



A Logical Analysis of the *Drosophila* Gap-gene System

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This manuscript focuses on the formal analysis of the gap-gene network involved in *Drosophila* segmentation. The gap genes are expressed in defined domains along the anterior–posterior axis of the embryo, as a response to asymmetric maternal information in the oocyte. Though many of the individual interactions among maternal and gap genes are reasonably well understood, we still lack a thorough understanding of the dynamic behavior of the system as a whole. Based on a generalized logical formalization, the present analysis leads to the delineation of: (1) the minimal number of distinct, qualitative, functional levels associated with each of the key regulatory factors (the three maternal Bcd, Hb and Cad products, and the four gap Gt, Hb, Kr and Kni products); (2) the most crucial interactions and regulatory circuits of the earliest stages of the segmentation process; (3) the ordering of different regulatory interactions governed by each of these products according to corresponding concentration scales; and (4) the role of gap-gene cross-interactions in the transformation of graded maternal information into discrete gap-gene expression domains. The proposed model allows not only the qualitative reproduction of the patterns of gene expression characterized experimentally, but also the simulation and prediction of single and multiple mutant phenotypes.

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Introduction

Epigenesis is the developmental process in which a single starting cell (zygote) gives rise to the gradual temporal and spatial formation of a set of different cell types arranged in a specific order (pattern formation/morphogenesis) characteristic of each species. During this process, cells lose their developmental potential and are irreversibly committed to develop along specific pathways (cellular determination). Each determination state (cell type) involves a particular combination of genes specifically activated as

a response to an external signal (morphogen). This combination of active genes is heritable at cell division.

One of the basic questions of pattern formation is how different cell types are generated within a uniform population of cells (field). Two mechanistic solutions to this problem have been proposed (Raff & Kauffman, 1983). In the *mosaic determinant* mechanism, the embryonic pattern results from the action of discrete morphogens in different regions of the egg. During cleavage, these morphogens are distributed into different blastomeres. Consequently, they develop along distinct developmental pathways that result in the formation of different cell types. In the *gradient determinant* mechanism, the pattern is

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established by one or more morphogens distributed in the egg in the form of a monotonic gradient. These morphogens organize the pattern of development by dictating distinct cellular responses to the different concentrations of the gradient. Genetic and molecular studies on *Drosophila melanogaster* have provided evidence for the use of the gradient determinant mechanism as the generator of various embryonic patterns. Two maternal morphogen gradients have been identified that are separately involved in the determination of the anterior–posterior and dorsal–ventral axes (reviewed in St. Johnston & Nüsslein-Volhard, 1992). The genes controlling pattern formation along the anterior–posterior and dorsal–ventral axes have been identified. This was possible thanks to the sophisticated degree of genetic analysis already performed with this organism. The genes were identified by the altered phenotypes produced by their mutations. Further, the epistatic relationships between the different mutations allowed the construction of gene networks that determine the pattern formation of the *D. melanogaster* embryo.

The knowledge acquired so far allows a formal analysis of these gene networks to be made. This will aid understanding of their dynamics and identification of the stable states corresponding to the different cell types. Different theoretical approaches have been attempted (Meinhardt, 1977, 1978, 1986, 1989; Kauffman, 1981; Goodwin & Kauffman, 1990; Hunding *et al.*, 1990; Kauffman & Goodwin, 1990; Lacalli, 1990; Burstein, 1995; Reinitz *et al.*, 1995, 1998; Reinitz & Sharp, 1995; Bodnar, 1997; Sánchez *et al.*, 1997; Sharp & Reinitz, 1998), and two main concepts are at the basis of these approaches. One is the idea of the *reaction–diffusion* mechanism, proposed long ago by Turing (1952) to account for the formation of periodic patterns in bound, homogeneous chemical mixtures. The other idea refers to the concept of *positional information*, which states that patterns result from different responses (positional values) of cells to the distribution of morphogens (positional information) (Wolpert, 1969, 1971, 1989).

In most theoretical approaches, differential equations are used to model biological processes. However, in the majority of cases, we still lack precise knowledge about the molecular interac-

tions between genes and/or their products controlling the process in question. In addition, when dealing with genetic systems, the information available is largely qualitative. This has led to the development of a qualitative or *logical* method to formalize regulatory gene networks (Thomas, 1973, 1991; Thomas & D'Ari, 1990; Thomas *et al.*, 1995). In brief, this associates a logical variable with the product of each gene of a regulatory network. In addition, a logical function is associated with each gene and qualitatively represents its actual level of transcription. Finally, logical parameters allow the qualification of the effects of each interaction or combination of interactions controlling the expression of a given gene.

Most previous applications of the logical method have emphasized the biological and dynamic importance of feedback circuits (i.e. closed chains of regulatory interactions). Whereas negative circuits allow the buffering of gene dosage effects, as well as tight control of the expression of key regulatory genes, positive regulatory circuits may constitute developmental switches, allowing alternative developmental pathways and/or encoding positional information. Moreover, whenever a circuit exists, even when buried in a more complex network, logical formalism allows the computation of the parametric constraints to be fulfilled for the circuit to generate its corresponding dynamic properties. When these conditions are fulfilled the circuit is said to be “functional”.

Originally conceived in the context of logical formalism, the requirement of positive feedback circuits for multistationarity, as well as the requirement of negative feedback circuits for sustained oscillations, have been recently demonstrated by several authors in much more general formal contexts (Plahte *et al.*, 1995; Gouzé, 1998; Snoussi, 1998).

We have previously applied such formalism to the modeling of dorso-ventral pattern in the segmented region of the *D. melanogaster* embryo (Sánchez *et al.*, 1997). The present report focuses on the formal analysis of the gap genes involved in anterior–posterior patterning in the *D. melanogaster* embryo.

The establishment of the *Drosophila* anterior–posterior pattern is introduced in the next section followed by the presentation of the gap-gene

network and its dynamic study. Later, the simulation of mutations in the maternal products and the gap genes is analysed. Finally, the manuscript ends with a general discussion and some concluding remarks.

The Embryonic Anterior–posterior Pattern in *Drosophila Melanogaster*

The embryonic anterior-posterior pattern in *D. melanogaster* refers to the segmentation process. This process takes place in syncytial embryos. The zygotic nuclei divide synchronously and migrate towards the periphery where they form the layer of blastoderm cells. This gives rise to the somatic component of the animal. Its organization along the anterior–posterior axis is metameric, i.e. the embryo is composed of serially repeated units (segments) each of which acquires a unique identity (morphology) depending on its position in the embryo. Along the anterior–posterior axis, the head, 11 trunk segments and the tail, form the embryo. The process of segmentation is genetically controlled by two systems formed, respectively, by the *segmentation genes*, which determine the number of segments along the anterior–posterior axis, and the *homeotic genes* that confer cellular identity upon each segment. The present analysis focuses on the segmentation genes.

Segmentation originates from the combined action of three maternal organizers on the zygotic genome (reviewed in St. Johnston & Nüsslein-Volhard, 1992; Pankratz & Jäckle, 1993; Sprenger & Nüsslein-Volhard, 1993; Rivera-Pomar & Jackle, 1996). The anterior organizer is the gene *bicoid* (*bcd*), the posterior organizer is *nanos* (*nos*), and the terminal organizer is *torso* (*tor*). The last of these is responsible for the formation of the head and the tail in the embryo. The maternal Tor product, which is a receptor distributed throughout the embryo, is selectively activated in both terminal regions of the embryo. The genes *tailless* (*tll*) and *huckebein* (*hkb*) are activated in the anterior and posterior regions of the embryo as a response to the activation of the Tor receptor. Since the segments in both the head and the tail are not so well characterized as those of the trunk, segmentation in the latter will only be considered here.

The zygotic segmentation genes can be classified into three categories depending on the number of segments affected by their mutations (Nüsslein-Volhard & Wieschaus, 1980). The gap genes affect several contiguous segments. The pair-rule genes affect complete alternate segments. Finally, the segment polarity genes affect each segment.

From the point of view of genetic interactions, the segmentation genes constitute a hierarchical system in which interactions take place in temporal order (see Fig. 1). The maternal genes *bcd* and *nos* are transcribed during oogenesis and their mRNAs are sequestered into the anterior and posterior poles of the oocyte, respectively. In addition, the oocytes contain *hunchback* (*hb*) and *caudal* (*cad*) mRNAs, distributed throughout the whole embryo. After fertilization, both *bcd* and *nos* mRNAs are translated and their proteins diffuse towards the posterior and the anterior regions, respectively. The Bcd protein prevents translation of *cad* mRNA (Dubnau & Struhl, 1996; Rivera-Pomar *et al.*, 1996), whereas the Nos protein prevents translation of the maternal *hb* mRNA (*hb_{mat}*) (Murata & Wharton, 1995). The result is the subdivision of the embryo into two broad regions: the anterior half containing Bcd and Hb_{mat} proteins, and the posterior half containing Cad protein. The next step in the segmentation process is the response of the zygotic genome to this maternal information, resulting in activation of the gap genes. In the anterior half, the genes *giant* (*gt*) and *hb* are activated as a response to Bcd and Hb_{mat}. In the posterior half, the gene *knirps* (*kni*) and *gt* are activated as a response to Cad. In the central region, the gene *Krüppel* (*Kr*) is activated. The combined action of maternal and gap gene products determines the activation of the pair-rule genes. These are expressed in seven stripes. Some pair-rule genes are needed for the formation of even segments and others for the formation of odd segments. The discovery of this class of segmentation genes was rather unexpected since it indicates that segmentation in *Drosophila* proceeds through a phase in which segments are first organized in double segments. Finally, the pair-rule genes determine the activity of the segment polarity genes. Each of these is expressed as 14 stripes, one stripe corresponding to each segment. During pair-rule gene

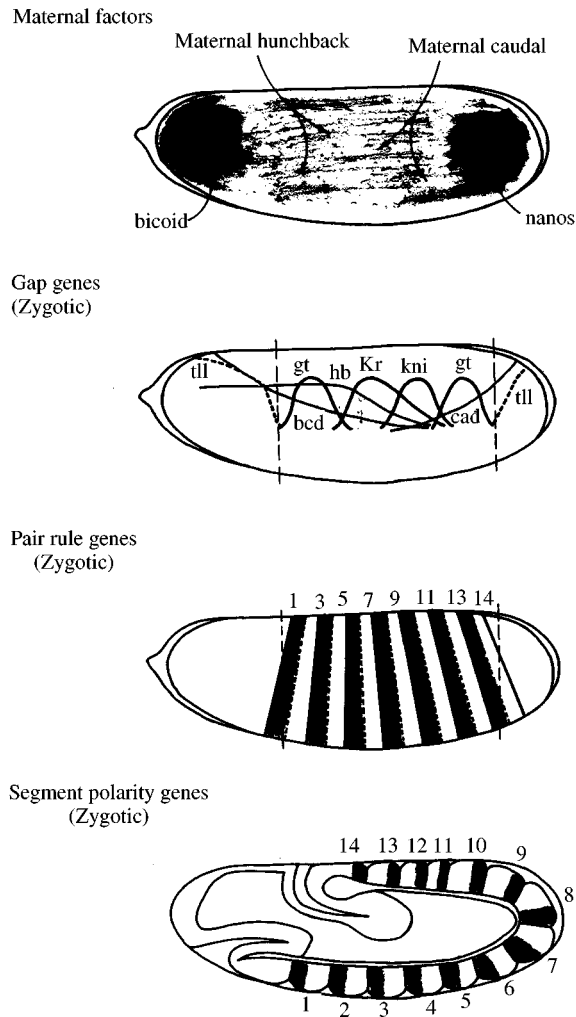


FIG. 1. Schematic representation showing the time course of the segmentation process in *Drosophila melanogaster* embryos (wild type). In the second panel, the vertical dotted lines delimit the trunk region of the embryo whose segmentation is studied here. The approximate expression pattern of the different classes of segmentation genes is shown. The symbols stand for *tll* = Tailless, *gt* = Giant, *hb* = Hunchback, *Kr* = Krüppel, *kni* = Knirps, *bcd* = Bicoid, *cad* = Caudal proteins. For further explanation see text.

expression, the blastoderm becomes cellularized. Therefore, the segment polarity genes act in a cellular rather than a syncytial environment. In contrast to the gap and the pair-rule genes, which are required transiently in the segmentation process, the segment polarity genes are required either continuously or over extensive periods for the maintenance of the segment pattern. The analysis reported here focuses on the expression pattern of gap genes.

A Logical Model for the Gap-gene System

DESCRIPTION OF RELEVANT INTERACTIONS

The name “gap genes” refers to their mutant phenotype—mutations in these genes affect several contiguous segments (Nüsslein-Volhard & Wieschaus, 1980). They are the first zygotic segmentation genes to respond to maternal positional information, and they transmit this morphogenetic information to the segmentation gene hierarchy. The result is the subdivision of the zygote space into subspaces defined by the gap-gene expression domains. From anterior to posterior, these overlapping domains are *hunchback* (*hb*), anterior-*giant* (*ant-gt*), *Krüppel* (*Kr*), *knirps* (*kni*) and posterior-*giant* (*post-gt*) (see Fig. 1). All these gap genes have been cloned and their products characterized. All encode transcription factors of different molecular natures (reviewed in Pankratz & Jäckle, 1993).

Figure 2 shows the proposed graph for cross-regulatory interactions between the gap genes as well as their regulation by the maternal morphogens. This scheme is a modified version of that reported by Rivera-Pomar & Jäckle (1996). Modifications have been introduced in the light of information gathered from the analysis of loss or gain-of-function mutations and further assumptions. A summary of the experimental results is presented below. For more exhaustive information, see the cited references. Roman numerals refer to the interactions shown in Fig. 2.

I. In embryos lacking *bcd* activity, the expression of the zygotic *hb* gene (*hb_{zyg}*) does not occur, and its activation by Bcd is concentration-dependent (Driever & Nüsslein-Volhard, 1989; Struhl *et al.*, 1989; Driever *et al.*, 1989b). Therefore, Bcd is the activator of *hb_{zyg}*.

