Variant forms of upstream stimulatory factors (USFs) control the promoter activity of *hTERT*, the human gene encoding the catalytic subunit of telomerase

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Abstract It is known that Myc regulates the expression of *TERT*, the telomerase catalytic subunit gene, by binding to E box. Here we show that another E box-binding protein, upstream stimulatory factor (USF), also regulates *TERT* expression. Specifically, the N-terminally truncated form of USF2 is present in telomerase-negative/resting human lymphocytes, but not in telomerase-positive/phytohemagglutinin-activated lymphocytes. In electrophoretic mobility shift assay, both full-length and truncated USF2s bound to the *TERT* E box. In a transient expression assay, the truncated USF had a dominant-negative effect on both exogenous full-length USF and endogenous positive regulators for activating *TERT* expression. These results suggest that the differential abundance of truncated USF2 may regulate telomerase activity during lymphocyte activation. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Telomerase; Telomerase reverse transcriptase; Promoter; Upstream stimulatory factor; Mini-upstream stimulatory factor

1. Introduction

Telomerase is a specialized reverse transcriptase that compensates for the telomere length reduction caused by the end replication problem (for recent review, see [1]). It is active in germ cells and 90% of cancer cells, but not in most normal somatic cells [2]. Two components, telomerase reverse transcriptase (TERT) and telomerase RNA (TR), are essential for the enzymatic activity measured in vitro. TR is expressed in a relatively wide range of cells, regardless of the presence or absence of telomerase activity. It is thought that the expression level of TERT primarily determines the activity [3–8].

Several transcription factors, including Myc, Sp1, ER (estrogen receptor), MZF-2, and E2F-1, have been proposed to regulate TERT expression [9–15]. Among them, TERT activation by Myc has been well demonstrated [9,11,16,17]. Myc belongs to the bHLH-zip (basic helix-loop-helix zipper) tran-

scription factor family (reviewed in [18]). Myc, as well as other members of this family, forms a heterodimer with Max to bind to a consensus sequence called E box (5′-CACGTG-3′), thereby activating gene expression. Because Max is ubiquitously present in cells, the Myc–Max activity is largely regulated by the amount of Myc. Myc is a short-lived protein, and its abundance is correlated with the cell proliferation rate. Therefore, Myc is an attractive candidate for regulating TERT expression, which is known to be high in proliferating cells [19].

Upstream stimulatory factor (USF) is another bHLH-zip protein that binds to E box. Two structurally and functionally related proteins derived from distinct genes, USF1 and USF2, are known [20]. In contrast to Myc, USF1 and USF2 are expressed ubiquitously in different types of cells. Accordingly, it was initially postulated that USF1 and USF2 are involved in regulating the expression of housekeeping genes [21]. However, there is evidence that USF1 and USF2 are also involved in tissue-specific and hormone- and nutrition-induced gene expression ([22] and references therein). It is not understood how ubiquitously expressed factors contribute to these controlled processes.

Several variant forms of USF1 and USF2 have been identified: these are derived from alternative splicing, and probably from alternative choice of the initiation codon or post-translational modification [23,24]. In vitro, USF1 and USF2 form both homo- and heterodimers at similar efficiencies, but in vivo they prefer to form heterodimers rather than homodimers [25]. Different types of USF1–USF2 heterodimers were found, depending on the different combinations of USF1 and USF2 variant forms.

Most studies that elucidated the transcriptional control of *hTERT* have been conducted using cell lines, and we know very little about how *hTERT* expression is controlled in normal cells. *hTERT* expression and telomerase activity are strictly regulated during proliferation and differentiation of normal human cells. For example, resting human lymphocytes, which are negative for telomerase activity, induce *hTERT* expression and become telomerase-positive upon being activated by a variety of stimuli, such as mitogenic lectin PHA (phytohemagglutinin). We do not know what transcriptional factors are involved in this regulated process. In this study, we examined whether USF proteins are involved

in the control of *hTERT* expression. The results revealed that differential abundance of variant forms of USF2 may contribute to *hTERT* induction in activated lymphocytes.

2. Materials and methods

2.1. Cell culture

Human normal peripheral blood mononuclear cells (PBMCs) were isolated from healthy volunteers by Ficoll–Conray density gradient centrifugation. Cells were initially cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) for 1 h in a 37°C, 5% CO_2 humidified incubator. Non-adherent cells, most of which do not contain monocyte/macrophages, were collected and washed twice with RPMI 1640 medium. Fractions of T cells and B cells were analyzed by FACS using magnetic beads bound to monoclonal antibodies against CD2/CD19. Cells were resuspended and cultured in RPMI 1640/10% FBS containing 3 μg/ml PHA (Gibco). HEK293, KMST6, SUSM1, WI38RA were cultured in Dulbecco's modified Eagle medium supplemented with 10% FBS.

