Dynamics of Higher-Order Interactions Define Transcriptomic Trajectories Associated with Human Embryonic Genome Activation

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Introduction: The newly fertilized embryo is transcriptionally quiescent. The embryo transitions from stored maternal transcripts to dependence on its own transcriptome through embryonic genome activation (EGA).

EGA is a fundamental developmental milestone and is a dynamic process, however the temporal regulation of the network of genes involved in EGA has not been quantified. In this study we use mathematical approaches to measure **i)** transcriptomic trajectories through the preimplantation period and **ii)** the dynamic organisation of the transcriptome associated with human EGA.

Methods: Transcriptomic network analysis measures co-ordination between expressed genes providing a model of the whole transcriptome. Networks define transcriptomic coordination at the level of pairwise relationships between genes (first order interactions [FOI]). We can include complex non-linear dynamic relationships between many genes (higher order interactions [HOI]) using a mathematical approach termed hypergraph analysis. Such properties measure wide-scale reorganizations of the transcriptome as happens during EGA.

A data set of human preimplantation single cell RNAseq data (n = 2280 cells, oocyte to day 14) was generated by meta-analysis of 4 studies [1-4] with batch correction using COMBAT and clustered using Leiden community detection. Trajectory analysis was performed using three approaches: Pseudotime inference, RNA velocity, and lineage tracing (scVelo and Monocle3).

Cells were aligned in developmental order (pseudotime) and gene networks modelled across the dataset using a moving window approach (window size of 10 and step size of 1). Transcriptomic networks were constructed through co-expression analysis within the individual windows. Both FOI and HOI were characterized in the networks using entropy as a measure of transcriptomic network structure.

Results: A total of 2046 genes were found to have developmental time dependent expression. Combining velocity and pseudotime inference defined trajectories characterised by high velocity at fertilization before dropping. An increase in velocity was observed in the 8-cell to morula transition (1.2-fold, p-value $<1x10^{-5}$).

Network entropy followed a similar pattern to velocity, in both the first- and higher-order networks. However, the 8-cell to morula transition demonstrated a significant increase in the entropy of HOI compared to FOI (adjusted p-value $<1x10^{-3}$). This increase in HOI entropy was associated with autophagy and oxidative phosphorylation pathways demonstrating co-ordinated regulation of these pathways following EGA. HOI were shown to specifically control cadherin pathway regulation.

Conclusion: These data show that the complex dynamics of the transcriptome in the early embryo can be precisely measured. The developmental trajectory marked by gene expression velocity confirms current understanding, i.e. that accelerated cell cycle during early cleavage results in reduced transcription but extends this by quantifying the dynamic process. The velocity observations provide evidence for this theory as a spike of activity was observed during the 8-cell to morula transition where a pause in proliferation is thought to occur [5].
The relative increase of HOI entropy associated with EGA suggests that the morula transcriptome is more coordinated when compared to the preceding blastomeres, explaining the differences between cleavage patterns at the blastomere and morula stages.
Associated gene ontology reveals that the first action of the embryonic transcriptome is to programme metabolism. Understanding transcriptomic dynamics in the early embryo is informative in defining lineage and stem cell specification.

[1] Cell, (2016) 165(4), 1012-1026
[2] Nat Struct Mol Biol, (2013) 20(9), 1131-1139.
[3] Development, (2015) 142(18), 3151-3165.
[4] Nature, (2020) 577(7791), 537-542.
[5] Nat Rev Genetics, (2019) 20, 221–234