Identification of mRNA targets of ETR-1/CELF that regulate Q neuroblast migration in muscle cells using fluorescence-activated cell sorting and RNA-seq

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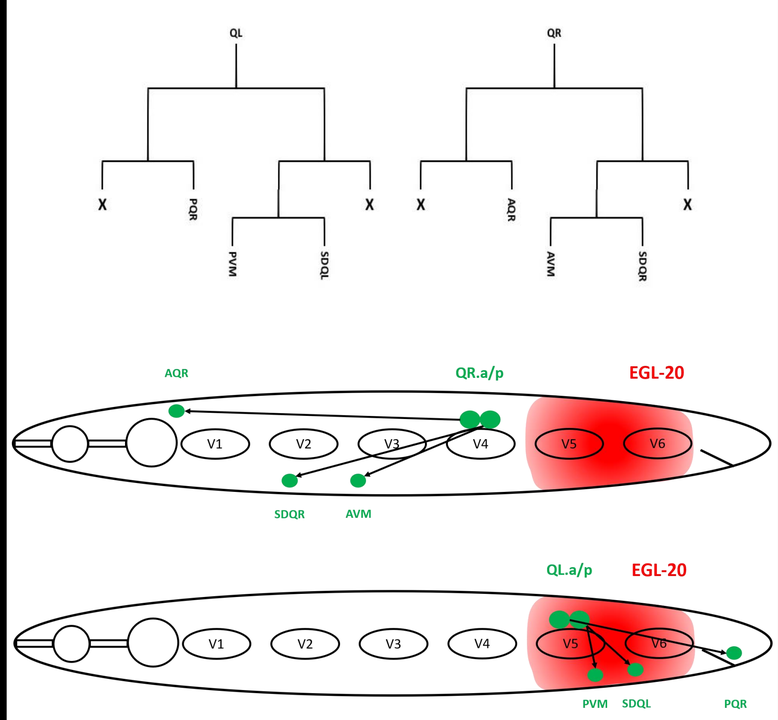
Introduction

Migration of neurons is a critical process for the development of the nervous system. *Caenorhabditis elegans* is an excellent model to study cell migration. The Q neuroblasts are bilaterally symmetrical, with QR and its descendants migrating anteriorly, and QL and its descendants on the left migrating posteriorly. These migrations are highly stereotyped. When QL begins its migration towards the posterior it is confronted with a Wnt signal, EGL-20. EGL-20 activates a canonical Wnt pathway, which expresses MAB-5 in QL. MAB-5 is not expressed in QR, which migrates anteriorly. Four other Wnt molecules in *C. elegans* are activated during Q neuroblast migration, CWN-1, CWN-2, LIN-44 and MOM-2. It is clear that Wnt signaling plays a critical role in the migration of the Q neuroblasts during development of the nervous system.

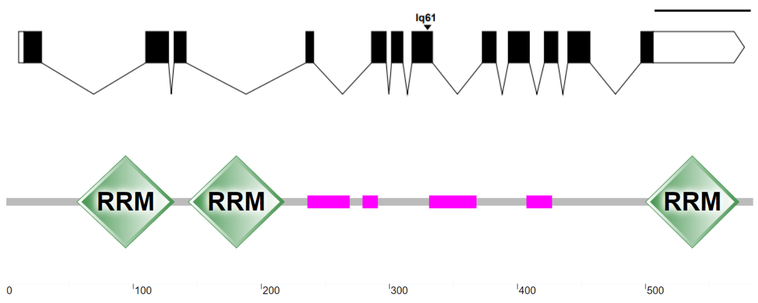
A forward genetic screen identified a mutation in the gene *etr-1*, which caused migration defects in the descendants of QR and QL. *etr-1* is an ELAV-type RNA binding protein, and it is known for its role in the development of muscle. The human homolog for *etr-1,* CUG-binding protein CELF1, has a central role in triplet nucleotide expansion diseases, including myotonic dystrophy type 1. In *C. elegans* mutations in *etr-1* usually result in severe muscle disorganization, paralysis at the two-fold stage, and lethality. We have isolated a viable mutation from our screen which results in a premature stop codon in an alternatively spliced exon. We have also generated another allele with CRISPR mediated knockdown, with a frameshift mutation in the same alternatively spliced exon.

