

From gradients to stripes: a logical analysis of the genetic network controlling early drosophila segmentation.

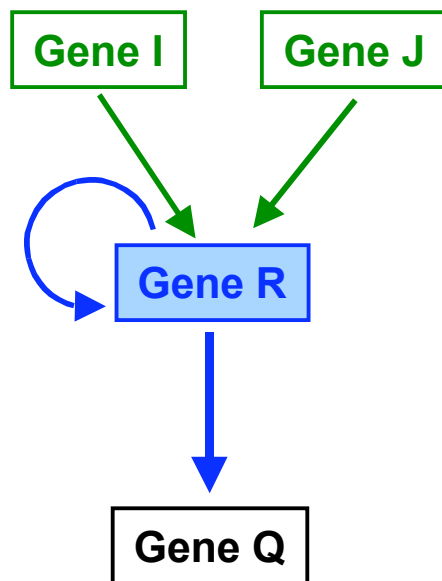
Denis THIEFFRY (LGPD-IBDM, Marseille, France)

Contents

1. Logical modelling of gene networks
2. Application to Drosophila segmentation
3. Conclusions and prospects

Logical formalisation (---> R. Thomas *et al.*)

Fragment of a **regulatory graph**

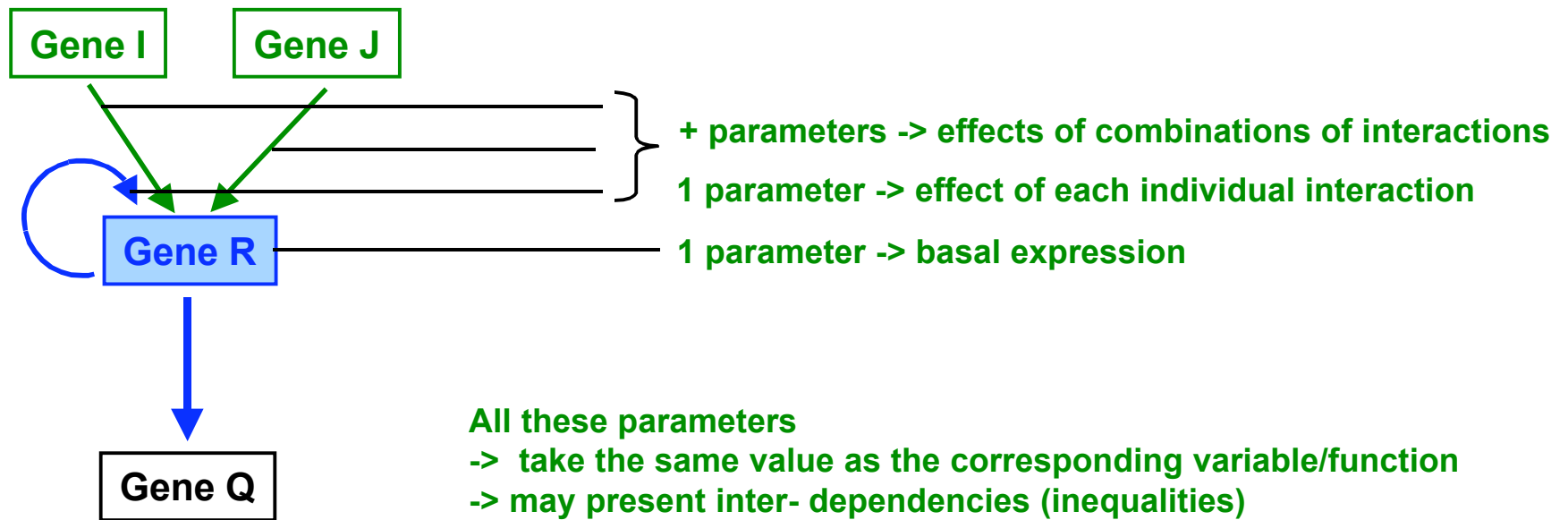


Multi-level logical variables and functions

Here, r , R takes their values in $\{0, 1\}$ or $\{0, 1, 2\}$

“Occam razor”: minimisation of the number of distinct logical values

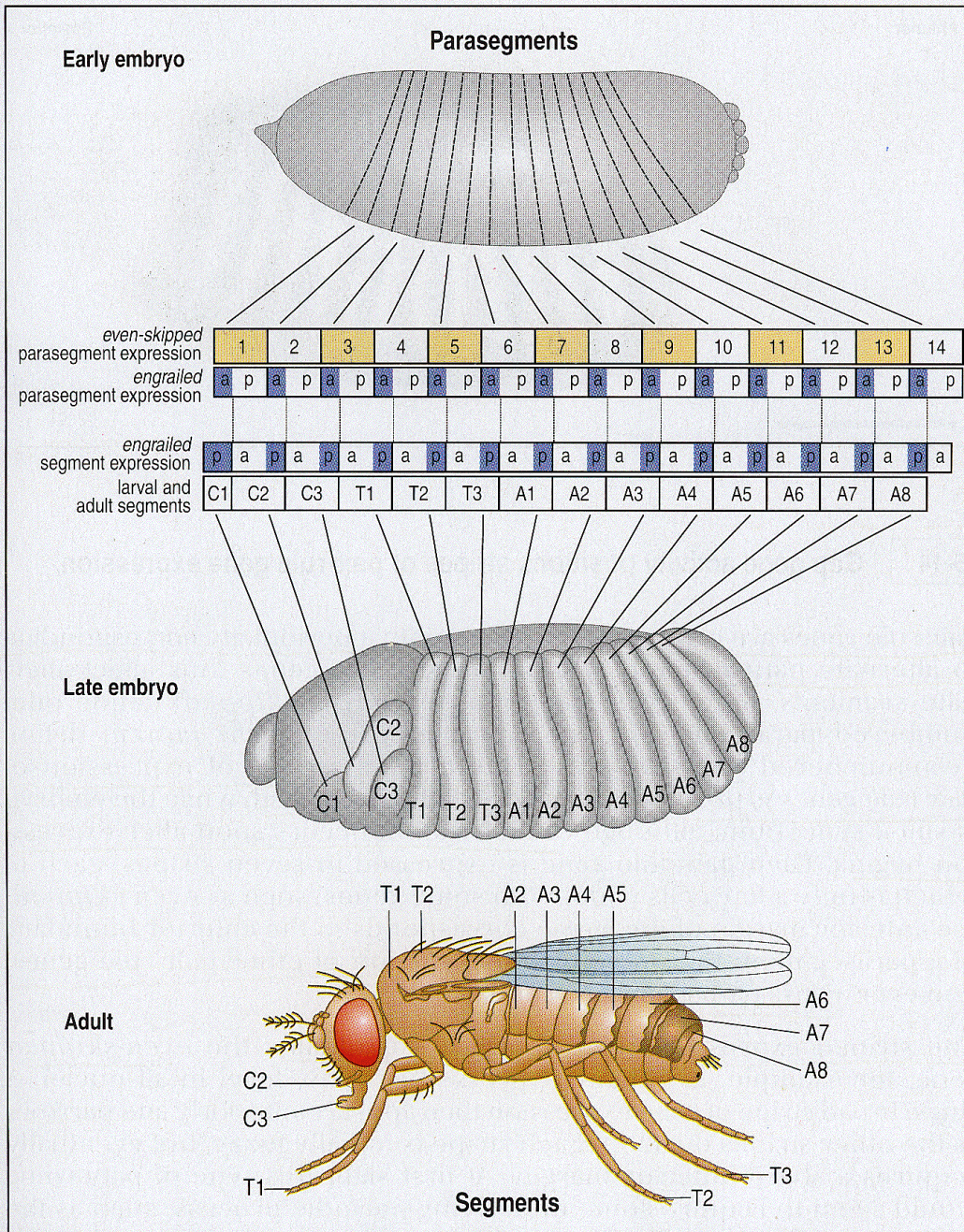
Logical parameters



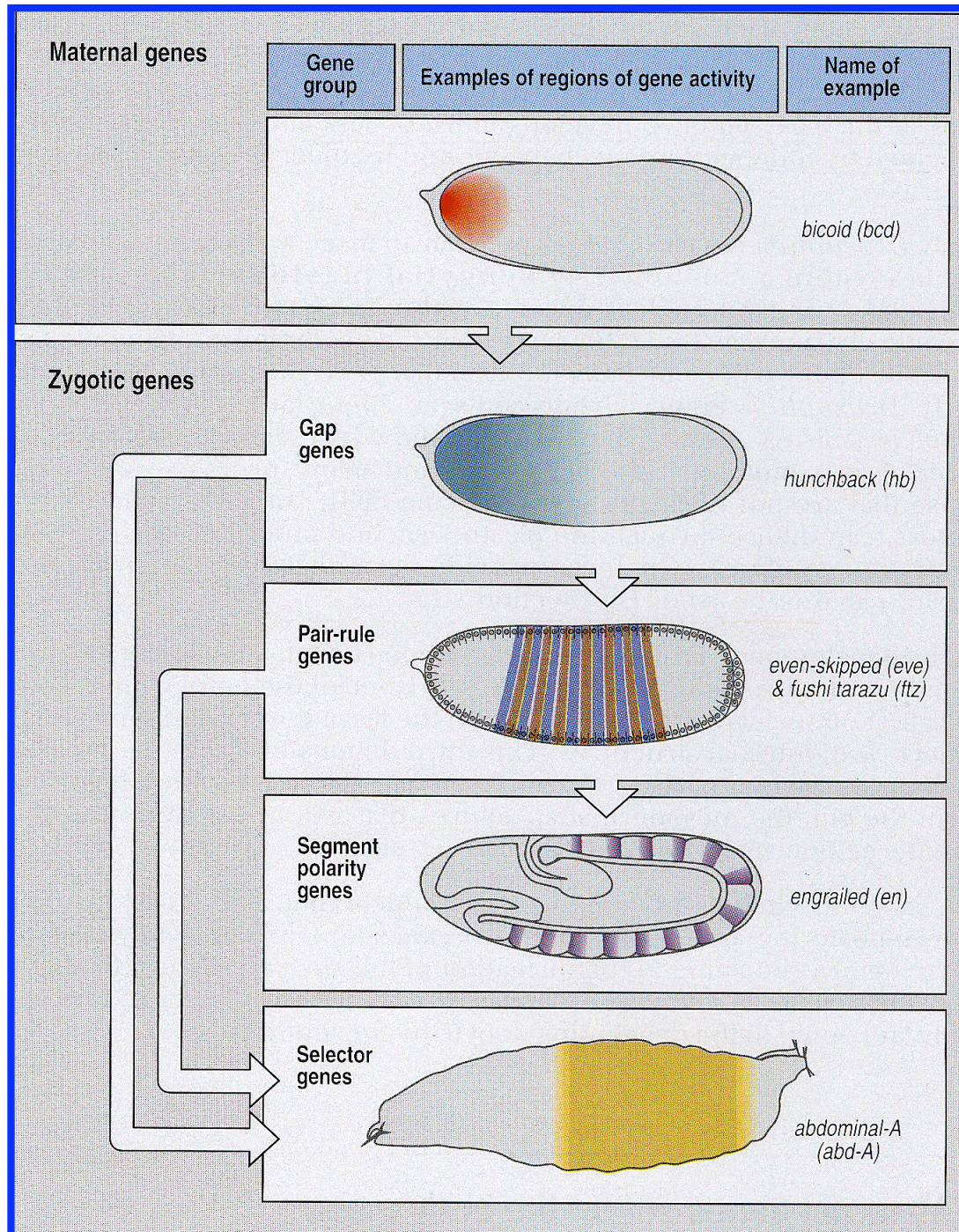
Default situation (“Occam razor”): logical parameters --> 0

Drosophila Segmentation

Collaboration with
Lucas SANCHEZ
