Metabolism of purine and pyrimidine nucleotides DNA replication

(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

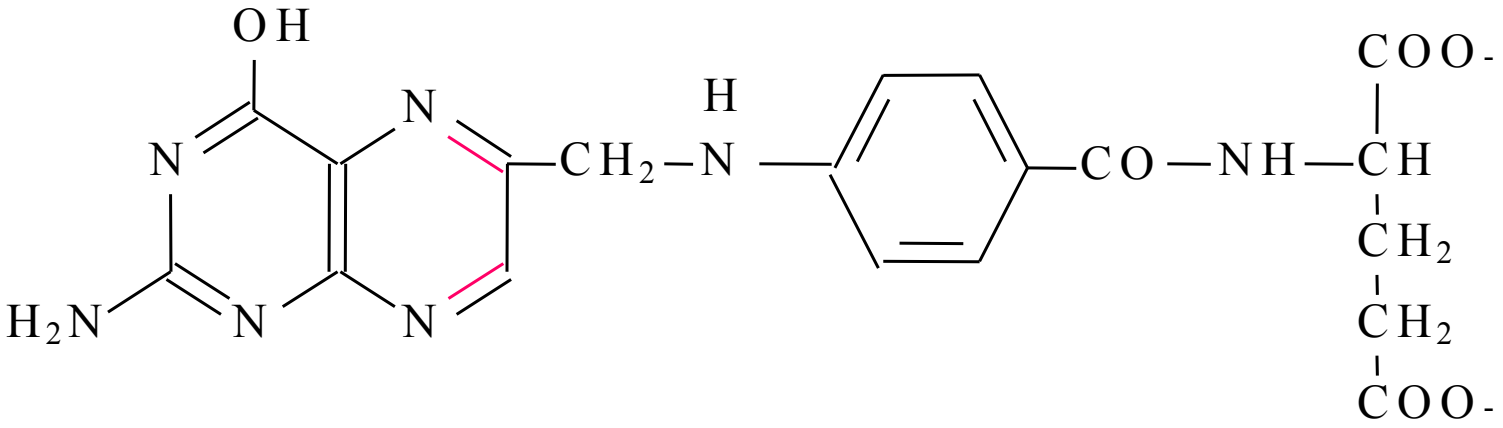
- Humans depend on the endogenous synthesis of purines and pyrimidines

Significance of folic acid for synthesis of bases

Folate

For human is essential:

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

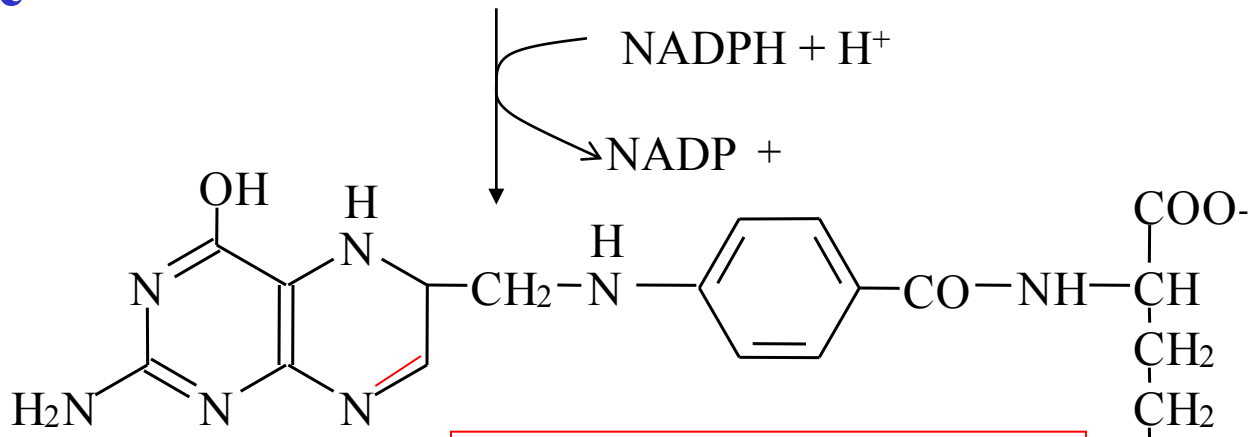


The effective form in organism of human is tetrahydrofolate.

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

Formation of tetrahydrofolate

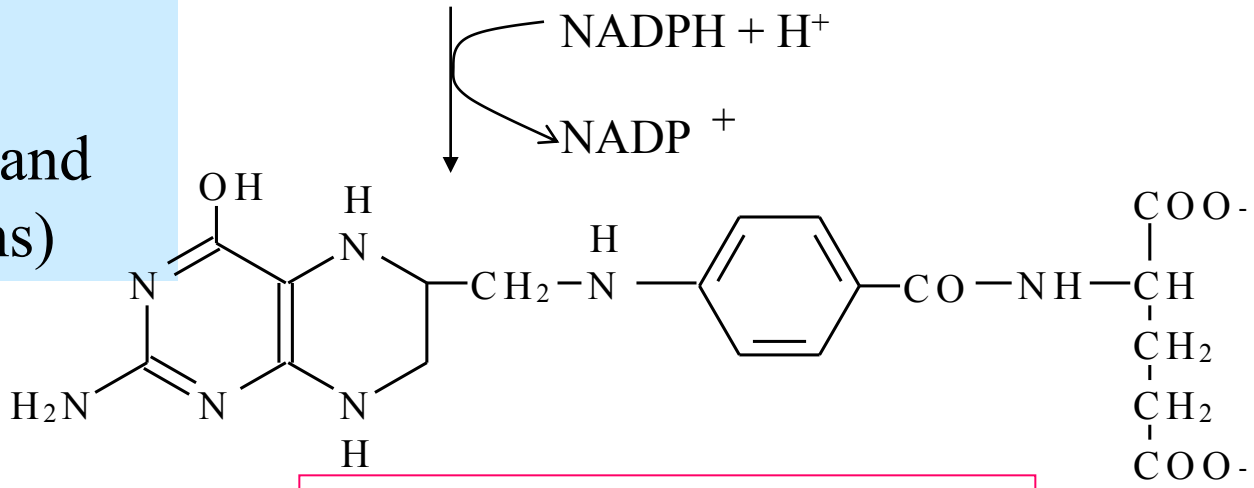
Folate



dihydrofolate

(dihydro)folate reductase

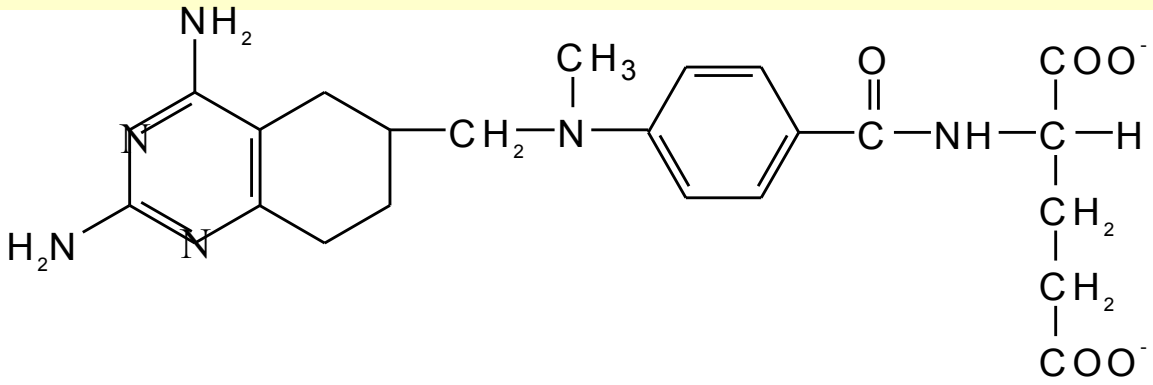
(catalyzes the both reactions in animals and some microorganisms)



tetrahydrofolate

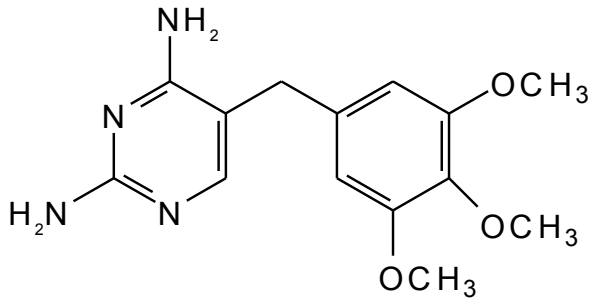
Inhibitors of (dihydro)folate reductase:

Methotrexate (anticancer drug)



Trimethoprim (bacteriostatics)

it inhibits bacterial dihydrofolate reductase

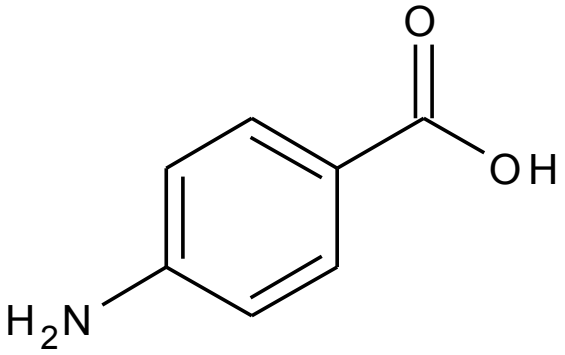


Inhibitors of folate synthesis

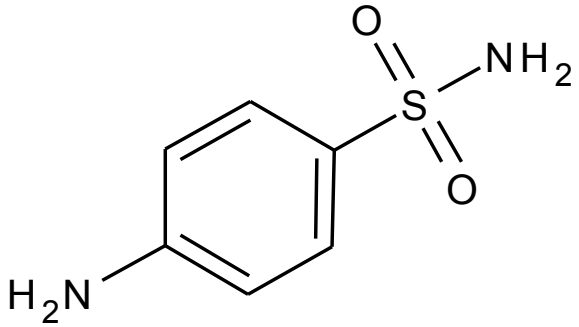
- Sulfonamides (e.g. Sulfamethoxazol) are structural analogs of p-aminobenzoic acid.
- p-aminobenzoic acid is necessary for bacterial synthesis of folic acid
- Folic acid is a growth factor for bacterias.

Sulfonamides act as competitve inhibitors of the synthesis.

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



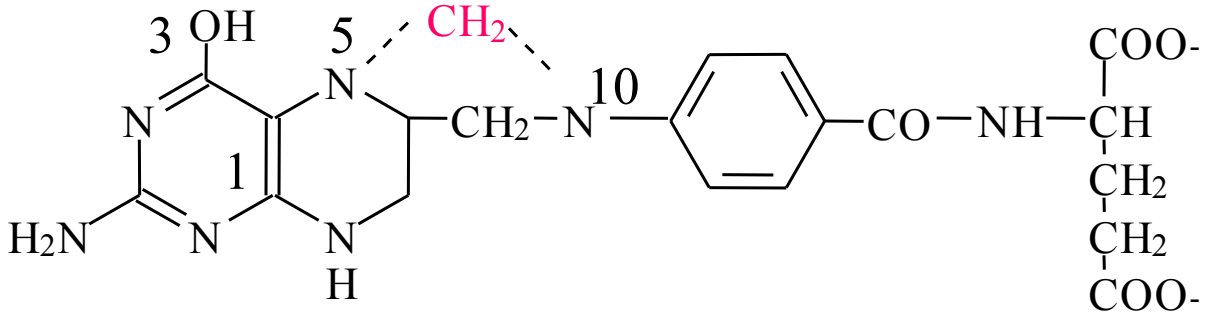
p-aminobenzoic acid (PABA)



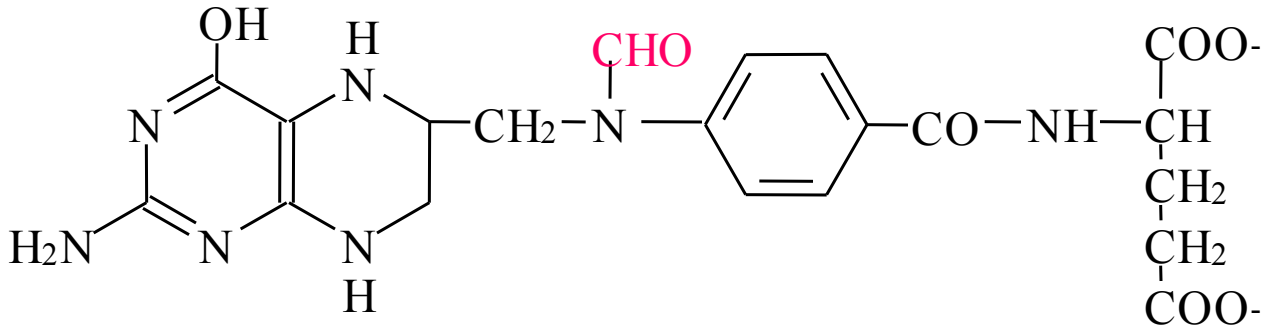
sulfanilamide

Using of tetrahydrofolate in synthesis of purines and pyrimidines

N-5,N-10- methylen H_4F – synthesis of thymine

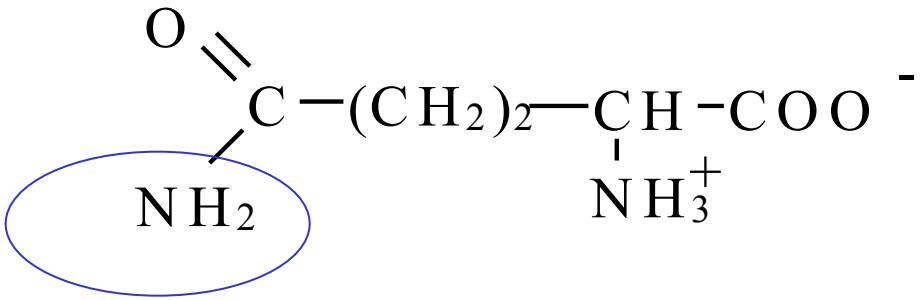


N-10-formyl H_4F – synthesis of purine



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

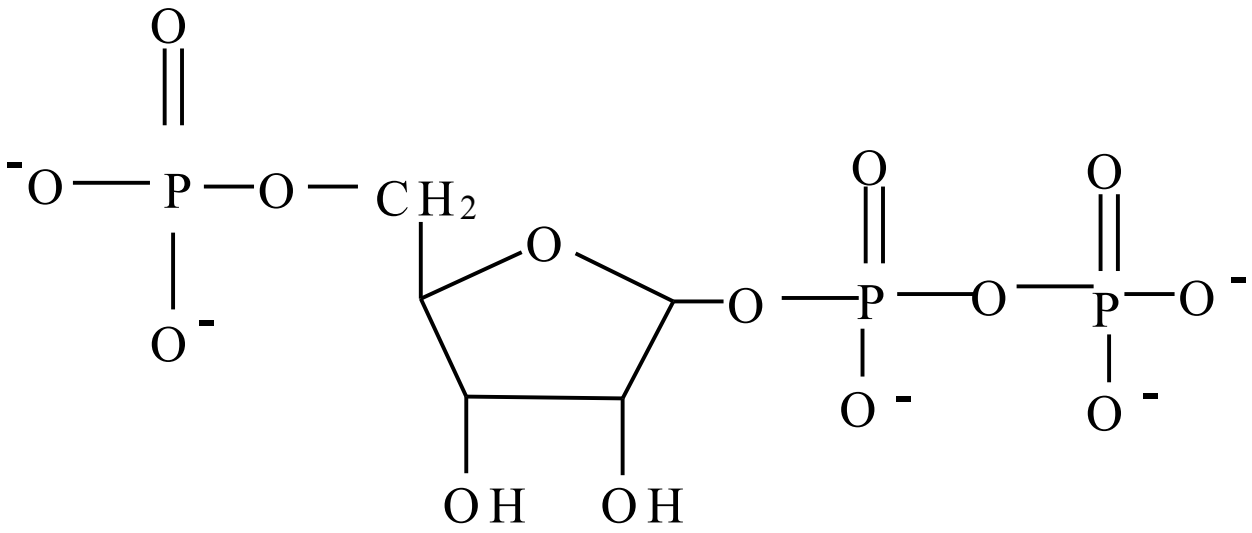
Required for synthesis of:

purine nucleotides

pyrimidine nukleotides

NAD^+, $NADP^+$

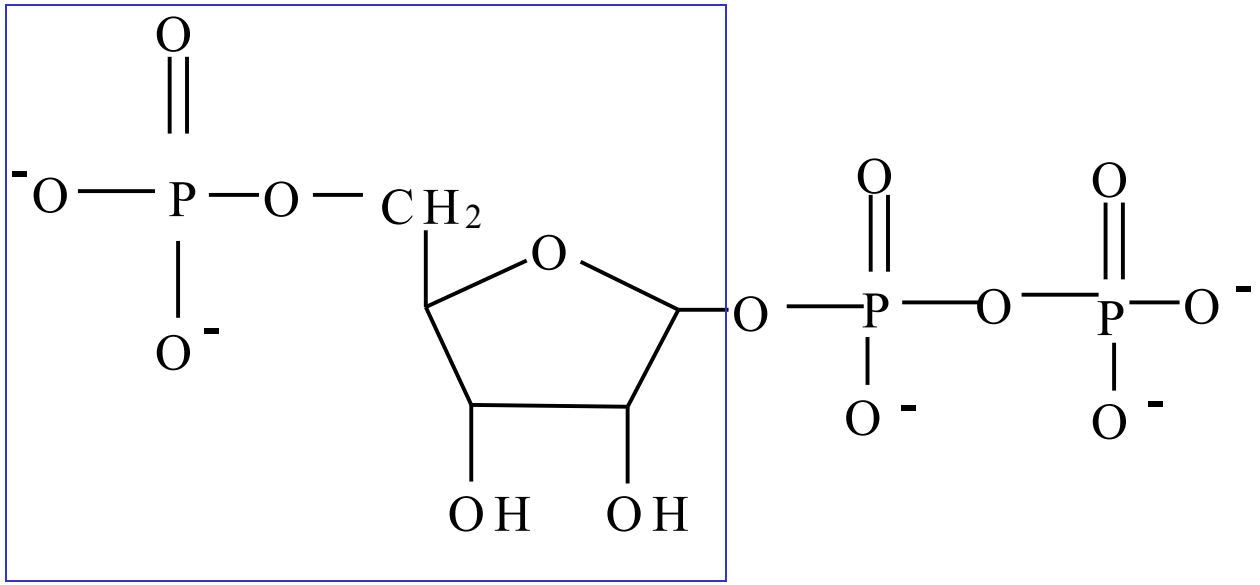
Activated pentose



Synthesis of phosphoribosyl diphosphate (PRPP)