II. In embryos lacking *Hb_{mat}*, Bcd causes activation of *hb_{zyg}* but this expression is transient and quickly disappears. This was observed when the expression of an *hb* mutation that produces a non-functional Hb protein was analysed (Simpson-Brose *et al.*, 1994). Constructs containing the *lacZ* gene under the control of the *hb*-promoter lead to higher expressions when the promoter includes three Bcd-binding sites and three Hb-binding sites than when it contains three Bcd-binding sites alone. There is no expression when the construct contains only three

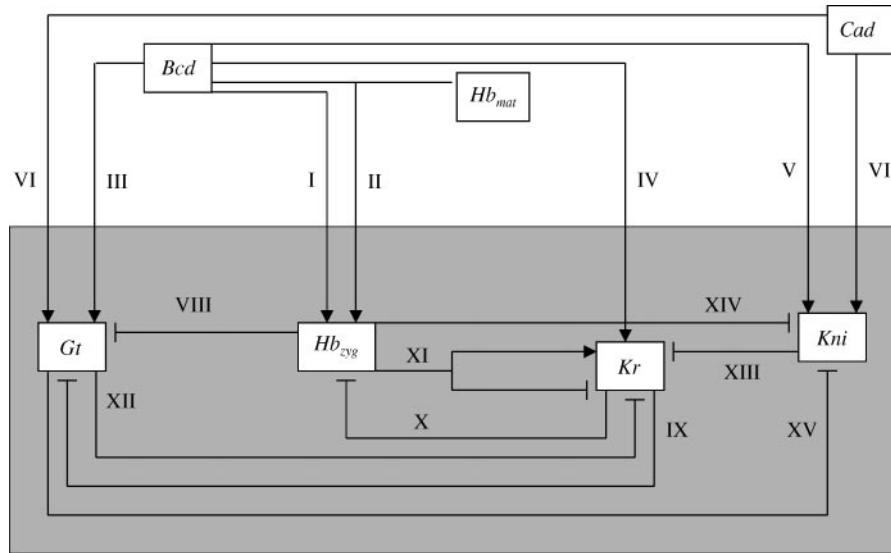


FIG. 2. Schematic representation of the functional relationships between maternal information and gap genes as well as cross-regulatory interactions among these genes. Arrows (\downarrow) and T (\perp) represent activation and repression, respectively, whether directly or indirectly. Roman numerals refer to explanations of the interactions in the text.

Hb-binding sites (Simpson-Brose *et al.*, 1994). Therefore, it is assumed here that the auto-activation of Hb requires the concerted action of Bcd.

III. Embryos from *bcd* homozygous mothers fail to initiate the anterior Gt-domain, whereas the posterior Gt-domain is still present (Eldon & Pirrotta, 1991; Kraut & Levine, 1991a; Capovilla *et al.*, 1992). Consequently, Bcd activates *gt*.

IV. In embryos lacking both *hb_{zyg}* and *hb_{mat}* activities but having *bcd* activity, *Kr* is expressed as a band near the central region of the embryo, and at the lower concentration of Bcd. In the simultaneous absence of *bcd* and *hb_{zyg}* and *hb_{mat}* activities, *Kr* is not activated (Gaul & Jäckle, 1987; Hoch *et al.*, 1990, 1992; Hülkamp *et al.*, 1990; Jacob *et al.*, 1991). Consequently, it can be assumed that Bcd activates *Kr*.

V. The *kni*-promoter contains an element, *kni64*, encompassing six Bcd-binding sites. In response to the Bcd gradient, this element drives the expression of a *lacZ* reporter gene according to an anterior-posterior gradient (Rivera-Pomar *et al.*, 1995). Therefore, it is assumed that Bcd activates *kni*. When combined with another element, *kni223*, the *kni64* element is unable to mediate Bcd-dependent activation of the *lacZ* reporter in the anterior region of the embryo. This is presumably due to the repression effect of

Hb exerted through the two binding sites present in this last element (Rivera-Pomar *et al.*, 1995).

VI. In embryos lacking *cad* activity, *gt* is not activated in its posterior domain (Rivera-Pomar *et al.*, 1995). Thus, Cad functions as an activator of *gt*.

VII. In embryos lacking *cad* activity, *kni* is not activated (Rivera-Pomar *et al.*, 1995). Consequently, Cad acts as an activator of *kni*.

VIII. Ectopic expression of *hb* abolishes both anterior and posterior expressions of *gt*. In *hb* mutants the posterior Gt-domain extends posteriorly (there is also expression of *hb* in the terminal posterior region). In the posterior half, and in the presence of *Hb_{mat}* due to the absence of *nos* activity, *gt* is not activated (Eldon & Pirrotta, 1991; Kraut & Levine, 1991b; Struhl *et al.*, 1992). Therefore, Hb functions as a repressor of *gt*.

IX. In *Kr* mutant embryos, the anterior Gt-domain expands posteriorly, and the posterior Gt-domain expands anteriorly, invading both the *Kr*- and *Kni*-domain, respectively. Ectopic *Kr* expression reduces *gt* expression (Mohler *et al.*, 1989; Eldon & Pirrotta, 1991; Kraut & Levine, 1991a, b; Capovilla *et al.*, 1992). Thus, *Kr* is a repressor of *gt*.

X. In *Kr* mutants, the Hb-domain expands posteriorly (Jäckle *et al.*, 1986). The *hb*-promoter

contains Kr-binding sites (Treisman & Desplan, 1989). Consequently, Kr acts as a repressor of *hb*.

XI. In embryos lacking *bcd* as well as *hb_{zyg}* and *hb_{mat}* activities, *Kr* is not activated. If these embryos, however, contain *Hb_{mat}*, *Kr* is activated and its domain is expanded towards the anterior pole. If the embryos also have *hb_{zyg}* activity, the anterior expansion of the Kr-domain does not occur (Gaul & Jäckle, 1987, 1989; Hoch *et al.*, 1990, 1992; Hülkamp *et al.*, 1990; Struhl *et al.*, 1992). This suggests that low levels of Hb activate *Kr* while high levels repress it.

XII. Ectopic expression of *gt* abolishes *Kr* expression. In *hb/gt* double mutants, the Kr-domain expands more anteriorly than in *hb* mutants. In *nos* mutant embryos, where *gt* is not expressed in the posterior half of the embryo, the Kr-domain extends posteriorly (Kraut & Levine, 1991b; Capovilla *et al.*, 1992). Thus, Gt acts as a repressor of *Kr*.

XIII. In *kni* mutant embryos, the Kr-domain expands posteriorly. Ectopic expression of *kni* suppresses *Kr* expression. The *Kr*-promoter contains Kni-binding sites (Jäckle *et al.*, 1986; Hoch *et al.*, 1992). Therefore, Kni functions as a repressor of *Kr*.

XIV. Ectopic expression of *hb* abolishes *kni* expression. In the presence of *Hb_{mat}* in the posterior half, and due to the absence of *nos* activity, *kni* is not activated (Hülkamp *et al.*, 1990; Kraut & Levine 1991b; Struhl *et al.*, 1992). The *kni*223 element of the *kni*-promoter contains two Hb-binding sites, which prevent Bcd-dependent activation of the *lacZ* reporter in the anterior region of the embryo (see V) (Rivera-Pomar *et al.*, 1995). Consequently, Hb represses *kni*.

XV. In *gt* mutant embryos, the Kni-domain expands posteriorly. Ectopic *gt* expression represses *kni* (Capovilla *et al.*, 1992). Gt thus functions as a repressor of *kni*.

It has been reported that the level of *kni* transcription is diminished in *Kr* mutants, though the spatial Kni-domain is not affected (Pankratz *et al.*, 1989). In addition, it was found that the *kni*-promoter contains Kr-binding sites, which can effect Kr-dependent activation of a reporter gene in tissue-cultured cells (Sauer & Jäckle, 1991). This activation was observed only at low levels of *Kr* activity. Increasing levels of this activity reduce the degree of transcription. Further, the

expression of the reporter gene under the control of the *kni*-promoter lacking Kr-binding sites is reduced when compared to the normal *kni*-promoter, though the reduction in expression is not complete. These results led to the idea that Kr protein was needed to activate the *kni* gene. This relationship, however, is probably only apparent and not really due to a direct positive effect of Kr upon *kni*. Rather, it seems to be a consequence of the repression effect of Gt protein on the expression of *kni* since in *Kr* mutants the posterior Gt-domain expands anteriorly and entirely invades the region in which *kni* is normally expressed, causing its repression. This is supported by the fact that in double *Kr/gt* mutants the expression of *kni* is normal in intensity (Capovilla *et al.*, 1992). Therefore, in the model proposed here, it is assumed that the expression of gene *kni* is independent of *Kr* activity.

LOGICAL FORMALIZATION OF THE GAP-GENE NETWORKS

A logical variable is associated with each maternal or gap product, so that one specific value is assigned to each functional level (i.e. functional product concentration). Whenever needed, multi-level logical variables are used to represent situations where distinct functional concentrations of the same regulatory product are involved. For example, a four-level variable is associated with the morphogen Bcd. Three different functional levels are considered for activation of the gene *hb_{zyg}*, leading to its graded expression. It is also considered that Bcd activates *gt*, *Kr* and *kni* at its first threshold concentration. A three-level variable is associated with Cad. This is considered to activate *kni* at its first threshold and *gt* at its second threshold. A four-level variable has been associated with Hb, with an activation on *Kr* at the first threshold but a repression of the same gene at the third threshold; Hb also represses *kni* at its second threshold. This assumption is based on the fact that the concentration of Hb needed to repress *kni* is higher than that needed to activate *Kr* (Hülkamp *et al.*, 1990; Struhl *et al.*, 1992). It is here assumed that Kr represses *hb* at the second threshold level, whereas all other interactions occur at the first threshold. Finally, all the interactions exerted by *gt* and *kni* are

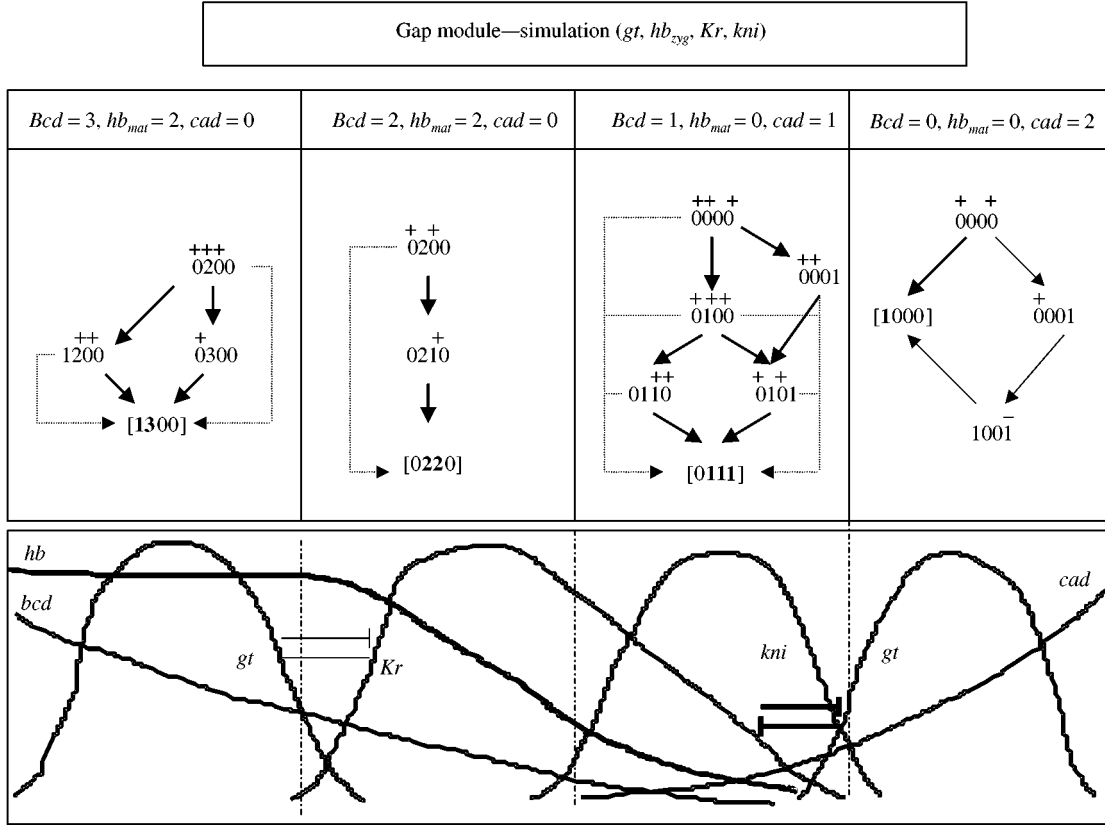


FIG. 3. Simulation of the dynamics of the gap system (wild type). In the lower part, the expression domains of the gap genes in the four regions of the trunk of *Drosophila* embryo are shown (see also Fig. 1). In the upper part, the logical state transitions from wild-type initial conditions are represented. In each region of the embryo, a specific final attractor is selected, characterized by particular maternal positional information made up of *Bcd*, *Cad* and *Hb_{mat}* products. A compact notation is used, consisting of writing the communication orders as variable superscripts instead of expliciting the difference between the variable and function values. A “+” means that the value of the function is greater than that of the variable whereas “−” means that it is smaller. When both values are equal, an attractor state exists which is emphasized by brackets (e.g. [1300] in the most anterior part of the trunk). Starting from wild-type initial conditions, the most biologically credible pathways are indicated by solid arrows, whereas dotted arrows represent theoretically possible but less likely pathways. For further explanation see text.

assumed to involve a single threshold concentration. The generalized logical equations for the interaction graph of Fig. 2 are given in the Appendix.

The response of the zygotic genome to the morphogenetic maternal information laid in the oocyte results in the activation of the gap genes in specific domains along the anterior–posterior axis of the embryo. Figure 3 shows a schematic representation of the expression domains of these genes. It is assumed that the maternal information is divided into four regions depending on the concentration of maternal morphogens. These regions are inferred from experimental data concerning the expression domains of the genes involved:

- Region A is defined by high levels of *Bcd* and medium-high levels of *Hb* (*Bcd* = 3, *Hb_{mat}* = 2 and *Cad* = 0).
- Region B is defined by medium-high levels of *Bcd* and medium-high levels of *Hb* (but see below) (*Bcd* = 2, *Hb_{mat}* = 2 and *Cad* = 0).
- Region C is defined by medium-low levels of *Bcd* and medium levels of *Cad* (*Bcd* = 1, *Hb_{mat}* = 0 and *Cad* = 1).
- Finally, region D is defined by high levels of *Cad* (*Bcd* = 0, *Hb_{mat}* = 0 and *Cad* = 2).

On the basis of the equations in the Appendix, a general “state table” (Appendix, Table A1) can be constructed, giving functions in terms of logical parameters (*K*’s) for all combinations of the

values of variables, i.e. for all qualitatively distinct combinations of regulatory product concentrations. Depending on the K values, the general state table encompasses a large but finite number of different dynamics.

The selection of the values of the logical parameters (K 's) is perhaps the trickiest part of the formalization process. However, in the context of logical formalism, only a small number of integer values are allowed for each parameter. To determine the parameter values and their constraints to have a functional feedback loop, a computer program was used that automates the dynamical analysis of the gene gap system (Thieffry *et al.*, 1993). To further simplify the procedure the lowest parametric values were adopted to enable the generation of expression states compatible with known wild type and mutant phenotypes, thus using a rational trial-and-error process. It is thus important to note that the K values included in Table A1 do not constitute a unique set of values compatible with available data, but rather a *minimalist set*. The non-zero parameters in Table 1 thus identify the most crucial interactions between

the maternal morphogenetic information and the gap genes as well as the interactions among these genes, with which the system can produce the different gap-gene-expression domains along the anterior–posterior axis of the embryo. Recall that each logical parameter (K 's) qualifies the effect of each interaction or combination of interactions controlling the expression of a given gene (for further information, see the Appendix).

