

DNA Damage-induced Translocation of the Werner Helicase Is Regulated by Acetylation*

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Werner syndrome is a rare autosomal recessive disorder involving the premature appearance of features reminiscent of human aging. Werner syndrome occurs by mutation of the WRN gene, encoding a DNA helicase. WRN contributes to the induction of the p53 tumor suppressor protein by various DNA damaging agents. Here we show that UV exposure leads to extensive translocation of WRN from the nucleolus to nucleoplasmic foci in a dose-dependent manner. Ionizing radiation also induces WRN translocation, albeit milder, partially through activation of the ATM kinase. The nucleoplasmic foci to which WRN is recruited display partial colocalization with PML nuclear bodies. The translocation of WRN into nucleoplasmic foci is significantly enhanced by the protein deacetylase inhibitor, Trichostatin A. Moreover, Trichostatin A delays the re-entry of WRN into the nucleolus at late times after irradiation. WRN is acetylated *in vivo*, and this is markedly stimulated by the acetyltransferase p300. Importantly, p300 augments the translocation of WRN into nucleoplasmic foci. These findings support the notion that WRN plays a role in the cellular response to DNA damage and suggest that the activity of WRN is modulated by DNA damage-induced post-translational modifications of WRN and possibly WRN-interacting proteins.

Werner syndrome, a rare autosomal recessive disorder, is considered a useful model for investigating human aging (1). Werner syndrome (WS)¹ patients exhibit a wide range of features typical of human aging and in addition are highly predisposed to the emergence of benign and malignant neoplasms (2). Cultured cells from WS patients exhibit a shortened life span and a prolonged S-phase of the cell cycle (3). WS occurs by mutation of the WRN gene, a member of the RecQ DNA helicase family (4). There are five known human recQ helicases (for

recent review, see Ref. 5). Mutations in two other family members, BLM and RecQ4, are responsible for the premature aging syndromes, Bloom's (6) and Rothmund Thomson (7), respectively. WRN is a double-stranded DNA unwinding enzyme that additionally possesses exonuclease activity (8–12). In normal human fibroblasts, WRN steady state protein levels are relatively constant throughout the cell cycle, and the WRN protein is localized mainly to the nucleolus (13, 14). Following treatment of cells with 4NQO, an alkylating agent, WRN is no longer detected in the nucleolus (13). WRN was found to interact directly with numerous proteins, including proliferating cell nuclear antigen and Topoisomerase I (15), Replication Protein A (RPA) (16), p53 (17, 18), the Ku complex (19), and DNA Pol δ (20). In this regard, WRN has been suggested to be a functional component of various cellular processes, including DNA replication, transcription, recombination, and repair. However, the precise physiological role of WRN remains to be elucidated.

DNA damage alters the subcellular localization of many proteins, as exemplified by ING1 (21), 53BP1 (22), and BLM (23). The effects of DNA damage are often exerted through post-translational modification of target proteins, as shown for instance for p53 and Mdm2 (24–28). Although most of the work addressing the role of post-translational modifications in the DNA damage response has focused on protein phosphorylation, there is now growing evidence that protein acetylation may also play an important role (25, 29–31). Furthermore, acetylation can modulate the function of many proteins, their interaction with other proteins, and their subcellular localization (25, 27, 32–35).

To explore possible links between WRN and the DNA damage response, we investigated the effect of several types of DNA damage on the subcellular distribution of WRN. We report that in response to UV exposure, WRN translocates from the nucleolus to nucleoplasmic foci. The rate and extent of WRN redistribution are correlated with the UV dose. Importantly, p300 promotes WRN acetylation *in vivo*, while cotransfection with p300 or treatment with Trichostatin A (TSA), a deacetylase inhibitor, mimics the effect of DNA damage and causes translocation of WRN to discrete nucleoplasmic foci. Similarly, TSA inhibits the reentry of WRN from such foci back into the nucleolus at late times after irradiation. The nucleoplasmic foci to which WRN is recruited upon irradiation or TSA treatment exhibit partial colocalization with PML nuclear bodies, which are known to contain protein acetyltransferases. Together, these findings support the notion that WRN may play a role in the DNA damage response pathway, perhaps in signaling or in facilitating specific types of DNA repair, in a manner that is regulated by protein acetylation.

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¹ The abbreviations used are: WS, Werner syndrome; TSA, Trichostatin A; GFP, green fluorescence protein; PCNA, proliferating cell nuclear antigen; ATM, ataxia telangiectasia mutation; IR, ionizing radiation; DAPI, 4',6-diamidino-2-phenylindole hydrochloride.

