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Original Article

Transgene-mediated co-suppression of DNA topoisomerase-1 gene in *Caenorhabditis elegans*

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Abstract: Ectopic expression of multi-transgenic copies can result in reduced expression of the transgene and can induce silence of endogenous gene; this process is called as co-suppression. Using a transgene-mediated co-suppression technique, we demonstrated the biological function of DNA topoisomerase-1 (*top-1*) in *C. elegans* development. Introduction of full-length *top-1* transgene sufficiently induced the co-suppression of endogenous *top-1* gene, causing embryonic lethality and abnormal germline development. We also found that the co-suppression of *top-1* gene affected morphogenesis, lifespan and larval growth that were not observed in *top-1(RNAi)* animals. Strikingly, co-suppression effects were significantly reduced by the elimination of *top-1* introns, suggesting that efficient co-suppression may require intron(s) in *C. elegans*. Sequence analysis revealed that the introns 1 and 2 of *top-1* gene possess consensus binding sites for several transcription factors, including MAB-3, LIN-14, TTX-3/CEH-10, CEH-1, and CEH-22. Among them, we examined a genetic link between *ceh-22* and *top-1*. The *ceh-22* is partially required for the specification of distal tip cells (DTC), which functions as a stem cell niche in the *C. elegans* gonad. Intriguingly, *top-1(RNAi)* significantly enhanced DTC loss in *ceh-22* mutant gonads, indicating that *top-1* may play an important role in CEH-22-mediated DTC fate specification. Therefore, our findings suggest that transgene-mediated co-suppression facilitates the silencing of the specific genes and the study of gene function *in vivo*.

Keywords: DNA topoisomerase I, co-suppression, *Caenorhabditis elegans*, morphogenesis, distal tip cells (DTCs), lifespan

Introduction

DNA topoisomerase 1 (TOP-1) is an essential enzyme, which eases the torsional stress involved in DNA replication, transcription and chromosome condensation [1, 2]. TOP-1 protein is also critical for cell proliferation in multicellular organisms [3-5]. TOP-1 is highly relevant in cancer as a number of anti-cancer drugs selectively target DNA TOP-1 [6]. For example, TOP-1 is a specific molecular target for anti-cancer drugs (e.g., Camptothecin), which are used in the treatment of many types of cancers, including ovarian and colorectal cancer [2, 7]. These drugs convert TOP-1 into cellular poisons by trapping the enzyme as it cleaves DNA [8]. Therefore, TOP-1 is considered to be an attractive target for chemotherapy in malignancies.

We previously identified two alternatively spliced products of *C. elegans top-1* (*top-1α* and *top-1β*) [9]. These two isoforms display differential subcellular localization during development: *top-1α* is mostly present in centrosomes, neuronal cells, excretory cells and centrosomes of germ cells, while *top-1β* with missing the second exon is broadly localized to the nuclei of many cells at all developmental stages and is concentrated in nucleoli of embryo gut and oogenic cells [10]. A reporter analysis and immunohistochemistry showed that *top-1* is strongly expressed in the *C. elegans* distal tip cells (DTCs) at the larval stages [10]. This is interesting because *C. elegans* DTC functions as a mesenchymal niche to promote germline stem cells self-renewal [11]. It also leads gonadal migration [12]. Notably, RNA interference (RNAi) targeted to *top-1* has consistently shown

germline proliferation defects and abnormal gonadal migration [10].

We further demonstrate in this report that *top-1* is also required for morphogenesis, stem cell niche (DTC) fate specification as well as normal lifespan and growth control using a transgene-mediated co-suppression. In plants and *Drosophila*, co-suppression can mediate transcriptional gene silencing or post-transcriptional gene silencing mechanism [13]. The transcriptional gene silencing mechanism involves DNA-related processes such as methylation [14, 15] and binding of polycomb-group proteins to co-suppressed genes [16, 17]. In *C. elegans*, RNAi and co-suppression are mediated at least in part by the same molecular machinery, possibly through RNA-guided degradation of mRNAs [18]. Although RNAi and transgene-mediated co-suppression share many key regulators, like *dcr-1* and *rde-2* genes [18], the transgene-mediated co-suppression has several benefits over RNAi-mediated knockdown; *First*, the transgene-mediated co-suppression can be inherited for several generations. *Second*, a somatic selection marker can be used to identify stable co-suppressed transgenic lines. *Third*, stable co-suppressed transgenic lines can be used to identify suppressor or enhancer genes through RNAi screening or classical mutagenesis. Therefore, our results may benefit studies related to the development of new genetic biotechnology and help in understanding the biological function of genes of interest.

Materials and methods

C. elegans culture and strains

All strains were derived from Bristol strain N2 and maintained at 20°C or 25°C as described [19]. The *ceh-22(q632)* mutant [20] was used for this work. The integrated transgene *qIs56 [lag-2::GFP]* was used as a DTC marker.

Embryo isolation

To isolate the embryos, avid worms at mixed stages were lysed in 10 volumes of a 1% NaOCl and 0.5 M NaOH solution, and embryos were precipitated from the lysate by a centrifugation at 140 × g for 1 min. The precipitated embryos were washed three times with an M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 ml 1 M $MgSO_4$, H_2O to 1 liter) and placed on nematode growth medium (NGM) or RNAi plates.