(CIB, Madrid)



Source: Wolpert *et al.* (1998)



Anterior-posterior patterning system

Collection and integration of regulatory data (GIN-db)



Graph analysis



Three strongly connected components:

Gap

Pair-rule

Segment-polarity

“cross-regulatory modules”

Source: Wolpert *et al.* (1998)

Models of the Gap Module

	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)

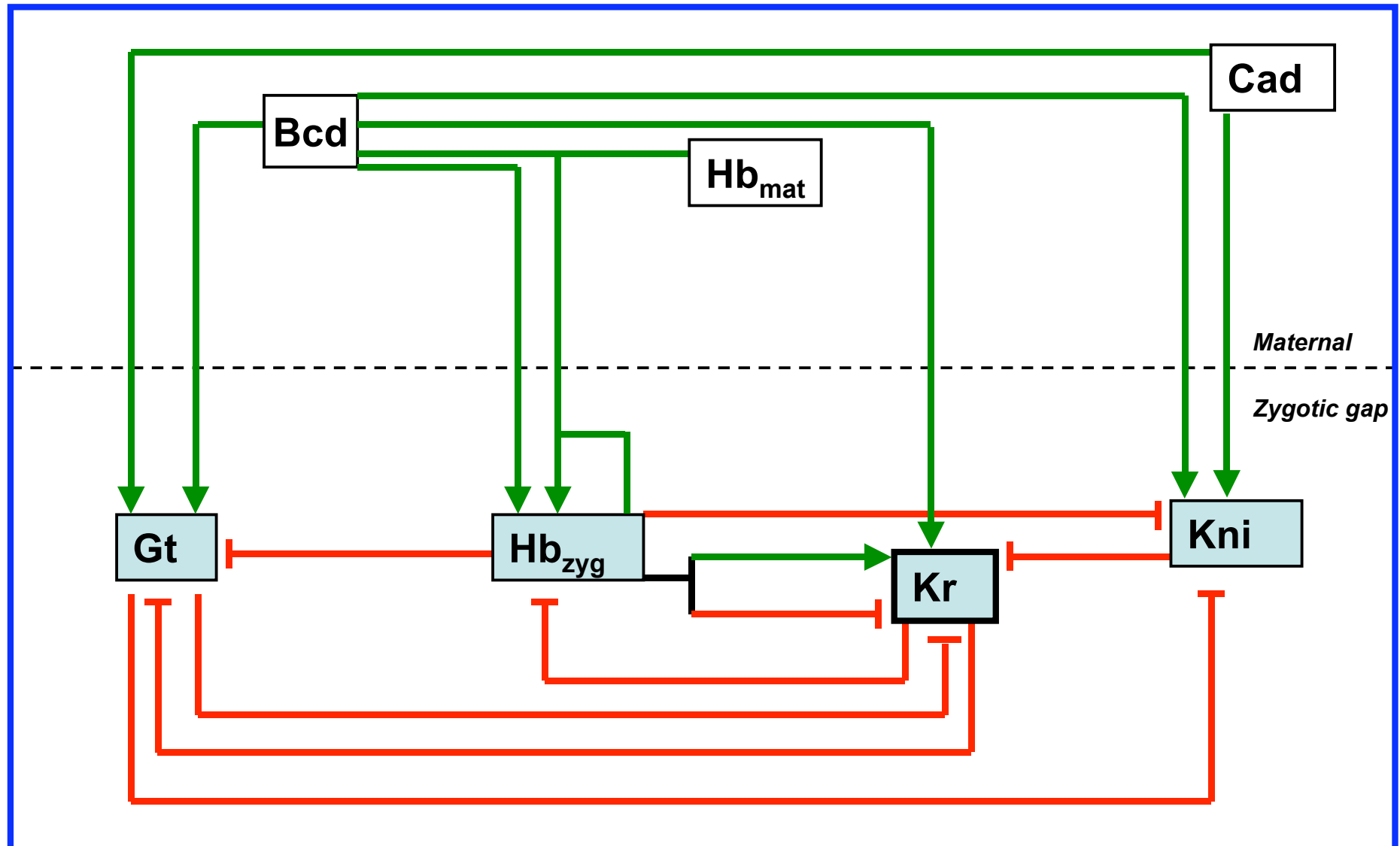
	gt	hb	Kr	kni
gt	0	0	—	—
hb	0	+	—	0
Kr	—	+/-	0	—
kni	—	—	+	0

Rivera-Pomar & Jäckle (1996)

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

Sánchez & Thieffry (2001)

Gap Module



Multi-level logical model for the Gap module

	gap network				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

- No restriction on multiple interactions
- Thresholds considered as parameters
- Sensitivity to context (K 's)

• 7 feedback circuits:

