ABSTRACT  The Q neuroblasts in Caenorhabditis elegans display left-right asymmetry in their migration, with QR and descendants on the right migrating anteriorly, and QL and descendants on the left migrating posteriorly. Initial QR and QL migration is controlled by the transmembrane receptors UNC-40/DCC, PTP-3/LAR, and the Fat-like cadherin CDH-4. After initial migration, QL responds to an EGL-20/Wnt signal that drives continued posterior migration by activating MAB-5/Hox activity in QL but not QR. QR expresses the transmembrane protein MIG-13, which is repressed by MAB-5 in QL and which drives anterior migration of QR descendants. A screen for new Q descendant AQR and PQR migration mutations identified mig-13 as well as hse-5, the gene encoding the glucuronyl C5-epimerase enzyme, which catalyzes epimerization of glucuronic acid to iduronic acid in the heparan sulfate side chains of heparan sulfate proteoglycans (HSPGs). Of five C. elegans HSPGs, we found that only SDN-1/Syndecan affected Q migrations. sdn-1 mutants showed QR descendant AQR anterior migration defects, and weaker QL descendant PQR migration defects. hse-5 affected initial Q migration, whereas sdn-1 did not. sdn-1 and hse-5 acted redundantly in AQR and PQR migration, but not initial Q migration, suggesting the involvement of other HSPGs in Q migration. Cell-specific expression studies indicated that SDN-1 can act in QR to promote anterior migration. Genetic interactions between sdn-1, mig-13, and mab-5 suggest that MIG-13 and SDN-1 act in parallel to promote anterior AQR migration and that SDN-1 also controls posterior migration. Together, our results indicate previously unappreciated complexity in the role of multiple signaling pathways and inherent left-right asymmetry in the control of Q neuroblast descendant migration.

KEYWORDS  MAB-5/Hox  MIG-13  Q neuroblasts  SDN-1/syndecan neuronal migration

Directed neuronal migration is an essential part of nervous system development. The Q neuroblasts, QR and QL, are an excellent system in which to study neuronal cell migration (Middelkoop and Korswagen 2014). The Q neuroblasts are born in the posterior lateral region of the worm ( Sulston and Horvitz 1977; Chalfie and Sulston 1981). QR, on the right, extends anterior protrusions over the seam cell V4 whereas QL, on the left, protrudes posteriorly over the seam cell V5. The cell bodies then migrate atop the respective seam cells, and the Q cells undergo their first division ( Honigberg and Kenyon 2000; Chapman et al. 2008). The daughter cells of QR and QL undergo further directional migration, divisions, and cell death to give rise to three neurons each. The QR descendants SDQR, AVM, and AQR migrate anteriorly, with AQR migrating the farthest to near the anterior deirid and first pharyngeal bulb (Chapman et al. 2008). The QL descendants SDQL, PVM, and PQR migrate posteriorly, with PQR migrating the farthest to behind the anus to the phasmid ganglion ( Sulston and Horvitz 1977; White et al. 1986; Chapman et al. 2008).

Q migration occurs in two distinct phases. The directional migration of early QR and QL is directed by transmembrane proteins UNC-40/DCC, PTP-3/LAR, MIG-21, and CDH-4/Fat (Honigberg and
Kenyon 2000; Middelkoop et al. 2012; Sundararajan and Lundquist
2012; Sundararajan et al. 2014). After this initial migration, QL and QR
descendant migration is governed by EGL-20/Wnt signaling. QR
descendants respond to an EGL-20/Wnt signal by activating expression
of the mab-5/Hox gene, which drives further posterior migration
(Kenyon 1986; Salser and Kenyon 1992; Challie 1993; Harris et al.
1996; Whangbo and Kenyon 1999; Korswagen et al. 2000; Herman
2001; Eisenmann 2005). QL descendants do not respond to the
EGL-20/Wnt signal and do not activate mab-5/Hox and thus con-
tinue anterior migration.

Previous work has shown that EGL-20/Wnt and MAB-5/Hox are
not required to direct early anteroposterior Q migrations (Chapman
et al. 2008). However, MAB-5 is both necessary and sufficient to direct
QL descendant migrations posteriorly (Kenyon 1986; Salser and
Kenyon 1992). In mab-5 loss-of-function, both AQR and PQR migrate
anteriorly to the normal location of AQR. In a mab-5 gain-of-function
background, both QR and QL descendants migrate posteriorly to the
normal position of PQR (Chapman et al. 2008; Tamayo et al. 2013).

