Chapter 1

Recombination Hot-Spots and Defense Players – Maintenance of Genomic Integrity

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54016

1. Introduction

Internal factors and external agents are a source of constant genomic stress in living organisms leading to instability in the form of chromosomal deletions, duplications and translocations. These erroneous rearrangements of the chromosome alter the normal functioning of the genes harbored on them leading to genetic birth defects, intellectual disabilities, premature ageing and even cancer predisposition in humans [1]. Such chromosomal aberrations occur at gaps within the genome or at breakpoint junctions on double stranded DNA motifs known as fragile sites. Preventing or repairing these DNA damages is pivotal for the normal physiological function of a human body. However, prevention or total abolition of DNA damage from an organism is impossible as it is a constant and spontaneous phenomenon occurring in a physiological environment, stalling DNA replication. Therefore, focus on mechanisms that could stabilize such breakage-prone motifs and repair the damage on DNA could grant an insight into understanding and enhancing them for maintenance of genomic integrity.

2. Mechanisms of DNA repair

Estimation studies suggest that each mammalian cell genome is subject to several hundreds of DNA strand breaks within the normal physiological setting [2]. Hence, prokaryotes and especially eukaryotes are equipped with defense mechanisms against genotoxic stress in order to constantly repair and restore the genome, bringing the replication process back in order from its attenuated state. Efficient timely repair can restore a near-zero status of damages at a steady-state level in a eukaryotic cell.

DNA repair pathways in higher eukaryotes such as yeast are constantly operational throughout the various phases of the cell cycle. Homologous Recombination (HR) is the most commonly known pathway to be predominant in the late S and G2 phases of the cell cycle [3]. It requires a pair of sister chromatids as template for adequate homology to recombine the broken DNA ends and hence is seen mostly in these two phases of the cell cycle where such templates are available. HR creates new combination of DNA sequences during sister chromatid exchange in meiosis, a cell division process carried out in germ cells in eukaryotes.

Intriguingly, mammalian DNA also undergoes constant damage and the first mechanism to sense these damages and respond to them is the Non-Homologous End-Joining pathway (NHEJ) [4]. As the name suggests, it has almost no regard for homology while rejoining the broken DNA ends and hence can occur throughout the cell-cycle regardless of the cycle phase. It is therefore depicted as an error-prone mechanism as opposed to HR which operates based on homology and hence is considered to be error-proof. A modified version of NHEJ is MMEJ (Microhomology-Mediated End-Joining) which requires a 5 – 25 bp homology for end-joining which is likely to be available in the S phase in contrast to G0/G1 and early S phase of the cell cycle where NHEJ is more predominant [5].

3. NHEJ

Mammalian system undergoes spontaneous DNA damage which is responded immediately by NHEJ, making it the first choice for DNA repair mechanism. The three core steps include detection, processing and ligation. According to the classical NHEJ pathway, Ku70/80 heterodimer detects these damages and is believed to act as early sensors binding the broken ends followed by recruitment of DNA dependent protein kinase (DNA-PK) which brings these ends in synapsis and activates the downstream substrates by phosphorylation. Several nucleases and polymerases then trim the overhangs or fill-in the gaps to create adequate homology for ligation by XRCC4-DNA Ligase IV-XLF complex [6].

NHEJ causes insertions or deletions of DNA sequences at the broken regions leading to chromosomal translocations which are frequently found in leukemia and lymphoid malignancies. Immunoglobulin (Ig)/T-cell receptor (TCR) recombinase is known to be involved in such aberrant chromosomal rearrangements because of its recognition of target heptamer-nonamer V(D)J signal sequences. Other non-resembling sequences also direct recombination. One such example was found in a patient with acute T-cell lymphoblastic leukemia (ALL) carrying t(8;14) (q24;q11) and t(1;14) (p32;q11) translocations [7]. The novel conserved sequences, GCAGC[A/T]C and CCCA[C/G]GAC, identified at recombination hot-spots led to the speculation that site-specific recombination events might occur mediated by proteins.

Recombination associated factor (ReHF-1) was identified to bind specifically to the 8q24 and 1p32 breakpoint junctions [8]. BCLF-1, another analogous protein was identified to bind to breakpoint clusters in Bcl-2 oncogene in patients with follicular lymphoma carrying t(14;18) (q32;q21) translocations [9]. Recombinant BCLF-1 protein demonstrated strong binding af-

http://dx.doi.org/10.5772/54016

finity towards single-stranded oligonucleotides representing the breakpoint junctions [10]. Thus the activity of the two proteins, ReHF-1/BCLF-1 was inseparable inferring them to be identical or nearly identical at consensus target sequences in chromosomal translocations in human lymphoid neoplasms. The protein was therefore renamed as Translin, derived from translocation [10].

4. Orthologues of Translin

4.1. Human Translin

Translin was identified to bind several breakpoint junctions, found in patients carrying chromosomal translocations t(8;14)(q24;q11), t(1;14)(p32;q11) and t(14;18)(q32;21), revealing similarity to consensus target sequences, ATGCAG and GCCC[A/T][G/C][G/C][A/T] [10]. The gene, assigned 2q21.1 as the chromosomal locus by fluorescence *in situ* hybridization (FISH) studies, was cloned and the cDNA predicted to code for a polypeptide chain consisting of 228 amino acids, whose sequence did not possess any significant similarity to then known proteins. Nucleotide and amino acid sequence analysis revealed a heptad repeat of hydrophobic amino acids, five leucines and one valine, which is consistent with the hypothetical structure of a "leucine zipper" [11]. Also, amino acids spanning from 54 – 64 and 86 – 97 were predicted as two basic regions upstream to the leucine zipper [12] (Figure1).

The purified recombinant protein migrated as a 27 kDa monomer under reducing conditions and as a 54 kDa dimer under non-reducing conditions on SDS-PAGE [10]. These results indicated that two polypeptide chains were bound together by disulphide bonds which could be easily separated under the presence of reducing agents such as β-mercaptoethanol or dithiotritol. Gel filtration analysis and native gel electrophoresis revealed the native state of translin as a 220 kDa octamer with the formation of higher order multimeric structure, probably connected via the leucine zipper motifs from each dimer [13].

4.2. Testis/Brain – RNA Binding Protein (TB – RBP)

Mouse testicular extracts revealed a RNA-protein complex that bound to the Y and H elements of 3′ UTR of protamine-2 [14]. A similar protein was also found in brain extracts and termed as Testis/Brain – RNA binding protein [15]. The open reading frame consisted of 228 amino acids coding for a molecular weight of 26 kDa. The heptad repeat of leucine zipper motif spanned from amino acids 177 – 212. Yeast 2-hybrid assays later confirmed that like translin, TB/RBP also dimerized via the C-terminal, housing the leucine zipper and a cystine at 225th position forming disulphide bridges [16].

It also shared a 90% and a 99% identity with Translin nucleotide and amino acid sequence respectively and was thus deemed as the mouse orthologue of the human protein [17]. Only three amino acids that differ in TB-RBP are alanine – threonine at 49th, glycine – serine at 66th and valine – glycine at 226th positions respectively. Analysis of the human and mouse translin revealed that each of them consisted of six exons, five introns and a GC-rich region

[12]. TB - RBP also harbors potential phosphorylation sites for protein kinase C and tyrosine kinase.

4.3. Drosophila Translin

The fly orthologue of translin was identified, cloned, purified and characterized by our group [18]. The gene from *Drosophila melanogaster* was recognized to have five exons as annotated by the Berkeley *Drosophila* Genome Project (BDGP). The 28 kDa monomer, established by MALDI-TOF, shared only 52% sequence identity with the corresponding human protein. As opposed to the 54 kDa dimer of human translin, the fly protein existed as a 56 kDa in its dimeric state. The dimer of translin existed in relative abundance as compared to that of the *Drosophila* protein laying differences in the stability of the two dimers. Although the fly protein shares a high sequential identity with that of the vertebrates, the extreme C-terminal varies in sequence and length (Figure 2). The putative leucine zipper domain may be responsible for multimerization, but the two basic regions are less conserved in *Drosophila* translin [18].

4.4. *S.pombe* Translin

Schizosaccharomyces pombe and human translin, both forming octameric ring, share an overall 36% identity and 54% similarity, with higher degrees in the C-terminal region [19].

4.5. Orthologues in other vertebrates

Another orthologue of translin was also identified in *Xenopus laevis*, annotated as X-translin [20]. Based on gel filtration studies, chicken translin was believed to exist as a decamer [13]. Translin was also identified as one of the structural proteins in 2D and MALDI-TOF profiling of the skeletal muscle of Takifugu rubripes, a kind of pufferfish [21]. It was thus established that translin was largely conserved across evolution, consisting of the leucine zipper and at least one of the short basic regions which was speculated as the DNA-binding domain.

