PROTEIN BIOSYNTHESIS

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DNA REPAIR

- During DNA synthesis, several chemicals (nitrous acid, etc.), or radiation (UV) may cause some errors.

- UV light: pyrimidin dimers
- High- energy ionizing radiation : double strand breaks
- Mismatch repair, etc.

DNA REPAIR
A. METHYL-DIRECTED MISMATCH REPAIR

- **Mut proteins** identify the mispaired nucleotide(s)
- Discrimination is based on the degree of methylation.
- GATC sequences, !!!
- an endonuclease nicks the strand and the mismatched nucleotide(s) is/are removed by an exonuclease.
- Additional nucleotides at the 5'- and 3'-ends of the mismatch are also removed.
- The gap is filled by a DNA polymerase.
- The 3'-hydroxyl of the newly synthesized DNA is joined to the 5'-phosphate of the remaining stretch of the original DNA strand by DNA ligase

MISMATCH REPAIR

- Mutation to the proteins involved in mismatch repair in humans is associated with hereditary nonpolyposis colorectal cancer (Lynch syndrome)
- only about 5% of all colon cancer is the result of mutations in mismatch repair.

B. REPAIR OF DAMAGE CAUSED BY ULTRAVIOLET (UV) LIGHT

- "Pyrimidine dimers"
- First, a UV-specific endonuclease recognizes the dimer, and cleaves the damaged strand on both the 5'-side and 3'-side of the dimer.
- The nick is then filled by DNA polymerase and DNA ligase.

UV RADIATION AND CANCER

- Pyrimidine dimers can be formed in the skin cells of humans exposed to unfiltered sunlight.

- Xeroderma pigmentosum (XP)

A rare genetic disease

The cells cannot repair the damaged DNA

The mutations extensively accumulated and numerous skin cancers develop.

C. CORRECTION OF BASE ALTERATIONS (BASE EXCISION REPAIR)

- The bases of DNA can be altered

A. Spontaneously, (formation of uracil form cytosine deamination)

B. By the action of deaminating or alkylating compounds (example: nitrous acid)

- Bases can also be lost spontaneously. For example, approximately 10,000 purine bases are lost this way per cell per day.

C. CORRECTION OF BASE ALTERATIONS

1. REMOVAL OF ABNORMAL BASES:

- Abnormal bases are the bases which must not be found in DNA

- For example, Uracil
- recognized by specific glycosylases that hydrolytically cleave them from the deoxyribose-phosphate backbone of the strand.
- This leaves an apyrimidinic site (or apurinic, if a purine was removed), both referred to as AP sites.

C. Correction of base alterations

1. Removal of abnormal bases:

- Specific AP-endonucleases recognize that a base is missing
- A deoxyribose phosphate lyase removes the single, base-free, sugar phosphate residue.
- A DNA polymerase and DNA ligase complete the repair process.

D. REPAIR OF DOUBLE-STRAND BREAKS

- High-energy radiation or oxidative free radicals
- Such breaks also occur naturally during gene rearrangements.

1. Nonhomologous end-joining repair
   -The ends of two DNA fragments are brought together by a group of proteins that effect their religation.
   -error prone and mutagenic.
2. Homologous recombination repair,

-Uses the enzymes that normally perform genetic recombination between homologous chromosomes during meiosis.

-much less error prone

RNA STRUCTURE AND SYNTHESIS

- The copying process, during which a DNA strand serves as a template for the synthesis of RNA, is called transcription.

STRUCTURE OF RNA

- There are three major types of RNA that participate in the process of protein synthesis:

1. ribosomal RNA (rRNA),
2. transfer RNA (tRNA),
3. messenger RNA (mRNA)

✓ RIBOSOMAL RNA

- rRNAs are found as components of the ribosomes
- serve as the sites for protein synthesis.
- In procaryotic cells, 23S, 16S, and 5S
- In the eukaryotic cytosol 28S, 18S, 5.8S, and 5S

- "S" is the Svedberg unit,

✓ tRNA

- the smallest (4S) of the three major types of RNA molecules.
- tRNAs make up about 15% of the total RNA in the cell.
- Each tRNA serves as an "adaptor" molecule that carries its specific amino acid—covalently attached to its 3'-end—to the site of protein synthesis. There it recognizes the genetic code sequence on an mRNA, which specifies the addition of its amino acid to the growing peptide chain

✓ MRNA

- 5% of the RNA in the cell,
- carries genetic information from the nuclear DNA to the cytosol, where it is used as the template for protein synthesis.
- Polycistronic mRNA is characteristic of prokaryotes.
- monocistronic mRNA is characteristic of eukaryotes.

TRANSCRIPTION OF PROKARYOTIC GENES

- In bacteria, one species of RNA polymerase synthesizes all of the RNA
- RNA polymerase is a multisubunit enzyme:

1. **Core enzyme:** Four of the enzyme's peptide subunits, 2α, 1β, and 1β', are required for enzyme assembly (2α), template binding (β'), and the 5'→3' RNA polymerase activity (β), and are referred to as the core enzyme
2. **Holoenzyme:** The σ subunit ("sigma factor") enables RNA polymerase to recognize promoter regions on the DNA. The σ subunit plus the core enzyme make up the holoenzyme.

