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SJSU Undergraduate Research Grants

Roles for pHi dynamics in cell cycle regulation

Harnoor Virk, Ismahan Chire and Bree Grillo-Hill, Ph.D.

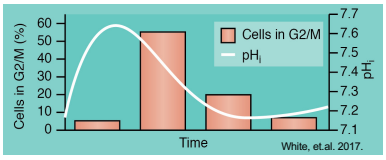
Department of Biological Sciences, San José State University, San Jose, CA

Abstract

Intracellular pH (pHi) is tightly regulated by cells, and emerging evidence suggests that regulated pHi dynamics modulate distinct cell behaviors, including regulated cell proliferation. Previous studies in cultured mammalian cells suggested that a transient increase in pHi at the end of S phase permits entry into the G2/M phase. Constitutively increased pHi is a conserved characteristic of cancers, and may facilitate hyperproliferation by permitting early entry into G2/M. However, these results have not been confirmed *in vivo* and the role for increased pHi in cell cycle progression remains unclear. Our research addresses these issues using methods we developed to increase pHi in developing *Drosophila* tissues by overexpression of the sodium-proton exchanger *DNhe2*. We confirmed that increased pHi increases proliferation *in vivo* and causes tissue overgrowth in both eye and wing imaginal discs. This observation led us to ask: how is the timing of the cell cycle regulated by increased pHi? To address this question, we are using Fluorescent Ubiquitination Cell Indicator (FUCCI) transgenic flies to directly visualize real time cell cycle dynamics *in vivo*. The FUCCI flies express different biosensors to indicate each stage of the cycle, which will allow us to measure the duration of each state of the cell cycle. *Drosophila* eye tissues expressing FUCCI will be dissected and live imaging performed to image the fluorophores. We will analyze the expression patterns and compare cell cycle kinetics at normal and elevated pHi. These studies combine the strength and utility of *Drosophila melanogaster* with cell biological techniques to elucidate pH-dependent mechanisms that influence the development of multicellular organisms.

Research Questions

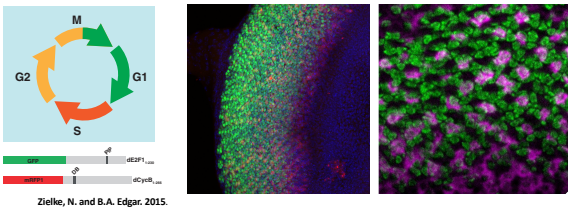
How is the timing of cell cycle progression regulated by pHi?



Cells with increased pHi have altered cell cycle kinetics, e.g. increased length of S phase

Increased pHi is a permissive signal that allows more cells to enter the cell cycle

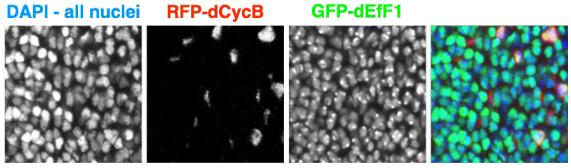
Generating flies that express FUCCI sensors in the developing fly eye

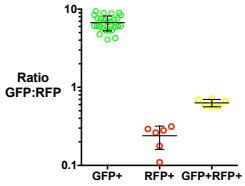


Zielke, N. and B.A. Edgar. 2015.

We generated flies that express FUCCI sensors in developing eye tissue. Expression of fluorescent reporters reflects the stage of the cell cycle as follows: green (late M/G1 = cytokinesis and rest); red (S = DNA synthesis); yellow (G2/M = mitosis).

Expression of FUCCI reports cell cycle stages

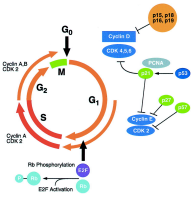




We generated flies that express FUCCI under the control of GMR and dissected the eye imaginal discs from third instar larvae. Eye discs were fixed and labeled with DAPI to label all nuclei, and FUCCI indicated cell cycle stage as follows: green (late M/G1); red (S); yellow (G2/M). We developed a quantative method to distinguish fluorescence levels between different stages of the cell cycle.

Project Activities

The Fluorescent Ubiquitination Cell Cycle Indicator (FUCCI) transgenic flies allow for visualization of the cells in each stage of the cell cycle through expression of fluorescently-tagged, cell cycle-regulated proteins. We use a specific driver to express FUCCI in the developing Drosophila eye (*GMR>FUCCI*). Previous studies have shown that increased intracellular pH causes increased proliferation, but we do not know the stage of the cell cycle or molecular mechanism affected by increased pHi. We will utilize FUCCI sensors to measure the duration of cells in each state of the cycle cycle at normal and increased pHi to determine differences. Here, we generated flies that express FUCCI sensors, and report quantitative analysis parameters that permit determination of cell cycle stage based solely on expression of FUCCI reporters.



Boehm and Nabel, 2001.

Citations & Funding

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