بسم الله الرحمن الرحيم

مادة البيو من أسهل وأمتع المواد في هذا العالم

لذلك قررنا أن نجعلها أسهل للجميع

* تعريف بالمذكرة :

- شاملة لجميع المواضيع . (كاملة)
- تركيز على النقاط الهامة .
- جداول تسهل عملية الحفظ .
- أسئلة عامة .

شعارنا : دعونا سويا نفك السوبر كويل

Telomerase & DNA repair

Done by :

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لا تنسونا من دعائكم

C. Telomerase :

- In eukaryotic cells , after removal of the RNA primer from the extreme 5`-end of the lagging strand **(NOT leading MCQ)**, there is no way to fill the remaining gap with DNA.

  ✓ To solve this problem & to protect the ends of the chromosomes from attack by nucleases noncoding sequences of DNA complexed with proteins are found at these ends, called telomeres.

◙ **Note :**

- telomeres = noncoding sequences of DNA + proteins (MCQ)
- Are found at the 5`-end of the lagging strand . (MCQ)

⌖ Telomeres

- DNA of telomeres consists of repetitive sequence of T`s & G`s, base paired to a complementary chain of A`s and C`s

✓ Tx Gy: where x and y are in the range of 1 : 4

◙ **The TG strand :**

◙ is **longer** than its complement leaving a region of single stranded DNA at the 3`-end (MCQ) of the double helix (few hundred nucleotides long).

✓ This single stranded region folds back on itself, forming a structure that is stabilized by protein to protect the ends of the chromosomes

► In aging cells **(SENESCENCE),** the ends of their chromosomes get slightly **shorter** with each cell division until the telomeres are gone and DNA essential for cell function is degraded.

✓ This phenomenon is related to cellular *aging & death.*

► Cells that *do not age* (as germ-line cells and cancer cells) contain an enzyme called **telomerase** .

✓ **telomerase** is responsible for replacing these lost ends of telomeres.

SO WE FIND TELOMERE IN ALL CELLS BUT TELOMERASE ONLY IN OLD CELLS (VERY imp MCQ)

◙ **Note :**

⌖ Telomerase : (IT EXTEND THE 3-END OF THE DNA)

- is a special kind of reverse transcriptase (MCQ) that carries its own RNA molecule of about 150 nucleotides long. (SO NOT ONLY VIRUSES HAVE REVERSE TRANSCRIPTASE)

  ✓ RNA contains copies of A/C sequence that is complementary to T/G repeat sequence , (MCQ)

  ✓ the RNA base pairs with the terminal nucleotides at the single stranded 3-end of DNA

