

7.03 Problem Set 4 Key

1. a. We can think of lac permease activity (from LacY⁺) and beta-galactosidase activity (from LacZ⁺) as reporters for Lac operon expression from the bacterial chromosome and the F', respectively. In other words, lac permease activity tells us whether the operon on the bacterial chromosome is regulated, constitutive, or uninducible; and beta-galactosidase activity tells us whether the operon on the F' is regulated, constitutive, or uninducible.

Lac1- is a dominant, cis-acting mutation. It is dominant because lac permease activity is still constitutive in the merodiploid with one wild-type allele and one Lac1⁻ mutation. Lac1⁻ is cis-acting because it can only confer constitutive expression on the reporter in cis with itself (LacY⁺), while the reporter is trans (LacZ⁺) is regulated.

Lac2- is a dominant, trans-acting mutation. It is dominant because lac permease activity is still constitutive in the merodiploid with one wild-type allele and one Lac2⁻ mutation. Lac2⁻ is trans-acting because it can confer constitutive expression on both the reporter in cis (LacY⁺) and the reporter is trans (LacZ⁺).

Lac1- best fits the properties of LacO^C mutations (cis-acting dominant) and Lac2- best fits the genetic characteristics of LacI^d mutations (trans-acting dominant).

- b. LacP⁻ mutations result in decreased expression of Lac operon genes, nevertheless, expression is still regulated by IPTG. A LacO^C mutation, on the other hand, would result in unregulated expression of Lac operon genes. In other words, the activity of both beta-galactosidase and lac permease would be independent of IPTG in LacO^C. Because the promoter is required for expression and the operator is involved for regulation of the operon, this implies that LacP and LacO represent functionally distinct sites on the DNA.

It is possible to imagine mutations in the operator that would give results like the LacP⁻ mutation, thus confounding the conclusion we just drew. For example, there could be a mutation in LacO that results in a greater binding affinity with the Lac repressor. In such a state, expression of the operon's genes can almost be completely off in the absence of IPTG and only slightly induced in the presence of IPTG (like the LacP⁻ mutations). We can, however, differentiate this from LacP⁻ with further experimentation. With a LacO mutation we can increase expression of the operon by increasing the amount of IPTG because the operator is again the region regulated by the inducer. On the other hand, LacP⁻ there will an insensitivity to IPTG inducer pass a particular point because its function is dependent on binding to RNA polymerase. In any case, this scenario is unlikely, as it is much more difficult to get a mutation in LacO that produces a higher binding affinity with Lac repressor than it is to get a mutation that produces a lower binding affinity.

c. **In Lac3-, a part of LacI is deleted in addition to the promoter.** From the data, we can deduce that the wild-type allele of Lac3- is a trans-acting factor, as beta-galactosidase activity becomes regulated when the wild-type allele (brought in on F') is in trans with wild-type LacZ. The wild-type LacI gene product can act to regulate the expression of both the chromosomal and the F' operon. The Lac3- mutation would result in a non-functional gene product (LacI-, which is consistent with the data.

In Lac4-, a part of LacO is deleted in addition to the promoter. From the data, we can deduce that the wild-type allele of Lac4- is a cis-acting factor, as beta-galactosidase activity is still unregulated when the wild-type allele (from the F') is in trans with the wild-type LacZ. The Lac4- mutation would result in an operator that the Lac repressor can no longer bind, so expression is constitutive (LacO^C), although at the decreased overall level of expression compared to wild-type owing to the promoter deletion.

Lac4- could possibly represent a LacI^d like mutation as judged from the data, but we know that such mutation affect the N-terminus of the protein product (the 5' part of the coding strand), so this is unlikely because the majority of the LacI gene would have to be deleted to reach the 5' region.

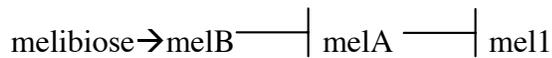
2. a. **We can classify MelA- as a recessive, loss of function mutation, whose wild-type allele codes for a trans-acting factor.** Because an amber suppressor can restore the wild-type phenotype, the MelA- mutation is most likely an amber mutation. Amber suppressors are only about 5% effective, thus the mutant allele is most likely recessive because 5% wild-type protein is unlikely to suppress the mutant phenotype for a dominant mutant allele. Because the wild-type allele codes for a functional protein product, we know that it is a trans-acting factor. We also know that MelA and Mel1 are different genes because they are unlinked.

