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Review

Translin: A multifunctional protein involved in nucleic acid metabolism

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Translin, a highly conserved, DNA/RNA binding protein, is abundantly expressed in brain, testis and in certain malignancies. It was discovered initially in the quest to find proteins that bind to alternating polypurines-polypyrimidines repeats. It has been implicated to have a role in RNA metabolism (tRNA processing, RNAi, RNA transport, etc.), transcription, DNA damage response, etc. Studies from human, mice, drosophila and yeast have revealed that it forms an octameric ring, which is important for its function. Translin is a cytoplasmic protein, but under genotoxic stress, it migrates into the nucleus, binds to the break point hot spots and therefore, thought to be involved in chromosomal translocation events as well as DNA damage related response. Its structure is known and DNA binding regions, GTP binding region and regions responsible for homotypic and heterotypic interaction are known. It forms a ball like structure with open central channel for accommodating the substrate nucleic acids. Besides this, translin protein binds to 3′ and 5′ UTR of certain mRNAs and probably regulates their availability for translation. It is also involved in mRNA transport and cell cycle progression. It forms a heteromeric complex with translin associated factor-X (TRAX) to form C3PO complex which is involved in RNA silencing process. Recently, it has been shown that translin is upregulated under starvation conditions in Drosophila and is involved in the integration of sleep and metabolic rate of the flies. Earlier studies classified translin as a DNA repair protein; however subsequent studies showed that it is a multifunctional protein. With this background, in this review we have summarized the translin biochemical activities, cellular function as well as structural properties of this important protein.

Keywords. DNA binding; DNA repair; RNA binding; Translin; translocations; TRAX

1. Introduction

DNA damage and repair is a continuous and dynamic process through which cells maintain their genomic integrity. In addition, DNA recombination process leads to generation of diversity during meiosis. Several proteins have been implicated in this vital process at different stages. Through various studies, Translin protein has emerged as one such important protein speculated to be involved in the process of DNA damage repair and recombination. Translin protein was identified with different names in separate studies on human and mouse systems (Aharoni *et al.* 1993; Kasai *et al.* 1994; Han *et al.* 1995). Subsequently, the encoding gene was isolated from T-ALL (T acute lymphoblastic leukaemia) cell line, DND41 and sequenced. Owing to the binding of the isolated protein specifically to the conserved chromosomal translocation breakpoint sequences, it was named Translin (Translocation) (Aoki *et al.* 1995). The first report on identification and characterization of Translin protein was by Aharoni *et al.* (1993). Translin was identified as a single stranded DNA binding protein, specifically single stranded d(GA)n/d(GT)n rich DNA, and was designated as PGB. It was identified during a search of proteins that bind to simple sequence DNA elements - alternating polypurines-polypyrimidines repeats d(TC)n.d(GA)n, that are spread across mammalian genomes. Such structures are known to transform to DNA triplexes that act as points of DNA replication arrest. PGB was isolated from nuclear extracts of human fibroblasts obtained from foreskins that were grown on microcarrier beads. It bound specifically to d(GA)n strand in the nuclear extract. Further, it was known to bind avidly to d(GT)n tracts and to other G rich ssDNA repeats, albeit with

