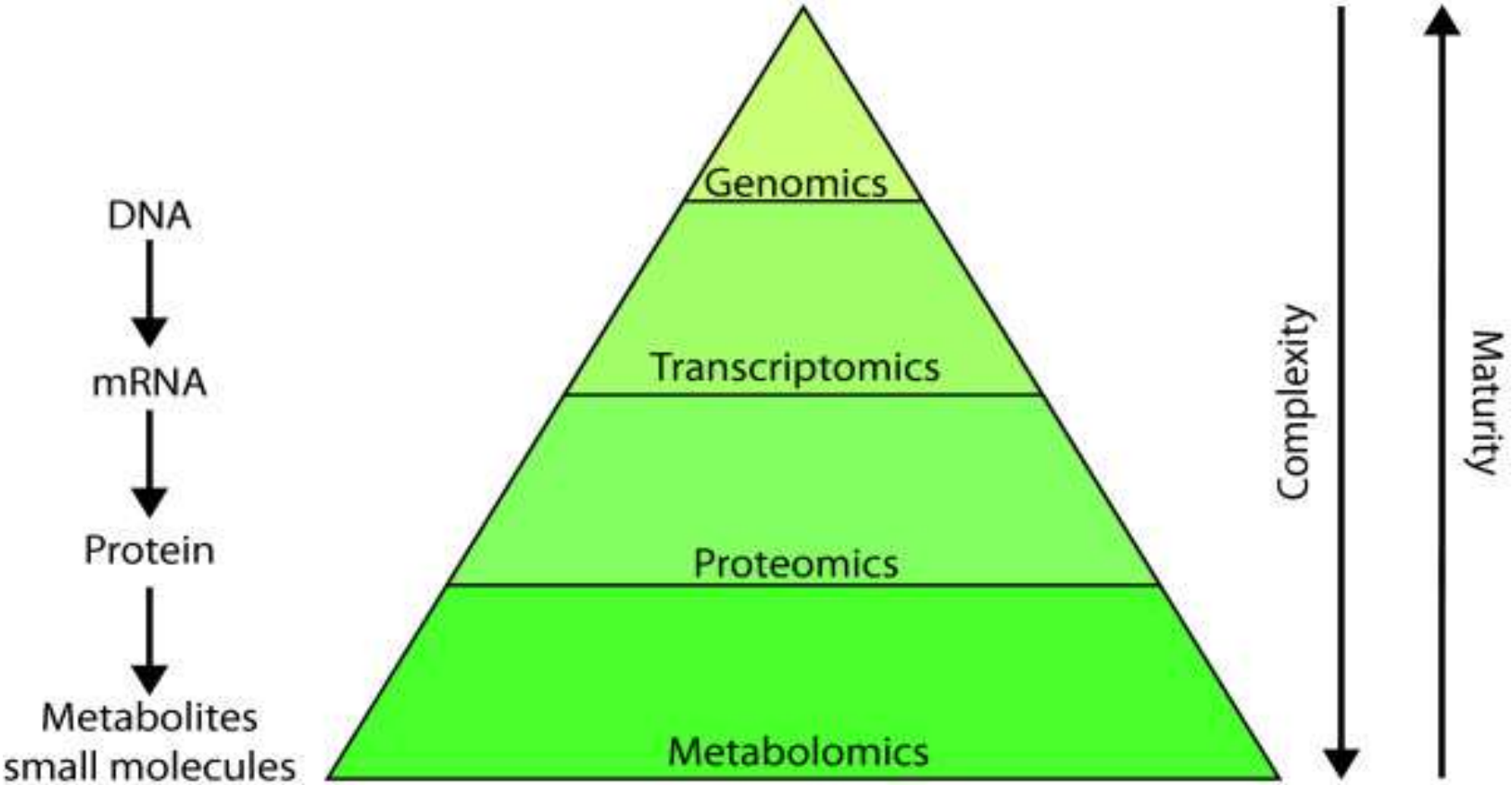
# 代谢基因组学

## Metabolic Genomics

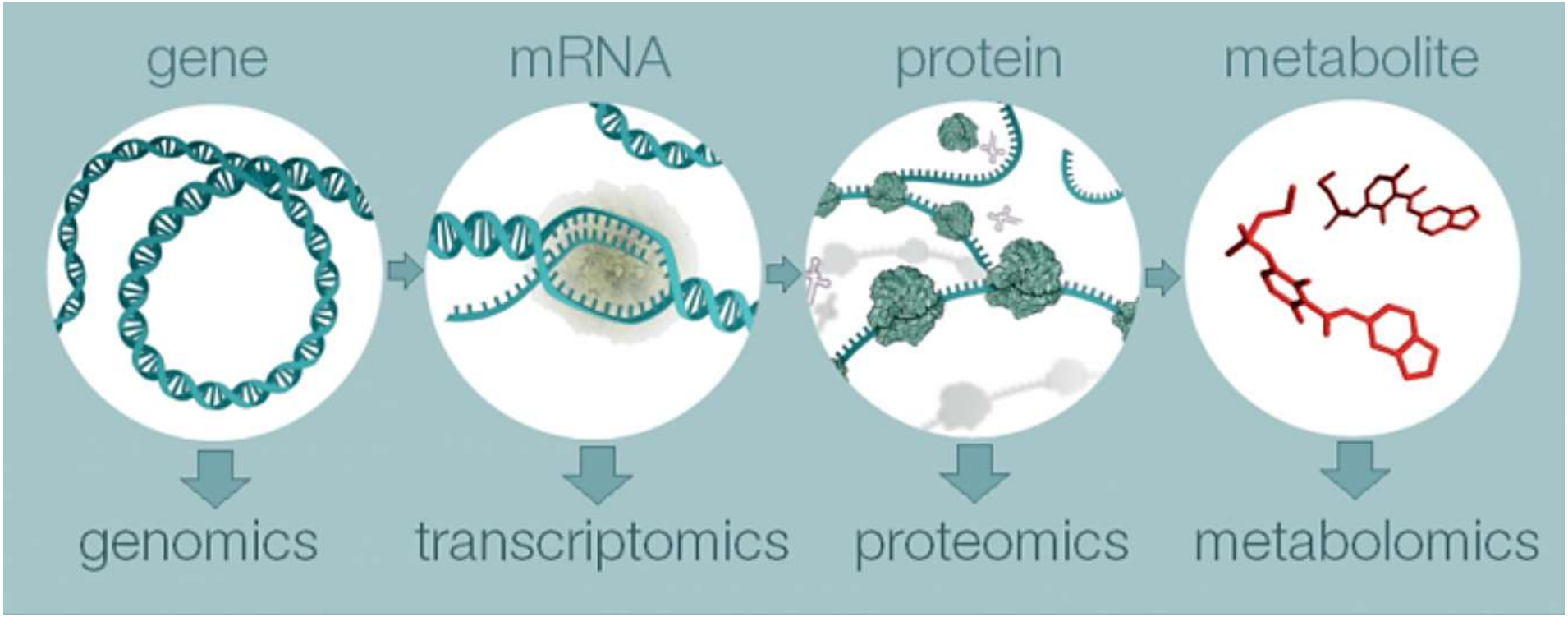
张 健

基础部 生物化学与分子生物学教研室

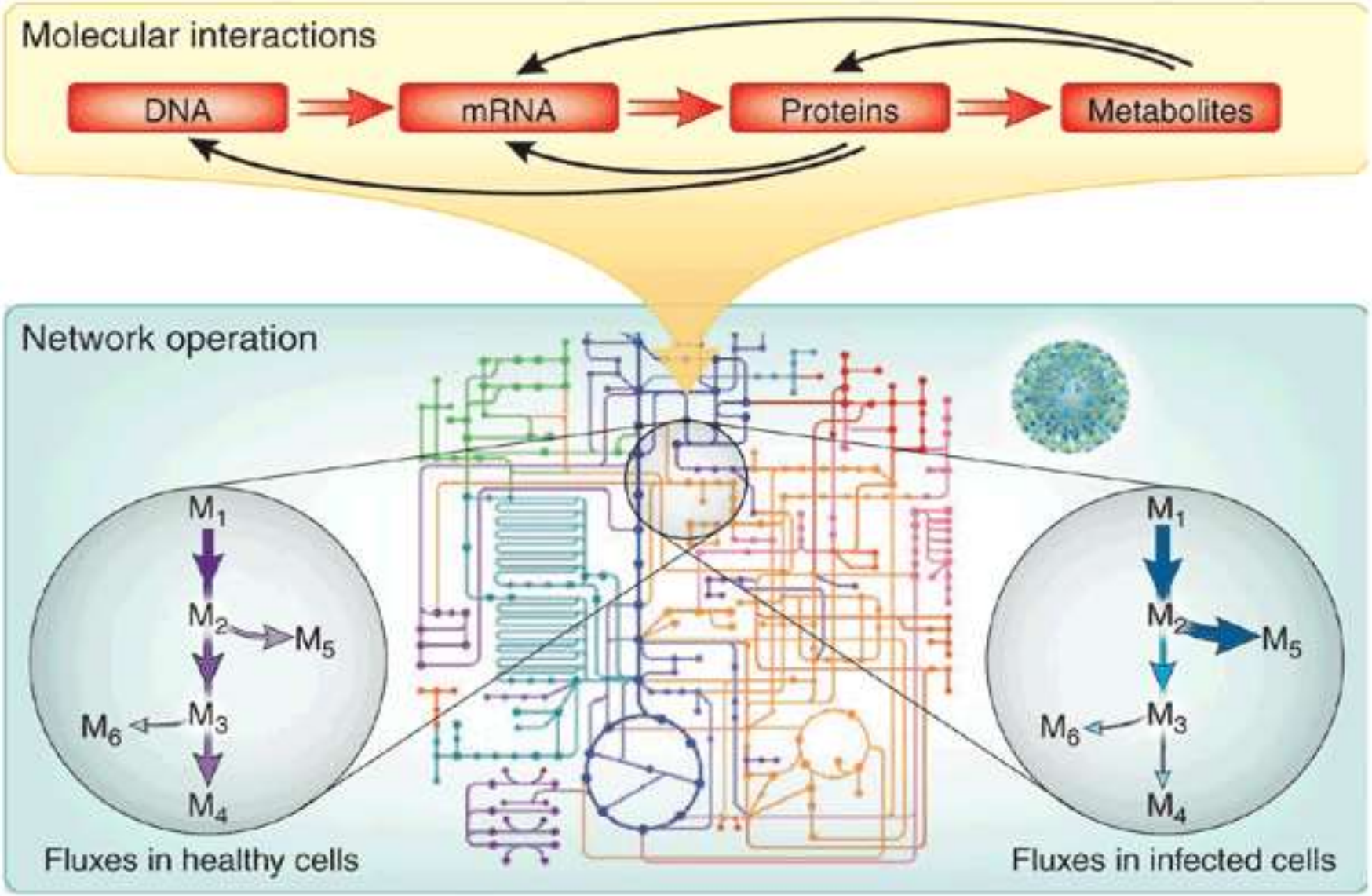
# Metabolic Genomics



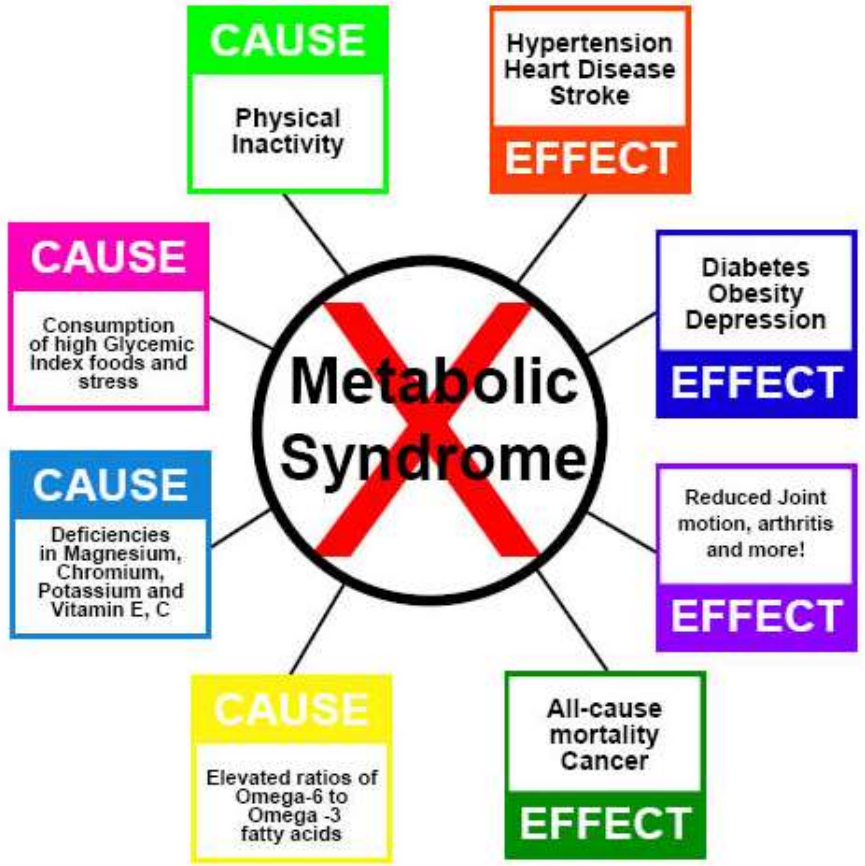
# From genomics to metabolomics



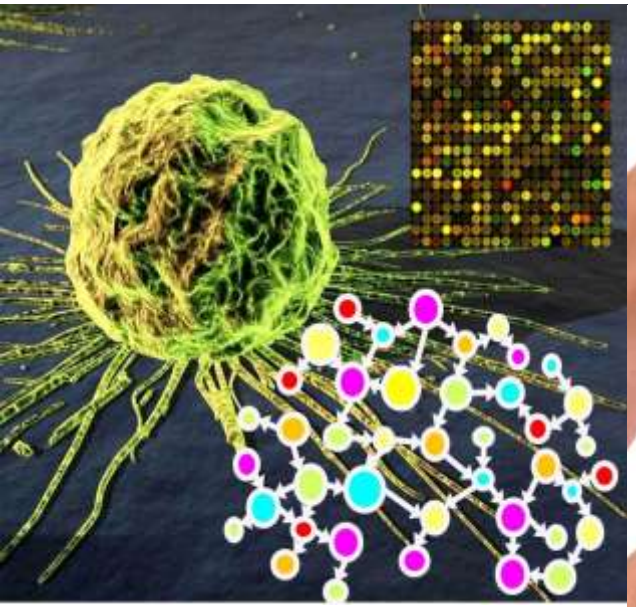

Evolution, development, disease diagnosis and treatment, drug development, et al.

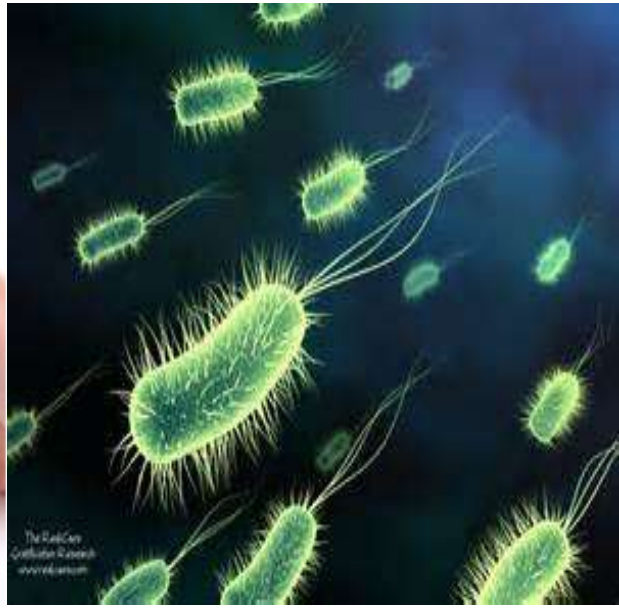



***Nature Biotechnology*** 26, 1090 - 1092 (2008)


CAUSE
Physical Inactivity
Hypertension Heart Disease Stroke
EFFECT
CAUSE
Consumption of high Glycemic Index foods and stress
Diabetes Obesity Depression
EFFECT
Metabolic Syndrome
CAUSE
Deficiencies in Magnesium, Chromium, Potassium and Vitamin E, C
Reduced Joint motion, arthritis and more!
EFFECT
CAUSE
Elevated ratios of Omega-6 to Omega -3 fatty acids
All-cause mortality Cancer
EFFECT

# Metabolic genomics



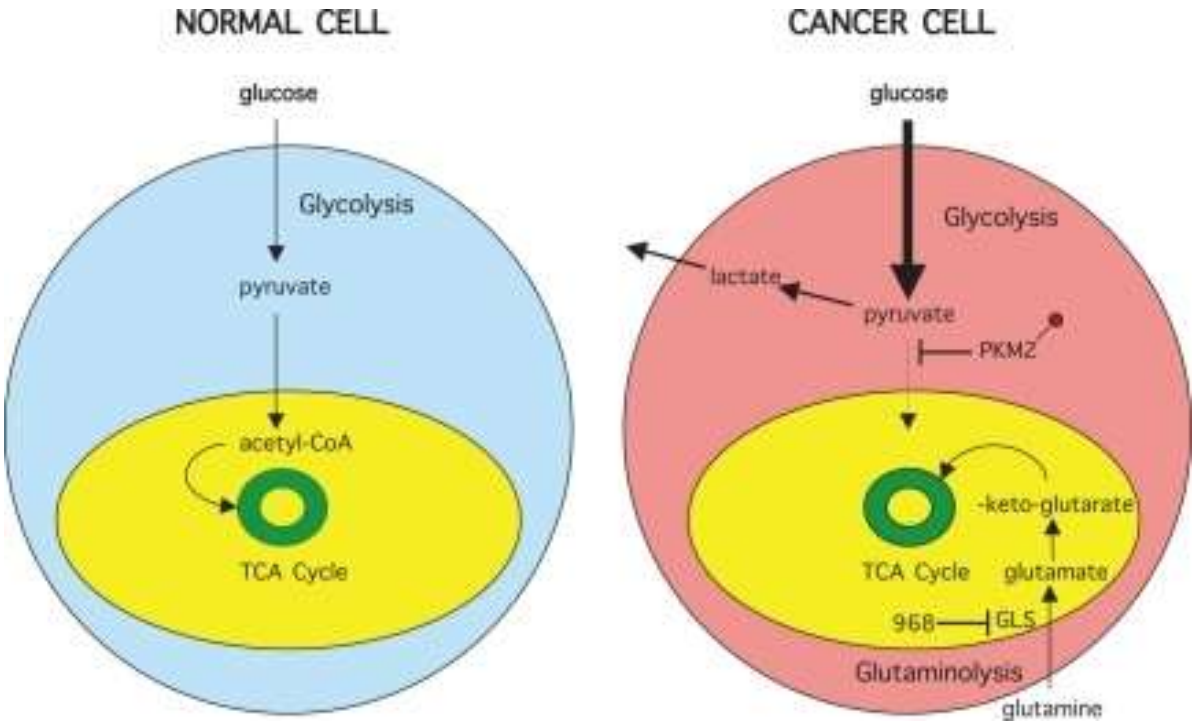



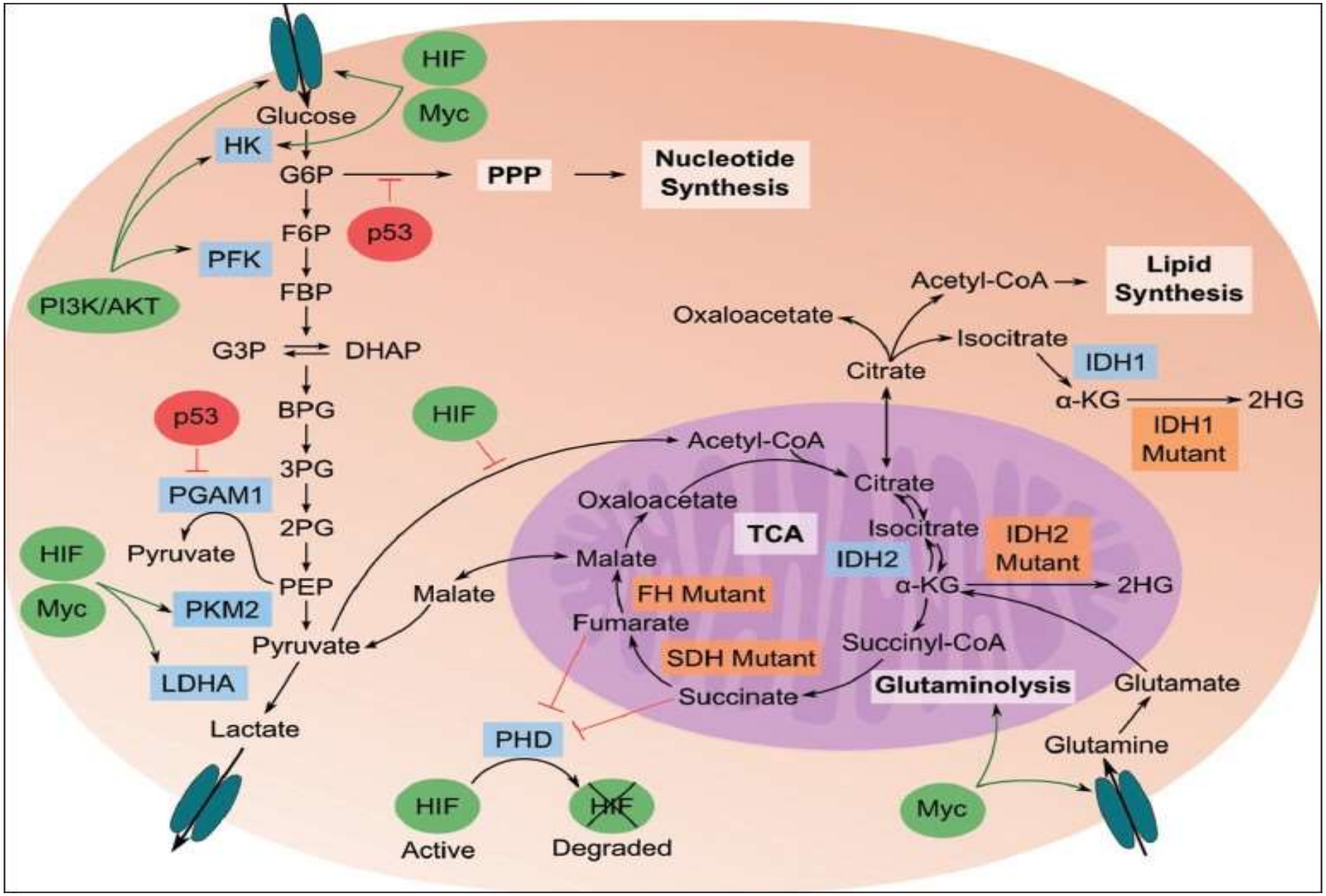
Cancer

Diabetes

Microbiome

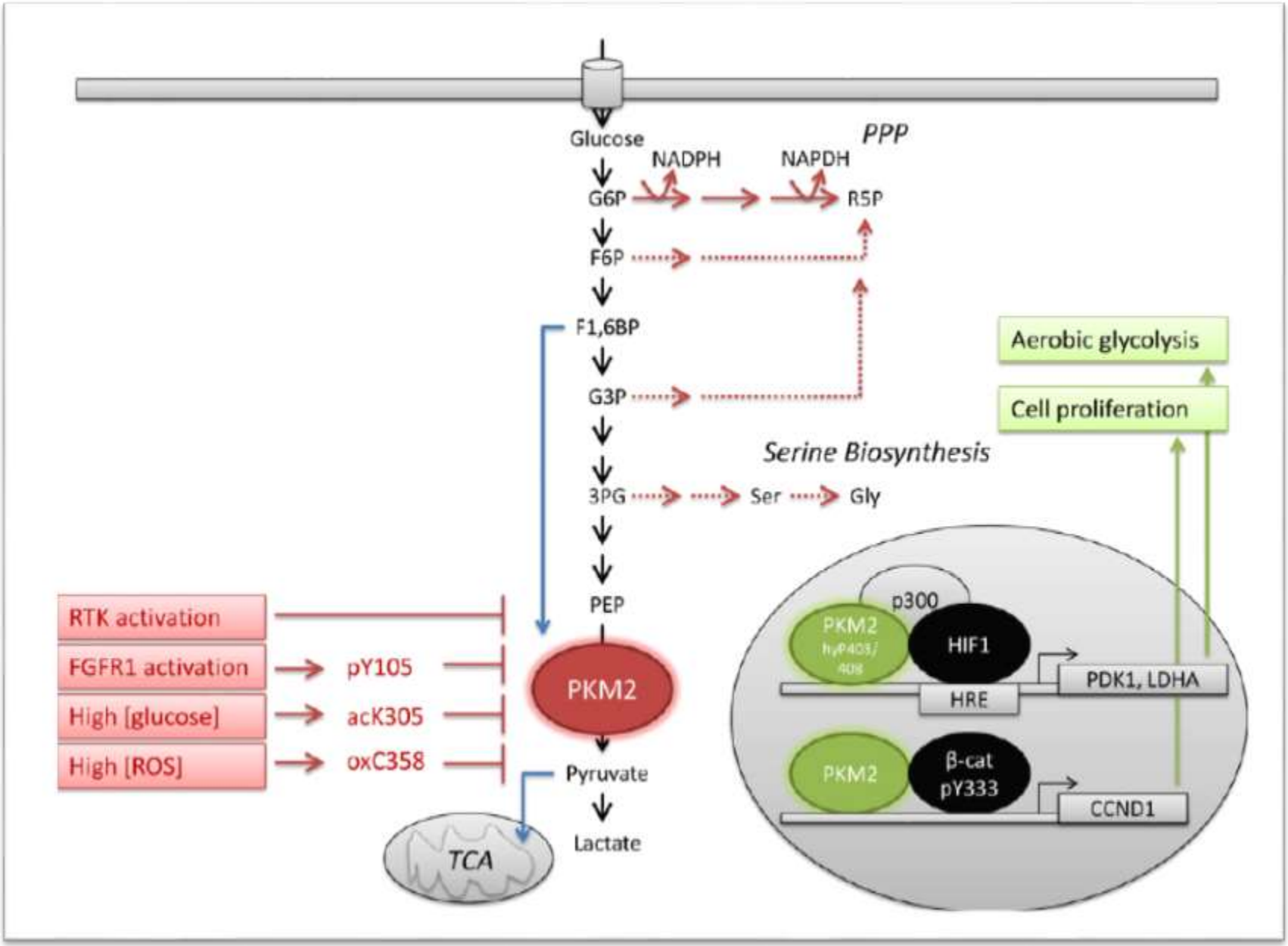
# 1. Metabolic genomics and Cancer






***J Carcinog***. 2013

# （1） PKM2 (Pyruvate kinase, 丙酮酸激酶)



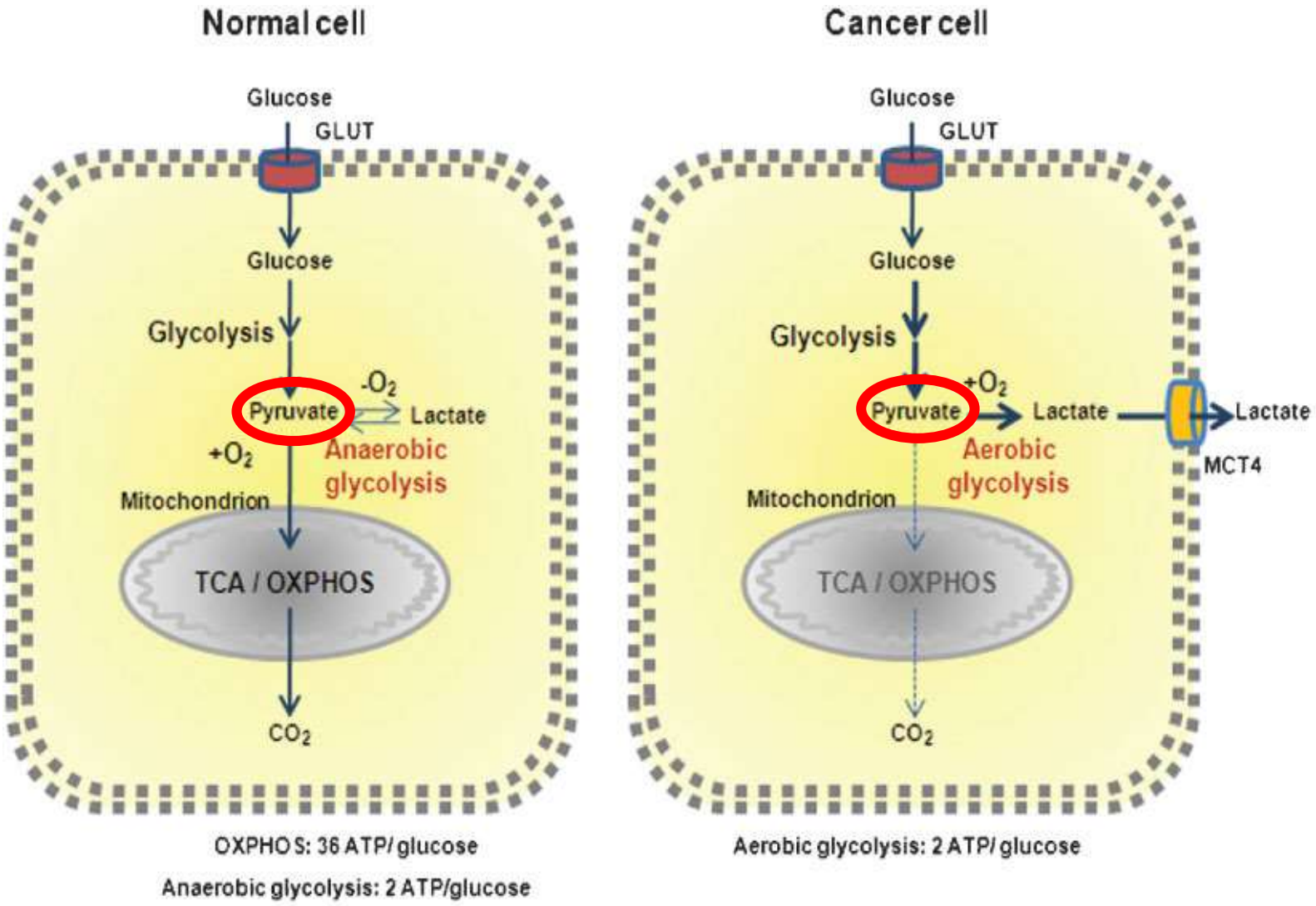
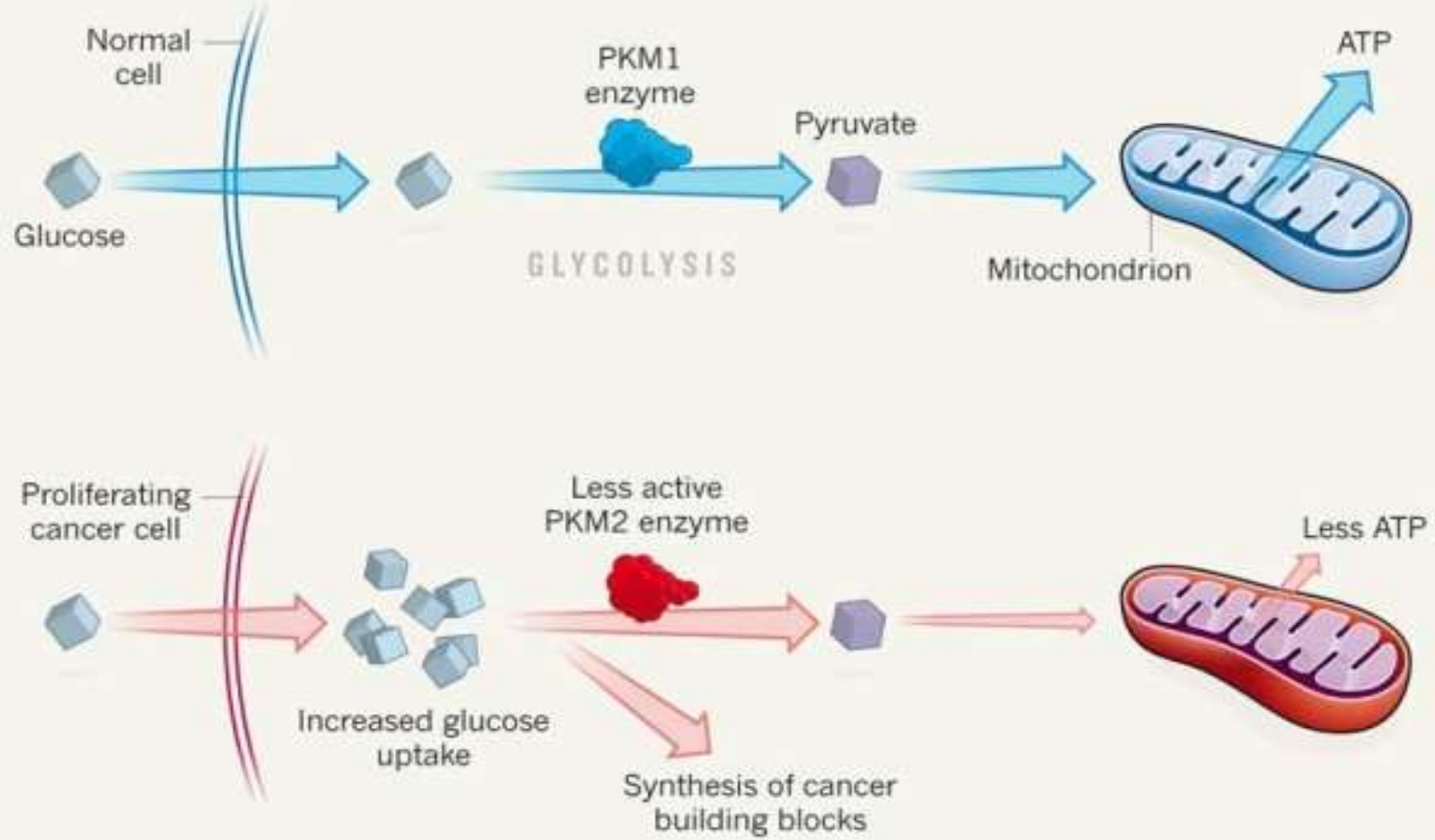