The transmembrane molecule MIG-13 drives QR descendant an-
terior migration (Sym et al. 1999; Wang et al. 2013). MIG-13 expres-
sion is dependent on LIN-39/Hox, expression of which is inhibited by
MAB-5/Hox (Sym et al. 1999; Wang et al. 2013). Thus, MAB-5 directs
poster ior migration by inhibiting lin-39 and mig-13, genes involved in
anterior migration. mig-13 drives anterior Q descendant migrations
in QR descendants that do not express mab-5, and in QL descendants
in mab-5 mutants (Sym et al. 1999; Wang et al. 2013). MIG-13 is a
single-pass transmembrane protein a CUB (C1r/C1s, Uegf, Bmp1) do-
main and a low-density lipoprotein receptor repeat (Sym et al. 1999).
mig-13 acts cell-autonomously in QR descendant migration and might
act as a receptor to polarize the actin cytoskeleton in response to guid-
ance cues (Wang et al. 2013).

We conducted a genome-wide screen for new mutations affecting
AQR and PQR migration. The screen identified hse-5, which encodes
the C. elegans ortholog of the glucuronyl C5-epimerase enzyme, which
catalyzes epimerization of glucuronic acid to iduronic acid in the
heparan sulfate side chains of heparan sulfate proteoglycans (HSPGs)
(Lindahl et al. 1998). Previous work has implicated HSPGs and modi-
yfing enzymes in cell migration and axon guidance (Merz et al. 2003;
Rhiner et al. 2005; Hudson et al. 2006; Bulow et al. 2008; Schwabiu-
ki et al. 2009; Wang et al. 2012; Gysis et al. 2013; Diaz-Balzac et al.
2014; Wang et al. 2015). HSPGs have long chains of differentially modi-
fied sugar side chains that play different roles in nervous system develop-
ment (Minniti et al. 2004; Rhiner et al. 2005; Hudson et al. 2006;
Johnson et al. 2006; Bulow et al. 2008; Wang et al. 2012). Indeed,
HSE-5 is required for early Q neuroblast protrusion and migration
along with the HSPG LON-2 (Wang et al. 2015).

The HSPG SDN-1 Syndecan is the only Syndecan in the C. elegans
genome (Rhiner et al. 2005). SDN-1 is a transmembrane protein with
heparan sulfate side chains attached to the extracellular domains and an
intracellular PDZ binding domain (Lindahl et al. 1998; Bernfield et al.
1999; Esko and Selleck 2002). SDN-1 is necessary for the migration of
different neuronal cell types such as the HSN, ALM, and CAN neurons
and is expressed extensively in the nervous system (Rhiner et al. 2005).
We found that sdn-1 affected AQR, similar to mig-13, sdn-1 also dis-
played weak PQR migration defects. This result combined with double
mutant analysis with mab-5 indicated that SDN-1 also regulates pos-
terior migration. No defects in initial Q neuroblast migrations were
observed in sdn-1 mutants, whereas hse-5 mutants displayed early
migration defects, suggesting the involvement of other HSPGs in initial
migration. Cell-specific expression revealed that SDN-1 can function
autonomously in Q to promote AQR anterior migration. The other
HSPGs LON-2, GPN-1/Glypican, and UNC-52/Perlecan had no de-
tectable role in Q descendant migrations, nor did the sdn-1 gpn-1 lon-2
triple mutant. We also identified an allele of mig-13 that had AQR
defects, as expected. Double mutant analysis revealed that MIG-13 and
SDN-1 act in parallel in Q to promote AQR anterior migration.

MATERIALS AND METHODS

C. elegans genetics

All experiments were conducted at 20°C using standard C. elegans
techniques ( Sulston and Hodgkin 1988). The following strains and
transgenes were used: LGI clc-1(e364), LGII hse-5(ok2463), hse-5
(lq49), hse-5(tm472), mab-5(e1239), mab-5(gk670), mab-5(e1751); LG
IV lqIs80[Pscm::gfp::caax]; LGV lqIs58[Pcyg-32::eGFP]; and LGX
sdn-1(ck20), sdn-1(ok244), mig-13(lq71), mig-13(lq71), lon-2(e678),
gpn-1(ok377). The presence of HSPG mutants were confirmed by poly-
merase chain reaction and/or sequencing. Transgenes were con-
structed using the standard gonadal microinjection techniques to
produce extra-chromosomal arrays (Mello and Fire 1995).

AQR/PQR mutant screen and mapping

L4 and young adult hermaphrodites of the strain LE2500, consisting
of the Pcyg-32::eGFP transgene lqIs58 and the Pscm::gfp transgene
lqIs80, were mutagenized with ethylmethane sulfonate using standard

tech-niques (Anderson 1995). Mutagenized animals were placed on single
seeded NGM plates and allowed to self-fertilize. F1 animals were placed
on plates, three animals per plate. The F2 progeny were screened, using
a fluorescence dissecting microscope, for animals with misplaced AQR
and/or PQR visualized by the lqIs58[Pcyg-32::eGFP]. Defective
animals were placed on single plates, and their progeny examined for
AQR and PQR defects to ensure germline mutation. Approximately
3000 haploid genomes were screened.

