Posttranslational modification of TEL and TEL/AML1 by SUMO-1 and cell-cycle-dependent assembly into nuclear bodies

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The E-26 transforming specific (ETS)-related gene, *TEL*, also known as *ETV6*, encodes a strong transcription repressor that is rearranged in several recurring chromosomal rearrangements associated with leukemia and congenital fibrosarcoma. TEL is a nuclear phosphoprotein that is widely expressed in all normal tissues. TEL contains a DNA-binding domain at the C terminus and a helix–loop–helix domain (also called a pointed domain) at the N terminus. The pointed domain is necessary for homotypic dimerization and for interaction with the ubiquitin-conjugating enzyme UBC9. Here we show that the interaction with UBC9 leads to modification of TEL by conjugating it to SUMO-1. The SUMO-1-modified TEL localizes to cell-cycle-specific nuclear speckles that we named TEL bodies. We also show that the leukemia-associated fusion protein TEL/AML1 is modified by SUMO-1 and found in the TEL bodies, in a pattern quite different from what we observe and report for AML1. Therefore, SUMO-1 modification of TEL could be a critical signal necessary for normal functioning of the protein. In addition, the modification by SUMO-1 of TEL/AML1 could lead to abnormal localization of the fusion protein, which could have consequences that include contribution to neoplastic transformation.

Posttranslational modifications of proteins are important signaling mechanisms during cell cycle, cell development, and cell differentiation. The ubiquitin system is one of the major protein-modification systems required for the highly selective turnover of specific proteins in eukaryotic cells (1). Ubiquitin conjugation involves a protein complex that includes the E1, E2, and E3 enzymes, and it results in formation of an isopeptidic bond between the C terminus of ubiquitin and the ε-amino group of a lysine in the target protein, leading to degradation of the protein through the 26S proteosome pathway. Eukaryotic cells also express a group of ubiquitin-like proteins, such as SUMO-1 (also known as PIC-1 or UBL1) and RUB-1 (2, 3), that are conjugated to the target protein by an isopeptidic bond similar to that of the ubiquitin system. In contrast to ubiquitination, however, the covalent attachment of ubiquitin-like proteins does not lead to protein degradation. Recently, it was shown that UBC9, a homologue of the E2 enzyme, conjugates SUMO-1 to the target protein (4). Only a few SUMO-1 target proteins have been identified, including PML and SP100, which when modified by SUMO-1 are found in the PML bodies, RanGAP1, IkBα, HIPK2, and p53 (5–8). The exact function of SUMO-1 modification is not known. In some cases (IkBα and p53), it was shown that conjugation of SUMO-1 could lead to protein stabilization and protection from degradation, but in other cases (PML, SP100, RanGAP1, and HIPK2), SUMO-1 conjugation could lead to different subcellular localization of the modified protein (5–8).

The *TEL* gene is also known as *ETV6. TEL* was originally cloned as one of the two genes rearranged in a t(5;12) chromosomal translocation associated with chronic myelomonocytic leukemia. This translocation also disrupts the platelet-derived growth factor receptor beta (*PDGFRβ*) gene on chromosome 5 (9). Further studies showed that *TEL* is involved in many chromosomal rearrangements, most often with genes that encode tyrosine kinases (9–13). The most frequent translocation involving *TEL* is t(12;21), which is the most common chromosomal rearrangement in childhood cancers and involves the transcription factor AML1 (also known as RUNX1; refs. 14, 15). *TEL* is also a frequent target of chromosomal deletions in patients with B cell leukemia (15).

