The presence of a long interspersed nucleotide element, named LiTc, which is actively transcribed in the parasite *Trypanosoma cruzi*, has been recently described. The open reading frame 1 of this element encodes the NL1Tc protein, which has apurinic/apyrimidinic endonuclease activity and is probably implicated in the first stage of the transposition of the element. In the present paper we show that NL1Tc effectively removes 3'-blocking groups (3'-phosphate and 3'-phosphoglycolate) from damaged DNA substrates. Thus, both 3'-phosphatase and 3'-phosphodiesterase activities are present in NL1Tc. We propose that these enzymatic activities would allow the 3'-blocking ends to function as targets for the insertion of LiTc element, in addition to the apurinic/apyrimidinic sites previously described. The potential biological function of the NL1Tc protein has also been evidenced by its ability to repair the DNA damage induced by the methyl methanesulfonate alkylating or oxidative agents such as hydrogen peroxide and t-butyl hydroperoxide in *Escherichia coli* (xth and xth, nfo) mutants.

Long interspersed nucleotide elements (LINE)\(^1\) are retrotransposons, which contain open reading frames similar to those present in retroviruses and long terminal repeat retrotransposons, that lack long terminal repeats (1). These elements, originally described in mammalian genomes, have been detected in a wide variety of species from protozoa to fungi, plants, and animals (2). Evidence exists that these elements are capable of transposition mediated by an RNA intermediate (3). Sequence analysis of LINEs shows that they encode for the enzymes involved in their own transposition. Several authors have suggested that integration of LINEs takes place at DNA breaks already existing in the chromosome produced by host-encoded products, probably during DNA repair or recombination (3). However, the exact integration site and the mechanisms of transposition of the LINEs remain unknown.

We have recently described a LINE, named LiTc, which shares high homology with the human L1 LINE (4), and is actively transcribed in the parasite *Trypanosoma cruzi* (4, 5). This element encodes enzymes that are probably involved in their own transposition (4). Interestingly, the ORF1 of LiTc has significant homology in the catalytic domains with the AP class II endonuclease family of DNA repair enzymes. This homology seems to be a common general feature of all nonsite-specific retrotransposon elements (4, 6). We have also described the existence of an endonuclease activity specific for AP sites, in the NL1Tc recombinant protein encoded by the ORF1 (7). The potential biological role of the LiTc protein was demonstrated by its ability to complement lethal *Escherichia coli* BW286, Δxth and Δyph and Δdut-I genotype, double mutant bacteria lacking the coding gene for the exonuclease III enzyme (7).

In the context of the integration mechanisms postulated for the nonsite-specific nonlong terminal repeat retrotransposons we proposed that the AP endonuclease activity of the NL1Tc recombinant protein may be connected with the formation of free 3'-OH ends into the DNA where integration of these elements would occur (6, 7). Feng *et al.* (8) have reported, on the other hand, that the protein encoded by the ORF2 NH\(_2\) terminus of the human element L1Hs has nuclease activity but shows no preference for AP sites. The high number of potential AP sites that could be generated along the chromosomal DNA by the NL1Tc protein can explain the high copy number and dispersion of the LiTc elements throughout the genome. We cannot, however, exclude the existence of other mechanisms for the generation of potential integration sites.

The AP endonuclease activity contributes to the repair of apurinic/apyrimidinic sites and carries 3'-phosphodiesterase and 3'-phosphatase activities as well (9, 10). The 3'-phosphatase and 3'-phosphodiesterase activities have been described to contribute to the repair of oxidative DNA damage (11). In the present paper we have analyzed whether those enzymatic activities are present in the recombinant protein NL1Tc. Thus, the existence of these activities in NL1Tc can contribute to a better understanding of the mechanisms by which these elements are integrated into the genome as well as their putative role in DNA repair processes. We show that both 3'-phosphatase and 3'-phosphodiesterase activities are associated with the endonuclease NL1Tc encoded by the nonlong terminal repeat retrotransposon LiTc. The biological function of the NL1Tc protein was examined by expression of the NL1Tc protein in *E. coli* null mutants lacking both exonuclease III and...
induced with 1 mM IPTG for 2 h. The experiments shown. Values shown are the average of
anti-6-histidine antiserum. The proteins were separated on 10% SDS-polyacrylamide gels, transferred to nitrocellulose membrane, and probed with protein samples. The proteins were incubated with varying concentrations of different DNA damaging agents (hydrogen peroxide (H₂O₂), tert-butyl hydroperoxide (t-BuO₂H), and methyl methanesulfonate (MMS)) at 37 °C for 15 min. 0.5 μCi of [methyl-³²P]dTMP were added and incubated at 37 °C for 15 min. Incorporation was terminated by adding 12.5% trichloroacetic acid. After 15 min at 4 °C, samples were transferred to a fiberglass filter and washed twice with 10% trichloroacetic acid. Thymidine incorporation was measured in a liquid scintillation counter. The expression level of the NL1Tc protein in transformed strains was determined using SDS-polyacrylamide gel electrophoresis and Western blot analysis (12).

