Metabolism of purine and pyrimidine nucleotides

DNA replication

Biochemistry I
Lecture 12

2008 (J.S.)

Purine and pyrimidine nucleotides are used in large quantities in the biosynthesis of nucleic acids. They are **synthesized *de novo*** by most organisms, the synthetic pathways are basically similar in all of them.

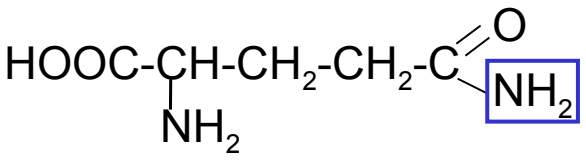
Some types of cell synthesize nucleotides from purines and pyrimidines salvaged from the degradation of nucleic acids by **scavenger pathways.**

Purine and pyrimidine bases released from the nucleic acids ingested as components of food are broken down in the gastrointestinal tract and are not utilized in the synthesis of nucleotides.

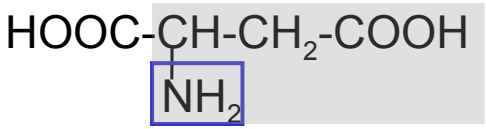
The allosteric feedback control mechanisms balance the synthesis of all purine and pyrimidine ribonucleotides and deoxyribonucleotides..

Glutamine, aspartate, tetrahydrofolate (H_4folate) and phosphoribosyl diphosphate (PRPP) are compounds of special importance in the biosynthesis of pyrimidine and purine bases.

Glutamine and **aspartate** are donors of amino groups for the synthesis of both pyrimidine and purine bases, and aspartate supplies three atoms of carbon for the pyrimidine ring. .

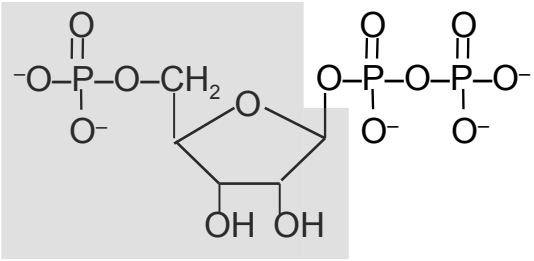


Glutamine



Aspartate

Phosphoribosyl diphosphate (**PRPP**, 5-phosphoribosyl 1-diphosphate) supplies the phosphorylated ribosyl for the synthesis of all nucleotides.
The synthesis of PRPP is under feedback control being inhibited allosterically by the intracellular nucleoside diphosphate concentration.



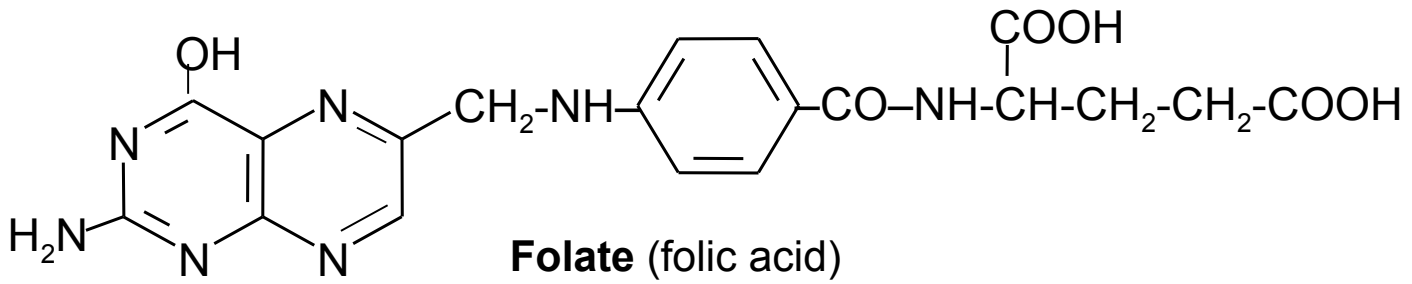
Ribose 5-phosphate + **ATP** → PRPP + AMP

PRPP synthetase

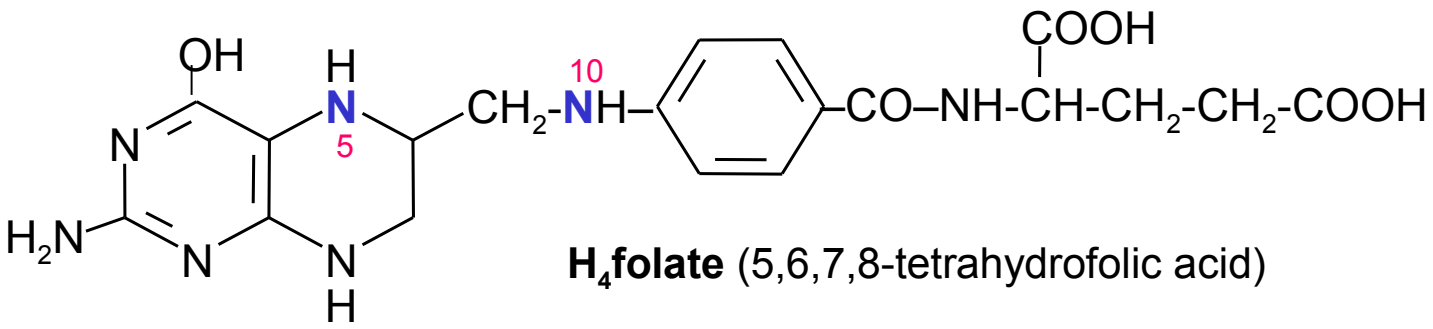
Tetrahydrofolate originates from folate by the reduction (dihydrofolate reductase). It serves as a coenzyme which transfers one-carbon units:

N^5,N^{10}-Methylene-H_4folate is the donor of methyl in the synthesis of thymine,

N^{10}-formyl-H_4folate supplies two C1-groups in the synthesis of purines.



Folate (folic acid)

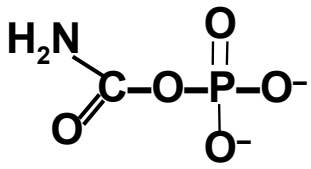


H_4folate (5,6,7,8-tetrahydrofolic acid)

Biosynthesis of pyrimidine nucleotides *de novo*

The heterocyclic ring of pyrimidine bases is synthesized from **carbamoyl phosphate** and **aspartate**. The pyrimidine derivative (orotic acid) is then attached to the ribosyl 5-phosphate.

Carbamoyl phosphate



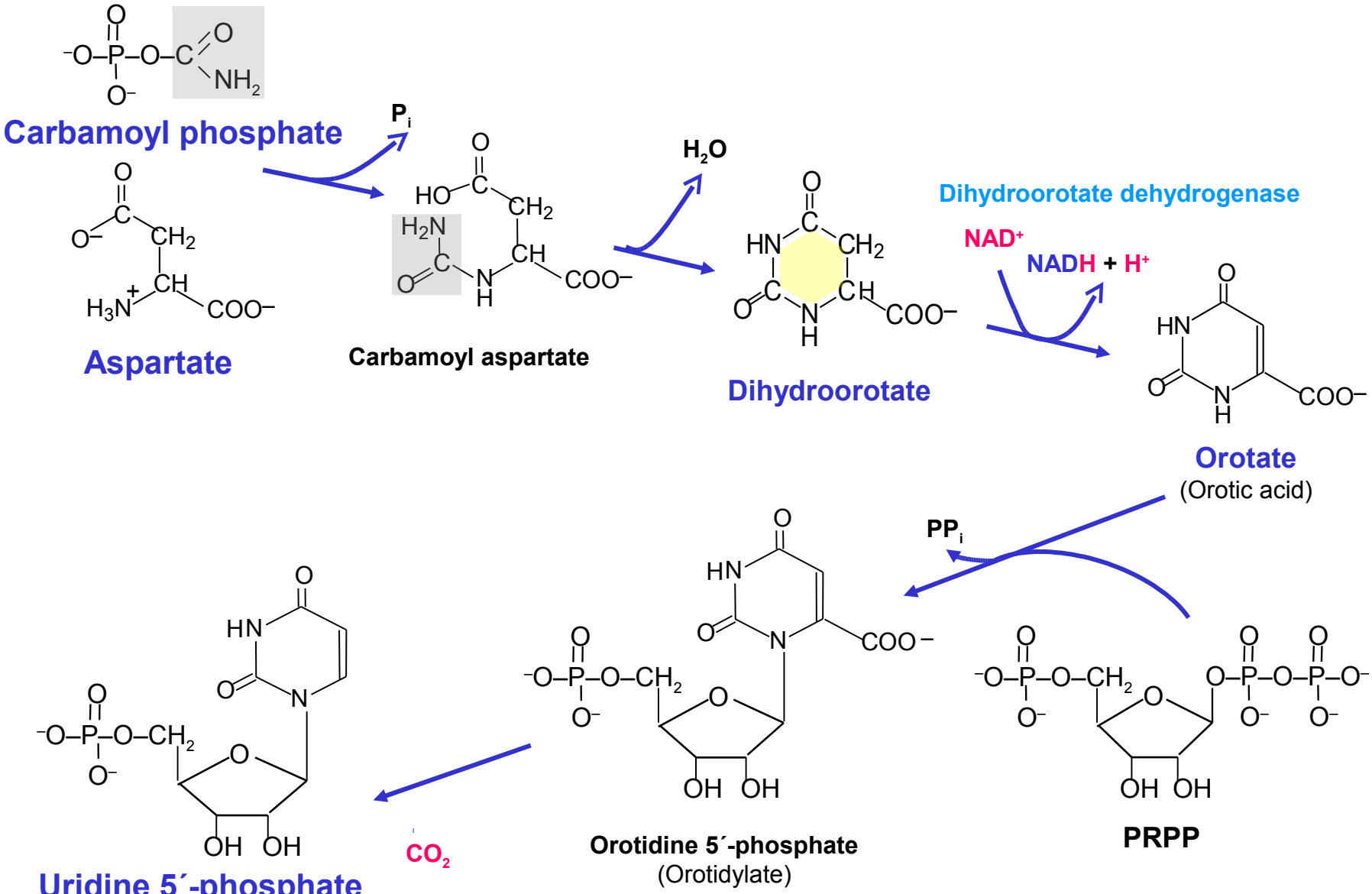
is formed in **cytosol**, the donor of an amido group is **glutamine:**

Glutamine + HCO_3^- + 2 ATP ⟶ Carbamoyl phosphate + Glutamate + 2 ADP + P_i

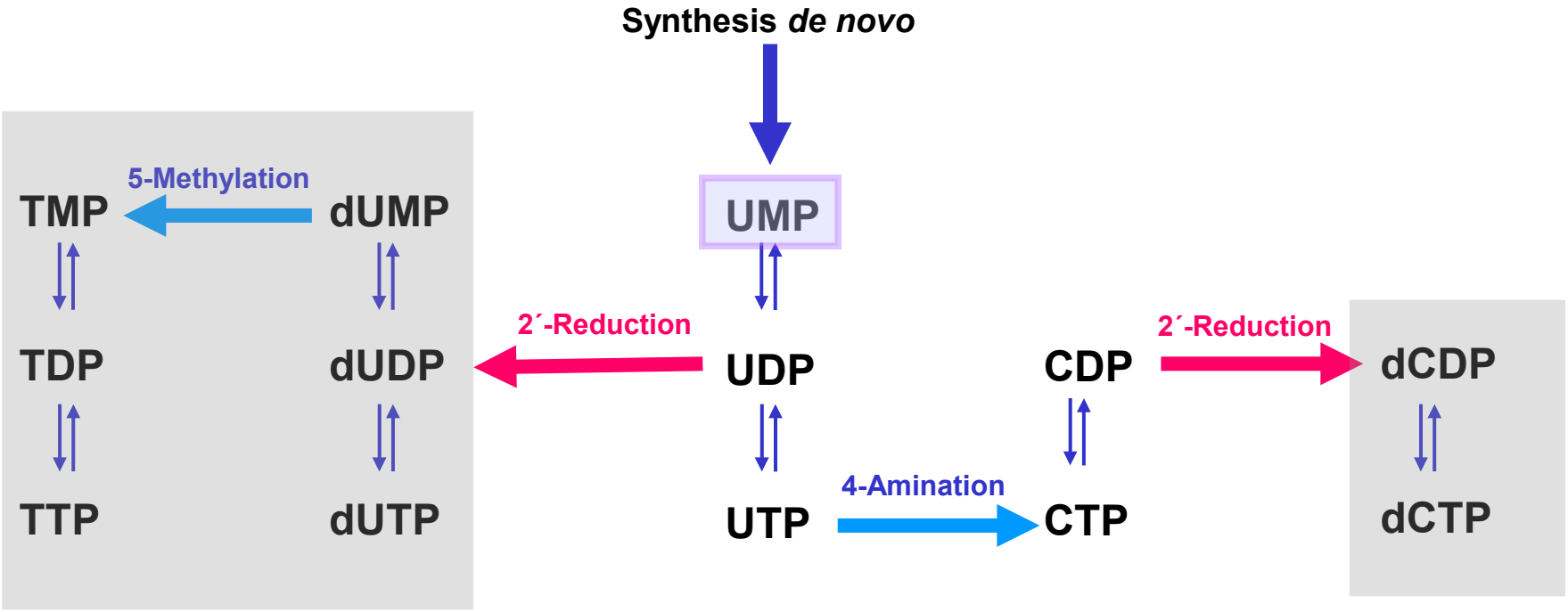
The reaction is catalysed by carbamoyl phosphate synthetase II, one of the three activities of the protein called **dihydroorotate synthase** that catalyses also the next two reactions of pyrimidine biosynthesis (aspartate transcarbamoylase, dihydroorotase).

In mitochondria, the enzyme **carbamoyl phosphate synthetase I** supplies carbamoyl phosphate for the ureosynthetic cycle, but it accepts the amido group from NH_4^+ and its activity depends essentially on the presence of *N*-acetylglutamate (V-type of allosteric activation).

Only two proteins catalyse the further reactions: **Dihydroorotate dehydrogenase**, an iron-containing flavoprotein which is located on the outer side of the **inner mitochondrial membrane** (two electrons reduce coenzyme Q). **Uridine phosphate synthase** catalyses the reaction with PRPP and the decarboxylation of orotidine phosphate to UMP.


Carbamoyl phosphate
Aspartate
Pi
Carbamoyl aspartate
H2O
Dihydroorotate
Dihydroorotate dehydrogenase
NAD+
NADH + H+
Orotate
(Orotic acid)
PPi
PRPP
Orotidine 5´-phosphate
(Orotidylate)
CO2
Uridine 5´-phosphate
(UMP, uridylate)

UMP is converted to other pyrimidine nucleotides:



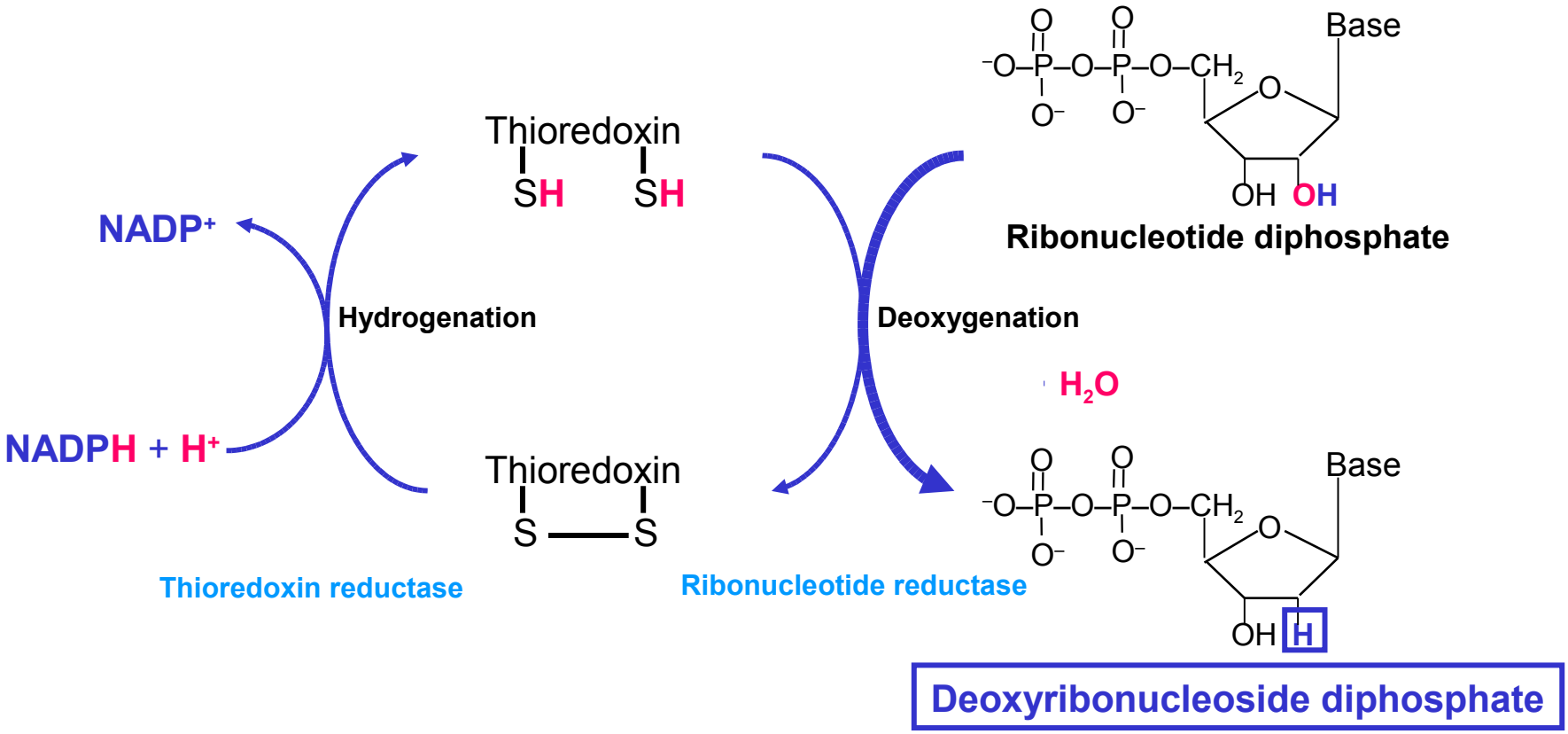
ATP serves as a donor of high-energy phosphoryl group in the successive **phosphorylations** of nucleoside phosphates to nucleoside diphosphates and triphosphates.

