Lebanese University **30 March 2019**
Faculty of Sciences **Partial exam**
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B3206 Microbial Genetics

N.B. The exam includes 10 questions on three pages. **You should answer all the questions on the question sheets.** Only answers on the question sheets will be taken into account and corrected. Your answer must not exceed the number of lines provided for each question. Note points/question and spend time accordingly. Please write legibly. [25 pts, 1 hour]
Alternative and/or complementary answers are given in brackets.

I- What are the features that make *E. coli* a good model system for scientific research? (2 pts)

Short generation time, fast growth in relatively cheap culture media, several molecular tools for manipulation, extensive knowledge of its genetics and genomics, easy mutant-hunting due to haploidy, etc.

II- What is auxotrophy? How can auxotrophic mutants be identified? (2 pts)

It is the inability of an organism to synthesize a particular organic compound required for its growth.
Auxotrophs or nutritional mutants can be identified by plating on special minimal medium.

III- Is there any difference between an F^+ and an HFR bacterium? Which one is used in interrupted mating technique? Why? (2 pts)

F^+ bacteria have a non-integrated F plasmid, while Hfr bacteria have an F plasmid that is integrated into their main chromosome.
HFR, since the integrated F factor enables the transfer of genetic material from a donor cell to a recipient by conjugation.

IV- How do generalized and specialized transductions in bacteria differ? (2 pts)

Generalized transduction is the process of transferring any bacterial gene to a second bacterium through a bacteriophage and specialized transduction is the process of moving restricted bacterial genes to a recipient bacterium.
While generalized transduction can occur randomly and more easily, specialized transduction depends on the location of the genes on the chromosome and the incorrect excision of the prophage.

V- What is gene conversion? Can the modified version of Meselson-Radding model for recombination account for this event? If yes, how? (3 pts)

It is the unidirectional transfer of genetic material from a 'donor' sequence to a highly homologous 'acceptor'.

[It is the process by which one DNA sequence replaces a homologous sequence such that the sequences become identical after the conversion event.]

[It is the process whereby, during meiosis, one allele in a heterozygote is converted to the other by a process of mismatch repair.]

[It is the unidirectional transfer of genetic material that results in the four products of meiosis displaying unusual segregation.]

In the modified version strand invasion is promoted by the 5' end rather than the 3' end, and the D-loop is degraded (not cleaved). DNA synthesis from the 3’ end accounts for the event.

VI- Explain briefly how the RecBCD pathway for homologous recombination in *E. coli* works. (3 pts)

The RecBCD complex binds to a DNA molecule and mediates unwinding DNA until it reaches a specific site called Chi site. The RecBCD complex cleaves the Chi site, 5 or 6 nucleotides to the 3' side. RecA and SSB proteins aid strand invasion of the 3'-ssDNA into the recipient homologous DNA molecule. Formation of a D-loop by displacement. Cleavage of the displaced strand and ligation of the free ends generates a cross stranded structure (Holliday junction) with heteroduplex regions.

[Step 1: RecBCD binds to a DNA double strand break. Step 2: RecBCD initiates unwinding of the DNA duplex through ATP-dependent helicase activity. Step 3: RecBCD continues its unwinding and moves down the DNA duplex, cleaving the 3' strand much more frequently than the 5' strand. Step 4: RecBCD encounters a Chi sequence and stops digesting the 3' strand; cleavage of the 5' strand is significantly increased. Step 5: RecBCD loads RecA onto the 3' strand. Step 6: RecBCD unbinds from the DNA duplex, leaving a RecA nucleoprotein filament on the 3' tail to initiate strand invasion and formation of a Holliday structure.]

VII- Why inteins have not they become extinct? What is meant by intein homing? (2 pts)

Though their loss is possible since they confer no benefit to the cell host, their presence is not affecting the fitness. Furthermore, inteins enhance their survival thanks to their homing endonuclease activity and their removal from genes proves very difficult.

It is the process of conversion of intein-minus allele into intein-plus allele by recombination.

VIII- The lytic cycle of a bacteriophage is regarded as a cascade of regulatory controls, how is that? (2 pts)

Phage genes are usually divided into three expression classes, early, middle, and late.

[Early phage genes are the most diverse

Middle genes usually encode viral DNA replication and recombination proteins
Late genes are involved in virion formation, DNA packaging, and host lysis.]

Control of gene expression is organized in a cascade-like way resulting in an ordered expression of genes belonging to different groups. Usually, host proteins are required for the transcription of early genes. Transcription factors required for middle gene expression are often encoded by early genes, while factors required for late transcription are usually the products of middle genes.

IX- How do genes *N, cII* and *cIII* help establish lysogeny following the infection of *E. coli* cells by phage Lambda? (3 pts)

Expression of *N* gene allows transcription of *cII* and *cIII* genes, and both gpcII and gpcIII are necessary for repressor gpcI synthesis; gpcIII and cAMP stabilizes gpcII. The protein gpcII acts as a positive regulator for the initiation of gpcI synthesis from PRE. The repressor activates its own expression from PRM. The repressor binds to operators O_L and O_R and blocks the expression of all phage genes.

X- The SOS response is controlled by the interplay of LexA and RecA. How does it work? What is the structure and function of the mutasome? (4 pts)

LexA repressor inhibits expression of the *SOS* genes during normal growth conditions, and RecA protein, following activation, leads to inactivation of LexA by specific proteolytic cleavage so that the SOS genes are derepressed. Turning on the *SOS* genes will help cell counteracting the damage to the DNA leading to resumption of normal cell growth.

The UmuD'2UmuCRecA mutasome reduces the proofreading activity of DNA polymerase III, thus enabling it to proceed past the damaged site and continue replicating the DNA.

Good Work