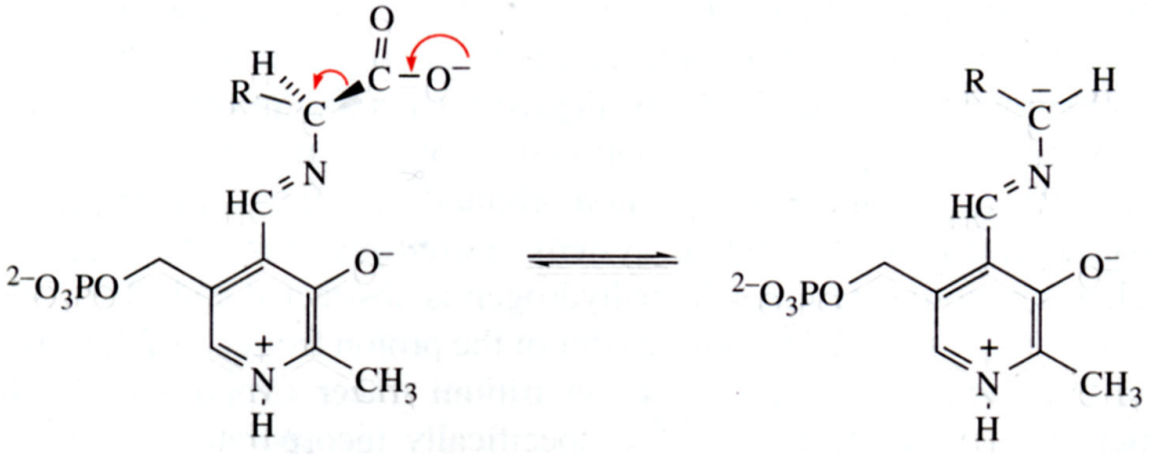
Rearrangement of an external aldimine: formation of an α-carbanionic PLP intermediate via α-decarboxylation



Scheme 3-3

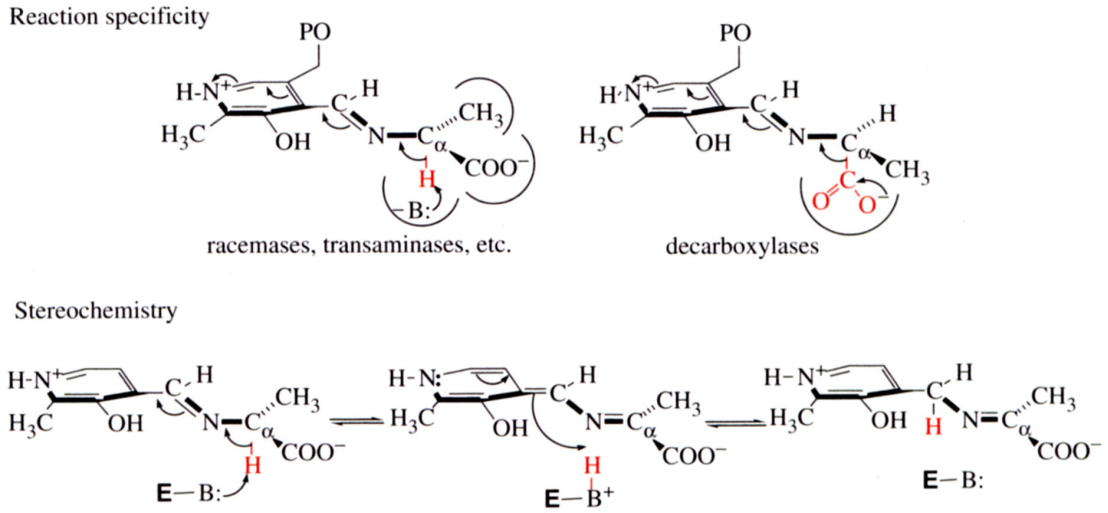


Fig. 3-20. Determinants of reaction specificities and stereospecificities of pyridoxal-5′-phosphate (PLP) enzymes. *Reaction specificity*: Orientation about the N—C_α bond in the external aldimine and the placement of catalytic groups determine whether decarboxylation or removal of the α-hydrogen will take (Floss and Vederas, 1982). Abstraction of the α-hydrogen is facilitated by the orientation at the left, in which the α-carbanion orbital developing from proton abstraction by a well-placed base is aligned for maximum overlap with the π-bonds of the imine and pyridinium ring. Decarboxylation is favored by the placement of the carboxylate group as at the right, in which the α-carbanion orbital developing from decarboxylation attains maximum overlap with the π-bonding system. *Stereospecificity*: In many PLP-dependent enzymes, such as aminotransferases, the α-hydrogen is abstracted and PLP-C4′ is temporarily protonated. In these cases, there is often transfer of the proton from C_α of the amino acid moiety to C4′ of the coenzyme. This is detected in tritium tracer experiments with [2-^{3}H]amino acids, in which tritium can be found stereospecifically incorporated into the C4′ carbon of pyridoxamine-5′-phosphate.

Specificities of PLP enzymes: α-hydrogen abstraction *vs* α-decarboxylation is determined by the binding mode in the enzyme active site

PLP enzyme mechanisms: conveniently studied because the intermediates can be observed spectrophotometrically

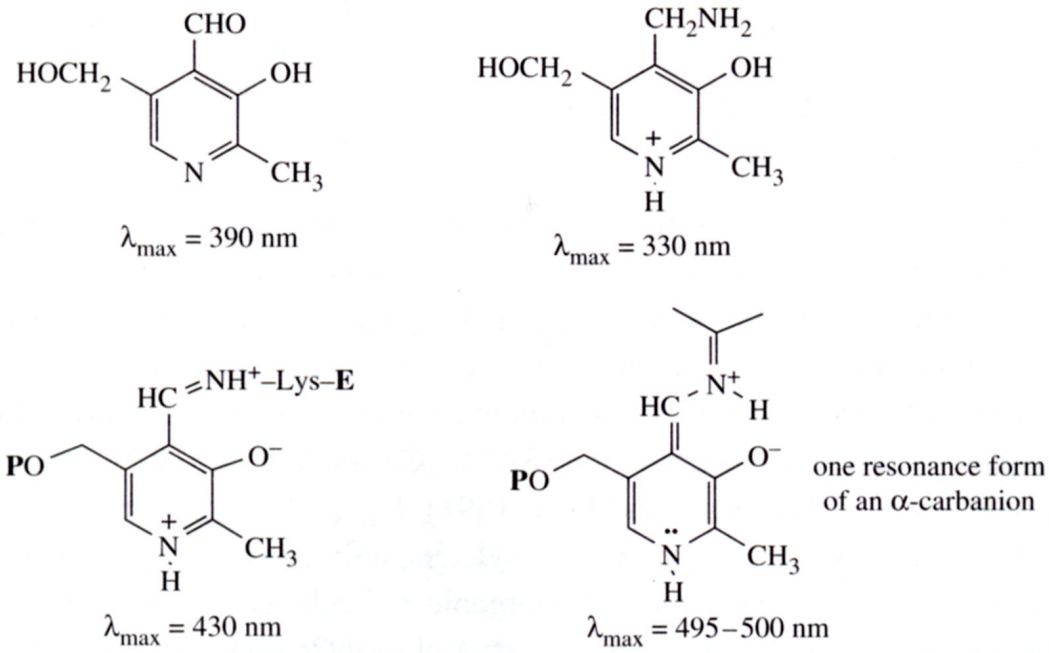


Fig. 3-21. Spectrophotometric properties of some pyridoxal-5′-phosphate compounds.

Nominal λ_{max}: vary somewhat from system to system

Mechanism of tryptophan synthase

Conversion of indole glycerol phosphate and serine to tryptophan and G3P

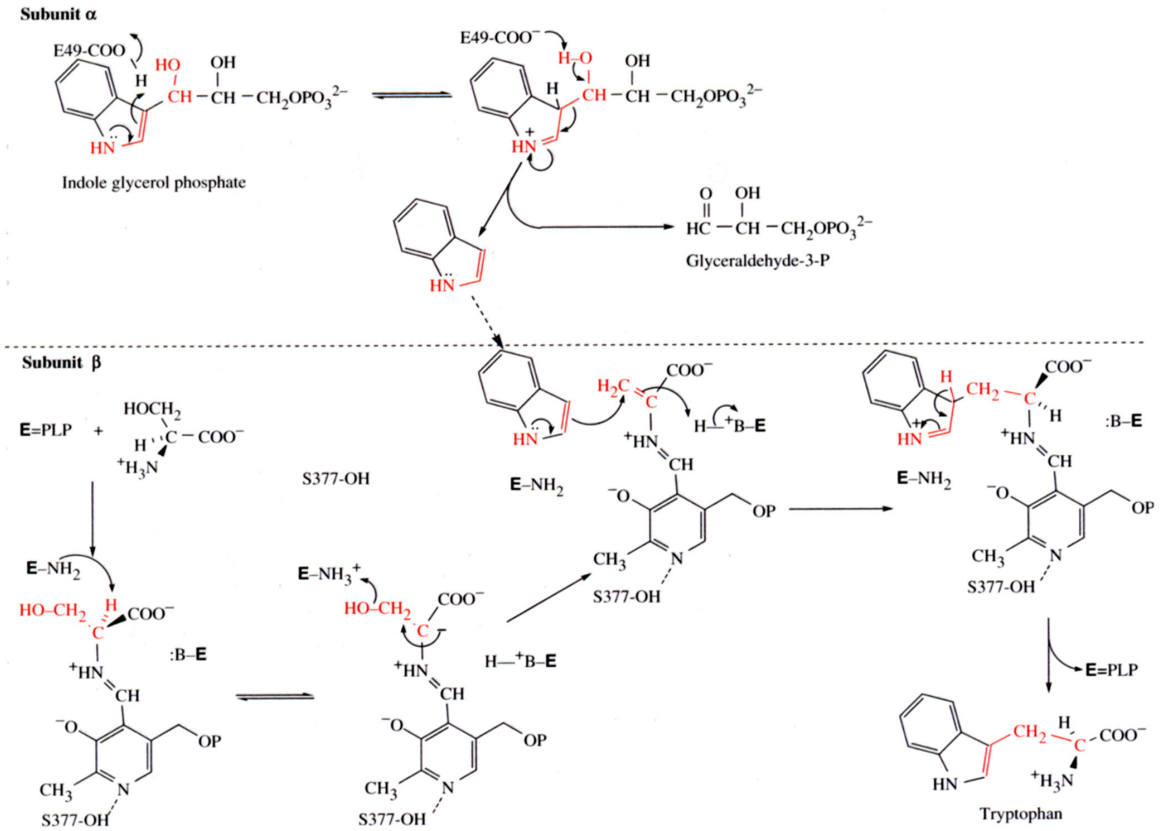


Fig. 3-22. A mechanism of the reaction of tryptophan synthase. The interface between the subunits symbolizes the tunnel shown in fig. 3-23. Indole is produced from indoleglycerol phosphate in the α subunit and migrates through the protein to the active site of the β subunit, where pyridoxal-5′-phosphate (PLP) catalyzes the dehydration of serine. Indole undergoes a β-replacement of the OH group of serine to form tryptophan. The ring nitrogen of PLP is not shown as protonated because the structure indicates that the nitrogen is hydrogen bonded to a serine residue, not an aspartate as in transaminases.

