Biological Feedback

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Chapter 17

EPIGENETIC DIFFERENCES AND MULTIPLE STEADY STATES

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I. ANTIGENIC VARIATION IN *PARAMECIUM AURELIA*

In the summer of 1948, the French Centre National de la Recherche Scientifique organized an international colloquium in Paris entitled "Biological Units Endowed with Genetic Continuity".[1] It was an exciting period for biologists. DNA had been identified a few years earlier as the *Pneumococcus* transforming principle but no one could say to what extent it carried the genetic information involved in nuclear and cytoplasmic inheritance. One major concern at the meeting, as the title suggests, was with autonomous cytoplasmic particles such as chloroplasts, kinetosomes, or the Kappa factor. Called "plasmagenes", these particles were known to conserve their properties independently of the nuclear genotype. There were several clear cases of cytoplasmic (non-Mendelian) inheritance correlated with cytologically observable entities. However, plasmagenes were also postulated, in a vaguer way, to explain a number of other cytoplasmically determined phenomena, even though they were not associated with any visible particle.

Sonneborn and Beale[2] presented observations of this type on antigenic variation in *Paramecium aurelia*. A given culture stably produced a specific surface antigen, but could segregate variants expressing another surface antigen. In crosses between different variants, the progeny all showed the "maternal" antigenic type, which was therefore postulated to be determined by a plasmagene. Each line of *P. aurelia* had its own repertoire of potential antigenic states. In a given culture, only one type was expressed, but limited treatment with the corresponding antiserum could cause up to 90% of the cells to express a different antigen of the repertoire. On the other hand, in crosses between independent lines, they found that the *potential* to produce a certain antigen exhibited Mendelian inheritance and thus was determined by a nuclear gene. This posed a paradox: the antigen actually being expressed in a culture was determined by cytoplasmic factors, whereas the potential ability to express it was determined by the nucleus. Sonneborn and Beale suggested that the nuclear genes determined the formation of the various plasmagenes, which then became essentially autonomous unless the antigen whose synthesis they directed were inactivated, e.g., by antiserum.

In the discussion following Beale's talk, Delbrück[3] pointed out that these observations could equally well be explained by the existence of multiple steady states, without postulating the presence of plasmagenes. Forty years later, it seems that antigenic variation is indeed epigenetic.[11] The cytoplasmic factors that determine the antigenic type being expressed (and exhibit "maternal" inheritance) are presumably regulators of the nuclear genes that code for the various antigens. The action of these regulators results in the expression of only the appropriate gene.

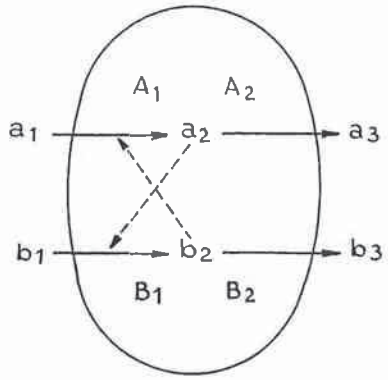
M. Delbrück's remark was so clearly formulated that we reproduce it here (our translation) in its entirety, both for historical interest and as a concise presentation of epigenetic regulation.

"In his discussion of the phenomena observed by Sonneborn and himself, Dr. Beale proposed considering that these phenomena result from the properties of a population of plasmagenes whose reproduction is favored or inhibited by the medium.

I do not intend to contest this conception but would like to draw attention to certain general properties of systems in so-called "steady state", properties which must be taken into consideration before postulating the existence of biological units endowed with genetic continuity in any or all cases in which the genetic continuity of a function is observed.

The argument I wish to develop is the following: *many systems in steady state can exhibit several different stable states under identical conditions. They can be shifted from one stable state to another by transient perturbations.*

This general proposition can be illustrated by a simple model. In the following diagram (Figure 1), the letters A_1, A_2, B_1, and B_2 stand for different enzymes in a cell, which is represented by the circle. The letters a_1, b_1 stand for substances in the medium. Via the action of A_1 and B_1, respectively, these substances are transformed into



intermediate metabolites a_2 and b_2. The latter, in turn, are the substrates of enzymes A_2 and B_2, which transform them into waste products a_3 and b_3. If the medium is constant, the cell will quickly reach a steady state characterized by a certain constant concentration of the intermediate products a_2 and b_2. In this model, there is only one stable state, determined by the medium and the cell's enzymatic properties.