THE DYNAMICS OF GENE EXPRESSION

The use of K values from Table 1 in Table A1 (not shown) leads to defined function values (gene expression levels) for each set of variable values (regulatory product concentrations). Whenever all variable values equal all function values, stable state gene expression exists, at least at the level of the gap subsystem. When one or several pairs of variable/function values differ, the system will tend to adopt new variable values (“commutation”). When, in a given state, several variables commute, the next state reached will depend on the relative magnitudes of the time delays

TABLE 1
*Parameter values**

Gene	(A) $b = 3, c = 0, h_m = 2$		(B) $b = 2, c = 0, h_m = 2$		(C) $b = 1, c = 1, h_m = 0$		(D) $b = 0, c = 2, h_m = 0$	
<i>giant</i>	$K_{g,b}$	0	$K_{g,b}$	0	$K_{g,b}$	0	$K_{g,c}$	0
	$K_{g,bh}$	0	$K_{g,bh}$	0	$K_{g,bh}$	0	$K_{g,ch}$	0
	$K_{g,br}$	1	$K_{g,br}$	1	$K_{g,br}$	1	$K_{g,cr}$	0
	$K_{g,bhr}$	1	$K_{g,bhr}$	1	$K_{g,bhr}$	1	$K_{g,chr}$	1
<i>hunchback</i>	$K_{h,c}$	3	$K_{h,\delta}$	2	$K_{h,b}$	1	K_h	0
	$K_{h,eh}$	3	$K_{h,\delta h}$	2	$K_{h,\rho}$	1	$K_{h,h}$	0
	$K_{h,er}$	3	$K_{h,\delta r}$	2	$K_{h,br}$	1	$K_{h,r}$	0
	$K_{h,chr}$	3	$K_{h,\delta hr}$	2	$K_{h,\rho r}$	1	$K_{h,hr}$	0
<i>Krüppel</i>	$K_{r,b}$	0	$K_{r,b}$	0	$K_{r,b}$	0	K_r	0
	$K_{r,bg}$	0	$K_{r,bg}$	0	$K_{r,bg}$	0	$K_{r,g}$	0
	$K_{r,bh}$	0	$K_{r,bh}$	0	$K_{r,bh}$	0	$K_{r,h}$	0
	$K_{r,bn}$	0	$K_{r,bn}$	0	$K_{r,bn}$	0	$K_{r,n}$	0
	$K_{r,bgh}$	1	$K_{r,bgh}$	1	$K_{r,bgh}$	1	$K_{r,gh}$	0
	$K_{r,bgn}$	0	$K_{r,bgn}$	0	$K_{r,bgn}$	0	$K_{r,gn}$	0
	$K_{r,bhn}$	1	$K_{r,bhn}$	1	$K_{r,bhn}$	1	$K_{r,hn}$	0
	$K_{r,bghn}$	2	$K_{r,bghn}$	2	$K_{r,bghn}$	2	$K_{r,ghn}$	0
<i>knirps</i>	$K_{n,b}$	0	$K_{n,b}$	0	$K_{n,bc}$	0	$K_{n,c}$	0
	$K_{n,bg}$	0	$K_{n,bg}$	0	$K_{n,bcg}$	0	$K_{n,cg}$	0
	$K_{n,bh}$	0	$K_{n,bh}$	0	$K_{n,bch}$	0	$K_{n,ch}$	0
	$K_{n,bgh}$	0	$K_{n,bgh}$	0	$K_{n,bcgh}$	1	$K_{n,cgh}$	1

*Logical parameters defining the effects of the interactions between maternal information and gap genes, plus the cross-regulatory interactions among these genes in the four regions of the anterior–posterior axis of wild-type *Drosophila* embryos. The meaning of the parameters (K 's) is explained in the legend of Table A1 and in the body of the Appendix. Left (A) to right (D) parts of the table correspond to the anterior-most to posterior-most regions of the trunk of the embryo. “ b ”, “ c ” and “ h_m ” represent the functional threshold concentrations of the maternal Bcd, Cad and Hb_{mat} products, respectively.

associated with these different commutations (see Thomas & D'Ari, 1990). Figure 3 shows a compact representation of the qualitative dynamics of the gap gene system in response to maternal positional information. Initially, all zygotic genes are "off" ($GHRN = 0000$, where G , H , R , and N denote functions associated with expression of genes *giant*, *hunchback*, *Krüppel* and *knirps*, respectively). However, some Hunchback product is found early in the two most anterior regions A and B of the embryo, as a result of the maternal expression of *hunchback*. Consequently, the initial states in the most anterior regions include non-zero values for the variable associated with the Hb product (h). The initial values of all the other variables are at zero.

Depending on the position of the nuclei in the embryo (i.e. the particular combination and/or concentration of maternal morphogens encountered) and on the genetic interactions between the gap genes, particular combinations of gap genes are activated. One or several spontaneous cascades of state transitions can then be followed, but all lead to a specific state of gene expression in each region of the embryo:

- In region A, the system reaches a state such that only *gt* and *hb* are activated, both at their maximal levels ($GHRN = 1300$).
- In region B, the system reaches a state where *hb* is activated at a high but not maximal level and *Kr* is activated at its maximal level ($GHRN = 0220$).
- In region C, the system reaches a state where *hb*, *Kr* and *kni* are all activated, the first two at their lowest significant levels, the last at its highest level ($GHRN = 0111$).
- Finally, in region D of the embryo, the system reaches a state characterized by the expression of the sole gene *gt* ($GHRN = 1000$).

Thus, in each trunk region of the embryo, a specific state of gene expression is selected. These four different logical states qualitatively match the patterns of gene expression in wild-type embryo, as shown schematically in the lower part of Fig. 3. At the moment, very little is known about the detailed kinetics of gene expression leading to these states. For the time being therefore, the state transition graphs represent several alternative pathways. It is important to keep in

mind, however, that the "final states" reached are, in reality, transient expression patterns due to the modification of the maternal gene product pattern and the later intervention of other regulatory products. In fact, the gap domains constitute the positional information for the activation of the next set of genes in the segmentation hierarchy, the pair-rule genes. As such, the particular combination of active gap genes in the different positions A–D along the anterior–posterior axis of the embryo should be considered to be "attractor" states rather than typical stable states.

Therefore, the logical method applied here allows us to identify the particular combination of active gap genes in the nuclei depending on their position along the anterior–posterior axis of the embryo, as a response to both the maternal information and the cross-regulatory interactions between the gap genes. The gap proteins diffuse in the syncytial blastoderm to form short-range gradients. This logical method also takes into consideration these short-range gradients, in relation to the final gap-gene expression domains, as well as in relation to the activation of pair-rule genes. The coexistence of nuclei with a given functional gap gene and its repressor protein, encoded by another gap gene, is possible if the amount of this repressor protein is below its functional threshold value so as to act upon the former gap gene (see the equations for the gap-gene system in the Appendix). On the other hand, the short-range gradients of the gap proteins may constitute a morphogenetic signal for the activation of the pair-rule genes, whenever these gradients correspond to any of the functional threshold concentration values of gap-gene proteins to activate these genes. Recall that the generalized logical equations explicitly represent the functional level of a gene depending on the concentration of products from their regulatory genes, these products being represented by the logical variables of the equations (for further information see the Appendix).

IDENTIFICATION OF THE MOST CRUCIAL INTERACTIONS

As mentioned earlier, the selection of the parameter values (Table 1) leading to the state transition graphs of Fig. 3 amounts to identifying

the interactions involved in the setting of specific gene expression domains. These interactions are briefly discussed for each gap-gene expression domain.

The Anterior Giant-domain

The formation of this domain depends on the positive effect of Bcd and the negative effect of Kr. Although Hb acts as a repressor of *gt*, it plays no role in the formation of the anterior Gt-domain in normal conditions. This is reflected in the value of the parameter representing the absence of Hb and the presence of Bcd and Kr ($K_{g,bh} = 0$). A crucial role is played by Bcd ($K_{g,br} = 1$, the parameter corresponding to the presence of Bcd in the absence of Kr and Hb) and Kr ($K_{g,b} = 0$, the presence of Bcd, Kr and Hb). This explains why this Gt-domain lies at the upper level of the Bcd gradient and coexists with the high concentration of the Hb-domain. High concentrations of Bcd can thus override the repressor effect of Hb upon *gt*. The posterior border of this Gt-domain is clearly positioned by the repression effect of Kr upon *gt*, whereas its anterior border is likely to be determined by the repression effect of the maternal and/or zygotic terminal system upon *gt* (which is not explicitly considered here).

The Hunchback-domain

The activation of *hb_{zyg}* requires the combined action of Bcd and Hb. There is no proper auto-regulation of *hb*, understood as the capacity of the Hb protein to activate its own gene ($K_{h,h} = 0$) by itself. But Hb does assist Bcd in *hb_{zyg}* activation. The expression level attained by gene *hb_{zyg}* is positively correlated with the different concentrations of the Bcd gradient. The posterior border of the Hb-domain is positioned by the lower part of the Bcd gradient.

The Krüppel-domain

The formation of this domain requires the activity of the anterior and posterior gap genes. The repression effect of either the Gt protein or the high concentration of Hb protein, positions the anterior border of Kr expression. The posterior border is due to repression by Kni and posterior-Gt. In particular, the formation of the Kr-domain

requires the activities of the Bcd and Hb proteins, as well as the absence of the Kni and Gt proteins ($K_{r,bghn} = 2$). The absence of these two repressors does not necessarily lead to the activation of the gene *Kr*, even in the presence of Bcd, since high concentrations of Hb protein function as a repressor ($K_{r,bgn} = 0$).

The Knirps-domain

The Hb protein, acting as a repressor, sets up the anterior border of this domain. The posterior-Gt protein sets up the posterior border. The activator is Cad. Although Bcd also behaves as an activator of *kni*, its contribution is dispensable in otherwise normal conditions. At the higher region of the Bcd gradient, repression by Hb and Gt overrides the activation by Bcd ($K_{n,b} = K_{n,bg} = 0$) and *kni* is consequently not activated.

The Posterior Giant-domain

The activator is Cad. The anterior border is set up by the repression effect of Kr upon gene *gt*. The posterior border is controlled by the repression effect of the posterior Hb-domain and the maternal and/or terminal system (which are not explicitly considered here).

CRUCIAL FEEDBACK CIRCUITS

The gap-gene system depicted in Fig. 2 encompasses seven feedback circuits in total: three positive circuits *hb*, *gt-Kr* and *gt-kni-Kr-hb*; three negative circuits *gt-Kr-hb*, *gt-kni-Kr* and *hb-kni-Kr*; and one dual (positive/negative) circuit *hb-Kr*. A formal analysis of these feedback circuits reveals that the only ones functional are the positive circuit *gt-Kr* and the negative circuit *gt-kni-Kr* (not shown). The first circuit operates in regions A, B and C of the embryo with the restriction that *hb* and *kni* must be expressed ($h = 1$ or 2 , $n = 1$). The second circuit operates in region C of the embryo as long as gene *hb* is active and adopts either value 1 or 2. This indicates that mutual negative interactions between gene *gt* and gene *Kr* are instrumental for the final expression pattern of the gap genes. However, the situation is asymmetric as the action of *Kr* upon *gt* is more important than vice versa. This is reflected in the

values of the corresponding logical parameters (K_g 's and K_r 's in Table 1). Indeed, among the parameters associated with *gt* expression, only those reflecting the absence of *Kr* protein (the K_g 's with "r" in the index) can adopt the value 1. In contrast, some of the logical parameters associated with the expression of *Kr* in the presence of *Gt* protein can adopt value 1 (e.g. $K_{r.bhn} = 1$), meaning that *Kr* can be activated even in the presence of its repressor *Gt*. From a biological point of view, this means that the negative effect of *Kr* upon *gt* is decisive for positioning the posterior border of the anterior *Gt*-domain and the anterior border of the posterior *Gt*-domain. However, the importance of gene *gt* in positioning the anterior border of the *Kr*-domain is diminished by the activity of gene *hb*, whereas the positioning of the posterior border of gene *Kr* is partly assumed by the expression of *kni*.

Simulation and Analysis of Loss-of-function Mutations

One way to test the qualitative robustness and consistency of the presented model consists in checking the effect of mutations. Below, the phenotypes predicted from the simulation of various mutations in the maternal and gap genes are analysed. Some of these predictions have already been experimentally supported, whereas other predictions still await experimental support. Figure 4 presents a summary of the expected mutant phenotypes corresponding to the main single and double loss-of-function mutant combinations in the maternal and gap genes.

BICOID MUTATIONS

Let us assume there is a loss-of-function mutation in gene *bcd*. The zygotes derived from



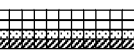

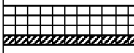
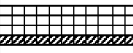
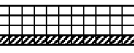



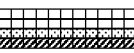
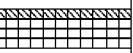


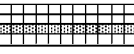
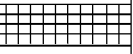


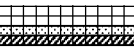
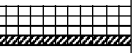






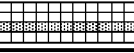


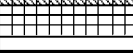








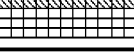
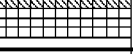

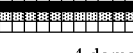

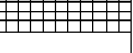
Genetic background (loss-of-function)	«Final state» <i>GHRN</i>				Functional predictions
	A	B	C	D	
<i>wild-type</i>					
<i>bicoid</i>					Loss of <i>giant</i> expression in A Loss of <i>hunchback</i> and <i>Krüppel</i> expression in B and C Activation of <i>knirps</i> in A and B
<i>hunchback_{mat}</i>					Wild type
<i>caudal</i>					Increase of <i>Krüppel</i> expression in C Loss of <i>knirps</i> expression in C Loss of <i>giant</i> expression in D
<i>giant</i>					Activation of <i>knirps</i> in D
<i>Krüppel</i>					Expression of <i>giant</i> in B and C
<i>knirps</i>					Increase of <i>Krüppel</i> expression in C
<i>hunchback_{mat+zyg}</i>					Activation of <i>giant</i> in all regions Loss of <i>Krüppel</i> expression in B and C Loss of <i>knirps</i> expression in C
<i>giant-Krüppel</i>					Activation of <i>knirps</i> in D
<i>Krüppel-knirps</i>					Activation of <i>giant</i> in B and C
<i>giant-knirps</i>					Increase of <i>Krüppel</i> expression in C
4 domains					
A —————> P					

FIG. 4. Simulation of loss-of-function mutations. *G*, *H*, *R* and *N* stand for genes *giant*, *zygotie hunchback*, *Krüppel*, and *knirps*, respectively. A–D correspond to the gap expression domains along the anterior (A) posterior (P) axis defined in the text. The small squares represent nuclei at the syncytial blastoderm stage populating A–D regions. The white squares indicate lack of expression of any gap gene. (▨) represents expression of gene *giant*. (■) represent expression of gene *hunchback* at levels 3, 2 and 1, respectively. (▩) represent expression of gene *Krüppel* at levels 2 and 1, respectively. (▧) represents expression of gene *knirps*.

oocytes produced by females homozygous for this mutation will thus lack functional Bcd protein. Formally, this amounts to saying that the logical parameters corresponding to *giant* expression (column G in Table A1) at positions A and B will lack Bcd contribution (absence of “b” subscript), so none of them can take a non-zero value. Similarly, the parameter corresponding to *hb* expression will also lack all the subscripts involving a positive contribution of Bcd (ε , δ , ρ and b subscripts) and thus all take the zero value. Consequently, neither *gt* (Eldon & Pirrotta, 1991; Kraut & Levine 1991a) nor *hb_{zyg}* (Driever & Nüsslein-Volhard, 1989; Struhl, 1989; Driever *et al.*, 1989a, b) genes can be activated in regions A and B of *bcd* mutants, as previously reported. However, *bcd* mutant embryos do contain *cad* mRNA distributed along the anterior–posterior axis, leading to significant Cad protein concentration—the activator of gene *gt* and *kni*—in all four trunk regions. This is in contrast to wild-type embryos where functional Bcd protein prevents *cad* mRNA translation in the anterior half (see Introduction). In addition, these mutant embryos also contain Hb_{mat} protein—the repressor of genes *gt* and *kni*—in the anterior half. Therefore, different developmental responses of the nuclei populating the anterior half of *bcd* mutant embryos are expected depending on their Hb_{mat} content.