5. Structure of Translin and orthologues

Electron microscopic studies and single-particle analysis reconstructed a three-dimensional structure of translin. The eight subunits appeared to assemble in an octameric ring with two distinct basic domains and a funnel shaped central channel [22] (Figure 1). This creates a binding interface for nucleotides. Ultracentrifugation and sedimentation equilibrium studies further established that the predominant species of translin was a hydrodynamic oblate ellipsoid structure of octamer which is also the basic binding unit for DNA at chromosomal breakpoints [23]. This was later confirmed by X-ray diffraction and crystallization at 2.2 Å that presented two tetramers to form an octamer by two-fold symmetry mainly brought about by hydrophobic interactions (Figure 1) [24]. These results suggest that the higher or-

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der structure of translin is not based on strong intermolecular hydrogen interactions rendering the whole molecule to be rather flexible in order to change the relative positions of monomer for nucleic acid binding with better accessibility of the central core. In the presence or absence of DNA or RNA, translin forms chiral or pin-wheel shaped rings which are similar to that of human Dmc1 protein, a meiosis specific recombinase [22]. Crystallization of TB-RBP resulted in the formation of orthorhombic crystals [25]. Dynamic light scattering (DLS) recognized equilibrium between tetramers and octamers of TB-RBP in solution. Wild-type *Drosophila* translin existed as an octamer/decamer whereas at high resolution crystallization parameters, the mutant P168S exhibited two identical tetrameric forms (Figure 2) [26].

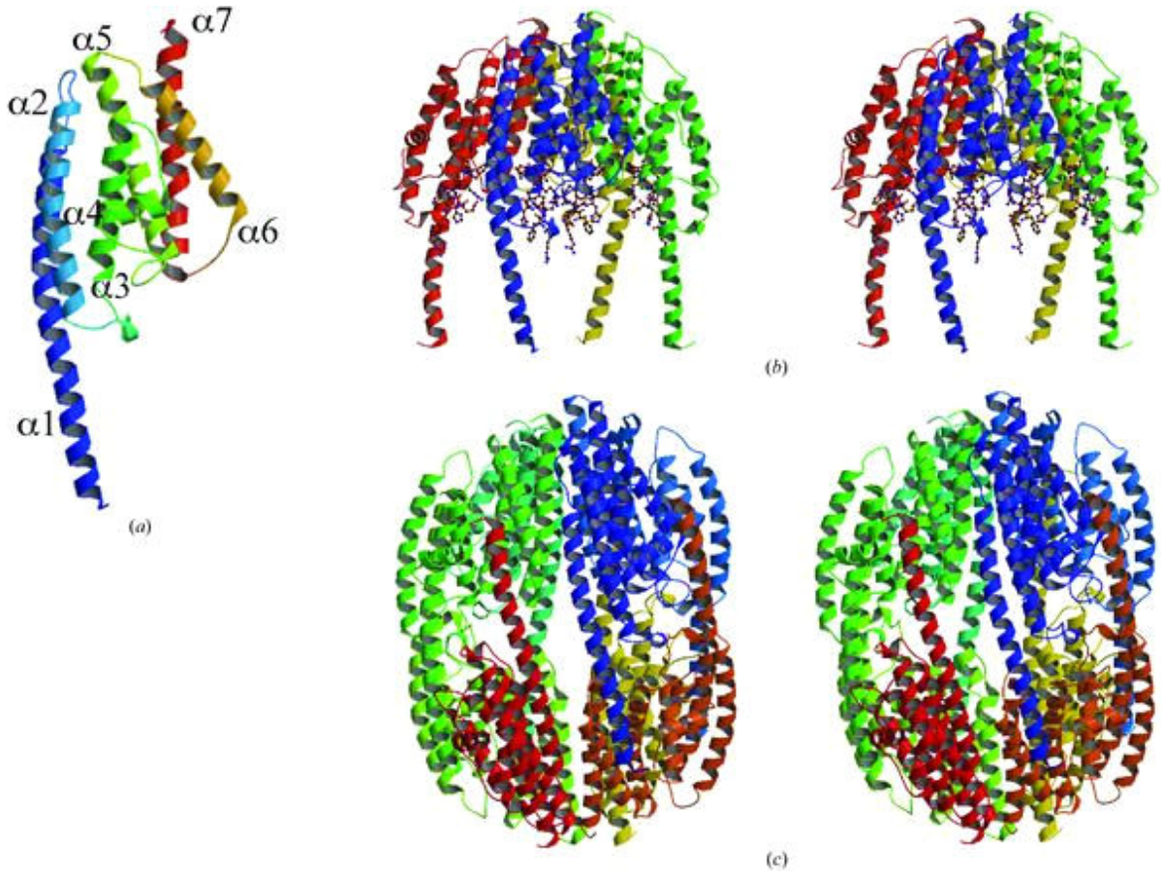

Figure 1. Overall architecture of human Translin. (a) Ribbon representation of residues Met1±Phe217 of a translin monomer. (b) Translin tetramer or `two dimers' in the asymmetric unit. The regions with side chains are `basic regions' that are supposed to bind to the target ©*2004 International Union of Crystallography* DNA/RNA. They are located in the inner surface of the tetramer. (c) Translin octamer, which is the two tetramers related by a crystallographic two fold symmetry. *Reproduced with permission from Acta Crystallographica Section D60, Sugiura et. al., 2004, 674-679 [24].*

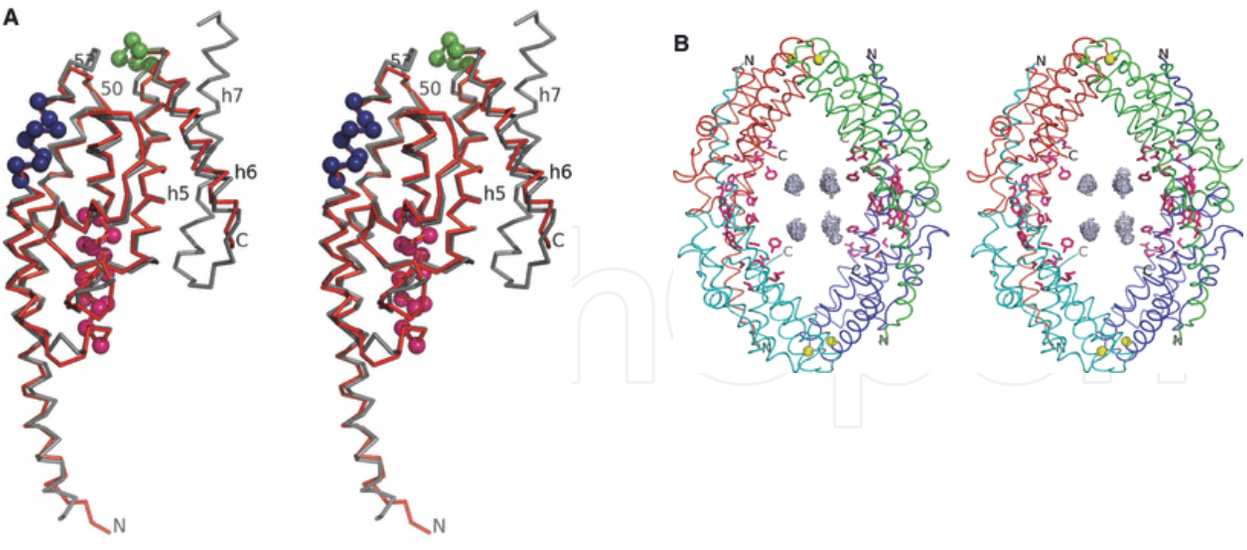

Figure 2. A) Stereo-view of the Cᵅ trace of the *Drosophila* P168S mutant translin monomer structure (red) (residues 3 and 187 are identified with N and C labels, respectively) super-posed onto the structure of human translin protein (grey). The basic-1, basic-2 and putative GTP-binding motifs of human translin sequence are shown as blue, magenta and green spheres, respectively. The shortened loop 1 of *Drosophila* translin is identified by residues numbered 50 and 53. The α 7 helix of human translin (labeled h7) is not modeled in *Drosophila* mutant translin structures as it is disordered in the crystals. Also marked are the α5 and α 6 helices (labeled h5 and h6). (B) Cartoon of the *Drosophila* P168S mutant translin tetramer. The translin molecules of the tetramer are shown in cyan, red, green and blue, respectively, with the N- and C-termini of each marked as N and C. The amino acids of the basic-2 motifs are shown in magenta. The positions of the four columns of unaccounted electron density observed at the center of the tetramer cavity are shown as gray contours. The amino acid residue at position 168 is marked with yellow sphere for each monomer. *Reproduced with permission from Dr.Vinay Kumar, BARC, FEBS Journal, Gupta et. al., 2008, 4235-4249 [26]. Journal compilation©2008 FEBS*

6. Localization of Translin and orthologues

Translin was mainly found to be localized in the cytoplasm as it comprises of a hydrophobic nuclear export signal in its C-terminal [10]. Both, the nuclear export signal and the putative GTP binding domain also seemed to be conserved among human, mouse and fly translin [27-29], although the GTP binding ability of the fly protein was lower than that of its mammalian counterpart [29].

