Inserm
Institut national
de la santé et de la recherche médicale

Technologies Avancées
TAGC
pour le Génome et la Clinique
ERM 206 INSERM

UNIVERSITÉ DE LA MÉDITERRANÉE
AIX-MARSEILLE II

Logical modelling of genetic regulatory networks

Contents

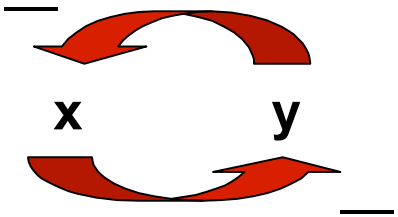
- Boolean modelling of gene networks
- Multilevel logical modelling
- Regulatory circuits
- Application to Drosophila segmentation

Biological regulatory networks

Abstraction levels *versus* biological questions:

- Molecular level: biochemical networks, signal transduction
- **Genetic level: genetic regulatory networks**
- Inter-cellular level: cell differentiation, tissues, patterns
- Macroscopic levels (organs, physiology...)

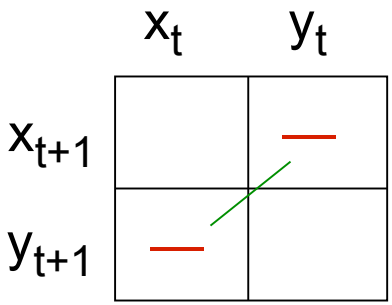
Boolean formalism : synchronous updating (1)



interaction graph

$$\begin{cases} x_{t+1} = \overline{y_t} \\ y_{t+1} = \overline{x_t} \end{cases}$$

logical equations



interaction matrix

$(xy)_t$	$(xy)_{t+1}$
00	11
[01]	01
[10]	10
11	00

state table

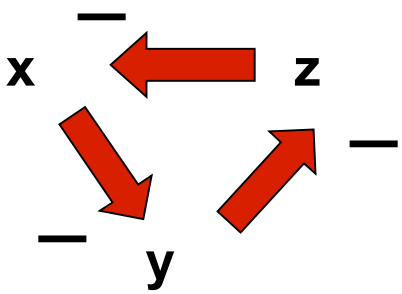
[10] [01]

00

11

attractors

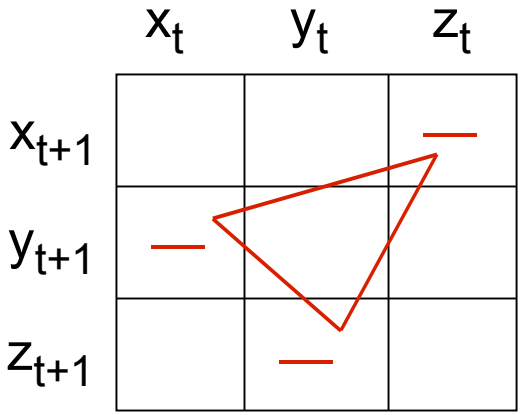
Boolean formalism : synchronous updating (2)



interaction graph

$$\begin{cases} x_{t+1} = \overline{z_t} \\ y_{t+1} = \overline{x_t} \\ z_{t+1} = \overline{y_t} \end{cases}$$

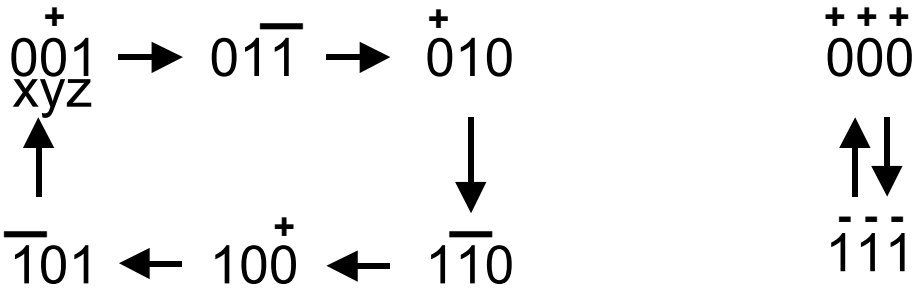
logical equations



interaction matrix

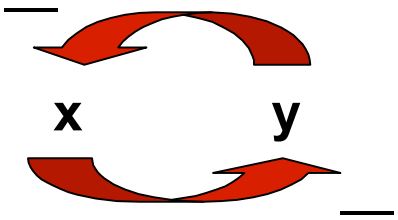
$(xyz)_t$	$(xyz)_{t+1}$
000	111
001	011
010	110
011	010
100	101
101	001
110	100
111	000

state table



spontaneous state transitions

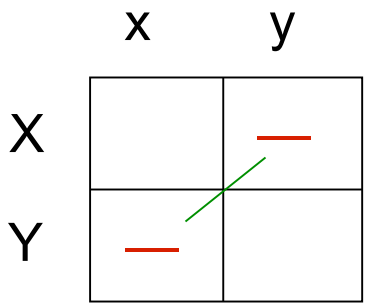
Boolean formalism : asynchronous updating (1)



interaction graph

$$\begin{cases} X = \overline{y} \\ Y = \overline{x} \end{cases}$$

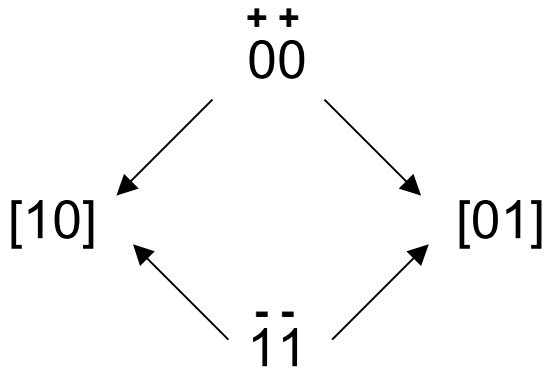
logical equations



interaction matrix

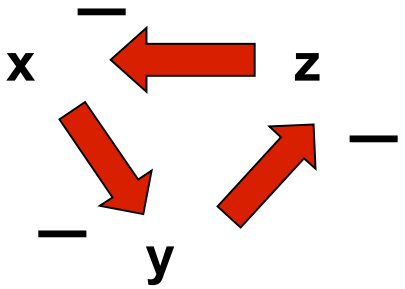
xy	XY
00	11
[01]	01
[10]	10
11	00

state table



spontaneous state transitions

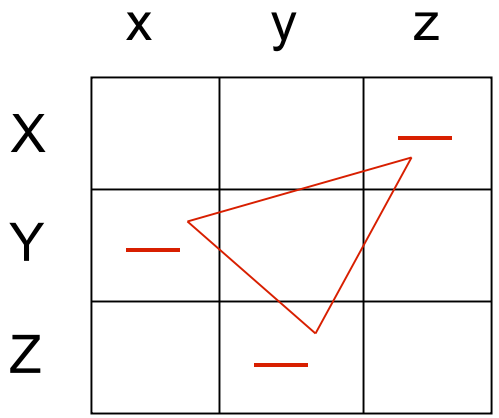
Boolean formalism: asynchronous updating (2)



interaction graph

$$\begin{cases} X = \overline{z} \\ Y = \overline{x} \\ Z = \overline{y} \end{cases}$$

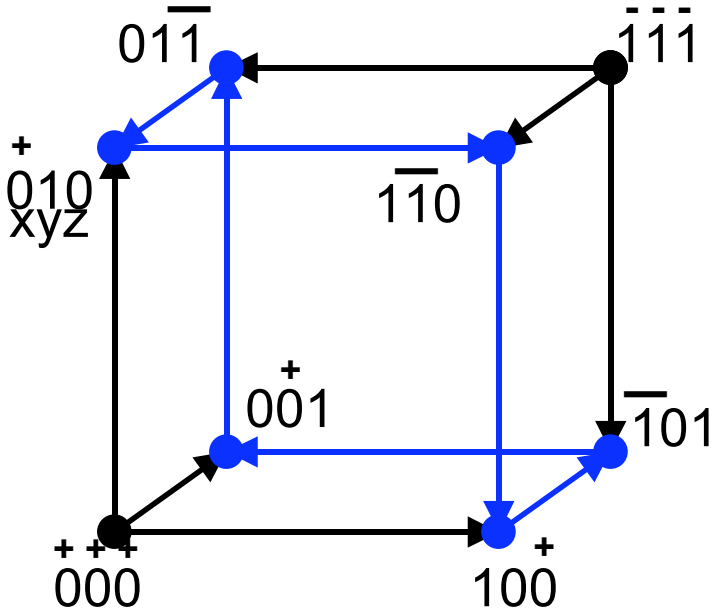
logical equations



interaction matrix

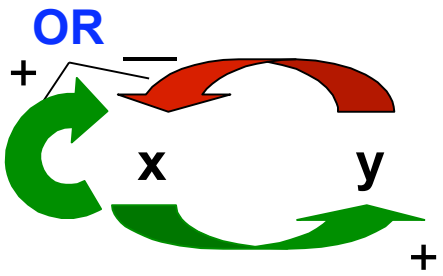
XYZ	xyz
000	111
001	011
010	110
011	010
100	101
101	001
110	100
111	000

state table



spontaneous state transitions

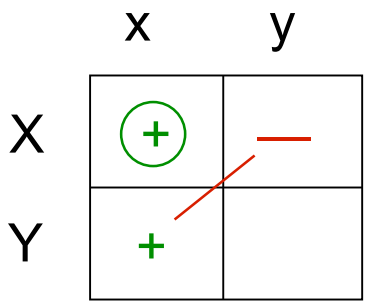
Boolean formalism : logical operators : OR



interaction graph

$$\begin{cases} X = x + \overline{y} \\ Y = x \end{cases}$$

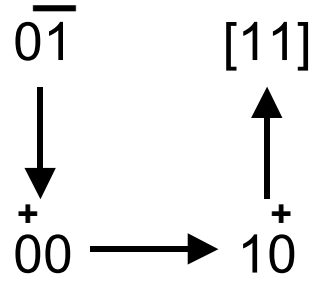
logical equations



interaction matrix

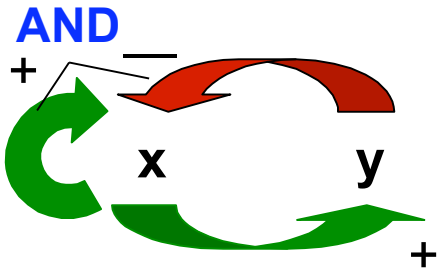
xy	XY
00	10
01	00
10	11
[11]	11

state table



spontaneous state transitions

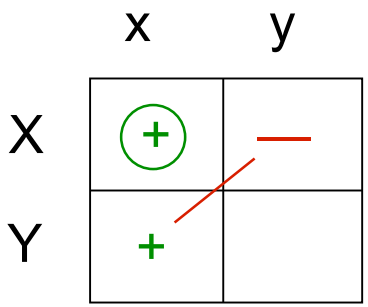
Boolean formalism : logical operators: AND



interaction graph

$$\begin{cases} X = x \,.\, \overline{y} \\ Y = x \end{cases}$$

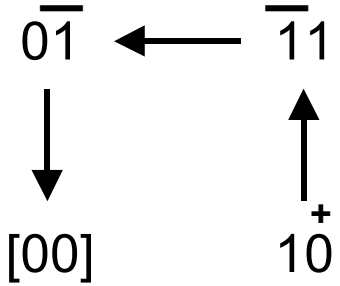
logical equations



interaction matrix

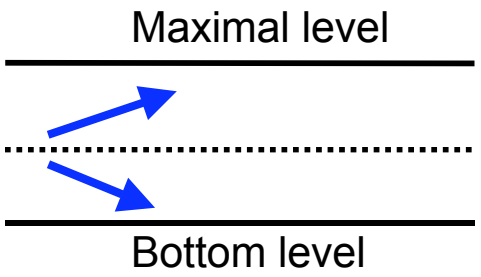
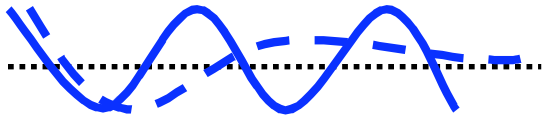
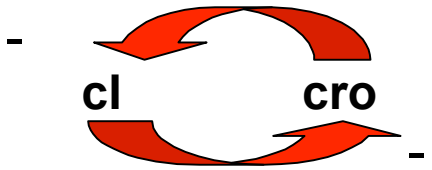
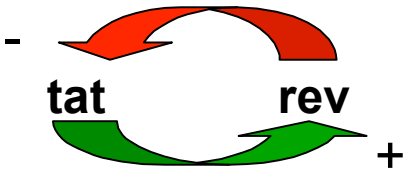
xy	XY
[00]	00
01	00
10	11
11	01

state table



spontaneous state transitions

Regulatory circuits

Characteristics	Positive circuits	Negative circuits
Number of negative interactions	**Even**	**Odd**
Dynamical property		
Biological property	**Differentiation**	**Homeostasis**
Examples		

Feedback circuits & Thomas' rules

- ✓ A **positive feedback circuit** is **necessary** to generate **multiple stable states or attractors**
- ✓ A **negative feedback circuit** is **necessary** to generate **homeostasis or sustained oscillatory behaviour**

Thomas R (1981). *Springer Series in Synergics* **9**: 180-193.

