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BJ Gene

Biochem. J. (2010) **429**, 225–234 (Printed in Great Britain) doi:10.1042/BJ20100273

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REVIEW ARTICLE

Biological roles of translin and translin-associated factor-X: RNA metabolism comes to the fore

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Translin, and its binding partner protein TRAX (translin-associated factor-X) are a paralogous pair of conserved proteins, which have been implicated in a broad spectrum of biological activities, including cell growth regulation, mRNA processing, spermatogenesis, neuronal development/function, genome stability regulation and carcinogenesis, although their precise role in some of these processes remains unclear. Furthermore, translin (with or without TRAX) has nucleic-acid-binding activity and it is apparent that controlling nucleic acid metabolism and distribution are central to the biological role(s) of this protein and its partner TRAX. More recently, translin and TRAX have together been identified as enhancer components of an RNAi (RNA interference) pathway in at least one organism and this might provide critical insight into the biological roles of this enigmatic partnership. In the present review we discuss the biological and the biochemical properties of these proteins that indicate that they play a central and important role in eukaryotic cell biology.

Key words: genome stability, mRNA trafficking, RNA interference (RNAi), translin, translin-associated factor-X (TRAX).

INTRODUCTION

The translin protein was first recorded in distinct biochemical studies that implicated it in a broad range of biological functions [1–3]. The gene was subsequently identified following the characterization of a novel protein that associated with chromosomal breakpoint junctions in human lymphoid malignancies [4], leading to proposals that translin was involved in genome instability and tumour formation. Indeed, the name translin derives from the word translocation. Shortly following these studies, the murine translin gene was independently identified as the gene encoding the TB-RBP (testis–brain RNA-binding protein), consolidating the earlier pointers that indicated translin may have diverse functional roles [5] (for clarity we shall refer to TB-RBP as translin in the review). Translin was found to have an intimate relationship with a binding partner protein that was termed TRAX (translin-associated factor-X) [6]. Both translin and TRAX proteins are highly conserved, with orthologues identified in species ranging from fission yeast through to humans, indicating that they are likely to play a biological role of fundamental importance. Analysis of null mutants in mouse, *Drosophila* and fission yeast demonstrated that these proteins are not essential [7–10], and thus the stage was set for 15 years of research in an array of organisms, resulting in these proteins being implicated in diverse biological processes, including the regulation of genome stability, neuronal regulation/development, spermatogenesis and, more recently, a central role in at least one RNAi (RNA interference) pathway. In the present review we will discuss the work leading to these findings and give an overview of our current understanding of the biochemical properties and the potential biological roles of these two proteins, which will be increasingly at the forefront of a number of research areas.

BIOCHEMICAL PROPERTIES OF TRANSLIN AND TRAX

Translin and TRAX are a paralogous pair of proteins, indicating a close co-evolution [6]. They are also highly conserved proteins, present within most eukaryotes, although *Saccharomyces cerevisiae* is a notable exception; moreover, Pfam-A translin domain proteins are found in some bacteria and archaea, although it remains unknown whether these proteins are functional orthologues of translin (see http://pfam.sanger.ac.uk/).

Human translin is 228 amino acids in length with a monomeric molecular mass of 26 kDa. Analysis of native translin indicates a molecular mass of approx. 220 kDa, suggesting that translin forms octomers in the native state [4]. EM (electron microscopy) and crystallographic studies support this, indicating that translin forms an octomeric toroidal structure [11–15]. However, these two analytical techniques give slightly distinct structures. Crystallographic analysis gives a quaternary ring structure with a 2-fold symmetry perpendicular to the ring based on two tetramers coming together [15]. This forms a central channel through the ring, ranging in aperture from 15 Å to 4.4 Å (1 Å = 0.1 nm) in diameter, with the basic residues from amino acids 86–92 facing inward. This 2-fold symmetry is not as clearly apparent in EM analysis [12]. The earliest study of the translin sequence predicted a leucine zipper motif at amino acid residues 177–212 (encoded by exon 6) [4]; however, the crystal structure data

Abbreviations used: Ago, Argonaute; BDNF, brain-derived neurotrophic factor; C3PO, component 3 promoter of RISC; Cb, C-basic domain; CDGS, chromatin-dependent gene silencing; CREM, cAMP-responsive-element modulator; Dcr, dicer; dsDNA, double-stranded DNA; dsRNA, double-stranded RNA; EM, electron microscopy; FMRP, fragile X mental retardation protein; GADD34, growth-arrest and DNA-damage-inducible protein 34; GRE, glucose-response-element; MBR, mammalian basic region; Mea2, male-enhanced antigen 2; MEF, mouse embryonic fibroblast; miRNA, microRNA; mRNP, mRNA ribonucleoprotein; Nb, N-basic domain; NES, nuclear export signal; NLS, nuclear localization signal; piRNA, piwi-interacting RNA; PTGS, post-transcriptional gene silencing; RdRP, RNA-dependent RNA polymerase; RISC, RNA-induced silencing complex; RLC, RISC-loading complex; RNAi, RNA interference; ssDNA, single-stranded DNA; ssRNA, single-stranded RNA; TB-RBP, testis–brain RNA-binding protein; Ter-ATPase, transitional endoplasmic reticulum ATPase; TRAX, translin-associated factor-X; TRC8, translocation in renal carcinoma on chromosome 8; UTR, untranslated region.

