Chapter I

Introduction

Embryogenesis and Transcriptional Regulation in Sea Urchins

Sea urchins as a developmental model system

Historically, sea urchins have been an important developmental model system, and many paradigms have been established using echinoid gametes. In particular, maternal determinants as polarity determinants (Boveri, 1901a; 1901b; Horstadius, 1928) and the existence of maternal mRNA (reviewed by Davidson, 1968) were first demonstrated in sea urchins. More recently, several labs have studied the development of sea urchins at the molecular level, revisiting classical experiments using new tools (Ransick and Davidson, 1998). For many of the processes observed in the late 19th and early 20th centuries, we now have access to the cell-to-cell signaling events and transcriptional regulators responsible for the establishment of asymmetric embryo fates (Oliveri and Davidson, 2004).

It has been known for a century now that sea urchin embryos have the ability to regulate their development when cells are separated up to the four-cell stage (longitudinal cleavage), but that several cell types are missing if embryos are split horizontally (equatorially), separating the animal and vegetal halves (Horstadius, 1939; Brandhorst and Klein, 2002). Animal halves give rise to dauer blastula, which are epithelial balls formed by ciliated and non-ciliated cells. Vegetal halves produce most cell types, and well-patterned albeit smaller-sized embryos.

Echinoderms are a good model system, as they have external fertilization and development allowing for continuous monitoring in sea water. Adults are very fertile, and

a single female can produce over 50 million eggs in a single spawning. More modest numbers of gametes are available year-round from several readily available species throughout the world. Being able to in vitro fertilize the oocytes allows for the synchronous culture of many embryos simultaneously, making the collection of embryological material simple (Leahy, 1986).

Several echinoderm species are amenable to embryological as well as molecular manipulations, including more traditional "cut-and-paste" experiments as well as the exogenous expression of mRNAs, use of antisense oligonucleotide technologies to block translation of mRNA messages in a sequence-specific way, and expression of DNA constructs. A major advantage is the relative ease with which DNA may be injected into the egg. Injected DNA becomes incorporated into the genome and replicates accordingly. This usually occurs within the first few cleavages, giving rise to a mosaic pattern in which only a subset of cells have the exogenous DNA (Hough-Evans et al., 1988; Livant et al, 1991). This requires that many embryos be scored to establish the expression pattern of a construct for a cis-regulatory analysis. However, clonal incorporation has been used as a tool to study cell-autonomous versus non-cell-autonomous use of genes (Rast et al., 2002).

A non-chordate deuterostome model system

Echinoderms are one of two extant non-chordate deuterostome groups, making them the sister group to the chordates. One of several long-lasting questions in

developmental biology involves the origin of morphological changes that lead to new body plans. It is natural that we focus on the origins of our own vertebrate lineage and the chordate body plan. Elucidating commonalities between sea urchins and vertebrates would shed light on ancient developmental processes and pathways. From the study of the three extant deuterostome groups, one could derive a deuterostome tool kit, i.e., what minimum set of genes is necessary to produce the deuterostome body plan. This question can be taken one or many steps further back to the split of the protostomes and the deuterostomes, or back to the origin of metazoans.

Comparative work within the echinoderms involving sea urchins and sea stars has already yielded interesting insights into the regulation of the endomesodermal territory and the origins of novel cell types. A gene regulatory network (GRN) of the endomesoderm was built for *Strongylocentrotus purpuratus* (Davidson et al., 2002a; 2002b) as well as for *Asterina miniata* (Hinman et al., 2003). During the course of the study, a "core" of regulators with conserved connections was found to be conserved across the approximately 500 million years since the split of the two groups. It is clear that there are several differences, but the similarity of a subnetwork involved in endomesoderm territory specification is remarkable. These predicted connections have been further-studied at the cis-regulatory level. Here, the conservation is perhaps even more striking and illuminating, as even though the nucleotide sequences in intergenic regions cannot be aligned, the DNA binding sites themselves are highly conserved with respect to type and number as well as relative distribution of neighboring sites (VF Hinman and EH Davidson, unpublished results).

Sea urchin embryogenesis

Very briefly, embryogenesis in the purple sea urchin occurs over a period of four days, the result of which is the swimming and feeding pluteus larva. Cleavages start out synchronously approximately every hour, initially forming blastomeres of the same size. However, a unique feature of sea urchins within the echinoderm group is apparent during the fourth cleavage. Although the animal half blastomeres cleave equally, the vegetal half blastomeres produce two tiers of daughter cells: 4 larger macromeres, and 4 smaller micromeres. The division immediately after is also asymmetric, as the micromeres divide unequally forming the large micromeres and small micromeres, while the macromeres and mesomeres cleave equally again (see 6 h panel in Figure 1). This initial difference between blastomeres is carried to all descendants and sets up the events from which the different embryonic territories will be derived. A summary of early development through the gastrula stage can be seen in Figure 1. For a detailed description of sea urchin development, see Davidson et al. (1998).

Several unique features of the micromeres and macromeres are discussed in the context of the EM-GRN in the gene regulatory network section. A stage-by-stage description of regulatory states that set up and maintain the cell specification program during embryogenesis is also described in the GRN section.

Studying Gene Regulation in Development

Sequence-specific transcriptional regulators play a central role in the differential expression of genes. These regulatory proteins bind to short specific DNA sequences and can affect the expression of large networks of downstream target genes that are often functionally related. Development of multicellular organisms occurs through many rounds of specification and differentiation of cells. This process is necessarily accompanied by changes in expression, the majority of which are causally linked to transcriptional regulation (Bulyk, 2003).

Different computational methods have been developed to analyze regulatory regions. Perhaps the most common is the transcription factor binding site search, where a position weight matrix is created for each transcription factor and compared to genomic non-coding sequences surrounding the gene being analyzed. High-throughput gene expression profiling allows for clustering of genes based on similar patterns of spatial-temporal expression. These co-expressed genes are more likely to be regulated by a common set of transcription factors than genes not observed to be expressed within these cohorts. Comparing the genomic sequences between different organisms has long been used to help predict coding regions of genes. This method can also be used to predict functional regulatory regions in non-coding sequences. A combination of the three methods listed is often employed during promoter analysis to narrow regions of interest for further analysis. Due to the widespread availability of genomic sequence and gene expression information, approaching gene regulatory networks has become possible (Qiu, 2003).

Gene Regulatory Network (GRN)

Development of multicellular animals has been described at many levels over the past century. Many of those phenomenological observations have been further pursued, while many remain to be elucidated at a mechanistic level.

A much more incisive approach involving perturbations of individual gene products followed by monitoring several putative downstream targets sheds light on the actual connections between genes. A larger framework was required to organize this data and maximize the information obtained while minimizing the effort and materials required for such a project. A list of likely players first needed to be compiled. For this subtractive hybridization screens were performed to isolate genes preferentially expressed in the vegetal plate territory (Ransick et al, 2002). This was further subdivided into mesodermal and endodermal derivatives (Calestani et al, 2003; Rast et al, 2002). A screen for oral ectoderm genes was also performed (Amore et al, 2003). From these and other studies, a list of genes was compiled. The spatial and temporal expression of the genes of this list was established by WMISH and QPCR. The function of regulatory genes was accessed either by morpholino knockdowns or overexpression of wildtype or dominant negative forms. From this, sets of predicted connections were organized into a logic frame that constitutes what we call the endomesoderm gene regulatory network (EM-GRN).

The EM-GRN also integrates data from looking at upstream connections through *cis*-regulatory analysis. Injection of reporter constructs containing genomic sequences with regulatory activities into sea urchin zygotes allows for assessment of their function (Yuh et al., 2002; Revilla-i-Domingo et al., 2004; Minokawa et al., 2005; Amore and Davidson, 2006). Isolation of individual binding sites that can be destroyed with mutations are used to assess the function of individual binding sites (Yuh et al., 2005).

Genomic libraries from several echinoderm species (including *S. purpuratus* and *L. variegatus*) were constructed in bacterial artificial chromosome vectors (BACs) and arrayed so that they could be probed (Cameron et al., 2000). BAC clones containing the genes encoding the chosen transcription factors were then fully sequenced (~140 kilobases) and used for computational analysis to find cis-regulatory elements (Brown et al, 2002, 2005; Yuh et al., 2002; 2004; Revilla-I-Domingo, 2004; Minokawa et al., 2005; Livi and Davidson, 2006b; C. Livi and E. Davidson, unpublished data).

