Developmental Biology 391 (2014) 43–53

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

The PAF1 complex is involved in embryonic epidermal morphogenesis in *Caenorhabditis elegans*

Yukihiko Kubota [a,1], Kenji Tsuyama [a,1], Yusuke Takabayashi [a,1,2], Nami Haruta [a], Rika Maruyama [b,3], Naoko Iida [b,4], Asako Sugimoto [a,b,*]

[a] Laboratory of Developmental Dynamics, Graduate School of Life Sciences, Tohoku University, Sendai 980-8577, Japan
[b] Laboratory for Developmental Genomics, RIKEN Center for Developmental Biology, Kobe 650-0047, Japan

ARTICLE INFO

Article history:
Received 2 March 2014
Received in revised form
29 March 2014
Accepted 2 April 2014
Available online 8 April 2014

Keywords:
PAF1 complex
Caenorhabditis elegans
Epidermal morphogenesis
Embryogenesis

ABSTRACT

The PAF1 complex (PAF1C) is an evolutionarily conserved protein complex involved in transcriptional regulation and chromatin remodeling. How the PAF1C is involved in animal development is still not well understood. Here, we report that, in the nematode *Caenorhabditis elegans*, the PAF1C is involved in epidermal morphogenesis in late embryogenesis. From an RNAi screen we identified the *C. elegans* ortholog of a component of the PAF1C, CTR-9, as a gene whose depletion caused various defects during embryonic epidermal morphogenesis, including epidermal cell positioning, ventral enclosure and epidermal elongation. RNAi of orthologs of other four components of the PAF1C (PAFO-1, LEO-1, CDC-73 and RTFO-1) caused similar epidermal defects. In these embryos, whereas the number and cell fate determination of epidermal cells were apparently unaffected, their position and shape were severely disorganized. PAFO-1::mCherry, mCherry::LEO-1 and GFP::RTFO-1 driven by the authentic promoters were detected in the nuclei of a wide range of cells. Nuclear localization of GFP::RTFO-1 was independent of other PAF1C components, while PAFO-1::mCherry and mCherry::LEO-1 dependent on other components except RTFO-1. Epidermis-specific expression of mCherry::LEO-1 rescued embryonic lethality of the *leo-1* deletion mutant. Thus, although the PAF1C is universally expressed in *C. elegans* embryos, its epidermal function is crucial for the viability of this animal.

Introduction

Temporally and spatially regulated gene transcription is essential during embryogenesis to produce diverse type of cells in a coordinated manner. For the tightly regulated transcription, recruitment of RNA polymerase II (Pol II) to the target genes and modulation of the Pol II activity is crucial. In addition, chromatin remodeling through alteration to nucleosomes by histone modification affects the DNA accessibility during transcription.

The Polymerase Associated Factor 1 (PAF1) complex, or PAF1C, is a protein complex conserved in eukaryotes, which is involved in multiple aspects of Pol II transcriptional regulation, including transcriptional elongation, 3′-terminal end processing, and histone modification (Jaehning, 2010; Tomson and Arndt, 2013). This complex was originally identified in *Saccharomyces cerevisiae* as an RNA pol II interactor (Shi et al., 1996; Wade et al., 1996), and consists of five proteins (Ctr9, Paf1/pancreatic differentiation 2, Leo1, Cdc73/parafibromin and Rtf1) (Mueller and Jaehning, 2002; Mueller et al., 2004). None of the PAF1C components are essential for the viability of *S. cerevisiae*, but functional loss of the PAF1C causes diverse phenotypes including sensitivity to cellular stresses, which is linked to defects in chromatin and transcriptional regulation (Kim and Levin, 2011).

Unlike in yeasts, the PAF1C in multicellular organisms are essential for viability, and implicated in a variety of developmental processes including the timing of flowering in plants (He et al., 2004; Oh et al., 2004), and development of somite, heart, neuronal

* Corresponding author at: Laboratory of Developmental Dynamics, Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan. Fax: +81 22 217 6194.
E-mail addresses: yu-kubota@m.tohoku.ac.jp (Y. Kubota), t-kenji@life.biology.tohoku.ac.jp (K. Tsuyama), takabayashi@seika.che.tohoku.ac.jp (Y. Takabayashi), nharuta@m.tohoku.ac.jp (N. Haruta), ymaruyama@ualberta.ca (R. Maruyama), naiida@nig.ac.jp (N. Iida), asugimoto@m.tohoku.ac.jp (A. Sugimoto).
[1] These authors contributed equally to this work.
[2] Present address: Department of Biomolecular Engineering, Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan.
[3] Present address: Department of Medical Genetics, University of Alberta, Edmonton, AB, Canada T6G 2H7.
[4] Present address: Genome Informatics Laboratory, National Institute of Genetics, Mishima 411-8540, Japan.

http://dx.doi.org/10.1016/j.ydbio.2014.04.002
0012-1606/

and craniofacial cartilage in zebrafish (Akanuma et al., 2007; Nguyen et al., 2010; Zhang et al., 2013a). Additionally, the components of the PAF1C affect Hedgehog, Notch and Wnt signaling (Akanuma et al., 2007; Mosimann et al., 2006, 2009; Tenney et al., 2006). It also has been shown that the PAF1C regulates cell proliferation, cell differentiation, cell morphology, cell migration, maintenance of stem cells and tumorigenesis (Bai et al., 2010; Carpten et al., 2002; Ding et al., 2009; Langenbacher et al., 2011; Lin et al., 2008; Moniaux et al., 2006; Ponnusamy et al., 2009; Shi et al., 1996; Zhang et al., 2013b). Although all five PAF1C components are conserved throughout eukaryotes, the human PAF1C contains another component Ski8/Wdr61 that plays a role in mRNA decay (Zhu et al., 2005a). In addition, Rtf1 in multicellular organisms is less tightly associated with other PAF1C components (Adelman et al., 2006; Rozenblatt-Rosen et al., 2005; Yart et al., 2005; Zhu et al., 2005a). Thus, during the evolution of the PAF1C, alteration of the interaction within components and with other interactors may have led to expansion of the PAF1C function.

The nematode *Caenorhabditis elegans* provides an excellent system to study genetic control of dynamic cellular behaviors because of its highly reproducible development (Sulston et al., 1983). The body elongation during *C. elegans* embryogenesis occurs through the coordinated shape change, migration and rearrangement of epidermal cells, in the absence of cell proliferation and cell death (Sulston et al., 1983). During this process, cytoskeletons of epidermal cells are dynamically reorganized, and cell-to-cell adhesion and cell-to-ECM interaction are modified (Chin-Sang and Chisholm, 2000; Labouesse, 2012; Lynch and Hardin, 2009; Michaux et al., 2001; Simske and Hardin, 2001; Zhang and Labouesse, 2012). However, gene regulations that control these dynamic behaviors of epidermal cells are still not well understood.

In this study, we identified an ortholog of the PAF1C components, Ctr9 (CTR-9) through an RNAi screen for genes involved in epidermal morphogenesis in late embryogenesis of *C. elegans*. We further identified other four orthologs of the PAF1C components (PAFO-1, LEO-1, CDC-73, and RTFO-1) and demonstrated that the PAF1C in *C. elegans* contributes to embryonic epidermal morphogenesis. This is the first functional analysis of the PAF1C in *C. elegans*, and will provide the bases for further studies on how this complex is involved in gene regulations in embryogenesis.

Materials and methods

C. elegans strains

C. elegans strains were derived from the wild-type Bristol strain N2 (Brenner, 1974). Worms were grown at 24.5 °C, except for *leo-1* (*gk1081*), which was maintained at 20 °C. The following alleles were used; *unc-119*(*ed3*), *leo-1*(*gk1081*) (*C. elegans* Gene Knockout Consortium), *nT1*[*qIs51*], *xnIs17*[*dlg-1*::*GFP*++*rol-6*(*su1006*)] (Firestein and Rongo, 2001; Totong et al., 2007), *tjIs57*[*pie-1p-mCherry*::*histone H2B* +*unc-119*(+)] (Toya et al., 2010), *mcIs50*[*lin-26p*::$_{vab\text{-}10}$*ABD*::*GFP*, *myo-2p*::*GFP*, *pBluescript*] (Gally et al., 2009), *ruIs32*[*pAZ132*::*pie-1p*::*GFP*::*histone H2B*] (Praitis et al., 2001), *edIs20*[*neuronal promoter*::*GFP*] (kindly provided by Dr. Joel Rothman), *ccIs4251*[*myo-3p*::*GFP-LacZ* (*NLS*), *myo-3p*::*mitochondrial*::*GFP*, *dpy-20*(+)] (Kostas and Fire, 2002), and *mcIs46*[*dlg-1*::*RFP*++*unc-119*(+)] (Diogon et al., 2007).

Transgenic worms were generated by microparticle bombardment transformation (for constructing integrated GFP/mCherry marker lines) (Praitis et al., 2001) or microinjection (for tissue specific rescue experiments) (Mello et al., 1991), using *unc-119*(*ed3*) as the host strain. For transformation markers, *Cbr-unc-119*(+) (Maduro and Pilgrim, 1996), *unc-119*(+) (pDP#MM061B) (Maduro and Pilgrim, 1995), or *sur-5*::*GFP* (pTG96) (Yochem et al., 1998) were used. The strains constructed in this study are listed in Supplementary Table S2.

Homology analysis

The amino acid sequence comparison was performed using the protein-protein BLAST. The amino acid sequences of *C. elegans* CTR-9, PAFO-1, LEO-1, CDC-73 and RTFO-1 correspond to GeneBank entries NP_499090.1, NP_505925.1, NP_502135.1, NP_500465.3 and NP_505473.1, respectively. The amino acid sequences of *H. sapiens* hCtr9, hPaf1, hLeo1, hCdc73 and hRtf1 correspond to GeneBank entries NP_055448.2, NP_061961.2, NP_620147.1, NP_078805.3 and NP_055953.3, respectively.

Plasmid construction

The list of the plasmids constructed in this study is listed in Supplementary Table S3.