3'-Phosphodiesterase Activity—125 ng/ml relaxed circular plasmid DNA (pSPFM55) was incubated for 10 min at 37 °C with 0.05 μM plocinexin in 25 mM sodium phosphate (pH 7.2), 10 mM NaCl, 5 mM MgCl₂, and 10 μM iron ammonium sulfate. This treatment produces ssDNA breaks with 3'-phosphoglycolate termini (13). The reaction was terminated by the addition of a volume of phenol/chloroform (1:1) equal to the volume of the reaction mixture. After extraction, it was ethanol precipitated. The primer activation reaction was carried out by incubation of the DNA substrate with 5 μM purified NL1Tc for 30 min at 30 °C in 50 mM Tris-HCl (pH 7.5), 50 μg/ml bovine serum albumin, and 5 mM MgCl₂, followed by 10 min at 65 °C. Exonuclease III enzyme was added to the negative control no enzyme was added to the reaction. Samples were cooled on ice. The nick translation reaction was carried out in buffer (50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 100 mM NaCl, 1 mM dithiothreitol) in the presence of 100 μM each dATP, dTTP, and dGTP, 1 μM dCTP, 0.5 μM [α-³²P]dCTP, and 0.01 unit of Klenow polymerase. The mixture was incubated for 30 min at 37 °C. The reaction was stopped by adding 5 μl of 2 mg/ml bovine serum albumin, 2 μl of 10 mg/ml herring sperm DNA, and 1 ml of ice-cold 10% trichloroacetic acid. Samples were washed with 1 ml of 10% trichloroacetic acid. The incorporated radioactivity present in the acid-insoluble fraction was measured in a liquid scintillation counter.

RESULTS

Complementation of the DNA Repair-deficient Strains BW9109 and BW528—To determine whether the NL1Tc protein can functionally complement the repair deficiency in vivo generated by oxidative and alkylating agents, pHisNL1Tc-transformed BW9109 and BW528 strains were made. As a negative control both strains were transformed with pTrcHisA. Expression analysis of the NL1Tc recombinant protein in the transformed bacteria was checked by Western blot. High expression of the NL1Tc recombinant protein was observed in both strains, even in the absence of the IPTG-inducing agent (Fig. 1). The E. coli strain BW9109, deficient in exonuclease III (xth), is described as a mutant sensitive to the alkylating agent MMS and to oxidative agents, such as H₂O₂, or t-BuO₂H (9, 15). The E. coli strain BW528, deficient in both exonuclease III (xth) and endonuclease IV (nfo), is known to be hypersensitive to the described oxidative and alkylating agents (9). The strain XL1-blue was used as a positive control. The results obtained show that NL1Tc expression in the mutant strains confers significant resistance to the damage caused by the alkylating agent MMS (Fig. 2A). MMS alkylates mainly the N groups of purine-generating AP sites via spontaneous and enzymatic hydrolysis of glycosydic bonds (16). In both mutant strains transformed with pHisNL1Tc, the survival levels following treatment with 10 mM MMS were very similar to the values observed for the control strain XL1-blue. The resistance to MMS of both NL1Tc transformed mutant strains did not require IPTG and was not enhanced by IPTG induction (data not shown).

The possible DNA repair activity of NL1Tc against damage by oxidative agents H₂O₂ and t-BuO₂H, which mainly cause ssDNA breaks with 3'-blocking groups, was also determined by complementation assays in E. coli BW9109 and BW528 mutant strains. The repair activity was measured by determining the level of [³²H]thymidine incorporation in the bacteria transformed with pHisNL1Tc or pTrcHisA (negative control) after
treatment with H$_2$O$_2$ and t-BuO$_2$H, in a similar way to the complementation assays mentioned above. The results obtained, shown in Fig. 2, B and C, reveal that expression of NL1Tc in the transformed bacteria produces a significant fall in the sensitivity to both oxidative agents. At 1 mM H$_2$O$_2$ concentrations, an oxidative agent that induces 3'-phosphate as terminal blocking groups, NL1Tc fully protects against the damage obtaining survival levels similar to those of the control bacteria XLI-1-blue (Fig. 2B). When the H$_2$O$_2$ concentration was increased, the complementation effect was more clearly observed in the double mutant strain, which is considerably more sensitive to the action of this oxidative agent. In the case of bacteria treated with t-BuO$_2$H, an agent that mainly generates 3'-phosphoglycolate as terminal blocking groups, expression of the NL1Tc protein effectively complements the absence of the gene coding for exonuclease III in the BW9109 strain. The survival indices were very similar to those of the wild-type strain. However, in the double mutant strain BW528, NL1Tc only marginally complements the repair activity (Fig. 2C).

