A preliminary investigation into the role of Sipa1l in bipolar tail neurons of Ciona robusta

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Abstract: Bipolar tail neurons (BTNs) in Ciona robusta offer a unique chance to assess regulatory networks that give rise to cell behaviors during development. Like most cells within the embryo of Ciona robusta, BTNs develop following highly stereotyped yet dynamic programs. Neurogenin, a transcription factor, was found to be necessary and sufficient for BTN specification, and SIPA1l was observed to be downstream of Neurogenin activation. The present study sought to elucidate the role of SIPA1l in BTN development, and hypothesized that it was required for collective cell migration of BTNs. The gene encoding SIPA1l, Sipa1l, was reconstructed prior to analyzing its domains and its relatedness to the human orthologs Sipa1l1, Sipa1l2, and Sipa1l3. It was found that this gene in Ciona closely resembles its human counterparts in both the domains present in the protein it encodes, as well as, its amino acid sequence alignment. Sipa1l was then targeted using CRISPR/Cas9 in an attempted knock out condition wherein mixed results were obtained that are limited in interpretation. The fluorescent protein used to assess BTN development only labeled anterior BTNs and thus collective cell migration could not be assessed. Moreover, it was observed that Sipa1l did not affect anterior BTN migration or morphology but did appear to affect axonal outgrowth in select embryos. In all, the results of this investigation provide insights into the role of Sipa1l in BTN development and serve as a preliminary study that will prove useful to future researchers seeking to understand BTN development.
Introduction

Background

*Ciona robusta* is considered a model organism in the study of chordate-specific neurodevelopmental processes. They are members of the tunicate family, which are the closest living invertebrate relatives to vertebrates.\(^1\) Tunicates are a large group of marine organisms, mostly sessile and suspension filter feeders, who possess a thick outer ‘tunic’ composed of crystalline cellulose fibrils.\(^2,3\) Tunicate embryos, many of them motile and capable of swimming a short time before settling on the sea floor, have been intensely investigated by experimental biologists for over 150 years ever since their influential description by embryologist Alexander Onufrievich Kovalevsky in 1866.\(^4\)

Recent interest in *Ciona* has steadily increased in the past decades thanks to advances in genomic techniques.\(^5\) With tunicates being the sister group of vertebrates, they possess many genes, organs, cell types, and processes that are also present in vertebrates.\(^5\) As such, uncovering conserved molecular and genetic mechanisms has relevancy to biomedical contexts. Further, the nervous system of *Ciona* is relatively simple, with its central nervous system containing 177 neurons and its peripheral nervous system containing 53 neurons.\(^7,8\) Given this relative simplicity, these organisms could be considered ideal for investigating gene regulatory networks that underly stereotypic neurodevelopmental processes like as axon migration, polarization, neurogenesis, etc.\(^2,5\)

Notably, *Ciona* is one of a handful of species whose entire genome has been sequenced.\(^9\) This genomic information enables researchers to design experiments using gene modification techniques such as CRISPR/Cas9 to selectively knock out or modify expression of genes believed to be associated with neurodevelopmental processes. A robust method using CRISPR/Cas9 to alter tissue-specific genes in *Ciona* was recently developed (Figure 1).\(^10,11\) This technique, combined with others, enables high-resolution functional genomics and *in vivo* imaging, and makes *Ciona* embryos a tractable model with which to determine conserved regulatory networks that control cell behavior during development.
Rationale and Preliminary Studies

