Introduction: Control of gene expression in phages

Phages, also known as bacteriophages are viruses that infect bacteria like E.coli. Bacteriophages either have DNA or RNA as their genetic material. They are linear or circular in the configuration as a single or double-stranded form. λ-phage has a dsDNA genome which is linear with a size of about 48.5kb in the capsid and circular when it is in the host. After infection, the linear genome is converted into a circular form by binding to two cos sites. Control of gene expression in phages is mainly driven by two kinds of cycles.

A phage usually follows one of the two life cycles called as lytic cycle and lysogenic cycle:

1)Lytic cycle -

It is a cycle of viral reproduction which **destroys the infected cell**. When only a lytic cycle is used by the bacteriophage it is called a **virulent phage.**

- Attachment - The first stage in the lytic cycle is an attachment which means the phage is attached surface of the host cell to inject its DNA into the bacterial cell.
- Penetration - Becteriophage injects its DNA into the host cell by penetration through the cell membrane.
- Transcription - Penetration is followed by the transcription in which the host cell DNA is degraded and the cell's metabolism is directed to initiate the phage biosynthesis. The phage uses the overall machinery of the host cell for the synthesis of different viral particles.
- Replication - In this process, new phage DNA and proteins are synthesized.
- Maturation - The replicated viral material is going to assemble to form a full viral phage. After maturation of phage, the lysis of the host cell takes place and the new phage that has been made inside the bacterial cell will be released into the environment.

2)Lysogenic cycle -

When a phage infects the surface of bacteria through attachment it penetrates its DNA into the bacterial cell. After penetrating its DNA into the bacterial cell, lysogeny is characterized by the integration of bacteriophage DNA into the host bacterium genome. In this condition, the bacterium is not destroyed and continues living and reproducing normally. When the genome of a bacteriophage is integrated into the host genome it is called a **prophage**. Prophage can be transmitted to the daughter cell at each subsequent cell division. This integrated prophage replicates when bacterial DNA replicates.

Control of gene expression in phages

How gene expression is regulated in phages?

In order to understand the Control of gene expression in phages, we need to know the temperate phage. The phage in which both lytic and lysogenic cycles are present is called **temperate phage**. The regulation of gene expression in phages is all about how the lytic cycle gets switched to the lysogenic cycle and vice-versa. **λ-phage** is the best example of a temperate phage. It can switch between the lytic cycle and the lysogenic cycle.

There are three classes of genes in the phage genome that regulate whether the lytic or lysogenic cycle will emerge.

- Immediate early genes - 1)Gene "N" -Antiterminator or activator of transcription. 2) Gene "cro" - Forms cro repressor protein.
- Delayed early genes - 1)cII - Activator of Transcription of cI 2)cIII - a structural mimic of cII and stabilizes cII 3)int (integrate) - specific for integration in the host genome 4)xis - excision of prophage from the host genome by enzyme Excisionase. 5)O & P - Replication of DNA 6) Q - for the expression of late genes.
- Late genes - 1) A to Z - Head and tail synthesis. 2)S and R - genes for lysis.

Promoters and operators: Phage lambda has two early transcription units i.e. Leftward and rightward.

1. PL - Left promoter (initiate transcription of N)
2. PR - Right promoter (initiate transcription of cro)
3. PRE - Promoter for repressor establishment (helps cI repressor for establishment)
4. PI - Promoter of integration
5. PRM - Promoter of λ-repressor maintenance
6. OR1, OR2, OR3 - operator regions of PR & PRM
7. OL1, OL2, OL3 - operator regions of PL

Let's see how these genes help in the regulation of gene expression in the lytic and lysogenic cycle:

When bacteriophage infects the host cell the promoter PL and PR start the transcription. Promoter PL and PR are responsible for the transcription of genes N and cro respectively. The main function of cro is to induce the lytic cycle by binding to the RNA polymerase and repressing the cI repressor and N acts as Antiterminator which binds to RNA polymerase and prevents transcriptional termination. Since the transcription of genes N and cro started early they are called immediate early genes.

After the transcription of N and cro, due to the action of the Antiterminator(N), cII and cIII genes also get transcribed by PR and PL respectively. These genes are called delayed early genes. Gene cII and cIII help in the lysogenic cycle.

Control of gene expression in phages

How is the lysogenic cycle established?

Promoter PRE establishes the cI repressor with the help of cII and cIII. cII is the activator of transcription of cI but it is more unstable so it will be easily degraded by cellular proteases made by the host bacterium. To avoid this, cIII binds to cII and degrade the cellular protease of the host bacterium and prevent the degradation of cII. Along with RNA polymerase, this cII-cIII complex synthesizes the cI repressor protein.

cI repressor protein is a dimer made up of 236 amino acids. It has C and N terminals. C terminal is responsible for dimerization and N terminal is for DNA-binding. As cI is a repressor protein its main function is to repress cro and induce a lysogenic cycle.

