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A Short History of a Genetic Paradigm

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Professor Benno Müller-Hill
Institut für Genetik an der Universität zu Köln
Weyertal 121, D-50931 Köln, Germany

With 20 figures and 2 tables.

Cover Illustration
Lac repressor-16 bp operator cocrystal in stabilizing buffer (left) and instabilizing buffer after adding IPTG (right).
(see: Pace et al. (1990) Proceedings of the National Academy of Sciences USA *87*, 1870–1873; courtesy of Ponzy Lu)

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To
Jim and Wally
who taught me science

Contents

Introduction

This book presents a short history of the *lac* system of *Escherichia coli* (*E. coli*). This is unusual. Molecular biology has no history for the young scientist. What happened ten years ago seems prehistoric and thus of no interest. Recently I asked a student of the "Max Delbrück Laboratory" during his doctor's exam to describe the experiment which made Max famous. The student who had produced an excellent thesis did not know. He had never heard of Luria. "Phage" was all he could say. And indeed, the Luria-Delbrück experiment is no longer described in many current textbooks.

This gives the young scientist the illusion that only the new exists and that everything new is true. Thus, for the student molecular biology has two faces. In the textbooks, almost everything is solved and clear. Most claims are so self-evident that no proofs are given. Old, classical experiments disappear. They are self-evident. Old errors in interpretation are not mentioned. Who cares? There is only one view, and this is the correct, modern view. Since molecular biology is virtually devoid of mathematical theory, multicoloured diagrams are the condensed essence. Thus, the student has to memorize multicoloured pictures as the essence of molecular biology. And I dare say they are sometimes misleading.

The other face of molecular biology is seen at scientific conferences or read in recent issues of *Nature*, *Science* or *Cell*. There, material is presented which has not yet been included in textbooks – and which most likely never will be. The mass of papers is growing exponentially. Textbook authors have to select the papers they will present. They will have to believe the abstracts, they have no time to carefully study the results. So, errors and mistakes enter the textbooks, veiled as truth. Their number will increase with time. Will there be total confusion at the end? Will molecular biology become a kind of art or advertising, where anything goes?

One has to grow old to understand the functioning of science. Then one may remember the papers which a long time ago aroused great interest and one may compare them to the present-day textbooks. It is virtually impossible to do this with all of molecular biology. One has to concentrate on the small field one knows well. I will present here the lactose operon of *E. coli*, the system I know best. I do not pretend that this is *the* history of the *lac* system. History becomes smooth, too smooth in writing. Right starts are forgotten, wrong expeditions into the desert disappear without a trace. Only a few papers will be cited. The reader may wish to consult a collection of the selected scientific papers of Jacques Monod (1) and

the commentaries of some of his collaborators (2). Another view is presented in the autobiography of François Jacob (3). In 1970, at the height of the classical era, a volume was dedicated solely to the *lac* operon (4). In 1978, at the beginning of the new era, about half of another volume (5) was dedicated to the *lac* operon, the rest dealt with other bacterial systems. Finally, the reader is alerted to the excellent book by Mark Ptashne on the competing λ system (6), a book I admire but did not want to copy. The reader will find that my description differs from the presentation in the textbooks. This clash is intended.

The peace and quiet of a sabbatical made it possible to concentrate on the writing of this book. I could not have written it without the support of the *Deutsche Forschungsgemeinschaft*, the *Ministerium für Forschung und Technologie* and the *Fonds der Chemie* who financed my research on this system for more than two decades. Many people read parts or all of the manuscript, typed meticulously by Elisabeth Stratmann and Anke Wagemann, and sent me their comments. I particularly thank Andrew Barker, Georges Cohen, Renate Dildrop, Walter Gilbert, Melvin Green, Jonathan Howard, George Klein, Peter Kolkhof, Howard Rickenberg, Maxim Schwartz and Agnes Ullmann. Any remaining errors are mine.

