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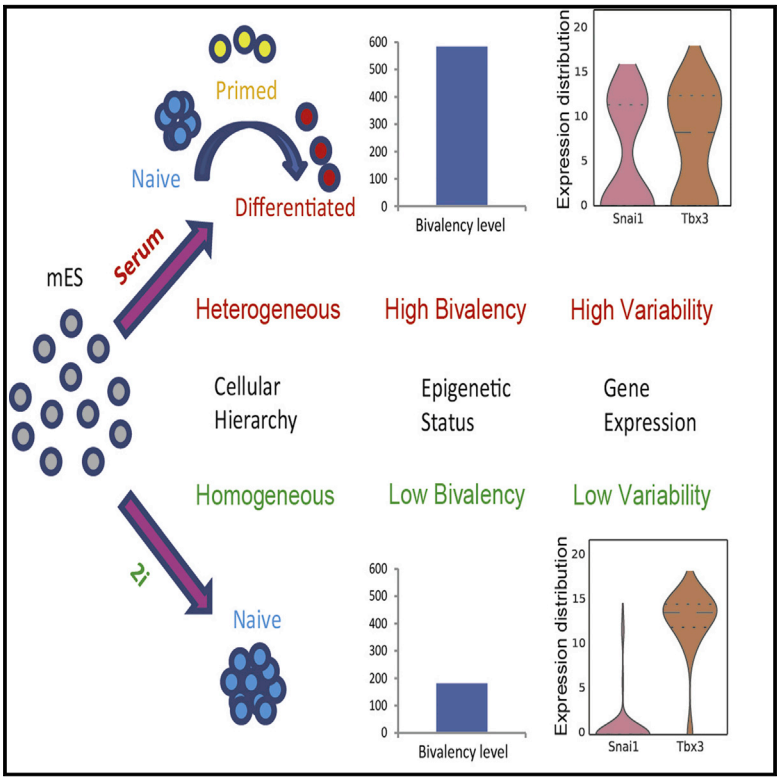
Serum-Based Culture Conditions Provoke Gene Expression Variability in Mouse Embryonic Stem Cells as Revealed by Single-Cell Analysis

Graphical Abstract



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In Brief

Guo et al. investigate mechanisms of gene expression variation and cellular heterogeneity in mouse embryonic stem cells using single-cell mRNA-seq analysis. They find a differentiation-priming pathway in the cell culture system and show that serum provokes gene expression variability in mouse embryonic stem cells.

Highlights

- Single-cell mRNA-seq analysis reveals heterogeneity in ESCs cultured in serum
- Gene expression variability is associated with distinct chromatin characteristics
- Computational analysis identifies an ESC-priming pathway
- External culture system affects gene expression variability of ESCs

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Serum-Based Culture Conditions Provoke Gene Expression Variability in Mouse Embryonic Stem Cells as Revealed by Single-Cell Analysis

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SUMMARY

Variation in gene expression is an important feature of mouse embryonic stem cells (ESCs). However, the mechanisms responsible for global gene expression variation in ESCs are not fully understood. We performed single-cell mRNA-seq analysis of mouse ESCs and uncovered significant heterogeneity in ESCs cultured in serum. We define highly variable gene clusters with distinct chromatin states and show that bivalent genes are prone to expression variation. At the same time, we identify an ESC-priming pathway that initiates the exit from the naive ESC state. Finally, we provide evidence that a large proportion of intracellular network variability is due to the extracellular culture environment. Serum-free culture reduces cellular heterogeneity and transcriptome variation in ESCs.

INTRODUCTION

Early mammalian development cells differentiate toward trophectoderm (TE) and inner cell mass (ICM). The ICM goes on to form the epiblast (EPI) and the primitive endoderm (PE). Embryonic stem cells (ESCs) can be derived from the ICM in the presence of leukemia inhibitory factor (LIF) and fetal calf serum (FCS) (Evans and Kaufman, 1981). ESCs have two important characteristics: the capacity for differentiation into all somatic cell types and the property of unlimited self-renewal in vitro.

Previous studies suggest that ESCs in culture are not homogeneous. Transcription factors associated with ESC identity may be expressed in a heterogeneous manner. For example, Nanog and Dppa3 are expressed in only a fraction of cells (Chambers et al., 2007; Hayashi et al., 2008). Variation in expression of these individual genes has been implicated in controlling the differentiation potential of different subpopulations. However, traditional methods are limited to the analysis of small number of genes. The mechanisms underlying genome-scale ESC variability are not fully characterized.

Single-cell gene expression analysis has been developed as a powerful tool for studying cellular heterogeneity and hierarchy. Several hallmark technical advances have been achieved. High-throughput single-cell qPCR is a dynamic approach for quantifying a set of target genes in systems of interest (Buganim et al., 2012; Dalerba et al., 2011; Guo et al., 2010, 2013; Moignard et al., 2013). Single-cell mass cytometry constitutes a complementary system for multiplexed gene expression analysis at the protein level (Bendall et al., 2011). Single-cell mRNA-sequencing strategies, which enable whole-transcriptome analysis from individual cells, have become increasingly mature and capable (Fan et al., 2015; Hashimshony et al., 2012; Islam et al., 2011; Jaitin et al., 2014; Klein et al., 2015; Macosko et al., 2015; Ramsköld et al., 2012; Sasagawa et al., 2013; Shalek et al., 2013; Tang et al., 2009, 2010; Treutlein et al., 2014; Xue et al., 2013; Yan et al., 2013).

Using single-cell technologies, several studies have reported transcriptomic analysis of mouse ESCs and uncovered signaling and microRNA pathways that influence heterogeneity of ESCs in culture (Grün et al., 2014; Kumar et al., 2014). More-recent studies have also examined transcriptional networks and cell-cycle regulators that contribute to transcriptional variation (Kolodziejczyk et al., 2015; Papatsenko et al., 2015). Epigenetic