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Functional Characterization of Drosophila Translin and Trax

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ABSTRACT

The vertebrate RNA and ssDNA-binding protein Translin has been suggested to function in a variety of cellular processes, including DNA damage response, RNA transport, and translational control. The *Tr*anslin-*a*ssociated factor *X* (Trax) interacts with Translin, and Trax protein stability depends on the presence of Translin. To determine the function of the Drosophila Translin and Trax, we generated a *translin* null mutant and isolated a *trax* nonsense mutation. *translin* and *trax* single and double mutants are viable, fertile, and phenotypically normal. Meiotic recombination rates and chromosome segregation are also not affected in *translin* and *trax* mutants. In addition, we found no evidence for an increased sensitivity for DNA double-strand damage in embryos and developing larvae. Together with the lack of evidence for their involvement in DNA double-strand break checkpoints, this argues against a critical role for Translin and Trax in sensing or repairing such DNA damage. However, Drosophila *translin* is essential for stabilizing the Translin interaction partner Trax, a function that is surprisingly conserved throughout evolution. Conversely, *trax* is not essential for Translin stability as *trax* mutants exhibit normal levels of Translin protein.

THE vertebrate RNA and single-stranded DNA (ssDNA)-binding protein, Translin/*t*estes-*b*rain–*R*NA-*b*inding *p*rotein (TB–RBP), binds to consensus sequences within recombination hotspot regions associated with chromosomal translocations in lymphoid malignancies and has been suspected of mediating chromosomal translocations in such tumors and in solid tumors (Aoki *et al.* 1995; Hosaka *et al.* 2000 and references therein).

Indications that Translin might be involved in sensing or repairing DNA damage were found while treating HeLa cells with DNA-damaging agents. After treatment with mitomycin C or etoposide, the amount of nuclear Translin greatly increased, suggesting a signaling pathway for the active nuclear transport of Translin that is initiated by exposure to DNA-damaging agents (Kasai *et al.* 1997). However, so far no evidence could be found for the direct involvement of Translin in DNA damage repair. Furthermore, exposure of mice embryonic fibroblasts (MEFs) from TB–RBP-deficient mice with DNA-damaging agents did not reveal differences between wild-type and TB–RBP null MEFs in terms of cell survival or number of DNA breaks and gaps (Yang *et al.* 2004).

*Tr*anslin-*a*ssociated factor *X* (Trax) was identified in a two-hybrid screen for Translin-interacting proteins and by immunoprecipitation experiments (Aoki *et al.* 1997; Wu *et al.* 1999). Trax shares conserved sequence similarities with Translin, and Trax orthologs have been found in virtually all species that also have Translin. The idea that Translin and Trax may play a role in cell proliferation is supported by a variety of studies that investigated the effect of Translin or Trax depletion in different cell types. MEFs cultured from TB–RBP-deficient mice grow more slowly than MEFs from heterozygous littermates (Yang *et al.* 2004). In addition, reduction of Translin or Trax by RNA interference slows cell growth rates of NIH3T3 cells, and reduction of Trax in HeLa cells slows growth rate and progression through G_2/M (Yang *et al.* 2004; Yang and Hecht 2004). Consistent with this observation, overexpression of Translin leads to the opposite effect—acceleration of cell proliferation (Ishida *et al.* 2002).

Translin has also been identified as an RNA-binding protein that binds a variety of brain and testes RNAs. Accordingly, it is thought to play a role in the subcellular transport and/or translational control of its target RNAs in these tissues (Han *et al.* 1995a; Kobayashi *et al.* 1998; Morales *et al.* 1998; Muramatsu *et al.* 1998; Wu and Hecht 2000; Yang *et al.* 2003). Unlike Translin, Trax does not bind nucleic acids directly, but might be part of the RNA- or DNA-binding complex, thereby modulating the nucleic-acid-binding affinity of Translin (Chennathukuzhi *et al.* 2001; Finkenstadt *et al.* 2002; Gupta *et al.* 2005).

Our interest in mRNA localization, cell cycle regulation, and DNA damage response led to our analyzing the role of these evolutionarily conserved genes in

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Drosophila. Because the results from vertebrate Translin and Trax revealed little concrete evidence about the function of these proteins *in vivo*, we wanted to analyze Translin in an invertebrate model system in which molecular pathways are often less redundant and where it may be simpler to reveal Translin and Trax functions. Furthermore, the availability of mutants in the two genes would allow us to directly test whether *translin* (*trsn*) and *Trax* are functionally redundant for an essential process.

MATERIALS AND METHODS

Generation of fusion genes, mutants, and fly stocks: Flies expressing C-terminal Translin and Trax GFP (or GFP derivatives) fusions were generated as described earlier (Pare and Suter 2000). A detailed description of cloning steps involved in generating constructs for transgenic flies is provided in the data supplement at http://www.genetics.org/supplemental/.

To create a *translin*^{null} mutant (Δ*trsn*), we mobilized an EPgy2 *P* element (pEY06981) that inserted in the last exon of the *translin* gene (Bellen *et al.* 2004). By bidirectional imprecise excision, a small deficiency was created that entirely removed the coding region of the gene as well as adjacent nontranscribed sequences. The neighboring genes *CPTI* and *CG17765* are not affected by this deletion. The deficiencies *Df(2R)stan2* and *Df(3R)Exel6174*, covering either *translin* or *trax* genomic loci were obtained from the Bloomington Stock Center (stock nos. 596 and 7653, respectively). *Translin* and *trax* mutant fly strains described in this article were of the following genotypes (unless otherwise noted) and were kept as stocks: *trax*^{W151*} has been described as *H813* by Schuetze *et al.* (2004), and hemizygous flies were analyzed as *w; trax*^{W151*} *ru st e ca/Df(3R)Exel6174*, Δ*trsn: w;* Δ*trsn/Df(2R)stan2 b pr*, and Δ*trsn;trax*^{W151*}*: w;* Δ*trsn/Df(2R)stan2 b pr; trax*^{W151*} *ru st e ca/Df(3R)Exel6174*.

Generation of antibodies against Drosophila Translin and Trax: The open reading frames of the *translin* and *trax* cDNAs were cloned into the pGEX-5X-1 (GE Healthcare) expression vector to produce GST-tagged fusion proteins, and the induced fusion proteins were purified using the GST fusion purification kit (GE Healthcare). Short C-terminal peptides of the Translin and Trax proteins were also synthesized (Sheldon Biotechnology Centre, McGill University, Montreal) and used to immunize rabbits. Anti-Translin sera were affinity purified against bacterially expressed full-length Translin–maltose binding-protein (MBP) and pMAL-vector (New England Biolabs, Beverly, MA) and conjugated to a cyanogen bromide-activated Sepharose column (GE Healthcare). Anti-Trax sera were purified against full-length Trax-MBP fusion protein immobilized on nitrocellulose membranes.