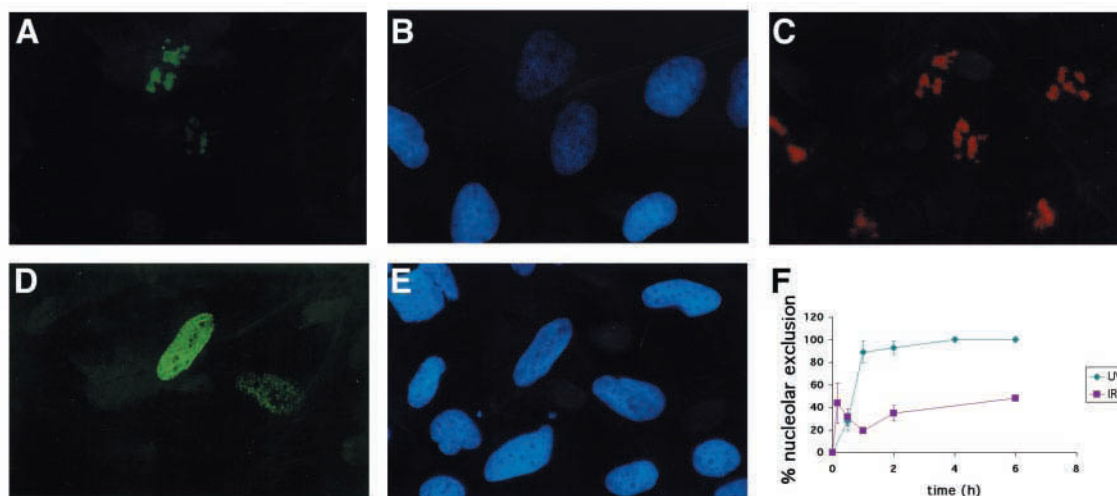


FIG. 1. Transfected WRN translocates from the nucleolus to the nucleoplasm in response to DNA damage. U2OS cells were transiently transfected with a GFP-WRN expression plasmid. Twenty-six hours post-transfection, cells were either exposed to 40J/m² UV radiation (D and E) or left untreated (A–C). Three hours later, cells were fixed and stained with DAPI (B and E) and an anti-B23 antibody to visualize nucleoli (C). GFP-WRN was detected with an appropriate filter (A and D). Panel F displays a kinetic analysis of the proportion of cells in which WRN has completely translocated from the nucleoli into nucleoplasmic foci, following exposure to UV (40J/m²) or ionizing radiation (10 Gray).

EXPERIMENTAL PROCEDURES

Cells, Plasmids, and Transient Transfections.—WI-38 normal human fibroblasts, as well as fibroblasts from individuals diagnosed as WS patients (AG00780), were obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (Coriell Institute, Camden, NJ). U2OS human osteosarcoma cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Human fibroblasts were maintained in Eagle's minimum essential medium containing 20% fetal calf serum. Cells were incubated at 37.0 °C in 5% CO₂. The GFP-WRN expression plasmid was a kind gift from Dr. S. M. Weissman (36). Transient transfection was performed by the calcium phosphate method as previously described (18).

Irradiation and Drug Treatment.—For UV irradiation, U2OS cells (300,000 per 60-mm dish) or human fibroblasts (60,000 per 35-mm dish) were washed with phosphate-buffered saline and irradiated in a UV Stratalinker 1800 (Stratagene), predominantly at 254 nm. Phosphate-buffered saline was then replaced with fresh medium. For ionizing radiation (IR) treatment, cells were exposed to a GB-150 cobalt-60 (Nordion Canada) γ -irradiation source. Cells were irradiated in their original medium. Camptothecin (CPT) and 4NQO (both from Sigma) were dissolved in dimethyl sulfoxide (Me₂SO) and used at 1 μ g/ml and 0.2 μ g/ml, respectively. TSA (Upstate) was dissolved in Me₂SO and used at 100 ng/ml.

Antibodies and Immunostaining.—The anti-WRN polyclonal antibody was used for immunofluorescence and confocal analysis as previously described (13). Images were acquired as single scans on a Zeiss confocal microscope using LSM software. Antibody against PML was a kind gift from Dr. A. Dejean.

Analysis of *in Vivo* Acetylation.—To detect WRN acetylation *in vivo*, 293T cells (2×10^6 per 10-cm dish) were transfected by the JetPEI method. WRN plasmid (3 μ g) was transfected either alone or in combination with a p300 expression plasmid (7 μ g). 48 h after transfection, 2 mCi/ml [³H]sodium acetate, TSA (10 μ M), and the proteasomal inhibitor MG132 (Sigma, 25 μ M) were added to the culture for 1 h. Cells were then washed twice with cold phosphate-buffered saline and lysed in Nonidet P-40 lysis buffer with freshly added protease inhibitor mixture (Sigma). The lysate was centrifuged at $17,000 \times g$ for 15 min at 4 °C. Supernatants were incubated with anti-WRN polyclonal serum, previously conjugated to protein A-Sepharose beads, for 1 h at 4 °C. The beads were washed three times with 1 ml of lysis buffer. Immunoprecipitated proteins were resolved on a 7.5% SDS-PAGE gel. Gels containing [³H]acetate-labeled WRN were first stained with Coomassie Brilliant Blue and then enhanced by impregnating with a commercial fluorography enhancing solution (Amplify, Amersham Biosciences) for 30 min. Dried gels were subjected to autoradiography at –70 °C for 3–7 days.

