Which GEF activates Rac1/CED-10 during epidermal morphogenesis?

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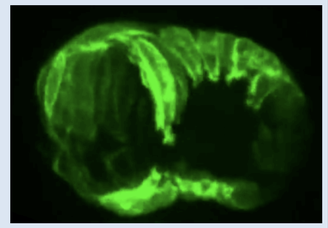
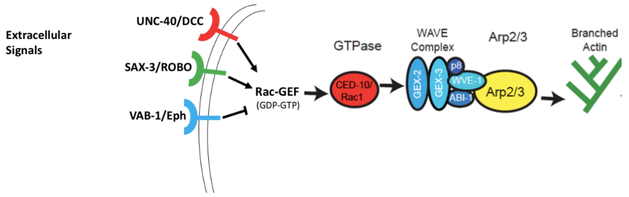


1. Abstract

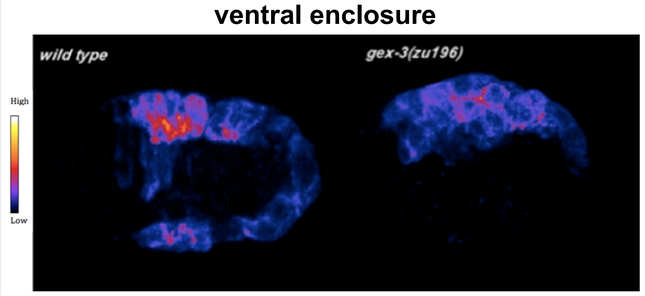
A properly organized actin cytoskeleton promotes cell migrations during embryonic development. Improperly organized actin promotes cell migrations during cancer metastasis. We study *C. elegans* embryonic cell motility and cell shape changes to better understand the transformation of newly differentiated cells into viable tissues during morphogenesis. We have described the morphogenesis role of Rac1/CED-10, a GTPase that activates the nucleation promoting factor WAVE/SCAR. Rac1/CED-10 promotes the collective cell migrations of epidermal ventral enclosure, by recruiting WAVE/SCAR to activate Arp2/3, to create branched actin that powers cell migration. Guanine Nucleotide Exchange Factors (GEFs) regulate GTPase activation and synchronize the position and level of their target GTPases, like CED-10/Rac1. We identified several upstream guidance receptors, SAX-3/ROBO, UNC-40/DCC and VAB-1/EphrinB, that regulate the distribution of Rac1/CED-10 and WAVE, thereby regulating actin levels and organization. Our current goal is to uncover how these receptors activate Rac1/CED-10 by identifying GEFs that connect the receptors to Rac1/CED-10 activation. Candidate Rac1 GEFs have been identified in other organisms, and in various processes in *C. elegans*. For example, TIAM-1 is thought to enable UNC40/DCC regulation of growth cone lamellipodial and filopodial protrusion while CED-5/DOCK180 has been proposed to be the GEF for Rac1/CED-10 during corpse engulfment and axonal migrations. The function of many GEFs is still unknown. While mammals have over 80 GEF proteins, *C. elegans* is predicted to have only 19, yet most have not been characterized for embryonic morphogenesis function. As part of a systematic search for the Rac GEF during embryonic morphogenesis, we have cloned all 19 GEF candidates into RNAi vectors for *C.* elegans and beginning CRISPR tagging and deletion of promising candidates. Surprisingly, we find that CED-5 is not the GEF for Rac1/CED-10 during ventral enclosure. Loss of CED-5 results in increased F-actin at the leading edge of the migrating cells, elevated ARP-2::GFP and elevated GFP::WVE-1, all opposite to what we expect from loss of a CED-10 GEF, and opposite to what we see for loss of *ced-10*.

2. Introduction

Model: pathway showing components involved in activation of branched actin

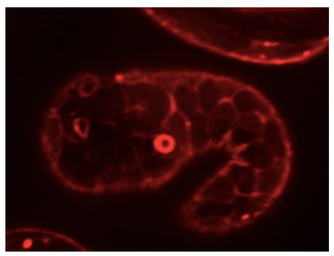


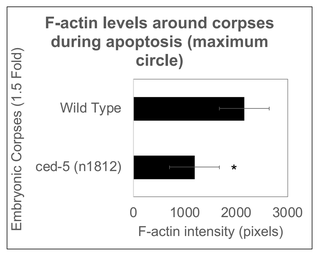
A defect in F-actin affects migration of cells during ventral enclosure

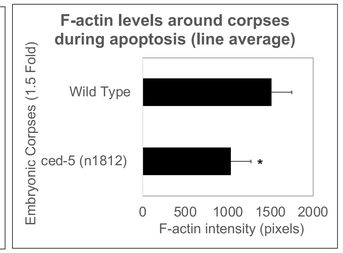


3. Results

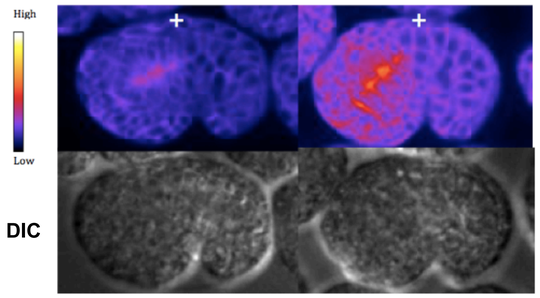
ced-5 mutants causes F-actin level around corpses to decrease during corpse engulfment in *ced-5(n1812); plin-LA-mCherry*