Immunostainings: Immunohistochemical stainings were done as described earlier (Suter and Steward 1991). Rabbit polyclonal antisera against full-length Translin and against a C-terminal peptide of Trax were used at a dilution of 1:400 and 1:200, respectively. The secondary OregonGreen488-conjugated anti-rabbit antibody (Molecular Probes, Eugene, OR) was used at a dilution of 1:2000. During the final washing steps, nuclei and actin filaments were stained for 20 min with 2.5 μg/ml Hoechst 33258 (Molecular Probes) and 1 unit/ml rhodamine-conjugated phalloidin (Molecular Probes), respectively. The ovaries were embedded in 60% glycerol and analyzed by confocal microscopy using a Leica TCS-SP2.

Western blots: Ovary and testis extracts were prepared by dissecting ovaries and testes in Ringer's solution and freezing them directly in 2× SDS sample buffer. Ovaries and testes were homogenized by vortexing and boiling for 10 min. Protein samples were separated on 12% SDS–PAGE gels and transferred onto nitrocellulose membranes. To detect three different proteins simultaneously on the blots, the membranes were cut according to the molecular weights of the proteins to be detected and probed with the appropriate antibodies. Rabbit polyclonal antisera against full-length Translin protein and the C-terminal peptide of Trax were used at a dilution of 1:750 and 1:250, respectively. Horseradish-conjugated anti-rabbit secondary antibody (GE Healthcare) was used at a dilution of 1:5000. The blots were probed with ECL reagents (GE Healthcare) for 1 min and the chemiluminescence was detected with ECL films.

***In situ* hybridization:** For *in situ* hybridizations, parts of *wingless* and *hairy* cDNAs corresponding to coding regions and 5′-UTR were cloned into pBS(KS+), linearized with *Sac*I, and transcribed with T3 polymerase (Stratagene, La Jolla, CA) in the presence of digoxygenin-rUTP (Roche) to generate digoxygenin-labeled antisense RNA probes. *In situ* hybridizations on embryos were in principle done according to Hughes and Krause (1999) with noted modifications. After hybridization, embryos were treated with RNAse A and T1 in 2× SSC for 15 min at 37°. The buffer was then changed in several steps from SSC to 1× maleic acid buffer (MAB) and embryos were blocked in 1× MAB containing 20% donkey serum (Chemicon) and 2% Roche blocking reagent (Roche). The digoxygenin-labeled probes were detected with a sheep-antidigoxygenin (Roche) antibody and Cy3-labeled donkey-anti-sheep F(ab′)2 fragments of IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). During the final washing steps the DNA-staining dye Hoechst 33258 (Molecular Probes) was applied. The embryos were embedded in 60% glycerol and analyzed by confocal microscopy.

Tests for recombination frequency and chromosome nondisjunction: To determine the recombination frequency of Trax-deficient flies, females of the genotypes *b pr cn bw*/+ + + +; *trax*^{W151*}*/Df(3R)Exel6174* and *b pr cn bw*/+ + + +; *Df(3R)Exel6174/ TM6B Tb ca* (control) were mated to *b pr cn bw* males and the progeny were scored for recombination events in the *b-pr*, *pr-cn*, and *cn-bw* intervals independently. Flies with *cn* and *bw* markers have white eyes, which mask the *pr* phenotype. Therefore, the recombined chromosomes + *pr cn bw* and + + *cn bw* cannot be discriminated from each other in this assay. They were scored as *b-pr* and also as *pr-cn* crossovers, so these recombination frequencies appear slightly higher than they actually were. Similarly, one result of a double crossover, namely *b* + *cn bw*, was scored as parental. However, because the distances between *b-pr* and *pr-cn* are small (6 and 3 MU, respectively), such events are rare and barely influence the overall result.

To determine the frequency of female chromosome nondisjunction in *translin* and *trax* mutant flies, females of the genotypes *w/w;* Δ*trsn/Df(2R)stan2 b pr, w/w; trax*^{W151*}*/Df(3R)Exel6174*, and *w/w; Df(3R)Exel6174/ TM6B Tb ca* (control) were crossed to wild-type males. Female and male progeny were scored for exceptional white-eyed *w/w*/Y (XXY) females and red-eyed +/0 (XO) males.

To determine the frequency of male chromosome nondisjunction in *translin* and *trax* mutant flies, males of the genotypes *w/B*^{s}*Y;* Δ*trsn/Df(2R)stan2 b pr,* w/*B*^{s}*Y;* Δ*trsn/Sp* or *Df(2R)stan2 b pr*/CyO (control), +/*B*^{s}*Y; trax*^{W151*}*/Df(3R)Exel6174*, and +/*B*^{s}Y; *Df(3R)Exel6174/ TM6B Tb ca* (control) were crossed to wild-type or *w*; +; + females, respectively. Progeny were scored for exceptional *Bar of Stone* +/*w/B*^{s}Y females and red-eyed +/0 (for *translin* mutants) or white-eyed *w*/0 (for *trax* mutants) males, respectively.

Irradiation experiments: Fly embryos were collected on apple juice plates at 25° and aged to reach the desired cell

cycle stages. Then they were irradiated with a dose of 6 Gy (half-lethal dose), transferred onto fresh standard corn food, and incubated at 25°. For survival tests, pupae and surviving flies were scored. Tests for the embryonic cell cycle checkpoint were done as described in Masrouha *et al.* (2003).

RESULTS

Structure and expression of Drosophila Translin and Trax: The amino acid sequences of human, mouse, and Xenopus Translin and Trax were aligned with the sequences from Drosophila (Figure 1, A and B). While the vertebrate Translin proteins share identities between 81% (human and Xenopus sequences) and 98% (human and mouse sequences), the Drosophila Translin protein is still well conserved and shares 52% identity with the vertebrate one. Note that the degree of identity between the Drosophila and vertebrate proteins is higher in the C-terminal part compared to the N-terminal half of the protein, except for the extreme C-terminal part of the Drosophila protein, which differs in sequence and length from the vertebrate proteins. The C-terminal half contains the putative leucine zipper domain (depicted in Figure 1), which is required for Translin homodimerization and probably also for nucleic acid binding (Wu *et al.* 1998; Aoki *et al.* 1999). Both the nuclear export signal (green) and the putative GTP-binding site (red) seem to be conserved in the Drosophila protein, although the GTP-binding activity of the Drosophila Translin seems to be lower than that of one of the mammalian orthologs (Sengupta *et al.* 2006). In contrast, the two basic regions (light and dark blue in Figure 1) that have been shown to be required for nucleic acid binding in the mouse protein (Chennathukuzhi *et al.* 2001) are less conserved in Drosophila Translin and a recent publication (Sengupta *et al.* 2006) found that, in contrast to the mammalian orthologs, Drosophila Translin does not exhibit ssDNA- or RNA-binding affinity under their experimental conditions.

