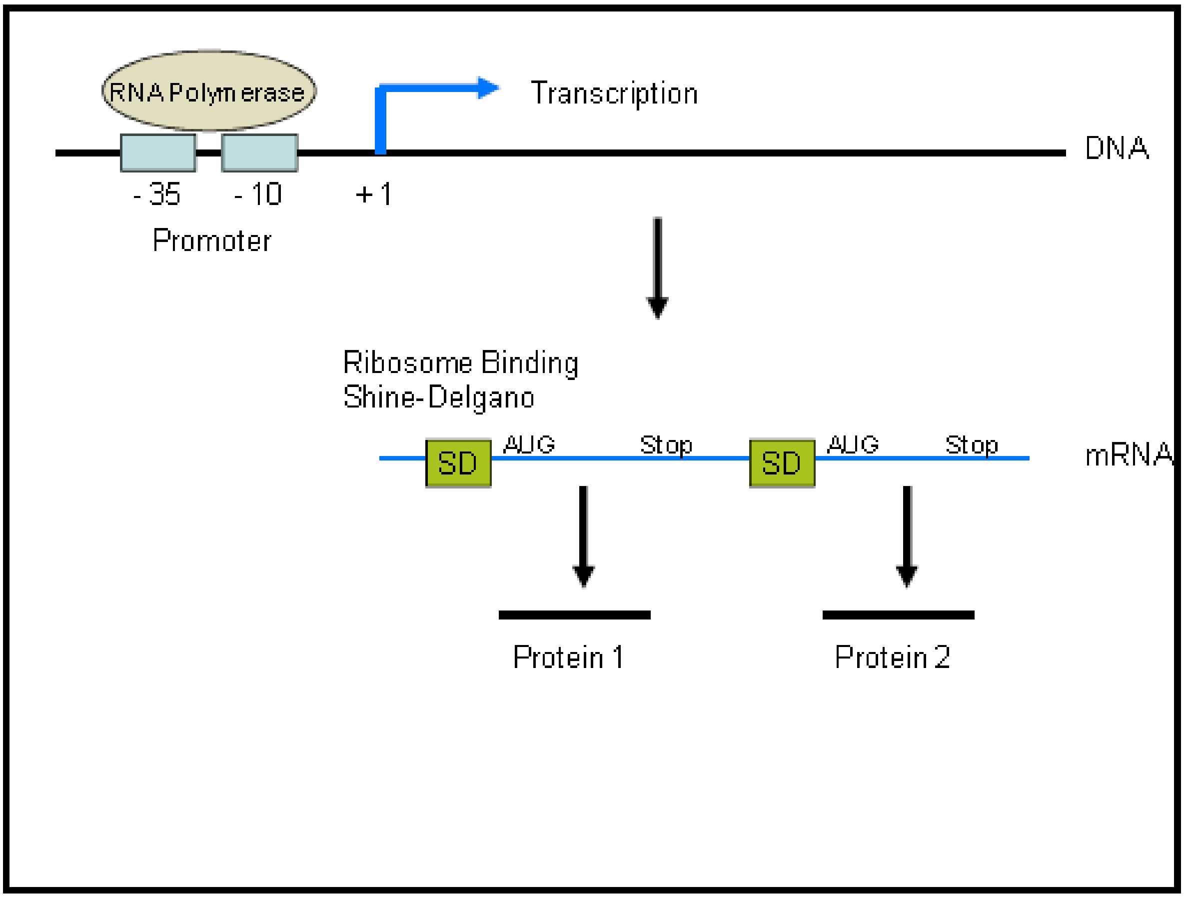
Gene expression

Gene regulation in prokaryotes

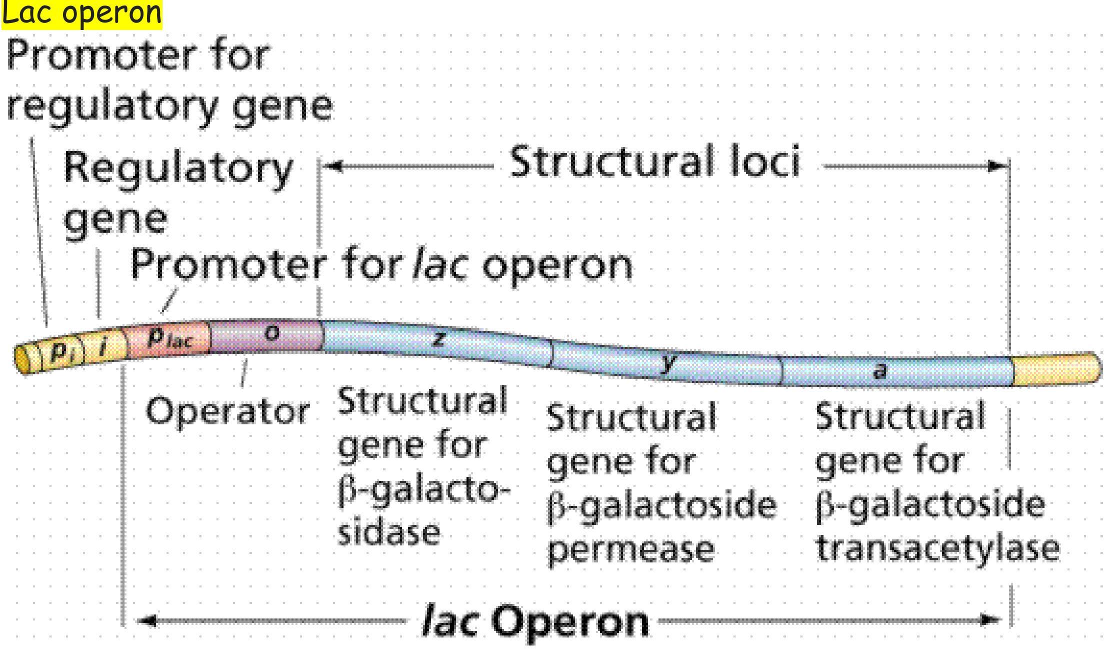
- The control occurs at the level of transcription
- How does the bacteria knows when to start transcription?
    - Promoter site. The sequence where the RNA polymerase binds. Start transcription on the place where it binds.
    - -10 region - consensus of TATAAT in E. coli
      -35 region - consensus of TTGACA in E. coli
- 

