

CHAPTER 17

JACOB/MONOD: HOW THE REPRESSOR PROTEIN CONTROLS THE *lac* OPERON

In 1961, François Jacob and Jacques Monod used mutations of the lac genes in E. coli to develop a general model of control of transcription in bacteria.

CONTROL OF TRANSCRIPTION

The same processes that permit cellular control of metabolism also provide the means of regulating how genes are expressed. At the metabolic level, cellular compounds bind to specific enzymatic proteins, changing their shape and therefore their function. At the level of mRNA synthesis, a similar series of allosteric controls (enzymes) exists, regulating which genes are transcribed and to what extent.

The influence of transcription controls can be very marked, and indeed has been observed since the turn of the century, but until recently the nature of these influences was not understood. In 1900, F. Dienert reported that the enzymes of galactose metabolism were present in yeast only when the yeast used galactose as a carbon source; it was as if the presence of galactose had “called forth” from the yeast the specific enzymes necessary to metabolize that sugar. Many similar reports of microbes that “adapted” to their growth medium followed. Microbial enzymes were grouped into two classes: *adaptive* enzymes, not normally present in cells and produced only when the substrate of the enzyme was present in the growth medium, and *constitutive* enzymes, produced normally by cells without regard to presence or absence of substrate.

YUDKIN'S THEORY

It was almost 40 years before a theory was advanced that could satisfactorily explain enzyme adaptation in bacteria. The *mass-action theory* proposed by John Yudkin in 1938, although later proven to be incorrect, seems surprisingly modern in retrospect. He suggested that enzymes exist within cells in dynamic equilibrium with their precursors, an equilibrium that favors enzyme formation for constitutive enzymes, but favors the inactive precursors in the case of adaptive enzymes. Binding of substrate to adaptive enzymes could stabilize them in the enzyme form. Observed from the outside, it would appear as if the substrate had magically ordered up its active enzyme.

Yudkin's mass-action hypothesis was disproved by two lines of evidence, both developed from the study of bacterial adaptation to the carbon source lactose, a system that has become the focus of intensive investigation. The bacterium *E. coli* can grow in media with the disaccharide lactose as the only carbon source. Lactose is cleaved into glucose and galactose by the enzyme beta-galactosidase, the galactose subsequently converted into more glucose, and the glucose used in primary metabolism. Bacterial cells actively growing on lactose each contain several thousand molecules of beta-galactosidase (up to 5 percent of the total cellular protein). However, when the same cells are grown on medium with a different carbon source (such as glucose), little beta-galactosidase is present—less than ten molecules per cell. Beta-galactosidase was thus a classic case of an “adaptive” enzyme.

The first line of evidence contradicting Yudkin's hypothesis was developed by Jacques Monod in the early 1950s. He pointed out that compounds other than lactose could induce the production of beta-galactosidase. Some of them, such as isopropylthiogalactoside (IPTG), were not even metabolizable

substrates of the enzyme. The existence of such *gratuitous inducers* argued against Yudkin's hypothesis, and suggested that the inducer might not interact directly with the enzyme after all.

The second line of evidence, repeated many times in different ways, was the demonstration that bacterial adaptation to lactose, the *enzyme induction* of beta-galactosidase by the inducer lactose, involves synthesis of new enzyme proteins rather than assembly of precursors as Yudkin had surmised. This was shown by growing *E. coli* for many generations in a ³⁵S medium without an inducer present, then transferring the cells to a nonradioactive medium and adding an inducer. The induced β -galactosidase did *not* contain any ³⁵S. This result proves that the induced β -galactosidase is newly-synthesized (*de novo*) and could not have been derived from preexisting subunits, which would have contained ³⁵S cysteine and methionine.

WHAT IS THE BASIS OF ENZYME INDUCTION?