The endoderm and mesoderm layers arise from the vegetal plate territory during initial stages, segregating from the rest of the sea urchin embryo as early as the third meridional cleavage, when the animal half and the vegetal half are first formed. A second important segregation step occurs during the 4^{th} cleavage. The animal half blastomeres divide equally longitudinally, while the vegetal half blastomeres divide equatorially again with the cleavage plane, moved toward the vegetal pole to form a smaller set of blastomeres, the micromeres, at the vegetal end of the embryo. The distinct properties of the two embryonic hemispheres and the micromeres have been known for

one and a half centuries, and modern molecular biological studies have elucidated some of the mechanisms responsible for the differencesbetween them. A predictive model that addresses the causal relationship between asymmetrically-distributed maternal determinants and the transcriptional state of their descendents in the early embryogenesis of purple sea urchin has been built and has been continuously updated over the last four years. Although much remains to be included in this model, several properties of development have been elucidated by this detailed model based on quantitative experimental data (Revilla-i-Domingo and Davidson, 2003, Oliveri and Davidson, 2004, Levine and Davidson, 2005).

A "view from the genome" (Bolouri and Davidson, 2002) that includes genes expressed during all stages in the EM-GRN can be seen in Figure 2. It shows all interactions simultaneously. A view from the nucleus takes into account the spatial temporal expression and shows signaling and transcriptional connections within their restricted territories on an hour-by-hour time scale. A detailed description of the model divided by spatial territories follows. To visualize the hour-by-hour description, please go to http://sugp.caltech.edu/endomes/ and select the BioTapestry Interactive Network Viewer (Longabaugh et al., 2005).

The oocyte and single celled zygote

In sea urchins, it is known that prior to fertilization, there are factors asymmetrically deposited in the egg. It is known that unfertilized as well as immature oocytes are transcriptionally active through most gametogenesis (reviewed by Davidson,

1986; Song and Wessel, 2005). Both proteins and mRNAs can be deposited within the eggs such that they end up asymmetrically distributed in the embryo. A small oocyte-GRN is shown in Figure 3. This is a portion of the basal transcriptional state from which development will proceed.

Upon fertilization, several cytoskeletal dependent processes further partition maternal factors within the fertilized egg. Differently from some other model organisms, sea urchin zygotes immediately initiate transcription, moving it from the oocyte basal transcriptional state to one that will give rise to the specification of cleavage-stage blastomeres to form the distinct territories.

Micromeres / The Primary Mesenchyme GRN

Hnf6, Tbr and Ets are all maternal factors that localize to the micromere territory after the 4th cleavage. Otx is another maternal factor that becomes nuclearized specifically in the micromeres immediately after the first asymmetric cleavage at the 16-cell stage (Chuang et al., 1996). This Otx input, together with the β-catenin/Tcf positive input, drives the expression of *Sppmar1* message in the micromeres by six hpf. The primary function of the *pmar1* gene (Oliveri et al, 2002; 2003) is to de-repress the micromere regulators by repressing a predicted ubiquitous repressor that is likely to be a zinc finger transcription factor. This initiates the expression of a battery of transcriptional regulators that drive the micromere and PMC differentiation gene battery. The restricted activation of *pmar1* exclusively in the micromeres is what determines their fate

differently from other vegetal plate cells that also have β-catenin nuclearization. Interestingly, *krl* is not downstream of the predicted Repressor of Micromeres and appears to be activated by the β-catenin /Tcf pathway and some ubiquitous activators only; however, no function for *krl* expression can be identified in the micromere lineage. *Delta*, and *alx1* message has also started to accumulate in the micromeres by nine hpf, being closely followed by *dri*, and *nrl* at 12 hpf, and a little later *foxB* and *gsc*. Around 15 hpf, the first differentiation genes start being expressed, including *msp-L*, *sm50* and *cyP*. At the same time, some of the very early phase regulators stop being expressed in the micromeres, particularly *pmar1* by 18 hpf (Oliveri et al, 2002). This is the end of the micromere transcriptional program and the beginning of the phase leading up to the ingression of PMCs, which occurs between 21 and 24 hpf. At this time, several other regulators also cease to be expressed in the PMC territory, including *hnf6*, *delta*, *dri* and *gsc*. All of the factors listed are later recruited for other functions during embryogenesis, and are likely to be used yet again afterwards (Figure 4).

During cleavage, the micromere lineage functions as a source of signals that is of central importance to embryogenesis. The Delta signal from the micromeres is essential for the separation of mesoderm fate from endoderm fate.

The micromere GRN does not display the positive regulatory loops observed in the endomesoderm. Instead, a single de-repression event appears to be responsible for the initiation of expression of several important factors that drive the PMC program with ubiquitous activators present. Speculatively, this could reflect the manner by which this

novel cell type was produced during evolution from a micromereless embryo. The basal state within echinoderms lacks a larval skeleton. Thus, the micromeres are a novel cell type, and it is not overly far-fetched to see them as a novel cell type dedicated to the early production of a skeleton.

The perturbation analysis from which the EM-GRN network is derived currently goes until the 30 hpf stages. At this point, many of the factors have more than one function, and distinguishing between them becomes increasingly difficult. However, from the continuing expression of *foxB*, *tbr*, *ets1*, *nrl* and *alx1* in the micromeres, they are likely to continue driving the expression of the skeletogenic mesenchyme program.

Several of the regulators described in the micromere EM-GRN go on to be expressed in other territories. Other regulators known to be expressed in the micromere territory during early development have yet to be linked to any target genes. Many of the genes without a known function within the micromeres follow a similar pattern of expression. They start being expressed in the micromeres, move to the veg2 tier, and are finally expressed in all or part of the veg1 tier of cells. Among them are *blimp1/krox* (the subject of this thesis), *eve*, and *wnt8* (however, *wnt8* does have known targets, as it is upstream of the β-catenin nuclearization pathway which is upstream of several micromere genes and many endomesodermal genes in the GRN).

Macromeres / The Endomesoderm

Endomesoderm – 6-11 hpf

At the fourth cleavage, an asymmetric cell division in the vegetal half of sea urchin embryos forms the macromeres. This tier will produce the secondary mesenchyme, the endoderm and some ectodermal cells. The sixth cleavage further subdivides the micromeres into two tiers: 1) the veg2 more vegetally, and 2) the veg1 more animally.

During the first 11 hpf, the veg2 territory is endomesodermal. A Delta signal from the micromeres between the 7^{th} and 9^{th} cleavage to Notch receptors expressed on the surface of neighboring cells segregates the mesoderm from the endoderm (this is discussed in the mesoderm EM-GRN; Figure 5).

One of the first maternal inputs into the endomesoderm GRN is the nuclearization of β-catenin, which, when bound to Tcf, converts this factor from a repressor to an activator. Therefore, all genes that have binding sites for Tcf in the regulatory regions will be repressed unless nuclear β-catenin is present. The higher level of stabilization of β-catenin in vegetal cells is mediated by disheveled (Weitzel et al., 2004). β-catenin nuclearization appears to occur as waves (Logan et al., 1999). The subsequent "waves" of β-catenin nuclearization are dependent on the secretion of Wnt8 and binding to its receptor, Frizzled. Since *wnt8* is itself downstream of β-catenin /TCF and is a short range ligand, cells that produce Wnt8 receive more Frizzled signaling, creating a community effect (Minokawa et al, 2005).

Another important early input that is operating on all cells before the Delta signal from the micromeres is the Repressor of Micromeres. This factor keeps all micromere genes from being activated in macromere and animal blastomere descendants. This repression of the micromere fate is very important, as the PMC lineage is determined to produce skeletogenic mesenchyme and is not susceptible to changing its potential. As can be observed when *pmar1* is ectopically expressed and the entire embryo transforms itself into PMCs, the ectopic activation of the micromere GRN in the rest of the embryo would have catastrophic consequences, and therefore, the tight regulation of genes that drive this program is essential. Among the target genes of the Repressor of Micromeres is the *delta* gene. A later Delta signal is important for the segregation of endoderm derived from the veg1 tier of cells from the neighboring ectoderm (Figure 6).

