Genetic Regulatory Mechanisms in the Synthesis of Proteins

François Jacob and Jacques Monod. ***Journal of Molecular Biology*** **(1961) 3: 318-356**

" In describing genetic mechanisms, there is a choice between being inexact and incomprehensible. In making this presentation, I shall try to be as inexact as conscience permits"

- Professor Sven Gard in Nobel Prize presentation speech.

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Author Biographies

In 1965, Jacob and Monod, shared along with André Lwoff, the Nobel Prize in Physiology or Medicine "for their discoveries concerning genetic control of enzyme and virus synthesis".

Francois Jacob
Born 1920 in Nancy, France.
Completed M.D. degree (1947) at Faculty of Paris, and a obtained doctorate in Science (1954) at the Sorbonne.
Joined Institute Pasteur as research assistant (1950), under Dr. André Lwoff.
Appointed Laboratory Director (1956) and Head of Dept. of Cell Genetics (1960).
Began collaboration with Monod (1958) at Institute Pasteur.
In 1964 became a Professor at the College de France and held chair of Cell Genetics.

Jacques Monod
Born 1910 in Paris, France. Died in 1976.
Lectured at Faculty of Science in Paris (1934) and spent time at Cal Tech (1936).
Obtained doctorate in Natural Sciences (1941) at Faculty of Paris.
Joined Institute Pasteur as Laboratory Director (1945) with André Lwoff.
Appointed Director of Cell Biochemistry Dept. (1954).
Began collaboration with Jacob (1958) at Institute Pasteur.
Professor at the College de France (1967).
Appointed Director of Institute Pasteur (1971).

Jacob and Monod proposed a series of **new concepts** in their paper, including, **messenger RNA**, **regulator/operator genes**, and **operons**.

The Operon Form of Gene Regulation

Operon: A group of genes which are transcribed together as a single *mRNA*.

Two types of genes found on operons:
Structural genes: Code for proteins and *RNA* molecules required for normal enzymatic functions in the cell.
Regulator genes: Code for proteins and *RNA* molecules which regulate the expression of structural genes.
Regulator genes are considered **trans-acting** since their activity can occur at a site other than where they are located ie. at another DNA molecule (as opposed to **cis-acting** where activity must occur on the same DNA molecule)

There can also be repressors and inducers in an operon:
Repressor: A protein produced by a regulator gene which binds to a site on the operon to prevent transcription of structural genes.
Inducer: A metabolite which prevents the repressor from binding to the DNA so that the structural genes can be transcribed.
The site at which the repressor binds is known as the **operator**.

The Lactose Operon

Structural genes:

lacZ - codes for **β-galactosidase**

lacY - codes for **β-galactosidase permease**

lacA - codes for **thiogalactoside transacetylase**

Regulator genes:
lacI - codes for the **lactose repressor**

Also the lactose operon has an **inducer** - **allolactose**

An overview of the *lac* operon

Genetic Analysis of the *lac* operon

Jacob and **Mo**nod along with **Pa**rdee studied various mutations in order to determine how regulation of the operon works.
This experiment was called the PAJAMO experiment, named after the three scientists.

In the PAJAMO experiment:
- Two strains of *E. coli* were used.
- One carried the **wild type** *lac* operon, a gene making it **sensitive** to streptomycin, and was a **donor** cell.
- The other carried the **mutation** of interest, a gene making it **resistant** to streptomycin and was an **acceptor** cell.
- Strains were mated and allowed to grow in medium containing streptomycin
- β-galactosidase synthesis was observed with and without inducer to determine relation of mutated gene to wild type gene.

Jacob and Monod looked at two types of mutations:

Constitutive: Mutations in which the production of β-galactosidase cannot be repressed.

Non-inducible: Mutation in which the production of β-galactosidase cannot be induced.

Looking at the mutations in order of the region of DNA they affect:

1. A 'faulty' regulator gene: I^-

In this type of mutation ***lacI*** cannot produce any repressor.

Without inducer: Since no repressor is made to bind to the operator, RNA polymerase can transcribe the structural genes, β-galactosidase is synthesized.

With inducer: There is no repressor for the inducer to bind to so there is synthesis of β-galactosidase.
This kind of mutation is **constitutive.**

In partially diploid cells: The functional ***lacI*** $^+$ produces repressor while ***lacI*** $^-$ does not. This repressor works transitively to bind both operators.

Without inducer: no β-galactosidase is synthesized.

With inducer: it binds to the repressor and β-galactosidase is synthesized.

Relation observed:
I^+ is dominant to I^-

How this mutation affects the *lac* operon

2. A dysfunctional operator: O^c

In this type of mutation the **operator** is changed so that the repressor cannot bind as well. However there is still some binding by the repressor depending on the severity of the change.

Without inducer: Some repressor can still bind in small amounts resulting in minimal synthesis of β-galactosidase.

With inducer: The inducer binds to all repressor synthesized so that β-galactosidase is synthesized in high amounts.
This kind of mutation is **constitutive**.

In partially diploid cells: Repressor binds to the O^+ operator, and only partially to the O^c operator.

Without inducer: there is β-galactosidase synthesized from operon with O^c.

With inducer: the level of synthesis increases as repressors are unable to bind either operator and both operons synthesize β-galactosidase.

Note: If the *lacZ* gene next to the O^c is mutated in this case, then no β-galactosidase will be synthesized since O^+ is repressed and *lacZ*$^-$ will not produce

β-galactosidase When inducer is added, the repressor is removed from both operators and the functional *lacZ* will produce β-galactosidase.

