12.2: The Use of Mutants to Study the lac Operon

12.2.1 Single mutants of the lac operon

The lac operon and its regulators were first characterized by studying mutants of *E. coli* that exhibited various abnormalities in lactose metabolism. Some mutants expressed the lac operon genes constitutively, meaning the operon was expressed whether or not lactose was present in the medium. Such mutant are called constitutive mutants.

The operator locus (lacO) - One example is O^c_, in which a mutation in an operator sequence and reduces or precludes the repressor (the lacI gene product) from recognizing and binding to the operator sequence. Thus, in O^c_ mutants, lacZ, lacY, and lacA are expressed whether or not lactose is present.

The lacI locus – One type of mutant allele of lacI (called I) prevents either the production of a repressor polypeptide or produces a polypeptide that cannot bind to the operator sequence. This is also a constitutive expresser of the lac operon because absence of repressor binding permits transcription.

Another type of mutant of lacI called I^s prevents the repressor polypeptide from binding lactose, and thus will bind to the operator and be non-inducible.. This mutant constitutively represses the lac operon whether lactose is present or not. The lac operon is not expressed and this mutant is called a “super-suppressor”.

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12.2.2 The F-factor and two lac operons in a single cell – partial diploid in *E.coli*

More can be learned about the regulation of the *lac* operon when two different copies are present in one cell. This can be accomplished by using the **F-factor** to carry one copy, while the other is on the genomic *E. coli* chromosome. This results in a partial diploid in *E. coli*.

The F-factor is an **episome** that is capable of being either a free plasmid or integrated into the host bacterial chromosome. This switching is accomplished by IS elements where unequal crossing over can recombine the F-factor and adjacent DNA sequences (genes) in and out of the host chromosome. Researchers have used this genetic tool to create partial diploids (merozygotes) that allow them to test the regulation with different combinations of different mutations in one cell.

For example, the F-factor copy may have a $I^S$ mutation while the genomic copy might have an $O^C$ mutation. How would this cell respond to the presence/absence of lactose (or glucose)? This partial diploid can be used to determine that $I^S$ is dominant to $I^+$, which in turn is dominant to $I^-$. It can also be used to show the $O^C$ mutation only acts in cis- while the *lacI* mutation can act in trans-.

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