Another maternal factor that activates transcription in the endomesoderm is Otx. Although it is initially only nuclearized in the micromeres (Chuang et al., 1996), at later stages, it becomes nuclearized in other cells as well, and is an important transcriptional regulator in multiple territories of the embryo.

Veg2 Mesoderm – 12-30 hpf

The endomesoderm starts as a combined territory, with the same GRN active in the cell nuclei. Between the 7^{th} and 9^{th} cleavage, this changes dramatically when the Delta/Notch signaling pathway is activated. The tier of cells in direct contact with the Delta expressing micromeres has its Notch receptors activated, and this leads to the

activation of the mesoderm GRN program. The first regulatory gene known to be directly downstream of the Notch/Su(H) input is *gcm*, whose expression starts around 12 hpf. After 15 hpf, *gataE* message is activated by the Notch/Su(H) input (Ransick and Davidson, 2002; and unpublished work). I will discuss the role of Delta signaling from the veg2 mesoderm when discussing the specification of the endoderm territory (Figure 7).

As in the micromere GRN, some factors are expressed in the veg2 mesoderm territory without being downstream of the Notch/Su(H) input, such as *foxA* and *blimp1/krox*. These two genes are downstream of Otx and are expressed across veg2 at this time. So, the inputs that drive their expression can not involve a mesoderm-specific signal given by Delta at this time. By 18 hpf, *blimp1/krox* expression is sharply downregulated in the mesodermal territory, and this repression is mediated by the Blimp1/Krox factor itself. It is likely that the difference in the behavior of Blimp1/Krox in the mesoderm and endoderm is due to the expression of different co-regulators, as Blimp1/Krox does not repress its own expression within the veg2 endoderm cells (see Livi and Davidson, 2006a, for data and discussion on *blimp1/krox* negative autoregulation). Another gene that is also downregulated early in the mesodermal territory, but retains its expression in the veg2 endoderm, is *foxA*. This is also due to a negative autoregulatory loop where FoxA represses its own transcription (Davidson et al, 2002; Oliveri and Davidson, 2004).

Otx expression also declines in the mesodermal territory concurrently with *blimp1/krox* expression. *Otx* does not form a positive cross-regulatory loop with *gataE* in the mesoderm as it does in the endoderm. It is possible that Blimp1/Krox is essential for the maintenance of *Otx* expression in this territory and that one of the functions of repressing *blimp1/krox* expression is to turn off *otx* expression as well.

What is referred to as the territorial subnetwork consists of territory-specific transcriptional regulators that either regulate the transcription of other regulators or regulate the transcription of differentiation gene batteries, e.g. structural genes. In the mesoderm territory this includes *gatac*, *gcm* and *not*. *nrl* is also a mesoderm-specific factor that is activated later at 30 hpf.

After 21 hpf, several differentiation genes start being expressed within the mesodermal territory including *sutx*, *capk*, *dpt*, *pks*, *fvmo1,2,3* and *decorin*, most of which as pigment-cell-specific genes. Very soon after (24 hpf), *orct*, *kakapo*, *apobec* and *gelsolin* expression also begins (Calestani et al, 2003).

Several of the genes expressed in the veg2 mesoderm are also expressed in the veg2 endoderm. Another variation in this expression pattern is that some genes are turned off in the veg2 mesoderm once they start being expressed in the veg2 endoderm. Obviously, all of these regulatory functions can be found in their respective cis-regulatory sequences, and can be visualized by studying these inputs in the GRN.

Early Endoderm – Veg2 – 12-17 hpf

blimp1/krox is shown in the first line of regulators. It is upstream of a battery of regulators that represents a territorial subnetwork. As in all embryonic stages studied, Blimp1/Krox is upstream of *wnt8,* driving its expression in the endomesoderm and renforcing the β-catenin pathway that is so important for the specification of vegetal fates. Blimp1/Krox is also a transcriptional activator of *eve*, and *hox11/13b* in the veg2 endoderm (Figure 8).

Endoderm with Veg1 – 18-30 hpf

As in the veg2 mesoderm, *otx* is expressed and activates the transcription of several genes including *blimp1/krox*, *bra*, *foxA*, *gataE* and *hox11/13b*.

As in the early endoderm, *blimp1/krox* is displayed in the first line of regulators, as it is upstream of the territorial subnetwork of regulatory proteins. *blimp1/krox* is again upstream of *eve*, and *hox11/13b* up to 21 hpf, when both of these genes stop being expressed in the veg2 endoderm and turn on in the veg1 endoderm. *blimp1/krox* itself also disappears from the veg2 endoderm and is turned on in veg1 endoderm at 24 hpf, where it is again upstream of *hox11/13b*. Perturbation analysis indicates that Blimp1/Krox isupstream of *eve* in the veg1 endoderm as well, but it is likely that this is an indirect connection mediated through Wnt8.

At 18 hpf, a major feature of the EM-GRN becomes apparent: a positive cross regulatory loop is established between *otx* and *blimp1/krox,* so that Otx activates *blimp1/krox* transcription and Blimp1/Krox activates *otx* transcription. This embrace will remain important for the maintenance of *otx* expression up to 24 hpf, when *otx* forms a positive cross-regulatory loop with *gataE* and the interdependence of *blimp1/krox* and *otx* disappears (Figure 9).

Mesomeres / The Ectoderm

At this time, there is no published network describing the undifferentiated ectoderm during early stages. From work by Coffman and Davidson, it is known that the oral/aboral polarity is specified early, but that the specification of this axis is not fixed (i.e., remaining labile) until the beginning of gastrulation (Coffman and Davidson, 2001; Coffman et al., 2004).

Oral Ectoderm

A limited oral ectoderm gene regulatory network (OE-GRN) has been previously published (Amore et al., 2003).

Aboral Ectoderm

Although one of the first cis-regulatory regions to be characterized in the lab is that of an aboral ectoderm specific gene, *SpcyIIIa* (Kirchhamer and Davidson, 1996), not much is known about the events that lead to the specification and differentiation of this territory. Perturbation analysis linking the regulators known to be expressed specifically in this territory has not been published to date.

Apical Plate Ectoderm

A distinct ectodermal territory that seems to be specified early in development is the apical plate. This zone is located at the animal pole of the embryo, and has a distinct set of transcriptional regulators expressed. A limited AP-GRN has been previously published (Takacs et al., 2004).

GRN as a testable model: Verifying predicted inputs

The network connections displayed are, for the most part, predictions of *cis*-regulatory interactions that can be tested to confirm or refute the linkages shown (Levine and Davidson, 2005). Analysis of regulatory regions of some of the genes from the EM-GRN have been carried out (Yuh et al., 2002; 2004; Revila-i-Domingo et al., 2004; Minokawa et al., 2005; Amore et al, 2006; Livi and Davidson, 2006b; C. Livi and E. Davidson, unpublished data). So far, a significant number of binding sites for the upstream regulators have been found within regulatory elements. Functional analysis has also been carried out, showing that these are necessary for the expression from the regulatory

regions (Yuh et al., 2004; Amore et al, 2006; Takuya et al., 2005; unpublished data from Davidson lab concerning *gatae* and *gcm* genes). This is a necessary step to assure that the connections between genes are indeed direct, and essential to investigate the level of completeness of the network model.

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Figure 1. Diagram of purple sea urchin development. Modified from Davidson et al., 2002a. This drawing shows the cell specification map of sea urchin development up to the pluteus stage. White areas indicate labile regions where specification has not yet occurred.

Fig. 1. Schematic diagrams of *S. purpuratus* embryos displaying specified territories (*10*). Drawings were traced off DIC images of embryos. The color coding shows the disposition of endomesoderm components, and refers also to the network diagrams that follow: lavender, skeletogenic lineage; darker purple, the small micromere precursors of adult mesoderm; light green, endomesodermal veg_2 lineage that later gives rise to endoderm, yellow, and to mesoderm, light blue. Light gray indicates oral ectoderm, darker gray, aboral ectoderm; white indicates regions yet to be specified at the stages shown. 10 h embryo: a median optical section of an early blastula, at about 7^{th} cleavage. 15 h blastula: a similar view, at about 9^{th} cleavage. There is now a single cell-deep ring of mesodermal precursors directly abutting the skeletogenic micromere lineage. 24 h mesenchyme blastula-stage embryo: specification of veg_2 endoderm and of mesodermal cell types completed. 55 h late gastrula stage embryo, about 800 cells: the drawing shows the later disposition of all the endomesodermal cell types about midway through embryonic morphogenesis.

