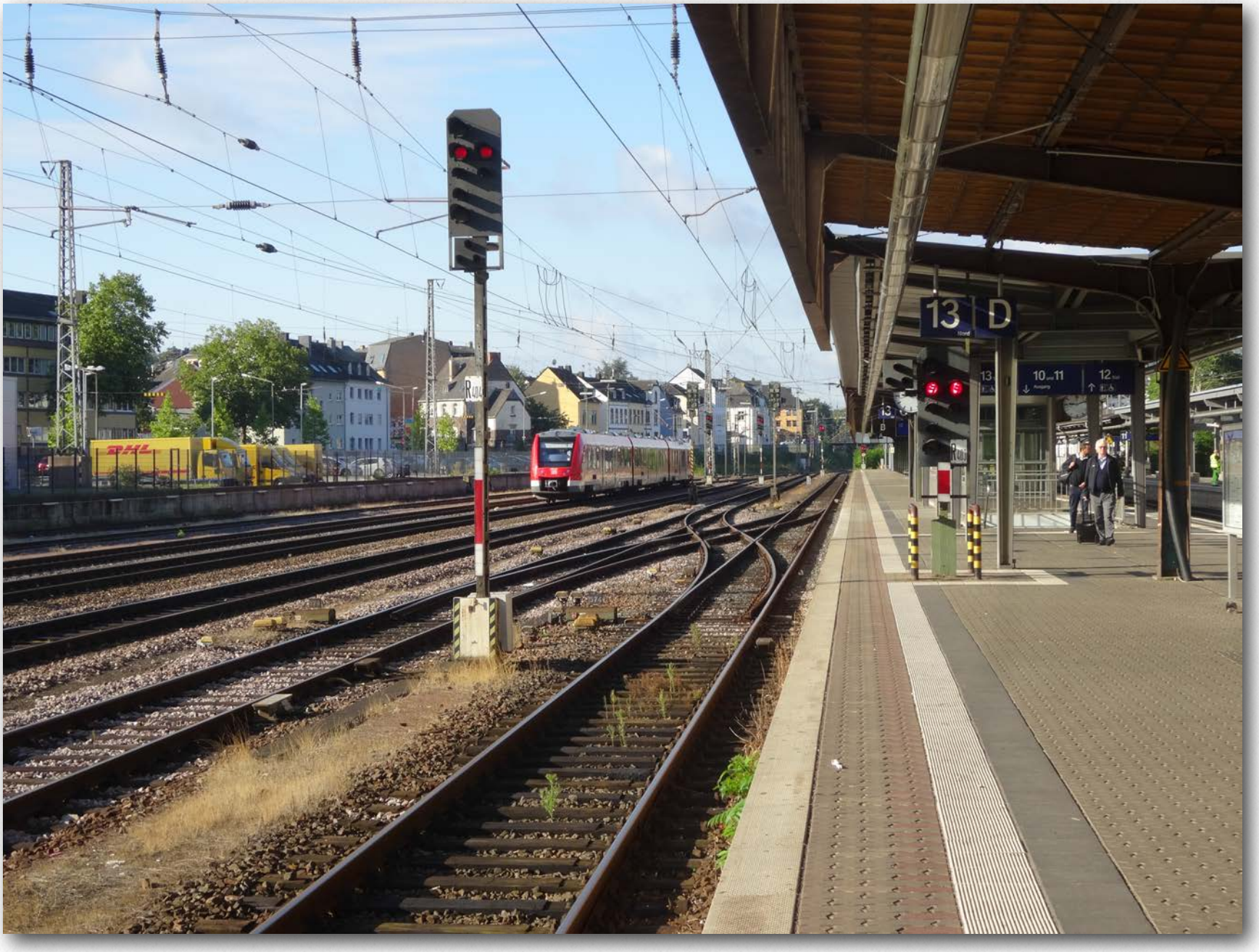
Metabolism: Nucleotides



Diverse functions of nucleotides

Nucleotides are most often thought of as the building blocks of the nucleic acids, DNA and RNA. While this, is, of course, a vital function, nucleotides also play other important roles in cells. Ribonucleoside triphosphates like ATP, CTP, GTP and UTP are necessary, not just for the synthesis of RNA, but as part of activated intermediates like UDP-glucose in biosynthetic pathways. ATP is also the universal “energy currency” of cells, and coupling of energetically unfavorable reactions with the hydrolysis of ATP makes possible the many reactions in our cells that require an input of energy. Adenine nucleotides serve as components of $NAD(P)^+$ and FAD. Nucleotides can also serve as allosteric and metabolic regulators. The synthesis and breakdown pathways for nucleotides and the molecules derived from them are thus, of vital importance to cells. Regulation of nucleotide synthesis, especially for deoxyribonucleotides, is important to ensure that the four nucleotides are made in the

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right proportions, as imbalances in nucleotide concentrations can lead to increases in mutation rates.

Pathways of nucleotide metabolism are organized in two major groups and one minor one. These include, respectively, metabolism of 1) purines; 2) pyrimidines; and 3) deoxyribonucleotides. Each group can be further subdivided into pathways that make nucleotides from simple precursors (*de novo* pathways) and others that use pieces of nucleotides to reassemble full ones (salvage pathways). Notably, *de novo* synthesis pathways for all of the nucleotides begin with synthesis of ribonucleotides. Deoxyribonucleotides are made from the ribonucleotides.

Purine nucleotide metabolism

Synthesis of purine nucleotides by the *de novo* pathway begins with addition of a pyrophosphate to carbon 1 of ribose-5-phosphate, creating phosphoribosylpyrophosphate (PRPP). The reaction is catalyzed by PRPP synthetase. Some number the purine metabolic pathway starting with the next reaction. We have therefore given this reaction the number of zero in Figure 6.172.

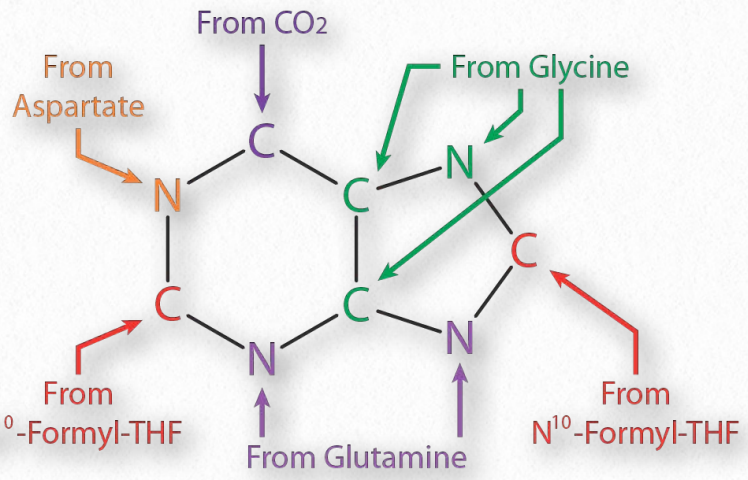

Figure 6.170 - Origin of atoms in purines

Image by Aleia Kim

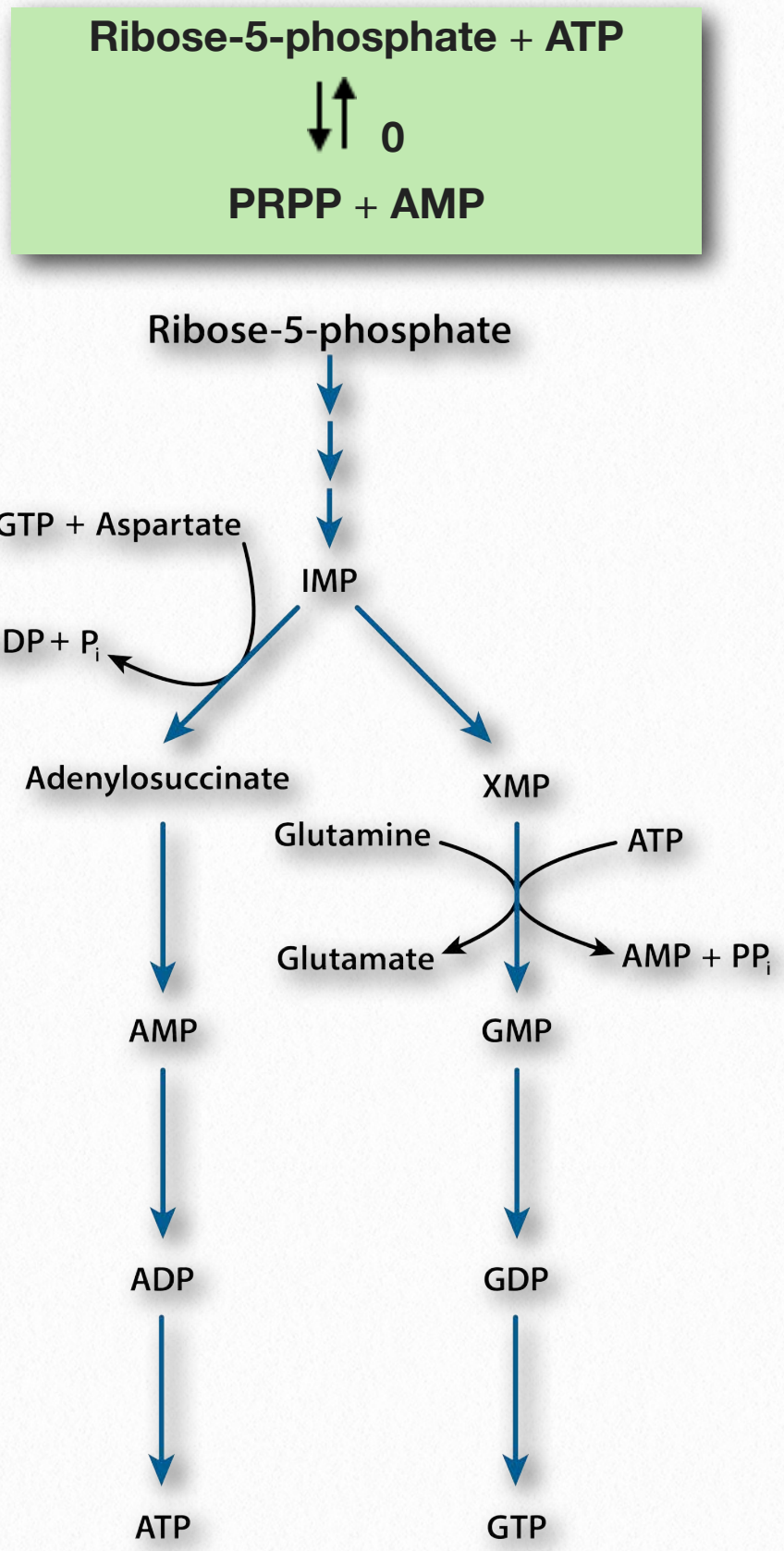

Figure 6.171 - Purine metabolism overview

Image by Pehr Jacobson

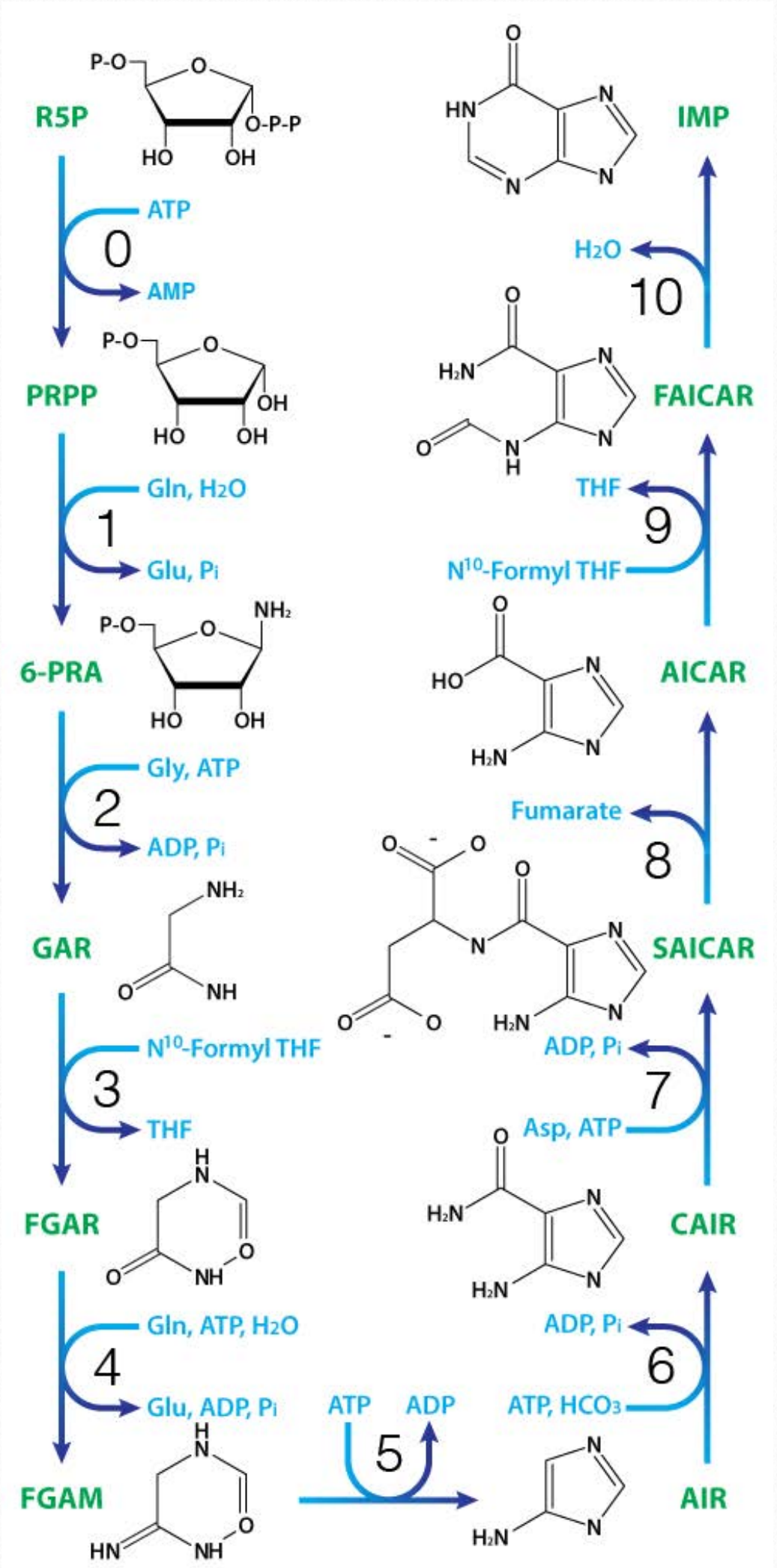

Figure 6.172 - Purine *de novo* synthesis

Image by Aleia Kim

In the next step (reaction 1 in Figure 6.172), the pyrophosphate is replaced by an amine from glutamine in a reaction catalyzed by PRPP amidotransferase (PPAT). The product is 5-phosphoribosylamine (5-PRA).

PRPP + **Glutamine** + H_2O

⇅ 1

5-PRA + **Glutamate** + **PP_i**

PPAT is an important regulatory enzyme for purine biosynthesis. The end products of the pathway, AMP and GMP both inhibit the enzyme and PRPP activates it. Interestingly, full inhibition of the enzyme requires binding of both AMP and GMP.

Binding of only one of the two nucleotides allows the enzyme to remain partially active so that the missing nucleotide can be synthesized. Through this enzyme, the relative amounts of ATP and GTP are controlled.

5-PRA is very unstable chemically (half-life of 38 seconds at

37°C), so it has been proposed that it is shuttled directly from PRPP amidotransferase to GAR synthetase for the next reaction.

In this reaction (#2), glycine is added to the growing structure above the ribose-5-phosphate to create glycineamide ribonucleotide (GAR). This reaction, which requires ATP, is catalyzed, as noted, by the enzyme GAR synthetase.

