



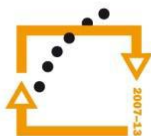
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EVROPSKÁ UNIE



MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



OP Vzdělávání
pro konkurenceschopnost

INVESTICE
DO ROZVOJE
VZDĚLÁVÁNÍ

„Propojení výuky oborů Molekulární a buněčné biologie a Ochrany a tvorby životního prostředí“

Reg. č.: CZ.1.07/2.2.00/28.0032



evropský
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fond v ČR



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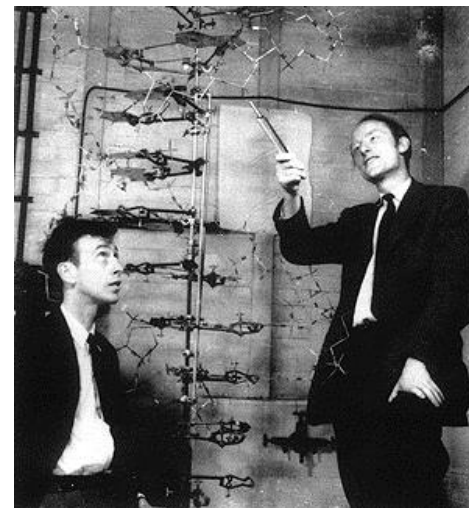


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VZDĚLÁVÁNÍ



Molekulární genetik (Molekulární základy dědičnosti)

Gen - historie

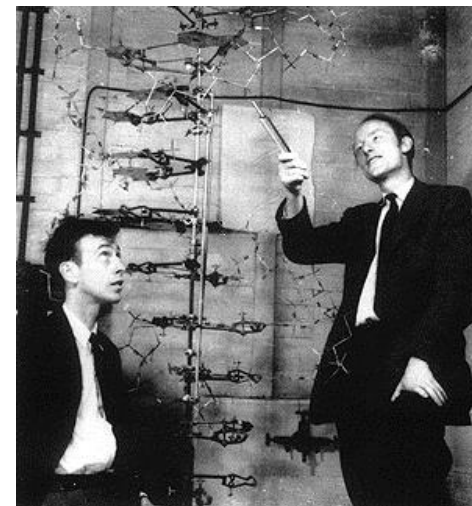
1909 Johanssen – Gen = Jednotka dědičnosti

1941 Beadle, Tatum - Hypotéza

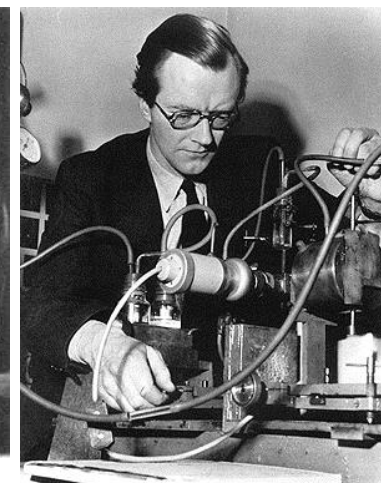
„Jeden gen = Jeden enzym“

1944 Avery a kol. Důkaz funkce DNA

1953 **Popis struktury DNA** (NC1962)



James Watson, Francis Crick; Rosalind Franklin, Maurice Wilkins

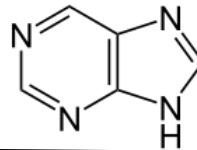


Struktura nukleové kyseliny

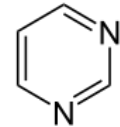
Cukerná pentóza: 2-deoxy-D-ribóza
D-ribóza

Kyselina fosforečná: H_3PO_4

Purin

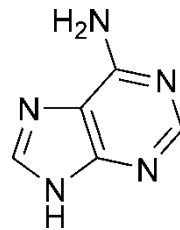


Pyrimidin

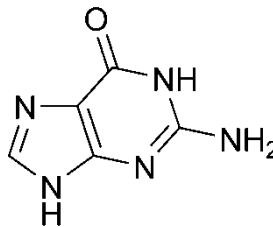


Dusíkatá
báze

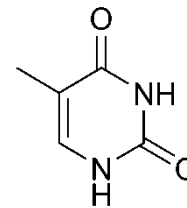
Adenin



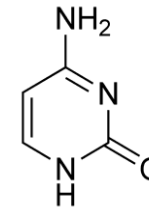
Guanin



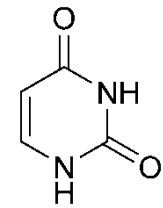
Tymin



Cytosin



Uracil



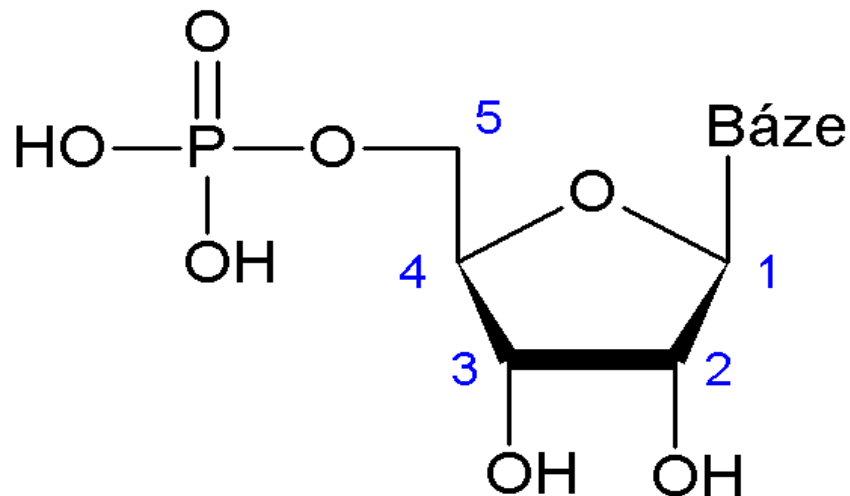
komplementarita bází: A - T

G - C

A - U

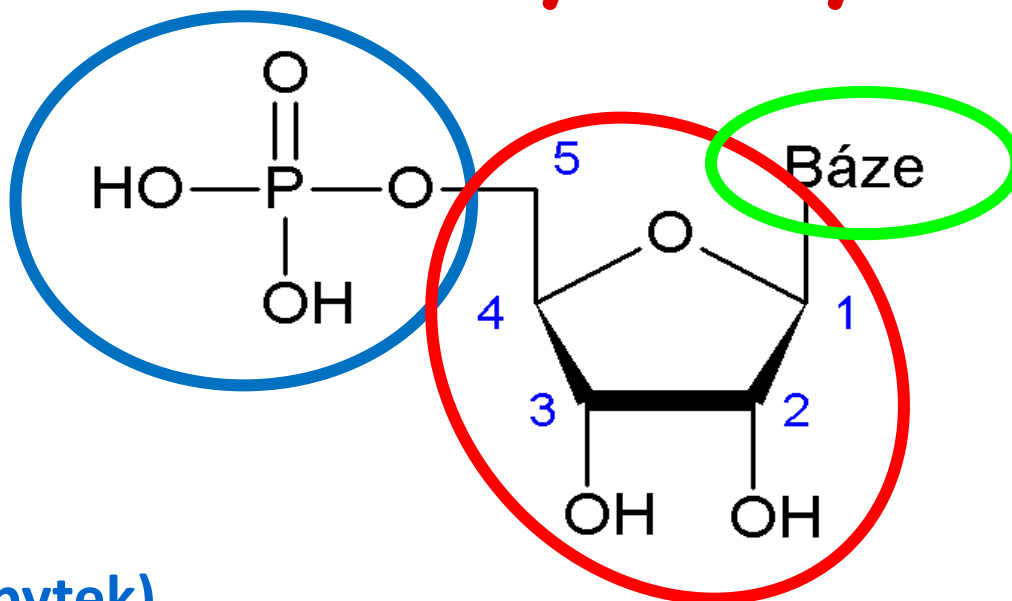
Struktura nukleové kyseliny

- Nukleotid:



Struktura nukleové kyseliny

- Nukleotid:



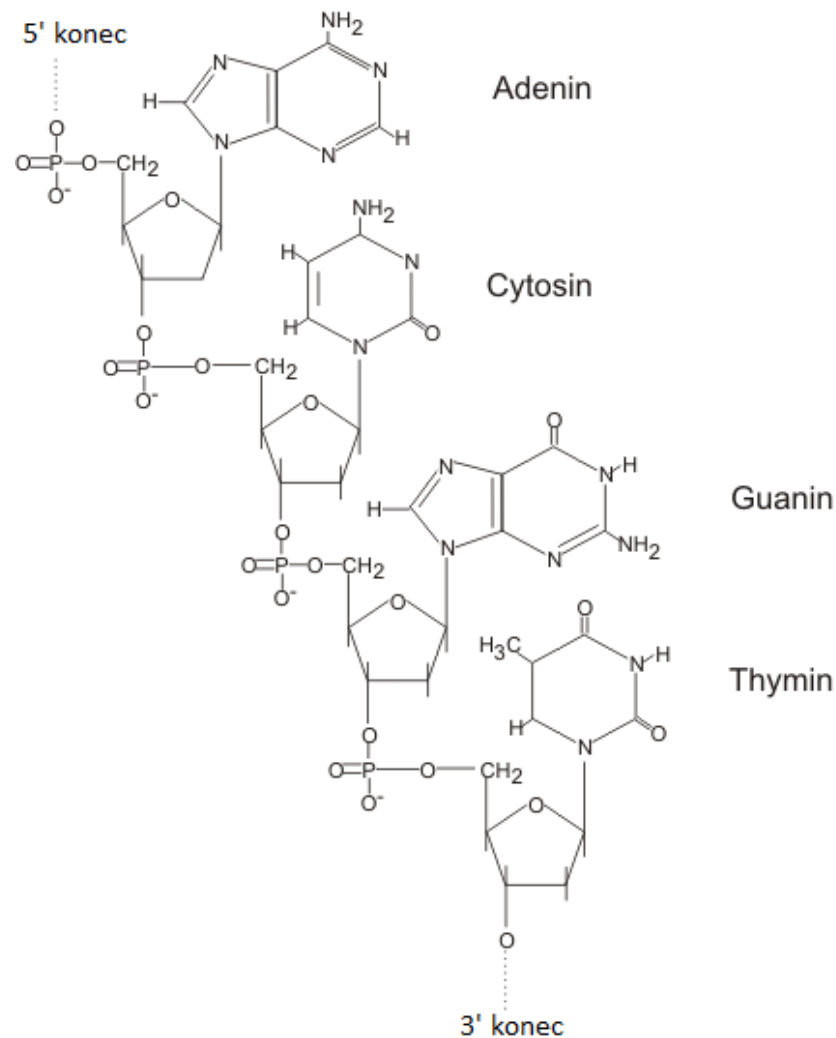
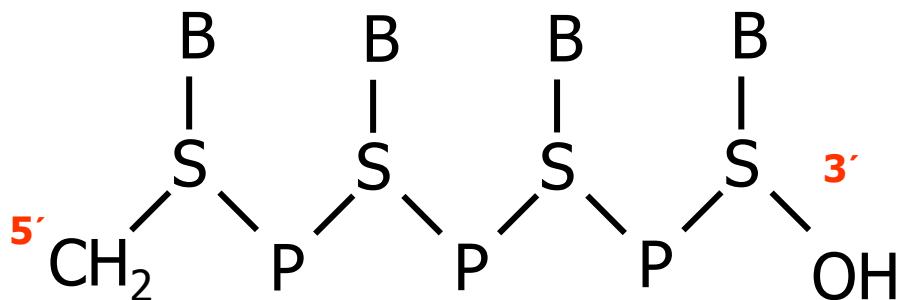
kyselina fosforečná (zbytek)

cukerná pentóza

dusíkatá báze

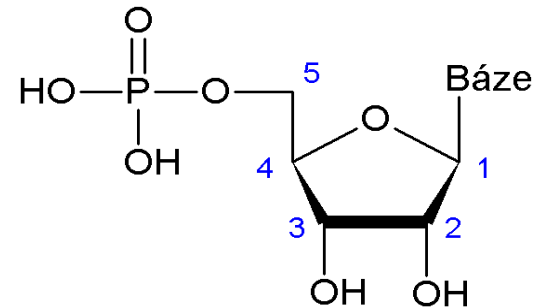
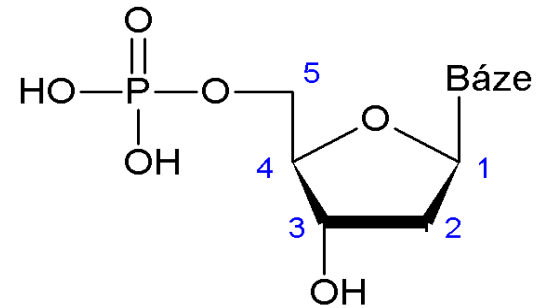
Struktura nukleové kyseliny

- (Poly)Nukleotidový
- fosfodiesterová vazba



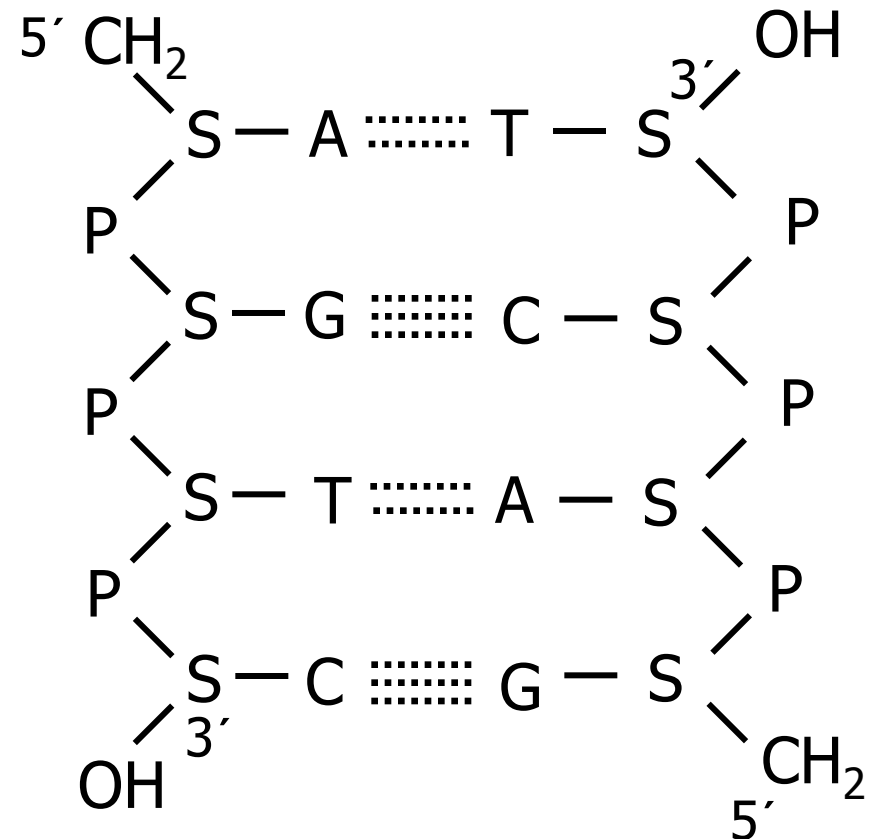
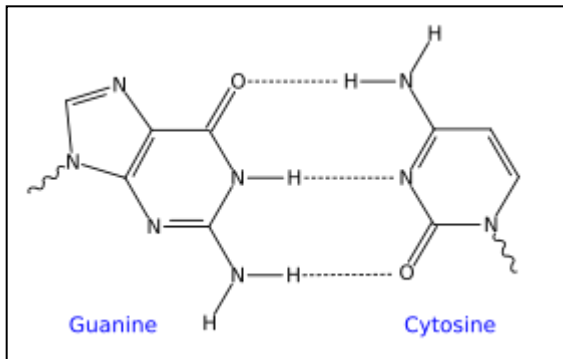
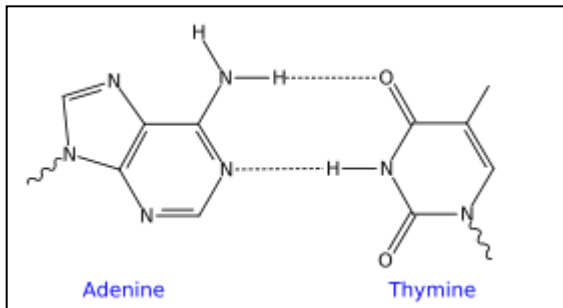
Nukleové kyseliny

- DNA – deoxyribonukleová kyselina
- RNA – ribonukleová kyselina



DNA - deoxyribonukleová kyselina

- 2-deoxy-D-ribóza
- A, G, T, C
- 2 vlákna, antiparalelní orientace = dvoušroubovice



