Cellular Biochemistry [918SM]

Module 1

Base and nucleotide metabolism

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BIOLOGICAL FUNCTIONS OF NUCLEOTIDES

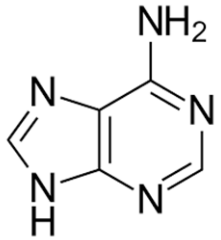
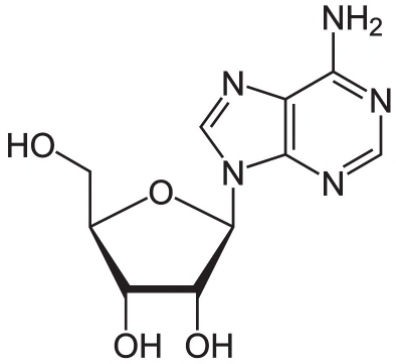
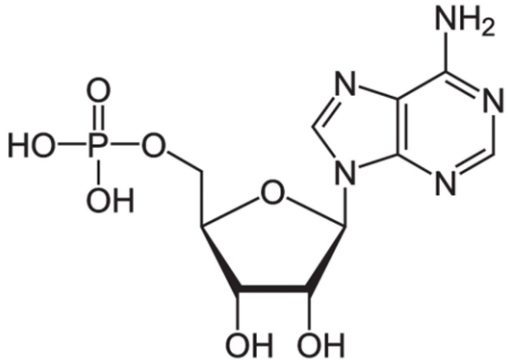
- **Nucleic acid syntheses** (DNA **and** RNA)
- **Metabolic intermediates in many metabolic pathways**
  - UDP-glucose (eg. Glicogen synthesis)
  - CDP-diacylglicerols / -etanolammine (phospholipid synthesis)
  - GDP-mannose (glycosylation)
  - S-Adenosyl-Met (SAM) (methylation)
- **Energy production & phosphorilation** (ATP, GTP)
- **Coenzyme structuress** (ATP, GTP, NAD, FAD, CoA,)
- **Signaling and regulation of metabolic pathways (**cAMP, cGMP**)**

Some enzymes in the methabolic pathways for base and nucleotide synthesis or degradation are:

- important drug targets

- if absent or malfunctioning cause serious pathologies

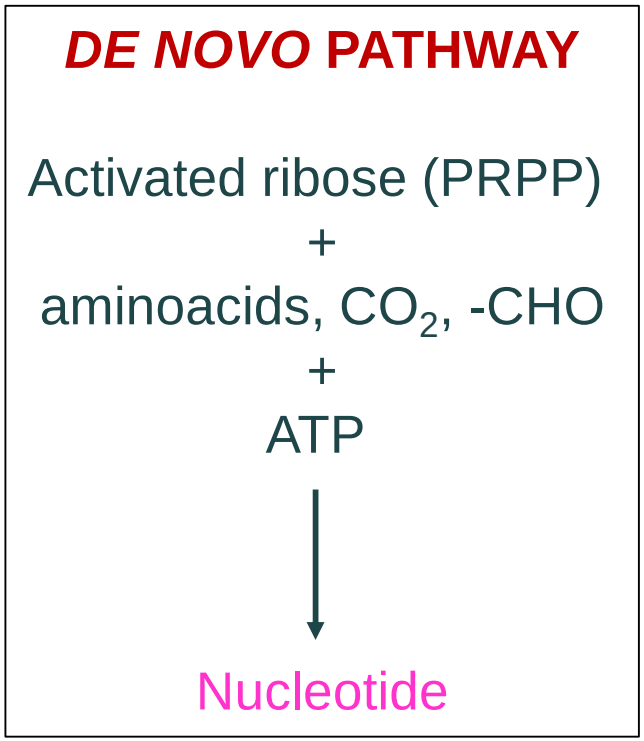
REVISION: NOMENCLATURE

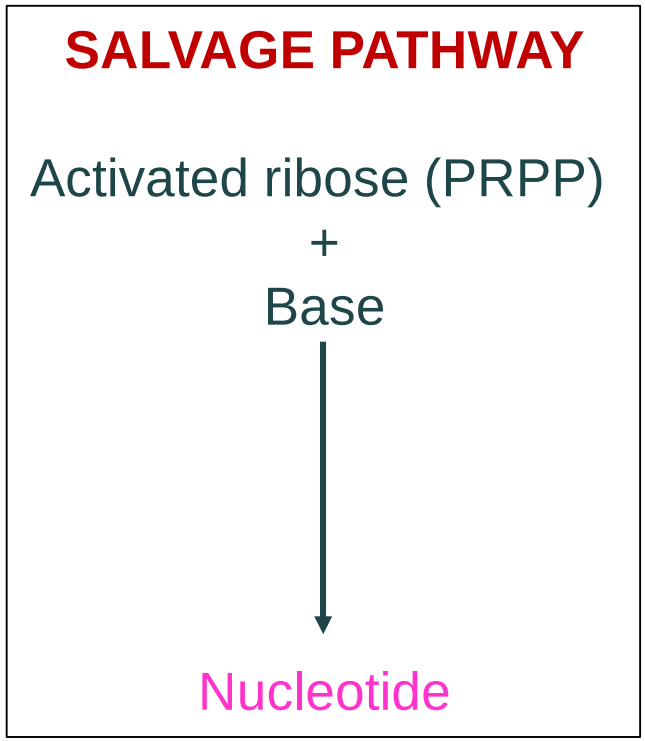
BASE	(RIBO)NUCLEOSIDE	(RIBO)NUCLEOTIDE
		
	ribose	ribose-5’-monophosphate
Adenine (A)	Adenosine	Adenilate (AMP, dAMP)
Guanine (G)	Guanosine	Guanilate (GMP, dGMP)
Citosine (C)	Citidine	Citidilate (CMP, dCMP)
Uracil (U)	Uridine	Uridilate (UMP, dUMP↓)
Thimine (T)	Timidine*	Timidilate* (dTMP)

* deoxy

NUCLEOTIDE BIOSYNTHESIS

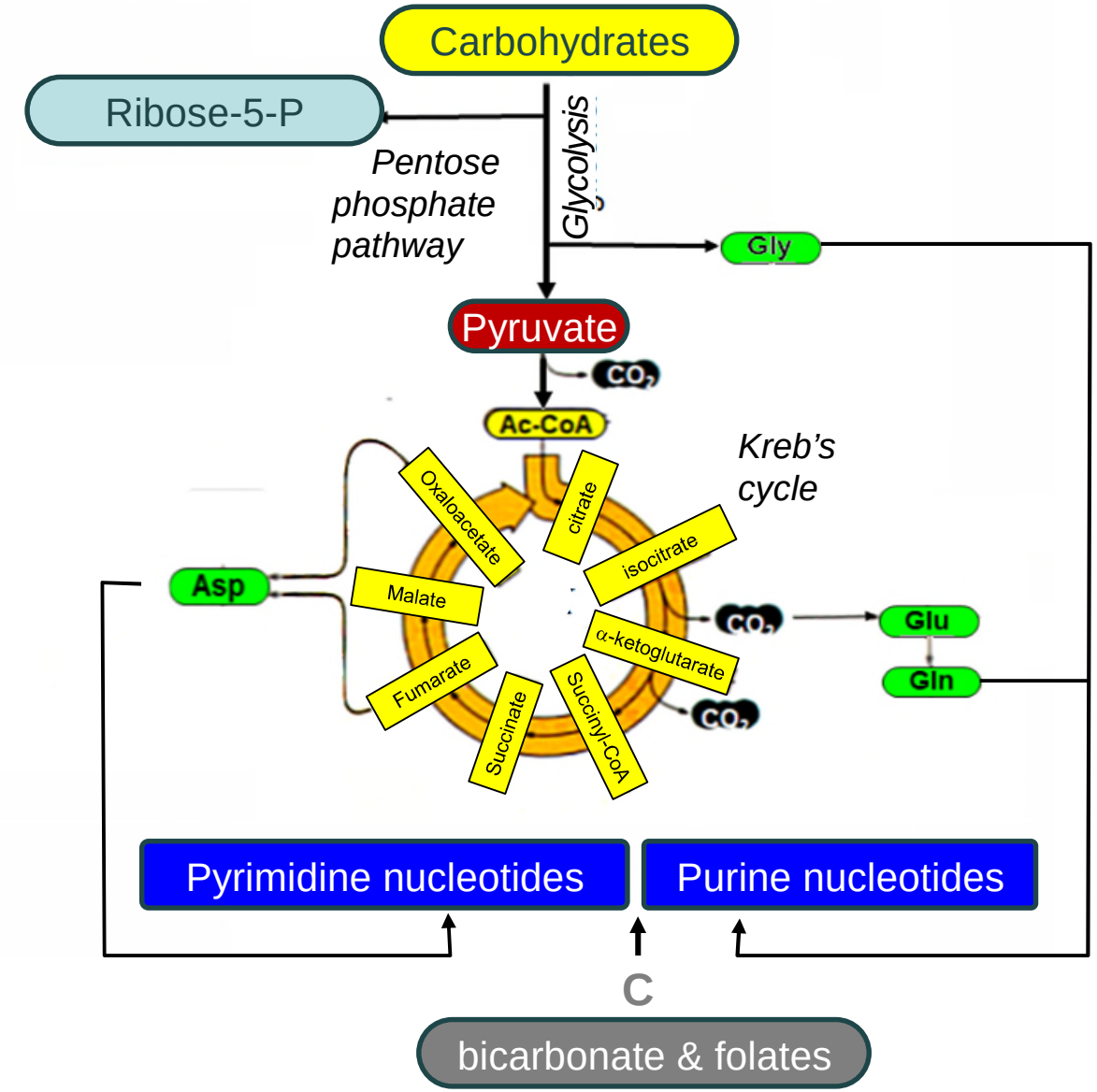
- **Nucleotides can be synthesized starting from simple precursors (*de novo*)or by recycling components (salvage)**



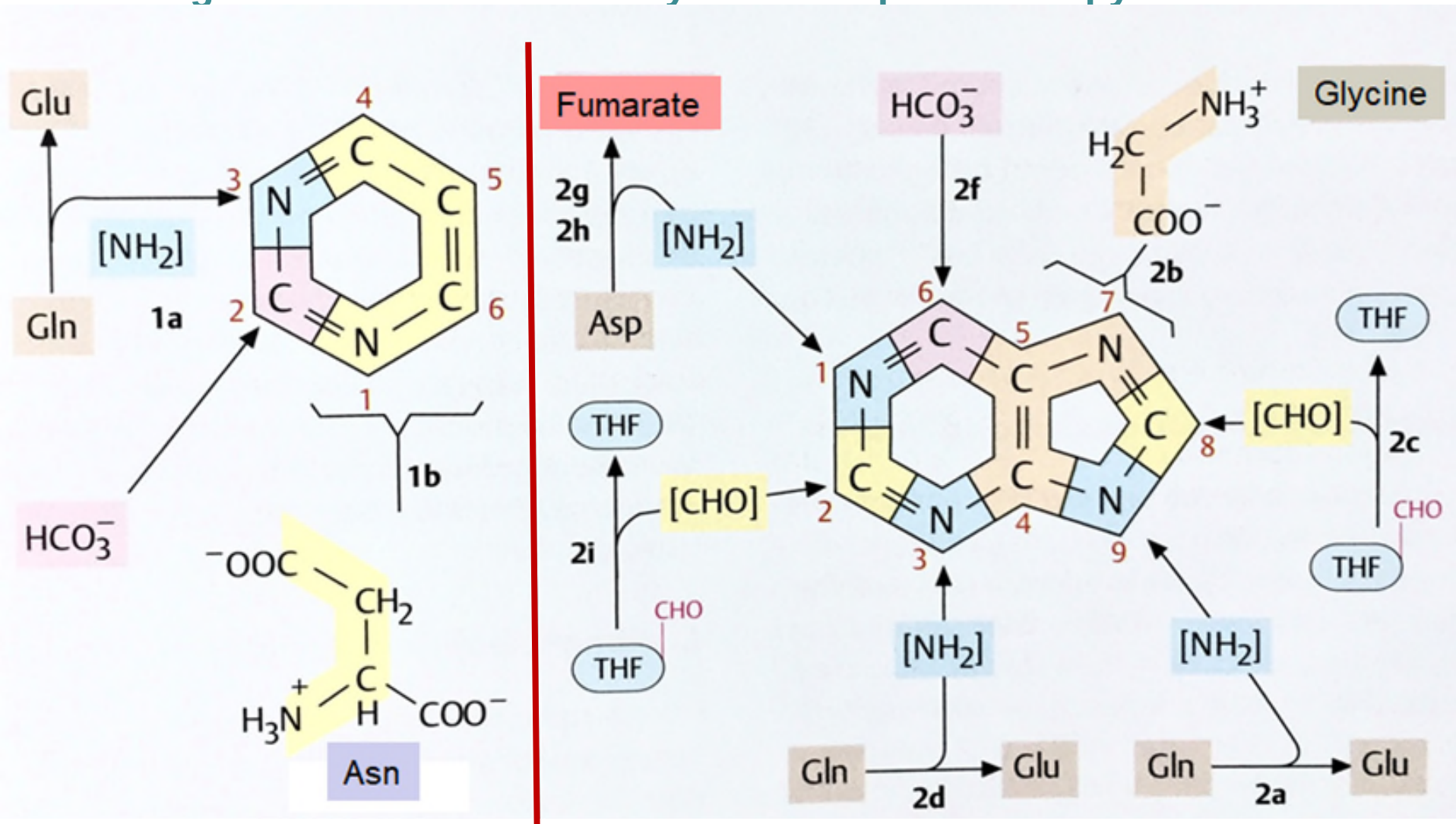


DE NOVO SYNTHESIS OF NUCLEIC ACIDS

- **The building blocks derive from intermediates of other metabolic pathways**



Building blocks for the de novo synthesis of purine and pyrimidine NA



Pyrimidines: Synthesis of the **base first** then **added to ribose**

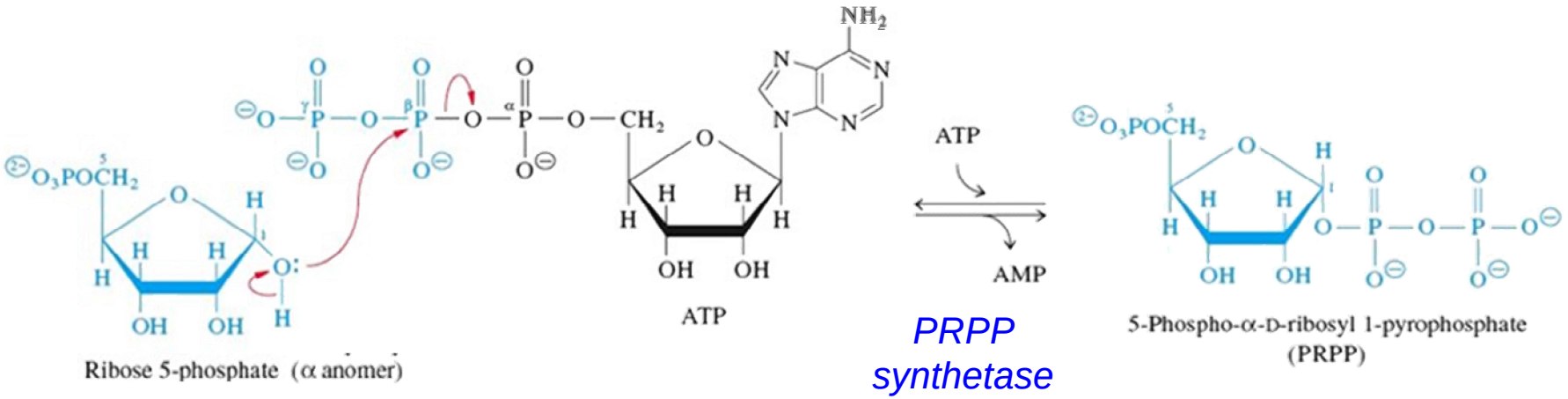
Purines: Synthesis of the base **directly on ribose**

In both cases it requires an activated form of ribose (PRPP)

5-PHOSPHORIBOSE-1-PYROPHOSPHATE (PRPP)

- **PRPP is an important intermediate in pathways involving nucleotides:**
  - *de novo* synthesis of purine nucleotides
  - *de novo* synthesis of pyrimidine nucleotides
  - synthesis of NAD and NADP

- **Synthesized by addition of Pyrophosphate to the α-anomer of Ribose-5-P**



R-5-P + ATP → PRPP + AMP

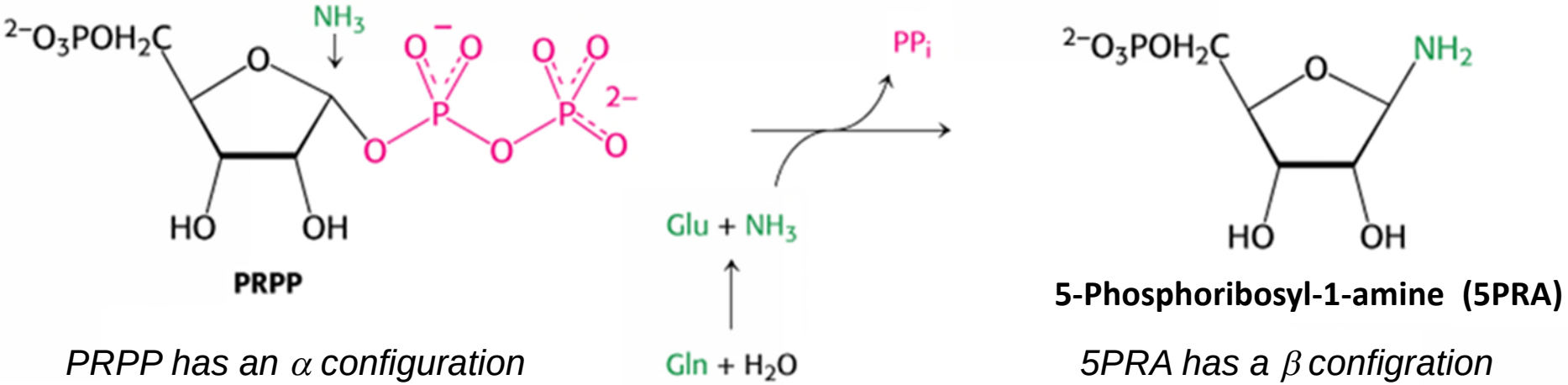
*NB: *synthases* (often *lyases)* don't require ATP
synthetase (often *ligases)* require ATP

DE NOVO SYNTHESIS OF PURINE BASES

- **The synthesis occurs directly on PRPP (on the pre-activated anomeric C)**

① γ-amino group transfer from Gln onto the pre-activated anomeric C1 of the sugar

PRPP amidotransferase (PPAT)



PRPP has an α configuration

5PRA has a β configration

NB. the equivalent (≡) of **two** phosphoanhydride bonds (2ATP) are spent

R-5-P + ATP → PRPP (+ AMP) + NH2 → 5PRA + PPi

NB: *amin**o**transferase (transaminase)* transfer of αNH_2 (ammine) from AA to α-ketoacid

*amid**o**transferase* transfer of γNH_2 (ammide) from Gln (>> Asn) to a substrate

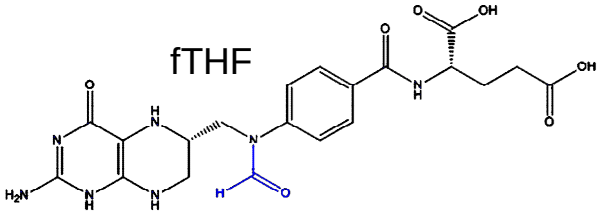
The purine synthetic pathway requires 10 reactions for IMP synthesis

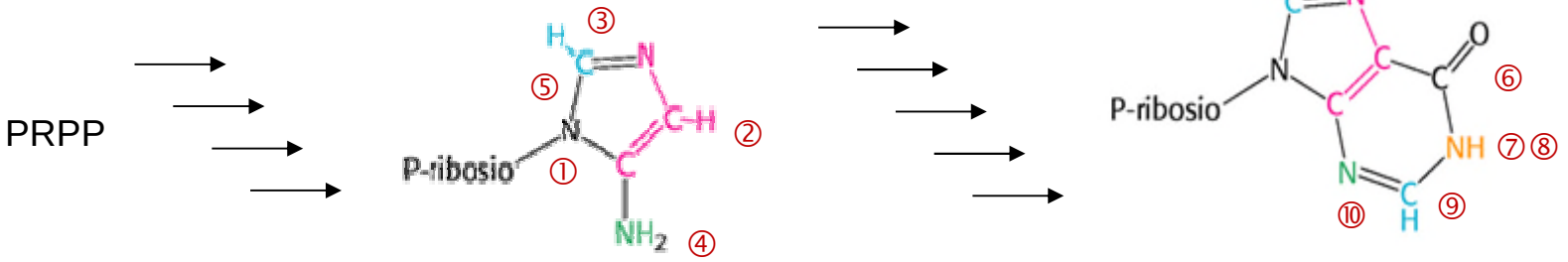
② a glycine is inserted onto the amine

③ a formyl group is inserted deriving from fTHF (10-formyl tetrahydrofolate).

④ a second amine (also from Gln) is inserted

⑤ the 5 atom aminoimidazole ring is closed

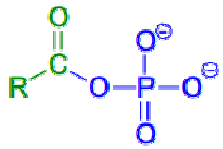




5-aminoimidazole ribonucleotide

Inosinate monophosphate (IMP)

⑥ a carboxylic group is inserted on the ring (from an activated bicarbonate)

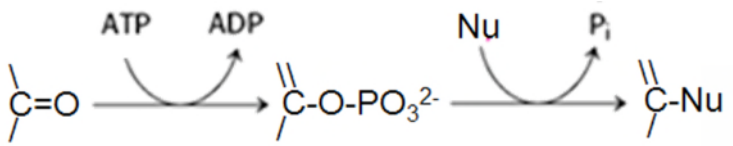


⑦ an third amine group is added (αNH_2 from Asp ⑧ leaving fumarate).