PRPP-synthase (kinase)

Ribose-5P + ATP → PRPP + AMP



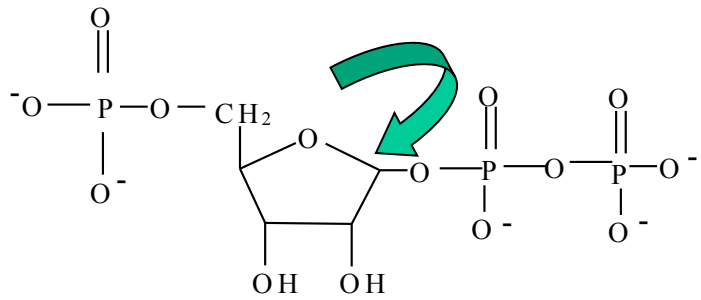
Differences in purine and pyrimidine synthesis

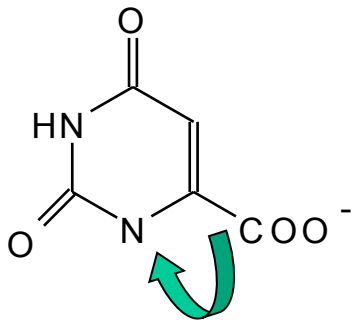
Purines

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

Pyrimidines

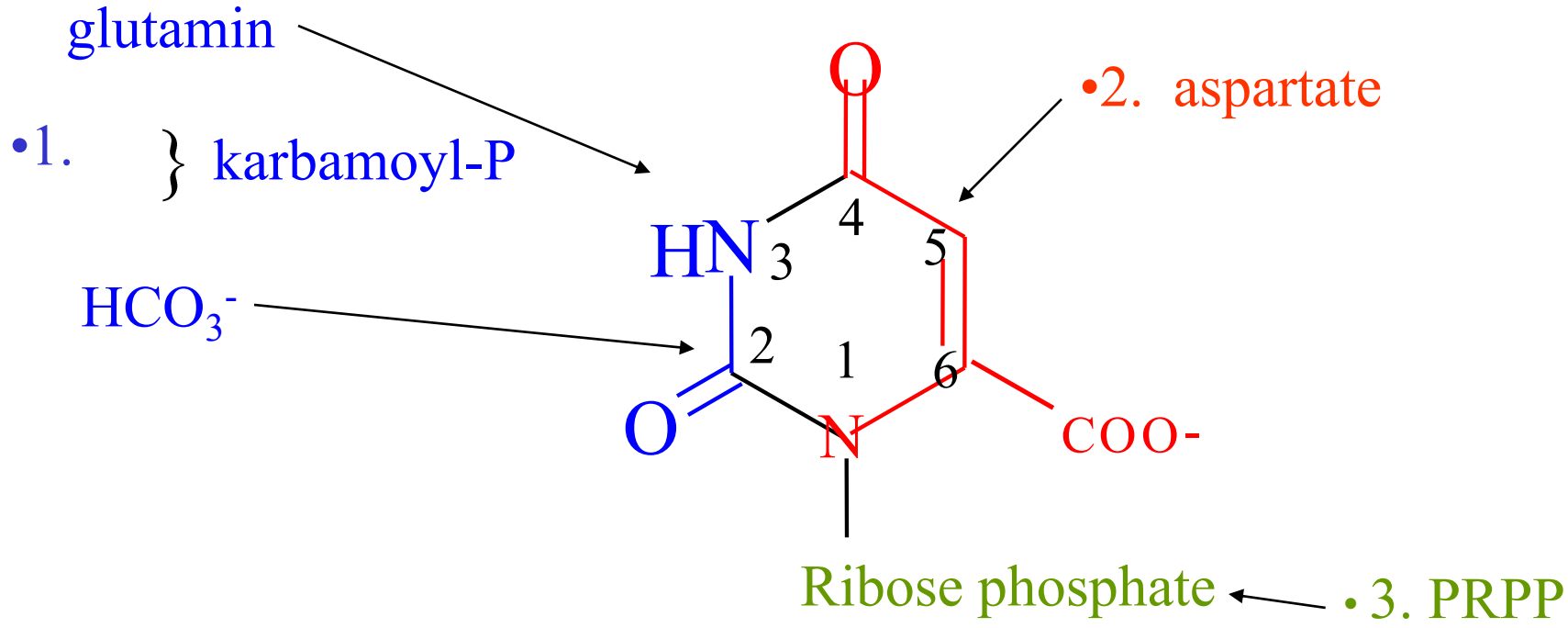
The pyrimidine ring is synthetized before ribose is added





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

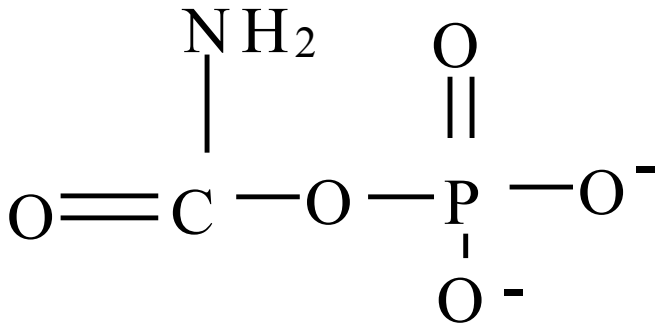
CYTOPLASMA

Carbamoyl phosphate synthase II

Compare with the reaction in the synthesis of urea - mitochondria

• 1 Glutamin + 2 ATP + HCO_3^-

→ carbamoylphosphate + glutamate + 2 ADP + P_i

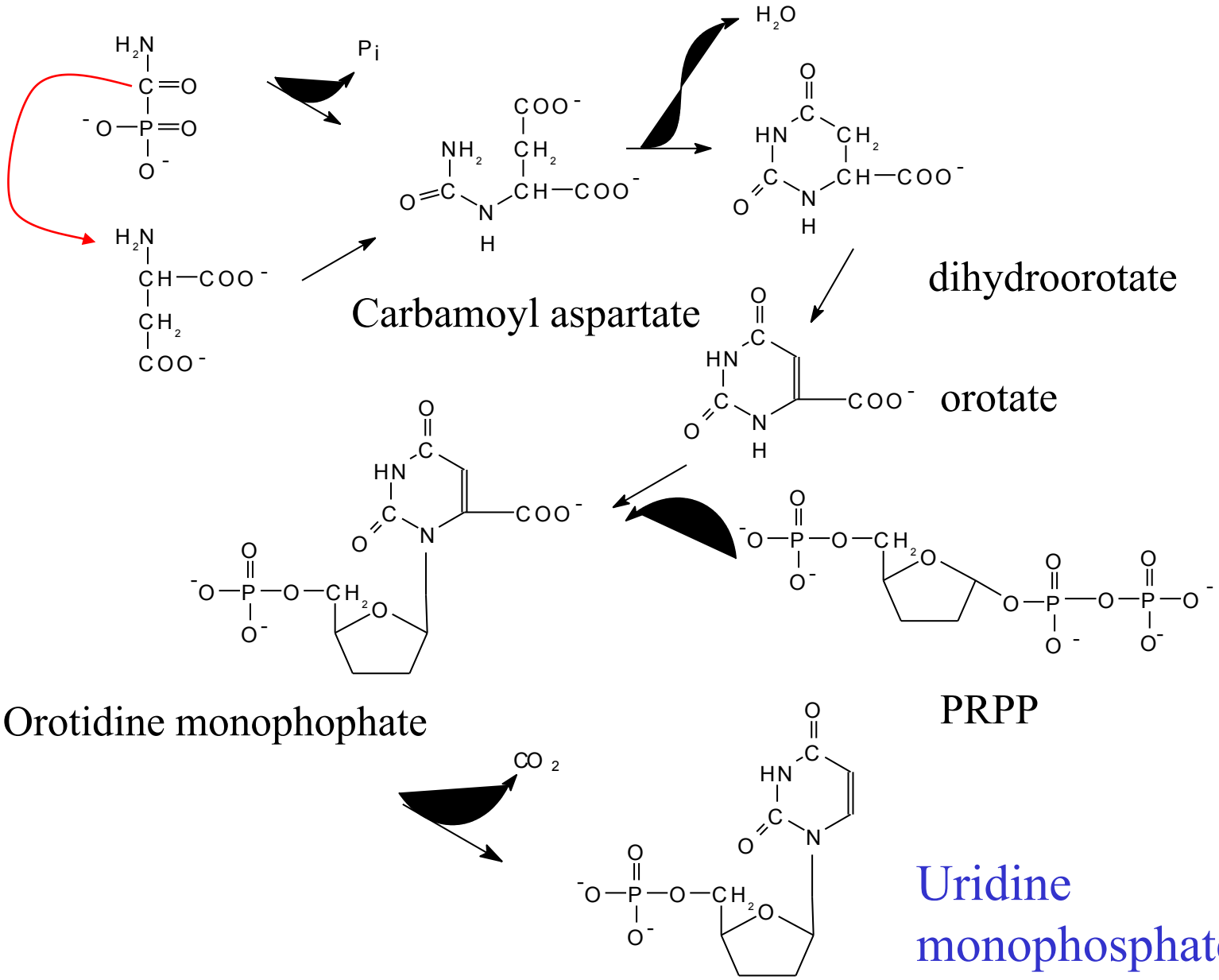


Reaction is the most regulated step

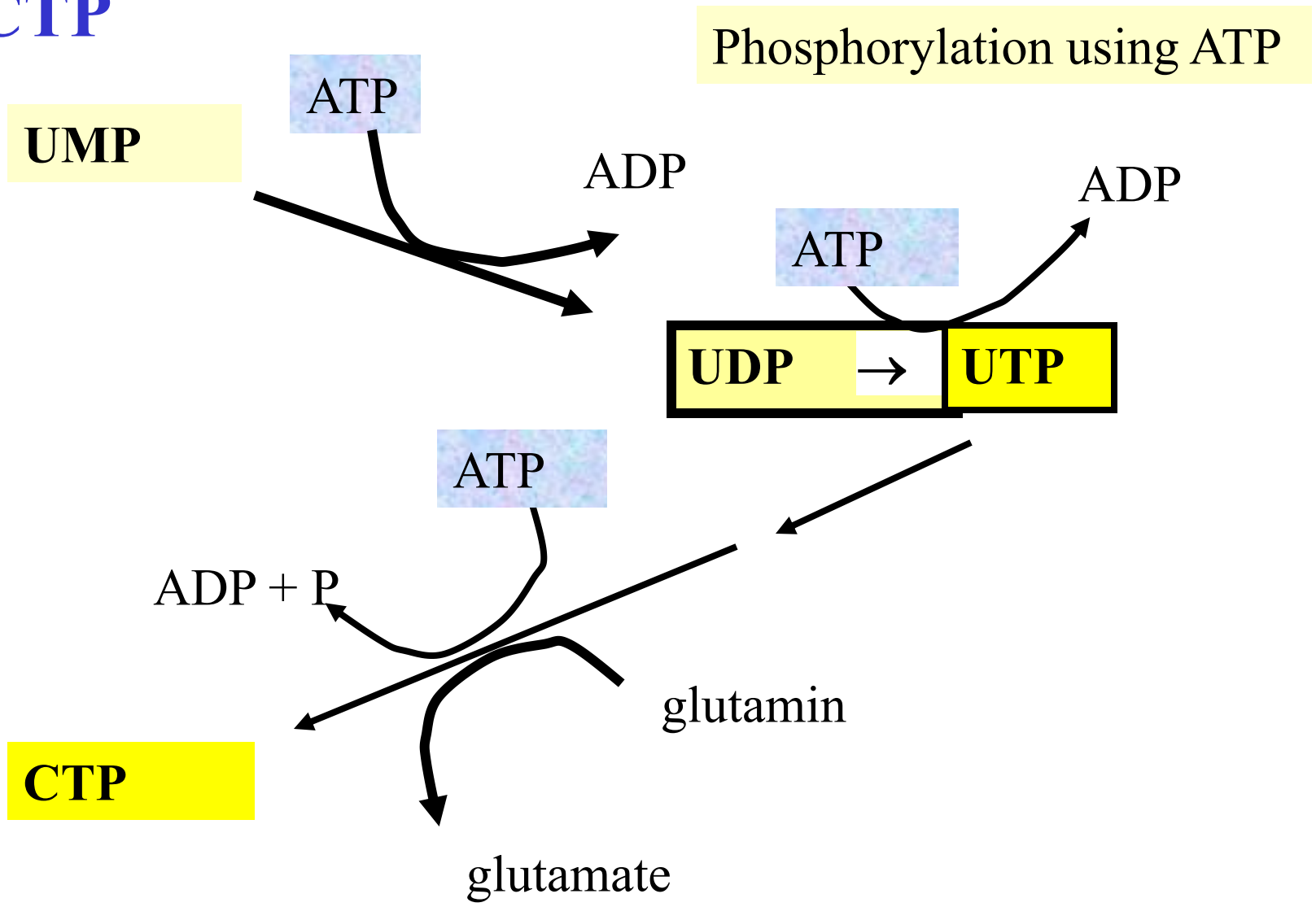
Comparision of carbamoyl phosphate synthetases

Enzyme typ	Carbamoyl phosphate synthetase I	Carbamoyl phosphate synthetase II
Localization in the cell	mitochondria	cytoplasma
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*

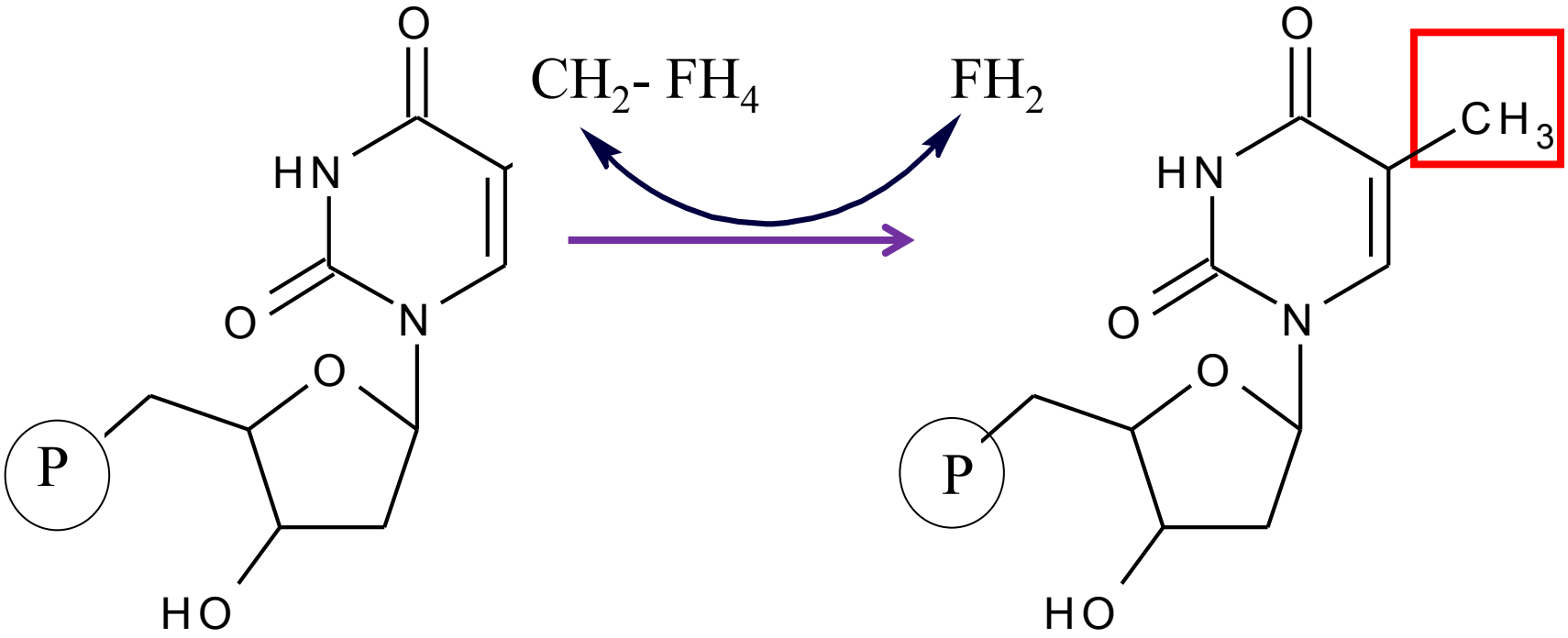


Biosynthesis of UTP a CTP



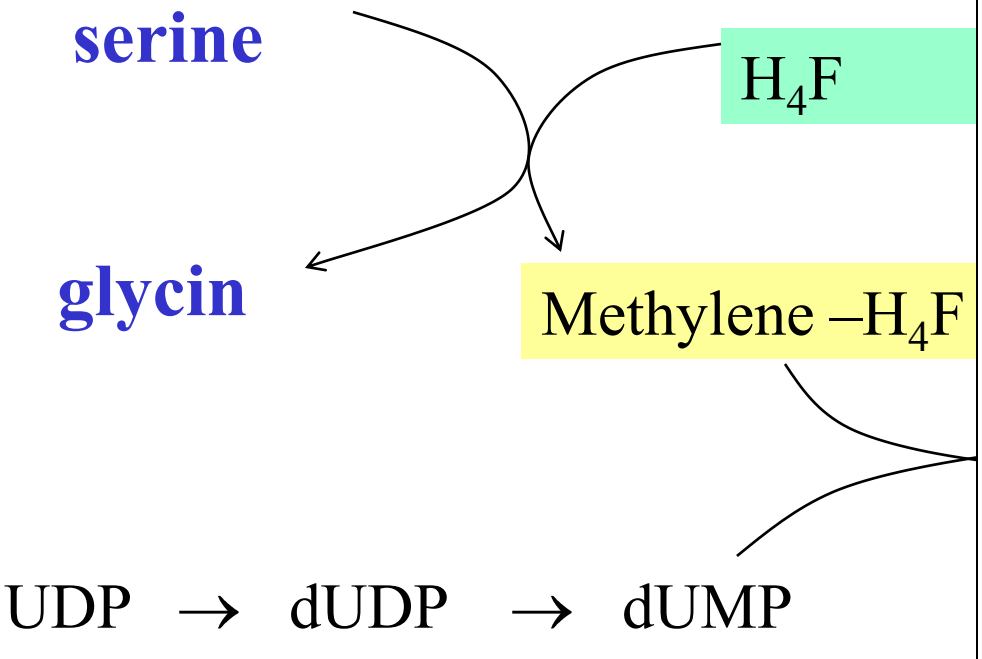
Formation of dTMP (methylation)

