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The splicing factor U2AF65 stabilizes TRF1 protein by inhibiting its ubiquitin-dependent proteolysis

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ABSTRACT

The human telomeric protein TRF1 is a component of the six-subunit protein complex shelterin, which provides telomere protection by organizing the telomere into a high-order structure. TRF1 functions as a negative regulator of telomere length by controlling the access of telomerase to telomeres. Thus, the cellular abundance of TRF1 at telomeres should be maintained and tightly regulated to ensure proper telomere function. Here, we identify U2 small nuclear ribonucleoprotein (snRNP) auxiliary factor 65 (U2AF65), an essential pre-mRNA splicing factor, as a novel TRF1-interacting protein. U2AF65 interacts with TRF1 *in vitro* and *in vivo* and is capable of stabilizing TRF1 protein by inhibiting its ubiquitin-dependent proteolysis. We also found that U2AF65 interferes with the interaction between TRF1 and Fbx4, an E3 ubiquitin ligase for TRF1. Depletion of endogenous U2AF65 expression by short interfering RNA (siRNA) reduced the stability of endogenous TRF1 whereas overexpression of U2AF65 significantly extended the half-life of TRF1. These findings demonstrate that U2AF65 plays a critical role in regulating the level of TRF1 through physical interaction and ubiquitin-mediated proteolysis. Hence, U2AF65 represents a new route for modulating TRF1 function at telomeres.

1. Introduction

Telomeres are protective nucleoprotein complexes found at the ends of linear eukaryotic chromosomes, and their deregulation has been implicated in aging and cancer [1]. Mammalian telomeric DNA consists of double-stranded TTAGGG tandem repeats as well as a single-stranded G-rich overhang and can form a higher order structure such as a t-loop [2]. The t-loop structure has been suggested to mask chromosome ends from being recognized as double-strand breaks [3]. Telomeric DNA is tightly associated with the six-subunit protein complex named shelterin, which consists of TRF1, TRF2, POT1, TIN2, TPP1 and Rap1 [4,5]. TRF1 and TRF2 are double-stranded telomeric DNA binding proteins whereas POT1 binds to the single-stranded G-rich overhang. Other shelterin components are recruited to telomeres through the interactions with TRF1, TRF2 and POT1 [6,7]. This shelterin complex plays an essential role in maintaining the chromosome integrity. In addition to shelterin complex, mammalian telomeres are associated with a number of accessory factors that are distinguished from the shelterin core components [8]. The accessory factors are involved in DNA metabolism such as DNA repair, DNA damage signaling, and chromatin structure [9–11]. Although these accessory factors play an important role in the maintenance of telomere integrity, the mechanism by which these factors regulate telomere function remains unclear.

TRF1 negatively regulates telomere length by controlling the access of telomerase to telomeres. Overexpression of TRF1 in telomerase-positive cells promotes telomere shortening whereas loss of TRF1 from telomeres has been shown to induce inappropriate telomere elongation [12,13]. Thus, the cellular abundance of TRF1 at telomeres should be maintained and tightly regulated to ensure proper telomere function. The levels of TRF1 at telomeres have been shown to be regulated through the interactions with various proteins. Tankyrase 1 has been shown to interact with and poly(-ADP-ribosyl)ate TRF1, which in turn dissociates TRF1 from telomeres, leading to the increased access of telomerase to telomeres [14,15]. Thus, tankyrase 1 serves as a positive regulator of telomere length. The dissociated telomere-unbound TRF1 is rapidly ubiquitinated by E3 ubiquitin ligases, such as Fbx4 [16] and RLIM [17], and subsequently degraded by the proteasome.

On the other hand, casein kinase 2 (CK2)-mediated phosphorylation is required for the efficient telomere binding of TRF1, suggesting a role of CK2 in determining the level of TRF1 at telomeres [18]. Polo-like kinase 1 (Plk1) has been also shown to interact with and phosphorylate TRF1 [19]. Upon phosphorylation by Plk1, the ability of TRF1 to bind telomeric DNA is dramatically increased, suggesting that Plk1-mediated phosphorylation is involved in its telomeric DNA binding ability. Pin1 has been shown to be an essential regulator of TRF1 stability [20]. Pin1 inhibition

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renders TRF1 resistant to protein degradation, enhancing TRF1 binding to telomeres. Guanine nucleotide-binding protein-like 3 (GNL3L) has also been reported to bind and stabilize TRF1 protein by inhibiting its ubiquitination and binding to Fbx4 [21].

In this study, we identify U2AF65 as a novel TRF1-interacting protein using a yeast two-hybrid screen. U2AF65 is an essential splicing factor that binds to the polypyrimidine tract at the 3′ splice site of the pre-mRNA and can form U2 snRNP auxiliary factor (U2AF) with U2AF35 that binds to the conserved AG dinucleotide at the intron 3′ end [22–24]. We showed that U2AF65 stabilizes TRF1 protein by inhibiting the interaction between TRF1 and Fbx4 and its ubiquitin-dependent degradation. These findings suggest that U2AF65 is a positive regulator of TRF1 protein level and represents a new pathway for modulating TRF1 function at telomeres.