It has been reported that embryos lacking *bcd* and *hb_{zyg}* activity from mothers containing either 0, 1 or 2 copies of *hb* show different phenotypes (Hülskamp *et al.*, 1990). Let us assume that the mother carries two doses of *hb*⁺ so that the embryo has a normal amount of Hb_{mat} protein ($h = 2$). In the absence of Bcd protein, there is no activation of *gt* or of *hb_{zyg}*. In addition, the Cad_{mat} protein present can neither activate *kni* nor *gt* because of the presence of Hb_{mat}. On the other hand, *Kr* is activated by the Hb_{mat}, although its activation is not maintained in the absence of the zygotic Hb product ($K_{r,gn} = 0$). The result is that in the anterior half of *bcd* mutant embryos the system is led to a state with no gap-gene expression at all ($GHRN = 0000$), even in the presence of normal levels of Hb_{mat} protein. Such a situation has been observed (Hülskamp *et al.*, 1990).

Now, let us assume that the mother carries one dose of *hb*⁺ so that the embryo contains half of the normal amount of Hb_{mat} protein ($h = 1$). In this situation, there is still no order to activate *gt* or *hb_{zyg}* since there is no Bcd protein. On the other hand, *Kr* is activated by Hb_{mat}, although its activation is not maintained in the absence of the zygotic Hb product. There is, however, an order to activate gene *kni*, because its activator Cad_{mat} is present, whereas the Hb_{mat} level is too low to repress *kni* ($h = 1$). Moreover, Cad_{mat} cannot activate *gt* because the amount of Hb_{mat} is sufficient to prevent this activation (see equations in the Appendix). The system will thus proceed to a state characterized by the sole expression of *kni* ($GHRN = 0001$). This agrees with experimental data showing an anterior expansion of the Kni-domain in such embryos (Hülskamp *et al.*, 1990).

Finally, let us assume that the mother is also mutant for *hb* so that the embryos lack Hb_{mat} as well as Bcd protein ($b = h = 0$). In this situation, though there is still no activation of *hb_{zyg}* or *Kr*, both *kni* and *gt* can be activated due to the presence of Cad_{mat} and the absence of Hb_{mat}. For the anterior half of these embryos, the simulations lead to a state where only *gt* is activated ($GHRN = 1000$). It has been reported, however, that in these embryos there is an anterior expansion of the Kni-domain (Hülskamp *et al.*, 1990). This discrepancy is further analysed in the Discussion.

Several authors have shown that increasing doses of *bcd* in the mother shifts the formation of the segments posteriorly, whereas low doses of this gene shifts the formation of segments anteriorly (Driever & Nüsslein-Volhard, 1988; Struhl *et al.*, 1989; Driever *et al.*, 1989a, b). Such increases or decreases in the dose of maternal *bcd* can be simulated by changing the appropriate parameters (e.g. converting $K_{h,\delta}$ into $K_{h,\varepsilon}$ for an increased dose of Bcd in region B, or converting $K_{h,\varepsilon}$ into $K_{h,\delta}$ for a decreased dose of Bcd in region A. This results in an anterior or posterior shift in the formation of segments.

CAD MUTATIONS

Let us assume a loss-of-function mutation in gene *cad* so that the embryos lack any functional

Cad product. In this situation, regions A and B are unaffected since there is no Cad expression in the wild type. In region C, hb_{zyg} and Kr will be activated as in wild-type embryos, whereas gene kni will not. Finally, in region D, our model predicts no expression of the gap genes at all (see below for further discussion).

GIANT MUTATIONS

Let us assume a loss-of-function mutation in gene gt . Formally, this amounts to the elimination of all rows with non-zero values for the “ g ” variable and puts a zero everywhere under the “ G ” function in the state table (Table A1). In region A, the system will be led to a state where hb is expressed at its maximal level ($GHRN = 0300$). In this region, none of the other gap genes is expressed, not even Kr , in spite of the fact that its main repressor (Gt) is not present. This is because high concentrations of Hb still prevent the activation of Kr . In regions B and C, the final states are the same as in the wild type. In region D, however, the system is led to a state characterized by the sole expression of kni ($GHRN = 0001$), corresponding to an expansion of the Kni-domain, as previously reported (Eldon & Pirrotta, 1991; Kraut & Levine 1991a,b; Capovilla *et al.*, 1992).

KRÜPPEL MUTATIONS

Let us now assume a loss-of-function Kr mutation. Final states of gap-gene expression are not affected in the regions A and D. In region B, however, the system will now be driven to a state where gt is activated in addition to hb ($GHRN = 1200$). This corresponds to a posterior expansion of the anterior Gt-domain reported by several authors (Mohler *et al.*, 1989; Eldon & Pirrotta, 1991; Kraut & Levine, 1991a,b; Capovilla *et al.*, 1992). Finally, in region C, our model predicts an anterior expansion of the posterior Gt-domain, invading the Kni-domain and consequently repressing gene kni (with $GHRN = 1100$ as the final state).

KNIRPS MUTATIONS

Let us assume a loss-of-function kni mutation. In this case, only region C is affected, giving rise

to a state where hb is expressed at a low level and Kr at a maximal level ($GHRN = 0120$). Accordingly, nuclei from region C in kni mutant embryos will now express Kr at a higher level than in wild-type embryos. This accounts for the posterior expansion of the Kr-domain reported by Jäckle *et al.* (1986) and Hoch *et al.* (1992).

HUNCHBACK MUTATIONS CAUSING LACK OF MATERNAL HB PRODUCT

It will be recalled that genes hb and nos are transcribed during oogenesis. The hb_{mat} mRNA is distributed throughout the oocyte whereas nos mRNA is sequestered into the posterior pole. After fertilization, the nos mRNA is translated and the protein diffuses towards the anterior region, preventing translation of the hb_{mat} mRNA in the posterior half of the embryo (see Introduction). Embryos derived from nos^- mutant females, however, will have Hb_{mat} protein in the posterior half. Consequently, kni will not be activated in region C ($K_{n.bcg} = 0$) and gt will not be activated in region D ($K_{g.cr} = 0$). This agrees well with experimental data (Hülskamp *et al.*, 1990; Eldon & Pirrotta, 1991; Kraut & Levine, 1991b; Struhl *et al.*, 1992).

It has been reported that the Hb_{mat} protein is dispensable for normal segmentation if the embryo receives a wild-type hb allele from its father (Lehman & Nüsslein-Volhard, 1987). Let us assume that we have such an embryo. Formally, this amounts to using the null state ($ghrn = 0000$) as the initial state in regions A and B. In these regions, however, the system will already be driven towards final states identical to the wild-type situation ($GHRN = 1300$ in region A, $GHRN = 0220$ in region B). Consequently, these embryos develop normally. Since the expression of gene hb_{zyg} requires not only Bcd but also the input of its own product (see above), the question arises as to how the expression of this gene occurs in an embryo without the Hb_{mat} product. In fact, in $hb/+$ embryos lacking Hb_{mat} , gene hb_{zyg} is initially activated by Bcd ($K_{h.b} = 1$). The resulting Hb_{zyg} protein can then act in concert with Bcd to amplify and maintain the expression of hb_{zyg} . In this way, the gene hb is expressed in its normal domain.

ZYGOTIC HUNCHBACK MUTATIONS

Let us assume that we have an embryo mutated in *hb_{zyg}* but containing the Hb_{mat} protein. In region A, *gt* expression is initially activated by the presence of Bcd, whereas *Kr* is activated by the presence of both Bcd and Hb_{mat} (see equations in the Appendix). However, according to our model, the system will move towards a state characterized by the sole expression of *Kr* (*GHRN* = 0020). In region B, *Kr* is still expressed. This agrees with the observation that in *hb_{zyg}* mutants, the Kr-domain expands anteriorly and represses gene *gt* in its normal domain, resulting in a shift of the anterior Gt-domain towards a more anterior position (Eldon & Pirrotta, 1991; Kraut & Levine, 1991a, b).

As Bcd activates both gene *gt* and *Kr*, and as these show mutual repression effects, one may ask why in *hb_{zyg}* mutants there must be an anterior expansion of Kr-domain into the Gt-domain with repression of *gt*, instead of the opposite (i.e. a posterior expansion of Gt-domain into the Kr-domain with repression of *Kr*). In this respect, the presented model predicts that this effect is a consequence of the presence of Hb_{mat}, which activates gene *Kr* in *hb_{zyg}* mutant embryos. Indeed, if we envisage a situation in which Hb_{mat} is also lacking, the model predicts that nuclei in region B will reach a state characterized by the sole expression of *gt* (*GHRN* = 1000). This means that gene *gt* is now activated in position B and, consequently, gene *Kr* is repressed. As in region A, *gt* would also be expressed. This amounts to a posterior expansion of the Gt-domain, resulting in *Kr* repression in both regions A and B. These predictions support the idea that Hb protein, rather than Bcd protein, plays the key role in the activation and maintenance of *Kr* expression. In the absence of both Hb_{mat} protein and *hb_{zyg}* activity, however, the activation and maintenance of *Kr* expression by Bcd is not sufficient to reach levels high enough to repress *gt* expression.

DOUBLE MUTANT CONDITIONS

Only the most interesting combinations are considered here. The predictions regarding other mutant combinations are straightforward.

- In double *gt/hb_{zyg}* mutant embryos, the present model predicts that the Kni-domain

expands posteriorly and invades the posterior Gt-domain. Moreover, the Kr-domain expands anteriorly to invade the anterior Gt-domain (region A of the embryo). Indeed, in region D, the system reaches a state where only *kni* is expressed (*GHRN* = 0001). In region A, only *Kr* is expressed (*GHRN* = 0020). Moreover, it may be predicted that the anterior expansion of the Kr-domain depends on the presence of Hb_{mat}, and that the activation of gene *Kr* in regions A and B is not stable. Both Kni and Kr expansions have been observed experimentally (Kraut & Levine, 1991b; Capovilla *et al.*, 1992).

- In double *gt/Kr* mutant embryos, the model predicts a posterior expansion of the Kni-domain, as a consequence of the absence of Gt. Indeed, in region D the system is driven to a state characterized by the sole expression of *kni* (*GHRN* = 0001). This agrees with experimental data (Capovilla *et al.*, 1992).
- In double *Kr/kni* mutant embryos, the model predicts an anterior expansion of the posterior Gt-domain. In region C, *gt* and *hb_{zyg}* are activated by the presence of Bcd and the system moves towards a state where both genes are simultaneously expressed (*GHRN* = 1100). Though Hb is also present in region C, *gt* activation is possible because its activator Bcd overrides the putative repressor effect of Hb (see above). This implies that Kr—not Hb—is the most crucial repressor involved in determining the normal *gt* expression domain.
- In double *gt/kni* mutant embryos, the model predicts that *Kr* expression increases in region C. Indeed, in this region, *Kr* is activated by the presence of Bcd, leading to a state characterized by the simultaneous expression of *hb* and *Kr* (*GHRN* = 0120). This suggests that *kni* plays the key role in determining the normal *Kr* expression domain.

Discussion

The model presented in this paper provides a consistent formal—though qualitative—representation of the roles of the various genes involved in the earlier steps of the segmentation process in *Drosophila melanogaster*. Not only

does this logical model allow the simulation of the qualitative patterns of gap-gene expression in response to maternal information in wild-type embryos, but can also simulate or predict the effects of various types of single and multiple loss-of-function mutations. In fact, the model also allows the simulation of other classes of mutants, involving for example *cis*-regulatory mutations, via the adjustment of the values of appropriate logical parameters (not shown).

Beyond such simulations, analyses using the model provide new insights into the ways an intertwined gene network functions in order to generate alternative patterns of expressions as a response to combined inputs (levels of maternal Bcd, Hb and Cad products). Indeed, such analyses contribute to the description of the role of the various interactions and their combinations (in particular those forming feedback circuits) in the system's dynamic behavior. In this respect, our analysis particularly emphasizes the crucial role of one single positive circuit, composed of the cross-inhibitory interactions between *gt* and *Kr*. In fact, several authors have already suggested that cross-inhibition between pairs of gap genes might play a crucial role in the establishment of their patterns of expression (see, e.g. Rivera-Pomar & Jäckle, 1996, and cites therein). The present analysis further suggests that only the interactions between *gt* and *Kr* would play such a crucial role, as their mutual inhibition would preclude any significant overlap between the expression domains of these two genes. This situation is reflected formally in the fact that, for the parameter values selected, the *gt-Kr* positive circuit is the only one found functional in most of the embryo.

Finally, a careful analysis of the details of the model (thresholds and logical parameters) and of the corresponding dynamic properties led to some interesting biological considerations. These are discussed below.

THE MATERNAL MORPHOGENETIC INFORMATION

The absence of Hb_{mat} protein does not preclude normal segmentation if the embryo receives a wild-type *hb* allele from its father (Lehman & Nüsslein-Volhard, 1987). Therefore, the presence of Hb_{mat} in the anterior half seems dispensable

for segmentation in wild-type embryos. This suggests that maternal Bcd plays the key role in normal conditions, with the Hb_{mat} product being of secondary importance. However, the model proposed here predicts that the presence of Hb_{mat} product might be the determinant in some gap mutants, such as double loss-of-function *gt/hb_{zyg}* mutants. Indeed, the experimental analysis of *Kr* expression in these mutants has revealed that the *Kr*-domain expands anteriorly (Kraut & Levine, 1991b). Our model predicts that if these double-mutant embryos also lack Hb_{mat}, the anterior expansion of the *Kr*-domain and its expression in region B are not established. Therefore, the presence of Bcd alone is insufficient for *Kr* to attain a significant expression state in the absence of both maternal and zygotic Hb products. This further indicates that segmentation in the anterior half of the embryo requires the cooperation of the Bcd and Hb products, in agreement with the proposal of Reinitz *et al.* (1995) and Rivera-Pomar & Jäckle (1996).