H_4F is required for methylation



dUMP dTMP

Formation of dTMP



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

Formation of TMP

NADP

Dihydrofolate reductase

serin

H_4F

NADPH

glycin

Methylene –H_4F

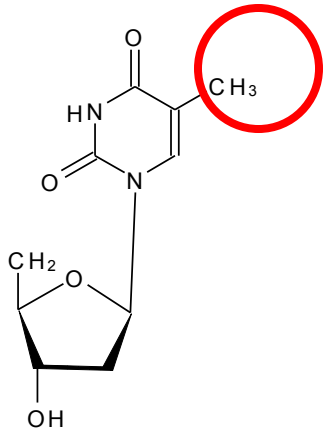
H_2F

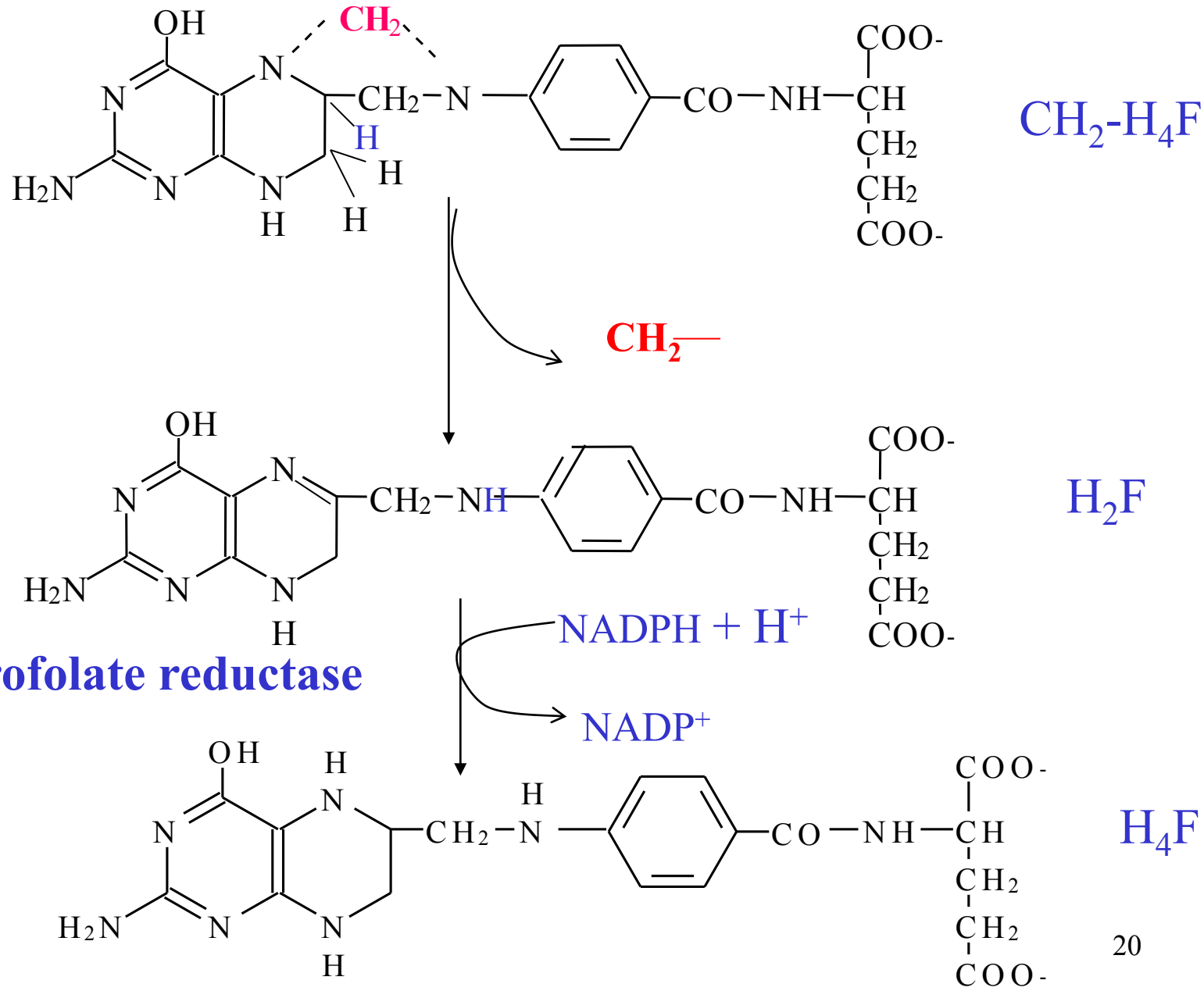
After release of methylene H_4F becomes oxidized to H_2F

UDP → dUDP → dUMP → TMP

Thymidylate synthase

(folate dependent enzyme)

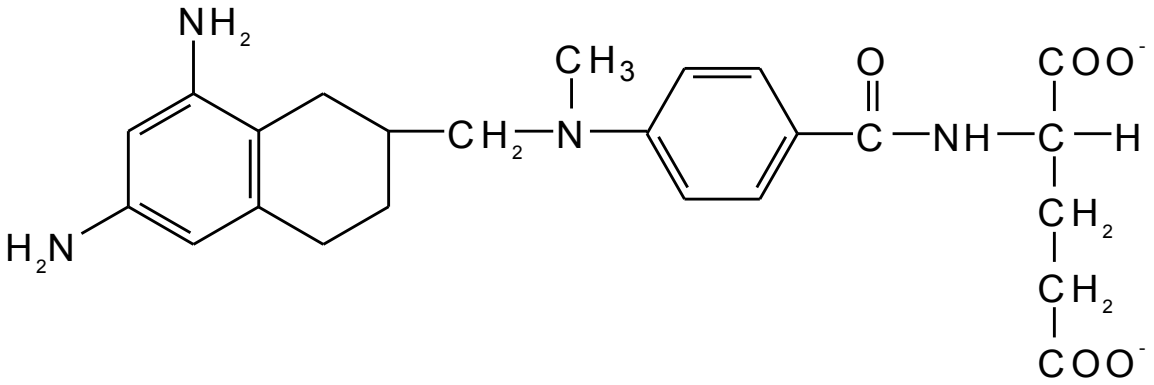



CH2-H4F
CH2—
H2F
NADPH + H+
NADP+
H4F
Dihydrofolate reductase

(Dihydro)folate reductase

reduces dihydrofolate (H_2F) back to tetrahydrofolate (H_4F)

Why metotrexate (amethopterine) functions as antineoplastic agent?



Many antineoplastic drugs inhibit nucleotide metabolism

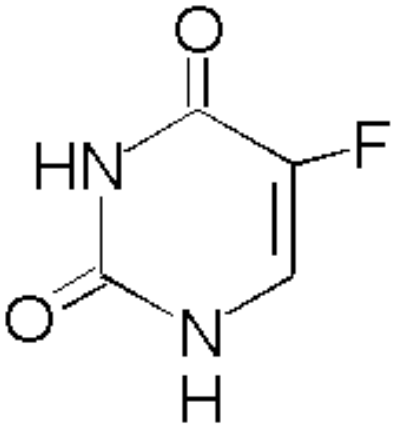
- The development of drugs with selective toxicity for cancer cells is difficult because cancer cells are too similar to normal cells
- Therefore, agents that are toxic for cancer cells are toxic also for normal cells
- Cancer cells do, however, have a higher mitotic rate than normal cells
- Therefore 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

↓

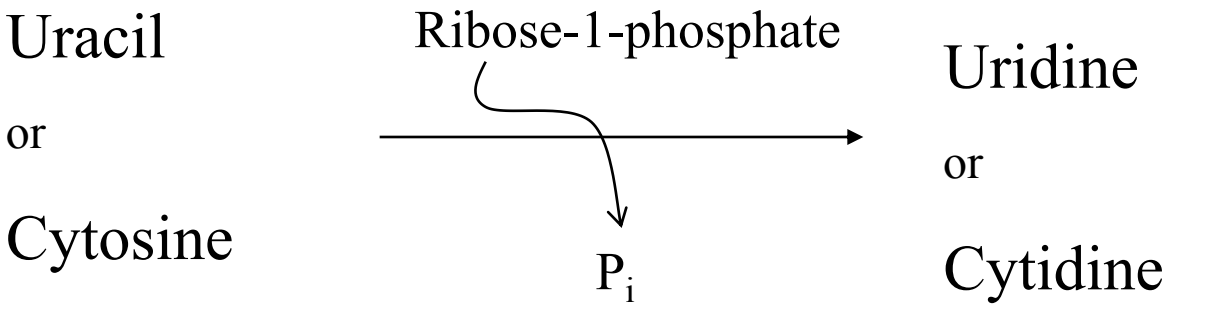
It irreversibly inhibits thymidylate synthase (suicide inhibition)

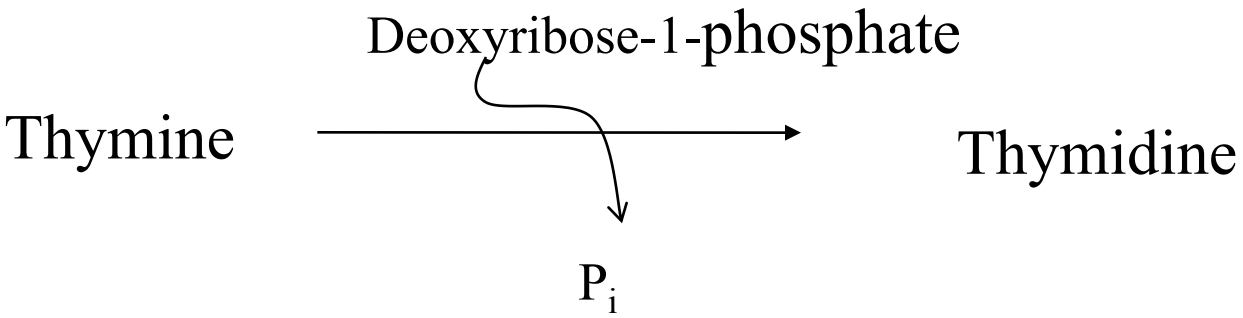
Cytostatic effect – cell division is stopped

All antineoplastic drugs are toxic not only for cancer cells but for all rapidly dividing cells, including those in bone marrow, intstinal 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

- thymidine + ATP → TMP + ADP
- cytidine + ATP → CMP + ADP
- deoxycytidine + ATP → dCMP + ADP
- uridine + ATP → UMP + 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 and of S-phase inhibition by UTP is more pronounced and activation by PRPP is reduced

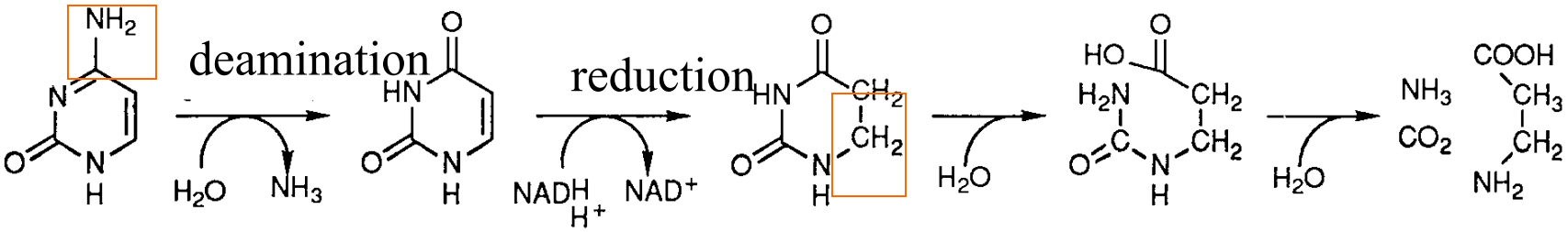
Degradation of pyrimidine nucleotides

Dephosphorylation and cleavage of nucleosides.

Free bases are converted to:

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

Soluble metabolites – excretion in urine



β-alanin

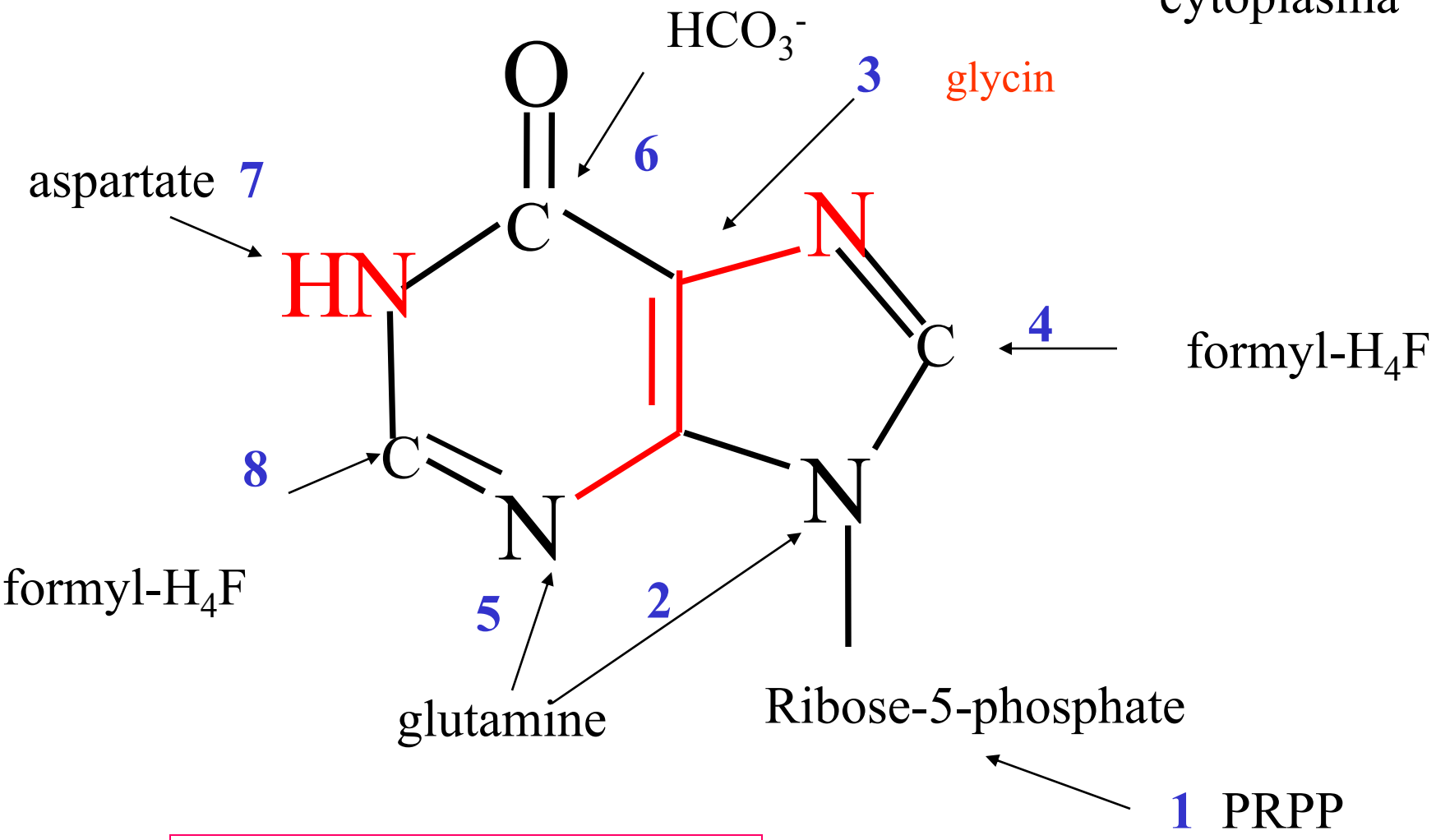
β-aminoisobutyrate from thymine

Biosynthesis of purine nucleotides

(multienzyme complex)

