

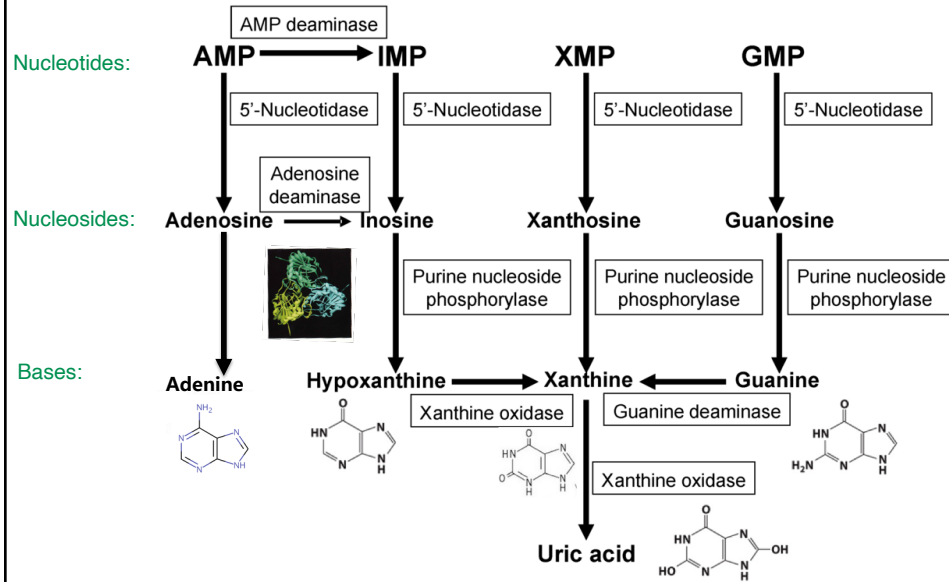
OUTLINE: BB 422/622	
<p>Introduction and review Transport Glycogenolysis Glycolysis Other sugars Pasteur: Anaerobic vs Aerobic Fermentations</p> <p>Pyruvate</p> <p>Krebs' Cycle</p> <p>Oxidative Phosphorylation Electron transport Chemiosmotic theory/ Phosphorylation</p> <p>Fat Catabolism</p> <p>Fatty acid Catabolism Mobilization from tissues (mostly adipose) Activation of fatty acids Transport; carnitine Oxidation: <math>\beta</math>-oxidation, 4 steps:</p> <p>Protein Catabolism Amino-Acid Degradation Dealing with the nitrogen; Urea Cycle Dealing with the carbon; Seven Families Nucleic Acid &amp; Nucleotide Degradation</p> <p><b>ANABOLISM I: PHOTOSYNTHESIS:</b> Overview and Key experiments: Light Reactions energy in a photon/pigments Reaction center &amp; Photosystems (PSII &amp; PSI) Motive Force – ATP Carbon Assimilation – Calvin Cycle Rubisco/Oxygenase (Glycolate cycle) remaking Ru 1,5P<sub>2</sub> Overview and regulation C4 versus C3 plants Kornberg cycle – glyoxylate Carbohydrate Biosynthesis in Animals precursors/Glu cycle Gluconeogenesis reversible steps irreversible steps – four Glycogen Synthesis UDP-glucose synthesis/branching Pentose-Phosphate Pathway oxidative-NADPH non-oxidative-Ribose 5-P Regulation of Carbohydrate Metabolism <b>Anaplerotic reactions</b> Biosynthesis of fatty acids contrast, location &amp; transport synthesis ACF FAS; ACP priming: 4 steps carnitine</p>	<p><b>ANABOLISM II:</b> Biosynthesis of Fatty Acids and Lipids Fatty Acids Triacylglycerides Membrane lipids Glycerophospholipids Sphingolipids Isoprene lipids: <b>Cholesterol</b> Ketone body synthesis Mevalonate <b>Cholesterol</b> bile acids steroids metabolism control of cholesterol biosynthesis</p> <p><b>ANABOLISM III:</b> Biosynthesis of Amino Acids and Nucleotides Nitrogen fixation nitrogenase Nitrogen assimilation Amino-acid Biosynthesis Nucleotide Biosynthesis <i>De novo</i> vs. salvage Purines Pyrimidines RNA precursors DNA precursors Control of nitrogen metabolism Biosynthesis and degradation of heme; Other 2° products of amino acids</p>
Exam-1 material	Exam-2 material
Exam-3 material	Exam-4 material
	Exam-5 material

## ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

- 1) Nitrogen fixation:  $N_2 \rightarrow NH_4$
- 2) Nitrogen assimilation: incorporation of ammonia into biomolecules
- 3) Biosynthesis of amino acids
  - a) non-essential
  - b) essential
- 4) Biosynthesis of nucleotides
  - a) sources
  - b) *de novo* purines (R)(as nucleotides\*); salvage; regulation
  - c) *de novo* pyrimidines (Y)(as bases); making nucleotide; regulation
  - d) deoxy-ribonucleotides, dTMP, and phosphorylation to NTP & dNTP
  - e) regulating levels for DNA synthesis
- 5) Control of nitrogen metabolism
- 6) Biosynthesis and degradation of heme; other 2° products of amino acids

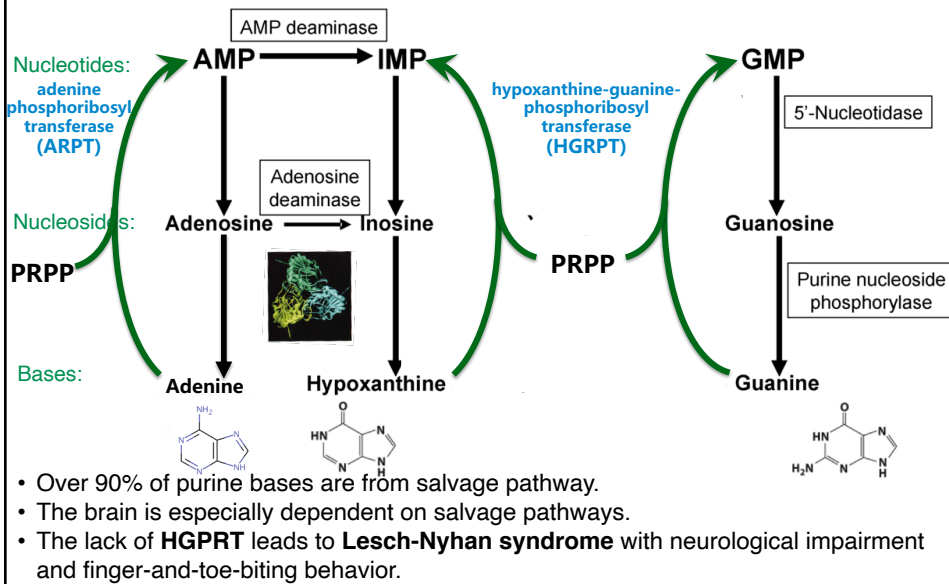
\*Bases synthesized *while* attached to ribose-5-P; products are RMP (R is one-letter code for purine, Y is one letter code for pyrimidine)

