Control of Ferredoxin and Gal/GalNAc Lectin Gene Expression in *Entamoeba histolytica* by a cis-Acting DNA Sequence

CAROL A. GILCHRIST, BARBARA J. MANN, AND WILLIAM A. PETRI, JR.

Departments of Internal Medicine, Microbiology, and Pathology,
University of Virginia, Charlottesville, Virginia 22908

Received 20 November 1997/Returned for modification 6 January 1998/Accepted 26 January 1998

The ferredoxin (fdx) and lectin (hgl5) promoters of *Entamoeba histolytica* contain the DNA sequence motif TATTCTATT (URE3). Previously we showed that mutation of the URE3 motif in the hgl5 lectin promoter results in an increase in promoter reporter activity. Mutation of this motif in the fdx promoter led to a 40-to-50% decrease in fdx promoter activity as measured by reporter gene activity and abundance of mRNA. *E. histolytica* nuclear proteins exhibited sequence-specific binding to the URE3 motif in electrophoretic mobility shift assays. These results support the regulation of both ferredoxin and lectin promoters by a nuclear protein(s) which recognizes the URE3 motif.

The protozoan *Entamoeba histolytica* is a major cause of morbidity and mortality worldwide. There are an estimated 50 million cases of invasive amebiasis annually. The most common clinical presentation of *E. histolytica* infection is amebic dysentery, but amebic abscesses in liver, lung, and brain also occur. Annual mortality in 1994 was estimated to be between 40,000 and 110,000.

The factors that promote invasive rather than commensal infection are not understood (24). Clonal lines derived from a single isolate of *E. histolytica* exhibit different levels of virulence (17). In addition, external factors alter the virulence of trophozoite cultures (1, 2, 8, 11, 13, 15). This difference in virulence is thought to be affected at least partly through altered expression of *E. histolytica* genes. The study of transcriptional regulation of *E. histolytica* genes is therefore important for understanding the virulent phenotype. Little is known about the regulation and control of gene expression in this pathogen. The recent development of transfection systems for the regulation and control of gene expression in this pathogen is therefore important.

Ferredoxin is the primary electron acceptor in the oxidation of pyruvate to acetyl coenzyme A, functioning as a cofactor for the amebic fermentation enzyme pyruvate-ferredoxin oxidoreductase. These electrons are used in the reduction of oxygen by this facultative anaerobe, and therefore this reaction is important for the survival of *E. histolytica* infection are not understood (24). Clonal lines derived from a single isolate of *E. histolytica* exhibit different levels of virulence (17). In addition, external factors alter the virulence of trophozoite cultures (1, 2, 8, 11, 13, 15). This difference in virulence is thought to be affected at least partly through altered expression of *E. histolytica* genes. The study of transcriptional regulation of *E. histolytica* genes is therefore important for understanding the virulent phenotype. Little is known about the regulation and control of gene expression in this pathogen. The recent development of transfection systems for the regulation and control of gene expression in this pathogen is therefore important.

The factors that promote invasive rather than commensal infection are not understood (24). Clonal lines derived from a single isolate of *E. histolytica* exhibit different levels of virulence (17). In addition, external factors alter the virulence of trophozoite cultures (1, 2, 8, 11, 13, 15). This difference in virulence is thought to be affected at least partly through altered expression of *E. histolytica* genes. The study of transcriptional regulation of *E. histolytica* genes is therefore important for understanding the virulent phenotype. Little is known about the regulation and control of gene expression in this pathogen. The recent development of transfection systems for the regulation and control of gene expression in this pathogen is therefore important.


0019-9567/98/$04.00 + 0

Copyright © 1998, American Society for Microbiology

The recent development of transfection systems for the regulation and control of gene expression in this pathogen is therefore important. Given that mutation of URE3 in both the fdx and hgl5 promoters affected reporter gene activity, we wished to determine whether an *E. histolytica* nuclear protein or proteins exhibited sequence-specific binding to URE3. Nuclear extracts were prepared by a modification of the method described by Esther Orozco, CINVESTAV, Mexico City, Mexico (17a): amebae (4 × 10⁷) were collected in growth medium, washed once in phosphate-buffered saline, and then collected by a 10-s spin at 7,000 × g. The amebae were resuspended in 4 volumes of 10 mM HEPES-KOH (pH 7.9), 1.5 mM MgCl₂, 10 mM KCl, 0.2% Nonidet P-40, 0.5 mM phenylmethylsulfonyl fluoride,
and 0.5 mM dithiothreitol and left to swell for 10 min on ice. After the mixture was vortexed for 10 s, the nuclei were collected by centrifugation for 10 min at 1,100 \( \times g \). The nuclei were then suspended in a solution of 150 mM of 20 mM HEPES-KOH (pH 7.9), 0.42 M NaCl, 1 mM EDTA, 1 mM EGTA, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM dithiothreitol. The nuclei were then incubated at 4°C for 30 min and spun for 20 min at 4°C at 10,000 \( \times g \). The supernatant containing the nuclear extract was aliquoted and flash frozen in liquid N\(_2\) and stored at \(-70°C\). Electrophoretic mobility shift assays were performed with the double-stranded oligonucleotides listed in Table 1. The oligonucleotides were labeled with \([\alpha^{32P}]dATP\) with the Klenow fragment of DNA polymerase I and purified from unincorporated nucleotide by a NucTrap column (Stratagene, La Jolla, Calif.). The radiolabeled probe was further purified by a polyacrylamide gel extraction procedure (7).