4-Amination of UTP to CTP is a glutamine-dependent reaction driven by ATP.

In the **reduction of ribonucleoside diphosphates to 2´-deoxyribonucleotides**, the direct reductant is thioredoxin, oxidized redoxin is reduced by NADPH + H^+.

5-Methylation of dUMP to TMP requires methylene-H_4folate as the one-carbon donor.

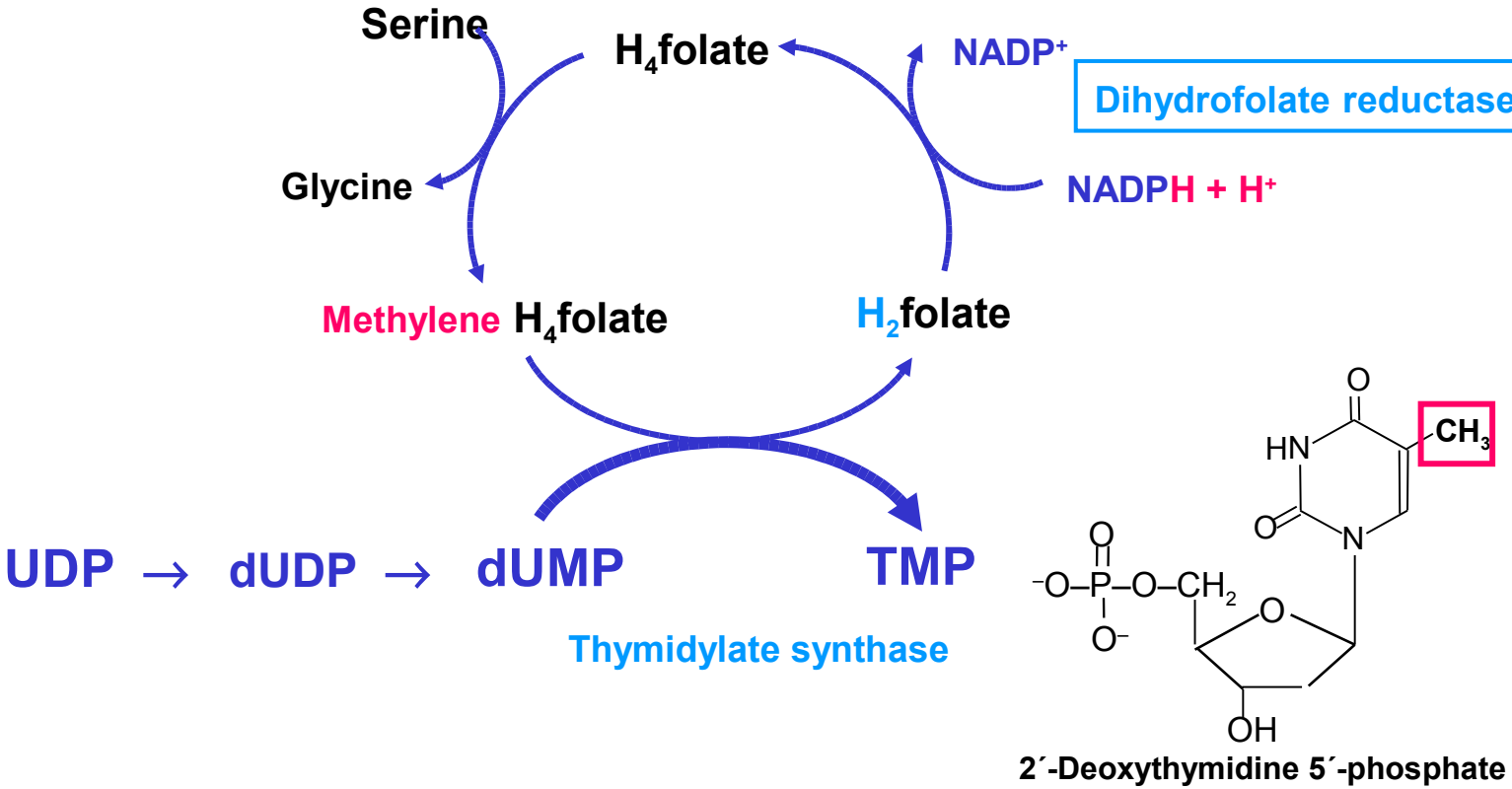
Synthesis of deoxyribonucleotides (both pyrimidine and purine) by the reduction of ribonucleoside diphosphates



Thioredoxin – a small protein – functions as a coenzyme in the reduction, it supplies two hydrogen atoms for the deoxygenation of ribosyl.

Synthesis of thymine nucleotides

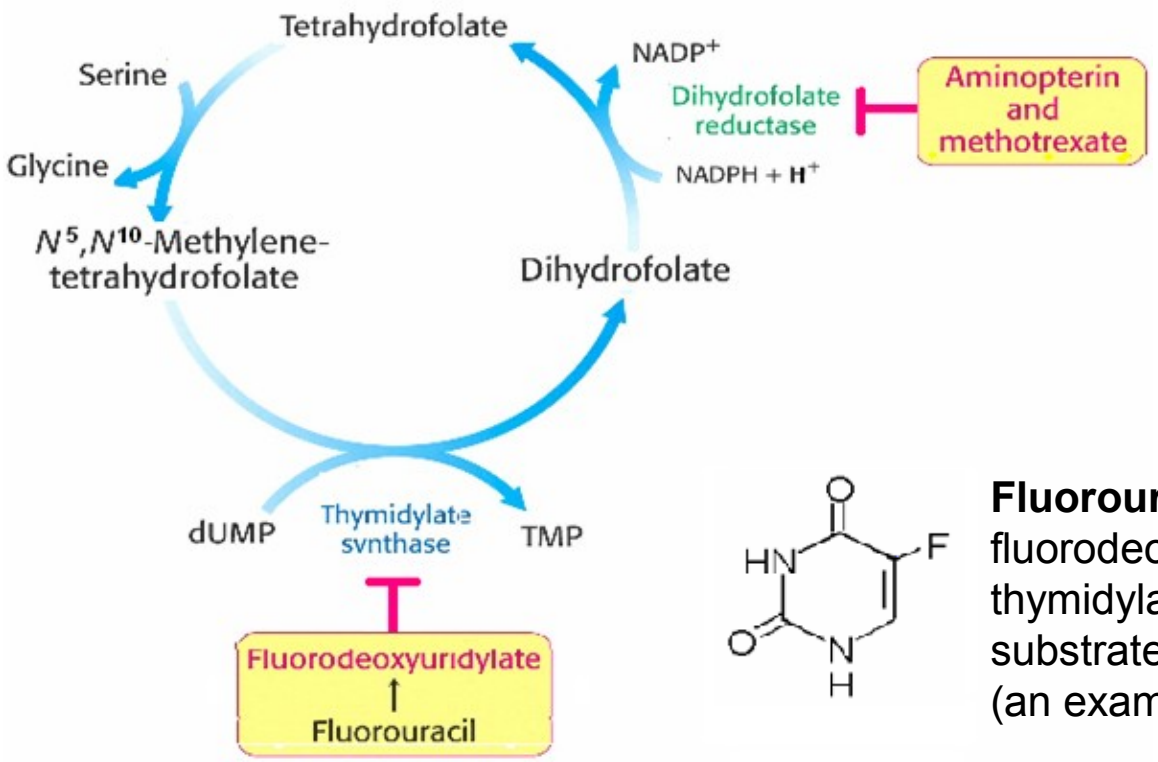
dUMP (deoxyuridylate) **is methylated to TMP** (deoxythymidine 5´-phosphate, thymidylate).
The methyl donor is methylene-H_4folate, which becomes oxidized to H_2folate during transfer. Dihydrofolate reductase catalyses the regeneration of tetrahydrofolate before it can be reused.



Inhibitors of the synthesis of thymidylate

are used in cancer chemotherapy. By inhibiting dihydrofolate reductase or thymidylate synthase, these compounds stop cell division in rapidly dividing cells so that are effective **anti-tumour drugs and immunosuppressants**.

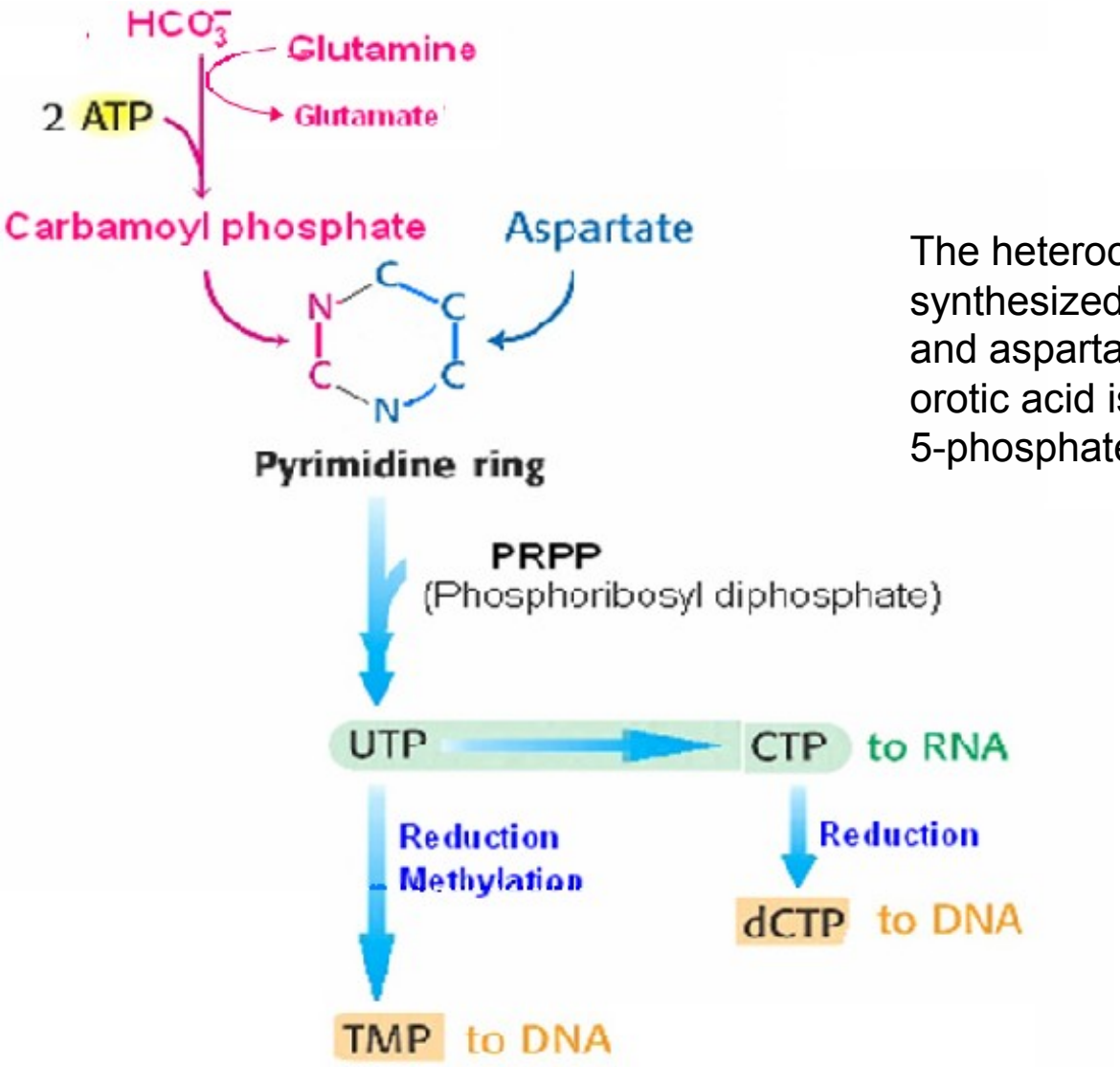
Aminopterin (4-amino-dihydrofolate) and **methotrexate** (amethopterin, 4-amino-10-methyl-dihydrofolate) are anti-folate drugs - potent competitive inhibitors of dihydrofolate reductase.



Trimethoprim, another folate analog, has potent antibacterial and antiprotozoal activity by inhibiting dihydrofolate reductases of many susceptible microorganism much more intensively than the mammalian enzymes. It is used (above all in combination with sulphonamides) to treat infections.

Fluorouracil is converted *in vivo* into fluorodeoxyuridylate that irreversibly inhibits thymidylate synthase after acting as a normal substrate through part of the catalytic cycle (an example of suicide inhibition).

Summary of the pyrimidine nucleotide synthesis:

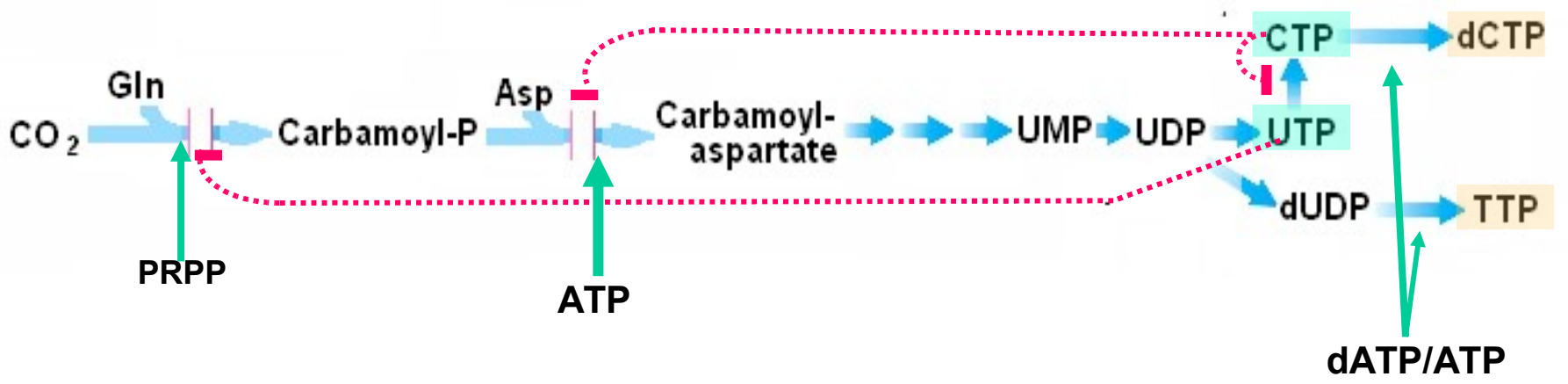


The heterocyclic pyrimidine ring is synthesized from carbamoyl phosphate and aspartate. The pyrimidine derivative orotic acid is then attached to the ribosyl 5-phosphate.

Regulation of the pyrimidine nucleotide biosynthesis

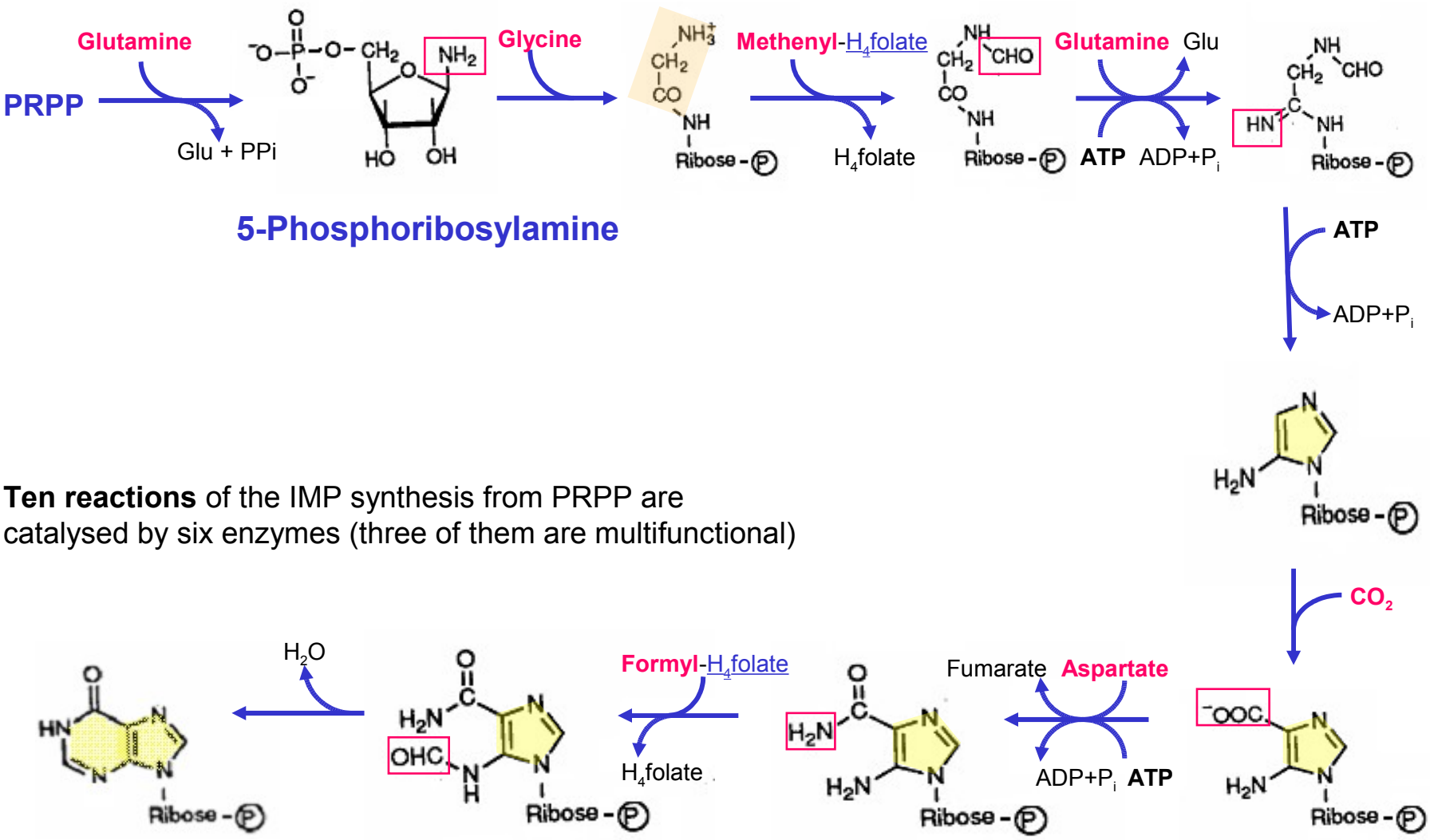
The key steps (catalysed by carbamoyl-P synthase and dihydroorotase activities) are regulated by feedback inhibition, **UTP and CTP act as allosteric inhibitors**.

