# GENE REGULATORY NETWORKS PROVIDE CAUSAL EXPLANATIONS FOR THE DEVELOPMENTAL PROCESS

KITP AUGUST 2011

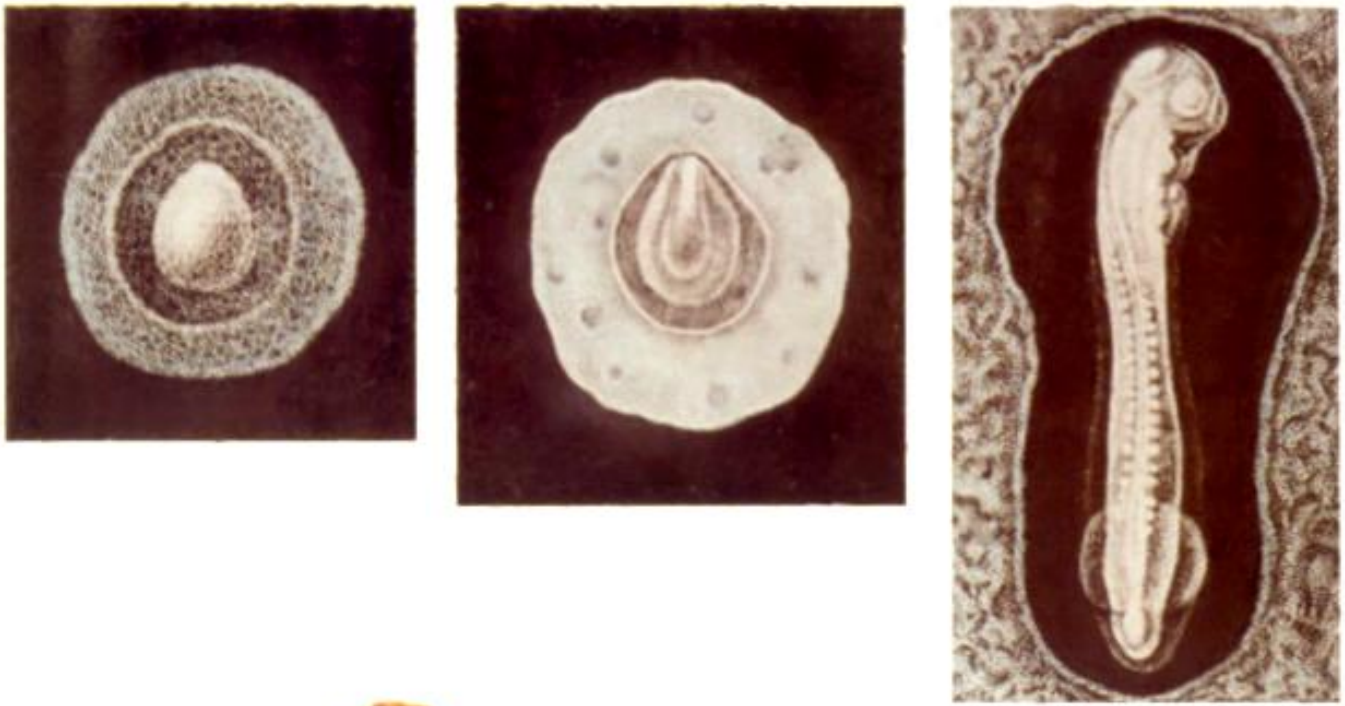
# CAUSALITY IN DEVELOPMENT BEGINS WITH SEQUENCE SPECIFIC RECOGNITION OF REGULATORY DNA SEQUENCES

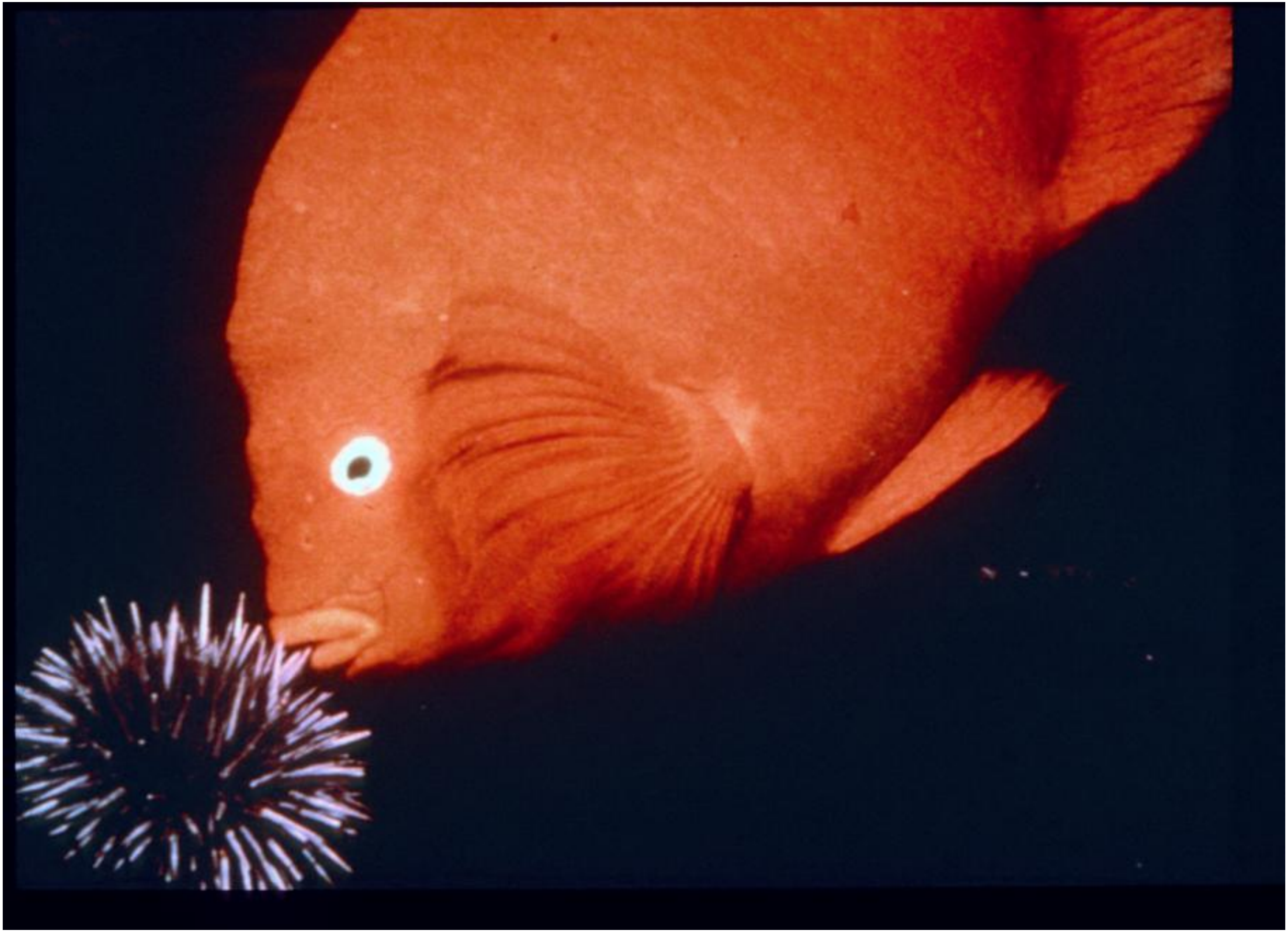
**(AND THEREFORE CAUSAL EXPLANATION OF DEVELOPMENTAL PHENOMENA MUST ULTIMATELY RELATE TO WHAT HAPPENS AT SPECIFIC REGULATORY DNA SEQUENCES!!)**

TWO FUNDAMENTAL ASPECTS OF DEVELOPMENT :

- INCREASE IN COMPLEXITY
- DIVERSITY IN HEREDITARY BODY PLANS

WHAT WE NEED TO UNDERSTAND IS THE CHAIN OF CAUSALITY FROM THE REGULATORY GENOME TO THESE PHENOMENOLOGICAL OUTPUTS





# THE ANIMAL REGULATORY GENOME:

NO. CIS-REGULATORY MODULES (CRM): 3-10X NO. OF GENES (?)

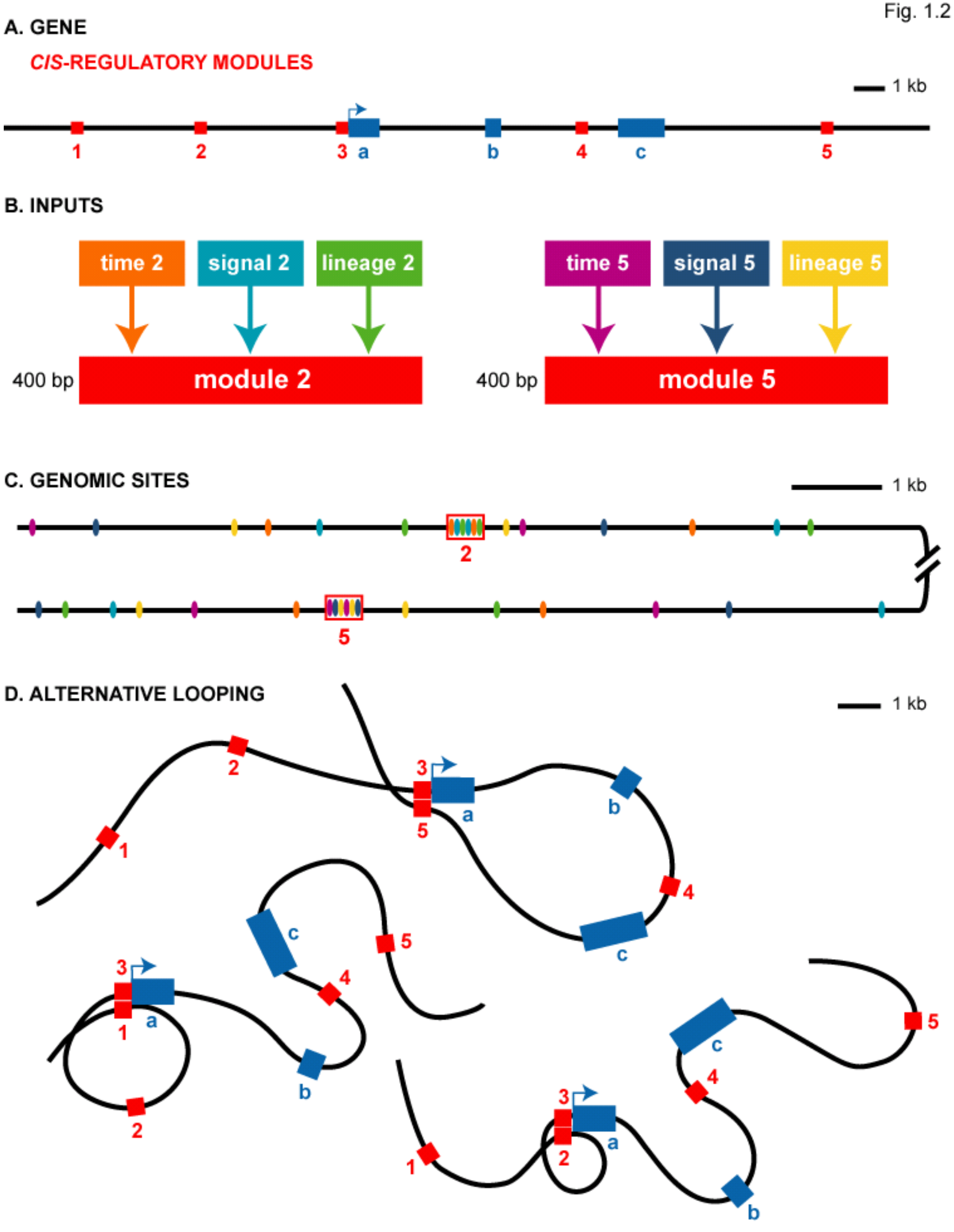
SIZE OF CRM: SEVERAL HUNDRED BASE PAIRS

TOTAL COMPLEXITY: AT LEAST AS MUCH DNA SEQUENCE LENGTH IS INCLUDED IN THE REGULATORY GENOME AS IN SUM OF PROTEIN CODING REGIONS OF GENES (MAYBE 2-3X AS MUCH)

MAIN FEATURE OF CRM: THEY COMBINATORIALLY PROCESS MULTIPLE INPUTS SO OUTPUT IS NEVER THE SAME AS ANY INPUT

IN REGARD TO DEVELOPMENT THE REGULATORY GENOME IS A VAST COMPUTATIONAL DEVICE

Fig. 1.2


A. GENE
CIS-REGULATORY MODULES
1 kb
1
2
3
a
b
4
c
5
B. INPUTS
time 2
signal 2
lineage 2
400 bp
module 2
time 5
signal 5
lineage 5
400 bp
module 5
C. GENOMIC SITES
1 kb
2
5
D. ALTERNATIVE LOOPING
1 kb
2
1
3
a
5
b
4
c
c
5
4
3
a
1
2
b
3
a
2
1
c
4
5
b

# A TYPICAL EXAMPLE OF SPATIAL INFORMATION PROCESSING

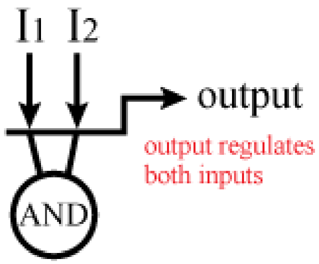
## 1. AND LOGIC

Spatial Specification by cis-regulatory & logic

LOGIC DIAGRAM

| $I_1$ | $I_2$ | output |
|---|---|---|
| 0 | 0 | 0 |
| 0 | I | 0 |
| I | 0 | 0 |
| I | I | I |

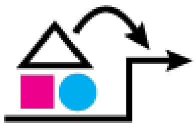
CIS-REGULATORY MODULE DIAGRAM



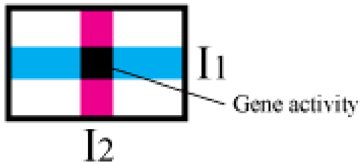
output regulates both inputs

BIOCHEMICAL DIAGRAM

“cis-regulatory device”



Field of cells



# AT THE SYSTEM LEVEL THE UNIQUE, FUNCTIONAL OUTPUTS OF THE REGULATORY GENOME IN ANIMAL DEVELOPMENT: REGULATORY STATES

BASIC DEFINITION: **REGULATORY STATE** IS THE SUM OF ALL ACTIVE TRANSCRIPTION FACTORS IN A CELL AT A GIVEN TIME, AND ALL THE CELL DOES DEPENDS ON REGULATORY STATE

