

Metabolism of purine and pyrimidine nucleotides DNA replication

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(E.T.)

Biosynthesis of purine and pyrimidine nucleotides

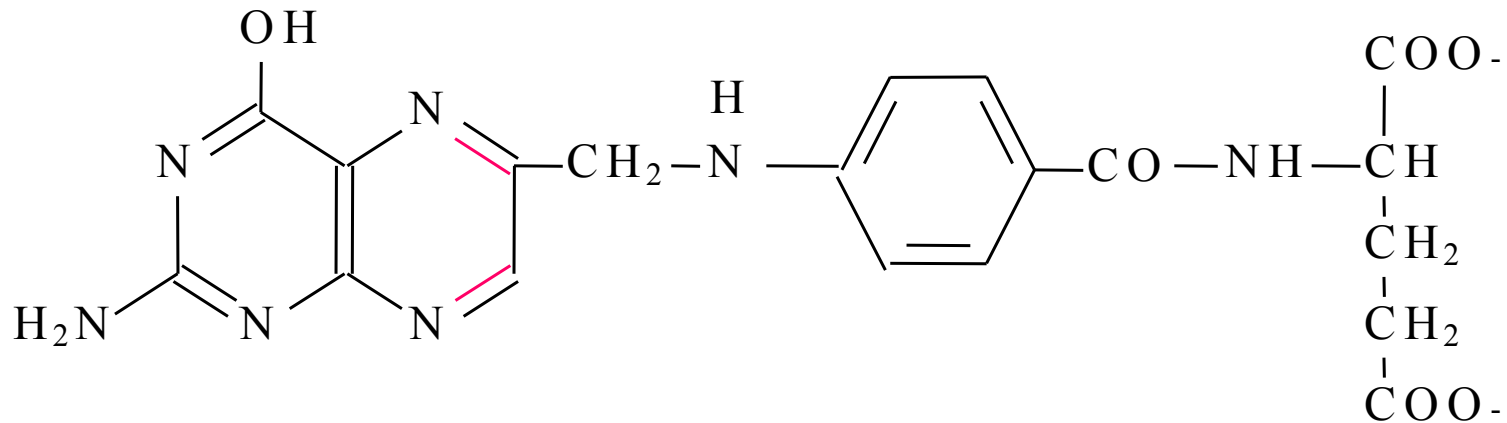
- All cells need ribonucleosides, deoxyribonucleosides and their phosphates
- Dietary purine and pyrimidine bases (nucleoproteins) are poorly absorbed and cannot be used for synthesis
- Humans depend on the endogenous synthesis of purines and pyrimidines

Significance of folic acid for synthesis of bases

For human is essential:

Sources: green food, liver,
food yeast, egg yelow

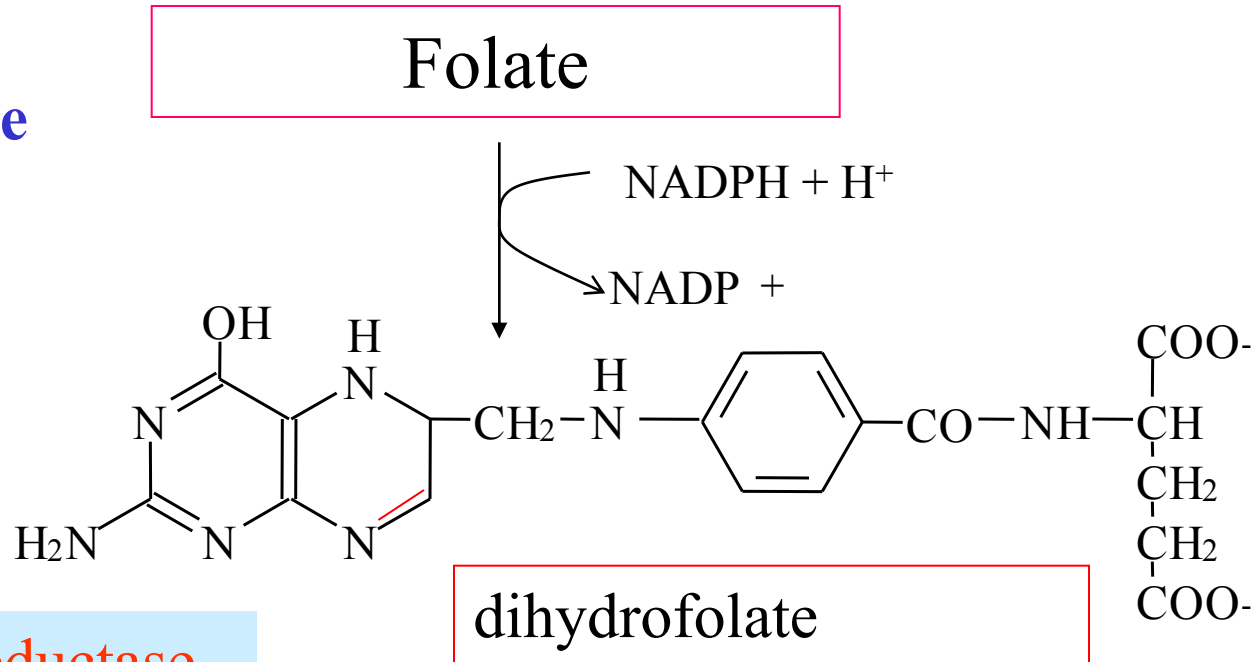
Folate



The effective form in organism of human is tetrahydrofolate.

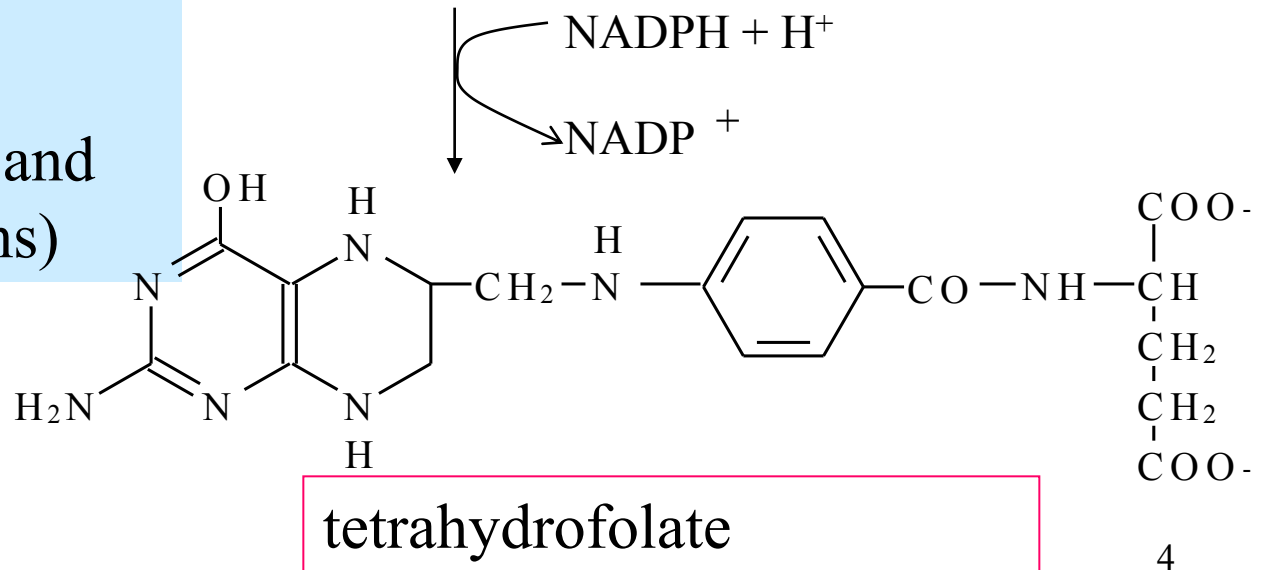
Some bacteria can synthesize folate. It is growth factor for them

Formation of tetrahydrofolate



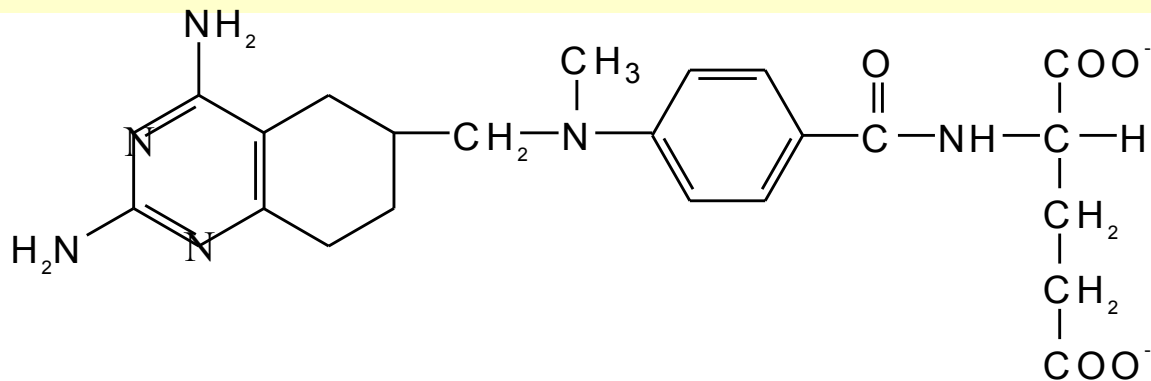
(dihydro)folate reductase

(catalyzes the both reactions in animals and some microorganisms)



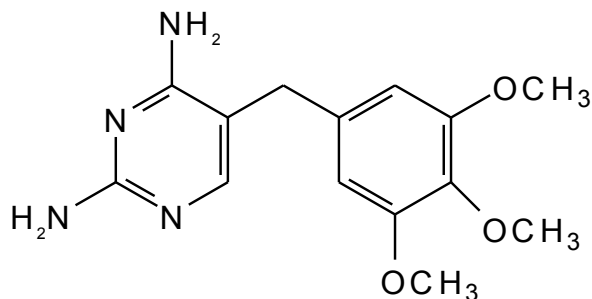
Inhibitors of (dihydro)folate reductase:

Methotrexate (anticancer drug)



Trimethoprim (bacteriostatics)

it inhibits bacterial dihydrofolate reductase



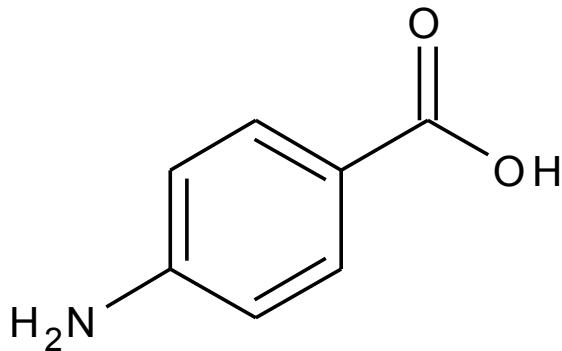
Inhibitors of folate synthesis

- Sulfonamides (e.g. Sulfamethoxazol) are structural analogs of p-aminobenzoic acid.
- p-aminobenzoic acid is necessary for bacterial synthesis of folic acid
- Folic acid is a growth factor for bacterias.

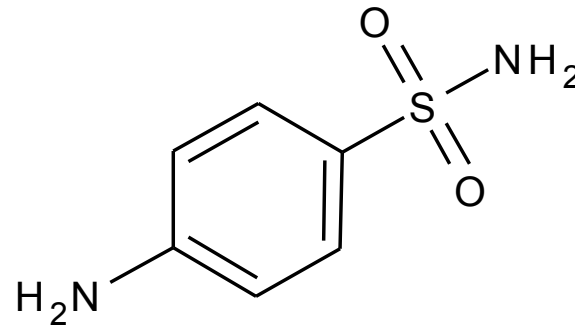


Sulfonamides act as competitive inhibitors of the synthesis.

Sulfonamides stop growth of bacterias dependent on folic acid (streptococcus, haemophilus etc.)



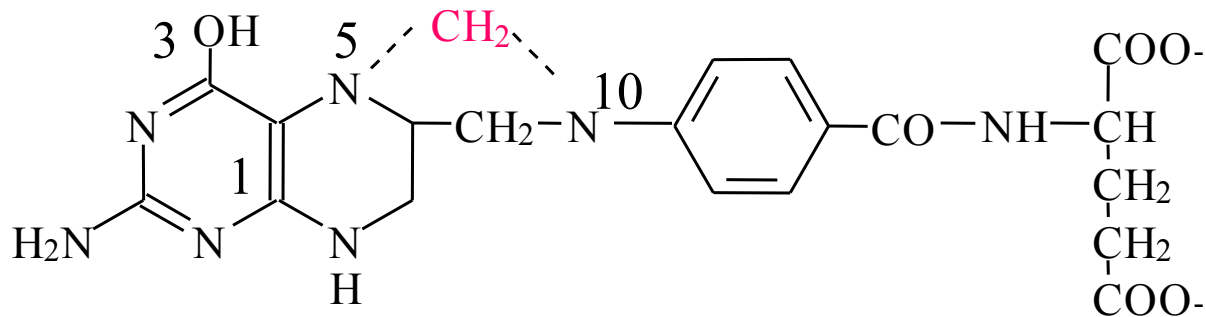
p-aminobenzoic acid (PABA)



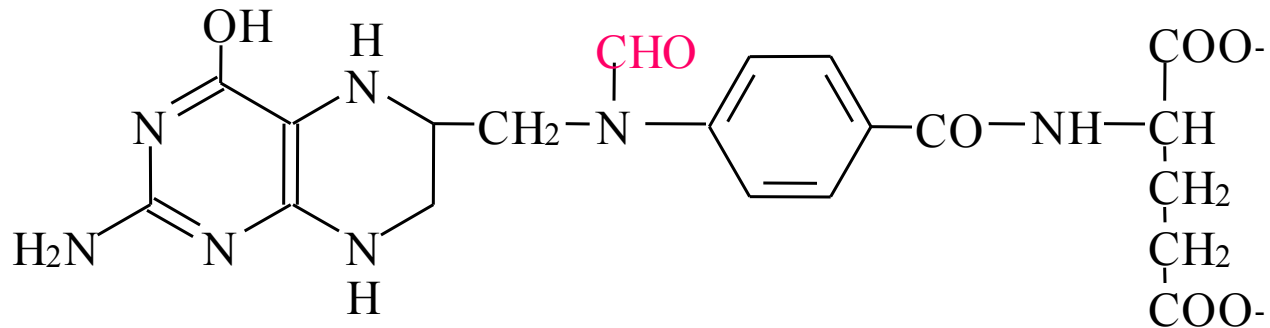
sulfanilamide

Using of tetrahydrofolate in synthesis of purines and pyrimidines

N-5,N-10- methylen H₄F – synthesis of thymine

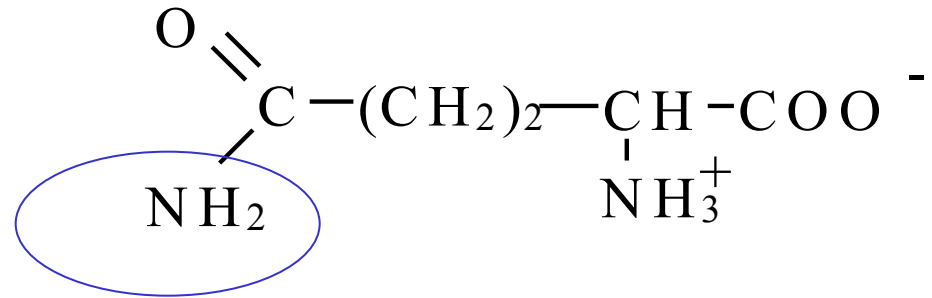


N-10-formyl H₄F – synthesis of purine



Significance of glutamine for biosynthesis of purines and pyrimidines

- It is donor of amino group



Why the cells with high mitotic rate consume high amounts of glutamine?



PRPP – phosphoribosyl diphosphate

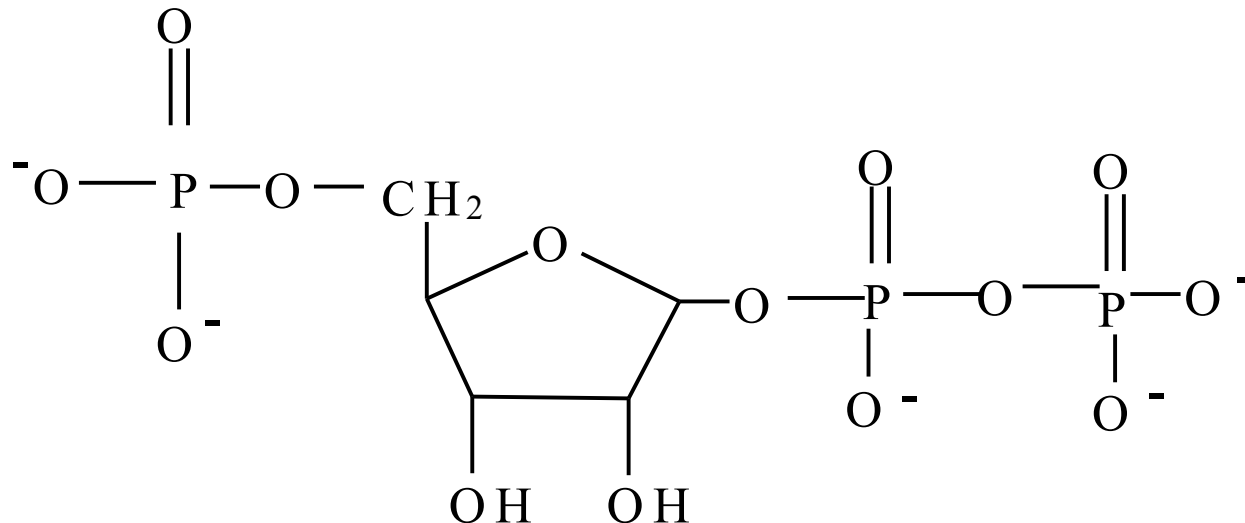
Required for synthesis of:

purine nucleotides

pyrimidine nukleotides

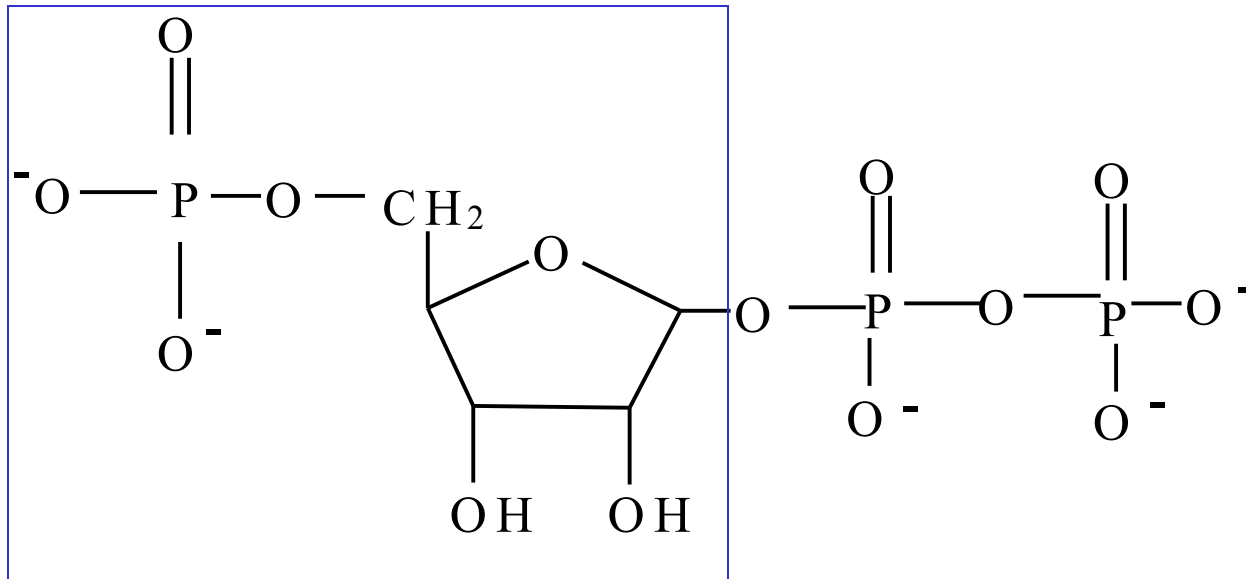
NAD⁺, NADP⁺

Activated pentose



Synthesis of phosphoribosyl diphosphate (PRPP)

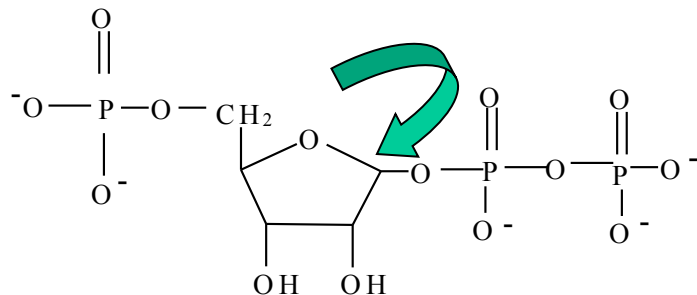
PRPP-synthase (kinase)



