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UV-induced ubiquitination of RNA polymerase II: A novel modification deficient in Cockayne syndrome cells

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ABSTRACT **Damage to actively transcribed DNA is preferentially repaired by the transcription-coupled repair (TCR) system. TCR requires RNA polymerase II (Pol II), but the mechanism by which repair enzymes preferentially recognize and repair DNA lesions on Pol II-transcribed genes is incompletely understood. Herein we demonstrate that a fraction of the large subunit of Pol II (Pol II LS) is ubiquitinated after exposing cells to UV-radiation or cisplatin but not several other DNA damaging agents. This novel covalent modification of Pol II LS occurs within 15 min of exposing cells to UV-radiation and persists for about 8–12 hr. Ubiquitinated Pol II LS is also phosphorylated on the C-terminal domain. UV-induced ubiquitination of Pol II LS is deficient in fibroblasts from individuals with two forms of Cockayne syndrome (CS-A and CS-B), a rare disorder in which TCR is disrupted. UV-induced ubiquitination of Pol II LS can be restored by introducing cDNA constructs encoding the *CSA* or *CSB* genes, respectively, into CS-A or CS-B fibroblasts. These results suggest that ubiquitination of Pol II LS plays a role in the recognition and/or repair of damage to actively transcribed genes. Alternatively, these findings may reflect a role played by the *CSA* and *CSB* gene products in transcription.**

The fidelity of the genetic material must be maintained to preserve normal cell function and to prevent disruption of genes that might lead to neoplastic transformation. The cell has developed several mechanisms for repairing DNA damage including the versatile nucleotide excision repair (NER) system (1). Transcription-coupled repair (TCR), a specialized form of NER, preferentially corrects damage to the transcribed strand of transcriptionally active DNA (1–4). Cells isolated from patients with Cockayne syndrome (CS), a rare autosomal recessive disorder, demonstrate defective TCR (1–4). Two complementation groups are recognized, CS-A and CS-B. The *CSA* and *CSB* genes have been cloned and the gene products are believed to play a mechanistic role in TCR (5, 6).

RNA polymerase II (Pol II), the polymerase that transcribes nearly all protein encoding genes, is also required for TCR. In yeast with a temperature-sensitive mutation of the large subunit of Pol II, TCR does not occur at the nonpermissive temperature (7, 8). In addition TCR can be blocked by inhibiting Pol II transcription with α-amanitin, and TCR of DNA under the transcriptional control of inducible promotors can be enhanced by pharmacologically stimulating the promotors (9, 10).

There is extensive evidence that transcription and NER are coordinated cellular events. Transcription factor TFIIH, which is required for initiation of transcription by Pol II, includes several proteins essential for NER (1–4). At least one of these proteins is required for both transcription and NER (11). When an elongating Pol II complex encounters a DNA lesion, it appears to stall, back up, and then resume elongating once the lesion is repaired (2, 12). The stalled Pol II complex, perhaps assisted by the *CSA* and *CSB* gene products, may play a role in efficiently identifying DNA lesions (1–6).

During transcription the C-terminal domain (CTD) of Pol II's largest subunit (Pol II LS), a subunit that directly contacts the DNA template, undergoes characteristic changes in its phosphorylation state. A 220-kDa form of Pol II LS (Pol IIa) with a hypophosphorylated CTD preferentially binds most promotors; Pol IIa shifts to a 240-kDa form (IIo) with a hyperphosphorylated CTD upon promotor clearance and transcript elongation (13). Using H14, a monoclonal antibody that recognizes Pol IIo and several less phosphorylated forms of Pol II LS (14), we have shown that UV-radiation and other DNA damaging agents induce a novel covalent modification of phosphorylated Pol II LS molecules.

MATERIALS AND METHODS

Cell Culture. HeLa cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium containing 7% fetal bovine serum (Sigma) and 1% glutamine in a 5% CO_2/95% air atmosphere at 37°C. They were grown to near confluence for experimental analysis. UV-irradiation was at 254 nm using a germicidal lamp with an incident dose rate of 1 J per m^2 per sec followed by incubation at 37°C for the indicated time period. Cisplatin treatment (20 μg/ml) was for 3 hr at 37°C. H_2O_2 treatment (0.25 or 2.0 mM) was for 1 hr at 37°C. Ionizing (γ) irradiation was administered in a ^{137}Cs irradiator; the dose was either 600 or 1800 rads (6 or 18 Gray) followed by a 60-min incubation at 37°C.