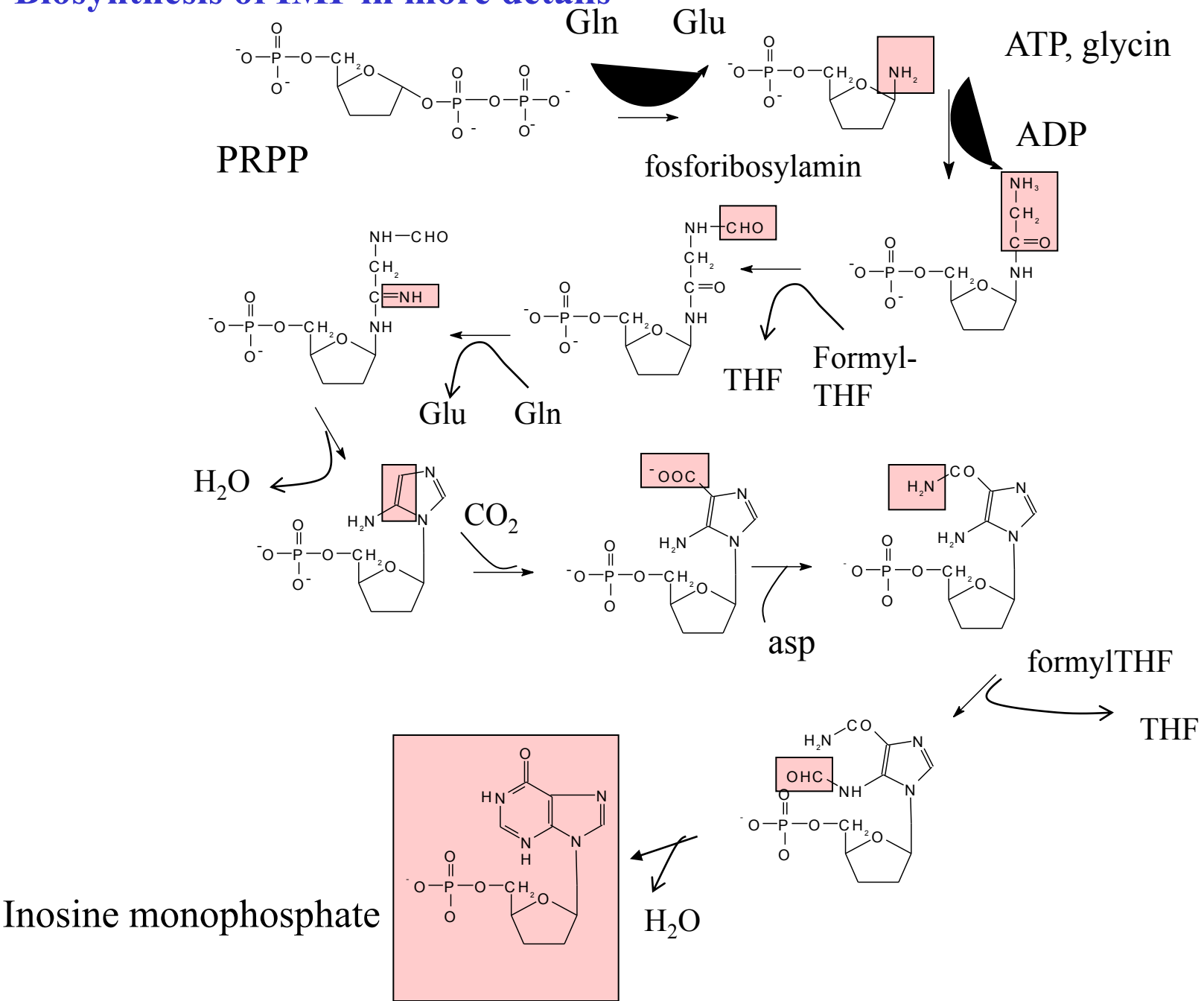
Mainly in liver

cytoplasma



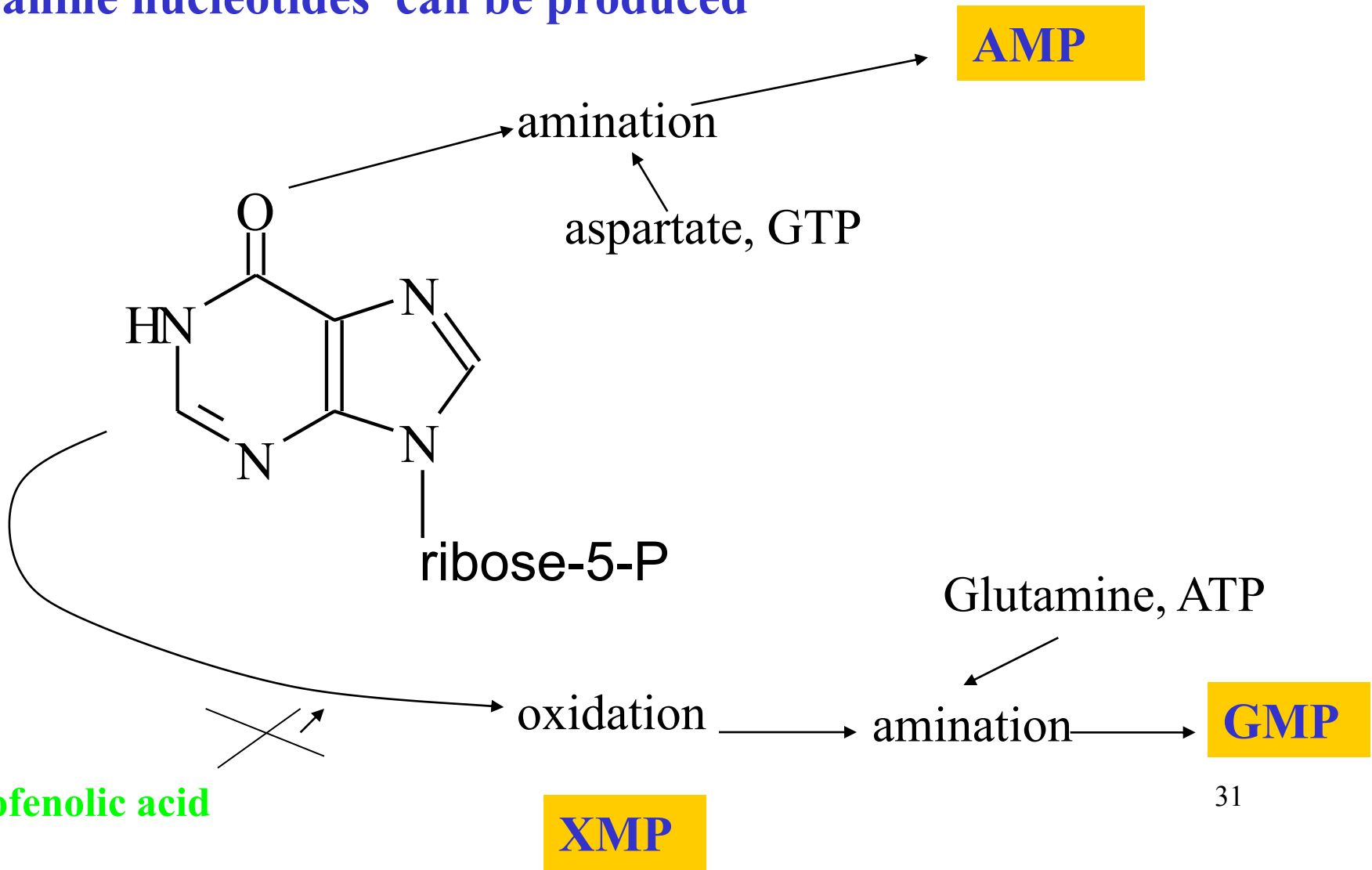
Inosine-5-P (IMP)

Biosynthesis of IMP in more details



Inosine-5-P (IMP)

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

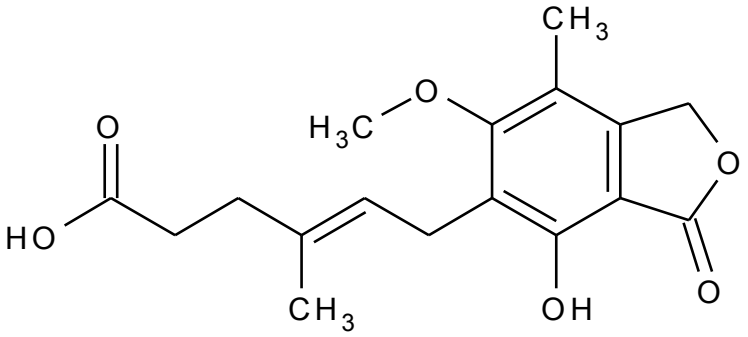


Mycophenolic acid

- Potent, reversible, uncompetitive inhibitor of IMP dehydrogenase

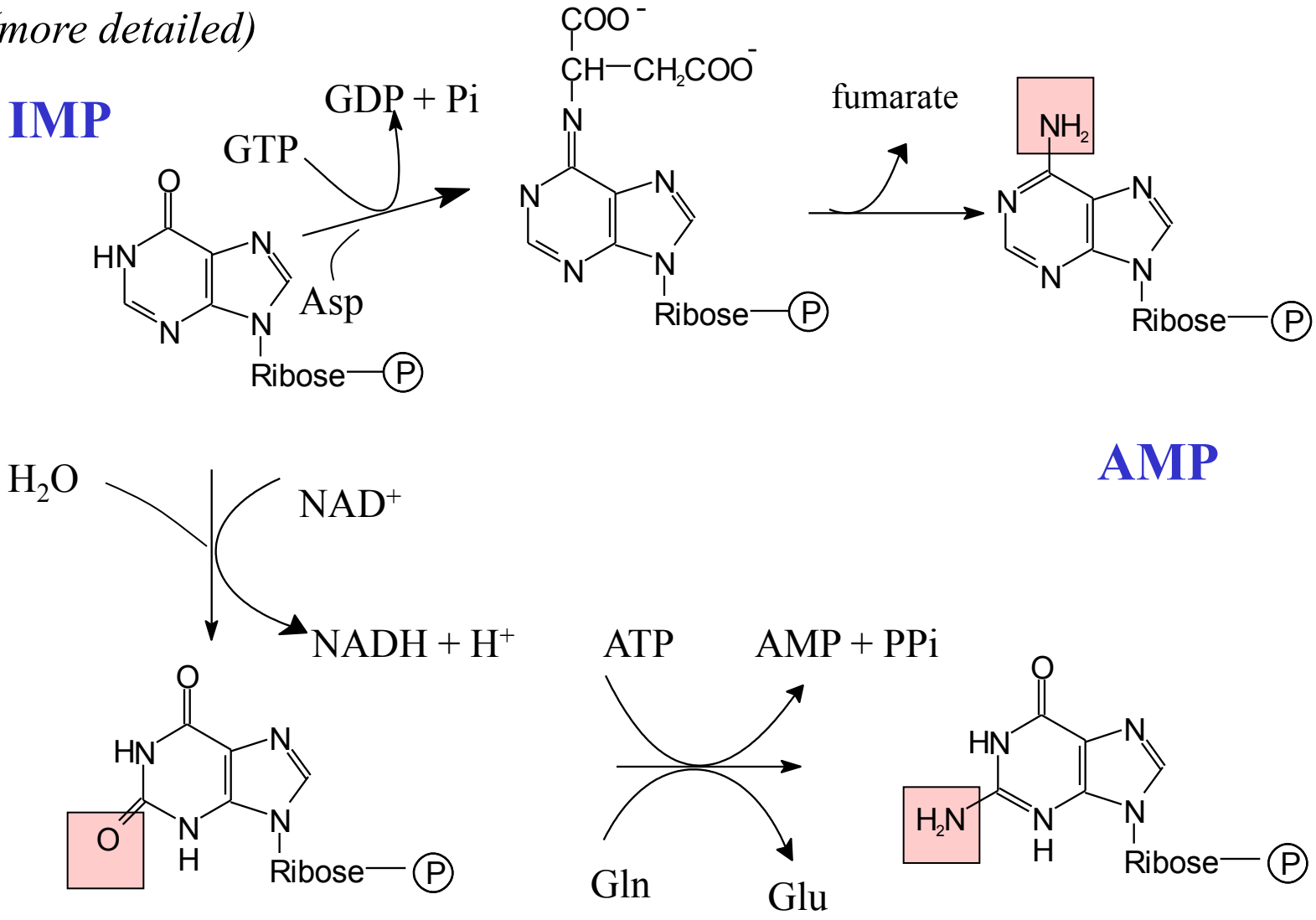
  Used in preventing graft rejection

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



Synthesis of AMP and GMP

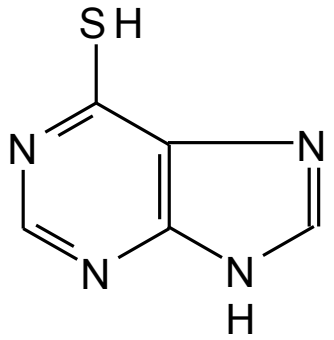
(more detailed)



Inhibitors of purine synthesis (antineoplastic agents)

- inhibitors of dihydrofolate reductase

- 6-mercaptopurine- inhibition of conversion of IMP to AMP and GMP



mercaptopurine

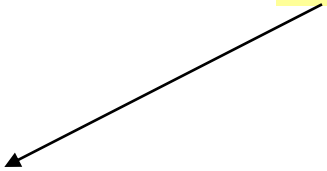
Synthesis of purine nucleotides by salvage pathway

Extrahepatal tissues

Recyclation of free bases

Phosphoribosyltransferases:

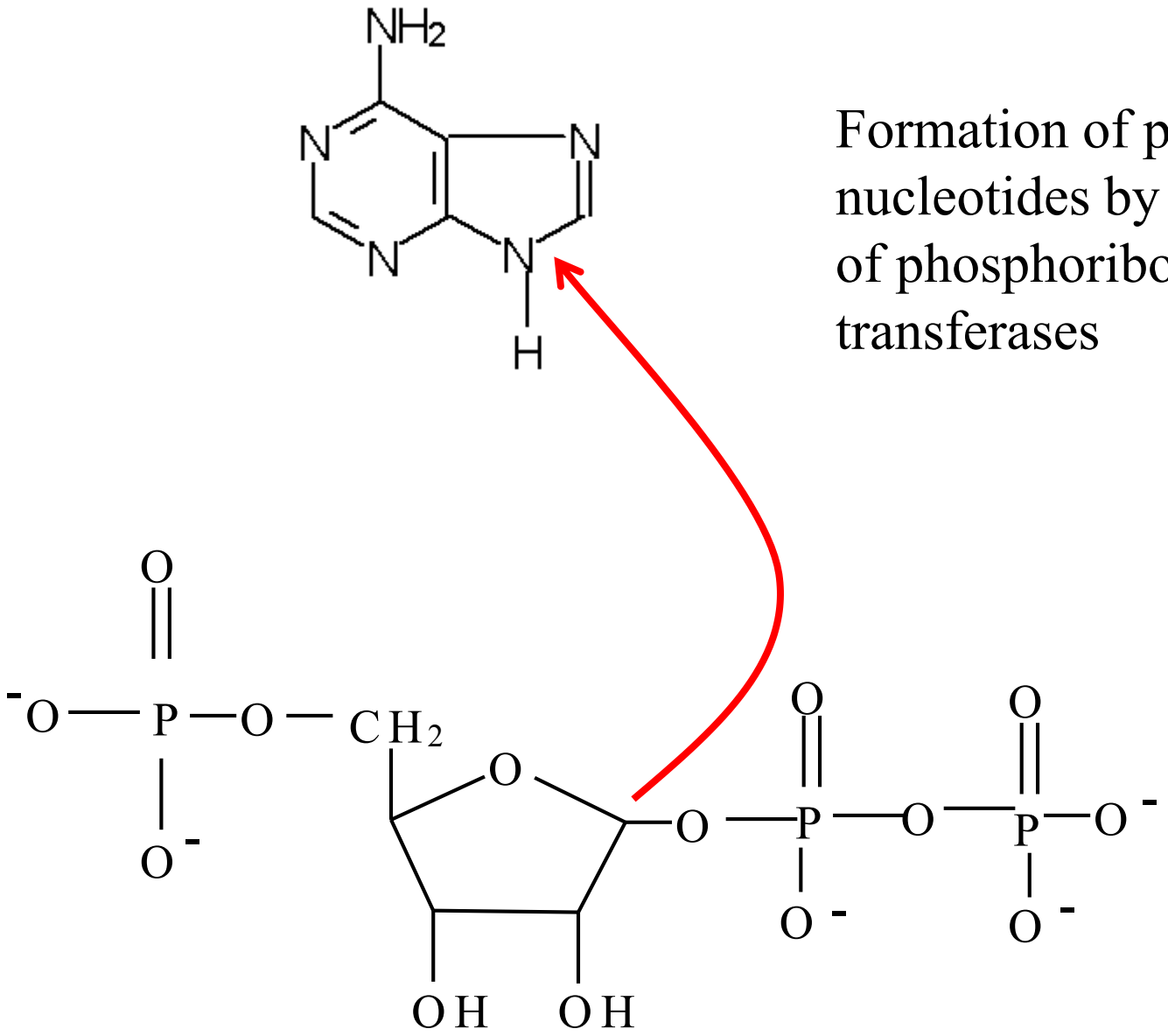
Adenine phosphoribosyltransferase
Hypoxantine phosphoribosyltransferase



Purine + PRPP → purine nucleotide monphosphate + PP

Recyclation of purine bases by phosphoribosyltransferase.

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

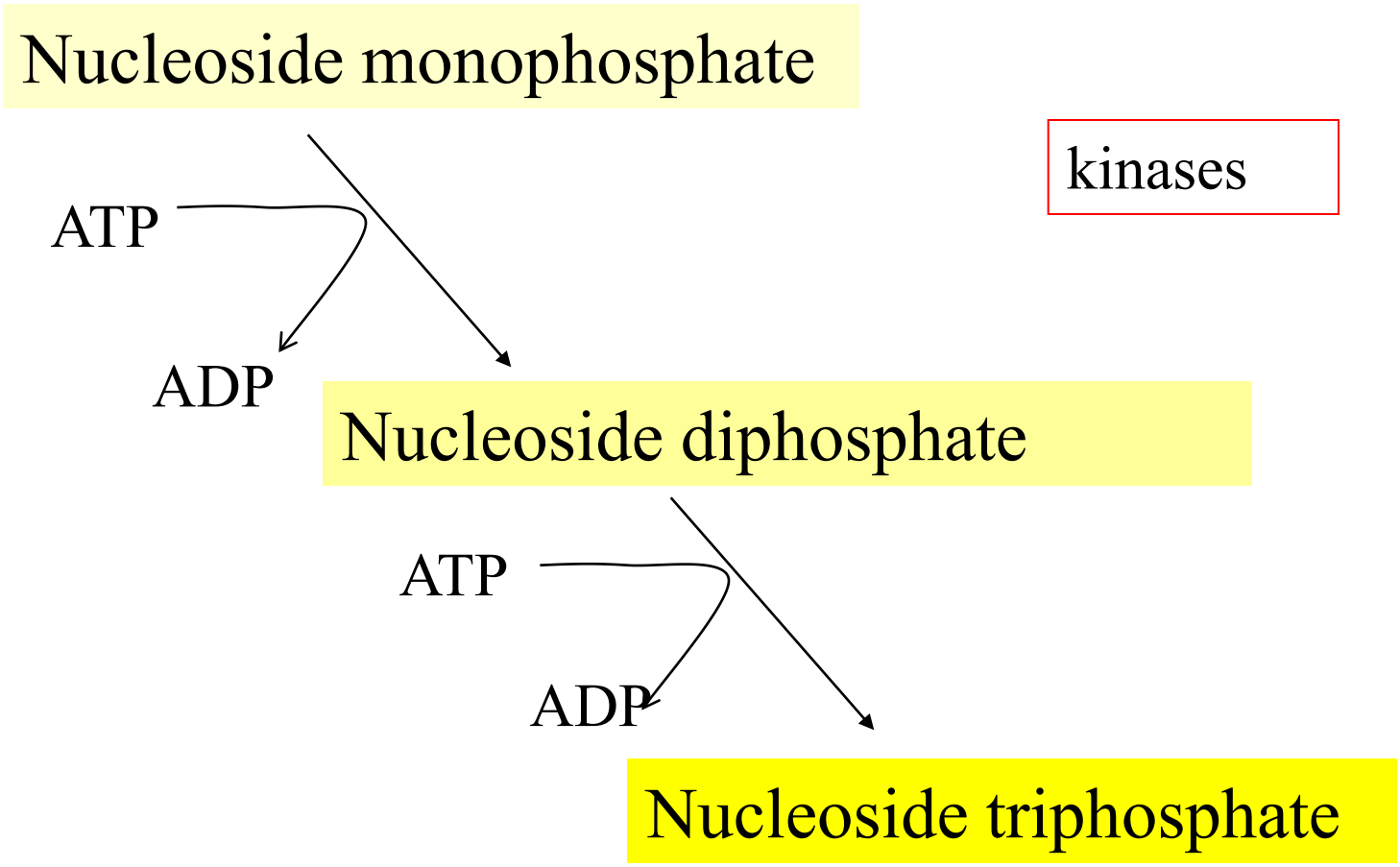


Formation of purine nucleotides by the action of phosphoribosyl transferases

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



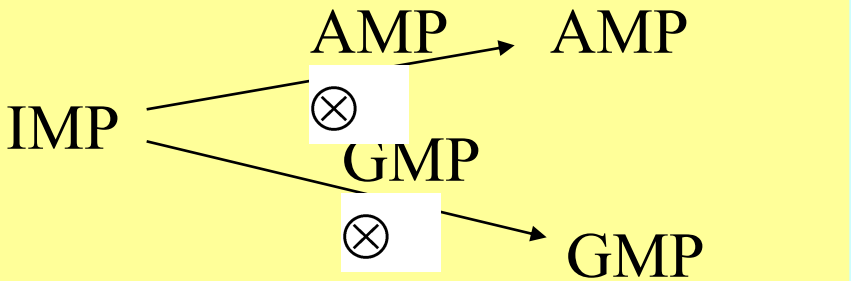
Regulation of purine nucleotide biosynthesis

The main factor is availability of PRPP

1.