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Part 1

A Short History of the *lac* System from its Beginning to 1978

1.1 From Noah to Pasteur: Adaptation in Yeast

The story begins four thousand years ago. Noah, having just escaped the deluge, planted a vineyard and produced wine (1). The sugar of grapes is turned into alcohol by omnipresent yeast. Later, yeast was isolated and used to make delicious bread. When the Jewish slaves fled from Egypt, they had no time to put yeast in their dough (2). When the Jews celebrate and commemorate the occasion on the Passover, they drink wine and eat unleavened bread (which is made without yeast). When Jesus celebrated the Passover with his students for the last time, he pointed to the bread and to the wine and said: "This is my body ... this is my blood" (3). In the Latin Christian service "this is my body" became "*hoc est corpus*". In the eighteenth century, at Dutch fairs and market places, the tricksters called their tricks "*hocus pocus*".

What happens when the juice of grapes turns into wine? What happens in the bread? Antony van Leeuwenhoek (1632-1723) was the first to look at yeast with a microscope he had constructed (Leeuwenhoek, 1680, quoted in 4). He and the biologists believed that yeast was alive. Some chemists later doubted that. In fact, Justus von Liebig (1803-1873), professor at Giessen University, who studied alcohol fermentation, stated in many papers that fermentation was a chemical process, not coupled to living matter. He presumed that the yeast broke down into non-living pieces which carried out the breakdown of glucose and smashed it into ethanol. Liebig kept his opinion until his death, as evident from his last paper on the subject (5). He had already been attacked most convincingly in 1861 by Louis Pasteur (1822-1895), who had presented solid evidence that fermentation is intimately coupled to the living state of the yeast cells (4). Pasteur was right with his experiments, but Liebig was right with his intuition that fermentation was simple chemistry.

Two years after Pasteur's death, the German chemist Eduard Buchner (1860-1917), then professor at Tübingen University, demonstrated that a yeast extract, which shows no sign of living cells, is able to break down glucose to ethanol (6). How does this breakdown of glucose to ethanol occur? The molecule responsible for this process was called "zymase". But what happens if one uses, instead of glucose, the related sugar galactose? In 1900 Frédéric Dienert, working at the Pasteur Institute, showed that yeast grown on glucose breaks down glucose but not galactose, but yeast grown on galactose breaks down galactose or glucose (7). If yeast is grown in the presence of glucose *and* galactose, then the glucose is broken down first. It takes time until the yeast adapts, "accustoms", to galactose. Die-

nert got similar results with other sugars. He also found yeast strains which were unable to break down galactose. Dienert discussed two possibilities to explain his observations: 1. there is just one fundamental molecule ("zymase") which is suitably modified to break down galactose; or 2. there is *de novo* synthesis of another zymase which now breaks down galactose (7). These experiments were repeated by the Nobel Prize winners Hans von Euler (1873-1964) and Richard Willstätter (1872-1942). There was no doubt about their correctness. But the emphasis shifted from regulatory aspects to the identification of the individual enzymes involved in these reactions.

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1.2 Adaptation in *Bacillus subtilis* and *Escherichia coli*

The *lac* story starts in the beginning of the winter of 1940. Paris was occupied by the German armed forces. Jacques Monod (1910-1976), then a young man of thirty years, asked André Lwoff (1902-1994) at the Institut Pasteur for advice. Four years earlier, he had returned from the USA where he had spent a year in the laboratory of Thomas Hunt Morgan working on genetics. *Drosophila* research seemed exhausted and had not become his subject. Now at the Sorbonne he had almost finished his thesis, dealing with bacterial growth. At that time, bacteria were generally thought not to have a nucleus and thus no genes. He had discovered a strange phenomenon. If he provided the bacteria with a mixture of two carbon sources, they sometimes used first one, then the other. What could it mean? "Adaptive enzymes", said Lwoff. "What are adaptive enzymes?" asked Jacques Monod (1,2).