2. Materials and methods

2.1. Cell culture and plasmids

The human embryonic kidney cell line HEK293 and the human cervical carcinoma cell line HeLa were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in 5% CO_2 at 37 °C. The expression vectors were transiently transfected using Lipofectamine-PLUS reagent according to the manufacturer's protocol (Invitrogen). The Myc-U2AF65 and V5-U2AF35 expression vectors were constructed by inserting the full-length U2AF65 and U2AF35 cDNA into pRK5-Myc and pRK5-V5 (Stratagene), respectively. The expression vector for GST-U2AF65 was constructed by cloning the full-length U2AF65 cDNA into pGEX-4T-1 (GE Healthcare).

2.2. GST pulldown, immunoprecipitation, and immunoblotting

For the GST pulldown assay, the cellular supernatants were precleared with glutathione-Sepharose 4B (Amersham Biosciences) and incubated with glutathione-Sepharose beads containing GST fusion proteins. For immunoprecipitation, the supernatants were preincubated with protein A-Sepharose (Amersham Biosciences) and incubated with primary antibodies precoupled with protein A-Sepharose beads. The precipitated proteins were washed extensively and subjected to immunoblotting analysis. Immunoprecipitation and immunoblotting were performed using anti-U2AF65 (Sigma), anti-TRF1 (Sigma), anti-TRF2 (Cell signaling), anti-Flag (Sigma), anti-V5 (Invitrogen), anti-HA (Santa Cruz Biotechnology) and anti-Myc (Santa Cruz Biotechnology) antibodies as specified. Immunoblotting experiments were repeated at least three times and a representative blot is shown.

2.3. In vivo ubiquitination assay

HEK293 cells were transfected with HA-ubiquitin, Flag-TRF1, and Myc-U2AF65 expression vectors, either alone or in combination, followed by MG132 treatment to inhibit proteasome function. Lysates were subjected to immunoprecipitation with anti-Flag antibody followed by immunoblotting analysis with anti-HA antibody to illuminate ubiquitin-modified TRF1.

2.4. RNA interference

The U2AF65-specific and control siRNAs were designed and synthesized by QIAGEN. Two different siRNA duplex sequences specific for U2AF65 were 5′-CAGCAAGUACGGGCUUGUCAATT-3′ for siU2AF65-1 and 5′-ACCCAACUACCUGAACGAUGATT-3′ for siU2AF65-2. The siRNA duplexes were transfected into HEK293 cells using RNAiMax transfection reagent (Invitrogen), according to the manufacturer's instructions. The scrambled sequence (5′-AATCGCATAGCGTATGCCGTT-3′) was used as a control and did not correspond to any known gene in the data bases.

3. Results

3.1. Identification of U2AF65 as a TRF1-interacting partner

To identify TRF1-interacting factors, we screened a HeLa cell cDNA library using a yeast two-hybrid system. With the full-length TRF1 as bait, we obtained a clone containing the cDNA encoding U2AF65 which is an essential pre-mRNA splicing factor during the assembly of the splicing complex [22,23]. Because U2AF65 was identified as a TRF1-interacting partner, we wished to examine the involvement of U2AF65 in telomere function. To confirm the direct interaction between TRF1 and U2AF65, we performed GST pulldown experiments. GST-TRF1, but not the control GST and GST-TRF2, bound to Myc-U2AF65 expressed in HEK293 cells, indicating that U2AF65 interacts with TRF1 *in vitro* (Fig. 1A). To determine whether TRF1 and U2AF65 associate *in vivo*, HEK293 cells were co-transfected with Flag-TRF1 (or Flag-TRF2) and Myc-U2AF65 expression vectors and subject to immunoprecipitation with anti-Flag antibody, followed by immunoblotting with anti-Myc antibody. Myc-U2AF65 was detected in anti-Flag immunoprecipitates when Flag-TRF1 was expressed, but not in anti-Flag immunoprecipitates when Flag-TRF2 was expressed (Fig. 1B). Endogenous TRF1, but not TRF2, was immunoprecipitated by endogenous U2AF65 (Fig. 1C), indicating that U2AF65 interacts with TRF1 in mammalian cells.

U2AF65 has been shown to interact with U2AF35 to form U2 snRNP auxiliary factor [24]. Therefore, we determined whether TRF1 binding to U2AF65 interferes with its ability to interact with U2AF35. HEK293 cells were co-transfected with Flag-TRF1, Myc-U2AF65 and V5-U2AF35 and subjected to immunoprecipitation. Myc-U2AF65, but not V5-U2AF35, was detected in anti-Flag immunoprecipitates when Flag-TRF1 was expressed (Fig. 1D, middle panel). However, V5-U2AF35 was detected in anti-Flag immunoprecipitates when both Flag-TRF1 and Myc-U2AF65 were expressed, suggesting that U2AF65 can interact with both TRF1 and U2AF35. We note that Myc-U2AF65 associates with Flag-TRF1 at a similar level with or without V5-U2AF35 overexpression (Fig. 1D), demonstrating that U2AF65 binding to TRF1 and U2AF35 could not be mutually exclusive. These results were further verified by immunoprecipitation with anti-Myc antibody. Flag-TRF1 was detected in anti-Myc immunoprecipitates only when Myc-U2AF65 was expressed (Fig. 1D, right panel). The amounts of Flag-TRF1 recovered with anti-Myc antibody were not significantly affected by overexpression of V5-U2AF35.

