



Characterizing muscle contraction strength in *Drosophila* embryos by intensity increase in GFP transgenically expressed in muscles.

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Abstract

Drosophila embryos provide a good model system for various developmental processes. They develop quickly, hatching with 24 hours post fertilization, thus allowing for high-throughput screens. They are also nearly transparent, and can thus be easily imaged while still alive. As part of the hatching process, embryos go through a series of muscle contractions that progress from either the anterior or posterior to the opposite end. Defects in these contractions have been characterized for a number of mutant and transgenic fly lines. However, the relative strength of these contractions has not been well characterized. Here we use a GFP construct expressed specifically in muscles as an indicator of contraction strength. We find that while individual contractions may increase or decrease in strength over the course of their progression, on average they get weaker as they move from one end to the other in wild type flies. This wild type profile provides a standard against which mutant lines may be compared.

Introduction

Development of *Drosophila* embryos from fertilization to hatching has been well characterized. Embryonic development has been divided into 17 separate stages. At the end of stage 17, embryos undergo a series of peristaltic contractions of the muscles just prior to hatching (Crisp, Evers *et al.*, 2008). Some *Drosophila* mutants undergo abnormal embryonic contraction patterns. These patterns can lead to defects including failure to hatch, and may be diagnostic of neurological disorders (Suster and Bate, 2002; Song, Onishi *et al.*, 2007). Most often embryonic contractions are measured in terms of frequency and direction. Here we use transgenic flies expressing *Myosin heavy chain-GFP* construct (MHC-GFP) in muscle-specific pattern to estimate the relative strength of contractions as they progress along the body axis in either direction in wild type embryos. Our results indicate that peristaltic contractions decrease in strength on average as they progress, regardless of direction. This information may be used as a point of comparison for relative contraction strengths and patterns in MHC-GFP flies with mutant genetic backgrounds.

Materials and Methods

A fly stock with MHC-GFP protein trap insertion was obtained from Cynthia Hughes (UC San Diego, CA). Adult flies were allowed to lay eggs on agar plates containing apple juice and yeast paste for three hour increments. After three hours, the flies were moved to a new plate and the first plate was incubated overnight. After incubation, stage 17 embryos with air-filled tracheae (when contractions begin) were collected and placed dorsal-side up on glass slides with a thin coating of petroleum jelly to hold them in place. Embryos were then immersed in PBS and imaged on a Zeiss Examiner D1 microscope using a 10× water immersion objective lens. Fluorescence excitation was produced by a X-Cite BDX LED with emission max at 460 nm, and fluorescence emission was captured by a Hamamatsu camera with an ET 525/50 emission filter. Video was taken for 1 hour after contractions began, and integrated fluorescence intensity over time was analyzed using ImageJ software. Embryonic cross-sections 5 μm thick were analyzed at positions 50 μm, 250 μm, and 450 μm from the posterior tip (designated posterior, middle, and anterior, respectively). GFP intensity over time was plotted for each position in Excel. Baseline GFP intensities were calculated by averaging all intensities between peaks. GFP intensity as a percentage above the baseline was calculated according to the equation $\% = 100(x-b)/b$ where x is the total intensity of a given point, and b is the baseline intensity.

Results and Discussion

We recorded several MHC-GFP embryos and were able to detect both forward (posterior to anterior) and backward (anterior to posterior) contractions. The GFP specifically expressed in muscles allowed us to visualize longitudinal shortening of individual muscle segments during contraction, as well as a coincident increase in GFP intensity (Figure 1). This intensity increase is due to the fact that as muscles contract, the concentration of GFP within the focal volume increases. Stronger contractions yield greater shortening of muscle segments than weaker ones, which corresponds to a greater increase in GFP concentration in vicinity of the focal position, and thus a greater GFP intensity. In other words, higher GFP intensity corresponds to stronger contractions.

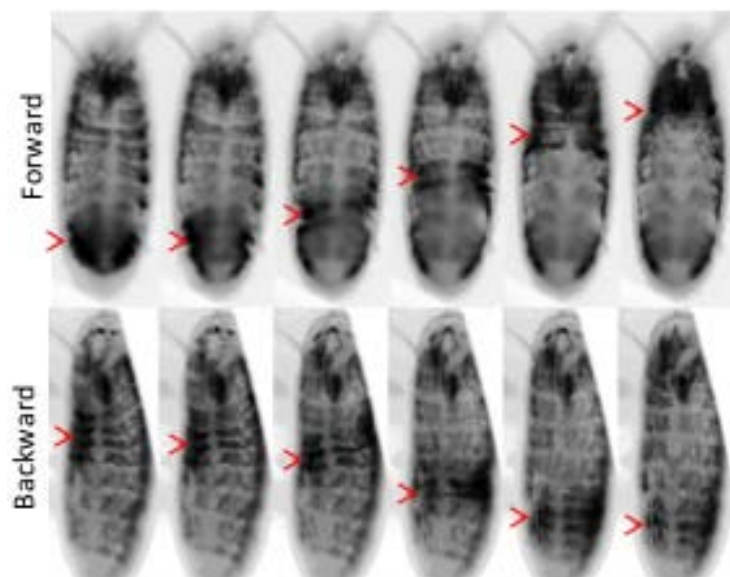
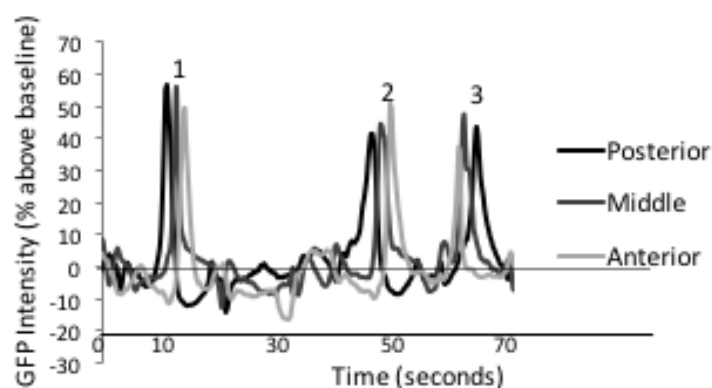


