Exploring Mitosis Phases

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Introduction

At the Graham Lab, my project served as an essential part of an extensive proteomics study, geared towards understanding the dynamic changes in proteins during the cell cycle, which consists of the G1, S, G2, and M phases. A pivotal aspect of my role involved synchronizing cells across these diverse phases of the cell cycle. To ascertain these phases accurately, we employed two vital techniques: Flow Cytometry and Western Blot. Flow Cytometry facilitated a deep examination of the DNA content of cells from different stages, offering critical insights into their respective cell cycle phases. Simultaneously, Western Blot analysis was utilized to probe cyclin proteins, known to be key biomarkers for various cell cycle phases. Together, these methods proved indispensable to the broader proteomics project, providing us with an in-depth understanding of the dynamic protein changes occurring throughout the cell cycle.

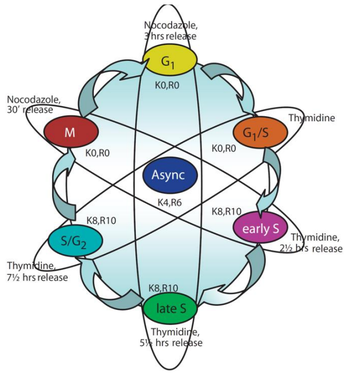

Figure 1: The dynamic of cell cycle. The experiment starts G1/S phase and follows with Early S, Late S, G2 phase, and then M and G1 phase.

Objective & Impact of Professor's Research

Professor Graham's lab pursues experimental and computational systems biology approaches to develop quantitative models of cancer and other human diseases. The lab draws biology, statistics, and engineering to build data-driven, predictive models of tumor phenotypes using quantitative data generated in-house. The genetic events that drive human disease must be implemented at the protein level. Using mass spectrometry, they are identifying and quantifying proteins and their post-translational modifications in cell lines, mouse models and human patient samples.

Method & Learning Process

Twenty-one dishes of our 293T cells (three replicates for each condition) were meticulously synchronized in the G1-S phase overnight using a 4 mM thymidine block (Sigma). Post-thymidine release, we collected cells at four distinct time intervals: immediately (G1-S phase), 2.5 hours (early S phase release), 5.5 hours (late S phase release), and 7.5 hours (S-G2 phase). We further arrested six dishes of cells with nocodazole overnight, following the 7.5-hour release from thymidine. The subsequent morning, these cells were released for either 0.5 hour (M phase) or 3 hours (G1 phase).

For Flow Cytometry, we prepared cell suspensions, fixed with 70% ethanol, and permeabilized using a 5-minute treatment with 0.25% Triton X-100 in phosphate-buffered saline (PBS). We then incubated them with 0.1% ribonuclease (RNase) and propidium iodide (10 mg/ml). The cellular DNA content was then accurately determined using a MACSQuant system and Flowlogic software.

For Western Blot, we washed the cells with ice-cold PBS containing 1 mM phenylmethylsulphonyl fluoride, scraped them off the plate, and resuspended them in ice-cold Ripa buffer. After a 15-minute incubation on ice, we centrifuged the lysed cells at 13,000 rpm for 15 minutes at 4°C. Using the BCA assay, we measured protein concentrations in the cleared lysate. We then loaded equal protein amounts onto SDS-PAGE gels. The separated proteins were transferred onto nitrocellulose membranes, and analyzed using a rabbit antibody against geminin, cyclin B1, cyclin D3, A-tubulin, and cyclin A2.

Figure 3 demonstrates results from a synchronized cell population, where the DNA content of individual cells was analyzed via a Flow Cytometer (FACS). This tool sorts cells based on fluorescence. Cells were stained with a dye that fluoresces upon DNA binding, linking fluorescence intensity to DNA content.

Results

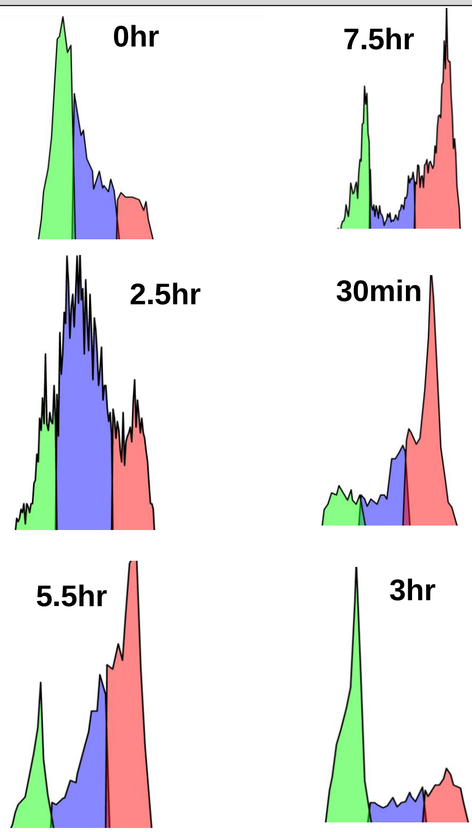

Figure 3. FACS profiles of the individual synchronized 293T Cell populations. Green color shows cells in G1 phase, Blue color Shows cells in S phase, and Red color shows cells in G2/M phase.

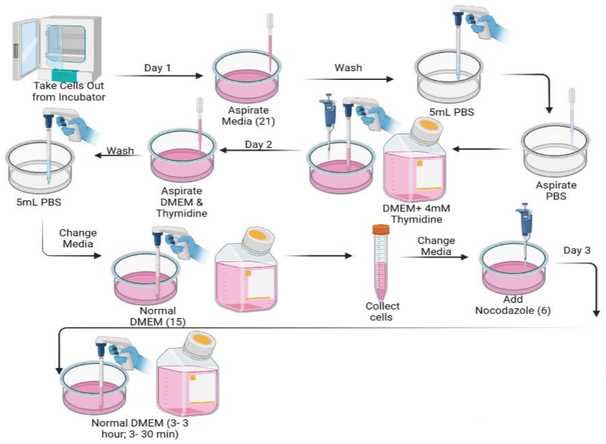

Figure 2: The Procedure of Cell Synchronization and Release from Thymidine and Nocodazole Blocks

Results Analysis

The distribution of cells highlights the three categories into which they fall: **1)** G1 phase cells that harbor an unreplicated DNA complement. **2)** G2 or M phase cells that contain a fully replicated DNA complement, doubling the G1 DNA content. **3)** S phase cells that possess an intermediate DNA quantity. For instance, at the 0hr, the distribution of cells in the G1 phase (marked green) and S phase (marked blue) indicates that the cells are as expected in the G1/S phase, attesting to the effectiveness of the thymidine block. Additionally, the distribution at the 30min shows a high prevalence of G2/M phase cells (marked red) compared to the S and G1 phases. This distribution confirms that most of the cells are in the M phase, indicating the successful implementation of the nocodazole block.

Next Steps for You & Advice to Future SHINE participants

My next steps as an upcoming junior will be to dig deeper into chemistry and biochemistry subjects in order to achieve my own research through the knowledge gained through SHINE. For future SHINE students, I recommend you meet new people and get out of your comfort zone. There are amazing scholars who share similar interests and having someone to persevere with in SHINE, is truly rewarding!

Acknowledgements

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