New mutations were mapped using single nucleotide polymor-
phism mapping or next generation sequencing combined with SNP
mapping using the Cloudmap pipeline and the polymorphic CB4856
Hawaiian strain (Davis et al. 2005; Minevich et al. 2012).

Plasmids

The cDNA yk139f3 encodes a full-length sdn-1 cDNA. The sdn-1
cDNA was positioned downstream of the seam cell promoter (Pscm)
expressed in the hypodermal seam cells and Q cells (Chapman et al.
2008), and the Q cell-specific cgl-17 promoter (Branda and Stern
2000; Cordes et al. 2006; Sundararajan and Lundquist 2012; Sundararaj-
ran et al. 2014). Sequences of these plasmids are available upon request.

Scoring AQR and PQR defects

Previously described quantification techniques were used (Chapman
et al. 2008; Sundararajan and Lundquist 2012). The gcy-32:: eGFP
transgene expressed in the URX, AQR, and PQR neurons was used to
visualize and score the final positions of AQR and PQR. The position
of AQR and PQR are scored in five positions along the body of the
worm (Table 1, Table 2, Table 3, Table 4, and Table 5). Position 1 is
the wild-type position of AQR in the anterior deirid just posterior to
the pharyngeal bulb. Position 2 represents the region posterior to the
pharyngeal bulb and anterior to the vulva. Position 3 represents the
region behind the anus in the phasmid ganglion, which is the wild-type
position of PQR. AQR and PQR were scored and analyzed in L4 larval
animals, which is after AQR and PQR undergo their final migrations.
At least 100 animals were scored for each genotype and statistical significance was determined using Fisher’s exact test. The predicted additive phenotype of double mutants for comparison in Fisher’s exact test was calculated by the formula p(A) = p1 + p2 – (p1p2), where p(A) is the predicted additive proportion, p1 is the proportion in single mutant 1, and p2 is the proportion in single mutant 2.

**Scoring early QR and QL defects**

Previously described larval synchronization techniques were used (Chapman et al. 2008; Dyer et al. 2010; Sundararajan and Lundquist 2012). All adult and larval worms were washed from plates using M9 buffer when the eggs remain adhered to the plates. These eggs were allowed to hatch and the larvae were collected every half an hour. All the larvae were collected between 0 and 0.5 hr after hatching. The larvae were then staged and visualized at 2–2.5, 3–3.5, and 4–4.5 hr posthatching using the Pscm:gfp:axaX transgene. The wild-type larvae were visualized at 2-2.5 hr posthatching, and they exhibited defined anterior QR protrusions and posterior QR protrusions. These protrusions extended over the seam cells V4 and V5, respectively. At approximately 3–3.5 hr posthatching, QR and QL follow the protrusions and migrate on top of the seam cells. QR migrates anterior on the seam cell V4 and QL migrates posterior on the seam cell V5. The larvae visualized at 4–4.5 hr posthatching showed QR and QL undergoing their first round of division. QR divides on V4 and QL divides on V5. Defects in direction of protrusion, migration, and division stages were scored for all the genotypes. QR that protruded, migrated, and divided posterior on the seam cell V5 were scored as defective. QL that protruded, migrated, and divided anterior on the seam cell V4 were scored as defective. Defects observed in QR and QL at 4–4.5 hr post hatching are represented in Figure 1. Previous work has shown that the defects seen in protrusion and migration stages do not differ significantly from the division stage. At least 25 cells were scored for each genotype and statistical significance was determined with the Fisher’s exact test.

**RESULTS**

**A screen for new mutations affecting AQR and PQR migration identified hse-5 and mig-13**

In a genome-wide screen for mutations affecting AQR and PQR migration (see the section Materials and Methods), we identified hse-5 (lq49) and mig-13 (lq71) (Table 1). hse-5 (lq49) caused directional migration defects in both AQR and PQR. The hse-5 (lq49) lesion was mapped and identified by whole-genome resequencing and the Cloudmap protocol (Minevich et al. 2012). lq49 was linked to linkage group III and resulted in a glutamine 172 to stop (C to T) in the hse-5 gene. The previously-isolated hse-5(ok2463) and hse-5(tm472) mutations also showed AQR and PQR directional defects similar to hse-5 (lq49) (Table 1). hse-5 encodes the C. elegans ortholog of the glucuronyl C5-epimerase enzyme, which catalyzes epimerization of glucuronic acid to iduronic acid in the heparan sulfate side chains of HSPGs. hse-5 was shown previously to affect axon guidance and cell migration (Lindahl et al. 1998; Rhiner et al. 2005; Bulow et al. 2008), including Q neuroblast migration (Wang et al. 2015). Our results indicate that hse-5 controls the direction and extent of AQR and PQR migration. mig-13 (lq71) was mapped to the X linkage group by the use of single-nucleotide polymorphism mapping (Davis et al. 2005). mig-13 (lq71) affected only AQR and not PQR migration (Table 1), similar to the known effects of the X-linked mig-13 on the QR but not QL lineage (Sym et al. 1999; Wang et al. 2013). As expected, the existing mig-13 (mu225) mutation showed AQR-specific defects (Table 1), and mig-13 (lq71) failed to complement mig-13 (mu225) for AQR defects (data not shown). mig-13 encodes a transmembrane protein with an extracellular C1r/C1s, Uegf, Bmp1 (CUB) domain and a low-density lipoprotein receptor repeat and a proline-rich cytoplasmic tail (Sym et al. 1999). These results indicate that HSPGs and the transmembrane protein MIG-13 regulate Q descendant migration.