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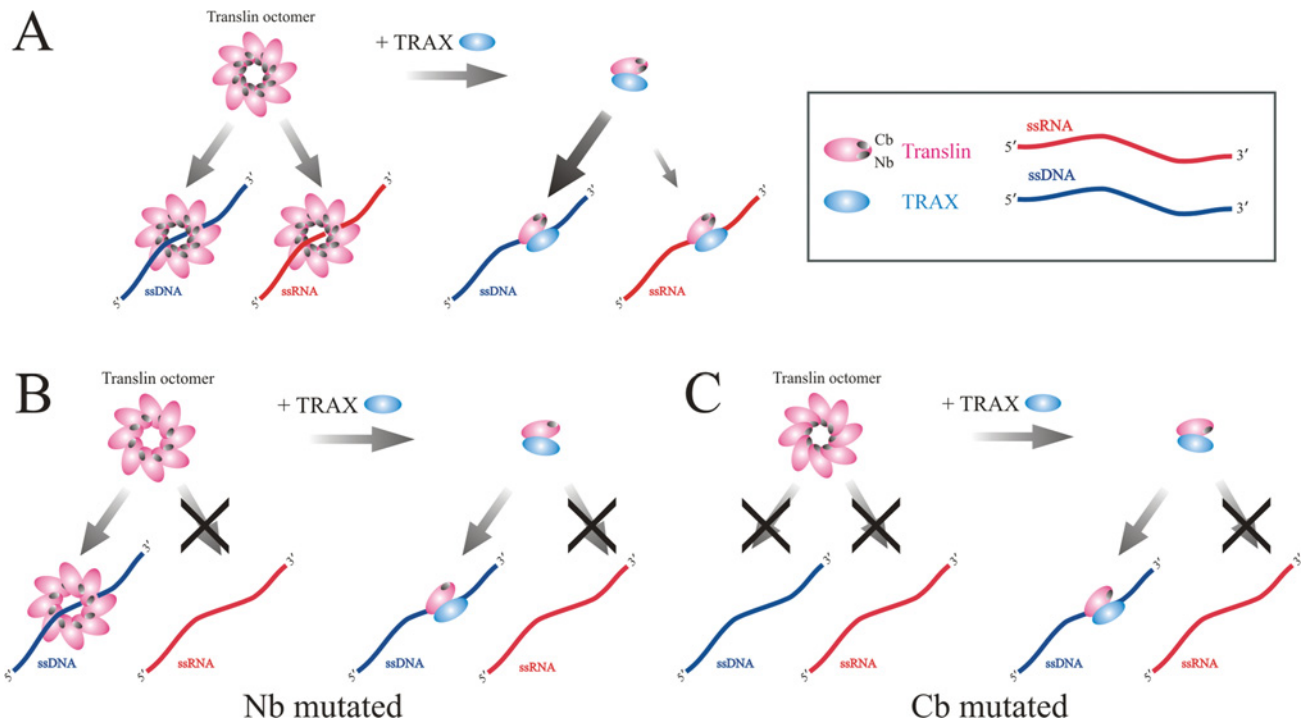

Figure 1 Schematic of the nucleic-acid-binding properties of wild-type and mutant versions of translin

(**A**) Translin (pink ovals) can bind to ssDNA and ssRNA and it is proposed that in the absence of an association with TRAX it does so in an octomeric toroidal form. On association with TRAX (blue oval), it is proposed that the toroidal form is disrupted and a translin–TRAX heterodimer is formed. Studies suggest that the translin–TRAX heterodimer has a greater affinity for ssDNA and a reduced affinity for ssRNA relative to the translin only octomer (the size of the arrows represents relative binding affinity). (**B**) Nucleic-acid-binding activity of translin carrying a mutation in Nb. Mutation of Nb does not alter the ability of translin to bind to ssDNA, but abolishes ssRNA binding. Moreover, the elevated binding to ssDNA is also lost when translin associates with TRAX [compare arrow sizes between (**A**) and (**B**)]. (**C**) Nucleic-acid-binding activity of translin carrying a mutation in Cb. Mutation of Cb prevents translin binding to ssDNA or ssRNA in the absence of TRAX. On addition of TRAX the ability to bind to ssDNA is restored, but not the ability to bind to ssRNA.

suggests these amino acids are within α-helices 6 and 7 and so are unlikely to form a functional leucine zipper [15]. TRAX also contains a putative leucine zipper domain, although, to date, no crystal structure for TRAX has been determined and so it remains unknown whether or not this is a functional leucine zipper.

The quaternary structures of human and mouse translin differ slightly, suggesting that multimerization might be relatively flexible, and could be associated with determining substrate specificity [14,15]. The monomers of the translin octomer appear to be held together by relatively weak hydrogen bonds and hydrophobic interactions, rather than stronger, more specific, interactions, thereby permitting the relative positions of the monomers to be readily altered. Specifically, amino acids within the C-terminal region of translin have been proposed to be important for monomer associations and the formation of the octomeric structure [16–18].

TRAX was first identified as a translin-binding partner by two-hybrid analysis [6]. Subsequently, it has been demonstrated that the stability of TRAX is dependent upon the presence of translin, indicating a close functional relationship between these two proteins. This feature of the protein pairing is conserved in mouse, *Drosophila* and fission yeast (*Schizosaccharomyces pombe*), suggesting that it is a key facet of their biological function [8–10,19]. The regulation of TRAX levels by translin has been shown to be post-transcriptional, as *TRAX* mRNA levels are not altered in translin-depleted cells compared with wild-type [8,19]. Consistent with this inter-dependence, all eukaryotes containing a translin orthologue also contain a TRAX orthologue. The domain within translin required for translin–translin interaction is also required for translin–TRAX association, leading to the possibility that an octomeric form containing only translin units has a distinct functional role (see below) [17]. Murine translin has a functional NES (nuclear export signal), which is conserved in fission yeast. TRAX, however, has a NLS (nuclear localization signal), which does not appear to be as highly conserved in fission yeast [7]. It has been proposed that the nuclear localization of the two proteins in mouse spermatocytes is regulated by the counteracting activities of the translin NES and the TRAX NLS (see below) [20].