5-PRA + Glycine + ATP
⇅ 2
GAR + ADP + Pi

In reaction #3, a formyl group is transferred onto the GAR from N^{10}-formyl-tetrahydrofolate (N^{10}-formyl-THF or fTHF) by phosphoribosylglycinamide formyltransferase (GART).

GAR + fTHF
⇅ 3
fGAR + THF

Next, the double bonded oxygen in the ring is replaced with an amine in a reaction catalyzed by phosphoribosylformylglycinamidine synthase (PFAS) that uses glutamine and produces glutamate. The reaction requires energy from ATP (top of next column).

fGAR + L-Glutamine + ATP
⇅ 4
fGAM + L-Glutamate + ADP + P_i

In humans the GAR synthetase, phosphoribosylglycinamide formyltransferase, and the enzyme catalyzing the next reaction (#5), AIR synthetase activities are all on the same protein known as trifunctional purine biosynthetic protein adenosine-3.

fGAM + ATP
⇅ 5
AIR + ADP + P_i + H_2O

In reaction #6, carboxylation of AIR occurs, catalyzed by phosphoribosylaminoimidazole carboxylase (PAIC)

AIR + HCO_3^-
⇅ 6
CAIR + $2H^+$

Aspartic acid is then added to donate its amine group and fumarate will be lost in the reaction that follows this one. The enzyme involved here is phosphoribosyl-

CAIR + L-Aspartate + ATP
⇅ 7
SAICAR + ADP + P_i

aminoimidazole-succinocarboxamide synthase (PAICS)

In the next reaction, the carbon shell of aspartate is released (as fumarate) and the amine is left behind. The reaction is catalyzed by adenylosuccinate lyase (ADSL).

SAICAR

⇅ 8

AICAR + **Fumarate**

Reaction #9 involves another formylation reaction, catalyzed by phosphoribosylaminoimidazolecarboxamide formyltransferase (ATIC-E1).

AICAR + **fTHF**

⇅ 9

FAICAR + **THF**

Next, inosine monophosphate synthase (ATIC-E2) catalyzes release of water to form the first molecule classified as a purine - inosine monophosphate or IMP).

FAICAR

⇅ 10

IMP + H_2O

Though it doesn't appear in DNA, IMP does, in fact, occur in the anticodon of many tRNAs where its ability to pair with numerous bases is valuable in reading the genetic code.

IMP is a branch point between pathways that lead to GMP or AMP. The pathway to GMP proceeds via catalysis by IMP dehydrogenase as follows:

IMP + **NAD^+** + H_2O

⇅

Xanthosine 5'-phosphate + **NADH** + H+

In the last step of GMP synthesis, GMP synthase catalyzes a transamination to form GMP using energy from ATP.

ATP + **Xanthosine 5'-P** + **Glutamine** + H_2O

⇅

AMP + **PP_i** + **GMP** + **Glutamate**

The energy source being ATP makes sense, since the cell is presumably making GMP because it needs guanine nucleotides. If the cell is low on guanine nucleotides, GTP would be in short supply.

Adenine nucleotide synthesis

Synthesis of AMP from IMP follows. First, adenylosuccinate synthetase catalyzes the addition of aspartate to IMP, using energy from GTP.

IMP + Aspartate + GTP

⇅

Adenylosuccinate + GDP + P_i

Then, adenylosuccinate lyase splits fumarate off to yield AMP.

Adenylosuccinate

⇅

AMP + Fumarate

In humans, the bifunctional purine biosynthesis protein known as PURH contains activities of the last two enzymes above.

Abbreviations used above

PRPP = Phosphoribosyl Pyrophosphate
5-PRA = 5-phosphoribosylamine
GAR = glycineamide ribonucleotide
fGAR = Phosphoribosyl-N-formylglycineamide
THF = Tetrahydrofolate
fTHF = N^{10}-formyl-Tetrahydrofolate
fGAM = 5'-Phosphoribosylformylglycinamidine
AIR = 5-Aminoimidazole ribotide
CAIR = 5'-Phosphoribosyl-4-carboxy-5-aminoimidazole
SAICAR = Phosphoribosylamino-imidazolesuccinocarboxamide
AICAR = 5-Aminoimidazole-4-carboxamide ribonucleotide
FAICAR = 5-Formamidoimidazole-4-carboxamide ribotide
IMP = inosine monophosphate

Regulation

It is worth repeating that synthesis of GMP from IMP requires energy from ATP and that synthesis of AMP from IMP requires energy from GTP. In addition, the enzymes converting IMP into intermediates in the AMP and GMP pathways are each feedback inhibited by the respective monophosphate nucleotide. Thus, IMP dehydrogenase is inhibited by GMP (end product of pathway branch) and adenylosuccinate synthetase is inhibited by AMP, the end product of that pathway branch.

Purine nucleotide levels are balanced by the combined regulation of PRPP amidotransferase , IMP dehydrogenase, adenylosuccinate synthetase and the nucleotides AMP and GMP. The importance of the regu-

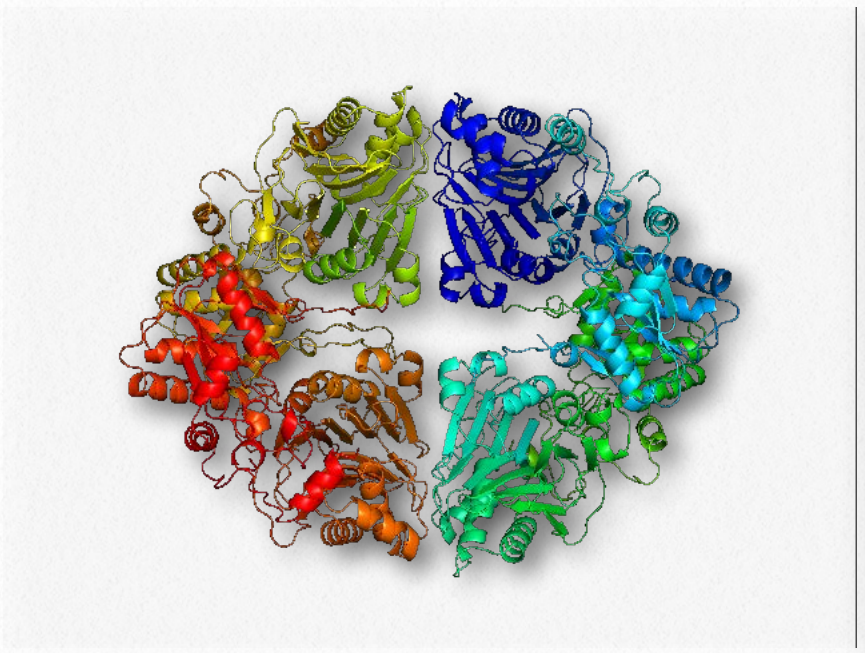

Figure 6.173 - PRPP amidotransferase
Wikipedia

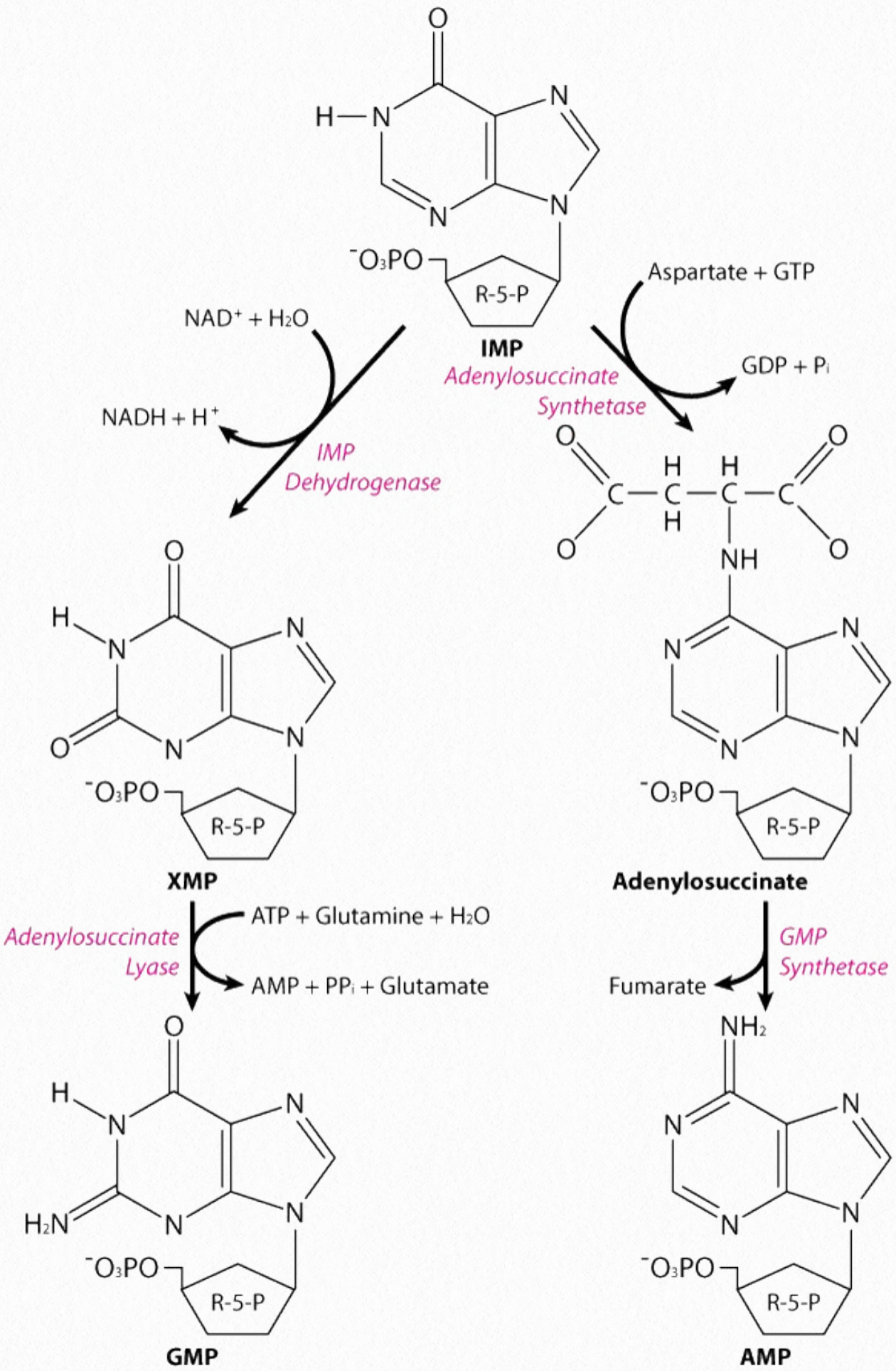

Figure 6.174 - The path from IMP (top) to AMP and GMP

Image by Aleia Kim

latory scheme of purines is illustrated by two examples. First imagine both AMP and GMP are abundant. When this occurs, PRPP amidotransferase will be completely inhibited and no purine synthesis will occur.

Partial activity

High levels of GMP and low levels of AMP would result in PRPP amidotransferase being slightly active, due to the fact GMP will fill one allosteric site, but low AMP levels will mean second allosteric site will likely be unfilled. This lowered (but not completely inhibited) activity of PRPP amidotransferase will allow for limited production of 5-PRA and the rest of the pathway intermediates, so it will remain active.

At the IMP branch, however, the high levels of GMP will inhibit

IMP dehydrogenase, thus shutting off that branch and allowing all of the intermediates to be funneled into making AMP. When the AMP level rises high enough, AMP binds to PRPP amidotransferase and along with GMP, shuts off the enzyme. A reversal will occur if AMP levels are high, but GMP levels are low.

Proper balance

Regulated in this way, AMP and GMP levels can be maintained in a fairly narrow concentration range. Properly balancing nucleotide levels in cells is critical. It is likely for this reason that cells have numerous controls on the amount of each nucleotide made.

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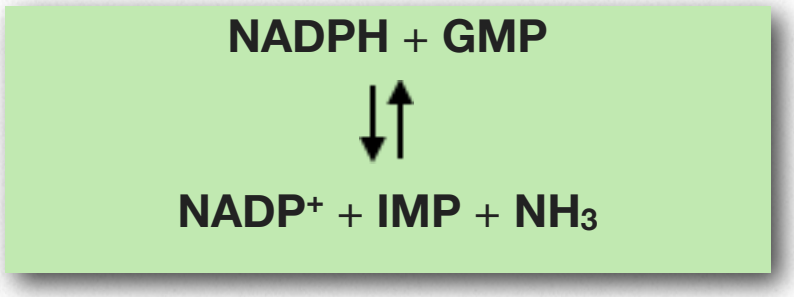
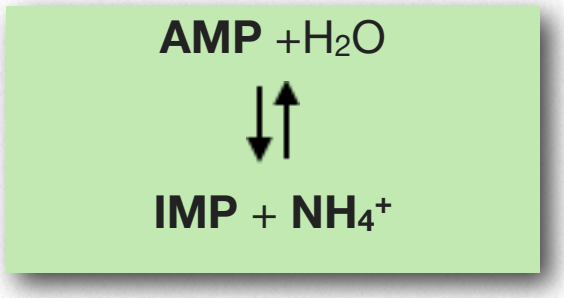
Other mechanisms

Cells have two other ways of balancing GMP and AMP nucleotides. First, the enzyme GMP reductase will convert GMP back to IMP using electrons from NADPH.

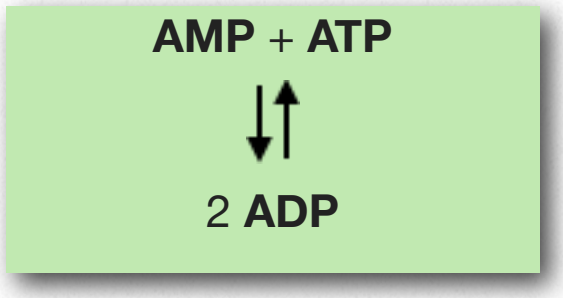


The IMP, in turn, can then be made into AMP if its concentration is low. Second, AMP can be converted back to IMP by the enzyme AMP deaminase. In this case, the IMP can then be made into GMP.



It is important to maintain appropriate proportions of the different nucleotides. Excess or scarcity of any nucleotide of any nucleotide can result in an increased tendency to mutation.

To convert AMP to ATP and GMP to GTP requires action of kinase enzymes. Each monophosphate nucleotide form has its own specific nucleoside monophosphate kinase. For adenine-containing nucleotides (ribose forms and deoxyribose forms), adenylate kinase catalyzes the relevant reaction.



The adenylate kinase reaction is reversible and is used to generate ATP when the cell's ATP concentration is low. When ATP is made from 2 ADPs in this way, AMP levels increase and this is one way the cell senses that it is low on energy.

Guanosine monophosphates also have their own kinase and it catalyzes the reaction at the top of the next page.

GMP + ATP

⇅

GDP + ADP

Other monophosphate kinases for UMP and CMP use ATP in a similar fashion.

In going from the diphosphate form to the triphosphate form, the picture is simple - one enzyme catalyzes the reaction for all diphosphates (ribose and deoxyribose forms). It is known as nucleoside diphosphate kinase or (more commonly) NDK or NDPK and it catalyzes reactions of the form

(d)XTP + (d)YDP

⇅

(d)XDP + (d)YTP

where X and Y refer to any base.

Purine salvage reactions

Not all nucleotides in a cell are made from scratch. The alternatives to *de novo* syntheses are salvage pathways. Salvage reactions to make purine nucleotides start with attachment of ribose to purine bases using phosphoribosylpyrophosphate (PRPP).

The enzyme catalyzing this reaction is known as hypoxanthine/guanine phosphoribosyltransferase (HGPRT - Figure 6.175) and is interesting from an enzymological as well as a medical perspective. First, the enzyme is able to catalyze both of the next two important salvage reactions - converting hypoxanthine to IMP or guanine to GMP.