Tunneling between the α and β subunits of tryptophan synthase

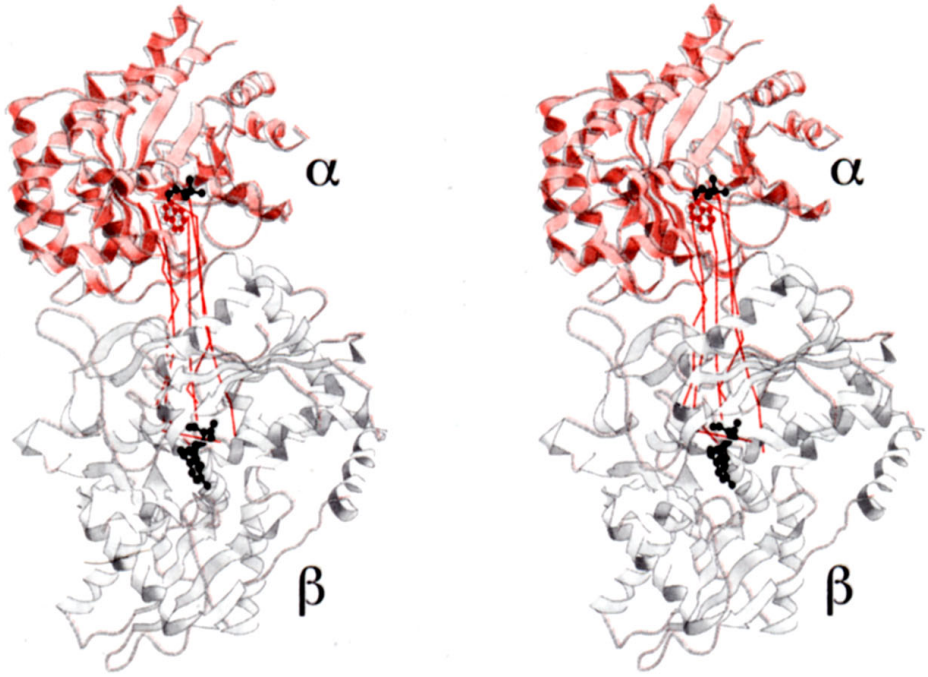
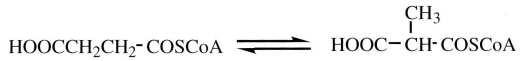
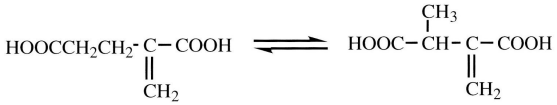
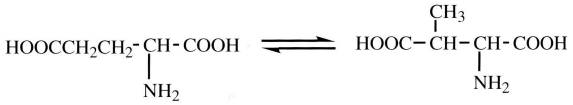
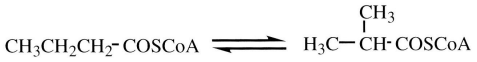
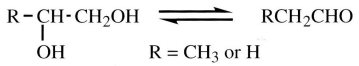
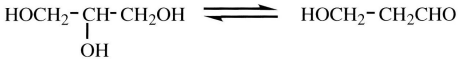
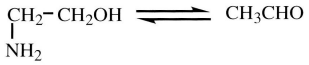
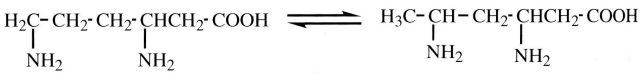
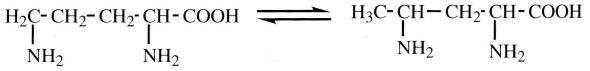
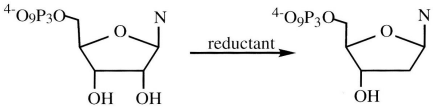


Fig. 3-23. A "tunnel" (red) connecting the active sites in tryptophan synthase runs between the two active sites in *Salmonella typhimurium* tryptophan synthase (EC 4.2.1.20), allowing indole generated in the α subunit to travel to the β subunit without being released into solution. Indole glycerol phosphate is shown as a ball-and-stick model in the α-subunit active site, with the indole moiety in red and the remainder in black. The pyridoxal-5′-phosphate internal aldimine is shown as a black ball-and-stick model in the active site of the β subunit. The illustration was generated using PDB 1QOQ (Weyand and Schlichting, 1999).

Enzyme	Reaction catalyzed
CARBON SKELETAL REARRANGEMENTS	
Methylmalonyl-CoA mutase	
2-Methyleneglutarate mutase	
Glutamate mutase	
Isobutyryl-CoA mutase	
ELIMINATIONS	
Diol dehydratase	
Glycerol dehydratase	
Ethanolamine ammonia lyase	
ISOMERIZATIONS	
L-β-Lysine-5,6-aminomutase	
D-Ornithine-4,5-aminomutase	
REDUCTION	
Ribonucleotide reductase	

Coenzyme B_{12}-dependent enzyme-catalyzed reactions

Also involve pyridoxal phosphate!

Radical-based rearrangements involving PLP: aminomutases

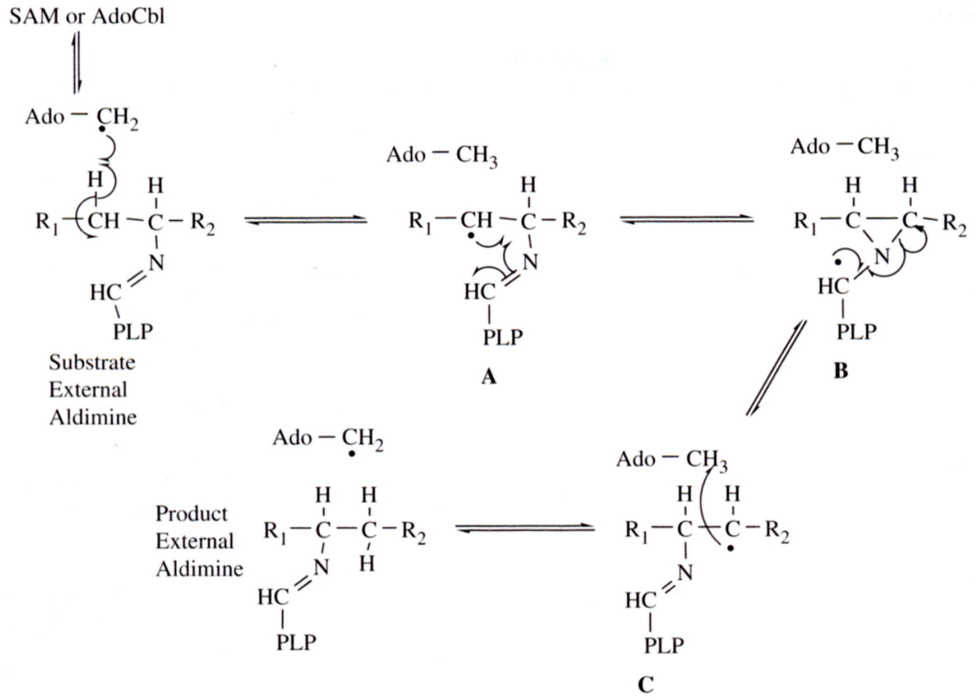


Fig. 3-25. The role of pyridoxal-5′-phosphate (PLP) in the radical-based rearrangement of aminomutases. PLP cannot facilitate the reaction of lysine 2,3-aminomutase by way of carbanionic intermediates. Instead, aminomutases induce radical formation through hydrogen abstraction by the 5′-deoxyadenosyl radical generated from *S*-adenosylmethionine or adenosylcobalamin (see chap. 4). PLP facilitates amino group migration in the external aldimine by means of radical isomerization.

