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ARTICLE

The *C. elegans* TIA-1/TIAR Homolog TIAR-1 Is Required to Induce Germ Cell Apoptosis

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Summary: In *Caenorhabditis elegans*, physiological germ cell apoptosis eliminates more than half of the cells in the hermaphrodite gonad to support gamete quality and germline homeostasis by a still unidentified mechanism. External factors can also affect germ cell apoptosis. The BH3-only protein EGL-1 induces germ cell apoptosis when animals are exposed to pathogens or agents that produce DNA damage. DNA damage-induced apoptosis also requires the nematode p53 homolog CEP-1. Previously, we found that heat shock, oxidative, and osmotic stresses induce germ cell apoptosis through an EGL-1 and CEP-1 independent mechanism that requires the MAPKK pathway. However, we observed that starvation increases germ cell apoptosis by an unknown pathway. Searching for proteins that participate in stress-induced apoptosis, we found the RNA-binding protein TIAR-1 (a homolog of the mammalian TIA-1/TIAR family of proteins). Here, we show that TIAR-1 in *C. elegans* is required to induce apoptosis in the germline under several conditions. We also show that TIAR-1 acts downstream of CED-9 (a BCL2 homolog) to induce apoptosis under stress conditions, and apparently does not seem to regulate *ced-4* or *ced-3* mRNAs accumulation directly. TIAR-1 is expressed ubiquitously in the cytoplasm of the soma as well as the germline, where it sometimes associates with P granules. We show that animals lacking TIAR-1 expression are temperature sensitive sterile due to oogenesis and spermatogenesis defects. Our work shows that TIAR-1 is required for proper germline function and demonstrates that this protein is important to induce germ cell apoptosis under several conditions. genesis 51:690–707. © 2013 Wiley Periodicals, Inc.

Key words: apoptosis; germline; germ cells; TIA-1; TIAR; TIAR-1; stress; *C. elegans*

INTRODUCTION

Germ cells produce gametes, which are the basis of organisms' continuity. To ensure gamete quality, germ cell apoptosis occurs normally as a highly conserved mechanism (Andux and Ellis, 2008; Baum *et al.*, 2005). In *C. elegans*, it is estimated that fifty percent of germ cells are eliminated during oogenesis in a response known as physiological germ cell apoptosis (Gumienny *et al.*, 1999). This process requires the core components of the apoptosis pathway: CED-3, CED-4, and CED-9 but it is activated by an unknown mechanism. The BCL2 family member CED-9 prevents apoptosis by sequestering the APAF1 homolog CED-4. During physiological germ cell apoptosis an unidentified mechanism inactivates CED-9. This stimulus liberates CED-4, which in turn activates the caspase CED-3 (Lettre and Hengartner, 2006).

Several types of environmental stress increase germ cell apoptosis by different pathways (Derry *et al.*, 2001; Salinas *et al.*, 2006; Schumacher *et al.*, 2001). DNA damage triggers germ cell apoptosis through the proapoptotic proteins EGL-1 (a BH3-only protein) and CEP-1 (the p53 homolog; Derry *et al.*, 2001; Schumacher *et al.*, 2001). Pathogen infections induced apoptosis in an EGL-

Additional Supporting Information may be found in the online version of this article.

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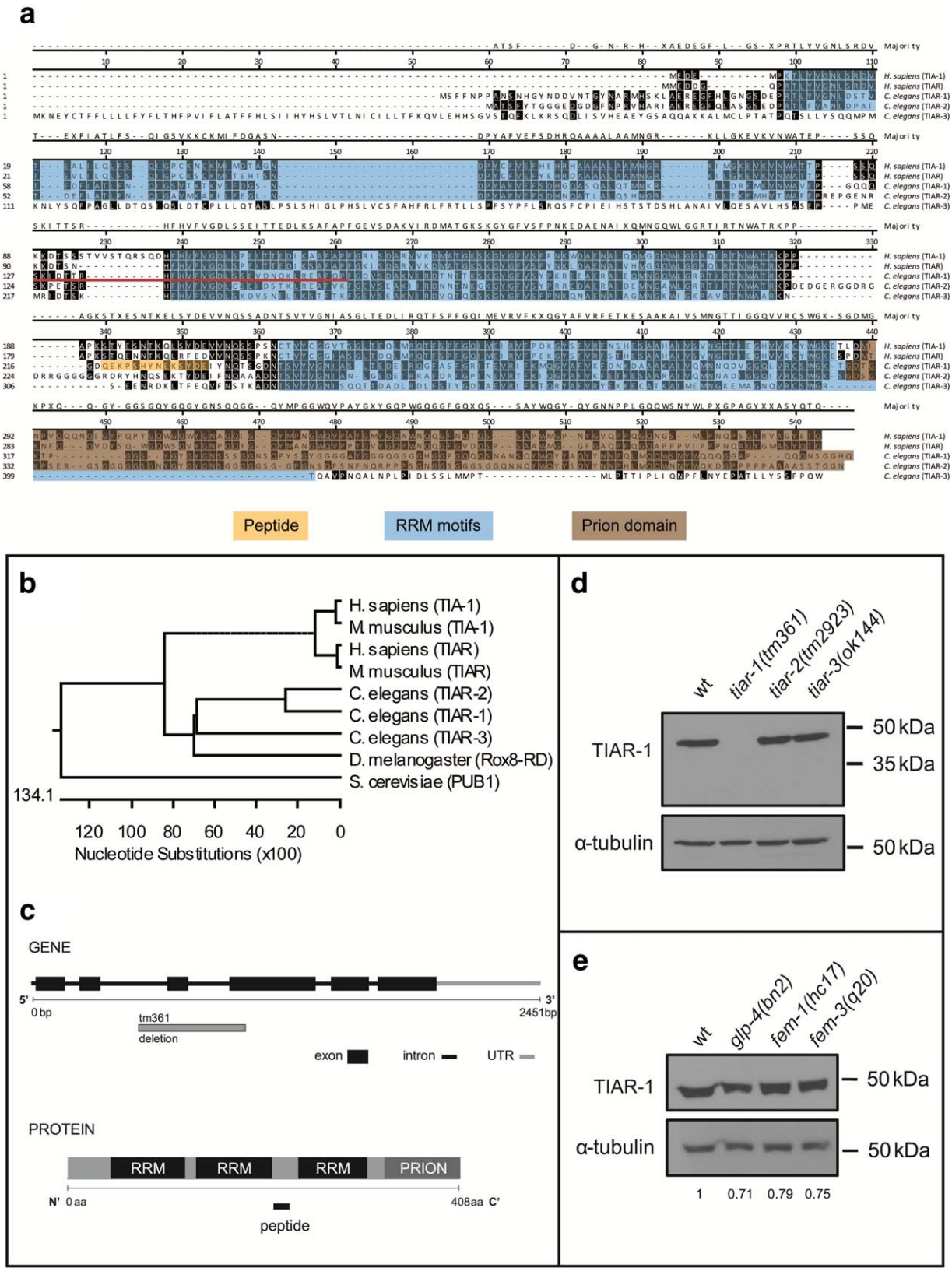
a
Majority
H. sapiens (TIA-1)
H. sapiens (TIAR)
C. elegans (TIAR-1)
C. elegans (TIAR-2)
C. elegans (TIAR-3)
Peptide
RRM motifs
Prion domain
b
H. sapiens (TIA-1)
M. musculus (TIA-1)
H. sapiens (TIAR)
M. musculus (TIAR)
C. elegans (TIAR-2)
C. elegans (TIAR-1)
C. elegans (TIAR-3)
D. melanogaster (Rox8-RD)
S. cerevisiae (PUB1)
134.1
120 100 80 60 40 20 0
Nucleotide Substitutions (x100)
c
GENE
5'
0 bp
tm361
deletion
2451bp
3'
exon
intron
UTR
PROTEIN
RRM
RRM
RRM
PRION
N' 0 aa
408aa C'
peptide
d
wt
tiar-1(tm361)
tiar-2(tm2923)
tiar-3(ok144)
TIAR-1
50 kDa
35 kDa
α-tubulin
50 kDa
e
wt
glp-4(bn2)
fem-1(hc17)
fem-3(q20)
TIAR-1
50 kDa
α-tubulin
50 kDa
1 0.71 0.79 0.75

FIG. 1.

1 dependent manner (Aballay and Ausubel, 2001). Previously, we found that oxidative and osmotic stress, as well as heat shock, activate germ cell apoptosis through the MAPKK pathway and independently of EGL-1 and CEP-1/p53 (Salinas *et al*., 2006). Unexpectedly, we observed that starvation induces germ cell apoptosis by an unknown mechanism that is independent of the EGL-1, CEP-1/p53 and the MAPKK pathways (Salinas *et al*., 2006). Characterization of the pathways that regulate stress-induced germ cell apoptosis will contribute to our understanding of how the germline stays healthy to pass genetic information for the next generation.

In this article, we show the role of TIAR-1, an RNA-binding protein from the TIA-1/TIAR family, in germ cell apoptosis. TIA-1/TIAR proteins were originally described as cytotoxic granule-associated RNA-binding proteins with proapoptotic functions. Purified recombinant TIA-1 and TIAR induced DNA fragmentation in digitonin-permeabilized thymocytes, suggesting that these proteins may be responsible for inducing apoptosis in cytolytic lymphocyte targets (Kawakami *et al*., 1992; Tian *et al*., 1991). Additionally, TIA-1 promotes Fas receptor exon 6 inclusion during splicing, resulting in the formation of a proapoptotic isoform involved in Fas-mediated apoptosis (Izquierdo *et al*., 2005). Also, TIA-1 interacts with Fas-activated serine/threonine kinase (FAST), which is a regulator of Fas-induced apoptosis (Li *et al*., 2004; Tian *et al*., 1995). Besides apoptosis, TIA-1/TIAR proteins are involved in other cellular functions, including splicing, translation, repression, degradation of specific mRNAs and stress granule formation (Anderson and Kedersha, 2002b; Del Gatto-Konczak *et al*., 2000; Förch and Valcárcel, 2001; Förch *et al*., 2000, 2002; Gilks *et al*., 2004; Izquierdo *et al*., 2005; Kawai *et al*., 2006; Kedersha *et al*., 1999; Kuwano *et al*., 2008; Le Guiner *et al*., 2001; Lopez de Silanes *et al*., 2005; Piecyk *et al*., 2000; Yamasaki *et al*., 2007; Zuccato *et al*., 2004).

Here, we show that in *C. elegans* TIAR-1 is required to induce germ cell apoptosis under different conditions, including starvation, heat shock, osmotic, oxidative stress, and UV irradiation. This nematode lacks the Fas apoptosis-inducing pathway, and in this article, we show that to trigger apoptosis TIAR-1 acts downstream of the BCL2 homolog CED-9 but apparently does not directly regulates the accumulation of *ced-4* or *ced-3* mRNAs. Our findings introduce TIAR-1 as a new component in the *C. elegans* germline whose function is important under normal growth and stress conditions.

RESULTS

TIAR-1 Is Ubiquitously Expressed

In an ongoing screen to identify genes that participate in starvation-induced germ cell apoptosis, we detected C18A3.5, a gene that encodes one of the three TIA-1/TIAR homologs in *C. elegans* (Fig. 1a,b). The other two homologs are *tiar-2* (*Y46G5A.13*) and *tiar-3* (*C07A4.1;* Fig. 1a,b). The predicted TIAR-1 protein is composed of 408 residues that contains three RNA recognition motifs and a prion-like domain (Fig. 1a,c). TIAR-1 displays 46.1 and 43.3% overall amino acid sequence identity with human TIA-1 and TIAR, respectively.