ATP and PRPP stimulate the biosynthetic pathway..



The overall activity of ribonucleotide reductase is diminished by dATP, which signals an abundance of deoxyribonucleotides, though **dATP or ATP enhances the reduction of UDP and CDP.** This complex pattern of regulation supplies the appropriate balance of the four deoxyribonucleotides needed for the synthesis of DNA.

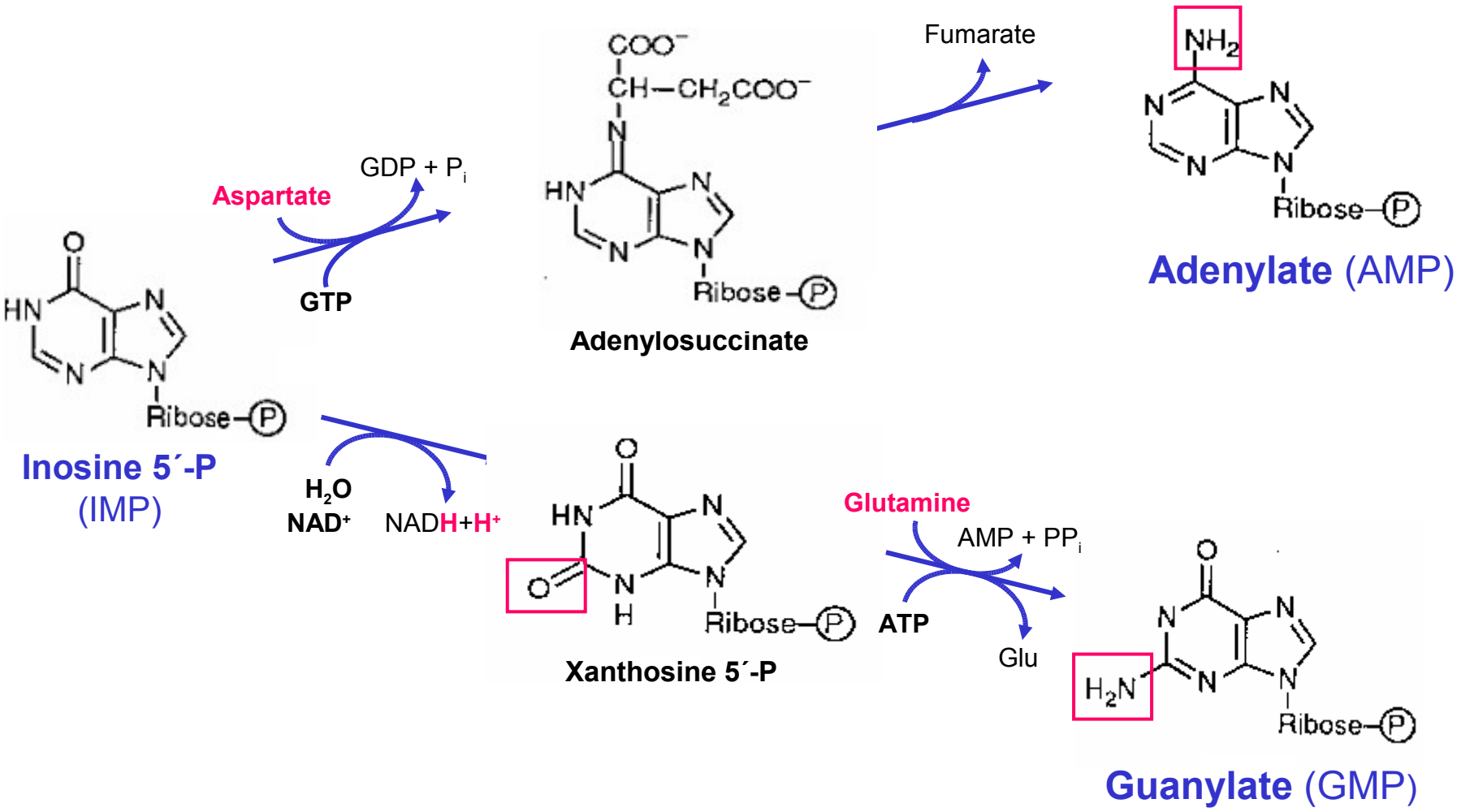
Biosynthesis of purine nucleotides *de novo*:



Ten reactions of the IMP synthesis from PRPP are catalysed by six enzymes (three of them are multifunctional)

(Two steps, the intermediate is *N*-succinate derivative)

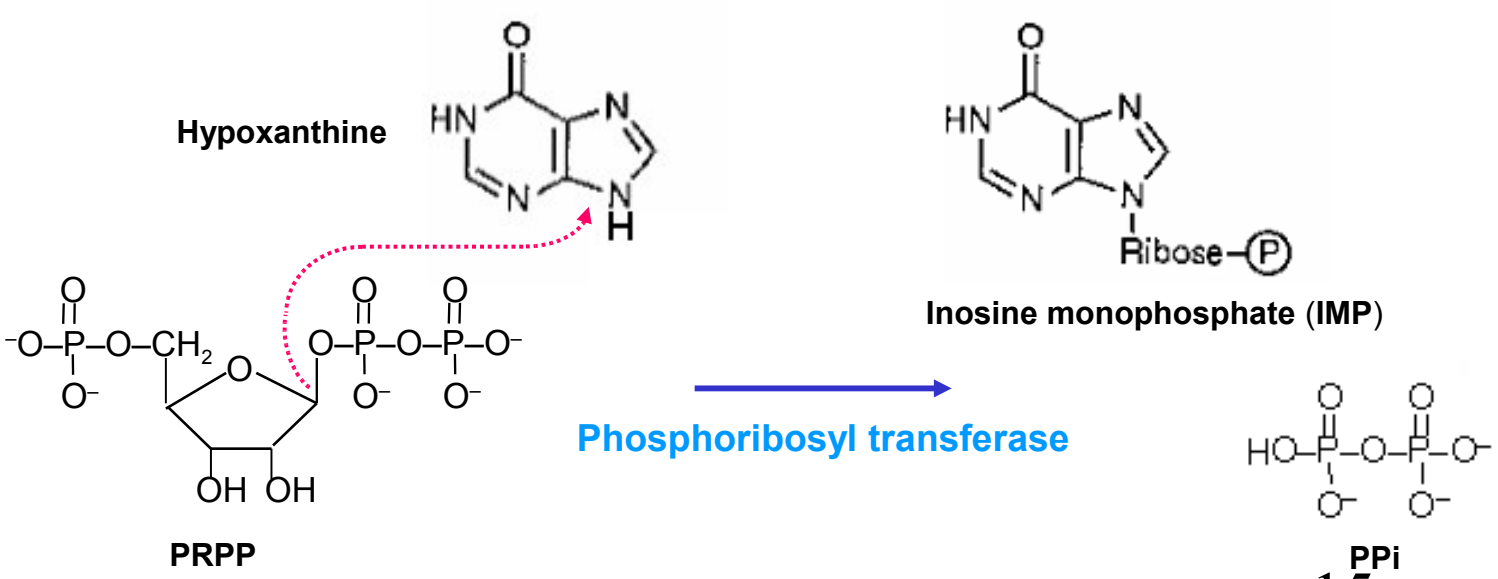
Inosine 5´-phosphate (IMP) is transformed
to **adenosine 5´-phosphate** (AMP) or **guanosine 5´-phosphate** (GMP):



Purine and pyrimidine nucleotides are synthesized ***de novo*** in many types of cell and used in large quantities in the biosynthesis of nucleic acids. The synthesis of one molecule AMP or GMP (incl. formation of PRPP) requires consumption of 7 and 8 ATP, resp.

The cells of some extrahepatic tissues are able to reconvert a part of free purine bases released by the action of 5´-nucleotidase and nucleoside phosphorylase from nucleotides (hypoxanthine and guanine above all) again to nucleotides, so that the bases are salvaged from their further degradation. Those reactions are called **scavenger pathways** and are catalysed by phosphoribosyl transferases.

For example:

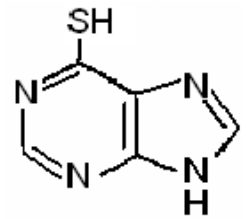


Inhibitors of the purine nucleotide synthesis

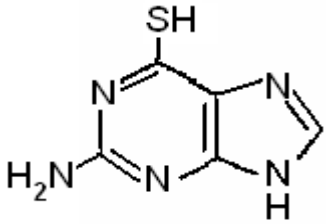
which act at control points in purine biosynthesis may be also of limited use in cancer chemotherapy or as immunosuppressants.

Glutamine analogs, e.g. antibiotics **azaserine** or diazooxonorleucine (DON), inhibit glutamine-dependent aminations of PRPP, formylglycinamidine ribotide, and xanthosine phosphate.

Purine analogs used in medicine, e.g. **6-mercaptopurine**, **6-thioguanine**, and **azathioprine**, are converted *in vivo* to their ribonucleotides, which act as inhibitors of the transformation of IMP to adenylate.



6-Mercaptopurine



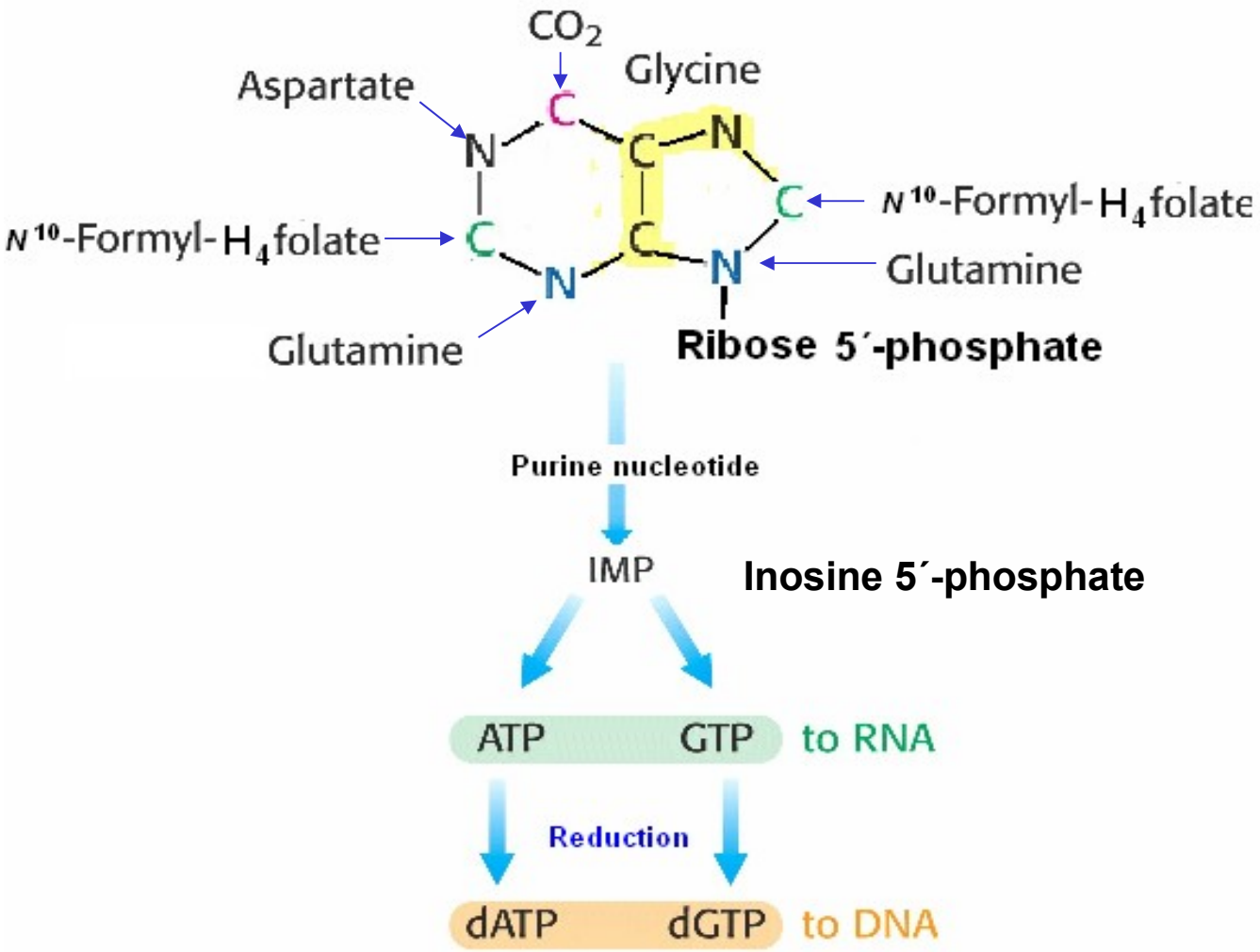
6-Thioguanine

Azathioprine

Anti-folate drug (folate analog) **methotrexate**, in addition to its inhibitive effect on methylation of dUMP to thymidylate, decreases the overall accessibility of H_4folate that supplies C1-groups for the synthesis of purines.

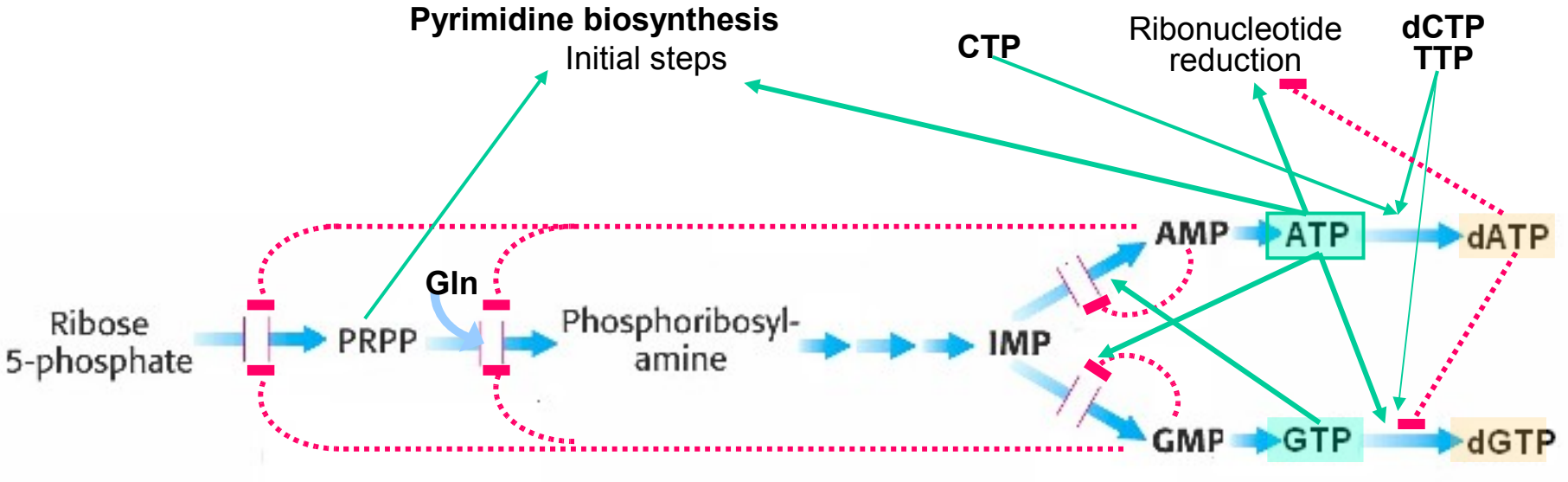
Summary of the purine nucleotide synthesis:

The heterocyclic ring systém is built up while attached to ribose 5-phosphate.
All intermediates are nucleotides.



Regulation of the purine nucleotide biosynthesis

Negative and positive feedback controls the overall rate of purine synthesis and ensures that all nucleotides required for RNA and DNA are synthesized in the correct proportions.
The committed step in the purine synthesis is the **conversion of PRPP into phosphoribosylamine** (glutamine phosphoribosyl amidotransferase). The enzyme is feedback-inhibited by synergistic action of **AMP** and **GMP**.



