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The *C. elegans* CDK8 Mediator module regulates axon guidance decisions in the ventral nerve cord and during dorsal axon navigation

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ABSTRACT

Receptors expressed on the growth cone of outgrowing axons detect cues required for proper navigation. The pathway choices available to an axon are in part defined by the set of guidance receptors present on the growth cone. Regulated expression of receptors and genes controlling the localization and activity of receptors ensures that axons respond only to guidance cues relevant for reaching their targets. In genetic screens for axon guidance mutants, we isolated an allele of *let-19/mdt-13*, a component of the Mediator, a large ~30 subunit protein complex essential for gene transcription by RNA polymerase II. LET-19/MDT-13 is part of the CDK8 module of the Mediator. By testing other Mediator components, we found that all subunits of the CDK8 module as well as some other Mediator components are required for specific axon navigation decisions in a subset of neurons. Expression profiling demonstrated that *let-19/mdt-13* regulates the expression of a large number of genes in interneurons. A mutation in the *sax-3* gene, encoding a receptor for the repulsive guidance cue SLT-1, suppresses the commissure navigation defects found in *cdk-8* mutants. This suggests that the CDK8 module specifically represses the SAX-3/ROBO pathway to ensure proper commissure navigation.

Introduction

The growth cone at the tip of outgrowing neuronal processes is the primary recipient of signals that guide axons to their target area. These guidance cues can only be recognized when the appropriate receptors are present at the surface of the growth cone (O'Donnell et al., 2009). In order to respond only to relevant cues growth cones are thought to express a limited subset of guidance receptors. Transcription factors, acting late during differentiation of neurons, are considered to be crucial for the correct expression of guidance receptors. One example is the combinatorial expression of LIM homeodomain transcription factors in spinal cord motoneurons. Motoneuron pools innervating muscle cells in a particular target area co-express a set of LIM proteins (Bonanomi and Pfaff, 2010). Misexpression of these transcription factors leads to changes in the innervation pattern, most likely due to changes in the expression of guidance receptors. Ectopic expression of the LIM protein Lhx3 in mouse, for example, induces FGF receptor 1 expression in additional motoneurons, rerouting them to the dermomyotome, the source of the attractive FGF signal (Shirasaki et al., 2006). A similar 'LIM-code' for neuronal differentiation has been described for *Drosophila* motoneurons (Thor et al., 1999) and for the differentiation of C. *elegans* thermo-sensory network interneurons (Hobert et al., 1997; Hobert and Ruvkun, 1998). In *C. elegans*, a number of different transcription factors are involved in axon navigation (Baran et al., 1999; Clark and Chiu, 2003; Doonan et al., 2008; Durbin, 1987; Esmaeili et al., 2002; Miller and Niemeyer, 1995; Prasad et al., 1998; Schmid et al., 2006; Wacker et al., 2003; Westmoreland et al., 2001; Wightman et al., 1997). However, target genes for these transcription factors remain largely unknown.

Misexpression of axon guidance receptors can reroute axons. Ectopic expression of UNC-5 in *C. elegans* mechanosensory neurons steers axons dorsally rather than ventrally (Hamelin et al., 1993). Similarly, ectopic expression of *Drosophila* UNC5 in all post-mitotic neurons prevents commissural axons in the ventral nerve cord (VNC) from crossing the midline (Keleman and Dickson, 2001). Overexpression of ROBO or ROBO2 in *Drosophila* (Simpson et al., 2000) also repels commissural axons from the midline. Therefore proper transcriptional regulation of guidance receptors is crucial for axon navigation since changes in guidance receptors present on the growth cone affect guidance decisions and pathway choices of axons.

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A key component of the transcription regulation machinery is the Mediator complex, a transcriptional co-regulator complex binding both transcription factors and RNA polymerase II (RNAP II). The Mediator complex was initially identified in yeast, where it consists of 25 subunits and interacts with RNAP II (Bourbon, 2008; Kim et al., 1994). In metazoans, the Mediator complex is composed of around 30 subunits (Boyer et al., 1999; Kwon et al., 1999; Malik et al., 2000; Mittler et al., 2001; Naar et al., 1999, 2002; Park et al., 2001; Sato et al., 2004; Taatjes et al., 2002; Wu et al., 2003). Some 26 subunits of the Mediator have homologs in plants, animals and fungi, suggesting that most Mediator subunits are of ancient origin. For inter-species comparison, a unified Mediator Nomenclature has been established (Bourbon et al., 2004). The Mediator consists of four distinct modules (Fig. 1A): head, middle, tail and the separable CDK8 module (Asturias et al., 1999; Davis et al., 2002; Dotson et al., 2000). The head and possibly the middle module contact RNAP II and contain most of the core Mediator subunits essential for basal transcription (Asturias et al., 1999; Dotson et al., 2000). The other modules, the tail module in particular, are considered entry points for regulatory information from transcription factors, making the Mediator complex a key integrator of transcriptional regulation at many RNAP II promoters (Kornberg, 2005).

The CDK8 module consists of MED12/DPY-22/MDT-12, MED13/LET-19/MDT-13, cyclin Cyc C/CIC-1 and the cyclin-dependent kinase CDK8/CDK-8 in a 1:1:1:1 stochiometry (Borggrefe et al., 2002; Knuesel et al., 2009b). The CDK8 module itself likely forms a stable complex in vivo. It binds Mediator via the MED13 subunit, whereas the MED12 subunit can regulate CDK8 kinase activity in vitro (Knuesel et al., 2009a, 2009b). Binding of the CDK8 module and RNAP II to the Mediator is mutually exclusive (Malik et al., 2000; Sun et al., 1998; Taatjes et al., 2002), indicating that the CDK8 module can function in transcriptional repression. CDK8 kinase activity is important for CDK8 module function in yeast (Surosky et al., 1994). However, the kinase activity of CDK8 is not always required for transcriptional repression in vitro (Knuesel et al., 2009a) and it has been suggested that the CDK8 module might sterically inhibit interactions between Mediator and RNAP II (Elmlund et al., 2006). Recent reports indicate that CDK8 is also critical for gene activation in several contexts (Donner et al., 2010, 2007; Firestein et al., 2008; Liu et al., 2004), suggesting that this module may have broader effects than originally anticipated. In mammals, paralogs of MED12, MED13 and CDK8 exist (Bourbon, 2008; Muncke et al., 2003; Sato et al., 2004), indicating that the functions of the non-kinase and kinase part of the CDK8 module diversified even further. The CDK8 module is the target of several signaling pathways, including Ras (Chang et al., 2004; Moghal and Sternberg, 2003), Shh (Zhou et al., 2006), Notch (Fryer et al., 2004), TGF-β (Alarcon et al., 2009) and Wnt (Carrera et al., 2008; Kim et al., 2006; Rocha et al., 2010; Yoda et al., 2005). In the above cases, the CDK8 module is involved in the regulation of specific target genes rather than acting as a general regulator of transcription. Thus the CDK8 module appears to be a versatile regulator of transcription, implicated in a number of signaling pathways in metazoans.

The CDK8 module is an important regulator of gene expression during development in invertebrates (Janody et al., 2003; Moghal and Sternberg, 2003; Treisman, 2001; Wang et al., 2004) and vertebrates (Hong et al., 2005; Rau et al., 2006; Rocha et al., 2010; Shin et al., 2008; Wang et al., 2006). In *Drosophila*, development of the eye-antennal disc is disrupted in MED12- and MED13-deficient flies (Treisman, 2001). Both subunits also regulate the

