Jacques Monod and the Advent of the Age of Operons

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"In Science, self satisfaction is death. Personal self satisfaction is the death of the scientist. Collective self satisfaction is the death of research. It is the restlessness, anxiety, dissatisfaction, agony of mind that nourish Science".

The profound statement cited above is one of the many famous quotes of Jacques Lucien Monod (1910–1976) who was unquestionably one of the great minds who laid the foundations of molecular biology in the middle of the 20th century. This year marks the centenary year of his birth and 2011 will be the Golden Jubilee year of the advent of the 'Operon' concept. The trio (Jacques Monod, Francois Jacob and André Lwoff) from the Institut Pasteur, Paris, shared the 1965 Nobel Prize in Physiology and Medicine for their "discoveries concerning the genetic control of enzyme and virus synthesis". The 'operon' hypothesis which Jacob and Monod postulated in 1961 after nearly 25 years of work, carried out in war-torn France, is hailed as the "last great discovery of molecular biology"[1]. "Even today, the concepts developed by Jacques Monod during his career remain at the core of molecular biology" [2]. The two major contributions of Monod are: (i) elucidation of the mechanism of regulation of gene expression (the operon concept) and (ii) allosteric behaviour of proteins through conformational transitions. In this article, I will focus on the first, more because of personal competence than any other reason. Monod himself considered his contributions on allostery to be more significant than the operon model. He is reported to have surprised his colleagues one day by saying that he had discovered the "second secret of life".

Monod's ideas on the philosophy of biology are enshrined in his book which appeared in French (*Le Hassard et la Necessite*) and also in English (*Chance and Necessity*). Both were bestsellers. In this article I will focus mainly on the lactose operon which is by

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far the most extensively studied system. The purpose of this article is not to reproduce all the information on operons, readily available in textbooks, but to trace the development of the concept from a historical perspective and try to show how simple and elegant experiments coupled with logical reasoning resulted in the advent of a new era of investigation in molecular biology, namely, regulation of gene expression.

Induced Enzyme Synthesis: The Forerunner of the Operon Model

Monod's epoch-making work started in the early 1940s during the dark years of World War II and continued till 1971 when he became the Director of the Institut Pasteur, Paris. The topic 'growth of bacterial cultures' on which Monod worked for his doctoral thesis and which ultimately led him to the glory of a Nobel Prize, was not very fashionable, even in those days. After Monod defended his thesis, the Director of his institute is reported to have remarked "Monod's work is of no interest to the Sorbonne" [2].

The main observation which ultimately blossomed into the Operon model and immortalised his name was the pattern of growth of *Escherichia coli*, on a mixture of two sugars (glucose and lactose or glucose and mannose, etc.). He observed two distinct phases of exponential growth intervened by a short period of stasis. He called this 'diauxic growth' (*Figure* 1). When Monod discussed this puzzling observation with his teacher and colleague, André Lwoff, the latter casually remarked that it could have something to do with "enzymatic adaptation", a term which Monod had not heard before! This was towards the end of 1940. Soon afterwards, Monod's work was interrupted by the War and he could resume his work only after the War ended, although he kept working clandestinely in between in André Lwoff's laboratory.

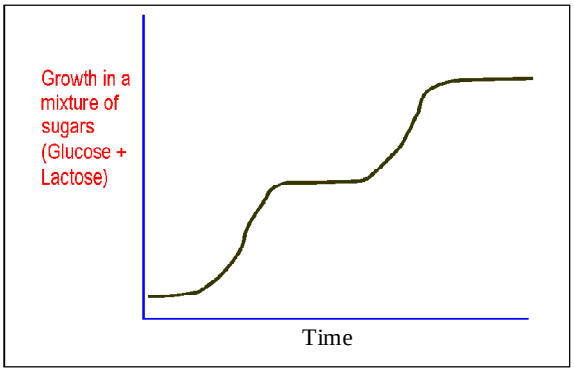

Figure 1. Diauxic growth curve.

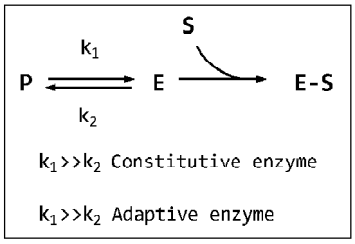

Figure 2. Adaptive versus constitutive enzymes.

