MORE ON DEVELOPMENTAL GENE REGULATORY NETWORKS

KITP AUGUST 26, 2011

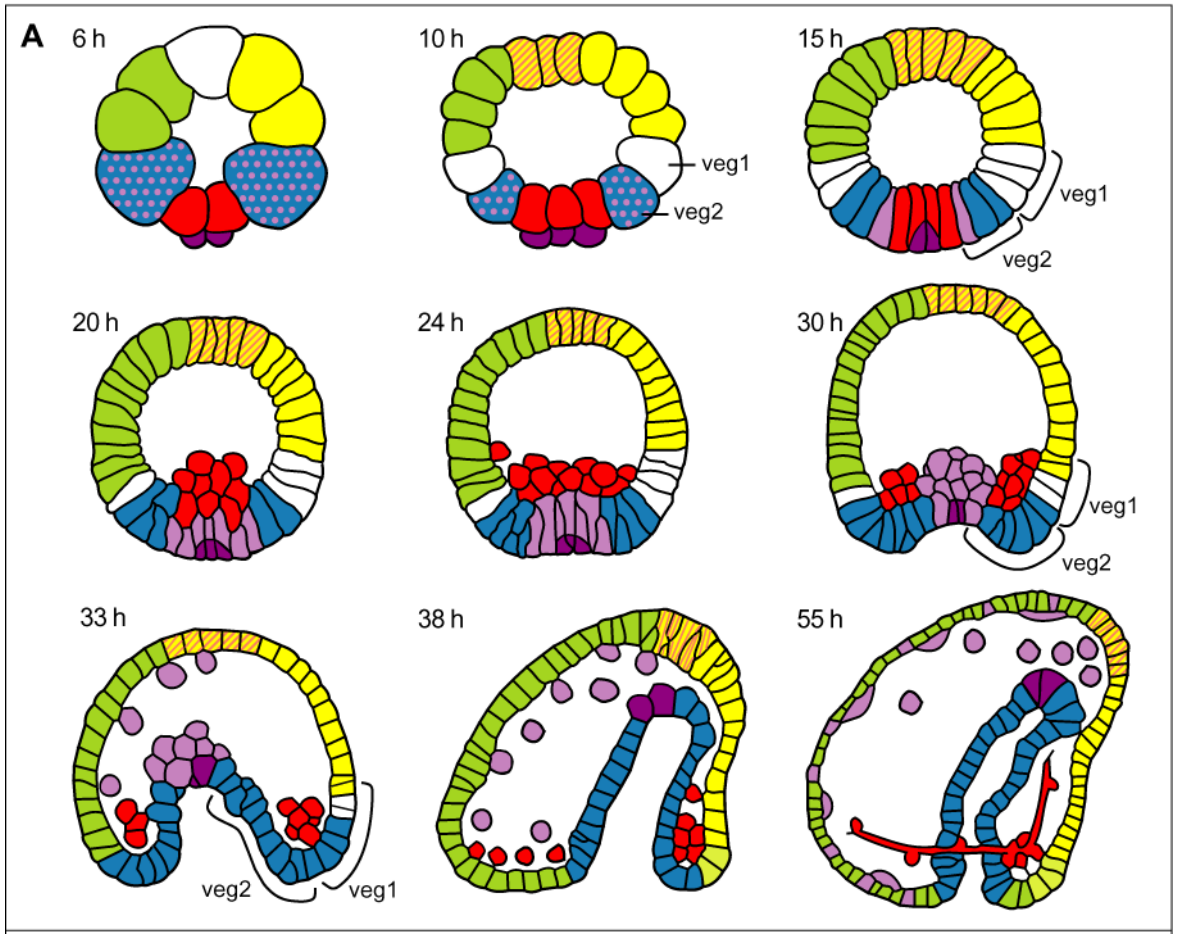
THE SEA URCHIN EMBRYO GRN'S

INCLUDES > 80 REGULATORY GENES IN VARIOUS EMBRYONIC TERRITORIES

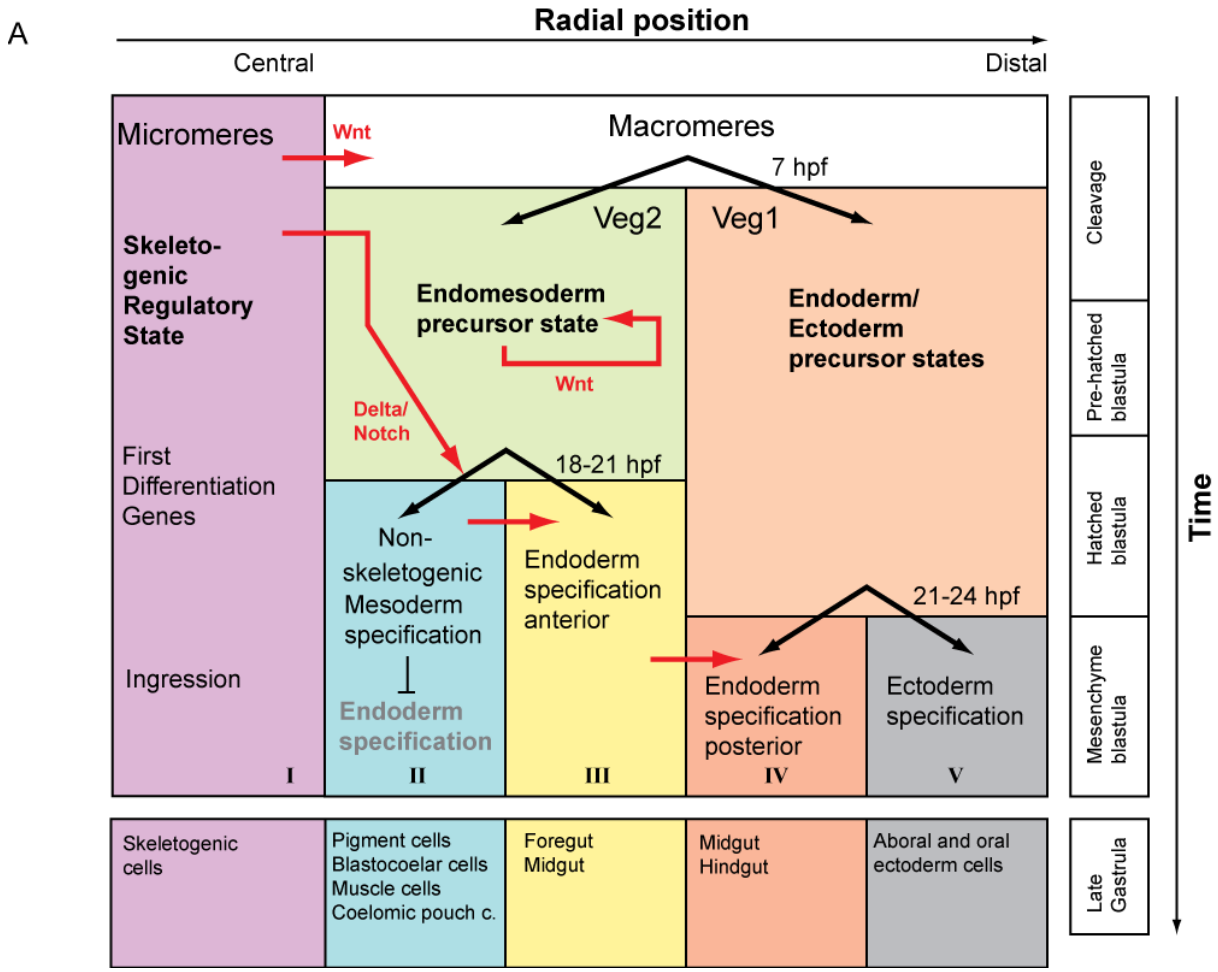
EXPERIMENTALLY BASED ON: (1) SPATIAL &TEMPORAL EXPRESSION DATA; (2) VERY LARGE SCALE MATRIX OF PERTURBATION RESULTS; (3) CIS-REGULATORY ANALYSES

GRN BUILT IN COMPUTATIONAL PLATFORM "BIOTAPESTRY"

COMPUTATIONAL BOOLEAN MODEL CAPTURES GENOMIC REGULATORY LOGIC AND PREDICTS SPATIAL OUTPUT FROM GRN TOPOLOGY



SPECIFICATION IN THE SEA URCHIN EMBRYO


A
Radial position
Central
Distal
Micromeres
Wnt
Macromeres
7 hpf
Veg2
Veg1
Skeleto-
genic
Regulatory
State
Endomesoderm
precursor state
Wnt
Endoderm/
Ectoderm
precursor states
Delta/
Notch
First
Differentiation
Genes
18-21 hpf
Non-
skeletogenic
Mesoderm
specification
Endoderm
specification
anterior
21-24 hpf
Ingression
Endoderm
specification
posterior
Ectoderm
specification
I
II
III
IV
V
Skeletogenic
cells
Pigment cells
Blastocoelar cells
Muscle cells
Coelomic pouch c.
Foregut
Midgut
Midgut
Hindgut
Aboral and oral
ectoderm cells
Cleavage
Pre-hatched
blastula
Hatched
blastula
Mesenchyme
blastula
Late
Gastrula
Time

A

	Time [h]	*blimp1b*	*brachyury*	*brn1/2/4*	*dac*	*eve*	*foxa*	*gatae*	*hnf1*	*hox11/13b*	*krl*	*myc*	*otx*	*soxc*	*tgif*
veg2 endoderm	12														
	15														
	18														
	21														
	24														
	27														
	30														
veg1 endoderm	12														
	15														
	18														
	21														
	24														
	27														
	30														

B

Input \ Output	*blimp1b*	*brachyury*	*brn1/2/4*	*dac*	*eve*	*foxa*	*gatae*	*hnf1*	*hox11/13b*	*krl*	*myc*	*otx*	*soxc*	*tgif*
Blimp														
Brachyury														
Dac														
Eve														
FoxA														
GataE														
Hnf1														
Hox11/13b														
Z13/Krl														
Mvc														
Otx														
SoxC														
Tgif														

Endomesoderm Specification up to 30 Hours

February 03, 2011

This model is frequently revised. It is based on the latest laboratory data, some of which is not yet published.

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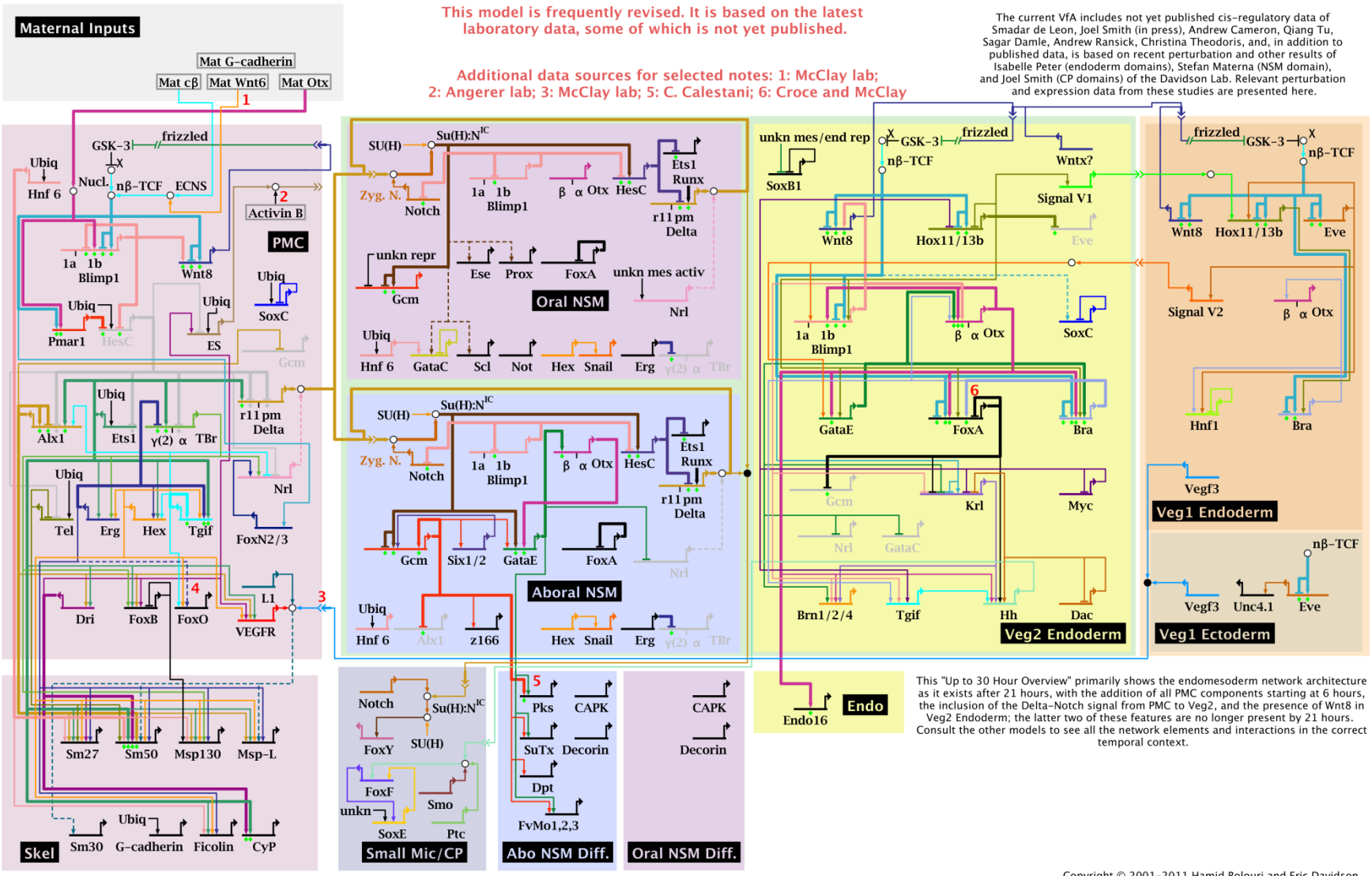
The current VfA includes not yet published cis-regulatory data of Smadar de Leon, Joel Smith (in press), Andrew Cameron, Qiang Tu, Sagar Damle, Andrew Ransick, Christina Theodoris, and, in addition to published data, is based on recent perturbation and other results of Isabelle Peter (endoderm domains), Stefan Materna (NSM domain), and Joel Smith (CP domains) of the Davidson Lab. Relevant perturbation and expression data from these studies are presented here.