## Recall: Nucleotide Degradation



## Biosynthesis Amino Acids & Nucleotides

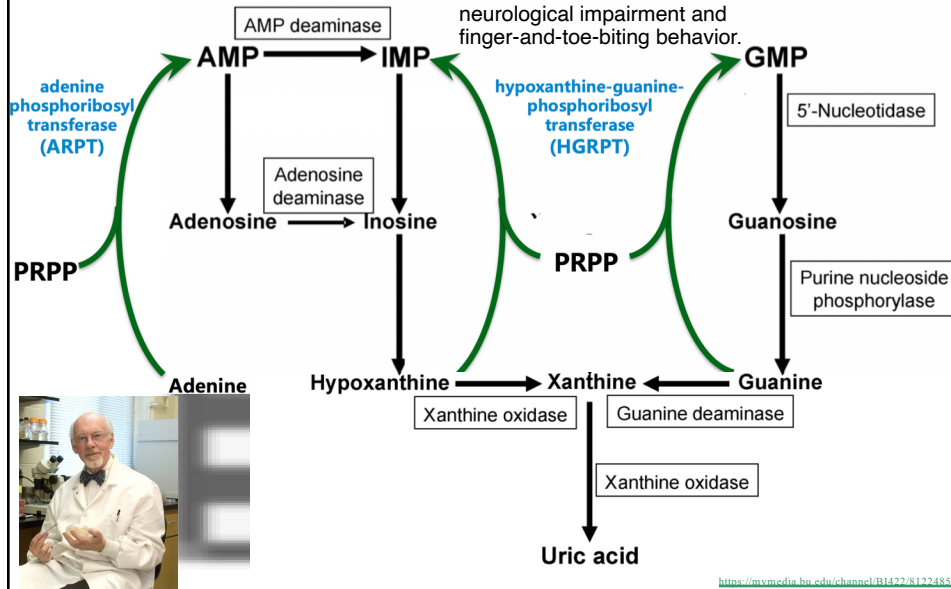
### Salvage Pathway of Purines



- Over 90% of purine bases are from salvage pathway.
- The brain is especially dependent on salvage pathways.
- The lack of **HGPRT** leads to **Lesch-Nyhan syndrome** with neurological impairment and finger-and-toe-biting behavior.

# Biosynthesis Amino Acids & Nucleotides

## Salvage Pathway

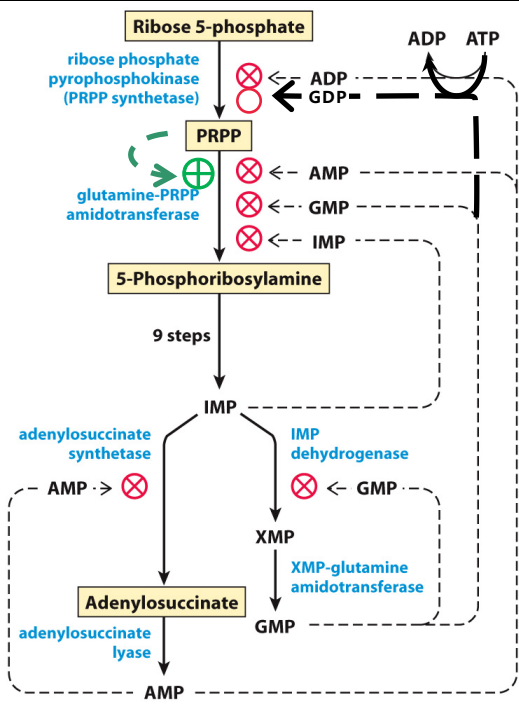


## Biosynthesis Amino Acids & Nucleotides

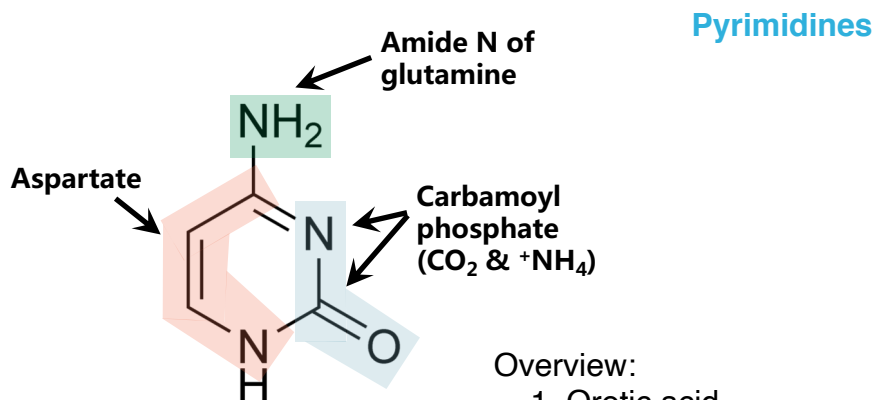
### Regulation of Purine Biosynthesis

#### Four Major Sites of Allosteric Regulation

1. **PRPP synthetase** is inhibited by ADP and GDP.
2. **Glutamine-PRPP amidotransferase** is inhibited by end-products IMP, AMP, and GMP.
3. Excess GMP inhibits formation of xanthylate from inosinate by **IMP dehydrogenase**.
4. Excess AMP inhibits formation of adenylosuccinate from inosinate by **adenylosuccinate synthetase**.