Q Neuroblasts are Bilaterally Symmetrical



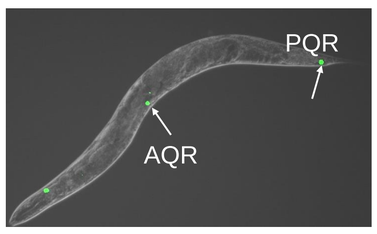
etr-1 encodes an RNA-binding protein

- *lq61* corresponds to a premature stop codon in an alternatively spliced exon
- Total of 84 different isoforms identified, *lq61* affects only ten. Genomic sequence is depicted below, showing all exons
- Protein is characterized by three RNA-recognition motifs



etr-1 causes AQR/PQR defects

Wild-type



etr-1(lq61)

	AQR						PQR						
Genotype	1	2	3	4	5	n	1	2	3	4	5	n	*p*
Wild-type	100	0	0	0	0	100	0	0	0	0	100	100	
etr-1(lq61)	85	12	2	0	1	100	3	5	4	0	88	100	<0.001
etr-1(lq133)	75	19	3	3	0	100	1	1	5	10	82	100	
etr-1(lq61);lqEx[etr-1(+)]	97	2	0	0	1	100	0	0	0	0	100	100	<0.01

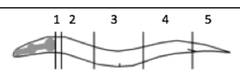
etr-1 acts non-autonomously in muscle cells

Pegl-17::etr-1(+)

	AQR						PQR					
Genotype	1	2	3	4	5	p	1	2	3	4	5	p
etr-1(lq61)	85	12	2	0	1		3	5	4	0	88	
etr-1(lq61) lqEx912	85	11	0	2	1	1	0	0	0	9	91	1
etr-1(lq61) no lqEx912	84	14	0	0	2		0	0	0	10	90	
etr-1(lq61) lqEx913	88	7	1	2	2	1	0	0	0	11	89	1
etr-1(lq61) no lqEx913	85	10	1	1	3		0	0	0	8	92	
etr-1(lq61) lqEx914	87	10	2	1	0	0.5530	1	0	5	4	90	1
etr-1(lq61) no lqEx914	83	13	2	2	0		1	1	3	6	89	

Pmyo-3::etr-1(+)

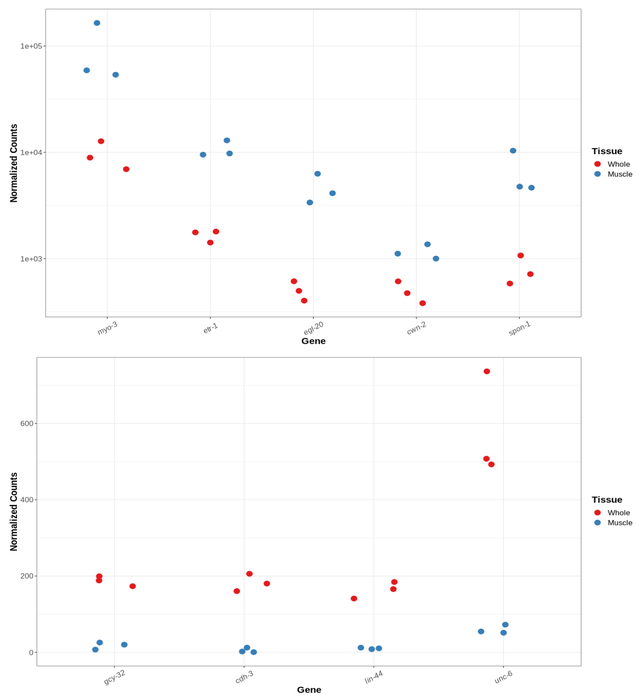
	AQR						PQR					
Genotype	1	2	3	4	5	p	1	2	3	4	5	p
etr-1(lq61)	85	12	2	0	1		3	5	4	0	88	
etr-1(lq61) lqEx944	100	0	0	0	0	<0.001	2	0	0	0	98	0.0055
etr-1(lq61) no lqEx944	70	22	5	1	2		2	1	3	7	87	
etr-1(lq61) lqEx945	86	14	0	0	0	0.0509	0	0	0	2	98	0.0101
etr-1(lq61) no lqEx945	74	24	1	1	0		4	3	0	5	88	
etr-1(lq61) lqEx946	94	6	0	0	0	0.0092	0	0	0	1	99	1.000
etr-1(lq61) no lqEx946	81	14	2	1	2		0	0	0	1	99	
etr-1(lq61) lqEx947	97	3	0	0	0	<0.001	1	0	0	0	99	0.0649
etr-1(lq61) no lqEx947	79	14	6	0	1		1	1	0	4	94	