Differences in purine and pyrimidine synthesis

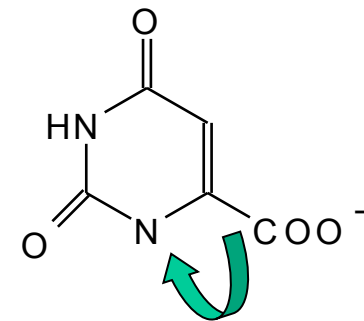
Purines

Synthesis starts with PRPP, purine ring is built step-by-step with C-1 of PRPP as a primer



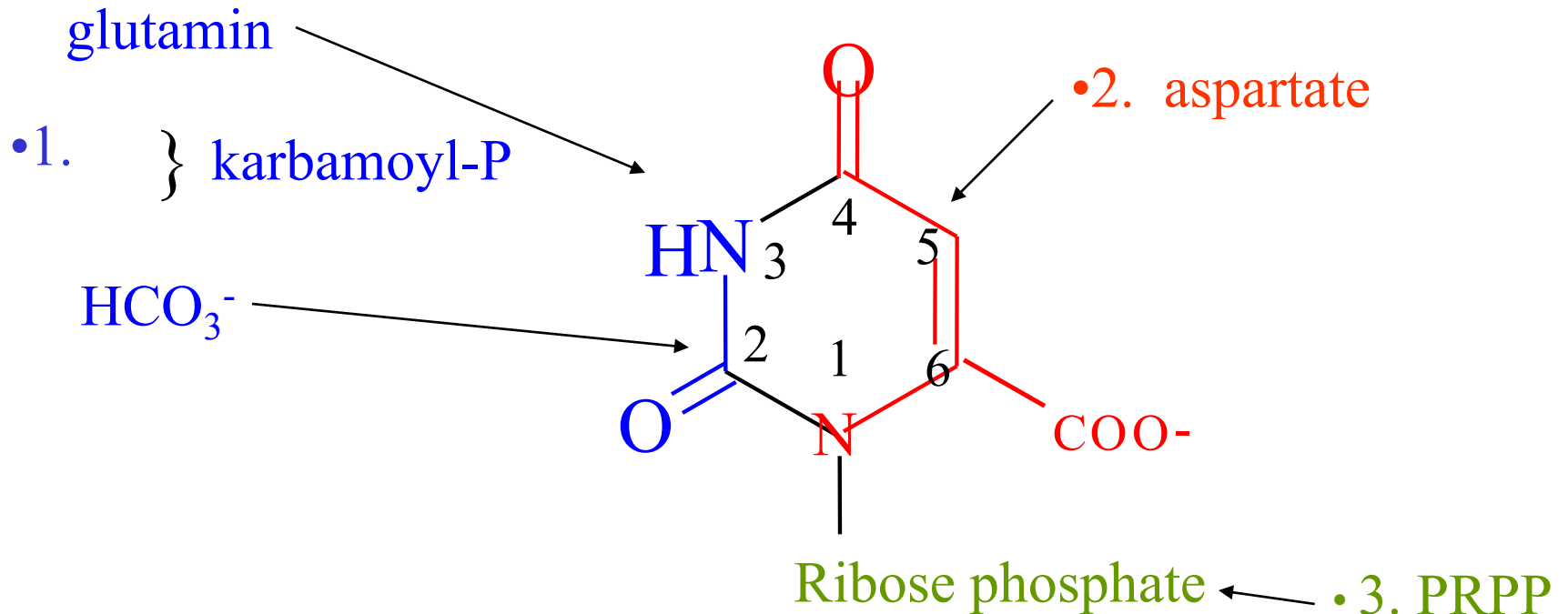
Pyrimidines

The pyrimidine ring is synthesized before ribose is added



Biosynthesis of pyrimidines

Origin of atoms in pyrimidine ring



Orotidin monophosphate is the first intermediate

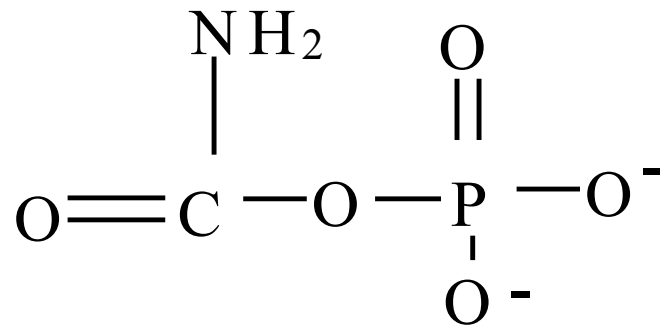
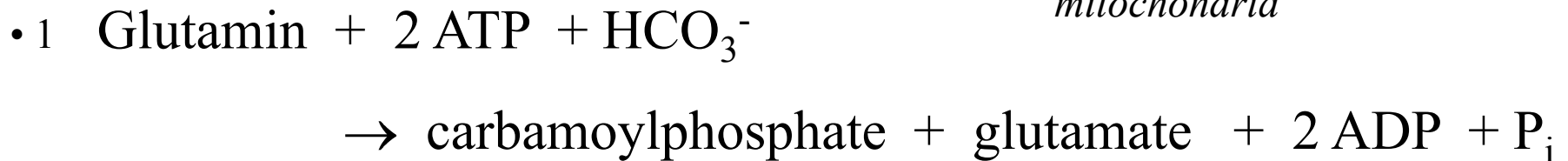
By decarboxylation is formed uridin monophosphate

• Synthesis of carbamoyl phosphate

CYTOPLASMA

Carbamoyl phosphate synthase II

Compare with the reaction in the synthesis of urea - mitochondria

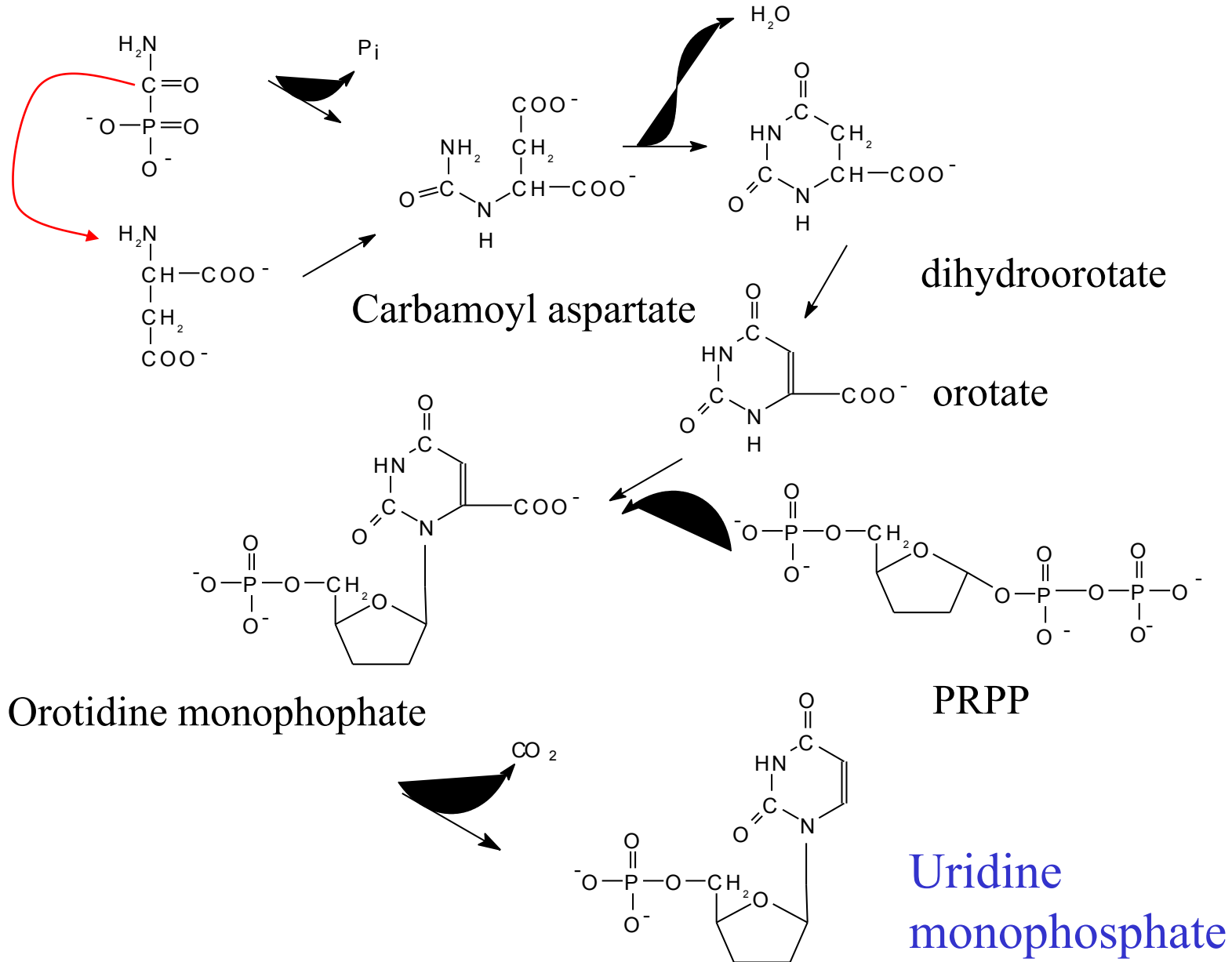


Reaction is the most regulated step

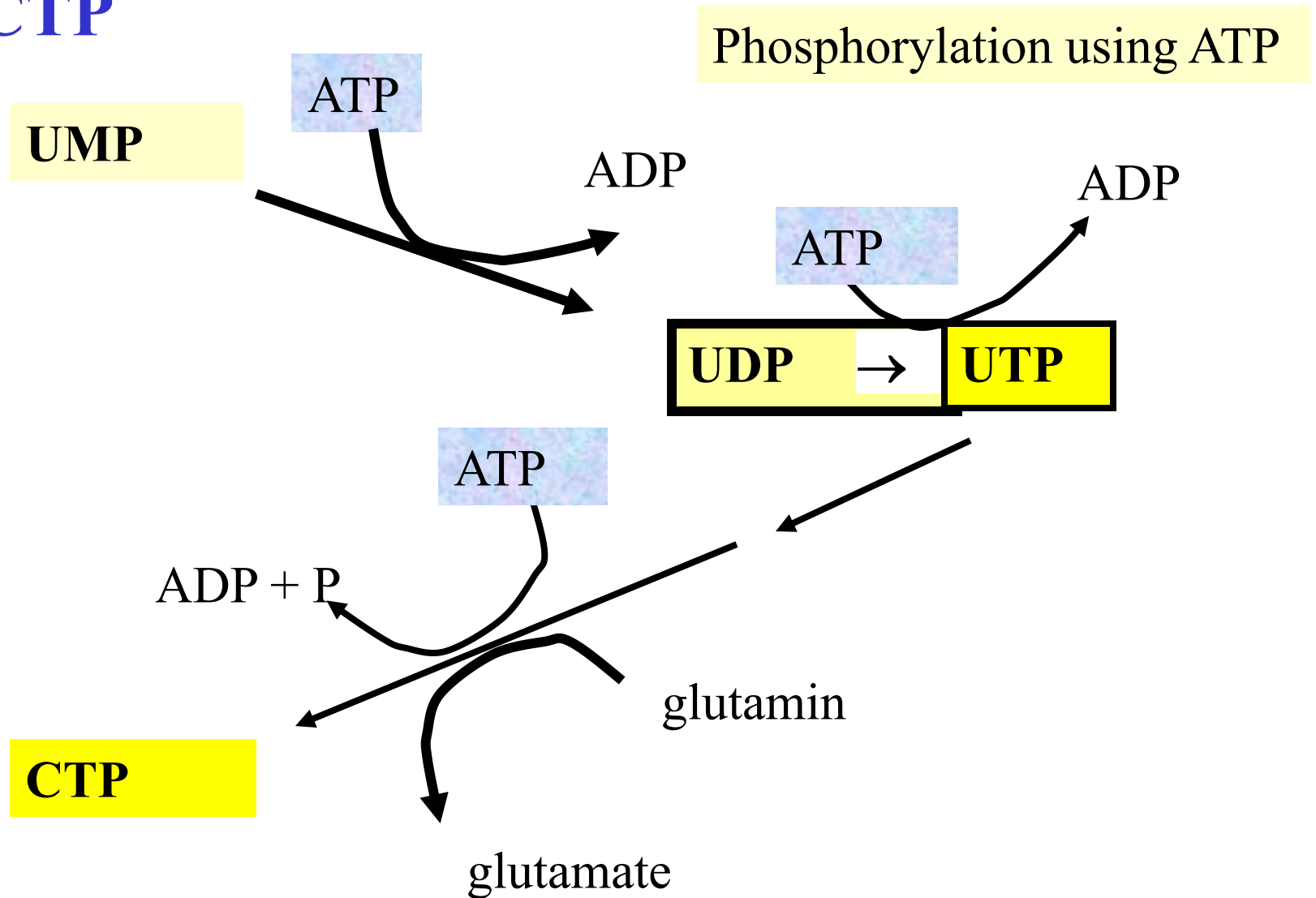
Comparison of carbamoyl phosphate synthetases

| Enzyme typ | Carbamoyl phosphate synthetase I | Carbamoyl phosphate synthetase II |
|--------------------------|----------------------------------|------------------------------------|
| Localization in the cell | mitochondria | cytoplasm |
| Metabolic pathway | synthesis of urea | synthesis of pyrimidine |
| Source of nitrogen | ammonia | glutamin |
| Regulation | activation: N-acetylglutamate | inhibition: UTP activation: ATP |

Steps in pyrimidine biosynthesis - *in detail*

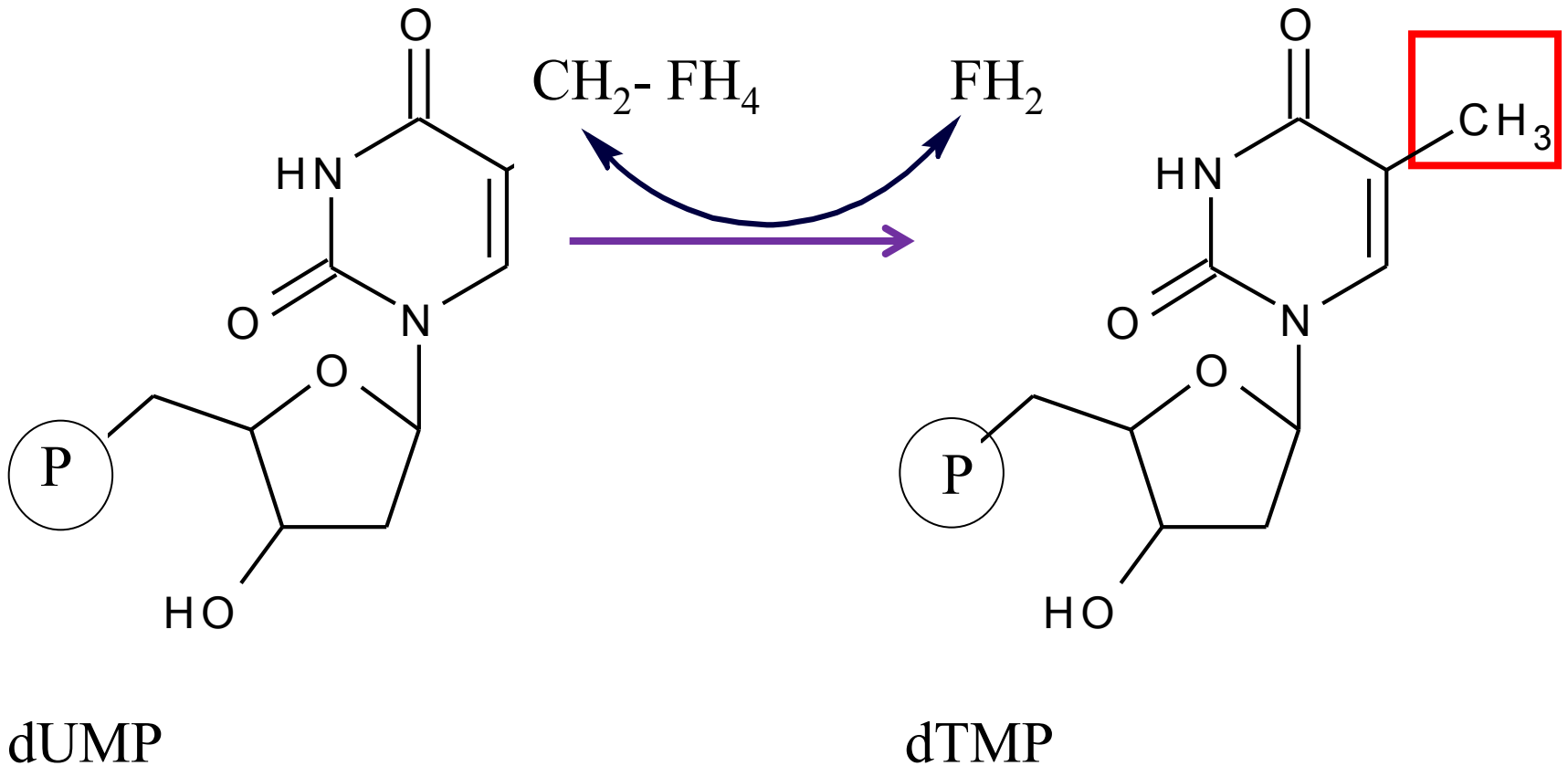


Biosynthesis of UTP and CTP



Formation of dTMP (methylation)

H₄F is required for methylation



Formation of dTMP

serine

H_4F

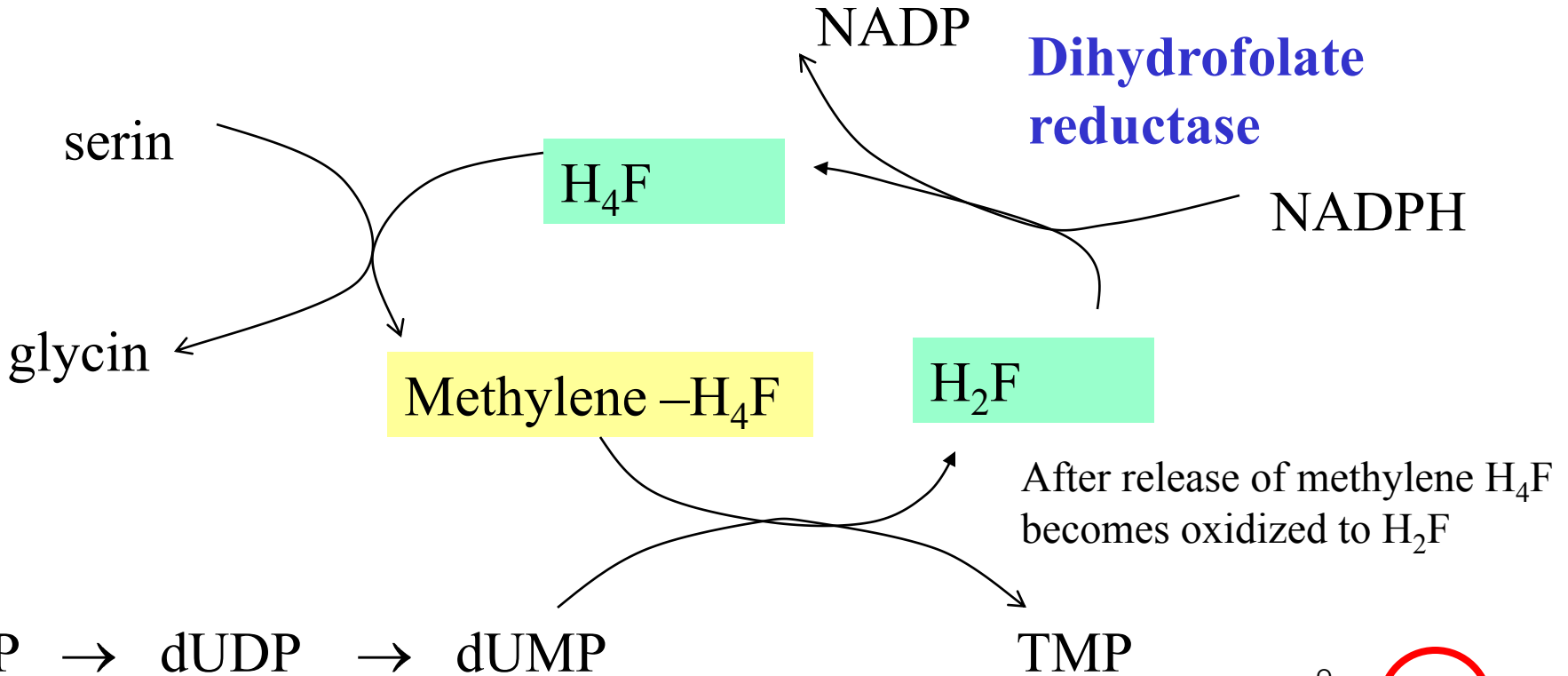
glycin

Methylene $-H_4F$

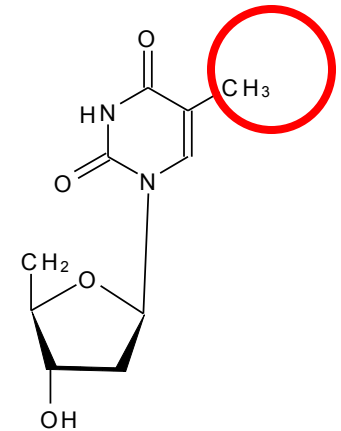
Methylene group bonded to H_4F is during the transfer to dUMP reduced to methyl group