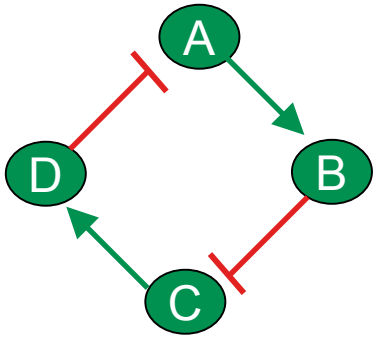
Mathematical theorems and demonstrations:

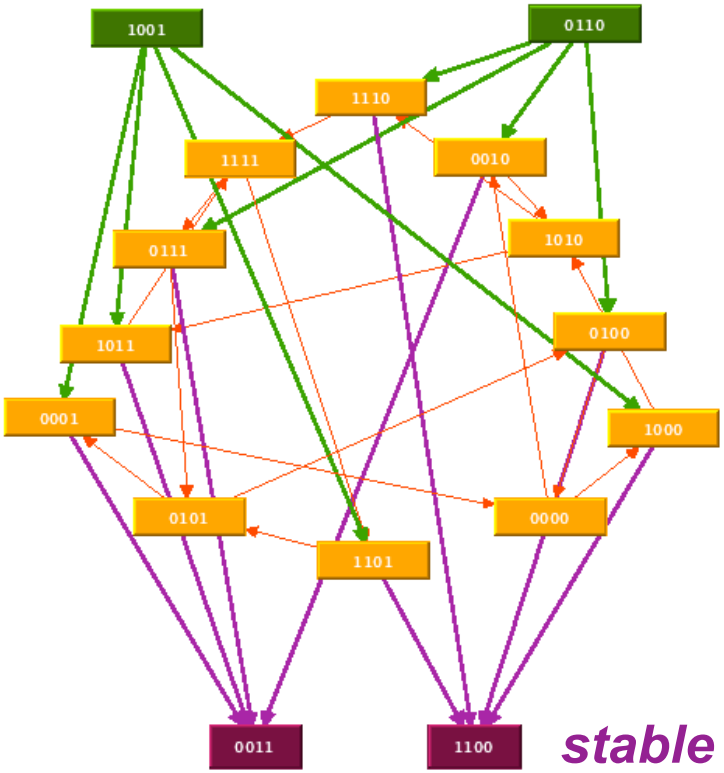
- ✓ **In the differential framework:**
  - Soulé C (2005). *ComPlexUs* **1**: 123–133.
- ✓ **In the discrete framework:**
  - Remy E, Ruet P, Thieffry D (2006). *LNCIS* **341**: 263-70.
  - Richard A (2006). *PhD thesis*, University of Evry, France.

Cf. also Thomas, Snoussi, Plahte, Aracena & Demongeot...

Discrete dynamics of simple feedback circuits

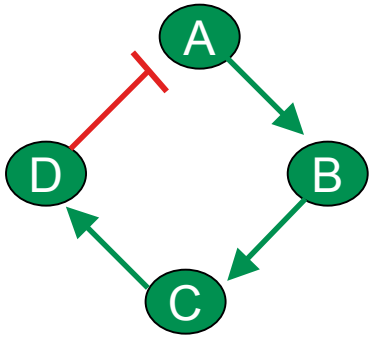
Positive circuit

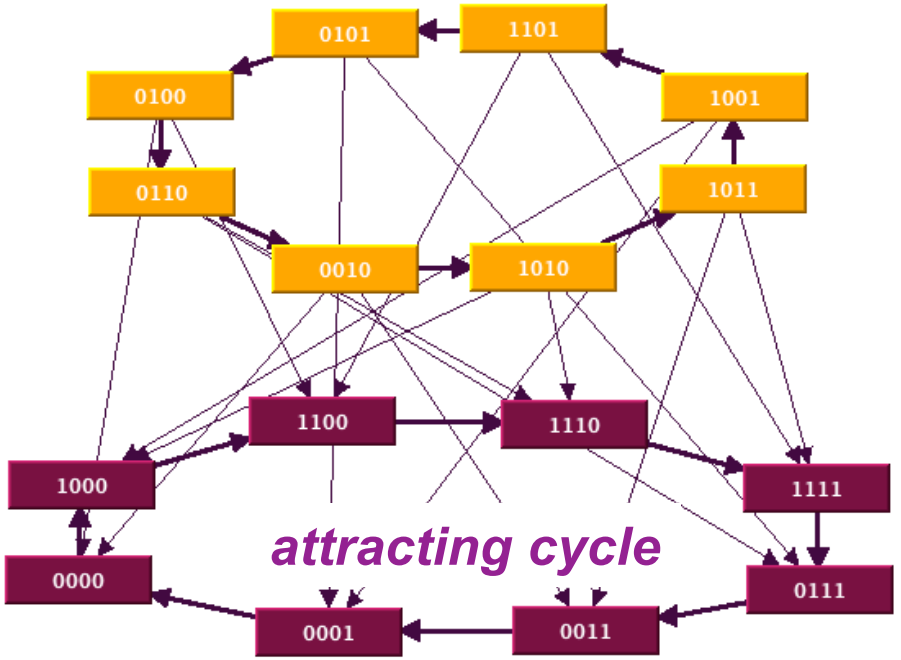




stable states

Negative circuit

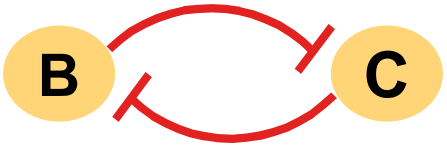




attracting cycle

Remy E, Mosse B, Chaouiya C, Thieffry D (2003). *Bioinformatics* **10**: ii172-8.

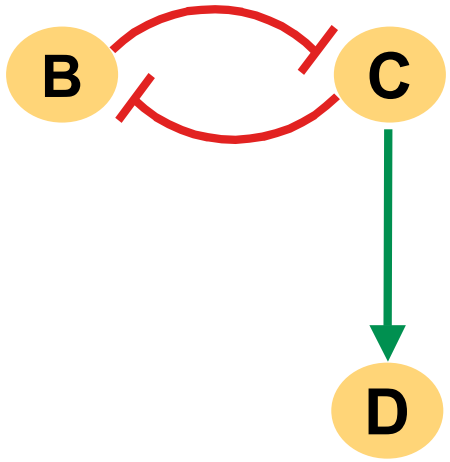
Circuit functionality context



This system typically gives two stable states

01 and **10**

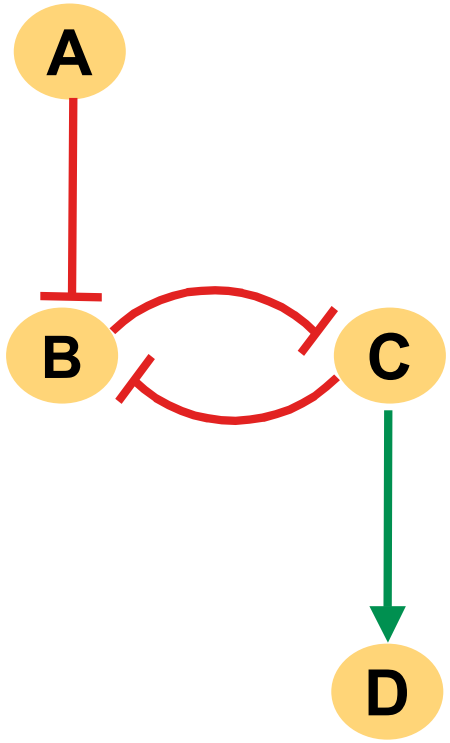
Circuit functionality context



This system typically gives two stable states,

now {B,C,D} = **011** and **100**

Circuit functionality context



Circuit behaviour depends on the effect of A on B

If A alone is able to switch OFF B:

- In the **presence** of **A**:

→ only **one stable state** with {A,B,C,D}= **1011**

- In the **absence** of **A**:

→ **two** stable states **0100** and **0011**

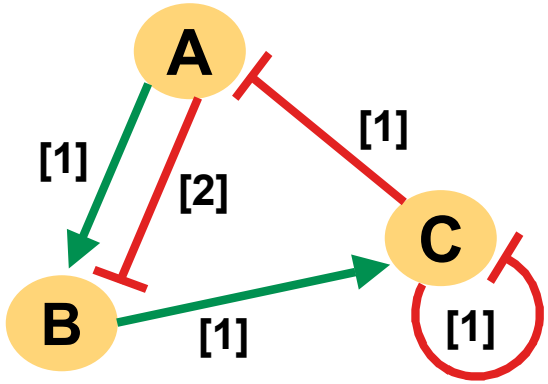
- The **positive cross-inhibitory circuit**

involving **B** and **C** is thus **functional**

only in the **absence of A**.

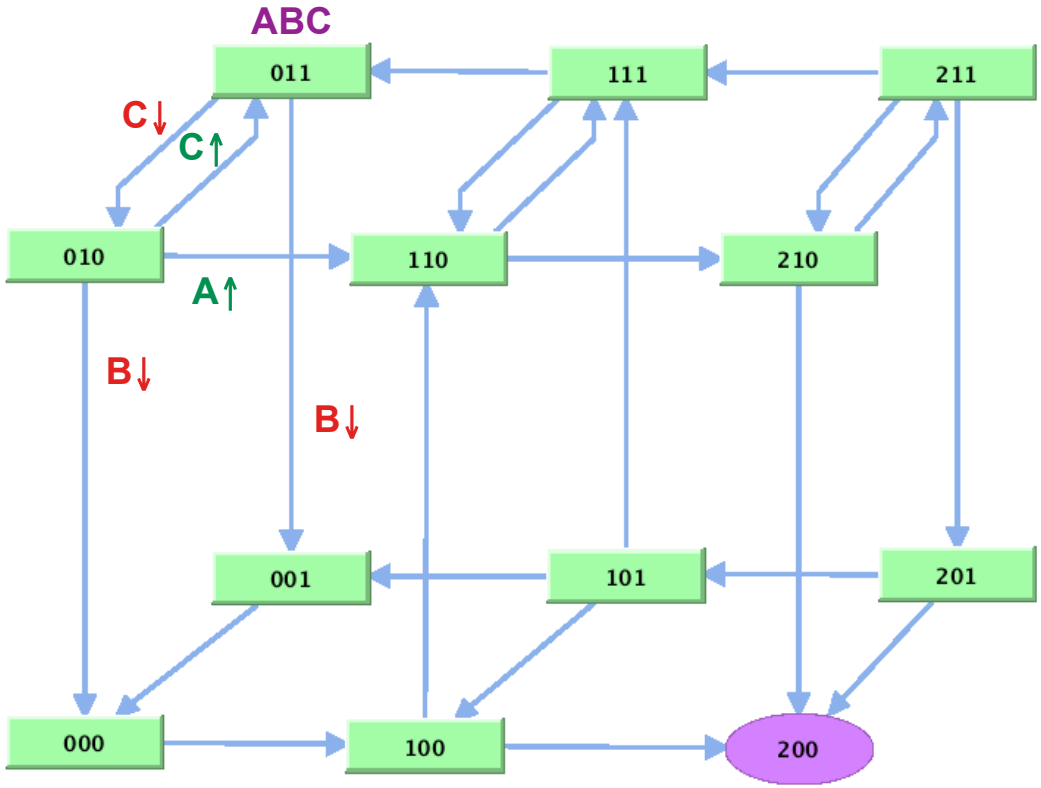
Development of a **computational algorithm** enabling the **analysis** of the **functionality** of **feedback circuits** in the **discrete case** (Naldi *et al*, in prep).

Multilevel modelling of regulatory networks



✓ A **graph** describes the interactions between genes or regulatory products

✓ **Discrete levels** of expression associated to each gene (logical variables) and interaction



✓ **Logical parameters**
define the effect of combinations of incoming interactions