To investigate TIAR-1 protein expression, we produced a rabbit antibody against a 14 amino acid peptide covering positions 218–231 (Fig. 1a,c). Western blotting with affinity-purified antiserum revealed a single ~46 kDa band, whereas the predicted size was 45.2 kDa (Fig. 1d). This band was not observed in *tiar-1(tm361)* mutant animals (Fig. 1d). In contrast, the band was detected in *tiar-2(tm2923)* and *tiar-3(ok144)* mutant animals (Fig. 1d), which indicates that the antibody specifically recognizes TIAR-1 protein. The *tm361* allele carries a 579 bp deletion that eliminates exon 3 and 33% of exon 4 (Fig. 1c). RT-PCR experiments revealed that *tiar-1(tm361)* animals expressed a transcript of 1054 bp. This transcript was not in frame and had lost 232 bp of the original ORF between positions 242 and 475.

TIAR-1 protein levels were slightly lower in mutants with a feminized germline [*fem-1(hc17)* (79%; Kimble *et al*., 1984)], a masculinized gonad [*fem-3(q20gf)* (75%; Barton *et al*., 1987)] or a reduced germline [*glp-4(bn2)* (71%; Beanan and Strome, 1992; Fig. 1e]. These data demonstrate that TIAR-1 is not exclusively expressed in the germline, and that its expression is abundant in somatic tissues. However, it is intriguing that TIAR-1 expression is similarly affected in mutants that do not have germline, sperm, or oocytes. A possible explanation for this is that perhaps whenever some

FIG. 1. The *C. elegans* TIA-1/TIAR homolog TIAR-1 is expressed in both somatic and germline. (**a**) Protein alignment in the TIA-1/TIAR family. The alignment was performed using human TIA-1 and TIAR and *C. elegans* TIAR-1/2/3 sequences, and the Clustal W Method. Conserved residues are marked with black boxes. The peptide against which the antibody was generated is shown in yellow. The conserved RRM motifs are shown in blue, and the prion-like domain is shown in brown. (**b**) Phylogenetic analysis of the TIA-1/TIAR family. *Saccharomyces cerevisiae* PUB1 protein is used as an out-group. The length of each pair of branches represents the distance between sequences, and the units at the bottom of the tree indicate the number of substitution events. (**c**) Schematic representation of the *tiar-1* gene and its protein. Peptide: QEKPSHYNEKSYDE. RRM: RNA recognition motifs. (**d,e**) Western blot analyses of whole-animal protein from 100 one-day-old animals of the indicated background. The blots were probed with the affinity-purified rabbit TIAR-1 antibody. A commercial mouse α-tubulin antibody (Sigma) was used for the loading control. Molecular markers are shown on the right, and expression levels are indicated at the bottom.

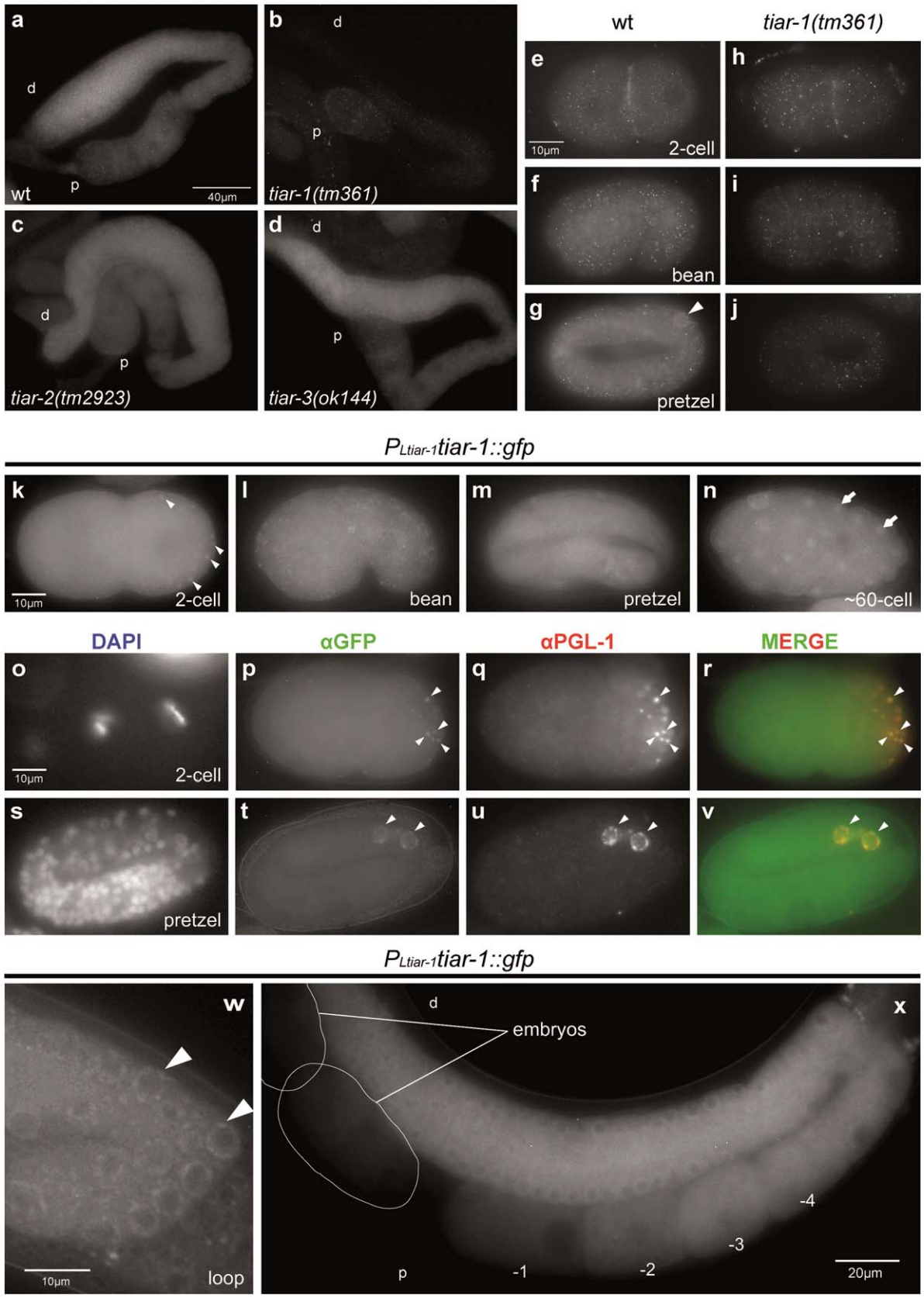
a
d
wt
p
40μm
b
d
p
tiar-1(tm361)
wt
tiar-1(tm361)
e
10μm
2-cell
h
f
bean
i
g
pretzel
j
c
d
p
tiar-2(tm2923)
d
d
p
tiar-3(ok144)
PLtiar-1tiar-1::gfp
k
10μm
2-cell
l
bean
m
pretzel
n
~60-cell
DAPI
αGFP
αPGL-1
MERGE
o
10μm
2-cell
p
q
r
s
pretzel
t
u
v
PLtiar-1tiar-1::gfp
w
10μm
loop
d
embryos
x
-4
-3
p
-1
-2
20μm

FIG. 2.

Table 1
tiar-1(tm361) Produce Fewer Offspring Than Wild Type Animals and Show Increased Embryonic Lethality

	% Offspring (n)		% Embryonic lethality (n)		% Sperm (n)	
Genotype	20°C	25°C	20°C	25°C	20°C	25°C
♀*N2*	100 (50)	100 (51)	0.4 (40)	1.2(41)	ND	100 (30)
♀*tiar-1(tm361)*	47.4 (39)	0.7 (42)	7.8 (39)	0.0 (42)	ND	94.2 (30)
♀*N2 vs* ♂ *him-8(e1489)*	100 (4)	ND	4.5 (4)	ND	ND	ND
♀ *tiar-1(tm361) vs* ♂ *him-8(e1489)*	43.6 (8)	ND	7.2 (8)	ND	ND	ND
♂ *him-8(e1489)*	ND	ND	ND	ND	100 (15)	100 (15)
♂ *tiar-1(tm361); him-8(e1489)*	ND	ND	ND	ND	48.7 (15)	34.7 (15)
♀ *fog-2(q71) vs* ♂ *him-8(e1489)*	100 (17)	ND	0.4 (17)	ND	ND	ND
♀ *fog-2(q71) vs* ♂ *tiar-1(tm361); him-8(e1489)*	22.7 (7)	ND	4.4 (7)	ND	ND	ND
♂ *tiar-1(tm361);* $P_{Stiar\text{-}1}$*gfp:tiar-1*	82.3 (22)	7.4 (23)	31.6 (22)	15.5 (23)	ND	ND
♂ *tiar-1(tm361);* $P_{pie\text{-}1}$*gfp:tiar-1*	26.1 (20)	12.1 (20)	1.4 (20)	0.6 (20)	ND	ND
♂ *tiar-1(tm361);* $P_{Ltiar\text{-}1}$*tiar-1:gfp*	59.8 (25)	35.6 (25)	2.7 (25)	0.6 (25)	ND	ND

Hermaphrodites were individually selected as L4 larvae and then transferred to new plates every 24 h over the course of 3 or 4 days, depending on the temperature (25 or 20°C, respectively). Plates were scored for dead embryos and surviving offspring every 24 h. Embryos that did not hatch within 24 h after laying were considered dead. To study oocytes contributions to fertility, we crossed *tiar-1(tm361)* hermaphrodites with *him-8(e1489)* males. To study male fertility, *tiar-1(tm361);him-8(e1489)* males were crossed with *fog-2(q71)* females. To determine sperm number, we stained extruded gonads from one-day-old males with DAPI and counted sperm under a fluorescent microscope. The percentages of each category are shown, with the number of animals tested is given in parentheses. ND: not determined.

alterations in the germline occur, TIAR-1 somatic expression is affected as a result.

Whole-mount immunostaining revealed that TIAR-1 had a diffuse pattern of expression in the cytoplasm of several tissues (Supporting Information Fig. S1a–d,i), and this expression was reduced in *tiar-1(tm361)* animals (Supporting Information Fig. S1e–h,j). TIAR-1 expression was particularly observed in germ cells where sometimes it has a perinuclear association (see insets in Supporting Information Fig. S1a–d,i). In dissected wild-type hermaphrodite gonads, TIAR-1 was observed in the cytoplasm (Fig. 2a), and this expression was reduced in *tiar-1(tm361)* animals but not affected in *tiar-2(tm2923)* or *tiar-3(ok144)* animals (Fig. 2b–d). TIAR-1 expression appears lower in the most proximal oocytes (Fig. 2a,c,d). In male gonads, TIAR-1 was expressed in the cytoplasm of all germ cells except mature sperm (Supporting Information Fig. S1k,l). Unexpectedly in extruded gonads, we did not see TIAR-1 associated with perinuclear granules, as was observed in whole-mount immunostaining. A possible explanation is that TIAR-1 may not associate with perinuclear granules very tightly therefore its association is easily lost during certain fixation methods or manipulations. Another possible explanation is that TIAR-1 associates to perinuclear granules transiently and only during certain conditions.

During embryogenesis, TIAR-1 was observed in all blastomeres and its levels seemed to increase at later stages relative to early stages (Fig. 2e–g). In late stages of embryogenesis, TIAR-1 was again observed in germ cell perinuclear granules (Fig. 2g; arrowhead). A granular pattern and cortex-like signal background artifacts were observed in *tiar-1(tm361)* embryos, but differences between specific TIAR-1 expression and background were clearer in older embryos (Fig. 2e–j), likely because TIAR-1 was expressed at low level at earlier stages.