2.2. Stretch PCR assay

Cellular telomerase activity was measured by stretch PCR assay as described [26].

2.3. Nuclear extract (NE) preparation

NEs of cells were prepared by the modified Dignam method [27]. In brief, cells were washed with phosphate-buffered saline twice, and suspended in 400 μl of buffer A (10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol (DTT), 0.5 mM PMSF). After 15 min of incubation on ice, cells were homogenized by passing them through a 23-gauge needle, and the sample was centrifuged for 1 min at 10 000 rpm. Nuclei pellets were resuspended in and salt-extracted with 50 μl of buffer B (20 mM HEPES (pH 7.9), 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF), and then incubated on ice for 30 min. The supernatant was collected, dialyzed against buffer C (20 mM HEPES (pH 7.9), 20% glycerol, 0.1 M KCl, 0.2 mM EDTA, 1 mM DTT) and used as NEs.

2.4. Electrophoretic mobility shift assays (EMSAs)

NE (5 μg/reaction for lymphocyte NE and 1 μg/reaction for 293 cell NE) was incubated with 30 000 cpm of end-labeled oligonucleotide probes in the presence of 2 μg poly(dI-dC) (Amersham Pharmacia Biotech) in a final volume of 25 μl buffer containing 50 mM KCl, 50 mM NaCl, 20 mM HEPES (pH 7.5), 5 mM DTT, 5 mM $MgCl_2$, and 12% glycerol. For competition assays, 100-fold molar excess of oligonucleotide was used in the binding reaction. For supershift assays, NEs initially preincubated with 1 μl of polyclonal antisera for 60 min were used for EMSA. The used polyclonal antibodies were: anti-c-Myc (sc-42X, sc-764X), anti-Max (sc-765X), anti-USF-1 (sc-229X), and anti-USF-2 (sc-861X, sc-862X) (Santa Cruz). Binding reactions were resolved by 5% polyacrylamide gel electrophoresis (PAGE) at 4°C.

2.5. Western blotting

Cell extracts were prepared by extracting lymphocyte cells with the lysis buffer (50 mM Tris–HCl, 1% NP40, 150 mM NaCl, 50 mM NaF, 1 mM Na_3VO_4, 5 mM EDTA, 1 mM PMSF, 3 mM β-glycerophosphate, 1 mM DTT, 1×complete cocktail protease inhibitor (Roche)). Western blotting was performed using an enhanced chemiluminescence kit (Amersham Pharmacia Biotech). NEs containing 50 μg of protein were analyzed using polyclonal antibodies (1:100 dilution).

2.6. Plasmid construction

The *hTERT* promoter region (−232 to +98 nucleotides from the transcription start site) was inserted into the pGL3-promoter vector (Promega) to produce the *hTERT*-luciferase reporter plasmid. cDNAs encoding mini-USF1 (Δ1–163) and mini-USF2 (Δ1–198) were produced by PCR. The nucleotide sequences of all plasmid constructs were confirmed.

2.7. Promoter activity assays

293, KMST6, WI38RA and SUSM1 cells were plated 24 h prior to transfection. DNA transfection was performed using FuGENETM6

transfection reagent (Boehringer Mannheim) or lipofectamine reagent (Life Technologies). At 40–48 h after transfection, cell NEs were prepared, and luciferase assays were performed using the dual luciferase assay system (Promega). The transfection efficiency was normalized by that of the co-transfected pRL-TK vector (Promega). All experiments were performed in triplicate.

3. Results

It is known that *hTERT* expression and telomerase activity are induced in normal human lymphocytes upon activation by PHA [28,29]. We chose this system to study the molecular mechanism of *hTERT* induction in normal somatic cells. Human PBMCs were isolated from healthy volunteers by Ficoll–Conray density gradient centrifugation. Monocyte/macrophages were removed by collecting non-adherent cells. The thus-prepared cells typically consisted of 70–85% T cells, 10–20% B cells. Cells were treated with 3 μg/ml PHA, cultured and harvested. As reported previously, telomerase activity, which was very low in unstimulated lymphocytes, became noticeably induced on day 1.5, and high on days 3.5 and 5.0 in PHA-treated lymphocytes. *hTERT* expression was hardly observed in unstimulated lymphocytes, but was significantly induced in lymphocytes activated by PHA with kinetics similar to that of telomerase activity. *hTERC*, the gene encoding human TR, was already expressed in unstimulated cells, and the expression level was largely unchanged during PHA stimulation. Therefore, as previously reported, it was highly possible that PHA induced telomerase activity in lymphocytes by activating *hTERT* expression. This idea is further supported by a recent result that stable expression of ectopic *TERT* results in the extension of the replicative life span in lymphocytes [30]. In the following experiments, we used unstimulated lymphocytes (resting lymphocytes) and activated lymphocytes that were obtained after 3 days incubation with PHA.

