

Adherence to Human Vaginal Epithelial Cells Signals for Increased Expression of *Trichomonas vaginalis* Genes

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Host parasitism by *Trichomonas vaginalis* is complex, and the adhesion to vaginal epithelial cells (VECs) by trichomonads is preparatory to colonization of the vagina. Since we showed increased synthesis of adhesins after contact with VECs (A. F. Garcia, et al., *Mol. Microbiol.* 47:1207–1224, 2003) and more recently demonstrated up-regulated gene expression in VECs after parasite attachment (A. S. Kucknoor, et al., *Cell. Microbiol.* 7:887–897, 2005), we hypothesized that enhanced expression of adhesin and other genes would result from signaling of trichomonads following adherence. In order to identify the genes that are up-regulated, we constructed a subtraction cDNA library enriched for differentially expressed genes from the parasites that were in contact with the host cells. Thirty randomly selected cDNA clones representing the differentially regulated genes upon initial contact of parasites with host cells were sequenced. Several genes encoded functional proteins with specific functions known to be associated with colonization, such as adherence, change in morphology, and gene transcription and translation. Interestingly, genes unique to trichomonads with unknown functions were also up-regulated. Semiquantitative reverse transcription-PCR (RT-PCR) confirmed expression of select genes. An increased amount of protein was demonstrated by immunoblotting with monoclonal antibody. Finally, we showed the transcriptional regulation of some genes by iron by using RT-PCR. To our knowledge, this is the first report addressing the differential regulation of *T. vaginalis* genes immediately upon contact with VECs.

Trichomonas vaginalis colonizes the urogenital tract of humans, causing trichomonosis, the number one nonviral sexually transmitted disease worldwide. Despite an estimated 8 million new cases per year in the United States alone (53), this health disparities disease (48) remains poorly studied. *T. vaginalis* infection is associated with adverse health consequences to both men and women, including infertility (20, 46), atypical pelvic inflammatory disease (35), and increased human immunodeficiency virus transmission (24, 49). Trichomonosis is also associated with preterm birth, low-birth-weight infants (16), predisposition to development of cervical neoplasia in women (51), and nongonococcal urethritis (9) and chronic prostatitis (10) in men. The complex interplay of trichomonad responses and that of the host cells during infection have not been investigated so far. Of the few reports concerning host-parasite interactions, one study showed that after short contact of trichomonads with host vaginal epithelial cells (VECs), but not HeLa cells, the parasite morphology is transformed from an ellipsoid to an amoeboid form (8), suggesting host-specific signaling of parasites. In addition, synthesis of all four adhesin proteins in the amoeboid forms bound to VECs was enhanced (8, 22). In a separate study using different forms of parasites grown in culture flasks, α -actinin was shown to be overexpressed in amoeboid parasites compared to batch-cultured ellipsoid trichomonads (1).

The transcriptional regulation of parasite genes in response to interactions with host cells using in vitro models has been

studied in *Neisseria meningitides* (23), *Porphyromonas gingivalis* (25, 41), and *Helicobacter pylori* (26). Due to the lack of a good animal model system to study *T. vaginalis* pathogenesis, we have used an in vitro model of immortalized human VECs (22) in our present study. Because of the unavailability of genome sequence data at the time this study was initiated, we used the subtraction cDNA library approach to identify the transcriptional changes in gene expression during the initial step of *T. vaginalis* attachment to VECs. Differentially expressed gene profiling using cDNA subtraction has been an ideal tool in identifying novel genes and transcripts of low abundance (13). Our data identify numerous *T. vaginalis* genes that are up-regulated upon contact, which was confirmed by semiquantitative reverse transcription-PCR (RT-PCR) and protein immunoblot analyses. We believe that functional analyses of up-regulated genes of both VECs, as done recently (29), and of the organisms after the adherence event will contribute to our understanding of the host-pathogen interrelationship and the elucidation of the mechanisms of pathogenesis.