To study TIAR-1 *in vivo*, we produced transgenic animals using microparticle bombardment (Praitis *et al.*, 2001). Our first attempt used a construct that carried 300 bp of the predicted *tiar-1* promoter, *gfp*, 1227 bp of the *tiar-1* ORF, and 485 bp of the downstream 3′ UTR ($P_{Stiar\text{-}1}$*gfp:tiar-1;* the S denotes the shorter promoter). This construct was expressed in the soma in a pattern similar to that observed for the native protein by immunostaining (compare Fig. 2e–g and Supporting Information Fig. S1i with S1m,p,r–v), but it was not expressed in the germline, possibly because of natural transgene silencing in this tissue (Kelly *et al.*, 1997). It

FIG. 2. TIAR-1 is mainly expressed in the cytoplasm of *C. elegans* gonads and embryos. Gonads (**a–d**) and embryos (**e–j**) from the indicated background were dissected and stained with the affinity-purified TIAR-1 antibody. TIAR-1 expression in wild-type (**a**), *tiar-1(tm361)* (**b**), *tiar-2(tm2923)* (**c**), and *tiar-3(ok144)* (**d**) gonads. TIAR-1 expression in wild-type (**e–g**) and *tiar-1(tm361)* (**h–j**) embryos. (**k–x**) $P_{Ltiar\text{-}1}$TIAR-1:GFP is localized in cytoplasm and sometimes is associated with P granules. (**k–n**) $P_{Ltiar\text{-}1}$*tiar-1:gfp* transgenic animals were mounted and observed by fluorescence microscopy. Arrowheads point to P granules in **k**. $P_{Ltiar\text{-}1}$TIAR-1:GFP expression in the nuclei of ~60-cell embryo (**n**; arrows). (**o–v**) $P_{Ltiar\text{-}1}$*tiar-1:gfp* transgenic embryos were dissected and stained with GFP and PGL-1 antibodies. (**w**) The loop area of a gonad arm of $P_{Ltiar\text{-}1}$*tiar-1:gfp* transgenic animals showing P granule localization (arrowheads). (**x**) $P_{Ltiar\text{-}1}$TIAR-1:GFP is expressed diffusely in the cytoplasm in the gonad. $P_{Ltiar\text{-}1}$TIAR-1:GFP expression is slightly decreased in the most proximal oocytes, especially in the −1 oocyte. This low expression continues throughout early embryogenesis. d: distal; p: proximal. Pictures from **k** to **n** have different exposure to ensure the visualization of P granules and the nuclear localization.

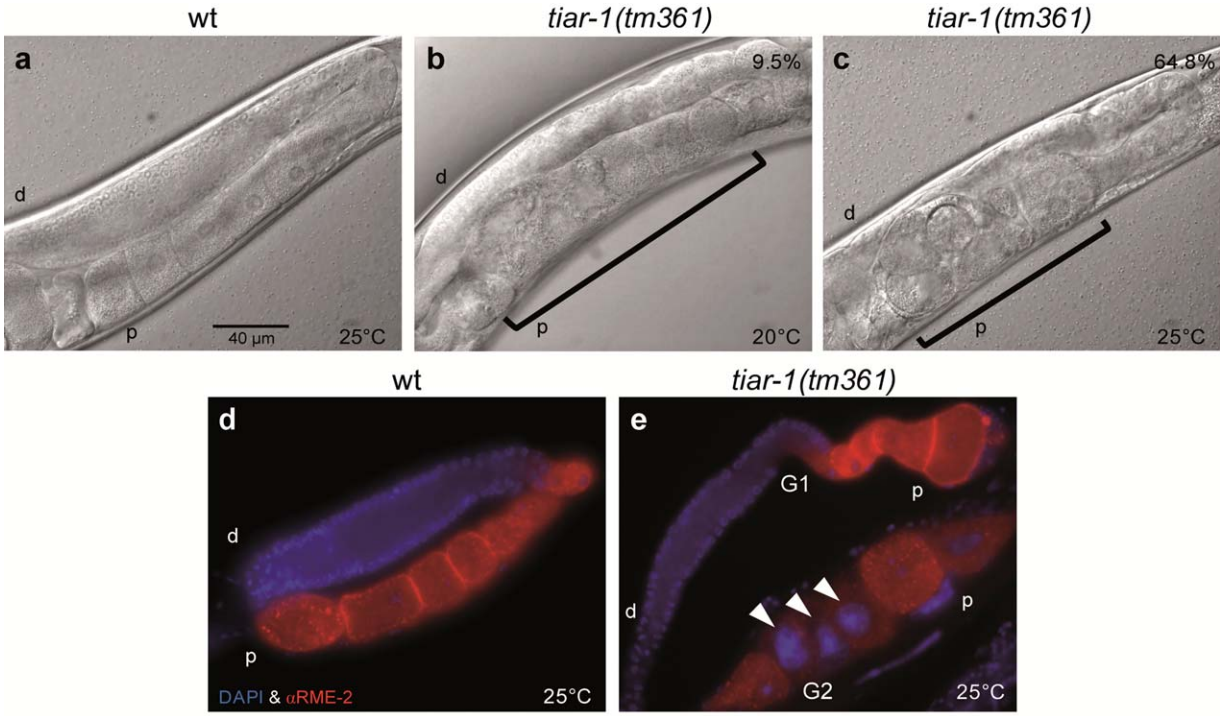

FIG. 3. Loss of *tiar-1* results in oogenesis defects. (**a–c**) Nomarski views of gonads from one-day-old wild-type (**a**) and *tiar-1(tm361)* (**b**,**c**) hermaphrodites grown at indicated temperatures. The percentage of animals with the depicted phenotype is shown in each picture. The clasps in **b**,**c** indicate abnormal oocytes. (**d,e**) Immunofluorescent images of extruded gonads from one-day-old wild-type and *tiar-1(tm361)* animals stained with an antibody against the yolk receptor protein RME-2 (red) and DAPI (blue). The arrowheads in **e** point to endomitotic oocytes. d: distal; p: proximal; G1: gonad 1; G2: gonad 2.

is also possible that the construct was lacking a DNA region required for germline expression.

Therefore, we generated a second construct carrying 1997 bp of the *tiar-1* promoter, 1966 bp of the *tiar-1* genomic sequence, *gfp* and 1500 bp of the *tiar-1* 3′ UTR ($P_{Ltiar\text{-}1}$*tiar-1:gfp;* the L denotes the longer promoter). $P_{Ltiar\text{-}1}$TIAR-1:GFP expression was similar to the expression of the native protein as measured by antibody staining in soma and germline, and also to the expression of the previous transgene (Supporting Information Fig. S1o,2k–n,x). Interestingly, we observed $P_{Ltiar\text{-}1}$TIAR-1:GFP associated with perinuclear granules (Fig. 2k,w; arrowheads). This association was only observed occasionally when we used antibody staining of the native protein (Fig. 2g and Supporting Information S1a–d). To test if observed perinuclear granules are P granules, we used PGL-1 as a marker (Kawasaki *et al.*, 1998). We immunostained $P_{Ltiar\text{-}1}$TIAR-1:GFP animals using GFP and PGL-1 antibodies and observed a colocalization between these two markers (Fig. 2o–v). These data suggest that TIAR-1 associates with P granules.

Occasionally, nuclear expression of $P_{Ltiar\text{-}1}$TIAR-1:GFP was observed in some embryos (Fig. 2n; arrows). Similar to what we observed using TIAR-1 antibody in inmunostainings, $P_{Ltiar\text{-}1}$TIAR-1:GFP expression appears reduced in the most proximal oocytes (−1 and −2 oocytes; Fig. 2x). This lower expression of TIAR-1 transgene continues through early embryogenesis (Fig. 2x).

To study solely TIAR-1 expression in the germline, we decided to use the *pie-1* promoter (Stitzel *et al.*, 2006). This construct carried 3845 bp of the *pie-1* promoter, *gfp*, 1966 bp of the *tiar-1* genomic sequence and 879 bp of 3′UTR ($P_{pie\text{-}1}$*gfp*:*tiar-1*), and it was expressed in the gonad in a manner similar to that of the native protein as measured by antibody staining (Supporting Information Fig. S1n,q,w–a′). The expression of this construct was observed occasionally associated with perinuclear granules in embryos (Supporting Information Fig. S1w,x). Similar to what we previously observed, the $P_{pie\text{-}1}$*gfp*:*tiar-1* transgene associates with perinuclear granules in certain conditions supporting the idea that the association of TIAR-1 to these structures is transient or easily affected.

TIAR-1 Is Important for Fertility

tiar-1(tm361) animals are shorter and move more slowly suggesting that TIAR-1 might have a somatic function, but in this manuscript, we will focus on its germline role. *tiar-1(tm361)* hermaphrodites show

temperature-sensitive sterility because they had 47.4% offspring and 7.8% embryonic lethality at 20°C, whereas at 25°C, they had only 0.7% offspring and almost no embryos were found (Table 1). We thus evaluated whether the low fertility was influenced by oocyte defects. We crossed *tiar-1(tm361)* hermaphrodites with *him-8(1489)* males. *him-8(e1489)* hermaphrodites produce 30% more males than wild-type animals, and they are often used for fertility experiments (Riddle *et al*., 1997). Crossed *tiar-1(tm361)* hermaphrodites produced 43.6% offspring and showed 7.2% embryonic lethality at 20°C (Table 1). This result suggests that oocyte defects are in part responsible for the low fertility observed in *tiar-1(tm361)* hermaphrodites.

Nomarski microscopy revealed that a low percentage of *tiar-1(tm361)* gonads presented atypical oocytes (9.5%) while the rest of gonads appeared wild-type. This phenotype increased at 25°C (64.8%; Fig. 3a–c). Using an antibody against the yolk receptor RME-2, which is an oocyte marker (Grant and Hirsh, 1999), and DAPI, we observed that some *tiar-1(tm361)* oocytes displayed enlarged nuclei with increased DNA content (Fig. 3d,e; arrowheads). This phenotype is known as Emo (endomitotic replication of the DNA within the oocyte) and is typically coupled with ovulation defects (Iwasaki *et al*., 1996). Our results suggest that TIAR-1 might play a role in oogenesis and/or ovulation.

We next investigated whether TIAR-1 is required for spermatogenesis. We first stained dissected gonads with DAPI to detect nuclei. *tiar-1(tm361)* hermaphrodites produced almost a wild-type number of spermatids at 25°C (94.2%; Table 1). We next analyzed the requirement for TIAR-1 in the male gonad. To obtain more males, we generated a double mutant: *tiar-1(tm361)*; *him-8(e1489)*. These double mutant males produced fewer sperm than *him-8(e1489)* males (48.7 and 34.7% at 20 and 25°C, respectively; Table 1). To assess the fertility of *tiar-1(tm361)*;*him-8(e1489)* males, we set up crosses with *fog-2(q71)* animals. *fog-2(q71)* animals exclusively produce oocytes, but they are fertile when crossed (Schedl and Kimble, 1988). *fog-2(q71)* females crossed with *tiar-1(tm361)*;*him-8(e1489)* males produced only 22.7% offspring and showed 4.4% embryonic lethality (Table 1).

Because *tiar-1(tm361)* animals exhibit a deficient locomotion, it is also possible that they cannot mate efficiently. To assess this possibility, we marked sperm mitochondria using Mitotracker (Life Technologies, Carlsbad, CA; Hill and L'Hernault, 2001). Only 11% of the *fog-2(q71)* females crossed with *tiar-1(tm361)*; *him-8(e1489)* males carrying labeled sperm, which indicates that these males could not cross efficiently. Despite this deficiency, whenever crosses were successful, animals had offspring although less than wild-type animals. Together these data suggest that TIAR-1 also plays a role during spermatogenesis.

We evaluated the fertility and embryonic lethality in the rescued animals. *tiar-1(tm361)*;$P_{Stiar\text{-}1}$*gfp*:*tiar-1* animals (somatic tissues expression) showed an improvement in fertility (82.3%) at 20°C; however, their embryonic lethality was high (31.6%; Table 1). These data suggest that there are some functions of TIAR-1 in somatic tissues, which can be related to the somatic gonad that influence fertility. *tiar-1(tm361)*;$P_{pie\text{-}1}$*gfp*:*tiar-1* animals (germline expression) had 26.1% offspring and 1.4% embryonic lethality at 20°C (Table 1). *tiar-1(tm361)*;$P_{Ltiar\text{-}1}$*tiar-1*:*gfp* animals (soma/germline expression) showed 59.8% fertility and 2.7% embryonic lethality at 20°C. The three rescued animals showed a slight improvement in fertility and embryonic development at 25°C (Table 1). It seems that TIAR-1 expression in the germline is important for embryo development because when an improvement in fertility was observed in *tiar-1(tm361)*;$P_{Stiar\text{-}1}$*gfp*:*tiar-1* animals (somatic tissues expression), embryonic lethality was higher.