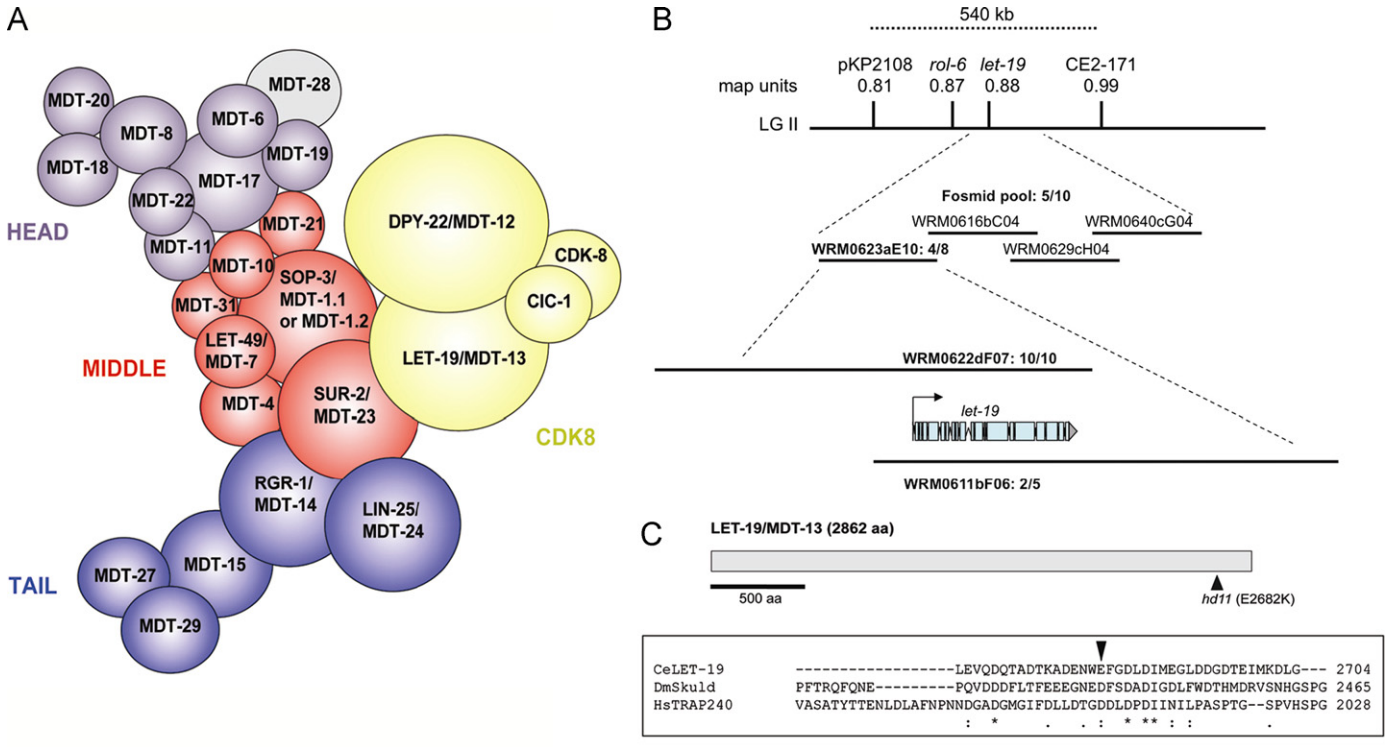

Fig. 1. The *C. elegans* Mediator complex and identification of *hd11* as an allele of *let-19/mdt-13*. (A) The *C. elegans* Mediator complex can be divided into a head (purple), middle (red), tail (blue) and CDK8 module (yellow). The module location of the subunits was taken from (Bourbon, 2008) with MDT-28 (grey) likely located in the head region (Beyer et al., 2007). Subunits were in part arranged according to Guglielmi et al. (2004). Interactions within the CDK8 module were taken from Loncle et al. (2007). The subunits MDT-9, -26 and -30 are not shown as their sequence identity and/or location was unclear. (B) The allele *hd11* was mapped to a region containing the *let-19/mdt-13* gene. Injection of fosmid pools and individual fosmids identified *let-19/mdt-13* as candidate gene. Two fosmids, with only *let-19/mdt-13* in their overlapping sequence, rescued the PVQ axon navigation defect in *let-19/mdt-13* mutants. The number of rescuing strains and the total number of analysed strains is indicated for each rescuing fosmid or fosmid pool. (C) Sequencing revealed the missense mutation E2682K in *let-19/mdt-13(hd11)*. ClustalW2 alignment of *C. elegans* LET-19/MDT-13 with its human and *Drosophila* homologs in the region affected by *hd11*. The affected amino acid in *hd11* is highlighted (arrowhead). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

establishment of compartment boundaries in the fly wing (Janody et al., 2003). In *C. elegans*, DPY-22/MDT-12 is involved in dosage compensation (DeLong et al., 1987; Meneely and Wood, 1987; Plenefisch et al., 1989) and the inhibition of Ras-dependent vulva fate specification (Moghal and Sternberg, 2003). LET-19/MDT-13 regulates expression of some early embryonic genes in *C. elegans* (Wang et al., 2004).

In zebrafish MED12 is required for development of the brain, neural crest, endoderm, heart and kidney (Hong et al., 2005; Rau et al., 2006; Shin et al., 2008; Wang et al., 2006). In mice, loss of MED12 leads to developmental arrest during embryogenesis and defects in neural tube closure, axis elongation, somitogenesis and heart development (Rocha et al., 2010).

Here we describe the function of the *C. elegans* CDK8 module during nervous system development. The complete CDK8 module and other Mediator subunits are required for correct axon navigation in several classes of neurons. We also found the Mediator is necessary to regulate a large number of genes in interneurons. Genetic interaction studies suggest that the CDK8 module regulates commissure navigation by suppressing the SAX-3/ROBO pathway in a subset of motoneurons.

Material and methods

Strains and phenotypic description

The *C. elegans* wildtype strain CB4856 and *unc-4(e120); dpy-10(e128)* animals were used for mapping of *let-19/mdt-13(hd11)*. For rescue experiments fosmids (Geneservice, Cambridge, UK) were injected into *let-19/mdt-13(hd11); pha-1(e2123ts)* animals as described (Mello et al., 1991) using *pha-1(+)* as a co-injection marker (Granato et al., 1994).

The following integrated GFP reporter constructs were used to characterize mutant phenotypes: *hdIs17 [glr-1::YFP; unc-47::YFP; unc-129::YFP; rol-6(su1006)]* I; *hdIs26[odr-2::CFP, sra-6::DsRed2]* III; *rhIs4[glr-1::GFP, dpy-20(+)]* III; *evIs111[rgef-1::GFP]* V; *hdIs22[unc-129::CFP, unc-47::DsRed2]* V; *hdIs29[odr-2::CFP, sra-6::DsRed2]* V; *hdIs30[glr-1::DsRed2]*.

The following alleles were used for phenotypic descriptions: *cdk-8(tm1238)* I; *sur-2/mdt-23(ku9)* I; *unc-40(e271)* I; *mdt-28(tm1704)* I; *max-2(ok1904)* II; *cic-1(tm3740)* III; *mdt-15(tm2182)* III; *mdt-29-(tm2893)* III; *unc-5(e53)* IV; *pak-1(ok448)* X; *sax-3(ky123)* X; *slt-1-(eh15)* X; *slt-1(ev741)* X; *slt-1(ok255)* X; *unc-6(ev400)* X and *dpy-22/mdt-12(sy622)* X. The balancer *hT2 [bli-4(e937) let-?(q782) qIs48]* (I;III) was used to maintain *cdk-8; sax-3* double mutants.

Strains were cultured at 20 °C using standard conditions (Brenner, 1974).

Axonal defects in the VNC are defined here as axons crossing the ventral midline into the contralateral axon tract or extending completely in the contralateral axon tract. Defasciculations within the same axon tract were not included.

RNAi screen

The bacterial RNAi clones, originally from an RNAi library created by the Ahringer Laboratory (Kamath and Ahringer, 2003), were obtained from Geneservice, Cambridge, UK. The strain *nre-1(hd20) lin-15b(hd126)* X was used for the RNAi screen (Schmitz et al., 2007). RNAi by feeding was performed as described (Kamath et al., 2001). Briefly, RNAi clones were grown over night in LB culture containing 50 μg/ml Ampicillin. Worm culture plates containing 1 mM IPTG and 50 μg/ml Carbenicillin were seeded with the bacteria. The plates were incubated at room temperature over night. Five or more L3 hermaphrodites were placed onto each plate the following day and incubate at 20 °C for five days. F1 progeny were analysed for phenotypes. Each clone was tested at least twice for axonal phenotypes.

Gene expression

The 5′-upstream region of *cic-1* was amplified from genomic DNA by using primers 5′-CGGGATCCGTTTATAGACGAAGAA-ATTGGCTG-3′ and 5′-CGGGATCCTTTTCAACTAAAATCATTAAAA-AAATG-3′. The 890 bp PCR fragment was cloned into the *Bam*HI site of pPD95.75, creating *cic-1p::GFP*. 604 bp *cdk-8* 5′-upstream region was PCR amplified from a vector, containing 1687 bp 5′-upstream region of *cdk-8*, cloned into the *Bam*HI site of pPD95.75, by using primers 5′-CGGGATCCTTTTCAGTTACTACAGC-GAAT-3′ and 5′-ATCACCGAAACGCGCGAGACG-3′. *cdk-8p::GFP* and *cic-1p::GFP* contain the 5′-upstream region of *cdk-8* and *cic-1* respectively up to the next upstream gene. The *cic-1p::GFP* construct and the *cdk-8p::GFP* PCR fragment were injected into *pha-1(e2123ts)* animals (Granato et al., 1994). The expression pattern of two independent lines per construct was analysed.

Microscopy

Confocal images of fluorescent protein containing mixed stage worms were acquired using a Zeiss Axioplan II microscope (Carl-Zeiss AG, Germany). Stacks of confocal images, with 0.3 to 0.5 μm distance between focal planes, were recorded with a Quorum WaveFX spinning disc system (Quorum Technologies, Canada). Image acquisition and analysis was done with the Volocity software package (Perkin-Elmer, Waltham, MA). Maximum intensity projections of all focal planes were used to generate images for the figures. Figures and GFP/Nomarski overlays were assembled with Adobe Photoshop CS 8.0 (Adobe, San Jose, CA).

Cell-specific RNAi depletion of sax-3 in DD/VD motoneurons

~1 kb *sax-3* 5′ sequence obtained from the *sax-3* cDNA (kind gift from the Chin-Sang lab) was cloned in both forward and reverse directions into the KpnI site of the pPD95.75 vector containing the promoter of *unc-25* (Jin et al., 1999), so that both sense and antisense transcripts can be expressed under the control of the *unc-25* promoter, which is active in DD and VD motoneurons. This approach was reported to be effective in depleting a gene of interest in *C. elegans* neurons (Esposito et al., 2007). The *sax-3* RNAi constructs were injected into worms with *nhr-25p::GFP* as a coinjection marker. The positive extra-chromosomal array lines were crossed into *cdk-8(tm1238); hdIs22* animals. The resulting animals with positive marker expression were evaluated for motoneuron commissure defects.

