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DYNAMICAL BEHAVIOUR OF BIOLOGICAL REGULATORY NETWORKS—II. IMMUNITY CONTROL IN BACTERIOPHAGE LAMBDA

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A number of bacterial and viral genes take part in the decision between lysis and lysogenization in temperate bacteriophages. In the lambda case, at least five viral genes (cI, cro, cII, N and cIII) and several bacterial genes are involved. Several attempts have been made to model this complex regulatory network. Our approach is based on a logical method described in the first paper of the series which formalizes the interactions between the elements of a regulatory network in terms of discrete variables, functions and parameters. In this paper two models are described and discussed, the first (two-variable model) focused on cI and cro interactions, the second (four-variable model) considering, in addition, genes cII and N.

The treatment presented emphasizes the roles of positive and negative feedback loops and their interactions in the development of the phage. The role of the loops between cI and cro, and of cI on itself (which both have to be positive loops) was discovered earlier; this group's contribution to this aspect mainly deals with the possibility of treating these loops as parts of a more extended network.

In contrast, the role of the negative loop of cro on itself had apparently remained unexplained. We realized that this loop buffers the expression of genes cro itself, cII, O and P against the inflation due to the rapid replication of the phage. More generally, negative auto-control of a gene appears an efficient way to render its expression insensitive (or less sensitive) to gene dosage, whereas a simple negative control would not provide this result.

1. Introduction. Temperate bacteriophages are viruses which can establish a permanent symbiosis with their bacterial host. Upon infection of a bacterial population most of the cells display a lytic response, i.e. the virus multiplies, kills and eventually lyses the cell, but a fraction of the cells become "lysogenic bacteria" (or, for short, "lysogens") in which the viral DNA has integrated into the bacterial chromosome and will be faithfully transmitted to the bacterial progeny. In this condition, a viral gene, called cI in the case of lambda, the best known of the temperate phages, produces a repressor which blocks the expression of all the other genes of the phage, thus making the viral genome harmless for the bacterium. Moreover, the presence of the repressor makes

lysogens immune toward infection with the same type of virus. The development of immunity is only one of the two crucial events necessary for lysogenization, the other being integration of the viral DNA into the bacterial chromosome.

Whether an infected bacterium will lyse or become lysogenic is a choice very similar to cell differentiation, in the sense that a given virus, infecting apparently identical cells, can behave in two extremely different ways. The decision of whether or not gene cI will be switched on, and, thus, whether or not immunity will be established, is subject to a subtle control in which four phage genes (cI, cro, cII and N) play a prominent role (Eisen *et al.*, 1970; Oppenheim *et al.*, 1970; Reichardt, 1975; Herskowitz and Hagen, 1980; Echols, 1986; Ptashne, 1986). A graph summarizing these regulations is given in Fig. 1.

First, a description is given, which will give an idea of the complexity of the situation. Gene cI is normally switched on by the product of gene cII, which operates as a trigger. Once on, gene cI will remain on, because the cI product activates its own synthesis, but, at the same time, gene cI will switch off the other lambda genes, including cII which had just switched it on! [In addition, it has been repeatedly suggested that the leftwards transcription initiated by the cII product might have a negative effect on cII and cro expression (C. Dambly, pers. comm.). We tested the inclusion of these effects in our model but, because no new dynamical properties followed, we choose to keep the simple model without an indirect negative effect of cII.] Gene cro exerts a negative control on cI, directly and indirectly, by impairing the expression of gene cII (more precisely, genes cro and cII, as well as the replicative genes O and P, are located

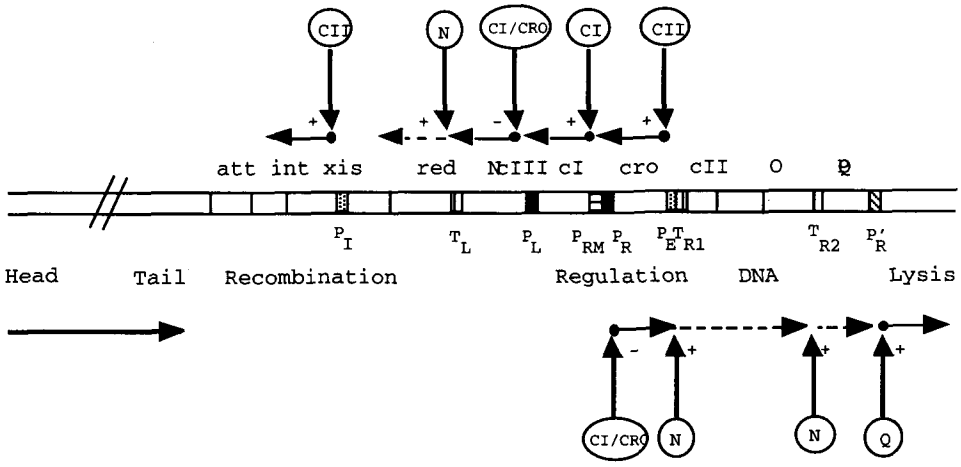


Figure 1. Schematic representation of bacteriophage lambda genome and its regulation. The circled names are the regulatory proteins; P represents the promotors and T the terminators. A description of the different regulations is given in the text.

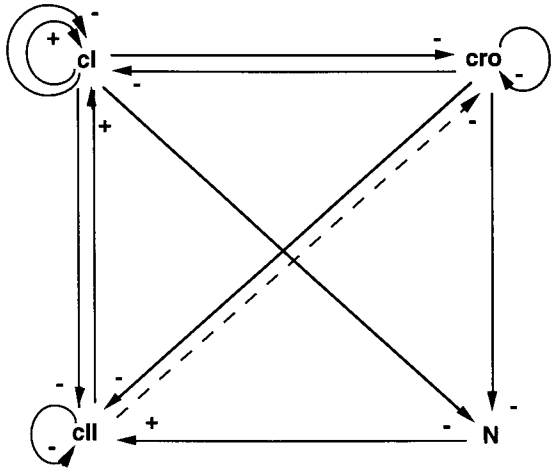


Figure 2. Graph of interactions for the bacteriophage lambda. The solid lines represent the main interactions of the system (with the sign − or + according to their inhibitory or activatory effect); the dotted lines represent accessory interactions (without real physiological function).

on an operon which is subject to negative control by cro). Finally, gene N, directly or indirectly, exerts a positive control on all other genes (except cro) and is itself under negative control of cI and cro. In fact, the repressor cI can also exert a negative control on its own synthesis; however, this interaction takes place only at very high concentrations and it does not seem to have a physiological role under normal conditions. For this reason this interaction will not be included in our formal description.