There was an improvement in fertility when TIAR-1 was expressed in solely in the germline or in a soma/germline combination; however, it is unclear why the $P_{Ltiar\text{-}1}$*tiar-1*:*gfp* construct was unable to fully rescue these phenotypes. It is possible that some elements required for TIAR-1 function were still missing in $P_{Ltiar\text{-}1}$*tiar-1*:*gfp* construct or that the genomic region where transgene was inserted is affected. Another possible explanation is that the addition of the GFP to the TIAR-1 construct could have an effect on its function. Regardless, our data suggest that TIAR-1 expression in the soma and the germline is important for both embryogenesis and fertility.

TIAR-1 Is Required to Induce Germ Cell Apoptosis Under Several Types of Stresses

Six hours of food deprivation induces germ cell apoptosis in *C. elegans* although the gonad still remains healthy (Salinas *et al*., 2006). We originally found *tiar-1* in an RNAi screen to identify genes that regulate starvation-induced germ cell apoptosis. To test whether *tiar-1*, *tiar-2*, and *tiar-3* are involved in apoptosis, we crossed each mutant with the MD701 strain ($P_{tim\text{-}7}$*ced-1*:*gfp*), which is used for detection of germ cell engulfment (Schumacher *et al*., 2005). To induce starvation, we exposed the animals to 6 h of bacteria deprivation and then analyzed them under a fluorescent microscope. According to our previous findings, germ cell apoptosis increases by up to 2.0-fold in starved *ced-1*:*gfp* control animals (Fig. 4a and Table 2; Salinas *et al*., 2006). In control conditions, *tiar-1(tm361);ced-1*:*gfp* animals showed a slightly decreased number of physiological germ cell corpses but did not exhibit increased apoptosis under starvation (Fig. 4a and Table 2). *tiar-2(tm2923);ced-1*:*gfp* and *tiar-3(ok144);ced-1*:*gfp* animals showed normal levels of physiological and

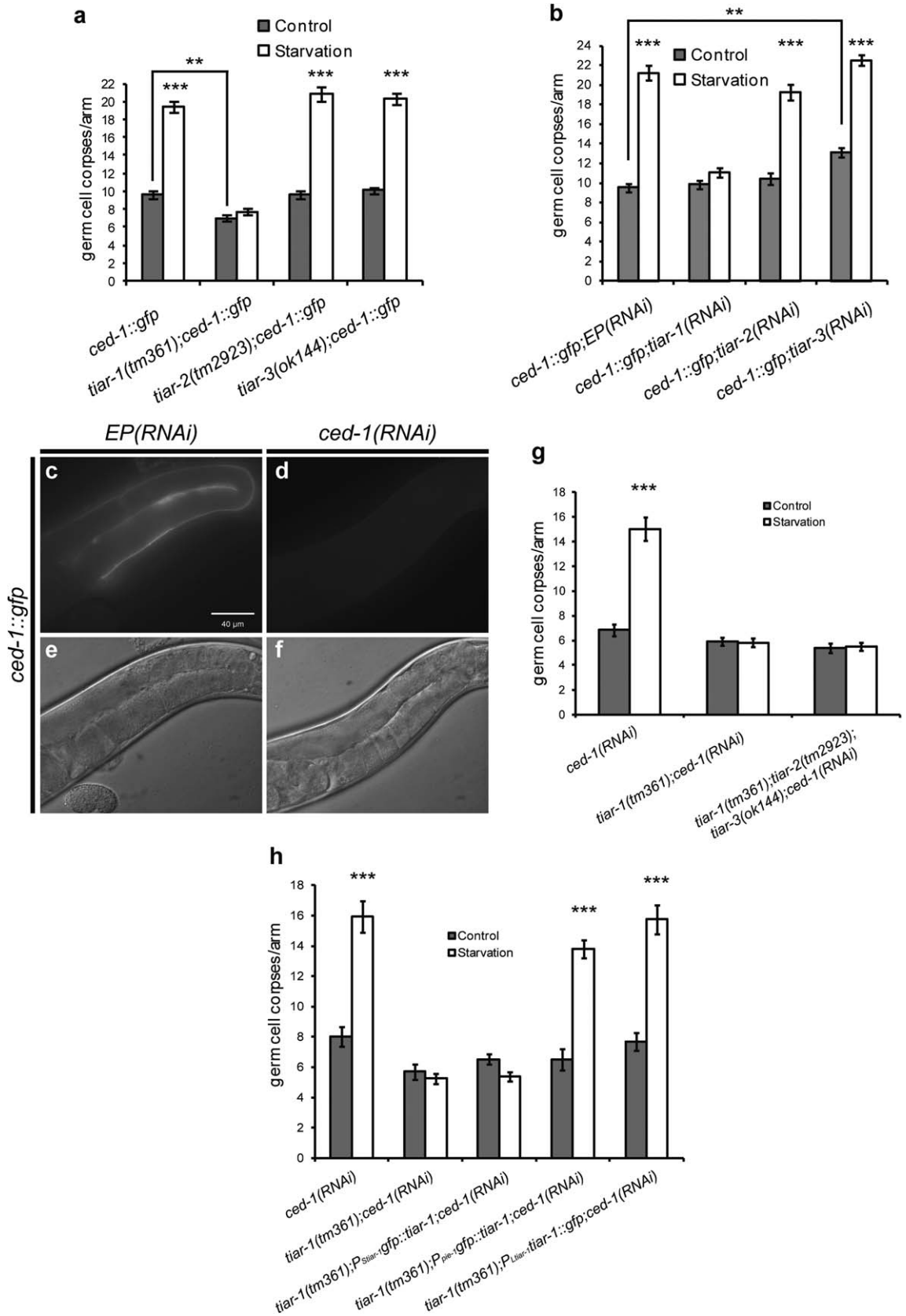
a
Control
Starvation
germ cell corpses/arm
ced-1::gfp
tiar-1(tm361);ced-1::gfp
tiar-2(tm2923);ced-1::gfp
tiar-3(ok144);ced-1::gfp
b
Control
Starvation
germ cell corpses/arm
ced-1::gfp;EP(RNAi)
ced-1::gfp;tiar-1(RNAi)
ced-1::gfp;tiar-2(RNAi)
ced-1::gfp;tiar-3(RNAi)
EP(RNAi)
ced-1(RNAi)
ced-1::gfp
c
d
e
f
40 µm
g
Control
Starvation
germ cell corpses/arm
ced-1(RNAi)
tiar-1(tm361);ced-1(RNAi)
tiar-1(tm361);tiar-2(tm2923);
tiar-3(ok144);ced-1(RNAi)
h
Control
Starvation
germ cell corpses/arm
ced-1(RNAi)
tiar-1(tm361);ced-1(RNAi)
tiar-1(tm361);Pstiar-1gfp::tiar-1;ced-1(RNAi)
tiar-1(tm361);Ppie-1gfp::tiar-1;ced-1(RNAi)
tiar-1(tm361);Ptiar-1tiar-1::gfp;ced-1(RNAi)

FIG. 4.

starvation-induced germ cell apoptosis (Fig. 4a and Table 2). To validate these data, we used RNAi to silence each gene in a *ced-1*:*gfp* background. We obtained similar results; except that we did not found a decrease number in physiological germ cell corpses in *ced-1:gfp;tiar-1(RNAi)* animals while *ced-1:gfp;tiar-3(RNAi)* animals showed a slightly increased number in this type of cell death (Fig. 4b and Table 2).

To test the redundancy of the *C. elegans* TIA-1/TIAR family of proteins in germ cell apoptosis, we silenced the *ced-1* gene in a triple mutant. Cell corpses can be detected under Nomarski microscopy directly and *ced-1* silencing or deletion aids their detection. CED-1 is a receptor involved in the engulfment process whose absence induces the accumulation of cell corpses (Zhou *et al*., 2001). First, we confirmed silencing efficiency by feeding *ced-1* dsRNA to MD701 animals and we observed that the *ced-1*:*gfp* expression decreased considerably its expression (Fig. 4c–f). Triple *tiar-1(tm361);tiar-2(tm2923);tiar-3(ok144);ced-1(gfp)* mutant animals did not show starvation-induced apoptosis even after two days of adulthood (Fig. 4g and Table 2) confirming that only TIAR-1 is required for this process.

We studied the somatic and germline contributions of TIAR-1 to starvation-induced germ cell apoptosis in animals rescued with transgenic lines. We found that two-day old *tiar-1(tm361);*$P_{Stiar\text{-}1}$*gfp:tiar-1;ced-1(RNAi)* animals (somatic tissues expression) did not show a significant increase in apoptosis in response to starvation (Fig. 4h and Table 2). In contrast, two-day old *tiar-1(tm361);*$P_{pie\text{-}1}$*gfp:tiar-1;ced-1(RNAi)* (germline expression) and *tiar-1(tm361);*$P_{Ltiar\text{-}1}$*tiar-1:gfp;ced-1(RNAi)* (somatic/germline expression) animals exhibited apoptosis during starvation (Fig. 4h and Table 2). Our results suggest that TIAR-1 germline expression is required for starvation-induced germ cell apoptosis.

We next evaluated whether TIAR-1 is necessary to induce apoptosis in other conditions like osmotic, oxidative, heat shock and UV stress (Derry *et al*., 2001; Salinas *et al*., 2006; Schumacher *et al*., 2001; see Materials and Methods for details). We found that TIAR-1 is required to induce apoptosis under all tested stress conditions (Fig. 5a and Table 2) suggesting that TIAR-1 might play a more general role during germ cell apoptosis.

TIAR-1 Induces Germ Cell Apoptosis Downstream of *ced-9*

Since TIAR-1 is required to induce germ cell apoptosis via several pathways that converge on CED-9, we decided to test if this protein is required for germ cell death when *ced-9* is silenced. CED-9 is the *C. elegans* homolog of BCL2, which protects cells from apoptosis by preventing the activation of CED-3/caspase by binding CED-4/APAF1 (Lettre and Hengartner, 2006). Thus, when CED-9 is absent, massive apoptosis is triggered, which in some cases can result in lethality (Hengartner *et al*., 1992). First, we evaluated *ced-9* silencing efficiency by semiquantitative RT-PCR and found that its expression was diminished ~50% in wild-type or *tiar-1(tm361)* backgrounds (Fig. 5b). This silencing efficiency is similar to that obtained by Ito *et al*. (2010).

As expected, *ced-9* silencing in *ced-1*:*gfp* control animals caused a significant increase in germ cell apoptosis (2.7-fold; Fig. 5c and Table 2). However, a less substantial increase (1.3-fold) in germ cell apoptosis was observed in *tiar-1(tm361);ced-1*:*gfp;ced-9(RNAi)* animals (Fig. 5c and Table 2). To confirm these results, we used acridine orange staining as an alternative apoptosis detection technique (Navarro *et al*., 2001). Wild-type control animals showed a mean count of 1.7 germ cell corpses per arm of the gonad, whereas *ced-9(RNAi)* animals contained 11.4 germ cell corpses/arm (a 6.7-fold increase; Fig. 5d and Table 2). In control *tiar-1(tm361)* animals, we detected a mean of 1.1 germ cell corpses/arm; however, when *ced-9* was silenced, we observed only 2.7 germ cell corpses/arm (a 2.5-fold increase) (Fig. 5d and Table 2). Our results demonstrate that TIAR-1 acts downstream of CED-9 to induce germ cell apoptosis.

