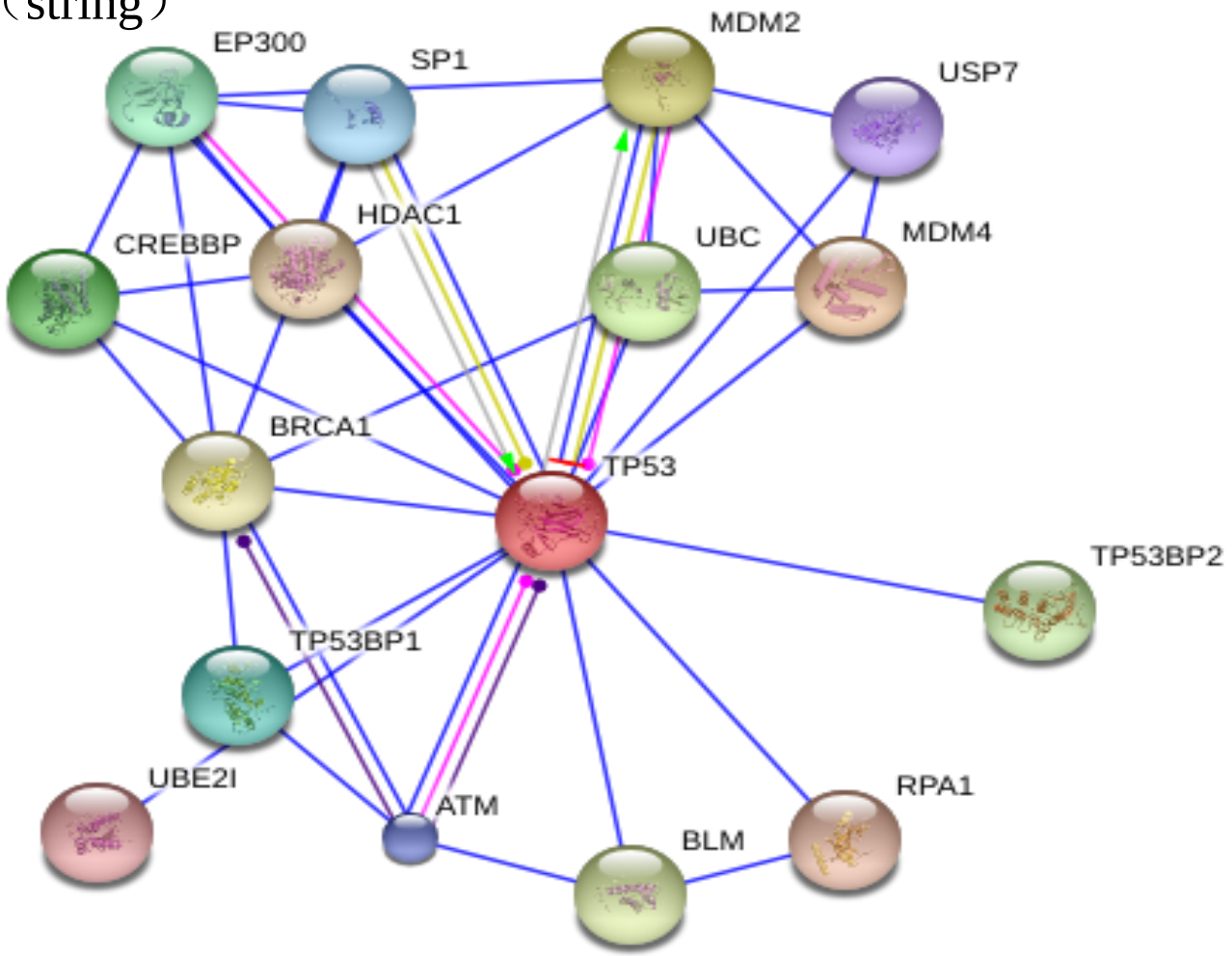

Cell stress
DNA damage
Hypoxia
Oncogene activation
Spindle damage
...
rNTP depletion
Signal mediator
ATM
ART
CHK2
...
ARF
Active p53
P
p53
transcription
Ubiquitylation
MDM2
Further protein modifications
Ac
Me
Binding
DNA
p53 RE
p53 target gene
Cofactor
HAT
Transcription
AMPK TSC2 mTOR GLUT2 GLUT4 NF-κB
Lif
Transactivation Transrepression
Bax Bid Bcl-2
Bak Bad Bcl-XL
Bcl-X1 Noxa MAP4
Apaf-1 Puma Survivin
Fas DR4/5
Gadd45 p48 p53R2 APE1 Pol β
p21Waf1/Cip1 14-3-3δ Gadd45
p21Waf1/Cip1 p66SHC
Metabolic homeostasis
maternal reproduction
Apoptosis
DNA repair
Cell-cycle arrest
senescence

8 p53相互作用蛋白网络

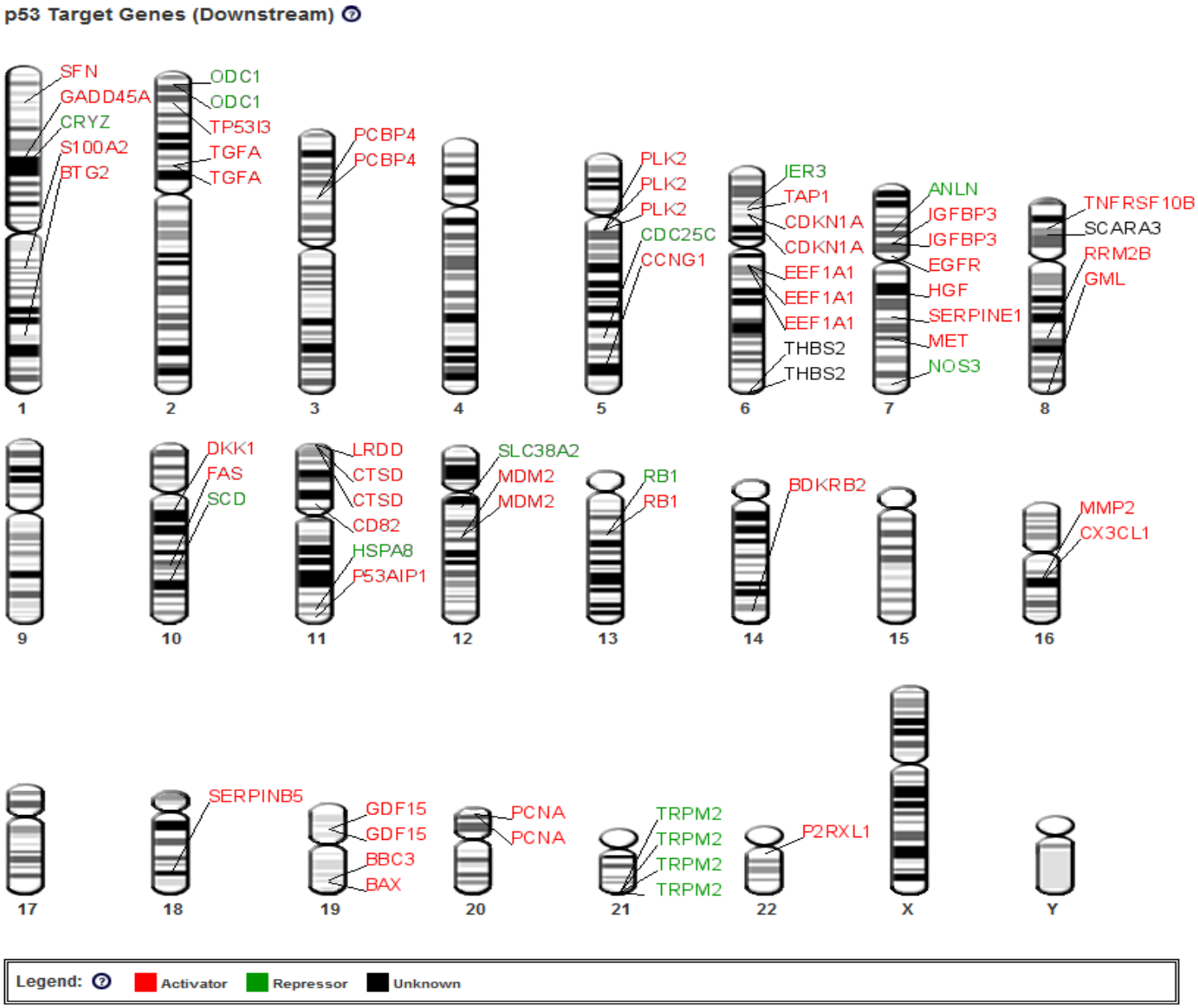
www.ebi.ac.uk/intact/pages/interactions/ （IntAct）