How cI repressor inhibits the genes required for the lytic cycle? The λ-repressor or cI repressor binds to operators that are adjacent to PR and PL. Both PR and PL have three operator regions i.e. OR1, OR2, OR3, and OL1OL1, OL2, OL3 respectively. λ-repressor has the highest affinity to OR1 then OR2 and OR3. (OR2 & OR3 is an operator for PRM also as it is adjacent to PR). OR1>OR2>OR3, OL1>OL2>OL3

λ-repressor binds to the OR1 region and inhibits PR to initiate transcription so there will be no production of cro protein and by repressing the expression of cro protein lytic cycle will be inhibited and the lysogenic cycle will be established.

When cI binds to the OR2 region it will stimulate the expression of cI with the help of PRM. (PRM & PR present adjacent to each other. If PR is blocked PRM initiates the expression. But they both can't express simultaneously). When cI binds to the OR3 region it represses its synthesis. This is why the cI repressor acts as an activator as well as a repressor because it activates its synthesis and represses it also when its amount becomes high.

For the maintenance of the lysogenic state, λ- repressor blocks PR and PL but activates PRM which initiates transcription by RNA polymerase and synthesis of cI mRNA continued.

In the lysogenic cycle, there is a synthesis of the prophage. Prophage is made by the integration of the viral genome into the bacterial genome by the enzyme *integrase*. This *integrase* protein is synthesized by the int gene present in the viral genome. The cII along with RNA polymerase bind to the promoter site of the int gene called PI (promoter of integration) and *integrase* protein is synthesized.

Why int gene is transcribed by PI promoter and not by PL promoter of N antitermination? This is because even though the N Antitermination complex transcribed the int gene, it was demonstrated that sib inhibits the expression of the int gene from PL but not from PI. *sib* is a cis-acting, retro regular downstream of the int gene.

The *sib* site forms the large stem-loop structure in the RNA which is sensitive to cleavage by RNaseIII. So the cleavage of the transcript initiated by PL promoter at *sib* by RNaseIII prevents int protein synthesis. Therefore *sib* is acting as a retroregulator by inhibiting PL to transcribe the int gene.

How is the lytic cycle established?

In the lytic cycle, two regulators cro and N transcribe by binding to RNA polymerase with the help of promoters PR and PL and form cro mRNA and N mRNA.

During the lytic cycle, the cro protein controls the switch. cro protein is a dimer & it has the highest affinity to OR3 then the same for OR2 and OR1. (OR3>OR2=OR1, OL3>OL2=OL1)

When cro protein bind to OR3, it will block transcription from PRM by repressing the expression of the cI repressor so the lysogenic cycle will be switched off. Late genes are expressed in the lytic cycle. Q gene is responsible for capsid formation and the lysis of the bacterial cell. It acts as an antiterminator that permits transcription of late genes through another promoter, PR'. PR' controls a very large operon that encodes the protein necessary for the assembly of phage coat, packaging of DNA, and lysis of bacterial cells.

To summarize all this, in the lysogenic cycle, the cI repressor blocks the transcription from PR and PL by binding to their operators and blocking expression of cro which allows RNA polymerase to bind to PRM and transcribe to form cI mRNA. While in the lytic cycle, cro represses the expression of the cI repressor by binding to the operator of PRM i.e.OR3 and inhibiting RNA polymerase to bind to PRM. So, RNA polymerase attaches to PR and PL and transcribes cro gene and N gene to form cro mRNA and N mRNA respectively.

Control of gene expression in phages

cI repressor ➞ repress cro ➞ Lysogenic cycle

cro repressor ➞ repress cI ➞ Lytic cycle

Factors that influence the choice between two cycles:

cII protein plays a key role in directing cI repressor to the lysogenic or lytic cycle. The cellular protein HFLA produced by bacteria forms FtsH protease that degrades the cII protein. These proteases are produced depending on environmental conditions. If the growth conditions are very favourable, the level of HFLA is high and it will degrade the cII protein and the cI repressor will not be synthesized so the lysogenic cycle will be switched off. Instead high levels of cro protein allow the lytic cycle to proceed. Thus, favourable **growth conditions** promote the **lytic cycle** because a sufficient supply of nutrients is necessary for the synthesis of new bacteriophages.

If the growth conditions are unfavourable that means if nutrients are limiting, the level of HFLA is low. Due to this cII protein builds up more quickly than cro and turns on PRE, forming the cI repressor. Thus, unfavourable **growth conditions** promote the **lysogenic cycle** because no sufficient nutrients are present for the production of new bacteriophages.

High nutrients(HFLA)➞ cII degraded➞ high cro level➞ Lytic cycle

Low nutrients (HFLA)➞ cII builds up quickly ➞activate PRE ➞cI made ➞ Lysogenic cycle

Certain conditions like exposure to UV light can also favour the induction of the lytic cycle. A cellular protein recA(a protein involved in DNA recombination) activates to become a protease by detecting the DNA damage. This protease cleaves cI repressor and inactivates it. Therefore, cro protein will accumulate and favours the lytic cycle.

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