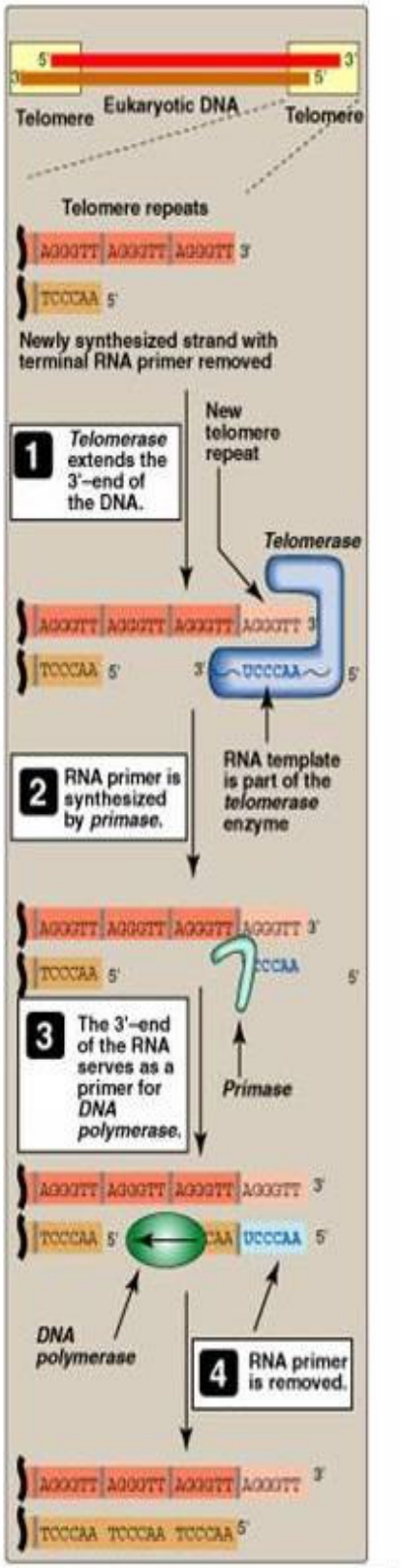

Figure 29.23
Mechanism of action of *telomerase*.

♣ Steps of telomere elongation

1. The telomerase RNA serves as template for extending **T/G** DNA strand (longer strand)
2. The 3`-end of the RNA serves as a primer for DNA polymerase to extend the **A/C** DNA strand (shorter strand)
3. Once the next repeat sequence is complete, telomerase RNA is translocated to the newly synthesized end of the DNA and the process is repeated.

◙ **Note : (From figure) (Very important)**
- TELOMERASE extend the 3-end of the DNA .
- RNA template that are part of telomerase enzyme from 5 to 3 .
- DNA polymerase work from 3 to 5 .

DNA Repair

- DNA repair is required in the following cases:

1- **Mismatches**: which occur after DNA replication including proofreading :

✓ incorrect base-pairing
✓ insertion of one to few extra nucleotides

2- **DNA damage** :

✓ due to environmental insults: & leads to alteration or removal of nucleotide bases

a) *chemical*: nitrous oxide
b) *radiation*: (MCQ)
   I. UV light :(can fuse 2 pyrimidines adjacent to each other in DNA)
   II. High-energy radiation: (can cause double-stranded breaks)

3- **Spontaneous alteration or loss of bases from mammalian DNA:**
✓ at a rate of thousands per cell per day

**** Effect of unrepaired mismatch and DNA damage:**

- A permanent mutation
- loss of control over the proliferation of the mutated cells ➔ **CANCER**

General Steps of Repair:

1. **Recognizing** the lesion
2. **Excision** of the damaged section of the DNA strand
3. **Fill the gap** using the sister strand as a template

A. Strand-directed mismatch repair system:

✓ Occur in cases of replication errors escaping the **proofreading function** (MCQ) during DNA synthesis

1. Recognizing the lesion

- endonuclease must be able to discriminate between the template strand and the newly synthesized strand containing the mistake.
- GATC sequences occurring every 1000 nucleotides are **methylated on the adenine**
- The methylation is not done immediately after synthesis ---- so, the newly synthesized DNA is temporarily hemimethylated (i.e. the parental strand is methylated but, the newly synthesized strand is not)

◙ **Note : (I see it in all exams) (MCQ)**
- endonuclease cut in the unmethylation GATC .(5 ال جهة الخطأ يسار على)

2. Excision of the damaged section of the DNA strand

- endonuclease cuts (nicks) the mismatched strand ➔ the mismatched base(S) are removed.

3. Fill the gap

- the gap left by removal of the bases is filled using the sister strand as a template by a 5`- 3` DNA polymerase. (*DNA polymerase I* in *E.coli* . *pol$_\beta$ and pol$_\varepsilon$ in eukaryotes*) (MCQ)
- The cut ends of the DNA are ligated (spliced) by DNA ligase **(3´-hydroxyl of new DNA spliced to 5´-P of remaining stretch of original DNA strand)** (this are the function of DNA ligase I see This question in one of the exam)

✓ A defect in mismatch repair in humans may cause herditary nonpolyposis colon cancer (HNPCC), common inherited cancer (MCQ)

B. Repair of damage caused by ultraviolet light☹:

- Exposure of a cell to **ultraviolet light** can result in the covalent joining of two adjacent pyrimidines (usually thymines), producing a dimer (**thymine dimer**) prevents DNA polymerase from replicating the DNA strand beyond the dimer formation. (MCQ)