The primary fibroblast cell lines (GM 5659C, GM 1856B, GM2965, GM 671, GM 1098B, and GM 1428) were from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (Camden, NJ) and were grown in RPMI 1640 medium containing 15% fetal bovine serum and 1% glutamine before being treated as indicated.

The simian virus 40 (SV40)-immortalized normal cell line (VH10) as well as SV40-immortalized CS-B cell lines (CS1AN Sv + *CSB* antisense and CS1AN Sv + *CSB* sense) were

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Abbreviations: Pol II, RNA polymerase II; Pol II LS, RNA polymerase II large subunit; CTD, C-terminal domain of Pol II LS; NER, nucleotide excision repair; TCR, transcription-coupled repair; CS, Cockayne syndrome; HA, hemagglutinin.

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maintained as described (5). The SV40-immortalized CS-A cell lines (CS3BE.S3.G1 + pDR2 and CS3BE.S3.G1 + pDR2-*CSA*) were also maintained as described (6).

Immunoblot and Immunoprecipitation Analysis. For immunoblot analysis of whole cell extracts (see Figs. 1, 3, and 4), cells were lysed in SDS/PAGE sample buffer at 95°C, and then the proteins were subjected to SDS/PAGE in 6% gels, Western immunoblot analysis with anti-Pol II LS (mAb H14), and visualization by "enhanced chemiluminescence" (Amersham) as described (14). For immunoblot analysis with rabbit anti-ubiquitin, the commercially prepared and lyophilized antiserum (Sigma) was reconstituted in 10 ml of 3% bovine serum albumin (Sigma) in Tris-buffered saline (50 mM Tris·HCl, pH 7.4/150 mM NaCl) and then stored in aliquots. An aliquot was diluted 1:20 in 3% bovine serum albumin in Tris-buffered saline before being used for immunoblot analysis as described (14). To demonstrate specificity of polyclonal anti-ubiquitin immunoblot analysis, the procedure was also carried out in the presence of ubiquitin from bovine red blood cells at 1 mg/ml (Sigma).

For immunoprecipitation with mAb H14 or monoclonal anti-ubiquitin (mAb 1510; Zymed), HeLa cells were lysed with "hot 1% SDS" as described (14). Lysates were incubated with antibodies coupled to protein G-Sepharose beads (Pharmacia LKB), washed, eluted with SDS/PAGE sample buffer, and subjected to SDS/PAGE in 6% gels and immunoblot analysis with the indicated antibody as described (14).

Transient Transfection with HA-UBI. HeLa cells grown to 50–70% confluence on 150-mm dishes were transfected with 50 μg of the cDNA expression construct HA-UBI (15) or control construct pSF with 0.25 ml of LipofectAmine (GIBCO/BRL) for 6 hr according to the manufacturers specifications. After another 24 hr in standard growth medium, the cells were subjected to UV-irradiation (where indicated). Cells were lysed with hot SDS buffer then the protein was immunoprecipitated as described (14). Immunoprecipitated material was subjected to SDS/PAGE in 6% gels and immunoblot analysis with the indicated antibodies as described above. The antibodies employed were mAb H14, control antibody H22 (C) (14), and monoclonal anti-HA (12CA5-Babco, Richmond, CA).

RESULTS

DNA Damaging Agents Induce a Novel Covalent Modification of Pol II LS. Immunoblot analysis of untreated HeLa cell extracts using mAb H14 yielded a broad band formed by hyperphosphorylated Pol IIo, hypophosphorylated Pol IIa, and numerous forms with an intermediate level of phosphorylation (Fig. 1*A*, end lanes). Exposure of the cells to UV-radiation followed by an 8- to 60-min "recovery" at 37°C led to the appearance of a Pol II LS species that migrated more slowly than Pol IIo (Fig. 1*A*, discrete bands denoted x and x′). A smear of bands that migrated more slowly than x′ was also observed.

