A role for the octameric ring protein, Translin, in mitotic cell division

Reiko Ishida[a,1], Haruo Okado[b,1], Hiroko Sato[a], Chieko Shionoiri[a], Katsunori Aoki[c], Masataka Kasai[a,*]

[a] *Department of Immunology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162, Japan*
[b] *Department of Neurobiology, Tokyo Metropolitan Institute for Neuroscience, 2-6 Musashidai, Fuchu, Tokyo 183, Japan*
[c] *Department of Hematology (Internal Medicine), The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

Received 9 July 2002; accepted 11 July 2002

First published online 24 July 2002

Edited by Jesus Avila

Abstract The octameric ring protein, Translin, demonstrates marked similarities to the family of helicase enzymes regarding its quaternary organization and dimerization of subunits. Here we show that the level of Translin closely parallels the proliferative state in various cell types. Expression is periodic during the cell cycle, with protein synthesis becoming maximal in the S and mitosis phases, consistent with a role in cell division. Moreover, induced overexpression of Translin was found to accelerate cell proliferation. Confocal microscopic analysis revealed that Translin is localized at the centrosomes at prophase and the mitotic spindle at metaphase, then translocating to the spindle midbodies during cytokinesis. This novel localization is attributable to specific interactions with microtubules of the mitotic spindles, and especially γ-tubulin. The results suggest that Translin participates in processes ensuring the segregation of chromosomes and cytokinesis.

Key words: Translin; Chromosome segregation; Cytokinesis

1. Introduction

Cells are generally capable of faithfully replicating chromosomal DNA during S phase so that a diploid complement of chromosomes is distributed equally to two identical daughter cells by mitotic division [1,2]. Overall defects in the underlying mechanisms result in genomic instability and are thought to be causes of cell proliferative diseases, including cancer exhibiting uncontrolled cell proliferation [3,4]. During the course of studies comparing basal expression levels of various proteins during mitotic cell division, we have found that there is a good correlation between the rate of cell proliferation and the level of Translin. This protein was originally identified as a single-stranded DNA binding protein exhibiting general binding activity to consensus sequences at chromosomal breakpoint junctions [5]. It was subsequently demonstrated to also bind to RNA in vitro, suggesting a multi-functional nature [6,7]. Crystallographic investigations to determine its three-dimensional character also indicated that human Translin is a ring-shaped structure formed by eight 27 kDa monomer subunits (228 amino acids), possibly connected by heptad repeats of hydrophobic amino acids (leucine zipper motifs) of four dimers [8]. Point mutation analyses of both the leucine zipper and upstream basic regions (amino acids 86–97) have revealed that creation of the DNA binding domain in the ring-shaped structure has essential functional significance [9]. Electron microscopic studies to generate a three-dimensional picture of Translin revealed the quaternary organization and the dimensions of subunits in the ring to be very similar to those observed for hexameric ring helicases [10]. While many of the ring proteins active in RNA and DNA metabolism are members of the RecA superfamily of proteins, Translin and the heptameric Rad52 recombination/repair protein [11] do not contain the highly conserved ATP binding core and have no sequence similarity. This finding suggests that the higher-order structure of these proteins may have arisen through convergent evolution to this ubiquitous ring form [10].

We previously identified a protein, Translin-associated factor X (TRAX), exhibiting extensive amino acid homology with Translin. Erdemir et al. [12] recently reported DNA damage-dependent interaction of TRAX with the nuclear matrix protein C1D, an activator of the DNA-dependent protein ki-