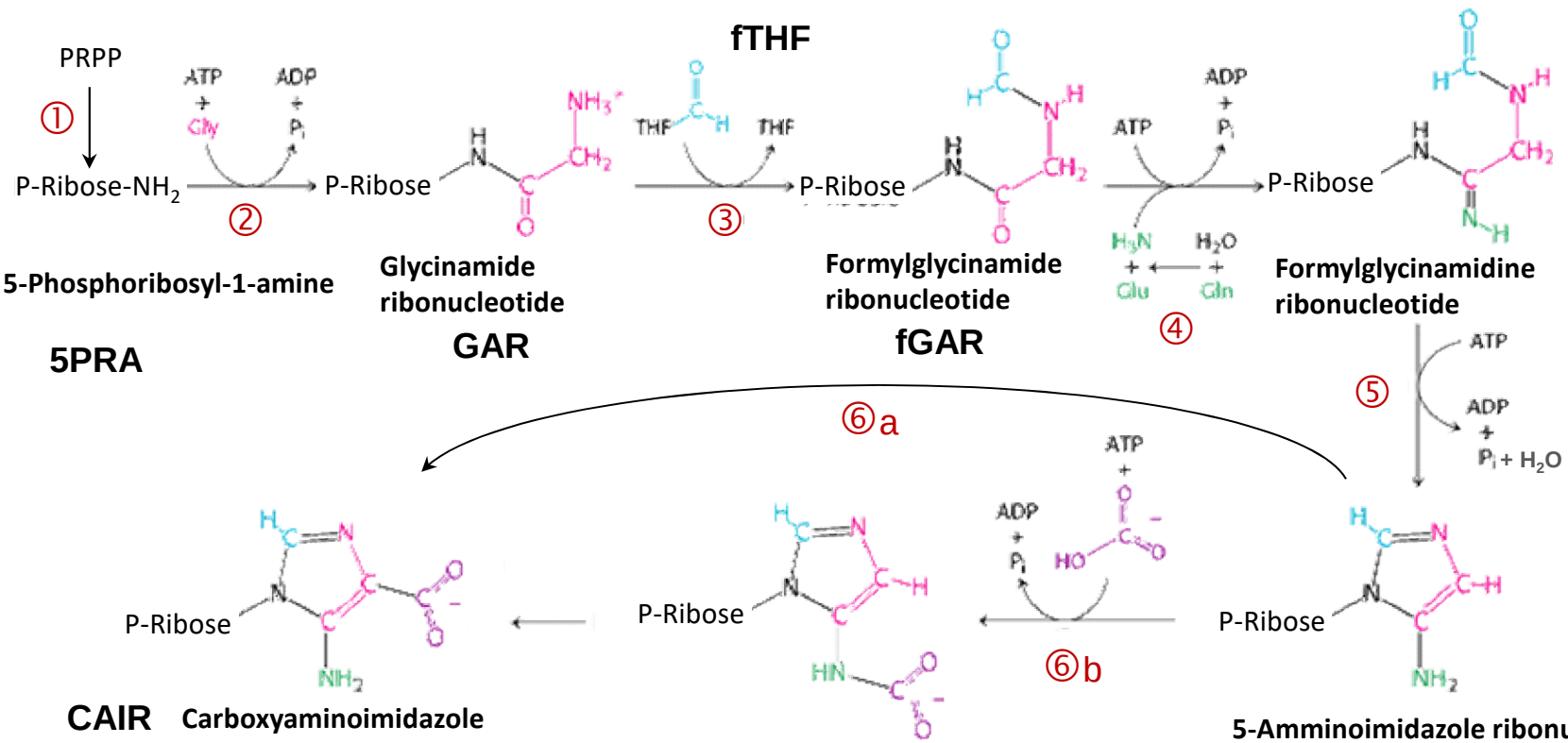
⑨ a second formyl group is added

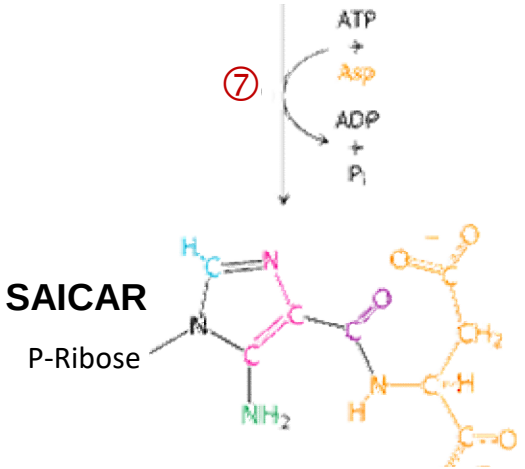
⑩ the 6-membered ring is closed to form the nucleotide **Inosinate monophosphate** (**IMP**).

altogether, 2 ≡ ATP are used for PRPP synthesis and 5 ATP for insertion of Gly, amine groups and carboxylic groups (preactivated incoming groups)



Synthesis of IMP (extra)



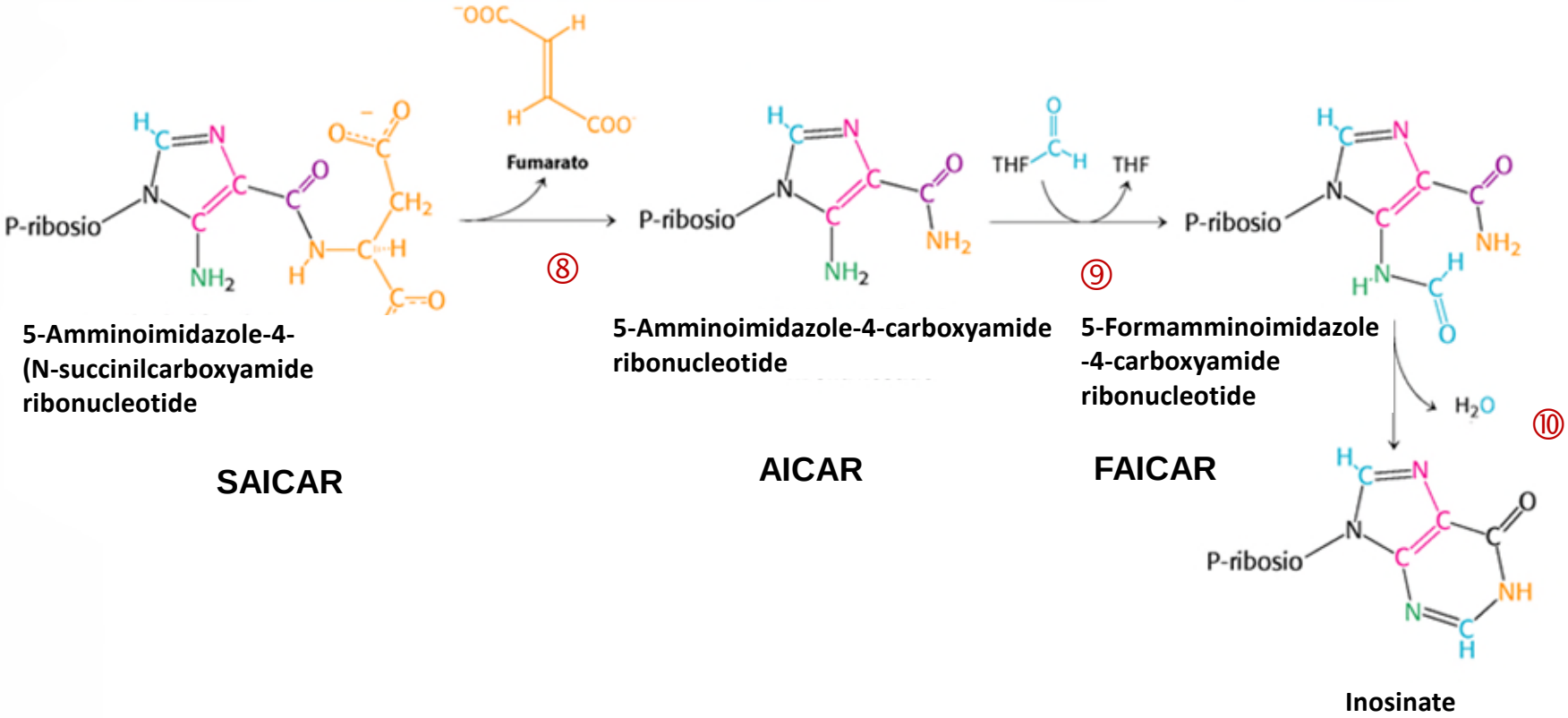


5-Amminoimidazole-4-(N-succinil_ carboxyamide ribonucleotide

1. *PRRP amidotransferase (PPAT)*
2. *GAR synthetase (ligase) (GARS)*¶
3. *GAR transformylase (GART)* ¶
4. *Phosforybosilglycinamide synthase (PFAS)*
5. *AIR synthase (AIRS)* ¶
6. a *CAIR synthetase* ¶ or b *AIR carboxylase* (bacteria, fungi)
7. *SAICAR synthetase* ¶

¶ part of bi- or trifunctional proteins in higher eukaryotes

GARS+*GART*+*AIRS* *CAIRS* + *SAICARS*



8. *Adenylosuccinate lyase (ASL)* (also catalyzes reaction 12.)
9. *AICAR transformilase (AICART)* ¶
10. *IMP synthase (ciclohydrolyase* domain, working backwards*)* ¶

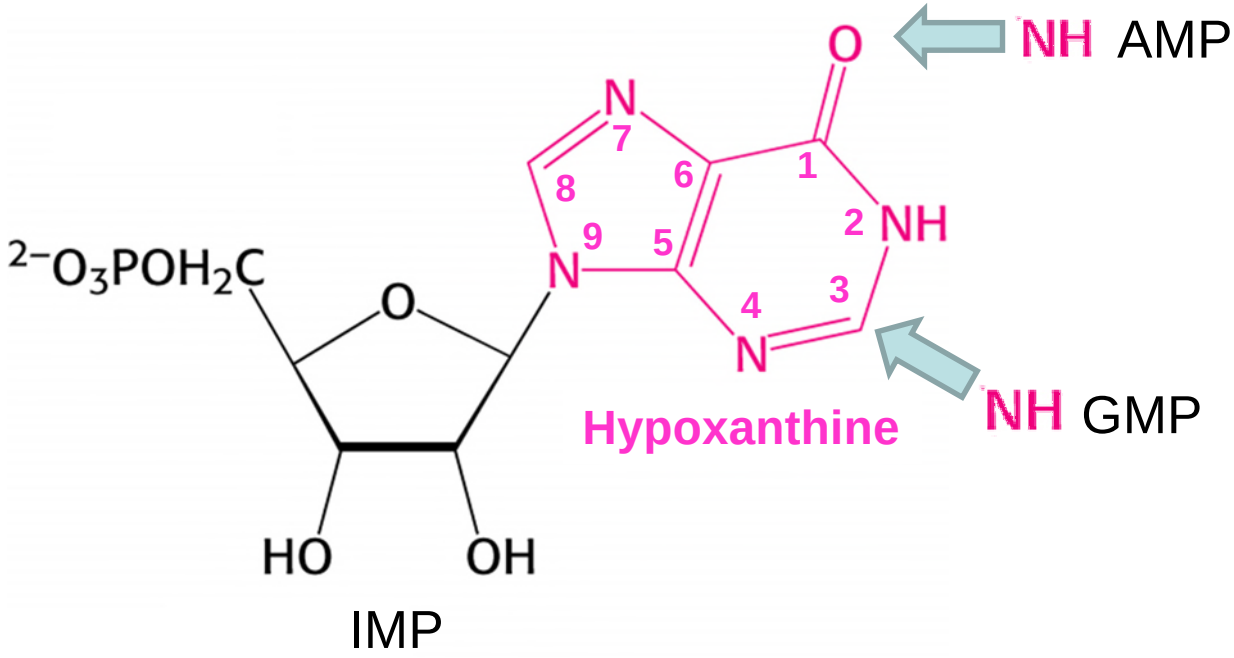
¶ *part of the bifunctional protein ATIC in higher eukaryotes*

*Inosinate contasins the base hypoxanthine

SYNTHESIS OF AMP & GMP

AMP and GMP are converted from IMP by amination of Hypoxanthine

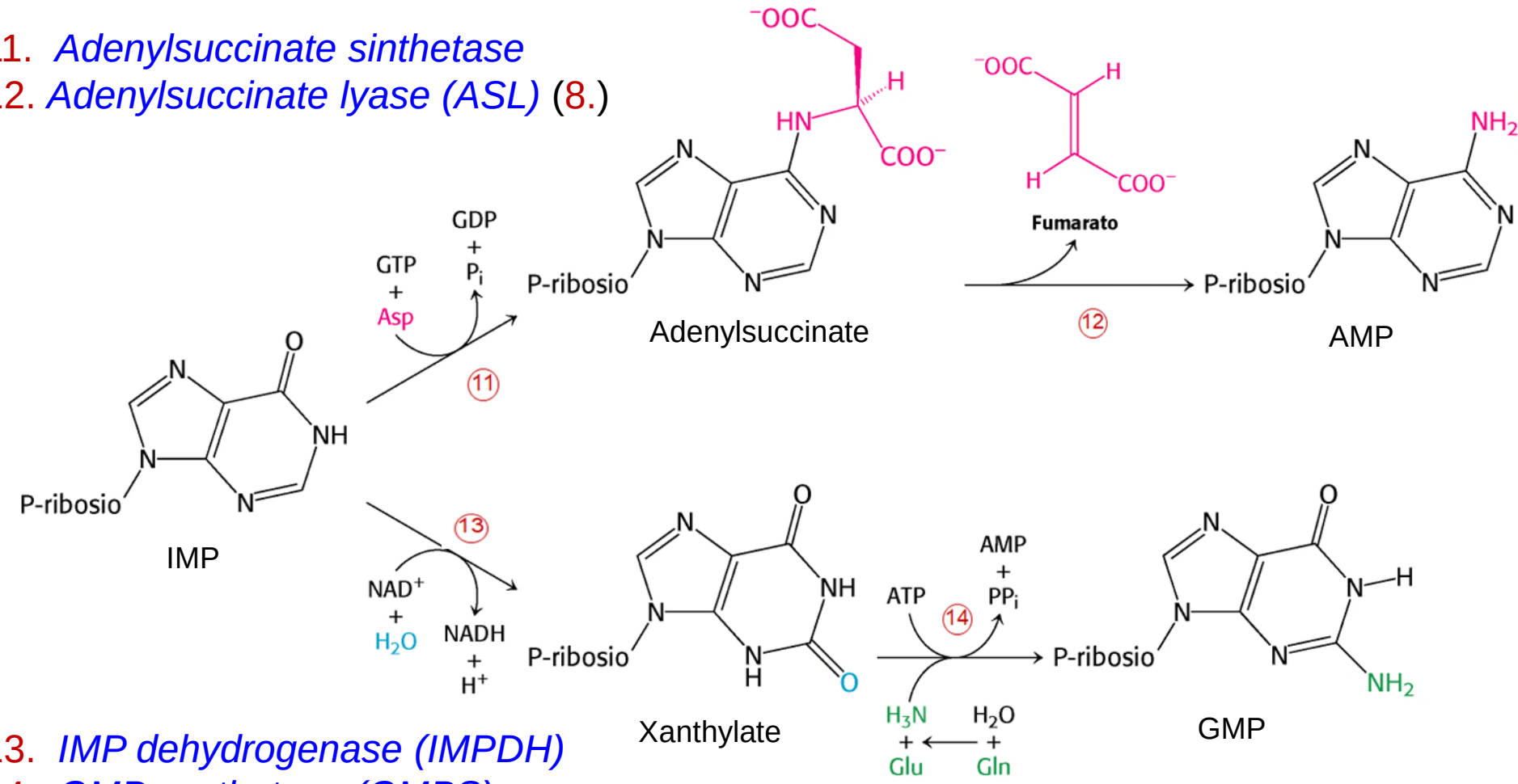
- The two patways are **complementary** and **reciprocally regulated**
- They depend on reciprocal energy sources GTP for AMP ATP for GMP)
- They have different sources of amine groups αNH_2 from Asp for AMP (similar to rxn 8.)
  γNH_2 from Gln for GMP
- Amination of Hypoxanthine C3 requires a prior oxidation



SYNTHESIS OF AMP & GMP (cont.)

AMP cost: 6≡ATP + 2≡ ATP for PRPP synthesis = **8ATP**

11. *Adenylsuccinate sinthetase*
12. *Adenylsuccinate lyase (ASL)* (8.)



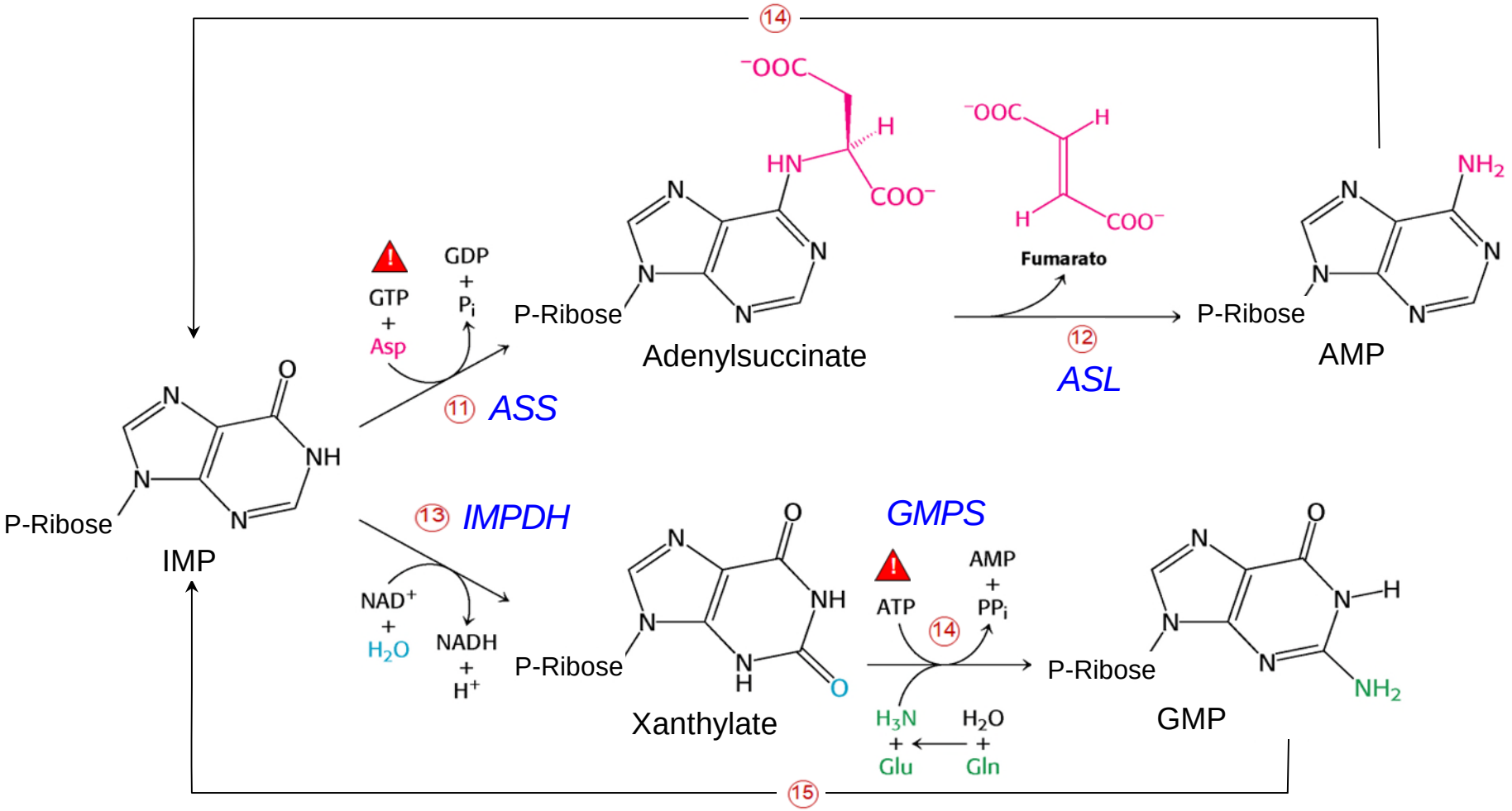
13. *IMP dehydrogenase (IMPDH)*
14. *GMP synthetase (GMPS)*

GMP cost: 7ATP + 2≡ ATP for PRPP synthesis = **9 ATP**

but 1 NADH formed

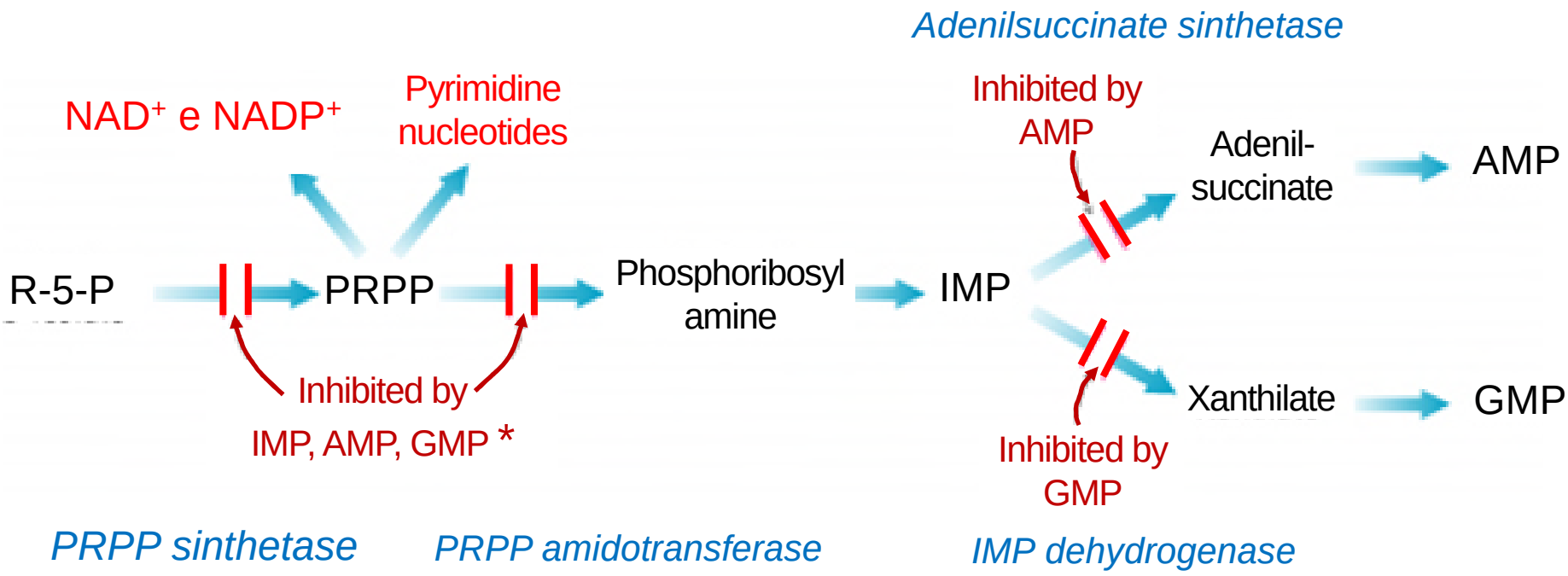
Complementarity & reciprocal regulation allow balancing purine nucleotides