Construction of top-1FL and top-1(ΔInt1&2) plasmid DNAs

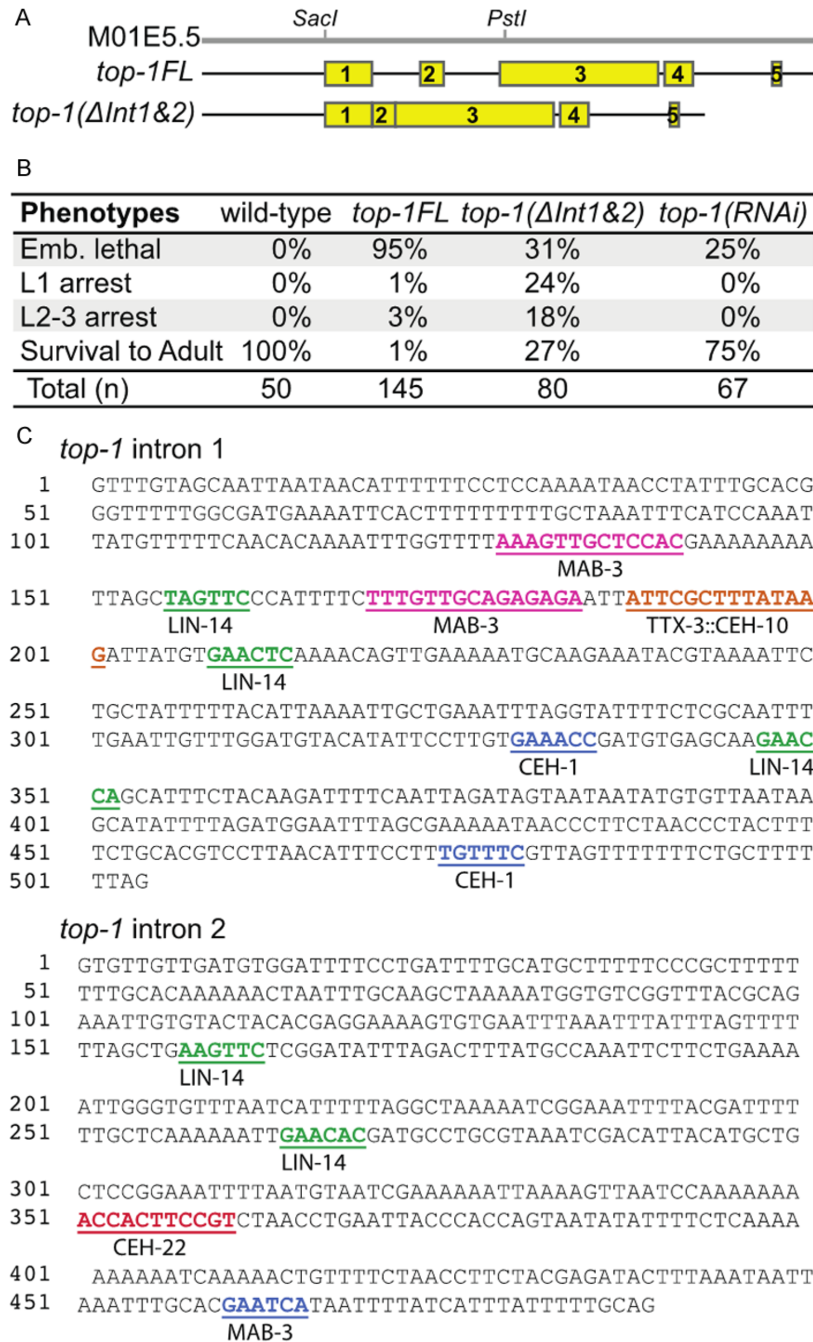
In order to construct a *top-1FL* plasmid DNA, an about 8 kb-long *top-1* full length (FL) DNA fragment was amplified by polymerase chain reaction (PCR) on *C. elegans* genomic DNA using gene-specific primers (nt 17546~17565, GGTACGAATGGAGAATACTG and nt 25576~ 25557 in the sequence of M01E5 genomic cosmid clone, CCTCTCACACTTATGAAATC). The amplified DNA fragment containing the *top-1* genomic DNA from the -3.0 kb upstream of the trans-splicing site to the +0.8 kb downstream of termination codon sequence was cloned into Topo TA cloning vector (Invitrogen) using a standard cloning procedure. For *top-1(ΔInt1&2)* plasmid DNAs, *top-1FL* plasmid DNA was digested with *Sac*I (78 bp downstream sequence from the trans-splicing site) and *PstI* (in the exon 3) restriction enzymes and then replaced with a cDNA fragment containing the exons 1, 2 and 3 (**Figure 1A**). The resulting plasmid DNAs were microinjected into wild-type worms with *pRF4* (*rol-6*) [21] or *csb-1::GFP* [22] as transformation-positive markers. Injected worms were grown at a lower temperature (18°C) due to embryonic lethality at 25°C, and an integrated transgenic line was generated by UV-irradiation (240 nm, 300 J/m^2) [23]. Phenotype of transgenic worms were observed under fluorescence microscope with a differential interference contrast (DIC) optics.

Measurement of embryonic lethality and germline phenotypes

In order to measure embryonic lethality, embryos were collected from wild-type or *top-1FL* transgenic worms and embryonic hatching rate was scored 24 h later at 20°C or 25°C. To determine the co-suppression phenotype of *top-1FL* gene in the *C. elegans* germline, L1 synchronized *top-1FL* transgenic worms were placed on NGM plates containing OP50 bacteria. 3 days later, the germline phenotypes were observed by staining dissected gonads with DAPI.

Antibody staining

Embryo staining was performed as described [24]. After freeze-cracking, embryos on a poly



Phenotypes	wild-type	top-1FL	top-1(ΔInt1&2)	top-1(RNAi)
Emb. lethal	0%	95%	31%	25%
L1 arrest	0%	1%	24%	0%
L2-3 arrest	0%	3%	18%	0%
Survival to Adult	100%	1%	27%	75%
Total (n)	50	145	80	67

Figure 1. Co-suppression effect of *top-1* gene. A: Structure of the *top-1FL* and *top-1(ΔInt1&2)* transgenes. The *top-1FL* transgene includes promoter, exons (yellow), introns, and 3' flanking region. The *top-1(ΔInt1&2)* transgene is removed two introns juxtaposing the second exon. Box, exon; connected line, intron. B: Phenotype analysis. The co-suppression of *top-1* gene copies the phenotype of *top-1(RNAi)* with a high efficiency. C: Prediction of transcription factor binding sites in intron 1 and 2 using ConTra v2 program.

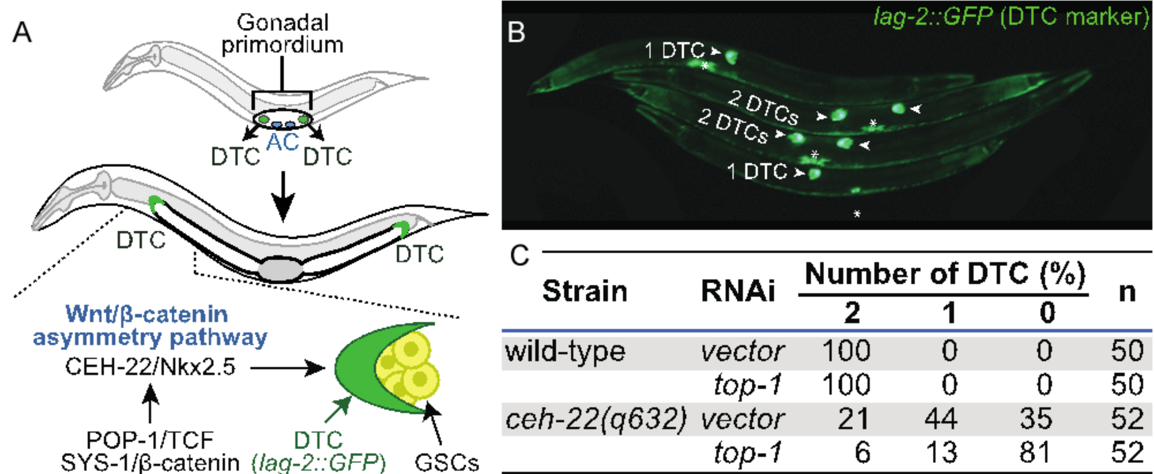
(lysine)-coated glass slide were serially immersed in methanol, acetone, and then methanol at -20°C for 5 min each. After leaving it in methanol at an ambient temperature for 5 min, the specimen was submerged in 90% methanol/H_2O, 70% methanol/PBS, and then in 50% methanol/PBS for 1 min each. After a pre-incubation in 1XPBS/0.5% BSA solution for 1 h, the embryos were incubated at room temperature for 1 h with MH27 mouse monoclonal antibodies (antibody against Jam-1, Developmental Studies Hybridoma Bank). After washing with 1XPBS/0.5% BSA solution three times, the embryos were treated with a secondary antibody (Molecular Probe, Alexa 488, 1:300 dilutions). After staining with DAPI, the embryos were observed under a fluorescence microscope.

