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Cellular Stress Triggers TEL Nuclear Export Via Two Genetically Separable Pathways

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Abstract TEL (translocation ets leukemia, also known as ETV6) is a repressor of transcription that is disrupted by the t(12;21), which is the most frequent chromosomal translocation in pediatric acute lymphocytic leukemia. TEL is modified by SUMOylation, and the lysine (Lys 99) that is conjugated to SUMO is required for TEL nuclear export. In addition, TEL is phosphorylated by p38 kinase, which is activated by cellular stress. Induction of cellular stress reduced the ability of TEL to repress transcription in vitro, but the mechanistic basis of this phenomenon was unclear. In this study, we show that osmotic stress causes re-localization of TEL to the cytoplasm and that p38-mediated phosphorylation of TEL is sufficient for this re-localization. However, impairment of both SUMOylation of Lys 99 and p38-dependent phosphorylation of Ser 257 of TEL were required to impair the re-localization of TEL in response to cellular stress induced by high salt, identifying two separate nuclear export pathways. Thus, alteration of the cellular localization of TEL may be a part of the cellular stress response and re-localization of TEL to the cytoplasm is an important step in the regulation of TEL. J. Cell. Biochem. 104: 488–498, 2008.

Key words: TEL; nuclear export; repression; ETV6; transcription; cellular stress

TEL (translocation ets leukemia), also known as ETV6 (ets translocation variant gene 6) is a frequent target in chromosomal translocations in both myeloid and lymphoid leukemia. TEL was initially identified as the gene fused to the PDGF receptor ß gene in the t(5;12), which is associated with chronic myelomonocytic leukemia [Golub et al., 1994]. Subsequently, the N-terminus of TEL containing the Pointed domain (also called the helix-loop-helix or SAM domain) was found fused to other tyrosine kinases including c-ABL and JAK2 by other translocations [Papadopoulos et al., 1995; Peeters et al., 1997]. Dimerization through the TEL Pointed domain led to constitutive activation of the tyrosine kinase domain, suggesting a mechanism for how these translocations induce leukemia [Golub et al., 1996b; Schwaller et al., 1998; Ho et al., 1999; Lacronique et al., 2000]. However, the most frequent chromosomal translocation involving TEL is the t(12;21), which is the most common genetic re-arrangement found in childhood pre-B acute lymphoblastic leukemia (ALL), accounting for up to 25% of the cases [Golub et al., 1995; Romana et al., 1995b; Shurtleff et al., 1995].

TEL is a member of the ETS family of transcription factors, but rather than activate transcription, TEL is a transcriptional repressor [Hiebert et al., 1996; Chakrabarti and Nucifora, 1999; Lopez et al., 1999]. The t(12;21) fuses the N-terminal repression domain of TEL to nearly all of RUNX1 [Romana et al., 1995a; Hiebert et al., 1996]. RUNX1 both activates and represses transcription depending on the cellular context, and the fusion of the repression domain of TEL to RUNX1 creates a constitutive transcriptional repressor of RUNX1 regulated genes.

Unlike the vast majority of genes that are disrupted by chromosomal translocations in acute leukemia, the second allele of *TEL* is

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deleted in up to 90% of t(12;21)-containing cases [Romana et al., 1995b; Golub et al., 1996a; Raynaud et al., 1996]. In addition, loss of at least one allele of *TEL* was detected in several solid tumor types including breast and ovarian cancers [Hatta et al., 1997]. Thus, the tumor genetics suggested that *TEL* has tumor suppressive functions. The founding members of the ETS family, *ETS1* and *ETS2*, are oncogenes and serve as endpoints in the Ras-MAPK signal transduction cascade to activate transcription [Yang et al., 1996; Wasylyk et al., 1998]. By contrast, TEL is a repressor and opposes the oncogenic action of Ras [Fenrick et al., 2000], perhaps by repressing genes that are activated by positively acting ETS family members. TEL represses transcription by recruiting the co-repressors mSin3A and N-CoR/SMRT [Chakrabarti and Nucifora, 1999; Fenrick et al., 1999; Guidez et al., 2000] and histone deacetylases 3 and 9 [Wang and Hiebert, 2001]. In Ras-transformed cells, TEL expression induced a cellular aggregation phenotype [Van Rompaey et al., 1999; Fenrick et al., 2000] that is at least partially due to repression of the *Stromelysin-1* matrix metalloproteinase [Fenrick et al., 2000]. In addition, TEL represses *Bcl-X_L*, which may contribute to TEL-induced apoptosis [Irvin et al., 2003].

TEL shares homology to ETS transcription factors through the C-terminal DNA binding region (the ETS domain), as well as an N-terminal "Pointed" domain. The Pointed domain is homologous with the *Drosophila* protein Pointed, and mediates multiple functional interactions. It is responsible for homo- and hetero-dimerization and this fragment can even polymerize in vitro [Golub et al., 1996b; Poirel et al., 2000; Potter et al., 2000; Kim et al., 2001]. In addition, the Pointed domain binds to mSin3A and mediates transcriptional repression when fused to a heterologous DNA binding domain [Fenrick et al., 1999]. The Pointed domain also interacts with the small ubiquitin like modifier (SUMO) ligase Ubc9 and is SUMOylated at Lysine 99 [Chakrabarti et al., 1999, 2000].