**Figure 1** - Metabolic differences between normal and cancer cells. Normal cells primarily metabolize glucose to pyruvate for growth and survival, followed by complete oxidation of pyruvate to $CO_2$ through the TCA cycle and the OXPHOS process in the mitochondria, generating 36 ATPs per glucose. $O_2$ is essential once it is required as the final acceptor of electrons. When $O_2$ is limited, pyruvate is metabolized to lactate. Cancer cells convert most glucose to lactate regardless of the availability of $O_2$ (the Warburg effect), diverting glucose metabolites from energy production to anabolic process to accelerate cell proliferation, at the expense of generating only two ATPs per glucose.

# ENERGY HUNGRY

Researchers are trying to find targets within cancer's metabolic cycles, such as one that breaks down glucose.

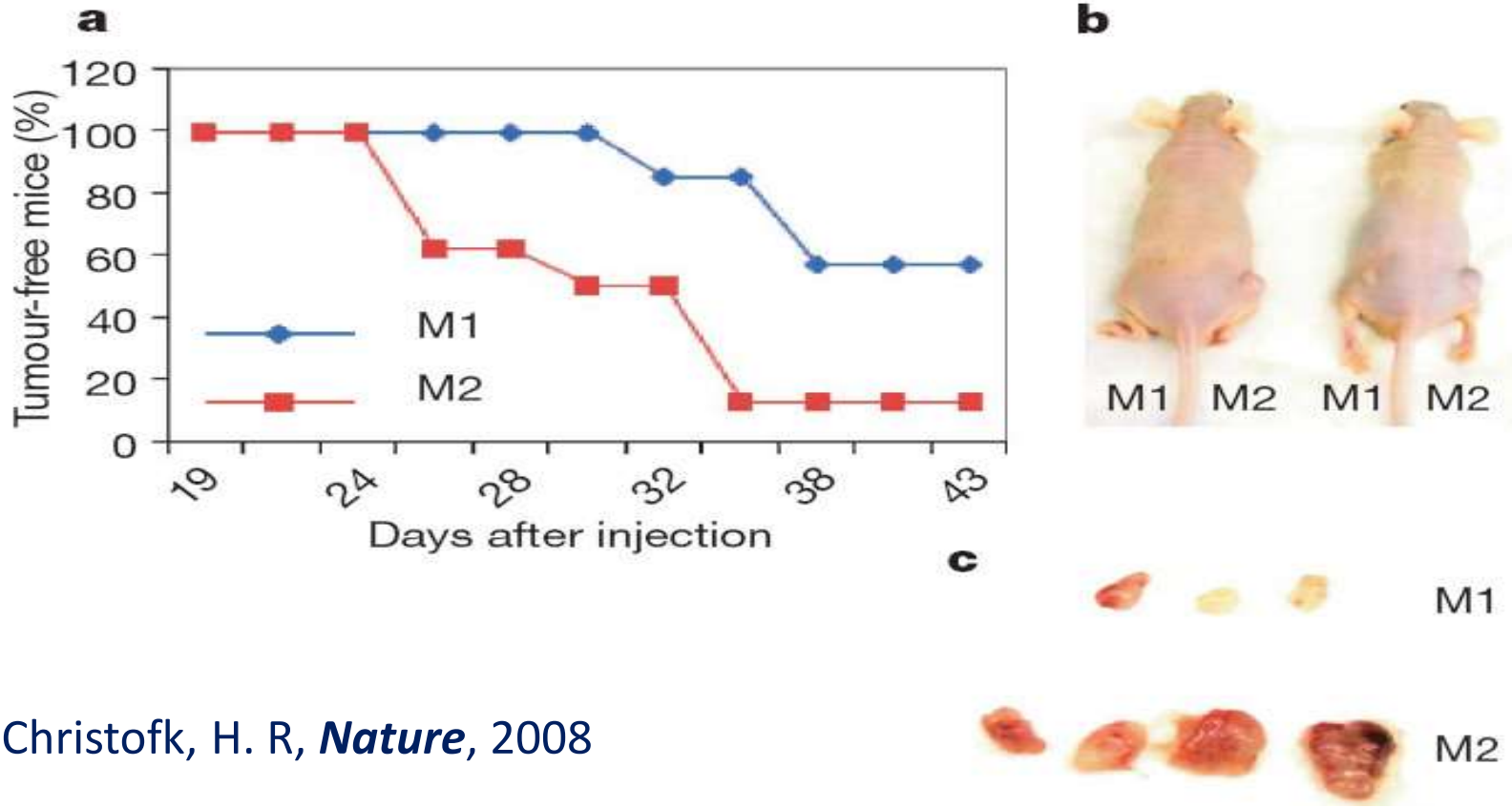


nature Vol 452|13 March 2008|doi:10.1038/nature06734

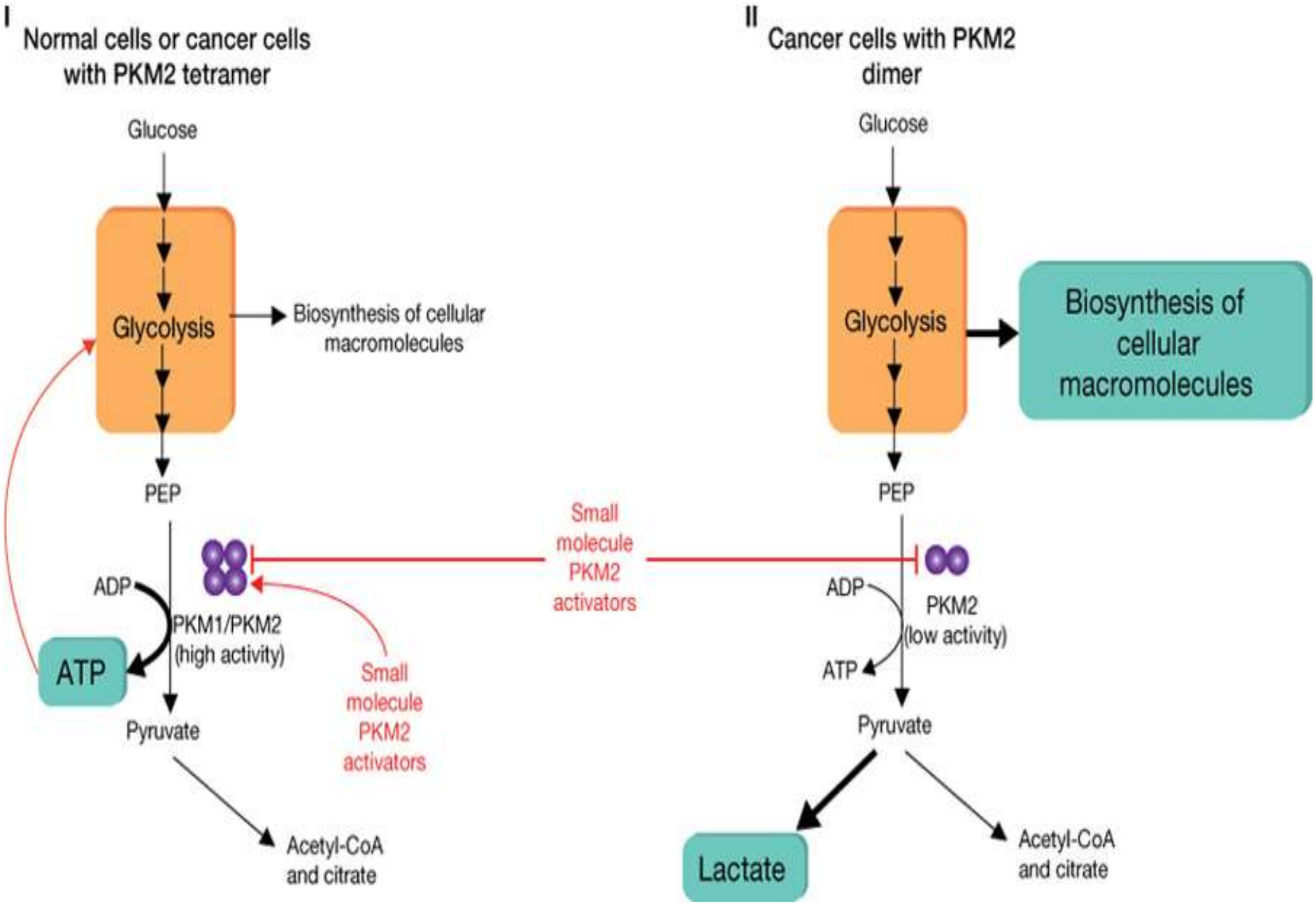
LETTERS

# The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth

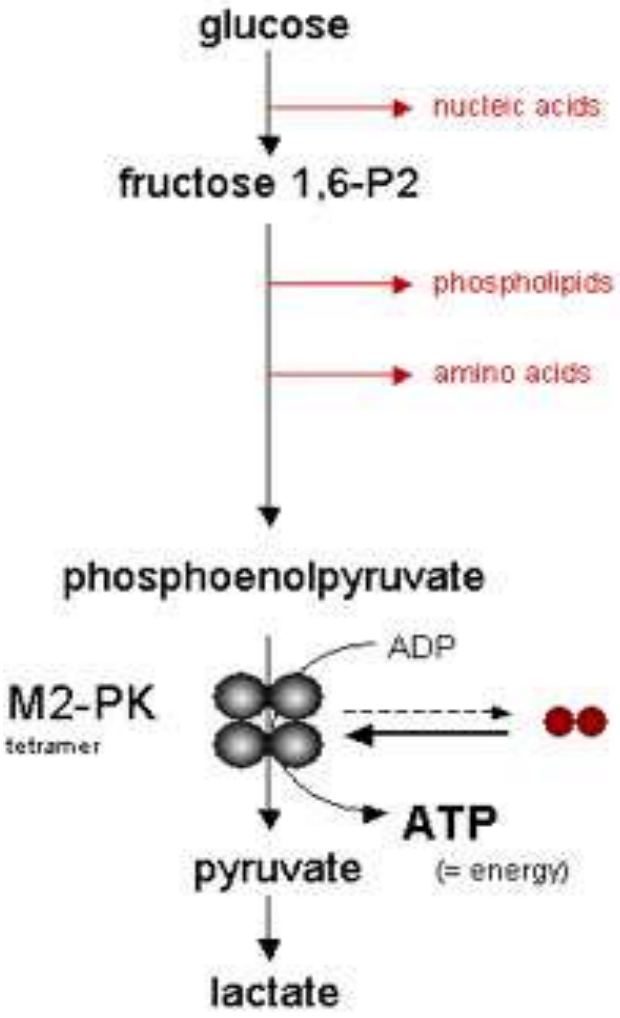
Heather R. Christofk[1], Matthew G. Vander Heiden[1,2], Marian H. Harris[3], Arvind Ramanathan[4], Robert E. Gerszten[4,5,6], Ru Wei[4], Mark D. Fleming[3], Stuart L. Schreiber[4,7] & Lewis C. Cantley[1,8]

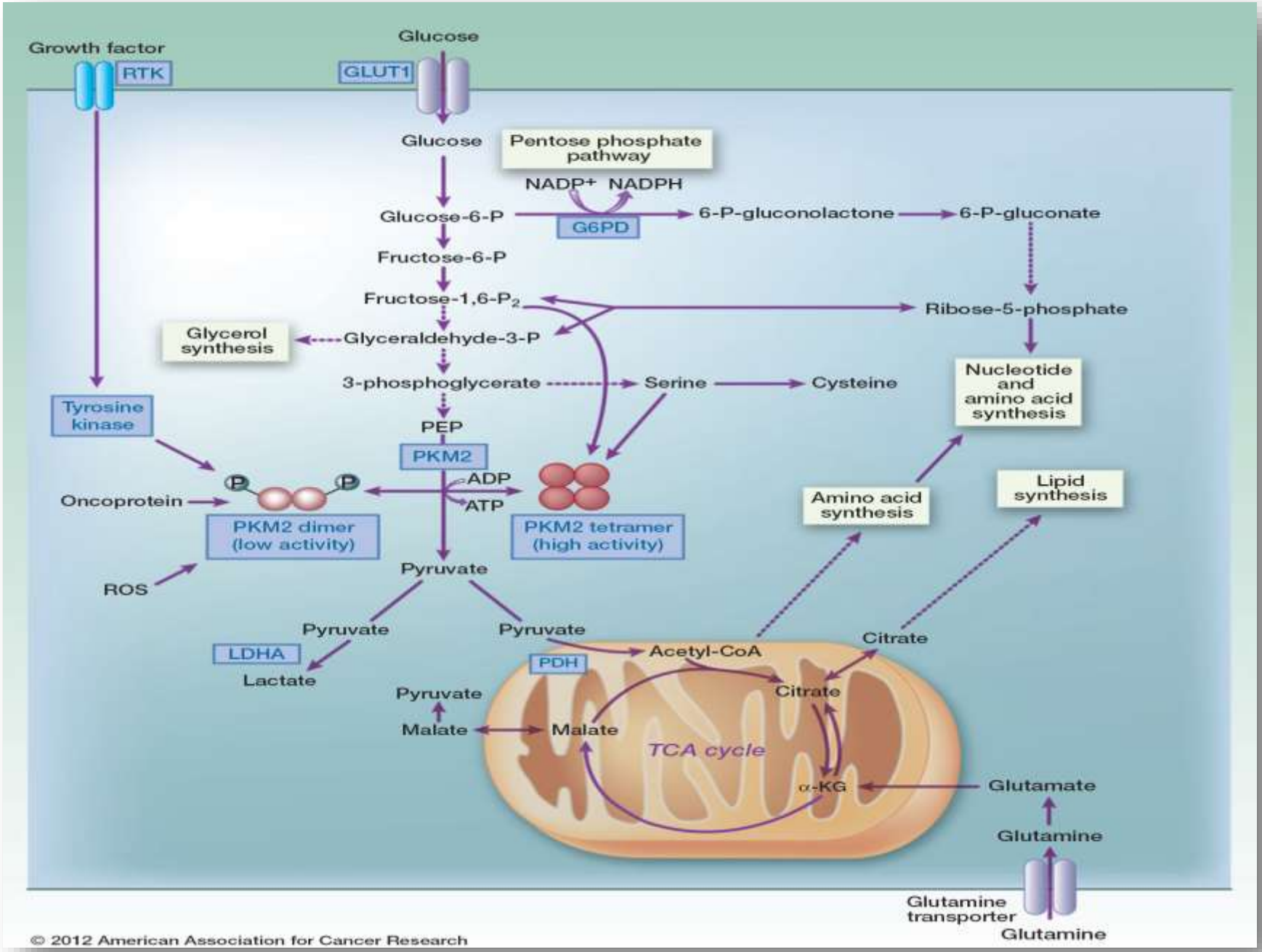



Christofk, H. R, ***Nature***, 2008


I
Normal cells or cancer cells with PKM2 tetramer
Glucose
Glycolysis
Biosynthesis of cellular macromolecules
PEP
ADP
PKM1/PKM2 (high activity)
ATP
Pyruvate
Acetyl-CoA and citrate
Small molecule PKM2 activators
Small molecule PKM2 activators
II
Cancer cells with PKM2 dimer
Glucose
Glycolysis
Biosynthesis of cellular macromolecules
PEP
ADP
PKM2 (low activity)
ATP
Pyruvate
Lactate
Acetyl-CoA and citrate

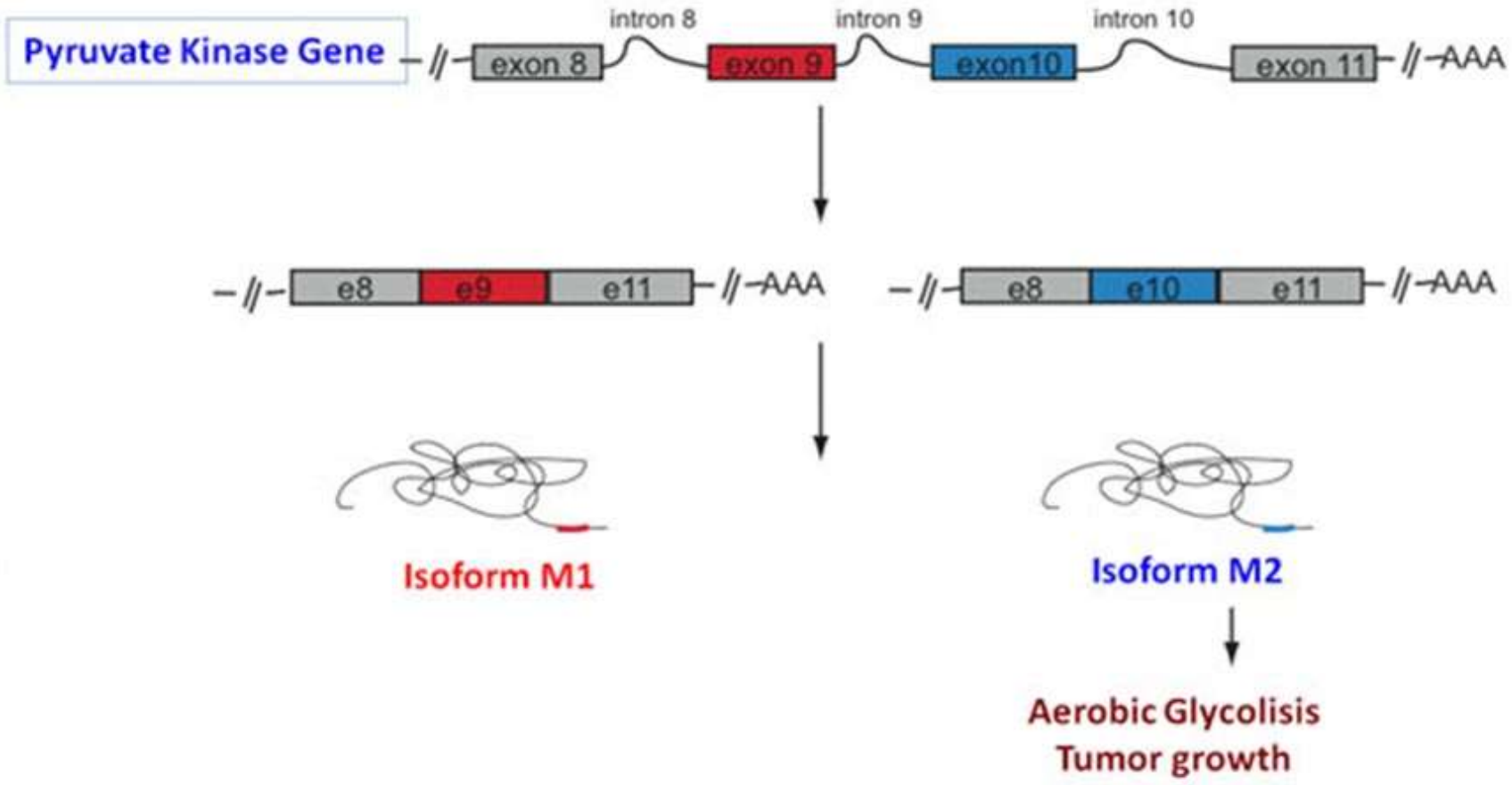
normal proliferating cells



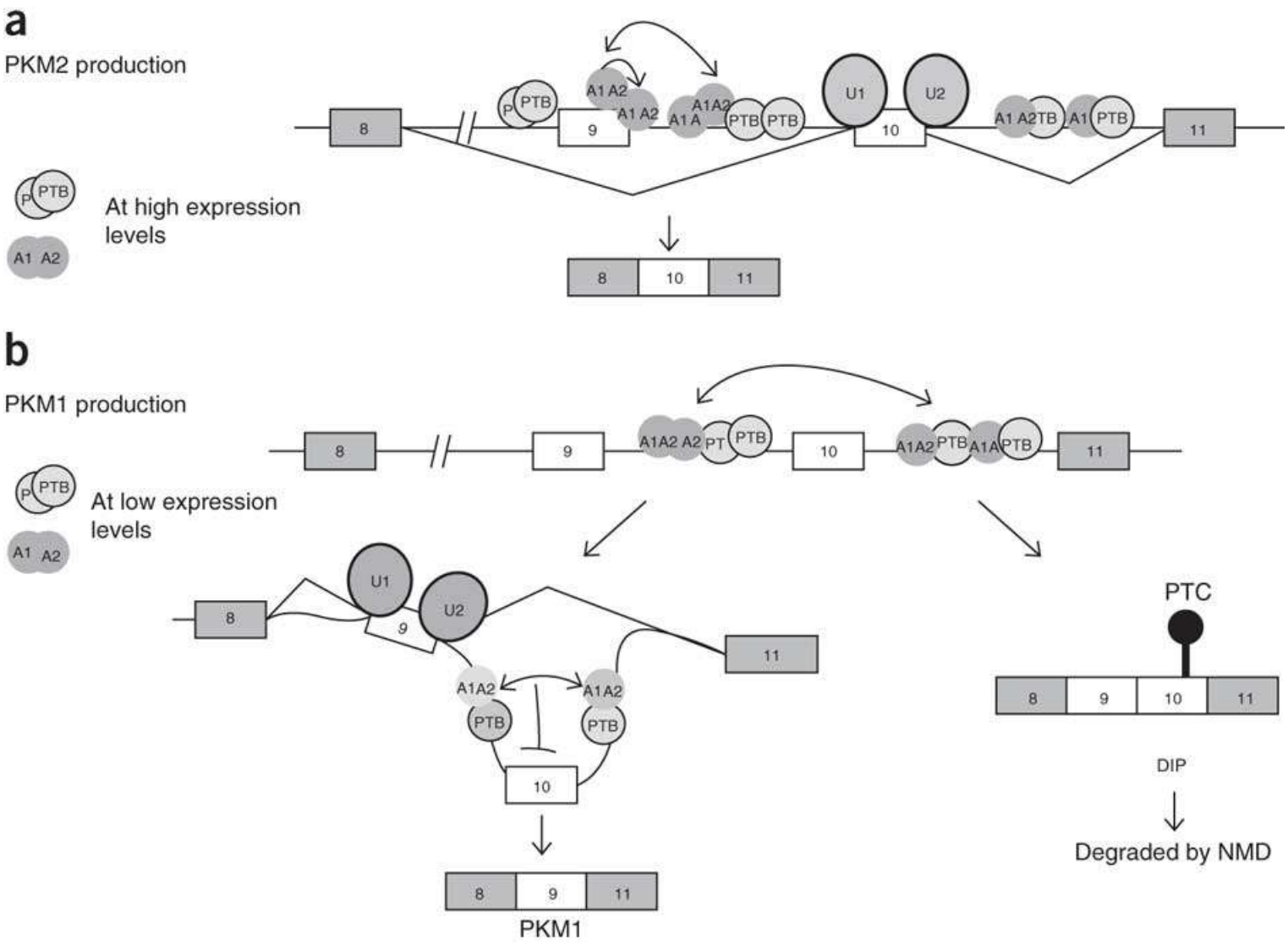



***Clin Cancer Res.*, 2012**

# PKM1 and PKM2 mRNA splicing



# PKM1 and PKM2 mRNA splicing



nature

Vol 463|21 January 2010|doi:10.1038/nature08697

LETTERS

# HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer

Charles J. David[1]*, Mo Chen[1]*, Marcela Assanah[2], Peter Canoll[2] & James L. Manley[1]

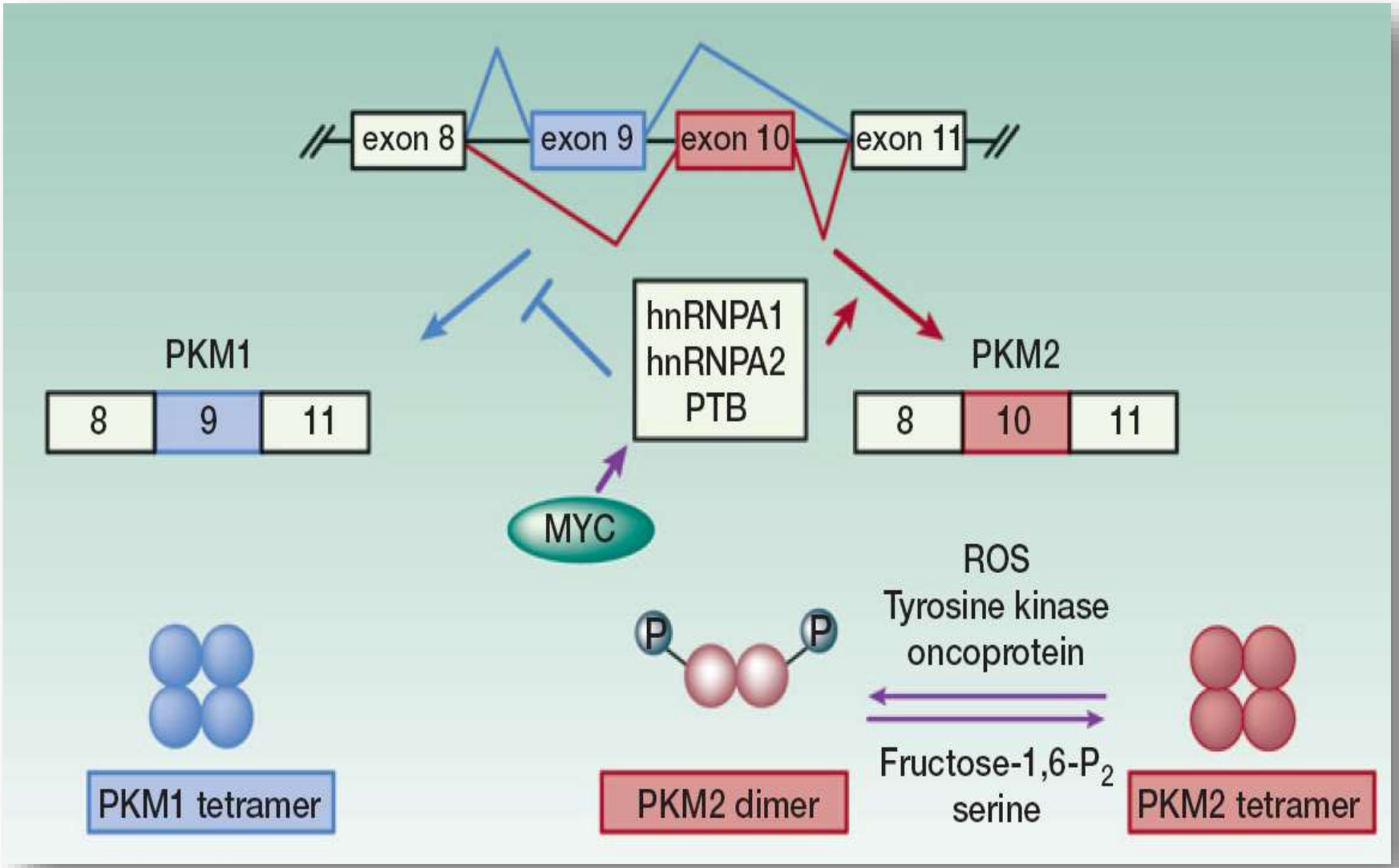
When oxygen is abundant, quiescent cells efficiently extract energy from glucose primarily by oxidative phosphorylation, whereas under the same conditions tumour cells consume glucose more avidly, converting it to lactate. This long-observed phenomenon is known as aerobic glycolysis[1], and is important for cell growth[2,3]. Because aerobic glycolysis is only useful to growing cells, it is tightly regulated in a proliferation-linked manner[4]. In mammals, this is partly achieved through control of pyruvate kinase isoform expression. The embryonic pyruvate kinase isoform, PKM2, is almost universally re-expressed in cancer[2], and promotes aerobic glycolysis, whereas the adult isoform, PKM1, promotes oxidative phosphorylation[2]. These two isoforms result from mutually exclusive alternative splicing of the PKM pre-mRNA, reflecting inclusion of either exon 9 (PKM1) or exon 10 (PKM2). Here we show that three heterogeneous nuclear ribonucleoprotein (hnRNP) proteins, polypyrimidine tract binding protein (PTB, also known as hnRNPI), hnRNPA1 and hnRNPA2, bind repressively to sequences flanking exon 9, resulting in exon 10 inclusion. We also demonstrate that the oncogenic transcription factor c-Myc upregulates transcription of PTB, hnRNPA1 and hnRNPA2, ensuring a high PKM2/PKM1 ratio. Establishing a relevance to cancer, we show that human gliomas overexpress c-Myc, PTB, hnRNPA1 and hnRNPA2 in a manner that correlates with PKM2 expression. Our results thus define a pathway that regulates an alternative splicing event required for tumour cell proliferation.

