THE OPERON

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Preface

Cold Spring Harbor Laboratory has been the site of two meetings on the lactose operon, the meeting organized by Jon Beckwith and David Zipser in September 1969 and the meeting we had the pleasure of organizing in July 1976. The first lactose operon meeting and its associated book served as a catalyst for an outpouring of molecular analyses of the *lac* operon. The second meeting presented the fruition of the studies, which are critically reviewed in the first half of this volume. We hope that these chapters will give students and scientists an up-to-date picture of the molecular basis of gene regulation in the *lac* system.

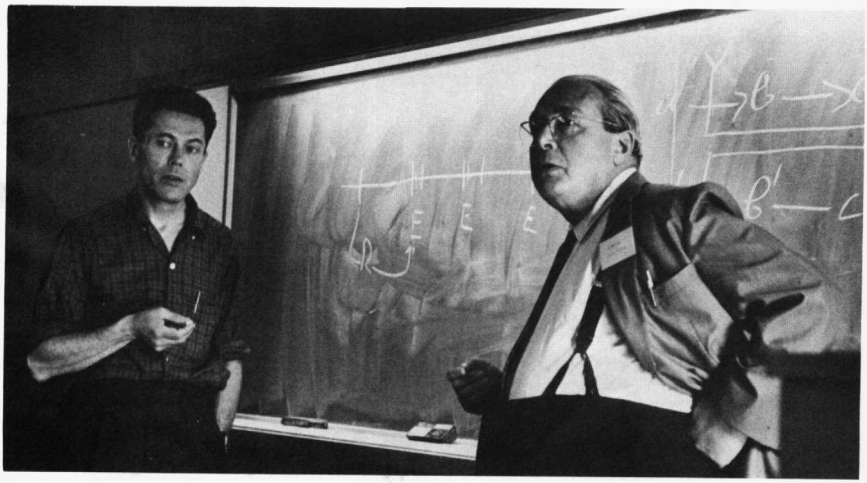
The second half of this volume presents analyses of other bacterial genetic regulatory systems. These chapters describe mechanisms which contrast markedly with that found in the *lac* operon. The examples presented will provide the reader with an overview of various alternative approaches to gene regulation.

We wish to express our gratitude to Jim Watson for making this entire venture possible and to thank the staff of the Cold Spring Harbor Laboratory meetings and publications offices for all of their help, especially Chris Nolan for her expert editing of the manuscript.

Tragically, Jacques Monod died on May 31, 1976, shortly before our meeting. His death was a profound loss to us all, for his studies were the origin of those described in this text. To those of us who had the honor of knowing him, he was a unique and stimulating personality. This book is dedicated to his memory.

Jeffrey H. Miller
William S. Reznikoff

(Top) *At the Cold Spring Harbor Symposium in August 1947, Monod with Dr. Barbara McClintock.* (Middle) *This 1951 photo shows Monod working at his desk in the Pasteur Institute, Paris.* (Bottom) *Returning to Cold Spring Harbor to speak at the 1961 Symposium, Monod discusses the Jacob-Monod model of genetic regulation with Dr. Leo Szilard.*



(Top) *Monod (with Dr. Suzanne Bourgeois) at the Lactose Operon Meeting held at Cold Spring Harbor in 1969.* (Middle) *A thoughtful Monod at the same meeting.* (Bottom) *In October 1969, Monod speaking before the Long Island Biological Association, at Cold Spring Harbor, on the occasion of its 50th Anniversary.*

Contents

In Memoriam

Did I take on that awesome gift when death parted my limp form from his protective clasp?

Mechkonik

When I was asked to trace, in a personal way, the contributions of Jacques Monod to the origins of our present concept of induced enzyme synthesis, I chose to deal with the Monod of the pre-operon era of induced enzymes because it is a largely unknown chapter which is particularly illustrative of his creativity. This is appropriate because, in the last years of his life, Monod was intensely preoccupied with the creative process. He set the study of it as one of the goals of the Salk Institute which he helped found. In Jacques Monod, this process was characterized by taste, elegance, and parsimony.

In writing his own rather personalized curriculum vitae, Monod begins by saying:

> I was born in 1910 in Paris but in 1917 my parents moved to the south of France where I spent my youth. Consequently I consider myself more of a southerner than a Parisian. My father was a painter, a vocation rare in a Hugenot family dominated by doctors, pastors, civil servants and teachers. My mother was American, of Scotch descent, born in Milwaukee; another anomaly when one considers the mores of the French bourgeoisie at the end of the last century. I came to Paris in 1928 to begin my studies in the Faculté des Sciences.

