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The UNC-83/UNC-84 LINC members are required for body wall muscle nuclei positioning in *C. elegans*

Andreas Ofenbauer, Baris Tursun
Berlin Institute for Medical Systems Biology, Max Delbrück Center for Molecular Medicine in the Helmholtz Association

Correspondence
andreas.ofenbauer@mdc-berlin.de
baris.tursun@mdc-berlin.de

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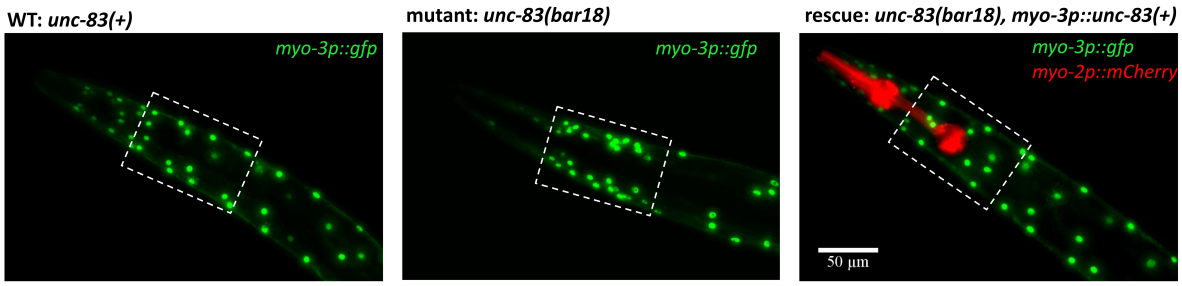
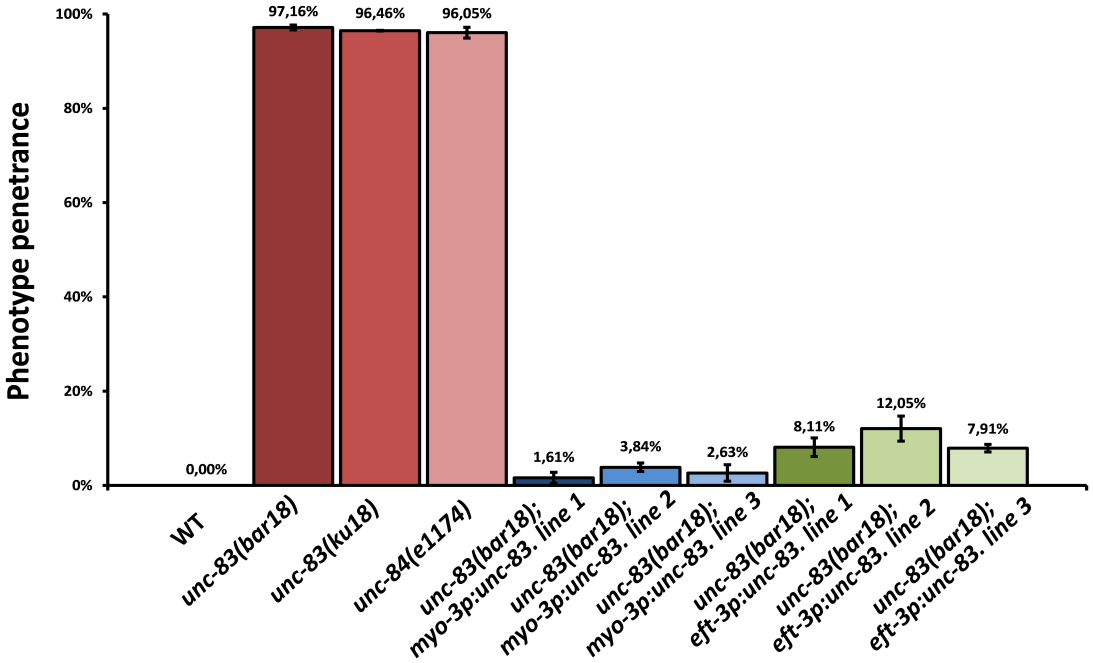
Abstract
From a mutagenesis screen in the nematode *C. elegans* we isolated the mutant *bar18,* showing an accumulation of muscle cell nuclei around the posterior pharyngeal bulb of the worm. Quantification of the overall amount of body wall muscle nuclei, based on the muscle-specific reporter *myo-3p::gfp::NLS*, revealed that the number of nuclei in *bar18* mutants is unchanged compared to WT worms. The accumulation of muscle nuclei around the posterior pharyngeal bulb is due to a positioning defect, which can be precisely quantified by subdividing the worm into head, neck, and posterior body segments.
Whole-genome sequencing revealed that *bar18* animals carry a mutation in the KASH-domain gene *unc-83* causing a premature STOP. An additional *unc-83* mutant allele recapitulates the phenotype, as does a mutant allele of UNC-84, a SUN-domain containing protein that interacts with UNC-83. UNC-83 and UNC-84 belong to a Linker of Nucleoskeleton and Cytoskeletonnuclear (LINC) complex that bridges the nuclear lamina with the cytoskeleton. SUN and KASH domain proteins are conserved in mammals and mutations in the corresponding genes have been linked to cancer, autism, muscular dystrophy and other diseases. Additionally, LINC complexes that function in nuclear migration have also been identified in mammals.
We were able to rescue the *unc-83* mutant phenotype by expressing the WT gene under a muscle-specific (*myo-3p*) promoter, demonstrating that the effect is cell autonomous. Mutations in either *unc-83* or *unc-84* have previously been linked to nuclear migration defects in P cells, intestinal cells and *hyp7* hypodermal precursors but not in muscles. Whether the mis-positioning of muscle nuclei is due to migration or anchoring defects still needs to be determined.