A conserved activity of translin is that it can bind to ssDNA (single-stranded DNA), ssRNA (single-stranded RNA) and dsRNA (double-stranded RNA), whereas TRAX has no measurable independent nucleic-acid-binding capability [1,4,5,7,11,18, 21–24]. Translin contains a highly conserved putative GTP-binding site, and the presence of GTP has been shown to negatively modulate the affinity of translin for specific mRNAs in the mouse testes (see below) [25]. Early work on mouse translin demonstrated that TRAX negatively modulates the affinity of translin for RNA and positively influences binding to specific ssDNA molecules *in vitro* [26] (Figure 1). There are two N-terminal basic domains in translin implicated in nucleic-acid-binding [the Nb (N-basic domain), KAREH; and the Cb (C-basic domain), RFHEH] (Figure 1). Mutation of either Nb or Cb significantly reduces or totally abolishes RNA binding *in vitro*, irrespective of the presence or absence of TRAX (Figure 1). Mutation of Nb does not appear to alter binding to ssDNA; however, whereas the addition of TRAX stimulates binding of unmutated translin to ssDNA, this stimulation is not observed in the Nb mutant (Figure 1B). In contrast, when Cb is mutated, DNA binding is abolished, but is restored on addition of TRAX [26] (Figure 1C). Taken together these observations

imply that the interaction between TRAX and translin may serve to control substrate specificity *in vivo* (see below).

In fission yeast, whereas the nucleic-acid-binding domains appear to be poorly conserved at the primary amino acid level, structural features seem to be well conserved [17]. In *S. pombe* the basic nucleic-acid-binding domains of translin have been designated MBRI and MBRII (mammalian basic region I and II). Mutational analysis of *S. pombe* MBRI and MBRII indicates that MBRI plays a lesser role, if any, in nucleic acid binding than MBRII.

Human translin has a higher affinity for $d(GT)_n$ and $d(TTAGGG)_n$ repeats, which has led to the suggestion that it may play a role in the metabolism of telomeres or microsatellites [27,28]. Interestingly, in contrast with human translin, octomeric *S. pombe* translin has a higher affinity for G-rich ssRNA than for G-rich ssDNA [7], so might be more likely to function in RNA metabolism [7]; indeed the distinct hydrophobicity of the *S. pombe* and human translin nucleic-acid-binding domains is consistent with the *S. pombe* translin having a greater affinity for RNA [17].

The minimum oligonucleotide length to which human translin can bind has been determined as 11 nucleotide residues [27]. The internal aperture of the translin octomer is too narrow to accommodate dsDNA (double-stranded DNA), which is 23.7 Å wide. The translin octomer requires free ssDNA for binding and while it can bind to the single-stranded ends of dsDNA with a single-stranded tail, it cannot bind to ssDNA flanked on either side by dsDNA *in vitro*, indicating that translin does not have a hinge for DNA loading [27]. These structural and biochemical studies strongly suggest that the biological role of translin relates to nucleic acid metabolism, either of ssDNA or ss/dsRNA (see below).

A ROLE IN GENOME STABILITY, CELL PROLIFERATION AND HUMAN GENETIC DISEASE?

The fact that translin was identified as a protein that binds to breakpoint junctions in lymphoid neoplasms suggests that it plays a role in carcinogenesis or genome stability regulation [4]. Translin-binding sites have subsequently been found in a range of human cancer-related chromosome translocation breakpoints [29–33], Sotos syndrome-associated chromosomal rearrangement breakpoints [34], balanced reciprocal translocation breakpoint junctions [35], human meiotic recombination hotspots [36], human chromosomal deletion breakpoints [37] and gene conversion/chromosomal rearrangement sites associated with inherited genetic disease [38,39]. The presence of translin-binding sites at chromosomal rearrangement breakpoints suggests an involvement in the regulation or initiation of recombination within eukaryotic genomes.

In constrast with this proposal, mouse, *Drosophila* and *S. pombe* translin-null mutants do not exhibit any obvious defects in processes in which recombination is involved, including DNA damage recovery, V(D)J (somatic) recombination (in mouse) and meiotic recombination [8–10]. Moreover, to date, there has been no report to suggest that mice lacking translin exhibit higher levels of neoplasms [9]. Mutants of *TRAX* have been generated in *S. pombe*, but like *S. pombe* translin mutants, these mutants exhibit no overt defect in recombinogenic processes [8]. Despite this lack of direct evidence for a role of translin in genome stability regulation, it remains to be tested whether or not translin and/or TRAX function in a redundant DNA repair/recombination pathway. There are a number of studies that provide evidence to indicate translin and TRAX do play some (as yet undefined) role in DNA damage recovery. First, translin-deficient mice have delayed haemopoietic colony formation in the spleen following irradiation with sub-lethal doses of ionizing irradiation [40]; the failure of other translin-deficient cells to exhibit sensitivity to DNA-damaging agents may indicate that translin (and possibly TRAX) have evolved tissue-specific roles in complex metazoans. Consistent with a haemopoietic-specific role for translin in DNA repair, translin is localized to the nucleus in haemopoietic cell lines, whereas it is predominantly cytoplasmic in many other tissues [4]. Secondly, translin has been shown to translocate from the cytoplasm to the nucleus of HeLa cells in response to the DNA-damaging agents mitomycin C and etoposide [11]. In contrast, however, *S. pombe* translin mutants do not exhibit sensitivity to DNA-damaging agents, including mitomycin C [8]. Thirdly, translin and TRAX have been shown to bind to other proteins involved in the DNA damage response. Translin has been demonstrated to bind to GADD34 (growth-arrest and DNA-damage-inducible protein 34) by yeast two-hybrid analysis [41]. GADD34 is a DNA-damage-inducible protein that, in conjunction with other proteins, serves to inhibit apoptosis in response to DNA damage [42–45]. However, given the finding that GADD34 has been implicated in translational initiation (for a review, see [46]), the role of translin in association with GADD34 could relate to an RNA-processing/binding activity and not a direct DNA damage association (see below). TRAX has also been shown to bind to C1D specifically in response to γ-irradiation [47]. C1D is an activator of DNA-PK (DNA-dependent protein kinase), a protein that is required for repair of DNA double-strand breaks during V(D)J recombination and non-homologous DNA end-joining repair [48–50]. C1D has also been implicated in other pathways where it interacts with distinct proteins; it has been found to associate with Cut3 in *S. pombe*, which is a SMC (structural maintenance of chromosome) protein involved in chromosomal condensation [51], Rac3, a small GTPase [52], and it has been implicated in exosome function, indicating a link between TRAX and RNA processing/degradation (see below) [53,54]. The interaction of TRAX with C1D and translin has been reported to be mutually exclusive, suggesting that TRAX may have a translin-independent function via C1D. If such translin-independent functions exist, dissecting them from translin-associated functions will be complicated by the fact translin-deficient cells are also depleted for TRAX [8–10].