The above description is summarized in Fig. 2. Other viral (cIII, Q) or bacterial (hflA/B, himA/D, recA, IHF, etc.) genes also play a role in the regulation of the expression of the virus without being clearly involved in a feedback loop including viral regulatory genes (Rattray *et al.*, 1984; Echols, 1986; Friedman *et al.*, 1989; Oppenheim *et al.*, 1991; Giladi *et al.*, 1992). In fact, such regulations, which do not take part in defined feedback mechanisms, can be treated as input variables. On the other hand, lambda structural genes, which are regulated by cI, cro or cII but do not take part into feedback mechanisms, can be represented by output functions. In view of its complexity, it is difficult to predict the behaviour of this regulatory system without a proper formalization.

2. A Simplified Experimental System. This system was first analysed in a simplified version. Eisen *et al.* (1970) used lysogens carrying a thermosensitive mutation on gene cI. At "high" temperatures (ca. 40°C) the repressor is reversibly inactivated and immunity disappears. Normally, this results in the development of the virus, which multiplies, kills and lyses the cell. However, in

the presence of appropriate additional mutations (two mutations inactivating gene N and one preventing replication), the cells survive at high temperature despite the loss of immunity.

The experiments can be described in a simplified way as follows:

(1) when the bacteria are exposed to high temperatures for a short time (minutes), they immediately recover immunity after being re-transferred to low temperature;
(2) when the bacteria are exposed to high temperatures for a long period (hours) and then returned to low temperature, immunity is not recovered immediately, suggesting that high temperatures not only inactivate the repressor but also impair its synthesis; the simplest interpretation is that active repressor is required for its own synthesis;
(3) whether immunity is ultimately recovered, or not, depends on the activity of gene cro (discovered on this occasion); in a cro^- mutant immunity is eventually recovered, but in a cro^+ strain it is not.

These results (Eisen, 1970) can be accounted for in terms of the interactions already mentioned; cro and cI exert a negative control on each other, and cI exerts a positive control on its own synthesis in addition to a negative control on all the other λ genes. These interactions have been beautifully analysed by Ptashne and co-workers (see Ptashne, 1986) at the level of the molecular mechanisms of the "λ switch".

3. Quantitative Models for Lambda Regulation. A number of quantitative models have been proposed for such simplified systems. A first series of models focused on the regulation of the right operator (O_R) and the two associated promoters (P_R and P_{RE}) (see, for examples, Ackers *et al.*, 1981; Lee and Bailey, 1984; Shea and Ackers, 1985; Womble and Rownd, 1986; Reinitz and Vaisnys, 1990). In this specific case, experimental studies led to an estimation of most relevant parameters (binding affinities, kinetic parameters). This allowed the building of statistical thermodynamic models for the operation of what is often called the "lambda switch".

In these analyses the emphasis is on the role of the cooperativity of CI binding and cro negative autoregulation. In contrast, the autocatalysis of CI seems to play a minor dynamical role. In the most recent of these studies, Reinitz and Vaisnys (1990) also point out that some levels of cooperativity are missing in the current description of the switch. Those authors therefore suggested that additional levels of regulation should be included in the model to account for the dynamical properties of the system. However, the development of a wider model, involving all or most of the regulatory genes (see Figs 1 and 2) of bacteriophage lambda, encounters two problems:

(1) quantitative estimation of the binding affinities and kinetic parameters corresponding to the additional interactions are missing;
(2) the resulting differential model would be too complicated to be analysed in the absence of the precise estimation of the parameters involved.

4. Qualitative Models for Lambda Regulation. Dealing with parts of lambda regulatory network, qualitative models have been proposed since the 1970s (see Thomas and Van Ham, 1974; Thomas *et al.*, 1976; Thomas, 1979; Thomas and D'Ari, 1990). These models were built using an asynchronous Boolean approach and led to several experimental predictions (see Thomas *et al.*, 1976).

In this paper, using a generalized logical formalism, presented elsewhere (see Thomas, 1991; and the first paper of this series), two models for lambda regulation are discussed: the first describes the simplified system, studied experimentally by Eisen *et al.* (1970); the second deals additionally with the interactions exerted by genes CII and N.

5. A Two-variable Model. Let us first propose a model of the simplified system in which the mutations affecting gene N knock out the expression of genes N and (indirectly) cII. The corresponding graph of interactions is given in Fig. 3. Cro prevents cI synthesis at sufficient concentrations (above threshold 1) and it represses its own synthesis at higher concentrations (above threshold 2). As for the repressor, it represses the synthesis of cro and activates its own synthesis. The same threshold (1) is used for these two interactions because Meyer *et al.* (1980) have shown that they depend on a common molecular mechanism and that the thresholds are indeed indistinguishable.

In view of these observations, a two-valued logical variable (x) is used for cI and a three-valued variable (y) for cro. Thus, the system can be described by the equations:

$$X = d_x(k_1 + k_{1.1}x^{(1)} + k_{1.2}\overline{y^{(1)}}),$$
$$Y = d_y(k_2 + k_{2.1}\overline{x^{(1)}} + k_{2.2}\overline{y^{(2)}}),$$

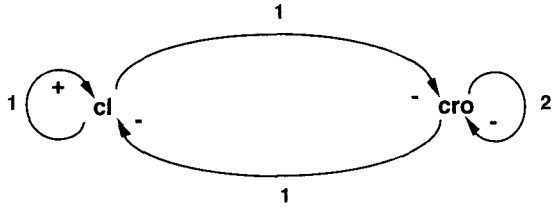


Figure 3. Graph of interaction for our two-variable model of the regulation of bacteriophage lambda expression. The digits refer to the relative magnitude of the thresholds.

in which X, Y and $x^{(i)}$, $y^{(i)}$ are the logical functions ("images") and the binary variables associated with the genes cI and cro, respectively; the d_is and the $k_{i.j}$s are the discretization operators and the real parameters introduced by Snoussi (see the first paper of the series and, for more detail, Snoussi, 1989; Thomas and D'Ari, 1990); the +s represent algebraic sums.

