Identifying regulators of directed neuroblast migration in *Caenorhabditis elegans*

Vitoria Paolillo and Erik A. Lundquist

Molecular Biosciences, The University of Kansas, Lawrence, KS, USA

Abstract

Guided neuronal migration is an essential process during nervous system development. The Q cell neuroblasts in *Caenorhabditis elegans* provide a simple and experimentally tractable model system for studies of directed neuronal migration. The Q neuroblasts are born in the same region of the animal and have similar differentiation patterns, yet undergo left-right asymmetric migration, with QR on the right migrating anteriorly and QL on the left migrating posteriorly. QL descendants encounter a posterior EGL-20/Wnt signal, which activates a canonical Wnt signaling pathway to induce expression of the Hox gene *mab-5* in QL and QL descendants, but not in QR and QR descendants. MAB-5 is both necessary and sufficient for posterior Q cell descendant migration, as QL descendants migrate anteriorly in *mab-5* loss-of-function (LOF) mutants, and QR descendants migrate posteriorly in *mab-5* gain-of-function (GOF) mutants. However, it is unknown what genes are regulated by MAB-5 in the Q cells to drive posterior migration. We isolated Q cells from wild-type and *mab-5* LOF animals via fluorescence-activated cell sorting and completed RNA-seq to generate novel Q cell transcriptomes. We identified 222 genes that were differentially expressed in the *mab-5* LOF Q cells versus wild-type Q cells. We predict that MAB-5 might affect Q cell migration by regulating other transcription factors, RNA processing factors, the cytoskeleton, and cell signaling factors. Thus, we have prioritized candidate transcription factors for functional studies, and identified 13 putative transcription factors that show decreased expression in the *mab-5* LOF Q cells versus wild-type Q cells. We predict that these 13 transcription factors require MAB-5 for their expression, and thus may regulate posterior Q cell migration. Indeed, our preliminary functional studies have revealed that several of these putative transcription factors regulate Q cell migration. We anticipate that further functional studies of these candidate *mab-5* targets will reveal new insights into directed Q neuroblast migration.

Background

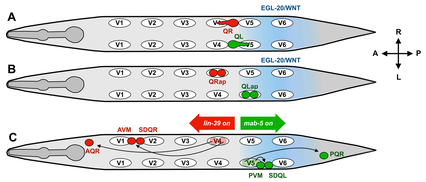


Figure 1: Migration of the Q neuroblasts and their descendants. A) Schematic of initial, Wnt-independent migration of QR and QL. B) First cellular division of the Q neuroblasts to generate QR.ap and QL.ap. C) Wnt-dependent migration of the Q neuroblast descendants. EGL-20/WNT signaling activates the Hox gene *mab-5* in QL descendants, but not in QR descendants. MAB-5 function is necessary and sufficient for posterior migration. MAB-5 inhibits expression of the Hox gene *lin-39*; thus, *lin-39* is expressed in the absence of MAB-5 in the QR descendants and is required for anterior migration.

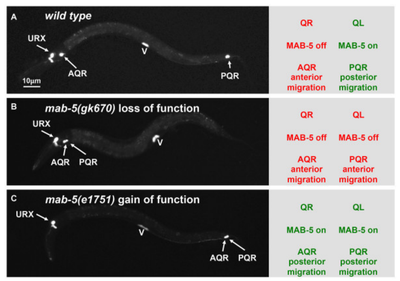


Figure 2: Migration of AQR and PQR in wild-type and *mab-5* mutant animals. Micrographs of L4 animals that express *gcy-32::cfp* (AQR, PQR, and URX neurons) and *egl-17::gfp* (vulva). A) AQR on the right migrates anteriorly, and PQR on the left migrates posteriorly in wild-type animals. B) In *mab-5* loss-of-function animals, both AQR and PQR migrate anteriorly. C) In *mab-5* gain-of-function animals, both AQR and PQR migrate posteriorly. Adapted from Tamayo *et al.,* 2013.

What genes are regulated by MAB-5 in the Q cells?
Which of these MAB-5 targets are required for Q cell migration?