- The two pathways compensate for each other.
- ⚠They use reciprocal energizing NTP (GTP to produce AMP, ATP to produce GMP)
- 14. excess AMP reconverted to IMP by *AMP deaminase* (catabolic enzyme, slide 34)
- 15. excess GMP reconverted to IMP by *GMP oxidoreductase* (nucleotide balancing, slide 34)



REGULATION OF PURINE NUCLEOTIDE SYNTHESIS

Regulation is by allosteric feedback inhibition

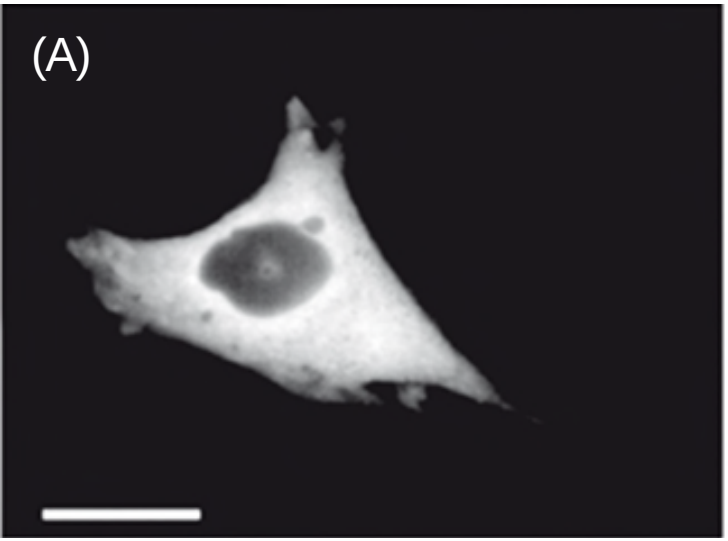


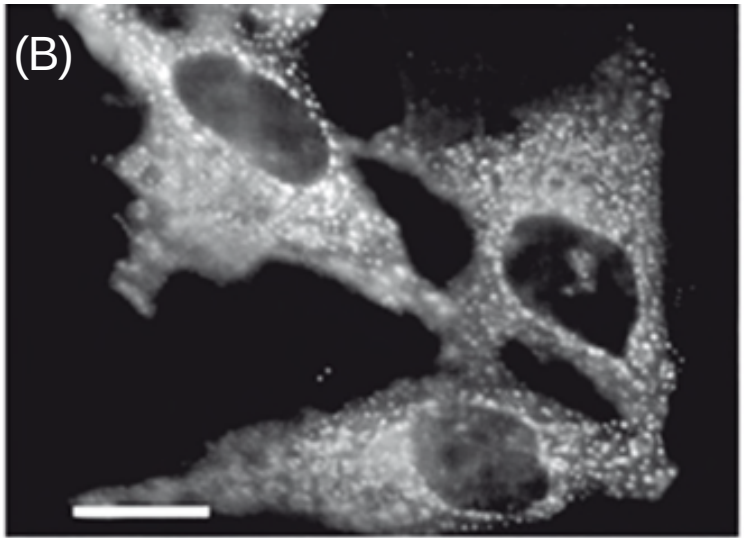
- For *PRPP synthetase* and *amidotransferase*, the effect is synergic; that of AMP + GMP is greater than the individual effect.
- Allosteric control favours selective regulation.

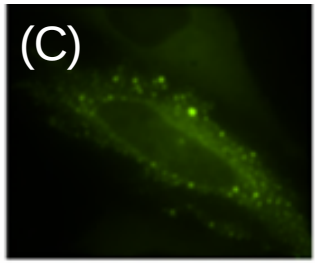
2 ore

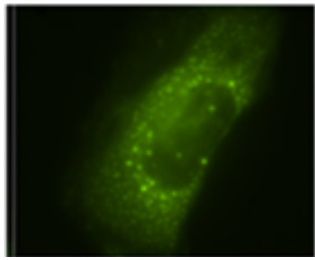
PURINOSOMES – dynamic purine factories

Multienzyme complexes with full functionality for *de novo* purine biosynthesis









- HeLa cells were transfected with ***PFAS***-**GFP** ④ constructs (intermediate enzyme).

(A) In the **presence of purines** (***PRPPS*** inhibited) **no synthesis**, **no fluorescence**.

(B) In the **absence of purines** (**synthesis** proceeds) non-diffuse, **point-like fluorescence**.

(C) In cells co-transfected with **PPAT**-**GFP** ① **fluorescence stays point-like**.

- **Function**: rapidly channel unstable, hydrophobic intermediates from one enzyme to the next
- **Location**: Cytoplasm near mitochondria (direct access to energy and precursors)
- **Regulation:** Assemble when purines are needed, then disassemble (dynamic)

Metabolon concept: transient assemblies of metabolic enzymes

SYNTHSIS OF NTP

NMP, NDP and NTP are interconvertible

- Synthesis of **NDP**: *Nucleoside monophosphate kinases*

Base specific & use ATP as donor

$$GMP + ATP \rightleftharpoons GDP + ADP$$

Guanilate kinase

$$AMP + ATP \rightleftharpoons ADP + ADP$$

Adenilate kinase

$$UMP + ATP \rightleftharpoons UDP + ADP$$

Uridilate kinase

- Synthesis of **NTP**: *Nucleoside diphosphate kinases* (ensures further balancing of NTPs)

Base unspecific & use any NTP as donor

$$NDP + XTP \rightleftharpoons NTP + XDP$$

NDK

DE NOVO SYNTHESIS OF PYRIMIDINE NUCLEOTIDES

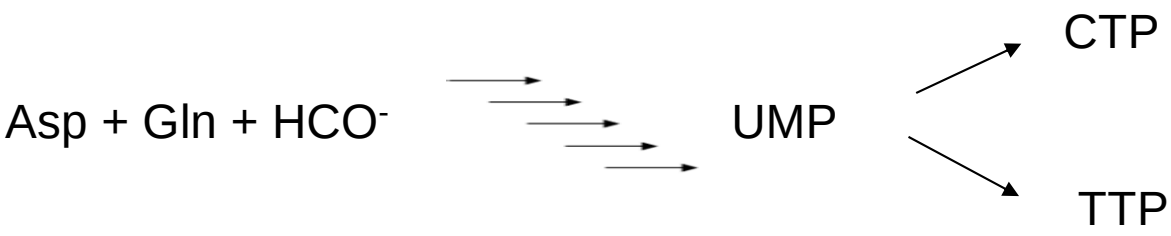
This pathway is shorter than for purine nucleotide synthesis

- Unlike purine biosynthesis, the **pyrimidine ring is synthesized first** and it is **subsequently linked to PRPP**

- **Two precursors (Asp** and **Gln**) are required that **with HCO^{3-}** contribute to the formation of the 6-membered ring.

- **Six reactions** are needed (compared to 10 + 2 for purines).

- **The final product is UMP** (uridine monophosphate).



SYNTHESIS OF PYRIMIDINE BASES – OROTATE INTERMEDIATE

① *Carbamoyl phosphate synthase II* (*CPS II*)

1st rate-limiting enzyme, catalyses the reaction of *L*-Gln, HCO_3^- & ATP to form carbamoyl phosphate

1st committed step in the pathway

② *Asp transcarbamoylase* (*ATCase*)

highly regulated enzyme that catalyses the condensation of *L*-Asp and carbamoyl phosphate to form N-carbamoyl-L-aspartate and Pi

③ *Dihydroorotase* (*DHO*)

catalyses ring formation and dehydration to 4,5-dihydroorotate

④ *Dihydroorotate dehydrogenase* (*DHODH*)

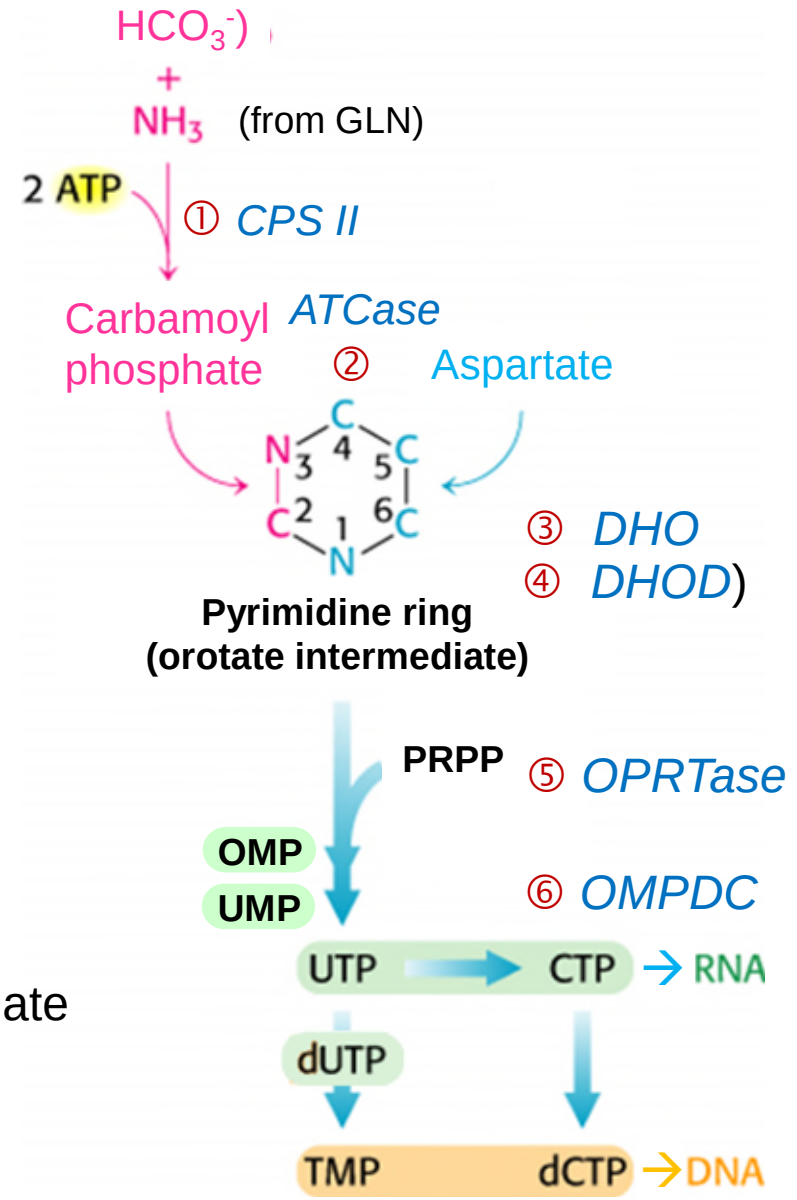
oxidises and desaturates ring to orotate

⑤ *Orotate phosphoribosyltransferase* (*OPRTase*)

catalyzes the formation of orotidine 5'-monophosphate (OMP) from orotate and PRPP

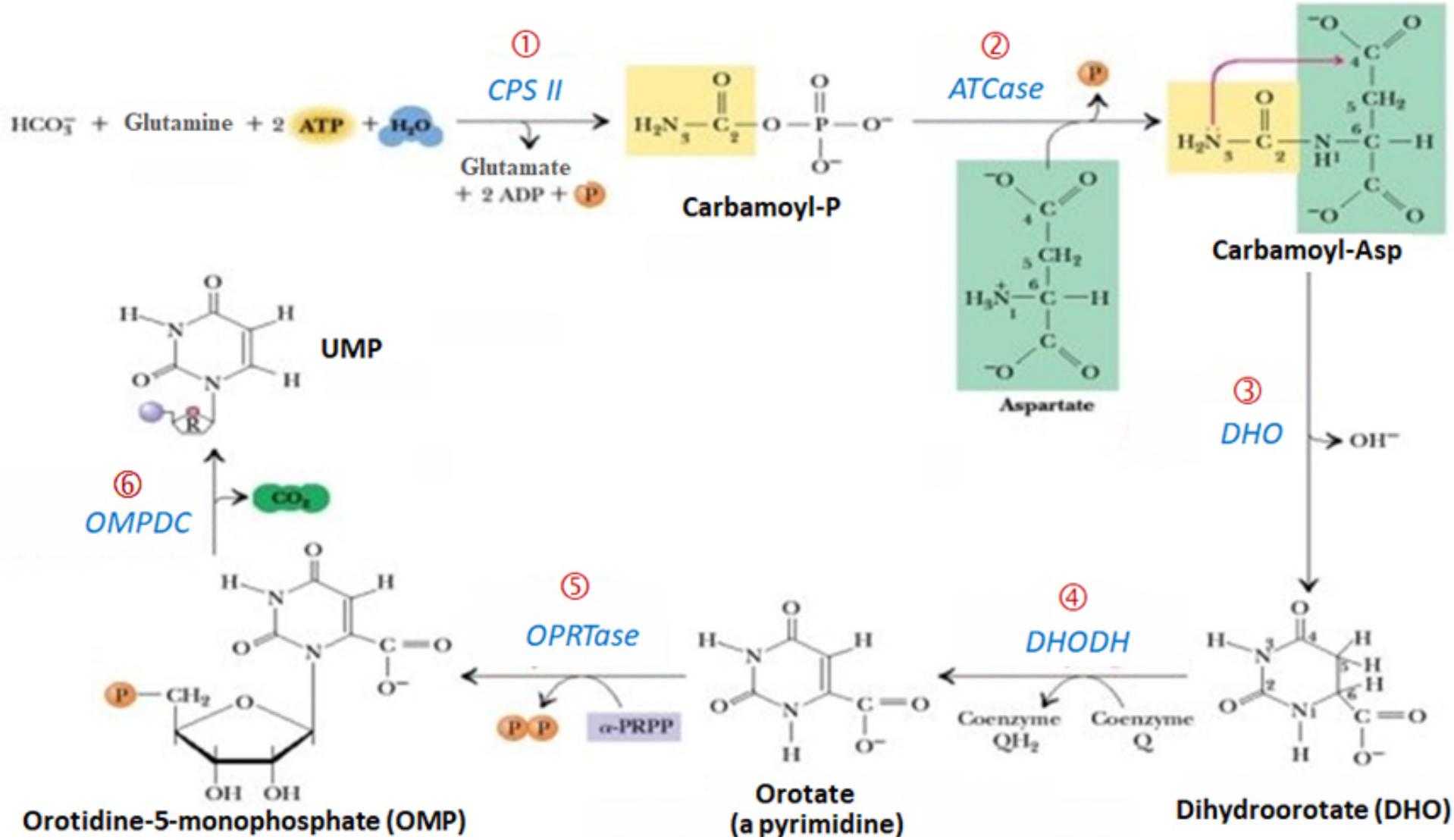
⑥ *OMP decarboxylase* (*OMPDC*)

removes carboxyl from OMP to form UMP

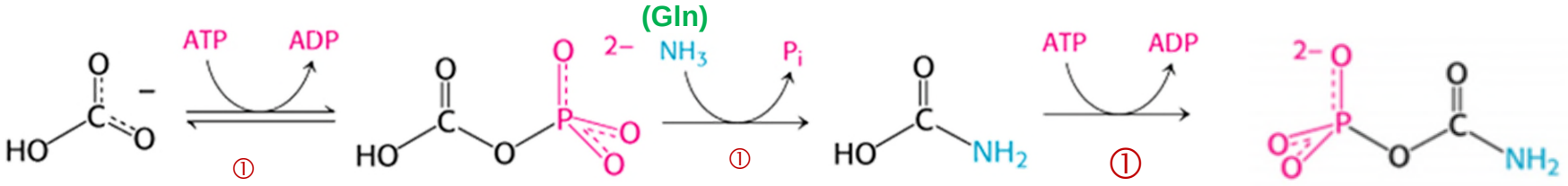


SYNTHESIS of OMP and UMP

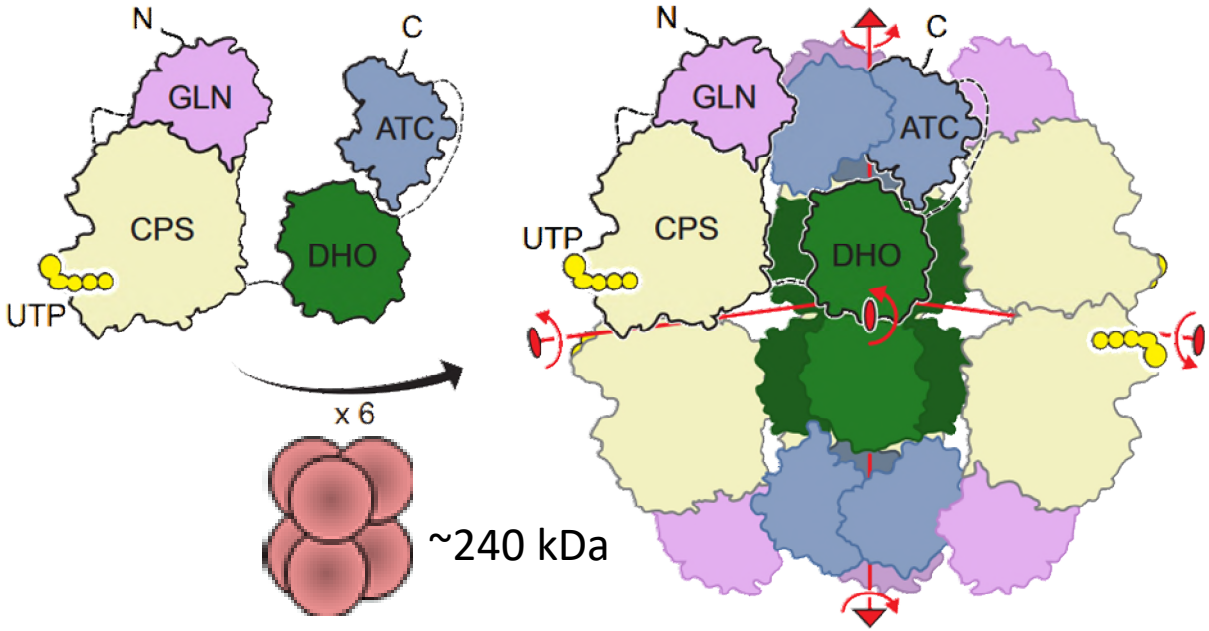
- **Two-step process of OMP synthesis (base 1st → nucleotide), followed by decarboxylation**
  - common to the *de novo* pyrimidine biosynthesis pathway in all three domains of life.



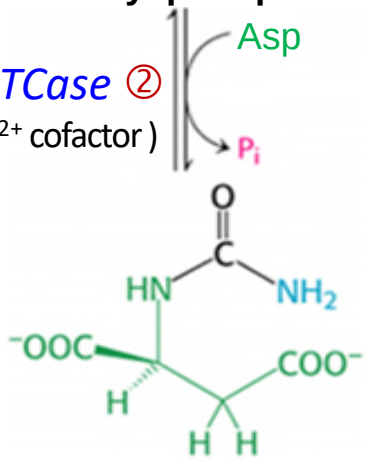
1st STEP: SYNTHESIS OF OROTATE



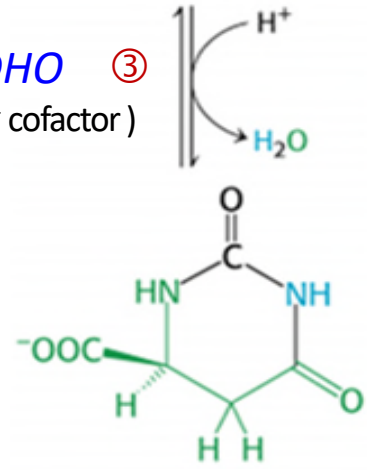
|----------*CPS II* (in eukaryotes)----------|



ATCase ② (Zn2+ cofactor)

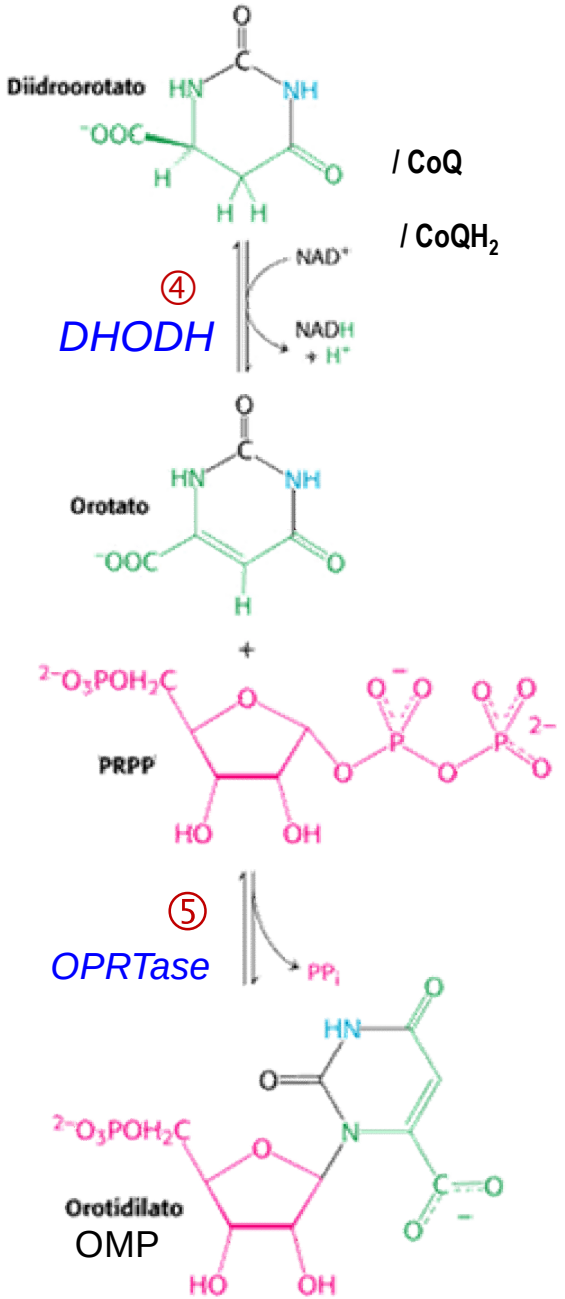


DHO ③ (Zn2+ cofactor)



- In bacteria, *CPS, ATCase* e *DHO* are individual proteins
- In higher eukaryotes a single protein chain (*CAD*) contains ***C**PS II, **A**TCase* and ***D**HO*. It forms a very large hexameric complex (6 identical chains)
- NB: *CPS I* mitochondrial isoform active in the urea cycle (uses NH_2 directly).

