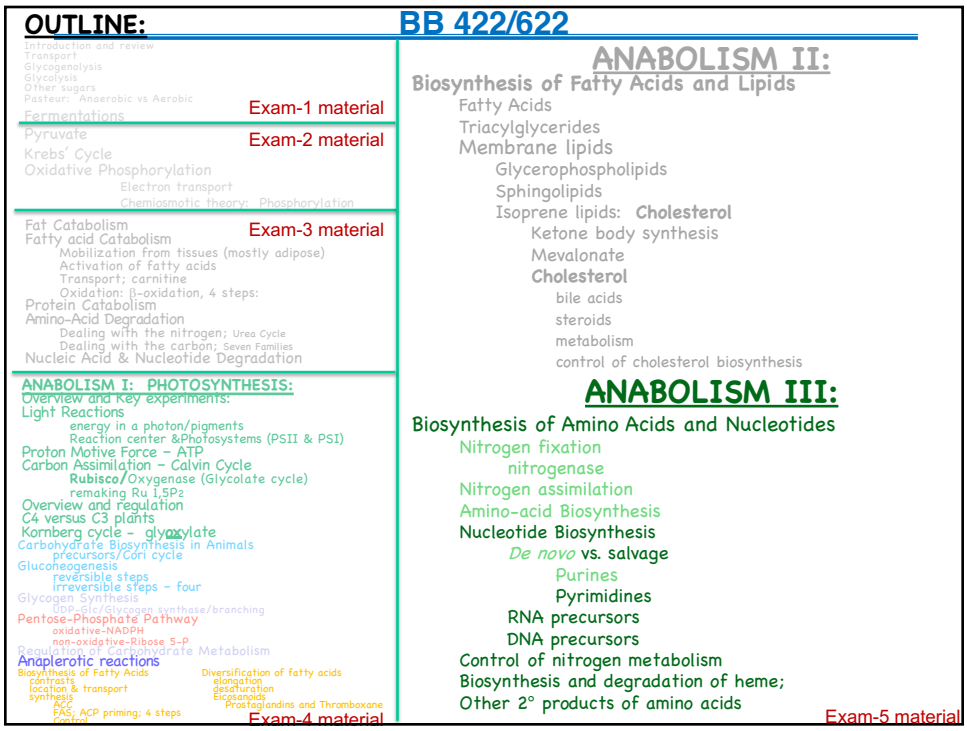
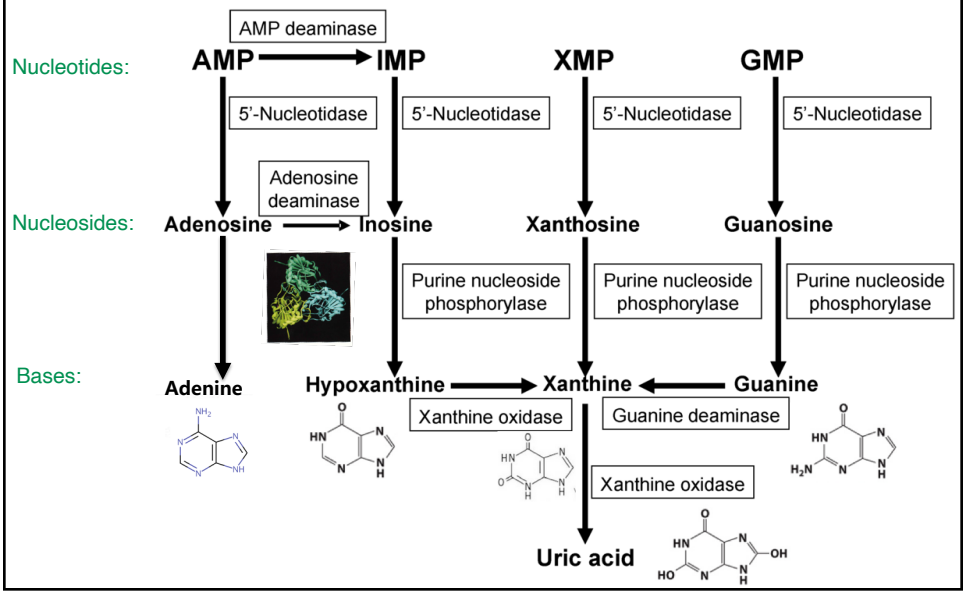


ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

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

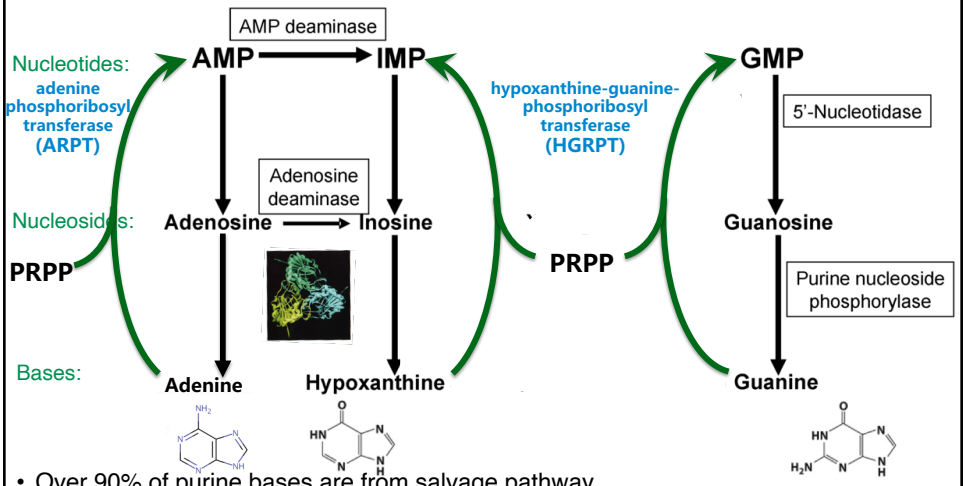
*Bases synthesized *while* attached to ribose-5-P; products are RMP (R is one-letter code for purine, Y is one letter code for pyrimidine)

Recall: Nucleotide Degradation



Biosynthesis Amino Acids & Nucleotides

Salvage Pathway of Purines

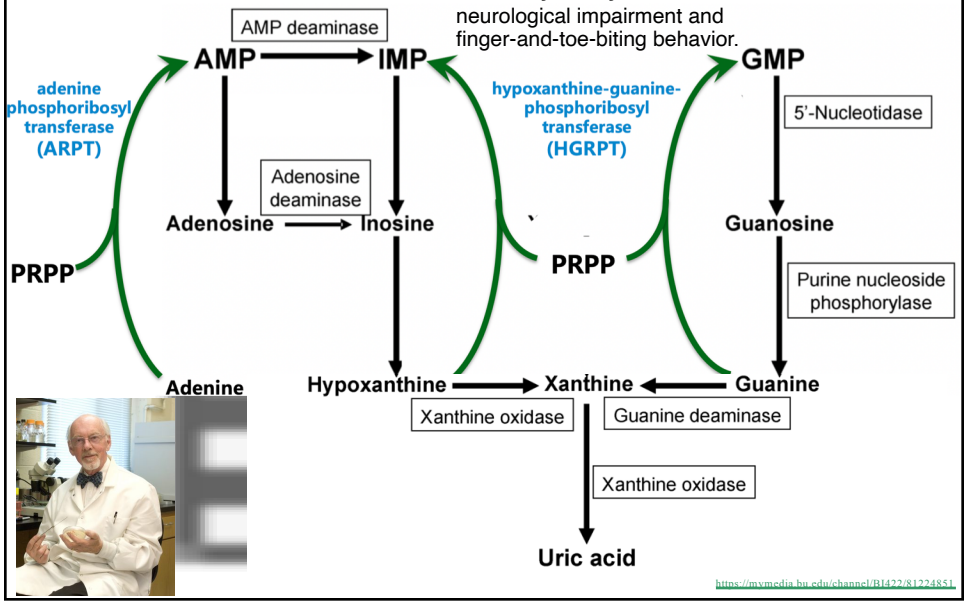


- Over 90% of purine bases are from salvage pathway.
- The brain is especially dependent on salvage pathways.
- The lack of **HGPRT** leads to **Lesch-Nyhan syndrome** with neurological impairment and finger-and-toe-biting behavior.