Involvement of PLP in deoxysugar biosynthesis

CDP-4-keto-
6-deoxyglucopyranose

CDP-4-keto-
3,6-dideoxyglucopyranose

2 one-electron
reductions

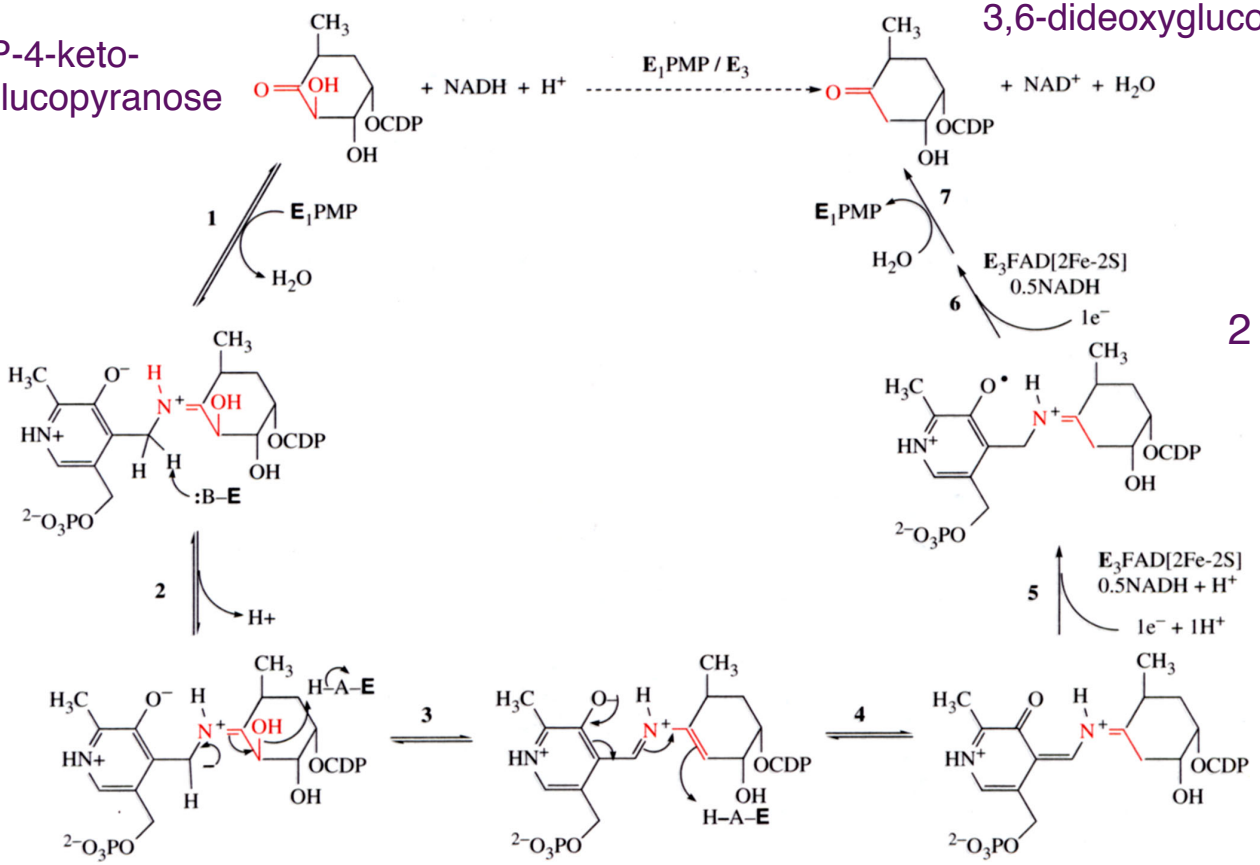
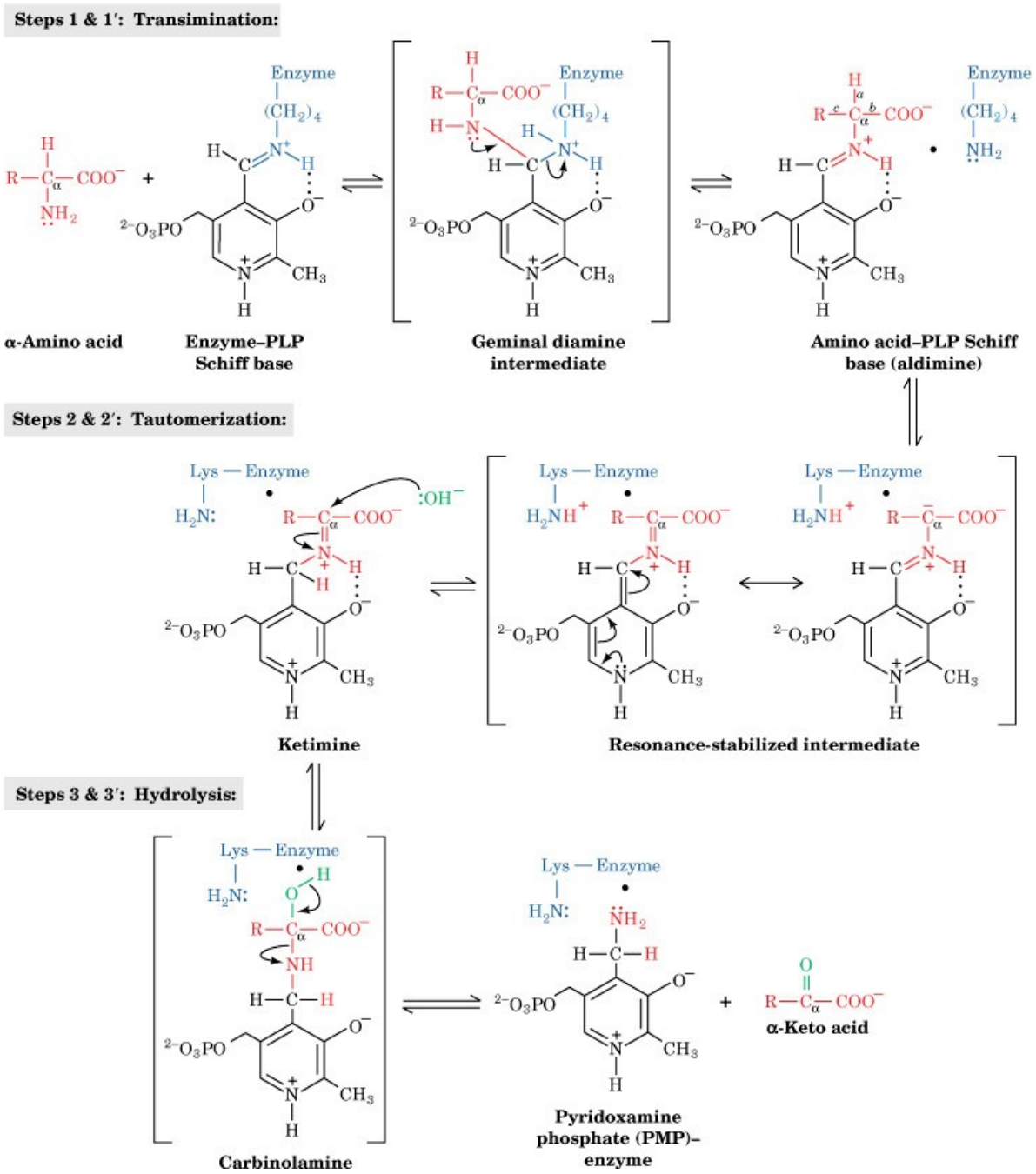


Fig. 3-26. Role of pyridoxal-5′-phosphate in deoxysugar formation. A hypothetical mechanism is shown for reduction of carbon 3 in 3,6-dideoxysugar biosynthesis.



The mechanism of PLP-dependent enzyme-catalyzed transamination

Folate coenzymes

Core structure: tetrahydrofolic acid

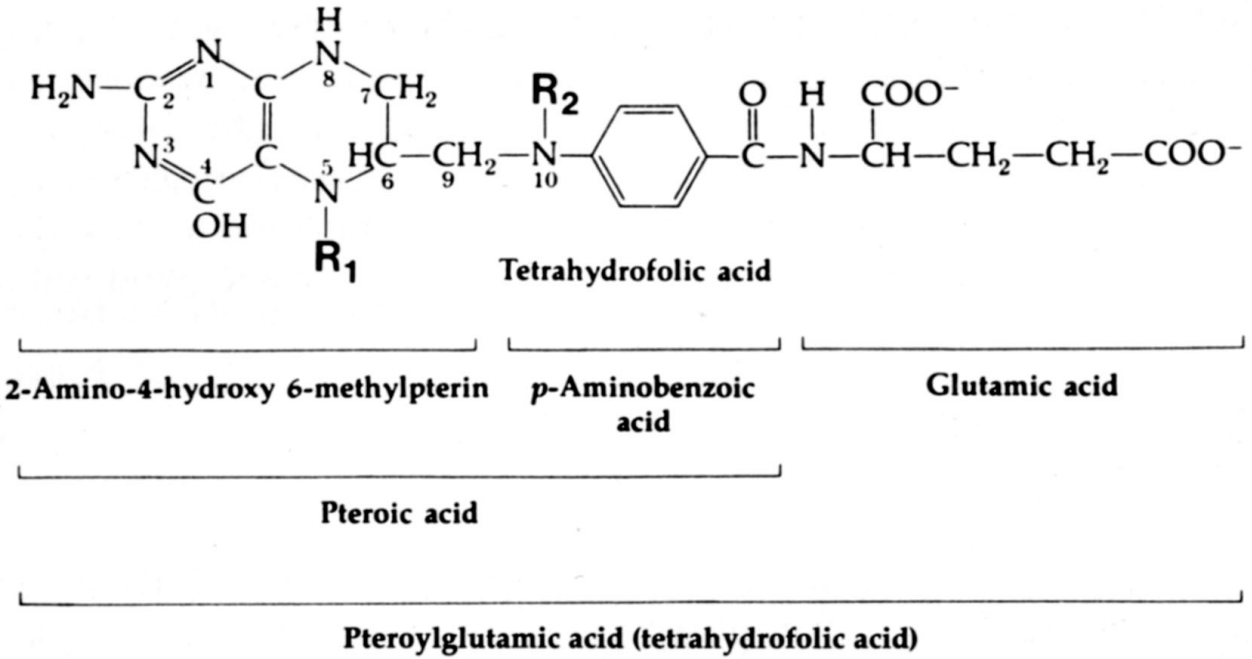
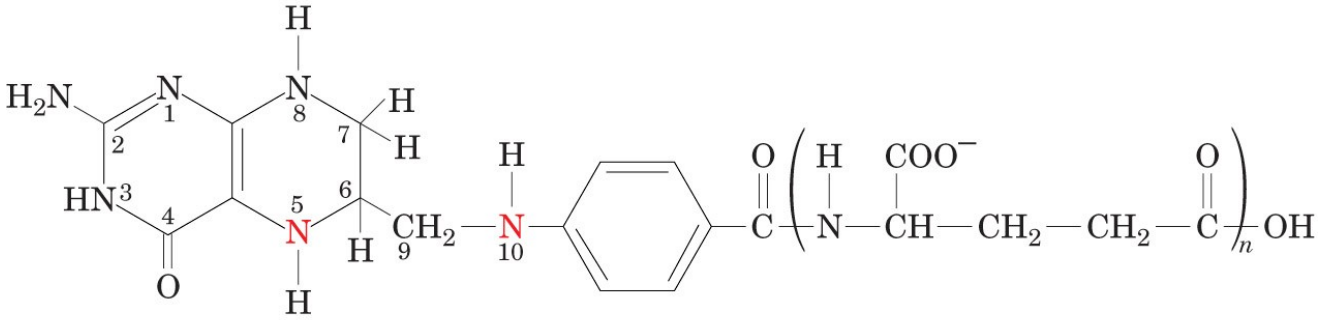


Figure 4.16 Generalized structure and nomenclature of tetrahydrofolate derivatives. The active, nucleophilic nitrogen atoms in the coenzyme are N^5 and N^{10}, both of which are activated by adjacent aromatic rings. The active coenzyme consists of three moieties: a reduced, substituted pterin ring, 2-amino-4-hydroxy-6-methylpterin, *p*-aminobenzoic acid, and a chain of from one to seven glutamyl residues. Only a single glutamyl residue is shown. R_1 and R_2 are either hydrogens (in FH_4 itself) or the one carbon fragments shown in Figure 4.17.