A series of clues rapidly emerged. The first has already been discussed: *induction involves de novo protein synthesis*. The second clue arose from the genetic studies of Joshua Lederberg on lactose induction. Like any good geneticist, he set out to obtain a collection of *lac*⁻ mutants, hoping that by comparing them to wild-type he could begin to describe the properties of the system. This approach of "looking to see what can go wrong" can be a very powerful one (it is surprising how much you can learn about the wiring of a house by removing fuses one at a time and looking for the effect). In the case of Lederberg's *lac*⁻ mutants, it became apparent after several years of screening that there was more than one class of mutant. Some were *lac*⁻ because they lacked β -galactosidase activity (called *lacZ* mutants), while others had β -galactosidase activity in cell extracts but not in intact cells (these were called *lacY* mutants). Activity in extracts was assayed by using a colorless analogue substrate called o-nitrophenyl- β -galactoside (ONPG), which is hydrolyzed by β -galactosidase to yield intensely yellow o-nitrophenol. Activity in intact cells was most conveniently assessed by growing cells on medium containing redox dyes eosin and methylene blue, a medium in which *lac*⁻ cells yield colorless colonies while normal *lac*⁺ cells (which release hydrogen ion when hydrolyzing lactose and so lower the pH of the surrounding medium) are red. It was shown that *lacZ* mutants had defective β -galactosidase enzyme. *LacY*⁻ mutants had normal β -galactosidase, but had an inactive *permease*, an enzyme necessary for transport of lactose into bacterial cells. The second clue was that *both enzymes were induced by lactose*: there were parallel changes in the activities of *both* enzymes. Later a third enzyme, galactosidase transacetylase (*lacA*), was also found to be induced by lactose. It too changed in concert with the others.

The third clue came in the later 1950s when Lederberg's three mutant types (*lacZ*⁻, *lacY*⁻, and *lacA*⁻) were subjected to genetic analysis. It is possible to derive a genetic map of bacterial chromosomes by analyzing recombination frequencies, with each mutation's relative position located. *Lederberg's three mutants all mapped together*.

What these three clues revealed was that enzyme induction involved the *de novo* synthesis of several enzymes contiguous to one another on the bacterial chromosome. This clearly suggested that the interaction of the inducer was at the chromosomal level.

THE INDUCER MUTANT

The key to the puzzle, as is so often the case, was a telltale mutant. Among the many Lederberg mutants was one with a most unusual phenotype: this mutant always made high levels of β -galactosidase, permease, and acetylase, even in the *absence* of lactose. The mutation had transformed the cell from being *adaptive* to being *constitutive*. Because it lacked the property of induction, this constitutive mutant was labeled *lacI*. Genetic mapping indicated that *lacI* was not part of the *lacZ lacY lacA* cluster, although it was located close by. *LacI* thus appeared to be a regulatory mutation of some sort. How might it work? The most obvious hypothesis was that *lacI*⁺ \rightarrow *lacI* leads to the production of an internal inducer, so that synthesis of *lacZ*, *lacY*, and *lacA* are always constitutive. This hypothesis was subject to a clear test: it predicted that

lacI⁻ would be dominant over *lacI*⁺. In a cell containing both *lacI*⁺ and *lacI*⁻ genes, *lacI*⁻ would still produce the internal inducer and the cell would still be constitutive.

JACOB AND MONOD'S HYPOTHESIS

Bacteria are haploid containing only one chromosome. François Jacob and Monod succeeded in obtaining cells in which the genes of the lactose cluster had been transferred from one bacterium to another (the two cells join, one donates its DNA to the other, replicating a copy via a "rolling circle" DNA replication process, and transferring the copy across a narrow cytoplasmic bridge to the recipient cell). This procedure was particularly important here, as it was thus possible to construct bacterial cell lines that were indeed partially diploid, by transferring donor DNA carrying the lactose gene to different bacterial recipients. When Jacob and Monod tested partial diploids that were *lac*⁻*Z*⁺/*lacI*⁺*Z*⁻, no beta-galactosidase was found unless lactose was added to the growth medium. Synthesis was thus inducible, not constitutive, and *lacI*⁻ was clearly not dominant.

