Examination of the Molecular Mechanisms Associated with Quiescence

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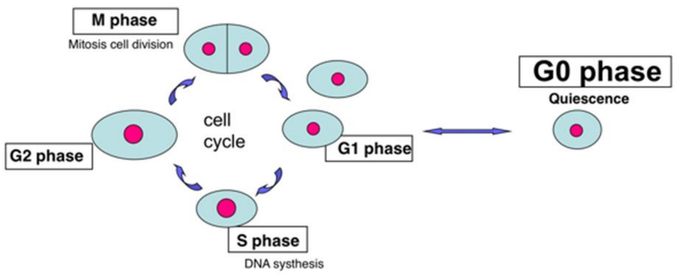
ABSTRACT

The overall aim of the research is to identify the sequence of molecular events during physiological cell cycle exit via both serum starvation and contact inhibition. The proliferation-quiescence decision is not well-understood. To examine this, time-lapse microscopy is being used in conjunction with live-cell fluorescent reporters and iterative indirect immunofluorescent imaging (4i).

BACKGROUND

The cell cycle refers to the series of events that occur in a cell as it develops and divides. Quiescence, denoted by G0, is the state in which the cell does not divide, but retains the ability to re-enter the cell cycle. This disruption of cell cycle machinery occurs in response to a lack of growth factor or nutrients. Inappropriate cell cycle exit can produce developmental abnormalities, aging, degeneration, and cancer.



Regarding the classifications of cell fate, proliferating cells are those actively continuing in the cell cycle; deciding cells (mothers) are those in their last cell cycle before making the decision to arrest; and arresting cells (daughters) are those that have decided to arrest. Arrest was concluded after 48 hours of no new division.

RESULTS

Figure 1. Ratio of Cytoplasmic to Nuclear Intensity of CDK1/2 in Proliferating and Deciding Cell

