METABOLISM
of
Nucleotides and Nucleic Acids

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Biosynthesis of pyrimidine nucleotides

Gln+CO_2+Asp

carbamoylphosphate synthetase II
aspartate carbamoil transferase
dihydroorotase

dihydroorotate dehydrogenase

+ PRPP

orotate phosphoribosil-transferase
OMP-decarboxylase

UMP

Multifunctional Proteins Catalyze the Reactions of Pyrimidine Biosynthesis

Five of the first six enzyme activities of pyrimidine biosynthesis reside on multifunctional polypeptides.

CAD-enzyme, a single polypeptide named for the first letters of its enzyme activities, catalazes the first three reactions

A second bifunctional enzyme (UMP-synthase) catalyzes two last reactions

UMP serves as precursor in the synthesis of cytidine nucleotides.

Firstly, kinases catalyze transfer of phosphoryl groups of the ATP molecules to UMP, and latter, to UDP:

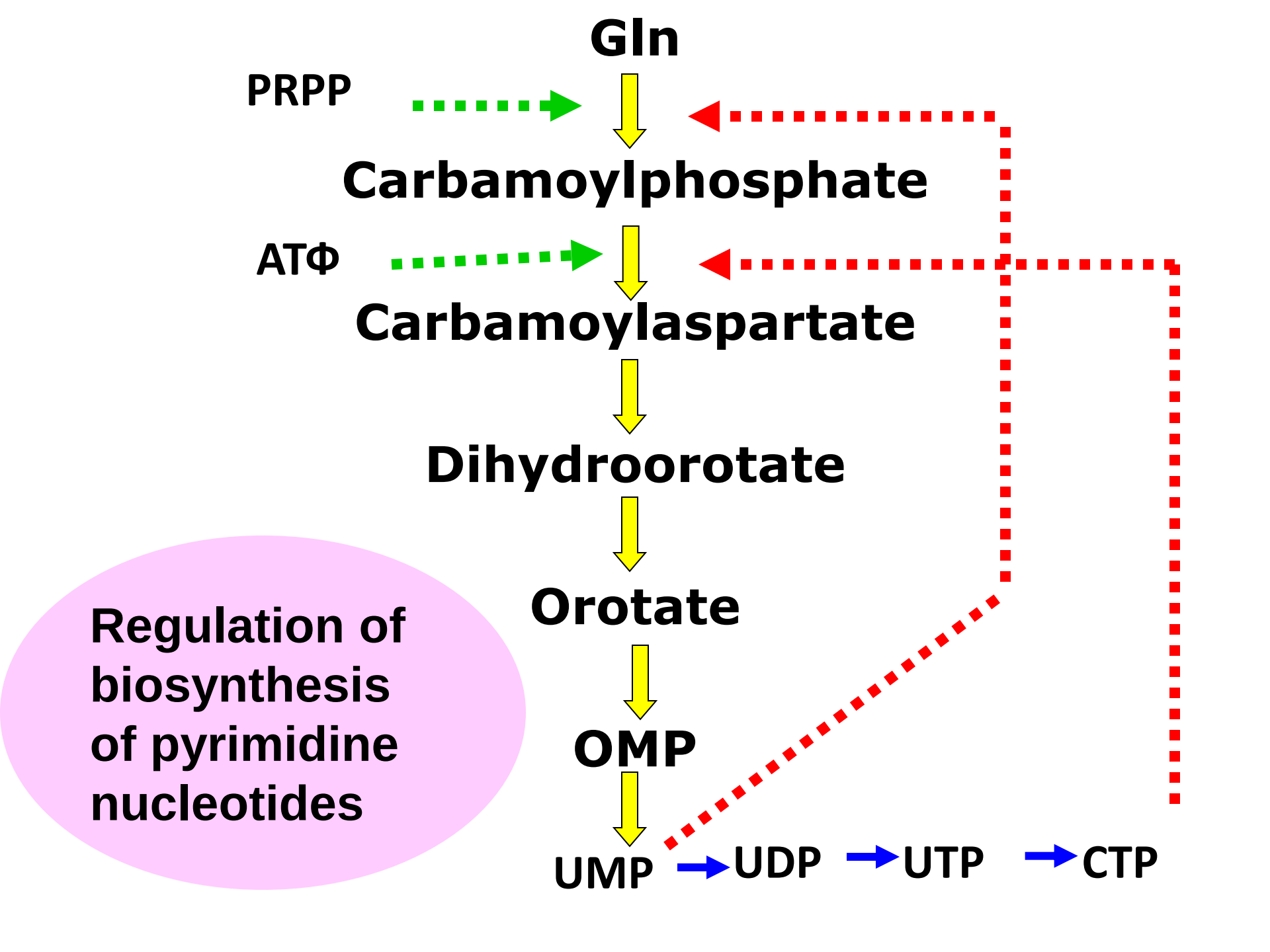
$$UMP + ATP \leftrightarrow UDP + ADP;$$

$$UDP + ATP \leftrightarrow UTP + ADP$$

Cytidine triphosphate is synthesized from UTP is the reaction, catalyzed by CTP-synthase:

$$UTP + glutamine + ATP \rightarrow$$

$$CTP + glutamate + ADP + P_i$$


Gln
PRPP
Carbamoylphosphate
АТФ
Carbamoylaspartate
Dihydroorotate
Orotate
OMP
UMP
UDP
UTP
CTP
Regulation of biosynthesis of pyrimidine nucleotides

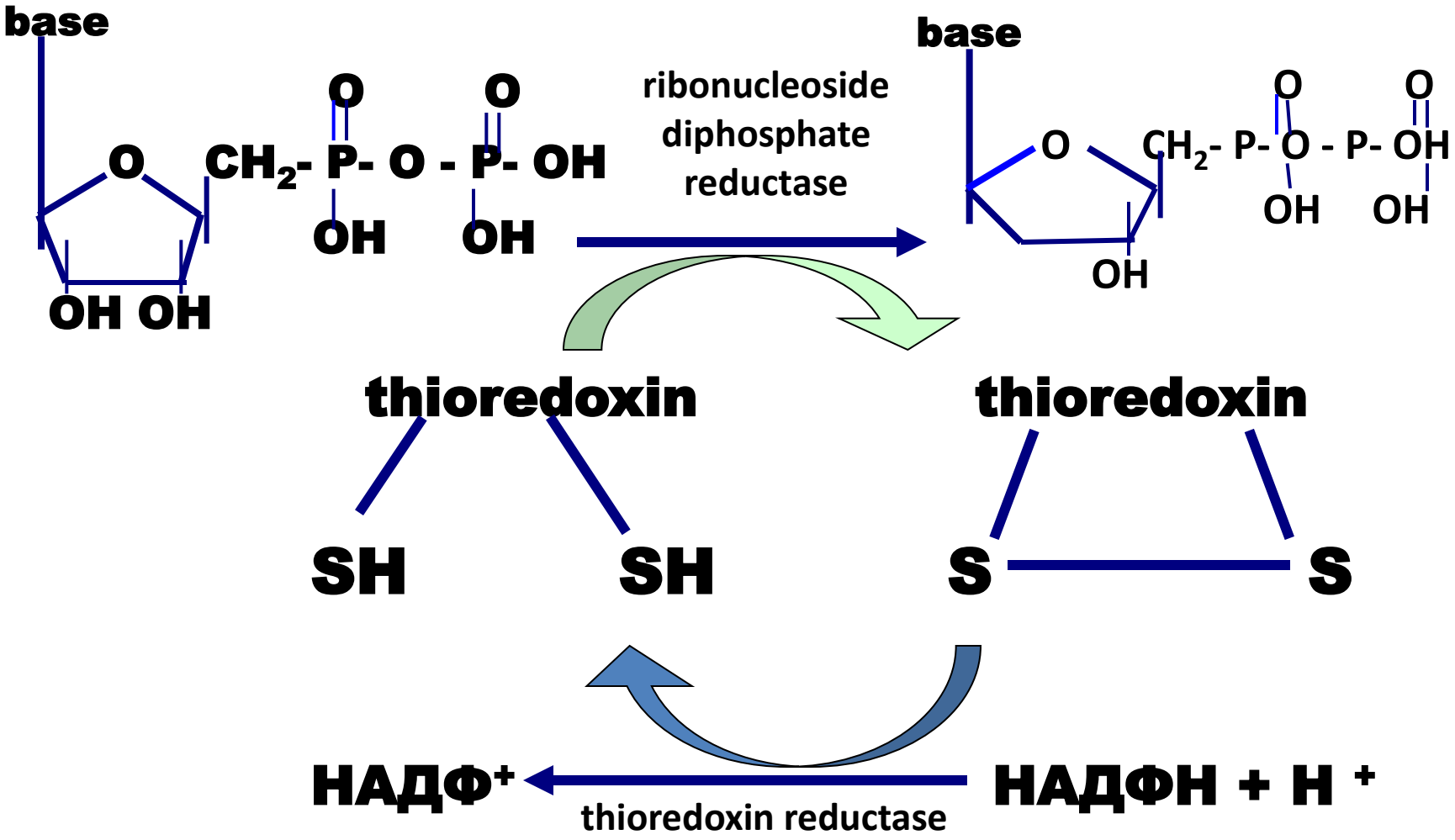
Synthesis of deoxyribonucleotides

Conversion of ribose to deoxyribose takes place within ribonucleoside diphosphates