TEL is a nuclear phosphoprotein that is widely expressed in all normal tissues and is a member of the E-26 transforming specific (ETS) family of transcription factors (16). TEL is characterized by a DNA-binding domain at the C terminus that is partially homologous to the ETS DNA-binding domain (10). TEL contains a helix–loop–helix (HLH) domain at the N terminus, also called a pointed domain (10), which is necessary for homotypic dimerization and for protein–protein interaction with the ubiquitin-conjugating enzyme UBC9 and with the ETS-family protein FLI1 (17, 18). By using chimeric mice as a model, it was shown that TEL is the first transcription factor specifically required for hematopoiesis within the bone marrow, confirming the importance of this gene in the normal development of the hematopoietic system (19). TEL also functions as a transcription repressor that represses the target gene through the histone–deacetylase pathway (20–22).

Previously, by using TEL as bait in a yeast two-hybrid screen, we identified UBC9 as a protein that interacts with TEL, and we showed that the interaction between TEL and UBC9 did not lead to the degradation of TEL (17). Here we show that the substrate of UBC9, SUMO-1, modifies TEL by conjugation to a specific lysine residue of the HLH domain and that the modification leads to localization of TEL to a type of nuclear speckle, the TEL bodies, which are cell-cycle dependent. The leukemia-associated fusion protein TEL/AML1 is also modified by SUMO-1 and localizes to the TEL bodies. In sharp contrast, AML1 is found in nuclear compartments that are quite distinct from those that contain TEL or TEL/AML1. That finding suggests that variation in posttranslational modification of TEL fusion proteins and subcellular localization might be additional factors contributing to leukemogenesis.

Materials and Methods

Plasmids Used. The full-length cDNAs of *TEL* and *UBC9* as well as the deletion mutants of *TEL* were subcloned in frame with the Gal4 DNA-binding domain in the pGBT9 (CLONTECH) or in the pCMV-HA (HA, hemagglutinin) or pCMV-Flag vectors as described elsewhere (17). Plasmids pCMV-Flag-SUMO-1 and

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Abbreviations: HLH, helix–loop–helix; HA, hemagglutinin.

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pCMV-Flag-AML1 were obtained by cloning the cDNAs in frame with the Flag epitope in pCMV (17). Plasmid pCMV-HA-TEL/AML1 was obtained by cloning the HA-tagged cDNA in plasmid pCMV. All cloning junctions were verified by DNA sequencing.

Yeast Two-Hybrid Analysis. To test the interaction between TEL and SUMO-1, we used the yeast two-hybrid system with pGBT9-TEL and pGAD-SUMO-1. The yeast strain pJA69, transformed with plasmid pGAD-SUMO-1 (1) and with the wild-type or one of the mutant pGBT9-TEL plasmids, was plated on selective medium (histidine or adenine-deficient medium) to isolate recombinant colonies according to the manufacturer's instructions (CLONTECH).

Site-Specific Mutagenesis of TEL. To introduce amino acid mutations, oligonucleotides that encode amino acids targeted for mutations (K99 to R99) were synthesized and used in PCRs. The PCR-synthesized fragments containing the mutations were inserted in the TEL cDNA by using existing convenient restriction sites. The replaced fragments and the restriction site junctions were sequenced.

Western Blot and Coimmunoprecipitation Assays. To detect the endogenous TEL protein by Western blot analysis, we used the human erythroleukemia cell line HEL, which expresses a high level of TEL. The TEL rabbit polyclonal antibody was a gift of O. Bernard (U434 Inserm-CEPH, Paris, France). To detect SUMO-1-modified TEL, COS7 cells (0.7×10^6 cells per 100-mm plate) were transiently transfected by the calcium phosphate precipitation method (Invitrogen) with plasmids encoding HA-TEL and/or Flag-SUMO-1. For immunoprecipitation analysis, the cells were lysed with 1 ml of Nonidet P-40 lysis buffer [20 mM Tris·Cl, pH 8.0/100 mM NaCl/1 mM EDTA/1% Nonidet P-40/0.1% SDS, and protease inhibitors (1 μg/ml aprotinin/1 μg/ml leupeptin/1 μg/ml pepstatin A/1.0 mM PMSF)]. The cell lysate was rocked gently for 3 h at 4°C with anti-Flag-M5 antibody (Eastman Kodak) and incubated with Protein G-Agarose beads (Sigma) for 1 h. After incubation, the beads were washed three times with Nonidet P-40 buffer and boiled for 3 min in 2× SDS/PAGE sample buffer. The proteins were separated by SDS/PAGE, transferred to a nylon membrane, and detected by using anti-HA antibody (Roche Molecular Biochemicals). For cellular fractionation, the cells were washed with cold PBS and resuspended in hypotonic buffer (10 mM Hepes, pH 7.9/1.5 mM MgCl2/10 mM KCl/1 mM DTT, and protease inhibitors). Nuclear and cytoplasmic fractions were obtained by treating the cells with 0.25% Nonidet P-40 for 10 min and were centrifuged to separate the nuclear fraction (pellet) from the cytoplasmic fraction (supernatant).