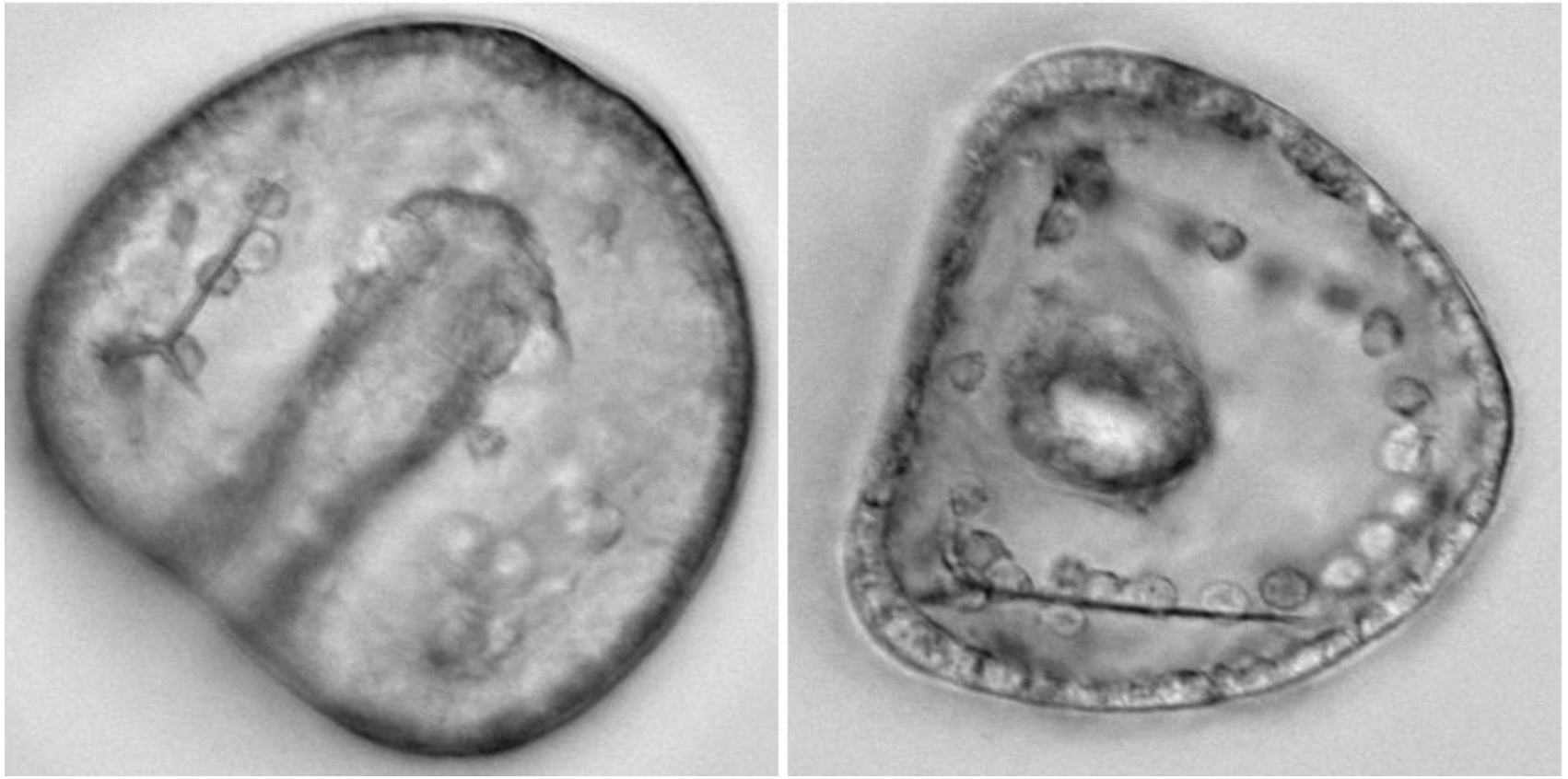


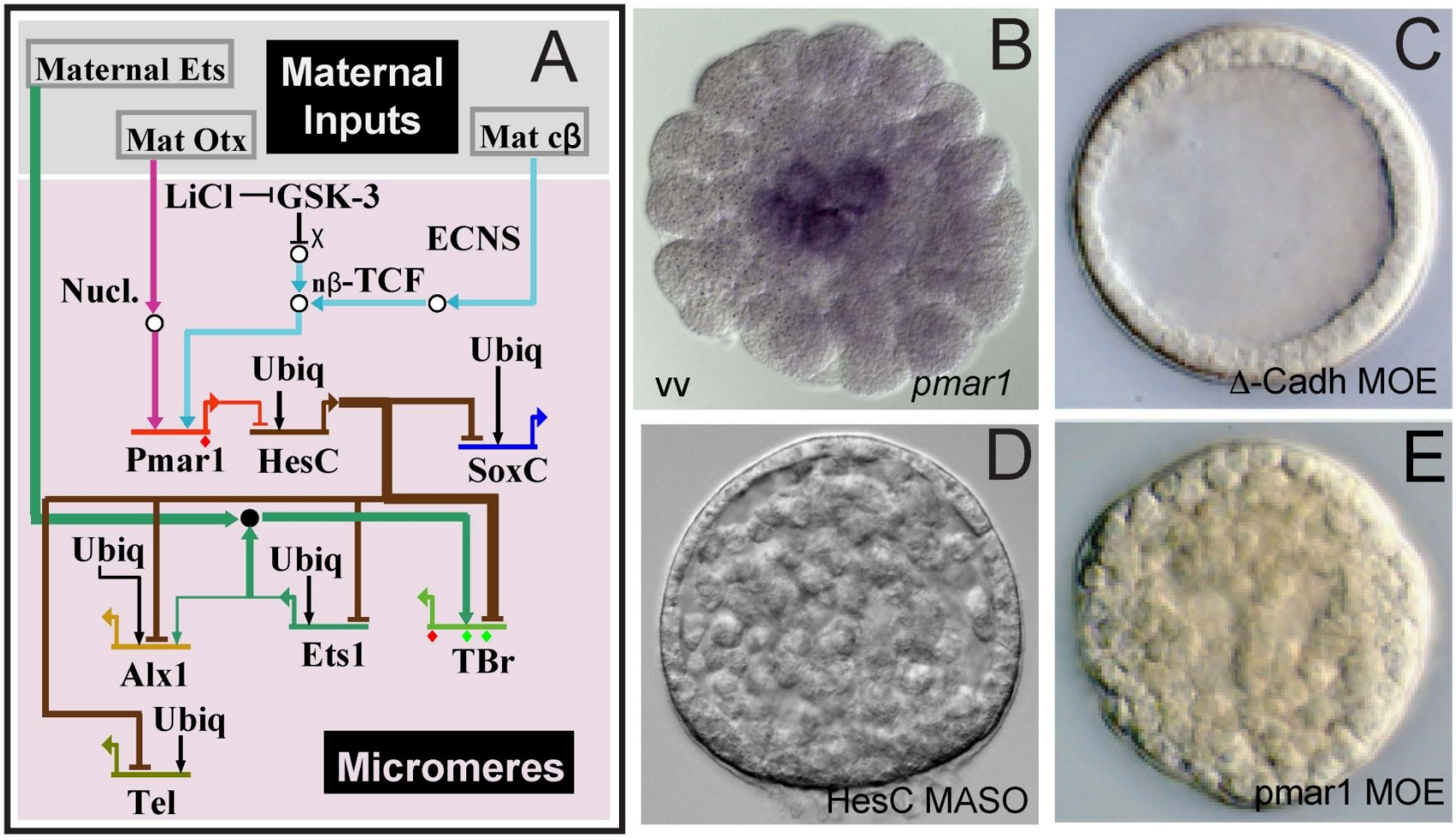
This "Up to 30 Hour Overview" primarily shows the endomesoderm network architecture as it exists after 21 hours, with the addition of all PMC components starting at 6 hours, the inclusion of the Delta-Notch signal from PMC to Veg2, and the presence of Wnt8 in Veg2 Endoderm; the latter two of these features are no longer present by 21 hours. Consult the other models to see all the network elements and interactions in the correct temporal context.

Ubiq=ubiquitous; Mat = maternal; activ = activator; rep = repressor;
unkn = unknown; Nucl. = nuclearization; χ = β-catenin source;
nβ-TCF = nuclearized b-β-catenin-Tcf1; ES = early signal;
ECNS = early cytoplasmic nuclearization system; Zyg. N. = zygotic Notch

HOW DEVELOPMENT IS ENCODED: EXAMPLE 1

SPECIFICATION OF SKELETOGENIC CELL LINEAGE



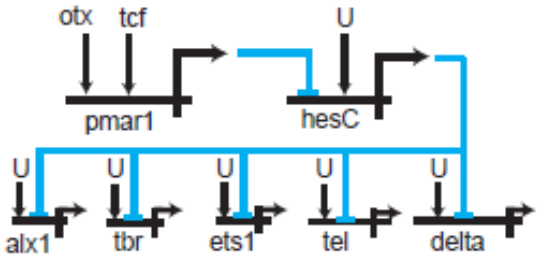

A
Maternal Ets
Maternal Inputs
Mat Otx
Mat cβ
LiCl ⊣ GSK-3
X
ECNS
Nucl.
nβ-TCF
Ubiq
Ubiq
Pmar1
HesC
SoxC
Ubiq
Ubiq
Alx1
Ets1
TBr
Ubiq
Tel
Micromeres
B
vv
pmar1
C
Δ-Cadh MOE
D
HesC MASO
E
pmar1 MOE

THE DOUBLE NEGATIVE GATE SUBCIRCUIT

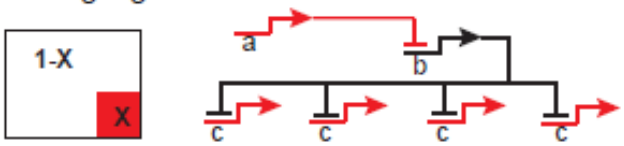
A Job: X, 1-X spatial specification function

A1 Subcircuit: double negative gate

From GRN:



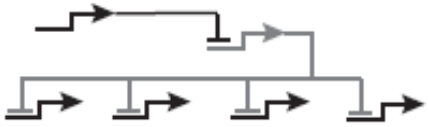
a: Repressor1
b: Repressor2
c: Target genes



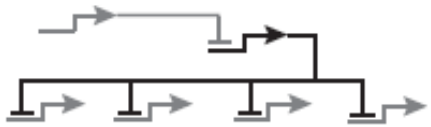
Subcircuit output

	a	b	c
X	1	0	1
1-X	0	1	0

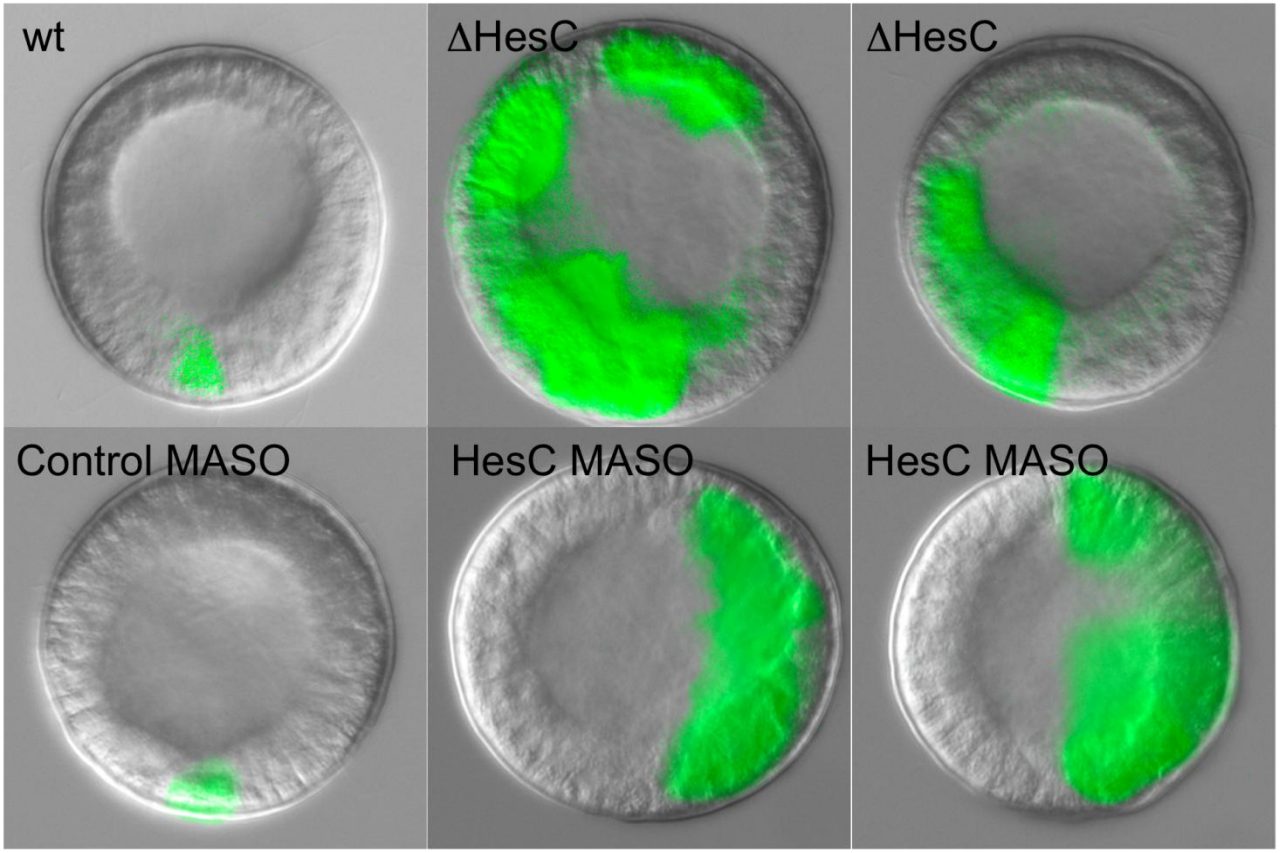
In X



In 1-X

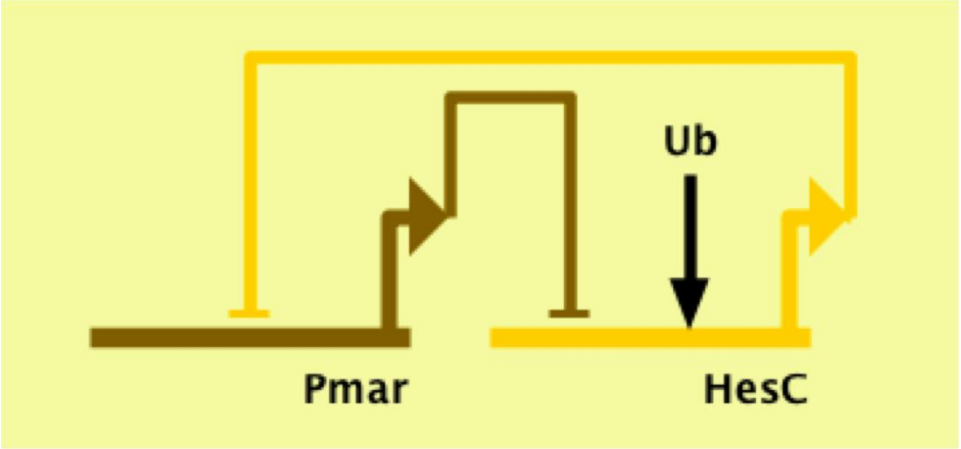


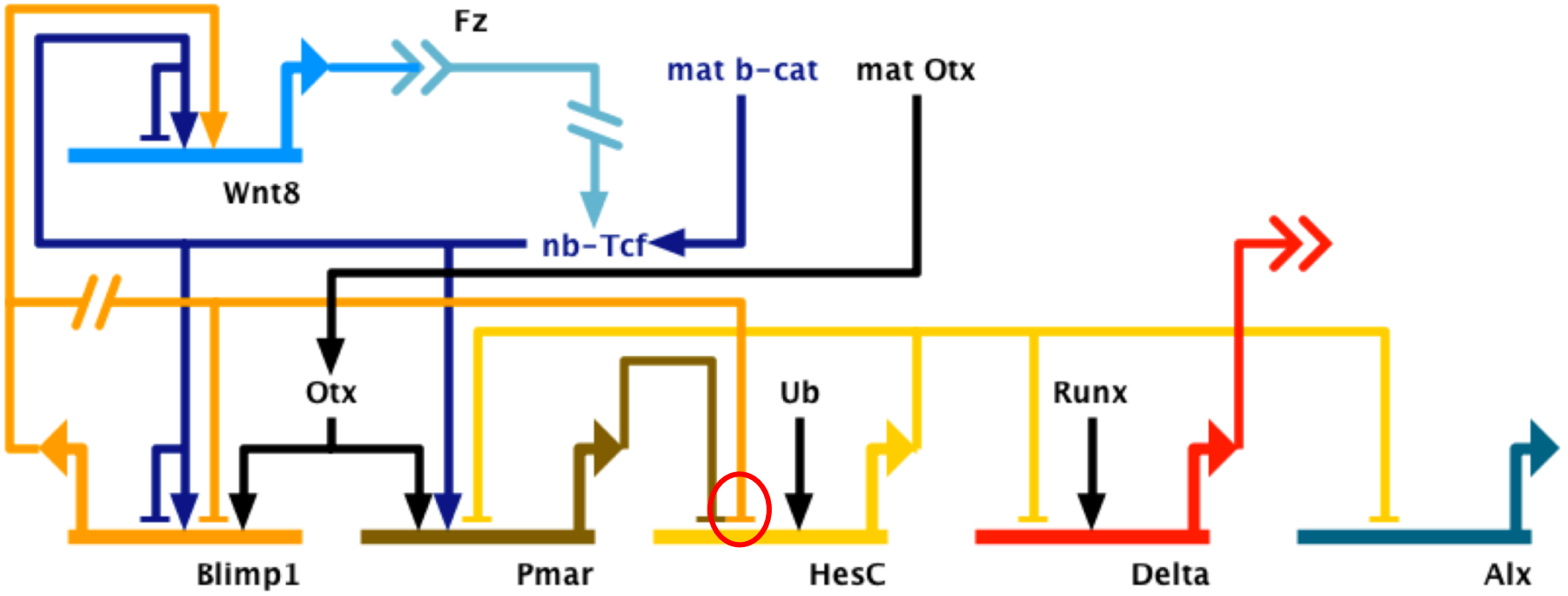
GENE REGULATORY NETWORKS FOR EMBRYONIC DEVELOPMENT ARE NOT "PARSIMONIOUSLY" WIRED: MULTIPLE SUBCIRCUITS OPERATE TO ENSURE THE RIGHT OUTCOME…



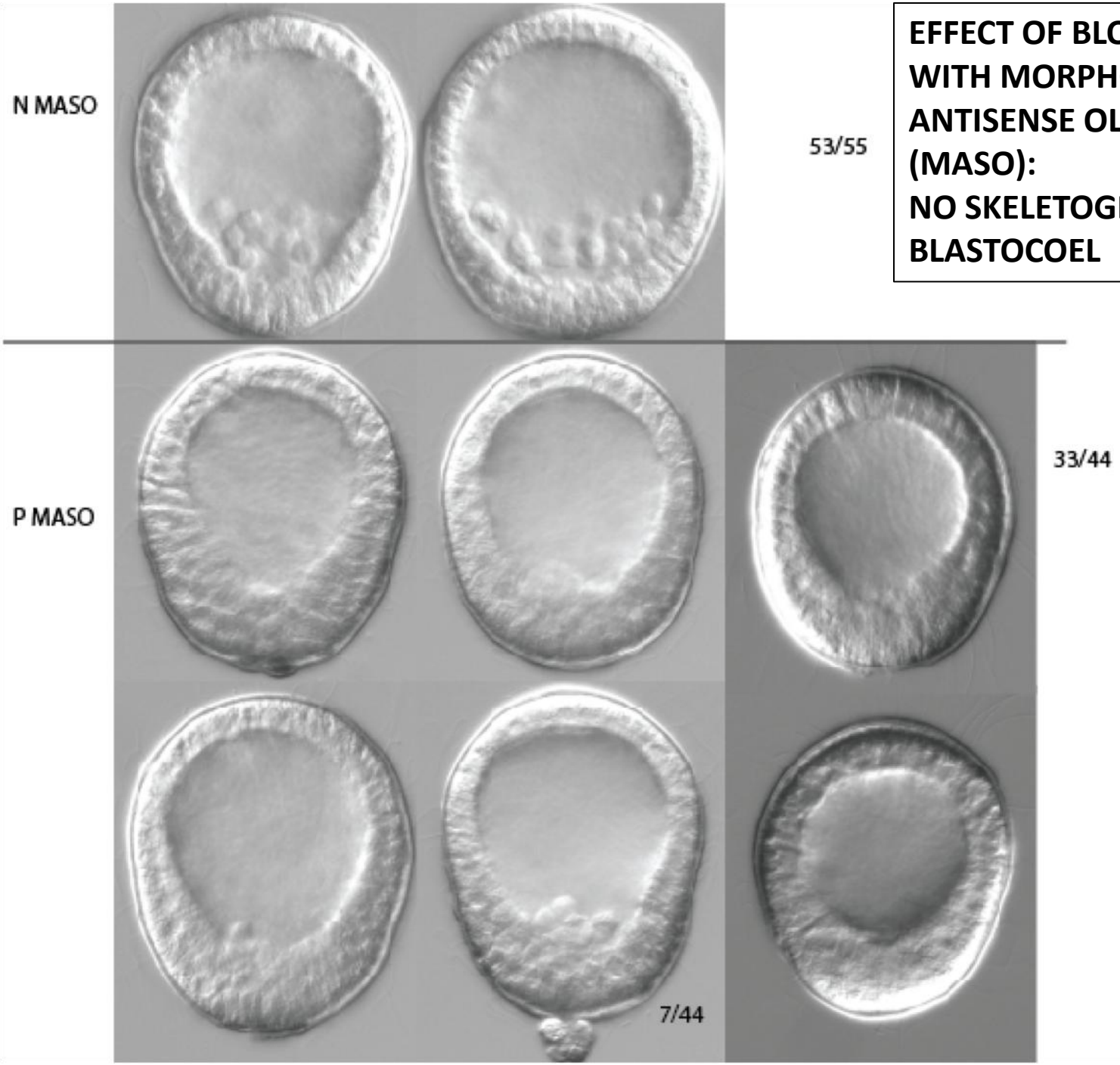
DESPITE APPEARENCES, NOT A "BISTABLE SWITCH" !