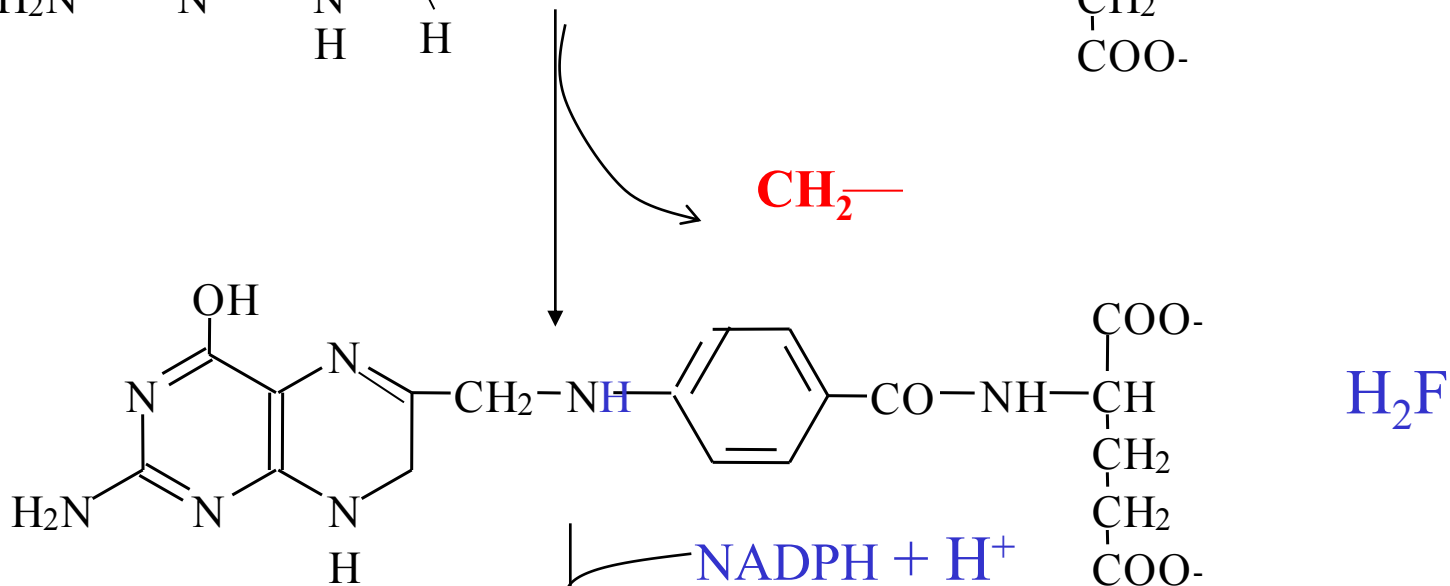
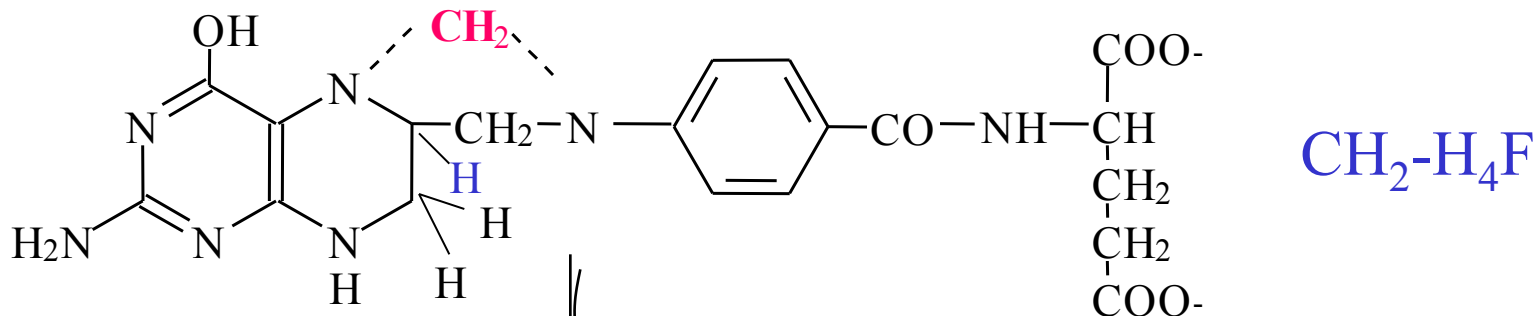
UDP \rightarrow dUDP \rightarrow dUMP

Formation of TMP

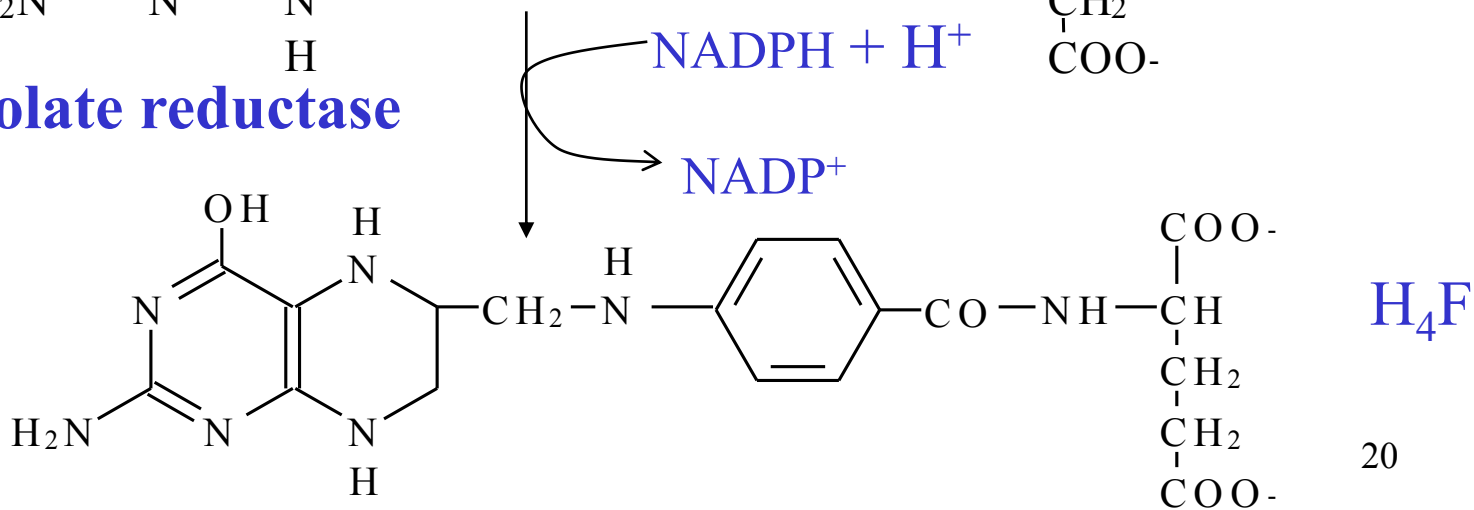


Thymidylate synthase
(folate dependent enzyme)





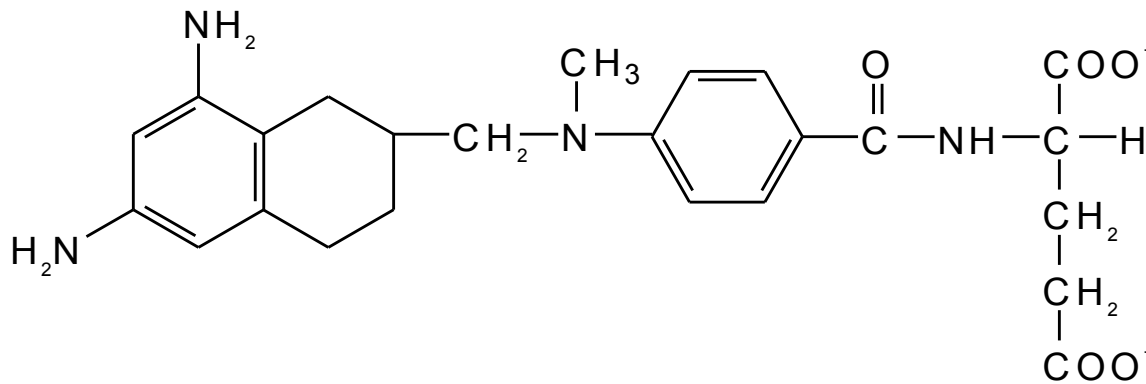
Dihydrofolate reductase



(Dihydro)folate reductase

reduces dihydrofolate (H_2F) back to tetrahydrofolate (H_4F)

Why metotrexate (amethopterin) functions as antineoplastic agent?



Many antineoplastic drugs inhibit nucleotide metabolism

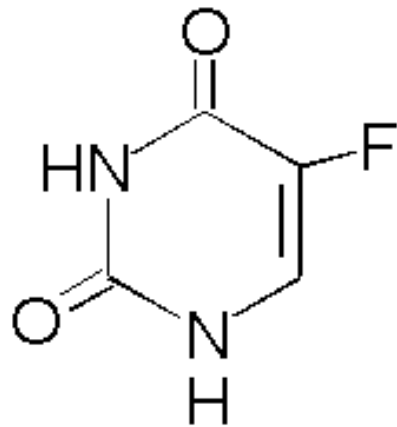
- The development of drugs with selective toxicity for cancer cells is difficult because cancer cells are too similar to normal cells
- Therefore, agents that are toxic for cancer cells are toxic also for normal cells
- Cancer cells do, however, have a higher mitotic rate than normal cells
- Therefore they have a higher requirement for DNA synthesis
- Most antineoplastic drugs act as antagonists of nucleotide synthesis

Dihydrofolate reductase - target of anti-tumour therapy.

Aminopterin (4-amino-dihydrofolate) and **methotrexate** (amethopterin, 4-amino-10-methyl-dihydrofolate) are anti-folate drugs - potent competitive inhibitors of dihydrofolate reductase.

They bind the enzyme 1000x more tightly than folate, they function as competitive inhibitors.

Also thymidylate synthase can be inhibited



5-fluorouracil

Fluorouracil is converted *in vivo* into fluorodeoxyuridylate



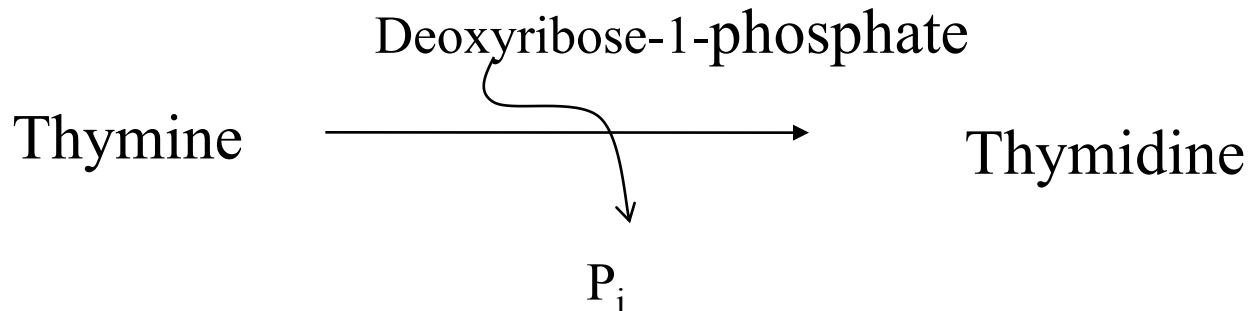
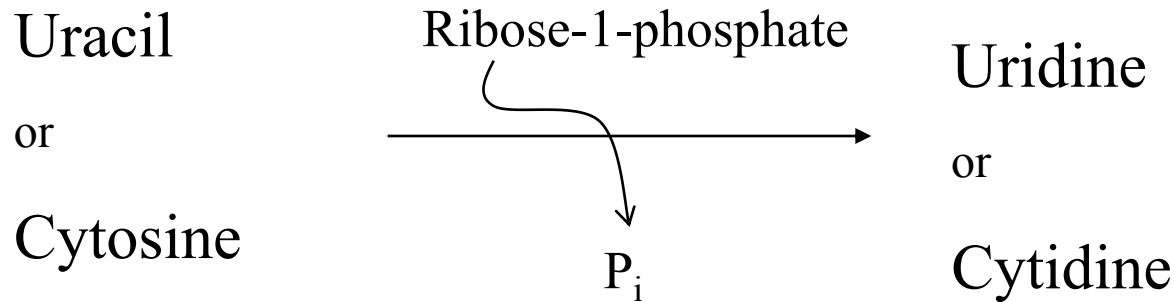
It irreversibly inhibits thymidylate synthase (suicide inhibition)

Cytostatic effect – cell division is stopped

All antineoplastic drugs are toxic not only for cancer cells but for all rapidly dividing cells, including those in bone marrow, intestinal mucosa and hair bulbs. Therefore, bone marrow depression, diarrhea, and hair loss are common side effects of cancer chemotherapy.

Formation of pyrimidine nucleotides by *salvage pathway (using of free bases for the synthesis)*

1. Relatively non-specific pyrimidine nucleoside phosphorylase converts the pyrimidine bases to their nucleosides



2. Formation of nucleotides from nucleosides by action of kinases

- $\text{thymidine} + \text{ATP} \rightarrow \text{TMP} + \text{ADP}$
- $\text{cytidine} + \text{ATP} \rightarrow \text{CMP} + \text{ADP}$
- $\text{deoxycytidine} + \text{ATP} \rightarrow \text{dCMP} + \text{ADP}$
- $\text{uridine} + \text{ATP} \rightarrow \text{UMP} + \text{ADP}$

Regulation of pyrimidine nucleotides biosynthesis

❑ **Allosteric inhibition:**

- Carbamoyl phosphate synthetase II (CPS II):
inhibition by UTP , activation by PRPP

Activity of carbamoyl phosphate synthetase is also regulated by the cell cycle.

At S-phase –CPS II becomes more sensitive to PRPP activation and less sensitive to UTP inhibition. At the end of S-phase inhibition by UTP is more pronounced and activation by PRPP is reduced

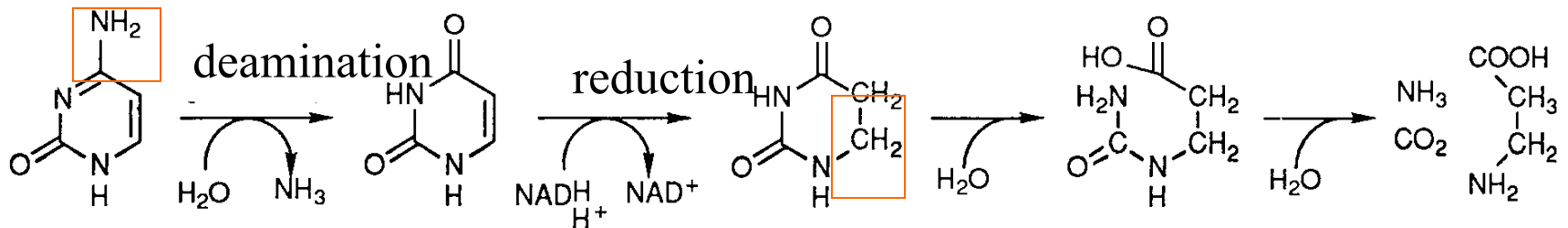
Degradation of pyrimidine nucleotides

Dephosphorylation and cleavage of nucleosides.

Free bases are converted to:

NH_3 , CO_2 , β -alanine, (β -aminoisobutyrate)

Soluble metabolites – excretion in urine

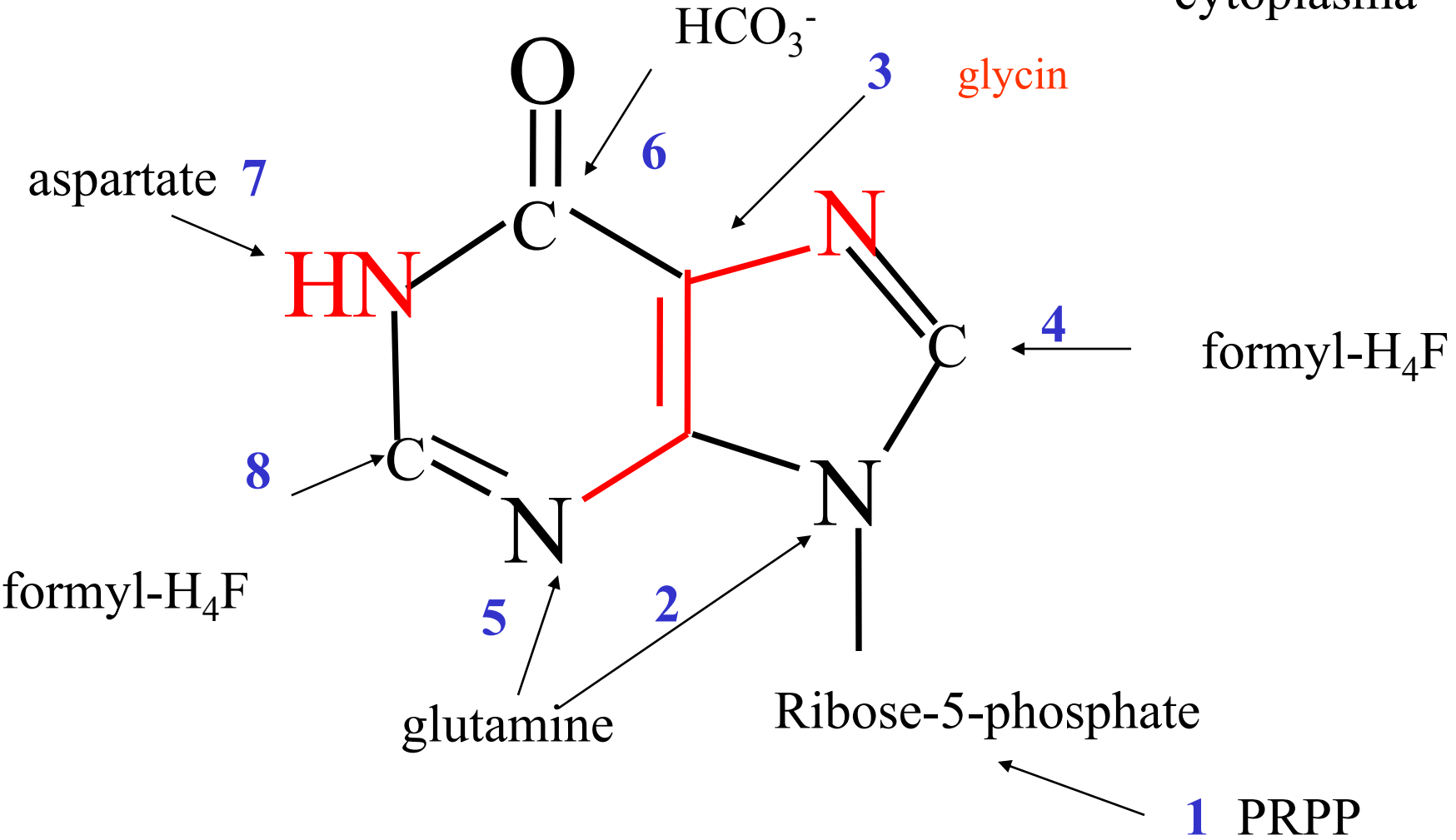


Biosynthesis of purine nucleotides

(multienzyme complex)