Hypoxanthine + PRPP

⇅

IMP + PP_i

Guanine + PRPP

⇅

GMP + PP_i

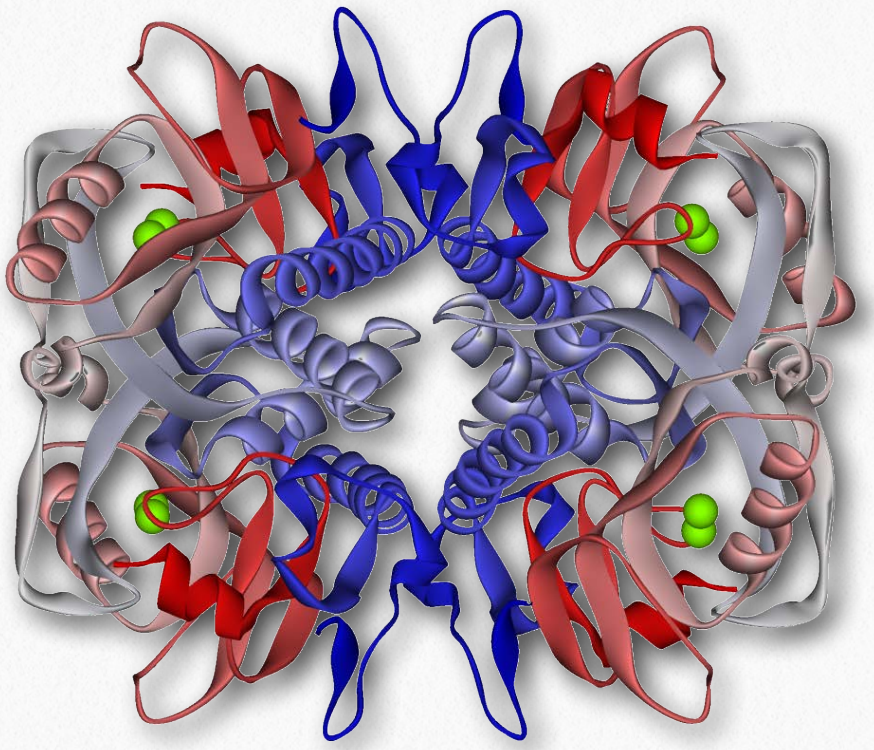
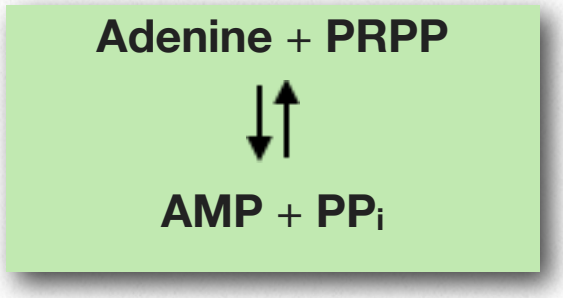


Figure 6.175 - HGPRT

HGPRT is able to bind a variety of substrates at its active site and even appears to bind non-natural substrates, such as acyclovir preferentially over its natural ones.



Medical perspective

From a medical perspective, reduction in levels of HGPRT leads to hyperuricemia, a condition where uric acid concentration increases in the body. Complete lack of HGPRT is linked to Lesch-Nyhan syndrome, a rare, inherited disease in high uric acid concentration throughout the body is associated with severe accompanying neurological disorders.

Reduced production of HGPRT occurs frequently in males and has a smaller consequence (gout) than complete absence. Interestingly, gout has been linked to a decreased likelihood of contracting multiple sclerosis, suggesting uric acid may help prevent or ameliorate the disease.

Expression of HGPRT is stimulated by HIF-1, a transcription factor made in tissues when oxygen is limiting, suggesting a role for HGPRT under these conditions.

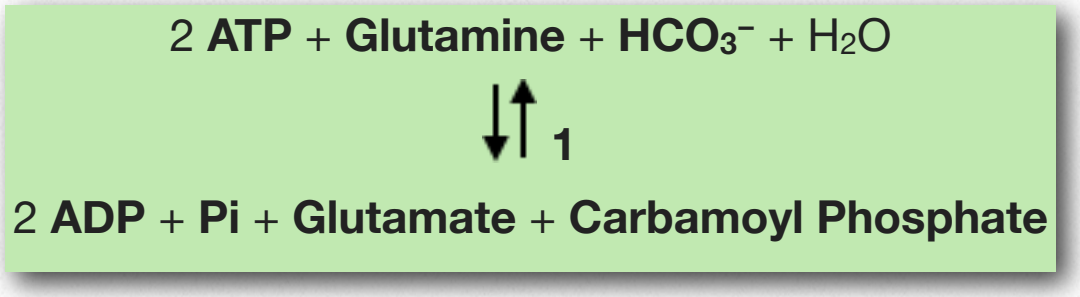
Adenine salvage

The enzyme known as adenine phosphoribosyltransferase (APRT) catalyzes the reaction corresponding to HGPRT for salvaging adenine bases.

Pyrimidine nucleotide metabolism

The *de novo* pathway for synthesizing pyrimidine nucleotides has about the same number of reactions as the purine pathway, but also has a different strategy. Whereas the purines were synthesized attached to the ribose sugar, pyrimidine bases are made apart from the ribose and then attached later.

The first reaction is catalyzed by carbamoyl phosphate synthetase (Figure 6.176).



Two different forms are found in eukaryotic cells. Form I is found in mitochondria and form II is in the cytoplasm.

The reaction catalyzed by carbamoyl phosphate synthetase is the rate limiting step in pyrimidine biosynthesis and corresponds to reaction 1 in Figure 6.178.

Balance

The enzyme is activated by ATP and PRPP and is inhibited by UMP. This helps to balance pyrimidine vs. purine concentrations. High concentrations of a purine (ATP) acti-

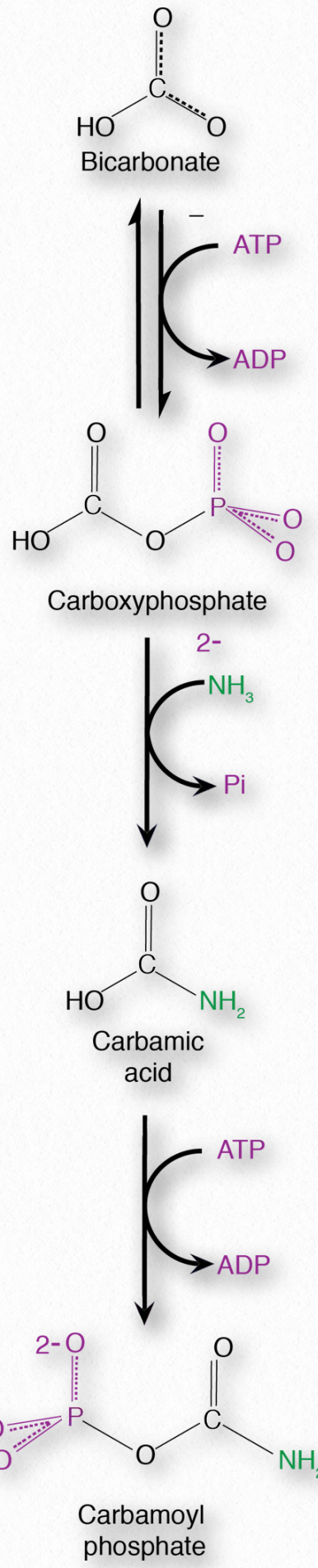


Figure 6.176 - Formation of carbamoyl phosphate in reaction 1

Image by Penelope Irving

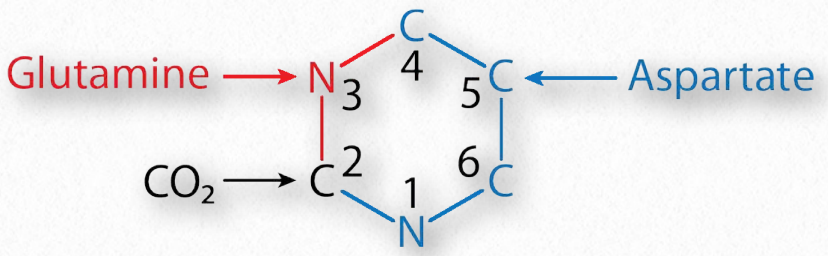


Figure 6.177 - Atom sources in pyrimidines

Image by Pehr Jacobson

vates the synthesis of pyrimidines. PRPP increases in concentration as purine concentration increases, so it too helps to establish that balance. UMP is an end product of pyrimidine metabolism, so the process is self-limiting. The next enzyme in the pathway, aspartate transcarbamoylase (ATCase) also plays a role in the same balance, as we will see. The reaction it catalyzes is shown below and is reaction 2 in Figure 6.178.

Carbamoyl Phosphate + Aspartate ⇅ 2 **Carbamoyl Aspartate + P_i**

ATCase is a classic enzyme exhibiting allosteric regulation and feedback inhibition, having both homotropic and heterotropic effectors (Figure 6.179 and see HERE). With 12 subunits (6 regulatory and 6 catalytic units), the enzyme exists in two states - a low activity T-state and a high activity R-state. Binding of the aspartate substrate to the active site shifts the equilibrium in favor of the R-state.

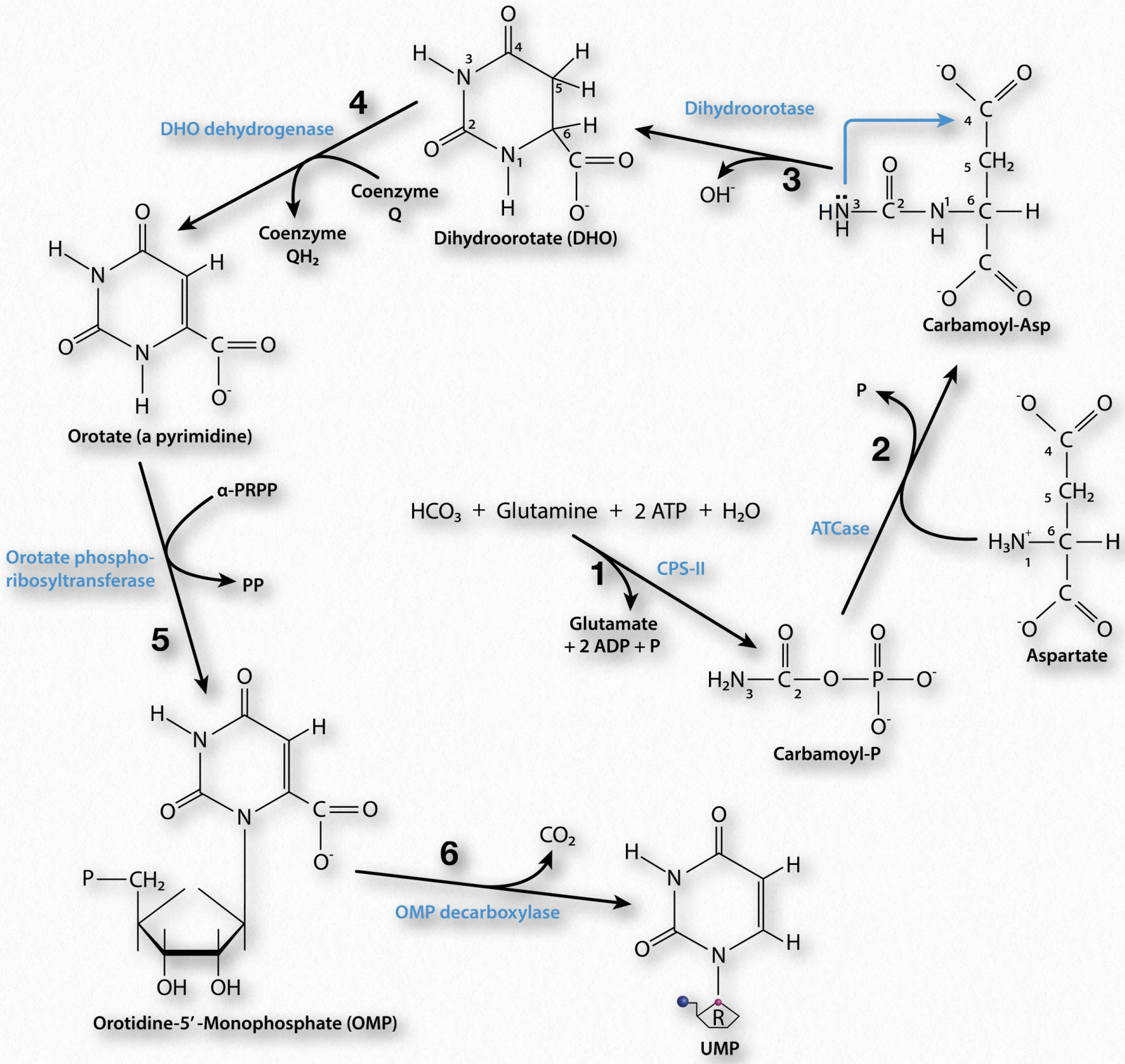


Figure 6.178 - Pyrimidine synthesis by the *de novo* pathway

Image by Pehr Jacobson

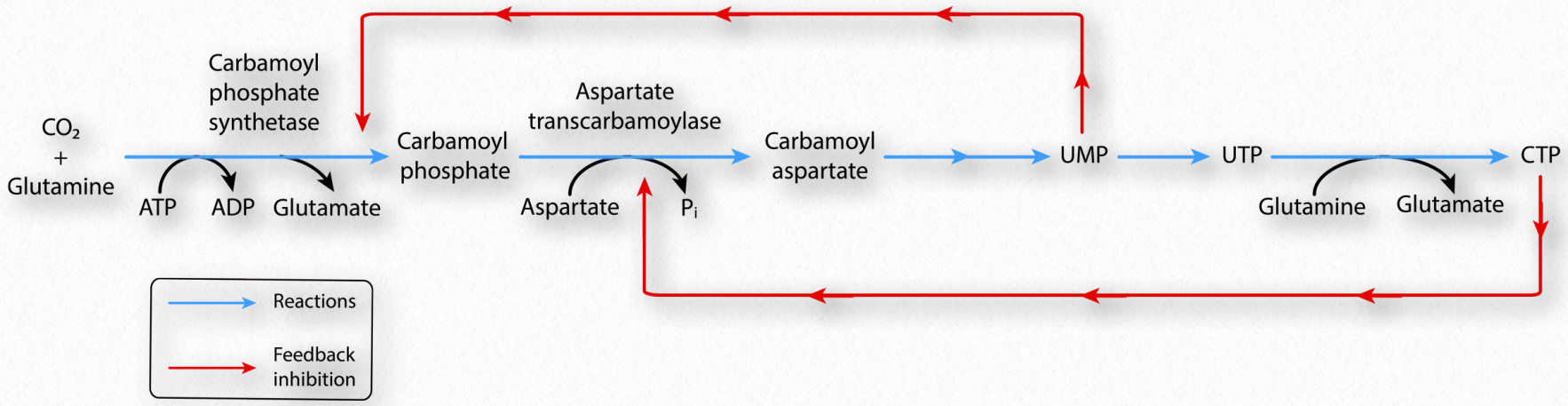


Figure 6.179 - Overview of pyrimidine metabolism feedback regulation

Image by Pehr Jacobson

Aspartate is a homotropic effector of the enzyme, because it acts allosterically on the enzyme and is a substrate for it as well. Similarly, binding of ATP to the regulatory units favors the R-state, whereas binding of CTP to the regulatory units favors the T-state. ATP and CTP are heterotropic effectors of the enzyme because they are not substrates for it, but act allosterically.

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Regulation

As was seen with the first enzyme of the pathway, high concentration of purine nucleotides stimulates synthesis of pyrimidines and high concentration of pyrimidines turns off the pathway that synthesizes them.

Dihydroorotase catalyzes reaction 3 and is found in the cytoplasm, as is ATCase.

Carbamoyl Aspartate

⇅ 3

Dihydroorotate + H_2O

Reaction 4 occurs in the mitochondrion, so the product of reaction 3, dihydroorotate, must be transported into the mitochondrion from the cytoplasm. In reaction 4, dihydroorotate is oxidized to orotate. The enzyme catalyzing the reaction is dihydroorotate dehydrogenase.