Biosynthesis Amino Acids & Nucleotides

Salvage Pathway

- The lack of **HGPRT** leads to **Lesch-Nyhan syndrome** with neurological impairment and finger-and-toe-biting behavior.



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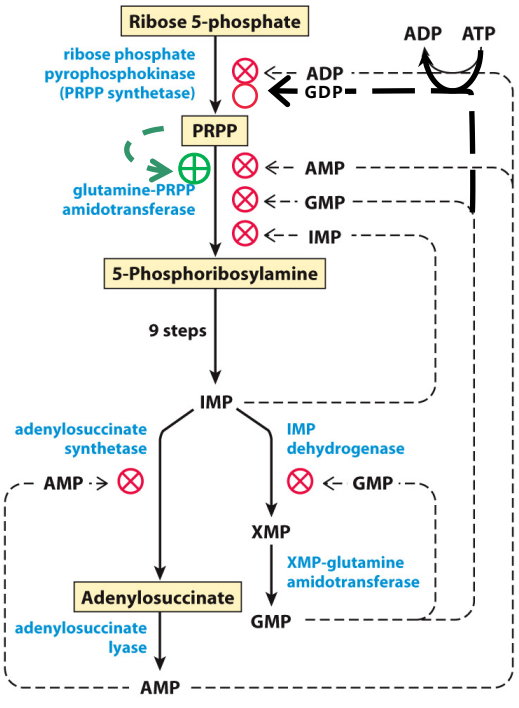
Biosynthesis Amino Acids & Nucleotides

Regulation of Purine Biosynthesis

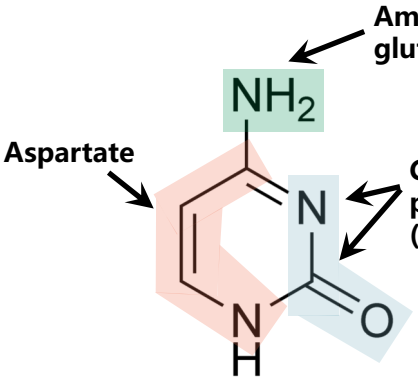
Four Major Sites of Allosteric Regulation

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 adenysuccinate from inosinate by *adenylsuccinate synthetase*.



Biosynthesis Amino Acids & Nucleotides

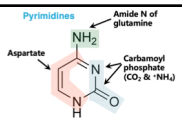
Pyrimidines



Overview:

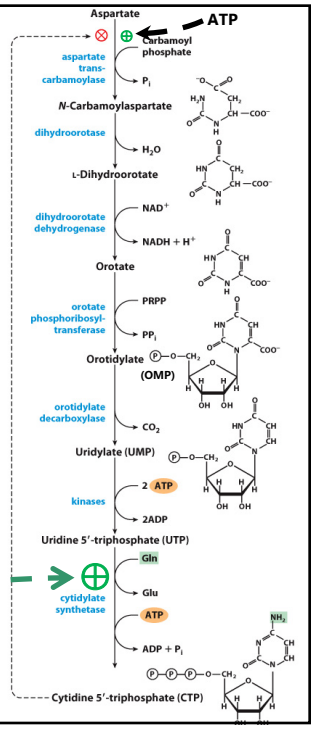
1. Orotic acid
2. Add ribose (PRPP), make UMP
3. CTP made from UTP

Biosynthesis Amino Acids & Nucleotides



De Novo Biosynthesis of Pyrimidines

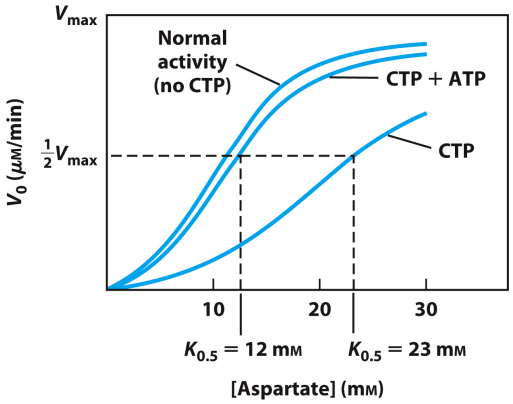
- Unlike purine synthesis, pyrimidine synthesis proceeds by *first making the pyrimidine ring* (in the form of orotic acid) and *then* attaching it to ribose 5-phosphate using PRPP.
- Aspartate and carbamoyl phosphate provide all the atoms for the heterocycle or pyrimidine. The first pyrimidine is Orotate.
- 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