lower affinity. Competition assays of PGB with various DNA substrates revealed that it was not a general single stranded DNA binding protein and it bound most avidly to d(GA)n/(GT)n tracts of n> 5, more to oligonucleotides with repetitive G residues (Aharoni *et al.* 1993). The nuclear localization of translin and its specificity to particular DNA substrates that are widespread across genomes suggested its biological role associated with DNA replication. However, subsequent report by Kasai *et al*. (1994) found a similar protein, REHF-1 (REcombination Hotspot-associated Factor-1) to be associated with chromosomal rearrangements, especially TCR delta locus. Translin was identified as a cytoplasmic phosphoprotein, TB-RBP (Testis-Brain RNA binding protein) from mouse testicular and brain tissues (Kwon and Hecht, 1991 and 1993). It specifically bound to 3′ UTR regions of protamine-2 mRNA in testis and 3′UTR of brain Tau mRNA of mouse. The 3′ UTR of protamine-2 mRNA contains highly conserved elements, and such conserved elements are present in several translationally regulated brain mRNAs, which could be a substrate of TB-RBP. Therefore, its role was suggested in storage, translocation and/or localization of specific mRNAs of brain and testis of mouse (Han *et al.* 1995). The mRNA population of brain and testicular tissue is expected to be different, suggesting a diverse, yet specific role of TB-RBP. However, like human translin, TB-RBP also showed specific binding to the target sequences of ssDNA, suggesting its role in DNA recombination/repair in male germ cells (Wu *et al.* 1997). Taken together, the initial biochemical analyses suggested Translin to be involved in DNA replication, repair or recombination, DNA unwinding which possibly exposes it to nuclease cleavage (nuclear functions) and also in metabolism of specific RNAs (extra nuclear functions, figure 1). Both the functions would obviously be in unison with several other factors, one of the significant partners is TRAX (TRanslin Associated Factor X). TRAX was identified to be the interacting partner of translin using yeast two hybrid system. The presence of nuclear localization signal on TRAX indicated its role in nuclear transport of translin, which would be triggered by events like recombination at Ig and TCR loci in lymphoid cells (Aoki *et al.* 1997). In addition, there is evidence of conformational changes brought about in DNA targets on translin binding that lead to its exposure to cleavage by DNase, suggesting the role of translin in DNA repair/recombination process (Sengupta and Rao 2002). However, while dissecting the proteins in the RISC complex, Wang *et al.* (2004) established that TB-RBP/Translin possess both single strand RNase and double stranded RNase activities but not DNase activity.

2. Structural aspects of Translin protein

Initial studies on translin native structure were reported by Kasai *et al.* (1997). Electron microscopic and X-ray crystallographic studies on human translin protein revealed that the translin in its native form assembles as an octamer. This assembly is required for its DNA binding activity. Sengupta and Rao (2002) showed the presence of a higher oligomeric structure along with the octamer form. Leucine zipper at C-terminal region of translin was initially hypothesized to participate in subunit-subunit interaction to form a ring like structure. In rice, deletion of seven amino acids at C-terminal makes the protein inactive (Gupta *et al.* 2017). In support of this, TEM analysis of the translin native complexes revealed a ring like structure (Kasai *et al.* 1997). Mutational analysis in the C-terminal region of rice translin revealed that they are critical for octamer formation as mutant proteins failed to form ring like structures (Gupta *et al.* 2017). Further, X-ray crystallography also revealed a two-fold symmetry in translin native structure. Based on molecular mass, TEM and X-ray crystallography data, it was concluded that translin forms octameric ring in its native state. Subsequently, electron microscopy of human, mouse and chicken translin revealed that the ring shape is conserved across the three species (Aoki *et al.* 1999). However, chicken translin (9.4 Å) is little bigger than human (8.5 Å) and mouse (8.6 Å) translin. It was suggested that chicken translin ring harbours 10 subunits in contrast to human and mouse translin, where both the proteins are octamers. Single particle analysis of Translin, Translin-DNA/RNA complexes revealed that translin octamer assembles to form a ring with 30 Å and 50 Å open channel at both the ends, in which nucleic acid molecule is accommodated (Vanloock *et al.* 2001). Like that of other nucleic acid binding proteins (ring shaped helicases and recombinases), translin also conserved the ring shaped quaternary structure for its function. A 2.2 Å resolution X-ray crystallographic structure was solved by Sugiura *et al.* (2004). It was observed that two asymmetric tetramer units with four-fold axis symmetry interacted one on the other to form an octameric structure with two-fold symmetry. These interactions were flexible and thought necessary for accommodating the substrate nucleic acid molecule.