$K_B(\varnothing)=0$

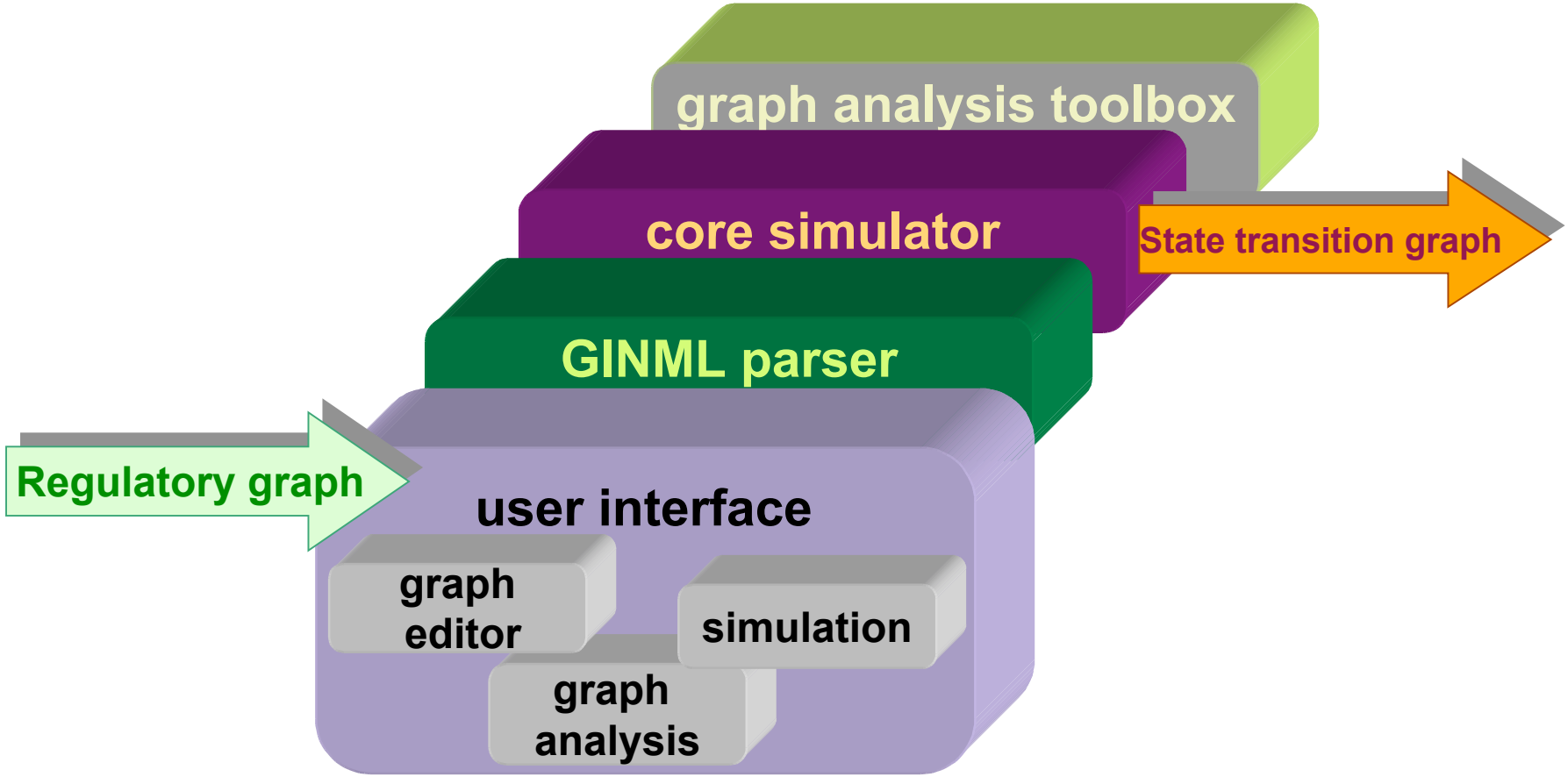
$K_B(\{A,1\})=1$

$K_B(\{A,2\})=0$

✓ The dynamics is represented by a **State Transition Graph** (here, all possible trajectories)

Chaouiya C, Remy E, Mossé B, Thieffry, D (2003). *LNCIS* **294**: 119-26.

GINsim (Gene Interaction Networks simulation)



Available at **http://gin.univ-mrs.fr/GINsim**

Gonzalez A, Naldi A, Sánchez L, Thieffry D, Chaouiya C (2006). *Biosystems* **84**: 91-100.

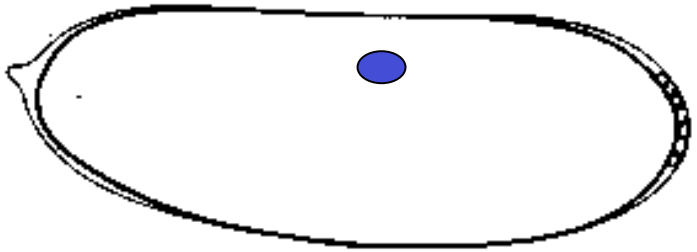
Applications

- **Drosophila development** (IBDML)
  - **Genetic control of segmentation** (L Sánchez, C Chaouiya)
  - Compartment formation in imaginal disks (A. Gonzalez)
- **Cell cycle** (DIAMONDS STREP) (A Fauré)
  - Yeast (*S. cerevisiae*)
  - Mammalian cells
- **T cell differentiation and activation** (A Naldi)
  - Differentiation: Th1/Th2, Regulatory T cells, lymphoid lineages
  - TCR signalling

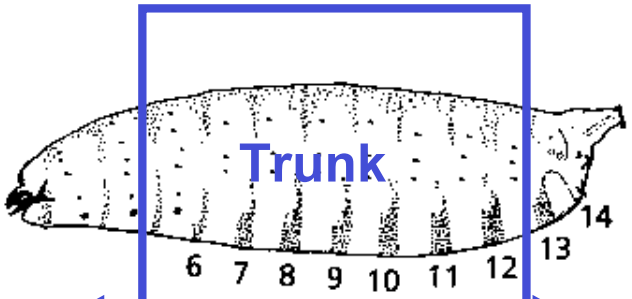
Drosophila Development

Zygote

24 hours

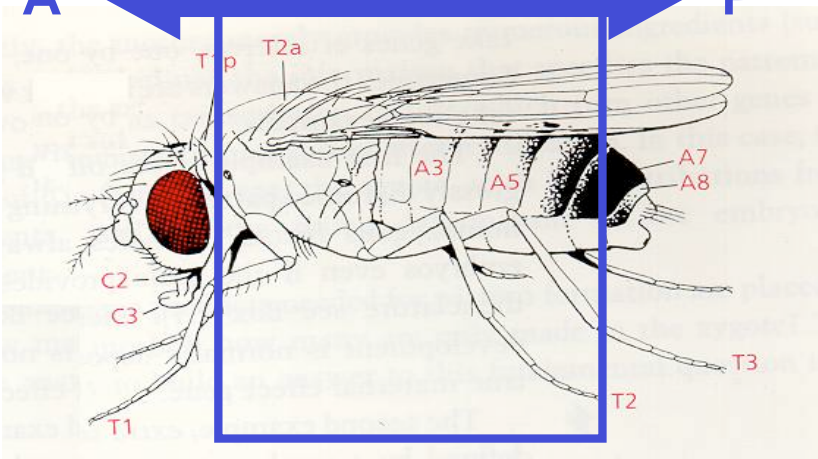


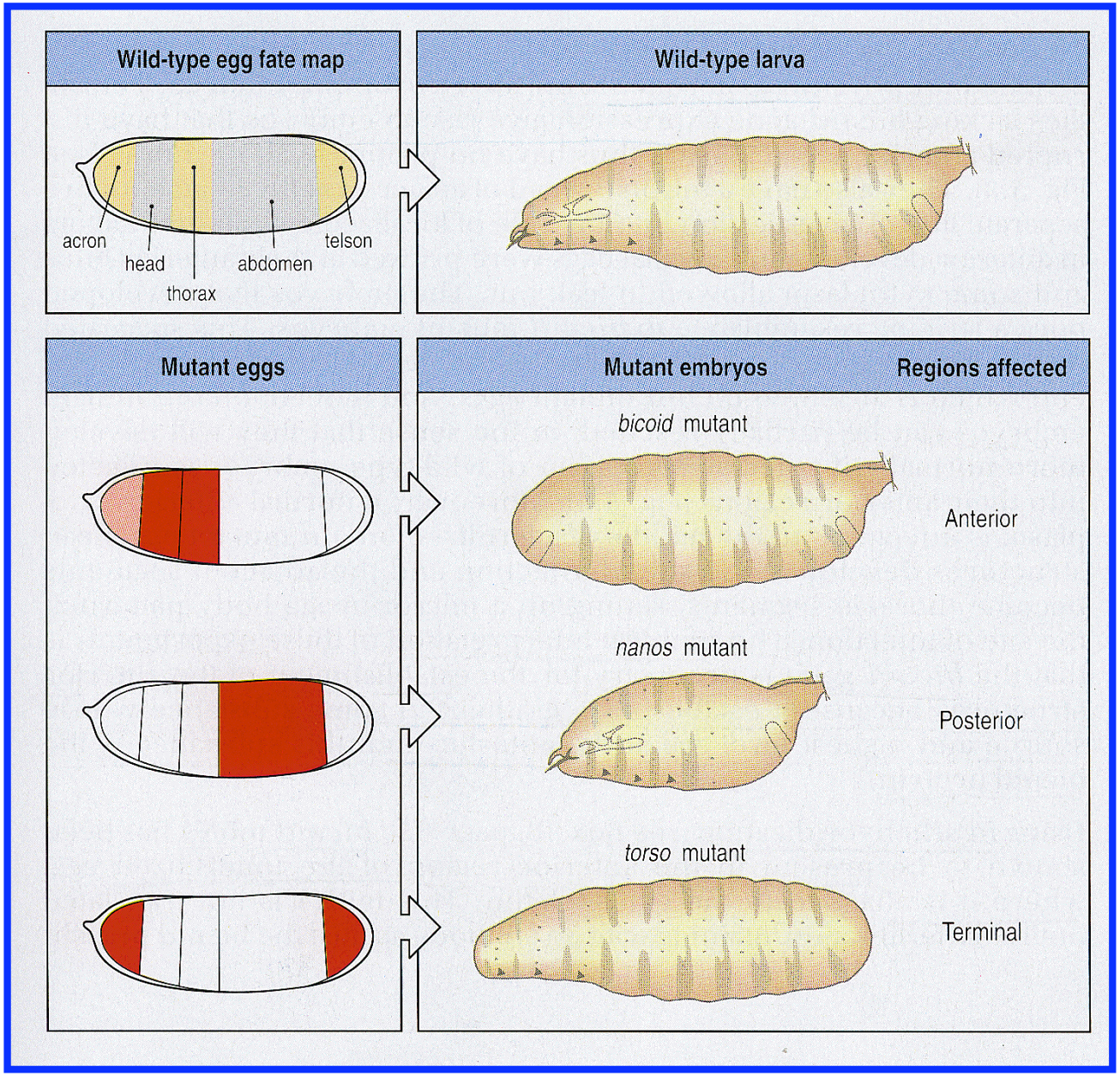
Larva



9 days

Adult





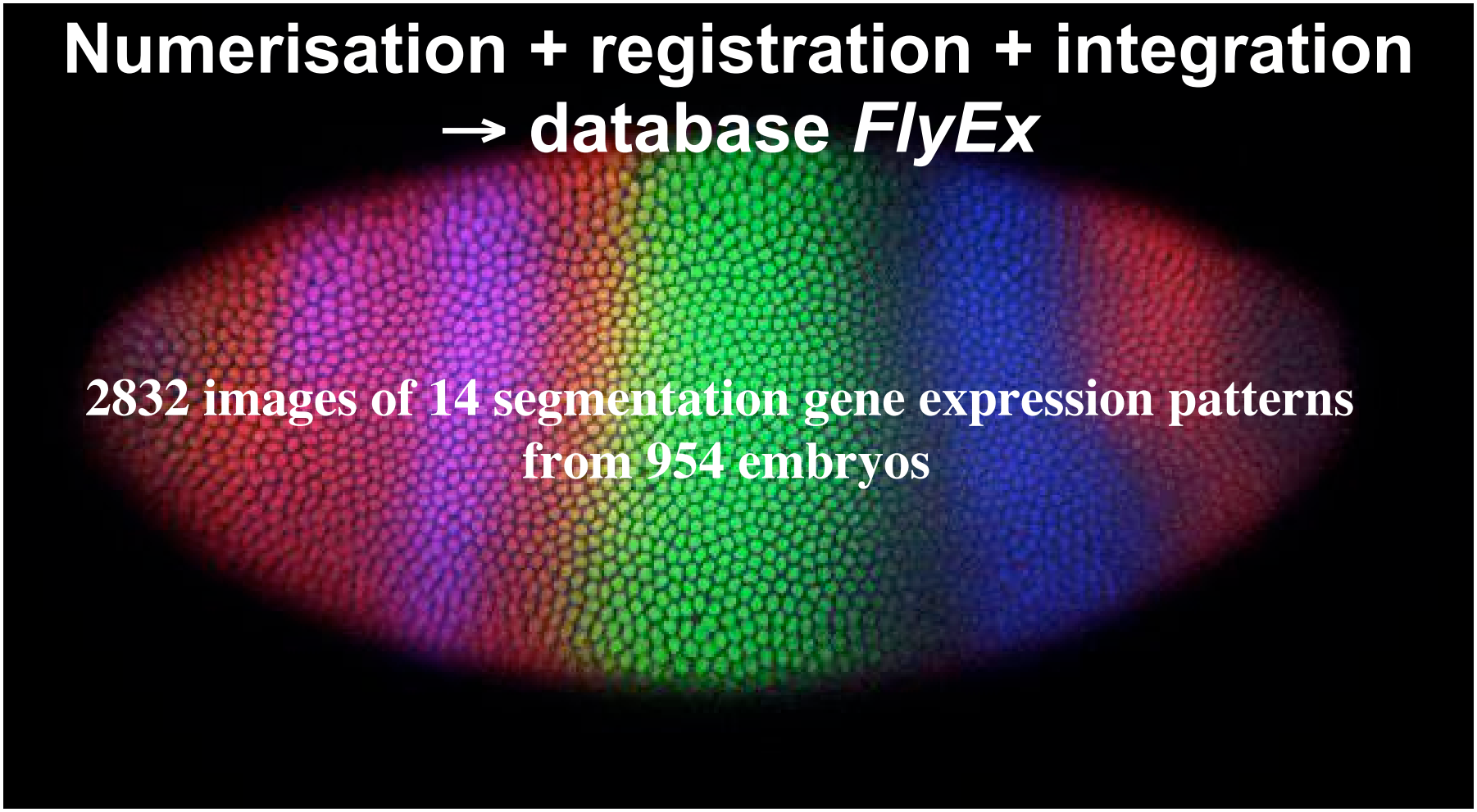
Genetic data

Maternal mutants

High troughput functional arrays: lof mutants, RNAi...