3.2. Identification of the domains in TRF1 and U2AF65 required for their interactions

To map the domain of TRF1 that is important for U2AF65 binding, we generated several deletion constructs of TRF1 and performed a GST pulldown experiment (Fig. 2A). TRF1 fragment containing the C-terminal Myb domain bound efficiently to GST-U2AF65 whereas other domains showed no detectable binding activity (Fig. 2B). To confirm the binding ability of the Myb domain to U2AF65, HEK293 cells were co-transfected with Myc-U2AF65 and various Flag-TRF1 fragments and subjected to immunoprecipitation. Myc-U2AF65 was immunoprecipitated only by TRF1 fragment containing the Myb domain (Fig. 2C). We next determined the TRF1-binding domain in U2AF65. The results showed that

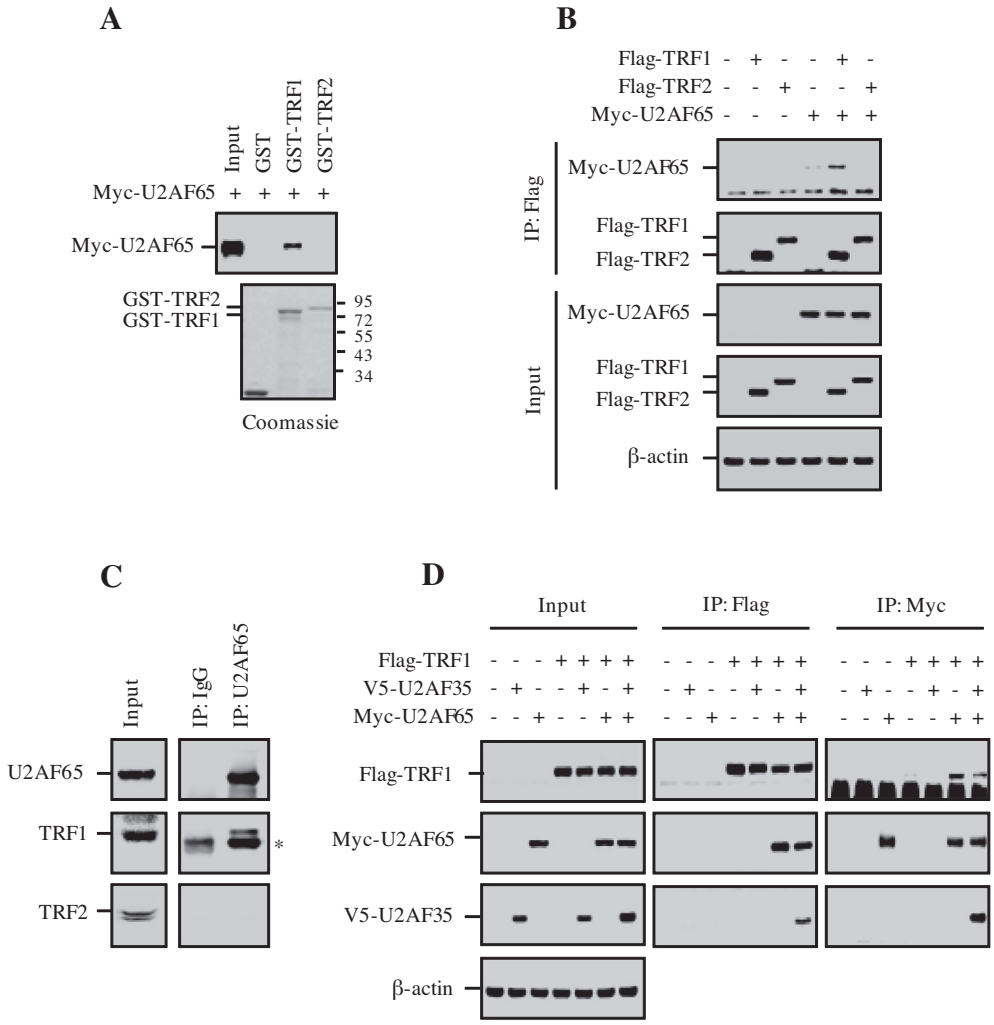

Fig. 1. U2AF65 interacts with TRF1 *in vivo* and *in vitro*. (A) GST, GST-TRF1 or GST-TRF2 were immobilized on glutathione-Sepharose and incubated with ectopically expressed Myc-U2AF65. Bound proteins were detected by immunoblotting with anti-Myc antibody. The purified GST fusion proteins were visualized by Coomassie staining. Molecular mass markers are shown in kilodaltons. (B) HEK293 cells were co-transfected with Flag-TRF1 (or Flag-TRF2) and Myc-U2AF65 and subjected to immunoprecipitation with anti-Flag antibody, followed by immunoblotting with anti-Myc antibody. An antibody against β-actin was used as a loading control. (C) HEK293 cell lysates were immunoprecipitated with anti-U2AF65 antibody, followed by immunoblotting as indicated. IgG was used as a negative control. The *asterisk* marks the position of nonspecific immunoglobulin chains. (D) HEK293 cells were co-transfected with Flag-TRF1, Myc-U2AF65 and V5-U2AF35 and subjected to immunoprecipitation with anti-Flag and anti-Myc antibodies, followed by immunoblotting to detect precipitated proteins as indicated.