DEVELOPMENT IS DIRECTLY PROGRAMMED BY CONTINUOUS INCREASE IN COMPLEXITY OF SPATIAL REGULATORY STATES

THIS LEADS TO FUNCTIONAL AND STRUCTURAL INCREASE IN COMPLEXITY, AND ACCOUNTS FOR ANIMAL MORPHOLOGY

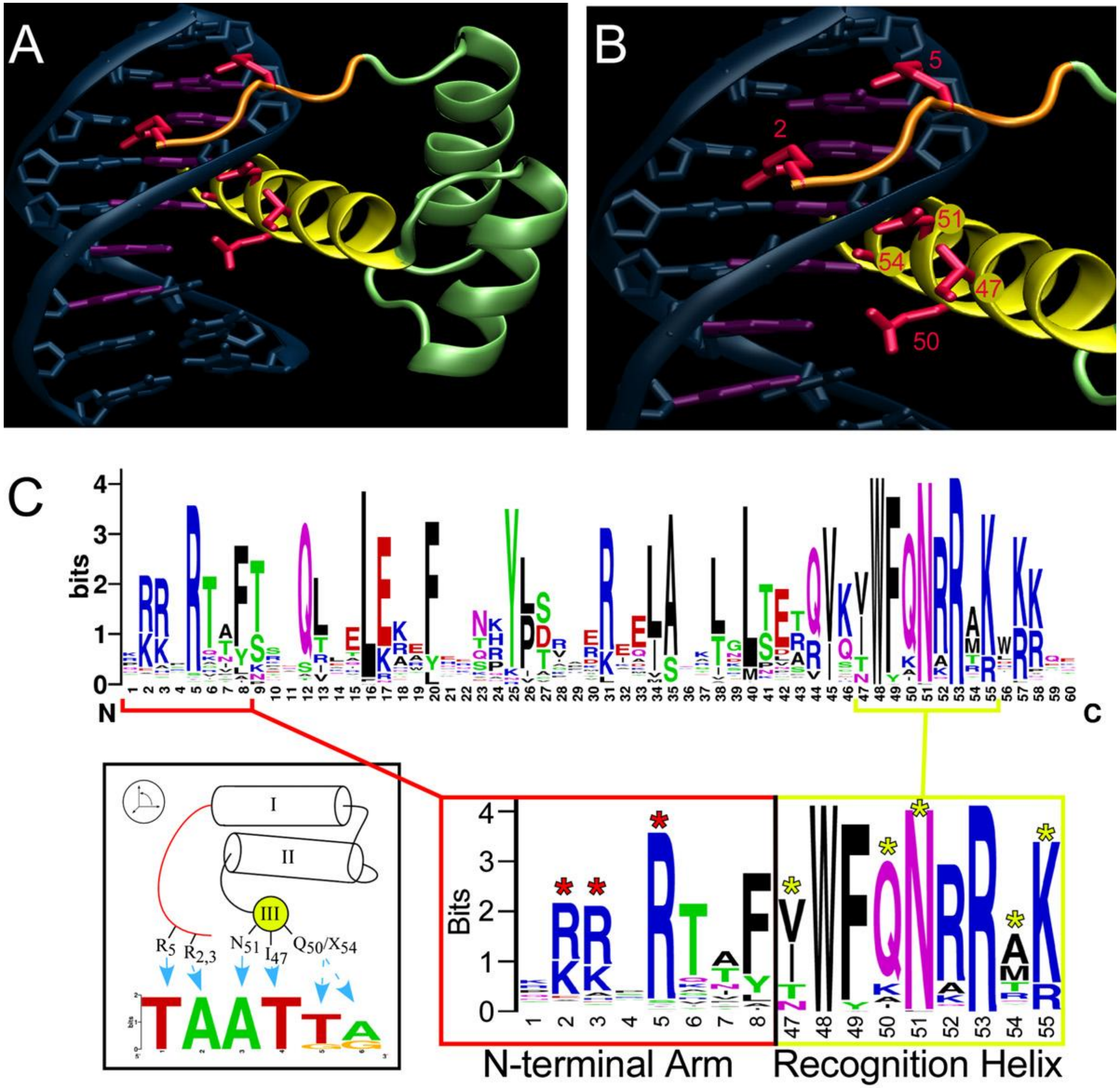
CHANGE IN THE REGULATORY GENOME IN GEOLOGICAL TIME LEADS TO EVOLUTIONARY CHANGE IN BODY PLAN, AND ACCOUNTS FOR MORPHOLOGICAL DIVERSITY OF ANIMALS

# GENOMIC CONTROL OF DEVELOPMENTAL PROCESS:

PARTICULAR NETWORKS OF INTERACTING REGULATORY GENES ENCODING TRANSCRIPTION FACTORS GENERATE PARTICLAR REGULATORY STATES

THE KEY TO CAUSAL UNDERSTANDING OF HOW GENOMES CONTROL DEVELOPMENTAL PROCESS LIES IN THE TOPOLOGY OF THESE GENE REGULATORY NETWORKS (GRN'S), I.E., IN THE FUNCTIONAL LINKAGES AMONG GENES

GRN TOPOLOGY IS DETERMINED DIRECTLY BY THE A'S, C'S, G'S, AND T'S THAT DEFININE THE TRANSCRIPTION FACTOR TARGET SITES OF THE REGULATORY GENOME



**NOYES ET AL., CELL, 2008**

# GENERAL CHARACTERISTICS OF DESIGN IN GRN’S FOR EMBRYONIC DEVELOPMENT:

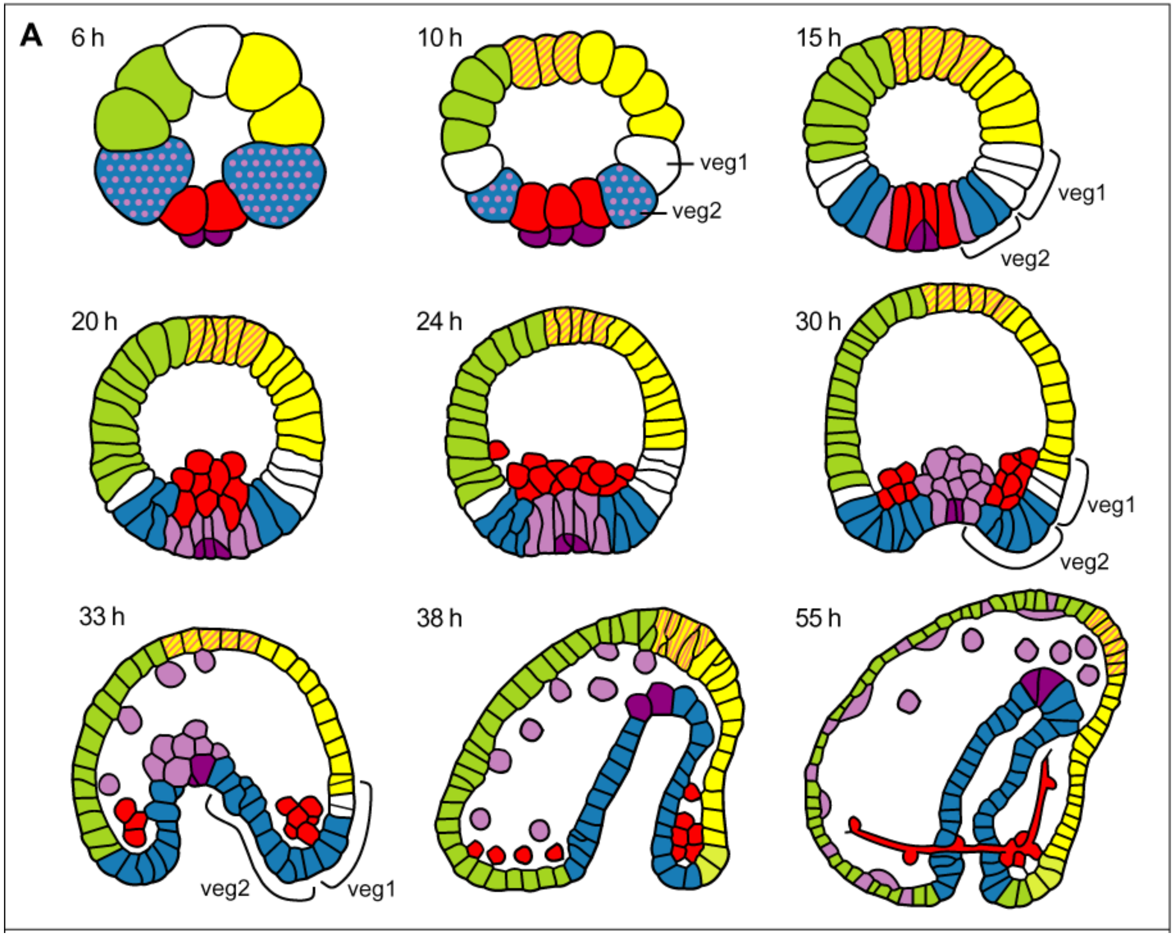
**EMBRYONIC GRNS PRODUCE IRREVERSIBLE, PROGRESSIVE SEQUENCE OF REGULATORY STATES; OSCILLATIONS OF REGULATORY STATE IN GIVEN DOMAINS RARELY OCCUR**

**MULTIPLE DIFFERENT MODULAR SUBCIRCUITS ENSURE THE CORRECT OUTCOME AT EACH STEP: “OVER-WIRED” CIRCUITRY**

**PROCESS IN GENERAL INDEPENDENT OF EXTERNAL ENVIRONMENTAL INPUT (WITHIN LIMITS OF VIABILITY)**

**PATTERN FORMATION (SPATIAL REGULATORY STATES) IS FAR UPSTREAM OF CELL TYPE SPECIFICATION, WHICH IS A NEAR-TERMINAL PROCESS ENCODED AT PERIPHERY OF GRN**

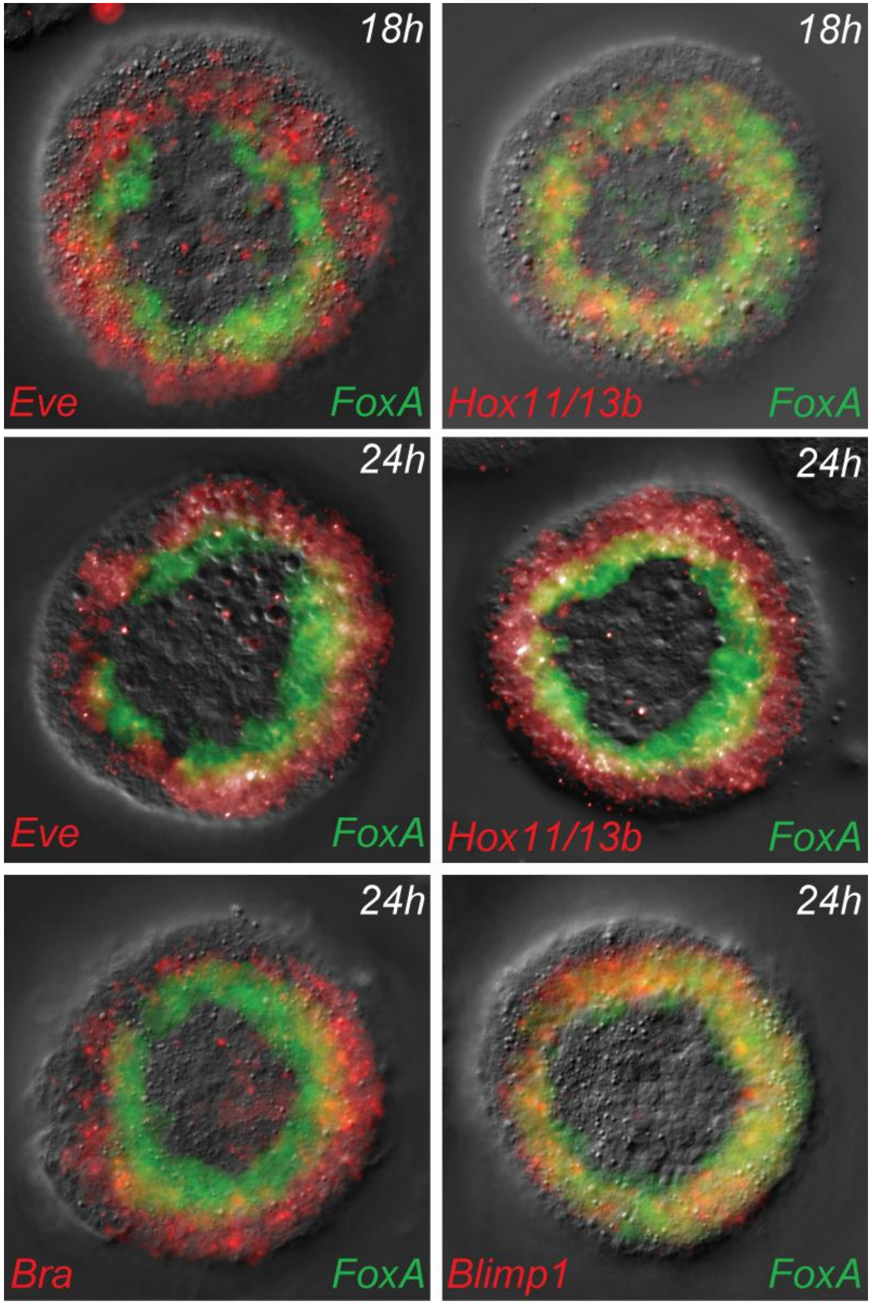
**MAIN FUNCTION OF ENCODED GRN LOGIC TRANSACTIONS IS SPATIAL SPECIFICATION OF REGULATORY STATE**

