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Nuclear deformability depends on H3K9-methylated heterochromatin anchorage to the nuclear periphery in *Caenorhabditis elegans*

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Nuclei adjust their deformability while migrating through constrictions to enable structural changes and maintain nuclear integrity. The effect of heterochromatin anchored at the nucleoplasmic face of the inner nuclear membrane on nuclear morphology and deformability during in vivo nuclear migration through constricted spaces remains unclear. Here, we show that abolishing peripheral heterochromatin anchorage by eliminating *CEC-4*, a chromodomain protein that tethers H3K9-methylated chromatin to the nuclear periphery, disrupts constrained P-cell nuclear migration in *Caenorhabditis elegans* larvae in the absence of the established linker of nucleoskeleton and cytoskeleton (LINC) complex-dependent pathway. This effect was suppressed by mutations that stabilize the lamin *LMN-1*. *CEC-4* acts in parallel to an actin and *CDC-42*-based pathway. We also demonstrate the necessity for the chromatin methyltransferase *MET-2* and the demethylase *JMJD-1.2* during P-cell nuclear migration in the absence of functional LINC complexes. We conclude that H3K9-methylated chromatin needs to be anchored to the nucleoplasmic face of the inner nuclear membrane to help facilitate nuclear migration through constricted spaces in vivo.

Keywords: nuclear envelope; heterochromatin; LINC complex; nuclear migration; WormBase

Introduction

Migrating cells must squeeze through constrictions as part of developmental and pathological processes, including immune cell extravasation and cancer metastasis (McGregor *et al.* 2016; Paul *et al.* 2017). Deformation of the nucleus, the largest and stiffest organelle, is often the rate-limiting step of cell migration through tight spaces (Davidson *et al.* 2014; Thiam *et al.* 2016; Renkawitz *et al.* 2019). Most studies of nuclear migration through constrictions are limited to in vitro models (Bone and Starr 2016; Denais *et al.* 2016; Raab *et al.* 2016; Xia *et al.* 2019). Here, we use an in vivo model, where *Caenorhabditis elegans* P-cell nuclei migrate through a constricted space as a normal part of larval development (Chang *et al.* 2013; Bone *et al.* 2016; Bone and Starr 2016). During this process, nuclei of neuronal and vulval precursor cells, referred to as P cells, move from lateral positions to the ventral cord of the organism (Sulston and Horvitz 1977; Bone *et al.* 2016; Fridolfsson *et al.* 2018). To complete this migration, P-cell nuclei must pass through a constriction between the cuticle and the body wall muscle. This space is only 100–200 nm, or ~5% of the diameter of P-cell nuclei prior to migration (Bone *et al.* 2016). Following nuclear migration to the ventral cord, P cells divide and differentiate to form the vulva and 12 of 19 gamma-aminobutyric acid (GABA)-producing (GABAergic) neurons (Sulston and Horvitz 1977).

Forward genetic screens for mutants missing P-cell-derived lineages identified 3 parallel pathways that move P-cell nuclei through constrictions. The first pathway is the linker of nucleoskeleton and cytoskeleton (LINC) complex pathway. The LINC pathway includes the Klarsicht/*ANC-1*/SYNE homology (KASH) protein *UNC-83* in the outer nuclear membrane and the Sad1/*UNC-84* (SUN) protein *UNC-84* in the inner nuclear membrane (Malone *et al.* 1999; Starr *et al.* 2001; Starr 2019). *UNC-83* and *UNC-84* interact with one another in the perinuclear space to create a force-transducing bridge across the nuclear envelope (McGee *et al.* 2006). *UNC-84* interacts with lamins in the nucleoplasm. The cytoplasmic domain of *UNC-83* recruits cytoplasmic dynein to the outer nuclear membrane to couple the nucleoskeleton to the microtubule cytoskeleton (Fridolfsson *et al.* 2010; Bone *et al.* 2014; Gregory *et al.* 2023). Dynein then generates the forces to move P-cell nuclei toward the minus ends of microtubules (Bone *et al.* 2016; Ho *et al.* 2018). The second pathway is an actin-based pathway that involves the small GTPase cell division control protein 42 (*CDC-42*) (Chang *et al.* 2013). This pathway also includes *CDC-42* guanine nucleotide exchange factor-1 (*CGEF-1*), a predicted GEF for *CDC-42* (Ho *et al.* 2023). Auxin-induced degradation of *CDC-42* or the actin-branching actin-related protein 2/3 (Arp2/3) complex subunit *ARX-3* in larval P cells also induced strong nuclear migration defects. This suggests that these actin regulators work together in a common pathway to help move

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P-cell nuclei through constrictions (Ho et al. 2023). In the third pathway, mutations in filamin-2 (*fln-2*) led to an increase in nuclear rupturing. This suggests that FLN-2 functions to maintain and/or repair the nuclear envelope during P-cell nuclear migration (Ma et al. 2024).