Chemical structure of tetrahydrofolate (THF)



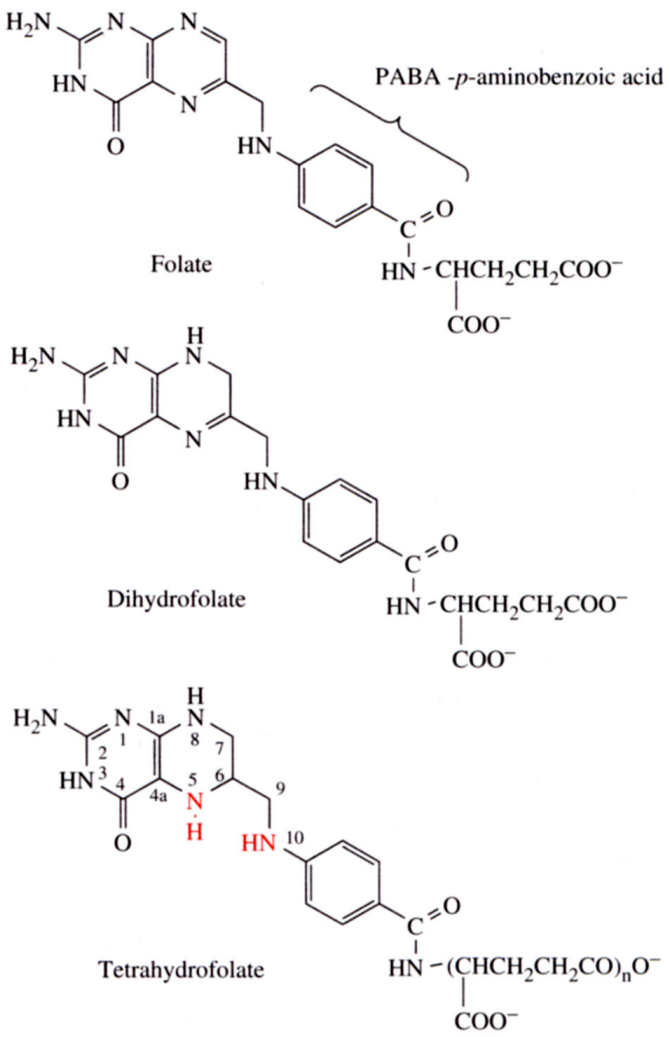
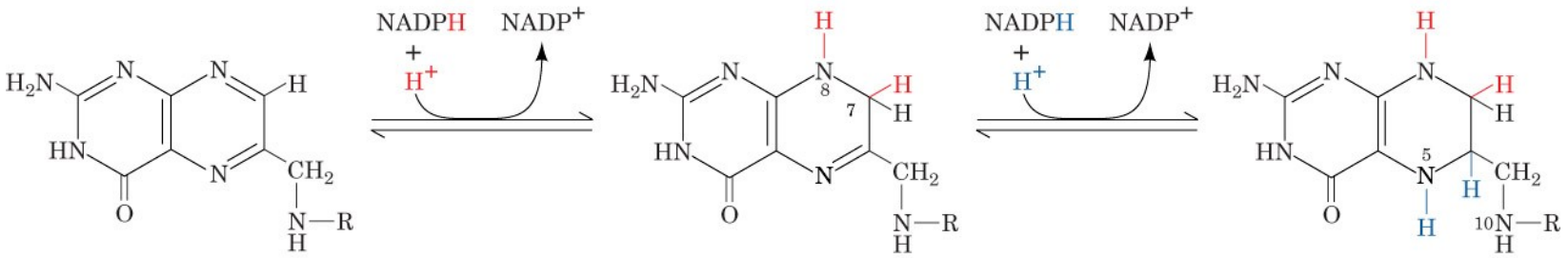


Fig. 3-33. Structure and oxidation states of folic acid. The biologically important forms of the vitamin folic acid are dihydrofolate and tetrahydrofolate, shown at the bottom of the figure. *p*-Aminobenzoic acid (PABA), a nutritional factor, is incorporated in the biosynthesis of folate. The highlighted N^5 and N^{10} in tetrahydrofolate participate directly in biological reactions of folic acid. A polyglutamate is shown in the structure of tetrahydrofolate because the polyglutamate species with five of six glutamates are the most biologically active.

Chemical structures of folate, dihydrofolate, and tetrahydrofolate

The two-stage reduction of folate to THF



Folate | **7,8-Dihydrofolate (DHF)** | **Tetrahydrofolate (THF)**

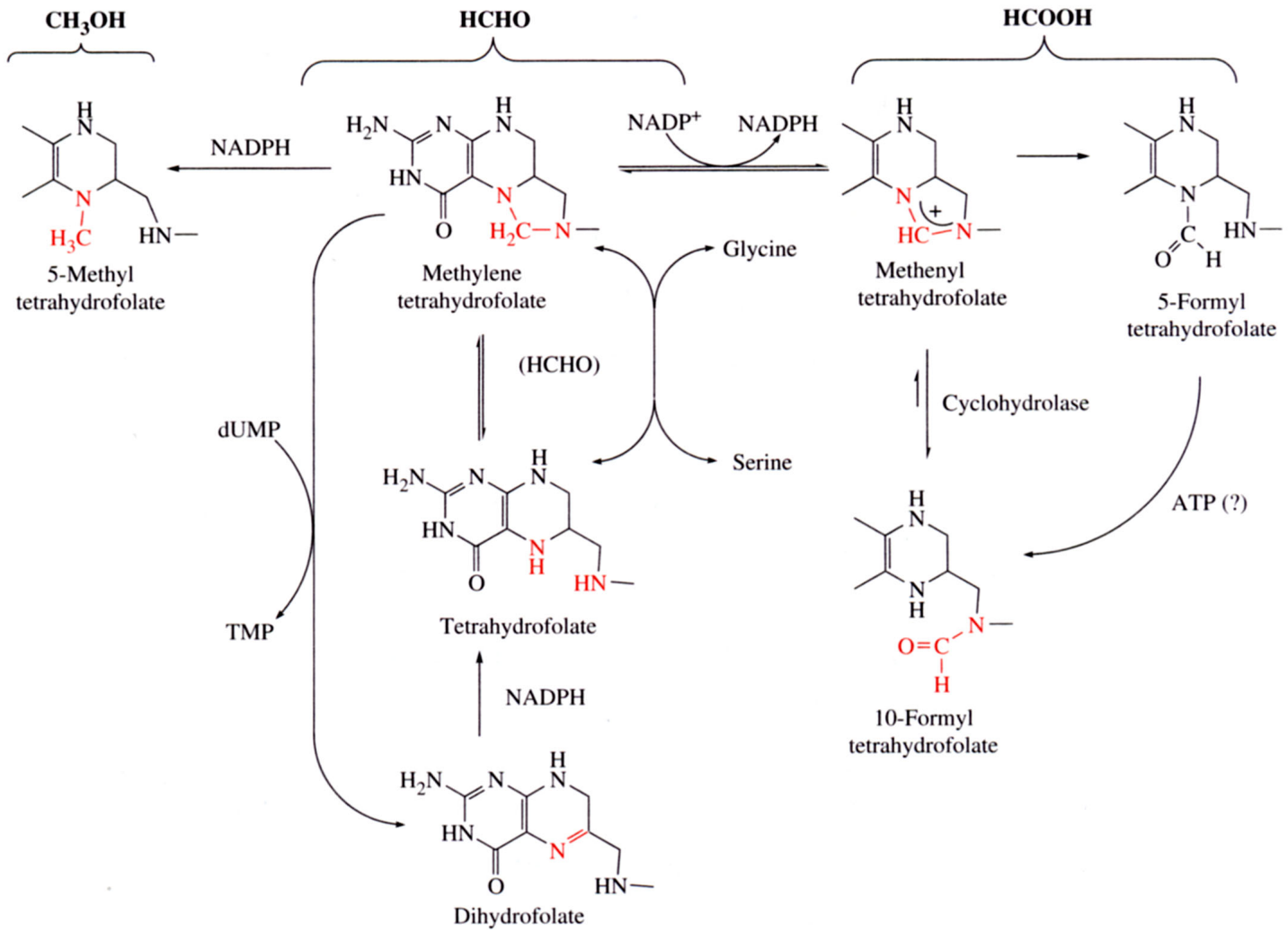
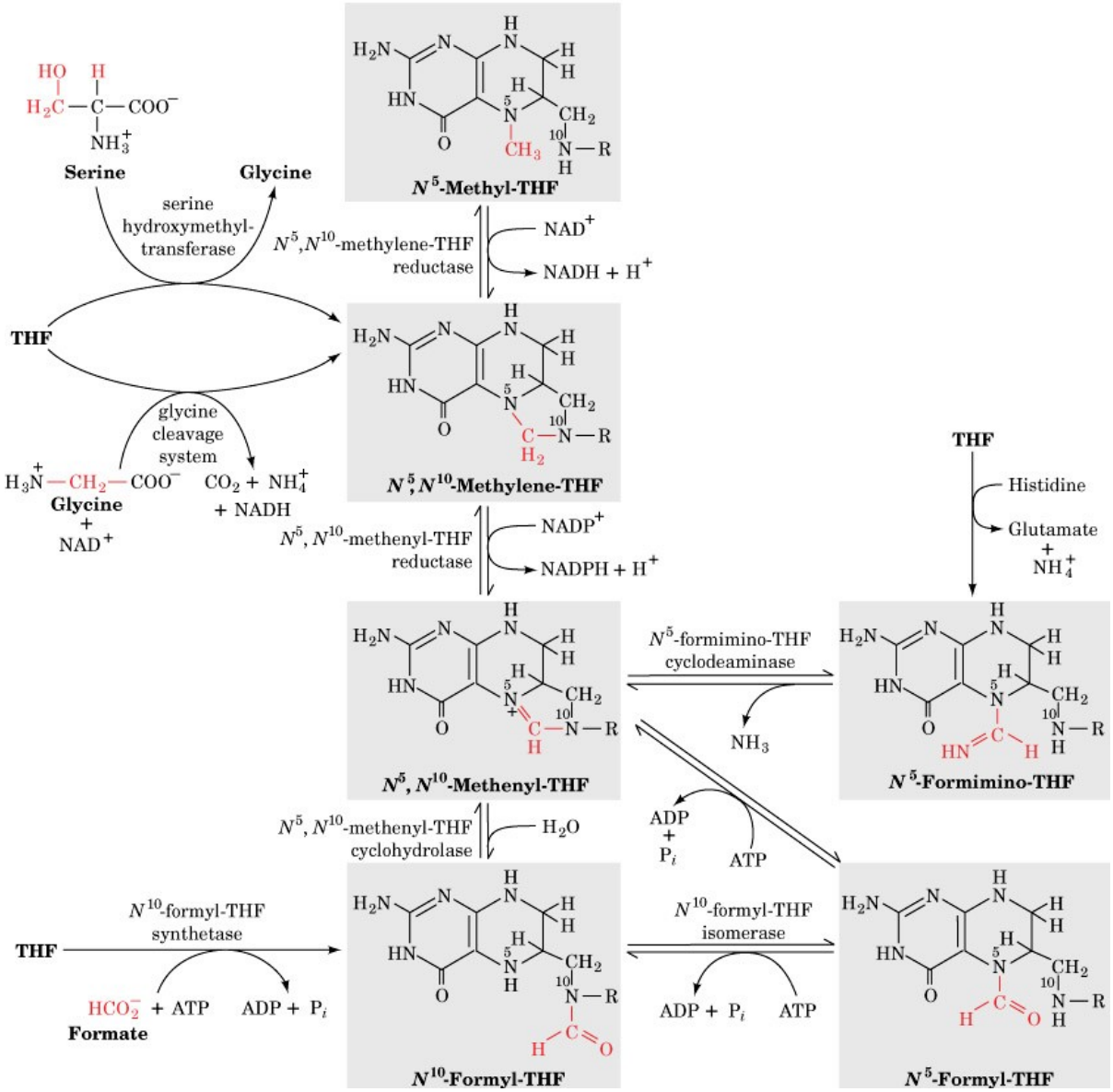


