Chem 352 - Lecture 10
Lipid, Amino Acid, and
Nucleotide Metabolism
Part III: Nucleotide Metabolism

1

Lipid Metabolism

2-1

Lipid Metabolism

Question:

Draw a general pathway for converting carbohydrates to fatty acids in a liver cell, and indicate which processes occur in the cytosol and which occur in mitochondria.

2-2

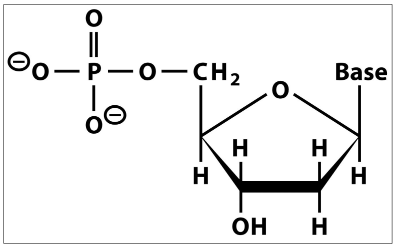
Lipid Metabolism

2-3

3-1

Introduction

- The nucleotides



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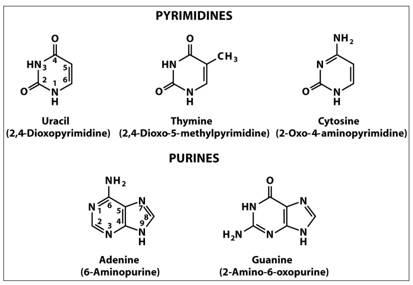
Introduction

- The nucleotides



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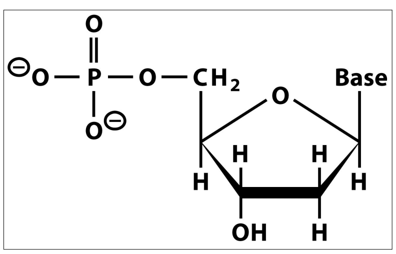
Introduction

- The nucleotides



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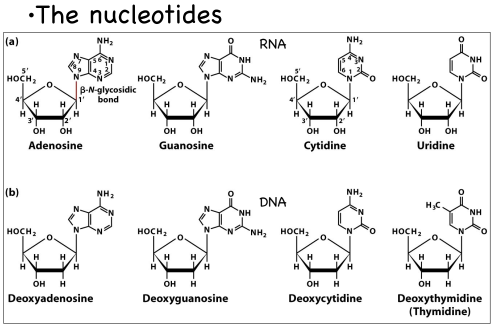
Introduction

- The nucleotides



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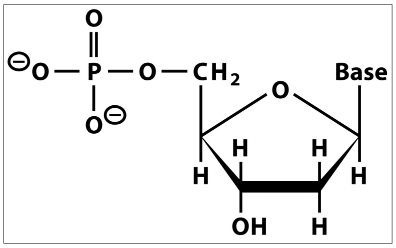
Introduction

- The nucleotides



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Introduction

- Nucleotide metabolism provides us with some nice examples of biochemically intricate and creative pathways.
- We will focus on a couple of examples
  - 18.1 Synthesis of Purine Nucleotides (Inosine Monophosphate, IMP)
  - 18.2 Other Purine Nucleotides are Synthesized from IMP
  - 18.3 Synthesis of Pyrimidine Nucleotides (Uridine monophosphate, UMP)
  - 18.4 CTP is Synthesized from UMP
  - 18.5 Reduction of Ribonucleotides to Deoxyribonucleotides

5

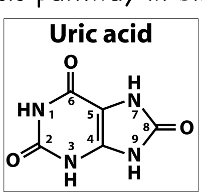
Introduction

- We will not cover nucleotide degradation or the salvage pathways
  - As we will see, the nucleotide biosynthesis pathways are very energy intensive.
  - The salvage pathways are used to recycle nucleotides and conserve energy in rapidly growing cells.

6

Synthesis of Purines

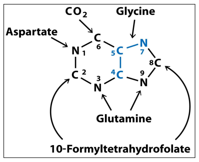
- Working out the details of purine biosynthesis started with the investigation the uric acid biosynthesis pathway in birds.