3.1. USF1 and USF2 bind to hTERT E boxes

It is well established that the two E boxes present in the *hTERT* promoter (at −210 bp and −34 bp from the translation initiation site, hereafter called the upstream and downstream E box, respectively, Fig. 1A) play a pivotal role in the regulation of *hTERT* expression by Myc [9,11]. To examine whether these E boxes are also involved in the *hTERT* induction by PHA in normal human lymphocytes, we performed EMSA using two oligonucleotide DNA probes containing the upstream or downstream E box (upstream and downstream probes, respectively, Fig. 1A). The upstream and downstream probes had the same nucleotide length. In the absence of NEs, no shifted band was observed (data not shown, and see Fig. 2A, lane 7). Both upstream (Fig. 1B, lanes 1–6) and downstream probes (Fig. 1B, lanes 7–10) produced retarded bands upon incubation with NEs from resting and activated lymphocytes. Resting cell NEs produced two different bands: slow-migrating (thin arrows) and fast-migrating (thick arrows) bands. The fast-migrating band had a significantly greater abundance than the slow-migrating band. Interestingly, only a single retarded band was produced by activated cell NEs, which migrated to the same position as that of the slow-migrating band produced by resting cell NEs. These probe–protein complexes were specific since they were not detected when a 100-fold molar excess of the cognate probe was added (lanes 3, 6, 8 and 10). This competition was not observed when
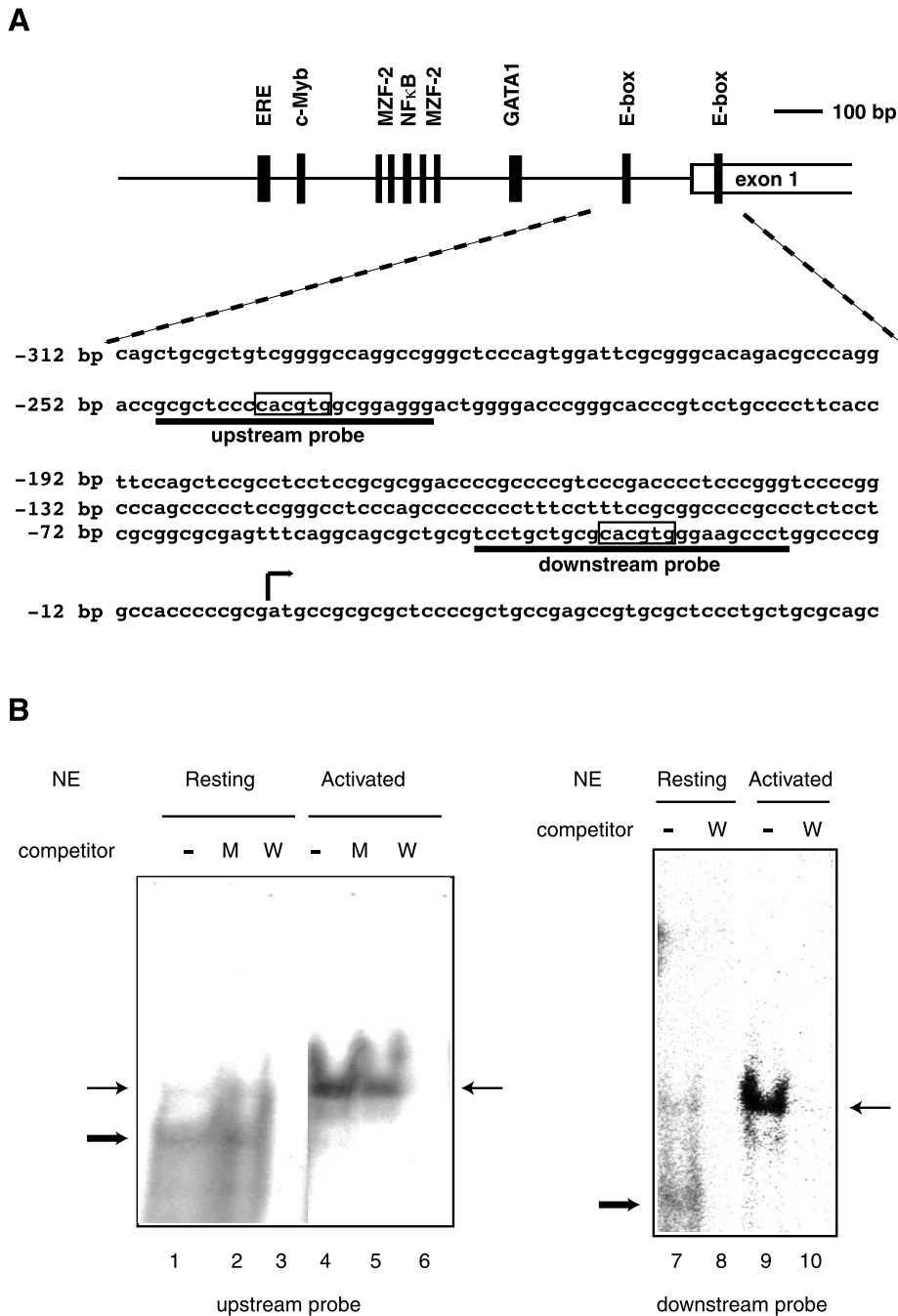
A