Monod then recalls his debt to his teachers, André Lwoff, Boris Ephrussi, and Louis Rapkine. He tells us that in 1934 he was a Fellow of the Rockefeller Foundation at Caltech working with Thomas Hunt Morgan. In 1936 he returned to France, soon to be faced with the second world war—terrible years which he never mentions, leaving it as a blank in his curriculum vitae (during this time he was in the French Underground). After the liberation, in 1945, Monod joined André Lwoff's laboratory at the Pasteur Institute.

I met Monod in 1947 at a Cold Spring Harbor symposium. He presented a paper entitled "The Phenomenon of Enzymatic Adaptation and Its Bearings on Cellular Differentiation." He made the explicit point in his talk that we would have to understand enzymatic adaptation before we could understand differentiation, in particular, antibody synthesis.

This allusion, plus the enthusiastic support of my teacher, Alvin Pappenheimer, Jr., is what sent me packing for Paris.

In the winter of 1948 I began my postdoctoral work at the Pasteur Institute in Paris. We were housed in an attic. At one end was André Lwoff's closed laboratory and on the door was a cartoon showing the Duke of Wellington addressing his officers after the Battle of Waterloo. The caption read, "Tea cleared my head and left me with no misapprehensions." At the other end of the attic was the laboratory which Jacques Monod, Anna Maria Torriani, and I occupied. That year the Paris winter without heat was merciless. The glacial acetic acid remained frozen on the shelf until noon, at which time I had the distinct feeling that it was the heated discussion at the lunch table that thawed it out. Jacques was a choirmaster and during a good deal of that winter spent afternoons rehearsing the Bach Requiem he was to conduct that Christmas. On Sundays we practiced rock climbing at Fontainbleau. There were many things to decide about the direction of the work, but we simply could not settle down to any problem.

The most important preoccupation was that Monod, who symbolized reactionary Mendel-Morgan genetics, came specifically under vitriolic attack by French Marxist biologists who looked upon the very existence of adaptive enzymes as proof that the substrate induced a directed mutation or a permanent hereditary modification in the cell. This position had a certain respectability, for Sir Cyril Hinshelwood was defending the same point. Even J. B. S. Haldane felt constrained to write only apologetic essays in defense of genetics. We spent one Thursday evening of every month at the meeting of the Mitchurin-Lysenko Society, at the Sorbonne, superficially debating the facts of genetics, but in reality what concerned us was the meaning of the scientific method. For Jacques Monod, who was "engagé" in the Sartre sense, the debates were ugly and degrading and they stomped on his sense of elegance and parsimony. He was moved to make his life's goal a crusade against antiscientific, religious metaphysics whether it be from Church or State. The last time we strolled together on the beach at Torrey Pines, in 1974, he was bitter. "The battle against such ignorance will never be won," he said. "All that one can do is die without calling a priest to the bedside."

In the spring of 1949, we settled down to work. I remember that I felt like Alice in Wonderland when Monod identified three key characteristics of adaptive enzymes for study:

1. The response to a given substrate was specific for that substrate, i.e., the phenomenon was adaptive. The consequences of the existence of systems which paradoxically seem to have a purpose yet arise blindly by variation and selection were a constant theme in his thinking, culminating in his book *Chance and Necessity.*

2. The ability to metabolize a new substrate appeared as an autocatalytic function of time. This had led to the plasmagene hypothesis of Spiegelman in which a gene produced a cytoplasmic self-replicating unit which in turn synthesized the adaptive enzyme.
3. Substrates competed for each other in the induction of given enzymes. This was the striking "diauxie" phenomenon where an organism faced with two growth substrates metabolized one or the other preferentially. Today we call this *catabolite repression*. There was competition between substrates for the attention of the cell. For Monod, this implied competition for precursor subunit molecules.

Given what we now know, it seems remarkable that these three facts could have provided a solid basis for us to begin because they were so misleading. Yet Monod singled them out as he brought exquisite taste to bear on complexity. Today we know that of all of the misleading truths at the time, only these three could have led to the creation of the modern field of regulatory biology.

