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Ref-1 Protein Enhances the IL-2-Stimulated Telomerase Activity

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Abstract Telomerase is an important ribonucleoprotein enzyme involved in cellular proliferation and senescence. Activation of telomerase has been detected in a vast majority of human cancer cells. In this article, we demonstrated that Interleukin-2 (IL-2) which is the pivotal cytokine in the immune system could stimulate the activity of telomerase in the cultured BA/F3β cells. It was also found that the level of IL-2-induced telomerase activity was decreased by the treatment with chemical oxidant in vitro. Since IL-2 stimulation produces a oxidative shift of the intracellular environment, the activation and maintenance of telomerase in this oxidative circumstance requires particular protection. Here we proved the redox factor-1 (Ref-1) protein was involved in this process. The addition of GST–Ref-1 protein increased the level of IL-2-induced telomerase activity in the TRAP assay, while elimination of the endogenous Ref-1 protein by immunodepletion decreased it. Consistent with these in vitro results, IL-2-induced telomerase activity could be enhanced by transient overexpression of Ref-1 protein in BA/F3β cells. Taken together, these findings proved that Ref-1 protein benefits the activation of telomerase activity in the oxidative microenvironment of the BA/F3β cells stimulated by IL-2. J. Cell. Biochem. 88: 1120–1128, 2003.

Key words: IL-2; Ref-1; telomerase; redox regulation

Telomerase is a specialized ribonucleoprotein DNA polymerase containing an integral RNA with a short template element that directs the synthesis of tandem repeats of hexanuleotides [5′-TTAGGG-3′] at chromosome ends to form telomere [Morin, 1989]. Telomeres, together with its various binding proteins, play an important role in the maintenance of chromosome structure integrity [Chong et al., 1995; Rhyu, 1995]. Telomerase is active during early embryonic development, but inactive in most adult tissue. It actually makes the telomere the 'counter' of the replication of chromosome DNA. The shortening of telomeres results in stagnation of cell cycles at the checkpoint of DNA damage, and finally leads to apoptosis [Blackburn, 1991]. It has thus been hypothesized that for a tumor cell to undergo sustained proliferation beyond the limits of cellular senescence, it must reactive telomerase or use an alternative mechanism to maintain the telomerase activity [Kim et al., 1994]. Therefore, the activity of telomerase could be regulated in many ways. Recent studies have indicated that telomerase is also regulated by the cellular redox state. Antiapoptotic protein Bcl-2 upregulates telomerase activity as anti-oxidant [Hockenbery et al., 1993; Mandal and Kumar, 1997]. And hypoxia was found to be able to increase telomerase activity in human solid tumor cells [Seimiya et al., 1999; Minamino et al., 2001]. Summing up these data, the oxidative state seems to be disadvantageous to telomerase activity.

Cytokine Interleukin-2 (IL-2) has been studied extensively for its central role in the immune

Abbreviations used: IL-2, Interleukin-2; Redox, reduction/oxidation; Ref-1, redox factor-1; TRAP, telomeric repeat amplification protocol.

Weijing Xu and Mingda Yan have contributed equally in this study.

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system, having a spectrum of biological activities on the growth, differentiation, and functioning of a variety of immune and nonimmune cells [Smith, 1988]. IL-2 also has tight relationship with the intracellular redox state, whose stimulation produces a oxidative shift of the intracellular environment in the cultured BA/F3β cells [Yan et al., 2000]. According to the data described above, it seems that the IL-2-induced cellular oxidative shift could somewhat inhibit the telomerase activity. However, it has been reported that the cultured IL-2-dependent T cell CTLL-2 possess telomerase activity [Ogoshi et al., 1997], indicating IL-2 is one of the stimulator of telomerase. Therefore, there should be complicated mechanisms in the IL-2-stimulated cells to ensure the telomerase activity in such a disadvantageous oxidative intracellular environment.

As we reported previously, reduction/oxidation (redox) factor-1 (Ref-1) ensures the activation of the "redox-sensitive" transcription factor AP-1 in the disadvantageous oxidative environment induced by IL-2 [Yan et al., 2000]. Ref-1 is an important mediator of the cellular redox regulation, whose expression is stimulated by IL-2 [Lu et al., 1996]. Thus, the simultaneous appearance of the telomerase activity and Ref-1 in response to IL-2 stimulation gives us the hint that IL-2-induction of Ref-1 protein may be involved in the regulation of telomerase activity. However, it remains unclear whether there is any linkage between Ref-1 function and IL-2-stimulated telomerase activity.