**SDN-1/syndecan controls AQR anterior migration**

HSPGs have a well-characterized role in axon pathfinding and cell migrations (Minniti et al. 2004; Rhiner et al. 2005; Hudson et al. 2006; Schwabiuik et al. 2009). Because hse-5 caused AQR and PQR migration defects, we wanted to analyze the effect of other C. elegans HSPGs in AQR and PQR migrations. Mutations in glp-2/Glypican, lon-2, cle-1/CollagenXVIII, and unc-52/Plercan did not affect AQR or PQR migration (Table 2). However, mutations in sdn-1/Syndecan affected the extent and direction of AQR migration and had a weak effect on the extent of PQR migration. The two well-characterized mutant alleles of sdn-1, zh20 and ok244 (Rhiner et al. 2005), are deletions that affect exons 1–5 and exons 2 and 3, respectively. zh20 is a candidate for a null mutant, and ok244 is thought to function as a hypomorph (Rhiner et al. 2005), but we observed similar defects in both. In sdn-1 mutants, AQR failed to migrate all the way to its anterior wild-type position (23–24%) and in fact migrated posteriorly in 3–5% of animals. PQR migrations defects were weaker (11–16%), with defective cells failing to migrate away from their birthplace and possibly slightly to the anterior, but never to the normal anterior position of AQR as observed in hse-5 (Table 2).

**sdn-1 expression in Q cells is sufficient for anterior migration**

We drove expression of the sdn-1 cDNA under the control of the Pscm expressed in the hypodermal seam cells and Q cells (Chapman et al. 2008), and the Q cell-specific egl-17 promoter (Brand and Stern 2000; Cordes et al. 2006; Sundararajan and Lundquist 2012;
Both Pscm::sdn-1 and Pegl-17::sdn-1 significantly rescued the AQR defects of sdn-1(zh20) (Table 3), indicating that sdn-1 function in QR descendants is sufficient for anterior migration. Neither transgene rescued PQR migration defects observed in sdn-1(zh20). In some sdn-1(zh20); Pegl-17::sdn-1 animals, PQR migrated anteriorly to the normal position of AQR, a defect not observed in sdn-1 alone. Although sdn-1 can act in QR for anterior descendant migration, its role in QL is more complex. sdn-1 might act nonautonomously in QL descendant migration, or transgenic expression in QL might drive anterior migration.

### HSE-5 and SDN-1 act redundantly in AQR and PQR migration

Previous work has shown that in axon pathfinding and cell migration, HSE-5 functions with other HSPGs redundantly with SDN-1 (Rhiner et al. 2005). hse-5 and sdn-1 alone had comparable effects on AQR migration (Table 2). In hse-5; sdn-1 double mutants, we observed a significant increase in the percentage of AQR migration defects compared with the predicted additive effects of the double mutant (Table 2). PQR migration defects also were significantly enhanced (Table 2). These data suggest that hse-5 and sdn-1 act redundantly to control AQR and PQR migration.

HSPGs and modifying enzymes have been shown to act additively and redundantly in some migration events (Rhiner et al. 2005; Diaz-Balzac et al. 2014; Kinnunen 2014). To investigate redundancy in AQR and PQR migration, we analyzed compound mutants of the genes encoding the other transmembrane HSPGs in the C. elegans genome, lon-2 and gpn-1. Neither the sdn-1(zh20) lon-2(e678) double nor the sdn-1(zh20) lon-2(e678) gpn-1(ok377) triple mutant enhanced AQR and PQR defects of sdn-1(zh20) (Table 2).