The comparison of the DNA damage resistance in strains expressing NL1Tc relative to strains not expressing NL1Tc are shown in Table I. Expression of NL1Tc protein in BW9109 and BW528 increases the resistance to the DNA damage caused by the alkylating agent MMS to 120 and 390%, respectively. The expression of NL1Tc in the BW9091 mutant induces a survival increase of 290 and 120% in the BW258 double mutant strain exposed to 1 mM H$_2$O$_2$. The increase was 79 and 18% in both strains, respectively, after exposure to 3.5 mM t-BuO$_2$H. In summary, the NL1Tc protein repairs the DNA damage caused by the MMS alkylating agent and the H$_2$O$_2$ oxidative agent more efficiently than the DNA damage caused by t-BuO$_2$H. 3'-Phosphatase and 3'-Phosphodiesterase Activities on NL1Tc—To determine the presence of 3'-phosphatase and 3'-phosphodiesterase activities associated with the recombinant protein NL1Tc, DNA samples treated with specific drugs that cause strand breaks with 3'-blocking lesions (3'-phosphate and 3'-phosphoglycolate) were used as substrates. The ability of NL1Tc to eliminate the 3'-blocking ends generated in the DNA template was observed indirectly by measuring the stimulation of DNA synthesis following pretreatment of the substrate with the recombinant protein. Hydrolysis carried out by the repair enzyme generated 3'-OH ends suitable for being used as primers by the Klenow fragment of DNA polymerase I, which was immediately added to the medium. The enzymatic activity was determined by measuring dCTP $^{32}$P incorporation to the DNA template used as substrate. As a positive control exogenous exonuclease III was added to a sample. Micrococcal nuclease and phleomycin were used to create 3'-phosphate and 3'-phosphoglycolate-blocked ssDNA breaks, respectively. In each case, the conditions were those previously described (13, 14), which favor ssDNA breaks over double-stranded DNA breaks producing substrates having as average 1–4 nicks/molecule. The results obtained reveal that NL1Tc effectively removes 3'-blocking groups (3'-phosphate and 3'-phosphoglycolate) from the damaged DNA substrates, as shown in Fig. 3, A and B. Thus, similar to the exonuclease III enzyme, our data indicate that NL1Tc has the potential to hydrolyze DNA when the substrate is phosphate-blocked.

**TABLE I**

Comparison of the sensitivity to alkylating and oxidative agents of AP mutant strains expressing NL1Tc against to the strains not expressing NL1Tc

| Survival increase ratio* | 10 mM MMS | 1 mM H$_2$O$_2$ | 3.5 mM t-BuO$_2$H |
|--------------------------|-----------|---------------|-----------------|
| BW9109-NL1Tc            | 120       | 290           | 79              |
| BW9109                  |            |               |                 |
| BW528-NL1Tc             | 390       | 120           | 18              |
| BW528                    |            |               |                 |

* The survival increase ratio means the ratio of the survival percentage against the alkylating and oxidative agents induced by expression in the indicated mutant strains of NL1Tc protein relative to the survival percentage of the mutant strains not expressing NL1Tc expressed as percentages.