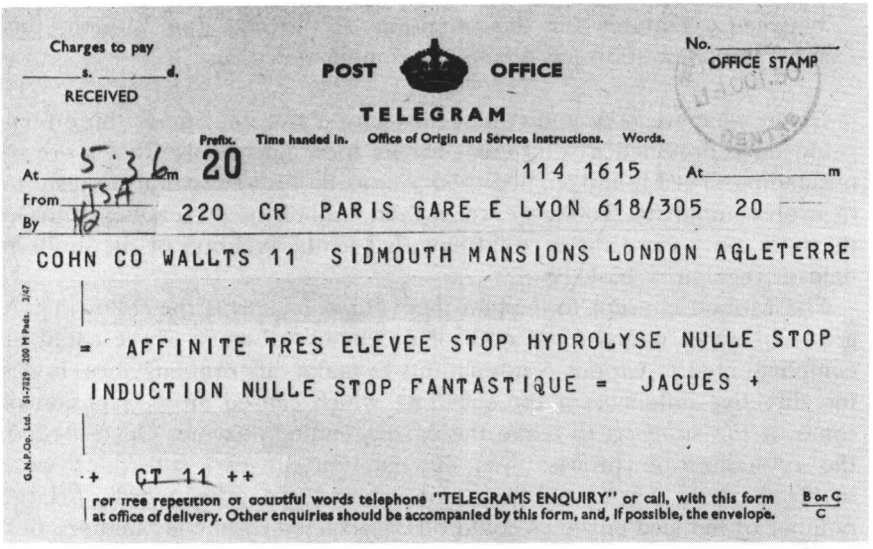
The Monod concept to explain these three facts was the following. A group of genes coded for a pool of precursor subunits. These could be complemented in various combinations to make different enzymes. It was the directive influence of the substrate which caused an aggregation of some of the subunits to make the corresponding enzyme. Once seeded, the crystallization process was autocatalytic. If two substrates were involved, there was competition for subunits. In other words, a large number of induced enzymes could be constructed from combinations of a smaller number of subunits which preexisted the appearance of the substrate in the milieu.

The way to test this hypothesis was to show that all substrates as well as competitive inhibitors were inducers. The hypothesis limited the choice of systems for study. *Escherichia coli* had ideal growth properties as well as an emerging genetics analyzable by mating and viral transduction. It expressed an adaptive enzyme, β-galactosidase, which had a substrate whose analogs were reasonably easy to synthesize. In 1950 I went to Bell's laboratory in Cambridge, England, and later to Helferich's laboratory in Bonn, Germany, to make the compounds which were sent back to Paris to test. By 1951, four findings changed our entire perspective:

1. Excellent substrates were *not* necessarily inducers, e.g., orthonitrophenyl-β-D-galactoside.
2. Excellent nonmetabolizable competitive inhibitors were *not* inducers, e.g., phenyl-β-D-thiogalactoside.
3. Poor nonmetabolizable competitive inhibitors could be excellent inducers, e.g., methyl- or isopropyl-β-D-thiogalactosides.

4. Noncompetitive inhibitors could be excellent inducers, e.g., the α-galactoside, melibiose.

The realization that his hypothesis was false had already crossed Monod's mind when, on October 14, 1950, he sent a telegram to me in England concerning phenyl-β-D-thiogalactoside which I had last given him to test.



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VERY HIGH AFFINITY STOP HYDROLYSIS
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FANTASTIC = JACQUES.

I show this telegram to illustrate the pleasure Jacques Monod derived in proving that his favorite idea was wrong; *fantastique* was the exact word. He was one of Karl Popper's greatest admirers and, like Popper, he insisted that scientific advance consisted in the falsification of hypotheses. I wish now that I could have realized that the Monod hypothesis on subunit complementation, which proved wrong for induced enzymes, would later prove correct for induced antibodies.

The existence of nonsubstrate inducers had a profound philosophical impact, for, like Ionesco, Monod had created a theater of the absurd. A bacterium growing on succinate was producing a useless enzyme, β-galactosidase, in response to a substance it could not metabolize. Monod, with great humor, invented the renowned Scottish philosopher, McGregor (his mother's maiden name), whom he quoted in all of his later writings. This time he attributed to McGregor the following quote: "Each

of science's conquests is a victory of the absurd." The vitalist Hinshelwood-Mitchourin-Lysenko position which irked him had been answered with experimental vengeance. For this reason he decided to drop the term "enzymatic adaptation" and use instead "induced enzyme synthesis," a term which was adopted eventually in an encyclical (*Nature 172*: 1096 [1953]) issued by the Adaptive Enzyme's College of Cardinals, Monod, Pollock, Spiegelman, and Stanier.

These four findings provoked Monod to toy with an idea which was very daring for 1951. The inducer had to be recognized by a stereochemically specific molecule which was *not* the induced enzyme itself. However, this idea left unexplained the autocatalytic nature of the response to lactose, a fact which now pointed strongly to a self-replicating gene product, the plasmagene, postulated by Spiegelman.

