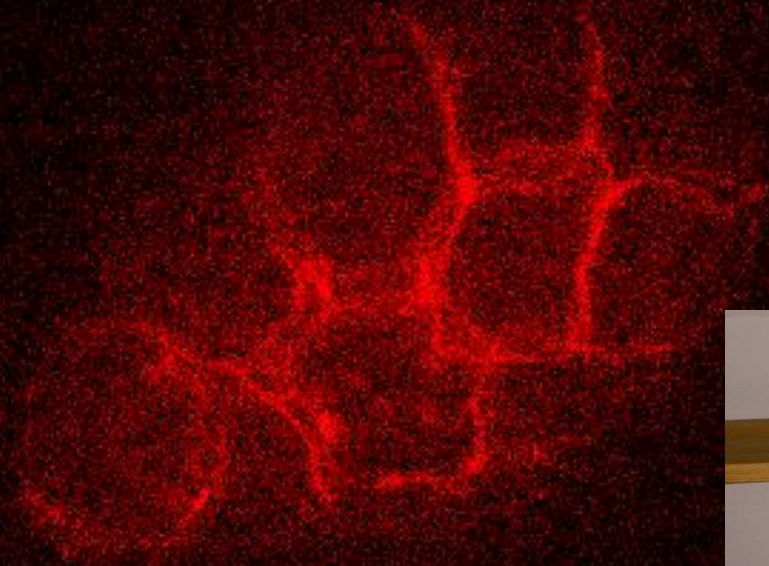


The role of an adhesion based molecular clutch in *C. elegans* gastrulation



Joseph McClellan, Biology

Faculty Advisor: Bob Goldstein, Biology

Summer Undergraduate Research Fellowship, 2009

Background/Goals

- Gastrulation in *C. elegans*
 - Internalization of the two endodermal precursors (E cells) (**Fig. 1**)
 - A cortical actomyosin contraction results in apical constriction
- Gastrulation occurs in 2 phases:
 - A slow phase, in which contact zone dynamics are not coupled to cortical myosin movements
 - A fast phase, in which contact zone dynamics are coupled to myosin movements (**Fig. 2**)
 - The contact zone is where the E cell membrane meets a neighboring cell
- In embryos with compromised cell adhesion, gastrulation fails to occur, and myosin movements are unaffected
 - This suggests that there may be an adhesion based molecular “clutch” linking actomyosin contraction to apical constriction
 - These adhesion deficient embryos lack both HMR-1, an adhesion protein, and CED-5, a Rac activator.

Fig. 1: Gastrulation, ventral view

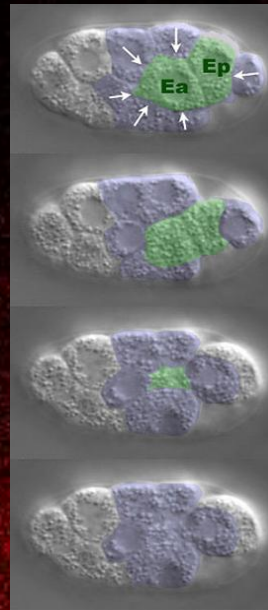
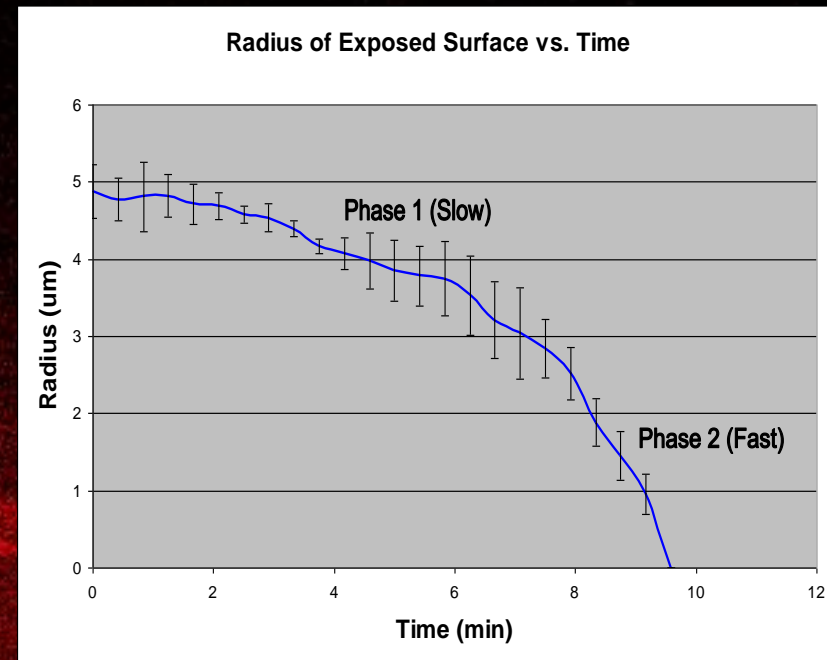