SAGE analysis

SAGE libraries were generated as described (Meissner et al., 2009). Briefly, mixed stage embryos, wildtype and *let-19/mdt-13-(hd11)* animals, containing the command interneuron marker *glr-1::GFP*, were harvested and dissociated. GFP-positive interneurons were isolated by FACS sorting. mRNA from those cells was extracted, small sequence tags were created and sequenced using a Solexa Sequencer at the BC Genome Science Centre in Vancouver, BC. Primary sequence information was processed, and SAGE tags were mapped to genes using multiSAGE (McKay et al., 2003). Tag frequencies were downloaded from multiSAGE and incorporated into the GExplore database for further analysis (Hutter et al., 2009). Data represent the average of two biological replicas each for wildtype and *let-19/mdt-13(hd11)*. FACS-sorting of GFP-labeled interneurons generally reaches a purity of around 90%. Tags present with low frequency, therefore, might have

originated from contaminating tissues. We found tags from genes that are known to be expressed in either epidermis, germ line or intestine (but not neurons) present in a range from 0.03 to 2.41 tags per 10^5 tags. To eliminate spurious expression tags from other tissues, we applied a threshold of 2 tags per 10^5 tags as minimum expression level resulting in a set of 4133 genes we considered to be expressed in wildtype interneurons. Genes at least 5 or 10 times up- and downregulated in *let-19/mdt-13(hd11)* were grouped into functional categories according to their gene ontology (GO) annotation.

RNA isolation and quantitative PCR analysis

Total RNA was prepared similar to the protocols described previously (Taubert et al., 2008, 2006), with the only modification being a 30 s sonication of the Trizol worm suspension to improve mRNA yield (Branson Sonifier S-450D, 3 pulses of 10 s, output 30%). Total 1 μg RNA from each sample was used to generate first strand complementary DNA with Superscript II reverse transcriptase (Invitrogen 18064-014), random primers, dNTPs (Fermentas R0186), and RNAseOUT Recombinant Ribonuclease Inhibitor (Invitrogen 10777-019). qPCR was performed in 30 μl reactions using Invitrogen Taq (Invitrogen 18038-240) and an Applied Biosystems StepOnePlus machine, and the data analyzed using the ΔΔCt method. mRNA levels were normalized to the levels of three normalization genes, namely act-1, ubc-2, and tba-1. Primers for qPCR were designed using Primer3. Primers were tested on dilution series of cDNA, and analyzed for PCR efficiency; Primers used in qPCR are listed in Supplementary data 1.

Results

let-19/mdt-13 was identified in a genetic screen for ventral nerve cord axon guidance defects

We isolated *ast-7(hd11)* in a genetic screen for axon navigation defects of command interneurons in the ventral nerve cord (VNC) using a *glr-1::GFP* reporter to visualize the interneurons and initially mapped it to the centre of chromosome II (Hutter et al., 2005). Further single nucleotide polymorphism (SNP) mapping narrowed the region to a 540 kbp region between the SNPs pKP2108 and CE2-171 (Fig. 1B). We were able to rescue the PVQ axon guidance defects of hd11 animals with fosmids WRM0622dF07 and WRM0611bF06, which share only the *let-19/mdt-13* gene. Sequencing of the coding region of *let-19/mdt-13* in *hd11* mutants revealed a missense mutation resulting in the change of a negatively charged glutamic acid to a positively charged lysine (E2682K) in the C-terminus of LET-19/MDT-13 (Fig. 1C). *let-19/mdt-13(os33)* did not complement *hd11* for interneuron and commissure navigation defects and the dumpy (Dpy) and egg-laying (Egl) phenotypes seen in *hd11* mutant animals, confirming that *hd11* is an allele of *let-19/mdt-13*. Independently three additional Mediator subunits, *sur-2/mdt-23*, *mdt-18* and *mdt-21*, were identified in a large-scale RNAi screen for genes involved in axon navigation (Schmitz et al., 2007), indicating that the Mediator complex plays an important role in axon guidance.

RNAi against Mediator subunits reveals a role for the CDK8 module in axon navigation

To investigate whether additional Mediator subunits are involved in the regulation of axon guidance, we used RNAi to deplete other *C. elegans* Mediator subunits (Table 1). As expected, we observed pleiotropic defects, including embryonic and larval lethality. Surviving animals were often Egl or uncoordinated (Unc).

Table 1
DD/VD commissure outgrowth defects after RNAi against selected Mediator subunits.

Gene	(%) Animals with DD/VD commissure defects[a]	*n*	Other phenotypes
Wild type	14	100	
dpy-22/mdt-12	82**	432	few Emb, Dpy, Egl, Unc
cdk-8	81**	333	Emb, Lvl, Egl
let-49/mdt-7	61**	277	Emb, Lvl, Egl
sur-2/mdt-23	59**	359	Emb, Lvl, Egl
mdt-10	44**	185	Emb, Lvl, Egl
mdt-18	43**	88	Emb, Let (~40%)
mdt-15	40*	15	Emb, Let (~85%), arrest in L1
mdt-8	28*	202	Emb, Lvl, Egl
cic-1	24*	365	Emb, Lvl, Egl
mdt-1.2	20	517	Emb, Lvl, Egl, Unc
mdt-21	19	351	Emb
mdt-28	16	256	Emb, Egl
mdt-17	15	67	Emb, Let (~50%), Egl, arrest in L2

Other phenotypes: Emb, embryonic lethal; Dpy, dumpy; Egl, egg-laying defective; Lvl, larval lethal; Let, lethal with percentage in brackets when noticeably strong; Marker used: *hdIs22*.

[a] Animals with at least one commissure affected.
* Significantly different with $p < 0.05$, compared to wild type (χ^2 test).
** Significantly different with $p < 0.01$, compared to wild type (χ^2 test).

As a monitor of neural wiring integrity the outgrowth and navigation of DD/VD commissures, extending circumferentially from the VNC to the dorsal nerve cord (DNC), was analysed in surviving animals. We found that RNAi against CDK8 module subunits (*cdk-8*, *dpy-22/mdt-12*, *cic-1*) as well as some other Mediator subunits resulted in DD/VD commissure navigation defects (Table 1). Several Mediator subunits did not show any axon navigation defects, suggesting that they are dispensable for axonal navigation. We did not detect navigation defects in PVP, PVQ and command interneuron axons in the VNC in any RNAi experiment (data not shown). This might be due to the fact that RNAi does not work efficiently in some neurons (Timmons et al., 2001). Taken together, our results suggest that the Mediator, most notably the CDK8 module, is required to regulate expression of genes involved in axon navigation in at least a subset of *C. elegans* neurons.

The CDK8 module regulates anterior–posterior and dorso-ventral axon navigation

To further characterize the role of the Mediator subunits in axon guidance, we obtained mutant alleles where possible. Mutations of several Mediator subunits result in early embryonic lethality, which makes it difficult to characterize axonal defects. However, we were able to obtain viable alleles for all four CDK8 module components. The *cdk-8(tm1238)* deletion eliminates the first three exons of *cdk-8*, including parts of the kinase domain, suggesting that no functional protein is made. In *cic-1(tm3740)* animals, the N-terminal part of the cyclin domain is deleted, leading to a frame shift and a stop codon after ~50 aa, likely resulting in a null allele as well. *dpy-22/mdt-12(sy622)* is a nonsense mutation that truncates half of the protein, probably leading to a strong loss-of-function (Moghal and Sternberg, 2003). In addition, we analysed a possible null allele of *mdt-28* and partial loss-of-function alleles of *mdt-15*, *sur-2/mdt-23* and *mdt-29*. With the exception of *mdt-29(tm2893)*, all strains exhibited pleiotropic defects (e.g. small, Dpy, Egl, Unc), indicating that these genes are involved in several developmental pathways. We focused our analysis on the axonal phenotypes of these subunits, in particular axons extending in or originating from the VNC.