TB-RBP was encoded by a single copy gene in mouse, but three different RNA transcripts of 1.2, 1.7 and 3 kb each were found during spermatogenesis in pre-pubertal and sexually active testes. Additionally, expression of the different sizes of TB-RBP mRNAs was also found in other tissues such as heart, liver, kidney and spleen [30]. Translin staining was found to be intense in the cytoplasm of cerebral cortex/purkinje cells establishing its somatodendritic localization which is also consistent with the studies performed in spermatocytes [31]. Non-hematopoietic mammalian cells, eg., HeLa treated with DNA damaging agents such as mitomycin C and etoposide increased the nuclear localization of TB-RBP, thereby indicating a

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signaling cascade that initiated the nuclear transport of the molecule following exposure to DNA damaging agents [32].

Drosophila translin was ubiquitously localized in the cytoplasm, in the early embryonal syncytial stage, but later enriched in ventral neuroblasts as demonstrated from our florescence studies; probably depicting the metaphase of the cell cycle. Cells around the tracheal pits in the embryo and oenocytes in the third instar larva also exhibited elevated levels of protein. RNA in situ hybridization displayed an increased expression in the ventral midline cells of the larval brain, suggesting a neuronal expression, which was corroborated by protein immunostaining. In adult flies, translin was localized in the brain neuronal cell bodies and in early spermatocytes. Interestingly, *Drosophila* translin mutants exhibited a sex specific impaired motor response i.e. only in females [18].Taken together, the multiple cellular localizations, the high neuronal expression and the attendant locomotor defect of the translin mutant indicated that the fly protein may have multiple roles in neuronal development.

7. Interactors of Translin and their physiological significance

Choromosomal translocations are widespread among a diverse group of neoplasms and other genetic disorders. Hence, the mechanism to repair and stabilize such anomalous rearrangements at recombination hot-spots presumably involved several other factors which could act as functional interactors of translin.

7.1. Translin associated factor – X (TRAX)

In order to isolate other recombination hot-spot proteins, yeast 2-hybrid system was employed by Aoki and co-workers using human translin as bait. It led to the identification of a 33 kDa protein which shared 28% overall and 38% C-terminal sequence identity with translin It was therefore nomenclatured as the Translin-associated factor – X (TRAX) [33]. The N-terminal encased the bipartite nuclear targeting signal comprising of two basic regions separated by a spacer sequence and supposed to be responsible for the nuclear transport of translin. Human TRAX gene composed of six exons with a putative CpG island at the 5′ end, mapped at least 35 kb proximal to DISC1 and within approximately 150–250 kb of the translocation breakpoint at 1q42.1 [34]. Mouse trax gene was later isolated by Devon et. al. and mapped by FISH on chromosome locus 1q41. It was identified as a gene encoding for a 290 amino acid protein and because of its shared sequential identity with that of the human protein, was presumed to be the mouse orthologue [35]. It was expressed in various tissues, such as spleen, testis, ovary, thymus, etc. and primarily cytoplasmic in localization [36]. The isoforms were transcribed at equal ratios in kidney, testis and heart. The open reading frame was largely conserved across evolution sharing 90%, 35%, 34% and 30% identity with human, *D. melanogaster*, *A. thaliana* and *S. pombe* respectively. In addition to coding sequence, conservation was also observed in the 3′UTR region. Mouse trax being an interactor, could also be considered as a paralog of translin due to its 29% identity and 41% similarity to the

mouse protein. The bipartite nuclear localization signal in human trax was absent in the fly protein.

7.2. GADD34

DNA damage, in mammalian cells, is capable of activating a cascade of cell-cycle checkpoints and also triggering an apoptotic response in the cells. GADD34 or growth-arrest and DNA-damage inducible gene is one such example which was inducted in response to rapid UV radiation in Chinese hamster ovary (CHO) cell lines. *In vitro* as well as *in vivo* studies established a strong interaction with translin in the cytoplasm, although the exact role of GADD34 in translin regulation is unclear [37]. However, one possibility could be the transport of translin from cytoplasm to nucleus as observed in lymphoid lineages with rearranged Ig/TCR loci. Evidence supports that GADD34 and translin play a role in stress response since DNA damaging drugs elevate the GADD34 levels in mammalian cultured cells.

7.3. TER-ATPase

Transcriptional endoplasmic reticulum ATPase (TER-ATPase), γ-actin and Trax co-immunoprecipitated with TB-RBP from mouse testicular extracts [38]. TB-RBP was further found to interact with mRNAs encoding for myelin basic protein and calmodulin kinase II as well as protamines 1 and 2 in brain and testicular extracts respectively. Based on confocal microscopic investigation, TB-BRP co-localized with microtubules throughout the cytoplasm in mouse germ cells [39].TER-ATPase is known to transport membrane vesicles to Golgi apparatus [40] whereas actin is a cytoskeletal structural protein of microfilaments [41]. Immunocytochemical analyses in the nervous system indicate that the interactions between TB-RBP and TER-ATPase facilitate them as two components of a larger complex facilitating mRNA transport and localization. Another possibility is that TB-RBP functions as an anchoring protein for RNA to dock onto microtubules, and, in association with other proteins such as the TER-ATPase and Trax, it translocates specific mRNAs [38].Thus, it is likely that TB-RBP functions in both intracellular and intercellular mRNA transport in testis [30] as well as facilitating its storage until the time of translation.

7.4. HCV

Another interactor of translin found by yeast assays and confirmed *in vitro* by co-immunoprecipitation was Hepatitis C virus core protein from liver hepatocellular carcinoma Hep G2 cell lines [42].Translin function can be triggered by chromosomal translocations in normal lymphocytes [43].Therefore, the interaction between HCV core protein and translin protein may trigger the B-cell progressing into lymphoma in patients infected with HCV. This molecular mechanism could at least partially explain for tumorigenesis of HCV.

7.5. GRBP

Glycolytic pathway is co-ordinated by a key enzyme L-type pyruvate kinase that is regulated by carbohydrates at transcriptional and post-transcriptional levels [44, 45]. A novel pro-

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tein binding factor bound to glucose response element (GRE) was isolated from rat liver cytosol and nuclei [46]. This glucose response binding protein (GRBP) complex revealed translin/trax 240 and 420 kDa heteromeric complex in the nuclear and cytosolic extracts respectively. The amount of GRBP complex was increased in liver nuclear extract by a high carbohydrate diet and decreased by starvation, high fat, and high protein diet. The levels in cytosol were dependent on carbohydrate availability [47]. The constituents of the GRBP complex could be designated to bind to the glucose response element of the pyruvate kinase gene as a function of high fat diet.

7.6. KIF17b and KIF2Aβ

A testis-enriched kinesin KIF17b coimmunoprecipitated with TB-RBP in a RNA–protein complex containing specific cAMP-responsive element modulator (CREM)-regulated mRNAs. This complex was temporally and sequentially expressed indicating a separation of the processes of transport and translation in mammalian male germ cells [48]. Another kinesin KIF2Aβ, also enriched in testes, colocalized with trax in the perinuclear region such as Golgi complex, thereby indicating some role in spermatogenesis [49].Testis mRNAs encoding protamine 1 and 2 (Prm1 and 2), transition protein 1 and 2 (Tnp1 and 2), A-kinase anchoring protein 4 (Akap4), and glyceraldehyde 3-phosphate dehydrogenase-S (Gapds), and brain mRNAs encoding tau, Ca2+-calmodulin-dependent protein kinase II, and myelin basic protein have been reported to be target mRNAs of translin [39, 50-52]. These are transcribed in post meiotic germ cells by (CREM)-tau and are subcellularly transported in association with the kinesin KIF17b [53]. Other examples of translin target mRNAs encoding diazepam-binding inhibitor–like 5, arylsulfatase A, a tetratricopeptide repeat structure-containing protein and ring finger protein 139 were initially expressed in pachytene spermatocytes [54]. In addition, two non-coding RNAs, Nct1 and 2, abundant in nuclei of the spermatocytes were also identified adjacent to recombination hot-spot motif GGA [55].