Immunostaining of Cells. To identify the intracellular localization of the proteins by confocal microscopy, 293 cells lacking the simian virus 40 T antigen were cultured on cover slips and transiently transfected by the calcium phosphate precipitation method. The cells were fixed and immunostained directly on the slides, as described (17). PML antiserum was purchased from Santa Cruz Biotechnology. Confocal laser scanning microscopy was performed with a Zeiss LSM510 microscope.

Cell-Cycle Synchronization and Flow Cytometry Analysis. For these assays, we used 293 cells lacking the simian virus 40 T antigen. The transiently transfected cells were arrested in G_1/S boundary by using thymidine-aphidicoline block as described by Everett *et al.* (23). The release from the block was obtained by removal of the drugs from the culture medium. The cells were collected at different time points for cytochemical analysis as described (17) and for flow cytometry analysis.

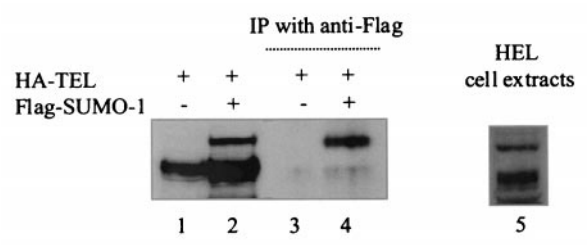

Fig. 1. TEL is covalently modified by SUMO-1. COS7 cells were transfected with HA-TEL alone (lanes 1 and 3) or with HA-TEL and Flag-SUMO-1 (lanes 2 and 4). Cell lysates (lanes 1 and 2) and the anti-Flag coimmunoprecipitation reactions (lanes 3 and 4) were subjected to Western blot analysis with anti-HA antibody. The higher molecular size band in lane 2 corresponds to SUMO-1-modified TEL, as confirmed by the coimmunoprecipitation results of lane 4. Lane 5, untransfected HEL cell lysates were separated, transferred to nylon membrane, and probed with anti-TEL antibody. Two bands were identified corresponding to unmodified and modified endogenous TEL. IP, immunoprecipitation.