583 binary interactions were found in IntAct.

http://string-db.org （string）



9 p53的靶基因（chip找到276条结合DNA片段，现试验证实有60多个下游靶基因）

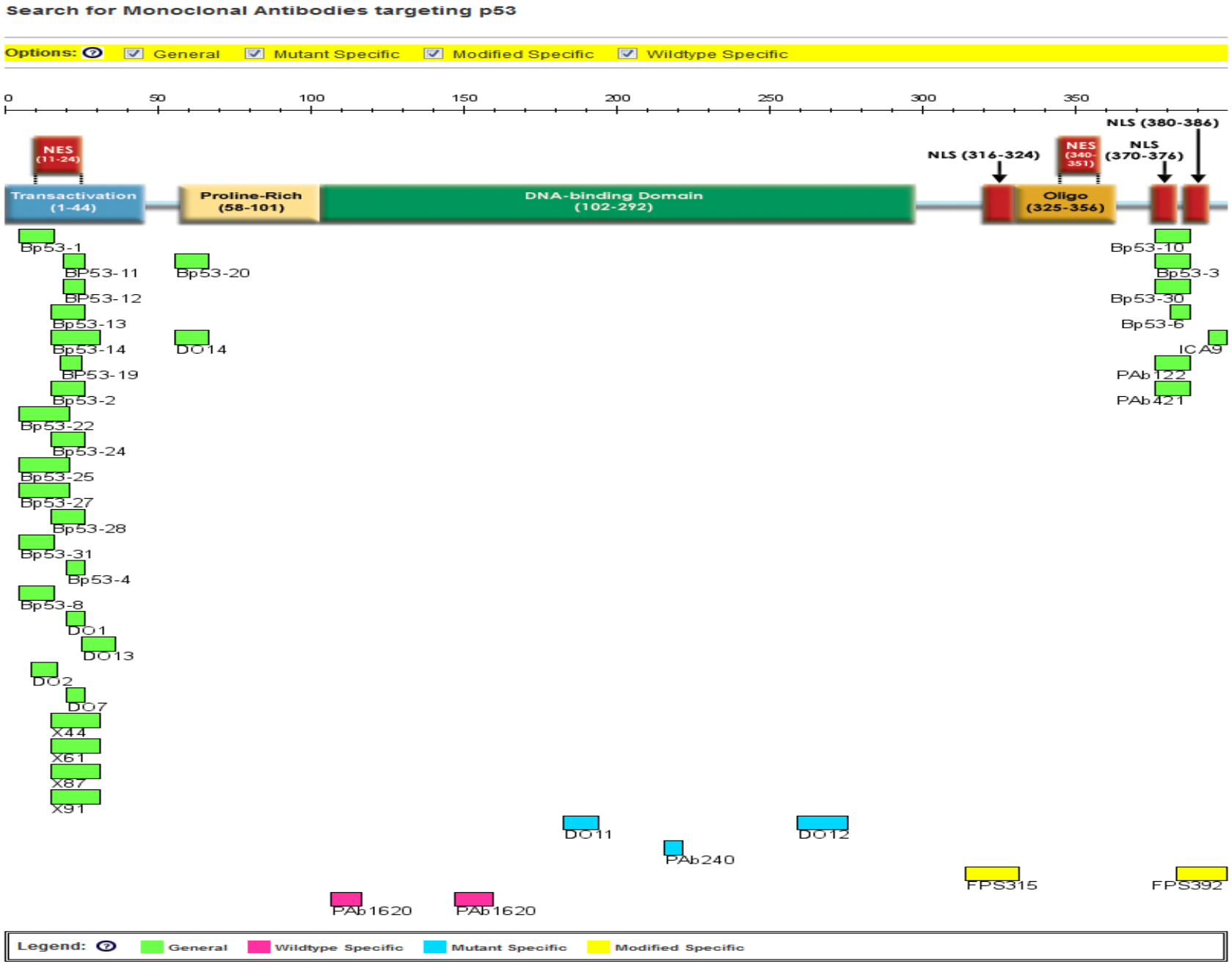


10 p53的转录因子

p53 Transcription Factors (Upstream) in the P1 region and Their Pathways

Binding Molecule	Binding Location	Interaction Type	Remarks	
ETS1	446-459	Uncharacterized		Details
ETS2	446-459	Uncharacterized		Details
BCL6	455-464	Repressor		Details
JUN/FOS	650-676	Cell-type specific activator/ repressor		Details
JUN/FOS	653-679	Cell-type specific activator/ repressor		Details
EGR1	657-704	Activator		Details
ATF3	660-666	Repressor		Details
SP1	674-680	Activator		Details
HOXA5	712-719	Activator		Details
P53	715-734	Cell-type specific activator/ repressor		Details
YY1	719-757	Activator		Details
NFIC	719-757	Activator		Details
KLF4	736-771	Repressor		Details
CBE-BP I	769-788	Uncharacterized		Details
NFKB1/RELA	776-804	Activator		Details
USF1	800-805	Activator		Details
MYC/MAX	800-805	Activator		Details
E2F4	812-823	Uncharacterized		Details
PAX2	901-950	Repressor		Details
PAX5	901-950	Repressor		Details
PAX8	901-950	Repressor		Details