For epidermal specific expression, we made a GATEWAY (Invitrogen) vector pYKN1R by substituting the *pie-1* promoter and the *pie-1* 3′–UTR of pMTN1R (Toya et al., 2010) to the *lin-26* promoter (Landmann et al., 2004) and the *let-858* 3′–UTR. The epidermal mCherry::TBB-2 expression plasmid (pYKN1R-tbb-2) was constructed by inserting the *tbb-2* coding sequence into pYKN1R by LR reaction (Supplementary Table S3). To construct transgenes that express mCherry or GFP fusion proteins from authentic promoter, genomic fragments were PCR-amplified and fused with the mCherry/GFP encoding DNA fragments. The details about the genomic fragments are described in Supplementary methods.

Tissue-specific rescue experiments

Transgenic worms were made by microinjection (Mello et al., 1991) using the following combination of plasmids: the mCherry-tagged transgene (*leo-1p*::*mCherry*::*leo-1*, *lin-26p*::*mCherry*::*leo-1*, *hlh-1p*::*mCherry*::*leo-1* or *kal-1p*::*mCherry*::*leo-1*), 5 μg/ml; an injection marker *sur-5*::*GFP* plasmid pTG96, 70 μg/ml; an *unc-119*(+) plasmid pDP#MM016B (Maduro and Pilgrim, 1995), 30 μg/ml; and pBluescript II KS(−), 45 μg/ml. The extrachromosomal arrays of the resultant transgenic worms were transferred to *unc-119*(*ed3*);*leo-1*(*gk1081*)/*nT1*[*qIs51*] animals by mating (Supplementary Table S2). To score embryonic lethality, embryos of the *gk1081* homozygotes were obtained by dissecting the gonads, and after incubating for 24 h at 20 °C, arrested embryos among the SUR-5::GFP-positive progeny were scored.

Antibody production

The DNA fragment coding the N-terminal region (1–91 a.a.) of LEO-1 was PCR amplified from the cDNA clone yk1402b12 (kindly provided by Dr. Yuji Kohara) and cloned into an expression vector pColdI (Takara) containing the *cspA* promoter and 6xHis-tag. The 6xHis-LEO-1(91 a.a.) protein was expressed in *E. coli* BL21 (Invitrogen) at 15 °C, purified and used as the antigen. Affinity purified rabbit anti-LEO-1 antibody was generated by Medical and Biological Laboratories (Nagoya, Japan).

Western blot analysis for LEO-1

Young adult worms were placed on agar plates without bacteria to reduce bacterial contamination. Then, eight wild-type worms, eight *leo-1*(*RNAi*) worms and twenty *leo-1*(*gk1081*) worms were added to 15 μl each of 1× sample buffer. The tubes were frozen and boiled for 5 min, then, the lysates were electrophoresed on a 4–15% gradient SDS–PAGE TGX gel (Bio-Rad). Rabbit anti-LEO-1 antibody (1:1000) and rabbit anti-histone H3 antibody (1:20,000, ab1791, Abcam) were used as primary antibodies. Horseradish peroxidase (HRP)-conjugated donkey anti-rabbit antibody (1:50,000, Jackson) was used as secondary antibodies, and the signals

were detected with chemilluminescence (Chemi-Lumi One Super Western blotting detection reagent, Nacalai Tesque). Signal was detected by ImageQuant 400 (GE Healthcare) and processed by Adobe Photoshop CS6 (Adobe).

RNAi

RNAi was carried out by the soaking method as described (Maeda et al., 2001). dsRNA was prepared by in vitro transcription from cDNA clones (yk1575g06 for *ctr-9/B0464.2*, yk725d11 for *pafo-1/C55A6.9*, yk1402b12 for *leo-1/B0035.11*, yk1503c9 for *cdc-73/F35F11.1*, yk843e11 for *rtfo-1/F25B3.6*; all cDNA clones were gifts from Yuji Kohara). L4 worms were soaked in 2 mg/ml dsRNA soaking solutions and incubated at 24.5 °C for 24 h. The worms were then recovered and cultured at 24.5 °C. Phenotypes of the embryos were analyzed at 24 h after the recovery.

Microscopy

Fluorescence and Nomarski images of *C. elegans* embryos were acquired as described (Toya et al., 2011). For time-lapse microscopy, embryos expressing fluorescently tagged proteins in Egg Buffer were mounted on 2% agarose pads. For each embryo, 15–60 Z-series images (0.5 μm steps or 1 μm steps) were acquired. The fluorescent Z-series images were projected using a maximum intensity algorithm of MetaMorph software (Molecular Devices) to produce a single integrated image. Images were processed with image J (NIH) or Adobe Photoshop CS6.

Time-lapse Nomarski microscopy was performed using Olympus BX63 microscope with iXon^{EM}+ EM-CCD camera (ANDOR) and PlanApo N X60 oil NA1.42 objective lens. Fluorescence confocal microscopy was performed using the CSU-X1 spinning-disk confocal system (Yokogawa Electric Corp) mounted on Zeiss Axioplan2 microscope with iXon^{EM}+ EM-CCD camera (ANDOR) and C-Apochromat X63 water NA1.2 objective lens. Images of nuclear localization of fluorescently tagged proteins were acquired using the CSU-X1 spinning-disk confocal system mounted on Olympus IX71 microscope with Orca-R2 12 bit/16 bit cooled CCD camera (Hamamatsu Photonics), UPlanSApo 60X silicone oil NA1.3 objective lens and UPlanSApo 100X NA1.4 objective lens. For imaging of actin and tubulin, the Olympus DSU (Disk Scanning Unit) system attached to Olympus BX61 microscope with PlanApo N 60X oil NA1.42 objective lens was used. All microscope systems were controlled by MetaMorph (Molecular Devices) software.

Results

The PAF1C is required for late embryogenesis of C. elegans

To identify genes involved in embryonic morphogenesis in *C. elegans*, an RNAi screen was performed. We previously identified ~800 embryonic lethal genes by a large-scale RNAi analysis (Maeda et al., 2001, and our unpublished data). The terminal RNAi phenotypes of these embryonic lethal genes were analyzed with the differential interference contrast (DIC) microscope and 60 of them showed late morphogenetic defects, such as limited elongation or body rupture. These genes were further analyzed by DIC live imaging, and the *B0464.2* gene was identified as a gene that caused a reproducible body elongation defect at a high penetrance.

A homology analysis revealed that B0464.2 is a *C. elegans* ortholog of Ctr9 (hereafter, CTR-9), a component of the PAF1 complex (PAF1C) (Chu et al., 2013) (Table 1). The PAF1C is an evolutionarily well conserved protein complex, and consists of five components, Paf1, Ctr9, Leo1, Cdc73 and Rtf1 (Mueller and Jaehning, 2002; Mueller et al., 2004). In addition to CTR-9, all other components of the PAF1C were identified in the *C. elegans* genome based on the sequence similarity (Paf1 ortholog C55A6.9/PAFO-1, Leo1 ortholog B0035.11/LEO-1, Cdc73 ortholog F35F11.1/CDC-73, and Rtf1 ortholog F25B3.6/RTFO-1) (Table 1).

Table 1
Components of the PAF1C in *C. elegans* are required for embryogenesis.

Name of the PAF1C component			Homology[a] (%)	Emb[b] (%) (*N*)
S. cerevisiae	*H. sapiens*	*C. elegans*		
Ctr9	hCtr9	CTR-9 (B0464.2)	43	98.2% (*N*=395)
Paf1	hPaf1	PAFO-1 (C55A6.9)	34	98.6% (*N*=217)
Leo1	hLeo1	LEO-1 (B0035.11)	55	26.2% (*N*=432)
Cdc73	hCdc73	CDC-73 (F35F11.1)	29	100% (*N*=351)
Rtf1	hRtf1	RTFO-1 (F25B3.6)	36	94.1% (*N*=461)

[a] Homology between *H. sapiens* and *C. elegans* orthologs.
[b] Embryonic lethality by RNAi knockdown in *C. elegans*. Control RNAi caused 2.0% embryonic lethality (*N*=304); *N*: numbers of embryos scored.

To test whether the components of the *C. elegans* PAF1C work as a complex in late embryogenesis, RNAi phenotypes for each component were compared by the time-lapse DIC microscopy. Similar to the RNAi of the *ctr-9* gene, RNAi knockdown of the four other genes caused morphogenesis defects in late embryogenesis (Fig. 1, Table 1, Supplementary Table S1). For all five genes, embryogenesis proceeded slower than the control embryos; eventually the embryos reached to morphogenesis stage, but their body elongation process was severely affected (Fig. 1, Supplementary Table S1). While the majority of *ctr-9*, *pafo-1*, *leo-1*, and *rtfo-1* RNAi embryos exhibited aberrant body elongation (Fig. 1B–D and F), ~60% of the *cdc-73*(*RNAi*) arrested at earlier stage without significant body shape change (Fig. 1E, Supplementary Table S1). Thus, all five components of the PAF1C are essential for the progression of late embryogenesis. The slight differences of the morphogenesis phenotypes and penetrance imply that each component of the PAF1C may have distinct functions, either as a component of the PAFC or in addition to their role as a PAF1C component. It is also possible that the phenotypic differences were caused by the variable efficiency of RNAi, but at least for *pafo-1*, *leo-1* and *rtfo-1*, RNAi reduced the corresponding proteins to the undetectable level (see below), thus partial RNAi effects were unlikely.

Loss of the PAF1C function does not reduce general gene expression

In other organisms, it has been shown that the PAF1C interacts with Pol II to modulate gene transcription (Jaehning, 2010; Tomson and Arndt, 2013). To examine whether the late embryonic defects by RNAi knockdown of the PAF1C components were caused by general reduction of gene expression, expression of cell-type specific GFP markers were examined in these embryos. In all *ctr-9*(*RNAi*), *pafo-1*(*RNAi*), *leo-1*(*RNAi*), *cdc-73*(*RNAi*) and *rtfo-1*(*RNAi*) embryos, the GFP signals of the cell type specific markers (neuronal marker, neuronal promoter::GFP; muscle marker, *myo-3p*::NLS::GFP; and epithelial marker, *dlg-1p*::DLG-1::GFP) were detected at the equivalent levels with the wild type (Fig. 2A–F, and data not shown). Thus, the loss of the PAF1C component did not cause general reduction of gene expression, nor affect cell specification at least for neurons, muscles and epithelia.

