Electronic Journal of Biotechnology 32 (2018) 1–5

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research article

Development and characterization of polyclonal antibodies against the linker region of the telomere-binding protein TRF2

Nadya V. Ilicheva [a,*], Aleksandra O. Travina [b], Alexey P. Voronin [a,c], Olga I. Podgornaya [a,c,d]

[a] Institute of Cytology RAS, Tichoretsky pr. 4, 194064 Saint Petersburg, Russia
[b] Saint Petersburg State Institute of Technology, Moskovsky pr. 26, 190013 Saint Petersburg, Russia
[c] Saint Petersburg State University, Universitetskaja nab. 7/9, 199034 Saint Petersburg, Russia
[d] Far Eastern Federal University, Sukhanova 8, 690091 Vladivostok, Russia

ARTICLE INFO

Article history:
Received 14 August 2017
Accepted 1 December 2017
Available online 7 December 2017

Keywords:
Chromosomes
Molecular cloning
Nuclear lamina
Nucleoprotein complexes
Polyclonal antibodies
Recombinant polypeptide
Shelterin
Telomere-binding protein TRF2
Telomeres
Telomeric DNA
TTAGGG repeats

ABSTRACT

Background: TRF2 (telomeric repeat binding factor 2) is an essential component of the telomere-binding protein complex shelterin. TRF2 induces the formation of a special structure of telomeric DNA and counteracts activation of DNA damage-response pathways telomeres. TRF2 has a poorly characterized linker region (udTRF2) between its homodimerization and DNA-binding domains. Some lines of evidence have shown that this region could be involved in TRF2 interaction with nuclear lamina.
Results: In this study, the fragment of the *TERF2* gene encoding udTRF2 domain of telomere-binding protein TRF2 was produced by PCR and cloned into the pET32a vector. The resulting plasmid pET32a-udTRF2 was used for the expression of the recombinant udTRF2 in *E. coli* RosettaBlue (DE3). The protein was isolated and purified using ammonium sulfate precipitation followed by ion-exchange chromatography. The purified recombinant protein udTRF2 was injected into guinea pigs to generate polyclonal antibodies. The ability of anti-udTRF2 antibodies to bind endogenous TRF2 in human skin fibroblasts was tested by western blotting and immunofluorescent staining.
Conclusions: In this study, the recombinant protein udTRF2 and antibodies to it were generated. Both protein and antibodies will provide a useful tool for investigation of the functions of the udTRF2 domain and its role in the interaction between TRF2 and nuclear lamina.

1. Introduction

Telomeres are nucleoprotein complexes at the ends of eukaryotic chromosomes. They protect chromosomes from degradation and prevent activation of reparative systems of the cell, which can recognize chromosome ends as double-stranded breaks. Telomeres' role in cancerogenesis and aging has been the object of numerous studies. Mammalian telomeres consist of TTAGGG repeats. The G-rich strand of telomere contains a single-stranded overhang on its 3′-end. This overhang invades the double-stranded telomeric DNA, base pairing with the C-strand and displacing the G-strand. The strand invasion results in the formation of a large duplex lariat structure named the t-loop. Telomeric DNA is bound with a specific protein complex called shelterin, which is composed of the following six subunits: TRF1, TRF2, POT1, TIN2, TPP1 and Rap1 [1]. Telomere-binding protein TRF2 binds directly to the telomeric DNA. TRF2 is required for the t-loop formation and protects telomeres from non-homologous end joining (NHEJ) and ATM-kinase pathway [2]. TRF2 contains a DNA-binding Myb/SANT domain, homodimerization domain (TRF homology [TRFH]), a basic N-terminal domain (B domain or GAR domain) and a poorly characterized linker region, which is named udTRF2 in this study (Fig. 1a). Computer analysis of TRF2 sequence has shown that this domain has a limited similarity to several sequences of rod domains of intermediate filaments, including nuclear lamins [3]. Lamins are the components of the nuclear envelope, so the domain of TRF2 with unknown functions is likely to be responsible for the interaction of telomeres with the nuclear envelope. Investigation of this interaction may shed light on the processes of aging because a mutation in the *LMNA* gene encoding lamin A leads to Hutchinson–Gilford progeria syndrome (HGPS), which is characterized by premature aging, abnormalities of nuclear membrane and telomeres shortening [4,5].