One project our lab has focused on recently is uncovering the cell fate decisions and cell behaviors of Bipolar Tail Neurons (BTNs) in *Ciona*. BTNs are proposed homologs to dorsal root ganglia (DRG) neurons in vertebrates. Similar to DRG neurons, BTNs migrate along the paraxial mesoderm after having delaminated from the dorsal midline ectoderm. They project two extensions during migration: first, one is extended anteriorly from the leading edge; then following a reversal in polarity, another is extended posteriorly. We have shown that expression of the transcription factor Neurogenin (Neurog) is necessary and sufficient for BTN specification. Neurog is known to be important for regulating neural crest and DRG development in vertebrates. Using RNAseq, our lab uncovered a list of candidate genes downstream of Neurog expression that are thought to be important for encoding rate-limiting effectors of neuronal migration, polarity, and axonal outgrowth in BTNs; one of these is Sipa1l (Figure 2).
Sipa1l was found to be upregulated by Neurog in BTN during delamination. In Ciona, Sipa1l is a member of the SIPA1-like (also known as SPAR-like) family of proteins, orthologous to the SIPA family of vertebrates; Sipa1l is a GTPase-activating protein for Rap1, a small GTPase protein. Rap1 has been known to be important for a wide variety of developmental processes, as will be reviewed below. Importantly, Rap1 was recently shown to support collective cell migration in Drosophila by regulating leader-trailer polarity of border cell protrusions. It is important to note that unlike these border cells, BTN display no signs of leader-trailer polarity during delamination. This leads to the question: What influence would Rap1 have on migration if it were active in BTN? Since SIPA1 is known to regulate Rap1, perhaps its expression is inhibiting Rap1 activity in BTN.

The present study provides preliminary results that serve to guide future explorations into the role of Sipa1l and Rap1 during BTN development. CRISPR/Cas9 via electroporation was employed in an attempt to disrupt expression of Sipa1l in Ciona embryos. While it was found that a minority of embryos developed abnormalities associated with axonal extensions, other aspects of BTN development were not observed to be different from the control. The experimental design employed, however, provides uncertainty regarding the success of CRISPR/Cas9 in perturbing Sipa1l expression (substantial limitations with in-person lab work was experienced due to COVID-19). Thus, the role of Sipa1l in BTN development remains to be elucidated in future studies.
Figure 2. *In Situ* hybridization showing Sipa1l expression in *Ciona* embryo. Precursors of aBTN and pBTN are indicated. BTNs delaminate and migrate along the paraxial mesoderm during development. pBTN arise from precursors in the tail tip and migrate anteriorly to meet the aBTN; once joined, they continue migrating as a pair. Image captured 9.5 hours past fertilization (h.p.f.) at 20°C. Source: *Regulation of Neurogenesis by FGF Signaling and Neurogenin in the Invertebrate Chordate Ciona*, Stolfi et al. 2020.
Methods

**Sipa1l Gene Reconstruction, Protein Domain Analysis, and Phylogenetic Analysis**

Analysis showed *Sipa1l* was erroneously split in the genomic databases ([http://ghost.zool.kyoto-u.ac.jp/](http://ghost.zool.kyoto-u.ac.jp/)). The *Sipa1l* gene was therefore reconstructed prior to construct design and experimentation. Additionally, the reconstructed gene was analyzed using protein domain and phylogenetic analyses. Protein domains were assessed using SMART ([http://smart.embl-heidelberg.de/](http://smart.embl-heidelberg.de/)) while its sequence conservation was analyzed using BLAST ([st.ncbi.nlm.nih.gov/Blast.cgi](http://st.ncbi.nlm.nih.gov/Blast.cgi)) and MAFFT ([ft.cbrc.jp/alignment/server/](http://ft.cbrc.jp/alignment/server/)). The results from these analyses were compared to proteins encoded by the *Sipa1l* genes in humans (SIPA1L1, SIPA1L2, SIPA1L3).

**Sipa1l sgRNA Design**

sgRNA targets were selected using CRISPOR ([http://crispor.tefor.net/](http://crispor.tefor.net/)), a web-based portal which generates sgRNA targets using predictive algorithms. The generated sgRNA targets were chosen based on specificity, predicted efficacy, position within the gene, and predicted off-target effects. Three sgRNAs were previously designed and made by the lab prior to commencement of this project (Table 1), all targeting regions of exon 4; in addition to these three, three more sgRNAs were designed and tested to use in the event that the premade ones did not work.

