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Identification of *Escherichia coli* DNA helicase I as the *traI* gene product of the F sex factor

(DNA-dependent ATPase/unwinding/conjugation/DNA transfer)

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ABSTRACT **Active DNA helicase I (M_r 180,000) can be isolated from *Escherichia coli* F^+ strains but not F^- strains. The transfer of the F sex factor to F^- strains by conjugation permits the purification of the enzyme from the transconjugant strains. We conclude from this that helicase I is coded for by a portion of the F factor. Results also obtained by using recombinant plasmids carrying different DNA fragments of the F factor transfer region suggest that DNA helicase I is identical to the product of *traI*, one of the transfer genes of the F factor.**

DNA helicases are enzymes capable of unwinding double-stranded DNA in energy-dependent reactions requiring ATP. Four such enzymes have been isolated from *Escherichia coli* strain K-12, namely DNA helicase I (1), DNA helicase II (2), *rep* protein (3), and DNA helicase III (4). The biological role of the helicases in the normally growing cell is not clearly understood (5, 6). Studies with *in vitro* replication systems with purified antibody raised against DNA helicase I have shown that this protein is not involved in DNA replication (7).

Helicase I (M_r 180,000 by $NaDodSO_4$ gel electrophoresis) is the largest peptide detectable in *E. coli* K-12 extracts. The enzyme is a DNA-dependent ATPase active in *in vitro* DNA unwinding and can be specifically inactivated by anti-helicase I antibody. Preliminary experiments have suggested that active helicase I with the above characteristics is present only in strains bearing the F sex factor (F^+) but not in strains without the F factor (F^-). This result was confirmed by using isogeneic F^+ and F^- strains. We also have found that transfer of the F factor to F^- recipient strains by conjugation enables purification of helicase I from the transconjugant cells.

Among the mapped cistrons of the transfer operon of the *E. coli* F sex factor, there is one gene (*traI*) that codes for a protein of $M_r \approx 180{,}000$ (8), which on the basis of its size might correspond to helicase I; *traI* is one of the approximately 20 so-called transfer genes of F factor that are needed for sexual contact between bacteria and for transfer of DNA between the conjugating cells. Our hypothesis that the *traI* product could be identical to helicase I was tested by the transformation of F^- cells with different plasmids carrying cloned DNA fragments of the F *tra* region, including the *traI* gene. F^- cells harboring these plasmids were indeed found to express helicase I.

MATERIALS AND METHODS

Bacteria and Plasmids. *E. coli* 1100 F^+ (*endA2 supE*), 1100 F^-, MO676 F^- (*endA2 recB21 recC22 xth1 sbcB15 supE*; from M. Oishi), and W208 F^+ (*leu thr lacZ supE*) are K-12 substrains kept in this laboratory. H560 F′ (*endA2 polA1 hsd*$^+$; F agent derived from Hfr313) is a K-12-C hybrid. Plasmids were kindly provided by M. Achtman, Berlin, and have been described elsewhere. The pRS chimeric plasmids (9) carry a fragment of the F factor *tra* region cloned in pSC101. The pPM series (10) are insertion (pPM320) or deletion (pPM3, pPM55) derivatives of pRS31.

Antibody Against Helicase I. Antibodies were purified from sera of immunized rabbits by ammonium sulfate precipitation, followed by DEAE-Sephadex chromatography (11).

DNA Duplexes. ^{32}P-Labeled partially double-stranded fd phage DNA was prepared by *in vitro* polymerization with single-stranded fd DNA as template and [α-^{32}P]dCTP (80 Ci/nmol; 1 Ci = 3.7×10^{10} Bq) for labeling the complementary DNA. Approximately 1,400 nucleotides were incorporated per DNA (1).

Other Materials. S1 nuclease from *Aspergillus oryzae* was purified through the DEAE-cellulose step (12) by H. Dürwald in this laboratory. Other proteins came from sources as described (13). Phosphocellulose P11 was a Whatman product. [γ-^{32}P]ATP was a gift from M. Makinose, and ^{125}I-labeled *Staphylococcus* protein A was from A. Stern. Buffer A is 50 mM Tris·HCl, pH 7.6/0.1 mM EDTA/0.5 mM EGTA/1 mM dithiothreitol/20% glycerol.

