Degradation and Biosynthesis of Nucleotides

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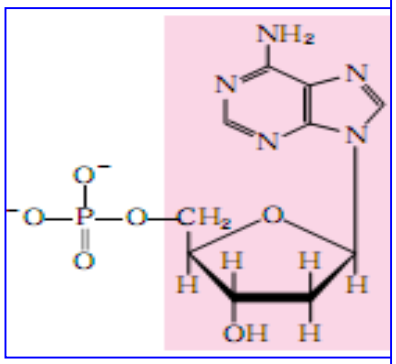
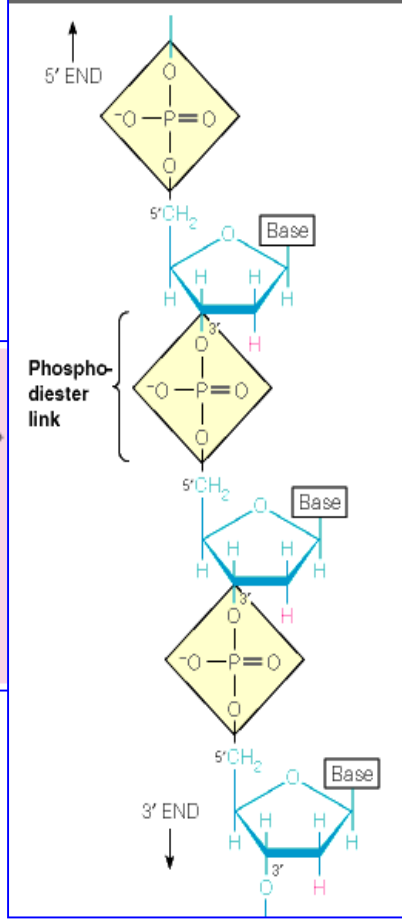
outline

- **Nucleotide Degradation**
  - Purine (嘌呤)
  - Pyrimidine (嘧啶)
- **Nucleotide (核苷酸) Biosynthesis**
  - Purine (嘌呤) Biosynthesis and regulation
    - *de novo*
    - Purine Salvage
  - Pyrimidine (嘧啶) biosynthesis
  - Deoxyribonucleotide (脱氧核苷酸) Biosynthesis

DNA and RNA degradation





DNA/RNA

DNases, RNases
Endonuclease,
Exonucleolase

nucleotide

phospholipase

phosphate

Nucleoside

Nucleosidase:
Nucleoside phosphorylase
Nucleoside hydrolase

Pentose-1-P
Pentose

base

Purine Degradation

Purine catabolism leads to uric acid

核苷酸酶 核苷酶 尿酸

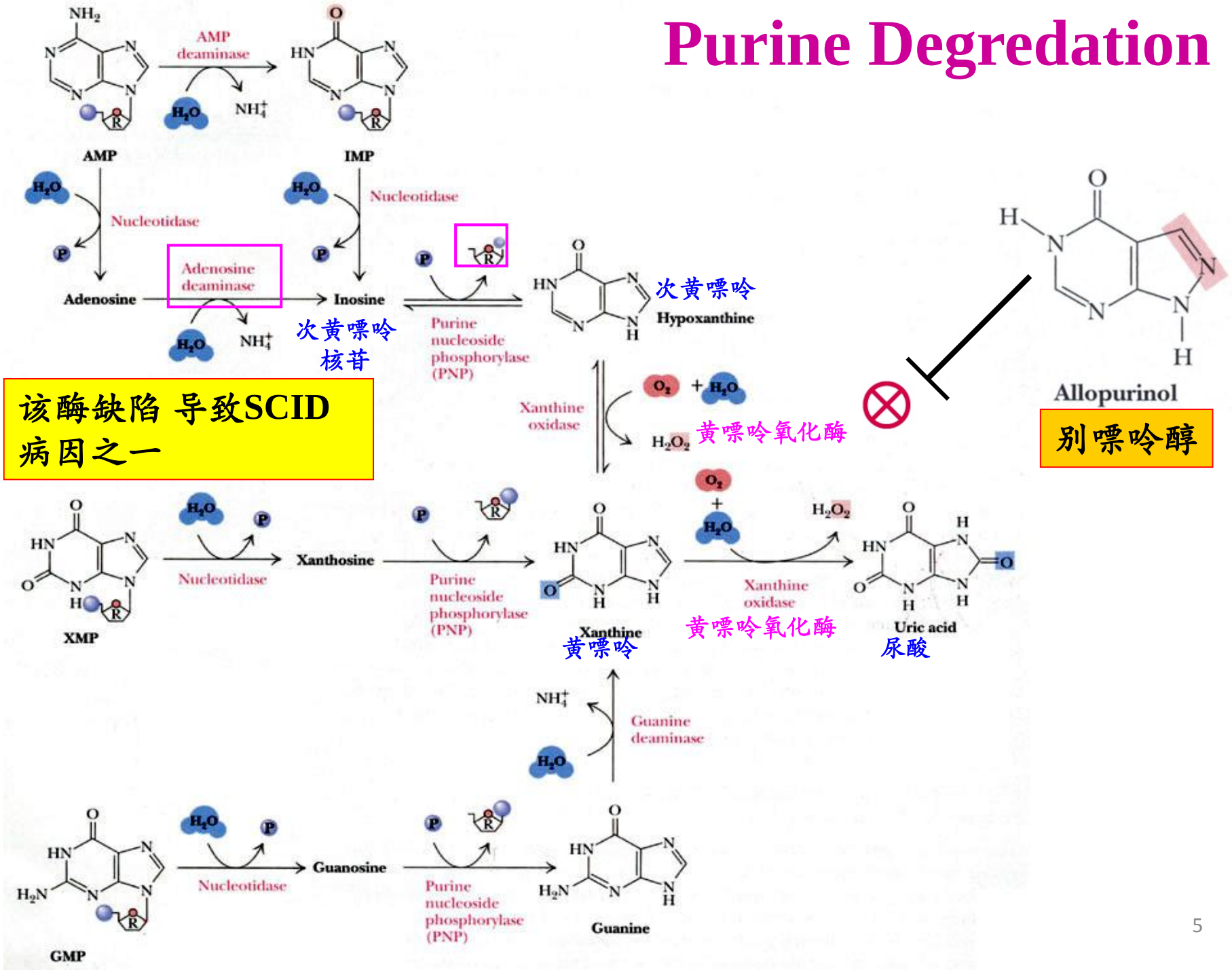
- Nucleotidases， nucleosidases → ribose + Pi + **bases**

黄嘌呤氧化酶 鸟嘌呤脱氨酶

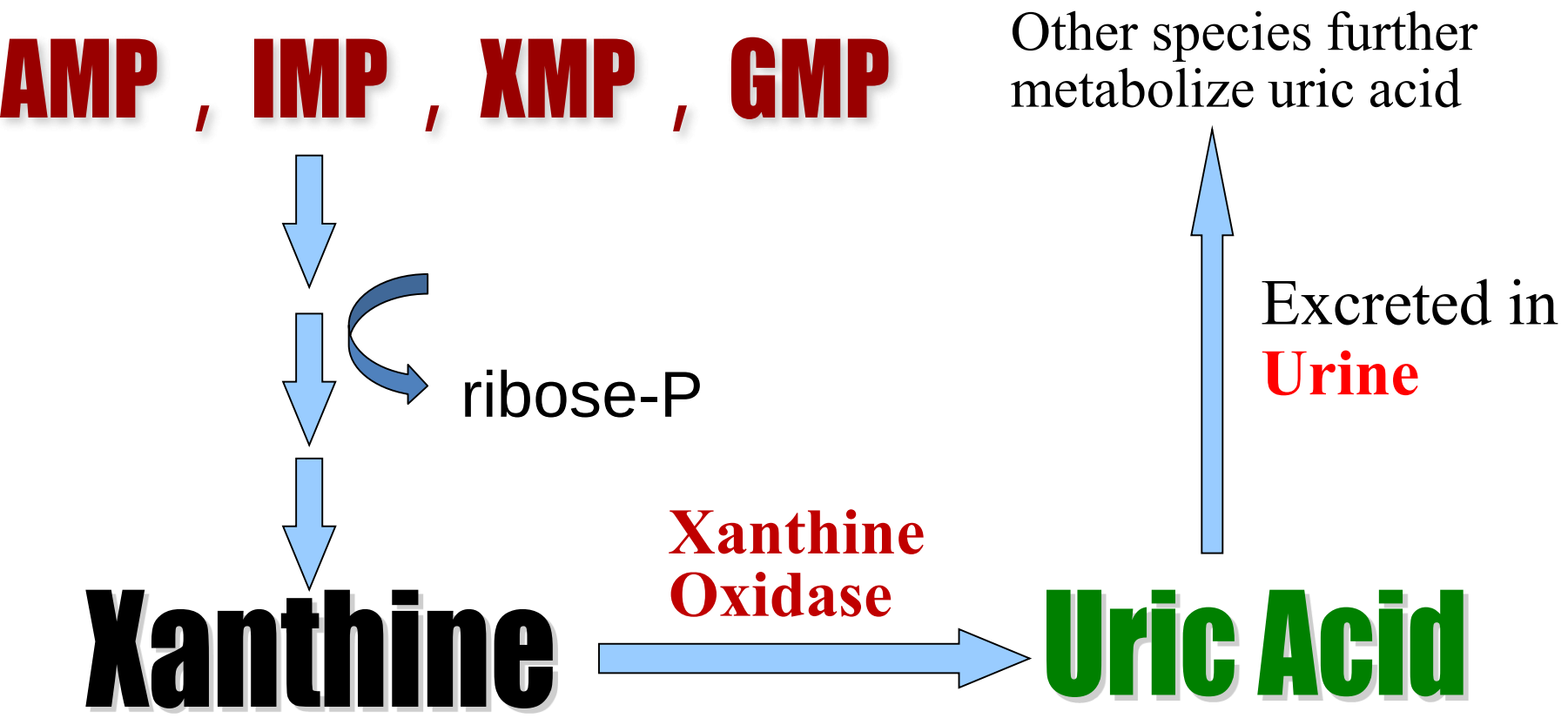
- Xanthine oxidase， guanine deaminase → **xanthine**
- **Xanthine oxidase** converts xanthine → uric acid

- **xanthine oxidase** can oxidize two different sites on the **purine** ring

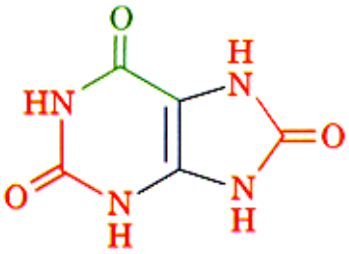
Purine Degredation



Purine Degredation



Excreted by

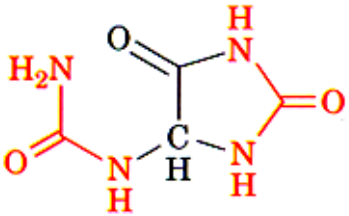


Uric acid

- Primates
- Birds
- Reptiles
- Insects

灵长类、鸟类、爬虫类、昆虫

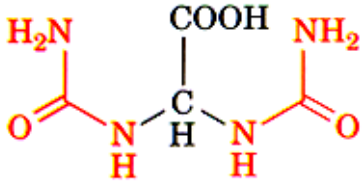
$2\,H_2O + O_2$ → $CO_2 + H_2O_2$: urate oxidase



Allantoin 尿囊素

- Other mammals

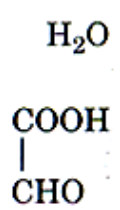
H_2O : allantoinase



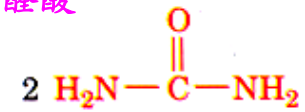
Allantoic acid 尿囊酸

- Teleost fish 硬骨鱼

H_2O : allantoicase



Glyoxylic acid 乙醛酸



Urea

- Cartilaginous fish
- Amphibia

大多数鱼类、两栖类

$2\,H_2O$ → $2\,CO_2$: urease

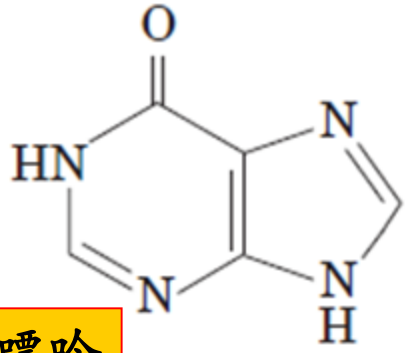
$4\,NH_4^+$

- Marine invertebrates

甲壳类和咸水辫鳃类

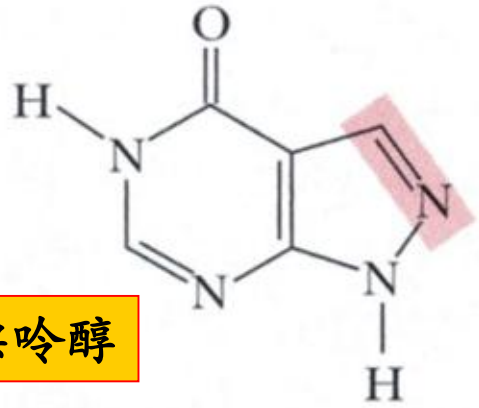
Xanthine Oxidase and Gout (痛风)

- **Impaired excretion** or **overproduction** of uric acid
- **Allopurinol** (hypoxanthine analog) binds to **Xanthine Oxidase** to dec roduction



次黄嘌呤

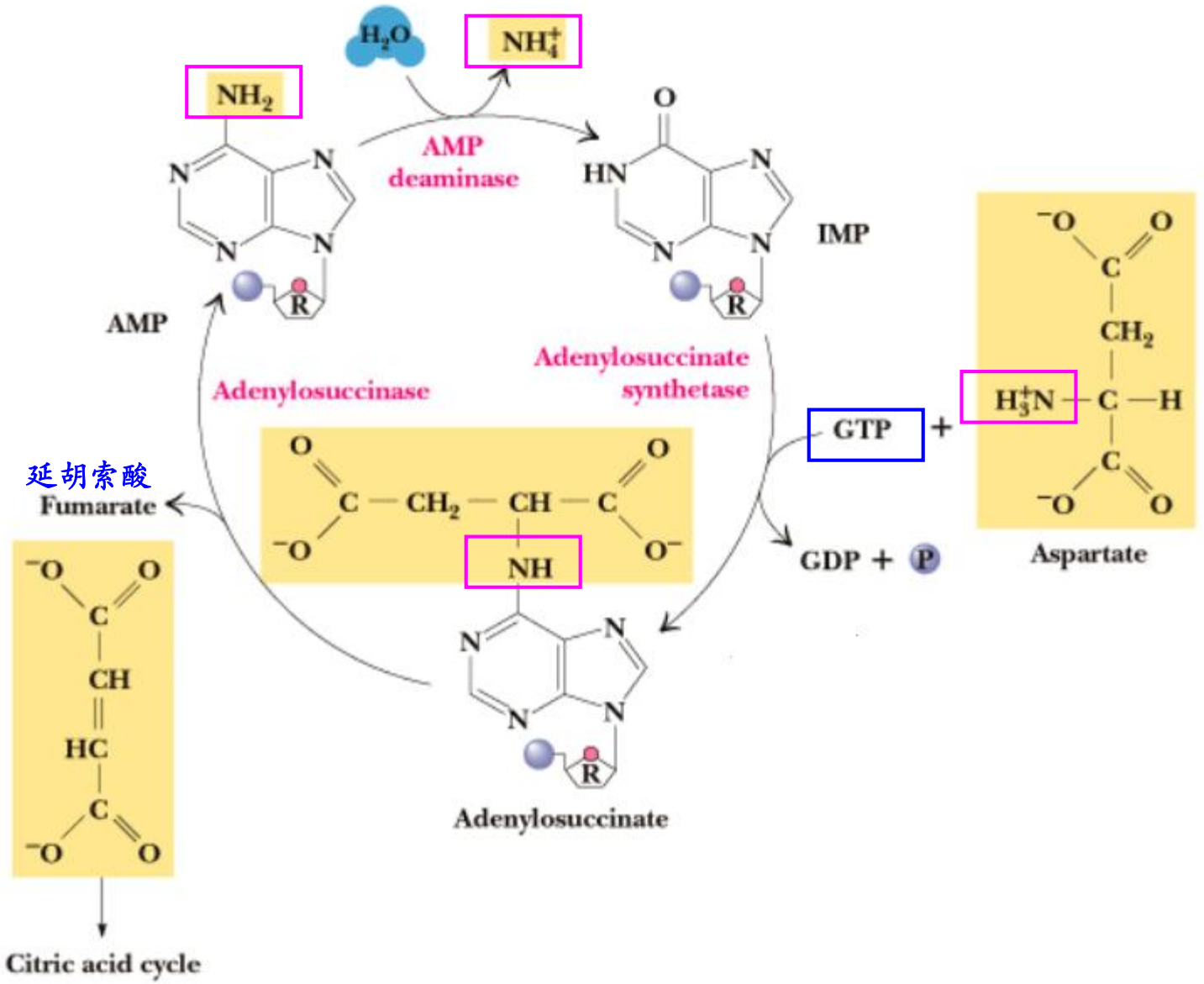
Hypoxanthine



别嘌呤醇

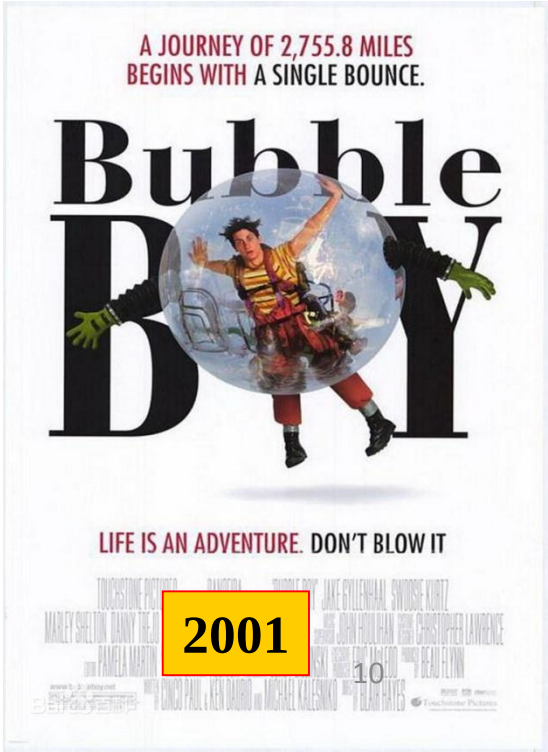
Allopurinol

The purine nucleoside cycle for anaplerotic TCA intermediates in skeletal muscle



Immunodeficiency Diseases Associated with Purine Degradation

- ☹ Defect in **adenosine deaminase**
  腺苷脱氨酶
  - Removes $-NH_2$ from adenosine
- ☹ SCID- severe immunodeficiency
- ☹ Defect in both B-cells and T-cells
- ☹ Extremely susceptible to infection
- ☹ "Bubble Boy" Disease