In the current work, the recombinant protein corresponding to udTRF2 was obtained, and polyclonal antibodies against it were raised in guinea pigs.

* Corresponding author.
E-mail address: nad9009@yandex.ru (N.V. Ilicheva).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2017.12.001

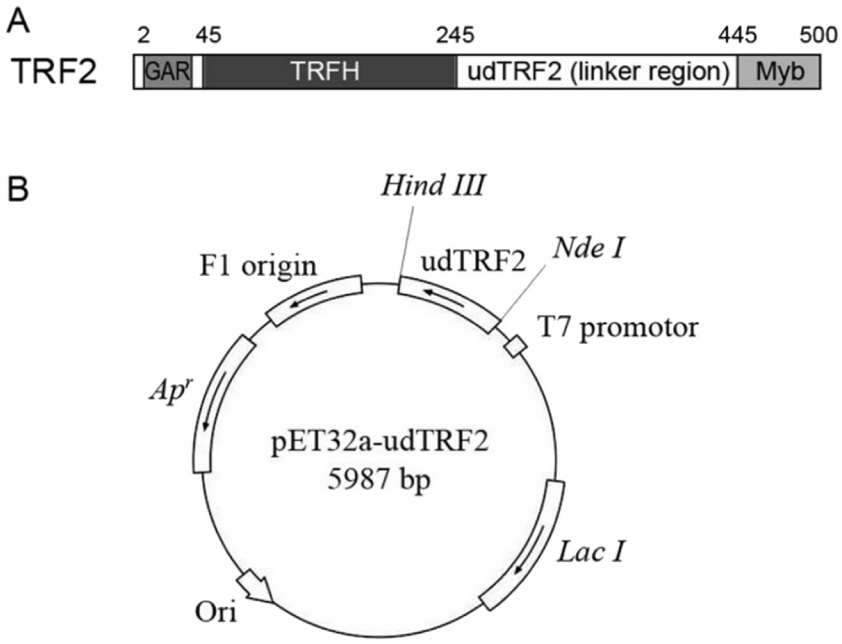

Fig. 1. Cloning of the udTRF2 domain (linker region) of the telomere-binding protein TRF2. A. TRF2 domain structure. GAR–N-terminal glycine-arginine rich domain; TRFH–homodimerization domain; Myb–C-terminal DNA-binding Myb-domain. B. pET32a-udTRF2 vector map.

2. Materials and methods

2.1. Plasmid construction

The fragment of the *TERF2* gene (GenBank accession no. **NM_005652.4**) encoding the udTRF2 domain of telomere-binding protein TRF2 was produced by PCR. The plasmid vector pCDNA3hTRF2Full containing the full-length human *TERF2* gene was used as a matrix for PCR. The plasmid was obtained from Dr. Eric Gilson (Department of Medical Genetics, CHU Nice, France). The following primers were used in PCR: TRF2udNDE (gccatatgtctgagtccgctgcctcaa) and TRF2udHind (gcaagcttttattttgttatattggttgtactgtct). The forward primer, TRF2udNDE, contained a restriction site for *Nde* I restrictase and start-codon ATG. The reverse primer, TRF2udHind, contained a restriction site for *Hind* III restrictase and stop-codon TAA. The PCR-product was cloned into the cloning vector pTZ57R/T (Thermoscientific). The plasmid was named pTZ57R-udTRF2. The cloned fragment was excised from the plasmid using restriction endonuclease *FauND* I, which is an isoschizomer of *Nde* I, and *Hind* III and ligated into the pET32a vector, which was digested with the same enzymes. The resulting plasmid was named pET32a-udTRF2 and its sequence was verified by sequencing on the ABI Prism 3100 Genetic Analyzer (Applied Biosystems) using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The plasmid was transformed into the expression strain *E. coli* RosettaBlue (DE3) (Novagen).