(Fig. 1b). Strong binding was mapped to a 19-nucleotide region we named EI9(50–68) that spans the E9 5′ splice site (Supplementary Fig. 1). To identify the bound proteins, we performed RNA affinity chromatography using a 5′ biotin-labelled RNA corresponding to EI9(50–68). After SDS–PAGE and Coomassie staining, the pattern of specifically bound proteins closely matched that observed after ultraviolet crosslinking (Fig. 1c). The four indicated proteins between 35–40 kDa were excised and identified by mass spectrometry as isoforms of hnRNPA1 and hnRNPA2, RNA binding proteins with well established roles as sequence-specific repressors of splicing (for example, see refs 7, 8). This result was confirmed by immunoblotting with antibodies against hnRNPA1 (Supplementary Fig. 2).

The sequence immediately downstream of the E9 5′ splice site contains a UAGGGC sequence that is highly related to the consensus hnRNPA1 high affinity binding site identified by SELEX, UAGGG(A/U)[9] (Fig. 1d). Consistent with previous mutational studies of an identical A1 binding site[8], mutation of the G3 nucleotide of this motif to C led to a large decrease in hnRNPA1 and hnRNPA2 binding (Fig. 1d and Supplementary Fig. 3). The G3C mutation resulted in increased splicing *in vitro* when introduced into a splicing substrate containing E9 (Supplementary Fig. 4), and led to increased E9 inclusion in a minigene construct *in vivo* (Supplementary Fig. 5). These data confirm the presence of an inhibitory hnRNPA1/hnRNPA2 binding site immediately downstream of the E9 5′ splice site.

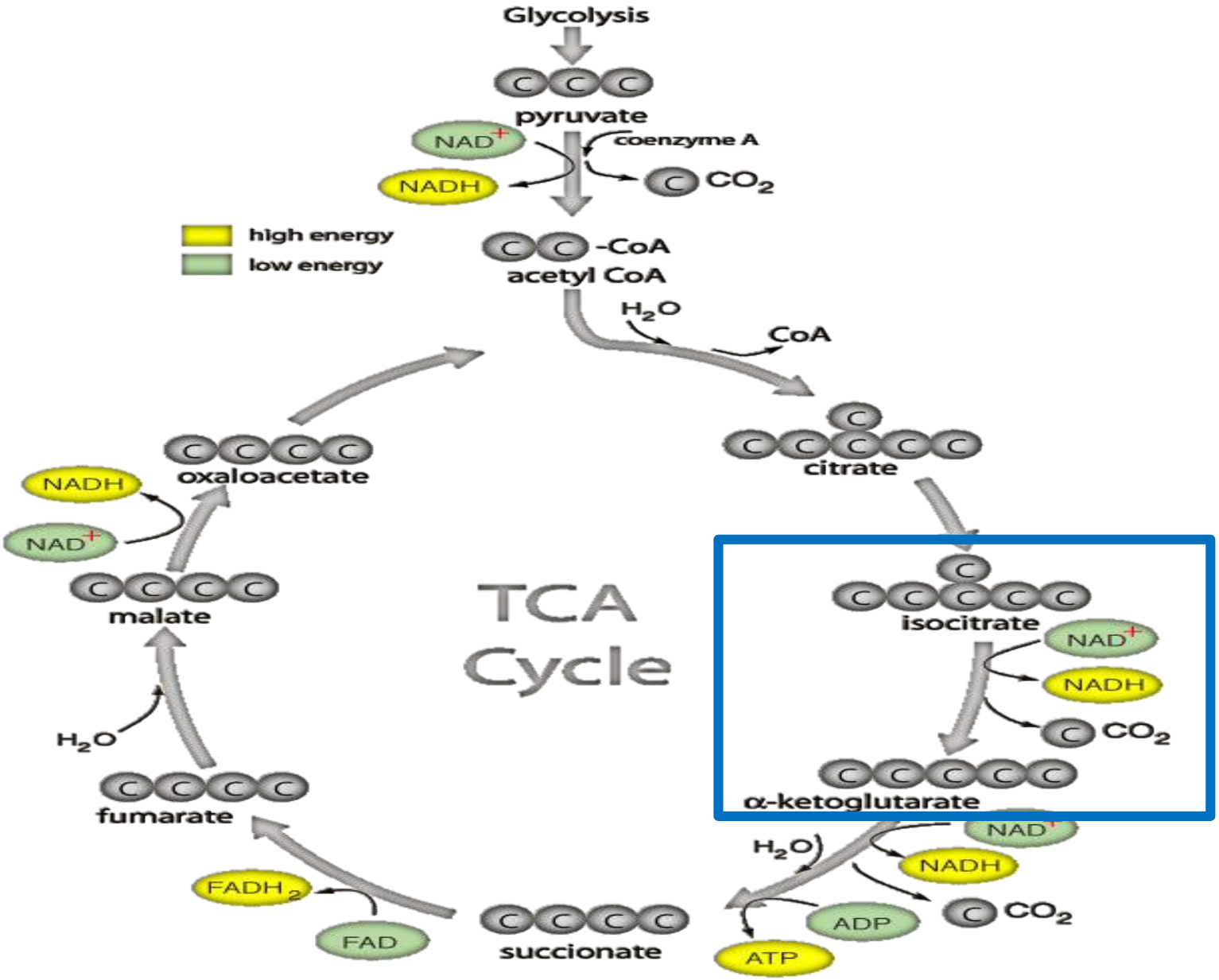
To explore the possibility that other splicing regulators bind

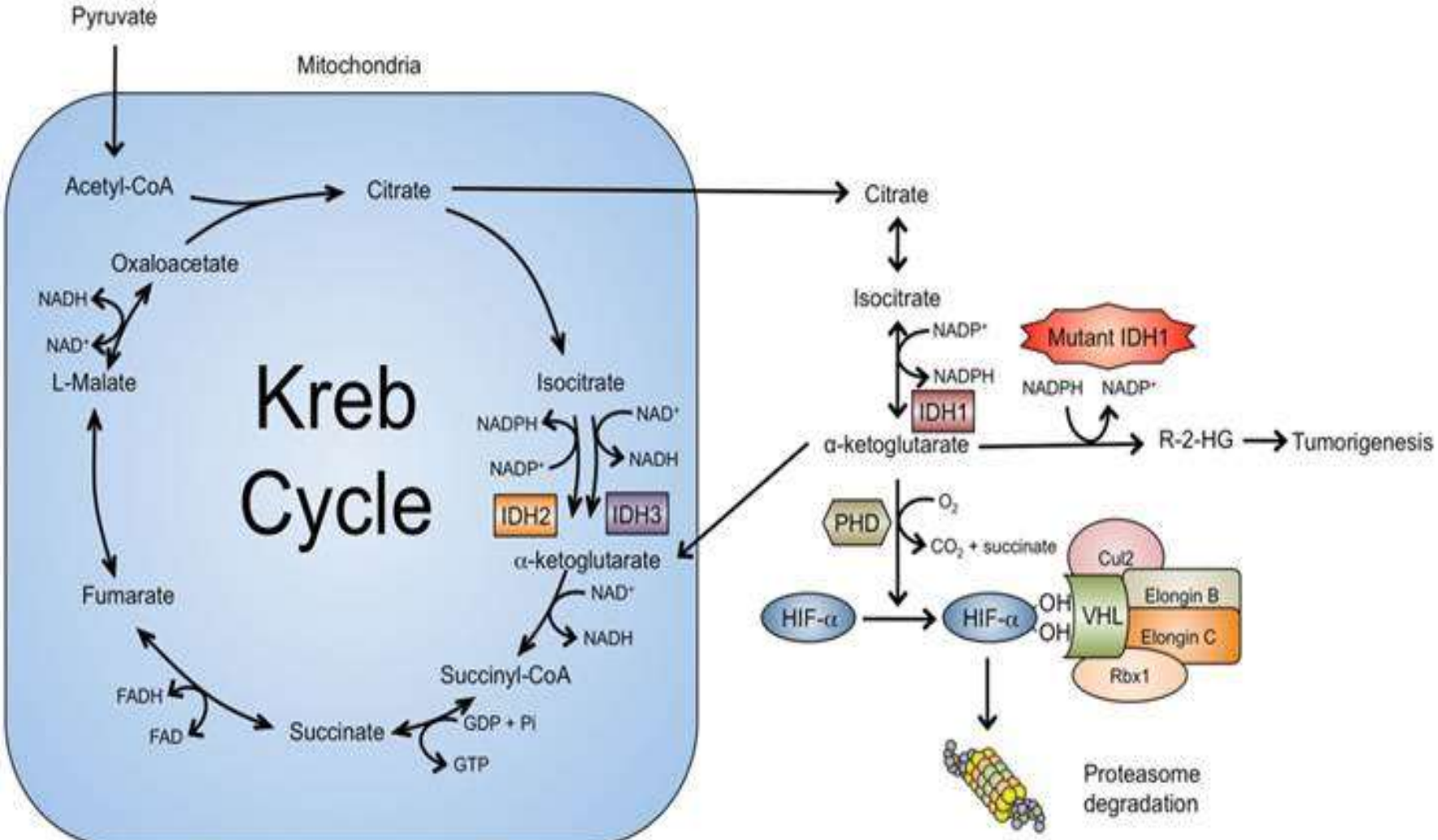
***Nature***, 2012



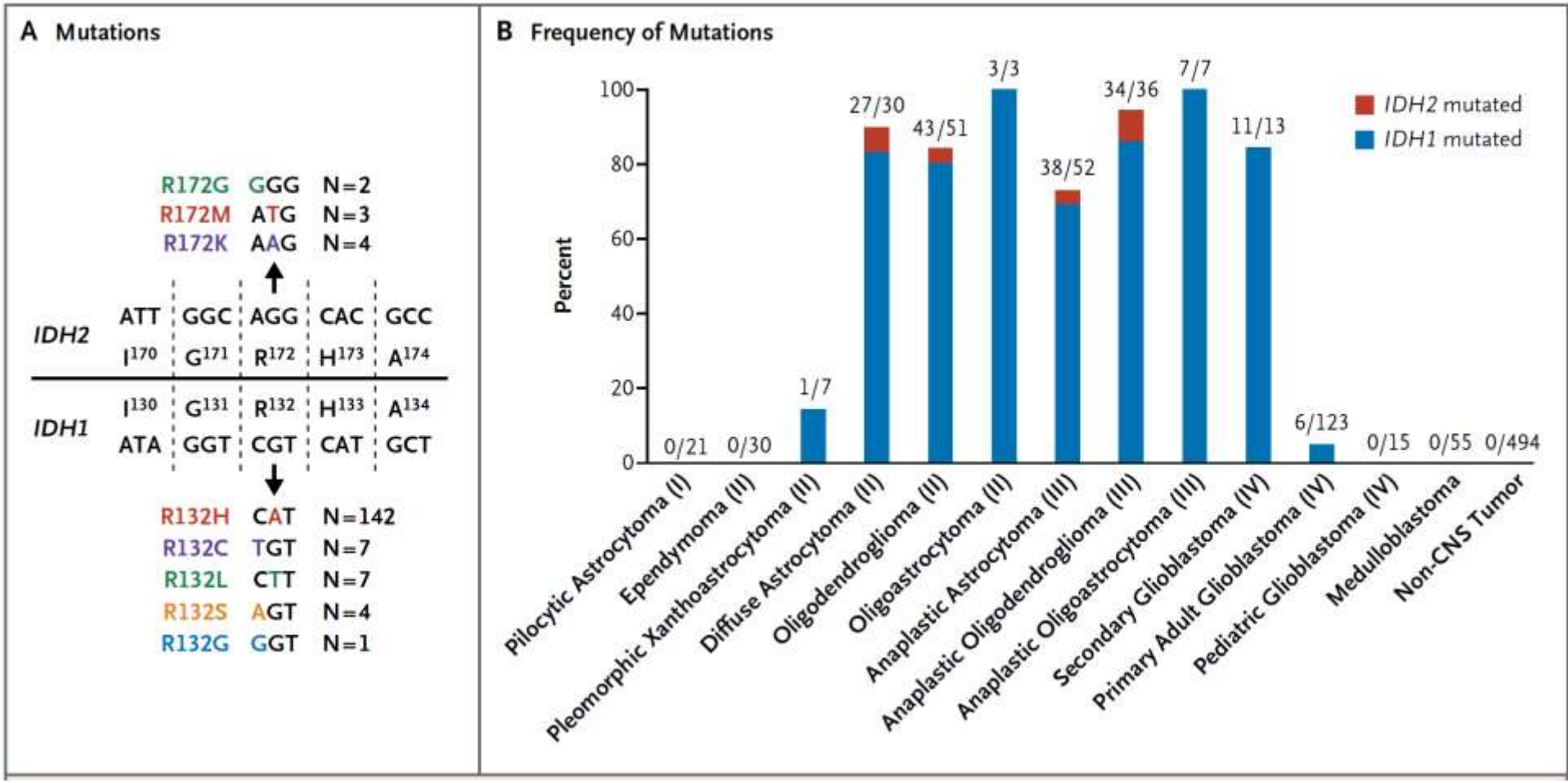

***Clin Cancer Res.,*** 2012

# （2） IDH (Isocitrate dehydrogenase, 异柠檬酸脱氢酶)



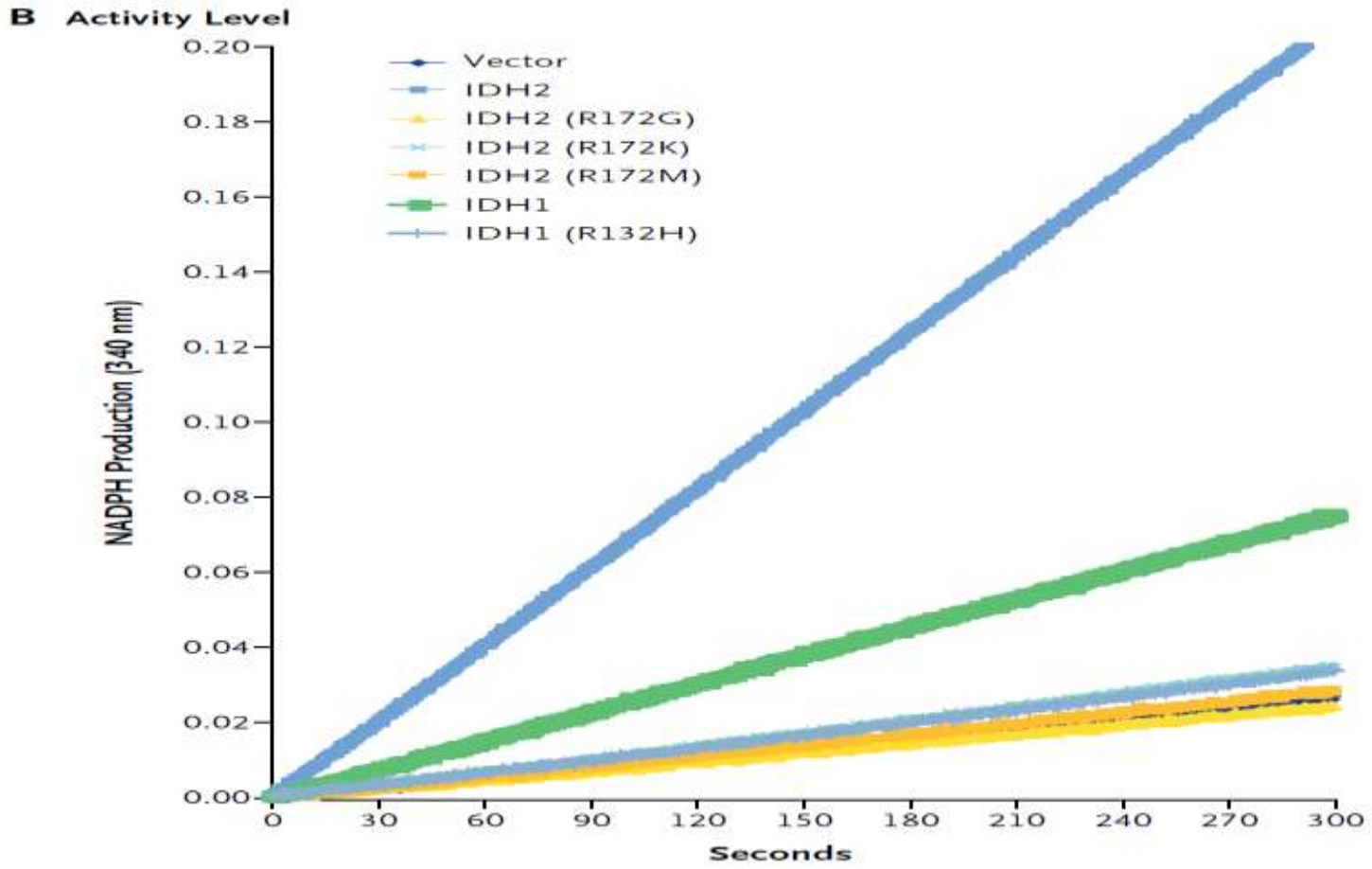

Pyruvate
Mitochondria
Acetyl-CoA
Citrate
Oxaloacetate
NADH
NAD+
L-Malate
Kreb Cycle
Isocitrate
NADPH
NAD+
NADP+
NADH
IDH2
IDH3
α-ketoglutarate
NAD+
NADH
Fumarate
Succinyl-CoA
FADH
FAD
Succinate
GDP + Pi
GTP
Citrate
Isocitrate
NADP+
NADPH
IDH1
Mutant IDH1
NADPH
NADP+
α-ketoglutarate
R-2-HG
Tumorigenesis
PHD
O2
CO2 + succinate
HIF-α
HIF-α
OH
OH
VHL
Cul2
Elongin B
Elongin C
Rbx1
Proteasome degradation