2st STEP: SYNTHESIS OF OMP and UTP



④ Dihydroorotate is oxidised to orotate by *DHODH,* localized on the outside of the mitochondrial inner membrane. In eukaryotes it reduces CoQ → $CoQH_2$

⑤ Orotate is then linked to ribose by the transferase *OPRT* and decarboxylated by *OMPDC*. In eukaryotes they are part of a single protein complex, *UMP synthase (UMPS). OMPDC* is one of the most efficient enzymes known – it increases the reaction velocity by 10^{17}

⑥ UMP synthesis therefore requires 3 gene products: ***CAD*** (*GAT, CPSII, DHO* and *ATCase*), ***DHODH*** and ***UMPS*** *(OPRT* and *OMPDC*).

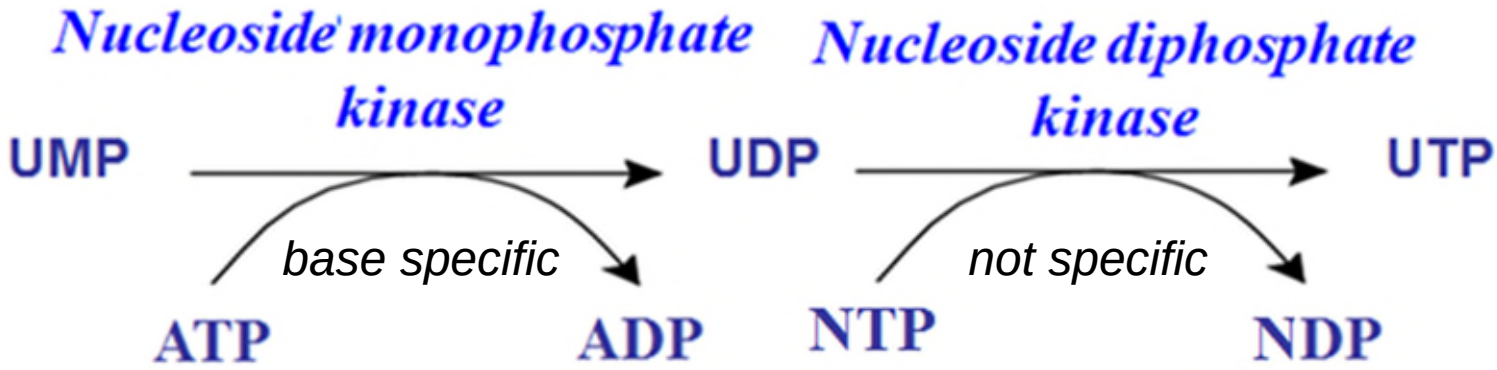
Energy cost: 2ATP≡ for PRPP synthesis +

2ATP≡ for Carbamoyl phosphate synthesis

= 4ATP

SYNTHESIS OF UTP & CTP

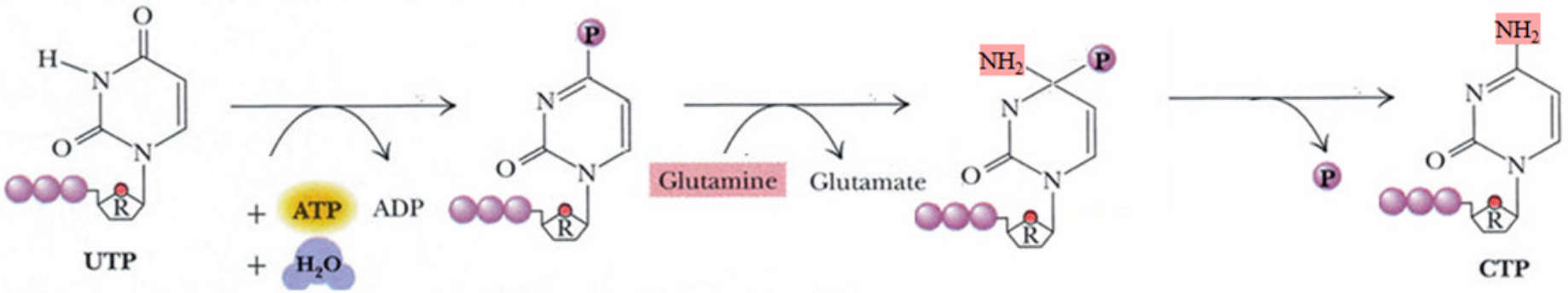
- **Synthesis of UTP requires *Monophosphate* and *Diphosphate kinases***



- **Synthesis of CTP from UTP, with γ-amine group transfer from Gln by *CTP synthase* (*CTPS*)**

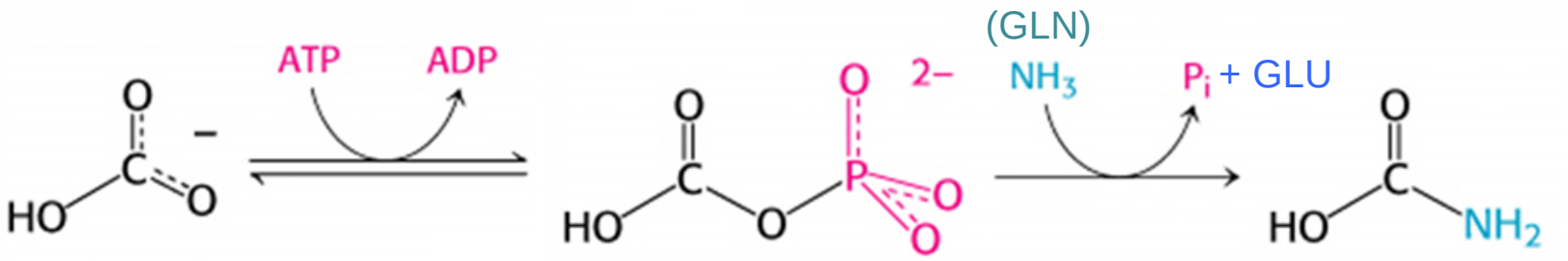
- *CTPS* has a domain with *amidotransferase* activity with a mechanism analogous to *CPS II*.

CTP synthase (*CTPS*)



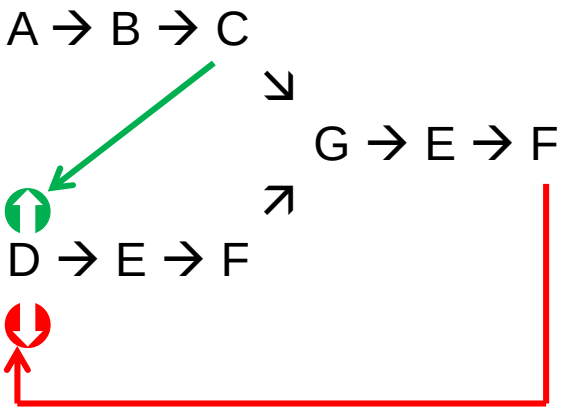
REGULATION of *DE NOVO* PYRIMIDINE PATHWAY

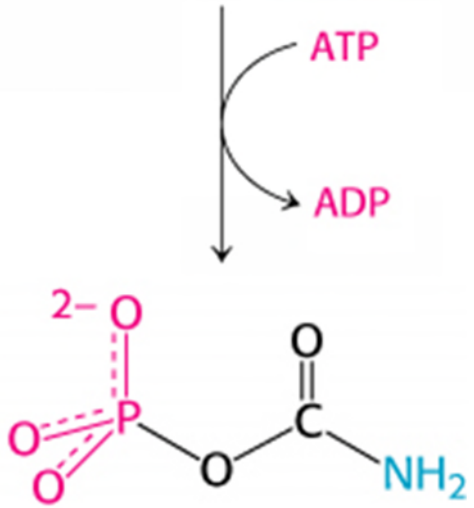
- **The reaction catalysed by *CPS II* is an important control step in eukaryotes**



Bicarbonate **Carboxyphosphate** **Carbamic acid**

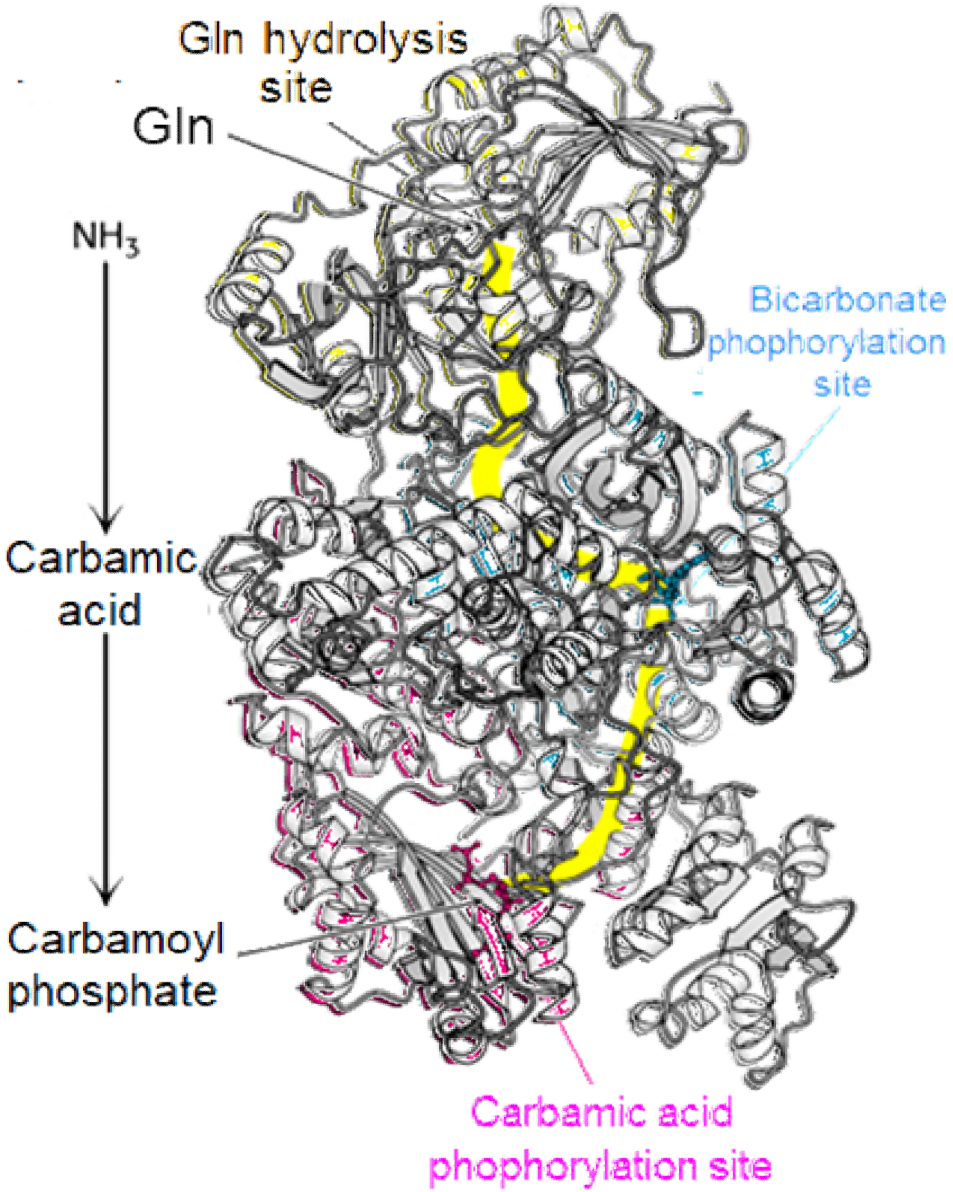
- Control step: feedback inhibition by the final product, UTP
  feedforward activation by PRPP





Carbamoyl phosphate

STRUCTURE and MECHANISM of BACTERIAL CPS



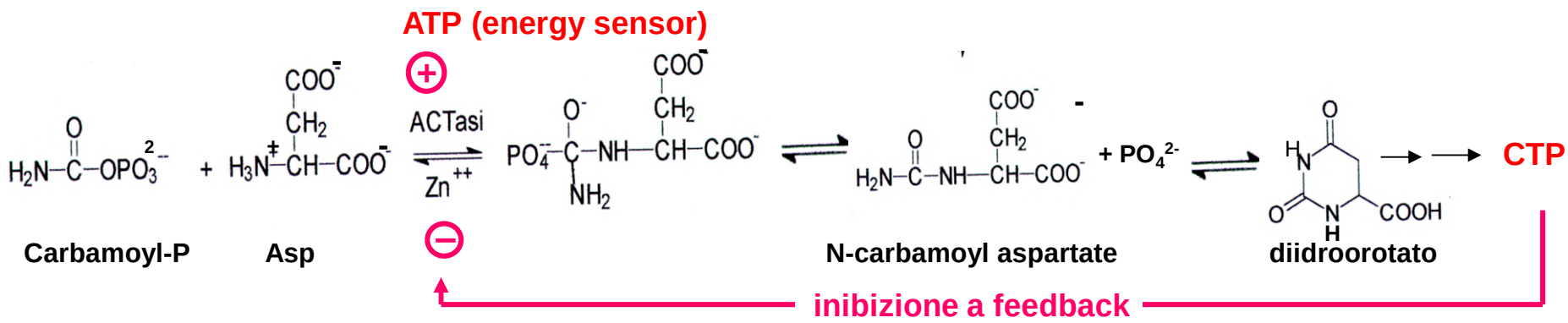
- Bacterial *CPS* is a dimeric enzyme
- The **smaller subunit** (yellow) binds Gln and **removes NH_2** by hydrolysis (*GAT* function)
- The **larger subunit** has **two homologous domains** (blu and red) with so called '**ATP-grasp folds**'
- In the 1st domain, **HCO_3^-** is activated for phosphorylation by ATP and **carboxyphosphate** then reacts with NH_2 to form **carbamic acid**
- This then moves to the 2nd domain where it is phosphorylated to **carbamoyl phosphate** (*CPS* function)
- Intermediates move in a 'molecular tunnel' inside the enzyme
- This ensures that:
  a) labile intermediates are protected
  b) intermediates do not separate by diffusion

REGULATION OF BACTERIAL ATCase

- **Bacterial *ATCase* is under allosteric control**

- Unlike eukaryotic *ATCase*, which is part of the *CAD complex,* **bacterial *ATCase*** is a stand-alone enzyme, and is **independently controlled**.

- It is regulated by: **heterotropic allosterism** (stimulated by ATP, feedback inhibited by CTP)

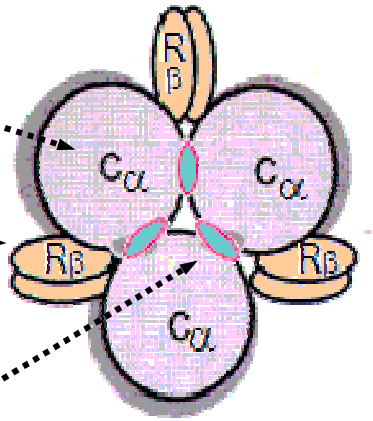


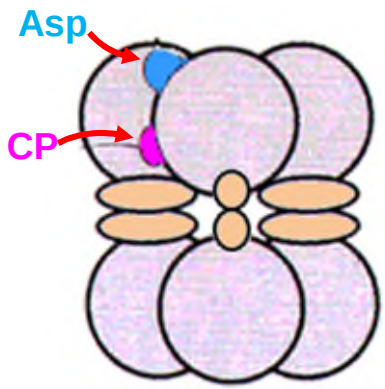
homotropic allosterism (Asp and Carbamoyl phosphate co-substrates)

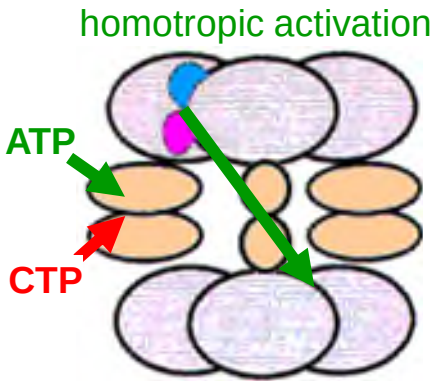
2 catalytic subunits (C)
(3 α chains each)

3 regulatory subunits (R)
(2 β chains each)

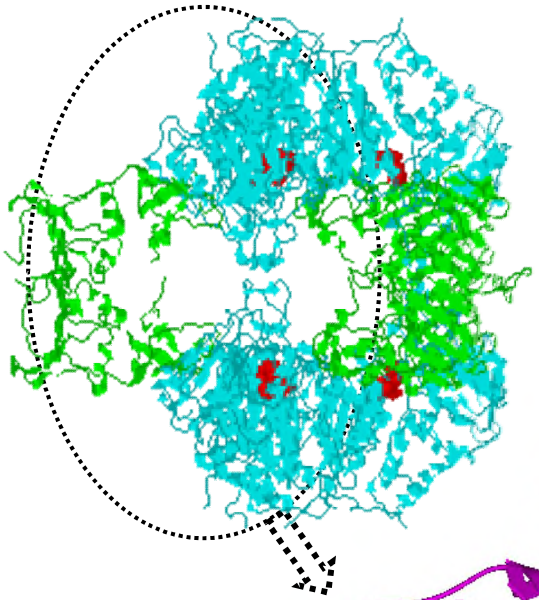
6 active sites at the interface of α-chains

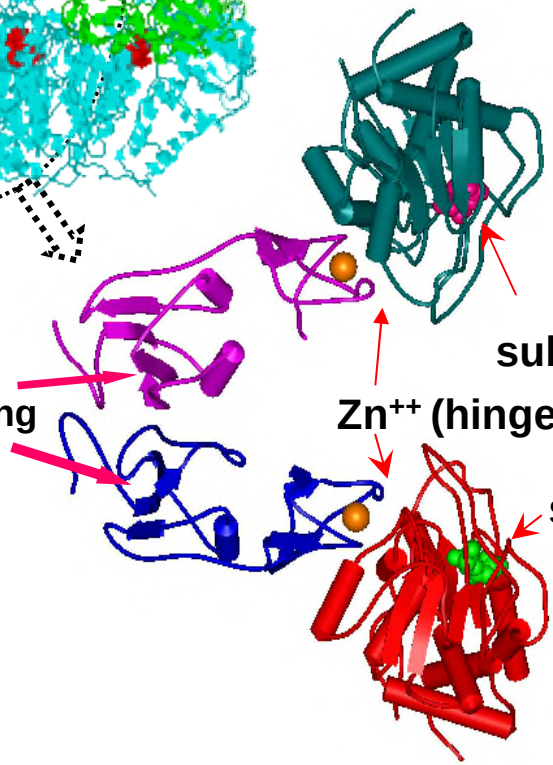






Mecchanism of *E. coli* *ATCase* allosteric control

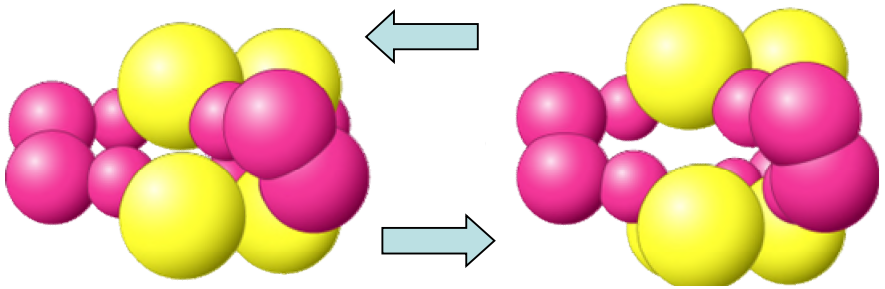




T (tense) state
(less active)

Heterotropic inhibition (CTP)

R (relaxed) state
(more active)



Homotropic activation (Asp, CP)
Heterotropic activation (ATP)