Assays. ATPase activity was determined in the presence of heat-denatured calf thymus DNA by the hydrolysis of [γ-^{32}P]-ATP (13). DNA unwinding was measured as the increase of sensitivity of ^{32}P-labeled partially double-stranded fd phage DNA to digestion with single strand-specific S1 nuclease (1). Inhibition with antibody against helicase I was carried out as described (7). Protein concentrations were measured as described by Bradford (14).

Gel Electrophoresis. $NaDodSO_4$ gel electrophoresis was performed on 7.5% polyacrylamide gels as described by Laemmli (15). The gels were stained with silver nitrate (16).

Purification of Helicase I. Protein of the crude extract was precipitated with 0.3 g of ammonium sulfate per ml. The precipitate was subjected to stepwise elution with buffer A containing 0.28 and 0.2 g of ammonium sulfate per ml as described (13). The pellet was then dissolved in buffer A. The dissolved material was dialyzed overnight against buffer A and applied to a phosphocellulose column (1.3 × 14 cm) equilibrated in buffer A. The column was washed with 150 ml of buffer A and eluted with a linear gradient (350 ml) of 0–1 M NaCl in buffer A. ATPase activity eluting as a peak between 240 and 260 mM NaCl was pooled, concentrated by precipitating with ammonium sulfate (13), and subjected to sucrose gradient sedimentation. The 12-ml 15–35% sucrose gradients contained 20 mM Tris·HCl (pH 7.6), 0.1 mM EDTA, 0.5 mM EGTA, 1 mM dithiothreitol, and

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Abbreviation: P_i/NaCl, phosphate-buffered saline.

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400 mM NaCl. Approximately 800 μg of protein in a 0.4-ml volume was layered onto the gradient. Centrifugation was at 40,000 rpm for 22 hr at 4°C in a Spinco SW 41 rotor. Peak fractions with ATPase activity (13) were pooled; 1- to 4-μl samples of this material were used to assay for DNA unwinding activity.

Immunoblotting. Proteins separated on $NaDodSO_4$/polyacrylamide gels were analyzed by immunoblotting essentially as described (17). The gel was electrophoretically blotted onto nitrocellulose filter, which was then incubated in 10 mM potassium phosphate, pH 7.4/140 mM NaCl (phosphate-buffered saline, P_i/NaCl) containing 3% bovine serum albumin at 37°C for 1 hr. Anti-helicase I IgG was added (25 μg/ml), and incubation was continued for another 16 hr. The filter was washed in P_i/NaCl (with six changes for a total of 3 hr) and was incubated for 12 hr at room temperature with *Staphylococcus* ^{125}I-labeled protein A diluted in P_i/NaCl containing 3% bovine serum albumin (10^6 cpm/ml). Unbound protein A was washed out twice with P_i/NaCl containing 0.1% $NaDodSO_4$, and the sheet was rinsed as described above. The filter was dried and exposed at −80°C to Kodak X-Omat AR film for 60 hr.

Analysis of Protein Expression in Minicells. Minicells from plasmid carrying derivatives of *E. coli* DS410 (*minA, minB, rpsL*) were purified and labeled with 40 μCi/ml [^{35}S]methionine (Amersham; 1,300 Ci/mmol) as described (18).

DNA Transfer Techniques. $CaCl_2$-treated *E. coli* cells were transformed as described in the literature (19) except that the transformation was carried out in 60 mM $CaCl_2$. F sex factor was transferred from donor strain W208 F^+ to recipient F^- strains by conjugation (20).

RESULTS

Purification of Helicase I. DNA helicase I was partially purified from 100 g of frozen cells by ammonium sulfate fractionation of the extract and passage through a phosphocellulose column. The purification procedure was carried out in both *E. coli* F^+ and F^- cells. Preparations obtained from F^+ cells showed two early eluting peaks of DNA-dependent ATPase activity as illustrated for *E. coli* 1100 F^+ cells in Fig. 1*A*. The later eluting ATPase peak (at 260 mM NaCl) corresponded to helicase I as judged from inhibition of its ATPase activity with anti-helicase I antibody (Table 1). The earlier eluting peak fraction (at 140 mM NaCl) was strongly inhibited by anti-helicase II antibody (data not shown).