The translin monomer folded into 7 alpha helices and six of them arranged in parallel to each other. The central channel had a wide (15 Å) and narrow (4 Å) openings. The nucleic acid binding residues are placed inside the channel cavity. Drosophila translin crystal structure was solved at 3.7 Å resolution (Gupta *et al.* 2008). During gel filtration analysis, it was observed that wild type translin forms decameric ring structure like that of chicken translin. In contrast, a point mutant translin protein (P168S) which fails to bind to DNA forms tetrameric structure. Subsequently, low resolution structure at 7.0 Å revealed that drosophila translin also forms a stable octameric ring like structure (Kumar and Gupta, 2012). This observation further emphasizes that the octameric structure is important for DNA binding properties of translin protein. C-terminal residues of Drosophila translin were disordered like that of human translin. Though reasonable amount of knowledge has emerged about the translin structure, it is not clear how it binds to nucleic acids. To address these unanswered

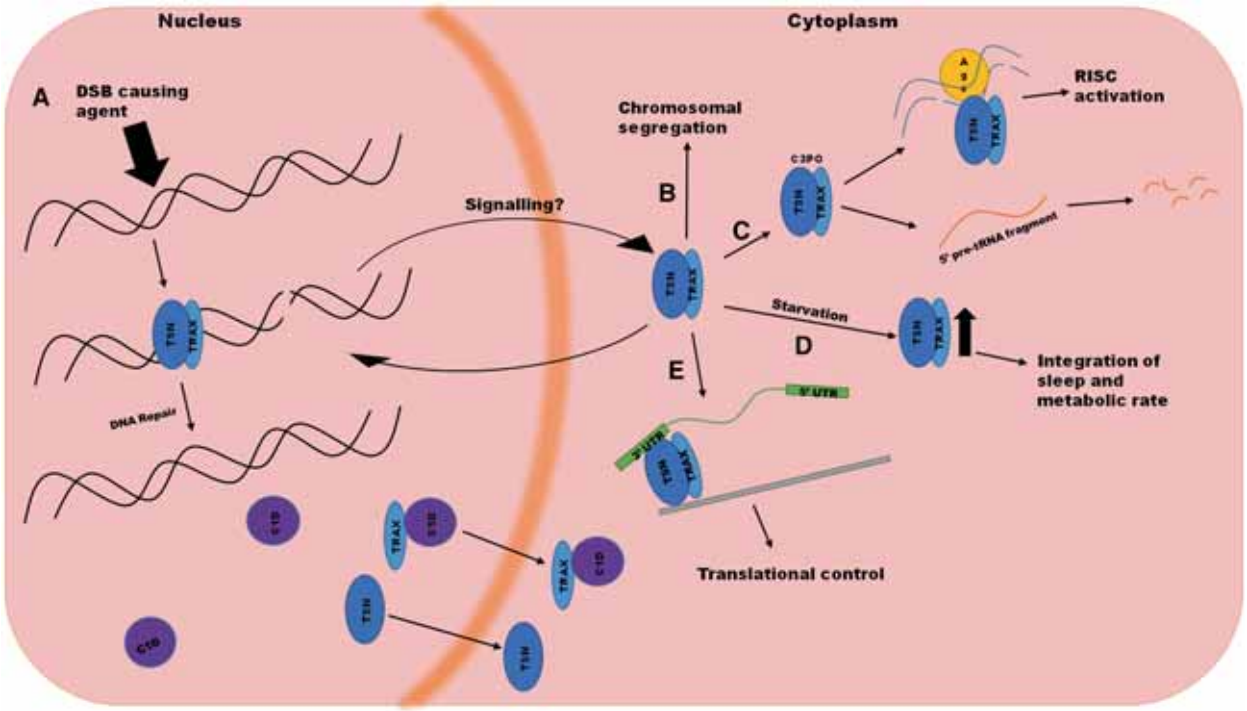

Figure 1. Diverse roles of Translin. A: In response to formation of DSB in DNA, Translin TRAX translocate into the nucleus as a hetero-octameric complex and probably participate in DDR. B: Expression of Translin has been shown to be cell cycle phase dependent, maximal expression being observed in G2/M stage. As such it has been observed to be important for proper chromosomal segregation. The knockdown of Translin reduces the proliferation rate of cells. C: Translin and TRAX as components of C3PO complex are involved in dicer activation. *Neurospora* Translin is involved in 5′ pre-tRNA processing. D: Under conditions of starvation, Drosophila Translin shows increased expression and has a role in integrating the sleep and metabolic rate of the fly. E: Translin exerts translational control on many different mRNAs by anchoring the conserved elements of 3′ UTR of mRNA to microtubules. It also carries out trafficking of mRNAs in neuronal cells by associating with microtubules.