MATERIALS AND METHODS

Parasites and host cells. *T. vaginalis* isolate T016 was grown in trypticase-yeast extract-maltose (TYM) medium supplemented with 10% serum at 37°C (19). For iron-replete parasites, TYM serum was supplemented with 200 μ M ferrous ammonium sulfate, and iron-depleted parasites were obtained by cultivation in medium depleted of iron with 50 μ M 2,2-dipyridyl (32). Immortalized MS-74 human VECs (22, 27, 29, 36) used for adherence experiments were grown in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum at 37°C in the presence of 5% CO₂.

Isolation of total RNA. The MS-74 VECs were used for adherence as recently detailed (22). Briefly, 6×10^5 VECs were seeded onto T75 culture flasks and allowed to form a monolayer for 2 days. VECs were then washed with a medium mixture of Dulbecco's modified Eagle's medium–TYM (2:1, vol/vol) without serum. Parasites at the mid-logarithmic phase of growth were added to the

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TABLE 1. Primers used for RT-PCR analyses

Gene	Primer sequence (5'-3')	Annealing temp (°C)	Amplicon size (bp)
α -Actinin	AAGTTCGGACGTGTTGTTTCGTCTGGGCCTGCTGGAGGTAGTTG	48	400
α -Tubulin	GAAATGACTGGTGCATAAGAGCACTCTGCTGCCTCGAGCACGGTA	48	700
AP33	CTCATTTCGTCC CAGCTCCAAACAATACCGATCTTACCG	45	300
AP65	CAGTTAGATATGGGTACAGACCGCTCGCAGTTAGCGCATGTAG	48	650
GTP-BP	ACACTT ACACCAGTTCCTGGCTTGTTCACACTAGAAGTAATTG	46	300
PDI	TACTCCTTCACAGAAATCACTATGAAGAACA CCGAATTTGTA	42	300

MS-74 monolayer at a parasite-VEC ratio of 10:1 and incubated at 37°C to allow for parasite adherence. Parasites were incubated with the VECs for 30 min, and nonadherent parasites were removed by aspiration. The monolayer with adherent parasites was further incubated for 2 h until the monolayer was completely lysed. The parasites after interaction with the monolayer are referred to as primed parasites. Total RNA from control and primed parasites after contact with VECs was isolated using Trizol reagent (Invitrogen).

Construction of subtraction cDNA library. The cDNA from primed (tester cDNA) and control (driver cDNA) parasites was prepared using a cDNA synthesis system (Roche Diagnostics Inc.). Briefly, 2 μ g of total RNA was reverse transcribed using an oligo(dT)₁₅ primer and avian myeloblastosis virus reverse transcriptase according to the manufacturer's protocol. The subtractive cDNA library was constructed according to the standard protocol (52). Briefly, each set of cDNA was digested with AluI and RsaI to generate shorter, blunt-ended cDNA fragments. The tester DNA and driver DNA were ligated with different sets of adaptors. Ligated cDNA was amplified by PCR using the adaptor primers to obtain large amounts of cDNA. The tester cDNA was labeled with [³²P]dCTP, and the driver cDNA was biotinylated using 0.5 mM bio-11-dUTP (Biotium Inc.). A first hybridization between an excess of driver and tester was carried out to enrich the differentially expressed sequences. Tester/driver and driver/driver hybrids and biotinylated single-stranded driver cDNA were removed by addition of streptavidin and extraction with phenol-chloroform. The percentage of tester cDNA removed was determined by the radioactive counts remaining after phenol-chloroform extraction. Further rounds of subtraction were performed using the cDNAs from the previous round as a template for PCR synthesis of tester and driver cDNAs. After 10 rounds of alternating long and short hybridizations, the subtracted cDNA fraction was cloned into a TA vector and transformed into *Escherichia coli* to create a primed *T. vaginalis* (pTv) cDNA library.

PCR analysis of the subtraction efficiency. PCR was performed on subtracted and unsubtracted cDNA with α -tubulin primers (Table 1) as an internal control. Each reaction was amplified for 30 cycles, and 5- μ l aliquots were removed after 15, 20, and 25 cycles. The PCR products were electrophoresed on 1% agarose gels followed by staining with ethidium bromide (EtBr). The efficiency of subtraction was reflected by the difference in the number of cycles required for equal amplification of the PCR product in subtracted and unsubtracted samples.