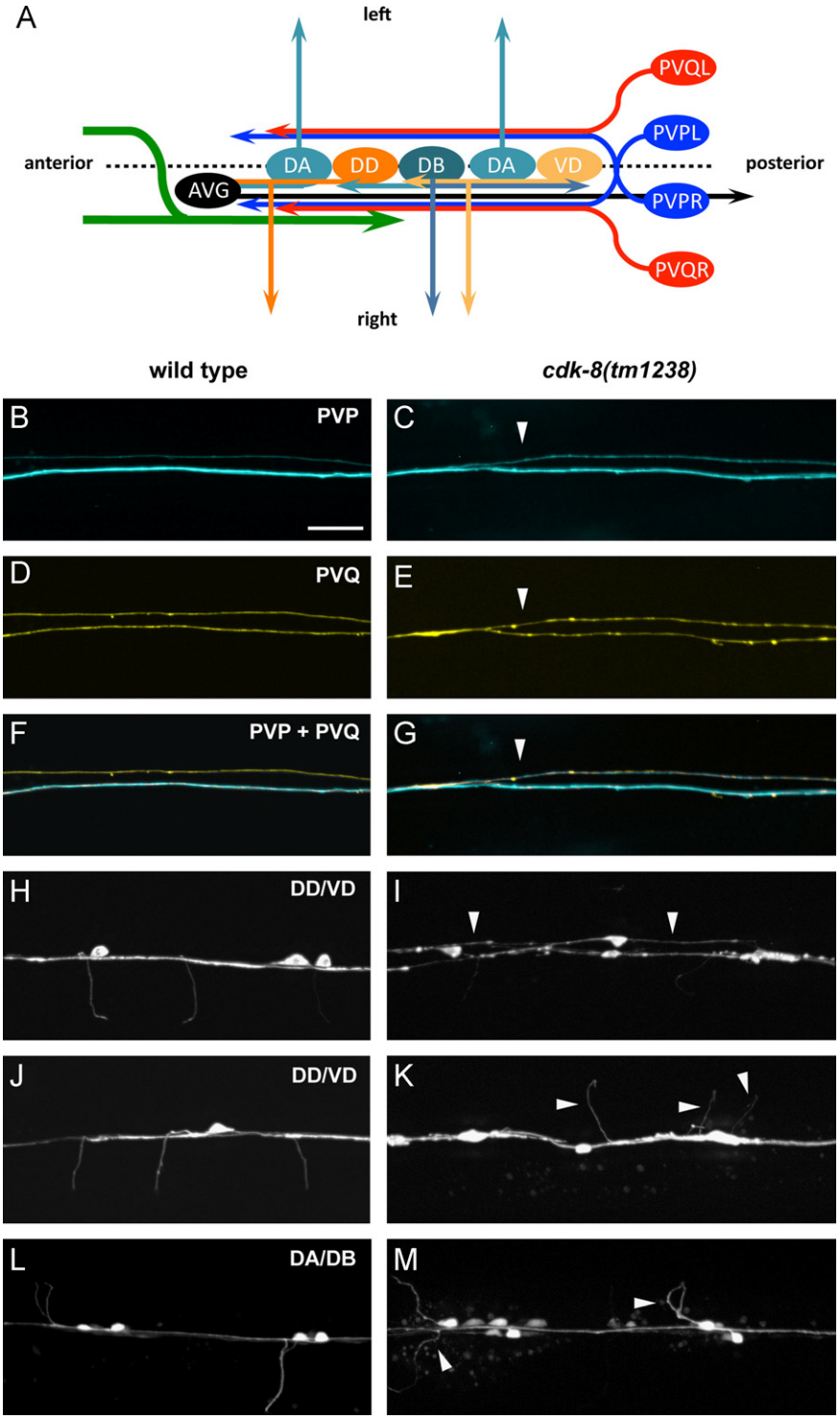

Fig. 2. Axonal defects in *cdk-8* mutants. (A) The *C. elegans* ventral nerve cord (VNC) consists of two axon tracts, with motoneuron cell bodies (light and dark orange, light and dark turquoise) lining the ventral midline (dashed line). The AVG axon (black) pioneers the right VNC axon tract and the PVPR axon pioneers the left VNC axon tract. Both PVP axons are closely followed by PVQ axons (red). DA, DB, VD and DD motoneurons (light and dark orange, light and dark turquoise) extend axons into the right axon tract and send commissures circumferentially to the dorsal nerve cord (DNC). Command interneuron axons (green), extending from the nerve ring, run in the right axon tract. Various axonal defects observed in *cdk-8(tm1238)* mutants ((C), (E), (G), (I), (K), (M)) are compared to the corresponding wildtype animal images ((B), (D), (F), (H), (J), (L)); (C) PVPR axon crosses the ventral midline (arrowhead); (E) PVQL axon crosses the ventral midline (arrowhead); (G) overlay showing that PVPR and PVQL cross the midline at the same point (arrowhead); (I) DD/VD axons, DD/VD axons extend into the left VNC axon tract (arrowhead); (K) DD/VD commissures erroneously extend along the left body side to the DNC (arrowhead); (L) In wild type, DA/DB commissure sidedness has a distinctive left-right pattern; (M) In *cdk-8* mutants, DA/DB commissures extend on the wrong body side (arrowhead); Ventral views, anterior to the left; ((F) and (G)) CFP/DsRed2 overlays; scale bar 10 μm; markers used: *hdIs26* (PVP, PVQ); *hdIs22* (DD/VD, DA/DB). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The *C. elegans* VNC is the main nerve bundle along the anterior-posterior body axis and consists of two axon tracts (Fig. 2A). Its development is characterized by the sequential outgrowth of pioneer and follower axons. The AVG axon is the first axon pioneering the right VNC axon tract from the anterior. Mild AVG axonal defects were observed only in *let-19/mdt-13(hd11)*

Table 2
Interneuron axon guidance defects of Mediator subunit mutants in the VNC.

Genes	(%) Animals with defects[a]					
	Left VNC axons		Right VNC axons			Command interneurons
	PVPR	PVQL	AVG	PVPL	PVQR	
wild type[b]	11	11	0	1	1	3
cdk-8	35**	35**	1	1	3	15**
cic-1	31**	32**	1	0	1	5
cdk-8; cic-1	27$	30$	n.d.	n.d.	n.d.	n.d.
dpy-22/mdt-12	31**	28**	5	1	2	21**
let-19/mdt-13	32**	32**	14**	0	0	31**
mdt-15	10	10	0	0	0	0
sur-2/mdt-23	29**	33**	4	0	2	12*
mdt-28	8	9	0	0	0	6
mdt-29	10	10	0	0	0	3

n.d.: not determined.
Marker used: *hdIs26* (AVG, PVP, PVQ), *rhIs4* (interneurons).

[a] Ventral midline cross-over defects or outgrowth into the wrong axon tract, $n=100–171$.
[b] For genes on chromosome III (*cic-1, mdt-15, mdt-29*) *hdIs29,* which has 13% PVPR/PVQL defects and no PVPL/PVQR, was used instead of *hdIs26. hdIs30* was used instead of *rhIs4*. Both show the same background defects.
* Significantly different with $p < 0.05$, compared to wild type.
** Significantly different with $p < 0.01$, compared to wild type.
$ Not significantly different with $p > 0.05$, compared to single mutants (χ^2 test).

Table 3
Motoneuron axon guidance defects in Mediator subunit mutants.

Genes	(%) Animals with defects in[a]						
	VNC axon navigation[b]		Commissure sidedness[c]		Commissure navigation[d]		No of defective comm. [e]
	DD/VD	DA/DB	DD/VD	DA/DB	DA/DB	DD/VD	DD/VD
Wild type	5	0	5	3	0	18	0.3 ± 1.5
cdk-8	37**	12**	45**	34**	1	100**	12.0 ± 2.3
cic-1	40**	5	37**	37**	3	100**	12.2 ± 2.7
cdk-8; cic-1	31$	10$	n.d.	n.d.	n.d.	100***	13.3 ± 2.1
dpy-22/mdt-12	46**	13**	49**	34**	0	100**	11.3 ± 2.4
let-19(hd11)	38**	8*	64**	57**	1	40**	1.5 ± 1.9
let-19(os33)[f]	n.d	n.d.	79**	n.d.	n.d.	100**	9.1 ± 2.7
mdt-15	9	2	6	2	0	5	-0.2 ± 1.0
sur-2/mdt-23	47**	2	35**	32**	0	65**	3.2 ± 3.2
mdt-28	9	1	7	0	0	12	0.2 ± 1.2
mdt-29	14	0	13	0	0	8	-0.2 ± 1.2

n.d.: not determined. Marker used: *hdIs22.*

[a] $n=100–111$.
[b] Animals with VNC axons crossing into left axon tract or extending in the left axon tract.
[c] Animals with one or more commissure leaving the VNC on the wrong side.
[d] Animals with at least one (DA/DB) or at least two (DD/VD) commissures not reaching the dorsal cord.
[e] Average number of DD/VD commissures ($\pm$ std) not reaching the dorsal cord; the number was calculated by subtracting the number reaching the dorsal cord from the average number reaching the dorsal cord in wild type.
[f] $n=57$.
* Significantly different with $p < 0.05$, compared to wild type.
** Significantly different with $p < 0.01$, compared to wild type.
$ Not significantly different with $p > 0.05$, compared to single mutants (χ^2 test).

animals but not in any of the other mutants (Table 2). The left VNC axon tract is pioneered by the PVPR axon, which is closely followed by the PVQL axon. We observed mild irregular PVPR axon midline cross over defects in the VNC only in animals with mutations in CDK8 module components and *sur-2/mdt-23* (Table 2, Fig. 2B–G). PVQL axons had corresponding defects (i.e. cross the midline together with the PVPR axon) in all mutant backgrounds, indicating that the tight pioneer-follower relationship of the PVPR/PVQL axon pair remained intact. Guidance of the PVPL/PVQR axon pair in the right VNC was unaffected (Table 2). Command interneuron axons extend in the right VNC. Mutations in any of the genes encoding CDK8 module components, except *cic-1*, caused irregular cross over of interneuron axons from the right into the left axon tract (Table 2). Similar defects were observed in *sur-2/mdt-23(ku9)* animals.

Navigation defects were also observed in some motoneurons. Cholinergic DA/DB and GABAergic DD/VD motoneurons extend neurites along the right VNC axon tract and send commissures circumferentially into the DNC. Motoneuron commissures extend either along the left or right body side in a highly invariable left-right pattern. In animals with mutations in CDK8 module subunits and in *sur-2/mdt-23* mutant animals, axon navigation of DD/VD motoneurons in the VNC was aberrant, with axons extending in or crossing into the left axon tract (Table 3, Fig. 2H and I). DA/DB axon

navigation was only mildly affected. In addition, we found strong defects in the invariant left-right choice of DD/VD and DA/DB commissures (Table 3, Fig. 2J–M). As expected from our RNAi analysis, DD/VD commissure outgrowth towards the DNC was severely disrupted in *cdk-8*, *cic-1* and *dpy-22/mdt-12* mutant animals (Table 3).