### HSE-5, but not SDN-1, affects early Q protrusion and migration

QR and QL undergo an initial anterior and posterior protrusion and migration controlled by the transmembrane proteins UNC-40/DCC, PTP-3/LAR, and CDH-4/Fat-like cadherin (Honigberg and Kenyon 2000; Sundararajan and Lundquist 2012; Middelkoop and Korswagen 2014; Sundararajan et al. 2014). hse-5(lq49) disrupted early QR and QL migrations (Figure 1), as was observed previously for other hse-5 mutations (Wang et al. 2015). In hse-5(lq49) mutants, QR migrated posteriorly and QL migrated anteriorly in some animals (Figure 1). sdn-1 alone had no effect on QL and QR early protrusion and migration, nor did it modify the effects of hse-5(lq49) in double mutants (Figure 1). These results indicate that hse-5 is required for early QR and QL migrations but sdn-1 is not. HSE-5 is likely acting via other HSPGs in regulating early Q migration as well as redundantly with SDN-1 in Q descendant migration. The HSPG LON-2 might be a target of HSE-5 in early Q protrusion and migration (Wang et al. 2015).

### Table 2 SDN-1/syndecan controls AQR and PQR migration

| Genotype                  | AQR position (%) |           |           |           | N  |
|---------------------------|------------------|-----------|-----------|-----------|----|
|                           | 1    | 2    | 3    | 4    | 5    | N  |
| lon-2(e678)               | 100  | 0    | 0    | 0    | 0    | 100|
| gpn-1(ok377)              | 100  | 0    | 0    | 0    | 0    | 100|
| unc-52(e444)              | 100  | 0    | 0    | 0    | 0    | 100|
| cle-1(kg364)              | 100  | 0    | 0    | 0    | 0    | 100|
| sdn-1(zh20)               | 79   | 10   | 6    | 3    | 2    | 100|
| sdn-1(ok244); hse-5(lq49) | 77   | 7    | 7    | 4    | 5    | 100|
| sdn-1(zh20); lon-2(e678)  | 69   | 15   | 10   | 5    | 1    | 100|
| sdn-1(zh20); lon-2(e678) gpn-1(ok377) | 72   | 14   | 9    | 8    | 1    | 100|
| hse-5(lq49)               | 76   | 4    | 1    | 2    | 17   | 100|
| sdn-1(zh20); hse-5(lq49)  | 26   | 19   | 15   | 25   | 15   | 100|
| sdn-1(ok244); hse-5(lq49) | 31   | 17   | 16   | 24   | 12   | 100|

*Comparison to the predicted additive effect of sdn-1 and hse-5 at AQR position 1, AQR position 5 (see Methods).

### Table 3 SDN-1 can act in the QR for anterior migration

| Genotype                  | AQR position (%) |           |           |           | N  |
|---------------------------|------------------|-----------|-----------|-----------|----|
|                           | 1    | 2    | 3    | 4    | 5    | N  |
| wild-type                 | 100  | 0    | 0    | 0    | 0    | 100|
| sdn-1(zh20)               | 79   | 10   | 6    | 3    | 2    | 100|
| sdn-1(zh20); [Pscm::sdn-1(+)] | 88   | 6    | 2    | 1    | 0    | 200|
| sdn-1(zh20); [Pegl-17::sdn-1(+)] | 93   | 3    | 3    | 0    | 1    | 100|
| sdn-1(zh20); [Pegl-17::sdn-1(+)] | 94   | 4    | 1    | 0    | 1    | 100|

*Comparison to sdn-1(zh20) at AQR position 1.
but did not act redundantly with \textit{sdn-1} in AQR and PQR migration (Table 2).

HSE-5 acts outside of the Q cells to direct early Q migrations (Wang et al. 2015), and we found that SDN-1 can act in the QR to promote anterior AQR migration (Table 3). Possibly, SDN-1 acts redundantly with another HSPG modified by HSE-5 outside of the Q cells.

**SDN-1 and MIG-13 function redundantly in AQR migration**

MIG-13 controls anterior migration of QR descendants (Sym et al. 1999; Wang et al. 2013). MIG-13 can act in the Q cells themselves to direct anterior migration (Wang et al. 2013). MIG-13 expression is inhibited in QL by the Hox transcription factor MAB-5 (Wang et al. 2013). QR descendants do not respond to the EGL-20/Wnt signal and thus do not activate MAB-5, resulting in MIG-13 expression in QR descendants (Wang et al. 2013). MIG-13 controls the anterior polarization and migration of the QR descendants (Wang et al. 2013). QL descendants respond to the EGL-20/Wnt signal by expressing MAB-5 and inhibiting MIG-13 expression, resulting in posterior migration.

\textit{mig-13} and \textit{sdn-1} both affected anterior AQR migration, with \textit{mig-13} having a more severe effect (Table 4). The double-mutant \textit{sdn-1}; \textit{mig-13} had significantly increased AQR migration defects compared to the predicted additive effect of the mutations (Table 3), indicating a synergistic interaction and redundancy between \textit{sdn-1} and \textit{mig-13} in AQR migration. \textit{mig-13} did not enhance PQR migration defects of \textit{sdn-1}, indicating that MIG-13 redundancy with SDN-1 is limited to AQR. MIG-13 and SDN-1 act in parallel to promote anterior migration in AQR.