The activity of TEL is regulated at multiple levels. TEL cellular localization is tightly regulated, as SUMOylation of TEL drives it into specific nuclear speckles and Lysine 99 is required for the nuclear export of TEL [Chakrabarti et al., 2000; Wood et al., 2003]. In addition, initiation of translation at Met43 of TEL creates a shorter isoform of TEL that is mostly nuclear [Lopez et al., 2003; Wood et al., 2003]. Phosphorylation of ETS factors is a common way to regulate their activity. For example, ETS2 is phosphorylated by the ERK kinases, which stimulates the ability of ETS2 to bind to DNA and activate transcription [Yang et al., 1996]. By contrast, when TEL is phosphorylated by p38, the ability of TEL to repress transcription is impaired [Arai et al., 2002], but the mechanistic basis of this regulation was unclear as Ser257 does not lie within the DNA binding domain or any other known functional domain. In addition, v-Src expression induced the re-distribution of TEL from the nucleus to the cytoplasm [Lopez et al., 2003]. However, v-Src did not phosphorylate TEL, suggesting that v-Src indirectly affected TEL activity.

In this study, we investigated how SUMOylation and p38-dependent phosphorylation of TEL affects its localization in response to cellular stress. Both cellular stress that triggers p38 activity and activation of p38 kinase activity by MAP/Kinase/Kinase 6 (MKK6) expression caused the re-localization of TEL from the nucleus to the cytoplasm. However, mutation of TEL at its p38 phosphorylation site (Serine 257) or disruption of SUMOylation of Lys99 was not sufficient to block re-localization of TEL in response to cellular stress. By contrast, re-localization was blocked when both Serine 257 and the SUMOylation site (Lysine 99) were mutated, suggesting that either pathway functions under cellular stress conditions.

MATERIALS AND METHODS

Cells and Plasmids

NIH 3T3 cells were obtained from ATCC. NIH 3T3 cells were grown in DMEM with 10% fetal calf serum. All medium contained glutamine and antibiotics. pCMV-TEL has been described [Fenrick et al., 2000]. N-terminal deletions of TEL were created by PCR using Kpn-linked 5′ primers and Xba-linked 3′ primers. The first ATG was not included in the primer design so that only HA tagged proteins would be produced. Purified PCR fragments were inserted into a modified pCMV5 vector that has the HA tag inserted after the EcoR1 site. Additional constructs were generated through subcloning the HA-TEL tagged insert into the pBabe puro vector. Point mutations

of pCMV-HA-TEL were made using the QuikChange site-directed mutagenesis kit (Stratagene, Inc., La Jolla, CA). Plasmid DNA was sequenced on an automated sequencer by the Vanderbilt Sequencing Core. Plasmids expressing activated MKK6E have been described [Han et al., 1997; Ludwig et al., 1998; Treinies et al., 1999].

Antibodies and Reagents

A polyclonal antibody made against HA was obtained from Santa Cruz, Inc. (Santa Cruz, CA). The monoclonal anti-HA antibody utilized was from Babco, Inc. (Berkeley, CA). Antibodies against the carboxy terminus of TEL and the amino terminus of TEL were as described [Fenrick et al., 1999]. Anti-rabbit and anti-mouse Alexa 546 or Alexa 488 antibodies were acquired from Invitrogen-Molecular Probes (Carlsbad, CA). Anisomyscin, normal goat serum (NGS), 6-diamidino-2-phenylindole (DAPI), and Hoescht No. 33258 were obtained from Sigma–Aldrich (St. Louis, MO).

Immunofluorescence Analysis

Cells were transfected using Superfect (Qiagen, Valencia, CA) or Lipofectamine (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Twelve hours after transfection, the cells were trypsinized and plated onto sterile glass coverslips. Thirty-six hours later, the cells were fixed in 3.7% formaldehyde/phosphate buffered saline (PBS), permeabilized in 0.1% NP-40/PBS for 10 min and blocked in 10% NGS/PBS for 30 min. Coverslips were rinsed in PBS between each step of the staining procedure. Primary antibodies were diluted in 10% NGS in PBS and incubated on the coverslip for 60 min. Endogenous TEL was visualized using a 1:1,000 dilution of the C-terminal TEL antibody. For localization of HA-TEL proteins, either a monoclonal antibody against the HA tag (Babco, Inc.) or a polyclonal antibody against the HA tag (Santa Cruz, Inc.) were used. For localization of FLAG-MKK6E a monoclonal M2 anti-FLAG (Stratagene, Inc.) was used. After washing, the coverslips were incubated for 60 min with the appropriate Alexa 488 or Alexa 568 conjugated secondary antibodies (Invitrogen-Molecular Probes) diluted in 10% normal goal serum in PBS. Coverslips were then incubated with 5 ng/ml Hoescht dye or 2 ng/ml DAPI for 10 min to visualize the DNA before the final wash. Coverslips were then inverted onto glass microscope slides with PBS/glycerol as mounting medium. Cells were analyzed on a Zeiss Axiophot microscope using Zeiss 40× or 63 × 1.4 oil immersion objectives.