The same information can be represented in a more compact way by the matrix ("matrix of interactions"):

$$\begin{array}{c} \\ \mathrm{X} \\ \mathrm{Y} \end{array} \begin{array}{c} \begin{array}{cc} \mathrm{x} & \mathrm{y} \end{array} \\ \begin{pmatrix} 1 & -1 \\ -1 & -2 \end{pmatrix} \end{array}$$

in which the first line indicates that gene X (cI) is under positive control of product x and under negative control of product y, acting above its first threshold; the second line indicates that gene Y (cro) is under negative control of product x and under negative control of its own product y, acting above its second threshold.

The state table of the system is:

x	y	X	Y
0	0	$K_{1.2}$	$K_{2.12}$
0	1	K_1	$K_{2.12}$
0	2	K_1	$K_{2.1}$
1	0	$K_{1.12}$	$K_{2.2}$
1	1	$K_{1.1}$	$K_{2.2}$
1	2	$K_{1.1}$	K_2

in which $K_{1.1}$, $K_{1.2}$, $K_{2.1}$ and $K_{2.2}$ are the logical parameters corresponding to the interactions located in the boxes 1-1, 1-2, 2-1 and 2-2, respectively, in the matrix of interactions; $K_{1.12}$ means that $K_{1.1}$ and $K_{1.2}$ are both represented (similarly for $K_{2.12}$), and K_1 (or K_2) represents the basal expression. Actually, this table covers several dynamical situations, depending on the values of the logical parameters.

The matrix of interactions shows three feedback loops, two positive and one negative, which may be labelled $x^{(+1)}$, $y^{(-2)}$ and $x^{(-1)}y^{(-1)}$, respectively. As multiple steady states are known to occur in the system at least one of the positive loops must be functional, and as far as the control exerted by cro on its own synthesis is functional, so must the negative loop $y^{(-2)}$ be also. Let us see

which conditions on the logical parameters validate the various feedback loops and to what extent these conditions are compatible with each other.

The conditions required for the loop $x^{(-1)}y^{(-1)}$ to be operative are those for which its characteristic state $s^{(1)}s^{(1)}$ is steady. The relevant regular states adjacent to $s^{(1)}s^{(1)}$ are 0 0 and 1 1 and their images are found in the table:

x	y	X	Y
0	0	$K_{1.2}$	$K_{2.12}$
1	1	$K_{1.1}$	$K_{2.2}$

The conditions for $s^{(1)}s^{(1)}$ to fall between the images of the adjacent states (and thus for the loop to be functional) are:

$$K_{1.1}=0, \quad K_{1.2}=1 \quad \text{(which implies } K_1=0 \text{ and } K_{1.12}=1),$$

$$K_{2.2}=0, \quad K_{2.12}\geqslant 1 \quad \text{(which implies } K_2=0).$$

This means that the parameters corresponding to the action of x or y on itself are subliminal, in contrast with $K_{1.2}$ (which deals with the action of y on x) and $K_{2.12}$ (which deals with the combined actions of x and y on y). If these parametric constraints are fulfilled, $s^{(1)}s^{(1)}$ is a steady state of the system and loop $x^{(-1)}y^{(-1)}$ is functional. Because the loop is positive, its characteristic state, when steady, corresponds to a saddle point in the differential description.

The conditions for loop $y^{(-2)}$ to be operative are that there be a steady state $x\,s^{(2)}$ in the subspace of variable y. The regular adjacent states are:

in the domain x = 0

x	y	X	Y
0	1	K_1	$K_{2.12}$
0	2	K_1	$K_{2.1}$

in the domain x = 1

x	y	X	Y
1	1	$K_{1.1}$	$K_{2.2}$
1	2	$K_{1.1}$	K_2

The conditions for the state $x\,s^{(2)}$ to be steady in the subspace y are:

in the domain x = 0	in the domain x = 1
$K_{2.1}\leqslant 1, \quad K_{2.12}=2$	$K_2\leqslant 1, \quad K_{2.2}=2$

To have a steady state in the whole variable space, additional parametric constraints have to be fulfilled:

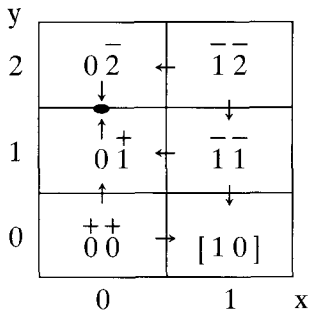
state $[0\,s^{(2)}]$	state $[1\,s^{(2)}]$
$K_1 = 0$	$K_{1.1} = 1$

It can be seen that for $x=0$ there is no incompatibility between the loops $x^{(-1)}y^{(-1)}$ and $y^{(-2)}$; they are both functional if:

$$K_{1.1}=0,\ K_{1.2}=1,\ K_{2.1}\leqslant 1,\ K_{2.2}=0 \quad \text{and} \quad K_{2.12}=2.$$

Using these parameter values, the following state table and trajectories are obtained:

x	y	X	Y
0	0	1	2
0	1	0	2
0	2	0	0 (or 1)
[1	0]	1	0
1	1	0	0
1	2	0	0



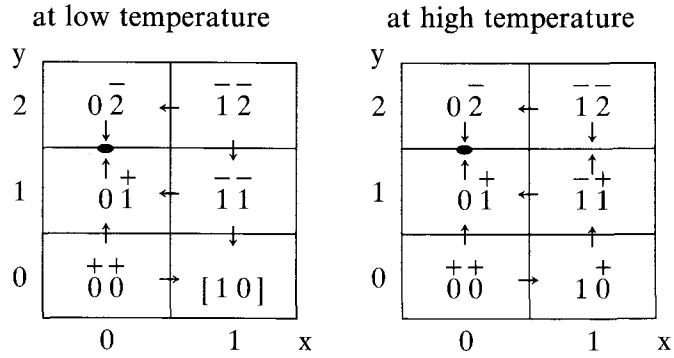
Thus, there are three steady states, $[1\,0]$, $[s^{(1)}s^{(1)}]$ and $[0\,s^{(2)}]$. States $[1\,0]$ and $[0\,s^{(2)}]$ correspond to stable nodes in the usual differential formalism and may account, respectively, for immunity (where gene cI is expressed and gene cro is not) and lytic development (where gene cI is not expressed and gene cro is expressed at its homeostatic level). State $[s^{(1)}s^{(1)}]$ corresponds to a saddle point and is not expected to be seen experimentally because it is unstable. This unstable steady state is nevertheless of fundamental importance, because the separatrix on which it is located separates the basins of the attractors $[1\,0]$ and $[0\,s^{(2)}]$.