RESULTS

To investigate the effects of DNA damage on the subcellular localization of WRN, human osteosarcoma U2OS cells were

transfected transiently with a plasmid encoding a fusion protein between WRN and green fluorescent protein (GFP). In agreement with what has been reported previously for endogenous WRN (13), GFP-WRN was localized almost exclusively to the nucleolus (Fig. 1A), as confirmed by staining for the nucleolar protein nucleophosmin/B23 (Fig. 1C). Exposure of the cells to high dose UV radiation (40J/m²) resulted in translocation of GFP-WRN from the nucleolus into a large number of small nucleoplasmic foci (Fig. 1D). A similar pattern of GFP-WRN translocation was also seen after treatment with either camptothecin (1 μ g/ml) or 4NQO (0.2 μ g/ml) (data not shown). Exposure to IR (10 Gray), had a milder effect with nucleoplasmic translocation seen in only some of the cells (Fig. 1F). Unlike the simple kinetics of induction by UV (Fig. 1F) or camptothecin (data not shown), IR elicited a biphasic response: a very rapid wave of GFP-WRN exit from the nucleolus followed by a slower, extended, and rather limited second wave (Fig. 1F).

Endogenous WRN protein manifested an essentially similar behavior. Thus, in untreated WI-38 diploid human fibroblasts (Fig. 2A), the vast majority of endogenous WRN was in the nucleolus, in agreement with earlier observations (13). A variable amount of nucleoplasmic staining could also be observed under those conditions, typically residing in diffuse small foci (Fig. 2A). This indicates that a minor fraction of WRN is present outside the nucleolus even without induced stress. Upon UV irradiation, WRN was effectively excluded from the nucleolus and translocated entirely to nucleoplasmic foci (Fig. 2C). Similar results were obtained with camptothecin and 4NQO (data not shown).

A more detailed time course analysis is shown in Fig. 3. In untreated cells, GFP-WRN was exclusively in the nucleolus (Fig. 3A). Shortly after irradiation at 40J/m², some of the GFP-WRN was still retained in the nucleolus, whereas the rest of the protein had already translocated into the nucleoplasm and was visible either in a relatively small number of large foci or as diffuse nucleoplasmic staining (Fig. 3, E and F). Later on, WRN became undetectable in the nucleoli, and much of the protein was found in a large number of small foci distributed throughout the nucleoplasm (Fig. 3G; quantitative analysis shown in Fig. 3H). At later times after irradiation, the GFP-WRN protein began to reaccumulate in the nucleoli, such that 24 h postirradiation the majority of the cells again exhibited only nucleolar GFP-WRN (see Fig. 5A, panel e).

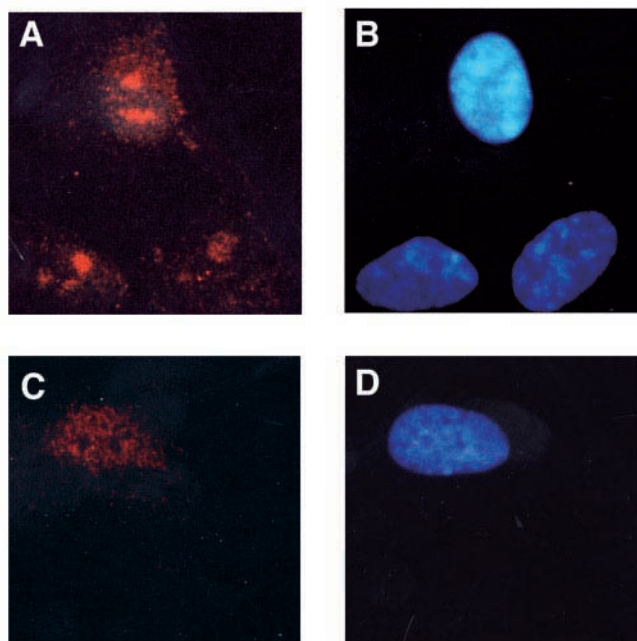


FIG. 2. Endogenous WRN translocates from the nucleolus to the nucleoplasm in response to DNA damage. WI-38 cells were fixed and stained with an anti-WRN polyclonal antibody (A and C) and with DAPI (B and D), either without treatment (A and B) or 3 h after exposure to 40 J/m² UV radiation (C and D).

The extent and pattern of WRN relocation depended on the dose of radiation. Thus, in cells exposed to 3 J/m² UV, much of the GFP-WRN was retained in the nucleolus, whereas the remainder was present in a relatively small number of rather large nucleoplasmic foci (Fig. 3B), similar to the picture seen very early after exposure to a high dose of UV irradiation. This limited translocation reached a maximum rapidly and did not progress significantly beyond 30 min postirradiation (Fig. 3, B–D; quantitative analysis in Fig. 3H). The correlation between UV dose and amount of nucleoplasmic WRN foci supports a role for WRN in the DNA damage response that ensues upon exposure of the cells to genotoxic agents. The progressive translocation following high dose irradiation may reflect the inability of the cell to deal successfully with such extensive DNA damage as fast as it does with a limited amount of damage.

The rapid DNA damage response elicited by IR is mediated, to a large extent, by the ATM kinase (37). To determine whether this was also the case for WRN translocation, the effect of IR on WRN localization was compared between normal human fibroblasts and cells derived from ataxia telangiectasia patients lacking ATM function. Detailed kinetic analysis revealed that in ATM-deficient cells, the first rapid phase of WRN translocation was completely abrogated (Fig. 4). This finding suggests that ATM can contribute to the initial response to IR-induced DNA damage by catalyzing the rapid mobilization of WRN into the nucleoplasm. It is conceivable that the extended, slow second phase of WRN translocation, which remains unaffected in ATM-deficient cells, may be due to the action of the ATM-related ATR kinase, although this remains to be formally proven.