Isolation of the Q neuroblasts for gene expression profiling

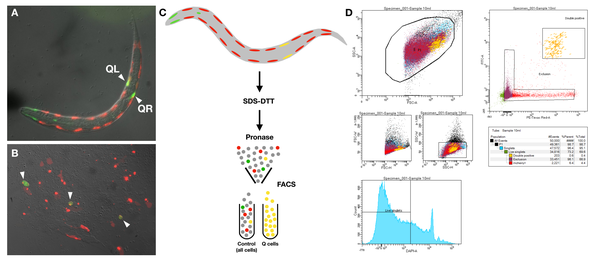


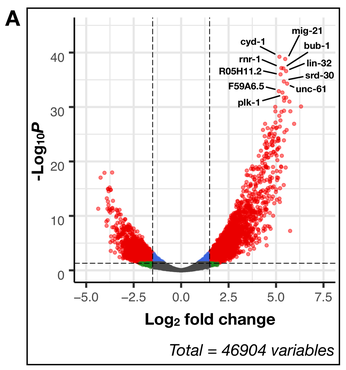
Figure 3: Isolation of the Q neuroblasts via fluorescent-activated cell sorting (FACS). A) L1 animal expressing *scm::rfp* and *Pegl-17::gfp*. Only the Q cells (arrows) express both *rfp* and *gfp*. B) Dissociated cells with double-positive Q cells (arrows). C) Schematic of the cell sorting work flow. D) Example of a Q cell sort.

Name	Genotype
wild-type	*lqIs97[scm::rfp]; ayIs9[Pegl-17::gfp]*
mab-5 LOF	*lqIs97[scm::rfp]; ayIs9[Pegl-17::gfp]; mab-5(gk670)*

Table 1: *C. elegans* strains utilized in FACS and RNA-seq analysis.

FastQC/fastp → HISAT2 → featureCounts → DESeq2

Figure 4: RNA-seq workflow. Quality control and preprocessing of FASTQ files were completed using FastQC and fastp. Reads were aligned to the *C. elegans* reference genome (release WBcel235) using HISAT2 and counted using featureCounts. Differential expression amongst samples was determined using DESeq2.



B

Genes enriched in the Q cells	
ani-1	*mig-5*
cam-1	*mig-13*
ceh-20	*mig-15*
cki-1	*mig-21*
ect-2	*mom-5*
egl-46	*nmy-2*
hlh-2	*pig-1*
lin-26	*pop-1*
lin-32	*pry-1*
lin-39	*toe-2*
mab-5	*unc-40*
mig-2	

Figure 5: Differential expression of wild-type Q-expressed genes versus whole animal. A) Volcano plot depicting 5,565 genes that were differentially expressed in wild-type Q cells relative to the whole animal (adj. p-value < 0.05, log2-fold change 0.58), with 3,084 of those genes enriched in the Q cells, and 2,481 genes depleted in the Q cells. B) Genes that have been previously shown to be expressed in the Q cells were also found to be enriched in our RNA-seq Q cell-specific analysis.

Differential expression analysis

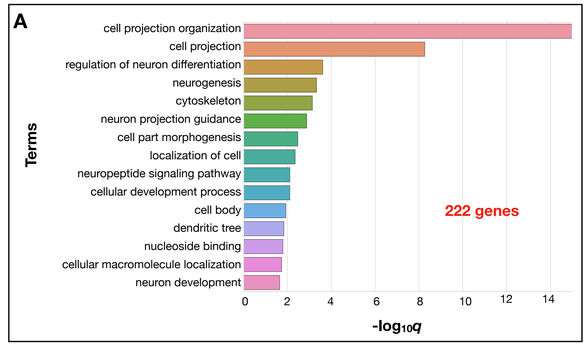


Figure 6: Gene ontology term (GO term) analysis. GO term analysis was performed via WormBase on the 222 differentially expressed genes identified from the *mab-5* LOF Q cells vs wild-type Q cells analysis. Top GO terms included cell projection organization, cell projection, localization of the cell, and the cytoskeleton, consistent with the role of *mab-5* in regulating cell migration. Neuron projection guidance, neurogenesis, and regulation of neuron differentiation were also significant GO terms, consistent with the neuronal identity of the Q cells and in the role of *mab-5* in their directed migration.

	UP (18 genes)	DOWN (204 genes)
Transcription factors	3	13
Transmembrane	5	55
Secreted	1	29

Table 2: Differentially expressed genes in *mab-5* LOF Q cells relative to wild-type Q cells. The number of predicted transcription factors, transmembrane molecules, and secreted molecules are indicated.

