

Lysogenic Bacteria as an Experimental Model at the Pasteur Institute (1915-1965)

Lysogenic bacteria, or virus-infected bacteria, were the primary experimental models used by scientists working in the laboratories of the Pasteur Institute in Paris, France, during the 1950s and 1960s. Historians of science have noted that the use of lysogenic bacteria as a model in microbiological research influenced the scientific achievements of the Pasteur Institute's scientists. François Jacob and Jacques Monod used lysogenic bacteria to develop their operon model of gene regulation, to investigate the cellular regulatory mechanisms of the lysogenic life cycle, and to infer the process of cellular differentiation in the development of more complex eukaryotes.

A lysogenic bacterium is a bacterium infected by a phage, or virus, called a bacteriophage. There are two phases of bacteriophagy: the lytic bacteriophage and the lysogenic bacteriophage. A bacteriophage can be in either phase depending on its environment. A lytic bacteriophage infects bacterial cells and reproduces its genetic material through the lytic life cycle, or the process by which the phage lyses the cell, which disintegrates the host cell. The process of lysis, or the lytic cycle, begins when a virus attaches itself to a receptor on the bacterial cell wall and inserts its DNA into the bacterial cell. The circular genetic material of the virus, which is composed of either RNA or DNA, then cleaves the host cell's DNA through the action of virus enzymes. The viral genome uses the host cell's resources to synthesize capsid proteins, or protein shells that protect the virus's nucleic acid, within the bacterium. The cell replicates the bacteriophage's linear double-stranded DNA, making many copies of the DNA that are packaged into the capsids. A phage enzyme then lyses the cell, killing the host cell, and the progeny phages disperse to infect other bacterial cells. The bacteriophages are in the lytic cycle typically when the host bacteria are in an environment in which the bacteria can reproduce at a constant rate. In such conditions, there are no shortages of host cells for the bacteriophage to infect.

When environmental conditions become unfavorable for the bacteria, the bacteriophage begins a lysogenic life cycle, during which the virus does not immediately destroy the host bacterial cell. A lysogenic bacteriophage is a virus that infects bacterial cells, but incorporates its DNA into the host cell's DNA to become a non-infectious phage, called a prophage. Consequently, a lysogenic bacteriophage is sometimes called a temperate bacteriophage, rather than a virulent bacteriophage. During the lysogenic cycle, when the virus has inserted its DNA within the bacterial cell, the viral DNA incorporates itself into the host cell's genetic material. The viral DNA is then replicated with the rest of the genetic material of the host cell as the cell reproduces itself. However, temperate or lysogenic bacteriophages maintain the ability to switch to the lytic cycle when conditions are favorable for the virus. Thus, the system contains a regulatory mechanism by which bacteriophages can modify their method of replication under the appropriate environmental conditions.

Frederick W. Twort from the UK and Félix d'Hérelle from France independently discovered bacteriophagy, or the phenomenon of a virus-infected bacterium, in 1915 and 1917 respectively. D'Hérelle, a French-Canadian working at the Pasteur Institute in Paris, hypothesized that a microbe caused lytic and lysogenic bacteria. In 1920, Jules Bordet, the director of a Pasteur Institute in Brussels, Belgium, challenged d'Hérelle's hypothesis. Contrary to d'Hérelle, Bordet argued that lysogenic bacteria were a particular strain of bacteria that had acquired the ability to lyse other bacteria by producing in excess a type of enzyme that could dissolve the cell walls of surrounding bacteria. In some instances, he claimed, these bacteria would overproduce the enzyme and lyse themselves. An individual bacterium that became immune to the effect of the enzyme gained a competitive advantage over its neighbours and passed on the immunity to its progeny. Thus, according to Bordet,

lysogenic bacteria were an example of an inherited acquired characteristic.