2.2. Protein expression and purification

The transformed bacteria *E. coli* RosettaBlue (DE3)-pET32a-udTRF2 were cultivated on the LB medium supplemented with 50 mg/l ampicillin at 37°C until the optical density of the culture at 500 nm reached 0.5. Expression was induced by adding 0.4 mM isopropyl-β-D-thiogalactoside (IPTG) (Fermentas), and cells were cultivated during 2.5 h at 37°C. The control culture was cultivated at the same conditions without IPTG. The bacteria were harvested by centrifugation at 3000*g* for 10 min. The cells were washed with 1× PBS and resuspended in the lysis buffer (20 mM Tris–HCl pH 7.5, 1% Triton X-100, 1 mM EDTA, 0.5 mM PMSF) and disrupted by three freeze–thaw cycles. The lysate was separated into soluble and insoluble fractions by centrifugation at 14000*g* for 10 min to determine whether the protein is expressed in a soluble state. All samples were analyzed using SDS-PAGE. The recombinant protein udTRF2 was extracted from the soluble fraction using the combination of ammonium sulfate precipitation and ion-exchange chromatography. Solid $(NH_4)_2SO_4$ was added to the precooled (0°C) bacterial lysate until the desired saturation (10%, 20%, 30%, 40%, 50%, 60%, 70%) was reached. The precipitated proteins were collected using centrifugation at 14000*g* for 10 min, and the next portion of $(NH_4)_2SO_4$ was added to the supernatant. On the next step, the proper ion-exchange chromatography conditions were determined. One milliliter of Q-sepharose (Amersham Biosciences) was loaded onto the chromatography column and equilibrated with the lysis buffer, and 1 ml of the lysate-soluble fraction was added to the column. The column was washed with three column volumes of the lysis buffer, and the protein fractions were eluted by a step-wise NaCl gradient (0.2–0.75 M) with the step of 0.05 M. When the proper conditions of precipitation and chromatography were chosen, the protein fraction precipitated between 30% and 40% ammonium sulfate saturation was diluted in 1 ml of the lysis buffer, loaded onto the column with Q-sepharose and udTRF2 was eluted by 0.25 M NaCl.

2.3. Polyclonal antibodies production

Two four-month-old male guinea pigs weighing 400–500 g (purchased from Rappolovo nursery, Leningrad region, Russia) were used for immunization. All animal procedures were performed according to the EU Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes. Pre-immunization sera were taken one week before immunization and tested by western blot. The animals were immunized with 20 μg of udTRF2 mixed with the Freund adjuvant three times with 10-day intervals. Injections were performed subcutaneously (first injection) and intramuscularly (second and third injections). The blood samples were taken 10 d after the last injection. The immune antiserum was obtained by centrifugation at 10,000*g* for 10 min.

2.4. Western blotting

Lysates of induced and control bacterial cultures and human skin fibroblast lysate were separated by 12% SDS-PAGE and transferred to the PVDF membrane (Millipore, Hertfordshire, UK) by Electroblot (BioRad Lab Ltd., Hertfordshire, UK) with the current density 1 mA × sm^2 for 1 h in an electrophoresis buffer containing 10% ethanol. After transfer, the membrane was incubated in a blocking solution (5% skimmed milk, 0.05% Tween 20, 1× PBS). The first antibodies were anti-TRF2 (ab4182, Abcam, dilution 1:500), preimmune serum (dilution 1:500) and immune serum anti-udTRF2 (dilutions 1:500, 1:1000, 1:2000). The blots were incubated with antibodies diluted in 1× PBS for 1 h RT, washed with PBS 3 × 10 min and incubated in the second antibodies (anti-guinea pig or anti-rabbit IgG (whole molecule)-alkaline phosphatase antibody produced in goat (Sigma), dilution 1:10,000). The blots were detected using BCIP-NBT in an alkaline-phosphatase buffer (50 mM Tris–HCl (pH 9.5), 5 mM $MgCl_2$, 100 mM NaCl) for around 30 min.