**Table 1.** sgRNAs used to target Exon 4 of *Sipa1l*. Cyan indicates protospacer-adjacent motif (NGG).

| Sipa.96.Fw  | CCCCCTAGTGTTGTGTTAGCT |
| Sipa.35.Fw  | CCACACTTACCTTCCATCGTT |
| Sipa.140.Rv | GAATTGAACATGGGACGATGG |

**Animal Handling and Electroporation of Plasmids into *Ciona* Embryos**

Adult *Ciona robusta* (intestinal Type A) were sourced from M-REP in San Diego California between March and April 2021 and maintained in aquariums containing artificial sea water (ASW) at 24°C until time of experimentation. Eggs and sperm were harvested by dissection and pooled together in filtered ASW at 20-24°C. Fertilized eggs were dechorionated by mixing in a solution containing 0.3 g sodium thioglycolate (in 40 ml of ASW), 2.5% pronase (in 1.2 ml of ASW), and 10N NaOH (168µl). Degradation of the chorion was confirmed through visual
inspection with a microscope and were then washed five times in ASW to remove chorion debris. Following these washes, fertilized eggs were transferred to low-adhesion 2-ml tubes using a Pasteur pipette and excess ASW was removed. Fertilized eggs were then added to a solution containing constructs in H2O with 0.96 M D-mannitol. This mixture was then added to a cuvette and electroporated with a time constant between 16-18 ms being noted. Electroporations occurred within 30 minutes after fertilization. Electroporated embryos were incubated at 20°C. Electroporations were used in the CRISPR experiment and the GFP reporter analysis (Table 2 and 3).

**Table 2.** Electroporated constructs for CRISPR/Cas9.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Mass (µg)</th>
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<tbody>
<tr>
<td>Fog&gt;Cas9</td>
<td>30</td>
</tr>
<tr>
<td>Fog&gt;H2B::mCherry</td>
<td>10</td>
</tr>
<tr>
<td>Islet-5915/-5356+bpfog&gt;Unc76::GFP (labels aBTNs)</td>
<td>80</td>
</tr>
<tr>
<td>U6&gt;Sipa1l-35 (sgRNA)</td>
<td>20</td>
</tr>
<tr>
<td>U6&gt;Sipa1l-96 (sgRNA)</td>
<td>20</td>
</tr>
<tr>
<td>U6&gt;Sipa1l-140 (sgRNA)</td>
<td>20</td>
</tr>
</tbody>
</table>

**Table 3.** Electroporated constructs for candidate GFP reporter.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Mass (µg)</th>
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<tbody>
<tr>
<td>Asic-3144/41::Msc</td>
<td>70</td>
</tr>
<tr>
<td>Coe-80&gt;Sipa:NTER::GFP</td>
<td>60</td>
</tr>
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**Fixation and Imaging of Electroporated Embryos**

Following incubation, embryos were added to a fixing solution containing. After incubation for 20 minutes at room temperature, embryos were washed with a solution Embryos were incubated again for 20 minutes at room temperature and washed a final time with a solution of 1X PBS/0.1% Triton-X100. Embryos were then added to a mounting medium. This was then transferred to glass slides using a glass pipette. A Leica DMI8 was used to image the mounted embryos.
Results

The expression and structure of SIpa1l in Ciona and humans

Protein domain analysis was conducted using SMART (http://smart.embl-heidelberg.de/), a web-based application that provides common protein domains given a protein sequence. The SIpa1l gene in Ciona encodes a protein that contains 1349 amino acids with a molecular mass of approximately 173.3 KDa. SMART revealed several domains found within this protein, and perhaps most notably, a PDZ domain (position 628-694), a RapGTPase-activating (RapGAP) domain (position 475-537), and a Coiled-coil domain (position 1459-1506) (Figure 3). In humans, the coiled-coil domain is a leucine zipper, and the Ciona ortholog is predicted to be the same.46

Figure 3. Protein Domain organization of SIpa1l in Ciona and humans. Illustrated are the domain organizations of SIpa1l in Ciona and humans. Pink regions indicate regions of low complexity; RAP and PDZ domains are as labeled; green regions represent Coiled-coil domains; Vertical dashes represent intron regions. (A) Domain organization of the reconstructed SIpa1l in Ciona. (B) SIpa1l1 organization in humans. (C) SIpa1l2 organization in humans. (D) SIpa1l3 organization in humans.