Fig. 3-34. Structures and interconversions of folate compounds in one-carbon metabolism.

Interconversion of the C_1 units carried by THF



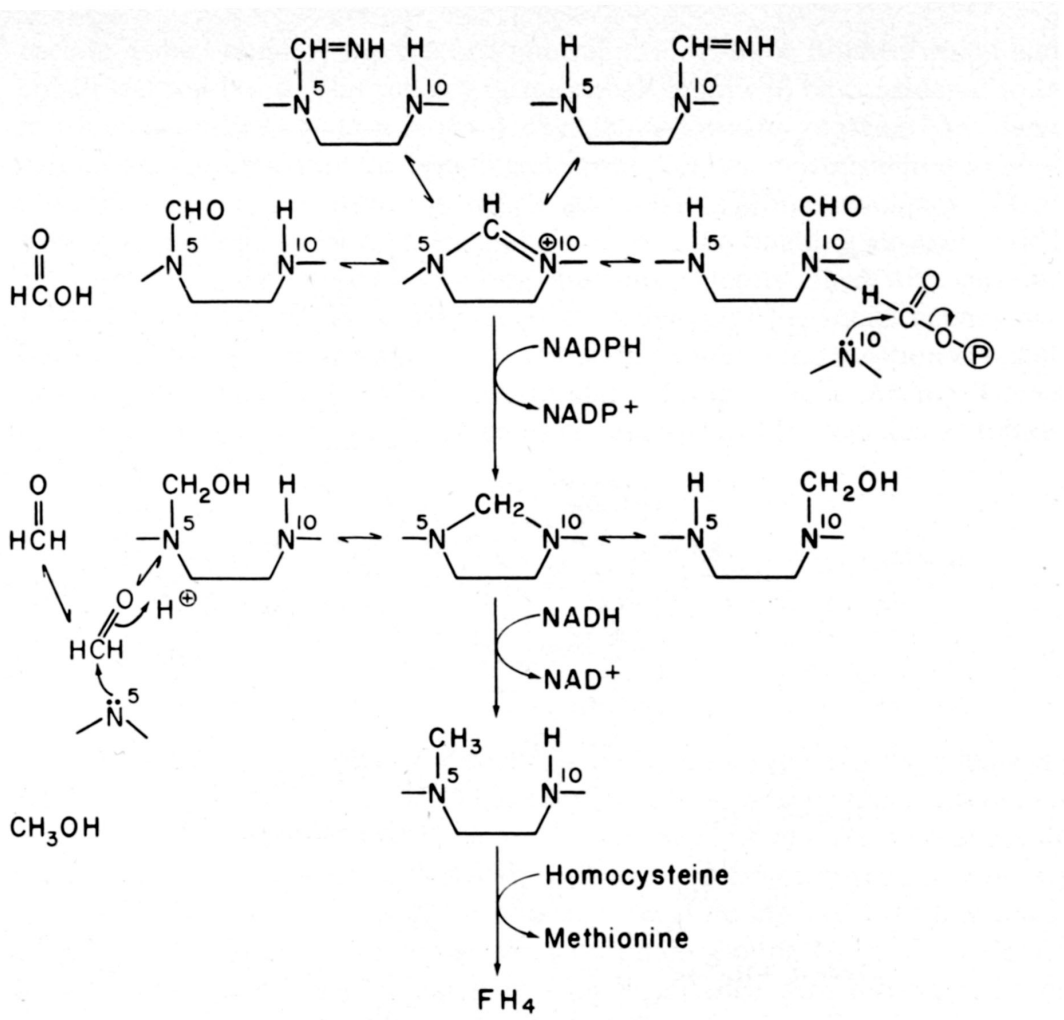
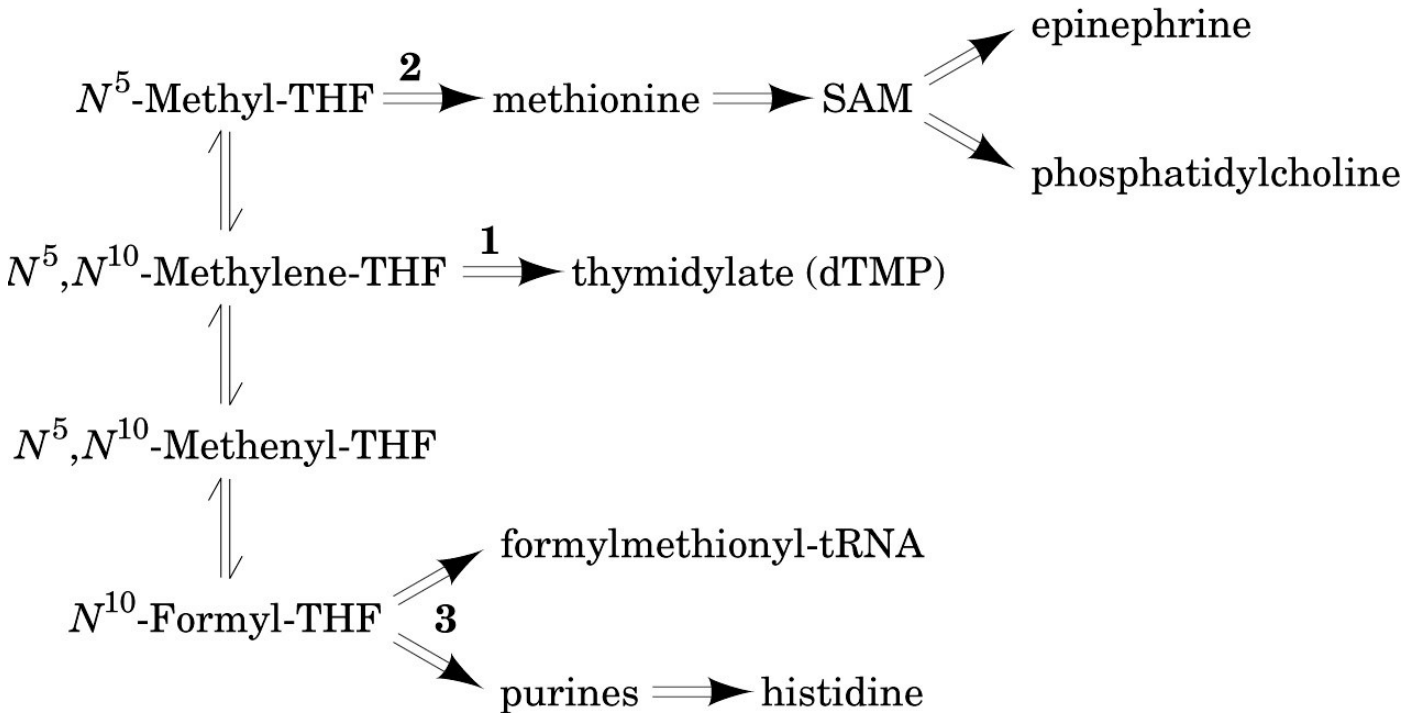
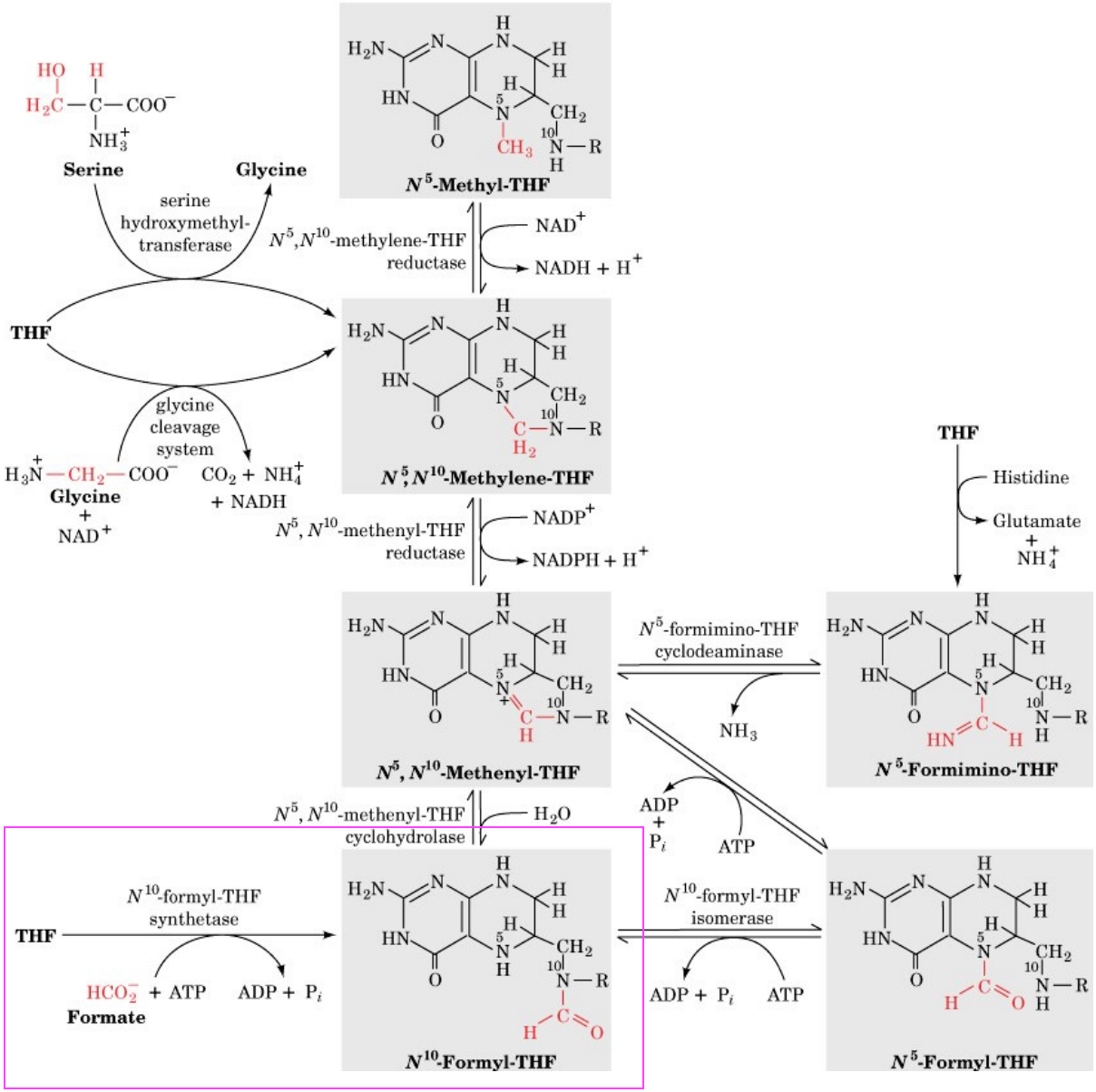