# IDH1 R132 and IDH2 R172 mutations in 70% of grade II and III Glioma



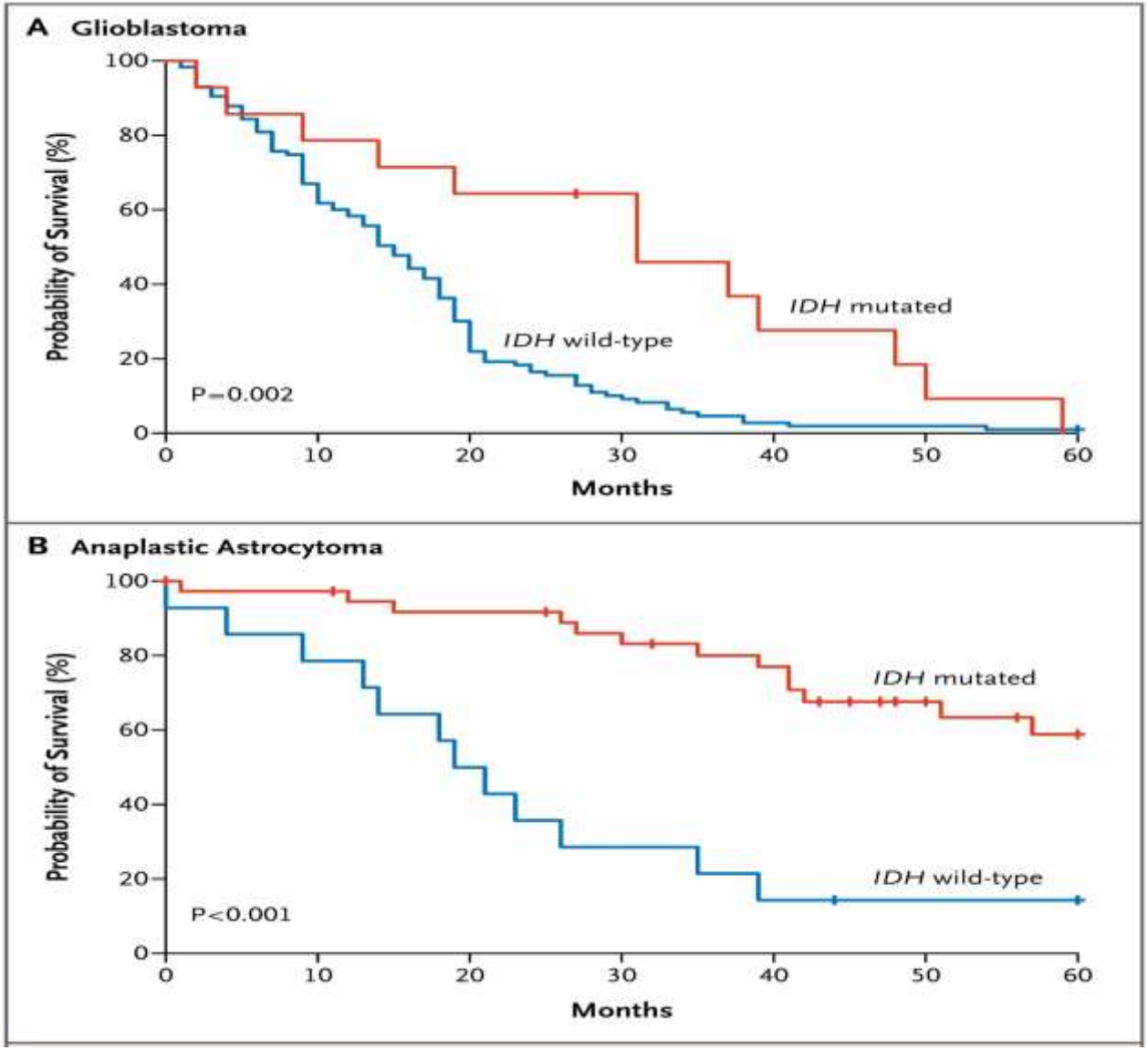

***N Engl J Med***. 2009

# IDH1 and IDH2 mutations reduced the enzymatic activity



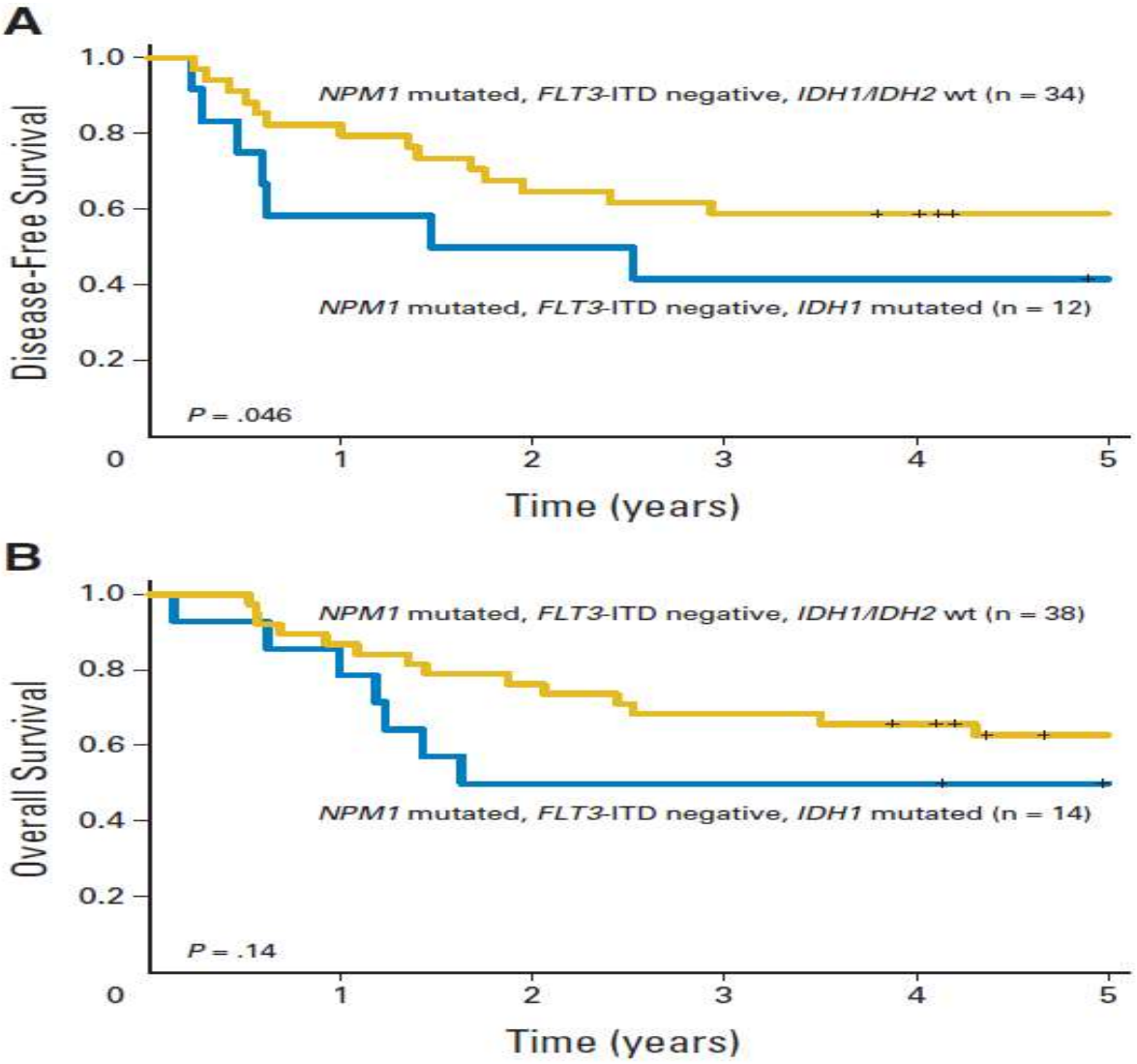

***N Engl J Med***. 2009

# Glioma patients with IDH mutations had a better outcome than those with wild-type IDH genes

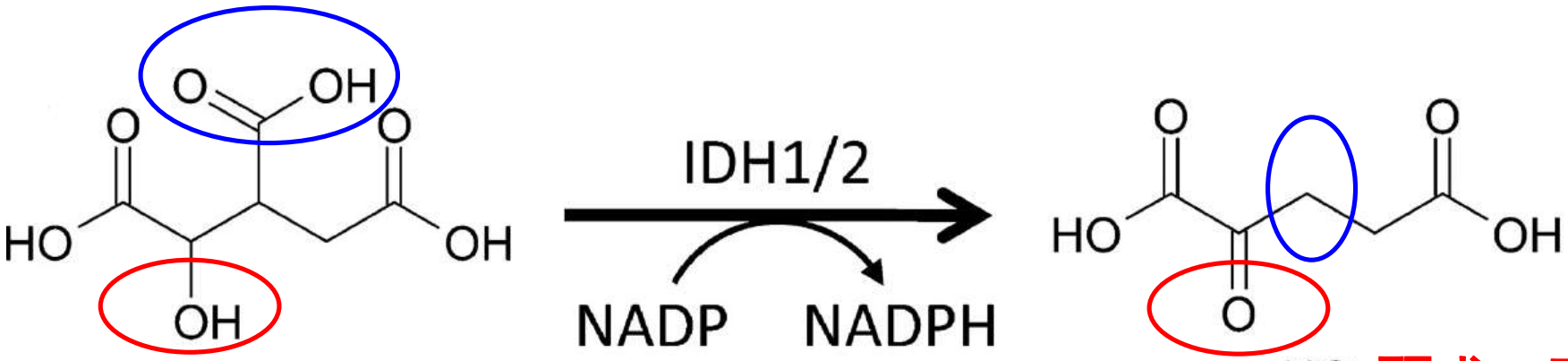



***N Engl J Med***. 2009

# IDH mutations were found in 33% of the Acute Myeloid Leukemia (AML)




***J Clin Oncol.*** 2010

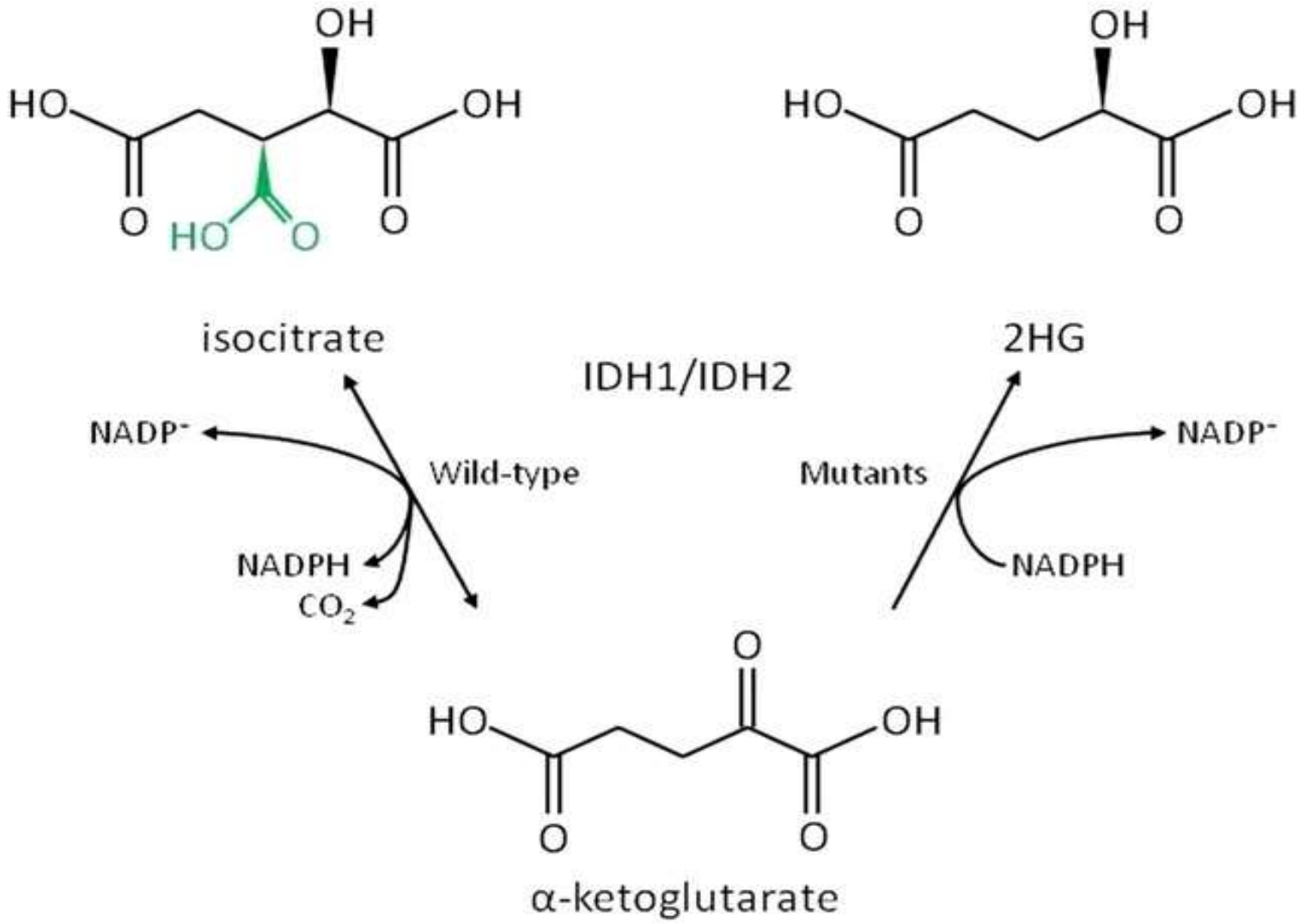



Isocitrate 异柠檬酸

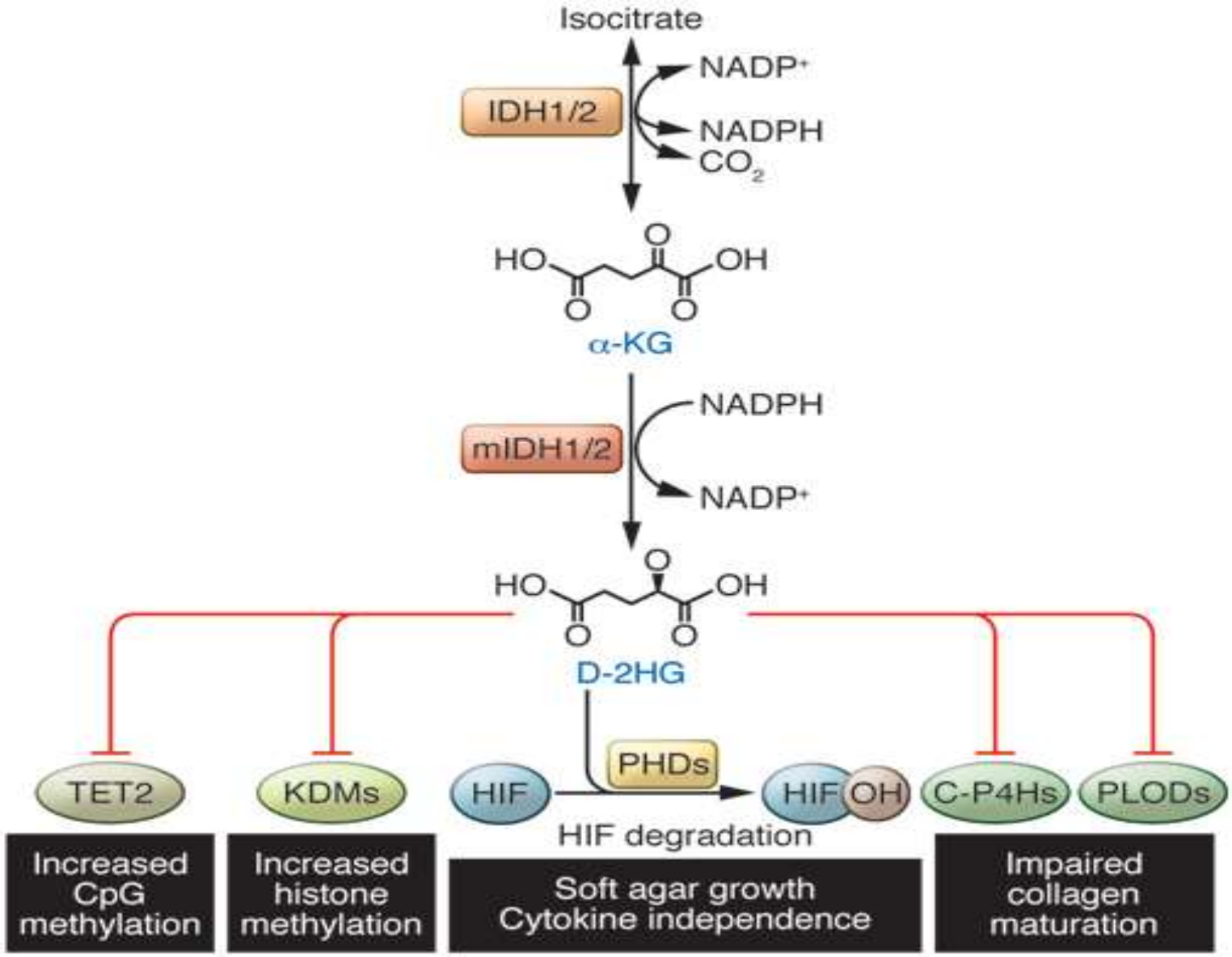
α-KG 酮戊二酸

*J Exp Med*., 2010

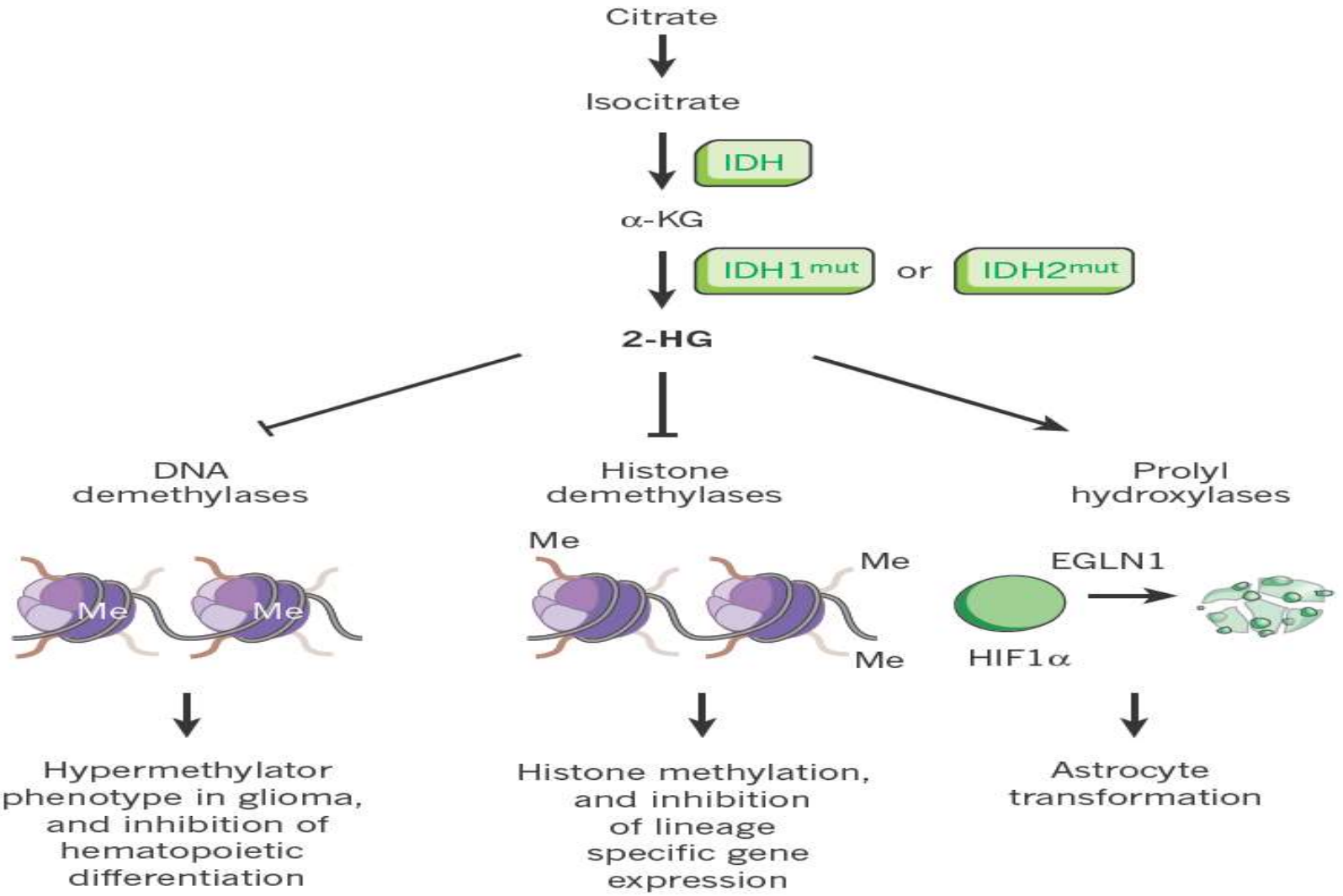
# Isocitrate, α-KG and 2-HG transformation



# 2-HG produced by mutant IDH1/2 affects metabolism and epigenetics by modulating activities of α-KG–dependent oxygenases

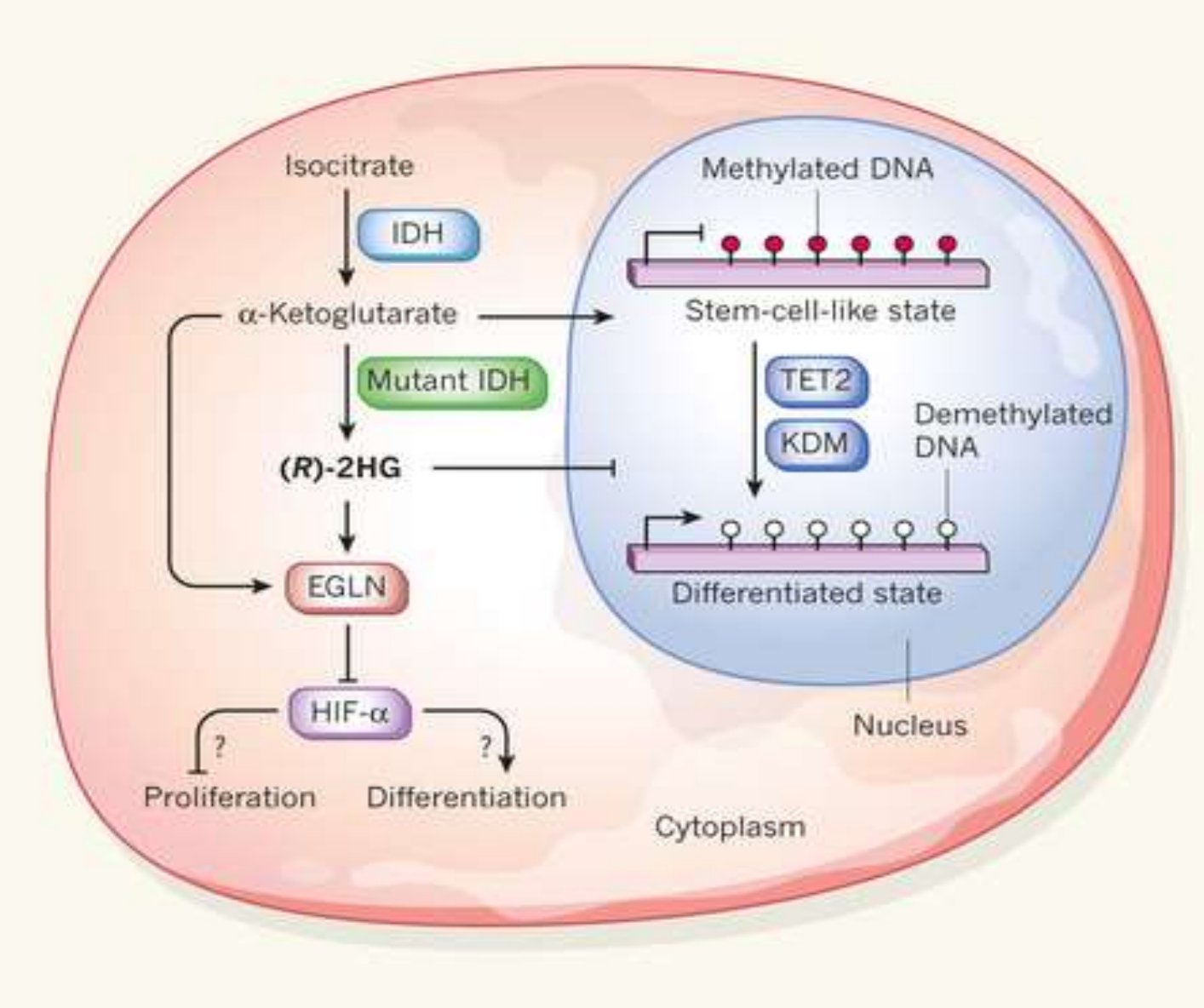


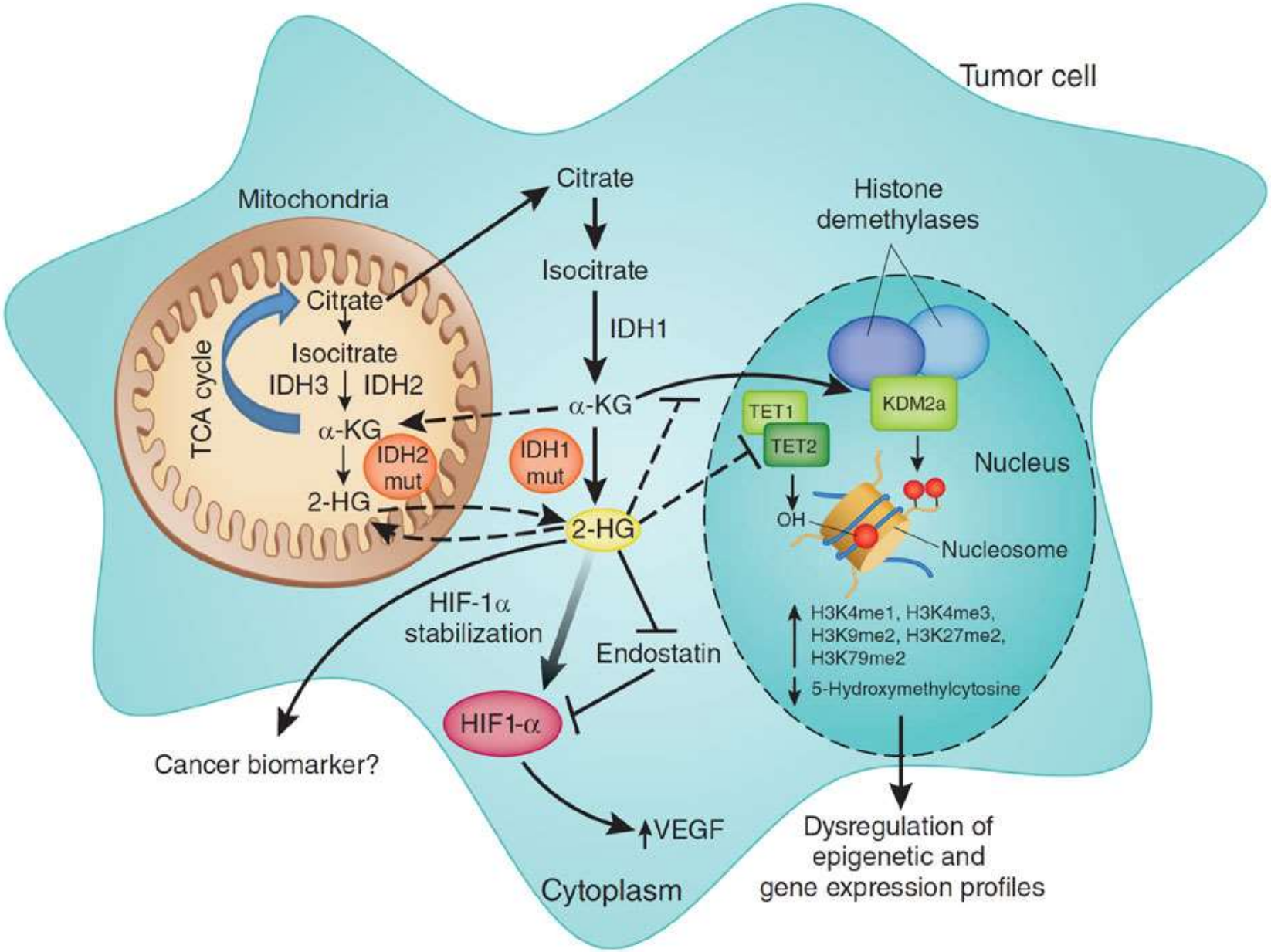
# Epigenetic regulation by 2-HG




***Nature***, 2012

# 2-HG promotes maintenance of stem-cell-like state



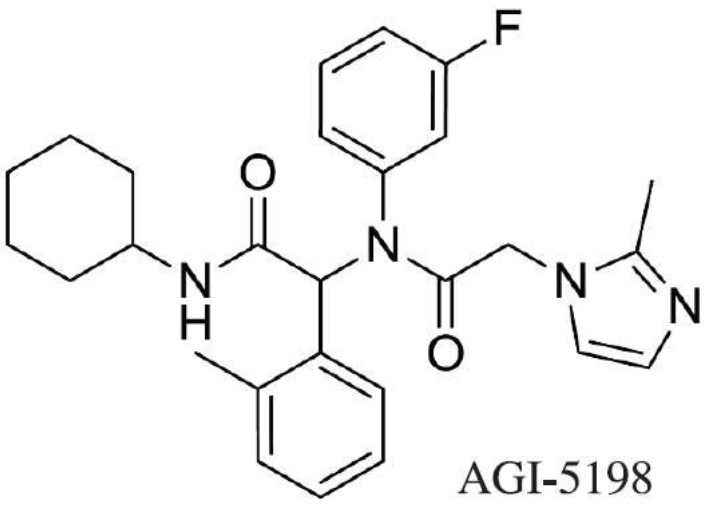



***Nat. Medicine.,*** 2011

3 MAY 2013 VOL 340 SCIENCE

# An Inhibitor of Mutant IDH1 Delays Growth and Promotes Differentiation of Glioma Cells

Dan Rohle,[1,2]* Janeta Popovici-Muller,[3]* Nicolaos Palaskas,[1]* Sevin Turcan,[1]* Christian Grommes,[4] Carl Campos,[1] Jennifer Tsoi,[8] Owen Clark,[1] Barbara Oldrini,[1] Evangelia Komisopoulou,[8] Kaiko Kunii,[3] Alicia Pedraza,[7] Stefanie Schalm,[3] Lee Silverman,[3] Alexandra Miller,[4] Fang Wang,[3] Hua Yang,[3] Yue Chen,[3] Andrew Kernytsky,[3] Marc K. Rosenblum,[6] Wei Liu,[3] Scott A. Biller,[3] Shinsan M. Su,[3] Cameron W. Brennan,[1,7] Timothy A. Chan,[1,5] Thomas G. Graeber,[8]† Katharine E. Yen,[3]†‡ Ingo K. Mellinghoff[1,2,4]†‡

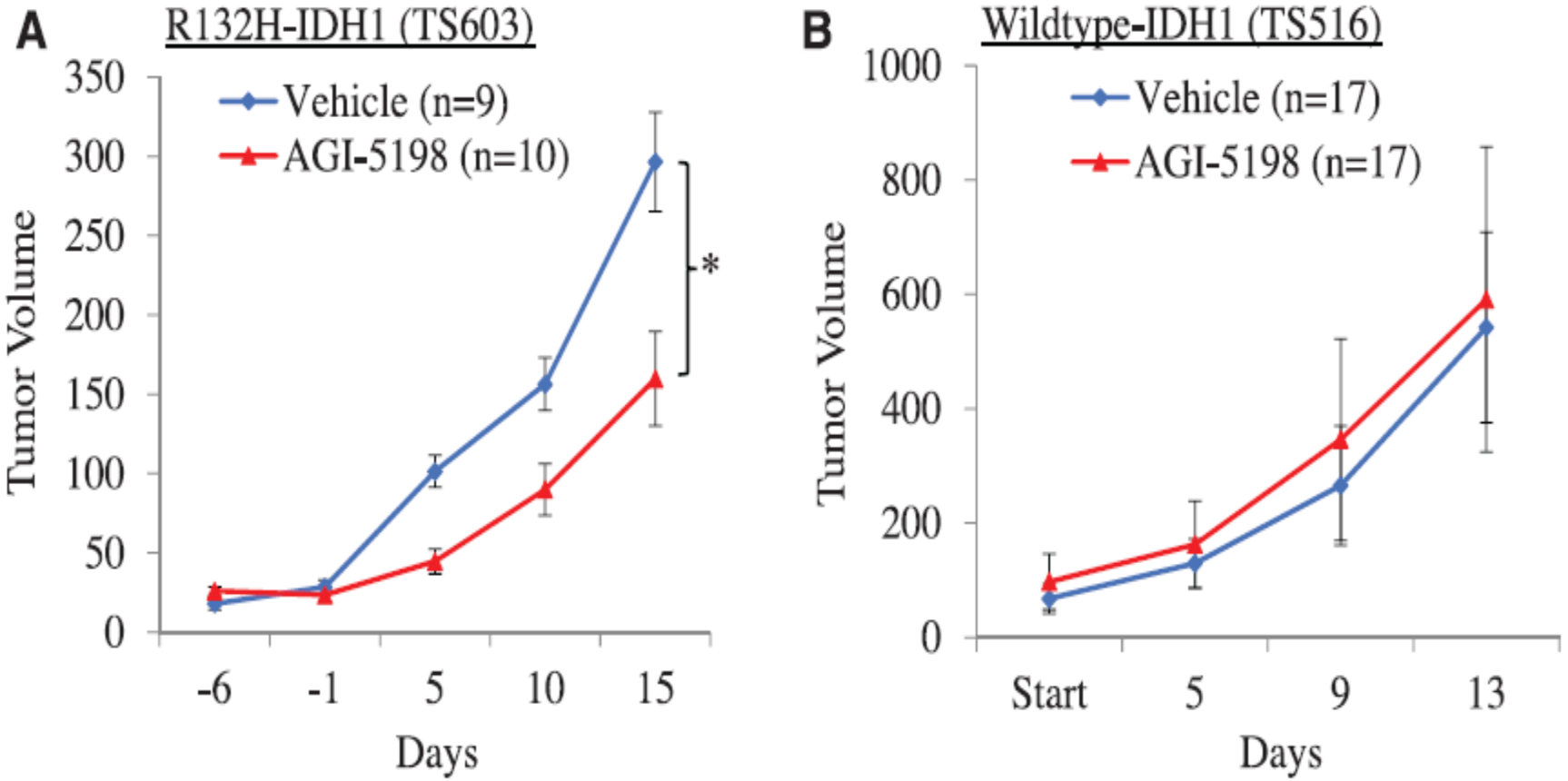



AGI-5198

| IDH1 | | |
|---|---|---|
| **R132H** | **R132C** | **wildtype** |
| $IC_{50}$ ($\mu$M) | $IC_{50}$ ($\mu$M) | $IC_{50}$ ($\mu$M) |
| 0.07 | 0.16 | > 100 |
| **IDH2** | | |
| **R140Q** | **R172K** | **wildtype** |
| $IC_{50}$ ($\mu$M) | $IC_{50}$ ($\mu$M) | $IC_{50}$ ($\mu$M) |
| > 100 | > 100 | > 100 |

***Science*, 2013**

# AGI-5198 impairs growth of IDH1-mutant glioma xenografts in mice

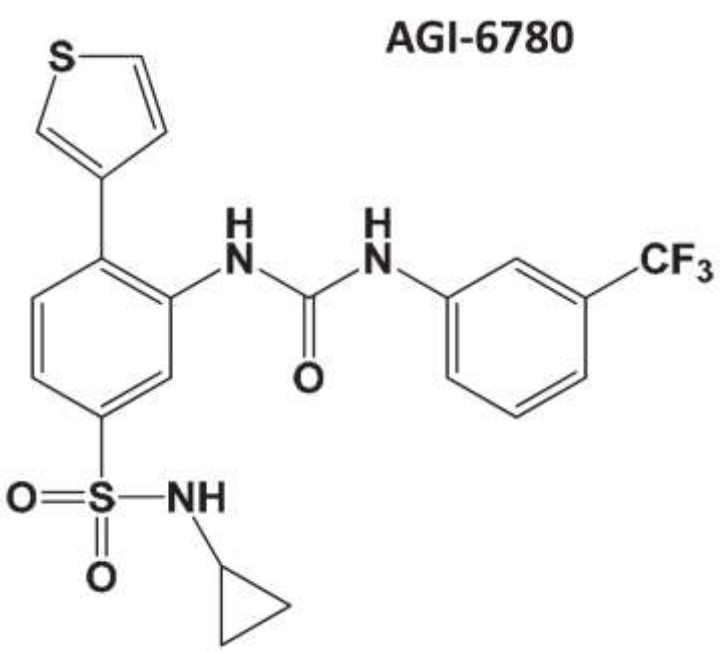


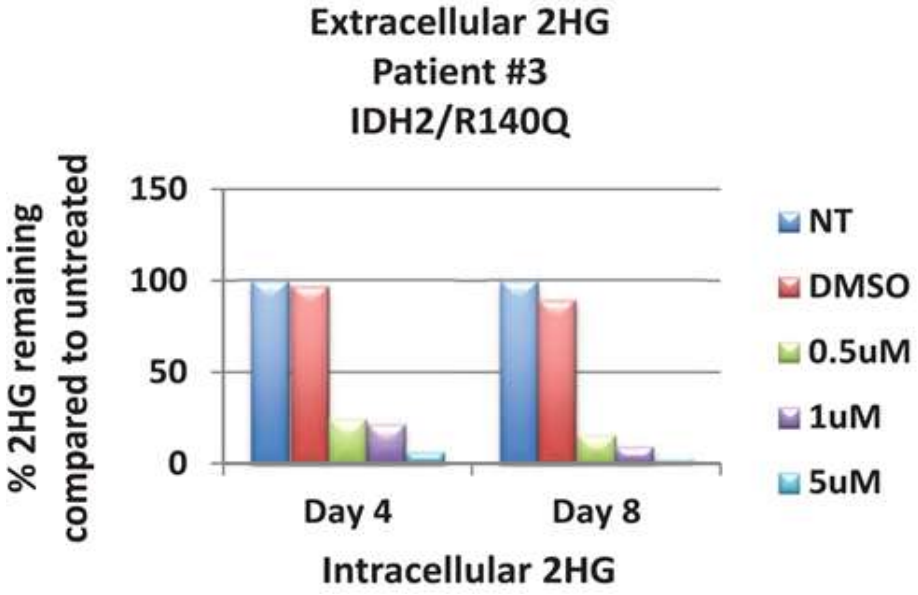

***Science,*** 2013

3 MAY 2013 VOL 340 SCIENCE

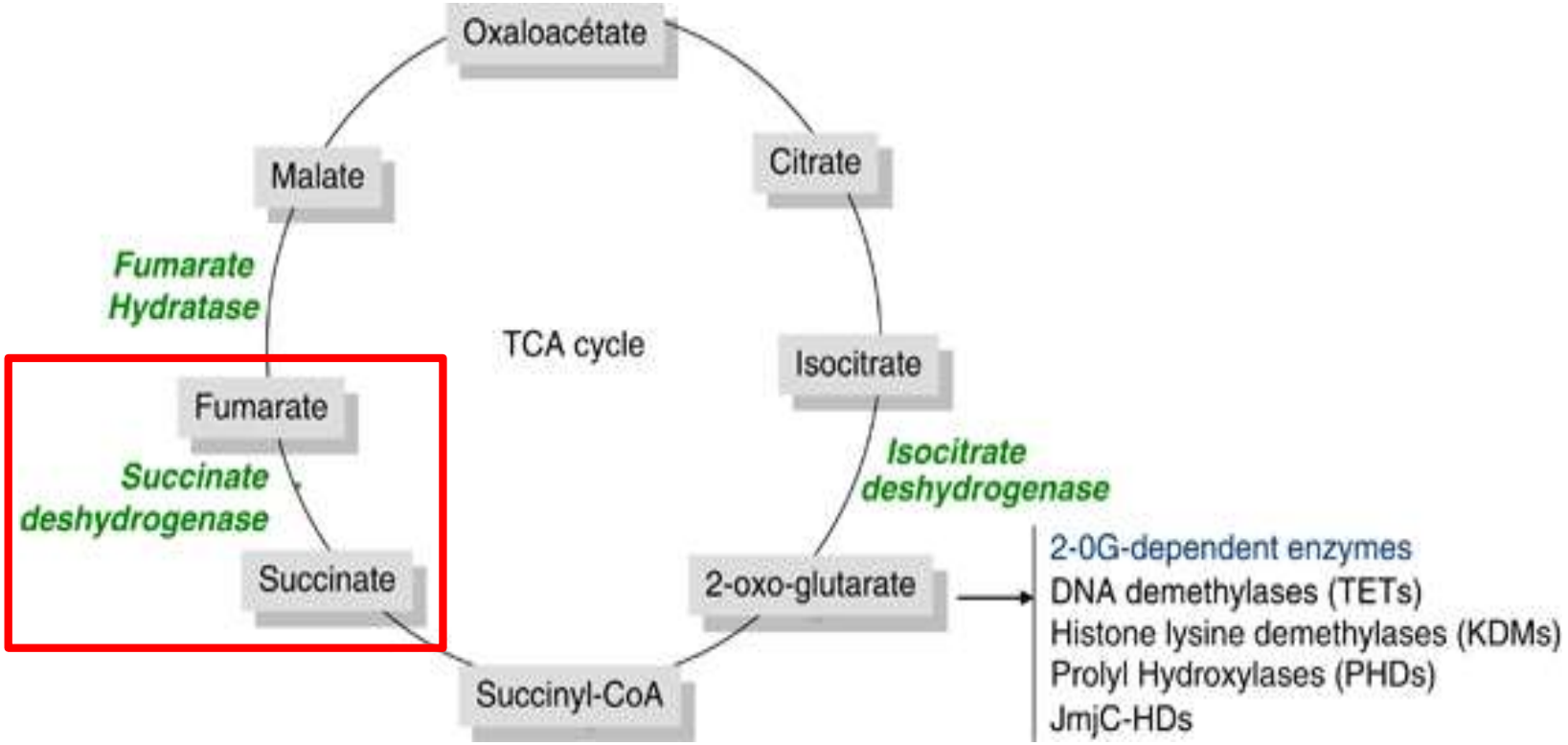
# Targeted Inhibition of Mutant IDH2 in Leukemia Cells Induces Cellular Differentiation