Pol IIx, x′, and the smear were also recognized by H5, another highly specific anti-Pol II LS mAb (14), as well as a polyclonal anti-Pol II LS antibody (results not shown). These upper molecular mass forms of Pol II LS were induced in all mammalian repair proficient cells tested including MDCK, COS, and several normal human fibroblast cell lines. Pol IIx, x′, and the smear were also induced by cisplatin (Fig. 1*A*), which causes bulky DNA lesions repaired by NER (16), and by mitomycin C and methyl methanesulfonate (results not shown). In contrast, ionizing radiation and hydrogen peroxide, which primarily cause oxidative DNA damage corrected by other repair mechanisms (17, 18), induced little or no Pol IIx and x′ (Fig. 1*A*). Thus, cells exposed to a subset of DNA damaging agents demonstrated a novel covalent modification of Pol II LS.

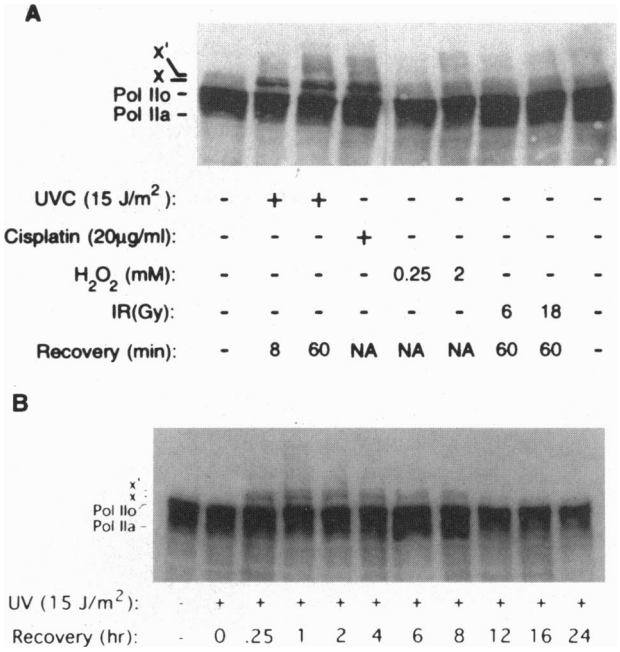

FIG. 1. DNA damaging agents induce novel slow-migrating forms of Pol II LS. Western immunoblots with H14, a mAb specific for Pol II LS, are shown. (*A*) HeLa cells were either untreated (−) or treated with UV-radiation, cisplatin, H_2O_2, or ionizing irradiation (IR) as indicated. Cells subjected to UV or ionizing radiation were subsequently allowed to recover at 37°C for the indicated period of time. Cisplatin and H_2O_2 were present during the entire incubation so the (stimulus free) recovery time is denoted NA (not applicable). (*B*) Kinetic analysis of Pol IIx and x′ induction. HeLa cells were either untreated (−) or irradiated with a UV pulse (15 J/m²) and then allowed to recover at 37°C for the indicated period of time. The UV pulses were timed so that all samples could be processed within a short time interval thus minimizing differences in cell number.

In an extended time course, the upper molecular mass forms of Pol II LS appeared within 15 min of the UV pulse, reached maximal levels by 1–3 hr, and were no longer detectable after 12–16 hr (Fig. 1*B*). The kinetics of this modification are similar to those of TCR as well as UV-induced transcriptional arrest in human cells (5, 19).

The Upper Molecular Mass Forms of Pol II LS Induced by UV Radiation Are Ubiquitinated. When Pol II LS from lysates of UV-irradiated cells was immunoprecipitated with mAb H14 then subjected to immunoblot analysis, the upper molecular mass forms were recognized by rabbit anti-ubiquitin antibodies (Fig. 2*A*, lane 2). Specific binding of anti-ubiquitin to Pol II LS was completely blocked by ubiquitin (1 mg/ml) (Fig. 2*A*, lane 4). In addition, upper molecular mass forms of Pol II LS could be immunoprecipitated from lysates of UV-irradiated HeLa cells by a monoclonal anti-ubiquitin antibody (mAb 1510) (Fig. 2*B*, lanes 2 and 3).