Prediction of transcription factor binding sites within top-1 introns

Transcription factor binding sites in the introns of *top-1* gene were searched using a ConTra v2 program (http://bioit.dmbr.ugent.be/contrav2/contradoc.php) as described [25]. ConTra v2 can analyze promoter regions, 5'UTRs (untranslated regions), 3'UTRs, introns or any other genomic region of interest.

RNA interference (RNAi)

RNAi was performed essentially as described [26]. In brief, synchronized L1s were placed on RNAi plates (NGM/Amp/IPTG) seeded with *ceh-22* double stranded RNA (dsRNA)-expressing or



Strain	RNAi	Number of DTC (%)			n
		2	1	0	
wild-type	*vector*	100	0	0	50
	top-1	100	0	0	50
ceh-22(q632)	*vector*	21	44	35	52
	top-1	6	13	81	52

Figure 2. TOP-1 is required for CEH-22-mediated DTC specification. A: Schematic of wild-type DTC specification. Wnt/β-catenin asymmetry pathway, including POP-1/TCF and SYS-1/β-catenin controls the transcription of *ceh-22/Nkx2.5* to specify DTC fate. The DTC maintains germline stem cell (GSC) self-renewal. B: The expression of *lag-2::GFP* transgene in DTCs. Wild-type animals have two DTCs, but some *ceh-22(q632)* mutants fail to generate DTCs. Arrowheads indicate DTCs (*lag-2::GFP*, DTC marker). Asterisk indicates vulva. C: A table quantifying the percentage of DTC loss.

empty vector control bacteria, and were incubated at 25°C.

Lifespan measurement

Adult wild-type and *top-1FL* worms were allowed to lay eggs overnight on separate plates. The adult worms were then removed from the plates to measure the lifespan of their progeny. About one hundred worms were picked at L2 or L3 larval stage and were transferred to new plates at the density of about 25 worms per plate. Dead worms were counted daily after the transfer of live worms to a new plate. The average lifespan and its deviation were calculated using Microsoft Excel program and the measurement was repeated three times.

Results and discussion

Transgene array induces top-1 loss-of-function phenotype

To determine the co-suppression phenotype of *top-1*, we generated a transgenic line carrying the multicopies of a full-length (FL) *top-1* transgene (about 8 kb-long), called *top-1FL* (**Figure 1A**) and an injection marker, *pRF4* plasmid DNA (*rol-6*) [21] or *csb-1::GFP* DNA [22]. Extrachromosomal array was integrated into the *C. elegans* genome using UV irradiation to establish non-mosaic transgenic strains with high transmission rate [27]. Interestingly, the integrated *top-1(FL)* transgenic worms exibited temperature sensitive phenotypes: 58% of transgenic worms (n=496) carrying the multicopies of *top-1FL* transgene died at embryo or early larval stage at permissive temperature (20°C) (not shown). At restrictive temperature (25°C), most transgenic animals showed embryonic lethality or larval arrest (**Figure 1B**), phenocopying *top-1(RNAi)* [10] or loss-of-function *top-1* mutant (not shown). Notably, the co-suppression of *top-1* gene also affected the germline development, including germ cell proliferation, gonadal migration, germ cell survival in the adult at 20°C (Figure S1). The overall phenotypes of *top-1* co-suppression showed much stronger than that of *top-1(RNAi)* [10] (**Figures 1B** and S1). These results suggest that *top-1FL* transgene arrays efficiently induce the co-suppression of *top-1* gene.

The effect of top-1 introns on co-suppression

Transgene-mediated co-suppression depends on the presence of a functional promoter [18]; constructs lacking a promoter cannot induce co-suppression [18]. In this report, we tested whether transgene-mediated co-suppression is also required for functional introns because introns and their removal by the spliceosome can influence gene expression and enhance mRNA metabolism [28]. To do so, we generated