Inhibiting any of the LINC complex, actin/CDC-42, or FLN-2-dependent pathways individually leads to mild or no nuclear migration defects (Chang et al. 2013; Bone et al. 2016; Ho et al. 2023; Ma et al. 2024). Moreover, simultaneously inhibiting all 3 pathways leads to a severe, but still incompletely penetrant defective nuclear migration phenotype (Ma et al. 2024). Therefore, we hypothesized that another pathway functions in parallel to these 3 previously identified pathways to help move P-cell nuclei through constricted spaces.

In other systems, heterochromatin is thought to safeguard nuclear integrity from mechanical stresses during nuclear movements and shape changes without significant changes in gene expression (Kalukula et al. 2022). Heterochromatin facilitates mammalian tissue culture cell migration through micropores in a transcription-independent manner (Gerlitz and Bustin 2010; Krause et al. 2019; Hsia et al. 2022). Similarly, inhibiting histone H3K4 methyltransferases affected the physical properties of nuclei and abrogated interstitial T cell migration through a 3D matrix as well as in a zebrafish xenotransplantation model (Wang et al. 2018). The peripheral localization of heterochromatin at the nucleoplasmic face of the inner nuclear membrane also appears to be critical for its function in regulating nuclear dynamics (Penagos-Puig and Furlan-Magaril 2020). Untethering heterochromatin from the nuclear envelope increases nuclear deformability in the fission yeast *Schizosaccharomyces pombe* (Schreiner et al. 2015). Based on these previously reported findings, we explored how peripheral heterochromatin might function independently of gene expression during *C. elegans* P-cell nuclear migration. Here, we identify a fourth pathway where the inner nuclear membrane protein CEC-4, which anchors heterochromatin at the nuclear periphery (Gonzalez-Sandoval et al. 2015), facilitates larval P-cell nuclear migration through constricted spaces.

Results and discussion

Untethering H3K9-methylated heterochromatin from the inner nuclear membrane contributes to P-cell nuclear migration failure

We hypothesized that heterochromatin anchored at the nuclear periphery affects nuclear deformability, thus impacting the capability of P-cell nuclei to migrate through narrow spaces. To test this hypothesis, we asked if P-cell nuclear migration occurred normally in *cec-4* null animals. The *C. elegans* inner nuclear membrane protein CEC-4 anchors H3K9-methylated chromatin to the periphery of the nuclear envelope (Gonzalez-Sandoval et al. 2015). Mutating *cec-4* untethers H3K9-methylated heterochromatin from the nucleoplasmic face of the inner nuclear membrane without affecting global transcription (Gonzalez-Sandoval et al. 2015). This approach enabled us to assess the influence of inner nuclear membrane-anchored heterochromatin on in vivo nuclear migration independently of any alterations in gene expression.

We assay P-cell nuclear migration by scoring missing cells in the P-cell lineage after nuclei move to the ventral cord (Fridolfsson et al. 2018). Normally, 12 of 19 GABAergic neurons are derived from P cells. Thus, defects in P-cell nuclear migration are expected to have significantly fewer GABAergic neurons than wild-type animals. We examined mutant lines at 15, 20, and 25°C because it is known that *unc-84*(null) and *unc-83*(null) mutants

cause a temperature-dependent defect in P-cell nuclear migration where migration is disrupted at 25°C but mostly normal at 15°C (Malone et al. 1999; Starr et al. 2001). The reason for the temperature-sensitive phenotype is not clear. It could be due to the length of time that fibrous organelles are cleared for nuclear migration to occur (Bone et al. 2016). Alternatively, it could be due to the loss of phase-separated heterochromatin-maintenance domains at the nuclear periphery at higher temperatures (Delaney et al. 2019). Regardless, examining the phenotypes at a range of temperatures allows us to compare the wide range of penetrance of the phenotype, with 12 missing neurons representing complete penetrance.

cec-4(ok3124) null mutant animals had an average of 19 GABAergic neurons at 15, 20, and 25°C, indicating that untethering H3K9-methylated heterochromatin from the nuclear periphery, on its own, does not disrupt P-cell nuclear migration (Fig. 1). We then crossed the *cec-4* mutant into the sensitized, LINC complex-defective *unc-84*(n369) null background. *unc-84*(null) mutants are missing 3–5 GABAergic neurons at 25°C, and are nearly wild-type at 15°C. In *unc-84*(n369) animals, loss of *cec-4* significantly increased the number of missing GABA neurons relative to controls. These phenotypes were comparable to those observed in the *cgef-1*(yc3) *unc-84*(n369) double mutant (Ho et al. 2023), in which both the LINC complex and actin/CDC-42-dependent P-cell nuclear migration pathways are disrupted.