- 2 positive circuits: **gt-Kr**, gt-kni-Kr-hb
- 1 conditional auto-catalytic circuit: hb
- 3 negative circuits: gt-Kr-hb, **gt-kni-Kr**, hb-kni-Kr
- 1 dual (+/-) circuit: hb-Kr

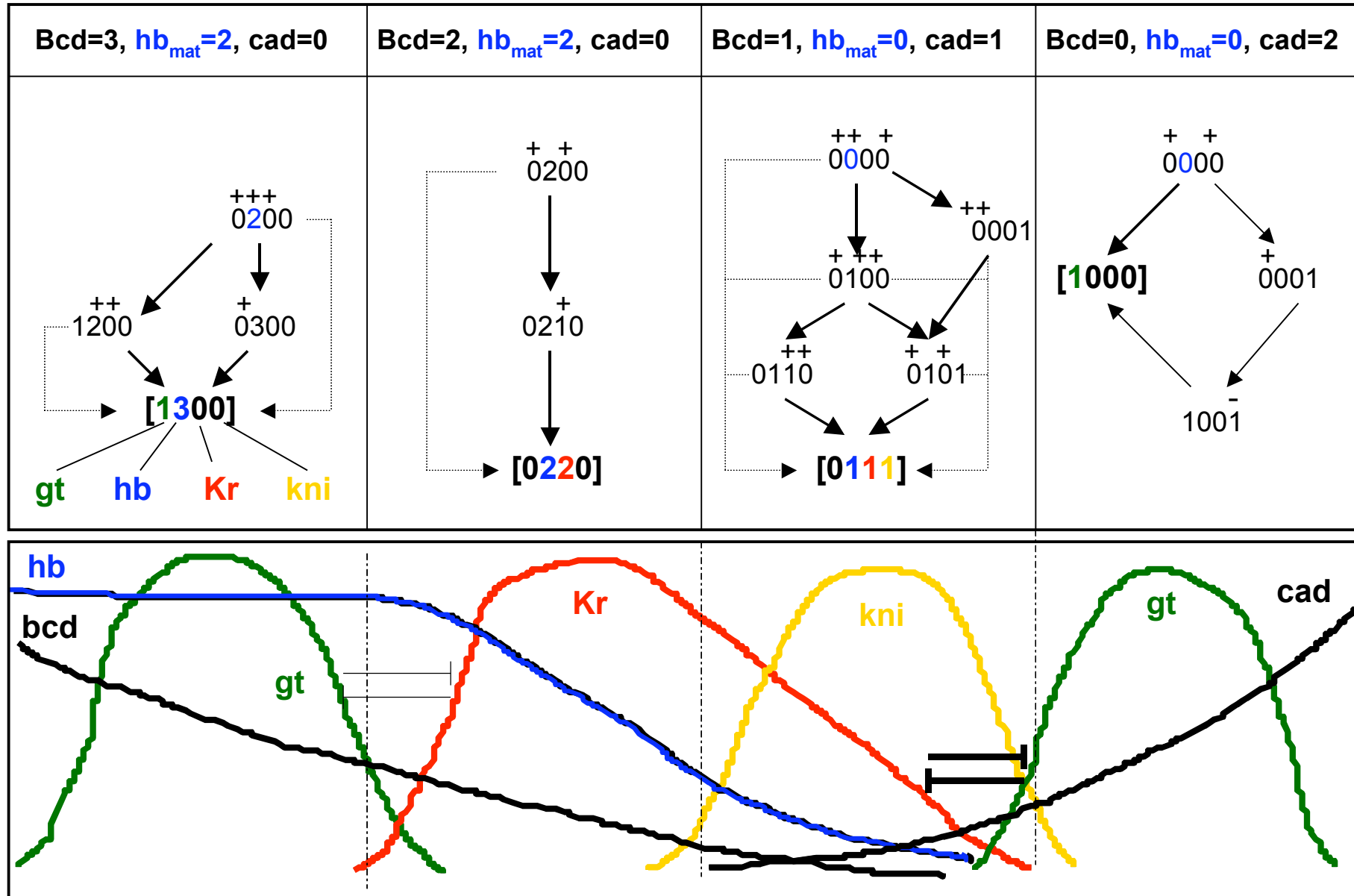
Gap module: logical parameter values

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) $b=0, c=2, h_m=0$	
giant	$K_{g,b}$	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
hunchback	$K_{h,\epsilon}$	3	$K_{h,\delta}$	2	$K_{h,b}$	1	K_h	0
	$K_{h,er}$	3	$K_{h,\delta r}$	2	$K_{h,\rho}$	1	$K_{h,h}$	0
					$K_{h,br}$	1	$K_{h,r}$	0
					$K_{h,\rho r}$	1	$K_{h,hr}$	0
Krüppel	$K_{r,b}$	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
knirps	$K_{n,b}$	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

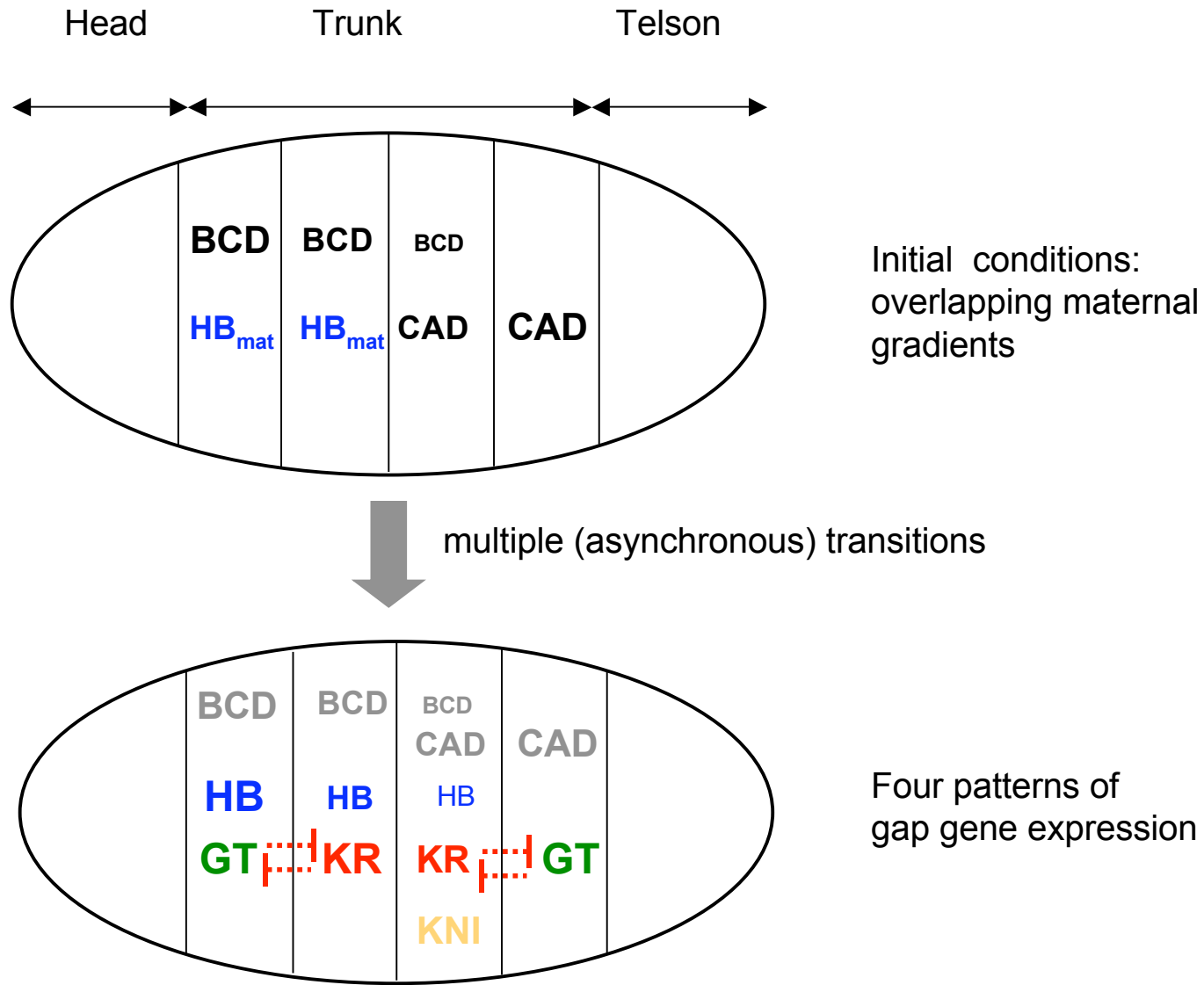
Anterior pole

Posterior pole

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



Gap Module - Simulation (**gt**, hb, **Kr**, **kni**)