Later during the 1920s, Eugène Wollman at the Pasteur Institute in Paris attempted to reconcile the views of D'Hérelle and Bordet on bacteriophagy. Wollman posited that the phenomenon was a trait that bacteria acquired through infection or through inheritance. Wollman claimed that lysogenic bacteria involved a form of what he called paraheredity, whereby traits could transmit both vertically, through the genetic material passed from parent to offspring, and horizontally, through genetic material transmitted by infection within the same generation. To test his theory, Wollman conducted experiments on the bacterium, *Bacillus megatherium* (*B. megatherium*), with his wife Elisabeth Wollman. They published several papers on this work between 1925 and 1940. Their work on lysogeny included the experimental replication of bacteriophagy and the production of bacteriophages in non-contaminated bacterial cultures. They also showed that, contrary to d'Hérelle's theory, there were many distinct species of bacteriophages. The Wollmans' work ended in 1943 when the Nazis took them to the Nazi extermination camp in Auschwitz, Nazi Germany, and executed them.

In 1953, André Lwoff, who also worked at the Pasteur Institute in Paris and was familiar with the Wollmans' work, published a review on lysogeny that later became one of the most cited papers on the subject. Lwoff speculated that bacteriophages are not simply microbes. Instead, they were types of unusual genes because they were involved in both the process of induction, in which cells interact with other cells via signals to modify cell behaviour, and the process of cell division. In his work, Lwoff also established the life cycle of bacteriophages in lysogenic bacteria, showing that the genetic material of prophages integrated into the DNA of its bacterial host cell, and then the prophages somehow controlled the cell and produced enzymes that could lyse the bacterial cell walls. After developing theories and experiments on the nature of viruses and their life cycles for several years, Lwoff began a new project in 1955 in which he developed methods to neutralize the polio virus.

In 1955 two Pasteurian scientists employed in Lwoff's laboratory, François Jacob and Élie Wollman, Eugène and Elisabeth Wollman's son, continued to work on bacteria and bacteriophages, with a focus on the physiology and genetic control of the phage life cycle. They studied the stages of the life cycle of bacteriophage in *Escherichia coli* (*E. coli*) K-12, a particular strain of the bacteria *E. coli*, and they used the temperate lambda phage (λ -phage). The switch from *B. megatherium* to *E. coli* enabled Jacob and Wollman to study the cellular and genetic properties of lysogenization and virulence, and to map the *E. coli* K-12 genome using cross-breeding in their studies of bacterial sexuality. In 1959, Jacob and Wollman published this work in *La sexualité des bactéries* (Sexuality and the Genetics of Bacteria).

During this time, Jacob began to develop a model of gene regulation, based on the experimental induction of different phases in the life cycle of lysogenic bacteria and enzyme synthesis. In 1958, Wollman left for a research trip to the California Institute of Technology (Caltech) in Pasadena, California. Jacob then collaborated with Jacques Monod, another Pasteur scientist working on enzymatic adaptation in *E. coli*.

In his early work, Monod studied the process by which enzymes form in bacteria. He hypothesized that enzymes were genetically determined in the cell and that sometimes cells can control the production of certain enzymes in response to particular environmental conditions. This latter phenomenon was called enzymatic adaptation, and it was the focus of Monod's project. He hypothesized that the phenomenon of enzymatic adaptation in bacteria would serve as a model for the process of cell differentiation in the development of more complex, multi-cellular eukaryotes.

Monod, who had worked in Thomas Hunt Morgan's laboratory at Caltech from 1936 to 1937, and had learned about classical genetics, did not favor the hypothesis that viewed lysogenic bacteria as an instance of the inheritance of acquired characteristics. Instead, he argued that the cells' genetic materials produced the enzymes. In 1958, he proposed that the genetic material in bacteria sometimes produced enzymes in the absence of particular inhibitors that blocked the production of those enzymes in the cell. To test his proposal, Monod conducted experiments on the *E. coli* K-12 system. This system, he claimed, would enable him and his colleagues to understand the physiological and regulatory processes of enzyme production.