The intracellular localization of proteins can be modulated by a variety of post-translational modifications. This also relates to protein acetylation, as exemplified by hepatocyte nuclear factor 4 (34). Furthermore, changes in acetylation in response to DNA damage can also modulate the functional state of proteins, as exemplified by p53 (25, 30). We therefore investigated whether protein acetylation may play a role in the observed relocation of WRN. To that end, U2OS cells were

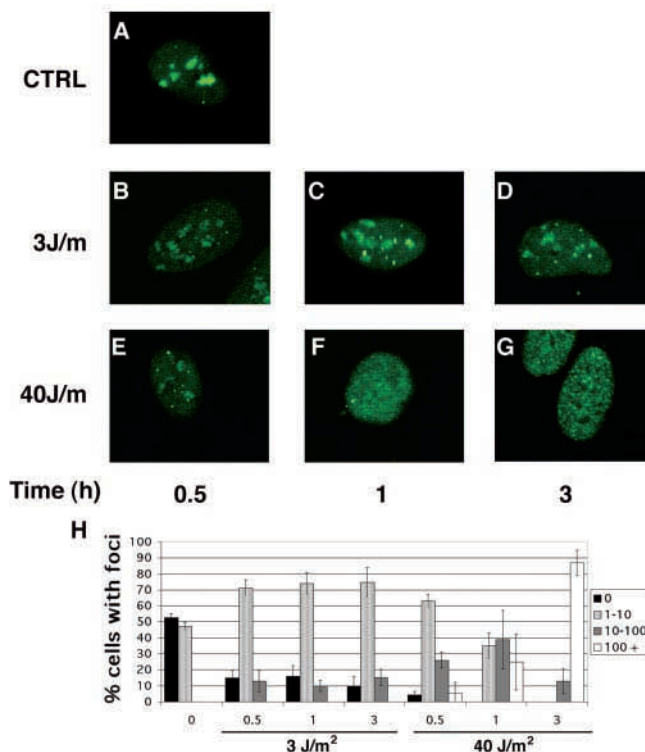


FIG. 3. The extent and kinetics of WRN translocation are UV dose-dependent. U2OS cells were transiently transfected with a GFP-WRN expression plasmid. Twenty-six hours later, cells were exposed to the indicated dose of UV. At various times thereafter, as indicated beneath the respective panels, cells were fixed and green fluorescence was monitored. Panel H displays a quantitative analysis of the redistribution of WRN into nucleoplasmic foci; cells were grouped according to the total number of nucleoplasmic foci (large plus diffuse small) present per cell.

transfected with GFP-WRN and incubated for 30 h in the presence of the histone deacetylase inhibitor TSA. As shown in Fig. 5A, panel c, TSA induced a prominent translocation of GFP-WRN from the nucleolus into discrete, rather large nucleoplasmic foci, similar to those observed early after exposure to high dose UV. Moreover, TSA also blocked the return of GFP-WRN from the nucleoplasmic foci back into the nucleolus. Thus, although 24 h after exposure to high dose UV the majority of the cells again displayed only nucleolar WRN, treatment with TSA significantly slowed this return and maintained much of the WRN in nucleoplasmic foci for longer periods (Fig. 5A, panels e and g, and Fig. 5B). These findings indicate that protein acetylation, augmented by TSA, plays a positive role in enabling the translocation of WRN from the nucleolus to nucleoplasmic foci. Similar results were obtained when FLAG-tagged WRN was used instead of GFP-WRN (data not shown).

The effect of TSA could also be observed on the endogenous WRN protein. Incubation of WI-38 cells with TSA for 30 h led to a substantial increase in the number of cells in which WRN was excluded from the nucleoli with concurrent accumulation in nucleoplasmic foci (Fig. 5C). Collectively, the data imply that the subcellular localization of WRN is regulated at least in part by protein acetylation. Furthermore, acetylation appears to play a role both in the exit of WRN from the nucleolus and in its subsequent return to the nucleolus, probably after the DNA damage response has subsided. In the absence of acetylation, WRN presumably retains its baseline nucleolar residence.

The simplest way to account for these observations is by assuming that the regulatory acetylation events occur on the WRN protein itself. It was therefore important to determine

FIG. 4. The ATM kinase is required for rapid translocation of endogenous WRN in response to IR. WI-38 cells and fibroblasts derived from an ataxia telangiectasia patient (ATM^{-/-}) were fixed and stained with anti-WRN polyclonal antibody either without treatment (time 0) or at the indicated times after exposure to 10 Gray of IR. The results represent the averages plus S.D. from three separate S.D. experiments.