In the studies presented here, we investigated the IL-2 responsiveness and the redox sensitivity of telomerase activity in the murine BA/F3β cells by the use of 'telomeric repeat amplification protocol' (TRAP) assay [Kim et al., 1994]. The impact of Ref-1 on the level of IL-2-induced telomerase activity was also examined. It was demonstrated that the addition of exogenous expressed Ref-1 protein could increase the telomerase activity in the in vitro TRAP assay, while immunodepletion of endogenous Ref-1 had some inhibitory effect. Consistent with in vitro results, IL-2-induced telomerase activity could be enhanced by transient overexpression of Ref-1 protein in BA/F3β cells. Taken together, these results established a close linkage between the Ref-1 protein and IL-2-stimulated telomerase activity.

MATERIALS AND METHODS

Materials

The IL-2, cell culture medium, and Cell Porator™ were purchased from Gibco BRL (Grand Isle, NY). α-^{32}P-dATP was obtained from Amersham, Piscataway, NJ, and X-ray film was from Kodak, Shantou, China. The plasmid of pBluescript SK was obtained from Stratagene (Cedar Creek, TX). pGEX-2T and GSH–Sepharose 4B resin were from Pharmacia, Piscataway, NJ. The antibody and Protein A-agarose were from Santa Cruz Biotech (Santa Cruz, CA).

Cell Culture and IL-2 Starvation/Restimulation

BA/F3β cells were from murine pro-B BA/F3 cells which were stably transfected with IL-2R β subunit and became IL-2-dependent [Doi et al., 1989]. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), penicillin (50 U/ml), streptomycin (50 U/ml), and IL-2 (100 U/ml) in a humidified 5% CO_2 atmosphere at 37°C. For IL-2 starvation/restimulation experiments, cells were deprived of IL-2 by washing twice with RPMI 1640 medium and suspended in RPMI 1640 with 10% FBS for 12 h, then restimulated with 200 U/ml IL-2 for the indicated time.

CHAPS Extract and Telomeric Repeat Amplification Protocol (TRAP) Assay

BA/F3β cells were collected by spin and washed twice sequentially by PBS and ice-cold wash buffer (10 mM Hepes–KOH pH 7.5, 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM dithiothreitol). Then the cells were lysed by CHAPS lysis buffer (10 mM Tris-HCl pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM phenylmethylsulfonyl fluoride, 5 mM β-mercaptoethanol, 0.5% CHAPS, 10% glycerol). After 30 min of incubation on ice, the lysate was centrifuged at 15,000 *g* for 15 min at 4°C, and the supernatant was stored at − 70°C until use. The protein concentration was determined by Bradford method.

CHAPS extract (0.1 μg) was assayed in 20 μl reaction mixture containing 1 × TRAP reaction buffer (20 mM Tris-HCl pH 8.8, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 1.75 mM $MgCl_2$, 0.005% Tween-20, 1 mM EGTA), 0.2 mM dNTP, 1 μCi α-^{32}P-dATP, 1 U Taq DNA polymerase, and 0.2 μM TS primer (5′-AATCCGTCGAGCAGAGTT-3′) at 25°C for 25 min for telomerase-mediated extension. After 0.2 μM CX primer

(5′-CCCTTACCCTTACCCTTACCCTAA-3′) was added, the samples were subjected to 30 PCR cycles of 94°C for 20 s, 48°C for 20 s, 72°C for 40 s, followed by 72°C for 5 min. The TRAP reaction products were separated by 10% nondenaturing polyacrylamide gel electrophoresis at 8 V/cm, dried, and autoradiographed [Kim et al., 1994; Wright et al., 1995].

Plasmid Constructs

pUC19-Ref-1 harboring *ref-1* gene was obtained by RT-PCR from the total RNA of BA/F3β cells using the primer set according to a GenBank record (D90374): ref-1-5′ (5′-AGAATTCACAGCGATGCCAAAGCG-3′) ref-1-3′ (5′-CGGATCCTCACAGTGCTAGGTAAAG-3′).

For the eukaryotic expression plasmid pcDNA3–Ref-1, the ref-1 cDNA in pUC19–Ref-1 was subcloned into pcDNA3. For prokaryotic expression of Ref-1, pGEX-2T harboring the *GST* gene was used. The cDNA of ref-1 in pUC19–Ref-1 was firstly subcloned into pBluescript SK by *Eco*R I and *Sal* I, then into pGEX-2T by *Bam*H I and *Sal* I (the *Bam*H I site was from pBluescript SK). The open read frame of *ref-1* gene matches with the ORF of upstream *GST* gene, thus GST–Ref-1 fusion protein can be expressed driven by P_{lac} promoter.