GST-TRF1 interacts with U2AF65 fragment containing the N-terminal RS domain (Fig. 2D and E). When HEK293 cells were co-transfected with Flag-TRF1 and various Myc-U2AF65 fragments, Flag-TRF1 was immunoprecipitated by the RS domain (Fig. 2F). It has been shown that the U2AF35-binding domain in U2AF65 is within amino acid residues 85–112 [25]. Because the RS domain encompassing amino acid residues 1–63 binds to TRF1, these results suggest that U2AF65 may associate simultaneously with both TRF1 and U2AF35 (see Fig. 1D).

3.3. U2AF65 inhibits ubiquitination of TRF1

It has been well documented that TRF1 negatively regulates telomere length by inhibiting access of telomerase at telomere termini [12,13]. Thus, the cellular abundance of TRF1 should be tightly regulated to ensure proper function of telomeres. To examine whether U2AF65 affects the stability of TRF1 protein, we perform the *in vivo* ubiquitination assay. HEK293 cells were co-transfected with Flag-TRF1, HA-ubiquitin and Myc-U2AF65. To illuminate ubiquitin-modified TRF1, anti-Flag immunoprecipitates were evaluated by immunoblotting with anti-HA antibody. Ubiquitinated TRF1 was detected even in the absence of MG132 (Fig. 3A). Interestingly, co-transfection of Myc-U2AF65 markedly reduced the amounts of ubiquitinated TRF1. However, the ubiquitination status of TRF1 was not affected by co-transfection of U2AF65 lacking the RS domain (Myc-U2AF65ΔRS). The similar trend was observed in the presence of MG132 although this modification was greatly enhanced by MG132 treatment (Fig. 3A). These results suggest that U2AF65 binding to TRF1 is required for inhibiting the ubiquitination of TRF1.

It has been reported that Fbx4, a member of the F-box family of proteins, targets TRF1 for ubiquitination [16]. To examine whether U2AF65 inhibits the Fbx4-dependent ubiquitination of TRF1, HEK293 cells were co-transfected with Flag-TRF1, HA-ubiquitin, Myc-U2AF65 (or Myc-U2AF65ΔRS) and V5-Fbx4 prior to MG132 treatment and subjected to immunoprecipitation to detect ubiquitinated TRF1. As shown in Fig. 3B, ubiquitinated TRF1 was greatly enhanced when V5-Fbx4 was expressed. The levels of ubiquitinated TRF1 were markedly reduced by overexpression of Myc-U2AF65 but not significantly influenced by overexpression of Myc-U2AF65ΔRS. These results suggest that U2AF65 may interfere with FBX4-binding to TRF1. To investigate this possibility, we expressed