André Lwoff may have told him that microorganisms such as bacteria and yeast adapt their metabolism, and thus their enzymes to the carbon source to which they are exposed. Many people had already worked on adaptation. A classical paper had been published in 1900 by Frédéric Dienert (3) on a particular case in yeast. There was even earlier work with *Aspergillus* (4). Dienert was a Pasteurian. Was he still working at the Institute? Could he be asked? Now, it is impossible to find out. However, I was told that in the late fifties a *very* old man whom nobody recognized came to Monod's office: he introduced himself as Dienert. He wanted to see Monod, the man who was solving in *Escherichia coli* the problem he had attacked in yeast: yeast grown on glucose lacks the enzymes which break down galactose, but yeast grown on galactose contains these enzymes.

Lwoff must have shown Monod the review on adaptation written in German by the Finnish biochemist Henning Karström (1899-1969) (5). There Monod saw a table (Table 1), which contained the major results of this part of his own thesis and which he duly reproduced. Bacteria grown on glucose cannot continue to grow on certain sugars, *e.g.* lactose, whereas bacteria grown on those sugars can continue to grow on glucose. Please note that Karström used the same carbon sources as Monod would use later. He also described similar, but less extensive experiments with *Escherichia coli*. He called the glucose degrading enzymes "constitutive" and the lactose, galactose, arabinose and maltose degrading enzymes "adaptive".

To study adaptation, Monod mainly used *Bacillus subtilis*, and to a lesser extent *E. coli*. Like Karström, he used all the carbon sources which he could find. First, he checked and confirmed that growth of both bacteria is strictly propor-

Table 1: Constitutive and adaptive enzymes of a lactic acid bacterium (*Betacoccus*).

Bacteria	ferment					
grown in the presence of	Glucose Fructose Mannose	Galactose	Arabinose	Saccharose	Maltose	Lactose
Glucose	+	0	0	(+)	0	0
Galactose	+	+	0	+	0	0
Arabinose	+	0	+	+	0	0
Saccharose	+	0	0	+	0	0
Maltose	+	0	0	+	+	0
Lactose	+	+	0	+	0	+
no carbohydrate	+	0	0	+	(+)	0

+ = fermentation; 0 = no fermentation. Translated from Karström (5).

tional to the amounts of the carbon sources in the mineral medium. Then he confirmed the findings of Dienert (3) and Karström (5) that the microorganisms grow first in glucose, and then and only then on the other added carbon source (*i.e.* galactose, maltose etc.). This was demonstrated easily by using various amounts of glucose and of the other carbon source (Fig. 1). The more glucose is present, the higher the first plateau of growth. He also invented a catchy new name for this phenomenon – diauxie.

Before Monod began to work on this problem, a tentative explanation was given by John Yudkin (Cambridge, England). He postulated an equilibrium between a hypothetical pre-enzyme and the active enzyme and that binding of the active enzyme by its substrate displaced the equilibrium in the direction of more active enzyme. So, Yudkin proposed that enzymes folded around their substrates and that the speed of enzyme synthesis depended on substrate assisted folding, *i.e.* the presence of the substrates (6). This theory – Yudkin called it the *mass action theory* - was generally accepted. Karl Landsteiner adapted it without quoting it, to explain antibody production (7). The great Linus Pauling used it too, without quoting it as part of his detailed explanation for the synthesis of specific antibodies (8). The concept was also called the *instructive theory.*

I pause here to recall that Paris was occupied by the German Army from 1940 to 1944. It was a time of war. Monod finished his work on his thesis at the Sorbonne in 1941 (9). Then, he followed the example of one of his professors, Mar-