Therapies for SCID

- Bone marrow transplant- **adenosine deaminase**
- Gene therapy
- 1990 NIH- Dr. Willi Anderson- Ashanti
- 2016.5 Strimvelis-

sina医药新闻 首页 药企 药店 医院 综合资讯 专栏 两票制 分级诊疗

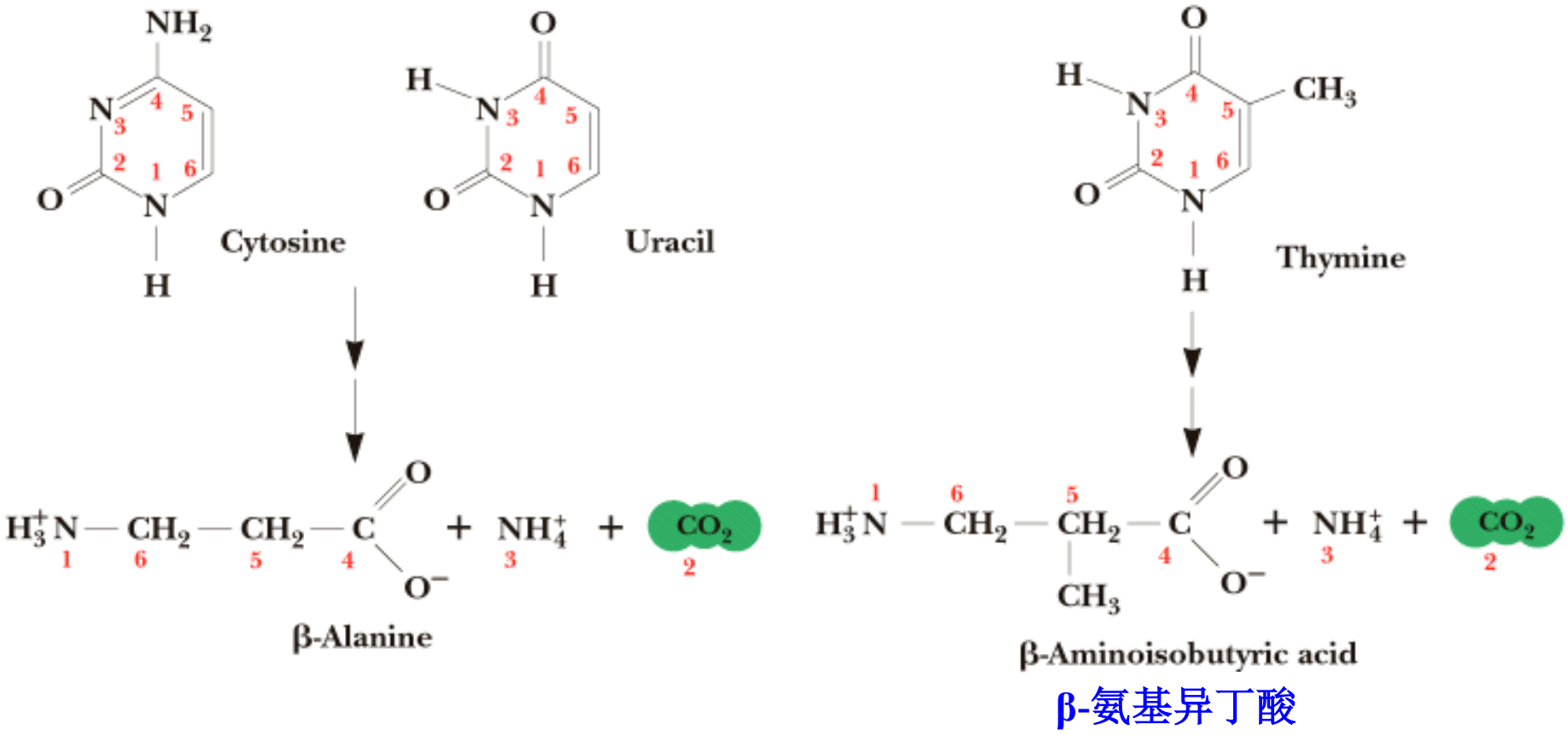
GSK基因疗法Strimvelis获英国NHS批准 治疗“泡泡宝宝”

原创 来源：新浪医药新闻 2017-10-25 A+ A-

近日，英国国家医疗服务系统（NHS）在英国国立健康与临床优化研究所（NICE）做出推荐批准**基因疗法Strimvelis**用于**“泡泡宝宝综合征”**治疗之后，决定将该基因疗法纳入到国家医疗系统，即使这款药物的治疗费用将高达**53万英镑**。

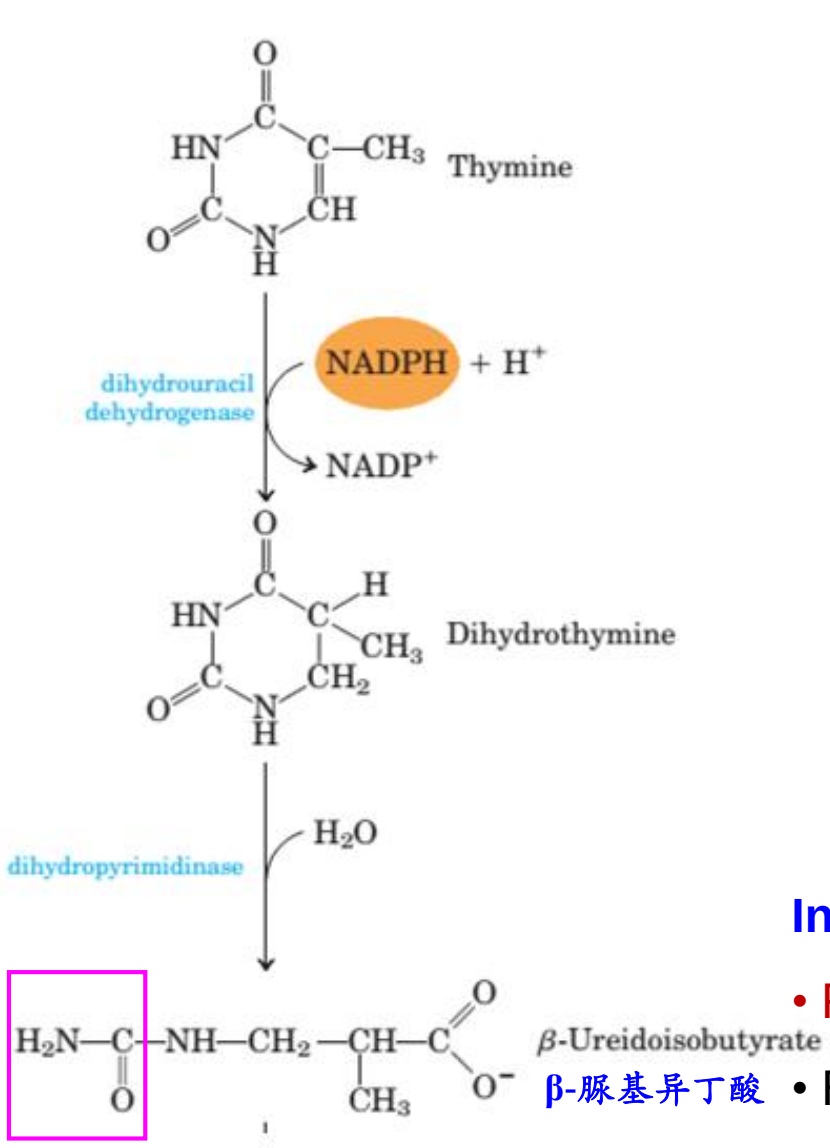
2016年5月，**GSK制药**的Strimvelis被欧盟批准用于**治疗腺苷脱氨酶（ADA）缺乏性重度联合免疫缺陷症（ADA-SCID）**儿科患者。ADA-SCID是一种非常罕见的疾病，在欧洲该疾病大约每年会影响**15位**患儿的健康，患有该疾病的新生儿**不具备健康、健全的免疫系统功能**，来自父母机体中错误的基因会影响患儿机体**ADA蛋白的产生**，而该蛋白是**产生淋巴细胞**所必需的，患者对日常感染失去抵抗力，必须生活在无菌环境中，所以该病又称之为“泡泡宝宝综合征”。

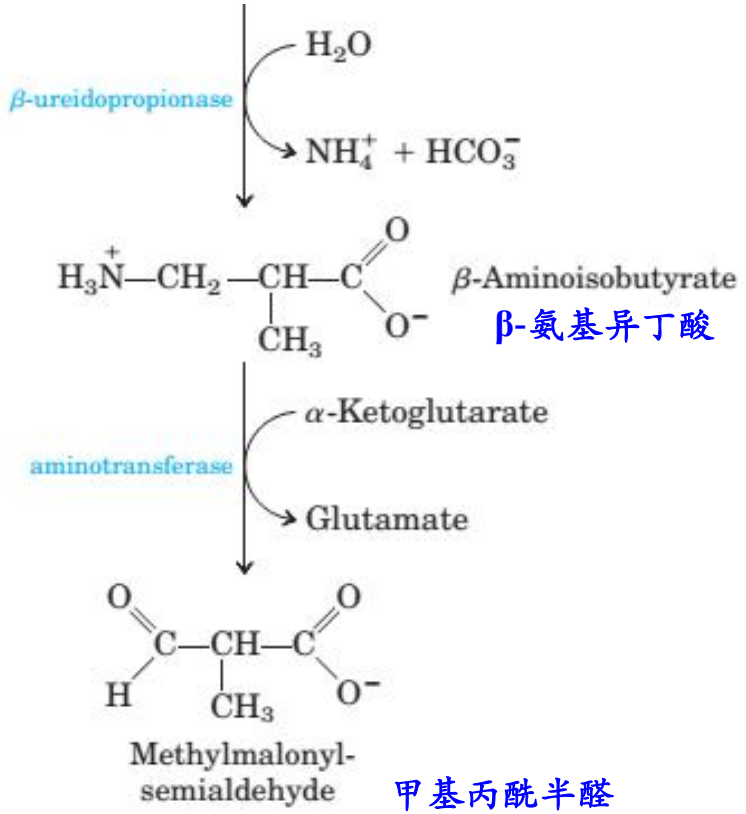
Pyrimidine Degradation



Final： amino acid, NH_3, CO_2

Pyrimidine Degradation





In humans

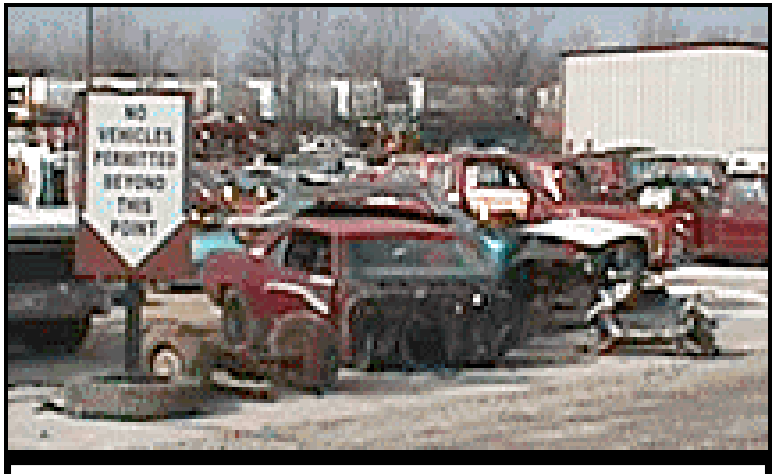
- Pyrimidines are recycled at nucleosides level
- Free pyrimidine are not salvaged→be degraded

Synthesis Pathways

★ Two means of synthesis for **purines** & **pyrimidines**

- *de novo*
- Salvage (recycle from pre-existing nucleotides)

***de novo* Pathway**



Salvage Pathway

Two types of pathways lead to nucleotides

核苷酸

➢***De novo*** begins with metabolic precursors: ribose 5-phosphate（HMP）, AAs, CO_2, NH_3.

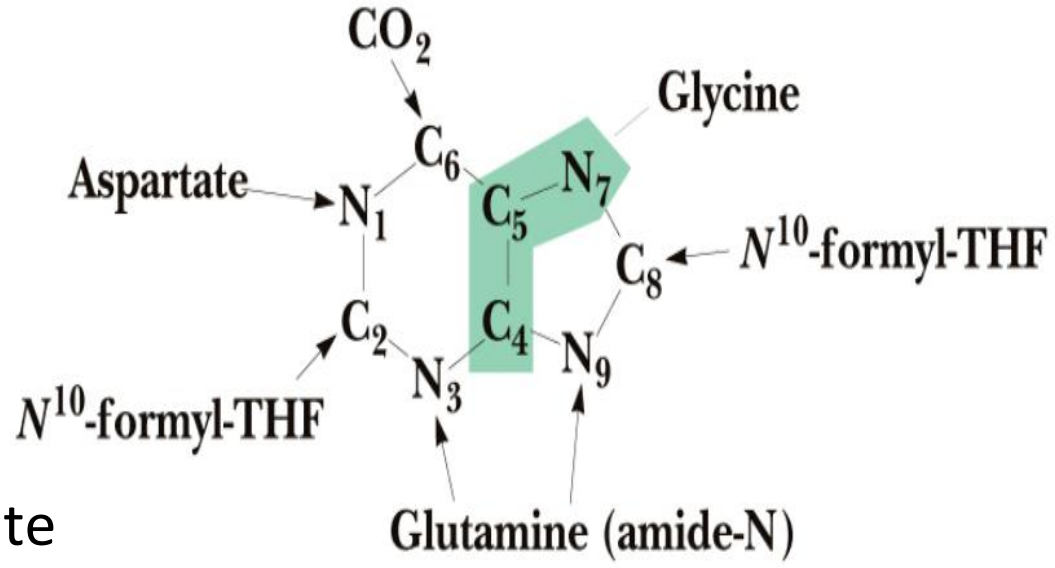
➢**Salvage** pathways recycle the free bases and nucleosides from nucleic acid breakdown.

purine synthesis （*de novo*）

John Buchanan (1948) "traced" the sources of all nine atoms of purine ring

★ **Atoms** derived from:

- Asp
- Glycine
- Gln
- CO_2
- N^{10}-CHO-Tetrahydrofolate



★ Also requires

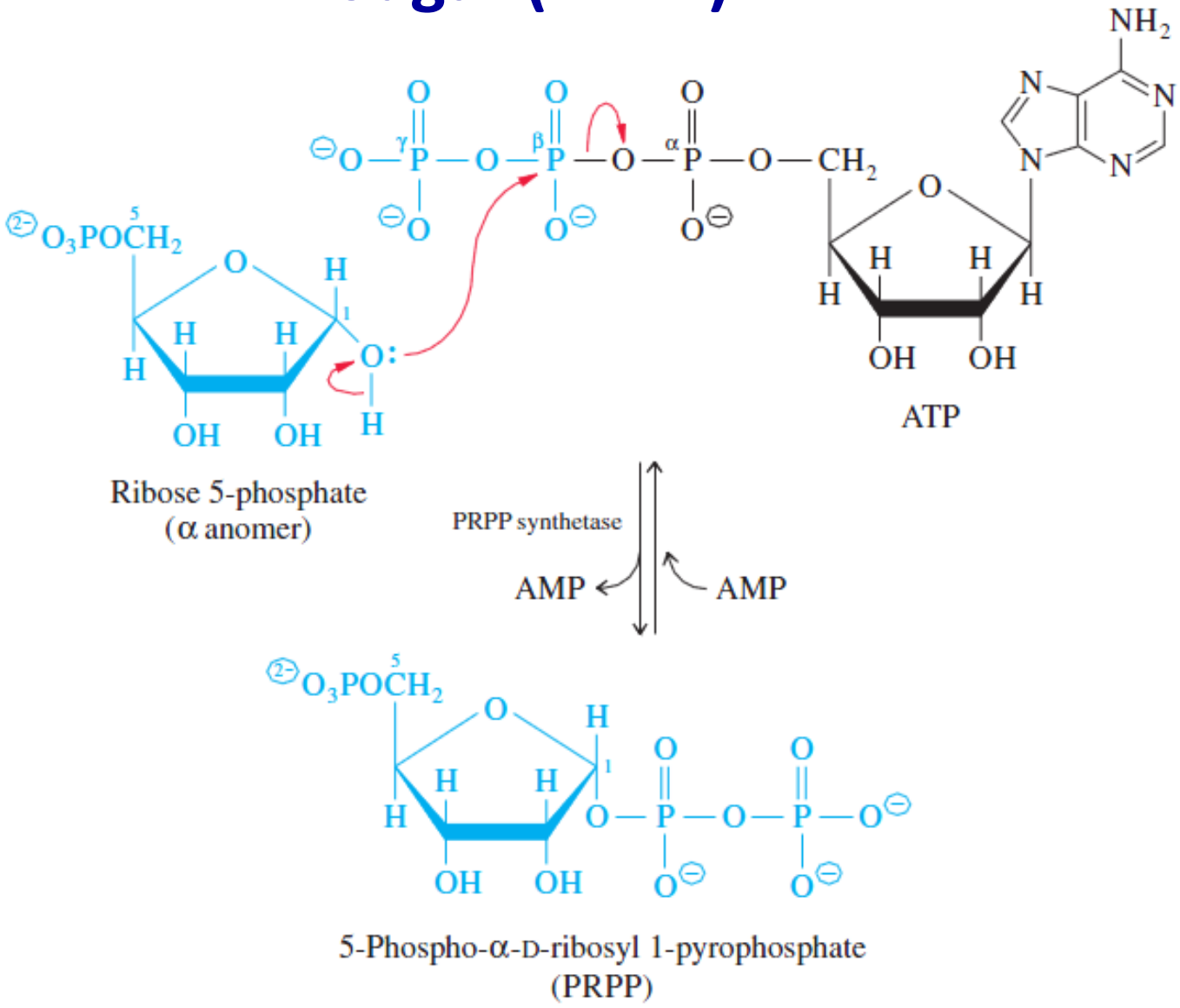
- 4 ATPs

Purines are synthesized on the Ribose ring

The purine ring is built on a ribose-5-P foundation

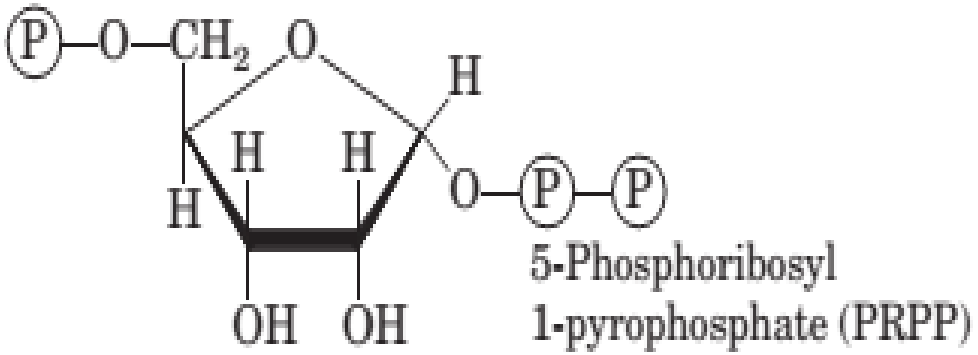
- **ribose-5-P must be activated → PRPP**
- **PRPP is the limiting substance for purine synthesis**
- **PRPP is a branch point so next step is the committed step- Gln PRPP amidotransferase （酰胺转移酶）**
- **Note that this step changes C-1 configuration (α→β)**