Sequencing and analysis. Colonies were randomly selected, and plasmids were prepared using a Miniprep kit (QIAGEN, Valencia, CA). The cDNA inserts were verified by restriction digestion, and the clones were sequenced in our institutional DNA-sequencing facility. First, the sequence data were compared with data in GenBank using a BLAST program. Since there were not many matches, we then used the TIGR database, which consisted of partially annotated *T. vaginalis* genome sequences (<http://tigrblast.tigr.org/er-BLAST/index.cgi?project=tv>).

RT-PCR analysis of selected genes. Differential expression of a subset of cloned genes was confirmed by semiquantitative RT-PCR. Total RNA from control nonprimed and primed parasites was reverse transcribed with the oligo(dT)₁₅ primer using Superscript II reverse transcriptase (Invitrogen), according to the manufacturer's protocol. PCR amplification of cDNA was carried out using gene-specific primers (Table 1). The trichomonad α -tubulin gene was used as an internal control. RT-PCR from iron-depleted and iron-replete organisms was also performed as explained above. Twenty-five cycles were used for amplification of specific genes (see Fig. 2), except for the protein disulfide isomerase (PDI) gene, which was amplified for 35 cycles because of its low levels of

expression. As a control, all reactions were spiked with a known amount of human DNA, and RT-PCR was performed using α -tubulin primers. This assured the abundance of PCR product detected under these experimental conditions with the known primers. In addition, all RNA samples without reverse transcription were also used for PCR to detect genomic DNA contamination, if any. PCR products were visualized on EtBr-stained agarose gels, and the band intensity was quantitated using the Scion image beta program. The PCRs were carried out at four different times to verify the reproducibility of results. The result from one of the experiments is used for the data presentation.

Production of MAb HA423 against α -actinin. Hybridomas producing monoclonal antibodies (MAbs) against the α -actinin protein were obtained using standard protocols, as we have described previously (4). The MAb HA423 was identified by screening supernatants of antibody-producing hybridomas with different preparations of high- M_r protein antigens eluted from acrylamide gels after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and using anti-mouse immunoglobulin G followed by alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G. After subsequent single-cell cloning, the hybridomas were then reacted with *E. coli* expressing the recombinant protein from the full-length α -actinin gene (1).

SDS-PAGE and immunoblotting. Lysates from identical numbers of control and primed parasites were separated by 10% SDS-PAGE, and the proteins were blotted onto nitrocellulose membranes (Bio-Rad, Hercules, CA). The nitrocellulose blots were then incubated in a solution containing 0.1% Tween 20 and 5% bovine serum albumin prior to probing with the MAbs F5, DM116, and HA423, specific for adhesin protein 33 (AP33), AP65, and anti- α -actinin, respectively, at a dilution of hybridoma supernatant of 1:1,000. As a protein loading control, an identical blot was probed with human α -tubulin MAb clone B512 (Sigma, St. Louis, MO) at a dilution of 1:50. The blots were further incubated with goat anti-mouse secondary antibody (1:1,500 final dilution) conjugated with horseradish peroxidase (Bio-Rad). The blots were washed well and incubated in horseradish peroxidase substrate (Bio-Rad) to visualize the reactive band.

RESULTS

PCR-based subtraction cDNA library. We used a subtractive cDNA library to obtain *T. vaginalis* genes that were up-regulated in response to contact with VECs. Total RNA from the primed parasites free of host cell RNA was isolated as detailed in Materials and Methods. Figure 1A shows the RNA without any apparent degradation on an agarose gel. Importantly, there was no contamination of host RNA in the trichomonad RNA preparations. Following reverse transcription, a cDNA library was constructed by subtracting the cDNA of *T. vaginalis* parasites incubated with VECs (referred to as primed *T. vaginalis*, or pTv) from control cDNA of *T. vaginalis* handled identically. The subtraction methodology involved suppression of PCR amplification of the common sequences. The amplified cDNAs were ligated to the TA vector to create the library. About 250 clones were obtained in the first round of plating

