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Co-expressed recombinant human Translin-Trax complex binds DNA

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Abstract Trax, expressed alone aggregates into insoluble complexes, whereas upon co-expression with Translin becomes readily soluble and forms a stable heteromeric complex (~430 kDa) containing both proteins at nearly equimolar ratio. Based on the subunit molecular weights, estimated by MALDI-TOF-MS, the purified complex appears to comprise of either an octameric Translin plus a hexameric Trax (calculated MW 420 kDa) or a heptamer each of Trax and Translin (calculated MW 425 kDa) or a hexameric Translin plus an octameric Trax (calculated MW 431 kDa). The complex binds single-stranded/double-stranded DNA. ssDNA gel-shifted complex shows both proteins at nearly equimolar ratio, suggesting that Translin "chaperones" Trax and forms heteromeric complex that is DNA binding competent.

Keywords: Trax-Translin complex; Co-expression; Purification; DNA-binding activity; Oligomers

1. Introduction

The human Translin, a 27 kDa protein, is a single stranded DNA and RNA binding protein with proposed function in chromosomal translocations and in regulating the expression of a variety of RNA sequences both in the brain and the testes. Translin recognizes chromosomal breakpoint sequences at single-stranded DNA ends and is observed to be recruited in the nucleus where double strand breaks are induced by DNA damaging drugs and in many cases of lymphoid neoplasms involving T-cell receptor or immunoglobulin gene rearrangements [1,2]. It has been proposed that GTP modulates the binding affinity of mouse orthologue of human Translin, TB-RBP, towards RNA or DNA. For instance, in the presence of GTP, the binding affinity of TB-RBP towards RNA decreases by more than 50% while its DNA binding affinity remains largely unaltered [3]. The Translin selectivity towards RNA or DNA is also modulated on interactions with another Translin-like protein, Trax. An interaction with Trax lowers the RNA binding ability and marginally enhances the DNA binding ability of Translin [4]. Trax, a 33 kDa protein, was earlier identified using yeast two-hybrid system [5]. It has recently been suggested that both Trax and Translin proteins are needed for normal cell proliferation in rat [6]. Variety of studies from Hecht and Baraban groups suggest that the biological functions of Translin as well as Trax are intimately linked via a protein complex involving both proteins [4,7]. However, the molecular basis of the same is unclear.

One of the approaches towards understanding the properties of Translin-Trax complex is to isolate and biochemically characterize its functions. Large amounts of purified complex is required for the same. Since Trax protein is post-transcriptionally stabilized by rat orthologue of human translin and is unstable by itself [6], we explored the possibility of purifying the heteromeric protein complex in bacteria, following co-expression of both the proteins. Further, we surmised that if a stable heteromeric complex is formed in situ in bacteria, following the co-expression, the same may mimic the physiological form, in contrast to the complex generated in vitro by mixing the individual protein components at arbitrary ratios, since the exact stoichiometry of Trax and Translin in the complex is not known. Here, we demonstrate that following co-expression in *Escherichia coli*, Translin and Trax proteins form a stable and functionally active heteromeric complex, which can be purified in large amounts. The complex contains nearly equimolar levels of either protein and may exist as a stable assembly of 430 kDa size. Moreover, the purified complex is proficient in binding single-stranded as well as double-stranded DNA targets. The gel-shifted complex formed on ssDNA reveals the presence of both proteins at nearly equimolar ratio, thereby suggesting that the heteromeric complex, which also contains both proteins at similar ratio, may be the DNA binding unit. The availability of large amount of recombinant complex is expected to facilitate detailed biochemical, biophysical and structural studies. The detailed structural characterization of the complex may provide clues about how Trax might modulate the nucleic acid selectivity of Translin. The crystal structures of the Translin protein from mouse and humans have recently been reported [8,9]. In view of the ease of crystallization of Translin, we expect that Translin may act as a scaffold for the crystal lattice and thus may facilitate structure determination of an otherwise unstable Trax protein. The use of molecular scaffolds to crystallize difficult or unstable proteins has recently been reviewed by Derewenda [10].