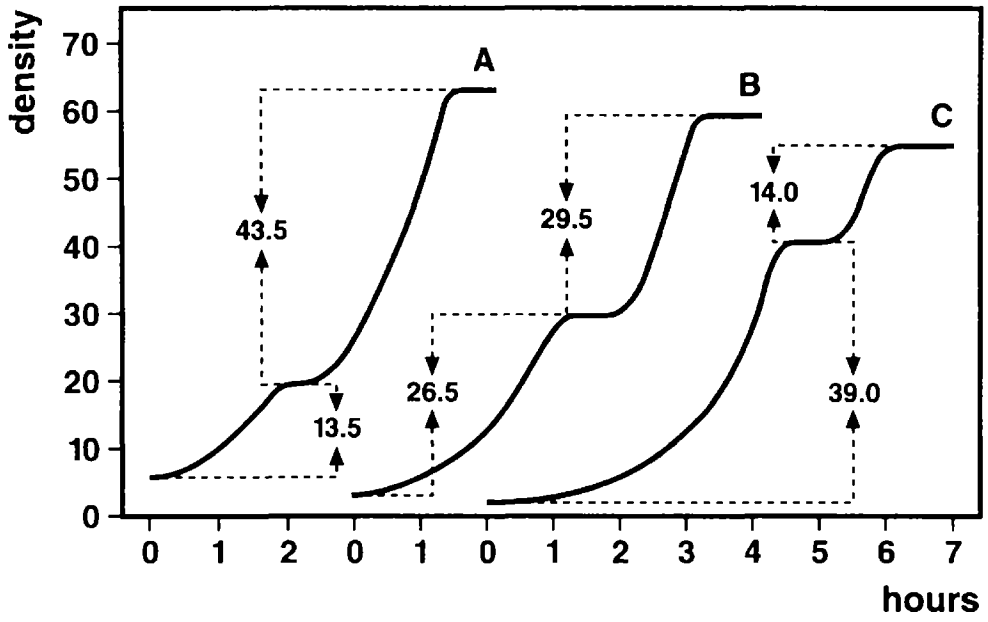

Fig. 1.: An example of diauxie (Fig. 51) taken from Monod's thesis (9). Growth of *E.coli* in a mixture of glucose and sorbite in the proportion 1/3 (A); 2/2 (B) and 3/1 (C). Abscissa: time in hours. Ordinate: bacterial density in relative units. The numbers inside the figure indicate total growth corresponding to each "growth cycle".

cel Prenant. He joined the illegal Communist party and went underground. Later, when asked about this period he remained rather silent (10).

Those were bitter years. I mention here only the fate of some of the people who later will appear in this book. I begin with Élisabeth and Eugène Wollman, a French couple working at the Institut Pasteur. They did research on lysogenic *Bacillus megatherium* and its phage (11). The phage and the bacteria behave exactly like λ and *E. coli*. Lysogenic bacteria are "immune" to attack by the phage. When grown in liquid media their culture supernatant always contains some phage which will attack non-lysogenic bacteria. This *was* the forerunner of the phage λ. The Wollmans were Jewish. So the Germans deported them to Auschwitz in 1944, where they were murdered. They had a son, Elie, who was 22 years old when the war began. He went underground and survived. His name will turn up later when I discuss bacterial mating. There was another young French Jewish student, Georges Cohen, who, like Monod, went underground and joined the Communist party. His name will turn up with Lac permease and *met* and *trp* control. And finally there was a very young medical student, François Jacob, also Jewish. He was just about to begin his medical studies when the war began. He

went to England, joined the French army under De Gaulle, marched from Chad via Libya to Tunisia, participated on D-day in the landings in Normandy and was severely wounded there. When he saw his French classmates, who had studied during the war and who had taken their exams, he must have felt like a loser. He joined Lwoff's laboratory in 1950 (12).

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1.3 Mutants in the *lac* System of *Escherichia coli*

The tacit assumption of Monod's work had been that during diauxie all or most of the bacterial cells adapt quickly, within hours, to the new carbon source. Yet Karström also mentions (1) "slow adaptation by mutation in the sense of de Vries". He cites a 1907 paper by Rudolf Massini (2) who worked as a postdoc in the laboratory of Max Neisser at the university of Frankfurt, and who had isolated a variant of *E. coli* from human feces which was unable to grow on lactose. When Massini plated these bacteria on lactose indicator plates the colonies were white (lac^-). After some days, red papillae formed on the white colonies (when lactose is degraded, the pH changes in and around the bacteria and this changes the colour of a dye added to the plate and so present in and around the bacteria). Bacteria picked from the red papillae were lac^+: they grew like ordinary *E. coli* on lactose, the rest of the colony remained lac^-. He repeated this process many times, always with the same results. Thus, he came to the conclusion that the lac^- bacteria were rare mutants of stable lac^- bacteria. To differentiate these bacteria from ordinary *E. coli*, he called them *Bacterium coli mutabile*.