In order to check whether the model accounts for the experiments of Eisen *et al.* (1970), let us see how the equations are modified at high temperature or in the presence of a mutation inactivating gene cro. At high temperatures the cI product is inactivated in the thermosensitive mutant. Thus, we have:

$$X = d_x(k_1 + k_{1.2}\overline{y^{(1)}}),$$

$$Y = d_y(k_2 + k_{2.1} + k_{2.2}\overline{y^{(2)}}).$$

For the parameter values already used ($K_{1.1}=0$, $K_{1.2}=1$, $K_{2.1}\leqslant 1$, $K_{2.2}=0$ and $K_{2.12}=2$), we obtain:



Note: whether $K_{2.1}=0$ or 1 does not change the situation; $K_{2.1}=1$ is used here.

It is apparent that there are two stable states at low temperatures but only one at high temperatures. Starting at a low temperature from the immune state [1 0], a shift to a higher temperature leads to the transient state 1 0/1 2, which leads, after a (rapid) synthesis of cro and a (slow) disappearance of cI to the stable state $[0\,s^{(2)}]$. If the lower temperature is then returned to then the system remains trapped in the non-immune state $[0\,s^{(2)}]$, in agreement with the observed non-recovery of immunity.

In the strains which are also impaired in the expression of gene cro the system reduces to:

$$X=d_x(k_1+k_{1.1}x^{(1)}+k_{1.2}),$$
$$Y=0.$$

At high temperatures product x is inactivated, giving:

$$X=d_x(k_1+k_{1.2}),$$
$$Y=0.$$

The state tables are:

at low temperature

x	X	Y
0	$K_{1.2}$	0
1	$K_{1.12}$	0

at high temperature

x	X	Y
0	$K_{1.2}$	0
1	$K_{1.2}$	0

Using the same parameters as above, one would have a stable state [1 0] not

only at low but also at high temperatures. This would mean that at high temperatures cI is synthesized (due to the absence of cro); it would, of course, be inactive at high temperatures, but one would expect immunity to be recovered immediately on returning to a low temperature. In order to account for the experimental situation—delayed recovery of immunity—one should have $K_{1.2}=0$ at high temperatures. This is, in fact, reasonable in the case of the thermosensitive mutant of gene cI; it is well-known that denatured proteins are usually much more sensitive to proteolytic degradation than native ones, and it is therefore likely that even though the rate of synthesis of cI is the same at high and low temperatures (in the cro^- condition), the steady state level is notably lower at high temperatures in view of accelerated degradation. This situation, for $cI^{ts}cro^-$ prophages, gives:

at low temperature

x	X	Y
0	1	0
1	1	0

(steady state 1 0)

at high temperature

x	X	Y
0	0	0
1	0	0

(steady state 0 0)

This accounts for the delayed reappearance of immunity in the cro^- lysogens. Note that if $K_{1.2}=0$ at high temperatures for the $cI^{ts}cro^-$ phage, it must also be the case for the $cI^{ts}cro^+$ phage. In fact, changing $K_{1.2}=1$ into $K_{1.2}=0$ does not change the fate of the system.

This type of analysis can be fully automated. A program has been developed by this group which computes the constraints on the parameters to be respected for each feedback loop to be functional, as well as the possibilities of coexistence of two or more functional loops (Thieffry *et al.*, 1993). On the basis of the logical structure of the system (matrix of the interactions), this program finds the elementary loops and their characteristic steady states, calculates the associated constraints on the parameter values and gives their compatibilities. Using this program enables a table to be constructed containing the constraints on the logical parameters for each loop to be functional:

Loops	Thresholds	K_1	$K_{1.1}$	$K_{1.2}$	$K_{1.12}$	K_2	$K_{2.1}$	$K_{2.2}$	$K_{2.12}$
1. $x^{(1)}$ $(y>0)$	$s^{(1)}$	0	1	–	1	–	–	–	–
2. $x^{(1)}$ $(y=0)$		0	–	0	1	–	–	–	–
3. $y^{(-2)}$ $(x=1)$	$s^{(2)}$	–	–	–	–	0 or 1	–	2	2
4. $y^{(-2)}$ $(x=0)$		–	–	–	–	0 or 1	0 or 1	–	2
5. $x^{(-1)}y^{(-1)}$	$s^{(1)}$, $s^{(1)}$	0	0	1	1	0	–	0	1 or 2

The compatibilities between the different sets of parametric constraints are also be computed and a table containing the resulting constraints constructed:

Compatibilities	K_1	$K_{1.1}$	$K_{1.2}$	$K_{1.12}$	K_2	$K_{2.1}$	$K_{2.2}$	$K_{2.12}$
C(1, 2, 3, 4)	0	1	0	1	0, 1	0 or 1	2	2
C(4, 5)	0	0	1	1	0	–	0	1 or 2

Note: Only the compatibilities involving a maximum number of original sets of parameter constraints are transcribed here ("maximal compatibilities"). For example, C(1,2,3,4) understand C(1,2), C(1,3), . . . , C(2,4), . . . , C(1,2,3), etc.

In both of the above tables "–" indicates that there is no constraint on the corresponding parameter. Row C(4,5) in the second table corresponds to the parameter constraints resulting from both lines 4 and 5 in the preceding table [similarly, for C(1,2,3,4)]; these constraints are consistent with the parameter values which were chosen above.