The timing of P-cell defects in development follows a clear progression. After nuclear migration, P cells divide to produce 2 distinct lineages: anterior daughters that form GABA neurons and posterior daughters that contribute to vulva development (Sulston and Horvitz 1981). Failed nuclear migration leads to P-cell death, resulting in both egg-laying defective (Egl) and uncoordinated (Unc) phenotypes (Sulston and Horvitz 1981). We count the loss of GABA neurons as a proxy for failed nuclear migrations (Fridolfsson et al. 2018). We previously demonstrated that counting missing GABA neurons are a good proxy to measure P-cell death, and that we are not inadvertently measuring something later in the development of GABA neurons for the LINC, actin/CDC-42, and FLN-2 pathways (Starr et al. 2001; Chang et al. 2013; Ho et al. 2023; Ma et al. 2024). Thus, it is probable that the *cec-4* enhancement observed here is also functioning at the time of P-cell nuclear migration and not later in GABA neuron development. If the *cec-4* pathway is enhancing *unc-84* function during P-cell nuclear migration, all later P-cell lineages should be defective, and the mutants should be Egl due to missing vulval cells. To test this, we examined egg-laying in strains carrying the *ycEx60[unc-84(+); odr-1::RFP]* extrachromosomal array, where RFP expression distinguishes *cec-4 cgef-1* double mutants (RFP+) from *cec-4 cgef-1 unc-84* triple mutants (RFP-) at the restrictive temperature (25°C). It is difficult to quantify percent Egl, as eventually wild-type animals are Egl when plates are close to starved, making percent Egl a qualitative trait. Nonetheless, we looked at Egl and non-Egl animals from this strain. We found that 85% of Egl animals were RFP- (and therefore *unc-84* mutant; *n* = 60), while only 13% of non-Egl adults were RFP- (*n* = 99). Thus, the absence of *unc-84* in a *cec-4 cgef-1* background leads to defects in both the GABA and vulval lineages, suggesting that the enhanced defect is due to P cells death at the time that nuclear migration would normally occur, prior to the division of P cells into neuronal and vulval lineages. We conclude it is likely that the anchorage of heterochromatin to the nucleoplasmic face of the inner nuclear membrane is critical for P-cell nuclear migration in LINC complex-defective larvae. Future studies are required to directly visualize heterochromatin organization at the nuclear periphery in LINC mutants.