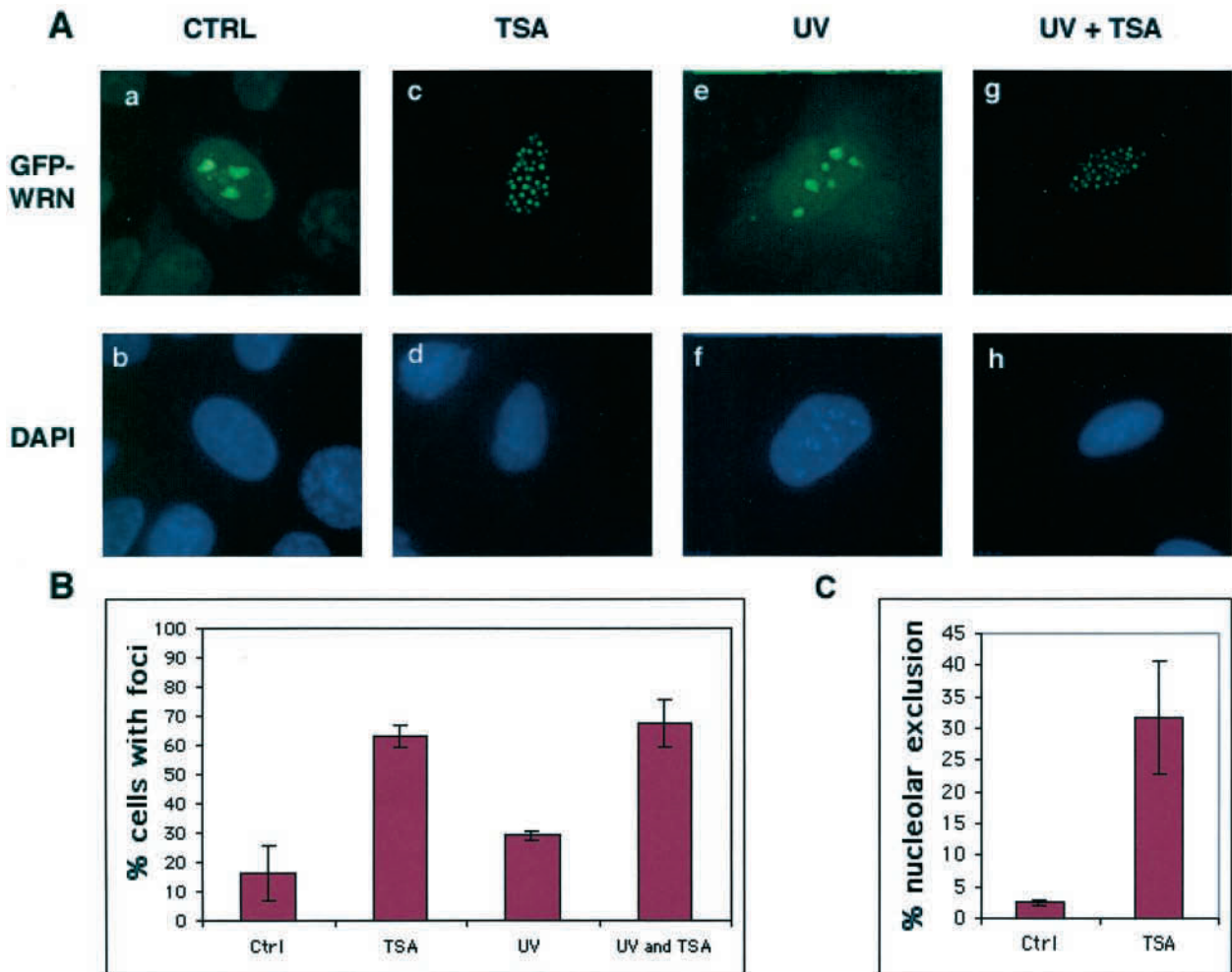
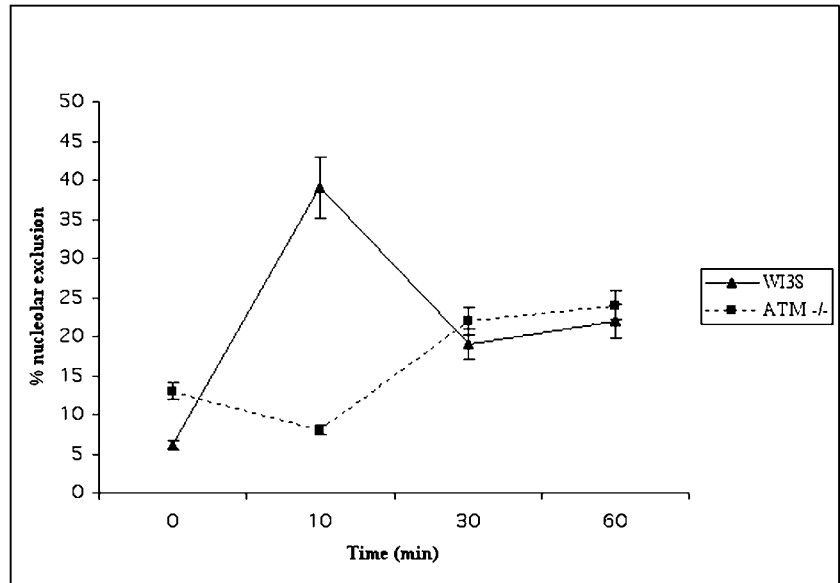


FIG. 5. Trichostatin A regulates the subnuclear localization of GFP-WRN. A, U2OS cells were transiently transfected with a GFP-WRN expression plasmid. 24 h post-transfection, TSA (*c, d, g, h*) or Me₂SO as a solvent control (*a, b, e, f*) was added for an additional 30 h, at which time the cells were fixed and stained with DAPI (*b, d, f, h*). GFP-WRN was monitored as in Fig. 1 (*a, c, e, g*). In *e-h*, cells were exposed to 20J/m² UV 6 h after the addition of TSA or Me₂SO, such that the experiment was terminated 24 h after irradiation. B, quantitative analysis of the percentage of cells exhibiting prominent nucleoplasmic localization of GFP-WRN as a function of culture history (see panel A). C, WI-38 cells were incubated for 30 h with TSA or with Me₂SO as a control (*Ctrl*) before being processed as in Fig. 2. Shown is a quantitative analysis of the percentage of cells in which the endogenous WRN protein was exclusively in the nucleoplasm.