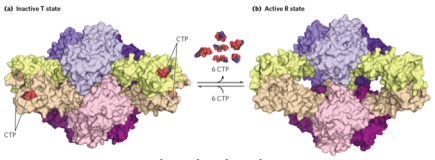


Biosynthesis Amino Acids & Nucleotides

Regulation of Pyrimidine Biosynthesis via Feedback Inhibition

Aspartate Transcarbamoylase (ATCase)





$(\alpha_3)_2(\beta_2)_3$

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/BI422/81224851

Biosynthesis Amino Acids & Nucleotides

So far:

GMP→GDP→GTP

AMP→ADP→ATP

UMP→UDP→UTP

CDP←CTP

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)

GDP→dGDP

ADP→dADP

UDP→dUDP

CDP→dCDP

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*

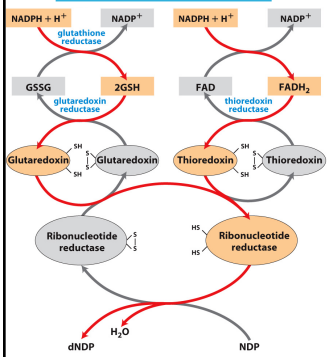
Very unique enzyme in all of biochemistry - use of free radicals (without B_{12} cofactor)

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

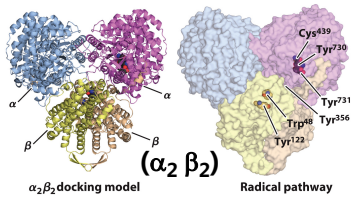


- NADPH serves as the electron donor.
- Funneled through glutathione or thioredoxin pathways

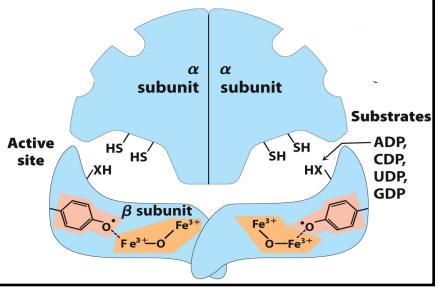
Structure of Ribonucleotide Reductase

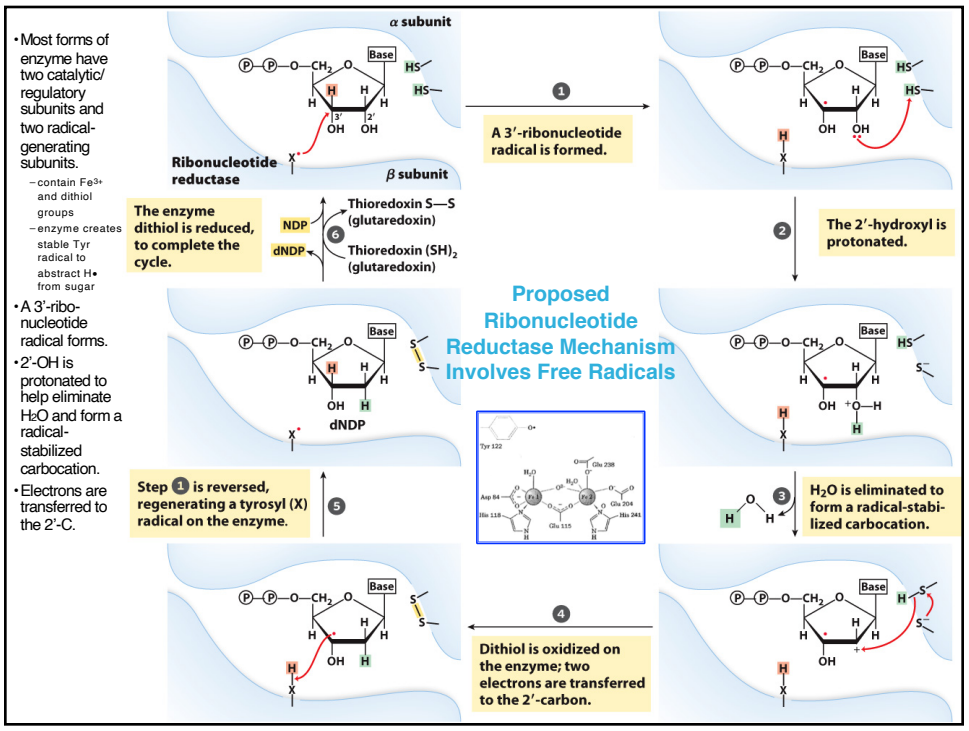
α_2 are regulatory and half the catalytic site; need to be reduced.