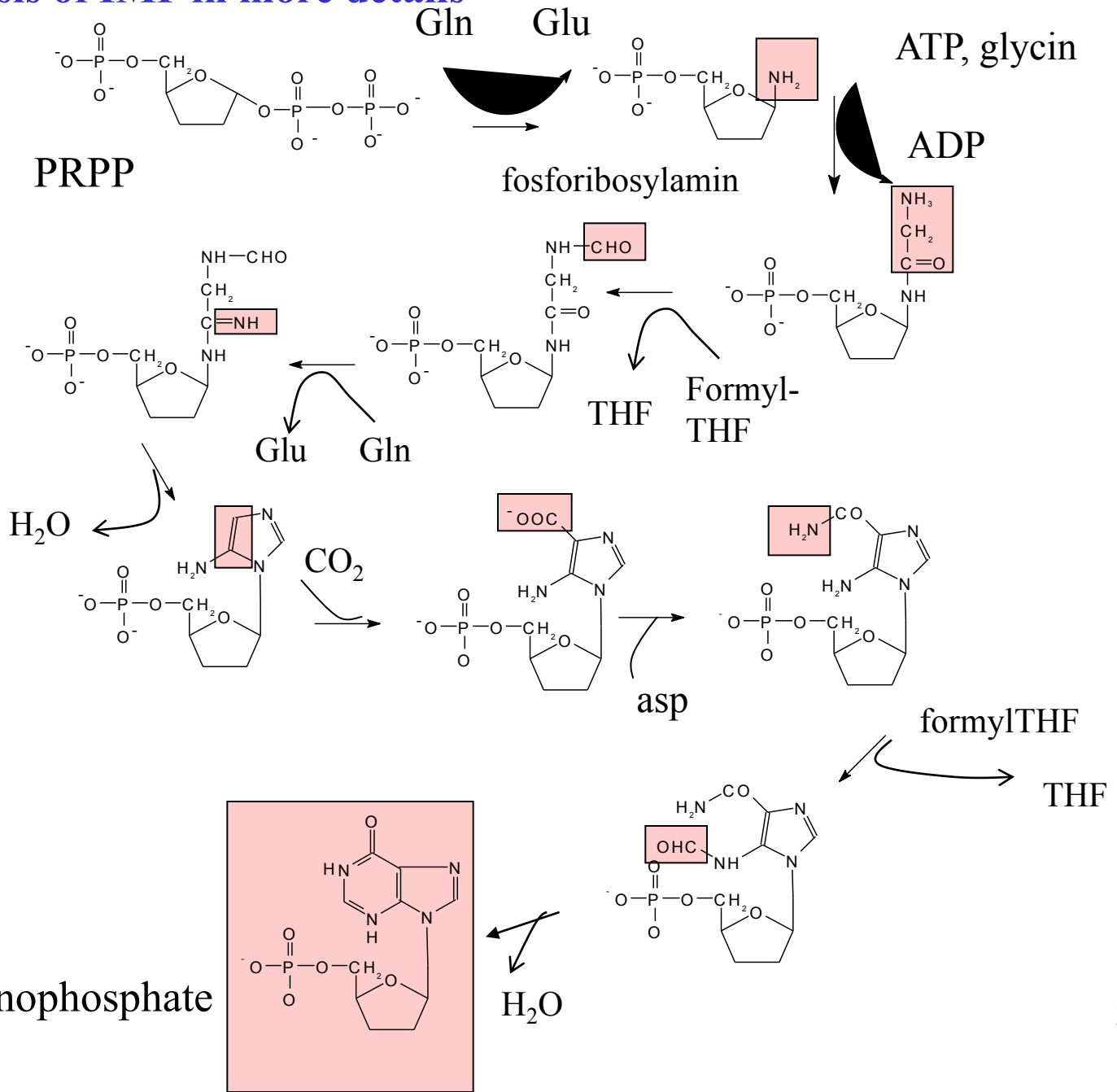
Mainly in liver

cytoplasm



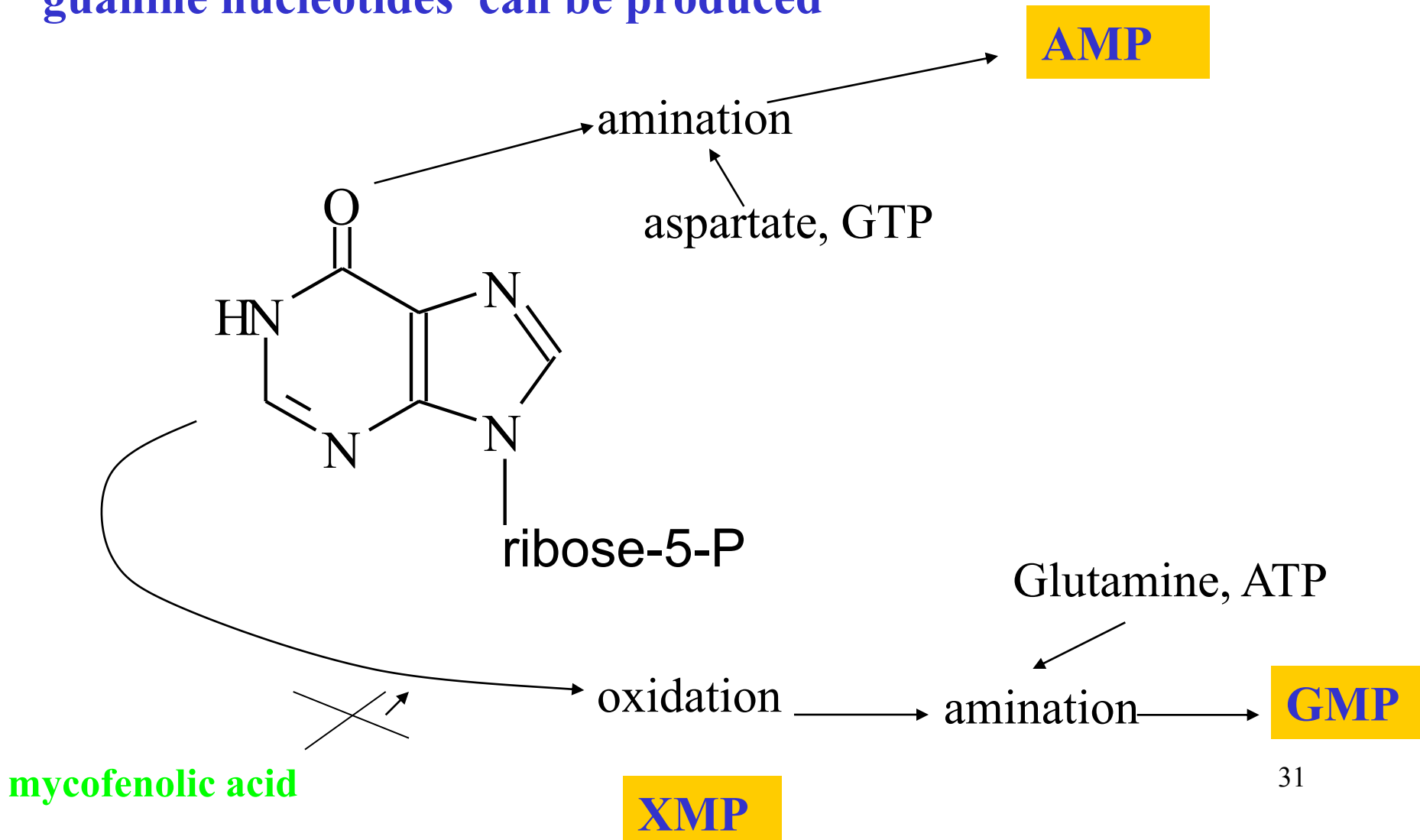
Inosine-5-P (IMP)

Biosynthesis of IMP in more details



Inosine-5-P (IMP)

Serves as the branchpoint from which adenine and guanine nucleotides can be produced

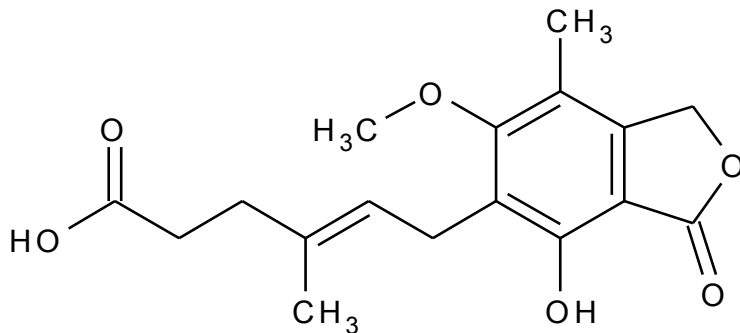


Mycophenolic acid

- Potent, reversible, uncompetitive inhibitor of IMP dehydrogenase

Used in preventing graft rejection

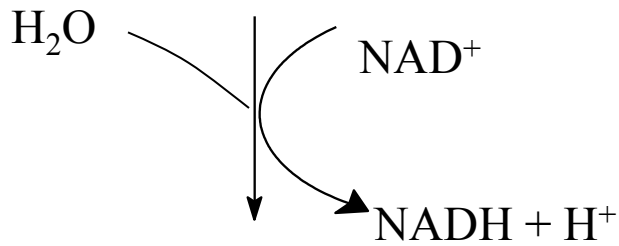
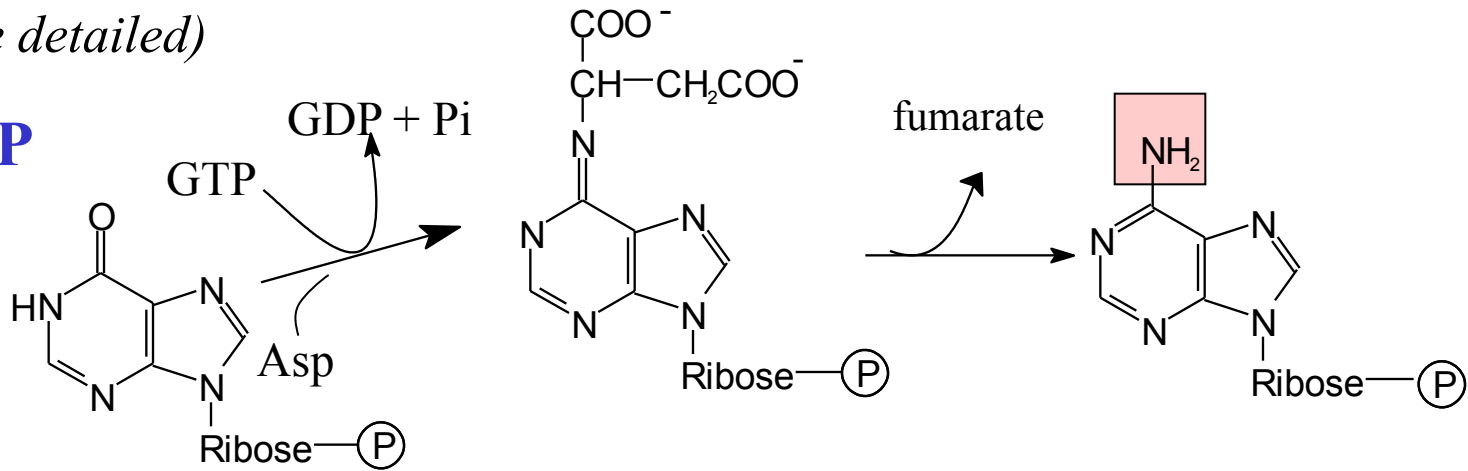
It blocks de novo formation of GMP → suppress the proliferation of T and B cells



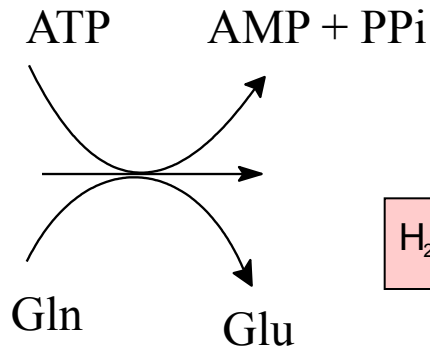
Synthesis of AMP and GMP

(more detailed)

IMP



XMP

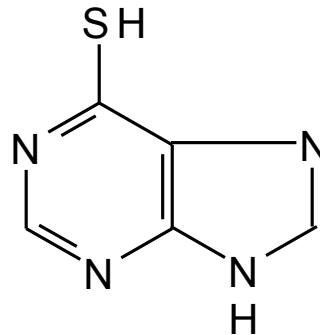


GMP

AMP

Inhibitors of purine synthesis (antineoplastic agents)

- inhibitors of dihydrofolate reductase
- 6-mercaptopurine- inhibition of conversion of IMP to AMP and GMP



mercaptopurine

Synthesis of purine nucleotides by salvage pathway

Extrahepatal tissues

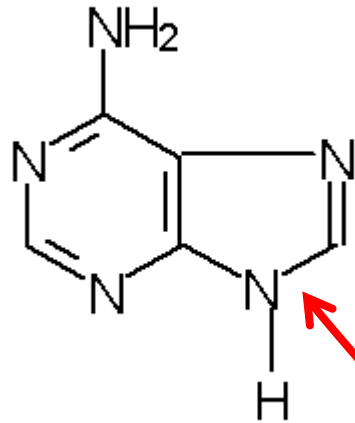
Recyclation of free bases

Phosphoribosyltransferases:
Adenine phosphoribosyltransferase
Hypoxanthine phosphoribosyltransferase

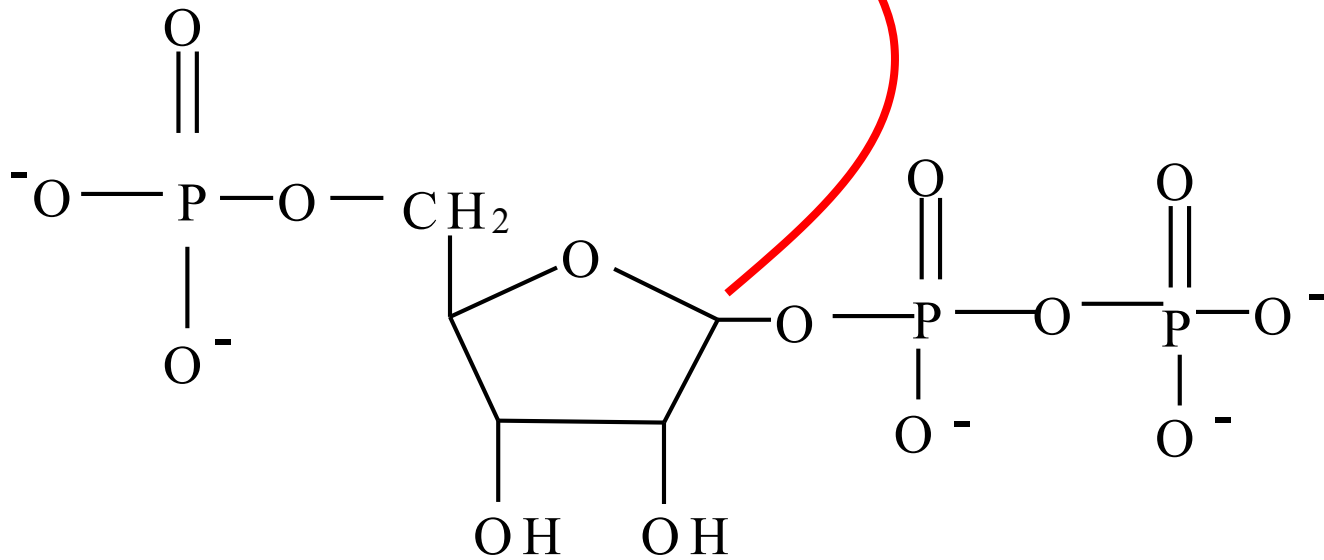


Recyclation of purine bases by phosphoribosyltransferase.

Purine nucleotides are synthesized preferentially by salvage pathway, so long as the free bases are available.



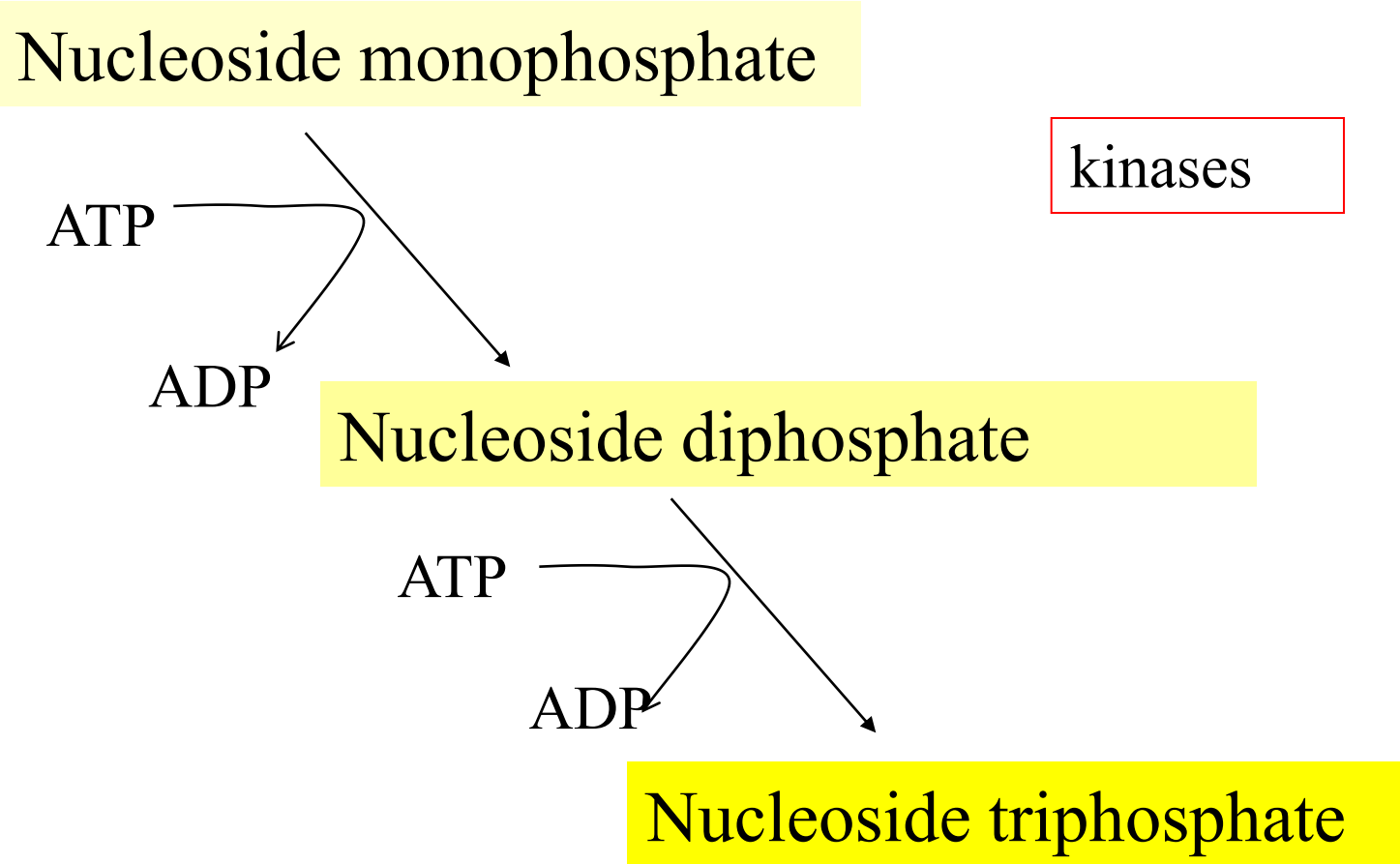
Formation of purine nucleotides by the action of phosphoribosyl transferases



Deficiency of phosphoribosyl transferase results in Lesch-Nyhan syndrom

- X-linked hereditary disease
- purine bases cannot be salvaged
- accumulation of PRPP
- overproduction of purine bases that are degraded to uric acid
- accumulation of uric acid – gout
- neurologic problems : mental retardation, self-mutilation

Synthesis of nucleoside diphosphates and nucleoside triphosphates

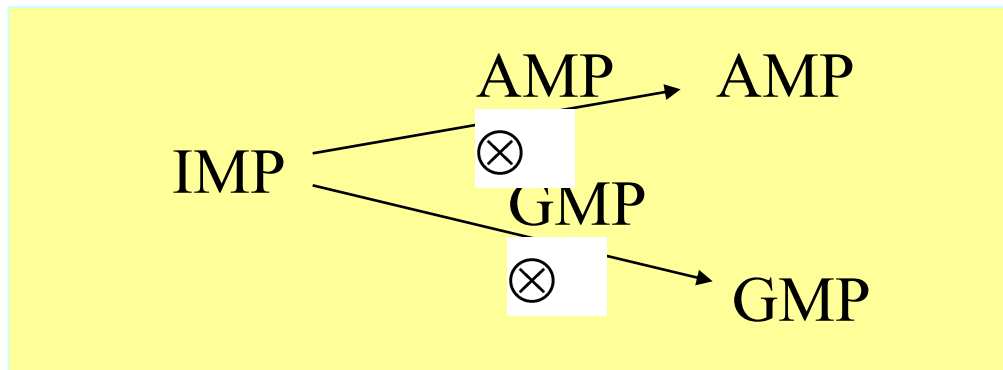


Regulation of purine nucleotide biosynthesis

The main factor is availability of PRPP

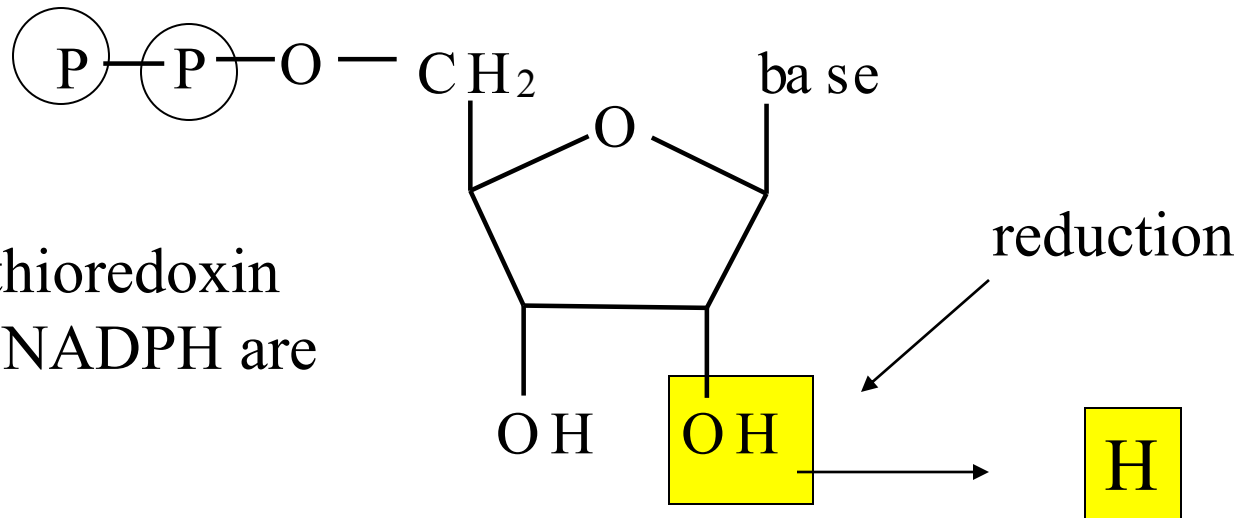
1.
 - inhibition of PRPP-glutamylamidotransferase by AMP, GMP, IMP (end-products), activation by PRPP

2.