Dihydroorotate + **NAD^+**

⇅ 4

Orotate + **NADH** + H^+

Reaction #5, catalyzed by orotate phosphoribosyl transferase, involves connection of orotate to ribose to yield a nucleotide - orotidine-5'-monophosphate (OMP).

Orotate + **PRPP**

⇅ 5

OMP + **PP_i**

Last, OMP is converted to uridine-5'-monophosphate (UMP) by action of a fasci-

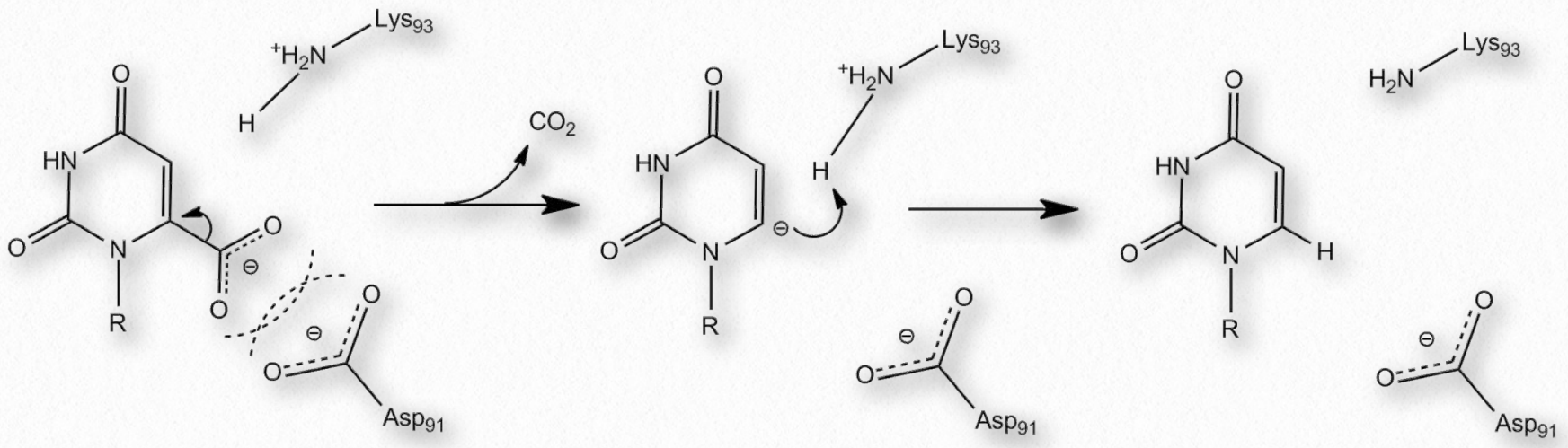

Figure 6.180 - Mechanism of action of OMP decarboxylase

Wikipedia

nating enzyme known as OMP decarboxylase.

OMP

⇅ 6

UMP + CO_2

OMP decarboxylase is frequently cited as an example for the incredible ability of an enzyme to speed a reaction. The decarboxylation of OMP, if allowed to proceed in the absence of an enzyme takes about 78 million years. In the presence of OMP decarboxylase, the reaction takes place in 18 milliseconds, a speed increase of about 10^{17}. Remarkably, the enzyme accomplishes this without any cofactors or coenzymes of any kind.

The mechanism of action of the enzyme is shown in Figure 6.180. In mammals, the activities of OMP decarboxylase and orotate phosphoribosyl transferase are contained on the same protein.

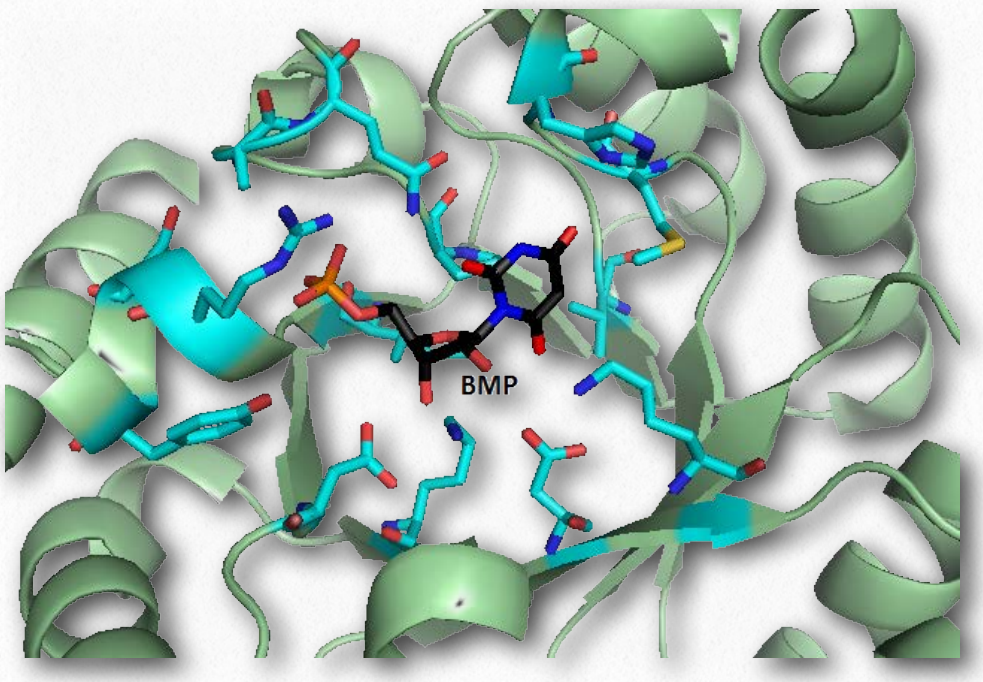

Figure 6.181 - OMP decarboxylase bound to an OMP analog at the active site

Wikipedia

A monophosphate kinase (UMP/CMP kinase) catalyzes conversion of UMP to UDP.

The same enzyme will also phosphorylate CMP to CDP and dCMP to dCDP. Like

UMP + ATP

⇅

UDP + ADP

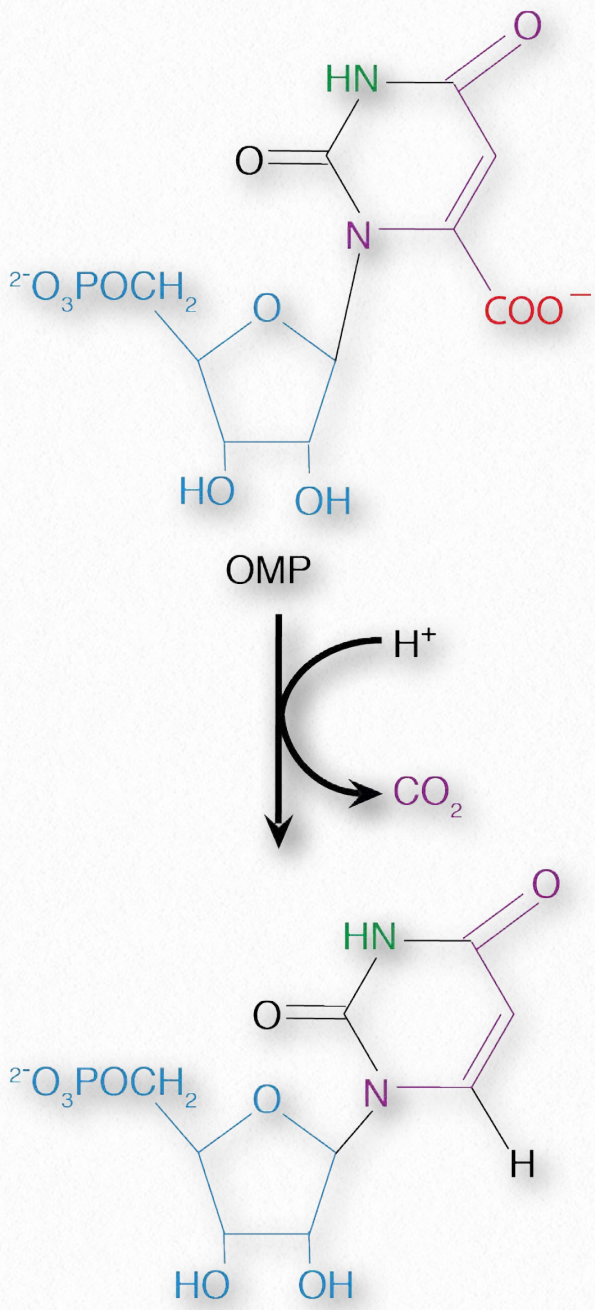

Figure 6.182 - Conversion of OMP to UMP

Image by Penelope Irving

the reaction of adenylate kinase, the reaction above, when run in the reverse direction, can be a source of ATP when the cell is low on energy.

The next step, catalyzed by NDPK, uses energy of any triphosphate nucleotide (XTP) to produce UTP from UDP.

UDP + XTP

⇅

UTP + XDP

CTP Synthase

UTP is the substrate for synthesis of CTP via catalysis by CTP synthase.

UTP + **Glutamine** + **ATP**

⇅

CTP + **Glutamate** + **ADP** + **P_i**

This enzyme is inhibited by its product, ensuring too much CTP is not made and activated by physiological concentrations of ATP, GTP, and glutamine. One human isozyme, CTPS 1, has been shown to be inactivated by phosphorylation by glycogen synthase kinase 3.

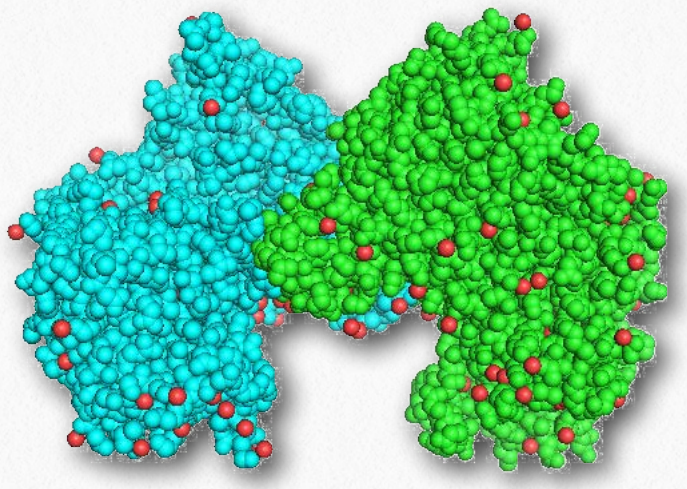

Figure 6.183 - CTP synthase dimer

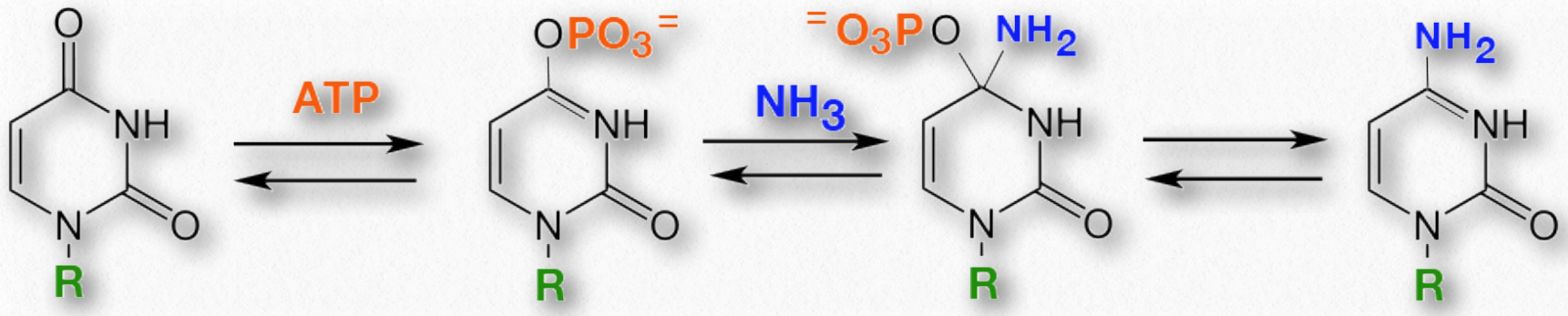

Figure 6.184 - Mechanism of CTP synthase action

CTP synthase has two domains and is a heterodimer (Figure 6.183). It exists as an inactive monomer at low enzyme concentrations or in the absence of UTP and ATP. One domain of the enzyme cleaves the amine group from glutamine and transfers it internally to the UTP. The other domain (synthase domain) binds ATP and initiates the mechanism shown in Figure 6.184 for making CTP.

CTP is the only nucleotide synthesized *de novo* directly as a triphosphate, since it arises directly from UTP. Since deoxyribonucleotides are made from ribonucleoside diphosphates, it means deoxycytidine nucleotides must either be made preferentially from salvage nucleotides or CTP must be dephosphorylated first.

One enzyme that can do this is a membrane-bound enzyme known as apyrase, which sequentially converts CTP to CDP and then CMP.

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Pyrimidine salvage reactions

Pyrimidine salvage synthesis allows cells to remake pyrimidine triphosphate nucleotides starting from either the C or U pyrimidine bases, nucleosides, or nucleotides. Figures 1.85 & 6.186 depict salvage pathway reactions. As is apparent in Figure 1.86, there are multiple ways of making the same molecules. For example, uracil can be made into uridine by reaction 11 or by reaction 12.

The figure depicts not only the synthesis of CTP and UTP from basic components, but also shows how these nucleotides can be broken down into smaller pieces.

In many cases, the same enzyme works on cytidine, uridine, and deoxycytidine molecules.

Enzymes of note

There are several enzymes of note in the salvage pathway. Seven enzymes, for example, work on both uracil and cytosine contain-

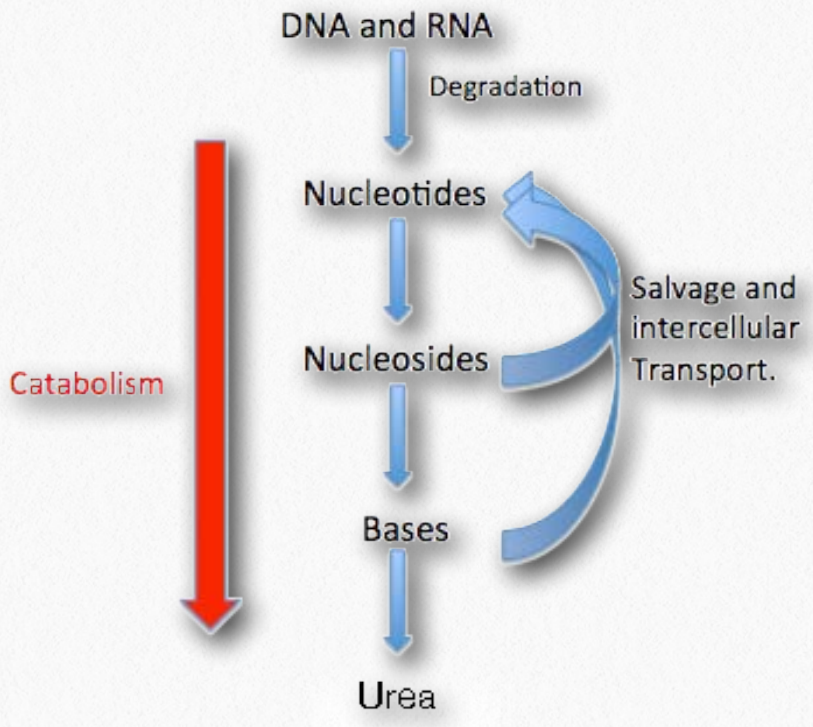


Figure 6.185 - Catabolism and salvage of nucleotides from DNA and RNA

Wikipedia

ing nucleosides/nucleotides. These include NTP phosphatase (reaction 2), NDPK (reaction 3), apyrase (reaction 4), NDP phosphatase (reaction 5), UMP/CMP kinase (reaction 6), pyrimidine-specific 5' nucleotidase (reaction 7), and uridine/ cytidine kinase (reaction 8). The enzymes for reactions 6 and 8 can also use deoxyribonucleosides/ deoxyribonucleotides as substrates.