Compared to Translin, the vertebrate Trax proteins are less conserved with identities ranging from 64% (between human and Xenopus sequences) to 90% (human and mouse sequences). We isolated a *Trax* cDNA from an ovary cDNA library (Larochelle and Suter 1995). Interestingly, this clone differs from one isolated from adult heads (GH01922; Rubin *et al.* 2000) by the removal of an intronic sequence and by a usage of a different polyadenylation site. Furthermore, the annotated fly genome also lists a transcript in which this intron is partially removed, but with a different splice acceptor site (CG5063-PB). Because of the splice differences, the corresponding predicted TRAX proteins also differ in size and sequence. The ovarian cDNA described here encodes the largest protein and the one with the best match to the human sequence (Figure 1B). This Drosophila Trax amino acid sequence shares 36% identity with human Trax. Vertebrate Trax contains a bipartite nuclear localization sequence (NLS), which maps to amino acid sequences 11–27 (orange in Figure 1) and was shown to be required for nuclear localization of the mouse protein (Cho *et al.* 2004). This feature is not conserved in Drosophila Trax, and NucPred (Heddad *et al.* 2004) and PredictNLS programs (Cokol *et al.* 2000) also did not reveal any NLS in Drosophila Trax.

The Drosophila *translin* and *trax* genes are located on the second and third chromosome, respectively. Schematics of the genomic organization of the *translin* and *trax* regions are depicted in Figure 2, A and B. To analyze the *in vivo* expression and localization of Translin and Trax, we generated C-terminal Translin–cyan fluorescent protein (CFP) and Trax–yellow fluorescent protein (YFP) fusion constructs. Since vertebrate Translins have been implicated in the functioning of RNA localization processes, we focused our expression analysis on ovaries and young embryos, in which well-documented transport of RNAs and proteins takes place. Analysis of Translin and Trax protein distribution revealed that they are expressed during oogenesis with the highest rates in the germarium (Figure 3A). The primarily cytoplasmic Translin and Trax signal decreases in the egg chambers during later oogenesis stages. But in blastoderm-stage embryos relatively high levels of Translin and Trax can be detected in the cytoplasm (Figure 3B).

Functional analysis of Translin and Trax: Strain *pEY06981* carries a *P*-element insertion in the 3′ exon of *translin* (Bellen *et al.* 2004). As judged from Western blotting and immunohistochemistry using polyclonal antisera against the full-length Drosophila Translin, these flies do not express detectable amounts of Translin. Nevertheless, we created a true *translin*-deficient mutant by mobilizing this *P* element and isolating an imprecise *P*-element excision that created a short deficiency that removed the entire *translin* gene (Δ*trsn* Figure 2). The analysis of the *translin P*-element insertion and the *translin* null allele revealed that homo- or hemizygous *translin* mutant flies are viable and fertile and exhibit no obvious mutant phenotype. Under standard laboratory conditions Drosophila *translin* is thus dispensable for viability and fertility.

Schuetze *et al.* (2004) mapped the *H813* female-sterile mutation from the Tübingen collection (Tearle and Nüsslein-Volhard 1987) to the *trax* region. We therefore sequenced the *trax* allele in this mutant and found a point mutation that generates an in-frame stop codon at amino acid position 151 ($trax^{W151*}$, Figures 1 and 2). No Trax protein can be detected in homo- or hemizygous *trax* mutant flies by Western blot or immunostaining using polyclonal antisera against full-length Trax (data not shown). Semiquantitative RT–PCR analysis revealed very low *trax* mRNA levels in mutant flies, suggesting that the premature stop codon leads to the at least partial degradation of the mutant mRNA (data not shown). However, the female-sterile effect is not due to the absence of functional Trax protein, as hemizygous