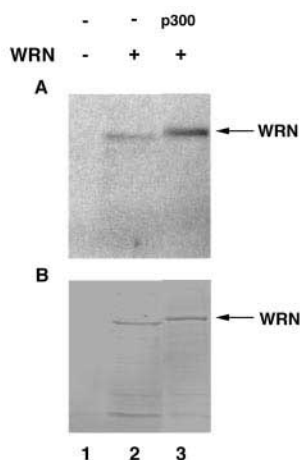


FIG. 6. WRN undergoes *in vivo* acetylation, which is enhanced by p300. 293T cells were transfected with a WRN expression plasmid, either alone or in combination with p300 expression plasmid, as indicated above each lane. Acetylated proteins were radiolabeled *in vivo* in the presence of radioactive acetate, as described under "Experimental Procedures." Cell extracts were prepared and subjected to immunoprecipitation with anti-WRN polyclonal antibody (Ab3). The immunoprecipitated proteins were resolved by SDS-PAGE and visualized by staining with Coomassie Brilliant Blue (panel B). The same gel was then subjected to autoradiography to visualize radioactive proteins (panel A). The cells in lane 1 were transfected with a control plasmid only.

whether WRN is subject to acetylation in living cells. To that end, 293T cells were transiently transfected with WRN expression plasmids, either alone or in combination with plasmids encoding the protein acetyltransferase, p300. Acetylated proteins were radiolabeled *in vivo* by incubation of the cultures with [3 H]sodium acetate under conditions that are expected to restrict protein labeling largely to direct acetylation only. As shown in Fig. 6A, lane 2, the immunoprecipitated WRN protein was specifically labeled in the presence of [3 H]acetate, implying that it is indeed acetylated *in vivo*. Cotransfection of WRN with p300 significantly increases WRN protein levels, presumably because of increased transcription from the transfected plasmids (data not shown). On the basis of previous calibration experiments, larger amounts of extract were therefore taken from cells transfected with WRN only. This indeed resulted in comparable amounts of total WRN protein in the different samples, as detected by Coomassie Blue staining (Fig. 6B). As seen in Fig. 6A, cotransfection with p300 substantially increased the extent of WRN acetylation (lane 3). These data imply that WRN is acetylated *in vivo* and suggest that p300 can contribute to this acetylation.

If acetylation of WRN plays an important role in facilitating its exit from the nucleolus into nucleoplasmic foci, it might be predicted that p300, which augments this acetylation *in vivo*, will also increase WRN translocation. This prediction was tested in WRN-transfected U2OS cells. As expected, TSA treatment led to a marked enhancement of the translocation of GFP-WRN from the nucleolus into large nucleoplasmic foci (Fig. 7, WRN+TSA). Importantly, cotransfection with p300 also resulted in a significant increase in the number of cells where GFP-WRN was present in such foci (WRN+p300); moreover, the microscopic pattern of these foci was indistinguishable from that elicited by TSA treatment (data not shown). Significantly, there was no apparent additive effect of p300 cotransfection and TSA treatment, supporting the notion that both agents were acting to increase WRN translocation via the same molecular mechanism, most probably acetylation.

PML nuclear bodies (38) constitute one well studied type of nucleoplasmic foci. It was of interest to find out whether any of the foci to which WRN relocated in irradiated cells represented

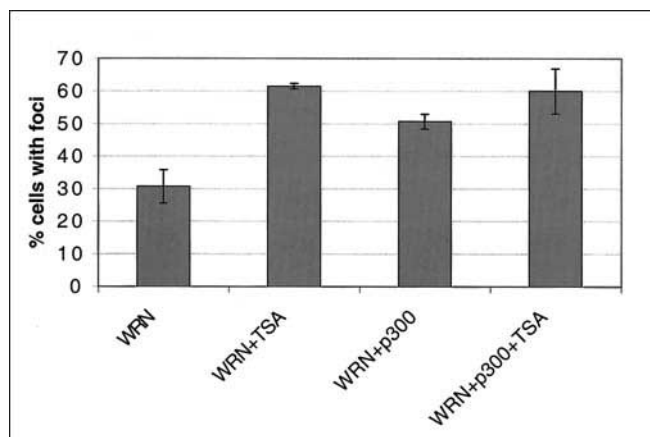


FIG. 7. p300 increases the translocation of WRN from the nucleolus to nucleoplasmic foci. U2OS cells were transiently transfected with a GFP-WRN expression plasmid, either alone or in combination with a p300 expression plasmid. 24 h post-transfection, TSA or Me₂SO as a solvent control was added for an additional 19 h, at which time the cells were fixed. GFP-WRN was monitored as in Fig. 1. Shown is a quantitative analysis of the percentage of cells exhibiting prominent nucleoplasmic foci of GFP-WRN in each type of culture.