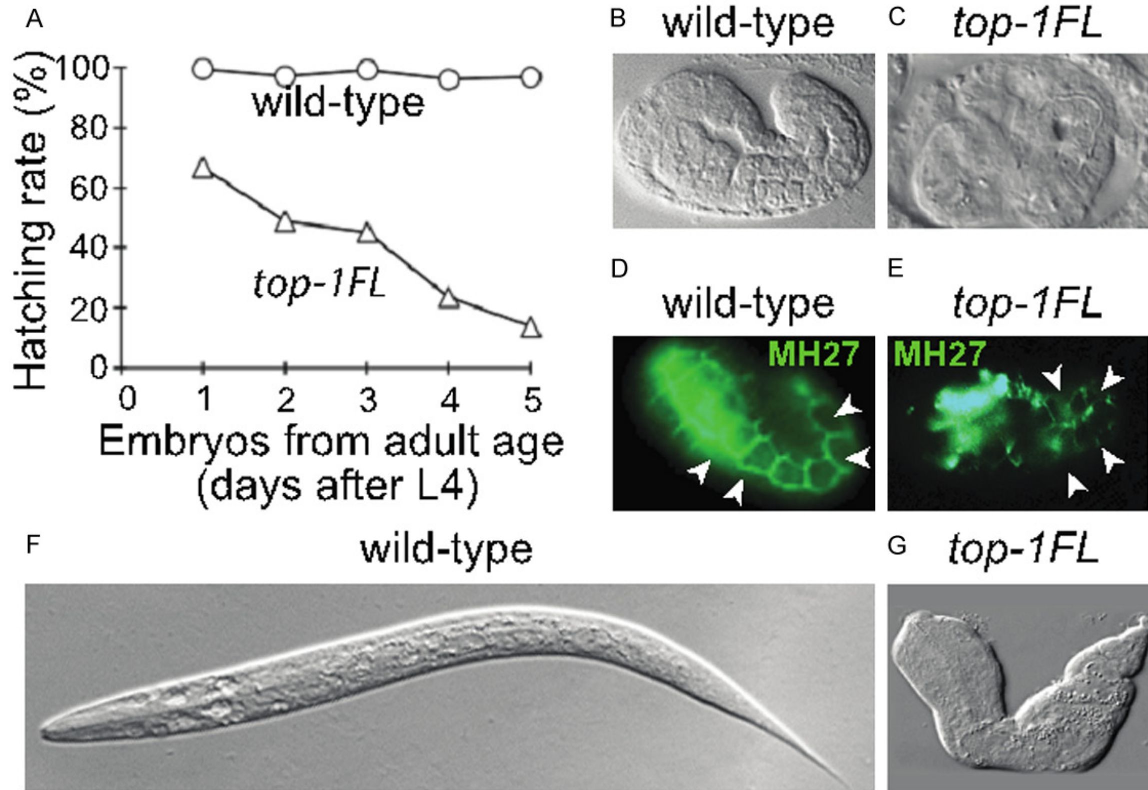


Figure 3. Phenotypes in embryos produced by wild-type and *top-1FL.* (A) Maternal effects on embryonic hatching produced by *top-1FL* co-suppression. *Top-1FL* worms were allowed to lay embryos, which were collected every day, and their hatching rate was measured 15 h later. Each experiment was carried out for 10 worms and repeated three times. (B) Comma-staged wild-type embryo (C) Comma-staged *top-1FL* embryo that has abnormal enclosure, elongation and morphogenesis. The lateral focal planes of comma-staged wild-type (D) and *top-1FL* (E) embryos stained with MH27 monoclonal antibody. (F) L1 staged wild-type. (G) L1 staged *top-1FL* with an abnormal morphology.

a transgenic line carrying a *top-1* transgene missing both intron 1 and 2, called *top-1(ΔInt1&2)* (**Figure 1A**). The majortity of embryos obtained from *top-1FL* transgenic worms at 25°C died at the early embryonic stage. However, embryos from *top-1(ΔInt1&2)* transgenic lines died at a relatively later stage of embryogenesis (31%) or were arrested at early larval stages (24% in L1 and 18% in L2/L3) (**Figure 1B**). This result suggests that transgene-mediated co-suppression may be influenced by the elimination of introns. It is important to know how introns affect the efficiency of the transgene-mediated co-suppression. One of the possible ideas is that introns may regulate the expressional level of transgenes in *C. elegans*. The mechanisms of intron-mediated enhancement of gene expression have been explored in several model systems [29]. For example, the gene expression in *C. elegans* is specifically regulated by first or second intron, which have conserved sequences for transcription factor interaction and are bound by more transcription factors when compared to other introns [30]. We therefore searched for potential transcription factor binding sites in the intron 1 and 2 of *top-1* gene using ConTra v2 program [25]. Interestingly, the program predicted eight consensus binding sites for four transcription factors in the intron 1, and four consensus binding sites for three transcription factors in the intron 2 (**Figures 1C** and S2). Therefore, we speculate that intron may influence the effects of co-suppression probably by regulating the expression levels of a specific gene in *C. elegans*.

In general, the silencing of specific genes can be induced by the introduction of multi-copies of homologous transgenes (called by co-suppression) [31-34] or by the delivery of double stranded RNA to target the specific gene (called by RNAi) [35, 36]. Although gene silencing by co-suppression is technically more difficult

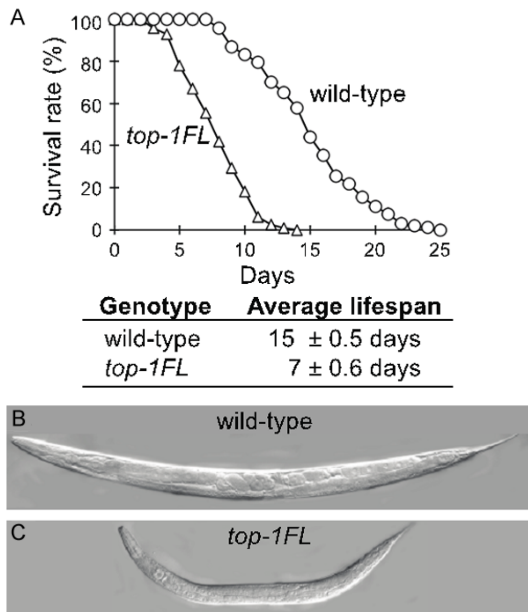


Figure 4. Shortened lifespan and slow growth of *top-1FL* worms. (A) Average lifespan of wild-type and *top-1FL* worms. Over 100 worms were tested in a single set of experiment, and each experiment was repeated three times. Growth rate of wild-type (B) *top-1FL* (C) worms. The growth rate was represented by the relative body length of *top-1FL* worms when wild-type worms grew up to the adult stage (3 days from L1). Growth rate of *top-1FL* worms corresponded to 59% of the wild type growth rate.

than that by RNAi, it provides notable experimental advantages over RNAi, including a reproducible and inheritable outcome, a germline specific functional analysis, and a source for genetic screening. Germline silencing of a specific gene can indeed result in a maternal effect lethal phenotype in the early embryos, causing decreased or absent transmitting lines. In this case, transgenic lines can be obtained by the injection of transgene into *mut-7(pk204)* mutant, which have a defective co-suppression response, and then be crossed out to yield the co-suppression effect [37]. In this report, we demonstrated that the presence or absence of intron could influence the co-suppression effects. Therefore, this genetic manipulation of intron may provide an additional experimental advantage for understanding the mechanism of co-suppression and the biological function of essential gene *in vivo*.