7-1

Synthesis of Purines

- Radioactively labeled precursors were fed to pigeons to see where the labels ended up in uric acid.
  - $^{13}CO_2$
  - $H^{13}COO^-$ (formate)
  - H_3N^+–CH_2–$^{13}COO^-$ (glycine)



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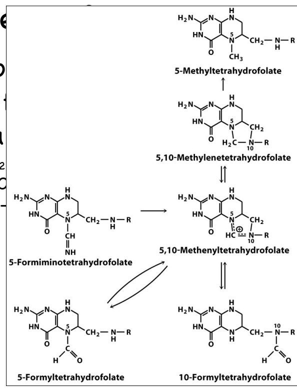
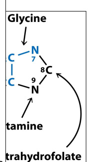
- Radio ...ors were ... ere the la... id.
  - $^{13}CO_2$
  - $H^{13}CO$...
  - H_3N^+...





7-3

Synthesis of Purines

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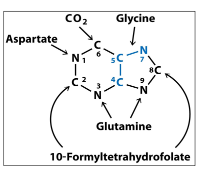

8-1

Synthesis of Purines

- Purines are synthesized on top of the ribose phosphate.
  - Ribose 5-phosphate
  - This starts with the activation of ribose 5-phosphate to 5-phospho-α-D-ribosyl 1-pyrophosphate (PRPP)
- The final product is inosine-5'-monophosphate.

8-2

Synthesis of Purines

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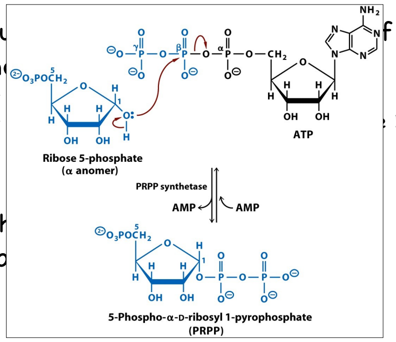

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8-3

Synthesis of Purines

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Synthesis of Purines

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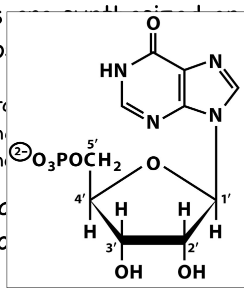

•The fin ... -5′-
monopho

8-5

Synthesis of Purines

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Synthesis of Purines

- Starting with PRPP, the complete synthesis of IMP is done in 10 steps.

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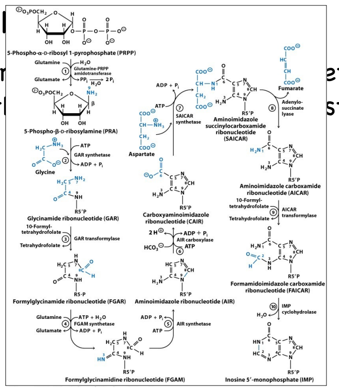

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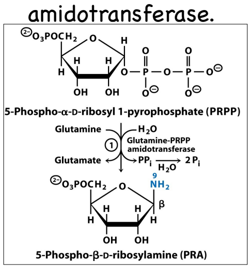
Synthesis of Purines

- Step 1: Glutamine-PRPP amidotransferase.



- An amido nitrogen is transferred form glutamine to the C1 position of PRPP.
- Note the inversion of the chirality of the ribose from α to β.
- Reaction is driven by the hydrolysis of the pyrophosphate

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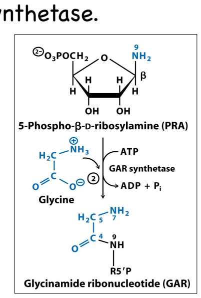
Synthesis of Purines

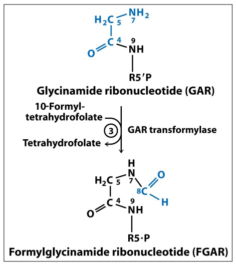
- Step 2: Glycinamide ribonucleotide synthetase.



- A peptide bond is formed between the glycine and the ribosylamine.
- The reaction requires activation of the glycine carboxylate with ATP.

Synthesis of Purines

•Step 3: Glycinamide ribonucleotide transformylase.