These two tables, called the table of parametric constraints and compatibility table, respectively, contain all possible dynamics compatible with the original equations, together with the corresponding parametric constraints. Following the same procedure, and using another option of our program, similar tables can be compiled for the singular steady states consistent with the generalized logical equations.

6. A Four-variable Model. Let us now briefly discuss the more complete system, including the effects of genes cI, cro, cII and N (symbolized here by variables x, y, z and u, respectively). Rightly or not, it has been assumed that the operon comprising gene N is more sensitive to the product of cro than the operon comprising genes cro, cII, O and P; this is because: (1) they have no reason to have the same sensitivity; and (2) if the N operon was less sensitive it would escape cro control at its homeostatic level. Similarly, there is no reason why cI would act on these two operons with the same threshold. Figure 4 summarizes our knowledge and the hypotheses concerning the effects of increasing concentrations of cI and cro products. The corresponding graph of interaction is given in the Fig. 5. Consequently, the products of genes cI, cro, cII and N are represented by 3-, 4-, 2- and 2-valued variables (x, y, z and u), respectively.

The corresponding matrix of interaction is:

$$\begin{array}{c} \\ X \\ Y \\ Z \\ W \end{array} \begin{array}{c} \begin{array}{cccc} x & y & z & w \end{array} \\ \begin{pmatrix} 2 & -1 & 1 & 0 \\ -2 & -3 & 0 & 0 \\ -2 & -3 & 0 & 1 \\ -1 & -2 & 0 & 0 \end{pmatrix} \end{array}$$

[Protein]	Binding sites						Transcription		
	O_L1	O_L2	O_L3	O_R1	O_R2	O_R3	P_L	P_R	P_M
cI	+	+					Repressed		
CI	+	+		+	+		Repressed	Repressed	Actived
CI	+	+	+	+	+	+	Repressed	Repressed	Repressed
Cro					(+)	+			Repressed
Cro		(+)	+	(+)	+	+	Repressed		Repressed
Cro	+	+	+	+	+	+	Repressed	Repressed	Repressed

Figure 4. Binding sites occupancies for increasing concentrations of cI and cro products with the corresponding effects on transcription from the promotors P_L, P_R and P_M.

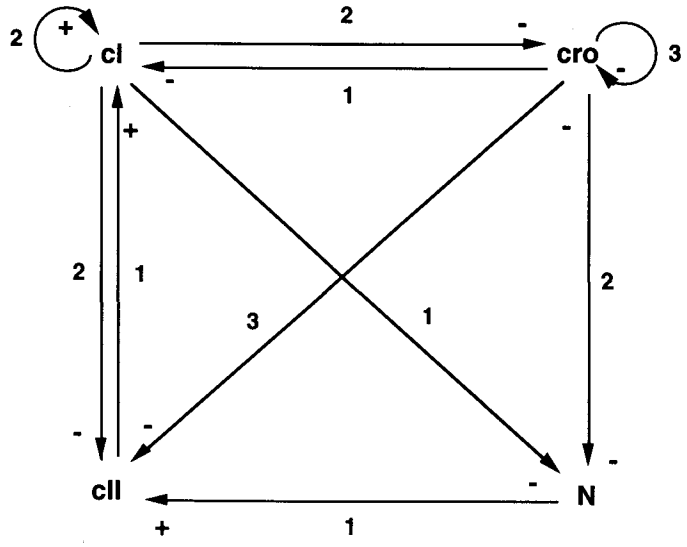


Figure 5. Graph of interactions for our four-variable model of the regulation of bacteriophage lambda expression. The digits refer to the relative magnitude of the thresholds.

The system comprises seven feedback loops, four positive and three negative:

Variables involved in the loop	Sign of the loop	Threshold(s)
x	+	$s^{(1)}$
y	−	$s^{(3)}$
xy	+	$s^{(2)}$, $s^{(1)}$
xz	−	$s^{(2)}$, $s^{(1)}$
xyz	+	$s^{(2)}$, $s^{(3)}$, $s^{(1)}$
xuz	−	$s^{(1)}$, $s^{(1)}$, $s^{(1)}$
xyuz	+	$s^{(2)}$, $s^{(2)}$, $s^{(1)}$, $s^{(1)}$

The state table is given in Appendix A. Now consistent values for the logical parameters must be determined. Two kinds of arguments have been used:

(1) Experimental arguments which give some constraints on the logical parameters:
   (a) To account for the immune state a stable steady state, with gene cI on and all other genes off, is needed. State 2 0 0 0 is stable if $K_{1.12}=2$ and $K_{2.2}=K_{3.2}=K_{4.2}=0$ (see the state table in Appendix A).
   (b) In the absence of CRO protein, cI gene should be fully expressed in order to repress promoters P_R and P_L (even in the absence of N and CII). Thus, it is proposed that $K_{1.2}=2$.
   (c) CII activates the synthesis of CI even, we think, in the presence of CRO (we are doing experiments to test this hypothesis). Accordingly, $K_{1.3}=2$, is used.
   (d) In the absence of CII and in the presence of CRO proteins, gene cI should be fully repressed. It is proposed that $K_1=0$.
   (e) CII is fully expressed only when N is present and CI and CRO concentrations are low. Accordingly, $K_{3.124}=1$ and $K_{3.12}=K_{3.14}=K_{3.24}=0$ are used.
   (f) N is expressed only when CI and CRO concentrations are low. Thus, $K_{4.1}=K_{4.2}=0$ and $K_{4.12}=1$ must hold true.

(2) Using our computer program it is possible to analyse the constraints on the parameters to be respected for each loop to be functional, in other words, for each of these loop to produce a singular steady state. The compatibilities between these different sets of constraints can also be checked. Among the seven loops of the system it is felt that the most important are the positive loop cI–cro and the negative loop cro. Let us determine the parameter constraints for which these loops are functional.

(a) If $K_{1.1} \leqslant 1$ and $K_{1.2} = 2$, $K_{2.2} = 0$ and $K_{2.12} \geqslant 1$, the positive loop cI–cro will be functional for low values of cII concentration (i.e. $z = 0$); if, in addition, $K_{3.2} = K_{3.12} = K_{4.2} = 0$, state $s^{(2)}s^{(1)}00$ is steady in the whole variable space.