β_2 are the other half of the active site, and the free-radical generators



JoAnne Stubbe (1946–)

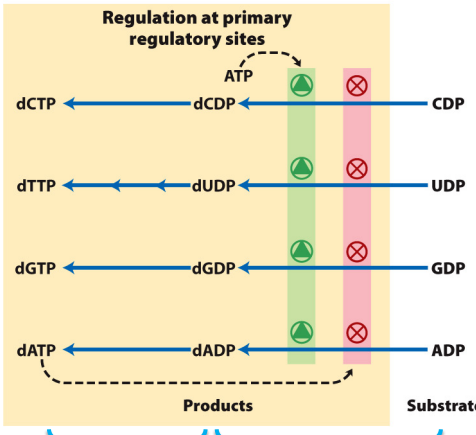


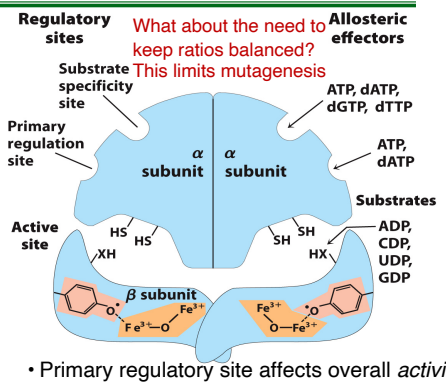


Biosynthesis Amino Acids & Nucleotides

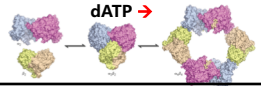
On/off Regulatory Site of Ribonucleotide

Feedback allosteric inhibition by dATP & activation by ATP





- 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.

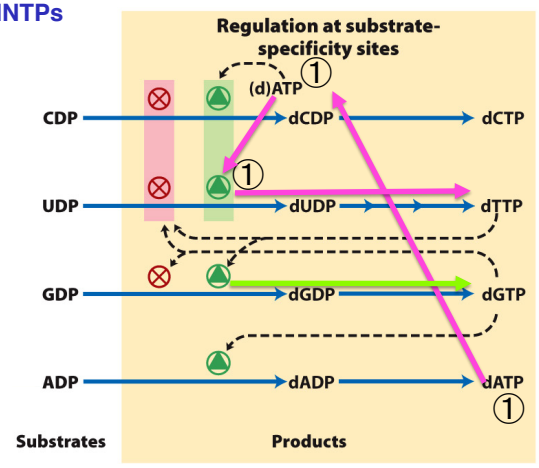


Biosynthesis Amino Acids & Nucleotides

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)



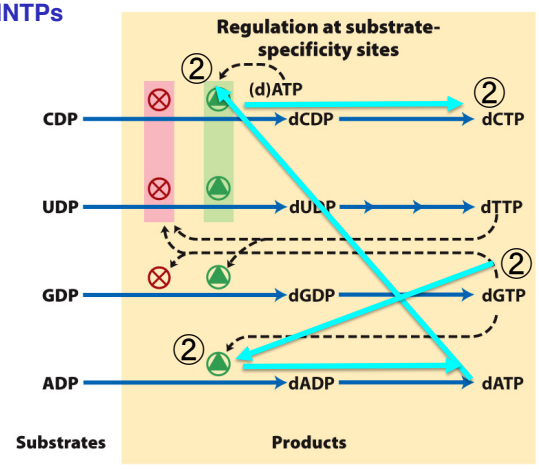
Biosynthesis Amino Acids & Nucleotides

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)