\textit{mig-13} and \textit{sdn-1} interact genetically with \textit{mab-5/Hox}

Previous work has shown that MAB-5/Hox inhibits MIG-13 expression in QL descendants (Wang et al. 2013). MAB-5/Hox expression is cell-autonomously required to direct posterior QL descendant migration, including PQR (Kenyon 1986; Chapman et al. 2008; Tamayo et al. 2013). In \textit{mab-5(e1239)} and \textit{mab-5(gk670)} loss-of-function backgrounds, both AQR and PQR migrated anteriorly to the wild-type AQR position (Table 5). In a \textit{mig-13(mu225)}; \textit{mab-5(e1239)} double mutant, AQR defects resembled \textit{mig-13} alone. \textit{mig-13} significantly suppressed anterior migration of PQR compared with a \textit{mab-5} loss-of-function background alone (Table 5), with some PQR (10%) migrating posteriorly. These results indicate that anterior migration of PQR in \textit{mab-5} mutants is partially dependent upon \textit{mig-13}. This result is consistent with previous observations that MIG-13 is ectopically expressed in QL descendants in \textit{mab-5} mutants through LIN-39/Hox

**Table 4 SDN-1 and MIG-13 act redundantly in AQR migration**

| Genotype               | AQR position (%) | PQR position (%) |
|------------------------|------------------|------------------|
|                        | 1    | 2    | 3    | 4    | 5    | N    | 1    | 2    | 3    | 4    | 5    | N    |
| wild-type              | 100  | 0    | 0    | 0    | 0    | 100  | 0    | 0    | 0    | 0    | 100  | 100  |
| \textit{mig-13(q71)}   | 54   | 18   | 13   | 10   | 5    | 100  | 0    | 0    | 0    | 0    | 100  | 100  |
| \textit{mig-13(mu225)} | 55   | 20   | 15   | 8    | 2    | 100  | 0    | 0    | 0    | 0    | 100  | 100  |
| \textit{sdn-1(zh20)}  | 79   | 10   | 6    | 3    | 2    | 100  | 0    | 0    | 0    | 0    | 100  | 100  |
| \textit{sdn-1(ok244)} | 77   | 7    | 4    | 5    | 100  | 0    | 0    | 0    | 0    | 100  | 100  |
| \textit{sdn-1(zh20); mig-13(mu225)} | 25   | 25   | 24   | 14   | 12   | 100  | 0    | 0    | 0    | 3    | 11   | 86   |

*p*compared to the predicted additive effect of \textit{sdn-1(zh20)} and \textit{mig-13(mu225)} at AQR position 1 (see Methods).

**Table 5 MAB-5 interacts genetically with SDN-1 and MIG-13**

| Genotype               | AQR position (%) | PQR position (%) |
|------------------------|------------------|------------------|
|                        | 1    | 2    | 3    | 4    | 5    | N    | 1    | 2    | 3    | 4    | 5    | N    |
| \textit{mig-13(q71)}   | 54   | 18   | 13   | 10   | 5    | 100  | 0    | 0    | 0    | 0    | 100  | 100  |
| \textit{mig-13(mu225)} | 55   | 20   | 15   | 8    | 2    | 100  | 0    | 0    | 0    | 0    | 100  | 100  |
| \textit{mab-5(gk670)} | 99   | 1    | 0    | 0    | 0    | 100  | 95   | 4    | 0    | 1    | 0    | 100  |
| \textit{mab-5(e1239)} | 100  | 0    | 0    | 0    | 0    | 100  | 96   | 3    | 0    | 1    | 0    | 100  |
| \textit{mig-13(q71)}   | 54   | 18   | 13   | 10   | 5    | 100  | 0    | 0    | 0    | 0    | 100  | 100  |
| \textit{mig-13(mu225)} | 55   | 20   | 15   | 8    | 2    | 100  | 0    | 0    | 0    | 0    | 100  | 100  |
| \textit{mab-5(e1239); mig-13(mu225)} | 51   | 26   | 12   | 7    | 4    | 100  | 29   | 37   | 18   | 6    | 10   | 100  |
| \textit{sdn-1(zh20)}  | 79   | 10   | 6    | 3    | 2    | 100  | 0    | 0    | 0    | 8    | 8    | 100  |
| \textit{sdn-1(ok244)} | 77   | 7    | 4    | 5    | 100  | 0    | 0    | 0    | 4    | 7    | 89   | 100  |
| \textit{sdn-1(zh20); mab-5(gk670)} | 65   | 18   | 11   | 6    | 0    | 100  | 34   | 27   | 17   | 15   | 7    | 100  |
| \textit{sdn-1(zh20); mab-5(e1239)} | 71   | 12   | 10   | 5    | 2    | 200  | 37   | 17   | 21   | 13   | 12   | 200  |
| \textit{mab-5(e1751)} | 0    | 0    | 0    | 8    | 92   | 100  | 0    | 0    | 0    | 0    | 100  | 100  |
| \textit{sdn-1(zh20); mab-5(e1751)} | 62   | 13   | 20   | 4    | 1    | 100  | 0    | 0    | 1    | 21   | 78   | 100  |