so formed

dADP, dGDP, dUDP, dCDP

Reduction of ribonucleoside diphosphates

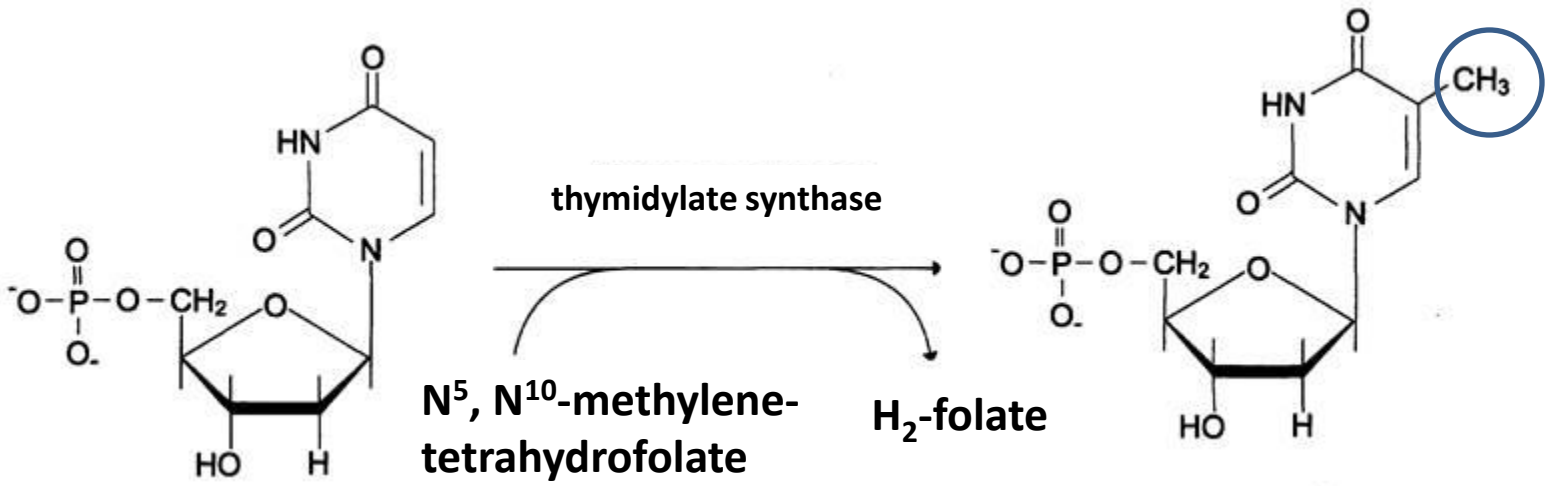


Synthesis of thymidylic acid

thymidylate synthase

dUMP → **dTMP**

N^5, N^{10}-methylene-tetrahydrofolate → H_2-folate



For synthesis of thymidylic acid, the molecule of deoxyuridine monophosphate (dUMP) is required:

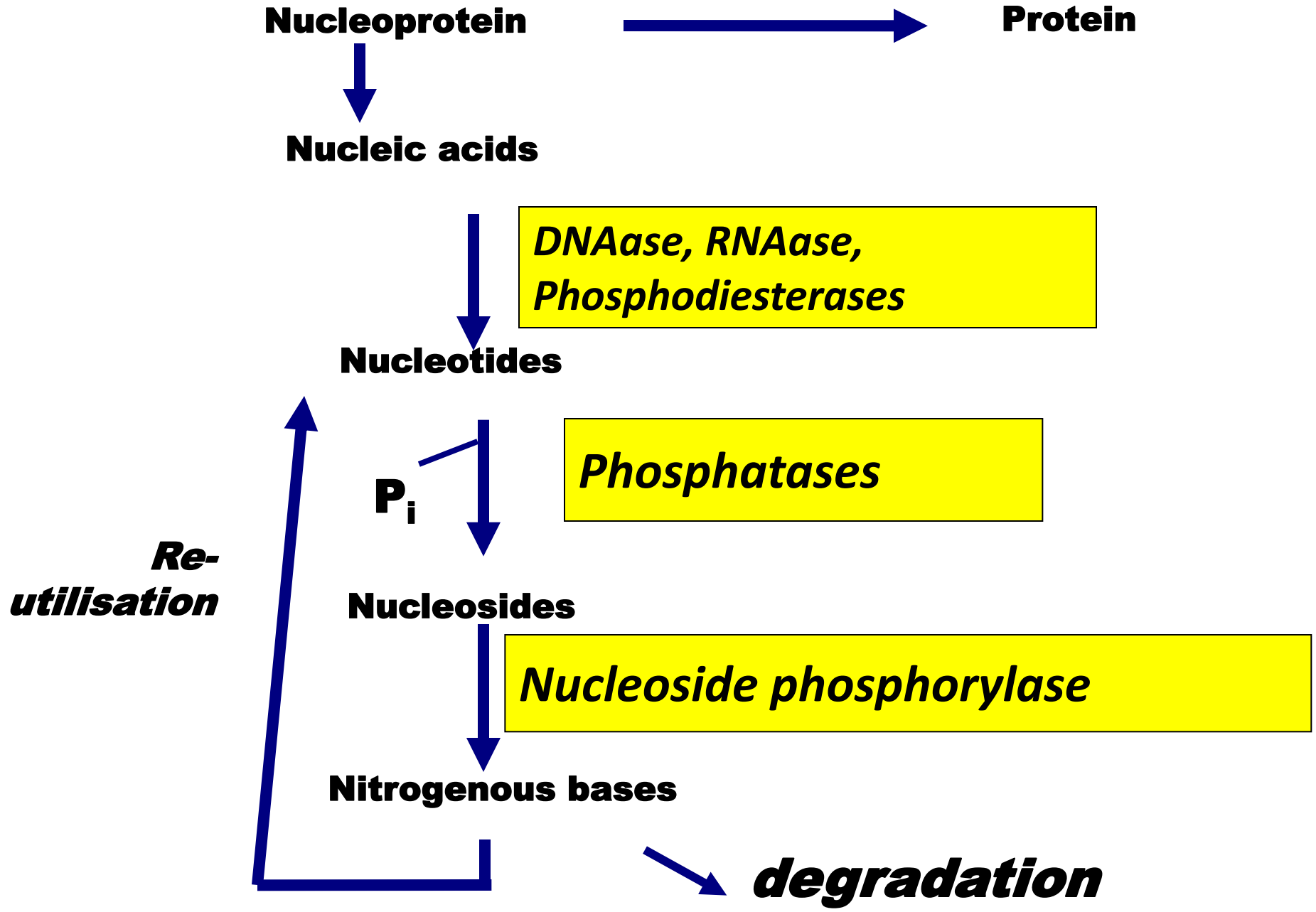
- **dUDP previously formed undergoes hydrolysis to dUMP:**

$$dUDP + H_2O \rightarrow dUMP + P_i$$

- **or or dUMP is formed by hydrolytic deamination of dCMP**

$$dCMP + H_2O \rightarrow dUMP + NH_3$$

Digestion of nucleic acids in the gastrointestinal tract



Degradation of nucleic acids in tissues

In tissues, nucleic acids are degraded by nucleases. There are several types of nucleases:

1) Endonucleases. They catalyze hydrolytic cleavage of inner phosphodiester bonds of DNA or RNA to produce oligonucleotides.

2) Exonucleases. They catalyze hydrolytic removal of terminal mononucleotides from DNA or RNA molecule.