Fig. 2: Two phases of gastrulation



Goals

- To determine whether knockdown of HMR-1 alone causes a defect in gastrulation
 - In the *hmr-1, ced-5* double knockdown, gastrulation fails to occur, but when only *hmr-1* is knocked down, gastrulation seems to proceed normally. My first goal was to look for a noticeable, although subtle, phenotype for *hmr-1* (RNAi).
- To further characterize cortical myosin movements during Phase 1 and 2
 - My second goal was to track individual myosin foci in an attempt to quantify centripetal myosin movements.
 - A better understanding of the cortical myosin dynamics will give us some insight into how the contractile actomyosin network induces apical constriction.

Results

hmr-1 RNAi experiments

- hmr-1 dsRNA was injected into adult *C. elegans*, and early embryos were imaged with confocal microscopy
- Using imageJ software, the radius of the exposed E cell surface was measured from early phase 1 to closure.
- Measurements for hmr-1 (RNAi) were compared to wild type measurements
 - The curve of the graph for wild type reaches a higher rate of change than that for hmr-1 (RNAi) (Fig. 3)
 - Wild type peak velocity: $1.0 \pm .2$ $\mu\text{m}/\text{timepoint}$
 - hmr-1 (RNAi) peak velocity: $0.6 \pm .2$ $\mu\text{m}/\text{timepoint}$
- Conclusion
 - Knockdown of hmr-1 alone does have a noticeable effect on phase 2 gastrulation dynamics, supporting the hypothesis that it plays a role in the molecular clutch.
- Future goals:
 - Compare wild type with CED-5 knockdown and knockdown of other adhesion proteins.

Myosin tracking

- I found and learned how to use an imageJ plugin (MtrackJ) that allowed me to manually track individual myosin foci and calculate their velocities (Fig. 4)
- The average velocities for myosin foci were:
 - Phase 1 – $4.7 \pm .3$ $\mu\text{m}/\text{min}$
 - Phase 2 – $4.3 \pm .2$ $\mu\text{m}/\text{min}$ ($p < .05$)
- Conclusion
 - Myosin foci do indeed move centripetally at the E cell apical cortex; Phase 2 foci move at a slower speed, due to either the drag of pulling the membrane with them or the fact that the cell is smaller at this stage.
- Future goals:
 - Compare myosin rates within a single cell, for example: outer foci and inner foci.
 - Quantify foci velocity compared to nearby membrane velocity between phase 1 and 2.

Fig. 3: hmr-1 (RNAi) rate of radius change vs. that of wild type embryos

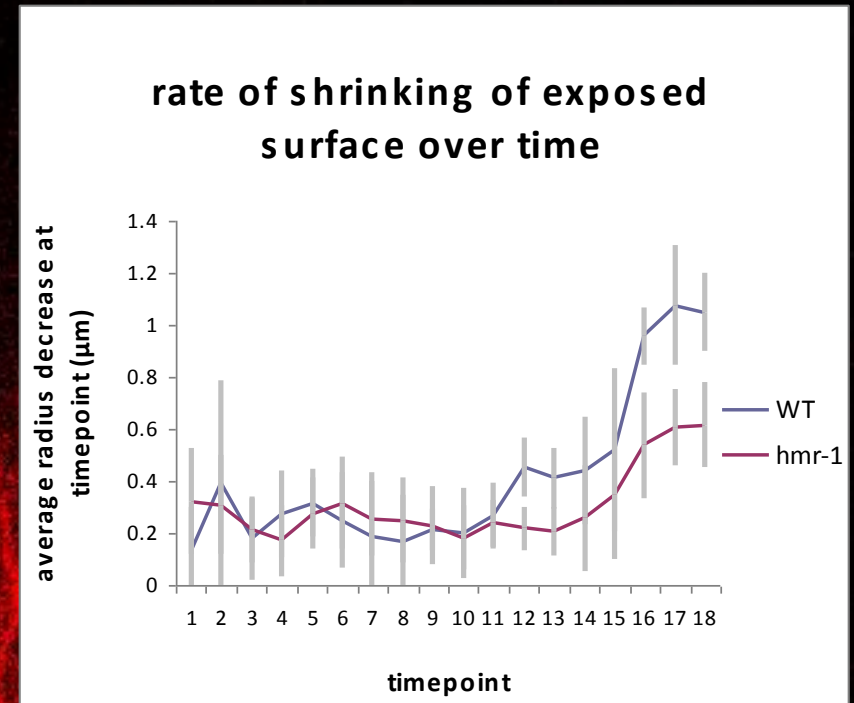


Fig. 4: Left: single frame from movie showing myosin (green) and the cell membrane (red). Right: individual myosin foci were tracked manually