The effect of top-1 on CEH-22-mediated DTC specification

In *C. elegans*, the distal tip cell (DTC) acts as a mesenchymal niche that maintains germline stem cells [38]. The *C. elegans* DTC fate is specified by Wnt/β-catenin signaling [39] (**Figure 2A**). One of the downstream targets of this signaling is *ceh-22* (a homeodomain transcription factor), which is required for DTC fate specification. Wild-type hermaphrodites normally have two DTCs, but 79% of *ceh-22* mutants often cause DTC loss (**Figure 2B** and **2C**). Since *top-1* is expressed in the *C. elegans* DTCs [10] and *top-1* intron 2 possesses a consensus binding site for the CEH-22 transcription factor (**Figures 1C** and S2), we examined whether *top-1* is genetically linked to *ceh-22* in the DTC fate specification. The depletion of *top-1* by RNAi did not affect DTC specification (**Figure 2C**) in wild-type hermaphrodites, but it significantly enhanced DTC loss and a consequent loss of germline stem cells in *ceh-22(q632)* mutants (**Figure 2C**). This result suggests that *top-1* may be linked genetically with *ceh-22* in the DTC specification. How does *top-1* control DTC fate specification in *ceh-22* mutant? One of the possible mechanisms is that *top-1* may control proper cell division timing of somatic gonadal precursor (SGP), which divides to generate one daughter with DTC potential and one daughter with non-DTC potential [11]. Similarly, *C. elegans* CYD-1/Cyclin D regulating G1/S phase transition, specifies DTC fate by regulating the cell divion timing of SGP [40]. Therefore, we speculate that a delayed cell division of SGP in *top-1(RNAi)* may contribute to DTC loss in *ceh-22* mutants.

The effect of top-1FL on embryonic survival and morphology

To analyze the co-suppression phenotype of *top-1* gene during embryogenesis, we observed embryos obtained from integrated *top-1FL* transgenic worms under DIC microscope. Most *top-1FL* embryos were arrested (or died) at different stages of embryonic development (**Figure 1B**), confirming the critical role of *top-1* in embryogenesis [10]. We recently reported that *top-1(RNAi)* showed chromosomal bridges, DNA fragmentation, and cell division defects in early embryos [10]. The *top-1FL* embryos also exhibited multiple nuclei per cells at the early embryonic stages (not shown). Next, to determine the maternal effects of *top-1* co-suppression on embryogenesis, we measured daily hatching rates of embryos obtained from wild-type and *top-1FL* transgenic worms at 20°C. Most embryos from wild-type adults survived

TOP-1 Functions	RNAi	Co-suppression	References
Embryonic cell division	O	O	[10] and this work
Chromosome segregation of spermaocyte	O		[53]
Morphogenesis		O	this work
Stem cell niche specification	O	O	this work
Gonadal migration	O	O	[10] and this work
Germline proliferation	O	O	[10] and this work
Apoptosis		O	[10]
Lifespan		O	this work
Growth control		O	this work

Figure 5. The role of *C. elegans top-1* in various developmental processes. The summary of *top-1* functions that were characterized by RNAi and/or the transgene-mediated co-suppression.

(**Figure 3A**). However, the hatching rate of embryos from adult *top-1FL* worms was gradually decreased in an age-dependent manner (**Figure 3A**). This suggests that the maternal effect of *top-1FL* co-suppression on embryogenesis may be associated with adult age.

C. elegans top-1 is predominantly expressed in the nuclei of most embryonic cells. Interestingly, *top-1*α isoform appears to localize in excretory cells of embryonic and larval stages [10]. The role of the excretory cell is to regulate osmotic/ionic regulation and waste elimination, which is analogous to the renal system of mammals [41-43]. In *C. elegans*, *ceh-6* (one of three POU homeobox gene) is the ortholog of vertebrate *Brn*, *Brn2*, *SCIP/Oct6* and *Brn4* and fly *Cf1a/drifter/ventral veinless* [44]. Importantly, *ceh-6(mg60)* mutants showed excretory canal defects, resulting in embryonic lethality with grossly malformed morphology [44]. Therefore, we examined whether the co-suppression of *top-1* gene affects embryonic morphogenesis. Notably, both *top-1FL* and *top-1(ΔInt1&2)* animals exhibited morphological defects (**Figure 3C** and **3E**). To further examine the morphology defects, we used the MH27 monoclonal antibody, which recognizes adherens junction surrounding hypodermal cells in the pharynx and intestine [45]. In wild-type comma stage embryos, cells on each side line-up from one end to the other (**Figure 3D**). By contrast, comma-staged *top-1FL* embryos exhibited abnormal MH27 staining (**Figure 3E**). It was also noted that the hatched L1 *top-1FL* worms had severe morphological defects (Compare **Figure 3F** and **3G**). Therefore, our results suggest that *top-1* may be involved in a normal cell division during early embryogenesis and in proper morphogenesis during late embryogenesis and early larval stages.

Co-suppression of top-1FL affects lifespan and growth

In eukaryotes, DNA topoisomerase I is critical for DNA replication, transcription, and genomic stability [46]. Interference between replication and transcription mainly causes sponteneous replication stress and DNA damages, which contribute to genomic instability during the early stages of tumorigenesis [46]. Interestingly, DNA topoisomerase I (TopA) is associated with telomere complex with DNA polymerase I (PolA) in *Streptomyces*. Moreover, both TopA and PolA regulate telomeric repeats by a highly efficient reverse transcriptase activity [47, 48]. However, the role of *top-1* controlling lifespan has not been adequately investigated in multi-cellular organisms. In order to determine whether *top-1FL* affects the lifespan, we measured the lifespan of both wild-type and *top-1FL* transgenic worms. As shown in **Figure 4A**, the average lifespan for *top-1FL* worms was 7 days at 20°C, which was 51% of the wild-type lifespan. Moreover, *top-1FL* transgenic worms showed a strikingly slow growth rate, which corresponded to 59% of the value of the wild-type (**Figure 4B** and **4C**).

The human Topoisomerase 1 (Top 1) physically interacts with Werner protien (WRN) [49], which belongs to the RecQ family of DNA helicase. This is required for the maintenance of genomic stability and aging in human cells [49-51]. In addition, mammalian Top 1 is associated with several DNA metabolism proteins that have been implicated in aging [49, 52]. Our results also suggest that *C. elegans top-1* may play a role in the maintenance of lifespan. Although further studies are necessary to understand mechanisms underlying aging, our findings provide valuable insight into the function of mammalian Top 1 in reguating senescence.

Conclusion

Our recent studies determined the differential subcellular localization of *C. elegans top-1* iso-

forms using immunohistochemistry and GFP reporter analysis [10]. The *top-1β* isoform is expressed in the nucleus of most tissues and cells, but *top-1α* isoform has tissue specificity [10]. RNAi targeted to both *top-1* isoforms demonstrated that *top-1* is essential for chromosome segregation in embryo [10] and spermatocyte [53] (**Figure 5**). It is also critical for germline proliferation and migration [10] (**Figure 5**). We here used a transgene-mediated co-suppression technique to investigate the further biological role of *top-1 in vivo*. We found that a full-length of *top-1* transgene sufficiently induced co-suppression of endogenous *top-1* genes, resulting in embryonic lethality and defects in germline proliferation and migration. Notably, transgene-mediated co-suppression copied the phenotypes of *top-1(RNAi)* with a stronger effect (**Figure 1A**). We also found that *top-1* may function in morphogenesis, stem cell niche (DTC) specification, normal lifespan, and growth control (**Figure 5**). Evidently, the roles of Topoisomerase II in development events, including neural [54], retinal [55], and embryonic development [56] have been reported in several model systems to date, but the biological roles of *top-1* in the specific developmental events are poorly understood. Therefore, our results may provide better understanding of the role of Topoisomerase I in other multicellular organisms, including humans.