Let us now add the hypothesis that there exist mutual interactions between the two series of enzymatic reactions. Explicitly, let us suppose that the metabolite a_2 affects the reaction catalyzed by enzyme B_1 such that at *high* concentrations of a_2 this reaction is inhibited.* We further postulate a similar effect of metabolite b_2 on enzyme A_1. These interactions are shown by dotted arrows in the diagram.

In this new model, it is still true that under constant conditions the cell will reach a steady state. However, there now exist three possible steady states for the same culture conditions, two stable and one unstable. Let us consider, for example, conditions in which substances a_1 and b_1 are at equal concentration. The steady state ultimately reached will depend on the order in which these substances were added to the medium. According to the conditions, the steady state will be characterized by:

a. Much a_2, little b_2, if a_1 was added first. This steady state is stable; we will call it state *a*.
b. Little a_2, much b_2, if b_1 was added first. This steady state is also stable. We will call it state *b*.
c. Equal low concentrations of a_2 and b_2 if the two substances were added simultaneously in equal quantities. This is a steady state, but it is an unstable state in which weak perturbations will cause a shift to state *a* or to state *b*.

The shift from state *a* to state *b* could be caused by strong *transient* perturbations. For example, if the initial state is *a*, a *temporary* interruption of the inhibition of B_1 by a_2 will cause a shift from state *a* to state *b*.

These alterations could occur via diverse mechanisms: *transient* treatment with anti-a_2 serum, *transient* change of temperature such that the activity of enzyme A_1 is selectively reduced, or *transient* transfer to a medium lacking substance a_1.

In summary, our model cell can exist in two functionally different steady states without this implying any change in the properties of the genes, plasmagenes, enzymes, or any other structural units. Shifts from one state to another can be caused by *transient* modifications in the medium.

Models of this type can be modified *ad infinitum* to account for a large number of different steady states endowed with any degree of stability. Shifts from one to another could, according to the case, be reversible or irreversible, as in differentiation, where the existence of plasmagenes has also been invoked, without any concrete proof.

I do not claim to propose a theory here explaining the phenomena described by Sonneborn and Beale. I simply wish to insist on the fact that, for systems in steady state (but not for systems at equilibrium), one can envisage diverse explanations of this type which from a general point of view are by no means outlandish or even improbable. The above proposition is not new, and many biologists have a fairly clear idea of what it implies. I thought this simple model would help illustrate and clarify the idea."

II. EPIGENETIC CHANGE IN THE *ESCHERICHIA COLI lac* OPERON

Delbrück's historic comment was a prelude for an entirely new way of looking at differentiation. Soon afterward, his ideas were borne out by experimental work. It was known from studies by Monod and co-workers that the bacterium *Escherichia coli* has the genes required

* Such a property could be due to reversible dimerization of a_2, with only the dimer being able to inhibit the reaction catalyzed by B_1.

to utilize the sugar lactose, but that these genes are expressed only in the presence of an "inducer". The natural inducer is a close derivative of lactose itself (produced by the cell from lactose), but there are a number of synthetic analogues that are also good inducers. Some of these cannot be metabolized by the cell and are called "gratuitous" inducers. The enzymes involved are β-galactosidase, which splits the disaccharide lactose into glucose and galactose, and β-galactoside permease, which sits in the membrane and actively pumps lactose and its analogues into the cell from the outside medium. The level of these enzymes is essentially nil in the absence of inducer, but becomes significant within minutes after the addition of inducer. A crucial observation was that in the presence of low external inducer concentrations, a cell that was already induced ("preinduced") would remain induced indefinitely, whereas a cell that was not induced would remain uninduced. We will call this range of inducer concentration a "maintenance" concentration.

This was the starting point for two admirable, complementary series of experiments by Novick and Weiner[4] and by Cohn and Horibata.[5] In simplified terms, one can describe their experiments as follows. Take an uninduced culture, add a high concentration of inducer, split the culture into two parts, and dilute them so that the inducer concentration falls to the "maintenance" range. This dilution is made immediately for subculture A, but only after 10 min for subculture B. Knowing the phenomenon of preinduction, one can predict the result: culture A was not exposed to a high inducer concentration (except for a few seconds) and thus remains uninduced, whereas culture B, exposed to a high inducer concentration for 10 min, was induced and will remain so since the residual inducer concentration suffices to maintain the induced state. This is exactly what was observed experimentally. What is absolutely striking is that the two cultures can be diluted indefinitely (more than 130 generations) in the same medium (containing a maintenance concentration of inducer) without changing their state: the subcultures derived from culture A remain uninduced (essentially, no β-galactosidase synthesis), whereas those derived from culture B remain induced (high level of β-galactosidase synthesis). Furthermore, it is readily shown that the populations have not changed genetically: if inducer is removed, all cells become uninduced, and if a high concentration of inducer is added, all cells are rapidly induced.