Results

***In Vivo* Modification of TEL by SUMO-1.** By using the yeast two-hybrid system with the entire TEL protein or the HLH domain only as baits, we determined that in yeast the HLH domain of TEL interacts with SUMO-1 (data not shown). To determine whether TEL is modified by SUMO-1, COS7 cells were transfected either with a plasmid encoding HA-TEL or with plasmids encoding HA-TEL and Flag-SUMO-1. The addition of a plasmid encoding UBC9 was not necessary, because this enzyme is expressed very abundantly in the cell (17). In contrast, a band corresponding to SUMO-1-modified TEL was detected only when the transfected DNA included the plasmid encoding SUMO-1 (Fig. 1), indicating that the level of endogenous SUMO-1 protein is limiting. Total cell extracts were analyzed by Western blot with anti-HA antibody. In cells transfected with HA-TEL only, a major band of about 55 kDa corresponding to HA-TEL was readily identified (Fig. 1, lane 1). In cells transfected with both plasmids, two bands were detected: a lower band corresponding to HA-TEL and a new, larger band of about 70 kDa corresponding to Flag-SUMO-1-modified HA-TEL (Fig. 1, lane 2). To confirm that the larger size band did indeed correspond to Flag-SUMO-1-modified HA-TEL, total cell lysate was immunoprecipitated with anti-FLAG antibody and incubated with Protein G-Agarose beads for 1 h. The precipitated proteins were then separated by electrophoresis, transferred to a nylon membrane, and visualized with anti-HA antibody. A band corresponding to SUMO-1-modified TEL was detected in cells cotransfected with HA-TEL and Flag-SUMO-1 (Fig. 1, lane 4) but not in cells transfected with HA-TEL only, confirming that the larger-size, slower migrating band shown in lane 2 is SUMO-1-modified TEL. We consistently found that the band corresponding to Flag-SUMO-1-modified HA-TEL was less intense than that of the unmodified HA-TEL. To confirm that the modification of TEL by SUMO-1 was a normal, biologic event, we analyzed the total cell lysate of untransfected HEL cells with TEL antibody. The results (Fig. 1, lane 5) show two bands identical in size and relative intensity to those of the transfected cells, confirming that the endogenous TEL is also partially modified by covalent conjugation to SUMO-1.

TEL and SUMO-1 Colocalize in Mammalian Cells. Previous studies speculated that one of the roles of SUMO-1 might be the translocation of target proteins to specific subcellular sites (3). To determine whether the subcellular localization of TEL is influenced by SUMO-1 conjugation, we analyzed the subcellular distribution of HA-TEL and Flag-SUMO-1 in 293 cells trans-

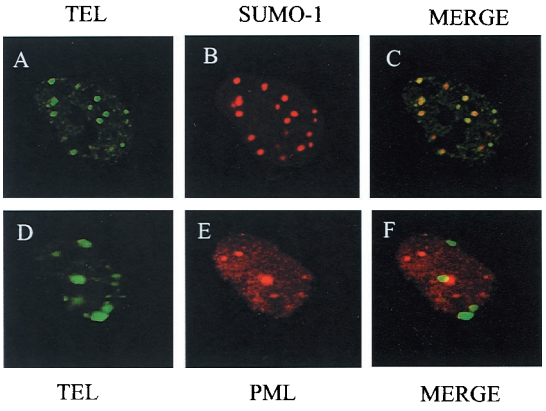

Fig. 2. SUMO-1-modified TEL localizes to the TEL bodies. 293 cells grown on coverslips were transiently transfected with HA-TEL and Flag-SUMO-1. The cells were fixed and immunostained with anti-HA rat/anti-rat-FITC antibodies and with anti-Flag mouse/anti-mouse Texas Red antibodies for HA-TEL detection (*A*) or for Flag-SUMO-1 detection (*B*). The images were overlapped to determine colocalization (*C*). The transfected 293 cells were treated as described and immunostained with anti HA antibody for TEL detection (*D*) and with anti-PML antibody for PML detection (*E*). (*F*) Overlap of *D* and *E*.

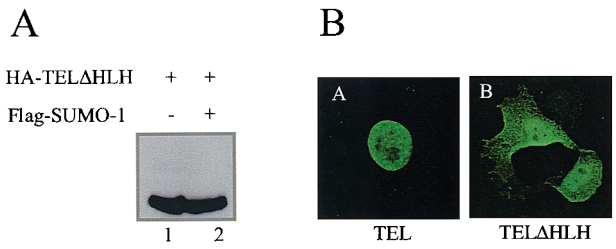

Fig. 4. The HLH domain of TEL is required for nuclear localization and SUMO-1 conjugation. (*A*) COS7 cells were transfected with HA-TELΔHLH alone (lane 1) or with HA-TELΔHLH and Flag-SUMO-1 (lane 2). After protein separation, the cell lysates were analyzed with anti-HA antibody. Absence of a SUMO-1-modified band in lane 2 indicates that the HLH domain of TEL is required for modification. (*B*) 293 cells transfected with HA-TEL (*A*) or with HA-TELΔHLH (*B*) were immunostained with anti-HA rat/anti-rat-FITC antibodies. TELΔHLH is present in the cytoplasm and nucleus of cells.