Prokaryotic Expression and Purification of GST Fusion Protein

pGEX-2T or pGEX-2T–Ref-1 plasmid was transformed into *E. coli* BL21(DE3) strain, and the fusion protein expression was induced by 0.5 mM isopropyl-1-thio-β-D-galactoside (IPTG) for 2 h. Cells were collected and lysed by ultrasonic and Triton X-100 (300 W for 3 s, stop for 3 s, repeat 100 times). Then GSH–Sepharose 4B resin was added to bind the GST fusion protein for 30 min. After the resin was washed 3 times by PBS, the bound protein was eluted with elution buffer (50 mM Tris-HCl (pH 8.0), 5 mM reduced glutathione).

Immunodepletion of Ref-1 Protein

Three micrograms Ref-1-specific antibody (Santa Cruz Biotech) was added into 10 μl (~25 μg) BA/F3β cell extract prepared for TRAP assay. After 1 h of incubation at 4°C, 20 μl agarose-conjugated protein A was added. The reaction mixture was shaken gently at 4°C overnight, and then spun at 12,000 *g* for 1 min. The supernatant was Ref-1-immunodepleted extract, the protein concentration was determined again by Bradford method. While the protein-A-agarose pellet was suspended in SDS–PAGE buffer, and Ref-1 protein level was detected by Immunoblotting. As the control, immunoprecipitations by other unrelated antibodies were also performed.

Immunoblotting

Cell lysate was resolved on a 10% SDS–polyacrylamide gel electrophoresis, followed by probing with Ref-1 specific antibody. As the internal control, the same blot was cut into two pieces, and the upper portion was probed with an unrelated heat shock protein (Hsp90) antibody. Low molecular mass maker was used as molecular weight standards.

Transient Eukaryotic Expression in BA/F3β Cells

Eukaryotic expression plasmid pcDNA3–Ref-1 in various amount was introduced into BA/F3β cells by electroporation using a Cell Porator™ at 675 V/cm, 17.9 ms in Opti-MEM medium containing 1.25% DMSO. After 24 h of recovery, the CHAPS extract was collected for the TRAP assay, and the protein level of Ref-1 was detected by immunoblotting. As the control, the cells were also transfected either with the vector plasmid pcDNA3 or with expression plasmid of unrelated tyrosine kinase pcDNA3–Jak3.

RESULTS

IL-2 Stimulation Increases Telomerase Activity

To examine the effect of IL-2 stimulation on telomerase activity, IL-2-dependent BA/F3β cells were deprived of IL-2 for 12 h and restimulated. After 5 h, the cellular telomerase activity was detected by TRAP assay. As shown in Figure 1, IL-2-starved sample only produced the internal TRAP assay standard band (ITAS) (lane 1), while IL-2-stimulated sample produced typical TRAP bands ladder (lane 3). And the observed telomerase activity was RNase-sensitive, as it could be inactivated completely by the pretreatment of extracts with RNase A (lanes 2 and 4). Therefore, the results in Figure 1 proved that IL-2-deprivation resulted in down-regulation of telomerase activity, while re-exposure to IL-2 led to significant upregulation. These results not only illustrated that IL-2 can stimulate the telomerase activity in BA/F3β cells but also indicated that the IL-2-dependent