Source: Wolpert *et al.* (1998)

Patterns of gene expression (mRNAs or proteins)

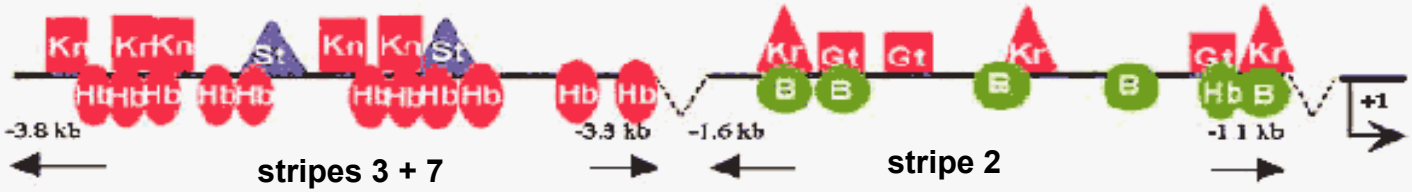


Simultaneous labelling of **HB**, **KR** & **GT** Proteins in *Drosophila* embryo around cell cycle 13 (courtesy John Reinitz).

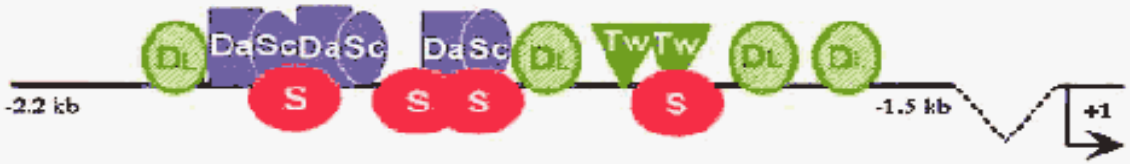
Information on *cis*-regulatory elements in *D. melanogaster*

ftz
zebra element

eve
stripes 2 & 3+7
elements

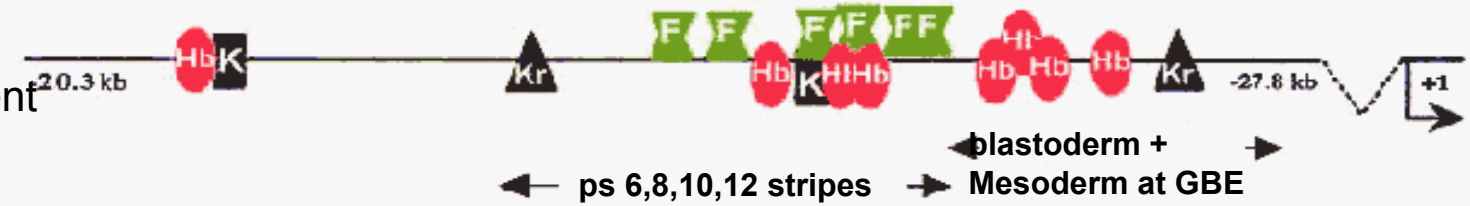


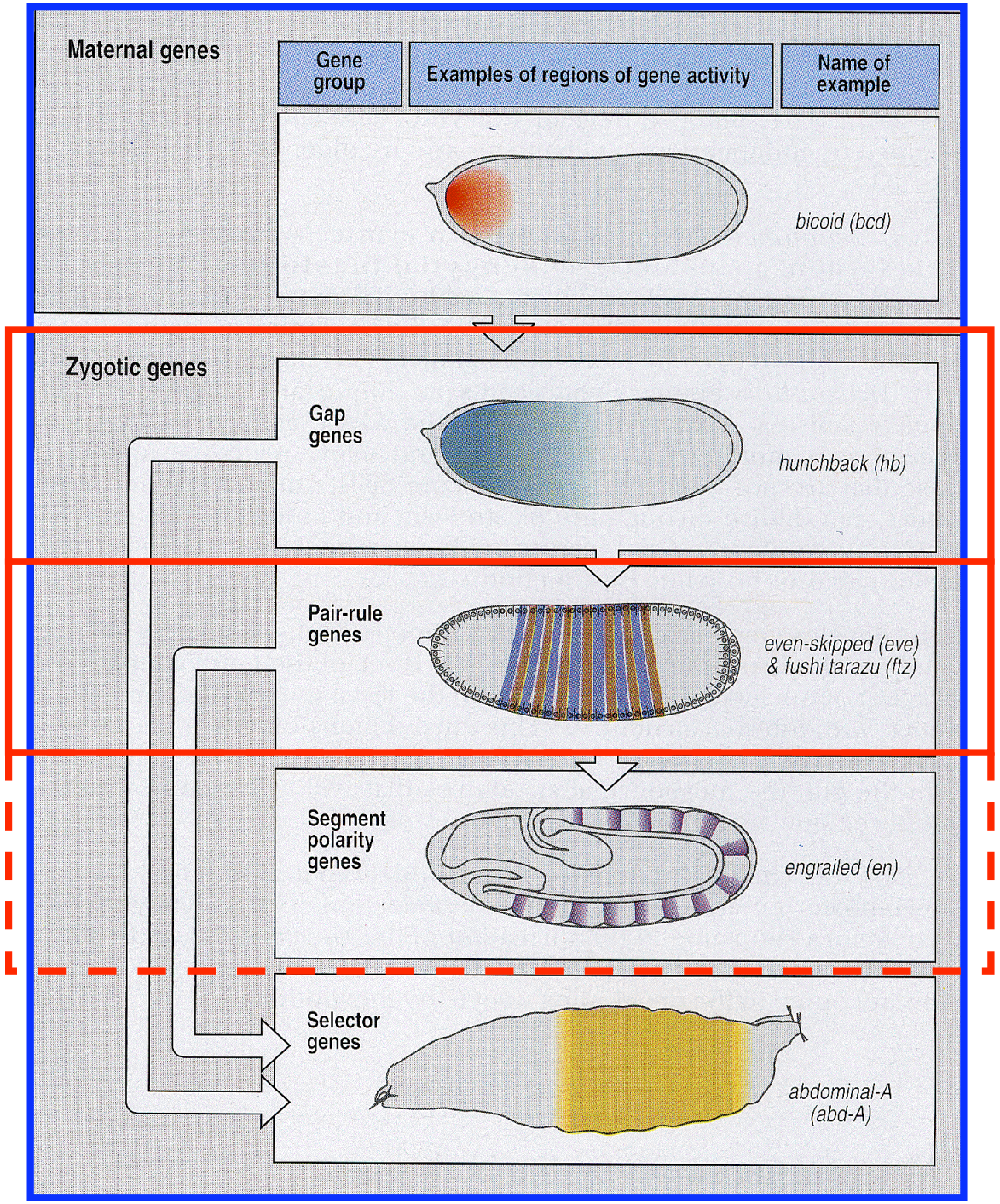
rho
lateral neurectoderm
stripe element



kni
posterior element

Ubx
PBX element





Initiation of segmentation in Drosophila

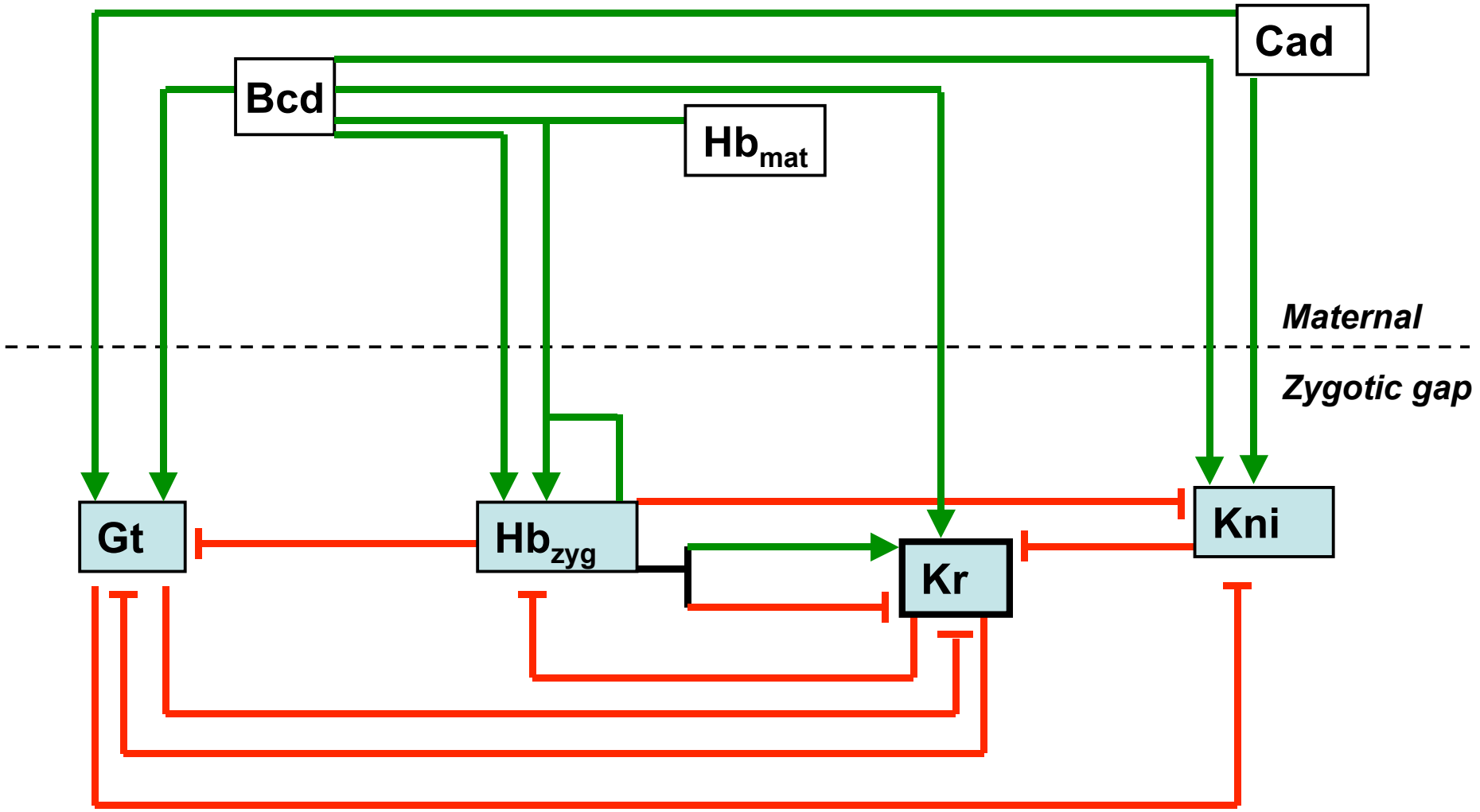
Sánchez L & Thieffry D (2001)
J theor Biol **211**: 115-41

Thieffry D & Sánchez L (2002)
An NY AcadSci **981**: 135-53

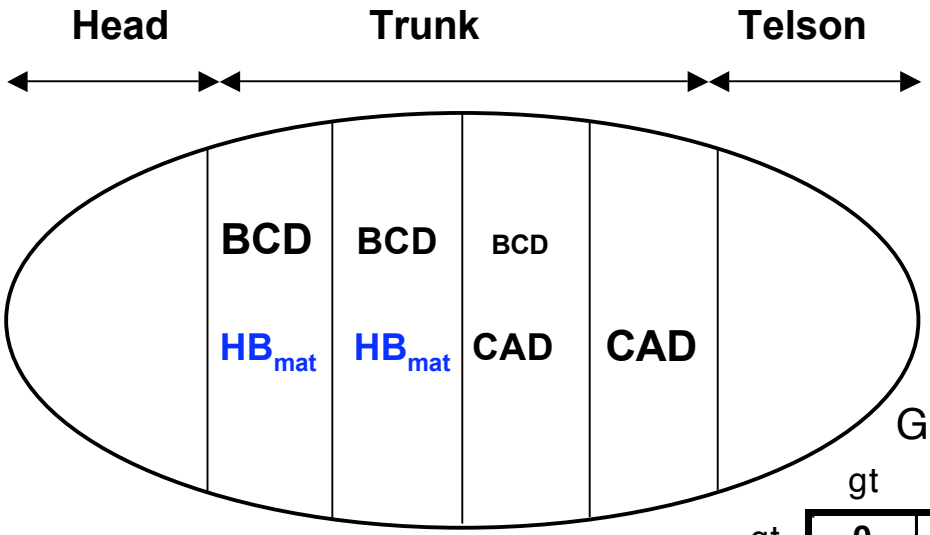
Sánchez L & Thieffry D (2003)
J theor Biol **224**: 517-37

Sánchez L, Chaouiya C
& Thieffry D (in prep.)