fected with one or both genes. Immunocytochemical analysis of cells transfected with HA-TEL showed a predominantly diffuse nuclear localization of the protein, which was excluded from subnuclear regions probably corresponding to the nucleoli. This pattern concurs with our previous results and those of other investigators (data not shown; refs. 16 and 17). In contrast, Flag-SUMO-1 was detected in brightly stained nuclear speckles corresponding to the PML nuclear bodies previously described (data not shown; refs. 5–8). When HA-TEL and Flag-SUMO-1 were coexpressed in 293 cells, each protein was localized to identical nuclear bodies in about 10% of the cells (Fig. 2 *Upper*). In the remaining cells, HA-TEL had a diffused nuclear pattern similar to that observed in the absence of SUMO-1. These bodies, which we termed TEL bodies, appeared to be dynamic structures varying in size and number in different cells (see Fig. 2 *A* and *D*). The TEL bodies were not identical to the PML bodies when the cells were costained with anti-TEL and anti-PML antibodies (Fig. 2 *Lower*).

The TEL Bodies Are Assembled During the S Phase. To determine whether the assembly of the TEL bodies is transient and is regulated by the cell cycle, we used confocal microscopy to analyze cell populations that had been synchronized at the G_1/S boundary and that were collected at different times after release. To correlate the TEL bodies with a specific time of the cell cycle, the cycling state of the cells was determined by flow cytometric analysis. The combined results of these analyses (Fig. 3) suggest that the TEL bodies are transient structures that are preferentially formed during the S phase.

Necessity of HLH Domain of TEL for SUMO-1 Modification and Nuclear Localization. In yeast, the HLH domain of TEL is essential for TEL to interact with both UBC9 and SUMO-1. Therefore, we examined whether the HLH domain is also necessary for *in vivo* modification by SUMO-1. Total extracts from COS7 cells transfected with HA-TELΔHLH and Flag-SUMO-1 were subjected to Western blot analysis with anti-HA antibody. Only a band corresponding to the unmodified deletion mutant was clearly detected in cells transfected only with TELΔHLH or with all of the plasmids (Fig. 4*A*, lanes 1 and 2), indicating that the HLH domain of TEL is required for SUMO-1 modification. To determine the pattern of cellular localization of a TEL mutant protein lacking the HLH domain, we performed immunocytochemical analysis of 293 cells transfected with HA-TELΔHLH. To our surprise, we found that in the absence of the HLH region, TELΔHLH was present in the nucleus and in the cytoplasm and did not colocalize with SUMO-1 (Fig. 4*B*). These results were confirmed by Western blot analysis of cytoplasmic and nuclear fractions of cells transiently transfected with either HA-TELΔHLH or HA-TEL (data not shown).