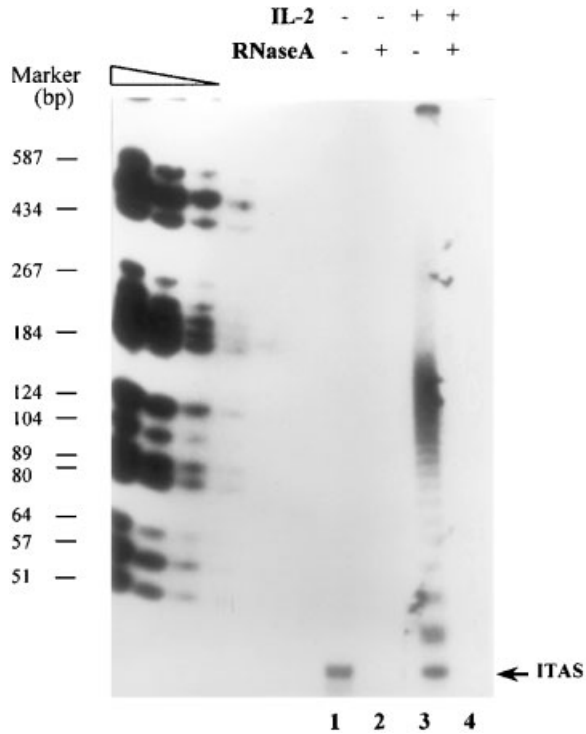

Fig. 1. IL-2 stimulation increases the telomerase activity. BA/F3β cells were IL-2-starved for 12 h and restimulated by IL-2 or not (IL-2 +/−) for 5 h. CHAPS extracts were analyzed for telomerase activity by TRAP assay (0.1 μg/reaction). Samples of **lanes 2** and **4** were pre-incubated with 0.5 μg RNase A at room temperature for 10 min to destroy template RNA before TRAP extension reactions. ITAS, internal TRAP assay standard. Marker is the terminal-labeled pBR322/*Hae* III. The results shown are representative of three similar experiments.

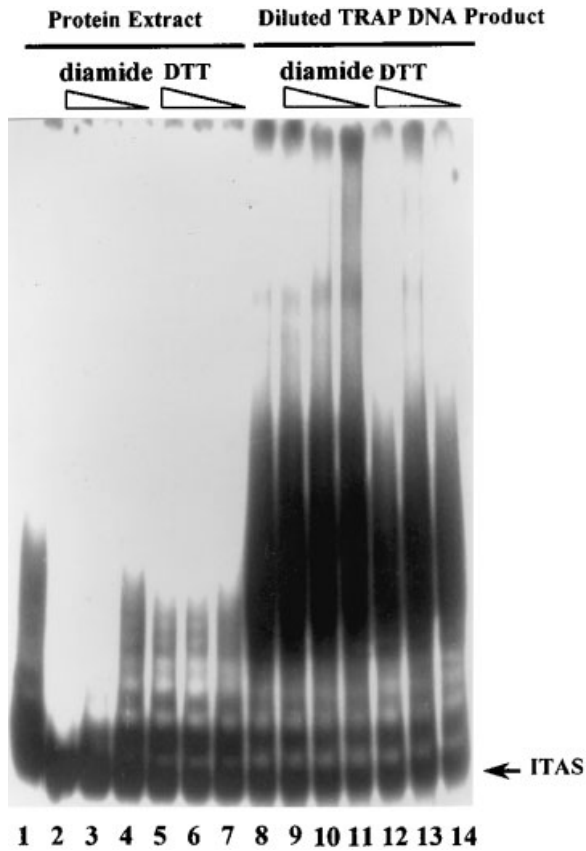

Fig. 2. Effects of DA and DTT on IL-2-induced telomerase activity in TRAP assay. BA/F3β cells were IL-2-starved for 12 h and restimulated for 5 h, CHAPS extracts were prepared and pre-incubated with oxidant diamide (**lane 2**: 10 mM; **lane 3**: 1 mM; **lane 4**: 0.1 mM) or reducing agent DTT (**lane 5**: 100 mM; **lane 6**: 10 mM; **lane 7**: 1 mM), followed by TRAP analysis (**lane 1**: untreated sample). In parallel as control, 100-fold diluted TRAP PCR products were treated with the same redox reagents and TRAP operation (**lanes 8–14**). The results shown are representative of three similar experiments.

BA/F3β cells could provide a good model to investigate the IL-2-stimulated telomerase activity.

Oxidant Inhibits the IL-2-Induced Telomerase Activity

According to the published work, telomerase activity is sensitive to the cellular redox state. To determine whether the IL-2-induced telomerase activity is affected by the change of redox status, we investigated the redox sensitivity of telomerase activity in vitro by the use of chemical reagents. The results in Figure 2 illustrated that addition of diamide (DA, a sulfhydryl oxidizing agent) into TRAP assay reaction mixture decreased the TRAP products in a dose-dependent manner (Fig. 2, lanes 2–4). While dithiothreitol (DTT, a sulfhydryl reducing agent) had no obvious effect (lanes 5–7). Since TRAP assay actually contains two separate reactions: the reverse transcription by telomerase, and the sequential PCR by Taq DNA polymerase. The potential effect of redox agents on Taq-mediated PCR was also examined using diluted TRAP DNA product as the sample, but no inhibitory effect was observed (lanes 8–14), indicating that the observed decrease in TRAP products was not due to Taq-mediated PCR, but caused by the loss of telomerase activity.

Taken together, the results in Figure 2 illustrated that oxidative status is disadvantageous to the IL-2-induced telomerase activity in vitro. However, according to our published work, IL-2 stimulation produces an intracellular oxidative shift in the cultured BA/F3β cells, which seems to be conflicted with the activation of telomerase under IL-2 treatment. Therefore, there must be some unknown mechanism to ensure the

telomerase activation under such an disadvantageous circumstances.