B

Fig. 1. Two E boxes in the hTERT promoter region and exon 1. A: Potential binding sites for transcription factors in the *hTERT* promoter region (upper panel) and the nucleotide sequences around the two E boxes. Numbers on the left refer to the distance from the translation initiation site (marked by an arrow). The two E boxes are enclosed by boxes, and the oligonucleotide probes used in this study are shown. B: EMSA using oligonucleotide probes containing the *hTERT* E box sequences. The upstream and downstream probes shown in (A) were used as probes in EMSA using resting and activated lymphocyte NEs. Binding reaction was performed without (competitor −) or with 100-fold molar excess of cold wild-type oligonucleotides (W) or mutated (the E box sequence, CACGTG, was mutated to TTTGTG) versions of oligonucleotides (M). Specific DNA–protein complexes are indicated by thick and thin arrows. To differentiate the two bands clearly, the electrophoresis was carried out for an extended time, and the free probe run away from the gel. However, in a separate experiment, we confirmed that the reaction took place under the condition that the free probe was present in excess of the DNA-binding activity (also see Fig. 2C).

mutant competitors that contain TTTGTG instead of CACGTG were used, further substantiating that the complex formation depends on the authentic E box sequence (lanes 2 and 5). These results indicated that resting cell NEs exhibited an additional binding activity to the E box probes compared to activated cell NEs. To examine whether Myc is involved in the formation of these complexes, three independent antibod-

ies against Myc or Max were included in the binding reactions. However, no supershift of the band mobility was observed, suggesting that Myc is not responsible for the complex formation (data not shown).