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

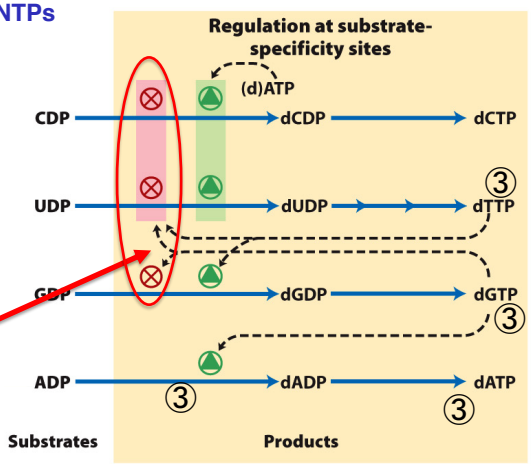


Biosynthesis Amino Acids & Nucleotides

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)
  - **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

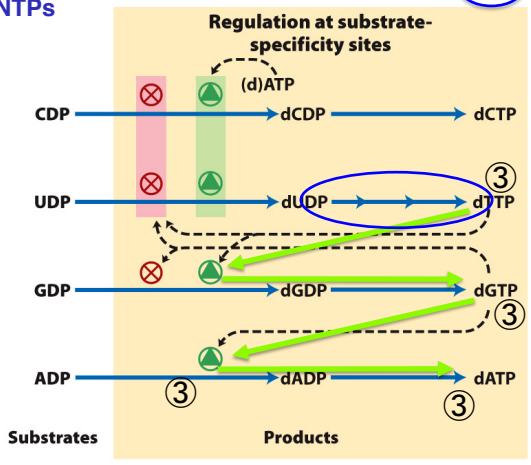
Specificity Regulatory Sites of Ribonucleotide Reductase

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

Feedback allosteric inhibition by dNTPs

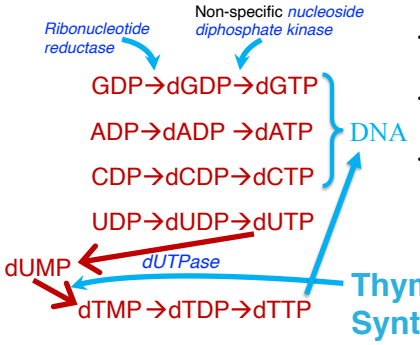
- The other site affects *substrate specificity* in order to maintain balanced pools of nucleotides.
- THREE LEVELS:
  - **A:T**: If dATP high → MORE specificity for UDP (A:T)
  - **G:C**: if dGTP high, more dATP→ more specificity for CDP

  - **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

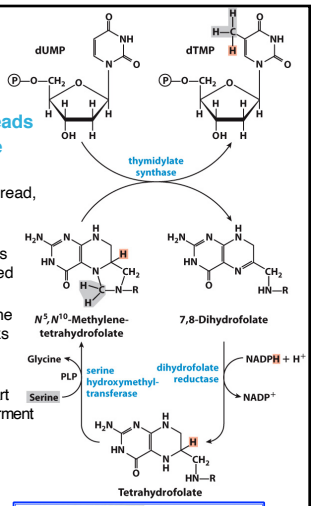


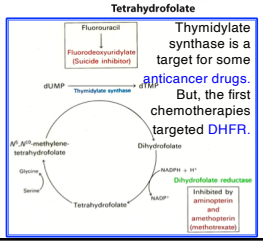
Biosynthesis Amino Acids & Nucleotides

dTTP is made from dUMP



- **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.

1. dUDP is made by *ribonucleotide reductase*
2. dUTP is made by *nucleoside diphosphate kinase*
3. dUMP is made by *dUTPase*, with PP_i as product
4. dUMP → dTMP by *thymidylate synthase*
   - adds a methyl group from *N^5,N^{10}-methylene-tetrahydrofolate*
5. dTMP → dTDP → dTTP