8. Interactors of TRAX and their physiological significance

Trax has been co-purified with translin on numerous occasions. In one of our own studies, we attempted the purification of recombinant human TRAX and were presented with a highly unstable and insoluble protein. It was stabilized by co-expression with translin and it co-purified as a soluble translin-trax heteromeric complex. This purified complex was assessed for its functional activity by DNA-binding gel shift assays. Translin gave rise to a distinct DNA gel-shift complex with duplex DNA unlike that of the translin-trax heteromeric complex. However, the complex like that to translin formed a stable protein-DNA complex demonstrating specific ssDNA binding activity. The gel shift complex was excised and analyzed for its composition on SDS-PAGE. Stoichiometrically, it was found that the minimum binding unit for ssDNA was a dimer of translin and monomer of trax which existed nearly in a ratio of 1:1, similar to that of a purified recombinant complex [56]. All these results, put together, suggested that heteromeric complex exhibits relatively more stable binding to ssDNA. trax contains three functional properties: a nuclear localization signal, RNA binding

activity, and the ability to interact with translin. The ability of trax to form a heteromeric complex with translin and the bipartite nuclear localization signal on trax may be the most vital properties to transport translin from cytoplasm to nucleus. Therefore, it will not be inconsequential to assume that there could be other molecules interacting with trax for translin transport and function in the nucleus.

8.1. C1D

A large number of proteins control gene expression by binding to repetitive sequences of genomic DNA and targeting a subset to nuclear matrix [57]. C1D is one such non-histone protein which is also an activator of DNA-PK [58], that plays an important role in DNA double-strand break (DSB) repair mechanism through NHEJ and V(D)J recombination, a process specific to lymphocytes for the development of immune system [59]. Yeast 2-hybrid screens established that trax interacts specifically with C1D via its putative leucine zipper. Immunoflorescence staining showed that C1D is predominantly localized in the nucleus with some diffused pattern observed in the cytoplasm. Moreover, whilst translin also interacts with trax via its leucine zipper, to stabilize the protein, C1D-TRAX interaction is enhanced or induced in response to γ-irradiation, thus deeming the interaction of both translin and C1D with trax as mutually independent events [60]. In this regard, it should be noted that Trax has been shown to enhance the DNA binding capacity of TB-RBP (translin), while decreasing its RNA-binding ability [36]. One biological consequence of trax-C1D interaction could be the regulation of translin – trax interaction rather than regulating any pre-formed heteromeric complex. Trax's ability to change protein partners between translin and C1D could act as a switch *in vivo* regulating the preference of translin binding to nucleic acids. This theory appeared to be consistent with the model proposed by Hecht and co-workers that trax is a vital regulator for Translin's sub-cellular locale [61].

Disruption of CID in yeast strains resulted in increased temperature sensitivity, but insensitivity towards DNA damaging agents such as methylmethanosulphate (MMS) or UV and only mild sensitivity to γ-irradiation. This phenomenon is highly reminiscent to that of YKu70p [62]. Further rejoining and recombination assays exhibited defects in NHEJ and HR pathways in yc1d mutants, thus implicating the role of C1D in both the DSB repair pathways [60]. This hypothesis is supported by the established interaction between C1D and DNA-PK [58].

8.2. A_2A adenosine receptor (A_2A-R)

p53 is a nuclear phosphoprotein and tumor suppressor that regulates the cell cycle [63]. $p53^{+/-}$ mice exhibit brain malformations whereas $p53^{-/-}$mice exhibit neuronal developmental abnormalities, including that of neural tube closure [64]. Adenosine with its four receptors is known to modulate neuronal function [65]. One of its receptorsA_2A-R can be stimulated in the presence of inhibitors against protein kinase A and C, in order to rescue the impairment of nerve growth factor (NGF) followed by inhibition of cell proliferation. Trax was found to be interacting with the cytoplasmic region of A_2A-R and its over-expression also demonstrated a similar rescue effect [66]. It can thus be inferred that A_2A-R might exert its rescue

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effect mediated as a function of negative proliferation signal by trax. It was later discovered that the p53 blockage rescue effect was critically dependent on the functional interaction of trax and KIF2A [67].

8.3. GAP – 43

Trax was shown to co-express and function as an operational switch to regulate the transcription of the growth-associated protein (GAP-43) during post-natal development. Following trax switch-off, axonal growth was upregulated as a result of increased levels of GAP-43 [68]. Thus, it can be speculated that trax may have potent therapeutic potential against neuronal injuries associated with the inability of axons to regrow, as usually occurs within long neuronal pathways such as the optic nerve and spinal tracts.

8.4. PLCβ1

Mammalian phospholipase Cβ1 (PLCβ1) is mainly localized on the cytosolic plasma membrane surface where it is associated with its membrane-bound activator $G\alpha_q$ [69]. PLCβ1 interacts with trax specifically through its C-terminal and allows trax to directly compete with its functional interactor $G\alpha_{q.}$ PLCβ1-trax complex is observed mostly in the cytosol and a small amount is seen in the nucleus thereby revealing yet another role of trax as a regulator for the cellular compartmentalization of its interactors [70]. The mode of stabilization of PLCβ1 could be attributed to two main factors; (i) activation of PKC through phosphorylation, which is directly downstream of PLCβ1 and (ii) interaction with trax [71] that regulates its cellular localization. A latest study also linked the interaction between PLCβ1 and trax in the regulation of genes by RNA interference [72].

9. Functional characterization of Translin/TB-RBP and Trax

9.1. DNA/RNA binding mode of Translin

Electron microscopic and X-ray diffraction studies have characterized translin as an assembly of eight polypeptide chains that form ring-shaped octameric structure (Figure 1) [22, 24]. Crystal structure showed that each monomer of translin/TB-RBP is composed of about 70% R-helices, 25% random coils, and 5% beta-sheets [24, 25, 73].The hydrophobic heptad repeats consisting of the leucine zippers form the core of the octamer. The DNA-binding activity is attributed to the two relatively short basic amino acid regions, 56 – 64 and 86 – 97, found upstream to the leucine zipper. The latter one was deemed responsible for creating the DNA binding domain on the ring structure and even a point mutation in this region could completely inhibit the DNA binding activity of the protein [13].

Amino acid sequence analysis of TB-RBP also demonstrated a leucine zipper and stretch of basic amino acid residues on two different peptides that were identical to the human protein and indexed for chromosomal translocation in lymphoid cells. GST–tagged TB-RBP recombinant protein also interacted *in vitro* with DNA oligo sequences representing the target

recognition motifs from clustered breakpoint region of Bcl2 oncogene found in follicular lymphoma patients [17]. These studies further confirmed single-stranded DNA binding ability of translin.

The DNA-RNA binding function of the protein is attributed to its C-terminal, encasing the motif of basic amino acids, with a minimum requirement of a dimer [16]. Additionally, the RNA-binding ability of the protein was observed only in brain and meiotic germ cells of mouse testis [30].

The mouse orthologues of human translin, and trax, respectively, also interact to form a heterodimer. This heterodimeric unit enhances the TB-RBP binding to ssDNA, but inhibits its interaction with RNA. In addition, analogous to translin-DNA binding, only one of the two basic regions is essential to bind to ssDNA interaction, but both the domains are required for RNA binding [36]. However, the absence of common RNA recognition motifs in TB-RBP sets it apart from other RNA-binding proteins [74]. Other RNA-binding proteins, such as the human teratocarcinoma protein p40, which binds to LINE-1 RNA [75], an AU-rich sequence-binding protein [76], thymidylate synthase [77], and one of the iron responsive element-binding proteins (IRE-BP1) [78], all lack common RNA-binding domains but are known to regulate stored mRNAs during spermatogenesis and facilitate transport of specific mRNAs in the nervous system [14, 50].

Electrophoretic mobility gel-shift assays (EMSA) based on interaction studies between translin and target DNA sequences from broken hot-spot regions on chromosome 18q21, clearly indicated that translin binds to DNA from ends and hence requires single-stranded ends to load onto staggered DNA break-points [43]. Studies from our lab further complied with these observations. According to our results, we were able to put forth a model stating that free translin octamer undergoes a conformational change, leading to either compaction or dissociation of the molecule and loads onto DNA duplexes via its free ends resulting into a tighter clamping of the duplex ends [79].