Catabolism of nucleotides

The nucleotides of a cell undergo continual turnover. Nucleotides are hydrolytically degraded to nucleosides by **5´-nucleotidase**:

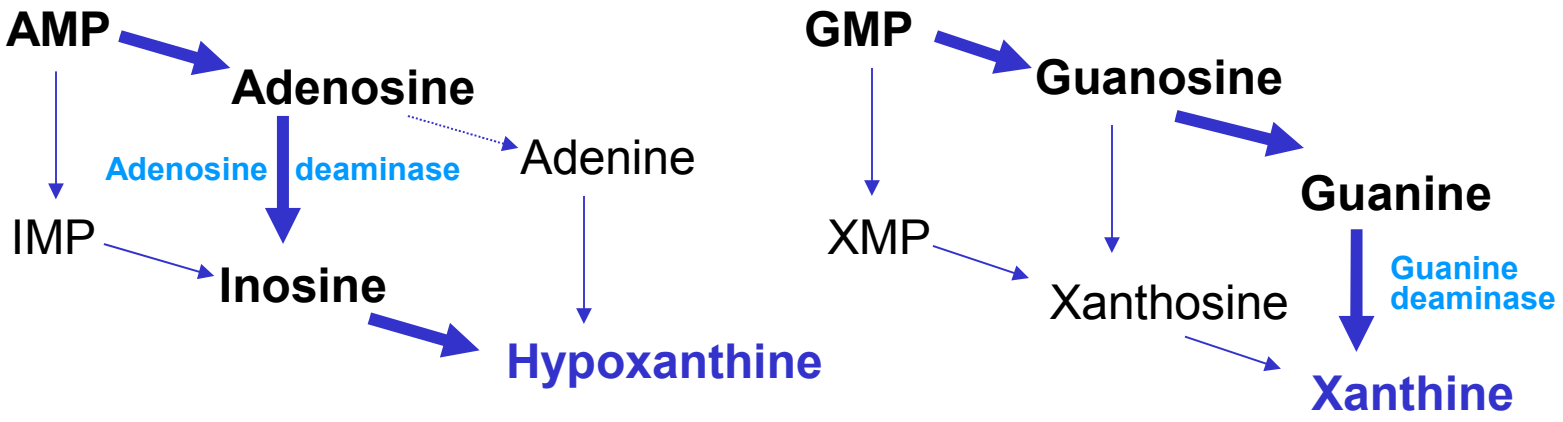
Nucleotide + H_2O ⟶ Nucleoside + P_i

The glycosidic bond of nucleosides are cleft in phosphorolytic reactions catalysed by **nucleoside phosphorylases**:

Nucleoside + $H_2PO_4^-$ ⟶ Free base + (Deoxy)ribose 1-phosphate

Ribose 1-phosphate is isomerized by phosphoribonutase to ribose 5-phosphate, a substrate in the synthesis of PRPP.

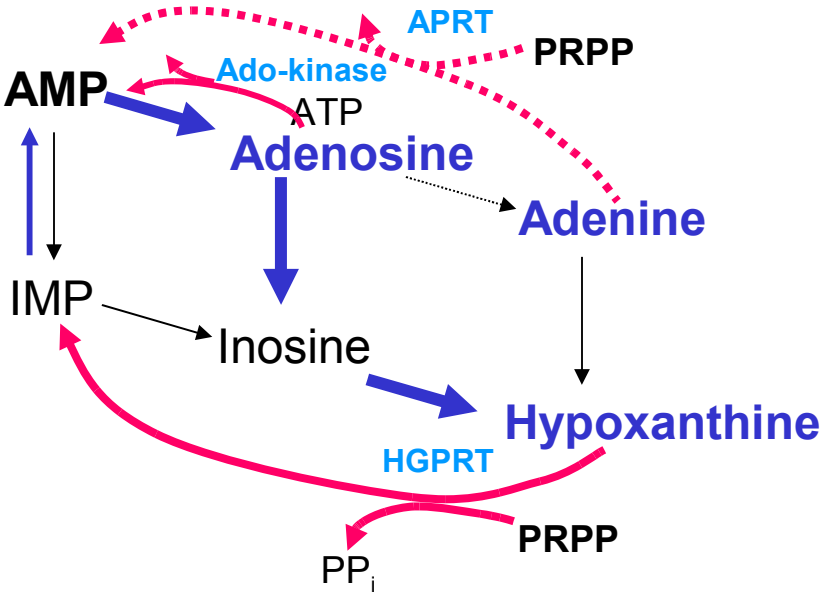
The deamination of purine bases adenine and guanine may be realized before the free bases are released (the usual pathways are highlighted): :

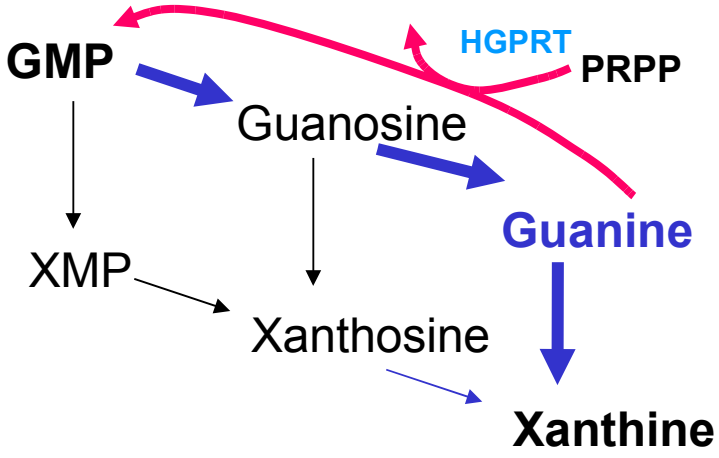


Some of the bases are reused to form nucleotides by **scavenger pathways** (named also **salvage reactions**).

The reincorporation of free **pyrimidine bases** into nucleotides is negligable, if it occurs at all, most of free pyrimidine bases are degraded to products that are excreted.

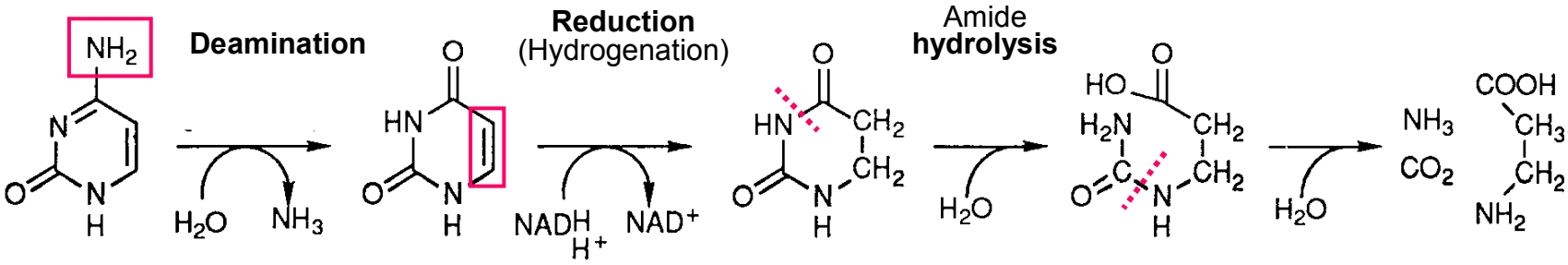
Free **purine bases** are reconverted to nucleotides in various extent. In many tissues much less than 30 % of purine bases enter the scavenger pathways, but those are very important in CNS and also in bone marrow and blood cells.
The reactions with PRPP are catalysed by **phosphoribosyl transferases** (**PRT**).





Catabolism of pyrimidine bases

Degradation of pyrimidines is essentially the reverse of synthesis:



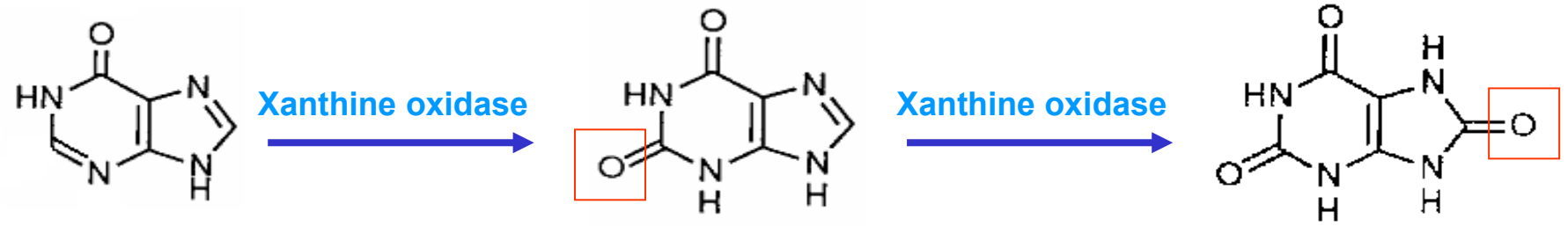
Cytosine

β-Alanine
(**β-Aminoisobutyrate** from thymine)

β-Alanine is deaminated to malonic semialdehyde (→ acetyl-CoA),
β-aminoisobutyrate to methylmalonic semialdehyde (→ succinyl-CoA).

Catabolism of deaminated purine bases

Xanthine oxidase oxidizes hypoxanthine to xanthine and this same enzyme oxidizes xanthine to uric acid. The lactam forms of those compounds are shown:



Hypoxanthine
(6-hydroxypurine)

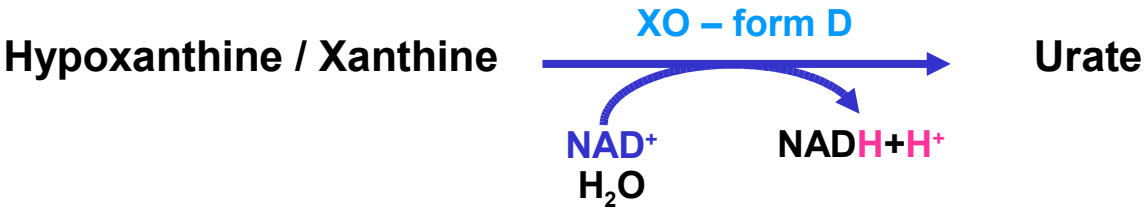
Xanthine
(2,6-dihydroxypurine)

Uric acid
(2,6,8-trihydroxypurine)

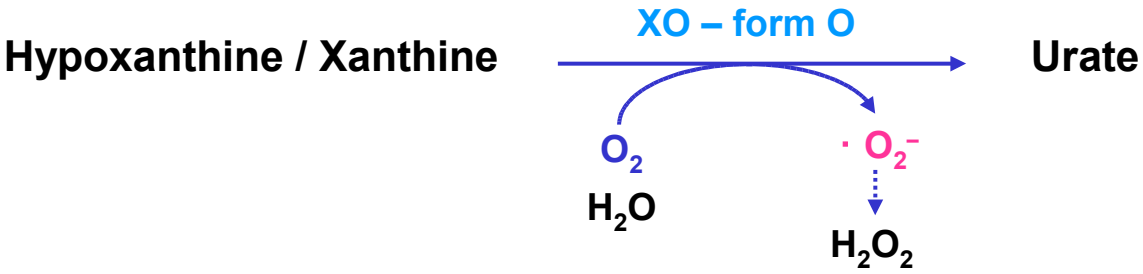
Uric acid is the **final metabolic product of purine catabolism in humans** and other primates (also in uricotelic animals - birds and land-dwelling reptiles), and is excreted in the urine.

Other organisms, incl. most mammals, metabolize urate to allantoin or other simpler forms.

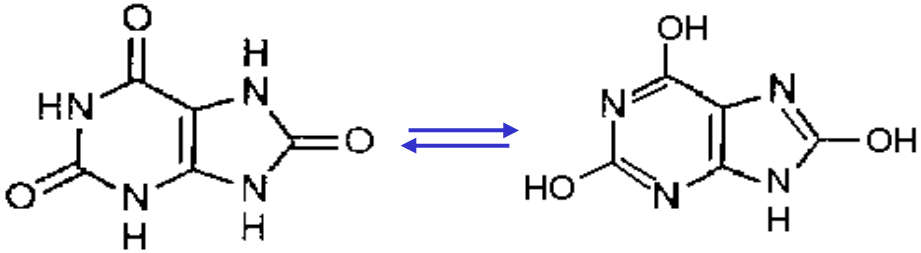
Xanthine oxidase ((XO) is a molybdenum- and iron-containing flavoprotein, which may exist in two forms – D-form and O-form.
Under physiological conditions, the **D-form** catalyses the oxidation (hydroxylation) as a **dehydrogenase**, the acceptor of electrons is NAD^+.



In tissue hypoxia, the D-form is transformed into the **O-form** by the proteolytic splitting of 20 amino acyl residues. The O-form is a **oxygenase**, the acceptor of electrons is dioxygen. Then the reaction produces superoxide anion-radicals which dismutase into hydrogen peroxide by the action of SOD (the cause of so-called "reperfusion injury" after the restoration of a sufficient oxygen supply to the ischaemic tissue).



Uric acid (2,6,8-trihydroxypurine)



Lactam form Lactim form

is a very weak diprotic acid. The pK_{a1} equals 5,75, therefore the predominant form of uric acid in body fluids is the **monovalent hydrogen urate anion**.

Unfortunately, uric acid and its urate salts have a **low solubility** in water. The average serum concentrations in humans (normal range 100-400 μmol/l) is close to the solubility limit, above which the precipitation of needle-shaped monosodium urate crystals may begin. Excessive accumulation of urate crystals results frequently in deposites in the soft tissues, particularly in interstitium of the kidney and in joints.

On the other hand, an increase in urate concentration in primates has a markedly beneficial action. Urate is a **highly effective antioxidant** - a scavenger of reactive oxygen species which is about as effective as ascorbate taken in the diet.

In the reaction with oxygen radicals, urate is changed into the radical that is non-enzymatically (spontaneously) transformed into allantoin. Primates thus excrete small amounts of allantoin proportionally to their exposure to oxidative stress, although they lack the uricacid oxidase.

Examples of defects in purine metabolism

Inborn gouty syndromes

Gout (primary gout) is a metabolic disease caused by accumulation of excess urate in body fluids due to the miscontrol of endogenous purine *de novo* synthesis supported by high intake of purines in the diet.
The crystals of monosodium urate are deposited in tissues, particularly in kidneys (may result in renal failure), and in joints (painful arthritis).

Lesch-Nyhan syndrome is a rare nearly complete deficit in hypoxanthine-guanine phosphoribosyl transferase (HGPRT, see scavenger pathways of nucleotide synthesis), that stimulates an overproduction of purines.

Children with this disease exhibit mental retardation, spasticity, compulsive self-destructive behaviour (biting their fingers and lips) and aggression toward others.
An incomplete deficit in HGPRT leads in adults only to the formation of renal stones followed by the gouty arthritis years later.

Familial hyperuricacidaemic nephropathy of juveniles is a heavy defect of urate renal excretion of young persons that leads progressively to the renal failure.

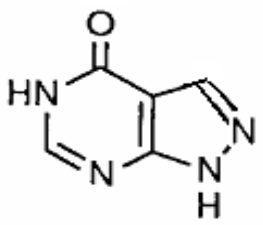
(*continued*)

"Secondary" hyperuricacidaemia and/or gout

may have its cause in

– an **intensified desintegration of cells**, e.g. in myelo- and lymphoproliferative diseases, during the cytostatic cancer chemotherapy, in hypercatabolic states (prolonged starvation, chronic alcoholism, high doses of corticosteroids),

– a **decreased renal excretion of urate** in renal diseases or due to the side-effect of some kind of diuretics (furosemide).



Allopurinol

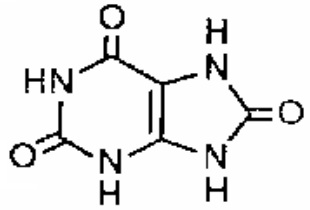
Hyperuricacidaemias and gouty syndromes are usually treated with **allopurinol**, a "suicide" **inhibitor of xanthine oxidase**.

Instead of urate, the final products of purine catabolism are then hypoxanthine and xanthine, which are more soluble and thus more easily excreted into the urine. Allopurinol is oxidized by xanthine oxidase to 2-hydroxyallopurinol (alloxanthine) that remains bound to the molybdenum atom of the enzyme, thereby inactivating it.

Immunodeficiency syndromes

The autosomal recessive form of severe combined immunodeficiency syndrome (**SCIDS**) has its cause in approximately 50 % of patients in a genetic deficiency of adenosine deaminase, which is oft associated with defects of 5˘-nucleotidase and nucleoside phosphorylase, too. All these enzymes také part in the purine salvage pathway.

