Pluripotency signatures in single embryonic stem cells

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A major conceptual question in developmental biology is how an embryonic stem cell maintains a pluripotent state that affords the stem cell the flexibility to turn into any major cell type in the body. We may obtain insights into this question by systematically perturbing the key players of the transcriptional network that underlies the maintenance of pluripotency. Using a nascent technique for measuring integer counts of individual mRNA molecules within single cells *in situ*, we examine cell-to-cell variability in transcriptional response to such perturbations in mouse embryonic stem cells.

***Keywords* — stochastic gene expression, *in vivo* single molecule biophysics, embryonic stem cells, pluripotency. phenomenological model.**

I. Motivation and Summary

PLURIPOTENCY, the ability of a cell to turn into any of the cell types that make up the organism, is one of the hallmarks of embryonic stem cells. A major conceptual question is how the complex network of transcriptional circuits maintains the pluripotent state that affords the stem cell the flexibility to turn into any major cell type in the body. To acquire a better understanding of this question, we systematically perturbed levels of some key proteins in the transcriptional circuitry involved in pluripotency maintenance. We then measured the cell's transcriptional response by measuring the integer counts of key transcripts, in single mouse embryonic stem cells. To do so, we used a nascent technique for directly visualizing individual mRNA molecules *in situ* within single cells, by fluorescence microscopy (known as "RNA FISH") [1]. In doing so, we discovered interesting patterns of cell-to-cell variability in RNA levels of key genes in the pluripotency network (notably Nanog, Sox2, and Oct4). Furthermore, we revealed relationships between the transcriptional response of each key gene to perturbations of the others and the role of cell-to-cell variability in this response. Finally, we developed a quantitative model that explains these observations and quantifies the simultaneously rigid and flexible nature of the transcriptional network involved in maintaining pluripotency of a stem cell.

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II. Results

Within an isogenic population of wild-type (V6.5) mouse embryonic stem cells, we measured the integer counts of transcripts of key genes involved in pluripotency maintenance, Nanog, Sox2, and Oct4, all within the same single embryonic stem cell using nascent RNA *in situ* hybridization technique [1]. We performed this measurement on many individual cells, thereby quantifying heterogeneity in the transcriptional state that defines pluripotency. For instance, our finding that an exponential distribution characterizes the single-cell distribution of Nanog transcript level shows that most of the cells within the ESC population have very low Nanog transcript levels. This is intriguing given that low Nanog expression is known to be associated with a cell that is on the verge of losing its pluripotent state and may be primed for differentiation into tissue-specific cells.

In addition, we systematically overexpressed Nanog, Oct4, and Sox2 genes individually. Such perturbations of the key players in the transcriptional network that underlies maintenance of pluripotency allowed quantitative study of the transcriptional regulatory network and revealed intricate transcriptional feedback regulations. In particular, overexpression of Nanog revealed a negative feedback response, in which a cell turns on a mechanism for repressing the overall Nanog transcription. But this mechanism still allows for the observed heterogeneity in Nanog, Oct4, and Sox2 transcript levels while at the same time ensuring that they remain within well-defined bounds that we were able to measure. We constructed a mathematical model to explain our measurements of perturbation-response in single stem cells, and quantified the simultaneously rigid and flexible nature of the key players in the transcriptional network involved in maintenance of pluripotency.

References

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