Uncovering the regulatory networks of gastrulation: A systems biology approach

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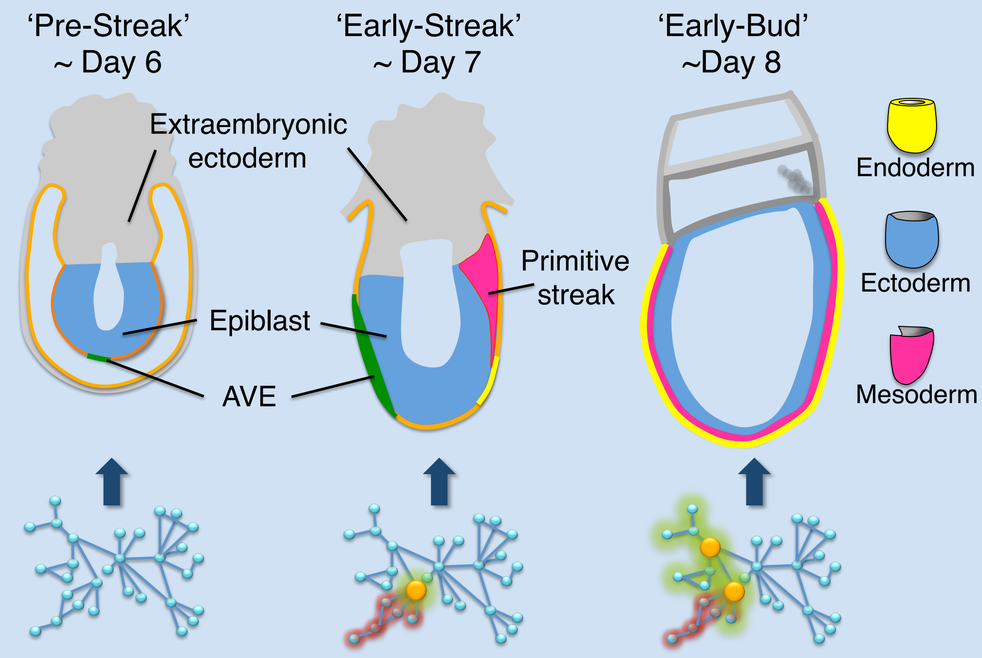
INTRODUCTION

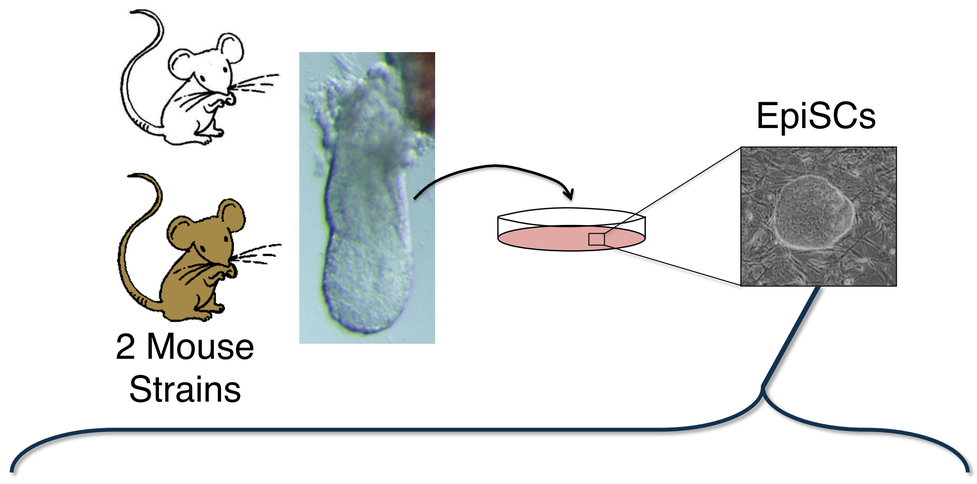
Gastrulation is the complex process during embryogenesis by which pluripotent epiblast cells give rise to the three primary germ layers: endoderm, ectoderm, and mesoderm. Despite extensive investigation of pre-gastrulation and gastrulation stages of mammalian development, relatively little is known about the regulatory network that controls these critical stages of development. Indeed, the known signaling events and master regulators (MRs) - genes that serve as central control points of the regulatory network for gastrulation - have generally been identified by mutational analyses and reverse-genetic approaches. To date, an unbiased methodology that is not dependent upon prior knowledge from the literature has not been used to identify a regulatory network ('interactome') or master regulators. Here we present a novel and innovative systems-based approach for modeling the genetic regulatory network for gastrulation.