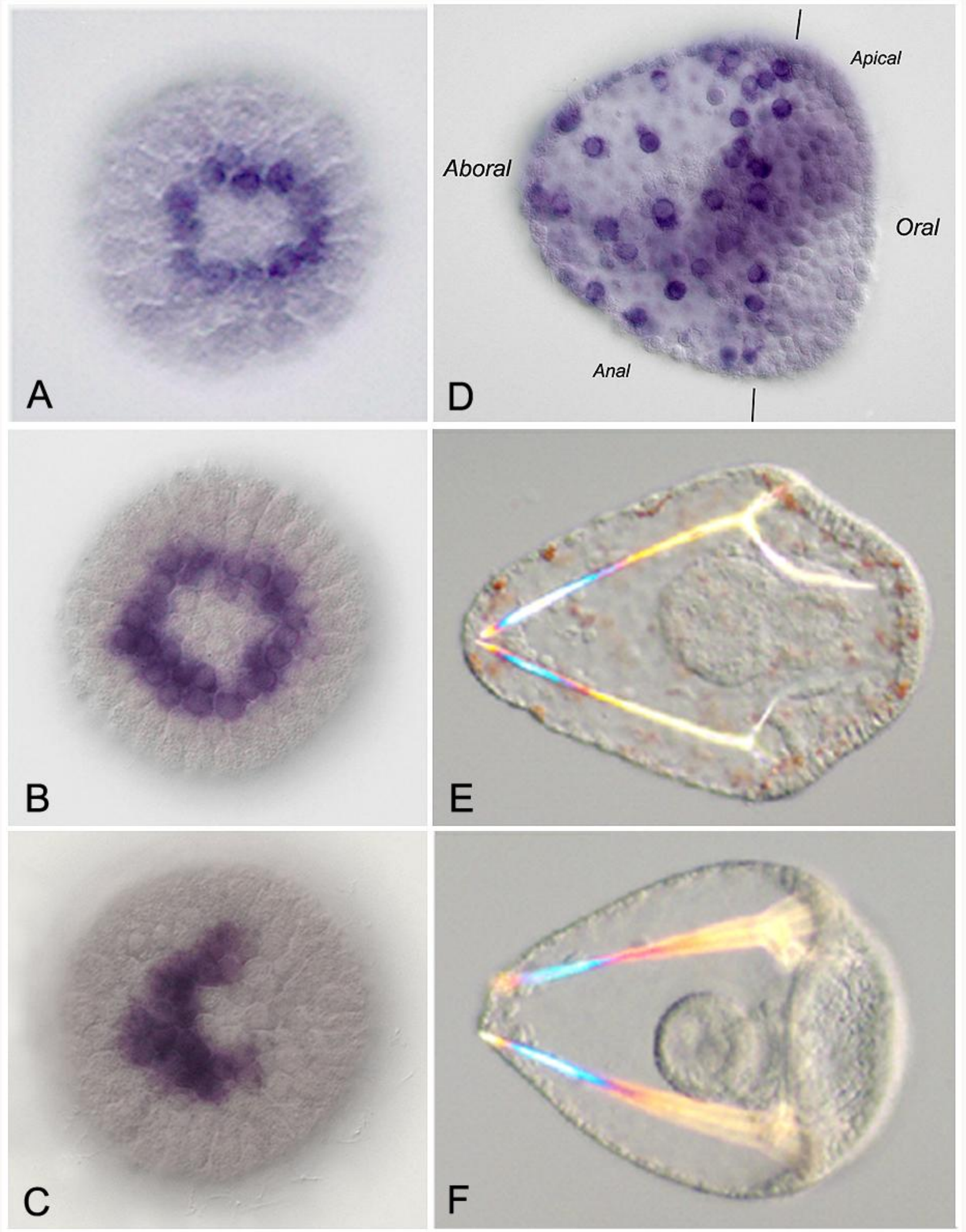


# SPECIFICATION IN THE SEA URCHIN EMBRYO

REPRODUCIBLE AND PRECISE SPATIAL GENE EXPRESSION PATTERNS

FOR EXAMPLE…


18h
Eve
FoxA
18h
Hox11/13b
FoxA
24h
Eve
FoxA
24h
Hox11/13b
FoxA
24h
Bra
FoxA
24h
Blimp1
FoxA


A
Apical
Aboral
Oral
Anal
D
B
E
C
F

# DYNAMICS AND BOOLEAN SPATIAL EXRESSION

IN DEVELOPMENT, REGULATORY STATE CHANGES DYNAMICALLY IN TIME

BUT REGULATORY STATE EXPRESSION IN SPATIAL CELLULAR DOMAINS OF THE EMBRYO IS BOOLEAN

REGULATORY STATES DO NOT MERGE INTO ONE ANOTHER; THEY ARE EITHER ON OR OFF IN ADJACENT REGIONS OF AN EMBRYO

THIS IS WHY TRANSCRIPTIONAL REPRESSION IS AS IMPORTANT IN DEVELOPMENTAL GRNS AS TRANSCRIPTIONAL ACTIVATION

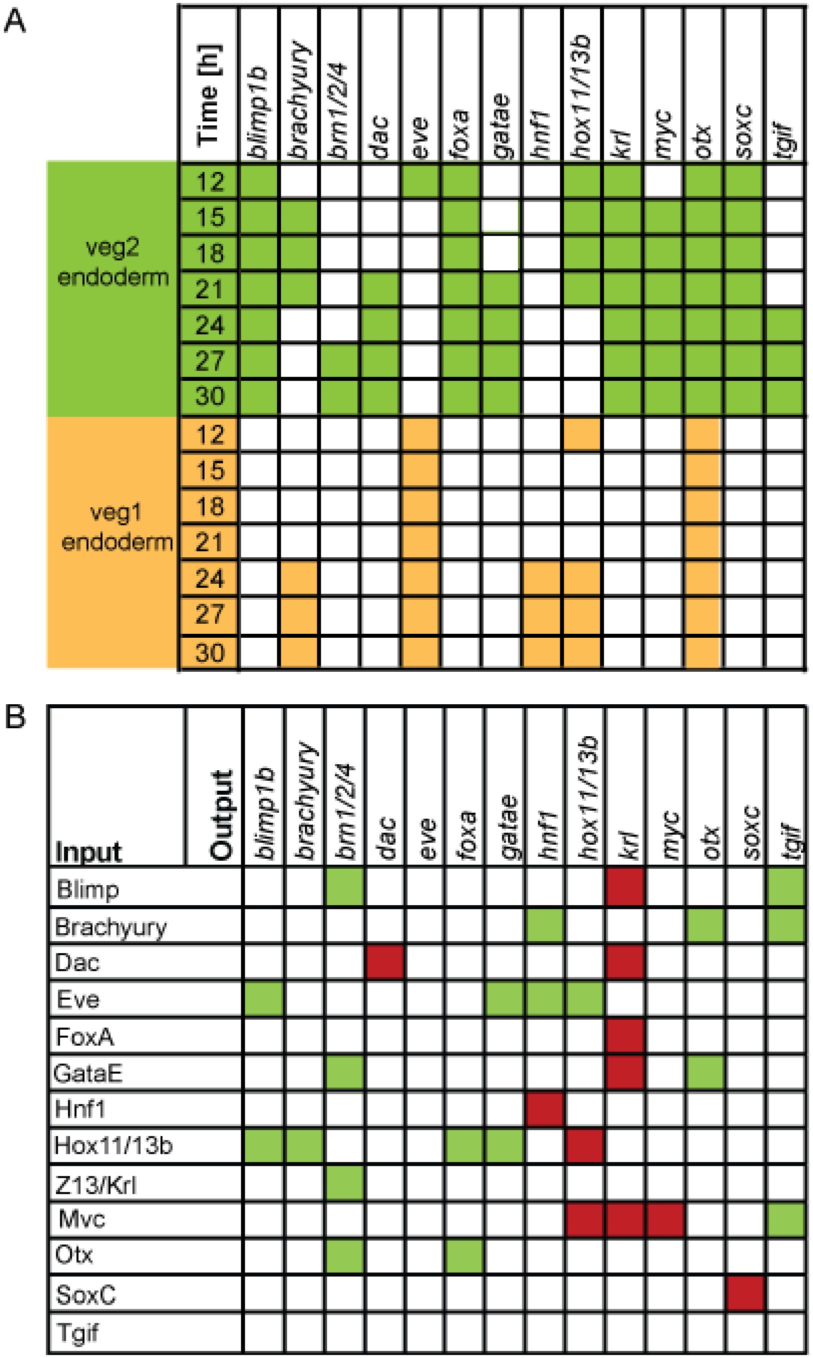
# THE SEA URCHIN EMBRYO GRN'S

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

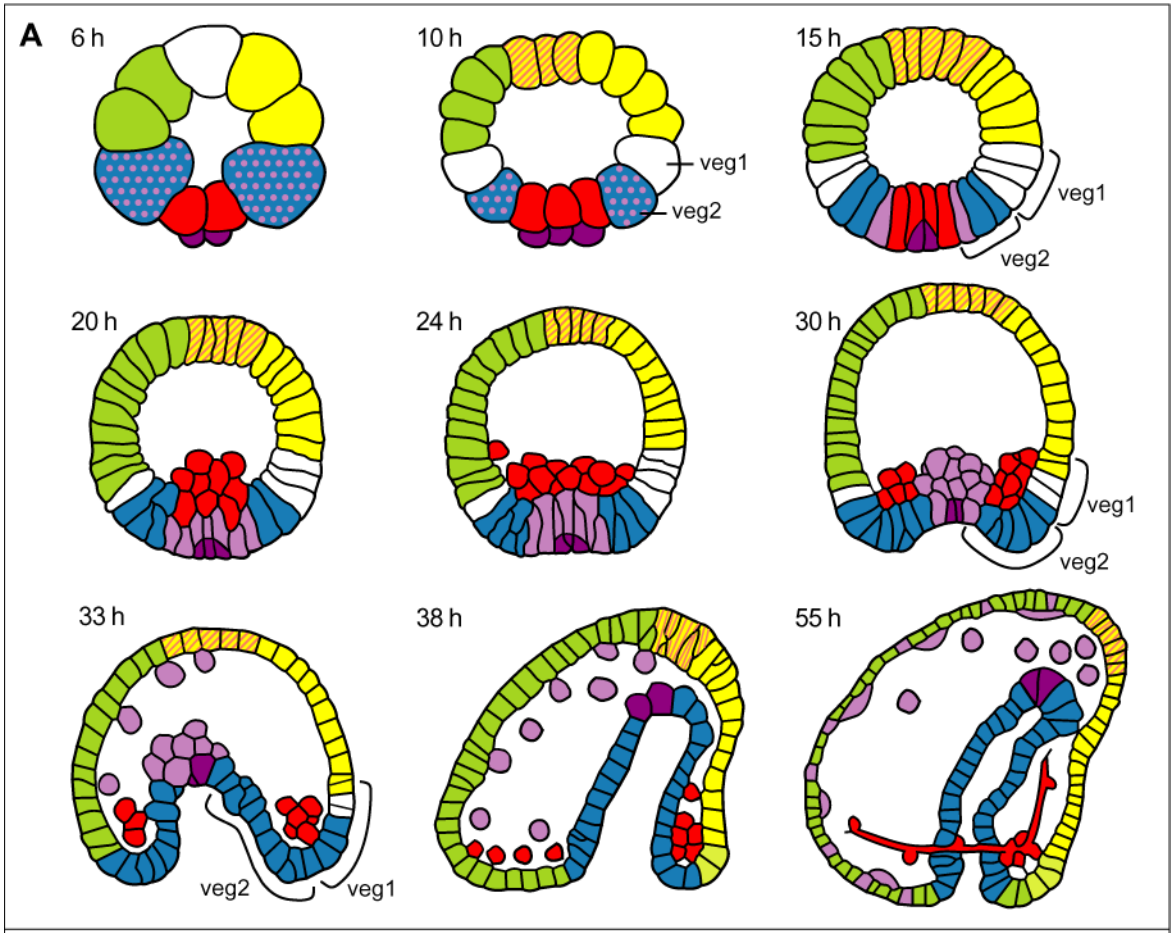


A

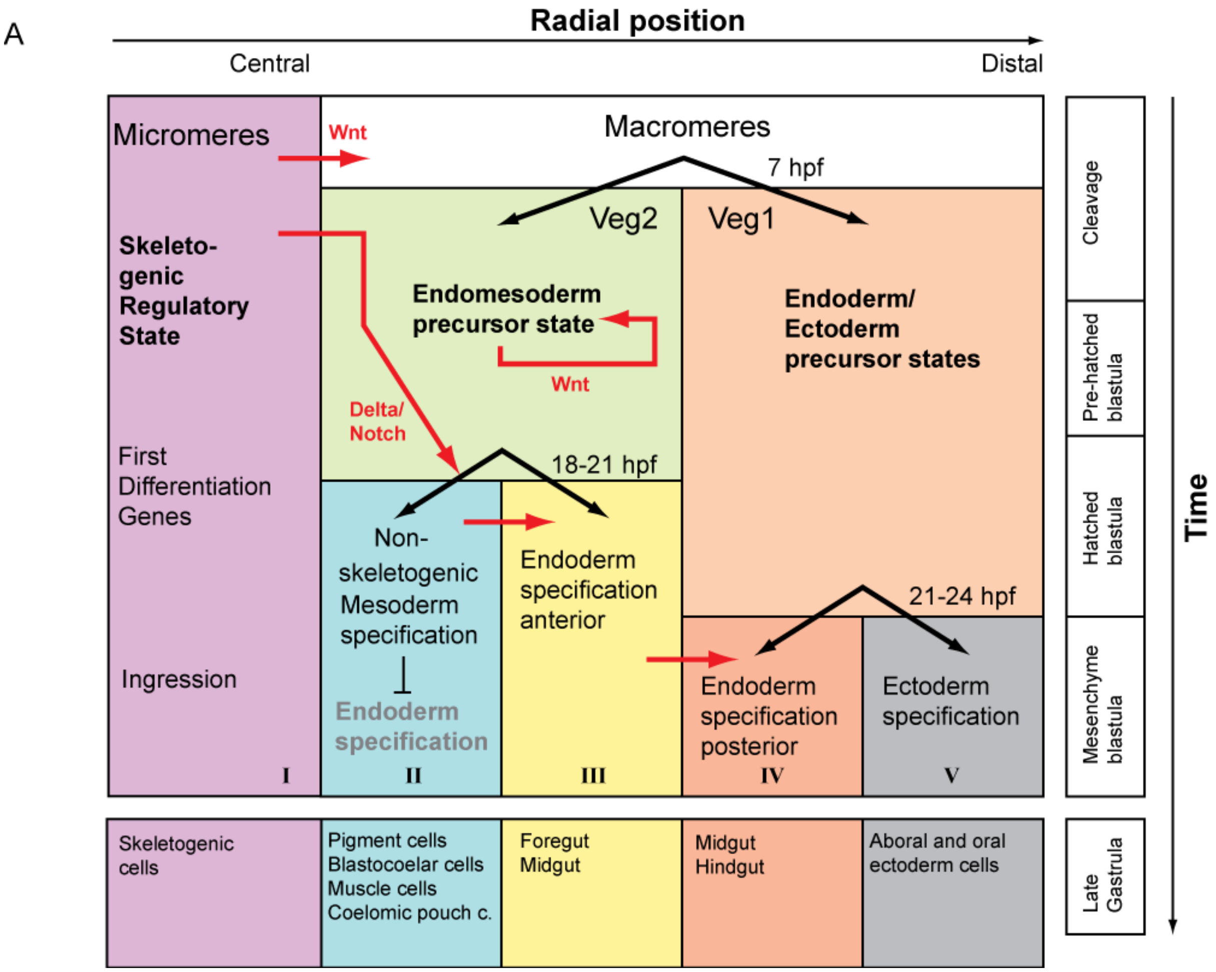
|  | Time [h] | *blimp1b* | *brachyury* | *brn1/2/4* | *dac* | *eve* | *foxa* | *gatae* | *hnf1* | *hox11/13b* | *krl* | *myc* | *otx* | *soxc* | *tgif* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| veg2 endoderm | 12 | 🟩 |  |  |  | 🟩 | 🟩 |  |  | 🟩 | 🟩 |  | 🟩 | 🟩 |  |
|  | 15 | 🟩 | 🟩 |  |  |  | 🟩 |  |  | 🟩 | 🟩 | 🟩 | 🟩 | 🟩 |  |
|  | 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 |  |  | 🟩 |  |  |  |  |  |  |  |  |  |  |  |
| Myc |  |  |  |  |  |  |  |  | 🟥 | 🟥 | 🟥 |  |  | 🟩 |
| Otx |  |  | 🟩 |  |  | 🟩 |  |  |  |  |  |  |  |  |
| SoxC |  |  |  |  |  |  |  |  |  |  |  |  | 🟥 |  |
| Tgif |  |  |  |  |  |  |  |  |  |  |  |  |  |  |