(b) If $K_{2.1} \leqslant 2$ and $K_{2.12} = 3$, the negative loop cro will be functional for low or middle range repressor concentrations (i.e. $x \leqslant 1$). If, in addition, $K_1 = K_{3.1} = K_{3.12} = K_{4.1} = 0$, $0s^{(3)}00$ is steady in the whole variable space.

Combining all these constraints gives the following parameter values:

$$K_1 = K_{1.1} = 0,\ K_{1.2} = K_{1.3} = K_{1.12} = K_{1.13} = K_{1.23} = K_{1.123} = 2;$$

$$K_2 = K_{2.2} = 0,\ K_{2.1} = 2,\ K_{2.12} = 3;$$

$$K_3 = K_{3.1} = K_{3.2} = K_{3.4} = K_{3.12} = K_{3.14} = K_{3.24} = 0,\ K_{3.124} = 1;$$

$$K_4 = K_{4.1} = K_{4.2} = 0,\ K_{4.12} = 1.$$

or, in matrix form (matrix of the logical parameters):

	K_i	.1	.2	.3	.4	.12	.13	.14	.23	.24	.34	.123	.124	.134	.234	.1234
X	0	0	2	2	/	2	2	/	2	/	/	2	/	/	/	/
Y	0	2	0	/	/	3	/	/	/	/	/	/	/	/	/	/
Z	0	0	0	/	0	0	/	0	/	0	/	/	1	/	/	/
U	0	0	0	/	/	1	/	/	/	/	/	/	/	/	/	/

in which "/" means that the corresponding logical parameter has no physiological meaning.

For the parameter values selected, it is possible to check which loops are functional, and which regular or singular states are steady (see Appendix B). As expected, there is one regular stable state, [2000] and two singular steady states $[s^{(2)}s^{(1)}00]$ and $[0s^{(3)}00]$. State [2000] corresponds to the immune state, with gene cI on and all other genes off. State $[0s^{(3)}00]$ corresponds to the lytic state, with low concentrations for cI, cII and N products, and an homeostatically regulated concentration of protein CRO. It can be shown that these two states correspond to stable nodes in the differential description, whereas state $[s^{(2)}s^{(1)}00]$ corresponds to a saddle point. Multi-stationarity is created by the positive loop cI–cro. Homeostasis (for variable cro) is ensured by loop $y^{(-3)}$. The other feedback loops have only a local effect.

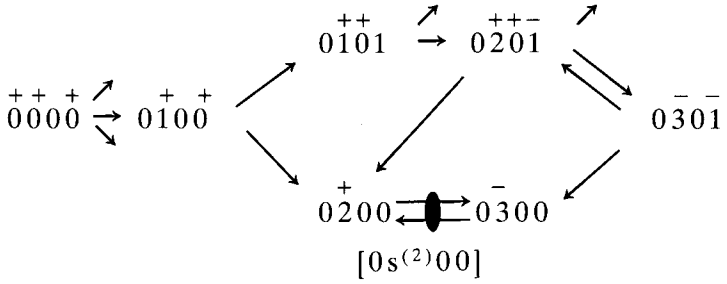
Starting from 0000, the system can go first to 1000, 0100 (cro on) or to 0001 (N on). In view of the low expression of cI in the absence of cII, the two latter cases are more likely. If state 0001 is chosen either the cI, cro or cII

product might appear next. In order to have immunity, cII product then has to be expressed. Following Reichardt and Kaiser (1971), as soon as the cII product is present, the rate of synthesis of cI becomes massive. Once the cI product is present the situation becomes irreversible, i.e. immunity is established and the other genes are switched off.

Thus, the more likely pathway to immunity is:

$$0000 \rightleftarrows 0001 \rightleftarrows 0011 \rightarrow 1011 \rightleftarrows 2011 \rightarrow 2010 \rightarrow [2000]$$

For the other decision (leading to phage growth and cell lysis), the simplest pathways are:



When different transitions are possible, starting from a single state, the pathway followed depends on the values of the corresponding transition delays (Thomas and D'Ari, 1990). There is then a "race" for the executions of simultaneous commutation orders. This race plays a crucial role in the functional choice of one among the paths which are compatible with the matrix of interaction and the parameter values.

As is the case of this two-variable model, various mutation(s) of the regulatory genes (see Fig. 6) have been simulated and the corresponding dynamics checked with the experimental knowledge of the system. The agreement between theoretical predictions and experimental results already published is qualitatively very good.

7. Discussion. There has been, in the past, intense experimental activity in the field of temperate bacteriophages, principally lambda. For some time, lambda has been an invaluable tool in biotechnology, and fashion has shifted from fundamental to applied interests. Does this mean that lambda is almost fully understood, in particular, the essential features of its regulation? The authors are convinced that this is not the case, and that much more than details are still to be found; for example, the complete sequence of lambda DNA has been known for more than 10 years, but there has been little interest in why so many lambda genes are linked by a short overlap.

Mutants	Steady states Low temperatures	Steady states High temperatures
λcI^-	$0S^{(3)}00$	$0S^{(3)}00$
λcro^-	2000	2000
λcII^-	2000, $S^{(1)}S^{(1)}00$, $0S^{(3)}00$	2000, $S^{(1)}S^{(1)}00$, $0S^{(3)}00$
λN^-	2000, $S^{(1)}S^{(1)}00$, $0S^{(3)}00$	2000, $S^{(1)}S^{(1)}00$, $0S^{(3)}00$
λcI^-cro^-	0011	0011
λcII^-N^-	2000, $S^{(1)}S^{(1)}00$, $0S^{(3)}00$	2000, $S^{(1)}S^{(1)}00$, $0S^{(3)}00$
λcro^-N^-	2000	2000
λcro^-cII^-	2000	2000
$\lambda cro^-cII^-N^-$	2000	2000
λcI^{ts}	2000, $S^{(1)}S^{(1)}00$, $0S^{(3)}00$	$0S^{(3)}00$
$\lambda cI^{ts}cro^-$	2000	0011
$\lambda cI^{ts}cro^-cII^-N^-$	2000	0000

Figure 6. Steady states corresponding to a series of single and multiple mutations for our four-variable model, at low and high temperatures. In the case of a cII^- or/and N^- mutation(s), the steady states of the system are the same, but it is expected that the path to the immune state should be slower and/or less probable.

