15.E: Positive and negative control of gene expression (Exercises)

Q15.1

Amber mutations are one class of nonsense mutations. They lead to premature termination of translation by alternation of an amino acid-encoding codon to a UAG terminator, e.g. CAG (Gln) may be changed to UAG (stop; amber). The phenotype of such amber mutants can be suppressed by amber-suppressor genes, which are mutant tRNA genes that encode tRNAs that recognize UAG codons and allow insertion of an amino acid during translation. Which genes or loci in the lac operon can give rise to amber-suppressible mutations?

15.2 (POB) Negative regulation.

In the lac operon, describe the probable effect on lacZ gene expression of:

a. Mutations in the lacoperator
b. Mutations in the lacI gene
c. Mutations in the promoter

Q15.3

Consider a negatively controlled operon with two structural genes (A and B, for enzymes A and B) an operator gene (O) and a regulatory gene (R). In the wild-type haploid strain grown in the absence of inducer, the enzyme activities of A and B are both 1 unit. In the presence of an inducer, the enzyme activities of A and B are both 100 units. For parts a-d, choose the answer that best describes the enzyme activities in the designated strains.
Uninduced Induced

Enz A Enz B Enz A Enz B

a) R+0CA+B+ a) 1 1 100 100
b) 1 100 100 1
c) 50 50 100 100

b) R-0+A+B- a) 1 100 100
b) 100 100 100 100
c) 100 0 100 0

c) R+0CA+B+/R+0+A+B+ a) 2 2 200 200
b) 51 51 200 200
c) 200 2 2 200
d) R-0+A+B+/R+0+A+B+ a) 2 2 200 200
b) 2 101 2 101
c) 200 200 200 200

Q15.4 (POB) Positive regulation.

A new RNA polymerase activity is discovered in crude extracts of cells derived from an exotic fungus. The RNA polymerase initiates transcription only from a single, highly specialized promoter. As the polymerase is purified, its activity is observed to decline. The purified enzyme is completely inactive unless crude extract is added to the reaction mixture. Suggest an explanation for these observations.

Q15.5

Consider a hypothetical regulatory scheme in which citrulline induces the production of urea cycle enzymes. Four genes (citA, citB, citC, citD) affecting the activity or regulation of the enzymes were analyzed by assaying the wild-type and mutant strains for argininosuccinate lyase activity and arginase activity in the absence (-cit) or presence (+cit) of citrulline. In the following table, wild-type alleles of the genes are indicated by a + under the letter of the cit gene and mutant alleles are indicated by a - under the letter. The activities of the enzymes are given in units such that 1 = the unininduced wild-type activity, 100 = the induced activity of a wild-type gene, and 0 = no measurable activity. In the diploid analysis, one copy of each operon is present in each cell.

Strain lyase activity arginase act.
number genes - cit + cit - cit + cit

Haploid: A B C D
1 + + + + 1 100 1 100
2 - + + + 100 100 100 100
3 + - + + 0 0 1 100
4 + + - + 100 100 100 100
5 + + + - 1 100 0 0

Diploid: A B C D / A B C D
6 + + + - / + - + + 1 100 1 100
7 - + + / + - + + 1 100 2 200
8 + + - + / + - + - 100 100 100 100
9 + - + / + + - 1 100 100 100

Use the data in the table to answer the following questions.

a) What is the phenotype of the following strains with respect to lyase and arginase activity? A single word will suffice for each phenotype.

<table>
<thead>
<tr>
<th>Lyase activity</th>
<th>Arginase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain 2</td>
<td></td>
</tr>
<tr>
<td>Strain 3</td>
<td></td>
</tr>
<tr>
<td>Strain 4</td>
<td></td>
</tr>
<tr>
<td>Strain 5</td>
<td></td>
</tr>
<tr>
<td>Strain 6</td>
<td></td>
</tr>
</tbody>
</table>

b) What can you conclude about the roles of \( \text{citB} \) and \( \text{citD} \) in the activity or regulation of the urea cycle in this organism? Brief answers will suffice.

c) What is the relationship (recessive or dominant) between wild-type and mutant alleles of \( \text{citA} \) and \( \text{citC} \)? Be as precise as possible in your answer.

d) What can you conclude about the roles of \( \text{citA} \) and \( \text{citC} \) in the activity or regulation of the urea cycle in this organism?
Brief answers will suffice.

Q15.6

Consider a hypothetical operon responsible for synthesis of the porphyrin ring (the heterocyclic ring that is a precursor to heme, cytochromes and chlorophyll). Four genes or loci, \(\text{porA, porB, porC, and porD}\) that affect the activity or regulation of the biosynthetic enzymes were studied in a series of haploid and diploid strains. In the following table, wild-type alleles of the genes or loci are indicated by a \(+\) under the letter of the \(\text{por}\) gene or locus and mutant alleles are indicated by a \(-\) under the letter. The activities of two enzymes involved in porphyrin biosynthesis, d-aminolevulinic acid synthetase and d-aminolevulinic acid dehydrase (referred to in the table as ALA synthetase and ALA dehydrase), were assayed in the presence or absence of heme (one product of the pathway). The units of enzyme activity are \(100 = \text{non-repressed activity of the wild-type enzyme, } 1 = \text{repressed activity of the wild-type enzyme (in the presence of heme), and } 0 = \text{no measurable activity. In the diploid analysis, one copy of each operon is present in each cell.}\)

<table>
<thead>
<tr>
<th>Strain number</th>
<th>porA heme</th>
<th>porB heme</th>
<th>porC heme</th>
<th>porD heme</th>
<th>ALA synthetase</th>
<th>ALA dehydrase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haploid:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+ + + + +</td>
<td>100</td>
<td>1</td>
<td>100</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>- + + + +</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>+ - + + 0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>+ + - + +</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>+ + + - +</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Diploid:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+ - + + /</td>
<td>+ + - + +</td>
<td>100</td>
<td>1</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>+ + + + /</td>
<td>+ + - + +</td>
<td>200</td>
<td>101</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>+ + + - /</td>
<td>+ + - + +</td>
<td>200</td>
<td>2</td>
<td>100</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>- + - + /</td>
<td>+ + - + -</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Use the data in the table to answer the following questions.

a) Describe the phenotype of the following the strains with respect to ALA synthetase and ALA dehydrase activities. A single word will suffice for each phenotype.

ALA synthetase ALA dehydrase

- + + + +
- - - - +
100 100 1
Strain 2 ______________________ ________________________
Strain 3 ______________________ ________________________
Strain 4 ______________________ ________________________
Strain 5 ______________________ ________________________
Strain 6 ______________________ ________________________

b) What is the relationship (dominant or recessive) between wild-type and mutant alleles of the four genes, and which strain demonstrates this? Please answer in a sentence with the syntax in this example: "Strain 20 is repressible, which shows that mutant grk1 is dominant to wild-type."

*porA* Strain ___ is ____________, which shows that ________
*porA* is ________________________________________________.

*porB* Strain ___ is ____________, which shows that ________
*porB* is ________________________________________________.

*porC* Strain ___ is ____________, which shows that ________
*porC* is ________________________________________________.

*porD* Strain ___ is ____________, which shows that ________
*porD* is ________________________________________________.

c) What is the role of each of the genes in activity or regulation of porphyrin biosynthesis? Brief phrases will suffice.

d) Is this operon under positive or negative control?