Induction of Cellular Stress

NIH 3T3 cells were transfected and plated to glass coverslips. Thirty-six hours post-transfer the media were removed and samples were rinsed with 1× PBS and new media were added containing 0.7 M NaCl for 15 min or 100 μg/ml Anisomycin for 30 min. Cells were then rinsed twice with 1× PBS before further analysis. In addition, some samples were allowed to recover from the NaCl treatment. Media containing the salt was removed, samples were rinsed twice with 1× PBS and complete media was added back to the cells. Samples were then incubated for 2 h at 37°C, 5% CO_2 prior to analysis. Additional samples underwent 2.5 h of ultra violet (UV) light irradiation (180 J/m^2) or were subjected to heat shock by incubation at 42°C, 5% CO_2 for 30 min.

Reporter Assay

NIH 3T3 cells were transiently transfected with indicated amounts of pCMV plasmids encoding HA-TEL or HATEL(K99R/S257A) with or without FLAG-MKK6E and the firefly luciferase reporter *pGL2-754TR*. A plasmid encoding Renilla luciferase (pRL-null, Promega) was used as an internal control for the transfection efficiency and the pCMV vector was used as a control. pBlueScript was used to balance the DNA content in each sample. Samples were assayed 48 h after transfection according to manufacturer's directions using the Dual-Luciferase Assay System (Promega) and the levels of firefly relative luciferase unit activity were normalized to renilla luciferase activity.

Statistical Analysis

The Prism software package was used to determine the statistical significance of individual samples using the Student's *t*-test. For these comparisons, 100–200 cells were manually counted per sample and each experiment was performed in biological triplicates to allow statistical analysis.

RESULTS

Cellular Stress Induces Cytoplasmic Localization of TEL

TEL is phosphorylated directly by p38 and ERK kinases, and co-expression of p38 and TEL decreased TEL-mediated repression [Arai et al., 2002; Maki et al., 2004]. Therefore, we tested whether TEL cellular localization was affected by p38-dependent phosphorylation during cellular stress. NIH 3T3 fibroblasts that stably express HA-TEL were stressed using anisomycin, a trigger of a p38-mediated stress response, ultraviolet irradiation, or a heat shock. Indirect immunofluorescence indicated that there was an increase in the cytoplasmic localization of TEL only in response to anisomycin (Fig. 1).

Although anisomycin activates both the p38 and JNK1 MAP kinases [Cruzalegui et al., 1999], TEL does not appear to be phosphorylated by JNK1 [Arai et al., 2002]. Given that high salt concentrations in the media preferentially triggers p38 activation and that high salt enhanced the phosphorylation of TEL [Arai et al., 2002], we tested the effect of high salt stress on TEL cellular localization. Indirect immunofluorescence of TEL-expressing cells that were cultured in DMEM containing 0.7 M NaCl for 15 min, indicated that TEL was efficiently exported from the nucleus (Fig. 2A). When the high salt was washed from the cells and the cells were cultured in normal media for 2 h, TEL re-entered the nucleus (Fig. 2B), indicating that this treatment is reversible. These data suggest that cellular stress regulates the subcellular localization of TEL.

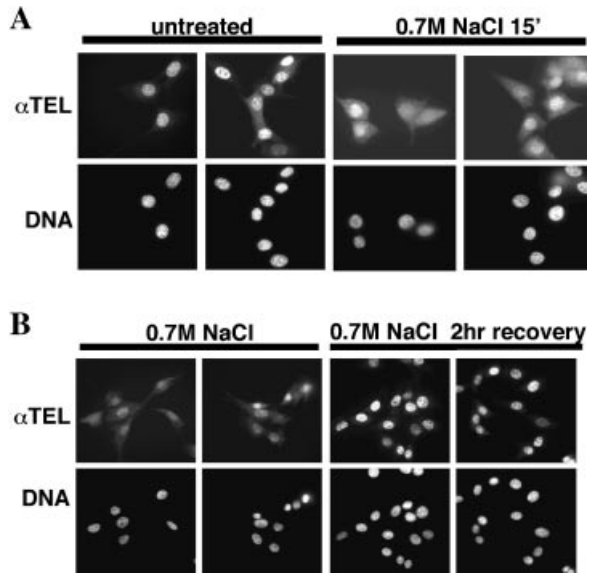

Fig. 2. High salt induces the reversible nuclear export of TEL. **A**: Osmotic stress triggers the nuclear export of TEL. NIH 3T3 cells that stably express an HA-TEL were treated with 0.7 M NaCl for 15 min and TEL was localized using immunofluorescence. Duplicate panels are shown. **B**: High salt inducible nuclear export of TEL is reversible. Cells were treated with 0.7 M NaCl and analyzed after 15 min or the cells were washed and allowed to recover for 2 h prior to immunofluorescence. TEL was identified using anti-HA and cells were counterstained with a Hoechst dye to stain DNA.