Formation of 2-deoxyribonucleotides (purine and pyrimidine)

Nucleoside diphosphate → 2-deoxynucleoside diphosphate



Thioredoxin, thioredoxin reductase and NADPH are required

Thioredoxin reductase is selenoenzyme

deoxygenation

Hydroxyurea

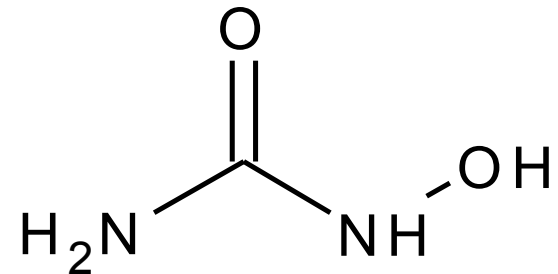
Hydroxyurea inhibits ribonucleotide reductase



Synthesis of deoxyribonucleotides is blocked

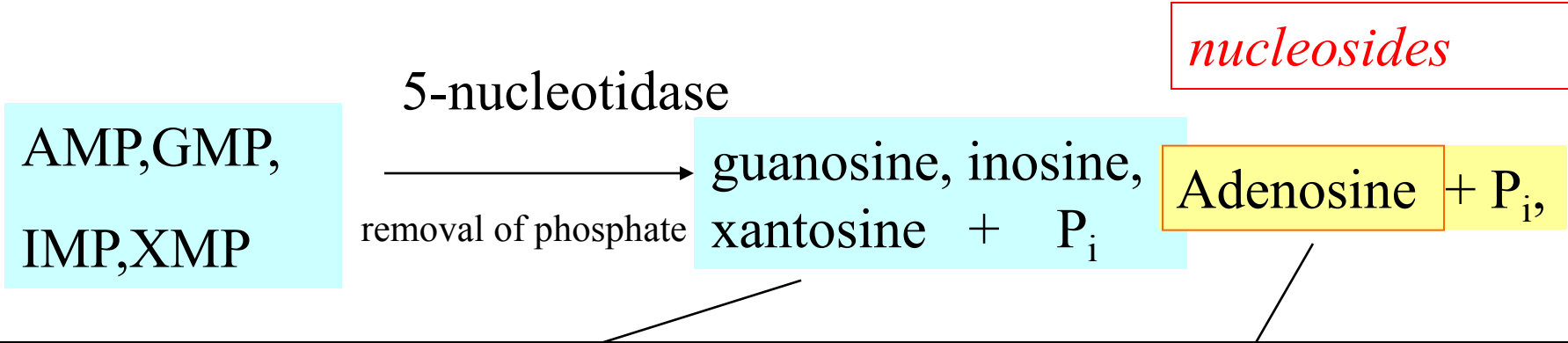


Treatment some of some cancers

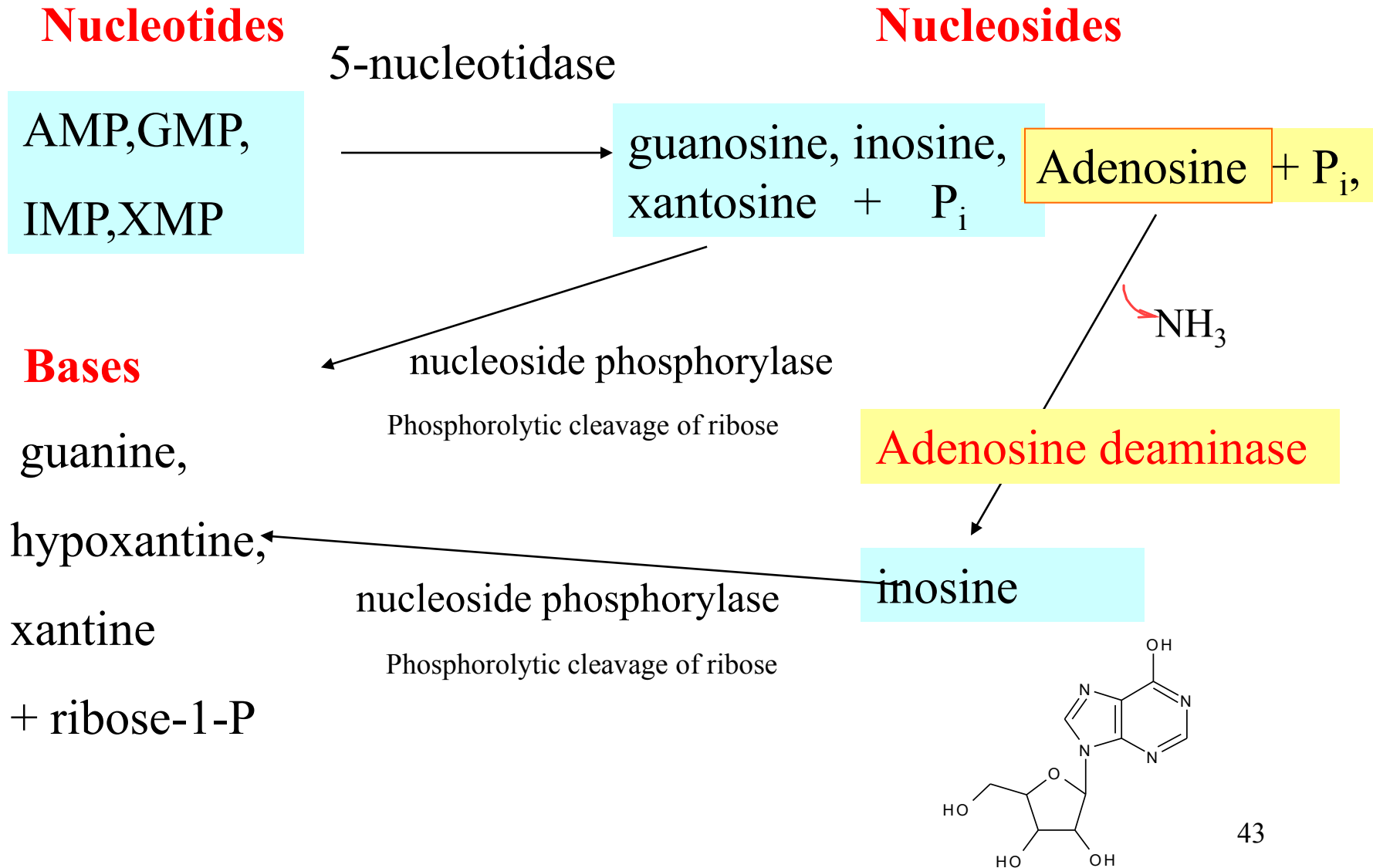


Degradation of purine nucleotides

In liver



Degradation of purine nucleotides



Adenosine deaminase deficiency

Enzyme deficiency → accumulation of adenosine in cells (esp. lymphocytes) → conversion to AMP, dAMP, ADP by cellular kinases.

Inhibition of ribonucleotide reductase

Findings of deoxyadenosine in urine

Synthesis of other deoxynucleotides drops

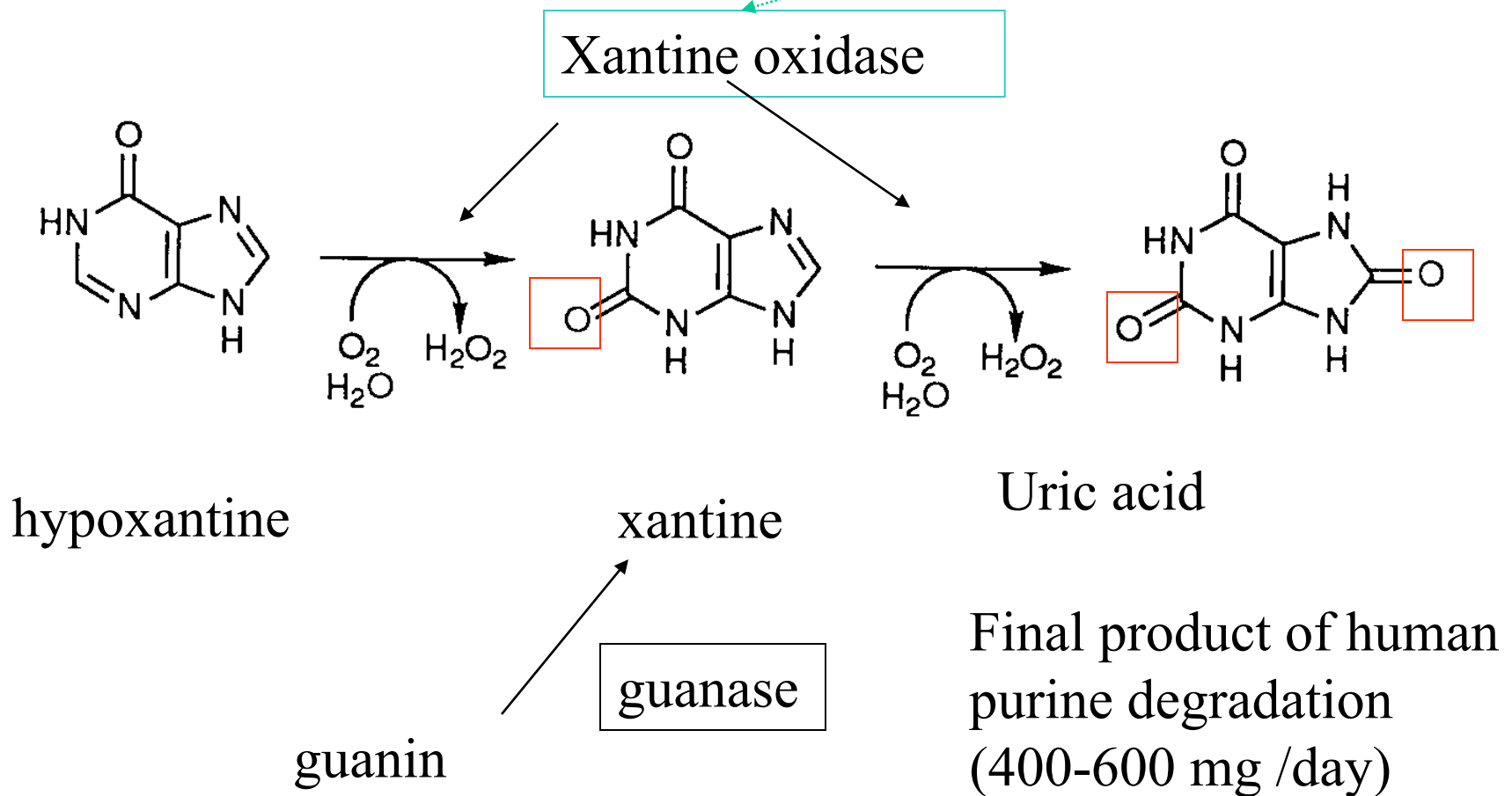
Cells cannot make DNA and divide.

One of the causes severe combined immunodeficiency disease (SCID).

Treatment by gene therapy

Degradation of purine bases

Inhibition by oxypurinol

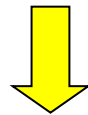


Gout

Gout is a disorder connected with high levels of uric acid in blood - hyperuricemia.

Causes:

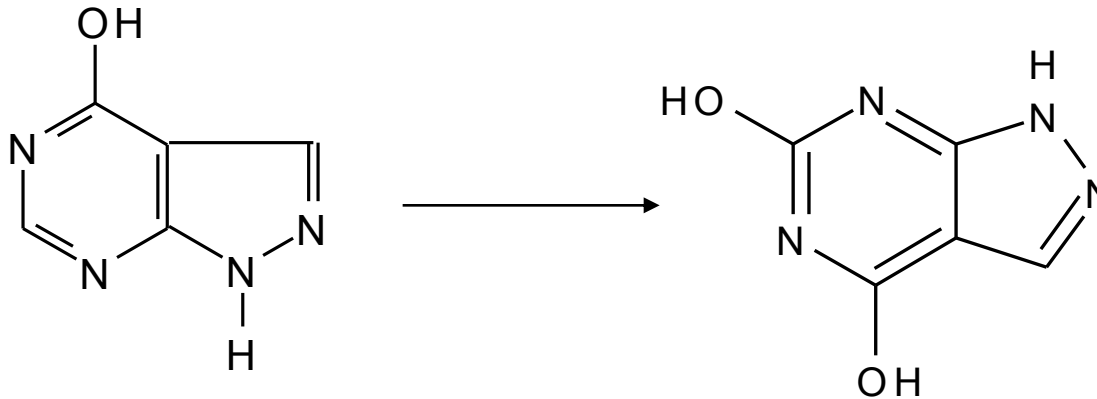
- overproduction of uric acid
- Lesch-Nyhan syndrome
- underexcretion of uric acid in kidneys



Deposition of urate crystals in joints → inflammatory response to the crystals → gouty arthritis.

Formation of uric acid stones is also possible.

Allopurinol – in the body is converted to oxypurinol - competitive inhibitor of xanthinoxidase



Treatment of gout: oxypurinol inhibits xanthine oxidase

More soluble xanthine and hypoxanthine are accumulated.

Hypoxanthine can be „salvaged“ in patients with normal level of hypoxanthine phosphoribosyltransferase.

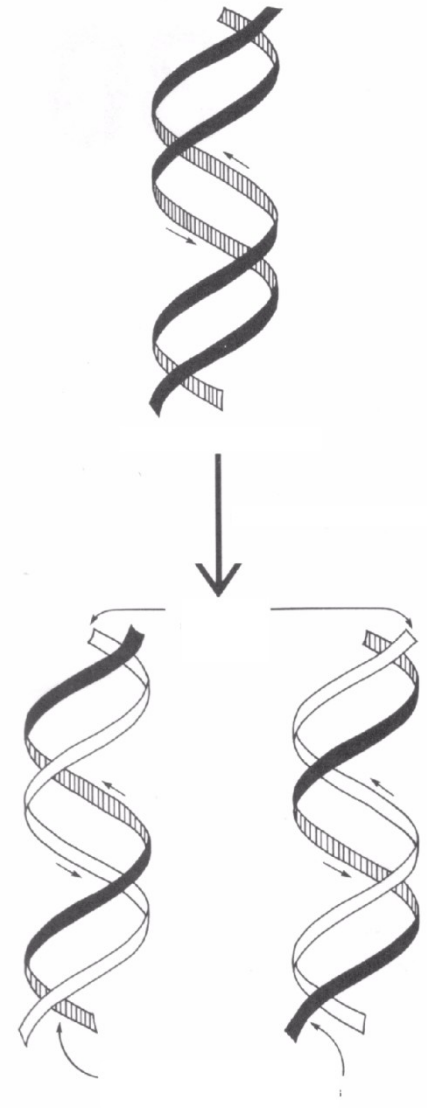
Replication of DNA

Replication of DNA

Each of the two parental strands serves as a template for the synthesis of complementary strand

Bases in the new strand are attached on the principle of complementarity to the bases in the template strand

Location: nucleus



Non protein substrates necessary for replication

| Compound | Function |
|------------------------|---------------------------|
| dATP, dCTP, dGTP, dTTP | High energy substrate |
| Mg ²⁺ | cofactor |
| primer RNA | Initiation of replication |
| Parental strand of DNA | template |

Enzymes and other proteins involved in replication (different for prokaryotes and eukaryotes)

| Enzyme | Function |
|-------------------------------|--|
| Helicase | Unwinding enzyme (ATP is required) |
| RNA polymerase (primase) | RNA primer formation |
| DNA-dependent DNA polymerases | catalyzes joining of nucleotides to 3'-terminal of the growing chain |
| DNA-ligase | Catalyzes joining of DNA fragments |

Enzymes and other proteins involved in replication (different for prokaryotes and eukaryotes) cont.

| Enzym | Function |
|---------------|---|
| SSB-proteins | prevention of reannealing |
| Topoisomerase | Relieve torsional strain on parental duplex caused by unwinding |
| RNA-nuclease | Hydrolyzes RNA from RNA-DNA hybrids |
| Sliding clamp | prevents this DNA polymerase from dissociating from the template DNA strand |
| Telomerase | enables replication at the 3'-ends of linear chromosomes (not present in all cells) |

Chemical reaction of DNA synthesis

Synthetic process is catalyzed by DNA-polymerases

Already formed strand (DNA or RNA) reacts with deoxyribonucleoside triphosphate (dNTP)

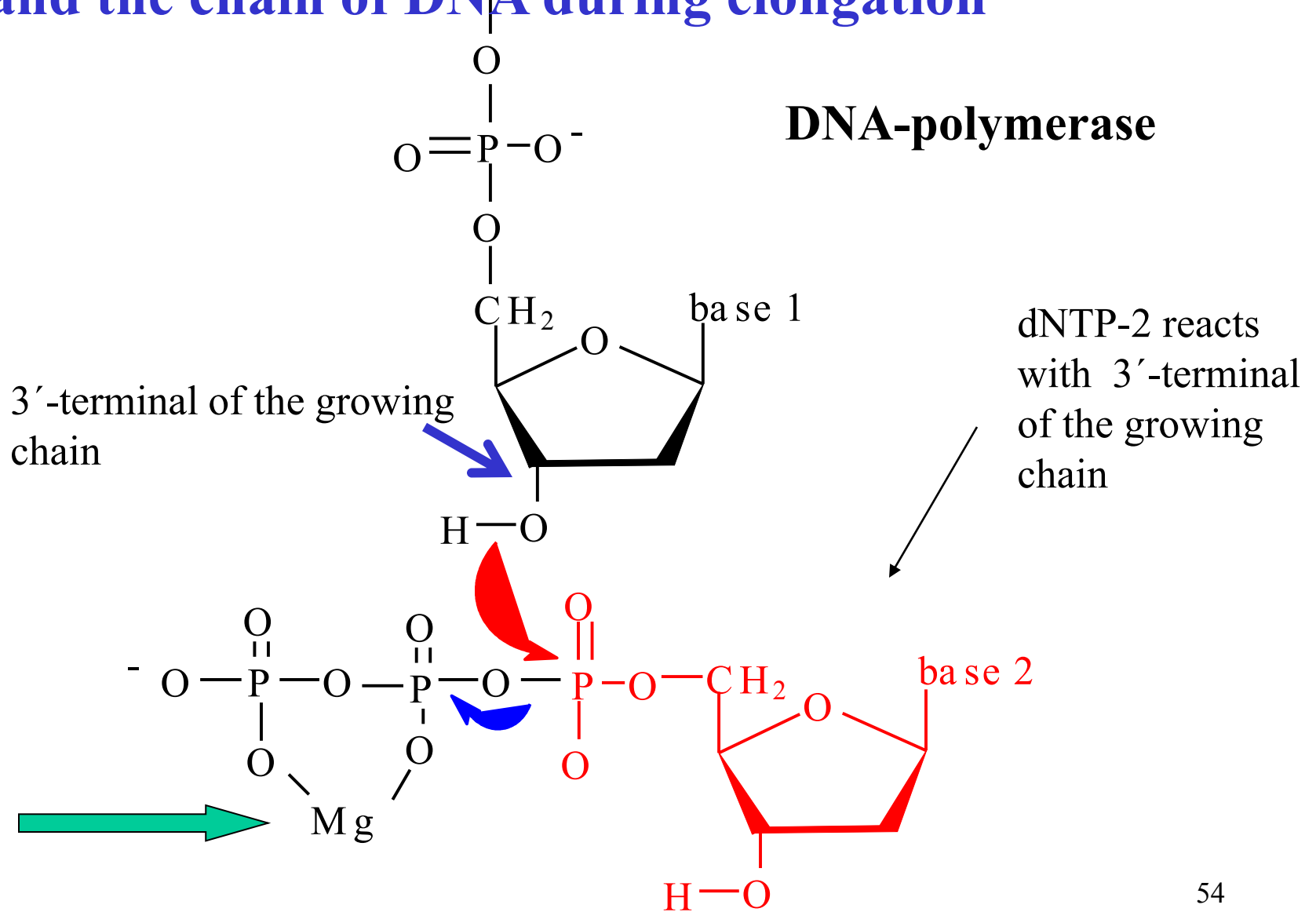
Diphosphate is released and dNMP is attached by ester bond