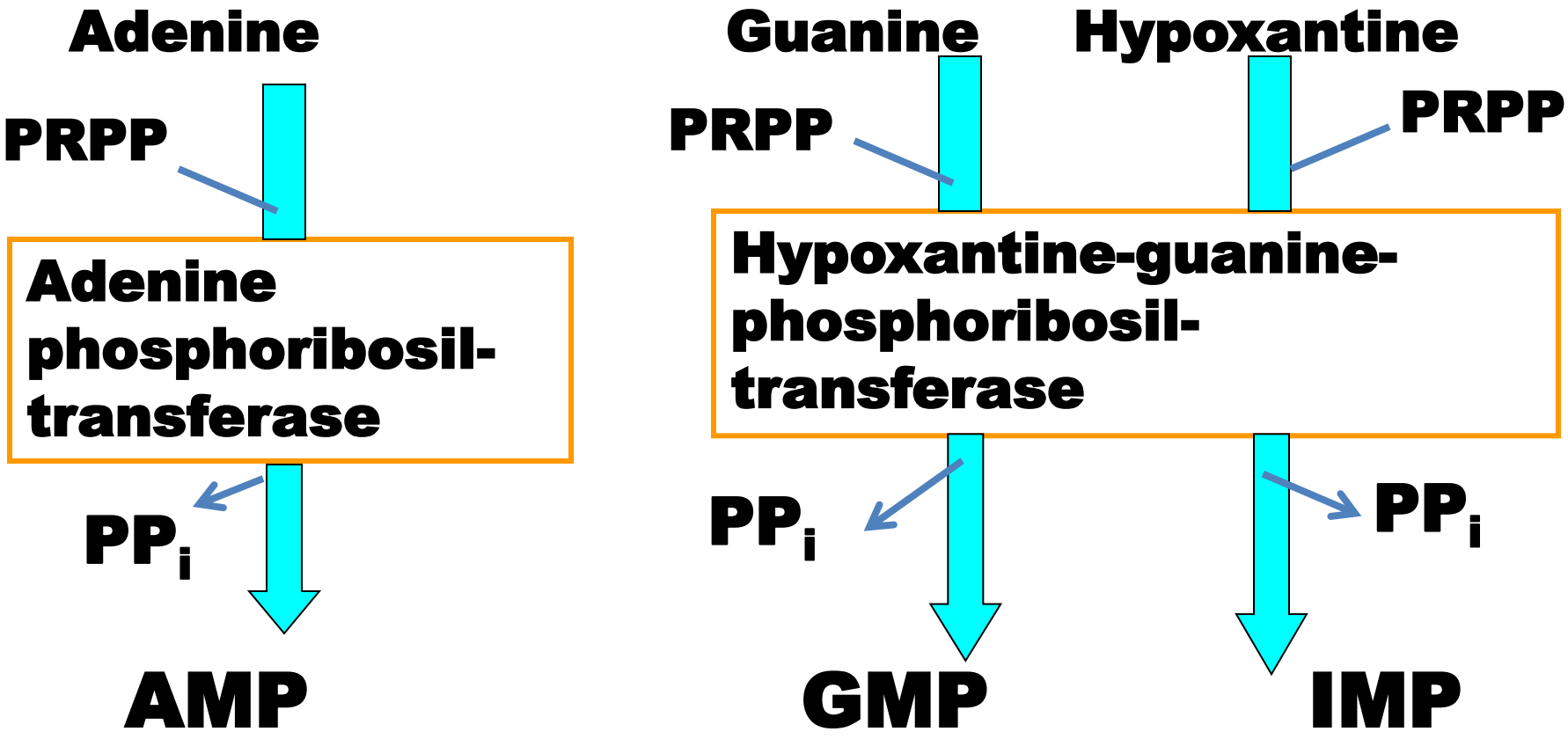
There are also specific nucleases involved in the breakdown of DNA or RNA molecule:

- **Deoxyribonucleases I. They catalyze cleavage of phosphodiester bonds within one of the two strands of DNA.**
- **Deoxyribonucleases II . They catalyze cleavage of phosphodiester bonds within both DNA strands.**
- **Ribonucleases . They catalyze cleavage of phosphodiester bonds within RNA.**
- **Restrictases. They catalyze cleavage of DNA at strictly defined regions of the DNA molecule**
- **Polynucleotide phosphorylase. It catalyzes phosphorolytic breakdown of RNA by adding inorganic phosphate to a mononucleotide cleaved from RNA to produce ribonucleoside diphosphate**
- **DNA-glycosidases . They catalyze hydrolysis of modified nitrogenous bases in a DNA molecule. DNA-glycosidases play an important role in the repair of DNA**

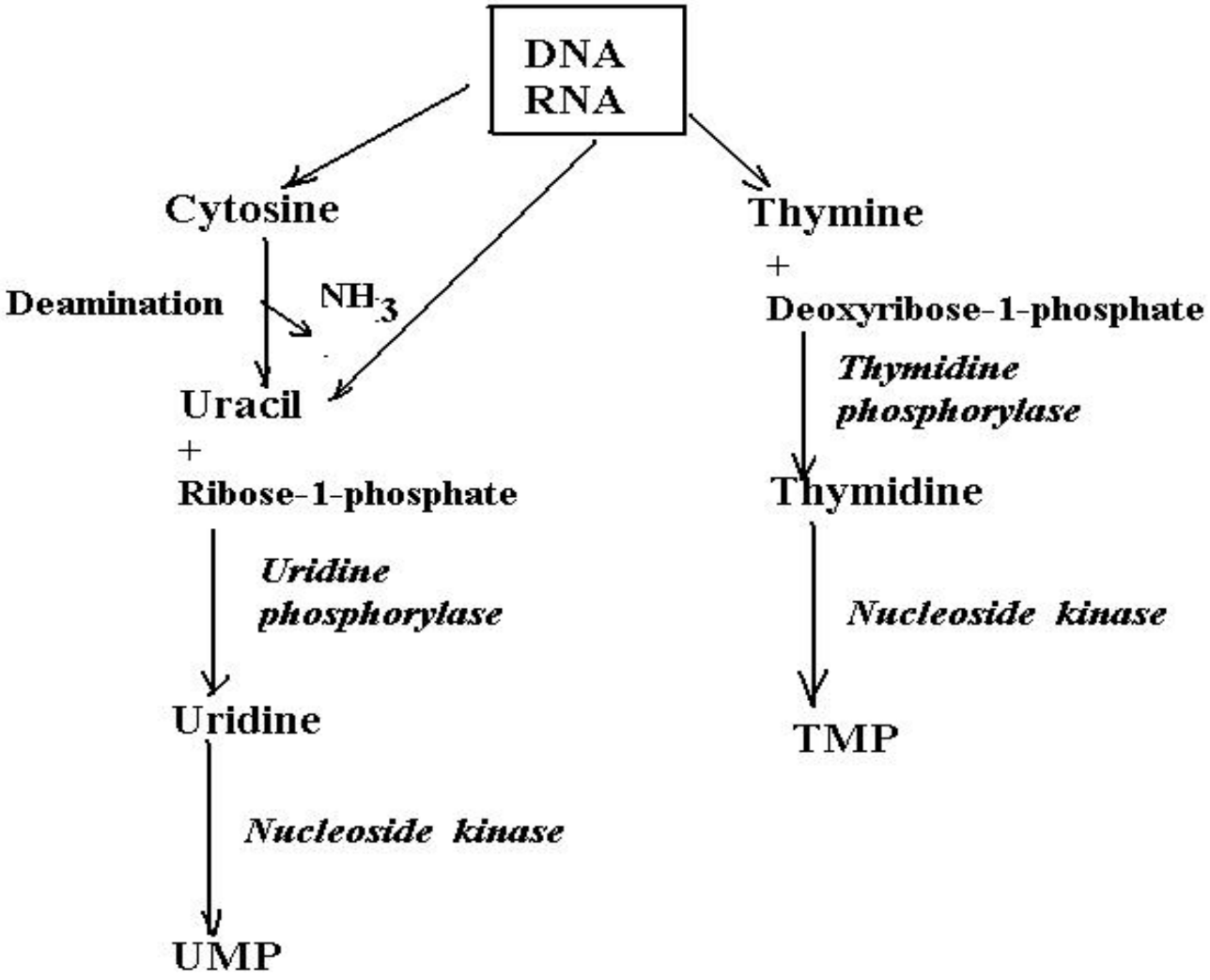
Re-utilization of nucleosides and nitrogenous bases for synthesis of nucleotides (salvage pathways)

Salvage pathways are used to recover nucleotides from bases and nucleosides that are formed during degradation of nucleic acids