Many Steps Require an Activated Ribose Sugar (PRPP)



Formation of Phosphoribosylamine

磷酸-核糖胺



glutamine-PRPP amidotransferase

酰胺转移酶

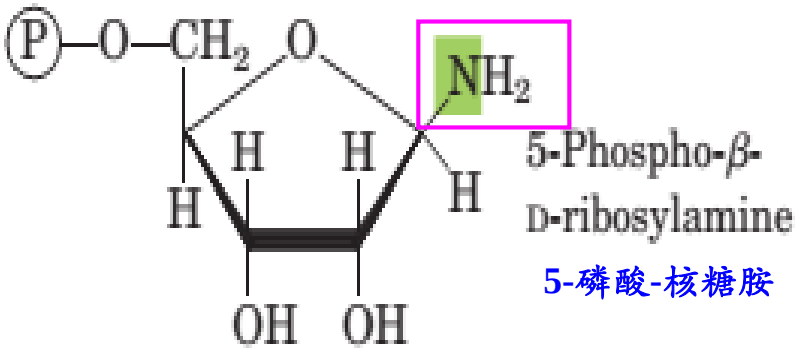
Glutamine

Glutamate

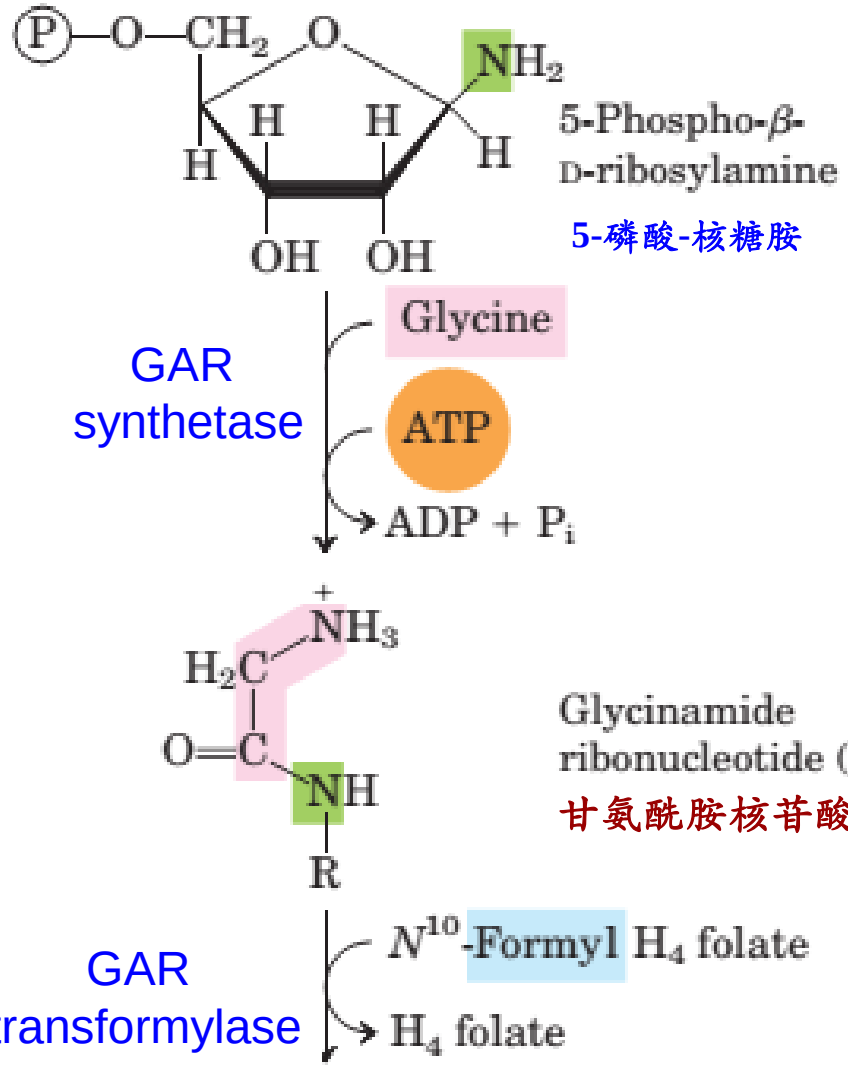
PP_i

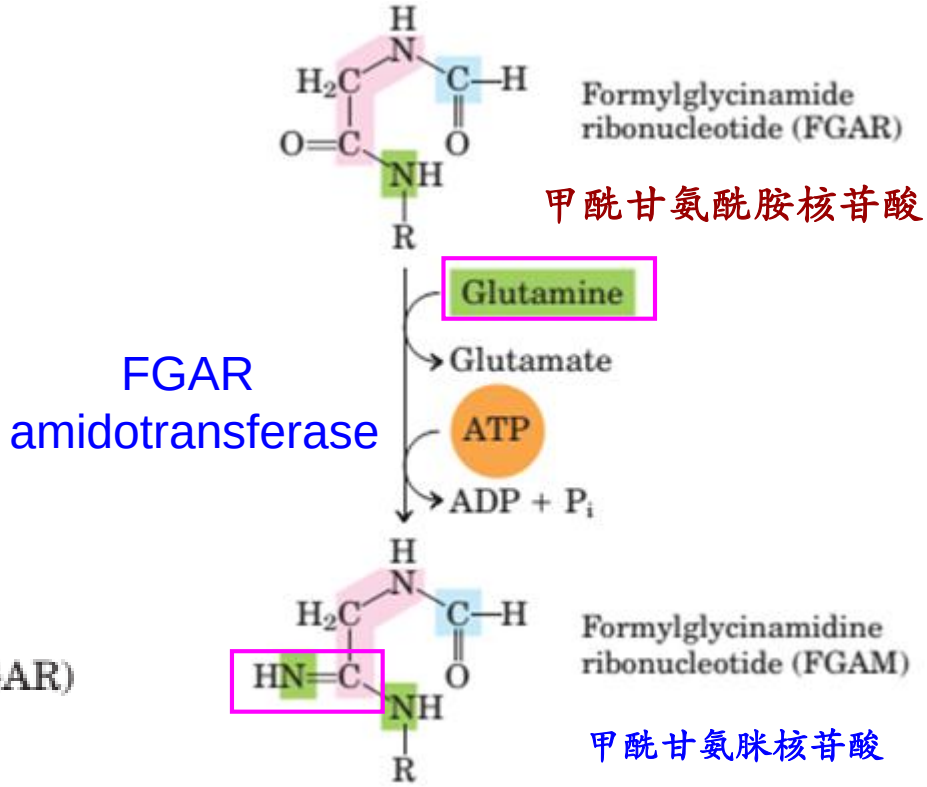
Azaserine（重氮丝氨酸）-

Gln analog- inhibitor/antitumor

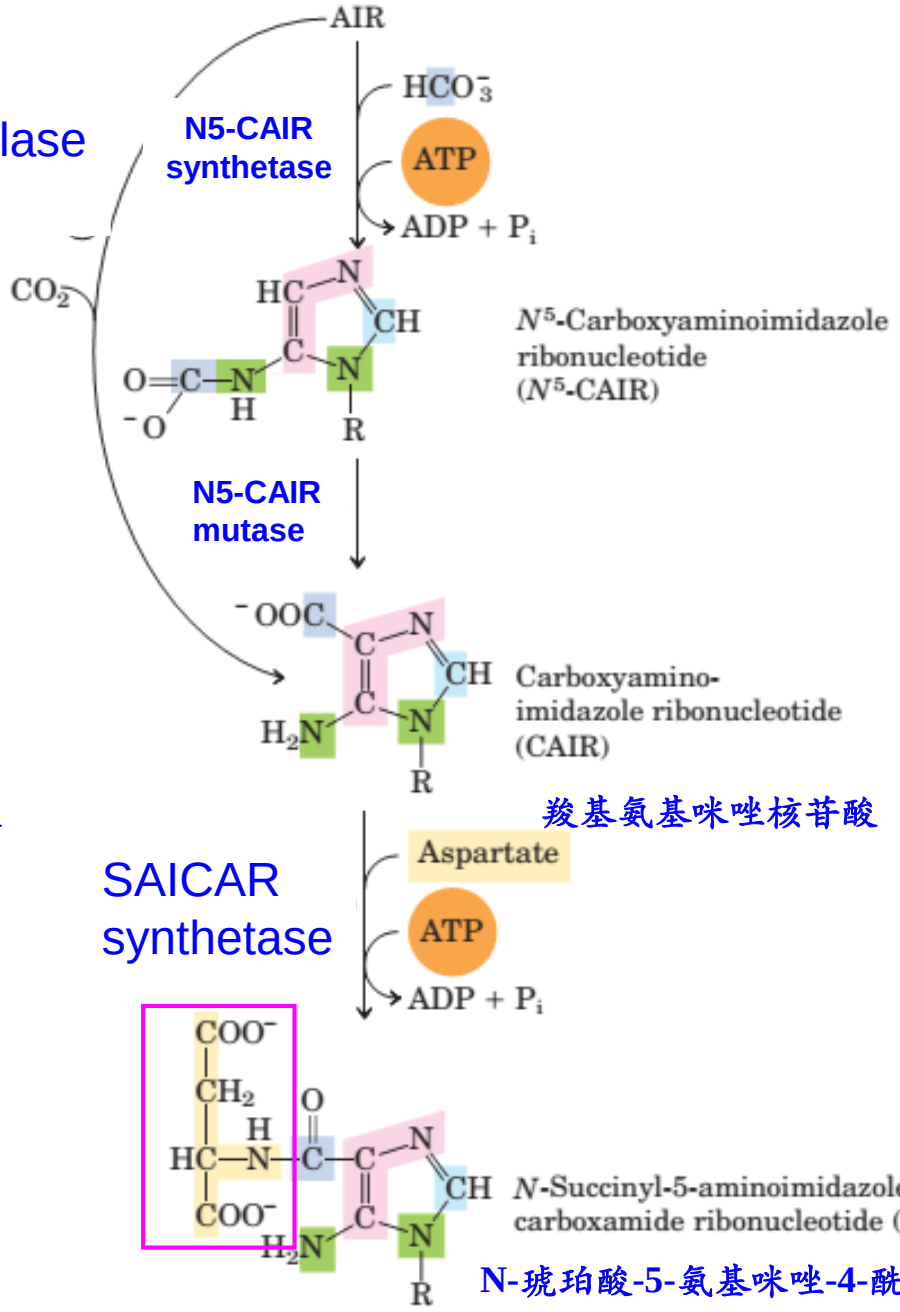


5-磷酸-核糖胺

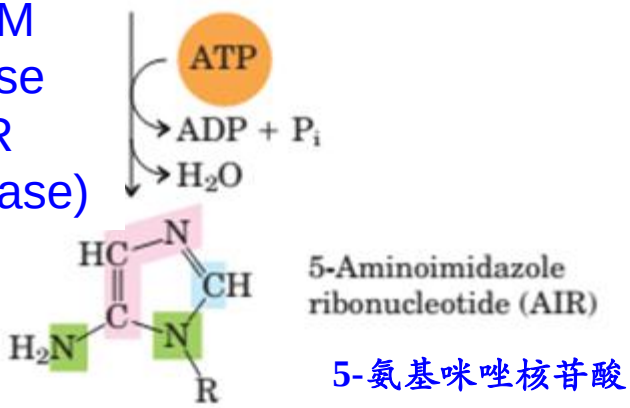

5-Phospho-β-D-ribosylamine
5-磷酸-核糖胺
GAR synthetase
Glycine
ATP
ADP + Pi
Glycinamide ribonucleotide (GAR)
甘氨酰胺核苷酸
GAR transformylase
N10-Formyl H4 folate
H4 folate

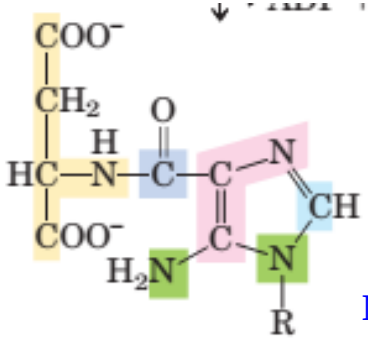

Formylglycinamide ribonucleotide (FGAR)
甲酰甘氨酰胺核苷酸
FGAR amidotransferase
Glutamine
Glutamate
ATP
ADP + Pi
Formylglycinamidine ribonucleotide (FGAM)
甲酰甘氨脒核苷酸

AIR carboxylase
In mammal



FGAM cyclase (AIR synthetase)



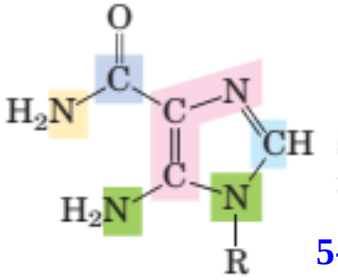


N-Succinyl-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR)

N-琥珀酸-5-氨基咪唑-4-酰胺核苷酸

SAICAR lyase

Fumarate



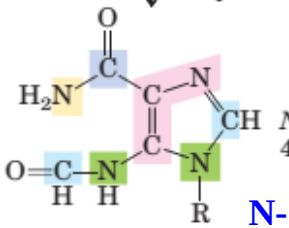
5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)

5-氨基咪唑-4-酰胺核苷酸

AICAR transformylase

N^{10}-Formyl H_4 folate

H_4 folate

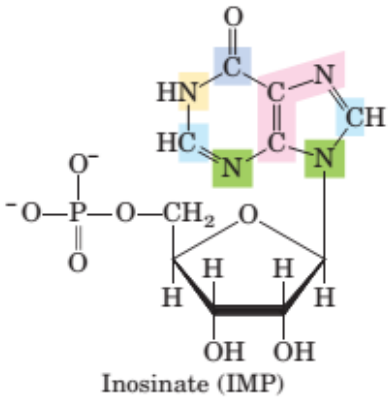


N-Formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)