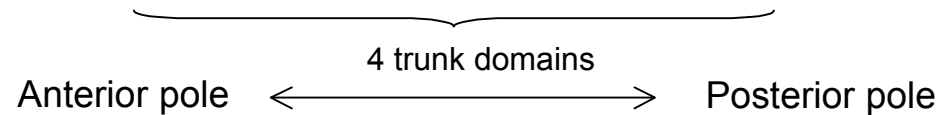
Simulation of maternal and gap loss-of-function mutations

Genetic background	Final state (GT, HB, KR, KNI)				Observations/predictions
	A	B	C	D	
Wildtype	1300	0220	0111	1000	
<i>Bicoid</i>	0001	0001	0001	1000	loss of GT in region A loss of HB in ABC and of KR in BC KNI expands anteriorly into region AB
<i>Hunchback_{mat}</i>	1300	0220	0111	1000	No significant effect
<i>caudal</i>	1300	0220	0120	0000	increase of KR in region C loss of KNI in region C loss of GT in region D
<i>giant</i>	0300	0220	0111	0001	KNI expands posteriorly into D
<i>Krüppel</i>	1300	1200	1100	1000	GT expands into regions B and C Loss of KNI in region C
<i>knirps</i>	1300	0220	0120	1000	increase of KR in region C
<i>Hunchback_{mat&zyg}</i>	1000	1000	1000	1000	GT expands into regions B and C loss of KR in regions B and C loss of KNI in region C
<i>giant-Krüppel</i>	0300	0200	0101	0001	KNI expands posteriorly into region D
<i>Krüppel-knirps</i>	1300	1200	1100	1000	GT expands into regions B and C
<i>giant-knirps</i>	0300	0220	0120	0000	increase of KR in region C

Anterior pole ←
4 trunk domains
 → Posterior pole

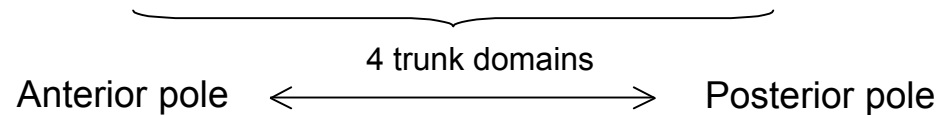
Simulation of gap gene ectopic expression

Genetic background	Final state (GT, HB, KR, KNI)				Observations/predictions
	A	B	C	D	
Wildtype	1300	0220	0111	1000	
<i>giant</i>	1300	1210	1110	1000	lowering of KR in region B loss of KNI in region C.
<i>Hunchback-level 1</i>	1300	0220	0[1-2]11	0101	loss of GT and activation of KNI in D loss of GT in region D activation of GT in BD but not in D
<i>Hunchback-level 2</i>	1300	0220	0211	0200	
<i>Hunchback-level 3</i>	1300	1300	1300	0300	
<i>Krüppel-level 1</i>	0310	0220	01[1-2]1	0011	loss of GT in AD, KNI activated in D loss of GT in AD, KNI activated in D
<i>Krüppel-level 2</i>	0320	0220	0121	0021	
<i>knirps</i>	1301	1201	0111	1001	loss of KR and activation of GT in B
<i>giant + knirps</i>	1301	1201	1101	1001	Loss of KR in region B and C