Figure 1

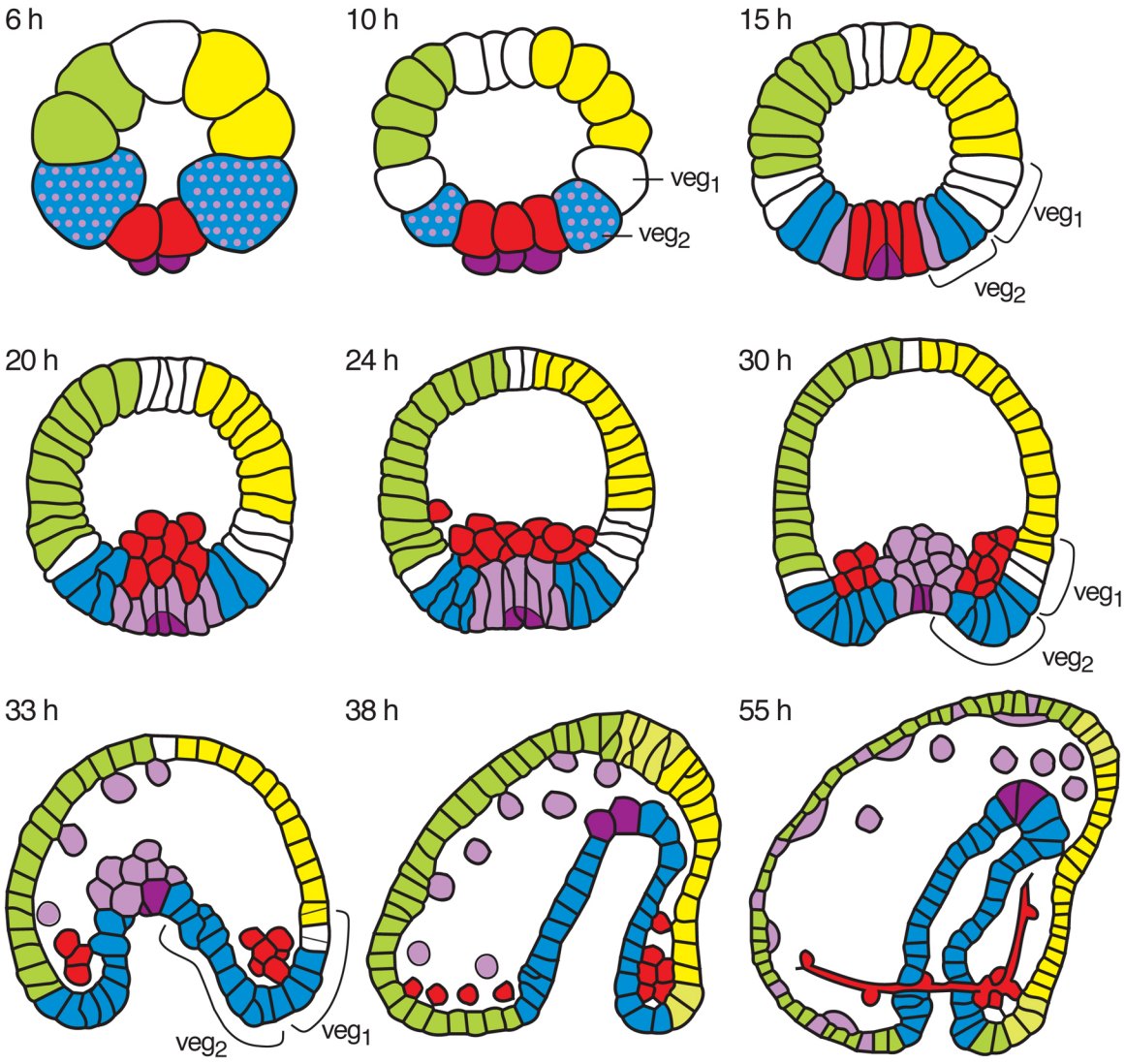
6 h
10 h
veg1
veg2
15 h
veg1
veg2
20 h
24 h
30 h
veg1
veg2
33 h
veg2
veg1
38 h
55 h

Figure 2. A view from the genome EM-GRN. Genes are displayed with their linkages regardless of their spatial temporal expression pattern. It is a view from the perspective of what is encoded in the DNA sequence.

Figure 2

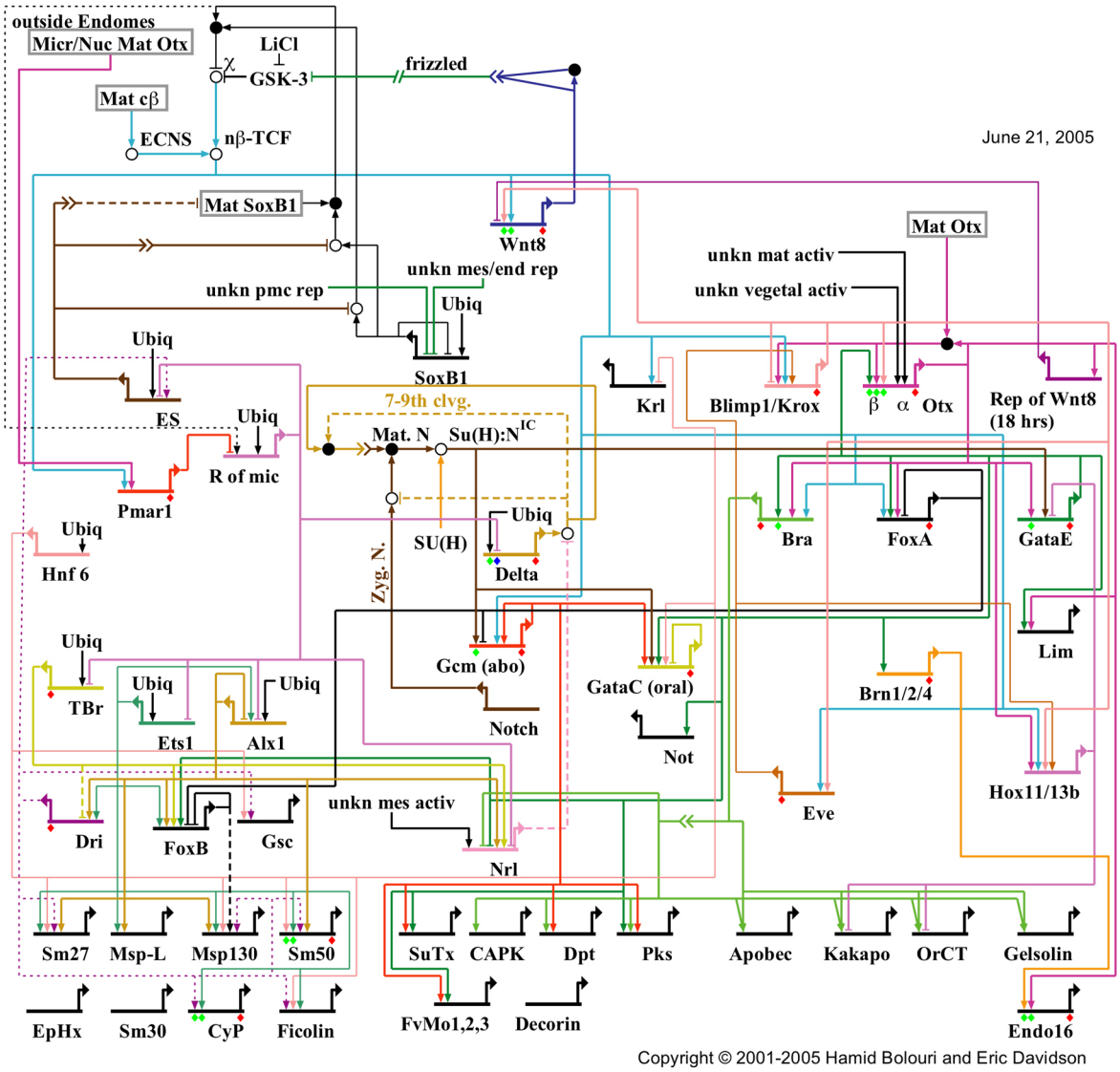
outside Endomes
Micr/Nuc Mat Otx
LiCl
GSK-3
frizzled
Mat cβ
ECNS
nβ-TCF
June 21, 2005
Mat SoxB1
Wnt8
Mat Otx
unkn mat activ
unkn vegetal activ
unkn mes/end rep
unkn pmc rep
Ubiq
SoxB1
Ubiq
ES
Ubiq
7-9th clvg.
Mat. N
Su(H):N^IC
Krl
Blimp1/Krox
β α Otx
Rep of Wnt8 (18 hrs)
R of mic
Pmar1
Ubiq
Hnf 6
SU(H)
Ubiq
Delta
Zyg. N.
Bra
FoxA
GataE
Lim
Ubiq
TBr
Ubiq
Ets1
Ubiq
Alx1
Gcm (abo)
Notch
GataC (oral)
Not
Brn1/2/4
Hox11/13b
Eve
Dri
FoxB
Gsc
unkn mes activ
Nrl
Sm27
Msp-L
Msp130
Sm50
SuTx
CAPK
Dpt
Pks
Apobec
Kakapo
OrCT
Gelsolin
EpHx
Sm30
CyP
Ficolin
FvMo1,2,3
Decorin
Endo16
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Figure 3. The oocyte and single celled zygote EM-GRN. The distribution of maternal factors within the single-celled organism.