The phenomenon called enzymatic adaptation has been around since the beginning of the 20th century but the underlying mechanisms were unknown. The early microbiologists (Declaux, Dinert, Went *et al*) knew that some enzymes could be detected in bacteria only when the cells were grown in the presence of their substrates while some others could be detected irrespective of the presence or absence of substrates. This was particularly true of enzymes involved in the catabolism of sugars. In 1938, Karstrom classified the sugar catabolic enzymes as *adaptive* (found only in the presence of substrates) and *constitutive* (found in the presence or absence of substrates). The same year, Yudkin proposed that enzymes existed in equilibrium between two forms, namely, the (inactive) precursor form (P) and the (active) enzyme form (E). In the case of the so-called adaptive enzymes the equilibrium was in favour of the P-form in the absence of substrates. In the presence of substrates, the formation of the enzyme–substrate complex (ES) shifted the equilibrium towards the E-state (*Figure* 2). This was the state of knowledge on enzymatic adaptation in 1948 when Monod resumed active work and decided to explore the phenomenon of diauxic growth in greater detail.

It seemed possible that the period of stasis separating the two growth phases could be the time taken to produce some enzyme(s) needed for the utilisation of the second sugar, after the first one was used up. In the case of a glucose–lactose mixture, glucose could be utilised first and lactose later. Therefore enzyme adaptation could actually be a case of enzyme induction by the substrate which could be an inducing agent as well as a substrate. (It turned out later on that this need not be true always; see below). It occurred to Monod that an important requirement was to find a suitable experimental system in which the activity of the concerned enzyme could be assayed easily and reliably. The enzyme β-galactosidase which hydrolyses β-galactosides such as lactose into the constituent sugars (glucose and galactose) appeared promising. A substrate that generates a coloured product upon hydrolysis would be useful in measuring enzyme activity. Such a

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substrate, o-nitrophenyl-β-D-galactoside (ONPG) which yields yellow-coloured o-nitrophenol upon hydrolysis, was introduced by Lederberg just then. Using ONPG to measure the level of enzyme activity, Cohn and Monod showed that *Escherichia coli* grown on lactose as the sole source of carbon had >1000-fold more β-galactosidase activity than when grown on other carbon sources. Therefore lactose seemed to act as an inducer of β-galactosidase by some mechanism.

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As a first step to unravel this phenomenon, Monod and co-workers purified the enzyme from *Escherichia coli* and raised an antiserum against the pure protein. Thus it was possible to follow the appearance of β-galactosdase both by following enzymatic activity (ONPG hydrolysis) and the protein (even if inactive) immunologically. The first major observation to emerge was that induction involved new (*de novo*) protein synthesis. Another significant observation to emerge at this time was that all inducers need not be substrates and all substrates need not be inducers. For instance, synthetic β-galactosides such as thiomethyl-β-D-galactoside (TMG) or isopropyl-β-D-thiogalacoside (IPTG) are excellent inducers but are not substrates; they are actually competitive inhibitors of the enzyme and act as gratuitous inducers. Similarly, compounds such as ONPG and phenyl-β-D-galactoside are good susbstrates but are not inducers. These two observations disproved the then prevalent hypothesis that induction involved the interaction between the inducers (substrates) with the inactive precursor form of the enzyme. By this time, Monod's laboratory had gained international reputation and people from the world over joined him. Together they made several important contributions towards understanding the mechanism of induced enzyme synthesis.

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Genetic Analyses of *lac* Mutants

Once the biochemical approach to understand diauxie was exhausted, Monod realized that further progress could be achieved only by employing a genetic approach. Monod's colleagues isolated several mutants of *Escherichia coli* altered in lactose

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utilisation and also used several already available ones isolated by Lederberg. Wild-type cells of *Escherichia coli* can grow in media containing lactose as the sole source of carbon and energy. These are called Lac^+ cells. Mutants which cannot do so are called Lac^-. Soon it was discovered that Lac^- mutants are of two types: those which cannot synthesise enzymatically active β-galactosidase and therefore cannot utilise lactose for growth and those which could synthesise the enzyme but still cannot utilise lactose. The latter class was called 'cryptic' mutants at that time. It was thought (rightly so, as it turned out later) that cryptic mutants were defective in the uptake (transport) of lactose or other inducers such as TMG from the medium into the cells. Fortunately, around this time, radioactively-labelled compounds became available for use in metabolic experiments. It was found that wild-type (Lac^+) cells could accumulate labelled TMG; however, uninduced wild-type cells or the cryptic mutants could not. This showed that a specific protein was necessary for the transport of TMG (or lactose) across the cell membrane. This protein was named lactose 'permease' and the gene responsible for its synthesis was designated as *lacY*. The gene encoding β-galactosidase was named *lacZ*. (In retrospect, one could wonder how the story would have turned out had they used IPTG which is not obligatorily dependent on the permease for transport)!