2.5. Immunocytochemistry

Human skin fibroblasts were obtained from the Russian Cell Culture Collection (Institute of Cytology RAS, St. Petersburg). The cells were cultivated on glass coverslips in Petri dishes in DMEM supplemented with glutamine, glucose, 10% fetal calf serum and antibiotics. Cells were washed with PBS and fixed in 2% paraformaldehyde with 0.1% Triton X-100 for 20 min at 4°C. After washing with PBS, cells were incubated in the blocking solution (3% BSA in 1× PBS) for 1 h and in antibodies solution (anti-udTRF2 and anti-TRF2 (Abcam), dilutions 1:100). The second antibodies were Alexa-488 goat anti-guinea pig IgG antibody (Molecular Probes). The preparations were mounted in Antifade Gold with DAPI (Molecular Probes) and analyzed with an LSM 5 PASCAL confocal laser-scanning microscope (Carl Zeiss) equipped with argon (488 nm) and helium–neon (543 nm) laser sets.

3. Results

3.1. Production and purification of recombinant udTRF2

Fragments of the *TERF2* gene encoding the linker region of telomeric protein TRF2 (udTRF2-domain) were amplified by PCR. This fragment encodes amino-acid residues from 245 to 445 situated between the homodimerization domain (TRFH) and C-end DNA-binding domain (Myb-domain) (Fig. 1a). Initially, we digested the PCR fragment by restrictases and tried to insert the fragment directly into the expression vector, but we failed to obtain the desired clones. Therefore, the PCR fragment was cloned into the cloning vector pTZ57R/T and then subcloned into the expression vector pET32a. This vector contains tags (two His-tags, Trx-tag and S-tag) which may interfere with the peptide of interest during the production of polyclonal antibodies. To eliminate nucleotide sequences encoding these tags, we used restriction endonuclease *FauND I*, which has two restriction sites in pET32a flanking the tag sequences. The resulting plasmid pET32a-udTRF2 (Fig. 1b) was transformed into *E. coli* RosettaBlue (DE3) competent cells.

Protein expression was carried out as described in Materials and methods. After 2.5 h of cultivation, the optical density OD_{500} of the induced culture was half that of the control culture (0.75 vs. 1.5), which meant good expression level. SDS-PAGE analysis of bacterial lysates revealed a major protein band in the induced culture that was not present in the control culture.

The apparent molecular weight of the protein determined by SDS-PAGE was found to be 25 kDa (Fig. 2), which approximately corresponds to the calculated molecular weight of udTRF2. To investigate whether the recombinant protein is expressed in the soluble or insoluble state, the supernatant and the pellet obtained after lysis were analyzed by SDS-PAGE. The analysis showed that the protein is mostly expressed in a soluble form and only a small part of it accumulates in inclusion bodies (Fig. 2).

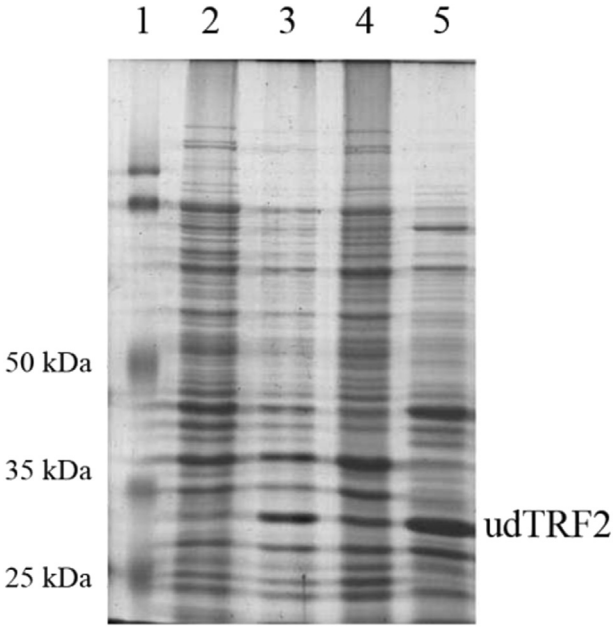

Fig. 2. 10% SDS-PAGE analysis of the recombinant udTRF2 expression in *E. coli* RosettaBlue (DE3)-pET32a-udTRF2. Lane 1–protein ladder; lane 2–control bacteria lysate (RosettaBlue (DE3)-pET32a-udTRF2 cultivated without adding IPTG); lane 3–induced bacteria lysate. Apparent molecular weight of the recombinant protein is about 25 kDa; lane 4–insoluble fraction of the induced bacteria lysate; lane 5–soluble fraction of the induced bacteria lysate. The protein is expressed mainly in the soluble form.