Several eukaryotic proteins contain ubiquitin-like domains that might cross-react with anti-ubiquitin antibodies. These include the human homologue of *Saccharomyces cerevisiae* RAD23 (20), which is involved in NER, and the 18-kDa subunit of elongin, which helps Pol II elongation complexes to transcribe past intragenic pause sites (21). To demonstrate that the upper molecular mass forms of Pol II LS resulted from bona fide ubiquitination, we transiently transfected HeLa cells with HA-UBI, a construct that expresses eight tandem copies of ubiquitin, each tagged with a hemagglutinin (HA) epitope (15). HA-tagged ubiquitin was incorporated into Pol IIx in transfected cells after UV-irradiation followed by incubation at 37°C for 8 or 60 min, (Fig. 2*C*, lanes 2 and 3). No HA epitope was incorporated into Pol IIx in cells transfected with a control plasmid pSF (Fig. 2*C*, lane 6) although such cells showed

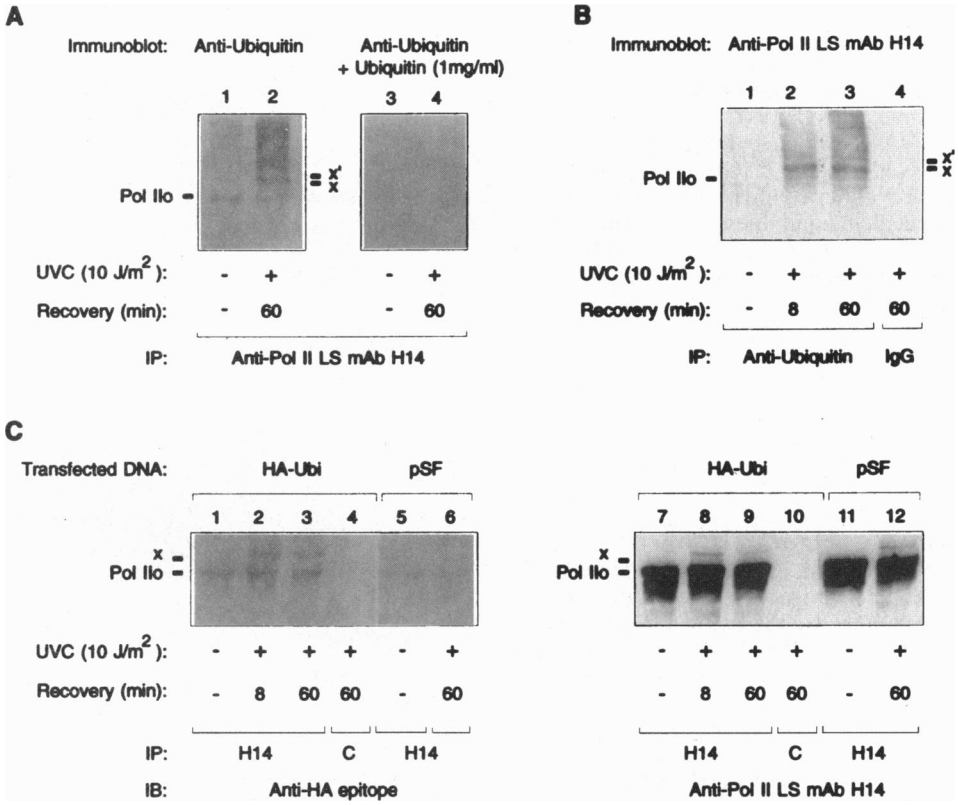

FIG. 2. Pol II LS is ubiquitinated in cells exposed to DNA damaging agents. (*A*) HeLa cells were treated as indicated and extracts were immunoprecipitated (IP) with anti-Pol II LS (H14) followed by immunoblot analysis with a polyclonal anti-ubiquitin antiserum in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of ubiquitin at 1 mg/ml. (*B*) Extracts of HeLa cells treated as indicated were immunoprecipitated with either monoclonal anti-ubiquitin or a control antibody (IgG) before being subjected to immunoblot analysis with anti-Pol II LS (H14). (*C*) HeLa cells were transfected with HA-UBI, a construct expressing HA-tagged ubiquitin, or pSF, a construct expressing an unrelated polypeptide then exposed to UV-radiation (+) 24 hr later. Proteins immunoprecipitated by anti-Pol II LS (H14) or control antibody (C) were subjected to immunoblot (IB) analysis with anti-HA (mAb 12CA5, lanes 1–6) or anti-Pol II LS (H14, lanes 7–12).

normal UV-induction of Pol IIx as demonstrated by immunoblot analysis with mAb H14 (Fig. 2*C*, lane 12).