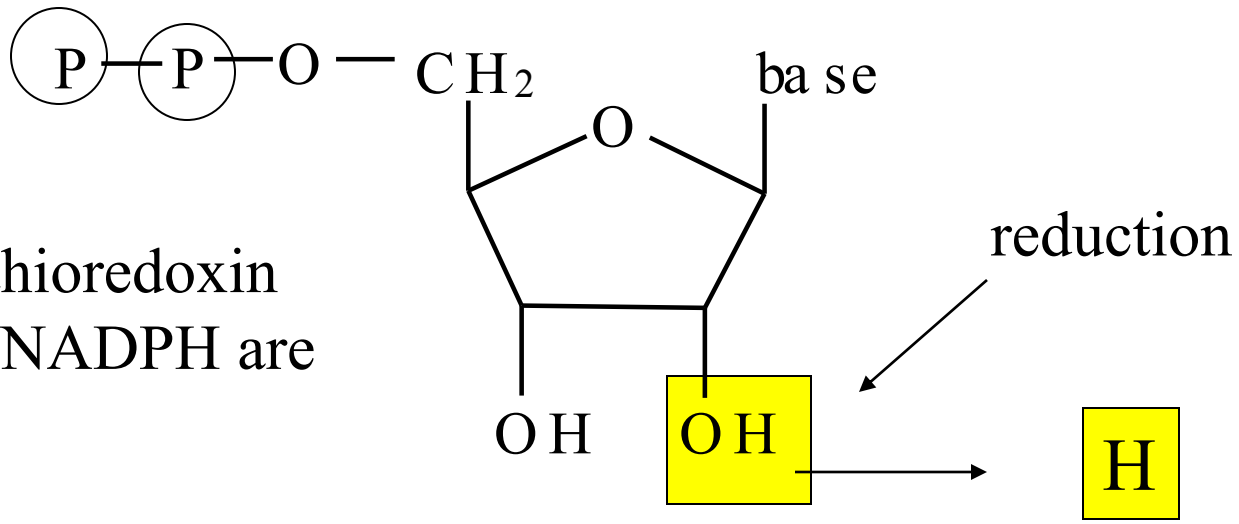
- inhibition of PRPP-glutamylamidotransferase by AMP, GMP, IMP (end-products), activation by PRPP

2.



Formation od 2-deoxyribonucleotides (purine and pyrimidine)

Nucleoside diphosphate → 2-deoxynucleoside diphosphate



Thioredoxin, thioredoxin reductase and NADPH are required

deoxygenation

Thioredoxin reductase is selenoenzyme

Hydroxyurea

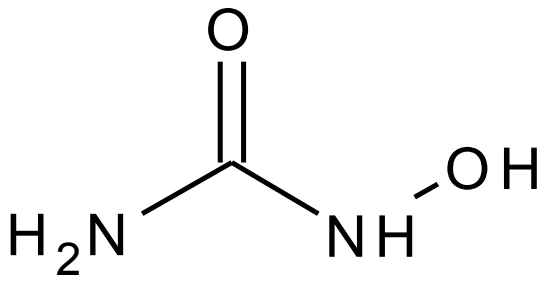
Hydroxyurea inhibits ribonucleotide reductase

↓

Synthesis of deoxyribonucleotides is blocked

↓

Treatment some of some cancers



Degradation of purine nucleotides

In liver

nucleosides

AMP,GMP,

IMP,XMP

5-nucleotidase

removal of phosphate

guanosine, inosine,
xantosine + P_i

Adenosine + P_i,

Degradation of purine nucleotides

Nucleotides

AMP,GMP,

IMP,XMP

5-nucleotidase

Nucleosides

guanosine, inosine,
xantosine + P_i

Adenosine + P_i,

NH_3

Adenosine deaminase

inosine

nucleoside phosphorylase

Phosphorolytic cleavage of ribose

Bases

guanine,

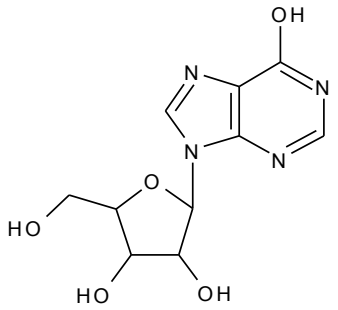
hypoxantine,

xantine

+ ribose-1-P

nucleoside phosphorylase

Phosphorolytic cleavage of ribose



Adenosine deaminase deficiency

Enzyme deficiency → acummulation 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 devide.

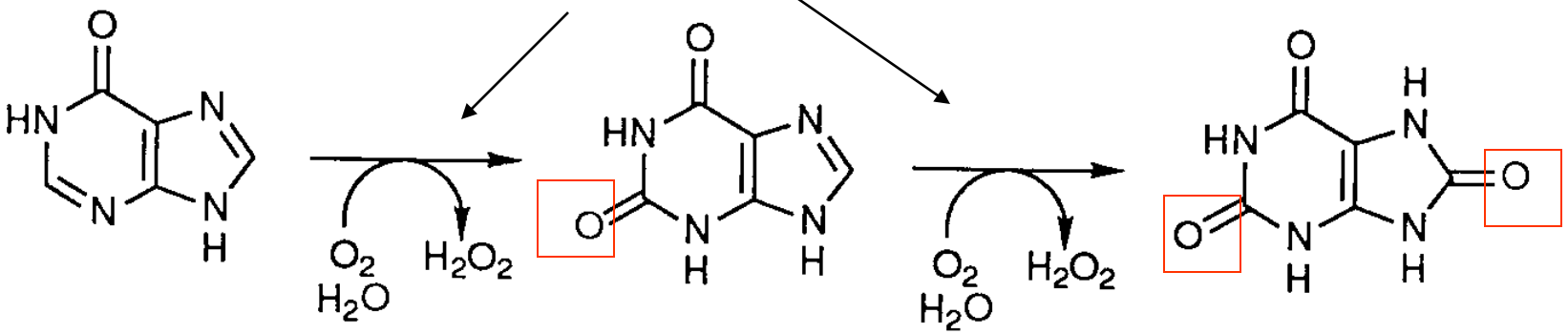
One of the causes severe combined immunodeficiency disease (SCID).

Treatment by gene therapy

Degradation of purine bases

Inhibition by oxypurinol

Xantine oxidase



hypoxantine

xantine

Uric acid

guanin

guanase

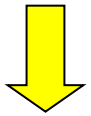
Final product of human purine degradation (400-600 mg /day)

Gout

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

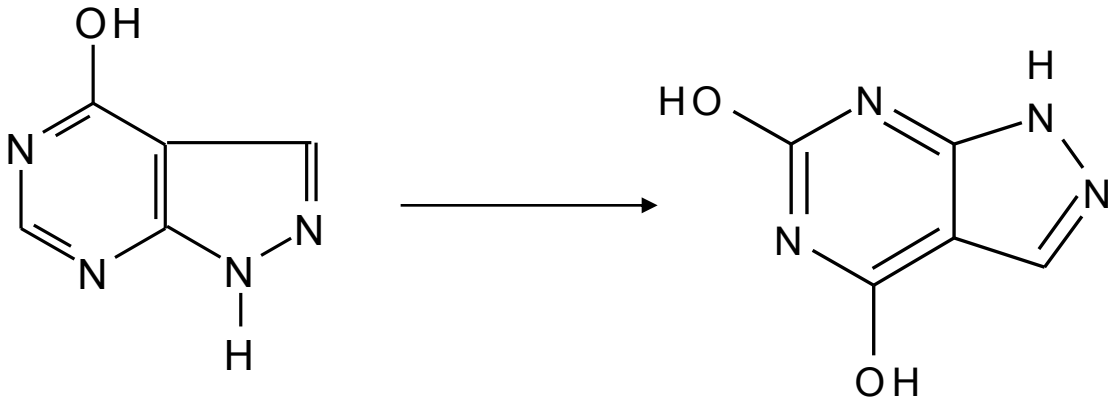
Causes:

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



Deposition of urate crystals in joints→ infammatory 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 xantinoxidase



Treatment of gout: oxypurinol inhibits xantine oxidase

More soluble xantine and hypoxantine are accumulated.

Hypoxantine can be „salvaged“ in patients with normal level of hypoxantine phosphoribosyltransferase.

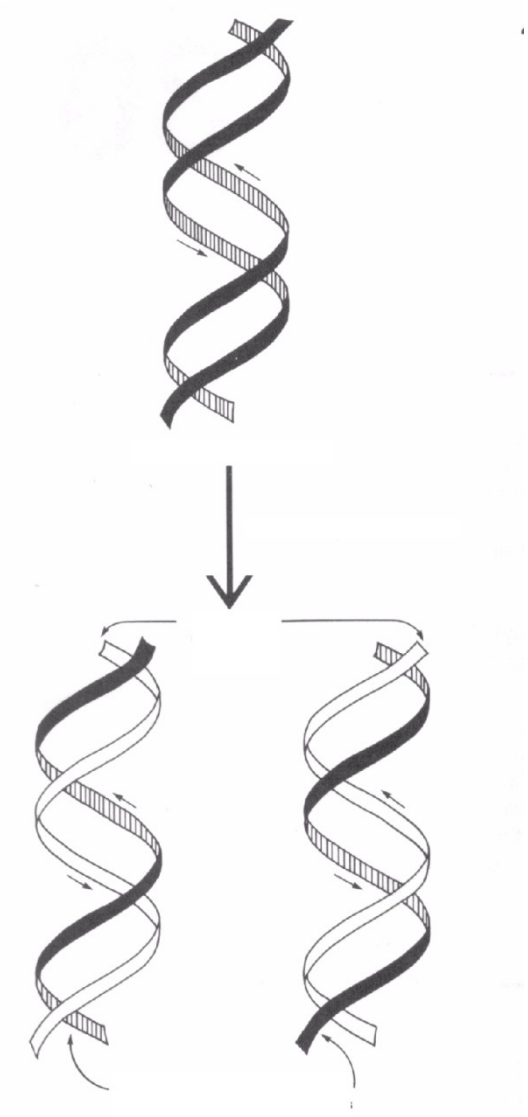
Replication of DNA

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)

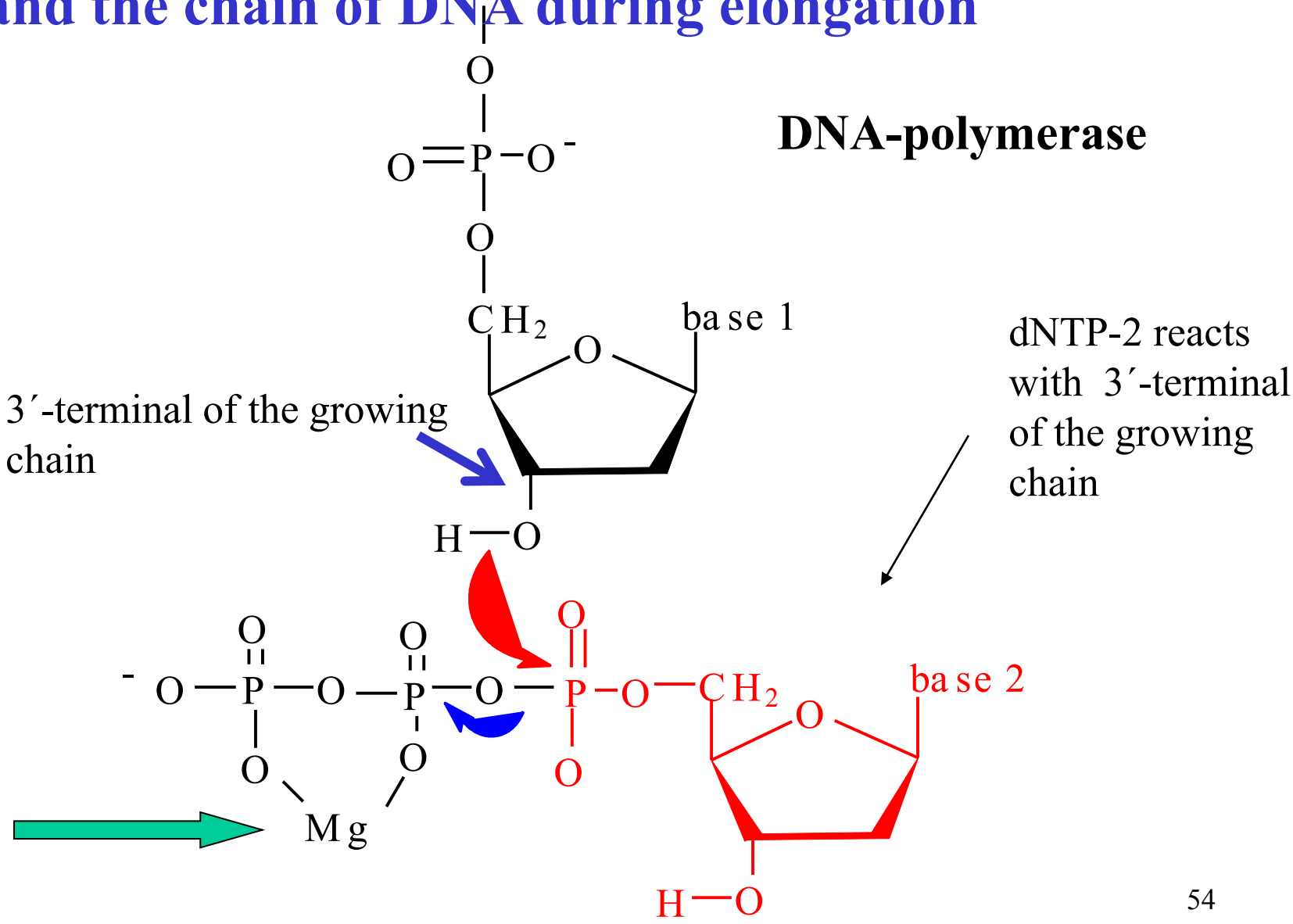
Diphosphate is released and dNMP is attached by ester bond

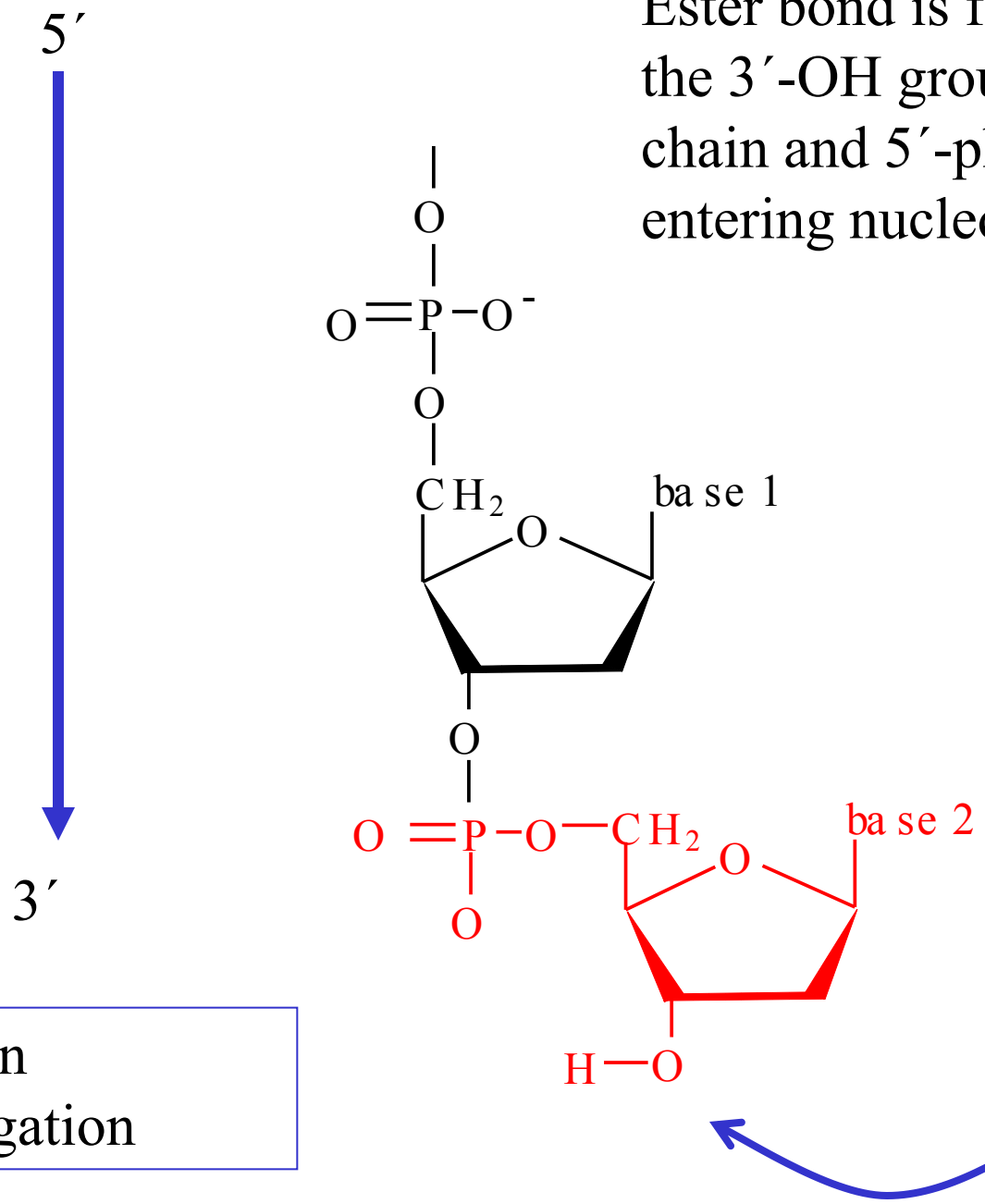
$$(DNA)_n + dNTP \rightarrow (DNA)_{n+1} + PP_i$$

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 Mg^{2+} ions).

dNTP3

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.