By the end of the 1950s, the team of scientists at the Pasteur Institute, part of a group they called le Club de Physiologie Cellulaire (the Cellular Physiology Club), were all studying the genetic control and regulation of the physiological processes in lysogenic bacteria. In the summer of 1958, Jacob noted an analogy between his research and Monod's work on enzyme formation and adaptation. In both of their mechanistic models of how genes produced proteins, there existed an adjacent regulatory gene modulating the activity. In 1959, Jacob and Monod published "Gènes de structure et gènes de regulation dans la biosynthèse des protéines" (Genes of structure and genes of regulation in the biosynthesis of proteins), in which they distinguished between structural genes and regulatory genes. Jacob's student, Michel Morange, later argued that this distinction created a conceptual hierarchy among genes that enabled researchers to categorize genes and genetic elements according to the different functions they have in the cell.

In 1959, Jacob and Monod continued to experiment on the *E. coli* K-12 system, along with Arthur Pardee, a collaboration that led to the so-called PaJaMo experiments, named after the researchers. In these experiments, the scientists applied genetic techniques developed in the study of lysogeny—especially Wollman and Jacob's methods of induction using ultra-violet light and temperature changes—to identify the mechanism of the induction of the enzyme beta-galactosidase (β -gal) by lactose in *E. coli*. The three scientists studied how *E. coli* bacteria produce the enzyme β -gal, which *E. coli* requires to decompose the sugar lactose, only when that sugar is present in its environment. Jacob and Monod discovered that a protein, which they called lac repressor, binds to the gene that produces the required metabolic enzyme, β -gal, and suppresses the gene's transcription and translation when lactose is absent in the bacteria's environment. When lactose is present, the lac repressor protein detaches from the gene, and the gene produces the metabolic enzyme β -gal. Jacob and Monod concluded that regulatory proteins can act as on/off switches in gene expression, controlling cell physiology at different times during the cell cycle and under different environmental conditions.

Jacob and Monod named this kind of structural-regulatory gene system an operon, and they hypothesized that it represented a fundamental mechanism that would be discovered in the cells of all living organisms. In 1961, Jacob and Monod published "Genetic regulatory mechanisms in the synthesis of proteins", in which they introduced the logic of gene regulation and expression through their discovery of enzyme induction in *E. coli* and their experiments on lysogenic bacteria.

Their discovery also led the Pasteurian researchers to posit the existence of an intermediate molecular entity that helped to synthesize enzymes in the cell. They called this entity the tape and later renamed it messenger RNA (mRNA). They claimed that it somehow associated with the ribosomal function in the cell's cytoplasm. In 1961, while at the Pasteur Institute, Sydney Brenner, Jacob, and Matt Meselson proved the existence of mRNA using phage-infected *E. coli*, and they showed that there was an intermediate stage between the transcription of the genetic code, or DNA, and its translation into proteins and enzymes.

In 1965, Jacob, Lwoff, and Monod won the Nobel Prize in Physiology or Medicine for their discovery of the genetic control and regulation of protein synthesis. From the 1910s to the 1960s, other groups of scientists in the United States also studied the mechanisms involved in protein synthesis. For example, Paul Zamecnik and his colleagues at Harvard University in Cambridge, Massachusetts, developed a cell-free system in which to study the biochemical basis of protein synthesis. They separated the cellular components in an ultracentrifuge and analyzed the function of each cellular component. In contrast, Jacob and Monod studied the physiological processes and the regulatory states of the entire cell during protein synthesis.

The tools and techniques in molecular biology developed at the Pasteur Institute during the mid-twentieth century developed from the experimental work conducted on lysogenic bacteria. The collaboration between Jacob, Monod, and their colleagues was a product of both Jacob's knowledge of the genetic control and regulation of lysogeny and Monod's work on the induction of enzymes in *E. coli*. As a result of this synthesis of knowledge, they constructed a model of regulation that, as Monod remarked, was as true for *E. coli* as for the elephant.

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