# Biosynthesis Amino Acids & Nucleotides



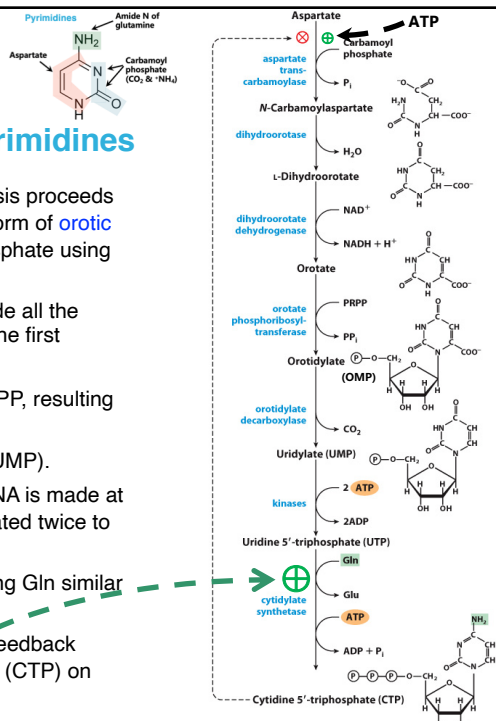
Overview:

1. Orotic acid
2. Add ribose (PRPP), make UMP
3. CTP made from UTP

## Biosynthesis Amino Acids & Nucleotides

### De Novo Biosynthesis of Pyrimidines

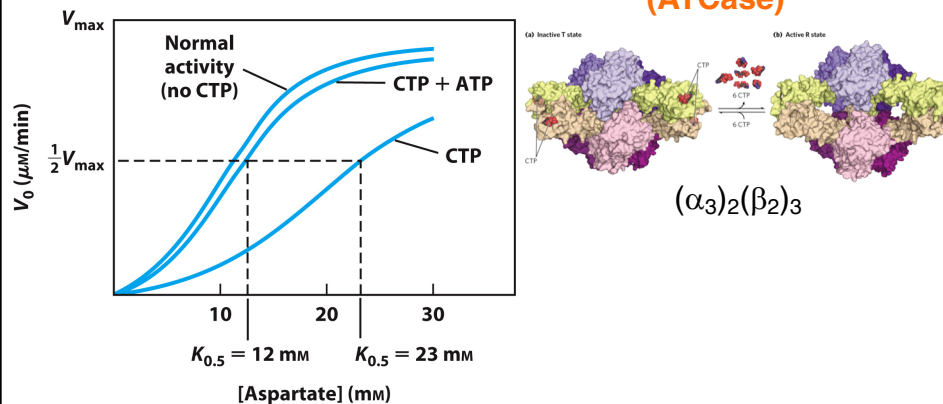
- Unlike purine synthesis, pyrimidine synthesis proceeds by *first making the pyrimidine ring* (in the form of **orotic acid**) and *then* attaching it to ribose 5-phosphate using PRPP.
- **Aspartate** and **carbamoyl phosphate** provide all the atoms for the heterocycle or pyrimidine. The first pyrimidine is **Orotate**.
- This is converted to a nucleotide using PRPP, resulting nucleotide (orotidylate; OMP).
- OMP is decarboxylated to form uridylate (UMP).
- The other pyrimidine nucleotide used in RNA is made at the triphosphate level; UMP is phosphorylated twice to make UTP.
- UTP is converted to CTP by amination using Gln similar to making AMP from XMP.
- The biosynthesis of CTP is the **CLASSIC** feedback inhibition by the allosteric negative effector (CTP) on ATCase. Also, activation by GTP



## Biosynthesis Amino Acids & Nucleotides

### Regulation of Pyrimidine Biosynthesis via Feedback Inhibition

#### Aspartate Transcarbamoylase (ATCase)



Recall from 421: ATCase is inhibited by end-product CTP and is accelerated by ATP.

## ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

### Involvement of ribonucleotide-derivatives in all of biology

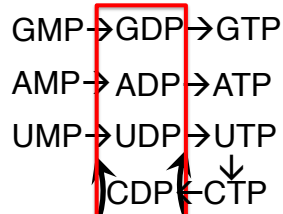


Dr. Kornberg  
Lecture 04.26.17 (0:00-5:06) 5 min

<https://mymedia.bu.edu/channel/B1422/81224851>

# Biosynthesis Amino Acids & Nucleotides

So far:



Specific kinases,  
e.g., *UMP kinase*,  
*GMP kinase*,  
*Adenylate kinase*  
etc.



Non-specific kinase,  
*nucleoside diphosphate kinase*  
(works on both oxy- and deoxy-ribose nucleosides)

How are Ribonucleic Acid Precursors converted to Deoxyribonucleic Acid Precursors?