questions, small angle X-ray scattering (SAXS), mutational, biochemical and computational analysis were carried out using human translin (Pérez-Cano *et al.* 2013). It was observed that in solution, translin exists in two different conformations. A less populated tightly closed octameric conformation and a more populated open octameric conformation. Translin nucleic acid binding cavity accommodates RNA with different conformations. It is necessary for translin to have conformational flexibility for accommodating more flexible, structurally heterogeneous nucleic acid substrates. The information thus generated still could not address the issue that what is the entry point for DNA/RNA for translin loading. Translin forms an open barrel structure which may be entry point for substrate nucleic acid molecules (Eliahoo *et al.* 2015). It was observed that translin notwithstanding it binding to DNA/RNA ends, also binds to single stranded regions between two dsDNA regions. This may be due to the translin disassembly/assembly mode of binding to DNA molecules.

Human C3PO complex involved in Dicer independent RISC activation process is a hetero-octameric structure consisting of 6 monomers of translin and two monomers of TRAX. Subunit assembly structure was solved for C3PO complex and two homo dimers of translin and two hetero dimers of translin-TRAX interact side by side to form octameric assembly (Ye *et al.* 2011). C3PO complex forms a football like structure with a hollow interior for accommodating the RNA passenger strand nicked by Ago2. Mutational analysis showed that this hetero-octameric assembly is indeed required for RNase activity. Reconstituted Drosophila C3PO complex structure was reported by Tian *et al.* (2011). Truncated translin forms hexameric structure containing four copies of translin and two copies of TRAX. These subunits are arranged as trimer of dimers to form concave, bowl like structure. In contrast, TEM studies and model building structures of full length Drosophila C3PO complex showed that it forms an octameric structure with translin to TRAX ratio of 6:2 or 5:3. However, 6:2 form of C3PO is predominant and active form when compared with that of 5:3 form of C3PO. However, the exact mechanism of RNA cleavage by C3PO complex is not known. Interestingly, *Archaeoglobus fulgidus* codes for a single poly peptide named as AfTRAX due to its closer resemblance to existing TRAX than that of translin (Parizotto *et al.* 2013). AfTRAX assembles into an octamer, which is an active form of *Archaeoglobus fulgidus* C3PO complex and shows highly similar structure with that of human C3PO complex. This octamer encapsulates a 13 base pair RNA duplex and

cleaves both the strands 7 base pairs apart. Like translin structure, C3PO complex structure is also conserved across the different species. In *Nanoarchaeum equitans*, C3PO complex forms octamer structure identical to other reported C3PO complexes (Zhang *et al.* 2016). NeC3PO-RNA complex structure was solved and it was found that after encapsulating the RNA, C3PO adopts a closed ball like structure. It was observed that substrate molecule enters in to the cavity using the gap between Translin-Trax dimer rather gap between than translin-translin dimer. It was speculated that in solution, this gap may be wider to allow the other nucleic acids substrates. Though lot of structural information is available for translin and C3PO complex, exact mechanism of DNA binding and function, RNA cleavage are yet to be explored in detail.