Figure 3

June 21, 2005

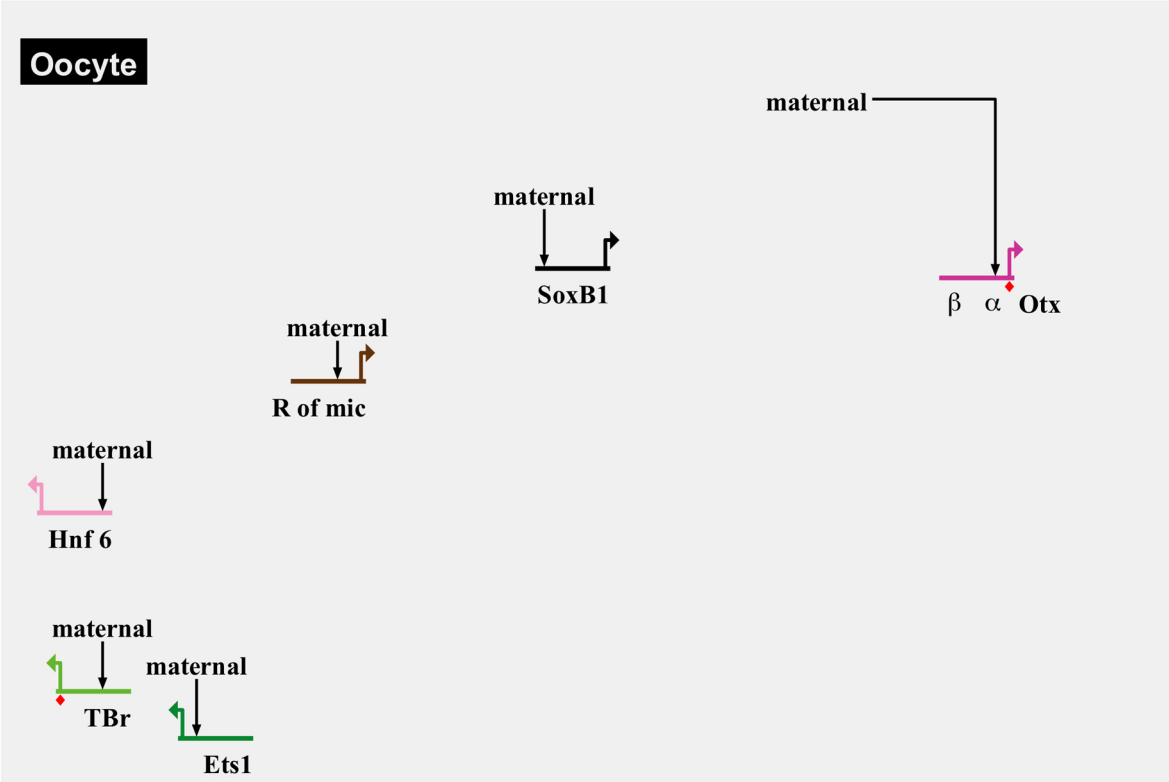

Figure 4. Micromeres / The Primary Mesenchyme EM-GRN. Micromeres, and The Primary Mesenchyme cells (PMCs). (A) cleavage stages (preingression) GRN; (B) blastula stage (post ingression) GRN; larval spiculogenesis GRN.

Figure 4

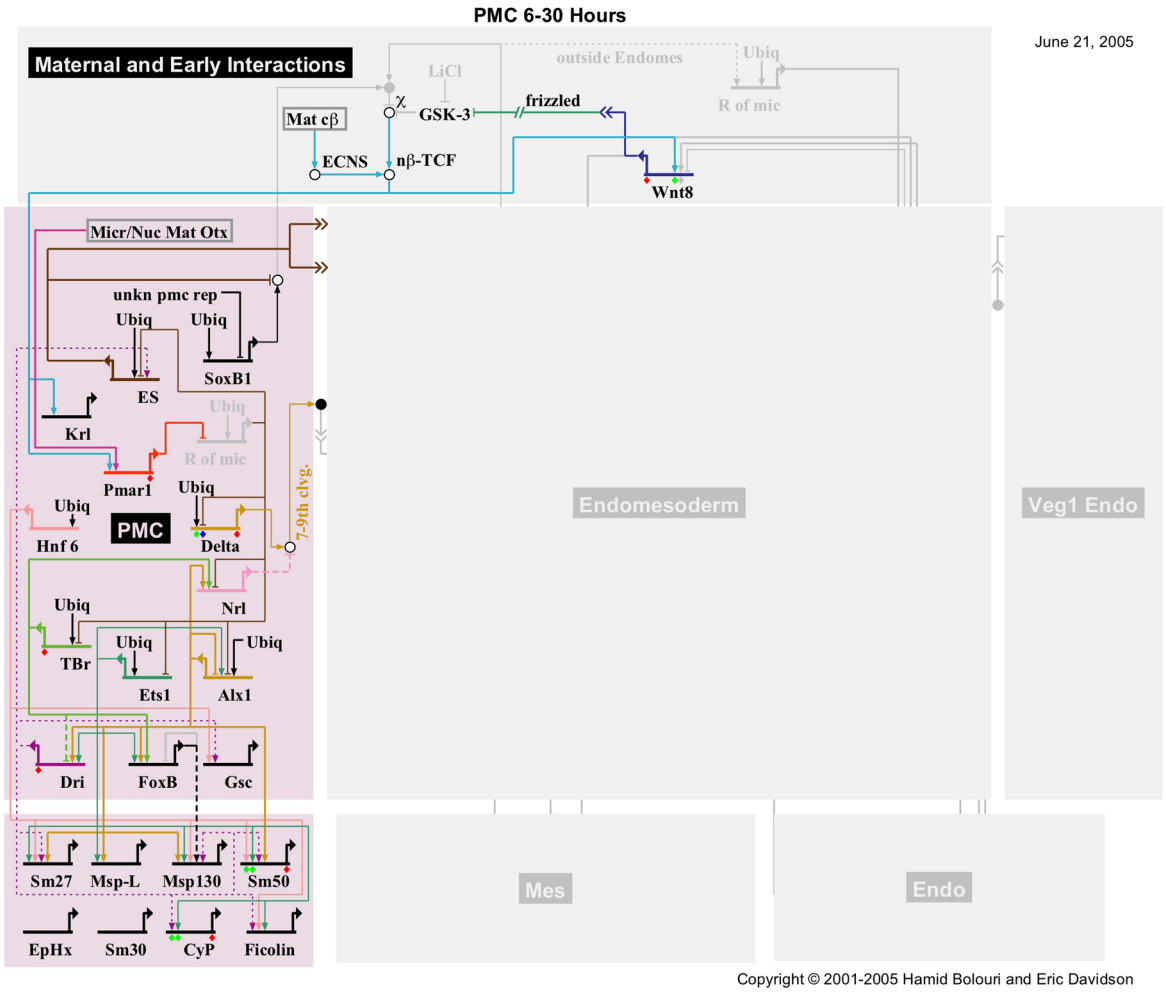
PMC 6-30 Hours
June 21, 2005
Maternal and Early Interactions
LiCl
outside Endomes
Ubiq
R of mic
Mat cβ
GSK-3
frizzled
ECNS
nβ-TCF
Wnt8
Micr/Nuc Mat Otx
unkn pmc rep
Ubiq
Ubiq
SoxB1
ES
Krl
Ubiq
R of mic
Pmar1
Ubiq
Ubiq
PMC
Hnf 6
Delta
7-9th clvg.
Endomesoderm
Veg1 Endo
Ubiq
Nrl
Ubiq
Ubiq
TBr
Ubiq
Ets1
Alx1
Dri
FoxB
Gsc
Sm27
Msp-L
Msp130
Sm50
EpHx
Sm30
CyP
Ficolin
Mes
Endo
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Figure 5. Endomesoderm – 6-11 hpf EM-GRN. Early stages of endomesoderm specification.