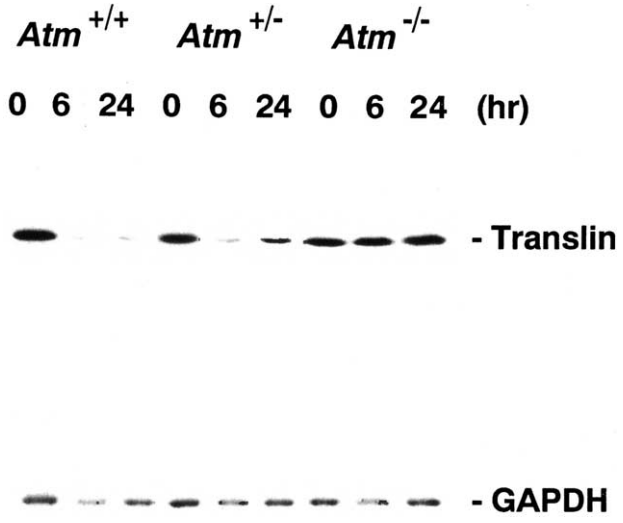


Fig. 1. Expression of Translin is associated with cell cycle checkpoint defects. Mice with the genotypes Atm(+/+), Atm(+/−) and Atm(−/−) were exposed to a 5 Gy dose of irradiation, and then total spleen cell lysates were assessed at the indicated times for Translin levels by immunoblotting. Control: GAPDH.

*Corresponding author. Fax: (81)-3-5285 1150.
E-mail address: masataka@nih.go.jp (M. Kasai).

[1] These authors contributed equally to this work.

PII: S0014-5793(02)03095-8

nase which is essential for the repair of DNA double-strand breaks and V(D)J recombination.

Numerous functions of Translin have thus already been reported with reference to DNA and RNA metabolism. In the present investigation, we provide further insight into the significance of Translin during mitosis, with potential roles in chromosome segregation and cytokinesis.

2. Materials and methods

2.1. Immunohistochemical examination

Cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 30 min at 4°C and incubated in 2 N HCl for 20 min at room temperature (RT). After neutralization with 0.1 M boric acid (pH 8.5), they were incubated in PBS containing 2.5% normal goat serum and 0.4% Triton X-100 for 20 min at RT, and double-stained with affinity-purified rabbit anti-Translin (1:200) and mouse anti-bromodeoxyuridine (BrdU) monoclonal antibodies (Boehringer Mannheim) for several hours at RT. Binding was visualized with Alexa 546 goat anti-rabbit IgG and Alexa 466 goat anti-mouse IgG conjugates (Molecular Probes). After nuclear staining with 0.5 mg/ml 4,6-diamino-2-phenylindole dihydrochloride (DAPI) for 5 min at RT, coverslips were mounted with PermaFluor (Lipshow/Immunon).

2.2. Translin–tubulin interactions

HeLa cell extracts in lysis buffer (50 mM Tris–HCl pH 7.4, 300 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, 10 mM iodoacetamide) were incubated with recombinant anti-Translin-conjugated Protein A Sepharose CL-4B overnight at 4°C. The beads were washed extensively with wash buffer (50 mM Tris–HCl pH 7.4, 300 mM NaCl, 5 mM EDTA, 0.1% Triton X-100) and boiled in sodium dodecylsulfate (SDS) sample buffer. After centrifugation, the supernatant was analyzed for the presence of tubulin by immunoblotting.

2.3. Immunoblotting

For detection of Translin, cells were centrifuged at 1000×*g* for 5 min and the pellets were dissolved in SDS sample buffer (62.5 mM Tris–HCl pH 6.8, 2% SDS, 5% glycerol, 0.01% bromophenol blue). Approximately 20 μg aliquots of total protein were run on 10% acrylamide SDS–PAGE under reducing conditions, transferred to Hybond polyvinylidene difluoride membranes (Amersham Pharmacia Biotech), and probed with affinity-purified rabbit anti-Translin antibody (1:500) followed by horseradish peroxidase-conjugated