In 1951 Seymour Benzer, François Jacob, and Elie Wollman (returning from sabbatical leave) joined the laboratory. Jacob and Wollman viewed adaptive enzymes with great suspicion and by exploring elsewhere paved the way for the era of the operon. It was only in 1953, when Max Delbruck visited Paris and demanded accountability, that the suspicion was diffused and our endeavors became respectable. Seymour Benzer, on the other hand, nettled by Stanier's published statement that it could never be done, decided to tackle the question of the cause of the S-shaped autocatalytic induction curve. Using Monod's and Wollman's finding that certain *E. coli* bacteriophages could block enzyme induction, he followed the appearance of enzyme induced by lactose as the sole carbon source under conditions where only cells which contained enzyme could be lysed. It became obvious that the S-shaped curve was due to the heterogeneity of response of individuals in the population. A bacterium with one molecule of enzyme could metabolize lactose to make more enzyme and therefore had a great advantage. In other words, the postulated *E. coli* plasmagene turned out to be the bacterium itself. For Monod, the second paradox was resolved.

From these studies Monod now developed the concept of *gratuitous induction.* Under conditions where the carbon source and the inducer were separated, the heterogeneity and the S-shaped induction kinetics disappeared.

At this point Monod was ready to face his third basic fact, the competition between substrates. This implied competition for precursors which had led him to the subunit hypothesis that preformed subunits were shared between different enzymes. It became necessary that he know whether the enzyme was made de novo after induction or from preformed precursor subunits.

The answer required an isotope experiment in a laboratory that had never seen even the shadow of a Geiger counter. Fortunately he captured the interest of a Canadian physicist, Lou Siminovitch, who had been

working with Louis Rapkine and André Lwoff since 1947. Siminovitch had discovered ^{35}S and proposed its use as a general protein marker. Siminovitch scrounged through the physics laboratories of Paris collecting junked parts which he checked off on his scribbled wiring diagram. He handed the precious do-it-yourself kit to Monod who, like a child with a tinker toy, put it together and made it work. At the Christmas party that year, I joshed Jacques in a skit which cast him as a bicycle repairman (*réparateur de vélos*).

David Hogness, now in the laboratory, began the experiment which required purification of very small amounts of β-galactosidase to greater than 95% purity. The only way to do this at the time was by immunologic methods. Six months later, Dave Hogness completed the definitive experiment, nervously counting each point on the tinker toy through the night, while Monod played his cello and I uncorked André Lwoff's best properly chilled Sancerre wine which he had carefully hidden in the cold room.

The result was clear. The enzyme was made from amino acids de novo after induction, at a maximum rate, virtually without lag.

This led Monod to formulate a new parameter which we christened as Monod's law, symbolized by $\Delta Z/\Delta B$ (the differential rate of synthesis), the basic unit of which was physiological time.

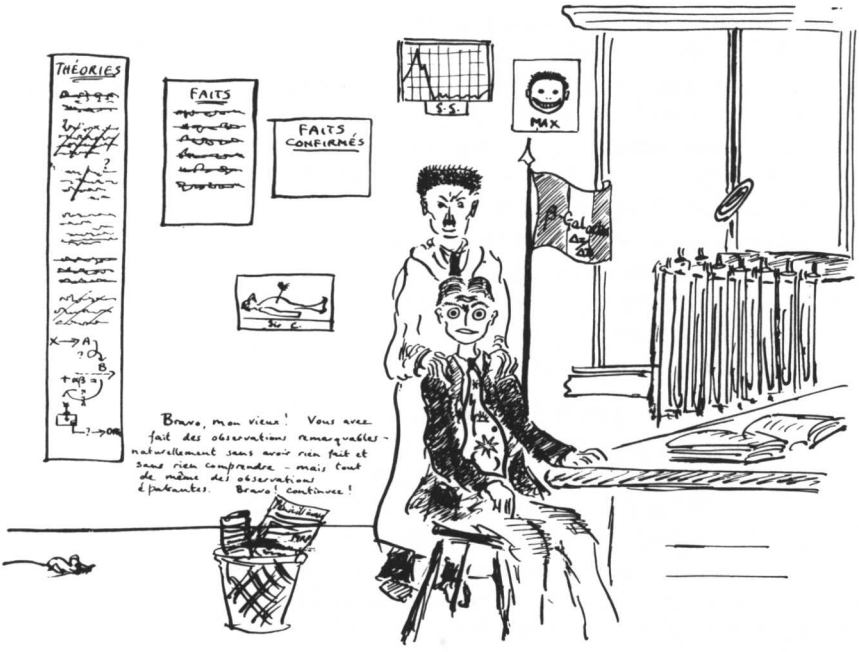
With hindsight it is easy to appreciate taste in science.

The three most important characteristics of induced enzyme synthesis formulated in 1949, misleading as they were, had led by 1953 to a clear definition of the problem, and Monod was prepared to pursue it virtually alone.