Figure 4.17 Interconversion of the various derivatives of tetrahydrofolic acid (FH_4). Vertical interconversions are catalyzed by $NADP^+$- and NAD^+-dependent enzymes as shown. Horizontal interconversions may occur spontaneously or be enzyme-catalyzed. The structures of formic acid (HCO_2H), formaldehyde (HCHO), and methanol (CH_3OH) are depicted on the left to indicate the oxidative state of the 1-carbon fragment(s) attached to FH_4. Reaction of an activated form of formic acid, the mixed anhydride derivative (top, right), and of formaldehyde (middle, left) with the free coenzyme (FH_4) are also shown.

The biosynthetic fates of the C_1 units in the THF pool



Interconversion of the C_1 units carried by THF



Formyltetrahydrofolate synthetase: production of 10-formyl-tetrahydrofolate

$$H_4\text{folate} + \text{MgATP} + HCOO^- \rightarrow \text{10-Formyl-}H_4\text{folate} + \text{MgADP} + P_i \quad (3\text{-}30)$$

Mechanism

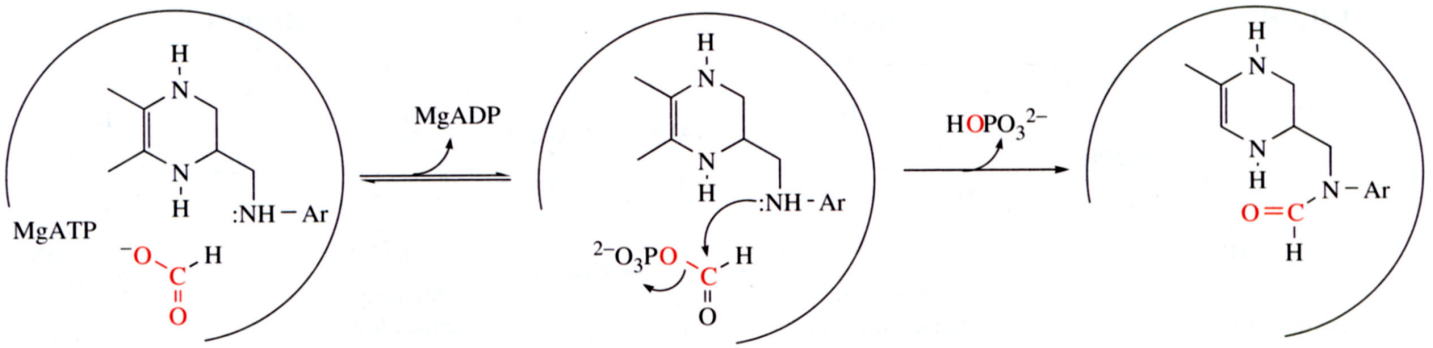
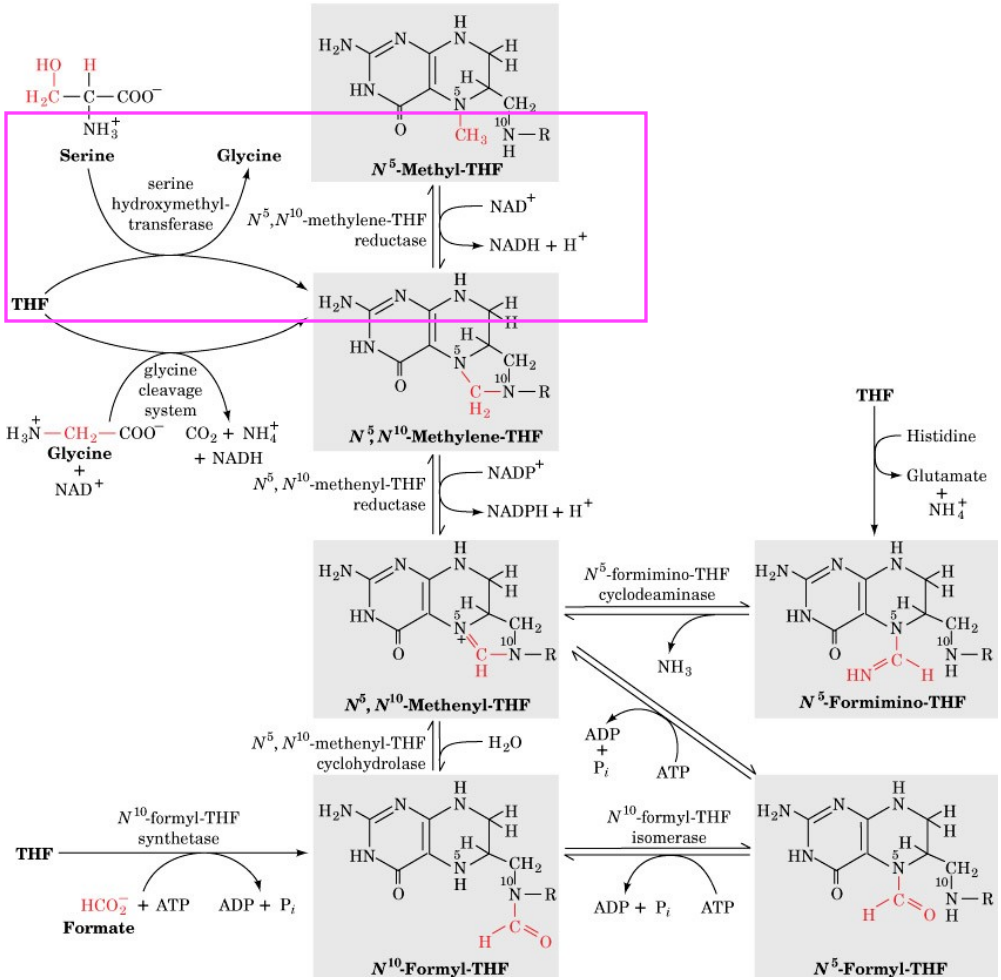
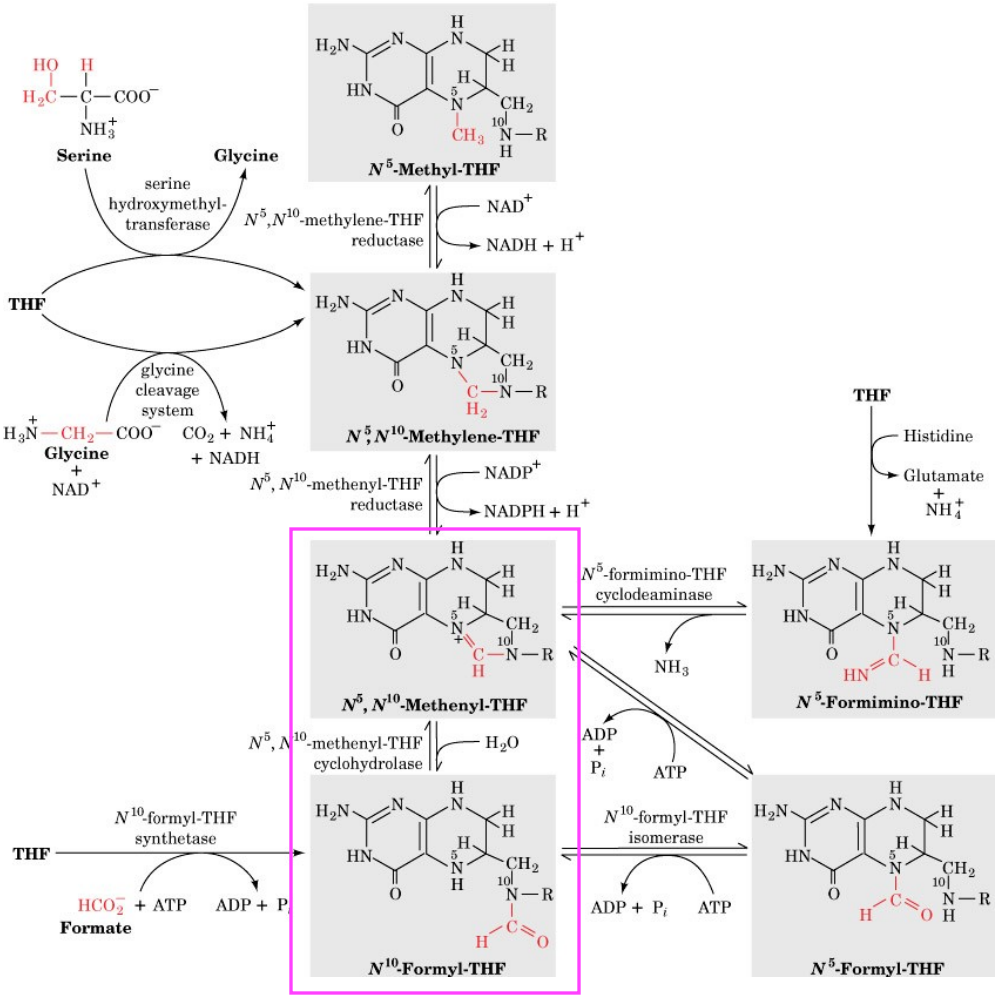