Further purification of the ATPase peak at 260 mM NaCl by sucrose gradient sedimentation eliminated contaminating nucleases and allowed the assay of DNA unwinding activity. This activity also was inhibited strongly by the addition of anti-helicase I antibody (Table 1). Similar fractionation of *E. coli* F^- extracts showed no DNA-dependent ATPase activity eluting from the phosphocellulose column at the salt concentration corresponding to elution of helicase I, as shown for strain 1100 F^- in Fig. 1*B*. The fractions eluting at 260 mM NaCl were pooled, assayed for DNA unwinding activity after further purification, and found to be negative (Table 1). The elution profile obtained for H560 F′ cells, from which helicase I normally has been purified (13), corresponded to that obtained for 1100 F^+ cells (Fig. 1*C*). The peak fraction at 260 mM NaCl also was analyzed on $NaDodSO_4$ gels with silver staining of the proteins. The largest band visualized on the gels ($M_r \approx 180,000$) corresponded to helicase I purified by the standard method (13) from strain H560 F′ (Fig. 2, lane B). This band was absent from the analogous fraction from 1100 F^- cells (Fig. 2, lane C) but was present in 1100 F^+ cells (Fig. 2, lane D).

To prove that synthesis of helicase I was associated with the F sex factor of *E. coli*, we transferred the F factor of donor strain

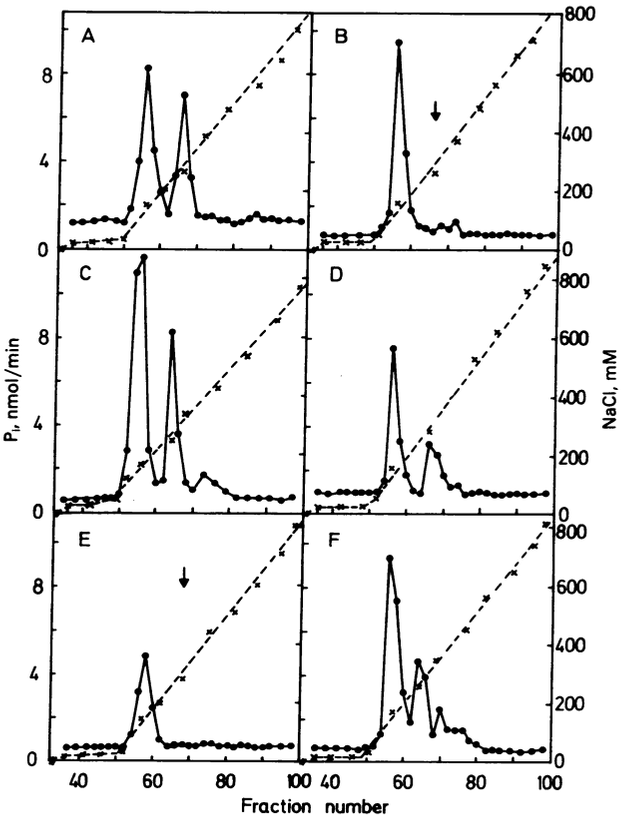

FIG. 1. Fractionation of DNA helicases on phosphocellulose column. Ammonium sulfate fraction was applied to a phosphocellulose column, the proteins were eluted, and fractions were collected and assayed for ATPase activity with single-stranded DNA (●) as described (13). Salt concentration was determined by conductivity (×). *E. coli* strains: 1100 F^+ (*A*); 1100 F^- (*B*); H560 F′ (*C*); 1100 F^-(pRS31) (*D*); 1100 F^-(pPM320) (*E*); and 1100 F^-(pPM55) (*F*).

W208 F^+ to recipient F^- strains by conjugation and purified helicase I as above. The elution profile from phosphocellulose chromatography of recipient cells containing the F factor was practically identical with that of 1100 F^+ cells: the later eluting ATPase peak supported DNA unwinding *in vitro* in a reaction inhibitable by antibody against helicase I. Furthermore, in electrophoretic gels the presence of the largest protein comigrating with authentic helicase I could be observed (data not shown). These experiments were carried out with 1100 F^- and MO676 F^- as recipient cells.

