Nucleic acids

Replication

Protein	Funkcja w replikacji DNA
Helikaza	Rozplata podwójną helisę
Prymaza	Syntetyzuje starterowy odcinek RNA
SSB	Stabilizuje regiony jednoniciowe
Gyraza DNA	Wprowadza ujemne skręty superhelikalne
Polimeraza DNA III	Syntetyzuje DNA
Polimeraza DNA I	Usuwa startery i wypełnia brakujące fragmenty nici DNA
Ligaza DNA	Łączy końce DNA

Telomerases

Ribonucleoprotein enzymes, which catalyse the elongation of 3` end DNA strand. They have the character of reverse transcriptase which possesses its own matrix.

Conseqences of the presence of tautomeric forms - pyrimidines

Keto-enol tautomery resulting from the movement of H protons causes the presence of different tautomeric forms of nitrogen bases:

Lactam (ketone form =O), or

Lactim (enol form –OH)

In physiological conditions lactam of thymine and uracil dominate while cytosine is present in form of lactim.

Mutagenic effect of pyrimidine bases tautomery results from the fact that lactim of thymine formates complementary pair with guanine instead of adenine.

Conseqences of the presence of tautomeric forms - purines

In physiological conditions main tautomeric forms of guanine and hypoxantine are lactams while dominating form of adenine is lactim.

Lactam tautomeric form of adenine formates pair with cytosine what may be related to mutagenesis.

Factors that modify the structure of DNA

- NItrous acid causes deamination of C ($\rightarrow$U), G, and A ($\rightarrow$hypoxanthine). The exchange of base pairs may occur
- Hydroxylamine converts C into compound similar to U – the exchange of base pairs may occur
- Alkylating compounds cause mainly alkylation of N7 and N1 of G as well as N1 and N3 of A and N3 of C. It may lead to transition and transversion: AT$\rightarrow$TA, AT$\rightarrow$GC, GC$\rightarrow$CG, GC$\rightarrow$AT, GC$\rightarrow$TA. Cisplatin used in cancer treatment acts in similar way.
- Acridine dyes penetrate between nitrogen bases and spread them apart $\rightarrow$ DNA matrix deformation and errors in replication

Factors that modify the structure of DNA

- Polychain aromatic hydrocarbons (from cigarette smoke, coffee, red meat) formate adducts with DNA leading to mutations
- Heterocyclic aromatic amines (are created during thermal processing of protein products) formate adducts
- Base analogs which are erroneously incorporated into strand may cause mutations
- Free radicals cause base modifications leading to mutations (8-hydroxy-guanine, thymine glycol). They modify also sugar residues.

Factors that modify the structure of DNA

- Ionizing radiation initiates free radical reactions and may lead to DNA strand break (the loss of genetic information)
- Ultraviolet radiation leads to the formation of pyrimidine dimers and in consequence deletions

Congenital diseases

- Congenital developmental disorders (spinal fission, heart defects, harelip) – genetic background and external factors
- Chromosomal abnormalities – are formated during gametogenesis (recombination of chromosomes) – are inherited
- One-gene diseases (metabolic defects) – around 3500 are known

Prophylaxis and detection

- Reducing the risk of exposure of pregnant females to external mutagenic and teratogenic factors
- Screening of endangered families
- Prenatal diagnostics

The essence of inheritance is the transmission of information about protein synthesis, their structure and function. This information is coded in the sequence of DNA bases.

Gene expression is understood as reading of this information and its realisation by the synthesis of appropriate protein.

Full gene expression is done by DNA replication, transcription, posttranscriptional modifications of precursor RNA as well as translation and posttranslational modifications of newly synthesized protein.

Mutations

Mutations rely on smaller or larger changes in the structure of genetic material.

- Base substitution – exchange of one base into another
- **Deletions** – (base falling out), **insertions** - (introduction of additional base)

Consequences depend on the location of mutation in DNA chain – changes in genetic information → changes in amount and properties of synthesized proteins, impaired protein function, the decrease in initiation velocity, elimination of gene activity, the lack of gene transcription,

Symptomatic mutations arising in the coding sequences are inherited. Asymptomatic mutations which are located in regions between genes are without any meaning.

Transcription

RNA polymerase from E. coli

Subunit	Number	Mass (kDa)	Function
α	2	37	Binds regulatory proteins
β	1	151	Formates phosphodiester bonds
β`	1	155	Binds DNA template
δ (sigma)	1	70	Recognizes the promotor and initiates the synthesis

RNA polymerase from E. coli

- Searches the fragments of transcription initiation on DNA
- Unwinds short fragment of double stranded DNA formating one stranded template for transcription
- Selects appropriate ribonucleotide triphosphate and catalyses the formation of phosphodiester bond
- Detects the signals of transcription termination
- Reacts with represor and activation proteins which modulate the velocity of transcription

Transcription starts at promotor signals on DNA template

After finding promotor signal double stranded DNA is separated on the distance of around 17 bp

Template DNA strand (complementary to transcript strand, anty-sense) selects complementary ribonucleotide triphosphates.