11 p53的单克隆抗体

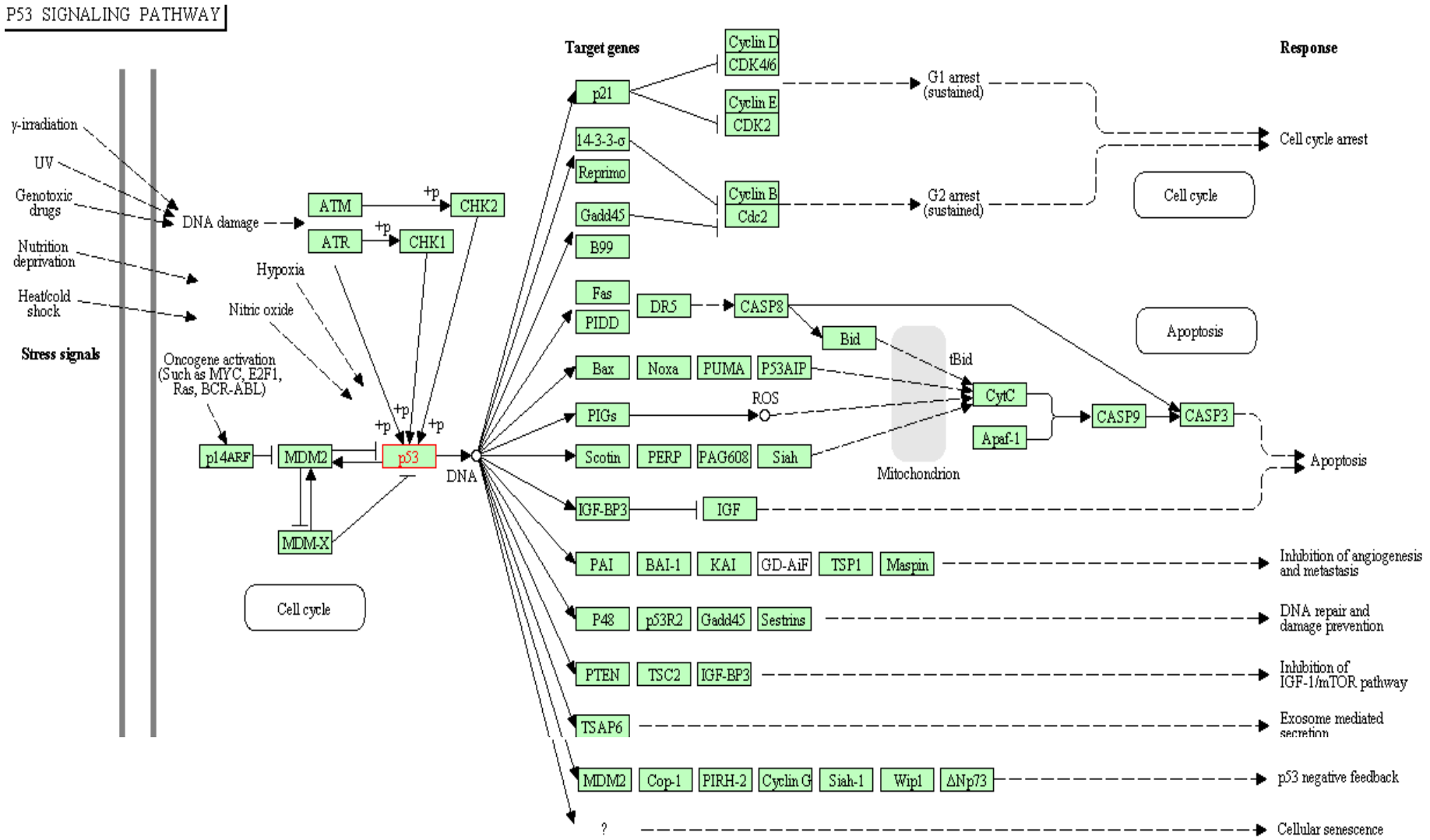


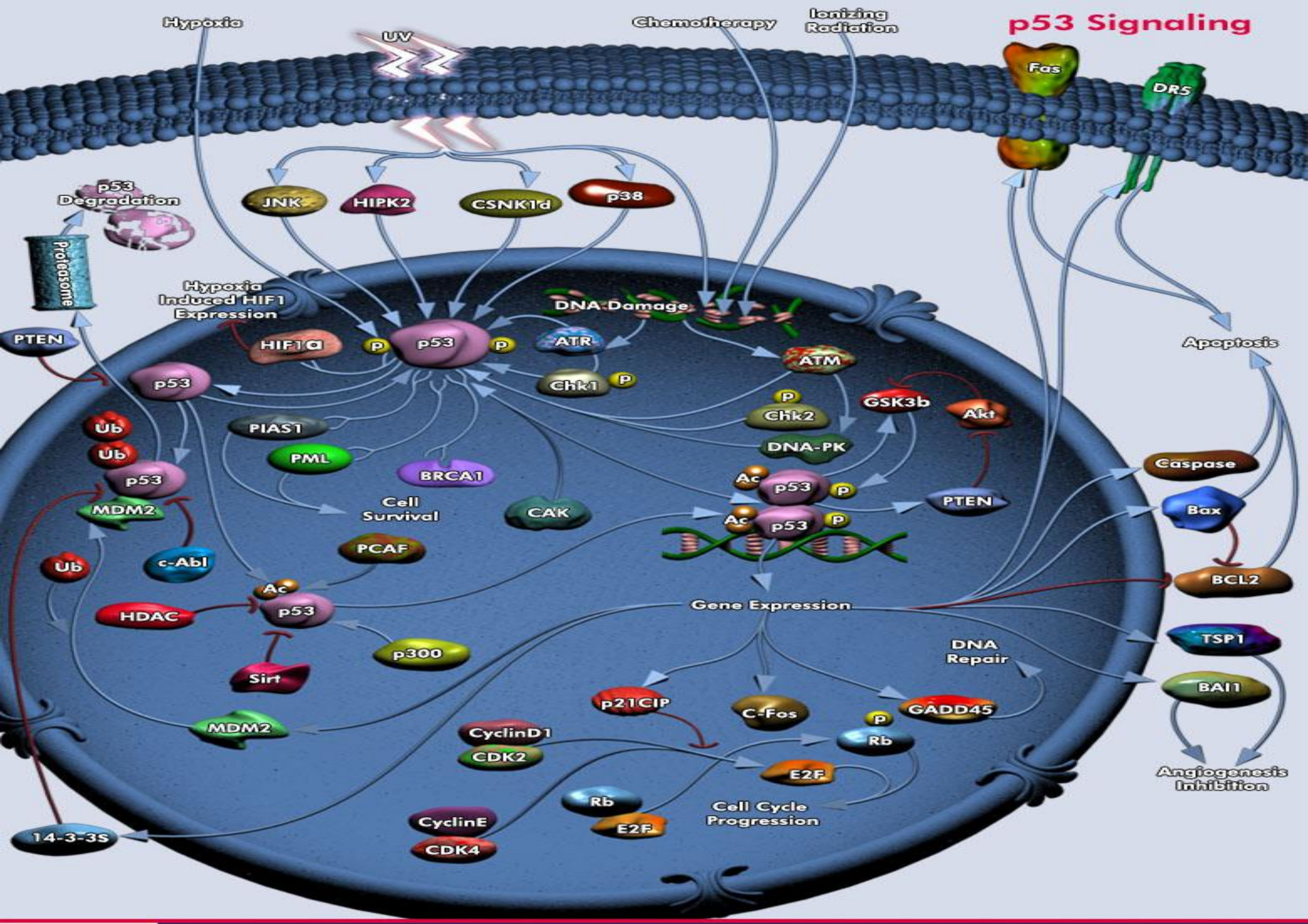
12 细胞信号通路

http://www.genome.jp/ keggdatabase及的信号通路有30个

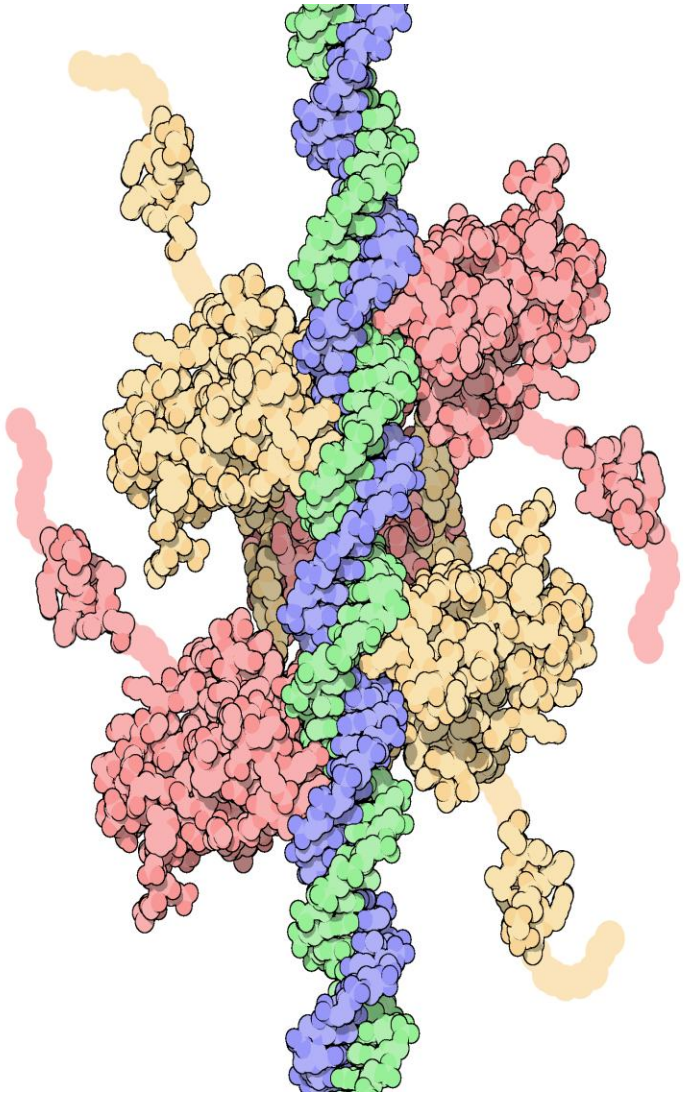
http://pid.nci.nih.gov pathway interaction database