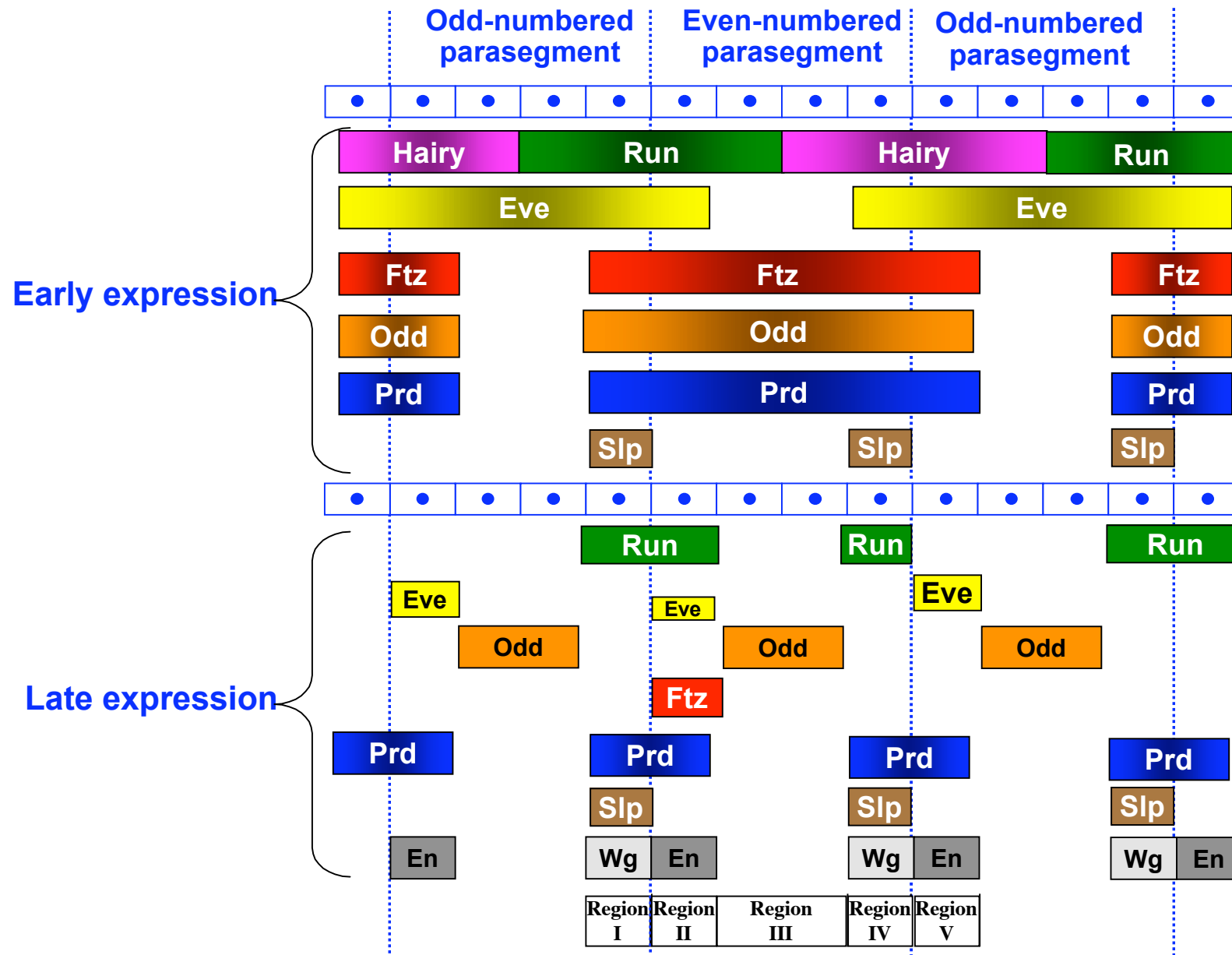


Simulation of *cis*-regulatory mutations

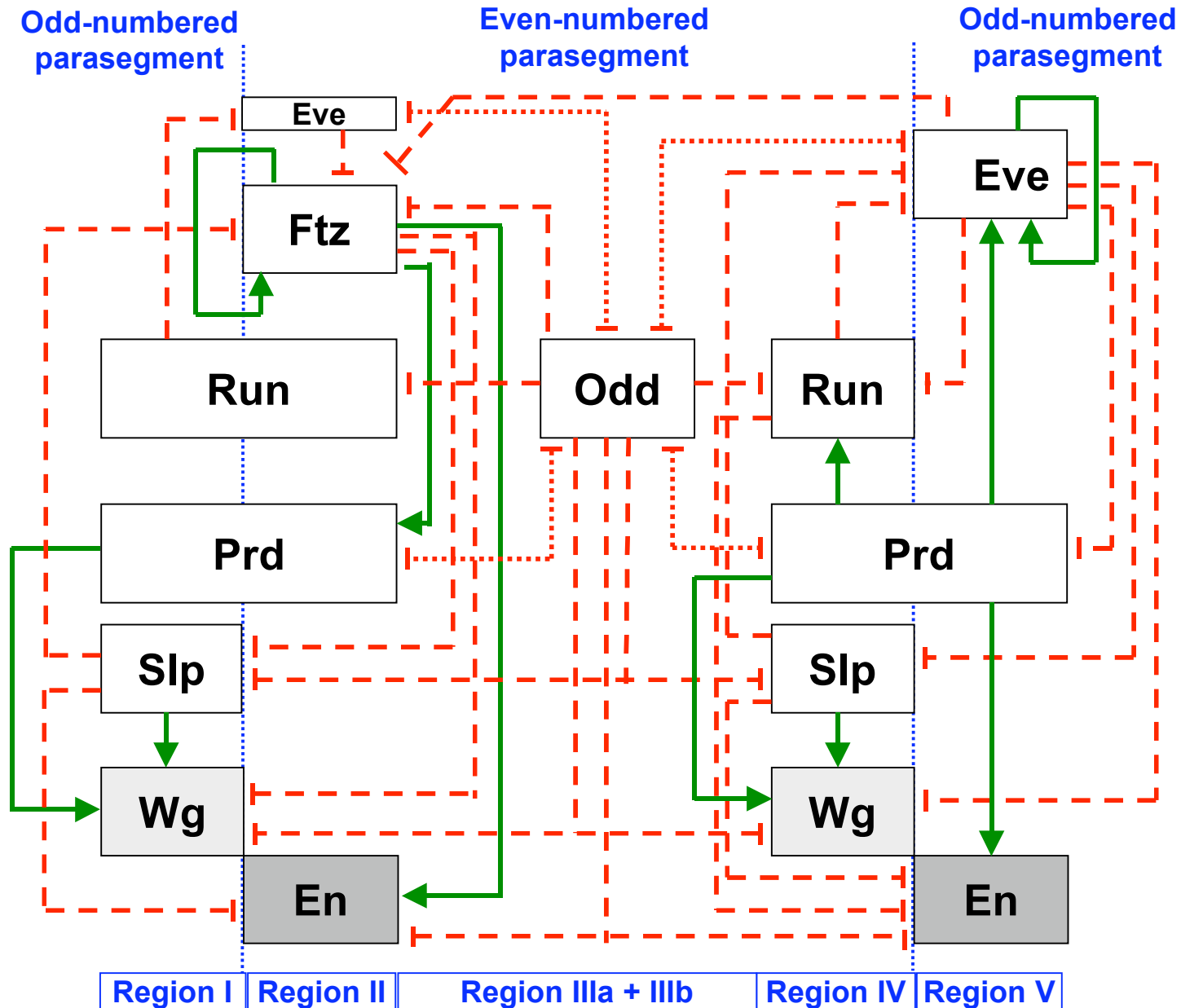
Genetic background	Final state (GT, HB, KR, KNI)				Observations/predictions
	A	B	C	D	
Wildtype	1300	0220	0111	1000	
<i>Hb autoregulation</i>	0[1-2]20	0[1-2]20	0111	1000	activation of KR and repression of GT in region A
<i>Kr BS controlling Gt</i>	1300	1210	1110	1000	expansion of GT in regions B and C, -> further repression of KR and KNI in regions B and C, respectively



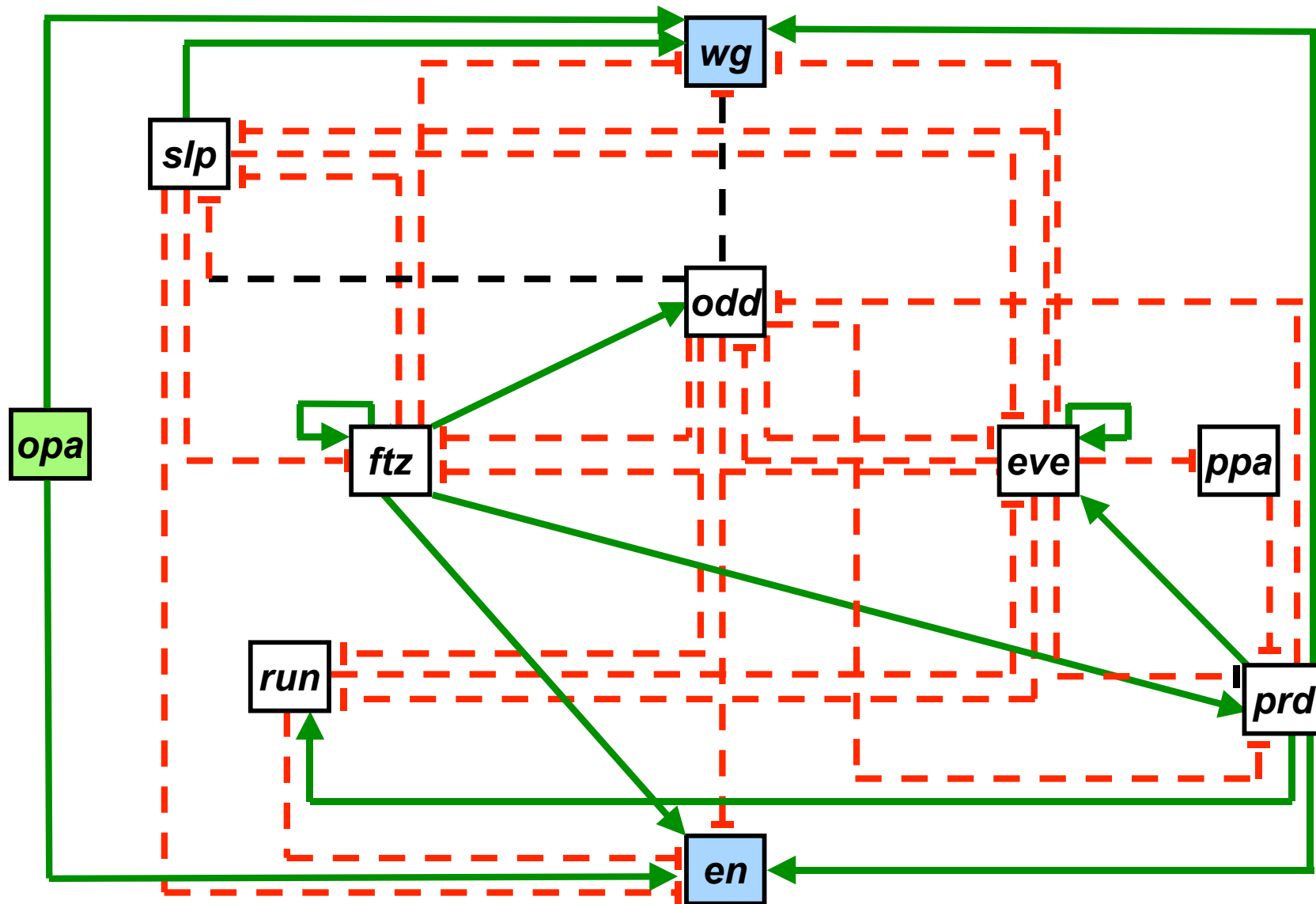
Pair-rule genes expression patterns



Pair-rule cross-regulations



Pair-rule cross-regulatory gene network



Pair-rule cross-regulatory matrix with thresholds

Input
(ubiquist
expression)