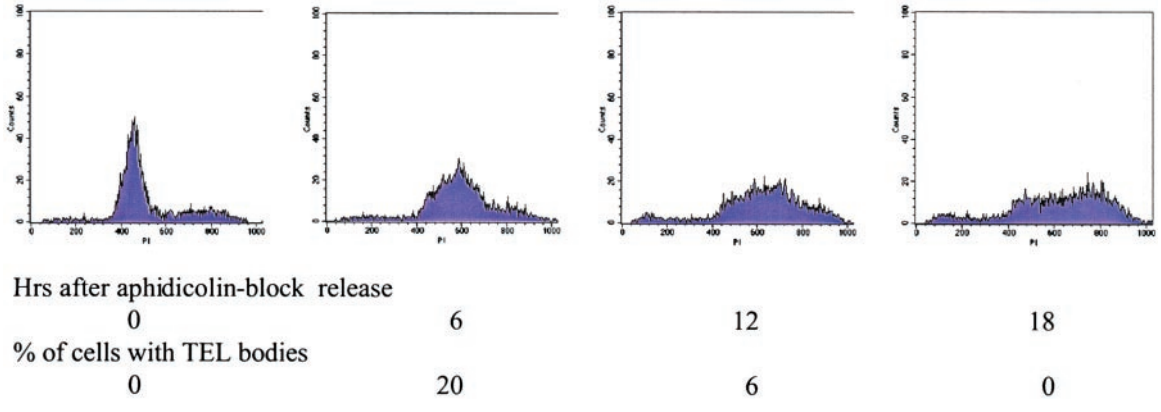

Fig. 3. The TEL bodies are assembled during the S phase. The growth of 293 cells transfected with HA-TEL and Flag-SUMO-1 was arrested in G_1/S by addition of aphidicolin to the culture medium. After removal of the drug, the cells were cultured in normal medium and collected at the times indicated. An aliquot of the cells was observed by confocal microscopy, and the number of cells with TEL bodies was counted. The remaining cells were analyzed by flow cytometry.

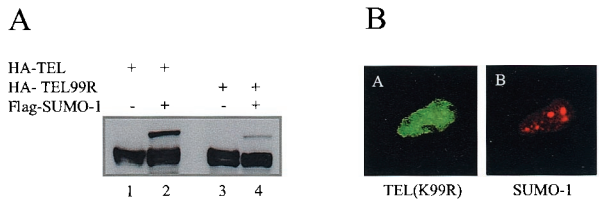

Fig. 5. Lysine residue K99 is preferentially targeted for SUMO-1 conjugation. (*A*) COS7 cells were transfected with HA-TEL (lane 1) or HA-TELK99R (lane 3) alone or with Flag-SUMO-1 (lanes 2 and 4). The cell lysates were subjected to Western blot analysis with anti-HA antibody. The higher molecular size band in lane 2 corresponding to SUMO-1-modified TEL is less abundant in lane 4. (*B*) 293 cells transfected with HA-TELK99R and Flag-SUMO-1 were immunostained and analyzed by confocal microscopy. The K99R mutant TEL shows a predominantly diffuse pattern (*A*) that does not affect the speckled pattern of SUMO-1 (*B*).

Lysine 99: A Target of SUMO-1 Modification. It was reported that the covalent modification of a target protein by SUMO-1 occurs at a preferential lysine residue (24). Although there is no known amino acid consensus for the lysine residues that are modified by SUMO-1, we noted that in IkBα and in SP100 the targeted lysine is next to another lysine and a glutamate. Therefore, for site-directed mutation we selected the lysine residue (K99), which is followed by a glutamate in the HLH region of TEL. Total extracts from COS7 cells transfected with HA-TEL(K99R) and Flag-SUMO-1 were analyzed by Western blot with anti-HA antibody. We observed that the relative abundance of SUMO-1-modified TEL was reduced compared with wild-type TEL (Fig. 5*A*, lanes 2 and 4), indicating that K99 could be the preferential lysine residue for SUMO-1 conjugation. Densitometric analysis of the bands indicated that, although the ratio between SUMO-1-modified and unmodified TEL bands was 1:6, the ratio for TEL(K99R) bands was 1:15, confirming that the extent of SUMO-1 modification was reduced in the mutant. In agreement with this result, we observed diffuse nuclear staining and rare speckles for cells cotransfected with both the K99R TEL point mutation and SUMO-1 (Fig. 5*B*), confirming that K99 is one of the predominant modification sites for SUMO-1.