Introduction
The nuclear lamina is connected to the cytoskeleton via different 'Linker of Nucleoskeleton and Cytoskeleton' (LINC) complexes with a variety of functions. LINC complexes are widely conserved over various phyla, which include organisms such as plants, slime molds, yeast, roundworms, fruit flies and mammals. LINC complexes cross the nuclear membrane and are composed of SUN and KASH domain-containing proteins, which interact in the perinuclear space between the inner and outer nuclear membrane. KASH proteins are located at the outer nuclear membrane and may interact with actin filaments, microtubules (via dynein and kinesin), intermediate filaments (via spectrin), centrosomes and other cytoplasmic organelles. SUN proteins are located at the inner nuclear membrane and are associated with both chromatin and nuclear lamins. Functions include nuclear movement and anchoring, moving meiotic chromosomes and telomeres and sensing mechanic stimuli [1] [2] [3].
The KASH protein UNC-83 and the SUN protein UNC-84 form a LINC complex in *C. elegans*, which is required for migration of nuclei in P cells, intestinal cells and *hyp7* hypodermal precursors, by recruiting dynein and kinesin-1 to the nuclear surface [4] [5] [6] [7] [8] [9]. Furthermore, UNC-84 has been implicated in maintaining the nuclear architecture of force-bearing cells, like body wall muscles [10].

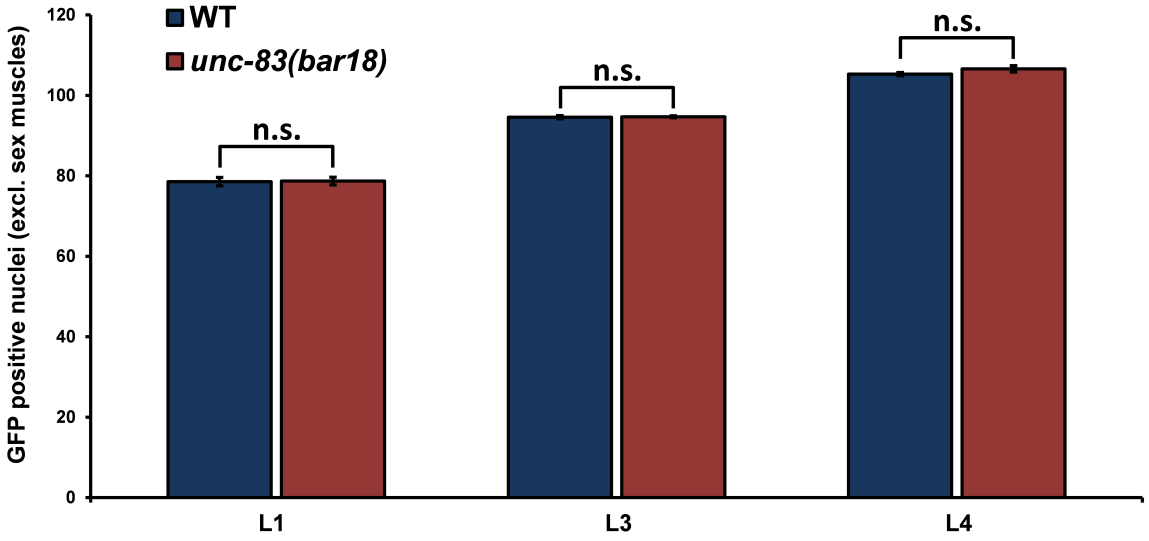
Objective
Our aim was to describe and quantify the novel observation of mis-positioned body wall muscle nuclei upon loss of the UNC-83/UNC-84 LINC complex and to address the question of whether the effect was cell autonomous or not.