Fig. 3-35. Synthesis of 10-formyltetrahydrofolate by formyltetrahydrofolate synthetase.

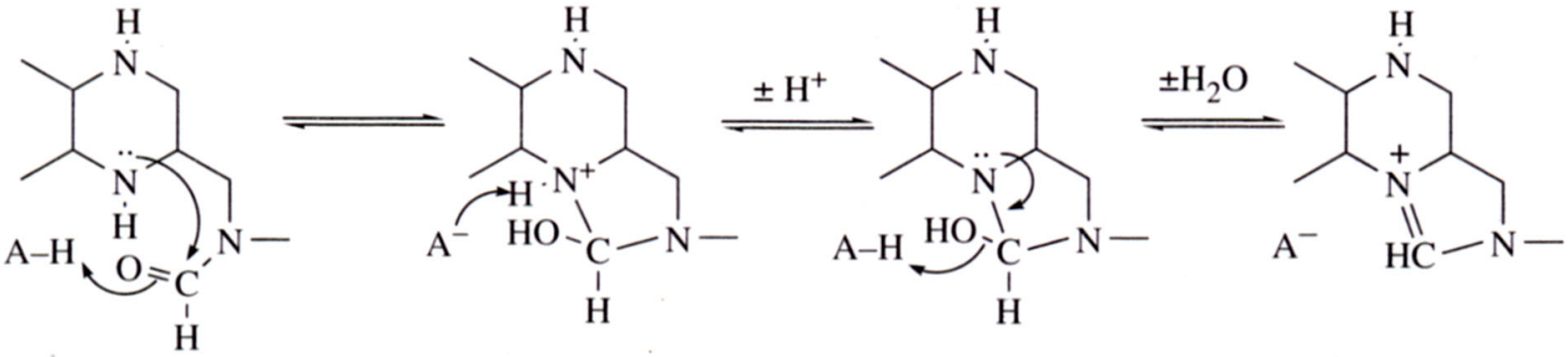
Methylene-H_4folate is produced mainly by serine hydroxymethyltransferase and methenyl-H_4folate reductase.



Cyclohydrolase dehydrates 10-formyl-H_4folate to give methenyl-H_4folate; a reasonable mechanism follows:



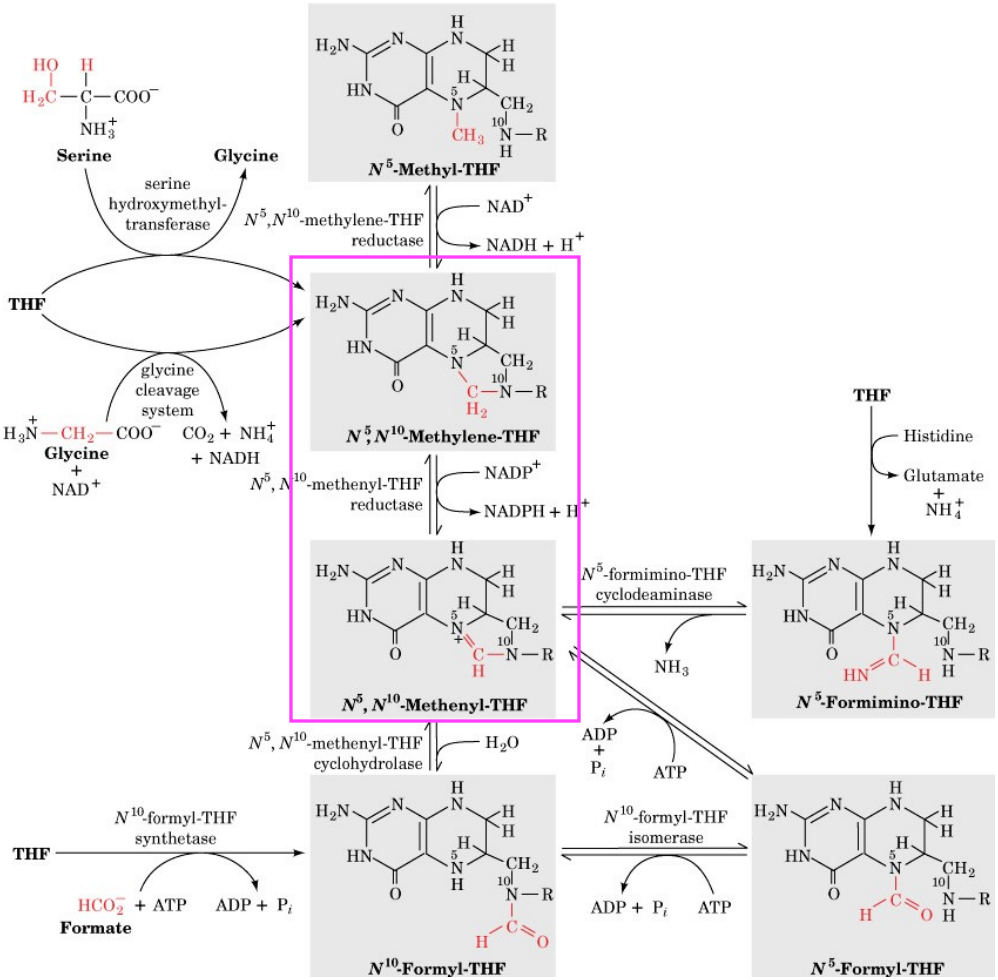
Cyclohydrolase mechanism:



Scheme 3-4

Reduction of the imine by NADPH catalyzed by methenyl-H_4folate reductase produces methylene-H_4folate.

Reduction of the imine by NADPH catalyzed by methenyl-H_4folate reductase produces methylene-H_4folate.



The enzymatic conversion of dUMP to dTMP requires methylene-H_4folate.