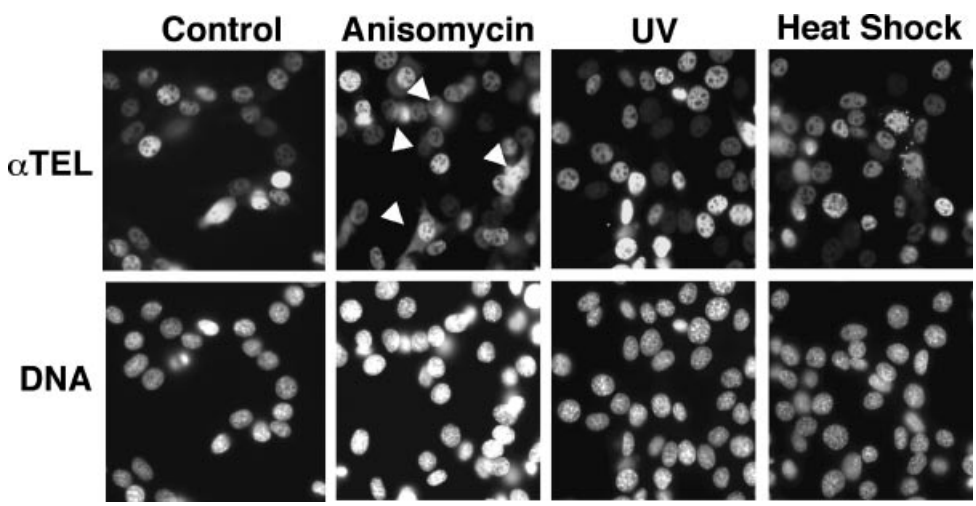

Fig. 1. Cellular stress induces the cytoplasmic localization of TEL. NIH 3T3 cells that stably express an HA tagged form of TEL were treated with anisomyosin (100 μg/ml) for 30 min, irradiated with ultra violet light (180 J/m^2) for 2.5 h, or incubated at 42°C for 30 min. TEL was identified using anti-HA and cells were counterstained with a Hoechst dye to stain DNA. White arrowheads in the anisomycin treated panel indicate cells with cytoplasmic TEL.

TEL Re-Localization Is Induced by Co-Expression of Activated MKK6

The cellular stress data pointed toward p38 as a key regulator of TEL cellular localization. Because a constitutively active form of p38 is not available, we asked whether co-expression of an activated MAP Kinase upstream of p38 could regulate the subcellular localization of TEL. Flag tagged constitutively active Map Kinase Kinase-6 mutant (MKK6E) was co-expressed by transient transfection with TEL, and the subcellular localization of TEL was defined by indirect immunofluorescence. MKK6E, a direct activator of p38 [Enslen et al., 1998; Roux and Blenis, 2004], caused the re-distribution of TEL to the cytoplasm (Fig. 3A,B; $P < 0.01$). These data, coupled with the cellular stress data, suggest that p38 mediated phosphorylation of TEL regulates the nuclear export of TEL to the cytoplasm.

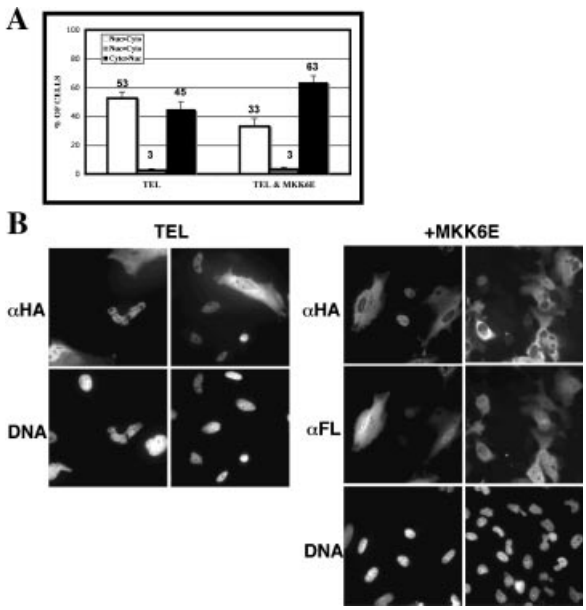

Fig. 3. TEL accumulates in the cytoplasm in the presence of activated MKK6. **A**: Activated MKK6 triggers the cytoplasmic accumulation of TEL. NIH 3T3 cells were transiently transfected with an HA-tagged form of TEL with or without a FLAG tagged form of active MKK6 (MKK6E). The nuclear and cytoplasmic localization of TEL was examined manually for each sample and shown as a bar graph. Two hundred cells were counted per sample and the results were verified independently by two individuals. Individual cells were placed in one of the following categories: Nuclear > Cytoplasmic if localization to the nucleus was >80%, Nuclear = Cytoplasmic if localization was <80% in one compartment or the other, or Cytoplasmic > Nuclear if localization to the cytoplasm was >80%. Results represent the average of three individual experiments with error bars representing standard deviation. **B**: An example of the immunofluorescence data used in (A). Immunofluorescence was performed using polyclonal anti-HA (Santa Cruz) and/or anti-FLAG to visualize TEL and the co-expressed kinase, respectively. Duplicate panels are shown for each condition tested. The cells were stained with a Hoechst dye to visualize the DNA.