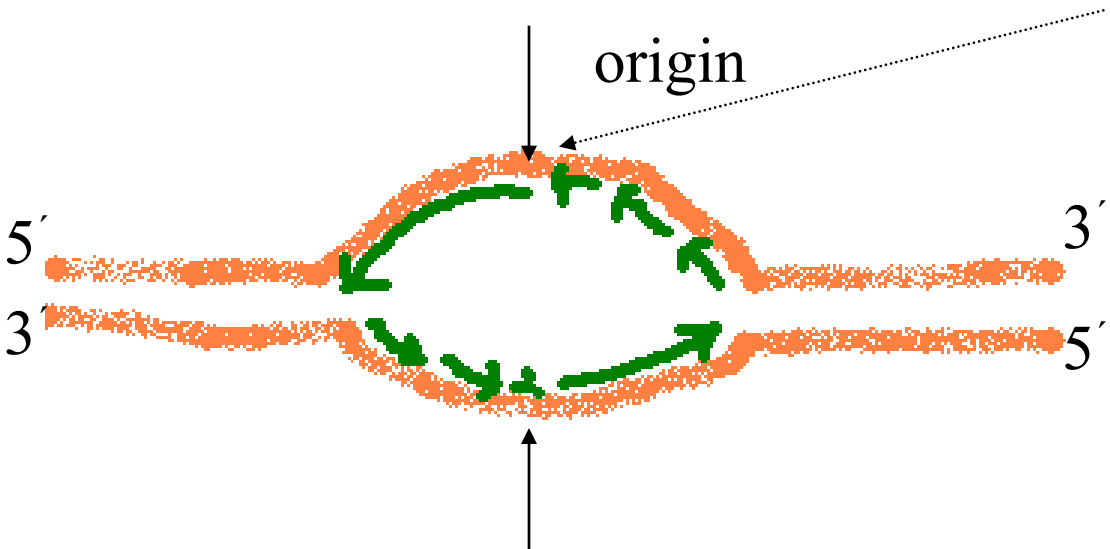
Replication proceeds on both strands

- 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

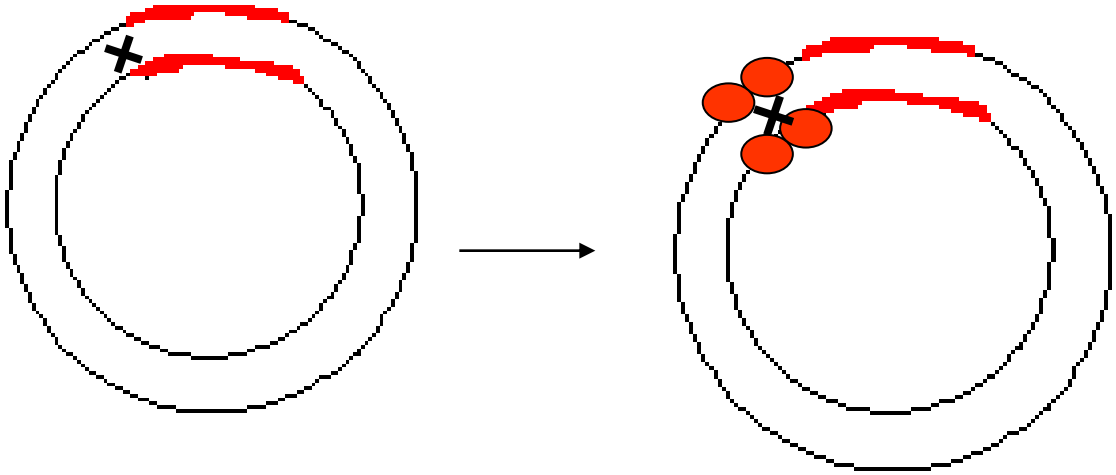


Beginning with one parental double helix two newly synthesized stretches of nucleotide chains must grow in opposite direction – one in 5→3 direction toward the replication fork and the other 5→3 direction away from replication fork

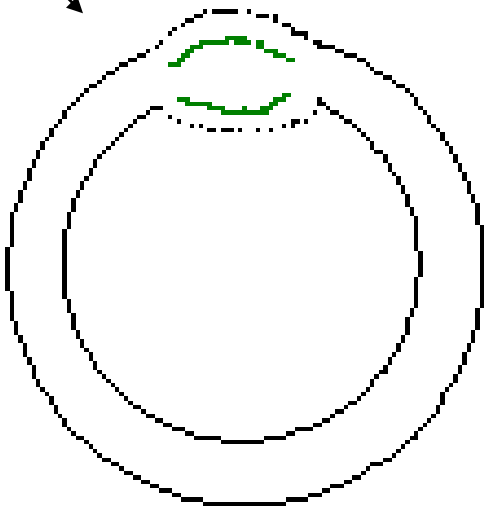
Initiation in prokaryotes

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.

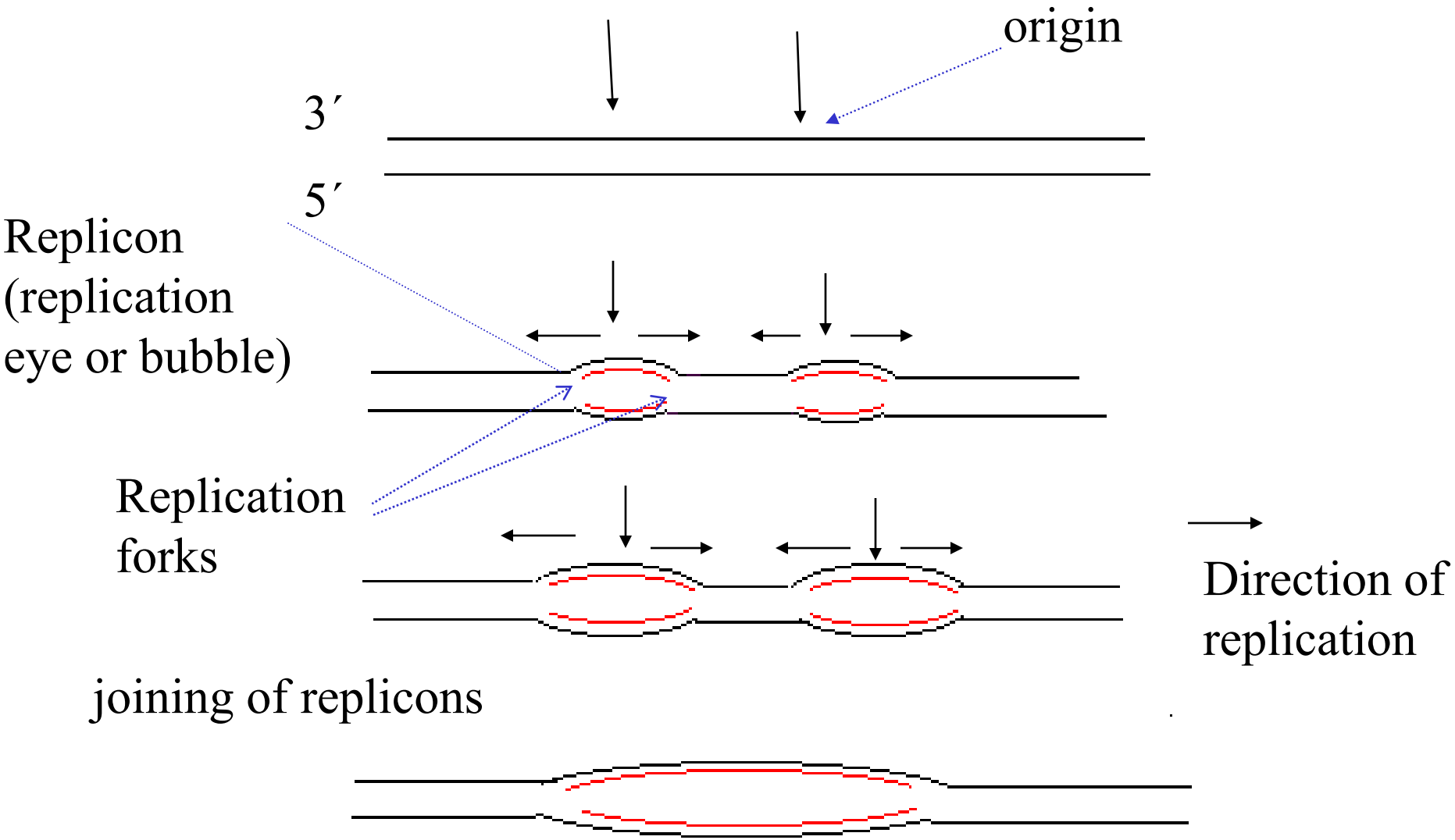
•**Replication** is initiated at **multiple origins** (up to several hundred in each chromosome, one every 30 to 300 kbp) **in both directions**.

• Initiation is controlled by time and space,

• Replication rate is lower then in eukaryotes, Okazaki fragments are much smaller in eukaryotes (200 of bases) then prokaryotes (1000-2000 of bases)

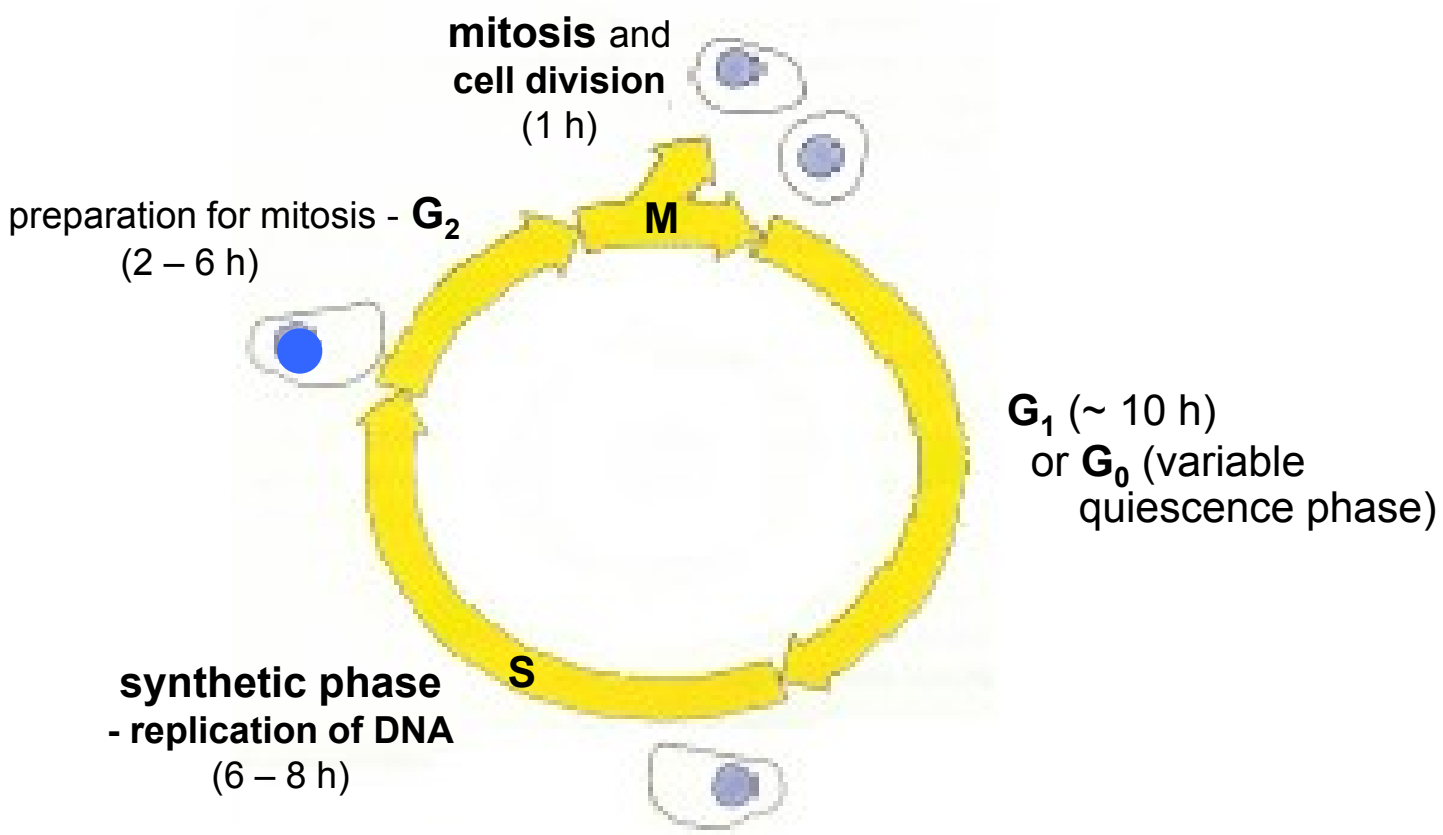
•Occurs in S-phase

Eukaryotic DNA replication

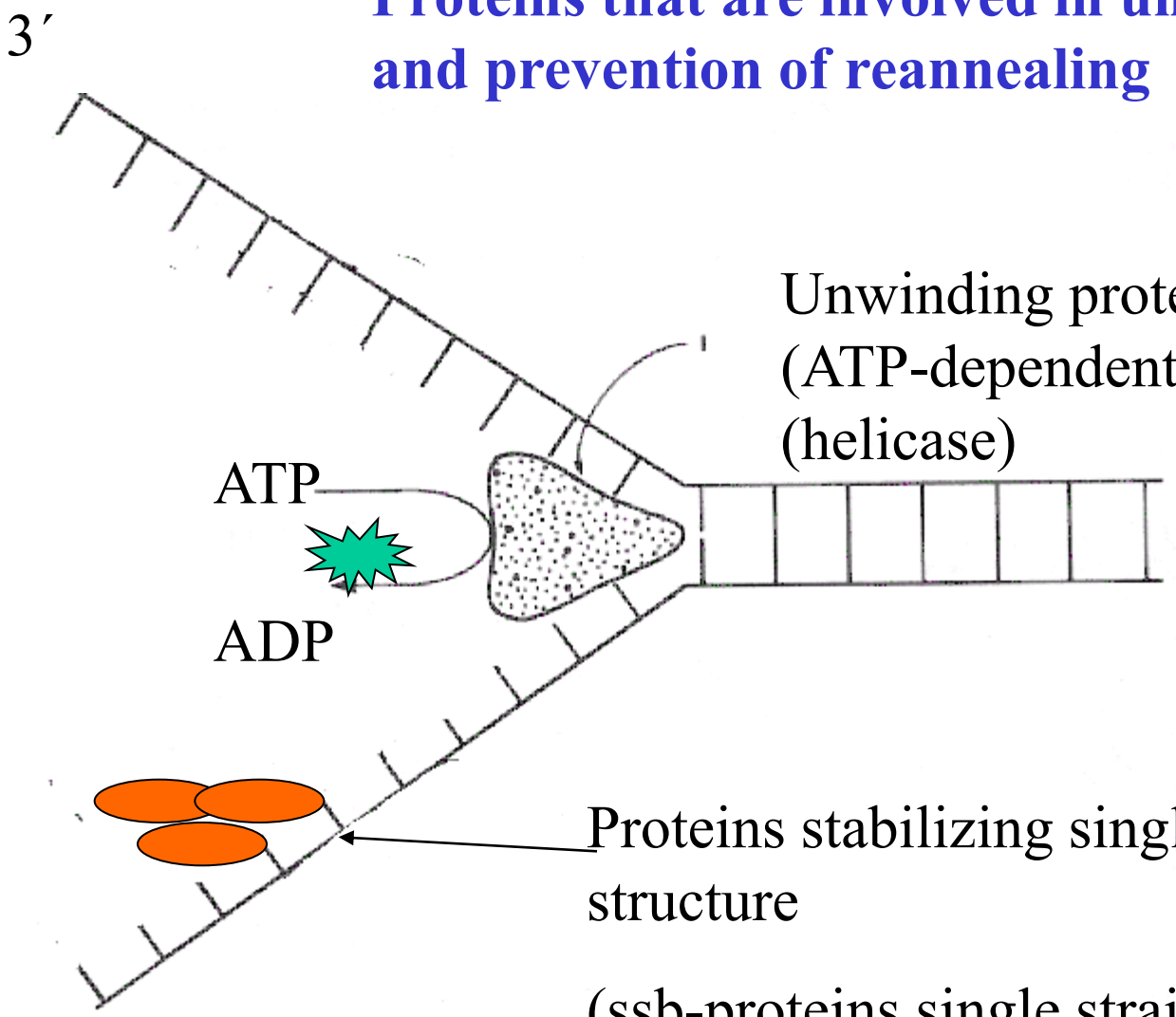


Eukaryotic DNA replication

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



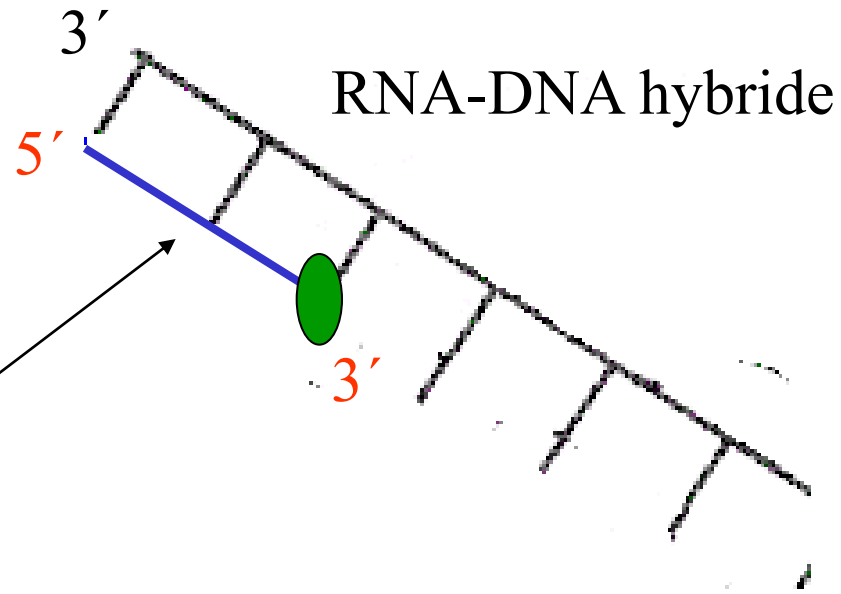
RNA primer is necessary for DNA synthesis