TEMPORALLY AND SPATIALLY NON-COINCIDENT; SEQUENTIAL AND NON-OVERLAPPING; ENTIRELY DETERMINISTIC



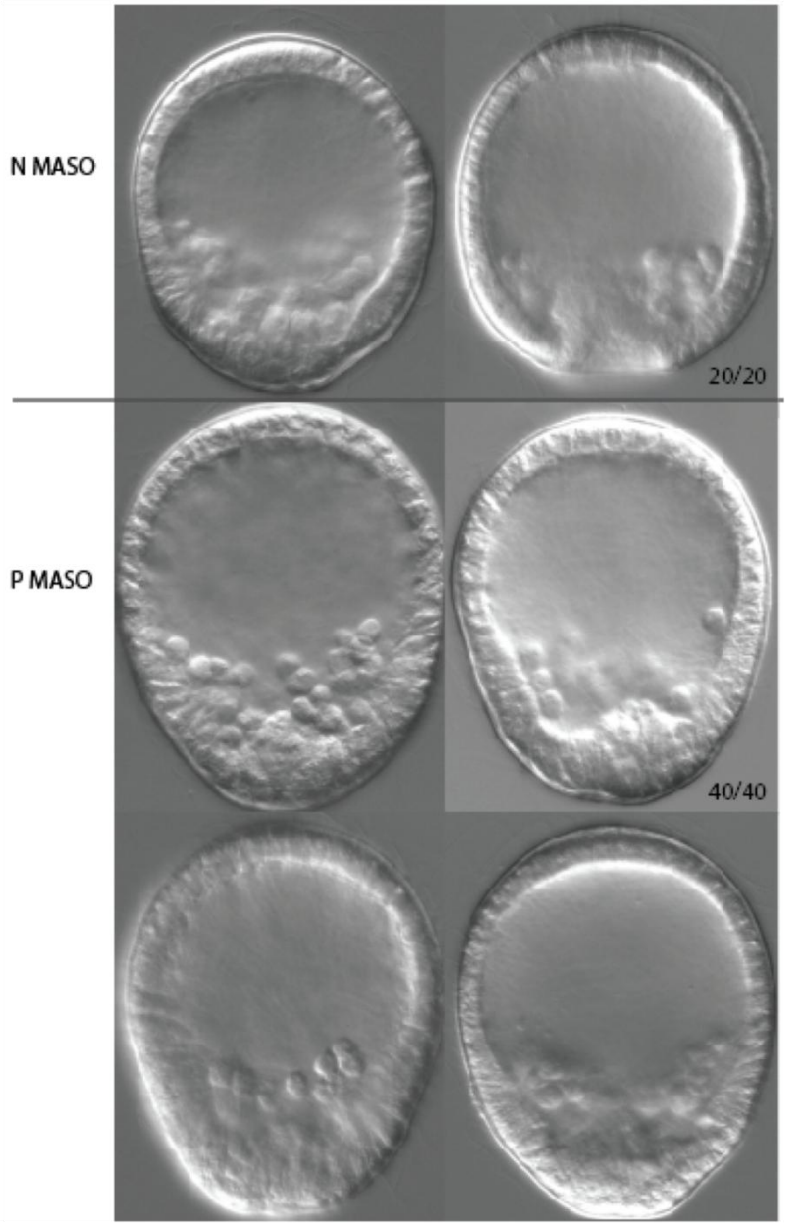

Fz
mat b-cat
mat Otx
Wnt8
nb-Tcf
Otx
Ub
Runx
Blimp1
Pmar
HesC
Delta
Alx

EFFECT OF BLOCKING PMAR1 WITH MORPHOLINO ANTISENSE OLIGONUCLEOTIDE (MASO):
NO SKELETOGENIC CELLS IN BLASTOCOEL



30 h

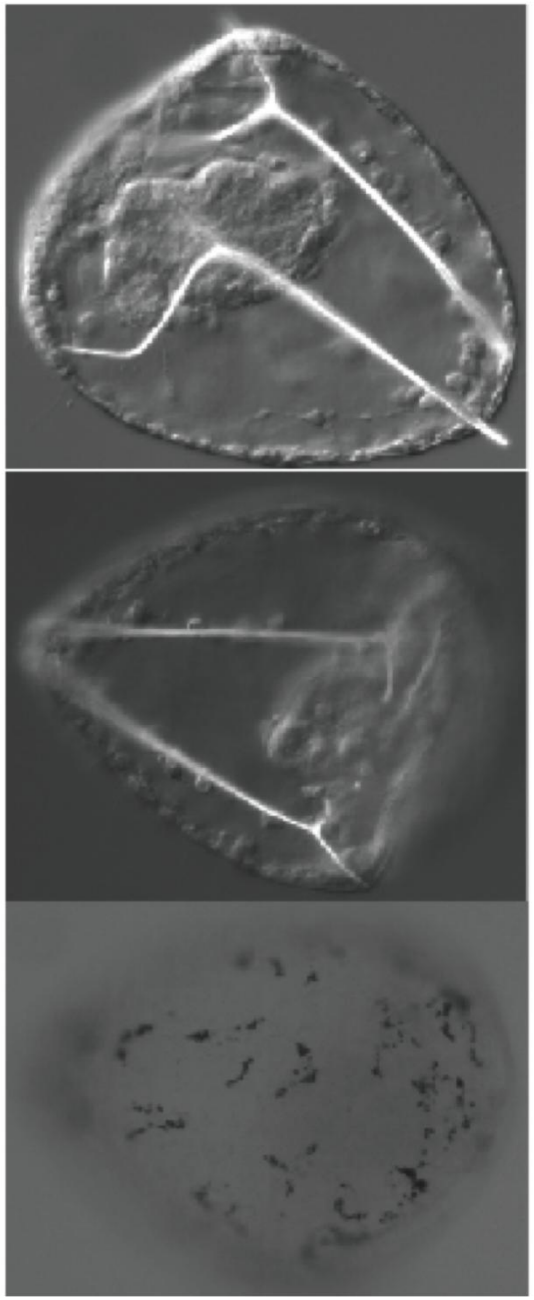
72 h



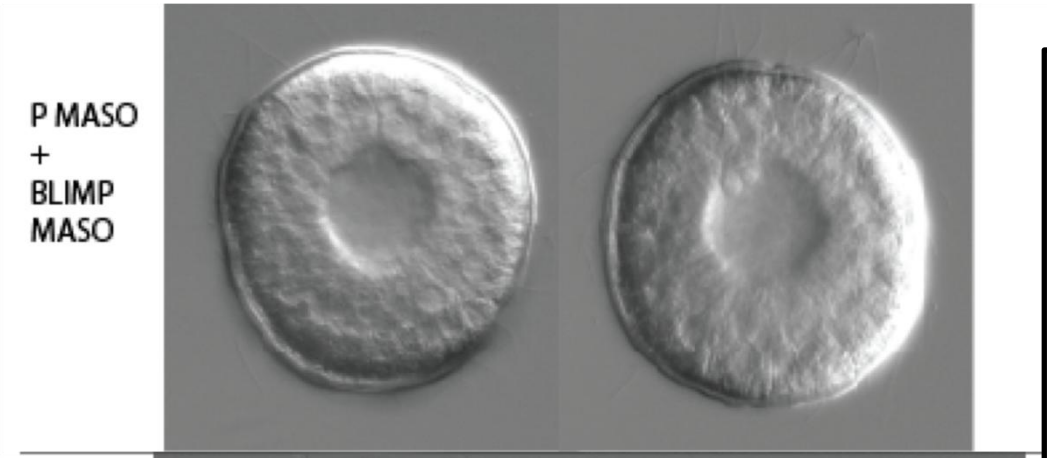
N MASO

REGULATIVE RECOVERY!

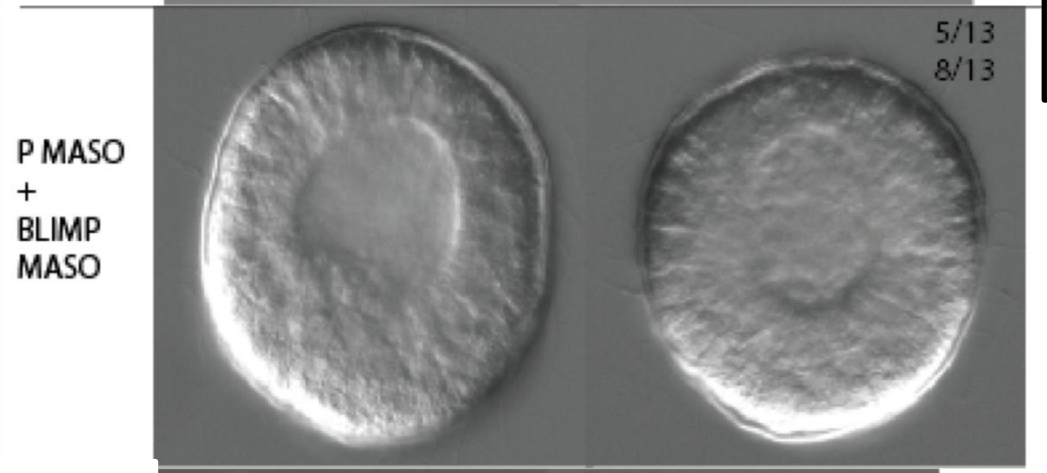
P MASO



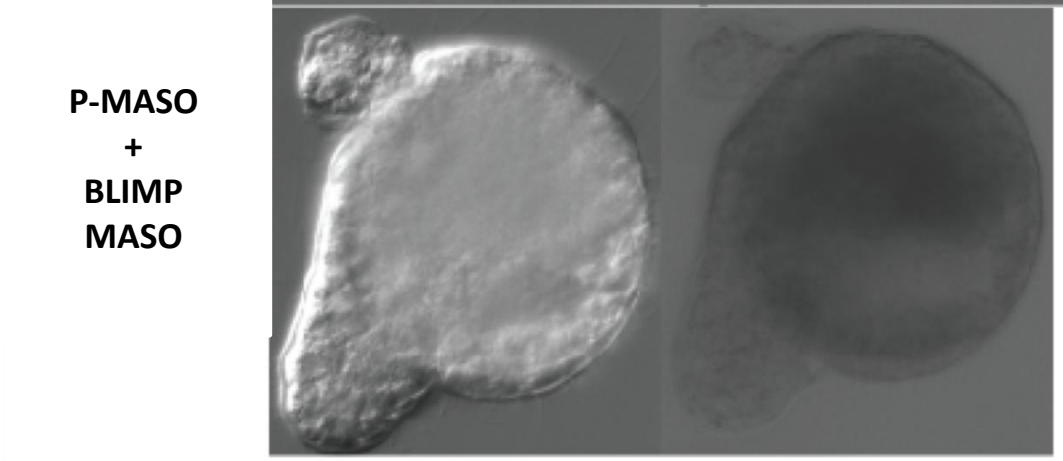
24 h



30 h



72 h



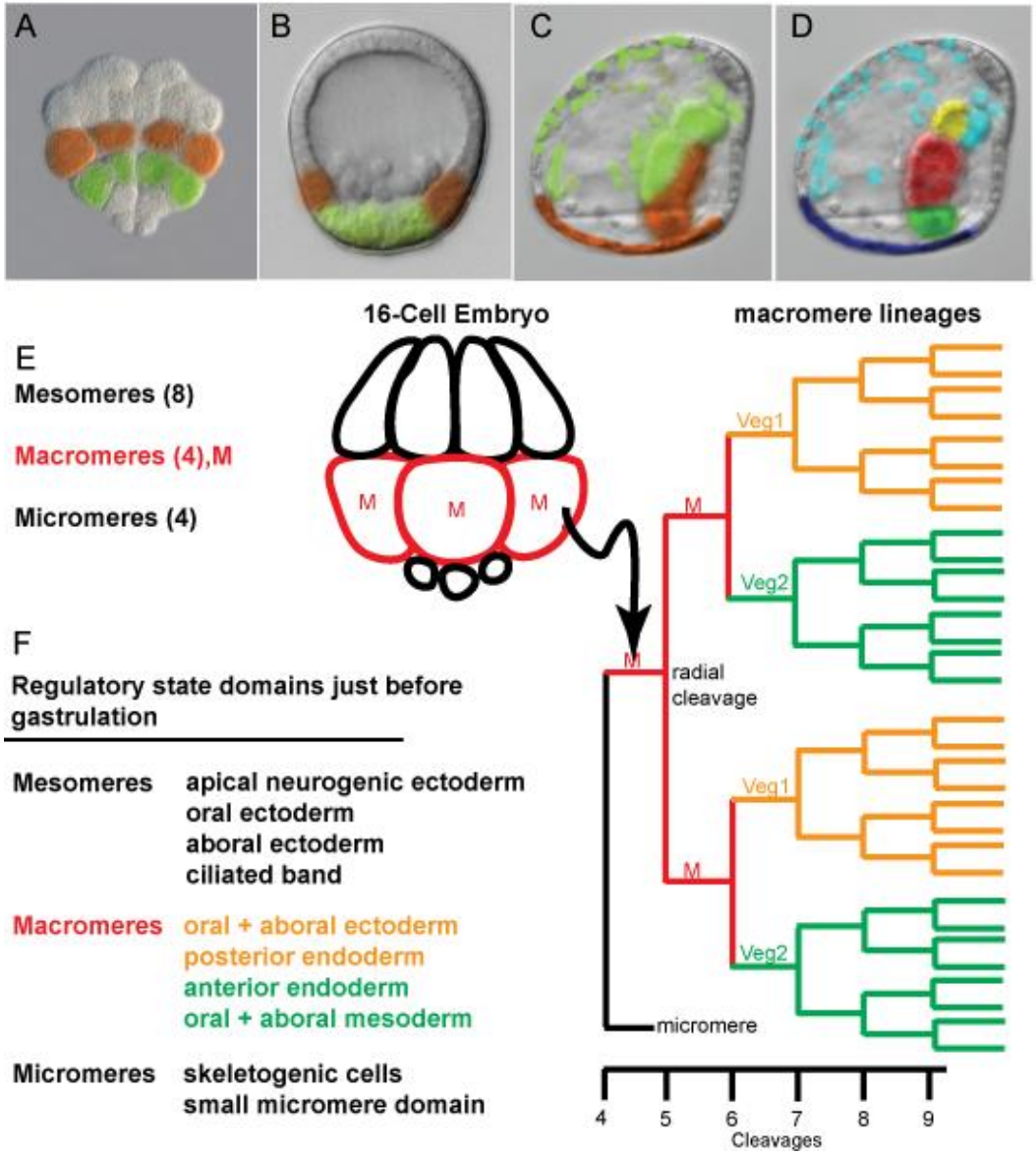
MINUS FAIL-SAFE LINKAGE: DEVELOPMENTAL DISASTER

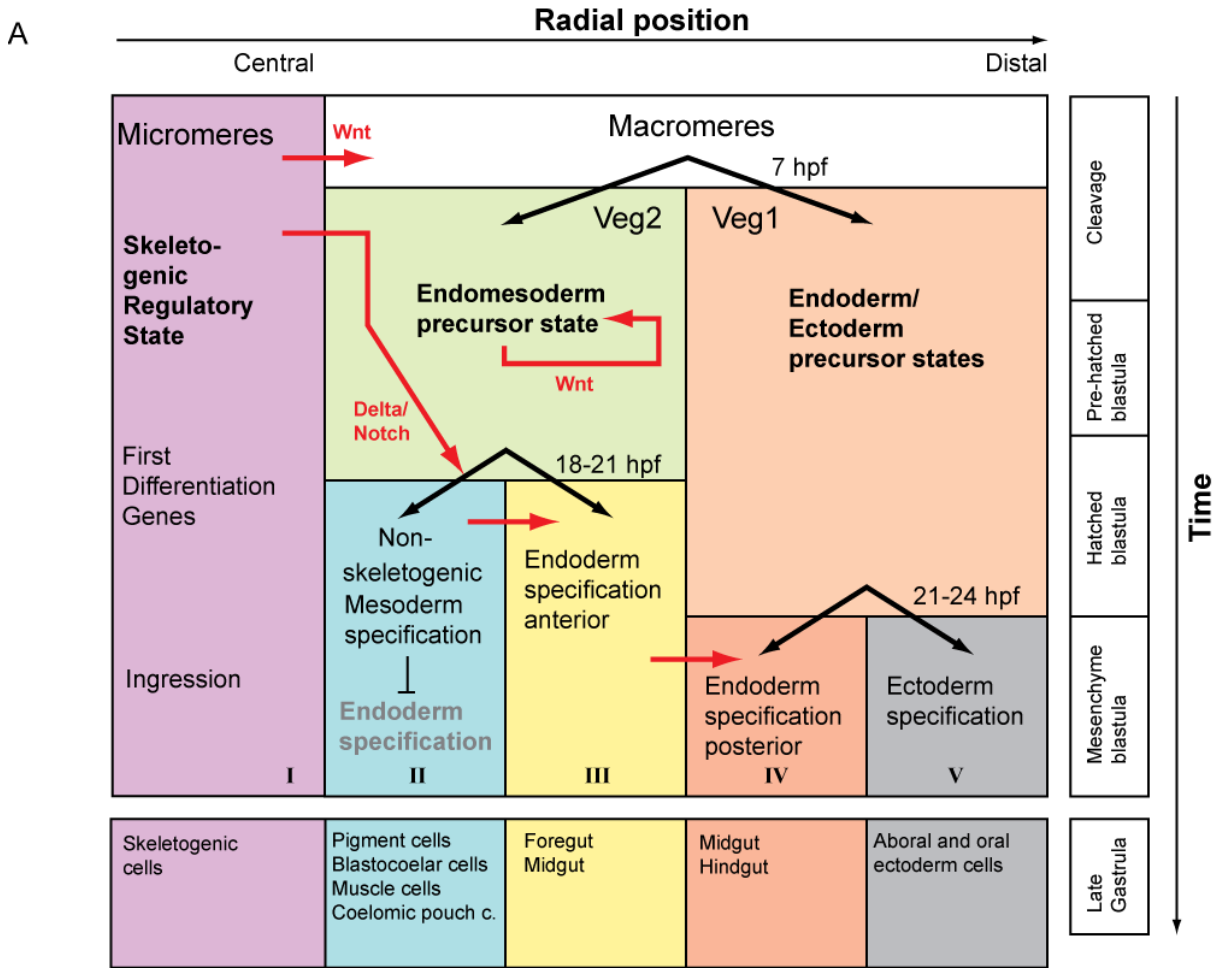
HOW DEVELOPMENT IS ENCODED: EXAMPLE 2

DYNAMIC SEPARATION OF FATES:

ENDODERM VS MESODERM REGULATORY STATE SPECIFICATION

ANTERIOR ENDODERM (FUTURE FOREGUT & MIDGUT) VS POSTERIOR ENDODERM (FUTURE HINDGUT) REGULATORY STATE SPECIFICATION


A
B
C
D
16-Cell Embryo
macromere lineages
E
Mesomeres (8)
Macromeres (4),M
Micromeres (4)
M
M
M
F
Regulatory state domains just before gastrulation
Mesomeres
apical neurogenic ectoderm
oral ectoderm
aboral ectoderm
ciliated band
Macromeres
oral + aboral ectoderm
posterior endoderm
anterior endoderm
oral + aboral mesoderm
Micromeres
skeletogenic cells
small micromere domain
Veg1
Veg2
Veg1
Veg2
M
M
M
radial cleavage
micromere
4
5
6
7
8
9
Cleavages


A
Radial position
Central
Distal
Micromeres
Wnt
Macromeres
7 hpf
Veg2
Veg1
Skeleto-
genic
Regulatory
State
Endomesoderm
precursor state
Wnt
Endoderm/
Ectoderm
precursor states
Delta/
Notch
First
Differentiation
Genes
18-21 hpf
Non-
skeletogenic
Mesoderm
specification
Endoderm
specification
anterior
21-24 hpf
Ingression
Endoderm
specification
posterior
Ectoderm
specification
I
II
III
IV
V
Skeletogenic
cells
Pigment cells
Blastocoelar cells
Muscle cells
Coelomic pouch c.
Foregut
Midgut
Midgut
Hindgut
Aboral and oral
ectoderm cells
Cleavage
Pre-hatched
blastula
Hatched
blastula
Mesenchyme
blastula
Late
Gastrula
Time