GST–Ref-1 Fusion Protein Enhances the IL-2-Induced Telomerase Activity

Our laboratory has previously found that the *Ref-1* gene is one of the enhanced transcribing genes in response to IL-2-stimulation in the murine CTLL-2 cells [Lu et al., 1996]. The observation that IL-2 stimulation could also increase the protein level of Ref-1 in BA/F3β cells would suggest that the induction of Ref-1 is an universal phenomenon of IL-2 signaling [Yan et al., 2000]. Located mainly in nuclei, Ref-1 protein is an important cellular redox regulator. Our laboratory previously discovered that Ref-1 ensures the activation of the ubiquitous transcription factor AP-1 in the IL-2-induced oxidative intracellular environment [Yan et al., 2000]. Thus, it was decided to examine whether Ref-1 could benefit IL-2-induced telomerase activity as well.

To examine the function of Ref-1, the bacterial expression system was applied: the mouse *ref-1* gene was cloned and the prokaryotic expressing plasmid pGEX-2T–Ref-1 was constructed and transfected into *E. coli*. Then the IPTG-induced GST–Ref-1 fusion protein was purified by glutathione–sepharose resin (Fig. 3, lanes 5–8), so did GST protein as the control (Fig. 3, lanes 1–4). Afterwards, GST–Ref-1 protein was added into TRAP reaction mixture. It was found that the TRAP products increased in the presence of GST–Ref-1 protein (Fig. 4, lanes 2 and 3). While as the control, neither the glutathione-containing elution buffer (lane 4) nor the GST protein (lanes 6, 7 compared with lane 5) caused any enhancement. The observation that the addition of exogenous Ref-1 protein lead to significant enhancement in the level of telomerase activity gave us the hint that Ref-1 may be involved in the regulation of telomerase activity, as it does on the transcription factor AP-1.

Immunodepletion of Ref-1 Decreases the IL-2-Induced Telomerase Activity

We next examined the role of endogenous Ref-1 protein in the maintenance of IL-2-induced telomerase activity. TRAP assay was performed when Ref-1 protein was eliminated by immunodepletion. The control experiments using unrelated antibody were also performed in parallel to exclude the potential effect of non-specific immunoadsorption. The results of immunoblotting in Figure 5B showed Ref-1 protein level in the immunoprecipitates, indicating Ref-1 protein was specifically immunodepleted by its antibody.

The results of TRAP assay in Figure 5A illustrated that the Ref-1-immunodepleted CHAPS extract had decreased level of telomerase activity (Fig. 5A, lane 4) compared with

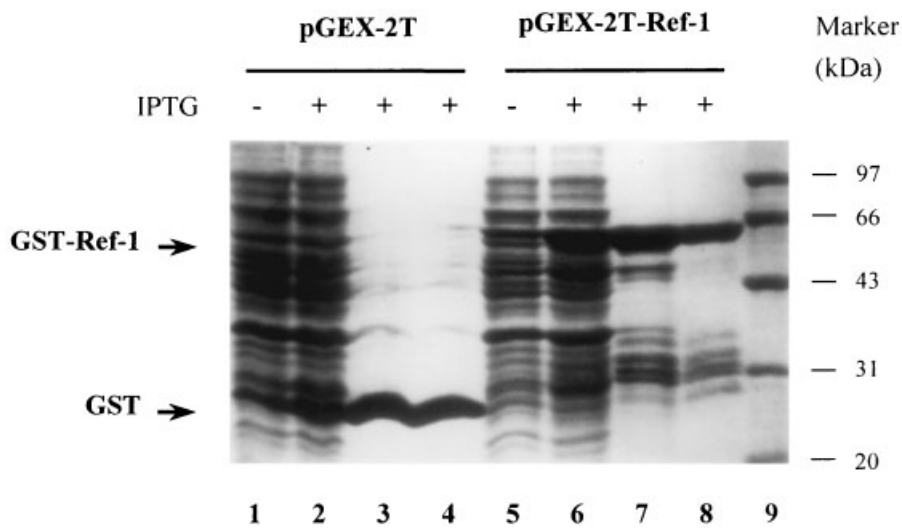

Fig. 3. Inducible prokaryotic expression and purification of GST and GST–Ref-1 proteins. Prokaryotic expression plasmid pGEX-2T–Ref-1 (**lanes 5–8**) or the control empty plasmid pGEX-2T (**lanes 1–4**) was transformed into *E. coli*. BL21 (DE3) strain and 0.5 mM IPTG was added for 2 h to induce the expression (lanes 2–4, 6–8). Cells were then lysed by ultrasonic and Triton X-100, and spun to collect the supernatants (lanes 1, 2, 5, 6). For purification, GSH–Sepharose 4B resin was added to the supernatants. Pellets were spun down (lanes 3, 7), which were then eluted (lanes 4, 8) by GSH. Samples were separated by SDS–PAGE, stained by Coomassie Blue. The results shown are representative of two similar experiments.