DD/VD commissures showed a variety of different defects (Fig. 3). Some commissures stopped prematurely (Fig. 3C), others turned before reaching the DNC (Fig. 3D), or branched ectopically (Fig. 3B). Occasionally commissures eventually reached the DNC after having first deviated significantly from their normal, straight trajectory (Fig. 3B). When counting the number of commissures, we noticed that fewer than the expected number of commissures was visible (an average of three commissures were missing in *cdk-8* mutants). The number of motoneuron cell bodies expressing the fluorescent reporter gene was not reduced in these mutants, suggesting that lack of reporter gene expression is not the cause for the reduced number of commissures. We noticed, however, a strong variability in fluorescence intensity in *cdk-8* mutants (Fig. 3B) with some commissures barely detectable; so expression levels might be below the detection limit in some commissures. In addition it is possible that some commissures never leave the VNC. *let-19/mdt-13(hd11)* and *sur-2/mdt23(ku9)* animals exhibited similar, but less penetrant DD/VD commissure defects (Table 3). Interestingly, DA/DB commissure outgrowth towards the DNC was entirely unaffected in all mutants tested (Table 3). Distal tip cell migration, which depends on the same guidance cues used by commissural axons (Hedgecock et al., 1990), was also unaffected in *cdk-8*- and *cic-1*-deficient animals (data not shown). The general architecture of the *C. elegans* nervous system, analysed with a pan-neuronal marker, was intact in all mutant strains (data not shown), suggesting that the CDK8 module regulates axon navigation only in specific neurons.

Axonal phenotypes of mutants in different CDK8 module components were highly similar, indicating that these subunits function together to regulate axon navigation. Navigation defects of motoneurons axons and PVPR/PVQL axons in the VNC in *cdk-8*; *cic-1* double mutant animals were not significantly different from either single mutant (Table 2, Table 3), consistent with both genes acting together in the same pathway. No axonal defects were detected in *mdt-15*, *mdt-28* and *mdt-29* mutant animals, again suggesting that these subunits are not important for the regulation of axon navigation.

cdk-8 and cic-1 are expressed ubiquitously in C. elegans

Axonal defects found in mutants of CDK8 module subunits might be limited to specific neurons because some of these subunits are expressed only in affected neurons. The expression patterns of *dpy-22/mdt-12* and *let-19/mdt-13* were published

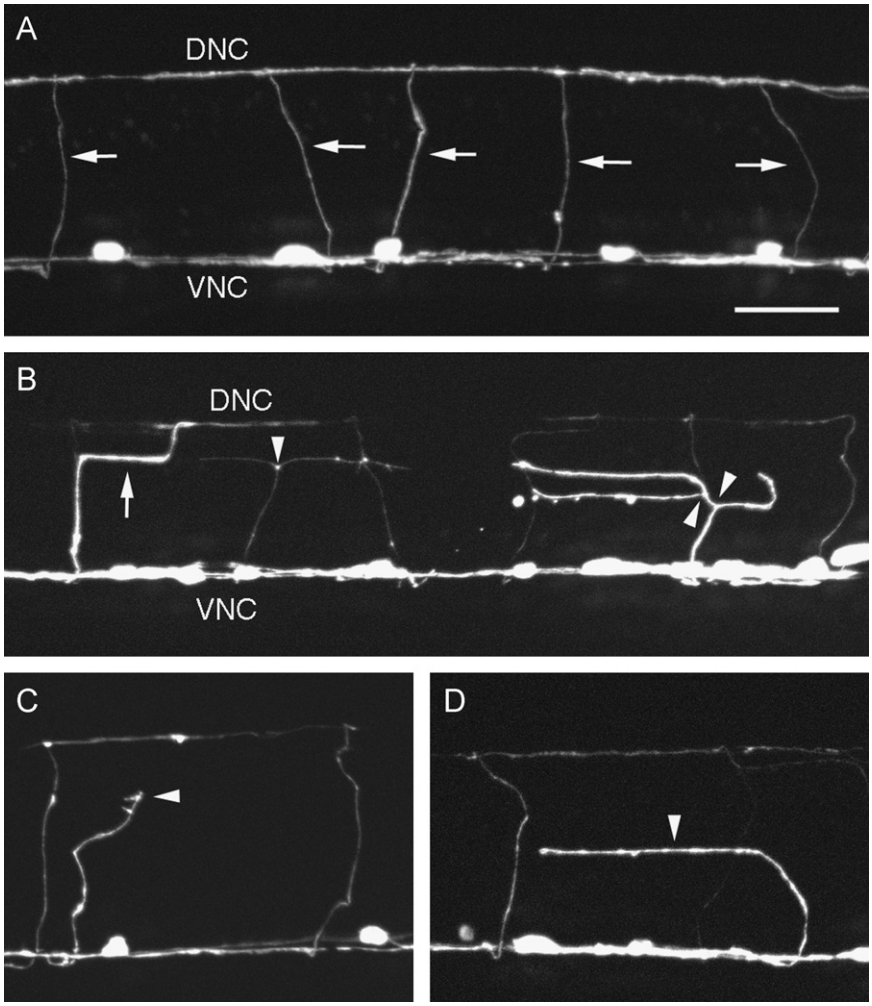

Fig. 3. DD/VD commissure outgrowth defects in *cdk-8* mutants. (A) DD/VD commissures (arrows) extend straight from the VNC to the DNC in wild type, ((B)–(D)) DD/VD commissure defects in *cdk-8(tm1238)* mutants; the arrow in B marks a commissure, which makes two 90° turns, but eventually arrives at the DNC. The arrowhead in B marks a commissure that branches before reaching the DNC. The arrowhead in C points to a commissure stopping prematurely and the arrowhead in D marks a commissure turning and never reaching the DNC. Lateral views, anterior to the left; scale bar 20 μm; marker used: *hdIs22*.

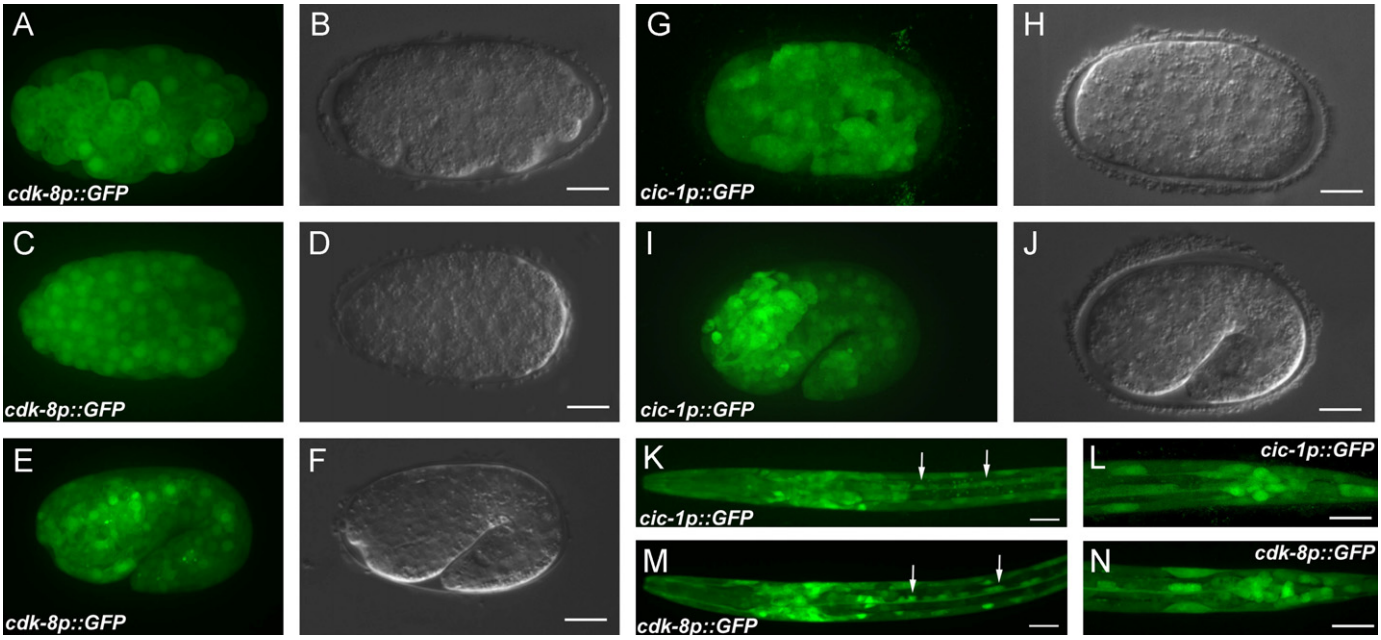

Fig. 4. Expression pattern of *cdk-8* and *cic-1* in *C. elegans*. Confocal images taken from embryos or L1 animals with *cdk-8p::GFP* transgene ((A), (C), (E), (M), (N)) and *cic-1p::GFP* transgene ((G), (I), (K), (L)), with corresponding Nomarski images ((B), (D), (F), (H), (J)); ((A), (B), (G), (H)) early gastrula-stage embryos; ((C) and (D)) ~200 cell-stage embryo; ((E) and (F), (I), (J)) 1.5-fold stage embryos; ((K), (M)) L1 larvae with motoneuron cell bodies visible (arrows); ((L), (N)) L1 larva tails; ((A)–(J)) lateral views, ((K)–(N)) ventral views, anterior is to the left; scale bars 10 μm.

previously and both genes are expressed ubiquitously (Wang et al., 2004; Zhang and Emmons, 2000). To test whether *cdk-8* and *cic-1* have a more restricted expression pattern in *C. elegans* we generated transgenic animals expressing GFP under the control of the putative promoter regions of these genes. In both cases, GFP expression was visible in embryogenesis starting in early gastrulation stages (Fig. 4). Expression was observed in a majority of cells, including many neurons throughout embryogenesis as well as post-embryonically. Thus the cell-specific function of the CDK8 module is likely regulated by mechanisms other than transcriptional regulation of its subunits.