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The discovery of permease is very significant because for the first time a specific protein was implicated in the transport of a small molecule. Subsequently, Georges Cohen established that permeases play a vital role in metabolism by showing that amino acid transport is permease-mediated. Since uninduced wild-type cells synthesised neither β-galactosidase nor permease whereas induced cells synthesised both, the *lacZ* and *lacY* genes are regulated coordinately, a key feature of the operon concept. Monod did not succeed in his attempts to purify the permease but in the process isolated another enzyme, trans-acetylase, whose synthesis is also coordinately regulated along with *lacZ* and *lacY*. The gene for the transacetylase was named *lacA*; its physiological role still remains unknown. Thus it emerged that the three genes

(*lacZ, lacY* and *lacA*) constitute one block of expression. This finding was a major breakthrough. (From now on the present article will focus only on *lacZ* and *lacY*).

Conjugation using F′ factors were brilliantly exploited by Monod in his experiments.

Around this time two major advances occurred in the genetics of *Escherichia coli* through the pathbreaking work of Joshua Lederberg, William Hayes, Francois Jacob and Elie Woolman. The first was the discovery of bacterial conjugation which involved the transfer of parts of the chromosome from a donor (male) bacterium into the cytoplasm of a recipient (female) cell through physical contact between the two. The donor which can do this very efficiently is called an Hfr cell and the recipient is denoted as F^- cell. The second was the discovery of what are known as F-prime (F′) factors which are derivatives of the conjugative (transferable) plasmid called the F factor. The F′ factors contain a small fragment of chromosomal DNA in the place of their own DNA (type-I F′s) or in addition to their own DNA (type-II F′s). Conjugation using F′ factors were brilliantly exploited by Monod in his experiments, as we shall see below.

An F′ factor, say F′ *lacZ lacY*, can be transferred into an F^- cell simply by mixing cultures of the donor and recipient. Once transferred, the F′ will establish a partial diploid status (merodiploidy) between the chromosomal *lac* genes and the F′-borne *lac* genes (F^-*lac* / F′*lac)*. By choosing appropriate allelic combinations in the relevant genes in the donor and the recipient (the *lac* genes in this case) the dominance–recessive relationship between mutations can be inferred by looking at the phenotypes of the merodiploids. Using this simple yet powerful technique Monod and coworkers made some remarkable discoveries. For example a merodiploid of the type F^- *lacZ*$^-$ *lacY*$^+$ / F′*lacZ*$^+$*lacY*$^-$ was phenotypically Lac$^+$. Likewise, a merodiploid of the type F^- *lacZ*$^+$ *lacY*$^-$ / F′ l*acZ*$^-$ *lacY*$^+$ was also Lac$^+$. Note that in both cases the recipient was Lac$^-$, the first due to inability to synthesise active β-galactosidase and the second because of inability to transport lactose. Both the defects could be complemented in trans by the wild-type alleles carried on the donor F′. Thus *lacZ*

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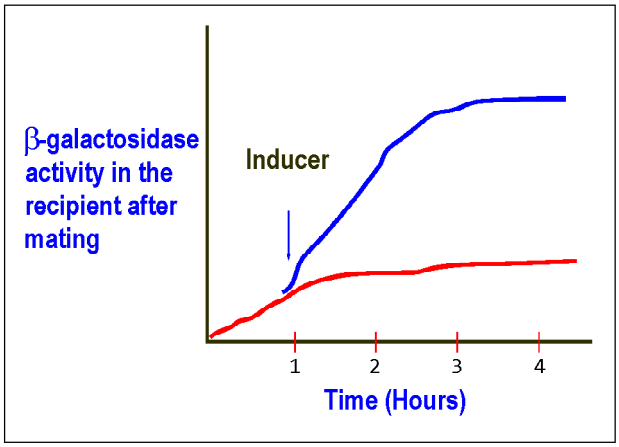

Figure 3. The PaJaMo experiment.

and *lacY* (also *lacA*) define 'structural' genes, mutations in which are (usually) recessive to their wild-type alleles.