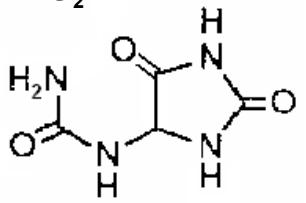
Final metabolic products of purine catabolism in animals other than primates



Urate is the final product in **humans and other primates** (benefitial antioxidant), as well as in **birds and terrestial reptiles** (embryogenesis in enclosed space)

Urate oxidase

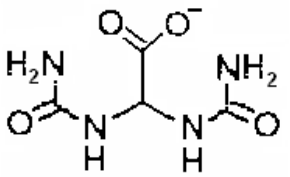
CO_2



Allantoin - most mammals other than primates, turtles, molluscs

H_2O

Allantoinase



Allantoic acid - many bony fish

Allantoicase

$2\ NH_2–CO–NH_2 + OCH–COO^-$

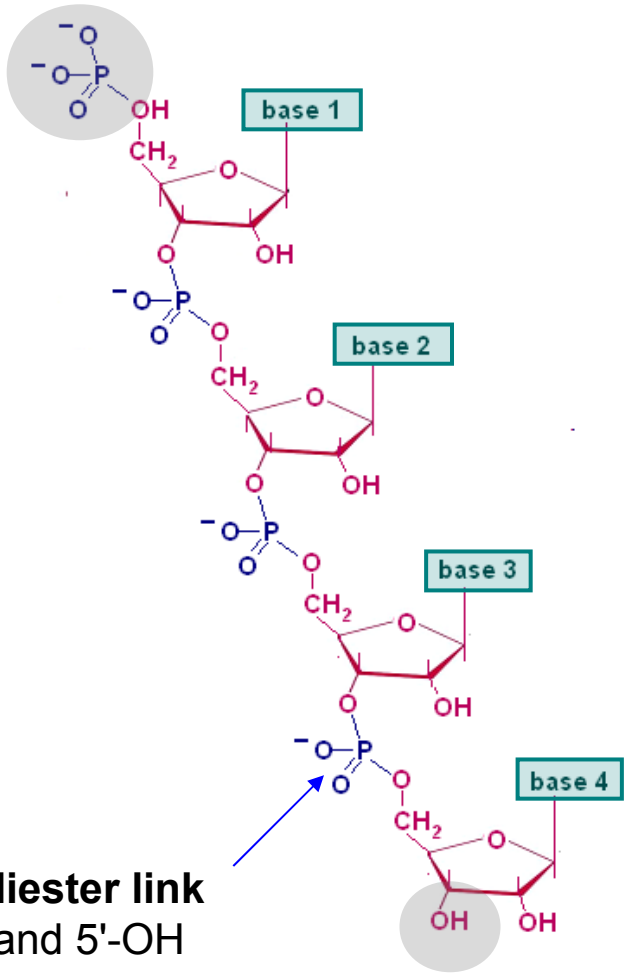
Urea and glyoxylate - many fish and amphibians; in many invertebrates urea is hydrolytically decomposed by **urease**.

DNA replication

Recapitulation of basal facts

Polynucleotide chain structure (RNA depicted)

5'-phosphate end



3'→5' phosphodiester link
between 3'-OH and 5'-OH

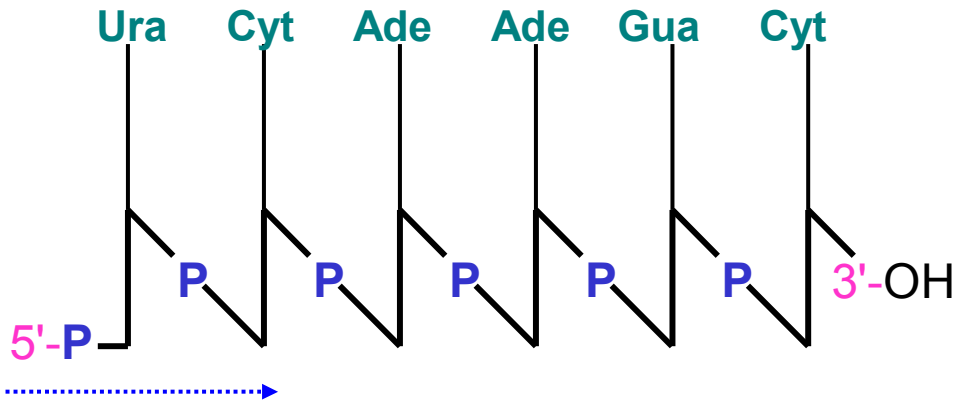
Bases are attached to the sugar-phosphate backbone through β-*N*-glycosidic bonds.

By convention, direction of reading is from 5'-end to 3'-end.

free 3'-OH end
(event. 3'-phosphate end)

Primary structure of single strand nucleic acid - abbreviated notation

RNA chain:



Symbolic notation of the base sequence

From the 5'-end: p**U**→**C**→**A**→**A**→**G**→**C**

5'-**UCAAGC**

UCAAGC

Identical chain from the 3'-end:

C←**G**←**A**←**A**←**C**←**U**p

3'-**CGAACU**

DNA chain

p**dG-dC-dT-dT-dG-dA**

d(p**GCTTGA**)

GCTTGA

or **d**(**A**←**G**←**T**←**T**←**C**←**G**)

3'-**AGTTCG**

Nuclear deoxyribonucleic acid of eukaryotes

is **linear double-stranded** DNA (dsDNA).

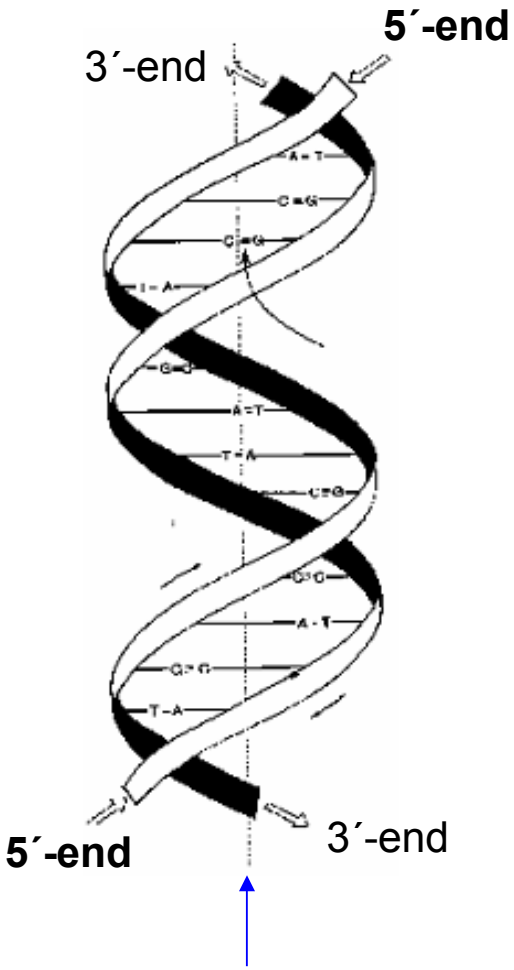
Most sequences of nucleotides on DNA (about 70 %) are quite unique, but only 3 % code for proteins. The other are either moderately and highly repetitive (20 %) or in the form of inverted repeats (10 %, called satellite sequences).

Mitochondrial DNA (mtDNA) is double-stranded and **circular**.

Human mtDNA consists of only 16 500 base pairs, almost entirely without non-coding regions.

Bacterial DNA is linear or circular dsDNA in the form of chromosome or plasmids. Some **viruses** contain single stranded DNA.

Secondary double helical structure of DNA



Two polynucleotide chains wind about a common axis with a right-handed twist. Two **strands are antiparallel**, they run in opposite direction.

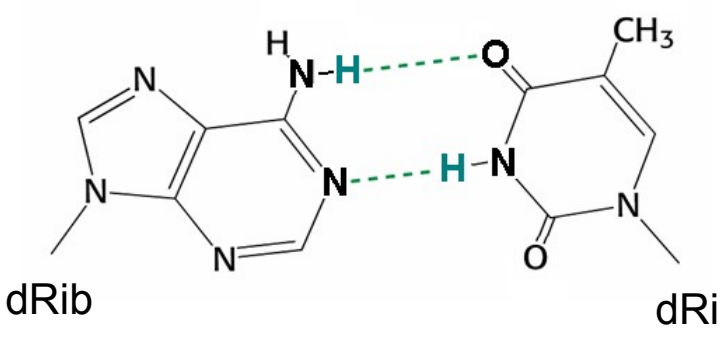
The coiling is plectonemic – the strands cannot be separated without unwinding the helix.

The hydrophilic **ribose-phosphate chains** are coiled about its periphery ("sugar-phosphate backbone"); negatively charged phosphate groups bind positively charged groups of proteins and simple cations.

The bases fill the inner of the helix as **complementary base pairs** – each base forms hydrogen bridges to the complementary base on the opposite strand. Hydrogen bonds originate spontaneously, without enzymatic catalysis.

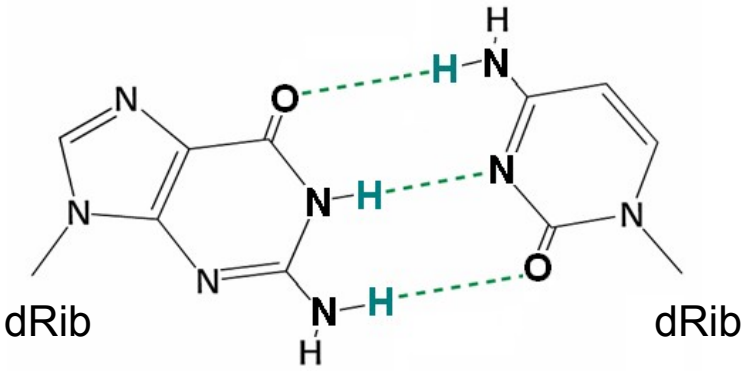
Pairing of bases (Watson-Crick geometry)

is the principle of chain complementarity:



d**A**=d**T**

(**A=U** in RNAs)



d**G**≡d**C**

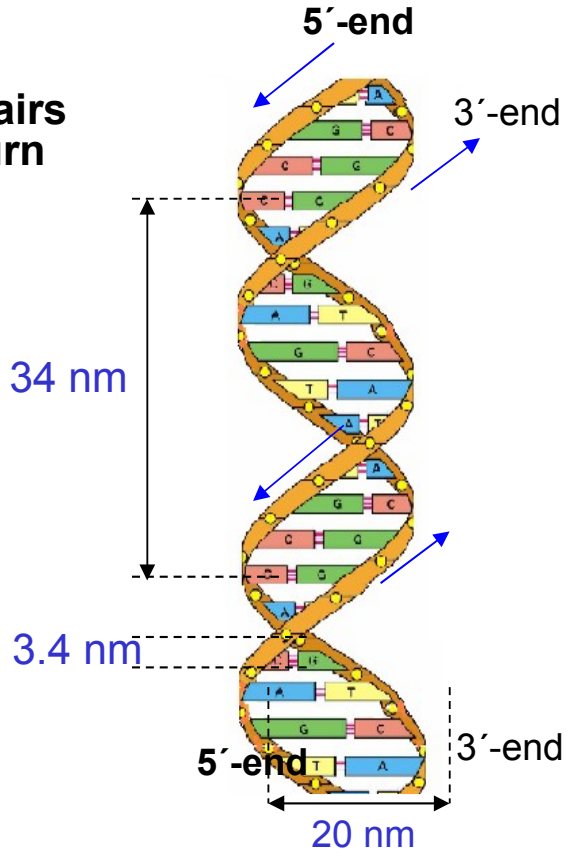
(**G≡C** in RNAs)

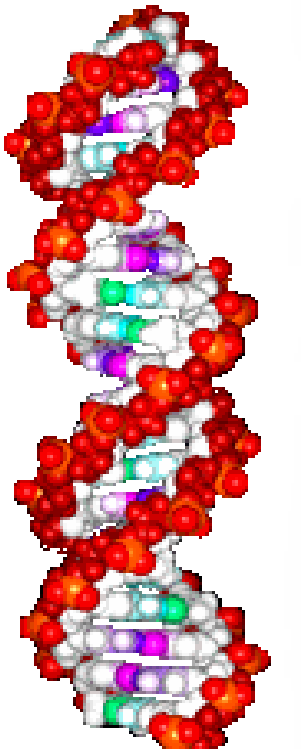
B-form of DNA (B-DNA)

is the **predominant form** of dsDNA - the regular right-handed helix of Watson and Crick.

The "ideal form":

Two unequal grooves arise on the surface because the glycosidic bonds of a base pair are not diametrically opposite each other.





Human nuclear genome

consists of circa 3×10^9 base pairs.

70 % of this number are **unique sequences**, which occur mostly in one copy in the haploid genome. Among those unique sequences, approx. 25 000 **structural genes coding for proteins** are included, as well as **genes coding for structures of rRNA and tRNA**.

DNA sequences that code for proteins represent only 3% of genome.

Moderately **repetitive sequences** (less than 10^6 copies) and highly repetitive sequences (6 – 100 bp, over 10^6 copies in the haploid genome, called **satellite DNAs**) represent about 20 % of genome and are clustered in several locations (e.g. at centromers, as telomers).
They are **not transcribed** and exhibit individual specifity so that they may be used for personal identification (DNA fingerprinting).

10 % of the genome are inverted repeats (palindromes with twofold axis of symmetry) and other non-classified (junk) DNA.

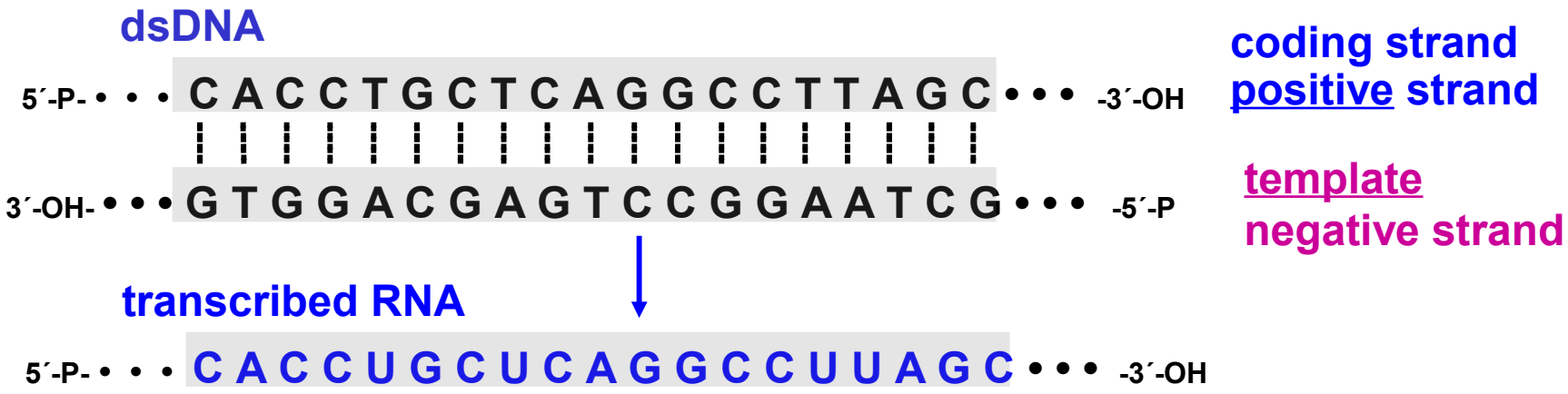
Human mitochondrial genome

has a highly compact structure consisting almost entirely of coding regions with genes for 13 protein subunits, 22 tRNAs and 2 rRNAs.

DNA as a template

In **DNA replication**, both DNA strands act as templates to specify the complementary base sequence on the new chains, by base-pairing.

In **transcription of DNA into RNA** *in vivo*, only one DNA strand of dsDNA acts as template that is called the negative strand. The base sequence of the transcribed RNA corresponds to that of the coding (positive) strand, except that in RNA thymidine is replaced by uridine.



Higher levels of DNA organization – chromatin

Human nuclear genome (23 chromosomes, each ≈ 1.3 x 10^8 bp) consists of circa 3 x 10^9 bp. There are 23 pairs of chromosomes in diploid cells.
In the nuclei, DNA is present in a condensed form as **chromatin**.

Three higher levels of DNA organization into chromatin:

"Bare" double helical DNA

1st level – fibrils of **nucleosomes,**

2nd level – **superhelix of nucleosome fibrils**, solenoid,

3rd level – radial loops of solenoids surrounding a central nuclear protein scaffold form the **fibres of intermitotic chromatin.**

In the course of mitosis, chromatin fibres are rearranged into the **metaphasic chromosomes**.

dsDNA (bare double helix, 10 bp per turn)

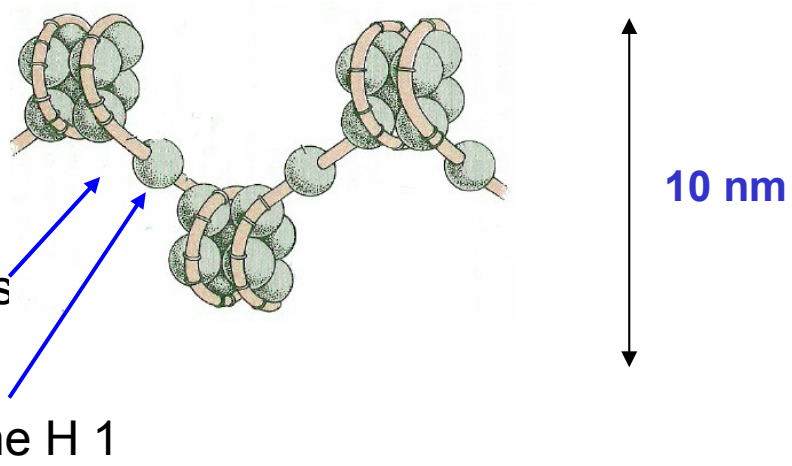
Fibrils of nucleosomes

Nucleosomes – two turns of DNA duplex (circa 160 bp) wound around the cluster of histones (octamer)

histone octamer contains two each of histones H 2A,H 2B,H 3, and H 4

the sequences of ≈ 60 bp – the **linker DNAs**

histone H 1 seals off the nucleosome



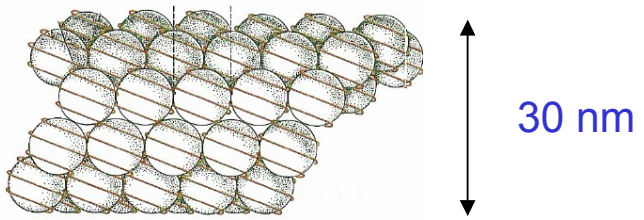
Histones are basic proteins that comprise about 100 aminoacyl residues, from which approx. 25 % is lysine and arginine. The histone octamers contain molecular types H 2A, H 2B, H 3, and H 4; type H 1 binds on the linker DNA.