For the sake of simplicity, it was assumed that middle-high levels of Hb_{mat} equals 2 in regions A and B of the embryo. However, the possibility exists that the Hb_{mat} product may be distributed according to a gradient, which might be more appropriately formalized by two different qualitative values (2 and 1) in regions A and B. Indeed, it will be recalled that the Nos protein diffuses anteriorly from the posterior pole and destroys *hb_{mat}* mRNA. Therefore, it is possible that the concentration of Nos in the middle region of the embryo is insufficient to destroy all the *hb_{mat}* mRNA. There may therefore exist an overlap between some Hb_{mat} and some Nos protein. This results in a high concentration of Hb_{mat} protein (value 2) in the anterior region A—where there is no Nos protein—whereas in region B the amount of Hb_{mat} is lower (value 1). A formal analysis of the model, assuming a gradient distribution of Hb_{mat}, suggests that the basic behavior of the system is unaffected (not shown). This result is consistent with the former statement that the Hb_{mat} present in the anterior half is dispensable for proper segmentation in wild-type embryos.

The model also assumes that Cad has two functional threshold concentrations, which activate genes *kni* and *gt* in its posterior domain. The

level associated with *kni* activation is lower than that for *gt*. Such a functional threshold gradient of Cad can be understood in molecular terms by supposing that a certain amount of Bcd protein is needed to destabilize *cad* mRNA. In the lower part of the Bcd gradient, not enough of this protein would be present to destabilize all the *cad* mRNA in that region of the embryo. Only a certain amount of this RNA would thus be degraded, the remainder would be translated. Consequently, a certain amount of Cad protein would be present in that region, coexisting with a low amount of Bcd protein. This would correspond to the region where gene *kni* is expressed.

The assumption of two functional threshold concentrations of Cad might explain the anterior expansion of the Kni-domain observed in mutant embryos lacking Bcd protein as well as the maternal and zygotic Hb products (see above). Indeed, the presented model predicts different quantities of Cad protein in the anterior and posterior halves of such mutant embryos, with sufficient Cad to activate *kni*, but not *gt* protein, in the anterior half. This difference may be due to an initial heterogeneous distribution of *cad* mRNA along the anterior-posterior axis. Indeed, in these mutants, the activation of *hb_{zyg}* does not occur and the *cad* mRNA located in the anterior half can be translated (see Introduction). Cad protein can then reach the level required to activate *kni* but not that needed to activate *gt*. If, on the contrary, *cad* mRNA is homogeneously distributed along the anterior-posterior axis of the embryo, Cad protein would reach the level needed to activate *gt* in the anterior half of the embryo. The model would then predict the activation of *gt*, preventing the expression of *kni*, and thus no anterior expansion of the Kni-domain. This is contrary to the observation of Hülkamp *et al.* (1990). However, since the gene *cad* shows maternal and zygotic expression (Mlodzik *et al.*, 1985; Macdonald & Struhl, 1986; Mlodzik & Gehring, 1987), another mechanism could be involved which produces different amounts of Cad protein in the anterior and posterior halves of the embryo. Indeed, Cad protein could result from the combination of maternal and zygotic *cad* activities, with later expression only occurring in the posterior half. Whatever the molecular mechanism may be, the model predicts

that the amount of Cad protein is lower in the anterior than in the posterior half of mutant embryos lacking Bcd protein as well as maternal and zygotic Hb products.

Remarkably, *hb* and *cad* show similar behavior. Both have maternal and zygotic expression, and both maternal products are dispensable if the embryos receive a wild-type copy of each gene. However, the impact of Hb_{mat} and Cad_{mat} on *Drosophila* segmentation are quite different. Cad_{mat} plays the key role in segmentation of the posterior half of the embryo, whereas Hb_{mat} alone is unable to trigger normal segmentation in the anterior half of the embryo. Indeed, the maternal Bcd product is essential for the activation of *hb_{zyg}* and *gt* in the anterior domain. Therefore, segmentation in *Drosophila* is mainly based on maternal information provided by maternal Bcd for the anterior half, and Cad_{mat} for the posterior half. This relevant role of Bcd, and the superfluous role of Hb_{mat}, seem to be a segmentation strategy that arose in flies since genes homologous to *bcd* have not been found outside higher Dipterans (Sommer & Tautz, 1991; Schröder & Sander, 1993; Stauber *et al.*, 1999). It has recently been shown in *Drosophila* that Hb can control partial formation of the thoracic segments in the absence of Bcd, thus supporting the view that in the flies' ancestors lacking *bcd* Hb_{mat} would control the *hb_{zyg}* gene (Wimmer *et al.*, 2000).

CROSS-REGULATORY INTERACTIONS BETWEEN GAP GENES

It has been proposed that gene *kni* acts as a repressor of gene *gt*, thereby contributing to the fixation of the anterior border of the posterior Gt-domain (Jäckle *et al.*, 1992). However, this Gt-domain is only affected very slightly, if at all, in *kni* mutant embryos (Eldon & Pirrotta, 1991; Kraut & Levine, 1991a), contrary to the expectation if *kni* were repressing *gt* expression. Nevertheless, this weak effect might be a consequence of the posterior expansion of the Kr-domain invading the Kni-domain since Kni is a repressor of gene *Kr*. This would prevent the anterior expansion of posterior Gt-domain, since the Kr protein is a repressor of gene *gt*. Thus, the negative effect of *Kr* upon *gt* could mask the negative effect of *kni* upon *gt*. In the model proposed here, it has

been assumed that *kni* is not a repressor of *gt*. However, the opposite has been also analysed. Let us assume that *kni* acts as a repressor of *gt*. This amounts to introducing a blunt-ended arrow between N and G in Fig. 2, and a new term ($k_{g,n}\bar{n}$) in the equation for gene *gt* (equation G in the Appendix). This leads to other logical parameters in the general state table for regions C ($K_{g.brhn}$, $K_{g.brn}$ and $K_{g.bn}$) and D ($K_{g.crh}$) of the embryo. However, the analysis of the potential values of these parameters and their relationships with other related parameters (cf. Table A1 and parameter inequalities in the Appendix) lead us to dismiss any significant role of *kni* in the establishment of the posterior Gt-domain ($K_{g.brhn} = K_{g.brn} = K_{g.crh} = 1$; $K_{g.bn} \leq 1$; $K_{g.bn} = 0$; $K_{g.brh} = K_{g.crh} = 1$).

In the model proposed here, we ignore the possibility of an auto-regulatory function of *Kr*, understood in terms of a direct feedback of *Kr* upon its own gene—a possibility questioned and discarded by Warrior & Levine (1990). Instead, we assume indirect auto-regulation of *Kr* via the repression of its repressor Hb, as favored by these authors. Nevertheless, we have also analysed the possibility that the gene *Kr* shows a proper auto-regulatory function. This implies the addition of a new term ($K_{r,r}$) in the equation for *Kr* (R) (see the Appendix). But here also, to obtain the right qualitative patterns of gene expression in the different regions of the embryos, we have to select parameter values amounting to the dismissal of any significant role of *Kr* auto-regulation in the determination of the expression domain of this gene in normal conditions.

Several authors proposed that *Kr* could act as a repressor of *hb* since in *Kr* mutant embryos the Hb-domain expands posteriorly (Jäckle *et al.*, 1986). This proposition has been taken into consideration, but our model predicts that this negative effect of *Kr* upon *hb* is of secondary importance and that the maternal Bcd product plays the key role in determining the Hb-domain in the anterior half of the embryo. This can be observed in the values of the logical parameters associated with the activation of hb_{zyg} in the absence of sufficient Bcd (parameters $K_{h,b}$'s with no "b" term in their indices), which all have the value 0. Such a weak inhibitory effect of *Kr* on *hb* is supported by the observation that the posterior

expansion of the Hb-domain in *Kr* mutant embryos seems rather weak and occurs too late in development to be instrumental in the establishment of the Hb-domain (Jäckle *et al.*, 1986; Gaul & Jäckle, 1989).

The values of the logical parameters governing the behavior of the gap-gene system, and the analysis of the feedback circuits, indicate that cross-regulatory interactions between the gap genes is crucial for the formation of their final expression pattern along the anterior-posterior axis of *Drosophila* embryos. With the exception of the positive effect of low concentrations of Hb protein on *Kr* expression, the interactions between the gap genes are negative, whereas those of the maternal morphogens upon these genes are positive. Activators and repressors can be simultaneously present in the expression domains of the gap genes that regulate. For example, the anterior Hb-domain entirely encompasses the anterior Gt-domain. In this domain, both the Bcd and Hb proteins, activator and repressor, respectively, of the gene *gt*, are simultaneously present. In normal conditions, the amounts of Bcd and Hb proteins in the anterior Gt-domain result in the activation of *gt*. However, this relationship depends on the relative concentrations of Bcd and Hb. A change in the ratio of these two proteins in favor of Hb can lead to repression of *gt*. In fact, it has been reported that ectopic expression of *hb* prevents *gt* activation (Eldon & Pirrotta, 1991; Kraut & Levine, 1991b; Struhl *et al.*, 1992).

COMPARISONS WITH OTHER FORMAL APPROACHES

The fascinating establishment of alternate stripes of gene expression along the antero-posterior axis in *Drosophila* has already stimulated a wealth of theoretical studies (see e.g. Meinhardt, 1977, 1978, 1986, 1989; Kauffman, 1981; Goodwin & Kauffman, 1990; Hunding *et al.*, 1990; Kauffman & Goodwin, 1990; Lacalli, 1990; Burstein, 1995; Reinitz *et al.*, 1995, 1998; Reinitz & Sharp, 1995; Bodnar, 1997; Sharp & Reinitz, 1998). A fundamental concept lies at the basis of these studies; namely, that the formation of these stripes is the result of the response of zygotic segmentation genes to maternal positional information (which adopts the form of gradients),

plus the cross-regulatory interactions among these genes (Meinhardt, 1977, 1989). When comparing the regulatory structures corresponding to the different models for the sole gap regulatory module, many differences appear. These differences reflect the difficulty in interpreting the numerous genetic and molecular data obtained with different *in vitro* or *in vivo* methodologies. Nevertheless, focusing on qualitative aspects, it is striking that as few as four interactions are assigned the same sign (i.e. repression vs. activation) in all models, for a total of 16 potential interactions between the four gap genes.

From our point of view, the most impressive results to date are those obtained by Reinitz and co-workers (Reinitz *et al.*, 1995, 1998; Reinitz & Sharp, 1995; Sharp & Reinitz, 1998). The interactions between the four gap genes (*giant*, *hunchback*, *Krüppel*, and *knirps*) are represented by a pre-defined set of generic, nonlinear, differential equations. According to these, all gap genes receive inputs from the main antero-posterior morphogen, Bicoid, and are allowed to interact with each other. A series of real parameters quantify all these interactions as well as the spontaneous decay and the diffusion of all the regulatory factors involved. In the face of a lack of quantitative data, they developed a computationally intensive algorithm (using the physicist's method of "simulated annealing") to obtain a square root fit of their equations with experimental patterns of expressions. In the process, the interactions between gap genes become both qualitatively (signs) and quantitatively (values of the corresponding parameters) defined. Though very attractive, this sort of reverse-engineering strategy implies a series of assumptions required to render the problem computationally tractable. The authors consider only monotonic terms and a single dimension (the antero-posterior axis). In particular, their generic equations exclude beforehand potential context-sensitive interactions since they formalize all regulatory contributions affecting the expression of a given gene as a sum of nonlinear, but monotonous, terms. Similarly, these equations do not allow for multiple interactions between two factors, *a fortiori* multiple interactions with different signs. On the other hand, the strength of the "simulated annealing" method rests on precise knowledge of quantitative data

on gene expression at the cellular level. This led Reinitz and collaborators to develop a set of specific antibodies which revealed the expression of most segmentation genes with nuclear precision (Kosman *et al.*, 1998). The development of computer routines enabling the calibration, classification (according to developmental stages) and enumeration of the hundreds of embryo images produced should soon lead to a very complete, homogeneous and precise set of kinetic data, particularly suited to the needs of the modeler. Although this strategy allows the characterization of the expression of only a limited set of known factors, it does so across whole embryos, revealing crucial regional differences, something impossible with DNA arrays or 2D protein gels.

To a large extent, our theoretical approach, and that of Reinitz and collaborators, complement each other. Our logical analysis should help delineate the roles of the feedback circuits found (in particular that of the positive *gt-Kr* circuit). But quantitative spatio-temporal simulations are also needed for a detailed explanation of the patterns of expression observed experimentally. The combination of the two approaches should lead to our reconsidering conflicting assumptions in the light of new experimental data.

Concluding Remarks

The subject of this manuscript is the formal analysis of the gap-gene network involved in *Drosophila* segmentation. The gap genes are expressed in defined domains along the anterior-posterior axis of *Drosophila* embryos as a response to asymmetric maternal information in the form of several protein concentration gradients in the oocyte. One of the first outcomes of our formal analysis is the proposition of definite numbers of functional concentration ranges for the different maternal protein gradients involved. Indeed, we propose that the maternal Bcd has three functional threshold concentrations, and Cad two, which act upon their target genes. Similarly, we propose that the Hb product has at least three functional threshold concentrations, though the highest levels are only attained through the expression of *hb_{zyg}* in response to the

concentration of maternal Bicoid. These different functional concentrations of Hunchback protein would thus result from the distinct functional concentrations of Bicoid. In this respect, our model also accounts for the fact that the stable activation of gene *hb_{zyg}* requires the concerted action of Bcd and its own Hb product.

A second step in the formalization process consists in ordering the different functional interactions along the scales of regulatory product concentrations. In this respect, our model accounts for the fact that the concentration of Hb protein needed to repress gene *kni* is higher than that needed to repress *gt*. Similarly, our analysis supports the hypothesis that the Cad concentration needed to activate *kni* is lower than that to activate *gt*. Finally, we propose that higher levels of Kr are needed to repress *hb* than to repress *gt*. It is important to note, however, that: (1) these different levels correspond to qualitative, functional distinctions; (2) whenever a single level suffices to explain the different relevant qualitative properties of the system, we opt for the simplest (Boolean) logical representation. This is the case, for example, in the stated formalization of *gt* inhibitory interactions.

In a third step, the interactions between the maternal genes and the zygotic genes, as well as the cross-regulatory interactions among the gap genes, are further specified by logical parameters (*K*'s). Interestingly, the selection of specific values for these logical parameters leads to the identification of the interactions playing the most crucial roles in the formation of the gap-gene expression pattern. In particular, the present analysis emphasizes that the cross-regulatory interaction between *gt* and *Kr* plays a predominant role, but that the interaction of *Kr* upon *gt* is clearly more important than that of *gt* upon *Kr*. The positive and negative interaction of gene *hb* upon gene *Kr* is also found to be crucial, whereas the negative effect of *Kr* upon *hb_{zyg}* is of secondary importance. The negative interactions of *hb* and *gt* on *kni* seem to determine the expression domain of the latter. Finally, *Kr* seems to play no direct role in regulating *kni* expression.

Several authors have previously shown that auto-catalytic loops can transform continuous morphogenetic positional information into discrete genetic signals made up of particular combi-

nations of active genes which, in turn, drive cells into specific developmental pathways (Lewis *et al.*, 1977; Meinhardt, 1978). More recently, we have proposed that such positive feedback circuits can ensure the maintenance of the developmental choice when triggered by a transient morphogenetic signal (Sánchez *et al.*, 1997). The present analysis supports the idea that cross-regulatory interactions between the gap-genes are of paramount importance in transforming the continuous maternal information into discrete gap gene expression domains. In this respect, the main biological consequence of the activity of gap genes consists in the subdivision of the embryo into regions where only one gap protein is present, whereas in other regions two or more gap proteins can overlap to a certain extent. These areas constitute the basic positional information system for activation of the pair-rule genes.