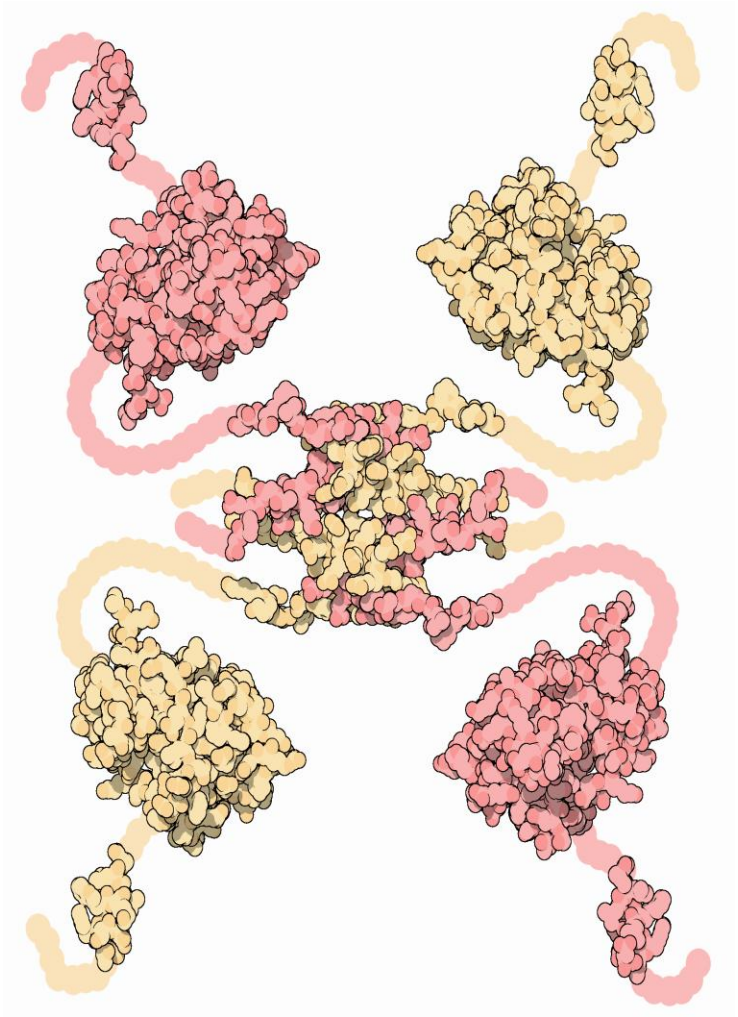
GeneGlobe Pathway Central qiagen公司



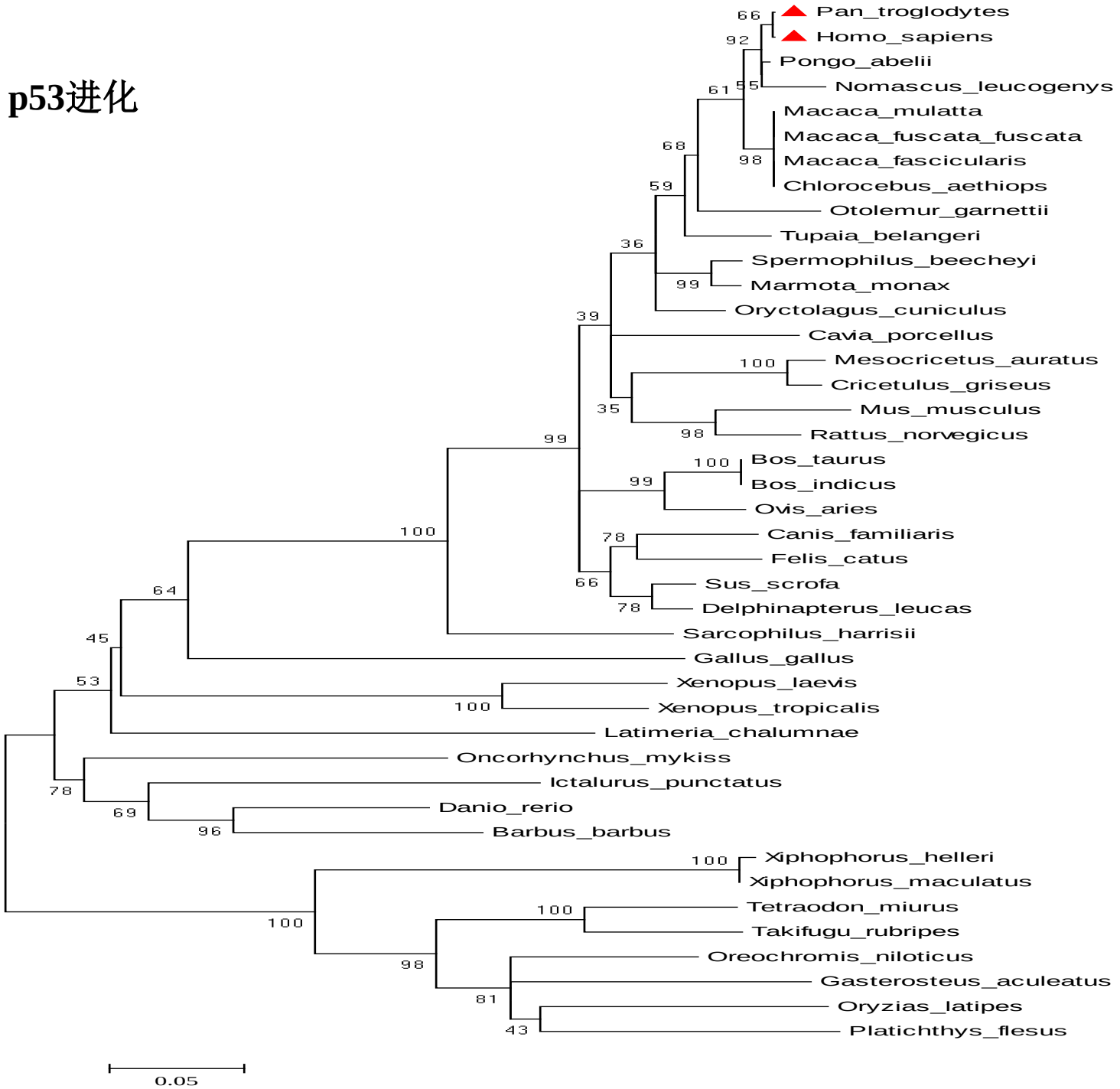


13 p53结构





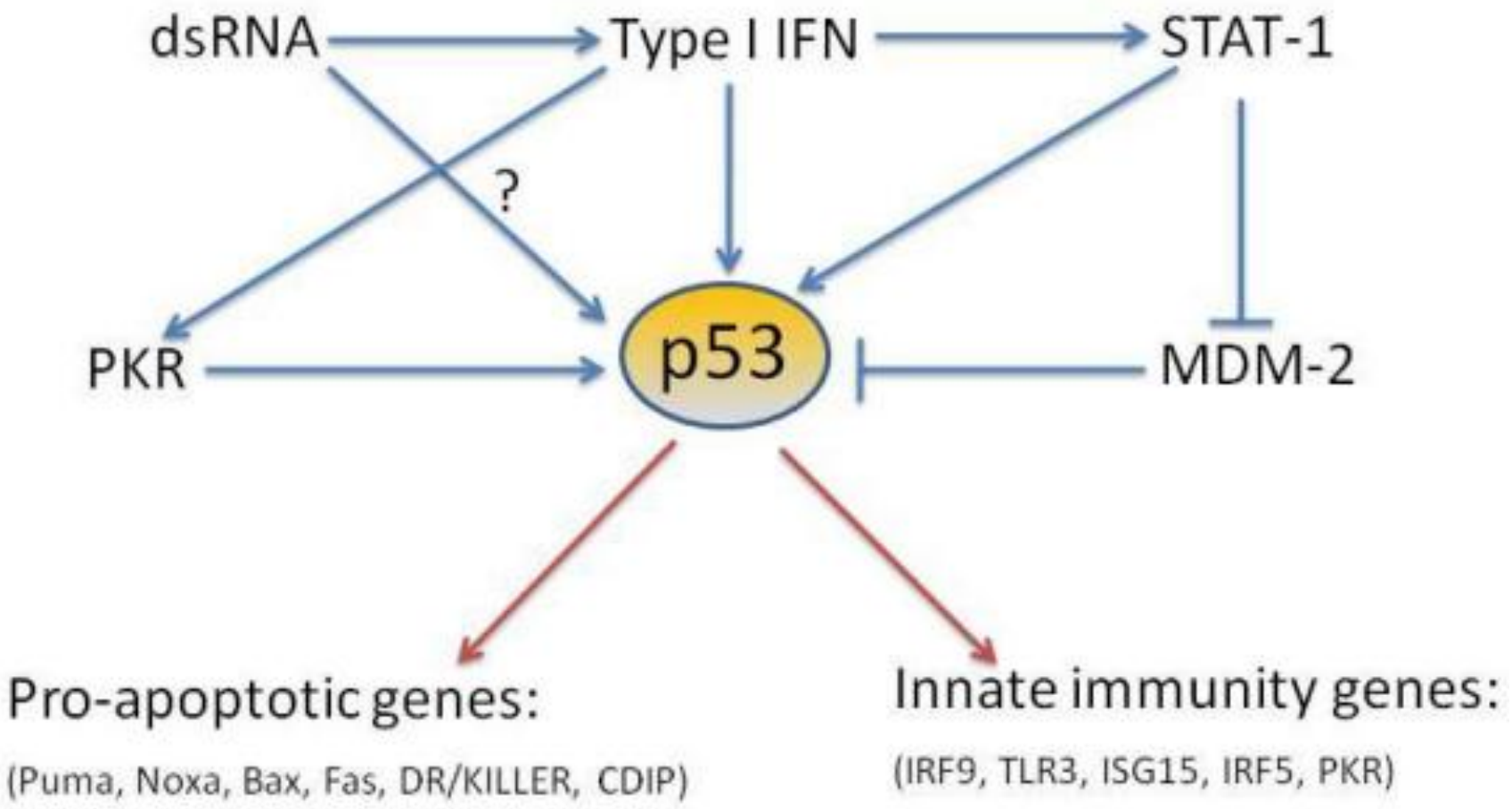
14 p53进化


Pan_troglodytes
Homo_sapiens
Pongo_abelii
Nomascus_leucogenys
Macaca_mulatta
Macaca_fuscata_fuscata
Macaca_fascicularis
Chlorocebus_aethiops
Otolemur_garnettii
Tupaia_belangeri
Spermophilus_beecheyi
Marmota_monax
Oryctolagus_cuniculus
Cavia_porcellus
Mesocricetus_auratus
Cricetulus_griseus
Mus_musculus
Rattus_norvegicus
Bos_taurus
Bos_indicus
Ovis_aries
Canis_familiaris
Felis_catus
Sus_scrofa
Delphinapterus_leucas
Sarcophilus_harrisii
Gallus_gallus
Xenopus_laevis
Xenopus_tropicalis
Latimeria_chalumnae
Oncorhynchus_mykiss
Ictalurus_punctatus
Danio_rerio
Barbus_barbus
Xiphophorus_helleri
Xiphophorus_maculatus
Tetraodon_miurus
Takifugu_rubripes
Oreochromis_niloticus
Gasterosteus_aculeatus
Oryzias_latipes
Platichthys_flesus
0.05

15 p53结合DNA基序

a consensus binding site with a striking internal symmetry, consisting of two copies of the 10 base pair motif 5'-PuPuPuC(A/T)(T/A)GPyPyPy-3' separated by 0-13 base pairs. One copy of the motif was insufficient for binding, and subtle alterations of the motif, even when present in multiple copies, resulted in loss of affinity for p53.