2. Materials and methods

2.1. Materials

Recombinant plasmids, pQE9 harboring human translin gene (pQE9-translin) and pET28a containing human trax gene

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(pET28a-trax), were obtained from Prof. Masataka Kasai, National Institute of Infectious Diseases, Japan and Prof. Norman Hecht, University of Pennsylvania (USA), respectively. In the pQE9-translin construct, the translin gene was cloned at the site that codes for 6xHis tag fused at the N-terminus of Translin, but has no thrombin cleavage site. Cloning of trax gene in pET28a, in addition to His-tag, provided protease cleavage site such that digestion with Thrombin removes the tag, leaving three additional amino acids at the N-terminus of the encoded protein.

2.2. Expression and purification of Trax

E. coli BL21(DE3) cells with His-tagged human trax cDNA cloned in pET28a vector (Novagen) was grown in 2.5 l Luria Bertani (LB) medium containing kanamycin (100 μg/ml) to an A_{600} of 0.2 at 25 °C (no IPTG induction needed) and harvested by centrifugation. The cell pellet was resuspended in 15 ml sonication buffer (20 mM Tris–HCl pH 8.0, 0.75 M NaCl, 0.05% imidazole and 10% glycerol), sonicated (5 output × constant × 15 s × 8) on ice and the lysate was centrifuged at 16 000 × *g* for 40 min at 4 °C. The supernatant (15 ml) was equilibrated with 1.0 ml of 50% slurry of Ni-NTA-agarose (previously equilibrated and suspended in sonication buffer) in a column for 1.5 h at 4 °C under constant shaking condition. The column was drained, followed by resuspension of the matrix in 20 ml of 500 mM imidazole prepared in above sonication buffer. The column was subjected to constant shaking in a rotary inverter for 60 min at 4 °C, followed by draining the protein sample unbound in these conditions. The bound protein was eluted as 1 ml fractions using 10 ml of 1 M imidazole in the same buffer, following a similar equilibration step on the rotary inverter. The eluted fractions were analyzed on an SDS–PAGE gel. The fractions containing the pure protein were pooled and dialyzed against 3 × 2 l dialysis buffer (20 mM Tris–HCl pH 8.0, 50 mM KCl, 0.5 mM EDTA and 1 mM DTT). Dialyzed sample was concentrated 10-fold by polyethylene glycol concentration method, aliquoted and stored at −20 °C. The protein concentration was estimated by Bradford's method using BSA as a standard.

2.3. Co-transformation and co-expression

Host strain *E. coli* BL21 (DE3) harboring pET28a-trax was made competent and co-transformed with pQE9-translin construct. The transformed cells were screened on the LB agar plate containing 50 μg/ml of carbenicillin and 25 μg/ml of kanamycin (carb-kan plate). The clones were transferred on the fresh carb-kan plate. The isolated fresh colony of co-transformed cells was inoculated in 25 ml of liquid LB medium containing 50 μg/ml of carbenicillin and 25 μg/ml of kanamycin. The expression of Trax and Translin proteins were induced by adding 0.5 mM of IPTG when A_{600} of the growing culture reached 0.6. Two hours after IPTG induction, the cells were harvested by centrifugation at 4000 × *g* at 4 °C. The co-expression of both the proteins was checked by SDS–PAGE of induced and uninduced cell pellets. The co-transformed cells under the selection pressure of carbenicillin and of kanamycin antibiotics were found to be stable at 37 °C at least for 20 h. The freshly transformed clones were stored as a glycerol stab at −70 °C. For large-scale purification of the proteins, the inoculum was always prepared with clones streaked from −70 °C stabs on carb-kan plate.