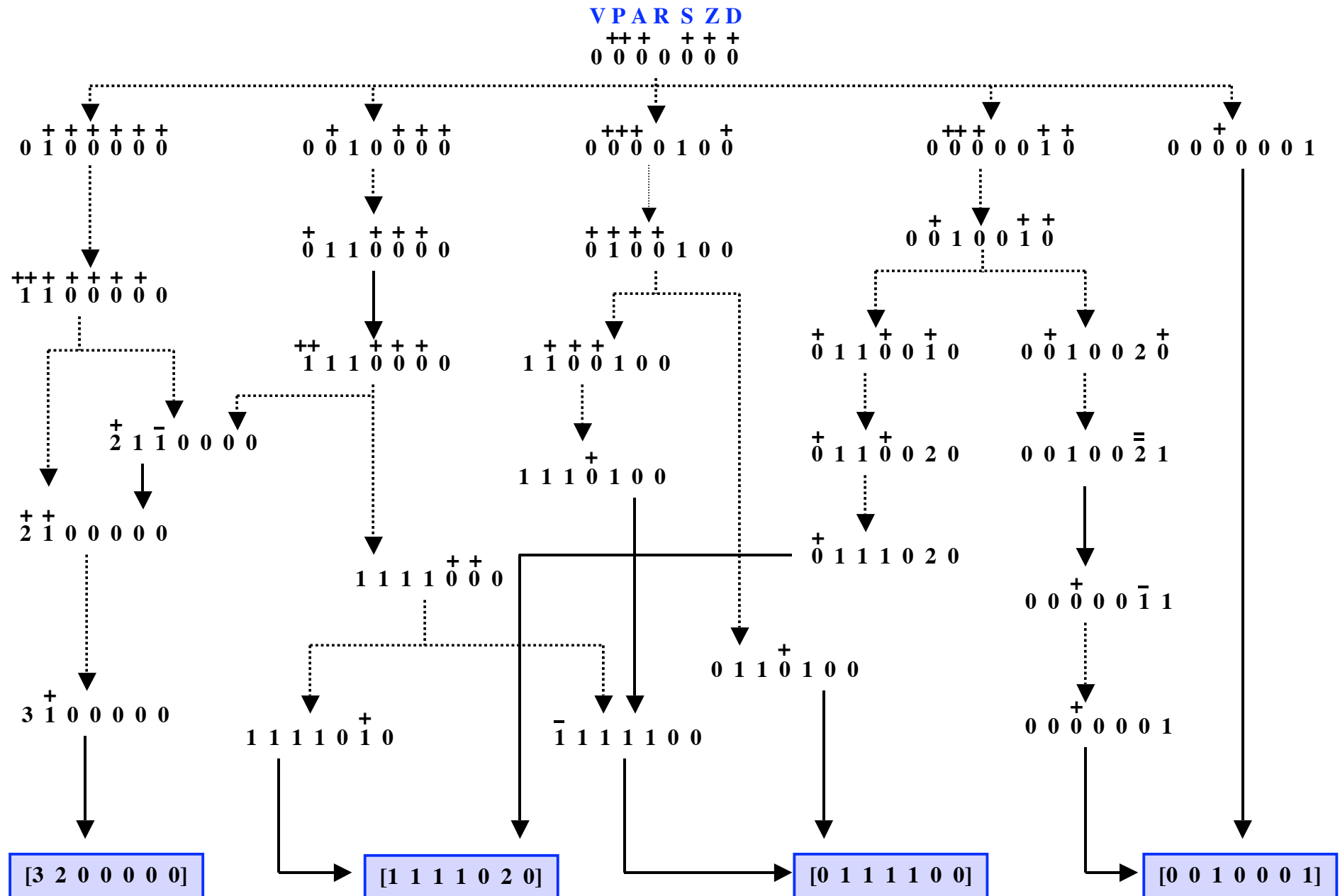
	<i>eve</i>	<i>prd</i>	<i>ppa</i>	<i>run</i>	<i>slp</i>	<i>ftz</i>	<i>odd</i>	<i>opa</i>
<i>eve</i>	+1	+1	0	-1	-1	0	-1	0
<i>prd</i>	-3	0	-1	0	0	+1	-1	0
<i>ppa</i>	-2	0	0	0	0	0	0	0
<i>run</i>	-2	+1	0	0	0	0	-1	0
<i>slp</i>	-2	0	0	0	0	-1	-1	0
<i>ftz</i>	-2	0	0	0	-1	+1	-1	0
<i>odd</i>	-1	-1	0	0	0	+1	0	0
<i>en</i>	0	+2	0	-1	-1	+2	-1	+1
<i>wg</i>	-1	+1	0	0	+1	-1	-1	+1

Ouputs
(Segment
polarity
genes)

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	$K_{z.vsd}$	$K_{z.zvzd}$	
odd	$K_{d.vp}$ $K_{d.zvp}$		
en	$K_{e.yzs}$ $K_{e.yprsd}$ $K_{e.yzrsd}$ $K_{e.ypszsd}$ $K_{e.ypzrsd}$		
wg	$K_{w.ypsvzd}$		

Logical parameter
values

Intrinsic dynamical properties of the pair-rule module



Pair-rule system and positional information

The state reached by the pair-rule system depends on **which of the pair-rule genes become first activated (3 rules)**

→ depends on the **position** along the A/P axis of the embryo's trunk

→ depends on **particular combination of maternal & gap products**
along the A/P axis of the embryo's trunk

Logical simulations for the wild type

	Region I	Region II	Region IIIa	Region IIIb	Region IV	Region V
Activation of primary pair-rule genes	V P A R S Z D 2 0 0 1 0 1 1 ↓ C1 C2 2 0 0 1 0 0 0 ↓ 1 0 0 1 0 0 0	V P A R S Z D 1 0 0 1 0 1 1 ↓ C1 1 0 0 1 0 1 0 ↓ 1 0 0 1 0 2 0	V P A R S Z D 0 0 0 1 0 1 1 ↓ 0 0 0 1 0 0 1 ↓ 0 0 0 0 0 0 1	V P A R S Z D 0 0 0 0 0 1 1 ↓ C3 0 0 0 0 0 0 1 ↓ 0 0 0 0 0 0 1	V P A R S Z D 1 0 0 0 0 1 1 ↓ C1 C3 1 0 0 0 0 0 0 ↓ 1 1 1 0 1 0 0	V P A R S Z D 2 0 0 0 0 1 1 ↓ C1 C3 2 0 0 0 0 0 0 ↓ 2 1 0 0 0 0 0
Activation of secondary pair-rule genes and refinement of pair-rule stripes	activation of <i>prd</i>, <i>ppa</i> and <i>slp</i> ↓ 1 1 1 1 1 0 0 ↓ [0 1 1 1 1 0 0]	activation of <i>prd</i>, <i>ppa</i> and <i>slp</i> ↓ [1 1 1 1 0 2 0] ↓ [1 1 1 1 0 2 0]	activation of <i>prd</i>, <i>ppa</i> and <i>slp</i> ↓ [0 0 1 0 0 0 1] ↓ [0 0 1 0 0 0 1]	activation of <i>prd</i>, <i>ppa</i> and <i>slp</i> ↓ [0 0 1 0 0 0 1] ↓ [0 0 1 0 0 0 1]	activation of <i>prd</i>, <i>ppa</i> and <i>slp</i> ↓ 1 1 1 0 1 0 0 ↓ 0 1 1 0 1 0 0 ↓ [0 1 1 1 1 0 0]	activation of <i>prd</i>, <i>ppa</i> and <i>slp</i> ↓ 2 1 0 0 0 0 0 ↓ [3 2 0 0 0 0 0]
Final state	[0 1 1 1 1 0 0]	[1 1 1 1 0 2 0]	[0 0 1 0 0 0 1]	[0 0 1 0 0 0 1]	[0 1 1 1 1 0 0]	[3 2 0 0 0 0 0]
	wg = ON & en = OFF	wg = OFF & en = ON	wg = OFF & en = OFF	wg = OFF & en = OFF	wg = ON & en = OFF	wg = OFF & en = ON
	odd parasegment	even parasegment				odd parasegment