Relation observed:
O^c is dominant to O^+ (But only in a **cis-acting** form)

How this mutation affects the *lac* operon

3. A repressor which cannot bind to the operator: I^{-D}

In this type of mutation repressor is still made from the ***lacI*** gene, but it is unable to bind to the operator. This will not block the transcription of structural genes.

Without inducer: Since the repressor cannot bind to the operator, β-galactosidase is still synthesized.
With inducer: Whether inducer binds to repressor or not makes no difference as repressor still doesn't bind to the operator. There is still synthesis of β-galactosidase.
This kind of mutation is **constitutive**.

In partially diploid cells: Functional repressor made from I^+ will begin to bind to the operator. Since the repressor is transcribed as a monomer, but forms a tetramer, non-functional repressor made from I^{-D} will begin to form tetramers with functional repressor and cause them to become non-functional.

Relation observed:
I^{-D} is dominant to I^+

View an image of this mutation

4. A repressor which cannot bind the inducer: I^S

The **S** stands for **Super-repressor**! Since this mutation is unable to bind to the inducer, it is **permanently bound to the operator** resulting in no synthesis of β-galactosidase.

Without inducer: Repressor is bound to the operator and transcription is blocked.
With inducer: Regardless of inducer added, repressor is bound to the operator and transcription remains blocked.
This type of mutation is **non-inducible**.

In partially diploid cells: Repressor made from I^+ and I^S will bind to operators in non-induced systems. When inducer is added, repressor from I^+ will leave the operator, but the I^S repressor will take its place. There will be no β-galactosidase synthesized with or without inducer.

Relation observed:
I^S is dominant to I^+

How this mutation affects the *lac* operon

Conclusions

In their original paper, Jacob and Monod had to introduce many speculative assumptions in their paper in order to come up with model for the specific control of protein synthesis.

In any case, they came up with several experimentally established conclusions from their speculations:
1. The existence of **regulator genes**.
2. **Regulator gene** acts via the specific cytoplasmic substance (ie. **repressor**) whose role is to **inhibit** expression of structural genes.
3. Product of regulator gene acts directly as **repressor** (rather than indirectly).
4. Chemical identification showed **repressor** is a **RNA fraction** not a protein. *
5. Existence of **operator** as site of action for repressor.
6. Interactions of **repressors** with **inducers** or co-repressor.
7. **Structural messenger** is an unstable intermediate. *

(* We did not discuss the details of these in this presentation)

Operon theory accounted for many unexplained observations and conflicts in classic genetic theory.
Did not apply to organisms other than bacteria or even all bacteria systems.

"The discovery of regulator and operator genes, and of repressive regulation of the activity of structural genes, reveals that the

genome contains not only a series of blue-prints, but a co-ordinated program of protein synthesis and the means of controlling its execution."

- Jacob and Monod 1961

Links of Interest

Biology 2250 Lecture Notes - Dr. Steven Carr
Biochemistry 3107 Lecture Notes - Dr. Martin Mulligan
Biochemistry 4103 Lecture Notes - Dr. Martin Mulligan
Modern Genetic Analysis

Questions or Comments? Please email Corinne Wilkerson or Jennifer Slade.

PAJAMO Experiment

Arthur **Pa**rdee, François **Ja**cob and Jacques **Mo**nod

Experiment was key to understanding induction of **β-galactosidase**.

Looked at expression of lactose operon in a partially diploid cell, in particular, whether **I $^{-}$** or **I $^{+}$** was dominant.
Proved Monod's "internal inducer" hypothesis wrong.

Genes

lacZ $^{+}$ (*can* synthesize **β-galactosidase**)

lacZ $^{-}$ (*cannot* synthesize **β-galactosidase**)

lacI $^{+}$ (no internal inducer) fully inducible by external inducer

lacI $^{-}$ (with internal inducer) already partly induced

Two strains of E. Coli

Donor strain (F^{+} or Hfr) is **$Sm^{S}T6^{S}$** (**sensitive** to streptomycin and bacteriophage T6).

Recipient strain (F^{-}) is and **$Sm^{R}T6^{R}$** (**resistant** to streptomycin and bacteriophage T6).
Synthesis of β-galactosidase could only be measured in recipient strain.

Experiment 1

Z $^{+}$ I $^{+}$ --> Z $^{-}$ I $^{-}$

Expected that *if* I $^{-}$ is dominant, and *if* I $^{-}$ provokes synthesis of internal inducer,
then **β-galactosidase** will be synthesized immediately (i.e. as soon as *lacZ* $^{+}$ gene enters recipient).

This was observed, BUT...

Experiment 2

Z $^{-}$ I $^{-}$ --> Z $^{+}$ I $^{+}$ (reverse of Exp 1)

Expected that *if* no external inducer present,
then no **β-galactosidase** will be synthesized until ***lacI*** $^{-}$ is transferred to the recipient and starts to make an internal inducer.

This was not observed, β-galactosidase was only made if an external inducer was added.

Important Findings

1. The **lacZ** gene, which codes for **β-galactosidase**, is expressed very fast and maximum from the beginning but it soon levels off and stops.
   Further synthesis requires addition of external inducer.

2. ***lacI*** $^{+}$ is dominant to *lacI* $^{-}$
   lacI $^{-}$ does not code for an internal inducer.

These findings led to the following ideas :

The **inducer** does not provoke synthesis of the enzyme, rather it inhibits synthesis of the "**repressor**" (i.e.. negative control mechanism).
The **"repressor"** is responsible for regulating expression of β-galactosidase and lactose permease.
The **"repressor"** is the product of the ***lacI*** gene and its function depended on the presence or absences of the external inducer.