Endomesoderm Specification up to 30 Hours

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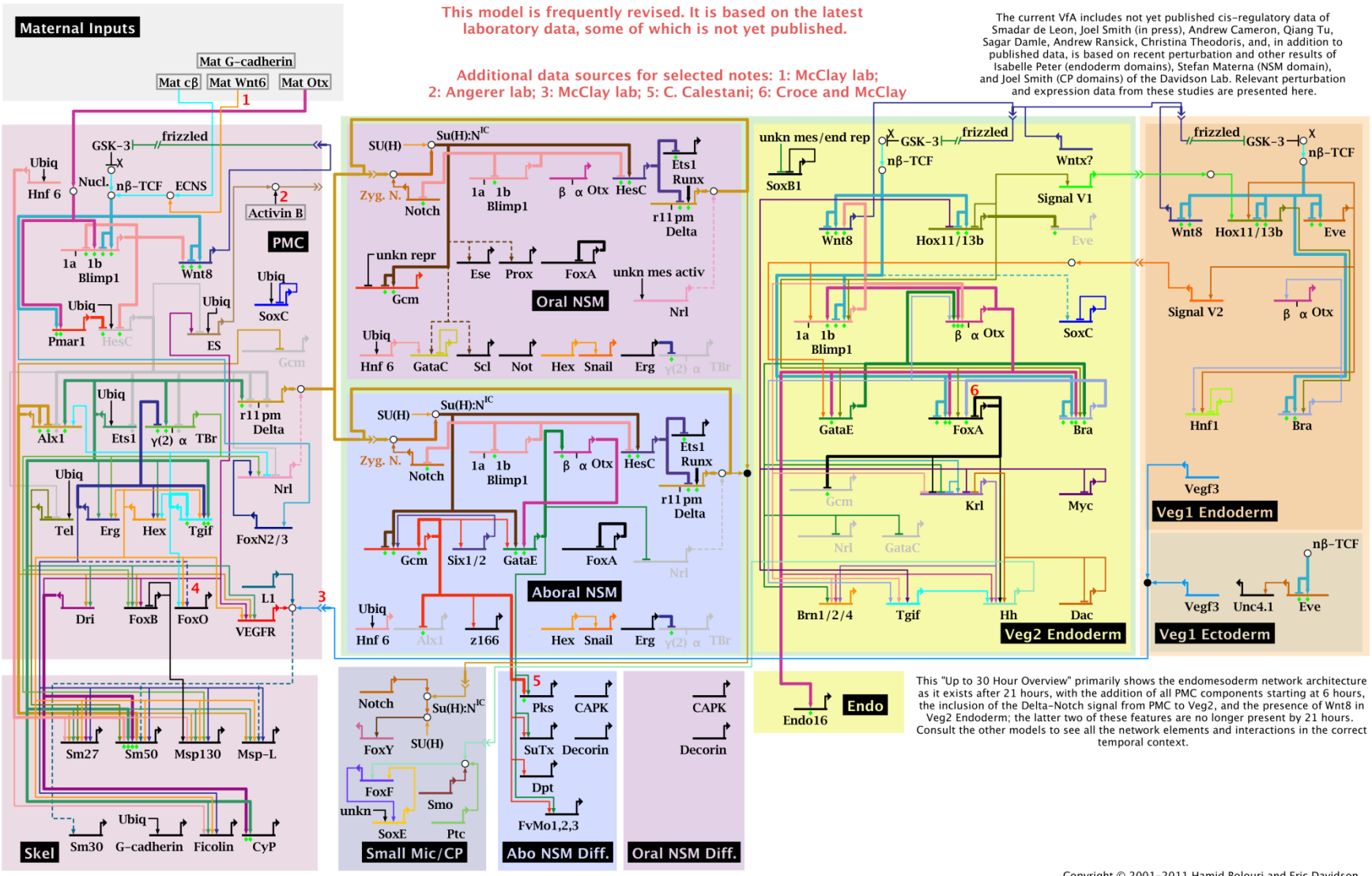

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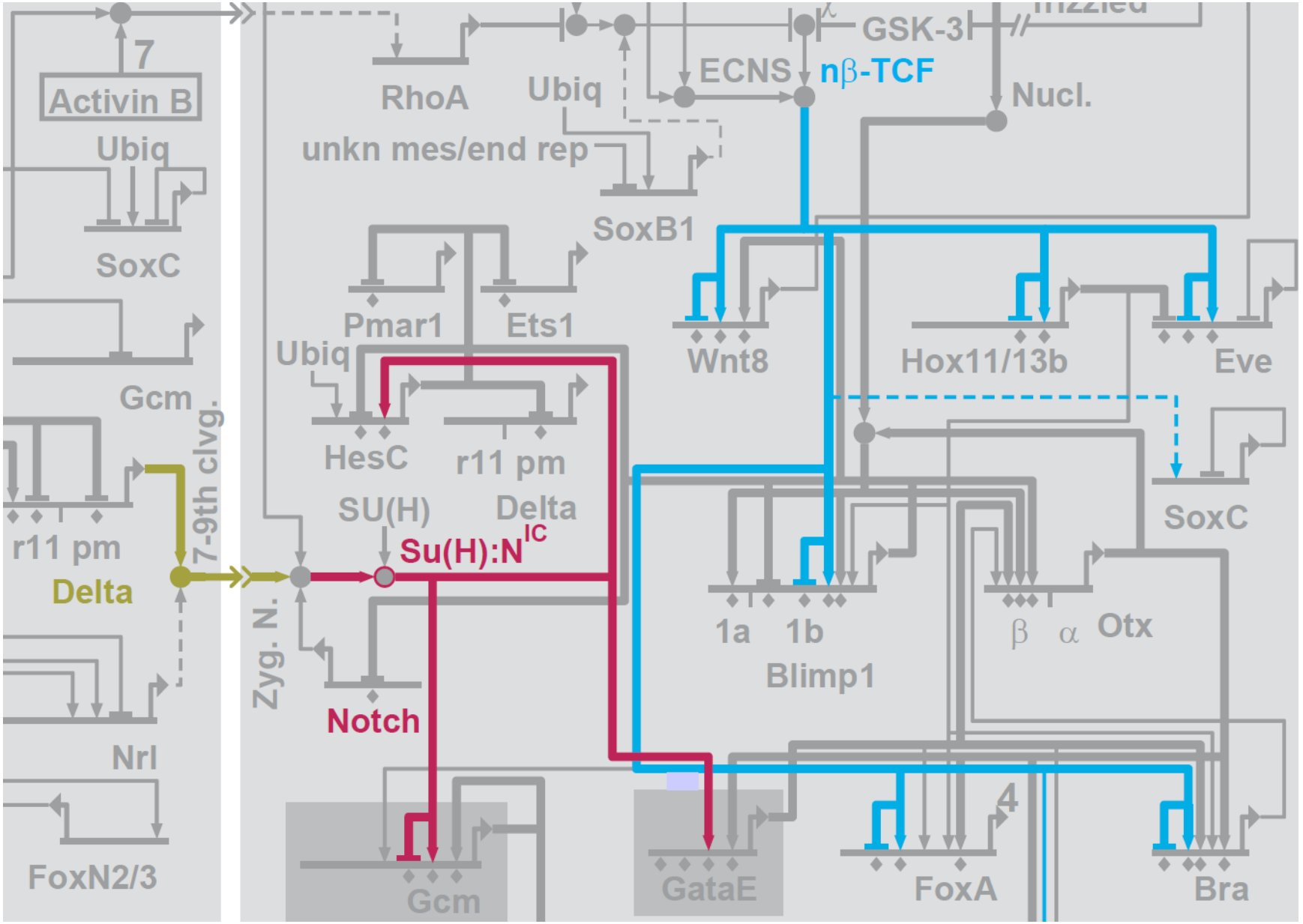
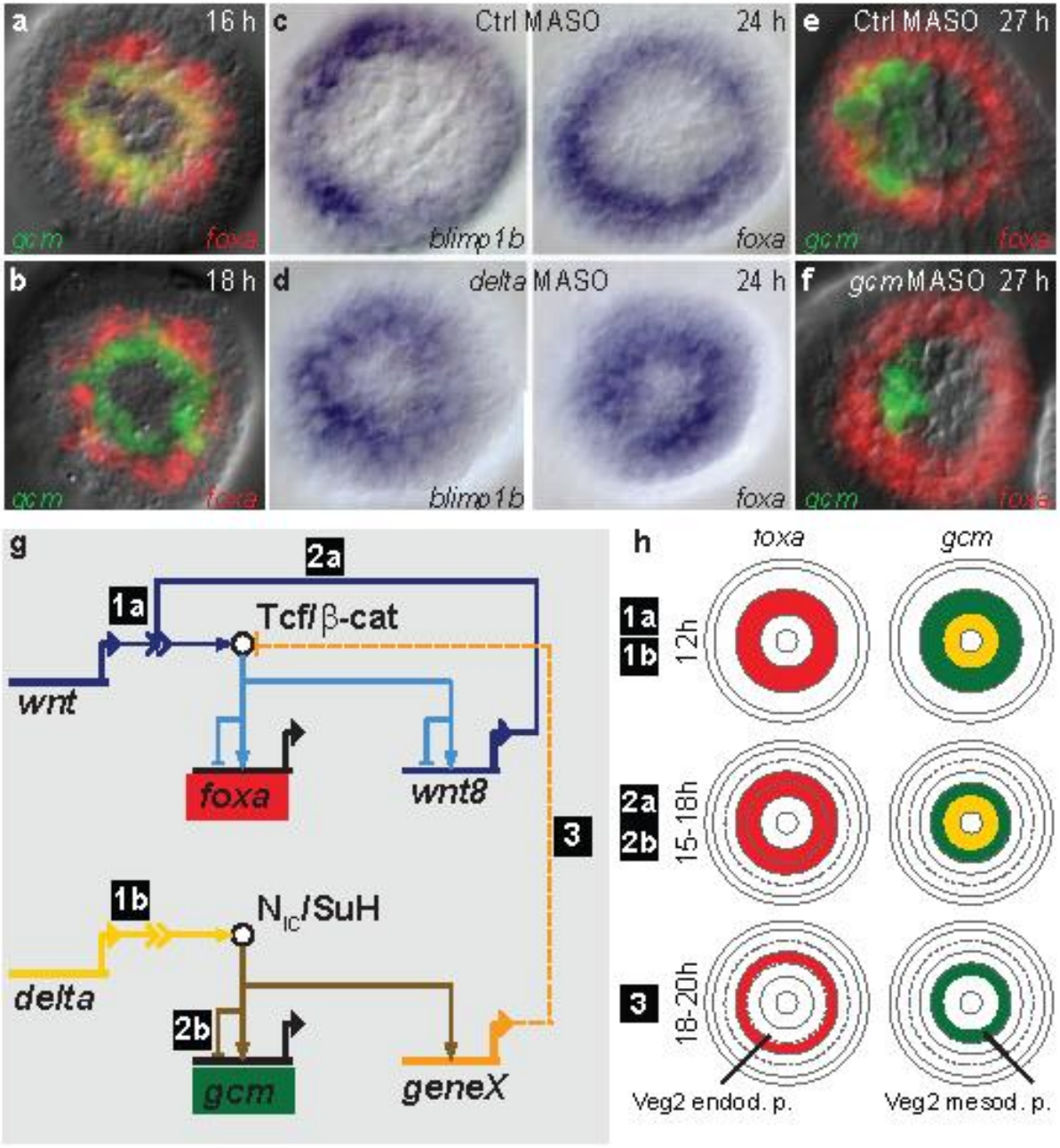
ENDODERM VS MESODERM

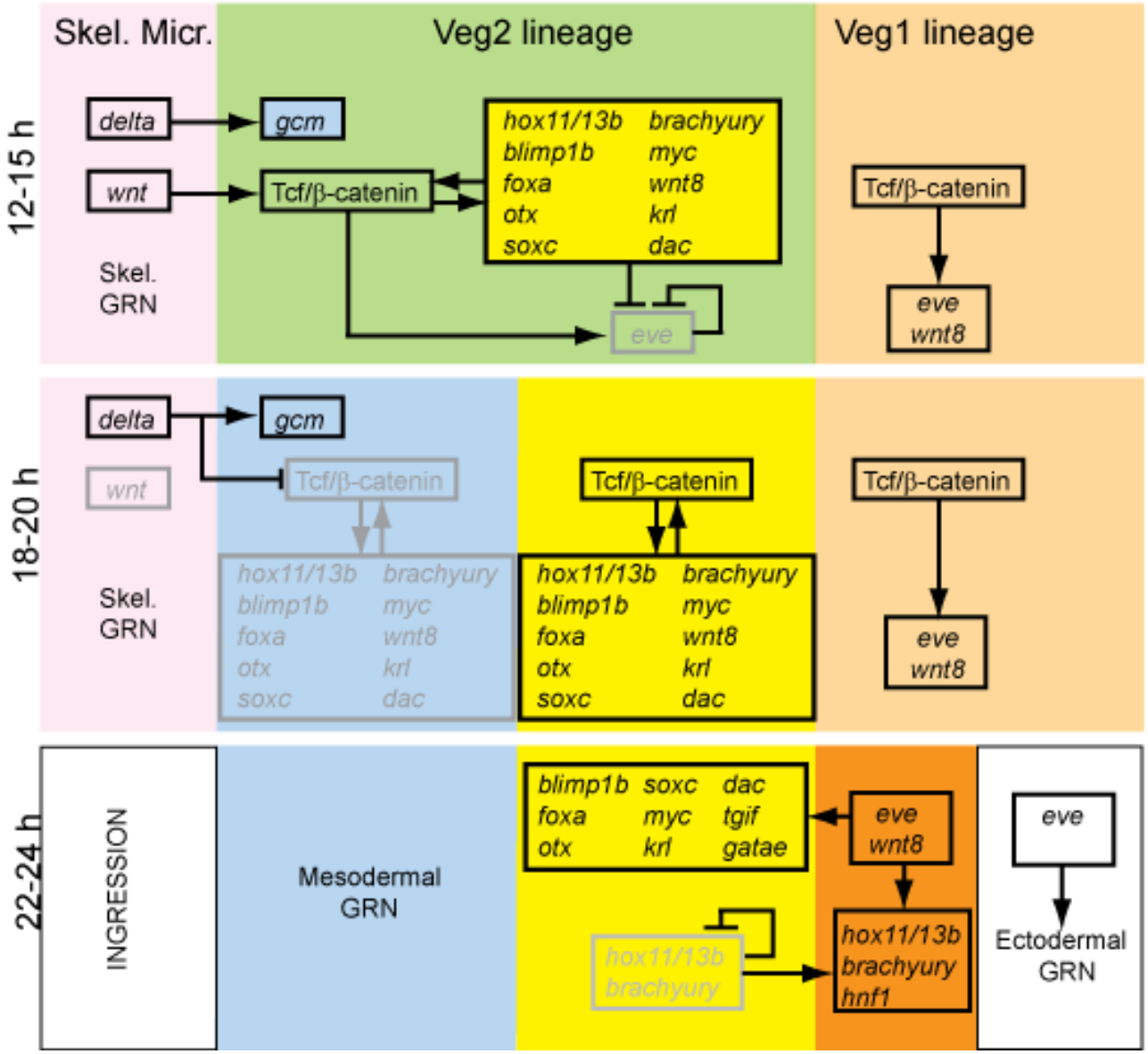
NON-INTERACTING GRNS OPERATING IN SAME CELLS: VEG2 MESODERM AND VEG2 ENDODERM SPECIFICATION


7
Activin B
Ubiq
SoxC
Gcm
r11 pm
Delta
7-9th clvg.
Nrl
FoxN2/3
RhoA
Ubiq
unkn mes/end rep
GSK-3
ECNS
nβ-TCF
Nucl.
SoxB1
Pmar1
Ets1
Ubiq
HesC
r11 pm
SU(H)
Delta
Su(H):N^IC
Zyg. N.
Notch
Gcm
Wnt8
Hox11/13b
Eve
SoxC
1a
1b
Blimp1
β
α
Otx
GataE
4
FoxA
Bra

CLEARANCE OF ENDODERM GENE EXPRESSION FROM MESODERM FOLLOWS:

DEPENDS ON SAME NOTCH SIGNALING AS IS USED TO SPECIFY MESODERM ORIGINALLY


a
16 h
gcm
foxa
c
Ctrl MASO
24 h
blimp1b
foxa
e
Ctrl MASO
27 h
gcm
foxa
b
18 h
gcm
foxa
d
delta MASO
24 h
blimp1b
foxa
f
gcm MASO
27 h
gcm
foxa
g
2a
1a
Tcf/β-cat
wnt
foxa
wnt8
3
1b
N_IC/SuH
delta
2b
gcm
geneX
h
foxa
gcm
1a
1b
12h
2a
2b
15-18h
3
18-20h
Veg2 endod. p.
Veg2 mesod. p.


Skel. Micr.
Veg2 lineage
Veg1 lineage
12-15 h
delta
wnt
gcm
Tcf/β-catenin
hox11/13b
blimp1b
foxa
otx
soxc
brachyury
myc
wnt8
krl
dac
eve
Skel.
GRN
Tcf/β-catenin
eve
wnt8
18-20 h
delta
wnt
gcm
Tcf/β-catenin
hox11/13b
blimp1b
foxa
otx
soxc
brachyury
myc
wnt8
krl
dac
Skel.
GRN
Tcf/β-catenin
hox11/13b
blimp1b
foxa
otx
soxc
brachyury
myc
wnt8
krl
dac
Tcf/β-catenin
eve
wnt8
22-24 h
INGRESSION
Mesodermal
GRN
blimp1b
foxa
otx
soxc
myc
krl
dac
tgif
gatae
eve
wnt8
hox11/13b
brachyury
hox11/13b
brachyury
hnf1
eve
Ectodermal
GRN

ANTERIOR ENDODERM VS POSTERIOIR ENDODERM (different regulatory state in veg2 from that in veg1)