Cytidine deaminase (reaction #9) converts cytidine to uridine by removing an amine group from the cytosine base and thus is a counter for the UTP to CTP reaction catalyzed by CTP synthetase. Countered reactions allow cells to balance concentrations of nucleosides/nucleotides in either direction if they should get out of balance.

Two other reactions in the figure are worth mentioning. Both UTP and CTP are converted in the breakdown process to UMP and CMP, respectively. Both of these reactions are important for deoxyribonucleotide metabolism. In each case, the monophosphate derivatives are phosphorylated, creating diphosphate derivatives (UDP and CDP) that are substrates for RNR that yield dUDP and dCDP, respectively. dUDP is phosphorylated to dUTP and then pyrophosphate is removed by dUTPase to yield dUMP.

Enzymes of Figure 6.186

1 = **CTP Synthase**
2 = **NTP Phosphatase**
3 = **NDPK**
4 = **Apyrase**
5 = **NDP Phosphatase**
6 = **UMP/CMP Kinase**
7 = **Pyrimidine-specific 5' Nucleotidase**
8 = **Uridine / Cytidine Kinase**
9 = **Cytidine Deaminase**
10 = **Uridine Nucleosidase**
11 = **Uridine Phosphorylase**
12 = **Uracil Phosphoribosyltransferase**

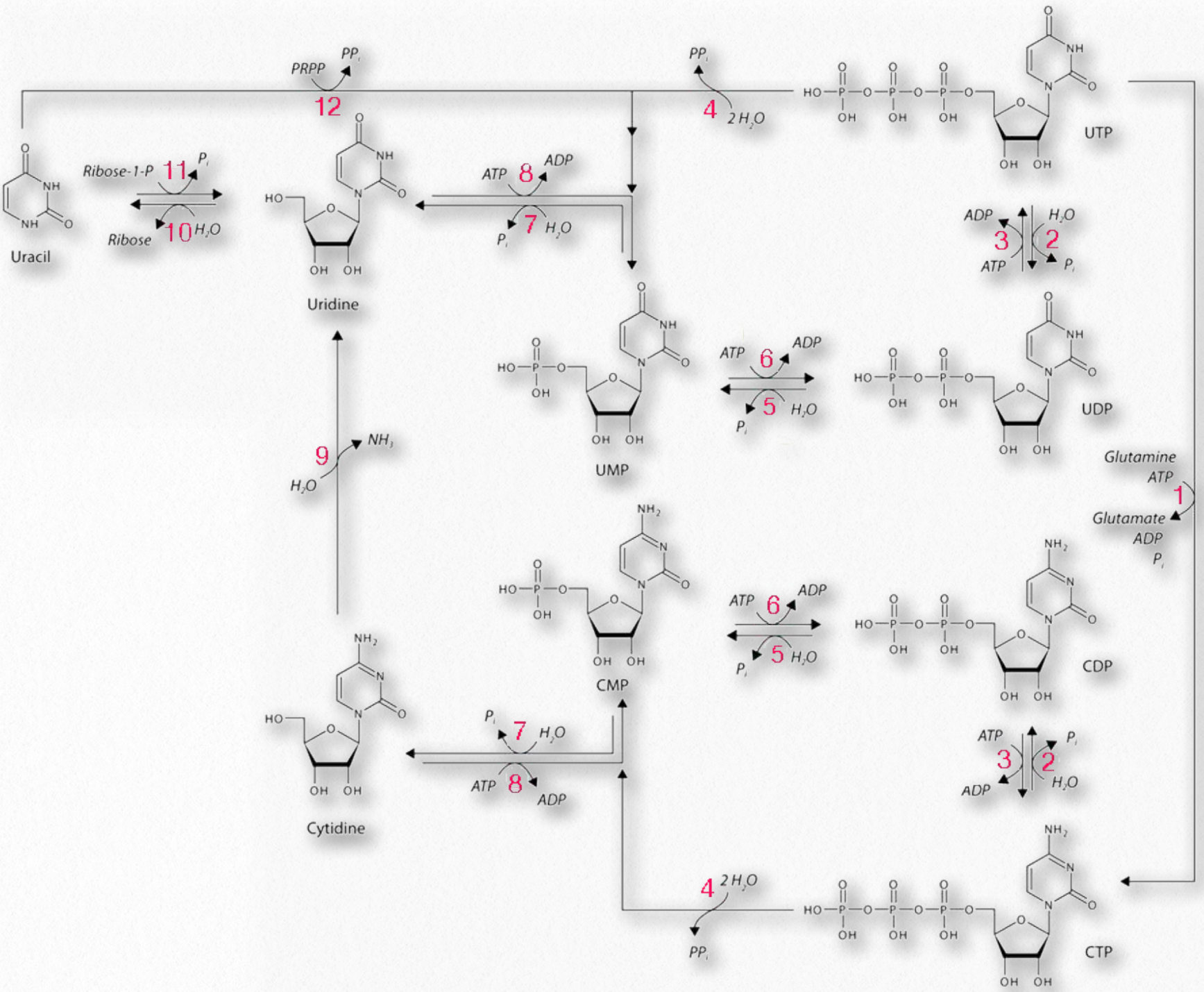


Figure 6.186 - Salvage pathways of pyrimidine nucleotides

dUMP is a substrate for thymidine synthesis (see HERE). dCDP is converted to dCTP by NDPK

Deoxyribonucleotide metabolism

Deoxyribonucleotides, the building blocks of DNA, are made almost exclusively from ribonucleoside diphosphates. A single enzyme called ribonucleotide reductase (RNR) is responsible for the conversion of each of these to a deoxy form (Figure 6.187). The enzyme's substrates are ribonucleoside diphosphates (ADP, GDP, CDP, or UDP) and the products are deoxyribonucleoside diphosphates (dADP, dGDP, dCDP, or dUDP). Thymidine nucleotides are synthesized from dUDP.

RNR has two pairs of two identical subunits - R1 (large subunit) and R2 (small subunit). R1 has two allosteric binding sites and a catalytic site. R2 forms a tyrosine radical necessary for the reaction mechanism of the enzyme.

There are three classes of RNR enzymes and they differ in the nature or means of generating a radical used in the enzyme's catalytic mechanism. Class I RNRs are found in eukaryotes, eubacteria, bacteriophages, and viruses. They all use a ferrous iron center that loses an electron (converting to ferric iron) to generate a free radical on a tyrosine ring. These enzymes only work in aerobic conditions.

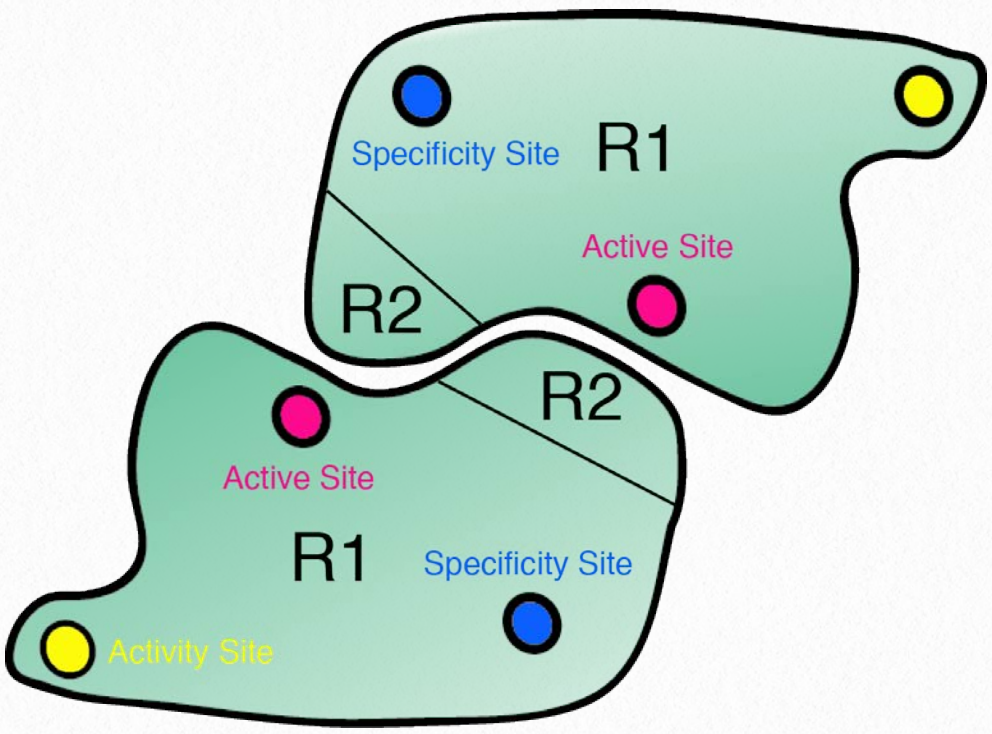


Figure 6.187 Ribonucleotide reductase
Image by Penelope Irving

Class II RNRs use 5'-deoxyadenosyl cobalamin (vitamin B_{12}) to generate a radical and work under aerobic or anaerobic conditions. They are found in eubacteria, archaebacteria, and bacteriophages. Class III RNRs generate a glycine radical using S-adenosyl methionine (SAM) and an iron-sulfur center. They work under anaerobic conditions and are used by archaebacteria, eubacteria, and bacteriophages. Substrates for class I enzymes are ribonucleoside diphosphates. Class II enzymes work on ribonucleoside diphosphates or ribonucleoside triphosphates. Class III enzymes work on ribonucleoside triphosphates.

In class I enzymes, RNR is an iron-dependent dimeric enzyme with each monomeric unit containing a large subunit (known as α or R1) and a small subunit (known as β or R2). The R1 subunit contains regulatory binding sites for allosteric effectors (see below), whereas the R2 subunit houses a tyrosine residue that forms a radical critical to the reaction mechanism of the enzyme. Electrons needed in the reaction are transmitted from NADPH to the enzyme by one of two pathways, reducing a disulfide bond in the enzyme to two sulfhydryls. In the first transfer mechanism, NADPH passes electrons to glutathione, which passes them to glutaredoxin, which then donates them to the RNR enzyme used

in the reaction. In the second mechanism, NADPH passes electrons to FAD, which uses them to reduce thioredoxin, which then passes the electrons to RNR with the same end result as in the first pathway - reduction of a suflhydryl in RNR.

In the reaction mechanism (Figure 6.188), a tyrosine side chain in the R2 unit must be radicalized to start. This electronic change is transmitted through the small R_2 subunit to the active site of the large R1 subunit. Several aromatic amino acid side chains are thought to play a role in that process. Iron atoms in the R2 subunit assist in creation and stabilization of the radical. The tyrosine radical contains an unpaired electron delocalized across its aromatic ring.

Transfer of the electronic instability to the R1 unit results in radicalization of a cysteine (to form a thiyl radical) at the active site. The thiyl radical, thus formed, abstracts a hydrogen atom (proton plus electron) from carbon 3 of ribose on the bound ribonucleoside diphosphate, creating a radical carbon atom. Radicalization of carbon #3 favors release of the hydroxyl group on carbon #2 as water. The extra proton comes from the sulfhydryl of the enzyme's cysteine. In the next step of the process, a proton and two electrons from the same cysteine are transferred to carbon #2 and then carbon #3 takes back the proton originally removed from it to yield a deoxyri-

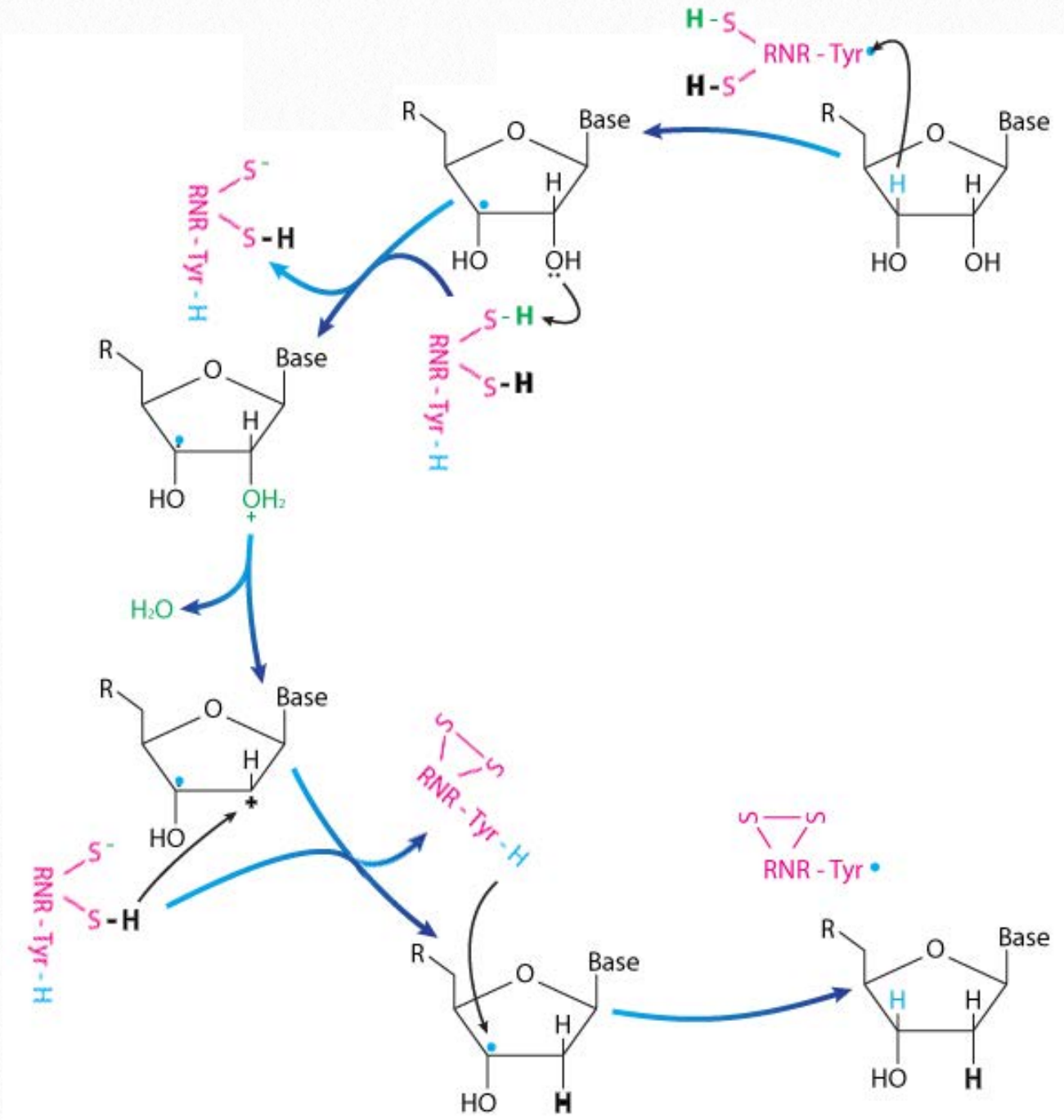


Figure 6.188 Ribonucleotide reductase reaction mechanism
Image by Aleia Kim

bonucleoside diphosphate. The enzyme's thiyl group gains an electron from R2 and the disulfide bond created in the reaction must be reduced by electrons from NADPH again in order to catalyze again.

Regulation

In addition to RNR's unusual reaction mechanism, the enzyme also has a complex system of regulation, with two sets of allosteric binding sites, both found in the R1 subunit. Because a single enzyme, RNR, is responsible for the synthesis of all four deoxyribonucleotides, it is necessary to have mechanisms to ensure that the enzyme produces the correct amount of each dNDP. This is a critical consideration, since imbalances in DNA precursors can lead to mutation.