The PAF1C is involved in positioning and shape change of epidermal cells

Since embryonic body elongation is driven by the epidermal cell shape change and migration, we examined the epidermal cell behaviors by live-imaging using a worm strain expressing an epithelial junction marker, DLG-1::GFP (Fig. 2E and F, Supplementary Fig. S1). In the wild-type embryos, DLG-1::GFP-positive epidermal

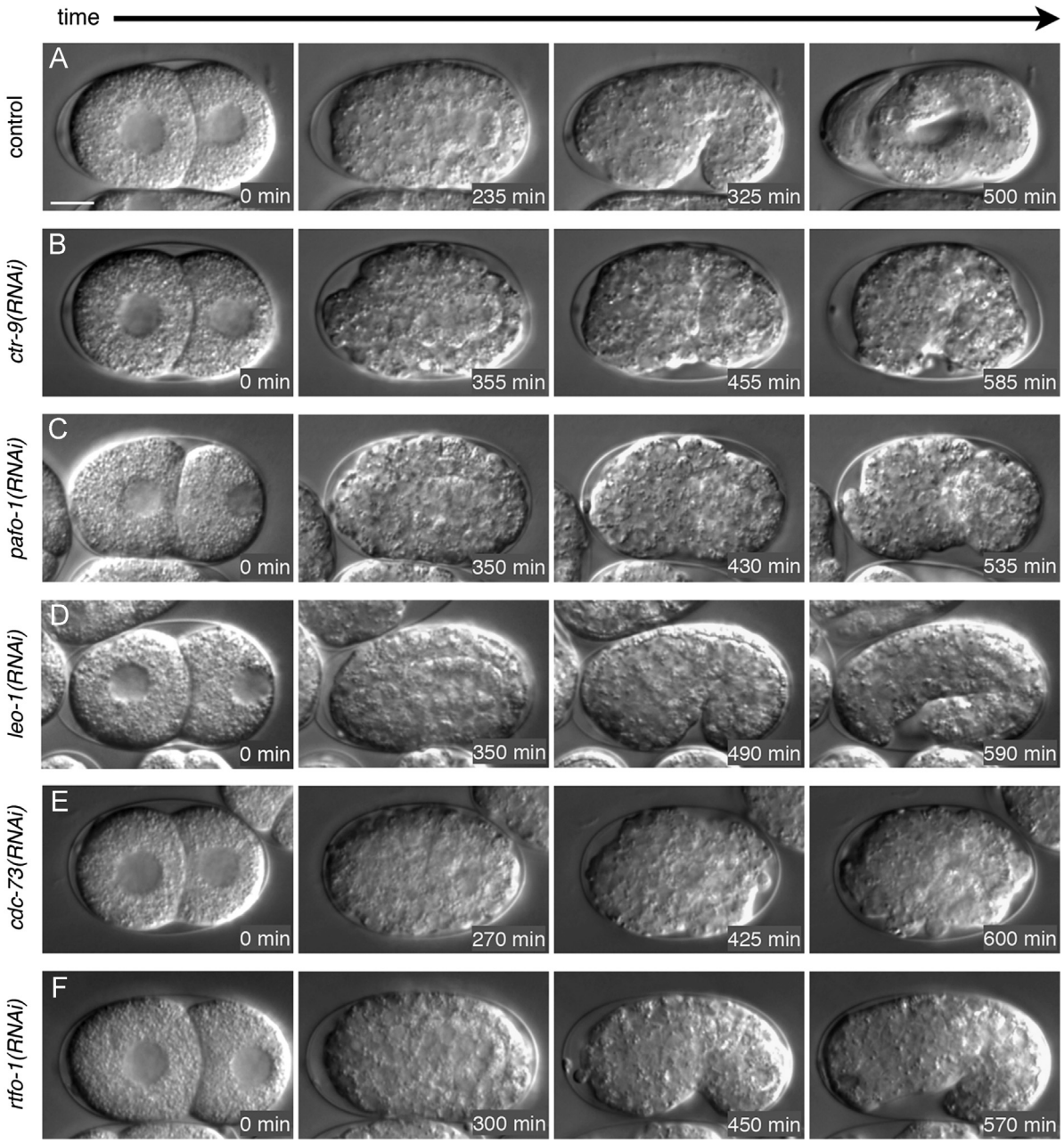

Fig. 1. The PAF1C is required for late embryogenesis of *C. elegans*. Time-laps DIC micrographs of (A) control, (B) *ctr-9*(*RNAi*), (C) *pafo-1*(*RNAi*), (D) *leo-1*(*RNAi*), (E) *cdc-73* (*RNAi*) and (F) *rtfo-1*(*RNAi*) embryos. Scale bar: 10 μm. Times in the pictures indicate minutes after the first picture (left panel, two-cell stage embryos).

cells were formed dorsally, and they move ventrally to enclose the embryos. After enclosure, epidermal cells elongate in the anterior-posterior direction, which leads to the body elongation. In the *ctr-9*(*RNAi*), *pafo-1*(*RNAi*), *leo-1*(*RNAi*), *cdc-73*(*RNAi*) and *rtfo-1*(*RNAi*) embryos, position and cell shape of epidermal cells were aberrant during epidermal morphogenesis (Fig. 2F, Supplementary Fig. S1). The severity and the timing of epidermal defects were variable. While most embryos showed defects in ventral enclosure of epidermal cells and epidermal elongation, some RNAi embryos showed leading cell movement to ventral midline.

To further analyze the defects in epidermal cell patterning, nuclei of seam cells, the lateral subset of epidermal cells, were visualized with CEH-16::GFP (Cassata et al., 2005). In the wild type, nuclei of ten seam cells are linearly aligned in each lateral side of embryos (Fig. 2G, I, and K). In *ctr-9*(*RNAi*) embryos, the number of the seam cell nuclei was equivalent to the wild type (Fig. 2H, J, and K). However, they are not linearly arranged from the onset of the morphogenesis stage (Fig. 2H), and the cell misplacement became more severe in later stages (Fig. 2J). Misplacement of epidermal cells was already evident at the time when the epidermal cells started expressing CEH-16::GFP or DLG-1::GFP, thus the cell positioning defect might have initiated before differentiation of epidermal cells.

These results suggest that the PAF1C does not affect epidermal cell proliferation and specification, but is required for cell shape change and positioning of epithelial cells.

CTR-9 is required for epidermal microtubule organization during morphogenesis

Actin and microtubule cytoskeletons are involved in epidermal cell shape change and migration in *C. elegans* embryogenesis (Diogon et al., 2007; Gally et al., 2009; Piekny et al., 2003; Priess and Hirsh, 1986; Williams-Masson et al., 1997, 1998; Wissmann et al., 1997). To examine whether cytoskeleton in epithelial cells was

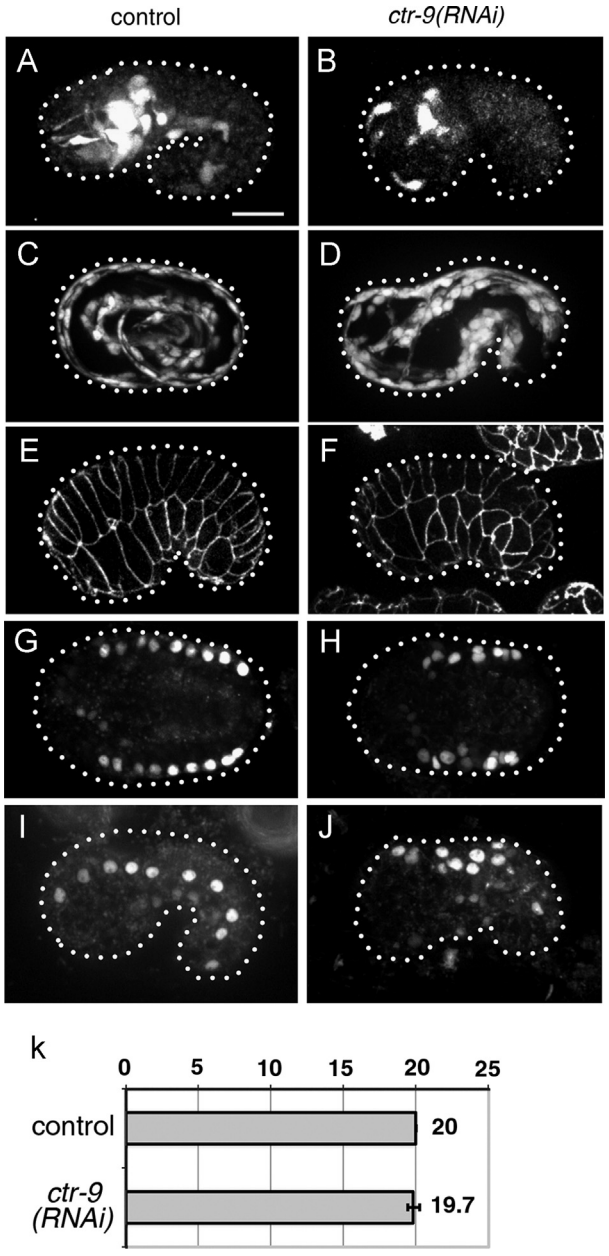

Fig. 2. The PAF1C is involved in positioning and shape change of epidermal cells. GFP marker expression in control (A, C, E, G, and I) and *ctr-9*(*RNAi*) (B, D, F, H, and J) embryos. (A and B) Neuronal marker (neuronal promoter::GFP) at comma ~1.5-fold stage. (C and D) Muscle cell marker (myo-3p::NLS::GFP) at 2-fold~pretzel stage. (E and F) Epithelial cell junction marker (DLG-1::GFP) at bean~comma stage. Seam cell nuclei marker (CEH-16::GFP) at ventral enclosure stage (G and H) and comma ~1.5-fold stage (I and J). Images of the control and *ctr-9*(*RNAi*) embryos were captured and processed with the same condition. In (G) and (H), two CEH-16::GFP-positive caudal seam cells (T cells) are out of focus, thus 18 CEH-16-positive cells are visible. (K) Average number of seam cells scored using the CEH-16::GFP signal at comma stage in the control and *ctr-9*(*RNAi*) embryos ($n=10$). The data represent the mean ± standard deviation. Scale bar: 10 μm.

affected in the RNAi embryos, we analyzed the actin and microtubule organization in epidermal cells by live-imaging during embryonic morphogenesis.