Early insect development is characterized by the occurrence of many nuclear divisions within the egg cytoplasm. The nuclei migrate to the periphery, giving rise to a syncytial layer from which the cellular blastoderm arises. Only some of the blastoderm cells are destined to form the embryo and these cells coalesce to form the germ primordium, which then matures into the germ band (Sander, 1976). Although the germ-band stage is conserved throughout all insects, variation occurs in the size of the germ primordium relative to that of the egg, as well as in the morphological process by which the germ primordium changes into the germ band. In the so-called long germ-band insects (such as *Drosophila*), the embryo develops from most of the blastoderm, and divides up more-or-less synchronously into segments. By contrast, in short germ-band insects (such as *Tribolium*), the small early embryo appears to comprise only the prospective anterior head and a growth zone. The remaining segments arise from the posterior growth zone, consisting of uncommitted cells that continue to proliferate and acquire a segmental identity later in development. (Sander, 1976). What differs in the molecular network that regulates segmentation in these different insects? Are any features of the segmentation gene pathway conserved? The expression patterns of genes homologous to *Drosophila* segmentation genes in

extant Diptera, Lepidoptera, and Coleoptera have been observed (reviewed in Nagy, 1994; Patel, 1994). Although the picture is far from being complete, the actual data strongly suggest that the patterning mechanisms at the end of the segmentation process in *Drosophila* are more conserved than those of earlier steps of the regulatory cascade. For example, in *Tribolium*, the expression domains of the gap, pair-rule and segment-polarity genes in the extending germ-band resemble those of their *Drosophila* homologs (Sommer & Tautz, 1993; Patel *et al.*, 1994; Nagy & Carroll, 1994). The *Tribolium* homologs of the gap genes *hunchback* and *Krüppel* are expressed in regions that give rise to several adjacent segments in the head and thorax, similar to their expression in *Drosophila* (Sommer & Tautz, 1993). This suggests that these conserved genes may serve a similar function in both types of insects. However, the blastoderm expression patterns of these genes are different. As mentioned above, in *Tribolium* both the thorax and the abdomen appear to arise from the most posterior parts of the germ primordium. Consistent with this, the *Krüppel* domain initially forms a cap at the posterior end of the *Tribolium* egg. But later in development, after the embryo has begun to elongate, the *Krüppel* expression domain is restricted to the thorax and the anterior abdomen (Sommer & Tautz, 1993). Thus, with the data currently available, it is not clear whether the posterior boundary of *Krüppel* expression in the *Tribolium* blastoderm corresponds to the same posterior boundary seen once the embryo begins to elongate. Nevertheless, these observations from the short-germ *Tribolium* suggest that the gap-patterning system might function here in a cellular, as opposed to a syncytial environment. This could be possible if junctions that allow the diffusion of various segmentation gene products connect the cells of *Tribolium*, as proposed by Sommer & Tautz (1993). Some gap-gene products belong to steroid receptor superfamily that contains additional members that are dependent on small ligand molecules. Therefore, an alternative possibility is that some of the products of the gap genes in *Tribolium* may act as receptors for small ligands that diffuse between cells, obviating any need from gap-gene products themselves to diffuse between cells (Pankratz & Jäckle, 1993).

Finally, gradients could also be established by the dilution of gene products as cells proliferate and the embryo elongates.

Whatever molecular mechanisms are at the basis of segmentation in the short germ-band insects (such as *Tribolium*), the cross-regulatory interactions between the gap genes and the global properties of the *Drosophila* gap-gene system that have been defined in this manuscript might be also operating in *Tribolium*. As mentioned above, feedback circuits can transform continuous morphogenetic positional information into discrete genetic signals made up of particular combinations of active genes and, in addition, they can also ensure the maintenance of the developmental choice when triggered by a transient morphogenetic signal (Lewis *et al.*, 1977; Meinhardt, 1978; Sánchez *et al.*, 1997). This may represent a general underlying property of developmental genetic systems.

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REFERENCES

- BODNAR, J. W. (1997). Programming the *Drosophila* embryo. *J. theor. Biol.* **188**, 391–445.
- BURSTEIN, Z. (1995). A network model of developmental gene hierarchy. *J. theor. Biol.* **174**, 1–11.
- CAPOVILLA, M., ELDON, E. & PIRROTTA, V. (1992). The *giant* gene of *Drosophila* encodes a b-ZIP DNA-binding protein that regulates the expression of other segmentation gap genes. *Development* **114**, 99–112.
- DRIEVER, W., MA, J., NÜSSLEIN-VOLHARD, C. & PTASHNE, M. (1989b). Rescue of *bicoid* mutant *Drosophila* embryos by Bicoid fusion proteins containing heterologous activating sequences. *Nature* **342**, 149–154.
- DRIEVER, W. & NÜSSLEIN-VOLHARD, C. (1988). The Bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* **54**, 95–104.
- DRIEVER, W. & NÜSSLEIN-VOLHARD, C. (1989). The bicoid protein is a positive regulator of *hunchback* transcription in the early *Drosophila* embryo. *Nature* **337**, 138–143.
- DRIEVER, W., THOMAS, G. & NÜSSLEIN-VOLHARD, C. (1989a). Determination of zygotic domains of gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen. *Nature* **340**, 363–367.
- DUBNAU, J. & STRUHL, G. (1996). RNA recognition and translational regulation by a homeodomain protein. *Nature* **379**, 694–699.
- ELDON, E. D. & PIRROTTA, V. (1991). Interactions of the *Drosophila* gap gene *giant* with maternal and zygotic pattern forming genes. *Development* **111**, 367–378.

- GAUL, U. & JÄCKLE, H. (1987). Pole region-dependent repression of the *Drosophila* gap gene *Küppel* by maternal gene products. *Cell* **51**, 549–555.
- GAUL, U. & JÄCKLE, H. (1989). Analysis of maternal effect mutant combination elucidates regulation and function of the overlap of *Krüppel* and *hunchback* gene expression in the blastoderm *Drosophila* embryo. *Development* **107**, 651–662.
- GOODWIN, B. C. & KAUFFMAN, S. A. (1990). Spatial harmonics and pattern specification in early *Drosophila* development. Part I. Bifurcation sequences and gene expression. *J. theor. Biol.* **144**, 303–319.
- GOUZÉ, J. L. (1998). Positive and negative circuits in dynamical systems. *J. Biol. Systems* **6**, 11–15.
- HOCH, M., GERWIN, N., TAUBERT, H. & JÄCKLE, H. (1992). Competition for overlapping sites in the regulatory region of the *Drosophila* gene *Krüppel*. *Science* **256**, 94–97.
- HOCH, M., SCHRÖDER, C., SEIFERT, E. & JÄCKLE, H. (1990). Cis-acting control elements for *Krüppel* expression in the *Drosophila* embryo. *EMBO J.* **9**, 2587–2595.
- HÜLSKAMP, M., PFEIFLE, C. & TAUTZ, D. (1990). A morphogenetic gradient of hunchback protein organizes the expression of the gap genes *Krüppel* and *knirps* in the early *Drosophila* embryo. *Nature* **346**, 577–580.
- HUNDING, A., KAUFFMAN, S. A. & GOODWIN, B. C. (1990). *Drosophila* segmentation: supercomputer simulation of prepattern hierarchy. *J. theor. Biol.* **145**, 369–384.
- JÄCKLE, H., HOCH, M., PANKRATZ, M. J., GERWIN, N., SAUER, F. & BRÖNNER, G. (1992). Transcriptional control by *Drosophila* gap genes. *J. Cell Sci.* **16** (Suppl.), 39–51.
- JÄCKLE, H., TAUTZ, D., SCHUH, R., SEIFERT, E. & LEHMANN, R. (1986). Cross-regulatory interactions among the gap genes of *Drosophila*. *Nature* **324**, 668–670.
- JACOB, Y., SATHER, S., MARTIN, J. & OLLO, R. (1991). Analysis of *Krüppel* control elements reveals that localized expression results from the interaction of multiple subelements. *Proc. Natl Acad. Sci. U.S.A.* **88**, 5912–5916.
- KAUFFMAN, S. A. (1981). Pattern formation in the *Drosophila* embryo. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **295**, 567–594.
- KAUFFMAN, S. A. & GOODWIN, B. C. (1990). Spatial harmonics and pattern specification in early *Drosophila* development. Part II. The four colour wheels model. *J. theor. Biol.* **144**, 321–345.
- KOSMAN, D., SMALL, S. & REINITZ, J. (1998). Rapid preparation of a panel of polyclonal antibodies to *Drosophila* segmentation proteins. *Dev. Genes Evol.* **208**, 290–294.
- KRAUT, R. & LEVINE, M. (1991a). Spatial regulation of the gap gene *giant* during *Drosophila* development. *Development* **111**, 601–609.
- KRAUT, R. & LEVINE, M. (1991b). Mutually repressive interactions between the gap genes *giant* and *Krüppel* define middle body regions of the *Drosophila* embryo. *Development* **111**, 611–622.
- LACALLI, T. C. (1990). Modelling the *Drosophila*-rule pattern by reaction-diffusion: gap input and pattern control in a 4-morphogen system. *J. theor. Biol.* **144**, 171–194.
- LEHMAN, R. & NÜSSEIN-VOLHARD, C. (1987). *hunchback*, a gene required for segmentation of an anterior and posterior region of the *Drosophila* embryo. *Dev. Biol.* **119**, 402–417.
- LEWIS, J., SLACK, J. M. & WOLPERT, L. (1977). Thresholds in development. *J. theor. Biol.* **65**, 579–590.
- MACDONALD, P. M. & STRUHL, G. (1986). A molecular gradient in early *Drosophila* embryos and its role in specifying the body pattern. *Nature* **324**, 537–545.
- MEINHARDT, H. (1977). A model of pattern formation in insect embryogenesis. *J. Cell Sci.* **23**, 177–189.
- MEINHARDT, H. (1978). Space-dependent cell determination under the control of morphogen gradient. *J. theor. Biol.* **74**, 307–321.
- MEINHARDT, H. (1986). Hierarchical inductions of cell states: a model for segmentation in *Drosophila*. *J. Cell Sci.* **4** (Suppl.), 357–381.
- MEINHARDT, H. (1989). Models for positional signalling with application to the dorsoventral patterning of insects and segregation into different cell types. *Development* **107** (Suppl.), 160–180.
- MLODZIK, M., FJOSE, A. & GEHRING, W. J. (1985). Isolation of *caudal*, a *Drosophila* homeo box-containing gene with maternal expression, whose transcripts form a concentration gradient at the pre-blastoderm stage. *EMBO J.* **4**, 2961–2969.
- MLODZIK, M. & GEHRING, W. J. (1987). Expression of the *caudal* gene in the germ line of *Drosophila*. Formation of an RNA and protein gradient during early embryogenesis. *Cell* **48**, 465–478.
- MOHLER, J., ELTON, E. & PIRROTTA, V. (1989). A novel spatial transcription pattern associated with the segmentation gene, *giant*, of *Drosophila*. *EMBO J.* **8**, 1539–1558.
- MURATA, Y. & WHARTON, R. P. (1995). Binding of pumilio to maternal *hunchback* mRNA is required for posterior patterning in *Drosophila* embryos. *Cell* **80**, 747–756.
- NAGY, L. M. (1994). Insect embryogenesis: a glance posterior. *Curr. Biol.* **4**, 811–814.
- NAGY, L. M. & CARROLL, S. (1994). Conservation of wingless patterning functions in the short-germ embryos of *Tribolium castaneum*. *Nature* **367**, 460–463.
- NÜSSEIN-VOLHARD, C. & WIESCHAUS, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795–801.
- PANKRATZ, M. J., HOCH, M., SEIFERT, E. & JÄCKLE, H. (1989). *Krüppel* requirement for *knirps* enhancement reflects overlapping gap gene activities in the *Drosophila* embryo. *Nature* **341**, 337–340.
- PANKRATZ, M. J. & JÄCKLE, H. (1993). Blastoderm segmentation. In: *The Development of Drosophila melanogaster* (Bates, M. & Martinez-Arias, A., eds), pp. 467–51. New York: Cold Spring Harbor Laboratory.
- PATEL, N. H. (1994). Developmental evolution: insights from studies of insect embryogenesis. *Science* **266**, 581–590.
- PATEL, N. H., CONDRON, B. G. & ZINN, K. (1994). Pair-rule expression of *even-skipped* arwe found in both short- and long-germ beetles. *Nature* **367**, 429–434.
- PLAhte, E., MESTL, T. & OMHOLT, S. W. (1995). Feedback loops, stability and multistationarity in dynamical systems. *J. Biol. Systems* **3**, 409–413.
- RAFF, R. A. & KAUFFMAN, T. C. (1983). *Embryos, Genes and Evolution: the Developmental Genetic Basis of Evolutionary Change*. London: Collier Macmillan Publishers.
- REINITZ, J., KOSMAN, D., VANARIOALONSO, C. E. & SHARP, D. H. (1998). Stripe forming architecture of the gap gene system. *Dev. Genet.* **23**, 11–27.
- REINITZ, J., MJOLSNES, E. & SHARP, D. H. (1995). Model for cooperative control of positional information in *Drosophila* by *bicoid* and maternal *hunchback*. *J. Exp. Zool.* **271**, 47–56.