TIAR-1 mammalian counterparts are well-characterized RNA binding proteins that act on mRNA to regulate splicing (Del Gatto-Konczak *et al*., 2000; Förch and Valcárcel, 2001; Förch *et al*., 2000, 2002; Izquierdo *et al*., 2005; Le Guiner *et al*., 2001; Zuccato *et al*., 2004) or stability (Yamasaki *et al*., 2007). Therefore, we tested whether the expression of genes that are downstream of *ced-9*, like *ced-4* and *ced-3*, was affected in the absence of *tiar-1*. We used *ced-3* and *ced-4* specific oligonucleotides to perform semiquantitative RT-PCR of cDNA obtained from wild-type and *tiar-1(tm361)* one-day-old animals that were well-fed or starved for 6 h. While *ced-3* does not seem to have alternative splicing forms, *ced-4* has two known as short (S) and long (L), which have opposite functions (Shaham and Horvitz, 1996). *ced-4S* (the predominant transcript) induce apoptosis, while *ced-4L* has antiapoptotic function (Shaham and Horvitz, 1996).

In wild-type animals and during starvation, *ced-4L* showed a minor increase in its accumulation (Fig. 5e)

FIG. 4. Starvation-induced germ cell apoptosis requires TIAR-1 germline expression. Animals of the indicated backgrounds were exposed to starvation conditions as described in the Materials and Methods. Cell corpses were counted under fluorescent (**a,b**) or DIC (**g** and **i**) microscopy. The numbers of germ cell corpses per gonad arm were counted in one-day-old (**a,b**) or two-day-old (**g,h**) living animals. We used RNAi to knock down *tiar-1*, *tiar-2*, and *tiar-3* (**b**) and *ced-1* (**c–h**), and empty plasmid (EP) was used as a control. (**c–f**) Fluorescence (**c, d**) or DIC (**e,f**) images of *ced-1*:*gfp;EP(RNAi)* and *ced-1*:*gfp;ced-1(RNAi)* animals. Error bars represent the SEM. *P*-values were determined by unpaired *t*-tests (Mann Whitney). See Table 2 for germ cell apoptosis number and statistics.

Table 2
Stress-Induced Germ Cell Apoptosis in Different Genetic Backgrounds

	Mean ± SE (n)					
Genotype	Control	Starvation	Salt	Paraquat	Heat Shock	UV
TIAR-1 is required to induce germ cell apoptosis under several stress conditions[a]						
ced-1:gfp	9.6 ±0.25 (180)	19.4 ± 0.56 (60) $P = < 0.0001$	16.2 ± 0.56 (40) $P = < 0.0001$	15.7 ± 0.70 (40) $P = < 0.0001$	15.7 ± 0.57 (40) $P = < 0.0001$	16.5 ± 0.73 (40) $P = < 0.0001$
tiar-1(tm361);ced-1:gfp	7.8 ± 0.21 (235) $P = < 0.0001$	7.7 ± 0.36 (60) $P = 0.9911$	8.8 ± 0.48 (60) $P = 0.0451$	8.0 ± 0.36 (60) $P = 0.4587$	7.6 ± 0.34 (60) $P = 0.9898$	7.0 ± 0.32 (60) $P = 0.1149$
tiar-2(tm2923);ced-1:gfp	9.7 ± 0.44 (60)	20.9 ± 0.81 (60) $P = < 0.0001$	ND	ND	ND	ND
tiar-3(ok144);ced-1:gfp	10.1 ± 0.44 (60)	20.3 ± 0.59 (60) $P = < 0.0001$	ND	ND	ND	ND
ced-1:gfp;EP(RNAi)	9.6 ± 0.46 (35)	21.3 ± 0.75 (34) $P = < 0.0001$	ND	ND	ND	ND
ced-1:gfp;tiar-1(RNAi)	9.9 ± 0.48 (53)	11.1 ± 0.48 (60) $P = 0.1265$	ND	ND	ND	ND
ced-1:gfp;tiar-2(RNAi)	10.5 ± 0.60 (30)	19.3 ± 0.77 (28) $P = < 0.0001$	ND	ND	ND	ND
ced-1:gfp;tiar-3(RNAi)	13.2 ± 0.48 (60) $P = < 0.0001$	22.6 ± 0.52 (60) $P = < 0.0001$	ND	ND	ND	ND
Only TIAR-1 is required for starvation-induced germ cell apoptosis[b]						
ced-1(RNAi)	6.8 ± 0.45 (30)	15.0 ± 0.96 (30) $P = < 0.0001$	ND	ND	ND	ND
tiar-1(tm361);ced-1(RNAi)	5.9 ± 0.32 (30)	5.8 ± 0.36 (30) $P = 0.7583$	ND	ND	ND	ND
tiar-1(tm361);tiar-2(tm2923); tiar-3(ok144);ced-1(RNAi)	5.4 ± 0.38 (30)	5.5 ± 0.34 (30) $P = 0.7247$	ND	ND	ND	ND
Starvation-induced germ cell apoptosis requires TIAR-1 germline expression[b]						
ced-1(RNAi)	8.0 ± 0.63 (30)	15.9 ± 1.0 (30) $P = < 0.0001$	ND	ND	ND	ND
tiar-1(tm361);ced-1(RNAi)	5.7 ± 0.63 (30)	15.9 ± 1.0 (30) $P = 0.8747$	ND	ND	ND	ND
tiar-1(tm361);$P_{Stiar\text{-}1}$*gfp:tiar-1;ced-1(RNAi)*	6.5 ±0.32 (30)	5.4 ± 0.31 (30) $P = 0.0142$	ND	ND	ND	ND
tiar-1(tm361); $P_{pie\text{-}1}$*gfp:tiar-1;ced-1(RNAi)*	6.5 ±0.69 (30)	13.8 ± 0.60 (30) $P = < 0.0001$	ND	ND	ND	ND
tiar-1(tm361);$P_{Ltiar\text{-}1}$*tiar-1:gfp;ced-1(RNAi)*	7.7 ±0.60 (30)	15.8 ± 0.95 (30) $P = < 0.0001$	ND	ND	ND	ND
TIAR-1 is required to induce germ cell apoptosis in the absence of CED-9[a]						
ced-1:gfp;EP(RNAi)	5.5 ±0.55 (50)	ND	ND	ND	ND	ND
ced-1:gfp;ced-9(RNAi)	14.9 ±0.59 (60) $P = < 0.0001$	ND	ND	ND	ND	ND
tiar-1(tm361); ced-1:gfp;EP(RNAi)	5.4 ± 0.75 (60)	ND	ND	ND	ND	ND
tiar-1(tm361); ced-1:gfp;ced-9(RNAi)	6.8 ± 0.49 (60) $P = 0.0295$	ND	ND	ND	ND	ND
N2;EP(RNAi)	1.7 ± 0.25 (50)	ND	ND	ND	ND	ND
N2;ced-9(RNAi)	11.4 ± 0.56(60) $P = < 0.0001$	ND	ND	ND	ND	ND
tiar-1(tm361);EP(RNAi)	1.1 ± 0.15 (60)	ND	ND	ND	ND	ND
tiar-1(tm361);ced-9(RNAi)	2.7 ± 0.31 (60) $P = 0.0002$	ND	ND	ND	ND	ND

[a]One- or [b]two-day-old animals of the indicated backgrounds were subjected to different stress conditions (see details in the Materials and Methods). When *ced-1:gfp* animals or acridine orange assay were used, cell corpses were detected by fluorescence microscopy. When the *ced-1(RNAi)* background was used, cell corpses were detected by DIC microscopy. The average number of cell corpses per gonad arm is shown with the SEM. The number of gonads evaluated (one per animal) is shown in parentheses. *P*-values were determined by unpaired *t*-tests (Mann Whitney). EP: empty plasmid; ND: not determined.

while *ced-4S* and *ced-3* did not show a significant difference on their expression. Unfortunately, we did not find significant differences in the accumulation levels of *ced-4S*, *ced-4L*, and *ced-3* mRNAs when *tiar-1* was absent neither in control or starvation conditions (Fig. 5e). Furthermore, we did not observe differences in the accumulation of the splicing forms of *ced-4L* vs *ced-4S* (Fig. 5e). These results suggest that TIAR-1 does not regulate the splicing or mRNA accumulation levels of *ced-4S*, *ced-4L*, and *ced-3* during normal or starvation conditions.

DISCUSSION

The TIA-1/TIAR protein family has been studied for several years in tissue culture (Anderson and Kedersha, 2002a,b; Buchan and Parker, 2009; Förch and Valcárcel, 2001; Kedersha and Anderson, 2007); however, relatively few studies have been performed in the context of a whole organism (Beck *et al*., 1998; Kharraz *et al*., 2010; Piecyk *et al*., 2000). Here, we show that the TIA-1/TIAR family has three homologs in *C. elegans* and demonstrate that TIAR-1 is the only one that has a conserved role in apoptosis. We showed that TIAR-1 is expressed in the cytoplasm of soma and germ cells where it occasionally associates with P granules. Although TIAR-1 appears to play important roles during larval development, locomotion, and embryogenesis, in this work, we focused on its function in the germline. We found that TIAR-1 is important for fertility and promotes germ cell apoptosis downstream of the *C. elegans* BCL2 homolog CED-9 under different stress conditions.

TIAR-1 Belongs to the TIA-1/TIAR Family of Proteins

TIAR-1 displays slightly higher amino acid structural identity with the human TIA-1 and TIAR (46% and 43%, respectively) than TIAR-2 (40% and 40%) or TIAR-3 (31% and 33%). Similar to the human TIA-1/TIAR proteins, TIAR-1 and TIAR-2 contain a prion-like domain and three RRM motifs. In contrast, TIAR-3 does not contain a prion-like domain and possesses only two RRM motifs (Fig. 1a). Furthermore, here we show that TIAR-1 is the only member of the *C. elegans* TIA-1/TIAR family of proteins that is required to induce germ cell apoptosis under different stress conditions (Figs. 4 and 5). TIAR-1 is ubiquitously expressed at all *C. elegans* developmental stages. Diffuse cytoplasmic expression was observed in most cells (Fig. 2 and Supporting Information S1). We found TIAR-1 occasionally associated with P granules in the germline (Fig. 2 and Supporting Information S1). Because we do not see, TIAR-1 always associated with P granules using immunostainings and/or in transgenic animals, we suggest that its association with these structures might be transient or sensitive to certain conditions or manipulations.

Members of the TIA-1/TIAR family localized to nuclei and cytoplasm in mammalian cells (Kedersha *et al*., 1999; Zhang, 2005). Although TIA-1 and TIAR proteins do not contain a NES (nuclear exportation sequence) domain, some of these proteins shuttle between the cytoplasm and the nucleus (Kedersha *et al*., 1999; Zhang, 2005). In the nuclei, members of the TIA-1/TIAR family participate in splicing (Del Gatto-Konczak *et al*., 2000; Förch and Valcárcel, 2001; Förch *et al*., 2000, 2002; Izquierdo *et al*., 2005; Le Guiner *et al*., 2001; Zuccato *et al*., 2004); while in the cytoplasm they regulated mRNA stability and translation (Kawai *et al*., 2006; Kuwano *et al*., 2008; Lopez de Silanes *et al*., 2005; Piecyk *et al*., 2000; Yamasaki *et al*., 2007).

Although in *C. elegans* TIAR-1 is primarily expressed in the cytoplasm and does not contain a NES domain; this protein was occasionally observed in embryonic nuclei (Fig. 2n), which suggests that it might shuttle between the cytoplasm and the nucleus under certain conditions. Similarly, a previous report showed nuclear accumulation of TIAR-2 in oocytes (Jud *et al*., 2008). The main localization of TIAR-1 into the cytoplasm suggests that in *C. elegans* this protein could play a more important role in functions related to mRNA stability or repression and not to splicing. Although it is possible that TIAR-1 needs a specific interaction or condition to be located in the nuclei.