Regulatory network and MRs implementing morphogenetic changes?

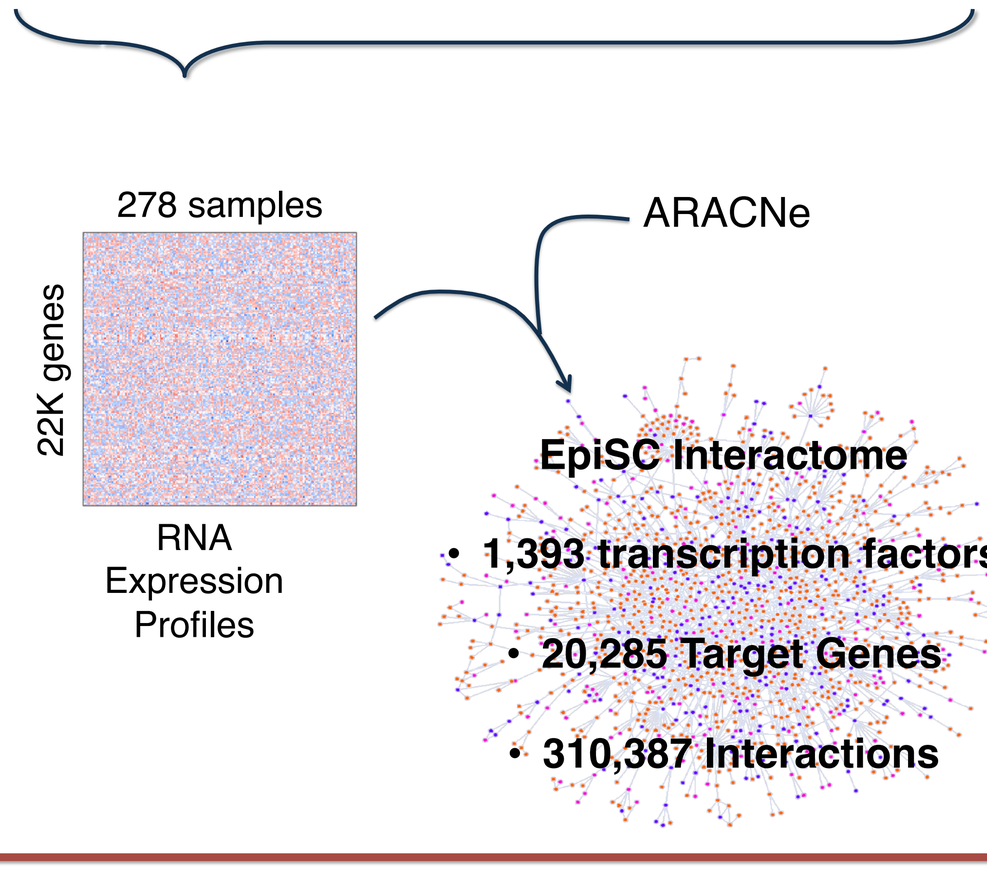
1. Constructing the first epiblast stem cell interactome

Epiblast stem cells (EpiSCs) are a pluripotent cell type that are derived from (and resemble) pre-gastrulation mouse epiblast tissue *in vivo*. Hence, we used ARACNe (Algorithm for the accurate reconstruction of cellular networks) to construct an unbiased interactome for mouse EpiSCs, which we hypothesized can be used to model the regulatory network for the epiblast at pre-gastrulation and gastrulation stages.



