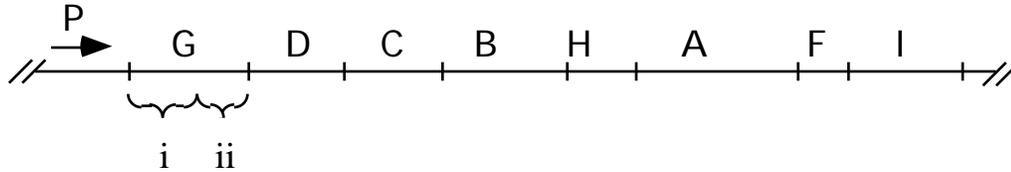


1. The *his* operon encodes eight enzymes required for the biosynthesis of the amino acid histidine. The organization of the *his* operon in *Salmonella typhimurium* is shown below:



The *hisD* gene encodes the enzyme which catalyzes the last step of histidine biosynthesis, the conversion of histidinol to histidine. Nonsense mutations in region “i” of the *hisG* gene prevent cells from making histidine from histidinol. However, missense mutations located at any position of the *hisG* gene or nonsense mutations in region “ii” of the *hisG* gene do NOT prevent the cells from making histidine from histidinol.

- What would be the phenotype of a mutant with a loss of function missense mutation in *hisG*? [3 points]

minimal – histidine = no growth (1 point)

minimal + histidine = growth (1 point)

minimal + histidinol = growth (1 point)

- (a) What would be the phenotype of a mutant with a loss of function missense mutation in *hisD*? [3 points]

minimal – histidine = no growth (1 point)

minimal + histidine = growth (1 point)

minimal + histidinol = no growth (1 point)

- (b) What is the most likely explanation for the different phenotypes observed for nonsense mutations in region “i” vs region “ii” of *hisG*? [8 points]

(i) **nonsense mutants are polar because Rho factor binds to naked mRNA prior to next translational start site, resulting in transcription termination and hence no expression of downstream genes (3 points for describing polarity, 2 points for explaining mechanism)**

(ii) **nonsense mutants are NOT polar because next translational start site is close enough that there Rho factor cannot effectively bind to naked mRNA, hence no transcription termination and downstreams are expressed (3 points for describing why not polar)**

- (c) Describe one way of testing an experimental prediction that could confirm or refute your explanation. [4 points]

Several answers were accepted: could determine if nonsense suppressors allow expression of downstream genes; could determine if *rho* mutation allows expression of downstream genes; could quantitate mRNA upstream and downstream of nonsense codon.

2. The *putP* gene product transports proline and the toxic proline analog dehydroproline (DHP) into the cell. Thus, *putP* mutants are unable to use proline as a sole carbon source and are resistant to DHP on minimal medium with succinate as a carbon source. [For each of the following questions, draw a cartoon showing what you would do and indicate the approximate number of bacteria or colonies expected at each step.]

- How could you select for *putP* mutants? [6 points]

Plate about 10^8 cells on minimal + succinate + DHP plates; expect about 10-100 colonies on this plate; selection because only *put* mutants will grow (2 points for describing numbers expected; 2 points for correctly describing concept of method; 2 points for actual selection)

- How could you screen for *putP* mutants? [6 points]

Dilute a culture of bacteria and spread about 200 cells onto multiple rich medium plates; incubate to obtain colonies; replicate onto (i) minimal + succinate plates and (ii) minimal + proline plates; compare the two types of media for rare colonies (about $1/10^6$) that fail to grow on proline as a carbon source; this is a screen because most colonies will grow and *put* mutants are identified by failure to grow (2 points for describing numbers expected; 2 points for correctly describing concept of method; 2 points for actual selection)

Note that you could also start with mutagenesis prior to doing the screen. In this case the number of mutants expected would typically be about $1/10^3$.

- How would you do a penicillin enrichment for *putP* mutants? [6 points]

- (i) **Grow a culture of bacteria in minimal medium + succinate (about 10^8 cells per ml). Expect about 1 *put* mutant per 10^6 cells.**
- (ii) **Transfer to minimal medium + proline + penicillin and incubate. About 99% of the *put*⁺ cells will be killed by the penicillin while the *put* mutants cannot grow and thus survive. Expect about 1 *put* mutant per 10^4 cells.**
- (iii) **Subculture the cells into minimal medium + succinate to allow *put* mutants to grow.**
- (iv) **Repeat the enrichment by subculturing into minimal medium + proline + penicillin. About 99% of the *put*⁺ cells will be killed by the penicillin while the *put* mutants cannot grow and thus survive. Expect about 1 *put* mutant per 10^2 cells.**
- (v) **Dilute the culture and plate about 200 cells onto minimal + succinate plates. Replicate onto minimal + proline plates; compare the two types of media for colonies (about $1/10^2$) that fail to grow on proline as a carbon source (2 points for describing numbers expected; 2 points for correctly describing concept of method; 2 points for actual selection)**

- Compare and contrast the advantages and disadvantages of each of these three approaches. [6 points]

