Investigation of Single-Cell pH Homeostasis Using Fluorescence Ratio Imaging Microscopy

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Abstract

Escherichio coli cells experience rapid and drastic shifts in pH during its passage through a host’s digestive system, and must be able to grow in ranges from pH 4.5 to pH 9 (Wiks and Slonczewski, 2007). How E. coli responds to rapid pH shifts is of interest in understanding intestinal colonization by pathogenic bacteria.

A new way to measure cell pH allows single cell measurements by using a GFP variant (ratiometric GFP) with an excitation spectrum dependent on surrounding pH (Olsen et al., 2002). The ratio of fluorescence intensities of two major peaks in the spectrum can be used as a reliable measure of intracellular pH (Meesenbrock et al., 1998).

E. coli is extremely acid resistant and can survive at pH as low as pH 2 for hours. Several different mechanisms protect E. coli from acid stress, including an antipHase and the others relying on deacetalization and antipporter activity (Foster, 2004). These mechanisms, in combination, allow for the acid resistance and rapid pH recovery shown by E. coli cells.

Cultures: Cells were grown overnight in unbuffered Luria broth with KCl (LBK) with 0.2% L-arabinose and 50 µg/ml ampicillin, and rotating at 16.20 rpm. Cultures were then diluted 1:50 into baffled flasks with LBK at pH 7.5 with 0.2% L-arabinose and 0.2% KCl (Miller, 1972) buffered at pH 6.0 (50 mM HOMOPFES), 5.5 (50 mM MES), 6.0 (50 mM MOPS), 7.0 (50 mM MOPS), 7.5 (50 mM MOPS), 8.0 (50 mM MOPS), 8.5 (50 mM MOPS) and 9.0 (50 mM AMPSO). An aliquot of cell culture was placed on a 40 mm round chamber (Sutter Instrument LB-150-TF7). Excitation intensity ratios were calculated using the software MetasFlour 7.6.5.0. Multiple images were taken before the illumination.

Materials and Methods

Introduction

The gram-negative bacterium Escherichio coli experiences rapid and drastic shifts in pH during its passage through a host’s digestive system, and must be able to grow in ranges from pH 4.5 to pH 9 (Wiks and Slonczewski, 2007). How E. coli responds to rapid pH shifts is of interest in understanding intestinal colonization by pathogenic bacteria.

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Conclusions

• A standard curve was generated for ratiometric GFP with an effective range from pH 5.0 to pH 9.0. The ratio of fluorescence intensities of two major peaks in the spectrum can be used as a reliable measure of intracellular pH (Meesenbrock et al., 1998).

• Cytoplasmic pH was measured over time in single adherent cells using ratiometric GFP and fluorescence microscopy. The responses of multiple cells to rapid environmental acidification were tracked over the course of several minutes (Fig. 2).

• Logarithmic and stationary phase cells showed similar responses to rapid acid shift. Both types of cultures showed different responses, but these responses were limited to just one culture type.

• A great deal of heterogeneity existed within single cultures, with adjacent cells responding to rapid acid shift. Both types of cultures showed different responses, but these responses were limited to just one culture type.

• Within a biofilm, cells showed diverse pH values. Most cells maintained homeostasis normally, but areas of both higher and lower pH were observed (Fig. 3). This could be due to varying degrees of access to fresh media, dependent on the biofilm architecture.

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References