Systems analysis of human pluripotent stem cells during self renewal and differentiation

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Human pluripotent Stem Cells (hPSCs) are an attractive raw material for regenerative medicine due to their unique properties of self-renewal and lineage specific differentiation. Delicate balance of signaling pathways decide on hPSC cell fate. Current experimental efforts have successfully identified the primary signaling pathways maintaining the balance between self-renewal and differentiation. Our objective is to develop mathematical modelsof the signaling pathway along with systems level analysis to identify key sensitive nodes controlling information flow through the pathway. This analysis will result in identification of targeted perturbations to maintain self-renewal or induce lineage specific differentiation.

We have developed a suite of modeling techniques specifically targeting to capture the dynamics of hPSCs. We first described the signaling dynamics of self-renewing hPSCs by mathematical model of the Insulin induced PI3K pathway. Performing global sensitivity analysis of the pathway using a meta-modeling approach we identified negative regulation through PKCζ to be the dominant signaling node controlling expression level of pAKT. This was further experimentally verified by inhibition of PKCζ which resulted in significant enhancement of pAKT levels in self-renewing hPSCs. Further, analysis of noise propagation through the pathway revealed that the negative feedback through PKCζ helps in noise elimination and protects pAKT levels against upstream variations.

While pAKT supports self-renewal, they were observed to be detrimental for Activin induced endoderm differentiation. Analysis of the dynamics of the multiple pathways during Activin induced differentiation by Dynamic Bayesian Network (DBN) analysis identified significant crosstalk between pAKT and pSMAD molecules. Suppression of pAKT by inhibition of the PI3K pathway successfully removed the interactions and enhanced endoderm differentiation. Current modeling efforts are targeted towards representing these interactions between PI3K and TGFβ pathway and identification of key nodes regulating endoderm differentiation.

In addition, hPSCs are known to have a unique cell cycle behavior, with a shortened G1 phase resulting in shorter doubling time. This G1 phase lengthens with differentiation, leading to an overall longer doubling time.We have developed a stochastic cell population model to track the dynamic and heterogeneous cell cycle behavior during self-renewal and differentiation of hPSCs. Thisstochastic model is able to accurately predict the statistical phase resident time distributions from experimentally synchronized hESC. The primary finding of this algorithm is the possible existence of a lag between cell commitment to differentiation and lengthening of G1 phase.