PML bodies, particularly because the interaction of the PML protein with the cAMP response element-binding protein binding-protein (CBP)/p300 acetyltransferase (39) could potentially underlie the coupling between WRN translocation and protein acetylation. Along this line, PML was shown to increase the acetylation of p53 by acetyltransferases residing in PML-NBs (40). Therefore, U2OS cells were transfected with GFP-WRN, exposed to 4J/m² UV, and subjected to immunofluorescence analysis by confocal microscopy, employing antibodies directed against PML. As seen in Fig. 8, panels D–F, there was indeed a partial colocalization of the large nucleoplasmic GFP-WRN foci with PML bodies. A similar pattern of partial colocalization was also revealed in cells analyzed 2 h after exposure to 40J/m² UV (data not shown). In contrast, PML did not colocalize with the many minute nucleoplasmic speckles harboring GFP-WRN at later times after exposure to 40J/m² UV. A pattern of partial colocalization of GFP-WRN with PML bodies was also observed in cells treated with TSA in the absence of UV (Fig. 9, D–F). The nature of the remainder of the large nucleoplasmic foci, which contain GFP-WRN but do not stain positive for PML, still remains to be determined.

DISCUSSION

Proper WRN activity is required for the efficient maintenance of genomic integrity, as reflected by the augmented genetic instability of cells derived from WS patients and by the increased rate of cancer in these patients (2). In the present study, we report that several types of DNA damage mobilize WRN and cause its translocation from the nucleolus into discrete nucleoplasmic foci in a manner apparently dependent on the extent of DNA damage. These observations support a role for WRN in the DNA damage response, at least in some types of DNA damage. It is noteworthy that translocation of WRN from the nucleolus into the nucleoplasm is also induced by other types of stress, such as serum starvation (41).

It is conceivable that WRN plays a role in the initial recognition of DNA damage or in the opening of the DNA damage site. Such a possibility is consistent with the fact that the helicase activity of WRN does not require the presence of a DNA terminus and particularly with the observations that WRN interacts with bubble structures (42), which are good substrates for its exonuclease activity (10). Alternatively, WRN may play a role in later stages of the DNA repair process. For

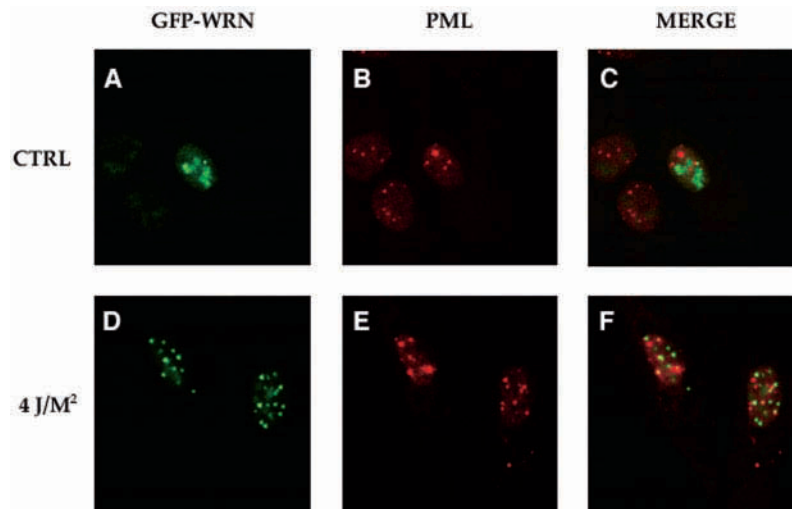


FIG. 8. **Partial colocalization of WRN and PML in UV-irradiated cells.** U2OS cells were transiently transfected with a GFP-WRN expression plasmid. 48 h later the cells were exposed to 4 J/m² UV radiation (*D–F*) or left untreated (*A–C*). Two hours later the cells were fixed and stained with anti-PML primary antibody, followed by incubation with Cy3-conjugated secondary antibody (*B* and *E*), and visualization by confocal microscopy. GFP-WRN was monitored by green fluorescence (*A* and *D*). Merging of the PML and the GFP-WRN staining patterns is shown in *C* and *F*, where colocalization of the two proteins yields a yellow signal.