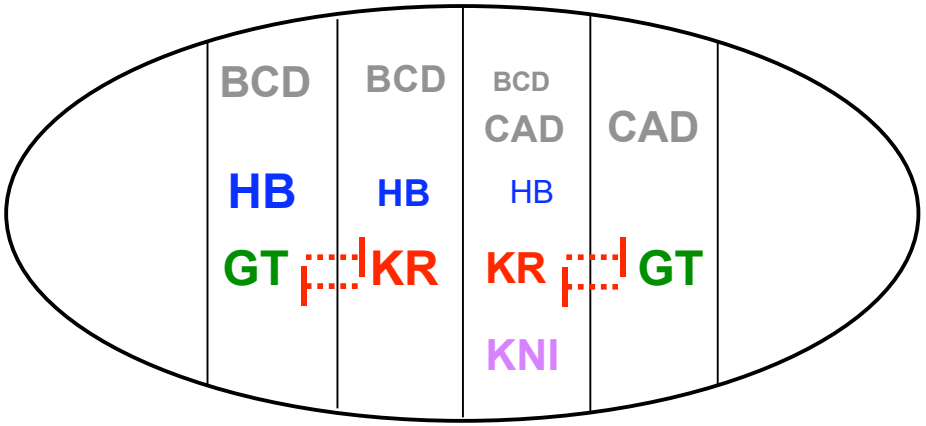
Gap Module



Simulation of the Gap Module



Multiple asynchronous transitions



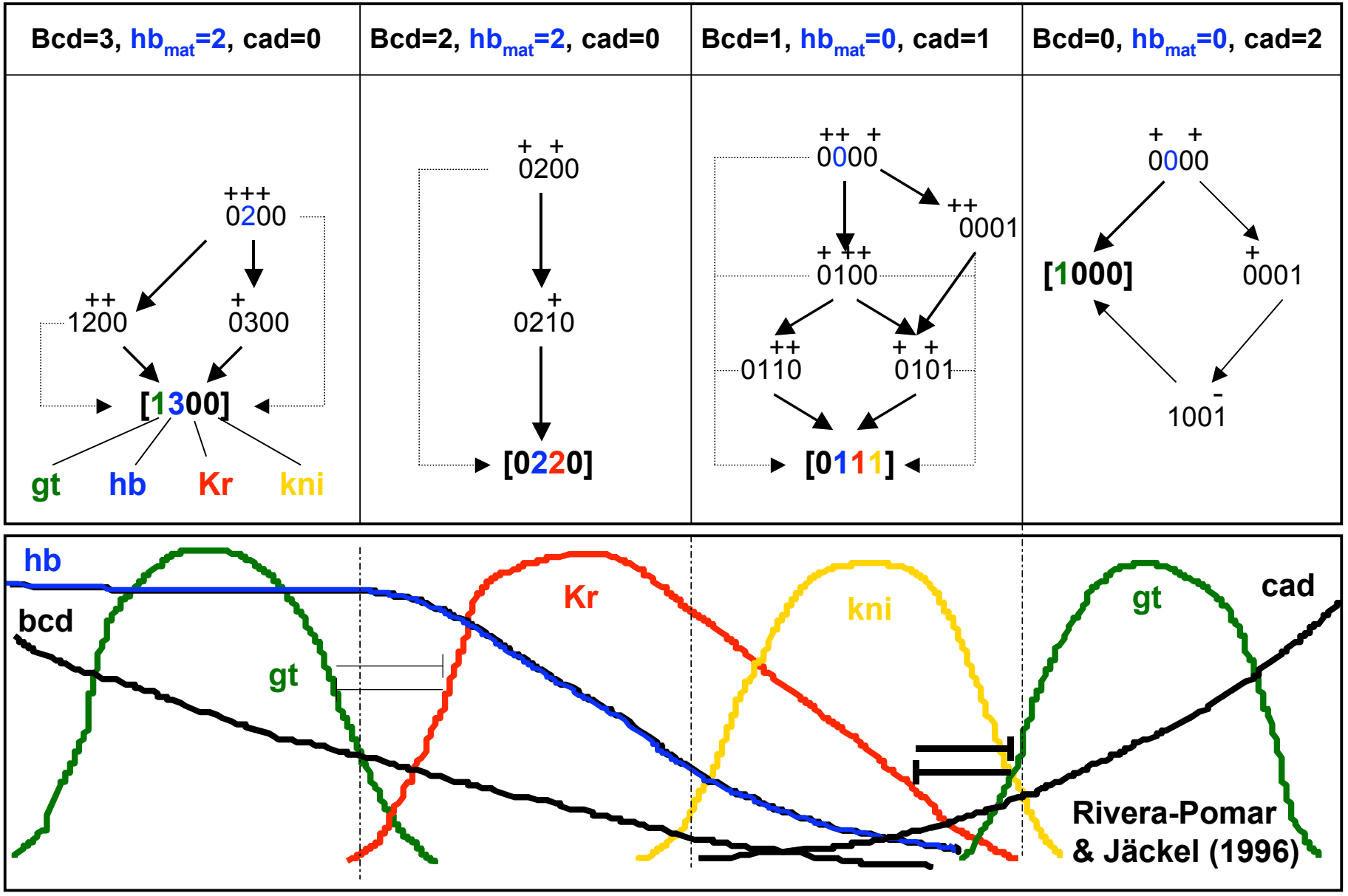
Input:
Initial maternal gradients

Gap module					Maternal inputs		
	gt	hb	Kr	kni	bcd	cad	hb_{mat}
gt	0	-1	-1	0	+1	+2	0
hb	0	(+1)	-2	0	+[1...3]	0	(+1)
Kr	-1	+1/-3	0	-1	+1	0	0
kni	-1	-2	0	0	+1	+1	0

+ Logical rules (parameters)

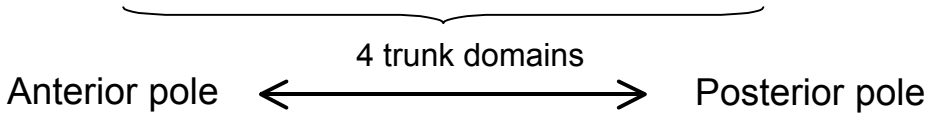
Output:
Four distinct gap gene expression combinations

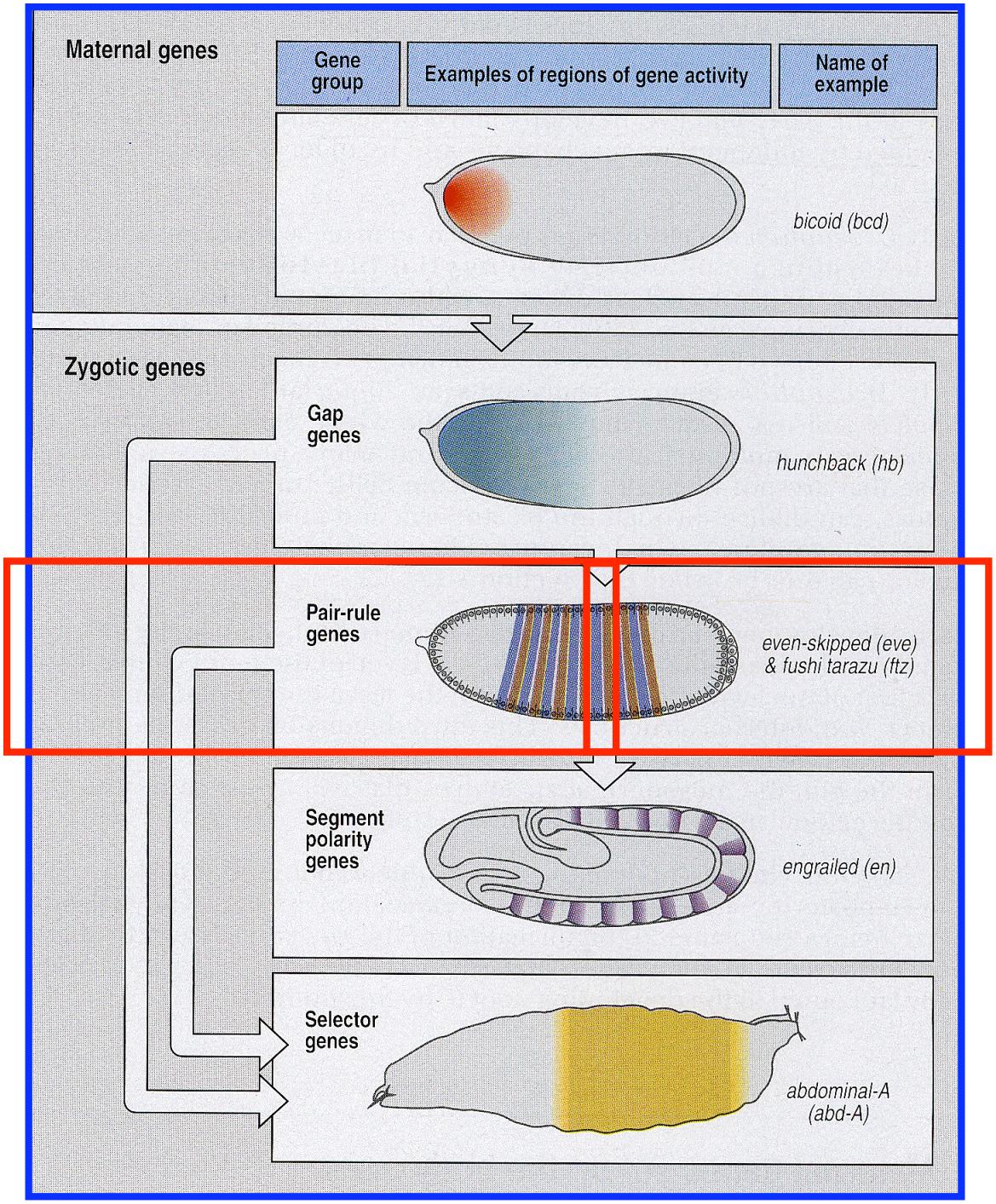
Gap Module - Simulation (gt, hb_{zyg}, Kr, kni)



Simulation of maternal and gap loss-of-function mutations

Genetic background	Final state (GT, HB, KR, KNI)				Observations/predictions
	A	B	C	D	
Wildtype	1300	0220	0111	1000	
Bicoid	**00**0**1**	0**000**1	0**00**1	1000	loss of GT in region A loss of HB in ABC and of KR in BC KNI expands anteriorly into region AB
Hunchback$_{mat}$	1300	0220	0111	1000	no significant effect
caudal	1300	0220	01**20**	**0**000	increase of KR in region C loss of KNI in region C loss of GT in region D
giant	**0**300	0220	0111	**000**1	KNI expands posteriorly into D
Krüppel	1300	1**2**00	1**10**0	1000	GT expands into regions B and C Loss of KNI in region C
knirps	1300	0220	01**2**0	1000	increase of KR in region C
Hunchback$_{mat\&zyg}$	1**0**00	1**00**0	1**00**0	1000	GT expands into regions B and C loss of KR in regions B and C loss of KNI in region C
giant-Krüppel	**0**300	0**2**00	0**1**01	**000**1	KNI expands posteriorly into region D
Krüppel-knirps	1300	1**2**00	1**10**0	1000	GT expands into regions B and C
giant-knirps	**0**300	0220	01**2**0	**0**000	increase of KR in region C

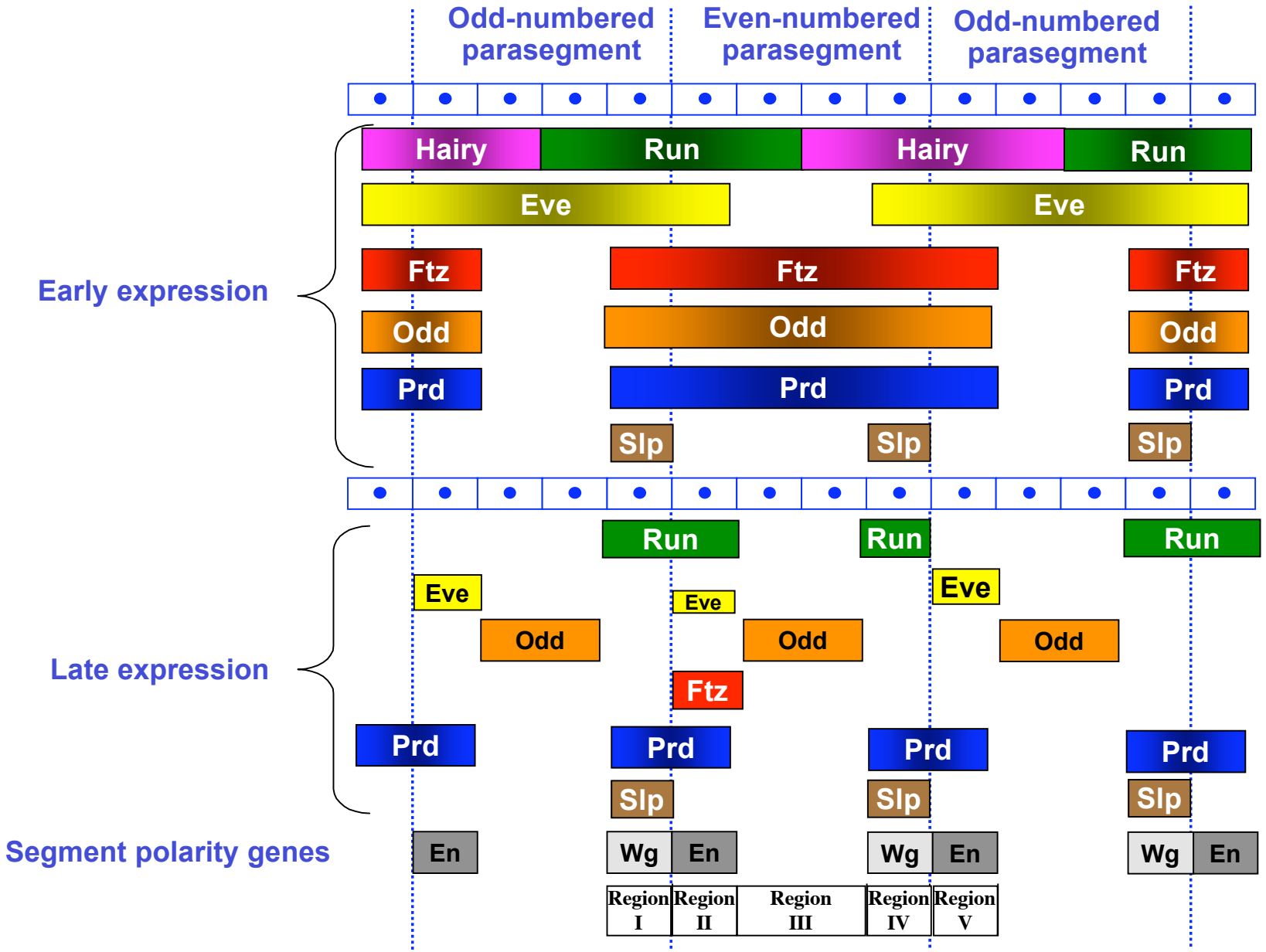




Drosophila segmentation control

Sánchez L & Thieffry D (2003)
J theor Biol **224**: 517-37

Pair-rule genes expression patterns



Pair-rule logical model

Input
(ubiquitous expression)