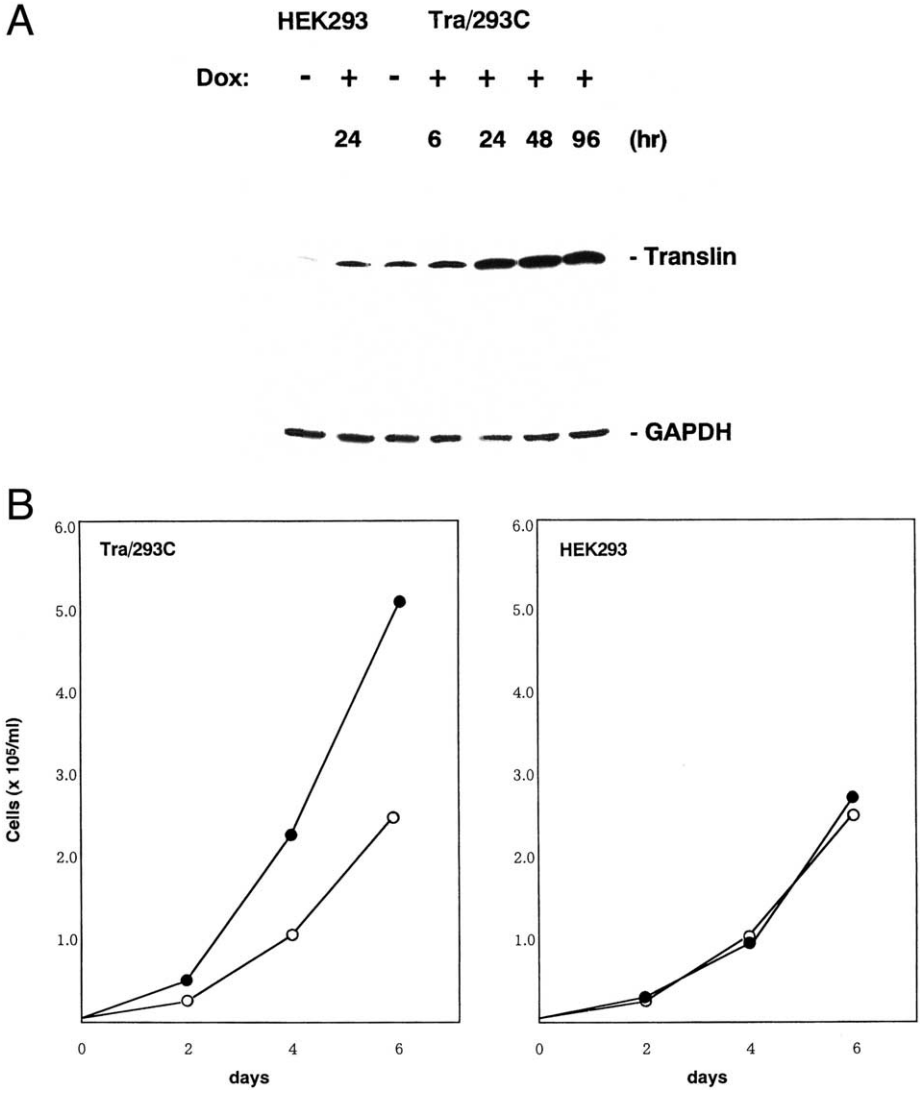


Fig. 2. Acceleration of cell division by overexpression of Translin. A: Cells of the Tra/293C clone, were incubated with doxycycline (1 μg/ml) and then total cell lysates were assessed at the indicated times for Translin levels by immunoblotting. Control: GAPDH. B: Tra/293C cells (left) and parental HEK293 cells (right) were seeded into culture medium with or without doxycycline. At the time points indicated, cell numbers were determined and a quarter of the cells was reseeded into fresh medium.

Fig. 3. Flow cytometry analysis of Translin in G0/G1, S, and G2/M phases. Tra/293C cells cultured with or without doxycycline for 4 days were stained with anti-Translin antibody and examined for protein levels in G0/G1, S, and G2/M phases by FACS. →

goat anti-rabbit IgG (1:1500). Antibody binding was detected by enhanced chemiluminescence according to the manufacturer's instructions (Amersham Pharmacia Biotech).