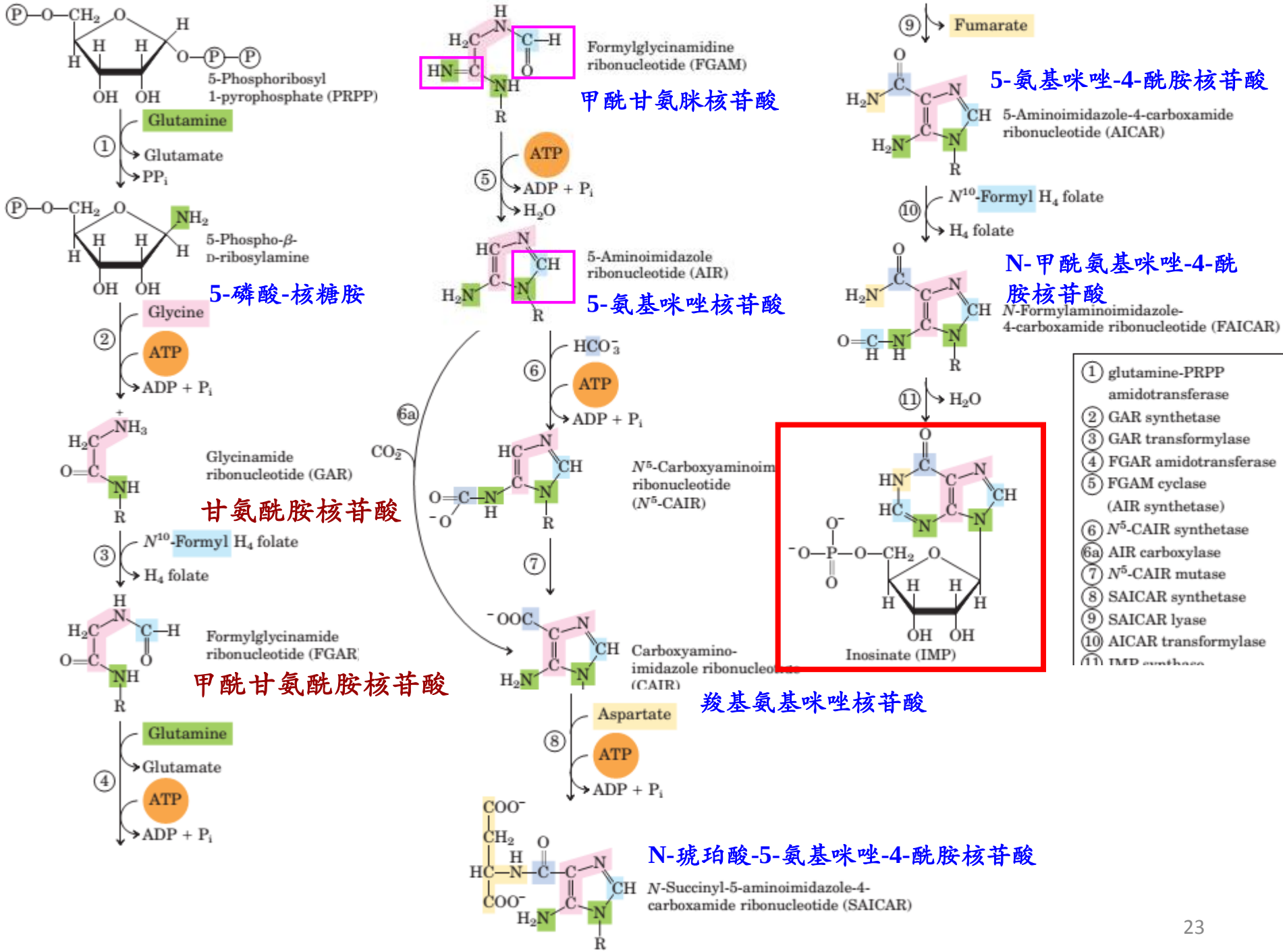
N-甲酰氨基咪唑-4-酰胺核苷酸

IMP synthase

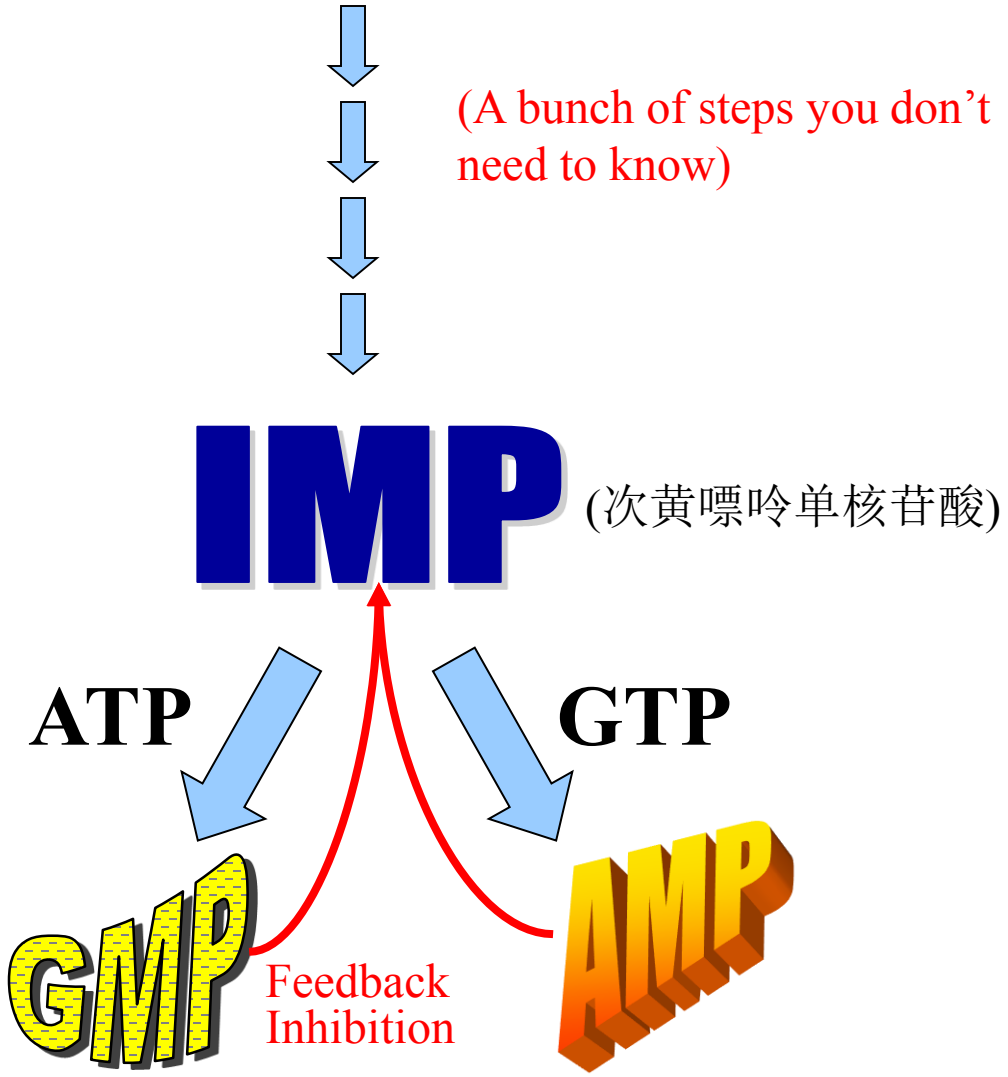
H_2O



Inosinate (IMP)


5-Phosphoribosyl 1-pyrophosphate (PRPP)
Glutamine
Glutamate
PPi
5-Phospho-β-D-ribosylamine
5-磷酸-核糖胺
Glycine
ATP
ADP + Pi
Glycinamide ribonucleotide (GAR)
甘氨酰胺核苷酸
N10-Formyl H4 folate
H4 folate
Formylglycinamide ribonucleotide (FGAR)
甲酰甘氨酰胺核苷酸
Glutamine
Glutamate
ATP
ADP + Pi
Formylglycinamidine ribonucleotide (FGAM)
甲酰甘氨脒核苷酸
ATP
ADP + Pi
H2O
5-Aminoimidazole ribonucleotide (AIR)
5-氨基咪唑核苷酸
HCO3−
ATP
ADP + Pi
6a
CO2
N5-Carboxyaminoimidazole ribonucleotide (N5-CAIR)
Carboxyamino-imidazole ribonucleotide (CAIR)
羧基氨基咪唑核苷酸
Aspartate
ATP
ADP + Pi
N-琥珀酸-5-氨基咪唑-4-酰胺核苷酸
N-Succinyl-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR)
Fumarate
5-氨基咪唑-4-酰胺核苷酸
5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)
N10-Formyl H4 folate
H4 folate
N-甲酰氨基咪唑-4-酰胺核苷酸
N-Formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)
H2O
Inosinate (IMP)
1 glutamine-PRPP amidotransferase
2 GAR synthetase
3 GAR transformylase
4 FGAR amidotransferase
5 FGAM cyclase (AIR synthetase)
6 N5-CAIR synthetase
6a AIR carboxylase
7 N5-CAIR mutase
8 SAICAR synthetase
9 SAICAR lyase
10 AICAR transformylase
11 IMP synthase

Purine Biosynthesis (*de novo*)



The synthesis of AMP and GMP from IMP

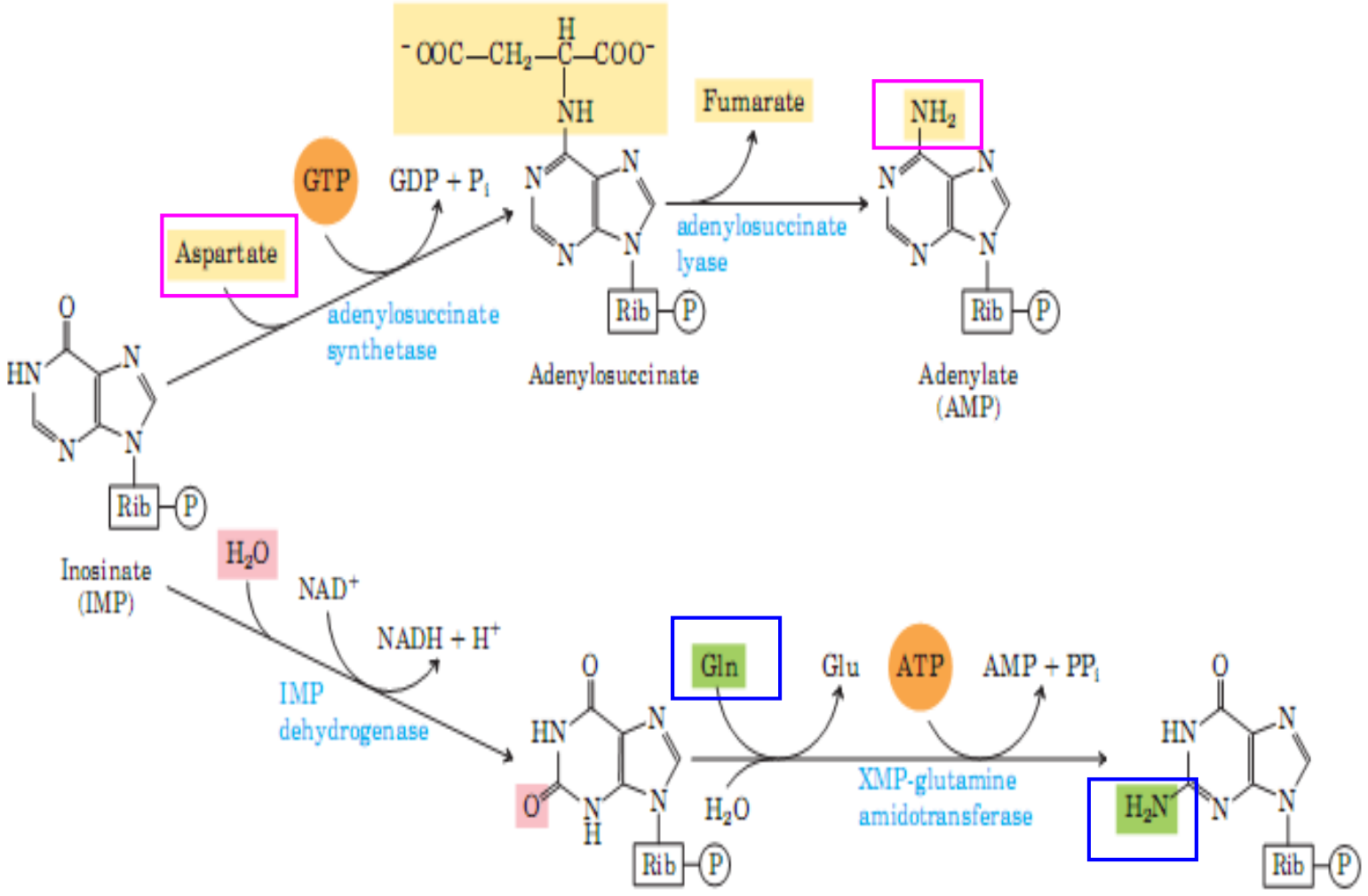


FIGURE 22-34 Biosynthesis of AMP and GMP from IMP.

purine synthesis（Salvage Pathway）

1. **Hypoxanthine**（次黄嘌呤）and **guanine** recombine with **PRPP** to form nucleotides in the **HGPRT** (Hypoxanthine-Guanine-Phospho-Ribosyl-Transferase) reaction

2. **Adenine** + **PRPP** → **AMP**

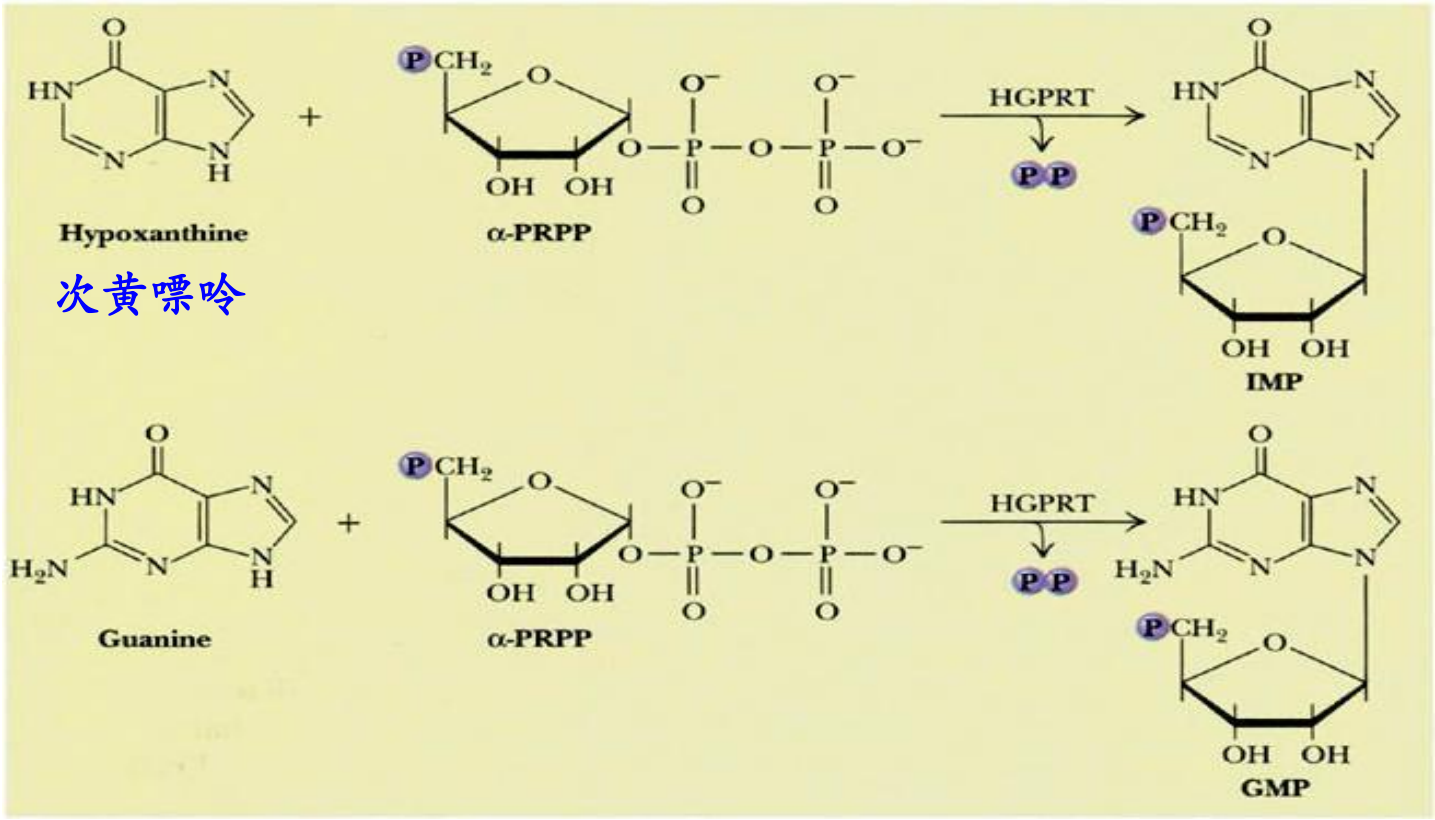
APRTase

（腺嘌呤磷酸核糖转移酶）

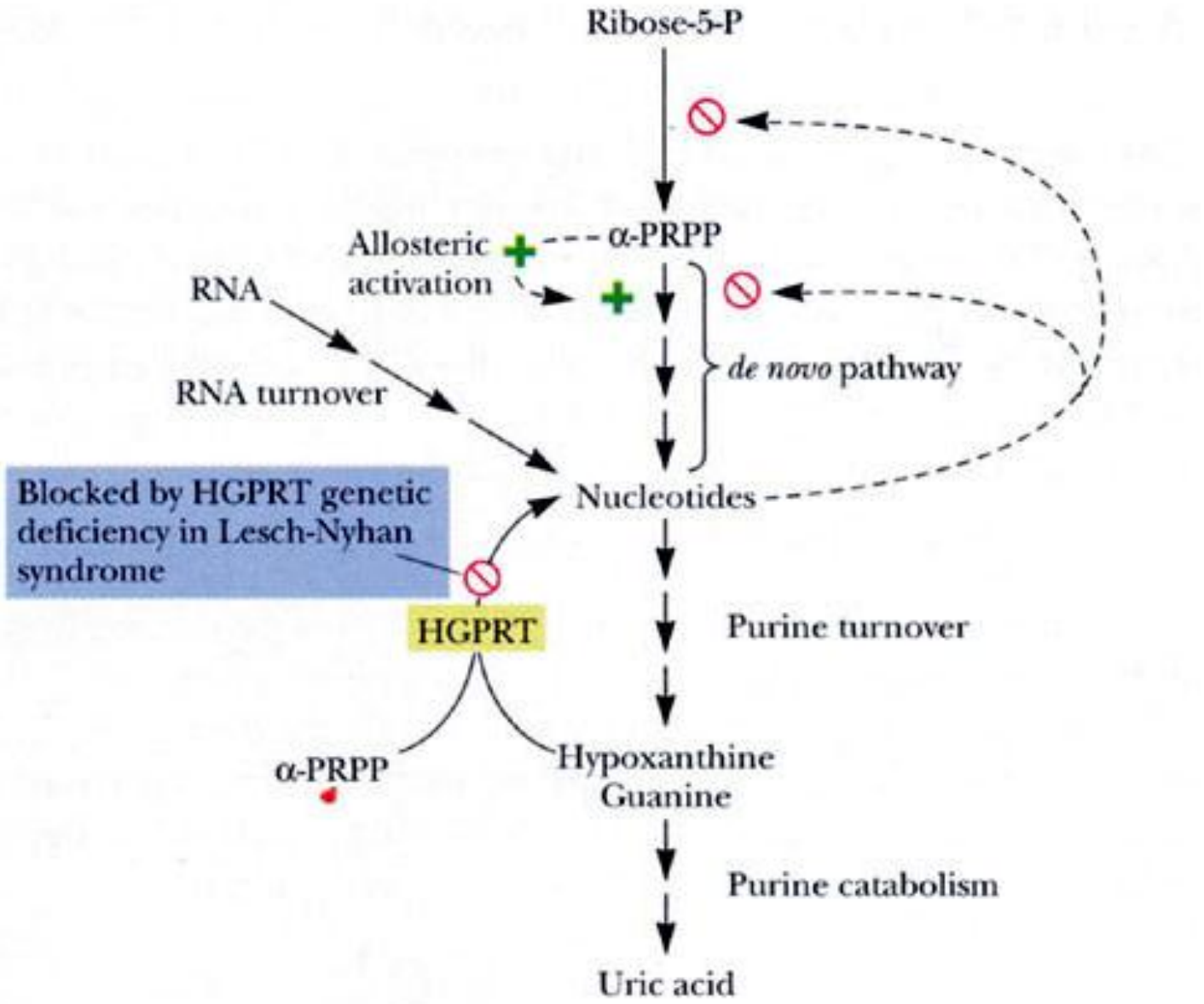
purine synthesis （Salvage Pathway）

1. $$\text{Adenin} + \text{PRPP} \xrightleftharpoons{\text{APRT}} \text{AMP} + PP_i$$

APRT = Adenin-Phosphoribosyl Transferase

2. 