We then investigated whether USF is involved in the DNA–protein complex formation. Fig. 2A shows the results obtained by EMSA using the downstream probe. The same re-
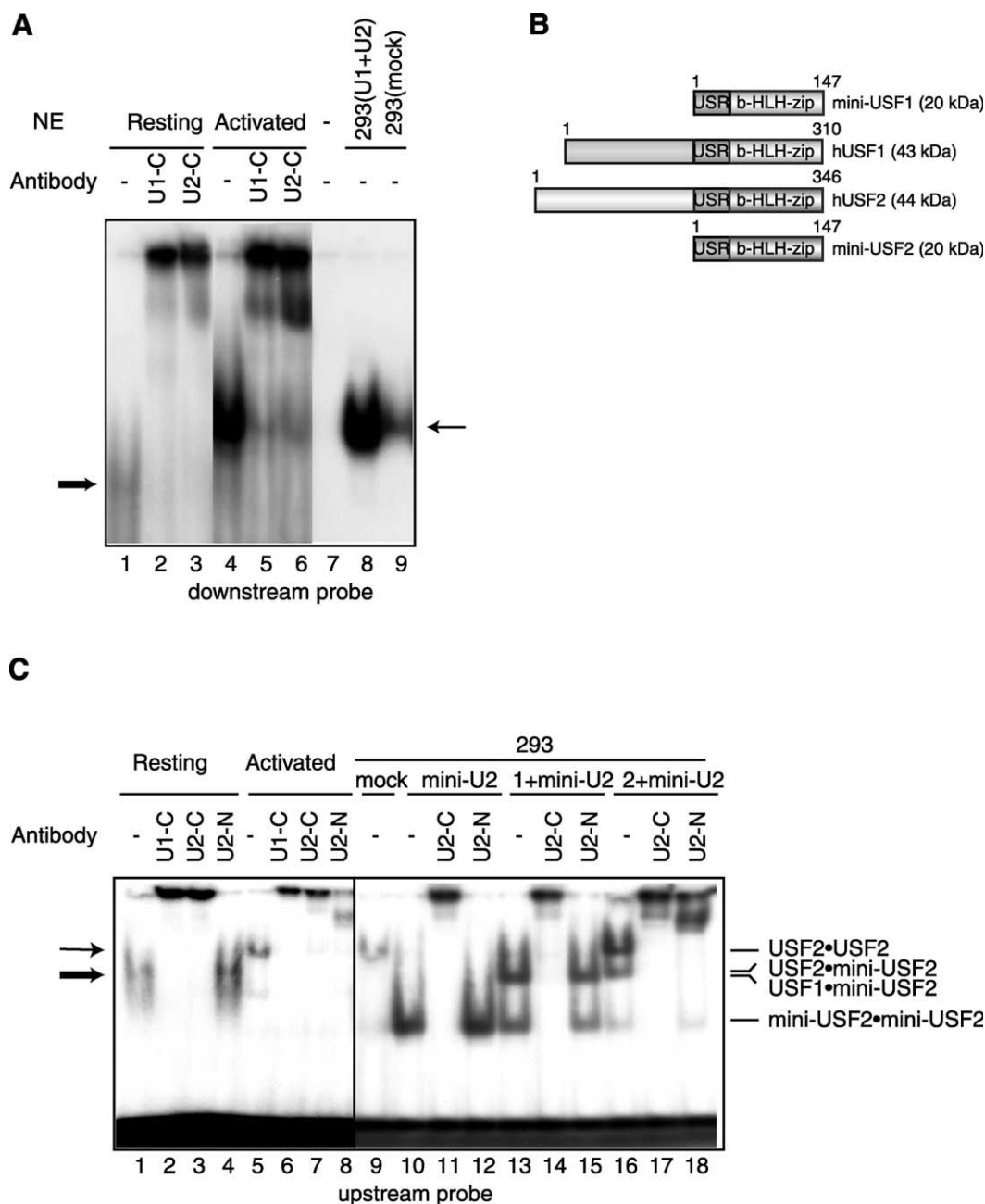
Fig. 2. USF1 and USF2 are responsible for the E box-binding activity present in lymphocyte extracts. A: Supershift assay of retarded bands using anti-USF antibodies. EMSA was performed using the downstream E box oligonucleotide probe, and NEs derived from resting (Resting) or activated (Activated) lymphocytes. NEs from 293 cells over-expressing full-length USF1 and USF2 simultaneously (U1+U2) or mock-transfected (mock) were also used. To identify the protein contained in the complexes, antibodies indicated were added to the binding reaction and the supershift of the electrophoretic mobility was monitored. The two retarded bands found in Fig. 1B are shown by thick and thin arrows. B: Schematic structures of human full-length USF1 (hUSF1), USF2 (hUSF2), mini-USF1 and mini-USF2 [23]. Numbers show the amino acid positions. USR, USF-specific region, and bHLH-zip, basic helix-loop-helix zipper. C: Supershift of retarded bands in EMSA using the upstream E box probe. EMSA was performed using the upstream probe, as well as NEs from resting (Resting) and activated (Activated) lymphocytes, and 293 cells mock-transfected (mock), over-expressing mini-USF2 (mini-U2), USF1+mini-USF2 (1+mini-U2), or USF2+mini-USF2 (2+mini-U2). The antibodies indicated were added to the binding reaction and the supershift of the band mobility was monitored. Fast- and slow-migrating bands produced by resting and activated cell NEs, respectively, are shown by thick and thin arrows. Because the transfected 293 cell NEs contained significantly higher binding activities than those in the lymphocyte NEs, lanes 1–8 and lanes 9–18 were obtained after longer and shorter exposures to X-ray films, respectively.

sults were obtained using the upstream probe (part of the results is shown in Fig. 2C). Antibodies against the C-terminus of USF1 (anti-U1-C) supershifted both the fast-migrating band produced by resting cell NEs and the slow-migrating band produced by activated cell NEs (lanes 2 and 5). Similarly, antibodies against the C-terminus of USF2 (anti-U2-C) changed the mobility of these two bands (lanes 3 and 6). In this experiment, no slow-migrating band was observed in the reaction with the resting cell NEs. These results suggested that the complexes produced by the resting and activated lympho-

cyte NEs contained both USF1 and USF2. To confirm this, ectopically expressed USF proteins were examined in the same EMSA. NEs from 293 cells simultaneously over-expressing full-length USF1 and USF2 generated in the presence of the downstream probe a band having exactly the same mobility as those produced by the NEs from activated lymphocytes (lane 8). Because the band intensity was significantly higher than that obtained by mock-transfected 293 cell NEs (lane 9), the shifted band should be produced by ectopically expressed USF1 and USF2. Accordingly, we concluded that the slow-migrating band observed with activated lymphocyte NEs was generated by the full-length USF1·USF2 heterodimer.