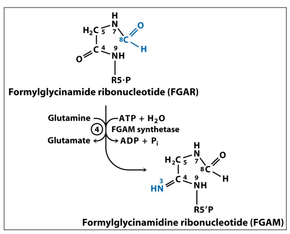


- A formyl group is transferred from 10-formyl-tetetrahydrofolate

12

Synthesis of Purines

•Step 4: Formylglycinamidine ribonucleotide synthetase.



- The amide is converted to an amidine
- The nitrogen is donated by glutamine.
- This reaction requires the hydrolysis of ATP

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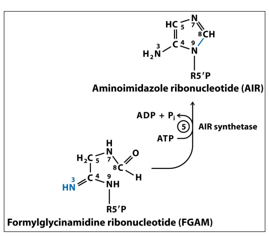
Synthesis of Purines

•Step 5: Aminoimidazole ribonucleotide synthetase.

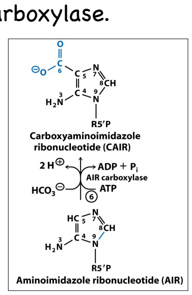


- Ring closure requires the hydrolysis of ATP

14

Synthesis of Purines

•Step 6: Aminoimidazole ribonucleotide carboxylase.



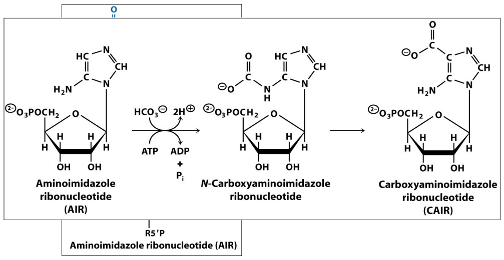
- Surprisingly, this carboxylase does not involve the use of biotin.
- The reaction does require the hydrolysis of ATP

15-1

15-2

Synthesis of Purines

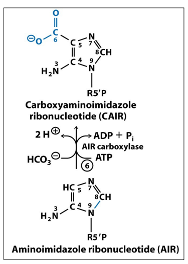
- Step 6: Aminoimidazole ribonucleotide carboxylase.



15-3

Synthesis of Purines

- Step 6: Aminoimidazole ribonucleotide carboxylase.

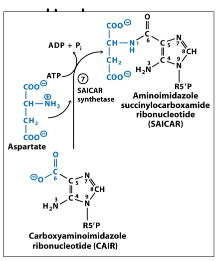


- Surprisingly, this carboxylase does not involve the use of biotin.
- The reaction does require the hydrolysis of ATP

16

Synthesis of Purines

- Step 7: Aminoimidazole succinylcarboxamide ribonucleotide

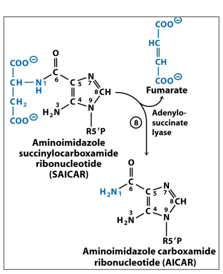


- The newly added carboxylate group is activated with ATP and condensed with aspartate to become succinylated.

17-1

Synthesis of Purines

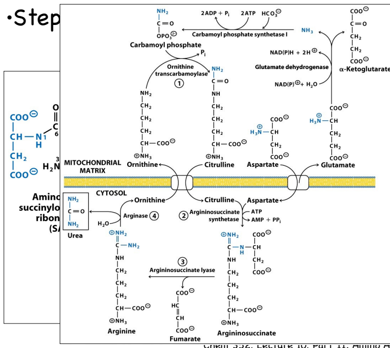
- Step 8: Adenylosuccinate lyase.



- Coupled to the last reaction, these two reactions resemble two that we saw in the urea cycle.

Synthesis of Purines

•Step

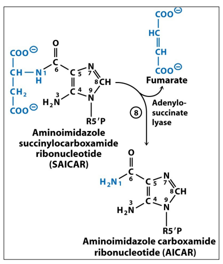


reaction, resemble the urea

17-2

Synthesis of Purines

•Step 8: Adenylosuccinate lyase.