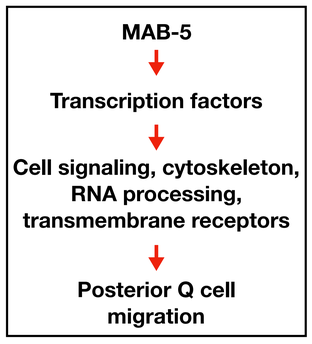


Figure 7: MAB-5 might regulate Q cell migration by acting through transcription factors, cell signaling factors, and/or transmembrane receptor molecules.

Table 3: Genes of interest. 16 predicted transcription factors were identified in the *mab-5* LOF Q cells vs wild-type Q cells analysis. As expected, *mab-5* (red, bold) displayed decreased expression in the *mab-5* LOF.

Gene	log_2 Fold Change (LOF vs WT)
sem-2	-2.955
hlh-14	-2.246
pag-3	-2.189
fkh-5	-2.098
mnm-2	-1.908
atf-2	-1.577
fkh-8	-1.575
elf-1	-1.570
mab-5	-1.477
egl-13	-1.328
ceh-48	-1.208
ztf-11	-0.938
attf-4	-0.865
nhr-150	1.710
nhr-39	2.054
nhr-290	2.173

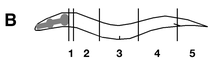
Functional analysis of predicted transcription factors

A

Genotype	AQR position (%) 1	2	3	4	5	PQR position (%) 1	2	3	4	5
wild-type	100	0	0	0	0	0	0	0	0	100
sem-2(n1343)	97	2	1	0	0	0	0	0	1	99
sem-2(ok2422)	97	2	0	0	1	1	0	0	11	88
pag-3(ls20)	100	0	0	0	0	1	0	0	1	98
pag-3(n3098)	100	0	0	0	0	0	0	0	2	98
ceh-48(ok1395)	100	0	0	0	0	0	0	0	2	98
attf-4(ok1390)	98	2	0	0	0	1	0	0	0	99
ztf-11(ok646)	99	1	0	0	0	0	0	0	2	98
elf-1(tm801)	97	3	0	0	0	0	1	0	2	97
egl-13(lq94)	96	3	1	0	0	0	1	0	6	93
egl-13(ku194)	97	3	0	0	0	0	0	0	94	6
egl-13(n483)	94	5	1	0	0	0	0	0	9	91

Significantly different ($p < 0.01$, $p < 0.001$) compared to wild-type (Fisher's Exact Test)

B



C

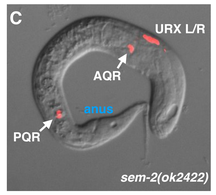


Figure 8: Predicted transcription factors regulate Q cell migration. A) Table displaying the percentage of AQR and PQR migration defects in mutant animals. n ≥ 100 B) Positions along the body used to score AQR and PQR migration defects in mutants in (A). C) A *sem-2(ok2422)* animal with anterior PQR migration defect.

Summary and Future Work

Summary

- Differential expression analysis between the *mab-5* LOF and wild-type genotypes revealed 222 genes that were differentially expressed in the Q cells, with 204 genes downregulated in the LOF and 18 genes upregulated in the LOF.
- We determined that 16 predicted transcription factors are differentially expressed in the LOF Q cells versus wild-type Q cells.
- Functional analysis revealed that the transcription factors SEM-2 and EGL-13 have a significant effect on Q cell migration, particularly in posterior Q cell migration. However, all transcription factor mutants analyzed have weak AQR and/or PQR migration defects compared to wild-type animals.
- Our data indicate that these transcription factors play a role in regulating Q cell migration. We predict that MAB-5 functions by inducing expression of these transcription factors, and that they, in turn, function to promote Q cell migration.

Future Work

- RNA-seq and differential expression analysis of additional *mab-5* mutant strains.
- Functionally characterize all transcription factors identified in the LOF versus wild-type differential expression analysis, including generating double and triple transcription factor mutants.

Support and References

Support

- Kansas INBRE, P20 GM103418
- CMADP-COBRE
- Thank you to Dr. Erik Lundquist and the Lundquist lab for support of this project.

References

Tamayo, J.V., Gujar, M., Macdonald, S.J., and Lundquist, E.A. (2013). Functional transcriptomic analysis of the role of MAB-5/Hox in Q neuroblast migration in Caenorhabditis elegans. BMC Genomics *14*, 304.