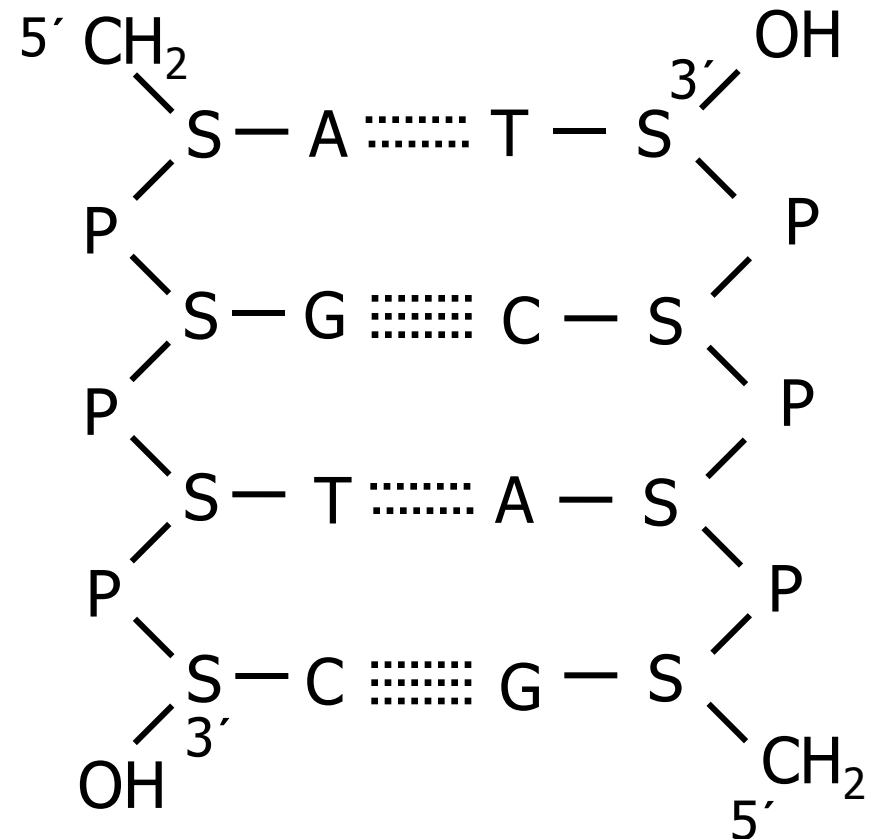
DNA - deoxyribonukleová kyselina

- 2-deoxy-D-ribóza
- A, G, T, C
- 2 vlákna, antiparalelní orientace = dvoušroubovice

Chargaffovo pravidlo:

$\Sigma \text{Purinů} = \Sigma \text{Pyrimidinů}$

$\Sigma A = \Sigma T ; \Sigma G = \Sigma C$



DNA - deoxyribonukleová kyselina

Dvoušroubovice

1. Pravotočivá B

10 bp na závit

Průměr 23.7 Å

2. Pravotočivá A

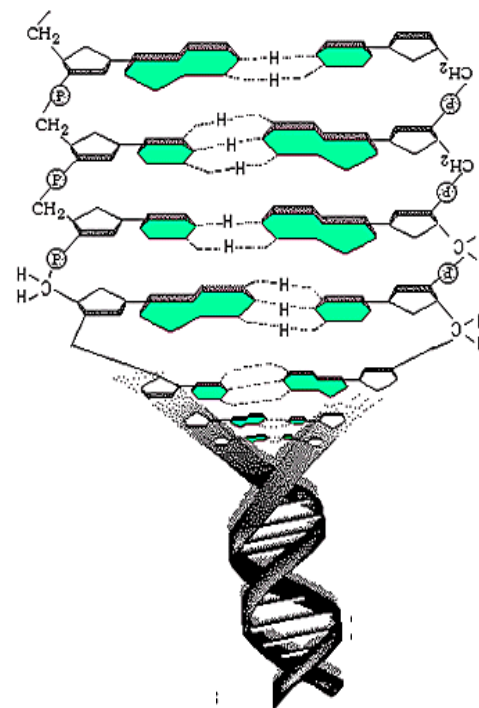
11 bp na závit

Průměr 25.5 Å

3. Levotočivá Z

12 bp na závit

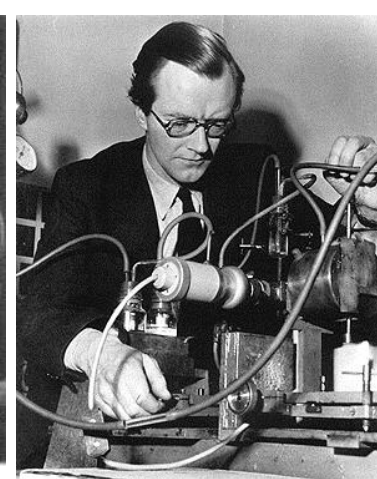
průměr 18.4 Å



Gen - historie

1953 Popis struktury DNA (NC1962)

James Watson, Francis Crick; Rosalind Franklin, Maurice Wilkins



equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

¹ Young, F. D., Gerard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1925).

² Longuet-Higgins, M. S., *Mon. Not. Roy. Astr. Soc., Geophys. Supp.*, **5**, 285 (1949).

³ Von Atz, W. S., *Woods Hole Papers in Phys. Oceanog. Meteor.*, **11** (3) (1950).

⁴ Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1908).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey¹. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β -D-deoxy-ribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's² model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate-sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.

is a residue on each chain every 3.4 Å. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 Å. The distance of a phosphorus atom from the fibre axis is 10 Å. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally^{3,4} that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data^{5,6} on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge, April 2.

¹ Pauling, L., and Corey, R. B., *Nature*, **171**, 348 (1953); *Proc. U.S. Nat. Acad. Sci.*, **29**, 81 (1953).

² Furberg, S., *Acta Chem. Scand.*, **6**, 394 (1952).

³ Chargaff, E., for references see Zamenhof, S., Trauterman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).

⁴ Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).

⁵ Astbury, W. T., *Symp. Soc. Exp. Biol.*, **1**, Nucleic Acid, 69 (Camb. Univ. Press, 1947).

⁶ Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 102 (1953).

Molecular Structure of Deoxyribose Nucleic Acids

WHILE the biological properties of deoxyribose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury¹) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

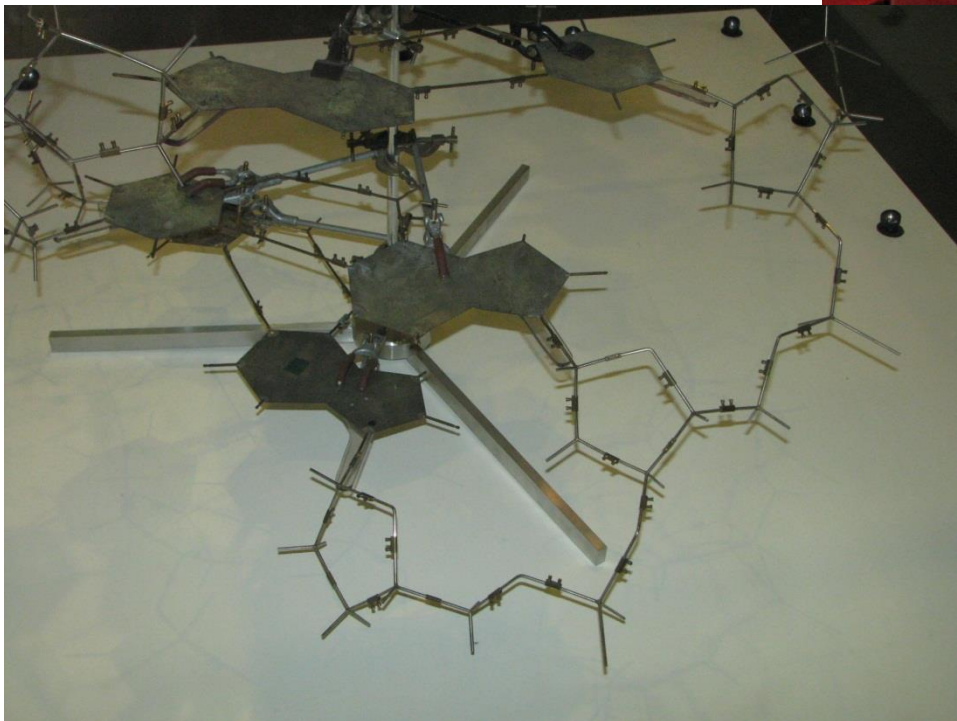
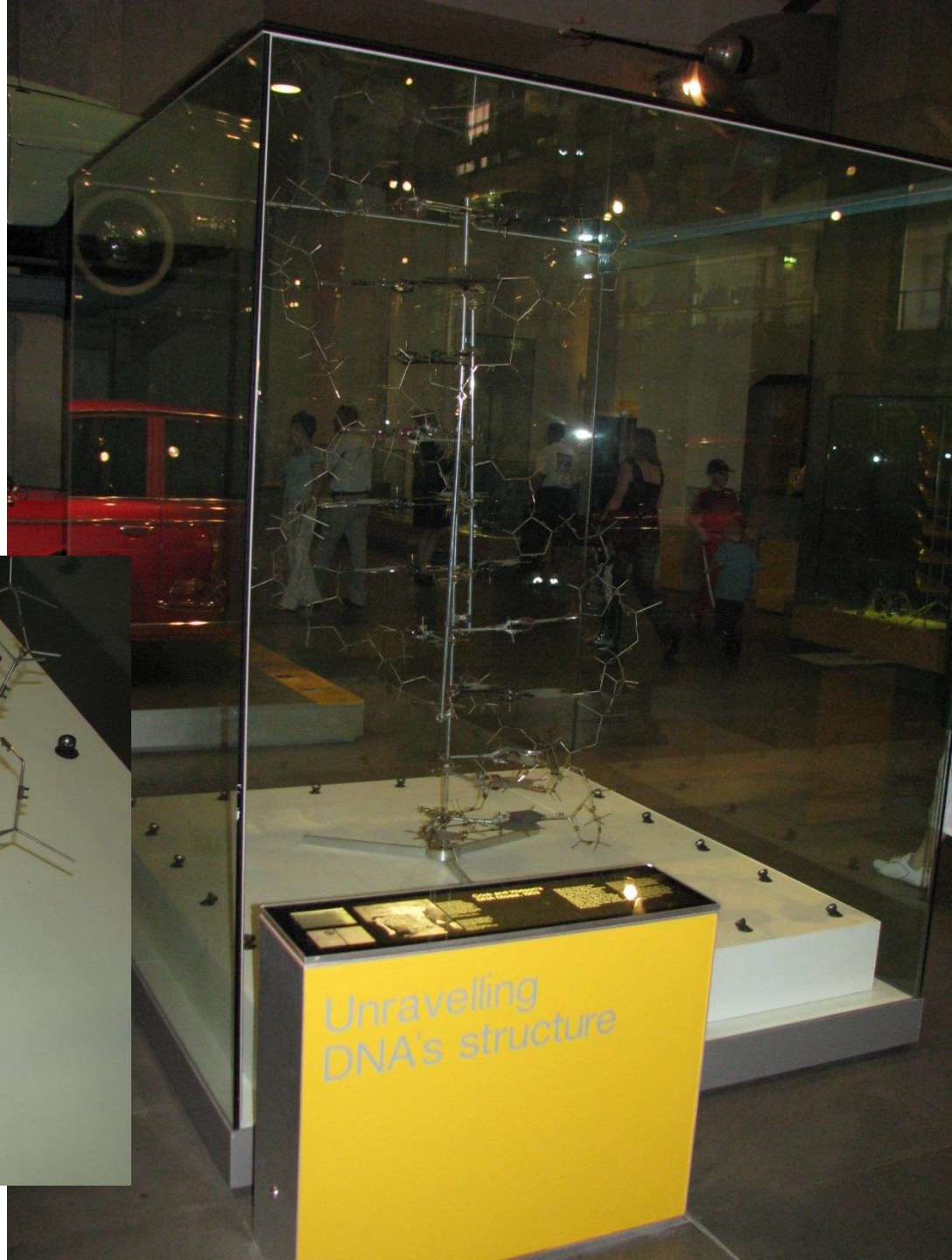
The structure of deoxyribose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably in nucleoprotein, extracted or in cells, and in purified nucleate). The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline¹⁻³, semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the regular spacing of nucleotides along the chain, and the other by the longer spacings of the chain configuration. The sequence of different nitrogen bases along the chain is not made visible.

Oriented paracrystalline deoxyribose nucleic acid ('structure B' in the following communication by Franklin and Gosling) gives a fibre diagram as shown in Fig. 1 (cf. ref. 4). Astbury suggested that the strong 3.4-Å. reflexion corresponded to the internucleotide repeat along the fibre axis. The ~34 Å. layer lines, however, are not due to a repeat of a polynucleotide composition, but to the chain configuration repeat, which causes strong diffraction as the nucleotide chains have higher density than the interstitial water. The absence of reflexions on or near the meridian immediately suggests a helical structure with axis parallel to fibre length.

Diffraction by Helices

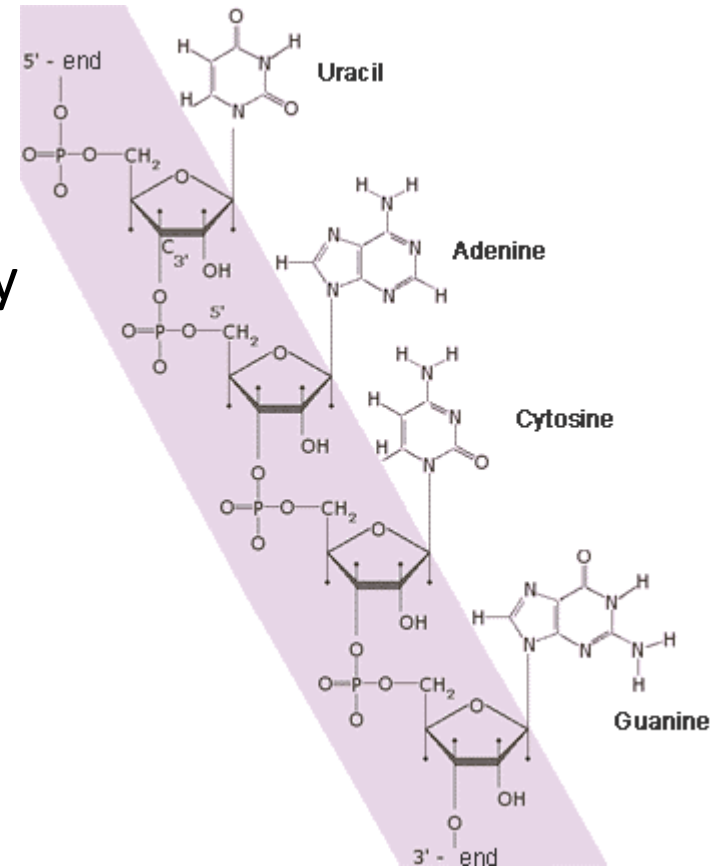
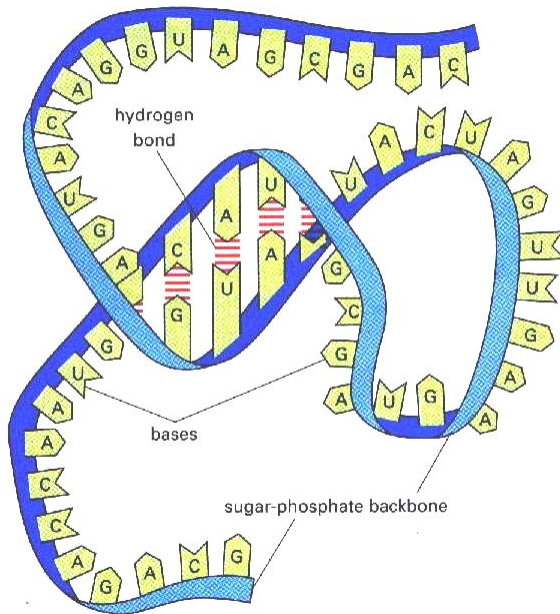
It may be shown⁵ (also Stokes, unpublished) that the intensity distribution in the diffraction pattern of a series of points equally spaced along a helix is given by the squares of Bessel functions. A uniform continuous helix gives a series of layer lines of spacing corresponding to the helix pitch, the intensity distribution along the *n*th layer line being proportional to the square of J_n , the *n*th order Bessel function. A straight line may be drawn approximately through

model DNA



RNA - ribonukleová kyselina

- D-ribóza
- A, G, U, C
- Jednovláknová, místní vlásenky



RNA - ribonukleová kyselina

- *mRNA*
- *rRNA*
- *tRNA*

- *snRNA*
- *miRNA*
- *siRNA*
- *tasiRNA, rasiRNA ...*

Definice:

Gen je konkrétní úsek DNA (u RNA virů RNA), daný sekvencí (pořadím) nukleotidů, kódující strukturní (polypeptid) nebo funkční (např. rRNA, tRNA) produkt.

gen – alela – lokus

Genom je veškerá genetická informace organismu, kompletní sekvence (pořadí dusíkatých bází), soubor veškeré kódující a nekódující informace (jaderné i nejaderné) v jedné kopii.