Figure 5

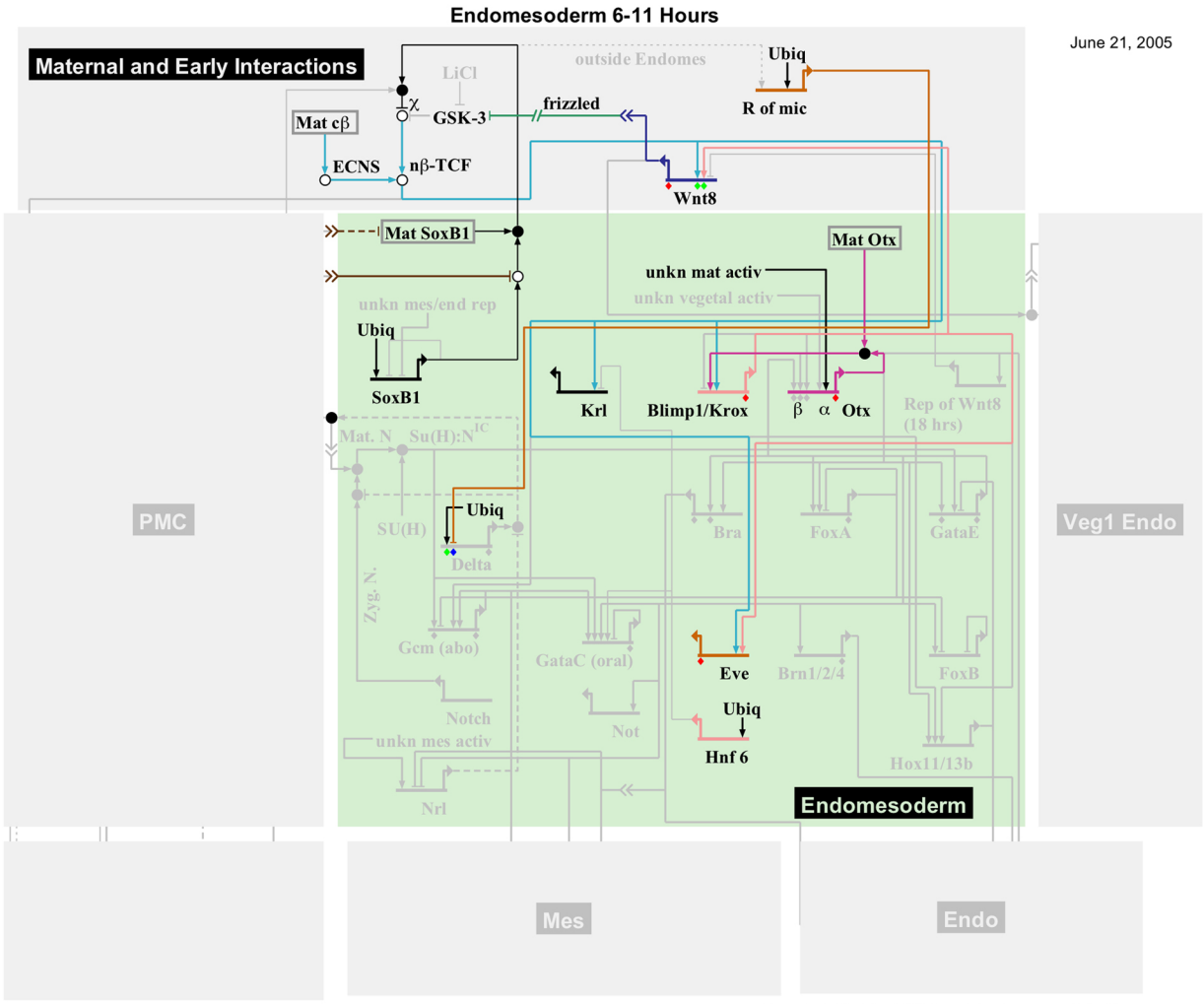
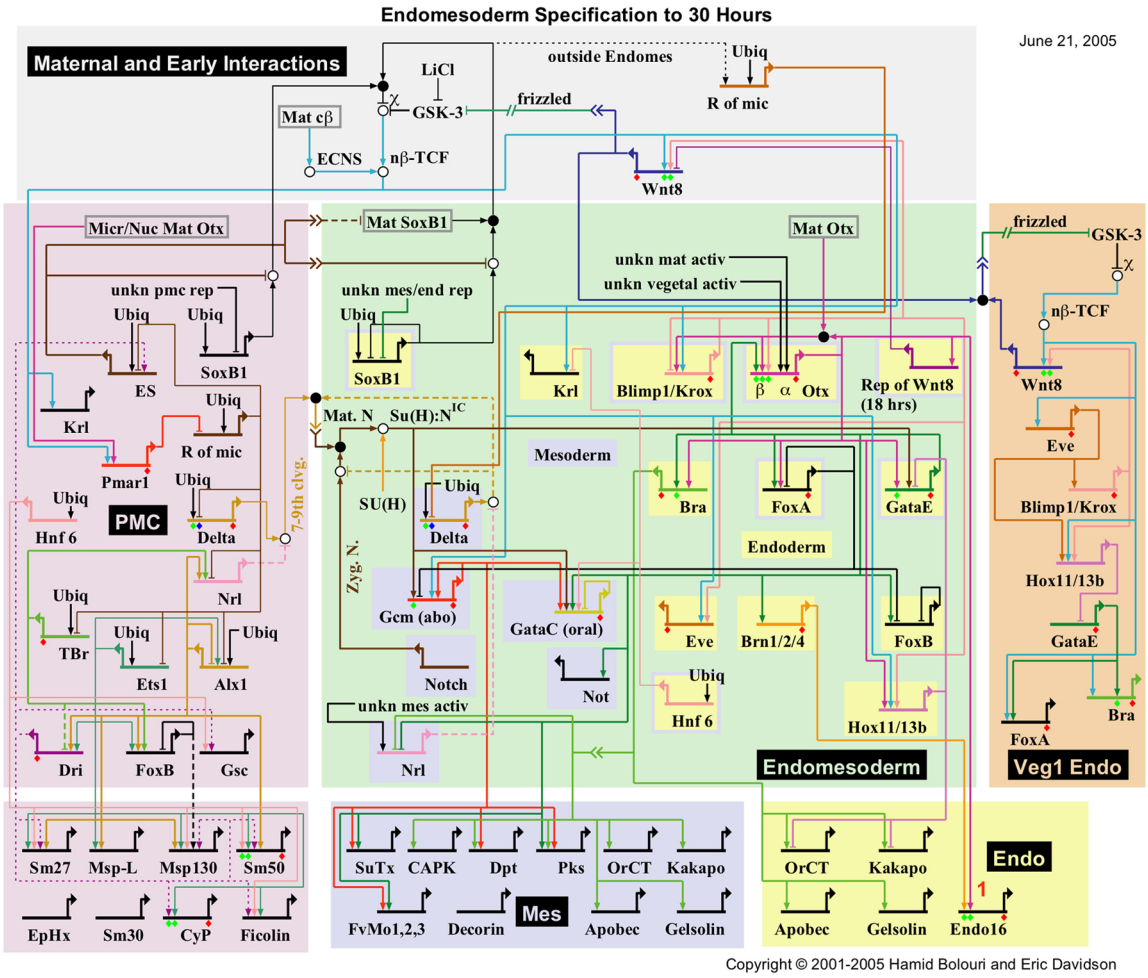


Figure 6. Endomesoderm up to 30 hpf EM-GRN. All endomesoderm interactions up to 30 hpf. See Figures 7, 8 and 9 for an individualized view of the mesoderm and endoderm respectively.