MelA is likely a negative regulator of Mel1 expression. The MelA- mutation causes constitutive activity, which is consistent with loss of function of a negative regulator.

b. **MelB- is a recessive mutation whose wild-type allele codes for a trans-acting factor that is not Mel1.** We know that MelB- is recessive because MelB-/F' Mel+ results in regulated melibiose activity, rather than uninducible. Mel1- is also a recessive mutation using the same reasoning as with MelB-. With two recessive mutation, a complementation test is then performed (MelB-/F' Mel1-). The result is that MelB- and Mel1- complement each other, meaning that they are mutations in different genes. This strain (MelB-/F' Mel1-) also allow us to deduce that MelB's wild-type gene product is trans-acting because we observe regulated activity when wild-type MelB is placed in trans with wild-type Mel1.

MelB is likely a positive regulator of Mel1 expression. The MelB- mutation causes uninducible activity, which is the result of a recessive mutation in a positive activator.

2c) We know that melibiose and MelB both have a net positive effect on MelI activity, and MelA has a net negative effect on MelI activity. Therefore there are two possible models:



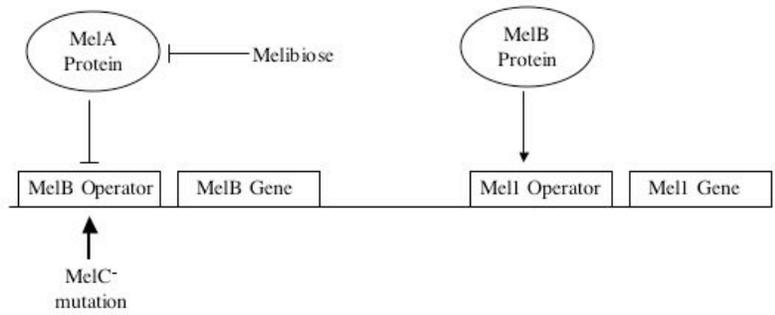
There could be other models consistent with this data, but these two models are the simplest.

2d) The phenotype of the MelA⁻ MelB⁻ double mutant is identical to the MelB⁻ mutant. Since MelB⁻ is epistatic to MelA⁻, the correct pathway must be:

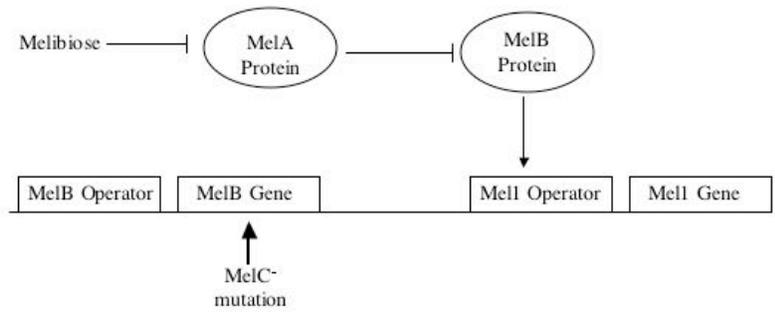


2e) Because MelC⁻ / F⁺ Mel⁺ has melibiase activity in both the presence and absence of melibiose, we know that MelC⁻ is dominant and constitutive. Furthermore, because MelC⁻ causes constitutive melibiase activity in either cis or trans to MelI⁺, it is a trans-acting factor. Therefore the two possibilities consistent with this data are MelC⁻ being either a dominant negative mutation in a negative regulator (repressor^d) or a super activator mutation (activator^s).

2f) If we assume that MelB⁻ and MelC⁻ are mutations in the same activator gene, then MelB⁻ must cause a loss-of-function in the activator, while MelC⁻ must cause a gain-of-function that renders the activator unresponsive to the MelA negative regulator protein. One possibility is that the MelA protein normally binds to the MelB protein, and the MelC⁻ mutation affects the protein such that MelA can no longer bind. Alternatively, MelA may be a repressor that binds to an operator in front of the MelB gene, and the MelC⁻ mutation is a mutation in the operator of MelB, causing constitutive MelB expression. The easiest way to distinguish these two possibilities from each other would be by sequencing the MelB gene and the upstream sequences in both wt and MelC⁻ bacteria. By comparing the sequences from wt and MelC⁻ it would be possible to determine where the MelC⁻ mutation lies. If the mutation lies in the operator for the MelB gene then we would know that MelA is a repressor that binds to an operator in front of MelB. If the mutation lies in the MelB gene itself then we would know that MelA protein negatively regulates the MelB protein.



MelC⁻ is a mutation in MelB operator



MelC⁻ is a mutation in MelB protein coding sequence