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
18-21 hpf
First Differentiation Genes
Non-skeletogenic Mesoderm specification
Endoderm 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

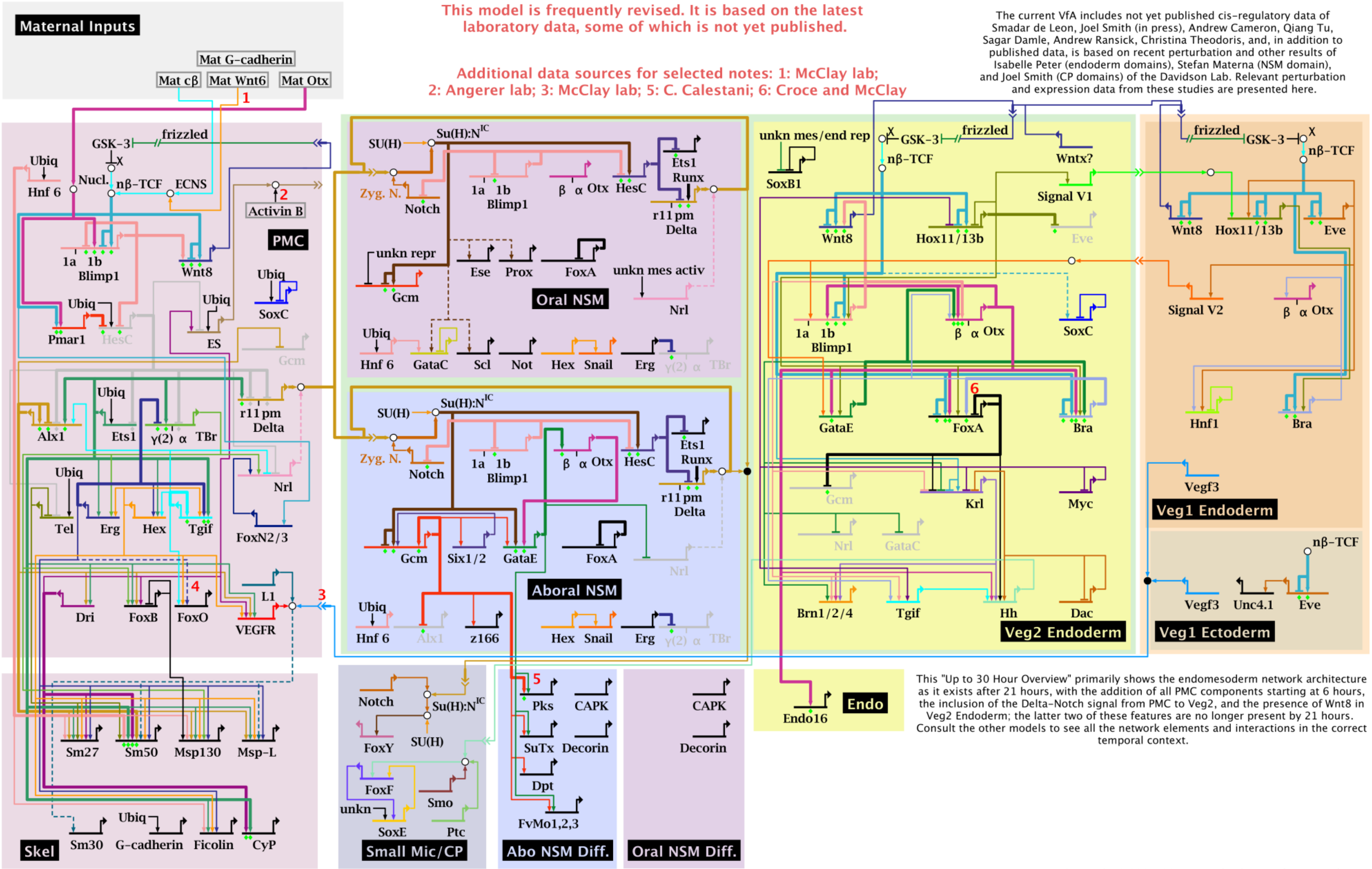
## Endomesoderm Specification up to 30 Hours

February 03, 2011

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

Additional data sources for selected notes: 1: McClay lab; 2: Angerer lab; 3: McClay lab; 5: C. Calestani; 6: Croce and McClay

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.

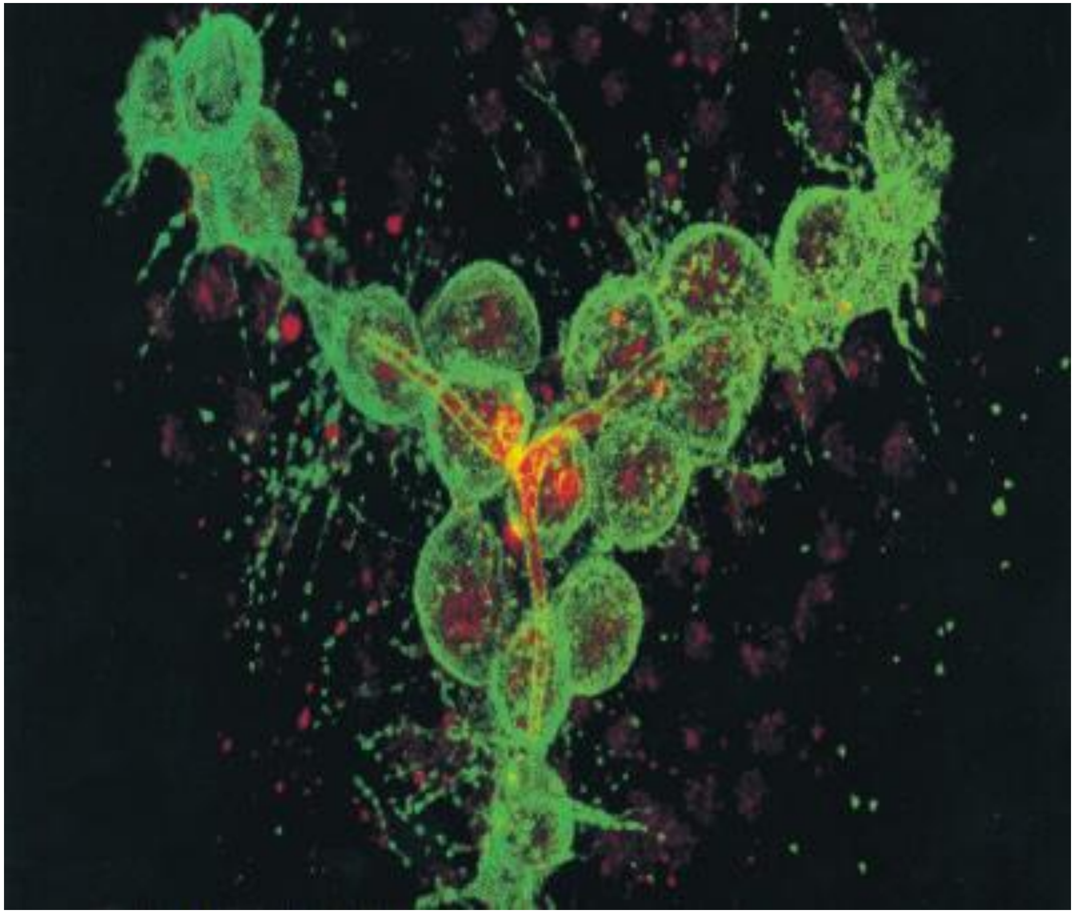


This "Up to 30 Hour Overview" primarily shows the endomesoderm network architecture as it exists after 21 hours, with the addition of all PMC components starting at 6 hours, the inclusion of the Delta-Notch signal from PMC to Veg2, and the presence of Wnt8 in Veg2 Endoderm; the latter two of these features 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