Gen

- Strukturní gen – nese informace o struktuře aminokyselinového vlákna / peptidu
- Gen pro RNA – rRNA, tRNA
- Regulační oblasti (funkční gen)

Centrální (ústřední) dogma

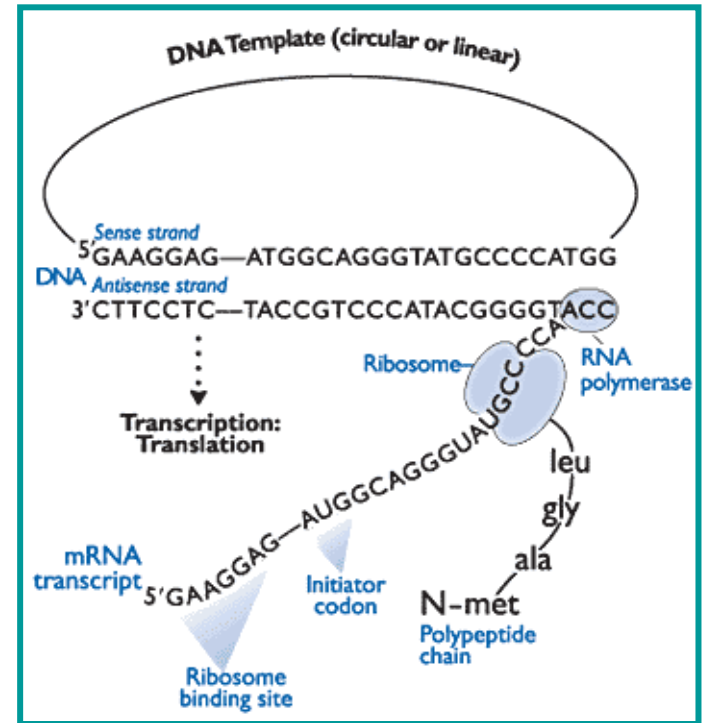
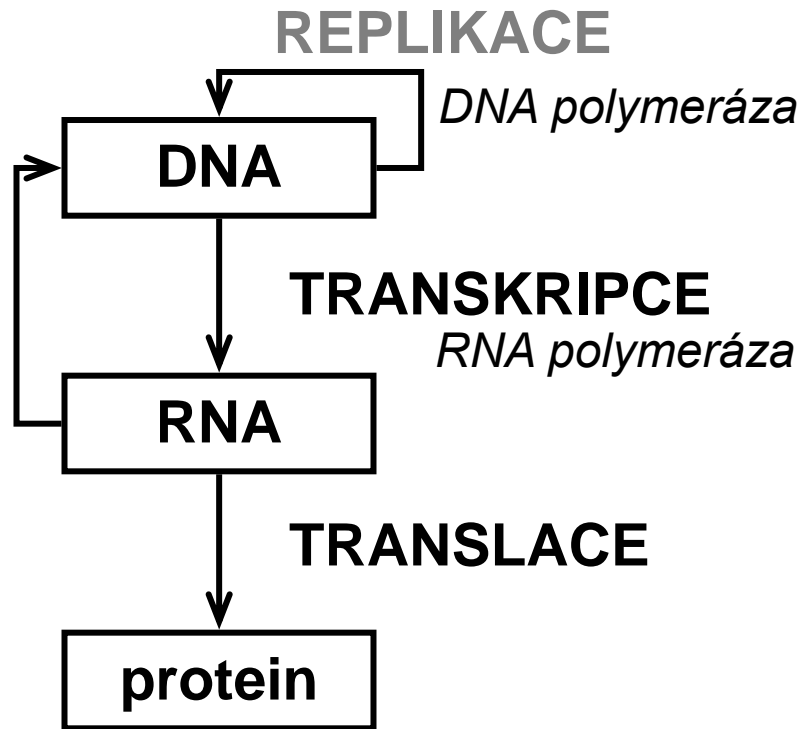
Realizace = exprese genetické informace?

Centrální (ústřední) dogma

1958 F. Crick – **expres genetiké informace**

REVERZNÍ TRANSKRIPCE

Reverzní transkriptáza



Transkripce

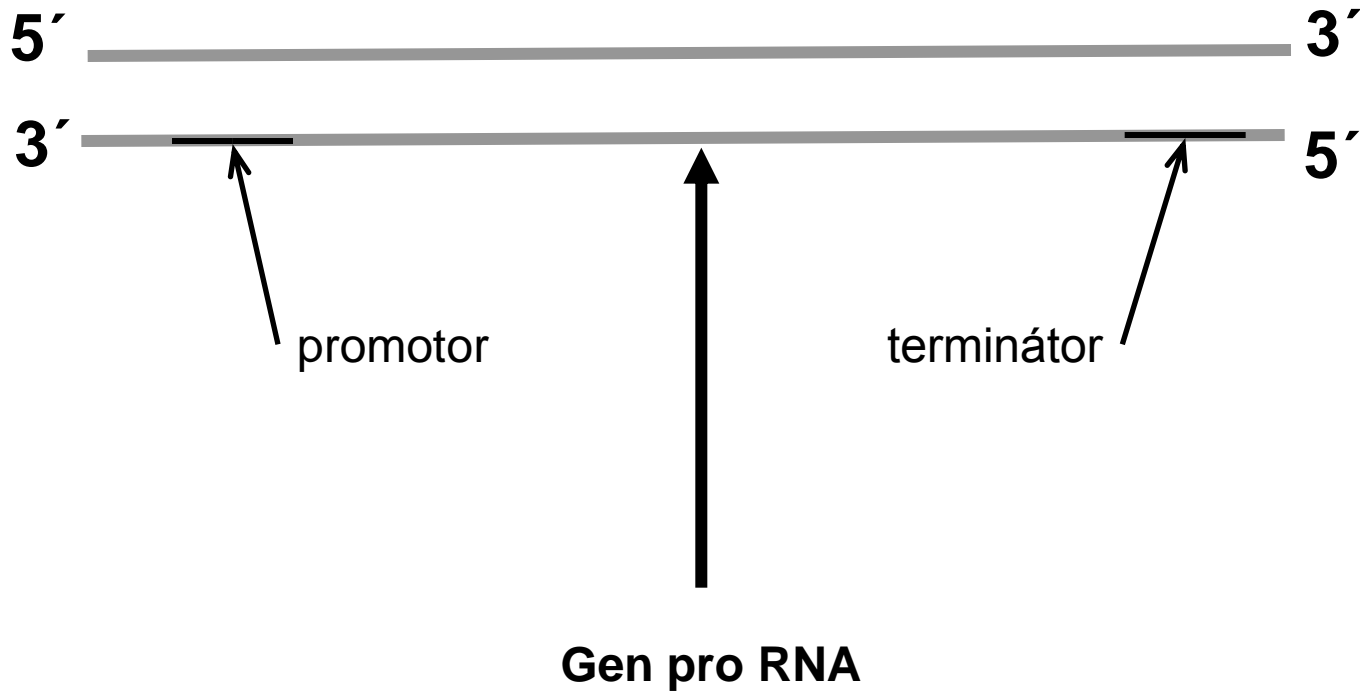
Přepis genetické informace DNA do molekuly (pre)RNA

- Probíhá v jádře
- Templátem je NEKÓDUJÍCÍ vlákno DNA v orientaci 3' - 5'
- Enzym RNA polymeráza

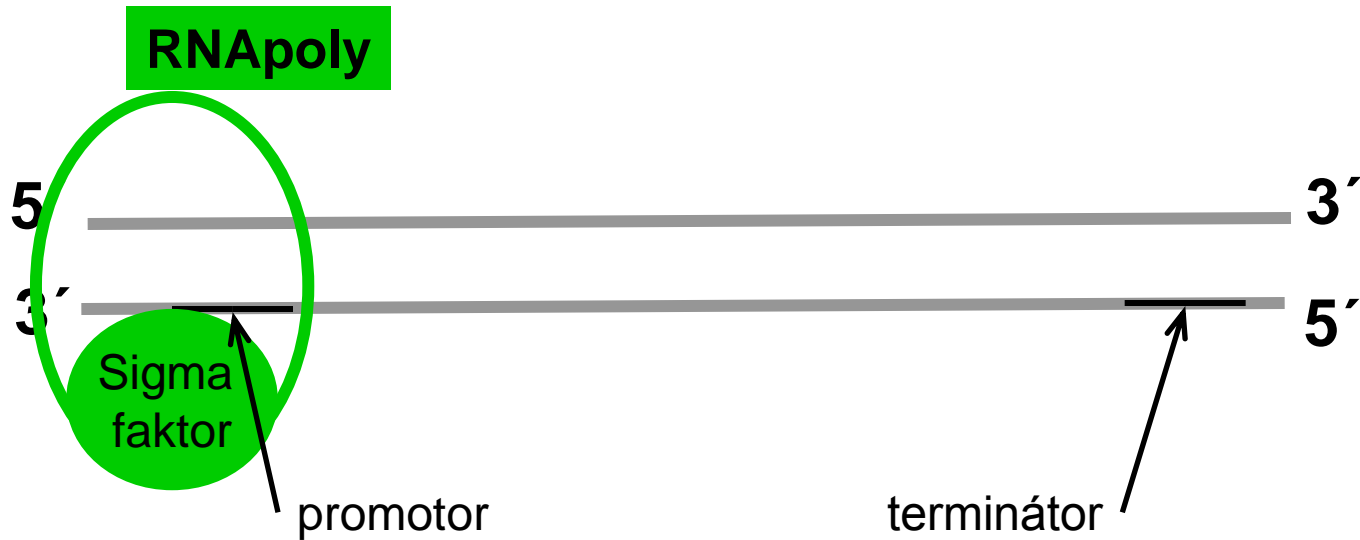
Transkripce - RNA polymerázy

- RNA polymeráza prokaryot:
 - Sigma faktor
 - Jádru / holoenzym (α , β podjednotky)
- RNA polymeráza eukaryot:
 - RNA pol I : rRNA
 - RNA pol II : mRNA, malé regulační RNA
 - RNA pol III : tRNA a další malé RNA

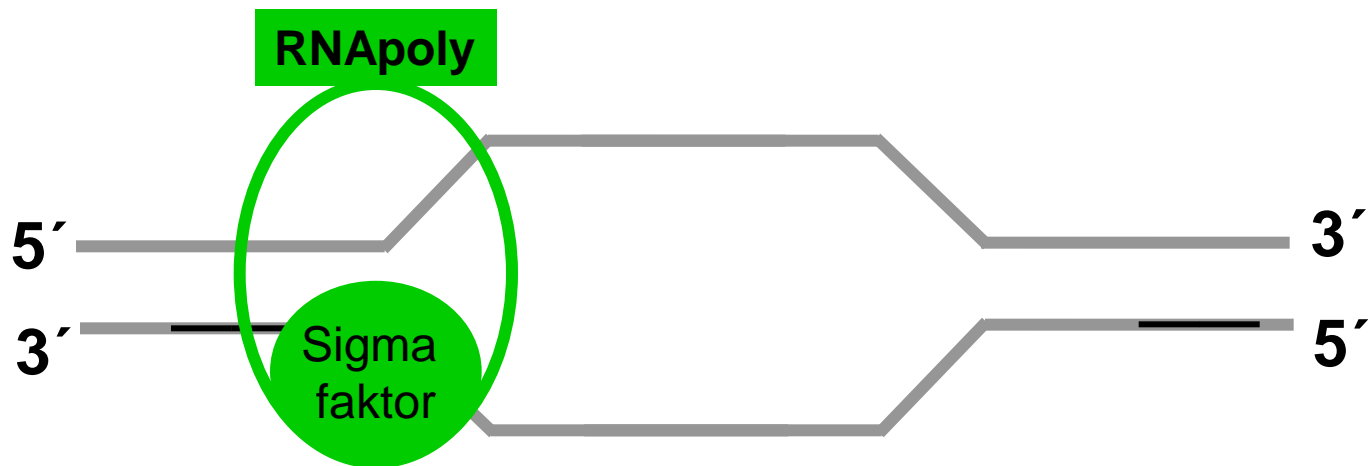
Transkripce - iniciace



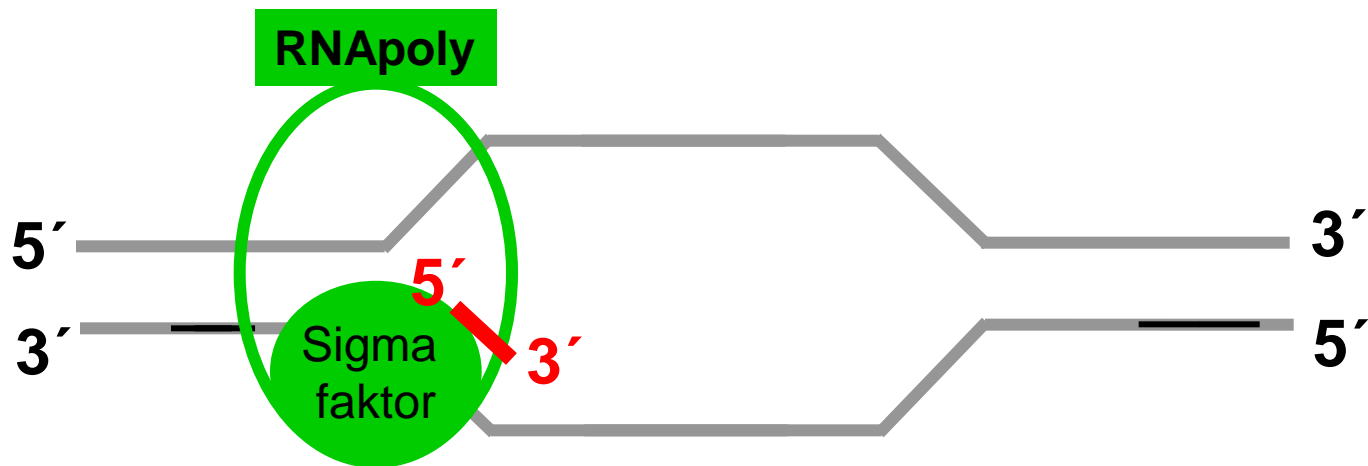
Transkripce - iniciace



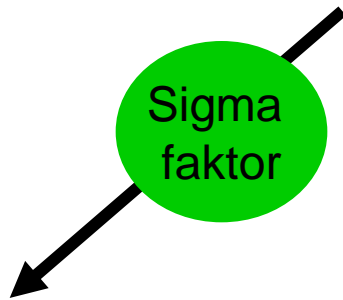
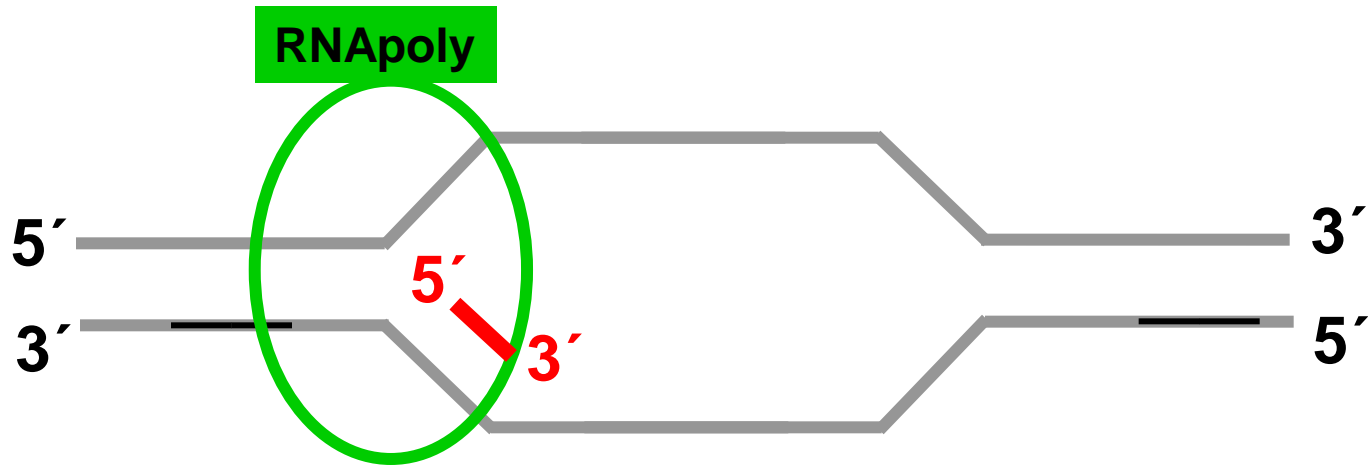
Transkripce - iniciace