Mediator regulates the expression of a large number of neuronal genes in interneurons

In order to identify neuronal target genes of the Mediator, in particular the genes causing axonal defects, we compared neuronal expression profiles of a mediator mutant and wildtype. We decided to use *let-19/mdt-13(hd11)* for this analysis, because defects in this particular mutant are largely limited to axonal navigation defects. We obtained expression profiles of embryonic *glr-1::GFP* positive interneurons from *glr-1::GFP* and *let-19/mdt-13(hd11)*; *glr-1::GFP* animals using SAGE (Velculescu et al., 1995). We first compared the expression levels of genes known to affect interneuron axon navigation (Table 4). These genes showed either no or only a minor reduction in expression levels (*sax-3*, *vab-15*), suggesting that interneuron axon defects are not due to the lack of expression of any of these genes. Some of the genes including the transcription factors *ast-1* and *zag-1*, as well as the cadherin *cdh-4* and the IgCAM *lad-2* were moderately (3–4 fold) upregulated. Two genes, the flamingo homolog *fmi-1* and the ena/VASP homolog *unc-34* were more than 5-fold upregulated. While loss-of-function mutants in those genes have interneuron axon guidance defects (Clark and Chiu, 2003; Schmid et al., 2006; Schmitz et al., 2008; Steimel et al., 2010; Wacker et al., 2003; Wang et al., 2008), there is currently no evidence that moderate overexpression would cause navigation defects.

Table 4
Expression of axon guidance genes in dissociated interneurons from *let-19/mdt-13(hd11)* mutant animals.

Gene	Expression level in wt[a]	Expression level in *let-19*[a]	Fold change in *let-19*[b]
Genes known to affect interneuron axon navigation			
unc-34	0.30	11.12	37.65
fmi-1	11.46	76.01	6.63
let-19	5.77	28.52	4.94
cdh-4	19.78	93.45	4.72
lad-2	6.01	22.89	3.81
zag-1	1.18	4.25	3.59
ast-1	33.69	111.91	3.32
unc-71	4.45	5.35	1.20
unc-130	1.12	1.13	1.01
sax-3	155.59	114.71	0.74
vab-15	0.54	0.30	0.55
Other known guidance genes			
max-2	0.03	4.64	176.35
unc-115	17.50	77.30	4.42
unc-73	11.50	38.24	3.32
mig-10	18.47	25.38	1.37
unc-53	37.87	50.51	1.33
vab-8	61.75	81.79	1.32
mig-2	4.71	4.46	0.95
unc-5	5.06	4.73	0.93
unc-40	188.18	23.93	0.13
pak-1	166.01	7.5	0.05

[a] Expression level measured in tags per 10^5 tags.
[b] Calculated as expression in *let-19* divided by expression in wt.

We analysed the expression of several other genes known to affect axon navigation, in particular components of signal transduction pathways and cytoskeletal adaptors. We found that *unc-40* is almost 10-fold downregulated, but since *unc-40* mutants have almost no interneuron axon navigation defects, it seems unlikely that this is the cause of the axonal defects we observe in *let-19/mdt-13(hd11)*. *pak-1*, encoding one of the p21-activated

kinases, is the only other gene in this list that is substantially downregulated. The second p21-activated kinase gene, *max-2*, is upregulated and could compensate for a lack of *pak-1* activity, since *pak-1* is known to act redundantly with *max-2* in commissure navigation (Lucanic et al., 2006). However, *pak-1(ok448)* single mutants have no interneuron defects suggesting that the down-regulation of *pak-1* does not cause the interneuron defects (Fig. 5). In contrast *max-2(ok1904)* single mutants show interneuron axon guidance defects similar to *let-19/mdt-13(hd11)* mutants (Fig. 5). Defects in *max-2(ok1904); let-19/mdt-13(hd11)* are additive, suggesting that *max-2* and *let-19/mdt-13(hd11)* act in different pathways (Fig. 5).

To gain a broader understanding of how LET-19/MDT-13 affects transcriptional regulation, we analysed the entire dataset for misregulated genes. A total of 4133 genes were found to be expressed in *glr-1::GFP* interneurons in wildtype animals. A comparable number of genes (4172) was expressed in *let-19/mdt-13(hd11)*. Overall 589 genes were at least 5-fold upregulated in *let-19/mdt-13(hd11)* and of these, 295 genes were more than 10-fold upregulated (Supplementary data 2). Conversely 719 genes were at least 5-fold downregulated with 275 genes more than 10-fold downregulated (Supplementary data 2). We used gene ontology (GO) annotations to group all genes that were at least 10-fold misregulated (Fig. 6). About one-third of these genes are implicated in developmental control, with few genes known to mediate axon guidance or cell migration. Several signaling pathway components were upregulated. Notable is the presence of many integral membrane proteins of unknown function, some

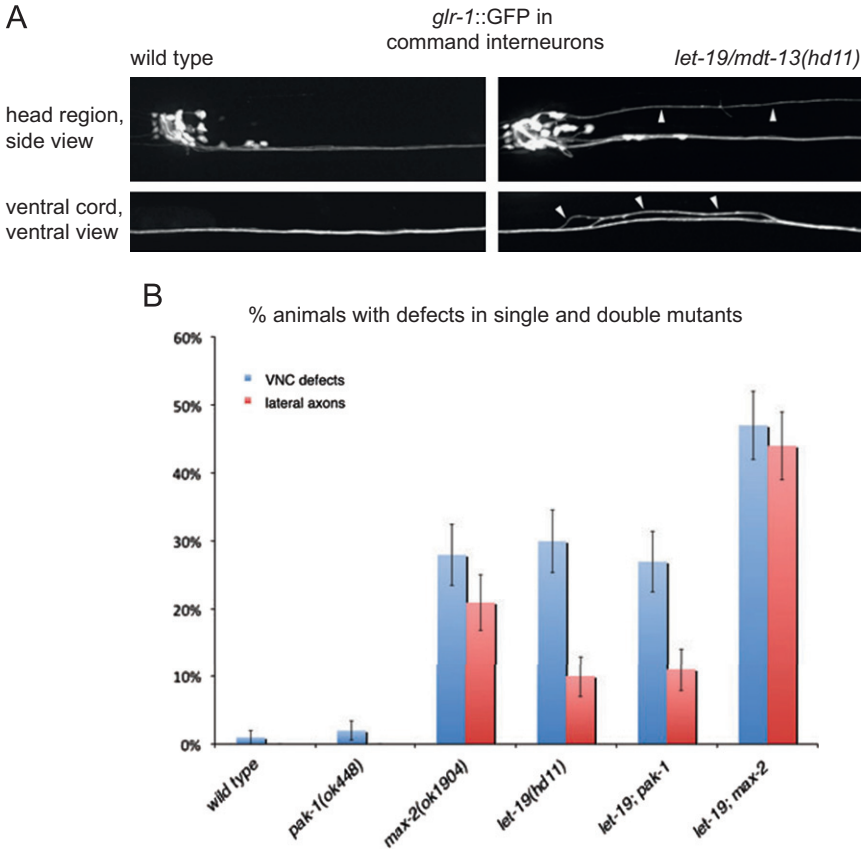

Fig. 5. Interneuron defects in *let-19/mdt-13(hd11)*. (A) In *let-19/mdt-13(hd11)* mutant animals some interneuron axons extend erroneously in lateral positions (arrows in upper panel). In the ventral cord axons cross into the left axon tract (arrows in lower panel). (B) Quantification of interneuron defects in *let-19/mdt-13(hd11)* single and double mutants ($n=100$).

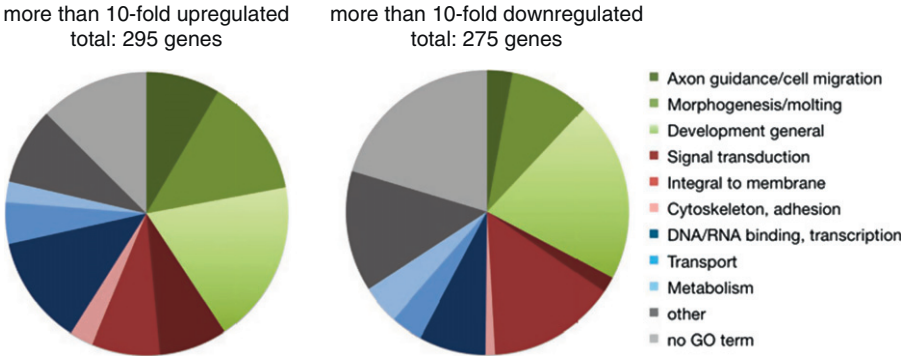

Fig. 6. Categories of genes misregulated in *let-19/mdt-13(hd11)*. Genes that are more than 10-fold up- or downregulated in *glr-1::GFP* expressing interneurons were grouped using Gene Ontology (GO) annotations.