Fang Wang,[1*] Jeremy Travins,[1*] Byron DeLaBarre,[1*] Virginie Penard-Lacronique,[2,3,4*] Stefanie Schalm,[1*] Erica Hansen,[1] Kimberly Straley,[1] Andrew Kernytsky,[1] Wei Liu,[1] Camelia Gliser,[1] Hua Yang,[1] Stefan Gross,[1] Erin Artin,[1] Veronique Saada,[3] Elena Mylonas,[2,3,4] Cyril Quivoron,[2,3,4] Janeta Popovici-Muller,[1] Jeffrey O. Saunders,[1†] Francesco G. Salituro,[1‡] Shunqi Yan,[5] Stuart Murray,[1] Wentao Wei,[6] Yi Gao,[7] Lenny Dang,[1] Marion Dorsch,[1] Sam Agresta,[1] David P. Schenkein,[1] Scott A. Biller,[1] Shinsan M. Su,[1] Stephane de Botton,[2,3,4] Katharine E. Yen[1§]

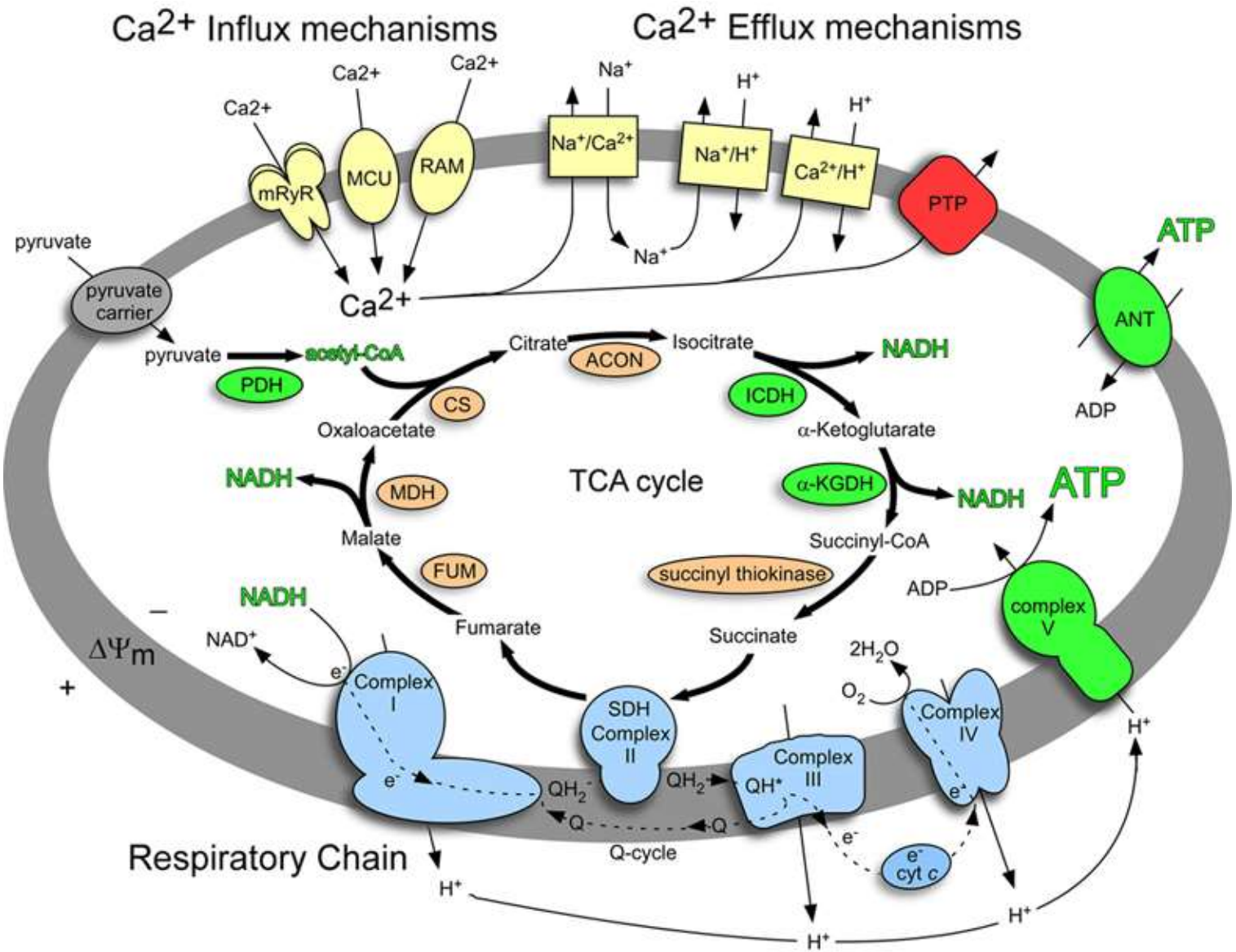


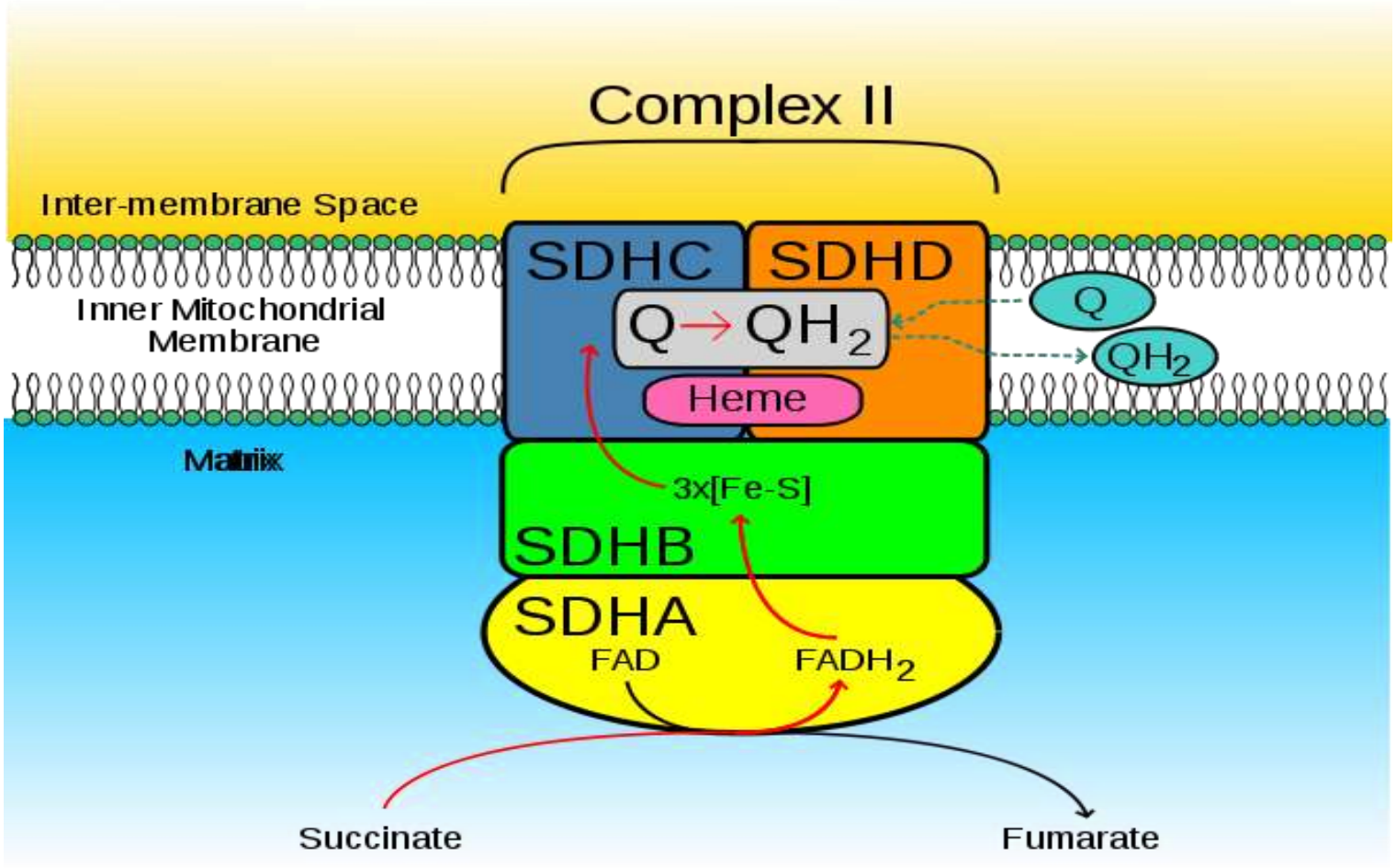
# （3） SDH (Succinate dehydrogenase，琥珀酸脱氢酶)



# SDH coupled with TCA cycle and Respiratory Chain

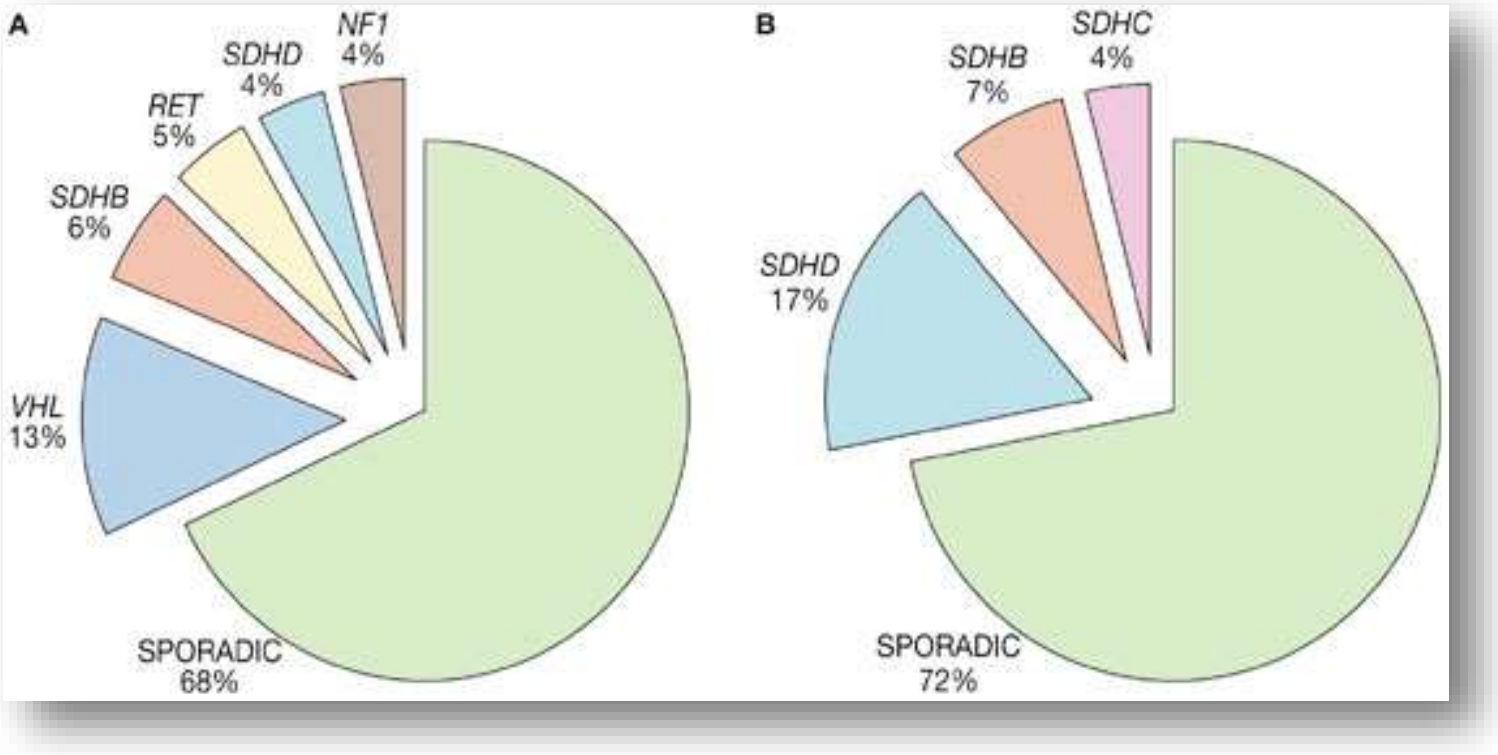


# SDH contains four subunits (SDHA, SDHB, SDHC, SDHD)



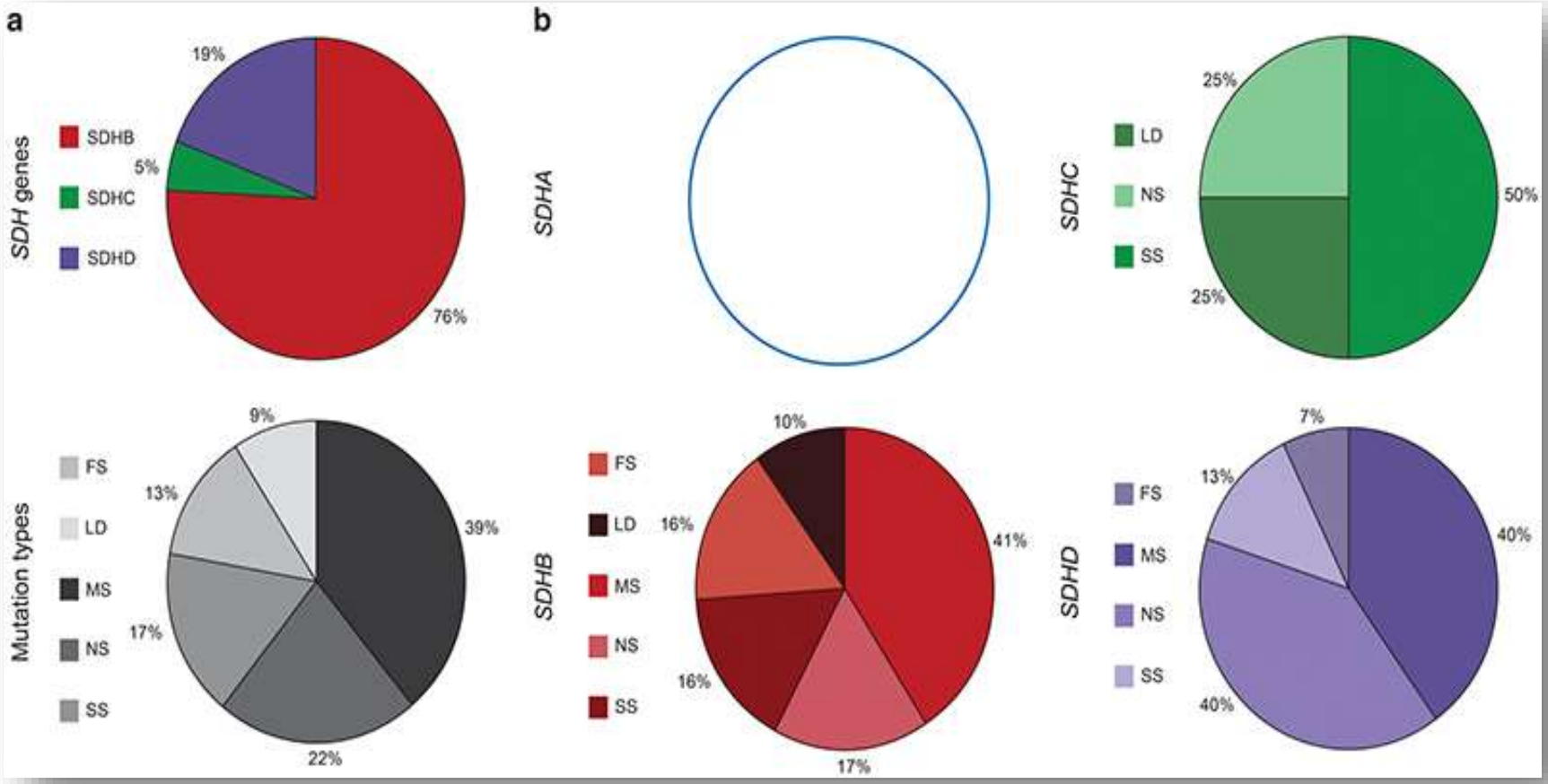
Paraganglioma–Pheochromocytoma syndrome was caused by germline mutations in SDHD, SDHB and SDHC

**副神经节瘤/嗜铬细胞瘤** (PGL/PCC)




***Nat Clin Pract Endocrinol Metab*** (2008)

# SDH germ-line mutations in malignant pheochromocytoma (PCC)/paragangliomas (PGL)



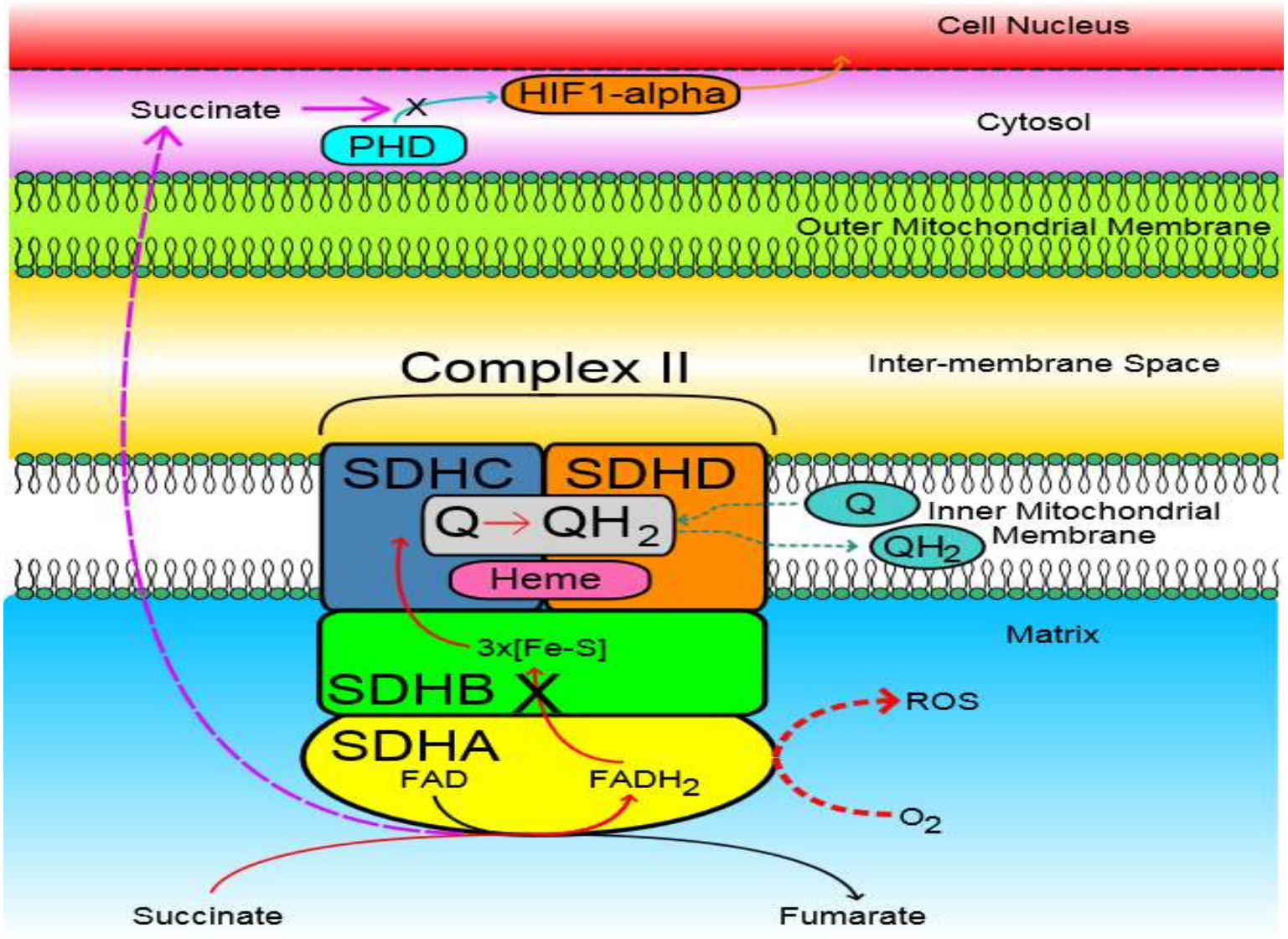

Frameshift (**FS**): deletion/duplication/insertion large deletion (**LD**), missense (**MS**), nonsense (**NS**), and splice site (**SS**)

***Genetics in Medicine*** (2014)

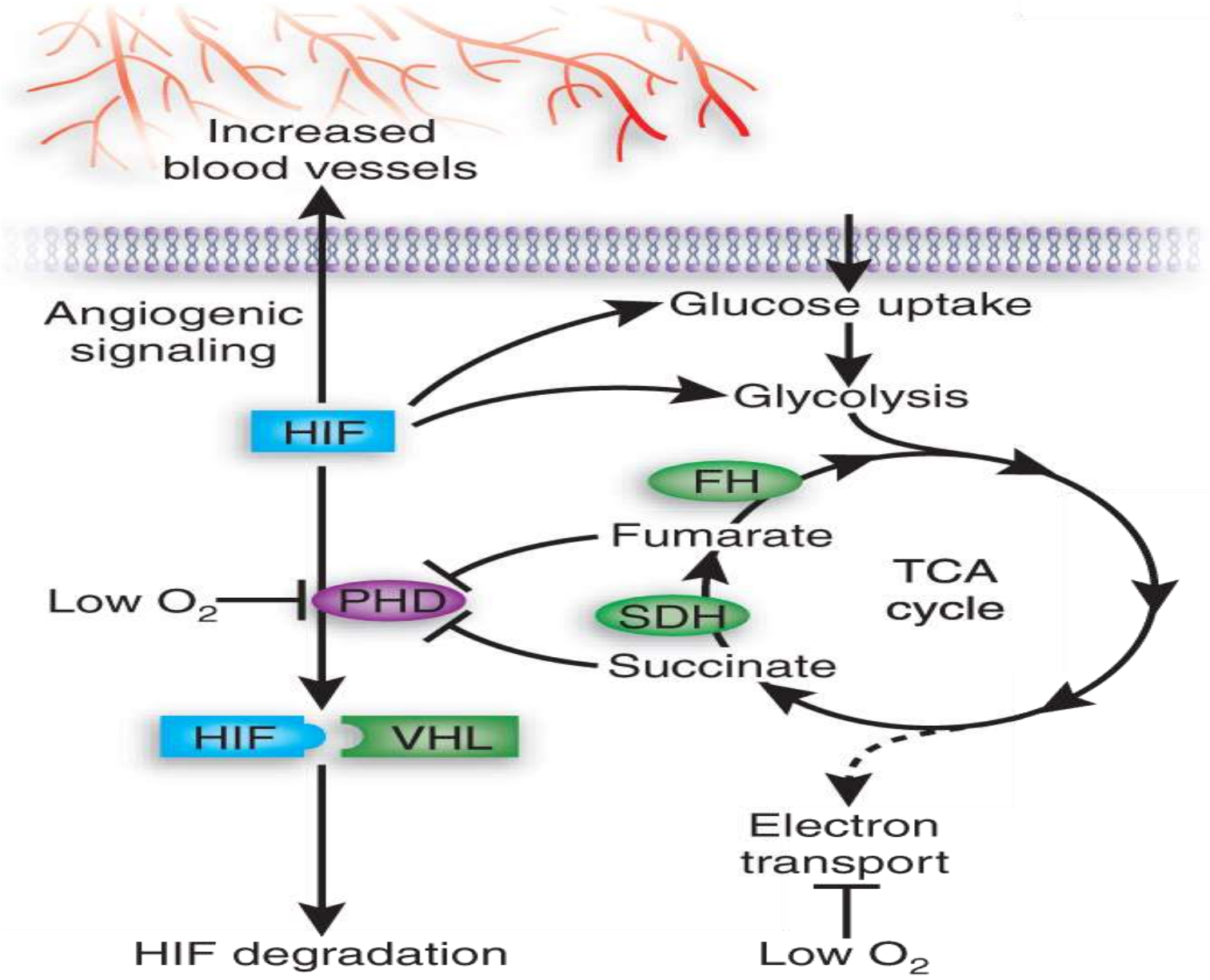
## Summary of Molecular Genetic Testing

| Gene (Syndrome) | Proportion of Hereditary PGL/PCC Attributed to Mutations in this Gene | Molecular Testing Method | Mutations Detected |
|---|---|---|---|
| **SDHA (PGL5)** | 0.6-3% | Sequence analysis | Sequence variants |
| **SDHB (PGL4)** | 22%-38%<br>12-20% of skull base and neck PGL<br>24%-44% of chest, abdomen, pelvic PGL/PCC | Sequence analysis<br><br>Deletion/duplication | Sequence variants<br><br>Partial and whole gene deletions |
| **SDHC (PGL3)** | 4%-8% | Sequence analysis<br><br>Deletion/duplication | Sequence variants<br><br>Partial and whole gene deletions |
| **SDHD (PGL1)** | 30%<br>40%-50% of skull base and neck PGL<br>15% of chest, abdomen, pelvic PGL/PCC | Sequence analysis<br><br>Deletion/duplication | Sequence variants<br><br>Partial and whole gene deletions |
| **SDHAF2 (PGL2)** | Unknown | Sequence analysis | Sequence variants |

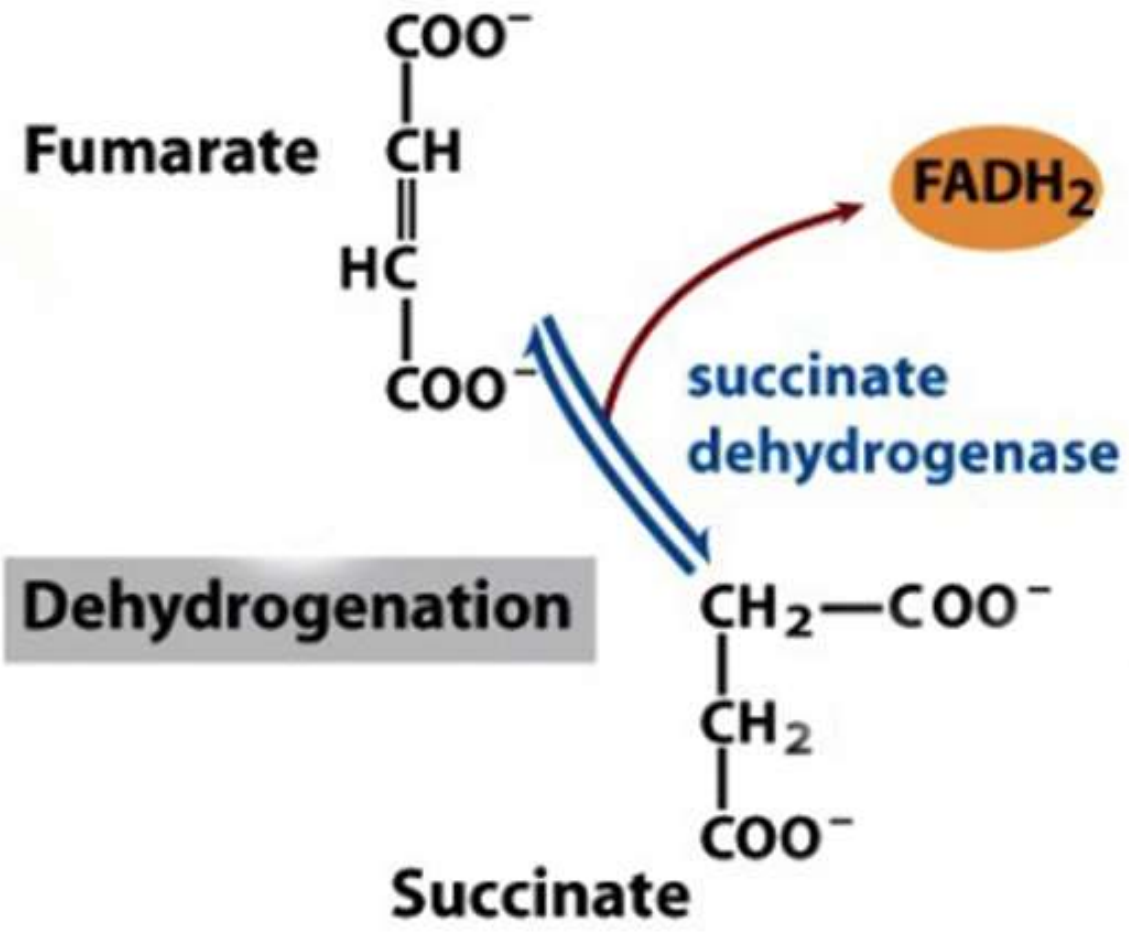
# SDH mutations promotes HIF-1α stability and tumorigenesis