N-Terminal Deletion Analysis of TEL Localization

The N-terminal portion of TEL is important for its cytoplasmic localization, as TEL is alternatively translated at Methionine 43 and this N-terminally truncated form of TEL is predominantly nuclear [Lopez et al., 2003; Wood et al., 2003]. In addition, there are 12 potential Ser/Thr/Tyr residues located in the TEL amino-terminus, which could regulate localization of TEL. Although TEL is phosphorylated on Ser22, mutation of this residue to Alanine did not affect its localization (data not shown). Therefore, we first examined this domain for regulation of nuclear export by making several amino-terminal truncations to identify the motif(s) in this domain that regulates cytoplasmic localization of TEL (Fig. 4A). These deletion constructs were made with an amino terminal HA-epitope tag and expressed transiently to define their intracellular trafficking. When TEL was transiently expressed it localized to both the nucleus and the cytoplasm, but mostly to the latter (Fig. 4B,C) [Wood et al., 2003]. When the mutants were transiently expressed, deletion to residues −14, or −24 did not greatly alter TEL localization. However, further deletion to −30 caused a moderate shift in the cytoplasmic to nuclear ratio and further deletion to −38 caused TEL to localize predominantly to the nucleus in much the same manner as the −43 isoform (Fig. 4B,C; $P = 0.01$ for −38 and $P = 0.008$ for −43 compared to TEL). Thus, −30 marks the amino-terminal boundary of a domain that is required for TEL cytoplasmic localization. However, sequence analysis of the region from −30 to the pointed domain did not reveal a nuclear export signal sequence (Fig. 4A) and fusing of this domain to GFP did not lead to exclusion of GFP from the nucleus, suggesting that this domain contains a cytoplasmic retention motif rather than an export signal. In addition, mutation of Thr 31 (within a MAP kinase consensus sequence) to alanine did not affect the subcellular localization of TEL and the −38 deletion responded like wild type TEL to osmotic stress (data not shown).

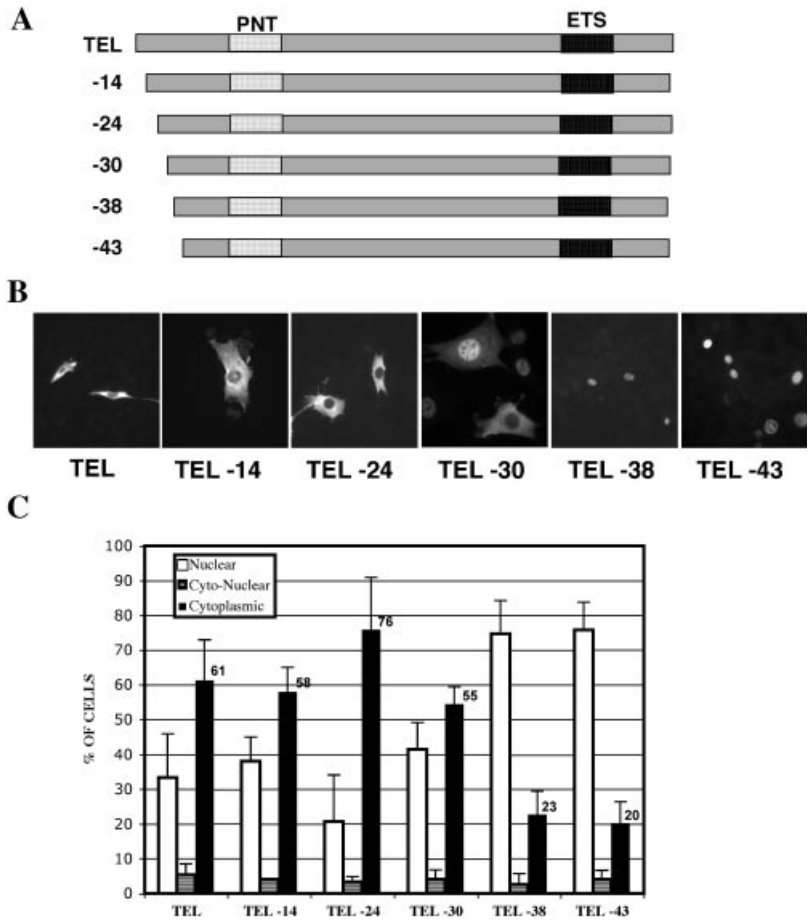

Fig. 4. N-terminal truncations of TEL affect its subcellular localization. **A**: Schematic diagram of the N-terminal deletion constructs of TEL that were generated with a HA-epitope tag. **B**: Localization of N-terminal deletion mutants of TEL. The mutants displayed in (A) were transiently transfected into NIH 3T3 cells and immunofluorescence was used to localize TEL. **C**: Quantification of the cytoplasmic/nuclear localization of the expression of the TEL N-terminal deletion mutants. Two hundred cells were counted per sample and results were verified independently by two individuals. Cells were manually counted and placed in the follow categories: Nuclear > Cytoplasmic if localization to the nucleus was >80%, Nuclear = Cytoplasmic if localization was between <80% nuclear or cytoplasmic, or Cytoplasmic > Nuclear if localization to the cytoplasm was >80%. Results represent the average of three individual experiments with error bars representing standard deviation, except for the −14 and −30 samples where the data represent two separate experiments with standard error.