	Advantage	Disadvantage
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Selection	best way to find rare mutants	sometimes too stringent to obtain leaky mutants
Screen	does not make stringent demands on cells so if the screen is sensitive can obtain both tight and leaky mutants	must search through a large number of colonies to find rare mutants
Enrichment	allows isolation of rare mutants that lack a good selection	the mutants from a single enrichment will include siblings with the same mutation

1. A *proB* mutant is unable to synthesize proline.

- How could you isolate suppressors of a *proB* mutation? [3 points]

Plate the cells on minimal medium – proline to select for growth without proline (“prototrophy”)

- List 5 potential ways that a second mutation might suppress the *proB* mutation. Briefly explain how each suppressor might work. [5 points]

There are many possible answers to this question. A few include:

- (i) **reversion within the same codon to restore original amino acid (true reversion or a second site intragenic suppressor),**
 - (ii) **a nonsense suppressor that allows translation of a nonsense mutation within the *proB* gene,**
 - (iii) **a bypass suppressor that allows the synthesis of proline via a different pathway,**
 - (iv) **an overproduction suppressor that increases the amount of a leaky, mutant ProB protein and thereby allows sufficient enzyme activity for growth,**
 - (v) **an interaction suppressor possibly restoring ProB activity by producing a tighter complex between ProB and another enzyme in the pathway.**
- If the original strain had a *proB* deletion mutation, what is the most likely mechanism of suppression? Explain your answer. [4 points]

A bypass suppressor because the other classes of suppressor act upon the gene transcript (informational suppressors) or the gene product itself and, thus, cannot suppress a mutation that removes the gene.

4. Although a suppressor mutation that restores functional interactions with a mutant protein may suppress some alleles of the protein much better than others, very few interaction suppressors are absolutely allele-specific.

- What is an allele specific suppressor? [4 points]

A second site mutation that restores activity of a mutant when combined with only one particular allele of the first mutation.

- How would you test for an allele specific suppressor? [4 points]

First, test vs the wild-type allele at the first position. If it fails to suppress the wild-type allele, then test many different mutant alleles.

- Why are so few interaction suppressors absolutely allele specific? [4 points]

Most protein-protein interactions have a certain amount of permissiveness for different combinations of interacting amino acids with an approximately appropriate size and charge.

5. The frequency of obtaining a spontaneous *pyrC* mutation is 10^{-6} per generation. The frequency of obtaining a spontaneous *pyrD* mutation is 10^{-7} per generation.

- If these are random events, what is the expected frequency of obtaining a *pyrC pyrD* double mutant? [4 points]

$$10^{-6} \times 10^{-7} = 10^{-13}$$

- What would you conclude if reversion of the *pyrC pyrD* double mutant occurred at a frequency of 10^{-8} per generation? Explain the rationale for your answer. [4 points]

The reversion is probably due to a single change that simultaneously suppresses both mutations – for example, it could be a bypass suppressor that provides a new route for the synthesis of pyrimidines, or it could be a nonsense suppressor if the two *pyr* mutants were both the same class of nonsense mutants (e.g. Amber mutants).

It is not likely to be due to a “hotspot” or a “mutator strain” because either of these two explanations would also increase the frequency of the single mutation to a proportional extent.

6. Describe a specific use for 3 of the following 5 types of mutations: missense mutations, nonsense mutations, frameshift mutations, deletion mutations, or insertion mutations. [6 points]

There are many possible answers to this question, but the answer should describe a specific function for that particular class of mutation. Only one function was needed per class of mutation. Some examples include:

Missense = can be used to study protein-protein interactions (e.g. by isolating allele specific interaction suppressors), can use TS and CS mutations to determine the order that gene products act in a pathway, can use TS and CS mutations to control the expression of a gene product in vivo to study the accompanying physiological processes.

Nonsense = provides a type of conditional mutation that allows gene expression in cells that contain an appropriate suppressor but not in cells that lack an appropriate suppressor (e.g. phage with amber mutations in essential replication genes can be used as vectors for chromosomal genes – in an amber suppressor strain the phage can reproduce and amplify the accompanying chromosomal genes, but in a strain lacking an amber suppressor the phage cannot replicate, allowing selection for delivery of the

chromosomal genes into the recipient bacteria), can be used to study the order of genes in an operon by testing for polarity.

Frameshift = historically they were used to establish that codons are comprised of three nucleotides, can be used to study strand slippage during DNA replication, reversion of frameshift mutations can be used to assay for potential carcinogens (intercalating agents)

Deletion = because they cannot undergo true reversion, they are good for constructing mutants that will be released into nature (e.g. in vaccines), they can be used to select for bypass suppressors that may identify new metabolic pathways, they can be used for genetic mapping

Insertion = because they provide a physical and often genetic marker, they can be used to clone genes or as a selectable marker to move genes between cells

7. *Salmonella typhimurium* has three independent restriction-modification systems, called LT, SA, and SB. It is possible to test the effect of these restriction-modification systems on DNA from other strains of *Salmonella* by using phage P22, as shown in the table below.