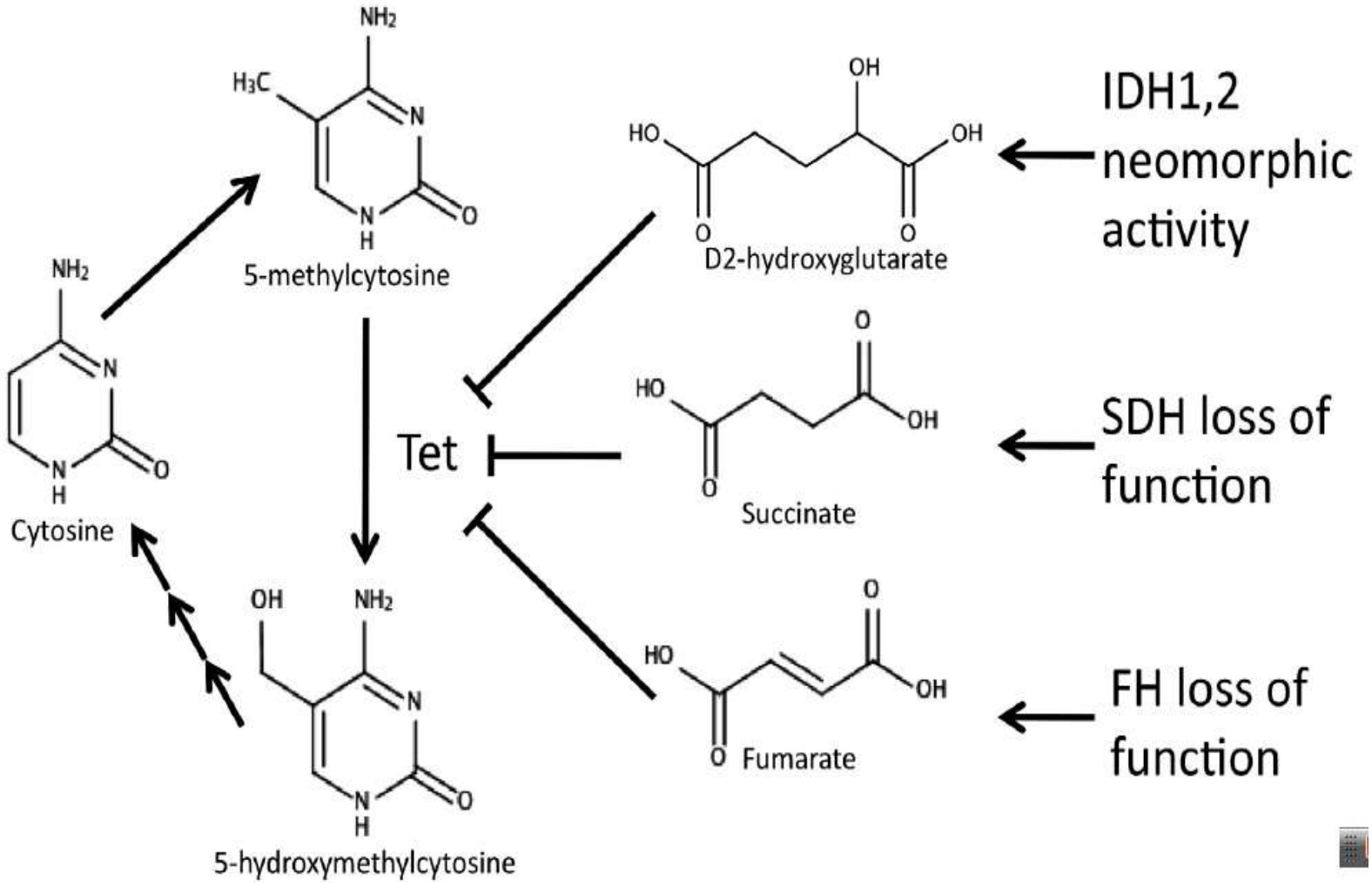


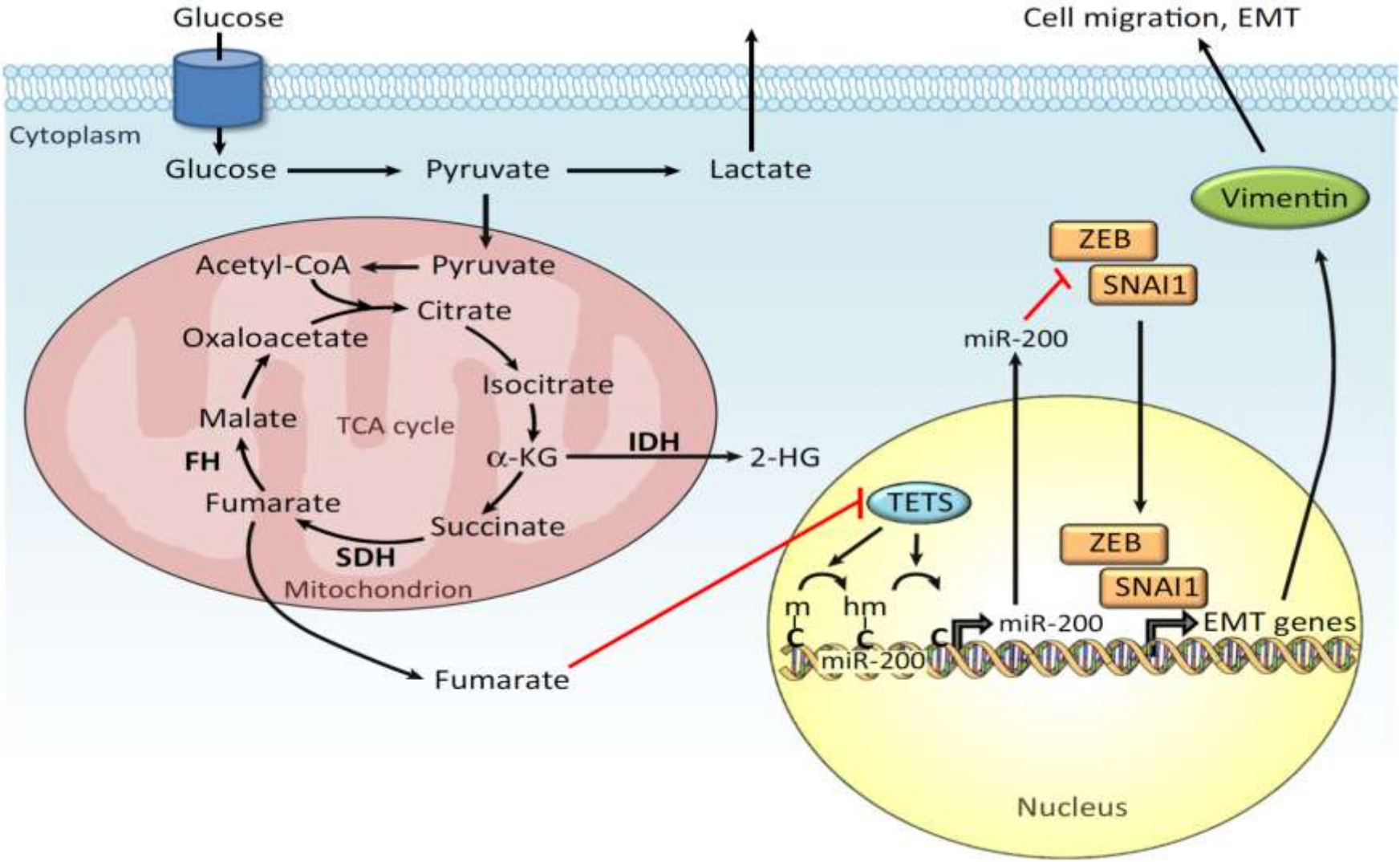
# SDH mutations promotes HIF-1α stability and tumorigenesis



# （4） Fumarate (延胡索酸)




NH2
H3C
N
N
H
O
5-methylcytosine
OH
HO
OH
O
O
D2-hydroxyglutarate
IDH1,2
neomorphic
activity
NH2
N
N
H
O
Cytosine
Tet
O
HO
OH
O
Succinate
SDH loss of
function
OH
NH2
N
N
H
O
5-hydroxymethylcytosine
O
HO
OH
O
Fumarate
FH loss of
function




Trends in Molecular Medicine

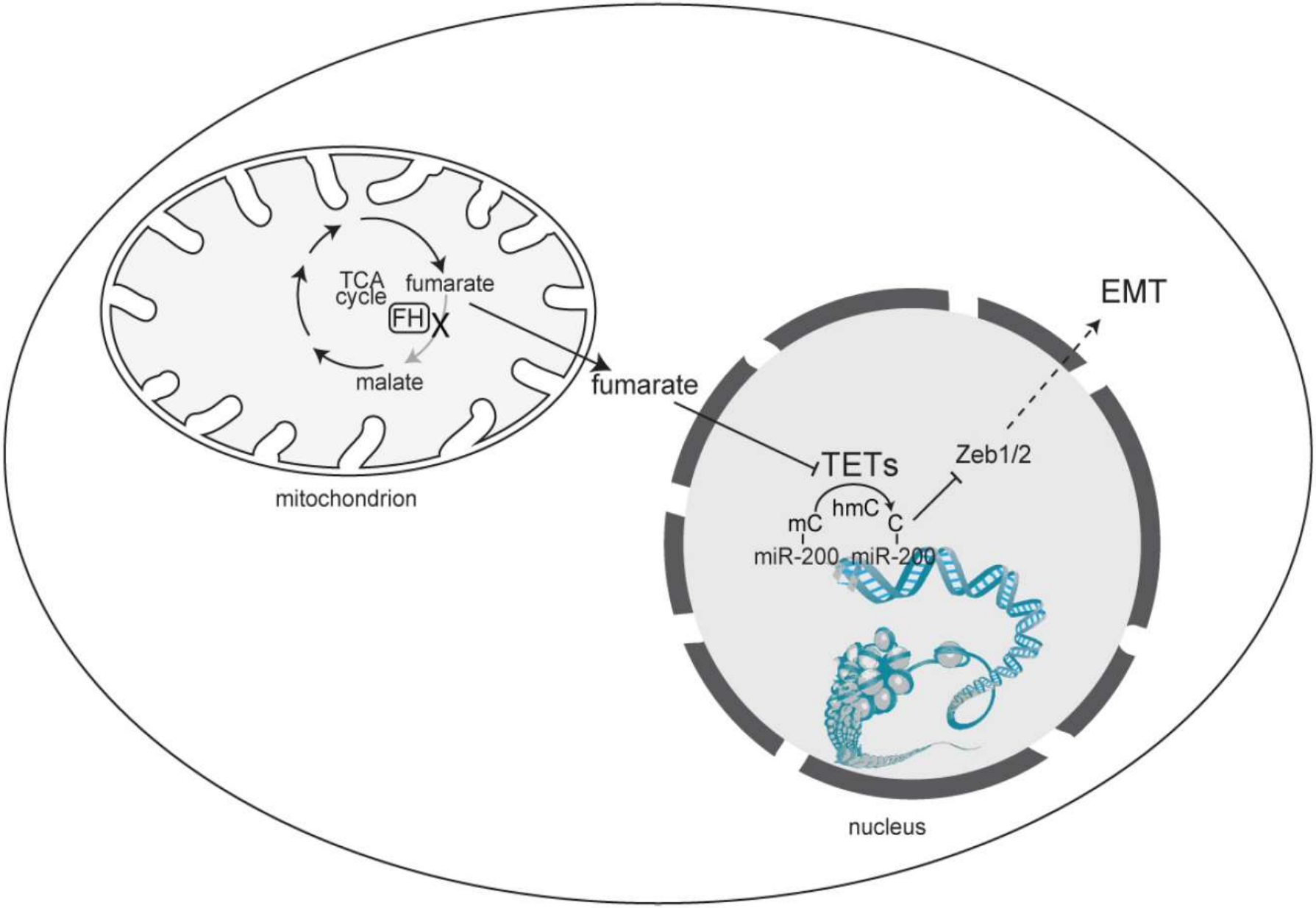
LETTER

doi:10.1038/nature19353

# Fumarate is an epigenetic modifier that elicits epithelial-to-mesenchymal transition

Marco Sciacovelli[1], Emanuel Gonçalves[2], Timothy Isaac Johnson[1], Vincent Roberto Zecchini[1], Ana Sofia Henriques da Costa[1], Edoardo Gaude[1], Alizee Vercauteren Drubbel[1], Sebastian Julian Theobald[1], Sandra Riekje Abbo[1], Maxine Gia Binh Tran[3]†, Vinothini Rajeeve[4], Simone Cardaci[5], Sarah Foster[6], Haiyang Yun[7], Pedro Cutillas[4], Anne Warren[8], Vincent Gnanapragasam[9], Eyal Gottlieb[5], Kristian Franze[6], Brian Huntly[7], Eamonn Richard Maher[10,11], Patrick Henry Maxwell[12], Julio Saez-Rodriguez[2,13] & Christian Frezza[1]

***Nature*** 537, 544–547 (22 September 2016)

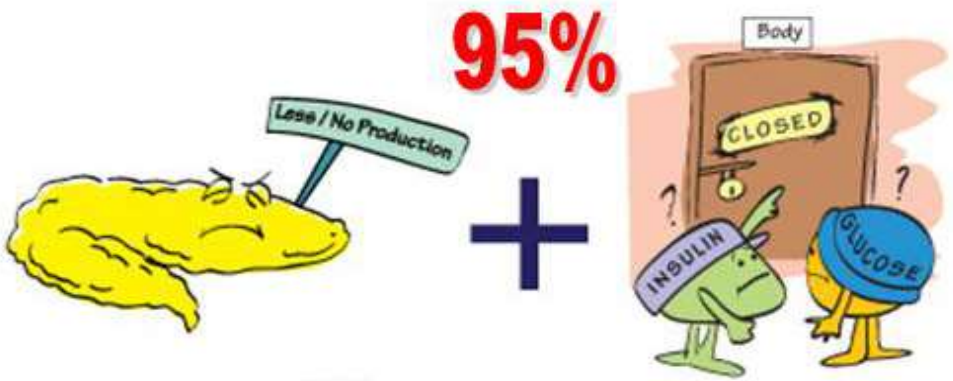



***Nature*** 537, 544–547 (22 September 2016)

# 2. Metabolic genomics and Diabetes

Type 1 – No Insulin


Type 2- Less insulin+ Insulin resistance

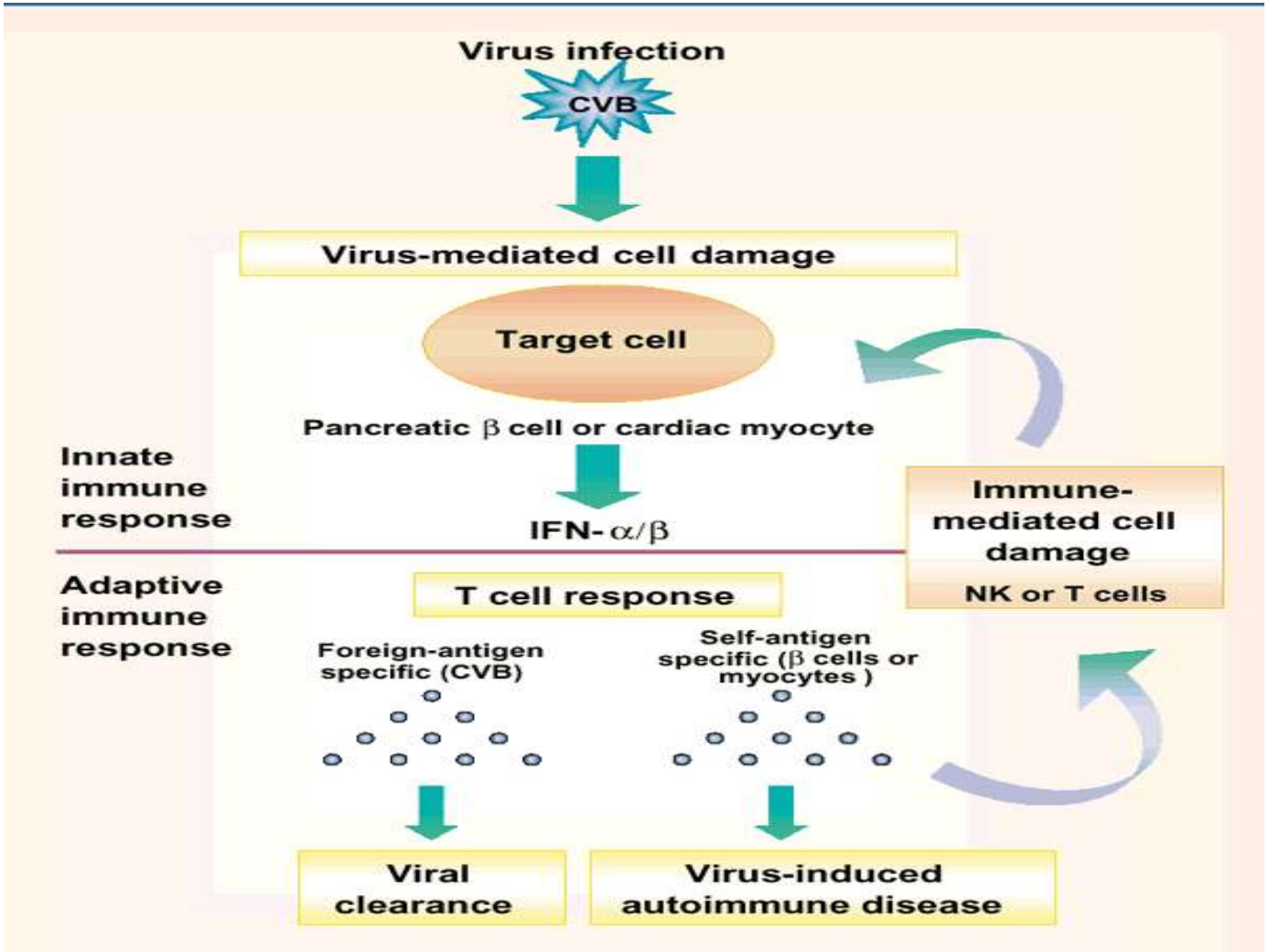



Gestational Diabetes

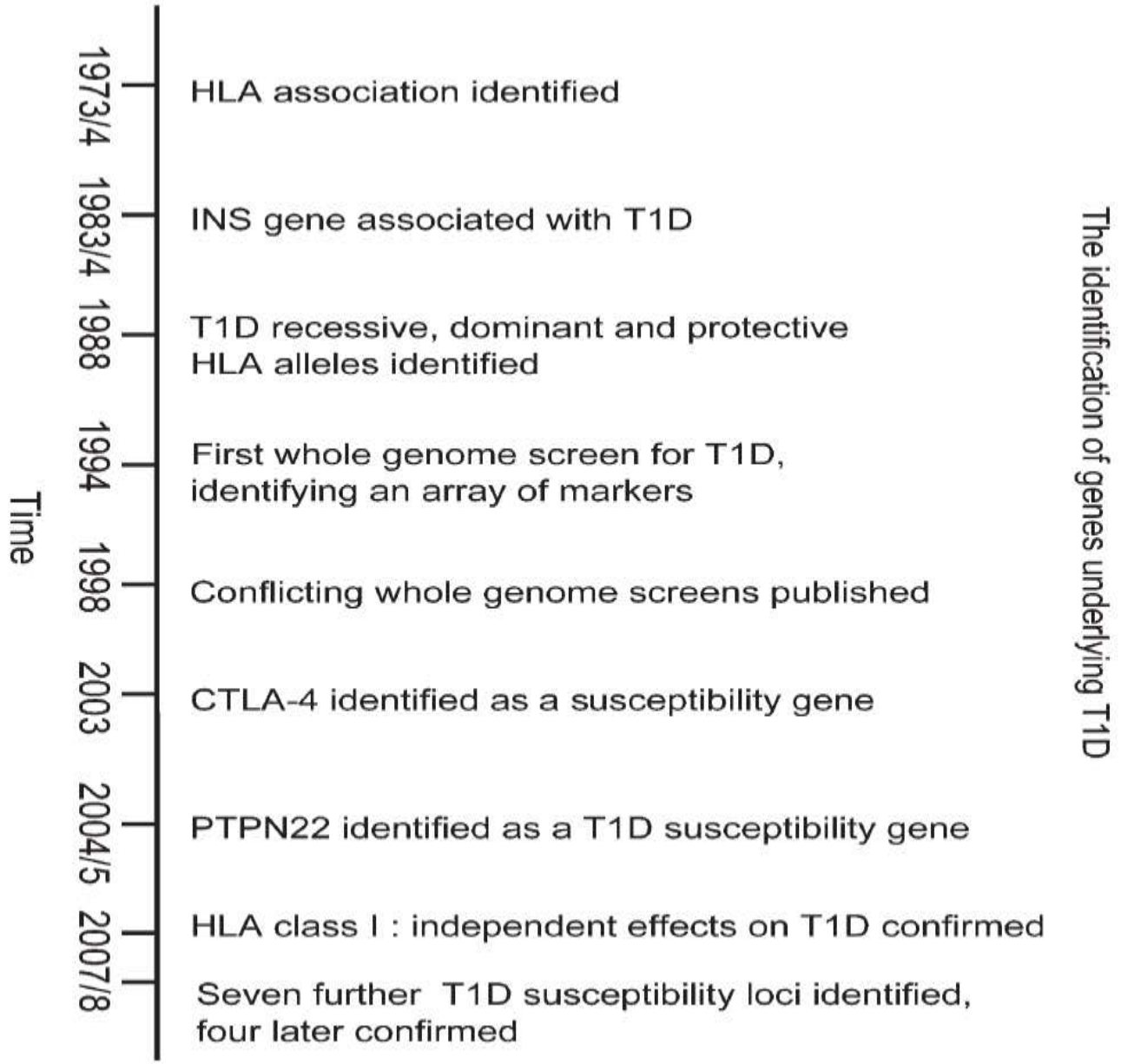
# Type I Diabetes and Genetics

- 直系亲属中有患IDDM的人群，患IDDM的危险性为5%
- 父亲患IDDM，其子女以后患IDDM的危险性为7%
- 母亲患IDDM，其子女患该病的机率为2%
- 同卵双生中一个患IDDM，另一个患病危险性为1/3

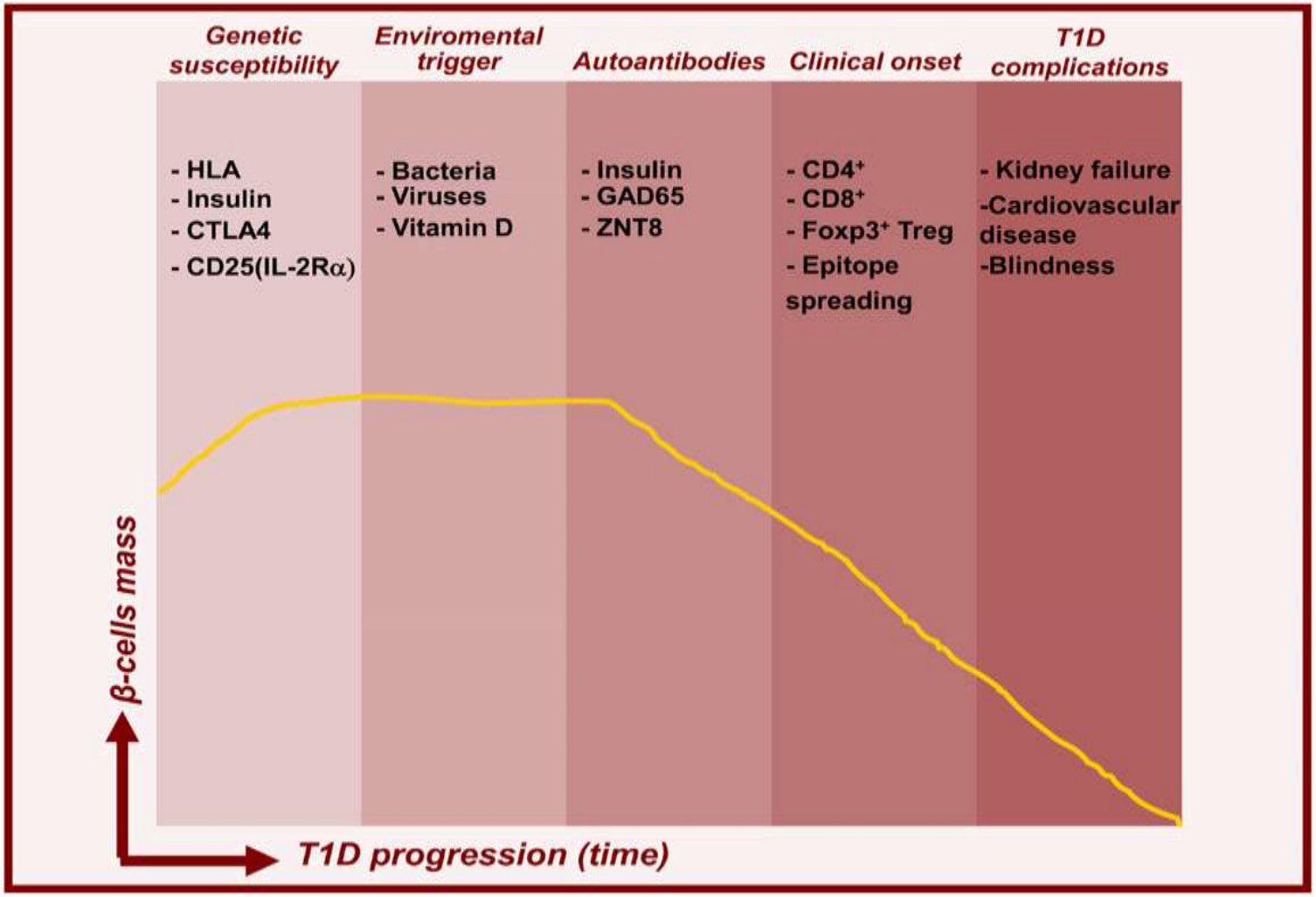
# Type I Diabetes and autoimmune disease