SMART analysis was also conducted on human orthologs of SIpa1l: SIpa1l1, SIpa1l2, and SIpa1l3. As in the Ciona, all three contained a PDZ domain and a coiled-coiled domain. Curiously, SMART displayed no Rap-GAP domain in any of the human proteins, but further investigation using Uniprot (Uniprot.org) showed these domains are indeed present. This is further supported by the literature wherein it is shown that all proteins belonging in the SIpa1l
family possess a RapGAP domain.\textsuperscript{46,47} This absence is expected to be an oversight of the algorithms used by SMART. Moreover, these domains are found in similar position along the protein in each species: the RapGAP domains appear at approximately the first third of the sequence, the PDZ domain is located approximately midway, while the leucine zipper is located at the very end for all four.

\textbf{Figure 4. Phylogenetic comparison of SIPA1l in Ciona and human orthologs.} MAFFT was used to create a phylogenetic tree to assess the relatedness between human orthologs and SIPA1l in Ciona. SIPA1l2 in \textit{Trichinella nelson} (a nematode) was included as an outgroup. Comparison showed Ciona Sipa1l being equally related to its human counterparts. SIPA1l1 and SIPA1l2 diverged from SIPA1l3 following a duplication event, then diverged from each other following an additional duplication.

MAFFT analysis revealed alignment of protein domains shared between between Ciona and humans (Figure 5). Alignment of these domains exist despite there being only a 47.65\% identity in the human proteins, according to BLAST. Construction of a phylogenetic tree was also accomplished using MAFFT (Figure 4). It can be seen that SIPA1l in Ciona is equally related to SIPA1l1, SIPA1l2, and SIPA1l3. Also, SIPA1l1 and SIPA1l2 emerged following two duplication events and are more related to one another than to SIPA1l3. In all, it is clear the SIPA1l in
Ciona is orthologous to SIPA1l1, SIPA1l2, and SIPA1l3 and that it shares similar domains that may perform analogous functions in certain contexts.

SIPA1l KO may result in abnormal development of BTNs

CRISPR/Cas9 was used in an attempt to KO expression of SIPA1l in BTNs. To assess the efficacy of this targeted KO, Islet-5913/-5356+bpfog>Unc76::GFP (green) was used to label
the aBTNPs and Fog>H2B::mCherry was used to label the H2B protein in cell nuclei (magenta) (Figure 6). The treatment group, in which sgRNA plasmids were co-electroporated with the reporters and Fog>Cas9, did not display any substantial alterations in morphology or position along the tail, nor was the overall number of aBTNPs affected compared to the negative control (Figure 6, A, B, and C). Select embryos did, however, show signs of abnormal axonal outgrowth (Figure 6, D). Indeed, unusual placement of portions of the axons with sharp kinks were observed in these embryos in addition to apparent synapsing with neighboring axons. Taken together, these results suggest that Sipa1l appears to have no apparent effect on morphology or migration of aBTNPs, but it may affect axonal outgrowth.

Figure 6. CRISPR/Cas9-mediated knockout of Sipa1l. (a) Negative control condition in which no Sipa1l sgRNAs were electroporated. (b) & (c) Representative images showing embryo in treatment possessing BTNPs indistinguishable from those of the negative control. (d) Representative Image showing abnormal
axon outgrowth in a select few embryos electroporated. Red Arrow indicates possible synapsing with axon of neighboring BTN. Embryos were fixed 17 hours after fertilization.

**Localization of SIPA1l Fragment in BTNs**

Coe-80>Sipa:NTER::GFP was designed and tested as a fluorescent reporter to label expression of Sipa1l in BTNs and to assess subcellular localization (Figure 7). Asic-3144/41::Msc was coelectroporated to label the nucleus. Resulting images show this construct effectively labeled BTNs and expression of the Sipa1l fragment appeared uniformly distributed across the cell body, as well as, portions of the axons.