We next examined the identity of the fast-migrating band produced by the resting lymphocyte NEs. USF1 and USF2 share highly homologous C-terminal sequences that comprise the bHLH-zip region, which is important for DNA-binding and dimerization (Fig. 2B). Immediately upstream of this region is a short sequence conserved in USF proteins, called USR (USF-specific region). USR is essential for the transcriptional activation activity of the protein [24]. The N-terminal regions differ markedly between USF1 and USF2. It has been suggested that smaller variants of USF1 and USF2 that consist of USR+bHLH-zip but lack the N-terminal regions may exist [23]. These putative variant forms are called mini-USF1 and mini-USF2, respectively.

To examine the possibility that mini-USF2 is involved in the complex formation in resting lymphocyte NEs, antibodies against the N-terminus of USF2 (anti-U2-N) were included in the reaction (Fig. 2C). Fig. 2C shows the results obtained in EMSA using the upstream probe. The same results were obtained in EMSA using the downstream probe (data not shown). Both fast- and slow-migrating bands generated by resting and activated lymphocyte NEs, respectively, were sensitive to the supershift by anti-USF1 C-terminus and anti-USF2 C-terminus antibodies (lanes 2, 3, 6 and 7). Anti-U2-N supershifted the slow-migrating band produced by activated lymphocyte NEs, but not the fast-migrating band produced by resting lymphocyte NEs (lanes 4 and 8). These results suggested that the fast-migrating band does contain an epitope reactive with anti-U2-C but not an epitope reactive with anti-U2-N. One possible explanation for these results is that the fast-migrating band consists of the N-terminally truncated variant form of USF2, such as mini-USF2, instead of the full-length USF2. To test this possibility, we over-expressed mini-USF2, USF1+mini-USF2 and USF2+mini-USF2, individually in 293 cells, and the resultant cell NEs were used in EMSA (lanes 9–18). Several shifted bands showing different mobilities were observed in experiments using different NEs. It is known that mini-USF2 forms a homodimer capable of binding to DNA. Therefore, the fastest band found in the experiment using 293 cells over-expressing mini-USF2 alone most likely represented the mini-USF2·mini-USF2·DNA complex (lane 10). Indeed, anti-U2-C, but not anti-U2-N, supershifted this band (lanes 11 and 12). Accordingly, the additional fast-migrating bands observed in experiments using 293 cells over-expressing USF1+mini-USF2 or USF2+mini-USF2 were attributed to USF1·mini-USF2 and USF2·mini-USF2, respectively. Finally, the two bands that were produced by 293 cells over-expressing USF2+mini-USF2 and sensitive to the supershift by anti-U2-N antibodies were thought to represent USF2·USF2 (or USF1·USF2) and USF2·mini-USF2. In these experiments, we found that the fast-migrating
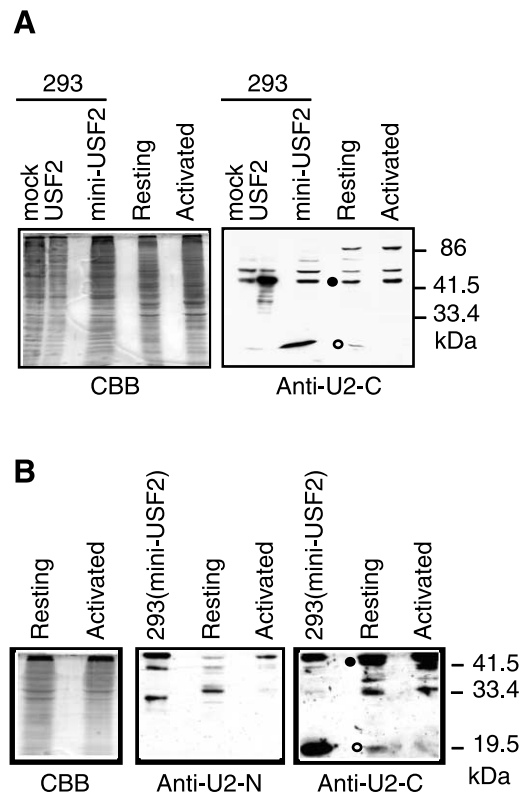

Fig. 3. N-terminally truncated form of USF2 is present in resting, but not in activated lymphocytes. Crude cell extracts containing the same amounts of protein derived from resting (Resting) and activated (Activated) lymphocytes, mock-transfected 293 cells (mock), USF2-over-expressing 293 cells, and mini-USF2-over-expressing 293 cells were examined by Western analysis using anti-USF2 C-terminus antibodies (anti-U2-C) or anti-USF2 N-terminus antibodies (anti-U2-N). Relative amounts of protein in lymphocyte extracts were five-fold larger than those in 293 cell extracts. Gels stained with Coomassie brilliant blue (CBB) are also shown. A and B were run in 10% and 15% SDS–PAGE, respectively. Closed and open circles represent the full-length USF2 and the N-terminally truncated USF2, respectively.