The Concept of Regulatory Genes

Another major landmark event in the story is the discovery of regulator(y) genes that control the expression of other (structural) genes. Among the several mutants Monod and associates had collected, there were some in which *β*-galactosidase (as well as permease and transacetylase) were synthesised constitutively, that is, even in the absence of inducers. These were called *lacI*$^-$ (constitutive) mutants as against the wild-type (inducible) parent which was called *lacI*$^+$. It was thought initially that the *lacI*$^-$ mutants produced an internal inducer such that no externally added inducer was necessary for induction. That this was not the case was shown by an elegant experiment done by Pardee, Jacob and Monod in 1959. This experiment has gone down in history as the PaJaMo experiment (*Figure* 3) (sometimes called the Pajama experiment in a lighter vein!). It involved a conjugational cross between an Hfr donor and an F$^-$ recipient wherein the donor's chromosome (usually parts of it) is transferred into the recipient cytoplasm. In a cross of the type:

Hfr *lacI*$^+$ *lacZ*$^+$ Str^s T6^s X F$^-$ *lacI*$^-$ *lacZ*$^-$ Str^r T6^r,

the donor is inducible and the recipient is genetically constitutive but makes inactive *β*-galactosidase due to the *lacZ*$^-$ mutation. Str^{sr} and T6$^{s/r}$ refer to sensitivity/resistance to sreptomycin and bacteriophage T6, respectively. A short time after mixing the donor and recipient, streptomycin and phage T6 in the form of a lysate were added to kill and lyse the donor cells and *β*-galactosidase activity was measured at intervals. It was found that the enzyme activity could be detected within a couple of minutes, which continued to rise for about 60 minutes and then levelled

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off. If an inducer (IPTG) was added at this point of time, the enzyme activity cotinued to rise for several minutes.

The PaJaMo experiment was extremely significant and, as Ullmann notes [2], was a "point of departure" for proposing the Operon Model a few years later. Briefly, it showed that when the donor *lacZ*$^+$ gene entered the cytoplasm of the *lacI*$^-$ *lacZ*$^-$ recipient it was expressed immediately because the recipient had no *lacI* gene product to start with. At the same time the product of the donor *lacI*$^+$ gene also accumulates in the recipient and after an hour or so shuts off constitutive expression. At this point addition of inducer becomes necessary for continued synthesis of the enzyme. This showed that the product of the *lacI* gene shuts off the expression of *lacZ*, in a sense acting as an inhibitor of expression. Therefore it was named 'repressor'. The role of the inducer seemed to be 'inhibition of an inhibitor'. Cohn and Monod jocularly called this the 'theory of double bluffs', two negatives making a positive [3]. The control of *lac* gene expression is thus a negative control. (Positively controlled expression systems, exemplified by the regulation of the genes of the arabinose utilisation pathway, were discovered later on). The PaJaMo experiment led to the emergence of three basic concepts in the regulation of gene expression, namely, concepts of a repressor, negative control and messenger RNA. (The last could be inferred from the fact that constitutive expression levelled off after some time implying whatever was responsible was an unstable entity. This led to the postulate of messenger RNA being an intermediate in the transfer of information from DNA to protein).

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While *lacI*$^-$ mutations generally lead to constitutive expression of the three genes (*lacZ*, *Y* and *A*) some *lacI* mutants were negative for the induction of all the three genes. These were called *LacI*^{s} (super-repressed) mutants. Merodiploid analyses showed that *lacI*$^+$ was dominant over *lacI*$^-$ but recessive to *lacI*^{s}, that is, *lacI*$^+$/ *lacI*$^-$ = I^+ and *lac* I^+ / *lac* I^s = I^s. In this case, the mutation turned out to be in the inducer-binding region of the repressor, excluding the interaction of the inducer with the repressor.

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The Operator: Site of Action of the Repressor

If Lac I, the repressor, turns off expression of the lactose utilisation genes, there must be a chromosomal site at which it acts to do so. Monod reasoned that if there is such a site, a mutation in that site inhibiting the interaction with the repressor should result in constitutive expression, analogous to a *lacI*$^-$ mutation. Monod named the putative site as Operator and the constitutive mutants as O^c mutants.

There is a fundamental difference in the constitutivity due to *lacI*$^-$ and *lacO*^{c} mutations: the *lacI* gene, in its own right, is a structural gene giving rise to a diffusible product which can act in *trans* as a repressor; therefore a mutation in it should be recessive to the wild-type allele. On the other hand, the putative *lacO* is a site at which the repressor acts; a mutation in it (O^c) should be dominant over O^+ since providing a good site cannot overcome the effect of a defective site. One important point to remember though is that the dominance of the *lacO*^{c} mutations can be exerted only on the *lacZ lacY* genes located downstream on the same DNA segment; a phenomenon known as *cis*-dominance.