Figure 6


Endomesoderm Specification to 30 Hours
June 21, 2005
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LiCl
GSK-3
Mat cβ
ECNS
nβ-TCF
outside Endomes
Ubiq
R of mic
frizzled
Wnt8
Micr/Nuc Mat Otx
Mat SoxB1
Mat Otx
unkn mat activ
unkn vegetal activ
unkn pmc rep
Ubiq
SoxB1
ES
Krl
Pmar1
R of mic
PMC
Hnf 6
Delta
Nrl
TBr
Ets1
Alx1
Dri
FoxB
Gsc
7-9th clvg.
unkn mes/end rep
Mat. N
Su(H):N^IC
SU(H)
Zyg. N.
Gcm (abo)
Notch
unkn mes activ
Nrl
Mesoderm
Krl
Blimp1/Krox
Otx
Rep of Wnt8 (18 hrs)
Bra
FoxA
GataE
Endoderm
GataC (oral)
Not
Eve
Brn1/2/4
FoxB
Hnf 6
Hox11/13b
Endomesoderm
frizzled
GSK-3
nβ-TCF
Wnt8
Eve
Blimp1/Krox
Hox11/13b
GataE
Bra
FoxA
Veg1 Endo
Sm27
Msp-L
Msp130
Sm50
EpHx
Sm30
CyP
Ficolin
SuTx
CAPK
Dpt
Pks
OrCT
Kakapo
FvMo1,2,3
Decorin
Mes
Apobec
Gelsolin
OrCT
Kakapo
Apobec
Gelsolin
Endo16
Endo
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Figure 7. Veg2 Mesoderm – 12-30 hpf EM-GRN. Veg2 macromere descendants in direct contact with the micromeres receive a Delta signal that is responsible for segregating the mesoderm away from the endoderm.

Figure 7

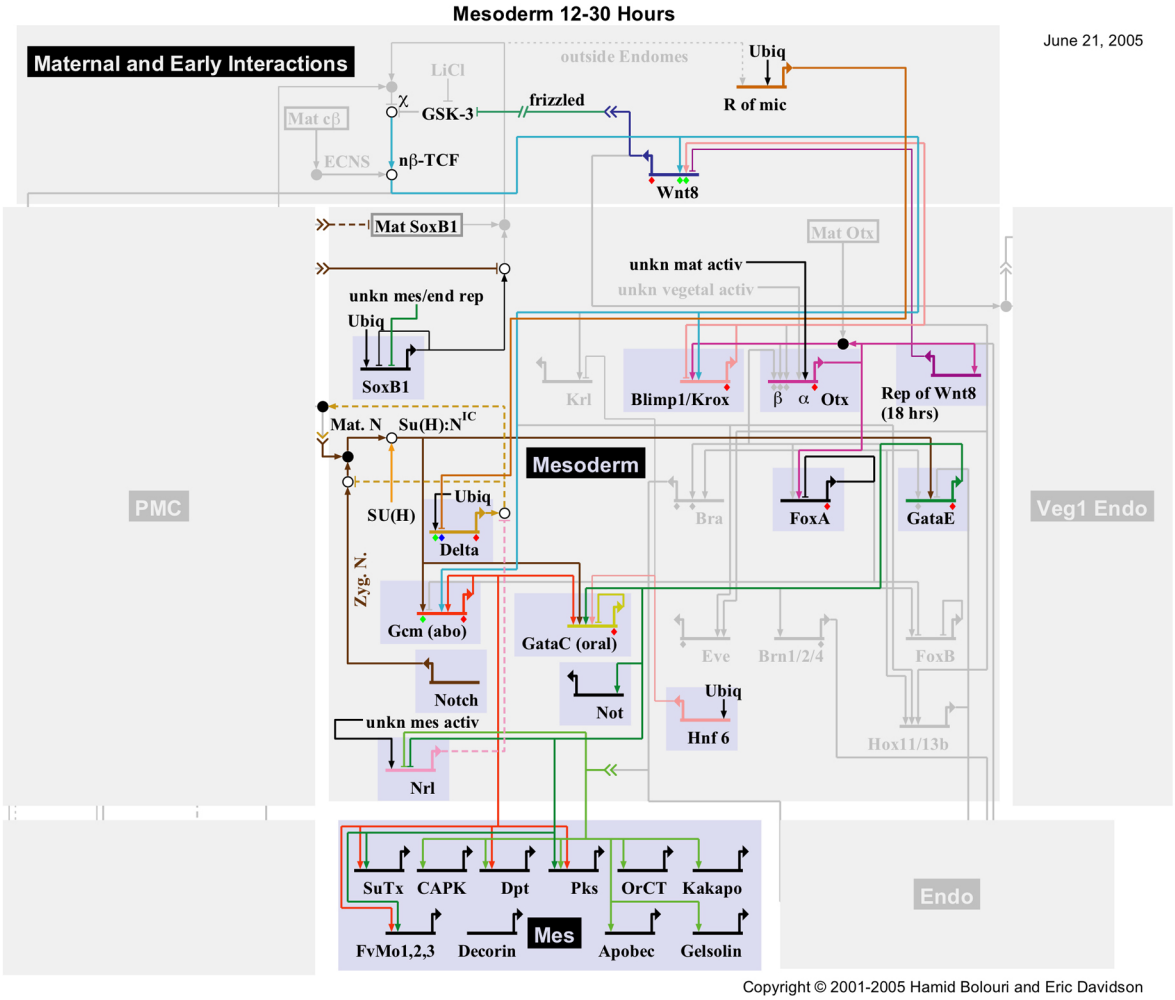
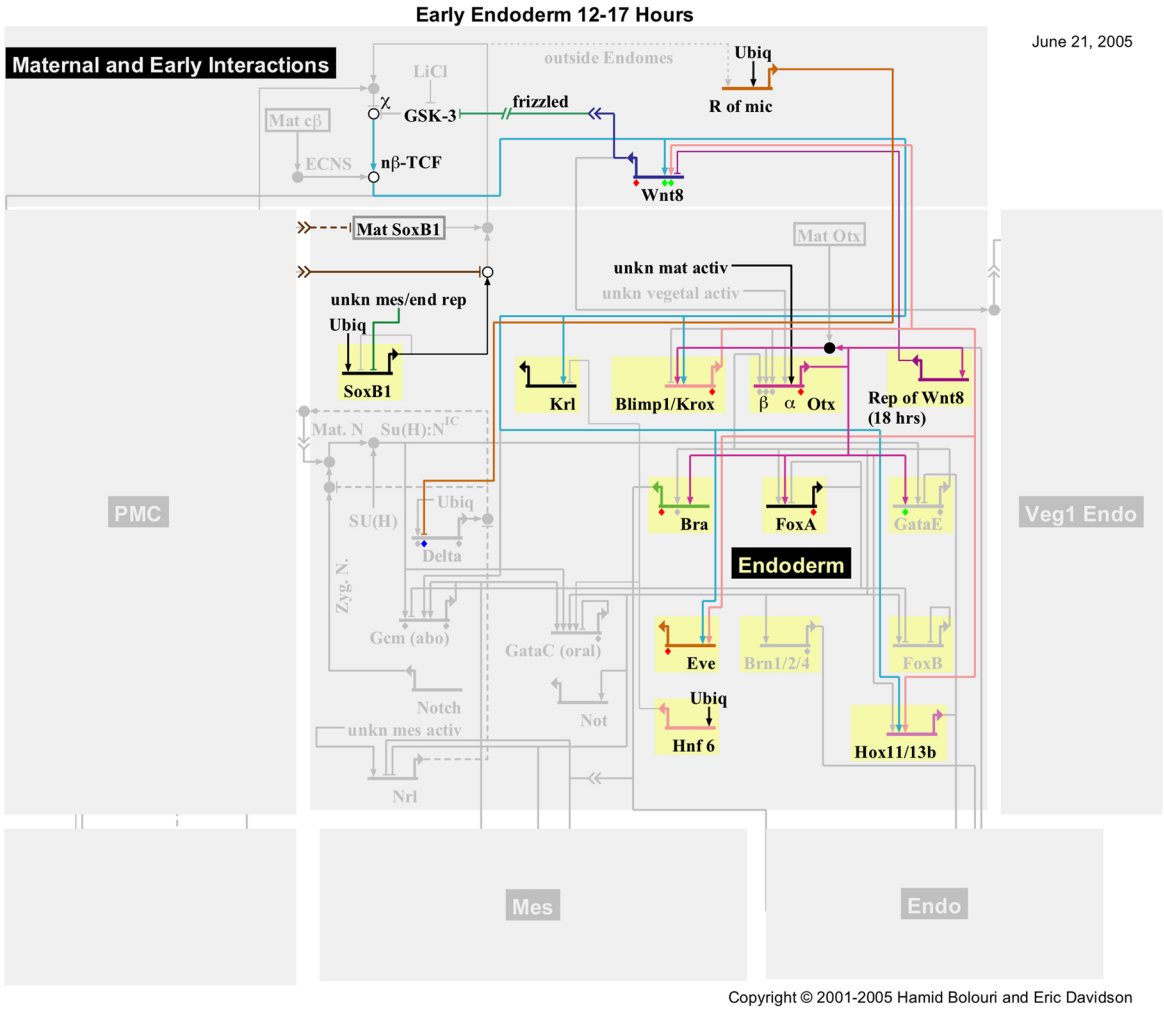