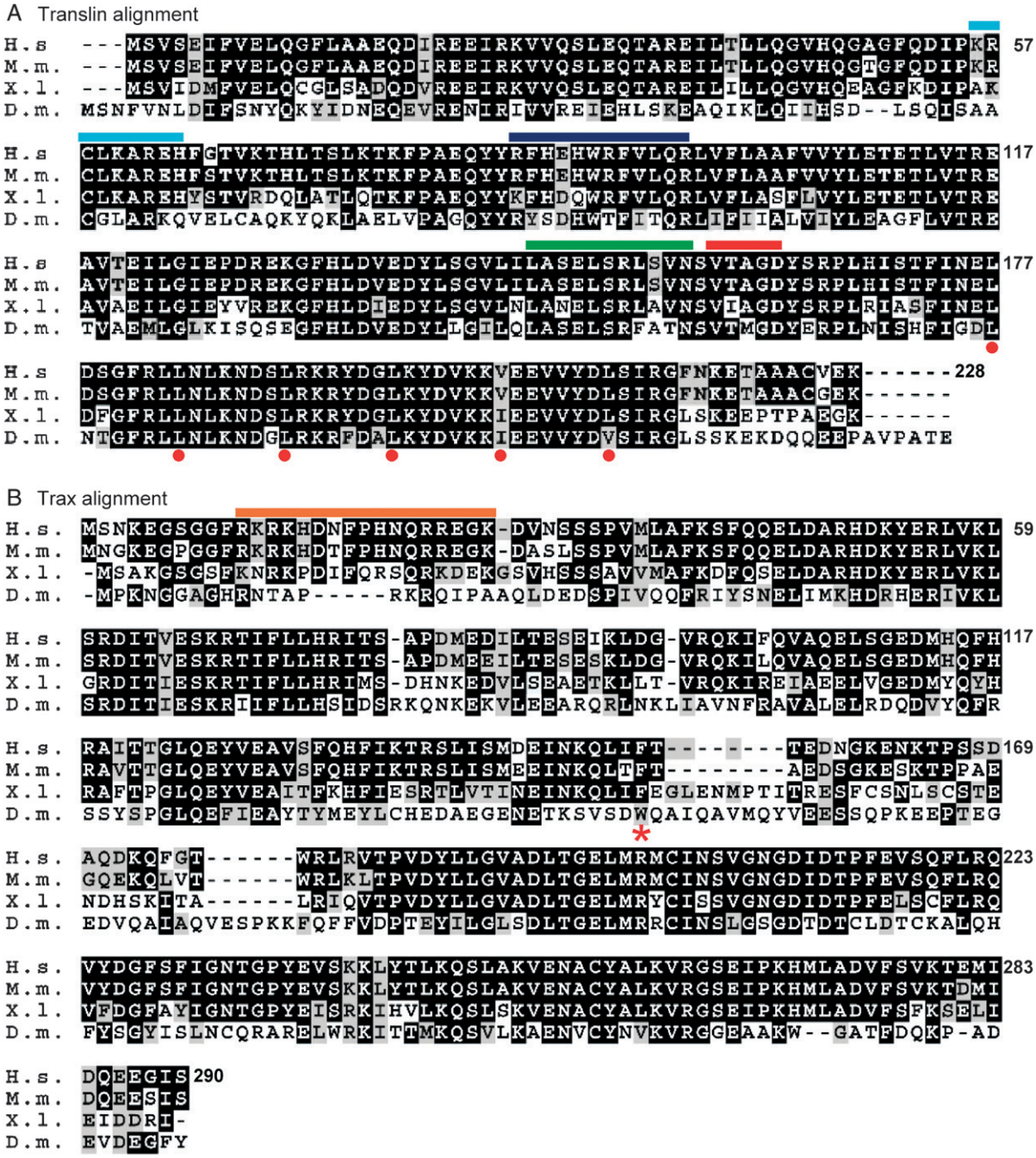

FIGURE 1.—Translin and Trax amino acid alignments. The amino acid sequences of human (NP_004613), mouse (NP_035780), Xenopus (AAF65620), and Drosophila (AAM50730). (A) Translin proteins were aligned using Multalin (CORPET 1988) and edited using the public-domain program BOXSHADE. The human (NP_005990), mouse (NP_058605), and Xenopus (AAH54180) (B) Trax amino acid sequences were similarly aligned with the Drosophila Trax protein sequence (DQ448818). The following protein residues have been marked in the alignment: nuclear export signal (NES) (green), GTP-binding site (red), basic regions in Translin (light and dark blue), and NLS (orange). Leucine residues of the Translin leucine zipper are marked by red dots. The tryptophane 151, which is replaced by a stop codon in the *trax*^{W151*} nonsense mutation, is marked by an asterisk. Amino acid identities given in the text were revealed by Blast 2 sequences (TATUSOVA and MADDEN 1999).

trax mutant flies are fertile in contrast to homozygous females. Similar to the *translin* mutant, the analysis of the hemizygous *trax* point mutation revealed that *trax* is also dispensable under standard laboratory conditions. Since Drosophila Trax shares homology with Translin (31% identity over large parts, 22% over the entire protein; data not shown), these two proteins may be functionally redundant and replace each other in single mutants. However, the analysis of the Δ*trsn*;*trax*^{W151*} double mutant revealed that these are viable and fertile, indicating that the two proteins do not have any essential role for which they function redundantly.

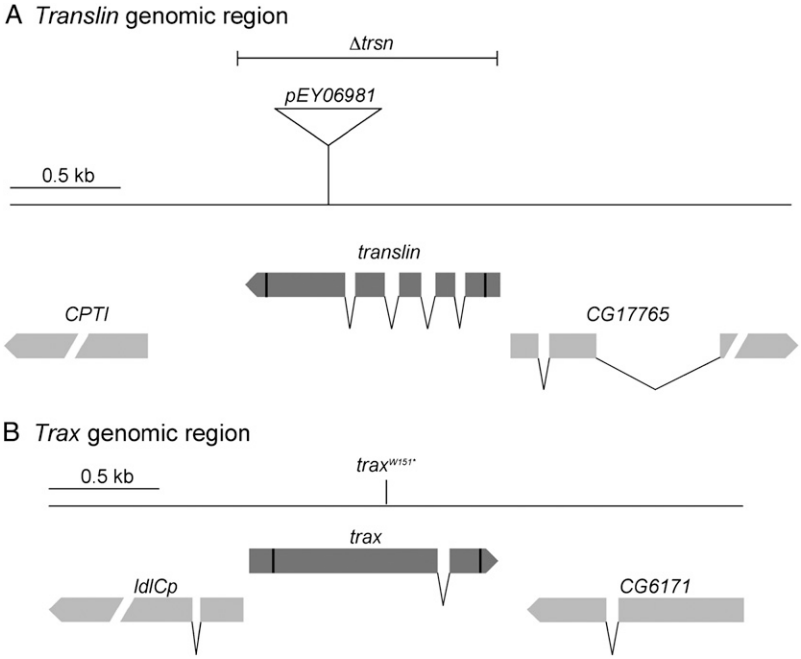

FIGURE 2.—Genomic organization of (A) *translin* (chromosome 2R; 47A11) and (B) *trax* (chromosome 3R; 88F1) regions. The location of the *pEY06981 P*-element insertion and the region deleted in the *translin*^{null} excision mutant (Δ*trsn*) are depicted. Note that the excision of the *P* element was bidirectional. The position of the *trax*^{W151*} nonsense point mutation is depicted in the schematic.

Drosophila *translin* functions to stabilize Trax: We next analyzed the distribution of Translin and Trax in ovaries from wild-type and *translin* and *trax* mutants using antibodies against these proteins (Figure 4). In wild-type ovaries, Translin can be detected throughout oogenesis with highest expression levels in the germarium and early stage egg chambers, similar to what has been observed for the Translin–CFP fusion protein (compare Figures 3A and 4A). During later stages, Translin staining can still be detected in the follicle cells that surround the ovariole, whereas the staining in the germline decreases. Similar to the Translin–CFP fusion protein, the endogenous protein seems to reside mainly in the cytoplasm and only weak nuclear staining can be detected. We note that the Translin signal is not enriched in the developing oocyte. A similar distribution is apparent for Trax (Figure 4B). In ovaries from *translin*-deficient flies (Δ*trsn*), no specific staining for Translin can be detected anymore, indicating that our antibody is very specific in immunostainings (Figure 4C). In addition to the loss of Translin staining, Trax staining also disappeared in Δ*trsn* ovaries (Figure 4D). It thus appears that Drosophila *translin* is essential in obtaining normal Trax levels and loss of Translin protein leads to loss of Trax proteins. Interestingly, this function of *translin* has been conserved through evolution, because similar results have also been reported for Translin knockout mice (CHENNATHUKUZHI *et al.* 2003). In contrast to loss of Drosophila *translin*, loss of Drosophila Trax protein does not lead to a reduction of Translin protein (Figure 4, E and F). Neither Translin nor Trax can be detected by immunofluorescence in Δ*trsn*;*trax*^{W151*} double-mutant ovaries (Figure 4, G and H).