If the hypothesis on the existence of the operator is correct, it should be possible to isolate *cis*-dominant constitutive mutants. Monod and coworkers obtained such mutants by selecting for constitutive mutants (colony formers on media having phenyl-β-D-galactoside as the sole source of carbon) from a merodiploid harbouring two copies of *lacI*$^+$ *lacZ*$^+$, one on the chromosome and one on an F′. As noted earlier, phenyl-β-D-galactoside is a substrate but not an inducer. Therefore only constitutive mutants will be able to utilise it in the absence of an inducer. The use of a strain having two copies of *lacI* will avoid selection of *lacI*$^-$ constitutives since they will be recessive to the I^+ allele and hence cannot grow on phenyl-β-D-galactoside. (The O^c mutants are weakly constitutive relative to I^- mutants).

Monod originally thought that the operator is the site of action of not only the repressor but also the site where the transcribing

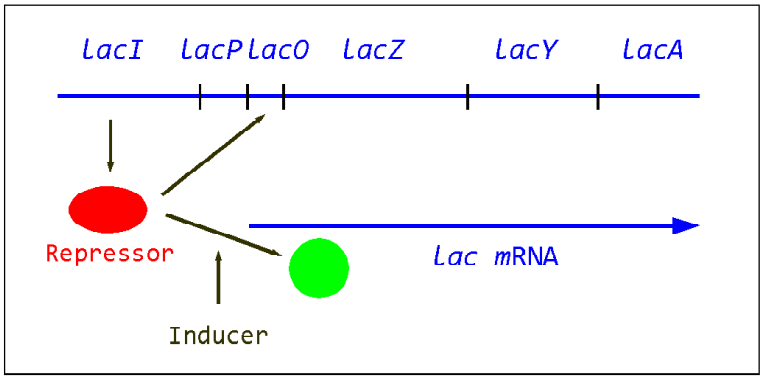

Figure 4. The lactose operon.

enzyme (RNA polymerase) could bind and transcribe the three genes. It is therefore logical to expect that a mutation in *lacO* which allows interaction with the repressor but inhibits the binding of RNA polymerase will be Lac$^-$. He named such mutants as O^0 mutants. Indeed such mutants were isolated but it turned out subsequently that the mutational lesion in the so-called O^0 mutant was not in *lacO* but very close to the beginning of the *lacZ* gene. Its pleiotropically negative phenotype is due to a phenomenon called polarity. (The mechanism of polarity is beyond the scope of this article). Subsequent work by Jonathan Beckwith and coworkers led to the identification of a distinct site (the Promoter) located between *lacI* and *lacO*.

Monod also believed that the Lac repressor could be RNA although its interaction with a ligand such as IPTG suggested it could be a protein. This problem was left unresolved when the Operon Model was proposed by Jacob and Monod in 1961 [5]. Subsequently, many lines of evidence accumulated suggesting that the Lac repressor could be a protein. The issue was finally settled in 1966 when Walter Gilbert and Benno Muller-Hill isolated the Lac repressor protein. Thus was born the concept of Operon which has influenced almost every branch of biology (*Figure* 4).

Repressible Systems of Gene Expression

While proposing the operon model, Jacob and Monod considered two other systems in which the control mechanisms are identical.

One is the biosynthesis of amino acids and the other is the maintenance of the lysogenic or dormant state by bacterial viruses that infect the host bacterium. A brief description of these two is presented below.

Wild-type cells of *Escherichia coli* do not have β-galactosidase and its synthesis has to be induced. On the other hand, wild-type cells growing in a glucose-ammonium salt minimal medium have to meet all their nutritional needs from the available sources of carbon and nitrogen. Therefore the genes for the enzymes of all anabolic pathways are expressed in such cells. However, even if a single nutrient, say tryptophan, is readily available from the medium, it would be a wasteful exercise to express the genes involved in tryptophan biosynthesis. Therefore they are shut off by the addition of tryptophan. This phenomenon is called enzyme repression, as opposed to enzyme induction. Monod pointed out that both induction and repression are mechanistically identical but work in opposite directions. (In order to avoid confusion Monod suggested that compounds such as tryptophan, arginine, etc., which turn off their respective biosynthetic pathways be called co-repressors). Just as an inducer like IPTG turns on the expression of three genes of the lactose pathway, a co-repressor like tryptophan turns off the expression of all the five genes involved in tryptophan synthesis. In some mutants, tryptophan (the co-repressor of the *trp* genes) does not turn off the expression of the concerned genes; they are constitutively expressed (analogous to $lacI^-$ mutations). In some other mutants, the genes of the pathway always remain shut off; that is, there is no need for a co-repressor to effect repression (analgous to $lacI^s$ mutations). Thus the control mechanisms are identical bur their directions are opposite to each other.