Figure 8. Early Endoderm – Veg2 – 12-17 hpf EM-GRN. Veg 2 macromere decendants that do not receive Delta signal give rise to the veg 2 endoderm, which will form the foregut and midgut of the gastrula-stage embryo.

Figure 8


Early Endoderm 12-17 Hours
June 21, 2005
Maternal and Early Interactions
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outside Endomes
Ubiq
R of mic
Mat cβ
GSK-3
frizzled
ECNS
nβ-TCF
Wnt8
Mat SoxB1
Mat Otx
unkn mat activ
unkn vegetal activ
unkn mes/end rep
Ubiq
SoxB1
Krl
Blimp1/Krox
β α Otx
Rep of Wnt8
(18 hrs)
Mat. N
Su(H):N IC
PMC
SU(H)
Ubiq
Delta
Zyg. N.
Bra
FoxA
GataE
Veg1 Endo
Endoderm
Gcm (abo)
GataC (oral)
Eve
Brn1/2/4
FoxB
Notch
unkn mes activ
Not
Ubiq
Hnf 6
Hox11/13b
Nrl
Mes
Endo
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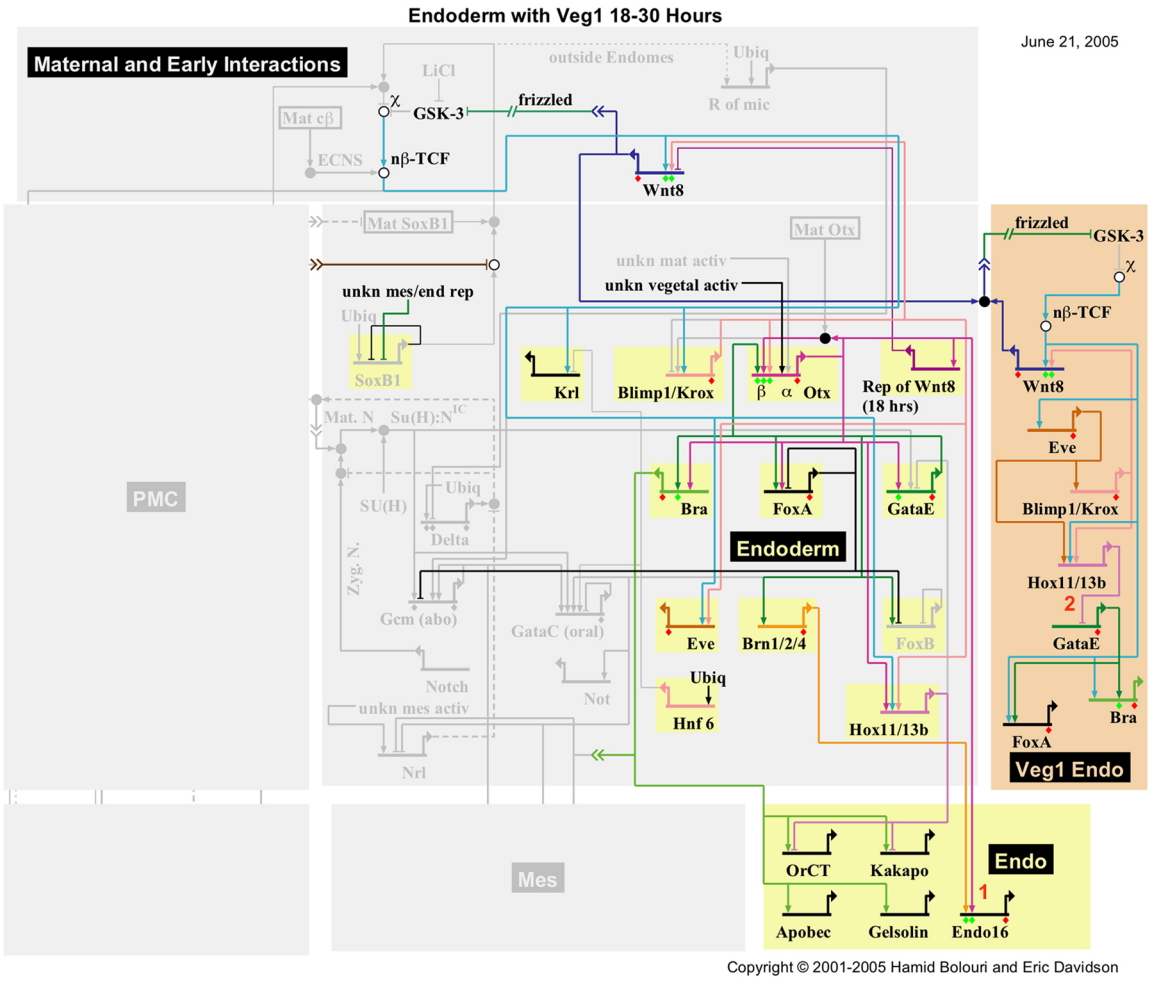
Figure 9. Endoderm with Veg1 – 18-30 hpf EM-GRN. The Veg 1 macromere descendents will form endoderm as well as ectoderm. The genes described within the veg 1 endoderm are responsible for recruiting these cells to form portions of the hindgut of the gastrula-stage embryo.

Figure 9


Endoderm with Veg1 18-30 Hours
June 21, 2005
Maternal and Early Interactions
LiCl
outside Endomes
Ubiq
R of mic
Mat cβ
GSK-3
frizzled
ECNS
nβ-TCF
Wnt8
Mat SoxB1
Mat Otx
unkn mat activ
unkn vegetal activ
unkn mes/end rep
Ubiq
SoxB1
Krl
Blimp1/Krox
β α Otx
Rep of Wnt8
(18 hrs)
Mat. N
Su(H):N^IC
PMC
SU(H)
Ubiq
Delta
Zyg. N.
Bra
FoxA
GataE
Endoderm
Gcm (abo)
GataC (oral)
Eve
Brn1/2/4
FoxB
Ubiq
Notch
Not
Hnf 6
Hox11/13b
unkn mes activ
Nrl
Mes
OrCT
Kakapo
Endo
Apobec
Gelsolin
Endo16
1
frizzled
GSK-3
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Wnt8
Eve
Blimp1/Krox
Hox11/13b
2
GataE
Bra
FoxA
Veg1 Endo
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