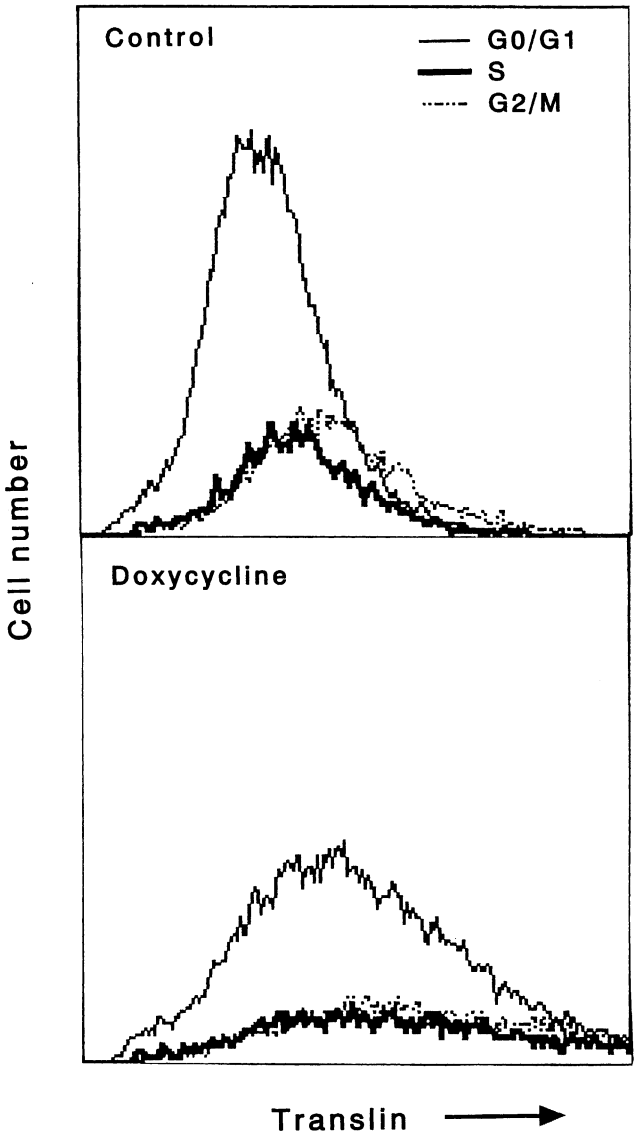
2.4. Establishing a doxycycline-regulated Translin-expressing cell line

To construct the plasmid expressing Translin protein, human Translin cDNA was subcloned into a tetracycline-inducible vector, pTRE (Clontech). To obtain a doxycycline (a tetracycline derivative)-regulated Translin-expressing cell line, pTRE-Translin and pTK-Hyg were cotransfected into the HEK293 (human embryonic kidney 293) Tet-On cell line (Clontech) with Superfect Transfection Reagent (Qiagen), and then the cells were selected in hygromycin. Positive clones were screened according to the manufacturer's instructions and then selected for their ability to express Translin in the presence of doxycycline by immunoblotting analysis.

3. Results and discussion

3.1. Translin expression correlates with cell division

To cast light on the link between Translin levels and cell proliferation, we asked whether the expression ceases with cell cycle arrest after exposure to ionizing irradiation. Ataxia telangiectasia (AT) is a recessive human genetic disorder resulting from mutations of the Atm gene, characterized by progressive neurodegeneration, immunologic defects, a cancer predisposition, and hypersensitivity to ionizing radiation [13,14]. AT cells show irradiation-induced cell cycle checkpoint defects [15] since wild type Atm activates p53, known to induce $p21^{WAF1/CIP1}$. The observed link between Translin and cell proliferation in various systems prompted us to ask whether cell cycle checkpoint defects of AT cells are associated with altered expression of the Translin protein. To address this issue, we examined levels in Atm-deficient mice [16]