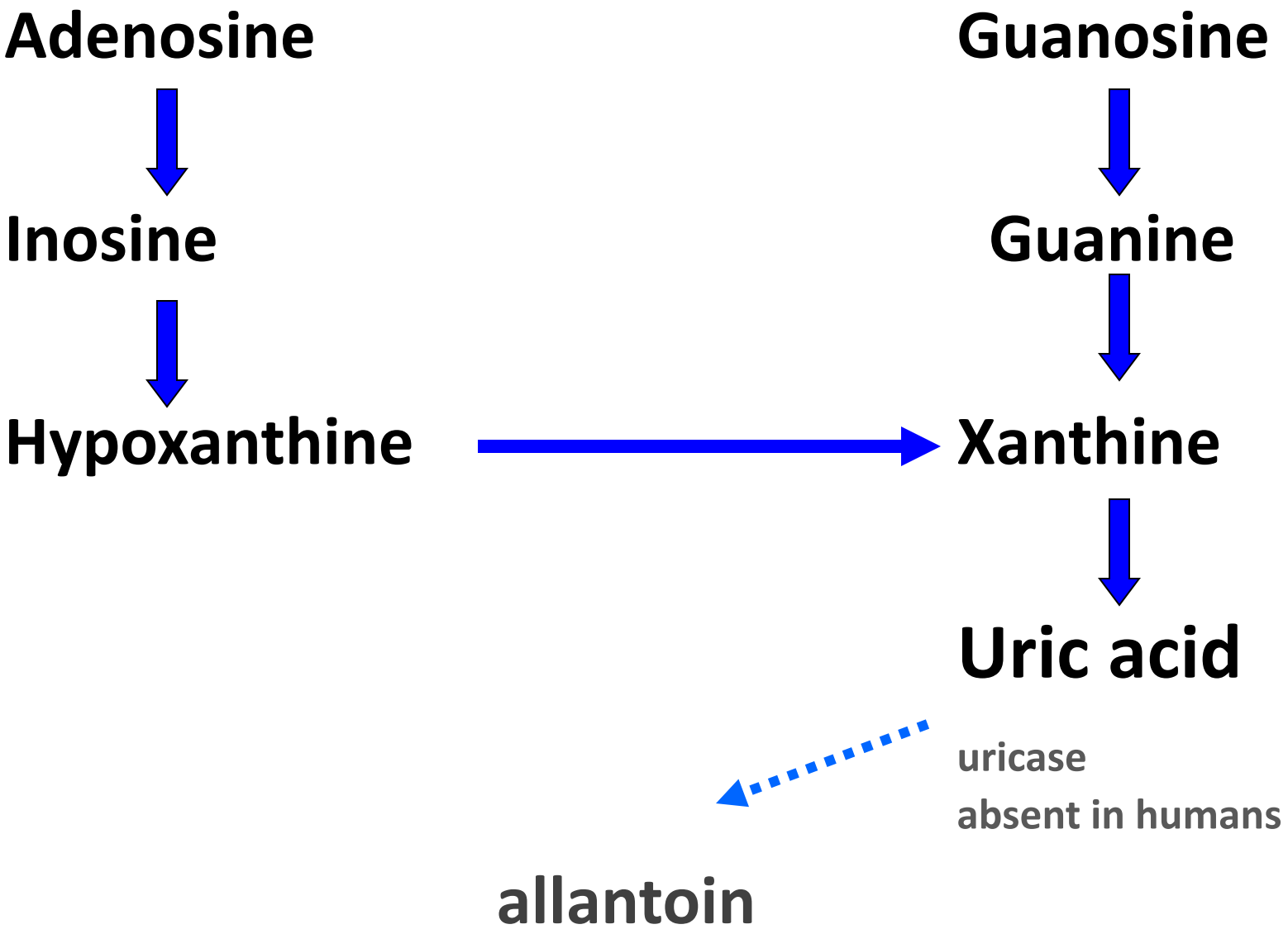
Purine salvage pathways



Pyrimidine salvage pathways



Degradation of purines



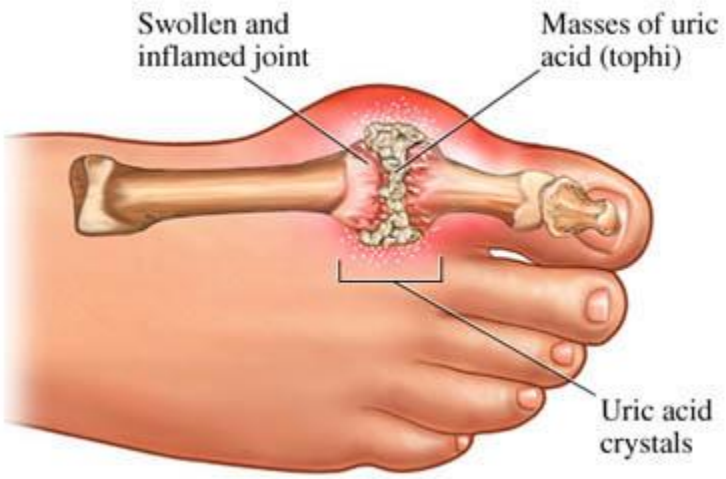
Disorders of purine metabolism

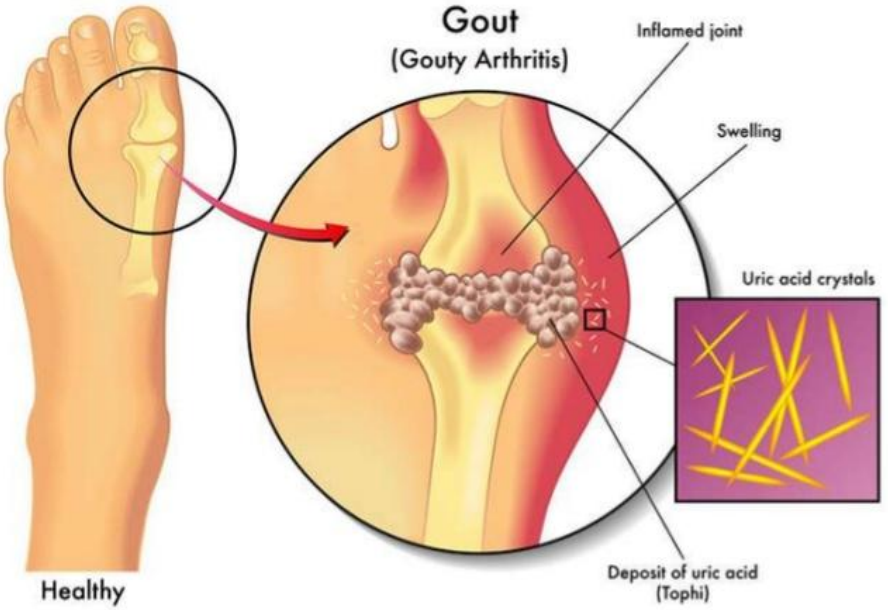
- **Gout**
- **Lesch-Nyhan Syndrome**
- **Xanthinuria**