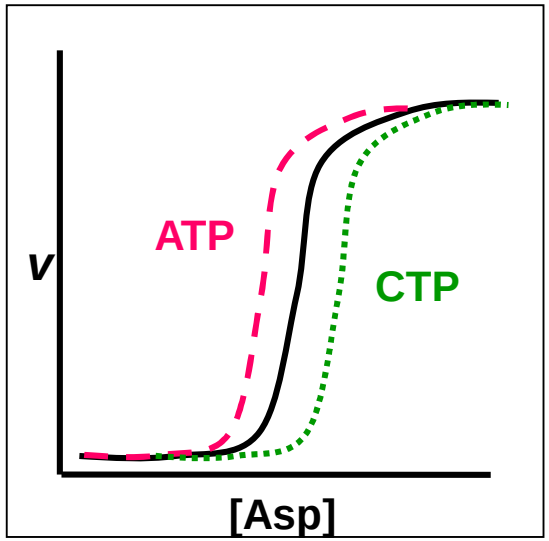
Allosteric effector binding site

ATP ↑
CTP ↓

substrate

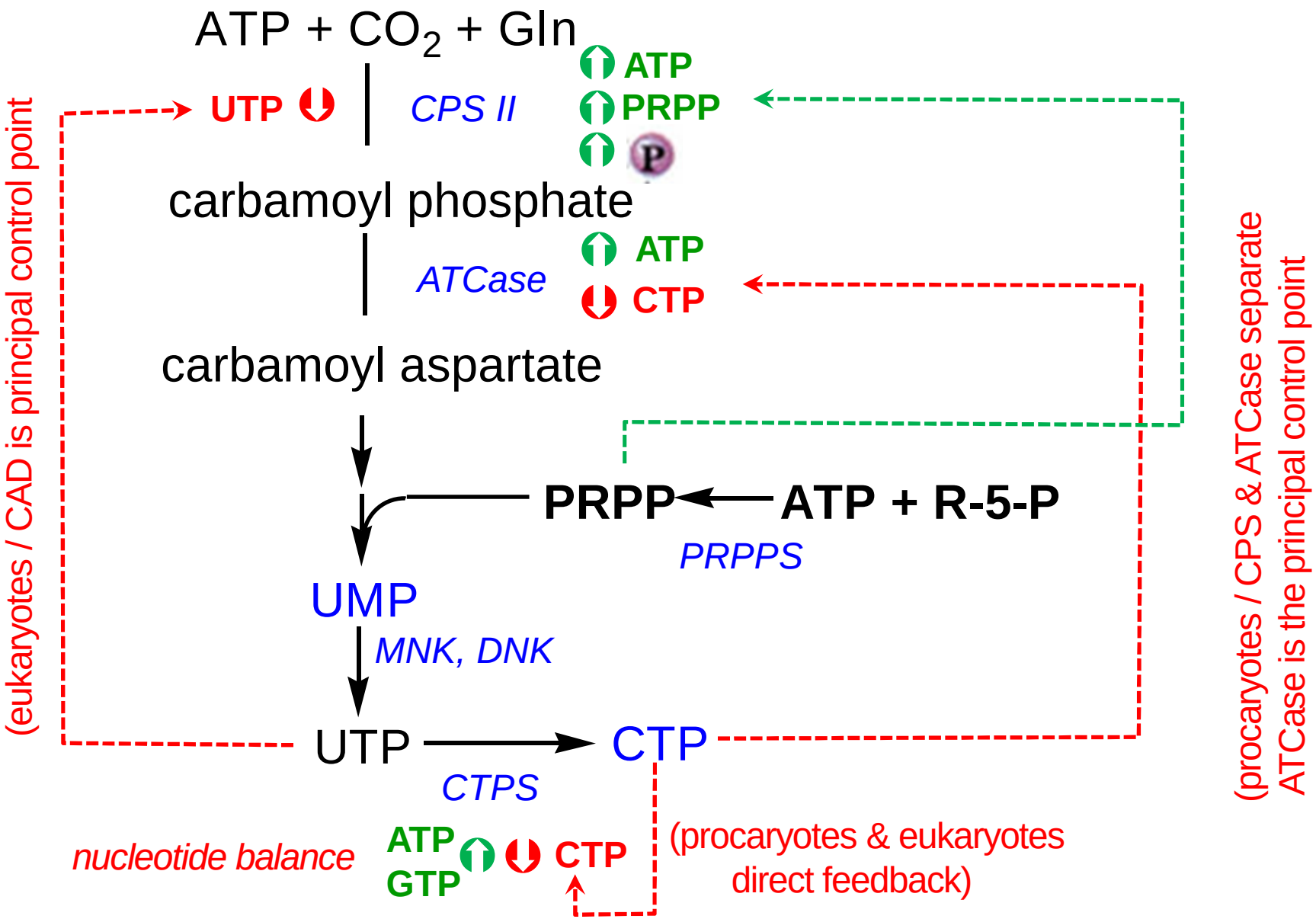
Zn^{++} (hinge, structural role)

substrate

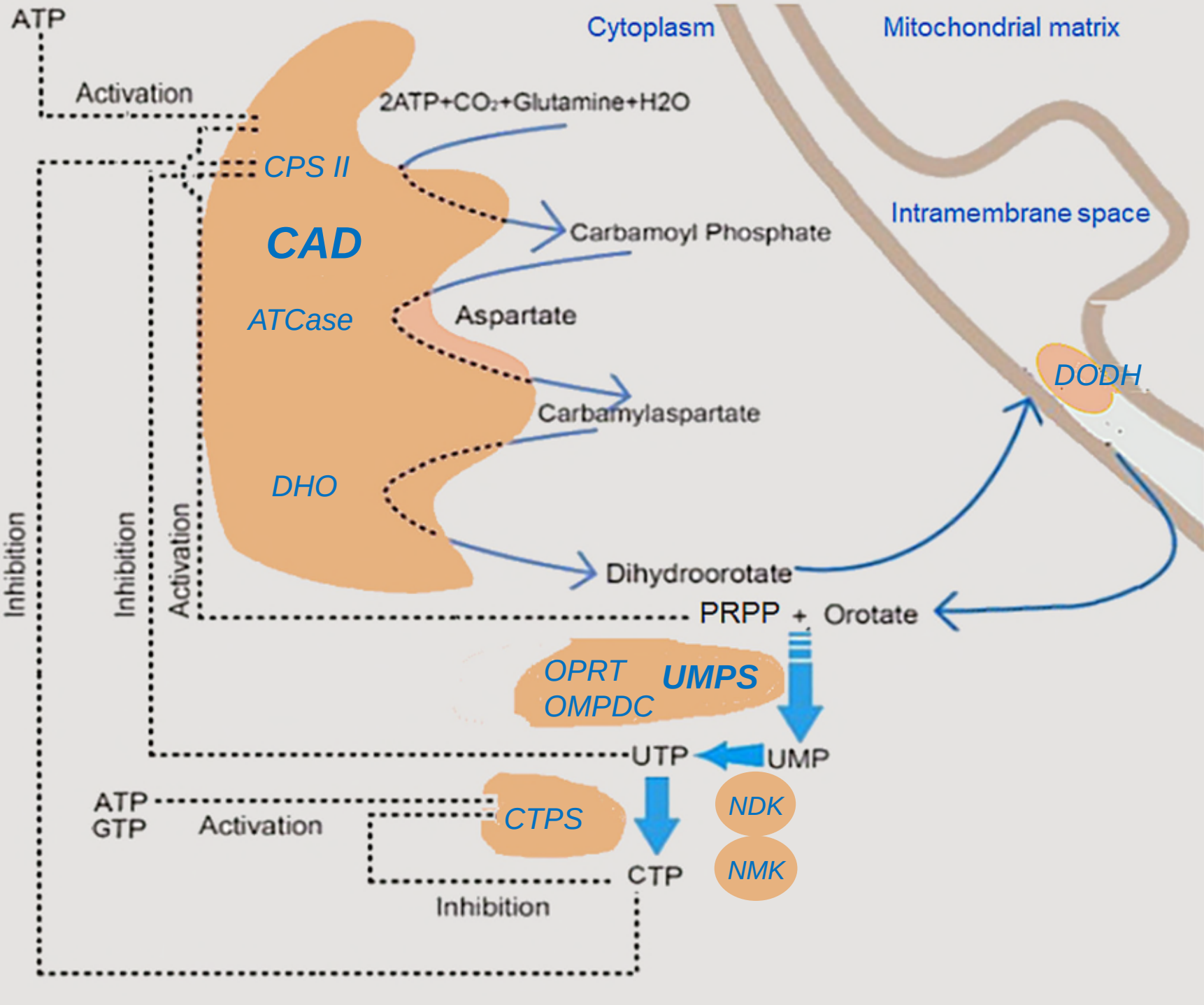


- **Sigmoidal behaviour** of *v* with respect to [S]
- ATP shifts curve towards lower [S], CTP shifts curve towards higher [S]

REGULATION OF THE *DE NOVO* PYRIMIDINE NT SYNTHESIS PATHWAY



EUKARYOTIC PYRIMIDINE NUCLEOTIDE *DE NOVO* SYNTHESIS - PATHWAY and REGULATION SUMMARY

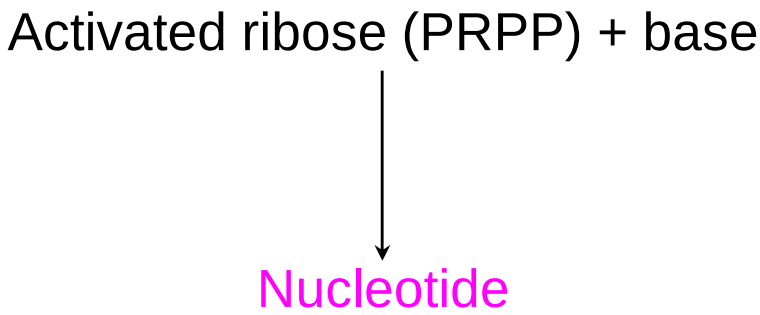


SALVAGE PATWAYS FOR NUCLEOTIDE SYNTHESIS

Salvage pathways

- Pathways in which a biological product is **produced from intermediates in the degradative pathway** of its own or a similar substances
- The term often refers to **nucleotide salvage** in particular, in which purine or pyrimidine nucleotides are synthesized from intermediates in their degradative pathway
- They **recover bases and nucleosides** formed during degradation of RNA and DNA.
- They are particularly important for some **tissues that don't have *de novo* synthesis**.
- Salvage pathways are **targets for drug development**.

NUCLEOTIDE SALVAGE PATHWAY



Nucleotide synthesis preference by cell type

Cell Type / Category	Primary Pathway	Why
Rapidly Proliferating Cells (embryonic, bone marrow, gut epithelia)	*De Novo*	- high demand for NT to support DNA replication
Hepatocytes	*De Novo*	- major site of *DN* synthesis - supplies NT to other tissues
Neoplastic cells (cancers, tumors)	*De Novo* & Salvage	← for rapid growth ← for metabolic flexibility
Mature Erythrocytes (RBCs)	Salvage only	- lack *PRPP amidotransferase* - recylce bases from blood
SNC / mature neurons	Salvage	- low *DN* activity - receive salvage precursors from liver
Differentiated/Resting Cells (muscle, bone, adipocytes, etc.)	Salvage	- low DNA synthesis
Immune Cells (leukocytes)	Salvage	- critical for maintaining NT pools

SALVAGE PATWAYS FOR PURINE NUCLEOTIDES

- **Source of purine bases**
  - degradation of endogenous RNA/DNA
  - degradation of exogenous nucleic acids derived from the diet
- **Direct conversion to corresponding nucleotides by linking to ribose (PRPP)**
- **The salvage pathway is significant because:**
  - It allows to save energy
  - It is the only way that some tissues (e.g. erythrocytes, brain and bone marrow) can synthesize nucleotides.
- **Two *Phosphoribosyl transferases* are involved:**
  - For Adenine: *APRT* (*Adenine phosphoribosyl transferase*).
  - For Guanine: *HGPRT* (*hypoxanthine-guanine phosphoribosyl transferase*) (or Hypoxanthine)

SALVAGE PATWAYS FOR PURINE NUCLEOTIDES

Purine + PRPP ⟶ Purine nucleotide + PPi

1) Adenine + PRPP ⟶ AMP + PP_i

APRT *(Adenine phosphoribosyl transferase)*

– **less important** as Adenine can be recovered also from Hypoxanthine

2) Hypoxanthine + PRPP ⟶ IMP + PP_i —*DN*→ **AMP** (*slide 13*)

HGPRT

DN ↓

Guanine + PRPP ⟶ GMP + PP_i

(Hypoxanthine/Guanine phosphoribosyl transferase)

– *HGPRT* is regulated by its own products - inhibited by both IMP and GMP.

3) AMP —*D* (*Nucleotidase*)→ **Adenosine + Pi** —*D* (*Adenosine deaminase*)→ **Inosine** —*D* (*Purine nucleoside phosphorylase*)→ **Hypoxanthine**

Hypoxanthine —*S* / *HGPRT*→ **IMP**

IMP —*DN* / *IMPDH* / *GMPS*→ **GMP**

– AMP can be converted to GMP by combining Degradation (*D*), Salvage (*S*) and *De Novo* (*DN*) pathways

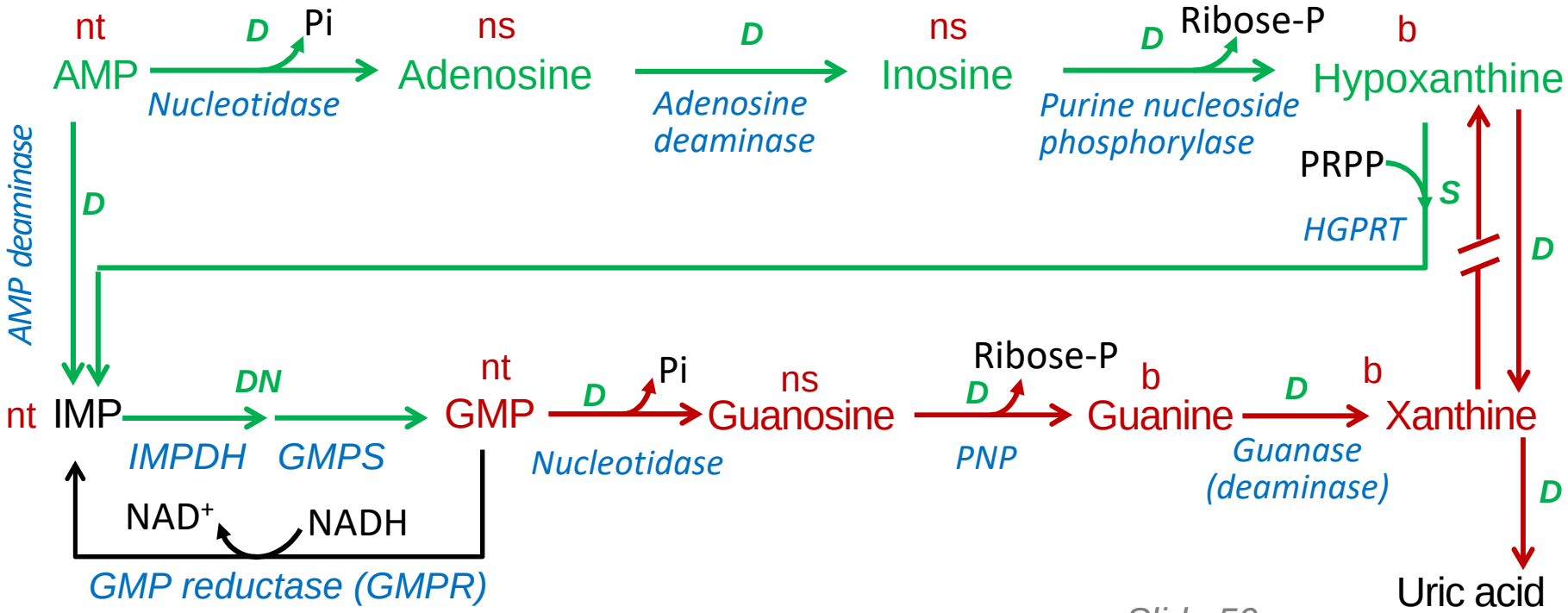
Slide 13

Slide 50

INTERCONVERSION OF PURINE NUCLEOTIDES (see also slide 14)

AMP & GMP can be interconverted, but the process is not symmetrical

– **For AMP** this involves using degradative (catabolic), salvage and *de novo* reactions



Slide 50

– **For GMP**, xanthine (a crucial catabolic intermediate) cannot be converted to hypoxanthine

– GMP can be directly converted to IMP, but is costly and not a major pathway in humans

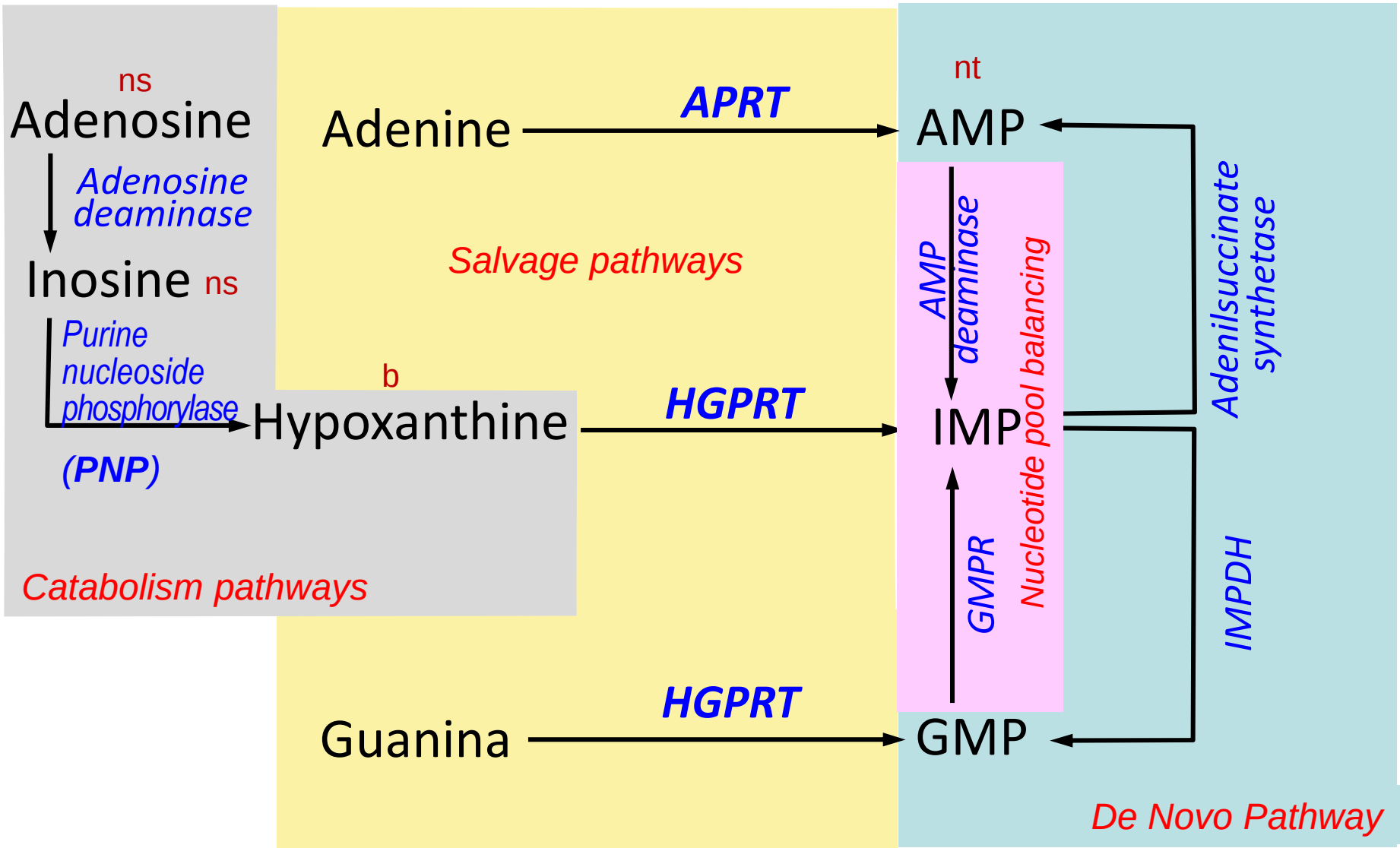
– *GMPR* is primarily involved in nucleotide pool balancing, not salvage or degradation.

DN = de novo, S = salvage, D = degradative PB = pool balancing

nt = nucleotide, ns = nucleoside, b = base

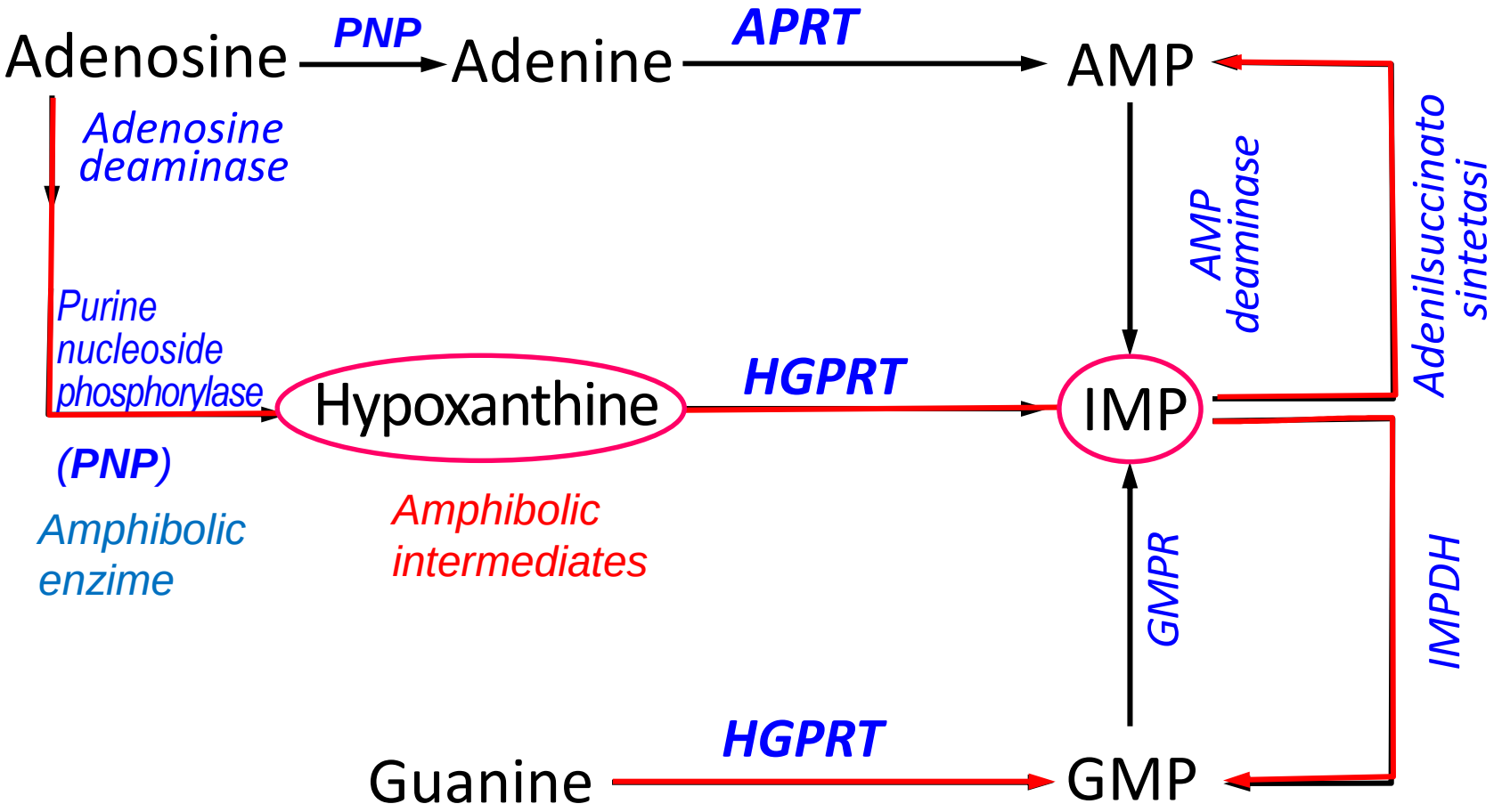
SALVAGE, DE NOVO & DEGRADATIVE PATHWAYS ARE CONNECTED

- **Different pathways connect through some common intermediates**



SALVAGE PATHWAY – IMPORTANCE OF *HGPRT*

- **Hypoxanthine & IMP are key intermediates in purine nucleotide metabolism**



Carenza o scarsa attività di *HGPRT* → sindrome di Lesch-Nyhan.