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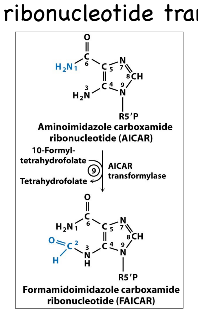
17-3

Synthesis of Purines

•Step 9: Aminoimidazole carboxamide ribonucleotide transformylase.

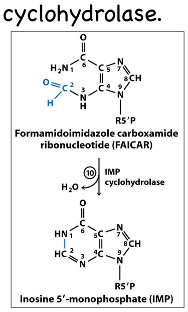


- Similar to Step 3, a formyl group is transferred from 10-formyl-tetrahydrofolate to an amino group

18

Synthesis of Purines

•Step 10: Inosine 5′-monophosphate cyclohydrolase.



- Like Schiff base formation, this is a condensation reaction between an aldehyde and an amine.

19

20-1

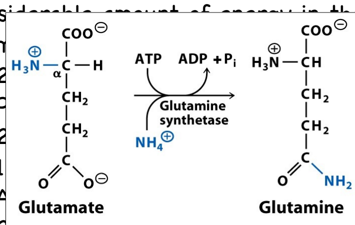
Synthesis of Purines

- The synthesis of IMP requires a considerable amount of energy in the form of ATP, (11 ATP's in all)
  - 2 ATP equivalents for the activation of PRPP
  - 2 for glutamine-PRPP amidotransferase
  - 1 each for steps 2, 4, 5, 6 & 7 (=5 ATP's)
  - 2 ATP for the two glutamine synthetase reactions.

20-2

Synthesis of Purines

- The synthesis of IMP requires a



synthetase reactions.

20-3

Synthesis of Purines

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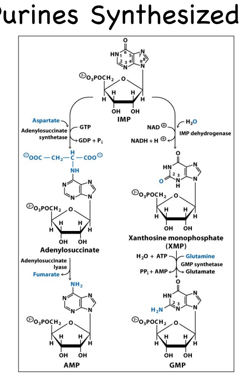
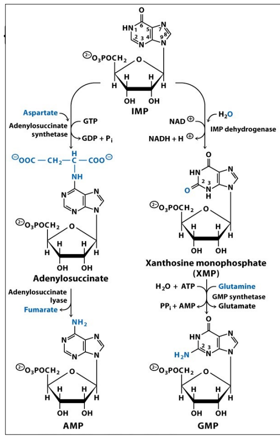
21-1

Other Purines Synthesized from IMP



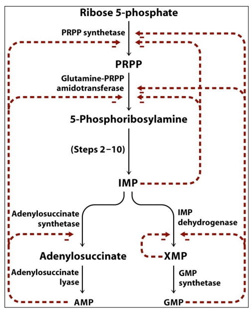
Other ... rom IMP



21-2

Regulation of Purine Synthesis

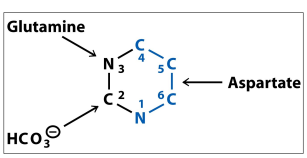
- Purine synthesis is regulated by a web of feedback inhibition of key branch-point reactions.



22

Synthesis of Pyrimidine Nucleotides

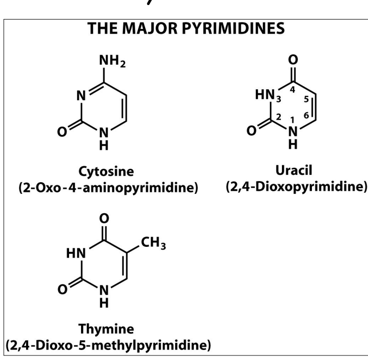
- Unlike purine synthesis, pyrimidines are synthesized first and then attached to the phosphoribose.
- Like purine synthesis, the atoms in the pyrimidine ring come from a number of different sources.