**DISCUSSION**

In previous studies we reported that the amino acid sequences from the ORF1 of LITc and the consensus sequence of the AP nuclease family (17) show 30% identity, which extends to all nonsite-specific LINEs described (4, 6). It was also shown that the recombinant protein encoded by the ORF1 of the LITc LINE is capable of hydrolyzing a 37-mer double-stranded DNA fragment containing an internal AP site and nicking super-coiled plasmids containing apurinic/apyrimidinic sites (7). The NH$_2$-terminal end of the ORF2 of the human L1 element, which has high sequence homology with the ORF1 of the *T. cruzi* LITc element, has also nuclease activity but there is no evidence for AP endonuclease activity (8). Recent studies have shown that L1 endonuclease is specific for the unusual DNA structural features found at the TpA junction of the 5'-d(T$_n$A$_n$)5'-d(T$_n$A$_n$) (18). We believe that the endonuclease activity encoded by LINEs might be involved in the integration mechanisms of these LINEs into the host genome as it would be responsible for generating free 3'-OH sites required as primers for integration (4, 7, 8). The present paper reveals that the NL1Tc protein encoded by the ORF1 of the mobile LINE LITc from *T. cruzi* has the ability to repair the DNA damage induced by alkylating and oxidative agents in *E. coli* (xth and xth, nfo) mutants. NL1Tc expression in these repair-deficient cells provides resistance to both alkylating (MMS-induced) and oxidative (H$_2$O$_2$- and t-BuO$_2$H-induced) DNA damage. Quantitative analysis of the repair capacity of NL1Tc shows that NL1Tc expression in BW9109 (xth) and BW528 (xth and nfo) completely reversed the MMS sensitivity of the mutants. NL1Tc had a moderate effect on sensitivity to H$_2$O$_2$ and only a very modest effect on sensitivity to t-BuO$_2$H. It was interesting to observe that the endonuclease activity encoded in a LINE of *T. cruzi* could substitute for the prokaryotic enzyme of *E. coli*, demonstrating that NL1Tc is endowed with potent AP endonuclease activity.
The AP endonuclease family is made up of a group of multifunctional proteins with four principal nuclease functions, AP endonuclease, 3'-exonuclease, 3'-phosphodiesterase, and 3'-phosphatase. The most distinctive feature of the members of this protein family is to have an efficient AP endonuclease activity (9). Studies with Drosophila Rrp1 mutants have established a strong correspondence between sensitivity to one of these chemical compounds (H$_2$O$_2$, t-BuO$_2$H, or MMS) and deficiency in one of the tested enzymatic functions (3'-phosphatase, 3'-phosphodiesterase, or AP endonuclease). H$_2$O$_2$ sensitivity corresponds to a deficiency in phosphatase activity, t-BuO$_2$H sensitivity corresponds to a deficiency in phosphodiesterase activity, and MMS sensitivity corresponds to a deficiency in AP endonuclease activity (9, 19). The ability of NL1Tc to repair 3'-terminal damage in DNA has also been demonstrated using two distinct activity assays similar to those reported for AP repair enzymes (13, 14): a 3'-phosphodiesterase assay that directly measures the removal of terminal phosphoglycolate and a 3'-phosphatase assay that directly measures the removal of terminal phosphate. It has been demonstrated that 3'-phosphatase and 3'-phosphodiesterase activities are essential for the repair of the oxidative damage that causes 3'-blocking ends (11). The results obtained showed that NL1Tc efficiently repairs oxidative damage that includes 3'-phosphatase-blocked termini but only a small amount of the damage that includes 3'-phosphoglycolate-blocked termini. These results are consistent with those obtained in the complementation assays where a significantly higher repair index was observed for H$_2$O$_2$-induced damage than for t-BuO$_2$H-induced damage. The higher 3'-phosphatase activity relative to the 3'-phosphodiesterase activity detected in the NL1Tc protein together with the ability to repair H$_2$O$_2$-induced damage to a higher extent than to repair t-BuO$_2$H-induced damage in mutant bacteria in repair enzymes cause the NL1Tc protein to be more similar to the exonuclease III enzyme than to other endonucleases such as Rrp1 protein from Drosophila or endonuclease IV from E. coli. The reported phylogenetic analysis made by comparison of the conserved domains of the AP proteins and those of LINEs showed that the L1 (L1hs, L1ms, L1mm, L1m, and L1md), the cin4 and the Tad1-1 are closer in evolution to the AP family proteins than to the rest of the LINEs (7). Interestingly, the exonuclease III protein is clearly closer in evolution to the LINEs than to endonuclease IV.

Given the potential involvement of the nucleases encoded by the LINEs in their own integration mechanism (8) we propose that the 3'-phosphatase and 3'-phosphodiesterase enzymatic activities detected in NL1Tc would allow the 3'-blocking ends to function as targets for the insertion of L1Tc element, in addition to the AP sites previously described (7). On the other hand, it should not be excluded that the presence of the 3'-repair activities associated with NL1Tc could be indicative of a possible repair role of the L1Tc element. In fact, repair of double-stranded DNA breaks because of the insertion of the Ty1 element from Saccharomyces cerevisiae in the presence of functional reverse transcriptase (from human L1, yeast Ty1, or Crithidia CRE1) (20, 21) has recently been described.

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