Because *lacI*⁻ was not dominant, and no internal inducer could ever be isolated, it seemed likely that the action of the *lacI* gene was at the level of mRNA synthesis itself. How might it work? As *lacI*⁺ was then known to be dominant over *lacI*⁻, it was assumed that *lacI*⁺ actively produced a protein product that acted to regulate the *lacZ*, *lacY*, and *lacA* genes. There were two alternatives, opposites really, that had to be considered:

1. Under one hypothesis, the *lacI*⁺ gene product might be an essential element in transcribing the lactose genes (perhaps an RNA polymerase factor?) with the lactose inducer necessary to activate the process. The *lacI*⁻ mutation would be constitutive if it freed the *lacI* gene product from the *positive control* of the inducer.
2. Under the alternative hypothesis, the *lacI*⁺ gene product might actually prevent the transcription of the lactose genes (perhaps binding the DNA at the RNA polymerase recognition site?), with the lactose inducer binding and inactivating the *lacI*⁺ "repressor," so that transcription of the lactose genes could proceed. The *lacI*⁻ mutation was constitutive because the *lacI*⁻ repressor was defective and could not bind DNA to exercise negative control.

JACOB AND MONOD'S EXPERIMENT

In attempting to understand the mode of action of Lederberg's *lacI*⁻ mutation, Jacob and Monod first had to determine whether or not the *lacI* gene made a product that in turn acted upon the *lac* operon (rather than some structural effect of the gene itself), and whether the *lacI* gene acted in a positive (stimulatory) or negative (inhibitory) manner.

They addressed the first question by determining the dominance behavior of the *lacI* mutation. They reasoned that a *lacI*⁺/*I*⁻ heterozygote should be constitutive if the *lacI* gene were *cis*-acting (limited in its action to the same chromosome), as the genes of the *lacI*⁻ chromosome would have the *lacI*⁻ (constitutive) phenotype, being unaffected by the *lacI*⁺ of the other chromosomes. Alternatively, a *lacI*⁺/*I*⁻ heterozygote should be inducible if the *lacI* gene produced a diffusible product, as the genes of the *lacI*⁻ chromosome would be exposed to that product and so would have the *lacI*⁺ (inducible) phenotype. In the first case, *lacI*⁻ would appear dominant over *lacI*⁺ in the heterozygote, while in the second case, *lacI*⁻ would appear recessive to *lacI*⁺.

The bacterium *E. coli* used in the study is normally haploid, but Jacob and Monod succeeded in getting partial diploids for *lac* by taking advantage of two facts:

1. Hfr strains of *E. coli* could transfer a copy of their chromosome into the F⁻ recipient strain of *E. coli* in a process known as conjugation. This provided, at least temporarily, a diploid organism containing both recipient and donor DNA. These conjugation products were called *meri-diploids*, and provided Jacob and Monod with a means of examining diploid gene combinations within *E. coli*.
2. Not all of the donor chromosome was transferred during conjugation. This means Jacob and Monod needed a means of identifying and isolating those cells that had indeed transferred the *lac* genes. They might of course have isolated and tested individual cells, but this would have been a tedious process. Instead, they located another gene, *proline*, situated quite near *lac* on the bacterial chromosome. When the recipient cells were *PRO*⁻, it was a simple matter to select for *PRO*⁺ recipients. In obtaining the *PRO*⁺ gene, these cells almost certainly had the *lac* genes transferred as well.

And what about the *PRO*⁺ donor cells? If one uses *PRO*⁺ to pick out the recipient cells that have obtained the *lac* genes, how are they distinguished from the original *PRO*⁺ donor cells? Jacob and Monod used another marker—sensitivity to the virus T6. They chose a recipient strain that lacked the proper cell surface T6 recognition protein and so was resistant to infection and lysis by the T6 virus. After the *PRO*⁺, T6^s (Hfr) was mixed with *PRO*⁻, T6R (F⁻), and conjugation proceeded for a while. Virus T6 was then added, killing all of the donor cells. The remaining cells were then spread out and allowed to grow on a surface of synthetic medium lacking proline. Only those cells that had received the *PRO*⁺ portion of the donor chromosome could grow to form visible colonies. Thus, this procedure produced colonies that were predominantly diploid for the *lac* region.

The dominance behavior of the *lacI* was tested by mating constitutive *lacI*⁻ Z⁺ (Hfr) to a *lacI*⁺ recipient that was also beta-galactosidase negative: *lacI*⁺ Z⁻ (F⁻). The resulting diploid *lacI*⁻ Z⁺/I⁺ Z⁻ had the *lacI*⁺ phenotype. It was inducible rather than constitutive. *LacI* was thus recessive to *lacI*⁺, indicating that the *lacI*⁺ gene produced a diffusible product, later shown to be a protein.