To visualize F-actin localization in epidermal cells, an actin marker VAB-10(ABD)::GFP was used (Gally et al., 2009). During ventral enclosure, F-actin detected with VAB-10(ABD)::GFP was accumulated at the leading edge of the ventral epidermal cells (Patel et al., 2008) (Fig. 3A). During the elongation stage, actin filaments in the dorsal and ventral epidermal cells were oriented circumferentially (Gally et al., 2009; Priess and Hirsh, 1986) (Fig. 3B). Although the epidermal cell shape and migration were affected in the *ctr-9*(*RNAi*) embryos, VAB-10(ABD)::GFP accumulation at the leading edge during ventral closure and circumferential actin bundles in epidermal cells were detected (Fig. 3C and D).

Microtubules in epidermal cells during embryonic morphogenesis were visualized by mCherry::TBB-2 (β-tubulin) driven by the epidermal promoter, *lin-26p* (Landmann et al., 2004). As reported previously, microtubules aligned circumferentially in dorsal and ventral epidermal cells in wild-type embryos at the elongation stage (Priess and Hirsh, 1986) (Fig. 3E). In contrast, randomly oriented microtubules and aggregated mCherry::TBB-2 signals were often observed in *ctr-9*(*RNAi*) embryos during epidermal elongation process (Fig. 3F).

These results imply that the loss of the PAF1C function affects the alignment of microtubules more severely than actin filaments in epidermal cells during the morphogenesis stage.

A deletion allele of leo-1 causes maternal effect embryonic lethality

To further analyze the requirement of the PAF1C in embryogenesis, a deletion allele for *leo-1*, *gk1081*, was used, which was isolated by the *C. elegans* Gene Knockout Consortium. The *leo-1* locus encodes a predicted polypeptide of 430 amino acids, and the *gk1081* allele deleted 627 bp that would result in a C-terminally truncated protein product of 137 amino acids (intrinsic 132 a.a. with extra 5 a.a.) (Fig. 4A). A Western blot analysis using the anti-LEO-1 antibody raised against N-terminus of LEO-1 detected a 65 kDa band that migrated slower than the predicted molecular masse (48 kDa) in wild-type animals (Fig. 4B). This band apparently corresponded to the LEO-1 protein because it was not detected in *leo-1*(*RNAi*) and the *leo-1*(*gk1081*) animals (Fig. 4B). Slower mobility of Leo1 protein in SDS–PAGE was also reported for Leo1 orthologs in yeast and humans (Magdolen et al., 1994; Mueller and Jaehning, 2002; Rozenblatt-Rosen et al., 2005). In the Western blot of the *leo-1*(*gk1081*) mutant extract, a weak 22 kDa band was detected, which was likely to correspond to the truncated LEO-1(*gk1081*) polypeptide (Fig. 4B).

We found that *leo-1*(*gk1081*) mutant is maternal effect embryonic lethal. The *gk1081* homozygotes from *gk1081*/+ heterozygotes were viable (embryonic lethality; 1.8%, $n=552$) but showed partial sterility and defective vulvae (data not shown). The *gk1081* homozygous progeny obtained by dissecting the gonads of these animals were mostly embryonic lethal with epidermal morphogenesis defects (82%, $n=101$) (Fig. 4D), which is consistent with the phenotype of *leo-1*(*RNAi*) embryos. This maternal effect embryonic lethality was rescued by integrated transgenes *tjIs279*[*leo-1p*::*mCherry*::*leo-1*] or *tjIs308*[*leo-1p*::*GFP*::*leo-1*] that contains the *leo-1* genomic region with an in-frame mCherry or GFP insertion, confirming that this phenotype is caused by the loss of the LEO-1 function.

Epidermal expression of mCherry::LEO-1 rescues embryonic lethality of the leo-1(gk1081) mutant

As mentioned above, *tjIs279*[*leo-1p*::*mCherry*::*leo-1*] transgenes (Fig. 4A) rescued embryonic lethality of *leo-1*(*gk1081*) homozygous animals, thus, the fusion proteins were regarded functional and their expression pattern was expected to reflect that of the endogenous LEO-1. In embryos that have *tjIs279*[*leo-1p*::*mCherry*::*leo-1*], mCherry::LEO-1 was detected in the nuclei of virtually all cells throughout embryogenesis from the one-cell stage (Figs. 5B and 6B).

Since *leo-1*(*RNAi*) and *leo-1*(*gk1081*) both exhibited defects in epidermal morphogenesis, we next asked whether expression of LEO-1 in epidermal cells was sufficient to rescue the mutant

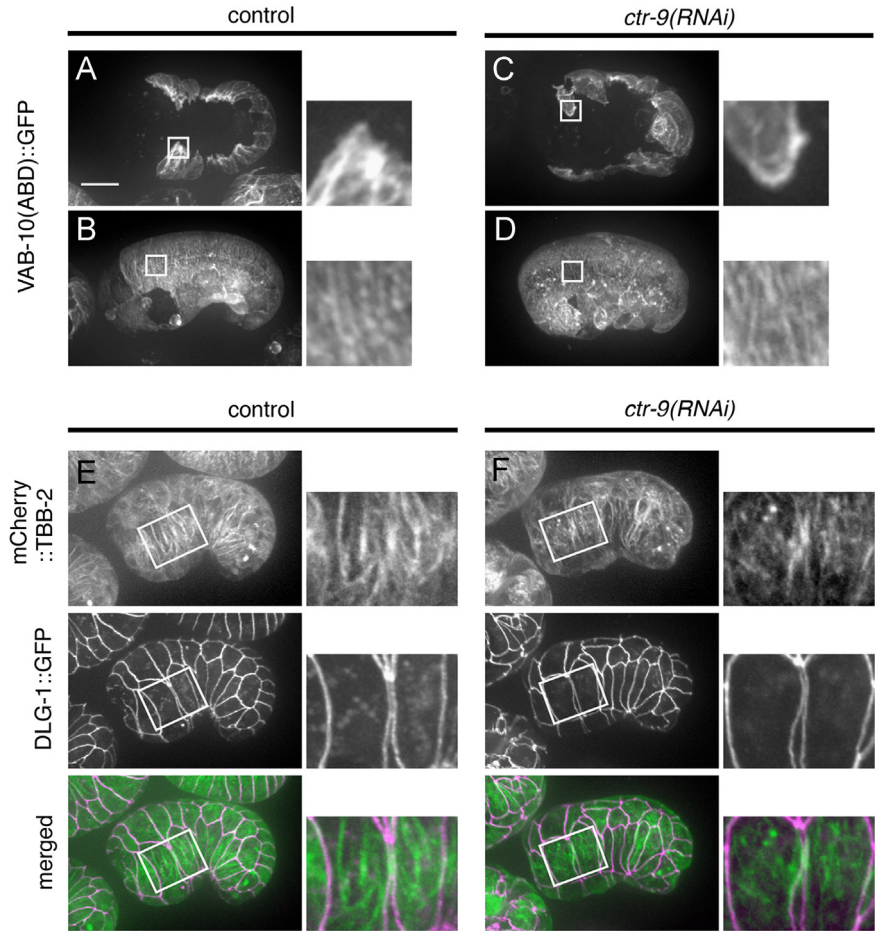

Fig. 3. F-actin and microtubule organization in epidermal cells of control and *ctr-9(RNAi)* embryos. Images of F-actin marker, VAB-10(ABD)::GFP in the control (A and B) and *ctr-9*(*RNAi*) (C and D) embryos at ventral enclosure (A and C) and bean-comma (B and D) stage. Magnified images of the boxed areas are shown in the right panels. (E and F) Images of mCherry::TBB-2 (β-tubulin) (top), DLG-1::GFP (epidermal cell junction) (middle), and merged images of the mCherry::TBB-2 (green) and DLG-1::GFP (magenta) (bottom) in the control (E) and *ctr-9*(*RNAi*) (F) embryos at comma stage. Magnified images of the boxed areas are shown in the right panels. Scale bar: 10 μm.

phenotype. The *mCherry*::*leo-1* coding fragment was expressed under promoters that drive expression in specific cell types (epidermal promoter, *lin-26p*; neuronal precursor promoter, *kal-1p*; and muscle promoter, *hlh-1p*). Expression of mCherry::LEO-1 under the control of the epidermal promoter (*lin-26p*) rescued the *leo-1*(*gk1081*) lethality, as efficient as the authentic *leo-1* promoter (Fig. 4C). On the other hand, expression of mCherry::LEO-1 under the muscle promoter or the neuronal promoter did not rescue embryonic lethality (Fig. 4C). Thus, although LEO-1 is expressed in a wide range of cells during embryogenesis, its expression in epidermal cells is crucial for the viability of the animal.

Components of the PAF1C localize to the nuclei of a wide range of cells during embryogenesis

In addition to LEO-1, expression patterns of PAFO-1 and RTFO-1 were analyzed by constructing transgenic insertion strains in which PAFO-1::mCherry or GFP::RTFO-1 were expressed under the control of authentic promoters. Similar to LEO-1, PAFO-1::mCherry was detected in the nuclei of virtually all embryonic cells from the one-cell stage (Fig. 5A, and data not shown). GFP::RTFO-1 was also detected in nuclei of a wide range of cells but only after ~30-cell stage and except for germ cells (Fig. 5C, and data not shown). Thus, these three components of the PAF1C were co-expressed in the majority of somatic cells during embryogenesis.

Subnuclear localization of PAFO-1::mCherry, mCherry::LEO-1 and GFP::RTFO-1 were examined using GFP::histone H2B or mCherry::histone H2B as a comparison. PAFO-1::mCherry, mCherry::LEO-1 and GFP::RTFO-1 were not uniformly present within the nuclei, but enriched in discrete nuclear regions (Fig. 5A–C). Notably, these proteins were only partially colocalized with histone signals within the nuclei (Fig. 5A–C). A larger fraction was colocalized within the nuclei for the combinations [GFP::LEO-1 and PAFO-1::mCherry] and [GFP::RTFO-1 and PAFO-1::mCherry], whereas GFP::RTFO-1 appeared more uniformly distributed in the nuclei than PAFO-1::mCherry (Fig. 5D and E).