The recombinant protein udTRF2 was purified from the soluble fraction of bacterial lysate using ammonium sulfate precipitation followed by ion-exchange chromatography (Fig. 3). The purity of the recombinant protein determined by gel analysis in ImageJ was about 95%.

3.2. Generation and characterization of antibodies

The purified recombinant protein udTRF2 was injected into guinea pigs to produce polyclonal antibodies. The immune sera were tested by western blotting as described in Materials and methods. Commercial rabbit polyclonal antibodies against TRF2 (ab4082, Abcam) were used as a control. These antibodies recognize an

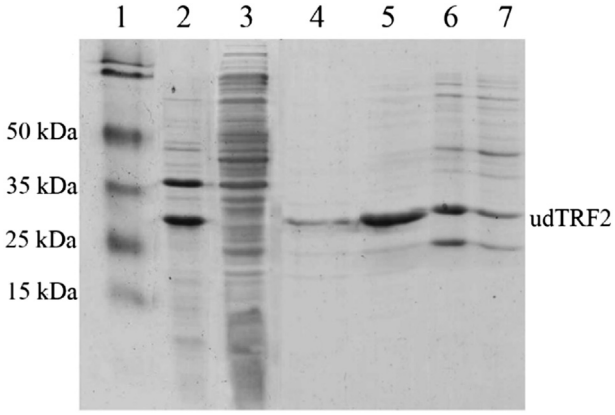

Fig. 3. Purification of the recombinant udTRF2 using ion-exchange chromatography (14% SDS-PAGE). Lane 1–protein ladder; lane 2–control bacteria lysate; lane 3–induced bacteria lysate; lanes 4–7–proteins of the fraction precipitated between 30 and 40% SA saturation eluted with 0.2 M, 0.25 M, 0.3 M and 0.35 M NaCl, respectively.

amino-acid sequence within TRF2 that partly overlaps udTRF2. Anti-udTRF2 antiserum appeared to reveal two proteins with apparent molecular weights of 25 kDa and 30 kDa in the induced bacterial culture lysate and one protein of 30 kDa in the control bacterial lysate. Commercial antibodies revealed one 25-kDa protein in the induced bacterial culture lysate (Fig. 4). Hence, the 25-kDa protein is udTRF2. The immune serum also recognized a 70-kDa protein in the human skin fibroblast lysate. The apparent molecular weight of this protein corresponds to the documented molecular weight of TRF2 [6].

The ability of anti-udTRF2 antibodies to bind endogenous TRF2 in human skin fibroblasts was tested by immunofluorescent staining (Fig. 5). Preimmune serum was used as a negative control (data not shown). All experiments were performed in triplicate and repeated at least twice. It was shown that the immune serum could reveal native TRF2 in normal human cells.

4. Discussion

In the current work, we obtained the recombinant protein udTRF2, which represents the still uncharacterized linker region (udTRF2) of the telomere-binding protein TRF2 and used it to generate domain-specific polyclonal antibodies. The antibodies were shown to recognize udTRF2 in the induced bacteria lysate on western blot and the wild-type TRF2 in human cells both on western blot and immunostained preparations.

The telomere-binding proteins TRF1 and TRF2 are far homologs; they bind double-stranded TTAGGG telomeric repeats and have similar domain structure, but they significantly differ by their N-terminal domains and linker regions located between the homodimerization and DNA-binding domains. The TRF2 linker region (udTRF2) differs from that of TRF1 both in amino-acid sequence and length, the TRF2 linker region being nearly 100 amino acids longer. This region contains Rap-binding motif (RBM) [7], TIN2-binding motif (TBM) [8] and a potential nuclear localization signal, but the majority of this region is still uncharacterized. Some lines of evidence have shown that this region could be involved in TRF2 interaction with nuclear lamina.