WT: unc-83(+)
myo-3p::gfp
mutant: unc-83(bar18)
myo-3p::gfp
rescue: unc-83(bar18), myo-3p::unc-83(+)
myo-3p::gfp
myo-2p::mCherry
50 µm

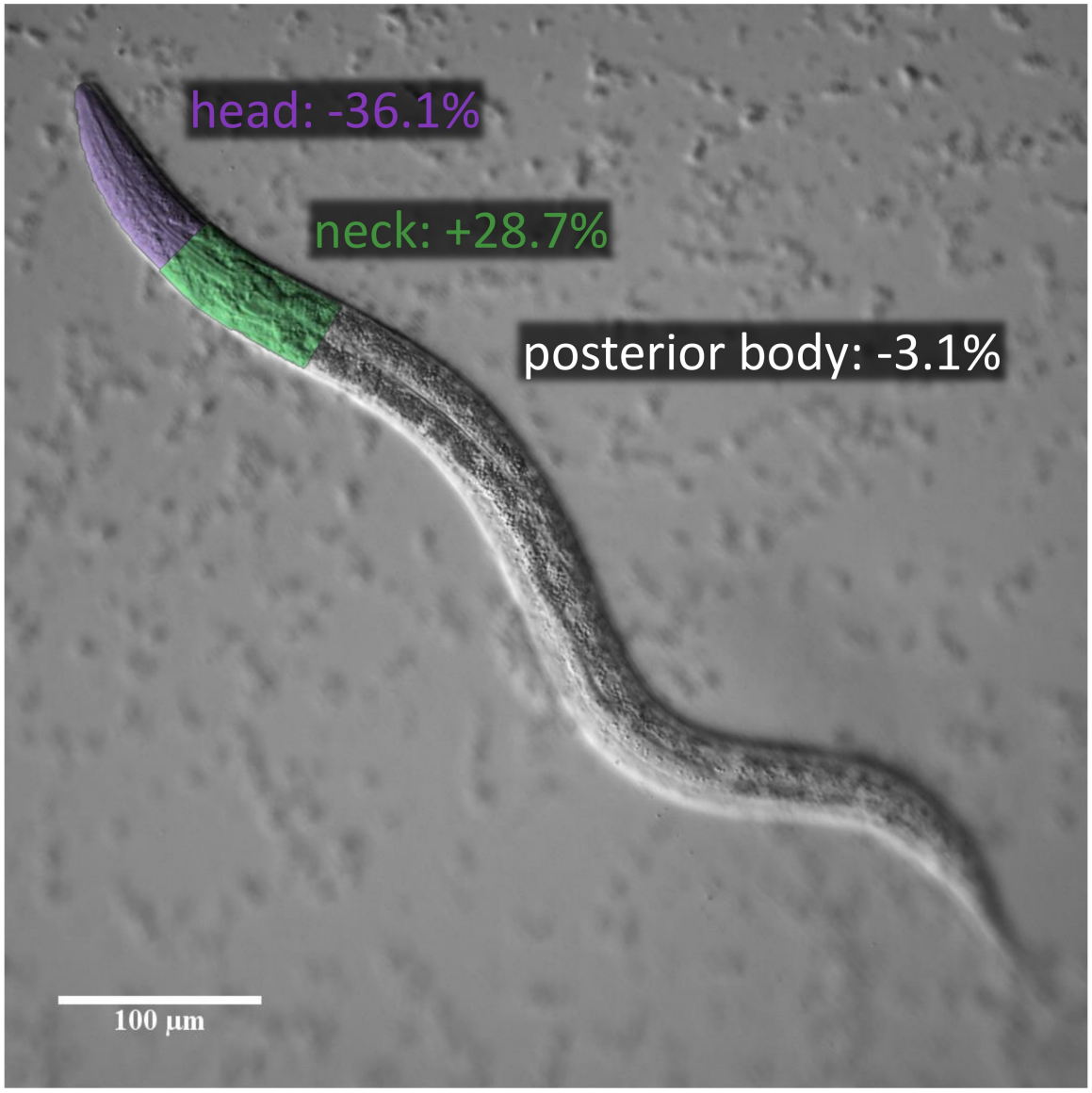
a


Phenotype penetrance
100%
80%
60%
40%
20%
0%
0,00%
97,16%
96,46%
96,05%
1,61%
3,84%
2,63%
8,11%
12,05%
7,91%
WT
unc-83(bar18)
unc-83(ku18)
unc-84(e1174)
unc-83(bar18); myo-3p:unc-83. line 1
unc-83(bar18); myo-3p:unc-83. line 2