Is Helicase I Identical to *traI* Gene Product? *E. coli* 1100 F^- cells were transformed with plasmid pRS31, which contains the *Eco*RI fragments f17, f19, and f2 of the transfer region of F factor (Fig. 3). These fragments include the *traS*, *traT*, *traD*, *traI*, and *traZ* cistrons of the transfer operon. In addition, pRS31 carries the insertion sequence IS3, which delimits the transfer region. Fractionation of the pRS31-transformed cells enabled the purification of helicase I with both ATPase and DNA unwinding activities inhibitable by anti-helicase I antibody (Fig. 1, Table 1). The yield of helicase I corresponded to 30–80% of the average obtained from 1100 F^+ cells, variations depending on the preparation. The presence of pRS31 in F^- cells also led to the appearance of a M_r 180,000 peptide band on $NaDodSO_4$ gels (Fig. 2, lane E) as already shown by Achtman and co-workers (10). The level of ATPase activity recovered from 1100 F^-(pRS31) cells appears much higher than the level of electrophoretically detectable helicase I protein. The former corresponds to approximately one-third of that obtained from 1100 F^+ cells (cf. Fig. 1 *A* and *D*), whereas the latter is considerably

Table 1. DNA-dependent ATPase activity eluting as peak II from phosphocellulose column and the DNA unwinding activity after sucrose gradient*

	ATPase activity		DNA unwinding activity	
	Arbitrary units	Inhibition by anti-DHI, %	Duplex DNA unwound, %	Inhibition by anti-DHI, %
1100 F^+	100	83	40	92
1100 F^-	<1	—	<1	—
H560 F′	110	90	41	90
1100 F^-(pRS31)	40	88	51	91
1100 F^-(pPM320)	<1	—	<1	—
1100 F^-(pPM3)	90	86	53	93
1100 F^-(pPM55)	87	82	48	90

*Comparison of different *E. coli* strains and sensitivity of the activity to anti-helicase I-antibody (anti-DHI, 15 μg).

lower than expected (Fig. 2, lanes D and E). Low recovery of helicase I protein relative to the ATPase was observed in three out of four preparations from 1100 F^-(pRS31) cells. We have no explanation for this discrepancy.

Helicase I activity was also detected in F^- strains bearing pPM3 (Table 1), a deletion derivative of pRS31 that has lost *traS*, *traT*, and part of *traD* but retains the complete *traI* and *traZ* genes as well as insertion sequence IS3 (Fig. 3). Both *traD* and *traZ* gene products have been assigned M_rs ≈ 78,000 (9, 10). Two preparations from 1100 F^-(pPM3) cells showed levels of helicase I protein (Fig. 2, lane H) and ATPase (not shown) similar to those obtained from 1100 F^+ cells (Fig. 2, lane G).

Direct evidence that helicase I is determined by *traI* was provided by using insertion and deletion plasmids available. Plasmid pPM320 was constructed by the insertion of transposon Tn*5* into the *traI* gene of pRS31. The insertion derivative had a reduced ability in complementing an F′*lac*$^+$*traI* mutation (10). Also, it did not encode the M_r 180,000 protein in minicells, in which polarity effects of the transposon insertion were not detected (10). Upon transformation of F^- cells with pPM320, neither active helicase I (Fig. 1*E*; Table 1) nor the M_r 180,000 electrophoretic band were observed (Fig. 2).

Plasmid pPM55 is a derivative of pRS31 deleted for *traS*, *traT*, *traD*, and ≈40% of the NH_2-terminal part of *traI* as determined by heteroduplex analysis (10). It is *traI*$^-$ in a genetic complementation test and does not encode the M_r 180,000 protein in minicells (10). An extract from 1100 F^-(pPM55) cells was processed as described for helicase I, and the purified protein was analyzed by $NaDodSO_4$ gel electrophoresis followed by blotting. Blotting helicase I antigen was radiographically visualized by reaction with helicase I antibody and subsequent binding of radioiodinated *Staphylococcus* protein A. A peptide of M_r ≈160,000 antigenically related to helicase I was found in the material prepared from 1100(pPM55) cells (Fig. 4). This peptide is shorter than the wild-type helicase purified from 1100 cells carrying either the F factor or plasmid pRS31. A protein fraction from F^- cells processed similarly did not show any band on the autoradiograph (not shown). A pPM55-encoded peptide of similar size was also detected in minicells (not shown). Considering the extent of the *traI* deletion present on pPM55, the detected peptide is probably derived from a gene fusion between the NH_2-terminal portion of a vector-encoded gene with the COOH-terminus of *traI*.