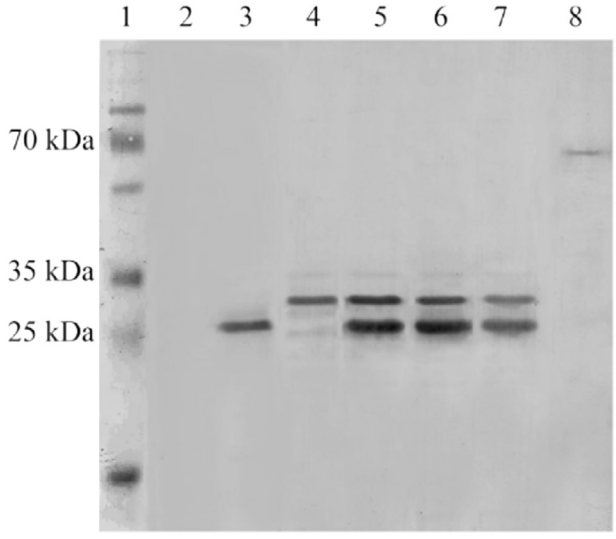

Fig. 4. Western blotting (WB) analysis of the bacterial lysates and human skin fibroblast lysate with antibodies to TRF2 (Abcam) and anti-udTRF2. 1–protein ladder; 2,3–WB analysis of the lysates of control (uninduced) and induced bacteria with commercial antibodies to TRF2 (Abcam) (dilution 1:1000). The antibodies reveal a 25-kDa protein corresponding to udTRF2 in the induced bacteria lysate; 4–WB analysis of the control bacterial cell lysate with anti-udTRF2 antibodies (dilution 1:1000); 5,6,7–WB analysis of the induced bacterial cell lysate stained with anti-udTRF2 antibodies (dilutions 1:500, 1:1000, 1:2000). Anti-udTRF2 antiserum reveals two proteins with apparent molecular weights of 25 kDa (udTRF2) and 30 kDa in the induced bacterial culture lysate and one protein of 30 kDa in the control bacterial lysate; 8–Anti-udTRF2 antiserum reveals TRF2 in the human skin fibroblast lysate.

In frog oocytes, protein MTBP/TRF2 appeared to be tightly bound to the nuclear envelope [9]. Antibodies generated against a complex of frog MTBP/TRF2 with telomeric DNA displayed cross-reactivity with keratins, which belong to intermediate filament proteins, as well as nuclear lamins. In further studies, it was found that in mouse embryonic fibroblasts, TRF2 co-localized with lamin B in the nuclear envelope remnants during mitosis [3]. Computer analysis of the TRF2 sequence has shown that the linker region has a limited similarity to several sequences of rod domains of human neurofilament and rat lamin A. This similarity with the rod domain may provide a mechanism for the telomere association with lamins. It has been shown that TRF2 can oligomerize in the presence of telomeric DNA, and this oligomerization is driven by the linker region [10]. Because the rod domain participates in lamin dimerization and network formation [11], oligomerization of TRF2 may be partly specified by sequences resembling the rod domain. Wood et al. [12] have shown that the wild-type TRF2 can interact directly with lamin A, whereas TRF2ΔBΔM, a mutant allele lacking the DNA-binding domain of TRF2, cannot. As TRF2 binding to telomeric DNA is indispensable for oligomerization, it is possible that TRF2 oligomerization plays some role in the interaction between lamin A and TRF2.

The interaction between nuclear lamina and telomeres is of great interest in the light of genetic disorders called progerias. Some of these diseases, such as HGPS [13] and atypical Werner syndrome (AWS) [14], are caused by mutations in the *LMNA* gene and characterized by premature aging phenotype and nuclear envelope abnormalities. In HGPS, a mutation in the *LMNA* gene leads to the production of progerin, the mutant form of lamin A. It is noteworthy that progerin is expressed in small amounts in healthy individuals [15]. HGPS patients' cells exhibit altered nuclear morphology (blebbings), loss of peripheral heterochromatin and premature senescence [4]. Furthermore, fibroblasts isolated from patients with HGPS exhibit reduced replicative capacity and rapid telomere attrition in comparison with age-matched controls [5]. Moreover, there seems to be a reciprocal relationship between progerin production and altered telomere biology. For example, induction of telomere dysfunction by expression of a dominant negative TRF2 protein (TRF2ΔBΔM) leads to elevated progerin production. Replicative senescence of normal human fibroblasts is also accompanied by an increased level of progerin mRNA, while this effect is not observed during telomere-independent senescence [16].