band generated by the resting lymphocyte NEs showed a very similar, if not identical, behavior with the band produced by USF1·mini-USF2: both showed similar mobilities, and were resistant to the supershift by anti-U2-N antibodies. It was therefore suggested that the binding activity present in resting lymphocytes to the *hTERT* E box probe is attributed to the heterodimer between USF1 and N-terminally truncated USF2 having a similar structure to mini-USF2.

3.2. N-terminally truncated USF2 is present in resting lymphocytes and not in activated lymphocytes

The differential usage of N-terminally truncated USF2 and full-length USF2 between resting and activated lymphocytes in binding to the *hTERT* E box sequence may be caused by the differential abundance of these two types of USF2 proteins. Previously, it was described that variant USF proteins exist differentially in various cell types [25]. Full-length USF2, identified by its mobility in SDS–PAGE and reactivity to both anti-U2-N and anti-U2-C, was found in almost the same abundance in both resting and activated lymphocytes (Fig. 3, marked by a closed circle). In contrast, mini-USF2, identified by its mobility and reactivity to anti-U2-C but not anti-
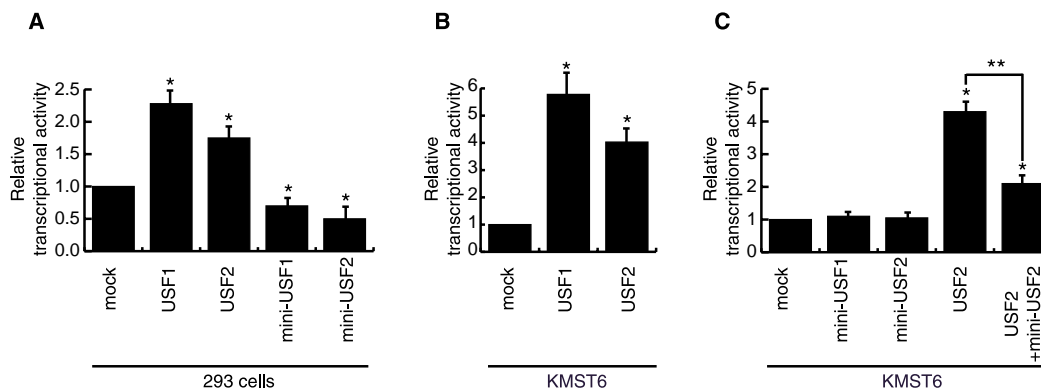
Fig. 4. Luciferase promoter assay of hTERT. A: Plasmids over-expressing the indicated modulators were co-transfected into 293 cells with the reporter plasmid containing the *hTERT* promoter−luciferase chimeric gene. Fold increases of luciferase activity compared to that in the mock-transfected cells are indicated. Relative transcriptional activities of mock- and USF-transfected samples were evaluated using the Dunnet's test. A statistically significant difference ($P < 0.05$) is indicated by an asterisk. B,C: Similar experiments were conducted in KMST6 cells using the indicated modulators. The total amount of transfected DNA was the same in these experiments. When USF2 and mini-USF2 were simultaneously transfected, the same molar amount of each plasmid was used. A statistically significant difference ($P < 0.05$) between values obtained with the mock- and USF-transfected samples is indicated by an asterisk. Values obtained with the USF2- and USF2+mini-USF2-transfected samples were statistically significant ($P < 0.05$) using the Student *t*-test (double asterisk).

U2-N, was detected in resting lymphocytes, but not in activated lymphocytes (marked by open circles). These results indicated that mini-USF2 is present in resting lymphocytes, and contributes to binding to the *hTERT* E box sequence.

3.3. mini-USF2 does not activate hTERT promoter on its own, and shows a dominant-negative effect on full-length USF2

To determine whether the N-terminally truncated form of USF2 found in resting lymphocytes has any biological role in *hTERT* expression, mini-USF2 was over-expressed, and its effect on a reporter plasmid containing the *hTERT* promoter+luciferase chimeric gene was examined. First, we used 293 cells that are telomerase-positive and actively express the endogenous *TERT* gene. Co-transfection of full-length USF1- or USF2-expressing vector together with the reporter plasmid resulted in an enhanced expression of the *hTERT* promoter compared to that found in the absence of over-expressed USF1 or USF2 (approximately two-fold) (Fig. 4A). This relatively weak *hTERT*-inducing activity by over-expressed USF1 and USF2 may be explained by the hypothesis that 293 cells have abundant endogenous positive regulators that saturate the *hTERT* promoter activity (including USF1 and USF2) (see below). When mini-USF1 or mini-USF2 was over-expressed, the *hTERT* promoter activity was significantly lower compared to that in mock-expressing cells. This result is most simply explained by the hypothesis that the introduced hTERT promoter was activated by the endogenous full-length USF1 and USF2, but this activation was inhibited by the dominant-negative effect of mini-USF1 and mini-USF2 (see below).