However, why we were so insufferably sure of ourselves is not clear to me. Given what we know today, one might say that we had not advanced very far. Justifiably annoyed by our arrogance, Martin Pollock produced a cartoon in 1953 which was upsetting to me but brought pleasure to Monod.

Pollock's cartoon shows Monod standing over a starry-eyed American (myself) symbolized by an outlandish tie, to whom he is saying, "Bravo my fine fellow! You have made remarkable observations—naturally without having done or understood anything—but nevertheless spectacular. Bravo! Continue the good work."

In the wastepaper basket are the papers of Pollock on penicillinase; on the wall is "Who killed cock robin (Sir Cyril Hinshelwood)?"; above that is Max Delbrück smiling approval; next to Max is plotted Monod's temperature as a function of Sol Spiegelman's publications (notice how normal it is after the Benzer experiment); Monod's law ($\Delta Z/\Delta B$) is inscribed on the French tricolor behind us; and on the left is Pollock's evaluation of our accomplishments: we had destroyed all existing so-called facts, replacing them with nothing he was willing to believe



(*Faits confirmés*), and we had produced nothing but wild theories. This is how Pollock saw us in 1953. (He had a personal piece of advice for me—symbolized by the mouse in the left corner—which did not escape my notice: Go back to the study of antibody synthesis in mice! In fact, long before molecular biology could influence immunology, Pollock proposed as the key, the study of the clonal distribution of antibodies [1 cell–1 antibody].)

Today, I understand Monod's reaction of pleasure because such understanding could only have been the consequence of profound friendship.

Just before the modern era of the operon, one striking fact that we had generated had been ignored. With George Cohen and Germaine Stanier, Monod had shown that the end product of a biosynthetic pathway, in this case tryptophan and methionine, repressed the *synthesis* of the corresponding enzymes on that pathway. Not only was function inhibited as Novick and Szilard had shown, but constitutive enzyme synthesis itself was also repressed by its end product—a remarkable energy-saving device.

In his Nobel lecture, Monod muses about this:

> I had learned like any schoolboy that two negatives are equivalent to a positive statement. Mel Cohn and I debated this logical

> possibility which we called the "theory of double bluff" recalling the subtle analysis of poker by Edgar Allan Poe. How blind I was not to take this hypothesis seriously sooner above all since several years earlier we had discovered that tryptophan inhibits the synthesis of tryptophan synthetase. I had always hoped that the regulation of constitutive and inducible systems would be explained by a similar mechanism. Why not suppose that induction could be effected by an anti-repressor rather than by repression of an anti-inducer? This was precisely the thesis which Leo Szilard proposed to us in a seminar. The preliminary results of the injection experiment (PaJaMo experiment) confirmed Leo Szilard's penetrating intuition and my doubts about "the theory of double bluff" were removed.

In a parallel world next door to us, Elie Wollman and François Jacob were creating the basis for genetic analyses which was soon to merge with induced enzymes to reveal what we know today as operon theory.

I did not participate in the merger which began in 1956 after I left Paris. This period is modern operon history: the discovery of the permease and transacetylase; the PaJaMa experiment; operator constitutive and promoter mutations, coordinate induction, polarity, and that remarkable insight, messenger RNA—all part of the 1961 Jacob-Monod Cold Spring Harbor paper. It was another great classic written like Monod's 1947 Cold Spring Harbor paper in that simple and direct Anatole France style. It took only one more concept formulated in 1965, that of allosteric interactions, to round out the story of regulation at the physiological level.

The key to the power of these Monod theories (1947, 1961, or 1965) was simply that they were physiological-level theories capable of reductionism, that is, they were capable of an analysis at the level of chemistry. They were truly theories of molecular biology, and this was the basis of their elegance and their parsimony.

Monod and I never finished our 1974 discussion on the Torrey Pines Beach. What was the next problem of regulation to be? Monod was concerned with the universality of the elements used in the regulation of the *lac* operon. Was there a limited number of elements which required minor rearrangements or was the number going to be large? Did we have to search for new generalizing rules on how they had to be organized? Were there any new laws which would come from the wiring diagrams, the logic of the circuitry? Were both positive and negative regulation fundamental to the integrated organism or could individuals have been constructed using only one switch or the other?

I believe that Jacques Monod had one of the most creative minds of our time, but not because he was a leader of righteous causes, a creator of molecular biology, or a founder and director of institutes of learning. He

had one of the most creative minds simply because he thought deeply, ascetically, and in a Socratic way about how knowledge is acquired, and it is this process that he insisted should be the only basis for a system of ethical and aesthetic values.

Melvin Cohn
The Salk Institute