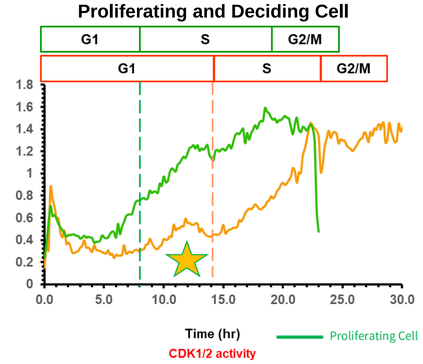


Figure 2. Nuclear Intensity of p27 in Proliferating and Deciding Cell

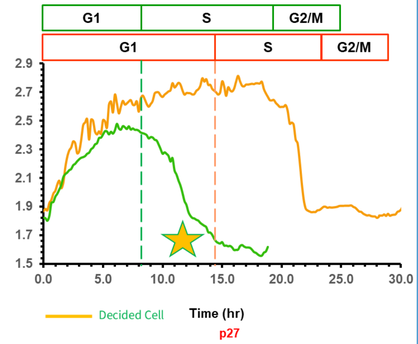


Figure 3. Cell Crowdedness At Mother's Mitosis Across Cell Fates

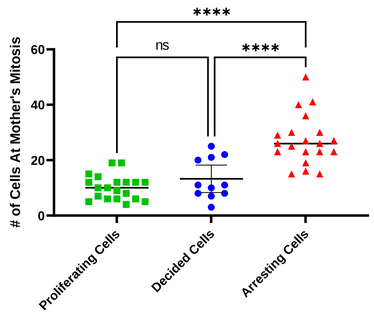


Figure 4. Maximum p27 Intensity Level Across Cell Fates

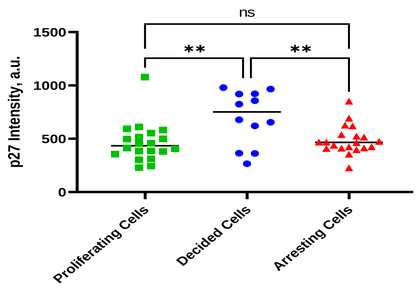
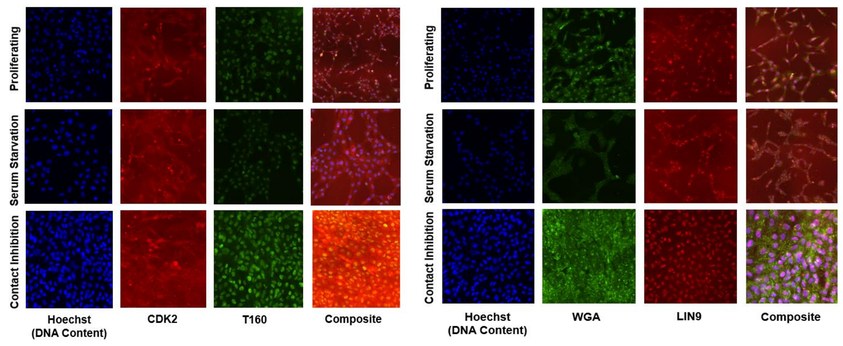
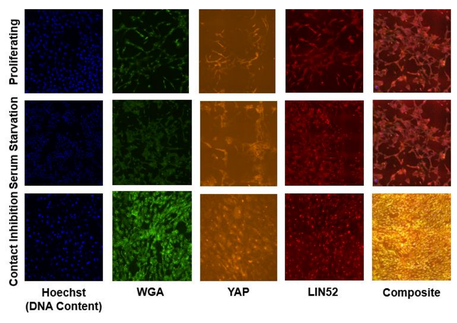


Figure 5. Antibody Validation Across Cell Conditions

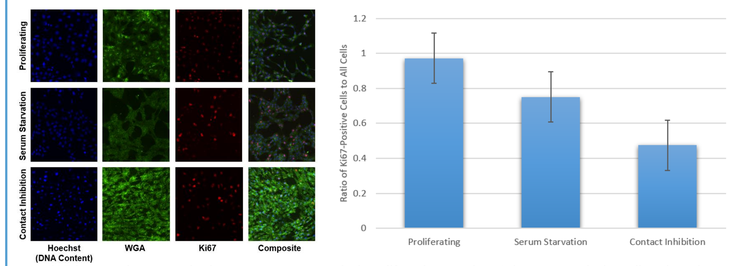




Hoechst is a blue fluorescent dye used to stain DNA. Wheat Germ Agglutin (WGA) is a carbohydrate-binding lectin that labels the plasma membrane and Golgi complex. The CDK2 antibody detects endogenous levels of total CDK2 protein while the T160 antibody detects CDK2 protein phosphorylated at the T160 site. LIN9 and LIN52 are proteins associated with the DREAM complex. YAP is a transcriptional regulation protein that promotes cell proliferation and suppresses apoptosis. The composite is composed of every channel in an individual condition overlaid on top of each other.

RESULTS (CONT.)

Figure 6. Antibody Validation for Ki67 and Comparison of Ratio of Ki-67 Positive Cells Across Cell Fates



Ki67 is a nuclear protein that serves as a graded proliferation marker. It is present during all active phases of the cell cycle, but degraded continuously in quiescence.

CONCLUSIONS

There is a sequence of molecular events that occurs when a cell decides to exit the cell cycle. 4i will empower prediction of this sequence of quiescent markers. Deciding cells (mothers of daughters who arrest) have longer cell cycle phases than proliferating cells.

FUTURE DIRECTION

Once antibody validation is completed, the appropriate concentrations will be used in 4i to identify at what time various markers rise and fall in the transition to quiescence. A proximity ligation assay will be conducted to examine the DREAM complex as a unique molecular marker of G0. Antibody validation will be conducted for acetylated tubulin, another marker of quiescence.

REFERENCES/ACKNOWLEDGEMENTS

Special Thanks to Dr. Martha Johnson, Dr. Jean Cook, Tessa Hill, Amy Song, and all members of the Cook Lab.