UV-Induced Ubiquitination of Pol II LS Is Deficient in CS Cells. If ubiquitination of Pol II LS is induced by DNA damaging agents, it might be altered in cells with TCR deficiencies. Fibroblasts from four different CS cell lines showed absent or drastically reduced induction of Pol IIx and x′ (Fig. 3, lanes 4–9 and 13–18). In contrast, normal fibroblasts and XP-C fibroblasts deficient in "genome overall" NER (22) show induction of discrete Pol IIx and x′ species (Fig. 3, lanes 1–3 and 10–12). UV-induction of Pol IIx and x′ has also been demonstrated in XP-A, XP-B, and XP-D fibroblasts, as well as several additional normal fibroblast lines (results not shown). Although faint smears could be observed in the lanes representing the UV-treated CS cells (lanes 6, 8, 9, 14, 15, 17, and 18), the discrete Pol IIx and x′ bands were markedly diminished.

Restoration of UV-Induced Ubiquitination of Pol II LS in CS-A and CS-B Fibroblasts With *CSA* and *CSB* cDNA. Immortalized CS-A and CS-B fibroblasts are available that have been stably transfected with *CSA* and *CSB* expression constructs, respectively (5, 6). CS-A fibroblasts stably expressing the *CSA* expression construct demonstrated full restoration of UV-induced Pol II LS ubiquitination and CS-B fibroblasts stably expressing the *CSB* construct showed restoration of a substantial amount of UV-induced ubiquitination (Fig. 4). Thus, UV-induced ubiquitination of Pol II LS appears to require normal *CSA* and *CSB* gene products.

DISCUSSION

DNA damaging agents induce the ubiquitination of Pol II LS. The ubiquitinated forms of Pol II LS are also phosphorylated on the CTD because they are recognized by H14 and H5, both of which bind to CTD epitopes in a phosphorylation-dependent manner (14; E. Kim, L. Du, D.B.B. and S.L.W., unpublished data). Elongating Pol II, the form that might accumulate at intragenic DNA lesions, has a phosphorylated CTD (13). A transcriptionally inactive fraction of Pol II LS also has a highly phosphorylated CTD (14).

The prominent Pol II LS species denoted x and x′ are likely to represent large subunits with one and two copies of ubiquitin, respectively. The smear of slower migrating forms that can be seen in addition to Pol IIx and x′ (Figs. 1*A* and 2 *A* and *B*) are probably due to multiubiquitination of Pol II LS. A low level of ubiquitination was detected in bands migrating at the position of Pol IIo in samples from unirradiated cells (Fig. 2, lanes 1). This may represent constitutive ubiquitination of Pol II or it may represent low-level cross-reactivity of the antibodies with the large amount of Pol IIo that is present (see Fig. 2*C*, lane 7). The amount of ubiquitination was clearly increased after UV-irradiation. Similarly, the weak anti-HA immunoreactive bands migrating at the position of Pol IIo in Fig. 2*C*, lanes 5 and 6 (sham transfected), are probably due to nonspecific binding of the anti-HA antibody to the large amount of Pol II LS immunoprecipitated by H14 (compare Fig. 2*C*, lanes 5 and 6 to lanes 11 and 12).

Analysis of digitalized scans of the UV-induction data using the IMAGE program from the National Institutes of Health revealed that approximately 7–10% of Pol II LS was modified. Although the UV dose employed for the studies presented in this report (10–15 J/m^2) would be expected to kill approximately half of the cells, Pol IIx and x′ could also be induced with 2 J/m^2 (results not shown), a dose that does not appreciably reduce survival (5, 23).