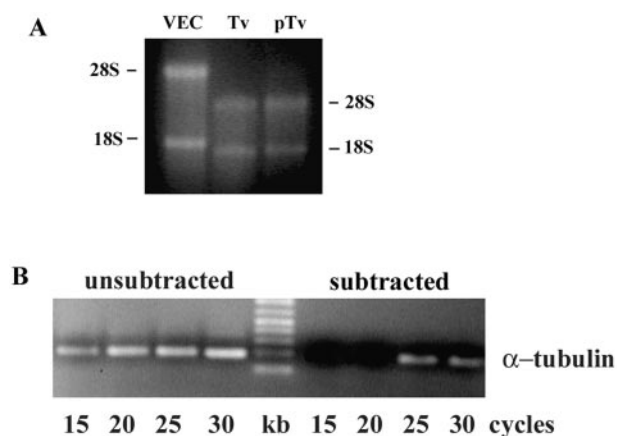


FIG. 1. RNA isolation and PCR analysis of subtraction efficiency. (A) Total RNA was isolated from primed parasites after contact with VECs (pTv) and control organisms handled identically (*T. vaginalis* [Tv]). Total RNA (3 μ g) was separated on a 1.2% agarose gel followed by staining with EtBr to visualize the purity and assess degradation of RNA. 28S and 18S refer to the rRNA bands of the VECs and the parasites. (B) PCR using α -tubulin primers was performed on unsubtracted and subtracted cDNAs. Aliquots (5 μ l) were removed at a predetermined number of cycles and analyzed by EtBr-stained gels after electrophoresis in 1.2% agarose. The α -tubulin product appeared only after 25 cycles in the subtracted sample compared to 15 cycles in the unsubtracted sample.

from the transformed cDNA library. The average size of cDNA inserts was \sim 200 bp. The subtraction efficiency was estimated by comparing the abundance of a known gene prior to and after subtraction. We amplified the *T. vaginalis* α -tubu-

lin housekeeping gene as a control from subtracted and unsubtracted cDNA. Figure 1B shows that the PCR product for α -tubulin is detectable only after \geq 25 cycles in the subtracted sample, while it is readily detectable after \leq 15 cycles in the unsubtracted sample, indicating successful subtraction efficiency.

***T. vaginalis* gene expression regulated by attachment to VECs.** A total of 30 rescued plasmid clones selected at random were sequenced. The sequences were used to perform BLAST searches of the GenBank database and the incomplete TIGR *T. vaginalis* genome database. The list of genes that were identified is shown in Table 2. The up-regulated genes exhibited homologies with the genomic sequences or expressed sequence tags encoding various functional classes of proteins. Interestingly, only 33% of the genes were previously documented in GenBank, and these included the adhesins AP65 and AP33, α -actinin, and enolase. A putative PDI gene, a phosphoglucomutase family protein involved in glucose metabolism, and a conserved GTP-binding protein (GTP-BP) were up-regulated. Genes involved in transcription and protein translation in addition to six genes with unknown functions were also elevated in expression. The identification of adhesin genes in the subtraction library validates the increased amounts of adhesins after adherence (8, 22).

RT-PCR confirms increased gene expression. Relative levels of transcription of specific genes were analyzed by semiquantitative RT-PCR. As mentioned above, AP65 and AP33 were selected because of increased synthesis of adhesins upon contact with host cells (7, 22). In addition, we chose α -actinin, GTP-BP, and PDI genes based on a possible role in adherence and the functional role played by these enzymes in other or-

TABLE 2. Genes upregulated in *T. vaginalis* upon early stages of cytoadherence to MS-74 VECs