After an intensive experimental study (Thomas, 1971), one of us realized that in this field the models imposed by experiments were becoming too complex to be grasped in their generality; for this reason the authors turned to theoretical biology. In the meantime, a number of highly valuable theoretical studies have been published on the subject. In particular, much is understood concerning the mechanism of immunity and its establishment.

So, what is the contribution of the present work? The authors do not consider it an achievement, but rather a new starting point. For obvious reasons, the preceding theoretical treatments were mostly detailed analyses of small parts of the network. The recent progress of the logical description now permits extension of the analysis to larger fragments of the network, with, in counterpart, less detail in the molecular mechanisms. In addition to the analysis of the cI and of the cI–cro loops, it has been possible to consider other feedback loops involving other genes, and, more importantly, the interactions between these loops. One of the next steps will be to explicitly include integration–excision in the scheme [in an early attempt to formalize integration and excision, stochasticity was introduced in the form of time delays endowed with a mean and a distribution (Thomas, 1979)]. This is of real interest because, although immunity and stable integration are both required for lysogenization

by a normal lambda, immunity exerts a negative control on the genes involved in integration–excision.

As regards these results, little has been added to preceding works concerning the operations of the positive loops and the decision for or against the establishment of immunity. Nevertheless, whereas the two-variable model accounts for the existence of two states of expression, the four-variable model accounts, in addition, for the crucial role of cII and N products in the choice between lysis and lysogeny in bacteriophage lambda.

For realistic parameter values, in addition of the cI–cro positive loop and the cro negative loop, three other loops are functional: the negative loops cI–cII and cI–N–cII, and the positive loop cI–cro–cII. These loops might increase the level of cooperativity of the system but, apparently, play no fundamental role in the decision for or against immunity.

The robustness of these models has been checked by simulating mutations in one or more of the genes involved, which resulted in dynamical behaviour (state transitions) in good qualitative agreement with the experimental knowledge of the system. Moreover, novel experimental predictions have been made during the process of modelization.

For example, in an early attempt to formalize the decision between immune and lytic responses, it was noticed that the model imposed by experiments implied a non-trivial consequence; while N^- and cII^- mutations almost completely prevent the establishment of immunity, it was predicted that, when combined with a cro^- mutation (admittedly known to increase the frequency of this process), an N^- and even an N^-cII^- phage should systematically establish immunity in spite of its defectiveness. N^- derivatives of lambda are so defective that they cannot establish immunity or integrate, but they replicate a little, so that they can be propagated as a plasmid. In order to easily recognize the presence of such a phage, a marker is required; for this reason, one uses a $\lambda N^-N^-gal^+$, which, when present, renders a gal^- strain gal^+. It was found experimentally that a cro^- mutation completely prevented the establishment of this phage as a plasmid but efficiently permitted its establishment as a true prophage (integrated and expressing immunity). Even when there is a cII^- mutation, the phage lysogenizes at a reasonable rate. That this rate is lower than in the cII^+ is due to the contribution of gene cII to integration, as immunity is established in virtually all cells (Thomas *et al.*, 1976).

The model presented here, which uses the generalized logical description, has the same implications as regards the behaviour of N^-cro^- and $N^-cro^-cII^-$ mutants.

On the other hand, the elucidation of the physiological role of negative auto-control of cro might be of fundamental interest. Let us re-state the point. A simple negative control (not looped) will reduce the expression of the gene considered, but this expression will remain proportional to the number of

copies of the gene. In contrast, negative autoregulation stabilizes the level of the gene product near its threshold of activity, which is probably little influenced by gene dosage. If the cro operon (which comprises genes cro, cII O, P and, after a second terminator, Q) was not regulated, a cell infected by lambda would soon produce an unnecessary amount of P and Q products, and a fatal amount of cro and cII products. The negative autoregulation of cro buffers the system against the effect of rapid replication. More generally, it is assumed that the main physiological meaning of negative autoregulation is buffering of gene dosage. In order to challenge this postulate experiments have been undertaken in this laboratory.

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APPENDIX A

State table for our four-variable model of the regulation of lambda expression.