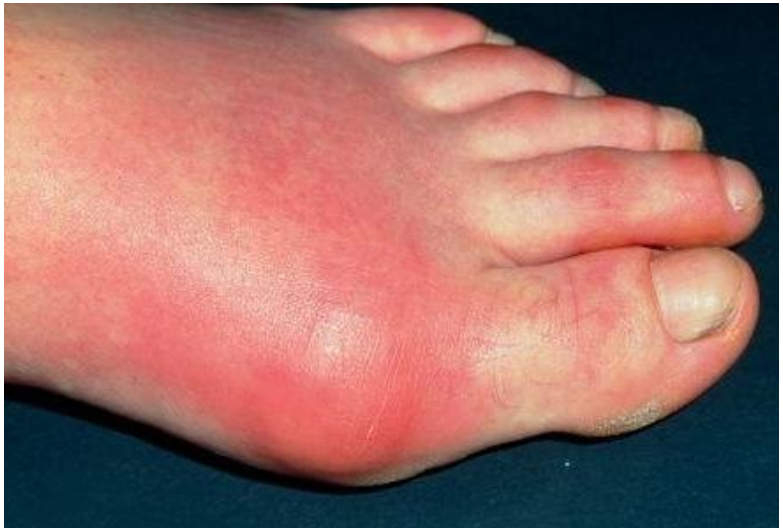
Gout

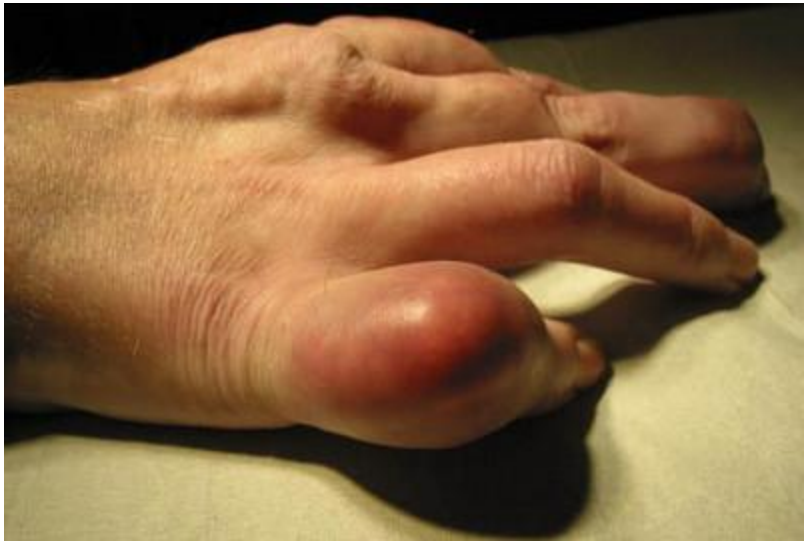
- Gout may occur in persons with persistent elevated levels of uric acid in the blood.
- The most common clinical manifestation of gout is repeated painful attacks of acute joint inflammation (arthritis).
- Depositions of uric salts around joints are called **tophi**. More often, tophi are located in small joints. Tophi cause deformity of joints and impair their function.
- Increased excretion of uric acid may cause uric acid crystals to be deposited in the collecting tubules of kidney and lower urinary tract, leading to stone formation (urolithiasis).

Gout


Swollen and inflamed joint
Masses of uric acid (tophi)
Uric acid crystals


Gout
(Gouty Arthritis)
Inflamed joint
Swelling
Uric acid crystals
Healthy
Deposit of uric acid (Tophi)





Lesch-Nyhan Syndrome

an overproduction hyperuricemia characterized by frequent episodes of uric acid lithiasis and a bizarre syndrome of self-mutilation, reflects a defect in hypoxanthine-guanine phosphoribosyl transferase, an enzyme of purine salvage

Xanthinuria

Xanthinuria is a rare genetic disorder caused by inherited deficiency of xanthine oxidase. The disease is characterized by decreased production of uric acid (hypouricemia) and increased excretion of hypoxanthine and xanthine.

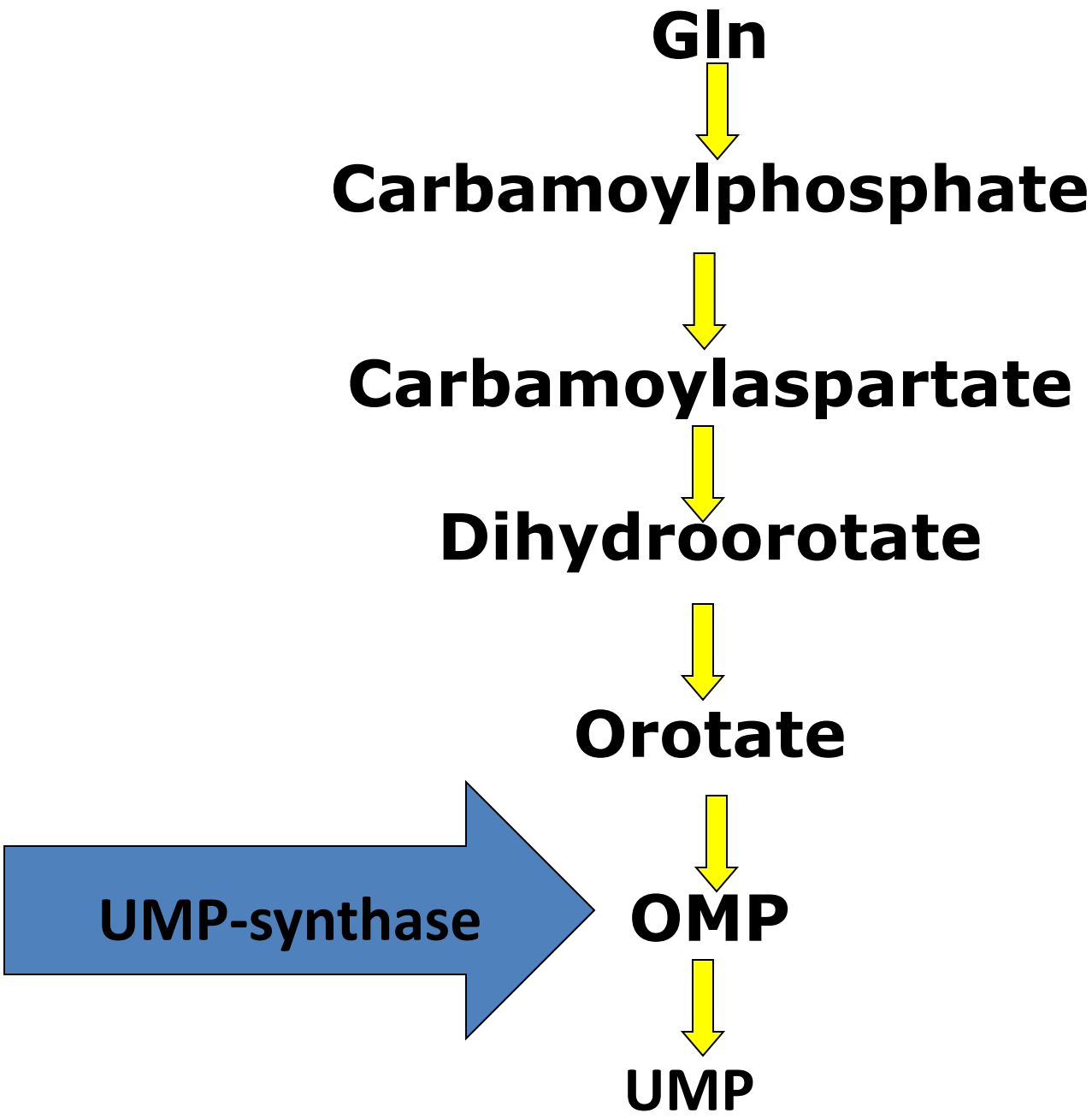
Disorders of pyrimidine metabolism :

Orotaciduria (orotic aciduria)

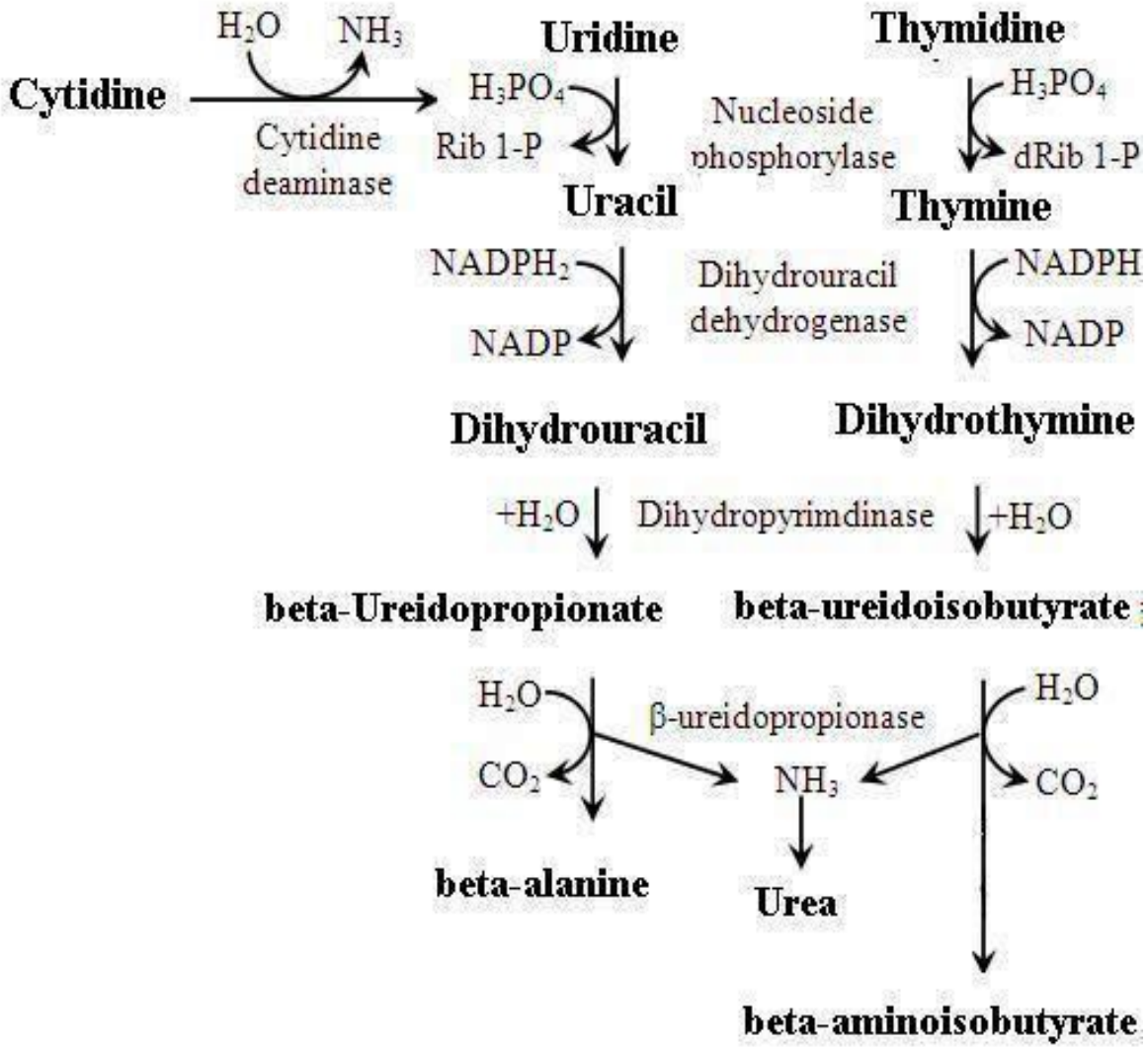
rare metabolic disorder results from loss of functional **UMP-synthase**