2.4. Purification of Translin-Trax complex

The purification was achieved using low-pressure liquid-chromatography system (Bio-Rad Biologic Lp) with manually packed columns at 25 °C, except where stated otherwise. The purification progress was monitored by SDS–PAGE. The recombinant clone BL21 (DE3) was grown in LB media containing 50 μg/ml carbenicillin and 25 μg/ml kanamycin. The cells (7 g) harvested from 2.5 l culture by centrifugation at 4000 × *g* at 4 °C were suspended in 100 ml ice-cold lysis buffer [25 mM Tris–HCl pH 8.0, 100 mM NaCl, 1 mM DTT, 5% Glycerol, 0.3 mg/ml lysozyme and one tablet of protease-inhibitor cocktail (Roche) per 100 ml]. The suspended cells were disrupted by sonication for 5 min in 25 ml portions maintained on ice. The lysate was cleared of cellular debris by centrifugation at 20 000 × *g* for 30 min and the supernatant was loaded onto Chelating-Sepharose FF column (Ni-IDA; Amersham-Pharmacia) charged with 100 mM $NiSO_4$ and pre-equilibrated with buffer H1 [20 mM Tris–HCl (pH 8.0), 100 mM NaCl, 50 mM imidazole and 5% glycerol]. The bound proteins were eluted with a linear gradient of 50–600 mM imidazole over six column volumes in buffer H1 at a flow rate of 1.0 ml/min. Both the proteins eluted at the same concentration of imidazole (~200 mM). The proteins eluted from Ni-IDA column were mixed with Thrombin protease (10 units/mg of fusion protein) and maintained at 17 °C for overnight for the cleavage of poly-histidine tag from 6xHis-Trax fusion protein expressed using pET28a vector. SDS–PAGE and amino acid sequencing of the N-terminus using a Shimadzu model PPSQ-10 protein sequencer confirmed complete removal of 6xHis tag from Trax, while poly-histidine tag of 6xHis-Translin fusion protein was not cleaved as the protein expressed using pQE9-translin construct lacks thrombin cleavage site. Thrombin treated proteins were dialyzed against the buffer H1 followed by loading onto Chelating-Sepharose FF column pre-equilibrated with buffer H1. Both Trax and Translin bound to Ni-IDA matrix and coeluted at imidazole concentration of ~150 mM in the buffer H1. The peak was dialyzed against buffer H2 [10 mM phosphate buffer (pH 7.0), 100 mM NaCl, 1 mM $MgCl_2$ and 1 mM EDTA] and loaded onto denatured DNA immobilized matrix (DNA-CELLULOSE) pre-equilibrated with the start buffer H2. The bound proteins were eluted with a linear gradient of 100–500 mM NaCl in buffer H2. The denaturating SDS–PAGE of the single peak eluted at ~150 mM NaCl confirmed presence of both the Translin and Trax proteins. The two bands on SDS–PAGE were quantified by densitometry using GENETOOLS software (SynGen) that suggested 1.1 ± 0.07 ratios of Translin and Trax. It is important to note that the Translin-Trax complex is stably maintained in solution in the presence of nearly 100 mM NaCl and removal of NaCl from the storage buffer leads to the formation of insoluble aggregates containing both the Translin and Trax proteins.

2.5. DNA and protein sequencing

The amino acid sequencing of the N-terminus of Thrombin digested partially purified Trax (from Ni-IDA column) was done using a Shimadzu model PPSQ-10 protein sequencer. The amino acid sequence however differed from the sequence deduced from published gene sequence (Genbank Accession No. X-95073) with our construct coding for an extra amino acid (MASNK... as compared to MSNK... of the chromosomal sequence). The presence of additional Ala residue was confirmed by DNA sequencing of trax insert in the recombinant plasmid (pET28a-trax) using an ABI automated DNA sequencer.

2.6. Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry analysis (MALDI-TOF-MS)

The purified Translin-Trax complex was diluted to 10–15 pmoles/μl in 10 mM phosphate buffer (pH 7.0), 100 mM NaCl, 1 mM $MgCl_2$ and 1 mM EDTA, 1 μl was mixed with Sinnappinic acid matrix (prepared in trifluoroacetic acid). This mixture was spotted on a target MALDI plate and allowed to dry at RT and fired and analyzed by the MASSLYNX program.

2.7. Gel filtration analysis

The peak eluted from the DNA-CELLULOSE column was loaded onto Superdex™ 200 column (1.5 × 135 cm) (equilibrated with buffer H1) and both Trax and Translin eluted as a single peak, which confirmed the heteromeric complex of the two proteins. The molecular weight of the complex was determined to be nearly 430 kDa based on the elution time of the complex on the molecular sieve column pre-calibrated using gel filtration calibration proteins (Pharmacia; Bovine Albumin, 66 kDa; Aldolase, 158 kDa; Ferritin, 440 kDa). The absorbance was measured at 280 nm in a cell of 5 mm path length. An aliquot from the peak fraction was electrophoresed on an SDS–PAGE, which showed the presence of both proteins at nearly 1:1 molar ratio.

