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Systematic Analysis of Embryonic Gap Phase Introduction through the Real-time Visualization of Cell Cycle Progression in Metazoan Embryos

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Abstract

Early embryonic cell cycles usually progress with S and M phases without any gap phase. When and how the gap phases are developmentally introduced remains poorly defined. To systematically establish cell-specific introduction of gap phases throughout *Caenorhabditis elegans* embryogenesis, I first generated multiple transgenic strains expressing a single copy of fluorescent ubiquitin cell cycle indicators (FUCCIs) along with my colleagues. Functional and biochemical validations show expected degradation dynamics of the fluorescence reporters (indicators). Aided by time-lapse 3 dimensional live-cell imaging and automated lineage analysis, I next systematically evaluated developmental introduction of cell cycle gaps through profiling lineal accumulation of each reporter, which indicates that all embryonic terminal cells enter G1 or G0 phase. To increase the sensitivity of these FUCCIs in detecting G1 phase, I examined the G1 phase introduction with these reporters upon depletion of cyclin D or E that is required for progression of G1 to S phase. Remarkably, the accumulation dynamics revealed a G1 phase during the last two rounds of embryonic division in most cells. Intriguingly, the G1 progression involves lineage- and stage-specific use of the two cyclins. The cyclin D is only used in the asymmetric division of "AB" and "EMS" descendants when one of their daughters become terminal cell, suggesting a coupling between fate differentiation and cyclin use. Finally, I characterized G2 phase introduction with the FUCCIs through the depletion of Cdc25, which provides preliminary evidence of G2 phase introduction after initiation of robust zygotic transcription in early embryo. In summary, I developed a method for systematic monitoring of developmental introduction of gap phases with cellular resolution, and established G1 phase introduction in numerous cells before their last or second last round of embryonic division. The FUCCIs and the findings of gap phase introduction pave the way for further characterization of the relationship between gap phase introduction and fate differentiation during development.

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