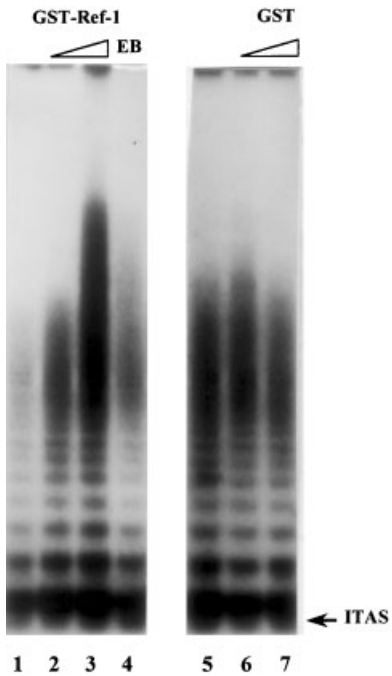

Fig. 4. Addition of exogenous Ref-1 protein to TRAP assay enhances the IL-2-induced telomerase activity. BA/F3β cells were IL-2-starved for 12 h and restimulated for 5 h. CHAPS extracts were analyzed for telomerase activity by TRAP assay (0.1 μg/reaction). Purified GST–Ref-1 (**lane 2**: 0.2 μg; **lane 3**: 1 μg), elution buffer (EB, **lane 4**), or GST (**lane 6**: 0.2 μg; **lane 7**: 1 μg) protein was added into TRAP reaction. The results shown are representative of three similar experiments.

the sample without treatment (lane 1). While the control immunodepletion with other unrelated antibodies had no inhibitory effect, either (lanes 2, 3, 5). Therefore, the functional importance of endogenous Ref-1 protein was assessed, further indicating that Ref-1 benefits the IL-2-induced activity of telomerase.

Transient Overexpression of Ref-1 in BA/F3β Cells Enhances the Telomerase Activity

To determine whether Ref-1 benefits IL-2-stimulated telomerase activity in vivo, we examined the function of overexpressed Ref-1 protein in the BA/F3β cells. In order to change the cellular Ref-1 protein level, the eukaryotic expression plasmid of Ref-1 (pcDNA3–Ref-1) was introduced into BA/F3β cells and Ref-1 was transiently overexpressed. The plasmid of pcDNA3 and pcDNA3–Jak3 were also introduced as the control. By detecting Ref-1 protein level in the various transformants, the results in Figure 6A not only verified the specific overexpression of Ref-1 protein, but also indicated that Ref-1 protein level was correspond-