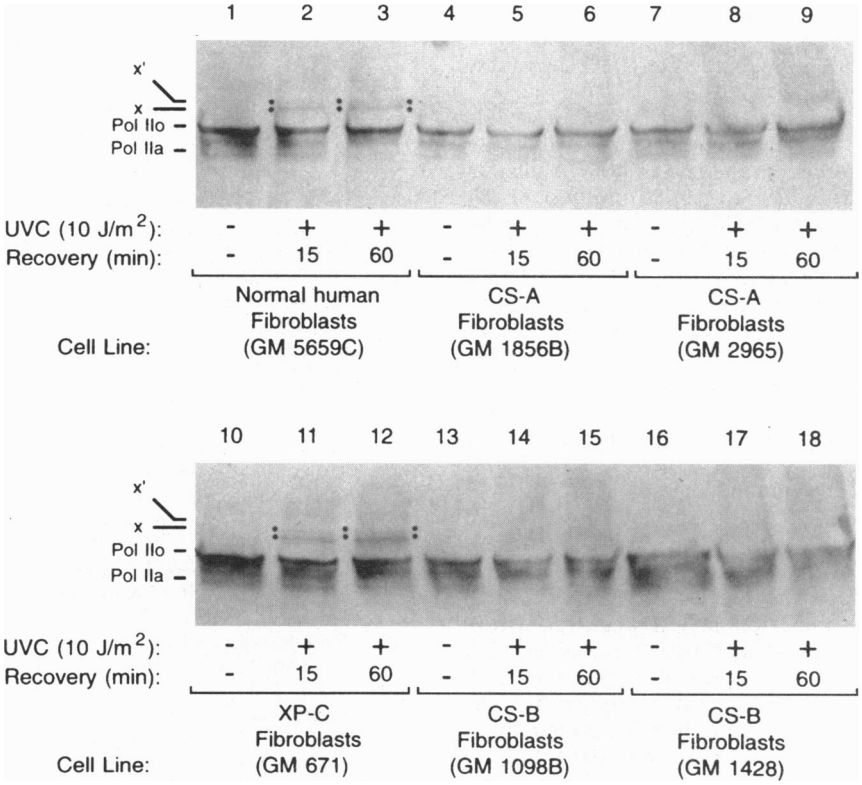

FIG. 3. UV-induced ubiquitination of Pol II LS is deficient in CS cells. Normal fibroblasts, CS-A and CS-B fibroblasts deficient in TCR, and XP-C fibroblasts deficient in "genome overall" NER were subjected to UV-irradiation (+) and allowed to recover at 37°C as indicated. Western immunoblots probed with anti-Pol II LS (H14) are shown. Pol IIx and x' in lanes 2, 3, 11, and 12 are highlighted with dots.

The fate of the Pol II LS that becomes ubiquitinated after UV-irradiation has not been determined in the present study. The major function of ubiquitination is to target proteins for degradation in multisubunit complexes called proteasomes (24, 25). Ubiquitin-mediated cleavage of Pol II LS stalled at sites of DNA damage could help the NER machinery to rapidly gain access to lesions located within actively transcribed genes. In support of this idea, multiubiquitinated substrates are usually targeted to the proteasome (24). However, we detect no significant increase in H14-immunoreactive Pol II LS breakdown products after UV treatment (Fig. 1*B*). This could be due to loss of the H14 epitope upon Pol II LS cleavage.

Significantly, several ubiquitinated proteins are not targeted for proteolysis including histones H2A and H2B, actin, and the platelet-derived growth factor (PDGF) receptor (24, 26–28). The ability of colony-stimulating factor 1 (CSF-1) to induce ubiquitination and membrane localization of the cytoplasmic protooncogene c-*cbl* (29) and the dependence of extracellular signal-induced IκBα kinase activity upon its ubiquitination (30) indicate that ubiquitination can sometimes play a signaling function.

Ubiquitination has already been implicated in DNA repair. The *S. cerevisiae* RAD6 protein, which is an enzyme involved in transferring ubiquitin molecules to their substrates, is required for certain forms of DNA repair (31). The RAD6 protein may be targeted to DNA damage sites by complex formation with the RAD18 protein (32). Human homologues of RAD6 have been cloned (33). In addition, DNA repair defects were observed when the *S. cerevisiae* ubiquitin genes were replaced with a mutated ubiquitin gene that would limit