unc-83(bar18); myo-3p:unc-83. line 3
unc-83(bar18); eft-3p:unc-83. line 1
unc-83(bar18); eft-3p:unc-83. line 2
unc-83(bar18); eft-3p:unc-83. line 3

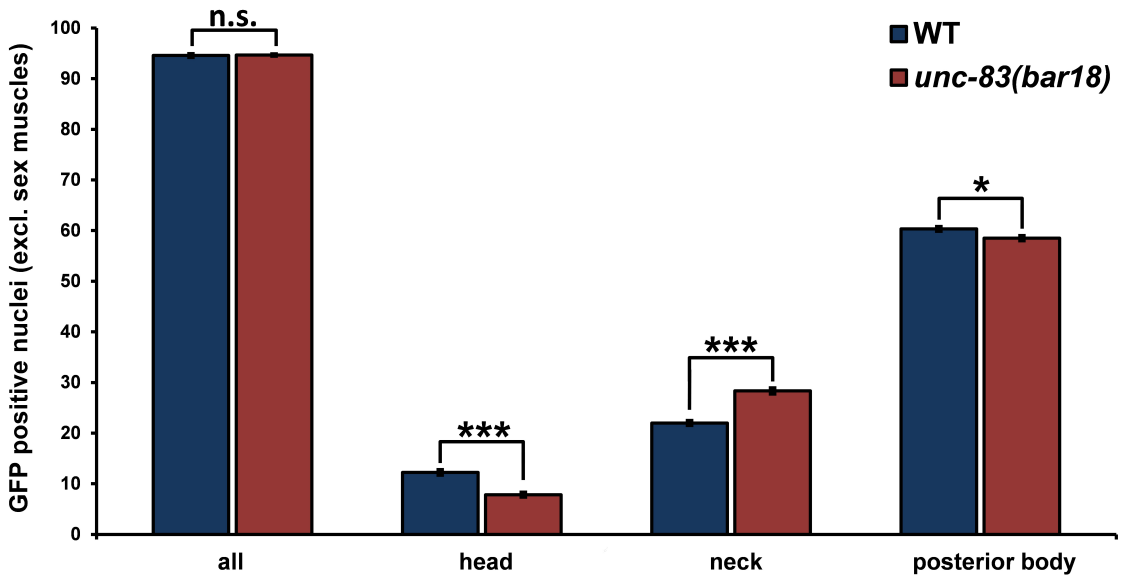
b


WT
unc-83(bar18)
GFP positive nuclei (excl. sex muscles)
120
100
80
60
40
20
0
n.s.
n.s.
n.s.
L1
L3
L4

c



d



e

Figure Legend

Figure 1. A non-functioning UNC-83/UNC-84 LINC complex leads to displaced body wall muscle nuclei in *C. elegans*.