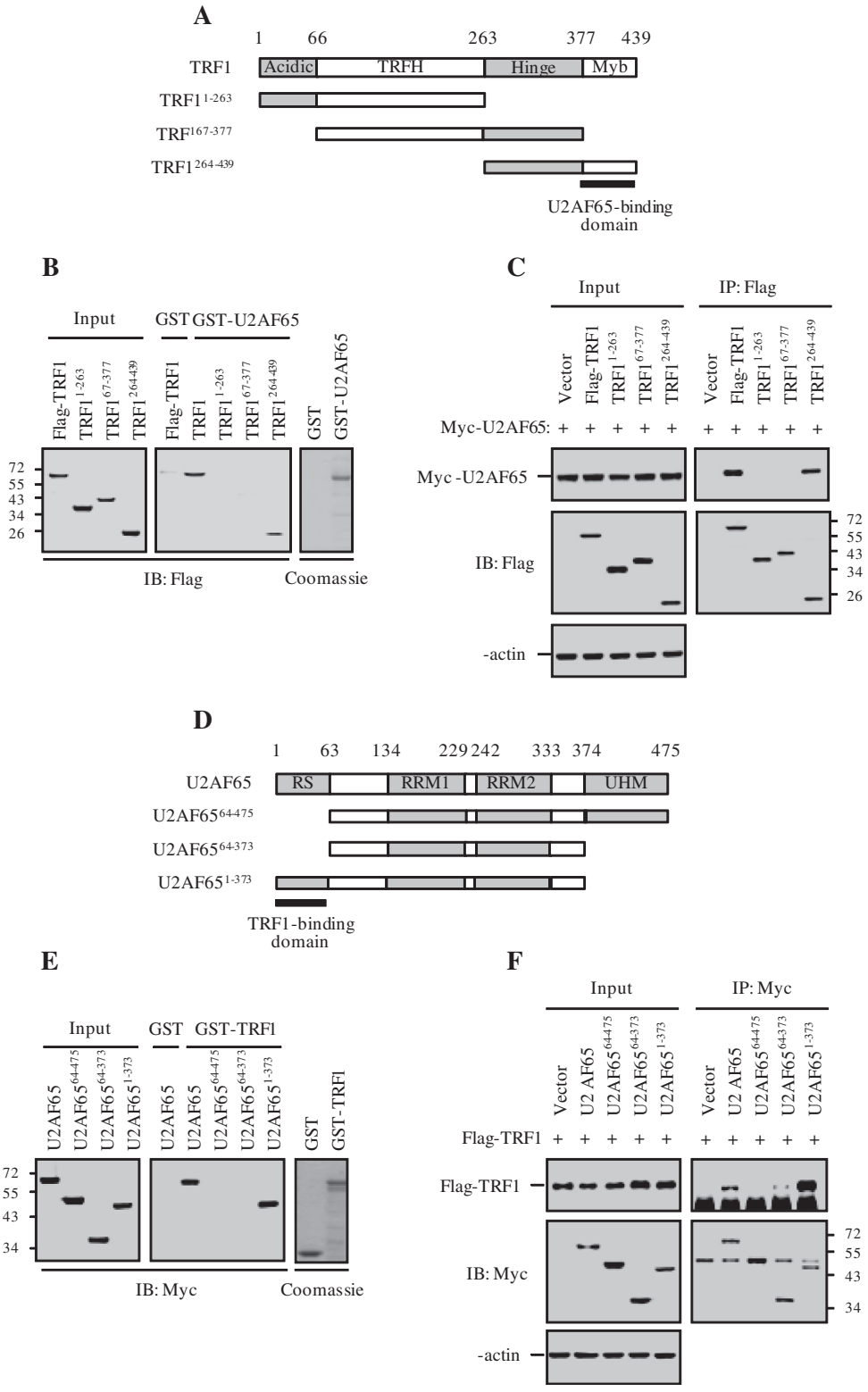

Fig. 2. Identification of the domains in TRF1 and U2AF65 required for their interaction. (A) Schematic representation of the region of TRF1 involved in binding to U2AF65. (B) GST or GST-U2AF65 were affinity-purified and incubated with ectopically expressed Flag-TRF1 fragments, followed by immunoblotting with anti-Flag antibody. The purified GST fusion protein was visualized by Coomassie staining. Molecular mass markers are shown in kilodaltons. (C) HEK293 cells were co-transfected with Myc-U2AF65 and various truncated Flag-TRF1 and subjected to immunoprecipitation with anti-Flag antibody, followed by immunoblotting with anti-Myc antibody. The various truncated Flag-TRF1 proteins were detected by immunoblotting with anti-Flag antibody. An antibody against β-actin was used as a loading control. Molecular mass markers are shown in kilodaltons. (D) Schematic representation of the region of U2AF65 involved in binding to TRF1. (E) GST or GST-TRF1 were incubated with ectopically expressed Myc-U2AF65 fragments, followed by immunoblotting with anti-Myc antibody. The purified GST fusion protein was visualized by Coomassie staining. Molecular mass markers are shown in kilodaltons. (F) HEK293 cells were co-transfected with Flag-TRF1 and various truncated Myc-U2AF65 and subjected to immunoprecipitation with anti-Myc antibody, followed by immunoblotting with anti-Flag antibody. The various truncated Myc-U2AF65 proteins were detected by immunoblotting with anti-Myc antibody. An antibody against β-actin was used as a loading control. Molecular mass markers are shown in kilodaltons.