Consequently, the enzyme must be responsive to the levels of the each deoxyribonucleotide, selectively making more of those that are in short supply, and preventing additional synthesis of those that are abundant. These demands are met by having two separate control mechanisms on the enzyme - one that determines which substrate will be acted on, and another that controls the enzyme's activity.

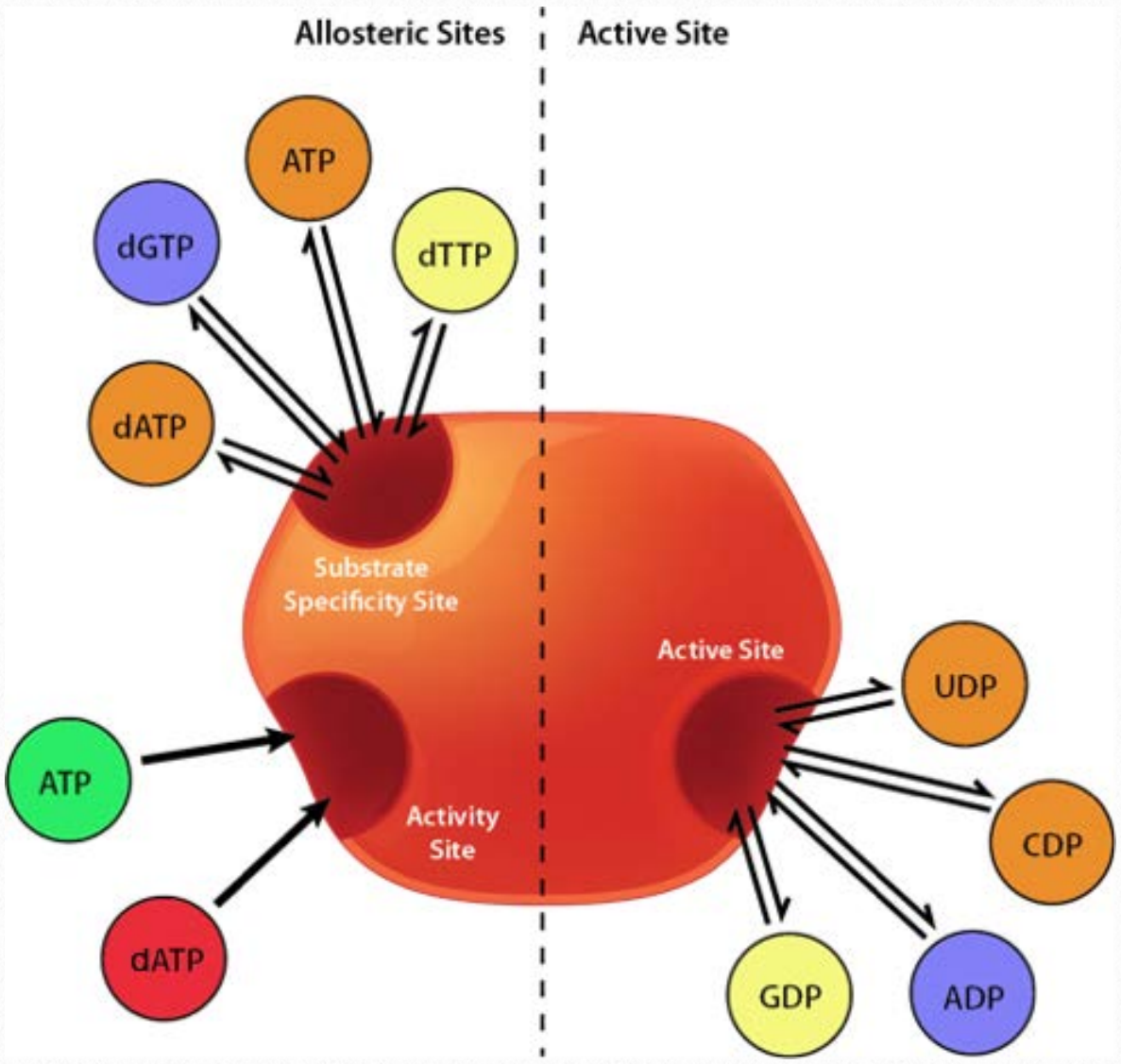


Figure 6.189 Allosteric regulation of RNR activity
Image by Aleia Kim

Two allosteric sites

RNR is allosterically regulated *via* two molecular binding sites - a specificity binding site (binds dNTPs and induces structural changes in the enzyme that determines which substrates preferentially bind at the catalytic site and an activity control site (controls whether or not enzyme is active). The activity control site functions like a simple on/off switch - ATP activates catalysis, dATP inactivates it. (One subset of class I enzymes, however, is not affected by dATP.)

The inactivation of RNR by dATP is an important factor in the disease known as Severe Combined Immunodeficiency Disease (SCID). In SCID, the salvage enzyme adenosine deaminase is deficient, leading to a rise in concentration of dATP in cells of the immune system. dATP shuts down RNR in these cells, thus stopping their proliferation and leaving the affected individual with a very weak or no immune system.

YouTube Lectures by Kevin HERE & HERE

Allosteric effectors

When dTTP is abundant (Figure 6.189), it binds to RNR's specificity site and inhibits binding and reduction of CDP and UDP but stimulates binding and reduction of GDP at the active site of the enzyme. Conversely, binding of ATP or dATP at the specificity site stimulates binding and reduction of CDP and UDP at the active site. Last, binding of dGTP to the specificity site (specificity site B) induces binding and reduction of ADP at the active site.

Students sometimes confuse the active site of RNR with the activity control site (sometimes called the activity site). The active site is where the reaction is catalyzed, and could better be called the catalytic site, whereas the activity site is an allosteric binding site for ATP or dATP that controls whether the enzyme is active. High levels of dATP are an indicator that sufficient dNTPs are available, so the enzyme gets inhibited to stop production of more. Low levels of dATP allow binding of ATP and activation of the enzyme.

In addition to regulation by deoxyribonucleotides and ATP, RNR can be directly inhibited by hydroxyurea.

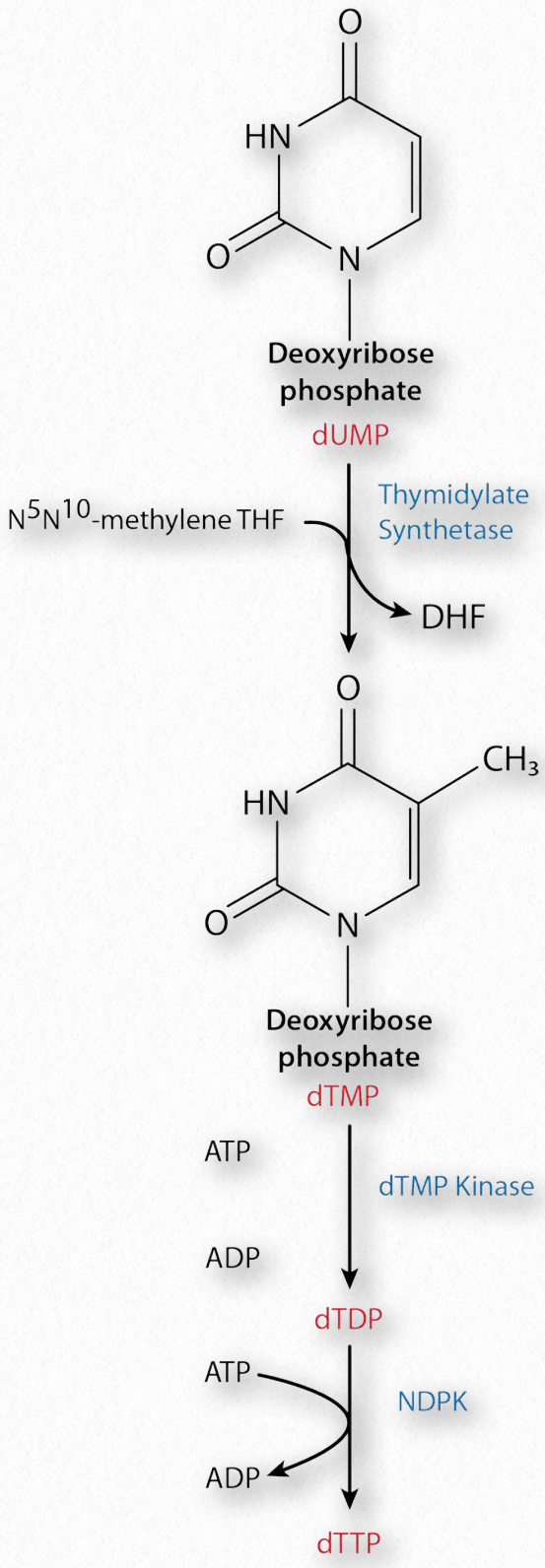


Figure 6.190 - Pathway from dUMP to dTTP

Image by Pehr Jacobson

dTTP synthesis

Synthesis of dTTP by the *de novo* pathway involves a multi-step process from UDP to dTTP. It begins with UDP, which is converted to dUDP by RNR. dUDP is phosphorylated by NDPK to yield dUTP, which is quickly broken down by dUTPase to produce dUMP. The remaining reactions are shown in Figure 6.190.

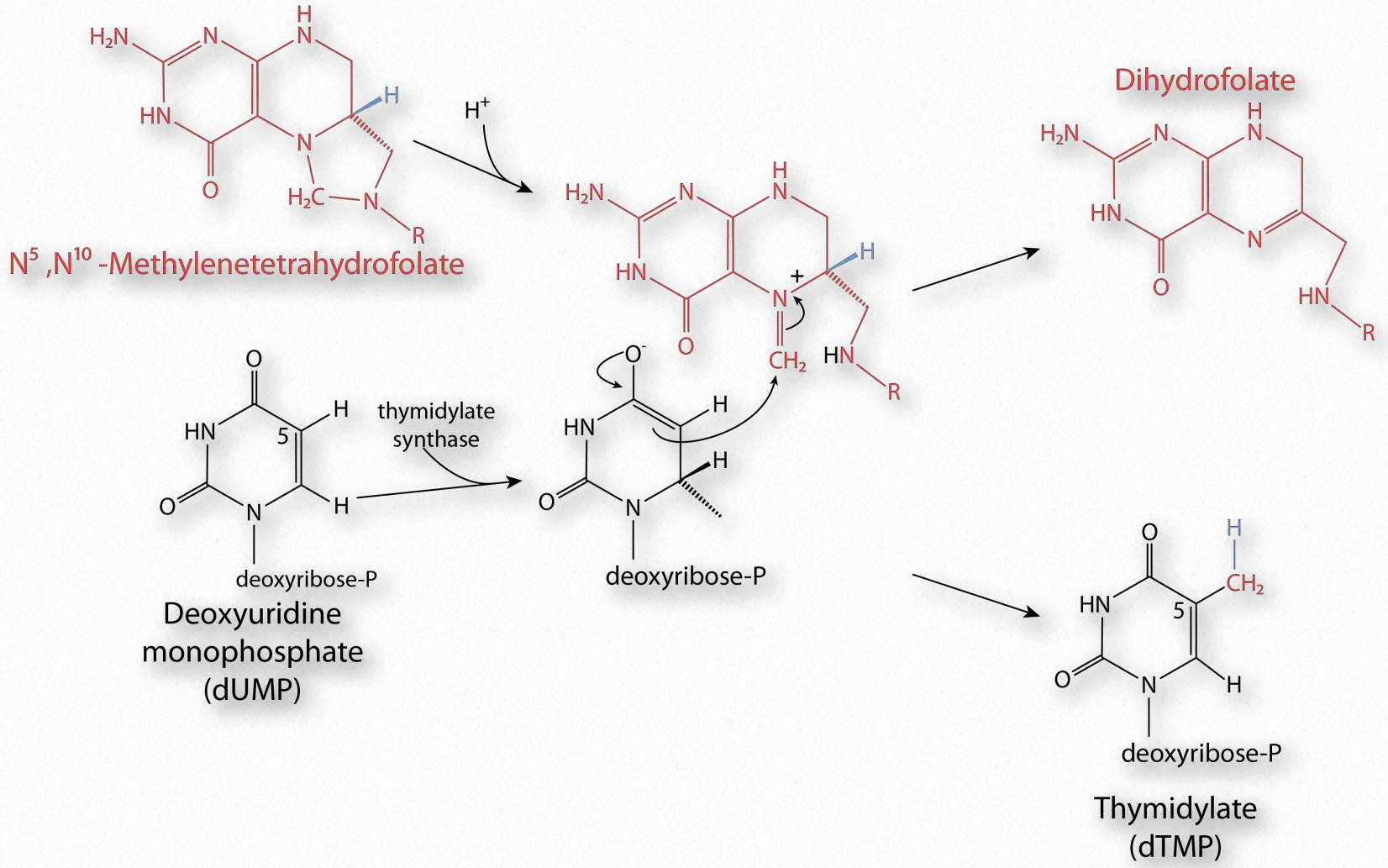


Figure 6.191 - Mechanism of synthesis of dTMP from dUMP

Image by Pehr Jacobson

Important enzymes in the pathway include dUTPase and thymidylate synthetase. dUTPase is important for keeping the concentration of dUTP low so it does not end up in DNA. DNA polymerase can use dUTP just as it does dTTP, and incorporate it into a DNA strand, across from adenine nucleotides.

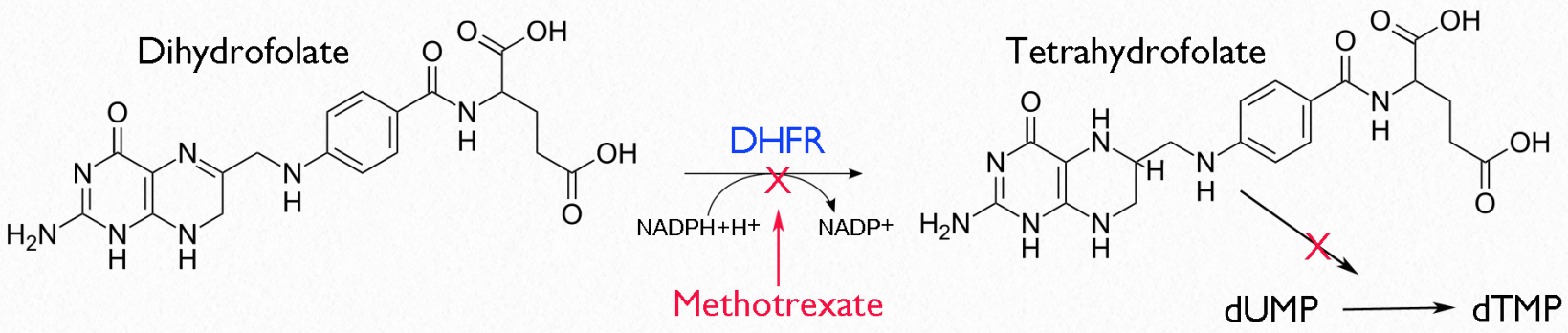


Figure 6.192 Recycling of dihydrofolate to tetrahydrofolate in cells can be inhibited by the drug methotrexate, thus stopping synthesis of thymidine nucleotides