.....and how is dTTP made?

2'C-OH bond is directly reduced to 2'-H bond ...without activating the carbon for dehydration, etc.!

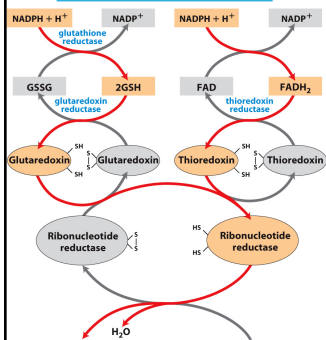
catalyzed by *ribonucleotide reductase*

*Very unique enzyme in all of biochemistry - use of free radicals (without B<sub>12</sub> cofactor)*

**Mechanism:** Two H atoms are donated by NADPH and carried by thioredoxin or glutaredoxin to the active site.  
-Substrates are the NDPs and the products are dNDP.

# Biosynthesis Amino Acids & Nucleotides

## Source of Reducing Electrons for Ribonucleotide Reductase

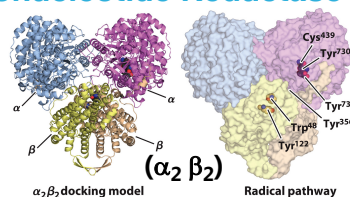


- NADPH serves as the electron donor.
- Funneled through glutathione or thioredoxin pathways

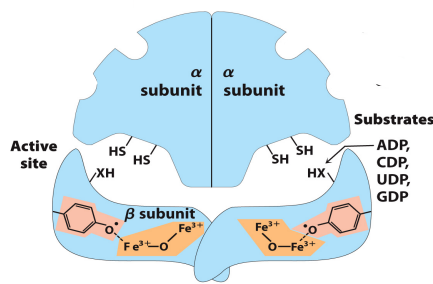
## Structure of Ribonucleotide Reductase

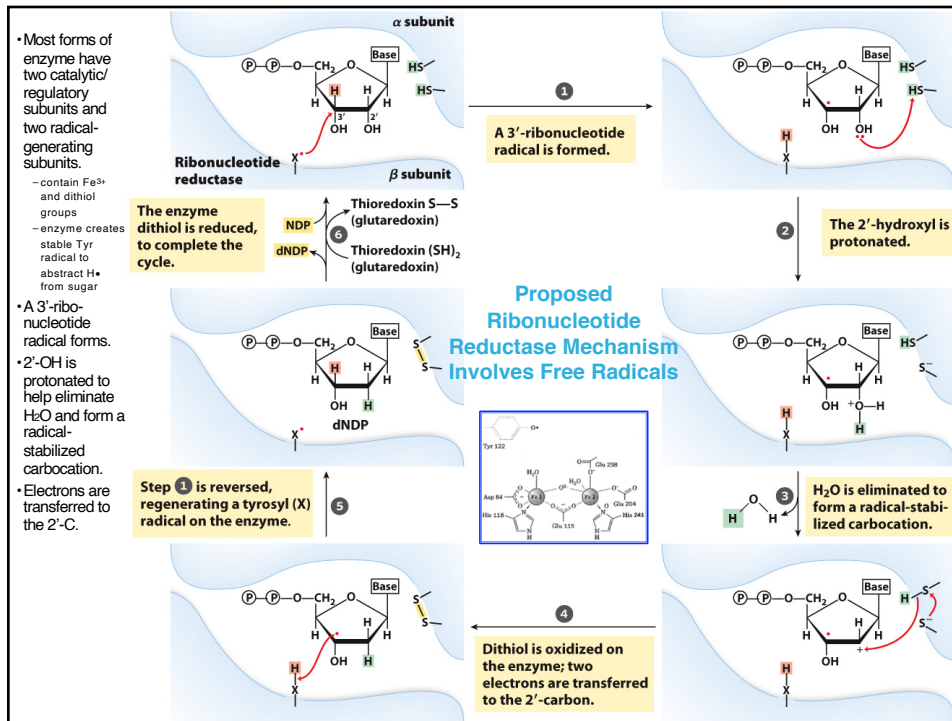
$\alpha_2$  are regulatory and half the catalytic site; need to be reduced.