To analyze the cell cycle-dependent change of the localization of the PAF1C, mCherry::LEO-1 was observed by live-imaging of 1-cell stage embryos, in which the nuclear size is much larger than late embryos and localization change can be readily observed. Before mitosis, the intense mCherry::LEO-1 signal was detected within the nuclei. Upon mitotic nuclear envelope breakdown (NEBD), the nuclear mCherry::LEO-1 signal apparently diffused within the cytoplasm (Fig. 5F). During metaphase through anaphase, mCherry::LEO-1 was not detected on condensed chromosomes. In telophase when nuclear envelope is reassembled, mCherry::LEO-1 signal became detectable again in the nuclei (Fig. 5F). These results suggest that the vast majority of LEO-1 is not tightly associated with chromatins, and it is actively transported to the nucleus from the cytoplasm.

Interdependency of the nuclear localization of the PAF1C components

In yeast, it has been shown that the overexpression or depletion of each component of the PAF1C can influence the level of other

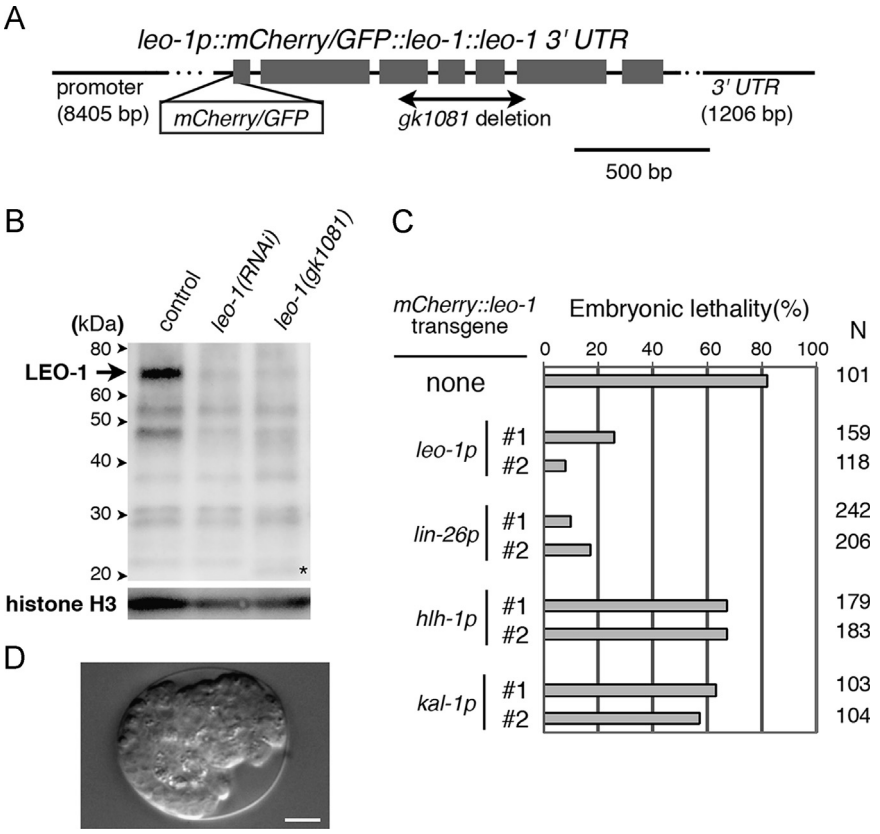

Fig. 4. Analysis of the *leo-1(gk1081)* mutant. (A) Structures of genomic translational mCherry/GFP fusion constructs of *leo-1* and the deleted region of the *gk1081* mutant. (B) Western blotting of the LEO-1 protein. Expression of the LEO-1 protein in young adult worms were detected by rabbit anti-LEO-1 antibody. Extracts from control (lane 1), *leo-1*(*RNAi*) (lane 2) and *leo-1*(*gk1081*) (lane 3) young adult worms were used. Rabbit anti-histone H3 antibody was used as a loading control. The asterisk indicates a faint band that corresponds to the truncated LEO-1 protein in the *gk1081* mutant. (C) Tissue-specific rescue experiments of the *leo-1*(*gk1081*) mutant. mCherry::LEO-1 was expressed in the *gk1081* mutant under the control of the authentic promoter (*leo-1p*), an epidermal specific promoter (*lin-26p*), a muscle specific promoter (*hlh-1p*) and a neuronal cell specific promoter (*kal-1p*). Two independent transgenic lines (♯1 and ♯2) were scored for each transgene construct. *N*: number of animals analyzed. (D) Terminal phenotype of the progeny of *leo-1*(*gkl081*) homozygotes. Scale bar: 10 μm.

components (Mueller et al., 2004; Shi et al., 1997). Therefore, we asked whether the depletion of each PAF1C component affect the protein level or subcellular localization of other components in *C. elegans*. The subcellular localization of PAFO-1::mCherry, mCherry::LEO-1 and GFP::RTFO-1 were analyzed in embryos in which one of the components of the PAF1C was depleted by RNAi (Fig. 6). In *pafo-1*(*RNAi*), *leo-1*(*RNAi*) and *rtfo-1*(*RNAi*) embryos, corresponding fluorescently tagged proteins (PAFO-1::mCherry, mCherry::LEO-1 and GFP::RTFO-1, respectively) became undetectable, confirming the efficiency of RNAi depletion (Fig. 6G, K, and R).

In *ctr-9*(*RNAi*) and *leo-1*(*RNAi*) embryos, nuclear PAFO-1::mCherry was significantly decreased and while cytoplasmic fraction increased (Fig. 6D and J). In *cdc-73*(*RNAi*) embryos, PAFO-1::mCherry was evenly detected in cytoplasm and nuclei (Fig. 6M). In contrast, in *rtfo-1*(*RNAi*) embryos, nuclear localization of PAFO-1::mCherry was unaffected (Fig. 6P). Similarly, in *ctr-9*(*RNAi*) and *pafo-1*(*RNAi*) embryos, mCherry::LEO-1 was mainly detected in the cytoplasm, but not in the nuclei (Fig. 6E and H). In *cdc-73*(*RNAi*) embryos, nuclear mCherry::LEO-1 was decreased and it was detected evenly both in cytoplasm and nuclei (Fig. 6N). As seen for PAFO-1::mCherry, the nuclear localization of mCherry::LEO-1 was unaffected in *rtfo-1*(*RNAi*) embryos (Fig. 6Q). These results suggest that PAFO-1 and LEO-1 localize to the nuclei independently of RTFO-1, but dependent on other three components.

Unlike mCherry::LEO-1 and PAFO-1::Cherry, nuclear localization of GFP::RTFO-1 was unaffected in *leo-1*(*RNAi*), *pafo-1*(*RNAi*), *ctr-9*(*RNAi*) and *cdc-73*(*RNAi*) embryos (Fig. 6F, I, L, and O). These results suggest that RTFO-1 localizes in nuclei independently of other components of the PAF1C.

Taken together, we speculate that four components of the PAF1C except RTFO-1 form a subcomplex to be transported to nuclei in an interdependent manner; on the other hand, RTFO-1 can localize to nuclei independently of other components (Fig. 6S).

Discussion

The PAF1C is an evolutionarily conserved protein complex that consists of Paf1, Ctr9, Rtf1, Cdc73 and Leo1 (Mueller and Jaehning, 2002; Mueller et al., 2004). The studies mainly in yeasts and mammalian culture cells demonstrated that the PAF1C is involved in multiple aspects of RNA pol II transcriptional regulation and histone modifications (Jaehning, 2010; Tomson and Arndt, 2013). Whereas the PAF1C is dispensable for yeast (Chang et al., 1999; Porter et al., 2002; Shi et al., 1996, 1997), recent studies revealed that it is required in diverse biological processes including stem cell pluripotency, cell cycle regulation, and development (Ding et al., 2009; Mosimann et al., 2009; Ponnusamy et al., 2009; Porter et al., 2002; Tenney et al., 2006). In this study, we performed the first functional analysis of the PAF1C in the development of *C. elegans*, and demonstrated its requirement in various processes of epidermal morphogenesis, including epidermal cell positioning, ventral enclosure and epidermal elongation.

While components of the PAF1C were present in the majority of cells throughout *C. elegans* embryogenesis, its expression in epidermal cells was crucial for the viability of this animal. While epidermal cell