The orotic acid accumulated in the organism is excreted into the urine.

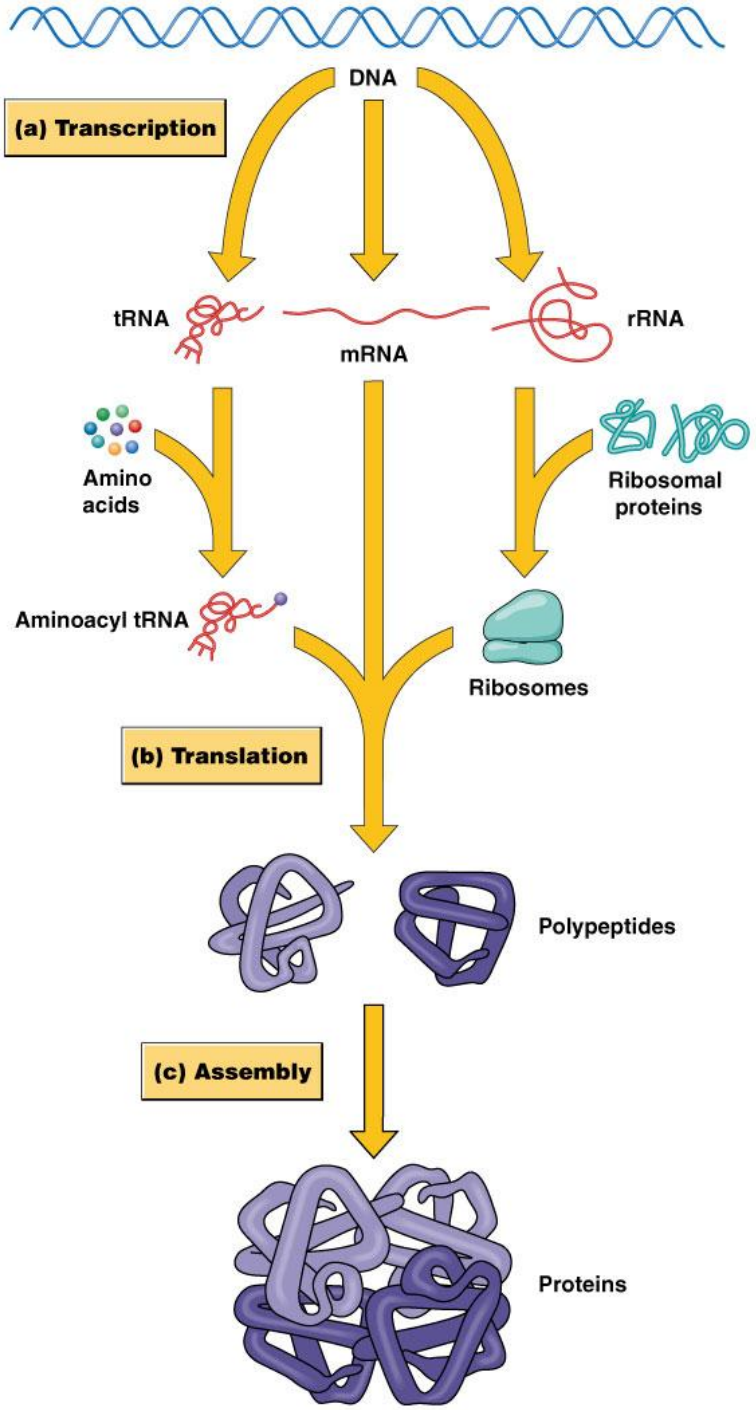
This disorder is followed by insufficient synthesis of pyrimidines and DNA due to lack of UMP and TMP. That leads to the state of "**pyrimidine starvation**" of tissues. The disease is characterized by the **retardation of growth, impairment of mental development**, as well as **megaloblastic anemia.**


Gln
Carbamoylphosphate
Carbamoylaspartate
Dihydroorotate
Orotate
UMP-synthase
OMP
UMP

Degradation of pyrimidines



GENE EXPRESSION



BIOSYNTHESIS OF DNA

(replication)

BIOSYNTHESIS OF DNA

occurs during the S phase

of the cell cycle

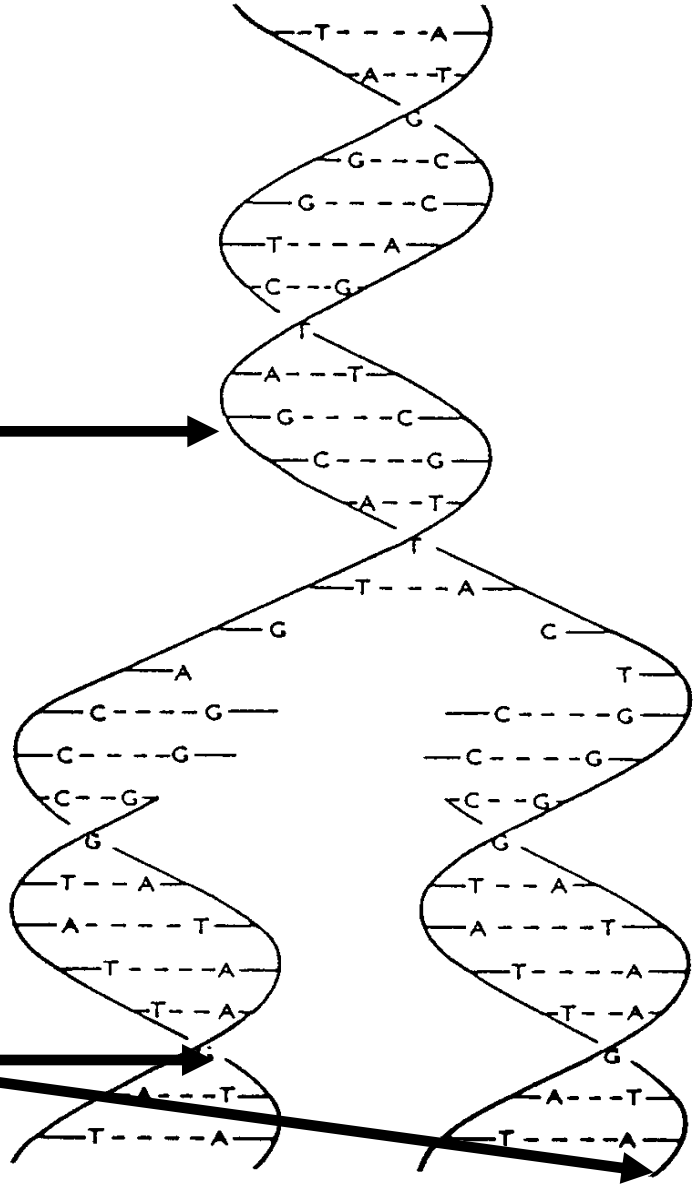
DNA Replication Is Semiconservative

each DNA strand serves as a template for the synthesis of a new strand,producing two new DNA molecules, each with one new strand and one old strand.