$\beta_2$  are the other half of the active site, and the free-radical generators



JoAnne Stubbe (1946-)

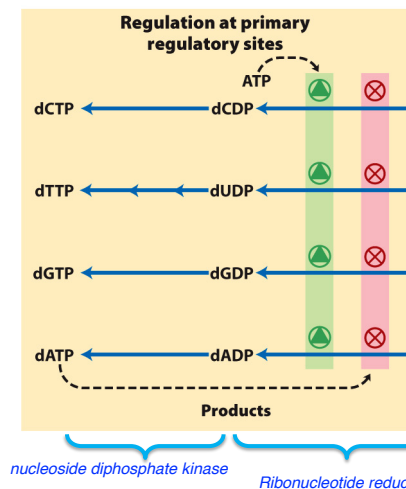




## Biosynthesis Amino Acids & Nucleotides

### On/off **Regulatory Site** of Ribonucleotide

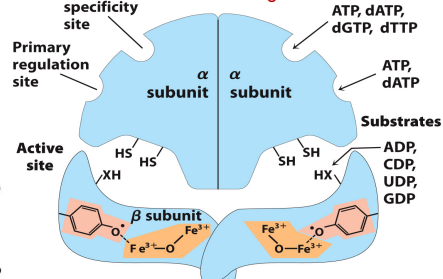
Feedback allosteric inhibition by dATP & activation by ATP



Regulatory sites: Substrate specificity site, Primary regulation site, Active site

What about the need to keep ratios balanced? This limits mutagenesis

Allosteric effectors



• Primary regulatory site affects overall activity

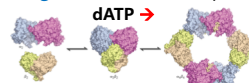
– ATP **activates**

• If energy sufficient, get ready for DNA replication

– dATP **inhibits**

• If too much dATP, don't make any of the others as well

• Enzyme **oligomerizes** to accomplish this change.

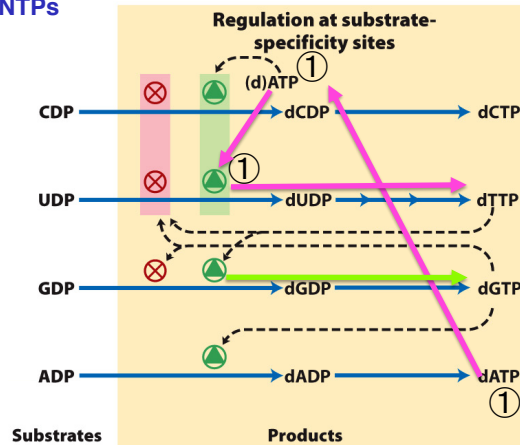


# Biosynthesis Amino Acids & Nucleotides

## Specificity Regulatory Sites of Ribonucleotide Reductase

### Feedback allosteric inhibition by dNTPs

- The other site affects *substrate specificity* in order to maintain balanced pools of nucleotides.
- THREE LEVELS:
  - **A:T**: If dATP high → MORE specificity for UDP (A:T)

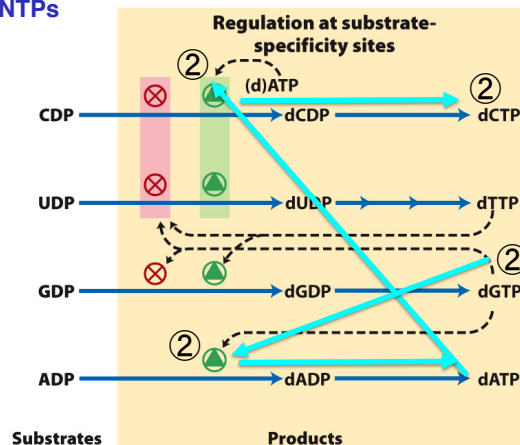


# Biosynthesis Amino Acids & Nucleotides

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  - **G:C**: if dGTP high, more dATP → more specificity for CDP