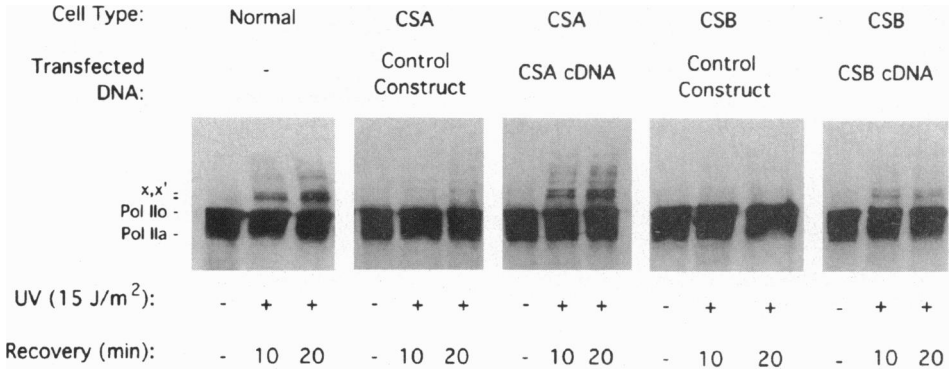

FIG. 4. UV-induced ubiquitination of Pol II LS can be restored in CS-A and CS-B fibroblasts with *CSA* or *CSB* cDNA, respectively. TCR-competent (VH10) human fibroblasts, CS-A fibroblasts stably transfected with the expression vector pDR2 (control construct) or with a *CSA* expression construct, and CS-B fibroblasts stably transfected with a *CSB* expression construct in antisense orientation (control construct) or a properly oriented *CSB* expression construct were subjected to UV-irradiation (+) and allowed to recover at 37°C as indicated. Western immunoblots probed with anti-Pol II LS (H14) are shown.

the patterns of multiubiquitin chains that could form on multiubiquitinated substrates (34). Although these data implicate ubiquitination in DNA repair, the substrates relevant to DNA repair have not been identified.

Ubiquitination of Pol II LS stalled at DNA lesions could alter its attachment to the DNA template so that repair enzymes can gain easier access to the lesion. Pol II stalled at a DNA lesion is believed to back up (2, 12), and loss of this response could help explain the TCR deficiency in CS cells. Similarly, ubiquitination of histones may alter chromatin structure by changing histone–DNA interactions (25, 35). Another possible role of ubiquitination could be to "flag" Pol II elongation complexes that are stalled at DNA lesions thereby promoting recruitment of the NER machinery to lesions encountered by the polymerase (2, 3). Alternatively, ubiquitination of Pol II LS could signal that transcribed DNA has been damaged so that the cell cycle and/or other regulatory systems can respond appropriately.

Ubiquitination of Pol II LS subsequent to UV-irradiation or cisplatin treatment could be a consequence of transcriptional arrest rather than activation of the TCR pathway. The dependence of UV-induced Pol II LS ubiquitination upon normal *CSA* and *CSB* gene products could reflect a role the CSA and CSB proteins may play in transcription. Several characteristics of these proteins are consistent with such a role. The *CSB* sequence includes helicase motifs present in the SWI/SNF family of proteins, which help regulate transcription by reorganizing nucleosome structure (5, 36); the CSA protein binds to the 44-kDa subunit of transcription factor TFIIH (6). It has been proposed that some of the clinical manifestations of CS could result from a generalized transcriptional defect rather than a DNA repair deficiency (3, 37).