TEL S257 and TEL K99 Contribute to the Regulation of TEL Nuclear Export

Lysine 99 of TEL is required for its nuclear export, as mutation of this residue causes the nuclear accumulation of TEL [Wood et al., 2003]. Therefore, we asked if the TEL K99R mutant was affected by high salt treatment and/or co-expression with constitutively activated MKK6 in NIH 3T3 fibroblasts. As observed previously, TEL localized to both the nucleus and cytoplasm, while the K99R mutant preferentially localized to the nucleus (Fig. 5A,B) [Wood et al., 2003]. High salt treatment or co-expression with MKK6E resulted in an increase in the cytoplasmic localization of TEL as previously observed (Fig. 5A,B; $P = 0.022$). When high salt was added to the culture medium or MKK6E was co-expressed with TELK99R, it also accumulated in the cytoplasm, suggesting that SUMOylation was dispensable for nuclear export in response to cellular stress signals and indicating that TEL can be exported via a second nuclear export pathway [Fig. 5A (upper panels), B; $P = 0.011$].

Serine 257 of TEL is a major p38-dependent phosphorylation site and when this amino acid was changed to an alanine (S257A), TEL phosphorylation induced by salt treatment was

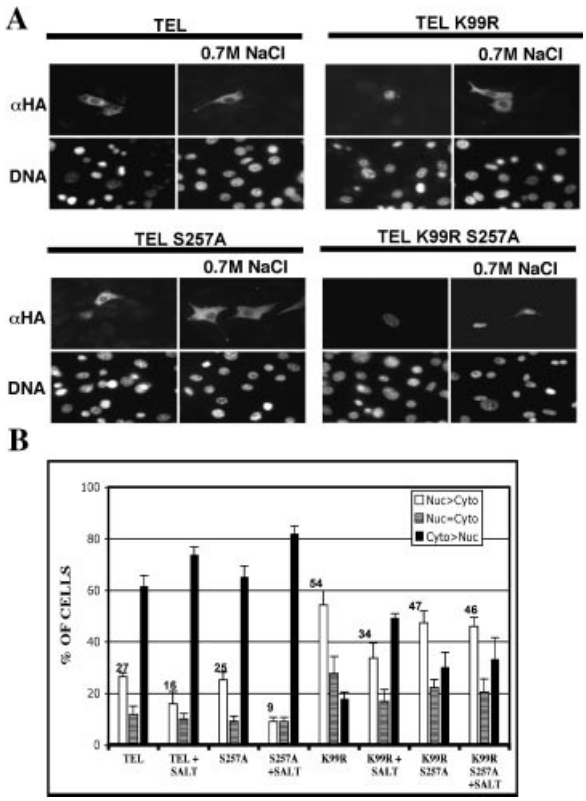

Fig. 5. K99 and S257 contribute to the regulation of TEL in response to high salt. NIH 3T3 fibroblasts were transiently transfected with various HA tagged point mutant TEL constructs. **A**: A representative example of the localization of the TEL mutants using immunofluorescence. Immunofluoresence assays were performed using a polyclonal antibody against the HA tag and cells were stained with DAPI to visualize DNA. **B**: Quantification of TEL localization. Each transfected or cotransfected cell was examined manually and placed in one of the following categories: Nuclear > Cytoplasmic if localization to the nucleus was >80%, Nuclear = Cytoplasmic if localization was <80% if either compartment or Cytoplasmic > Nuclear if localization to the cytoplasm was >80%. At least 100 cells were counted per sample and results represent the average of three individual experiments with error bars representing standard deviation.

impaired [Arai et al., 2002]. Moreover, p38-dependent phosphorylation of TEL impaired its ability to repress transcription even though Ser257 does not reside within a known functional domain. When the S257A mutant of TEL was transiently expressed, its subcellular localization mirrored that of TEL and upon high salt treatment it too was exported from the nucleus [Fig. 5A (lower left panel), B; $P = 0.001$]. However, TEL containing both the SUMOylation and p38 specific phosphorylation mutations (TEL K99R/S257A) localized predominantly in the nucleus and when these cells were treated with high salt there was no change in localization [Fig. 5A(lower right panel),B]. Similar results were obtained for these mutants when co-expressed with MKK6E (Fig. 6A,B;