Transkripce - iniciace

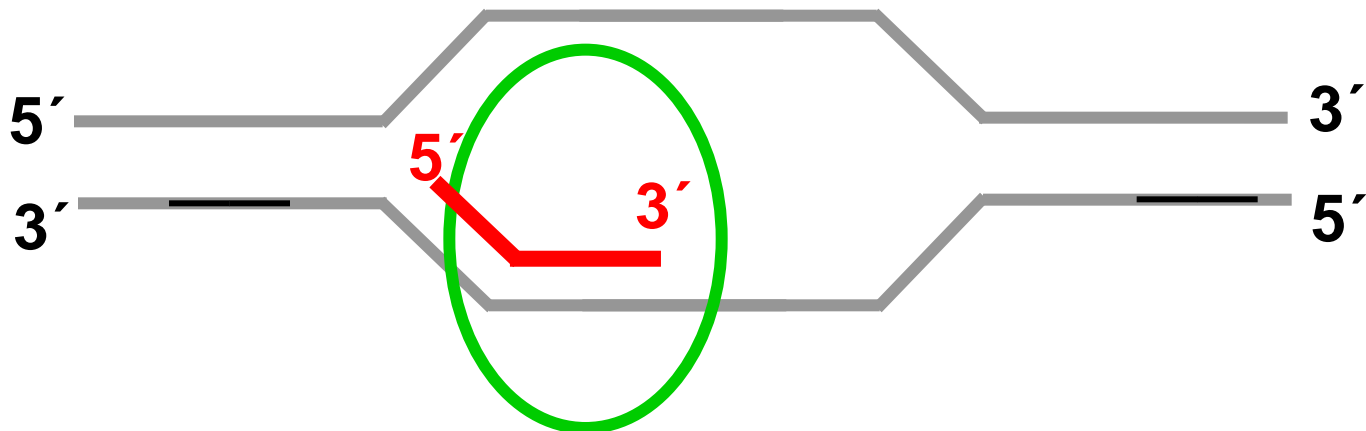


Transkripce - iniciace

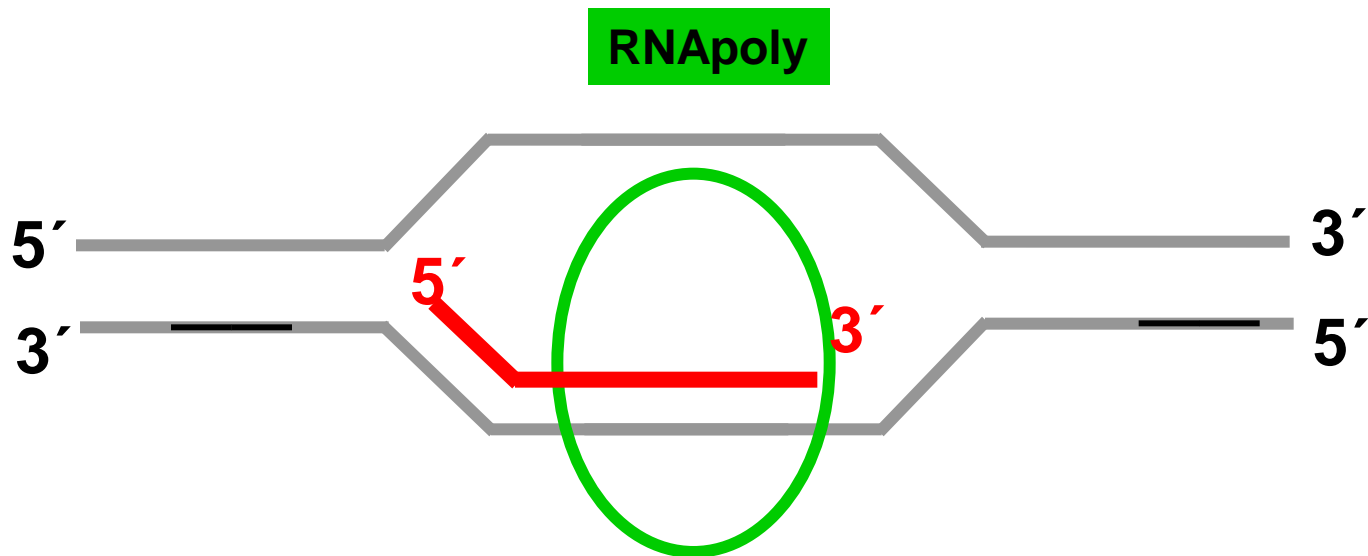


Transkripce - elongace

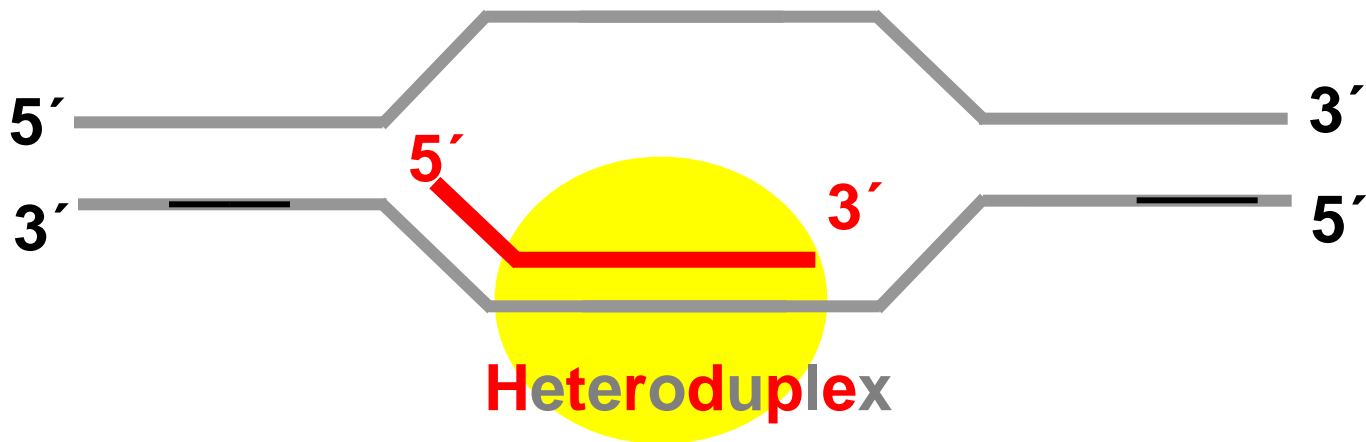
RNApoly



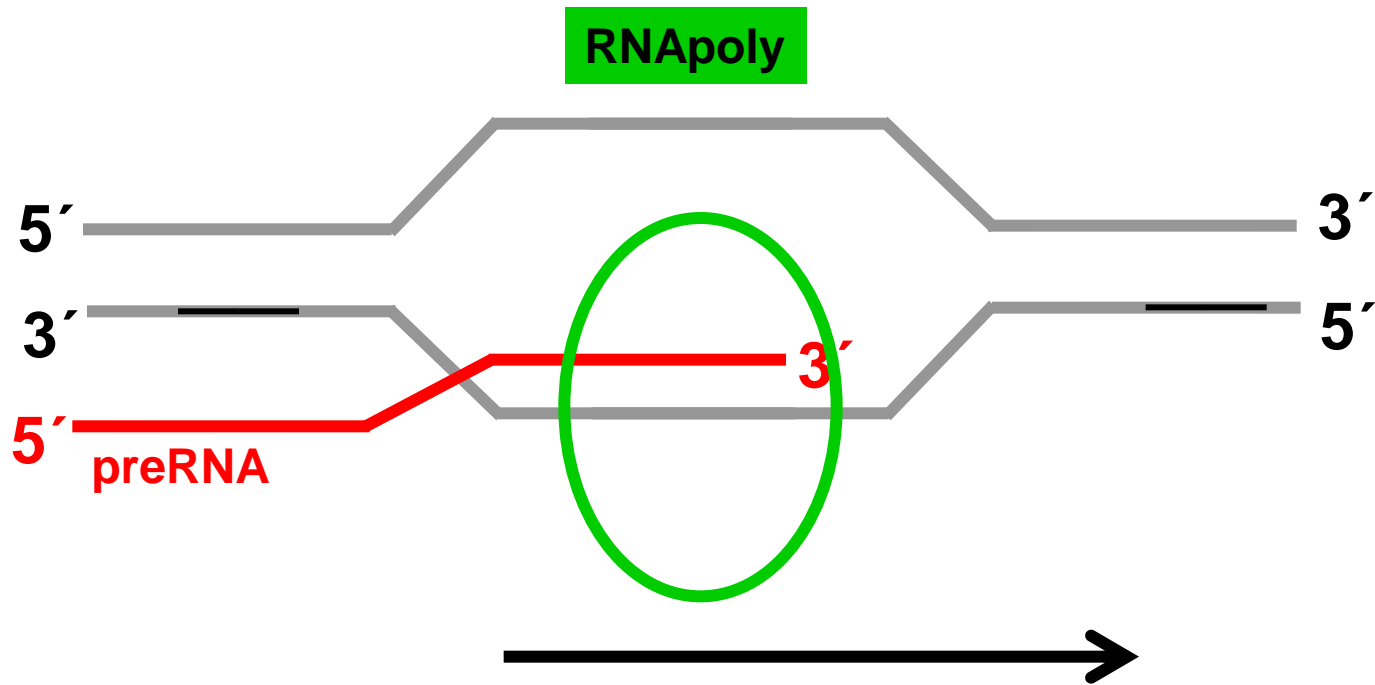
Transkripce - elongace



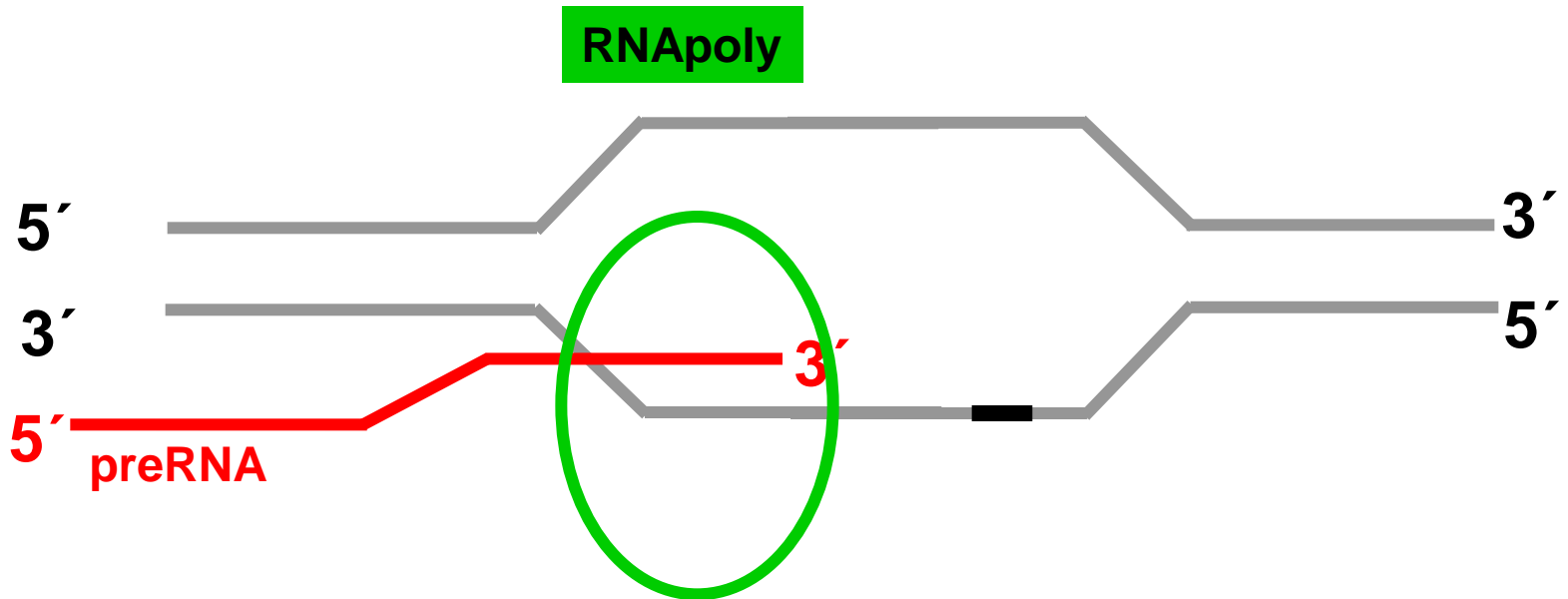
Transkripce - elongace



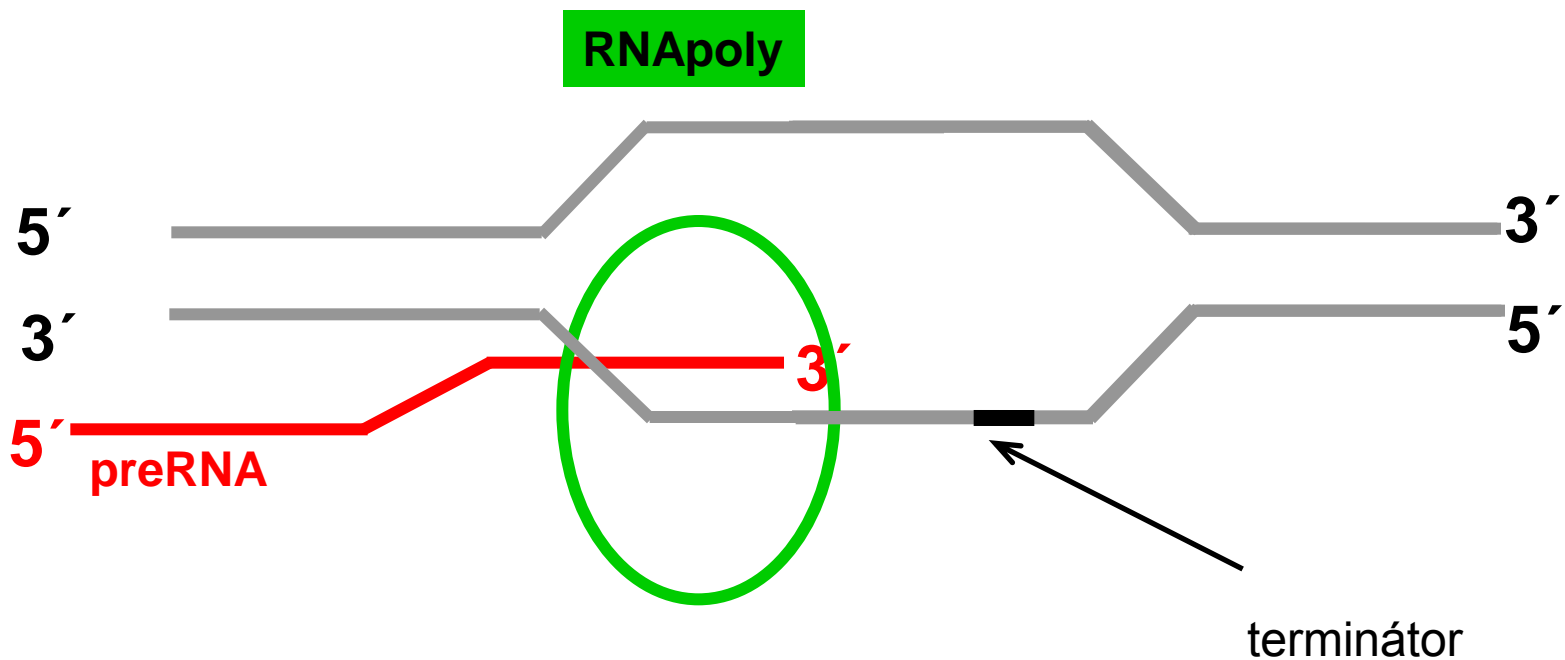
Transkripce - elongace



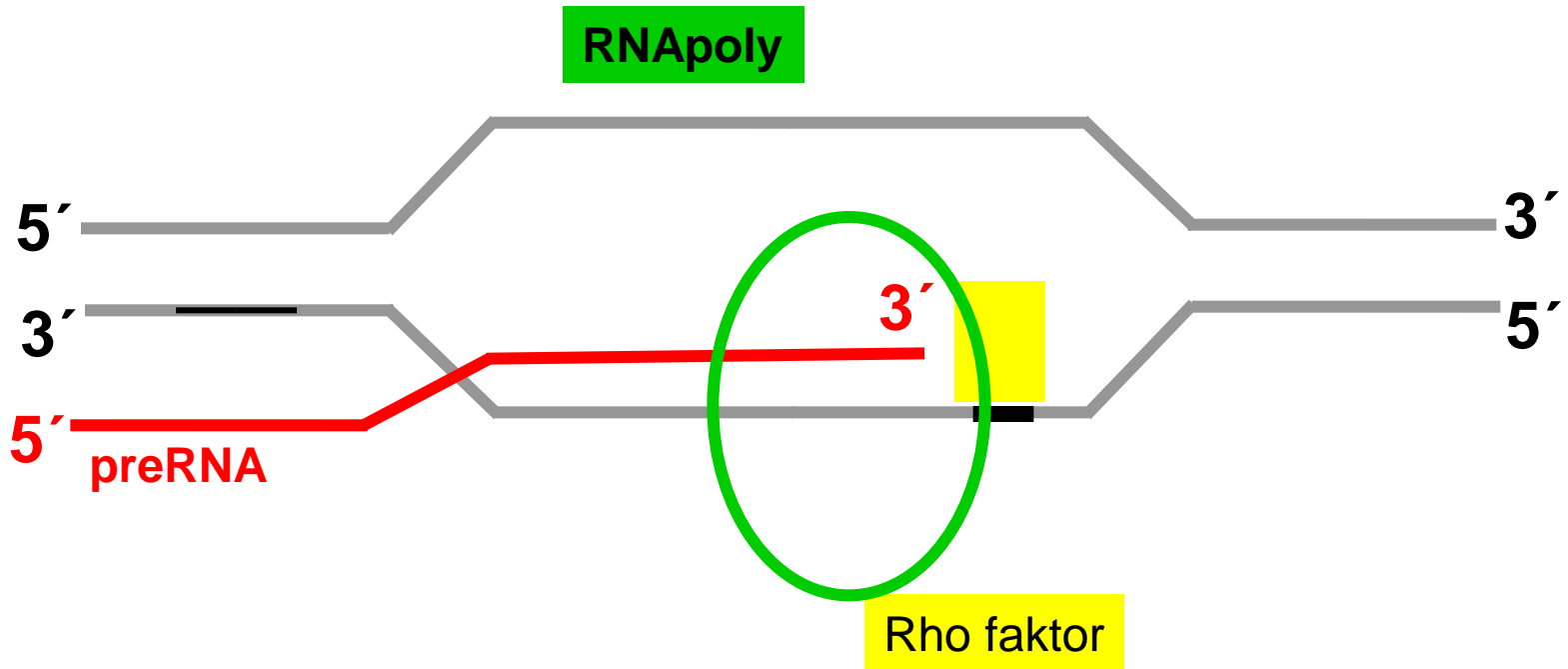
Transkripce - terminace



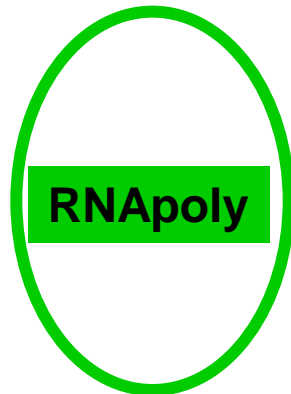
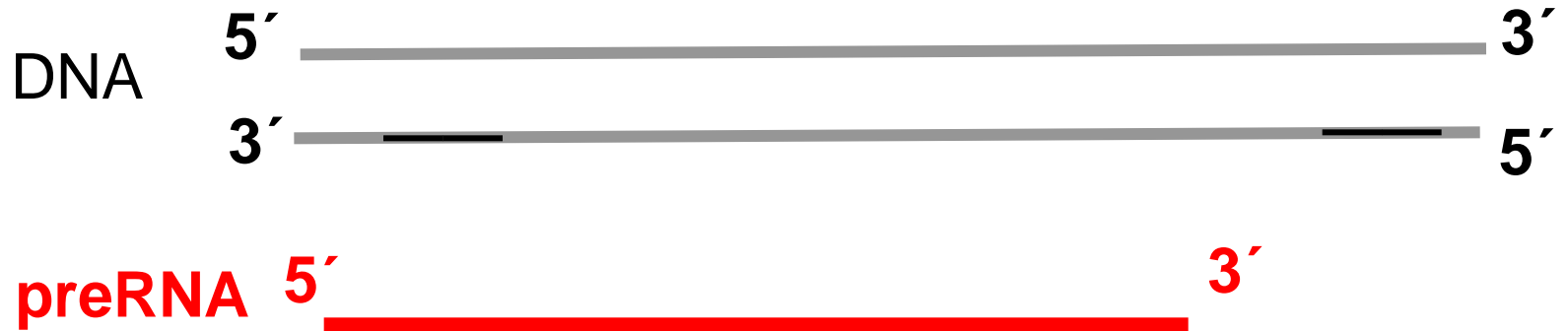
Transkripce - elongace



Transkripce - terminace



Transkripce - terminace



Postranskripční úpravy



1) Sestřih exonů

- odstranění nekódujících sekvencí, intronů

2) Úprava pre-RNA na RNA

mRNA metylguanozinová čepička 5'
polyadenylace 3'

tRNA

rRNA

3) Editace RNA

Postranskripční úpravy

1) Sestřih exonů

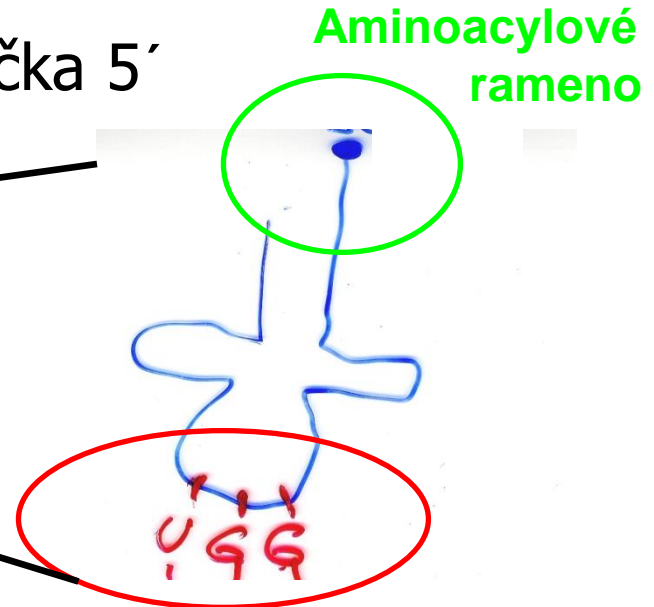


- odstranění nekódujících sekvencí, intronů

2) Úprava pre-RNA na RNA

mRNA metylguanozinová čepička 5'
polyadenylace 3'

tRNA
rRNA



3) Editace RNA

Translace

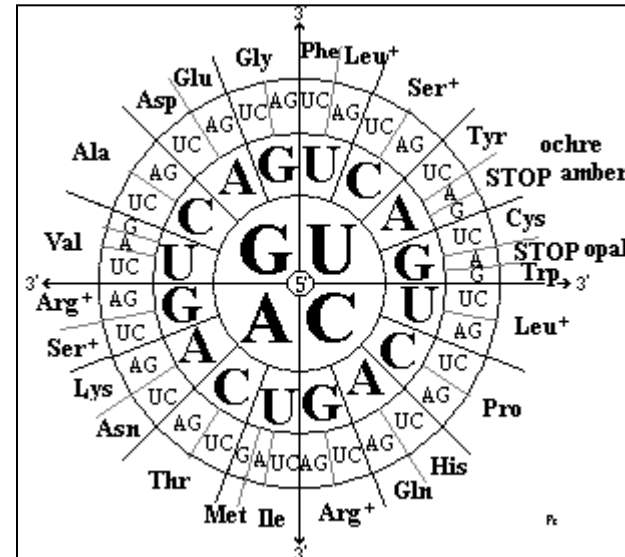
Překlad genetická informace mRNA do sekvence aminokyselin, tj. do primární struktury polypeptidu = proteosyntéza

- Probíhá v cytoplazmě, na ribozómu
- Realizace na základě genetického kódu

Translace - Genetický kód

Aminokyseliny proteinu kódovány trojicemi (triplety/kodony) dusíkatých bází

- Univerzální
- Degenerovaný
- Nepřekryvný
- Bez interpunkce



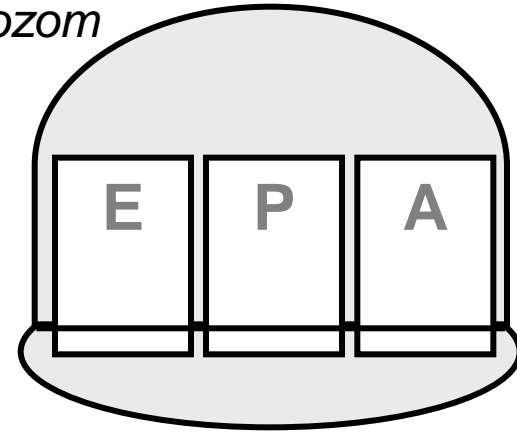