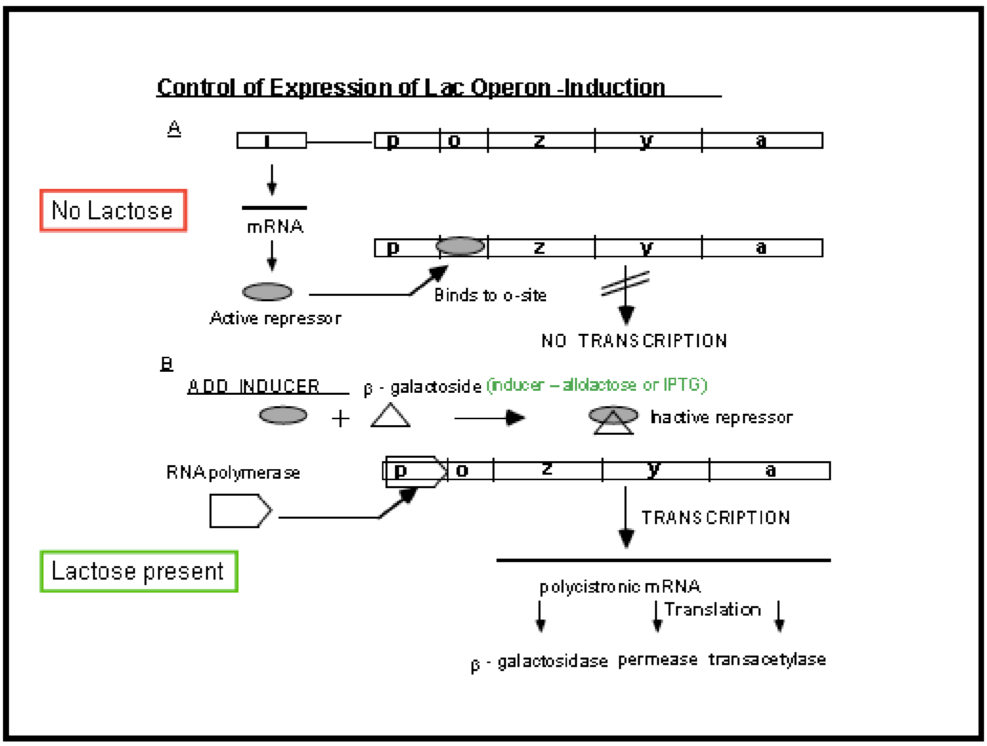
    - Start transcription at +1
    - Ribosome binds onto Shine Dalgarno sequence
    - In bacteria we have **Polycistronic mRNA**, we can produce more than one protein from one messenger RNA. Eukaryotes only have monocistronic mRNA.
- **Why is controlling gene expression important?**
    - 10 ATPs per amino acids are required to transcribe and translate DNA to protein.
    - Hence it is important to conserve energy and only express when needed.
- **Main mechanism used**

- The binding of proteins to DNA will determine if the gene is expressed or not
- The ability of these proteins to interact with DNA is controlled by small molecules.