Gene product	Accession no. ^b	Description and/or putative function
Genes with known function		
AP65 (2) ^a	U35243	Adhesin, decarboxylating malic enzyme
AP33 (3)	U87098	Adhesin, α -subunit of succinyl coenzyme A synthetase
α -actinin (3)	AF072678	Cell structure maintenance
Enolase (2)	AF159525	Glycolytic enzyme
PDI (1)	74220.m00007	Disulfide formation, isomerization, chaperone
(GTP-BP) (2)	40053.m00110	Protein sorting, endocytosis
Phosphoglucomutase family protein (1)	43341.m00129	Glucose metabolism
Transcription		
ATP-dependant RNA helicase (Dbp5) (1)	61898.m00047	Gene transcription
Protein kinase domain protein (2)	52196.m00022	Gene transcription/cell signalling
Translation		
Acidic ribosomal protein p2- β	39445.m00044	Protein synthesis
Glutamyl tRNA synthetase (1)	40147.m00031	Protein synthesis
Ribosomal protein L-16, cytosolic (1)	38379.m00089	Protein synthesis
Translation elongation factor EF-1 (3)	43366.m00104	Protein synthesis
Unknown		
Conserved hypothetical protein (1)	55466.m00002	Unknown
Hypothetical protein (1)	56189.m00008	Unknown
Hypothetical protein (2)	40839.m00207	Unknown
Large supernatant protein 1 (1)	37893.m00027	Unknown
Pollen-specific glycine-rich protein (1)	52891.m00042	Unknown
Unnamed protein product (1)	41853.m00030	Unknown

^a Numbers of copies of the clone picked up out of total 30 clones sequenced are shown in parentheses.

^b Accession numbers listed are from GenBank and the TIGR database.

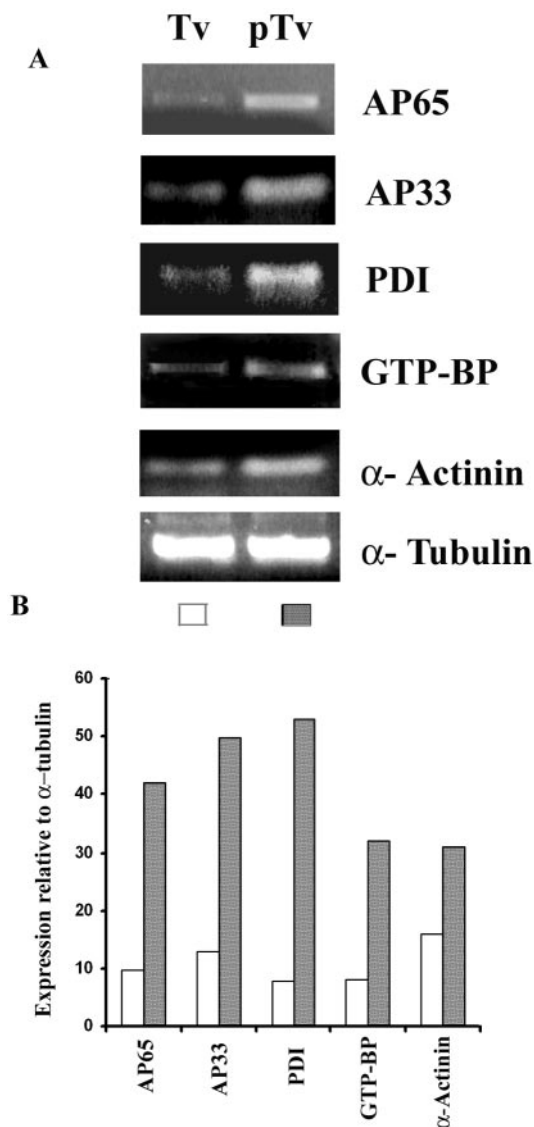


FIG. 2. Representative experiment showing confirmation of gene expression patterns in primed (pTv) and control (*T. vaginalis* [Tv]) parasites by semiquantitative RT-PCR analyses. Total RNA from pTv and *T. vaginalis* was isolated as detailed in Materials and Methods. RNA was reverse transcribed using oligo(dT) primer, and PCR was performed using gene-specific primers (Table 1). (A) RT-PCR products separated on EtBr-stained gels after electrophoresis in 2% agarose. (B) Gene expression pattern relative to the housekeeping trichomonad α -tubulin gene used as a control. The values were obtained by scanning the intensities of bands from pictures of the gels in A using the Scion image beta program. The expression for each gene was relative to baseline density for α -tubulin plotted on the graph. This experiment was repeated on four separate occasions with similar results.

ganisms. RT-PCR products of the selected genes were separated and visualized on ethidium bromide-stained gels, and as shown in Fig. 2A, band intensities were greater in PCR products from primed parasites compared to those of controls. Furthermore, the bar graph in Fig. 2B illustrates the higher levels of increased expression for these genes relative to α -tubulin, as quantitated by the Scion image beta program. Com-