SALVAGE PATWAYS FOR PYRIMIDINE NUCLEOTIDES

- **There are three alternative salvage pathways**

A) Starting from the nucleoside

(d)Nucleoside + ATP ⟶ (d)Ribonucleotide monophosphate + ADP

Nucleoside kinase *(NK)*

uridine / cytidine + ATP ⟶ UMP / CMP + ADP

Uridine-cytidine kinase

(deoxy)thymidine + ATP ⟶ TMP + ADP

Thymidine kinase

deoxycitidine + ATP ⟶ dCMP +ADP

Deoxycytidine kinase

NB: Nucleoside + ATP → NMP	***Nucleoside kinase[1]***	***NK***
NMP + ATP → NDP	***Nucleoside monophosphate kinase[1]***	***NMK***
NDP + NTP → NTP	***Nucleoside diphosphate kinase[2]***	***NDK***

[1] Specific – targeted metabolic reactions

[2] Nonspecific – free exchange of NTPs and NDPs

B) Starting from bases → nucleoside → A) NMP **active in humans**

Base + (d)Ribose-**1**-P ⟶ (d)Ribonucleoside + P_i

Phosphorylase

Uracil + Ribose-1-P ⟶ Uridine + P_i

Uridine phosphorylase

Timina + dRibosio-1-P ⟶ dThymidine + P_i

Thymidine phosphorylase

C) Starting from bases → nucleotide (similar to purines) **limited in humans**

Base + PRPP ⟶ NMP + ADP

Ribosyl transferase

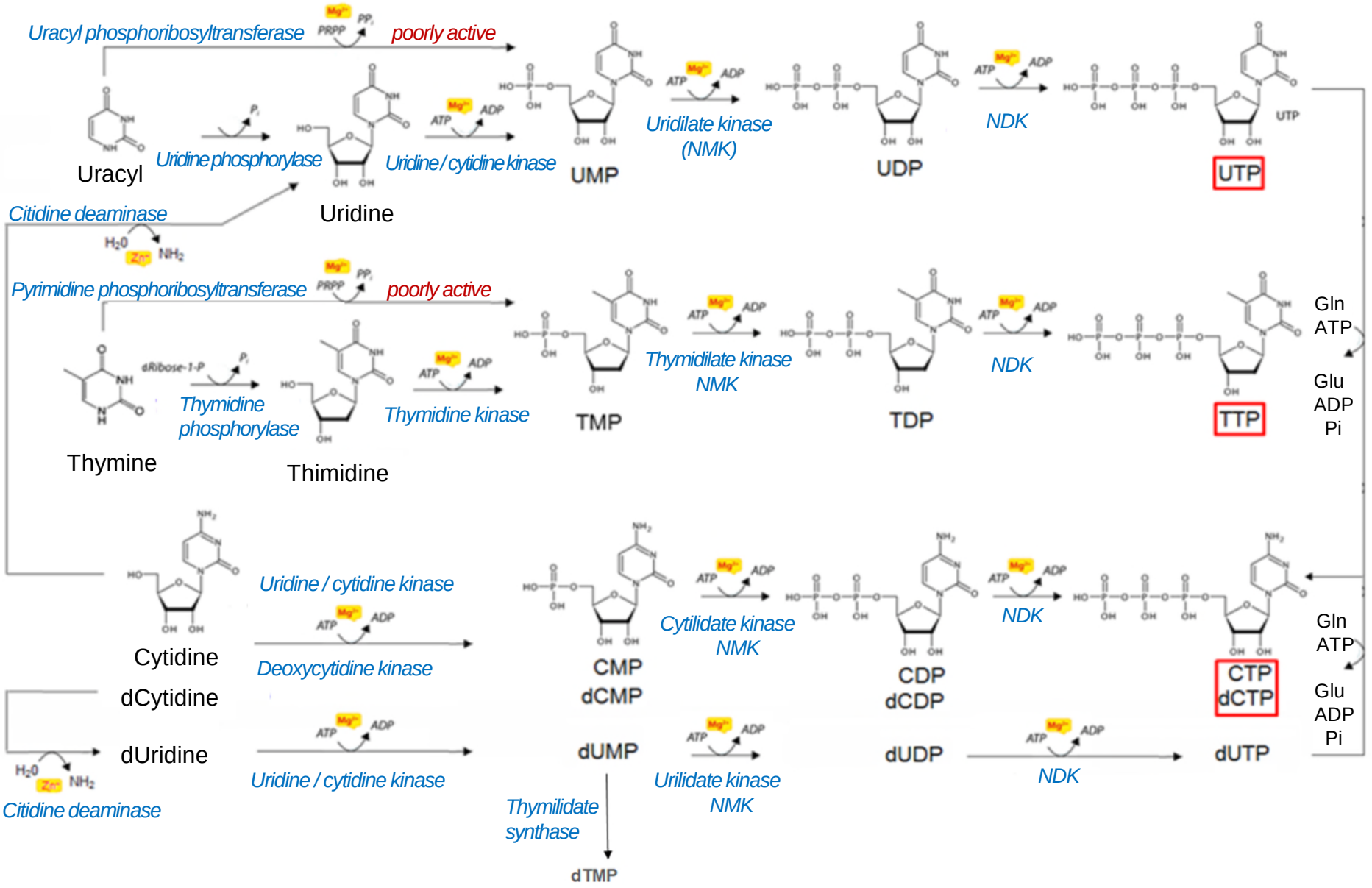
Uracyl + PRPP ⟶ UMP + PPi **limited in humans**

Uracyl phosphoribosyltransferase

Orotate + PRPP ⟶ OMP + PPi **active in humans,**

Pyrimidine phosphoribosyltransferase **link to *De Novo* PW**

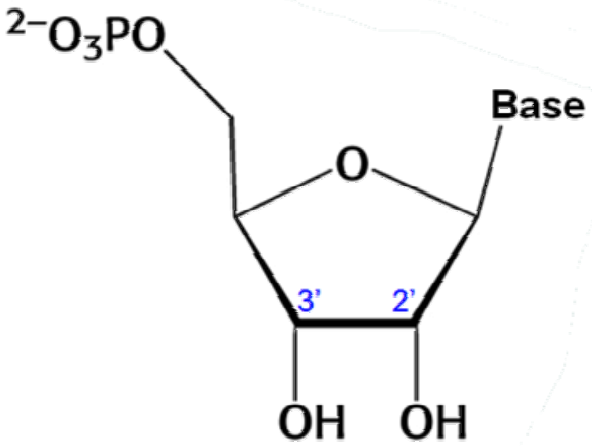
PYRIMIDINE NUCLEOTIDE RECOVERY PATHWAYS (SUMMARY)



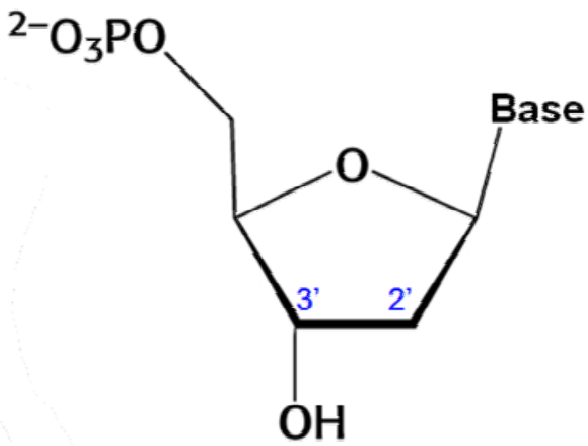
(Slide 47)

BIOSYNTHESIS OF DEOXYRIBONUCLEOTIDES

- This involves the reduction of ribose at C2' in NDP (ADP, GDP, CDP, UDP).



Ribose-P

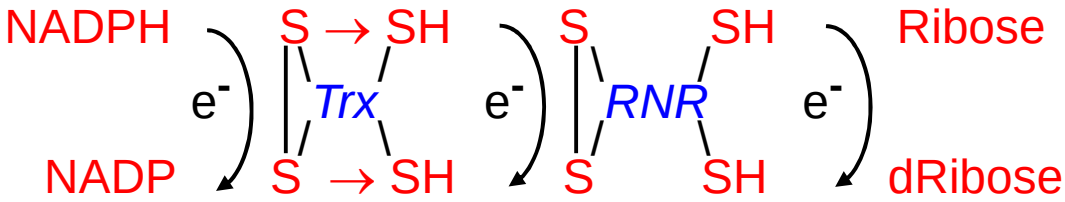


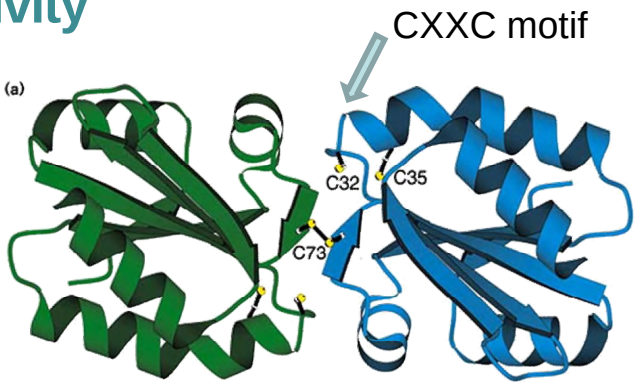
Deoxyribose-P

- A **single enzyme**, ***Ribonucleotide reductase***, catalyzes the reaction
- It appears to be a single enzyme capable of **acting on all four ribonucleotides**
- This enzyme is therefore **essential for DNA biogenesis** and **proofreading**.

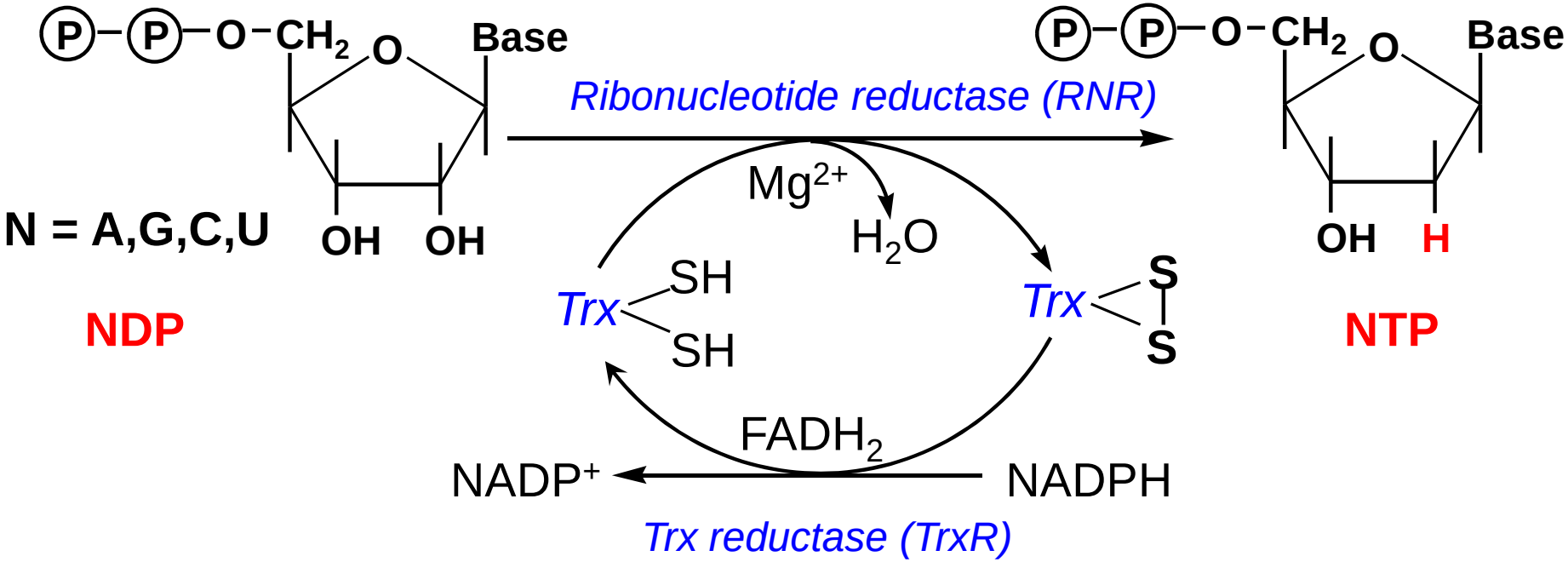
NUCLEOTIDE REDUCTION OCCURS IN NDPs

- **Removal of OH at C2' requires tandem enzyme activity**
  - ***Ribonucleotide reductase (RNR)*** **+** *Thioredoxin (Trx)* **+** *Trx reductase* (oxidation-reduction of S-S bridges)

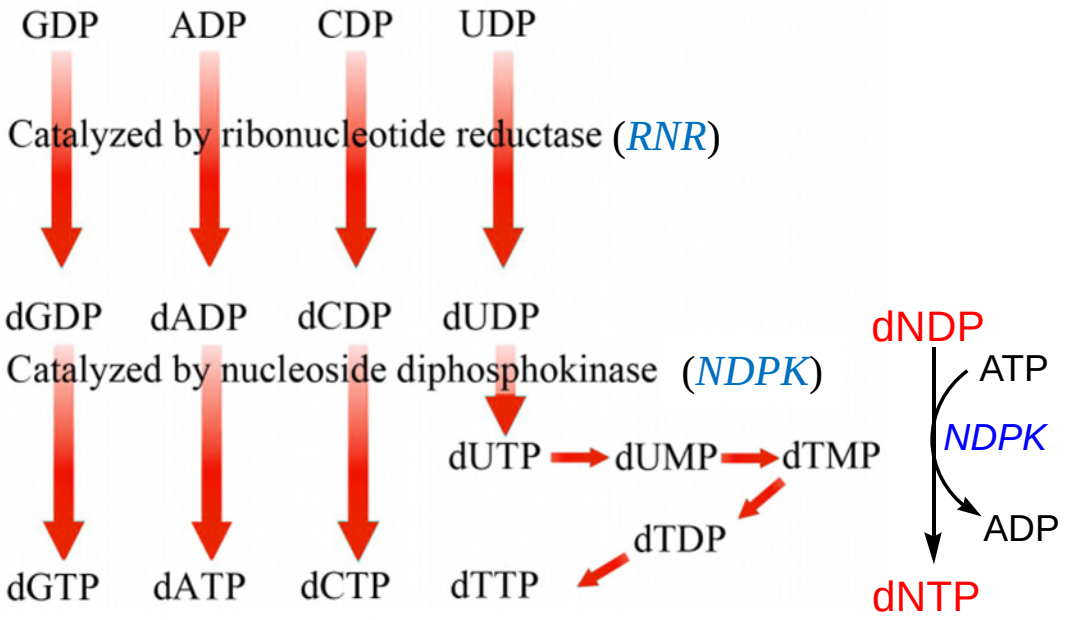


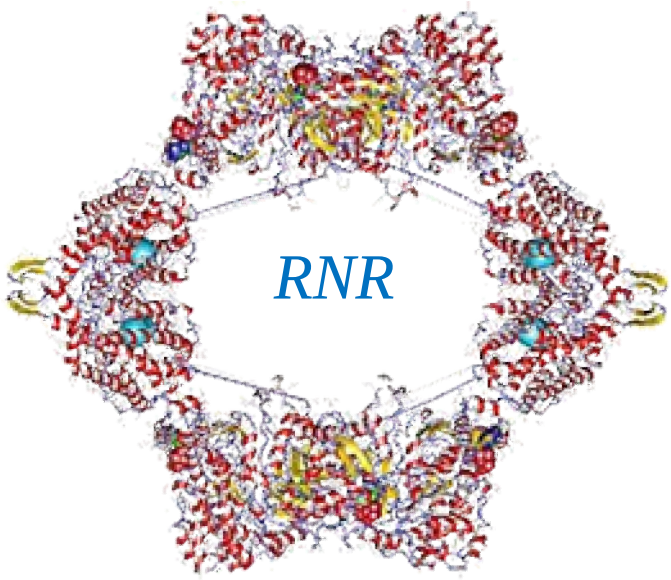


Thioredoxin (Trx)



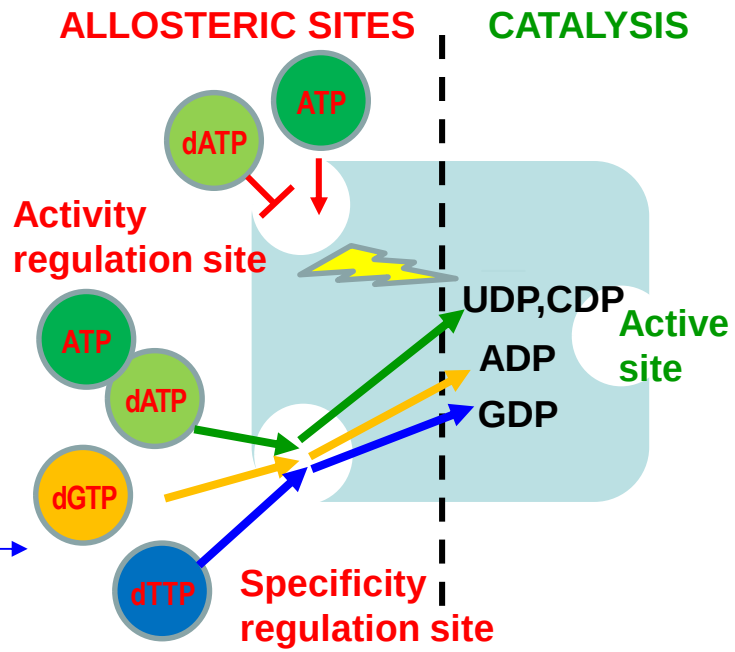
BIOSYNTHESIS OF dNTP





Regulation of *RNR*

- *Ribonucleotide reductase* is allosterically regulated via two binding sites:
- activity binding site (control of enzyme efficiency; ATP ↑, dATP ↓)
- substrate specificity binding site dNTPs control substrate binding with a reciprocal balancing effect



MODE-of-ACTION of *RIBONUCLEOTIDE REDUCTASE*

Ribonucleotide reductases **use a stable radical (e.g. Tyr•) to form a Cys radical**

– The thiyl radical* -S• participates with two reduced Cys residues in the mechanism of action

❶ *Radical transfer* to the sugar C³’ ❷ a reduced Cys transfers a proton to the hydroxyl in C²’

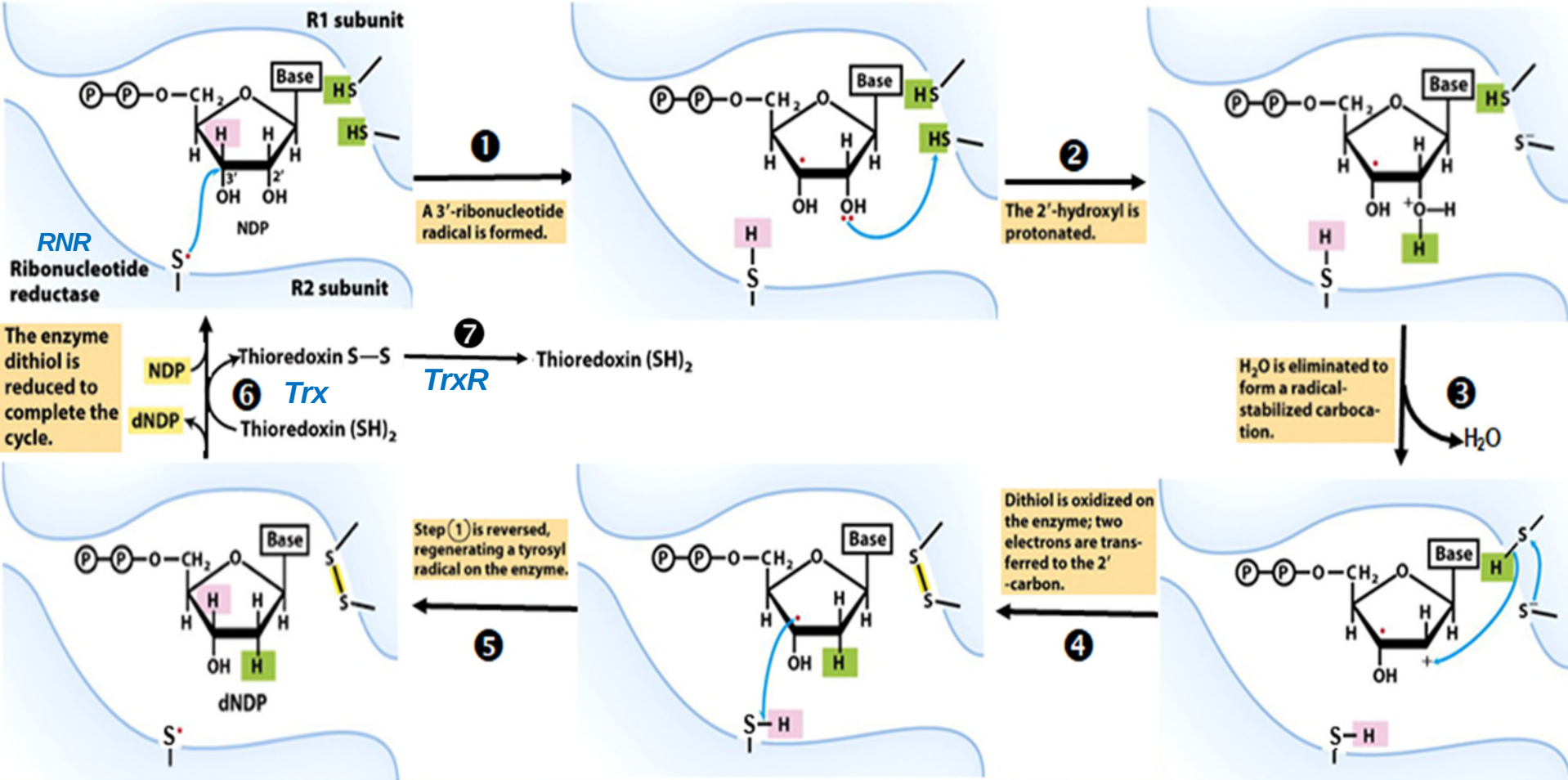
❸ Elimination of H_2O leaves a carbocation ❹ a second reduced Cys transfers a proton

– The complete oxidation of the thiols transfers $2e^-$ and H^+ to C²’ and allows formation of an S-S bridge)

❺ The radical is transferred back to *RNR* ❻ *Trx* reduces S-S bridge and the cycle can restart