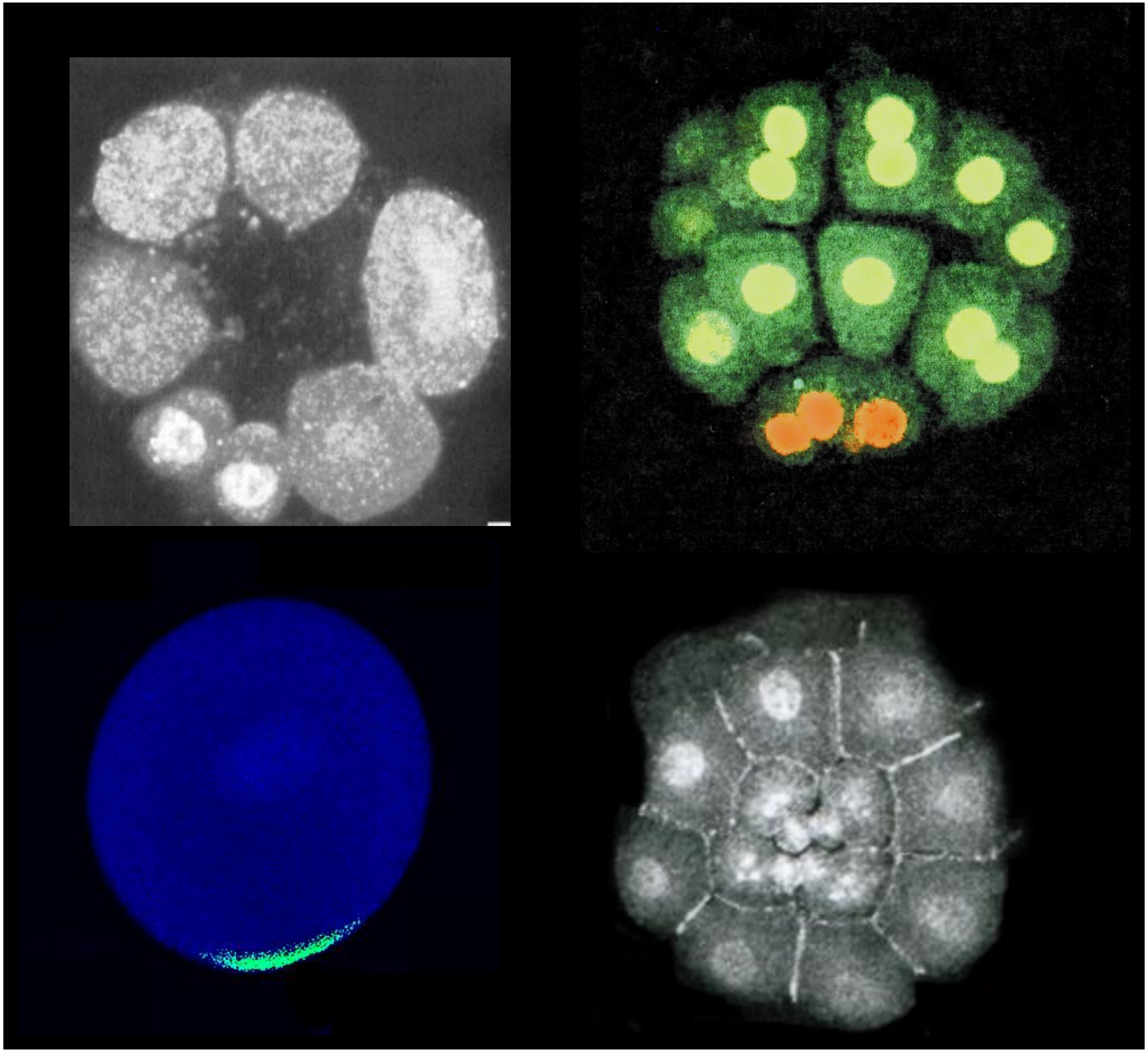
# HOW DEVELOPMENT IS ENCODED: EXAMPLE 1

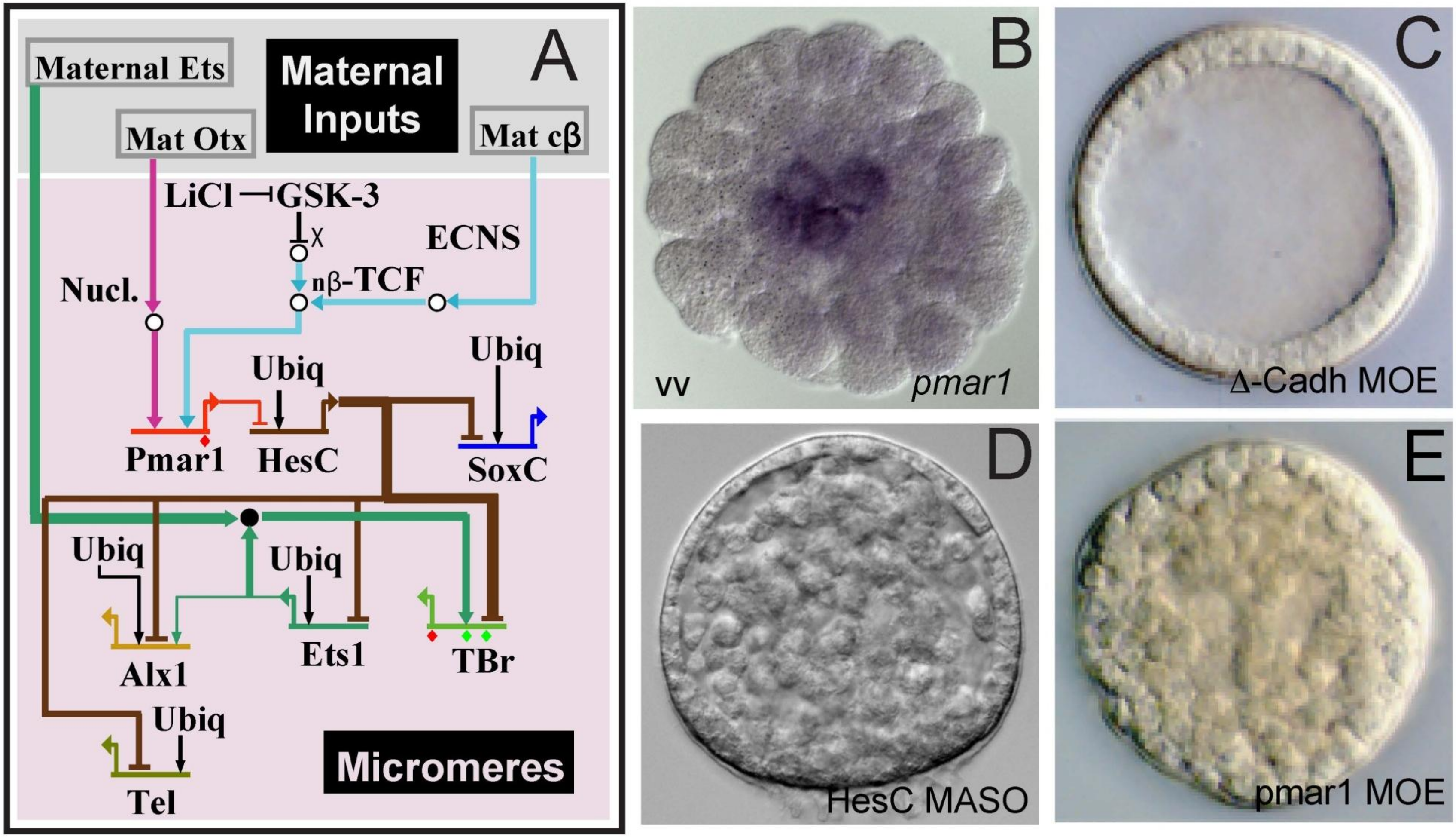
## SPECIFICATION OF SKELETOGENIC CELL LINEAGE

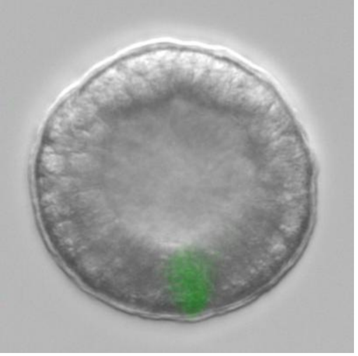


# THE FIRST JOB: HOW DO FUTURE SKELETOGENIC CELLS CELLS KNOW WHAT THEY ARE TO BE, AND HOW DO THEY SET UP SPECIFIC REGULATORY STATE????

A GENOMICALLY ENCODED DOUBLE NEGATIVE GATE




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

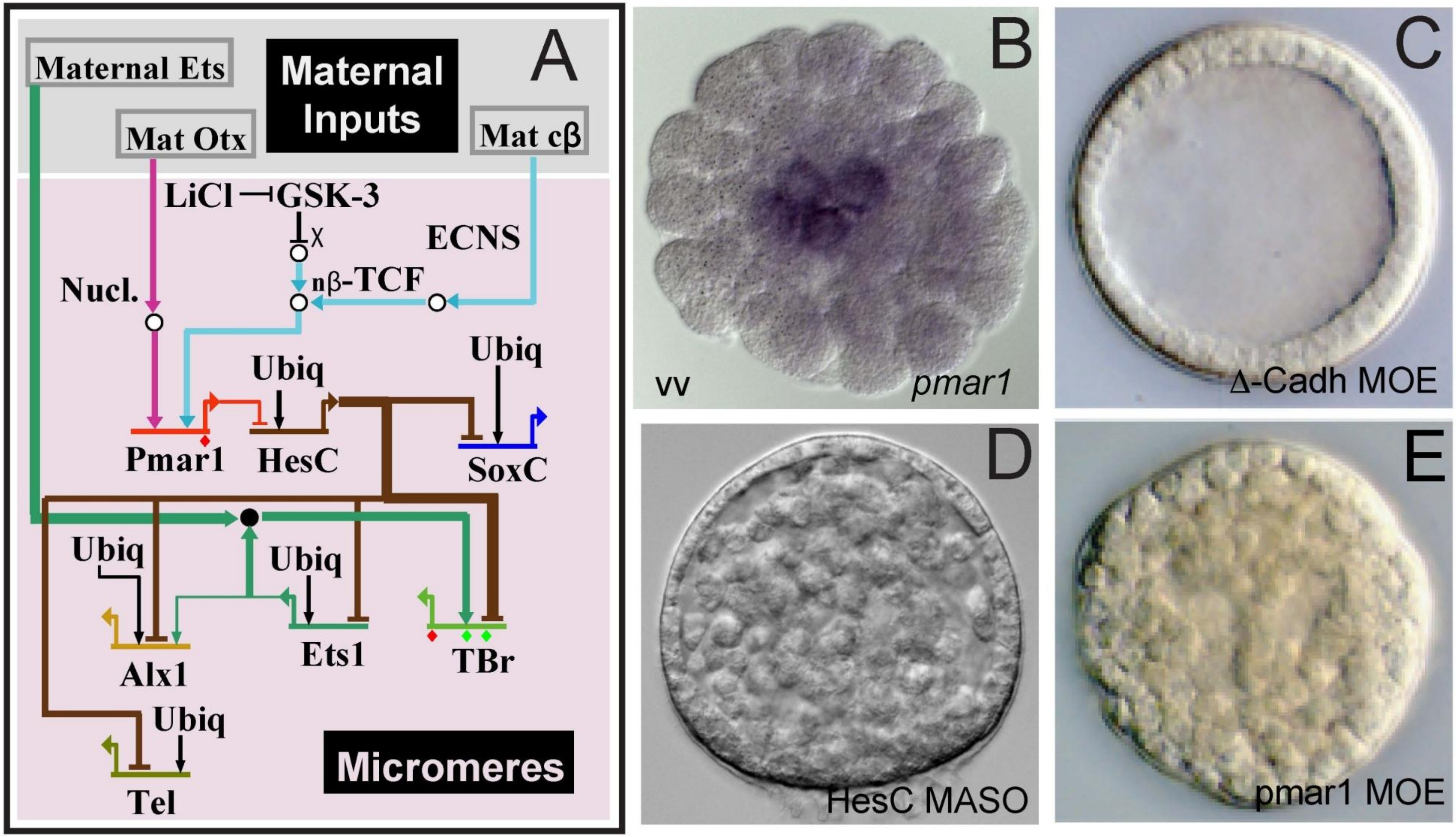


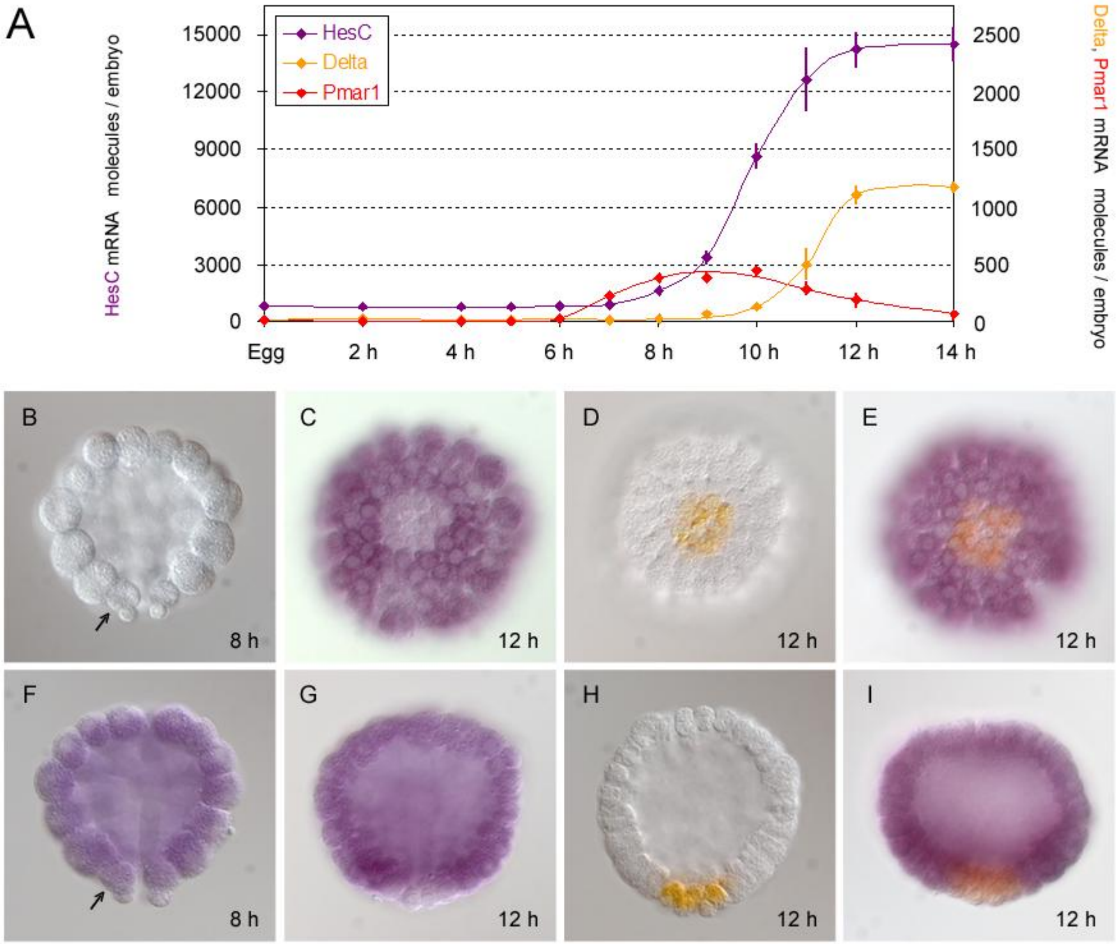
# Reporter construction: *pmar1* 2.5 kb reporter

## AS PREDICTED, THE *PMAR1* GENE REGULATORY SEQUENCE IS DRIVEN BY OTX AND TCF/BCATENIN INPUTS

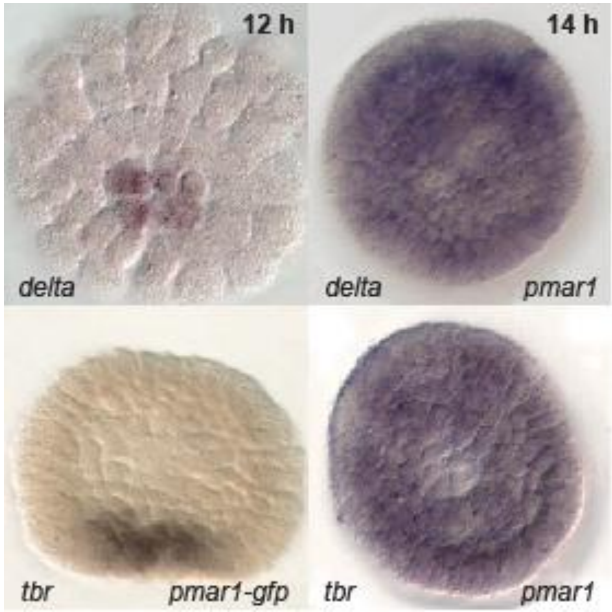
CGTAGTGATCC**TCCGGTTACTCAGCCTTAAT**TGTACATGTAGTGTCTCTGTTGATTCTCCGCCCTATTA
TTCTCTCATTCTCTTTTTTCTAAAATCTTAGTTTCATTCTTTCCCGCTCTTCCGCGTGAACGGCGTAT
GTTGGGATTATGACAAAAAATGAAATTGCGCCCCGCTCAAAAATGTCGCCATAACTGTACAAGCGTGTG
GCAACGCGACCGAGCAGTACTTTTGTTTCTTGTCAAACTGTTAAATACAGTAATTATTATGCATATATTA
ACTAGAGAAAGATTTGCAAGTGAGCACTGCAAACTAGCTGTAGAGTTTCTTAATGTGAACATTGATAAG
GATCTTCTACTCAAATTAGGCTGAAATTAACAAAAACTATTGAGAAATGTAGACTGAGACCTTTTCAAT
CAAACAATATTATTTTCATAACTCAATCAAACCATTAAAACAAATGAATATCAAATGACATTTGCAATT
TAACATACAAGTTGAAACTTGGTTGTAATGATACAATCTTAAATGTCGATAAGTAATAAATAAAAACAT
CACAGAATATGTGAGCATATAATAAATTGAGTCGAATTTAAAATGAAAAGAGAAAACTGATAATCAAAA
GCAGAGCTCGTATTTTTGAGAATAACTAAACATTTTAAAAAGGAAAGAAAGAGAGAAAGAAAAAAAAA
CCCCTAAAAAATATTGTTTACTTAATTAGTTTTAAGATATTTTATATTAGAGATTTCTATACTTCGATC
GATTATCGTGTGCATCATTATATAGCGCTTAATCGTAAAATCAAGCATTACACTTTTTTTCAGTAAAGT
GATACATGATGTAAAGCGCGTATACTATCCTAATTTGTTTTACTACATTGTATTATAGAAAATGATCAA
ATGTATGTTGATTATTAAAGTGAACAAATTTCAGTCCCTTAGGCTCTACATGTAAACAATTATACATTT
TCGTAAAATACCGTATTGTGGATAAAACATAGTTTCGATGTTTAGCTCTATTTCATTTGTGATTGTCACA
TTGAATGTAATTGACGGTGAAGGATTTGGCCTACTATGTAATTTGATTTTCCATGATAGGCCTACACAA
CTGCAATTTTTTATCGAGAATCATACTTCTTGTTCTAACACCAAATTTCCATTCATTTATAAAGCTAT
GATTGGGAAGACACAGACTGACAGTTTCTAATGAAAGACAGCTTTGGCGTTTGAAACGCCGCGGTCAC
AATGACGAGTTATTGTGTCTCTAATCTCTCCTTATGTAATTGACGAATCAGGAGGATCTGTTTATCTTCG
GCACAATTCAAAGTACTTCTTTCCTAAAAGGGCACATTCCCTAATCCTATTGGCTACCTTAAATGACCA
ATAGAAAAGTGCGTCAACGCTGCAGAGAGCGGATAAGTGGTTTCTGTACTCATGGTAATCATTCGCTAA
AGCTGCTAAGTCAATCAACAAGATTAATTATTCGCTCCAAACAAGTAATCACCTATCATCAATCGGTTC
CAACTAGTTATCAGAATG