- REINITZ, J. & SHARP, D. H. (1995). Mechanism of *eve* stripe formation. *Mech. Dev.* **49**, 133–158.
- RIVERA-POMAR, R. & JACKLE, H. (1996). From gradients to stripes in *Drosophila* embryogenesis: filling in the gaps. *Trends Genet.* **12**, 478–483.
- RIVERA-POMAR, R., LU, X., PERRIMON, N., TAUBET, H. & JÄCKLE, H. (1995). Activation of posterior gap gene expression in the *Drosophila* blastoderm. *Nature* **376**, 253–256.
- RIVERA-POMAR, R., NIESSING, D., SCHMIDT-OTT, U., GEHRING, W. J. & JÄCKLE, H. (1996). RNA binding and translational suppression by bicoid. *Nature* **379**, 746–749.
- SÁNCHEZ, L., VANHELDEN, J. & THIEFFRY, D. (1997). Establishment of the dorso-ventral pattern during embryonic development of *Drosophila melanogaster*: a logical analysis. *J. theor. Biol.* **189**, 377–389.
- SANDER, K. (1976). Specification of the basic body pattern in insect embryogenesis. *Adv. Insect Physiol.* **12**, 125–238.
- SAUER, F. & JÄCKLE, H. (1991). Concentration-dependent transcriptional activation or repression by KRÜPPEL from a single binding site. *Nature* **353**, 563–565.
- SCHRÖDER, R. & SANDER, K. (1993). A comparison of transplanted *bicoid* activity and partial *bicoid* homeobox sequences in several *Drosophila* and blowfly species (Calliphoridae). *Roux's Arch. Dev. Biol.* **203**, 34–43.
- SHARP, D. H. & REINITZ, J. (1998). Prediction of mutant expression patterns using gene circuits. *Biosystems* **47**, 79–90.
- SIMPSON-BROSE, M., TREISMAN, J. & DESPLAN, C. (1994). Synergy between the hunchback and bicoid morphogens is required for anterior patterning in *Drosophila*. *Cell* **78**, 855–865.
- SNOUSSI, E. H. (1989). Qualitative dynamics of piece-linear differential equations: a discrete mapping approach. *Dyn. Stability Systems* **4**, 189–207.
- SNOUSSI, E. H. (1998). Necessary conditions for multistationarity and stable periodicity. *J. Biol. Systems* **6**, 3–9.
- SNOUSSI, E. H. & THOMAS, R. (1993). Logical identification of all steady states: the concept of feedback loop characteristic states. *Bul. Math. Biol.* **55**, 973–991.
- SOMMER, R. & TAUTZ, D. (1991). Segmentation gene expression in the housefly *Musca domestica*. *Development* **113**, 419–430.
- SOMMER, R. & TAUTZ, D. (1993). Involvement of an orthologue of the *Drosophila* pair-rule gene *hairy* in segment formation of the short germ-band embryo of *Tribolium* (Coleoptera). *Nature* **361**, 448–450.
- SPRENGER, F. & NÜSSLEIN-VOLHARD, C. (1993). The terminal system of axis determination in the *Drosophila* embryo. In: *The Development of Drosophila melanogaster* (Bates, M. & Martínez-Arias, A., eds), pp. 365–387. New York: Cold Spring Harbor Laboratory.
- ST. JOHNSTON, D. & NÜSSLEIN-VOLHARD, C. (1992). The origin of pattern and polarity in the *Drosophila* embryo. *Cell* **68**, 201–220.
- STAUBER, M., JACKLE, H. & SCHMIDT-OTT, U. (1999). The anterior determinant bicoid of *Drosophila* is a derived Hox class 3 gene. *Proc. Natl Acad. Sci. U.S.A.* **96**, 3786–3789.
- STRUHL, G. (1989). Differing strategies for organising anterior and posterior body pattern in *Drosophila* embryos. *Nature* **338**, 741–744.
- STRUHL, G., JOHNSTON, P. & LAWRENCE, P. A. (1992). Control of *Drosophila* body pattern by the *hunchback* morphogen gradient. *Cell* **69**, 237–249.
- STRUHL, G., STRUHL, K. & MACDONALD, P. M. (1989). The gradient morphogen bicoid is a concentration-dependent transcriptional activator. *Cell* **57**, 1259–1273.
- THIEFFRY, D., COLET, L. M. & THOMAS, R. (1993). Formalization of regulatory networks: a logical method and its automatization. *Math. Modelling Sci. Comput.* **2**, 144–151.
- THOMAS, R. (1973). Boolean formalization of genetic control circuits. *J. theor. Biol.* **42**, 563–585.
- THOMAS, R. (1991). Regulatory networks seen as asynchronous automata: a logical description. *J. theor. Biol.* **153**, 1–23.
- THOMAS, R. & D'ARI, R. (1990). *Biological Feedback*. Boca Raton, FL: CRC-Press.
- THOMAS, R., THIEFFRY, D. & KAUFMAN, M. (1995). Dynamical behaviour of biological regulatory networks. I. Biological role of feedback loops and practical use of the concept of the loop-characteristic state. *Bul. Math. Biol.* **57**, 247–276.
- TREISMAN, J. & DESPLAN, C. (1989). The products of the *Drosophila* gap genes *hunchback* and *Krüppel* bind to the *hunchback* promoters. *Nature* **341**, 335–337.
- TURING, A. M. (1952). The chemical basis of morphogenesis. *Philos. Trans. R. Soc. Lond. B* **237**, 37–72.
- WARRIOR, R. & LEVINE, M. (1990). Dose-dependent regulation of pair-rule stripes by gap proteins and the initiation of segment polarity. *Development* **110**, 759–767.
- WIMMER, E. A., CARLETON, A., HARJES, P., TURNER, T. & DESPLAN, C. (2000). bicoid-independent formation of thoracic segments in *Drosophila*. *Science* **287**, 2476–2479.
- WOLPERT, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. theor. Biol.* **25**, 1–47.
- WOLPERT, L. (1971). Positional information and pattern formation. *Curr. Top. Dev. Biol.* **6**, 183–224.
- WOLPERT, L. (1989). Positional information revisited. *Development* **107** (Suppl.), 3–12.

APPENDIX

The generalized logical equations for the interactions graph (Fig. 2) are

$$\begin{aligned}
 G &= d_g(k_g + k_{g,b}b^{(1)} + k_{g,c}c^{(2)} + k_{g,h}\overline{h^{(1)}} + k_{g,r}\overline{r^{(1)}}), \\
 H &= d_h(k_h + k_{h,\varepsilon}\varepsilon + k_{h,\delta}\delta + k_{h,\rho}\rho \\
 &\quad + k_{h,b}b^{(1)} + k_{h,r}\overline{r^{(2)}}), \quad (\text{A.1})
 \end{aligned}$$

$$R = d_r(k_r + k_{r,b}b^{(1)} + k_{r,h}h + k_{r,g}\bar{g} + k_{r,n}\bar{n}),$$

$$N = d_n(k_n + k_{n,b}b^{(1)} + k_{n,c}c^{(1)} + k_{n,g}\bar{g} + k_{n,h}\overline{h^{(2)}}),$$

with

$$\begin{aligned}
 \varepsilon &= b^{(3)} \wedge h^{(1)}, \\
 \delta &= b^{(2)} \wedge h^{(1)}, \\
 \rho &= b^{(1)} \wedge h^{(1)}, \\
 h &= h^{(1)} \wedge \overline{h^{(3)}}. \quad (\text{A.2})
 \end{aligned}$$

Symbols and Definitions

1. “*G*”, “*H*”, “*R*” and “*N*” are the logical functions corresponding to the genes *giant*, *hunchback*, *Krüppel* and *knirps*, respectively.

2. “*b*”, “*c*”, “*g*”, “*h*”, “*r*” and “*n*” are the logical variables associated with the concentrations of proteins Bicoid, Caudal, Giant, Hunchback, Krüppel and Knirps, respectively. As explained in the text, for biological reasons, three distinct functional thresholds are considered for Bicoid (*b*) and Hunchback (*h*), two for Caudal (*c*) and Krüppel (*r*), and one only for Giant (*g*) and Knirps (*n*). Whenever a superscript is appended to a variable, as in equation set (A.1), it refers to one specific threshold, leading to the definition of a specific Boolean variable. For example, $b^{(2)} = 0$ when the concentration of the Bicoid protein is below its second functional (i.e. $b \leq 1$) threshold, $b^{(2)} = 1$ in the opposite case (i.e. $b \geq 2$).

3. “*k*”s are real parameters, which quantify the weight of each interaction. For example, k_g represents the expression of *giant* in the absence of its activators (Bicoid, Caudal) and in the presence of its repressors (Hunchback and Krüppel), whereas $k_{g,b}$ quantify the sole positive effect of a significant concentration of the activator Bicoid ($b \geq 1$) on the expression of *giant* (*G*).

4. d_g, d_h, d_r and d_n are scaling operators, which transform the bracketed terms into logical parameters. These logical parameters take values according to the scale of the corresponding variables (see below).

5. “ ε ”, “ δ ” and “ ρ ” in the equation for *H*, and “*h*” in equation for *R*, are auxiliary variables whose values are defined as follows: $\varepsilon = 1$ iff $b \geq 3$ and $h = 1$; $\delta = 1$ iff $b \geq 2$ and $h = 1$; $\rho = 1$ iff $b \geq 1$ and $h = 1$; and $h = 1$ iff *h* has a value 1 or 2. Otherwise it has value 0.

6. The terms k_g, k_h, k_r and k_n in eqn. A1 represent the basal expression levels of the corresponding genes *giant*, *hunchback*, *Krüppel* and *knirps*, respectively. Since these genes show no basal expression in wild-type conditions, these basal parameters all take zero values.

From Continuous to Logical Parameters

Point 3 above refers to (positive) real parameters, which quantify the effect of a given factor in the expression of a regulated gene. Of real

interest, however, is the qualitative effect on gene expression, i.e. whether this effect would lead to an expression high enough to reach protein concentrations that allow further interactions in the network. In other words, we only require to know how these real parameters, and some of their sums (representing combinations of interactions converging on a given gene), are located with respect to the functional threshold of the variable corresponding to the regulated gene. This led Snoussi (1989) to define “logical parameters” as briefly explained hereafter.

Depending on the values of the Boolean variables in eqn (A.1), the bracketed term in each equation results in a combination of real parameters. This is then transformed into a specific logical parameter as a result of the application of the scaling operator. For example, the bracketed term in the first equation can lead to

$$K_g = d_g(k_g) \\ \text{for } b = 0, c \leq 1, h \geq 1 \quad \text{and} \quad r \geq 1,$$

$$K_g = d_g(k_g + k_{g,h}) \\ \text{for } b = 0, c \leq 1, h = 0 \quad \text{and} \quad r \geq 1,$$

$$K_g = d_g(k_g + k_{g,b} + k_{g,r}) \\ \text{for } b \geq 1, c \leq 1, h \geq 1 \quad \text{and} \quad r = 0,$$

$$K_g = d_g(k_g + k_{g,b} + k_{g,c} + k_{g,r}) \\ \text{for } b \geq 1, c = 2, h \geq 1 \quad \text{and} \quad r = 0,$$

$$K_g = d_g(k_g + k_{g,b} + k_{g,c} + k_{g,h} + k_{g,r}) \\ \text{for } b \geq 1, c = 2, h = 0 \quad \text{and} \quad r = 0.$$

Note that in the case of negative regulatory effects (e.g. repression of *giant* by Hunchback or Krüppel), a subscript (e.g. *h* or *r* subscript) is included in the logical parameter only when the corresponding regulatory product is below its relevant threshold. In total, 16 different parameters can thus be defined, representing the effects of none, one, two, three, or all four of the different factors affecting *giant* expression. These parameters are not independent, however, as they

TABLE A1
General state stable for the gap-gene network

				(A) $b = 3, c = 0, h_m = 2$				(B) $b = 2, c = 0, h_m = 2$				(C) $b = 1, c = 1, h_m = 0$				(D) $b = 0, c = 2, h_m = 0$			
g	h	r	n	G	H	R	N	G	H	R	N	G	H	R	N	G	H	R	N
0	0	0	0	$K_{g,bhr}$	$K_{h,br}$	$K_{r,bgn}$	$K_{n,bgh}$	$K_{g,bhr}$	$K_{h,br}$	$K_{r,bgn}$	$K_{n,bgh}$	$K_{g,bhr}$	$K_{h,br}$	$K_{r,bgn}$	$K_{n,bcgh}$	$K_{g,chr}$	$K_{h,r}$	$K_{r,gn}$	$K_{n,cgh}$
0	0	0	1	$K_{g,bhr}$	$K_{h,br}$	$K_{r,bg}$	$K_{n,bgh}$	$K_{g,bhr}$	$K_{h,br}$	$K_{r,bg}$	$K_{n,bgh}$	$K_{g,bhr}$	$K_{h,br}$	$K_{r,bg}$	$K_{n,bcgh}$	$K_{g,chr}$	$K_{h,r}$	$K_{r,g}$	$K_{n,cgh}$
0	0	1	0	$K_{g,bh}$	$K_{h,br}$	$K_{r,bgn}$	$K_{n,bgh}$	$K_{g,bh}$	$K_{h,br}$	$K_{r,bgn}$	$K_{n,bgh}$	$K_{g,bh}$	$K_{h,br}$	$K_{r,bgn}$	$K_{n,bcgh}$	$K_{g,ch}$	$K_{h,r}$	$K_{r,gn}$	$K_{n,cgh}$
0	0	1	1	$K_{g,bh}$	$K_{h,br}$	$K_{r,bg}$	$K_{n,bgh}$	$K_{g,bh}$	$K_{h,br}$	$K_{r,bg}$	$K_{n,bgh}$	$K_{g,bh}$	$K_{h,br}$	$K_{r,bg}$	$K_{n,bcgh}$	$K_{g,ch}$	$K_{h,r}$	$K_{r,g}$	$K_{n,cgh}$
0	0	2	0	$K_{g,bh}$	$K_{h,b}$	$K_{r,bgn}$	$K_{n,bgh}$	$K_{g,bh}$	$K_{h,b}$	$K_{r,bgn}$	$K_{n,bgh}$	$K_{g,bh}$	$K_{h,b}$	$K_{r,bgn}$	$K_{n,bcgh}$	$K_{g,ch}$	K_h	$K_{r,gn}$	$K_{n,cgh}$
0	0	2	1	$K_{g,bh}$	$K_{h,b}$	$K_{r,bg}$	$K_{n,bgh}$	$K_{g,bh}$	$K_{h,b}$	$K_{r,bg}$	$K_{n,bgh}$	$K_{g,bh}$	$K_{h,b}$	$K_{r,bg}$	$K_{n,bcgh}$	$K_{g,ch}$	K_h	$K_{r,g}$	$K_{n,cgh}$
0	1	0	0	$K_{g,br}$	$K_{h,er}$	$K_{r,bghn}$	$K_{n,bgh}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bghn}$	$K_{n,bgh}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bghn}$	$K_{n,bcgh}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,ghn}$	$K_{n,cgh}$
0	1	0	1	$K_{g,br}$	$K_{h,er}$	$K_{r,bgh}$	$K_{n,bgh}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bgh}$	$K_{n,bgh}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bgh}$	$K_{n,bcgh}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,gh}$	$K_{n,cgh}$
0	1	1	0	$K_{g,b}$	$K_{h,er}$	$K_{r,bghn}$	$K_{n,bgh}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bghn}$	$K_{n,bgh}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bghn}$	$K_{n,bcgh}$	$K_{g,c}$	$K_{h,r}$	$K_{r,ghn}$	$K_{n,cgh}$
0	1	1	1	$K_{g,b}$	$K_{h,er}$	$K_{r,bgh}$	$K_{n,bgh}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bgh}$	$K_{n,bgh}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bgh}$	$K_{n,bcgh}$	$K_{g,c}$	$K_{h,r}$	$K_{r,gh}$	$K_{n,cgh}$
0	1	2	0	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bghn}$	$K_{n,bgh}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bghn}$	$K_{n,bgh}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bghn}$	$K_{n,bcgh}$	$K_{g,c}$	K_h	$K_{r,ghn}$	$K_{n,cgh}$
0	1	2	1	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bgh}$	$K_{n,bgh}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bgh}$	$K_{n,bgh}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bgh}$	$K_{n,bcgh}$	$K_{g,c}$	K_h	$K_{r,gh}$	$K_{n,cgh}$
0	2	0	0	$K_{g,br}$	$K_{h,er}$	$K_{r,bghn}$	$K_{n,bg}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bghn}$	$K_{n,bg}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bghn}$	$K_{n,bcg}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,ghn}$	$K_{n,cg}$
0	2	0	1	$K_{g,br}$	$K_{h,er}$	$K_{r,bgh}$	$K_{n,bg}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bgh}$	$K_{n,bg}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bgh}$	$K_{n,bcg}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,gh}$	$K_{n,cg}$
0	2	1	0	$K_{g,b}$	$K_{h,er}$	$K_{r,bghn}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bghn}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bghn}$	$K_{n,bcg}$	$K_{g,c}$	$K_{h,r}$	$K_{r,ghn}$	$K_{n,cg}$
0	2	1	1	$K_{g,b}$	$K_{h,er}$	$K_{r,bgh}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bgh}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bgh}$	$K_{n,bcg}$	$K_{g,c}$	$K_{h,r}$	$K_{r,gh}$	$K_{n,cg}$
0	2	2	0	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bghn}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bghn}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bghn}$	$K_{n,bcg}$	$K_{g,c}$	K_h	$K_{r,ghn}$	$K_{n,cg}$
0	2	2	1	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bgh}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bgh}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bgh}$	$K_{n,bcg}$	$K_{g,c}$	K_h	$K_{r,gh}$	$K_{n,cg}$
0	3	0	0	$K_{g,br}$	$K_{h,er}$	$K_{r,bgn}$	$K_{n,bg}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bgn}$	$K_{n,bg}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bgn}$	$K_{n,bcg}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,gn}$	$K_{n,cg}$
0	3	0	1	$K_{g,br}$	$K_{h,er}$	$K_{r,bg}$	$K_{n,bg}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bg}$	$K_{n,bg}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bg}$	$K_{n,bcg}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,g}$	$K_{n,cg}$
0	3	1	0	$K_{g,b}$	$K_{h,er}$	$K_{r,bgn}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bgn}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bgn}$	$K_{n,bcg}$	$K_{g,c}$	$K_{h,r}$	$K_{r,gn}$	$K_{n,cg}$
0	3	1	1	$K_{g,b}$	$K_{h,er}$	$K_{r,bg}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bg}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bg}$	$K_{n,bcg}$	$K_{g,c}$	$K_{h,r}$	$K_{r,g}$	$K_{n,cg}$
0	3	2	0	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bgn}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bgn}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bgn}$	$K_{n,bcg}$	$K_{g,c}$	K_h	$K_{r,gn}$	$K_{n,cg}$
0	3	2	1	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bg}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bg}$	$K_{n,bg}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bg}$	$K_{n,bcg}$	$K_{g,c}$	K_h	$K_{r,g}$	$K_{n,cg}$
1	0	0	0	$K_{g,bhr}$	$K_{h,br}$	$K_{r,bn}$	$K_{n,bh}$	$K_{g,bhr}$	$K_{h,br}$	$K_{r,bn}$	$K_{n,bh}$	$K_{g,bhr}$	$K_{h,br}$	$K_{r,bn}$	$K_{n,bch}$	$K_{g,chr}$	$K_{h,r}$	$K_{r,n}$	$K_{n,ch}$
1	0	0	1	$K_{g,bhr}$	$K_{h,br}$	$K_{r,b}$	$K_{n,bh}$	$K_{g,bhr}$	$K_{h,br}$	$K_{r,b}$	$K_{n,bh}$	$K_{g,bhr}$	$K_{h,br}$	$K_{r,b}$	$K_{n,bch}$	$K_{g,chr}$	$K_{h,r}$	K_r	$K_{n,ch}$
1	0	1	0	$K_{g,bh}$	$K_{h,br}$	$K_{r,bn}$	$K_{n,bh}$	$K_{g,bh}$	$K_{h,br}$	$K_{r,bn}$	$K_{n,bh}$	$K_{g,bh}$	$K_{h,br}$	$K_{r,bn}$	$K_{n,bch}$	$K_{g,ch}$	$K_{h,r}$	$K_{r,n}$	$K_{n,ch}$

