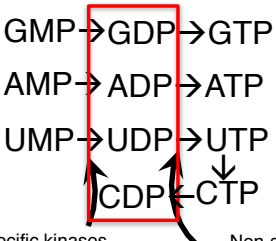
# ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

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)

GDP→dGDP
ADP→dADP
UDP→dUDP
CDP→dCDP

**How are Ribonucleic Acid Precursors converted to Deoxyribonucleic Acid Precursors?**
**……..and how is dTTP made?**

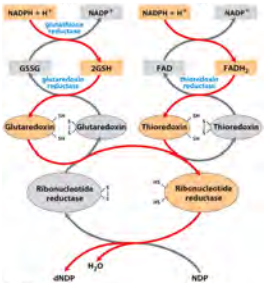
2'C-OH bond is directly reduced to 2'-H bond …without activating the carbon for dehydration, etc.!
catalyzed by *ribonucleotide reductase*

*Very unique enzyme in all of biochemistry - use of free radicals*

*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



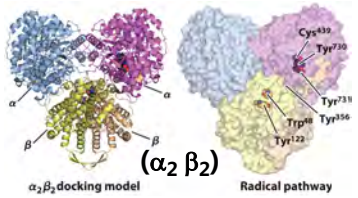

- NADPH serves as the electron donor.
- Funneled through glutathione or thioredoxin pathways

JoAnne Stubbe (1946– )

## Structure of Ribonucleotide Reductase

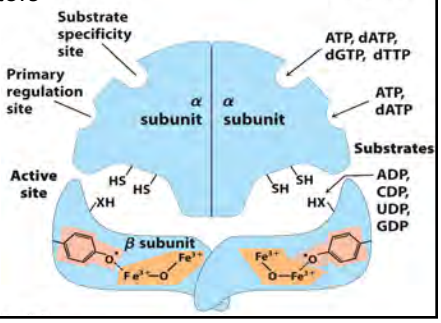
$\alpha_2$ are regulatory and half the catalytic site; need to be reduced.

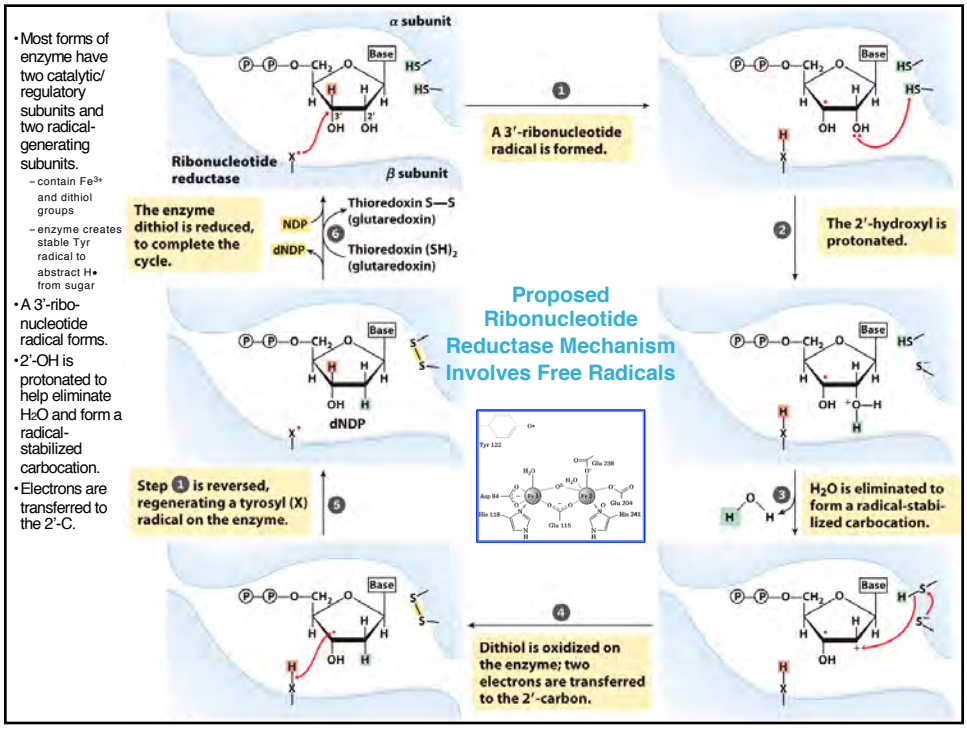
$\beta_2$ are the other half of the active site, and the free-radical generators