Translin has also been proposed to have a role in cell proliferation control. Comparisons of the levels of various proteins during the mitotic cell division cycle in mouse spleen cells appears to indicate that levels of translin correlate with the rate of cell proliferation. In agreement with this, translin levels are reduced following irradiation-induced cell-cycle arrest, and expression of the translin gene appears to exhibit some cell-cycle-dependent periodicity, peaking during S-phase [55]. Furthermore, overexpression of the translin gene gives an apparent increase in cell proliferation levels [55]. Indeed, in a mouse model for lung cancer, overexpression of translin was linked with an invasive phenotype, suggesting that translin levels might directly influence cell proliferation during tumour progression [56]. The proposal that translin (and TRAX) influence cell proliferation is supported by a further study in which translin-deficient MEFs (mouse embryonic fibroblasts) show a reduced proliferative rate relative to MEFs derived from their translin-proficient litter mates. Consistent with this, reductions in translin and/or TRAX levels using RNAi also reduced cell proliferation [19,57]. Localization of translin in HeLa cells demonstrates an enrichment of translin at the centrosomes, bipolar mitotic spindles and mid-bodies [55], a finding paralleled by the observation that some cellular translin has centrosomal localization in *Xenopus* [58]. So it is possible that

cell proliferation is influenced by translin via a regulatory link to the spindle apparatus [55]. Further evidence linking translin to cell-cycle regulation comes from the observation that translin-null mice have a reduced rate of cell proliferation in the first few months of development, although by 6 months they exhibit the same body weights as their translin-proficient litter mates [9]. However, this elevated body weight is ascribed to higher levels of body fat [9] and an independent screen for mouse genes involved in obesity identified translin mutants to have obesity characteristics, the molecular basis of which remains unknown [59].

Mouse translin has been demonstrated to bind to microtubules and distinct mRNAs (see below) and so it is possible that proliferative capability is reduced indirectly due to reduced mRNA trafficking associated with microtubules (see below) [60,61]. Despite the evidence from mammalian studies, it appears that no such role exists for translin or TRAX in cell proliferation in *S. pombe* or *Drosophila*, and it is possible that translin (and TRAX) from distinct organisms might have evolved distinct functional roles in cell proliferation control [7,8,10].

There is also evidence that translin plays a role in meiosis. First, translin-binding sites have been located within human male meiosis recombination hot-spots [36], although no defects in meiotic recombination have yet been identified in fission yeast or *Drosophila* [8,10]. Secondly, translin-null mice have reduced fertility, with males producing fewer spermatozoa and many germ cells do not proceed beyond the first meiotic metaphase during spermatogenesis; furthermore, translin-null females have reduced litter sizes [9]. These observations can possibly be explained by a role for translin in germ cell mRNA processing (see below). In *Drosophila* early spermatocytes, translin levels are elevated, although loss of translin does not reduce fertility [10].

REGULATION OF mRNA DYNAMICS: KEY ROLES IN GERM LINE DEVELOPMENT AND NEURONS?

As previously described, the mouse orthologue of translin is more frequently referred to as TB-RBP [5,62]. Early work using the mouse model suggested that translin was present at higher levels in the brain and the testes, indicating a possible tissue-specificity [3]. However, the genes encoding translin and TRAX appear to be more ubiquitously expressed than believed previously, as illustrated by the localization to haemopoietic cells (see above). They are also expressed in kidney cells, and translin and TRAX proteins have been identified as components of the previously characterized GRBP [GRE (glucose-response-element)-binding protein] found within the liver which binds to the MLTF (major late transcription factor)-like site within the GRE of the liver-type pyruvate kinase gene [63,64]. Findings such as these not only indicate a broader functional role for translin and TRAX, but also that they have roles in a diverse array of tissues.

The different roles of translin and TRAX in distinct tissue types have been linked to the translocation of mRNAs or translational regulation. It was proposed that translin bound only to Y- and H-elements of 3′-UTRs (untranslated regions) of mRNAs found in the brain and testis [3,5,60,65,66]. However, these elements are G-rich and translin preferentially binds to most G-rich ssRNAs, and so the biological importance of the 3′-UTR mRNA binding specificity remains unclear. However, the binding of translin to mRNA 3′-UTRs in neurons and in germ cells has been demonstrated to prevent their translation, suggesting this binding is an important feature of their functional role [62,65,67]. Translin is localized to the nucleus during meiosis, but has a cytoplasmic localization in subsequent developmental stages, indicating a possible temporal regulation of mRNA processing, that to some degree might be governed by the ratio of translin and TRAX levels [68].