TIAR-1 Is Important for Fertility

tiar-1 ablation results in short animals with slow locomotion, embryonic lethality and germline defects. Among germline phenotypes are temperature-sensitive fertility defects due to oogenesis and spermatogenesis problems (Fig. 3 and Table 1). At 25°C some *tiar-1(tm361)* animals showed oocytes that appeared abnormal and present an Emo phenotype (Fig. 3e), which typically occurs in cases of oocyte maturation/ovulation defects (Iwasaki *et al*., 1996). *tiar*$^{-/-}$ mice are also sterile, but this deficiency is attributed to a lack of spermatogonia/oogonia (Beck *et al*., 1998). In *tiar*$^{-/-}$ mice, primordial germ cells are reduced in number but still migrate to the genital ridge, although they are not eliminated by apoptosis (Beck *et al*., 1998). Accordingly, with this, we did not observe an increase in physiological germ cell apoptosis in *tiar-1(tm361)* hermaphrodites (Fig. 4a). The variability in the somatic and germline phenotypes of *tiar-1(tm361)* animals and their mammalian counterparts may be due to the variety in the downstream targets of affected proteins (Kim *et al*., 2007; Lopez de Silanes *et al*., 2005).

TIAR-1 Induces Germ Cell Apoptosis Downstream of CED-9

TIA-1 and TIAR were originally described as cytotoxic granule-associated RNA-binding proteins with pro-

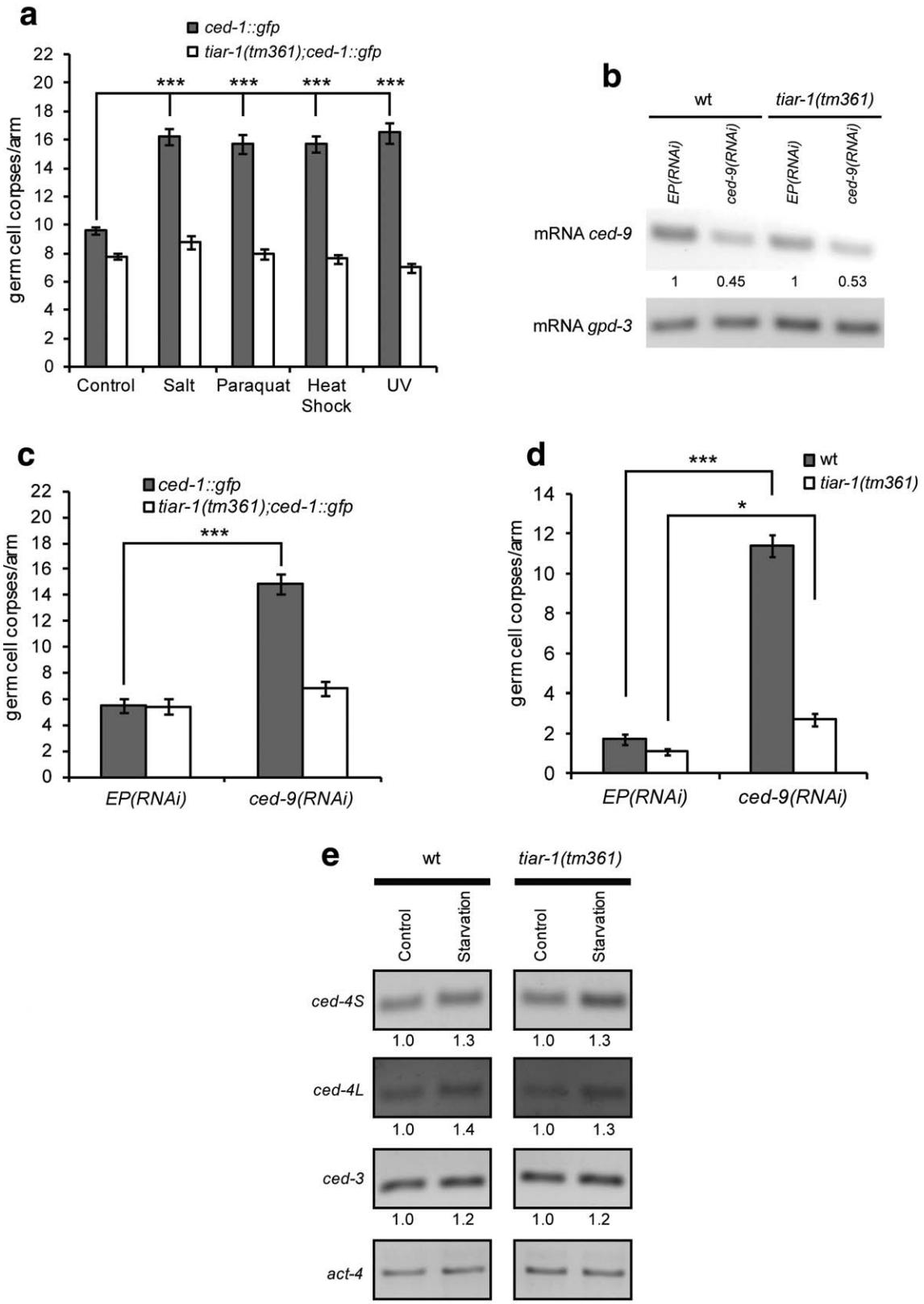
a
ced-1::gfp
tiar-1(tm361);ced-1::gfp
germ cell corpses/arm
***
Control
Salt
Paraquat
Heat Shock
UV
b
wt
tiar-1(tm361)
EP(RNAi)
ced-9(RNAi)
mRNA ced-9
1
0.45
1
0.53
mRNA gpd-3
c
ced-1::gfp
tiar-1(tm361);ced-1::gfp
germ cell corpses/arm
EP(RNAi)
ced-9(RNAi)
d
wt
tiar-1(tm361)
*
e
wt
tiar-1(tm361)
Control
Starvation
ced-4S
1.0
1.3
ced-4L
1.0
1.4
ced-3
1.0
1.2
act-4

FIG. 5.

apoptotic functions (Kawakami *et al*., 1992; Tian *et al*., 1991). One of the mechanisms by which TIA-1 induces apoptosis in mammals is by promoting an alternatively spliced form of the pro-apoptotic Fas receptor (Förch *et al*., 2000; Izquierdo *et al*., 2005). To our knowledge, *C. elegans* has no Fas homolog; therefore, TIAR-1 must function through a different pathway and here we demonstrate that TIAR-1 acts downstream of CED-9/BCL2 to induce germ cell apoptosis (Fig. 5d). We also show that TIAR-1 is required to induce germ cell apoptosis under several stress conditions that are activated via different pathways (Figs. 4 and 5; Derry *et al*., 2001; Salinas *et al*., 2006; Schumacher *et al*., 2001) suggesting that TIAR-1 might have a general role regulating germ cell death.

Although mammalian TIA-1 and TIAR had been implicated as splicing regulators (Del Gatto-Konczak *et al*., 2000; Förch and Valcárcel, 2001; Förch *et al*., 2000, 2002; Izquierdo *et al*., 2005; Le Guiner *et al*., 2001; Zuccato *et al*., 2004), we found that TIAR-1 does not seem to regulate the alternative splicing forms of *ced-4* to induce germ cell apoptosis (Fig. 5e). This idea is supported by the fact that we barely found TIAR-1 in germ cells nuclei (Figs. 2 and Supporting Information S1). Members of the TIA-1/TIAR family of proteins can also regulate the stability of certain mRNAs (Yamasaki *et al*., 2007); however, TIAR-1 does not seem to be regulating the accumulation of *ced-4L*, *ced-4S* or *ced-3* mRNAs under normal or stress conditions (Fig. 5e).

In mammals, during normal conditions the cytochrome *c* mRNA is being actively translated, but when cells are subject to stress TIA-1 represses its translation (Kawai *et al*., 2006). Therefore, a possible scenario is that in *C. elegans* TIAR-1 could be regulating *ced-4L*, *ced-4S*, or *ced-3* mRNA translation. Finally, it is also possible that TIAR-1 is regulating the splicing, stability or translation of one of the *ced-4L*, *ced-4S*, or *ced-3* regulators to induce stress-induced germ cell apoptosis.

Despite the fact that the *tiar-1(tm361)* phenotype is pleiotropic, these animals did not have increased physiological germ cell apoptosis (Fig. 4). Indeed, we observed that *tiar-1(tm361);ced-1*:*gfp* animals showed slightly lower physiological germ cell apoptosis (Fig. 4a) suggesting that might play a minor role in physiological germ cell apoptosis. Although it has been shown that sometimes somatic tissues can influence germ cell apoptosis (Ito *et al*., 2010), we found that TIAR-1 expression solely in the germline is sufficient to trigger germ cell apoptosis (Fig. 4h).

Our data suggest a scenario where TIAR-1 converges on the same regulatory point downstream of CED-9 to induce germ cell apoptosis under conditions like starvation, heat shock, DNA damage, osmotic and oxidative stress, which are controlled via different regulatory pathways (Fig. 6). Since TIAR-1 is a putative RNA-binding protein whose mammalian homologs regulate mRNA splicing, expression or degradation, it is possible that this protein regulates one of these aspects in a key component of the apoptosis machinery to induce apoptosis under stress.

MATERIALS AND METHODS

Nematode Culture and RNAi

The maintenance and genetic manipulation of *C. elegans* strains (Supporting Information Table S1) derived from the wild-type Bristol strain N2 were following standard conditions (Brenner, 1974) at indicated temperatures (20, 24, or 25°C).

RNAi was performed by feeding as previously described (Timmons *et al*., 2001). RNAi constructs for the *tiar-1*, *tiar-2* and *ced-1* genes were obtained from the *C. elegans* RNAi v1.1 feeding library (Open Biosystems). The following genes were cloned using the indicated primers: *tiar-3* (5′-ATGTGCTTACCAACTGCG ACTCC-3′ and 5′-TCACCATTGTGGGAAGGAACTG-3′) and *ced-9* (5′-GGAATTCCCGATATTCGAGAAGAAGCA CG-3′ and 5′-GGGGTACCCCTTACTTCAAGCTGAAC ATCATC-3′). The products were digested with EcoRI and KpnI and cloned into the feeding vector (PD129.36), and the resulting plasmid was transformed into *Escherichia coli* strain HT115.

Antibody Production and Western Blot Analysis

Rabbit polyclonal TIAR-1 antibodies were raised against the TIAR-1 peptide QEKPSHYNEKSYDE (residues 218 to 231). The peptide was synthesized (Sigma) and injected into two rabbits according to Sigma protocols. The TIAR-1 antibody was purified from the rabbit antisera by TIAR-1 peptide affinity column (Sulfolink, Pierce).

For Western blot analysis, 100 *C. elegans* hermaphrodites from the indicated backgrounds were grown at 20 or 25°C, collected and washed in PBS 1× with loading

FIG. 5. TIAR-1 is required to induce germ cell apoptosis under several stress conditions and in the absence of CED-9. Animals of the indicated backgrounds were exposed to stress conditions as described in the Materials and Methods. (**a**,**c**,**d**) Germ cell corpses were counted under fluorescent microscopy using a *ced-1*:*gfp* background (**a,c**) and acridine orange (**d**). The numbers of germ cell corpses per gonad arm were counted in one-day-old living animals. (**b**) Semi-quantitative RT-PCR to evaluate *ced-9* silencing efficiency. (**b–d**) We used RNAi to knock down *ced-9* expression and an empty plasmid (EP) was used as a control. Error bars represent the SEM. *P*-values were determined by unpaired *t*-tests (Mann Whitney); see Supporting Information Table S1. (**e**) Semiquantitative RT-PCR to evaluate mRNA expression levels of *ced-4S*, *ced-4L*, and *ced-3* in wild-type and *tiar-1(tm361)* animals in control and starvation conditions.