Other functions of Drosophila *translin* and *trax* are less evident as suggested by the viability and fertility of the mutants. Loss of Translin (and Trax) proteins does

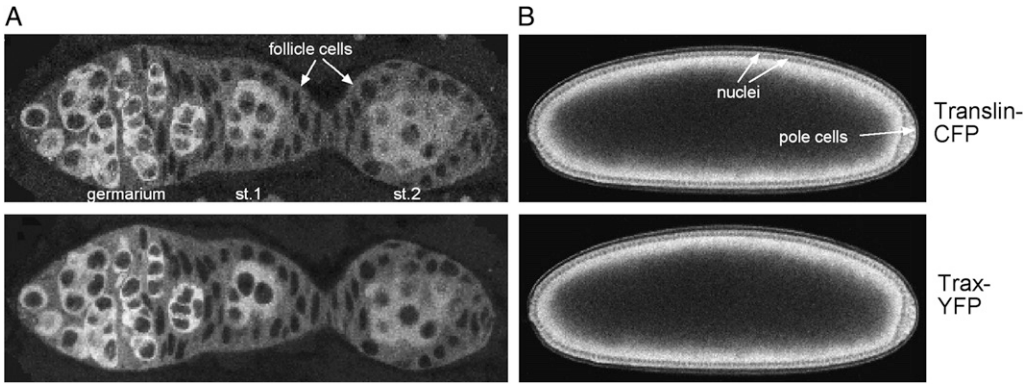

FIGURE 3.—*In vivo* localization of Translin-CFP and Trax-YFP in (A) early oogenesis and in (B) syncytial blastoderm-stage embryos. Germarium, stage 1 and 2 egg chambers, and somatic follicle cells are marked. Nuclei and pole cells are indicated by arrows. (Top) Translin–CFP. (Bottom) Trax–YFP. Ovaries were dissected in halocarbon oil and immediately analyzed by confocal microscopy (left). Embryos were dechorionated before imaging (right).

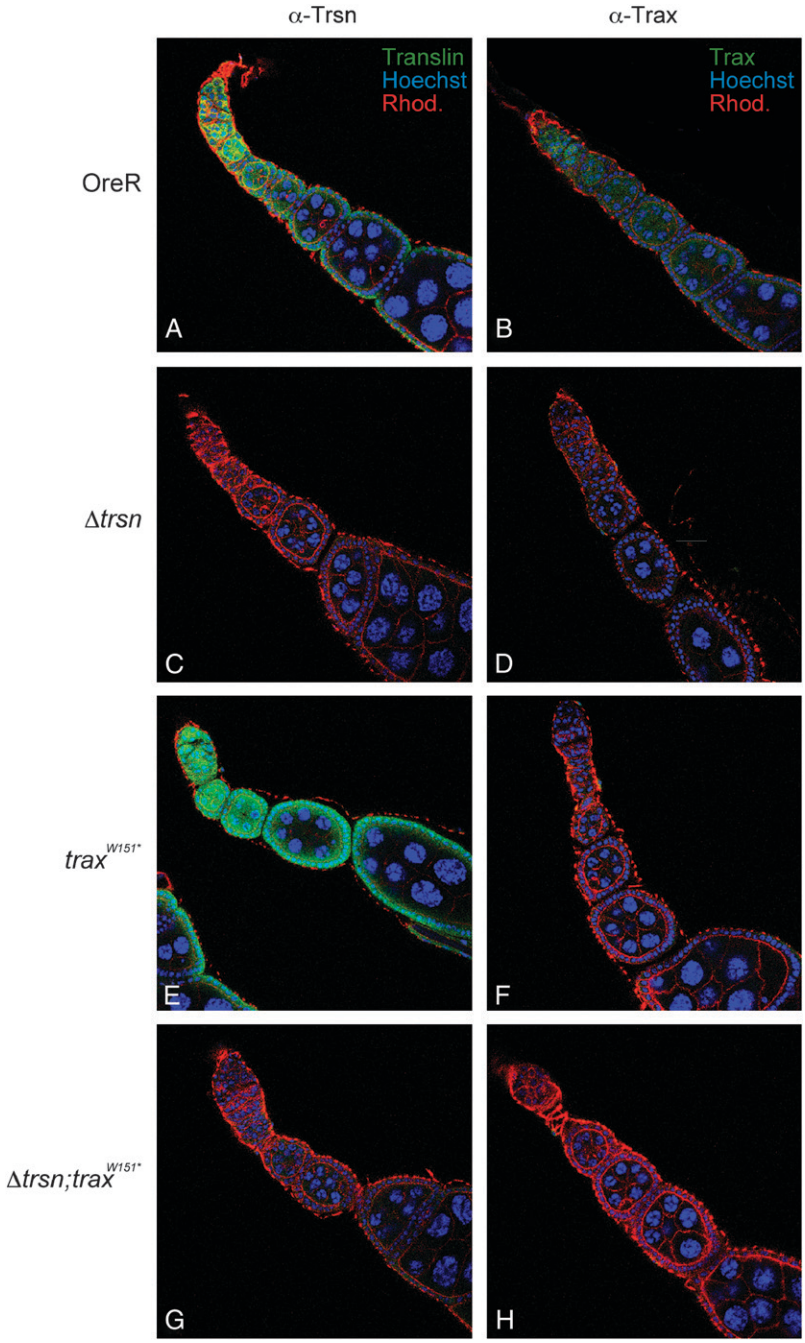

FIGURE 4.—Translin and Trax immunostainings with ovaries. Ovaries from wild-type, Δ*trsn*, *trax*^{W151*}, and Δ*trsn*; *trax*^{W151*} mutant females (indicated on the left) were subjected to immunostainings with either α-Translin or α-Trax antibodies (shown in green and indicated at the top). The nuclei were stained with Hoechst (blue) and actin filaments are visualized by rhodamine–phalloidin staining (red).

not seem to have an effect on oogenesis. Ovarioles from mutant flies look normal and the egg-laying rate of Translin-deficient females is not reduced compared to that of wild-type females. This result contrasts with results obtained in mice, where TB–RBP knockout females have been reported to be subfertile with reduced litter sizes (CHENNATHUKUZHI *et al.* 2003).