Lack of HGPRT to cause self-destruction face disease



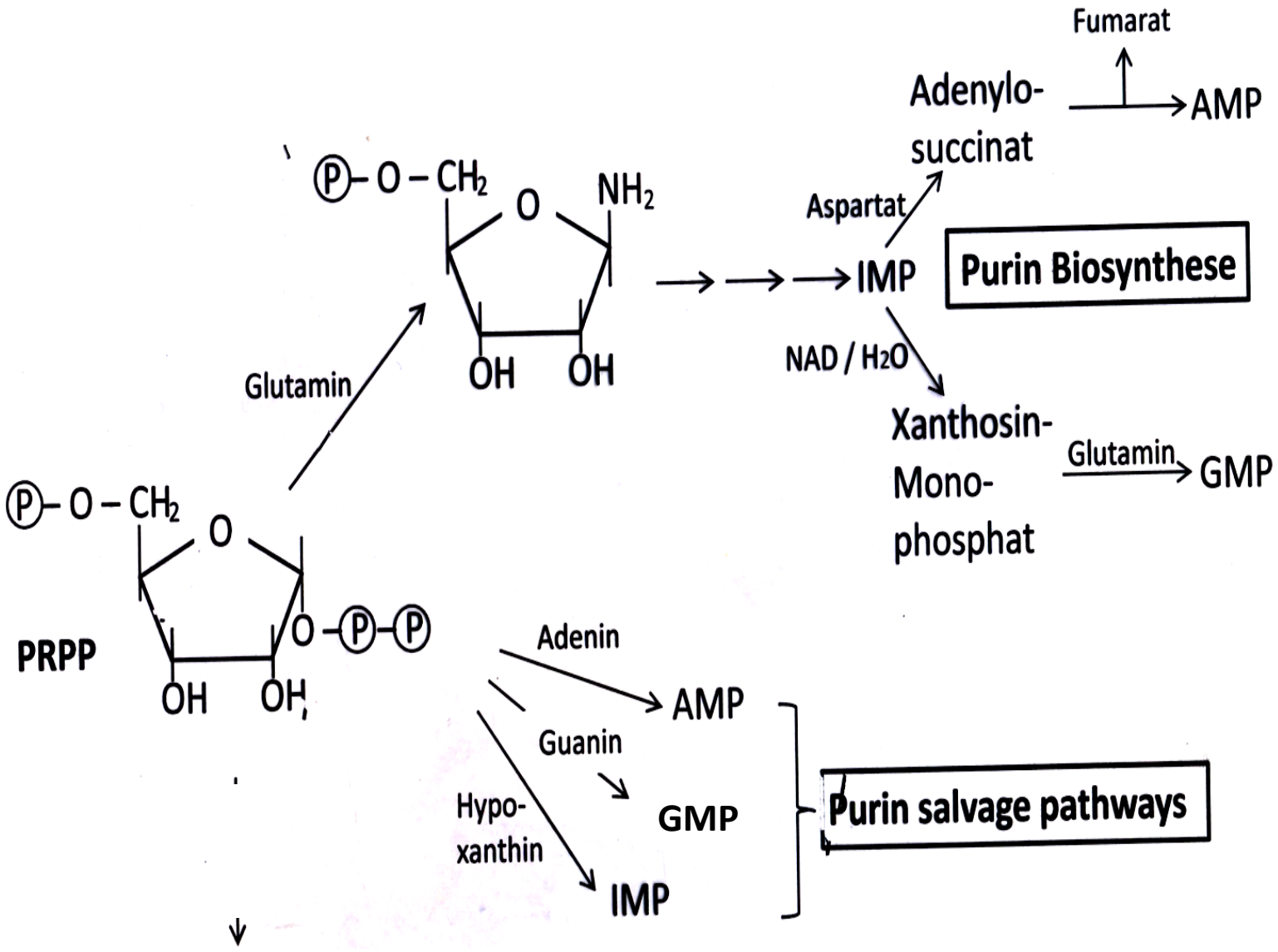
Lesch-Nyhan Syndrome

- ☹ Absence of **HGPRTase**
- ☹ X-linked - boys
- ☹ Characterized by:
  - Increased uric acid
  - Spasticity （痉挛）
  - Neurological defects
  - Aggressive behavior
  - Self-mutilation（自残）

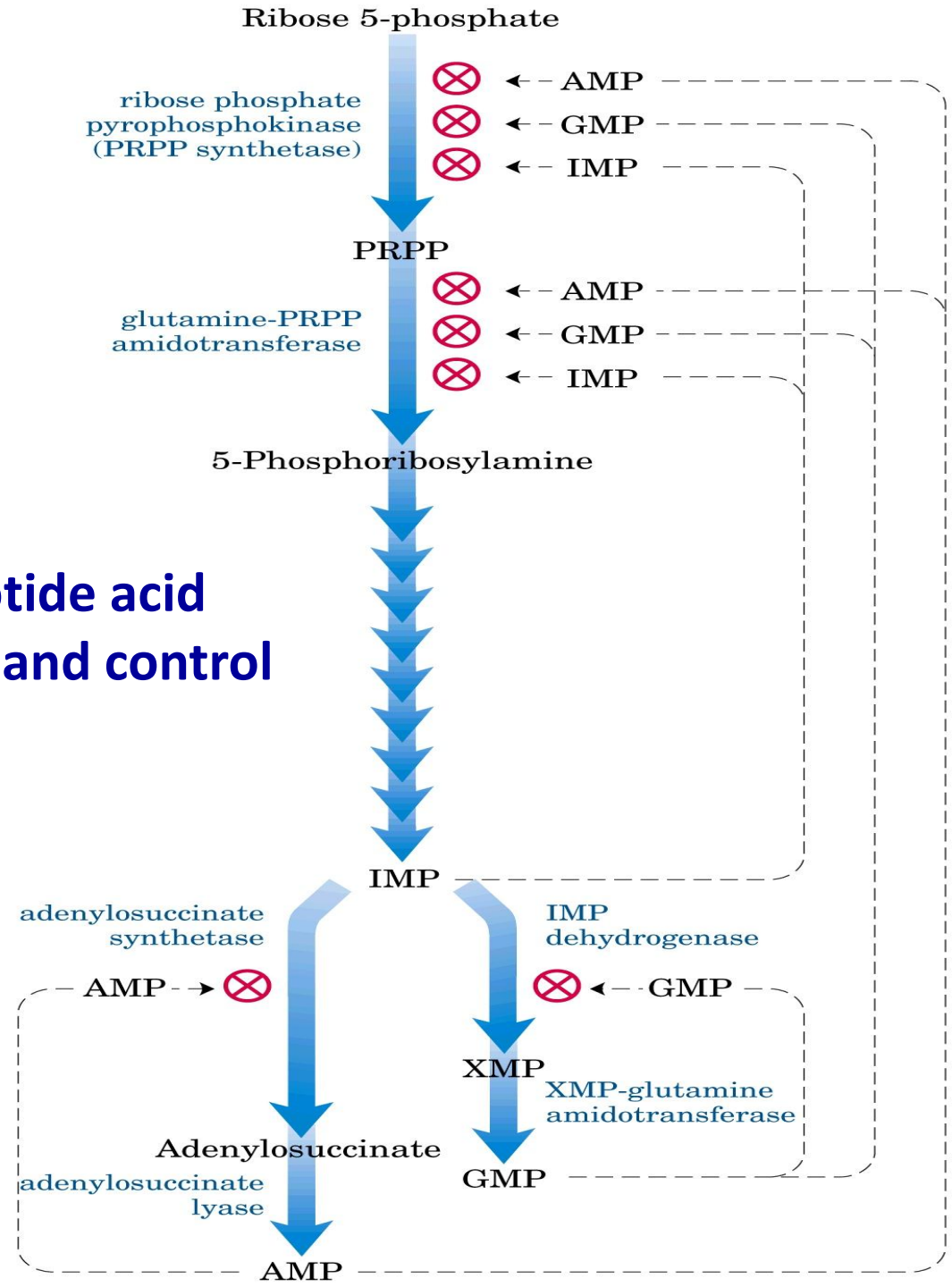
自咬嘴唇，手指面致残。痉挛，手足舞蹈样徐动，生长发育迟缓，智力低下。

自毁容貌综合征

purine nucleotide biosynthesis



Purine ribonucleotide acid synthesis regulation and control



Pyrimidines synthesis （*de novo*）

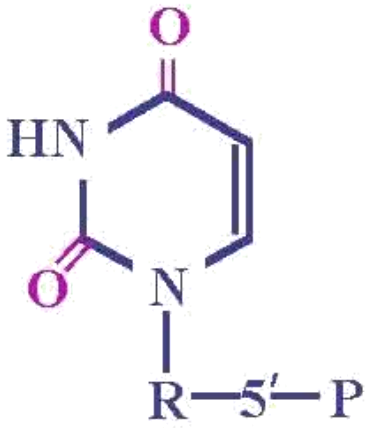
★ Synthesized from:

- **Gln**
- **CO_2**
- **Asp**
- **Requires ATP**

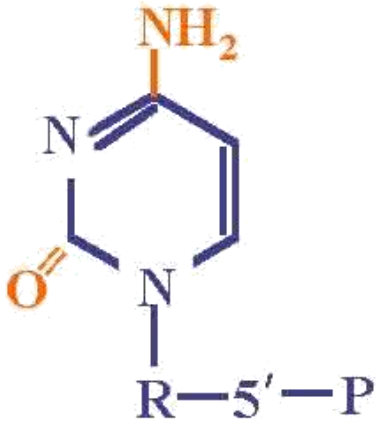
★ Pyrimidine rings are synthesized **independent** of the ribose and transferred to the PRPP (ribose)

★ Generated as **UMP**

Pyrimidine Biosynthesis （*de novo*）



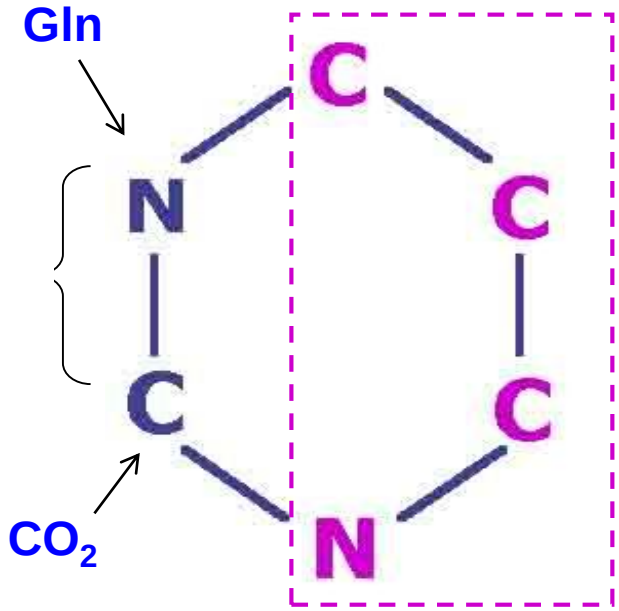
UMP



CMP

Carbamoyl phosphate

氨甲酰-P

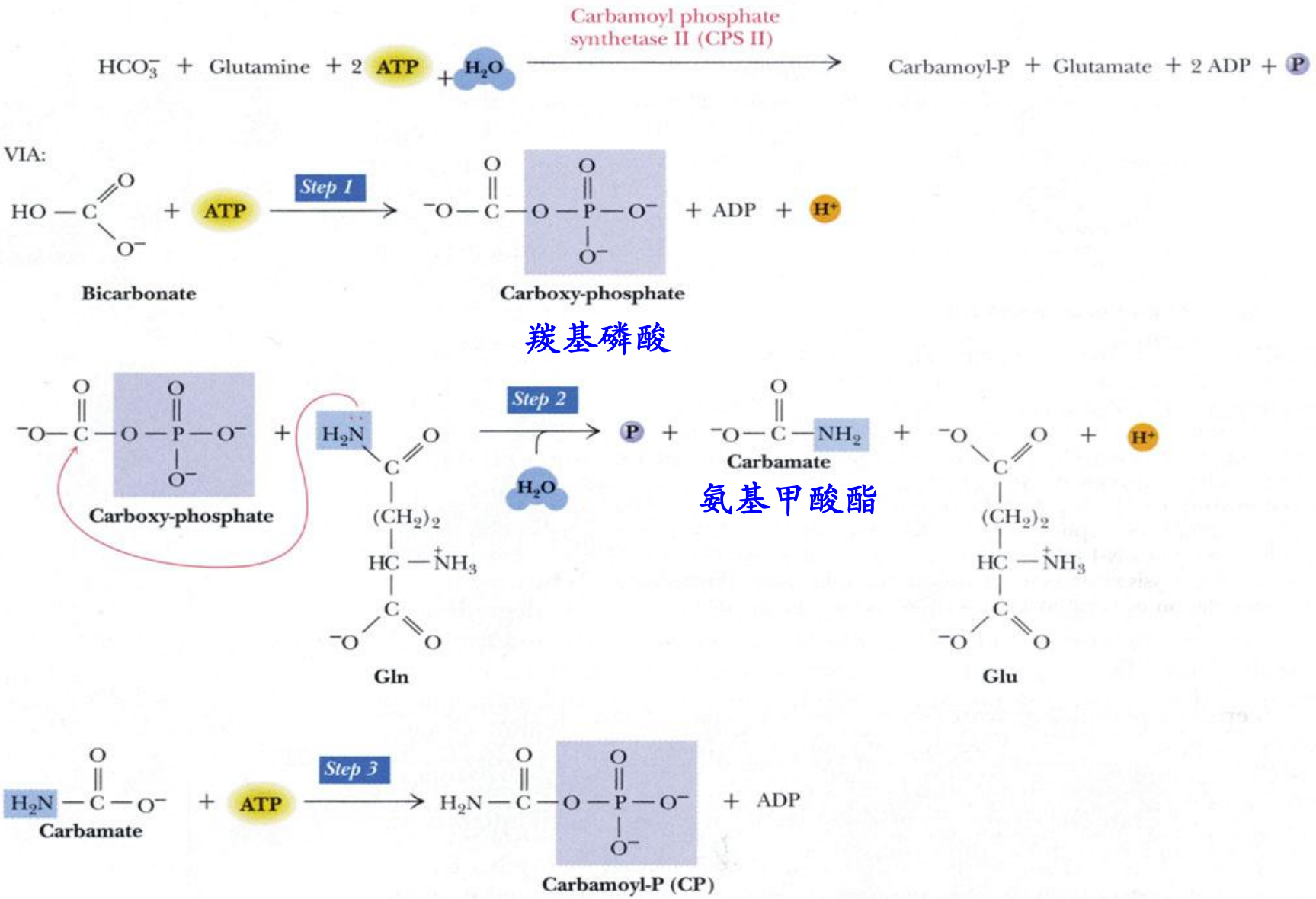


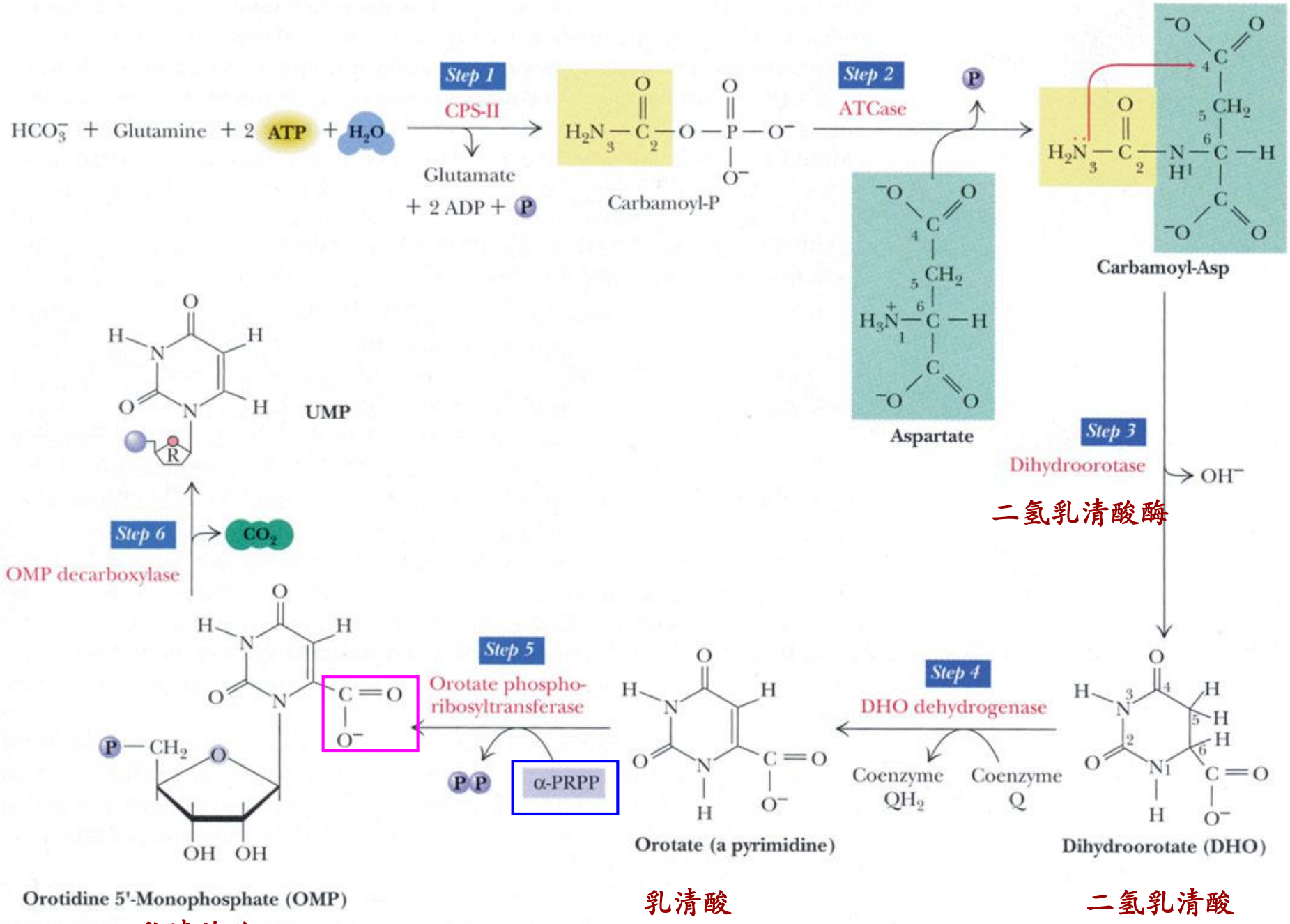
Asp

Pyrimidine Biosynthesis （*de novo*）

- **Step 1:** Carbamoyl phosphate for pyrimidine synthesis is made by 氨甲酰-P
  carbamoyl phosphate synthetase II (CPSII)
  - In **CP**
  - **HCO_3^-, Gln, 2ATP**
  - carbamoyl phosphate represents an 'activated' carbamoyl group

