Abstract

Intracellular pH (pHi) is tightly regulated by cells, and emerging evidence suggests that regulated pH 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 bicistronic reporters to indicate each stage of the cell 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 metanorganizer 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?

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 address IC and B.A. Edgar. 2015. “FUCCI sensors: powerful new tools for analysis of cell proliferation.” WIREs Dev Biol 2015, 4:469–487. doi: 10.1002/wdev.109

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Citations & Funding