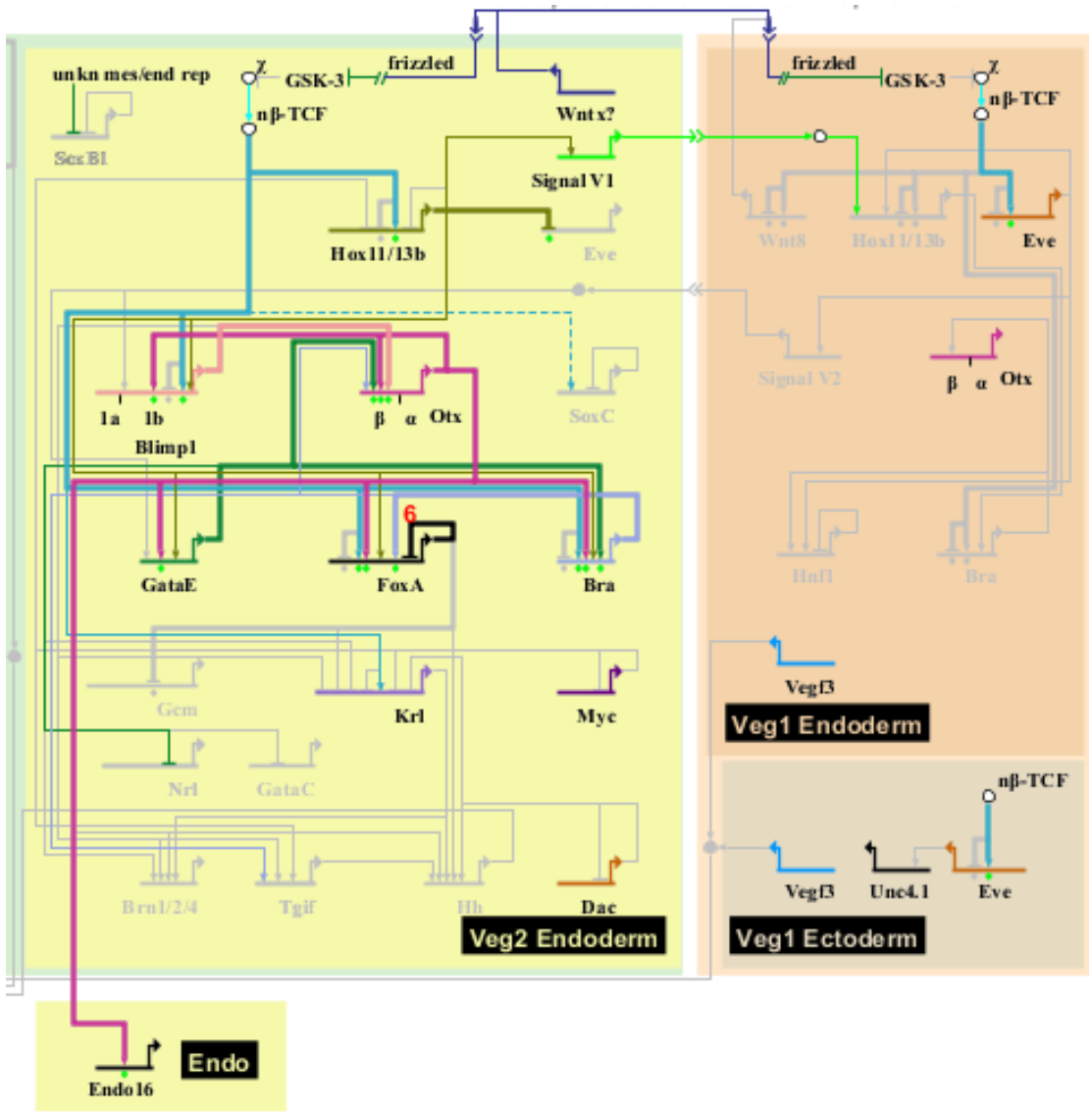
A

	Time [h]	*blimp1b*	*brachyury*	*brn1/2/4*	*dac*	*eve*	*foxa*	*gatae*	*hnf1*	*hox11/13b*	*krl*	*myc*	*otx*	*soxc*	*tgif*
veg2 endoderm	12														
	15														
	18														
	21														
	24														
	27														
	30														
veg1 endoderm	12														
	15														
	18														
	21														
	24														
	27														
	30														

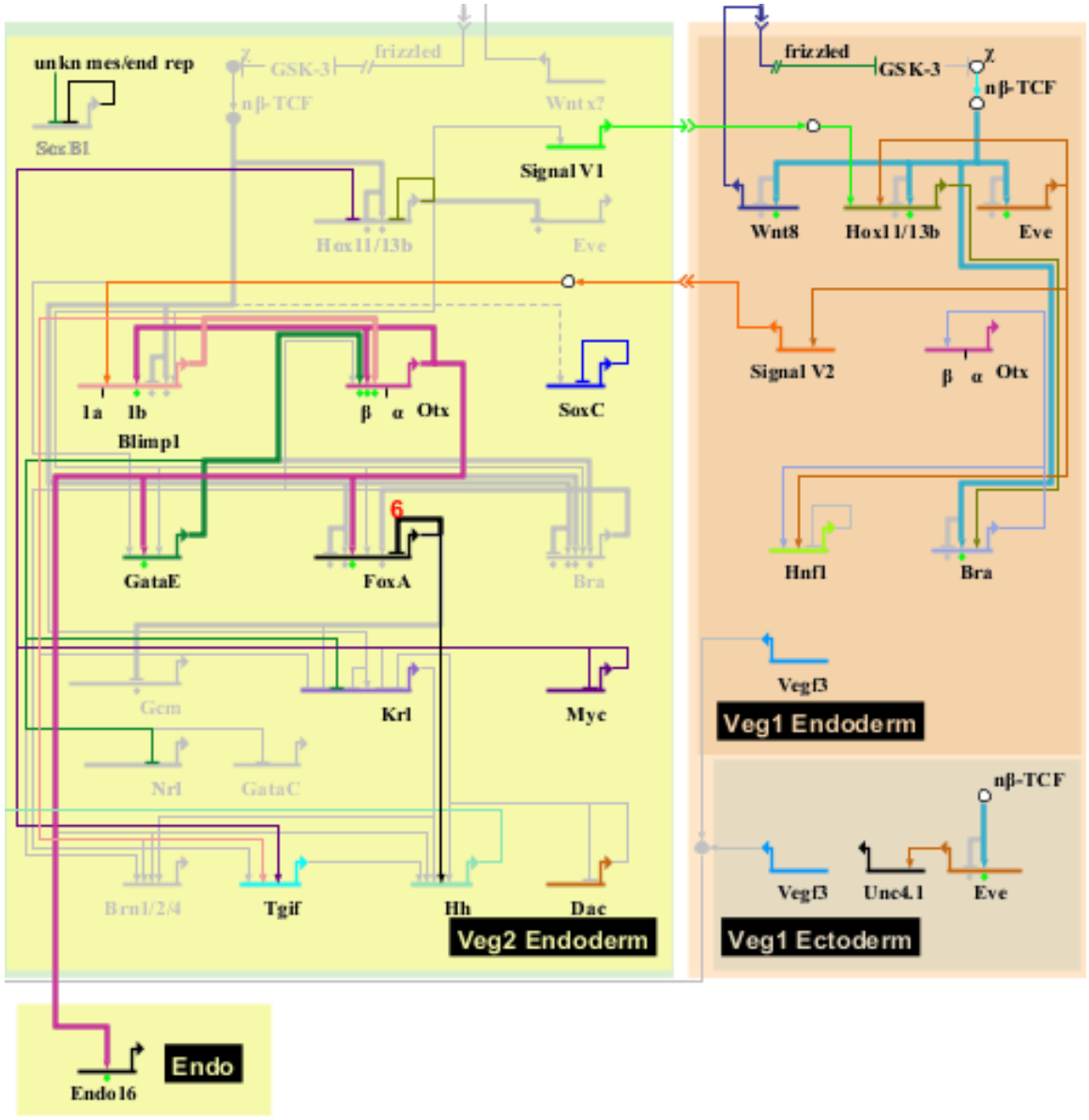
B

Input \ Output	*blimp1b*	*brachyury*	*brn1/2/4*	*dac*	*eve*	*foxa*	*gatae*	*hnf1*	*hox11/13b*	*krl*	*myc*	*otx*	*soxc*	*tgif*
Blimp														
Brachyury														
Dac														
Eve														
FoxA														
GataE														
Hnf1														
Hox11/13b														
Z13/Krl														
Mvc														
Otx														
SoxC														
Tgif														

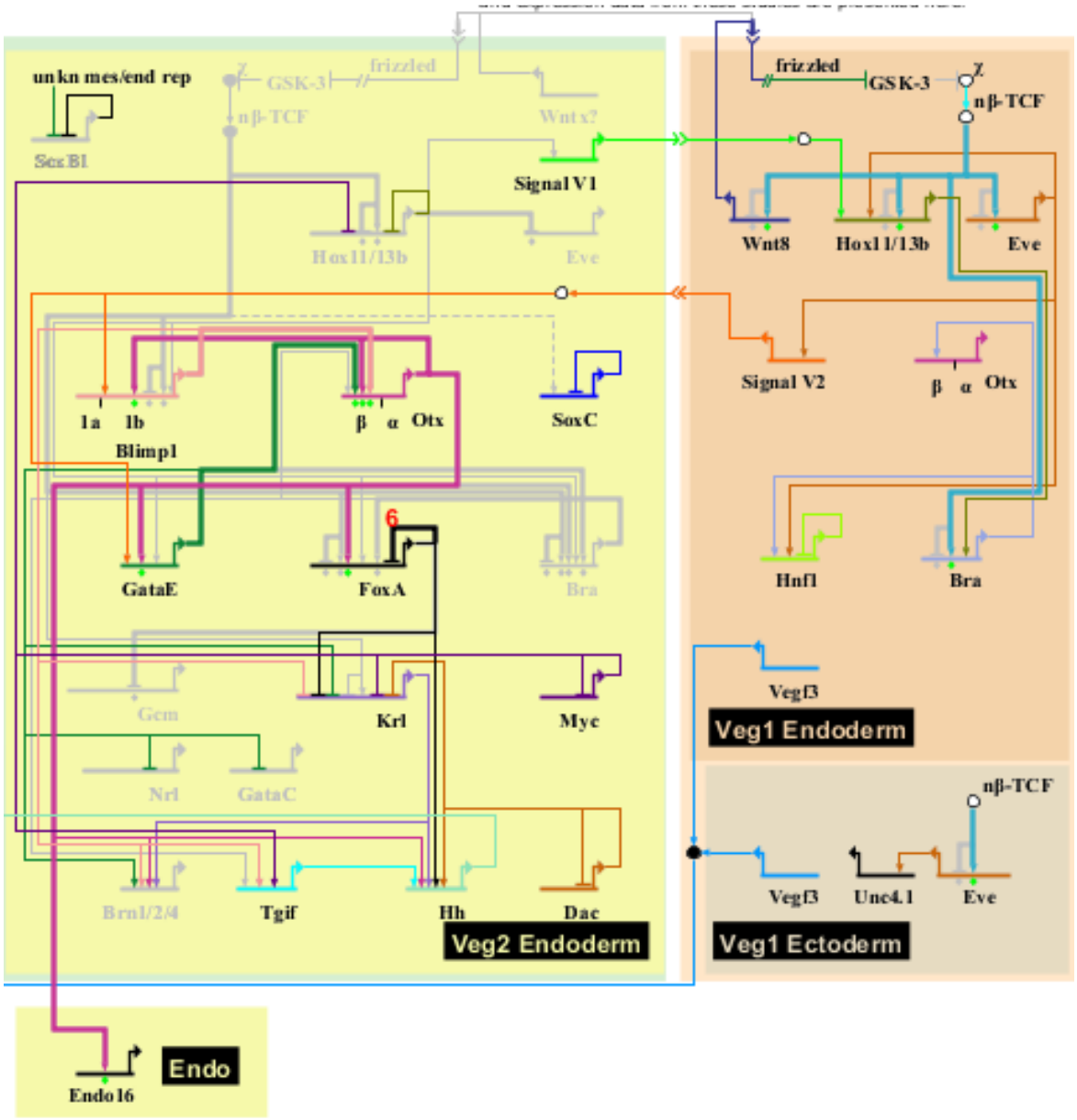
21h


unkn mes/end rep
GSK-3
frizzled
nβ-TCF
Wnt x?
SoxB1
Signal V1
Hox11/13b
Eve
1a
1b
Blimp1
β
α
Otx
SoxC
GataE
FoxA
6
Bra
Gcm
Krl
Myc
Nrl
GataC
Brn1/2/4
Tgif
Hh
Dac
Veg2 Endoderm
Endo
Endo16
frizzled
GSK-3
nβ-TCF
Wnt8
Hox11/13b
Eve
Signal V2
β
α
Otx
Hnf1
Bra
Vegf3
Veg1 Endoderm
nβ-TCF
Vegf3
Unc4.1
Eve
Veg1 Ectoderm

24h


unkn mes/end rep
SoxB1
GSK-3
frizzled
nβ-TCF
Wnt x?
Signal V1
Hox11/13b
Eve
1a
1b
Blimp1
β
α
Otx
SoxC
GataE
6
FoxA
Bra
Gcm
Krl
Myc
Nrl
GataC
Brn1/2/4
Tgif
Hh
Dac
Veg2 Endoderm
Endo
Endo16
frizzled
GSK-3
nβ-TCF
Wnt8
Hox11/13b
Eve
Signal V2
β
α
Otx
Hnf1
Bra
Vegf3
Veg1 Endoderm
nβ-TCF
Vegf3
Unc4.1
Eve
Veg1 Ectoderm

27h

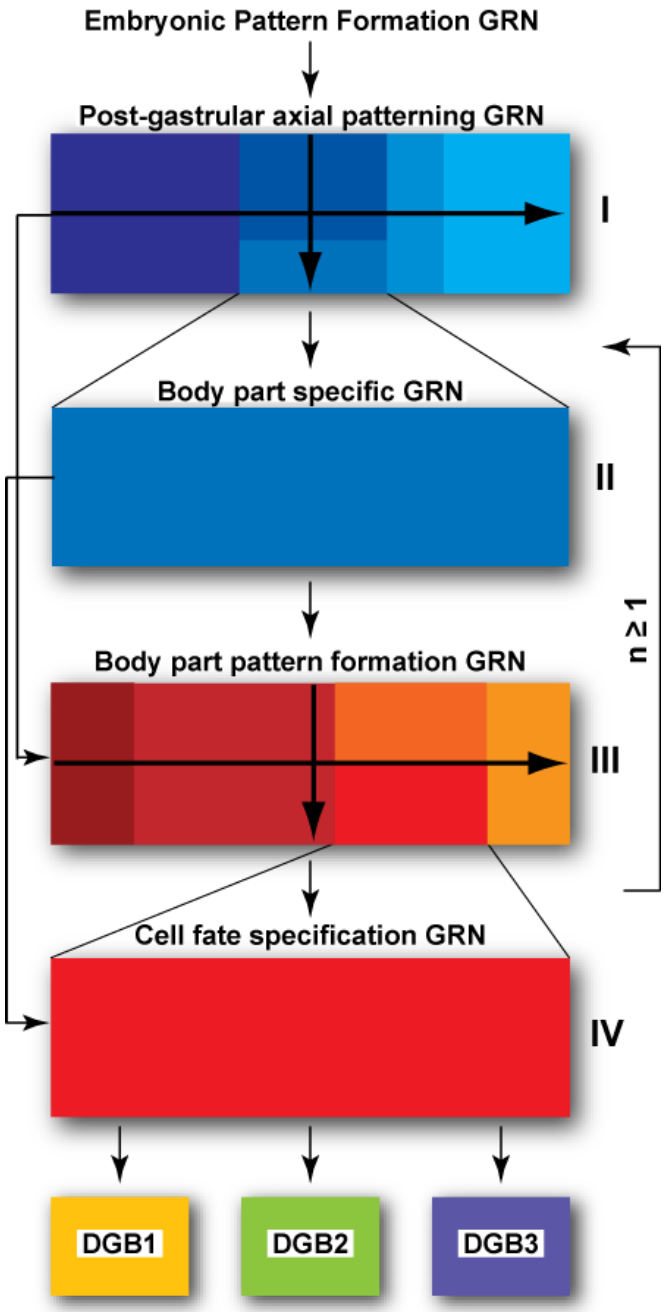

unkn mes/end rep
SoxB1
GSK-3
frizzled
nβ-TCF
Wnt x?
Signal V1
Hox11/13b
Eve
1a
1b
Blimp1
β
α
Otx
SoxC
GataE
6
FoxA
Bra
Gcm
Krl
Myc
Nrl
GataC
Brn1/2/4
Tgif
Hh
Dac
Veg2 Endoderm
Endo16
Endo
frizzled
GSK-3
nβ-TCF
Wnt8
Hox11/13b
Eve
Signal V2
β
α
Otx
Hnf1
Bra
Vegf3
Veg1 Endoderm
nβ-TCF
Vegf3
Unc4.1
Eve
Veg1 Ectoderm

UPSTREAM AND DOWNSTREAM IN GRN'S

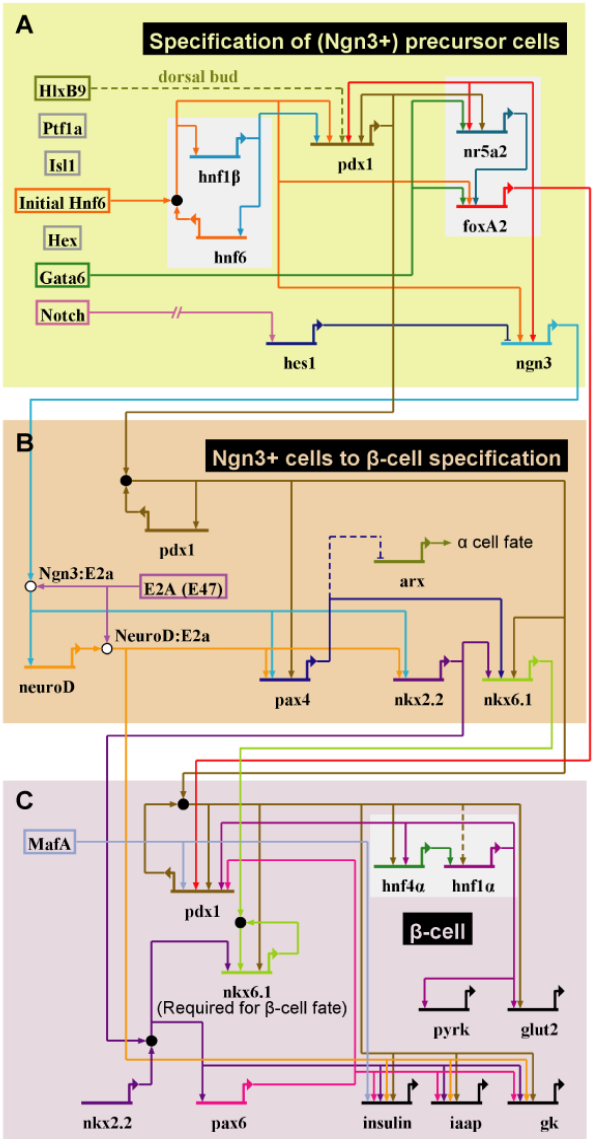
HIERARCHY

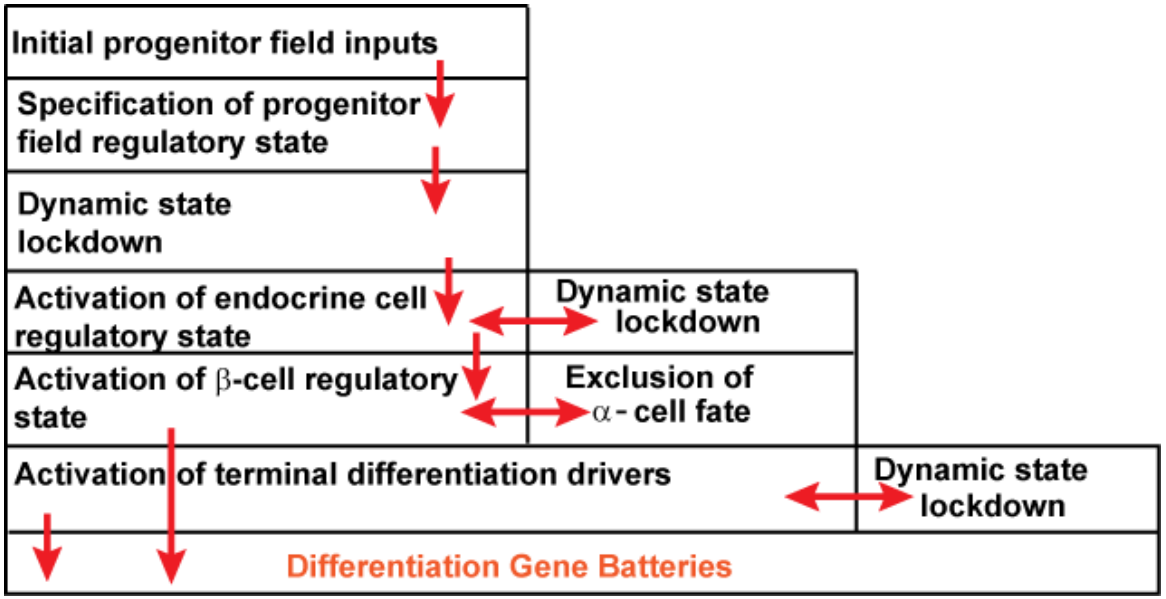
TERMINAL/PERIPHERAL CIRCUITRY

A DIAGRAM OF GRN HIERARCHY



PANCREATIC β– CELL DEVELOPMENT





TYPES OF DOWNSTREAM GRN MODULES, THE STRUCTURE OF WHICH IS ENCODED IN THE GENOME:

DEVELOPMENTAL REGULATORY MODULES:

-DIFFERENTIATION GENE BATTERIES

-CELL BIOLOGY CASSETTES

-MORPHOGENESIS CASSETTES

PHYSIOLOGICAL RESPONSE MODULES:

-PATHOGEN RESPONSES

-STRESS RESPONSES

-NUTRIENT FLUCTUATION RESPONSES, ETC

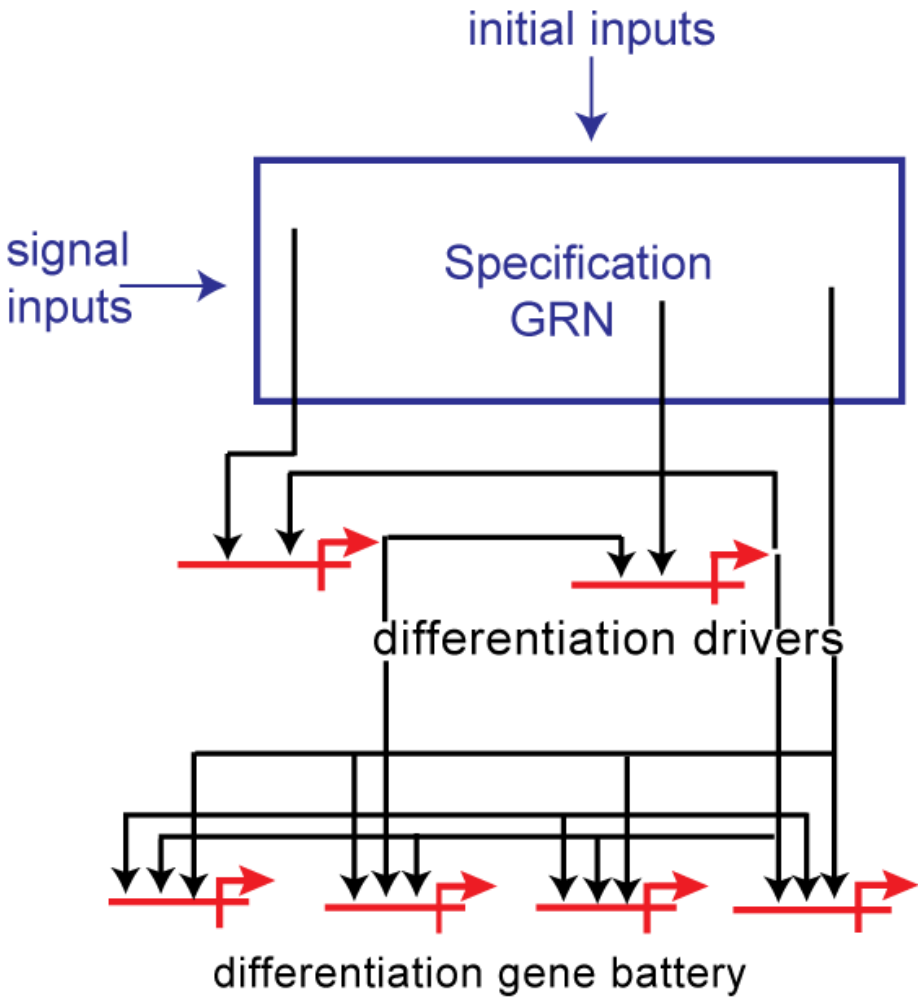
GLOBAL GRN: UPSTREAM AND DOWNSTREAM LINKED!

DIFFERENTIATION GENE BATTERIES

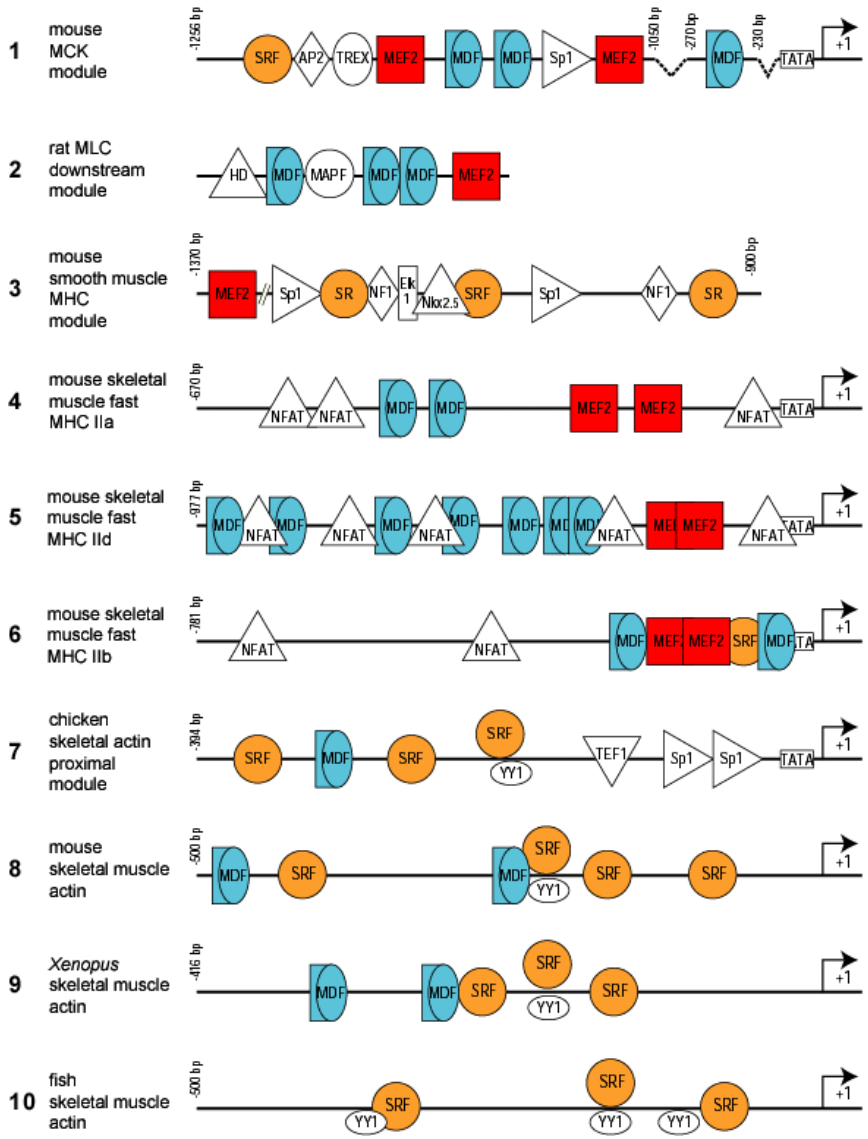
Differentiation genes are always controlled by MULTIPLE REGULATORY GENES, which themselves cross-regulate, directly or indirectly

Differentiation gene batteries are at the periphery of GRNs

They are large but repetitively organised subcircuits


initial inputs
signal
inputs
Specification
GRN
differentiation drivers
differentiation gene battery

A. MUSCLE CONTRACTILE PROTEIN GENES



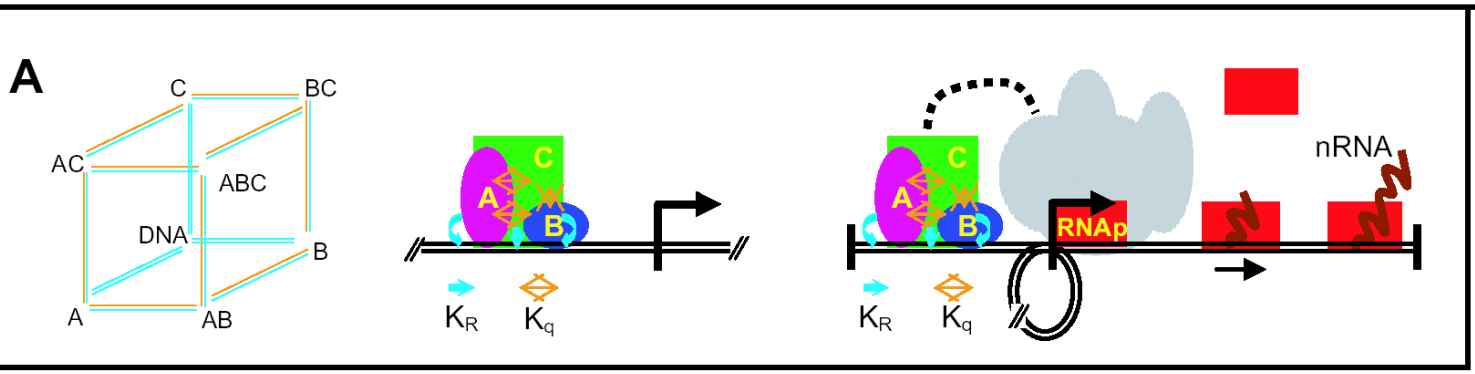
THE OUTPUT OF EMBRYONIC SPECIFICATION GRNS IS A BOOLEAN CHECKERBOARD OF SPATIAL REGULATORY STATES

KINETIC O.D.E. MODELS ARE A SQUARE PEG IN A ROUND HOLE FOR THIS PROBLEM:

- **FAR TOO MANY PARAMETERS FOR LARGE SYSTEMS**
- **ALSO ILL SUITED FOR PRIMARILY SPATIAL TRANSACTIONS RELATED TO EMBRYO GEOMETRY**
- **ALSO BOUNDARIES ARE ALL CLIFF-LIKE**

YET TO CAPTURE THE LOGIC AND TO COMPARE OBSERVED AND PREDICTED GRN SPATIAL REGULATORY STATES, A FORMAL SPECIFIC COMPUTATIONAL MODEL OF SOME KIND IS CLEARLY REQUIRED…

TIME HAS ALSO TO BE CONSIDERED: WE APPLIED RESULTS OF AN EARLIER O.D.E. ANALYSIS OF SEA URCHIN CASCADE DYNAMICS (BOLOURI AND DAVIDSON, 2003)



$$(1) \quad Y_{AB} = \frac{A.K_{RA}.B.K_{RB}.K_q}{D_N^2 + A.D_N + B.D_N + A.K_{RA}.D_N + B.K_{RB}.D_N + A.K_{RA}.B.K_{RB}.K_q}$$

$$(2) \quad I = M.(1 - e^{-k_b.Y_{AB}/M})$$

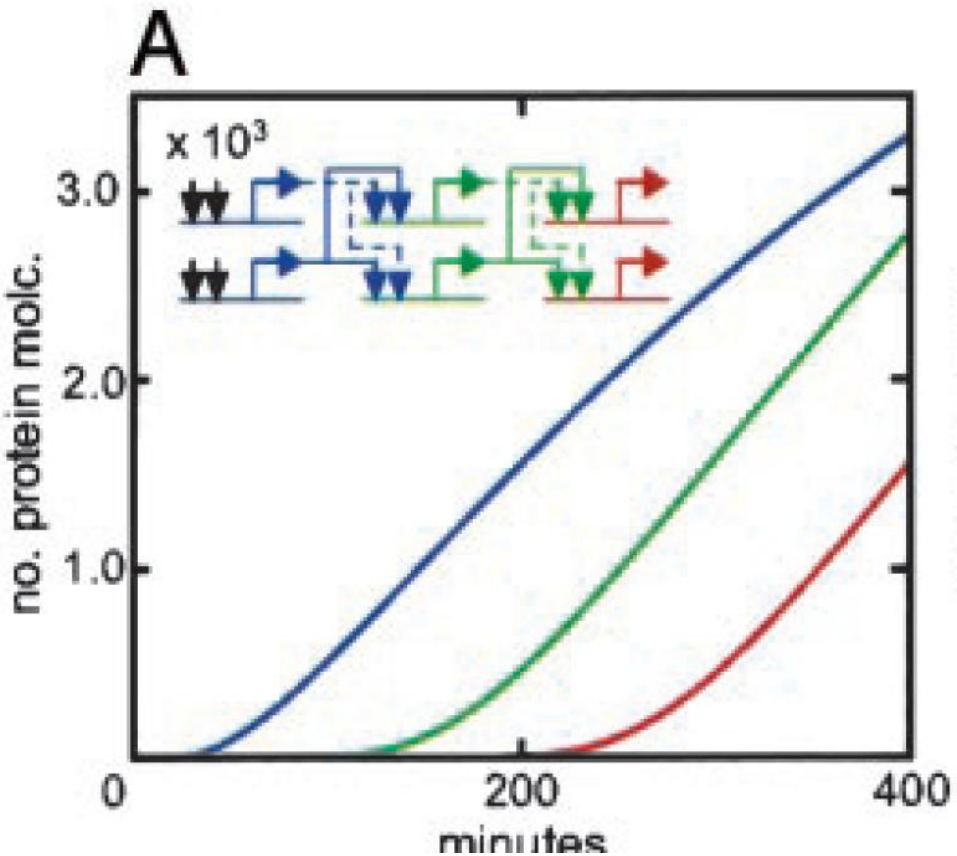
$$(3) \quad \frac{d(nRNA)}{dt} = I - k_{dn}.nRNA$$

$$(4) \quad \frac{d(mRNA)}{dt} = I_{t+\Delta t1} - k_{dm}.mRNA$$

$$(5) \quad \frac{d(P)}{dt} = k_t.mRNA_{t+\Delta t2} - k_{dP}.P$$

K_R	= relative equilibrium constant
K_q	= coefficient of cooperativity
k_b	= CRM activating strength
Y_{AB}	= DNA occupancy by A & B
I	= no. of transcription initiations per minute
M	= max. no. of initiations per minute
$I_{t+\Delta t1}$	= initiations per minute, $\Delta t1$ mins delayed
$mRNA_{t+\Delta t2}$	= mRNA molecules, $\Delta t2$ mins after transcription
k_t	= rate of mRNA translation
k_d's	= rates of degradation

Cascade behavior



BASIC STRUCTURE OF MODEL-1:

IN LIFE THE REGULATORY GENOME CONSISTS OF AN UNORDERED, LINEAR ARRAY OF INFORMATION PROCESSING CIS-REGULATORY MODULES

WE CAPTURED THIS BY CONSTRUCTING FOR EACH GENE A BOOLEAN EQUATION STATING THE INPUTS AND HOW THESE INPUTS ARE PROCESSED BY CIS-REGULATORY LOGIC FUNCTIONS; THESE EQUATIONS NEVER CHANGE, LIKE THE DNA SEQUENCE

BUT THE INPUTS TO WHICH THEY MAY RESPOND CHANGE SPATIALLY AND TEMPORALLY, LIKE THE REGULATORY STATES IN LIFE

THE INPUTS WERE DERIVED FROM THE STRUCTURE OF THE GRNS, WHICH PREDICTS THEM (IN MANY CASES VALIDATED)

CONCLUSIONS

-REMARKABLE CONVERGENCE OF COMPUTED AND OBSERVED RESULTS

-ALL OBSERVED DIFFERENTIALLY EXPRESSED GENES ARE EXPRESSED IN THE CORRECT DOMAIN IN COMPUTED MODEL

-SUFFICIENT CAUSALITY OF GENOMICALLY ENCODED LOGIC TRANSACTIONS TO EXPLAIN SPATIAL SPECIFICATION

-INFORMATIONAL CONTENT OF GRN IS NEARLY SUFFICIENT (AND WE SEE EXACTLY WHAT WE DON'T YET KNOW)

-SHOWS WHY SYSTEM LEVEL EXPLANATION IS THE ONLY WAY TO SOLVE PROBLEM, SINGLE GENE FUNCTIONAL ANALYSIS WILL NEVER BE SUFFICIENT

-PROVIDES APPROACH TO RE-ENGINEERING REGULATORY SYSTEM IN SILICO

SUMMARY: ***A CHAIN OF VERTICAL CAUSAL LINKS EXTENDS COMMUTATIVELY BETWEEN THE EMBRYO AND THE GENOME***

- **EMBRYOGENESIS IS CAUSALLY DEPENDENT ON TEMPORAL AND SPATIAL EXPRESSION OF LARGE SETS OF GENES**
- **GENE EXPRESSION DEPENDS CAUSALLY ON REGULATORY STATE IN EACH TEMPORAL AND SPATIAL ELEMENT OF THE EMBRYO**
- **REGULATORY STATE IS PRODUCED BY NETWORKS OF INTERACTING REGULATORY GENES (GRNS)**
- **WHAT GRNS DO, WHAT REGULATORY GENES THEY INCLUDE, HOW THEY ENCODE DEVELOPMENTAL PROGRESSION, ARE CAUSALLY DEPENDENT ON THEIR CIRCUITRY**
- **GRN CIRCUITRY IS CAUSALLY DEPENDENT ON THE INHERITED GENOMIC REGULATORY SEQUENCE**

THE PEOPLE WHO MAKE THE DISCOVERIES

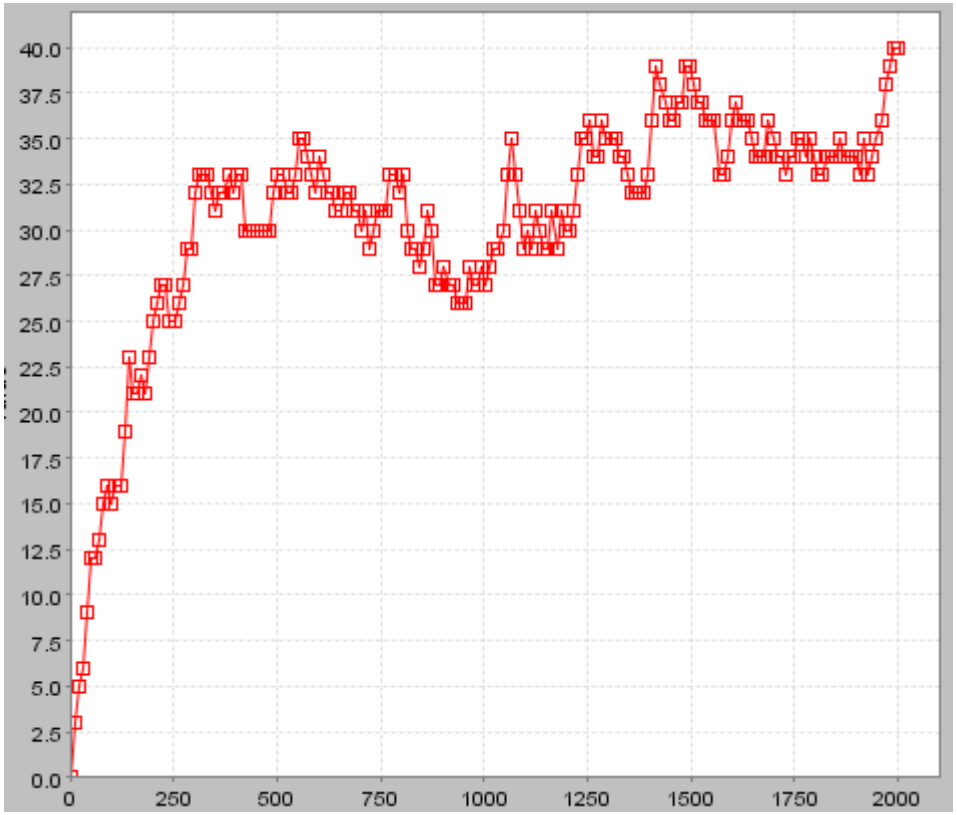
For these talks:

- ISABELLE PETER: Spatially dynamic Endoderm/Mesoderm specification GRNs; The sox21 project; Evolution of gene regulatory networks, Boolean logic model
- EMMANUEL FAURE: Boolean logic model; computational virtual embryo
- PAOLA OLIVERI and ROGER REVILLA, The skeletogenic double negative gate
- QIANG TU: Feedback circuitry in skeletogenic GRN
- ANDY RANSICK: Gcm cis-reg, N signaling pathway
- JOEL SMITH: delta, hesC, Pmar1 cis-reg, non-parsimonious design
- FENG GAO: Hijacking skeletogenesis; Cis-reg basis of cooption;The sox21 project
- SMADAR DE LEON: FoxA cis-reg; Kinetic modeling
- JONGMIN NAM: Revolutionizing cis-reg analysis
- ENHU LI: The new Oral/Aboral Ectoderm GRN
- JULIUS BARSI: The ciliated band GRN; late gene expression blockade
- SAGAR DAMLE: Lineage fate transformation; Alx cis-reg
- STEFAN MATERNA: Expanding the Mesoderm GRN
- ANDY CAMERON: The S. purpuratus GENOME CENTER and database; brachyury cis-reg; cis-reg module evolution; BI COMPUTATIONAL CENTER FOR REGULATORY GENOMICS
- ERIC ERKENBRACK: Eucidaris developmental GRN
- JON VALENCIA: Later endoderm cis-reg project
- HAMID BOLOURI, BILL LONGABAUGH: Computational collaboration
- DOUG ERWIN; DAVID BOTTJER; JL SKARMETA ET. AL: Important collaborators

- SUPPORT: NICHD; also, NIGMS, NCRR, NSF, Beckman Institute; HGRI, Richard Gibbs, & the Baylor HGSC

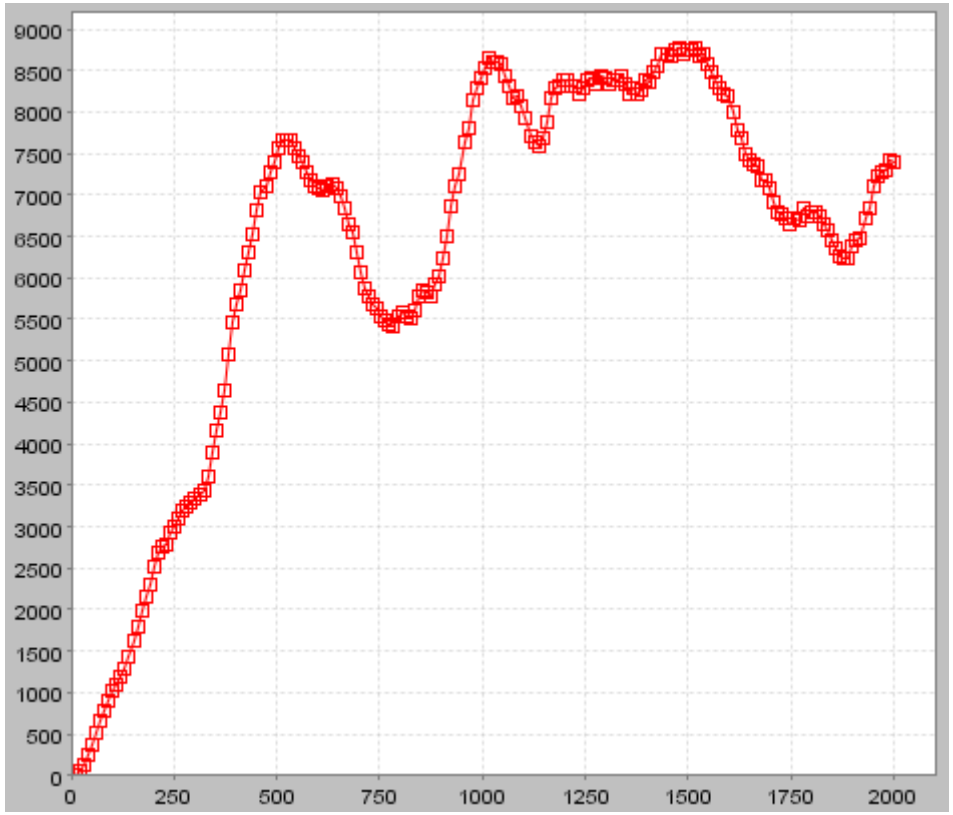
GR

mRNA molecules in a single cell



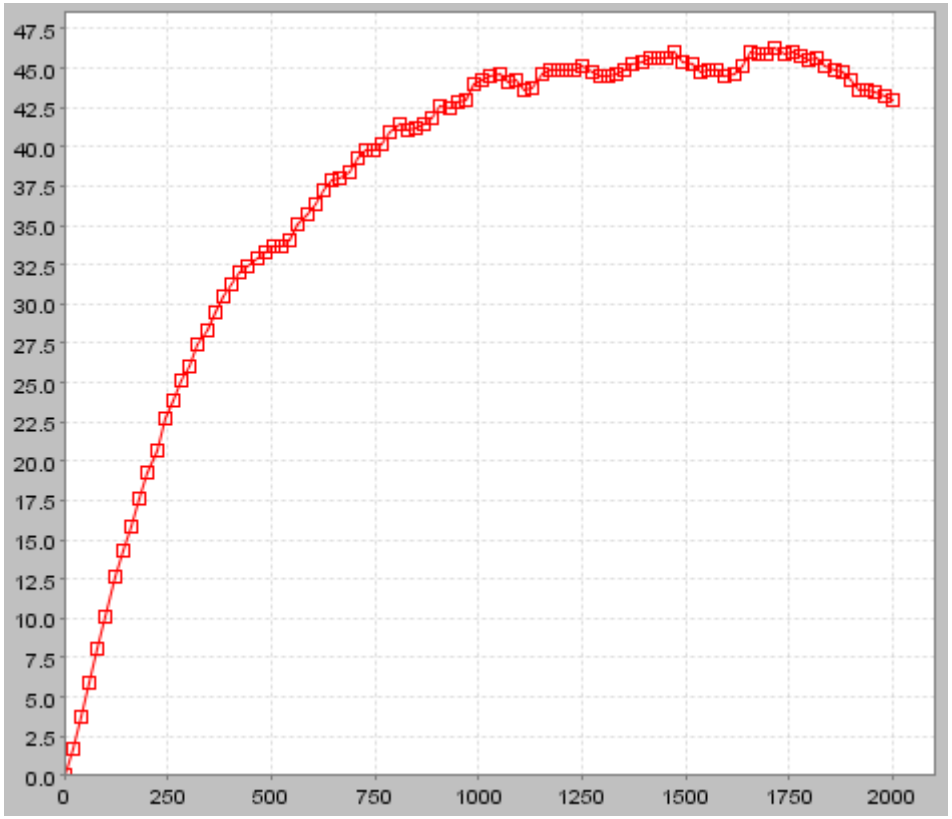
Time (minutes after activation)

protein molecules in a single cell



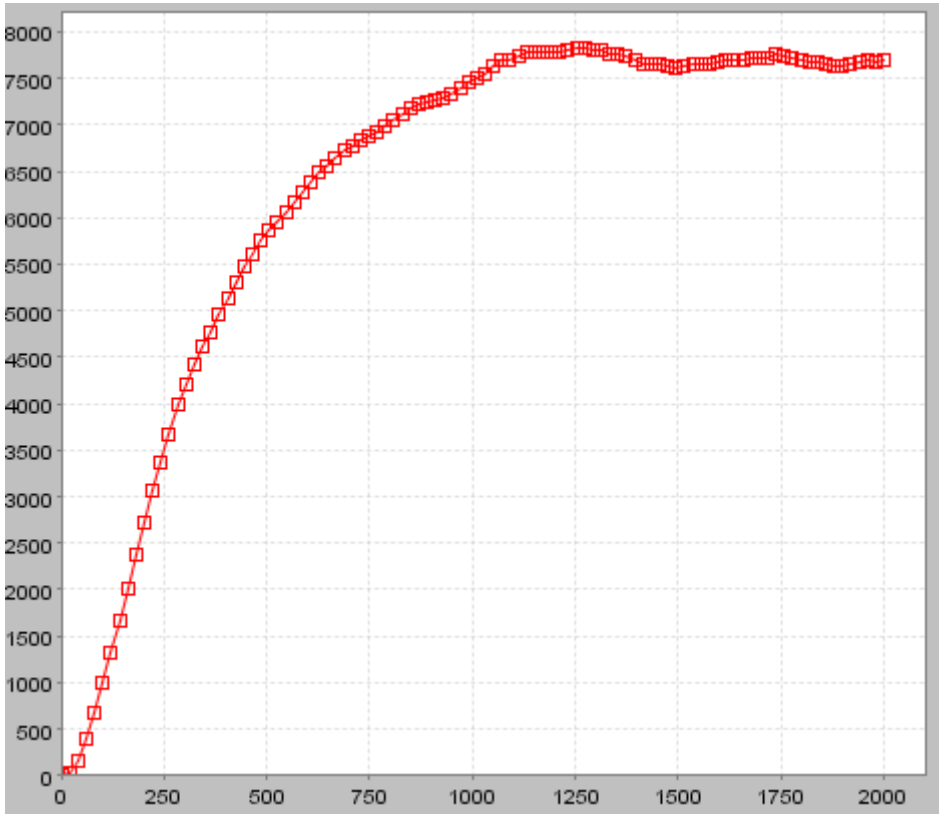
Time (minutes after activation)

Number of mRNA molecules per cell averaged over 50 cells



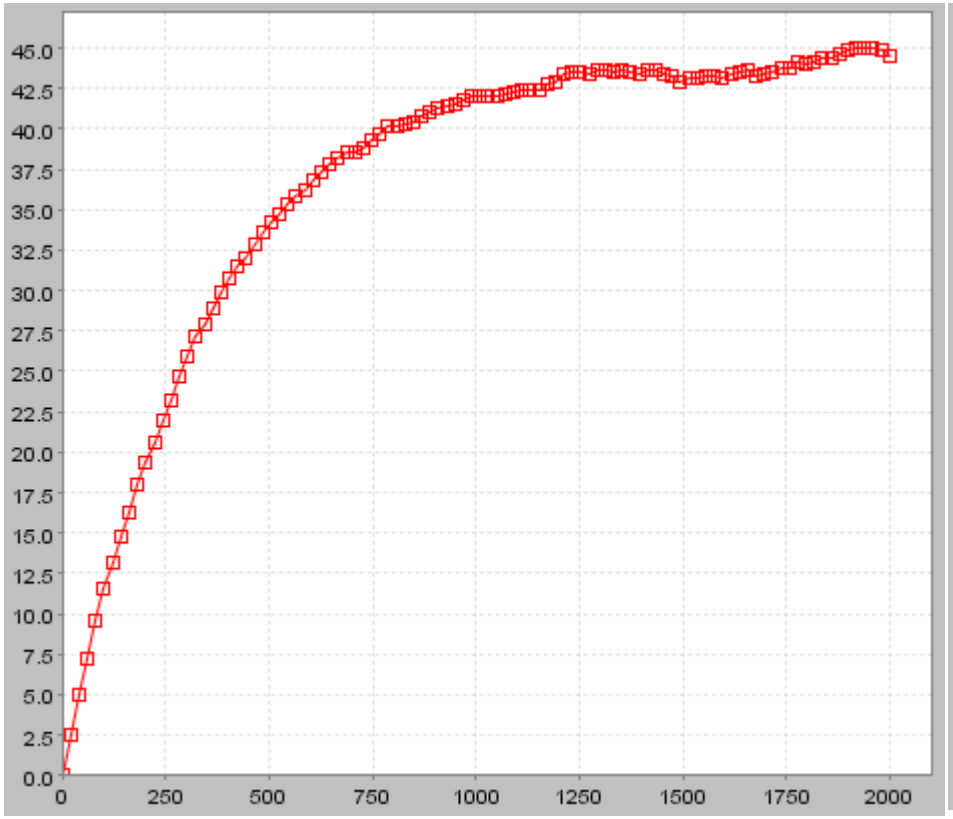
Time (minutes after activation)

Number of protein molecules per cell averaged over 50 cells



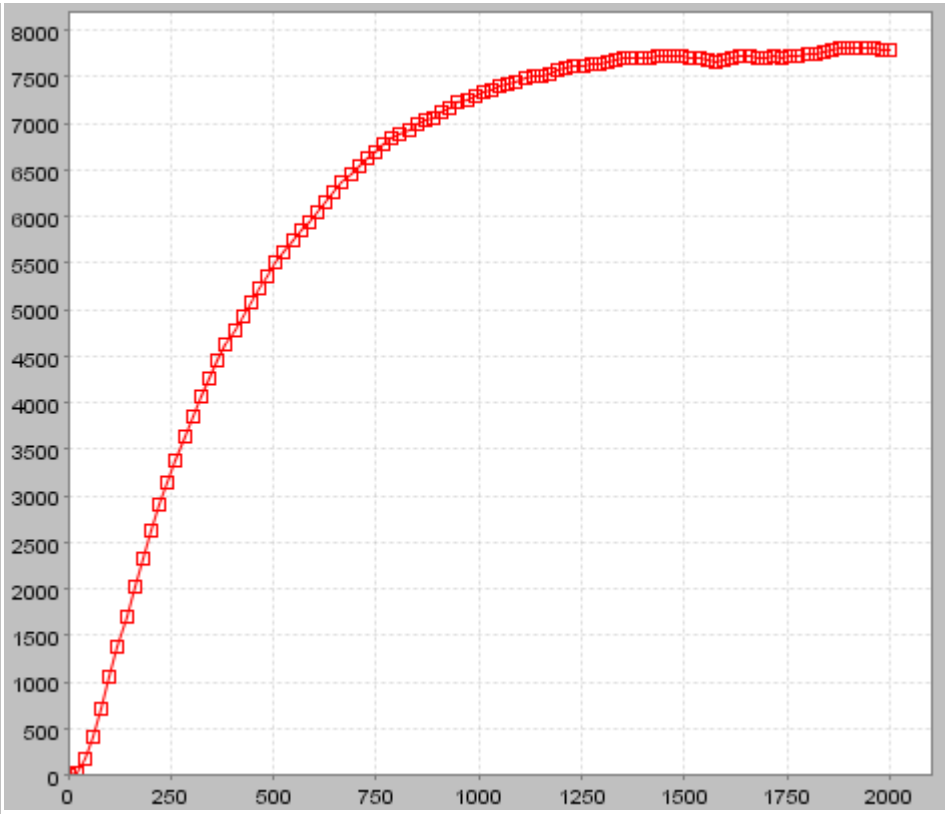
Time (minutes after activation)

Number of mRNA molecules per cell averaged over 200 cells



Time (minutes after activation)

Number of protein molecules per cell averaged over 200 cells



Time (minutes after activation)

Gene		Vector Equation
Alx1		if AT-2 Ets1=1 AND AT-2 NOT HesC=1 then=1 else=0
Blimp1b	Mod1	if [AT-0 J(Tcf):ModH=1 OR AT-0 J(Tcf):ModL=1] AND PERM-0 J(Tcf):ModH=0 AND AT-2 Otx=1 then=1 else=0
	Mod2	if AT-2 Hox11/13b:ModH=1 AND AT-0 J(Tcf):ModH=1 AND PERM-0 J(Tcf):ModH=0 then=1 else=0
	Mod3	if AT-0 J(V2)=1 then=1 else=0
		if Mod1=1 OR Mod2=1 OR Mod3=1 then=1 else=0
Bra		if AT-3 Hox11/13b=1 AND AT-0 J(Tcf)=1 AND PERM-0 J(Tcf):ModH=0 AND AT-3 Otx=1 AND [AT-3 GataE=1 OR AT-3 GataE=0] then=1 else=0
Brn1/2/4		if AT-3 Otx=1 AND AT-3 Blimp1b=1 AND AT-3 GataE=1 AND [AT-3 z13/Krl=1 OR AT-3 z13/Krl=0] then=1 else=0
Dac		if >17 AND IN V2.Endoderm then=1 else=0
Delta	ModA	if AT-2 Runx=1 AND AT-2 NOT HesC=1 then=1 else=0
	ModR11	if AT-3 Ets1=1 then=1 else=0
		if [ModA=1 OR ModR11=1] AND AT-2 NOT HesC=1 then=1 else=0
Dri		if AT-3 Alx1=1 AND AT-3 Ets1=1 then=1 else=0
Erg		if AT-3 Ets1=1 AND AT-3 Tbr=1 OR AT-3 Hex=1 then=1 else=0
Ets1		if AT-0 U1=1 AND AT-2 NOT HesC=1 then=1 else=0
Eve		if AT-2 J(Tcf)=1 AND PERM-0 J(Tcf):ModH=0 AND PERM-3 [Hox11/13b:ModH=1 AND Eve=1] then=1 else=0
FoxA	Mod1	if [AT-0 J(Tcf):ModH=1 OR AT-0 J(SuH)=1] AND PERM-0 J(Tcf):ModH=0 then=1 else=0
	Mod2	if AT-0 J(Tcf):ModH=1 AND AT-3 Hox11/13b:ModH=1 AND AT-3 Otx=1 AND [AT-3 Bra=1 OR AT-3 Bra=0] AND PERM-0 J(Tcf):ModH=0 then=1 else=0
		if Mod1=1 OR Mod2=1 OR Mod3=1 then=1 else=0
	Mod3	if IN V2.Endoderm AND >23 then=1 else=0
FoxB		if AT-3 Alx1=1 AND AT-3 Dri=1 AND AT-3 Ets1=1 AND AT-3 Tbr=1 then=1 else=0
FoxN2/3		if AT-3 Tbr=1 then=1 else=0
FoxO		if AT-3 Tgif=1 AND AT-3 Erg=1 then=1 else=0
GataE		if [AT-3 Otx=1 AND AT-0 J(SuH)=1 AND AT-3 Gcm=1] OR [AFTER-3 Hox11/13b:ModH=1 AND AT-0 J(V2)=1 AND AT-3 Otx=1] then=1 else=0
Gcm	ModE	if AT-0 J(SuH)=1 AND PERM-0 J(SuH)=0 then=1 else=0
	ModG	if AT-2 Gcm=1 AND AT-2 NOT FoxA=1 then=1 else=0
		if ModE=1 OR ModG=1 then=1 else=0
GeneX		if >15 AND AT-0 J(SuH)=1 then=1 else=0
HesC		if [AT-0 U1=1 OR AT-0 J(SuH)=1] AND PERM-0 Pmar1=1 then=1 else=0
Hex		if AT-2 Erg=1 AND [AT-2 Ets1=1 OR AT-2 Tgif=1] then=1 else=0
Hh		if AT-3 Dac=1 AND AT-3 FoxA=1 AND AT-3 Tgif=1 AND AT-3 Otx=1 AND [AT-3 z13/Krl=1 OR AT-3 z13/Krl=0] then=1 else=0
Hnf1		if AT-2 Bra=1 AND AT-2 Eve=1 then=1 else=0
Hox11/13b	ModE	if AT-0 J(Tcf):ModH=1 AND NOT [AT-3 J(Wnt16)=1 AND AT-3 Hox11/13b:ModH=1] AND PERM-0 Hox11/13b:ModH=0 AND [AT-2 Myc=1 OR AT-2 Myc=0] then=1 else=0
	ModH	if ModE=1 AND PERM-0 J(Tcf):ModH=0 then=1 else=0
	ModW	if AT-2 Eve=1 AND AT-0 J(Wnt16)=1 then=1 else=0
		if ModH=1 OR ModW=1 then=1 else=0
J(SuH)		if AT-1 Delta=1 IN CC R then=1 else=0
J(Tcf)	ModH	if AT-0 N_b-cat:ModH=1 OR AT-1 N_b-cat:ModH=1 then=1 else=0
	ModL	if AT-0 N_b-cat:ModL=1 OR AT-1 N_b-cat:ModL=1 then=1 else=0
	ModV1	if AT-0 N_b-cat:ModV1=1 OR AT-1 N_b-cat:ModV1=1 then=1 else=0
		if ModH=1 OR ModL=1 OR ModV1=1 then=1 else=0
J(V2)		if AT-0 SignalV2=1 IN CC R then=1 else=0
J(Wnt16)		if AT-3 Wnt16=1 IN CC R OR AT-3 Wnt16=1 IN R then=1 else=0
Mat-n_b-cat	ModSkel	if >4 AND <10 AND IN Skel.Micromere then=1 else=0
	ModV2	if >5 AND <13 AND IN V2.Endoderm then=1 else=0
	ModV1	if [IN V1.Endoderm.A OR IN V1.Endoderm.O] AND >12 AND <10 then=1 else=0
	ModMeso	if [IN Oral.Mesoderm OR IN Aboral.Mesoderm] AND >5 AND <13 then=1 else=0
		if ModSkel=1 OR ModV2=1 OR ModV1=1 OR ModMeso=1 then=1 else=0