Three rules for the formation of the pair-rule pattern

C1) Eve —| odd **overcomes** Odd —| eve

C2) maternal-gap inputs --> run **overcomes** Eve —| run

C3) Hairy —| ftz & Hairy —| run **are needed**

Simulation of loss-of-function pair-rule mutants

Genetic background	stable states	Embryo regions	EN/WG expression	(partially) functional circuits	Comments
	v p a r s z d				
<i>wild-type</i>	0 0 1 0 0 0 1 0 1 1 1 1 0 0 1 1 1 1 0 2 0 3 2 0 0 0 0 0	III I, IV II V	- Wg En En	eve (+), eve/run (+), eve/slp (+), prd/odd (+), slp/ftz (+), eve-ftz-slp (-), prd/odd/ftz (+)	
<i>eve</i>	0 0 1 0 0 0 1 0 1 1 1 1 0 0 0 1 1 1 0 2 0	I-V Not reached Not reached	- (Wg) (En)	prd/odd (+), slp/ftz (+), prd/odd/ftz (+)	Loss of all En - and Wg -stripes
<i>prd</i>	0 0 1 0 0 0 1 1 0 1 0 0 2 0	I, III-V II	- En	eve (+), slp/ftz (+)	Loss of odd-numbered En -stripes and all Wg -stripes
<i>ppa</i>	0 0 0 0 0 0 1 0 2 0 1 1 0 0 1 2 0 1 0 2 0 3 2 0 0 0 0 0	III I, IV II V	- Wg En En	eve (+), eve/run (+), eve/slp (+), prd/odd (+), slp/ftz (+), eve-ftz-slp (-), prd/odd/ftz (+)	En - and Wg -stripes are normally formed (though three of the wild-type stable states are modified).
<i>run</i>	0 0 1 0 0 0 1 0 1 1 0 1 0 0 3 2 0 0 0 0 0	III IV I, II, V	- Wg En	eve (+), eve/slp (+), prd/odd (+), slp/ftz (+), eve-ftz-slp (-), prd/odd/ftz (+)	Loss of odd-numbered Wg -stripes
<i>slp</i>	0 0 1 0 0 0 1 1 1 1 1 0 2 0 3 2 0 0 0 0 0	III I, II, (IV) (IV), V	- En En	eve (+), eve/run (+), prd/odd (+), prd/odd/ftz (+)	Replacement of Wg -stripes by En -stripes
<i>ftz</i>	0 1 1 1 1 0 0 3 2 0 0 0 0 0	I-IV V	Wg En	eve (+), eve/run (+), eve/slp (+), prd/odd (+)	Loss of even-numbered En -stripes and expansion of Wg -stripes
<i>odd</i>	0 1 1 1 1 0 0 1 1 1 1 0 2 0 3 2 0 0 0 0 0	I, IIIb, IV II, IIIa V	Wg En En	eve (+), eve/run (+), eve/slp (+), slp/ftz (+), eve-ftz-slp (-)	Even-numbered En - and Wg -stripes expand posteriorly and anteriorly into the parasegment, respectively

Prediction of double loss-of-function mutants

Genetic background	stable states	Embryo regions	EN/WG expression	(partially) functional circuits	Comments
wild-type	0 0 1 0 0 0 1 0 1 1 1 1 0 0 1 1 1 1 0 2 0 3 2 0 0 0 0 0	III I, IV II V	- Wg En En	eve (+), eve/run (+), eve/slp (+), prd/odd (+), slp/ftz (+), eve- ftz-slp (-), prd/odd/ftz (+)	
<i>eve;odd</i>	0 1 1 1 0 2 0 0 1 1 1 1 0 0	I, II, IIIa IIIb, IV, V	En Wg	slp/ftz (+)	Odd Wg -stripes are replaced by En -stripes; even En -stripes expand posteriorly; odd En -stripes are replaced by Wg -stripes; even Wg -stripes expands anteriorly.
<i>ftz;odd</i>	0 1 1 1 1 0 0 3 2 0 0 0 0 0	I-IV V	Wg En	eve (+), eve/run (+), eve/slp (+)	Even En -stripes are lost and the Wg -stripes are expanded.
<i>prd;slp</i>	0 0 1 0 0 0 1 1 0 1 0 0 2 0	III I, II, IV, V	- En	eve (+)	Loss of odd-numbered En -stripes and of all Wg -stripes

Simulation of ectopic expression of pair-rule genes

Genetic background	stable states	Embryo regions	EN/WG expression	(partially) functional circuits	Comments
	v p a r s z d				
<i>wild-type</i>	0 0 1 0 0 0 1 0 1 1 1 1 0 0 1 1 1 1 0 2 0 3 2 0 0 0 0 0	III I, IV II V	- Wg En En	eve (+), eve/run (+), eve/slp (+), prd/odd (+), slp/ftz (+), eve-ftz-slp (-), prd/odd/ftz (+)	
<i>eve</i>	3 2 0 0 0 0 0	I-V	En	None!	The Wg -stripes are lost and the En stripes are expanded
<i>prd</i>	0 2 1 1 1 0 0 1 2 1 1 0 2 0 3 2 0 0 0 0 0	I, IIIb, IV II, IIIa V	Wg En En	eve (+), eve/run (+), eve/slp (+), slp/ftz (+), eve- ftz-slp (-)	Anterior expansion of Wg -stripes
<i>ppa</i>	0 1 1 1 1 0 0 1 1 1 1 0 2 0 0 0 1 0 0 0 1 3 1 1 0 0 0 0	I, IV II III V	Wg En - -	eve (+), eve/run (+), eve/slp (+), prd/odd (+), slp/ftz (+), eve-ftz-slp (-), prd/odd/ftz (+)	Loss of odd En -stripes
<i>run</i>	0 0 1 1 0 0 1 0 1 1 1 1 0 0 1 1 1 1 0 2 0	III I, IV, V II	- Wg En	eve (+), prd/odd (+), slp/ftz (+), prd/odd/ftz (+)	Replacement of odd En -stripes by Wg -stripes
<i>slp</i>	0 0 1 0 1 0 1 0 1 1 1 1 0 0	III I, II, IV, V	- Wg	prd/odd (+)	Replacement of En -stripes by Wg -stripes
<i>ftz</i>	0 0 1 0 0 2 1 1 1 1 1 0 2 0 3 2 0 0 0 2 0	III I, II, IV V	- En En	eve (+), eve/run (+), prd/odd (+)	Replacement of Wg -stripes by En -stripes
<i>odd</i>	0 0 1 0 0 0 1 1 0 1 0 0 0 1	I-V Not reached	- (-)	eve (+)	Loss of En and Wg stripes

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				
<i>wild-type</i>	0 0 1 0 0 0 1 0 1 1 1 1 0 0 1 1 1 1 0 2 0 3 2 0 0 0 0 0	III I, IV II V	- Wg En En	eve (+), eve/run (+), eve/slp (+), prd/odd (+), slp/ftz (+), eve-ftz-slp (-), prd/odd/ftz (+)	
<i>eve</i> auto-regulation	0 0 1 0 0 0 1 0 1 1 1 1 0 0 1 1 1 1 0 2 0	III I, IV, (V) II, (V)	- Wg En	prd/odd (+), slp/ftz (+), prd/odd/ftz (+)	Possible replacement of odd En -stripes by Wg -stripes
<i>Ftz</i> auto-regulation	0 0 1 0 0 0 1 0 1 1 1 1 0 0 1 1 1 1 0 1 0 3 2 0 0 0 0 0	III I, IV II V	- Wg - En	eve (+), eve/run (+), eve/slp (+), prd/odd (+), slp/ftz (+), eve-ftz-slp (-), prd/odd/ftz (+)	Loss of even En -stripes