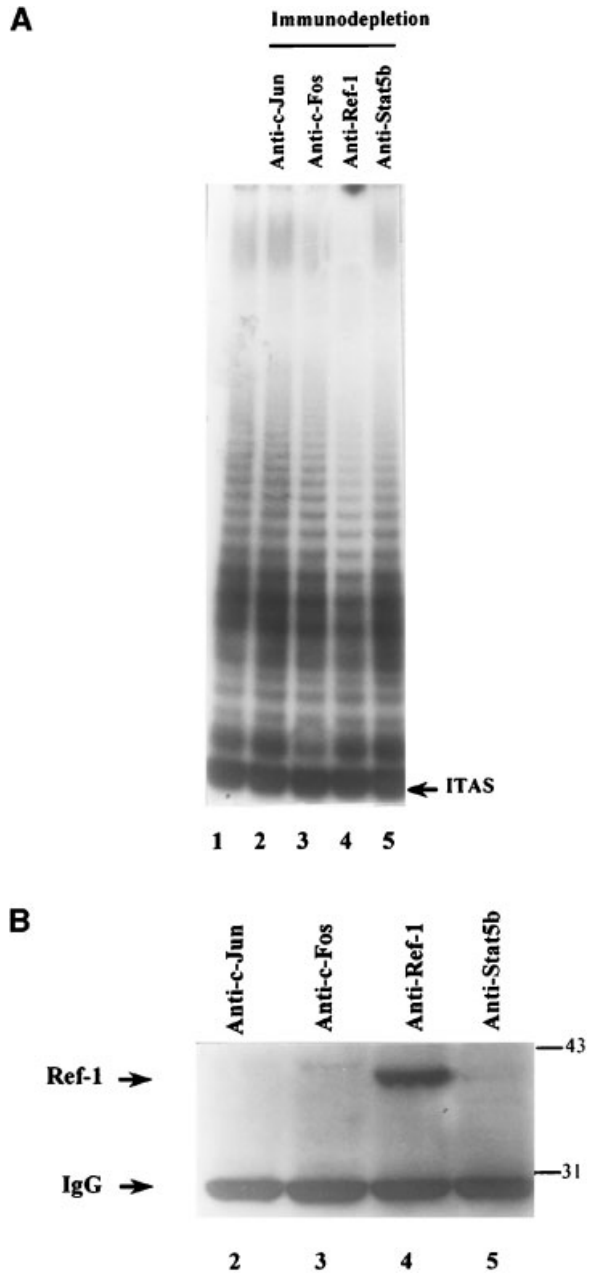

Fig. 5. Immunodepletion of Ref-1 decreases the IL-2-induced telomerase activity. BA/F3β cells were IL-2-starved for 12 h and restimulated for 5 h, then CHAPS extracts were prepared (**lane 1**). CHAPS extracts were then incubated with c-Fos- (**lane 2**), c-Jun- (**lane 3**), Ref-1- (**lane 4**), or Stat5b- (**lane 5**) specific antibody and agarose-conjugated protein A. After shaking gently at 4°C overnight, the mixture was spun to separate the supernatant and pellet. The supernatants were analyzed by TRAP assay **(A)**. While the pellets were analyzed by Western blot to detect the immunoprecipitated Ref-1 protein **(B)**, IgG indicates the light chain of antibodies. The shown results are representative of three similar experiments.

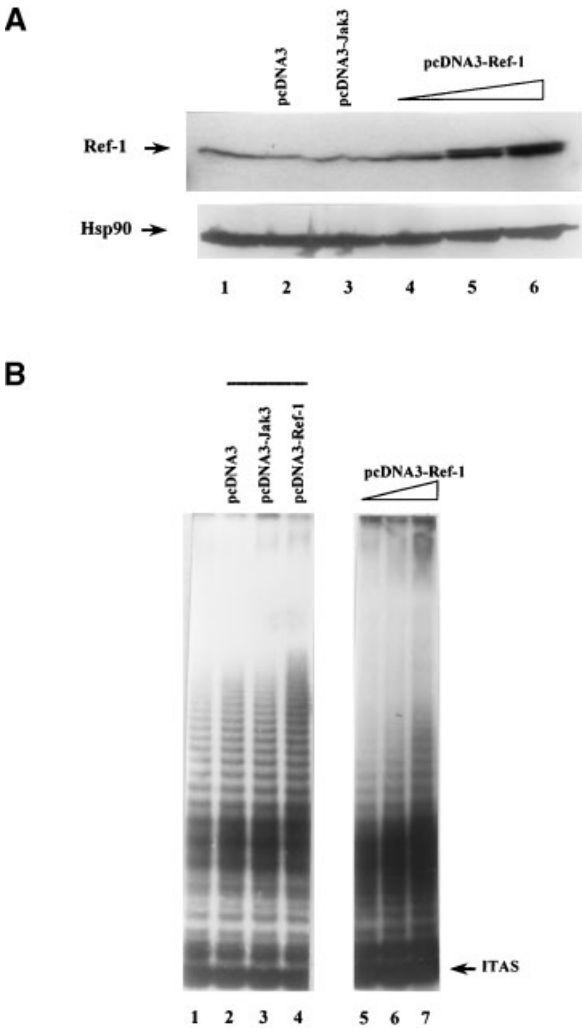

Fig. 6. Transient overexpression of Ref-1 enhanced the IL-2-stimulated telomerase activity in BA/F3β cells. BA/F3β cells were transfected by eukaryotic expression plasmid pcDNA3–Ref-1 (**lane 4**, 5 μg; **lane 5**, 10 μg; **lane 6**, 20 μg), 10 μg pcDNA3 (**lane 2**), and 10 μg pcDNA3–Jak3 (**lane 3**) or not (**lane 1**). After 24 h, CHAPS extract was collected. **A**: The protein level of Ref-1 was detected by immunoblotting, and Hsp90 protein was also detected as internal control. **B**: The CHAPS extracts were analyzed for telomerase activity by TRAP assay (lane 1, untransfected control, lanes 2, 3, 4, transfected with 10 μg pcDNA3, pcDNA3–Jak3, pcDNA3–Ref-1, respectively). And another TRAP assay with PCR cycles decreased to 28 was performed to show the Ref-1-level-dependent increase of telomerase activity (lanes 5, 6, 7, CHAPS extract from BA/F3β cells transfected with 5, 10, 20 μg pcDNA3–Ref-1, respectively). The results are representative of three similar experiments.

ing to the amount of plasmid pcDNA3–Ref-1 transfected.