9.2. GTP acts as a "switch" to regulate Translin-DNA/RNA binding

Sequence analysis of mouse TB-RBP revealed several domains, one of it being the putative GTP-binding domain, VTAGD, in the C-terminal, that shares substantial homology with sequence, DTAGQ on G-proteins [27]. This domain is also fairly conserved differing in only one amino acid among *Drosophila*, human & *Xenopus* translin. Radiolabeled EMSA revealed that only GTP, but neither GDP, GDP-γS nor ATP, decreased the RNA binding ability of TB-RBP. A mutation in the GTP binding site altered only the RNA binding ability of the protein but did not influence its DNA binding ability. This mutation also did not interfere with the dimerization of the protein, its interaction with wild-type TB-RBP or also with trax, since these interactions are mostly dependent on the leucine zipper. Moreover, mammalian cell lines transfected with the GTP mutated TB-RBP resulted in cell death indicating a dominant negative role in cultured cells [28].

In order to further understand the mechanism of GTP modulation on translin, we performed several biochemical and biophysical experiments on human translin and its *Droso-*

http://dx.doi.org/10.5772/54016

phila orthologue which was cloned and characterized in our laboratory [18]. Our studies using circular dichroism (CD) spectroscopy showed that addition of GTP reduced the ellipticity from the secondary structure of human translin whereas the response was not similar for that of the *Drosphila* orthologue. MALDI-TOF analyses of the total tryptic profile for both the proteins showed that the liberated proteolyzed fragments predominantly belonged to 24 – 27 and 6 – 8 kDa size categories. GTP addition further enhanced the C-terminal cleavages in the former category, specifically in translin. Isothermal calorimetric studies probed the heat changes associated with GTP-mediated effects, distributed in two distinct phases for human and fly translin protein. In the first phase, the GTP : protein monomer ratio increased from 0:1 to 1:1 showing an initial exothermic curve followed by an endothermic change. However, in the final phase of titration, as the ratio increased beyond 1:1, the heat changes observed with translin were markedly different from that of the *Drosophila* protein. Human translin showed an exponential decrease in enthalpy, whereas the *Drosophila* protein showed a monotonic rise in enthalpy. These two sets of sites seemed independent models as per curve-fitting analysis and hence their binding patterns could not be correlated as either parallel or sequential. Our findings led us to hypothesize a model. As the GTP : protein ratio increases beyond 1:1, the occupancy of the putative final site on translin with GTP induces dissociative change within the translin octamer, as evidenced by the exponential decrease in the enthalpy curve in the second stage of titration. Interestingly, under similar conditions, the heat changes recovered from GTP titration with *Drosophila* translin were similar to that of human translin in the first part but different in the second stage affirming that the fly translin oligomer may be smaller than the octamer, perhaps a tetramer or hexamer, which can dissociate into stable dimers as evidenced from gel filtration studies. Human translin exhibits a stable octameric state and binds ssDNA/RNA/dsDNA targets, in sequential order of binding ability, all of which get attenuated when GTP is added. Conversely, *Drosophila* translin exhibits a stable dimeric state that assembles into a sub-octameric (tetramer/hexamer) form and fails to bind ssDNA and RNA targets [29]. We predicted that this phenomenon could likely be a manifestation of a structural dissociation, i.e., "loosening or slackening" in the ellipsoidal ring that lowers the nucleic acid tethering by the protein. These observations were compliant with our earlier hypothesis for translin loading onto free DNA ends due to conformational changes [79]. Furthermore, enhanced C-terminal cleavages by the protease action in the presence of GTP are a reflection of structural reorganization in the human translin ring, and the lack of the same in *Drosophila* protein is consistent with the model that oligomeric status may be critical for the "switchability" by GTP. A parallel inference has been drawn from the well-characterized RAG1and RAG2 proteins that perform critical DNA recognition and cleavage functions in V(D)J recombination, where physiological concentrations of GTP strongly and selectively inhibit the RAG-mediated transposition reaction [80]. This further encouraged us to believe that GTP binding might similarly impinge on the proposed chromosomal breakage-rejoining function of translin, *in vivo*.

Not only translin, but also tanslin-trax complex has been investigated substantially for its nucleic acid binding properties, but trax, a rather unstable protein was not known to bind DNA or RNA independently. Very recently, Gupta and Kumar successfully identified two novel nucleic acid binding motifs in trax, nomenclatured as B2 and B3 (B2,115QFHRA119;

B3,237YEVSKKL243) [81]. Intriguingly, the binding activity displayed by the translin-traxB2 complex was comparable to that of the wild type translin-trax complex,, but that of the translin-traxB3 complex was markedly reduced. The motifs seemingly contributed towards the DNA-binding ability of the tanslin-trax heteromeric complex.

10. Physiological role of Translin and its implications in genetic disorders

Translin was identified as a novel DNA binding protein at chromosomal breakpoint junctions in several lymphoid malignancies [10]. Since then several biochemical and molecular studies have been carried out in order to characterize the protein for its physiological relevance across evolution.

Cellular processes such as cell signaling, trafficking, and targeting are governed by protein interactions occurring through short peptide segments that share a common "motif". Two such protein binding modules are; DxxDxxxD protein phosphatase 1 binding motif and a VxxxRxYS motif that binds to translin [82].

10.1. Cell cycle proliferation

Translin was contemplated as a part of cell division machinery when mammalian cells were treated with DNA damage inducing agents, such as, doxycycline, which led the protein synthesis to become maximal during the G2/M phase. The protein was also found to be associated with γ-tubulin and less markedly with α-tubulin, in agreement with the presence of γ -tubulin in the centrosome, the spindle poles and the microtubule bundles of the mid-bodies during mitosis. Translin localizes to mitotic spindle microtubules during metaphase and shifts to mid-bodies in late telophase [83].

Mutation in Atm gene leads to a recessive human genetic disorder, Ataxia telangiectasia (AT), characterized by progressive neurodegeneration, immunologic defects, cancer predisposition, and hypersensitivity to ionizing radiation [84]. AT cells show irradiation-induced cell cycle checkpoint defects, since wild type ATM activates p53, which in turn is known to induce the downstream apoptosis cascade $p21^{WAF1/CIP1}$ [85]. Intriguingly, mice spleen cells, defective in ATM gene exhibited intermediate translin levels in response to γ-irradiation, associating altered protein expression with cell cycle proliferation.

TB-RBP heterozygous mice were phenotypically indistinguishable from their wild-type littermates. Normal T-cell development and V(D)J recombination supported that absence of TB-RBP was not essential for its function but had an influence on the behavioral pattern. However, the birth weight was 10-30% lower for TB-RBP deficient homozygotes with a coordinated reduced sperm count and high level of apoptosis indicating abnormal spermatogenesis. Also, the females produced smaller and fewer litters [53]. The TB-RBP-deficient mouse embryonic fibroblasts (MEFs) exhibited a reduced growth rate compared with MEFs from littermates which was remedied with the reintroduction of TB-RBP. Trax was also

found to be absent in these cells in spite of normal mRNA levels, probably a consequence of ubiquitination [86]. Complementing the deficiency with a wild-type TB-RBP molecule regulated the trax protein expression levels, indicating that they both existed proportionally for normal cell proliferation. This phenomenon was also corroborated by shRNA against trax in HeLa cells that exhibited sluggish proliferation due to loss of trax mRNA [86]. On the other hand, deletion of translin in the yeast gene, did not alter the growth rate or phenotypic changes in cell morphology or size, but rather a double mutant of translin-trax slightly stimulated the cell growth [19]. Thus, both these genes can be deemed non-essential in *S.pombe*. Translin also exhibited a higher affinity for homologous RNA sequences, such as (GU)n and (GUU)n, suggesting its primary role in functions related to RNA metabolism [19]. In addition, X-translin exhibited a weak and diffused nuclear staining, but a prominent granular cytoplasmic staining during interphase. Interestingly, it refers that a part of the protein underwent a remarkable redistribution throughout mitosis and associated with centrosomes, thus mystifying its role in cell cycle [20].

10.2. mRNA regulation

Immunocytochemical studies showed that translin/TB-RBP was distributed in the nucleus and the cytoplasm of the developing rat hippocampal cells whereas it localized only in the nuclei of the glial cells [87]. Mouse cerebellar extracts demonstrated that both translin and trax were predominantly localized in the cytosolic fraction as components of the GS1 complex, which also consists of RNA oligonucleotides [88]. The translin-trax heteromeric complex was found to be enriched in brain following UV radiation. This led to a speculation that the complex may somehow be responsible for increase in the basal levels of GS1, thus implying a role in DNA repair.

Paradoxically, immunoblot analysis demonstrated levels of translin and trax in kidney, lung and cerebellum equal to that of brain and testis. Interestingly, gel-shift analysis of kidney extracts revealed that the expression of these proteins was masked by endogenous RNA; asserting that the TB/RBP-trax complex bound to RNA *in vivo*, implicating its role in RNA processing [89]. Translin knock-out mice exposed multiple behavioral abnormalities and alterations in levels of transcripts encoding synaptic proteins [90].