3. Biochemical properties of Translin protein

Molecular studies revealed human Translin peptide to be 228 amino acids long having molecular mass of 27 kDa. It was shown to bind to specific conserved nucleotide sequences as an octamer. Its amino acid sequence led to prediction of several unique features, *viz.* predominance of leucine, glutamic acid and arginine, a periodic repetition of hydrophobic amino acids (five leucine and one valine), several phosphorylation sites, a glycosylation site and a putative leucine zipper (Aoki *et al.* 1995). Mouse homolog of translin, TB-RBP showed 99% identity to human translin. The optimum pH and salt concentration for RNA-TBRBP complex formation was 7.5 and 100mM KCl; variation in pH and higher salt concentrations disrupted the complex formation. Ca^{2+} and ATP enhanced the TBRBP-transcript complex formation indicating phosphorylation dependent binding of TBRBP (Han *et al.* 1995). In mouse testis and brain cells, possibly kinases regulate the specific mRNA storage and translocation functions of TBRBP.

The TSN gene is widespread throughout the eukaryota, although translin like proteins have been reported in bacteria also (Gupta *et al.* 2012). Notably, *Saccharomyces cerevisiae* lacks the translin gene (Jaendling and McFarlane 2010). In plants, although the presence of translin like genes is reported in tomato and Arabidopsis, biochemical properties were reported for translin protein from rice (Chittela *et al.* 2014; Gupta *et al.* 2017). However, its role in plants is not established as yet. Translin protein is not essential for the growth of fission yeast, *Saccharomyces pombe*. It is suggested that *S. pombe* Translin may be primarily involved in RNA metabolism as it bound to $(GU)_n$ and $(GUU)_n$ with higher affinities in comparison to human Translin (Laufman *et al.* 2005). Human Translin also binds to G rich regions and microsatellite repeats, in addition to chromosomal breakpoint sequences, suggesting its role in telomere maintenance. The minimum length of oligonucleotide required for translin binding has been worked out to be 11 bases (Jacob *et al.* 2004).

The translin protein is highly conserved and typical sequence features are present in various orthologues. Two nucleic acid binding regions, rich in basic amino acids, are designated as mammalian basic region I and II (MBRs) in *S. pombe* translin (Eliahoo *et al.* 2010). The C - terminal region of translin protein has been suggested to be involved in protein-protein interactions based on both bioinformatic and experimental studies (Eliahoo *et al.* 2010; Gupta *et al.* 2017). Interestingly, plant systems also conserved this octameric ring structure, which is essential for its function (Gupta *et al.* 2017). There is strong evidence of highly conserved GTP binding site 'VTAGD' toward the C-terminal region, known to determine substrate specificity. Presence of GTP reduced the binding of TB-RBP to RNA by about 50%, and did not affect DNA binding significantly. In addition, the VTAGD to VTNSD mutant of TB-RBP showed interaction with itself, with wild type TB-RBP and with TRAX (Chennathukuzhi *et al.* 2001). However, GTP attenuated binding of human translin to both DNA and RNA. Drosophila translin, where, the alanine is replaced by methionine in the VTAGD sequence, did not exhibit any effect of GTP. Moreover, Drosophila Translin shows 52% identity and 67% similarity with human Translin at amino acid level, and has a comparable CD spectra signature, has lower order oligomers and low affinity to the ssDNA/RNA (Sengupta *et al.* 2006). Translin is expressed in many tissues at embryonic stages of Drosophila and highly expressed in neuronal tissue in the adult fly (Suseendranathan *et al.* 2007). These studies suggest subtle modifications in the translin protein across species according to functional requirement. These functions are altered on interaction with other proteins. For instance, Tranlsin-TRAX complex has been implicated in pre-micro RNA degradation revealed by *in vitro* biochemical assays, in-cell and mouse models. This finding suggested possible use of Translin/TRAX complex as a target for diseases in which Dicer is deficient (Asada *et al*. 2014). In addition, Drosophila Dicer and other proteins of RISC were used to purify C3PO, a RISC regulatory complex composed of Translin/TRAX complex (Liu *et al.* 2009). Put together, Translin protein binds to DNA and involves in DNA repair process. In addition, it binds to RNA and regulates RNA transport, translation and RNA degradation.