23-1

Synthesis of Pyrimidine Nucleotides

- ... e ... the
- ... f



23-2

Synthesis of Pyrimidine Nucleotides

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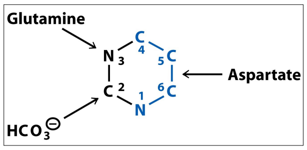
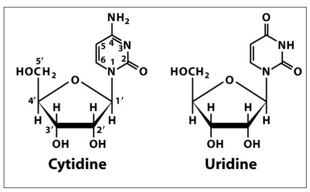


23-3

Synthesis of Pyrimidine Nucleotides

•Pyrimidine synthesis is a 6-step process that leads to UMP



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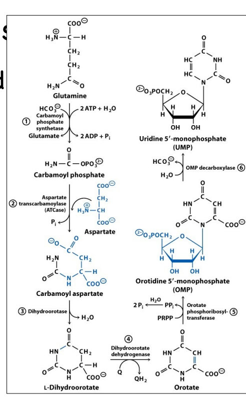
Synthesis of Pyrimidine Nucleotides

•Pyrimidine synthesis is a 6-step process that leads to UMP

25-1

Synthesis ... cleotides

•Pyrimid ... step process



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25-3

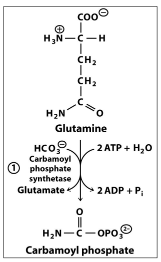
Synthesis of Pyrimidine Nucleotides

- Pyrimidine synthesis is a 6-step process that leads to UMP

26-1

Synthesis of Pyrimidine Nucleotides

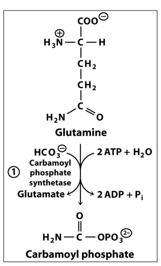
- Step 1: Carbamoyl phosphate synthetase II.



26-2

Synthesis of Pyrimidine Nucleotides

- Step 1: Carbamoyl phosphate synthetase II.

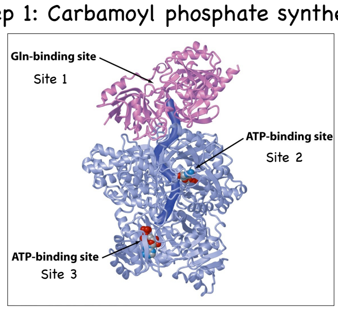


- This reaction is synthesized by carbamoyl phosphate synthetase II in mammals.
- This enzyme is found in the cytosol instead of the mitochondrial matrix
- Unlike the reaction in the urea cycle, the sources of the nitrogen is glutamine instead of free ammonia

27-1

Synthesis of Pyrimidine Nucleotides

- Step 1: Carbamoyl phosphate synthetase II.



Synthesis of Pyrimidine Nucleotides

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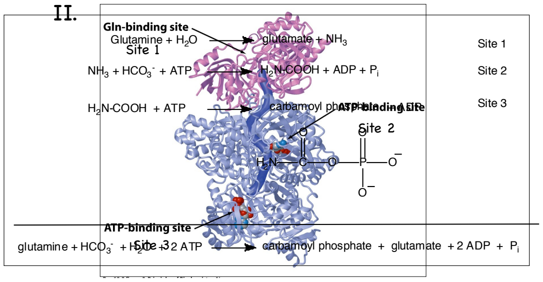

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Synthesis of Pyrimidine Nucleotides

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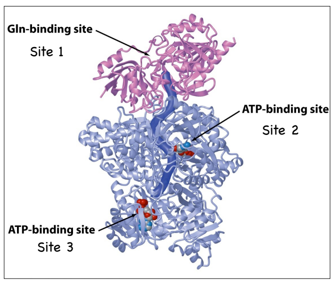
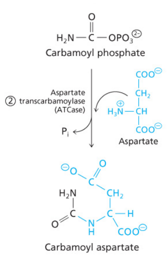


27-3

Synthesis of Pyrimidine Nucleotides

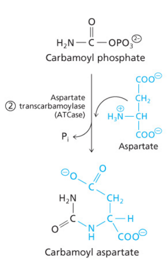
•Step 2: Aspartate transcarboylase (ATCase).



28-1

Synthesis of Pyrimidine Nucleotides

•Step 2: Aspartate transcarboylase (ATCase).