One would think that the subject of the analysis of bacterial mutants could now be attacked in a rational manner. This was far from so. Twenty four years later, I.M. Lewis, from the University of Texas, came to the conclusion (3): "The subject of bacterial variation and heredity has reached an almost hopeless state of confusion. Almost every possible view has been set forth and there seems no reason to hope that any uniform consensus of opinion may be reached in the immediate future. There are many advocates of a Lamarckian mode of bacterial inheritance while others hold the view that it is essentially Darwinian". Lewis himself again analysed *E. coli* mutabile strains, which could mutate from lac^- to lac^+. He showed convincingly that mutations from lac^- to lac^+ occur spontaneously in the absence of lactose (3).

Some years later, Lwoff isolated a similar *E. coli* strain (ML3 = Mutabile Lwoffi, or according to knowledgable sources, more prosaically *merdae Lwoffi*). Monod and his student, Alice Audureau, repeated the old experiments of Massini and Lewis with these bacteria in 1946, and reproduced their results (4). When they plated about 5×10^8 such bacteria on lactose plates, they found about 300 colonies which grew on lactose. If one grew such lac^+ mutants on a mixture of glucose and lactose, one observed the phenomenon of diauxie, as in ordinary *E. coli*. The bacteria grew first on glucose, and then and only then on lactose (4). One lac^+ revertant was kept and named ML30, and used for all subsequent ex-

periments. The nature of the defect in the *lac* system was unknown. It took ten more years before it became clear that the *lac*$^-$ bacteria carry a defect in the lactose permease, the pump which transports lactose into the cells and the presence of which is essential for growth on lactose. Even today, the mutated *lac* DNA of ML3 has not been sequenced.

During the same year, 1946, Monod attended the conference at Cold Spring Harbor on Heredity and Variation in Microorganisms. There he met Max Delbrück and Salvadore Luria, who had analysed the nature of the *E. coli* mutation to *T1* resistance. They had shown that it involved a change in the genetic material which occurred by chance (5). Also present was Sol Spiegelman, who believed he had demonstrated that in yeast the precursors of the galactose metabolising enzymes multiplied during adaptation (6). Present, too, was Joshua Lederberg, who had shown in his doctoral thesis that one could isolate recombinants from crosses of *E. coli* strains carrying various auxotrophic mutations (7).

One year later, Lederberg isolated mutants of *E. coli* which could not grow on lactose. Some were defective in β-galactosidase, the enzyme which breaks down lactose. They were called *z*$^-$ (z is arbitrary) mutations. Other mutants had different unexplained defects (8). And perhaps even more startling, he isolated a mutant (9) which did not need to adapt to lactose, since it always produced large amounts of β-galactosidase. This mutant was called constitutive. In this mutant, the mechanism which governs adaptation was clearly damaged. All these *lac* mutations appeared to be tightly linked; they recombined poorly when they were crossed to each other.

Both *lac*$^-$ and *lac* constitutive mutations are rare, spontaneous events. They occur about once in a million cell divisions. Thus it became important to devise techniques to *select* for such mutants. Such a technique for isolating *lac* constitutive mutants was designed in 1953 at the Institute Pasteur (10). During diauxie *lac* constitutive mutants have a growth advantage. If one uses several growth cycles, one strongly enriches for such *lac* constitutive mutants.

"Adaptation", the phenomenon Monod had set out to resolve, apparently consisted of two radically different phenomena: 1. the rapid increase of activity or concentration of an enzyme after "induction" with its substrate, the "fast adaptation" according to Karström; 2. the "selection of mutants" under suitable selection pressure, the "slow adaptation" according to Karström. Monod was a man who disliked sloppy language in science. He was also most apt at introducing new words to define phenomena. Thus together with Melvin Cohn, Martin Pollock, Sol Spiegelman and Roger Stanier he published a letter in Nature on the "terminology of enzyme formation" in 1953. There they proposed the use of the specific