Ouputs (Segment polarity genes)	*eve*	*prd*	*ppa*	*run*	*slp*	*ftz*	*odd*	*opa*
eve	+1	+1	0	-1	-1	0	-1	0
prd	-3	0	-1	0	0	+1	-1	0
ppa	-2	0	0	0	0	0	0	0
run	-2	+1	0	0	0	0	-1	0
slp	-2	0	0	0	0	-1	-1	0
ftz	-2	0	0	0	-1	+1	-1	0
odd	-1	-1	0	0	0	+1	0	0
en	0	+2	0	-1	-1	+2	-1	+1
wg	-1	+1	0	0	+1	-1	-1	+1

Gene	value 1	value 2	value 3
eve	$K_{v.vrs}$ $K_{v.psd}$ $K_{v.vsd}$ $K_{v.prsd}$ $K_{v.vpsd}$ $K_{v.vprs}$ $K_{v.vrsd}$		$K_{v.vprsd}$
prd	$K_{p.d}$ $K_{p.vd}$ $K_{p.zd}$ $K_{p.zvd}$	$K_{p.ad}$ $K_{p.vad}$ $K_{p.azd}$ $K_{p.zvad}$	
ppa	$K_{a.v}$		
run	$K_{r.pvd}$		
slp	$K_{s.vzd}$		
ftz	$K_{z.vsd}$	$K_{z.zvsd}$	
odd	$K_{d.vp}$ $K_{d.zvp}$		
en	$K_{e.yzs}$ $K_{e.yprsd}$ $K_{e.yzrsd}$ $K_{e.ypzsd}$ $K_{e.ypzrsd}$		
wg	$K_{w.ypsvzd}$		

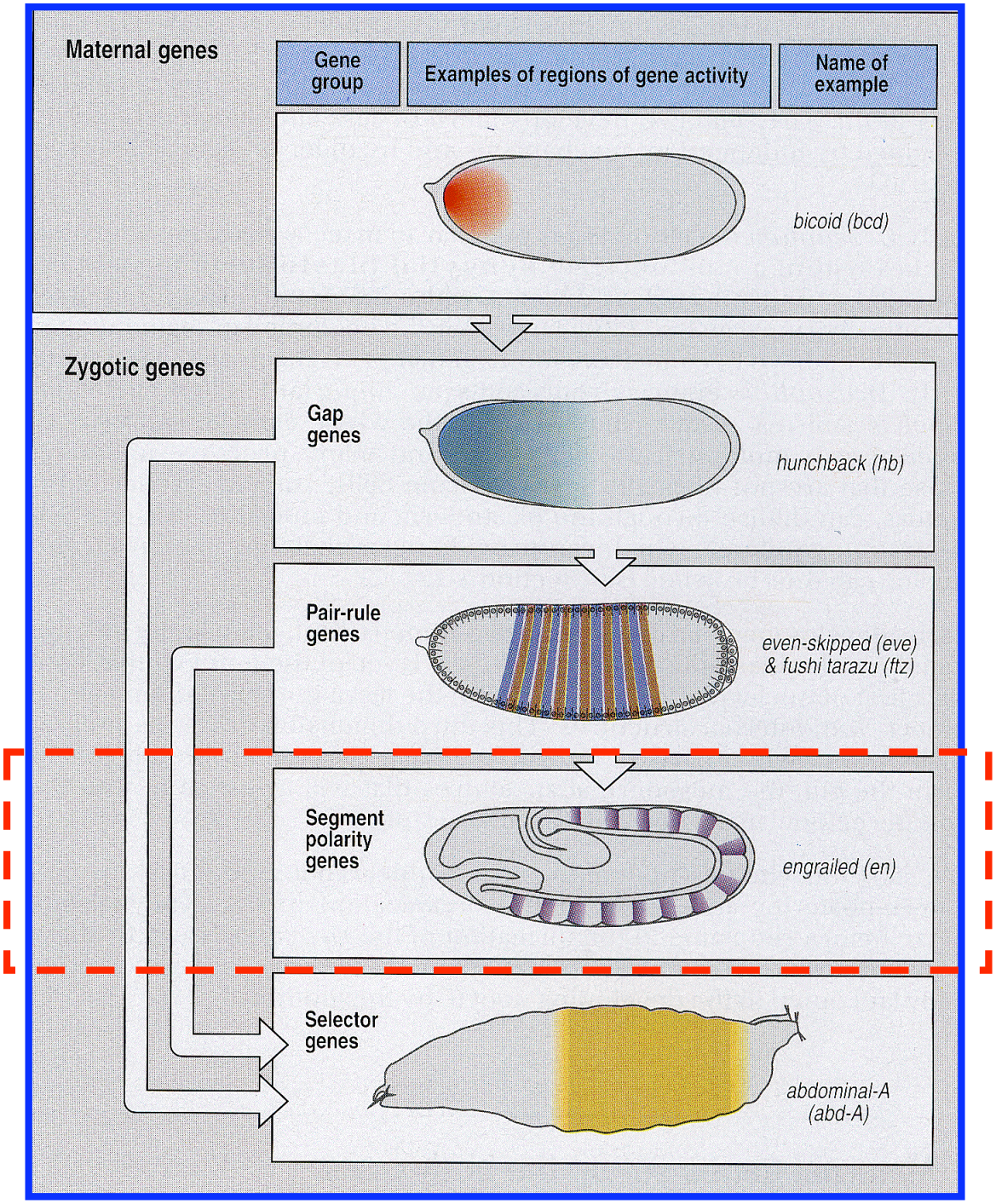
Four stables states :

- One **Wg** expressing state
- Two different **En** expressing states
- One state with no Wg, nor EN, but **Odd** expression

Prediction of pair-rule *cis*-regulatory mutants

Genetic background	stable states							Embryo regions	EN/WG expression	(partially) functional circuits	Comments
	v	**p**	**a**	**r**	**s**	**z**	**d**				
wild-type	0	0	1	0	0	0	1	III	-	eve (+), eve/run (+), eve/slp (+), prd/odd (+), slp/ftz (+), eve-ftz-slp (-), prd/odd/ftz (+)	
	0	1	1	1	1	0	0	I, IV	**Wg**		
	1	1	1	1	0	2	0	II	**En**		
	3	2	0	0	0	0	0	V	**En**		
eve **auto-regulation**	0	0	1	0	0	0	1	III	-	prd/odd (+), slp/ftz (+), prd/odd/ftz (+)	Replacement of odd **En**-stripes by **Wg**-stripes
	0	1	1	1	1	0	0	I, IV, (V)	**Wg**		
	1	1	1	1	0	2	0	II, (V)	**En**		
Ftz **auto-regulation**	0	0	1	0	0	0	1	III	-	eve (+), eve/run (+), eve/slp (+), prd/odd (+), slp/ftz (+), eve-ftz-slp (-), prd/odd/ftz (+)	Loss of even **En**-stripes
	0	1	1	1	1	0	0	I, IV	**Wg**		
	1	1	1	1	0	**1**	0	II	-		
	3	2	0	0	0	0	0	V	**En**		