4. Cellular functions of Translin protein

Translin is a cytosolic protein but it specifically localizes to the nucleus of cells of lymphoid lineage cell lines as opposed to cytoplasmic localization in non-lymphoid lineage and non-haematopoietic cell lines (Aoki *et al.* 1995). In response to DNA damaging agents such as etoposide and mitomycin C, Translin translocated into the nucleus of HeLa cells (Kasai *et al.* 1997). This suggested a probable role of Translin in DNA damage response. The signalling that stimulates the nuclear translocation of cytosolic Translin and the functions it performs upon nuclear localization remains

to be deciphered. Soon after, Translin was found to be present in alveolar rhabdosarcoma cell lines, which contained Translin binding sequences flanking the chromosomal translocation breakpoint junction (Chalk *et al.* 1997). This was the first report in case of solid tumour cell lines and further deepened the need to determine the role of Translin in DNA damage response and genesis of neoplasm involving translocation events. TB-RBP interacts with conserved H and/or Y elements in the 3′ untranslated sequences of many different mRNAs specific to the brain and testes cells followed by attaching these mRNAs to microtubules. This specifically led to the determination of its role as an RNA anchoring molecule (Wu *et al.* 1997). The requirement of these elements for binding of Translin was later reported dispensable, where binding could also be seen in the presence of particular string of G rich sequence. These findings paved way for research to understand the roles that Translin plays in translational control, transport and localization of specific mRNAs which causes differential expression of protein in different tissues depending on the life cycle stage of cells. Expression of TB-RBP in different tissues was assessed and it was found that the mRNA expression levels were different in different tissues, predominantly high in brain and testes. Such expression pattern of translin in different tissues suggests evolution of tissue specific role for the protein. Gu *et al.* (1998) studied the expression of TB-RBP in different stages of spermatogenesis and reported the expression to be highest in meiotic and post meiotic cells especially in pachytene spermatocytes and round spermatids. Thus, TB-RBP expression level varies according to the state of differentiation of the cells. Contrary to the nature of testis, in which cells divide during spermatogenesis, brain is largely composed of long lived post mitotic cells. And thus, maintaining the genomic integrity of the brain cells is of utmost importance. Taira *et al.* (1998) found the DNA interacting GS1-protein complex enriched in the brain extracts and suggested three possible roles for the complex. It was suggested that GS1-protein complex could be involved in DNA damage response since maintenance of genomic stability is important, it could be involved in the translational control of mRNAs in the brain or it could function as transcription regulatory factor. With ideas of such different roles that the complex probably played, the group characterized the protein components of the GS1 complex. They determined Translin and TRAX to be a part of the complex further deepening the interest to understand the exact role of Translin in tissues where it is found enriched.

A study to determine the interacting partners of GADD34 (growth arrest and DNA damage inducible) using Yeast two hybrid system revealed Translin as one of its interacting partners (Hasegawa and Isobe, 1999). *In vitro* binding experiments followed by *in vivo* mammalian two hybrid system analysis showed the interaction between GADD34 and Translin. Though the biological significance of this interaction remains unclear, it could shed light on downstream roles of Translin after its DNA damage induced nuclear translocation. Another independent study determined TER-ATPase, TRAX and γ-actin as interacting partners of Translin/TB-RBP using immuno-precipitation analysis. The interaction between TER-ATPase and Translin was confirmed by an *in vitro* assessment (Wu *et al.* 1999). Also, Translin-γ-actin interaction is in conjunction with the finding that Translin interacts with components of the microtubules and attaches specific mRNAs to the same. Identical level of *Xenopus* Translin expression during gametogenesis and early development of embryo was seen by western blot analysis. Though the expression levels are identical, X-Translin undergoes redistribution during mitosis and it was also seen to associate with centrosomes. It was suggested to have a role in mitotic cell cycle control and appropriate redistribution of some mRNAs among the two daughter cells (Castro *et al.* 2000). Followed by the presence of Translin binding consensus sequences in rhabdosarcoma cell lines, such sites were also observed in liposarcomas consisting TLS-CHOP reciprocal translocation. The binding of recombinant Translin and cytosolic extracts of HeLa cells to the consensus sequences obtained from analysis of TLS-CHOP translocation flanking sequences was tested using gel shift assay. It revealed differential binding affinity of translin to different sequences. Based on these findings, Hosaka *et al.* (2000) questioned the random presence of such Translin binding sequences at flanking regions in different cancers studied and attributed it to a yet un-deciphered role of Translin in such translocation events. Apart from the studies exploring role of Translin as translocation hotspot binding protein in tumours, its role in mitotic cell division in normal cells was assessed revealing association between the cell cycle phase and protein levels. Translin levels correlate with the mitotic cell cycle phase with protein expression starting from S phase and reaching maximum level in G2/M phase. Such cell cycle phase-based expression of Translin was observed in model systems such as K562 and PC12 cell lines where Translin levels were high in dividing cells and downregulated in case of cell cycle arrest accompanied by differentiation (Ishida *et al.* 2002). Induction of Translin expression in a tetracycline responsive Translin stable transfectant clone accelerated cell proliferation reiterating its role in cell division. Translin associates with centrosome and with advancing cell cycle stage, associates with mitotic spindle microtubules and later with the mid bodies. As such, translin is important for chromosome segregation during mitosis and in microtubule organization. (Ishida *et al.* 2002). The generation and study of Translin null mice revealed that the size of null mice litter was small owing to the previously mentioned finding that Translin levels modulate cell proliferation rate. But T and B cell development was normal contrary to the expectation of their altered development due to absence of Translin for all the recombination related functions. This suggests that alternative pathways exist for fulfilment of functions that Translin performs. Phenotypic manifestation