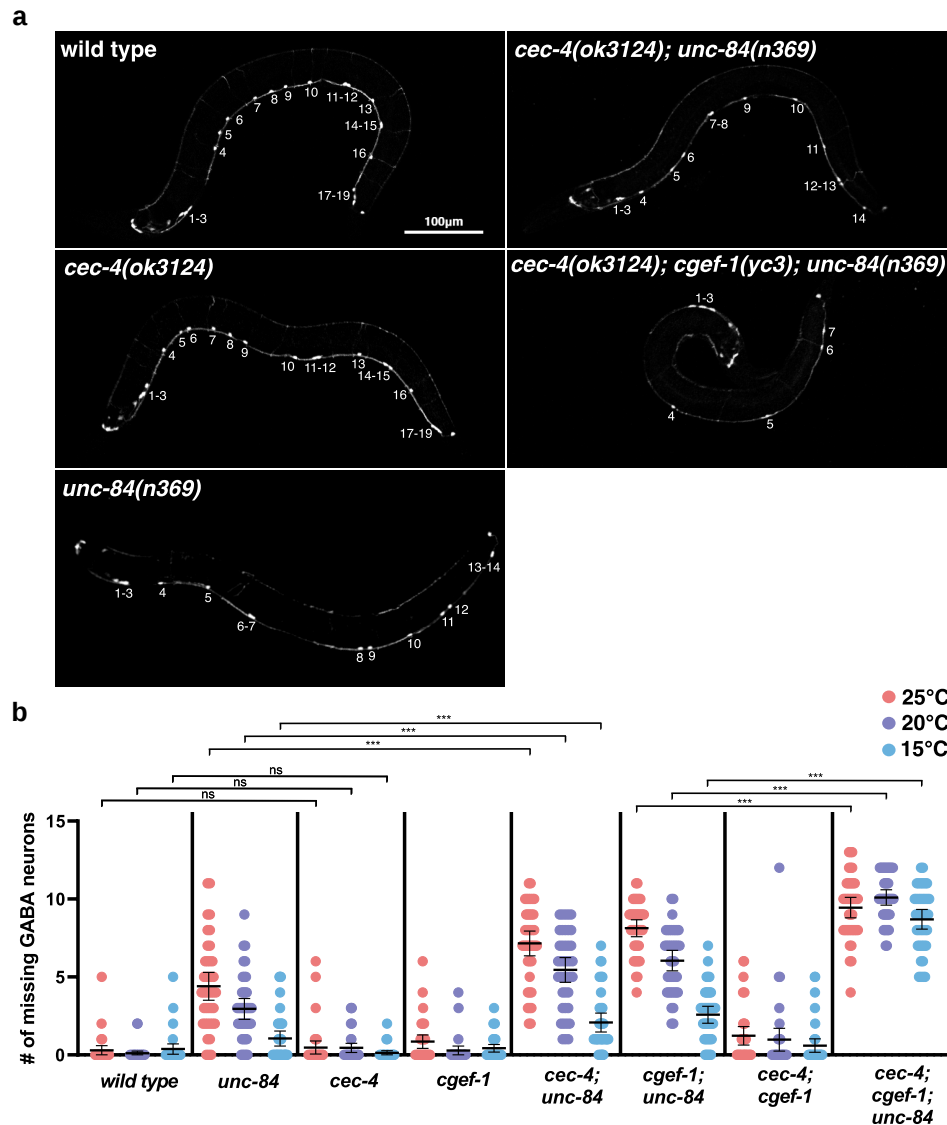


Fig. 1. The *cec-4* null mutation enhances the P-cell nuclear migration defect observed in *unc-84* and *unc-84; cgef-1* null mutants. a) Images of L4 animals grown at 25°C expressing GFP from the *oxIs12[p_{unc-47}::gfp]* marker in P-cell derived GABAergic neurons. Dorsal is up, ventral is down. The numbers of GABAergic neurons present in the ventral cord were scored and are numbered from anterior (left) to posterior. Scale bar: 100 μ m. b) Plot of the number of missing GABAergic neurons from the 19 observed in wild-type (UD87 RFP+) and null mutant lines *unc-84* (UD87 RFP–), *cec-4* (UD462 RFP+), *cgef-1* (UD285 RFP+), *cec-4; unc-84* (UD462 RFP–), *cgef-1; unc-84* (UD285 RFP–), *cec-4; cgef-1* (UD795 RFP+), and *cec-4; cgef-1; unc-84* (UD795 RFP–). Each dot represents a single worm. Three different temperatures are shown: 25°C (salmon), 20°C (violet), and 15°C (blue). $n = 40$. Means and 95% confidence intervals (CIs) are shown. Tests for statistical significance are shown to compare *cec-4* to wild-type, *cec-4; unc-84* double mutants to *unc-84* single mutants, and *cec-4; cgef-1; unc-84* triple mutants to *cgef-1; unc-84* double mutants. Results were compared using a Tukey HSD test. *** $P \leq 0.001$.

We next simultaneously disrupted all 3 pathways (i.e. the LINC complex, the actin/CDC42, and the *CEC-4*-dependent pathways). The triple *cec-4; cgef-1; unc-84* mutant had a nearly complete nuclear migration failure at 15, 20, and 25°C, significantly worse than all 3 double mutant combinations (Fig. 1). These results suggest that the LINC complex, actin/CDC-42, and *CEC-4*-dependent pathways function in parallel.

Depleting H3K9-methylated heterochromatin in LINC complex-defective animals enhanced defects in P-cell nuclear migration

C. elegans encodes a pair of H3K9 methyltransferases, *MET-2* and *SET-25* (Ahringer and Gasser 2018). *MET-2*, a homolog of mammalian SET domain bifurcated histone lysine methyltransferase 1 (SETDB1), is the primary methyltransferase for H3K9me1 and

me2 (Towbin et al. 2012). *SET-25* plays a secondary role in H3K9me1 and me2 but is the only methyl transferase for H3K9me3 (Towbin et al. 2012). Furthermore, *met-2(n4256); set-25(n5021)* double mutant animals exhibit disrupted anchorage of heterochromatin to the nuclear periphery, resulting in a phenotype similar to what was observed in *cec-4(ok3124)* mutant animals (Towbin et al. 2012; Gonzalez-Sandoval et al. 2015).

To test the hypothesis that the methyltransferases *met-2* and *set-25* function in the *cec-4* pathway, we examined their genetic interactions with mutations that disrupt the LINC complex and actin/CDC-42 pathways (Fig. 2). We generated double mutants by crossing the null alleles *met-2(n4256)* or *set-25(n5021)* into *cgef-1(yc3)* and *unc-84(n369)* backgrounds. Analysis of the double mutants revealed pathway-specific effects. *met-2(n4256); cgef-1(yc3)* and *set-25(n5021); cgef-1(yc3)* double mutants both showed normal