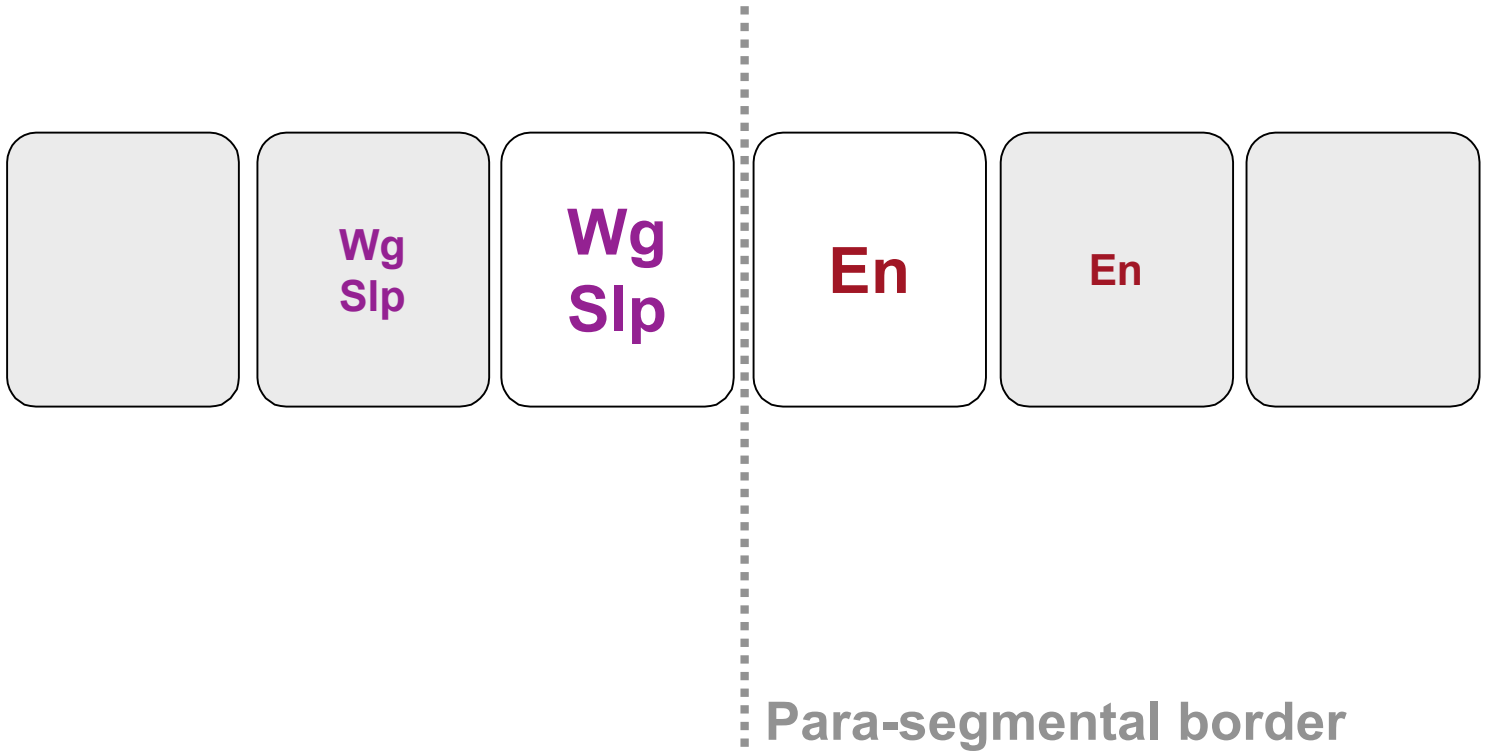
Eve Prd Ppa Run Slp Ftz Odd



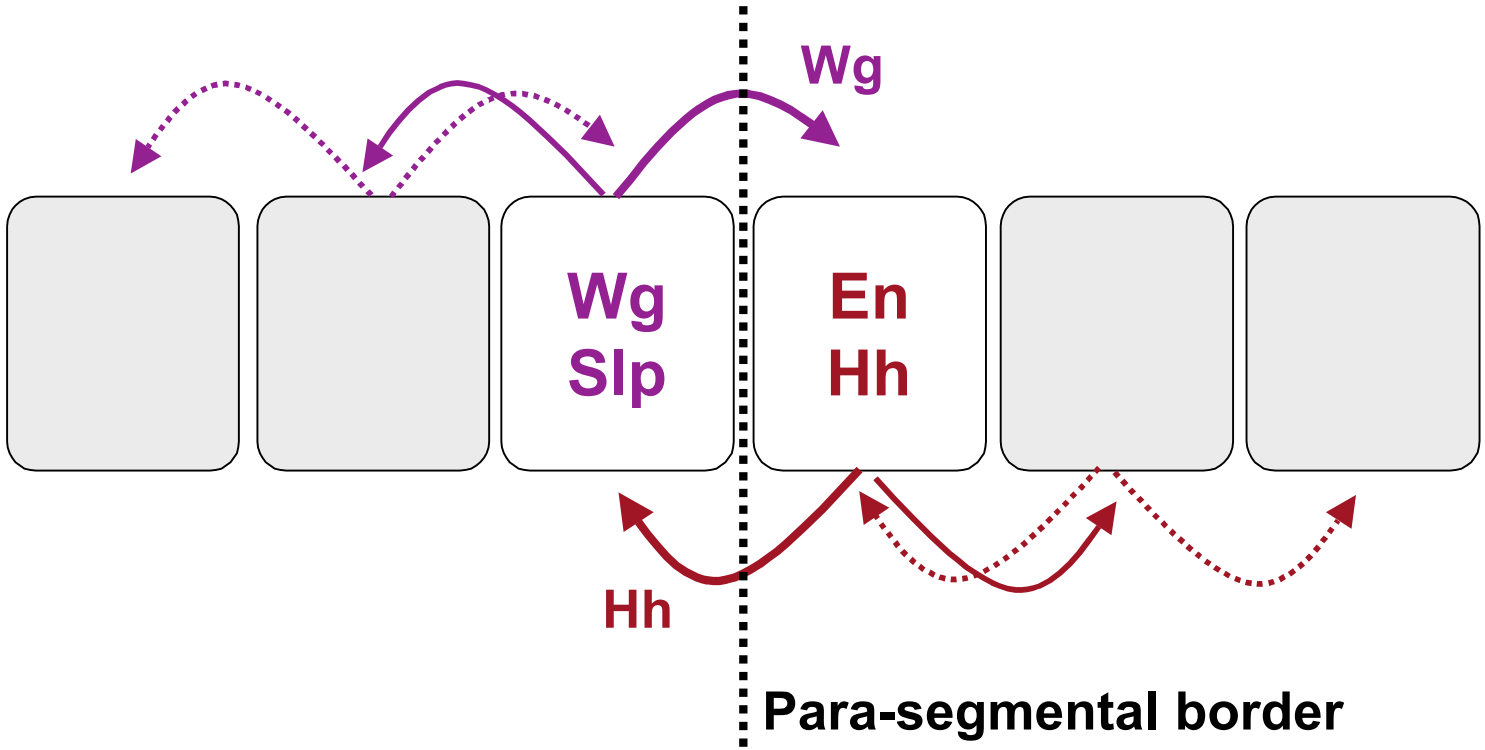
Drosophila segmentation control

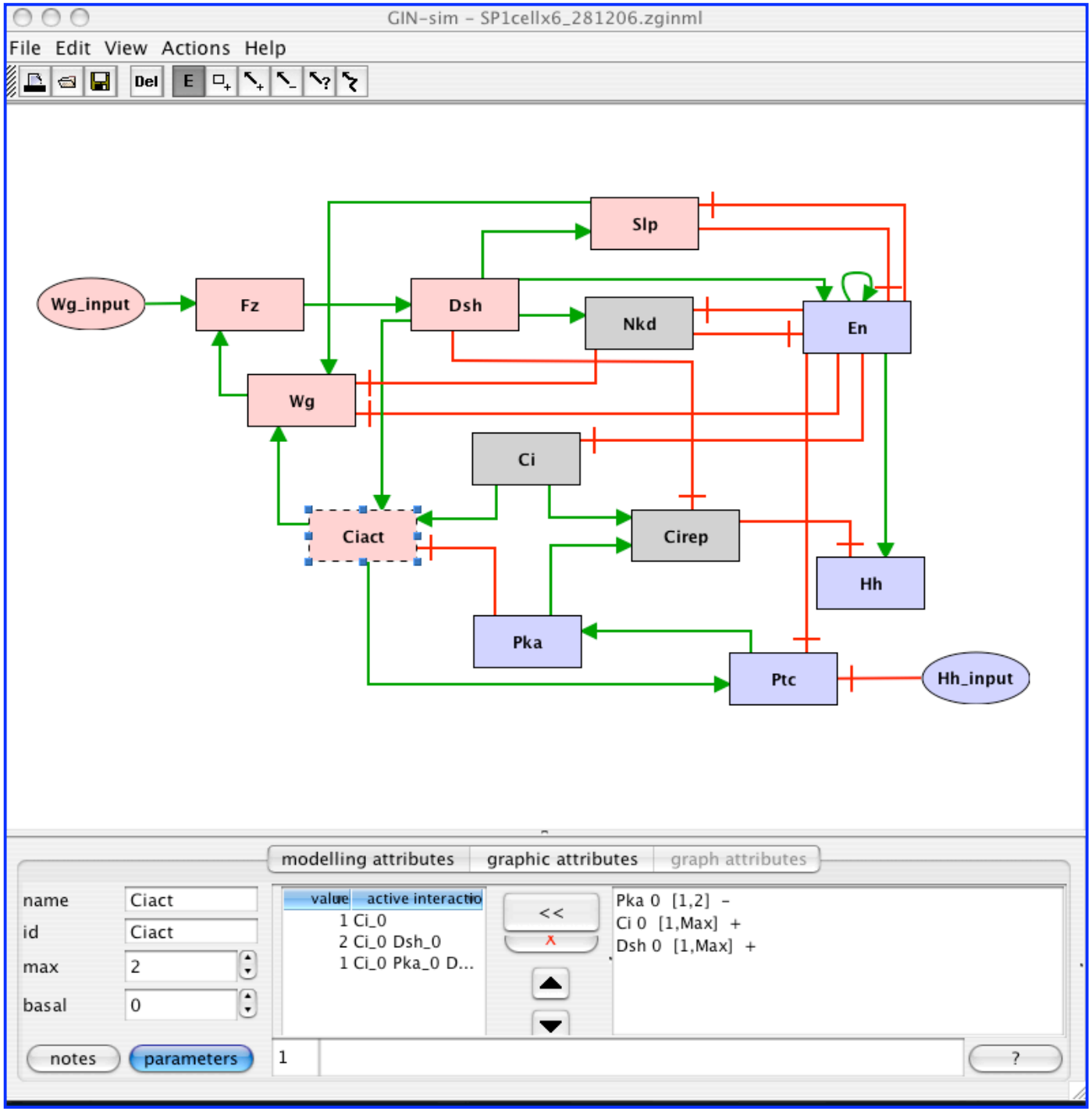
Sánchez L, Chaouiya C & Thieffry D (in prep.)

Segment Polarity system: pair-rule input



Segment Polarity system: intercellular signalling





Logical modelling of the Segment Polarity module

Collaboration with
Lucas SANCHEZ
(CIB, Madrid)

Dynamical analysis: strategy

- **Single cell** analysis -> delineation of possible **stable states** (= cellular states) for different Hh and Wg input configurations
- **Chaining of 6 cells** trough Wg and Hh signalling.
- Use of **constraint programming** (or **decision diagrams**) to identify all **multicellular stable states**
- **Classification** of multi-cellular stable patterns (symmetries)
- Use of **Petri net** (or **Model checking**) **tools** to assess the **reachability** of relevant differentiation states **from relevant initial conditions**
- **Feedback circuit analysis**

Wild type encapsulated cell

5 differentiation states depending on inputs combinations:

Wg	Hh	Wg	Fz	Dsh	Slp	Nkd	En	Hh	Ci	Ciact	Cirep	Pka	Ptc	State
0	0	0	0	0	0	1	0	0	1	0	1	2	1	**T**rivial
0	1	0	0	0	0	1	0	0	**1**	**1**	0	0	0	**C**i+Ci_act
0	1	**2**	1	1	1	2	0	0	1	2	0	0	0	**W**g
1	0	0	1	1	0	0	**1**	1	0	0	0	0	0	**E**n
1	0	0	1	1	1	2	0	0	1	1	0	2	2	**N**kd
1	1	**2**	1	1	1	2	0	0	1	2	0	0	0	**W**g
1	1	0	1	1	0	0	**1**	1	0	0	0	0	0	**E**n

Inputs coming from neighbouring cells

Wild type multicellular behaviour

- A priori, there are **5^6** possible combinations of the 5 unicellular stable states.
- The intercellular constraints enable only **59** possible combinations
- **37** remaining combinations after discarding the symmetrical ones (eg TTTTEW <=>EWTTTT)
- **Reachability analysis** -> two multi-cellular outcome accessible from a relatively broad range of initial conditions:

T	N	W	E	C	T
T	T	T	T	T	T

Simulation of perturbations

- Single **loss-of-functions** of Wg, En, Hh, or Ci give rise to a unique trivial (like) pattern:

- **Double loss-of function** of Wg/Ptc gives rise to a unique pattern:

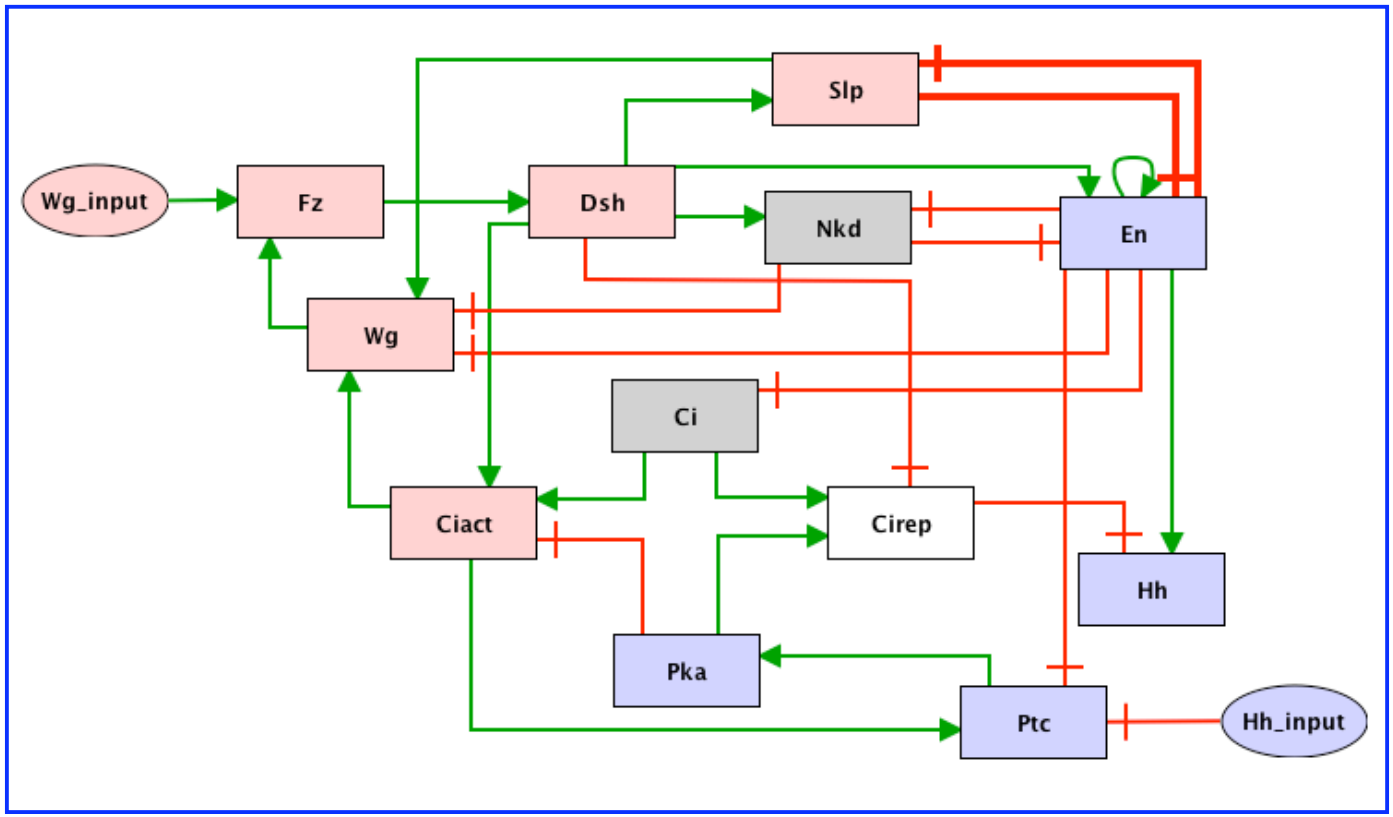
- **Ectopic En expression** gives also rise to a unique pattern:

Wg	Fz	Dsh	Slp	Nkd	En	Hh	Ci	Ciact	Cirep	Pka	Ptc
0	0	0	0	0	1	1	0	0	0	0	0

More complex perturbations...