# Progress in the identification of T1D susceptibility alleles



# Timelines for Type 1 Diabetes




***Front. Endocrinol.*** (2013)

# MHC genes on chromosome 6 confer almost 50% of genetic susceptibility to T1D

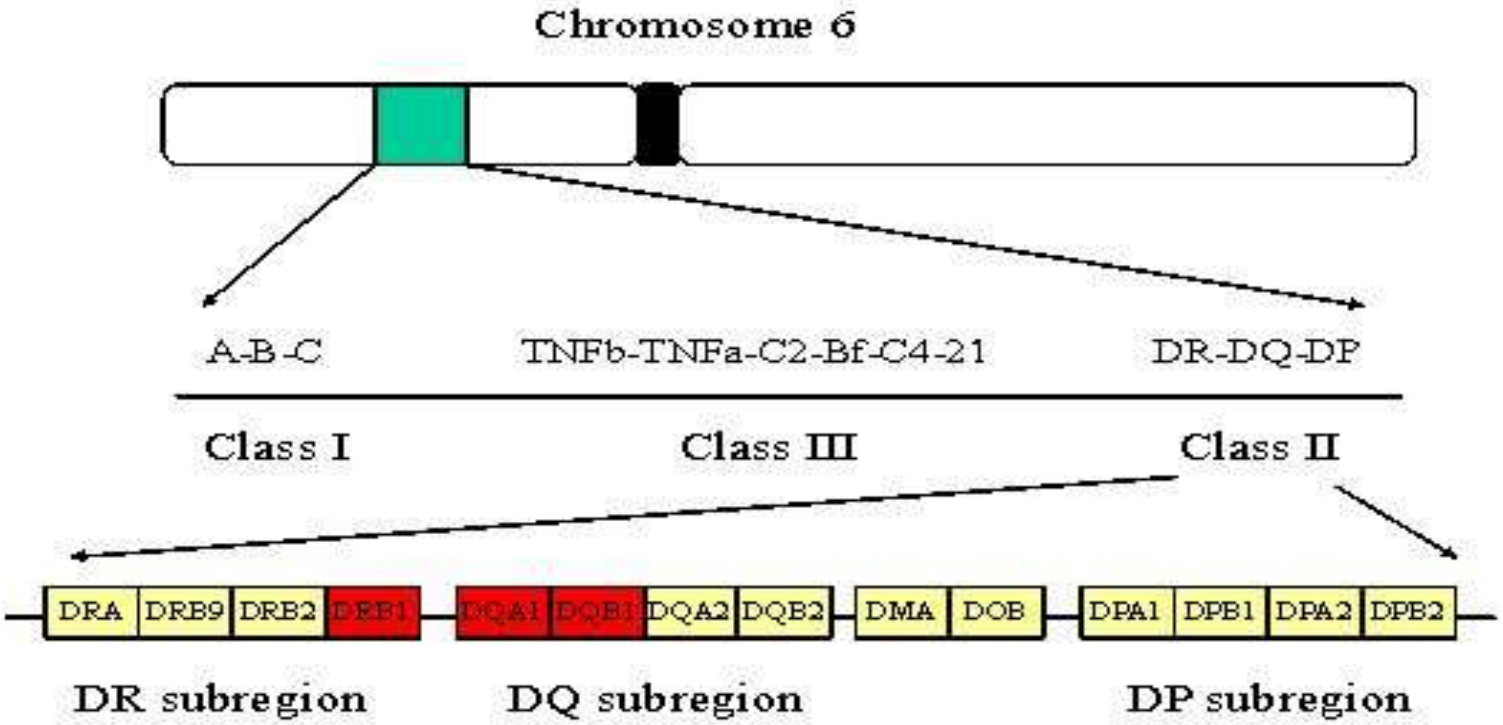
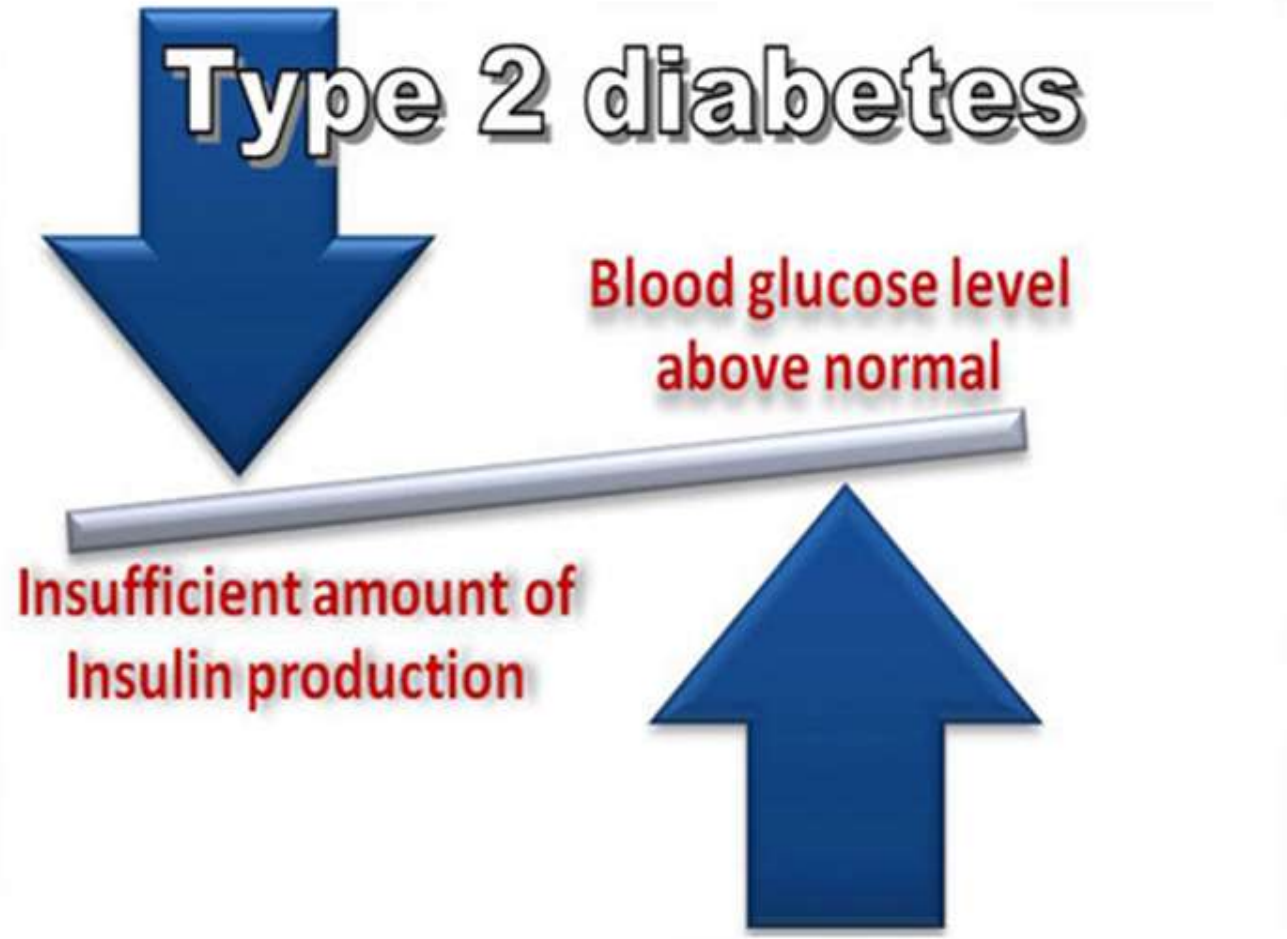
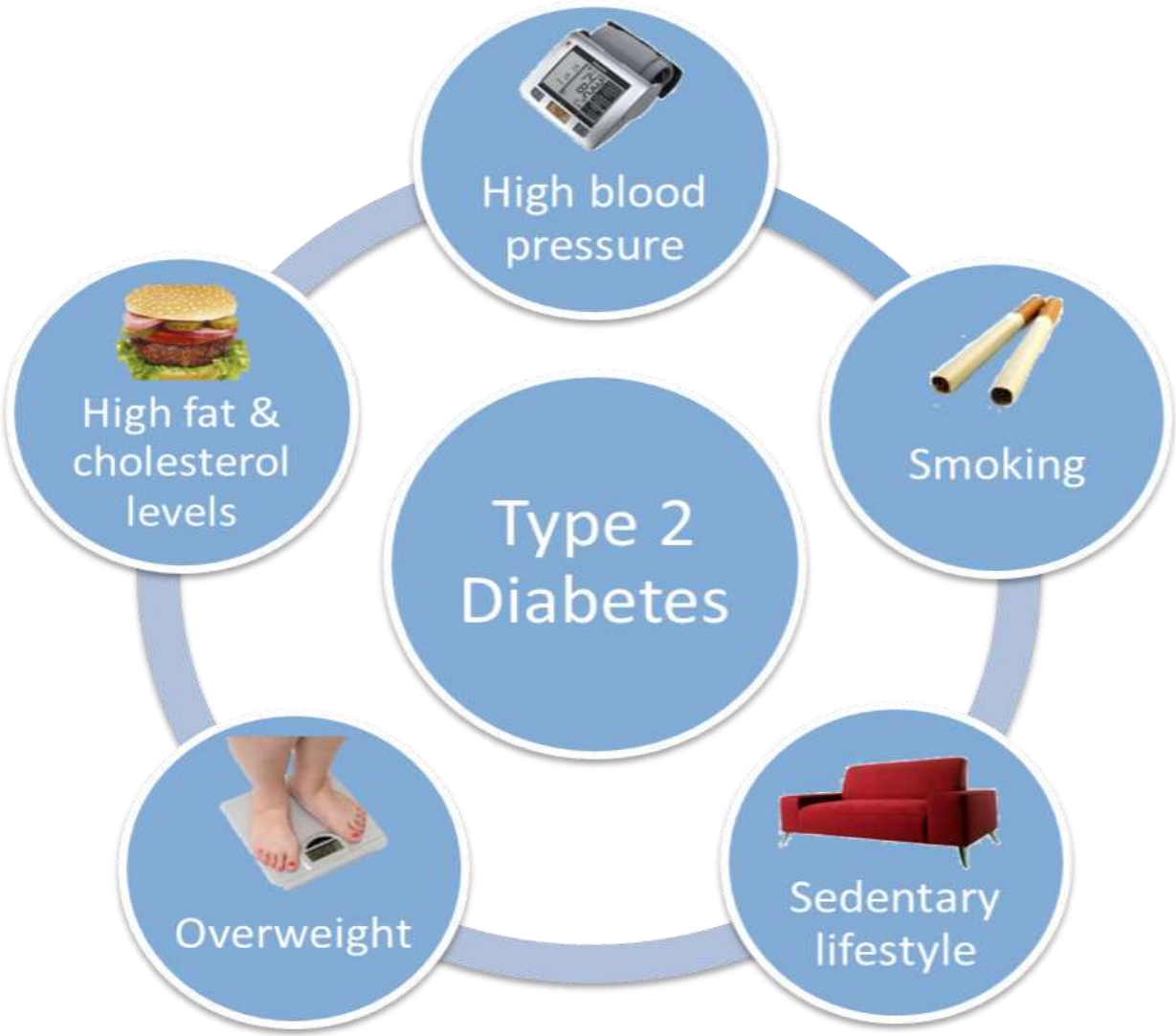
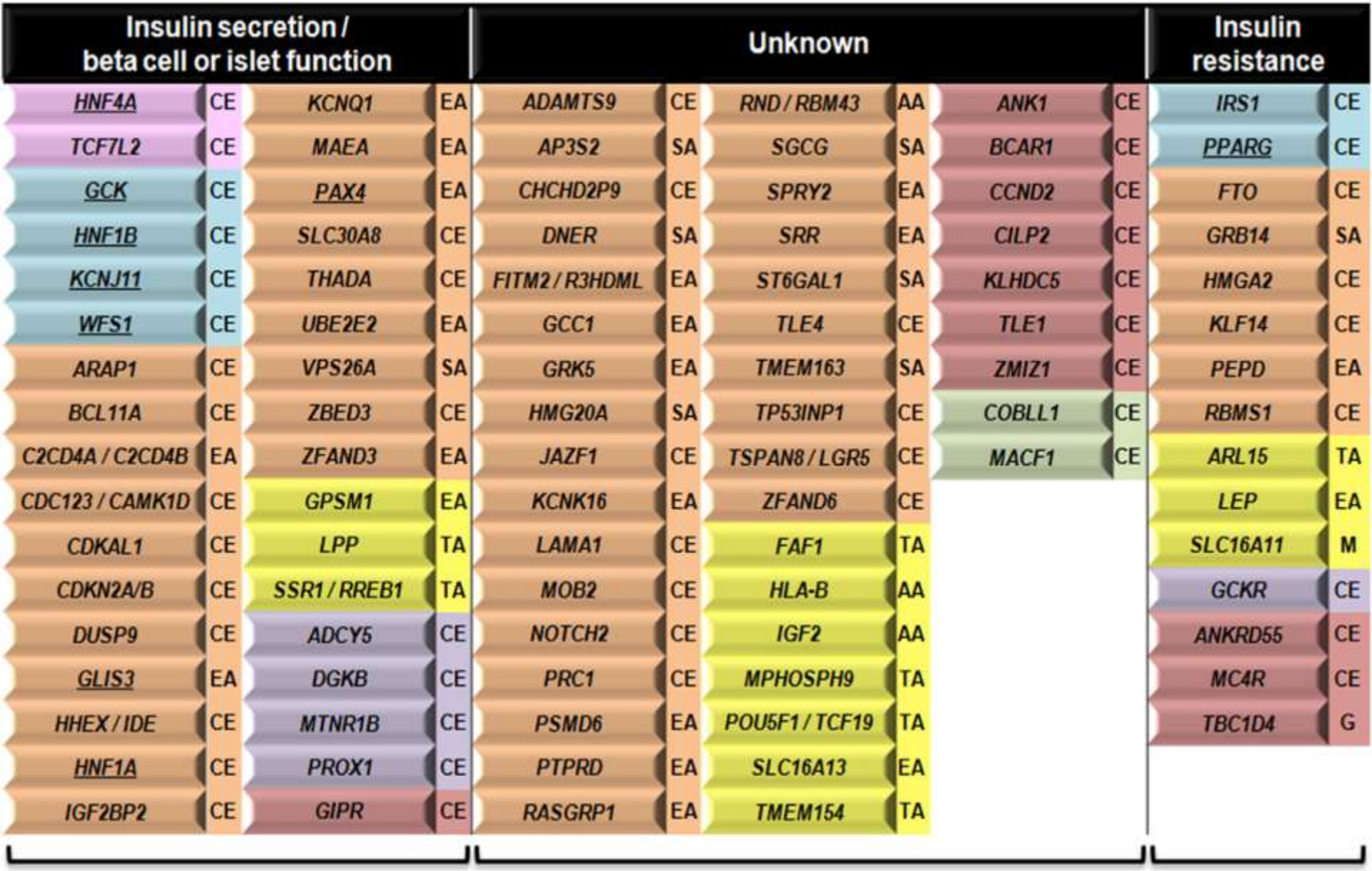



Figure 1: The HLA region on chromosome 6 (from Mehers and Gillespie 2008). The T1D associated haplotypes are DRB1*03-DQB1*02 and DRB1*04-DQB1*0302


Type 2 diabetes
Blood glucose level above normal
Insufficient amount of Insulin production

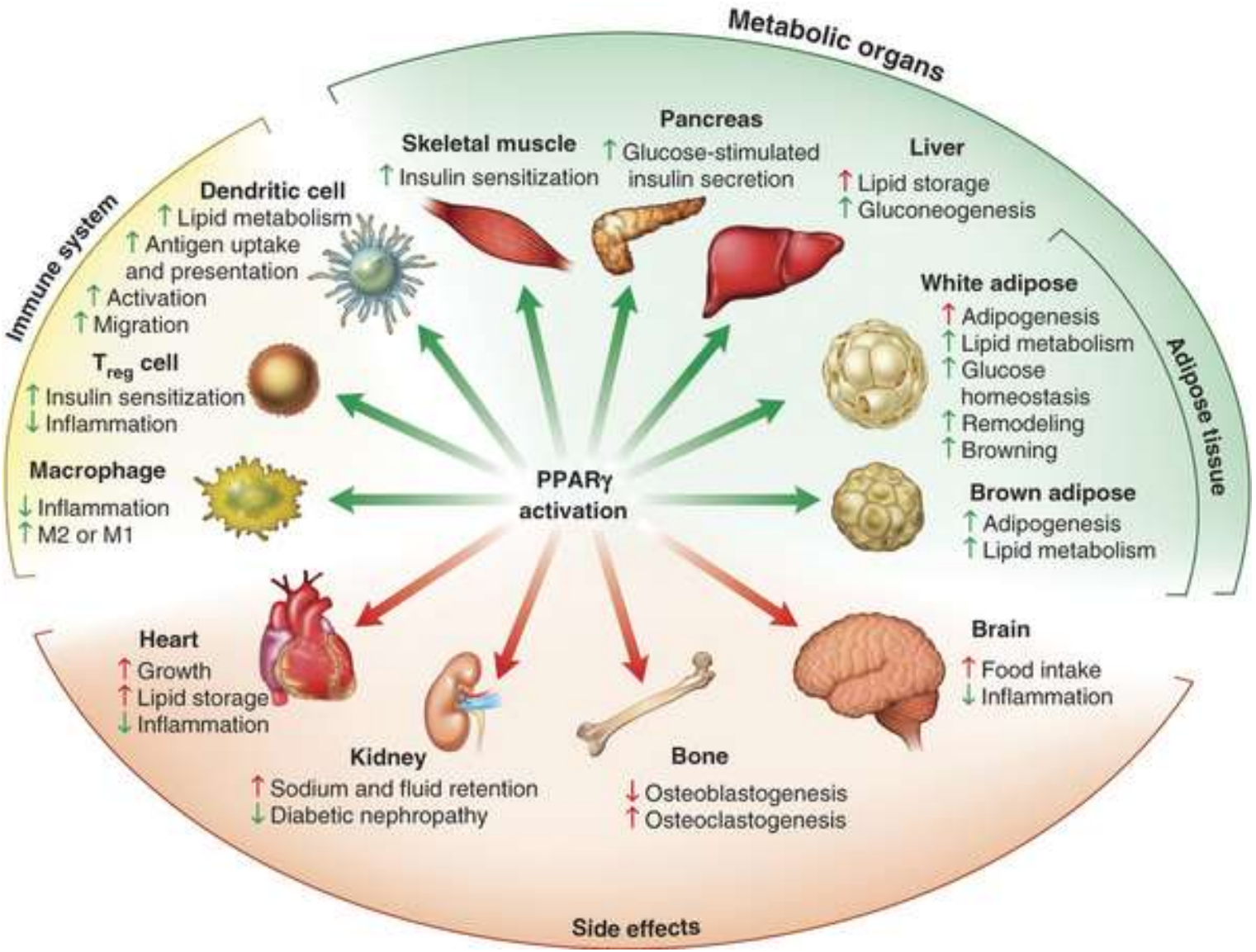

High blood pressure
Smoking
Sedentary lifestyle
Overweight
High fat & cholesterol levels
Type 2 Diabetes

# Common SNPs Contributing to T2D

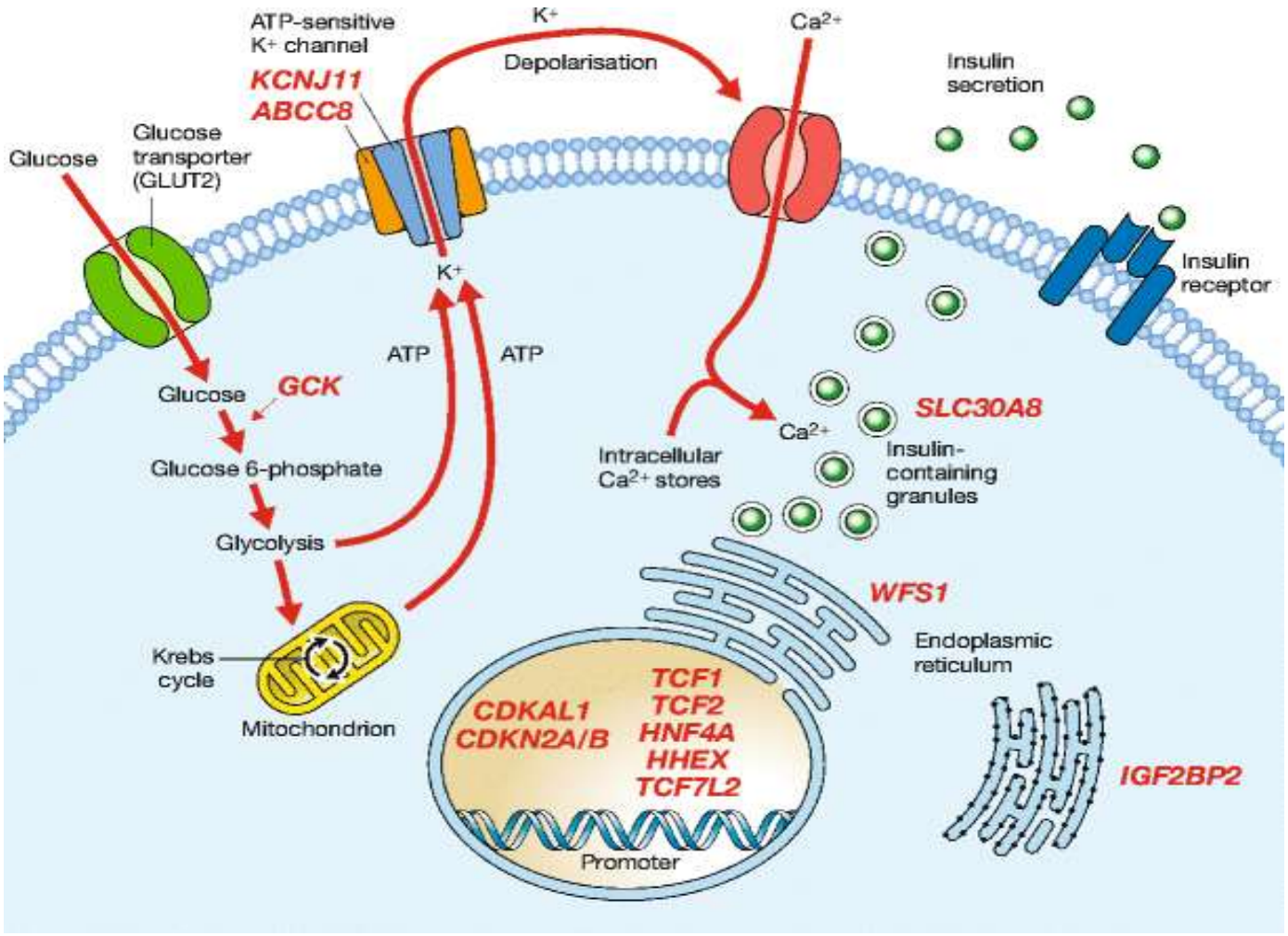



*Cell Metabolism* (2015)

# **PPARG** (Peroxisome proliferator-activated receptor gamma )



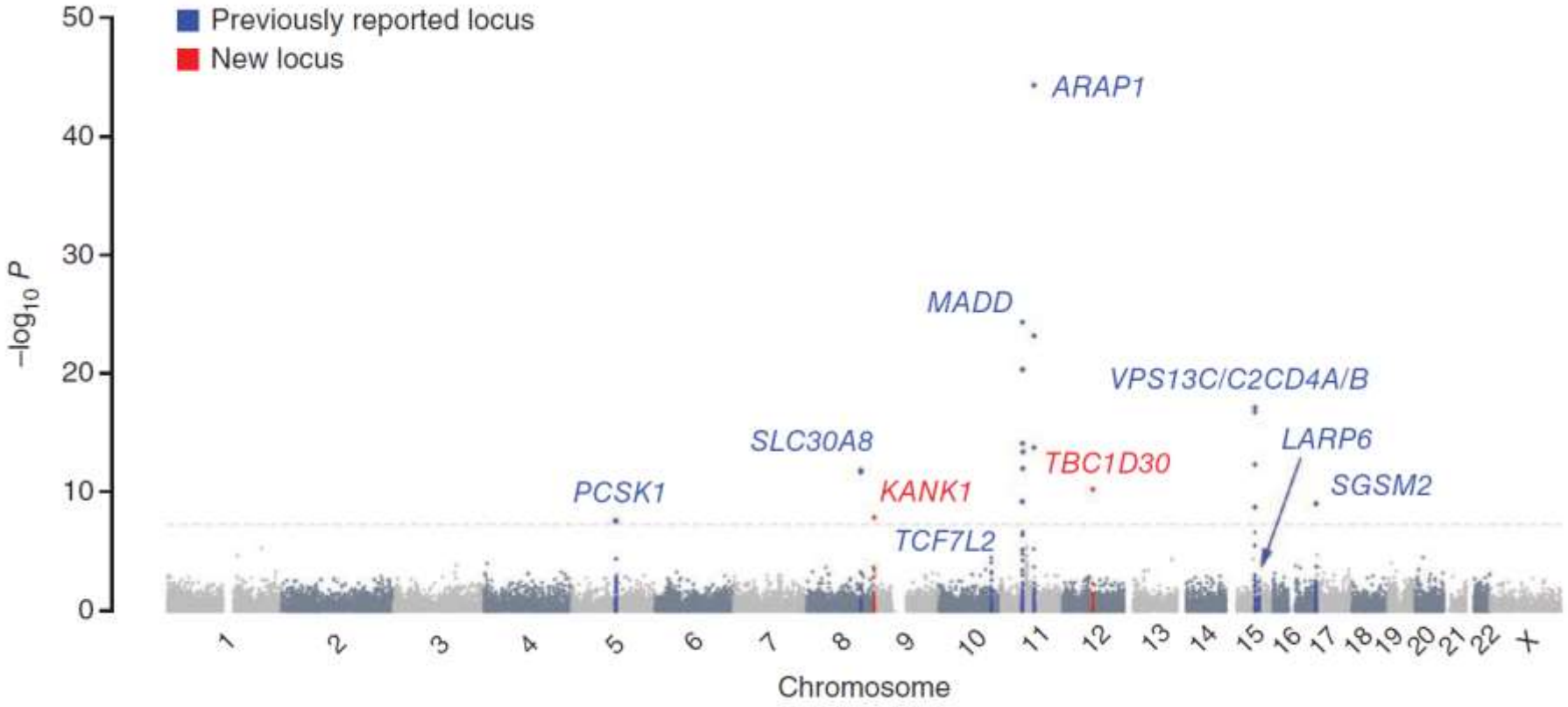
# **KCNJ11** (potassium channel, subfamily J, member 11)




***Diabetologia*** (2008)

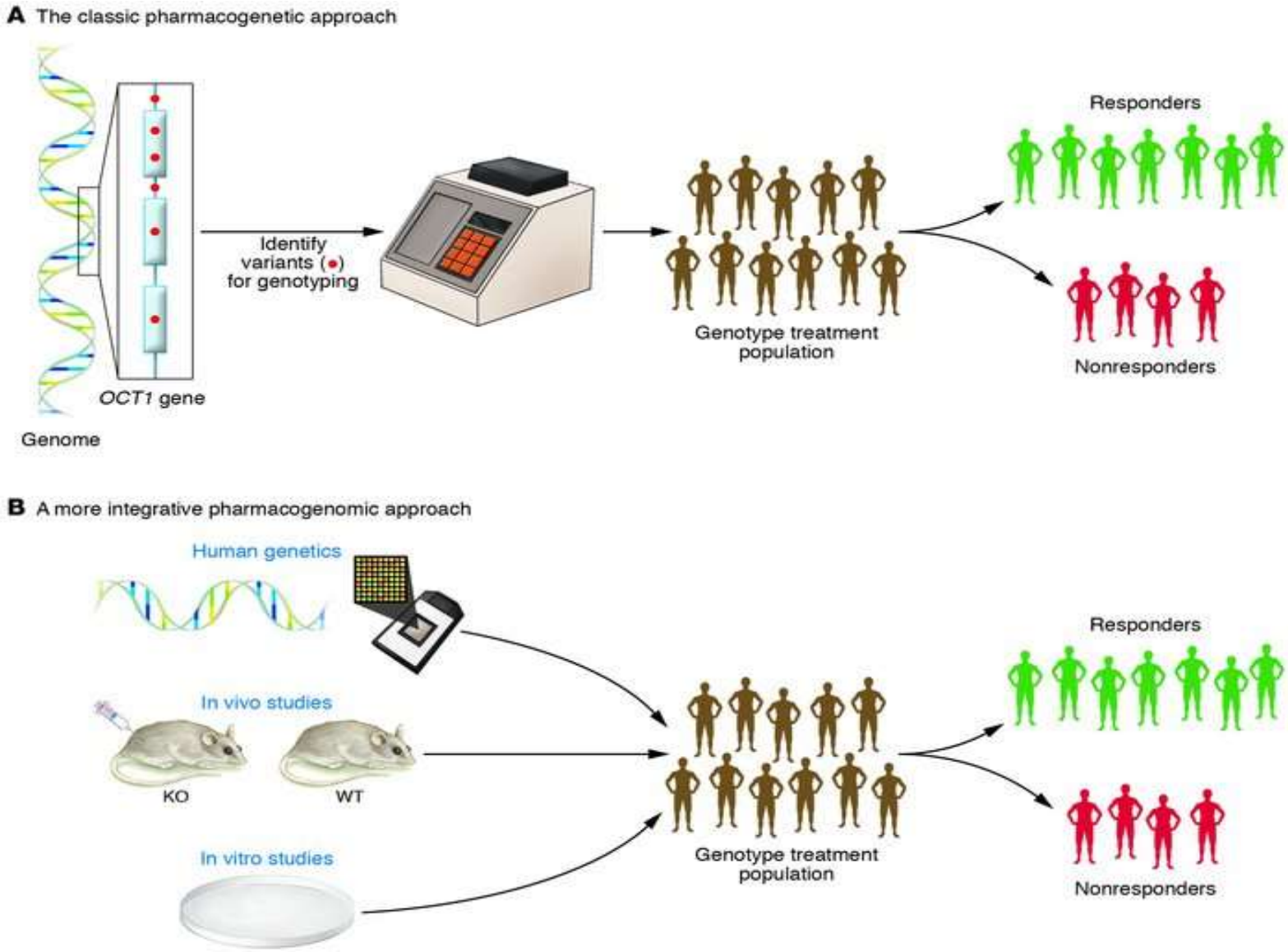
nature genetics

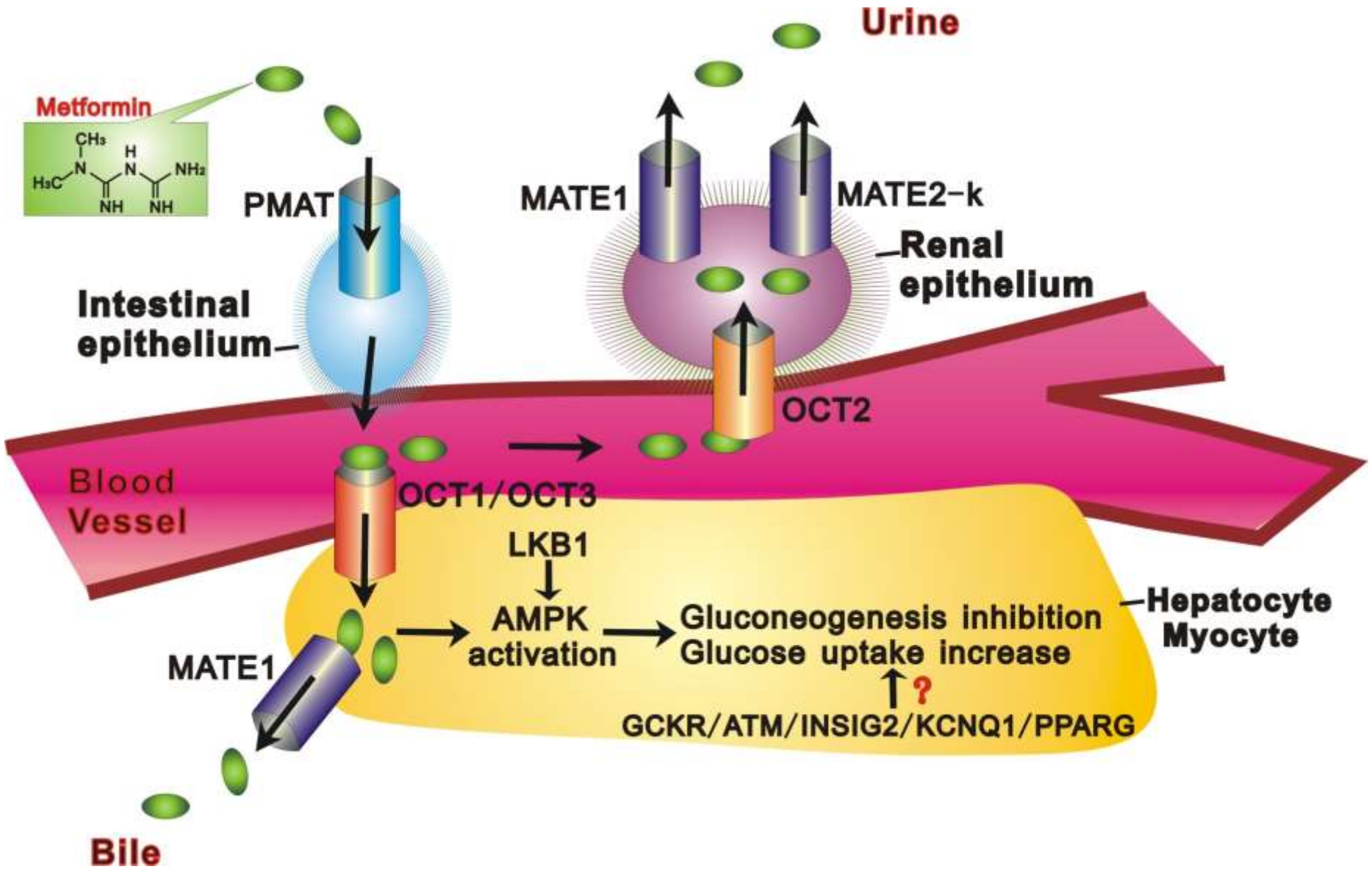
# Exome array analysis identifies new loci and low-frequency variants influencing insulin processing and secretion