Operon

1. A group of genes coding for related proteins that are arranged in units known as operons.
    a. Lac operon





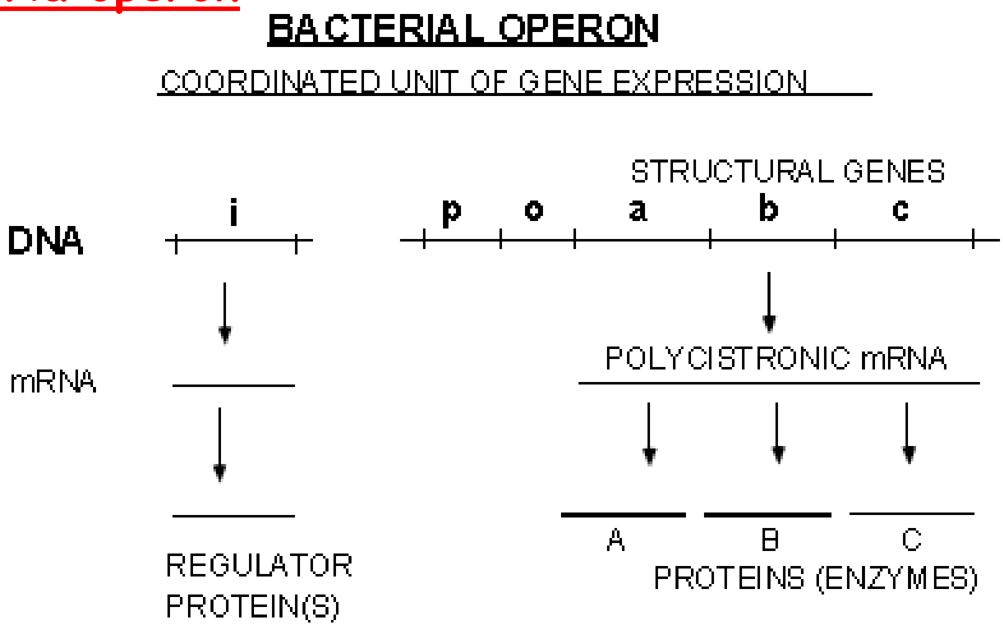
i. Uptake and metabolism of lactose requires galactoside permease and beta galactosidase
ii. Beta galactosidase breaks down lactose to galactose and glucose
iii. When lactose is present, RNA polymerase binds onto the promoter and we get a transcription of polycistronic mRNA, producing beta-galactosidase, permease and transacetylase.
iv. When there is no lactose, the repressor will binds onto the operator and RNA polymerase will not be able to bind to the promoter. Steric hindrance prevent RNA polymerase from binding to the promoter. No transcription occurs.
v. Adding lactose will cause the inducer (allolactose or IPTG) to bind to the repressor protein and change its shape so that repressor protein can't bind to the operator. This will mean that RNA polymerase can bind onto the promoter site and transcription can occur.
vi. The inducer does not have to be a substrate like allolactose for the enzyme to be synthesised. IPTG can be used as the inducer.
vii. **What happens when glucose and lactose are present?**
    1. It will cause catabolite repression
        a. This involves a mechanism whereby glucose is the preferred substrate and its presence represses the expression of the lac operon.
        b. It involves cyclic AMP which binds to a Catabolite Activator Protein (CAP).
        c. This complex binds to a region of the operon near the promoter and stimulates binding of the RNA polymerase, hence transcription proceeds. When the levels of glucose are high in the E. coli then the level of cAMP is low. Thus there is no active complex formed between CAP and cAMP and there is no binding of the RNA polymerase to the promoter site.
        d. When all the glucose is used up, the level of cAMP will increase and the CAP will form a complex which binds near the promoter stimulating binding of RNA polymerase.
        e. This is the process by which the catabolite, glucose, represses the expression of the lac operon and thus lactose utilisation.
        f. This is obviously economical for the cell as the enzymes for glucose utilisation are always present whereas

energy has to be expended to synthesise the enzymes for lactose utilisation (glucose is the preferred substrate).

g. Hence, cAMP.CAP is considered to be a positive effector as its presence promotes the binding of RNA polymerase to the promoter region of the operon and this has a positive effect on transcription.

h. The lac repressor is a negative effector because binding to the operator has a negative effect on transcription.

Bacteria operon



- I = regulatory gene
- P= promoter site- site on DNA where RNA polymerase binds
- O= operator site - site on DNA where regulator protein can bind
- A, b,c- structural genes- lead to proteins
- Requires polycistronic mRNA in order for the operon to work

How does glucose regulate the amount of cyclic AMP inside the cell?

- The entry of glucose into the cell causes inhibition of the lac permease (lac Y) and prevents entry of lactose into the cell
- When there is no glucose crossing the cell membrane, EIIA is phosphorylated and lac Y (permease) is active and lactose can enter cell. Phosphorylated EIIA activates Adenyl Cyclase (AC) and increase level of cAMP.

- The transport of glucose into the cell dephosphorylates EIIA. Dephosphorylated EIIA inhibits lac Y (permease) and hence stops entry of lactose into cell. Dephosphorylated EIIA cannot activate Adenyl Cyclase and hence there is low cAMP.

Mutation in lac operon

1. High level of expression of the lac operon
    a. I^- - Inactive repressor (mutated) cannot bind to operator
    b. O^- (O^c) - Mutation in the operator, does not allow repressor to bind
2. No induction of lac operon
    a. I^s- Inducer cannot bind to repressor, hence repressor permanently binds to operator
    b. P^-- mutation in promoter does not allow RNA polymerase to bind

Tryptophan operon

- **Purpose**
    - Repression of enzyme synthesis
        - If an organism requires a particular molecule that is not available 'externally', then it must synthesise this molecule to survive.
        - However, if the molecule is available or sufficient, then it is most economical for the organism the organism to repress synthesis of enzymes responsible for synthesis of that molecule.
        - Eg: Biosynthesis of amino acids in E. coli.