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activity (Wang et al. 2013), sdn-1(zh20) double mutants with mab-5 had a similar effect (Table 4) in that AQR and PQR often failed in their anterior migrations in the double mutants, indicating that SDN-1 is also required for anterior PQR migration in mab-5 mutants.

In a mab-5(c1751) gain-of-function background, AQR migrated posteriorly as previously reported (Table 5). Surprisingly, sdn-1 suppressed posterior AQR migration of the mab-5(c1751) gain-of-function mutant, indicating that sdn-1 is required for posterior migration of AQR in mab-5(c1751) gain-of-function. These data indicate that SDN-1 is required for both anterior and posterior migration, consistent with weak PQR defects observed in sdn-1 mutants.

**DISCUSSION**

**SDN-1/syndecan regulates Q descendant migrations**

The guidance of Q descendants in the anteroposterior axis involves intrinsic left-right asymmetries of the QL and QR cells, as well as extracellular signals, such as Wnts (Zinovyeva et al. 2008). HSPGs have been implicated considerably in nervous system development (Bulow et al. 2002; Minniti et al. 2004; Rhiner et al. 2005; Hudson et al. 2006; Johnson et al. 2006; Schwabiku et al. 2009; Diaz-Balzac et al. 2014; Kinnunen 2014). The two classes of HSPGs, the syndecans and glypicans, are known to regulate cell migration and axon path-finding in the nervous system (Rhiner et al. 2005; Hudson et al. 2006; Bulow et al. 2008). In C. elegans, SDN-1 is the only Syndecan encoded by the genome (Rhiner et al. 2005). Previous work has shown that SDN-1 is required to direct neuronal cell migration, including HSN, CAN, and ALM neurons (Rhiner et al. 2005; Kinnunen 2014). It also is required for proper axon path-finding (Rhiner et al. 2005). HSPGs have heparan side chains that require modifications in a function-dependent manner (Lindahl et al. 1998). HSE-5 inhibits N-sulfation and stimulates 2-O and 6-O-sulfation (Townley and Bulow 2011). SDN-1 interacts with HS-modifying genes including hse-5 in a context-dependent manner, acting in the same pathway or in parallel pathways (Lindahl et al. 1998; Bulow and Hobert 2004; Rhiner et al. 2005; Bulow et al. 2008; Kinnunen 2014).

Here we show that SDN-1/syndecan is required for proper extent and direction of migration of Q descendant AQR, with weaker effects on the migration of PQR. sdn-1 is expressed broadly in neuronal and non-neuronal cells, including hypodermis and pharynx (Minniti et al. 2004; Rhiner et al. 2005; Hudson et al. 2006). We show that SDN-1 can function in Q to promote anterior migration, suggesting a cell-autonomous function. hse-5 mutants also displayed both AQR and PQR migration defects, and hse-5; sdn-1 double mutants showed synergistically more severe defects in both AQR and PQR migration, suggesting redundant function. hse-5 is expressed in hypodermis (Bulow and Hobert 2004), is not expressed in Q cells, and is required in the hypodermis for early Q cell migration (Wang et al. 2015). Thus, hse-5 acts nonautonomously in Q migration. HSE-5 might modify another HSPG that acts...
redundantly with SDN-1. No other single mutations in the lon-2, gpn-1, unc-52, or cle-1 genes encoding HSPGs showed any effect on AQR and PQR. The HSPGs LON-2 and GPN-1/Glypican are both, like SDN-1, transmembrane molecules, and the HSPGs CLE-1, UNC-52, and AGR-1/Agrin are predicted secreted molecules. The "transmembrane" triple mutant sdn-1 lon-2 gpn-1 showed no more AQR defects than sdn-1 alone, arguing against redundant function of LON-2 and GPN-1 with SDN-1 in AQR migration. Possibly, HSE-5 modifies one or more of the HSPGs outside of the Q cells that interact redundantly with SDN-1 in AQR and PQR migration (Figure 2). This is distinct from the roles of sdn-1, lon-2, and gpn-1 in another cell migration event, that of the HSN neuron, in which each additively controls HSN migration (Kinnunen 2014). This indicates that different migration events use distinct mechanisms involving HSPGs to control migration.