Regulatory sites

Allosteric effectors

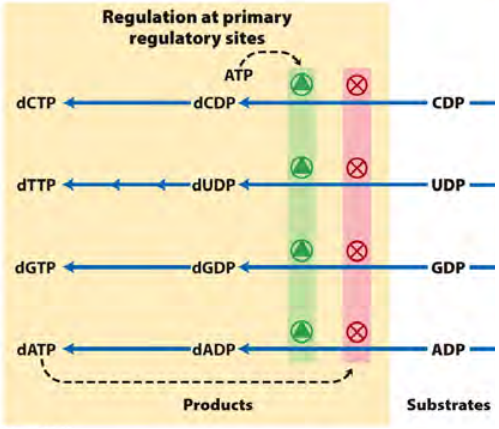


# Biosynthesis Amino Acids & Nucleotides

## Ribonucleotide Reductase Has Two Types of Regulatory Sites: On/off & Specificity

**Feedback allosteric inhibition by dATP & ATP**




- One type affects *activity*
  - –ATP activates
    - If no energy, not DNA replication
  - –dATP inhibits
    - If too much dATP, don't make any of the others as well

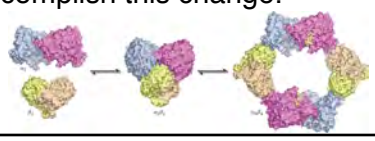
Need to keep ratios balanced to limit mutagenesis

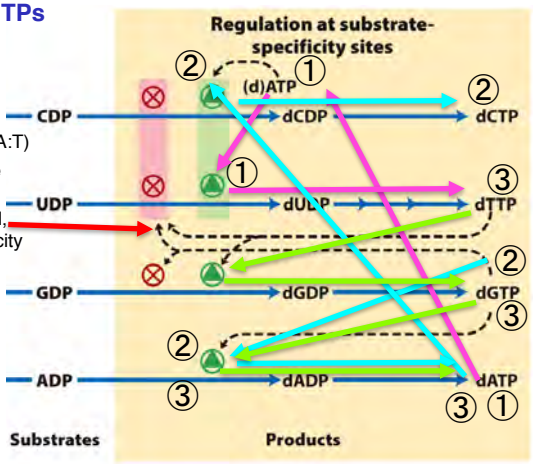
# Biosynthesis Amino Acids & Nucleotides

## Ribonucleotide Reductase Has Two Types of Regulatory Sites: On/off & Specificity

**Feedback allosteric inhibition by dNTPs**

- The other type affects *substrate specificity* in order to maintain balanced pools of nucleotides.
  - If dATP high → MORE specificity for UDP (A:T)
  - For (G:C); if dGTP high, more dATP→ more specificity for CDP
  - If dTTP or dGTP high, UDP & CDP inhibited, but dTTP will indrectly cause MORE specificity for ADP→more dATP
- Enzyme ***oligomerizes*** to accomplish this change.





# Biosynthesis Amino Acids & Nucleotides

## dTMP is Made from dUMP

Ribonucleotide reductase

Non-specific *nucleoside diphosphate kinase*

GDP→dGDP→dGTP

ADP→dADP →dATP

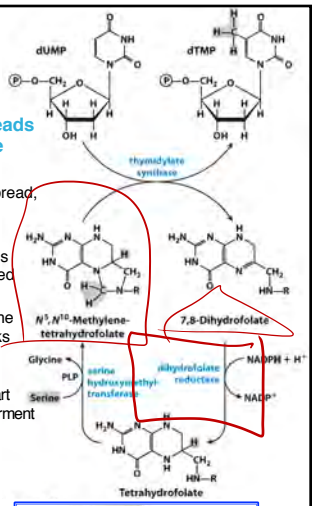
CDP→dCDP→dCTP

UDP→dUDP→dUTP

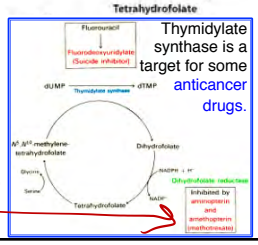
dUMP ← *dUTPase* ← dTMP→dTDP→dTTP

**Thymidylate Synthase**

- **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




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



Thymidylate synthase is a target for some anticancer drugs.

# ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

1) Nitrogen fixation: $N_2$ → $NH_4$
2) Nitrogen assimilation: incorporation of ammonia into biomolecules
3) Biosynthesis of amino acids
   a) non-essential
   b) essential
4) Biosynthesis of nucleotides and deoxynucleotides
5) Control of nitrogen metabolism
6) Biosynthesis and degradation of heme; other 2° products of amino acids

# Biosynthesis Amino Acids & Nucleotides

## Regulation of Nucleotide Metabolism:

**Purines**: Feedback inhibition by AMP, GMP, IMP at committed steps
**Pyrimidines**: Feedback inhibition by CTP at committed step
**PRPP synthesis**: Feedback inhibition by GDP, ADP, UMP, CTP
**RNA/DNA precursors**: GTP activates CTP synthetase from UTP; GTP used for AMP and ATP used for GMP; Ribonucleotide reductase

## Regulation of Overall Nitrogen Metabolism and Amino Acid Biosynthesis

Multilayered approach: Often, more than one mechanism of feedback regulation is utilized. There are FOUR modes of Feedback control

*De novo purine-GMP*

Sequential – every committed product feeds back

*homoserine dehydrogenase in Thr & Ile*

Concerted – all end-products must be present to inhibit at all

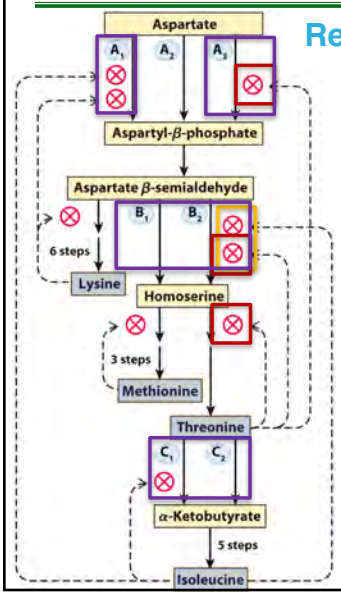
*Glutamine Synthetase*

Cummulative – all end-products inhibit the same percentage

*Aromatic amino acids*

Isozymes – use of isozymes for regulation of specific pathways; each end-product inhibits a specific isozyme

# Biosynthesis Amino Acids & Nucleotides




## Regulation of Aspartate/Pyruvate-Derived Pathways

**Classic** Feedback Inhibition in Lys, Met, Thr, and Ile Biosynthesis

**Sequential** Feedback Inhibition in Thr Biosynthesis

**Concerted** Feedback Inhibition of Homoserine Dehydrogenase in Ile and Thr Biosynthesis

**Use of Isozymes Is Another Important Means of Regulation:**

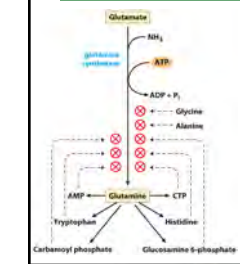
*Homoserine dehydrogenase*
*Serine/Threonine dehydratase*
*Aspartate Kinase*

*Asp Kinase* initiates biosynthsis of Lys, Met, Thr, and Ile. Use of isozymes, all regulated by different effectors, allows *E. coli* to produce the amino acids when needed.

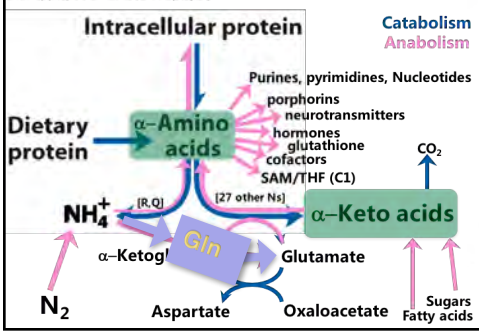
Example: *Asp Kinase* isozyme A1 is inhibited if Ile or Lys is high, but not if Met or Thr are high.
Only the A1 isozyme is inhibited by Ile at this step.