Using Western blots, we compared levels of Translin and Trax proteins in wild-type ovaries and testes with the ones in Translin and/or Trax-deficient tissues (Figure 5). Translin and Trax can be detected in ovaries (Figure 5A) and testes from wild-type flies (Figure 5B). No Translin can be detected in ovaries and testes from Translin-deficient flies and, similar to what has been observed in the immunostainings, Trax levels are lost or greatly reduced in these ovaries and testes. Translin levels are unaffected in ovaries from *trax*^{W151*} mutants. Therefore, as opposed to the requirement for *translin* for maintaining normal levels of Trax, Translin protein levels do not depend on *trax*. Neither Translin nor Trax can be detected in ovaries or testes from Δ*trsn*;*trax*^{W151*} double mutants. The low Trax protein levels found in *translin* mutants can be rescued by reintroducing a *translin*$^{+}$ construct into this mutant background (Figure 5, A and B). Trax levels can also be rescued to a certain extent by reintroducing a *Translin-GFP* fusion

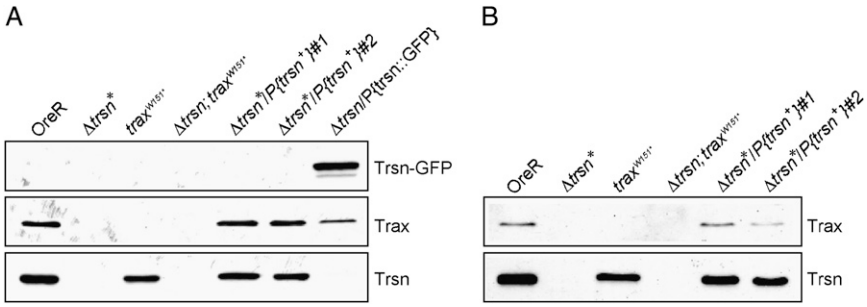

FIGURE 5.—Detection of Translin and Trax in ovaries and testes by Western blot. Ovarian (A) and testis (B) proteins from wild-type, Δ*trsn**, *trax*^{W151*} and Δ*trsn*; *trax*^{W151*} mutant flies as well as Δ*trsn**/*P*{s*trsn*$^{+}$}#1 and 2 and Δ*trsn*/*P*{*trsn*::GFP} flies were separated by SDS–PAGE and transferred to membranes. These were then cut into three pieces according to the expected sizes of the proteins to be detected. Individual sections were probed with either α-Translin or α-Trax antibodies. An asterisk indicates that flies were homozygous for the Δ*trsn* chromosome.

construct that is expressed under the control of the *translin* promoter (Figure 5A). This would indicate that Translin–GFP is at least partially functional. However, a very weak Translin band can be detected, which might correspond to a degradation product of the Translin–GFP fusion. Therefore, it cannot be ruled out that part of the Trax stabilization might be due to the presence of this wild-type size degradation product.

***Translin* is not required for the apical localization of *hairy* and *wingless* transcripts during embryogenesis:** Human and mouse Translin proteins were found to associate with a variety of different so-called Y- and H-element-containing RNAs from brain and testes, including α-*CAMKII*, *MBP*, *Tau*, and *Protamine* mRNAs as well as noncoding RNAs such as *BC1* (Han *et al.* 1995a,b; Kobayashi *et al.* 1998; Muramatsu *et al.* 1998; Severt *et al.* 1999). Translin has been suggested as being involved in the subcellular localization and translational regulation of its target RNAs (Morales *et al.* 1998; Severt *et al.* 1999; Yang *et al.* 2003). We set out to analyze this process in embryos, which contain relatively large amounts of Translin and Trax. During Drosophila embryogenesis, pair-rule and *wingless* transcripts become specifically localized to the apical cytoplasm above the nuclei (Figure 6, A and B). Apical *wingless* localization is not affected in *trsn*^{pEY06981} embryos derived from mutant mothers (Figure 6, C and D). Similarly, the proper apical localization of *hairy* mRNA also is not affected in Translin-deficient embryos (data not shown). *translin* is thus not essential for the proper apical localization of *hairy* and *wingless* transcripts during embryogenesis.

Meiotic recombination and DNA damage response: Translin and Trax have been implicated in playing a role during DNA double-strand break repair (DSBR) either by nonhomologous end joining (NHEJ) or homologous recombination (HR). In addition to the more general repair of double-strand breaks (DSBs), homologous recombination is also utilized during meiotic recombination. To test whether Drosophila Translin and Trax are involved in homologous recombination during meiosis, we analyzed whether the recombination frequency is reduced in *translin* or *trax* mutant flies. For these

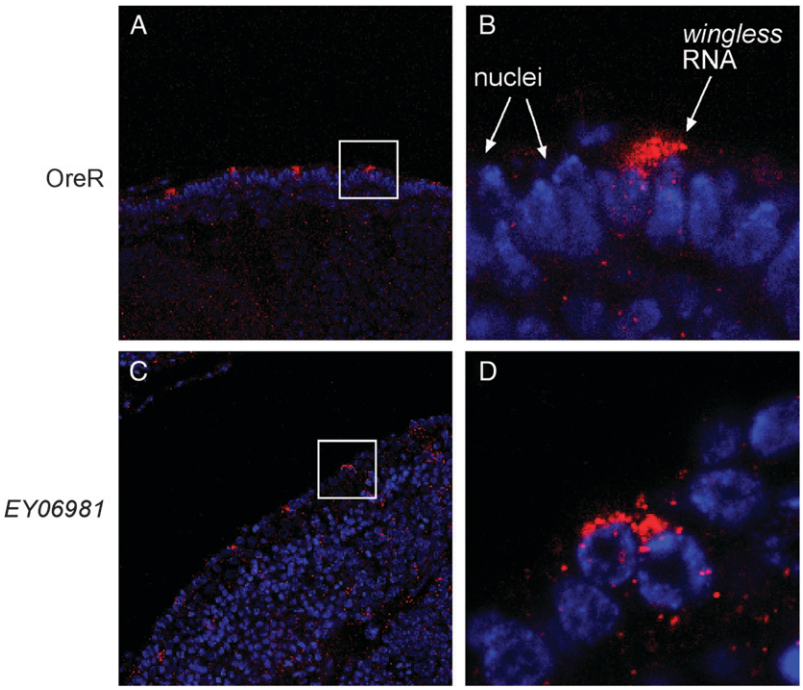

FIGURE 6.—Apical localization of *wingless* transcripts in wild-type and *translin* mutant embryos. Localization of endogenous *wingless* transcripts of wild-type (A and B) and *pEY06981*(C and D) embryos was detected by *in situ* hybridization and is shown in red. The nuclei are labeled by Hoechst staining and are shown in blue. B and D are magnifications of the depicted areas in A and C, respectively.