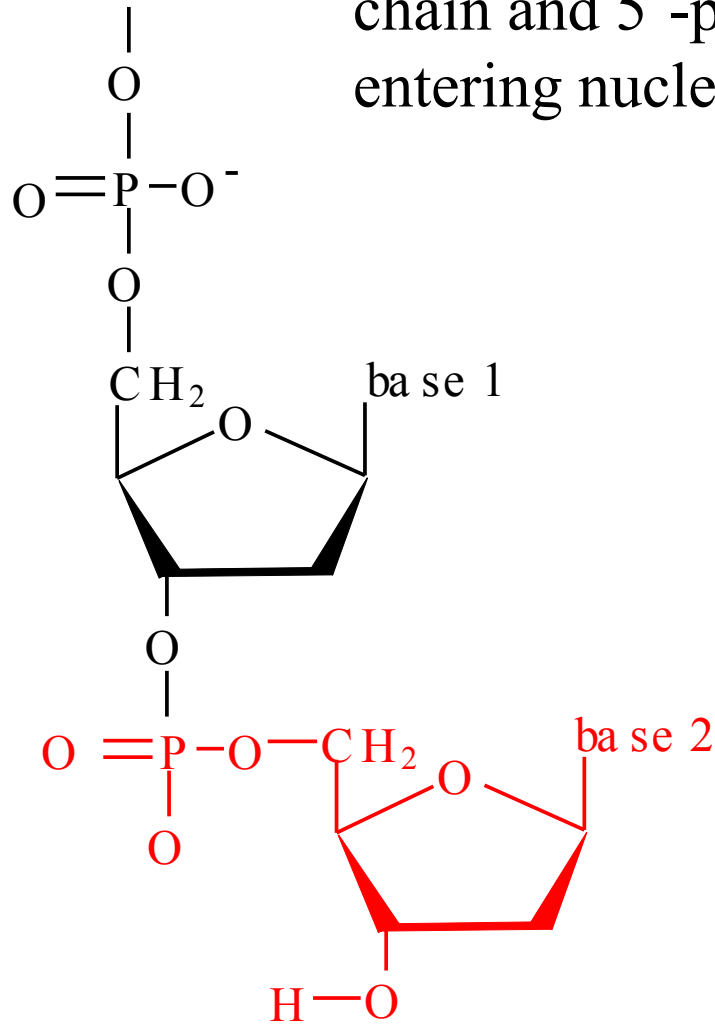
The all DNA polymerases attach nucleotides on 3'-end of a growing chain

(new DNA is formed in the direction 5'→3')

Formation of a bond between new deoxynucleotide and the chain of DNA during elongation



Ester bond is formed between the 3'-OH group of growing chain and 5'-phosphate of entering nucleotide



+ PPi

Diphosphate is released (complexed with Mg²⁺ ions).

Chain elongation

Significance of 3'OH group

Some anticancer and antiviral drugs are nucleotides missing the 3' OH.

Such "dideoxy" nucleotides shut down replication after being incorporated into the strand.

Fast-replicating DNA in cancer cells or viruses is inactivated by these drugs.

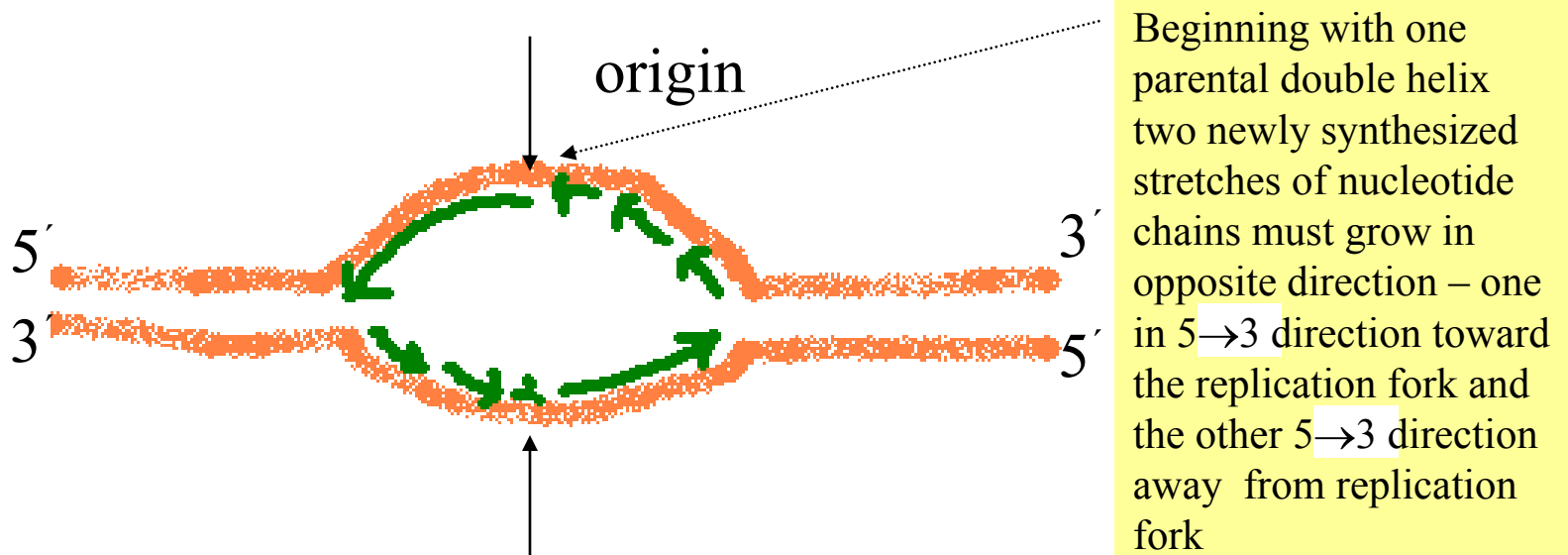
Replication proceeds on both strands

- double helix must be unwinded – enzyme helicase
- formation of **replication fork**
- reannealing of strands is prevented by ssb-proteins (single strain binding proteins)
- each newly synthesized strand of DNA base-pairs with its complementary parental template strand

Initiation of replication

Differences between prokaryotes and eukaryotes

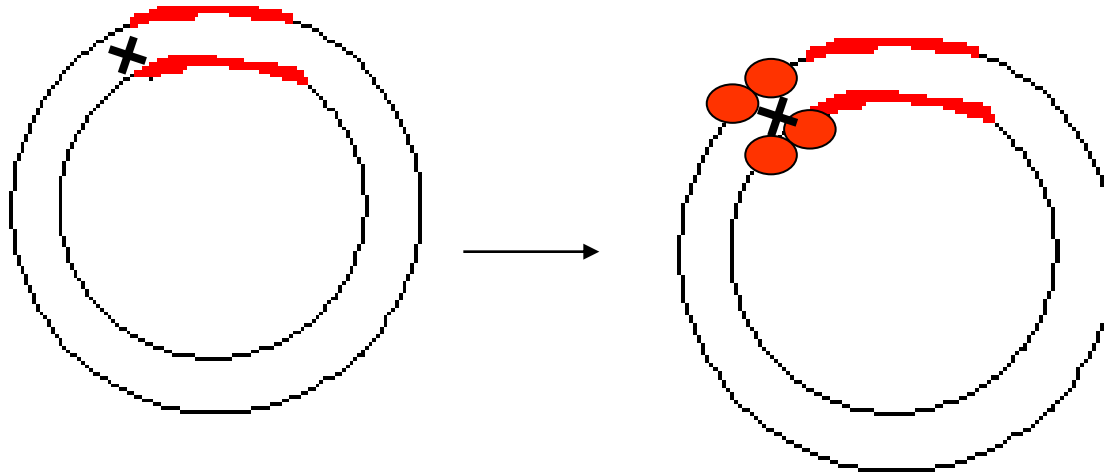
- replication in prokaryotes and eukaryotes starts at the given point → origin
- it occurs in both directions from the origin, two replication forks are formed that move away from the origin bidirectionally (in both direction at the same time)
- replication bubbles are formed - replicons



Initiation in prokaryotes

Origin (rich in A,T sequences)

Ori-binding proteins



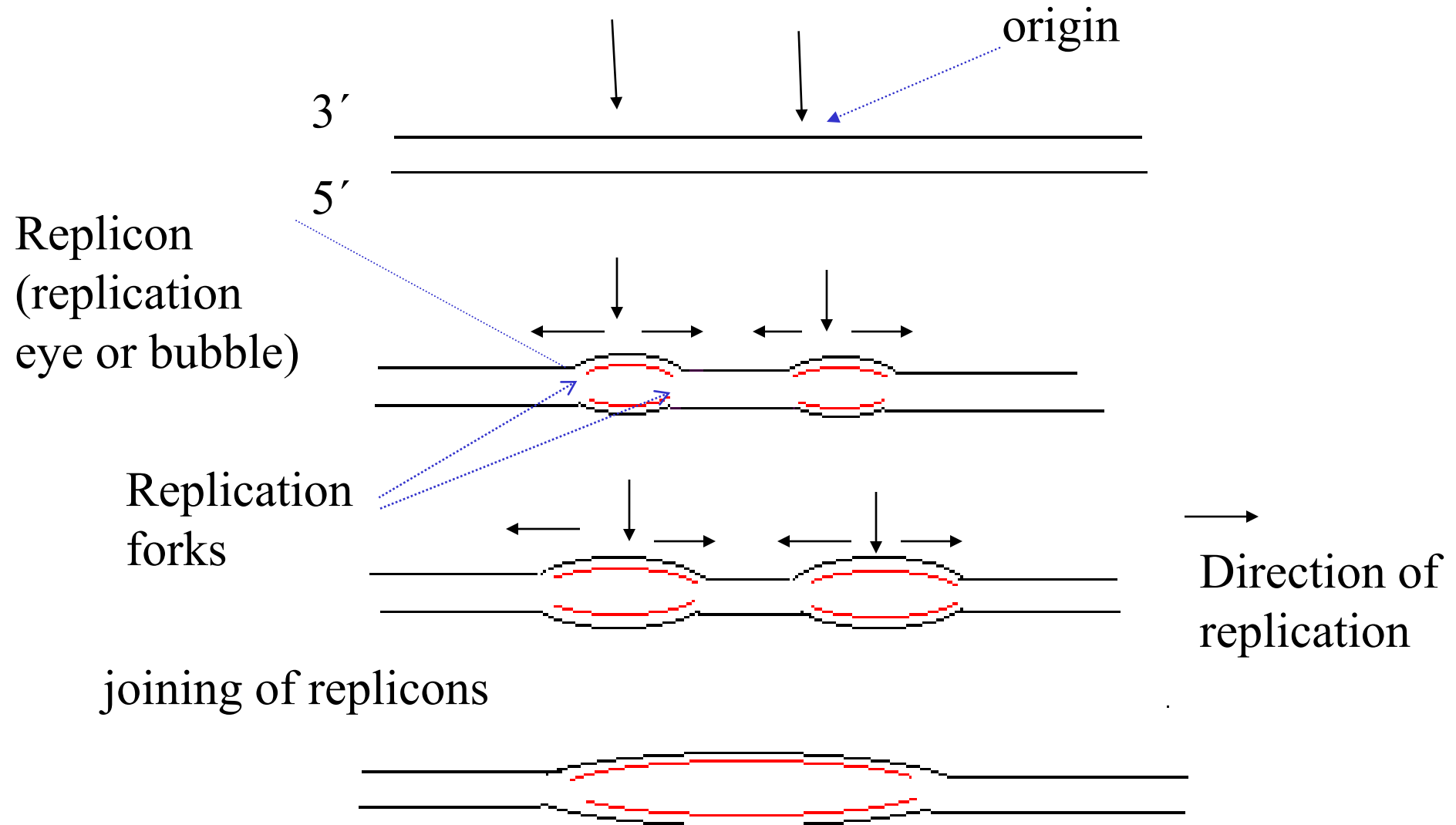
Denaturation in A,T site

Replication starts in the origine and continues untill both forks meets

Eukaryotic DNA replication

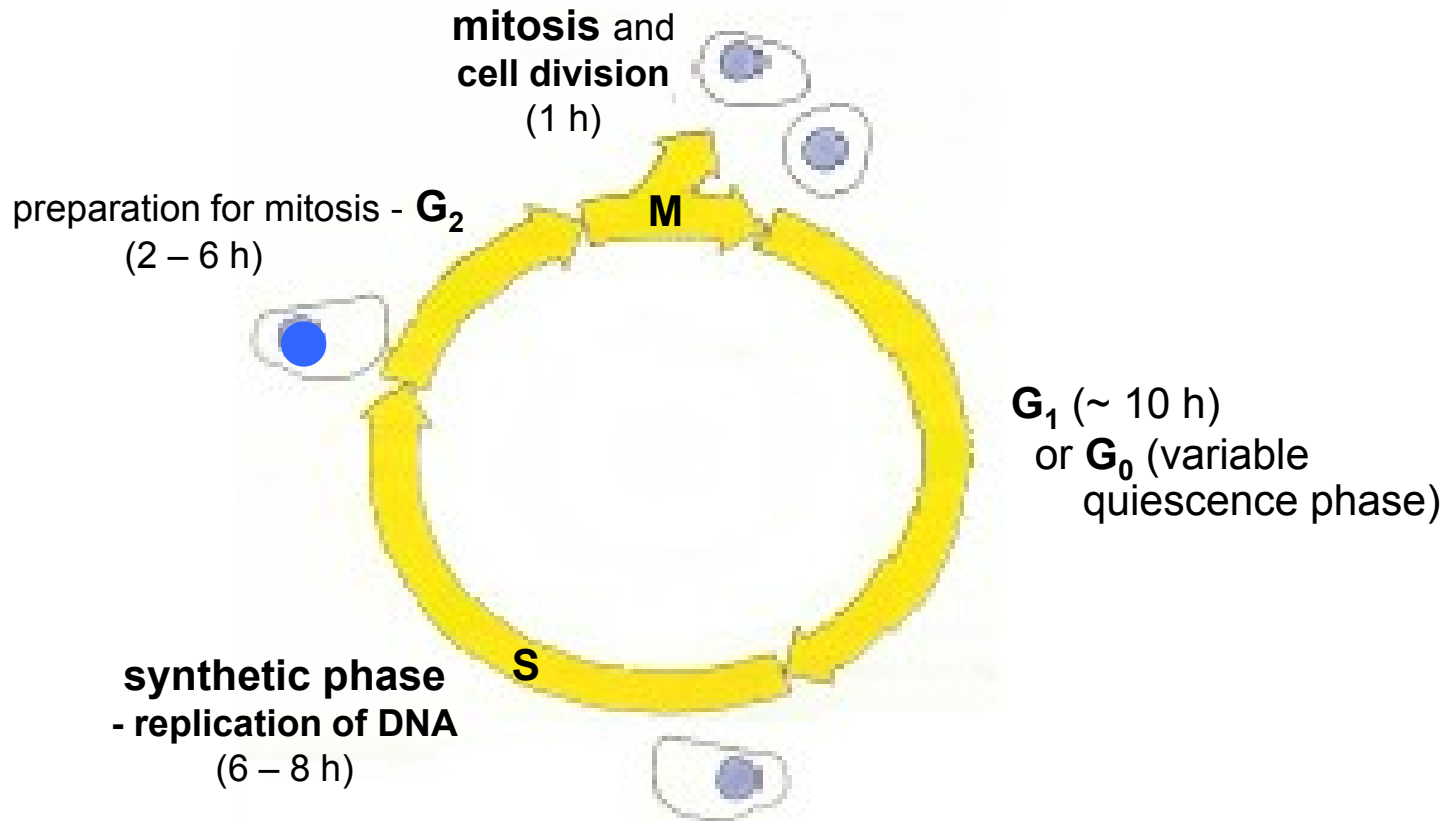
- Chromosomes in eukaryotes are very long DNA molecules that cannot be replicated continuously.
- **Replication** is initiated at **multiple origins** (up to several hundred in each chromosome, one every 30 to 300 kbp) **in both directions**.
- Initiation is controlled by time and space,
- Replication rate is lower than in prokaryotes, Okazaki fragments are much smaller in eukaryotes (200 of bases) than prokaryotes (1000-2000 of bases)
- Occurs in S-phase

Eukaryotic DNA replication

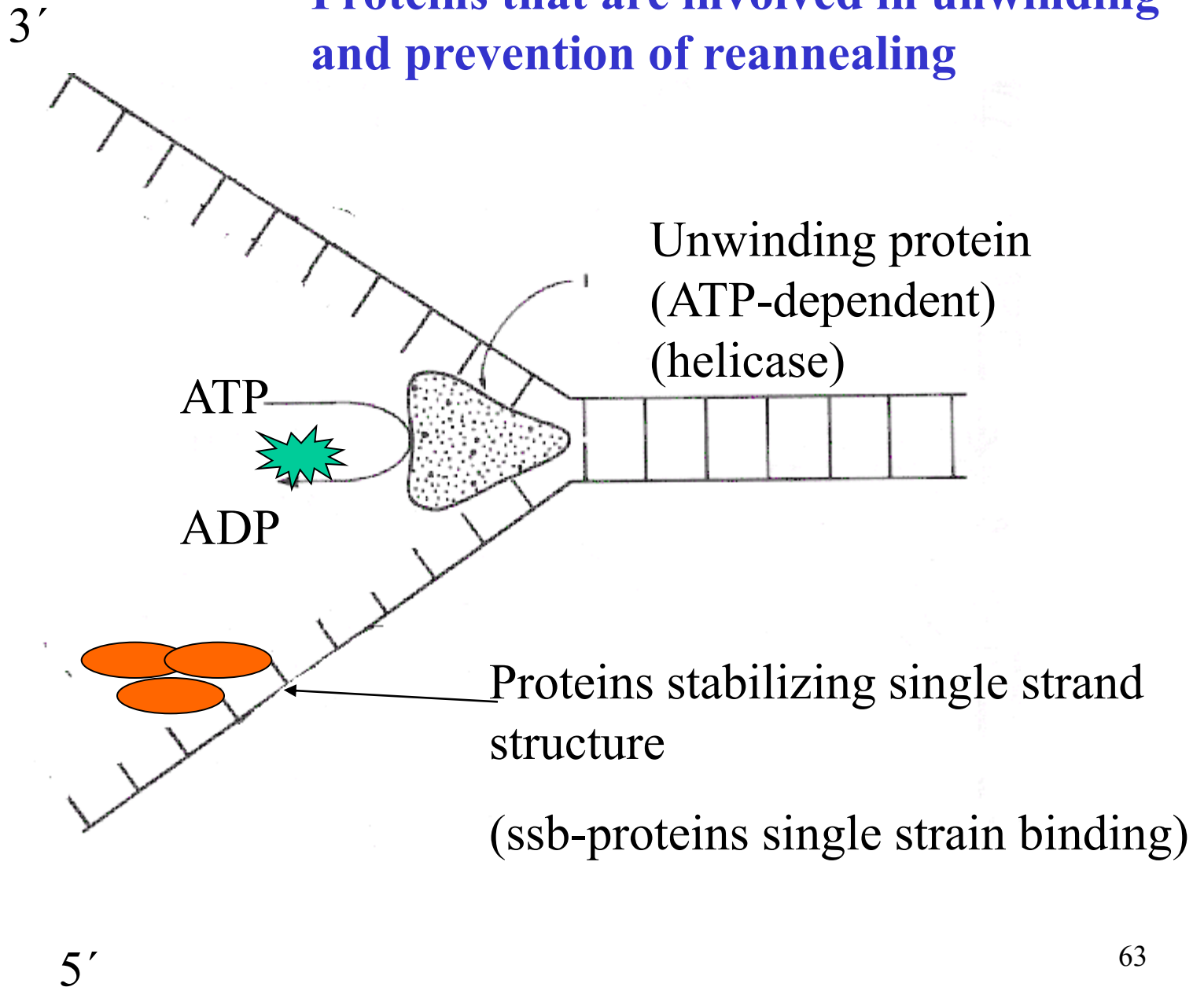


Eukaryotic DNA replication

Nuclear DNA is replicated only **in the S phase of the cell cycle**, mitosis takes place after the replication of all DNA sequences has been completed. Two gaps in time separate the two processes.