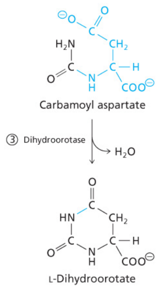


- The activated carbamoyl phosphate condenses with aspartate.

28-2

Synthesis of Pyrimidine Nucleotides

• Step 3: Dihydroorotase.



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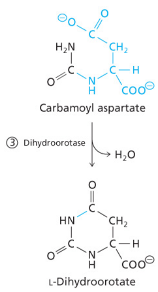
Synthesis of Pyrimidine Nucleotides

• Step 3: Dihydroorotase.

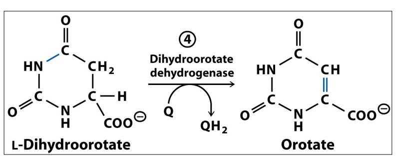


- The carboxylate and amide $-NH_2$ condense to close the ring and form a cyclic imide.

29-2

Synthesis of Pyrimidine Nucleotides

• Step 3: Dihydroorotate dehydrogenase



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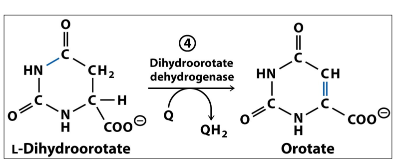
Synthesis of Pyrimidine Nucleotides

• Step 3: Dihydroorotate dehydrogenase



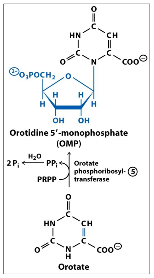
- The ring is oxidized to form the aromatic orotate ring
- In eukaryotes, this reaction occurs at the inner mitochondrial membrane

30-2

31-1

Synthesis of Pyrimidine Nucleotides

- Step 5: Orotate phosphoribosyl transferase



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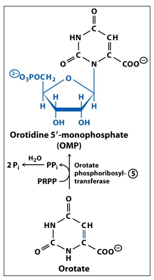
Synthesis of Pyrimidine Nucleotides

- Step 5: Orotate phosphoribosyl transferase

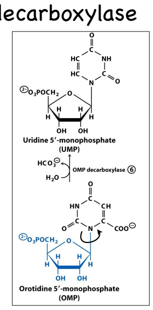


- The orotate is condensed with phosphoribosyl pyrophosphate (PRPP)

32

Synthesis of Pyrimidine Nucleotides

- Step 6: Orotidine 5′-monophosphate decarboxylase

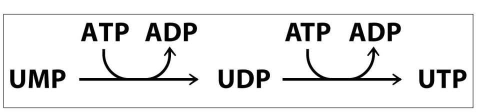


- Decarboxylation of orotidine 5′-monophosphate produces UMP

33

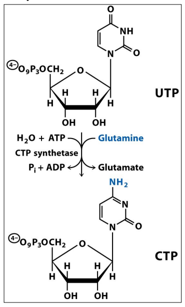
Synthesis of Pyrimidine Nucleotides

- UMP is phosphorylated to UTP



Synthesis of Pyrimidine Nucleotides

•UTP is converted to CTP by CTP synthetase



- This reaction is analogous to Step 4 in purine biosynthesis

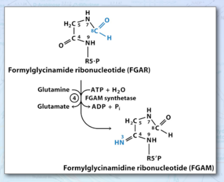
34-1

Synthesis of Pyrimidine Nucleotides

•UTP is converted to CTP by CTP

Synthesis of Purines

Step 4: Formylglycinamidine ribonucleotide synthetase.