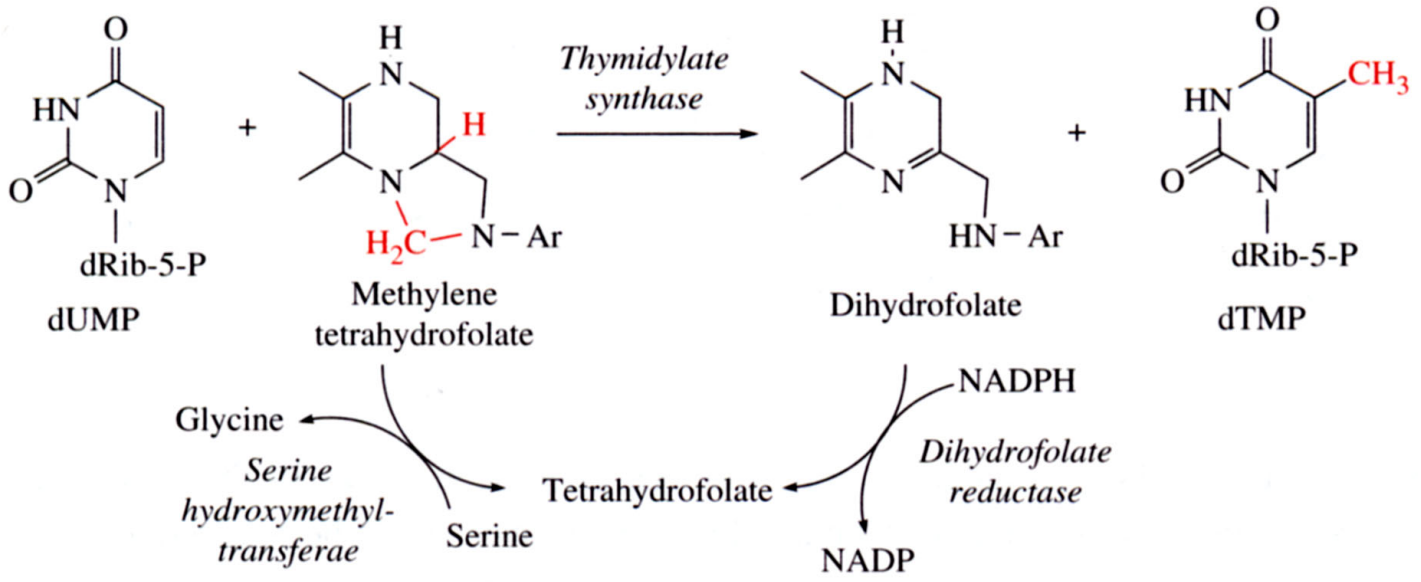
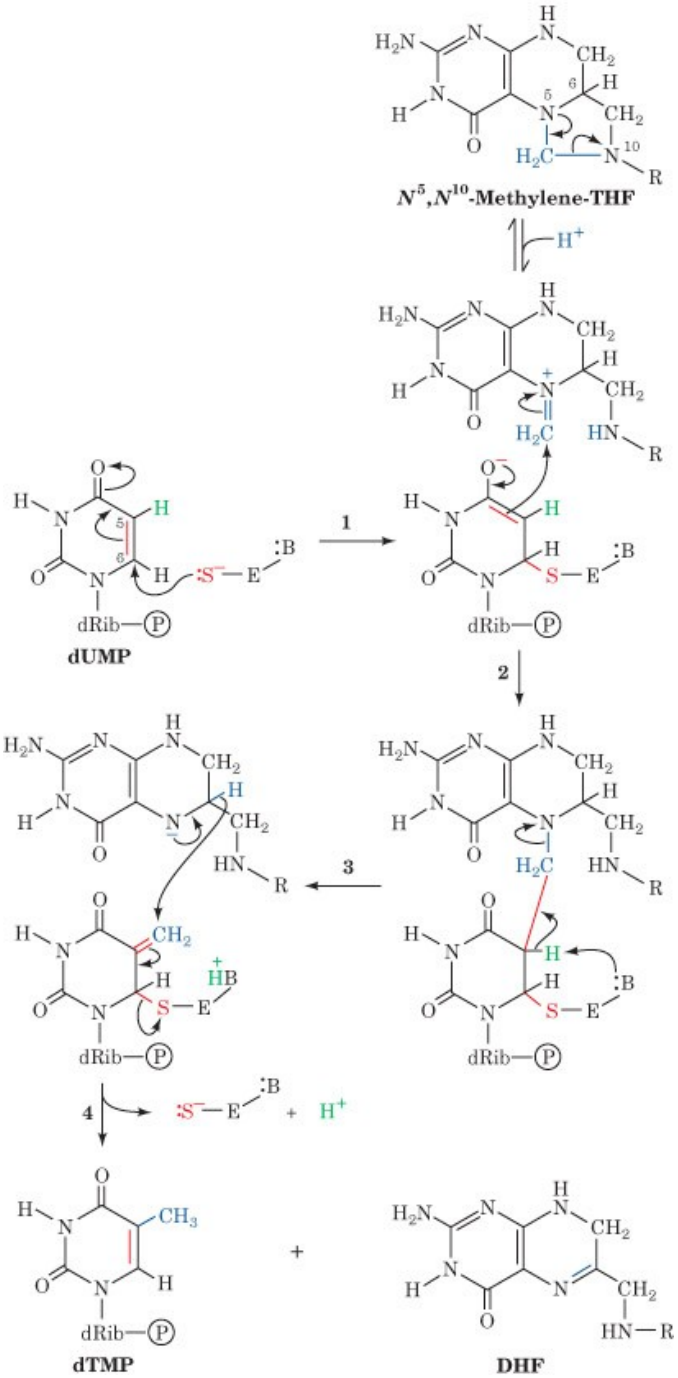
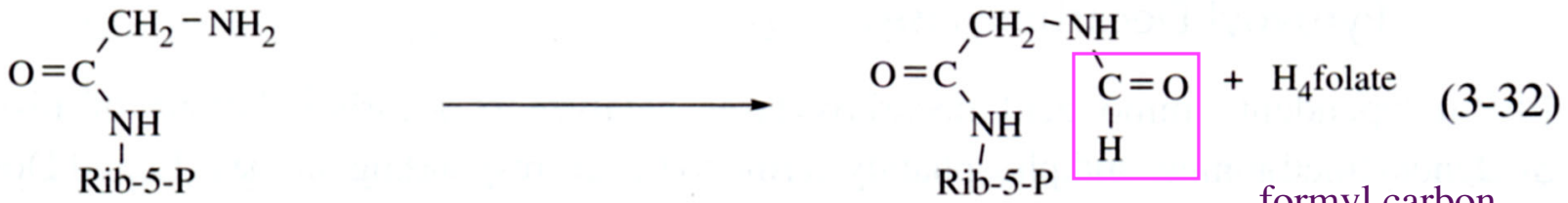


Fig. 3-36. The enzymatic conversion of dUMP into dTMP. Thymidylate synthase catalyzes the conversion of dUMP and methylenetetrahydrofolate into dTMP and dihydrofolate. The process would deplete the tetrahydrofolate pool were it not for dihydrofolate reductase, which catalyzes the reduction of dihydrofolate by NADPH. Serine hydroxymethyltransferase regenerates methylenetetrahydrofolate.

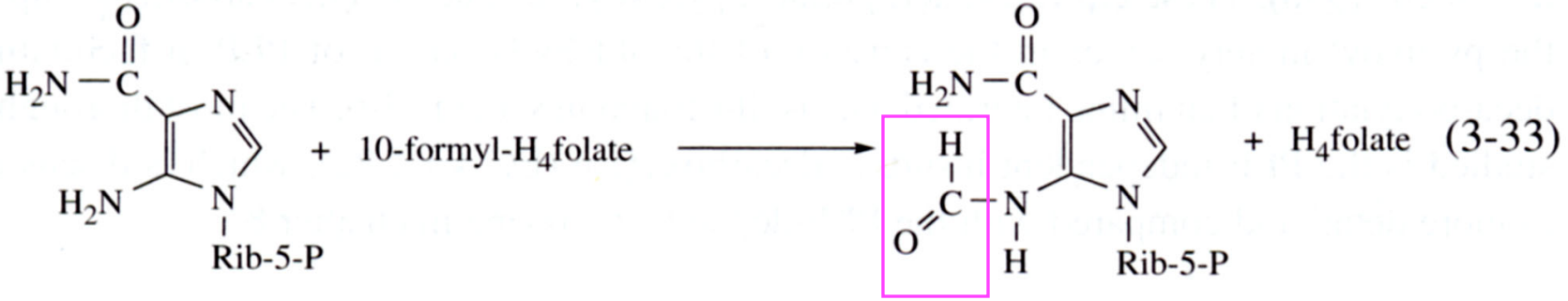


Mechanism of thymidylate synthase

Role of formyl transferases in purine biosynthesis



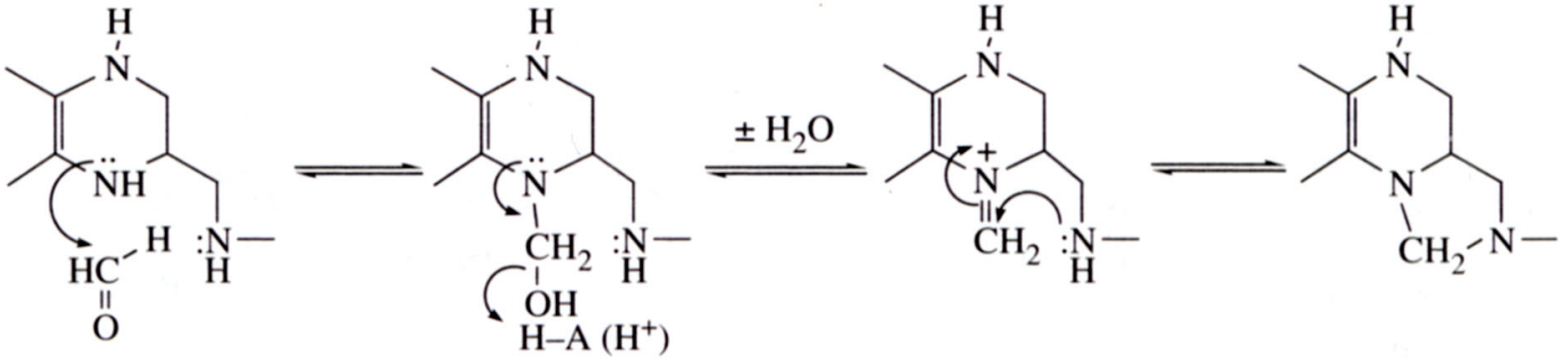
formyl carbon
becomes C8 of purine ring



formyl carbon
becomes C2 of purine ring

Biological importance of folate: masked methanol, formaldehyde, and formate functionalities

Free CH_3OH, HCHO and HCOOH are cytotoxic. HCHO is especially reactive towards amino groups (crosslinking).



Scheme 3-5