p53 与抗病毒先天性免疫



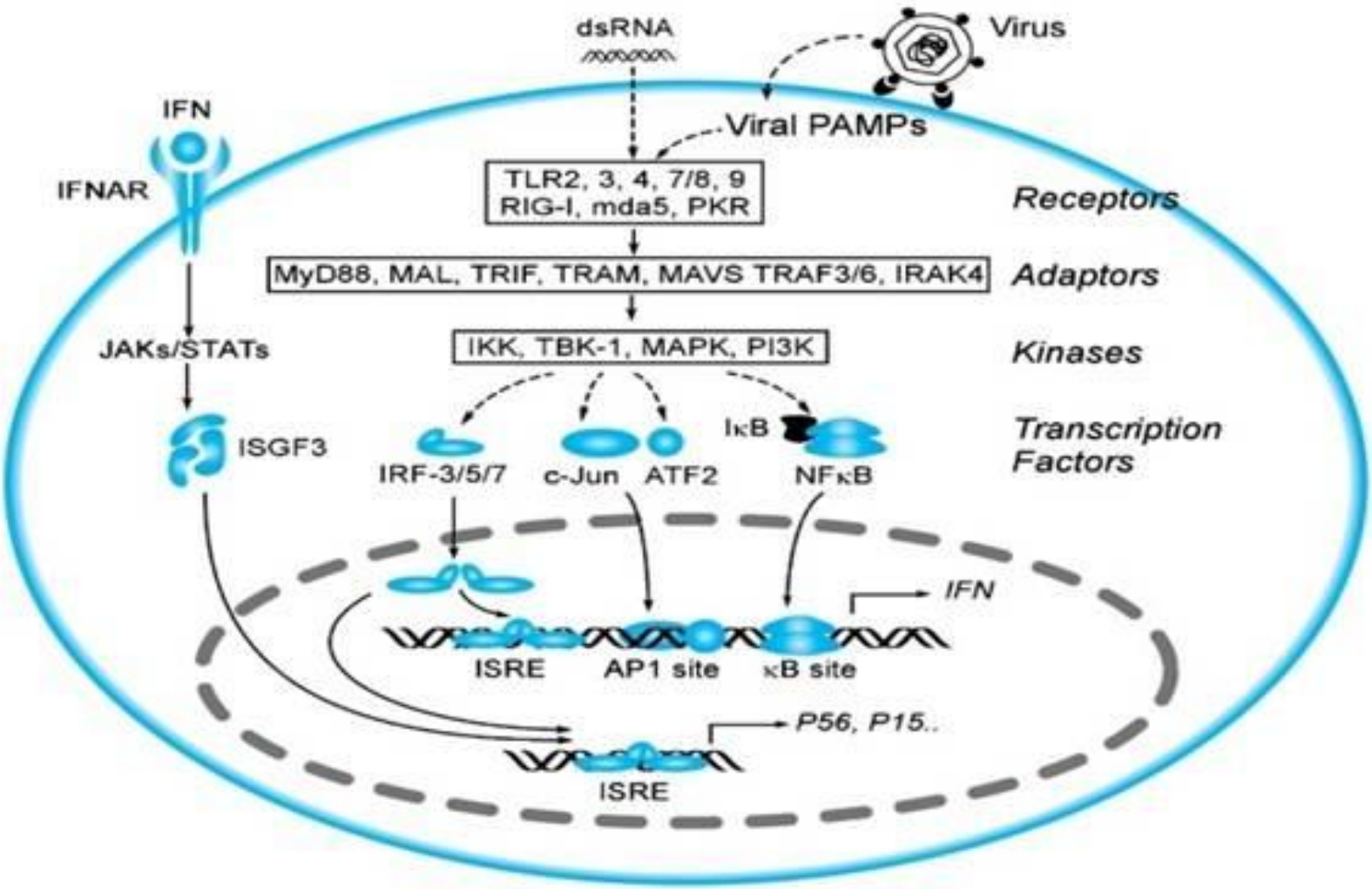
Carmen Rivas，2010

现阶段报道的与p53有关的非致瘤性病毒

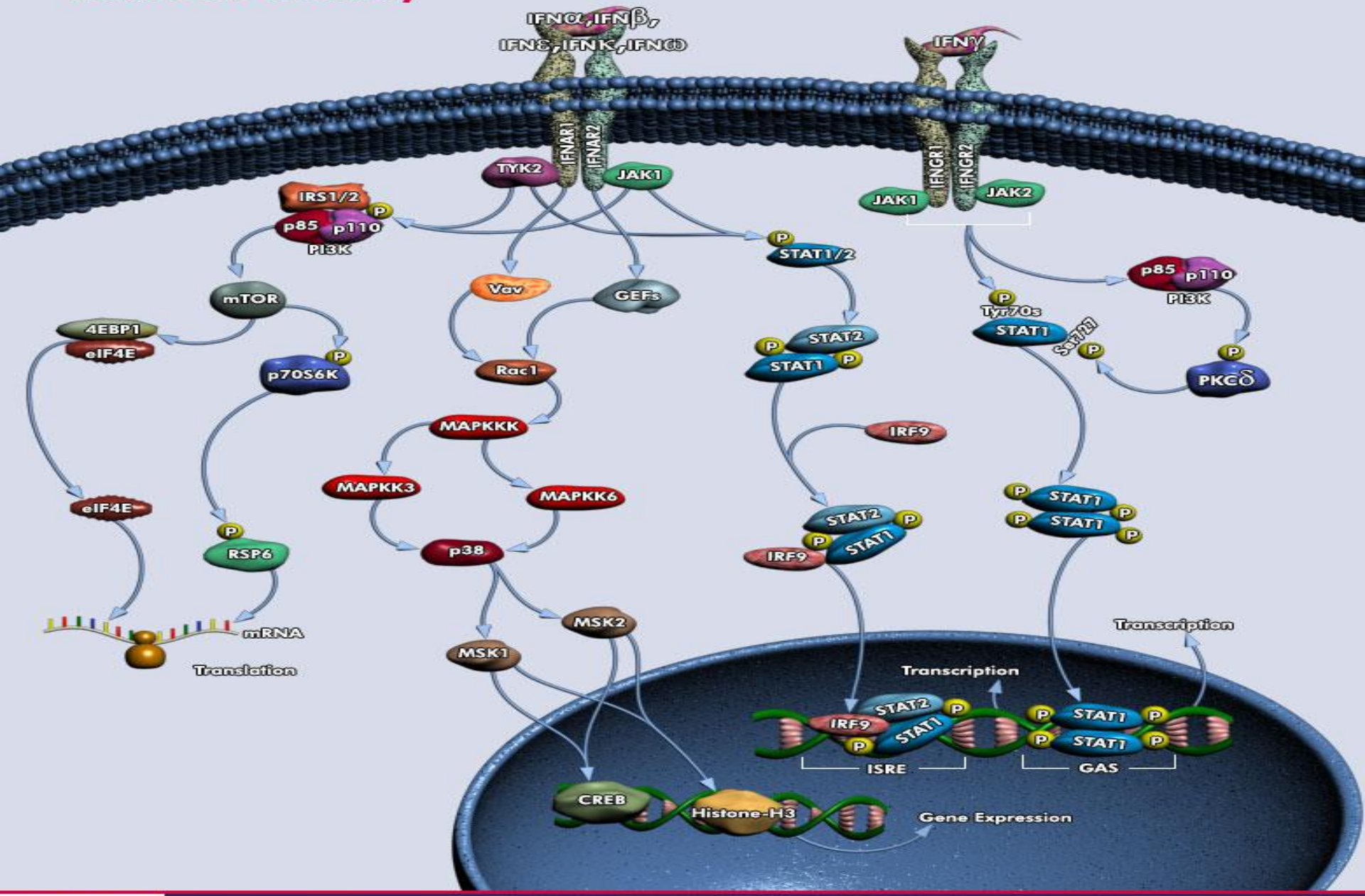
病毒名称	病毒英文名	报道杂志	作者	时间	文章题目
水泡性口炎病毒	Vesicular stomatitis virus	Nature	Akinori Takaoka et al	2003	Integration of interferon-a/b signalling to p53 responses in tumour suppressio and antiviral defence
新城疫病毒	New castle disease virus	Nature	Akinori Takaoka et al	2003	Integration of interferon-a/b signalling to p53 responses in tumour suppressio and antiviral defence
人流感病毒	Influenza virus	JOURNAL OF VIROLOGY	Elizabeth Turpin et al	2005	**Influenza Virus Infection Increases p53 Activity: Role of p53 in Cell Death and Viral Replication**
3型人副流感病毒	Human parainfluenza virus type 3	JOURNAL OF VIROLOGY	Joao T. Marques et al	2005	**Down-regulation of p53 by double-stranded RNA modulates the antiviral response**
人脑心肌炎病毒	Encephalomyo carditis virus	JOURNAL OF VIROLOGY	Joao T. Marques et al	2005	**Down-regulation of p53 by double-stranded RNA modulates the antiviral response**
脊髓灰质炎病毒	Poliovirus	JOURNAL OF VIROLOGY	Mathieu Pampin et al	2006	**Cross talk between PML and p53 during poliovirus infection: implications for antiviral defense**