Neural BC1 RNA complex is expressed in the brain and distributed in the dendrites in the form of ribonucleoproteins [91]. Pur α and β are single stranded DNA/RNA binding proteins that have been known to play a role in transcription and replication [92, 93]. These proteins linked the BC1 RNA, distributed in the neuronal dendrites as ribonucleoproteins (RNPs) and consisted of translin, to microtubules. Mouse translin and a like partner protein, assumingly trax co-purified, from brain, with BC1 RNA as a 138 kDa complex suggesting that it is a molecular scaffolding assembly required for translin transport along dendritic microtubules, probably with a transient interaction with RNPs [94] and with the ability to repress mRNA translation [14]. Thus translin could possibly play a role in regulation of mRNA translation within dendrites during transport. Another example of translin/trax complex binding to RNA is its interaction with 3'UTR of protamine-2 comprising of Y and H elements. Mutation studies found that a minimum cluster of 8 G residues with an oligo length

of 24 nucleotides was vital for high binding affinity [95]. This further supported the translin – BC1 RNA interactions at its 5′ end, rich in G-clusters. A confluence of localization, biochemical and RNA trafficking studies supports the view that this complex mediates dendritic trafficking of RNAs, a process thought to play a critical role in synaptic plasticity. Another study showed translin binding to ssDNA of Tetrahymena telomerase, (TTGGGG)n and human telomeric repeats, (TTAGGG)n also rich in G residues, probably by unwinding the hairpins formed by hydrogen bonding between non-canonical structures [96].

Another brain derived neurotropic factor (BDNF) mRNA is targeted to dendrites where it plays a key role in mediating synaptic plasticity [97]. Translin has been shown to bind to this mRNA and regulate its dendritic trafficking which is impaired due a mutation G196A (Val66Met) in BDNF [98]. Thus, the abnormal targeting can lead to pathologic neuropsychiatric disorders.

Similarly, TB-RBP was also observed in the nuclei of neurons and dendrons in the mouse hypothalamus [99]. Other RNA-binding proteins such as FMR1 and FXR1 and 2, responsible for mental retardation and Fragile X syndrome, are also expressed differentially in the cytoplasm of neurons during brain development [100]. This puts forth a theory based on co-existence of translin with factors accountable for mental disorders wherein TB-RBP/translin functions in the neurons binding mRNA for its cytoplasmic export followed by storage, localization and regulation of translation.

Translin also co-operates in the activation of steroidogenic factor – 1 (SF–1) for transcriptional regulation in rat leydig cells [101].

RNA interference (RNAi) is a biological mechanism in order to degrade the dsRNA and also the concomitant degradation of the homologous mRNA [102]. Mechanistic studies revealed that when dsRNA enters a cell, it is first digested into ~22 bp short dsRNA (small interference RNA or siRNA) by Dicer, a RNase III family member that is also responsible for miRNA formation. siRNA fragments, usually 5′-phosphorylated, then bind to the RNA-induced silencing complex (RISC) where they are unwound and directed to mRNA. One of the components of RISC is Argonaute2 (Ago2), which is believed to bind to the 3′ overhang of siRNA through a PAZ domain capable of binding single-stranded RNA with relatively low affinity [103, 104]. Another protein tightly bound to siRNA was identified to be TB-RBP and discovered to possess both ssRNase and dsRNase activities from two open ends of the corresponding RNA molecules [105]. A complex of translin-trax purified from the Dicer-R2D2-Ago2 reconstituted system from *Drosophila* was termed as C3PO. It enhanced the RISC activity of the recombinant complex [106], thus acting as a key activator in regulation of RNAi machinery. Only C3PO complex, neither translin nor trax alone, could function together with hAgo2 to reconstitute duplex siRNA-initiated RISC activity. Crystallization studies of hC3PO revealed two translin-trax heterodimers and two translin-translin homodimers tetramerizing side-by-side, in a stoichiometry of 6:2 to form an asymmetric octameric barrel. This asymmetric assembly proved pivotal for the function of C3PO as a novel endonuclease that cleaves RNA at the interior surface [107]. Truncated C3PO in *Drosophila* adopts a hexameric topology composed of four translin and two trax molecules according to the crystal structure, which is consistent with gel filtration and light scattering studies. The trun-

cated complex, like full-length, exhibits endoribonuclease activity on the siRNA passenger strand, leaving 3′ hydroxyl-cleaved ends in order to activate RISC [108].

R2D2, dsRNA-binding protein and an essential component in the siRNA pathway in *Drosophila*, was expressed at minimal levels in silk moth tissues. The silk moth-derived Bm5 cell line was also deficient in expression of mRNA encoding full-length Bm translin, an RNA-binding factor that has been shown to stimulate the efficiency of RNAi [109], thereby explaining variable success of RNAi technology in lepidopteram insects.

A most recent study in the filamentous fungus, *Neurospora crassa*, showed that C3PO does not play a significant role in RNAi, but rather functions as an RNase that removes the 5′ pre-tRNA fragments which were identified as the major substrates for translin-trax complex in the fungus [110]. In the translin knock-out and trax knock-out mutants, tRNA levels, protein translation efficiency and cell growth were elevated which was consistent with the increase of cell proliferation rates of translin knock-out and trax knock-out mutant cells observed in fission yeast [19]. In addition, both translin and trax are known to be required for normal cell proliferation of mouse embryonic stem cells [86]. Because the changes in tRNA levels can differentially affect expression of various proteins, the roles of translin and trax in tRNA processing and other RNA processing may provide a potential explanation for its many biological roles in several organisms.

10.3. Regulation in meiotic germ cells

Similar to the human and mouse protein, *Xenopus* translin also binds to single stranded DNA encompassing the chromosomal breakpoint consensus sequences. It has been described as capable to inhibit paternal mRNA translation, indicating that it could play an important role in maternal mRNA translation and control during *Xenopus* oogenesis and embryogenesis [20].

Interestingly, western blot analysis of germ cell protein extracts demonstrated an increased ratio of trax to TB-RBP in meiotic pachytene spermatocytes compared to the post-meiotic round and elongated spermatids, resulting in nuclear localization due to a functional nuclear localization signal on trax; whereas elevated levels of TB-RBP prompted trax to remain in the cytoplasm due to functional nuclear export signal on TB-RBP. This indicates that the localization of the two proteins in male germ cells is modulated by their relative ratios [111].

Based upon the specificity of translin binding to consensus sequences of breakpoints in chromosome translocations, it can be proposed that TB-RBP functions in the nuclei of germ cells in meiotic recombination or DNA repair in addition to serving as an RNA- and microtubule binding protein in the cytoplasm of testicular cells. Gapds mRNA was also found to be present in the adult testis extract and its translation was inhibited by the TB-RBP according to *in vitro* translation assays [52].

Drosophila translin was also found to be essential for normal trax expression substantiating a report in a parallel study that trax expression was lost in translin knock-out mice [53]. Loss of translin and trax in *Drosophila* did not seem to have an effect either on oogenesis or meiotic recombination rates and chromosome segregation. In addition, no evi-

dence was found for an increased sensitivity for DNA double-strand damage in embryos and developing larvae [32].

10.4. Hemotopoietic regeneration

Pluripotent human leukemia cell line K562 exhibited decrease in translin levels as a response to DNA damaging drugs such as etoposide and mitomycin C [43]. p53 is known to increase in response to ionizing radiation, but also nuclear levels of translin were elevated. This referred to the activation of signal transduction pathways to arrest cells at specific checkpoints in the cell cycle, allowing translin to localize in the nucleus and carry out the repair of damaged DNA [112]. In order to address the functional significance of translin in the hematopoietic generation system with reference to acute radiation-responses, translin homozygous and heterozygous mice were assessed for hematopoietic colony formation. In response to 4 Gy IR, 1 week later, extramedullary hematopoietic colonies were observed in translin$^{+/-}$ mice, whereas those in translin$^{-/-}$ mice were delayed for more than two weeks as compared to their wild type comtemporaries [113]. Thus, it can be assumed that translin somehow contributes to hematopoietic regeneration by acting as a sensor protein for radiation-induced damage. Neonatal translin $^{-/-}$ mice also exhibit delayed chondrocyte development linked to differentiation of mesenchymal stem cells. This can be further linked to the maintenance of constant number of hematopoietic progenitors by self renewal [114]. Their differentiation from hematopoietic stem cells, which is a critical phenomena in bone marrow hemtapoeisis, is shown to be perturbed in the absence of translin and trax [115].