The carbamoyl phosphate synthetase II (CPS-II) reaction




Step 1
CPS-II
Glutamate
+ 2 ADP + P
Carbamoyl-P
Step 2
ATCase
Aspartate
Carbamoyl-Asp
Step 3
Dihydroorotase
OH⁻
二氫乳清酸酶
Dihydroorotate (DHO)
Step 4
DHO dehydrogenase
Coenzyme QH₂
Coenzyme Q
Orotate (a pyrimidine)
Step 5
Orotate phospho-ribosyltransferase
α-PRPP
Orotidine 5'-Monophosphate (OMP)
Step 6
OMP decarboxylase
CO₂
UMP
乳清酸
乳清苷酸
二氫乳清酸

Hereditary Orotic Aciduria

乳清酸尿

☹ Loss of UMP related synthetase

- Defect in ***de novo*** synthesis of **pyrimidines**

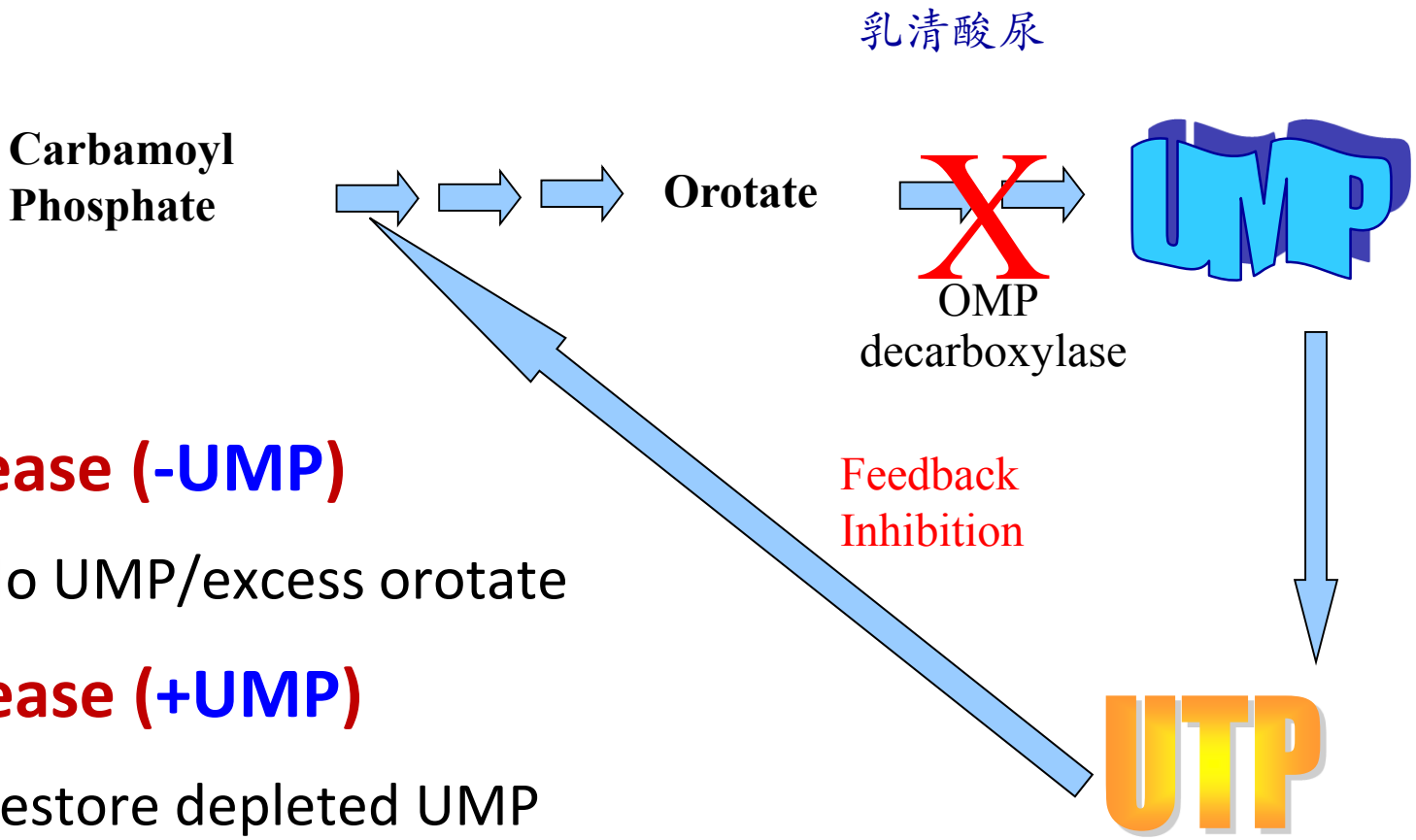
- Characterized by excretion of **orotic acid**

- Severe anemia（贫血）and growth retardation

☹ Extremely rare (15 cases worldwide)

☹ Treated by **feeding UMP**

Why does UMP Cure Orotic Aciduria?



☹ **Disease (-UMP)**

- No UMP/excess orotate

☺**Disease (+UMP)**

- Restore depleted UMP
- UTP feedback inhibition (Less orotate)

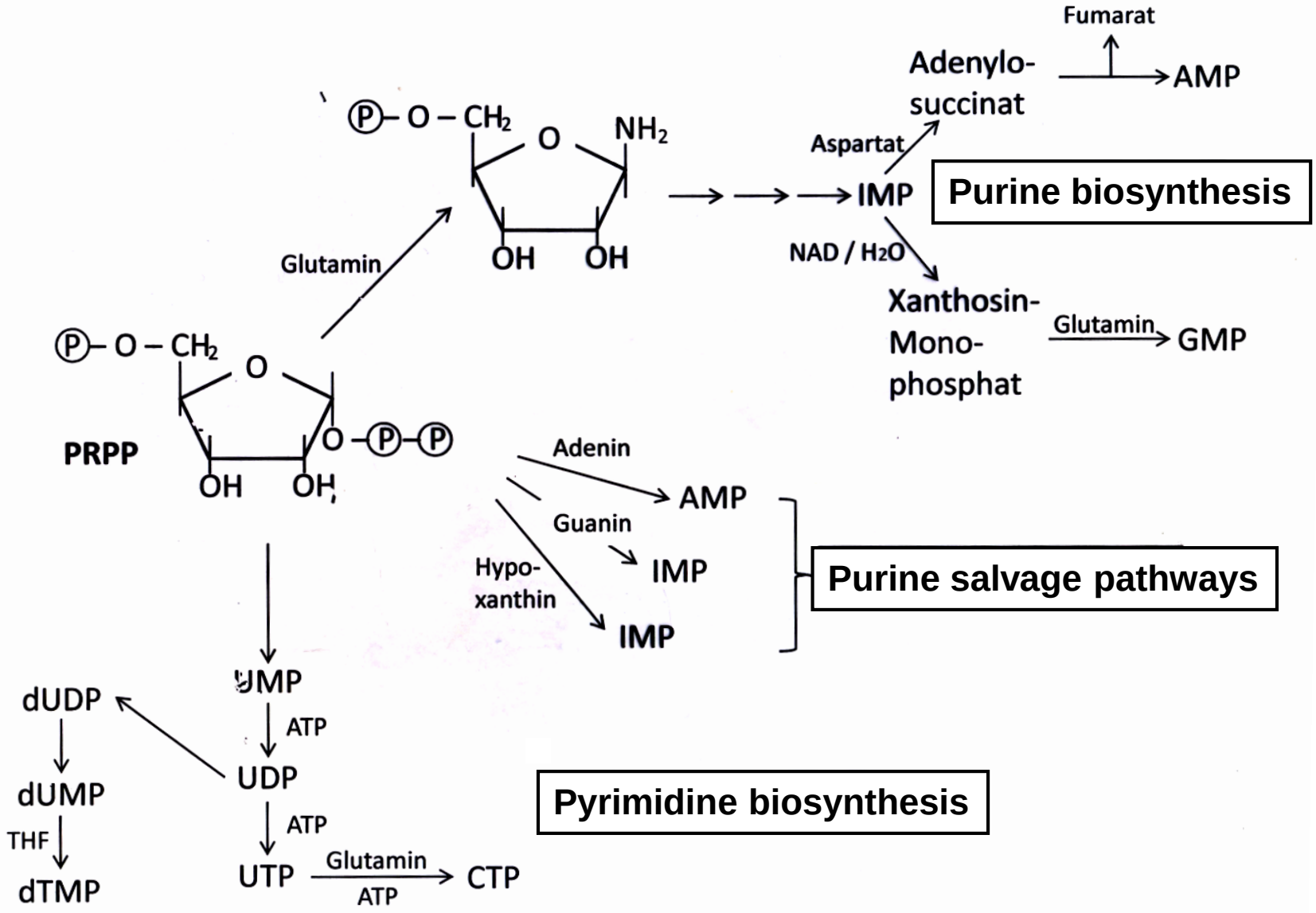
Pyrimidine Salvage

- ★ Can be salvaged by reactions with PRPP - **Pyrimidine phosphoribosyltransferase**
- ★ Nucleoside kinase

Biosynthesis: Purine vs Pyrimidine

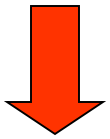
Purine	Pyrimidine
1. Synthesized **on PRPP**	1. Synthesized **then** added to **PRPP**
2. Regulated by GTP/ATP	2. Regulated by UTP
3. Generates **IMP**	3. Generates **UMP**
4. Requires Energy	4. Requires Energy

Overview of nucleotide biosynthesis



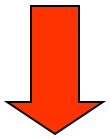
Beyond AMP, GMP and UMP

Purine Biosynthesis



AMP GMP

Pyrimidine Biosynthesis



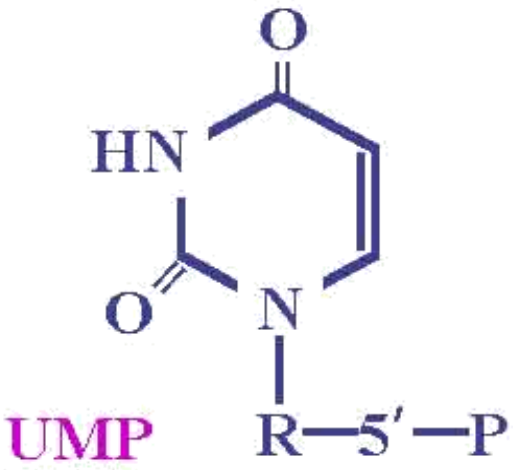
UMP

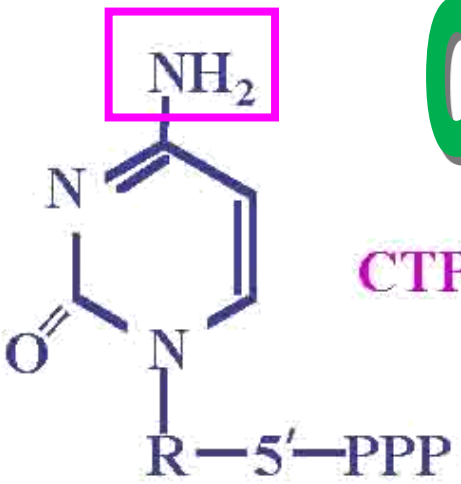
But other forms of these nucleotides are needed

Synthesis of UTP/CTP

UMP → (UMP kinase, ATP) → UDP → (UDP kinase, ATP) → UTP

UTP → (CTP synthetase, Gln + ATP) → CTP





CTP

Synthesis of ATP/GTP

- **ATP** serves as the phosphoryl donor for synthesis of the other nucleoside triphosphates

AMP kinase

Adenylate kinase: AMP + ATP → 2 ADP

- **ADP → ATP** by **oxidative phosphorylation**

- **GDP and GTP**

GMP kinase

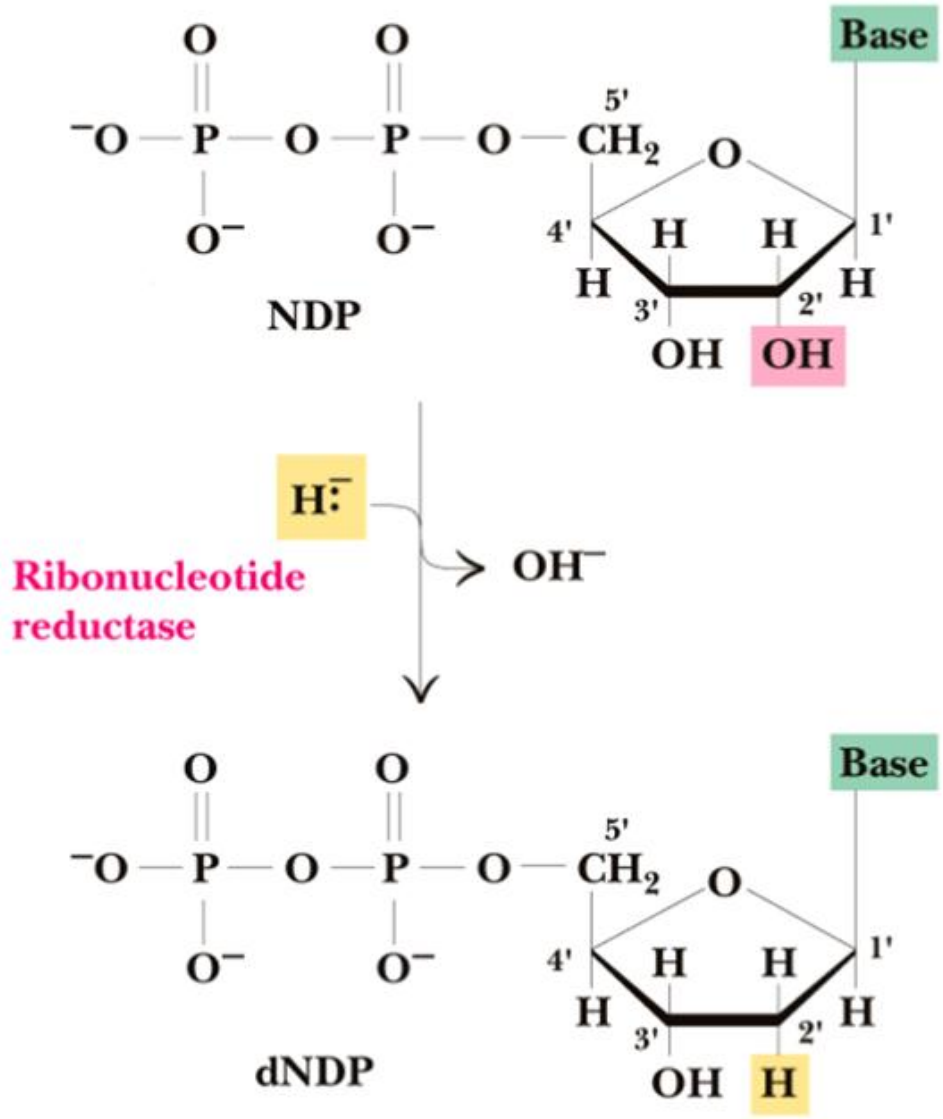
Guanylate kinase: GMP + ATP → GDP + ADP

GDP kinase

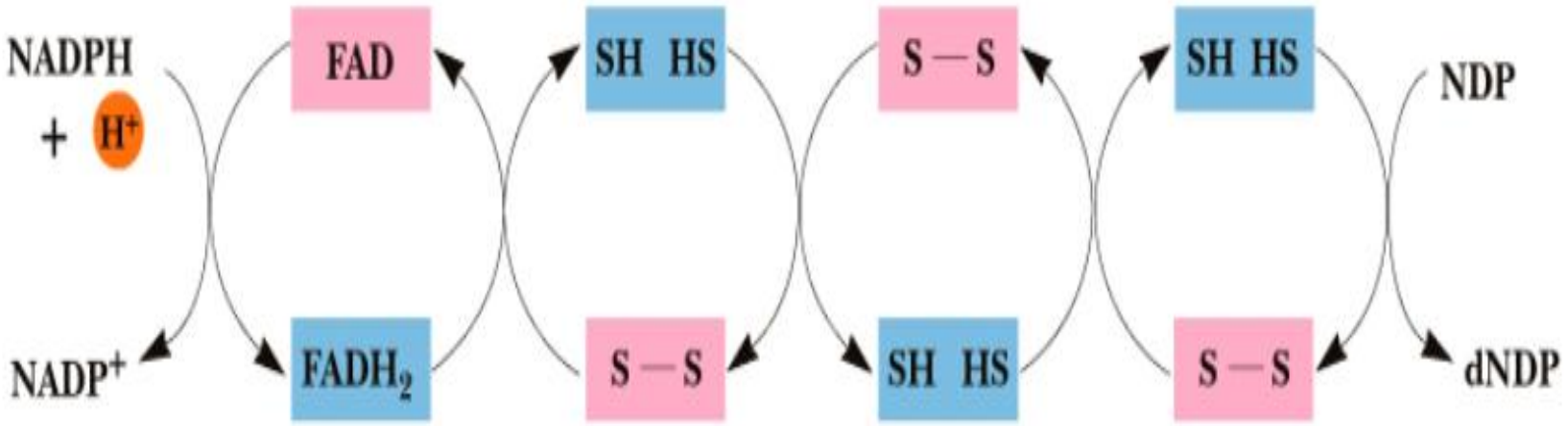
Nucleoside diphosphate kinase: GDP + ATP → GTP + ADP

Conversion of Ribonucleotides to Deoxyribonucleotides

- Reduction at **2'-OH** by **ribonucleotide reductase** (**Fe** cofactor)