Figure 1. Representative examples of forward (top) and backward (bottom) contraction wave visualization in MHC-GFP embryos in a wild type genetic background. Each image is a single frame from a live video, separated by 2.5 seconds. Images are inverted monochromes, and thus black pixels represent higher GFP intensity. Arrows indicate intensity peak positions for each frame.

backgrounds. Because embryos may not have been placed on slides in a perfectly level manner, it is possible that posterior, middle, and anterior portions did not all lie in exactly the same focal plane. This might lead to artifacts in which one portion of the embryo has a higher GFP baseline intensity than another. Indeed, we found this to be the case in many of the embryos analyzed (not shown). Thus the raw GFP intensities cannot be directly compared. We solved this problem by plotting each graph as percent increase above the baseline, as described. We found several modes of contraction strengths for both backward and forward waves. These included waves in which the GFP intensity decreases as the contraction progresses, waves in which intensity increases throughout the contraction, and waves in which the intensity reaches its peak in the middle of the embryo (Figure 2).



In order to quantitatively estimate relative contraction strength, we plotted GFP intensity over time for positions at the posterior, middle, and anterior regions of several MHC-GFP embryos with wild type

Figure 2. Representative plots of 3 types of contractions. 1) Forward contraction with decreasing intensity from posterior to anterior. 2) Forward contraction with increasing intensity. 3) Backward contraction. Peak intensity is in the middle of the embryo.

The ability to estimate relative contraction strengths at different positions along the embryo also allowed us to generate average contraction strength profiles. We separated backward waves from forward waves in 10 embryos and averaged their peak intensities at each position along the embryo. We found that in wild type embryos, contraction waves decrease in strength on average as they progress. Thus in forward waves the highest intensity is typically at the posterior end, while in backward waves the highest intensity is most frequently at the anterior (Figure 3).

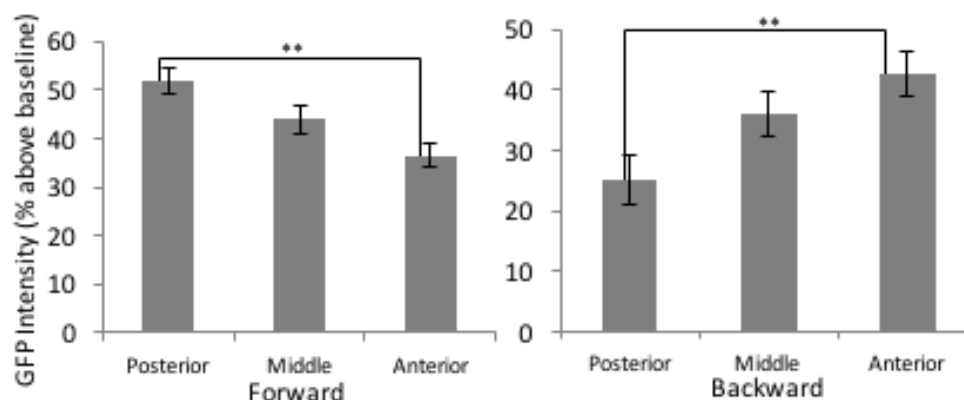


Figure 3. Average GFP intensity at posterior, middle, and anterior positions for forward (left panel) and backward (right panel) waves. In both wave types, contraction is stronger at the point of initiation than at the end point. Ten embryos were analyzed, yielding a total of 50 forward contractions and 24 backward contractions.

Comparison of posterior and anterior intensities by ANOVA analysis yielded p-values of less than 0.01 for both wave types.

Here we have shown that the use of a simple fluorophore transgenically expressed in the muscles of *Drosophila* embryos allows for a quantitative estimate of relative contraction strength within embryos. This technique can be used to examine relative strengths at various points within a given contraction and to generate average contraction profiles. Embryonic contraction waves are an important part of *Drosophila* development and are often affected by mutations, particularly those related to neurological disorders that could disrupt contraction coordination. Therefore the technique described here may be important in characterizing the phenotypes of *Drosophila* mutants by comparing their profiles to that of wild type, and may also help elucidate cellular and molecular mechanisms underlying these phenotypes.

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References: Crisp, S., J.F. Evers, A. Fiala, and M. Bate 1998, *Development* 22: 3707-3717; Hughes, C.L., and J.B. Thomas 2007, *Mol. Cell. Neurosci.* 35: 383-396; Song, W., N. Onishi, L.Y. Jan, and Y.N. Jan 2007, *Proc. Natl. Acad. Sci. USA* 12: 5199-5204; Suster, M.L., and M. Bate 2002, *Nature* 416: 174-148.



***Drosophilidae* (Diptera) attracted to fallen fruits of *cajá-manga* (*Spondias dulcis* Parkinson; Anacardiaceae) in the western region of the city of São Paulo, state of São Paulo, Brazil.**

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Spondias dulcis Parkinson (The Plant List 2015), vernacularly known as *cajá-manga* or *cajarana* in Brazil and as Polynesian plum, hog plum, ambarella, and many other names in other countries (Pio Corrêa,