In male mouse germ line cells translin is involved in the inter- and intra-cellular transport of specific mRNAs as a component of the mRNP (mRNA ribonucleoprotein) complex containing the Ter-ATPase (transitional endoplasmic reticulum ATPase); these include translationally delayed X-chromosome-encoded mRNAs [69]. Moreover, translin has been linked to the kinesin family member KIF17b, as it co-precipitates with KIF17b from mouse testes cell extracts [70]. The kinesin family of motor proteins are required for driving the movement of macromolecules, including mRNAs, along microtubules [71]. KIF17b is responsible for the nucleoplasmic localization and transcriptional co-activation of ACT (activator of cremin testes), the activator of CREM (cAMP-responsive-element modulator) in post-meiotic germ cells [72]. The mRNAs that are bound to translin are dependent upon CREM for their transcription, linking KIF17b and translin in transcriptional and translational regulation of post-meiotic mRNAs. This regulation can be associated with microtubule binding given the finding that translationally delayed mRNAs are linked to microtubules by translin, including mRNAs for protamines 1 and 2 [60,66].

The association with microtubules has resulted in the suggestion that translin can also act as a kinesin and this is supported by the fact that mammalian translin contains two putative domains (residues 9–35 and 14–47) with 55 % and 62 % similarity to human kinesin heavy-chain [5]. Loss of human kinesin heavy-chain function does not impair movement of all mRNAs, suggesting there might be multiple kinesin activities involved in trafficking of distinct mRNPs, some of which might be translin-specific [60,73]. A model has been proposed (Figure 2) in which a mRNP complex containing translin and KIF17b is exported out of the nucleus through nuclear pores, utilizing the translin NES that binds to the CRM1 exportin [20]. The mRNP is then anchored to and moves along microtubules, mediated by kinesin proteins (e.g. KIF17b) or translin, in conjunction with TRAX and Ter-ATPase. This is concomitant with translational suppression that is controlled in a temporal and spatial fashion by the dissociation of, first, KIF17b and then translin. Translin is then recycled back into the nucleus via association with TRAX, which might occur via interaction of the TRAX NLS with an importin, although this remains to be demonstrated [20] (Figure 2).

TRAX has also been linked to a kinesin family member protein in the testes. The kinesin family member KIF2Aβ was identified as a TRAX-interacting protein in a yeast two-hybrid screen using mouse testes cDNA, and TRAX and KIF2Aβ were shown to co-localize to the perinuclear region implicating TRAX in macromolecular movement in germ line cells, possibly of mRNAs [74,75]. No interaction was detected between translin and KIF2Aβ, and so it remains unclear whether this is a translin-independent function of TRAX. TRAX also associates with Mea2 (male-enhanced antigen 2) [75], which is required for spermatogenesis [76] and is the orthologue of the *S. pombe* meiotic spindle pole body-associated protein Spo15, although interaction between Spo15 and *S. pombe* TRAX remains to be tested [77]. TRAX and Mea2 localize to the Golgi complex during mid-to-late meiotic pachytene, but the biological significance of this interaction/localization is unknown [76].

Translin and TRAX have been shown to be present at high levels in neurons and have been implicated in neuronal function and development [5,65,67,78–80]. During development in *Drosophila* translin appears in ventral neuroblasts, and both mouse and *Drosophila* translin mutants exhibit neurological disorders, indicating translin plays a key role in neurological function [9,10,81,82]. Consistent with a role in gene expression,

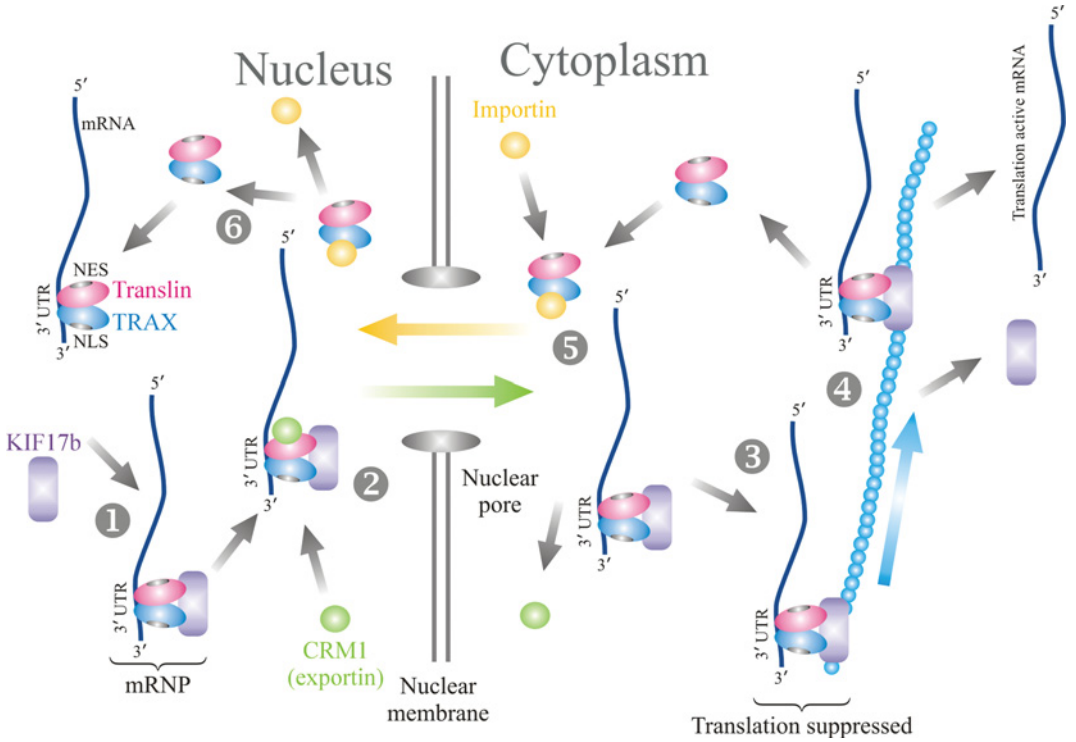

Figure 2 Schematic outline of the proposed role of translin and TRAX in mRNA trafficking and translational repression in mouse spermatocytes