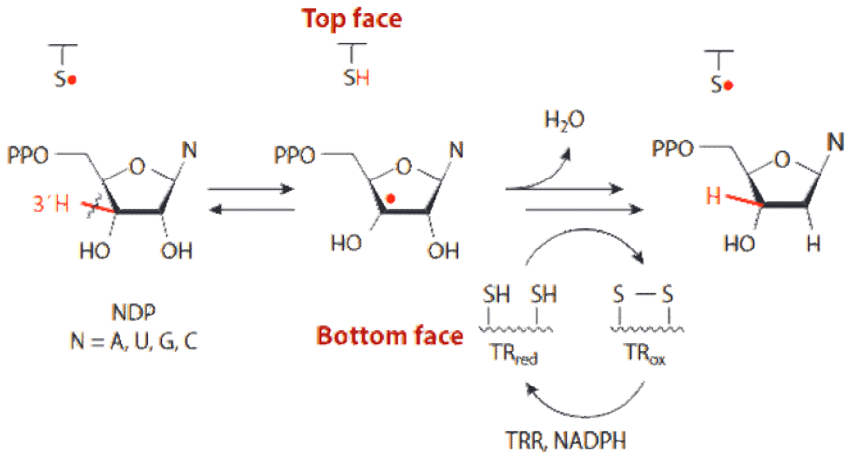
❼ *Trx* in turn is reduced by *TrxR* using reductive potential provided by NADPH

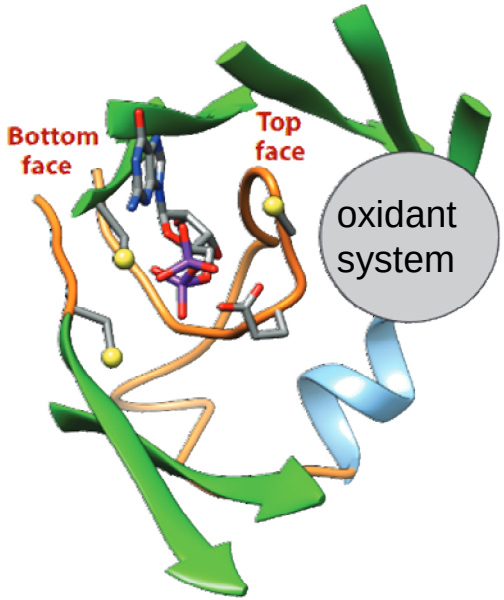
**radicale tiilico*



DIFFERENT *RIBONUCLEOTIDE REDUCTASES* IN DIFFERENT ORGANISMS

Common mechanism





3 classes of *RR*

- Class I (eukaryotes, bacteria)
- Class II & III (bacteria)

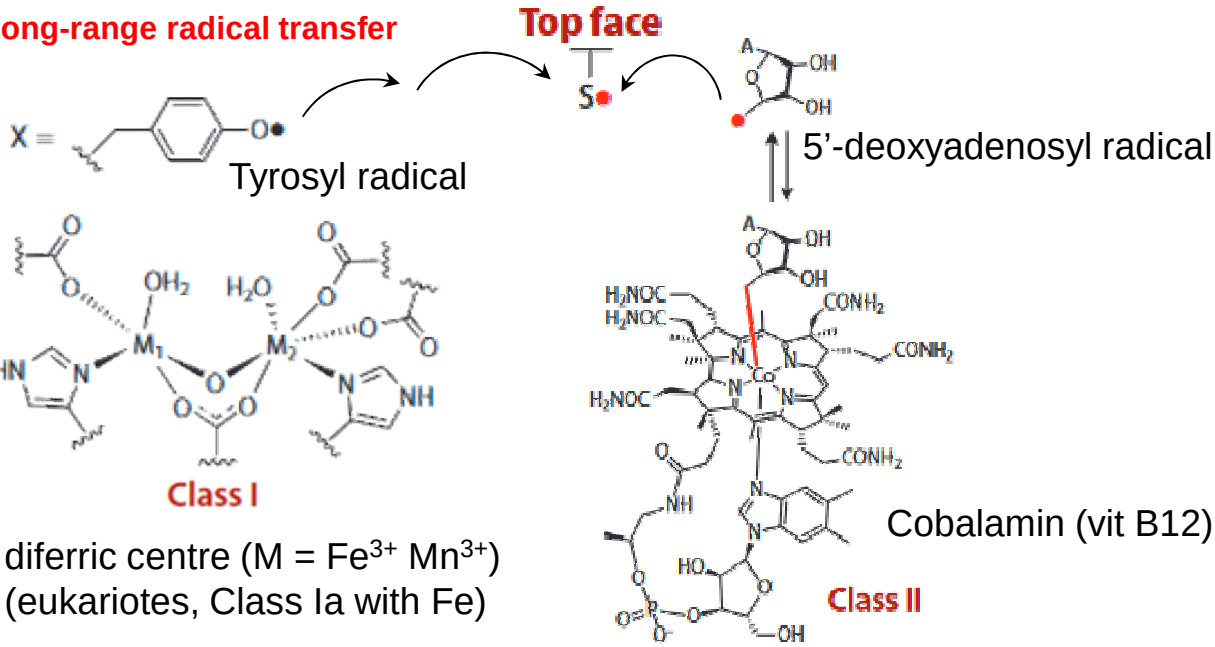
Class I – aerobic

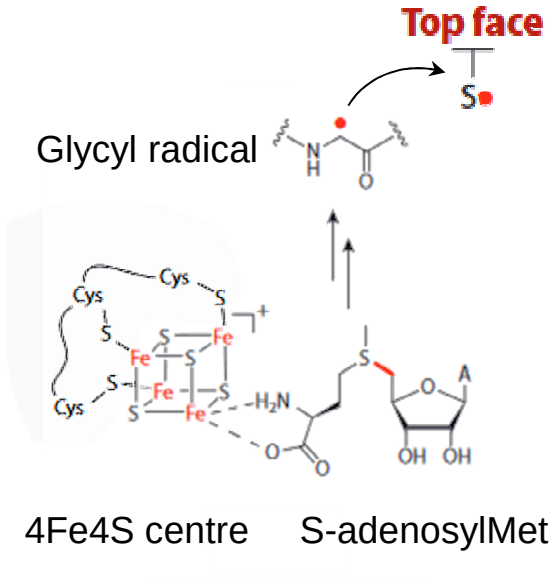
Class II – O_2 independent

Class II – anaerobic

Different oxidant systems

Long-range radical transfer

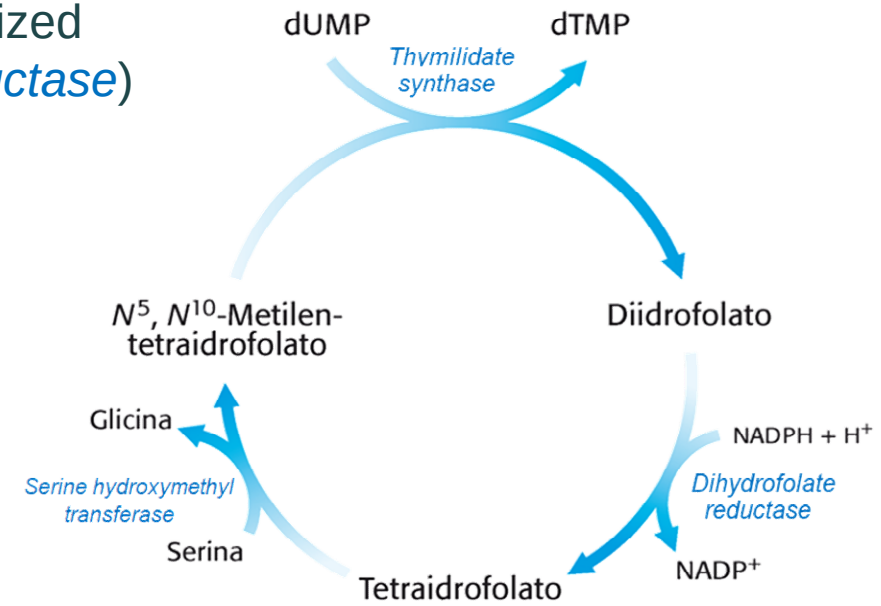




•–SH reformation: *TRX* + *TRXR* or else *Glutaredoxin (GRX)* + glutathione (GSH)

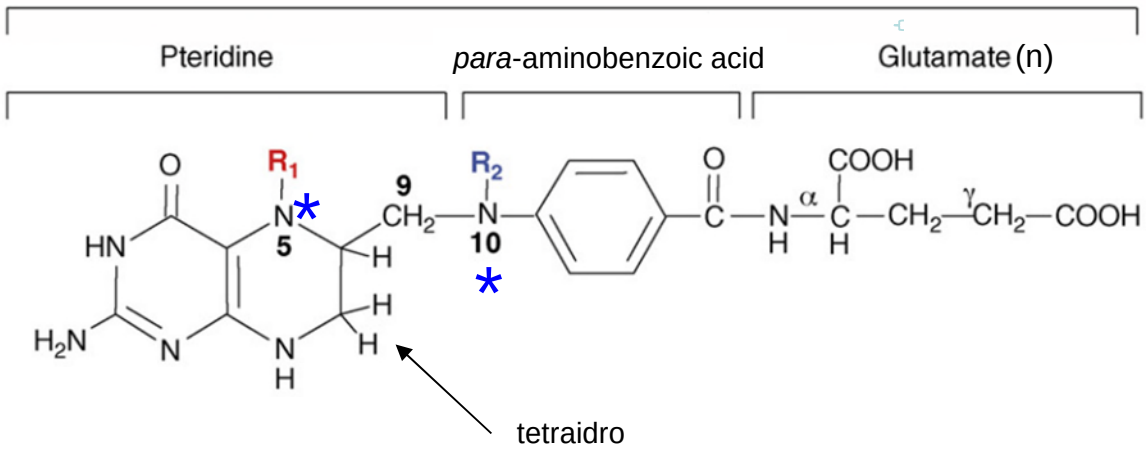
TMP SYNTHESIS

- **The synthesis of dTMP requires methylenetetrahydrofolate (*N*5,*N*10-CH_2-THF)**
  - *Thymidilate synthase* transfers the methylene onto the base in UMP
  - The remaining dihydrofolate must be re-oxidized to tetrahydrofolate (THF) (*Dihydrofolate reductase*) and the methylene group added from serine (*Serine hydroxymethyl transferase*)
  - In effect, in this context, THF acts both to *donate a carbon unit* and to *donate e⁻* (reducing power)



Tetrahydrofolic acid (THF, Vit B_9)

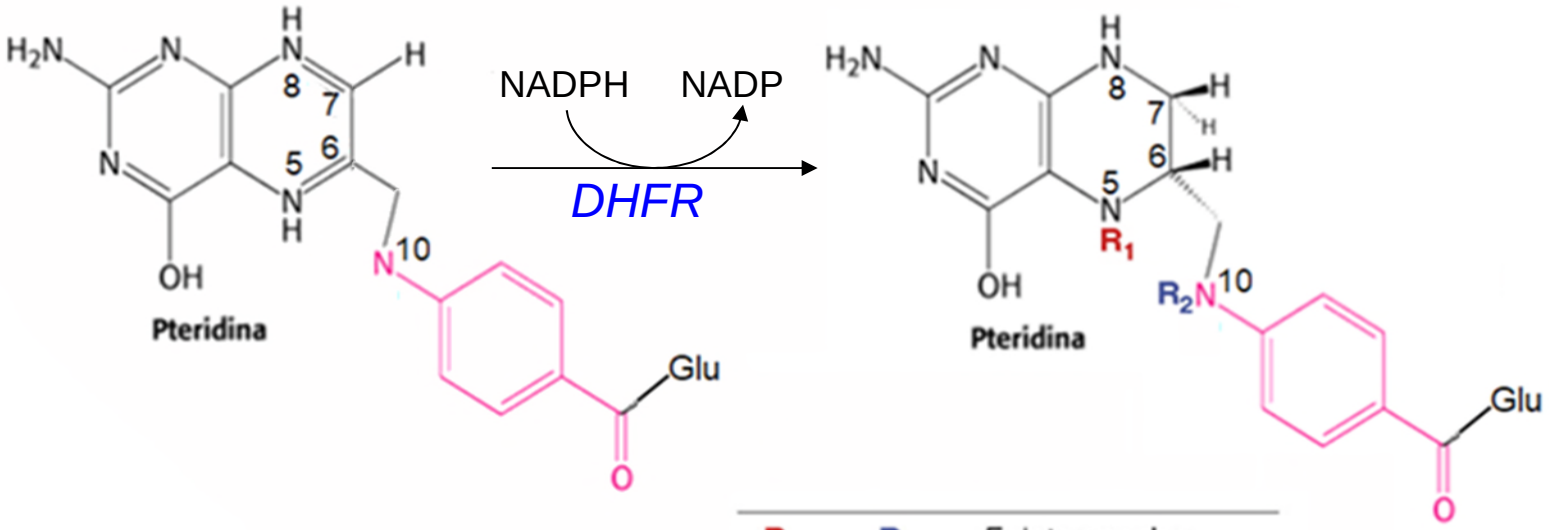
(THF)



- A complex of essential vitamins
- RDA: 0.2 – 0.8 mg
- Required both for nucleotide and amino acid metabolism
- Required for cell division

TETRAIDROFOLATO (THF, FH_4)

- **Synthesis of THF requires reduction of double bonds 5-6 e 7-8 in pteridine**
- *Dihydrofolate reductase* (*DHFR*) uses NADPH to first reduce the 7-8 double bond and then another NADPH to reduce the 5-6 double bond
- THF can then bind single-carbon units (methyl, formyl) to N^5 and/or to N^{10}



R_1	R_2	Folate species
H	H	THF
CHO	H	5-formyl-THF
H	CHO	10-formyl-THF
CH_3	H	5-methyl-THF
$-CH_2-$		5,10-methylene-THF
$=CH-$		5,10-methenyl-THF

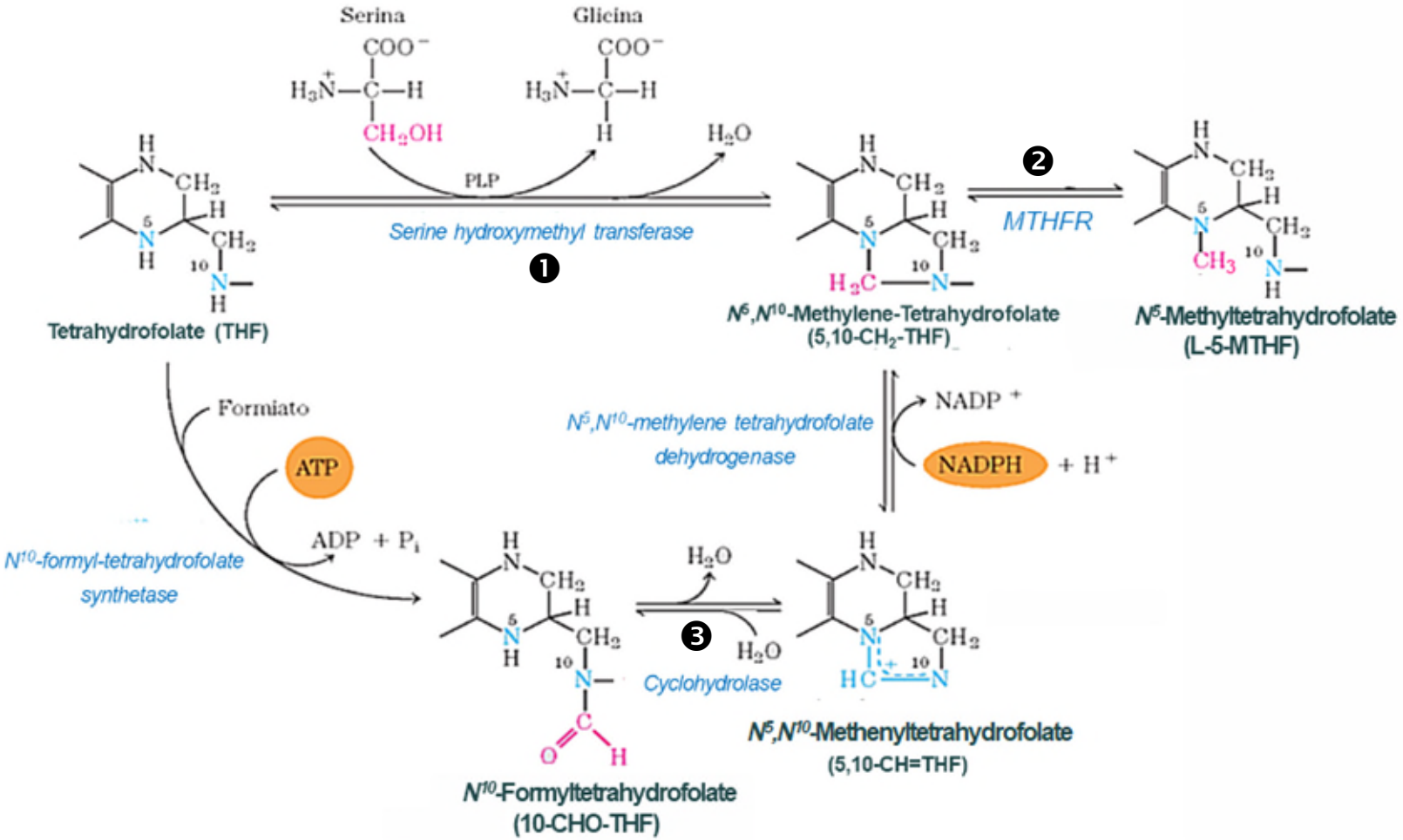
LOADING SINGLE-CARBON UNITS ONTO THF

❶ *Serine hydroxymethyltransferase* uses PLP to transfer CH_3 to THF $N^{5,10}$ forming 5,10-CH_2-THF

❷ 5,10-CH_2-THF is converted by a *Reductase (MTHFR)* to *N*^{5}-methylTHF (used in TMP synth.)

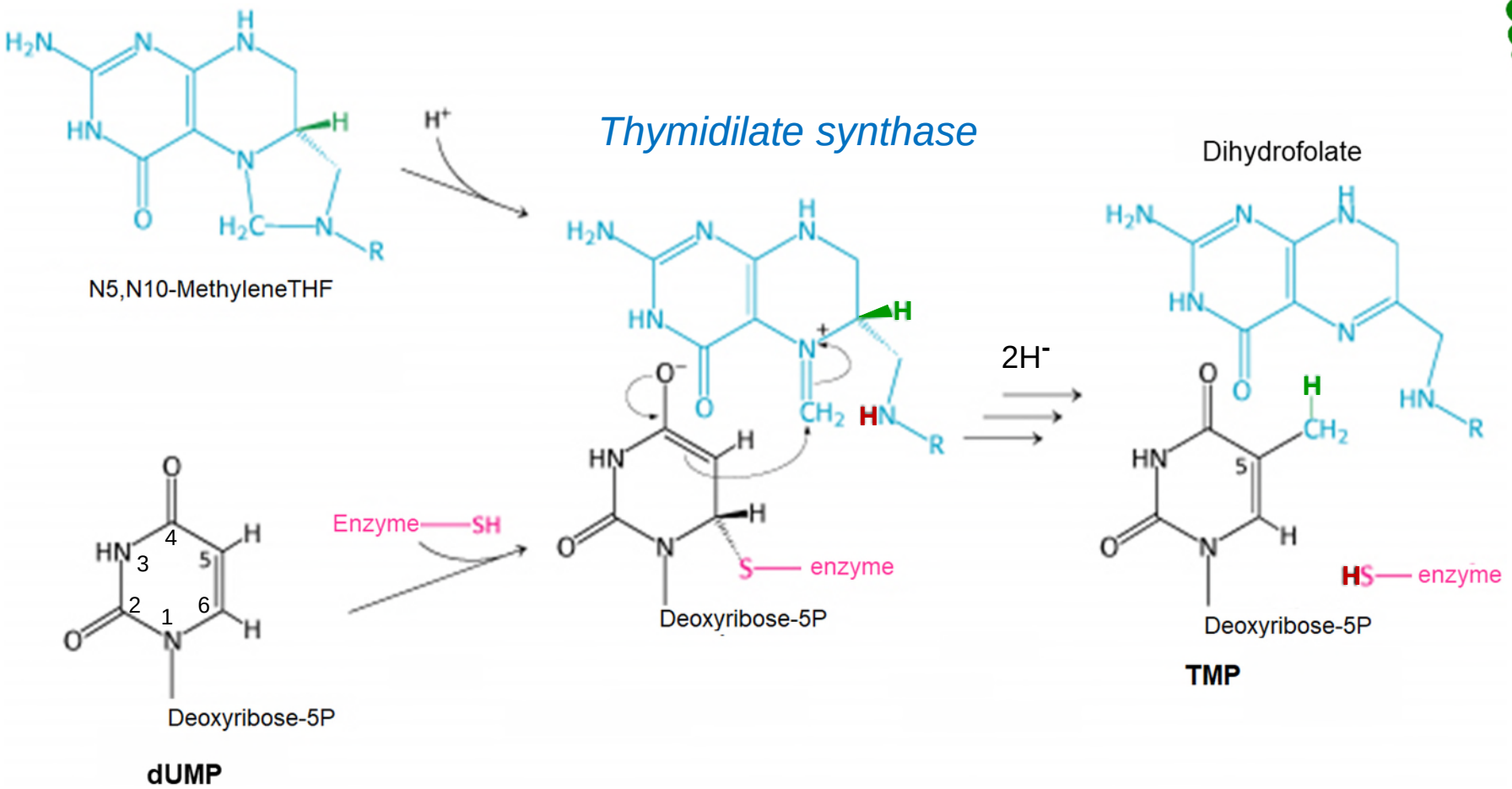
❸ 5,10-CH_2-THF can also be converted to MethenylTHF (5,10-CH=THF) by *MTDH* and then to *N*^{10}-FormylTHF (used in De Novo pathways) by *MethenylTHF dehydrogenase* (*MTHFD*, a *Cyclohydrolase*)

❹ *N*^{10}-FormylTHF can also be synthesised form THF by *N10-FormylTHF synthetase,* leading to 5,10-CH_2-THF



SYNTHESYS OF TMP

- ***Thymidylate synthase* modifies dUMP**
  - in the active site, dUMP binds to a Cys residue at C6 and an oxyanion forms
  - In the active site 5,10-CH_2-THF tautomerizes to from the iminium cation
  - This favours methyl transfer to the double bond at C5 of the base and DHF forms
  - This is the only *de novo* pathway for TMP biosynthesis – important drug target



NUCLEOTIDE BIOSYNTHESIS: AN OVERVIEW

1 *Ribonucleotide reductase* (acts on N**D**P)

2 *CTP synthase* →→ CTP

4 *Nucleoside monophosphate kinase* (base specific)