2.8. DNA-binding assay

Typically, the binding reactions were done in 20 μl volumes. Single stranded or double stranded DNA (121 mer, 366 μM nucleotides) with 28 μM of Translin or 12.5 μM Translin-Trax (both proteins at monomer concentrations), were incubated for 1 h at 4 °C in binding buffer [50 mM Tris–HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 10% glycerol and 0.1 mg/ml BSA]. The products were resolved by 1.5% agarose gel electrophoresis and stained with ethidium bromide. To resolve the components in the gel-shifted complex, the agarose regions containing gel-shifted complex were excised and electrophoresed on 12% SDS–PAGE and visualized with silver stain.

3. Results and discussion

3.1. Trax protein expression

Human Trax protein expresses in *E. coli* rather well (lane 3, Fig. 1A), but remains largely insoluble (lane 5, Fig. 1A). The meager amount of protein that is soluble in the extract can be purified on Ni-NTA column following the affinity capture of poly-histidine tail (lane 8). However, the overall yield of the protein (~0.1 mg/10^{11} cells) is about three orders of magnitude less than that of Translin (~52 mg/10^{11} cells), under similar conditions of expression and purification [11]. Purified Trax protein migrates as a smear in native PAGE, indicative of collection of protein forms that are perhaps highly disordered in structure (Fig. 1B). In the same conditions, Translin migrates as a distinct oligomer (Fig. 1B), demonstrated earlier as octamer [12]. We tested if the highly structured Translin facilitates stabilization of Trax into a structured form, when co-expressed together. This supposition was also motivated by the fact that the two proteins not only interact with each other, but also are known to regulate each others expression levels in the cells, post-transcriptionally [6].

3.2. Co-expression of Translin and Trax proteins

We co-expressed both proteins in *E. coli* BL21(DE3). The overproduction of heteromeric complexes in bacteria has been successfully exploited for several protein complexes recently [11,13]. The vectors pQE9-translin and pET28a-trax, harboring carbenicillin and kanamycin antibiotics resistance genes, respectively, were employed for co-expression. Both the ColE1-derived plasmids have same origin of replication and are thus expected to be incompatible. However, as observed by Yang et al. [14], overproduction of both proteins succeeded largely due to the combined selection pressure of carbenicillin and kanamycin. Both plasmids were found to be stable and maintained for more than 20 h at 37 °C, which gave enough time for the induced expression of the proteins. Moreover, the co-transformed cells were stable at least for 30 days, when maintained at 4 °C on LB agar plate with carbenicillin and kanamycin (data not shown).

3.3. Translin-Trax heteromeric complex

The purification of co-expressed Translin and Trax proteins, containing poly-His tails, was achieved using Ni-IDA, DNA-CELLULOSE, and gel filtration matrices. The protocol also included thrombin cleavage of the histidine-tail selectively from Trax, followed by purification with Ni-IDA matrix using intact histidine-tail of Translin. Thrombin cleavage site was present only in Trax fusion protein and not in Translin. The co-elution of histidine tail containing Translin with that of histidine-tailess Trax on Ni-IDA followed by DNA-CELLULOSE and gel filtration matrices confirmed in situ formation of the heteromeric Translin-Trax complex. The complex,