![Image of localization of SIPA1l Fragment in BTNs](image)

**Figure 7. Sipa1l GFP Reporter.** Coe-80>Sipa:NTER::GFP reporter was tested using electroporation. Representative image demonstrates this construct successfully labeled aBTN (top left). Asic reporter (top right) was previously shown to successfully label BTNs and was included here as a control. The bottom middle shows a merged image where overlapping areas are white. Embryos were fixed 14.5 hours after fertilization.
Discussion

The present study sought to provide a preliminary look at the role of Sipa1l during BTN development. Notwithstanding substantial limitations that were met with regards to in-person experimentation, due to COVID-19, meaningful insights were still gathered that will help guide future researchers seeking to understand Sipa1l’s role in cell behaviors of Ciona and humans. Elucidating these roles remain important and may help discern mechanisms underlying abnormal development of dorsal root ganglion neurons (proposed homologs to BTNs) or may be applicable to the development of therapeutics that target cancer metastasis (in which a better understanding of collective migration could prove vital).

Sipa1l is activated downstream of Neurog in Ciona, a transcription factor that was found to be necessary and sufficient for BTN specification.13 Genes downstream of Neurog are thought to be important for encoding rate-limiting effectors of migration, axonal outgrowth, and polarity in BTNs. At the onset, the present study hypothesized that Sipa1l might play an important role in the regulation of Rap1 (a small GTPase protein) in BTNs. Specifically, given that Sipa1l is known to act as a RapGAP and inhibit Rap1 activity in other contexts, it was thought that a Sipa1l KO might enable Rap1 to promote certain developmental processes that would otherwise not emerge due to inhibition by Sipa1l.48

After delamination from the dorsal midline ectoderm, BTNs migrate as a pair on either side of the neural tube. These pairs consist of an anterior BTN (aBTN) and a posterior BTN (pBTN) and migrate as a cell collection, exhibiting no leader-trailer polarity.13 Indeed, each cell within this pair contains its own leading edge when migrating, making it resemble epithelial sheet migration rather than other modes of collective migration, such as is found in cancer metastasis or the Trunk Ventral Cells of Ciona.36,49,52 This lack of leader-trailer polarity is notable because it may be, in part, due to the suppression of Rap1 by Sipa1l via its RapGAP domain (Figure 3). In Drosophila Border Cells, Rap1 was shown to support leader-trailer polarity through regulation of protrusive activity; wildtype border cells demonstrate polarity of protrusive activity in border cell clusters, while interference of Rap1 induced absence of this polarity and disruptions in collective cell migration.17

Given the experimental design employed in the current study, it was not possible to assess the effect which a Sipa1l KO would produce on collective cell migration of aBTN and pBTN. The GFP reporter used in the CRISPR/Cas9 experiment labeled only the aBTN making it
impossible to discern changes in collective migratory characteristics. Nevertheless, it was shown that a Sipa1l KO produced mixed effects in aBTN s, having no impact on the number, morphology, or migration of aBTN s but inducing apparent disruptions in axonal outgrowth (Figure 6). Indeed, select embryos possessed axons which made sharp deviations while migrating along the tail with some synapsing to neighboring axons (Figure 6, D). This may possibly be due to the activity of Rap1, which is known to be involved in promoting neurite outgrowth via regulation of cytoskeletal dynamics and the MAPK cascade. Without inhibition by Sipa1l, Rap1 could be accelerating outgrowth and causing disruptions in the cytoskeleton of BTN axons. This inference is supported by the results of the protein localization analysis wherein expression of Sipa1l fragments were observed in the axons of BTN s (Figure 7); though it should be noted that the Sipa1l fragment that was labeled was observed to be distributed uniformly within the cell.

Furthermore, an extensive comparison between SIPA1l in Ciona and the human orthologs Sipa1l1, Sipa1l2, and Sipa1l3 was made to determine relatedness and conserved characteristics. SMART analysis revealed SIPA1l contains three major domains: a RapGAP domain, a PDZ domain, and a coiled-coil domain. The presence of these domains was also observed in the three human Sipa1l proteins analyzed (Figure 3). Although, SMART failed to identify the RapGAP domain within the human proteins but follow-up analysis using Uniprot showed these domains are indeed present in SIPA1l1, SIPA1l2, and SIPA1l3.