Of interest is the fact that the shortened polypeptide encoded by the *traI* deletion mutant retained *in vitro* ATPase and DNA unwinding activities, both inhibitable by antibodies against helicase I (Table 1). In addition, the active helicase I fragment copurified with the wild-type enzyme as shown on the phosphocellulose elution profile (Fig. 1*F*). Apparently only the COOH-terminal part of the helicase I molecule is needed for *in vitro* enzymatic activity.

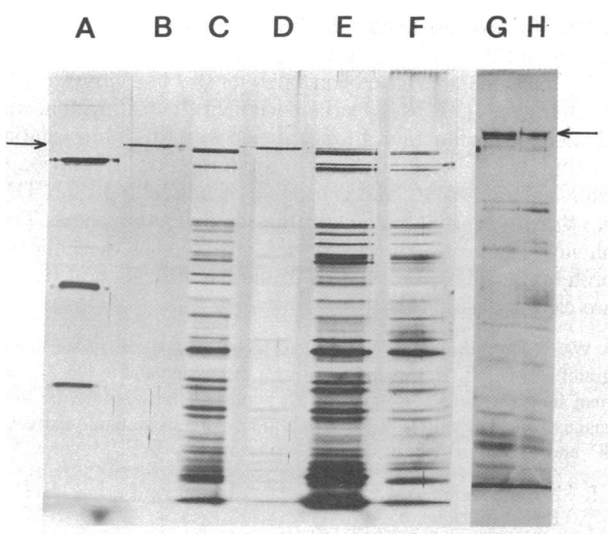

FIG. 2. $NaDodSO_4$/polyacrylamide slab gel electrophoresis (7.5%) from phosphocellulose fractions eluted at 260 mM NaCl (see Fig. 1). The gel was stained with silver nitrate (16). Lanes: A, *E. coli* RNA polymerase and bovine serum albumin as marker proteins; B, 0.2 μg of helicase I purified from *E. coli* H560 F′ cells as described (13); C–F, proteins from *E. coli* strains 1100 F^-, 1100 F^+, 1100 F^-(pRS31), and 1100 F^-(pPM320), respectively. Each lane carries 4 μg of protein with the exception of lane D (0.8 μg). Less protein was applied on lane D to prevent overloading of the M_r 180,000 band. The large difference in the number of other proteins in lane E compared to lane D is due to low recovery of helicase I protein from strain 1100 F^-(pRS31) (see text) and inherent variability in the efficiency of helicase purification in different preparations. On a separate gel were run 3 μg of protein from strains 1100 F^+ (lane G) and 1100 F^-(pPM3) (lane H). Arrows show the position of helicase I in the two gels: left margin, helicase I in lanes A–F; right margin, helicase I in lanes G and H.

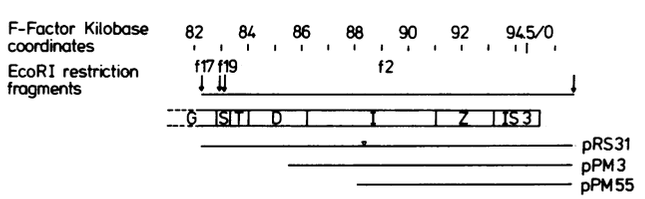

FIG. 3. Physical map of the F sex factor DNA encoded within the plasmids used (10). The F factor DNA shown is cloned in pSC101. ▽, Position of the Tn*5* insertion in pPM320. Boxes represent the amount of DNA required to encode the various proteins. Genes *S*, *T*, *D*, *I*, and *Z* code for transfer proteins; IS3 is an insertion sequence.