The protein-DNA interaction occurred in band shift buffer (10 mM Tris-HCl [pH 7.9], 50 mM NaCl, 1 mM EDTA, 0.05% nonfat milk powder [Carnation], 3% glycerol, 0.05 mg of bromophenol blue) to which 0.2 \( \mu \)g of poly(dIdC), 10 fmol of DNA probe, and 2 \( \mu \)g of nuclear extract were added (14). The reaction mixture was allowed to incubate at room temperature (20°C) for 1 h prior to electrophoresis on a nondenaturing polyacrylamide gel for 2 to 3 h (6). The gel was then fixed and dried, and the signal from the protein-DNA complex was quantitated after exposure of the gel to a phosphorimage screen as described previously.

A major and two minor protein-DNA complexes were observed when the nuclear extract was assayed with the \( fdx \) URE3 oligonucleotide (Fig. 2A). All three DNA-protein complexes were competed with self (the \( fdx \) URE3) but not with \( hgl5 \) MUT or the unrelated oligonucleotide. Only the major \( fdx \) URE3 DNA-protein complex was specifically competed by the addition of cold \( hgl5 \) URE3. In a converse experiment, we also assayed the \( hgl5 \) URE3 oligonucleotide (Table 1) by electrophoretic mobility shift. One major and several minor nuclear protein-DNA complexes were formed with \( hgl5 \) URE3 (Fig. 2B). Only the major (most prominent) band was competed by excess unlabeled \( hgl5 \) URE3 but not by the unrelated oligonucleotide or \( hgl5 \) MUT. The formation of the major \( hgl5 \) DNA-protein complex could be depleted by the addition of the \( fdx \) URE3 competitor but at a higher molar excess than that required to achieve competition by itself. The major gel shift band was not observed when the \( hgl5 \) MUT oligonucleotide was assayed by electrophoretic mobility shift (data not shown).

The cross competition for the nuclear protein binding by \( fdx \) URE3 and \( hgl5 \) URE3 is consistent with the recognition of both URE3 motifs by the same nuclear protein or proteins. The surrounding sequences of the \( fdx \) and \( hgl5 \) promoters may have an impact on the equilibrium or strength of the URE3 DNA-protein binding reactions, as competition by the homologous URE3 oligonucleotide was greater than that by the heterolo-

![Image](image.png)

**p=0.0041**

FIG. 1. Mutation of URE3 motif decreases \( fdx \) promoter activity. (A) Luciferase reporter gene activity in amebae transfected with wild-type or URE3-mutated \( fdx \) promoters in growth-arrested and exponentially growing trophozoites (\( \times \) standard error, \( n = 3 \) where \( n \) is the number of independent experiments, each of which was done in triplicate). (B) \( luc \) and \( neo \) mRNA in exponentially growing amebae transfected with wild-type or URE3-mutated \( fdx \) promoters. (The image was generated with the PhosphorImager [Molecular Dynamics model 425] in conjunction with the Adobe Photoshop 3 software program.)

![Table 1](table.png)

<table>
<thead>
<tr>
<th>Oligonucleotide or promoter</th>
<th>Sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( hgl5 ) URE3 ..........</td>
<td>TGTCCAAAAAGATAATTCTATT GAAAATAAAAGAG</td>
</tr>
<tr>
<td>( hgl5 ) MUT ..........</td>
<td>TGTCCAAAAAGATTAGAATTCCAA GAAAATAAAAGAG</td>
</tr>
<tr>
<td>( fdx ) URE3 ..........</td>
<td>ACTAACTAAATTTCTATTCTATT TAAATCACAAAA</td>
</tr>
<tr>
<td>Olig-1* ..........</td>
<td>AGAACGGTGATAGTC</td>
</tr>
<tr>
<td>( hgl5 ) promoter ..........</td>
<td>GTCACAAAAGAGATAATTCTATT GAAAATAAAAGAG</td>
</tr>
<tr>
<td>( fdx ) promoter ..........</td>
<td>ACTAACTAAATTTCTATTCTATT TAAATCACAAAA</td>
</tr>
</tbody>
</table>

* The URE3 motif is indicated in bold-faced type, and underlining indicates sequence similarity.

* Olig-1, unrelated oligonucleotide.
gous URE3 oligonucleotide. However, these results could also be due to the binding of two different proteins with different affinities for the URE3 sequence in the contexts of hgl5 and fdx promoters.

In summary, mutation of the URE3 motif in both fdx and hgl5 altered reporter gene activity. The electrophoresis mobility shift assays demonstrated recognition of the URE3 motif by an E. histolytica nuclear protein(s). The fact that the mutation of URE3 had opposite effects in different promoter contexts might seem unusual; however, there are a considerable number of precedents. The binding of transcription factor E2F, for instance, can affect promoter activity positively or negatively depending whether unphosphorylated pocket proteins are present (12, 18).

Identification of the protein or proteins which bind to URE3 will be important for understanding its function in transcriptional regulation. Future studies on URE3-mediated regulation will allow clarification of the role of the proteins which bind to URE3 in both trophozoite growth and host invasion.

We thank Esther Orozco and Janet Yee for advice on preparing nuclear extracts and Tim Bender, Alison F. Richardson, Lisa Palmer, and David Auble for helpful discussions.

This work was supported by NIH grant AI 37941. W.A.P. is a Burroughs Wellcome Fund Scholar in Molecular Parasitology.

REFERENCES

17. Orozco, E., Personal communication.  