3 *Thymidilate synthase* dTTP

5 *Nucleoside dinophosphate kinase* (aspecific) → (d)ATP, (d)GTP, UTP →→ dCTP

6 *dUTP diphosphohydrolase*

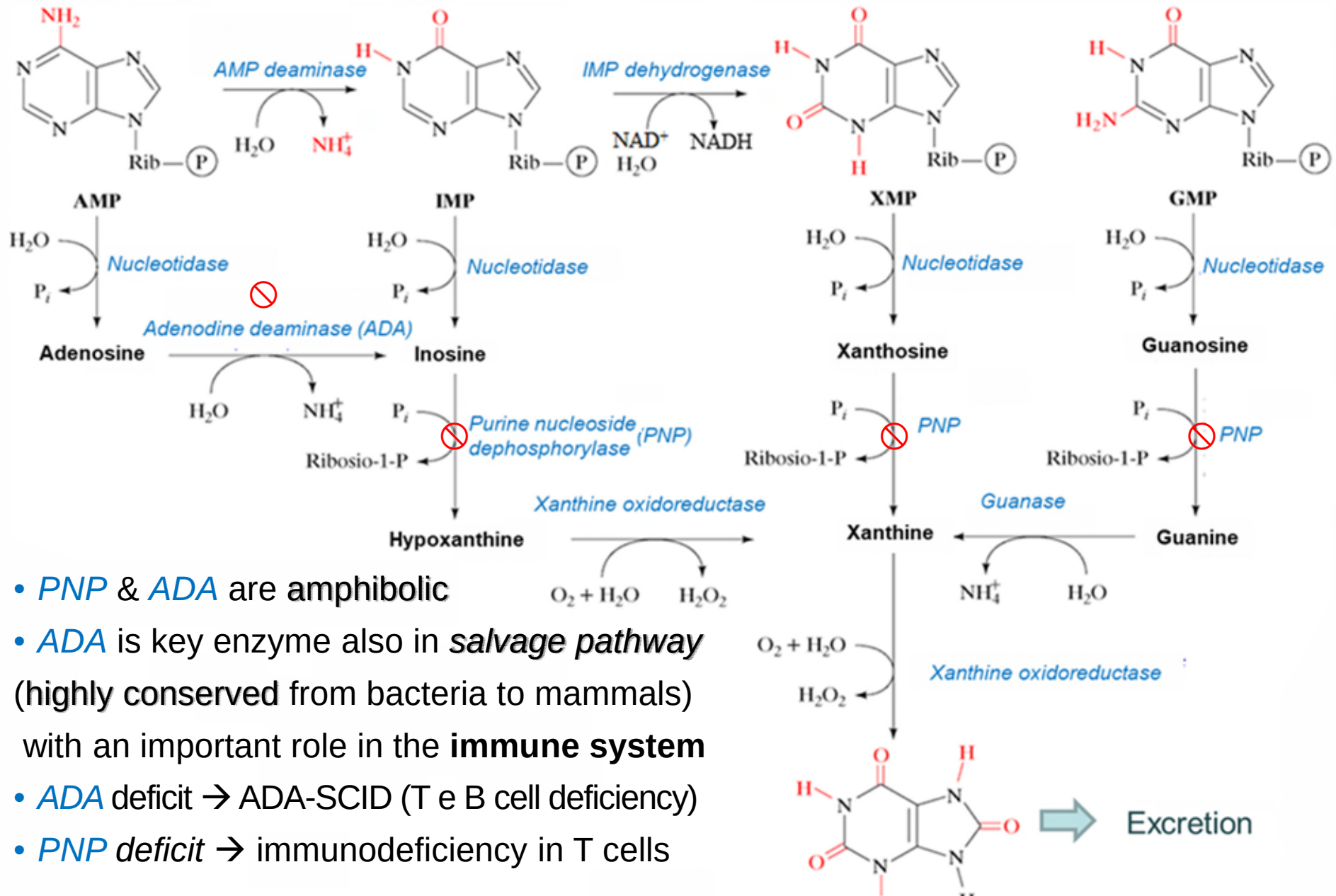
PURINE NUCLEOTIDE CATABOLISM

- **Degradaton of purine nucleotides requires several different enzymes**

- GMP, IMP, XMP and AMP are **dephosphorylated** by ***Nucleotidases*** forming the respective **nucleosides**; Adenosine, Inosine, Xantosine and Guanosine
- AMP can be **deaminated to IMP** by ***AMP deaminase (ADA)***
- IMP can be **converted to XMP** by ***IMP dehydrogenase***
- Adenosine can be deaminated to Inosine by ***Adenosine deaminase***
- Inosine, Xanthosine & Guanosine: bases are removed form ribose by ***Purine nucleoside phosphorylase*** (***PNP***) (**phosphorolysis)**, releasing Ribose-1-P and the bases **Hypoxanthine, Xanthine** and **Guanine**
- Guanine is deaminated by ***Guanine deaminase (Guanase)*** ↘
- Hypoxanthine is oxidized by ***Xanthine oxidoreductase*** ↗ to **Xanthine**
- Xanthine is converted by *Xanthine oxidase* to **Uric acid**, which is excreted

NMP → Nucleosides (I, X, G) → Ribose-1-P + Bases → Xanthine → Uric Acid

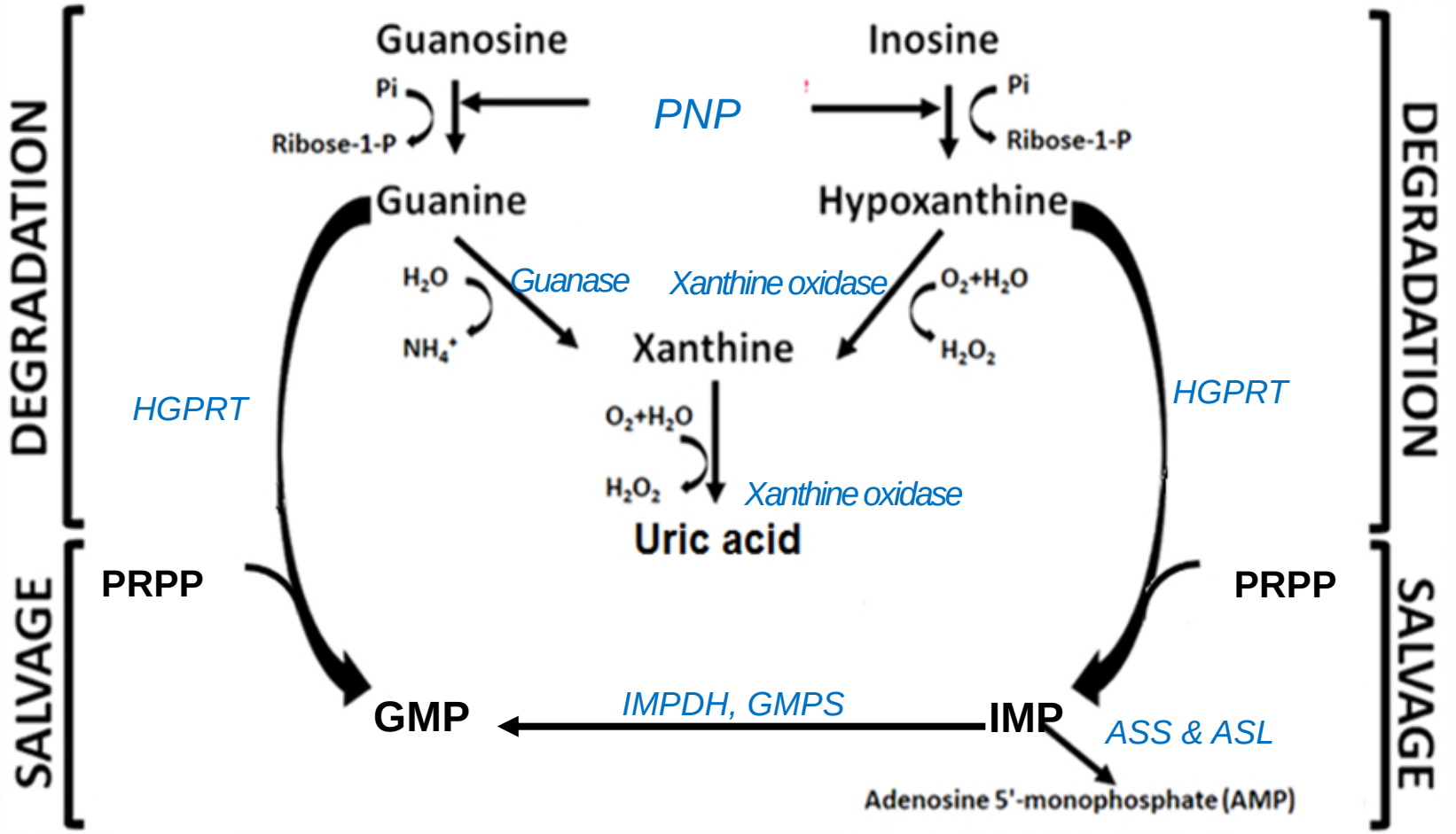
PURINE NUCLEOTIDE CATABOLISM- OVERVIEW



- *PNP* & *ADA* are amphibolic
- *ADA* is key enzyme also in *salvage pathway* (highly conserved from bacteria to mammals) with an important role in the **immune system**
- *ADA* deficit → ADA-SCID (T e B cell deficiency)
- *PNP deficit* → immunodeficiency in T cells

ROLE OF PURINE NUCLEOTIDE CATABOLIC ENZYMES

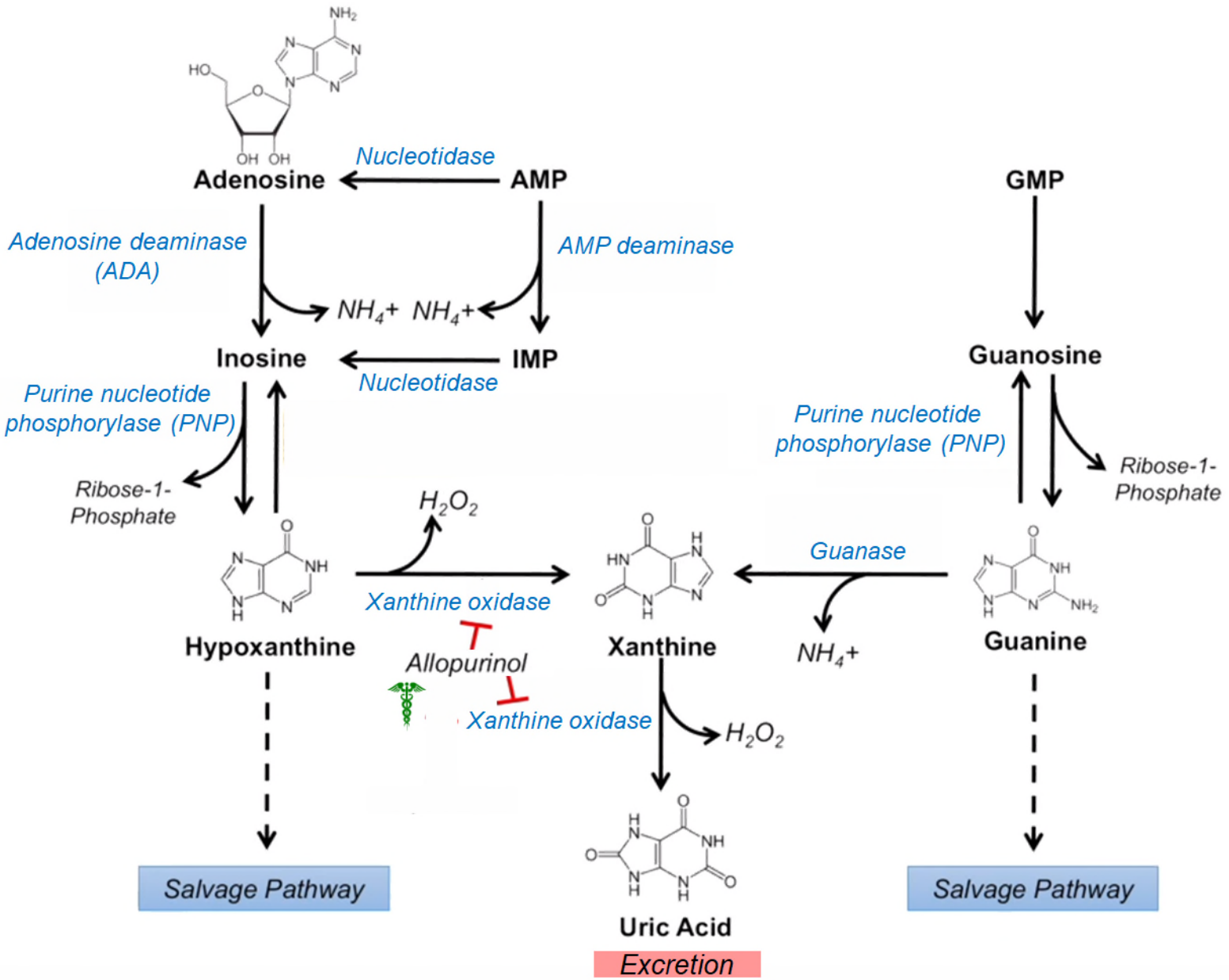
- *Purine nucleoside phosphorylase* (*PNP*) is **amphybolic**; it acts in both degradation and recovery.
- *Hypoxanthine-guanine phosphoribosil transferase* (*HGPRT*) determines **recovery**
- *Xanthine oxidase* and *Guanine deaminase* (*Guanase*) determine **degradation**



ASS = Adenilsuccinate synthetase; ASL = Adenilsuccinate liase; IMPDH = IMP dehydrogenase; GMPS = GMP synthase (SL13)

ELIMINATION VS SALVAGE

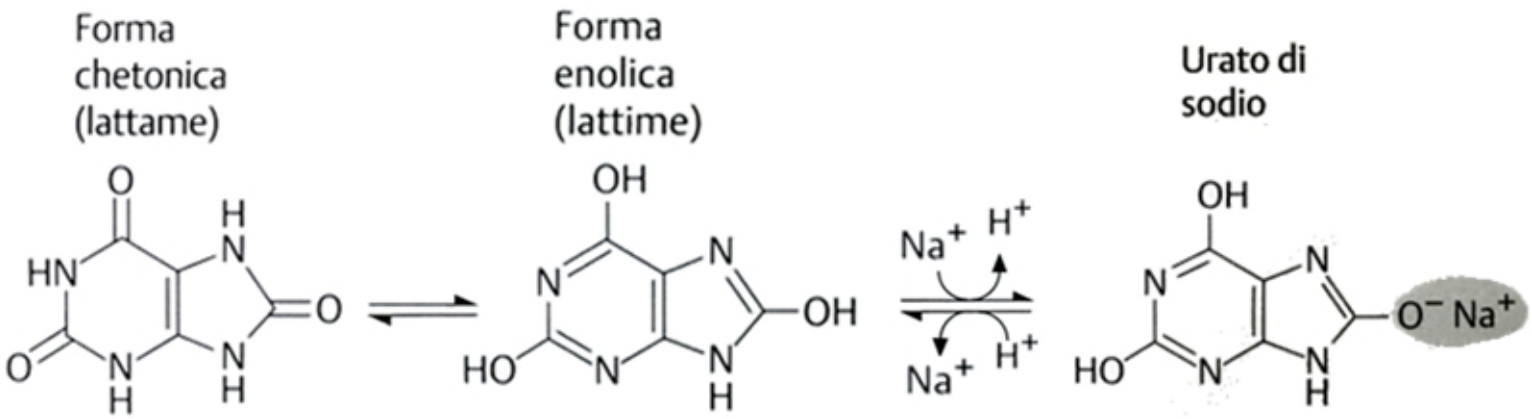
– *Xanthine oxydase* and *Guanase* catalyse the committing step for elimination



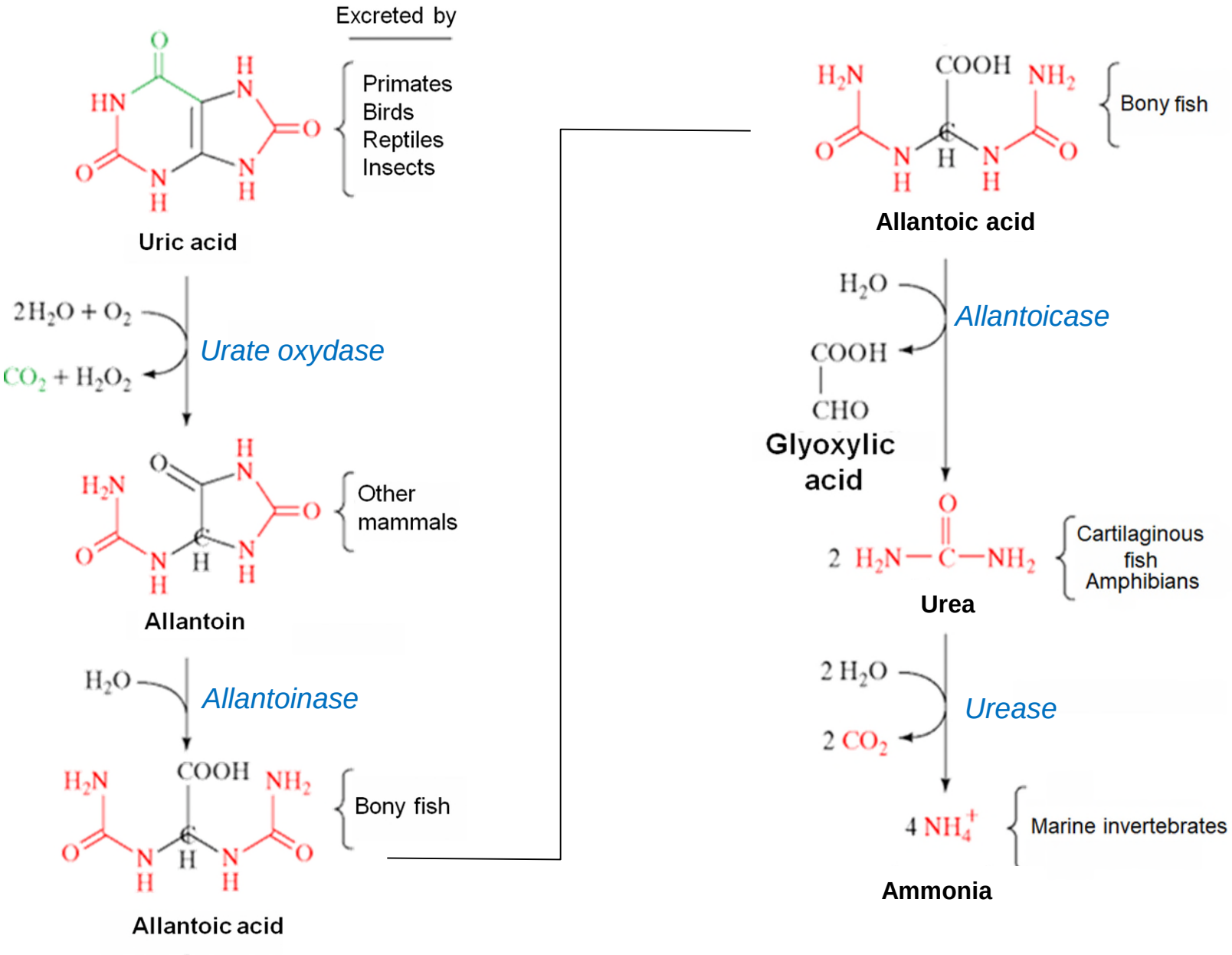
URIC ACID and ELIMINATION

Uric acid has diverse forms

- It is the final product of base catabolism of purines and is escreted as urate in primates, birds and some other animals.
- In man, it is eliminated at a rate of about 0.6 g/24hr
- It derives in part from exogenous purines obtaiend with the diet, and in part from the e in parte from purine nucleotide turnover in nucleic acids.
- The normal concentration in adults is between 3-7 mg/l. Above this it can cause health problems

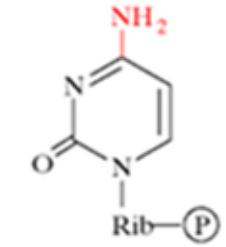


OXIDATION END PRODUCTS IN PURINE CATABOLISM OF ANIMALS



PYRIMIDINE NUCLEOTIDE CATABOLISM

① Nucleotide → Nucleoside
② Nucleoside → base
③ Ossidazione doppio legame
④ Apertura dell'anello



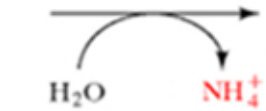
CMP

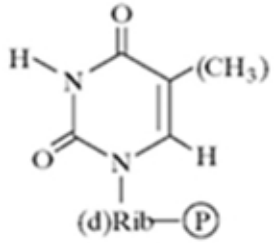
①

Cytidine

Salvage

①b

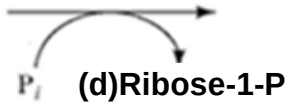




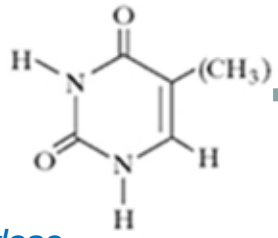
UMP (dTMP)

Uridine
(Deoxythymidine)

②

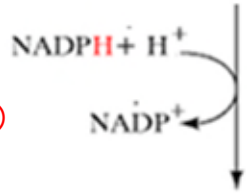


(d)Ribose-1-P



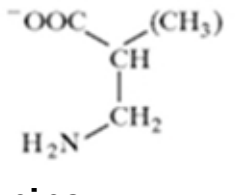
⇒ Salvage

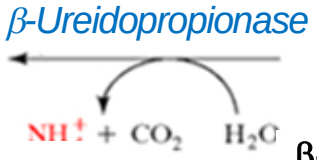
Uracyl
(Thymine)

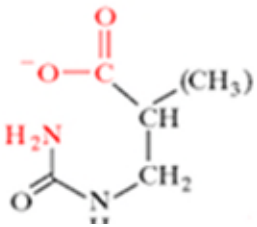


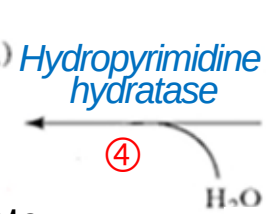
③

carnosine (βAla-His)

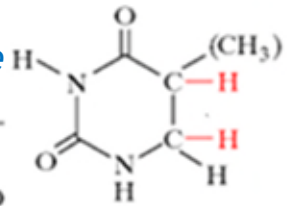








④



β-Alanine
(β-Aminoisobutyrate)

β-Ureidopropionate
(β-Ureidoisobutyrate)

Dihydrouracyl
(Dihydrothymene)

FA Metabolism (minor)

NH_3
CU
Excr

malonyl-CoA
(methylmalonyl-CoA)

Ac-CoA
(succinyl-CoA)

CK ⇒ **CO_2**

Pyrimidines →→→ NH_3, CO_2

ROLE OF PYRIMIDINE NUCLEOTIDE CATABOLIC ENZYMES