The essential logical mechanisms underlying these experiments were fully understood in the late 1950s by their authors. Internal inducer is required for the synthesis of permease, but unless the external inducer concentration is high, permease is required to build up a significant internal concentration. Thus, at low external inducer concentrations, we have a vicious circle: internal inducer is required for the synthesis of permease and permease is required for the internalization of inducer. A very simple logical formalization is given in Table 1. Clearly, there is a range of external inducer concentration (the maintenance range) in which *permease behaves autocatalytically* — a typical positive feedback loop — and this is the basis for the two distinct, heritable phenotypes observed.

In the above experiments, we had two cultures of genetically identical bacteria growing in identical environments, yet presenting a heritable phenotypic difference: one had a high level of β-galactosidase synthesis, the other, essentially none. An outside observer unaware of the history of the two cultures could easily conclude that the bacteria were genetically different. The formal analogy with the antigenic variation in *P. aurelia* discussed by Delbrück is obvious: there are two stable states, and the cells can be made to pass durably from one to the other by transient perturbations (temporary exposure to high or low inducer concentration). This is a perfectly unambiguous case of an *epigenetic difference*, the first to be clearly established and understood. It remains perhaps the simplest and most elegant illustration of an epigenetic change.

TABLE 1

The presence and synthesis of permease are represented by the Boolean variable and function y and Y, respectively, and the external inducer concentration by l. We consider three levels of external inducer:

$l = 0$, negligible;

$l = 1$, maintenance;

$l = 2$, inducing.

We use the Boolean variables ${}^1l$ and ${}^2l$: ${}^1l = 0$ iff $l < 1$, and ${}^2l = 0$ iff $l < 2$. The logical relation is

$$Y = {}^2l + {}^1l y.$$

It simply says that permease is synthesized if there is a high external inducer concentration or if there is a maintenance inducer concentration *and* permease is already present. In the state table, we treat l as an input variable.

$l = 0$

y	Y
⓪	0
$\bar{1}$	0

$(Y = 0)$

$l = 1$

y	Y
⓪	0
①	1

$(Y = y)$

$l = 2$

y	Y
$\bar{0}$	1
①	1

$(Y = 1)$

[a] A differential treatment of this system can be found in Reference 10.

III. EPIGENETIC MODELS OF MONOD AND JACOB

The very basis of today's ideas on gene regulation is the recognition that there exist regulatory genes whose products, in response to external stimuli, influence the expression of other genes by interacting with specific DNA sequences near the genes to be regulated.[6] In isolation, these elementary pieces of the regulatory puzzle permit reversible modulation of gene expression by environmental variables. As pointed out in Chapter 9, they form individual positive or negative controls, not feedback loops. However, as soon as Monod and Jacob discovered gene regulators, they began to speculate about the possible effects of looped sets of such elements.[7] This remarkable paper should be quoted *in extenso*. Here, we will simply show two of the models.

These authors envisaged a system in which a gene E, negatively regulated by a repressor, codes for an enzyme e that catalyzes the synthesis of a product p which, in turn, antagonizes the repressor; in other words, enzyme e produces its own inducer. The system is readily formalized:

$$E = p,$$
$$P = e,$$

ep	EP
⓪⓪	00
$\bar{0}\bar{1}$	10
①①	11
$\bar{1}\bar{0}$	01

confirming that the system does indeed constitute a positive loop, with two stable states: both enzyme and inducer present (⑪) or both absent (⓪⓪).

A second example involves two genes, $\underline{E}_1$ and $\underline{E}_2$, coding for enzymes $\underline{e}_1$ and $\underline{e}_2$, which catalyze the formation of products $\underline{p}_1$ and $\underline{p}_2$, respectively. Product $\underline{p}_1$ is a corepressor of gene $\underline{E}_2$ and $\underline{p}_2$ is corepressor of $\underline{E}_1$. Again, the system forms a positive loop:

$$\begin{array}{ccc} {}_{-}e_1 & \rightarrow & {}^{+}p_1 \\ \uparrow & & \downarrow \\ p_2{}^{+} & \leftarrow & {}^{-}e_2 \end{array}$$