Genetický kód

		2. báze				3. báze
		U	C	A	G	
1. báze	U	UUU fenylalanin	UCU serin	UAU tyrosin	UGU cystein	U
		UUC fenylalanin	UCC serin	UAC tyrosin	UGC cystein	C
		UUA leucin	UCA serin	UAA stop (Ochre)	UGA stop (Opal)	A
		UUG leucin	UCG serin	UAG stop (Amber)	UGG tryptofan	G
	C	CUU leucin	CCU prolin	CAU histidin	CGU arginin	U
		CUC leucin	CCC prolin	CAC histidin	CGC arginin	C
		CUA leucin	CCA prolin	CAA glutamin	CGA arginin	A
		CUG leucin	CCG prolin	CAG glutamin	CGG arginin	G
	A	AUU isoleucin	ACU threonin	AAU asparagin	AGU serin	U
		AUC isoleucin	ACC threonin	AAC asparagin	AGC serin	C
		AUA isoleucin	ACA threonin	AAA lysin	AGA arginin	A
		AUG methionin start	ACG threonin	AAG lysin	AGG arginin	G
	G	GUU valin	GCU alanin	GAU aspartát	GGU glycin	U
		GUC valin	GCC alanin	GAC aspartát	GGC glycin	C
		GUA valin	GCA alanin	GAA kys. glutamová	GGA glycin	A
		GUG valin	GCG alanin	GAG kys. glutamová	GGG glycin	G

Translace

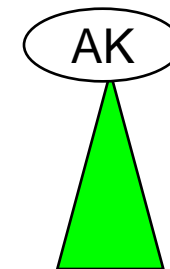
- mRNA
- rRNA → Ribozóm
- tRNA
- Energie (ATP, GTP)
- Pomocné faktory

ribozom



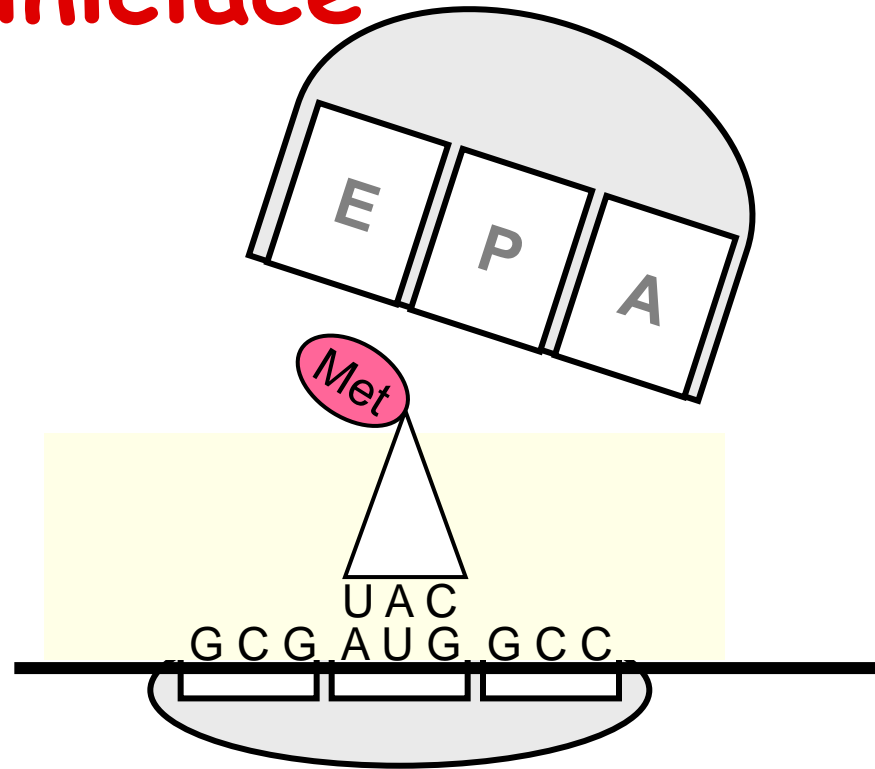
5' *mRNA* G C G A U G G C C 3'

tRNAs



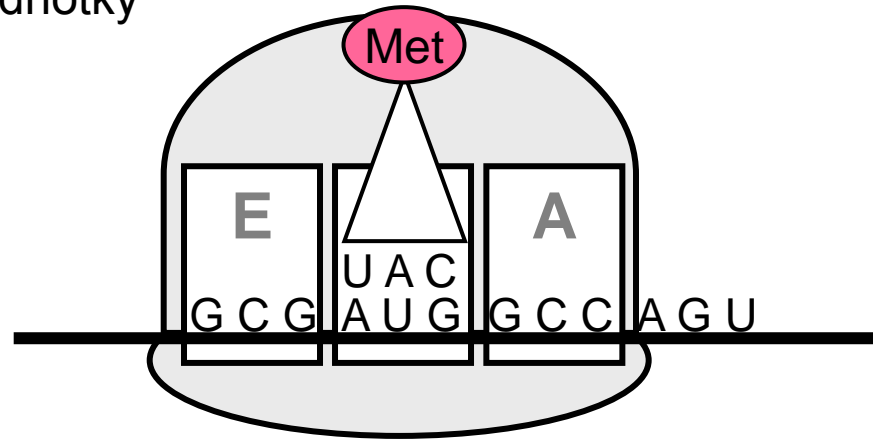
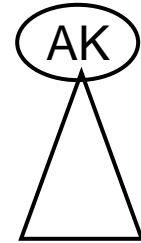
Translace - iniciace

- Navázání iniciační tRNA
na iniciační kodon mRNA



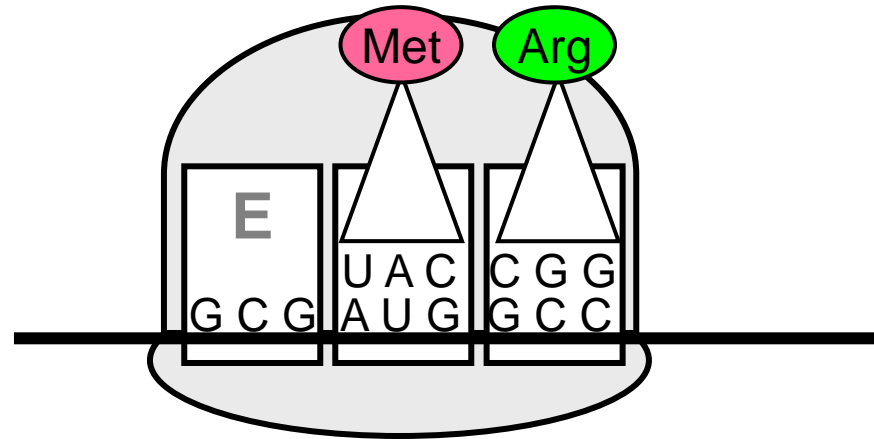
Translace - elongace

- Navázání iniciační tRNA na iniciační kodon mRNA
- Sestavení malé a velké podjednotky do funkčního ribozómu
- Iniciační tRNA v P-místě