included reduced fertility and altered expression of varied number of genes involved in different functions (Chennathukuzhi *et al.* 2003). Contrary to this study, knockout mice mesenchymal cells showed enhanced differentiation. Though this variation could be stage dependent, it was seen that Translin plays a role in mesenchymal cell proliferation and differentiation. An epigenetic based causal of such effect was also considered and remains to be explored (Ikeuchi *et al.* 2018). Translin expression affects response to irradiation and it is induced in presence of H_2O_2 indicating its possible role in signal transduction pathway post DNA damage induction (Fukuda *et al.* 2008). Translin/TB-RBP also possess single stranded RNA and double stranded RNA endonuclease activity and are components of Dicer complex involved in RNA interference (Wang *et al.* 2004). This endonuclease activity was not found against DNA. In Drosophila, Translin along with its major interacting partner TRAX forms the C3PO complex which activates RISC by removing the cleavage products of siRNA passenger strand. The complex is Mg^{2+} dependent and is involved in slicer activity which activates the Dicer core (Liu *et al.* 2009). Another important finding in conjunction with earlier reports was the binding and trafficking of BDNF in neuronal cells by Translin TRAX complex. Mutational analysis revealed the site at which Translin binds and siRNA-based knockdown of Translin revealed corresponding decrease in the mRNA's dendritic trafficking. This reduced BDNF mRNA trafficking may lead to phenotypic manifestations such as behavioural changes, reduced memory performance, etc. (Chiaruttini *et al.* 2009). Two contrasting functions exist for Translin, one where it is involved in dendritic trafficking of mRNA, exerts translational control and the other where it shows endonuclease activity to cleave the passenger siRNA strand (Wu et al. 2011). Other proteins, factors and conditions contributing to the selection of the function have to be studied. The Translin from *Neurospora crassa* showed no involvement in RNAi but it had a role in tRNA processing giving one other possible mechanism by which Translin affects cell proliferation rate (Li *et al.* 2012). Recently, a rather unique function of Translin came to the forefront when Murakami *et al.* (2016) published their study on Translin role in metabolic regulation of sleep in Drosophila. Under conditions of starvation in Drosophila, there is an upregulation of Translin. This overexpressed Translin is involved in response of the flies to starvation. Translin knockdown altered the response of the flies to starvation where loss in hyperactivity and lack of sleep suppression was observed. Translin/TRAX complex was shown to play a role in repression of miRNA based translational silencing of ACVR1C in response to synaptic stimulation. This is an important area of research as defects in complexes such as Translin/TRAX and other repressors of miRNA silencing are suspected to play roles in neuro-developmental and psychiatric disorders. The study of such complexes would help in understanding the pathophysiology of such conditions (Park *et al.* 2017).