[Coding strand has the same sequence as RNA transcript, is sense strand]

- Majority of newly synthesised RNA starts at pppG or pppA. The direction of synthesis is 5` $\rightarrow$ 3`.
- Starter is not required

Elongation

- Elongation occurs in transcription bubbles (regions containing RNA polymerase, DNA and newly synthesized RNA) and it starts after the formation of the first phosphodiester bond. δ subunit of RNA polymerase is lost.
- In parralel with unwinding before polymerase winding after the enzyme occurs.
- RNA polymerase does not check newly synthesized chain – it is less exact as DNA polymerase in replication.

Termination

- At the termination stage the formation of phosphodiester bonds stops, RNA dissociates from hybrid RNA-DNA and unwinded DNA winds again.
- Stop signal is usually palindrom region rich in GC pairs followed by region rich in AT and 4 or more U residues.
- Sigma subunit binds to RNA polymerase and searches for next promotor signal.

The model of trascription bubble

Posttranscriptional modifications – RNA maturation

In procaryotic cells

Excision and modification of some regions

Adding of nucleotides to the end of RNA chain

Modification of bases (methylation) and ribose residues

In eucaryotic cells

Cutting of the leader sequence from 5` side

Excision of intron (by endonuclease)

Substitution of UU on 3` end by CCA

Modification of some bases

Differences between Pro and Eucaryota

- In Eucaryota transcription and translation occur in different cell compartments what allows for multistep and precise regulation of gene expression.
- In Eucaryota RNA mautration is more complicated – almost all mRNA precursors undergo splicing what leads to the increase in protein diversity.
- In Eucaryota 3 kinds of RNA polymerases occur – they consist of 8-12 subunits and in addition subunits RPB1 and RPB2
- In Eucaryota transcriptional factors and TATA sequence at the start site are indispensable.

Splicing

- Introns are precisely excised from mRNA precursors.
- Main intron sequence starts with GU and ends with AG.
- Splicing is initiated by cutting of phosphodiester bond between exon above intron and 5` end of intron. Attacking group is 2`OH group of adenine residue in branched site. Between A residue and 5` phosphate intron end new 2`-5` phosphodiester bond is formated.

Splicing

In paralel A residue is linked with two other nucleotides via 5`-3` phosphodiester bond. In this way intermediate product in the shape of lasso is formated.

Then 3`OH end of exon 1 attacks phosphodiester bond between intron and exon 2. Exons 1 and 2 are bound and intron in the form of lasso is released.

Two reactions of transesterification occur in this process (not hydrolysis and ligation). The number of phosphodiester bonds remains the same during both stages.

Low-particle nuclear RNA (snRNA) together with specific proteins participate in the excision of introns from mRNA precursors.

Enhancer Sequences

They increase the activity of many promotors in Eucaryota but do not have promotor activity. They can stimulate the transcription in sites far away from each other, can appear before, after or even in the middle of transcribed gene.

rRNA splicing

- rRNA splicing is based on the autocatalytic excision of introns without the participation of proteins.
- rRNA precursors may express nuclease and polymerase activity.

Gene expression

DNA in eucaryotic chromosomes is tightly bound with basic proteins – histones and formates chromatin.

Histones can be separated from DNA by treatment with salt solution or diluted acid. Five types of histones can be formated : H1, H2A, H2B, H3 i H4.

Histones – their form and character may influence the regulation of the level of DNA packing and its availability for replication and transcription

Type	Lys/Arg	Number of aminoacid residues	Mass
H1	20.0	215	21.0
H2A	1.25	129	14.5
H2B	2.5	125	13.8
H3	0.72	135	15.3
H4	0.79	102	11.3

Nucleosomes

- Structural units of chromatin consisting of 2 molecules of histones H2A, H2B, H3 and H4 as well as DNA fragment containing 200 bp.
- Majority of DNA is wrapped around histone core. Remaining DNA called „connector” binds surrounding nucleosomes and gives chromatin fibers flexibility.
- This structure resembles the string of beads.

Nucleosomes are the first stage of DNA condensation in the cell. Wrapping the DNA on nucleosome core shortens its linear size.

Straight fragment of DNA consistining of 200 bp is 68 nm long while in nucleosome it is shortened up to 10 nm.

During replication old histones remain at complex with DNA duplex containing leading strand while new histones join to duplex with lagging strand.