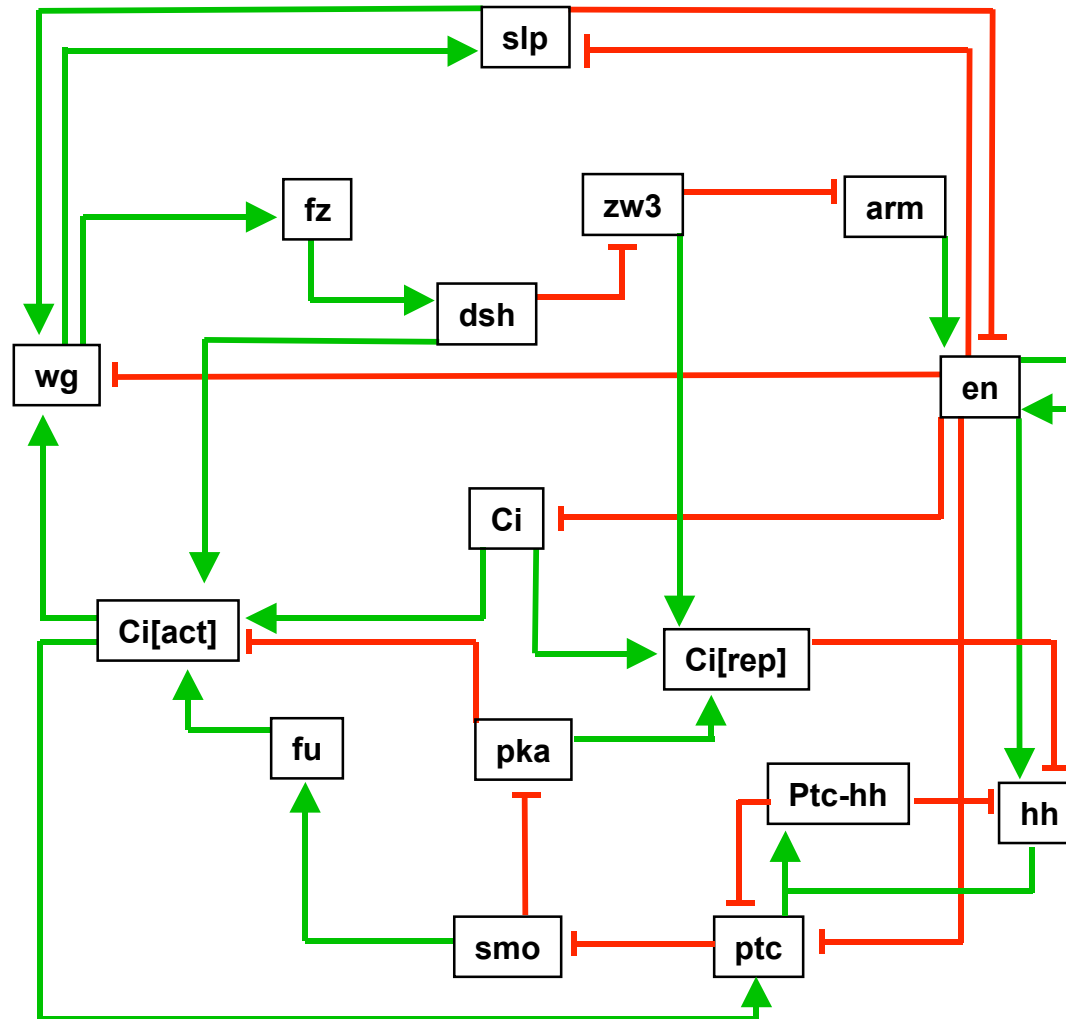
Feedback circuit analysis

- **51** intertwined, distinct **feedback circuits**
but only **7 (partly) functional feedback circuits**: **6 +**, **1 -**
- **Multiple positive circuits on a given gene** (e.g. eve)
→ **cooperation to generate a non-linear switch!?**
- **Perturbations at the feedback circuits**
→ **Loss of certain stable states** (cellular states)
→ **Miss-localization of some of the remaining states**

Four expression *modes*

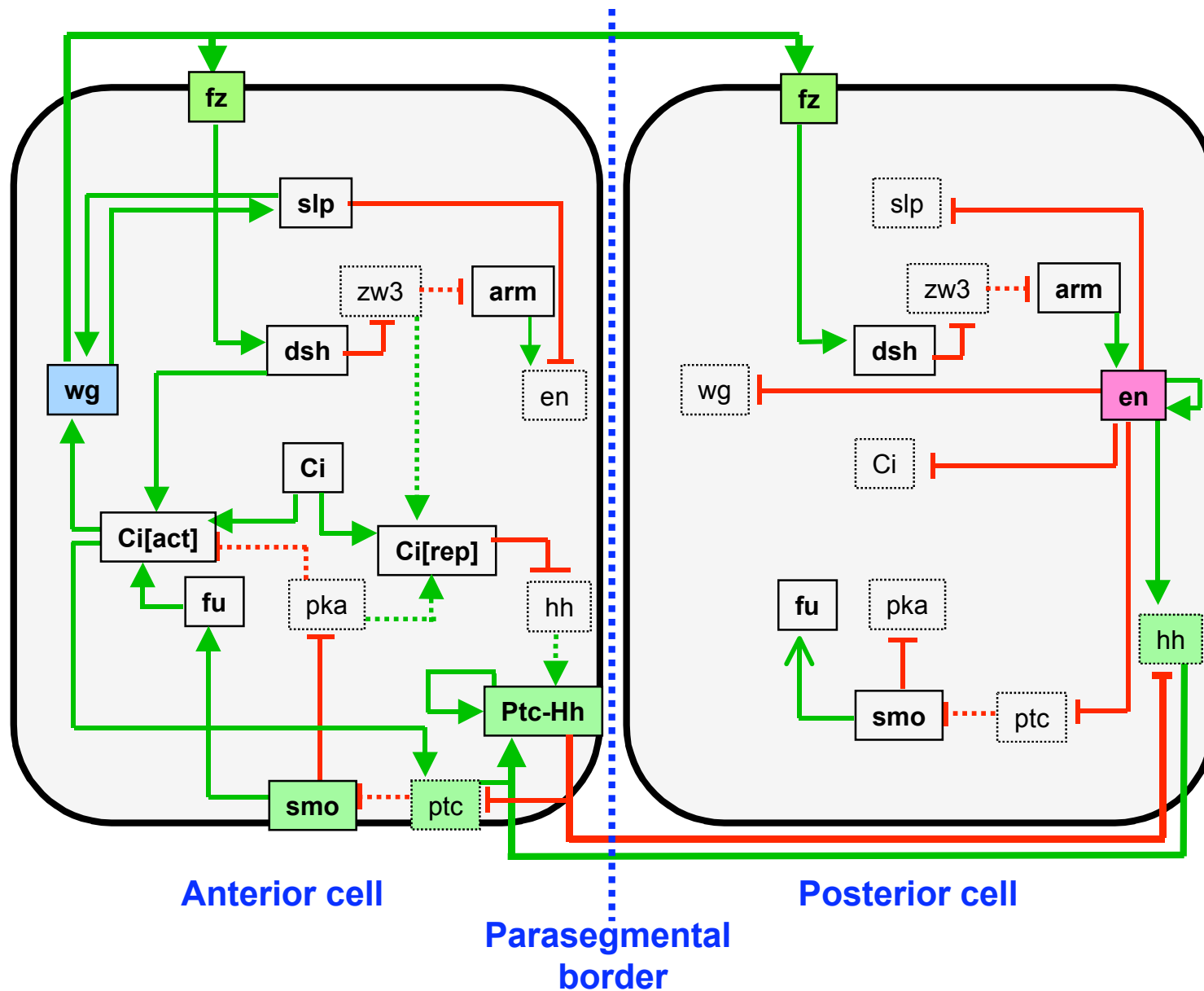
1. The ***eve***-expression mode
→ formation of odd-numbered **En**-stripes
2. The ***ftz***-expression mode
→ formation of even-numbered **En**-stripes
3. The ***prd/slp*** expression mode
→ formation of odd- and even-numbered **Wg** -stripes
4. The ***odd***-expression mode
→ prevention of ***en*** and ***wg*** expression in the middle of the parasegment

Segment polarity gene network



ptc, **hh**, **Ci[act]** are represented by **quaternary variables**; **Ptc-hh**, **wg**, **smo**, **pka**, **fz**, **dsh** are represented by **ternary variables**; the others 8 regulatory products are represented by **binary variables**.

Segment polarity **intercellular** network



Summing up

- Emphasis on the roles of **regulatory circuits** (+ and -) vs gene cascades
- Crucial role of **positive circuits** in **differentiative decisions** (Kr-gt)
- Principally **short circuits** are found **functional** (Kr-gt, eve, eve-run...)
- Notion of "**cross-regulatory modules**" = sets of intertwined circuits
- Notion of "**cross-regulatory modes**"
- Cross-regulatory **circuits** and **modules** as **dynamical building blocks**
- Qualitative **reproduction** of the **gene expression patterns** associated with the different **cell types**
- **Prediction** of the **phenotypes of various types of mutants**

Prospects

- **Coupling between the gap, pair-rule and segment polarity modules**
→ **towards a model of the whole segmentation hierarchy**
- Modelling of the network controlling **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**)
- **Methodological/computational developments** (**formalism**, **software**)
- From **Logical Regulatory Graphs** to **Petri Nets**

Multidisciplinary collaborations at Luminy