Unique to *pmar1*


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


A
HesC
Delta
Pmar1
HesC mRNA molecules / embryo
Delta, Pmar1 mRNA molecules / embryo
15000
12000
9000
6000
3000
0
2500
2000
1500
1000
500
0
Egg
2 h
4 h
6 h
8 h
10 h
12 h
14 h
B
8 h
C
12 h
D
12 h
E
12 h
F
8 h
G
12 h
H
12 h
I
12 h

Pmar1 over express



HesC morpholino

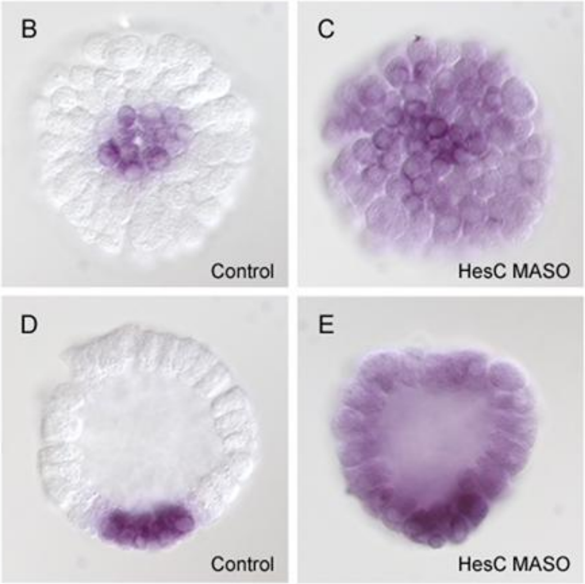


**Figure 5**

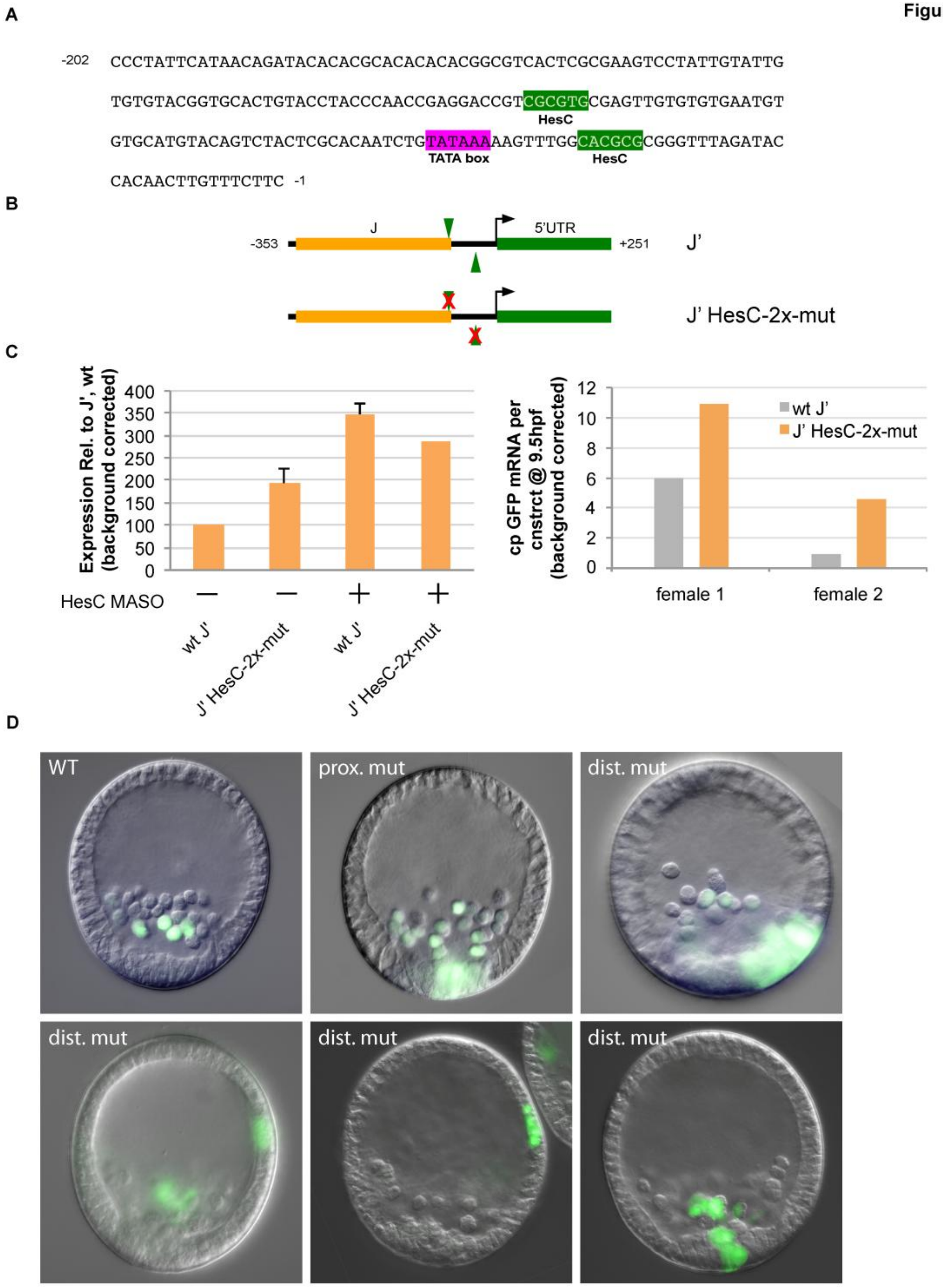
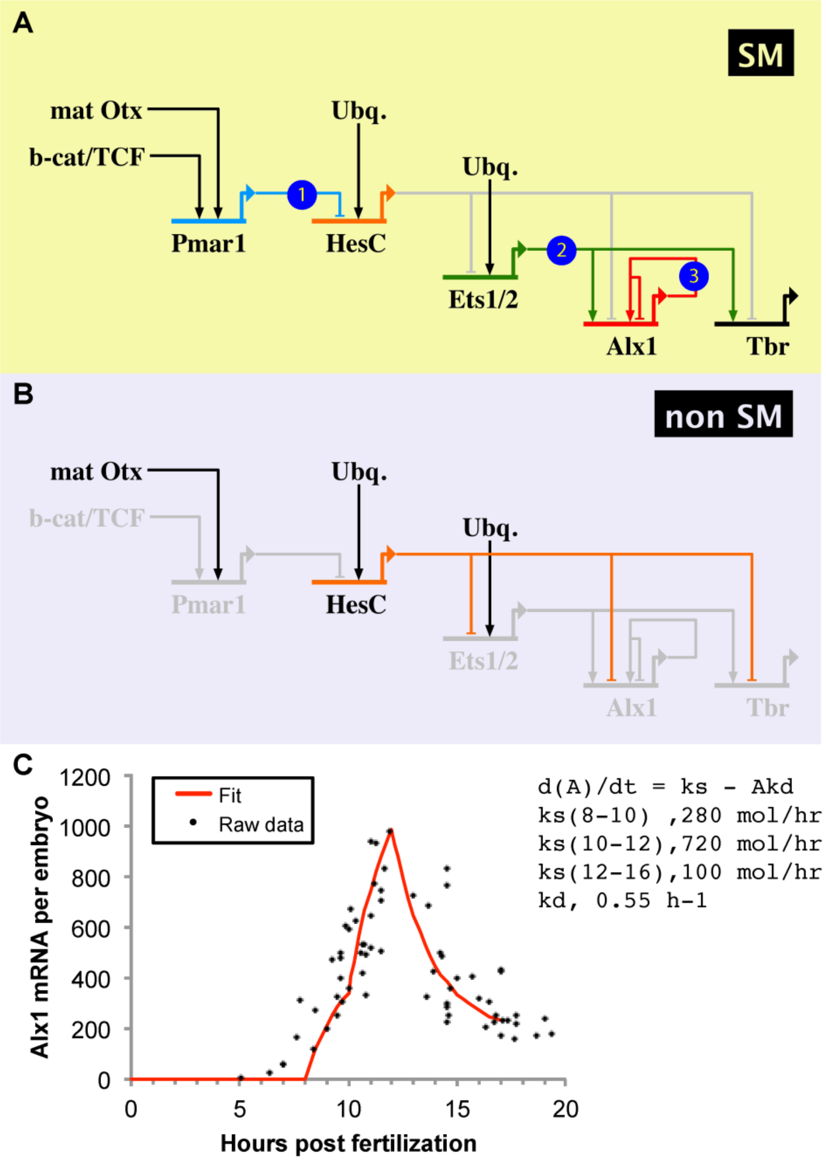
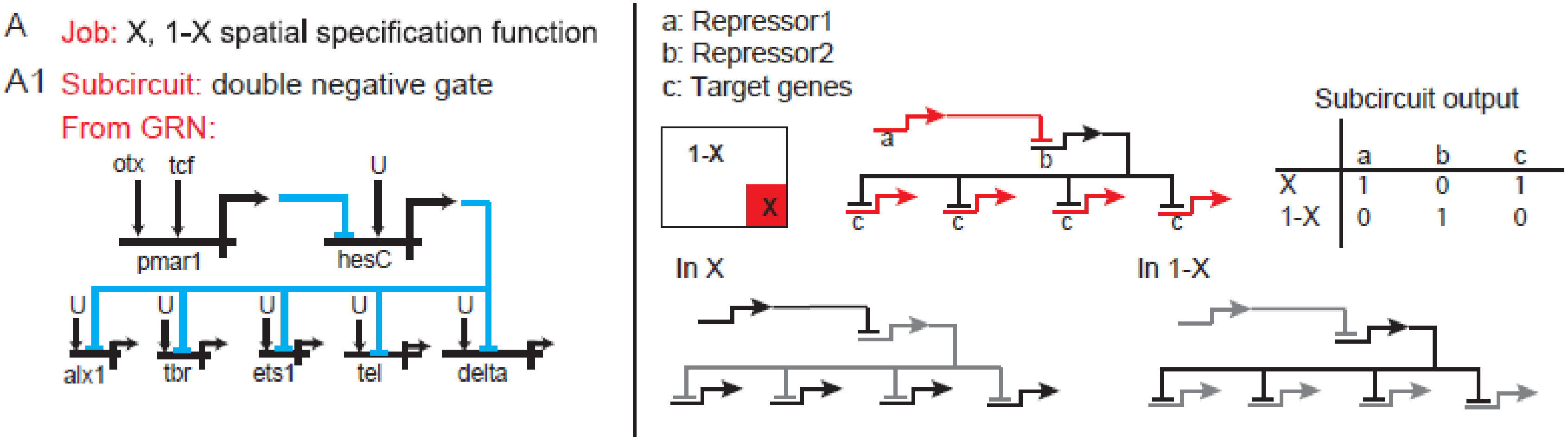

A
-202 CCCTATTCATAACAGATACACACGCACACACACGGCGTCACTCGCGAAGTCCTATTGTATTG
TGTGTACGGTGCACTGTACCTACCCAACCGAGGACCGTCGCGTGCGAGTTGTGTGTGAATGT
HesC
GTGCATGTACAGTCTACTCGCACAATCTGTATAAAAAGTTTGGCACGCGCGGGTTTAGATAC
TATA box
HesC
CACAACTTGTTTCTTC -1
B
J
5'UTR
-353
+251
J'
J' HesC-2x-mut
C
Expression Rel. to J', wt (background corrected)
400
350
300
250
200
150
100
50
0
HesC MASO
—
—
+
+
wt J'
J' HesC-2x-mut
wt J'
J' HesC-2x-mut
cp GFP mRNA per cnstrct @ 9.5hpf (background corrected)
12
10
8
6
4
2
0
wt J'
J' HesC-2x-mut
female 1
female 2
D
WT
prox. mut
dist. mut
dist. mut
dist. mut
dist. mut

Figure 9


A
SM
mat Otx
b-cat/TCF
Ubq.
Ubq.
1
2
3
Pmar1
HesC
Ets1/2
Alx1
Tbr
B
non SM
mat Otx
b-cat/TCF
Ubq.
Ubq.
Pmar1
HesC
Ets1/2
Alx1
Tbr
C
Alx1 mRNA per embryo
1200
1000
800
600
400
200
0
0
5
10
15
20
Hours post fertilization
Fit
Raw data
d(A)/dt = ks - Akd
ks(8-10) ,280 mol/hr
ks(10-12),720 mol/hr
ks(12-16),100 mol/hr
kd, 0.55 h-1

# 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

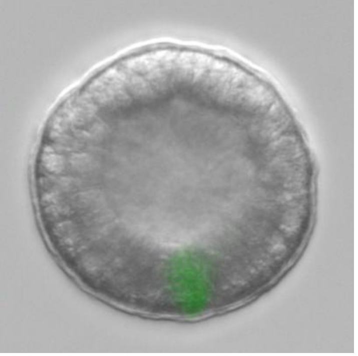
|  | a | b | c |
|---|---|---|---|
| X | 1 | 0 | 1 |
| 1-X | 0 | 1 | 0 |

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

# EXAMPLE: THE DOUBLE NEGATIVE GATE SUFFICES TO PRODUCE A LINEAGE-SPECIFIC REGULATORY STATE, BUT ON TOP OF THAT ARE TWO ADDITIONAL SUBCIRCUITS THAT FUNCTION IN DIFFERENT WAYS TO ENSURE THE OUTCOME:

(1) EXCLUSION OF SKELETOGENIC FATE IN MESODERM (AND EVERYWHERE ELSE) BY CIS-REGULATORY REPRESSION OF PMAR-1

(2) THE SAME INITIAL MATERNAL INPUTS, OTX AND TCF, ARE WIRED INTO ANOTHER GENE, BLIMP1, TO PRODUCE A BACKUP TO THE PMAR1 DOUBLE NEGATIVE GATE