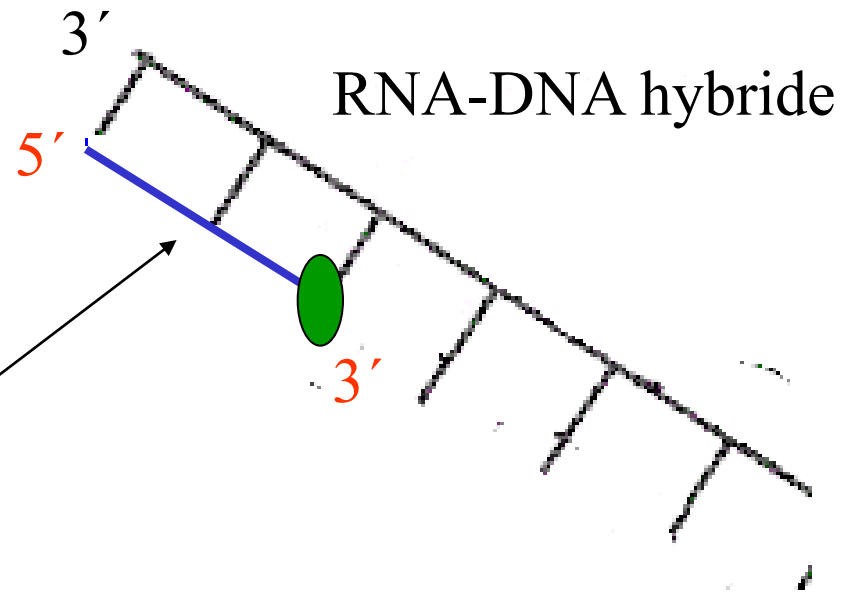


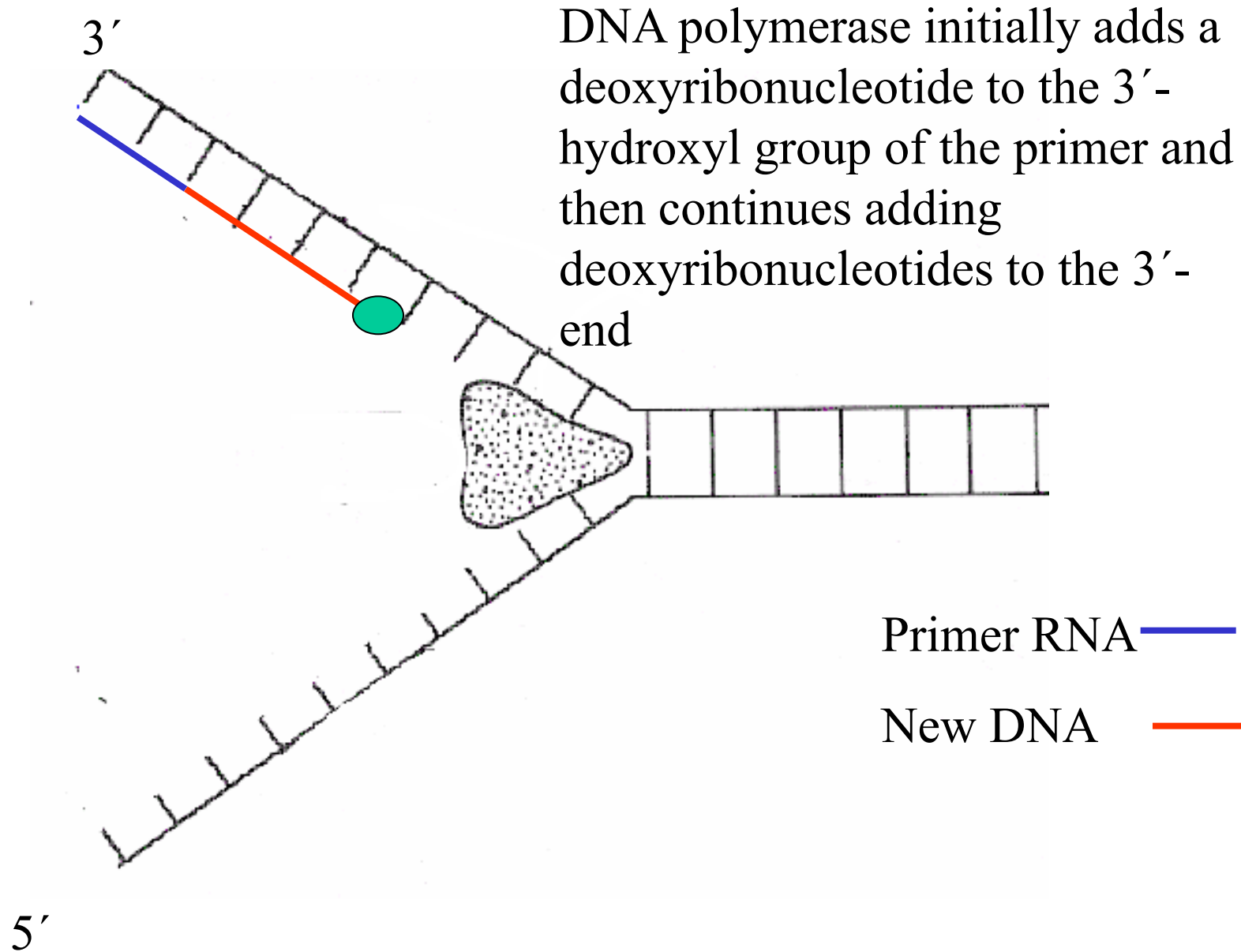
Proteins that are involved in unwinding and prevention of reannealing



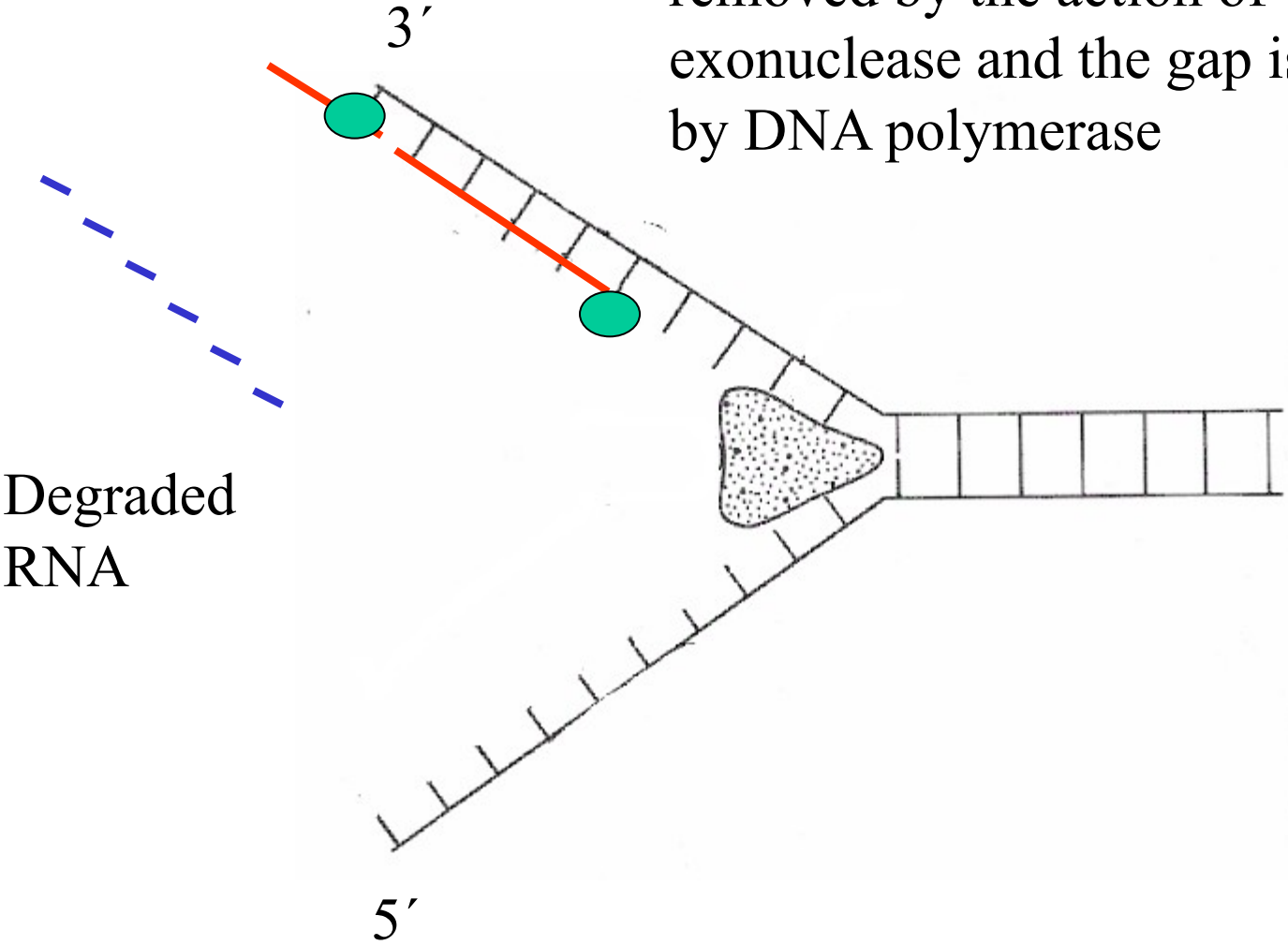
RNA primer is necessary for DNA synthesis

- DNA polymerase cannot initiate de novo synthesis of the chain, it requires free 3'-OH group for linking a new nucleotide.
- This primer is RNA oligonucleotide (10-20 bases)
- RNA primer is synthesized in direction 5' → 3' by the action of RNA polymerase (primase)
- Primer is coded according to template sequence



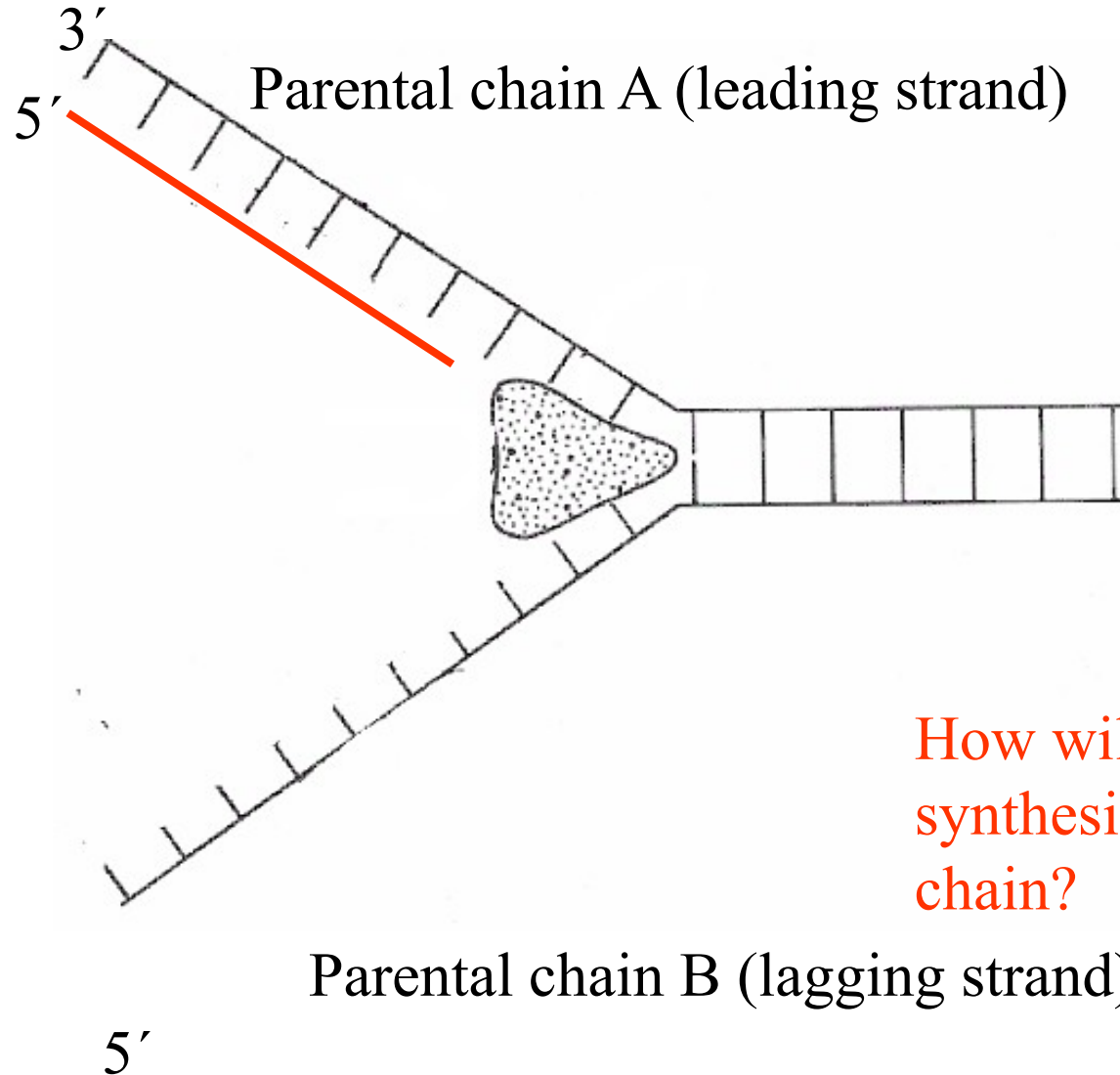


RNA primer is subsequently removed by the action of exonuclease and the gap is filled by DNA polymerase



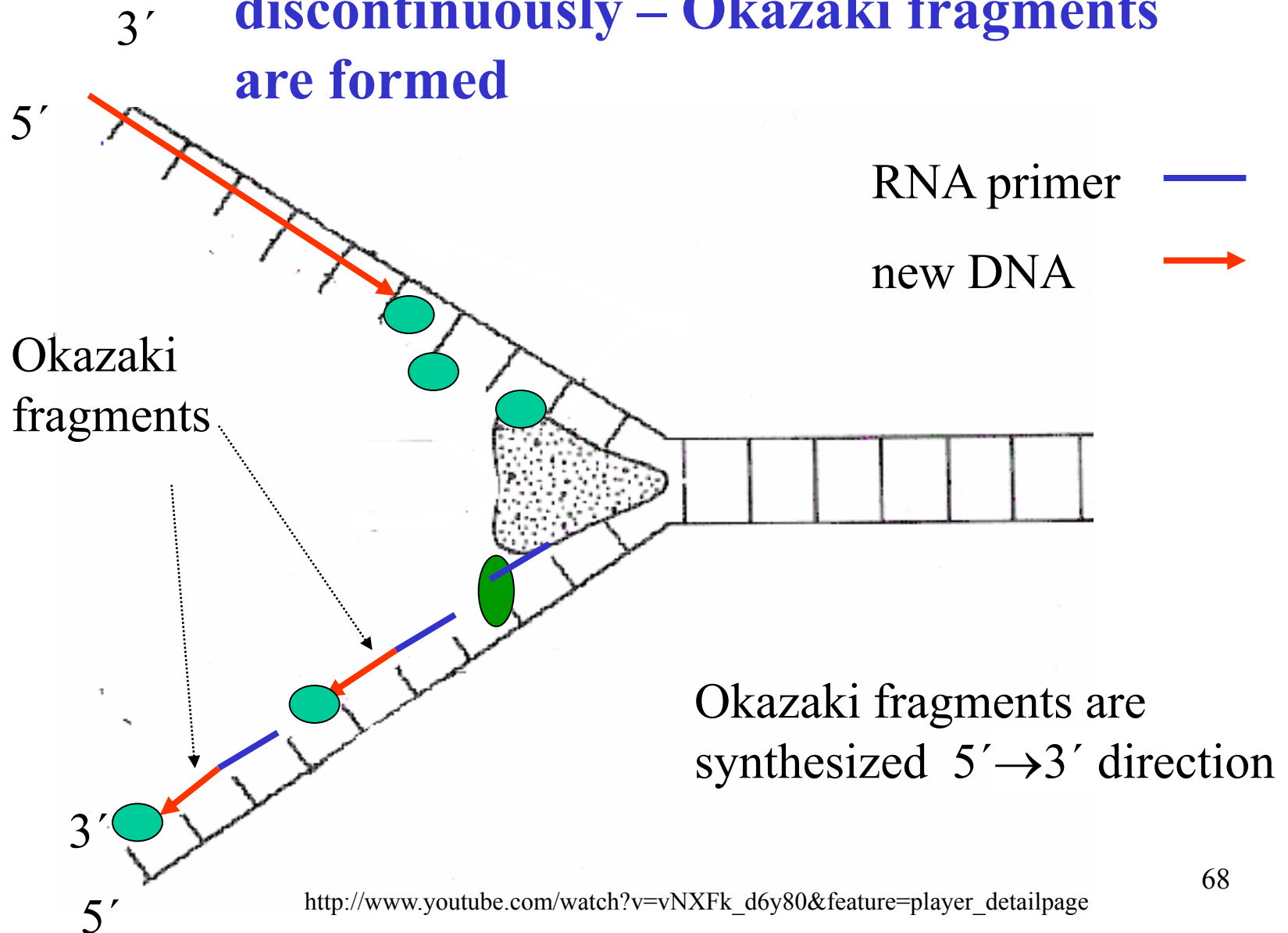
Synthesis of DNA proceeds always in 5' → 3' direction

The synthesis of new DNA along the A parental strand occurs without problems

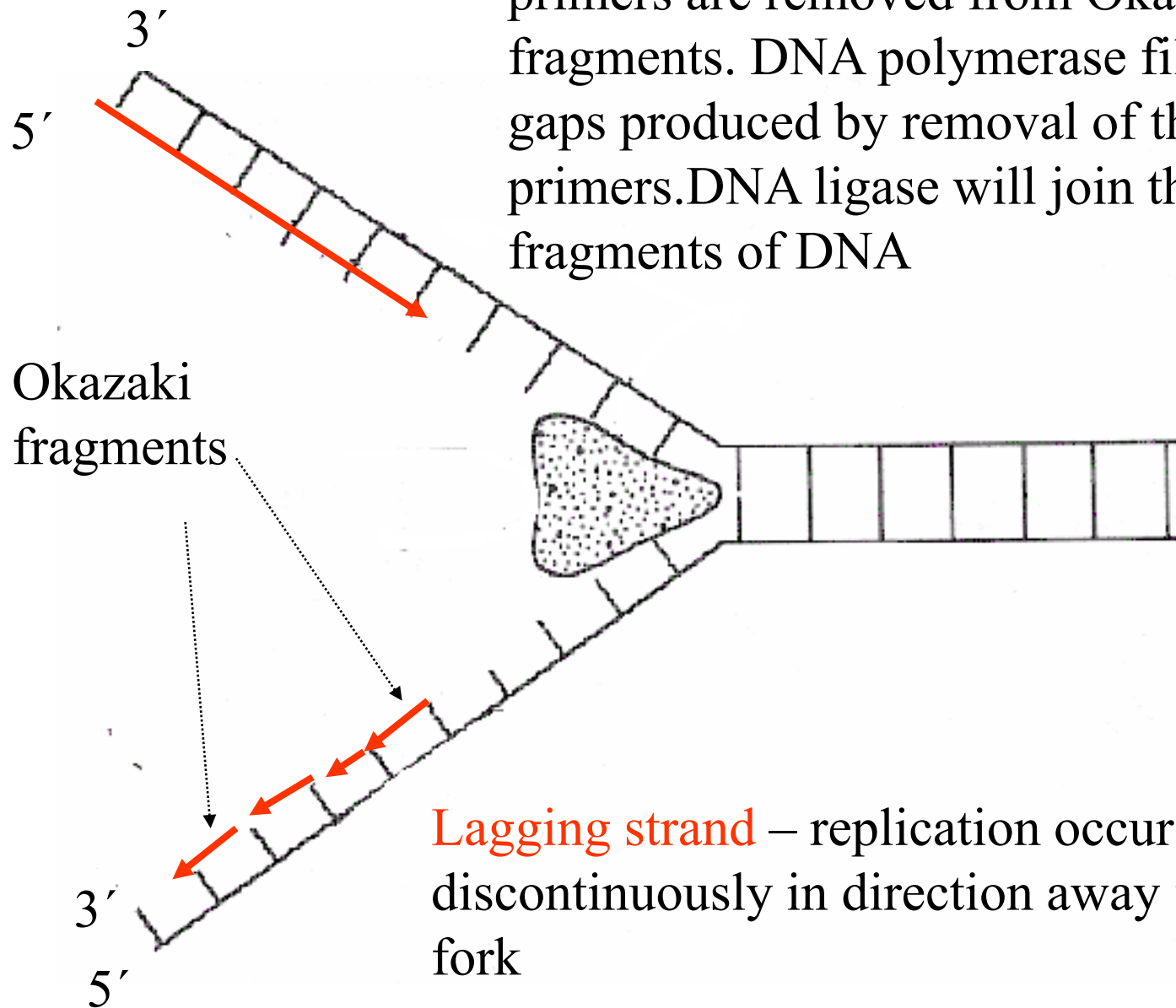


How will occur the synthesis among the B chain?