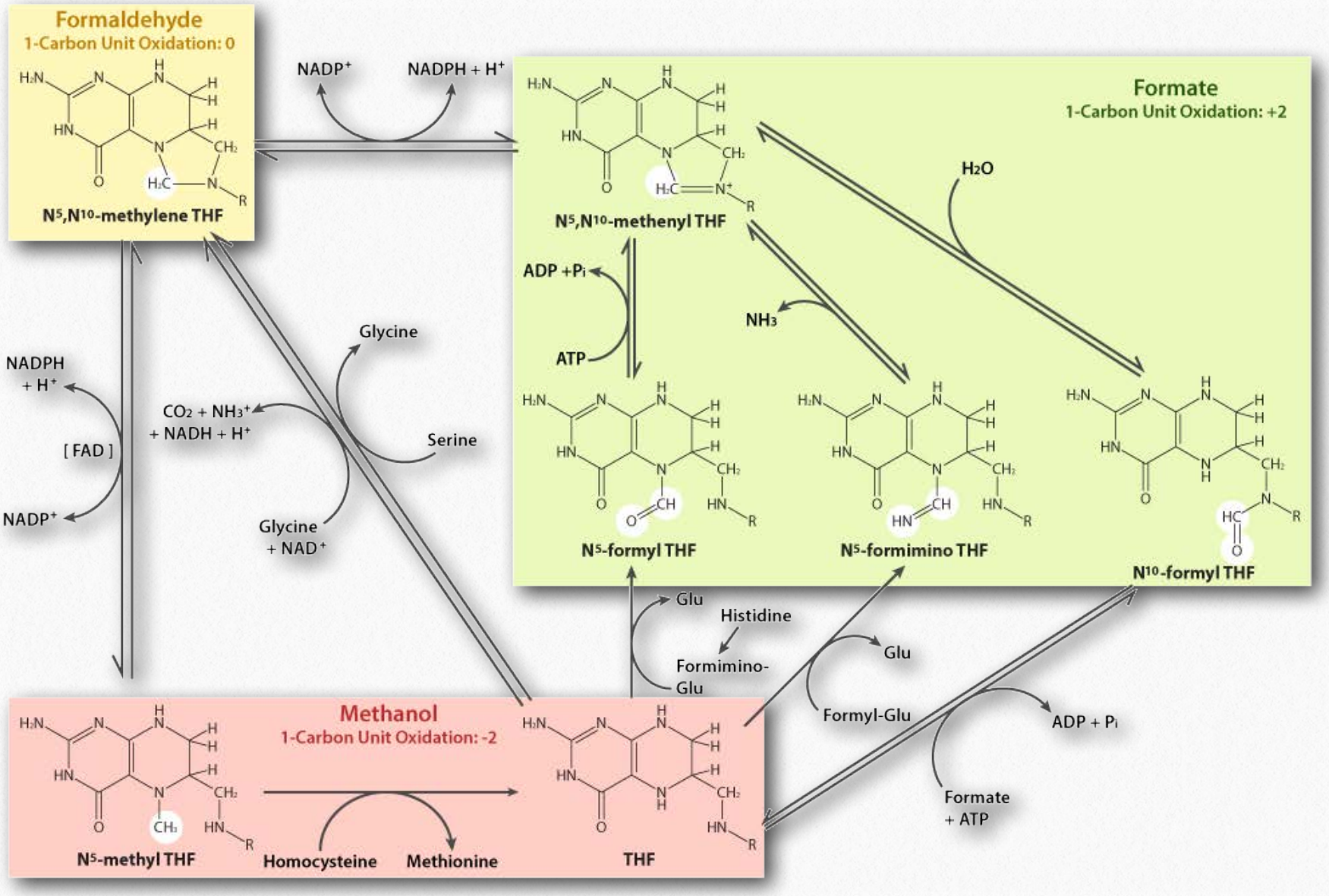


Figure 6.193 - Metabolism of folate molecules

Image by Aleia Kim

Thymidylate synthetase is important because it is a target (directly and indirectly) for anticancer therapies. As shown in Figure 6.191, a methyl group from N^5,N^{10}-methylene-tetrahydrofolate (often called tetrahydrofolate) is donated to dUMP, making dTMP and dihydrofolate (DHF). Folate molecules are in limited quantities in cells and must be recycled, because if they are not, then the reaction to make dTMP cannot occur. Recycling of dihydrofolate to tetrahydrofolate occurs by the reaction shown in Figure 6.192.

The enzyme involved in the conversion of dihydrofolate to tetrahydrofolate, dihydrofolate reductase (DHFR - Figure 6.192), is one target of anticancer drugs because by stopping the regeneration of tetrahydrofolate from dihydrofolate (otherwise a dead end), one can stop production of thymidine nucleotides and, as a result, halt DNA synthesis, thus preventing a cancer cell from dividing. Competitive inhibitors of DHFR include methotrexate (Figure 6.194) or aminopterin. Cells contain numerous folates for performing one carbon metabolism and the path-

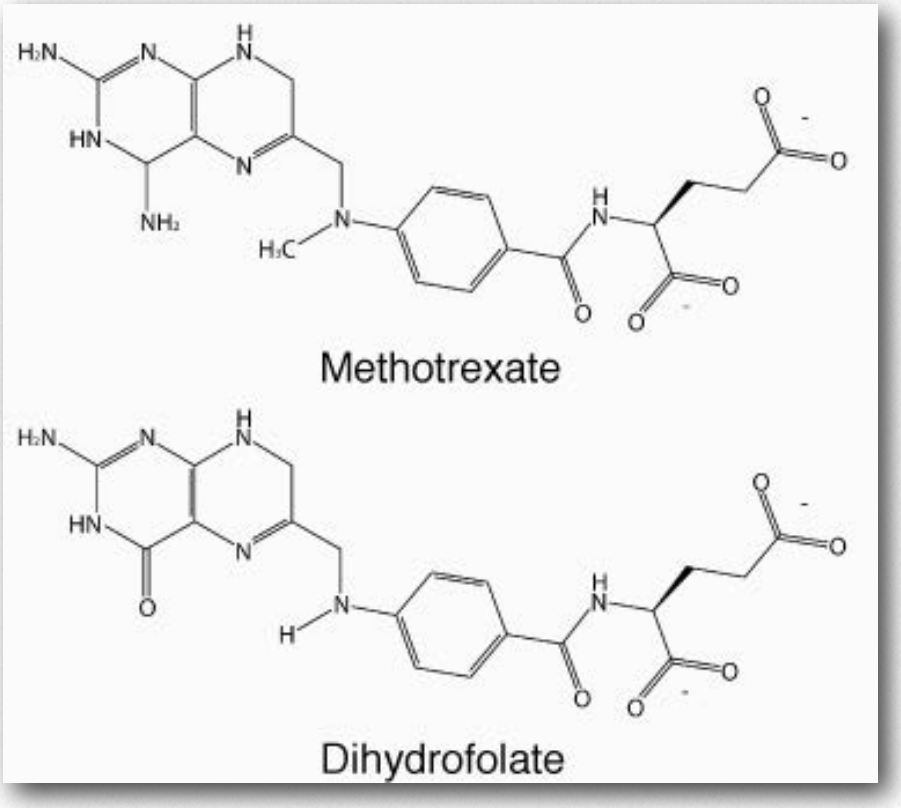


Figure 6.194 Dihydrofolate and competitive inhibitor methotrexate

Image by Ben Carson

ways by which they are all recycled is shown in Figure 6.193.

5-fluorouracil

Yet another important inhibitor of thymidine synthesis is used to treat cancer. This compound, 5-fluorouracil (Figure 6.195 and Movie 6.3) is a suicide inhibitor of thymidylate synthase.

Salvage synthesis

Besides synthesis from simple precursors, nucleotides can also be made from pieces of existing ones. This is particularly relevant, since consumption of food introduces to the

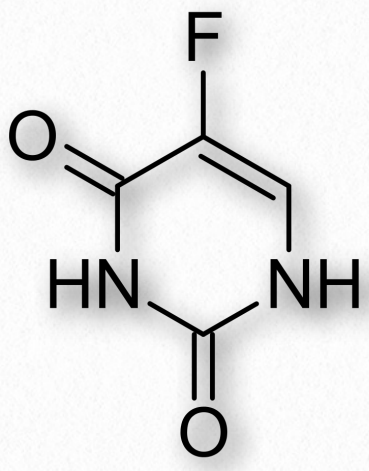
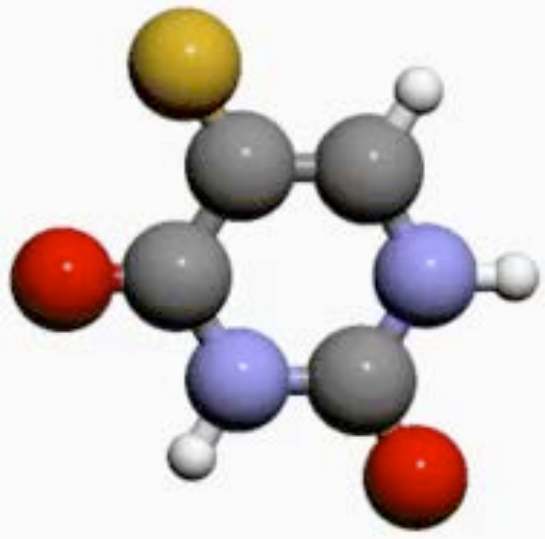


Figure 6.195 - 5-fluorouracil



Movie 6.3 - 5-fluorouracil

Wikipedia

body a large collection of proteins, lipids, and nucleic acids that are all more efficiently recycled than degraded. For proteins, the process is simple. Digestion converts them into constituent building blocks (amino acids) and these are re-assembled into proteins of the consuming organism using the genetic code.

Nucleotides

The multi-component structure of nucleotides, though (base, sugar, phosphate) means subsections of them may be re-utilized. Phosphate is recycled simply by entering the phosphate pool of the cell. It is typically built back into triphosphate forms (ultimately) by oxidative phosphorylation and kinase actions. Salvage of bases is different for purines and pyrimidines and is discussed separately HERE and HERE.

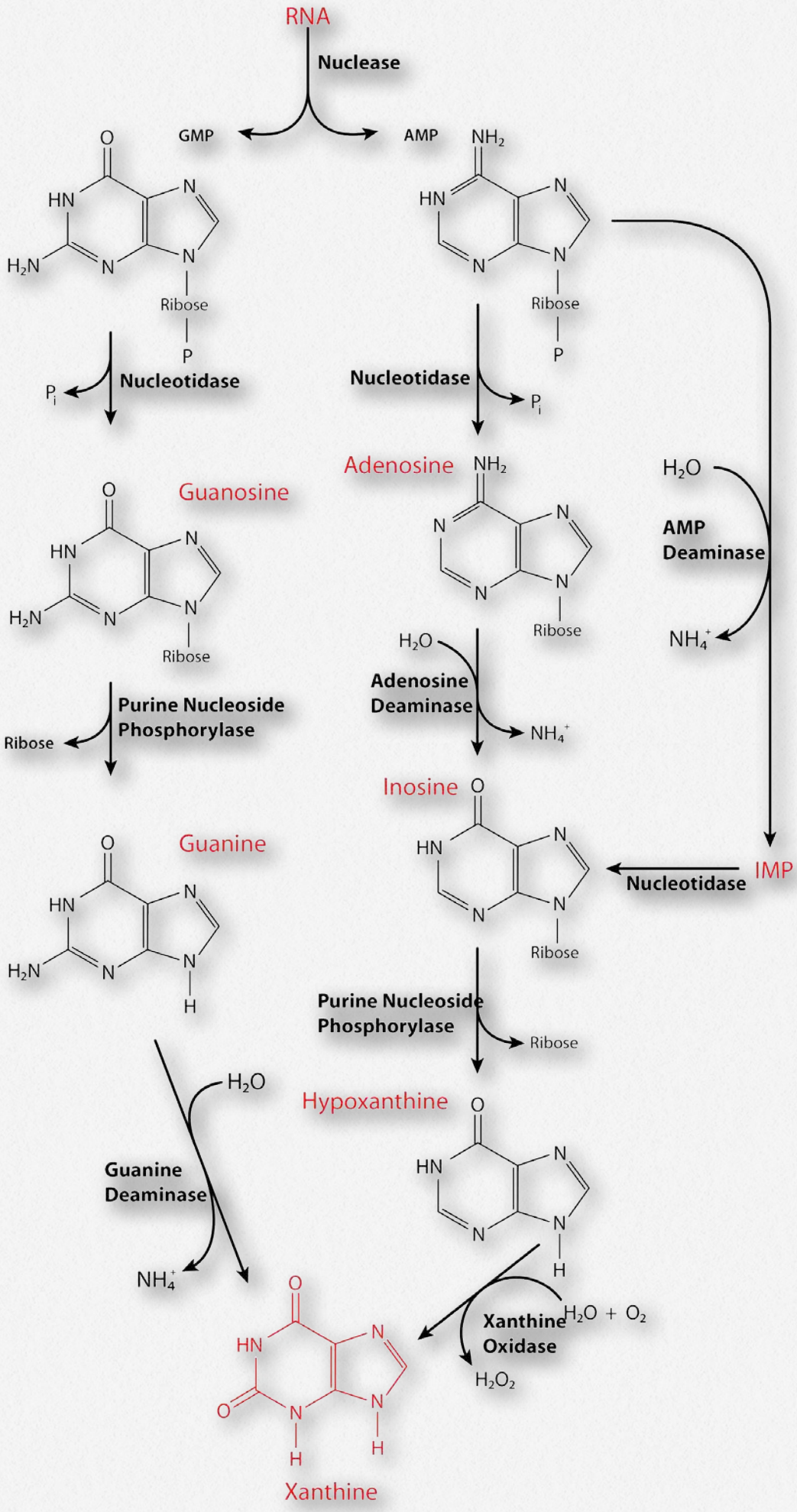


Figure 6.196 - Catabolism of purines - part I

Image by Pehr Jacobson

Nucleotide catabolism

Besides salvage and being built into nucleic acids, nucleotides can also be broken down into simpler component molecules. Some of these molecules, such as uric acid, can have significant impact on organisms (see HERE).

Purine catabolism

Breakdown of purine nucleotides starts with nucleoside monophosphates, which can be produced by breakdown of an RNA, for example, by a nuclease (Figure 6.196).

Metabolism of AMP and GMP converge at xanthine. First, AMP is dephosphorylated by nucleotidase to create adenosine, which is then deaminated by adenosine deaminase to yield inosine. Alternatively, AMP can be deaminated by AMP deaminase to yield IMP.

IMP is also an intermediate in the synthesis pathway for purine anabolism. Dephosphorylation of

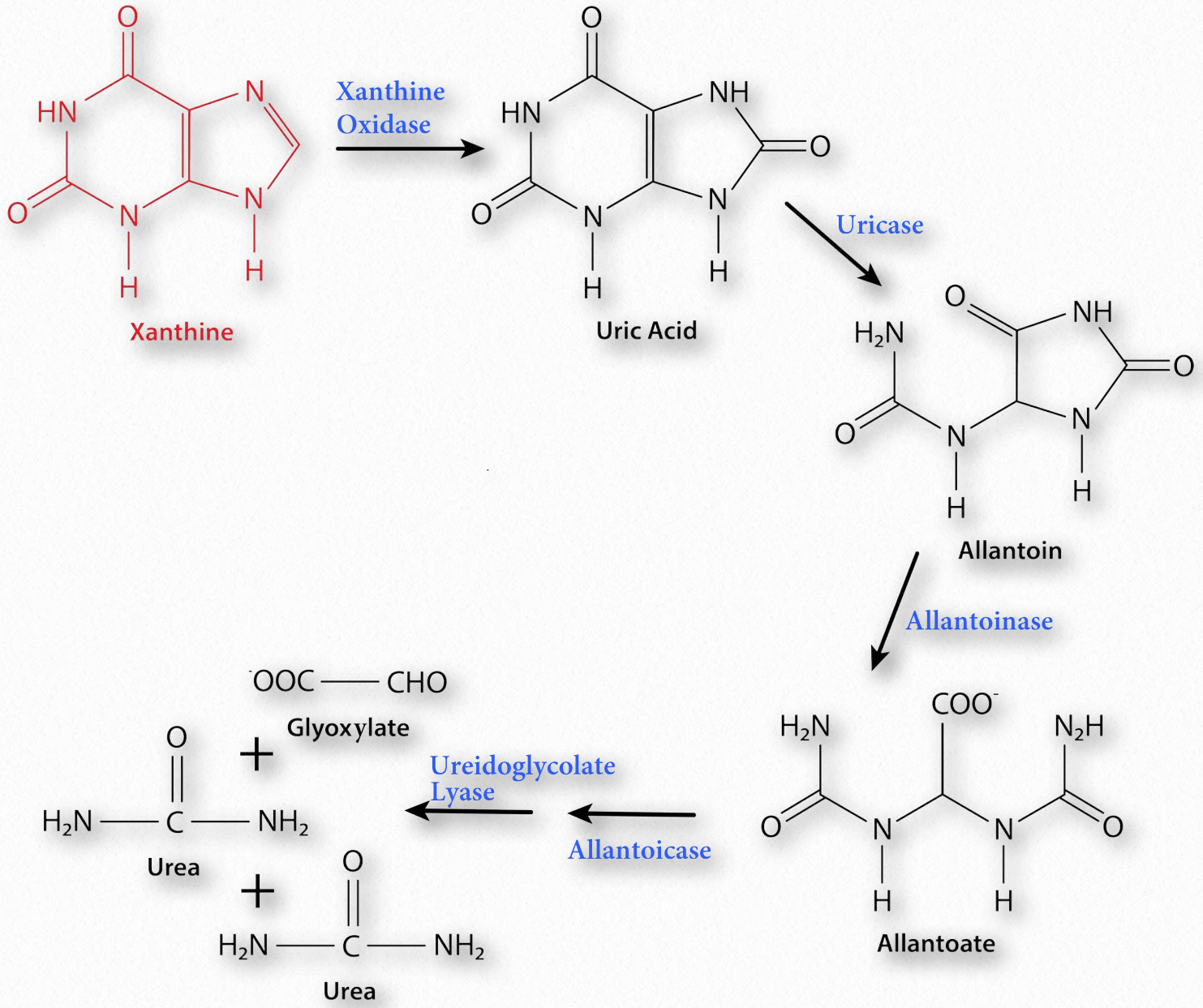


Figure 6.197 - Purine catabolism - part II

Image by Pehr Jacobson

IMP (also by nucleotidase) yields inosine. Inosine has ribose stripped from it by action of purine nucleotide phosphorylase to release hypoxanthine. Hypoxanthine is oxidized to xanthine in a hydrogen peroxide-generating reaction catalyzed by xanthine oxidase.