The Lactose Operon and Lysogeny: A Common Paradigm

Monod realised that the basic features of their model of regulation of gene expression could be extrapolated to a totally unrelated phenomenon, namely, maintenance of the lysogenic state. In lysogeny, the chromosome of a temperate phage such as λ is

stably integrated into that of the host (*Escherichia coli*) as a 'prophage'. A cell carrying an integrated prophage is called a lysogen. The potentially lethal genes of the phage are kept repressed in the prophage. However, they can be triggered into expression resulting in the excision of the prophage, expression of the phage genes, production of progeny phage, lysis and death of the cell.

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There is a remarkable similarity between the lactose operon and the lysogenic state. In the case of the phage λ, almost all of the viral genes are shut off by the repressor encoded by the *cI* gene in a lysogen. Only the CI repressor is expressed in this state and any additional λ virus infecting the lysogen will also be shut off, a phenomenon known as 'immunity to superinfection'. The lysogenic state can be de-repressed by treatment such as exposure to ultra-violet radiation that leads to the inactivation of the CI repressor. In addition, *cI* mutants show inability to form lysogens and are 'constitutively' lytic. Operator sites at which the CI repressor acts have also been identified and mutations within such sequences show *cis*-dominance similar to the O^c mutations in the *lac* operon. There is a point-to-point similarity between the two unrelated gene control systems. The operon model is thus not a specific mechanism operating to control the expression of only the *lac* genes. The same type of similarity can also be drawn for a repressible system, typified by the *trp* operon and also many other amino acid biosynthetic operons.

Epilogue

Nearly half a century has rolled by since the Operon Model was proposed in 1961. The model has undergone enormous refinements and advances in the subsequent years, but its core features remain the same. Some of the later developments include the isolation of the CI repressor by Mark Ptashne simultaneously with the Lac repressor, discovery of the involvement of cyclic AMP and its receptor protein, not only in *lac* gene expression but several others, multiple operators in many operons, discovery of positively regulated operons, validation of the mRNA concept,

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dissection of the intricacies in repressible (as opposed to inducible) operons, complexities of gene regulation in eukaryotes, etc. But the concept of operons as proposed by Monod is at the root of almost every later advancement. Nowadays words like operons, operators, repressors, negative regulators, positive regulators, transcription factors with fanciful names, have become familiar words even in the undergaduate/high school classrooms. Descriptions of a variety of operons with all their myriad molecular details have become textbook material. Present-day students of molecular biology may reel out such details of any operon at the wink of an eye but might be ignorant as to how the core ideas were developed by Monod. It is hoped that this article will tempt at least a few students to read, at least once, the classic paper of Jacob and Monod.

Suggested Reading

[1] **M Morange, The scientific legacy of Jacques Monod, *Research in Microbiology*, Vol.161, pp.77–81, 2010.**

[2] **A Ullmann, Jacques Monod, 1910–1976: his life, his work and his commitments, *Research in Microbiology*, Vol.161, pp.68–73, 2010.**

[3] **J Monod, From enzymatic adaptation to allosteric transitions. Nobel Lecture, *Science*,Vol.154, pp.1475–1483, 1966.**

[4] **F Jacob and J Monod, Genetic regulatory mechanisms in the synthesis of proteins, *J. Mol. Biol.*, Vol.3, pp.318–356, 1961.**

[5] **R Y Stainer, Obituary : Jacques Monod, 1910– 1976, *J. Gen. Microbiol.*, Vol.101, pp.1–12, 1977.**

[6] **A brief account of Monod's life can be found in the commemorative essay by one of his long-time associates, Agness Ullmann [2]. A detailed account of his life, work, achievements and personality can be found in the obituary article by Roger Stainer [5]. The Nobel Lecture of Monod gives an account of his life and work in his own words [3]. See also *Resonance*, July 2003.**

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