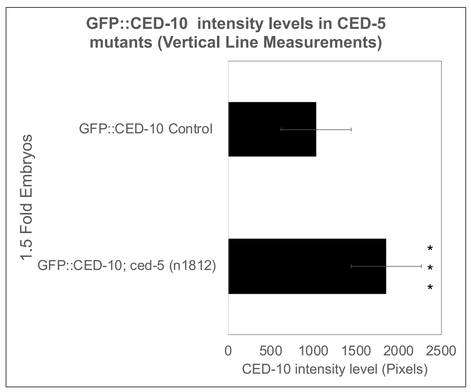


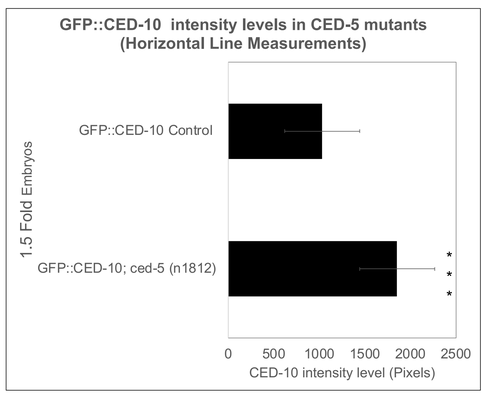


Increase in CED-10 levels in the pharynx in *ced-5* mutants



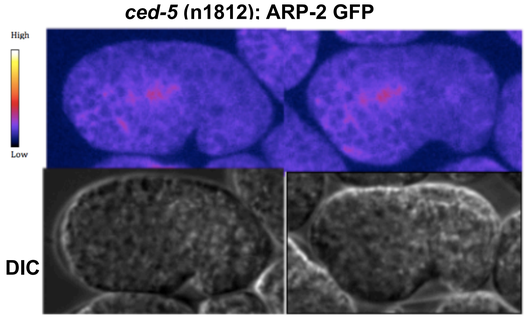
Wild Type **GFP::CED-10; *ced-5* (n1812)**

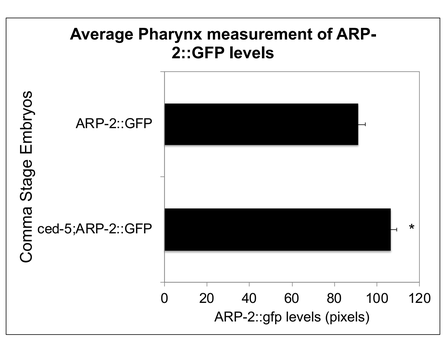




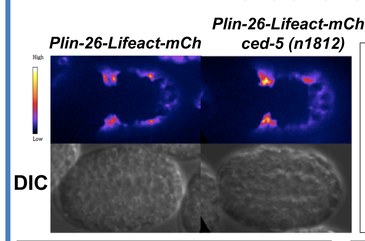
Increase in ARP-2 levels in pharynx of *ced-5* mutants

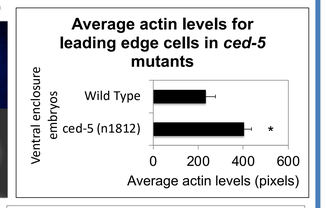
ced-5 (n1812): ARP-2 GFP

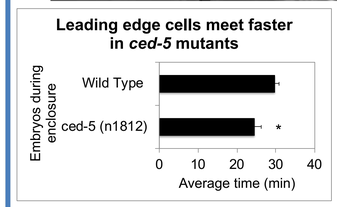


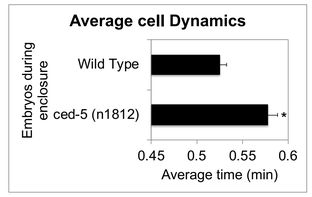


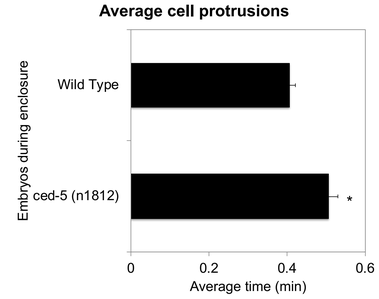
Increased F-actin level in *ced-5* mutants during ventral enclosure



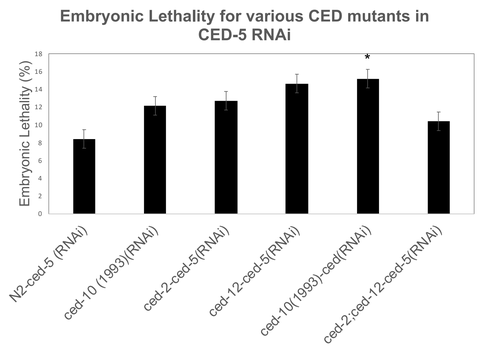








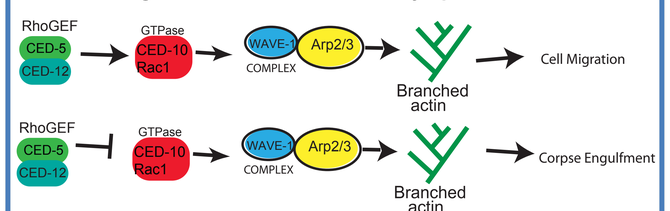
Genetic interaction of CED-5 GEF with other CEDs



C. elegans fed with ced-5 RNAi

4. Conclusion

Model: regulation of branched actin by upstream activators



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