Additionally, the placement of these domains along the protein and alignment, determined using MAFFT, closely matched the human orthologs (Figure 3, Figure 6). The location along the portion of the proteins where the RapGAP domain resides is approximately 233 amino acids long, and 138 of these were found to be identical across all four proteins (59%). Given the strong resemblance across these proteins, it is expected that their regulation of Rap1 developmental processes are likely to be highly conserved.

The PDZ domain also bore great similarity with matching sequence lengths and 38 out of 78 (49%) amino acids being identical. All proteins belonging to the Sipa1l family possess both a domain that interacts with RAP as well as a PDZ domain. One of the functions possessed by Sipa1l due to its PDZ domain is the ability to bind and regulate aquaporin-2, a channel in cell membranes that traffics water in and out of cells. It is believed that the tight conservation of this domain observed between humans, Ciona, and other species is due to it being essential for
maintaining homeostasis. These results domain analyses combined with the phylogenetic tree (Figure 4) (which showed Ciona being more closely related to humans than nematodes) demonstrate Ciona’s relatedness to humans and provide motivation for using it as a model organism to uncover conserved regulatory networks controlling cell behaviors.

**Future Directions and Conclusion**

Future studies seeking to understand the role of SIPA1l in collective migration will need to consider perturbing Rap1 expression. This may be done through: (1) expressing a constitutively active form of Rap1 (RapV1); (2) expressing a dominant-negative version (Rap1-N17); and (3) by over-expressing RapGAP, which inhibits Rap1 activity. These conditions should also be paired with and without a Sipa1l KO background. Additionally, the use of a fluorophore which labels both the aBTN and the pBTN will prove vital in ascertaining the impact of these conditions on collective cell migration.

In all, uncovering the role of Sipa1l in developmental contexts remains an aim worthy of consideration. The current investigation provided a bioinformatic background that described the structure and function of the SIPA1l protein in Ciona and humans; the reconstructed form of the Sipa1l gene in Ciona and the domains identified will prove valuable to future studies. Moreover, a simple, yet insightful, CRISPR/Cas9 experiment was conducted in which Sipa1l was found to have no effect on aBTN, aside from slight abnormalities present in axonal outgrowth of select embryos. Despite the limitations in the ability to interpret the results of this CRISPR experiment, this was an important first step in a much longer goal of elucidating the roles of SIPA1l and Rap1. Ciona is an excellent model with which to study the conserved developmental processes and characterizing the regulatory networks that give rise to BTN specification may prove immensely valuable to human contexts.
Literature Review

Cell behaviors such as migration, differentiation, and polarization arise in development due to complex interactions of gene regulatory networks. Cells may transiently enter regulatory states based on the activity of these networks and engage in behaviors at specific points in time during development. Many researchers seek to uncover the molecular mechanisms underlying these transitions to regulatory states and the subsequent effects exerted on behavior. Despite much progress being made in the past decade, thanks to advances in genomics and molecular techniques (e.g., CRISPR/Cas9), we have yet to come to a comprehensive understanding of how these processes are facilitated in even the simplest of nervous systems, as found in C. Elegans and Ciona Robusta. Here, a review is included to characterize Rap1 and Sipa1l, and their roles in regulating the motility of cells and cell collections during development.

Overview of Sipa, Rap, and Collective Migration

Raps are a family of small signaling molecules which drive a host of different cellular processes and importantly, in the context of cell migration, they are known to control cell-cell junction formation and adhesion. Structure, they consist of small guanosine tri-phosphate (GTP)-binding proteins and cycle between an active and inactive state by switching from binding GTP to binding guanosine di-phosphate (GDP) — i.e., they engage in GTPase activity. Raps GTPase activity is regulated by GTPase activating proteins (GAPs). The two main groups of RapGAPs are RapGA1 and the Sipa1-family of proteins (also known as Spa-family). The acronym “Sipa” stands for signal-induced proliferation-associated gene. These genes express proteins that share common characteristics such as possessing a RapGAP domain at the N-terminus, a C-terminal coiled-coil (leucine zipper), and a PDZ domain. Researchers seeking to understand the functions of Raps in collective cell migration must consider their regulation by the Sipa family of proteins.