Step 1: translin and TRAX bind to the 3′-UTR of the mRNA and recruit the kinesin, KIF17b, to form a mature mRNP complex, which also contains the Ter-ATPase (not shown). Step 2: CRM1 (exportin) binds to the translin NES and mediates the export of the mRNP out of the nucleus through the nuclear pore. Step 3: CRM1 dissociates and the mRNP is loaded on to microtublules (small conjoined blue circles) where the kinesin activity (or translin) mediates the microtubule-dependent trafficking to a region where translation of the mRNA is required. Step 4: the mRNA becomes dissociated from the microtubule and translation is de-repressed by the sequential loss of first the kinesin, and then translin and TRAX. Translation can now ensue in the appropriate cellular location. Step 5: importin associates with the nuclear localization domain on TRAX and translin is recycled into the nucleus with TRAX in a TRAX-dependent fashion. Step 6: importin is lost and the recycled translin and TRAX can re-initiate the export cycle by forming a new mRNP. This Figure is adapted with permission from [20].

microarray analysis of brain extracts determined that mRNA levels from 14 genes were elevated and levels of mRNAs from over 200 genes were reduced in the translin-null mouse relative to wild-type litter mates; a number of neurotransmitter receptors and ion channels were within the group of strongly down-regulated mRNAs [9].

Recently, translin and TRAX have been demonstrated to be required for the intracellular targeting of mRNA splice variants of the mammalian *BDNF* (brain-derived neurotrophic factor) gene [83] (Figure 3). These splice variants share a common exon, which contains the full-length *BDNF* open reading frame. Within this exon is a translin/TRAX-binding site and the association of translin with this site is required for targeting of *BDNF* mRNAs to dendrites (Figure 3). Reduction in translin levels with siRNA (small interfering RNA) results in a failure to achieve dendritic localization of *BDNF* mRNAs. However, not all splice variants containing the translin-binding site localize to dendrites as some variants contain unique 5′-UTRs that counter the translin-mediated *BDNF* mRNA dendritic localization [83] (Figure 3). Mutating the open reading frame sequence in the translin-binding region (G196A) inhibits dendritic targeting. The G196A mutation is associated with human neurological and psychiatric disorders, including reduced hippocampal dendritic complexity and volume, memory defects and mood disorders [84–86]. These findings directly link translin to the pathophysiology of complex human neuropsychiatric disorders [83]. The *TRAX* gene has also been genetically linked to human psychological disorders, supporting the proposal that mRNA processing by translin/TRAX is important for brain function in humans [87–92].

Interestingly, the neurological defects present in the translin-null mutant mice [9,81] are similar to those seen for mice mutated in the gene encoding the FMRP (fragile X mental retardation protein), *frm1* [93–95]. FMRP has similarities to translin in that it is found at comparatively high levels in the testes and the brain and is a selective RNA-binding protein that regulates protein synthesis at the neuronal synapse [96]. Collectively, these observations have resulted in the suggestion that translin and FMRP function in similar biological pathways [81].

Translin and TRAX have been further implicated in neuronal regulation in a number of distinct studies. First, translin functions to stimulate steroidogenic-factor-1-mediated transcription (2-fold) through unknown mechanisms [97]. Secondly, TRAX has been identified as a functional partner of the A_{2A}-R (A_{2A} adenosine receptor), which is involved in regulation of neuronal plasticity and development [98]. Thirdly, TRAX functions to regulate the production of the GAP-43 (growth-associated protein 43) during retinal ganglion cell development [99].

KEY PLAYERS IN RNAi PATHWAYS

It has been widely demonstrated that small RNA molecules (of approx. 20–30 nucleotides) play a key role in the governance of pathways that process larger polyribonucleotide molecules, controlling if and when they are translated and/or degraded. These small RNAs are primarily linked to two broad pathways for gene silencing known as: (i) PTGS (post-transcriptional gene silencing), where small RNA molecules direct translational inhibition and/or mRNA destruction; and (ii) CDGS

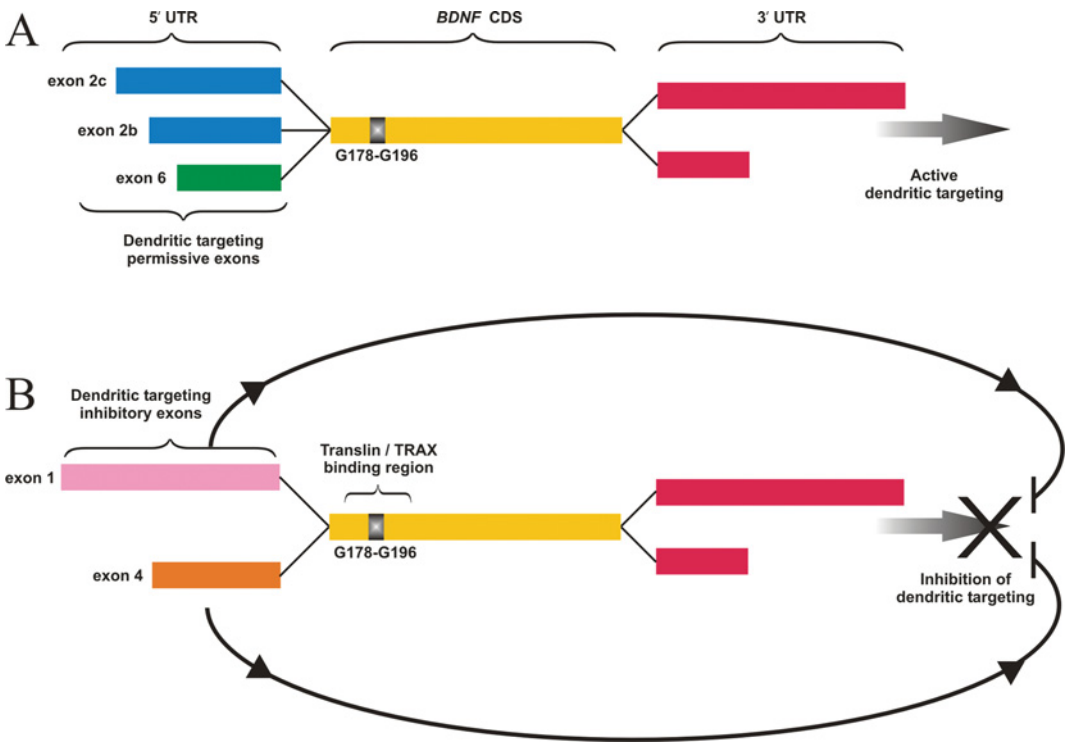

Figure 3 Distinct splice variants transcribed from the *BDNF* gene have distinct dendritic-targeting specificities

