Automated Live Cell Imaging of Green Fluorescent Protein Degradation in Individual Fibroblasts

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Quantitative measurements of dynamic processes in single, living cells are challenging, especially when the processes being studied are long-lived and the number of cells required to achieve an accurate determination of the distribution of responses in the population are large. The rates at which large numbers of individual living cells degrade green fluorescent protein (GFP) was examined using automated light microscopy. This study examined a clonal population of NIH3T3 fibroblasts that resulted from stable transfection with a construct containing the tenascin-C gene promoter ligated to a destabilized, enhanced GFP (dsEGFP) reporter gene. We used simple protein patterning methods based on soft microlithography to confine cells to discrete spatial locations coated with the extracellular matrix protein, fibronectin. Spatial confinement facilitated the analysis of GFP degradation rates by limiting the migration of the live cells during the 16 hours of the experiment. Cells continued to move within and slightly beyond the designated areas during the experiment. To generate a segmentation mask for cells which included the entire area that each cell explored, sequential phase images were subtracted, and the absolute difference in intensities of each pixel in the images were summed. Pixels above a manually set threshold provided a mask under which the GFP intensity for individual cells was determined (Figure 1).

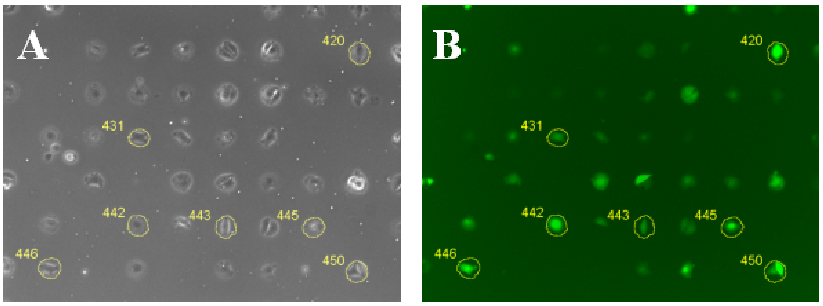

Figure 1. Representative phase contrast (A) and GFP (B) images. The segmentation masks for single cells are shown in yellow for both images. For each experiment, images from 24 different fields were collected at 15 minute intervals and GFP intensities were calculated from the fluorescence images for each single cell.

This method allowed us to examine GFP intensity over 14 h in 524 live cells and determine the degradation rate constants for GFP in those cells. To measure GFP degradation rates in the absence of new GFP production, protein synthesis was inhibited by treating the cells with cycloheximide. Results indicated a wide range of cell-to-cell variability in the GFP fluorescence within individual cells, and a non-Gaussian distribution of intensities. Cell-to-cell variability in degradation kinetics was relatively

narrow, and more symmetrical. Degradation for this reporter was analyzed as a first order rate process with a degradation half-life of 2.8 +/- 0.7 h, where the variance reflects the width of the distribution of rate constants (Figure 2).

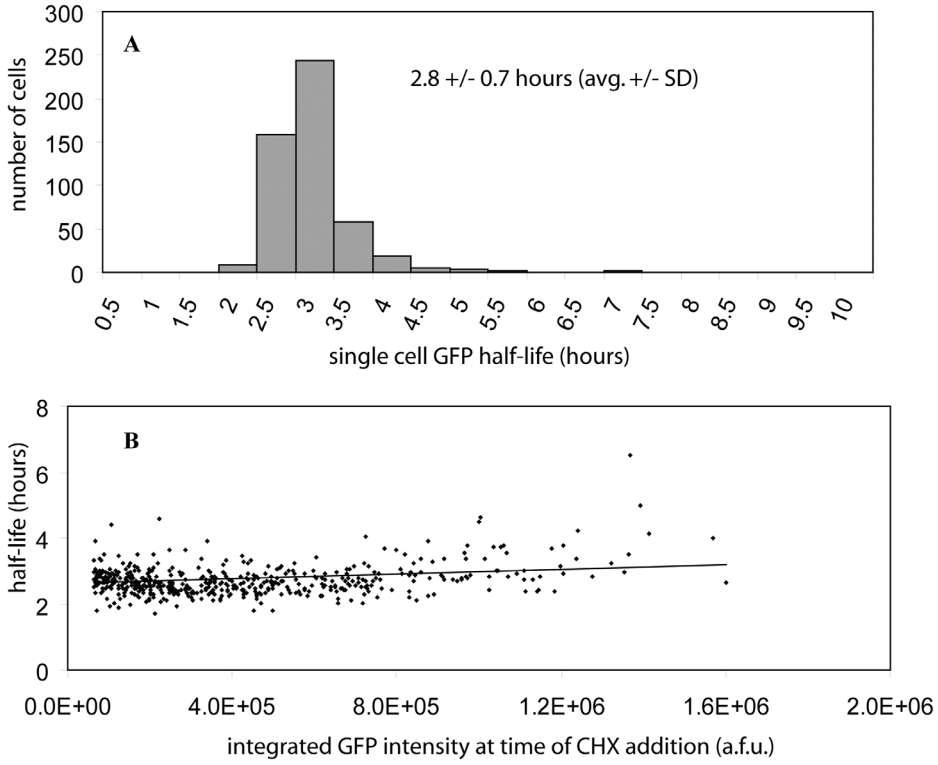

Figure 2. (A) Half-lives for GFP degradation in individual cells in the population were determined from single exponential fits to GFP intensity data collected over 8 h following addition of 100 μg/ml CHX. (B) Relationship between the GFP intensity measured in each individual cell prior to protein synthesis inhibition and the half-life determined for GFP degradation in that cell. Data from 499 individual cells are represented.

By examining degradation rate as a function of initial GFP intensity, we found that GFP degradation rate constants were independent of the initial intensity of GFP fluorescence within cells. This result suggests that higher GFP abundance in some cells is likely due to higher rates of gene expression, because it is not due to systematically lower rates of protein degradation.