STEPS OF RNA SYNTHESIS

The process of transcription of a typical gene of E. coli can be divided into three phases:

1. Initiation
2. Elongation
3. Termination

INITIATION

- Transcription begins with the binding of the RNA polymerase holoenzyme to a region of the DNA known as the **promoter**, which is not transcribed. The prokaryotic promoter contains characteristic consensus sequences

1. -35 SEQUENCE (TTGACA)

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2. PRIBNOW BOX (TATAAT)

NUCLEOTID SEQUENCES RECOGNISED BY RNA POLIMERASE

2. ELONGATION

- The recognition of promotor region and local unwinding of the DNA helix continues, mediated by the polymerase.

- The elongation phase is said to begin when the transcript (typically starting with a purine) exceeds ten nucleotides in length.

- Sigma is then released

- As with replication, transcription is always in the 5'→3' direction.

TERMINATION

- The elongation of the single-stranded RNA chain continues until a termination signal is reached.
- Termination can be intrinsic (spontaneous) or dependent upon the participation of a protein known as the ρ (rho) factor.

TRANSCRIPTION OF EUKARYOTIC GENES

- More complex than prokaryotes

- Eukaryotic transcription involves separate polymerases for the synthesis of rRNA, tRNA, and mRNA.

- In addition, a large number of proteins called transcription factors (TFs) are involved.

A. NUCLEAR RNA POLYMERASES OF EUKARYOTIC CELLS

- There are 3 classes of RNA polymerase in the nucleus of eukaryotic cells.

- ✓ 1. **RNA polymerase I:** This enzyme synthesizes the precursor of the 28S, 18S, and 5.8S rRNA in the nucleolus.

- ✓ **2. RNA polymerase II:** This enzyme synthesizes the nuclear precursors of mRNA that are subsequently translated to produce proteins.

PROMOTERS AND TRANSCRIPTION FACTORS FOR RNA POLYMERASE II

- -25 nucleotides upstream of the transcription start site "TATA (Hogness) box"
- -70-80 nucleotides upstream "CAAT box"
- In constitutive genes a "GC-rich region (GC box)"

RNA POLIMERASE III

- This enzyme synthesizes tRNA, 5S rRNA, and some snRNA and snoRNA.

Mitochondrial RNA polymerase

- Mitochondria contain a single RNA polymerase that more closely resembles bacterial RNA polymerase than the eukaryotic enzyme.

POSTTRANSCRIPTIONAL MODIFICATIONS OF RNA !!!!!!!!!!!!!!!!!

PROTEIN BIOSYNTHESIS

GENETIC CODE

Codons

- Codons are presented in the mRNA language of adenine (A), guanine (G), cytosine (C), and uracil (U).
- There are 64 different combinations of bases, taken three at a time (a triplet code)

- 5'-AUG-3' (Methionine) is the initiation (start) codon for translation !!!!

- **Termination (“stop” or “nonsense”) codons:**
- UAG, UGA, and UAA, do not code for amino acids,
- When one of these codons appears in an mRNA sequence, synthesis of the polypeptide coded for by that mRNA stops.

CHARACTERISTICS OF THE GENETIC CODE

- 1. Specificity
- 2. Universality
- 3. Degeneracy
- 4. Nonoverlapping and commaless

CONSEQUENCES OF ALTERING THE NUCLEOTIDE SEQUENCE:

- Point mutation

- Silent mutation
- Miscense mutation
- Nonsense mutation

COMPONENTS REQUIRED FOR TRANSLATION

- A large number of components are required for the synthesis of a protein !!!!!

1. Aminoacids
2. tRNA
3. Aminoacyl-tRNA synthetases
4. mRNA
5. Functional ribosomes
6. Protein factors
7. Energy (ATP and GTP)

STEPS IN PROTEIN SYNTHESIS

- mRNA is translated from its 5'-end to its 3'-end,
- Protein synthesis occurs from its amino-terminal end to its carboxyl-terminal end.

STEPS IN PROTEIN SYNTHESIS

1. INITIATION

- Initiation factors are needed /(in prokaryotes IF-1, IF-2, IF-3, in eukaryotes eIF)
- There are two mechanisms by which the ribosome recognizes the nucleotide sequence (AUG) that initiates translation:

1. Shine-delgarno sequence
2. Initiation codon

STEPS IN PROTEIN SYNTHESIS

2. ELONGATION

- Elongation of the polypeptide chain involves the addition of amino acids to the carboxyl end of the growing chain.
- Elongation factors are needed
- During elongation, the ribosome moves from the 5'-end to the 3'-end of the mRNA that is being translated.

STEPS IN PROTEIN SYNTHESIS

3. TERMINATION

- Termination occurs when one of the three termination codons moves into the A site.
- Termination factors are needed

POSTTRANSLATIONAL MODIFICATIONS

A. Trimming (Zymogen proteins)

B. **Covalent modifications**

- ✓ **Phosphorylation:** The phosphorylation may increase or decrease the functional activity of the protein.
- ✓ **Glycosylation:** N-linked or O-linked glycosylation
- ✓ **Hydroxylation:**

- **C. Protein folding**: "chaperones"
- **D. Protein Degradation**: "ubiquitin","proteasome"

REFERENCES

- Lippincott's Biochemistry, 5th Edition
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