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Referernces

[1] Champoux JJ. DNA topoisomerase I-mediated nicking of circular duplex DNA. Met Mol Biol 2001; 95: 81-87.

[2] Leppard JB and Champoux JJ. Human DNA topoisomerase I: relaxation, roles, and damage control. Chromosoma 2005; 114: 75-85.

[3] Lee MP, Brown SD, Chen A and Hsieh TS. DNA topoisomerase I is essential in Drosophila melanogaster. Proc Natl Acad Sci U S A 1993; 90: 6656-6660.

[4] Morham SG, Kluckman KD, Voulomanos N and Smithies O. Targeted disruption of the mouse topoisomerase I gene by camptothecin selection. Mol Cell Biol 1996; 16: 6804-6809.

[5] Zhang CX, Chen AD, Gettel NJ and Hsieh TS. Essential functions of DNA topoisomerase I in Drosophila melanogaster. Dev Biol 2000; 222: 27-40.

[6] Moukharskaya J and Verschraegen C. Topoisomerase 1 inhibitors and cancer therapy. Hematol Oncol Clin North Am 2012; 26: 507-525, vii.

[7] Garcia-Carbonero R and Supko JG. Current perspectives on the clinical experience, pharmacology, and continued development of the camptothecins. Clin Cancer Res 2002; 8: 641-661.

[8] Froelich-Ammon SJ and Osheroff N. Topoisomerase poisons: harnessing the dark side of enzyme mechanism. J Biol Chem 1995; 270: 21429-21432.

[9] Lee MH, Jang YJ and Koo HS. Alternative splicing in the Caenorhabditis elegans DNA topoisomerase I gene. Biochim Biophys Acta 1998; 1396: 207-214.

[10] Cha DS, Hollis SE, Datla US, Lee S, Ryu J, Jung HR, Kim E, Kim K, Lee M, Li C and Lee MH. Differential subcellular localization of DNA topoisomerase-1 isoforms and their roles during Caenorhabditis elegans development. Gene Expr Patterns 2012; 12: 189-195.

[11] Byrd DT and Kimble J. Scratching the niche that controls Caenorhabditis elegans germline stem cells. Semin Cell Dev Biol 2009; 20: 1107-1113.

[12] Wong MC and Schwarzbauer JE. Gonad morphogenesis and distal tip cell migration in the Caenorhabditis elegans hermaphrodite. Wiley Interdiscip Rev Dev Biol 2012; 1: 519-531.

[13] Bingham PM. Cosuppression comes to the animals. Cell 1997; 90: 385-387.

[14] Mette MF, van der Winden J, Matzke MA and Matzke AJ. Production of aberrant promoter transcripts contributes to methylation and si-

lencing of unlinked homologous promoters in trans. EMBO J 1999; 18: 241-248.
[15] Rountree MR and Selker EU. DNA methylation inhibits elongation but not initiation of transcription in Neurospora crassa. Genes Dev 1997; 11: 2383-2395.
[16] Pal-Bhadra M, Bhadra U and Birchler JA. Cosuppression in Drosophila: gene silencing of Alcohol dehydrogenase by white-Adh transgenes is Polycomb dependent. Cell 1997; 90: 479-490.
[17] Pal-Bhadra M, Bhadra U and Birchler JA. Cosuppression of nonhomologous transgenes in Drosophila involves mutually related endogenous sequences. Cell 1999; 99: 35-46.
[18] Ketting RF and Plasterk RH. A genetic link between co-suppression and RNA interference in C. elegans. Nature 2000; 404: 296-298.
[19] Brenner S. The genetics of Caenorhabditis elegans. Genetics 1974; 77: 71-94.
[20] Lam N, Chesney MA and Kimble J. Wnt signaling and CEH-22/tinman/Nkx2.5 specify a stem cell niche in C. elegans. Curr Biol 2006; 16: 287-295.
[21] Kramer JM, French RP, Park EC and Johnson JJ. The Caenorhabditis elegans rol-6 gene, which interacts with the sqt-1 collagen gene to determine organismal morphology, encodes a collagen. Mol Cell Biol 1990; 10: 2081-2089.
[22] Lee MH, Ahn B, Choi IS and Koo HS. The gene expression and deficiency phenotypes of Cockayne syndrome B protein in Caenorhabditis elegans. FEBS Lett 2002; 522: 47-51.
[23] Mariol MC, Walter L, Bellemin S and Gieseler K. A rapid protocol for integrating extrachromosomal arrays with high transmission rate into the C. elegans genome. J Vis Exp 2013; 82; e50773.
[24] Lee MH, Hollis SE, Yoo BH and Nykamp K. Caenorhabditis elegans DNA-2 helicase/endonuclease plays a vital role in maintaining genome stability, morphogenesis, and life span. Biochem Biophys Res Commun 2011; 407: 495-500.
[25] Broos S, Hulpiau P, Galle J, Hooghe B, Van Roy F and De Bleser P. ConTra v2: a tool to identify transcription factor binding sites across species, update 2011. Nucleic Acids Res 2011; 39: W74-78.
[26] Timmons L, Court DL and Fire A. Ingestion of bacterially expressed dsRNAs can produce specific and potent genetic interference in Caenorhabditis elegans. Gene 2001; 263: 103-112.
[27] Mariol MC, Walter L, Bellemin S and Gieseler K. A rapid protocol for integrating extrachromosomal arrays with high transmission rate into the C. elegans genome. J Vis Exp 2013; e50773.
[28] Le Hir H, Nott A and Moore MJ. How introns influence and enhance eukaryotic gene expression. Trends Biochem Sci 2003; 28: 215-220.
[29] Rose AB. Requirements for intron-mediated enhancement of gene expression in Arabidopsis. RNA 2002; 8: 1444-1453.
[30] Fuxman Bass JI, Tamburino AM, Mori A, Beittel N, Weirauch MT, Reece-Hoyes JS and Walhout AJ. Transcription factor binding to Caenorhabditis elegans first introns reveals lack of redundancy with gene promoters. Nucleic Acids Res 2014; 42: 153-162.
[31] Cogoni C and Macino G. Isolation of quelling-defective (qde) mutants impaired in posttranscriptional transgene-induced gene silencing in Neurospora crassa. Proc Natl Acad Sci U S A 1997; 94: 10233-10238.
[32] Ruiz F, Vayssie L, Klotz C, Sperling L and Madeddu L. Homology-dependent gene silencing in Paramecium. Mol Biol Cell 1998; 9: 931-943.
[33] Vaucheret H, Beclin C, Elmayan T, Feuerbach F, Godon C, Morel JB, Mourrain P, Palauqui JC and Vernhettes S. Transgene-induced gene silencing in plants. Plant J 1998; 16: 651-659.
[34] Jensen S, Gassama MP and Heidmann T. Taming of transposable elements by homology-dependent gene silencing. Nat Genet 1999; 21: 209-212.
[35] Guo S and Kemphues KJ. par-1, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell 1995; 81: 611-620.
[36] Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE and Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998; 391: 806-811.
[37] Ketting RF, Tijsterman M and Plasterk RH. Cosuppression in C. elegans. CSH Protoc 2006; 2006.
[38] Kimble JE and White JG. On the control of germ cell development in Caenorhabditis elegans. Dev Biol 1981; 81: 208-219.
[39] Kimble J and Crittenden SL. Controls of germline stem cells, entry into meiosis, and the sperm/oocyte decision in Caenorhabditis elegans. Annu Rev Cell Dev Biol 2007; 23: 405-433.
[40] Tilmann C and Kimble J. Cyclin D regulation of a sexually dimorphic asymmetric cell division. Dev Cell 2005; 9: 489-499.
[41] Nelson FK and Riddle DL. Functional study of the Caenorhabditis elegans secretory-excretory system using laser microsurgery. J Exp Zool 1984; 231: 45-56.
[42] Nelson FK, Albert PS and Riddle DL. Fine structure of the Caenorhabditis elegans secretory-