This is **semiconservative replication.**

“Old” strains

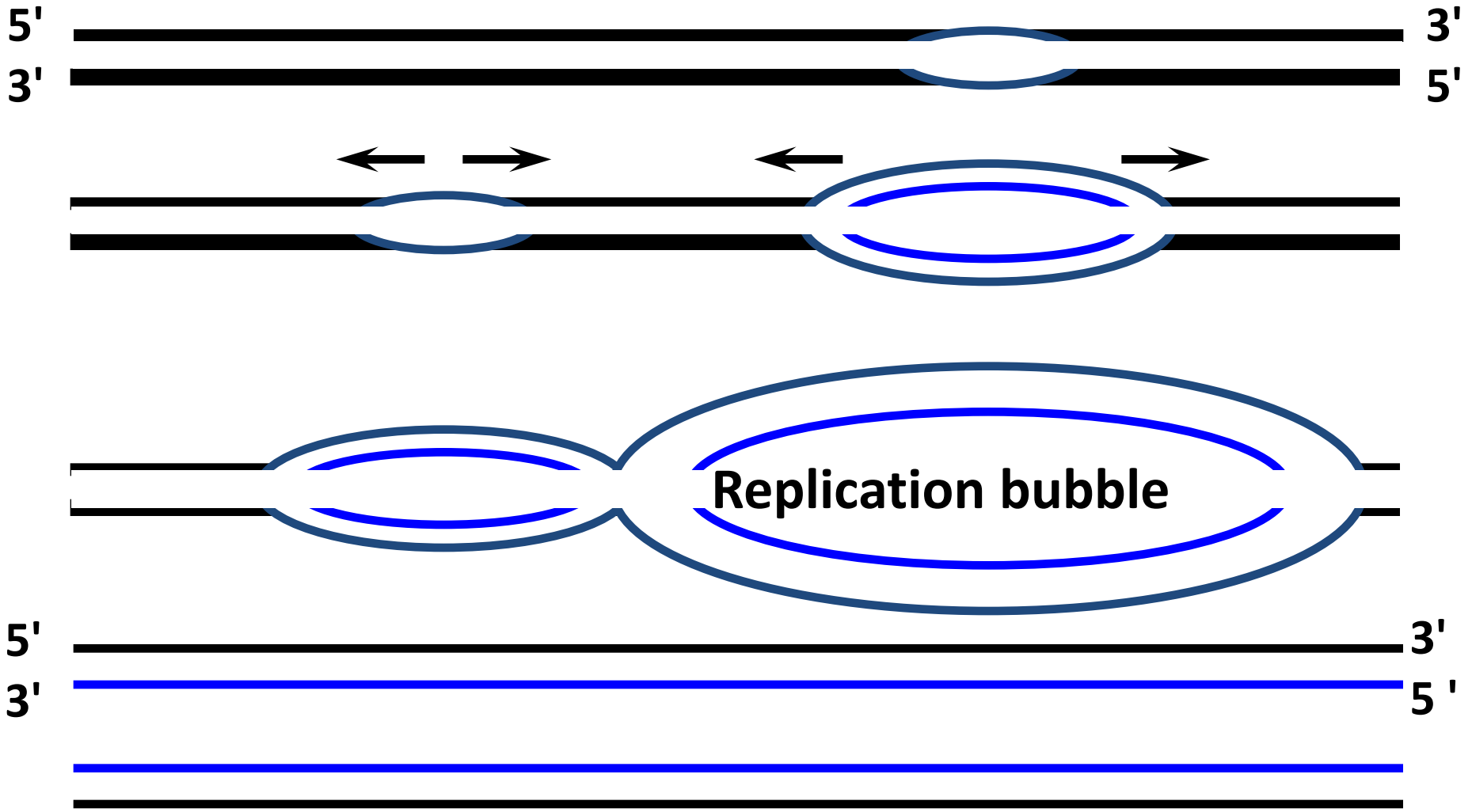
“New” strains



Major steps of DNA replication

1. Identification of the origins of replication
2. Denaturation of dsDNA to provide ssDNA template
3. Formation of the replication fork, synthesis of RNA primer
4. Initiation of DNA synthesis and elongation
5. Formation of replication bubbles with ligation of the newly synthesized DNA segments
6. Reconstitution of chromatin structure

Replication Begins at an Origin and Usually Proceeds Bidirectionally



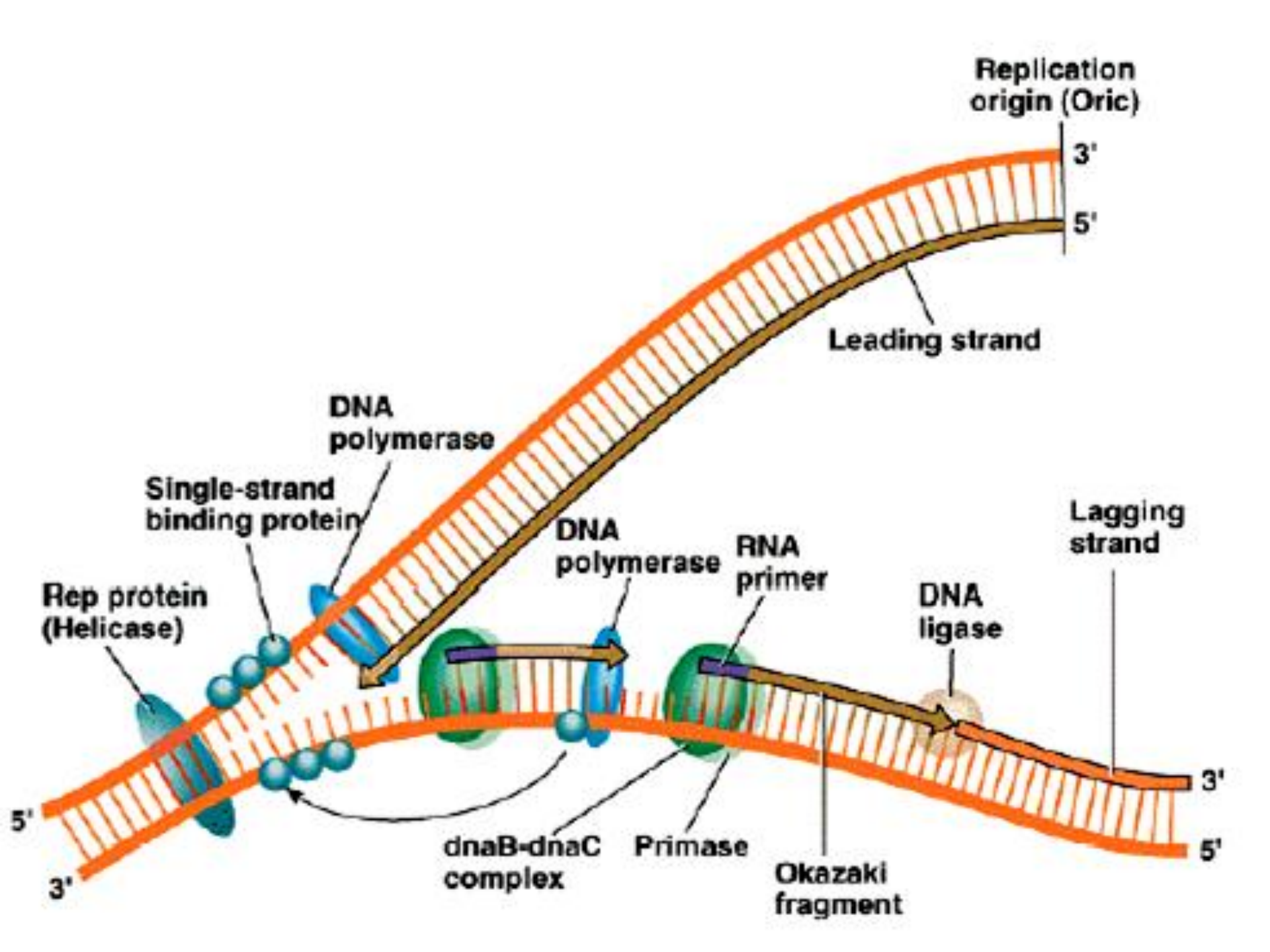
One or both ends of the bubble are dynamic points, termed