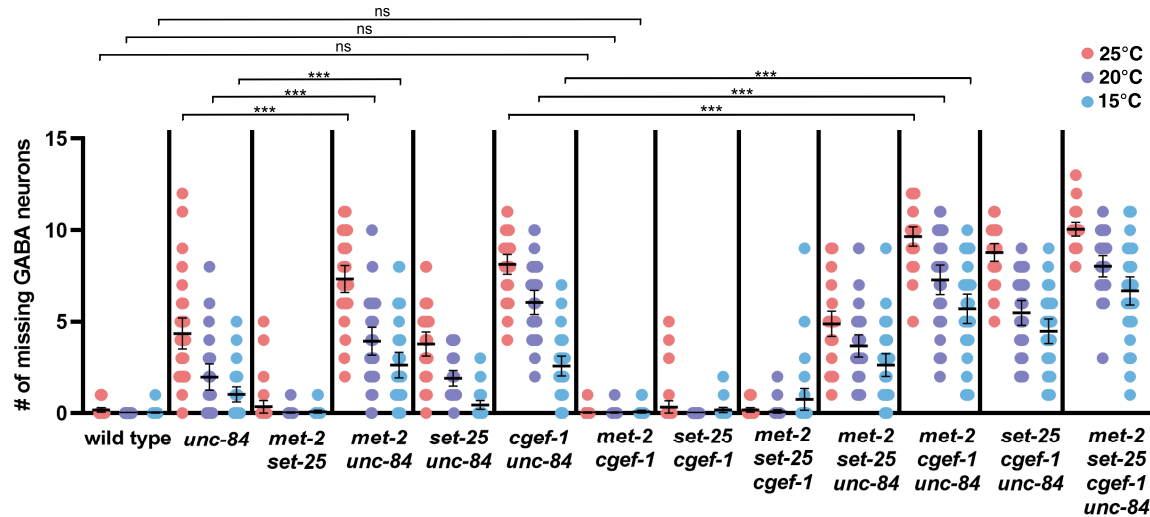


Fig. 2. Inhibiting H3K9 methylation enhances the P-cell nuclear migration defects observed in *unc-84* mutants. Plot of the number of missing GABAergic neurons observed in wild-type and indicated mutant lines. See Table 1 for strain names and full genotypes. Each dot represents a single worm. $n = 40$. Three different temperatures are shown: 25°C (salmon), 20°C (violet), and 15°C (blue). Means and 95% CIs are shown. Significance is indicated for select genotypes with *met-2* mutations compared with wild-type and *unc-84* mutants. The *set-25* mutants were not significantly different from wild-type counterparts, and the significance is therefore not shown. Results were compared using a Tukey HSD test. *** $P \leq 0.001$.

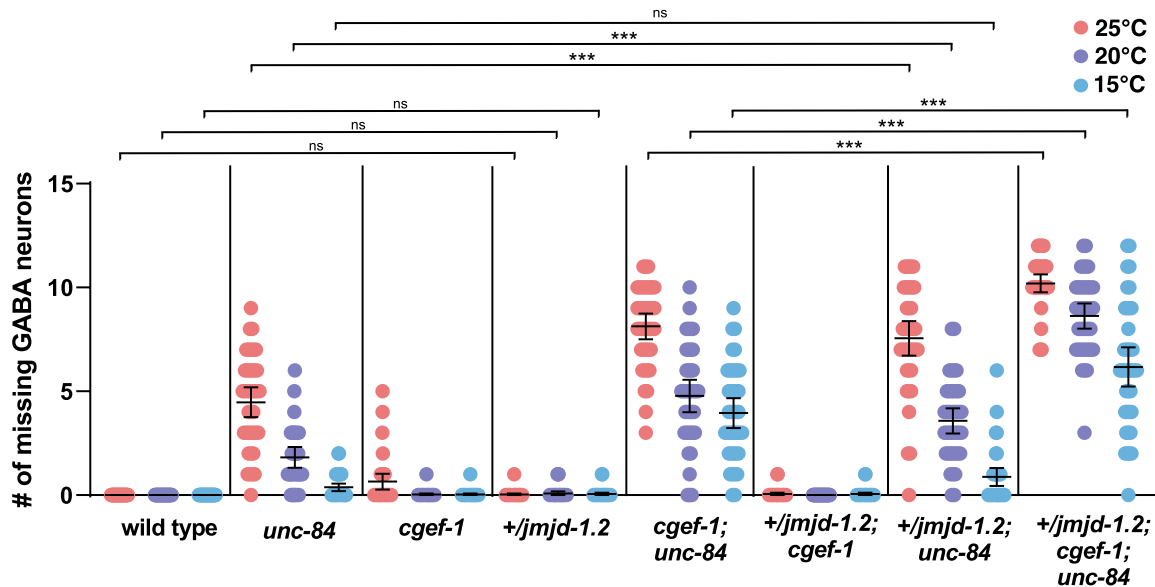


Fig. 3. The presence of one copy of the *jmd-1.2* mutation enhances the P-cell nuclear migration defect observed in *unc-84* mutant animals. A plot of the number of missing GABAergic neurons of wild-type (UD87 RFP+ animals) and the following null mutant lines: *unc-84* (UD87 RFP-), *cgef-1* (UD285 RFP+), *+/jmd-1.2* (UD996 RFP+), *cgef-1; unc-84* (UD285 RFP-), *+/jmd-1.2; unc-84* (UD996 RFP-), *+/jmd-1.2; cgef-1* (UD997 RFP+), and *+/jmd-1.2; cgef-1; unc-84* (UD997 RFP-). Each dot represents a single worm. Three different temperatures are shown: 25°C (salmon), 20°C (violet), and 15°C (blue). $n = 40$. Means and 95% CIs are shown. Significance indicated is compared to *unc-84*(n369) at each temperature. Results were compared using a Tukey HSD test. *** $P \leq 0.001$.

numbers of GABAergic neurons, consistent with having intact LINC complexes. However, mutants that had disrupted LINC complexes had numbers of missing GABA neurons consistent with, or enhanced compared to, the *unc-84*(n369) single mutant. *met-2*(n4256), *unc-84*(n369) doubles exhibited increased missing neurons compared with the single mutant strains. This phenotype was comparable to but slightly less severe than *cgef-1; unc-84* doubles (Ho et al. 2023). In contrast, *set-25*(n5021) had no significant enhancement of *unc-84*(n369) defects, consistent in acting in a stepwise fashion after the initial deposition of H3K9me1/2 by *MET-2*. These results indicate that *met-2* functions

parallel to *unc-84* in nuclear migration, while *set-25* appears dispensable for this process. The normal phenotype of *met-2; cgef-1* doubles prevented conclusions about *MET-2*'s relationship to the actin/CDC-42 pathway.