- Single **Ptc loss-of-function** gives rise to several possible multi-cellular stable states, including a pattern with Wg posterior extension and posterior ectopic En expression

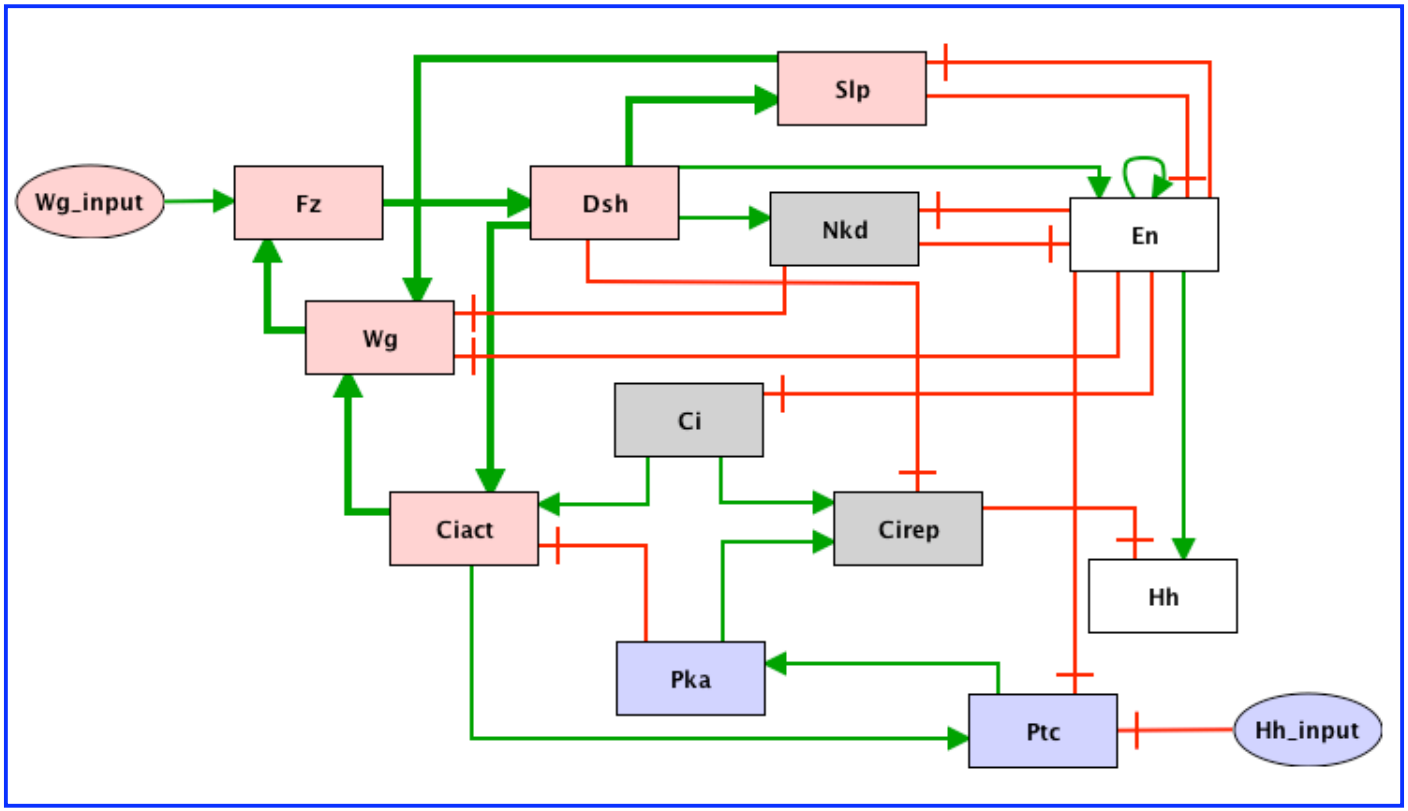
Feedback circuit analysis: functional intracellular circuits (1)



En-Slp circuit functional when Dsh=1

Enables **two different cellular states** in the presence of Wg signalling, one with En ON and Slp OFF, the other with En OFF and Slp ON

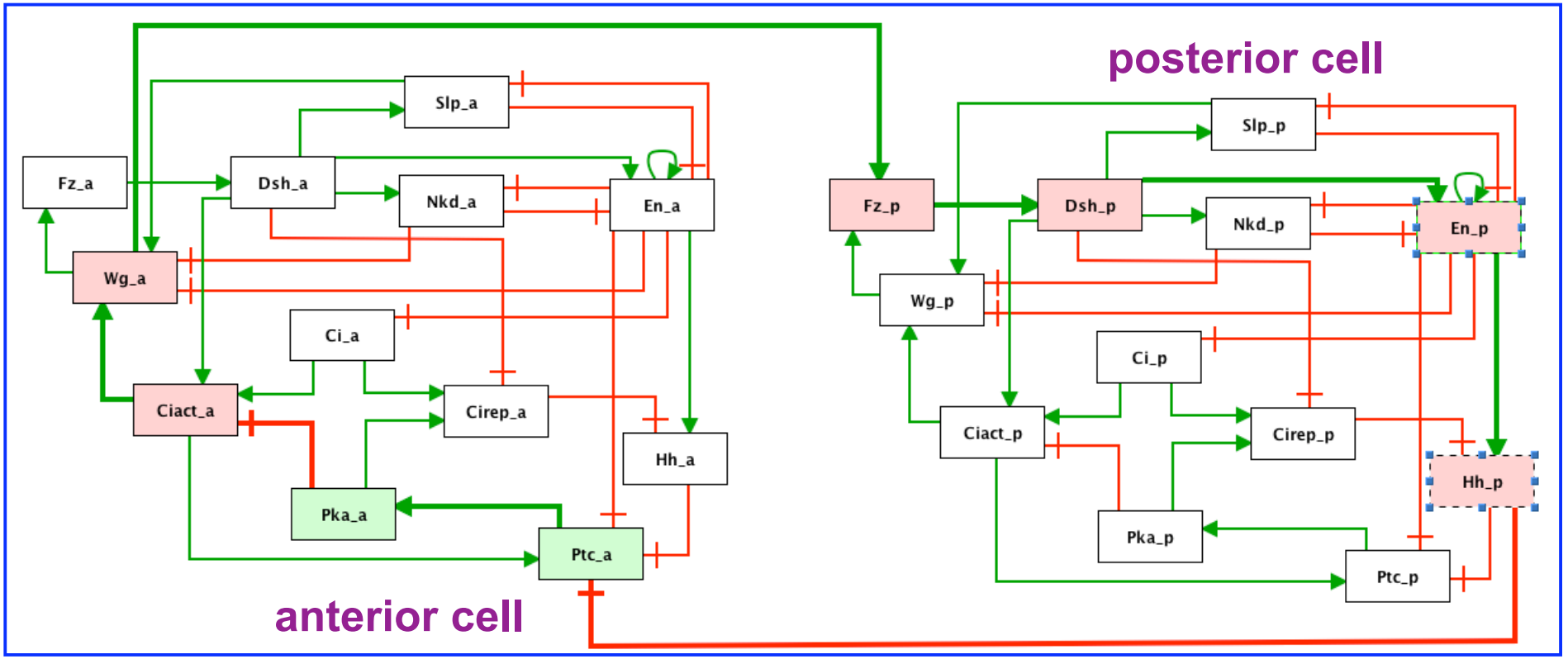
Feedback circuit analysis: functional intracellular circuits (2)



Wg circuits functional when En=0

Enables **different stable states**
with no, low or high Wg expression when En is OFF

Feedback circuit analysis: functional intercellular circuit



Forces the **combination** of specific neighbouring cellular states

Novel insights

- The **pair-rule signal** needs to be **operative** until the inter-cellular circuit become functional
- The **consolidation** of **Wg** and **En** expression pattern require the proper activity of both **autocrine** and **paracrine Wg pathways**
- **Dual role** played by the **Protein kinase A** (Pka) through phosphorylation of Cubitus interruptus, effector of Hh Pathway
- Important roles of **Slp** and **Nkd** during the transition from pair-rule to segment polarity expression patterns
- Novel insights in the roles of the various **feedback circuits**, in particular **positive circuits**, at the basis of **differentiation decisions**
- Consistency between the results of the **simulation of altered expression** of segment polarity genes with published data

Prospects

- **Coupling between the gap, pair-rule and segment polarity modules**
  → towards a model of the whole segmentation hierarchy
- **Modelling of control of the formation of the anterior-posterior boundary in wing imaginal disks**
  → comparative analysis of segment polarity network variants
- Modelling of the molecular network controlling embryonic **cell cycle**
  → analysis of the **coupling between cell cycle and cell differentiation**
- **Comparative and evolutionary analysis** of homologous regulatory networks (**graph topology**, **qualitative dynamics**, **redundancy**)

Ongoing methodological developments

A Naldi, F Lopez, C Chaouiya

- **Improved model definition** (logical rules, OMDDs)
- **Automated feedback circuit analysis**
- **Attractor identification** (constraint programming, OMDDs)
- **Model checking** (temporal logics + model checkers)
- Translation into **Petri net** formalism (quantitative extensions)
- Support of various **formats** for models/simulations:

  GINML (XML), SVG, INA & PNML (Petri nets), SBML, Prolog, NuSMV...
- Qualitative **regulatory interaction inference**

Main recent publications

- Chaouiya C, Remy E, Mossé B, Thieffry D (2004). *LNCIS* **294**: 119-126.
- Fauré A, Naldi A, Chaouiya C, Thieffry D (2006). *Bioinformatics* **22**: e124-31.
- Gonzalez A, Chaouiya C, Thieffry D (2006). *Genetics* **174**: 1625-34.
- Gonzalez A, Naldi A, Sánchez L, Thieffry D, Chaouiya C (2006). *Biosystems* **84**: 91-100.
- Remy E, Mossé B, Chaouiya C, Thieffry D (2003). *Bioinformatics* **10** : ii172-8.
- Remy E, Ruet P, Thieffry D (2006). *LNCIS* 341: 263-70.
- Sánchez L, Thieffry D (2003). *J theor Biol* **224**: 517-37.
- Thieffry D, Sánchez L (2002). *Ann NY Acad Sci* **981**: 135-153.
- Thieffry D, Sánchez L (2003). *Curr Op Genet Dev* **13**: 326-30.

ministère éducation nationale enseignement supérieur recherche
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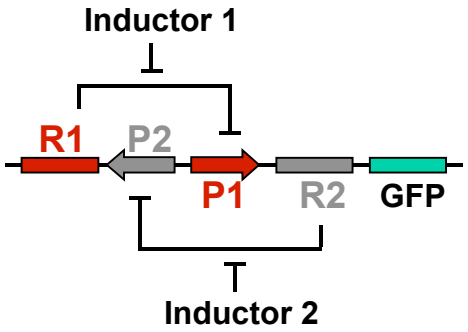
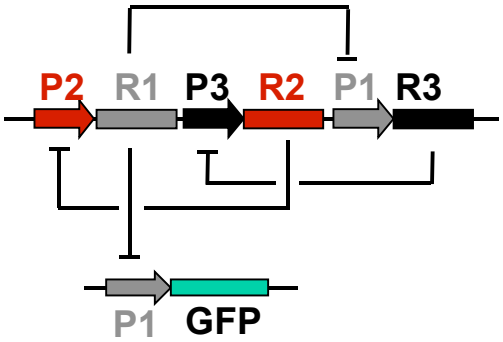
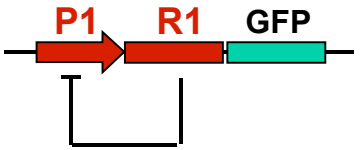
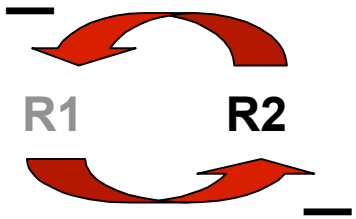
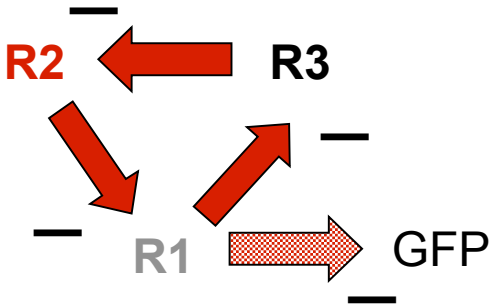
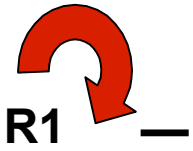
Synthesis of auto-regulated gene circuits

	Gardner *et al.* (2000) *Nature* **403**: 339-342	Elowitz & Leibler (2000) *Nature* **403**: 335-338	Becskei & Serrano (2000) *Nature* **405**: 590-593
Construction			
Logical scheme	**Positive circuit** 	**Negative circuit** 	**Negative circuit** 
Properties	- Stable and exclusive expression of one of the two repressors - Memorisation of induction - Stability and robustness against biochemical fluctuations	- Cyclic expression of the repressors and reporter gene - Transmission of this oscillating behaviour through bacterial divisions	- Increased stability and decreased variability of the repressor expression - Compensation of dosage effects due to the variation of the number of copies

Models of the Gap Module

	gt	hb	Kr	kni
gt	0	0	—	—
hb	0	+	—	0
Kr	—	+/-	0	—
kni	—	—	+	0

Rivera-Pomar & Jäckle (1996)

	gt	hb	Kr	kni
gt	+	—	—	—
hb	—	+	(-)	—
Kr	—	—	+	—
kni	—	—	—	(-)

Reinitz (1996)

	gt	hb	Kr	kni
gt	-2	-1/-3	**-2**	0
hb	0	0	**-3**	-1
Kr	**-3**	+2	0	0
kni	0	**-2**	+3	0

Bodnar (1997)

	gt	hb	Kr	kni
gt	0	—	—	0
hb	0	(+)	—	0
Kr	—	+/-	0	—
kni	—	—	0	0

Sánchez & Thieffry (2001)

	Von Dassow *et al.* (2000)	**Reinitz *et al.* (1998)** **Jaeger *et al.* (2004)**	**Sánchez *et al.* (2001, 2003)**
Formalism	Specified set of ODEs (continuous)	Generic set of ODEs (continuous)	Logical relationships Graphs (discrete)
Methodology	Simulations Random/directed parameter space exploration	Reverse engineering and model fitting (simulated annealing)	Logical analysis simulations
Initial data	Detailed knowledge of all components + interactions	List of key actors: the four gap genes + input + output genes	List of actors + qualitative characterisation of the interactions
Emphasis	Generic properties in relations with parameters values	Extracting interactive features from the knowledge of the dynamics	Role of specific feedback structures Simulations of mutants
Insights	Core interactions Robustness with respect to parameters and initial conditions	Gap genes specify a unique set of pair-rule stripes Diffusion coefficients Dynamics of stripe border setting	Identification of the crucial feedback circuits and delineation of their roles Prediction of new mutant phenotypes
Limitations	Variation of one parameter at a time Scaling up difficult	Standardisation of regulatory terms Scaling up difficult	Less standard maths… Transition towards more quantitative models?