x	y	z	u	X	Y	Z	U
0	0	0	0	$K_{1.2}$	$K_{2.12}$	$K_{3.12}$	$K_{4.12}$
0	0	0	1	$K_{1.2}$	$K_{2.12}$	$K_{3.124}$	$K_{4.12}$
0	0	1	0	$K_{1.23}$	$K_{2.12}$	$K_{3.12}$	$K_{4.12}$
0	0	1	1	$K_{1.23}$	$K_{2.12}$	$K_{3.124}$	$K_{4.12}$
0	1	0	0	K_{1}	$K_{2.12}$	$K_{3.12}$	$K_{4.12}$
0	1	0	1	K_{1}	$K_{2.12}$	$K_{3.124}$	$K_{4.12}$
0	1	1	0	$K_{1.3}$	$K_{2.12}$	$K_{3.12}$	$K_{4.12}$
0	1	1	1	$K_{1.3}$	$K_{2.12}$	$K_{3.124}$	$K_{4.12}$
0	2	0	0	K_{1}	$K_{2.12}$	$K_{3.12}$	$K_{4.1}$
0	2	0	1	K_{1}	$K_{2.12}$	$K_{3.124}$	$K_{4.1}$
0	2	1	0	$K_{1.3}$	$K_{2.12}$	$K_{3.12}$	$K_{4.1}$
0	2	1	1	$K_{1.3}$	$K_{2.12}$	$K_{3.124}$	$K_{4.1}$
0	3	0	0	K_{1}	$K_{2.1}$	$K_{3.1}$	$K_{4.1}$
0	3	0	1	K_{1}	$K_{2.1}$	$K_{3.14}$	$K_{4.1}$
0	3	1	0	$K_{1.3}$	$K_{2.1}$	$K_{3.1}$	$K_{4.1}$
0	3	1	1	$K_{1.3}$	$K_{2.1}$	$K_{3.14}$	$K_{4.1}$
1	0	0	0	$K_{1.2}$	$K_{2.12}$	$K_{3.12}$	$K_{4.2}$
1	0	0	1	$K_{1.2}$	$K_{2.12}$	$K_{3.124}$	$K_{4.2}$
1	0	1	0	$K_{1.23}$	$K_{2.12}$	$K_{3.12}$	$K_{4.2}$
1	0	1	1	$K_{1.23}$	$K_{2.12}$	$K_{3.124}$	$K_{4.2}$
1	1	0	0	K_{1}	$K_{2.12}$	$K_{3.12}$	$K_{4.2}$
1	1	0	1	K_{1}	$K_{2.12}$	$K_{3.124}$	$K_{4.2}$
1	1	1	0	$K_{1.3}$	$K_{2.12}$	$K_{3.12}$	$K_{4.2}$
1	1	1	1	$K_{1.3}$	$K_{2.12}$	$K_{3.124}$	$K_{4.2}$
1	2	0	0	K_{1}	$K_{2.12}$	$K_{3.12}$	K_{4}
1	2	0	1	K_{1}	$K_{2.12}$	$K_{3.124}$	K_{4}
1	2	1	0	$K_{1.3}$	$K_{2.12}$	$K_{3.12}$	K_{4}
1	2	1	1	$K_{1.3}$	$K_{2.12}$	$K_{3.124}$	K_{4}
1	3	0	0	K_{1}	$K_{2.1}$	$K_{3.1}$	K_{4}
1	3	0	1	K_{1}	$K_{2.1}$	$K_{3.14}$	K_{4}
1	3	1	0	$K_{1.3}$	$K_{2.1}$	$K_{3.1}$	K_{4}
1	3	1	1	$K_{1.3}$	$K_{2.1}$	$K_{3.14}$	K_{4}
2	0	0	0	$K_{1.12}$	$K_{2.2}$	$K_{3.2}$	$K_{4.2}$
2	0	0	1	$K_{1.12}$	$K_{2.2}$	$K_{3.24}$	$K_{4.2}$
2	0	1	0	$K_{1.123}$	$K_{2.2}$	$K_{3.2}$	$K_{4.2}$
2	0	1	1	$K_{1.123}$	$K_{2.2}$	$K_{3.24}$	$K_{4.2}$
2	1	0	0	$K_{1.1}$	$K_{2.2}$	$K_{3.2}$	$K_{4.2}$
2	1	0	1	$K_{1.1}$	$K_{2.2}$	$K_{3.24}$	$K_{4.2}$
2	1	1	0	$K_{1.13}$	$K_{2.2}$	$K_{3.2}$	$K_{4.2}$
2	1	1	1	$K_{1.13}$	$K_{2.2}$	$K_{3.24}$	$K_{4.2}$
2	2	0	0	K_{1}	$K_{2.2}$	$K_{3.2}$	K_{4}
2	2	0	1	K_{1}	$K_{2.2}$	$K_{3.24}$	K_{4}
2	2	1	0	$K_{1.13}$	$K_{2.2}$	$K_{3.2}$	K_{4}
2	2	1	1	$K_{1.13}$	$K_{2.2}$	$K_{3.24}$	K_{4}
2	3	0	0	$K_{1.1}$	K_{2}	K_{3}	K_{4}
2	3	0	1	$K_{1.1}$	K_{2}	$K_{3.4}$	K_{4}
2	3	1	0	$K_{1.13}$	K_{2}	K_{3}	K_{4}
2	3	1	1	$K_{1.13}$	K_{2}	$K_{3.4}$	K_{4}

APPENDIX B

Loop efficiencies and singular steady states compatible with the parameter values selected for our four-variable model of the regulation of lambda expression.

Variables of the loop	Loop sign	Domain of efficiency	Parametric constraints
y	−	[0 1]y[0 1] [0 1]	$K_{2.1}(0\,1\,2)\ K_{2.12}(3)$
xy	+	xy[0] [0 1]	$K_{1.1}(0\,1)\ K_{1.2}(2)$ $K_{2.2}(0)\ K_{2.12}(1\,2\,3)$
xz	−	x[1 2]z[1]	$K_{1.1}(0\,1)\ K_{1.3}(2)$ $K_{3.24}(0)\ K_{3.124}(1)$
xyz	+	xyz[1]	$K_{1.1}(0\,1)\ K_{1.3}(2)$ $K_{2}(0\,1\,2)\ K_{2.12}(3)$ $K_{3.4}(0)\ K_{3.124}(1)$
xwz	−	x[1]zw	$K_{1}(0)\ K_{1.3}(1\,2)$ $K_{3.12}(0)\ K_{3.124}(1)$ $K_{4.2}(0)\ K_{4.12}(1)$
Sum of parametric constraints			$K_{1}(0)\ K_{1.1}(0\,1)\ K_{1.2}(2)\ K_{1.3}(2)$ $K_{2.1}(0\,1\,2)\ K_{2.2}(0)\ K_{2.12}(3)$ $K_{3.12}(0)\ K_{3.24}(0)\ K_{3.124}(1)$ $K_{4.2}(0)\ K_{4.12}(1)$

Singular steady states	Loop variables	Loop sign	Parametric constraints
$0s^{(3)}00$	y	−	$K_{1}(0)$ $K_{2.1}(0\,1\,2)\ K_{2.12}(3)$ $K_{3.1}(0)\ K_{3.12}(0)$ $K_{4.1}(0)$
$s^{(2)}s^{(1)}00$	xy	+	$K_{1.1}(0\,1)\ K_{1.2}(2)$ $K_{2.2}(0)\ K_{2.12}(1\,2\,3)$ $K_{3.2}(0)\ K_{3.12}(0)$ $K_{4.2}(0)$
Sum of parametric constraints			$K_{1}(0)\ K_{1.1}(0\,1)\ K_{1.2}(2)$ $K_{2.1}(0\,1\,2)\ K_{2.2}(0)\ K_{2.12}(3)$ $K_{3.12}(0)$ $K_{4.1}(0)\ K_{4.2}(0)$