1	0	1	1	$K_{g,bh}$	$K_{h,br}$	$K_{r,b}$	$K_{n,bh}$	$K_{g,bh}$	$K_{h,br}$	$K_{r,b}$	$K_{n,bh}$	$K_{g,bh}$	$K_{h,br}$	$K_{r,b}$	$K_{n,bch}$	$K_{g,ch}$	$K_{h,r}$	K_r	$K_{n,ch}$
1	0	2	0	$K_{g,bh}$	$K_{h,b}$	$K_{r,bn}$	$K_{n,bh}$	$K_{g,bh}$	$K_{h,b}$	$K_{r,bn}$	$K_{n,bh}$	$K_{g,bh}$	$K_{h,b}$	$K_{r,bn}$	$K_{n,bch}$	$K_{g,ch}$	K_h	$K_{r,n}$	$K_{n,ch}$
1	0	2	1	$K_{g,bh}$	$K_{h,b}$	$K_{r,b}$	$K_{n,bh}$	$K_{g,bh}$	$K_{h,b}$	$K_{r,b}$	$K_{n,bh}$	$K_{g,bh}$	$K_{h,b}$	$K_{r,b}$	$K_{n,bch}$	$K_{g,ch}$	K_h	K_r	$K_{n,ch}$
1	1	0	0	$K_{g,br}$	$K_{h,er}$	$K_{r,bhn}$	$K_{n,bh}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bhn}$	$K_{n,bh}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bhn}$	$K_{n,bch}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,hn}$	$K_{n,ch}$
1	1	0	1	$K_{g,br}$	$K_{h,er}$	$K_{r,bh}$	$K_{n,bh}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bh}$	$K_{n,bh}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bh}$	$K_{n,bch}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,h}$	$K_{n,ch}$
1	1	1	0	$K_{g,b}$	$K_{h,er}$	$K_{r,bhn}$	$K_{n,bh}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bhn}$	$K_{n,bh}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bhn}$	$K_{n,bch}$	$K_{g,c}$	$K_{h,r}$	$K_{r,hn}$	$K_{n,ch}$
1	1	1	1	$K_{g,b}$	$K_{h,er}$	$K_{r,bh}$	$K_{n,bh}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bh}$	$K_{n,bh}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bh}$	$K_{n,bch}$	$K_{g,c}$	$K_{h,r}$	$K_{r,h}$	$K_{n,ch}$
1	1	2	0	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bhn}$	$K_{n,bh}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bhn}$	$K_{n,bh}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bhn}$	$K_{n,bch}$	$K_{g,c}$	K_h	$K_{r,hn}$	$K_{n,ch}$
1	1	2	1	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bh}$	$K_{n,bh}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bh}$	$K_{n,bh}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bh}$	$K_{n,bch}$	$K_{g,c}$	K_h	$K_{r,h}$	$K_{n,ch}$
1	2	0	0	$K_{g,br}$	$K_{h,er}$	$K_{r,bhn}$	$K_{n,b}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bhn}$	$K_{n,b}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bhn}$	$K_{n,bc}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,hn}$	$K_{n,c}$
1	2	0	1	$K_{g,br}$	$K_{h,er}$	$K_{r,bh}$	$K_{n,b}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bh}$	$K_{n,b}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bh}$	$K_{n,bc}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,h}$	$K_{n,c}$
1	2	1	0	$K_{g,b}$	$K_{h,er}$	$K_{r,bhn}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bhn}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bhn}$	$K_{n,bc}$	$K_{g,c}$	$K_{h,r}$	$K_{r,hn}$	$K_{n,c}$
1	2	1	1	$K_{g,b}$	$K_{h,er}$	$K_{r,bh}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bh}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bh}$	$K_{n,bc}$	$K_{g,c}$	$K_{h,r}$	$K_{r,h}$	$K_{n,c}$
1	2	2	0	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bhn}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bhn}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bhn}$	$K_{n,bc}$	$K_{g,c}$	K_h	$K_{r,hn}$	$K_{n,c}$
1	2	2	1	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bh}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bh}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bh}$	$K_{n,bc}$	$K_{g,c}$	K_h	$K_{r,h}$	$K_{n,c}$
1	3	0	0	$K_{g,br}$	$K_{h,er}$	$K_{r,bn}$	$K_{n,b}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,bn}$	$K_{n,b}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,bn}$	$K_{n,bc}$	$K_{g,cr}$	$K_{h,r}$	$K_{r,n}$	$K_{n,c}$
1	3	0	1	$K_{g,br}$	$K_{h,er}$	$K_{r,b}$	$K_{n,b}$	$K_{g,br}$	$K_{h,\delta r}$	$K_{r,b}$	$K_{n,b}$	$K_{g,br}$	$K_{h,\rho r}$	$K_{r,b}$	$K_{n,bc}$	$K_{g,cr}$	$K_{h,r}$	K_r	$K_{n,c}$
1	3	1	0	$K_{g,b}$	$K_{h,er}$	$K_{r,bn}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,bn}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,bn}$	$K_{n,bc}$	$K_{g,c}$	$K_{h,r}$	$K_{r,n}$	$K_{n,c}$
1	3	1	1	$K_{g,b}$	$K_{h,er}$	$K_{r,b}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\delta r}$	$K_{r,b}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\rho r}$	$K_{r,b}$	$K_{n,bc}$	$K_{g,c}$	$K_{h,r}$	K_r	$K_{n,c}$
1	3	2	0	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,bn}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,bn}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,bn}$	$K_{n,bc}$	$K_{g,c}$	K_h	$K_{r,n}$	$K_{n,c}$
1	3	2	1	$K_{g,b}$	$K_{h,\varepsilon}$	$K_{r,b}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\delta}$	$K_{r,b}$	$K_{n,b}$	$K_{g,b}$	$K_{h,\rho}$	$K_{r,b}$	$K_{n,bc}$	$K_{g,c}$	K_h	K_r	$K_{n,c}$

*The first column encompasses all possible combinations of the values of the four logical variables associated with the different functional levels of the products of *giant* (*g*), *hunchback* (*h*), *Krüppel* (*r*) and *knirps* (*n*). For example, *ghrn* = 0201 represents a state where the levels of Giant and Krüppel are both below their first functional thresholds, with a middle-high level of Hunchback and a high level of Knirps. The other columns give the values for the functions associated with the expression levels of these four genes (*G*, *H*, *R* and *N*) in terms of logical parameters (*K*'s). These parameters are defined as follows: K_g stands for the basal expression of *giant*; $K_{g,b}$ accounts for the activating effect of the sole Bicoid on the expression of *giant*; $K_{g,br}$ qualifies the effect of the combination of the presence of Bicoid (activator) and the absence of Krüppel (repressor) on the expression of *giant*, etc. For the sake of simplicity, “*b*” is omitted in *K*'s subscripts whenever a Greek letter is already present, as Greek symbols represent combinations of Bicoid and Hunchback products. To account for the different inputs from the maternal morphogens, slightly different sets of parameters are associated with the different regions of the trunk of the embryo (regions A–D corresponding to the most anterior through to the most posterior part of the embryo). Depending on the parameter values selected, this general state table may give rise to a large variety of different dynamics.

obey inequalities such as $K_g \leq k_{g,b} \leq k_{g,bc} \leq k_{g,bch} \leq k_{g,bchr}$. In addition, they can take only a limited number of integer values (in this case two, 0 or 1), according to the scale of the corresponding logical variable (g). For further explanations see Thomas & D'Ari (1990). To determine the parameter constraints to have a functional feedback loop, a computer program was used, which automates the dynamical analysis of the gene system (Thieffry *et al.*, 1993). This is based on the notion of loop characteristic state. Each feedback loop can be associated with a specific state, precisely located on the threshold of action of the variables involved in the loop. For specific parameter values, one can show that this characteristic state may be steady, and that at the same time the corresponding feedback loop will be functional (i.e. will generate multistationarity in the case of a positive circuit, homeostasis in the case of a negative circuit; see Snoussi & Thomas, 1993 and Thomas *et al.*, 1995).

On the basis of these equations, a state table can be built, giving, for each possible state of the system (i.e. combination of the values of the variables), the corresponding values for the functions in terms of logical parameters (Table A1). The placing of these values in Table A1 allows: (1) the location of all stable states of the systems (i.e. the states in which all the variable values equal all the corresponding function values, i.e. $ghrc = GHRC$); (2) the reconstruction of the qualitative pathways of gene expression compatible with the logical structure of the system (Fig. 3).

From Bicoid to Hunchback Gradient

The protein Bicoid (b), which is distributed in the form of a gradient, is an activator of *hunchback* (H). Therefore, one possibility would be to express this relationship in formal terms by means of the equation $H = d_h(k_{h,b}b)$. This implies that the expression level of gene H is the same at any point in the Bicoid gradient above the functional threshold concentration of this protein required to activate gene H . Further, the Hunchback protein has a single functional threshold concentration for all its interactions with the other gap genes. However, the activation level of gene H is proportionally related to the

concentration of Bicoid. In addition, the Hunchback protein has different functional threshold concentrations, which act upon their target gap genes. In other words, the gradient of Bicoid protein, with different functional threshold concentrations, is transformed into a gradient of Hunchback protein with its own different functional threshold concentrations. Therefore, the simple equation $H = d_h(k_{h,b}b)$ is incorrect for the representation of the particular relationship between the expression levels of gene H and the concentration of its activator Bicoid. To overcome this problem, a new formal concept is defined for the equation associated with the activation of gene *hunchback* (H) by the Bicoid protein. Since each of the functional threshold concentrations of Bicoid results in a different functional threshold concentration of Hunchback protein, the idea is to attach a Boolean variable to each functional Bicoid threshold. This results in a given functional Hunchback threshold. In other words, this is formally equivalent to the decomposition of the step function in the activation of *hunchback* gene by Bicoid, into three small step functions, each representing the activation level attained by the *hunchback* gene as a response to a given Bicoid concentration. Thus, the equation corresponding to the activation of *hunchback* in response to the Bicoid gradient, and the presence of its own product is

$$H = d_h(k_h + k_{h,\varepsilon}\varepsilon + k_{h,\delta}\delta + k_{h,\rho}\rho + k_{h,b}b^{(1)}), \quad (\text{A.3})$$

where $k_{h,\varepsilon}$ (for $\varepsilon = b^{(3)}$), $k_{h,\delta}$ (for $\delta = b^{(2)}$), and $k_{h,\rho}$ (for $\rho = b^{(1)}$) reflect the effects of different concentrations of Bicoid, ($b = 3, 2$, and 1) in combination with middle levels of Hunchback ($H_{\text{mat}} = 2$) protein on the final levels of zygotic *hunchback* product reached ($h = 3, 2$ and 1). The incorporation of term " $k_{h,b}b^{(1)}$ " may appear redundant. However, though Hunchback auto-activation is dependent on the presence of Bicoid, an activator effect of Bicoid can be observed even in the absence of Hunchback (e.g. in some *hunchback* loss-of-function mutants) (Simpson-Brose *et al.*, 1994). This Hunchback-independent effect of Bicoid on *hunchback* expression is made

explicit in the corresponding equation due to the introduction of the term $k_{h,b}b^{(1)}$. Finally, in addition to the sophisticated control of *hunchback* by combinations of Hb and Bcd proteins, *hunchback*

is also negatively regulated by *Krüppel*. After introducing this additional control, the final equation for *hunchback* (H) become that included in set (A.1).