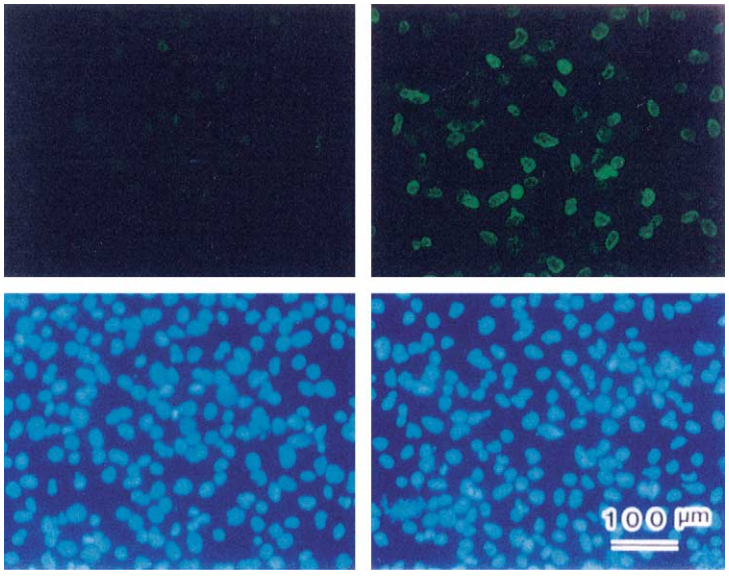


Fig. 4. Accelerated incorporation of BrdU with overexpression of Translin. Tra/293C cells untreated (left) and treated with doxycycline (right) for 4 days were immunolabeled with antibodies against BrdU (visualized with Alexa 466, green) and DAPI (blue).

after exposure to a 5 Gy dose of ionizing radiation. The results illustrated in Fig. 1 clearly indicate that down-regulation of the Translin levels in spleen cells of wild mice, Atm(+/+), occurred within 24 h of irradiation, while those in Atm(−/−) mice did not change at all, consistent with the lack of cell cycle arrest. Interestingly, the levels in Atm(+/−) were intermediate. Thus, expression of Translin is associated with cell cycle checkpoint defects in AT cells, providing further support for a general tight link with cell proliferation. In addition, similar lines of evidence for a link between Translin levels and cell proliferation were obtained using differentiation of PC12 cells by nerve growth factor and megakaryotic differentiation of K562 cells as model systems (data not shown). In all of these cases, Translin was abundantly expressed in proliferating cells and rapidly downregulated by cell cycle arrest accompanied by differentiation.

3.2. Translin protein per se is associated with cell division

The studies described above, pointing to a tight link between the expression of Translin and cell proliferation, raised the possibility that Translin protein per se is associated with cell division. To test whether overexpression might directly increase the rate of passage through the cell cycle, an experiment was designed with stable transfectants expressing inducible Translin under the control of a tetracycline-responsive promoter [17]. Among several clones of HEK293 cells expressing Translin in the presence (+) of doxycycline, one clone designated Tra/293C was obtained and used for further analysis. When Tra/293C cells were stimulated with doxycycline, Translin levels were found to increase several-fold, as monitored by immunoblotting (Fig. 2A), with strict dose-dependent acceleration of cell proliferation (Fig. 2B). Doxycycline had no such effect on the parental cell line, HEK293. These