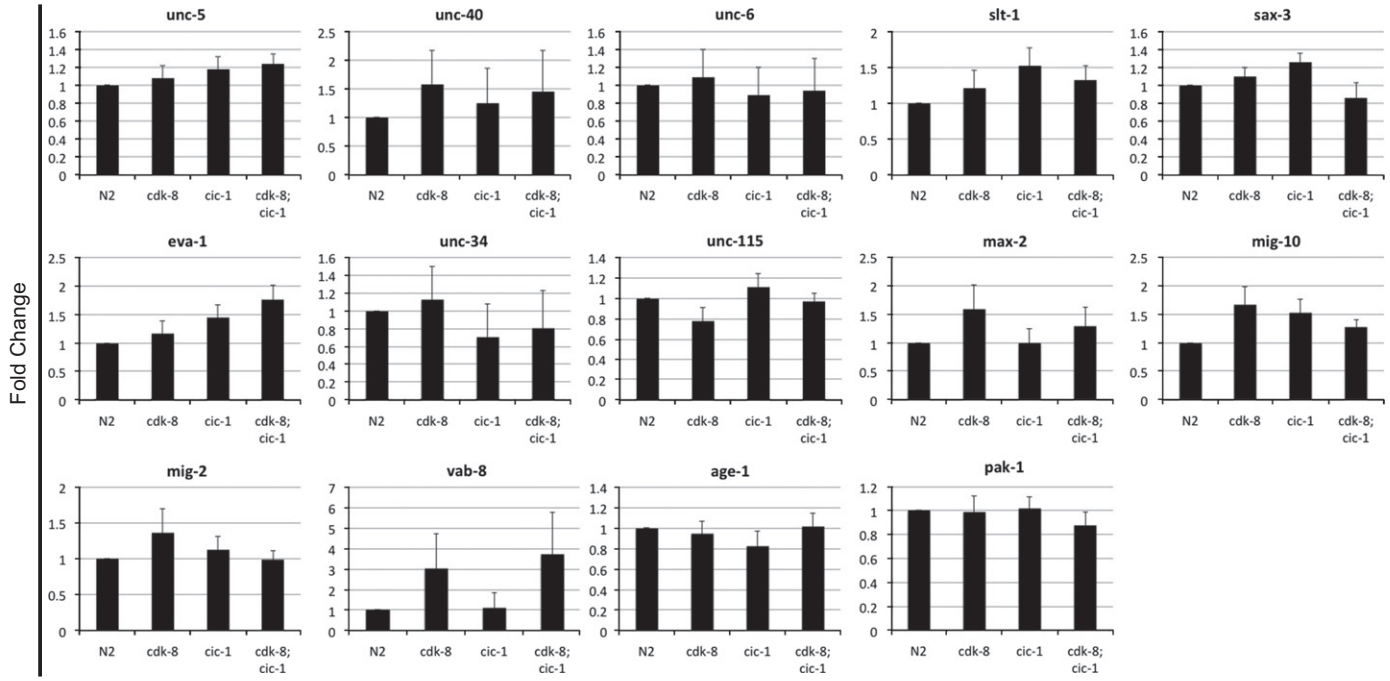

Fig. 7. qPCR experiments in *cdk-8* and *cic-1* mutant animals. The expression of genes known to affect commisure navigation was measured in L1/L2 animals at the time when postembryonic VD commissures grow out using quantitative PCR. The average of three independent experiments is shown as expression relative to wildtype. Error bars depict the standard error of the mean.

of which might be involved in signal reception. Comparable numbers of transcription factors and other DNA-binding proteins were found in both the up- and downregulated categories. In addition, expression of several metabolic genes and transporters was misregulated. Taken together, our results suggest that LET-19/MDT-13 positively and negatively regulates the expression of a large and diverse set of genes. It is possible that the axonal defects observed in interneuron axons of *let-19/mdt13* mutants are due to the misregulation of more than one gene.

We used a more targeted approach to identify potentially misregulated genes affecting commissure navigation. The expression of genes known to affect commissure navigation was examined using qPCR on samples derived from late L1 animals, a time shortly before postembryonic VD motoneuron commissures begin to grow out. We tested *unc-5*, *unc-6*, *unc-40*, *unc-34*, *unc-115*, *slt-1*, *eva-1*, *sax-3*, *max-2*, *pak1*, *age-1*, *mig-2*, *mig-10* and *vab-8* and found that expression levels were not significantly different from wildtype in *cdk-8* and *cic-1* single mutants as well as in *cic-1;cdk-8* double mutants (Fig. 7). Since we analysed expression of these genes from whole animal samples, not from DD/VD motoneurons, we cannot rule out the possibility that we might have missed some cell-specific expression changes.

A sax-3/Robo mutation suppresses commissure navigation defects in cdk-8-deficient animals

The axonal phenotypes observed in CDK8 module mutants resembled phenotypes of animals deficient in UNC-6/Netrin (Hedgecock et al., 1990; Hutter, 2003) and SAX-3/ROBO pathway components (Hedgecock et al., 1990; Hutter, 2003; Zallen et al., 1998). To identify potential genetic interactions with these pathways, we created double mutants with *cdk-8* and UNC-6/Netrin or SAX-3/ROBO pathway components. Double mutants with *cdk-8* were sometimes not viable, so that progeny from a balanced strain heterozygous for *cdk-8* had to be used in some cases (e.g. *sax-3*). Since the homozygous mutant progeny from such strains potentially has maternal CDK-8, we tested whether this affects

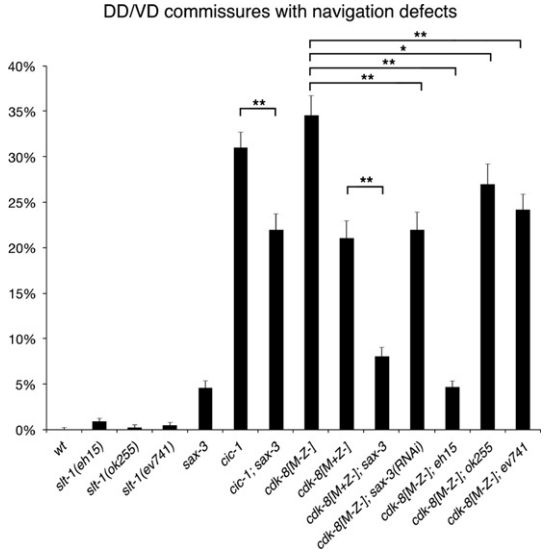

Fig. 8. Genetic interaction studies of *cdk-8* and *cic-1*. Percentage of DD/VD commissures per animal with navigation errors in various single and double mutants ($n=100$, error bars indicate the standard error of the mean); note that only visible commissures were evaluated, so that the percentage of commissures with defects is lower than the total defects reported in Table 3. Asterisks (*) indicate significant differenes in pair wise comparisons with $^{*}p<0.05$, $^{**}p<0.01$ (paired *t* test); alleles used: *cic-1(tm3740); cdk-8(tm1238); sax-3(ky123);* *cdk-8[M−Z−]* animals are progeny from homozygous mutant *cdk-8* parents, i.e. have neither maternal (M) nor zygotic (Z) CDK-8; *cdk-8[M+Z−]* are *cdk-8* homozygous mutant animals from heterozygous parents, i.e. have maternal (M), but not zygotic CDK-8. *cdk-8; sax-3* double mutant animals cannot be maintained as a double mutant strain. We therefore analysed double mutant progeny from a strain where *cdk-8* was balanced. All other strains could be maintained without balancer.

the penetrance of the commissure navigation defects. Indeed we found that commissure defects were less penetrant in *cdk-8* mutant progeny from heterozygous hermaphrodites (*cdk-8-[M+Z−]*) compared to progeny from a homozygous mutant parent (*cdk-8[M−Z−]*, Fig. 8). DD/VD commissure navigation

defects in *cdk-8* mutants were almost completely suppressed in a *sax-3*-mutant background (Fig. 8), suggesting that CDK-8 negatively regulates the SAX-3/ROBO signaling pathway. Commissure navigation defects in *cic-1; sax-3* double mutants were also significantly suppressed, although much less effectively compared to *cdk-8* (Fig. 8). The *cic-1; sax-3* double mutant could be maintained as a homozygous strain. It is possible that maternal CDK-8 affects the ability of SAX-3 to suppress the navigation defects. Expressing a *sax-3* RNAi construct in DD/VD motoneurons in *cdk-8* mutants leads to a significant suppression of commissure defects (Fig. 8), suggesting that the presence of SAX-3 in DD/VD motoneurons is responsible for the defects. Expression of a *cdk-8* cDNA under the control of a pan-neuronal promoter rescued the commissure as well as the interneuron axon defects in the VNC, (data not shown), suggesting that CDK-8 is required in neurons for correct navigation of commissures and interneuron axons in the ventral cord. SAX-3 is a receptor for SLT-1 and we expected that strong loss-of-function mutations in *slt-1* would equally suppress the commissure navigation defects of *cdk-8*. We tested three *slt-1* alleles and found that one of them, *slt-1(eh15)* strongly suppressed the navigation defects (Fig. 8), whereas other two alleles, *slt-1(ev741)* and *slt-1(ok255)*, only partially suppressed the defects. The *ev741* and *eh15* mutations lead to early stop codons in the *slt-1* coding sequence truncating the proteins after the first (*ev741*) or second (*eh15*) leucine rich repeat (Hao et al., 2001). *ok255* is an in-frame deletion removing the first and part of the second leucine rich repeat (Hao et al., 2001). *slt-1(eh15)* is the only allele that still would contain the putative SAX-3 binding site in the second leucine-rich repeat (Howitt et al., 2004). It is possible that binding of SAX-3 to a truncated SLT-1 ligand is required for efficient suppression.