One of the central exons of the *BDNF* gene contains the whole *BDNF* coding sequence (yellow). This exon is found in all *BDNF* mRNA splice variants. This exon also contains a specific translin/TRAX-binding region (grey box within the central yellow exon). Translin/TRAX binding to this region is required for appropriate *BDNF* mRNA dendritic targeting. Mutation of this region is linked to human neurological disorders (see the main text) linking translin to human neurological disease. Two distinct 3′-UTRs (red) can be found on distinct variants and these are linked to an inducible increase in targeting of *BDNF* mRNA species via uncharacterised mechanisms. (**A**) mRNA splice variants with three distinct 5′-UTRs are permissive for translin/TRAX-mediated dendritic targeting. These contain 5′ exons 2b (blue), 2c (blue) or exon 6 (green). (**B**) Alternatively, mRNAs containing 5′ exon 1 (pink) or exon 4 (orange) contain a signal which inhibits translin/TRAX-mediated dendritic targeting via unknown mechanisms. This Figure is adapted with permission from C. Chiaruttini, A. Vicario, Z. Li, G. Baj, P. Braiuca, Y. Wu, F.S. Lee, L. Gardossi, J.M. Baraban and E. Tongiorgi. Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. Proc. Natl. Acad. Sci. U.S.A. **106**, 16481–16486, © 2009, The National Academy of Sciences, © 2009 The Authors.

(chromatin-dependent gene silencing), where small RNA molecules direct the generation of heterochromatin, which results in transcriptional repression. Moreover, small RNA molecules are implicated in other processes, including having a role in the molecular mechanisms that confer specific chromatin activity to regions such as centromeres and as a genome defence mechanism against retrotransposition and viral infection. RNAi collectively refers to these small RNA molecules and the molecular machinery associated with their biological function (for reviews, see [100–102]). Small RNA molecules are divided into three distinct sub-classes, siRNAs, miRNAs (microRNAs) and piRNAs (piwi-interacting RNAs). siRNAs and miRNAs have been implicated in both PTGS and CDGS, whereas piRNAs are involved in the inhibition of parasitic DNAs, such as viruses and transposable elements [100]. siRNAs and miRNAs are generally distinguished by their mode of biogenesis. miRNA pathways are conserved in plants and animals, but are not apparent in lower eukaryotes. miRNAs are generated from intra-strand hairpin-forming single RNA molecules by the successive action of drosha and Dcr (dicer), two ribonuclease III enzymes located in the nucleus and cytoplasm respectively. siRNAs are generated from long dsRNAs which can be formed via a number of routes, including long-hairpin RNAs. In some cases dsRNA is synthesized from non-coding RNA molecules which are processed into dsRNA by an RdRP (RNA-dependent RNA polymerase), although RdRPs are absent from *Drosophila* and mammals (reviewed in [100]). The small RNA molecules are guided to their respective target RNAs via effector complexes known as the RISC (RNA-induced silencing complex) in PTGS or a nuclear form termed RITS (RNA-induced transcriptional silencing complex) in CDGS. Both complexes contain an Ago (Argonaute) family protein that mediates the cleavage of the target RNA via an endoribonuclease activity of the RNaseH-like piwi domain [100].

In *Drosophila*, it is proposed that siRNAs are loaded on to RISC via an RLC (RISC-loading complex), which contains Dcr-2 and a partner protein R2D2; the Dcr-2 activity in the RLC is genetically distinct from its role in generating siRNAs (reviewed in [102]) (Figure 4). Lui et al. [103] found that, when recombinant Dcr-2 and R2D2 were added to recombinant Ago2 and duplex siRNA, low levels of RISC activity were reconstituted, with Ago2 being responsible for mRNA cleavage activity. Subsequently they purified a RISC enhancer activity and termed it C3PO (component 3 promoter of RISC), the other two components being Dcr-2 and R2D2. Analysis of C3PO revealed that it consisted of translin and TRAX, and both were required for full activity [103]; the study took this further by demonstrating that C3PO was required for RNAi *in vivo*, indicating that, in *Drosophila* at least, translin and TRAX are key enhancers of the RNAi machinery. Further biochemical analysis revealed that C3PO possessed ribonuclease activity [103]. That group propose that C3PO associates with Dcr2–R2D2 during RLC activity to form the active RISC complex and serves within this complex to remove the passenger strand of the duplex siRNA, freeing the guide strand to target Ago2 to the target mRNA (Figure 4). This activity is associated with