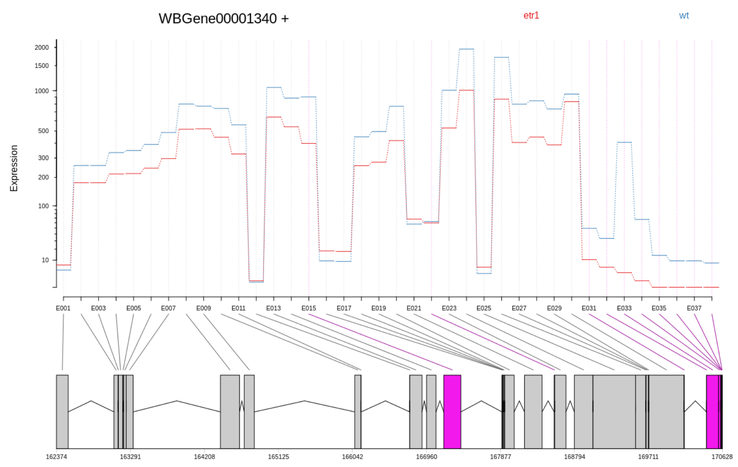
Fisher's Exact Test
Compared to matched *etr-1(lq61)* no *Ex* at AQR position 1 and PQR position 5

N=100

Normalized counts of genes to show proper enrichment and depletion in wild-type muscles

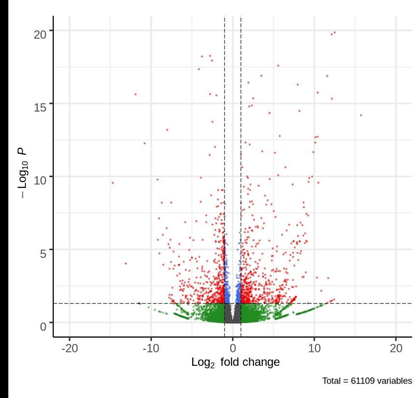


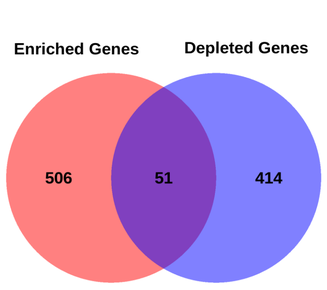
244 genes had differential exon usage when comparing wild-type muscle to *etr-1* muscle



Transcription Factors		Transmembrane Proteins		Secreted Proteins
hmg-5	*dhhc-4*	*hop-1*	*wht-1*	*lbp-2*
lin-26	*crh-2*	*lin-12*	*acs-17*	*zmp-1*
nhr-61	*nsy-7*	*pbo-4*	*C48E7.6*	*C28H8.8*
skn-1		*ver-1*	*E_BE45912.2*	*bgal-2*
ceh-79		*tbc-19*	*F13C5.5*	*W08A12.3*
F23B12.7		*scav-4*	*ZC449.5*	*hpo-38*
F43G9.12		*emre-1*	*ZC581.3*	
flh-1		*M05D6.6*	*C07A9.12*	
atg-4.1		*T01H3.3*		

1180 transcripts were differentially expressed when comparing wild-type to *etr-1* muscles



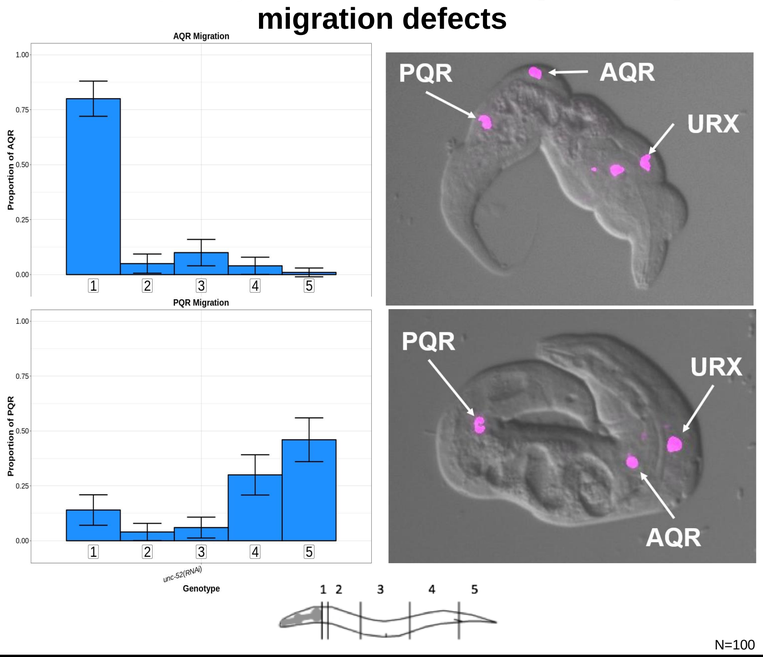


1180 transcripts corresponded to 971 genes. Of those 51 had transcripts enriched and depleted