Small molecule 'perturbagen'

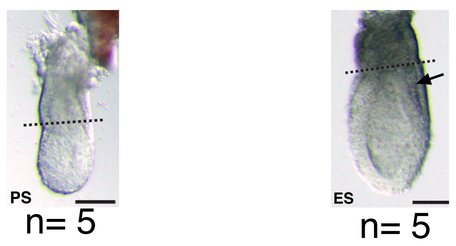
Morphogen			
BMP4	10058-F4	Forskolin	Olomoucine
Embryod body	(5Z)-7-Oxozeaenol	Genistein	Purmorphamine
PD0325901	BAY-11-7082	IDE1	Rapamycin
Retinoic acid	BIO	Indolactam	SB202190
SB431542	Bix01294	IPA-3	STAT3_inh
	CHIR99021	IWP-2	SU5402
	DAPT	KAAD-Cyclopamine	Telomerase_inh
	Dexamethasone	KT5720	Valproic_Acid
	Docetaxel	LDN-193189	wnt_agonist
	Dorsomorphin	LIF	wortmannin
	FGF2	Locostatin	Y-27632



2. Systematic discovery of regulatory events of mouse epiblast during gastrulation.

We used MARINa and FIRE algorithms to interrogate the EpiSC interactome with gene expression signatures corresponding to mouse epiblast tissue of different stages of gastrulation *in vivo.* MARINa and FIRE identify candidate MR genes and active RNA/DNA binding sites that putatively regulate the transition between two states, represented by the expression signatures.

Pre-streak ------> Early streak



n= 5 n= 5

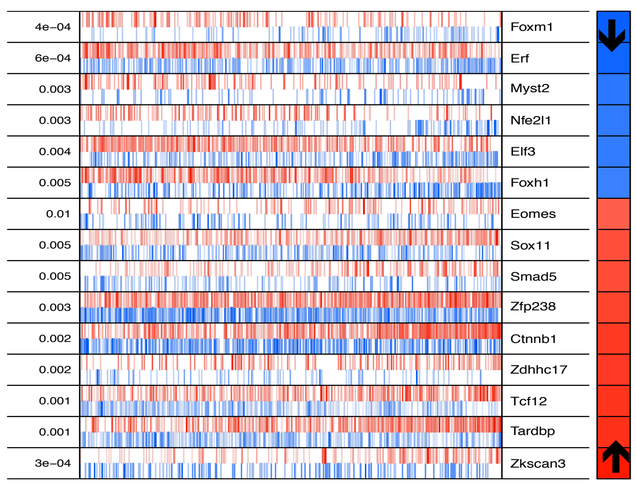
(4 pooled epiblasts per sample)

MARINa (Master Regulator Inference Agorithm)

RNA Seq

FIRE (Finding Informative Regulatory Elements)

15 of 76 candidate MRs of early gastrulation identified



Gene expression signature (22,000 genes)

Activity

Synergistic pairs of MRs of early gastrulation

Co-regulators	p-value
Zfp238--Zdhhc17	0.0006
Ctnnb1--Tcf12	0.0007
Nfe2l1--Zdhhc17	0.0011
Sox11--Ctnnb1	0.0016
Sox11--Zfp238	0.0026

4 Motifs active during onset of gastrulation

3' UTR	Target of unidentified RNA binding protein
5' ELK4	Target for ETS transcription factors
5'bZIP	Target for bZip transcription factors

Our preliminary data demonstrate that the EpiSC interactome will provide a basis for modeling the pre-gastrulation epiblast and that we can identify candidate MRs by interrogating the EpiSC interactome with signatures of pre-gastrulation and gastrulation stages. Functional analyses of these candidate MRs and active motifs will include validation of transcriptional targets by ChIP, and loss- and gain-of-function experiments in both EpiSCs and *in vivo.*