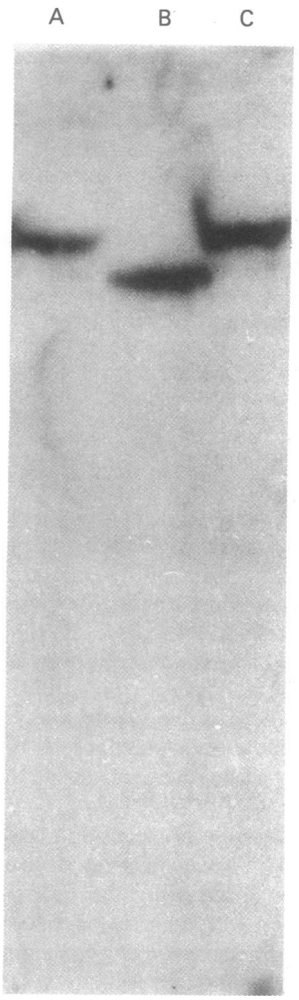

FIG. 4. Immunoblot analysis of the purified protein fractions from an F^+ strain and plasmid-carrying strains. Lanes: A, 1100 F^-(pRS31) strain, 6 μg of protein; B and C, 1100 F^-(pPM55) and 1100 F^+ strains, respectively, 3 μg each.

DISCUSSION

The presence of helicase I in partially purified fractions from bacterial cells can be ascertained by the following criteria: (*i*) the presence of a DNA-dependent ATPase activity and an *in vitro* DNA unwinding activity, both of which are completely inhibitable by anti-helicase I-antibody, (*ii*) chromatographic resolution of DNA-dependent ATPase activity on a phosphocellulose column, and (*iii*) visualization of a peptide band of M_r 180,000 on NaDodSO$_4$ gels.

Using these criteria, we have detected helicase I in F^+ cells but not F^- cells. After the F factor was transferred to F^- cells, activity and the M_r 180,000 electrophoretic band characteristic of helicase I could be identified in the transconjugant cells. Evidence that helicase I is encoded by the *traI* gene of the F factor was obtained by studying plasmids pRS31 and pPM3, each of which covers the *traI* gene of F factor. Because of the presence of these plasmids, helicase I could be detected in *E. coli* F^- strains. On the other hand, helicase I was not found in F^- cells carrying a *traI*::Tn5 mutated plasmid derived from pRS31. This insertion plasmid (pPM320) was shown to be *traI*$^-$ in genetic complementation tests (10). Furthermore, deletion of part of the *traI* gene in plasmid pPM55 resulted in the synthesis of a shortened helicase I peptide, thus providing direct evidence for the identity of helicase I as the *traI* gene product.

The conclusion that helicase I is specified by the *traI* gene of the F sex factor implicates a role for helicase I in bacterial conjugation, a process which has been characterized mainly genetically (21).

The product of *traI* gene is one of the few cytoplasmic gene products encoded by the transfer operon (21). Mutations in the gene are also known to prevent donor conjugal DNA synthesis and transfer of F factor plasmid DNA to the recipient cell (22). Functions concerning formation of stable mating aggregates are not impaired in these mutants (21). Thus, the product of the *traI* gene is shown to be required at the stage of DNA metabolism during conjugation.

The initiation of F factor plasmid transfer is presumably initiated by endonucleolytic cleavage of the DNA strand to be transferred. Involved in this origin-specific nicking event are the products of the transfer genes *traY* and *traZ* (23). The products of *traM* and *traI* have been proposed to function in the modulation of the *traYZ* endonuclease (23), allowing subsequent transfer of the nicked DNA strand to the recipient cell. Concomitantly, donor conjugal DNA synthesis normally replaces the transferred single strand.

Considering these results, we conceive helicase I acting after the endonuclease cleavage step that separates the DNA strands of the open F factor plasmid form (as required for transfer and for donor conjugal synthesis) at the expense of ATP hydrolysis. From the biochemical characterization of helicase I, it is known that the enzyme requires a single-stranded DNA region of 200 nucleotides for the start of unwinding and cannot start at a nick (24). Thus, we assume that prior to the unwinding action of helicase I, the nick made by endonuclease *traYZ* must be extended to form the required single strand. The *traM* protein, also required at the stage of DNA transfer and synthesis, might accomplish such a function, possibly acting as a DNA binding protein. Because helicase I unwinds DNA in the 5′-to-3′ direction by moving along the strand to which it is bound, the enzyme must act on the strand being transferred.

Very few mutants have been found for *traI* in contrast to other transfer genes (10), as if a large portion of the *traI* protein were not needed for function. In accordance with this interpretation, we find that helicase I deleted in the NH_2-terminal portion retains ATPase activity and is capable of unwinding short DNA *in vitro*. Interestingly enough, the same deletion shows TraI$^-$ phenotype *in vivo*. Studies on the structure of the helicase I protein in conjunction with controlled modification of its gene are clearly desirable.

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