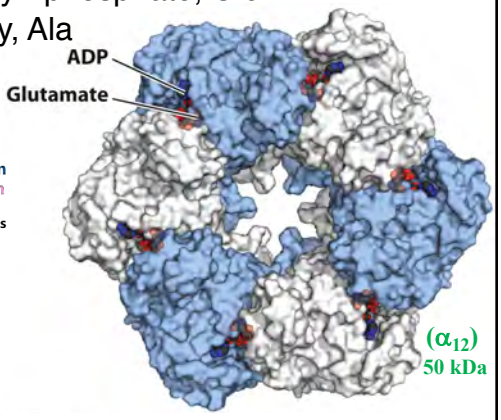
What is a good example of Cumulative feedback inhibition?

# Biosynthesis Amino Acids & Nucleotides




**Cumulative** allosteric inhibition by:
CTP, AMP, His,
Trp, Carbamoyl- phosphate, Glc-amine-6-P, Gly, Ala

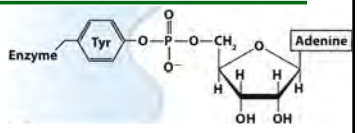



Glutamate + $NH_4^+$ + ATP → glutamine + ADP + $P_i$ + $H^+$

# Biosynthesis Amino Acids & Nucleotides

## Adenylylation of Glutamine Synthetase



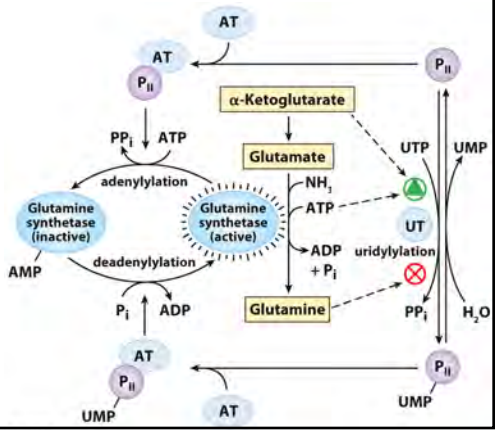

***Adenylylation*** (attachment of AMP) to Tyr-397 assists in inhibition.

- Increases sensitivity to feedback inhibitors
- Part of complex cascade that is dependent on [Glu], [α-ketoglutarate], [ATP], and [$P_i$]
- Activity of *adenylyltransferase (AT)* regulated by binding to regulatory protein $P_{II}$
- ***Adenylylation*** inhibits *Gln synthetase*.

When $P_{II}$ is uridylylated, *adenylyltransferase* stimulates deadenylylation of Gln synthetase (increasing the latter's activity).

- Uridylyation controlled by Gln (inhibits) and α-KG & ATP (activates).

ALSO, uridylylated $P_{II}$ upregulates transcription of Gln synthetase.