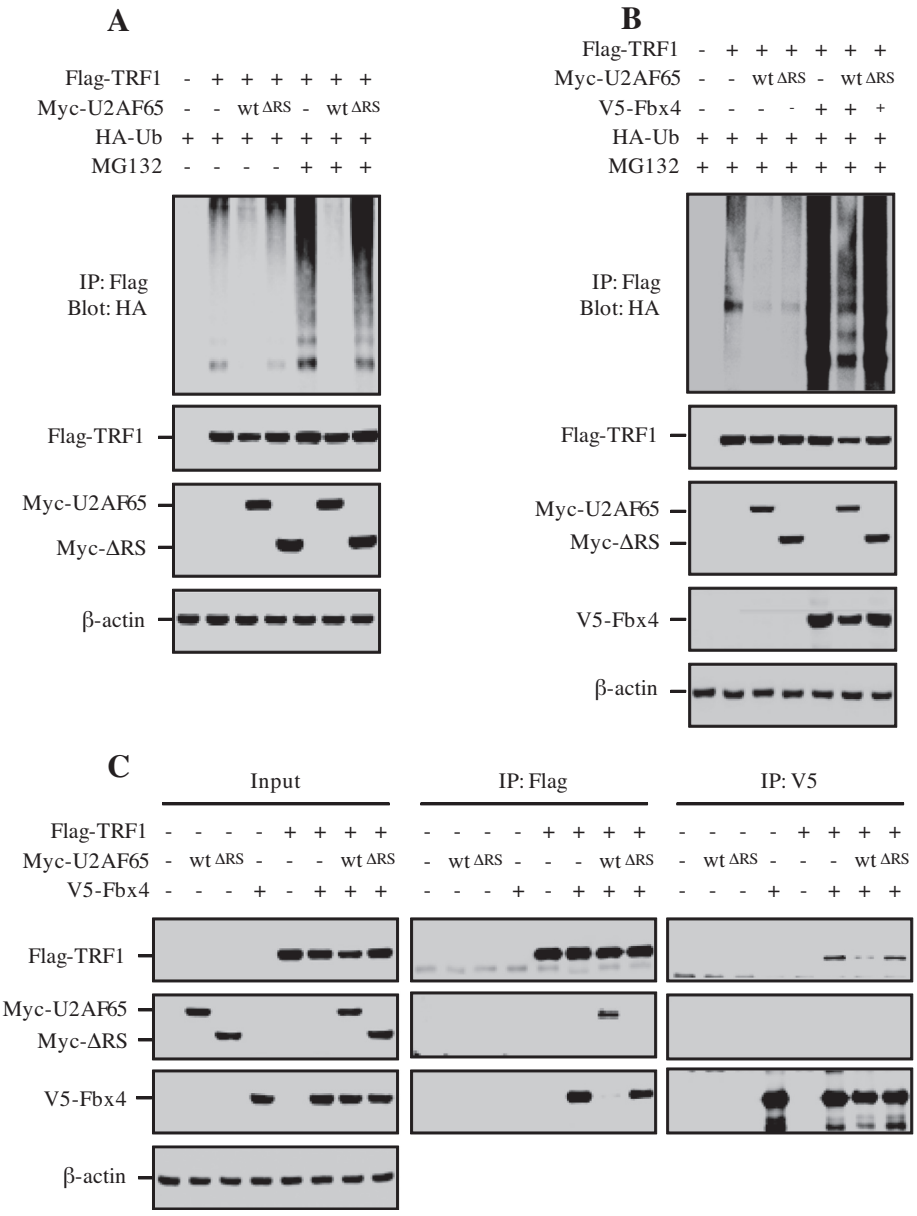

Fig. 3. U2AF65 inhibits Fbx4-dependent ubiquitination of TRF1. (A) HEK293 cells were co-transfected with Flag-TRF1, Myc-U2AF65 (or Myc-U2AF65ΔRS) and HA-ubiquitin (HA-Ub) as specified and treated with, or without, 10 μM MG132 for 2 h. Immunoprecipitation was carried out with anti-Flag antibody before probing with anti-HA antibody. An antibody against β-actin was used as a loading control. (B) HEK293 cells were co-transfected with Flag-TRF1, Myc-U2AF65 (or Myc-U2AF65ΔRS), V5-FBX4 and HA-ubiquitin (HA-Ub) as specified and treated with 10 μM MG132 for 2 h. Immunoprecipitation was performed with anti-Flag antibody before probing with anti-HA antibody. (C) HEK293 cells were co-transfected with Flag-TRF1, Myc-U2AF65 (or Myc-U2AF65ΔRS) and V5-FBX4 and subjected to immunoprecipitation with anti-Flag or anti-V5 antibodies, followed by immunoblotting to detect precipitated proteins as indicated.

Flag-TRF1, Myc-U2AF65 (or Myc-U2AF65ΔRS) and V5-Fbx4 in HEK293 cells and performed immunoprecipitation with anti-Flag antibody. V5-Fbx4 was detected in anti-Flag immunoprecipitates from cells expressing Flag-TRF1 (Fig. 3C, middle panel). When Myc-U2AF65 was co-transfected, V5-Fbx4 was not detected in anti-Flag immunoprecipitates. However, overexpression of Myc-U2AF65ΔRS did not affect the interaction between Flag-TRF1 and V5-Fbx4 (Fig. 3C, middle panel). When we performed immunoprecipitation with anti-V5 antibody, Flag-TRF1 was precipitated by anti-V5 antibody in cells expressing V5-Fbx4 (Fig. 3C, right panel). However, Flag-TRF1 binding to V5-FBX4 was inhibited when Myc-U2AF65 was expressed but not affected by overexpression of Myc-U2AF65ΔRS (Fig. 3C, right panel). These data are consistent with the idea that TRF1 interacts specifically with Fbx4 [16]; however, TRF1 does not appear to form a stable complex with Fbx4 in the presence of U2AF65. Therefore, TRF1 binding to U2AF65 and Fbx4 may be mutually exclusive.