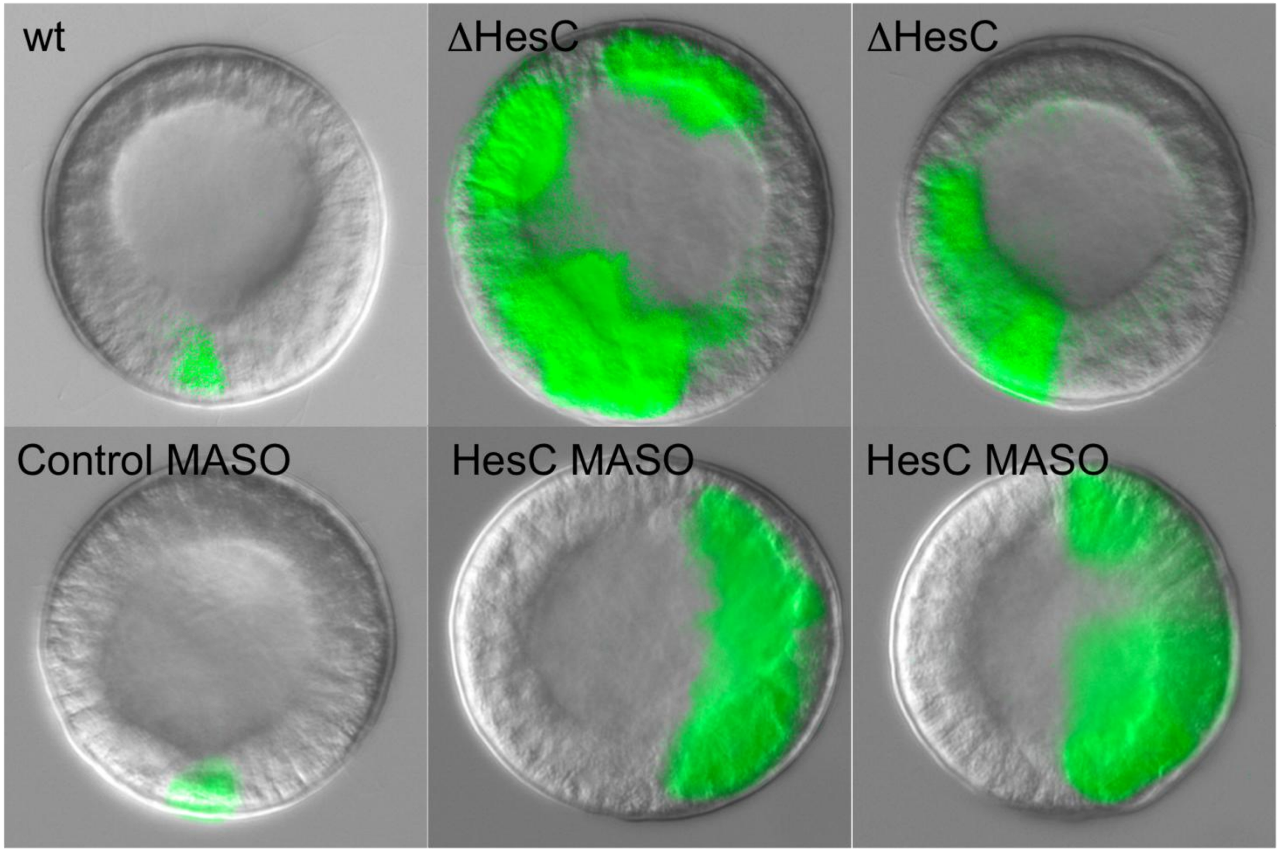
# *PMAR1* REPRESSES *HESC*, BUT, VIA THIS HESC SITE, EVERYWHERE ELSE IN THE EMBRYO HESC SHUTS DOWN *PMAR1*

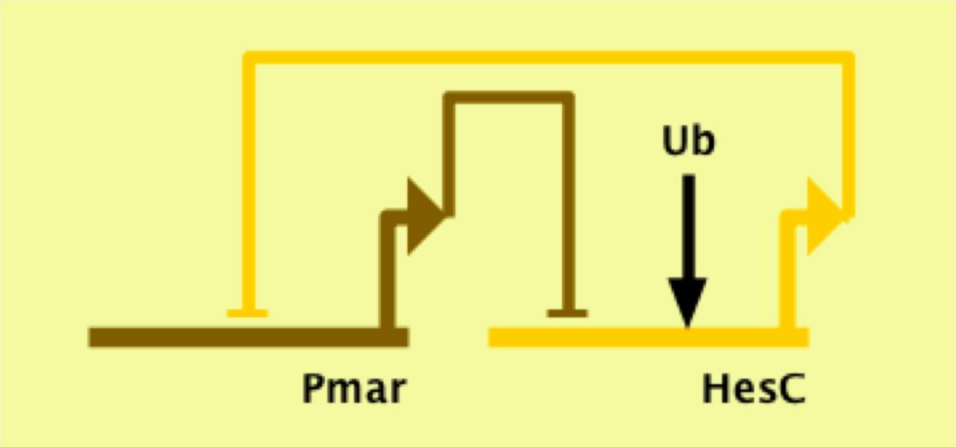
Reporter construction: *pmar1* 2.5 kb reporter

CGTAGTGATCC**TCCGGTTACTCAGCCTTAAT**TGTACATGTAGTGTCTCTGTTGATTCTCCGCCCTATTA
TTCTCTCATTCTCTTTTTTCTAAAATCTTAGTTTCATTCTTTCCCGCTCTTCCCGCGTGAACGGCGTAT
GTTGGGATTATGACAAAAAATGAATTGCGCCCCGCTCAAAAATGTCGCCATAACTGTACAAGCGTGTG
GCAACGCGACCGAGCAGTACTTTTGTTTCTTGTCAAACTGTTAAATACAGTAATTATTATGCATATATTA
ACTAGAGAAAGATTTGCAAGTGAGCACTGCAAACTAGCTGTAGAGTTTCTTAATGTGAACATTGATAAG
GATCTTCTACTCAAATTAGGCTGAAATTAACAAAAACTATTGAGAAATGTAGACTGAGACCTTTTCAAT
CAAACAATATTATTTTCATAACTCAATCAAACCATTAAAACAAATGAATATCAAATGACATTTGCAATT
TAACATACAAGTTGAAACTTGGTTGTAATGATACAATCTTAAATGTCGATAAGTAATAAATAAAAACAT
CACAGAATATGTGAGCATATAATAAATTGAGTCGAATTTAAAATGAAAAGAGAAAACTGATAATCAAAA
GCAGAGCTCGTATTTTTGAGAATAACTAAACATTTTAAAAAGGAAAGAAAGAGAGAAAGAAAAAAAAA
CCCCTAAAAAATATTGTTTACTTAATTAGTTTTAAGATATTTTATATTAGAGATTTCTATACTTCGATC
GATTATCGTGTGCATCATTATATAGCGCTTAATCGTAAAATCAAGCATTACACTTTTTTTCAGTAAAGT
GATACATGATGTAAAGCGCGTATACTATCCTAATTTGTTTTACTACATTGTATTATAGAAAATGATCAA
ATGTATGTTGATTATTAAAGTGAACAAATTTCAGTCCCTTAGGCTCTACATGTAAACAATTATACATTT
TCGTAAAATACCGTATTGTGGATAAAACATAGTTTCGATGTTTAGCTCTATTTCATTTGTGATTGTCACA
TTGAATGTAATTGACGGTGAAGGATTTGGCCTACTATGTAATTTGATTTTCCATGATAGGCCTACACAA

Unique to *pmar1*

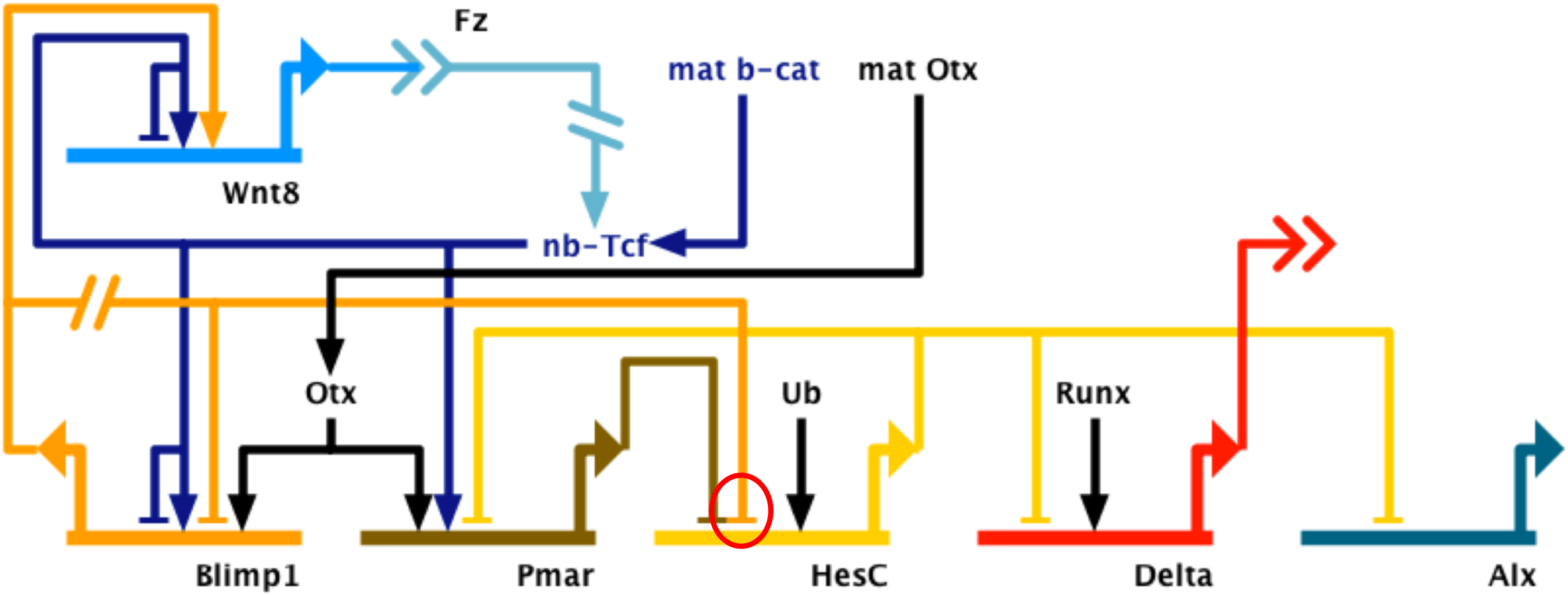
CTGCAATTTTTTATCGAGAATCATACTTCTTGTTCTAACACCAAATTTCCATTCATTCTATAAAGCTAT
GATTGGGGAAGACACAGACTGACAGTTTCTAATGAAAGACAGCTTTGGCGTTTGAAACGCCGCGGTCAC
AATGACGAGTTATTGTGTCTCTAATCTCTCCTTATGTAATTGACGAATCAGGAGGATCTGTTTATCTTCG
GCACAATTCAAAGTACTTCTTTCCTAAAAGGGCACATTCCCTAATCCTATTGGCTACCTTAAATGACCA
ATAGAAAAGTGCGTCAACGCTGCAGAGAGCGGATAAGTGGTTTCTGTACTCATGGTAATCATTCGCTAA
AGCTGCTAAGTCAATCAACAAGATTAATTATTCGCTCCAAACAAGTAATCACCTATCATCAATCGGTTC
CAACTAGTTATCAGAATG