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1. Friedberg, E. C., Walker, G. C. & Siede, W. (1995) *DNA Repair and Mutagenesis* (Am. Soc. Microbiol., Washington, DC).
2. Hanawalt, P. C. (1994) *Science* **266,** 1957–1958.
3. Chalut, C., Moncollin, V. & Egly, J. M. (1994) *BioEssays* **16,** 651–655.
4. Maldonado, E. & Reinberg, D. (1995) *Curr. Opin. Cell Biol.* **7,** 352–361.
5. Troelstra, C., van Gool, A., de Wit, J. Vermeulen, W., Bootsma, D. & Hoeijmakers, J. H. J. (1992) *Cell* **71,** 939–953.
6. Henning, K. A., Li, L., Iyer, N., McDaniel, L. D., Reagan, M. S., Legerski, R., Schultz, R. A., Stefanini, M., Lehmann, A. R., Mayne, L. V. & Friedberg, E. C. (1995) *Cell* **82,** 555–564.
7. Sweder, K. S. & Hanawalt, P. C. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10696–10700.
8. Leadon, S. A. & Lawrence, D. A. (1992) *J. Biol. Chem.* **267,** 23175–23182.
9. Leadon, S. A. & Lawrence, D. A. (1991) *Mutat. Res.* **255,** 67–78.
10. Leadon, S. A. & Snowden, M. M. (1988) *Mol. Cell. Biol.* **8,** 5331–5338.
11. Guzder, S. N., Sung, P., Bailly, V., Prakash, L. & Prakash, S. (1994) *Nature (London)* **369,** 578–81.
12. Donahue, B. A., Yin, S., Taylor, J.-S., Reines, D. & Hanawalt, P. C. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8502–8506.
13. Dahmus, M. E. (1995) *Biochim. Biophys. Acta* **1261,** 171–182.
14. Bregman, D. B., Du, L., van der Zee, S. & Warren, S. L. (1995) *J. Cell Biol.* **129,** 287–298.
15. Treier, M., Staszewski, L. M. & Bohmann, D. (1994) *Cell* **78,** 787–798.
16. Zamble, D. B. & Lippard, S. J. (1995) *Trends Biochem. Sci.* **20,** 435–439.
17. Seeberg, E., Eide, L., Bjoras, M. (1995) *Trends Biochem. Sci.* **20,** 391–397.
18. Jackson, S. P. & Jeggo, P. A. (1995) *Trends Biochem. Sci.* **20,** 412–415.
19. Mellon, I., Spivak, G. & Hanawalt, P. C. (1987) *Cell* **51,** 241–249.
20. Masutani, C., Sugasawa, K., Yanagisawa, J., Sonoyama, T., Ui, M., Enomoto, T., Takio, K., Tanaka, K., van der Spek, P. J., Bootsma, D., Hoeijmakers, J. H. J. & Hanaoka, F. (1994) *EMBO J.* **13,** 1831–1843.
21. Garrett, K. P., Aso, T., Bradsher, J. N., Foundling, S. I., Lane, W. S., Conaway, R. C. & Conaway, J. W. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7172–7176.
22. Venema, J., van Hoffen, A., Karcagi, V., Natarajan, A. T., van Zeeland, A. A. & Mullenders, L. H. F. (1991) *Mol. Cell. Biol.* **11,** 4128–4134.
23. Bohr, V. A., Okumoto, D. S. & Hanawalt, P. C. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3830–3833.
24. Jentsch, S. (1992) *Annu. Rev. Genet.* **26,** 179–207.
25. Hilt, W. & Wolf, D. H. (1996) *Trends Biochem. Sci.* **21,** 96–102.
26. Hochstrasser, M. (1996) *Cell* **84,** 813–815.
27. Ball, E., Karlik, C. C., Beall, C. J., Saville, D. L., Sparrow, J. C., Bullard, B. & Fyrberg, E. A. (1987) *Cell* **51,** 221–228.
28. Yarden, Y., Escobedo, J. A., Kuang, W.-J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ando, M. E., Harkins, R. N., Francke, U., Fried, V. A., Ullrich, A. & Williams, L. T. (1986) *Nature (London)* **323,** 226–232.
29. Wang, Y., Yeung, Y.-G., Langdon, W. Y. & Stanley, E. R. (1996) *J. Biol. Chem.* **271,** 17–20.
30. Chen, Z. J., Parent, L. & Maniatis, T. (1996) *Cell* **84,** 853–862.
31. Jentsch, S., McGrath, J. P. & Varshavsky, A. (1987) *Nature (London)* **329,** 131–134.
32. Bailly, V., Lamb, J., Sung, P., Prakash, S. & Prakash, L. (1994) *Genes Dev.* **8,** 811–820.
33. Koken, M. H., Reynolds, P., Jaspers-Dekker, I., Prakash, L., Prakash, S., Bootsma, D. & Hoeijmakers, J. H. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8865–8869.
34. Spence, J., Sadis, S., Haas, A. L. & Finley, D. (1995) *Mol. Cell. Biol.* **15,** 1265–1273.
35. Bradbury, E. M. (1992) *BioEssays* **14,** 9–16.
36. Peterson, C. L. & Tamkun, J. W. (1995) *Trends Biochem. Sci.* **20,** 143–6.
37. Vermeulen, W., van Vuuren, A. J., Chipoulet, M., Schaeffer, L., Appeldoorn, E., Weeda, G., Jaspers, N. G. J., Priestley, A., Arlett, C. F., Lehmann, A. R., Stefanini, M., Mezzina, M., Sarasin, A., Bootsma, D., Egly, J.-M. & Hoeijmakers, J. H. J. (1994) *Cold Spring Harbor Symp. Quant. Biol.* **59,** 317–329.