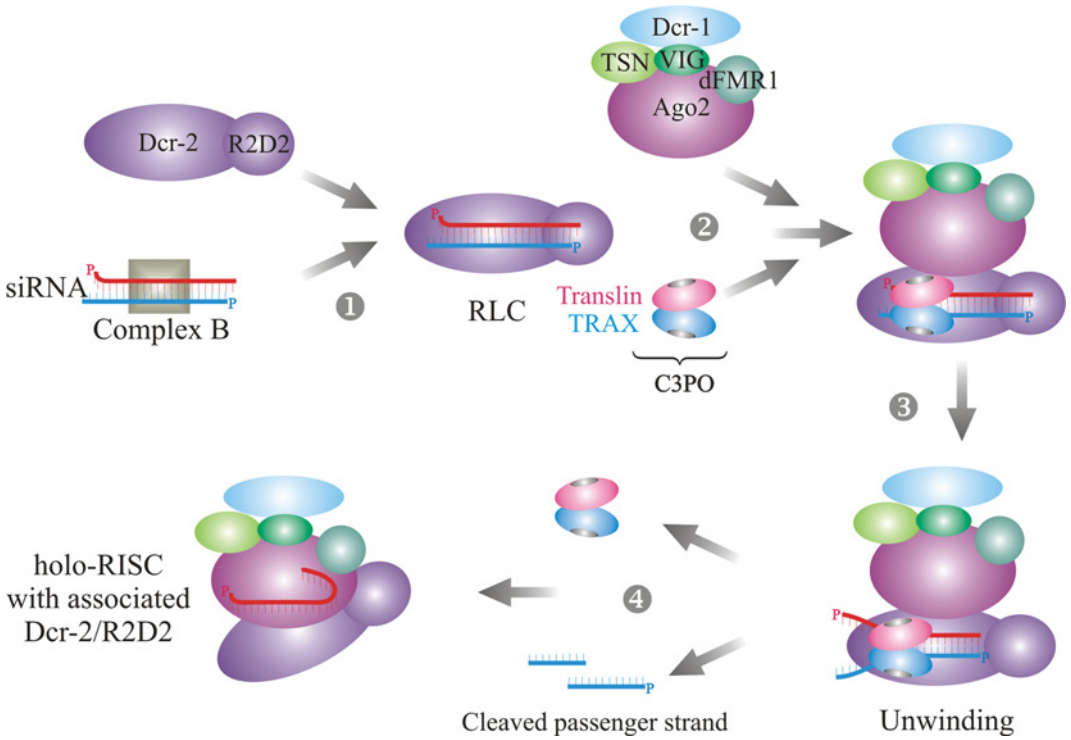

Figure 4 Schematic of the proposed role of translin and TRAX (C3PO) in *Drosophila* RNAi

Step 1: the siRNA duplex is transferred from complex B to the RLC, consisting of Dcr-2 and R2D2. Step 2: C3PO (translin and TRAX, grey ellipses are the NLS and NES; see Figure 1) are joined with the RLC and the RISC complex {consisting of the Dcr-1, TSN (Tudor-staphyloccal nuclease), VIG (vasa intronic gene), dFMR (*Drosophila* FMR) and Ago2 subunits; see [102] for further details} to generate the holoRISC by a Drc-2–Ago2 interaction. Step 3: the passenger strand is removed/endonucleolytically cleaved from the siRNA, which is enhanced by C3PO activity. Step 4: the holoRISC complex can proceed to associate with target mRNAs. It remains unclear whether C3PO remains associated with the holoRISC, or whether it dissociates, as depicted in the model shown. See [102] and [103] for more details. An animated version of this Figure is available at http://BiochemJ.org/bj/429/0225bj4290225add.htm.

endonucleolytic cleavage of the passenger strand, which was ascribed to an RNase activity of C3PO, and required conserved residues on TRAX. This model is consistent with previous work in which translin has been shown to bind to small RNA molecules and possess ss/dsRNase activity *in vitro* [10,24,104].

RNAi pathways are highly conserved and the expression of approximately one-third of all human genes is proposed to be controlled by miRNAs [105]. One model organism notable for lacking RNAi regulators is *S. cerevisiae* and this yeast also lacks orthologues of translin and TRAX; indeed other yeasts that possess the RNAi machinery, such as *S. pombe*, also possess translin and TRAX orthologues. Although *S. pombe* does not appear to contain an orthologue of R2D2, it remains to be seen whether translin and TRAX function in concert with the *S. pombe* Dcr orthologue, Dcr1 and/or in passenger strand removal.

Interestingly, a recent study on a human dysgerminoma found reduced levels of the potential tumour suppressor TRC8 (translocation in renal carcinoma on chromosome 8) concomitant with reduced levels of translin [106]. The 3′-UTR for the *TRC8* mRNA contains multiple conserved target sites for multiple miRNAs. The authors of that study postulate a possible link between the loss of translin and the reduced production of TRC8. That study might be a paradigm for a link between a mechanistic role for translin in RNAi and human cancerous disease [106].

CLOSING REMARKS

Translin and TRAX have provided a challenging series of puzzles over the last 15 years. They are highly conserved and yet are not essential for cellular life. They have appeared in disparate studies, which has made pinpointing specific functions difficult. They clearly regulate biological systems via interaction with nucleic acids, but it remains unclear whether or not they have similar substrate specificities in distinct species or distinct tissue types of more complex metazoans. The growing body of evidence, collated in the present review, strongly indicates a primary role for translin and TRAX in RNA metabolism, both mRNA processing and trafficking, and RNAi regulation of protein production, two functions that seem unlikely to be mutually exclusive. Despite the fact that there is little direct or conclusive evidence pointing to a role in DNA metabolism, it remains a compelling possibility that the octomeric ring formed by translin plays some direct role in chromosome dynamics. Such toroidal structures are frequently associated with DNA repair, recombination and replication processes, indeed key questions relating to the relationship between translin and genome stability remain unanswered by the current results. Although these two proteins seem to have retained an intimate functional relationship throughout evolution, the possibility remains that they have become promiscuous, functioning independently, taking on altered substrate preferences in the absence of their long-term partner. Finally, the discovery that C3PO, the RNAi RISC complex enhancer, is made up of translin and TRAX will, no doubt, result in accelerated research into the functions of these proteins, shedding light on the mechanisms of their many roles in cellular biology.

ACKNOWLEDGEMENTS

We thank David Pryce and Jane Wakeman for careful review of this manuscript prior to submission.

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Received 19 February 2010/20 April 2010; accepted 22 April 2010
Published on the Internet 28 June 2010, doi:10.1042/BJ20100273