Fibrils of nucleosomes

(„beads on a thread“)

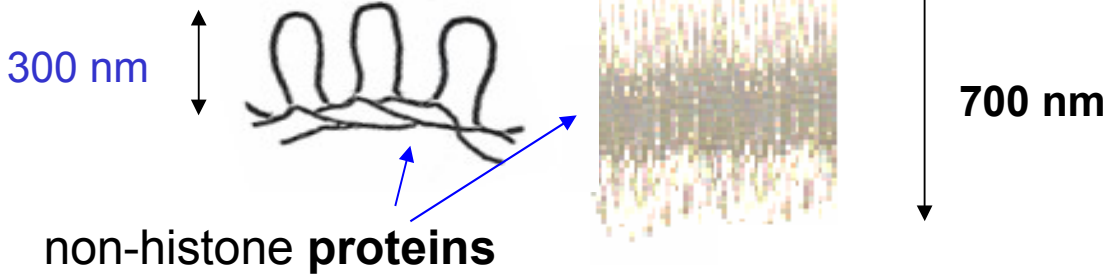
Solenoid - fibrils of nucleosomes are coiled in a superhelix

fibres – diameter 30 nm
1200 bp per turn
of the supercoil



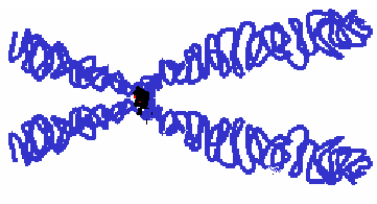
Fibres of intermitotic chromatin

radial loops of solenoids (20 000 – 80 000 bp per one loop are anchored to the nuclear protein scaffold



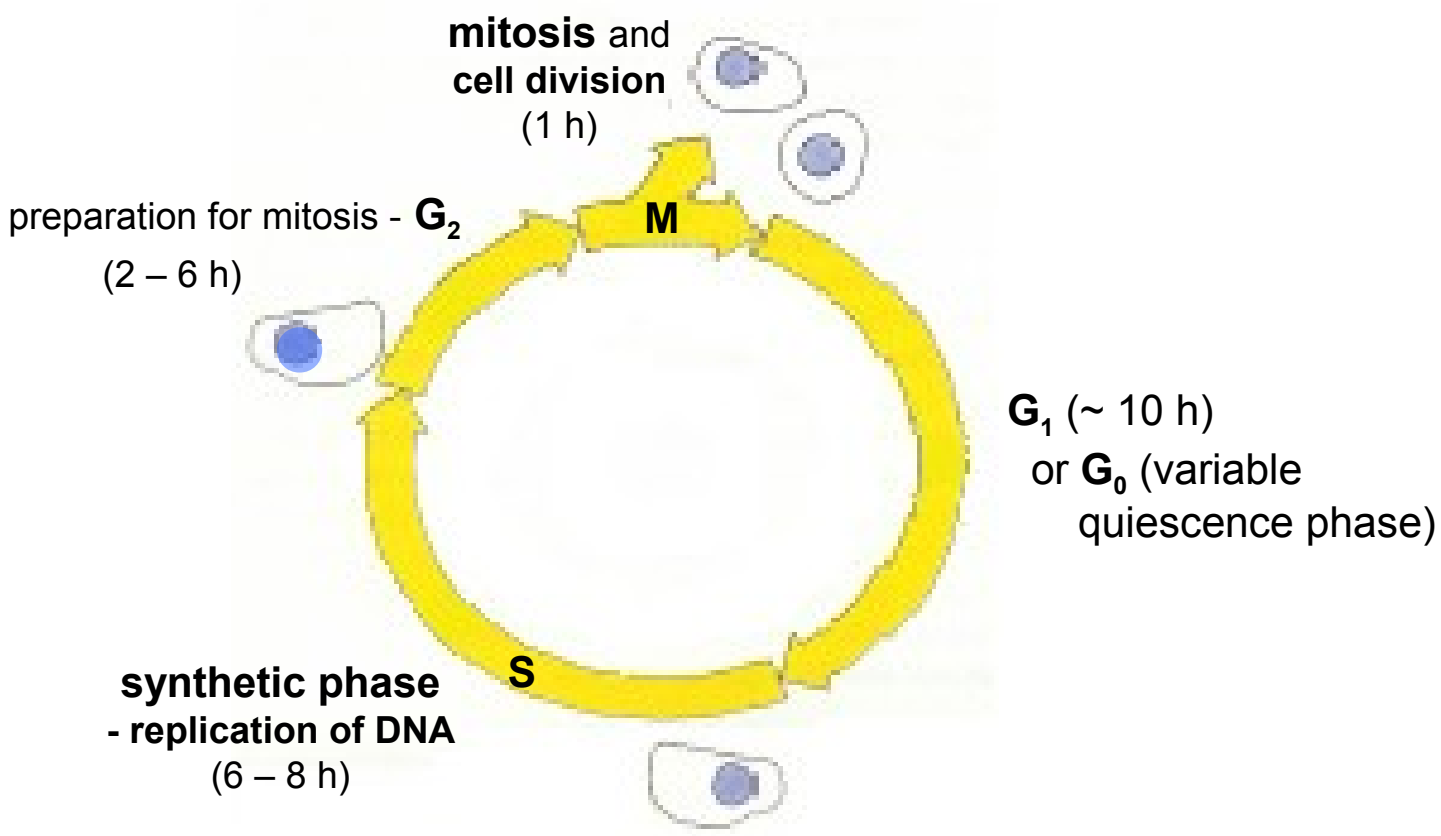
Metaphase chromosomes

originate by condensation of intermitotic chromatin fibres



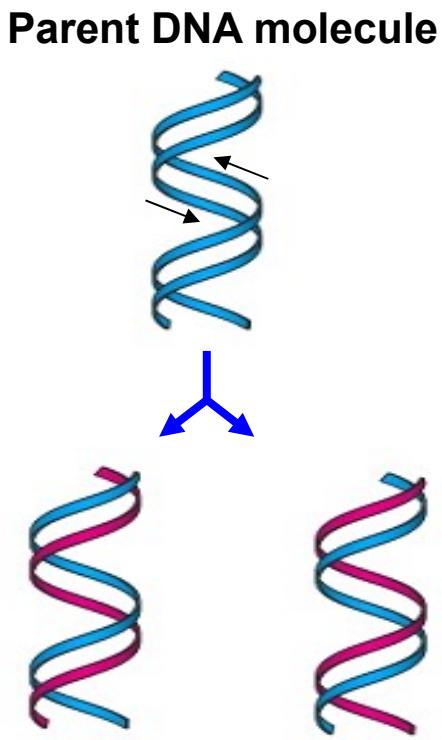
Eukaryotic DNA replication

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.



Each of the two strands of the DNA double helix serves as a template for the replication of a **new complementary strand**. Two daughter molecules are produced, each of which contains two DNA strands with an antiparallel orientation.

The replication is **semiconservative** – each of the parental strands is left intact in one of the two daughter molecules.

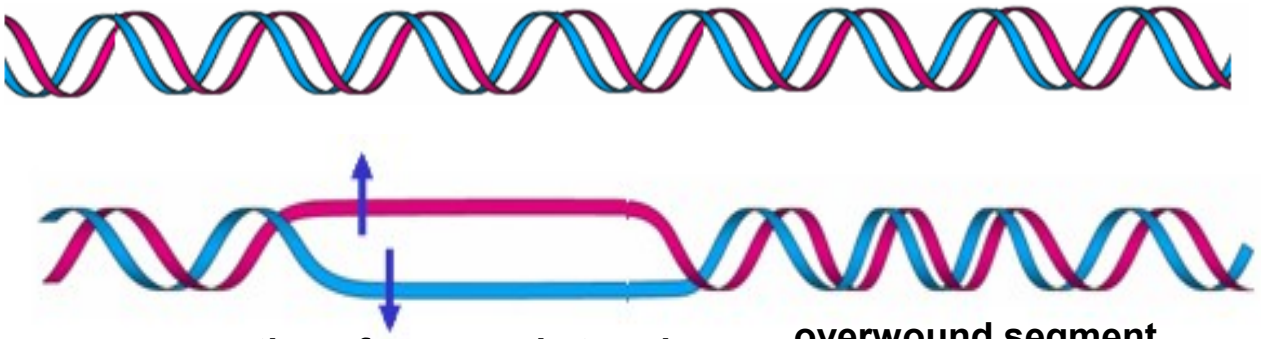


The separation of the two strands of the parental dsDNA must precede the binding of DNA polymerases, which use only single-stranded DNA as a template.

Unwinding of DNA double helix in the replicated segments causes **overwinding** (supercoiling) in other parts of the helix.

The resulting strain is removed by **topoisomerases**.

Helicases then separate single strands of the double helix and **single-strand binding proteins**, which cannot actively unwind the helix, stabilize the single strands.

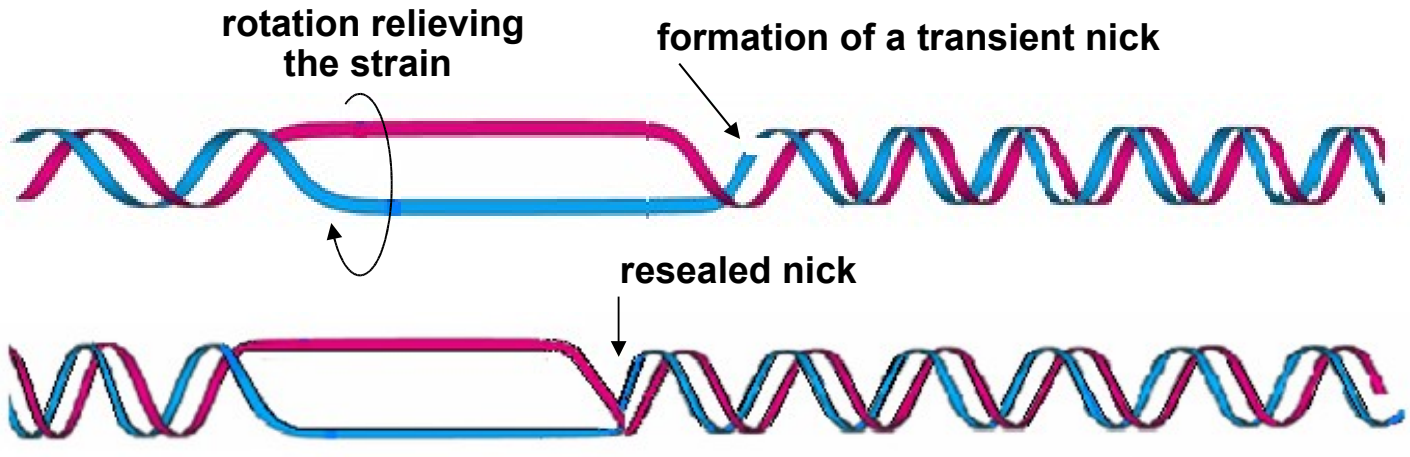


Positive supercoiling is the twisting the helix in the same direction as the original helix (overwinding, tightening the helix). It worsens further unwinding of the helix.

Topoisomerases

can repair overwound segments.

Topoisomerases type I exhibit endonuclease activity, they uncoil DNA by transient breaking a single strand of the double helix. After rotation of the helix on either side of the nick, they catalyze resealing of the strand; this reaction does not require ATP hydrolysis.



Topoisomerases type II make transiently two breaks in both strands of a DNA helix at a short distance. After another DNA helix has passed through the intervening space, the breaks are resealed.
These types of topoisomerases in eukaryotes don't require ATP.

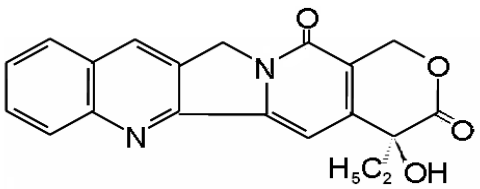
Inhibitors of topoisomerases

make replication impossible. Some inhibitors serve as **anti-cancer drugs**.

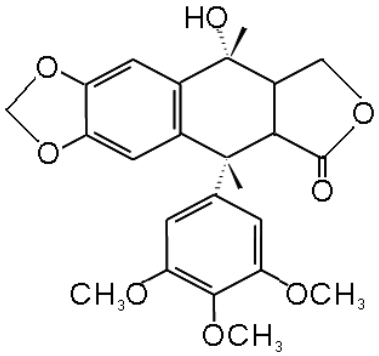
For example,

– **topotecan**, the semisynthetic derivative of toxic **camptothecin**, an alkaloid from *Camptotheca acuminata*, inhibits type I topoisomerases,

– **daunorubicin**, an anthracyclin, antibiotic product of some microorganism, or **etoposide**, the semisynthetic derivative of **podophyllotoxin**, produced by the plant *Podophyllum peltatum*, inhibit type II topoisomerases.



camptothecin



podophyllotoxin

Polydeoxyribonucleotide synthesis

DNA polymerases (**DNA-dependent deoxyribonucleotidyltransferases**) are able to recognize the nucleotide sequences in the template strands and catalyze the formation of 3´-5´ phosphodiester bonds in the replicated complementary strands. They **elongate** existing oligo- or polynucleotide chains in the 5´→3´ direction.

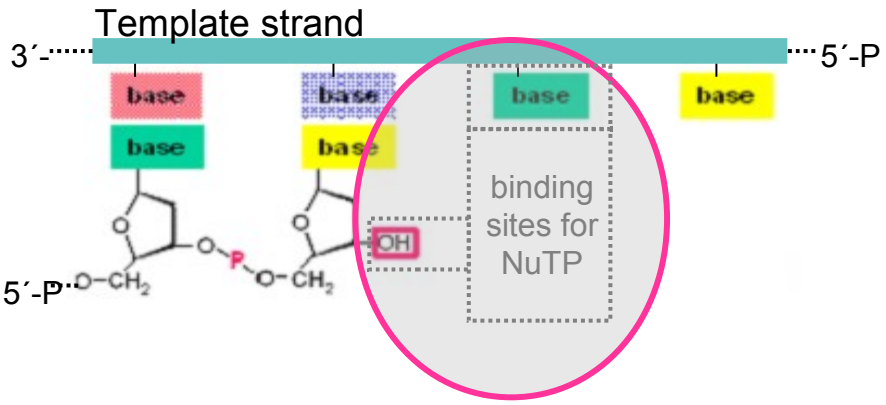
Nucleoside triphosphates are the substrates for the synthesis.

The new strands can grow only in the 5´→ 3´ direction, antiparallel to the template strand.

DNA polymerases have **binding sites**
- for the free 3´-OH group,
- for bases of the template strand,
- for nucleoside triphosphates.

They are **able**
- to cleave β-phosphate bond of NuTP and
  - to form 3´-5´ phosphodiester bond,
- to recognize improperly paired base,

and some of them exhibit
- 3´-5´ exonuclease (proofreading) activity.



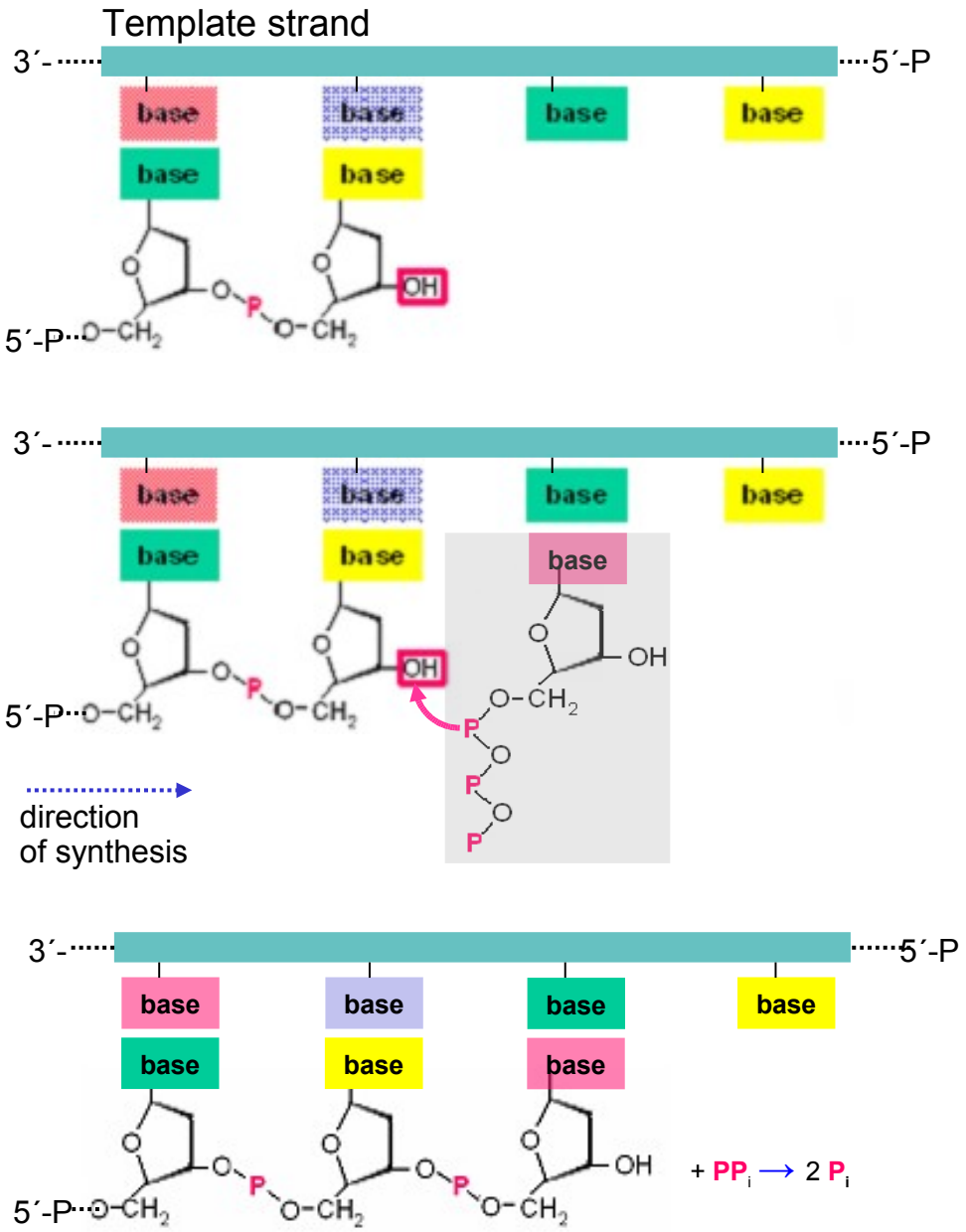
Nucleases catalyze hydrolytic splitting of phosphodiester bonds.