(A) Fluorescent images showing GFP-positive body wall muscle nuclei in WT (BAT661), *unc-83(bar18)* mutant (BAT1298, without the extrachromosomal rescue construct *barEx453*) and *unc-83(bar18)* mutant rescue animals (BAT1298, with the extrachromosomal rescue construct *barEx453*, driving WT *unc-83* from the muscle-specific promoter *myo-3p*). In the rescued animal, the pharynx expresses *myo-2p::mCherry* which is marking transgenic animals carrying *barEx453*. Dashed boxes highlight the area around the posterior pharyngeal bulb, where nuclei accumulate in the mutant. Anterior is to the left.

(B) Phenotype penetrance of body wall muscle nuclei mis-positioning penetrance in WT (BAT661), *unc-83(bar18)* mutant (BAT197), *unc-83(ku18)* mutant (BAT1980), *unc-84(e1174)* mutant (BAT968), *unc-83(bar18)* muscle-specific rescued (BAT1298, BAT1906, BAT1907) and *unc-83(bar18)* ubiquitously rescued (BAT1300, BAT1908, BAT1909) animals. n ≥300 for each sample; 3 biological repeats; Error bars represent SEM.

(C) Amount of GFP-positive nuclei (excluding sex muscles) in WT (BAT1488) and *unc-83(bar18)* mutant (BAT1099) animals at the larval stages L1, L3 and L4. For L1 and L3 stages, these are exclusively body wall muscle cells. For the L4 stage, nuclei of other *myo-3p::gfp*-positive muscles, like somatic sheath or enteric muscles, contribute to the overall number of counted cells. n ≥10 for each condition; n.s. = not significant according to a student's t-test ($p > 0.05$) between WT and mutant; Error bars represent SEM.

(D) Differential interference contrast (DIC) microscopic image of a L3 worm. The defined areas head (anterior part of the worm until after anterior pharyngeal bulb), neck (end of head region until after first pair of intestinal nuclei) and posterior body (end of neck region until posterior end of the worm) are highlighted. The differences in body wall muscle nuclei numbers between WT (BAT1488) and *unc-83(bar18)* mutant (BAT1099) worms, are shown (based on the values shown in (E)).

(E) Distribution of body wall muscle nuclei in WT (BAT1488) and *unc-83(bar18)* mutant (BAT1099) L3 animals. There is no significant difference for the total number of body wall muscle nuclei. For the head and posterior body region, mutant animals display reduced amounts of body wall muscle nuclei, while the number of nuclei in the neck region is increased. Statistical significance based on student's t-test. n =12 for each

condition; *p ≤0.02, ***p ≤0.0001; Error bars represent SEM.

Results & Discussion

We performed a forward genetics EMS screen to isolate mutants with an aberrant number of muscle nuclei in the nematode *C. elegans*. After mutagenizing worms carrying the muscle specific myosin reporter construct *ccIs4251 [myo-3p::gfp::NLS] I*, the F1 generation was analyzed for an atypical number of GFP-positive nuclei. A semi-automated high-throughput screen using a system that allows fluorescence-assisted sorting of large particles (Biosorter, Union Biometrica), yielded the isolation of a single mutant. This mutant was termed *bar18* and showed an accumulation of muscle nuclei around the posterior pharyngeal bulb (Fig. 1A).