Modification of TEL/AML1 by SUMO-1 and Localization of TEL/AML1 to the TEL Bodies. To determine whether the leukemia-associated fusion protein TEL/AML1, which contains the HLH domain of TEL, is also modified by conjugation of SUMO-1, we analyzed total extracts of COS7 cells transfected with HA-TEL/AML1 and Flag-SUMO-1 by Western blot analysis. The results (data not shown) indicate that TEL/AML1 is modified by SUMO-1 conjugation. To correlate the conjugation of SUMO-1 with the subcellular localization of the fusion protein, HA-TEL/AML1 was transiently coexpressed with Flag-SUMO-1 in 293 cells. In about 10% of the cells, HA-TEL/AML1 was found in nuclear bodies similar to those observed for TEL and SUMO-1. However, a minority of the nuclear speckles did not overlap with those identified by SUMO-1 (Fig. 6 *A–C*). Fluorescence-activated cell sorter analysis of synchronized cells transiently transfected with TEL/AML1 showed that the speckles containing TEL/AML1 are assembled during the S phase (data not shown).

TEL/AML1 and AML1 Have Distinct Nuclear Localization. TEL/AML1 contains the entire AML1 protein. To compare the localization of the two proteins, we coexpressed HA-TEL/AML1 and Flag-AML1 in 293 cells. Whereas in a subpopulation of the transfected cells TEL/AML1 has a speckled pattern that can be superimposed on that of SUMO-1 (Fig. 6 *Upper*), AML1 is consistently found in large nuclear speckles in all of the transfected cells (Fig. 6*E*). These speckles do not overlap with those containing TEL/AML1 (Fig. 6 *Lower*). Indeed, we observed consistently that TEL/AML1 and AML1 occupy nonoverlapping and mutually exclusive sites in the nucleus.

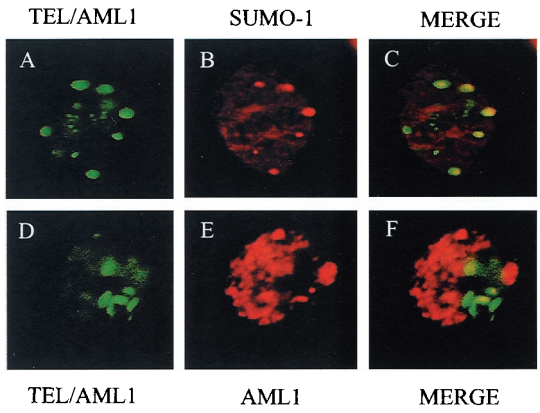

Fig. 6. TEL/AML1 is localized to the TEL bodies and not with AML1. COS7 cells transfected either with HA-TEL/AML1 and Flag-SUMO-1 (*A–C*) or with HA-TEL/AML1 and Flag-AML1 (*D–F*) were analyzed by confocal microscopy as described in the legend of Fig. 2. Immunostaining shows that TEL/AML1 colocalizes with SUMO-1 (*C*) but not with AML1 (*F*).

Discussion

In this study, we have shown that TEL is posttranslationally and covalently modified by conjugation of the small protein modifier SUMO-1. Our results show that this modification requires the HLH domain of TEL and involves mainly a specific lysine residue of the HLH domain, K99. In addition, we found that the HLH domain is necessary for specific and complete nuclear localization of TEL. The modification of TEL by SUMO-1 leads to the compartmentalization of TEL to specific nuclear speckles, the TEL bodies, which are distinct from other nuclear bodies containing SUMO-1, such as the PML bodies. Several different types of nuclear bodies have been described. In general, the nuclear bodies are considered active centers for nuclear functions, and it has been predicted that they have a role in transcription, chromatin structure, control of cellular growth, and apoptosis (25). The TEL bodies appear to be dynamic structures varying in size and number in each cell, and they apparently are formed and disrupted according to the cell cycle. In contrast to most nuclear bodies, the TEL bodies are observed only during a limited time of the cell cycle, according to our results during the S phase. Thus, it is possible that the assembly of the TEL bodies depends on signals that regulate a specific phase of the cell cycle. At this time, we have no conclusive data linking the observed cell-cycle-dependent assembly of the TEL bodies to the cell-cycle-specific modification by SUMO-1. It is possible that TEL is always present as a SUMO-1-modified protein. However, Western blot analysis has shown only partial modification of TEL suggesting that only a fraction of the cells contains SUMO-1-modified TEL. If the modification of TEL by SUMO-1 is cell-cycle regulated, then this case would be similar to the situations of PML and SP100, for which modification by SUMO-1 is regulated in a cell-cycle-dependent manner (25, 26). In agreement with this hypothesis, it was shown that when the yeast enzymes required for SUMO-1 conjugation and hydrolysis were inactivated, a G_2/M block in the yeast cell resulted (27), reinforcing the hypothesis that, as for PML, the modification of TEL by SUMO-1 depends on factors that are active during a specific phase of the cell cycle.