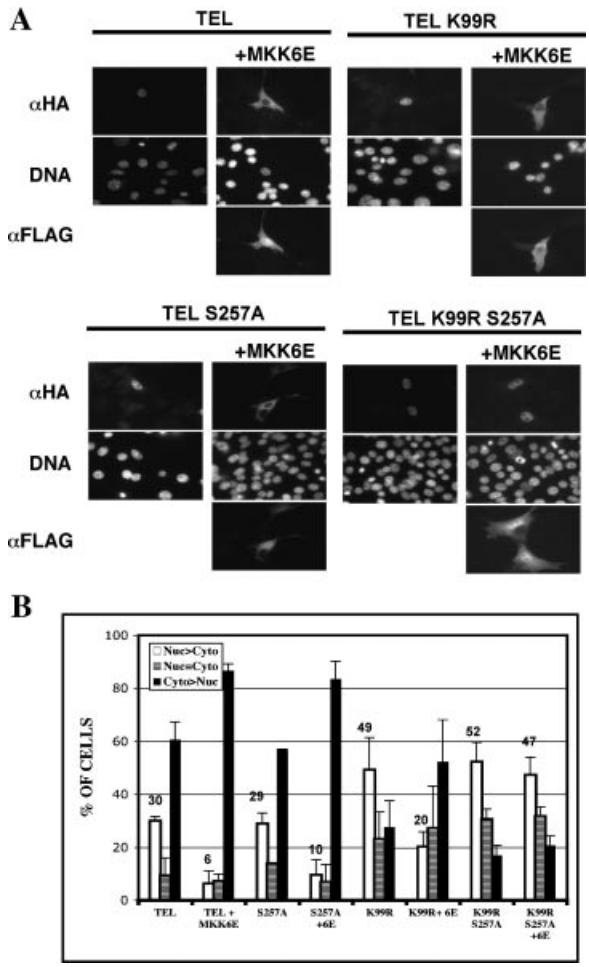

Fig. 6. Mutation of K99 and S257 impairs the redistribution of TEL induced by activated MKK6. NIH 3T3 fibroblasts were transiently transfected with HA tagged TEL and HA tagged TEL containing the indicated point mutations. These constructs were co-expressed with a FLAG tagged constitutively activated version of MKK6 (MKK6E). Immunofluoresence assays were performed using a polyclonal antibody against the HA tag and a monoclonal antibody against the FLAG tag. Cells were stained with DAPI to visualize DNA. **A**: A representative example of the immunofluorescence data. **B**: Quantification of TEL localization. Each transfected cell was examined manually and placed in one of the following categories: Nuclear > Cytoplasmic if localization to the nucleus was >80%, Nuclear = Cytoplasmic if localization was <80% in either compartment or Cytoplasmic > Nuclear if localization to the cytoplasm was >80%. At least 100 cells were counted per sample and results represent the average of three individual experiments with error bars representing standard deviation. The statistical significance for the change in nuclear localization upon expression of MKK6E for each construct is as follows: TEL, $P = 0.0011$; S257A, $P + 0.01$; K99R, $P = 0.02$; K99R/S257A, $P = 0.43$.

see legend for P values). Thus, nuclear export mediated by either Lys99 or Ser257 controls TEL in response to cellular stress.

A consequence of stress-induced re-localization of TEL to the cytoplasm would be the loss of TEL-mediated repression. Therefore, we tested the effects of cellular stress on TEL using co-expression of MKK6E and TEL. When TEL was transiently expressed with a plasmid containing the *Stromelysin-1* promoter linked to luciferase (pGL2-TR754) TEL repressed its activity as compared to the control vector, and no effects were observed on the internal control promoter (Fig. 7; $P = 0.037$). When constitutively active MKK6 (MKK6E) was co-expressed with TEL this repression was relieved, supporting the hypothesis that the ability of TEL to repress is impaired upon its re-localization to the cytoplasm. Although a double mutation of S213E/S257E impaired the ability of TEL to bind to DNA [Maki et al., 2004], TEL K99R/S257A repressed the *Stromelysin-1* promoter as well as wild type TEL (Fig. 7; $P = 0.021$). However, co-expression with MKK6E, did not affect TEL K99R/S257A-mediated repression, suggesting that nuclear export of TEL contributes to the regulation of TEL in response to cellular stress.