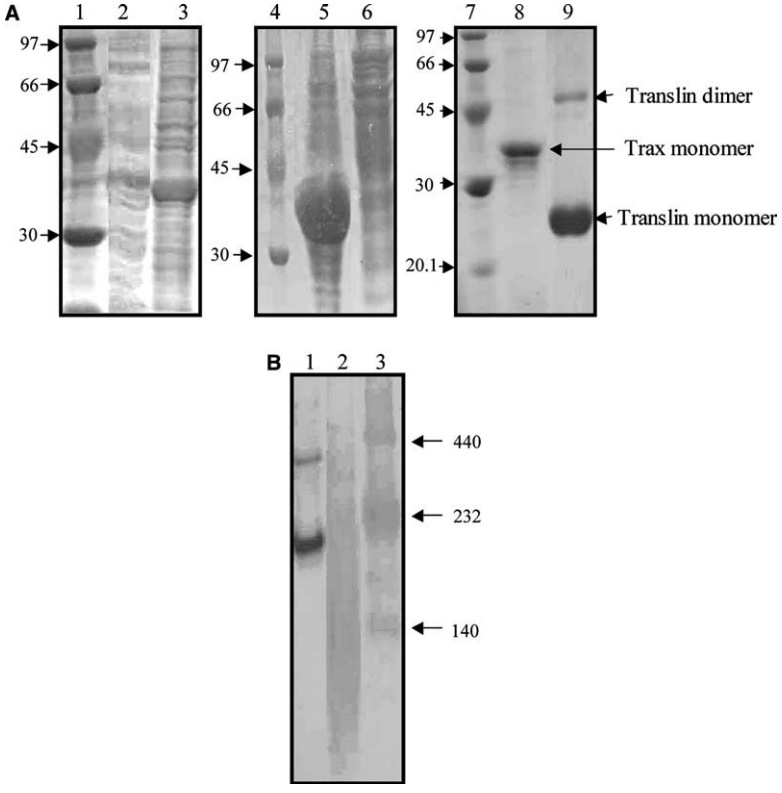

Fig. 1. (A) Poor solubility and purification of Trax protein. *E. coli* BL21(DE3) strain, transformed with human Trax cloned in pET28a vector, was grown for 16 h, lysed and the lysate centrifuged. Electrophoresis of the respective lysates and pellets was carried out on a 12% SDS–PAGE [uninduced total lysate (lane 2), expression of Trax in the lysate (lane 3), the pellet fraction of induced lysate (lane 5) and supernatant fraction of induced lysate (lane 6), Ni-NTA purified His-tagged Trax protein (lane 8) and Ni-NTA purified His-tagged Translin protein (lane 9)]. Protein molecular weight markers (lanes 1, 4, 7). (B) Oligomerization status of Trax and Translin proteins. Electrophoresis of purified Translin (7.1 μg) and Trax (4.6 μg) was carried out on a 4% non-denaturing gel electrophoresis and stained by Coomassie Blue. Note the diffused smear of protein in Trax lane. The last lane has native molecular weight markers.

monitored by SDS–PAGE during all steps of purification, revealed the presence of nearly equal intensity of stained bands corresponding to Translin and Trax proteins. The partially purified complex following Ni-IDA step appeared substantially pure as evidenced by SDS–PAGE analyses (lane 3, Fig. 2A). However, minor contaminating proteins were removed in the next steps involving DNA-CELLULOSE and gel filtration column steps. The purified complex thus obtained was