The logical relations are

$$P_1 = e_1 \qquad P_2 = e_2$$

$$E_1 = \bar{p}_2 \qquad E_2 = \bar{p}_1$$

and the state table is

$p_1e_1p_2e_2$	$P_1E_1P_2E_2$
$\bar{0}\bar{0}0\bar{0}$	0101
$0\bar{0}\bar{0}1$	0111
⓪⓪⑪	0011
$00\bar{1}\bar{0}$	0001
$\bar{0}\bar{1}\bar{1}\bar{0}$	1001
$\bar{0}\bar{1}11$	1011
$\bar{0}1\bar{0}1$	1111
$\bar{0}10\bar{0}$	1101

$p_1e_1p_2e_2$	$P_1E_1P_2E_2$
①①⓪⓪	1100
$11\bar{0}\bar{1}$	1110
$1\bar{1}1\bar{1}$	1010
$1\bar{1}\bar{1}0$	1000
$\bar{1}0\bar{1}0$	0000
$\bar{1}01\bar{1}$	0010
$\bar{1}\bar{0}\bar{0}\bar{1}$	0110
$\bar{1}\bar{0}00$	0100

The two stable states are ⓪⓪⑪ (only $\underline{E}_2$ and $\underline{p}_2$ are present) and ①①⓪⓪ (only $\underline{E}_1$ and $\underline{p}_1$ are present).

These and other examples were presented in 1961 as simple illustrations of the type of logical circuit capable of giving rise to the epigenetic changes that characterize differentiation. In all cases, a simple positive feedback loop was involved.

IV. THE CI-CRO SYSTEM OF BACTERIOPHAGE λ

This system will be described in more detail in Chapter 20 as part of the λ circuitry. Here, we will briefly describe the essential aspects of repressor regulation, elucidated by the Jacob group.[8]

Bacteria lysogenic for λ carry the phage DNA inserted in their own chromosome, where it is called a "prophage". The prophage cI gene directs the synthesis of a repressor, which directly or indirectly represses the transcription of other prophage genes. In particular, the genes coding for phage production and lytic growth are silent. The lysogenic bacterium is thus not inconvenienced by its prophage and, in fact, thanks to the repressor, is immune to infection by other λ phage. The cI gene is negatively regulated by the product of the cro

gene, which, in turn, is repressed by cI. If the λ repressor is temporarily inactivated, the situation quickly becomes irreversible. In the absence of active cI repressor, the cro product is synthesized and, once a threshold concentration is reached, it prevents the synthesis of new cI. Thus, *transient* inactivation of cI repressor can result in a *permanent* loss of immunity. This normally leads to lytic development of the phage and death of the cell. However, if the viral functions responsible for cell death are mutationally inactivated, lysogens can survive despite the loss of immunity. These lysogens can grow indefinitely in either of two stable states, immune or non-immune. This is, again, a beautiful example of an epigenetic difference.

The cI-cro system of phage λ has been elegantly exploited by Toman et al.[9], who replaced the genes distal to cro by the bacterial *gal* genes, coding for the enzymes involved in galactose utilization. In this way, cells in the "cro" (nonimmune) phase express the *gal* genes and are able to metabolize galactose, whereas cells in the "cI" (immune) phase do not express these genes and are unable to use galactose. Using appropriate "indicator" plates, the strain produces red or white colonies, according to whether the cells are in the "cro" or "cI" phase. (Indicator plates contain galactose and a pH indicator; colonies in which the galactose is fermented cause local acidification, producing a red color.)

This sophisticated bacterial strain has interesting properties. If, for example, it is exposed to UV light, the DNA will be damaged and the SOS response will be induced (cf. Chapter 19). One manifestation of SOS induction is the proteolytic cleavage of the λ cI repressor. This leads to a stable conversion of bacteria from the immune state (Gal^-, white colonies) to the nonimmune state (Gal^+, red colonies). The frequency of red colonies in the irradiated culture is a direct and highly sensitive measure of the degree of DNA damage. UV doses that have no effect on cell viability can cause a significant increase in the frequency of red colonies. The same strain can be — and, in fact, is — used to evaluate DNA damage caused by other agents.