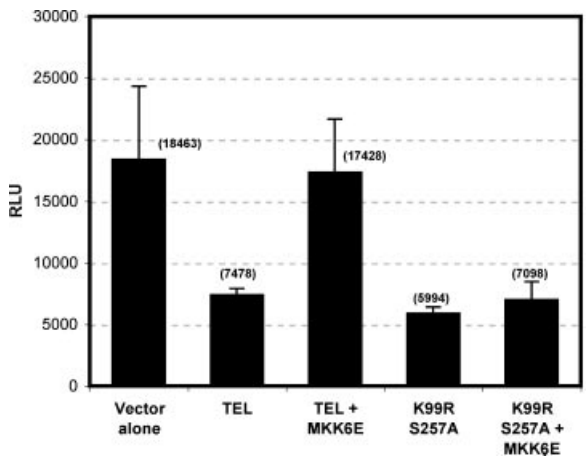

Fig. 7. Activated MKK6 impairs TEL, but not TEL(K99R/S257A)-dependent repression. NIH 3T3 cells were transiently transfected with 0.1 μg of luciferase reporter *pGl2-754TR* and 0.1 μg of pCMV5 HA tagged TEL or pCMV5 HA tagged K99R S257A with or without 0.05 μg of pCMV5 FLAG tagged MKK6E. To normalize for transfection efficiency each sample was also transfected with 0.01 μg of the pRL-null luciferase reporter and pBluescript II KS to balance DNA content. The pCMV5 empty vector was used as a further control and sample lysates were prepared and assayed 48 h after transfection using a Dual Luciferase Reporter Assay System (Promega) via measurement in a luminometer. Results are expressed as relative luciferase units (RLU) and shown is the average of three assays with the error bars representing standard deviation. Student *t*-tests comparing HA-TEL or HA-TEL mutant RLUs to pCMV5 empty vector alone were calculated to measure significance ($P < 0.05$).

DISCUSSION

TEL is regulated by both cytoplasmic retention/nuclear import and nuclear export [Lopez et al., 2003; Wood et al., 2003]. The studies presented here begin to shed light on the physiological significance of this regulation and place it in a cellular stress context. We found that TEL is exported from the nucleus to the cytoplasm in response to specific cellular stresses that characteristically activate the p38 signal transduction pathway. While constitutively active forms of p38 are not available, a constitutively active form of the upstream kinase, MKK6E, was sufficient to trigger TEL nuclear export. The most obvious effect of cytoplasmic localization of TEL is that TEL is unable to carry out its duties in the nucleus as a repressor of transcription and this was confirmed in transcription assays (Fig. 7). These results thus provide a cellular mechanism by which p38-mediated phosphorylation impairs TEL-dependent repression.

The regulation of TEL subcellular localization by high salt or anisomycin may have important implications for the overall cellular response to stress. The rapid nuclear export of TEL (less than 15 min, Fig. 2) would allow the immediate activation of genes repressed by TEL. ETS family proteins regulate many genes that stimulate growth and survival, including several genes that are induced in response to growth factors [Hsu et al., 2004]. TEL is a potent transcriptional repressor that negatively regulates some of these genes, as well as Bcl-X_L, which contributes to cell survival [Irvin et al., 2003]. One outcome of re-localizing TEL from the nucleus to the cytoplasm may be that genes such as *Bcl-X_L* are de-repressed, which would allow positively acting factors, such as ETS-2, to potently activate the expression of these genes to increase survival [Sevilla et al., 2001; Zhang et al., 2005]. Unfortunately, our conditions for inducing robust TEL cytoplasmic localization were too harsh to be maintained for extended periods of time such that an analysis of changes in gene expression profiles could be obtained. Nevertheless, it is notable that positively acting

ETS family members such as Elk-1 are also phosphorylated by p38 and this modification stimulates the ability of Elk-1 to activate transcription [Janknecht and Nordheim, 1996; Enslen et al., 1998; Li et al., 2003]. The coordinate regulation of both activating and repressing ETS family members should lead to rapid and robust changes in gene expression to allow the cell to survive the stress.

The mutation of K99R in the context of S257A points toward two independent pathways to regulate TEL nuclear export. Mutation of Lys99 within the Pointed domain of TEL impaired the nuclear export of TEL in the absence of stress [Wood et al., 2003], but this mutant was exported to the cytoplasm after high salt treatment or when it was co-expressed with MKK6E (Figs. 5 and 6). Thus, a second nuclear export pathway, which is independent of SUMOylation, can regulate TEL functions. Moreover, the N-terminal deletion analysis (Fig. 4) coupled with the demonstration that the N-terminus of TEL is sufficient to retain a heterologous protein in the cytoplasm [Lopez et al., 2003], suggests that phosphorylation of Ser257 contributes to nuclear export rather than cytoplasmic retention. That is, p38 dependent phosphorylation may direct the export of TEL, where it then can be retained by an N-terminal-dependent mechanism.

TEL is not only disrupted by the t(12;21) in B-cell ALL, but the second allele is also deleted in the vast majority of these cases, suggesting that it is a tumor suppressor [Romana et al., 1995b; Golub et al., 1996a; Raynaud et al., 1996]. Mechanistically, TEL may oppose the oncogenic action of Ras by repressing downstream targets of Ras-dependent activation, which is often mediated by ETS factors [Wasylyk et al., 1998; Fenrick et al., 2000]. The nuclear export of TEL in response to osmotic stress may provide further clues to how the loss of TEL contributes to tumorigenesis. That is, TEL may act as a key regulatory node to modulate genes that regulate the cellular stress response. In this model, TEL inactivation would de-repress a network of genes that are normally activated in the face of cellular stress and that promote cellular survival. The rapid reversibility of the high salt mediated nuclear export of TEL (Fig. 2) may suggest that TEL also functions in the termination of the stress response signal. If so, LOH of TEL would not only allow for more robust signaling by the ERK/p38 kinase pathways, either in response to growth factors or cellular stress, but also increase the duration of the signal once the growth factor or stress was removed.

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