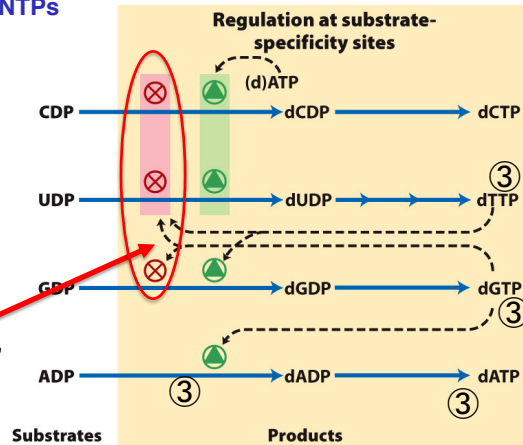


# Biosynthesis Amino Acids & Nucleotides

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- THREE LEVELS:
  - A:T**: If dATP high → MORE specificity for UDP (A:T)
  - G:C**: if dGTP high, more dATP → more specificity for CDP
  - Inhibition**: If dTTP or dGTP high, UDP, GDP (classic feedback) & CDP inhibited (balance G:C).



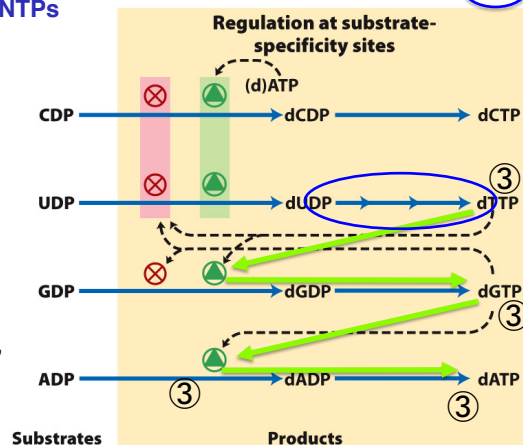
# Biosynthesis Amino Acids & Nucleotides

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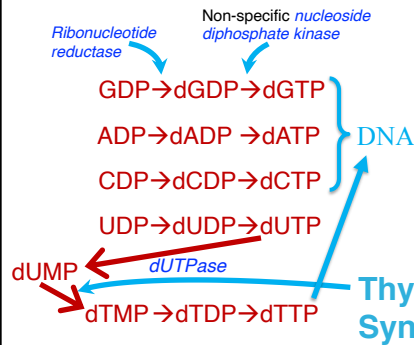
- The other site affects *substrate specificity* in order to maintain balanced pools of nucleotides.
- THREE LEVELS:
  - A:T**: If dATP high → MORE specificity for UDP (A:T)
  - G:C**: if dGTP high, more dATP → more specificity for CDP
  - Inhibition**: If dTTP or dGTP high, UDP, GDP & CDP inhibited.
  - T:A**: If dTTP high, it will indirectly cause MORE specificity for ADP → more dATP

Example:  
dGTP **inhibits** making less dCTP  
dATP **activates** making more dTTP



# Biosynthesis Amino Acids & Nucleotides

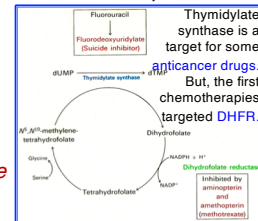
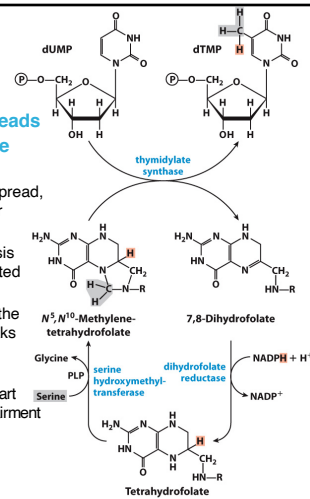
## dTTP is made from dUMP



1. dUDP is made by *ribonucleotide reductase*
2. dUTP is made by *nucleoside diphosphate kinase*
3. dUMP is made by *dUTPase*, with PP<sub>i</sub> as product
4. dUMP → dTMP by *thymidylate synthase*
  - adds a methyl group from *N*<sup>5</sup>,*N*<sup>10</sup>-methylene-tetrahydrofolate
5. dTMP → dTDP → dTTP

## Folic Acid Deficiency Leads to Reduced Thymidylate Synthesis

- Folic acid deficiency is widespread, especially in nutritionally poor populations.
- Reduced thymidylate synthesis causes uracil to be incorporated into DNA.
- Repair mechanisms remove the uracil by creating strand breaks that affect the structure and function of DNA.
  - associated with cancer, heart disease, neurological impairment



Thymidylate synthase is a target for some anticancer drugs. But, the first chemotherapies targeted DHFR.