	Bacteria that phage P22 was grown on:	Recipient bacteria infected with phage P22:	Efficiency of plating (EOP) observed:
a	<i>S. typhimurium</i>	<i>S. typhimurium</i>	1
b	<i>S. enteritidis</i>	<i>S. typhimurium</i>	10 ⁻⁶
c	"	<i>S. typhimurium</i> LT ⁻ SA ⁻ SB ⁻	1
d	"	<i>S. typhimurium</i> LT ⁻ SB ⁻	10 ⁻¹
e	"	<i>S. typhimurium</i> SA ⁻ SB ⁻	10 ⁻³
f	"	<i>S. typhimurium</i> LT ⁻	10 ⁻³
g	"	<i>S. typhimurium</i> SA ⁻	10 ⁻⁵
h	"	<i>S. typhimurium</i> SB ⁻	10 ⁻⁴
i	<i>S. gallinarum</i>	<i>S. typhimurium</i>	1
j	"	<i>S. typhimurium</i> LT ⁻ SA ⁻ SB ⁻	1
k	"	<i>S. typhimurium</i> LT ⁻ SB ⁻	1
l	"	<i>S. typhimurium</i> SA ⁻ SB ⁻	1
m	"	<i>S. typhimurium</i> LT ⁻	1
n	"	<i>S. typhimurium</i> SA ⁻	1

o	"	<i>S. typhimurium</i> SB ⁻	1
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- What can you conclude about the differences between the *S. typhimurium* restriction-modification systems and the *S. enteritidis* restriction-modification systems? Explain your logic. [7 points]

There are several notable differences between these two strains:

- Phage grown on *S. enteritidis* is restricted by *S. typhimurium* and therefore it must not be modified for the *S. typhimurium* R-M system. (You cannot tell if *S. enteritidis* has an R-M system itself, only that it does not have the *S. typhimurium* systems.)**
- All three R-M systems in *S. typhimurium* restrict phage from *S. enteritidis*, indicating that it lacks all three R-M systems. Note the difference between lane b which is fully restricted and lane c which shows no restriction. (You cannot tell if the *S. typhimurium* mutants have the corresponding modification systems, you can only infer the restriction phenotype for the three restriction systems.)**
- The three R-M systems restrict unmodified DNA to different extents. (The differential sensitivity to the three restriction systems does not tell you anything about how closely the systems are related – either the restriction site is modified and therefore protected or it is not modified. The differential sensitivity is simply due to the fact that the three systems recognize different sites and do so in different ways.) The LT system restricts unmodified DNA about 1000x (note lane f which lacks the LT system but has the other two systems). The SA system is much weaker and only restricts unmodified DNA about 10x (note lane d which only has the SA system). The SB system is intermediate and restricts DNA about 100x (e.g. note the difference between lanes d and f).**

- What can you conclude about the differences between the *S. typhimurium* restriction-modification systems and the *S. gallinarum* restriction-modification systems? Explain your logic. [7 points]

***S. gallinarum* is not restricted by any of the three *S. typhimurium* R-M systems, so it is probably modified by the same three R-M systems. (You cannot tell if it also has the restriction endonucleases, only that it is modified at the corresponding sites.)**

8. Wild-type *S. typhimurium* was infected with phage P22 from each of the *S. typhimurium* restriction-modification mutants. The results are shown below.

	Bacteria that phage P22 was grown on:	Recipient bacteria infected with phage P22:	Efficiency of plating (EOP) observed:
a	<i>S. typhimurium</i>	<i>S. typhimurium</i>	1
b	<i>S. typhimurium</i> LT ⁻ SA ⁻ SB ⁻	"	10 ⁻³
c	<i>S. typhimurium</i> LT ⁻ SB ⁻	"	10 ⁻³
d	<i>S. typhimurium</i> SA ⁻ SB ⁻	"	1

e	<i>S. typhimurium</i> LT ⁻	"	10 ⁻³
f	<i>S. typhimurium</i> SA ⁻	"	1
g	<i>S. typhimurium</i> SB ⁻	"	1

- What can you conclude about the modification phenotype of each of the three *S. typhimurium* restriction-modification systems? Explain your logic. [6 points]

Each of the three mutants is clearly r⁻ based upon the results in question #7. These results allow you to determine the modification phenotype of each of these mutants. The idea is that if the phage is modified in the donor, it will not be restricted by the corresponding system in the recipient. Phage grown on the SA mutant is not restricted (lane f), indicating that it has been modified and hence the SA mutant is r-m+. The same is true of the SB mutant (lane g), so the SB mutant is also r-m+. In contrast, phage grown on the LT mutant is restricted about 1000x, indicating that it has not been modified and the LT mutant must thus be r-m-.

(Look at the numbers. By comparing lanes b and e, it is clear that the LT mutant is responsible for the full restriction observed. It isn't critical to answer this question, but note that phage grown on the triple mutant is only restricted about 10³-fold, not 10⁶-fold as seen in question #7.)