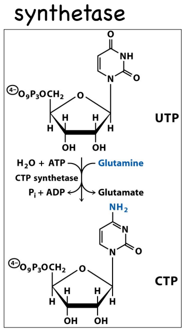


- The amide is converted to an amidine
- The nitrogen is donated by glutamine.
- This reaction requires the hydrolysis of ATP

34-2

Synthesis of Pyrimidine Nucleotides

•UTP is converted to CTP by CTP synthetase

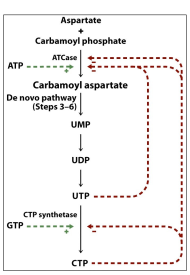


- This reaction is analogous to Step 4 in purine biosynthesis

34-3

Synthesis of Pyrimidine Nucleotides

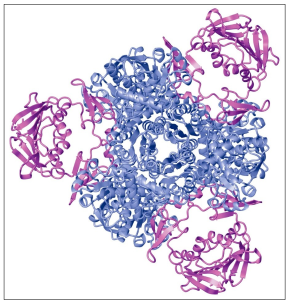
•Regulation of pyrimidine synthesis in prokaryotes.



35

Synthesis of Pyrimidine Nucleotides

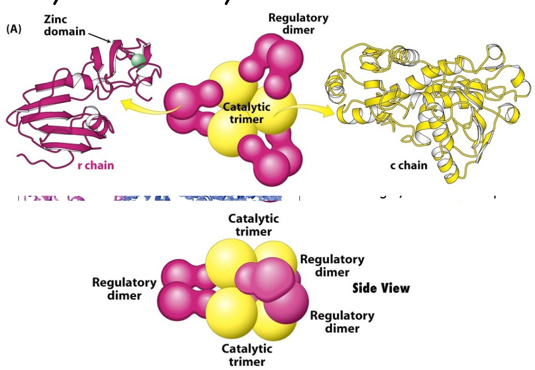
•Regulation ATCase in E.coli



- ACTase is one the most thoroughly studied examples of allosteric enzyme regulation.

36-1

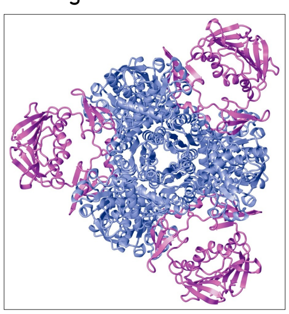
Synthesis of Pyrimidine Nucleotides



36-2

Synthesis of Pyrimidine Nucleotides

•Regulation ATCase in E.coli



- ACTase is one the most thoroughly studied examples of allosteric enzyme regulation.

36-3

Synthesis of Pyrimidine Nucleotides

- In prokaryotes ATCase is branch point between pyrimidine synthesis and arginine synthesis
  - Regulation of ATCase controls the flow of material in these two pathways
    - Both pathways share the same carbamoyl phosphate synthetase.
- In eukaryotes this is not the case
  - carbamoyl phosphate synthetase I
    - mitochondria (arginine synthesis)
  - carbamoyl phosphate synthetase II
    - cytoplasm (pyrimidine synthesis)

37-1

Synthesis of Pyrimidine Nucleotides

•In pr etween
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✦ Re w of
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•In eu
✦ ca
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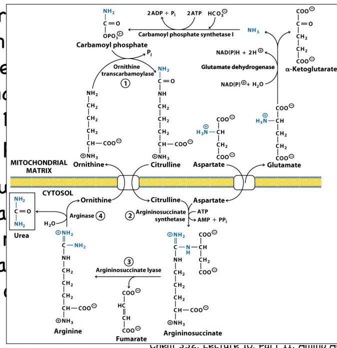


37-2

Synthesis of Pyrimidine Nucleotides

•In prokaryotes ATCase is branch point between pyrimidine synthesis and arginine synthesis
- ✦ Regulation of ATCase controls the flow of material in these two pathways
  - ‣ Both pathways share the same carbamoyl phosphate synthetase.

•In eukaryotes this is not the case
- ✦ carbamoyl phosphate synthetase I
  - ‣ mitochondria (arginine synthesis)
- ✦ carbamoyl phosphate synthetase II
  - ‣ cytoplasm (pyrimidine synthesis)

37-3

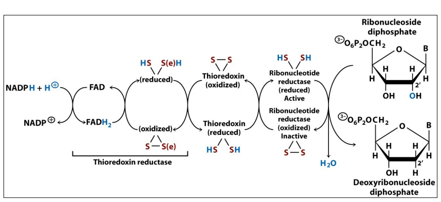
Reduction of Ribonucleotides to Deoxyribonucleotides.