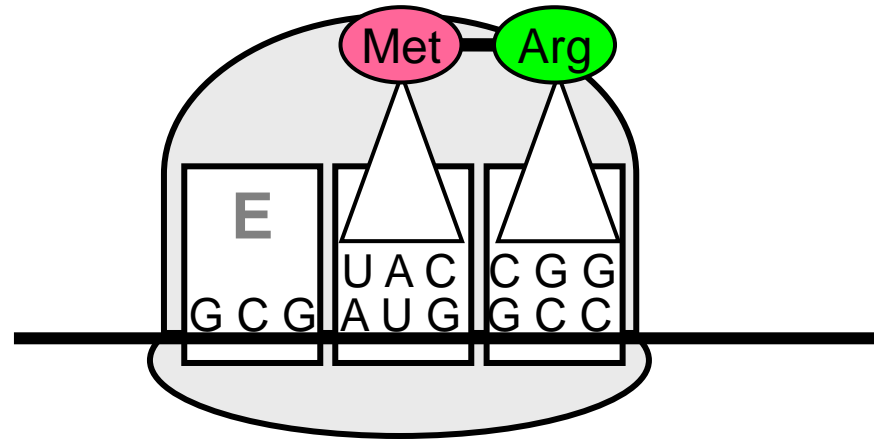
Translace - elongace

- Navázání druhé tRNA do A-místa



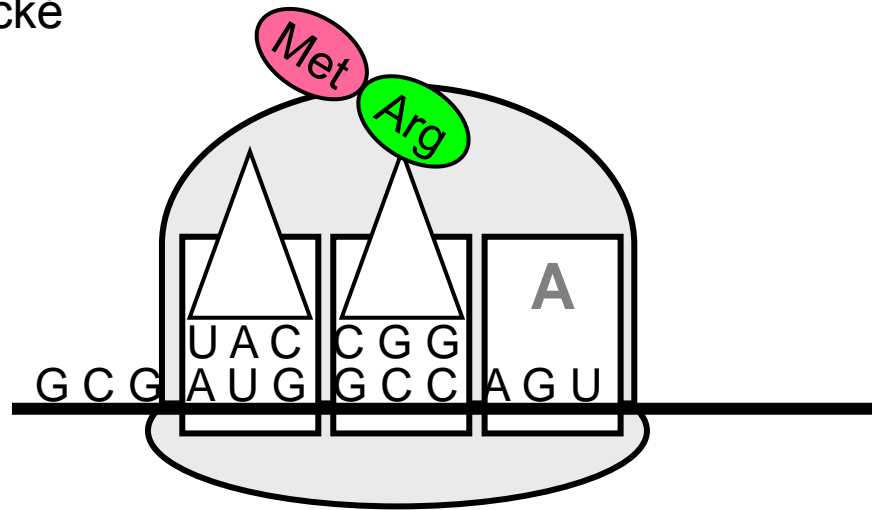
Translace - elongace

- Navázání druhé tRNA do A-místa



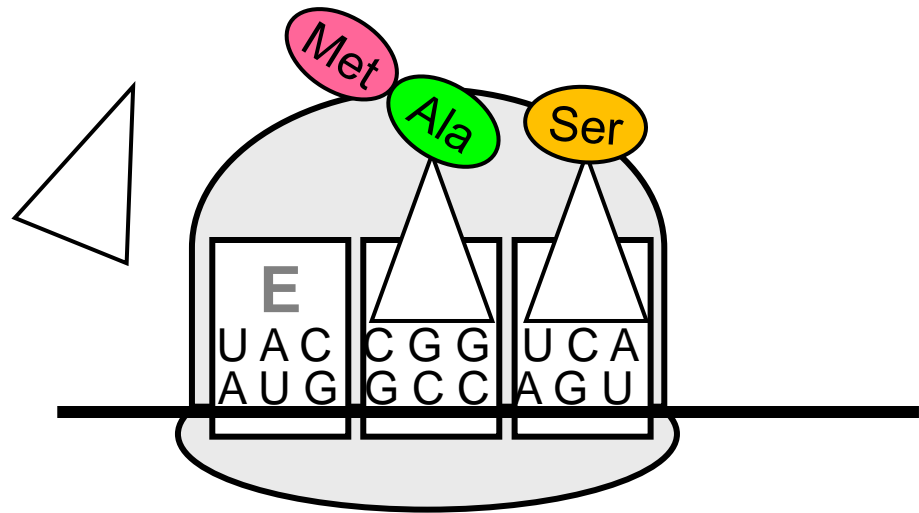
Translace - elongace

- Navázání druhé tRNA do A-místa
- Peptidizace : vytvoření peptidické vazby mezi AK P- a A-místa
- a Posun o jedno místo



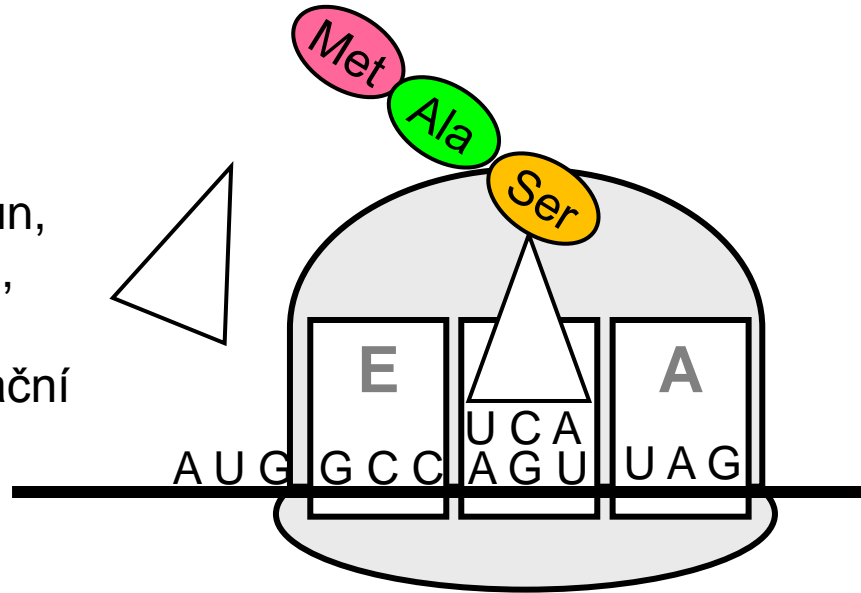
Translace - elongace

- Uvolnění tRNA (bez AK) z E-místa
- Zaplnění A-místa



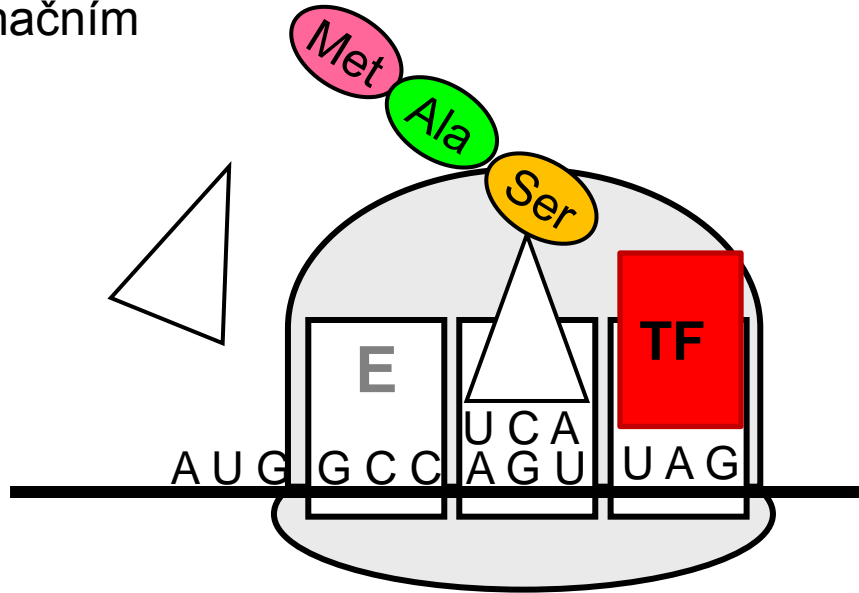
Translace - terminace

- Opakování: peptidizace, posun, zaplnění A-místa, peptidizace, posun,....
- Dokud není v A-místě terminační kodon

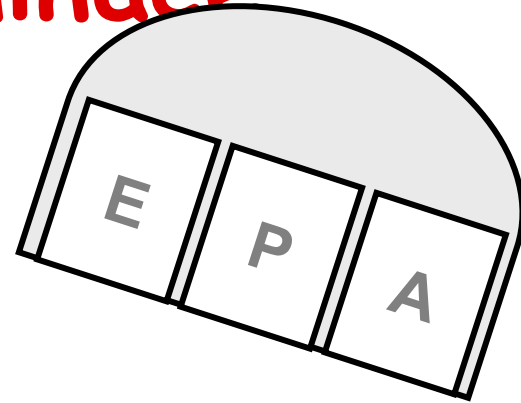


Translace - elongace

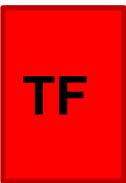
- Obsazení A-místa terminačním faktorem
- Ukončení AK řetězce



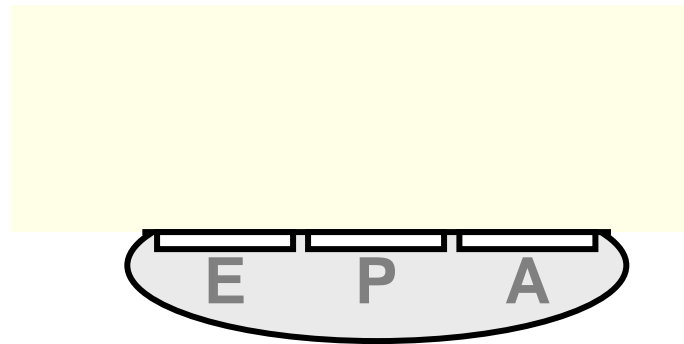
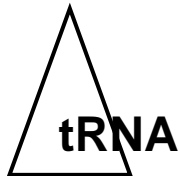
Translace - terminace



- Uvolnění primárního proteinu
- Rozpad translačního aparátu



mRNA AUG GCC AGU UAG



Užitečné odkazy - jednoduché animace

- <http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/transcription.swf>
- <http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/translation.swf>
- <http://www.stolaf.edu/people/giannini/flashanimat/molgenetics/dna-rna2.swf>

Replikace

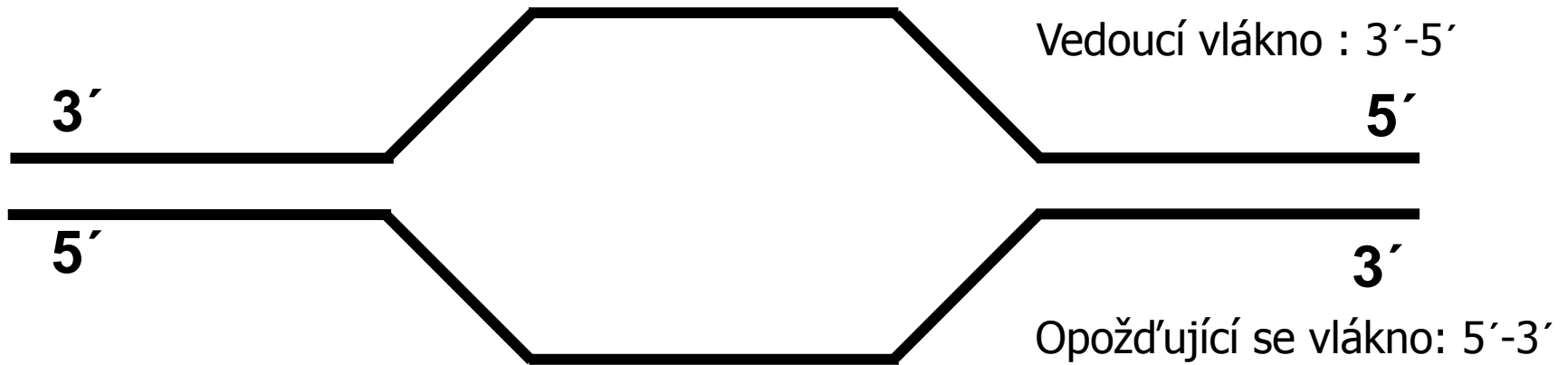
- Zdvojování DNA
- Primárně v jádře,

Replikace

- Dvoušroubovice DNA
- Primáza → primer (RNA fragment, 10-11 nt)
- DNA polymeráza (I, II, III; α , β , δ)
- Enzymy: exonukleáza, ligáza, gyráza, ...
- Nukleotidy
- Energie
- Pomocné faktory, ...