The lagging strand is synthesized discontinuously – Okazaki fragments are formed



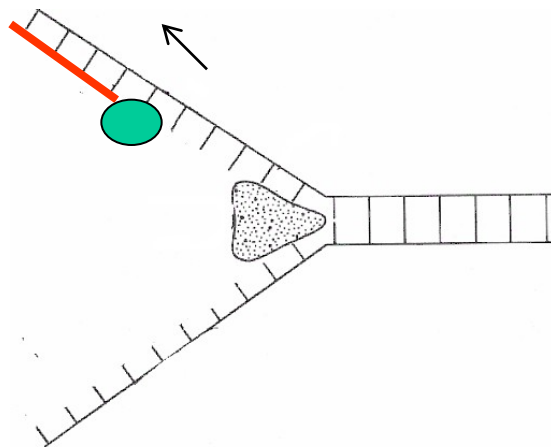
As replication progress, the RNA primers are removed from Okazaki fragments. DNA polymerase fills the gaps produced by removal of the primers. DNA ligase will join the fragments of DNA



Lagging strand – replication occurs discontinuously in direction away from the fork

Proofreading of newly synthesized DNA structure

- Precision of replication ~ 1 mistakes / 10^9 BP
- Enzymes proofreads the newly synthesized DNA
- As each nucleotide is added to the chain, DNA polymerase checks to make certain the added nucleotide is correctly matched to its complementary base.
- If it is not, the $3' \rightarrow 5'$ exonuclease activity edits the mistake.
- The $5' \rightarrow 3'$ polymerase then replaces it with the correct nucleotide.



DNA-polymerases have $5' \rightarrow 3'$ polymerase activity and $3' \rightarrow 5'$ exonuclease activity

Prokaryotic DNA-polymerases

| Polymerase | Polymerase activity (for all enzymes 5' → 3) | Exonuclease activity |
|---------------------|---|---------------------------------|
| DNA polymerase I | Filling if gap after removal RNA primer, DNA repair, removal of RNA primers | 5' → 3 and 3 → 5 |
| DNA polymerase II | DNA repair | 3 → 5 |
| DNA polymerase III* | Replication, proofreading and editing | 3 → 5 |

*The main enzyme of replication

Eukaryotic DNA-polymerases

| Polymerase* | Polymerase activity (for all enzymes 5' → 3') | Exonuclease activity |
|------------------------------|--|----------------------|
| DNA polymerase α | replication, DNA repair | no |
| DNA polymerase β | DNA repair | no |
| DNA polymerase γ | replication in mitochondria | 3 → 5 |
| DNA polymerase δ^{**} | replication, DNA repair | 3 → 5 |
| DNA polymerase ϵ | replication | 3 → 5 |

* At least 9 polymerases is known

**major replicative enzyme

Topoisomerase

(Topology of DNA = tridimensional structure of DNA)

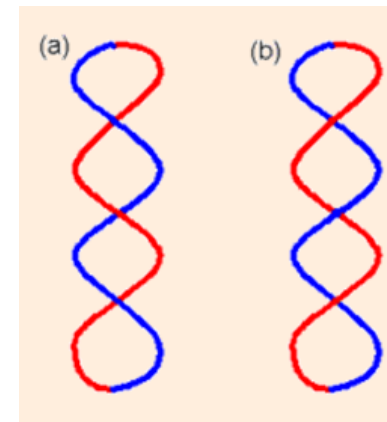
Topoisomerase regulates the formation of superhelices

These enzymes catalyze the concerted breakage and rejoining of DNA strands, producing a DNA that is more or less superhelical than the original

The precise regulation of the cellular level of DNA superhelicity is important to facilitate protein interaction with DNA

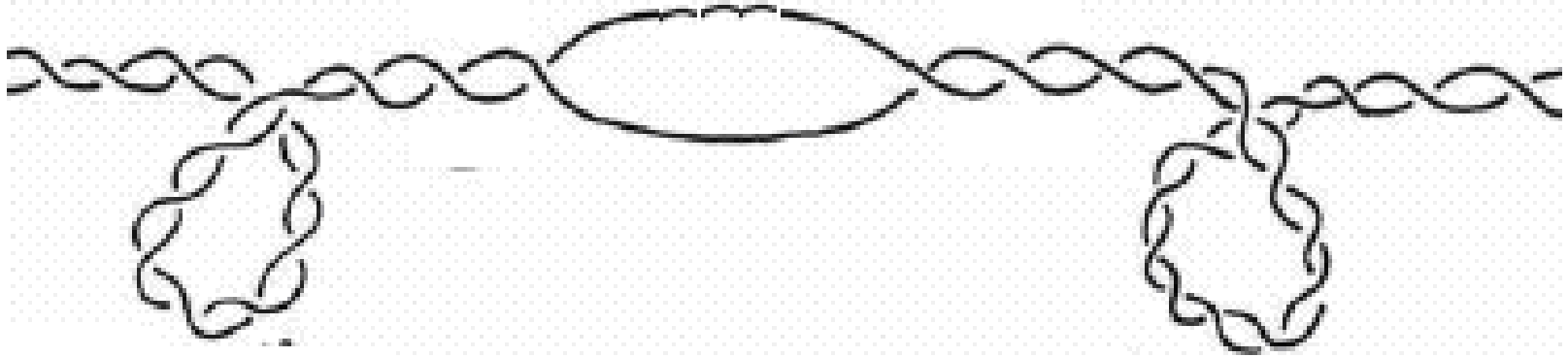
DNA topoisomerases have many functions (at replication, transcription, repairs, etc.)

positive negative
supercoiling



<http://www.youtube.com/watch?v=EYGrElVyHnU>

Supercoiling at unwinding double helix DNA



Negative supercoiling

Positive supercoiling

DNA topoisomerases have many functions (at replication, transcripti, repair, ...)

Topoisomerase I

Make a transient single-strand break in negatively supercoiled DNA double helix. Passage of the unbroken strand through the gap eliminates one supercoil from DNA.

Energy is not required.

Present in prokaryotes and eukaryotes.

Topoisomerase II

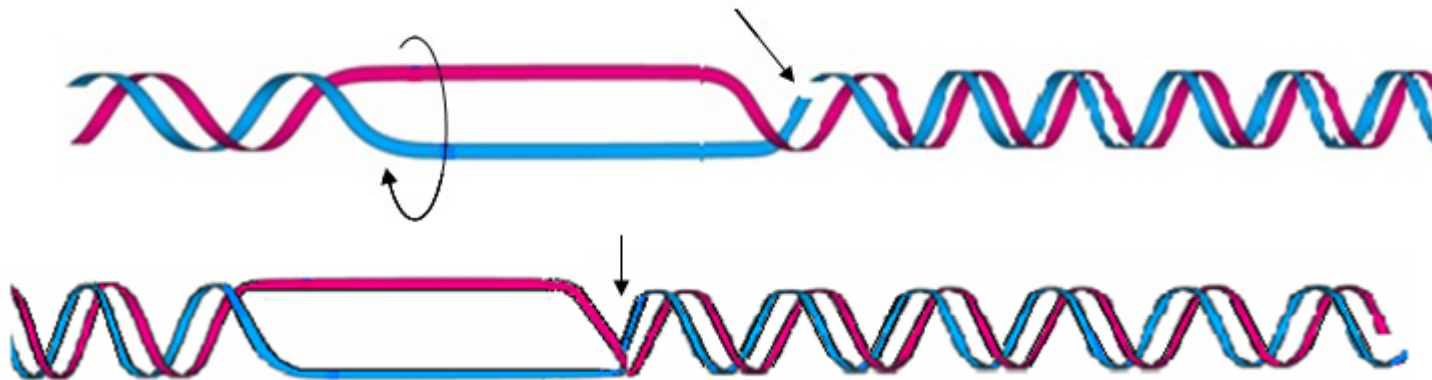
It binds to double helix DNA and cleave both strands. It can relax supercoiled DNA or introduce supercoil into DNA.

Present in prokaryotes and eukaryotes.

Requires ATP cleavage energy.

Action of topoisomerase I

Interruption of phosphodiester bond followed by rotation around the second strand and closing the break by ligation



Inhibitors of human topoisomerase prevent replication

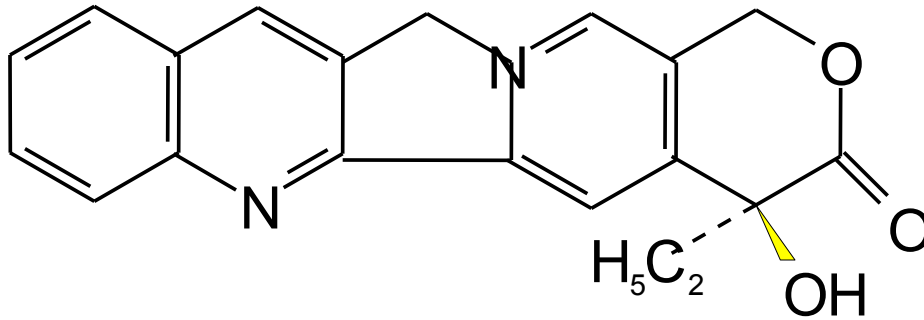
Antineoplastic drugs

Examples of topoisomerase inhibitors

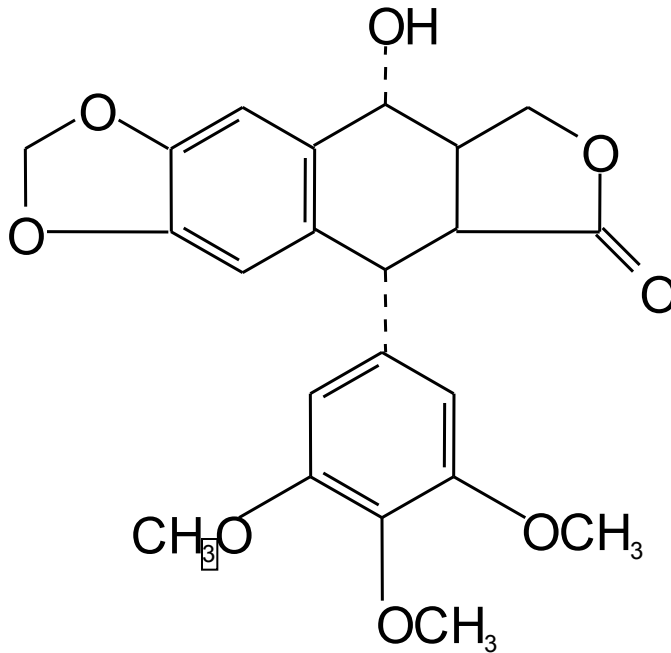
camptothecine – plant product

anthracyclines (daunorubicine) -bacterial products

podophyllotoxines-plant product



camptothecine
Camptotheca
acuminata



podophyllotoxine
Podophyllum
peltatum ad.

Telomeres

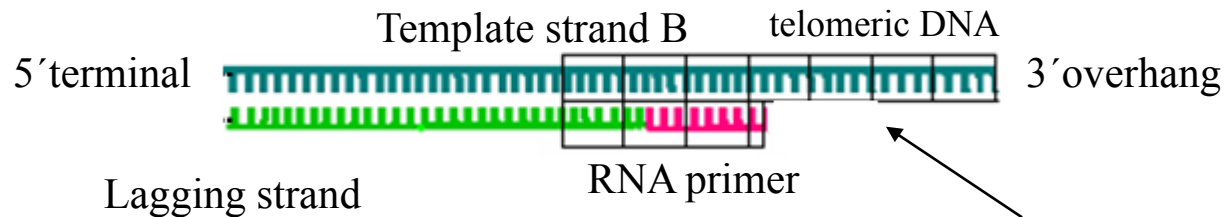
Eukaryotic chromosomes are linear. A solution must be found to two problems:

- First, the ends of the chromosomes must be protected from degradation.
- Secondly, there must be some mechanism to ensure replication of a complete chromosome

Telomeres

As DNA replication approaches the end of chromosome, a problem develops with lagging strand. Either primase cannot lay down a primer at very end of the chromosome, or after DNA replication is complete, the RNA at the end is degraded.

Consequently, the newly synthesized strand is shorter at the 5' end, and there is 3'-overhang in DNA strand being replicated.



Telomeres are special sequences at the ends of chromosomes

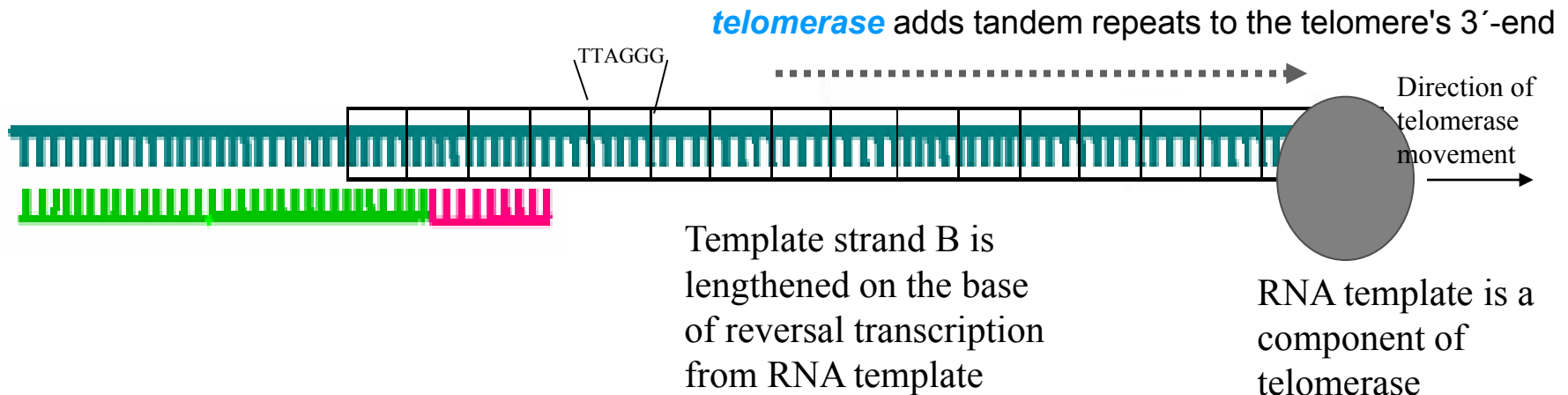
Tandem repeats of species-specific oligonucleotides, G-rich

(in human TTAGGG up to 1000x)

They protect the ends of chromosomes against nuclease activities.

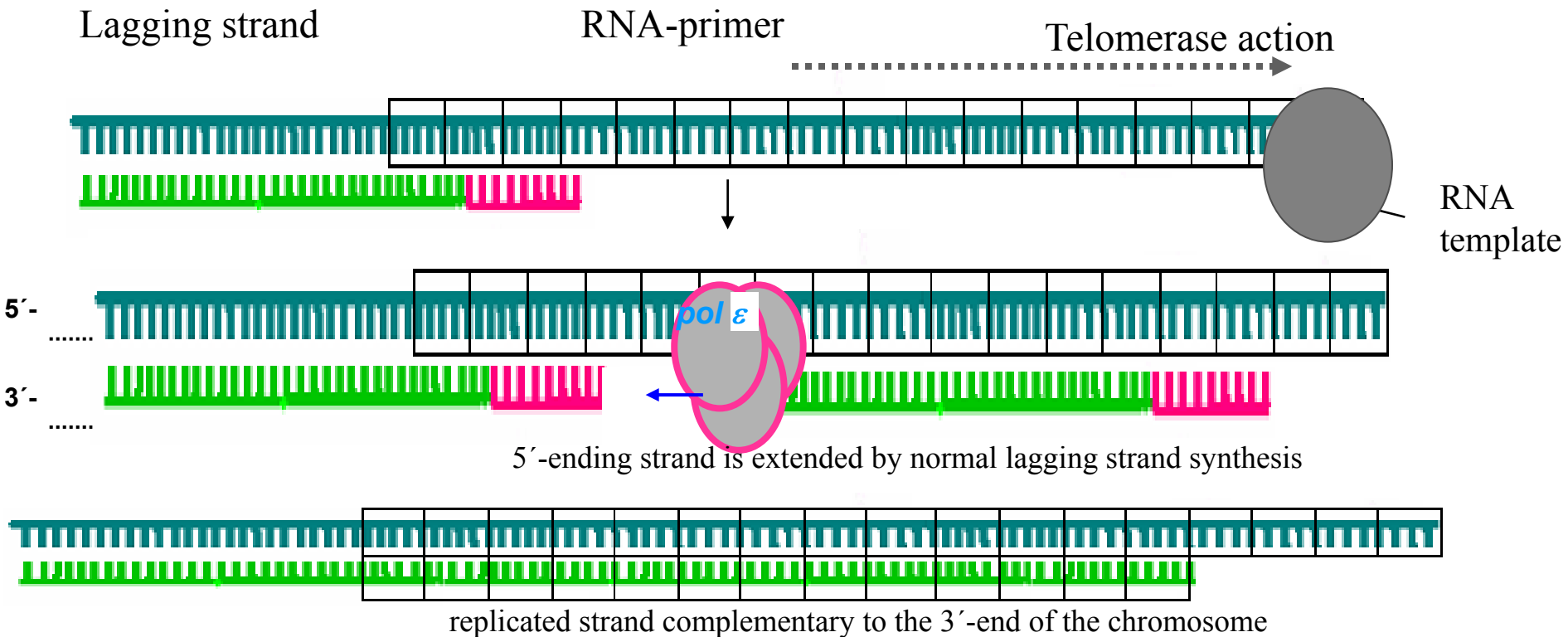
Telomerase

- completion of DNA synthesis
- adds newly synthesized hexanucleotide to 3'-end template strand
- it is reverse transcriptase – it carries its own RNA template (CA), this is added to 3'-end of DNA template and new DNA is synthesized that lengthens the 3'-end of DNA strand.
- Then the telomerase moves down the DNA toward the new 3' end and repeats the process a number of times.



Telomerase action

Replicating leading strand is not included in the scheme



? Does the length of telomers correlate with the age of the cell and its replication capacity?

- The inability to replicate telomeres has been linked to cell aging and death
- Many somatic cells do not express telomerase – when placed in culture, they survive a fixed number of population doublings, enter senescence and then die.
- Analysis has shown significant telomere shortening in those cells.
- In contrast, stem cells do express telomerase and appear to have an infinite lifetime in culture.
- Therefore research is focused on understanding of the role of telomeres in aging, growth and cancer

DNA damage and repair

It is estimated that the number of damaging interventions into the DNA structure in the human cell is about:

$\sim 10^4$ - 10^6 /day

\Rightarrow In adult human (10^{12} cells) it results in 10^{16} - 10^{18} repair processes per day.

DNA damage and repair

| Type of damage | Cause of damage |
|---------------------|--|
| Missing base | Depurination (10^4 purines/day) |
| Altered base | Ionizing radiation, alkylating agents |
| Non-correct base | Spontaneous deamination |
| Deletion-insertion | Intercalating drugs (acridines) |
| Formation of dimers | UV radiation |
| Strand breaks | Ionizing radiation, chemicals (bleomycine) |
| Cross-linkages | Chemicals (derivateves of psoralene, mitomycine C) |
| Tautomer formation | Spontaneous and temporary |

All cells are able to recognize damaged DNA and possess highly efficient mechanisms to repair modified or damaged DNA.

DNA repair enzymes:

Specific glycosylases

can eliminate altered bases by hydrolysis of the N-glycosidic bond between the base and deoxyribose;

specific endonucleases

cause breaks in the strand, **5'-3' exonucleases** excise one or more nucleotides from the strand

DNA polymerase β fills in the gap,

DNA ligase rejoins the DNA strand.

The two major repair pathways are

base excision repair and **nucleotide excision repair**.