TABLE 1

Recombination frequencies in *trax* mutant females

Maternal genotype	N^a total	Recombinants[b]			Frequency[c]		
		b-pr	*pr-cn*	*cn-bw*	*b-pr*	*pr-cn*	*cn-bw*
b pr cn bw/+ + + +; $trax^{W151*}$/*Df(3R)Exel6174*	457	29	17	161	6.35	3.72	35.23
b pr cn bw/+ + + +; *Df(3R)Exel6174/TM6B* (control)	550	36	12	246	6.55	2.18	44.73
Expected recombination frequencies					6	3	47

TM6B contains *Tb, ca.*

[a] *N*, the total number of progeny scored from a cross of females of the genotype listed and *b pr cn bw* males.

[b] Recombinants of the three intervals were scored independently and scored recombination events or frequencies include all recombinant progeny in a certain interval whether or not a second recombination event was scored in a different interval.

[c] The recombination frequency for each interval was calculated as follows: (recombinants) × 100/*N*. The expected recombination frequencies as deduced from the published genetic positions (Lindsley and Zimm 1992) are shown below.

experiments, multiply marked chromosomes were used and the meiotic recombination rate of these markers was determined by visual inspection of the phenotype of the offspring. The calculated recombination rates for mutant flies were compared to rates of control flies and to published map distances (Table 1). While the recombination frequency between *b* and *pr* is almost identical, the number of crossovers between *pr* and *cn* is slightly increased in *trax* mutant females compared to wild type controls. The observed recombination frequency between *cn* and *bw* does not take into consideration double recombination events and possible effects of the third chromosomal balancer that could explain the observed differences at least in part. Because the recombination frequencies in the *b-pr* and *pr-cn* intervals are similar in *trax* mutants and control flies and in agreement with the published map distances, we conclude that *trax* does not have a critical function in meiotic recombination. Similar results were obtained for *translin* mutants (data not shown). Therefore *translin* and *trax* are not essential for meiotic recombination in Drosophila.

As many genes involved in meiotic recombination and double-strand break repair in Drosophila are also required for proper chromosome segregation, mutations in these genes cause increased meiotic chromosome nondisjunctions (McKim *et al.* 2002). We therefore also tested whether the rate of chromosome nondisjunction is increased in *translin* and *trax* mutants. We analyzed the segregation of the sex chromosomes in a standard assay, which allowed us to score the exceptional females and males that were produced by such chromosome nondisjunction events. No differences in the rate of X chromosome nondisjunction were observed between control flies and *translin* or *trax* mutant females or males (Table 2). Furthermore, the overall frequency of nondisjunction events is comparable to the published wild-type nondisjunction frequency (Ghabrial *et al.* 1998; McKim *et al.* 2002 and references therein).

To test whether Translin and/or Trax function in a double-strand DNA damage pathway in Drosophila, we determined the survival rates of *translin* and *trax* double mutants with and without γ-irradiation during embryogenesis. *Translin*, *trax*, and *translin;trax* double mutants do not seem to be more sensitive to γ-irradiation as similar survival rates were observed in treated and untreated animals (data not shown). Similarly, embryos lacking Trsn and Trax (Δ*trsn;*$trax^{W151*}$ double mutants derived from mutant parents) display a normal cell cycle arrest during embryonic nuclear cycle 14 when double-strand breaks are induced by γ-irradiation, suggesting that Translin and Trax are dispensable for this DNA damage checkpoint. In addition, no nuclear transfer or enrichment of Translin–CFP and Trax–YFP fusion proteins was observed after embryo irradiation (data not shown).

DISCUSSION

Differences between Drosophila and mammalian Translin/Trax protein sequences: Sequence alignments show that Drosophila Translin shares 52% identity with human and mouse Translins (Figure 1). The degree of identity between Drosophila and vertebrate proteins is lower in the N-terminal part of the proteins. This includes the two basic regions required for nucleic acid binding (Chennathukuzhi *et al.* 2001). Thus, the weak conservation of the two basic regions in Drosophila might be the reason why no RNA or DNA-binding activity has been revealed for the Drosophila Translin in gel-shift assays (Sengupta *et al.* 2006). However, even though the *Schizosaccaromyces pombe* Translin is less conserved than the Drosophila ortholog (33% identity with human Translin), it still binds single-stranded oligodeoxynucleotide and oligoribonucleotide probes (Laufman *et al.* 2005). In addition, in contrast to human Translin, the fission yeast protein has much higher affinities for RNA sequences than for homologous DNA sequences. Since the two groups used different RNA and ssDNA probes in gel-shift assays, it cannot be ruled out that lack of nucleic acid binding of Drosophila Translin simply reflects that Drosophila Translin

TABLE 2

Female and male nondisjunction

Maternal genotype	Normal progeny (XX and XY)	Nondisjunction progeny (XXY and X0)	% nondisjunction[c]
	Female nondisjunction[a]		
w/w; Δ*trsn/Df(2R)stan2 b pr*	3591 (1819 + 1772)	3 (0 + 3)	0.08
w/w; *trax*^{W151*}*/Df(3R)Exel6174*	3037 (1709 + 1328)	3 (1 + 2)	0.1
w/w; *Df(3R)Exel6174/TM6B Tb ca* (control)	2753 (1474 + 1279)	3 (2 + 1)	0.11
Paternal genotype	Normal progeny (XX and XB^{s}Y)	Nondisjunction progeny (XXB^{s}Y and X0)	% nondisjunction[c]
	Male nondisjunction[b]		
w/B^s*Y*; Δ*trsn/Df(2R)stan2 b pr*	1778 (841 + 937)	3 (0 + 3)	0.17
w/B^s*Y*; Δ*trsn/Sp* or *Df(2R)stan2 b pr*/CyO (control)	2057 (1043 + 1014)	4 (1 + 3)	0.19
+/B^{s}Y; *trax*^{W151*}*/Df(3R)Exel6174*	3658 (1960 + 1698)	0 (0 + 0)	—
+/B^{s}Y; *Df(3R)Exel6174/TM6B Tb ca* (control)	2302 (1387 + 915)	1 (0 + 1)	0.04

[a] For female nondisjunction, progeny were scored from crosses of wild-type males with females of the genotype listed. Normal chromosome segregation in females gives rise to *w*/+ females and *w*/Y males. Chromosome nondisjunction gives rise to exceptional white-eyed *w/w*/Y (XXY) females and red-eyed +/0 (X0) males that can be distinguished from their wild-type siblings.

