DOCTORAL THESIS

Systematic characterisation of temporal and fate asymmetries and its regulation during Caenorhabditis elegans Embryogensis

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Date of Award:
2017

Abstract

It is well known that tight coordination of cell division timing is essential for proper cell fate specification and tissue growth during metazoan development. However, how cell divisions are coordinated *in vivo* is largely unknown. In this thesis, a high-content screening was conducted to identify genes responsible for temporal coordination of cell division during *Caenorhabditis elegans* embryogenesis. A total of 822 genes were depleted using RNA interference (RNAi). The genes were prioritized based on their degree of conservation in human, as well as their roles in development. In addition to RNAi, an experimental pipeline was established, including 3D time-lapse imaging of an RNAi perturbed *C. elegans* embryos followed by automated lineaging, which allows systematic quantification of division timing of each individual cells up to approximately 350-cell stage. To identify genes with a significant reduction in the asynchrony of division between sister cells (ADS) upon perturbation, average division timings of each cell between at least two replicate perturbed embryos was compared against the average division timings of 92 wild type embryos. It was found that cell fate determinants were not only important for maintaining fate asymmetries, but are also imperative for establishing ADS regardless of cellular context. Hence, the results demonstrate that fate and temporal asymmetries share a common genetic architecture. The temporal coordination appears to facilitate cell migration during fate specification or tissue growth.

Given the observation that perturbations of signalling pathways, especially Wnt and Notch pathways, are frequently associated with the ADS, it would be essential to map the exact signalling event that takes place at cellular level which is responsible for a given ADS or fate asymmetry. To this end, a miniMos transposon-meditated transgenic technique was adopted to insert a fusion between GFP and a promoter derived from individual components of the two signalling pathways into the *C. elegans* genome. The resulting insertion was crossed into a strain expressing lineaging markers, which allows automated lineaging and gene expression profiling to map the expression of each component with single cell resolution. A combination of cellular expression and cell-cell contact data would lead to a comprehensive map of each signalling event during *C. elegans* embryogenesis. Our preliminary results on cell-cell contacts and cellular expression validated the existing signalling events and identified potential novel ones that are associated with either ADS and/or fate asymmetry.

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