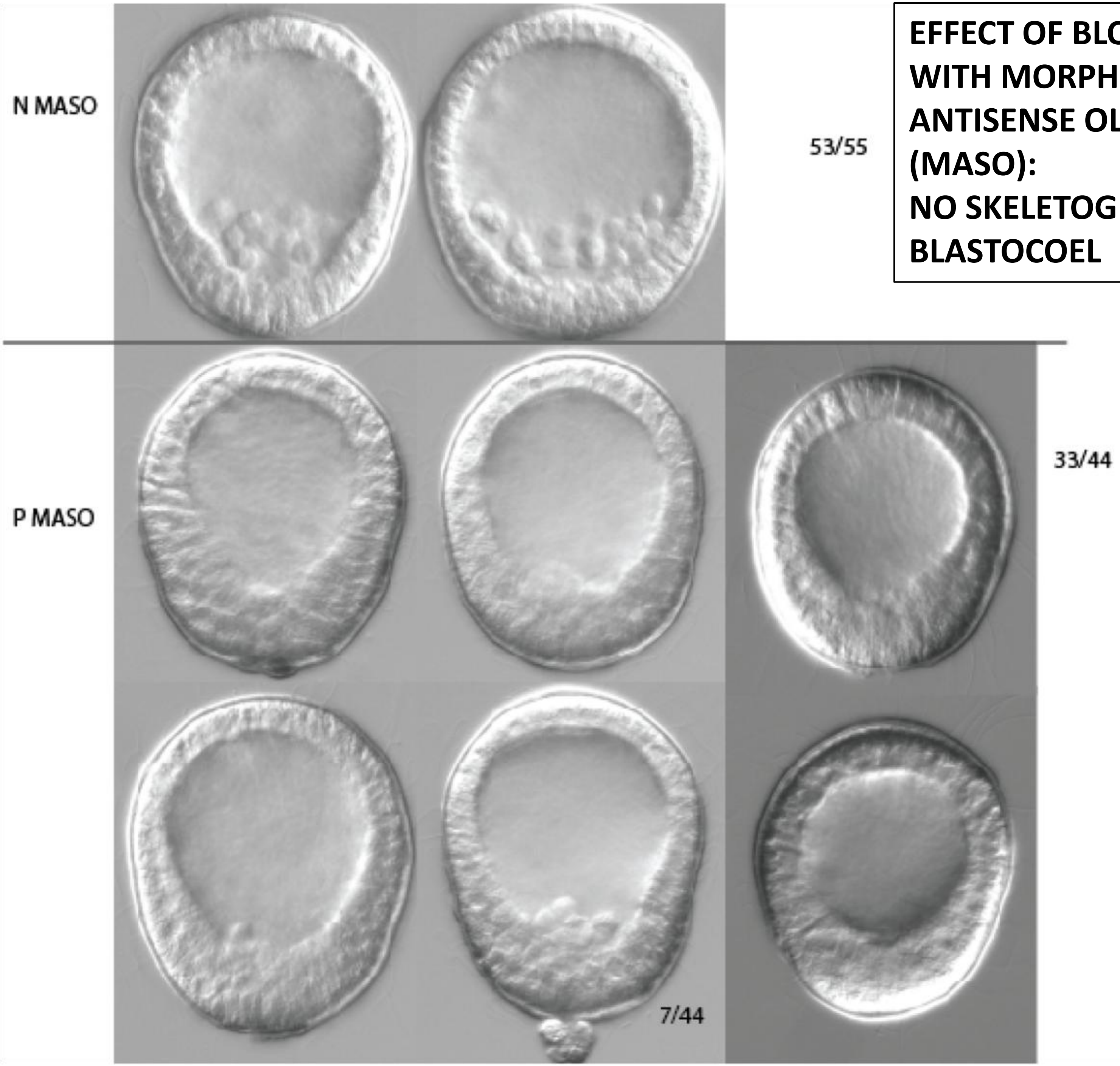


**DESPITE APPEARENCES, NOT A “BISTABLE SWITCH” !**

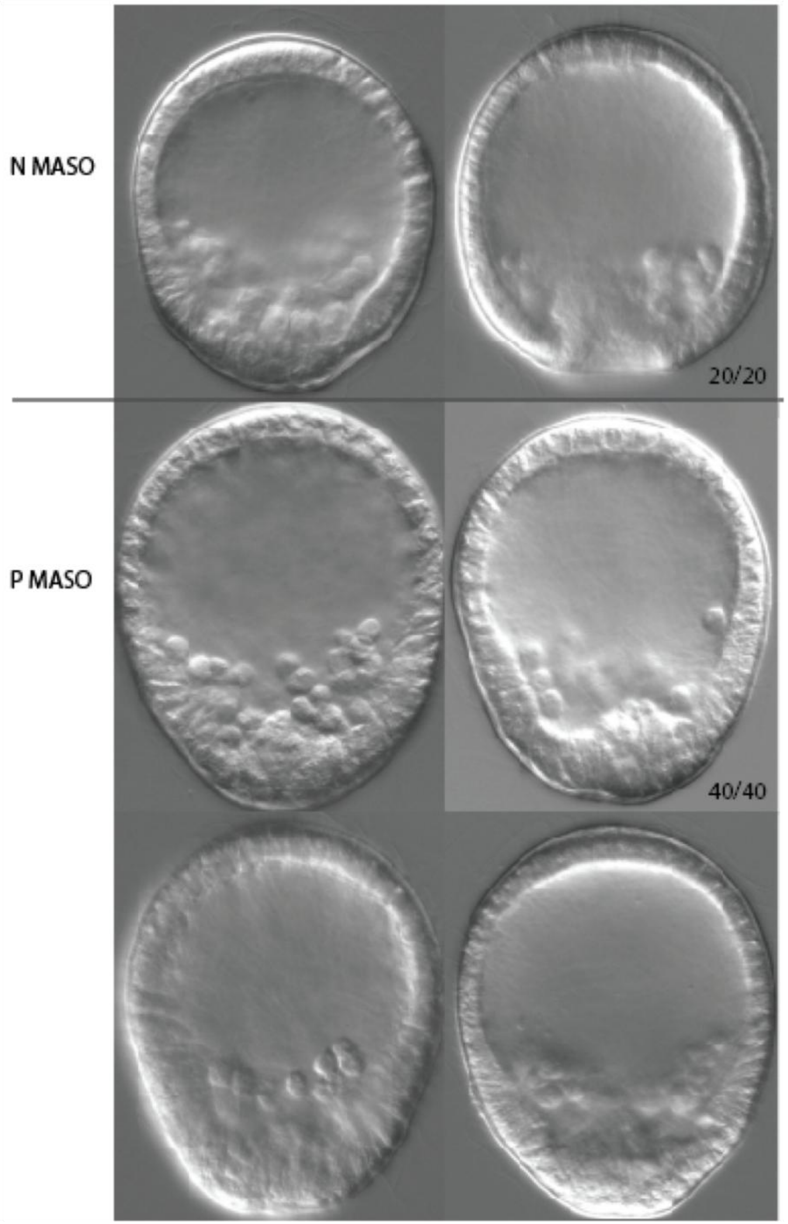
**TEMPORALLY AND SPATIALLY NON-COINCIDENT; SEQUENTIAL AND NON-OVERLAPPING; ENTIRELY DETERMINISTIC**


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

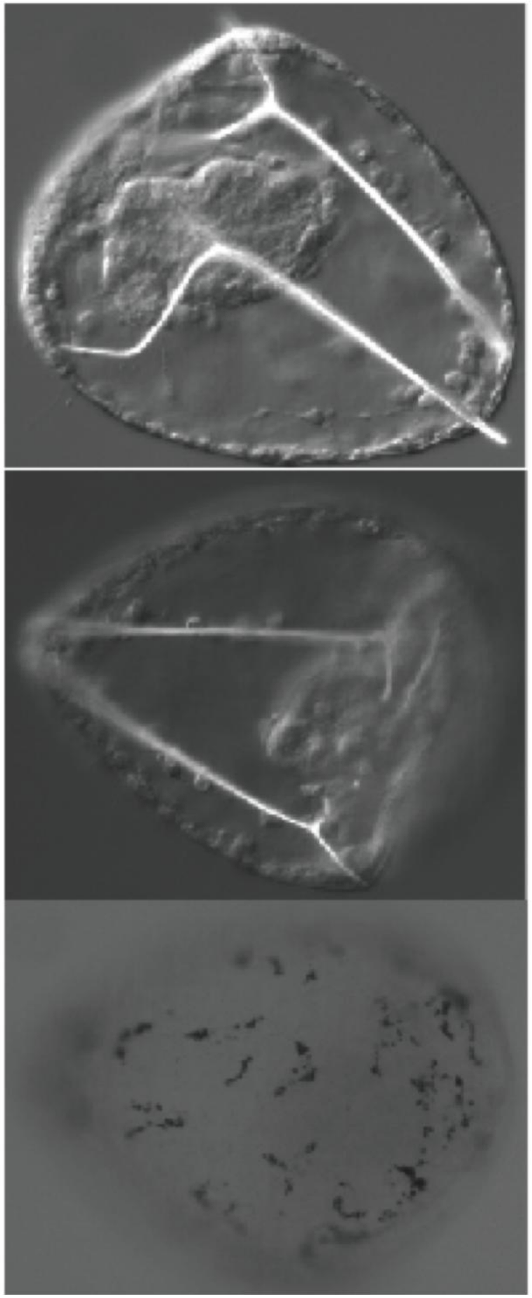


**REGULATIVE RECOVERY!**

72 h

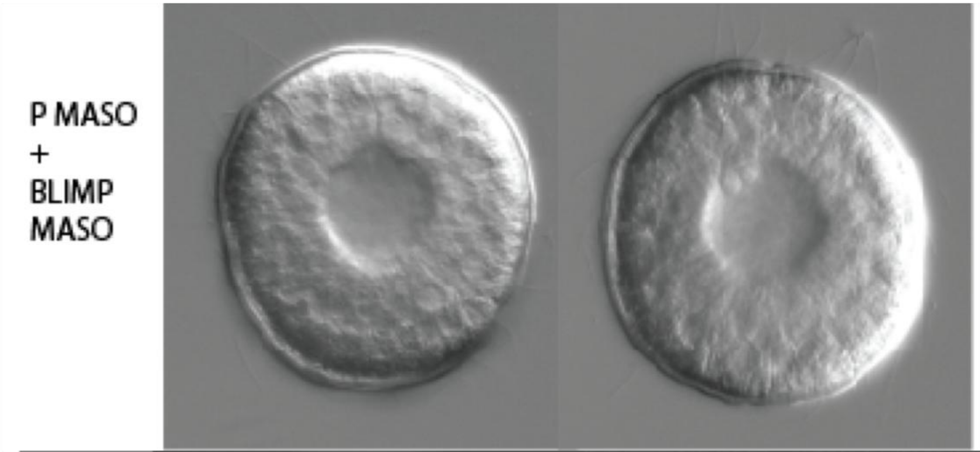
N MASO

P MASO

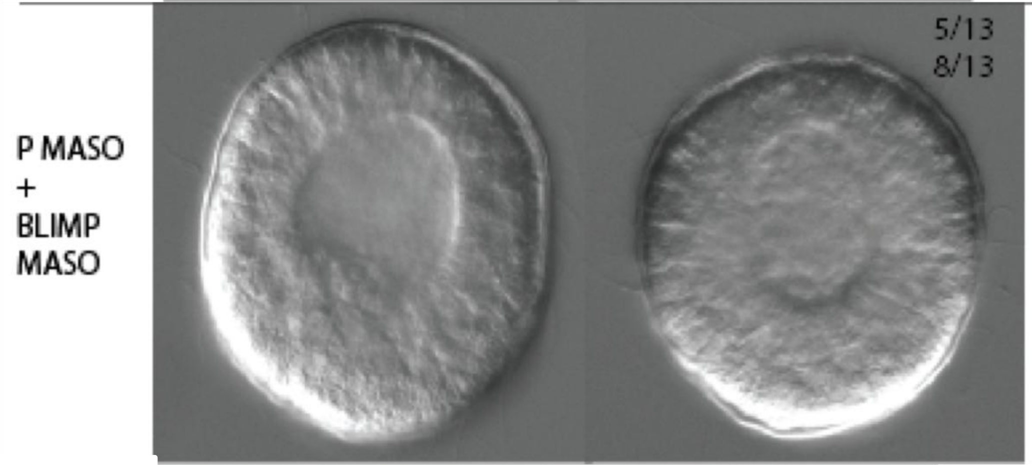


**MINUS FAIL-SAFE LINKAGE: DEVELOPMENTAL DISASTER**

24 h

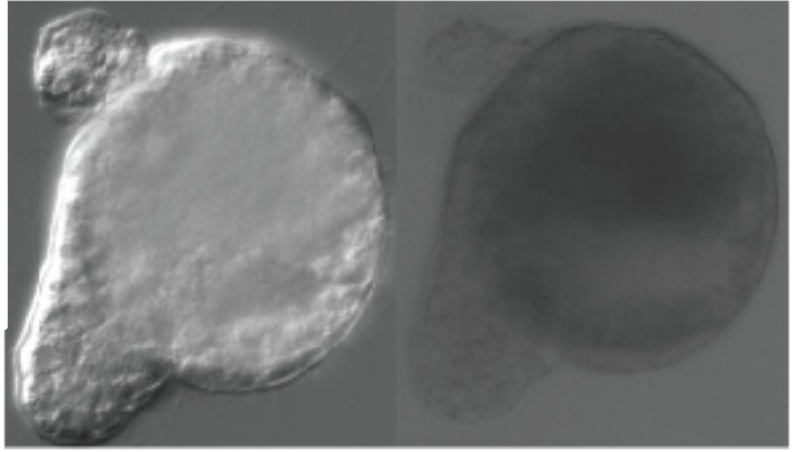


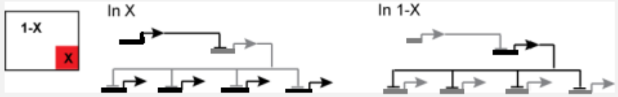
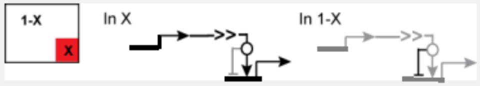
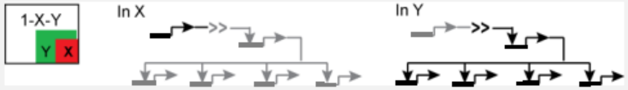
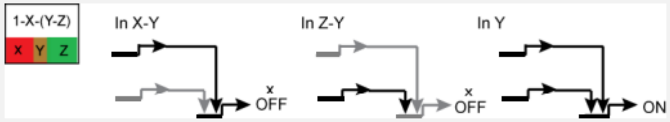
30 h

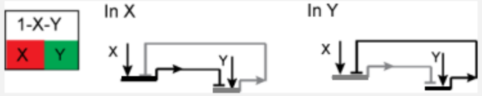
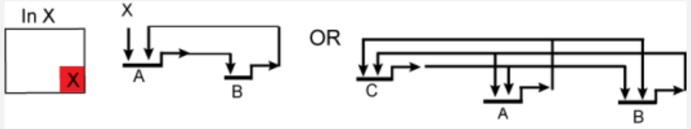
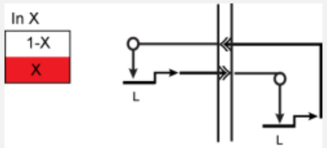


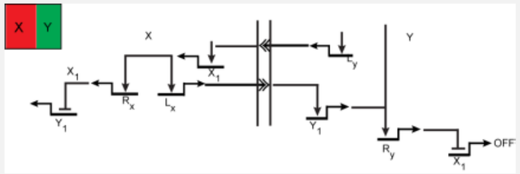
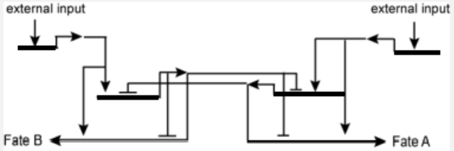
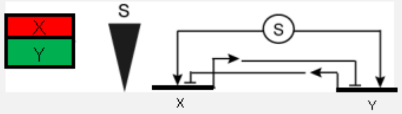
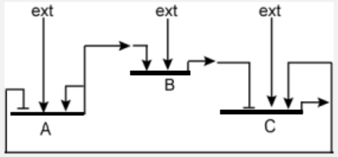
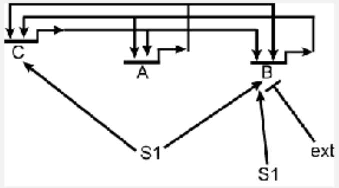
72 h

**P-MASO**
**+**
**BLIMP**
**MASO**



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

| **Regulatory State Specification Function** | **Subcircuits** | **What they do** | **Topologies** |
|---|---|---|---|
| | Spatial repression[2,8]<br>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]<br>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]<br>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]<br>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]<br>4 | Different signals are produced by apposing cells and their reception triggers repressive circuitry excluding the cross-boundary regulatory state. |  <br>i |
| Terminal binary cell fate choice | Alternate subcircuits driven by reciprocal repressors[8,15-18]<br>5 | External inputs tip the balance of repressor expression, resulting in activation of one differentiation program and exclusion of the other. |  <br>j |
| Discontinous transcriptional response to signal intensity and/or duration | Reciprocal repressor genes responding cooperatively to inducer[17,18,19,20]<br>6.1 | Circuitry generates differential stimulation of expression of reciprocal repressors in low vs. high signal intensity[k] |  <br>l |
| | Reciprocal repressor genes, one activating an additional repressor gene, each with variable external positive inputs[21]<br>6.2 | Circuitry generates irreversible transitions, in stem cell regulatory state, off vs. on in response to signals of different strength and duration |  <br>o |
| | Triple feedback linkage with asymmetric signal inputs[22]<br>6.3 | Produces alternative regulatory states, or low level indeterminate state, depending of different positive inputs[m] |  <br>n |

# THE PEOPLE WHO MAKE THE DISCOVERIES

## For this talk:

- ISABELLE PETER: Spatially dynamic Endoderm/Mesoderm specification GRNs; The sox21 project; Evolution of gene regulatory networks, Boolean GRN logic
- 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

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GR