To identify the relevant mutation, we used whole-genome sequencing in conjunction with a SNP Mapping Strategy [11] and a published CloudMap pipeline [12]. We identified a premature STOP in the KASH-domain containing gene *unc-83*. To test whether this mutation was causing the observed phenotype, we performed rescue experiments, driving WT *unc-83* from an extrachromosomal array either under the control of the muscle specific promoter *myo-3p* or the ubiquitous promoter *eft-3p* (Fig. 1A, 1B). Since driving WT *unc-83* from either promoter rescues this phenotype, we could not only confirm that the phenotype-causing mutation *bar18* indeed belongs to *unc-83*, but could also show that the effect is cell autonomous. Furthermore, we could phenocopy the dispositioning effect using the *unc-83(ku18)* premature STOP allele, which supports our rescue experiments (Fig. 1B). In addition, we tested the *unc-84(e1174)* deletion allele, which also shows this phenotype, supporting the assumption that a non-functioning UNC-83/UNC-84 LINC complex is responsible for the mis-positioning of muscle nuclei. Alleles are summarized in supplementary figure 1.

Next, we quantified the amount of GFP-positive muscle nuclei in WT and *unc-83(bar18)* worms at different larval stages using the reporter construct *rrrSi261 [myo-3p:: gfp::H2B] I* (in contrast to the reporter described above, a single copy of *rrrSi261* is integrated into the genome thereby making it very dim, but more stable without any tendency for mosaicism). Surprisingly, the overall number of GFP-positive cells remained unchanged for all larval stages (Fig. 1C). At L1 stage, we counted 78.5 vs. 78.6 nuclei, at L3 stage we counted 94.7 vs. 94.6 nuclei and at L4 we counted 106.6 vs. 105.3 nuclei in *unc-83(bar18)* or WT, respectively. Our results are comparable with previously reported numbers of muscle cells [13]: *C. elegans* has an invariant number of somatic cells, including 95 striated body wall muscles from the L2 stage onwards (81 in L1) and several other non-striated muscles, some of which are born at later larval stages.

In order to quantify the positioning defect of body wall muscle nuclei in *unc-83(bar18)* animals, we sub-divided animals into 3 different regions (head, neck, posterior body) and quantified the amount of body wall muscle nuclei in WT and *unc-83(bar18)* animals (Fig. 1D, 1E). The neck region was defined as the region between the anterior pharyngeal bulb and the first pair of intestinal nuclei. The anterior region was defined as head and posterior was defined as posterior body (Fig. 1D). Overall, *unc-83(bar18)* animals displayed 36.1% less nuclei in the head region (7.8 vs. 12.3), 28.7% more nuclei at the neck region (28.3 vs. 22.0) and 3.1% less nuclei in the remainder region 58.5 vs. 60.3). Our findings suggest that the nuclei accumulating in the neck region of *unc-83* mutants originate primarily from the head region (Fig. 1D, 1E).

Conclusions

We describe a so far uncharacterized phenotype of mis-positioned body wall muscle nuclei upon lack of a functioning UNC-83/UNC-84 LINC complex in *C. elegans*. *Unc-83/84* mutant animals display an accumulation of body wall muscle nuclei around the posterior pharyngeal bulb. Our data suggests that this cell autonomous effect is primarily due to nuclei that are displaced from the head of the worm towards the neck region. Our findings broaden our current understanding of the ubiquitously expressed LINC complex, which was so far described to ensure proper nuclei positioning in P cells, the intestine and *hyp7* hypodermal precursors, but not for muscle tissue.

Limitations

The reporters we used to show the nuclei mis-positioning effect in *unc-83(bar18)* animals are nuclear, so they cannot distinguish between mis-positioning of whole cells or of nuclei only. Since the somatic muscle tissue in *C. elegans* is not syncytial (unlike the epidermis), it's likely that whole cells are mis-positioned, but the current study does not address this question.

Furthermore, we did not analyze whether these mis-positioned nuclei have any physiological impact on the worm. For example, if they are linked to previously observed pleiotropic phenotypes [14] such as Unc (uncoordinated, impaired movement) or Egl (egg laying defect).

Finally, it remains to be determined when in development the defect occurs. Since we can rescue the mis-localization of body wall muscle nuclei by driving the WT gene from a *myo-3* promoter, it is likely that the defects are manifested after the *myo-3* promoter gets activated during embryonic development.

Our next steps will be adressing the questions that we have outlined in the 'Limitations' part.

Additional Information

Methods and Supplementary Material

Please see https://sciencematters.io/articles/201805000009.

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Ethics Statement

Not Applicable.

Citations

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