[b] For male nondisjunction, progeny were scored from crosses of wild-type (for *translin*) or *w*;+;+ (for *trax*) females with males of the genotype listed. Normal chromosome segregation in males containing the *B*^{s}Y-chromosome gives rise to *w*/+ females and +/*B*^{s}Y or *w*/*B*^{s}Y males, respectively. Male chromosome nondisjunction gives rise to exceptional *Bar of Stone w*/+/*B*^{s}Y females and red-eyed (+/0) or white-eyed (*w*/0) males which can be distinguished from their *Bar of Stone* siblings.

[c] Female and male nondisjunction was counted as follows: (nondisjunction progeny) × 100/total progeny.

recognizes RNA and DNA sequences different from the ones used.

The Translin nuclear export signal (NES), which resides in the more C-terminal part of the mammalian protein (Chennathukuzhi *et al.* 2001), is fairly well conserved in the Drosophila protein, suggesting that Translin might shuttle between the nucleus and the cytoplasm. In mammalian tissue culture cells, the subcellular localization of Translin and Trax seems to be interdependent and determined by their relative ratio (Cho *et al.* 2004). However, this shuttling interdependency is probably not conserved as Drosophila Trax does not seem to exhibit a functional NLS.

Post-transcriptional downregulation of Trax in the absence of Translin: In this study we showed that Translin is required to maintain normal Trax levels in Drosophila. Similar results were obtained previously in TB–RBP-deficient mice and embryonic fibroblasts from such mice (Chennathukuzhi *et al.* 2003; Yang *et al.* 2004). Since truncated Translin proteins, which do not homo- or heterodimerize, do not stabilize Trax, this function is probably directly dependent on protein interaction (Yang *et al.* 2004). There is evidence that excess Trax is ubiquitinated and degraded in the proteasome (Yang *et al.* 2004). In contrast, as we showed here, Translin stability is independent of *trax* and this feature may also be conserved over evolution as it is also the case in *S. pombe* (Laufman *et al.* 2005).

Role of *translin* and *trax* in RNA transport and localization: A role for Translin in RNA transport was suggested from *in situ* hybridization and EM–immunolocalization studies, which showed the colocalization of Translin with certain RNAs in the nuclei as well as in the cytoplasm and in intercellular bridges that interconnect developing male germ cells (Morales *et al.* 2002). Similarly, during Drosophila oogenesis, large amounts of RNAs, proteins, and other materials are synthesized in the nurse cells and transported through intercellular bridges, called ring canals, into the oocyte, which is (almost) transcriptionally quiescent and depends on the RNAs supplied by the nurse cells. These similarities in intercellular transport events in mouse testes and Drosophila egg chambers suggest that Translin might also be involved in RNA transport processes in Drosophila. However, the highest expression rates of Translin (and Trax) have been observed during the earliest stages of oogenesis, in the germarium, and the amount of Translin decreases continuously during the development of the egg chamber and is comparably low in stages in which the nurse-cell-to-oocyte transport is at its peak. Because RNA transport and localization processes also take place in Drosophila embryos and as these contain relatively large amounts of Translin and Trax, we focused our analysis on this developmental phase. However, the *translin* mutation *EY06981* did not affect apical localization of *wingless* and *hairy* transcripts and we therefore concluded that *translin* is not essential for the proper localization of these transcripts.

Role of Translin/Trax in DSBR, meiotic HR, and double-strand DNA damage response: Translin was initially shown to specifically bind to consensus sequences residing at the breakpoint regions of chromosomal translocations that are associated with lymphoid malignancies and solid tumors. Thus it was hypothesized that

Translin might be involved in mediating chromosomal translocations. As translocations involve the union of different chromosomes, it is thought that they arose from DNA DSBs that had been substrates for the cellular DNA repair machinery (Ferguson and Alt 2001). Translin binds preferentially to single-stranded or tailed duplex DNA structures, which also occur during double-strand DNA breakage events (Sengupta and Rao 2002). Therefore, Translin might function in NHEJ, a double-strand break repair pathway which is also crucial to V(D)J recombination in developing lymphocytes. However, mice deficient for the murine Translin homolog TB–RBP show a normal development of B- and T-cells, suggesting that Translin has no essential function in NHEJ processes required for immunoglobulin or TcR rearrangements (Chennathukuzhi *et al.* 2003). In addition, TB–RBP-deficient MEFs do not exhibit an increased sensitivity for DNA-damaging agents or irradiation (Yang *et al.* 2004). Similarly, Drosophila *translin* and *trax* single and double mutants also did not show an increased sensitivity for DNA double-strand breaks. In addition, *S. pombe translin* and *trax* single or double mutants also did not exhibit an increased sensitivity for different DNA-damaging drugs (M. Claußen and B. Suter, unpublished observations).

A second cellular DNA double-strand break repair pathway involves the HR of free DNA ends with intact homologous sequences. As this repair process is also initiated upon the occurrence of double-strand breaks or two nearby single-strand breaks, which often contain single-stranded DNA overhangs, Translin might be involved in homologous recombination. In addition to DSBR, HR is also utilized during meiotic recombination. The fact that Drosophila *translin* and *trax* are not essential for meiotic recombination and chromosomal segregation during meiosis argues against these hypotheses.

Very recently, Stein *et al.* (2006) reported alterations in learning and memory, locomotor activity, and anxiety-related behavior in *translin* knockout mice. Similar behavioral phenotypes have been reported for mice lacking the fragile X mental retardation protein (FMRP), suggesting that Translin and FMRP may have similar functions in neurons (Stein *et al.* 2006 and references therein). Similarly, mutations in the Drosophila homolog *dfmr1* have been implicated in affecting locomotor as well as courtship behavior (Dockendorff *et al.* 2002). Behavioral analysis of *translin* and *trax* mutant flies thus might show why *translin* and *trax* remained conserved during evolution even though they have no essential function under laboratory conditions. On the other hand, Translin and Trax might also be redundant with a third gene in an essential pathway, and genetic screens in a *translin* and *trax* mutant background might then reveal the factors that functionally replace Translin and Trax.

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