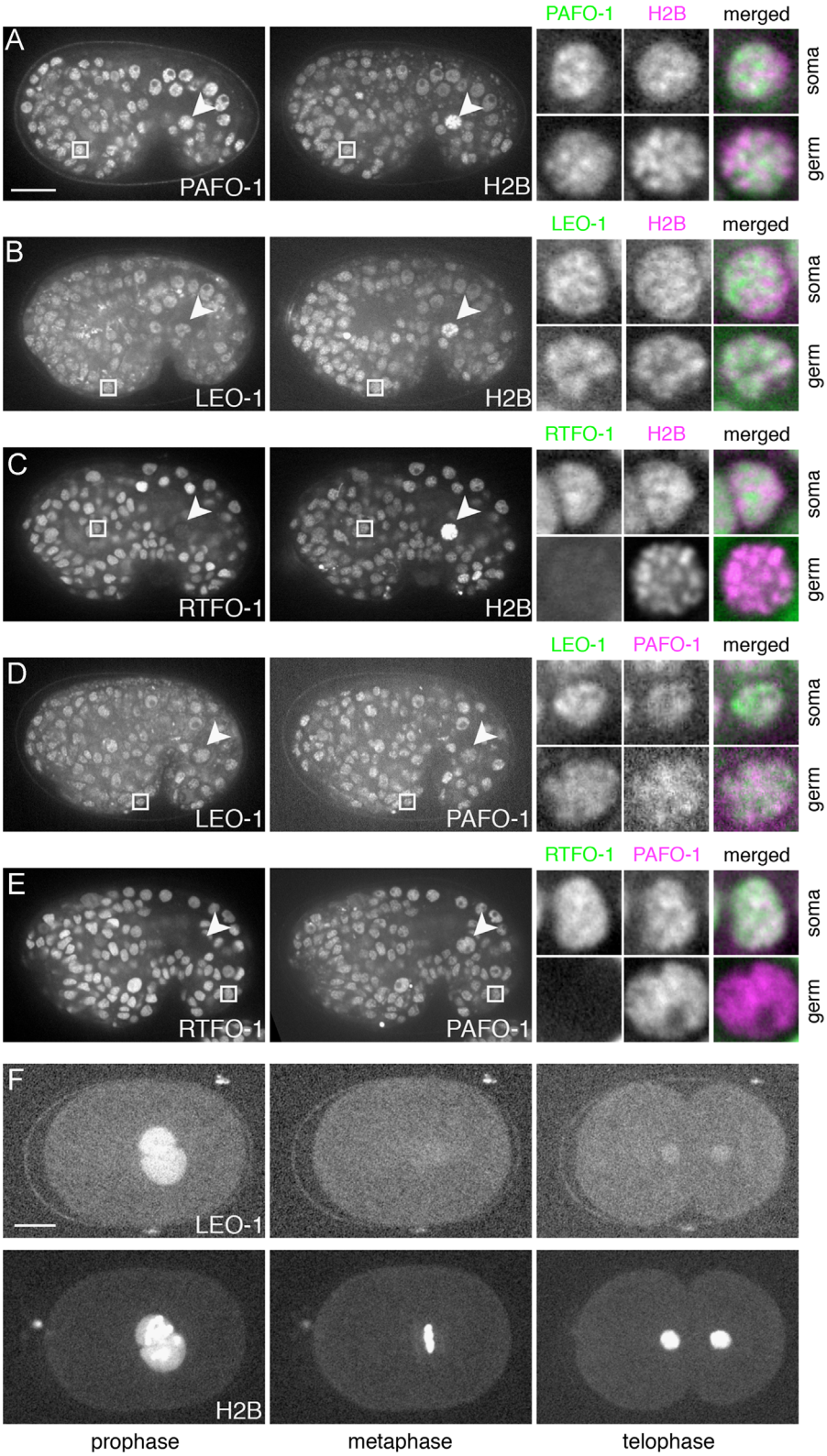

Fig. 5. Nuclear localization of PAFO-1::mCherry, mCherry/GFP::LEO-1 and GFP::RTFO-1 during embryogenesis. Live fluorescent images of the integrated transgenic worms that express mCherry or GFP-tagged PAF1C components under the control of authentic promoters. Comma stage embryos coexpressing GFP- and mCherry-tagged proteins: (A) PAFO-1::mCherry and GFP::histone H2B, (B) mCherry::LEO-1 and GFP::histone H2B, (C) GFP::RTFO-1 and mCherry::histone H2B, (D) GFP::LEO-1 and PAFO-1::mCherry, and (E) GFP::RTFO-1 and PAFO-1::mCherry. Magnified images of single somatic nuclei (boxed area) and single germ cells (arrowheads) are shown in the right panels. (F) Time series images of mCherry::LEO-1 (top) and GFP::histone H2B (bottom) during the first cell division. Prophase (left), metaphase (middle) and telophase (right). Scale bar: 10 μm.

fate determination was not apparently inhibited by the loss of the PAF1C components, circumferentially oriented microtubules in the dorsal and ventral epidermal cells during epidermal morphogenesis (Priess and Hirsh, 1986) were disorganized in *ctr-9*(*RNAi*) embryos. Thus, one possibility of the role of the PAF1C might be modulating expression of microtubule regulators by controlling RNA pol II

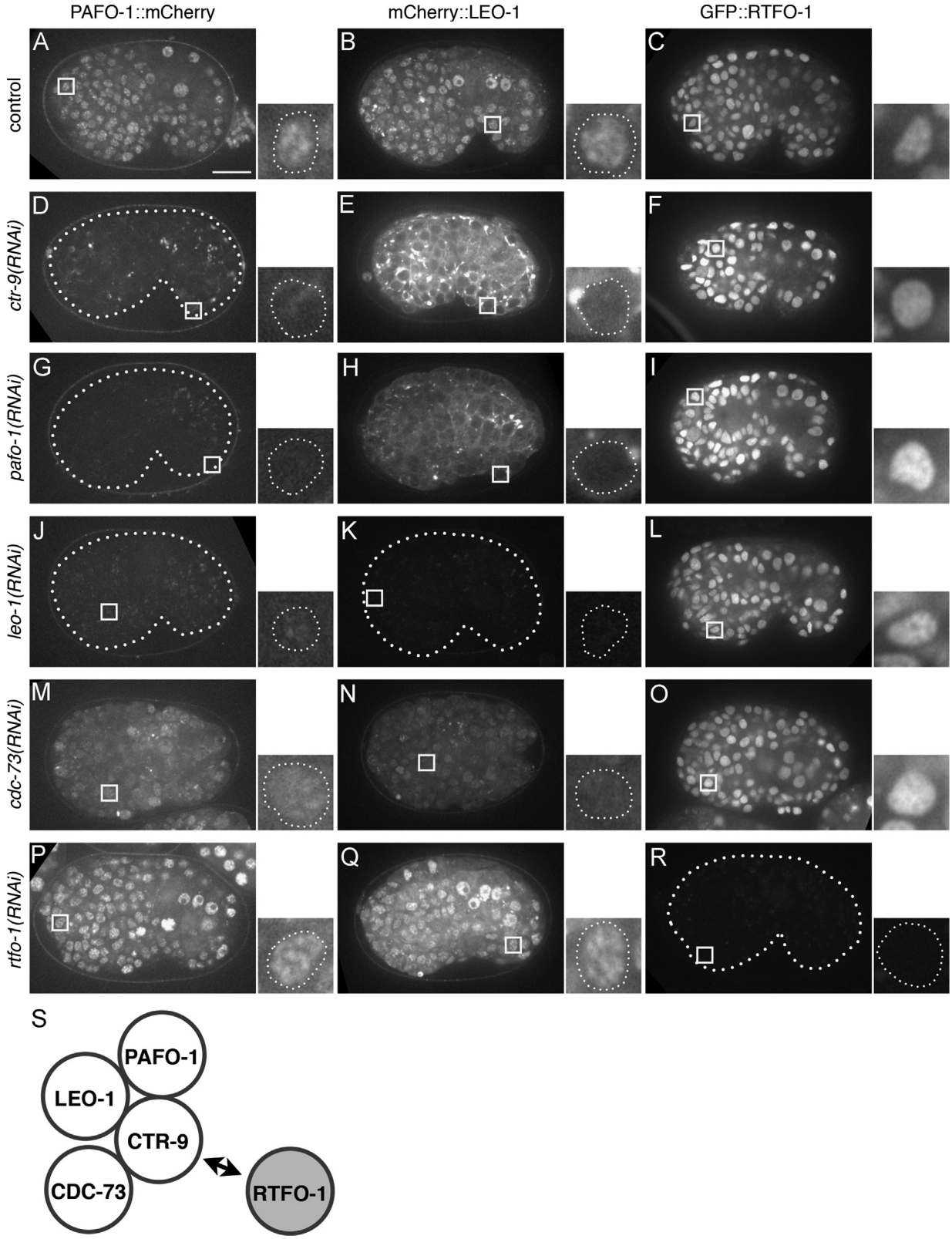

Fig. 6. Interdependency of nuclear localization of the PAF1C components. Fluorescent live images of the comma-stage embryos: PAFO-1::mCherry (A, D, G, J, M, and P), mCherry::LEO-1 (B, E, H, K, N, and Q) and GFP::RTFO-1 (C, F, I, L, O, and R). Control (A–C), *ctr-9*(*RNAi*) (D–F), *pafo-1*(*RNAi*) (G–I), *leo-1*(*RNAi*) (J–L), *cdc-73*(*RNAi*) (M–O) and *rtfo-1*(*RNAi*) (P–R). Magnified images of the boxed areas are shown in the right panels. Images of control and RNAi embryos were captured and processed with the same condition. Scale bar: 10 μm. (S) A model of the interaction between the PAF1C components. Four components of the PAF1C, CTR-9, PAFO-1, LEO-1 and CDC-73, make a subcomplex and localize to nucleus in an interdependent manner. RTFO-1 localizes to nucleus independently of other components.

transcription or by affecting histone modifications. It remains unclear whether the microtubule defects at elongation stage are causative or a result of earlier defects in epidermal cell positioning.

We showed in *C. elegans* late embryos that PAFO-1, LEO-1 and RTFO-1 tagged with GFP or mCherry were unevenly localized in the nuclei of a wide range of cells. This finding is consistent with the

report in other organisms that the PAF1C localizes at transcriptionally active chromatin loci (Adelman et al., 2006; Chen et al., 2009; Kim et al., 2010; Rozenblatt-Rosen et al., 2009). For example, in *Drosophila* salivary glands, Paf1 and Rtf1 are colocalized with RNA pol II at actively transcribed loci on chromatins (Adelman et al., 2006). On the other hand, the vast majority of nuclear PAF1C were not tightly associated with chromatins at least in early *C. elegans* embryos, which may correlate to the fact that transcription is globally repressed in early *C. elegans* embryos (Edgar et al., 1994).

The nuclear localization of PAFO-1 and LEO-1 was dependent on other PAF1C components except RTFO-1. On the other hand, RTFO-1 localized to nuclei independently of other PAF1C components. In addition, whereas PAFO-1 and LEO-1 were expressed in virtually all cells from the 1-cell stage, RTFO-1 was not expressed before ~30 cells and in germline cells. Thus, RTFO-1 and other four components apparently behave differently during embryogenesis. We speculate that nuclear localization of PAFO-1, LEO-1, CTR-9 and CDC-73 are interdependent, probably by forming a subcomplex. CDC-73 contains a nuclear localization signal that is conserved in Cdc73 orthologs in other organisms (Bradley et al., 2007; Hahn and Marsh, 2005), which may contribute to the nuclear localization of the potential subcomplex. RTFO-1 is likely to be independently transported to nuclei, then forms the complete PAF1C by associating with the subcomplex, which promotes epidermal morphogenesis in *C. elegans*.

