Generation and maintenance of robust cell fate proportions by FGF/ERK signaling

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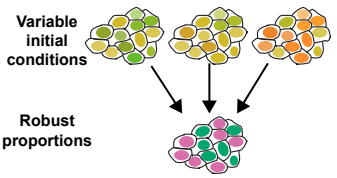
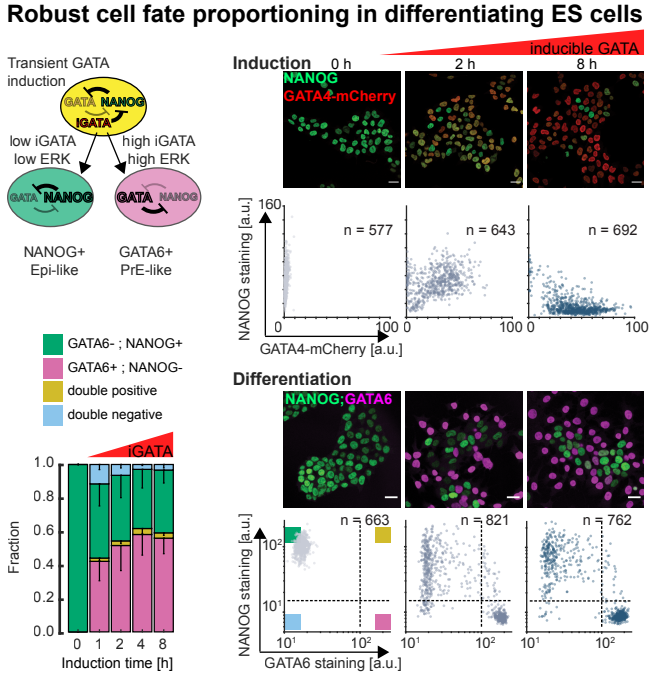
How are robust cell fate proportions established?

During embryonic development and tissue homeostasis, robust proportions of differentiated cell fates need to be specified from multipotent precursor populations. How this is achieved despite uncertainty in initial conditions in the precursor cells, and how robust proportions are regenerated upon perturbations, is not known.

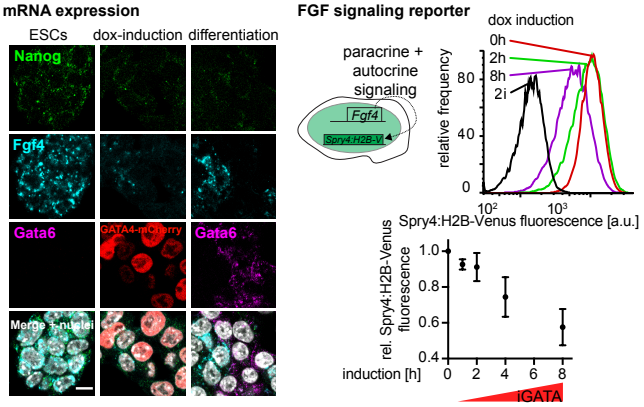


Robust cell fate proportioning in differentiating ES cells



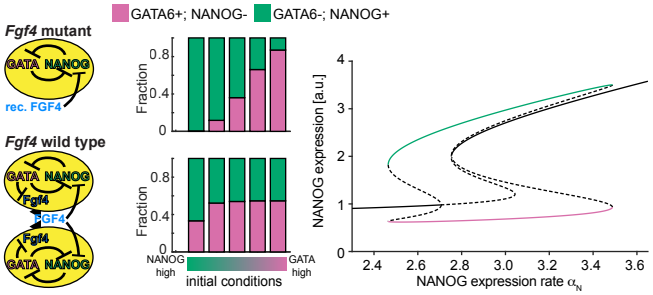
We study mechanisms of cell fate proportioning in an embryonic stem cell model for an early lineage decision of mammalian embryogenesis. In this system, GATA6-expressing primitive endoderm-like (PrE) cells and NANOG-expressing epiblast-like (Epi) cells are specified from a precursor state that we artificially generate through the transient expression of doxycycline-inducible GATA4-mCherry transgenes. We find that robust proportions of the differentiated cell fates are obtained for a wide range of initial conditions that we control by titrating GATA4-mCherry induction levels. This indicates that cell fate proportions are controlled at the population level.