To test whether *met-2* represents a distinct pathway, we examined *met-2*(n4256), *cgef-1*(yc3), *unc-84*(n369) triple mutants (Fig. 2). These animals showed significantly enhanced defects compared with all double mutant combinations, approaching the theoretical maximum of 12 missing neurons at higher temperatures. This nearly complete penetrance suggests the involvement of 3 parallel pathways.

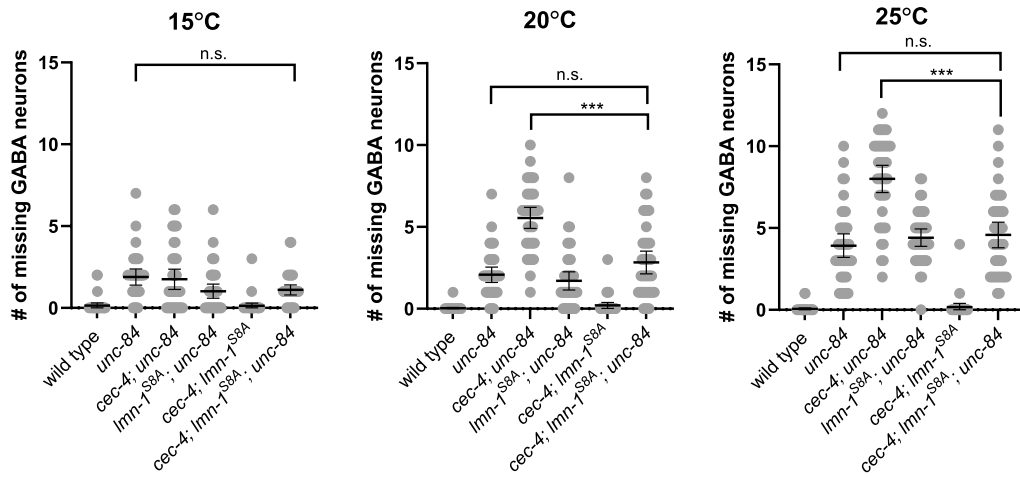


Fig. 4. A non-phosphorylatable allele of *lmn-1* suppresses the P-cell nuclear migration defect of *cec-4; unc-84* double mutants. A plot of the number of missing GABAergic neurons of wild-type (UD87 RFP+ animals) and the following null mutant lines: *unc-84* (UD87 RFP–), *cgef-1, unc-84* (UD285 RFP–), *lmn-1^{S8A}, unc-84* (UD624 RFP–), *cec-4, lmn-1^{S8A}* (UD674 RFP+), and *cec-4, lmn-1^{S8A}, unc-84* (UD674 RFP–). Each dot represents a single worm. Three different temperatures are shown: 15, 20, and 25°C because of the temperature-sensitive phenotype of *unc-84*. $n = 40$. Means and 95% CIs are shown. Significance indicated is compared to *unc-84(n369)* at each temperature. Results were compared using a Tukey HSD test. *** $P \leq 0.001$, n.s. $P > 0.05$.

Table 1. Strains used in this study.

Strain	Genotype	Reference
EG1285	<i>oxIs12[p_{unc-47::gfp}] X</i>	McIntire et al. (1997)
UD87	<i>unc-84(n369), oxIs12 X; yEx60[odr-1::rfp, WRM0617cH07 unc-84(+)]</i>	Chang et al. (2013)
RB2301	<i>cec-4(ok3124) IV</i>	Consortium (2012)
UD642	<i>cec-4(ok3124) IV; unc-84(n369), oxIs12 X; yEx60</i>	This study
UD285	<i>cgef-1(y3), unc-84(n369), oxIs12 X; yEx60</i>	Chang et al. (2013)
UD795	<i>cec-4(ok3124) IV; cgef-1(y3), unc-84(n369), oxIs12 X; yEx60</i>	This study
MT13293	<i>met-2(n4256) III</i>	Lee et al. (2019)
MT17463	<i>set-25(n5021) III</i>	Andersen and Horvitz (2007)
UD906	<i>met-2(n4256) III; unc-84(n369), oxIs12 X; yEx60</i>	This study
UD910	<i>set-25; unc-84(n369), oxIs12 X; yEx60</i>	This study
UD957	<i>met-2(n4256) III; cgef-1(y3), unc-84(n369), oxIs12 X; yEx60</i>	This study
UD952	<i>set-25(n5021) III; cgef-1(y3), oxIs12 X</i>	This study
UD922	<i>set-25(n5021) III; cgef-1(y3), unc-84(n369), oxIs12 X</i>	This study
UD874	<i>set-25(n5021), met-2(n4256) III; oxIs12 X</i>	This study
UD945	<i>set-25(n5021), met-2(n4256) III; cgef-1(y3), oxIs12 X</i>	This study
UD893	<i>set-25(n5021), met-2(n4256) III; unc-84(n369), oxIs12 X; yEx60</i>	This study
UD928	<i>set-25(n5021), met-2(n4256) III; cgef-1(y3), unc-84(n369), oxIs12 X</i>	This study
VC2878	<i>jmjd-1.2(ok3628) IV/nT1[qIs51] (IV;V)</i>	Consortium (2012)
UD996	<i>jmjd-1.2(ok3628) IV/nT1[qIs51] (IV;V); unc-84(n369), oxIs12 X; yEx60</i>	This study
UD997	<i>jmjd-1.2(ok3628) IV/nT1[qIs51] (IV;V); cgef-1(y3), unc-84(n369), oxIs12 X; yEx60</i>	This study
UV2059	<i>lmn-1(jf140)[S21,22,24,32,397,398,403,405A] I</i>	Velez-Aguilera et al. (2020)
UD624	<i>lmn-1(jf140) I; unc-84(n369) oxIs12 [unc-47::GFP] X; yEx60 [odr-1::RFP, unc-84(+)]</i>	This study
UD674	<i>lmn-1(jf140) I; cec-4(ok3124) IV; unc-84(n369) oxIs12 [unc-47::GFP] X; yEx60 [odr-1::RFP, unc-84(+)]</i>	This study