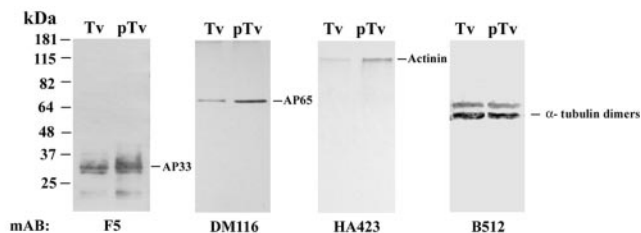


FIG. 3. Detection of protein expression in *T. vaginalis* upon adherence to VECs. Cell lysates were prepared from equivalent numbers of primed (pTv) and control (*T. vaginalis* [Tv]) parasites prior to SDS-PAGE and blotting onto nitrocellulose membranes, as described in Materials and Methods. Quadruplicate blots were probed with the MAbs F5, DM116, HA423, and B512, specific for AP33, AP65, α -actinin, and α -tubulin, respectively. The numbers indicated on the left-hand side are the molecular mass standards. A negative control without MAb and with only secondary antibody was always nonreactive in duplicate blots (data not shown).

pared to unprimed control RT-PCR products, the range of increased expression varied from twofold (α -actinin) to sevenfold (PDI). These data reaffirm the up-regulation of genes identified by the subtraction library.

Verification of protein expression of up-regulated genes. We felt that it was important to verify elevated levels of transcription with amounts of those proteins where MAbs were available. Figure 3 shows that MAbs F5, DM116, and HA423 against AP33, AP65, and α -actinin, respectively, readily detected each corresponding protein on nitrocellulose blots containing total proteins of control unprimed versus primed *T. vaginalis* parasites. Increased amounts of proteins based on the intensities of the bands for identical numbers of parasites loaded onto gels were obtained for the primed trichomonads. As an additional control, MAb that was reactive to the trichomonad α -tubulin was used similarly, and identical intensities of protein bands were obtained for control and primed parasites, indicating specific upregulation of expression of AP33, AP65, and α -actinin upon contact. Negative control experiments were performed on duplicate nitrocellulose blots using secondary antibody alone and were unreactive with any trichomonad proteins. These data suggest that signaling of parasites upon contact with VECs is evidenced by increased transcription of genes and corresponding translation of transcripts to protein.

Effect of iron on gene regulation. The genes for the AP65 and AP33 adhesins were up-regulated upon contact with the host cells, and both also are known to be transcriptionally regulated by iron (22, 31). We therefore wanted to examine whether other genes that were increased in expression upon contact were equally modulated by iron. For trichomonads grown in medium limited in or supplemented with iron, we performed RT-PCR on the four other genes with the AP65 and AP33 genes as controls. Only the adhesin genes for AP65 and AP33 gave decreased and increased amounts of transcript for parasites grown in the presence of low- compared to high-iron medium, respectively (Fig. 4A). The intensity of RT-PCR products for the internal control α -tubulin gene and the GTP-binding protein remained unaltered, although the gene for GTP-binding protein was expressed at higher levels above α -tubulin. Interestingly, the α -actinin gene had a 60% higher