One of the mechanisms by which lamin A can affect telomere biology is the formation of interstitial telomeric loops (ITLs) when telomeric DNA associates with interstitial telomeric sequences (ITSs) found within nontelomeric DNA [17]. It was shown that interaction between TRF2 and lamin A facilitates ITLs' formation. In contrast with lamin A, progerin does not interact with TRF2, and HGPS patients' fibroblasts exhibit a reduced number of ITLs [12]. Thus, further investigations of interaction between TRF2 and lamin A may provide new insights into the molecular mechanisms of aging.

In this study, we generated the recombinant protein udTRF2 and antibodies to it. Both protein and antibodies will provide a useful tool for investigation of the udTRF2 domain role in the interaction between TRF2 and nuclear lamina. For example, co-immunoprecipitation experiments with lamin A will show whether udTRF2 is responsible for the interaction between TRF2 and lamin A. The products obtained in this work will be used to uncover some other possible functions of the udTRF2 domain. In our future investigations, we are planning to test the anti-udTRF2 antibodies by immunoprecipitation and perform co-immunoprecipitation experiments of recombinant udTRF2 with cellular lysate, nuclear lysate and nuclear lamina extract to find proteins that specifically bind udTRF2. The proteins bound to the peptide could be identified using different approaches, such as MALDI and western blot. The antibodies can also be used in a variety

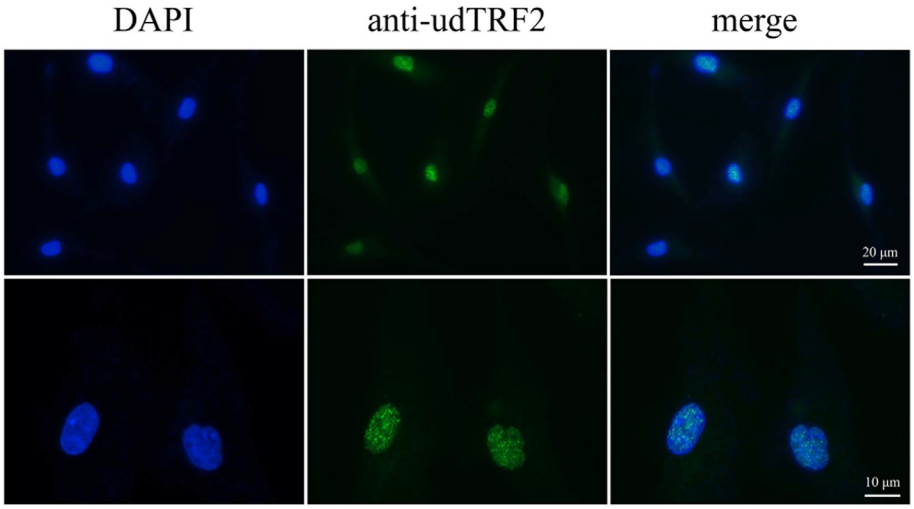

Fig. 5. Immunofluorescent staining of human skin fibroblast cell culture with anti-udTRF2 antibodies. TRF2 is revealed in the nucleus as expected.

of studies to visualize native TRF2 in cells by immunostaining and western blot.

Ethical approval

All animal procedures were performed according to the EU Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes.

Conflict of interests

The authors declare that they do not have potential conflict of interests.

Financial support

This work was supported by the RFBR (grant nos. 15-04-01857 and 16-34-00714), RSF no. 15-15-20026 (animals purchasing) and the granting program "Molecular and cell biology" of the Russian Academy of Sciences.

Acknowledgments

We acknowledge Dr. E. Gilson for providing the plasmid pCDNA3hTRF2Full, Dr. L.S. Adonin for help with animal procedures and Dr. A.M. Kiselev for help with sequencing.