- ✦ Reduction occurs at the diphosphate level
- ✦ The same system is used for all four ribonucleotides
  - ‣ ADP, GDP, CDP & UDP
- ✦ The system involves three enzymes
  - ‣ ribonucleotide reductase
  - ‣ thioredoxin
  - ‣ thioredoxin reductase

38

Reduction of Ribonucleotides to Deoxyribonucleotides.

- ✦ Ribonucleotide reductase

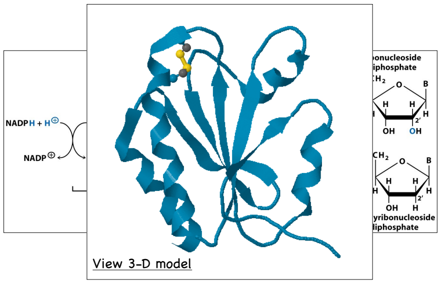


39-1

39-2

Reduction of Ribonucleotides to Deoxyribonucleotides.

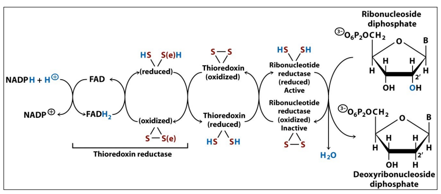
- Ribonucleotide reductase



39-3

Reduction of Ribonucleotides to Deoxyribonucleotides.

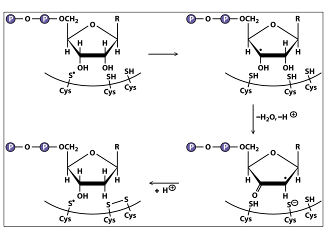
- Ribonucleotide reductase



40

Reduction of Ribonucleotides to Deoxyribonucleotides.

- Ribonucleotide reductase
  - The enzyme mechanism involves free radicals.



41

Reduction of Ribonucleotides to Deoxyribonucleotides.

- Ribonucleotide reductase has two regulatory sites
  - An activity site
  - A specificity site

TABLE 18.1 Allosteric regulation of eukaryotic ribonucleotide reductase

Ligand bound to activity site	Ligand bound to specificity site	Activity of catalytic site
dATP		Enzyme inactive
ATP	ATP or dATP	Specific for CDP or UDP
ATP	dTTP	Specific for GDP
ATP	dGTP	Specific for ADP

Methylation of dUMP to dTMP

- dUDP is first converted to dUMP in a way the prevents the buildup of dUTP.

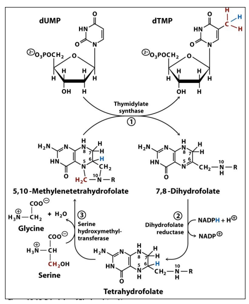
$$\mathrm{dUDP} + \mathrm{ATP} \xrightarrow{\;\to \mathrm{ADP}\;} \mathrm{dUTP} \xrightarrow{\;H_2O\;} \mathrm{dUMP} + \mathrm{PP_i}$$

  - This done to head off the incorporation of dUTP into DNA in place of dTTP.

42

Methylation of dUMP to dTMP

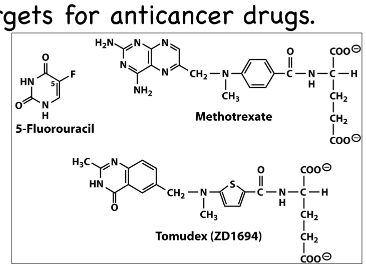
- dUMP is converted to dTMP by Thymidylate synthase.
  - The source of the methyl group is serine, by way of 5,10-methylenetetrahydrofolate



43

Methylation of dUMP to dTMP

- Both thymidylate synthase and dihydrofolate reductase are prime targets for anticancer drugs.



44

Next Up

- Exam III (Lectures 7-10)

45