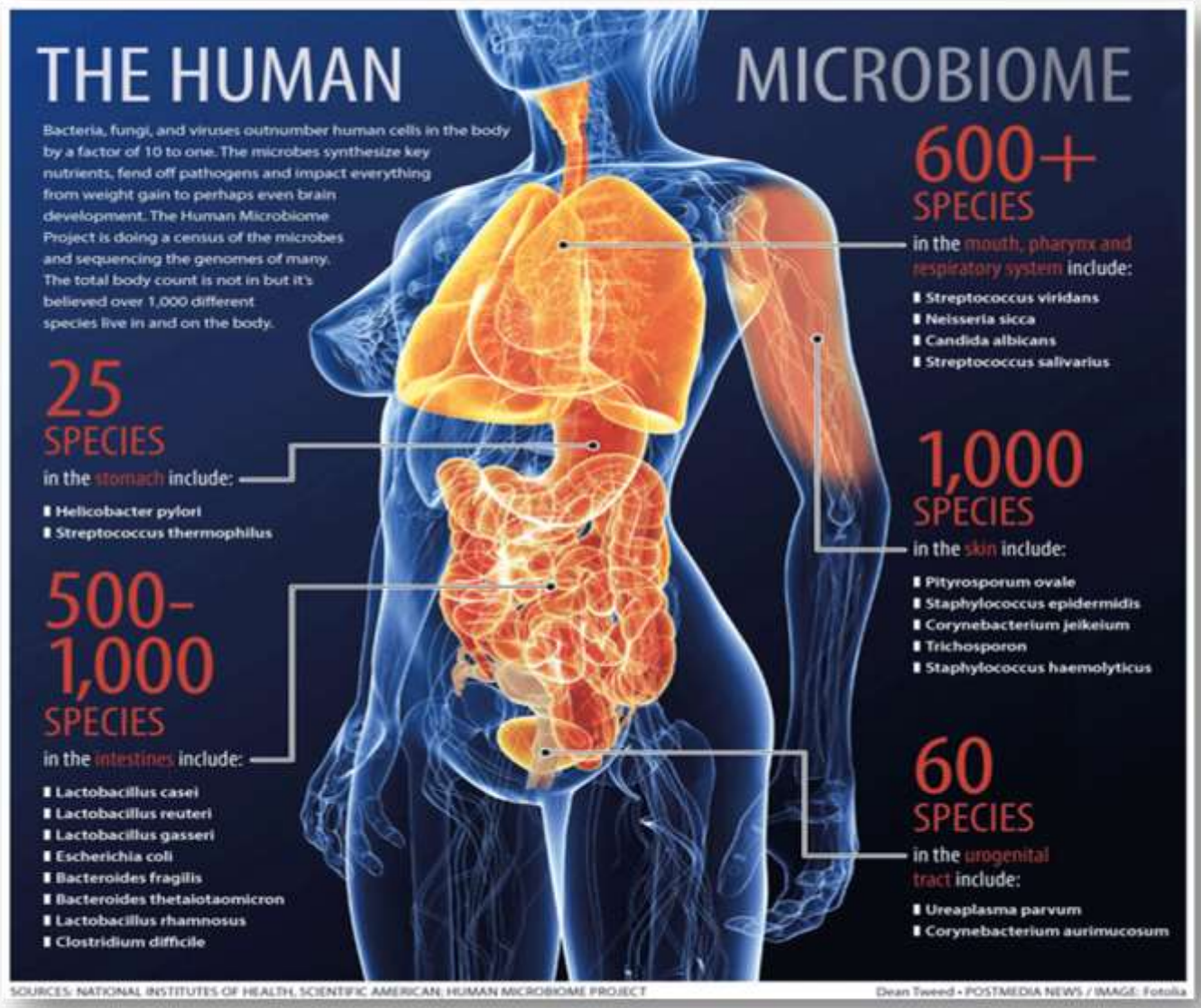

***Nature genetics*** (2013)

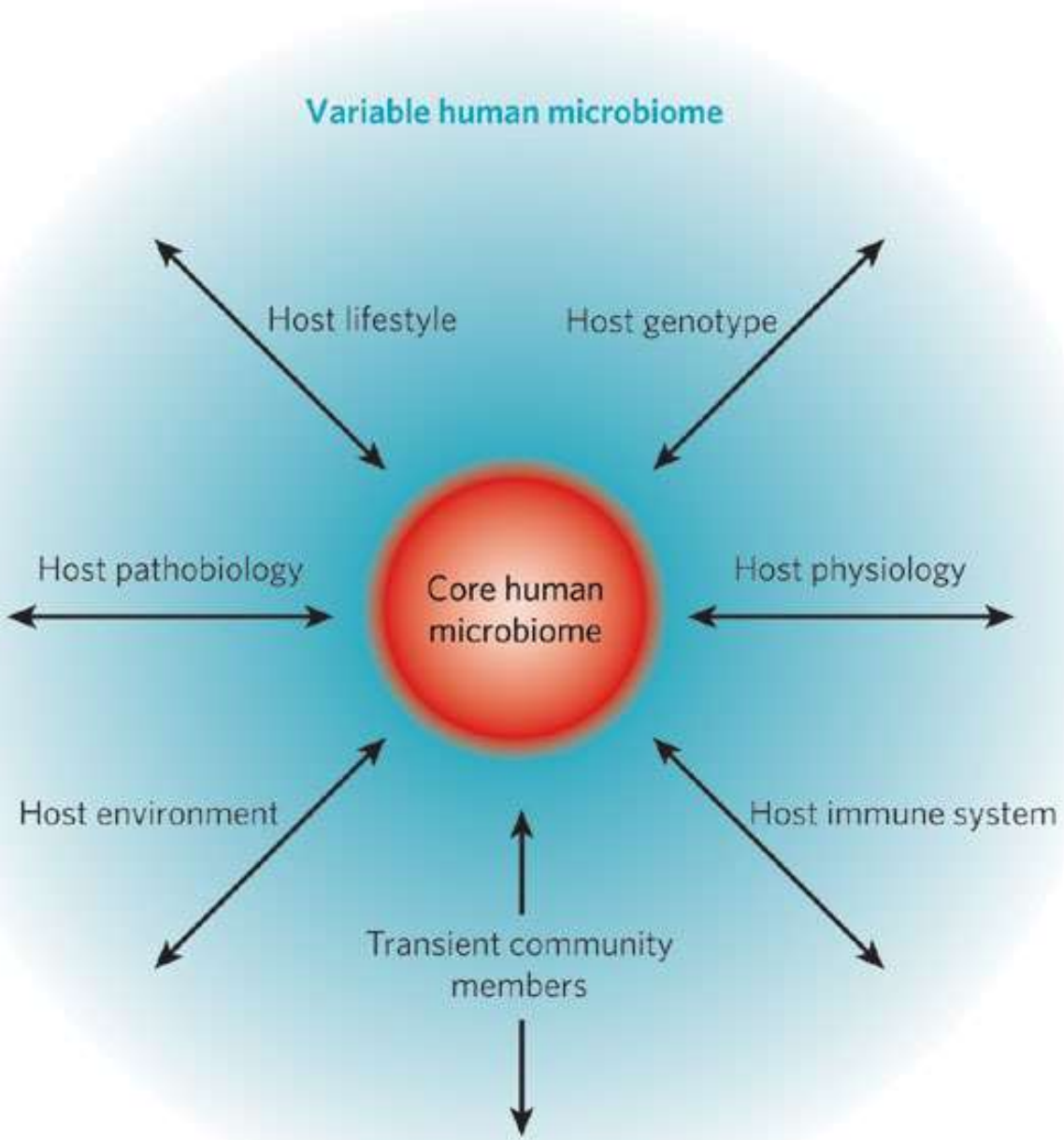
# Pharmacogenetic variation and metformin response




Urine
Metformin
PMAT
MATE1
MATE2-k
Renal epithelium
Intestinal epithelium
OCT2
Blood Vessel
OCT1/OCT3
LKB1
AMPK activation
Gluconeogenesis inhibition
Glucose uptake increase
Hepatocyte
Myocyte
MATE1
?
GCKR/ATM/INSIG2/KCNQ1/PPARG
Bile

# 3. Metabolic genomics and Microbiome




Variable human microbiome
Host lifestyle
Host genotype
Host pathobiology
Core human microbiome
Host physiology
Host environment
Host immune system
Transient community members


Chronic Disease,
Autoimmunes,
Obesity,
Diabetes
Mood
Disorders,
Depression,
Anxiety,
Autism
Age
Responsible
Sunlight
Mode
of birth
Food
Sensitivities
Allergies
Maternal
Gut Flora
GUT
MICROBIOME
Stress:
Physical
Exercise
Mental
emotional
Maternal
and Paternal
Nutrient
sufficiency
Breastfed?
Colicky?
Environmental
Exposures
Antibiotics
and Drugs
Sleep
Nutrient Dense,
Low Toxin,
Anti-inflammatory Diet,
Prebiotics and
Probiotics

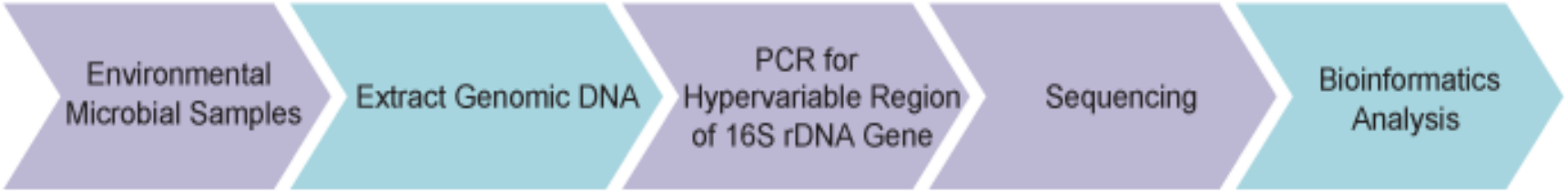
# 宏基因组学

## Metagenomics

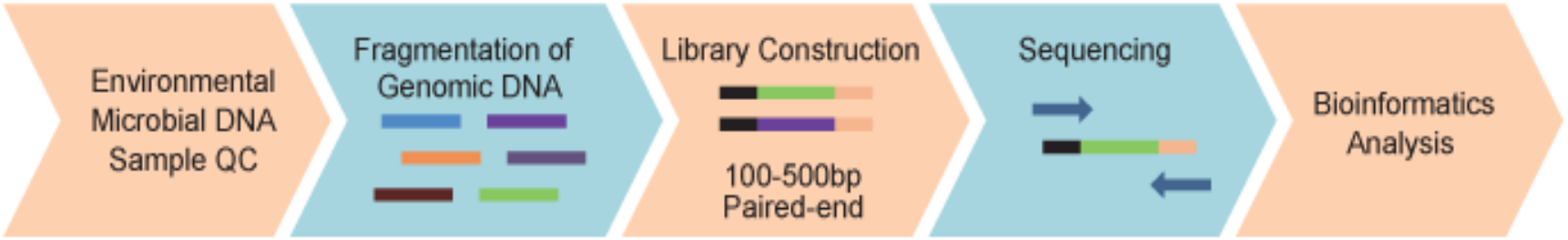
**Metagenomics** is the study of genetic material recovered directly from environmental samples. The broad field may also be referred to as environmental genomics, ecogenomics or community genomics.

# Metagenomics Sequencing

16S rDNA Metagenomics Sequencing




Whole Genome Metagenomics Sequencing



Vol 464|4 March 2010|doi:10.1038/nature08821



# A human gut microbial gene catalogue established by metagenomic sequencing

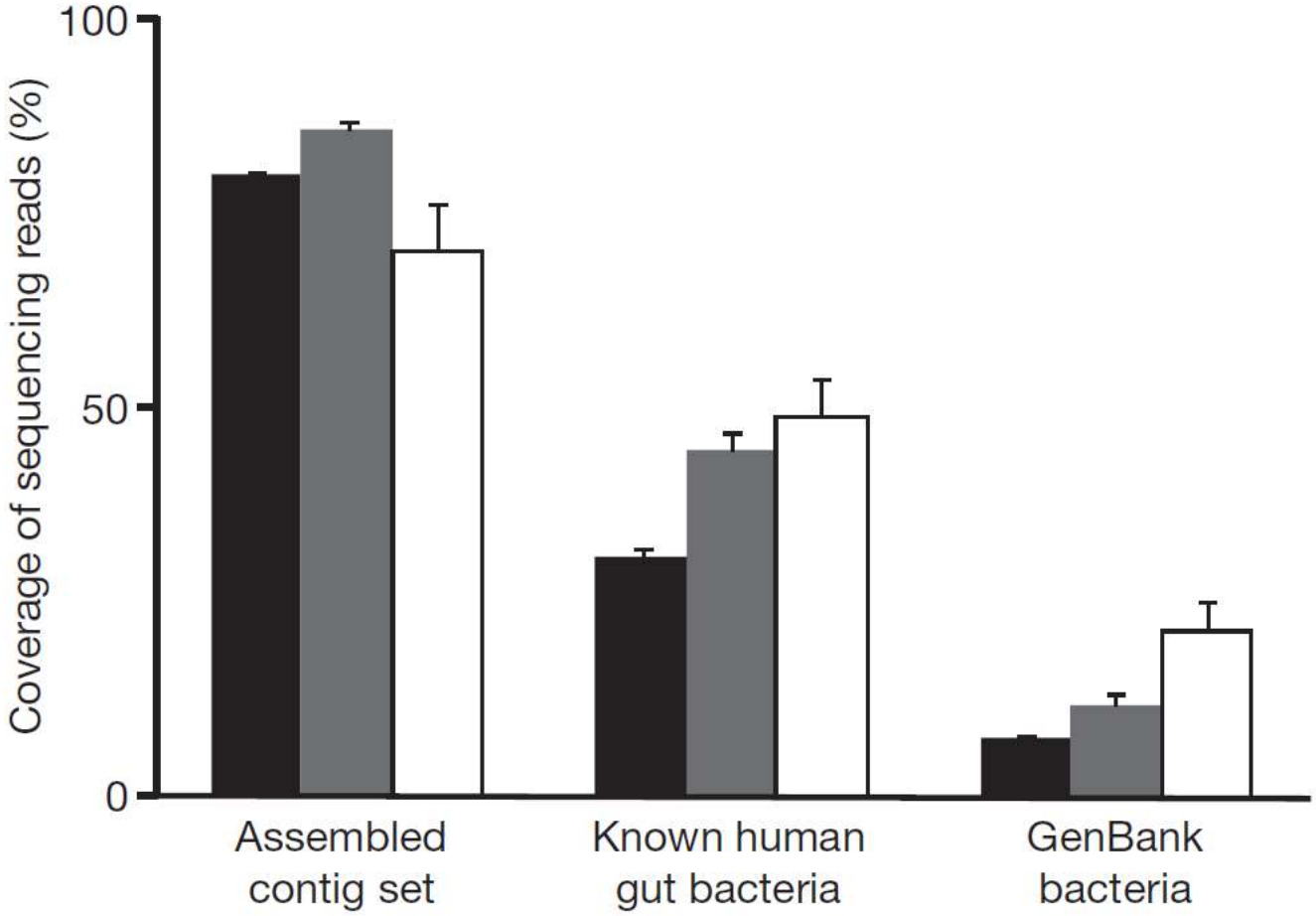
Junjie Qin[1]*, Ruiqiang Li[1]*, Jeroen Raes[2,3], Manimozhiyan Arumugam[2], Kristoffer Solvsten Burgdorf[4], Chaysavanh Manichanh[5], Trine Nielsen[4], Nicolas Pons[6], Florence Levenez[6], Takuji Yamada[2], Daniel R. Mende[2], Junhua Li[1,7], Junming Xu[1], Shaochuan Li[1], Dongfang Li[1,8], Jianjun Cao[1], Bo Wang[1], Huiqing Liang[1], Huisong Zheng[1], Yinlong Xie[1,7], Julien Tap[6], Patricia Lepage[6], Marcelo Bertalan[9], Jean-Michel Batto[6], Torben Hansen[4], Denis Le Paslier[10], Allan Linneberg[11], H. Bjørn Nielsen[9], Eric Pelletier[10], Pierre Renault[6], Thomas Sicheritz-Ponten[9], Keith Turner[12], Hongmei Zhu[1], Chang Yu[1], Shengting Li[1], Min Jian[1], Yan Zhou[1], Yingrui Li[1], Xiuqing Zhang[1], Songgang Li[1], Nan Qin[1], Huanming Yang[1], Jian Wang[1], Søren Brunak[9], Joel Doré[6], Francisco Guarner[5], Karsten Kristiansen[13], Oluf Pedersen[4,14], Julian Parkhill[12], Jean Weissenbach[10], MetaHIT Consortium†, Peer Bork[2], S. Dusko Ehrlich[6] & Jun Wang[1,13]

3.3 million non-redundant microbial genes

576.7 gigabases of sequence

124 European individuals (faecal samples)

# Coverage of human gut microbiome




***Nature,*** 2010

Article

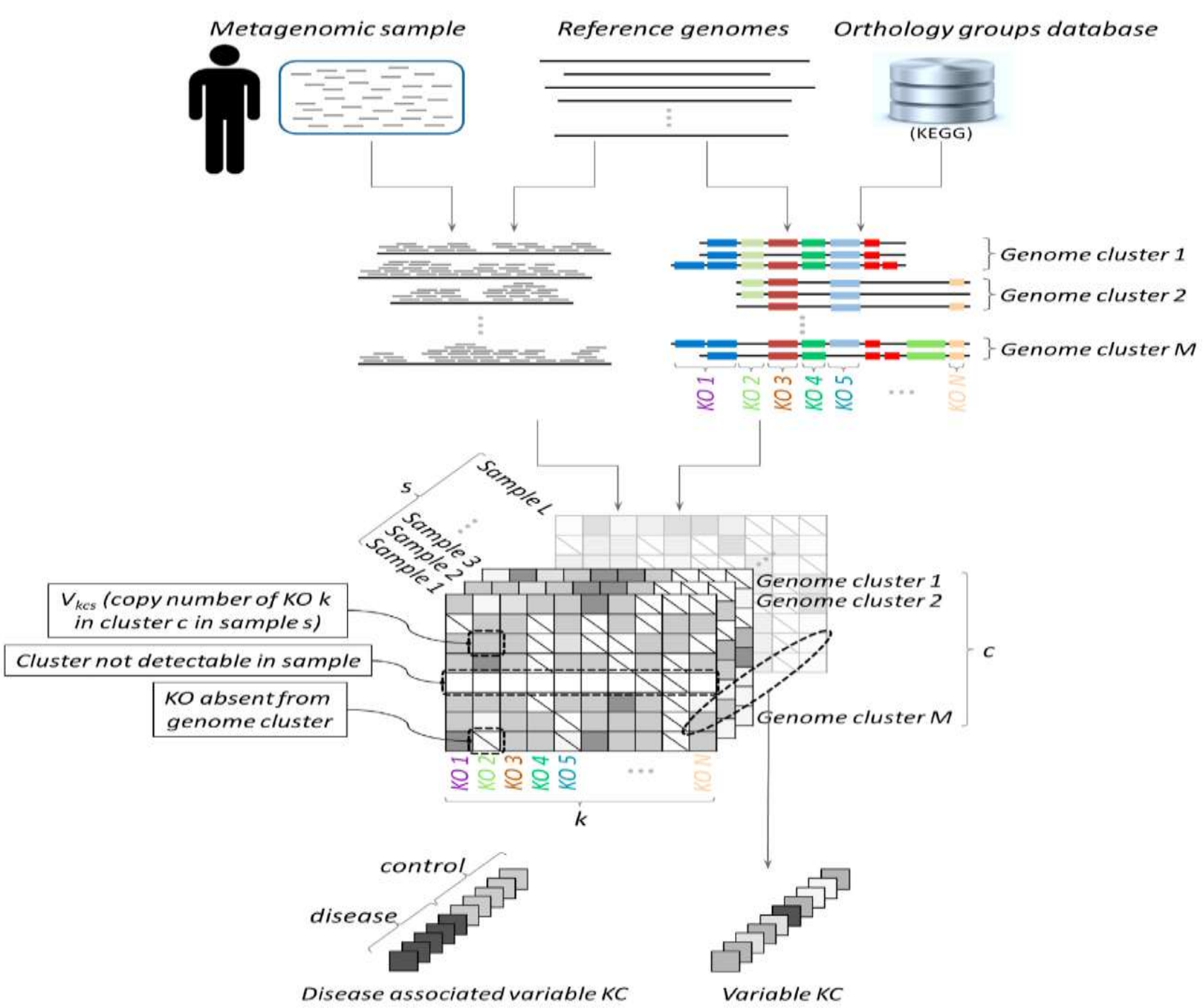
Cell

# Extensive Strain-Level Copy-Number Variation across Human Gut Microbiome Species

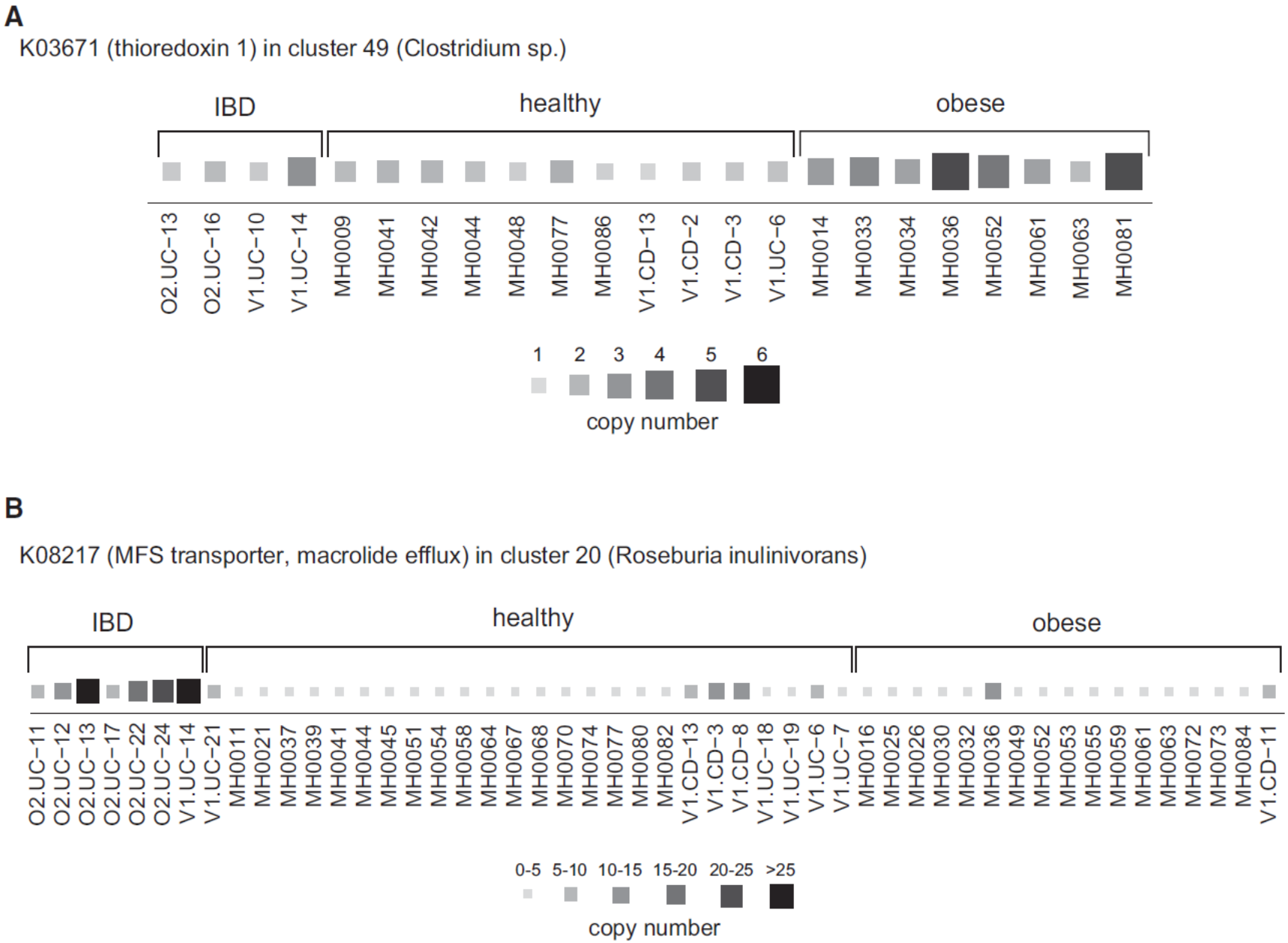
Sharon Greenblum,[1] Rogan Carr,[1] and Elhanan Borenstein[1,2,3,*]
[1]Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA
[2]Department of Computer Science and Engineering, University of Washington, Seattle, WA 98195, USA
[3]Santa Fe Institute, Santa Fe, NM 87501, USA
*Correspondence: elbo@uw.edu
http://dx.doi.org/10.1016/j.cell.2014.12.038

**属（Genus）– 种 （Species）– 菌株 （Strain）**

Taxonomic characterization of the human microbiota is often limited to the species level or to previously sequenced strains, and accordingly, the prevalence of intra-species variation, its functional role, and its relation to host health remain unclear.


Metagenomic sample
Reference genomes
Orthology groups database
(KEGG)
Genome cluster 1
Genome cluster 2
Genome cluster M
KO 1
KO 2
KO 3
KO 4
KO 5
KO N
s
Sample L
Sample 3
Sample 2
Sample 1
$V_{kcs}$ (copy number of KO k in cluster c in sample s)
Cluster not detectable in sample
KO absent from genome cluster
Genome cluster 1
Genome cluster 2
Genome cluster M
c
k
control
disease
Disease associated variable KC
Variable KC

# Copy-Number Variation of Host State-Associated KCs



Cell

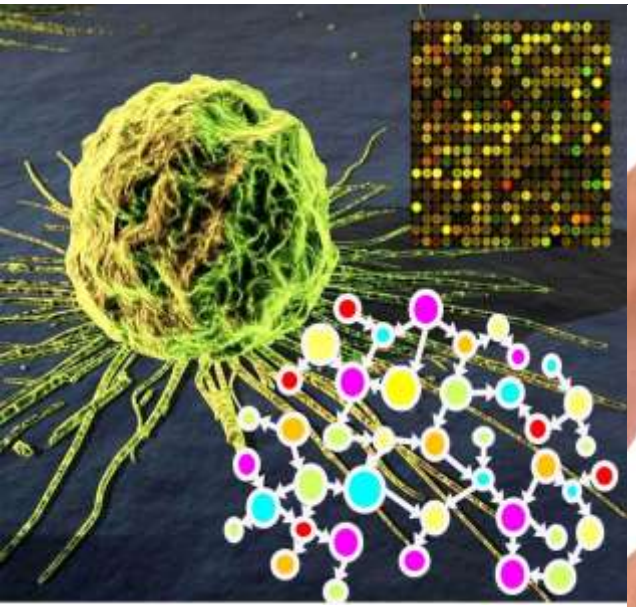
Volume 160
Number 4

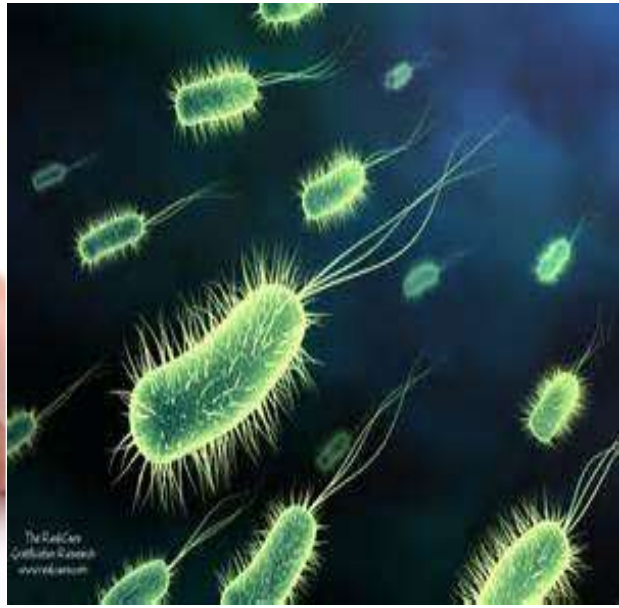
February 12, 2015

www.cell.com

# Strain-Level Variation in the Gut Microbiome

# Metabolic genomics

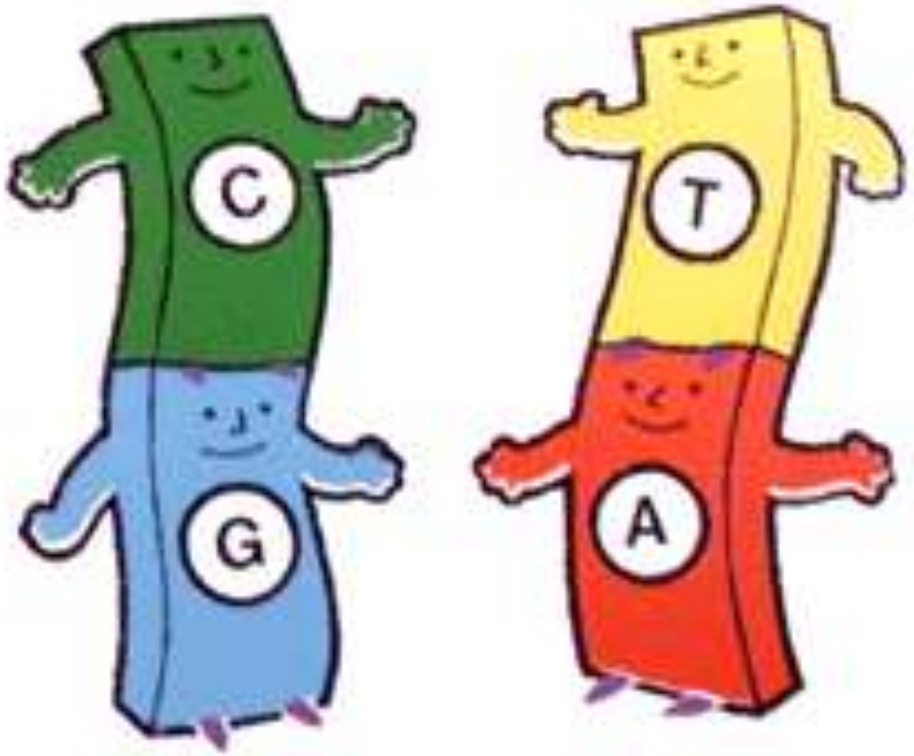


Cancer

Diabetes

Microbiome




The code contains only four letters


give
+hanks