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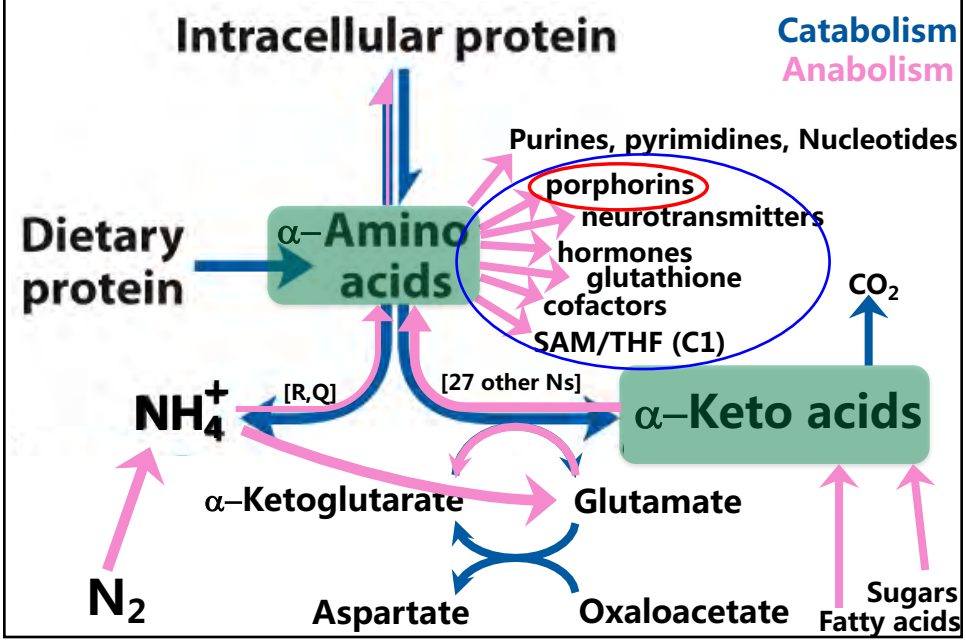
## Biosynthesis Amino Acids & Nucleotides



# Biosynthesis Amino Acids & Nucleotides

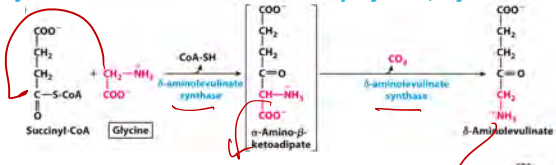
## Many Important Metabolites are Derived from Amino Acids

- Porphyrin rings (e.g., heme, cytochromes, chlorphylls, etc.)
- Phosphocreatine
- Glutathione
- Cofactors; niacin, biotin, folic acid
- Neurotransmitters (serotonin, GABA, adrenalin, DOPA, histamine)
- Signaling molecules
  - Hormones; melatonin, adrenaline
  - Paracrine signals; NO, leukotrienes
- Cell-wall constituents; Peptidoglycan, Lignin

# Biosynthesis Amino Acids & Nucleotides

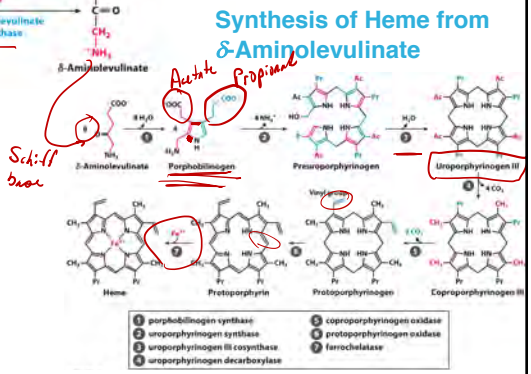
## Porphyrin Biosynthesis

### Glycine is the Precursor to Porphyrins; synthesis of δ-Aminolevulinate




- Porphyrin makes up the heme of hemoglobin, cytochromes, myoglobin.
- In higher animals, porphyrin arises from reaction of glycine with succinyl-CoA.
  - In plants and bacteria, glutamate is the precursor.
- The pathway generates two molecules of the important intermediate *δ-aminolevulinate.* ALA
- *Porphobilinogen* is another important intermediate.

### Synthesis of Heme from δ-Aminolevulinate




1. Two molecules of δ-aminolevulinate condense to form porphobilinogen.
2. Four molecules of porphobilinogen combine to form protoporphyrin.
3. Fe ion is inserted into protoporphyrin with the enzyme *ferrochelatase.*