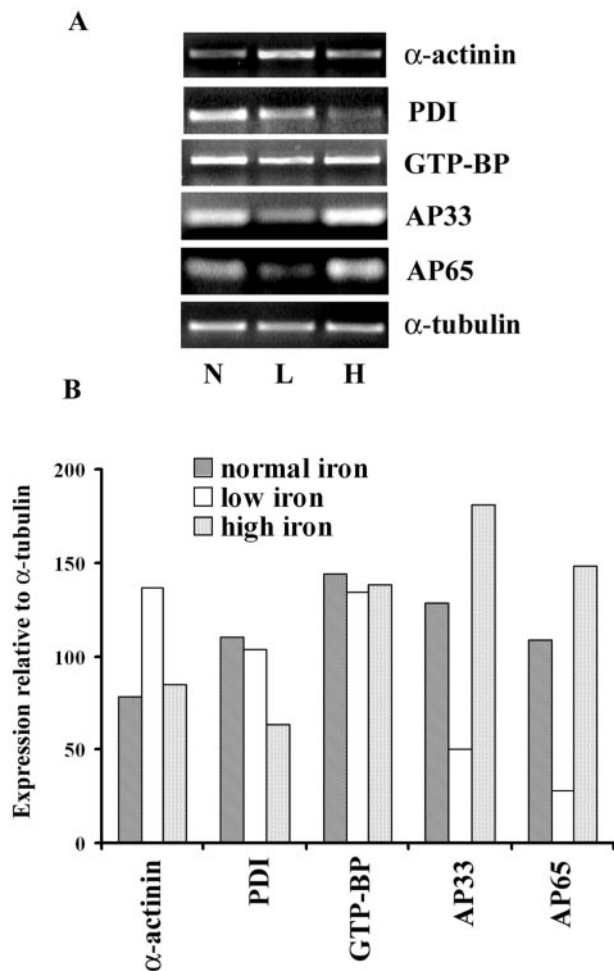


FIG. 4. Representative experiment showing the effect of iron on regulation of selected genes. Total RNA from parasites grown in normal TYM medium (N), iron-depleted medium (L), and iron-replete medium (H) was used in RT-PCR. (A) PCR products were separated on 2% agarose gels for visualization after EtBr staining. (B) Bar graph plotted using the relative values of band intensity obtained by Scion image beta scanning. Again, all values were normalized to the band intensity of α -tubulin as a control. This experiment was repeated on four separate occasions with similar results.

level of expression based on the amount of transcript in low-iron-grown parasites than those of normal or high-iron organisms (Fig. 4B). Last, the amounts of transcript for PDI were decreased when trichomonads were grown in high-iron medium. These results reinforce the idea of multiple signaling and/or regulatory pathways influencing the expression of genes induced by contact of organisms with host cells.

DISCUSSION

Specific adherence by *Trichomonas vaginalis* to epithelial cells of the vagina is mediated by surface proteins (7). We believe that dissection of the cross talk between the host cells and the adhering parasites is important to understand the mechanisms of pathogenesis, and this knowledge is prerequisite for intervention strategies. Recently, it was demonstrated that numerous genes of VECs are up-regulated in response to

contact by parasites (29). We now describe the use of subtraction cDNA hybridization to evaluate the signaling and subsequent transcriptional responses of the parasite after adherence to host cells, similar to what we reported recently for VEC genes (29). We compared gene expression in *T. vaginalis* adhering to human VECs with that of nonadherent parasites that were handled identically. Interestingly, the up-regulated genes with known functions appear to be related to events known to result from adherence. The fact that adhesin genes accounted for 5 of 30 randomly selected clones reinforces the important role of these proteins in infection and increased synthesis and placement of adhesins on the parasite surface after contact with VECs (8, 22). Indeed, these results strongly support past (5, 7, 21–23) and recent genetic studies using antisense RNA silencing of AP65 gene expression (36) and heterologous expression in *Trichomonas fetus* of AP65 to show a prominent role of this protein in adherence (30). Furthermore, 3 out of 30 clones were identified as α -actinin, which is known to have enhanced expression in the amoeboid forms of trichomonads (1).

We also picked up two clones of the poorly characterized *T. vaginalis* enolase gene. Importantly, recent studies have shown that enolase has functional diversity and is a plasminogen-binding protein in other pathogenic organisms, including *Fasciola hepatica* (12), *Streptococcus* spp. (11, 39), and *Staphylococcus aureus* (34). In addition, enolase acts as a spreading factor during tissue invasion of group A streptococci (40). Of particular interest to us will be future localization and functional analyses of the trichomonad enolase to determine whether there is an alternative, nonenzymatic role, if any, in surface expression and parasite adherence. The fact that the gene for GTP-binding protein was also up-regulated indicates an important role for this gene product during early events of infection. GTP-binding protein is mainly involved in the endocytic and vacuolar protein-sorting pathways (47) in higher eukaryotes and also is involved in signal transduction pathways (15). As both signaling and compartmentalization of adhesins are mediated by contact and iron (8, 22, 32), it is conceivable that this GTP-binding protein may likewise play an essential role in adherence.