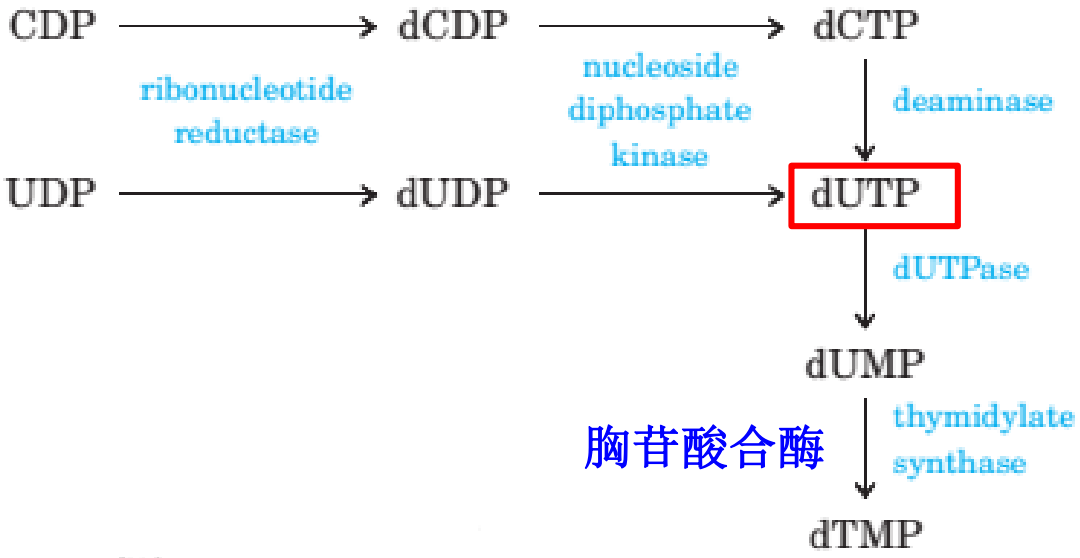


The (-S-S-)/(-SH HS-) oxidation-reduction cycle involving ribonucleotide reductase, thioredoxin, thioredoxin reductase, and NADPH



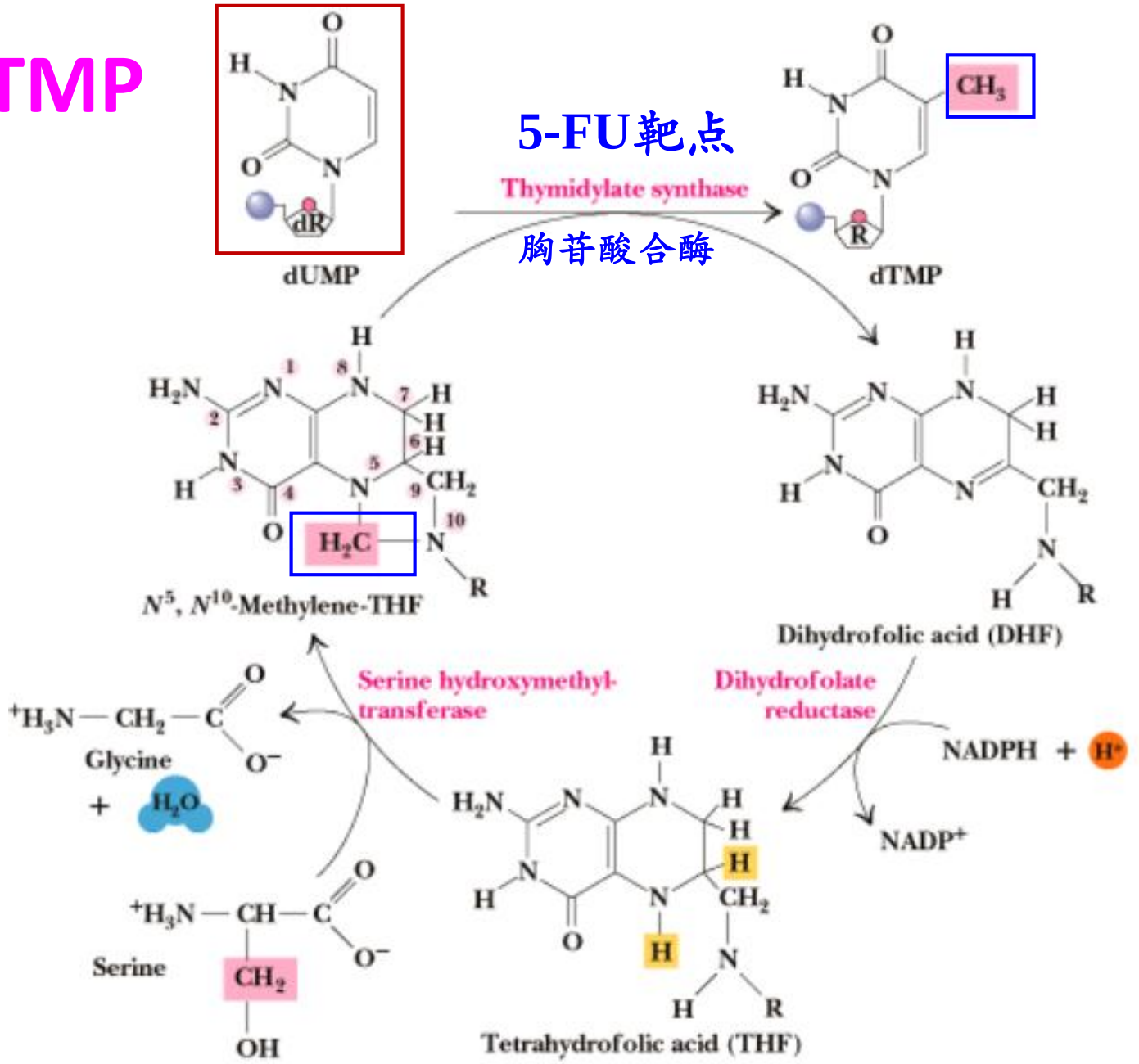
Synthesis of Thymine Nucleotides

- **dTMP** are made from **dUMP** （can from dUDP, dCDP）



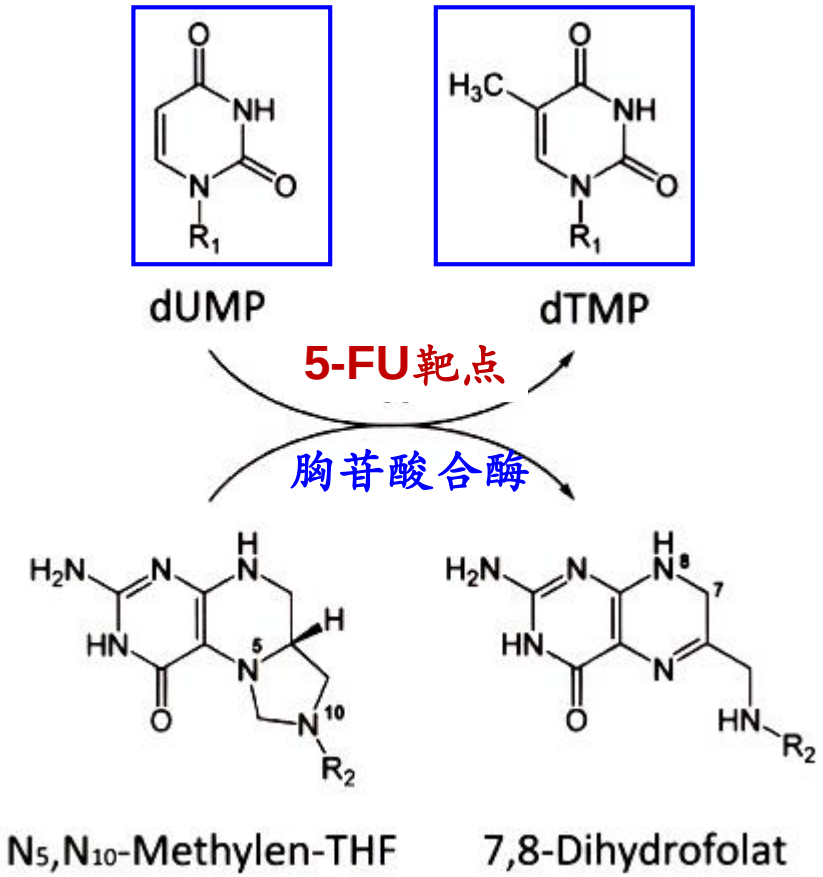
- **Thymidylate synthase**（胸苷酸合酶） methylates **dUMP** at 5-position to make **dTMP**
- **N^5,N^{10}-CH_2-FH_4** is 1-C donor

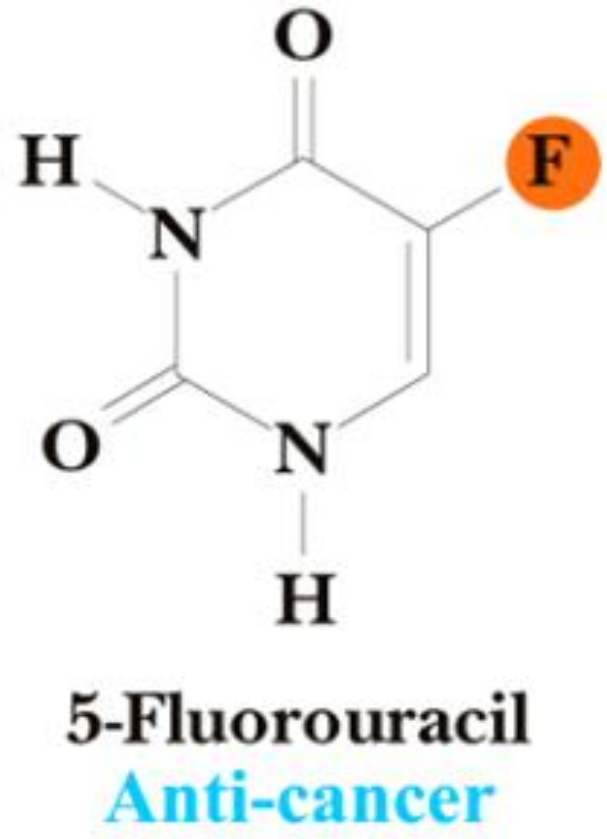
dUMP→ dTMP


5-FU靶点
Thymidylate synthase
胸苷酸合酶
dUMP
dTMP
N^5, N^{10}-Methylene-THF
Dihydrofolic acid (DHF)
Serine hydroxymethyl-transferase
Dihydrofolate reductase
NADPH + H+
NADP+
Glycine
Serine
Tetrahydrofolic acid (THF)

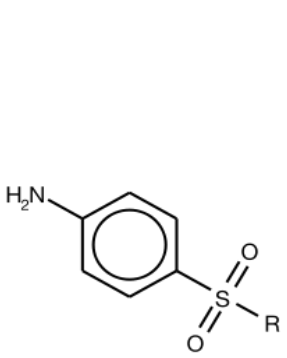
5-FU in cancer chemotherapy

- **Thymidylate synthase** leads to **methylation** of **dUMP → dTMP**.

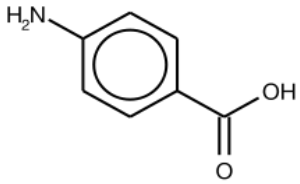




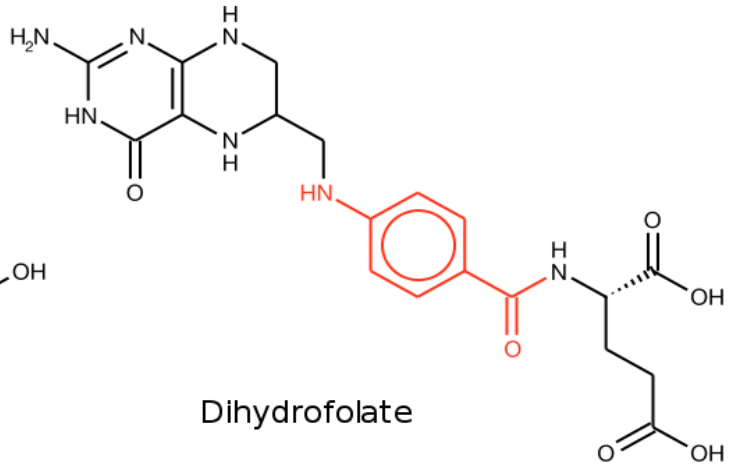
inhibitors of purine biosynthesis



Sulfanilamide

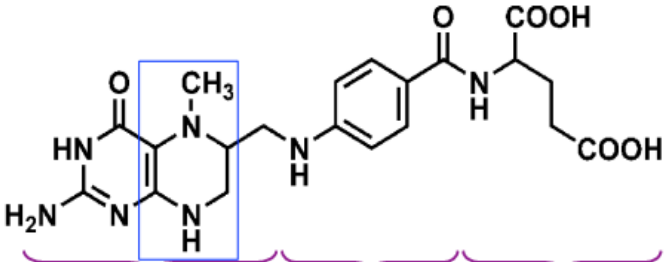


PABA



Dihydrofolate

Folates/VB9 Chemical structure



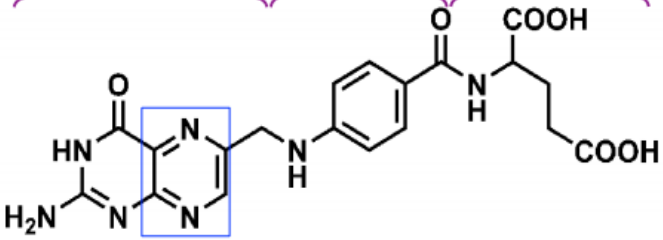
5-methyl-tetrahydrofolate
(Natural, Reduced, major form in circulation)

Pteridin ring

P-Aminobenzoic acid (PABA, 氨基苯酸)

Glutamic acid

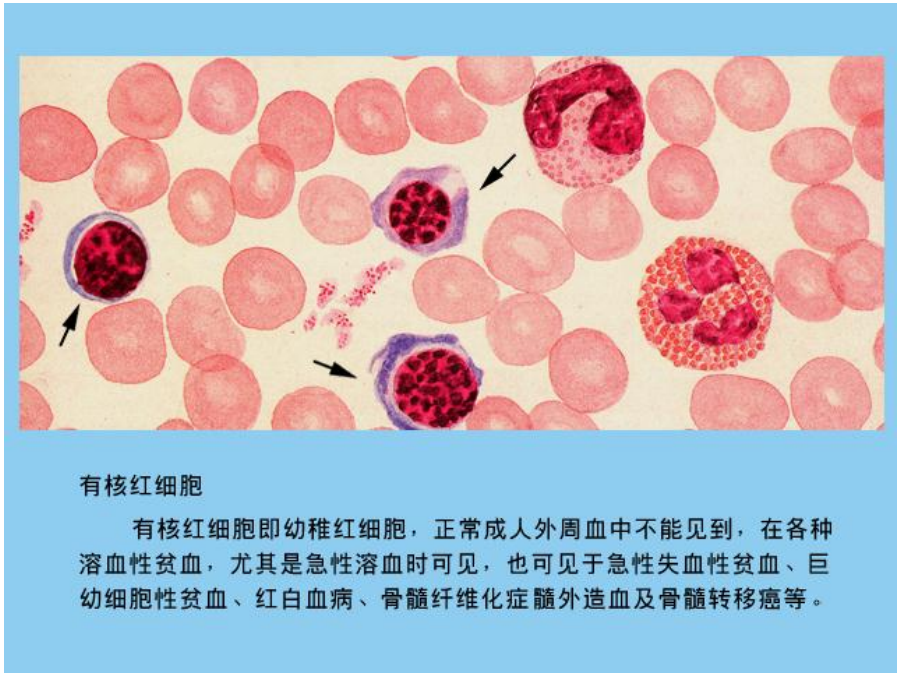
Pteroic acid (蝶酸)



Folic acid
(Synthesized, Oxidized)

Folate Deficiency in Human

- Neural tube defects – **embryos**
- Immune deficiency and neurological disorders – **infants**
- Megaloblastic anemia and diarrhea – **infants and adults**
  巨幼细胞性贫血　　痢疾
- Increased risk of cancer and cardiovascular diseases – **adult**



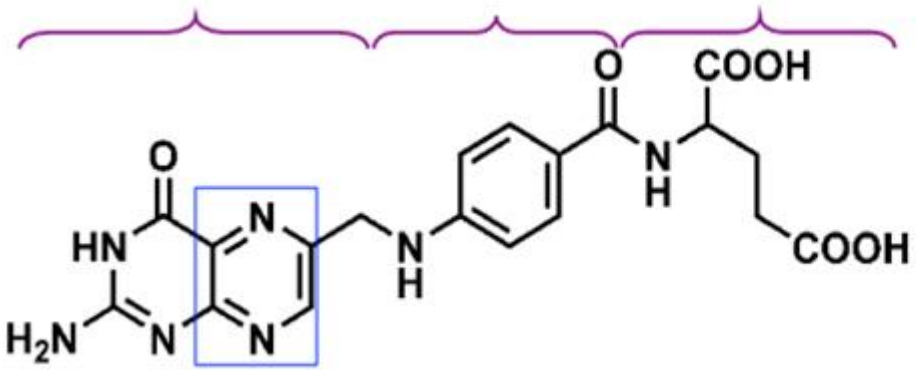
有核红细胞

有核红细胞即幼稚红细胞，正常成人外周血中不能见到，在各种溶血性贫血，尤其是急性溶血时可见，也可见于急性失血性贫血、巨幼细胞性贫血、红白血病、骨髓纤维化症髓外造血及骨髓转移癌等。