# Biosynthesis Amino Acids & Nucleotides

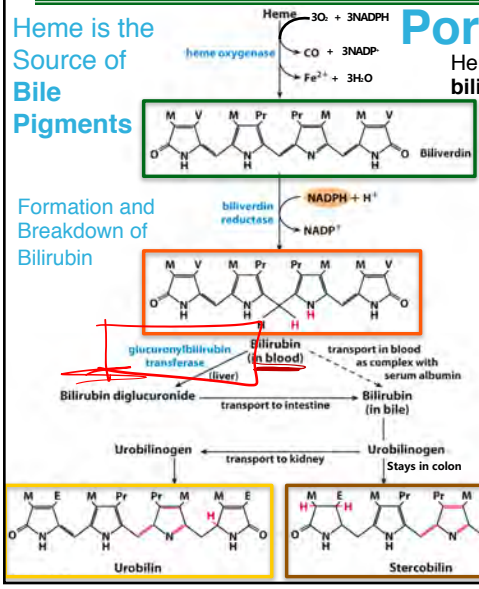
## Defects in Heme Biosynthesis

- Most animals synthesize their own heme.
- Mutations or mis-regulaton of enzymes in the heme biosynthesis pathway lead to **porphyrias** (pour-fear-ia).
  - Precursors accumulate in red blood cells, body fluids, and liver.
- Accumulation of precursor uroporphyrinogen I
  - Urine becomes discolored (pink to dark purplish depending on light, heat exposure).
  - Teeth may show red fluorescence under UV light.
  - Skin is sensitive to UV light.
  - There is a craving for heme.
- Explored as possible biochemical basis for vampire myths

# Biosynthesis Amino Acids & Nucleotides

Dr. Kornberg:
**Lecture 04.14.17 (23:27-28:08,-29:07) 4.5,5.5 min**
use the My Media

## Heme is the Source of **Bile Pigments**




## Porphyrin Degradation

Heme from degradation of erythrocytes is degraded to **bilirubin** in two steps:

1. *Heme oxygenase* linearizes heme to create **biliverdin**, a green compound (bruises).
2. *Biliverdin reductase* converts biliverdin to **bilirubin,** a red-orange compound that travels bound to serum albumin in the bloodstream.
3. Bilirubin is oxidized in the kidney to **urobilin**, the major pigment of urine.
4. Urobiligen not taken up by the kidney stays in the colon and is reduced by intestinal microbiota to **stercobilin.**

### **Jaundice** is Caused by Bilirubin Accumulation

Jaundice (yellowish pigmentation of skin, whites of eyes, etc.) can result from:

- impaired liver (in liver cancer, hepatitis)
- blocked bile secretion (due to gallstones, pancreatic cancer)
- insufficient *glucouronyl bilirubin transferase* to process bilirubin (occurs in infants)
  - treated with UV to cause photochemical breakdown of bilirubin

## ANABOLISM III: Biosynthesis Amino Acids & Nucleotides

## Summary

What we learned:

- Methods for fixation of molecular nitrogen to nitrates, nitrates, and **ammonia**
- Gln serves as the primary entry point of assimilation of ammonia via *Gln Synthetase* in animals; but made useful by *Glu Synthase* to make net Glu.
- The 20 common amino acids are synthesized from $\alpha$-ketoglutarate, 3-phosphoglycerate, oxaloacetate, pyruvate, phosphoenolpyruvate, erythrose 4-phosphate, and ribose-5-phosphate (through phosphoribosyl pyrophosphate (PRPP).
- About half are non-essential in humans and are made much like they are degraded
- About half are essential and are made through extensive and inter-related paths
- Nucleotides can be synthesized either *de novo* from simple precursors, or reassembled from the salvage pathway using PRPP.
- *De novo* purines are synthesized on the ribose, while pyrimidine rings are assembled prior to attachment to ribose
- Ribonucleotides (NDP) are converted to deoxyribonucleotides (DNTP) by *ribonucleotide reductase*, which is regulated in ways to ensure equal amounts of A:T & G:C.
- Regulation of amino-acid biosynthesis, as well as nucleotide synthesis, is by various types of feedback inhibition
- Porphyrin biosynthesis and degradation is one example of 2° product of amino acids

## Farewell to Biochemistry II (BI/CH 422 & BI/CH 622)

This course is Dedicated to the memory of Sir Hans Kornberg

January 14, 1928 ~ December 16, 2019

I want to thank Dr. Kornberg for all his help this semester and the inspiration!

Dr. Kornberg: **Lecture 04.26.17 (28:03-28:29) (½ min)**