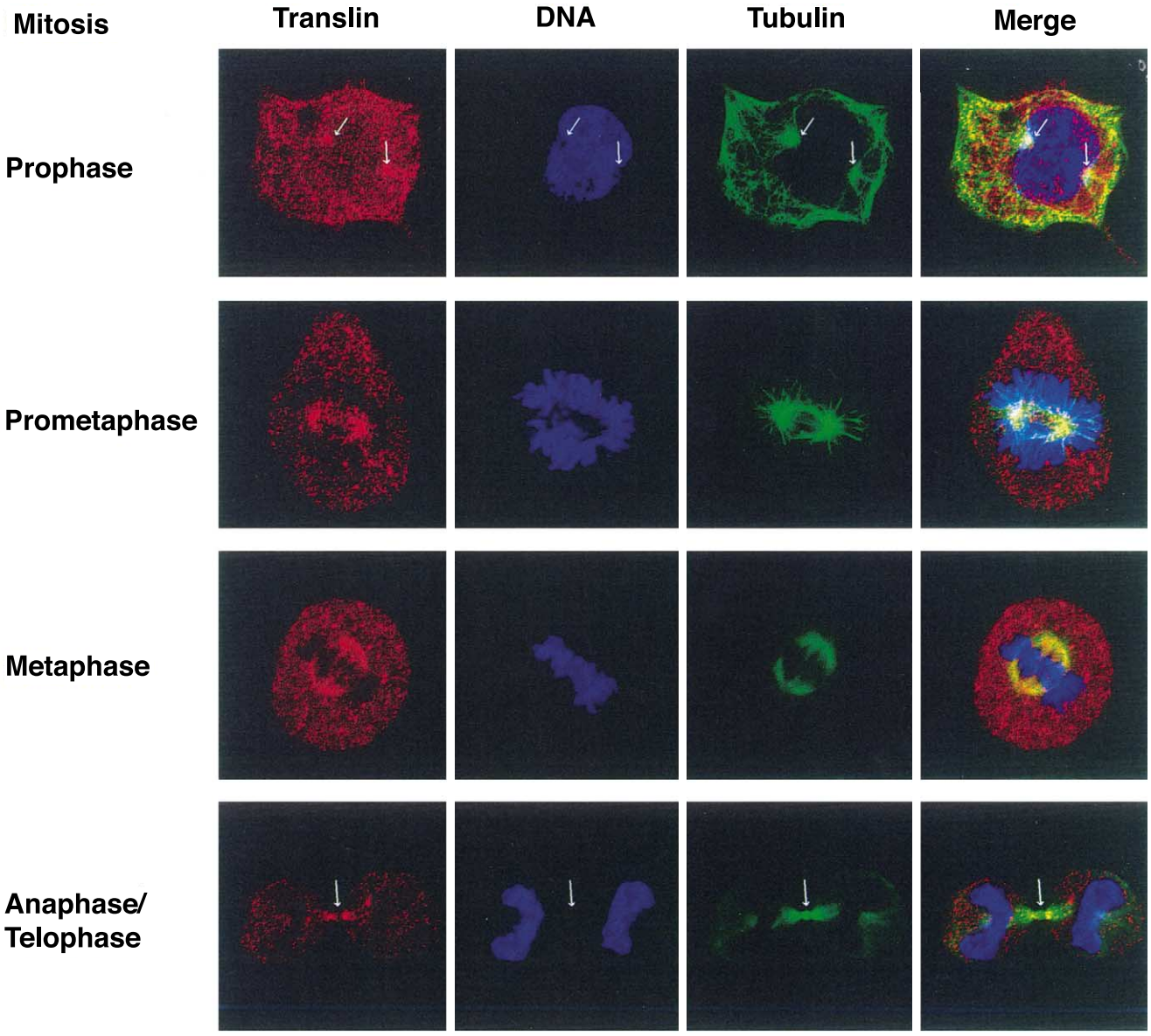


Fig. 5. Translin localizes to the centrosome, bipolar mitotic spindles and midzone. HeLa cells at prophase, prometaphase, metaphase and anaphase/telophase were stained with Hoechst 33258 for the nucleus (blue), FITC anti-α-tubulin (green), and anti-Translin followed by goat anti-rabbit IgG-TRITC (red). Localization of tubulin and Translin was examined by confocal laser scanning microscopy as described previously [5].