Fgf4 expression reflects cell state



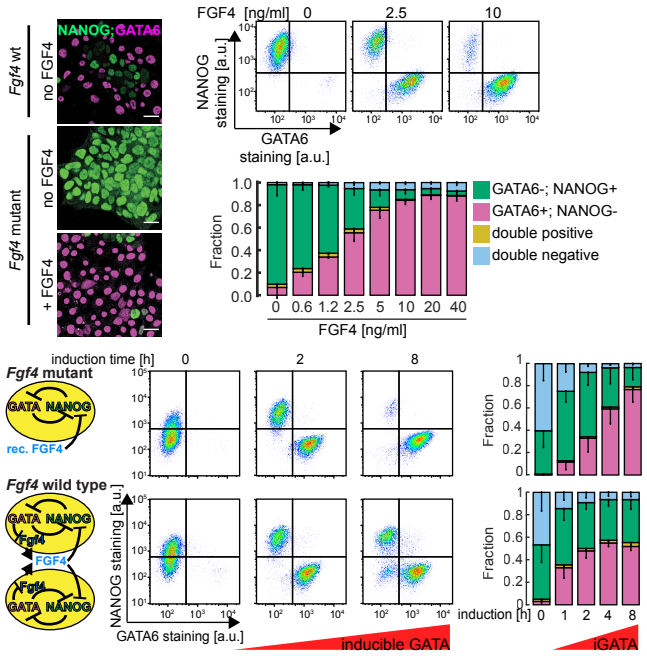
Consistent with a possible role of Fgf4 in cell fate proportioning, we found that Fgf4 mRNA was downregulated in GATA-expressing cells, and that expression levels of an Fgf-responsive transcriptional reporter negatively correlated with GATA4-mCherry induction levels. Thus, Fgf4 expression communicates the state of single cells to the population.

Cell fate proportioning emerges upon coupling single cell circuits



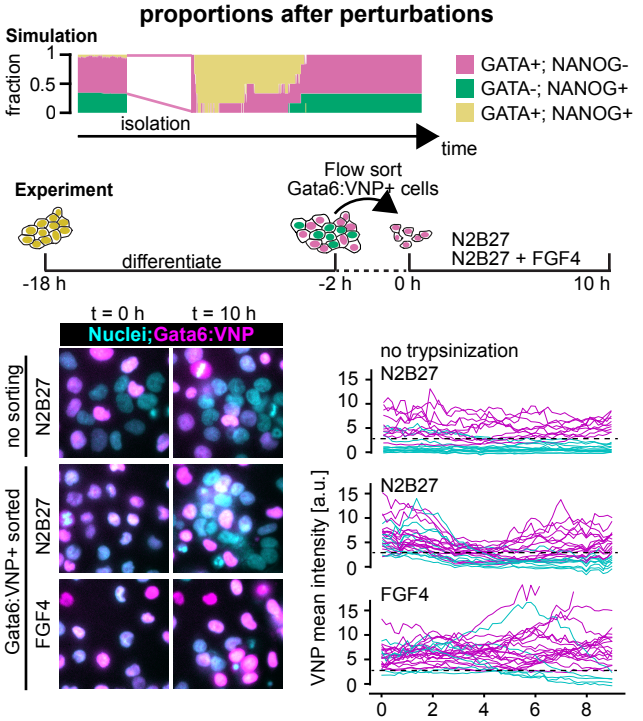
To test if a simple cell-cell communication mechanism via FGF4 is sufficient to explain cell fate proportioning, we simulated differentiation outcomes for different distributions of initial conditions, comparing populations of isolated cells to populations communicating via FGF4. The simulations closely recapitulated the experimentally oserved behavior of wild type and mutant cells. Through bifurcation analysis, we show that the emergent behavior of cell fate proportioning can be explained by an inhomogeneous steady state, a new solution of conjugate cell states that arises in the coupled system.

Communication via FGF4 mediates cell fate proportioning



To test a candidate molecular mechanism for cell fate proportioning, we mutated the *Fgf4* gene in inducible cells. This led to a complete loss of PrE-like differentiation which could be rescued by recombinant FGF4 in a dose-dependent manner. However, the robustness of cell fate proportions to GATA induction levels was lost in mutant cells treated with a constant dose of FGF4. This indicates that communication via paracrine FGF4 underlies cell fate proportioning.

Cell communication allows re-establishing cell fate proportions after perturbations



The theory of the inhomogeneous steady state predicts that cell fates are not only established, by also dynamically maintained through cell-cell communication. We test this prediction by flow sorting of live cells expressing a Gata6-reporter that indicates a PrE-like identity. In line with the theory, these populations regenerate a heterogeneous mix of cell identities.