抗病毒先天性免疫通路

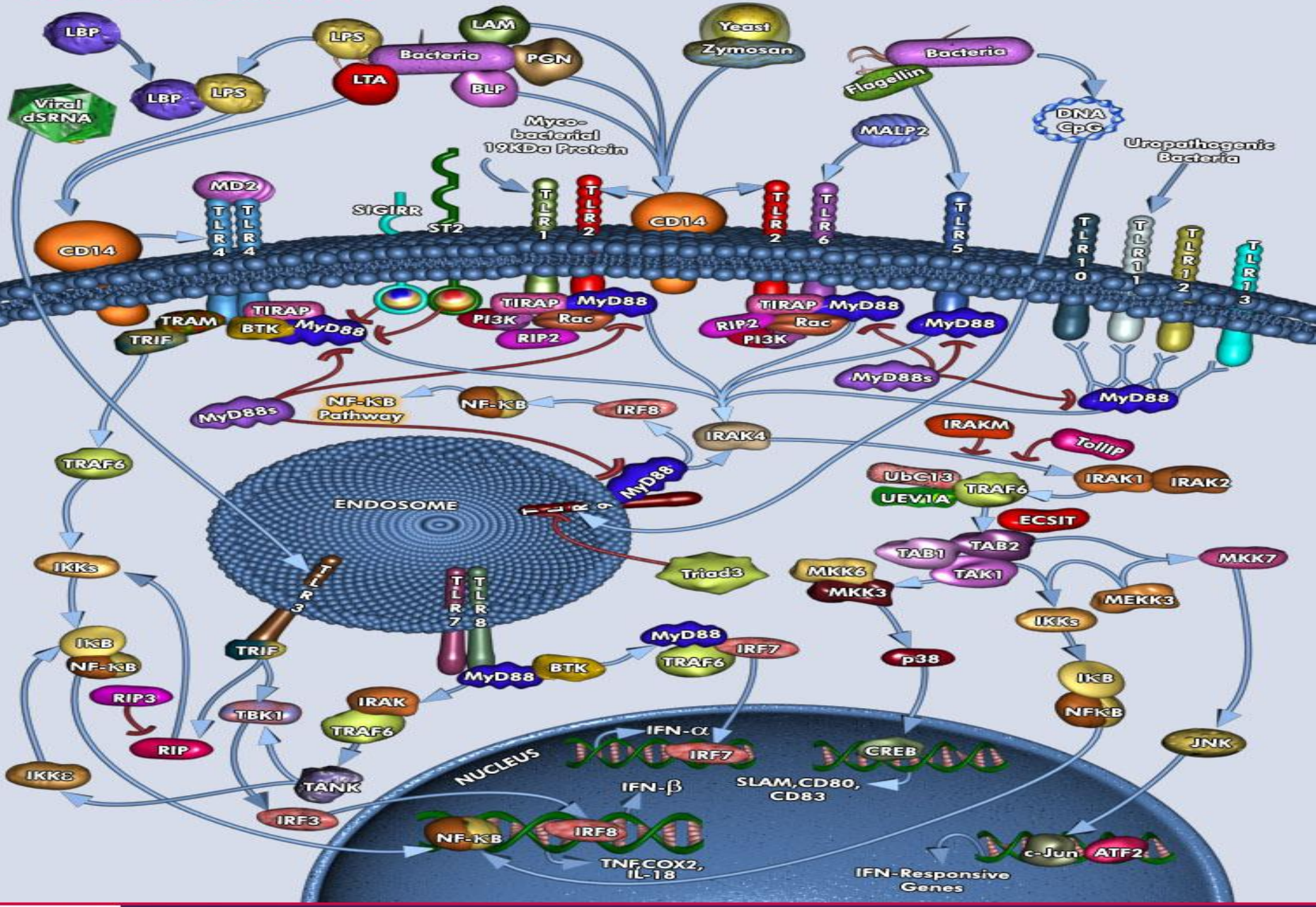
病毒感染激活IFN通路



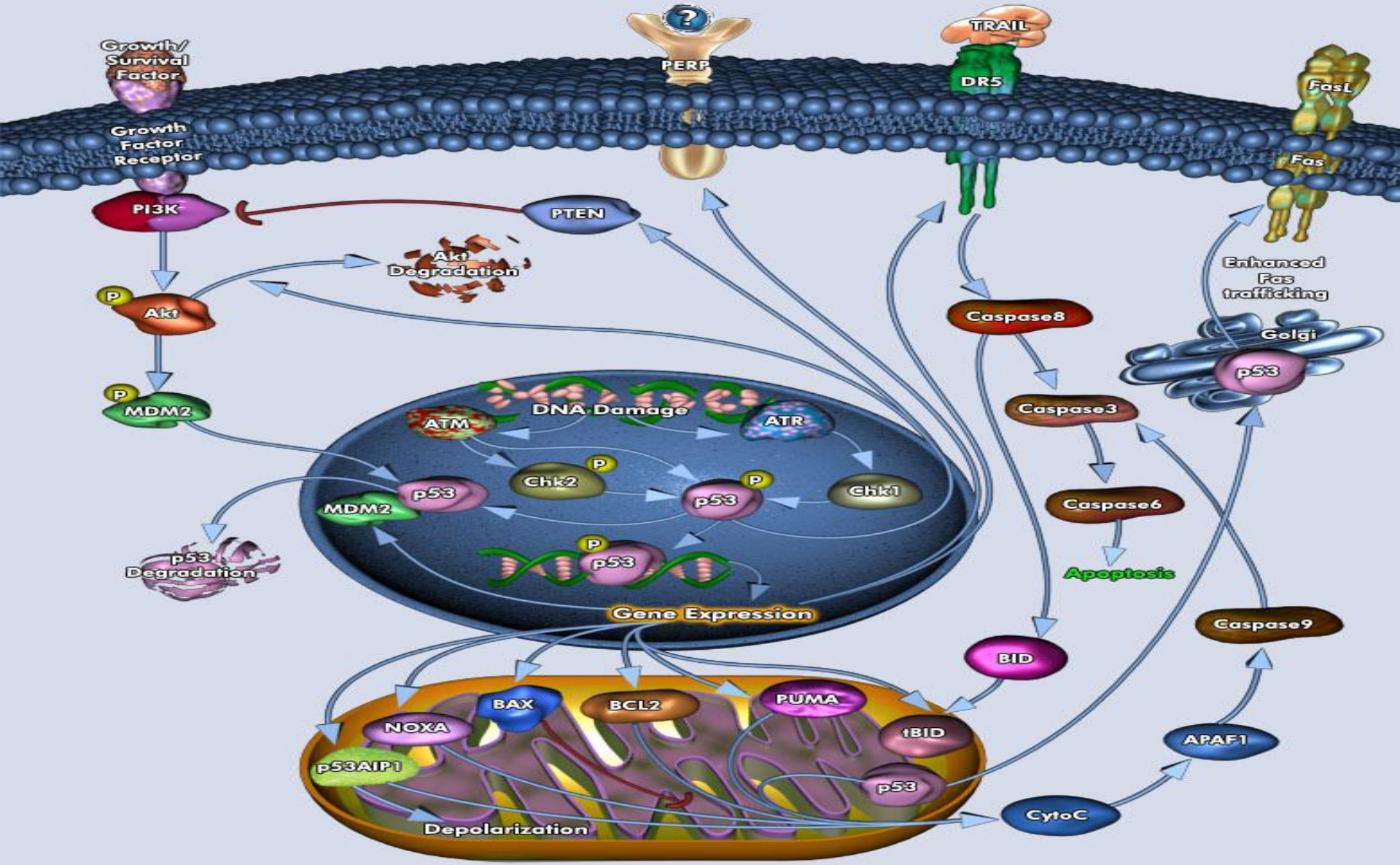
Interferon Pathway



Toll-Like Receptors

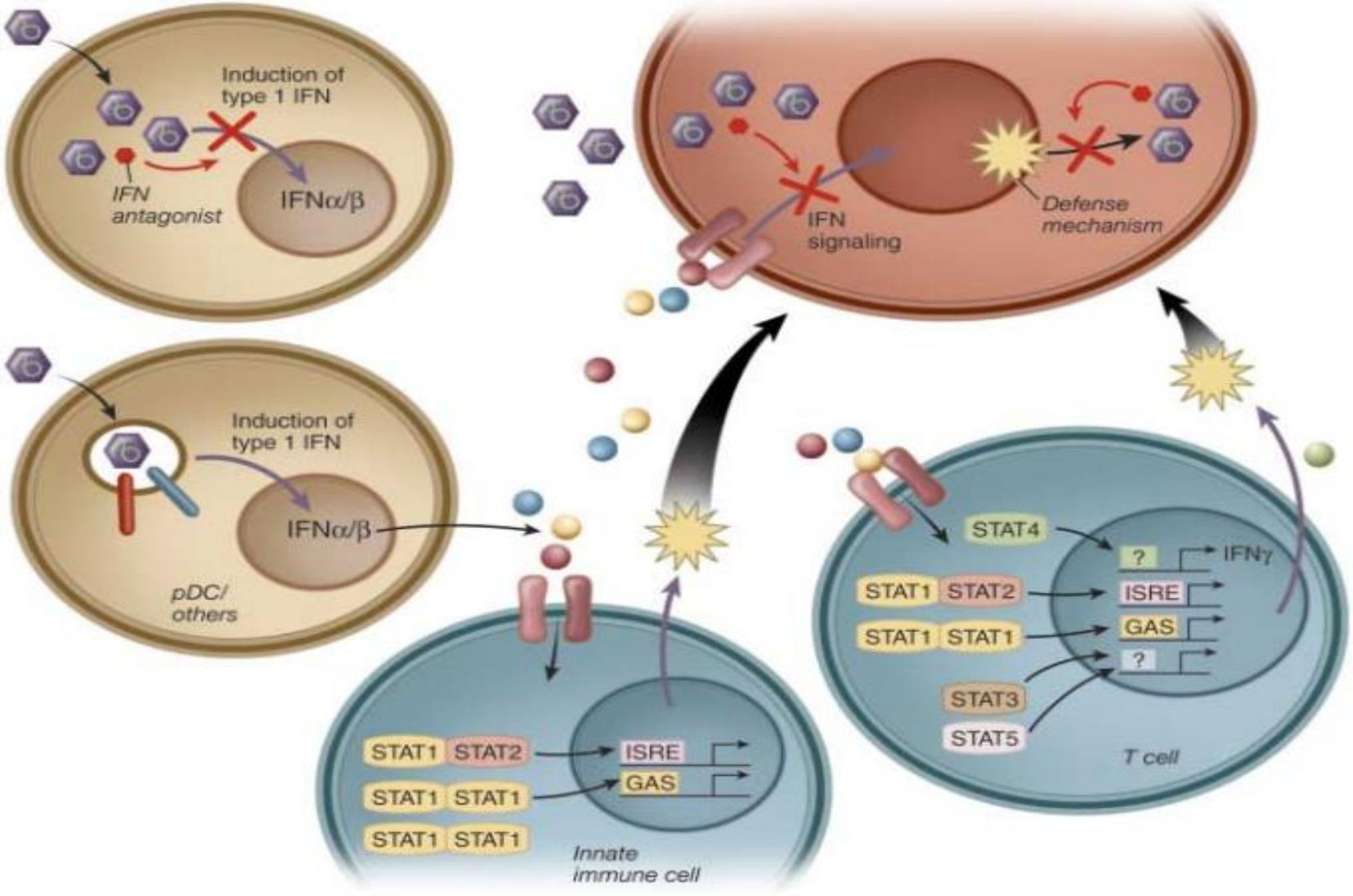


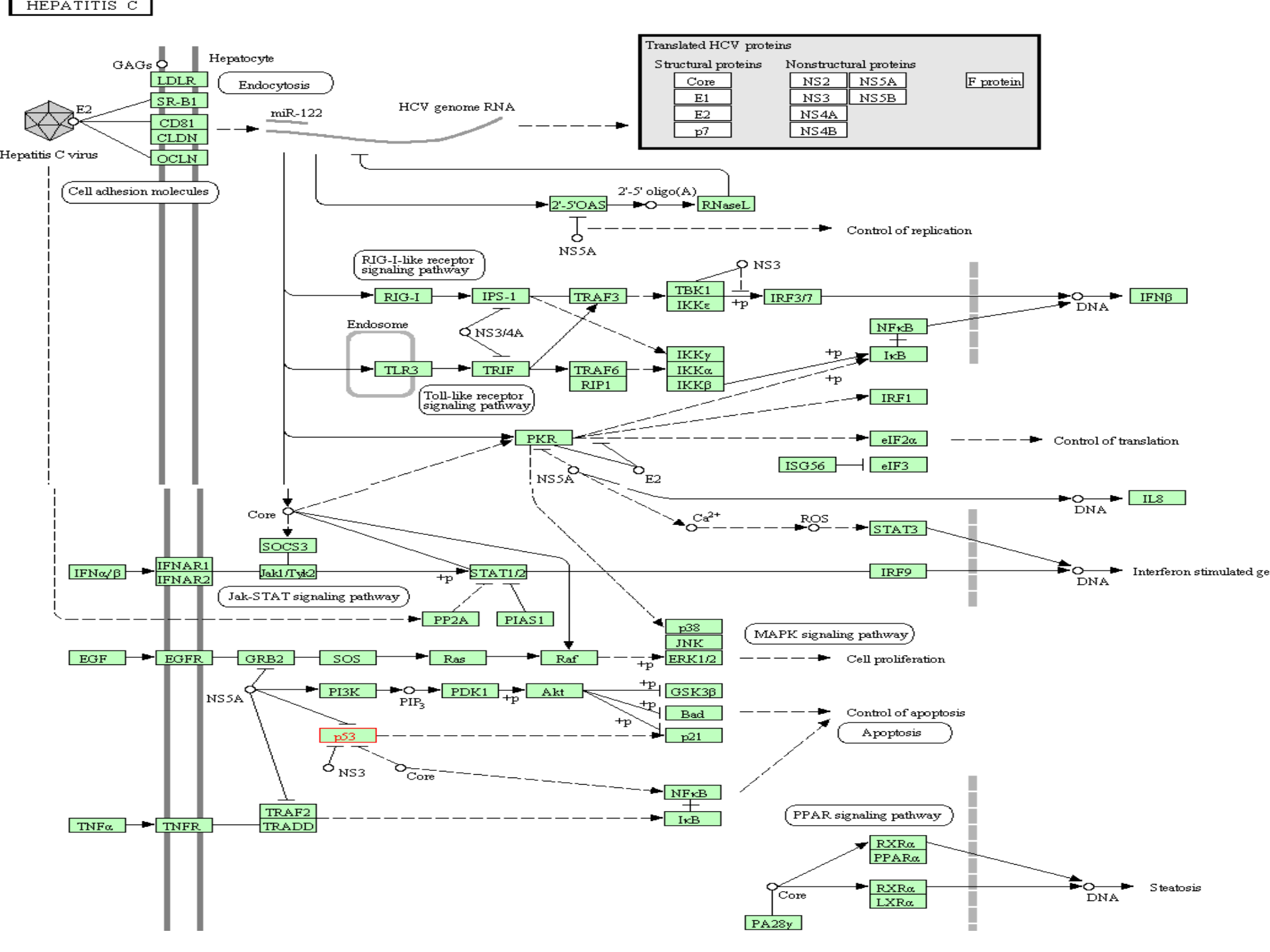
p53 Mediated Apoptosis



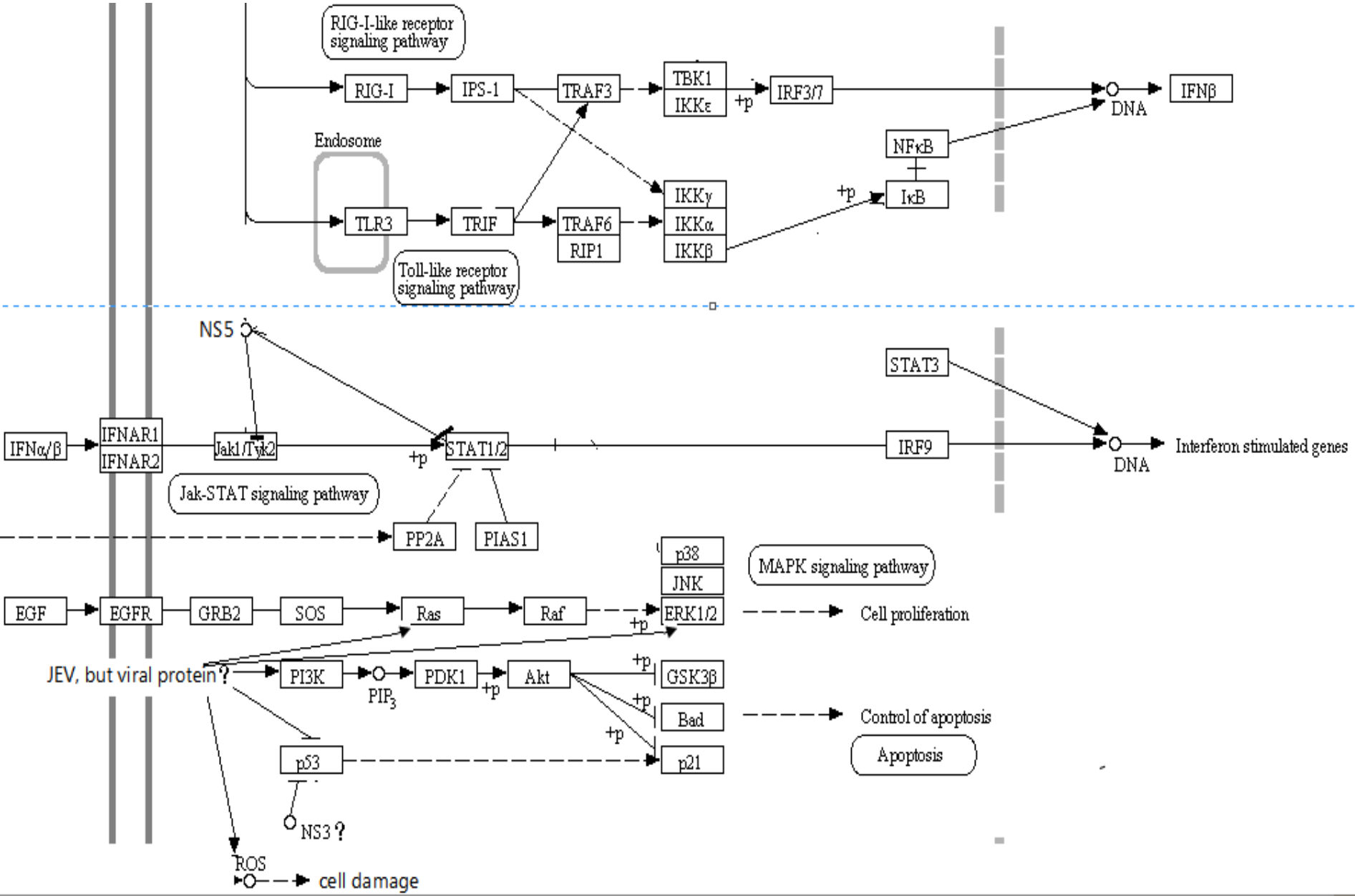
JEV与抗病毒先天性免疫

IFN抗病毒与病毒逃逸




HEPATITIS C
Translated HCV proteins
Structural proteins
Core
E1
E2
p7
Nonstructural proteins
NS2
NS3
NS4A
NS4B
NS5A
NS5B
F protein
GAGs
Hepatocyte
LDLR
SR-B1
CD81
CLDN
OCLN
E2
Hepatitis C virus
Endocytosis
miR-122
HCV genome RNA
Cell adhesion molecules
2'-5'OAS
2'-5' oligo(A)
RNaseL
NS5A