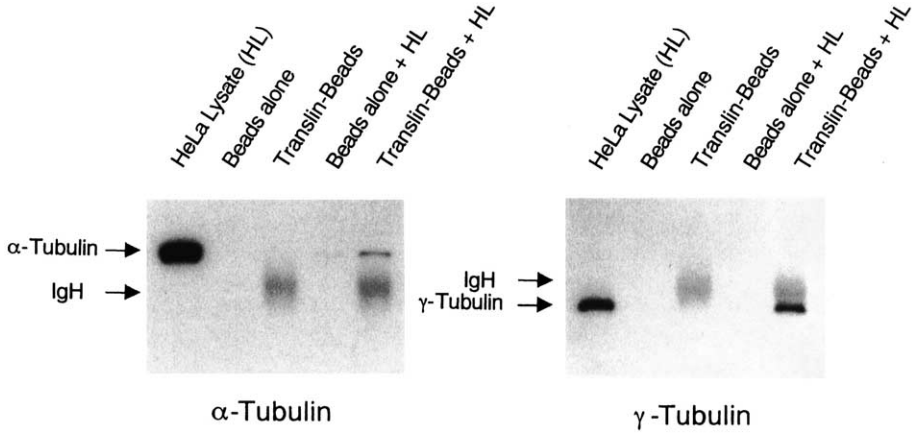


Fig. 6. Translin is associated with microtubules of the mitotic spindle. HeLa cell extracts were incubated with recombinant Translin-conjugated Sepharose 4B. The beads were washed extensively and boiled in SDS sample buffered and after centrifugation, the supernatant was analyzed for the presence of α-tubulin and γ-tubulin by immunoblotting.

results suggest that Translin protein per se is indeed associated with cell division machinery.

3.3. Expression of Translin is periodic during the cell cycle and initiates DNA synthesis

Our observations led us to ask whether Translin levels vary during the cell cycle. FACS analysis of the Tra/293C cells revealed expression to be periodic, with protein synthesis starting in S phase and becoming maximal during the G2/M phase (Fig. 3, upper panel). Interestingly, this pattern of periodic expression was maintained in Translin-overexpressing cells, suggesting that Translin protein levels are regulated under strict control during cell cycle progression (Fig. 3, lower panel). Maximal expression during S and mitosis phases is most likely associated with functions in the replication of chromosomal DNA or cell division control.

To address the roles of Translin in DNA metabolism, Tra/293C cells were stimulated with doxycycline and exposed to BrdU. DNA replication was then analyzed by immunofluorescence staining with anti-BrdU antibody. As shown in Fig. 4, Tra/293C cells treated with doxycycline (upper right) incorporated BrdU more efficiently as compared with untreated cells (upper left). Although the detailed mechanisms are obscure, Translin may participate in processes ensuring the replication of DNA as well as the acceleration of cell division.

3.4. Translin localizes to the centrosome, bipolar mitotic spindles and midbodies

To further explore whether Translin is involved in chromosome segregation and cell division, we studied its behavior in different stages of mitosis. Confocal microscopic analysis showed a dispersed, but punctate distribution in the cytosol, and in prophase Translin immunoreactivity was detected on spindle poles (centrosomes) (Fig. 5, top row). This result is in line with the recent observation that, in *Xenopus* oocytes, some Translin is localized in centrosomes [18]. When cells entered mitosis, the astral microtubules, which radiate in all directions from the centrosomes, were sharply stained in prometaphase/metaphase (Fig. 5, second and third rows). Then, intense fluorescent staining was transiently seen in the spindle midbodies during anaphase/telophase (Fig. 5, bottom row). All of these results suggest that significant roles of Translin in cell division can be ascribed to acceleration of microtubule organization and chromosome segregation during mitosis.

3.5. Translin associates with microtubules of the mitotic spindle

Localization to centrosomes, mitotic spindles and the midbody raised a possibility that Translin is associated with microtubules. Translin-conjugated beads were therefore mixed with HeLa cell extracts and Translin-associated molecules were detected by immunoblotting. In this co-sedimentation experiment, Translin was found to be associated with γ-tubulin and less markedly with α-tubulin (Fig. 6), in agreement with the presence of γ-tubulin in the centrosome, the spindle poles and the microtubule bundles of the midbody during mitosis [19]. Location of Translin at midbody extremities as shown in Fig. 5 also coincides with the behavior of γ-tubulin. Thus these results suggest that Translin is functionally important for cell division through interaction with the mitotic apparatus.