Hypoxanthine + H_2O + O_2

⇅

Xanthine + **H_2O_2**

Catabolism of GMP proceeds independently, though similarly. First, phosphate is removed by nucleotidase to yield guanosine. Guanosine is stripped of ribose to yield free guanine base, which is deaminated by guanine deaminase (also called guanase) to produce xanthine.

Xanthine oxidase enters the picture a second time in the next reaction catalyzing a second reaction by a similar mechanism to the hypoxanthine oxidation described previously. It is shown on the next page.

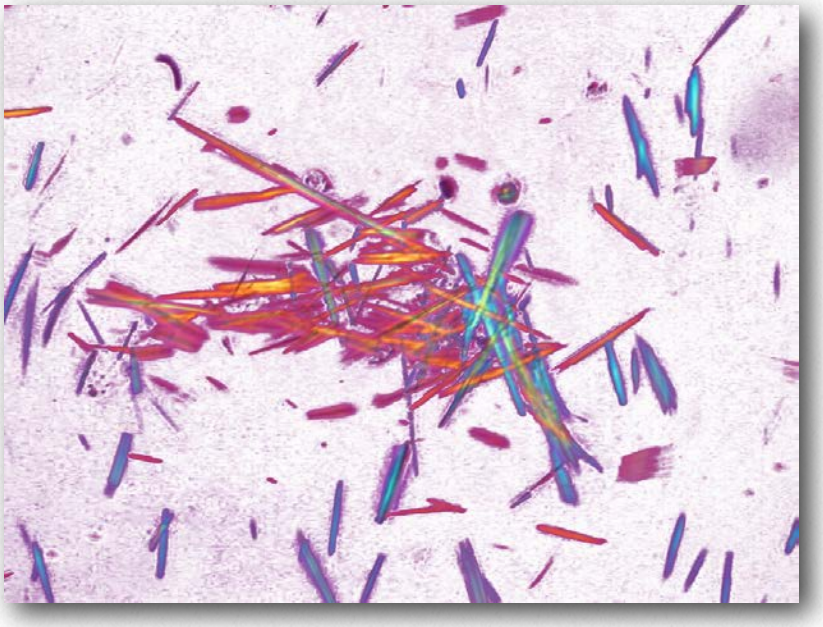


Figure 6.198 - Crystals of uric acid
Wikipedia

Xanthine + H_2O + O_2

⇅

Uric Acid + **H_2O_2**

Uric acid

Uric acid is problematic in some higher organisms (including humans) because it is not very soluble in water. Consequently it precipitates out of solution, forming crystals (Figure 6.198). Those crystals can accumulate in joints and (frequently) in the big toe. Such a condition is known as gout.

Interestingly, there may be a negative correlation between gout and contracting multiple sclerosis. This protective effect may be due to the antioxidant protection afforded by uric acid. Uric acid is the primary excretion form of nitrogen for birds. Dalmation dogs also excrete uric acid instead of urea and may suffer from joint pain as a result of gout-like conditions.

Gout is treated with a hypoxanthine analog known as allopurinol (Figure 6.199). It inhibits action of xanthine oxidase, which favors increase in the concentration of hypoxanthine. The latter is used in salvage synthesis to make additional purines.

Uric acid can be excreted into the urine (in humans) or broken down into allantoin by the uricase enzyme. Since humans lack the enzyme to make allantoin (urea in humans is produced by the urea cycle), its presence in the body means it was produced by non-enzymatic means. This is taken to be an indicator of oxidative stress, since it allantoin is produced non-enzymatically by oxidation of uric acid.

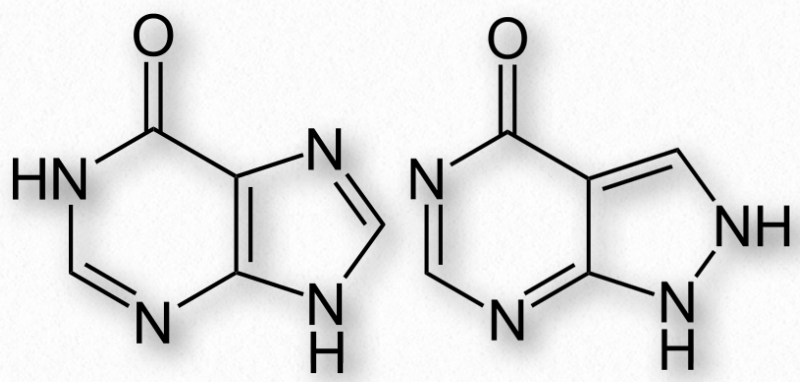


Figure 6.199 - Hypoxanthine (left) and allopurinol (right)
Wikipedia

Pyrimidine catabolism

Catabolism of uridine and thymidine nucleotides is shown above (Figure 6.200). Catabolism of cytidine nucleotides pro-

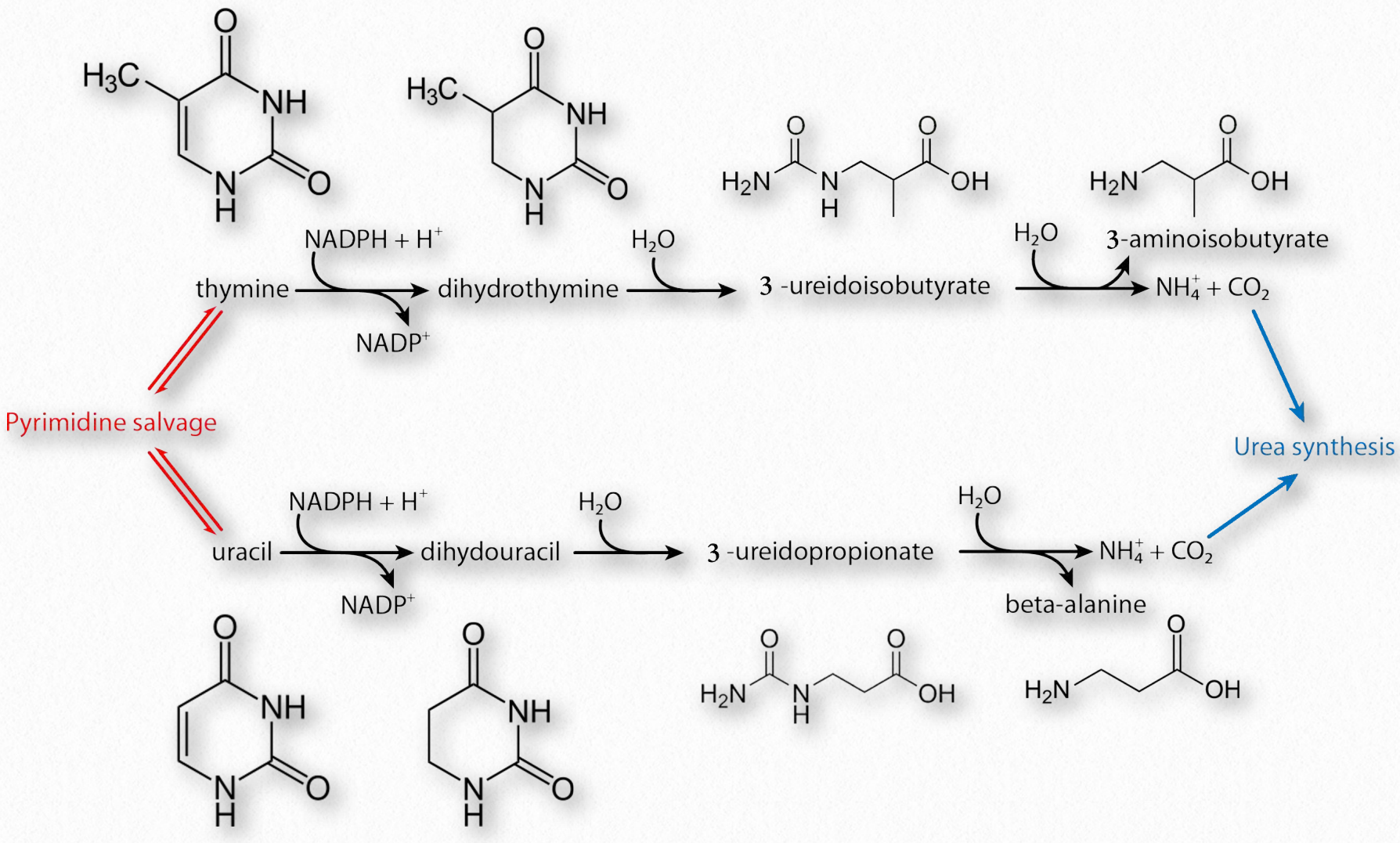


Figure 6.200 - Catabolism of pyrimidine nucleotides

Image by Pehr Jacobson

ceeds through uridine by deamination of cytosine. The free bases, thymine and uracil, are released by the enzyme ribosylpyrimidine nucleosidase In the reductive pathway, uracil and thymine reduction by NADPH gives dihydrothymine and dihydrouracil respectively. Addition of water to these creates 3-ureidoisobutyrate and 3-ureidopropionate respectively. Hydrolysis of both these intermediates yields ammonium ion and carbon dioxide (which are made into urea) plus 3-aminoisobutyrate for the thymine pathway and β-alanine for the product of the uracil pathway. 3-aminoisobutyrate is produced during exercise and activates expression of thermogenic genes in white fat cells.

YouTube Lectures by Kevin HERE & HERE

β-alanine is a rate-limiting precursor of carnosine, a dipeptide of histidine and β-alanine (Figure 6.201). Carnosine functions as an antioxidant that scavenges reactive oxygen species. It also acts as an anti-glycating agent to prevent against attachment of sugar molecules to proteins. These are factors in degenerative diseases and may play a role in aging.

Sugars

Last, but not least, the sugars ribose and deoxyribose can be recycled (ribose) or catabolized (ribose and deoxyribose). In the case of ribose, it can be reattached to bases by phos-

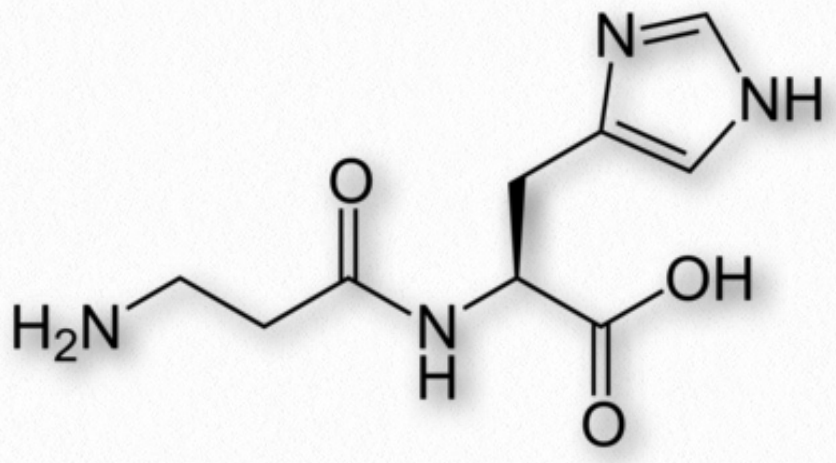


Figure 6.201 - Carnosine

phorylase enzymes, such as uridine phosphorylase, or converted into PRPP for the same purpose, to create nucleosides. Ribose-5-phosphate is an intermediate in the pentose phosphate pathway, allowing it to be converted into other sugars or broken down in glycolysis.

Deoxyribose-5-phosphate can be broken into two pieces by deoxyribose-5-phosphate aldolase. The products of this reaction are glyceraldehyde-3-phosphate and acetaldehyde. The former can be oxidized in glycolysis and the latter can be converted into acetyl-CoA for further metabolism.

Graphic images in this book were products of the work of several talented students. Links to their Web pages are below

Click HERE for Aleia Kim's Web Page

Click HERE for Pehr Jacobson's Web Page

Click HERE for Penelope Irving's Web Page

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Deoxynucleotides

(to the tune of "*Ticket to Ride*")

Metabolic Melodies Website HERE

Tonight I'm feeling quite glad
Because I can say (yeah)
My cells are working like mad
To make DNA

They're making nucleoti-ides
Way down deep and insi-i-ide
Deoxynucleotides
Are on the way

They activate RNR
With an ATP, yeah
Changing a T to an R
Deep inside of me

Deoxynucleoti - ides
That's what the enzyme provi-i-ides
Deoxynucleotides
Are on the way

The enzyme's mechanism's so sly
It is tactical
Using radical
You see
So when it kisses substrate goodbye
It seems magical
In its practical-i-ty

The two prime I will mention
In this exercise, yeah
Its one bit of oxygen
Has got to downsize, yeah

2-prime is de-ox-i-fying
That's what the enzyme is buy-i-ing
2-prime is deoxified
It's gone away

They're gonna go and make DNA
You gotta know that
They're gonna do that in me
They're gonna go and replicate
A polymerase
Is gonna do that for me

Because of structure decrees
In nucleotides, yay
DNA is in b's
But not much in a's

Oh deoxynucleotides
Deoxynucleoti-i-ides
Deoxynucleotides
Give B-forms
That oxygen's gone
That oxygen's gone
That oxygen's gone

Lyrics by Kevin Ahern

No Recording Yet For This Song

Things You Should Remember

To the tune of "*In My Life*"

Metabolic Melodies Website HERE

There are things you should remember
When you're stud-y-ing for this exam
All the pathways since September
And the mol-e-cules comprising them

Though that is an awful lot of information
I hope that you can retain it all
If you do you will avoid a grade deflation
When you study right, you will recall

Now in all your preparation
There is soooome-thing you should regard
How your brain stores information
So transcribe your notes onto a card

I assure you it will up your recollection
Of enzymes and com-plex Haworth rings
It will drive performance to perfection
Simply from the act of writing things

I assure you it will up your recollection
Of enzymes and com-plex Haworth rings
It will drive performance to perfection
Simply from the act of writing things

So go forward now . . . . . and write down things

Recording by David Simmons
Lyrics by Kevin Ahern