Control of replication
RIG-I-like receptor signaling pathway
RIG-I
IPS-1
TRAF3
TBK1
IKKε
NS3
+p
IRF3/7
DNA
IFNβ
Endosome
NS3/4A
TLR3
TRIF
TRAF6
RIP1
IKKγ
IKKα
IKKβ
NFκB
IκB
Toll-like receptor signaling pathway
IRF1
PKR
eIF2α
Control of translation
ISG56
eIF3
NS5A
E2
IL8
Core
Ca2+
ROS
STAT3
SOCS3
IFNα/β
IFNAR1
IFNAR2
Jak1/Tyk2
STAT1/2
IRF9
Interferon stimulated ge
Jak-STAT signaling pathway
PP2A
PIAS1
p38
JNK
ERK1/2
MAPK signaling pathway
EGF
EGFR
GRB2
SOS
Ras
Raf
Cell proliferation
NS5A
PI3K
PIP3
PDK1
Akt
GSK3β
Bad
Control of apoptosis
p53
p21
Apoptosis
NS3
Core
NFκB
IκB
TNFα
TNFR
TRAF2
TRADD
PPAR signaling pathway
RXRα
PPARα
Core
RXRα
LXRα
DNA
Steatosis
PA28γ

JEV的免疫逃逸途径


RIG-I-like receptor signaling pathway
RIG-I
IPS-1
TRAF3
TBK1
IKKε
+p
IRF3/7
DNA
IFNβ
Endosome
NFκB
IKKγ
+p
IκB
TLR3
TRIF
TRAF6
RIP1
IKKα
IKKβ
Toll-like receptor signaling pathway
NS5
STAT3
IFNα/β
IFNAR1
IFNAR2
Jak1/Tyk2
+p
STAT1/2
IRF9
DNA
Interferon stimulated genes
Jak-STAT signaling pathway
PP2A
PIAS1
p38
JNK
MAPK signaling pathway
EGF
EGFR
GRB2
SOS
Ras
Raf
ERK1/2
Cell proliferation
+p
JEV, but viral protein?
PI3K
PIP3
PDK1
+p
Akt
+p
GSK3β
+p
Bad
+p
Control of apoptosis
p53
p21
Apoptosis
NS3?
ROS
cell damage


昵图网 nipic.com/
诚挚感谢罗老师循循善诱的教导、
对我们课题的关心和建议
Thank You