10.5. Inherited genetic disorders and neoplasms

Inverted repeats, minisatellites, and the chi (χ) recombination hotspots are some of the DNA motifs that have been associated with gene conversion in human genes causing inherited diseases. DNA breakage could be more prominent in such gene conversion events that tend to occur within the G-rich or CpG-islands that can potentially form non-B-DNA structures [116]. Maximal converted tracts were enriched in a truncated version of the χ-element (TGGTGG motif), immunoglobulin heavy chain class switch repeats, translin target sites and several novel motifs including (or overlapping) the classical meiotic recombination hotspot, CCTCCCCT. It was thus postulated that the high density of recombination-related motifs served as target binding sites for protein complexes, such as translin and RAG-associated proteins, or arrest sites for DNA polymerases, which may assist, induce or indeed be required for the recombination-repair process [117].

10.5.1. Muscular dystrophy

Muscular dystrophies are allelic disorders caused by a mutation in the dystrophin gene [118]. Two deletion hot-spots in this gene locus were comprehensively analyzed for target recognition consensus sequences. Among other elements, such as chi, Pur α, minisatellite sequences, translin-binding sites were also identified in the muscular dystrophy gene at chromosomal breakpoint junctions [119]. This further validates the involvement of gene rearrangement in genetic disorders.

10.5.2. Sotos syndrome

Sotos syndrome (SoS), a rare congenital dysmorphic disorder, is characterized by overgrowth in childhood, distinctive craniofacial features, and mental retardation [120]. It is caused by mutations in NSD1 gene flanked by low copy repeats (LCRs). Translin target motifs were significantly higher in and around these breakpoint regions [121].

10.5.3. Fragile X syndrome

Mutations in the Fragile X mental retardation protein (FMRP) is responsible for Fragile X syndrome resulting in behavioral and neurochemical alterations in mice [122]. Like FMRP, translin is present in neuronal dendrites and associates with microtubules and motor proteins. Translin knock-out mice also exhibit behavioral, locomotor and sex-related variations [123]. These evidences suggest that both the proteins may act in the same neuronal pathway thus leading to a speculation that mutations in one or both the proteins are likely to contribute to neuronal illnesses such as fragile X-like syndrome, mental retardation, attention deficit hyperactivity disorder, epilepsy, and autism spectrum disorders in humans.

10.5.4. Schizophrenia

Disrupted in Schizophrenia 1 locus (DISC1) was first identified from a large Scottish family with a balanced translocation t(1;11) (q42.2;q14.3) responsible for schizophrenia and bipolar disorder (BP). Translin-associated factor X (TRAX), has been shown to undergo intergenic splicing with DISC1 and thus may also be affected by the translocation [124]. Locus 1q42 encompasses, DISC1 and 2 and trax that occur as an enriched complex with Translin in brain thus making it highly relevant for etiology of psychotic disorders [125]. These haplotypes were also associated with several quantitative endophenotypic traits including impairments in short- and long-term memory functioning and reduced gray matter density in the prefrontal cortex. The effects were consistent with their production of proteins that play roles in neurotic outgrowth, neuronal migration, synaptogenesis, and glutamatergic neurotransmission [126].

10.5.5. Sarcoma

Rhabdomyosarcoma occurs in connective tissue, presumably arising from progenitors of skeletal muscle. It is a common malignant tumor among young children and adolescents. Another variant of rhabdomyosarcoma is alveolar rhabdomyosarcoma and is characterized by a specific chromosomal translocation t(2;13)(q35;q14) [127] generating the PAX3-FKHR fusion gene. The t(2;13) breakpoint lies within the PAX3 and FKHR genes on chromosomes 2 and 13 respectively. The sequences flanking the breakpoint sites in these genes were found to be 62% homologous to the consensus sequence alleged to be the target recognition sequence of translin at the translocations [128].

Another example of reciprocal translocation, t(12;16)(q32;q16), is a common genetic event occurring in myxoid and round-cell liposarcomas, a malignant adipose tissue neoplasm. It is the result of a novel chimera formed by TLS/FUS and CHOP genes [129]. *In silico* sequence

analysis revealed more than 70% homologous sequences possessing translin-binding motifs adjacent to TLS/CHOP breakpoint junctions. Also, topoisomerase II consensus cleavage sites were found at these regions suggesting a role of the enzyme in creating staggered ends and recruiting one of the several factors such as translin in the process of chromosomal translocation. [130]. Furthermore, sequences highly homologous to consensus translin-binding motifs were also found at the breakpoints generated by translocation t(X;18) in synovial sarcoma [131].

10.5.6. Leukemia

Chronic myelogenic leukemia, associated with unregulated growth of myeloid cells in bone marrow is the result of a somatic gene rearrangement forming a fusion by two-way exchange between 2 genes; BCR on chromosome 22 and ABL on chromosome 9 to form BCR-ABL. Consequently, t(9;22)(q34;q11) is the chromosomal translocation and the small derivative chromosome 22 product is well known as the Philadelphia or Ph chromosome [132]. Clinical studies also demonstrated these breakpoints in most patients with topoisomerase II inhibitor therapy-related acute myeloid leukemia (tAML) [133]. Breakpoint sequence patterns on this region of the BCR gene shared 80% identity with the translin consensus recognition sites. These were also positively identified in acute lymphoblastic leukemia cases with BCR-ABL hybrid. Alu sequences, the most repetitive regions of the human genome possess a high frequency of involvement in BCR recombination. Surprisingly, they also shared a close homology to translin consensus sequences, thereby indicating that the protein might be able to bind to one of the most ubiquitous regions of the genome [134].

10.5.7. Lymphoma

Burkitt's lymphoma cells that are deficient in component(s) of NHEJ pathway exhibit a large number of translocations resembling the classic translocations [135]. Further investigation may lead to a novel pathway employing translin and interactors for rejoining the breakpoints at these junctions that resemble translin recognition motifs.

10.5.8. Carcinoma

Translin-like protein was also detected in the proteomic analysis of human colorectal carcinoma cell lines along with other proteins, such as endothelial cell growth factor 1 (platelet-derived), rhotekin protein (RTKN), septin 1, cyclin dependent kinase 1, and sialic acid binding Ig-like lectin 11, tyrosinase-related protein. All of these are known to be involved in cell growth, motility, invasion, adhesion, apoptosis and tumor immunity, which is associated with distinct aspects of tumour metastasis [136].

10.5.9. Dysgerminoma

Dysgerminoma, arising from gonad cells, is a rare form of ovarian tumor in adolescent women [137]. RNF139/TRC8 is a potential tumor suppressor gene and its post-transcription-

http://dx.doi.org/10.5772/54016

al regulation is disrupted by a balanced translocation t(8;22)(q24.13;q11.21). Translin was found to be involved in posttranscriptional regulation of TRC8, which could be related to the interaction between translin and TRC8 to dysgerminoma. Thereby, a model was proposed wherein one copy of TRC8 was disrupted by palindrome-mediated translocation followed by further loss of TCR8 expression through suppression by translin, thus setting the stage for deregulated proliferation [138].

Monosomy 1p36 is the most common terminal deletion in newborns [139]. Two interstitial deletions were further discovered within the same chromosome generating balanced reciprocal translocation t(1;9)(p36.3;q34). Alignments of these junctions did not exhibit any sequence similarities suggesting the involvement of NHEJ in the ligation of broken ends. Further analysis of the breakpoint regions, even from solid tumors, revealed sequences similar to that of translin consensus binding motifs, GCCCWSSW [140]. Although the translin recognition sequences are frequent in the human genome, due to their repetitive nature, DNA breakage can still not be considered a random event. These results could support the hypothesis that either the translin-binding sites are more prone to breakage or are involved in rejoining the broken chromosomes furthering the mechanism of NHEJ.

11. Translin and NHEJ

It will be worth investigating as to how translocations are generated in compromised cases of NHEJ. One theory proposed by our group states that molecules such as translin, trax and their partners/interactors who do not appear to directly function in either of the predominant repair pathways, NHEJ or HR, might somehow deceptively lead the cells into misrepair functions and leading to chromosomal translocations. A possible mechanism that could function like the NHEJ would involve recruitment of translin and parallel factors onto damaged DNA ends, rejoining the staggered DNA DSBs followed by ligation of broken ends. The result would be a DNA sequence comprising of deletions and insertions at the repaired breakpoint junctions [141]. The mechanism is analogous to the study which demonstrated XRCC4-DNA Ligase IV complex as the most critical factor in rejoining the broken DNA ends though NHEJ. However, the recruitment and assembly of the NHEJ core factors was strikingly diverse from the known classical hierarchy of the molecules [142].