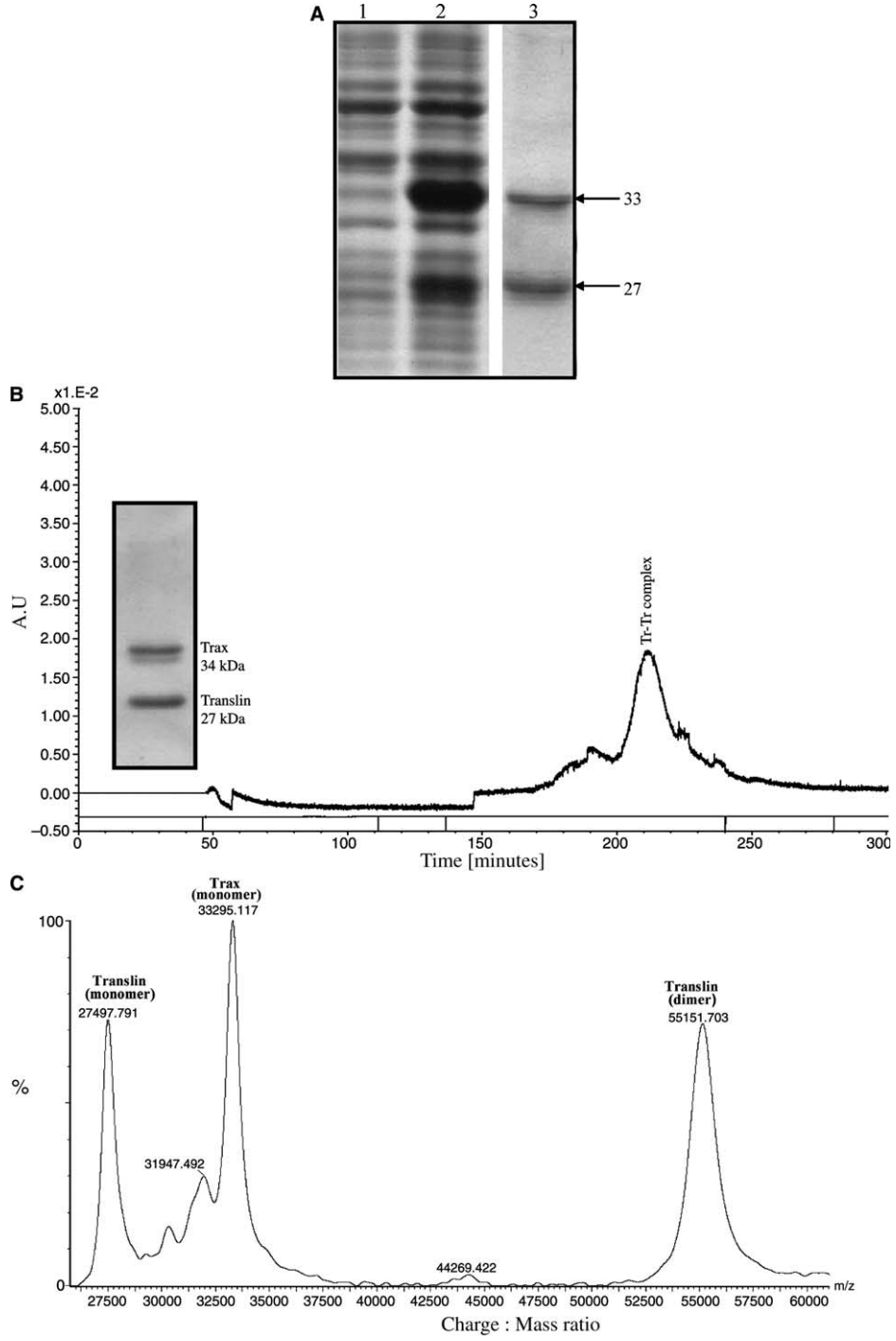

Fig. 2. (A) Purification of Translin-Trax complex. *E. coli* BL21(DE3) cells were co-transformed with human translin and trax. Lysates from uninduced (lane 1) and induced (lane 2) cells were analyzed on 12% SDS–PAGE. The Ni-IDA purified His-tagged Translin-Trax complex (lane 3) shows the presence of both Trax (33 kDa) and Translin (27 kDa) proteins. (B) Gel-filtration analysis of Translin-Trax complex. The complex was chromatographed onto Superdex^{TM} 200 column. The molecular weight of the peak (430 kDa) was determined from the elution time on the pre-calibrated column. The inset shows the SDS–PAGE analyses of the peak fraction obtained from Superdex^{TM} 200 elution and corresponds to both Translin and Trax proteins. (C) MALDI-TOF analysis of Translin-Trax complex. ~10 pmoles/μl of the Translin-Trax complex was subjected to MALDI-TOF analysis; well-resolved monomer (~27.5 kDa) and dimer (~55 kDa) of Translin and monomer of Trax (~33 kDa), were clearly evident. The shoulder peaks ahead of Trax monomer peak corresponds to its clipped products of Trax.