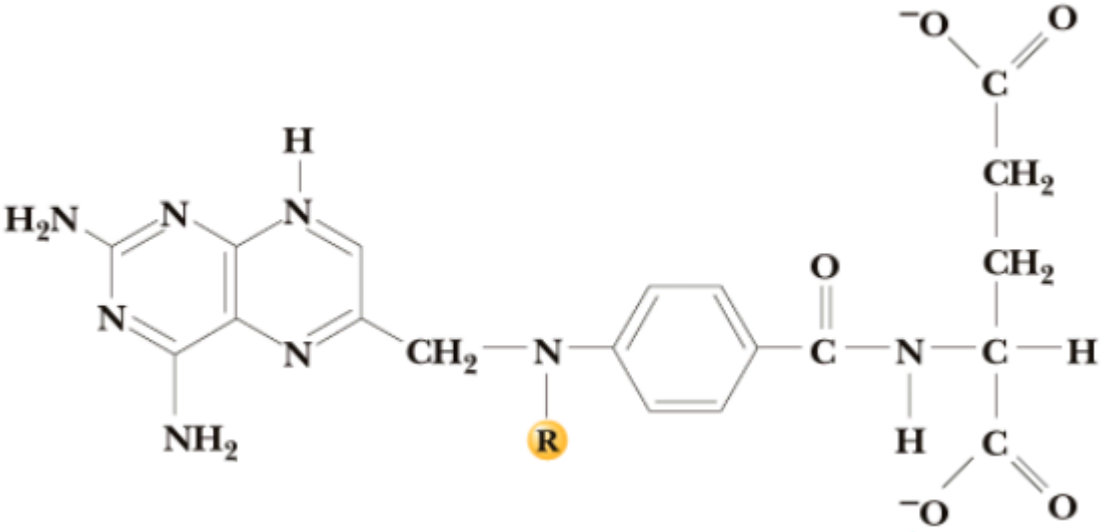
Antifolates for cancer

- In **1940s**, no specific treatment for children acute leukemia
- In **1945**, **aminopterin** (4-amino folic acid, 氨蝶呤) was synthesized
- In 1948, Children's Hospital in Boston – **aminopterin** in children acute leukemia
- In 1949, **methotrexate** (甲氨蝶呤) was clinically introduced to replace aminopterin due to lesser toxicity （**2nd generation**）
- In 2004, **pemetrexed** (培美曲塞) was approved for **malignant pleural mesothelioma** in combination with cisplatin by FDA （**3rd generation**）

Anti-folates



Folic acid
(Synthesized, Oxidized)

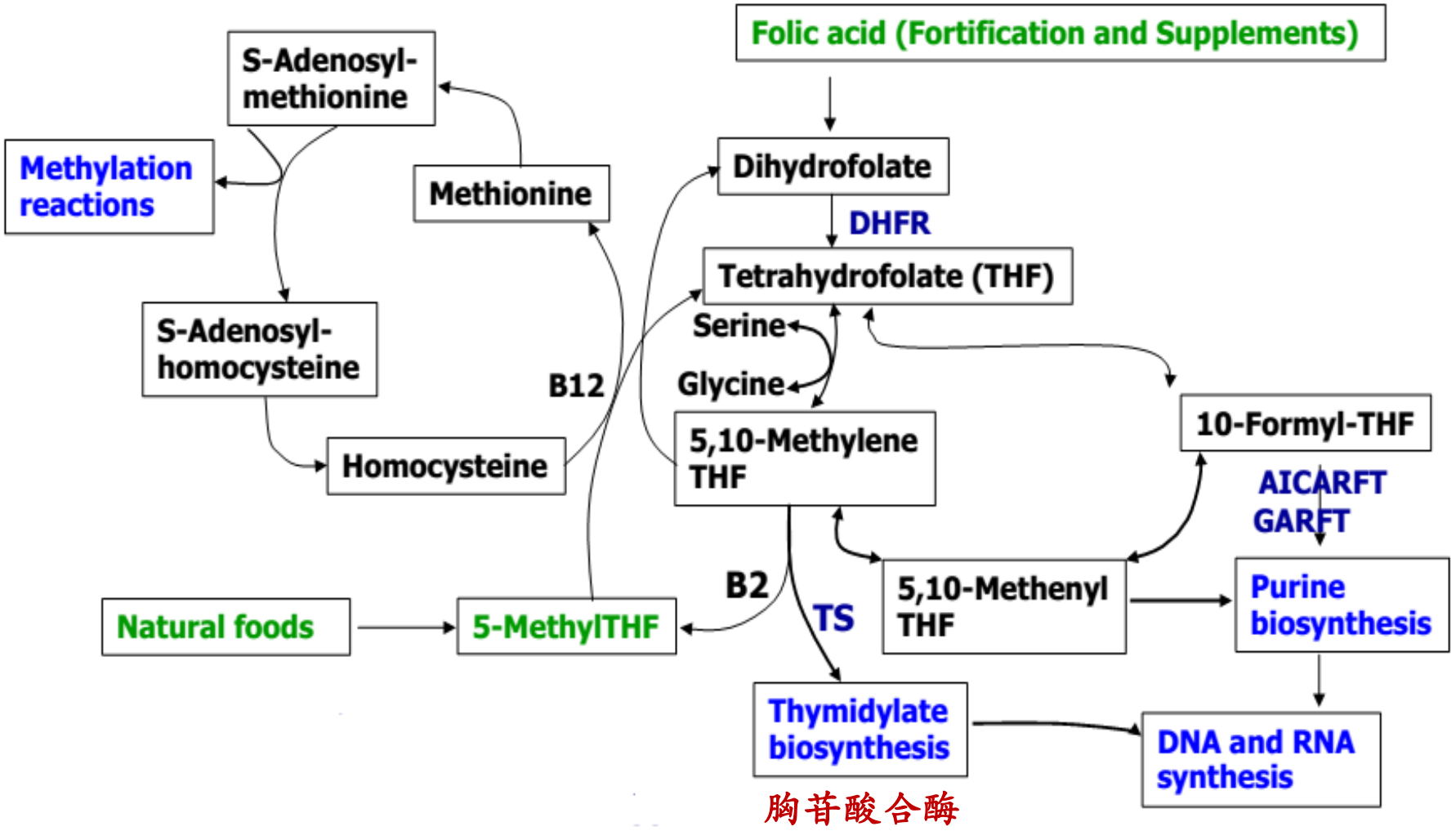


2-Amino, 4-amino analogs of folic acid

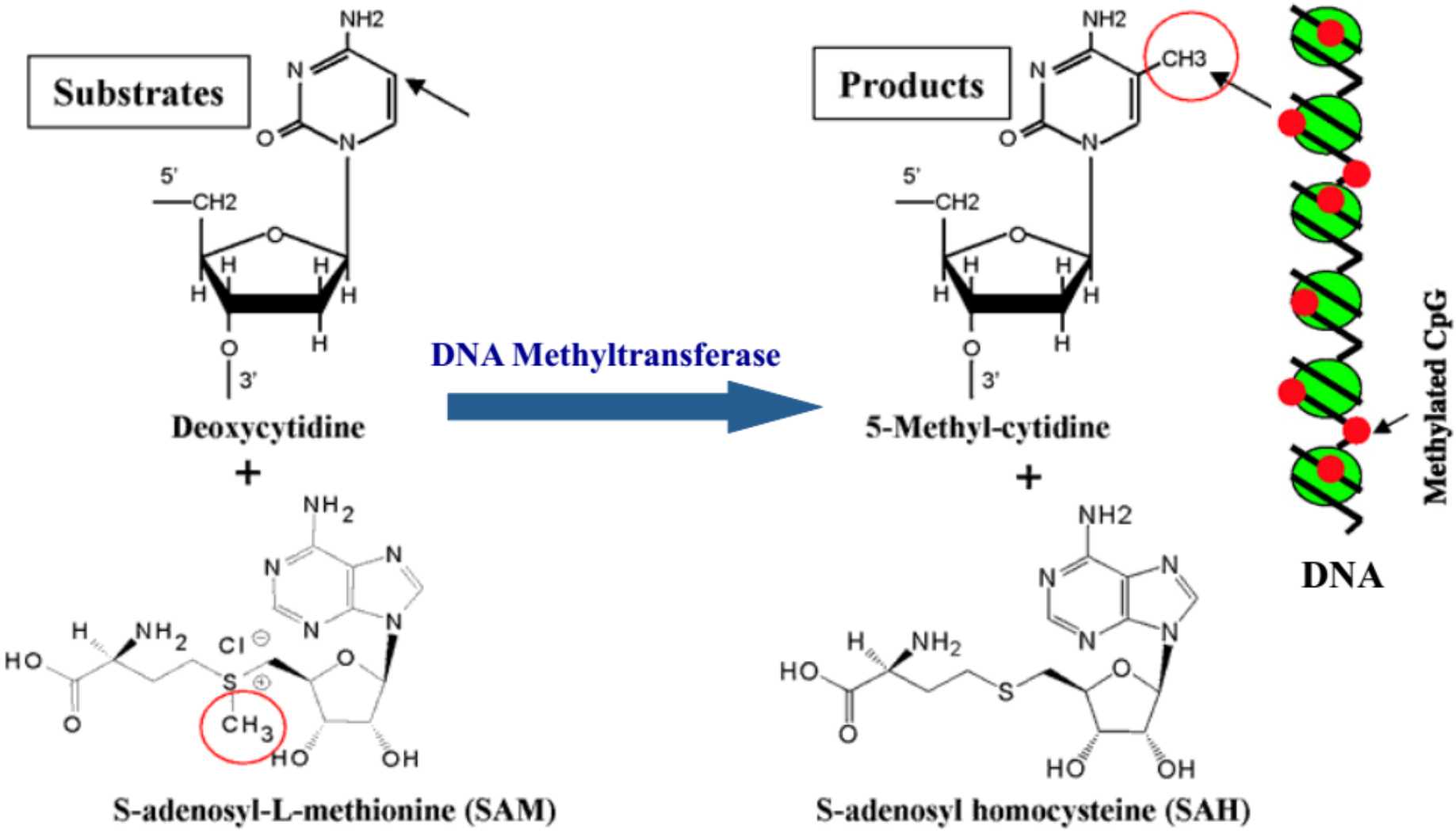
R = H Aminopterin 1st generation

R = CH_3 Amethopterin (methotrexate) 2nd generation

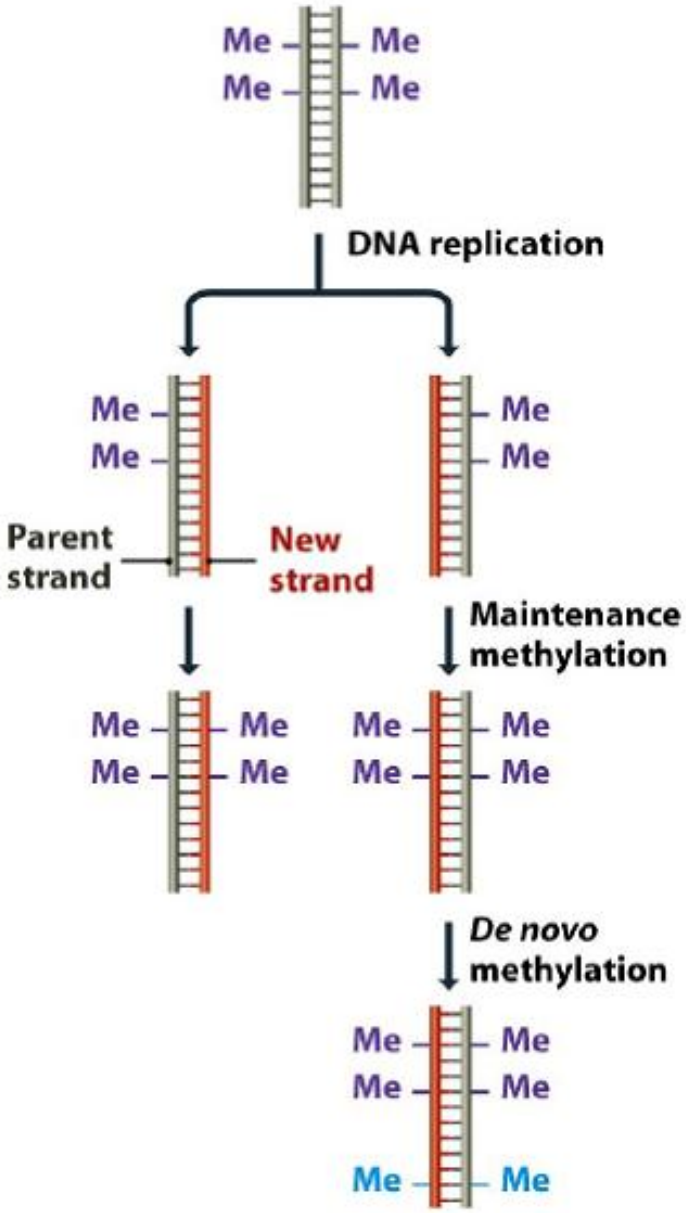
Folates Are Required for Nucleic Acid Synthesis and Methylation Reactions



Methylation of CpG island in DNA



DNA methylation pattern



- 维持型甲基化
- 重新甲基化

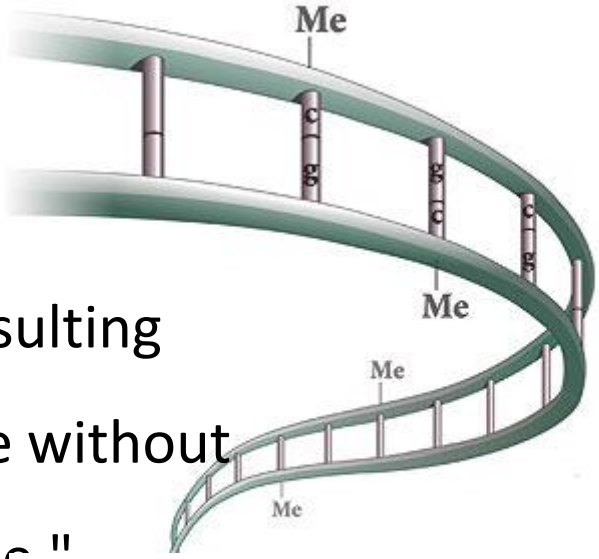
Dnmt1

- Methylates new DNA opposite a methylated site on the parent strand
- Both daughter strands retain parent DNA methylation pattern
- Pattern inherited after cell division

Dnmt3a
Dnmt3b

- Methylates totally new positions in the genome
- Changes the patterns of methylation
- Often recruited to genes for promoting gene silencing

Epigenetics

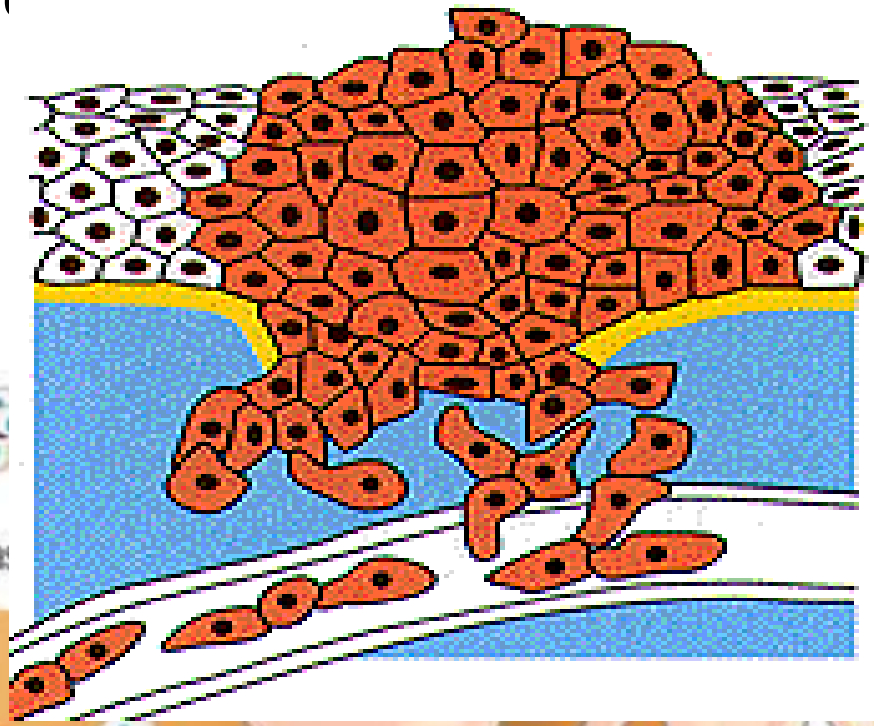


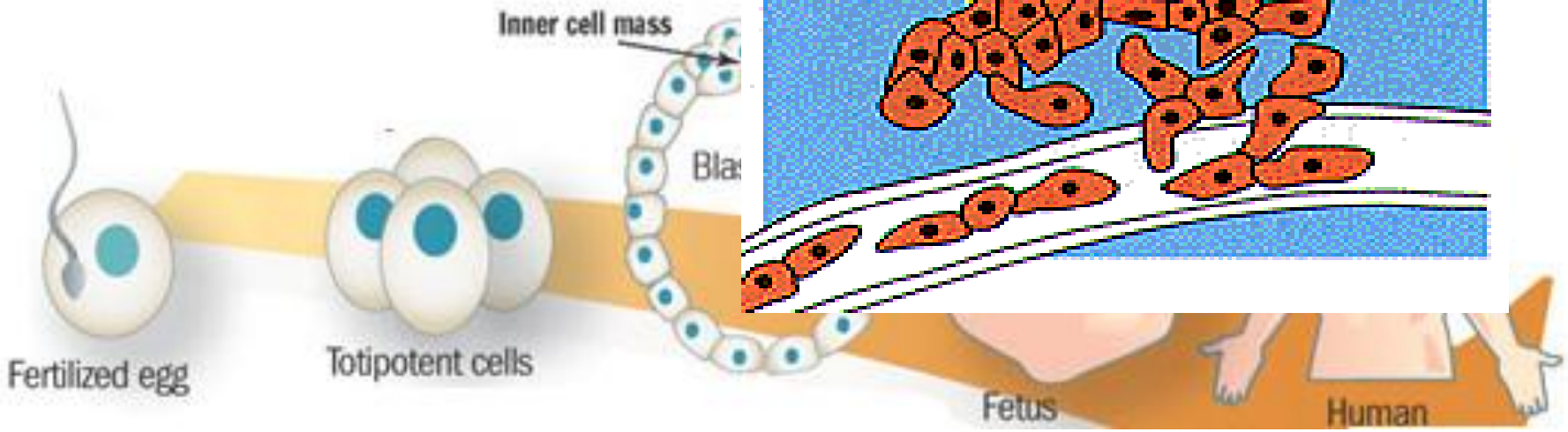
The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

"stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence "

- DNA methylation（CpG）
- Histone modifications





Summary

- > 90% of **purines** are salvaged
- Most ***de novo*** synthesis in **liver**, highly regulated
- Cross regulation of **purine** and **pyrimidine** nucleotide biosynthesis assures balanced levels of these metabolites
- Disruption of salvage or catabolism leads to disease

问题

1 细胞内嘌呤和嘧啶降解的终产物是什么？

2 嘌呤核苷酸和嘧啶核苷酸如何从头合成？各原子来源是什么？

3 与嘌呤从头合成和补救途径相关疾病的发病机理。