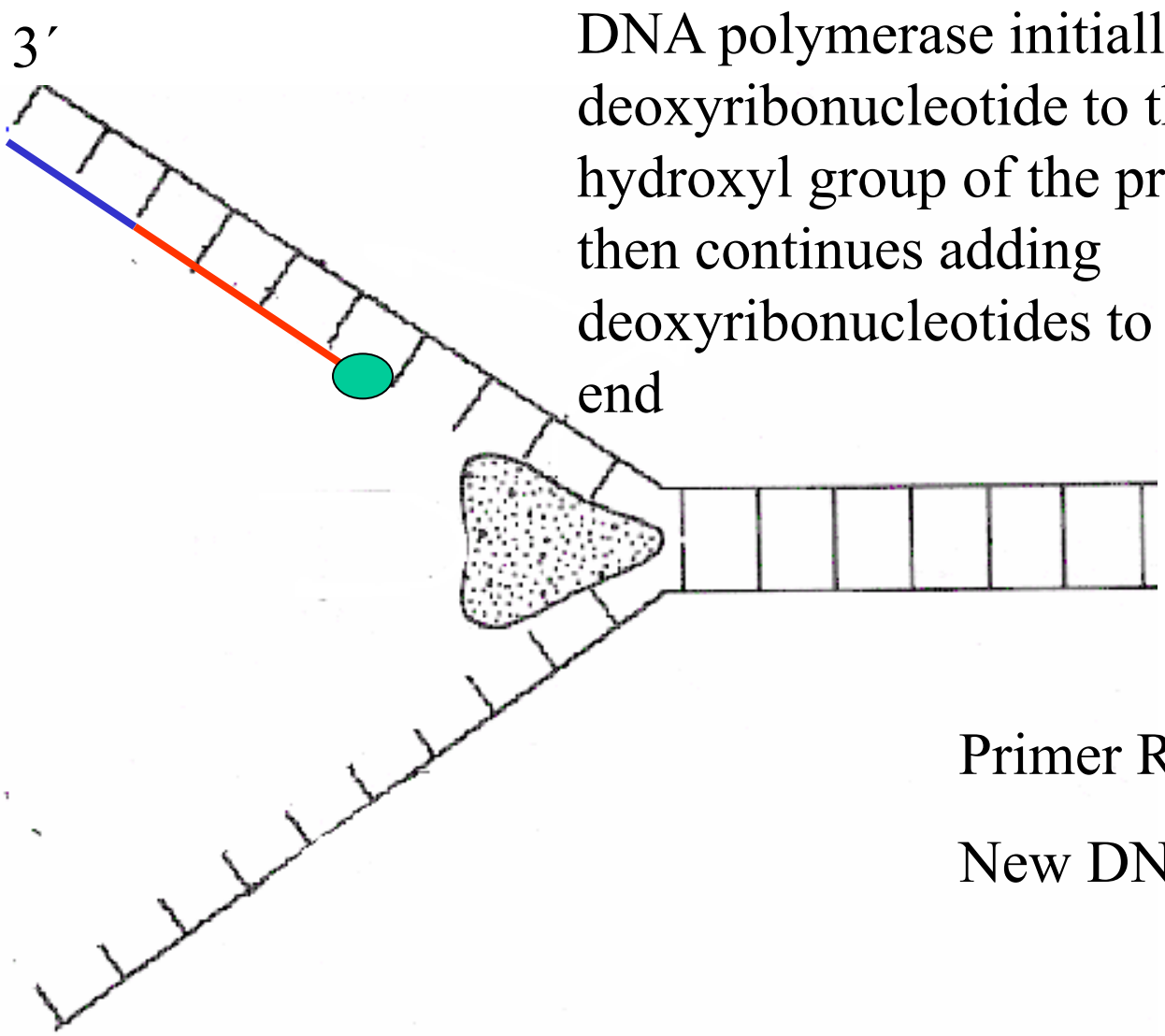
•DNA polymerase cannot initiate de novo synthesis of the chain, it requires free 3′-OH group for linking a new nucleotide.

• 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



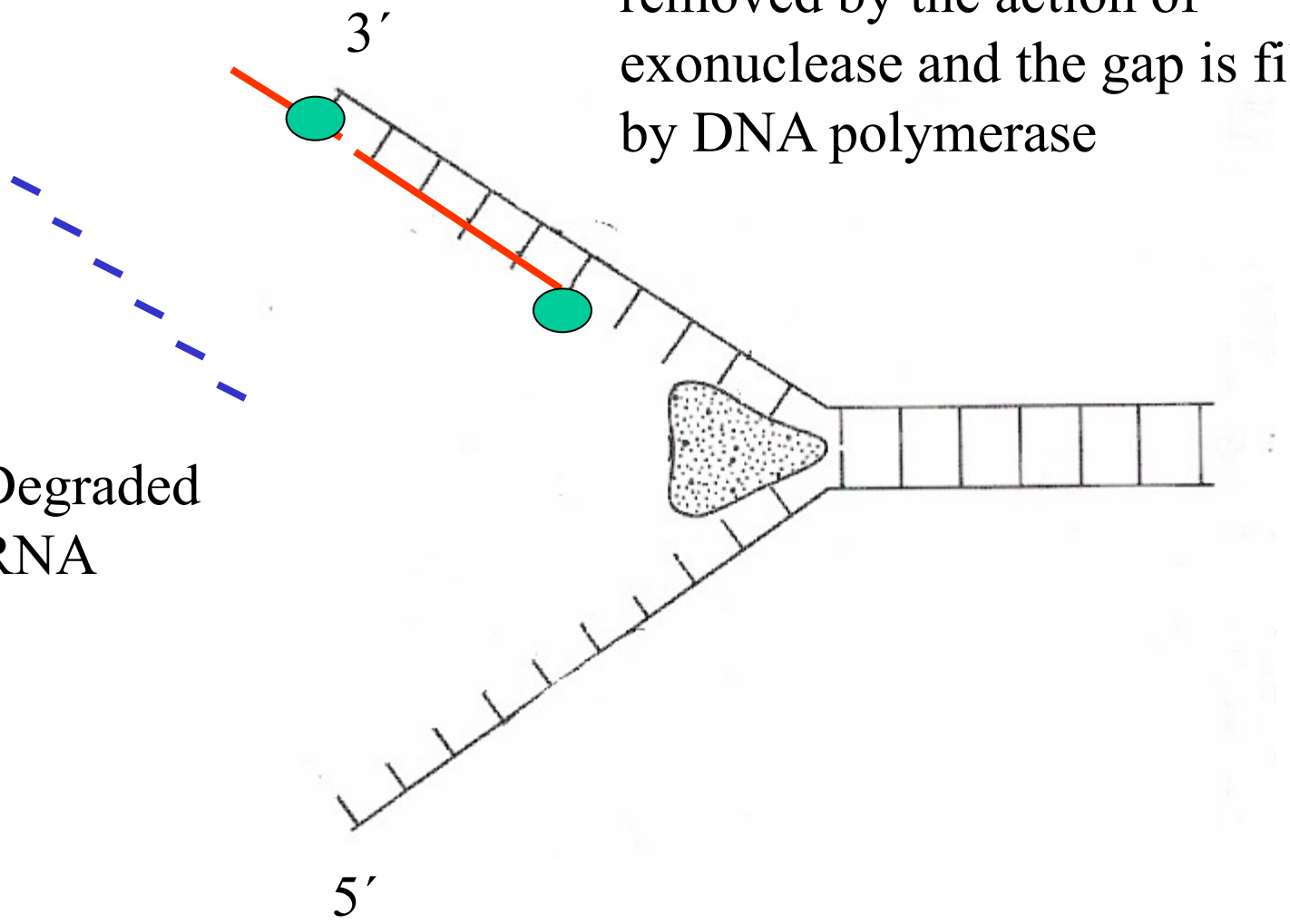


DNA polymerase initially adds a deoxyribonucleotide to the 3′-hydroxyl group of the primer and then continues adding deoxyribonucleotides to the 3′-end

Primer RNA

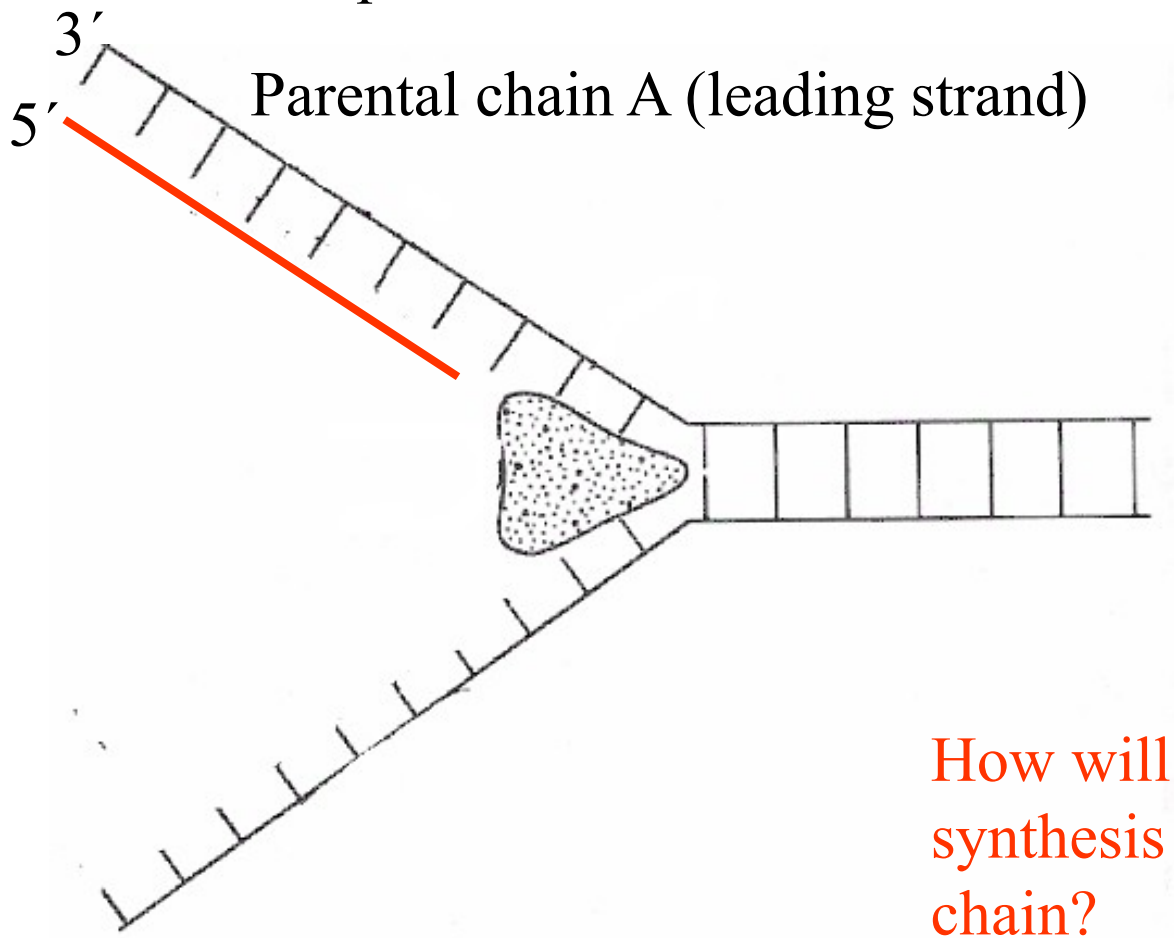
New DNA

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



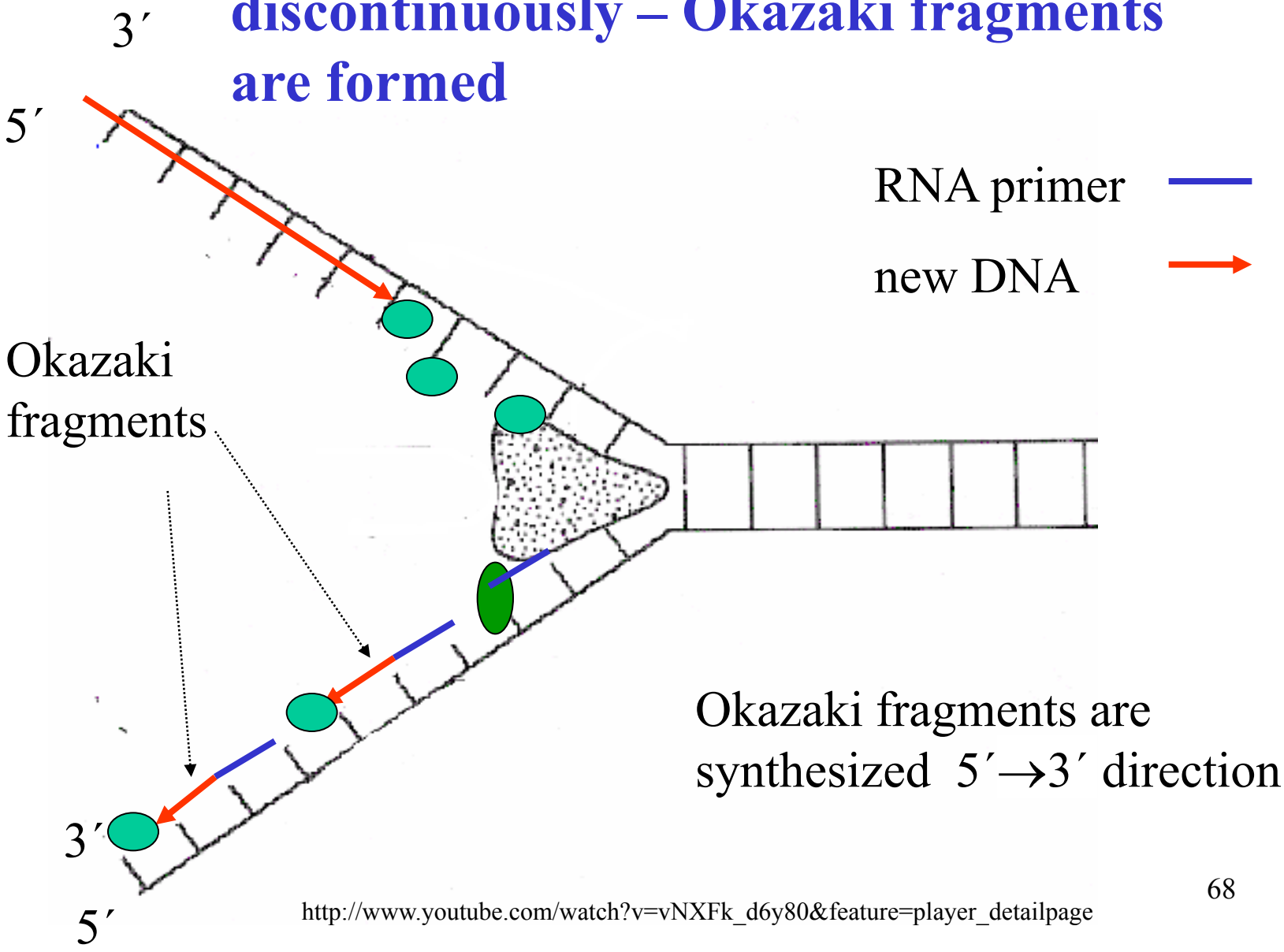
Synthesis of DNA proceeds always in 5′→ 3′ direction

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



How will occur the synthesis among the B chain?