subjected to SDS–PAGE, followed by densitometric scanning of stained bands. Such analyses, of three different concentrations and various independent preparations of complexes showed a ratio of Translin to Trax as 1.1 ± 0.07, which suggests that it is made up of 1:1 molar ratio of both proteins. The total yield of the purified complex from one liter of *E. coli* culture was typically 10–12 mg. The equimolar amount of the two proteins in extracts of male germ cells has also been suggested by Yang et al. [6], whereas Wu and colleagues proposed an average molar ratio of 2:1 for a mixture of purified heteromeric complexes of Translin-Trax from rat liver extracts [15]. Based on elution time on pre-calibrated molecular sieves, the molecular weight of the complex is estimated to be about 430 kDa for the complex (Fig. 2B). The predominant peak in such elution profile again showed a nearly 1:1 ratio of both proteins (inset, Fig. 2B). The estimated molecular weight of the purified complex was close to that of 420 kDa reported for the cytosolic Translin-Trax complex from rat liver extract [15], the difference was mainly due to additional histidine residues from the poly-histidine tag of Translin in our preparation. In order to estimate precise molecular weights as well as to analyze the monomer–dimer status of both the proteins in the isolated complex, the complex was subjected to MALDI-TOF analyses (Fig. 2C). The analyses, besides revealing the subunit molecular weights, showed that the preparation had a small fraction of Trax in truncated forms and Translin existed as a mixture of monomer–dimer units in the complex. Based on the estimated sub-unit molecular weights, it appears that one of the following three heteromeric complexes best represent the purified 430 kDa complex, with nearly equimolar levels of either proteins: octameric Translin plus a hexameric Trax (calculated MW: 420 kDa) or a heptamer each of Trax and Translin (calculated MW: 425 kDa) or a hexameric Translin plus an octameric Trax (calculated MW: 431 kDa). Since molecular weight and the molar ratio estimations are prone to some level of experimental inaccuracies, it is at present unclear to predict which of the three possible configurations of heteromer best represents the isolated Translin-Trax complex. Wu and co-workers reported also a nuclear Translin-Trax heteromeric complex of molecular weight 240 kDa from rat liver extract [15]. We however, did not observe such a complex in our preparation from the bacterial expression system. We observed that the purified 430 kDa complex tends to dissociate in solution conditions whenever the salt concentration was low, and a minimum of 100 mM NaCl was essential to maintain the stability of complex.

3.4. DNA binding ability of Translin-Trax heteromer complex

We tested the DNA binding ability of the complex and compared the same with that of Translin. A 121-mer single stranded oligo (ssDNA) as well as its duplex counterpart were tested in a gel-shift assay. Under identical conditions of assay, while Translin protein gave rise to a distinct gel-shifted complex with the duplex DNA, Transin-Trax complex yielded a smeared gel-shift indicative of unstable protein-DNA complexes (compare duplicate lanes 3, 4 with 1, 2; Fig. 3A). On the other hand, with ssDNA, Translin as well as the heteromeric complex yielded very similarly migrating and distinct gel-shifted complexes (compare duplicate lanes 9, 10 with 7, 8; Fig. 3A). In order to test whether the gel-shifted complex formed by the heteromeric protein represents a genuine binding of both proteins, we excised the gel piece containing the gel-shifted complex (thatched boxes of lanes 7, 8; Fig. 3A) and analyzed its composition using an SDS–PAGE. As a control, we also analyzed the protein composition of a corresponding gel-piece from a lane where free heteromeric complex (minus ssDNA control) was run (thatched box of lane 11, Fig. 3A). This control was intended to reveal any background protein signal coming from the DNA-unbound free protein fraction migrating in the gel at the region corresponding to gel-shifted complex (Fig. 3A). Protein composition analyses revealed that ssDNA gel-shifted complex formed by herteromeric complex contained both proteins, i.e. Translin and Trax, at nearly 1:1 ratio, very similar to that of purified complex itself (lanes 5, 6; Fig. 3B). As can be seen, the analyses were performed in duplicate, where samples from lanes 7, 8 (Fig. 3A) resulted in SDS–PAGE lanes 5, 6, respectively (Fig. 3B). However, interestingly enough, on SDS–PAGE, while purified complex showed a mixture of monomeric and