5. An integrated model for Translin functions

Translin is a protein having diverse functions and all the roles played by translin pertain to its nucleic acid binding ability. It forms an octameric complex with a central aperture into which the nucleic acid substrates fit. The dimensions of the channel present may regulate the kind and nature of nucleic acids Translin interacts with. Initially, it was speculated to be involved in translocation events due to its binding to chromosomal translocation breakpoint junctions. Also, it was shown by Sengupta and Rao (2002) that binding of Translin to DNA prevents fraying of DNA. In response to double strand break causing agents such as gamma radiation, Translin translocates into the nucleus. For this, it associates with TRAX, which has a nuclear localization signal (NLS). Another protein, GADD34 was hypothesized to be involved in nuclear translocation of Translin. Thus, Translin enters into the nucleus in response to DNA double strand breaks and probably acts as DNA damage response protein holding the DNA in place till the downstream proteins come into play and carry out their functions. These proteins could either carry out DNA damage repair or translocation events. Also, Translin has a GTP binding site and GTP could be the molecular switch responsible for bringing about conformational changes in the protein resulting in modulation of its function. A GTP based control or signalling pathway maybe involved in translocation into the nucleus or it's binding to nucleic acids.

Though Translin is found primarily in the cytosol, a DNA damage induced translocation of Translin is observed. This conditional presence of Translin in the nucleus seems important and well regulated. These findings suggest the involvement of Translin in the DNA damage repair responses but no concrete evidence supporting this line of thought has emerged. An alternate pathway does probably compensate for the absence of Translin and thus, seeing direct phenotypic effects of Translin knockdown has proved inconsequential. It remains to be seen how Translin networks with other nuclear proteins and DNA.

Also, Translin interacts with TRAX and forms a hetero-octameric complex. This hetero-octamer forms the C3PO complex which has RNAse activity and is involved in RNAi. The *Neurospora* Translin has been shown to be involved in 5′pre-tRNA processing where it is fragmented by Translin TRAX complex. On the other hand, Translin is involved in transport and anchoring of mRNA via interaction with microfilaments like actin. Thus, it can be said that it degrades specific set of RNAs and acts as an anchoring protein for particular set of RNAs. Whether the sequence and secondary structure of the RNA or other participating proteins confer the specificity to Translin to perform either of the contrasting roles remains to be seen.

6. Conclusion and future directions

With the knowledge from more than two decades of research on translin, it can be considered as a protein of ubiquitous, but well regulated expression and performing diverse

functions. Dedicated regions of protein–protein and protein–nucleic acid interactions have been mapped on the three dimensional structure (Eliahoo *et al.* 2010). This, in addition to the flexibility in its structure corroborate with diverse functionalities; all related to nucleic acid metabolism. At cellular level it is understood that the Tranlsin protein plays role of controlling protein expression by regulating specific mRNAs. On the other hand, although not clear, its role in maintenance of genomic integrity has emerged. Recent studies have enlightened the biological roles of translin, like sleep dysregulation, learning etc, which highlight its neuronal function. Even with the present information available, there are avenues open for research on translin which will enhance our understanding in unravelling more of its functions. For instance, more solid experimental evidence is needed on residues involved in protein-protein and protein–nucleic acid interaction. The high resolution structure Translin in solution is not available yet. It will lead to full understanding of dynamic nature of this multifunctional protein and enlighten on the indirect evidence available about presence of two forms in solution and also pinpoint the actual point of entry of DNA/RNA. Since its role in genomic integrity and regulation of RNA metabolism has been established, the new information generated may be useful for translational research. Deciphering the stimulus that Translin requires for performing any of the above mentioned functions, the role of other contributing proteins and complexes remains to be dissected. Inter connection between all these functions as to how such a similar and contrasting network of functions is coordinated and performed has to be determined setting the stage for investigators for years to come.

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