The following TRAP assay demonstrated the effect of Ref-1 overexpression on the level of telomerase activity. The results in Figure 6B showed that BA/F3β cells transfected with Ref-1 cDNA (Fig. 6B, lane 4) have elevated level of telomerase activity compared with the cells transfected with empty vector or unrelated kinase Jak3 (lanes 2, 3), indicating the overexpression of Ref-1 protein remarkably enhanced the activity of telomerase. This conclusion was further supported by the observation that the enhancement is dependent on the protein level of Ref-1 (Fig. 6B lanes 5, 6, 7).

DISCUSSION

Telomerase is an important ribonucleoprotein enzyme that is responsible for adding the telomeric repeats onto the 3′end of chromosomes [Morin, 1989]. It is minimally composed of an integral RNA template (TR) and a reverse transcriptase (TERT). In addition, there are many proteins associated with telomerase, which are also critical for its proper function [Chong et al., 1995]. In our laboratory, the human TERT promoter was used to target the suicide gene to the tumor cells [Liu et al., 2002], for telomerase activity has been stimulated in 85–90% of all human tumor cells but hardly in normal cells. Therefore, the inhibition of telomerase is widely investigated in many malignant cells and by various agents [White et al., 2001]. However, the effects of the cellular circumstances on telomerase function, especially the redox state, received relatively less attention.

It has been reported that hypoxia could increase the function of telomerase in some cell types [Seimiya et al., 1999; Minamino et al., 2001]. While treatment with reducing agent vitamin C could suppress the oxidative stress partly by slowing down the telomere shorten [Furumoto et al., 1998]. Although there are still some opposite suggestions about the effect of vitamin C [Reddy et al., 2001], it remains clear that telomerase is affected by the cellular redox state. In our experiment, it was also proved that the IL-2-induced telomerase activity in BA/F3β cells is inhibited by the oxidant treatment in vitro (Fig. 2).

Many signal molecules have been discovered to be involved in the regulation of telomerase activity under the redox conditions, which include Bcl-2, P^{53}, P^{21}, etc. [Mandal and Kumar, 1997; Li et al., 1999; Harada et al., 2000]. In this article, we presented the data that telomerase is under the protection of Ref-1 to maintain its activity in disadvantageous oxidative environment of IL-2-dependent BA/F3β cells. The

evidences were put forward both in vitro and in vivo. In the TRAP assay, the addition of Ref-1 protein increased the telomerase activity (Fig. 4), while the exclusion of Ref-1 protein weakened the activity of telomerase to some extend (Fig. 5). In the IL-2-stimulated BA/F3β cells, the telomerase activity was also enhanced by the overexpression of Ref-1 protein (Fig. 6). All the results confirmed the requirement of Ref-1 on the telomerase activity. The finding that there is a linkage between Ref-1 protein and telomerase is interesting, as it provides new evidence in support of the redox regulation of telomerase activity. Since the activation of telomerase activity has been shown to be associated with the development of human cancer, our finding may provide an important insight into the molecular mechanisms involved in the process of cell malignancies.

Moreover, redox regulation plays an important role in the cellular signaling. Recent studies have put forward the concept of cellular dual redox regulation: the upstream of signal pathway may produce and/or prefer oxidation, while the downstream of signal pathway need a reductive environment [Kamata and Hirata, 1999]. Therefore, our observation would suggest that Ref-1 plays an important role in the cellular signal transduction stimulated by IL-2 as the factor in the cellular redox regulation. Increasing evidences have shown that Ref-1 expression is regulated by various signals including the thyrotropin, hypoxia, and ROS [Yao et al., 1994; Asai et al., 1997; Ramana et al., 1998]. According to our work, Ref-1 is one of the key molecules that join the paradoxical dual redox regulation together in the cellular signal transduction, ensuring the function of downstream signal transducers and effectors, such as transcriptional factor AP-1 in our previous article [Yan et al., 2000], and telomerase in this article. However, the mechanism of Ref-1 function still remains to be determined, and it is a long way to go to investigate the particular role of Ref-1 in the redox-related signaling of IL-2-stimulated cells.

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