Discussion

The CDK8 module of the Mediator regulates axon navigation

In a genetic screen for axon navigation defects, we isolated a missense mutation located in the functionally important C-terminus of LET-19/MDT-13 (Wang et al., 2004). LET-19/MDT-13 orthologs in yeast, *Drosophila* and human are part of the CDK8 module of the Mediator, which also contains MED12, CDK8 and Cyc C (Borggrefe et al., 2002; Janody et al., 2003; Knuesel et al., 2009b; Leclerc et al., 1996; Loncle et al., 2007). Animals deficient in any CDK8 module component had similar axonal defects, indicating that this module contributes specifically to the regulation of axon navigation. Complete absence of *let-19/mdt-13* causes lethality (Herman, 1978; Wang et al., 2004; Yoda et al., 2005), whereas *cdk-8*- and *cic-1*-deficient animals are viable, pointing to additional roles for LET-19/MDT-13 in early embryonic development that are independent of CDK-8 and CIC-1. Different requirements for CDK8 module subunits were also observed in *Drosophila* development (Loncle et al., 2007).

The CDK8 module is able to detach from the Mediator complex and can be isolated as separate complex, suggesting that it forms a stable complex in vivo (Borggrefe et al., 2002; Knuesel et al., 2009b). Thus the CDK8 module could possibly function independently of the Mediator. However, Mediator subunits that are not part of the CDK8 module also exhibited axonal defects, indicating that the CDK8 module acts together with the Mediator here. For example, RNAi against *sur-2/mdt-23* caused strong axonal defects and *sur-2/mdt-23* loss-of-function animals had similar phenotypes as mutants of CDK8 module components. It was also shown that LET-19/MDT-13 physically interacts with SUR-2/MDT-23 in *C. elegans* (Yoda et al., 2005). The human CDK8 subcomplex binds to Mediator via MED13, the homolog of *C. elegans* LET-19/MDT-13 (Knuesel et al., 2009a). Thus SUR-2/MDT-23 could function as anchor for the CDK8 module to the rest of the Mediator complex in *C. elegans*. In addition, RNAi directed against Mediator subunits that are part of the middle (LET-49/MDT-7, MDT-10) and head modules (MDT-8, MDT-18), resulted in commissure defects as well. RNAi against the tail subunit *mdt-15* caused embryonic lethality, indicating that MDT-15 regulates important events in early embryonic development. Surviving animals also exhibited axonal defects, arguing for an additional role for MDT-15 in axon guidance. *mdt-15* is strongly expressed in the nervous system and intestine. It regulates fatty acid metabolism (Taubert et al., 2006; Yang et al., 2006) and the expression of detoxification genes (Taubert et al., 2008). We also identified subunits that did not show axon navigation defects, most notably a potential null-allele of MDT-28, which indicates that not all subunits of the Mediator are essential for regulation of axon navigation. Taken together, our results suggest that the CDK8 subcomplex acts as part of the Mediator complex to regulate several different axon guidance decisions in *C. elegans*.

Navigation defects in CDK8 module mutants are limited to certain classes of neurons. This could be due to the restricted expression of one or more of the subunits. This seems unlikely, since reporter constructs for all CDK8 module subunits showed ubiquitous expression throughout the developmental stages ((Wang et al., 2004; Zhang and Emmons, 2000) and Fig. 4). However, we currently cannot exclude the possibility that the activity of one or more of the subunits is regulated differently in neurons leading to cell-specific defects. On the other hand the CDK8 module could affect the expression of critical axon guidance genes in a subset of neurons. The specificity in this case would have to come from cell-type specific transcription factors interacting with the Mediator.

A comparison of expression profiles from *glr-1::GFP* positive interneurons in wildtype and *let-19/mdt-13(hd11)* mutant animals revealed a large number of genes that were significantly up- or downregulated. This raises the possibility that the axonal defects in Mediator component mutants are due to the misregulation of more than one gene. None of the genes currently known to affect interneuron navigation is substantially downregulated in *let-19/mdt-13(hd11)* mutants, so the target genes causing the defects remain to be identified. We found a number of transcription factors among the misregulated genes raising the possibility that some of the genes are indirectly affected by mutations in Mediator components. The number of primary target genes of the Mediator in these neurons is unclear.

The CDK8 module suppresses the SAX-3/ROBO pathway during dorsal axon navigation

Axons of DD/VD motoneurons frequently fail to reach the dorsal cord in Mediator component mutants. The molecular basis for dorso-ventral axon navigation in *C. elegans* is well understood (Killeen and Sybingco, 2008). UNC-6 is the major cue for migration in dorso-ventral direction (Ishii et al., 1992). It is thought to form a gradient, with its highest concentration on the ventral side (Wadsworth et al., 1996). Axons growing away from the ventral cord and towards the dorsal cord are repelled by UNC-6 (Hedgecock et al., 1990). Responses to UNC-6 are mediated by the receptors UNC-5 and UNC-40 and modulated by UNC-129, a member of the TGF-β family (Chan et al., 1996; Colavita et al., 1998; Hamelin et al., 1993; Leung-Hagesteijn et al., 1992; MacNeil et al., 2009). SLT-1, a repulsive cue, is expressed on the dorsal side (Hao et al., 2001) and repels axons towards the ventral cord. This repulsion is mediated by the receptor SAX-3/ROBO (Zallen et al., 1998). The ventral UNC-6 gradient is probably unaffected in *cdk-8*-deficient animals, because some axons that

require UNC-6/Netrin repulsion for dorsal-directed navigation such as DA/DB motoneuron commissures migrate normally. Mutations in *sax-3*/robo, encoding the receptor for SLT-1, suppressed the *cdk-8*-induced commissure defects, indicating that the SAX-3 pathway might cause these defects. Mutations in *slt-1* also suppressed the defects, suggesting that an inappropriate response to dorsal SLT-1 could prevent DD/VD commissures from reaching the dorsal cord. *slt-1(eh15)*, which retains the putative SAX-3 binding site, strongly suppressed the commissure defects, whereas two other alleles of *slt-1*, which eliminate the putative SAX-3 binding site, only weakly suppressed the defects. This raises the possibility that binding of SAX-3 to a truncated SLT-1 protein is required for efficient suppression of navigation defects, but suggests that activation of SAX-3 by SLT-1 is not required for suppression. SAX-3 can interact with UNC-40, which leads to repression of UNC-40 signaling (Stein and Tessier-Lavigne, 2001; Yu et al., 2002). It is possible that the DD/VD defects are in part the result of a failure in the response to UNC-6/Netrin due to inappropriate interaction between SAX-3 and UNC-40. Binding of SAX-3 to a truncated and functionless SLT-1 protein in *slt-1(eh15)* mutants might prevent the interaction with UNC-40, which could explain the more effective suppression by allele *slt-1(eh15)*. The molecular basis for the axonal defects in DD/VD commissure navigation in Mediator mutants remains to be elucidated.

The fact that the CDK module is selectively required in a subset of neurons is intriguing. The CDK module has also been implicated in the developmental of *C. elegans*, *Drosophila*, and zebrafish. Notably, MED12 is required for normal neuronal development in zebrafish, most likely by acting as a coactivator of the transcription factor Sox9; as in our study, some, but not all neurons were affected by med12 mutations (Hong et al., 2005; Wang et al., 2006). In *Drosophila* cdk8 mutants were isolated in genetic screens for wiring defects in the visual system (Berger et al., 2008). In humans, MED12 interacts with the chromatin remodeling complex REST to control neuronal gene expression (Ding et al., 2008) and MED12 mutations have been linked to various neuropsychiatric disorders (Risheg et al., 2007; Sandhu et al., 2003). Axon morphology was not investigated in any of these studies, and whether the cell biological process we identified, namely axon navigation, is linked to the signaling pathways discovered in vertebrates remains to be determined. Nevertheless, it appears likely that CDK module subunits represent an evolutionarily conserved entity that selectively drives the development of a specific subset of neurons.

In this study, we show that the *C. elegans* Mediator, in particular the CDK8 module, is required for certain axon guidance decisions during neuronal development. The CDK8 module regulates the expression of a large number of genes in interneurons showing navigation defects in the VNC. The commissure navigation defects in a subset of motoneurons can be suppressed by a mutation in the guidance receptor *sax-3*, suggesting that misregulation of a single guidance pathway might be responsible for the defects. These results show that the CDK8 module of the Mediator complex is involved in transcriptional regulation of specific target genes in certain subsets of neurons and emphasizes the importance of transcriptional control for proper axon navigation.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ydbio.2013.02.009.

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