Moreover, the finding that PDI was up-regulated in expression may be noteworthy, given the important function of these proteins in the complex processes of protein folding for biological functions (28). Little is known about protein folding in *T. vaginalis*, and, although speculative, it is likely that trafficking, compartmentalization, and surface placement of adhesins may require proteins such as PDI. It has been proposed that PDIs confer protection as an important survival mechanism against oxidative stress and a denaturing environment for *F. hepatica* (45) and *Giardia lamblia* (28). Equally noteworthy are the chaperone and nonchaperone activities of PDIs in addition to the disulfide bond isomerization. Our finding, therefore, highlights the possibly critical role of these multifunctional PDI proteins in initial steps of colonization. Finally, the fact that genes with unknown functions specific for *T. vaginalis* respond to parasite contact with VECs perhaps highlights the importance of these proteins both in the property of adherence and in pathogenesis. These findings make evident the need for structure-function characterization of these novel virulence factors heretofore not identified previously.

The finding that the genes for AP65, AP33, α -actinin, GTP-BP, and PDI were up-regulated in expression was consistent with the relative abundance of the clones in the subtraction library, and indeed, AP65, AP33, and α -actinin also had increased amounts of protein. The coordinated up-regulation of α -actinin with the adhesion genes is not surprising given the involvement of α -actinin in cytoskeletal rearrangements for transformation from ellipsoid to amoeboid forms and for recruitment and surface placement of adhesins following contact (1).

In the vagina, the menstrual cycle likely plays a role in modulating the availability of iron sources and other nutrients (14) essential for optimal parasite viability and colonization (5, 33, 42–44). Despite the fact that both high- and low-iron environments are found in the vagina (31), most trichomonads during infection are high iron (2, 3). Accordingly and not surprisingly, an iron-responsive promoter element regulating the expression of the prominent AP65 adhesion gene of *T. vaginalis* has been characterized previously (50). While the AP65 and AP33 genes had increased amounts of mRNA for parasites grown in high-iron medium (6, 21, 37), it was previously hypothesized that the increased synthesis of adhesins by parasites after attachment may be due to utilization of intracellular iron pools (7). Alternatively, the data may indicate the possibility of two distinct signaling pathways for up-regulation of expression of genes, i.e., iron and contact. The fact that the α -actinin gene had elevated amounts of transcript for low-iron trichomonads is also consistent with the notion of at least two signaling pathways modulating α -actinin gene expression (1, 7). Given the role of α -actinin in maintenance of the amoeboid form during adherence to VECs, it is conceivable that a dual signaling system for increased expression of α -actinin permits sustained adherence when organisms are in a low-iron niche. In this scenario, trichomonads are unable to synthesize new adhesins because of low iron yet retain the amoeboid morphology for maximum surface interaction and utilize existing adhesins for optimal attachment to VECs. In contrast, PDI had decreased amounts of transcript when organisms were grown in high-iron medium. To our knowledge, there are no reports of such regulation of PDI in other systems (28, 45). It is possible that this reduction in amounts of transcript may be a posttranscriptional event regulated in part by iron. Furthermore, since PDI is a highly stable protein with a half-life of approximately 7 days in rat liver (38), the decreased transcript amounts may not be problematic for PDI-mediated protein folding and other functions. The fact that GTP-binding protein remained unaltered under all the conditions tested may indicate a need for steady-state levels of this protein at all times to ensure the proper sorting and compartmentalization of proteins (47) regardless of environmental cues.

Collectively, these results indicate that there are different mechanisms of gene regulation for parasites in the complex, constantly changing environment of the vagina (14). That the gene regulation patterns are altered to achieve optimal host parasitism regardless of external stimuli represents a theme of the *T. vaginalis* organisms (2, 3, 5, 8, 17, 18, 31, 32). In conclusion, we have identified a set of known and unknown parasite genes that are up-regulated after contact with host cells. Although the functional role of some of these gene products in terms of parasite adherence and pathogenesis remains to be

determined, we feel that identification of both parasite and VEC-induced genes (29) after a crucial step preparatory for successful infection will help us elucidate and understand the complex host-parasite interrelationship.

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