Our observations of the distinct behavior of RTFO-1 from other four PAF1C components are consistent with the findings in other organisms (Adelman et al., 2006; Kim et al., 2010; Langenbacher et al., 2011; Rozenblatt-Rosen et al., 2005). In *Drosophila*, recruitment of Paf1 and Rtf1 to transcriptionally activated Hsp70 loci is independently controlled (Adelman et al., 2006). Biochemical analysis showed that hRtf1 was not included in the same fraction as that of hCdc73/hPaf1 in human cells (Rozenblatt-Rosen et al., 2005). Furthermore, in human cells, it was reported that some PAF1 complexes contain hSki8 instead of hRtf1 (Ponnusamy et al., 2009; Zhu et al., 2005b). Thus, in some *C. elegans* cells, such as the early embryonic cells before ~30 cell stage or in germline cells in which RTFO-1 is undetected, it is plausible the PAF1 subcomplex composed of PAFO-1, LEO-1, CTF-9 and CDC73 may function independently of RTFO-1, possibly by associating other factor(s).

Our study also demonstrated that the loss of each component of the PAF1C caused similar epidermal morphogenesis phenotypes, but their severity and penetrance were different for each gene. Interestingly, differential phenotypes by the loss of the each PAFC component have also been reported in zebrafish in which the PAF1C is involved in cardiac specification and heart morphogenesis (Nguyen et al., 2010). Phenotypes caused by the knockdown of individual component of the PAF1C were overlapping but distinct, and loss of Rtf1 function resulted in the most severe defects in the specification of cardiac precursors (Langenbacher et al., 2011). Our results and the report in zebrafish raise the possibility that, while all PAF1C components are required for the PAF1C function, each component may have distinct roles in regulating gene expression, possibly by affecting the stability of the complex or specific protein-protein/protein-DNA/RNA interactions. Alternatively, these proteins may have additional function(s) unrelated to the PAF1C. Further studies on the role of each component and that of the PAF1C as a whole will be needed to understand how these proteins participate in the regulation of gene expression during development.

Acknowledgments

We thank Y. Kohara for kindly providing cDNA clones, and members of the Sugimoto's lab for discussions. We thank Aya Katsuyama and Hiroko Sugawara for technical assistance. Some of the worm strains used in this study were provided by the *Caenorhabditis* Genetics Center, which is funded by the USA National Institutes of Health (NIH) National Center for Research Resources, the *C. elegans* Gene Knockout Consortium and the National Bioresource Project in Japan lead by S. Mitani. This work was supported by the NEXT Program LS006 from the Cabinet Office, the Government of Japan to A.S., by JSPS KAKENHI 23657139 to Y.K., and by Narishige Zoological Science Award to Y.K.

Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ydbio.2014.04.002.

References

Adelman, K., Wei, W., Ardehali, M.B., Werner, J., Zhu, B., Reinberg, D., Lis, J.T., 2006. Drosophila Paf1 modulates chromatin structure at actively transcribed genes. Mol. Cell. Biol. 26, 250–260.

Akanuma, T., Koshida, S., Kawamura, A., Kishimoto, Y., Takada, S., 2007. Paf1 complex homologues are required for Notch-regulated transcription during somite segmentation. EMBO Rep. 8, 858–863.

Bai, X., Kim, J., Yang, Z., Jurynec, M.J., Akie, T.E., Lee, J., LeBlanc, J., Sessa, A., Jiang, H., DiBiase, A., Zhou, Y., Grunwald, D.J., Lin, S., Cantor, A.B., Orkin, S.H., Zon, L.I., 2010. TIF1gamma controls erythroid cell fate by regulating transcription elongation. Cell 142, 133–143.

Bradley, K.J., Bowl, M.R., Williams, S.E., Ahmad, B.N., Partridge, C.J., Patmanidi, A.L., Kennedy, A.M., Loh, N.Y., Thakker, R.V., 2007. Parafibromin is a nuclear protein with a functional monopartite nuclear localization signal. Oncogene 26, 1213–1221.

Brenner, S., 1974. The genetics of *Caenorhabditis elegans*. Genetics 77, 71–94.

Carpten, J.D., Robbins, C.M., Villablanca, A., Forsberg, L., Presciuttini, S., Bailey-Wilson, J., Simonds, W.F., Gillanders, E.M., Kennedy, A.M., Chen, J.D., Agarwal, S.K., Sood, R., Jones, M.P., Moses, T.Y., Haven, C., Petillo, D., Leotlela, P.D., Harding, B., Cameron, D., Pannett, A.A., Hoog, A., Heath 3rd, H., James-Newton, L.A., Robinson, B., Zarbo, R.J., Cavaco, B.M., Wassif, W., Perrier, N.D., Rosen, I.B., Kristoffersson, U., Turnpenny, P.D., Farnebo, L.O., Besser, G.M., Jackson, C.E., Morreau, H., Trent, J.M., Thakker, R.V., Marx, S.J., Teh, B.T., Larsson, C., Hobbs, M.R., 2002. HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. Nat. Genet. 32, 676–680.

Cassata, G., Shemer, G., Morandi, P., Donhauser, R., Podbilewicz, B., Baumeister, R., 2005. ceh-16/engrailed patterns the embryonic epidermis of *Caenorhabditis elegans*. Development 132, 739–749.

Chang, M., French-Cornay, D., Fan, H.Y., Klein, H., Denis, C.L., Jaehning, J.A., 1999. A complex containing RNA polymerase II, Paf1p, Cdc73p, Hpr1p, and Ccr4p plays a role in protein kinase C signaling. Mol. Cell. Biol. 19, 1056–1067.

Chen, Y., Yamaguchi, Y., Tsugeno, Y., Yamamoto, J., Yamada, T., Nakamura, M., Hisatake, K., Handa, H., 2009. DSIF, the Paf1 complex, and Tat-SF1 have nonredundant, cooperative roles in RNA polymerase II elongation. Genes Dev. 23, 2765–2777.

Chin-Sang, I.D., Chisholm, A.D., 2000. Form of the worm: genetics of epidermal morphogenesis in *C. elegans*. Trends Genet. 16, 544–551.

Chu, X., Qin, X., Xu, H., Li, L., Wang, Z., Li, F., Xie, X., Zhou, H., Shen, Y., Long, J., 2013. Structural insights into Paf1 complex assembly and histone binding. Nucleic Acids Res. 41, 10619–10629, http://dx.doi.org/10.1093/nar/gkt819.

Ding, L., Paszkowski-Rogacz, M., Nitzsche, A., Slabicki, M.M., Heninger, A.K., de Vries, I., Kittler, R., Junqueira, M., Shevchenko, A., Schulz, H., Hubner, N., Doss, M.X., Sachinidis, A., Hescheler, J., Iacone, R., Anastassiadis, K., Stewart, A.F., Pisabarro, M.T., Caldarelli, A., Poser, I., Theis, M., Buchholz, F., 2009. A genome-scale RNAi screen for Oct4 modulators defines a role of the Paf1 complex for embryonic stem cell identity. Cell Stem Cell 4, 403–415.

Diogon, M., Wissler, F., Quintin, S., Nagamatsu, Y., Sookhareea, S., Landmann, F., Hutter, H., Vitale, N., Labouesse, M., 2007. The RhoGAP RGA-2 and LET-502/ROCK achieve a balance of actomyosin-dependent forces in *C. elegans* epidermis to control morphogenesis. Development 134, 2469–2479.

Edgar, L.G., Wolf, N., Wood, W.B., 1994. Early transcription in *Caenorhabditis elegans* embryos. Development 120, 443–451.

Firestein, B.L., Rongo, C., 2001. DLG-1 is a MAGUK similar to SAP97 and is required for adherens junction formation. Mol. Biol. Cell 12, 3465–3475.

Gally, C., Wissler, F., Zahreddine, H., Quintin, S., Landmann, F., Labouesse, M., 2009. Myosin II regulation during *C. elegans* embryonic elongation: LET-502/ROCK, MRCK-1 and PAK-1, three kinases with different roles. Development 136, 3109–3119.

Hahn, M.A., Marsh, D.J., 2005. Identification of a functional bipartite nuclear localization signal in the tumor suppressor parafibromin. Oncogene 24, 6241–6248.

He, Y., Doyle, M.R., Amasino, R.M., 2004. PAF1-complex-mediated histone methylation of FLOWERING LOCUS C chromatin is required for the vernalization-responsive, winter-annual habit in *Arabidopsis*. Genes Dev. 18, 2774–2784.

Jaehning, J.A., 2010. The Paf1 complex: platform or player in RNA polymerase II transcription? Biochim. Biophys. Acta 1799, 379–388.