3.4. U2AF65 regulates the levels of endogenous TRF1

To examine the effect of U2AF65 on the cellular abundance of endogenous TRF1, the expression of endogenous U2AF65 was depleted using two different small interfering RNA (siRNA) duplexes. Cytoplasmic and nuclear extracts were separately collected from U2AF65 knockdown cells. Both U2AF65 and TRF1 were exclusively detected in the nuclear fractions but not in the cytoplasmic fractions (Fig. 4A). U2AF65 knockdown resulted in a clear reduction in the levels of TRF1 but not significantly affect the levels of TRF2. However, no significant difference was observed in the steady-state levels of *TRF1* mRNA between U2AF65 knockdown cells

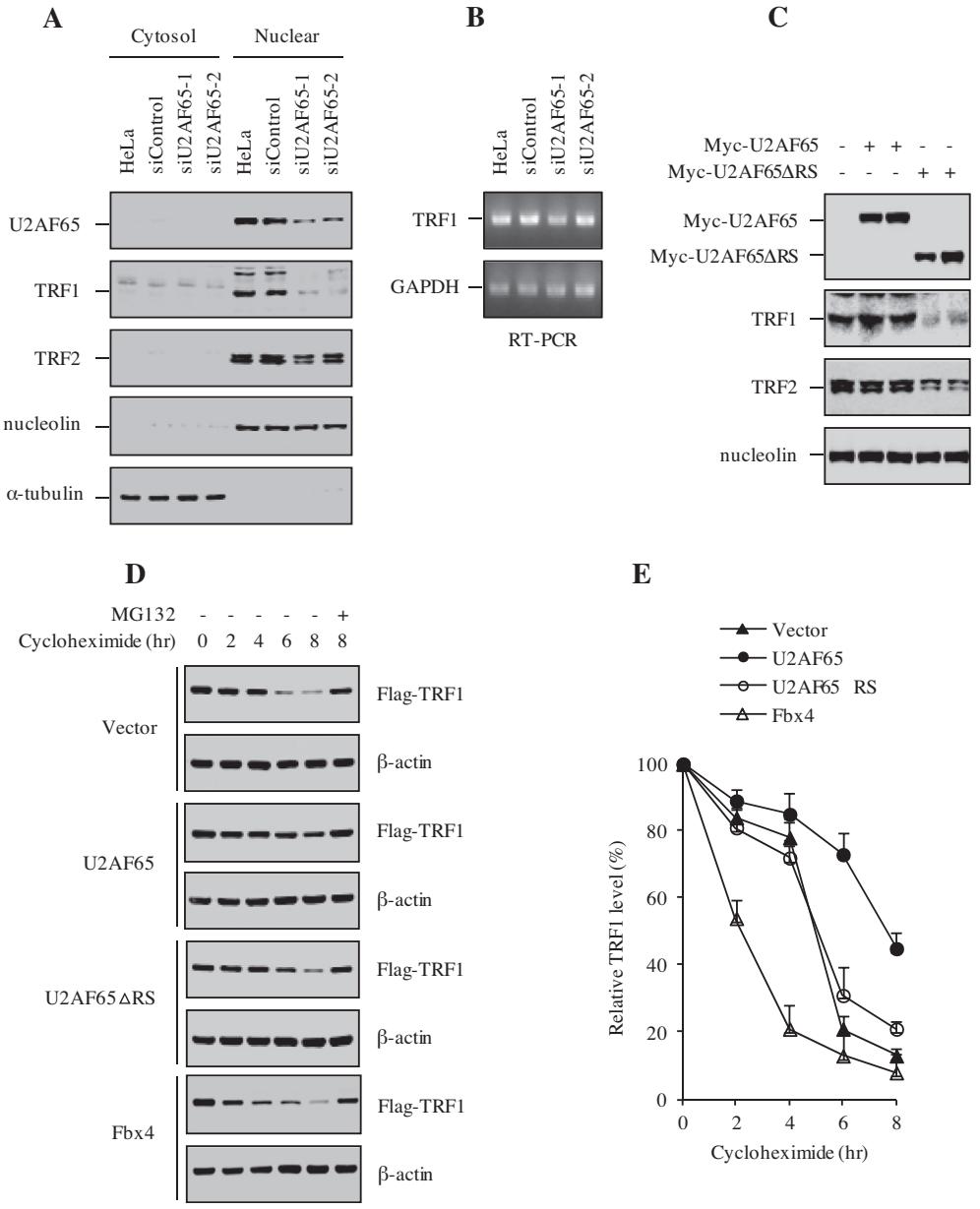

Fig. 4. U2AF65 regulates the levels of endogenous TRF1. (A) HeLa cells were transfected with two different U2AF65 siRNAs (siU2AF65-1 or siU2AF65-2) or scrambled control siRNA (siControl). Nuclear and cytosolic fractions were separately collected and subjected to immunoblotting for detection of endogenous TRF1 and TRF2. Duplicate blots were immunolabeled with anti-tubulin (for cytosolic fraction) and anti-nucleolin (for nuclear fraction) antibodies to confirm the absence of the cross contamination in each fraction. (B) HeLa cells were transfected with two different U2AF65 siRNAs or scrambled control siRNA and subjected to reverse transcription-PCR analysis for the expression of *TRF1* gene. (C) HeLa cells were transfected with Myc-U2AF65 or Myc-U2AF65ΔRS and subjected to immunoblotting for detection of endogenous TRF1 and TRF2. An antibody against nucleolin was used as a loading control. (D) HeLa cells were co-transfected with Flag-TRF1 and Myc-U2AF65 or Myc-U2AF65ΔRS or V5-Fbx4 and treated with 100 μg/ml cycloheximide together with, or without, 10 μM MG132 for the indicated times. Lysates were analyzed on immunoblots with anti-Flag or anti-β-actin antibodies. (E) Graphical representation of the relative TRF1 levels normalized against the β-actin loading control. The TRF1 expression levels were quantified with the average and standard deviation from three independent experiments.

and the control cells (Fig. 4B). To further verify the involvement of U2AF65 in regulating the levels of TRF1, we transiently expressed Myc-U2AF65 or Myc-U2AF65ΔRS in HeLa cells and examined the levels of endogenous TRF1. The levels of TRF1 was substantially increased in U2AF65-expressing cells compared to the empty vector-expressing cells but was decreased in U2AF65ΔRS-expressing cells (Fig. 4C). We also found that endogenous TRF2 was not significantly altered by overexpression of U2AF65.