*** similar in human & bacteria .

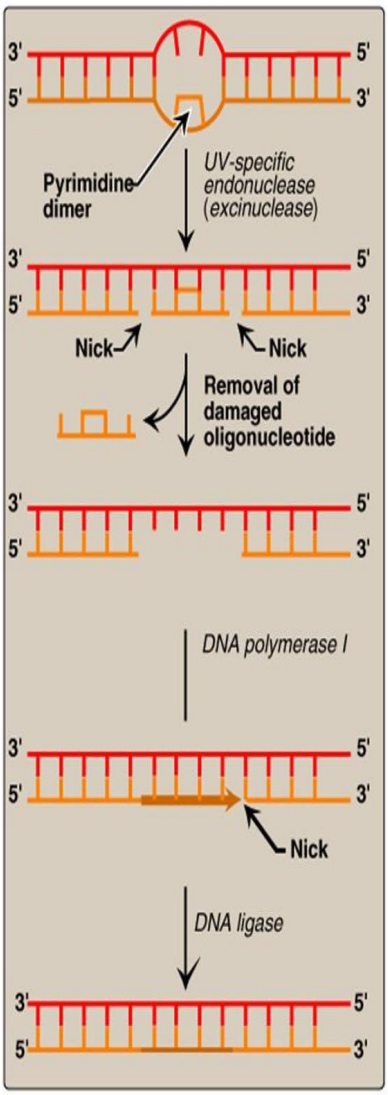

Figure 29.27
Excision repair of pyrimidine dimers in E. coli DNA.

1. Recognizing the lesion :

- UV-specific endonuclease (called: *uvrABC* excinuclease)(MCQ) (NOT exonuclease or endonuclease be careful ☺) recognize (thymine dimer).

2. Excision of the damaged section of the DNA strand :

- ***uvrABC* excinuclease cleaves at phospodiester bond on both 5` & 3` sides (2 cut not only one like mismatch) (very important it will come in exam) of** the dimmer making a gap by releasing the damaged oligonucleotides .

3. Fill the gap using the sister strand as a template

- the gap left by removal of the bases is filled using the sister strand as a template by a 5`-3` DNA polymerase. (*DNA polymerase I* in *E.coli* . pol_β *and* $pol\varepsilon$ *in eukaryotes*).

- The cut ends of the DNA are ligated (spliced) by DNA ligase (3´-hydroxyl of new DNA spliced to 5´-P of remaining stretch of original DNA strand)

✓ In xeroderma pigmentosum (a rare genetic disease), the cells cannot repair the damaged DNA resulting in extensive accumulation of mutations & consequently Skin Cancers (MCQ)

- **The most common form of this disease is caused by the absence of *UV-specific excinucleases.***

C. Correction of base alterations & losing (base excision repair):

► **The bases of DNA can be altered either :**

1- **spontaneously**

- ✓ (as the case of **c**ytosine which is spontaneously deaminated slowly to **u**racil)

2- by the action of deaminating or alkylating compounds as nitrous oxide

- ✓ nitrous oxide is formed by the cell from precursor as nitrosamines, nitrites & nitrates.
- ✓ nitrous oxide deaminates *cytosine*, *adenine* & *guanine* (NOT thymine – NOT uracil be careful)

*** cytosine is in both 1 & 2 (always come MCQ)

► **Bases are lost spontaneously**

- ✓ ~ 10,000 purine bases are lost per cell per day.

◙ **Note :** ☺

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لن تبلغ المجد حتى تلعق الصبرا

Removal of abnormal bases :

- Abnormal bases such as uracil or improper incorporation of dUTP instead of dTTP is **recognized** by specific glycosylases(MCQ) . **removal** is from the **deoxyribose phosphate** backbone of the strand , leave a apyrimidinic or apurinic site (AP-site)

1. Recognizing the lesion

- (AP-site) is recognized by specific **AP-endonuclease** هاالام that recognize that a base is Missing .