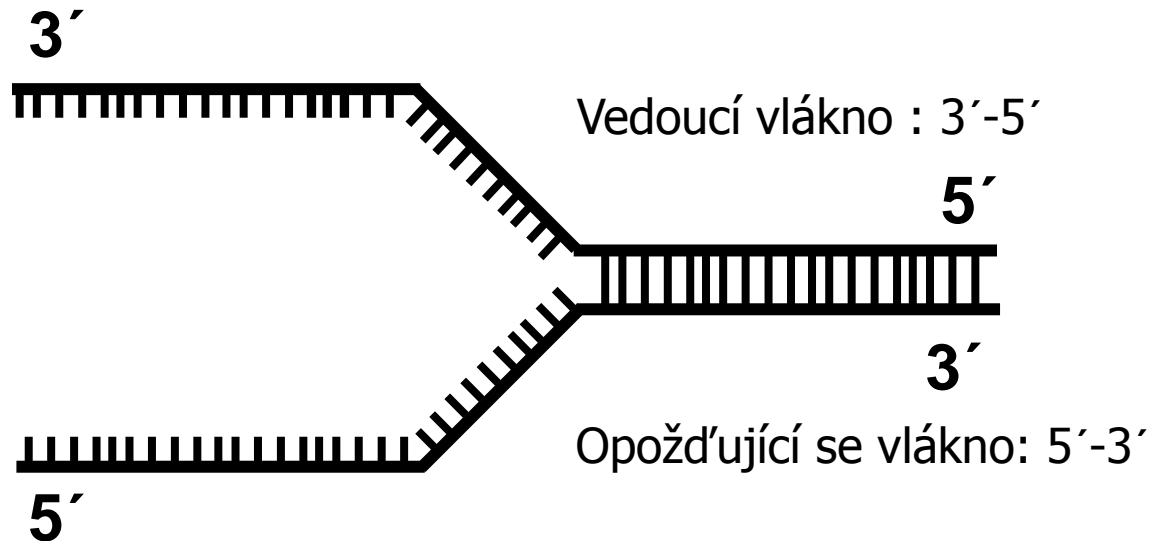
Replikace

Replikační bublina

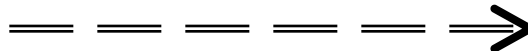


Replikace

Replikační vidlička

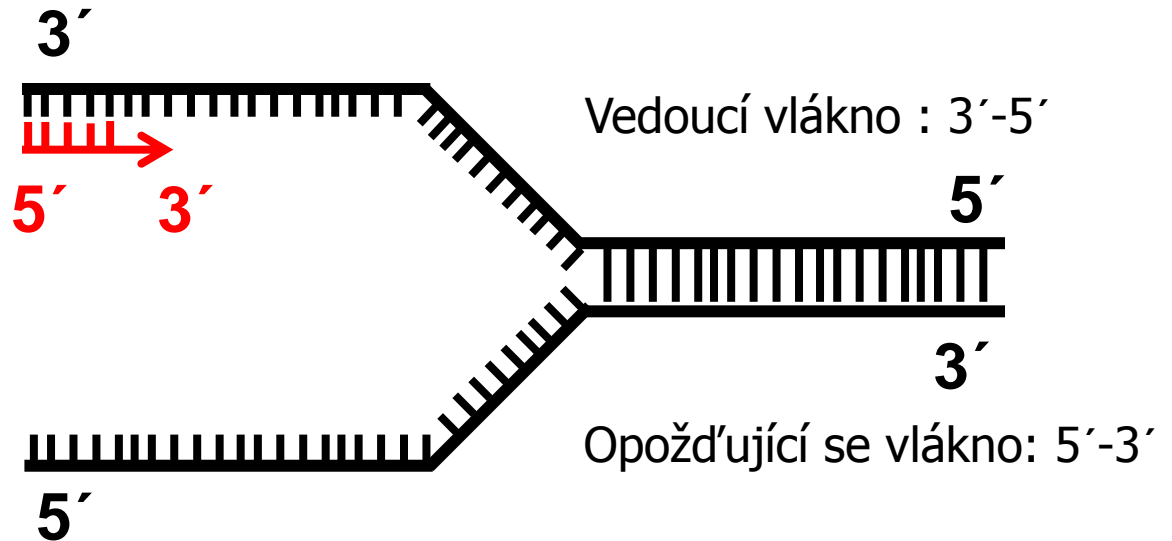


Směr rozvolňování dvoušroubovice

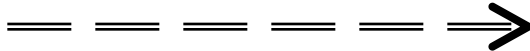


Replikace

primáza



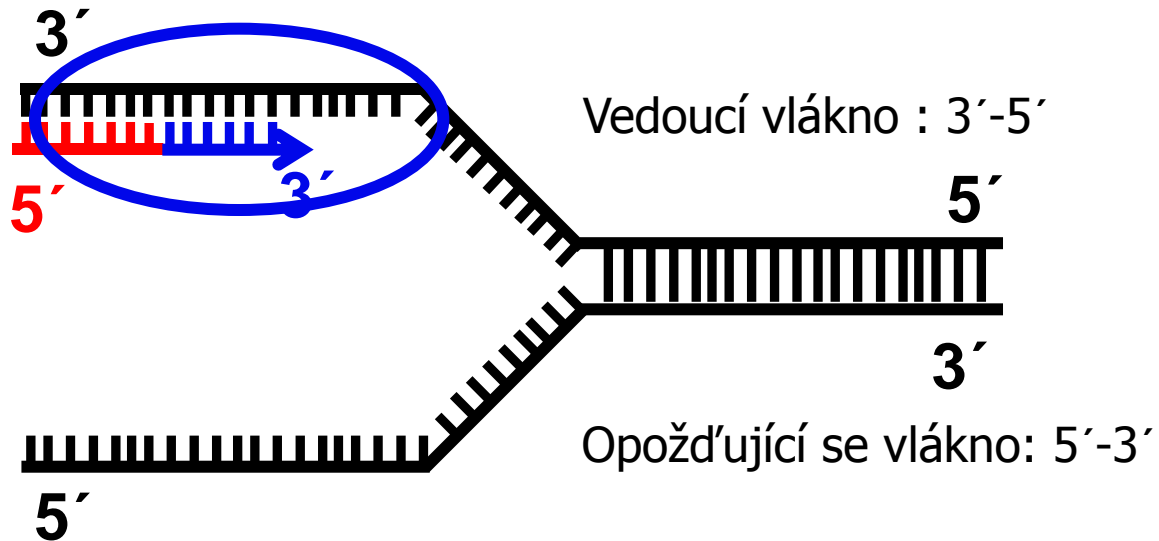
Směr rozvolňování dvoušroubovice



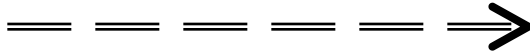
Replikace

primáza

DNA polymeráza



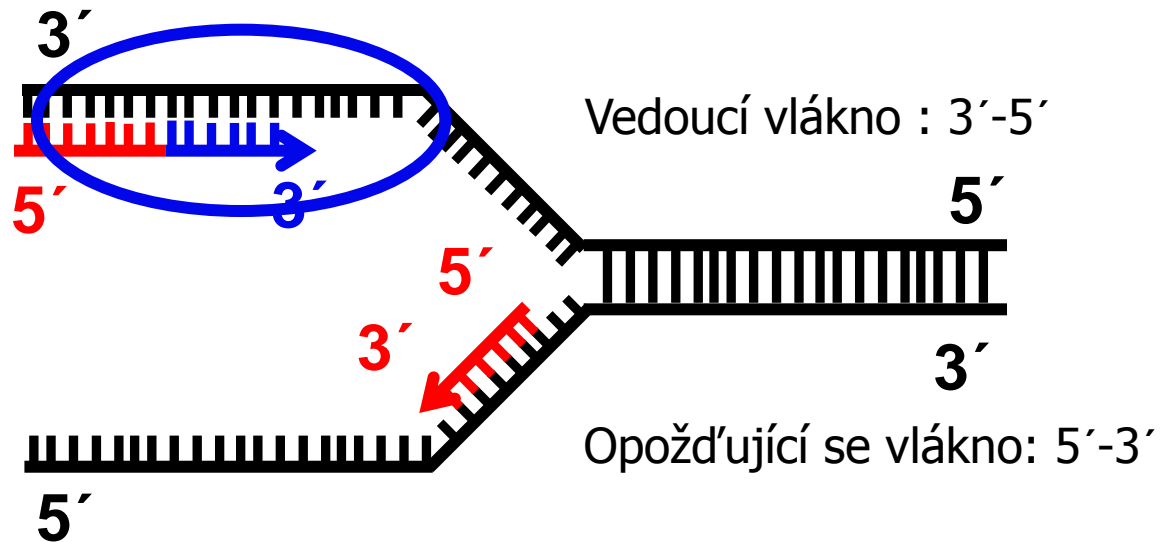
Směr rozvolňování dvoušroubovice



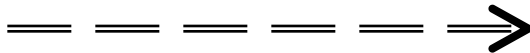
Replikace

primáza

DNA polymeráza



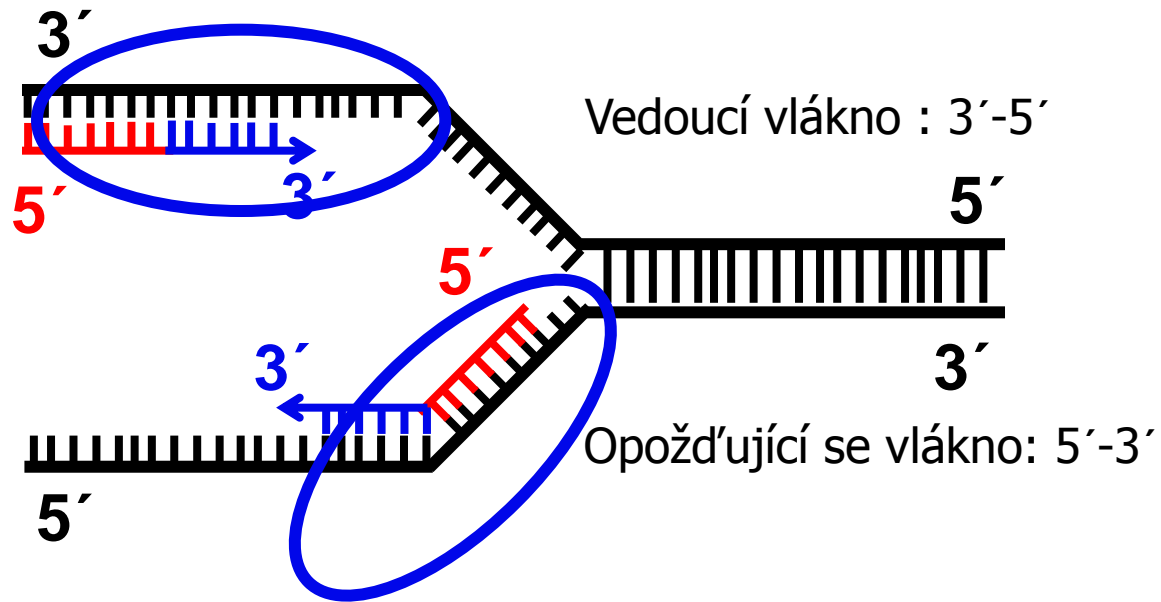
Směr rozvolňování dvoušroubovice



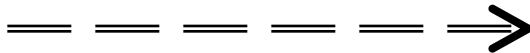
Replikace

primáza

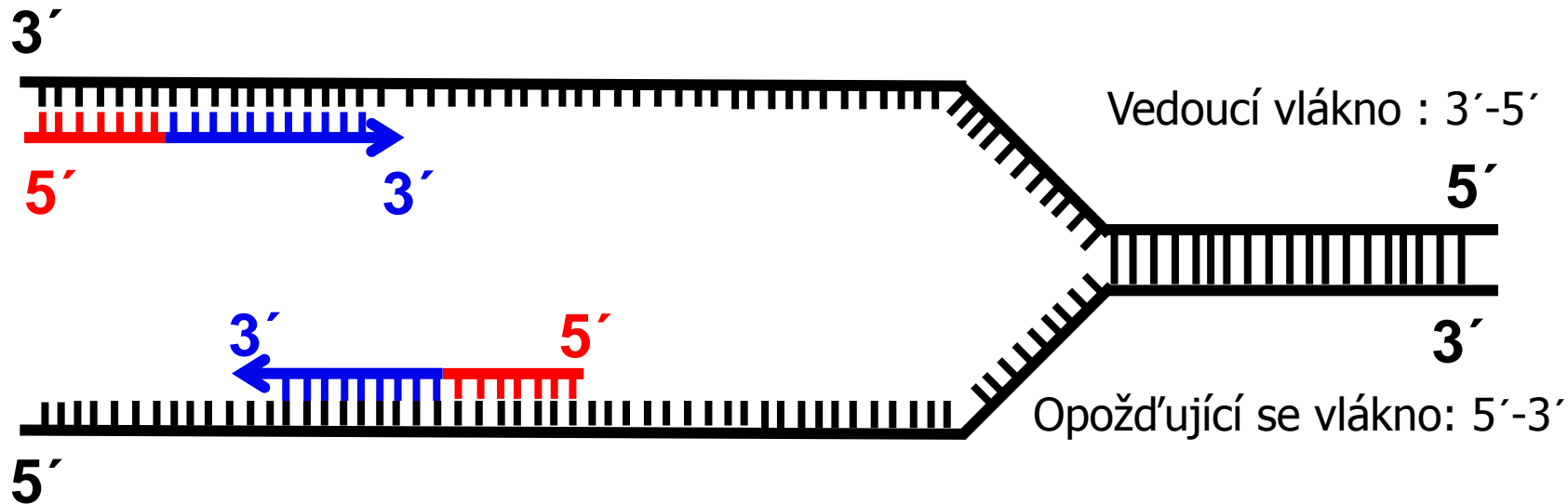
DNA polymeráza



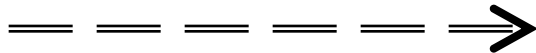
Směr rozvolňování dvoušroubovice



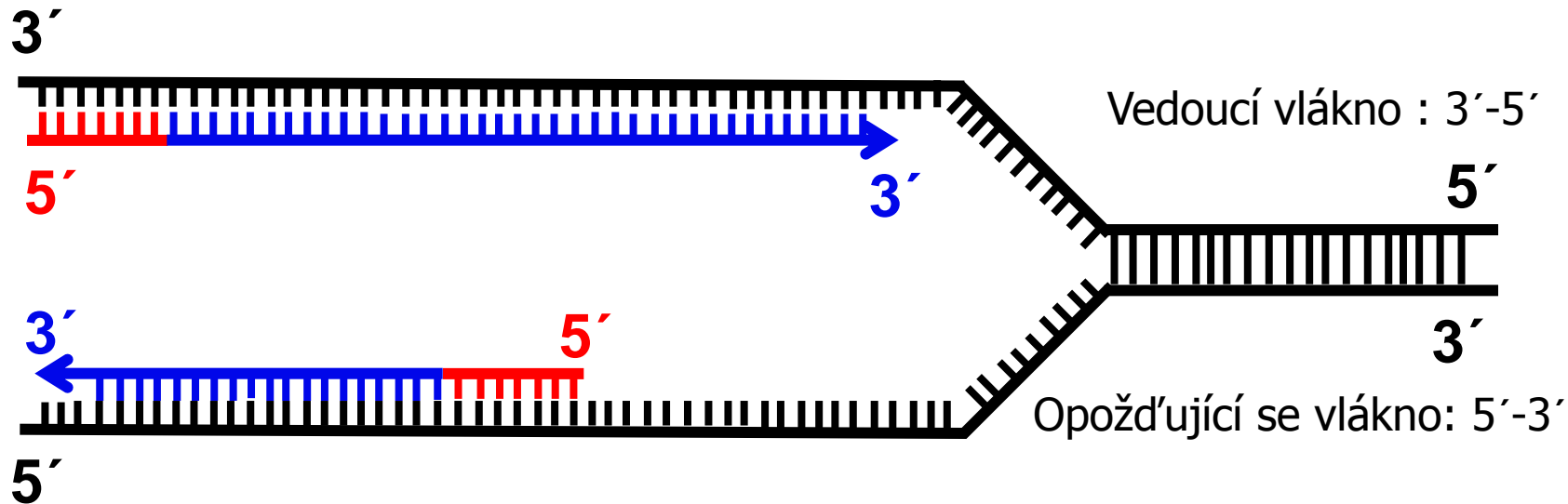
Replikace



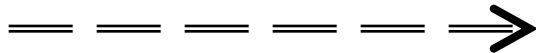
Směr rozvolňování dvoušroubovice



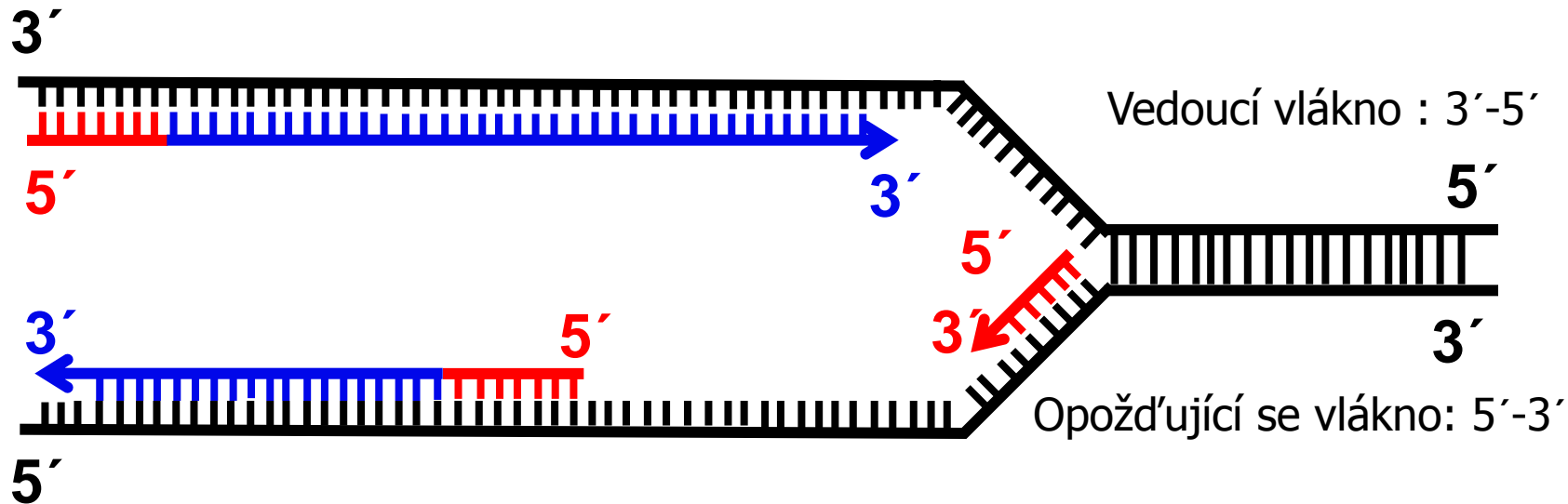
Replikace



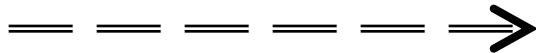
Směr rozvolňování dvoušroubovice



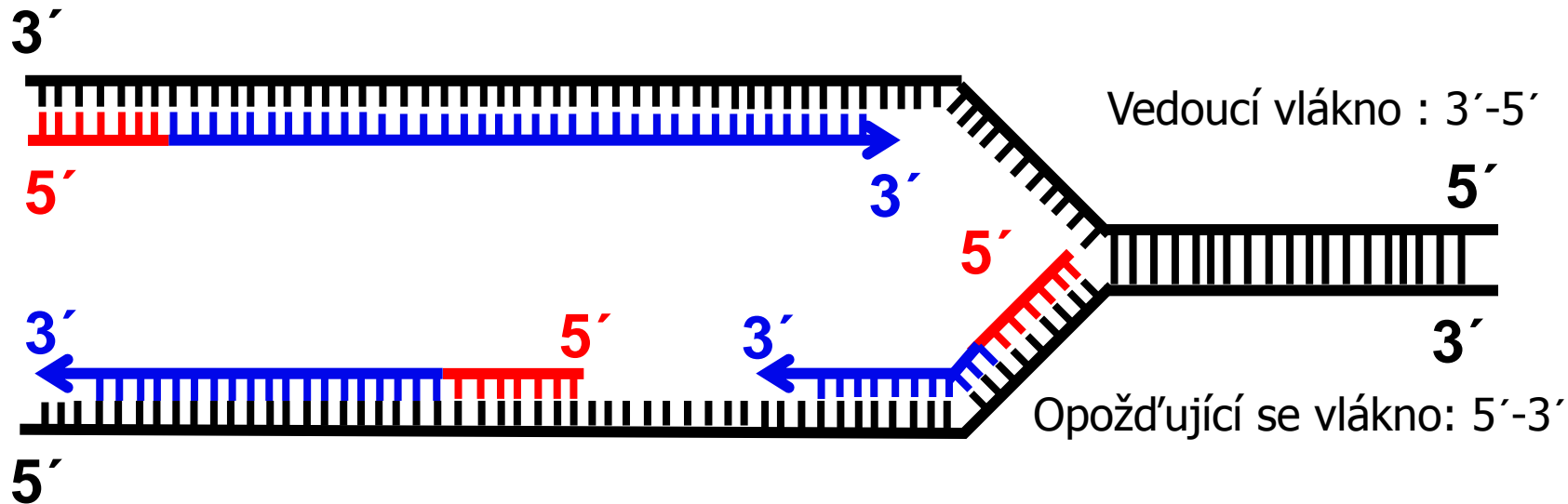
Replikace



Směr rozvolňování dvoušroubovice

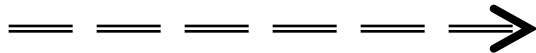