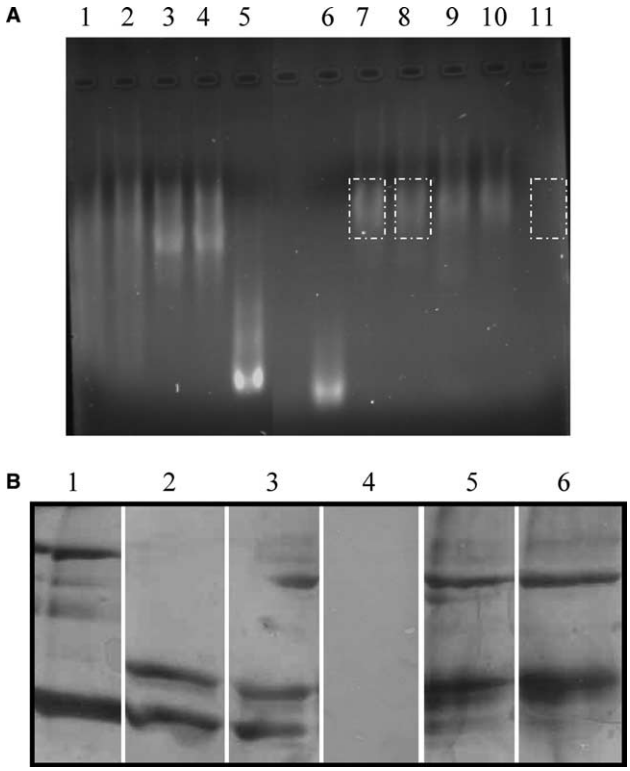

Fig. 3. DNA binding analysis of the Translin-Trax complex. (A) 121-mer duplex and ssDNA (2.56 μg each) was separately incubated with either purified Translin-Trax complex or with Translin (15 μg each) (Please see Section 2 for conditions), followed by gel-shift analyses on a 1.5% agarose gel and stained with ethidium bromide. Binding reactions were performed in duplicate. Duplicate sets of Translin-Trax were in lanes 1, 2 and 7, 8 for ds-DNA and ss-DNA, respectively. Similarly duplicate sets of Translin were in lanes 3, 4 and 9, 10 for ds-DNA and ss-DNA, respectively. The last lane (lane 11) only contained Translin-Trax and no DNA. (B) Analysis of the gel-shifted complex on SDS–PAGE. Translin-Trax gel shifted complex obtained with ssDNA was excised from the corresponding two lanes of stained agarose gel (from the regions shown as thatched boxes of lanes 7, 8 in Fig 3A), and the gel-pieces loaded onto the SDS–PAGE gel and subjected to electrophoresis (lanes 5, 6) along with the following controls in the same gel: purified Translin (not boiled in Laemmli buffer, lane 1), purified Translin-Trax complex [boiled (lane 2) or not boiled (lane 3) in Laemmli buffer], control agarose gel piece of the lane containing no ssDNA (shown as thatched box in lane 11 of Fig 3A) (lane 4).

dimeric Translin (lane 3, Fig. 3B), corroborating the matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) result (Fig. 2C), the gel-shifted complex revealed only dimeric form of Translin, with very low traces of the monomeric form (lanes 5, 6; Fig. 3B). The monomeric status of Trax in MALDI-TOF experiment was unchanged in both purified as well as gel-shifted complexes. Moreover, the protein signal associated with the gel-shifted complex was genuine, as the control gel piece revealed no traces of any free protein in that region of the gel (lane 4, Fig. 3B). All these results, put together, suggest that heteromeric complex exhibits relatively more stable binding to ssDNA than to dsDNA, where perhaps the heteromeric complex, as a unit, may be involved in stable ssDNA binding.

The current study has demonstrated that Translin and Trax co-expression system in *E. coli* offers a simple system to purify the heteromeric protein complex in large amounts. In addition, the purified complex is functionally active as assessed by ssDNA binding. We believe that this method will spur more complete physicochemical characterization of the complex. This protein complex has been implicated to mediate many diverse biological effects in the cell encompassing cell proliferation [6,16], RNA transport [7,17–20], cytoplasmic/nuclear localization [21], glucose responsive gene expressions [15], chromosomal functions [1,5,22] and possibly GTP-mediated allosteric effects [3]. Studies from Baraban group have shown that brain extracts contain single-stranded DNA as well as RNA binding proteins that were identified as Translin-Trax complex and suggested their plausible involvement in RNA sequestering function in vivo [7,17–20]. More recently Translin-Trax complex was isolated from rat liver extracts as glucose response element binding complex, whose native molecular weight was estimated to be 420 and 240 kDa for cytosolic and nuclear forms, respectively [15]. It is very likely that multiple forms of Translin-Trax complexes, stemming from various oligomeric states, supported by several different interactor proteins might be involved in bringing about such diverse biological effects. Although the relevance of the purified complex described in the current study vis-à-vis the diverse biological functions just stated above is unclear, we believe that the high stability and the associated DNA binding ability of the complex renders it an interesting protein assembly to focus further more deeply. And our current study offers for the first time, a simple system to isolate the complex in large-scale in pure form for such studies.

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