To further verify that the amount of U2AF65 affects the half-life of TRF1, HeLa cells were co-transfected with Flag-TRF1 and Myc-U2AF65 (or Myc-U2AF65ΔRS), incubated with cycloheximide to block new protein synthesis and analyzed by immunoblotting with anti-Flag antibody. As shown in Fig. 4D and graphically in Fig. 4E, overexpression of Myc-U2AF65 extended the half-life of TRF1 compared to the empty vector-expressing cells. However, overexpression of Myc-U2AF65ΔRS did not significantly alter the stability of TRF1. In contrast, overexpression of V5-Fbx4 reduced the half-life of TRF1. In all cases, turnover of TRF1 was blocked by the MG132 treatment, further indicating that degradation of TRF1 is mediated by the proteasome.

4. Discussion

TRF1 functions as a negative regulator of telomere length by controlling the access of telomerase to telomeres. Overexpression of TRF1 in telomerase-positive cells promotes telomere shortening

whereas loss of TRF1 from telomeres has been shown to induce inappropriate telomere elongation [12,13]. Because telomere length is maintained within a given size range in most human cancer cells, the cellular abundance of TRF1 should be tightly regulated at telomeres. The level of TRF1 at telomeres has been shown to be regulated by interacting with various proteins such as tankyrase 1, Fbx4, RLIM, CK2, Pin1 and Plk1 [26]. In this study, we report that U2AF65, in addition to its function as a pre-mRNA splicing factor, interacts with TRF1 and stabilizes TRF1 protein by inhibiting ubiquitin-dependent degradation of TRF1. Depletion of U2AF65 by RNA interference reduced the stability of endogenous TRF1 whereas overexpression of U2AF65 significantly extended the half-life of TRF1. These findings demonstrate that U2AF65 plays a critical role in regulating the level of TRF1 at telomeres.

U2AF65 binds to the Myb domain of TRF1 (residues 378–439) whereas Fbx4 associates with the TRFH domain of TRF1 (residue 48–155) [16], indicating that U2AF65-binding region in TRF1 does not overlap with Fbx4-binding region. We showed that TRF1 interacts specifically with Fbx4. However, TRF1 does not appear to form a detectable and stable complex with Fbx4 in the presence of U2AF65 even when both Fbx4 and U2AF65 were expressed to similar levels, suggesting that U2AF65 interferes with the interaction between TRF1 and Fbx4. Although it is not clear how these three proteins have opportunity to interact in intact cells, we could not detect formation of a ternary protein complex (TRF1/U2AF65/Fbx4), suggesting that TRF1 binding to U2AF65 and Fbx4 is mutually exclusive. Taken together, these results suggest that, although U2AF65 and Fbx4 are each capable of interacting with TRF1, U2AF65 appears to have much higher affinity for TRF1 binding than Fbx4.

It is interesting to note that TRF1 interacts with U2AF65 through the Myb domain which is responsible for binding double-stranded telomeric DNA [27]. This suggests that U2AF65 cannot interact with TRF1 that is bound to telomeric DNA. Therefore, U2AF65 may function primarily to stabilize TRF1 that is not associated with telomeres. This is consistent with previous findings that telomere-bound TRF1 is protected from ubiquitin-mediated degradation [28]. On the basis of previous reports and our data, we propose a model where two counteracting factors may determine the pool of telomere-unbound TRF1: U2AF65 as a stabilizing factor and Fbx4 as a degradation factor. When newly synthesized TRF1 is translocated into the nucleus or pre-existing TRF1 is dissociated from telomeres, the dynamic balance between these two proteins may determine the equilibrium level of telomere-unbound TRF1. Under conditions in which U2AF65 is kept below the threshold level, TRF1 could be available for Fbx4 binding, thus leading to rapid ubiquitination and subsequent degradation of TRF1 by the proteasome. In contrast, cells overexpressing U2AF65 displays stabilized TRF1 by inhibiting its Fbx4-dependent ubiquitination. Thus, these results suggest that U2AF65 is upstream of Fbx4 in the TRF1 degradation pathway.

Overall, our results provide evidence for the new cellular function of U2AF65 in modulating the cellular abundance of TRF1 at telomeres in addition to its role in pre-mRNA splicing process. Although many important questions about the biological significance and pathological consequence of the interaction between TRF1 and U2AF65, our data suggest that U2AF65 represents a new pathway for maintaining functional telomeres by acting as a positive regulator of TRF1 protein stability.

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