Replikace

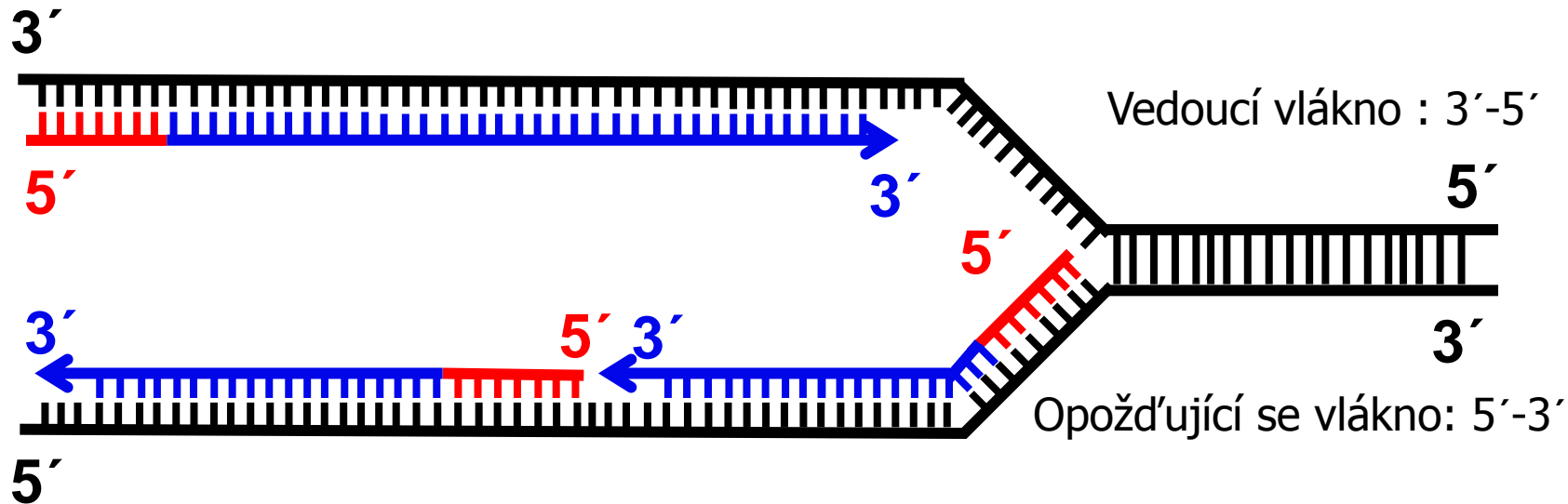


Okazakiho fragmenty

Směr rozvolňování dvoušroubovice

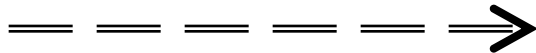


Replikace

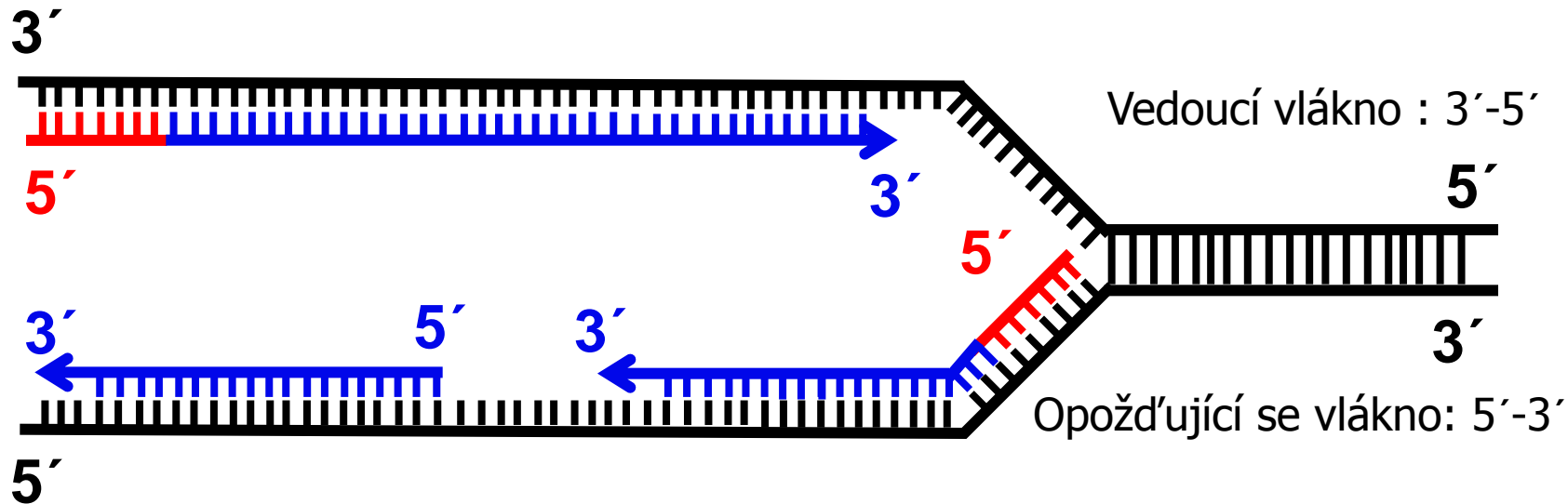


Okazakiho fragmenty

Směr rozvolňování dvoušroubovice

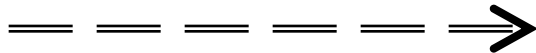


Replikace

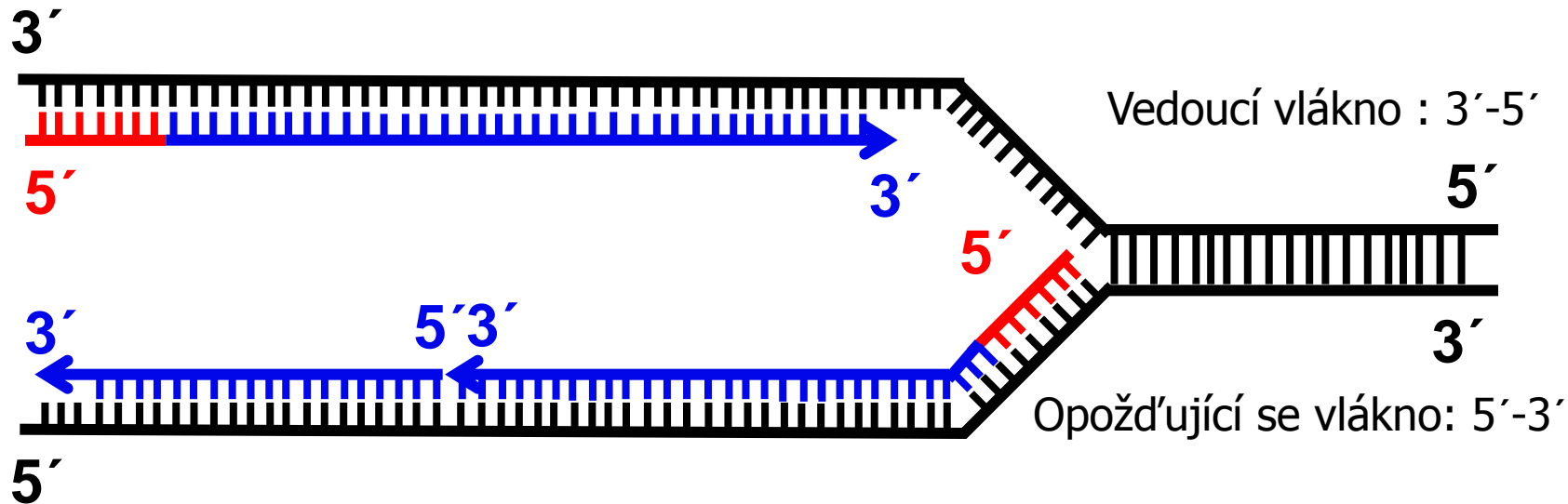


Okazakiho fragmenty

Směr rozvolňování dvoušroubovice

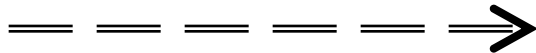


Replikace

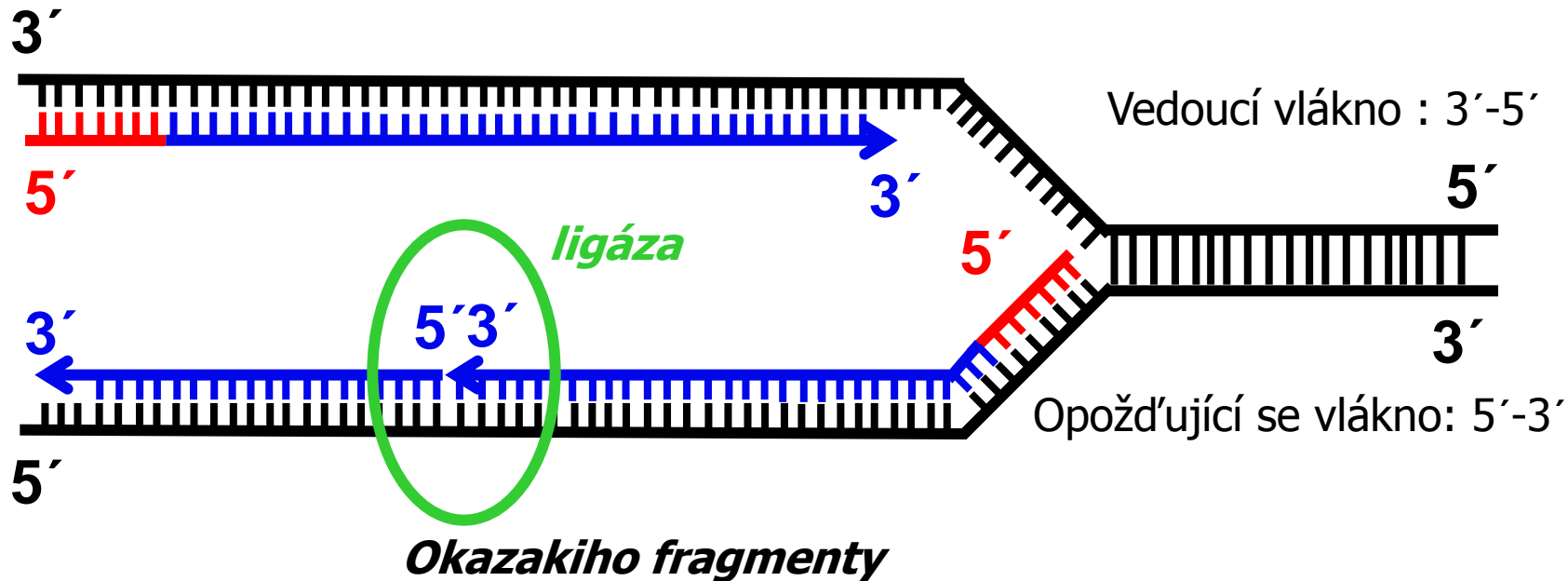


Okazakiho fragmenty

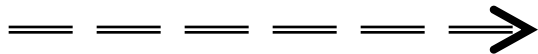
Směr rozvolňování dvoušroubovice



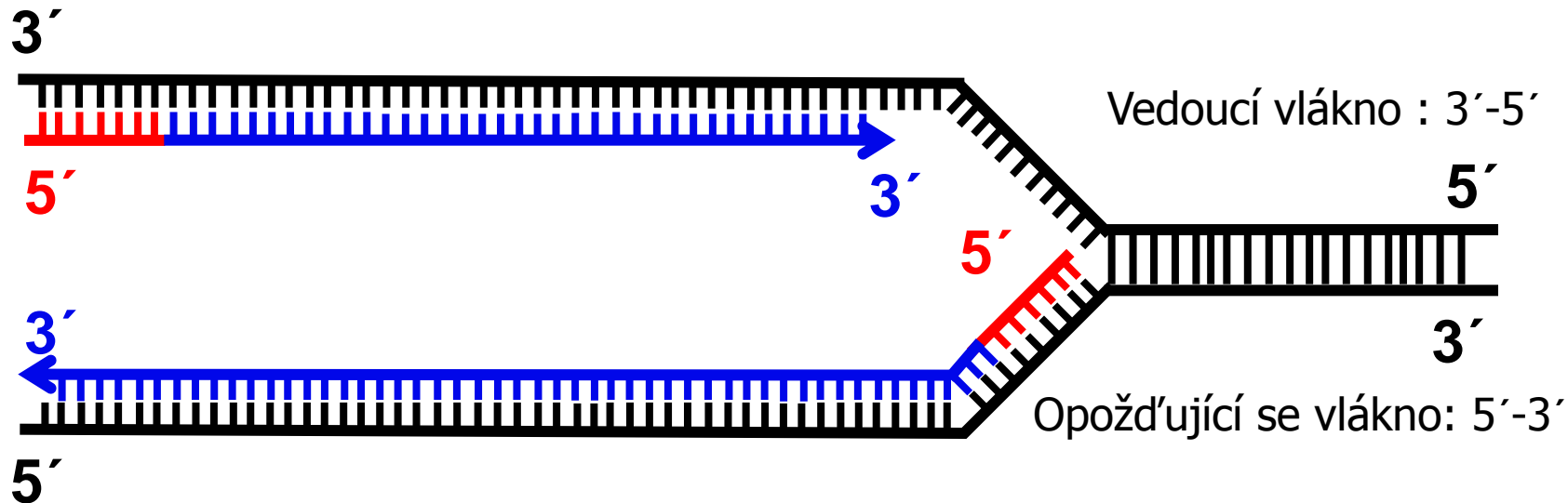
Replikace



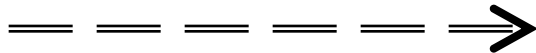
Směr rozvolňování dvoušroubovice



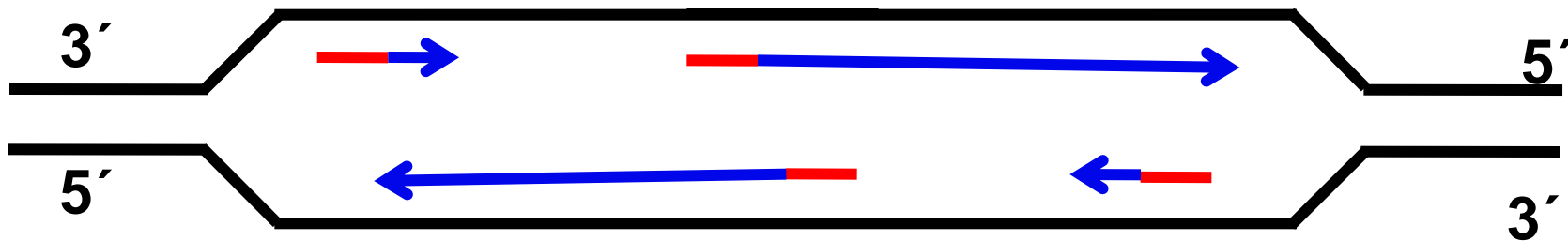
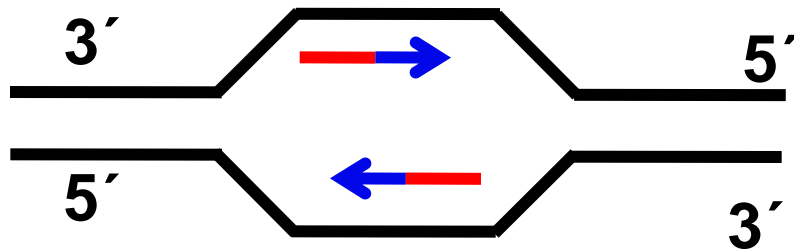
Replikace



Směr rozvolňování dvoušroubovice

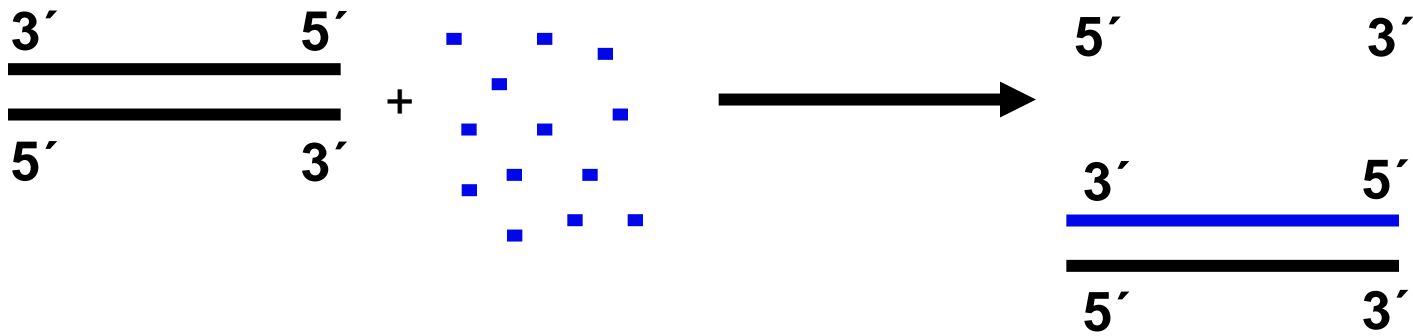


Replikace



Replikace

- semikonzervativní
- komplementární



Replikace

Prokaryota

Jeden replikační počátek, tj. jedna rep. bublina

- Rychlejší, 1000bp/s
- Více chyb/mutací

Eukaryota

- Více replikačních počátků, tj. více rep. bublin
- Pomalejší, cca 50bp/s
- Účinnější oprava chyb
- Konec dosyntetizován pomocí telomerázy