Rap1 is a highly conserved member of the Rap family and has been a primary focus for investigators since the mid 2000’s. The effects of Rap1 were summarized by Kooistra et al. in 2007, wherein they describe Rap1’s importance for the maturation of cell-cell junction adhesions. Junction formation plays an important role during migration. The three primary ways Rap1 regulates junction formation, as described by Kooistra et al. 2007, are: (1) by inhibiting endocytosis of E-cadherins; (2) by activating E-cadherins via Afadin6, an effector of Rap1; and (3) by remodeling the actin cytoskeleton.
Efforts have also been made to elucidate the role of Rap1 in collective cell migration. Collective cell migration is a process by which cells move as a single unit, maintaining attachment to one another. Three stereotypical processes which drive motility of most cell collections are: (1) the emergence of polarity-driven F-actin protrusions from the leading edge; (2) the establishment of adherence between the motile cells and the extracellular matrix (ECM) or surrounding cells; and (3) retraction of the trailing edge through adhesion breakage.\textsuperscript{17,36}

**Rap1 and Sipa1 in cell and collective migration**

In vertebrates, Rap1’s roles are highly conserved with its signaling being a known component in axonal pathfinding, neuronal differentiation, establishment of polarity, closure of the neural tube, and activin-induced convergent extension during gastrulation.\textsuperscript{38-42} Of particular relevance for the present proposed study, however, is Rap1’s influence on cell and collective migration. Although there exists of plethora of research regarding Rap1’s role in migration during cancer metastasis (see Zhang et al. 2017 for a review\textsuperscript{37}), the following paragraphs will briefly summarize the most important findings from the developmental literature relevant to the present study.

Rap1’s signaling pathway is known to be involved in several processes that support the motility of certain cells.\textsuperscript{20} Migration of single cells during chemotaxis requires a coordinated effort of F-actin protrusions from the leading edge, and contraction of the trailing edge in response to an external chemical gradient. In *Dictyostelium*, Rap1 mediates these outcome through its effector Phg2.\textsuperscript{43} Rap1 signaling results in asymmetrical reorganization of the actin cytoskeleton, disassembly of myosin at the leading edge, regulation of cell adhesion to the substratum, and formation of a gradient of cellular components.\textsuperscript{43-45} These effects can be regulated by GAPs, like SIPA1l, and ultimately result in motion toward a chemoattractant or away from a chemorepellant.\textsuperscript{43}

The roles of Rap1 and its regulator SIPA1l in collective migration is not well understood. A paper which motivated and guided the present study was published by Sawant et al. 2018 and looked at the role of collective migration of border cells in *Drosophila*.\textsuperscript{17} They demonstrate that Rap1 activity is maintained at precise, transiently-fluctuating levels through activation by PDZ-GEF and inactivation by RapGAP1.\textsuperscript{17} Gain-of-function of Rap1 was observed to increase E-cadherin formation between border cells and nurse cells and prevent detachment of some cells from the epithelium; Rap1 loss-of-function differentially impacted E-cadherin formation between
border cells resulting in some instances where cells detached from the border cell cluster.\textsuperscript{17} Additionally, proper Rap1 levels were shown to be critical for maintaining proper length and size of polarized protrusions from the leading edge of the border cell cluster.\textsuperscript{17}

This study by Sawant et al. 2018 is relevant to the present study due to the contrasts that exist between these border cells and the BTNs in \textit{Ciona}; a key difference being that BTNs display no leader-trailer polarity during delamination. We hypothesize that this is due to Rap1 inhibition by Sipa1l and will seek to test this in future studies using CRISPR/Cas9 to KO expression of Sipa1l and perturb expression of Rap1 by using a dominant-negative and constitutively active form.
References