Several cell lines and cancers are known to maintain telomeres independently of telomerase, and are collectively called ALT (alternative lengthening of telomeres). ALT cells do not express *TERT*, and presumably lack factors necessary for *TERT* gene expression [31]. We thought that ALT cells might reflect the effects in the promoter assay of exogenous *trans*-factors more directly than telomerase-positive cells do, because the *TERT* promoter should not be saturated by the weak endogenous activating activity. We then used KMST6 cells, an ALT cell line, in our promoter assay. Full-length USF1 and USF2 activated the promoter activity at signifi-

cantly higher levels (Fig. 4B, approximately 5.8- and 4.8-fold, respectively) than that observed in 293 cells, as expected. In contrast to 293 cells, mini-USF1 or mini-USF2 did not inhibit the endogenous activity to activate the *hTERT* promoter, suggesting that endogenous USF1 or USF2 is not functional in terms of activating the *hTERT* promoter in this cell line (Fig. 4C). Importantly, when full-length USF2 and mini-USF2 were simultaneously over-expressed, the transcription activation by full-length USF2 alone was significantly inhibited by mini-USF2 (Fig. 4C). Similar results were obtained using WI38RA and SUSM1 cells that are also ALT cells. This result indicates that mini-USF2 has a dominant-negative effect on full-length USF2 in activating the *hTERT* promoter.

4. Discussion

Because telomerase is active in proliferating cells including activated lymphocytes [19], and Myc is induced immediately after the addition of PHA to lymphocytes [32], it appears that Myc is a good candidate for explaining the physiological control of *TERT* expression in these cells. However, the correlation between Myc induction and *TERT* expression is not always observed. For example, primary human B lymphocytes activated by Epstein–Barr virus actively proliferate, yet do not show telomerase activity [33]. Papilloma virus E6 oncoprotein is known to induce *TERT* expression and telomerase activity [34]. However, correlation between the levels of Myc and *TERT* expression was not observed in experiments using a set of E6 mutants [35]. These results strongly suggest that in addition to Myc, there is another pathway for controlling *TERT* expression.

The presence of the N-terminally truncated form of USF2 has been originally suspected based on in vitro observation [23]. However, the implication that mini-USF2 may be involved in gene expression control in vivo is not unprecedented. DNA-binding activity to the PGHS-2 (prostaglandin G/H synthetase-2) promoter, which was attributed to mini-USF, was identified in bovine granulosa cells that were unstimulated by human chorionic gonadotropin (hCG), but not in hCG-stimulated cells. It was therefore proposed that hCG

induces PGHS-2 by removing the negative regulator, mini-USF2 [22]. In this study, we observed similar findings: the DNA-binding activity attributed to the N-terminally truncated form of USF2 was specifically found in telomerase-negative resting lymphocytes, and not in telomerase-positive activated lymphocytes. Furthermore, using a transient expression assay, we showed that mini-USF2 has a dominant-negative effect on full-length USF2 in activating the *hTERT* promoter. These results support the idea that the differential abundance of variant forms of USF2 may control the *hTERT* promoter during human lymphocyte activation.

Because USF and Myc bind to the same sequence (E box), it appears that they should associate with a particular promoter in a mutually exclusive manner. However, using the chromatin immunoprecipitation assay, it was found that the same E box sequence present in the CAD gene could be bound to Myc and USF in vivo in a given population of cells [36]. It is not known whether Myc and USF simultaneously bind to a particular E box in a single DNA molecule. It is, however, possible that a single E box sequentially associates with Myc and USF, and binding to one factor augments the effect of the other factor. Therefore, we do not exclude the possibility that Myc plays an important role in controlling *TERT* expression. Rather, we propose that Myc and USF coordinately regulate *TERT* expression. One attractive possibility is that chromatin structures of the E box are first opened by Myc, which is known to associate with histone acetylase, and then the general transcription factor USF proteins bind to the opened E box [18]. In this context, the USF complexes containing the N-terminally truncated form of USF2 may inhibit the association of the active USF complex formed by the full-length USF proteins. This type of negative regulator may be important in normal human cells in which telomerase is known to be strictly regulated.

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