3´-5´ exonuclease activity (a **"proofreading“ activity**)
cleaves phosphodiester bonds **from the 3´-OH end** of the chain, i.e. in the reverse direction from that of synthesis, **if there is a mismatch** (improperly paired base), and release single nucleotides.

If DNA polymerase mispairs a nucleotide with the template, the 3´-5´ exonuclease activity of the ***DNA polymerases δ*** and ***ε*** is utilized to excise the mismatched nucleotide.

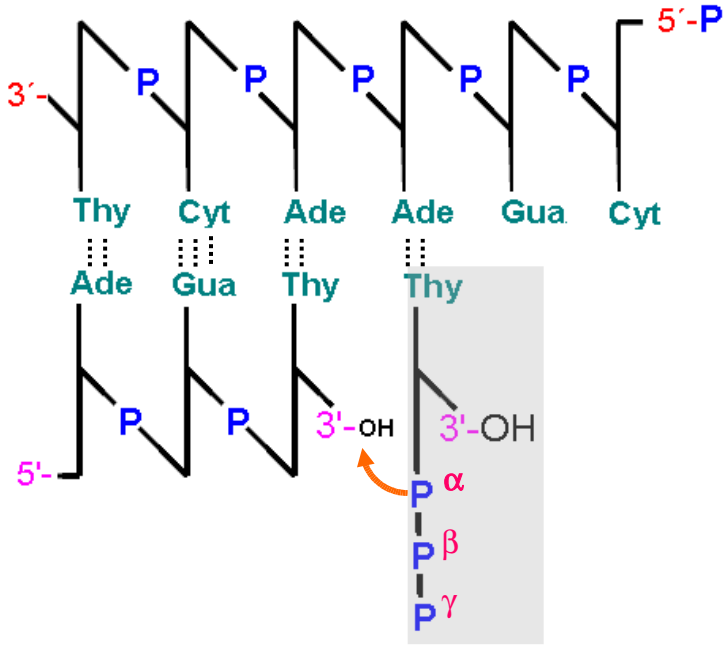
Among eukaryotic DNA polymerases, only ***DNA polymerase β*** has a **5´-3´ exonuclease activity** that is needed for splitting of RNA primers, or for repairs of DNA strands.

Endonucleases
cleave bonds **within the chains** and produce single-stranded nicks.



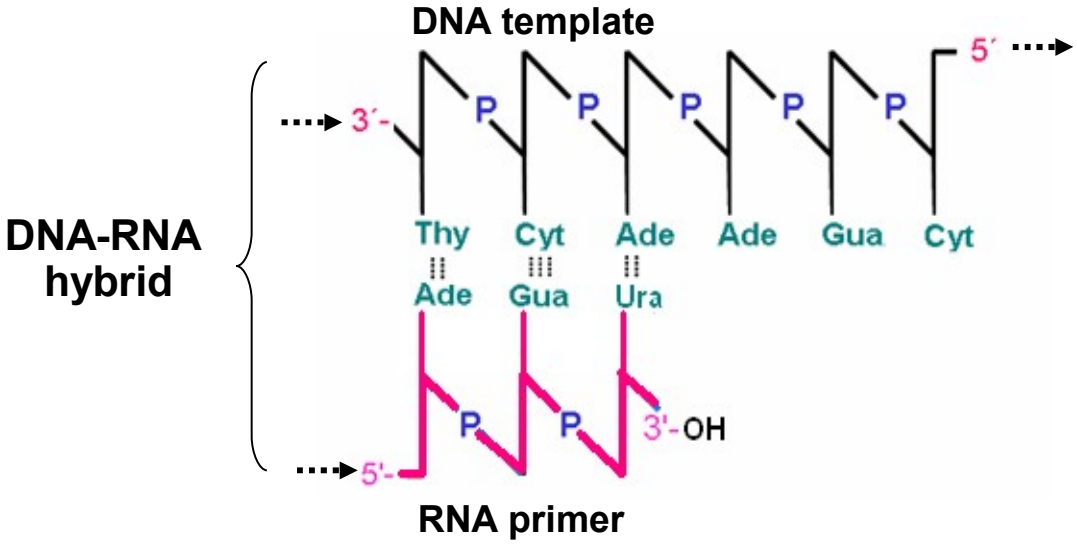
New 3´-5´ phosphodiester bond originates in the reaction between 3´-OH group of existing chain and α-5´-phosphate of the incoming nucleoside triphosphate.

Diphosphate is released (complexed with Mg^{2+} ions).



DNA polymerases cannot start the synthesis of DNA chains de novo. Because they require a free 3´-hydroxyl group for linking a new nucleotide, they are able to elongate only existing chains. . Before the DNA chain synthesis begins, a short stretch of RNA complementary to the template, **RNA primer**, is synthesized. It consists of about 10 – 20 ribonucleotides.

The synthesis of primers de novo is catalyzed by a specialized DNA-dependent RNA polymerase called ***primase***. In eukaryotes, ***DNA polymerase α*** has primase activity, used to synthesize RNA primers.

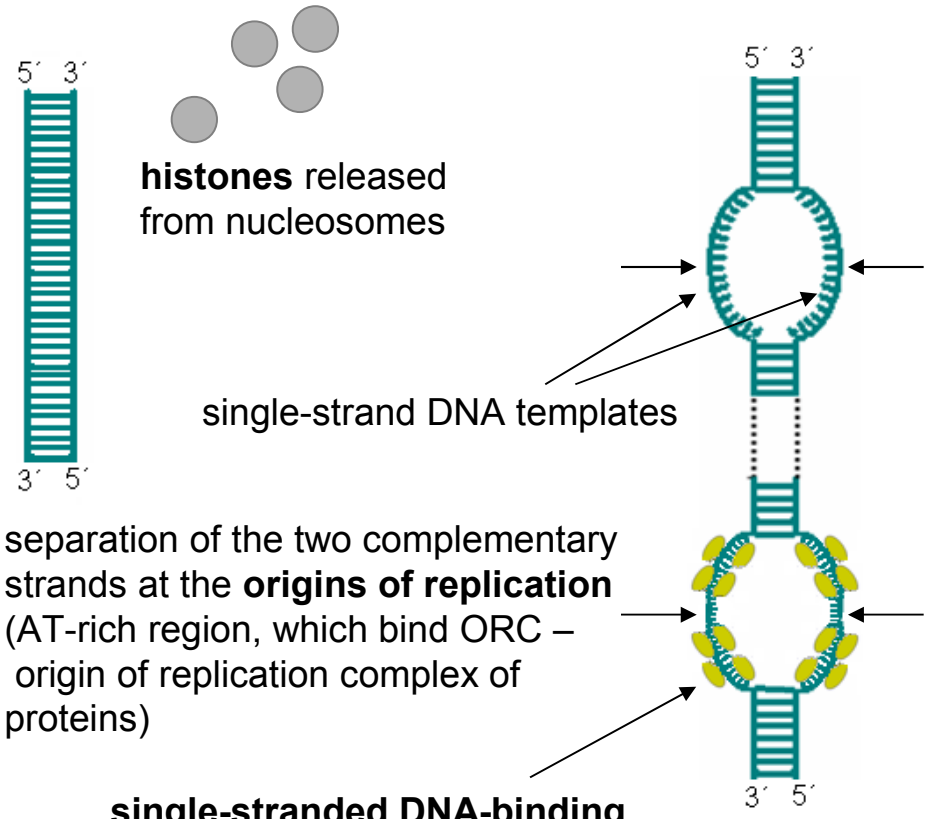


After a sequence of some deoxyribonucleotides has been added to the primer, DNA pol α is displaced. The DNA pol δ or ε bind onto the template and replication continues until adjacent replicons meet and fuse.

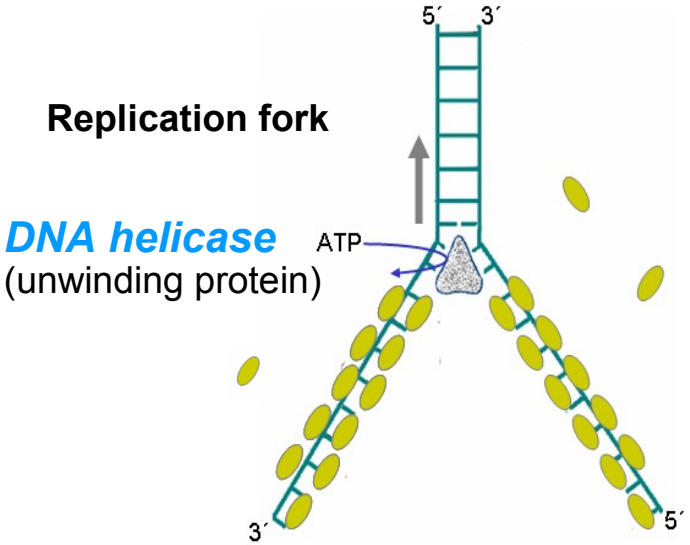
RNA primers are removed and the DNA fragments are joined.

Chromosomes in eukaryotes are very long DNA molecules that cannot be replicated continuously.

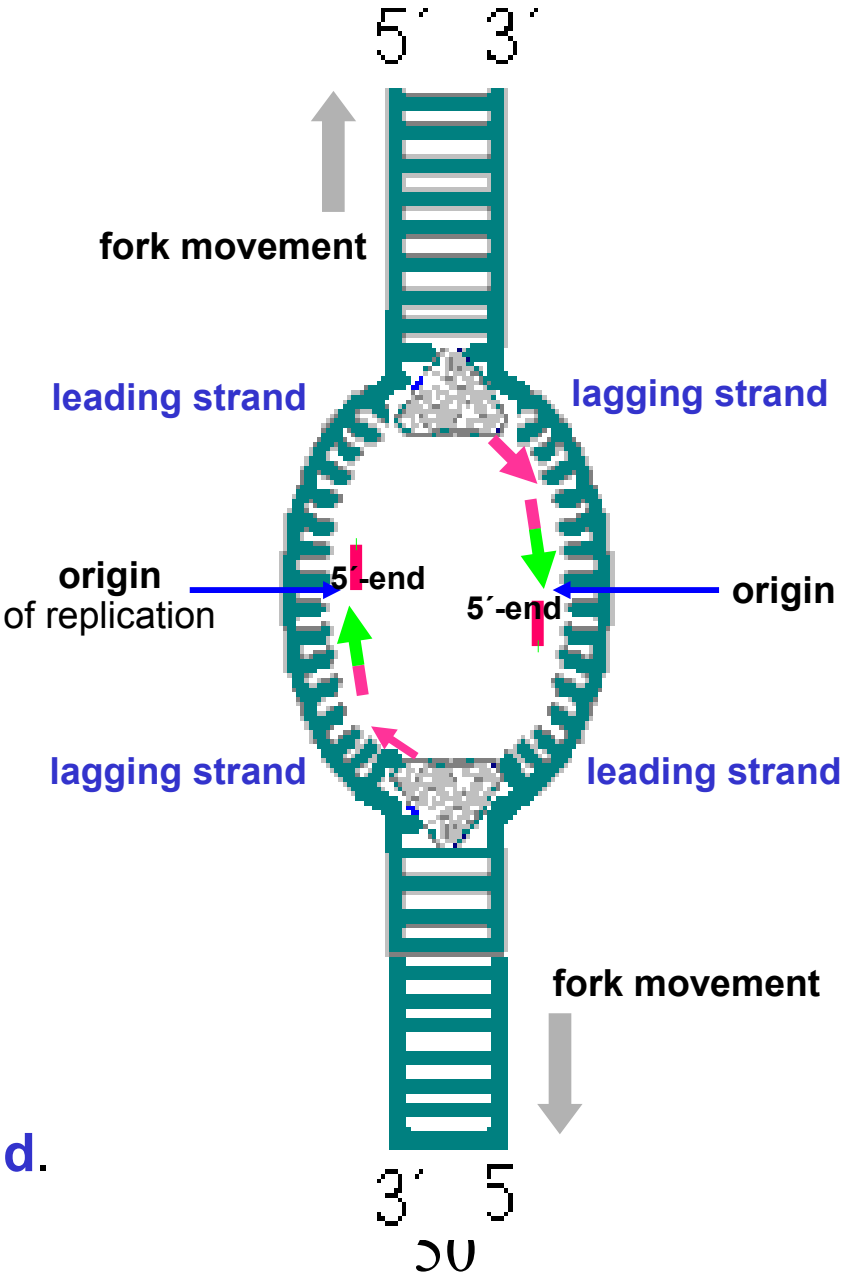
Replication is initiated at **multiple origins** (up to several hundred in each chromosome, one every 30 to 300 kbp) **in both directions**.



In each replicon (replication eye or bubble), there are two replication forks, which move in opposite directions.

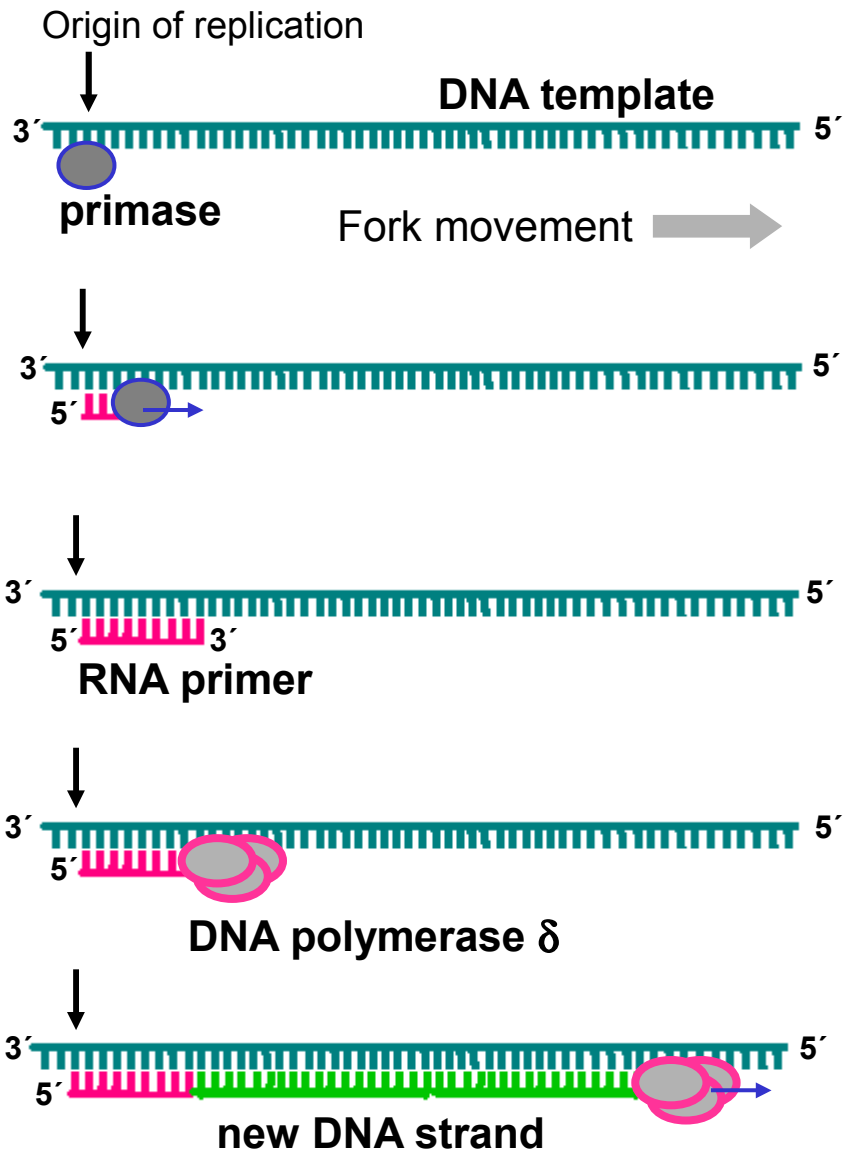


Since DNA polymerases add nucleotides only to 3´-ends, DNA synthesis can proceed in a simple continuous manner along one strand of the fork, that is called the **leading strand**.



The antiparallel strand of the fork is synthesized from short segments called the **Okazaki fragments** (about 100 – 200 nucleotides), which are joined after removal of primers and filling in the gaps. This DNA is called the **lagging strand**.

Continuous DNA synthesis of the leading strand



"Prepriming" proteins and primase bind onto single-strand DNA and displace SSB-proteins.

A short RNA primer is synthesized and (after polymerase "switching“) elongated by DNA polymerase synthesizing new DNA strand continuously until reaching adjacent replicon.

