Quantifying Protein Mobility in Living Drosophila Embryos Using Fluorescence Recovery After Photobleaching (FRAP)

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Overview

• Days 1 and 2
• Drosophila Introduction
• Protocol
• Results
• Conclusions
• Future Propositions
• References
• Acknowledgements
Fluorescence Microscopy

Calibration

E. Coli
Gel Electrophoresis

Ladder

10 Kilobases
5 Kilobases
3 Kilobases
2 Kilobases
1.5 Kilobases
1 Kilobases
0.5 Kilobases

Appx. 42ng

Ladder Info from:
FRAP Overview

• Excite Green Fluorescent Protein (GFP) fluorophore with low energy 488nm Argon laser
  – Detect emission of a lower wavelength signal
• Maximum laser power is used to photobleach GFP for analysis
• Returning to low energy emission allows quantification of the remaining unbleached GFP-coupled proteins
Drosophila Embryos

• FRAP assay performed on two transgenic types:
  – Wild-type embryos ubiquitously expressing histone (H2A-GFP)
  – Wild-type embryos ubiquitously expressing nuclear localization signal (NLS-GFP)
Nuclear Labeling of NLS-GFP

Supatto, W et al. PNAS 2005
Protocol

• Embryo Preparation
  – De-chorionated and placed on microscope slide after 14 developmental cycles (2.5 hours)
  – After isolation, the nuclei remain as a single epithelial layer at the edge of the yolk for approximately 30-40 minutes
    • Gastrulation occurs after this stationary period
Protocol

• Imaging
  – 63x, 0.9 NA water Acroplane Confocal Microscope objective
  – Seven 1.5 micron z-sections are taken between a nuclear layer to allow full planar photobleaching

• Bleaching and Time Lapse
  – Define a region of interest and bleach the middle section with 100% laser power
  – Take 7 z-sections every 10 seconds to quantify fluorescence recovery
H2A Experiment 1
H2A Experiment 1

\[ f(t) = (A - B) \left( 1 - e^{-(t - t_0)/\tau} \right) + B \]

\[ D_{eff} = \frac{\omega^2}{\tau} \]

Calculated \( D_{eff} \) was between 0.0094 and 0.0139 \( \mu m^2/sec \)
H2A Experiment 2

\[ f(t) = (A - B) \left(1 - e^{-(t - t_0)/\tau}\right) + B \]

\[ D_{\text{eff}} f = \frac{\omega^2}{\tau} \]

Calculated \( D_{\text{eff}} \) was between 0.0079 and 0.0117 \( \mu\text{m}^2/\text{sec} \)
NLS Experiment
NLS Experiment

Fluorescence Recovery for nls Experiment

Slope was calculated to be approximately 0.0012 sec\(^{-1}\)
Control 1: Linearity

\[ f_0 = \alpha C_0 \]
\[ C_n = \gamma^n C_0 \]
\[ f_n = \alpha C_n \]

\[ \ln(f_n) = n \ln(\gamma) + \ln(\alpha C_0) \]
Control 2: Time Bleach

- No significant photobleaching at 2% laser power for 690 seconds
Control 3: Single Nucleus Bleach
Control 3: Single Nucleus Bleach

- Signal is restored above background levels after one entire nucleus is photobleached

Calculated $D_{\text{eff}}$ was between 0.0056 and 0.0083 $\mu$m$^2$/sec
Control 4: Five Nucleus Bleach
Control 4: Five Nucleus Bleach

• Central nucleus no longer regains signal in a diffusion-like manner
Conclusions

• Motion of H2A within Drosophila embryo nuclei can be modeled by simple diffusion
  – $D_{eff}$ was calculated to be between 0.0079 and 0.0139 μm²/sec
  – $D_{eff}$ of free GFP is approximately 87 μm²/sec
  – Kicheva et al (2007) reported Dpp-GFP to have a $D_{eff}$ of approximately 0.10 μm²/sec during Drosophila wing development
Conclusions

• NLS diffusion is too rapid for manual FRAP experiments
  – NLS fluorescence returns to a stable level in less than 1 second
  – Relative size (kDa) between GFP and NLS may contribute (GFP is 27kDa)
  – Better time resolution may be needed to observe an exponential recovery
Conclusions

• Single cell control indicates that H2A can migrate between nuclei
  – Cell membranes are not fully formed between nuclei
  – Multiple cell control verifies that production is unlikely
  – Since H2A is expected to constantly bind DNA, why is it diffusing between nuclei in the embryo?
Future Propositions

• H2A experiments must be redone
  – Include diffusion between nuclei
  – Perform a complete embryo bleach to be sure of no generation
  – Perform for longer time intervals (20 minutes instead of around 10)
• NLS experiment must be performed with higher time resolution
Future Propositions

• Nuclear bleach experiments must be performed to better quantify diffusion between nuclei
  – Bleach 1, then 2, then 3, etc. to see whether the $D_{\text{eff}}$ is approximately the same
  – Perform full nuclei bleaches at different stages of development
    • Nuclei change size during different stages of development (larger at early stages)
References

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