References

[1] Erdel F, Kratz K, Willcox S, et al. Telomere recognition and assembly mechanism of mammalian Shelterin. Cell Rep 2017;18(1):41–53. https://doi.org/10.1016/j.celrep.2016.12.005.

[2] Ilicheva NV, Podgornaya OI, Voronin AP. Chapter three - telomere repeat factor 2 (TRF2) is responsible for the telomere attachment to the nuclear membrane. Adv Protein Chem Struct Biol 2015;101:67–96. https://doi.org/10.1016/bs.apcsb.2015.06.009.

[3] Voronin AP, Lobov IB, Gilson E, et al. A telomere-binding protein (TRF2/MTBP) from mouse nuclear envelopes with motives of an intermediate filament type rod-domain. J Anti Aging Med 2004;6(3):205–18. https://doi.org/10.1089/109454503322733054.

[4] Goldman RD, Shumaker DK, Erdos MR, et al. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. Proc Natl Acad Sci U S A 2004;101(24):8963–8. https://doi.org/10.1073/pnas.0402943101.

[5] Decker ML, Chavez E, Vulto I, et al. Telomere length in Hutchinson-Gilford progeria syndrome. Mech Ageing Dev 2009;130(6):377–83. https://doi.org/10.1016/j.mad.2009.03.001.

[6] Bilaud T, Brun C, Ancelin K, et al. Telomeric localization of TRF2, a novel human telobox protein. Nat Genet 1997;17(2):236–9. https://doi.org/10.1038/ng1097-236.

[7] Chen Y, Rai R, Zhou ZR, et al. A conserved motif within RAP1 has diversified roles in telomere protection and regulation in different organisms. Nat Struct Mol Biol 2011; 18(2):213–21. https://doi.org/10.1038/nsmb.1974.

[8] Chen Y, Yang Y, van Overbeek M, et al. A shared docking motif in TRF1 and TRF2 used for differential recruitment of telomeric proteins. Science 2008;319(5866): 1092–6. https://doi.org/10.1126/science.1151804.

[9] Podgornaya OI, Bugaeva EA, Voronin AP, et al. Nuclear envelope associated protein that binds telomeric DNAs. Mol Reprod Dev 2000;57(1):16–25. https://doi.org/10.1002/1098-2795(200009)57:1<16::AID-MRD4>3.0.CO;2-8.

[10] Khan SJ, Yanez G, Seldeen K, et al. Interactions of TRF2 with model telomeric ends. Biochem Biophys Res Commun 2007;363(1):44–50. https://doi.org/10.1016/j.bbrc.2007.08.122.

[11] Dechat T, Pfleghaar K, Sengupta K, et al. Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. Genes Dev 2008;22: 832–53. https://doi.org/10.1101/gad.1652708.

[12] Wood AM, Rendtlew Danielsen JM, Lucas CA, et al. TRF2 and lamin A/C interact to facilitate the functional organization of chromosome ends. Nat Commun 2014;5: 5467. https://doi.org/10.1038/ncomms6467.

[13] Eriksson M, Brown WT, Gordon LB, et al. Recurrent *de novo* point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature 2003;423(6937):293–8. https://doi.org/10.1038/nature01629.

[14] Chen L, Lee L, Kudlow BA, et al. LMNA mutations in atypical Werner's syndrome. Lancet 2003;362(9382):440–5. https://doi.org/10.1016/S0140-6736(03)14069-X.

[15] Scaffidi P, Misteli T. Lamin A-dependent nuclear defects in human aging. Science 2006;312(5776):1059–63. https://doi.org/10.1126/science.1127168.

[16] Cao K, Blair CD, Faddah DA, et al. Progerin and telomere dysfunction collaborate to trigger cellular senescence in normal human fibroblasts. J Clin Invest 2011;121(7): 2833–44. https://doi.org/10.1172/JCI43578.

[17] Wood AM, Laster K, Rice EL, et al. A beginning of the end: new insights into the functional organization of telomeres. Nucleus 2015;6:172–8. https://doi.org/10.1080/19491034.2015.104840.