Whether this product acted in a positive or negative manner could then be tested directly by repeating the analysis in the opposite gene configuration. Jacob and Monod mated a *lacI*⁺ Z⁺ (Hfr) with a *lacI*⁻ Z⁻ (F⁻) strain, and monitored the *lacZ* gene during the conjugation process. The cells were periodically removed, ruptured, and tested for the ability to hydrolyze ONPG (figure 17.1). At first, donor cells were not making beta-galactosidase because they were *lacI*⁺ and growing on a medium without lactose, while recipient cells were not making beta-galactosidase because they were *lacZ*⁻. When the *lacZ*⁺ gene was transferred to the recipient cell, that cell was then *lacZ*⁺ I⁻ and immediately initiated constitutive synthesis of beta-galactosidase. Shortly thereafter the *lacI*⁺ gene was transferred, constitutive synthesis stopped, and beta-galactosidase synthesis became inducible. The entry of *lacI*⁺ permitted the production of a substance that stopped constitutive synthesis despite the presence of *lacI*⁻. Not only did this result confirm that *lacI*⁺ was dominant over *lacI*⁻, it also demonstrated unambiguously that *the lacI gene product exercised negative control*, acting to inhibit synthesis of the *lac* genes in the absence of inducer.

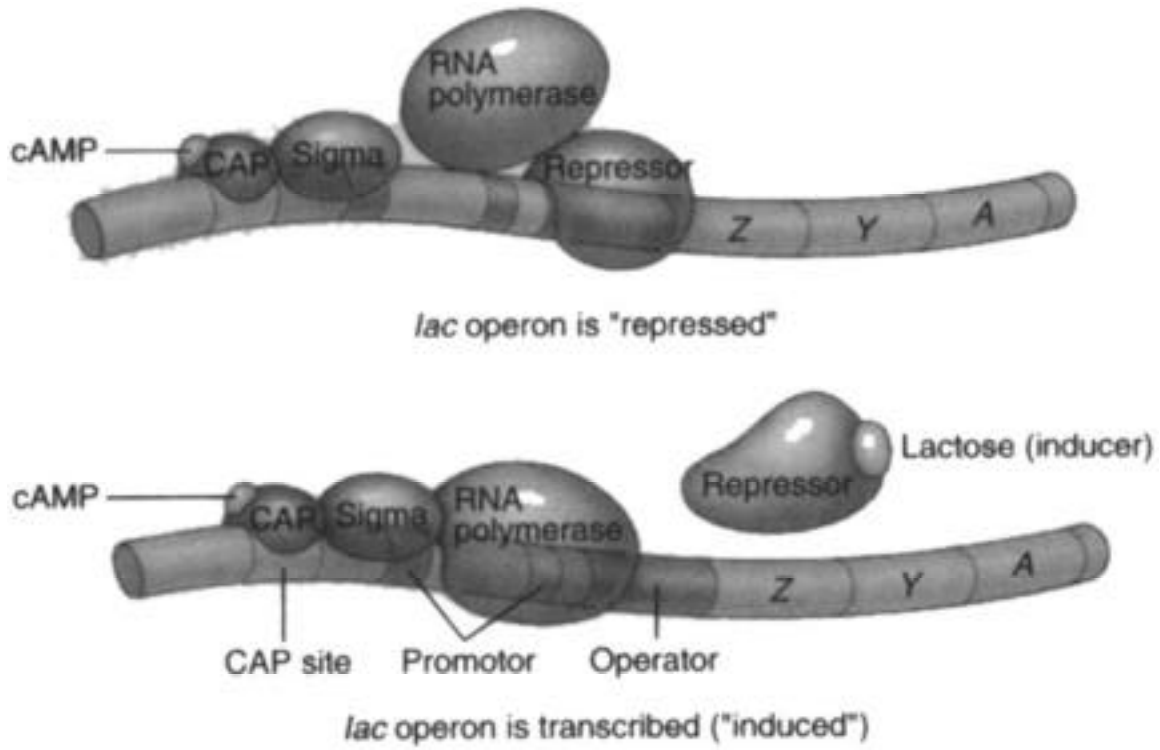


Figure 17.1
The Jacob-Monod experiment.