It is well known that UV irradiation and other DNA damaging treatments are mutagenic. In the experiment just described, the UV light could mutationally inactivate the cI gene and, of course, this would also result in conversion from the immune state to the nonimmune state. The mechanism, however, is completely different: SOS induction involves an epigenetic change, whereas mutational inactivation is a genetic change. In fact, the two types of events can be distinguished on the indicator plates. The epigenetic Gal^+ colonies are a paler red since the frequency of recovery of the immune (Gal^-) state is about 10^{-4}, whereas genetic Gal^+ colonies generally contain few, if any, Gal^- revertants and are thus dark red. In the case of UV irradiation, the epigenetic effect is about 100-fold higher than the genetic effect. Other mutagens behave differently. For example, for the powerful mutagen nitrosoguanidine, the genetic effect is more pronounced than the epigenetic effect, and ethylmethanesulfonate produces only the genetic effect.

V. AND SCRAPIE?

Scrapie is a transmissible, mortal disease in sheep that presents a curious paradox: the infectious agent of the disease, isolated from infected animals, contains no nucleic acid!. In fact, the active principle seems to be protein. Of course, a self-propagating protein would violate the paradigm, based on more than 40 years of intensive molecular biology research, that all genetic information is carried by the nucleic acids, DNA and RNA.

A simple hypothesis accounting for this paradox (and respecting orthodoxy) is that the gene coding for the scrapie protein is part of the normal sheep genome. How, then, can we explain that sheep are healthy unless infected by the scrapie protein? The reader will no doubt have guessed: it is sufficient to postulate an autocatalytic positive loop such that the scrapie gene can only be expressed if the scrapie protein is already present (much like lactose per-

mease in the conditions described in Section III above). This type of circuit will have the two stable states "gene off, scrapie protein absent", the normal, healthy state, and "gene on, scrapie protein present", the situation after infection by scrapie protein.

Such a hypothesis, although gratuitous at present, is testable and, if borne out by experiments, would provide a framework for disease control and for the development of resistant lines of sheep. It would also pose an interesting question: what is the normal role of this potentially lethal protein?

The examples of the *E. coli lac* operon and the cI-cro system of λ (Sections II and IV) are particularly striking because the underlying molecular mechanisms of the epigenetic changes are thoroughly understood and, indeed, form positive feedback loops. It seems virtually certain that other, more complex situations in which multiple stable states occur, such as antigenetic variation and possibly scrapie (Sections I and V) will similarly turn out to be based on positive feedback loops. In fact, we are convinced that this type of logical circuit will provide the ultimate explanation for the many stable states reached in the course of embryonic development.

REFERENCES

1. Unités Biologiques Douées de Continuité Génétique, Editions du CNRS, Lyon, 1949.
2. **Sonneborn, T. M. and Beale, G. H.,** Influence des gènes, des plasmagènes et du milieu dans le detérminisme des caractères antigéniques chez *Paramecium aurelia,* p. 25, in Unités Biologiques Douées de Continuité Génétique, Editions du CNRS, Lyon, 1949.
3. **Delbrück, M.,** Discussion p. 33, in Unités Biologiques Douees de Continuité Génétique, Editions du CNRS, Lyon, 1949.
4. **Novick, A. and Weiner, M.,** Enzyme induction as an all-or-none phenomenon, *Proc. Natl. Acad. Sci. U.S.A.,* 43, 553, 1957.
5. **Cohn, M. and Horibata, K.,** Inhibition by glucose of the induced synthesis of the β-galactoside-enzyme system of *Escherichia coli.* Analysis of maintenance, *J. Bacteriol.,* 78, 601, 1959.
6. **Jacob, F. and Monod, J.,** On the regulation of gene activity, *Cold Spring Harbor Symp. Quant. Biol.,* 26, 193, 1961.
7. **Monod, J. and Jacob, F.,** General conclusions: telenomic mechanisms in cellular metabolism, growth, and differentiation, *Cold Spring Harbor Symp. Quant. Biol.,* 26, 389, 1961.
8. **Eisen, H., Brachet, P., Pereira da Silva, L., and Jacob, F.,** Regulation of repressor expression in λ, *Proc. Natl. Acad. Sci. U.S.A.,* 66, 855, 1970.
9. **Toman, Z., Dambly-Chaudière, C., Tenenbaum, L., and Radman, M.,** A system for detection of genetic and epigenetic alteration in *Escherichia coli* induced by DNA-damaging agents, *J. Mol. Biol.,* 186, 97, 1985.
10. **Babloyantz, A. and Sanglier, M.,** Chemical instabilities of "all or none" type in β-galactosidase induction and active transport, *FEBS Lett.,* 23, 364, 1972.
11. **Capdeville, Y.,** Regulation of surface antigen expression in *Paramecium primaurelia, J. Cell Physiol.,* 99, 383, 1979.