Another important role of SUMO-1 modification is protection of the modified protein from degradation by the proteosome

pathway. This function is especially important for proteins that are essential for cell-cycle progression, such as p53, known to be rapidly degraded by the ubiquitin pathway but protected from degradation by SUMO-1 conjugation (28). If similar but distinct families of ubiquitinating enzymes regulate either protein degradation or stability, then the choice between the two opposite fates must be regulated by factors that are active when the protein must either carry out a signal or has completed its task.

TEL is one of the genes most often rearranged in human leukemia, and the inappropriate SUMO-1 modification of fusion proteins that result from chromosomal rearrangements with *TEL* could have several implications. The most frequent rearrangements of *TEL* involve genes encoding a tyrosine kinase and result in fusion proteins that maintain the oligomerization domain of HLH. Several investigators (29, 30) have shown that the homotypic interaction of the chimeric tyrosine kinase leads to constitutive self-activation and phosphorylation of a cascade of effectors ultimately resulting in continuous growth signals. Although there is no doubt that the inappropriate activation of the kinase is the critical mechanism leading to transformation, it is also possible that modification of the HLH domain by SUMO-1 stabilizes the fusion protein so it is not degraded, thus increasing the damage.

Another mechanism by which TEL could alter the normal functions of the fusion partner is by forcing the fusion protein to inappropriate subcellular domains after SUMO-1 modification. We have examined the leukemia-associated transcription factor TEL/AML1 and found that it was modified by SUMO-1 and present in the TEL bodies. Thus, through its fusion to the HLH domain and after SUMO-1 modification, TEL/AML1 could inappropriately carry along to the TEL bodies unknown proteins with which AML1 might normally interact.

Chromosomal translocations are commonly associated with a variety of human leukemias, and they often result in fusion transcription factors. Several investigators have proposed that one of the major consequences of the leukemia-associated rearrangements could be the alteration of the properties of the resulting fusion transcription factors, thus leading to inappropriate regulation of lineage-specific genes that are critical in controlling the growth and the correct differentiation of the hematopoietic cells. Whereas this hypothesis is very attractive and is supported by the results of many reporter gene studies, it is likely that the role of the fusion proteins could be much more complex, involving, at least in some cases, their localization to nonpertinent subcellular sites. This situation has been described for the PML protein, found in the PML bodies only after modification by SUMO-1 (31, 32). Similar to the situation with TEL, it was shown that SUMO-1-unmodified PML was unable to migrate to the PML bodies. However, in contrast to TEL/AML1, the leukemic fusion protein PML/RARα lost the ability to be modified efficiently by SUMO-1 and to localize efficiently to the PML bodies. In the case of acute promyelocytic leukemia (APL), on treatment with retinoic acid, which is the treatment of choice for this disease, the fusion protein PML/RARα reacquires the ability to be modified by SUMO-1 and to migrate to the PML bodies. Therefore, it seems that, at least for PML/RARα, there is a correlation between correct cellular growth and differentiation, and the ability to conjugate SUMO-1 and to migrate to the appropriate subnuclear structures. At this time, we do not know whether treatment with Daunomycin or any other drugs currently used for patients with TEL/AML1 would restore the proper migration of TEL/AML1 to the AML1 sites.

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