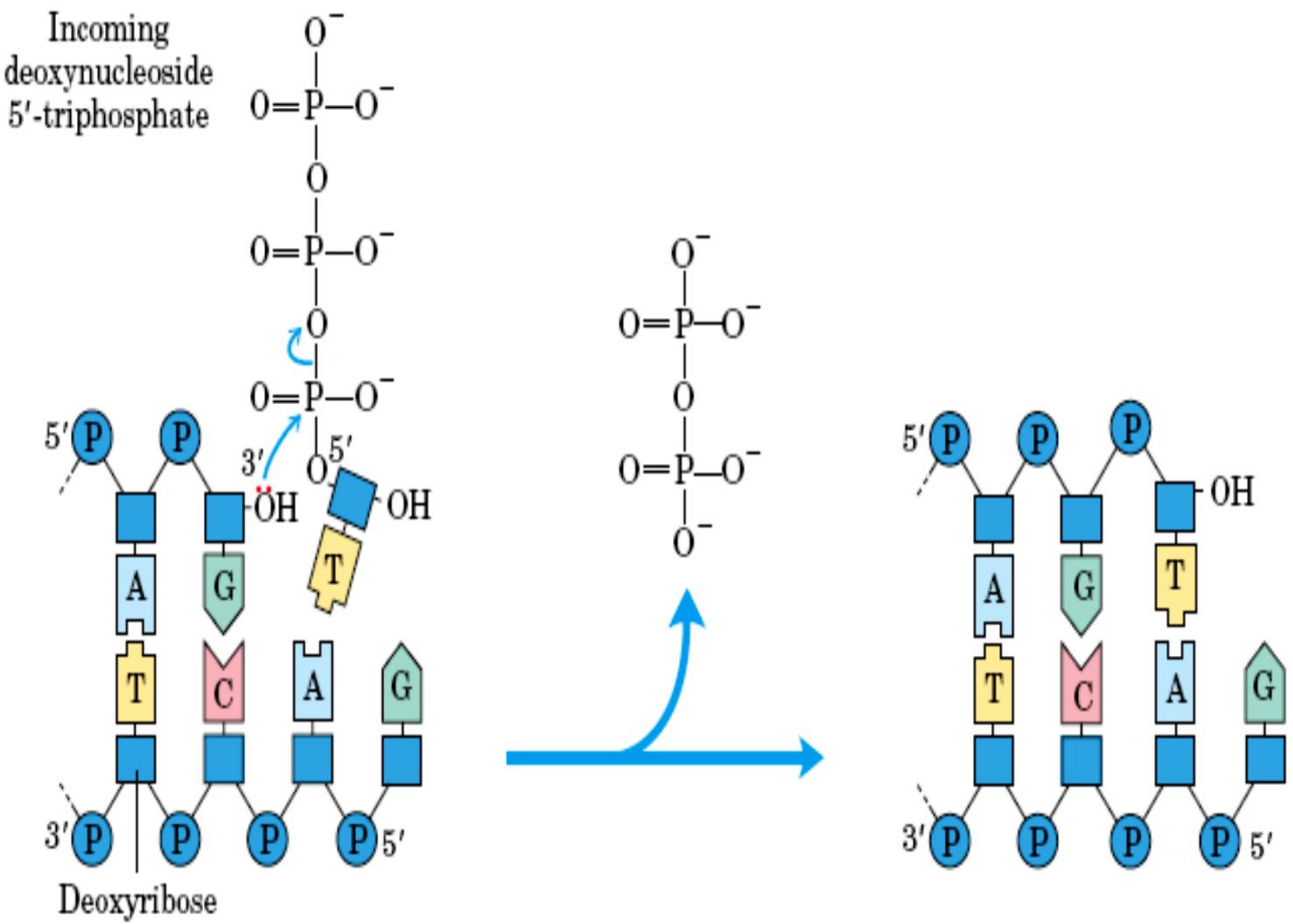
replication forks,

where parent DNA is being unwound and the separated strands quickly replicated

Proteins Involved in Replication:

Topoisomerases
Helicases
DNA polymerases α, β, δ, ε
DNA ligase
Single-strand binding proteins


Replication origin (Oric)
3'
5'
Leading strand
DNA polymerase
Single-strand binding protein
Rep protein (Helicase)
DNA polymerase
RNA primer
DNA ligase
Lagging strand
5'
3'
dnaB-dnaC complex
Primase
Okazaki fragment
3'
5'


Incoming
deoxynucleoside
5′-triphosphate
5′
P
P
3′
OH
5′
OH
A
G
T
C
A
G
T
3′
P
P
P
P
5′
Deoxyribose
P
P
P
OH
5′
3′

A new strand of DNA is always synthesized in the 5' →3' direction, with the free 3' - OH as the point at which the DNA is elongated.

Because the two DNA strands are antiparallel, the strand serving as the template is read from its 3' end toward its 5' end.

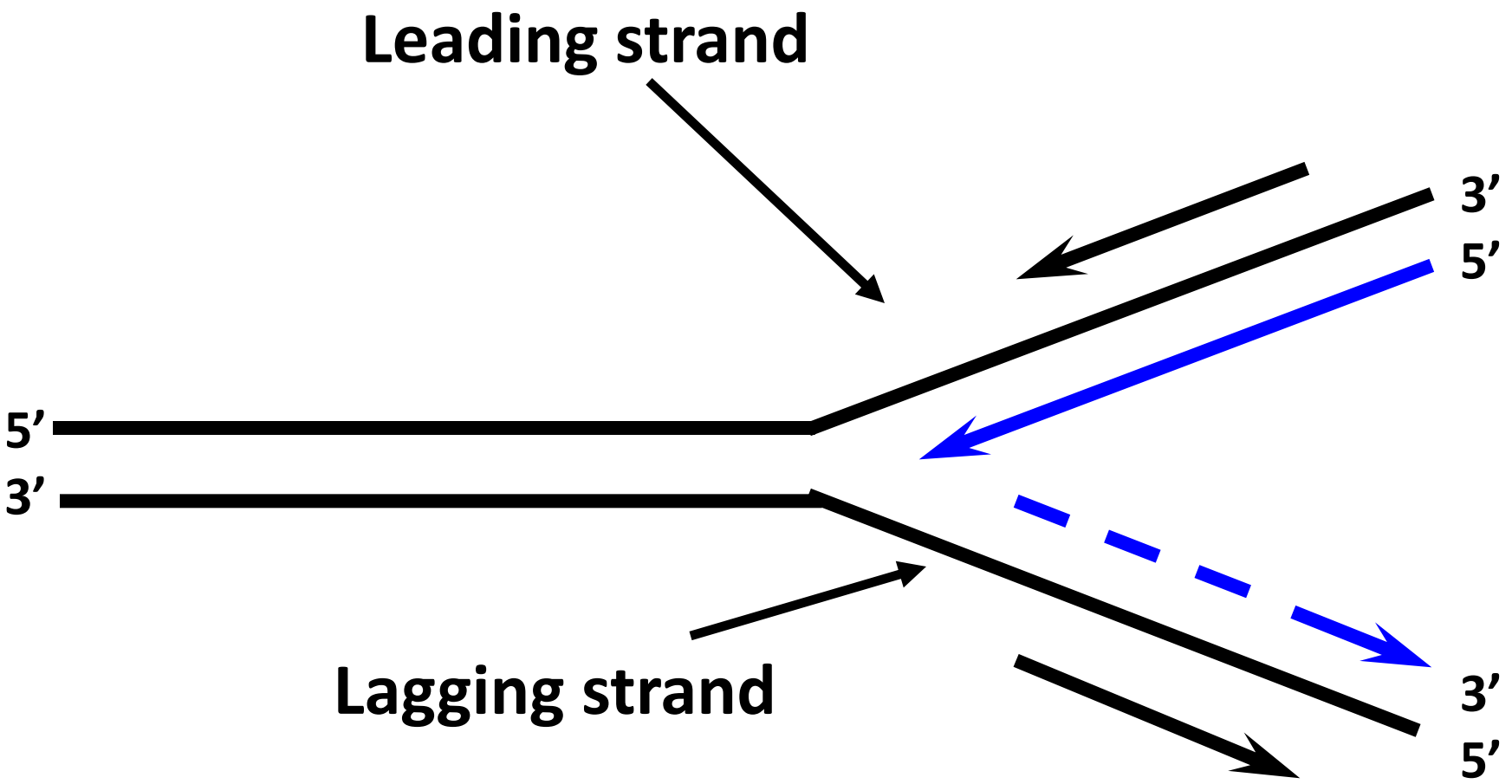
One strand is synthesized continuously and the other discontinuously.

Okazaki found that one of the new DNA strands is synthesized in short pieces, now called

Okazaki fragments.

The continuous strand, or leading strand, is the one in which 5' → 3' synthesis proceeds in the *same* direction as replication fork movement.

The discontinuous strand, or lagging strand, is the one in which 5' → 3' synthesis proceeds in the direction *opposite* to the direction of fork movement.


Leading strand
3’
5’
5’
3’
Lagging strand
3’
5’