102 genes were identified in both transcript and exon usage analyses

atn-1	*mrp-1*	*unc-73*	*cpna-2*	*ZK370.4*
avr-14	*nhr-61*	*unc-87*	*wht-1*	*nsy-7*
ccr-4	*ntl-2*	*unc-89*	*fln-2*	
ced-1	*num-1*	*unc-96*	*crh-2*	
ctn-1	*ketn-1*	*ver-1*	*C27H5.2*	
deb-1	*ret-1*	*zmp-1*	*pxl-1*	
dur-1	*rme-1*	*alx-1*	*tag-250*	
alp-1	*rpl-19*	*C06B3.6*	*acs-17*	
egl-10	*sli-1*	*ceh-79*	*fhod-1*	
etr-1	*slo-1*	*tbc-19*	*C56G2.9*	
gcy-31	*srp-7*	*pde-3*	*F13C5.5*	
gld-2	*lim-9*	*shw-1*	*F20A1.6*	
gon-1	*ttn-1*	*F23B12.7*	*dip-2*	
hsp-4	*dgk-4*	*pole-1*	*F44E7.4*	
kin-1	*cpna-1*	*mlcd-1*	*F46F11.1*	
lea-1	*tns-1*	*F40F8.5*	*F48E3.8*	
lec-2	*tax-6*	*F43G9.12*	*K09F6.10*	
lec-3	*tmd-2*	*F49E2.5*	*trx-3*	
lev-11	*tnt-3*	*yif-1*	*M01H9.3*	
lin-45	*tsp-17*	*magi-1*	*R11G1.6*	
lip-1	*unc-32*	*nep-21*	*T23E7.2*	
lsm-5	*unc-36*	*T28F4.5*	*zig-11*	
mai-1	*unc-52*	*grdn-1*	*fln-1*	
mel-11	*unc-64*	*Y105E8A.25*	*him-19*	
mlp-1	*unc-68*	*nit-1*	*ZC449.5*	

unc-52(RNAi) results in severe AQR and PQR migration defects



N=100

Most AQR and PQR migration defects are minor

	AQR					PQR					
Genotype	1	2	3	4	5	1	2	3	4	5	n
mel-11(RNAi)	94	6	0	0	0	0	0	0	0	100	100
unc-89(RNAi)	89	11	0	0	0	0	0	0	0	100	100
ced-1(RNAi)	92	8	0	0	0	0	0	0	1	99	100
atn-1(RNAi)	92	8	0	0	0	0	0	0	0	100	100
unc-32(RNAi)	98	2	0	0	0	0	0	0	0	100	100
lea-1(RNAi)	89	11	0	0	0	0	0	0	0	100	100
unc-87(RNAi)	96	3	1	0	0	0	0	0	0	100	100
tnt-3(RNAi)	95	5	0	0	0	0	0	0	0	100	100
hmg-5(RNAi)	80	20	0	0	0	0	0	0	0	100	100
ver-1(RNAi)	78	22	0	0	0	0	0	0	0	100	100
lin-45(RNAi)	85	15	0	0	0	0	0	0	0	100	100
ceh-79(RNAi)	92	8	0	0	0	0	0	0	0	100	100
mig-22(RNAi)	88	12	0	0	0	0	0	0	3	97	100
sop-2(RNAi)	87	12	1	0	0	0	0	0	1	99	100
unc-112(RNAi)	98	2	0	0	0	0	0	0	1	99	100
unc-71(RNAi)	91	4	0	0	5	5	0	0	0	95	100
noca-1(RNAi)	87	12	1	0	0	0	0	0	0	100	100
ani-1(RNAi)	89	11	0	0	0	0	0	0	2	98	100

Conclusions and future directions

- *etr-1* causes defects in the migration of the Q neuroblast descendants
- *etr-1* acts in the muscle cells
  - *Pmyo-3::etr-1* rescues defects
- We have identified 244 potential genes that are alternatively spliced by ETR-1
- We have identified 1180 transcripts that are potential targets of ETR-1
- RNAi knockdown of *unc-52* displays severe AQR and PQR migration defects
- RNAi knockdown of most genes identified resulted in minor AQR and PQR defects

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