Model
-> Data
V2.Endoderm
hpf
Alx1
Blimp1b
Bra
Brn1/2/4
Dac
Delta
Dri
Erg
Ets1
Eve
FoxA
FoxB
FoxN2/3
FoxO
GataE
Gcm
GeneX
HesC
Hex
Hh
Hnf1
Hox11/13b
J(SuH)
J(Tcf)
J(V2)
J(Wnt16)
Mat-n_b-cat
Myc
N_b-cat
Notch
Nrl
Otx
Otx-alpha
Otx-beta
Pmar1
Runx
SignalV2
Tbr
Tel
Tgif
U1
VEGFR
Wnt16
Wnt8
z13/Krl
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
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18
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21
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24
25
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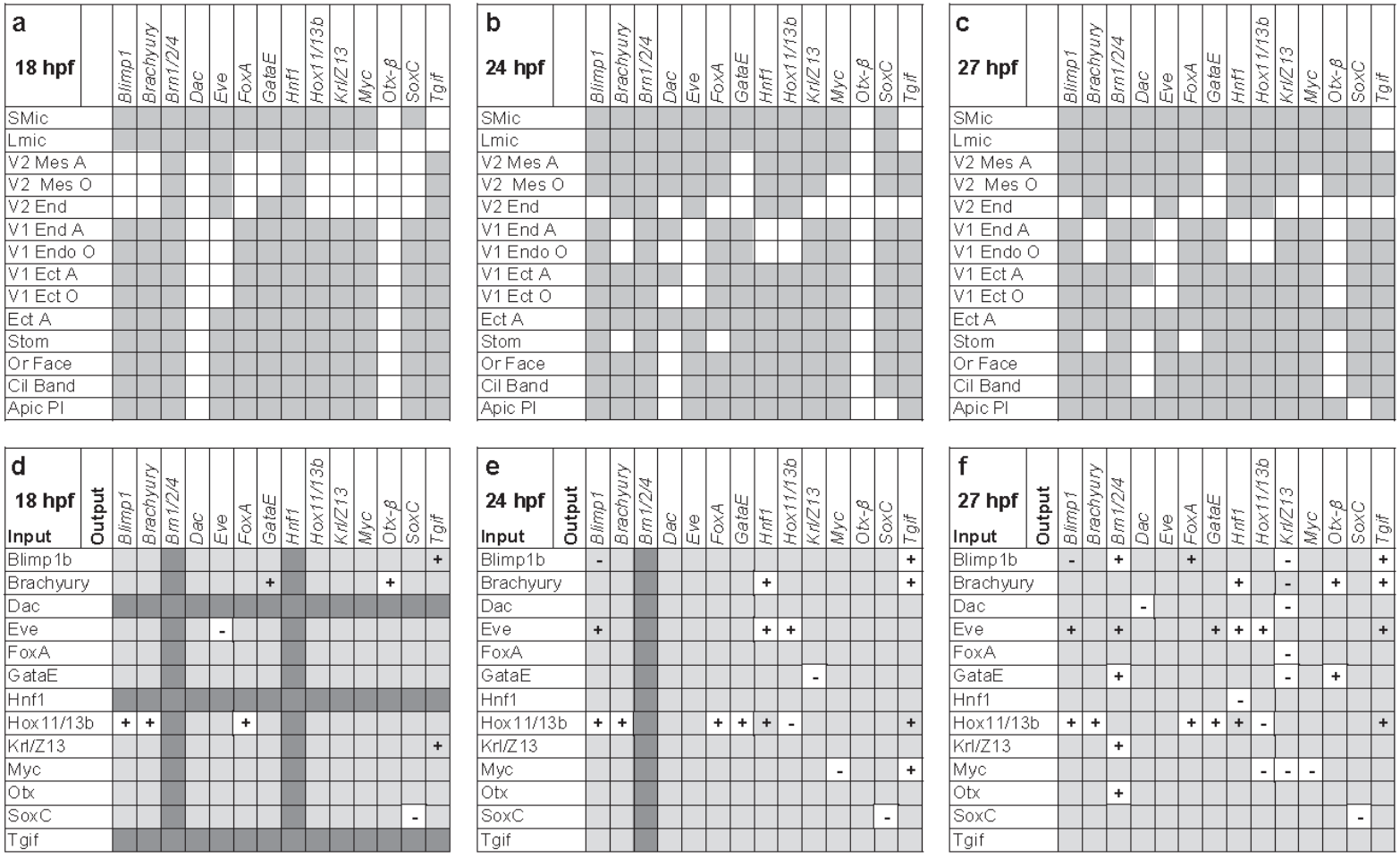
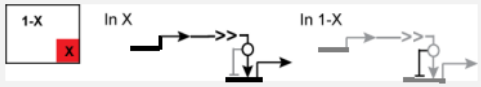
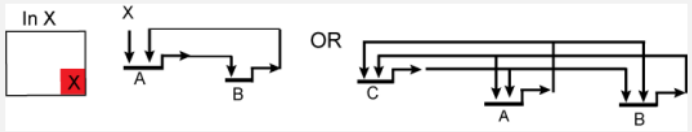
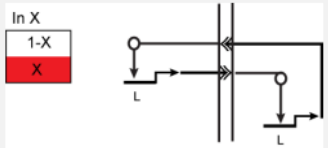
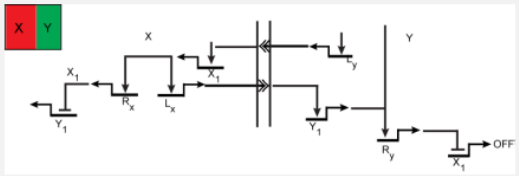
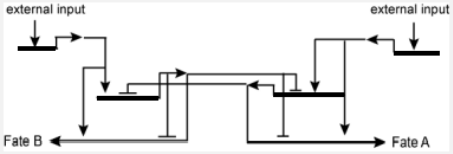
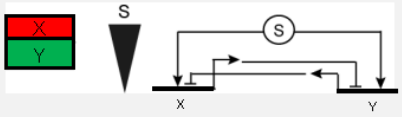
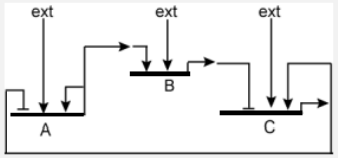
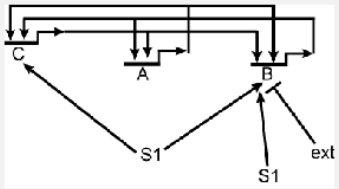
27
28
29
30
Egg
Macrom
V2
V2 Endo


a
18 hpf
Blimp1
Brachyury
Brn1/2/4
Dac
Eve
FoxA
GataE
Hnf1
Hox11/13b
Krl/Z13
Myc
Otx-β
SoxC
Tgif
SMic
Lmic
V2 Mes A
V2 Mes O
V2 End
V1 End A
V1 Endo O
V1 Ect A
V1 Ect O
Ect A
Stom
Or Face
Cil Band
Apic Pl
b
24 hpf
c
27 hpf
d
18 hpf
Input
Output
e
24 hpf
f
27 hpf

Regulatory State Specification Function	**Subcircuits**	**What they do**	**Topologies**
X,1-X processors	Double negative gate[1,2,6] **1.1**	Install regulatory state in X domain, prohibit same state everywhere else[b]	
	Signal mediated switch[2] **1.2**	Activate regulatory gene(s) in cells receiving signal, repress same genes everywhere else[c]	
Spatial subdivision	Inductive signaling[2] **2.1**	Activation of new regulatory genes in a cellular domain by transcriptional response to signal ligands produced by other cells	
	AND logic circuitry[2] **2.2**	Overlapping but spatially non-coincidental inputs are generated and both are required for regulatory gene activation, which occurs only in overlap subdomain	

Regulatory State Specification Function	**Subcircuits**	**What they do**	**Topologies**
	Spatial repression[2,8] **2.3**	Boundaries of spatial regulatory state domains controlled by transcriptional repression .	 g
Dynamic lockdown of regulatory state[d]	Reciprocal repression of state[1,2,9-11] **3.1**	In each spatial regulatory state domain key activators of alternative states are transcriptionally repressed by "exclusion" circuitry[e].	 g
	Feedback circuitry (Supplementary Table 1) [1] **3.2**	Two or three regulatory genes engage in positive intergenic feedback, stabilizing regulatory state irrespective of transient inputs	 g
	Community effect circuitry[2,12,13] **3.3**	Cells within a territory all signal to one another, driving continued uniform expression both of ligand gene and signal dependant regulatory genes[f].	 h

Boundary maintenance	Reciprocal repressive signaling across boundary[14] 4	Different signals are produced by apposing cells and their reception triggers repressive circuitry excluding the cross-boundary regulatory state.	i 
Terminal binary cell fate choice	Alternate subcircuits driven by reciprocal repressors[8,15-18] 5	External inputs tip the balance of repressor expression, resulting in activation of one differentiation program and exclusion of the other.	j 
Discontinous transcriptional response to signal intensity and/or duration	Reciprocal repressor genes responding cooperatively to inducer[17,18,19,20] 6.1	Circuitry generates differential stimulation of expression of reciprocal repressors in low vs. high signal intensity[k]	l 
	Reciprocal repressor genes, one activating an additional repressor gene, each with variable external positive inputs[21] 6.2	Circuitry generates irreversible transitions, in stem cell regulatory state, off vs. on in response to signals of different strength and duration	o 
	Triple feedback linkage with asymmetric signal inputs[22] 6.3	Produces alternative regulatory states, or low level indeterminate state, depending of different positive inputs[m]	n 

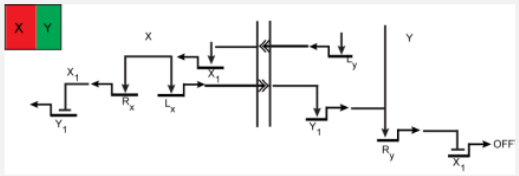
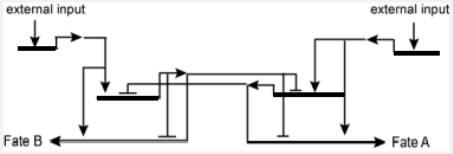
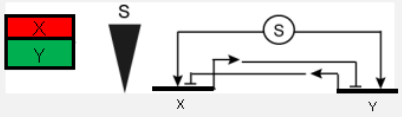
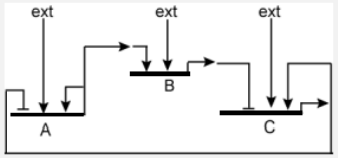
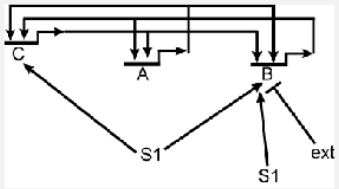
PREDICTIVE GRN MODEL FOR THE SEA URCHIN EMBRYO

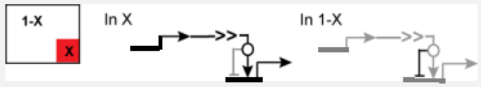
MATHEMATICAL MODELS OF DEVELOPMENTAL GRN DYNAMICS ARE OF DIVERSE TYPES. THE MOST COMMON TYPES ARE:

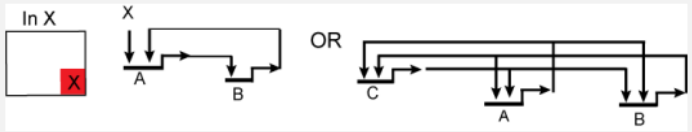
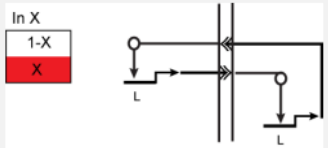
- **KINETIC O.D.E. MODELS OF TRANSITIONS OR SWITCHES MEDIATED BY KNOWN GRNS IN A GIVEN DOMAIN OVER TIME (E.G., IN MAMMALIAN STEM CELLS), OFTEN MODELS BASED ON GIVEN GRN SUBCIRCUITS**

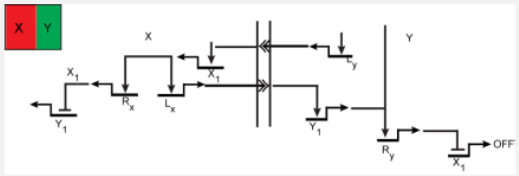
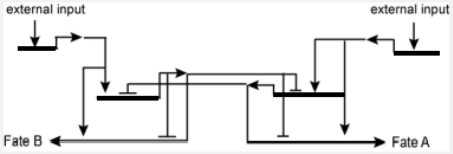
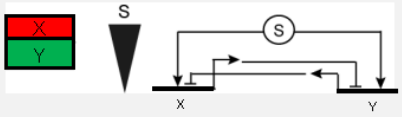
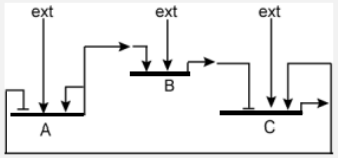
- **KINETIC O.D.E. MODELS OF LONG DISTANCE SIGNAL LIGAND DISTRIBUTION IN SPACE, WITH ASSUMED TRANSCRIPTIONAL RESPONSE GRNS (E.G., GRADED HH SIGNAL RESPONSE)**

- **PARAMETERIZED KINETIC O.D.E. MODELS OF SPATIAL PATTERNING FUNCTIONS, IN THE LATE SYNCYTIAL DROSOPHILA EMBRYO**

Boundary maintenance	Reciprocal repressive signaling across boundary[14] 4	Different signals are produced by apposing cells and their reception triggers repressive circuitry excluding the cross-boundary regulatory state.	i 
Terminal binary cell fate choice	Alternate subcircuits driven by reciprocal repressors[8,15-18] 5	External inputs tip the balance of repressor expression, resulting in activation of one differentiation program and exclusion of the other.	j 
Discontinous transcriptional response to signal intensity and/or duration	Reciprocal repressor genes responding cooperatively to inducer[17,18,19,20] 6.1	Circuitry generates differential stimulation of expression of reciprocal repressors in low vs. high signal intensity[k]	l 
	Reciprocal repressor genes, one activating an additional repressor gene, each with variable external positive inputs[21] 6.2	Circuitry generates irreversible transitions, in stem cell regulatory state, off vs. on in response to signals of different strength and duration	o 
	Triple feedback linkage with asymmetric signal inputs[22] 6.3	Produces alternative regulatory states, or low level indeterminate state, depending of different positive inputs[m]	n 

Regulatory State Specification Function	**Subcircuits**	**What they do**	**Topologies**
X,1-X processors	Double negative gate[1,2,6] **1.1**	Install regulatory state in X domain, prohibit same state everywhere else[b]	
	Signal mediated switch[2] **1.2**	Activate regulatory gene(s) in cells receiving signal, repress same genes everywhere else[c]	
Spatial subdivision	Inductive signaling[2] **2.1**	Activation of new regulatory genes in a cellular domain by transcriptional response to signal ligands produced by other cells	
	AND logic circuitry[2] **2.2**	Overlapping but spatially non-coincidental inputs are generated and both are required for regulatory gene activation, which occurs only in overlap subdomain	

Regulatory State Specification Function	**Subcircuits**	**What they do**	**Topologies**
	Spatial repression[2,8] **2.3**	Boundaries of spatial regulatory state domains controlled by transcriptional repression .	 g
Dynamic lockdown of regulatory state[d]	Reciprocal repression of state[1,2,9-11] **3.1**	In each spatial regulatory state domain key activators of alternative states are transcriptionally repressed by "exclusion" circuitry[e].	 g
	Feedback circuitry (Supplementary Table 1) [1] **3.2**	Two or three regulatory genes engage in positive intergenic feedback, stabilizing regulatory state irrespective of transient inputs	 g
	Community effect circuitry[2,12,13] **3.3**	Cells within a territory all signal to one another, driving continued uniform expression both of ligand gene and signal dependant regulatory genes[f].	 h

Boundary maintenance	Reciprocal repressive signaling across boundary[14] 4	Different signals are produced by apposing cells and their reception triggers repressive circuitry excluding the cross-boundary regulatory state.	i 
Terminal binary cell fate choice	Alternate subcircuits driven by reciprocal repressors[8,15-18] 5	External inputs tip the balance of repressor expression, resulting in activation of one differentiation program and exclusion of the other.	j 
Discontinous transcriptional response to signal intensity and/or duration	Reciprocal repressor genes responding cooperatively to inducer[17,18,19,20] 6.1	Circuitry generates differential stimulation of expression of reciprocal repressors in low vs. high signal intensity[k]	l 
	Reciprocal repressor genes, one activating an additional repressor gene, each with variable external positive inputs[21] 6.2	Circuitry generates irreversible transitions, in stem cell regulatory state, off vs. on in response to signals of different strength and duration	o 
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