Importantly, *met-2(n4256); set-25(n5021); cgef-1(y3) unc-84(n369)* quadruple mutants showed no enhancement beyond the triple mutant phenotype, supporting our model that *met-2* functions in the *cec-4* pathway, while *set-25* is dispensable (Fig. 2). Together, these findings establish *MET-2* as a key methyltransferase acting parallel to both the LINC complex and actin/*CDC-42*-dependent pathways in P-cell nuclear migration and support that H3K9-methylated heterochromatin is required for P-cell nuclear migration.

Inhibiting the JMJD-1.2 demethylase in the *unc-84(null)* background leads to P-cell nuclear migration defects

Decompaction of heterochromatin induced by histone methyltransferase or deacetylase inhibitors alters nuclear structure

and inhibits cell migration (Gerlitz and Bustin 2010; Stephens et al. 2018). Likewise, treatment with histone demethylase inhibitors can rescue abnormal nuclear morphology even in the presence of disrupted lamins (Stephens et al. 2018). Thus, we hypothesized that knocking down a H3K9 demethylase would suppress the P-cell nuclear migration defect of *unc-84; cgef-1* null mutants. To test this, we investigated the role of *jmjd-1.2*, which encodes a JmJC demethylase that targets H3K27me2 and H3K9me2 repressive marks (Kleine-Kohlbrecher et al. 2010), during P-cell nuclear migration. We used a heterozygous *jmjd-1.2(ok3628)* balanced line, referred to as *jmjd-1.2(ok3628)/+*, because the homozygous *jmjd-1.2* animals are embryonic lethal (Myers et al. 2018).

jmjd-1.2/+ animals in an otherwise wild-type background did not exhibit a P-cell nuclear migration defect (Fig. 3). However,

the *jmjd-1.2(ok3268)/+* mutation significantly enhanced the *unc-84(n369)* P-cell nuclear migration defects at 20 and 25°C (Fig. 3). Furthermore, *jmjd-1.2(ok3268)/+; cgef-1(yc3) unc-84(n369)* triple mutant animals had significantly more missing GABAergic neurons than the double mutants, approaching the maximum phenotype of 12 expected if they fully inhibited P-cell nuclear migration (Fig. 3). In conclusion, the elimination of one copy of the *jmjd-1.2*, a gene that encodes for a demethylase, resulted in a disruption of P-cell nuclear migration, resembling the pattern observed in *met-2*-null animals, which lack H3K9 methylation. Contrary to our hypothesis that more methylation could suppress P-cell nuclear migration defects, we found that either a lack of or an excess of methylation leads to similar P-cell migration failures. Perhaps there is a sweet spot of heterochromatin methylation at the periphery of nuclei that provides the right amount of mechanical support. Too much could lead to a stiff nucleus that blocks migration and too little could lead to a soft nucleus that cannot survive migration.

A stable nuclear lamina compensates for loss of heterochromatin tethering in P-cell nuclear migration

Our results suggest that *CEC-4* protects P-cell nuclei by anchoring methylated chromatin to the nuclear envelope. The nuclear lamina, formed by the single *C. elegans* lamin homolog *LMN-1*, provides additional structural support to the nuclear envelope, but its role in P-cell nuclear migration remained unexplored (Vahabikashi et al. 2022; Gregory et al. 2023). During meiosis, *LMN-1* undergoes phosphorylation at 8 serine residues, which solubilizes the lamin network to facilitate chromosome movement along the nuclear envelope (Link et al. 2018). When these phosphorylation sites are mutated to alanine (*lmn-1(jf140)*, hereafter *lmn-1^{S8A}*), the resulting stable nuclear lamina restricts chromosome movement and chromatin reorganization at the nuclear periphery (Link et al. 2018). We hypothesized that this non-phosphorylatable *LMN-1* might compensate for the loss of heterochromatin tethering in *cec-4* mutants by providing a more stable nuclear envelope. To test this, we examined *lmn-1^{S8A}* mutants carrying 8 serine-to-alanine mutations in endogenous *lmn-1* locus (Velez-Aguilera et al. 2020). It appears that *lmn-1^{S8A}* mutants do not cause a significant P-cell nuclear migration on its own, because *lmn-1^{S8A} cec-4* double mutants had no defect compared with wild-type. Indeed, *lmn-1^{S8A}* suppressed the nuclear migration defect in *cec-4(ok3124); unc-84(n369)* double null mutants (Fig. 4), suggesting that enhanced nuclear lamina stability can partially overcome the loss of *CEC-4*-mediated heterochromatin tethering.