hse-5 mutants also had defects in initial QL and QR protrusion and migration as described previously (Wang et al. 2015). sdn-1 mutations alone had no initial QL or QR migration defects, nor did sdn-1 enhance the initial QR and QL defects of hse-5 mutants. One interpretation of this result is that HSE-5 and SDN-1 act in the same pathway in early protrusion, and that HSE-5 also regulates another HSPG in parallel to SDN-1 in initial migration. Indeed, genetic interactions suggest that the HSPG LON-2 might be a target of HSE-5 in initial Q migrations: lon-2 mutants alone showed no phenotype but suppressed hse-5 (Wang et al. 2015). Alternately, SDN-1 might not affect early Q migrations, only later Q descendant migrations.

SDN-1/syndecan acts in parallel to MIG-13 in AQR to direct anterior migration

sdn-1 and mig-13 single mutants had similar AQR migration defects. sdn-1 mig-13 double mutants showed synergistically increased AQR migration defects (Table 4). This finding suggests that SDN-1 and MIG-13 act redundantly in anterior AQR migration. Previous work has shown that MIG-13 is required autonomously in QR descendants to direct their anterior migration (Wang et al. 2013) and is repressed by MAB-5 in QL to allow posterior migration. MIG-13 acts in QR descendants, possibly as a receptor, to drive anterior polarization and migration (Wang et al. 2013). SDN-1 might also act in parallel to MIG-13 to drive anterior migration (Figure 2). One model is that SDN-1 might be generally required for migration in both anterior and posterior directions, whereas MIG-13 cooperates with SDN-1 in anterior, but not posterior migration. This is consistent with weak PQR defects in sdn-1 mutants. However, transgenic expression of sdn-1 in QL did not rescue PQR defects, suggesting that SDN-1 might function nonautonomously in QL descendant migration. Pegl-17:sdn-1(+) expression caused a small percentage of complete PQR anterior migration not seen in sdn-1 alone (Table 2), suggesting that SDN-1 transgenic expression in QL might drive anterior migration. Although the role of SDN-1 in QL is unclear, our results show that SDN-1 can function in QR, in parallel to MIG-13, to promote anterior migration of the QR descendant AQR.

SDN-1 mediates responses to MAB-5/Hox

In mab-5 loss-of-function mutants, QL descendants migrate anteriorly, similar to QR descendants. We found that sdn-1; mab-5 double mutants had defects in anteriorly migrating PQRs, similar to AQRs in an sdn-1 single mutant. MIG-13 also was required for anterior migration of PQR in mab-5 loss-of-function. Previous work showed that MAB-5 represses LIN-39 expression in QL, and thus MIG-13 also is not expressed in QL, so the QL descendants do not migrate anteriorly (Wang et al. 2013). Our data are consistent with this model and implicate SDN-1 in acting in parallel to MIG-13 to promote anterior migration.

mab-5(e1751) gain-of-function results in posterior migration of both QR and QL descendants, including AQR and PQR. sdn-1(zh20); mab-5(e1751) showed much anterior AQR and PQR migration, suggesting that sdn-1 is required for posterior migration in the mab-5(e1751) gain-of-function mutant, which might represent sensitized backgrounds that reveal the effect of SDN-1 on posterior migration. In sum, these data indicate that SDN-1/syndecan can regulate both anterior and posterior Q descendant migration controlled by MAB-5/Hox.

Our results reveal the complexity of the decision of the Q descendants to migrate anteriorly or posteriorly. Wnts have been shown to redundantly control Q descendant migrations (Zinovyeva et al. 2008). Here we show that the HSPG Syndecan is also involved, possibly acting in Q along with the transmembrane molecule MIG-13 to promote anterior migration (Figure 2). The role of SDN-1 in QL is unclear, but it might act outside of QL for posterior migration. Alternately, transgenic expression of sdn-1 might perturb its function in QL. In dorsal migration of the gonadal distal tip cells, sdn-1 mutation results in misregulated EGL-20/Wnt signaling (Schwabik et al. 2009). SDN-1 also interacts with Wnt signaling to orient spindle positioning in the early embryo (Dejima et al. 2014). It is possible that SDN-1 governs AQR and PQR migration by interacting with the Wnt signals that redundantly guide their anterior-posterior migrations (Zinovyeva et al. 2008). We show that SDN-1 can function in Q autonomously, so it is possible that SDN-1 modifies the manner in which QR descendants respond to Wnt signals. It is becoming clear from our work and that of others that anteroposterior Q descendant migrations are due to the intersection of multiple signaling pathways and the inherent left-right asymmetry of the cells, which mandates how the cells respond to these pathways.

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