excretory system. J Ultrastruct Res 1983; 82: 156-171.

[43] Igarashi P. Overview: nonmammalian organisms for studies of kidney development and disease. J Am Soc Nephrol 2005; 16: 296-298.

[44] Burglin TR and Ruvkun G. Regulation of ectodermal and excretory function by the C. elegans POU homeobox gene ceh-6. Development 2001; 128: 779-790.

[45] Labouesse M. Deficiency screen based on the monoclonal antibody MH27 to identify genetic loci required for morphogenesis of the Caenorhabditis elegans embryo. Dev Dyn 1997; 210: 19-32.

[46] Tuduri S, Crabbe L, Tourriere H, Coquelle A and Pasero P. Does interference between replication and transcription contribute to genomic instability in cancer cells? Cell Cycle 2010; 9: 1886-1892.

[47] Bao K and Cohen SN. Reverse transcriptase activity innate to DNA polymerase I and DNA topoisomerase I proteins of Streptomyces telomere complex. Proc Natl Acad Sci U S A 2004; 101: 14361-14366.

[48] Kang MR, Muller MT and Chung IK. Telomeric DNA damage by topoisomerase I. A possible mechanism for cell killing by camptothecin. J Biol Chem 2004; 279: 12535-12541.

[49] Laine JP, Opresko PL, Indig FE, Harrigan JA, von Kobbe C and Bohr VA. Werner protein stimulates topoisomerase I DNA relaxation activity. Cancer Res 2003; 63: 7136-7146.

[50] Singh DK, Ahn B and Bohr VA. Roles of RECQ helicases in recombination based DNA repair, genomic stability and aging. Biogerontology 2009; 10: 235-252.

[51] Mohaghegh P and Hickson ID. Premature aging in RecQ helicase-deficient human syndromes. Int J Biochem Cell Biol 2002; 34: 1496-1501.

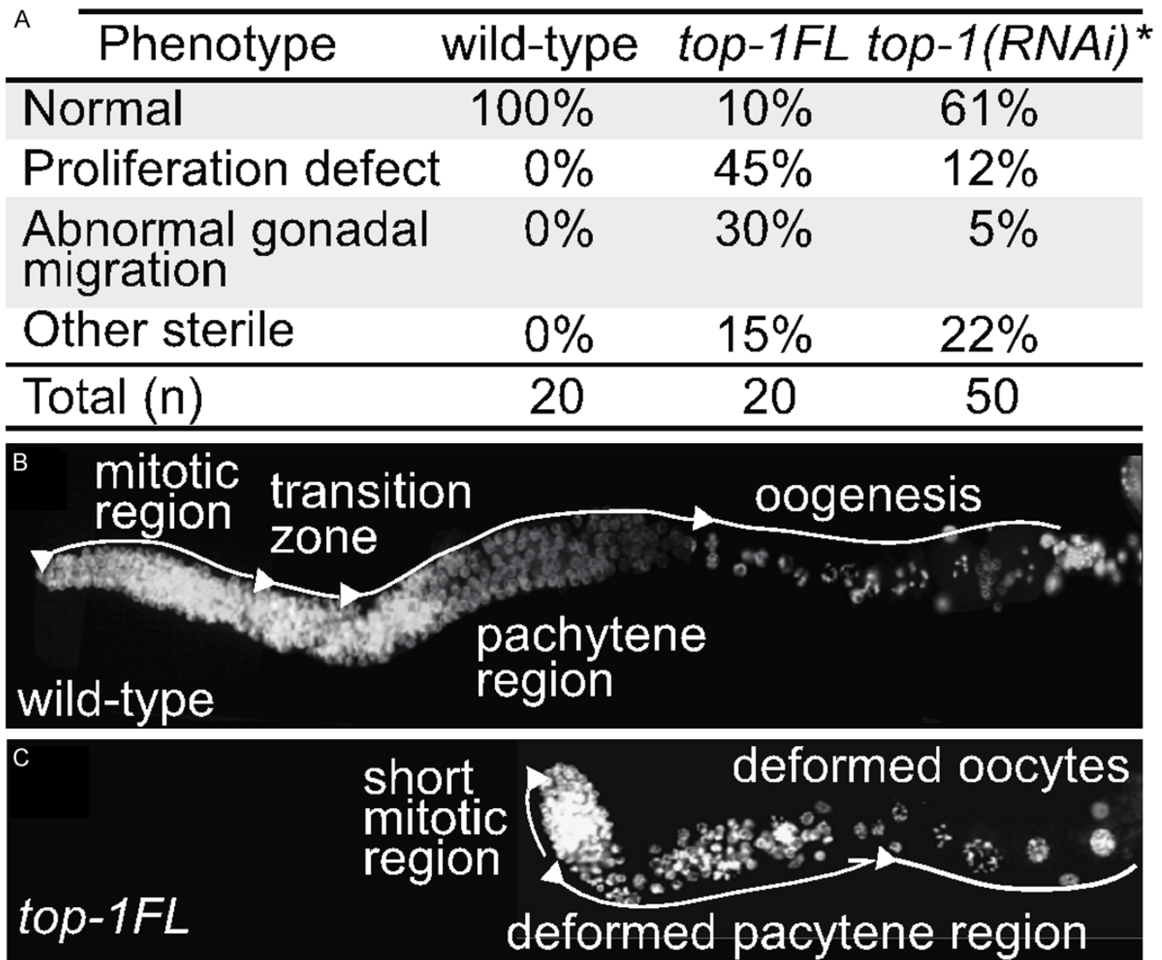
[52] Plaschkes I, Silverman FW and Priel E. DNA topoisomerase I in the mouse central nervous system: Age and sex dependence. J Comp Neurol 2005; 493: 357-369.