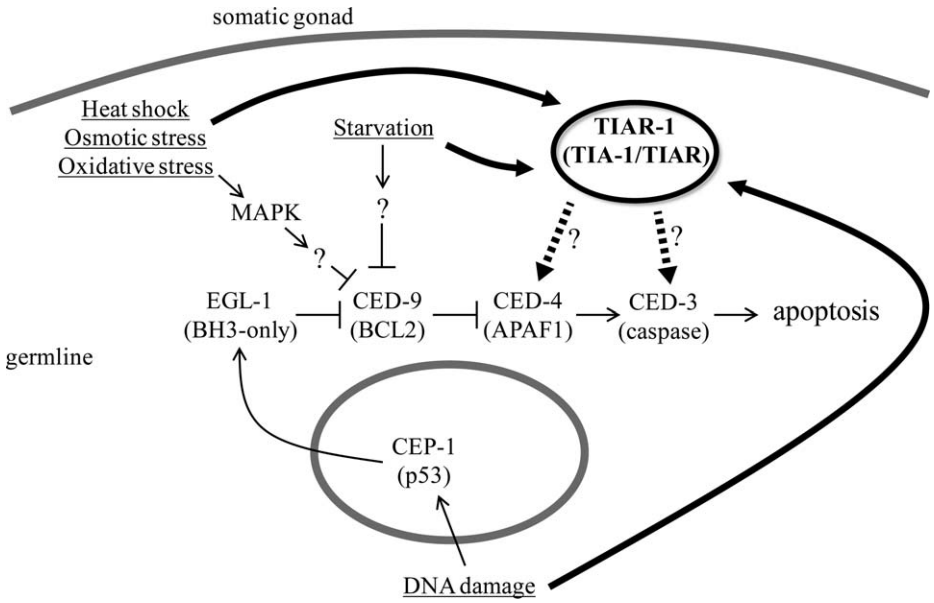

FIG. 6. Model depicting *tiar-1* germline requirement for stress-induced germ cell apoptosis. Because TIAR-1 acts downstream of CED-9 to induce apoptosis, we hypothesize that this protein induces germ cell apoptosis by regulating the mRNA splicing, stability, or translation of *ced-4*, *ced-3*, or some of their regulators (dotted lines).

buffer and DTT (174 mM). To extract protein, samples were boiled for 5 min and spun for 1 min at 13,000 rpm. The supernatant was loaded into 10% polyacrylamide gel, which was subsequently transferred onto an Immobilon-P PVDF membrane (Millipore) following standard procedures. Membranes were initially incubated with the TIAR-1 antibody (1:500) and then with a secondary HRP-conjugated goat anti-rabbit antibody (1:1000; Pierce). Bound antibody was detected using Super Signal West Pico Chemiluminescent Substrate (Pierce). An α-tubulin monoclonal antibody (Sigma, T9026) was used as a loading control. The TIAR-1 antibody obtained from the first rabbit only recognized one specific band in Western blotting experiments (Fig. 1c), but the TIAR-1 antibody obtained from the second rabbit recognized two main bands of ∼46 kDa and ∼65 kDa (Supporting Information Fig. S2). The ∼46 kDa band appeared to be the TIAR-1 protein, but the other band (∼65 kDa) appeared to be non-specific because it was still present in protein extracts from a triple *tiar-2(tm2923);tiar-1(tm361);tiar-3(ok144)* mutant (Supporting Information Fig. S2). Because this antibody did not specifically recognize the TIAR-1 protein, it was not used in subsequent experiments.

Immunofluorescence Analysis

For TIAR-1, PGL-1 and RME-2 immunolocalization in gonads and embryos, samples were processed as previously described (Salinas *et al.*, 2007). Briefly, extruded gonads or embryos from one-day-old gravid adults grown at 20 or 25°C were frozen in liquid nitrogen, freeze-cracked, fixed in methanol at −20°C for 1 min and treated with 1× PBS, 3.7% paraformaldehyde, 80 mM HEPES, 1.6 mM MgSO4, and 0.8 mM EGTA for 30 min at room temperature. The affinity-purified rabbit anti-TIAR-1 antibody was applied at a 1:100 dilution, the PGL-1 and RME-2 antibodies were diluted as in previous studies (Grant and Hirsh, 1999; Kawasaki *et al.*, 1998). Cy3-conjugated goat anti-rat (Jackson ImmunoResearch Laboratories) and Alexa Fluor 488-conjugated goat anti-rabbit secondary antibodies (Molecular Probes) were used at a concentration of 1:100, and 4′,6-diamidino-2-phenylindole DAPI was used at a concentration of 1 ng μl^{-1}.

Whole-mount immunofluorescence was performed using the Finney-Ruvkun protocol (Finney and Ruvkun, 1990). Briefly, animals grown at 20°C were suspended in Ruvkun's fixing solution (80 mM KCl, 20 mM NaCl, 10 mM EGTA, 5 mM spermidine, 15 mM PIPES, 25% methanol, and 1% paraformaldehyde) and frozen in a dry ice-ethanol bath. Samples were then treated with 1% β-mercaptoethanol followed by 10 mM DTT and finally by 0.3% hydrogen peroxide. Samples were initially incubated with the rabbit anti-TIAR-1 antibody (1:100) and then with 1 ng μl^{-1} DAPI and an Alexa Fluor 488-conjugated goat anti-rabbit antibody (1:100). Samples were mounted in Vectashield fluorescence medium (Vector Laboratories).

Transgenic fluorescent animals were anesthetized with 40 mM NaN_3, mounted on agar pads and observed under fluorescence microscopy. Images from immunostained samples and $P_{Stiar\text{-}1}$*gfp*:*tiar-1*, $P_{pie\text{-}1}$*gfp*:*tiar-1*, and $P_{Ltiar\text{-}1}$*tiar-1*:*gfp* transgenic animals were obtained

by MRGrab 1.0.0.4 program on a Nikon Eclipse E600 microscope equipped with an AxioCam MRc digital camera (Zeiss). All images were processed to improve their contrast and brightness in the ImageJ 1.47g program.

Phenotype Studies

To study the germline defects of *tiar-1(tm361)* mutants, one-day-old adults grown at 20 or 25°C were anesthetized (40 mM NaN_3), mounted on agar pads and observed under Nomarski microscopy [Nikon Eclipse E600 microscope equipped with an AxioCam digital camera (Zeiss)] to determine their phenotype. Because the *tiar-1(tm361)* animals are sterile at 25°C, we incubated L1 larvae at 25°C and allowed them to progress to adult stages.

To study the fertility of *tiar-1(tm361)* mutants, hermaphrodites were individually selected as L4 specimens and then transferred to new plates every 24 h over the course of 3 (25°C) or 4 (20°C) days. Plates were scored for dead embryos and surviving offspring. Embryos not hatched within 24 h after being laid were considered dead. N2 was used as the control.

To obtain *tiar-1(tm361)* males, we employed the *him-8(e1489)* strain, whose hermaphrodites produce ~30% male progeny. To test the fertility of *tiar-1(tm361);him-8(e1489)* males, we crossed three animals with one *fog-2(q71)* hermaphrodite for 24 h at 20°C and scored fertility, embryonic lethality and successful mating. *him-8(e1489)* animals were used as the control.

To count sperm, L4 males were incubated at 20 or 25°C for 24 h to allow them to reach adulthood. Male gonads were then dissected, and extruded gonads were fixed as described for immunofluorescence analysis. The samples were then incubated for 5 min in 1× PBT with 1 ng μl^{-1} DAPI. Stained gonads were visualized using a Nikon Eclipse E600 microscope equipped with an AxioCam digital camera (Zeiss).

Mating efficiency was evaluated as previously described (Hill and L'Hernault, 2001). Briefly, young adult males were incubated in a dark chamber for 3 h at 20°C in the presence of M9 medium containing 75 μM mitotracker (Invitrogen). These males were then crossed and left overnight at 20°C. Sperm transference into uteri and/or spermathecae was scored under fluorescence microscopy. The reported mating efficiency reflects the proportion of successful mating events (i.e., when fluorescent sperm was detected in the uterus of a female) relative to the total number of crosses.

Transgenic Line Constructions

The *tiar-1* transgenes were constructed by Gateway cloning (Invitrogen) using the following regions and primers: *tiar-1* short promoter (5′-GGGGACAACTTTGTATAGAAAAGTTGCTGGCTGAAACTTAAAATTATTTG-3′ and 5′-GGGGACTGCTTTTTTGTACAAACTTGTCATGGCTTAAATGATAGATCCCTG-3′; 300 bp), *tiar-1* ORF + *tiar-1* 3′UTR (485bp) (5′-GGGGACAGCTTTCTTGTACAAAGTGGCCATGTCCTTCTTCAACCCACCAGC-3′ and 5′-GGGGACAACTTTGTATAATAAAGTTGACTTTACCCTCGCATTTATTATTGTGC-3′; 1712 bp), *tiar-1* gene + *tiar-1* 3′UTR (879bp) (5′-GGGGACAGCTTTCTTGTACAAAGTGGCCATGTCCTTCTTCAACCCACCAGC-3′ and 5′-GGGGACAACTTTGTATAATAAAGTTGAGAAGCATTCTGTGTCACATTAATCAG-3′; 2845 bp), *tiar-1* long promoter + *tiar-1* gene (5′-GGGGACAACTTTGTATAGAAAAGTTGCTGGCTGAAACTTAAAATTATTTG-3′ and 5′-GGGGACTGCTTTTTTGTACAAACTTGTTTGATGTCCTCCAGAGTTCTGTTG-3′; 3963 bp), and *tiar-1* 3′UTR (1500bp) (5′-GGGGACAGCTTTCTTGTACAAAGTGGCCTAAACTCCAGTATTTTTTAAAAAC-3′ and 5′-GGGGACAACTTTGTATAATAAAGTTGACATATTAGTCACAACGGTTTCTCG-3′; 1500 bp). *tiar-1* constructs were introduced by microparticle bombardment (Praitis *et al.*, 2001).

Stress Conditions

Stress conditions were induced as reported previously (Salinas *et al.*, 2006). Briefly, to simulate starvation conditions, one-day-old animals were transferred to NGM plates with or without food and incubated for 6 h. One-day-old animals were incubated for 1 h in control M9 medium or M9 containing 10 mM paraquat (oxidative stress) or 70 mM sodium chloride (osmotic stress); following incubation, the animals were transferred to seeded NGM plates and allowed to recover for 1 h. To induce germ cell apoptosis by heat shock, the animals were incubated at 31°C in a water bath for 3 h and then allowed to recover for 4.5 h. To induce DNA damage, animals were exposed to 0.1 J of UV light (Stratalinker, model 1800) and then allowed to recover for 3.5 h. After every treatment, animals of the appropriate strain were anesthetized and mounted for visualization of cell corpses.

Apoptosis Assays

Cell corpses were counted using Nomarski optics (for *ced-1(RNAi)* experiments) or fluorescence microscopy (for *ced-1:gfp* strains and acridine orange assays) For acridine orange (AO) staining, animals were incubated in 200 ml of 100 μM AO in M9 for 2 h in the dark and then fed for 1 h to decrease background gut fluorescence. After treatment, the animals were anesthetized with 40 mM NaN_3, mounted on agar pads and observed to visualize cell corpses. All statistical analyses were performed using Prism (GraphPad Software). All error bars indicate SEM. All *t*-tests are two-tailed unpaired *t*-tests (Mann Whitney).

RT-PCR

Synchronized animals grown under the described conditions were collected in M9 medium and lysed in

TRIzol buffer (Invitrogen). RNA was extracted from each sample using standard phenol-chloroform-based procedures. cDNA synthesis was performed using the standard reverse transcription protocol from Promega. To analyze mRNA levels, RT-PCR was performed using specific primers for *ced-9*: 5′-AAACGGAATGGAATG GTTTG-3′ and 5′-AATTTTCCGCGTGCTTCTT-3′; *ced-4S* and *ced-4L*: 5′-TTCGAGAGTATCACGTGGATCGAG-3′ and 5′- TACAACTGACGTGACATGCTCCAC-3′; *ced-3*: 5′-GAGCAAGATCTCGT TCTCGATCG-3′ and 5′-GTCGA AAACACGGCTTATGGTTG-3′. Glyceraldehyde 3-phosphate dehydrogenase and actin genes (*gpd-3* and *act-4*) were used as loading control with the following primers: *gpd-3*: 5′-CAGCACAAGATCAAGGTCTACAAC-3′ and 5′-GAAGACTCCGGTGGACTCAAC-3′; and *act-4*: 5′-ATGT GTGACGACGAGGTTGCC-3′ and 5′-CTGGAAGAGAGC CTCTGGGC-3′. The sizes of amplified fragments were 100 bp for *ced-9*, 295 bp for *ced-4S*, 361 bp for *ced-4L*, 247 bp for *ced-3*, 792 bp for *act-4* and 100 bp for *gpd-3*.