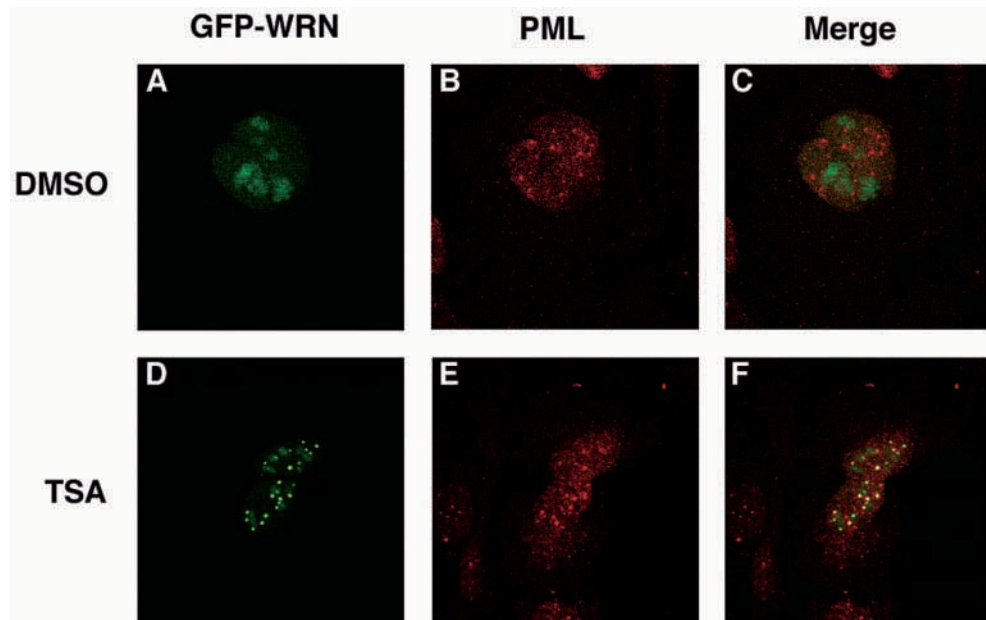


FIG. 9. **Partial colocalization of WRN and PML in TSA-treated cells.** U2OS cells were transiently transfected with a GFP-WRN expression plasmid. 24 h later the cells were incubated for 24 h in the presence of TSA (*D–F*) or Me₂SO (*A–C*) as a control. Cell staining and image processing were as in Fig. 7.

instance, WRN may act as a proofreading factor of the repair polymerase that seals up the DNA after removal of the initial damage-induced lesion. In line with such an idea, WRN has recently been shown to interact with the p50 subunit of DNA polymerase δ (Pol δ) and to stimulate the polymerization activity of Pol δ in the absence of PCNA (20) (43). The ability of WRN to act as a proofreading factor for Pol δ may rely on its helicase and exonuclease activities (8, 9). A role for WRN in the repair of DNA damage has also been deduced from studies exploring its functional interaction with the Ku protein (44) (45). It is of note, however, that we have not been able to demonstrate a colocalization of WRN with nuclear sites containing pyrimidine dimers, identified through the use of specific antibodies (data not shown). Furthermore, pyrimidine dimers were removed with similar kinetics in fibroblasts derived from WS patients as in their normal counterparts (data not shown). This suggests that if WRN indeed plays a role in the DNA repair process, its

activity is not obligatory and is not rate-limiting for the process, at least in the case of UV damage, but rather may be important for ensuring the quality of the repair.

An alternative possibility is that WRN is involved in the signaling events that follow the occurrence of DNA damage. This is consistent with the finding that proper WRN function is required for efficient induction of p53 by UV and several other types of genotoxic agents (46). For instance, WRN may transmit signals to upstream checkpoint control proteins, such as the CHK2 or ATM kinases (47, 48). The translocation of WRN from the nucleolus into the nucleoplasm may enable it to interact with such proteins and transmit the activating signal to them. In this scenario, it is proposed that nucleolar WRN may be in a “standby” state, at least as it pertains to the DNA damage response, and that its mobilization into nucleoplasmic foci reflects its recruitment into an active mode.

While this paper was under preparation, Sakamoto *et al.* (49)

also reported that WRN translocates to nucleoplasmic foci in response to several types of DNA damage. The findings implied that WRN plays a role in the recombination repair of double strand DNA breaks and in the processing of stalled replication forks. The rapid kinetics with which WRN translocates to nucleoplasmic foci after exposure to UV (our study) suggests that WRN also has a role in the response to other types of DNA damage, subject to nucleotide excision repair.

A novel aspect of the present study is the finding that exposure to TSA or excess activity of p300, both of which are known to increase the level of protein acetylation, can strongly enhance the recruitment of WRN to nucleoplasmic foci. Acetylation might either decrease the interaction of WRN with protein(s) that retain it in the nucleolus in the absence of genotoxic stress or enhance its interaction with proteins that anchor it to the nucleoplasmic foci; these two possibilities are not mutually exclusive. The regulatory acetylation events may occur on any of a number of proteins that control the intracellular localization of WRN. However, the findings that WRN is acetylated in living cells and the demonstration that p300, which increases WRN acetylation *in vivo*, also promotes its translocation from the nucleolus into nucleoplasmic foci, argue strongly in favor of the notion that at least some of the critical acetylation events occur on the WRN protein itself.

It is of note that nucleoplasmic WRN colocalizes partially with PML bodies. Interestingly, WRN can be modified by covalent attachment of SUMO-1 (50) and colocalizes with SUMO-1 in the nucleus (51). Modification of the PML protein by SUMO-1 is thought to target PML to nuclear bodies (38), raising the possibility that sumoylation of WRN is also involved in its recruitment to nuclear foci, particularly those coinciding with PML bodies. It is noteworthy that BLM, another member of the RecQ family, has also been shown to associate with PML bodies (52); in that case, p53 appears directly responsible for recruiting BLM to these bodies. Once recruited to PML bodies, WRN may be modified by acetyltransferases present within these nuclear bodies (40), including p300. Because p53 is also recruited to such PML bodies, at least under some circumstances (40, 53, 54), these sites may promote the interaction between WRN and p53 (17, 18) and enable the WRN-dependent activation of p53 in response to UV and other types of genotoxic agents (46).

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