Our results have shown that Translin and Survivin, a member of the inhibitor of apoptosis (IAP) family [20], share many similarities, including cell cycle-dependent expression during G2/M, localization to mitotic spindle microtubules during metaphase and shift to midbodies in late telophase. The available information suggests that Translin acts together with Survivin or other chromosomal passenger proteins to effect chromosome separation, spindle midzone formation, and cytokinesis.

Acknowledgements: We are grateful to Y. Gu for valuable suggestions and for providing Atm mice. This work was supported by Human Science Research Funds, awarded to M.K.

References

[1] Hartwell, L. (1971) J. Mol. Biol. 59, 183–194.
[2] Nurse, P. (1997) Cell 91, 865–867.
[3] Ross, R. (1993) Nature 362, 801–809.
[4] Sherr, C.J. (1996) Science 274, 1672–1677.
[5] Aoki, K., Suzuki, K., Sugano, T., Tasaka, T., Nakahara, K., Kuge, O., Omori, A. and Kasai, M. (1995) Nature Genet. 10, 167–174.
[6] Wu, X., Gu, W., Meng, X. and Hecht, N. (1997) Proc. Natl. Acad. Sci. USA 94, 5640–5645.
[7] Muramatsu, T., Ohmae, A. and Anzai, K. (1998) Biochem. Biophys. Res. Commun. 247, 7–11.

[8] Kasai, M., Matsuzaki, T., Katayanagi, K., Omori, A., Maziarz, R.T., Strominger, J.L., Aoki, K. and Suzuki, K. (1997) J. Biol. Chem. 272, 11402–11407.
[9] Aoki, K., Ishida, R. and Kasai, M. (1999) FEBS Lett. 443, 363–366.
[10] VanLoock, M., Yu, X., Kasai, M. and Egelman, E. (2001) J. Struct. Biol. 135, 58–66.
[11] Stasiak, A.Z., Larquet, E., Stasiak, A., Muller, S., Engel, A., Van Dyck, E., West, S.C. and Egelman, E.H. (2000) Curr. Biol. 10, 337–340.
[12] Erdemir, T., Bilican, B., Oncel, D., Goding, C.R. and Yavuzer, U. (2002) J. Cell Sci. 115, 207–216.
[13] Savitsky, K., Bar-Shira, A., Gilad, S., Rotman, G., Ziv, Y., Vanagaite, L., Tagle, D.A., Smith, S., Uziel, T. and Sfez, S. (1995) Science 268, 1749–1753.
[14] Swift, M., Morrell, D., Massey, R. and Chase, C. (1991) New Engl. J. Med. 325, 1831–1836.
[15] Thacker, J. (1994) Int. J. Radiat. Biol. 66, S87–S96.
[16] Borghesani, P.R., Alt, F.W., Bottaro, A., Davidson, L., Aksoy, S., Rathbun, G.A., Roberts, T.M., Swat, W., Segal, R.A. and Gu, Y. (2000) Proc. Natl. Acad. Sci. USA 97, 3336–3341.
[17] Gossen, M. and Bujard, H. (1992) Proc. Natl. Acad. Sci. USA 89, 5547–5551.
[18] Castro, A., Peter, M., Magnaghi-Jaulin, L., Vigneron, S., Loyaux, D., Lorca, T. and Labbe, J.C. (2000) Biochem. Biophys. Res. Commun. 276, 515–523.
[19] Julian, M., Tollon, Y., Lajoie-Mazenc, I., Moisand, A., Mazarguil, H., Puget, A. and Wright, M. (1993) J. Cell Sci. 105, 145–156.
[20] Li, F., Ambrosini, G., Chu, E.Y., Plescia, J., Tognin, S., Marchisio, P.C. and Altieri, D.C. (1998) Nature 396, 580–584.