Various biochemical and genetics studies have demonstrated that even in the absence of one or more core components of NHEJ, broken ends of DNA are joined. These mechanisms are referred to as alternative end-joining (EJ) or back-up pathways [143]. In this case, the rejoining of DNA DSBs occurs at slower kinetics and can be erroneous which is incompatible with the concept of HR mechanism. Hence, it can be inferred that there are two possible signaling cascades in an event of DNA DSB, once which is the classical NHEJ (C-NHEJ), also known as DNA-PK – dependent NHEJ (D-NHEJ) involving Ku-DNA-PK complex as well as XRCC4-DNA Ligase IV complex which is effective in class switch recombination (CSR) in normal B lymphocytes. The other is the back-up NHEJ (B-NHEJ) which takes over the repair

task on the occasion of deficiency of the core factors such as Ku heterodimer, DNA-PK and XRCC4-DNA Ligase IV [144]. Boboila et. al. have demonstrated that CSR is mediated by alternative end-joining (A-EJ) in the event of combined deficiency of Ku 70 and DNA Ligase IV. IgH-c-myc chromosomal translocations were also augmented in this case [145]. Another study demonstrated that the characteristics of translocation breakpoint junctions in wild-type mammalian cells and those deficient in XRCC4-DNA Ligase IV were similar, further implying that A-EJ pathway could be the primary mediator of chromosomal translocation in mammalian cells [146].

All of these recent evidences suggest that chromosomal translocations are rather suppressed when canonical NHEJ in involved in repair of DNA DSBs. But they become more common when A-EJ takes over. One of the speculation is that there is a rare probability for concurrent DSBs as one is usually repaired and restored to original chromosomal configuration by immediate sensing of Ku-DNA-PK-XRCC4-DNA Ligase IV complex before the next one occurs. Thus the temporal opportunity for translocations to occur is reduced [147]. On the occasion of inefficient C-NHEJ, the rejoining is slower by A-EJ, widening the time frame before each DSB closure, thus increasing the chance of two or multiple DSBs at the same time, leading to chromosomal translocations. However, which enzymes participate in this pathway is not quite certain. There are mounting evidences depicting Mre11-Rad50-NBS1, pol β, PARP, PALF and DNA Ligase I or III as some of the players carrying out A-EJ. However, no set rules governing the hierarchy of this mechanism are brought to light. Several of these translocations possess translin recognition motifs. Therefore, one theory could be postulated, wherein, translin might be the early sensory molecule binding to recognition sequences and recruiting the downstream nucleases and ligases.

Several other pathways which are ATM-dependent or MRN-dependent are also highlighted by other groups. Since any physiological mechanism is less likely to be exclusively independent, there is a high probability of cross-talk even among the DSB repair pathways: HR, C-NHEJ, A-EJ, or other pathways involving the core and alternative components. However, this discussion is beyond the scope of this review.

12. Conclusions

Detailed analysis of breakpoint junction consensus sequences suggested that they were not simple and could possess a diverse amount of variations. Translin has been found to bind at translocation break points and proposed to be involved in DNA recombination and repair and in the regulation of telomere length [148]. Surprisingly, AT and GC repeat sequences which had almost no homology with known breakpoint sequences such as ATGCAG and GCCC(A/T)(G/C)(G/C)(A/T) showed a high binding affinity to translin. Translin also binds d(GT)n and (TTAGGG)n overhangs linked to Ds DNA which forms unusual structures such as DNA quadruplexes or that inhibits their binding to the protein unless unwound and the binding domains are accessible per octamer [96]. This leads to a proposition that translin

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might be involved in the control of recombination at microsatellites and in the maintenance of telomeres which are highly repetitive structures. The binding of translin to oligonucleotides *in vitro* has been demonstrated to increase the extension of telomeres [96]. Amplification of telomerase and increased telomere length is associated with the invasive and metastatic potential of murine and human tumors [149]. Translin transcripts are also at an elevated level in mouse lung adenocarocinoma indicating an early event in carcinogenesis [150].

Other than DNA/RNA regulation, translin might also be considered a responsible factor in one of the benchmark obese phenotypes in mice [151].

13. Future directives

Long-term administration of imipramine, an anti-depressant drug, downregulated translin presumably playing a vital role in the segregation of chromosomes and cytokinesis as well as accelerating cell proliferation [152]. tsnΔ and traxΔ cells were not responsive to several DNA damaging agents indicating that neither protein was required for recovery from DNA damage, dispelling the suggestion that these proteins are evolutionarily conserved due to a fundamental role in the DNA damage response [153]. The finding that trax and translin seem to regulate cell proliferation in higher eukaryotes, but not in *S. pombe*, where the biochemical function is conserved, indicates that there is not a clear correlation between the conserved biochemical function and regulation of cell proliferation, suggesting that the two are not linked. Further analysis in this simple eukaryote will provide insight into the nature of this process.

Trax harbors a nuclear localization signal and interacts with translin to transport it to the nucleus when required. Once in the nucleus, translin–trax can interact with DNA to carry out the repair function along with several other co-factors. Thereafter, trax dissociates from the complex, exchanging translin for C1D, and freeing translin to interact with mRNAs marked for export from the nucleus via translin's nuclear export signal [60, 86]. Once translin has re-entered the cytoplasm, it can remain bound to the mRNA until a cellular signal for release and subsequent translation of the message has been received (Figure 3). The ability to act as a shuttling protein is a hallmark of the RNA-binding proteins that traffic mRNAs in neuronal cells [154]. Based on studies of translin-trax involved in dendritic targeting of BDNF mRNA, it is conceivable that heteromeric translin/trax complexes mediate dendritic trafficking of mRNAs, but that its nuclease activity is suppressed during mRNA transport [155] and activated when functioning as components of RISC complex. Accordingly, it will be of interest in future studies to test these models of translin's dual role in mRNA transport and silencing.

The influence of translin on proliferation, DNA repair, chromosome segregation and cytokinesis, RNA stability and transport, and translation of proteins as well as telomere elongation may be critical in tumor formation and progression.

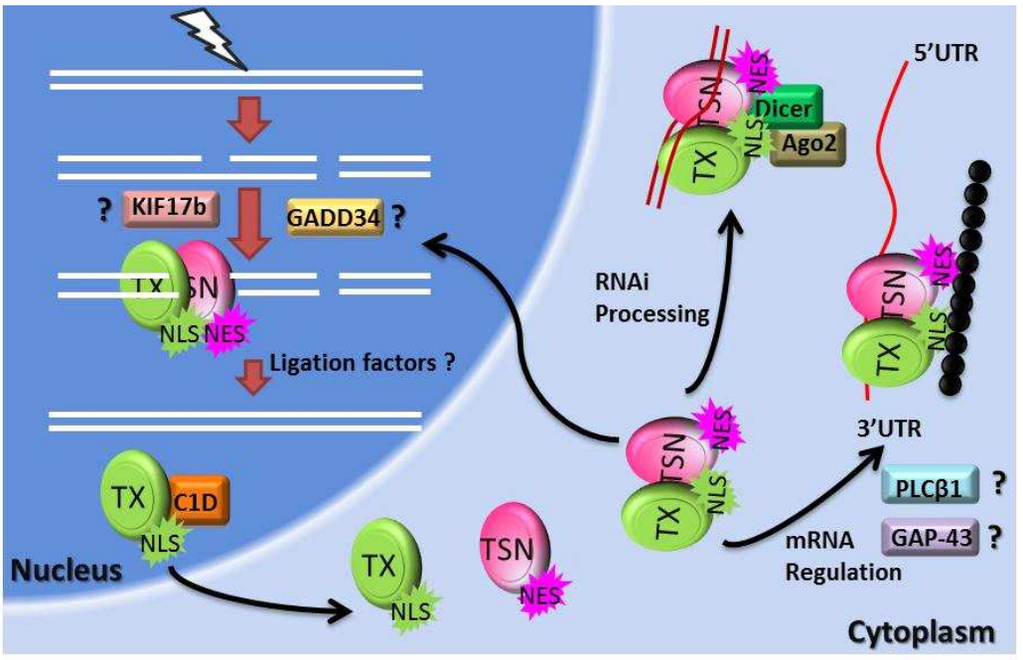

Figure 3. Proposed model for shuttling mechanism of Translin-Trax complex

Acknowledgements

I would like to thank the Journal of Acta Crystallographica Section D, Biological Crystallography and Dr. Masataka Kasai, for granting me permission to reproduce the crystal structure of Translin, in order to better emphasize the significance of the oligomeric state. RPK is also greatly appreciative of Dr. Vinay Kumar and his group, BARC, India, for consenting to the re-use of the crystal structure of the *Drosophila* mutant translin and its oligomeric status from his publication, Gupta et. al., (2008) FEBS Journal. I would also like to thank Dr. Sunil Saroj, Emory University, USA, for his kind help in graphic designing of the model.

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