2. Excision of the damaged section of the DNA strand

a. AP- endonuclease cuts just to the 5-side of (AP-site) هام

b. deoxyribose phosphate lyase removes the single empty sugar-phosphate residue هام

3. Fill the gap using the sister strand as a template

- the gap left by removal of the bases is filled using the sister strand as a template by a 5`-3` DNA polymerase. (*DNA polymerase I* in *E.coli* . *pol$_\beta$ and polε in eukaryotes*).

- The cut ends of the DNA are ligated (spliced) by DNA ligase (3´-hydroxyl of new DNA spliced to 5´-P of remaining stretch of original DNA strand)

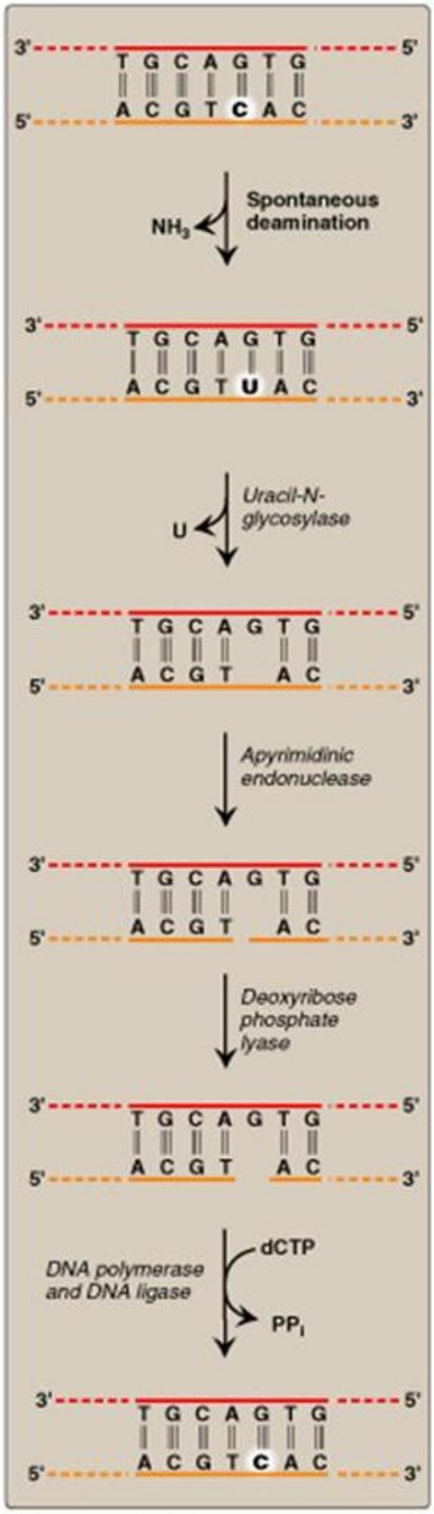

Figure 29.29
Correction of base alterations.

D. Repair of double-stranded breaks:

- High energy radiation or oxidative free radicals can cause **double stranded breaks in DNA** --- lethal to cells.(MCQ) (very important)
- Double-stranded breaks in DNA can occur naturally during gene rearrangement
- double-stranded breaks **cannot** be repaired by strategy of excising the damage on one strand & using remaining strand as template for replacing the missing nucleotide(s) as in (A,B &C) (MCQ)
- **double-stranded breaks are repaired by one of two systems:**

1- nonhomologous end-joining repair

- ✓ The ends of two DNA fragments are brought together by a group of **proteins** هام that cause their religation.
- ✓ This does not require that the two DNA sequences have any sequence homology
- ✓ Is the main repair mechanism in humans
- ✓ Is Error prone & mutagenic
- ✓ Defects in this repair system are associated with predisposition to cancer & immunodeficiency syndrome.

2- Homologous recombination repair

- ✓ Uses the **enzymes** هام لاحظ الفرق مع السابق that normally perform genetic recombination between homologous chromosomes during meiosis
- ✓ This system is used by the lower eukaryotes to repair double-stranded breaks.

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