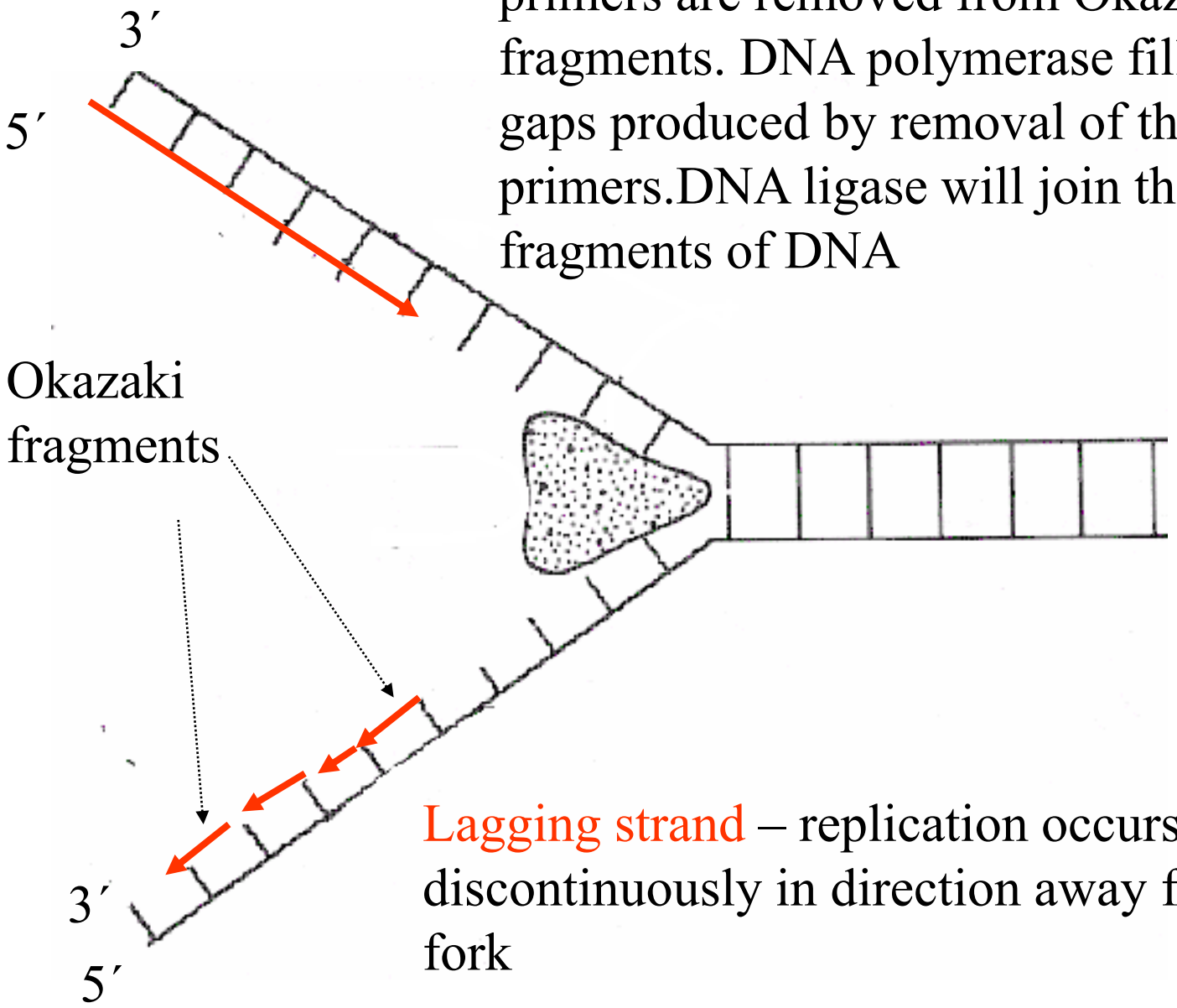
The lagging strand is synthetized discontinuously – Okazaki fragments are formed



Okazaki fragments are synthesized 5′→3′ direction

http://www.youtube.com/watch?v=vNXFk_d6y80&feature=player_detailpage

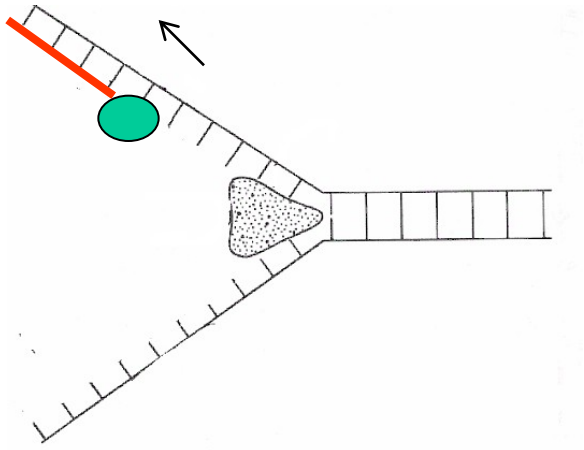
As replication progress, the RNA primers are removed from Okazaki fragments. DNA polymerase fills the gaps produced by removal of the primers.DNA ligase will join the fragments of DNA



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

Proofreading of newly syjthesized DNA structure

- Precision of replication ~ 1mistakes /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′→5′ exonuclease activity edits the mistake.
- The 5′→3′polymerase then replaces it with the correct nucleotide.



DNA-polymerases have 5′→3′polymerase activity and 3′→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

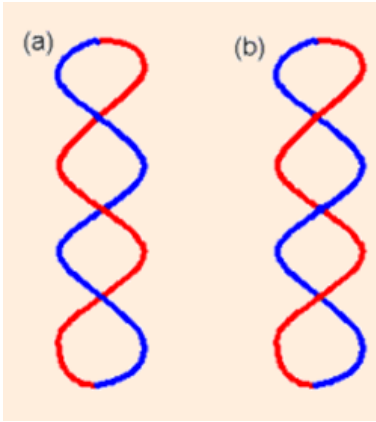
Polymerase*	**Polymerase activity** **(for all enzymes 5′ → 3)**	**Exonuclease activity**
DNA polymerase α	replication, DNA repair	no
DNA polymerase β	DNA repair	no
DNA polymerase γ	replication in mitochondria	3 →5
DNA polymerase δ**	replication, DNA repair	3 →5
DNA polymerase ε	replication	3 →5

* At least 9 polymerases is known **major replicative enzyme

Topoisomerase

(Topology od DNA = tridimensional structure of DNA)

positive negative supercoiling



Topoisomerase regulates the formation of superhelices

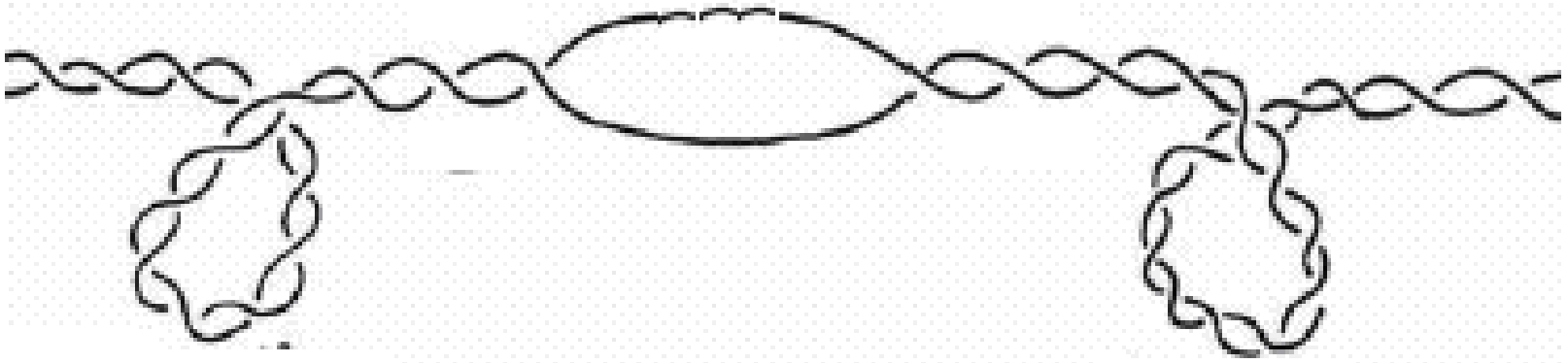
These enzymes catalyze the concerted breakage and rejoining of DNA strands, producing a DNA that is more or less superhelical than the original

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.)

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.

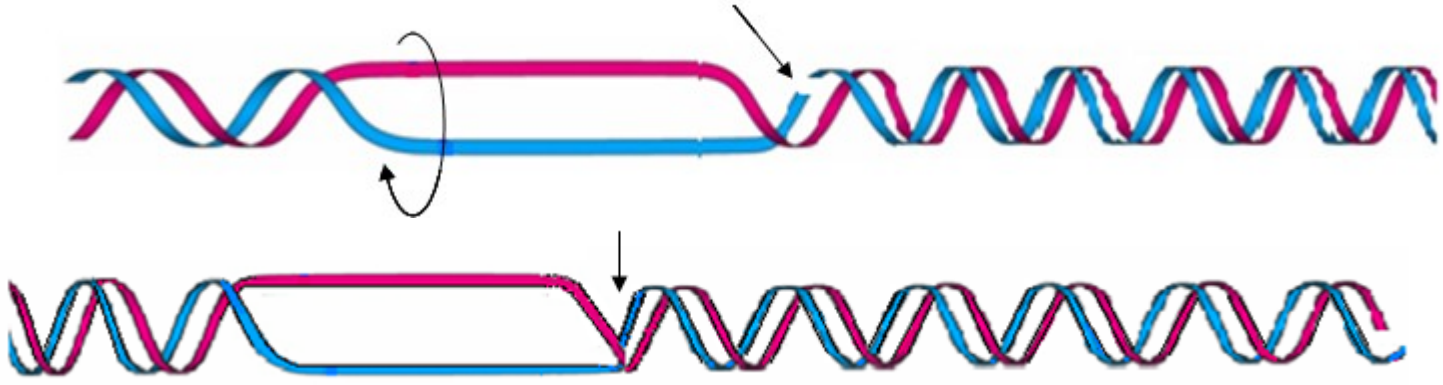
Present in prokaryotes and eukaryotes.

Requires ATP cleavage energy.

.

Action of topoisomerase I

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



Inhibitors of human topoisomerase prevent replication

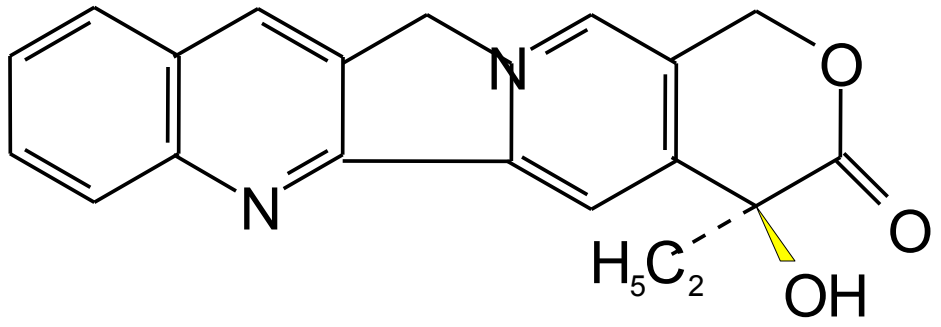
Antineoplastic drugs

Examples of topoisomerase inhibitors

camptothecine – plant product

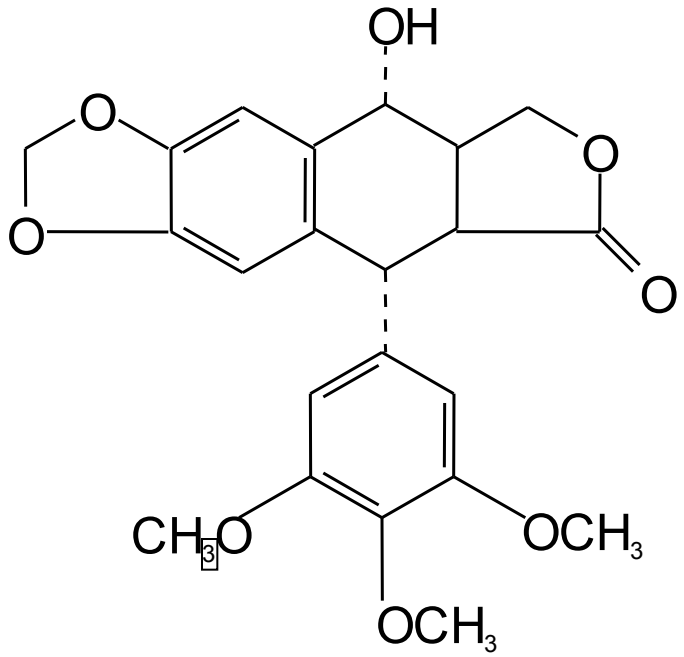
antracyclines (daunorubicine) -bacterial products

podophyllotoxines-plant product



camptothecine

Camptotheca acuminata



podofyllotoxine

Podophyllum peltatum ad.

Telomeres

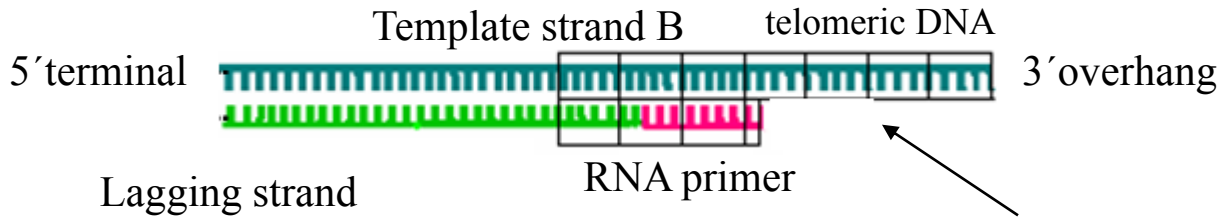
Eukaryotic chromosomes are linear. A solution must be found to two problems:

- 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 develop 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.



Telomers are special sequences at the ends of chromosomes

Tandem repeats od species-specific oligonucleotides, G-rich

(in human TTAGGG up to 1000x)

They protect the ends of chromosomes against nuclease activities.

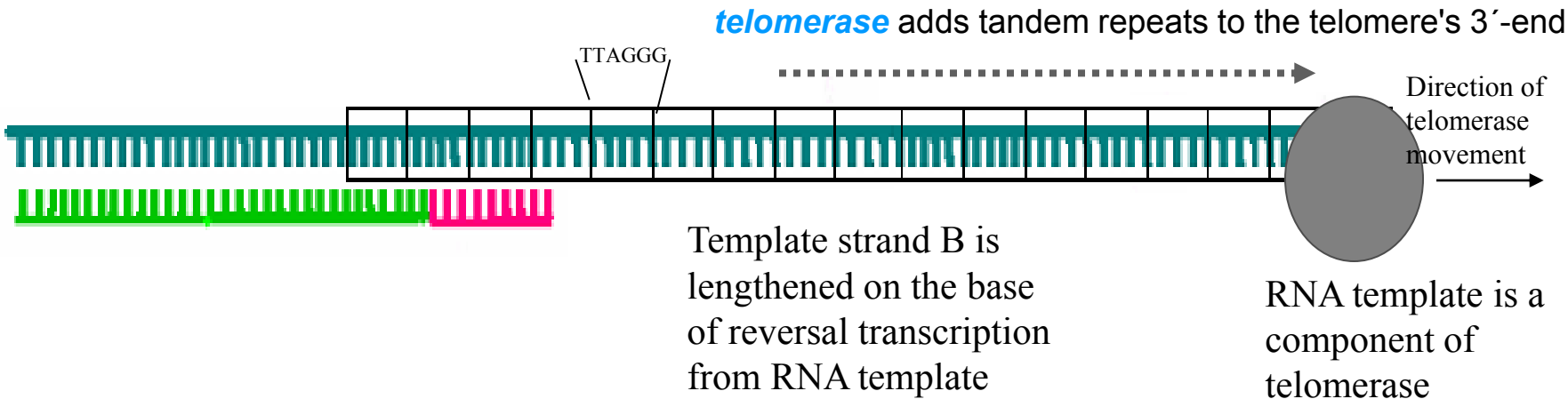
Telomerase

• completition 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.

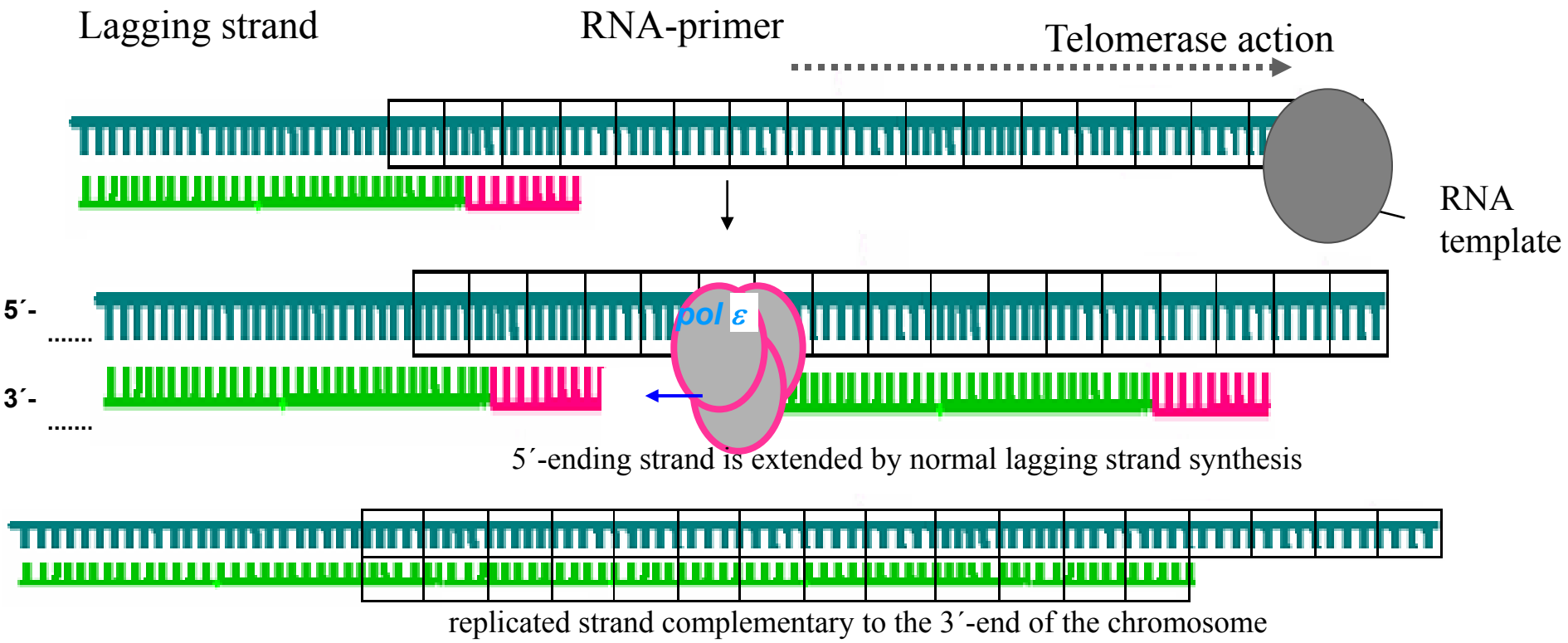
•Then the telomerase moves down the DNA toward the new 3´end and repeats the process a number of times.



http://faculty.plattsburgh.edu/donald.slish/Telomerase.html

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 addult 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 (10^4purines/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**.