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REFERENCES

Aballay A, Ausubel FM. 2001. Programmed cell death mediated by *ced-3* and *ced-4* protects *Caenorhabditis elegans* from *Salmonella typhimurium*-mediated killing. Proc Natl Acad Sci 98:2735–2739.

Anderson P, Kedersha N. 2002a. Stressful initiations. J Cell Sci 115:3227–3234.

Anderson P, Kedersha N. 2002b. Visibly stressed: The role of eIF2, TIA-1, and stress granules in protein translation. Cell Stress Chaperones 7:213–221.

Andux S, Ellis RE. 2008. Apoptosis Maintains Oocyte Quality in Aging *Caenorhabditis elegans* Females. PLoS Genet 4:1–13.

Barton MK, Schedl T, Kimble J. 1987. Gain-of-Function Mutations of *fem-3*, a Sex-Determination Gene in *Caenorhabditis elegans*. Genetics 115:107–119.

Baum JS, St. George JP, McCall K. 2005. Programmed cell death in the germline. Semin Cell Dev Biol 16: 245–259.

Beanan MJ, Strome S. 1992. Characterization of a germline proliferation mutation in *C. elegans*. Development 116:755–766.

Beck ARP, Miller IJ, Anderson P, Streuli M. 1998. RNA-binding protein TIAR is essential for primordial germ cell development. Proc Natl Acad Sci USA 95: 2331–2336.

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

Buchan JR, Parker R. 2009. Eukaryotic stress granules: The ins and outs of translation. Mol Cell 36:932–941.

Del Gatto-Konczak F, Bourgeois CF, Guiner CL, Kister L, Gesnel MC, Stévenin J, Breathnach R. 2000. The RNA-binding protein TIA-1 is a novel mammalian splicing regulator acting through intron sequences adjacent to a 5′ splice site. Mol Cell Biol 20:6287–6299.

Derry WB, Putzke AP, Rothman JH. 2001. *Caenorhabditis elegans* p53: Role in apoptosis, meiosis, and stress resistance. Science 294:591–595.

Finney M, Ruvkun G. 1990. The *unc-86* gene product couples cell lineage and cell identity in *C. elegans*. Cell 63:895–905.

Förch P, Puig O, Kedersha N, Martínez C, Granneman S, Séraphin B, Anderson P, Valcárcel J. 2000. The apoptosis-promoting factor TIA-1 is a regulator of alternative pre-mrna splicing. Mol Cell 6:1089–1098.

Förch P, Puig O, Martínez C, Séraphin B, Valcárcel J. 2002. The splicing regulator TIA-1 interacts with U1-C to promote U1 snRNP recruitment to 5′ splice sites. EMBO J 21:6882–6892.

Förch P, Valcárcel J. 2001. Molecular mechanisms of gene expression regulation by the apoptosis-promoting protein TIA-1. Apoptosis 6:463–468.

Gilks N, Kedersha N, Ayodele M, Shen L, Stoecklin G, Dember LM, Anderson P. 2004. Stress granule assembly is mediated by prion-like aggregation of TIA-1. Mol Biol Cell 15:5383–5398.

Grant B, Hirsh D. 1999. Receptor-mediated Endocytosis in the *Caenorhabditis elegans* Oocyte. Mol Cell Biol 10:4311–4326.

Gumienny TL, Lambie E, Hartwieg E, Horvitz HR, Hengartner MO. 1999. Genetic control of programmed cell death in the *Caenorhabditis elegans* hermaphrodite germline. Development 126:1011–1022.

Hengartner MO, Ellis RE, Horvitz HR. 1992. *Caenorhabditis elegans* gene ced-9 protects cells from programmed cell death. Nature 356:494–499.

Hill KL, L'Hernault SW. 2001. Analyses of reproductive interactions that occur after heterospecific matings

within the genus Caenorhabditis. Dev Biol 232:105–114.

Ito S, Greiss S, Gartner A, Derry WB. 2010. Cell-nonautonomous regulation of *C. elegans* germ cell death by kri-1. Curr Biol 20:333–338.

Iwasaki K, Mcearter J, Francis R, Schedl T. 1996. *emo-1*, a *Caenorhabditis elegans* Sec61p/homologue, is required for oocyte development and ovulation. J Cell Biol 134:699–714.

Izquierdo JM, Majós N, Bonnal S, Martínez C, Castelo R, Guigó R, Bilbao D, Valcárcel J. 2005. Regulation of Fas alternative splicing by antagonistic effects of TIA-1 and PTB on exon definition. Mol Cell 19:475–484.

Jud MC, Czerwinski MJ, Wood MP, Young RA, Gallo CM, Bickel JS, Petty EL, Mason JM, Little BA, Padilla PA, Schisa JA. 2008. Large P body-like RNPs form in *C. elegans* oocytes in response to arrested ovulation, heat shock, osmotic stress, and anoxia and are regulated by the major sperm protein pathway. Dev Biol 318:38–51.

Kawai T, Lal A, Yang X, Galban S, Mazan-Mamczarz K, Gorospe M. 2006. Translational control of cytochrome c by RNA-binding proteins TIA-1 and HuR. Mol Cell Biol 26:3295–3307.

Kawakami A, Tian Q, Duan X, Streuli M, Schlossman SF, Anderson P. 1992. Identification and functional characterization of a TIA-1-related nucleolysin. Proc Natl Acad Sci USA 89:8681–8685.

Kawasaki I, Shim Y-H, Kirchner J, Kaminker J, Wood WB, Strome S. 1998. PGL-1, a predicted RNA-binding component of germ granules, is essential for fertility in *C. elegans*. Cell 94:635–645.

Kedersha N, Anderson P. 2007. Mammalian stress granules and processing bodies. Methods Enzymol 431: 61–81.

Kedersha N, Gupta M, Li W, Miller I, Anderson P. 1999. RNA-binding proteins TIA-1 and TIAR link the phosphorylation of eIF-2α to the assembly of mammalian stress granules. J Cell Biol 147:1431–1441.

Kelly WG, Xu S, Montgomery MK, Fire A. 1997. Distinct requirements for somatic and germline expression of a generally expressed *Caenorhabditis elegans* gene. Genetics 146:227–238.

Kharraz Y, Salmand P-A, Camus A, Auriol J, Gueydan C, Kruys V, Morello D. 2010. Impaired embryonic development in mice overexpressing the RNA-binding protein TIAR. PLoS One 5:1–12.

Kim HS, Kuwano Y, Zhan M, Pullmann R, Mazan-Mamczarz K, Li H, Kedersha N, Anderson P, Wilce MCJ, Gorospe M, Wilce JA. 2007. Elucidation of a C-Rich Signature Motif in Target mRNAs of RNA-Binding Protein TIAR. Mol Cell Biol 27:6806–6817.

Kimble J, Edgar L, Hirsh D. 1984. Specification of male development in *Caenorhabditis elegans*: The fem genes. Dev Biol 105:234–239.

Kuwano Y, Kim HH, Abdelmohsen K, Pullmann R Jr., Martindale JL, Yang X, Gorospe M. 2008. MKP-1 mRNA stabilization and translational control by RNA-binding proteins HuR and NF90. Mol Cell Biol 28:4562–4575.

Le Guiner C, Galiana D, Kister L, Breathnach R, Stévenin J, Del Gatto-Konczak F. 2001. TIA-1 and TIAR activate splicing of alternative exons with weak 5′ splice sites followed by a U-rich stretch on their own Pre-mRNAs. J Biol Chem 276:40638–40646.

Lettre G, Hengartner MO. 2006. Developmental apoptosis in *C. elegans*: A complex CEDnario. Nat Rev Mol Cell Biol 7:97–108.

Li W, Simarro M, Kedersha N, Anderson P. 2004. FAST is a survival protein that senses mitochondrial stress and modulates TIA-1-regulated changes in protein expression. Mol Cell Biol 24:10718–10732.

Lopez de Silanes I, Galban S, Martindale JL, Yang X, Mazan-Mamczarz K, Indig FE, Falco G, Zhan M, Gorospe M. 2005. Identification and functional outcome of mRNAs sssociated with RNA-binding protein TIA-1. Mol Cell Biol 25:9520–9531.

Navarro RE, Shim EY, Kohara Y, Singson A, Blackwell TK. 2001. *cgh-1*, a conserved predicted RNA helicase required for gametogenesis and protection from physiological germline apoptosis in C. elegans. Development 128:3221–3232.

Piecyk M, Wax S, Beck ARP, Kedersha N, Gupta M, Maritim B, Chen S, Gueydan C, Kruys V, Streuli M, Anderson P. 2000. TIA-1 is a translational silencer that selectively regulates the expression of TNF-α. EMBO J 19:4154–4163.

Praitis V, Casey E, Collar D, Austin J. 2001. Creation of Low-Copy Integrated Transgenic Lines in *Caenorhabditis elegans*. Genetics 157:1217–1226.

Riddle DL, Blumenthal T, Meyer BJ, Priess JR, editors. 1997. C. elegans II, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Salinas LS, Maldonado E, Macías-Silva M, Blackwell TK, Navarro RE. 2007. The DEAD box RNA helicase VBH-1 is required for germ cell function in C. elegans. Genesis 45:533–546.

Salinas LS, Maldonado E, Navarro RE. 2006. Stress-induced germ cell apoptosis by a p53 independent pathway in *Caenorhabditis elegans*. Cell Death Differ 13:2129–2139.

Schedl T, Kimble J. 1988. fog-2, a Germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans*. Genetics 119:43–61.

Schumacher B, Hofmann K, Boulton S, Gartner A. 2001. The *C. elegans* homolog of the p53 tumor suppressor is required for DNA damage-induced apoptosis. Curr Biol 11:1722–1727.

Schumacher B, Schertel C, Wittenburg N, Tuck S, Mitani S, Gartner A, Conradt B, Shaham S. 2005. *C. elegans ced-13* can promote apoptosis and is induced in response to DNA damage. Cell Death Differ 12:153–161.

Shaham S, Horvitz HR. 1996. An alternatively spliced *C. elegans* ced-4 RNA encodes a novel cell death inhibitor. Cell 86:201–208.

Stitzel ML, Pellettieri J, Seydoux G. 2006. The C. elegans DYRK kinase MBK-2 marks oocyte proteins for degradation in response to meiotic maturation. Curr Biol 16:56–62.

Tian Q, Streuli M, Saito H, Schlossman SF, Anderson P. 1991. A polyadenylate binding protein localized to the granules of cytolytic lymphocytes induces DNA fragmentation in target cells. Cell 67:629–639.

Tian Q, Taupin J, Elledge S, Robertson M, Anderson P. 1995. Fas-activated Serine/Threonine Kinase (FAST) Phosphorylates TIA-1 during Fas-mediated Apoptosis. J Exp Med 182:864–874.

Timmons L, Court DL, Fire A. 2001. Ingestion of bacterially expressed dsRNAs can produce specifc and potent genetic interference in *Caenorhabditis elegans*. Gene 263:103–112.

Yamasaki S, Stoecklin G, Kedersha N, Simarro M, Anderson P. 2007. T-cell intracellular antigen-1 (TIA-1)-induced translational silencing promotes the decay of selected mRNAs. J Biol Chem 282:30070–30077.

Zhang T. 2005. Identification of the sequence determinants mediating the nucleo-cytoplasmic shuttling of TIAR and TIA-1 RNA-binding proteins. J Cell Sci 118:5453–5463.

Zhou Z, Hartwieg E, Horvitz HR. 2001. CED-1 is a transmembrane receptor that mediates cell corpse engulfment in *C. elegans*. Cell 104:43–56.

Zuccato E, Buratti E, Stuani C, Baralle FE, Pagani F. 2004. An intronic polypyrimidine-rich element downstream of the donor site modulates cystic fibrosis transmembrane conductance regulator exon 9 alternative splicing. J Biol Chem 279:16980–16988.