Kim, J., Guermah, M., Roeder, R.G., 2010. The human PAF1 complex acts in chromatin transcription elongation both independently and cooperatively with SII/TFIIS. Cell 140, 491–503.
Kim, K.Y., Levin, D.E., 2011. Mpk1 MAPK association with the Paf1 complex blocks Sen1-mediated premature transcription termination. Cell 144, 745–756.
Kostas, S.A., Fire, A., 2002. The T-box factor MLS-1 acts as a molecular switch during specification of nonstriated muscle in *C. elegans*. Genes Dev. 16, 257–269.
Labouesse, M., 2012. Role of the extracellular matrix in epithelial morphogenesis: a view from *C. elegans*. Organogenesis 8, 65–70.
Landmann, F., Quintin, S., Labouesse, M., 2004. Multiple regulatory elements with spatially and temporally distinct activities control the expression of the epithelial differentiation gene lin-26 in *C. elegans*. Dev. Biol. 265, 478–490.
Langenbacher, A.D., Nguyen, C.T., Cavanaugh, A.M., Huang, J., Lu, F., Chen, J.N., 2011. The PAF1 complex differentially regulates cardiomyocyte specification. Dev. Biol. 353, 19–28.
Lin, L., Zhang, J.H., Panicker, L.M., Simonds, W.F., 2008. The parafibromin tumor suppressor protein inhibits cell proliferation by repression of the c-myc proto-oncogene. Proc. Natl. Acad. Sci. U.S.A. 105, 17420–17425.
Lynch, A.M., Hardin, J., 2009. The assembly and maintenance of epithelial junctions in *C. elegans*. Front. Biosci. 14, 1414–1432.
Maduro, M., Pilgrim, D., 1995. Identification and cloning of unc-119, a gene expressed in the *Caenorhabditis elegans* nervous system. Genetics 141, 977–988.
Maduro, M., Pilgrim, D., 1996. Conservation of function and expression of unc-119 from two *Caenorhabditis* species despite divergence of non-coding DNA. Gene 183, 77–85.
Maeda, I., Kohara, Y., Yamamoto, M., Sugimoto, A., 2001. Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. Curr. Biol. 11, 171–176.
Magdolen, V., Lang, P., Mages, G., Hermann, H., Bandlow, W., 1994. The gene LEO1 on yeast chromosome XV encodes a non-essential, extremely hydrophilic protein. Biochim. Biophys. Acta 1218, 205–209.
Mello, C.C., Kramer, J.M., Stinchcomb, D., Ambros, V., 1991. Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J. 10, 3959–3970.
Michaux, G., Legouis, R., Labouesse, M., 2001. Epithelial biology: lessons from *Caenorhabditis elegans*. Gene 277, 83–100.
Moniaux, N., Nemos, C., Schmied, B.M., Chauhan, S.C., Deb, S., Morikane, K., Choudhury, A., Vanlith, M., Sutherlin, M., Sikela, J.M., Hollingsworth, M.A., Batra, S.K., 2006. The human homologue of the RNA polymerase II-associated factor 1 (hPaf1), localized on the 19q13 amplicon, is associated with tumorigenesis. Oncogene 25, 3247–3257.
Mosimann, C., Hausmann, G., Basler, K., 2006. Parafibromin/Hyrax activates Wnt/Wg target gene transcription by direct association with beta-catenin/Armadillo. Cell 125, 327–341.
Mosimann, C., Hausmann, G., Basler, K., 2009. The role of Parafibromin/Hyrax as a nuclear Gli/Ci-interacting protein in Hedgehog target gene control. Mech. Dev. 126, 394–405.
Mueller, C.L., Jaehning, J.A., 2002. Ctr9, Rtf1, and Leo1 are components of the Paf1/RNA polymerase II complex. Mol. Cell. Biol. 22, 1971–1980.
Mueller, C.L., Porter, S.E., Hoffman, M.G., Jaehning, J.A., 2004. The Paf1 complex has functions independent of actively transcribing RNA polymerase II. Mol. Cell 14, 447–456.
Nguyen, C.T., Langenbacher, A., Hsieh, M., Chen, J.N., 2010. The PAF1 complex component Leo1 is essential for cardiac and neural crest development in zebrafish. Dev. Biol. 341, 167–175.
Oh, S., Zhang, H., Ludwig, P., van Nocker, S., 2004. A mechanism related to the yeast transcriptional regulator Paf1c is required for expression of the *Arabidopsis* FLC/MAF MADS box gene family. Plant Cell 16, 2940–2953.
Patel, F.B., Bernadskaya, Y.Y., Chen, E., Jobanputra, A., Pooladi, Z., Freeman, K.L., Gally, C., Mohler, W.A., Soto, M.C., 2008. The WAVE/SCAR complex promotes polarized cell movements and actin enrichment in epithelia during *C. elegans* embryogenesis. Dev. Biol. 324, 297–309.
Piekny, A.J., Johnson, J.L., Cham, G.D., Mains, P.E., 2003. The *Caenorhabditis elegans* nonmuscle myosin genes nmy-1 and nmy-2 function as redundant components of the let-502/Rho-binding kinase and mel-11/myosin phosphatase pathway during embryonic morphogenesis. Development 130, 5695–5704.
Ponnusamy, M.P., Deb, S., Dey, P., Chakraborty, S., Rachagani, S., Senapati, S., Batra, S.K., 2009. RNA polymerase II associated factor 1/PD2 maintains self-renewal by its interaction with Oct3/4 in mouse embryonic stem cells. Stem Cells 27, 3001–3011.
Porter, S.E., Washburn, T.M., Chang, M., Jaehning, J.A., 2002. The yeast pafl-rNA polymerase II complex is required for full expression of a subset of cell cycle-regulated genes. Eukaryot. Cell 1, 830–842.
Praitis, V., Casey, E., Collar, D., Austin, J., 2001. Creation of low-copy integrated transgenic lines in *Caenorhabditis elegans*. Genetics 157, 1217–1226.
Priess, J.R., Hirsh, D.I., 1986. *Caenorhabditis elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. Dev. Biol. 117, 156–173.
Rozenblatt-Rosen, O., Hughes, C.M., Nannepaga, S.J., Shanmugam, K.S., Copeland, T.D., Guszczynski, T., Resau, J.H., Meyerson, M., 2005. The parafibromin tumor suppressor protein is part of a human Paf1 complex. Mol. Cell. Biol. 25, 612–620.
Rozenblatt-Rosen, O., Nagaike, T., Francis, J.M., Kaneko, S., Glatt, K.A., Hughes, C.M., LaFramboise, T., Manley, J.L., Meyerson, M., 2009. The tumor suppressor Cdc73 functionally associates with CPSF and CstF 3′ mRNA processing factors. Proc. Natl. Acad. Sci. U.S.A. 106, 755–760.
Shi, X., Chang, M., Wolf, A.J., Chang, C.H., Frazer-Abel, A.A., Wade, P.A., Burton, Z.F., Jaehning, J.A., 1997. Cdc73p and Paf1p are found in a novel RNA polymerase II-containing complex distinct from the Srbp-containing holoenzyme. Mol. Cell. Biol. 17, 1160–1169.
Shi, X., Finkelstein, A., Wolf, A.J., Wade, P.A., Burton, Z.F., Jaehning, J.A., 1996. Paf1p, an RNA polymerase II-associated factor in *Saccharomyces cerevisiae*, may have both positive and negative roles in transcription. Mol. Cell. Biol. 16, 669–676.
Simske, J.S., Hardin, J., 2001. Getting into shape: epidermal morphogenesis in *Caenorhabditis elegans* embryos. Bioessays 23, 12–23.
Sulston, J.E., Schierenberg, E., White, J.G., Thomson, J.N., 1983. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. 100, 64–119.
Tenney, K., Gerber, M., Ilvarsonn, A., Schneider, J., Gause, M., Dorsett, D., Eissenberg, J.C., Shilatifard, A., 2006. Drosophila Rtf1 functions in histone methylation, gene expression, and Notch signaling. Proc. Natl. Acad. Sci. U.S.A. 103, 11970–11974.
Tomson, B.N., Arndt, K.M., 2013. The many roles of the conserved eukaryotic Paf1 complex in regulating transcription, histone modifications, and disease states. Biochim. Biophys. Acta 1829, 116–126.
Totong, R., Achilleos, A., Nance, J., 2007. PAR-6 is required for junction formation but not apicobasal polarization in *C. elegans* embryonic epithelial cells. Development 134, 1259–1268.
Toya, M., Iida, Y., Sugimoto, A., 2010. Imaging of mitotic spindle dynamics in *Caenorhabditis elegans* embryos. Methods Cell Biol. 97, 359–372.
Toya, M., Terasawa, M., Nagata, K., Iida, Y., Sugimoto, A., 2011. A kinase-independent role for Aurora A in the assembly of mitotic spindle microtubules in *Caenorhabditis elegans* embryos. Nat. Cell Biol. 13, 708–714.
Wade, P.A., Werel, W., Fentzke, R.C., Thompson, N.E., Leykam, J.F., Burgess, R.R., Jaehning, J.A., Burton, Z.F., 1996. A novel collection of accessory factors associated with yeast RNA polymerase II. Protein Expression Purif. 8, 85–90.
Williams-Masson, E.M., Heid, P.J., Lavin, C.A., Hardin, J., 1998. The cellular mechanism of epithelial rearrangement during morphogenesis of the *Caenorhabditis elegans* dorsal hypodermis. Dev. Biol. 204, 263–276.
Williams-Masson, E.M., Malik, A.N., Hardin, J., 1997. An actin-mediated two-step mechanism is required for ventral enclosure of the *C. elegans* hypodermis. Development 124, 2889–2901.
Wissmann, A., Ingles, J., McGhee, J.D., Mains, P.E., 1997. *Caenorhabditis elegans* LET-502 is related to Rho-binding kinases and human myotonic dystrophy kinase and interacts genetically with a homolog of the regulatory subunit of smooth muscle myosin phosphatase to affect cell shape. Genes Dev. 11, 409–422.
Yart, A., Gstaiger, M., Wirbelauer, C., Pecnik, M., Anastasiou, D., Hess, D., Krek, W., 2005. The HRPT2 tumor suppressor gene product parafibromin associates with human PAF1 and RNA polymerase II. Mol. Cell. Biol. 25, 5052–5060.
Yochem, J., Gu, T., Han, M., 1998. A new marker for mosaic analysis in *Caenorhabditis elegans* indicates a fusion between hyp6 and hyp7, two major components of the hypodermis. Genetics 149, 1323–1334.
Zhang, C., Mejia, L.A., Huang, J., Valnegri, P., Bennett, E.J., Anckar, J., Jahani-Asl, A., Gallardo, G., Ikeuchi, Y., Yamada, T., Rudnicki, M., Harper, J.W., Bonni, A., 2013a. The X-linked intellectual disability protein PHF6 associates with the PAF1 complex and regulates neuronal migration in the mammalian brain. Neuron 78, 986–993.
Zhang, H., Labouesse, M., 2012. Signalling through mechanical inputs: a coordinated process. J. Cell Sci. 125, 3039–3049.
Zhang, K., Haversat, J.M., Mager, J., 2013b. CTR9/PAF1c regulates molecular lineage identity, histone H3K36 trimethylation and genomic imprinting during preimplantation development. Dev. Biol. 383, 15–27.
Zhu, B., Mandal, S.S., Pham, A.D., Zheng, Y., Erdjument-Bromage, H., Batra, S.K., Tempst, P., Reinberg, D., 2005a. The human PAF complex coordinates transcription with events downstream of RNA synthesis. Genes Dev. 19, 1668–1673.
Zhu, B., Zheng, Y., Pham, A.D., Mandal, S.S., Erdjument-Bromage, H., Tempst, P., Reinberg, D., 2005b. Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. Mol. Cell 20, 601–611.