Conclusions

Our genetic data support a model where the anchorage of H3K9-methylated heterochromatin at the nucleoplasmic face of the inner nuclear membrane via *CEC-4* plays an important role in nuclear migration through constricted spaces as a part of normal *C. elegans* development. Alternatively, hyper-stabilization of lamins can partially overcome the requirement of *CEC-4*. This *CEC-4*/heterochromatin/lamina nuclear anchorage-based pathway works with 3 other pathways: the LINC complex pathway (Bone et al. 2016), an actin/CDC-42-based pathway (Ho et al. 2023), and a *FLN-2*-based pathway (Ma et al. 2024).

It remains unclear how anchoring heterochromatin to the inner nuclear membrane is related to the function of LINC complexes. One possibility is that LINC complexes transmit mechanical strain to the peripheral heterochromatin (Kirby and

Lammerding 2018; Lityagina and Dobrova 2021; Kalukula et al. 2022). LINC complexes are required to stabilize the architecture of the nuclear envelope in cells subjected to increased mechanical forces (Crisp et al. 2006; Cain et al. 2014). Heterochromatin has also been implicated in regulating nuclear mechanics (Stephens et al. 2019), suggesting that the absence of peripheral heterochromatin could lead to decreased nuclear morphological changes and/or increased nuclear envelope rupturing (Starr 2012; Maciejowski and Hatch 2020). Disruption of LINC complexes upregulates the deposition of heterochromatic marks in *Drosophila* muscle nuclei (Pavlov et al. 2023). Alternatively, LINC may regulate the distribution or localization of *MET-2* phase-separated foci at the inner nuclear envelope. *MET-2* foci rely on *CEC-4* for localization at the nuclear periphery, but elimination of *CEC-4* does not affect foci formation or number (Delaney et al. 2019). However, *MET-2* foci disperse in response to higher temperature, resulting in germline developmental defects (Delaney et al. 2019). This could be related to the temperature-sensitive P-cell nuclear migration defects seen in LINC null mutant animals (Malone et al. 1999; Starr et al. 2001). We propose that LINC complexes regulate nuclear mechanics by modulating perinuclear heterochromatin. Since *CEC-4* is necessary for heterochromatin localization but not transcriptional repression (Gonzalez-Sandoval et al. 2015), our findings likely reflect the requirement for changes in heterochromatin organization rather than transcription during P-cell nuclei migration.

Materials and methods

C. elegans strains and genetics

Animals were grown on nematode growth medium plates spotted with *OP50 Escherichia coli* (Brenner 1974). Strains were maintained at room temperature (22–23°C), other than those carrying *met-2* and *set-25* mutations, which were kept at 15°C to avoid progressive germline sterility at higher temperatures (Zeller et al. 2016). The strains made and used in this paper are listed in Table 1. The WormBase knowledgebase was used for this research (Sternberg et al. 2024).

P-cell nuclear migration assay

Successful nuclear migration of larval P cells was scored by counting the number of GABAergic neurons marked with *oxIs12[p_{unc-47::gfp}]* (McIntire et al. 1997) in animals at the fourth larval stage of development, as described (Fridolfsson et al. 2018). GABAergic neurons and the *p_{odr-1}::RFP* marker were visualized using a wide-field epifluorescent Leica DM6000 microscope with a 63×Plan Apo 1.40 NA objective, a Leica DC350 FX camera, and Leica LAS AF software (Leica Microsystems, Inc., Deerfield, IL).

Statistics

Data were tested for significance at each temperature (15, 20, and 25°C) with one-way ANOVA. Results were statistically compared by Tukey honestly significant difference (HSD) tests calculated using the R multcomp package v1.4-23 (Hothorn et al. 2008). Graphs were made using Prism 9 software (GraphPad, Boston, MA). All raw data and statistics for all comparisons are given in Supplementary File 1.

Data availability

Strains are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

Supplemental material available at GENETICS online.

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Conflicts of interest

The author(s) declare no conflict of interest.

Author contributions

Conceptualization: E.F.G., G.W.G.L., and D.A.S.; Methodology: E.F.G. and D.A.S.; Validation: E.F.G.; Formal analysis: E.F.G.; Investigation: E.F.G.; Resources: E.F.G., G.W.G.L., and D.A.S.; Data curation: E.F.G.; Writing—original draft: E.F.G. and D.A.S.; Writing—review and editing: E.F.G., G.W.G.L., and D.A.S.; Visualization: E.F.G. and D.A.S.; Supervision: G.W.G.L. and D.A.S.; Project administration: D.A.S.; Funding acquisition: D.A.S.

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