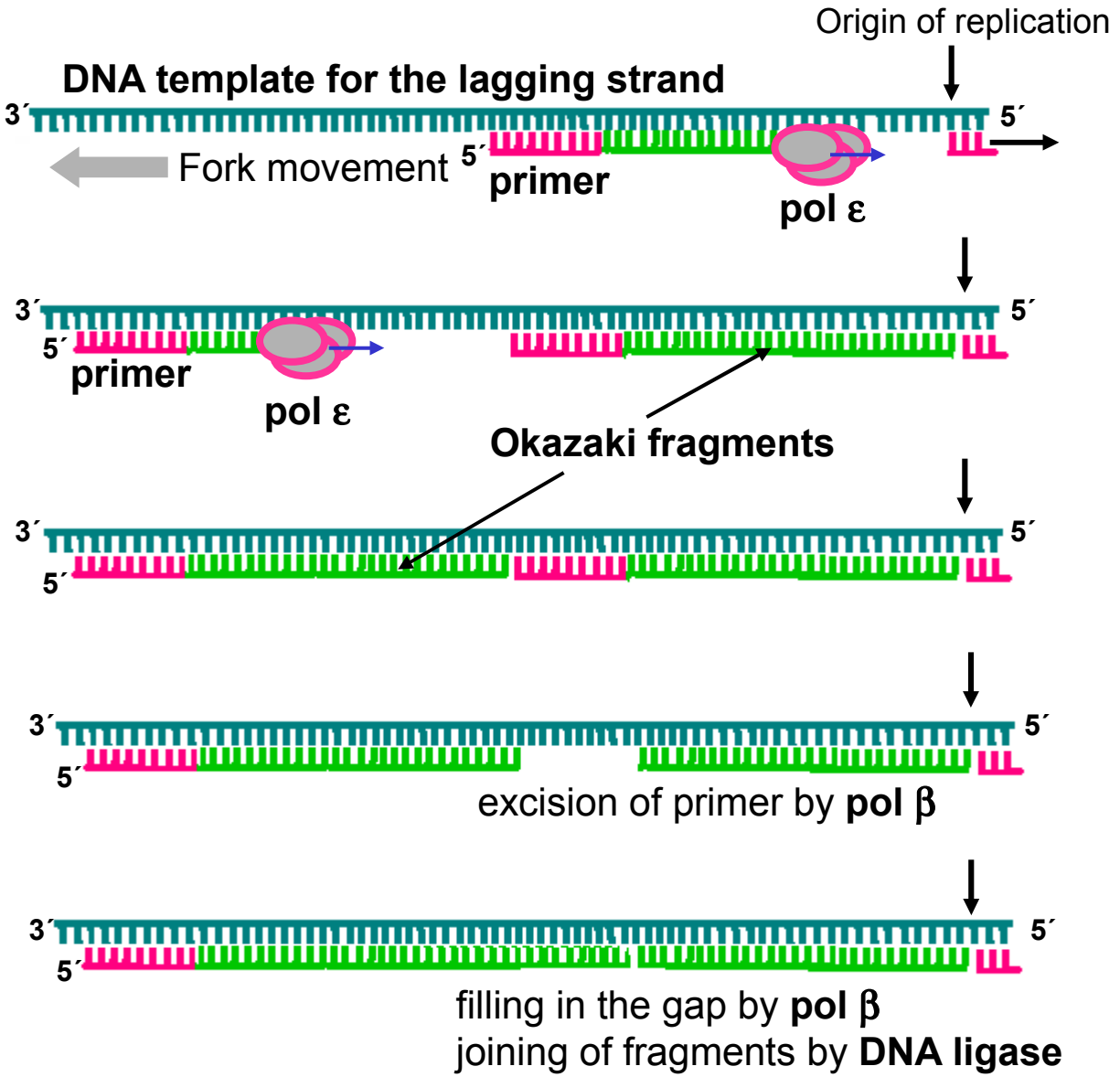
Discontinuous DNA synthesis of the lagging strand

Unwinding of the duplex allows the synthesis of new RNA primers.

Primers are elongated by DNA polymerase synthesizing short DNA strands.

RNA primer is removed by the 5´-3´ exonuclease activity of polymerase β. The same enzyme also replaces primer with DNA by elongating the Okazaki fragment, so that the gap is filled in. DNA ligase joins the fragments.

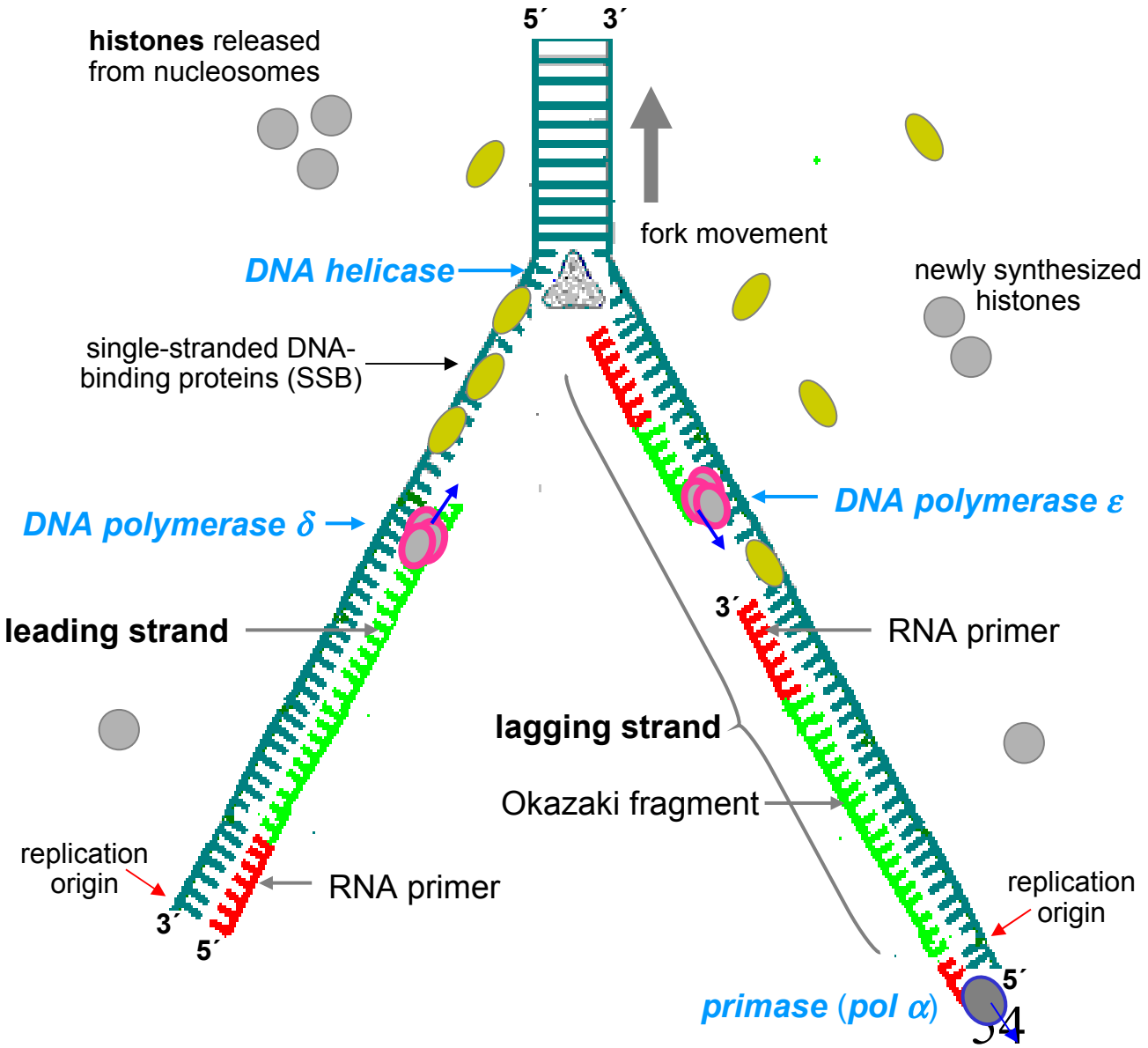
DNA polymerase moves back to initiate a new Okazaki fragment.



Enzyme activities involved in eukaryotic DNA replication

- **Topoisomerases** and **helicase** – unwinding of dsDNA.
- **Primase activity** (DNA-dependent RNA polymerase) catalyzing formation of RNA primers, exhibited by DNA polymerase **α**.
- DNA-dependent **DNA polymerases δ** and **ε** synthesizing leading DNA strands (pol δ) and Okazaki fragments in the lagging strands (pol ε); both enzymes have also **3´-5´ exonuclease (proofreading) activity**.
- **5´-3´ exonuclease activity** (excision of primers) and **DNA polymerase** activity (filling the gap), exhibited by DNA polymerase β.
- **DNA ligase** (with ATPase activity) that removes nicks by joining the fragments through phosphodiester bond.
- **Telomerase activity** (not present in some cell types) that enable replication at the 3´-ends of linear chromosomes.

DNA synthesis at the replication fork



Eukaryotic DNA polymerases

Major type	Function	Exonuclease activity	Relative activity
Polymerase α	**primase** activity (synthesis of the RNA primer)	none	
Polymerase δ	elongates the **leading** strand	3´ - 5´	80 %
Polymerase ε	elongates the **lagging** strand	3´ - 5´	
Polymerase β	excision of primers and filling the resulting gaps	5´ - 3´	10 – 15 %
Polymerase γ	replication of **mitochondrial DNA**	3´ - 5´	2 – 15 %

Telomeres and telomerases

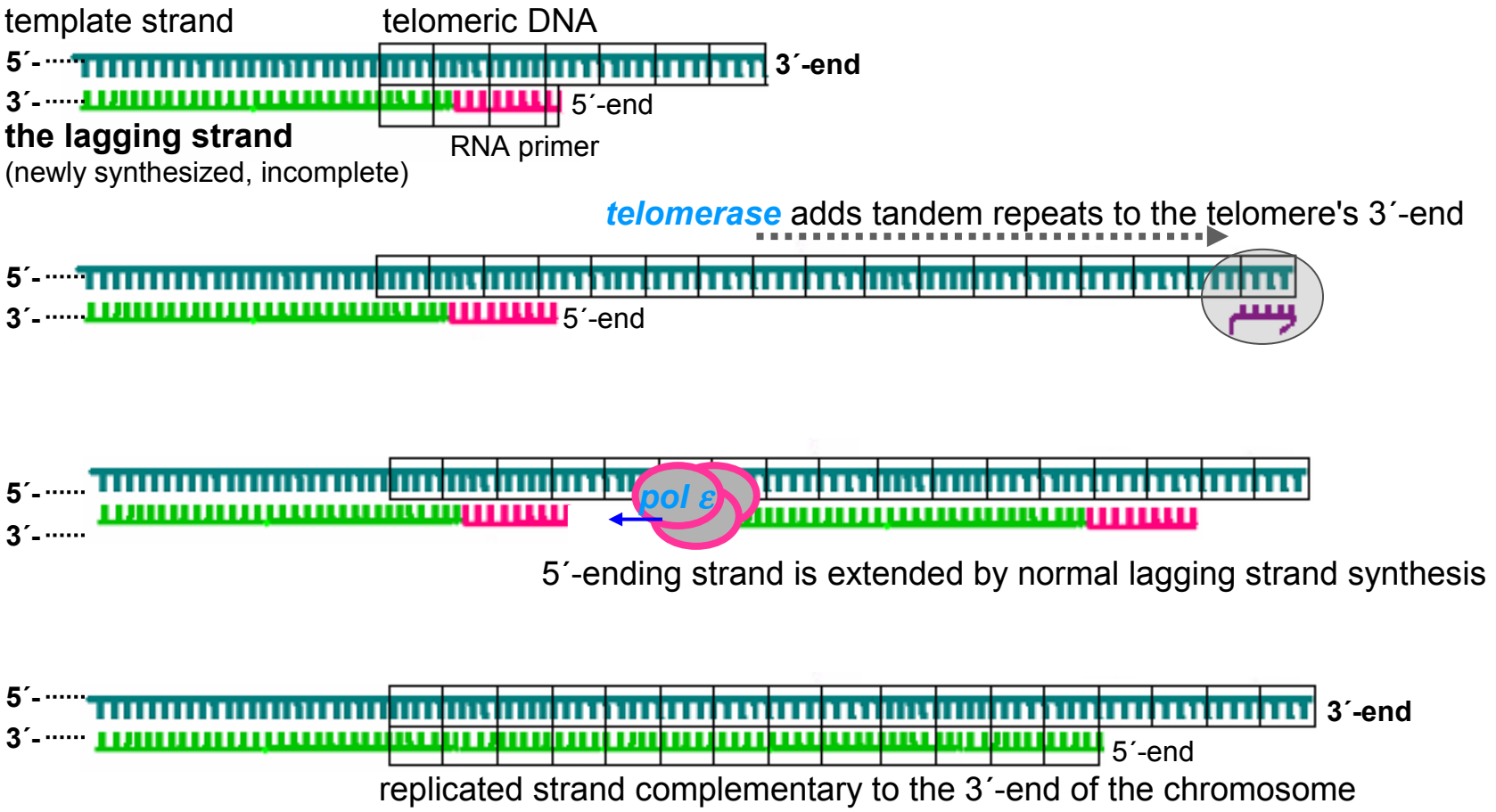
At the ends of eukaryotic linear chromosomes, there are the DNA sequences called **telomeres**. The telomeric DNA is unusual, it contains up to 1000 tandem repeats of a hexanucleotide sequence, G-rich (at the 3´-end, TTAGGG in humans). Telomeres protect the ends of chromosomes against nuclease activities.

It is difficult to fully replicate template DNA strands at their 3´-ends, because the syntheses of the lagging strands require parental strands longer than those replicated in fact (the lagging strand would have an incomplete 5´-end after removal of the last RNA primer). Unless long telomere sequences are attached to parental DNAs, each round of replication would shorten chromosomes.

Telomerases – not present in all cell types – elongate the telomeres by attaching the newly synthesized telomeric hexanucleotide repeats.
A telomerase is a specialized **reverse transcriptase** (RNA-dependent DNA polymerase) **that carries its own RNA template** - it is a nucleoprotein whose RNA component contains a segment that is complementary to the telomeric tandem repeats.

Telomerase in the replication of the chromosome's 3´-end

(the replication of the chromosome's 5´-end is not shown)



Somatic cells of multicellular organisms lack telomerase activity.
The chromosomes are shortened at both ends by the length of an RNA primer with every cycle of DNA replication and cell division, because the RNA primer at the 5´-end of a completed lagging strand cannot be replaced with DNA by normal lagging strand synthesis. After the essential genes located near the ends of chromosomes have been lost by this process, the descendent cells have to die.

There is a correlation between the initial telomere length of the cells in a cell culture and their proliferative capacity. The cells with longer telomeres undergo significantly more divisions than the cells with relatively short telomeres.

It seems to be obvious that telomere shortening is a significant cause of cellular senescence and hence aging.

Germ cells exhibit an active telomerase (at some stage of cell growth at least) and telomeres of stable length, as well as **cancer cells**, which are immortal and grow uncontrollably.

Inhibitors of telomerases might be useful antitumour drugs.

DNA repair

The bases of DNA can be altered or lost **spontaneously**:

- cytosine is slowly **deaminated** to form uracil,
- **depurination** occurs oft (about 10^4 purines are lost spontaneously per day).

DNA also suffers many **environmental insults**:

- **oxidative damage** produced by hydroxyl radical •OH, e.g. oxidation of guanine to 8-oxoguanine that results in G-to-T transversion (after replication, TA mutation is introduced instead of GC base pair),
- **UV** or **ionizing radiation**,
- **various compounds** (e.g. alkylating agents, acridines that can intercalate dsDNA) act as mutagens, teratogens, carcinogens, etc.

There are several **specific types of damage** to DNA, including

- **single-base alteration** (missed base due to depurination, deamination of cytosine or adenine, alkylation), deletion or insertion of a nucleotide, base-analogue incorporation, alterations of adjacent pairs of bases (thymine cyclobutyl dimers after UV radiation),
- **chain breaks** by ionizing radiation, bleomycin, etc.
- **cross-linkages** between bases in opposite chains (bifunctional alkylating agents, derivatives of psoralene, mitomycin C) or between bases and proteins (namely histones).

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, and

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**.

Examples:

Base excision repair

Cytosine has been deaminated to uracil:

5′-ATGCUGCATTGA
3′-TACGGCGTAACT

Uracil N-glycosylase removes the base, an AP site (apyrimidinic site) exists - the ribose phosphate backbone is intact, the base is missing.

5′-ATGC GCATTGA
3′-TACGGCGTAACT

AP endonuclease splits the phosphodiester bond adjacent to the missing base, the residual deoxyribose unit is excised by ***exonuclease*** (or deoxyribose phosphodiesterase).

5′-ATGC GCATTGA
3′-TACGGCGTAACT

The gap is filled in by insertion of cytidine phosphate by ***DNA polymerase β***

5′-ATGCCGCATTGA
3′-TACGGCGTAACT

and the corrected strand resealed by ***DNA ligase***.

5′-ATGCCGCATTGA
3′-TACGGCGTAACT

Nucleotide excision repair

Thymine dimer has been formed by UV radiation:

The distortion induced by the pyrimidine dimer is recognized by the multisubunit complex. ***Endonuclease*** called excinuclease cuts the strand at (not closely adjacent) phosphodiester bonds on the dimer's 5′- and 3′-sides, and

exonuclease catalyzes excision of the oligonucleotide.

DNA polymerase β carries out the repair synthesis.

The 3′-end of the newly synthesized DNA stretch and the original part of the DNA chain are joined by ***DNA ligase.***