[53] Chu DS, Liu H, Nix P, Wu TF, Ralston EJ, Yates JR 3rd and Meyer BJ. Sperm chromatin proteomics identifies evolutionarily conserved fertility factors. Nature 2006; 443: 101-105.

[54] Yang X, Li W, Prescott ED, Burden SJ and Wang JC. DNA topoisomerase IIbeta and neural development. Science 2000; 287: 131-134.

[55] Li Y, Hao H, Tzatzalos E, Lin RK, Doh S, Liu LF, Lyu YL and Cai L. Topoisomerase IIbeta is required for proper retinal development and survival of postmitotic cells. Biol Open 2014; 3: 172-184.

[56] Dovey M, Patton EE, Bowman T, North T, Goessling W, Zhou Y and Zon LI. Topoisomerase II alpha is required for embryonic development and liver regeneration in zebrafish. Mol Cell Biol 2009; 29: 3746-3753.



A

Phenotype	wild-type	*top-1FL*	*top-1(RNAi)**
Normal	100%	10%	61%
Proliferation defect	0%	45%	12%
Abnormal gonadal migration	0%	30%	5%
Other sterile	0%	15%	22%
Total (n)	20	20	50

Figure S1. Germline defects in *top-1FL* transgenic worms. A: Comparison of germline defects between RNAi and co-suppression of *top-1* gene. B and C: DAPI staining of dissected gonads. B: Wild-type germline. C: *top-1FL* germline with a proliferation defect. *RNAi results are from Cha et al (2012) Gene Expression Pattern.

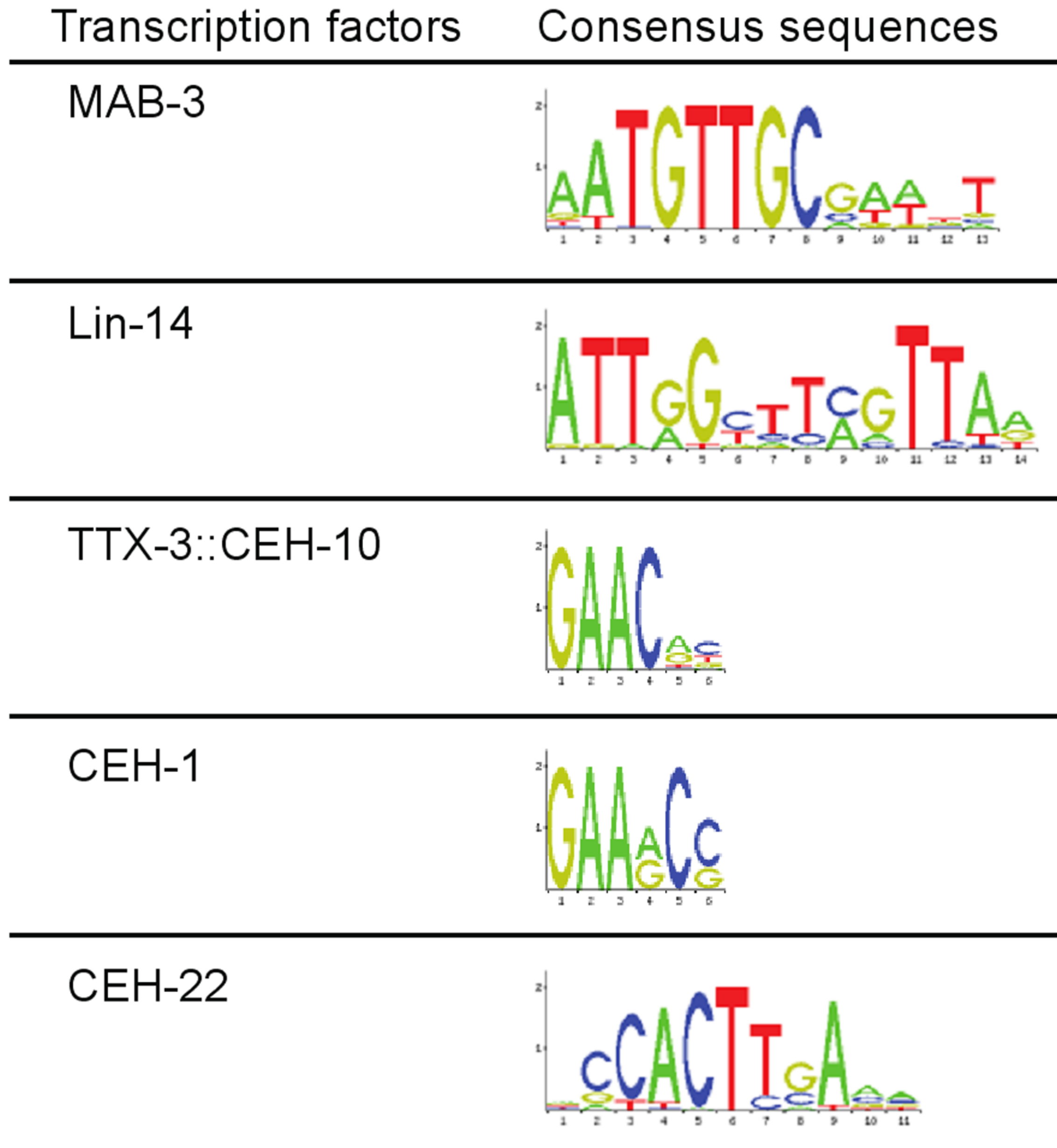


Figure S2. Consensus binding sequences of transcription factors. ConTra v2, a tool to identify transcription factor binding sites identified consensus sequences for the transcription factors (see **Figure 1C**), including MAB-3, LIN-14, TTX-3/CEH-10, CEH-1 and CEH-22.