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Trichomonas vaginalis: current understanding of host–parasite interactions

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Abstract

Trichomonas vaginalis is a sexually transmitted obligate extracellular parasite that colonizes the human urogenital tract. Despite being of critical importance to the parasite's survival relatively little is known about the mechanisms employed by *T. vaginalis* to establish an infection and thrive within its host. Several studies have focused on the interaction of the parasite with host cells and extracellular matrix, identifying multiple suspected *T. vaginalis* adhesins. However, with the exception of its surface lipophosphoglycan, the evidence supporting a role in adhesion is indirect or controversial for many candidate molecules. The availability of the *T. vaginalis* genome sequence paved the way for genomic analyses to search for proteins possibly involved in host–parasite interactions. Several proteomic analyses have also provided insight into surface, soluble and secreted proteins that may be involved in *Trichomonas* pathogenesis. Although the accumulation of molecular data allows for a more rational approach towards identifying drug targets and vaccine candidates for this medically important parasite, a continued effort is required to advance our understanding of its biology. In the present chapter, we review the current status of research aimed at understanding *T. vaginalis* pathogenesis. Applied experimental approaches, an overview of significant conclusions drawn from this research and future challenges are discussed.

Introduction

Trichomonas vaginalis is the most common non-viral sexually transmitted organism in humans, infecting more than 170 million people worldwide [1]. One of the challenges in estimating infection rates, diagnosing and ultimately treating *T. vaginalis* is the high proportion of asymptomatic infections [2]. When symptoms do occur, they are generally mild, manifesting as a general irritation and/or swelling of the urogenital tract and surrounding tissues. However, infections can lead to severe complications such as cervical erosion and premature birth during pregnancy [1]. Furthermore, *T. vaginalis* can cause infertility in both men and women [1,3]. Infection with this parasite also increases the risk of acquiring HIV, cervical cancer and aggressive prostate cancer [1,2,4]. These severe

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Note added in proof

Since this chapter was submitted for publication, detailed chemical analyses of LPG revealed a lack of phosphate in the glycan and this molecule has been renamed TvLG (*T. vaginalis* lipoglycan).

complications and the high incidence of infection support the need for more dynamic approaches to find new drug and vaccine targets.

As an extracellular organism, *T. vaginalis* must adhere to the epithelial lining of the host's urogenital tract to survive. Inflammation of the urethra, vagina and cervix in women as well as the urethra, epididymis and prostate in men reveal the ability of the parasite to inhabit several host environments [2]. These niches are continuously changing as a result of different host biological processes and outside forces. Such changes include natural hormonal shifts that can lead to epithelial shedding in the female urogenital tract, excretion that can alter salinity, pH and toxicity of the environment, and flux in communities of commensal and infectious micro-organisms. The parasite may turn this diverse environment to its advantage as a nutritional source. Several observations have shown that *T. vaginalis* has a cosmopolitan appetite and phagocytozes a variety of human cells and micro-organisms, including red blood cells, leucocytes, vaginal epithelial cells, bacteria, yeast and virus particles [5]. Interestingly, phagocytosis of leucocytes may be one way the parasite tempers the immune response, contributing to the lack of symptoms observed in the majority of infections. A host immune response may also be avoided by the parasite moving through cell junctions to dwell beneath the epithelial cell layer [6]. The secretion of proteases by the parasite that are used to degrade immunogenic cytokines and antibodies, or to activate a specific immune response, have also been proposed as mechanisms that favour survival of the parasite [7]. This myriad of interactions to obtain nutrients, establish infection, move within the urogenital tract, avoid or adapt to immune responses, and propagate infection is poorly understood in *T. vaginalis*.

Fortunately, the last few years have witnessed major advances in providing tools for *Trichomonas* researchers, including the sequence of the genome [8] and partial proteomes of defined subcellular compartments [9–12] (Table 1). Genome analyses [8] have revealed the existence of a vast number of multigene families, some of which are likely to play a role in *T. vaginalis* pathogenesis [13]. The massive size of the *T. vaginalis* genome, which is estimated to be ~165 Mb [8], will necessitate the use of multi-tiered approaches using complementary genomic, proteomic and biochemical analyses to resolve which proteins play central roles in pathogenesis. Proteomics is particularly useful, as global RNA expression tools have yet to be developed for *T. vaginalis* as a result of both the complexity and the repetition of its genome. In the present chapter, we review the literature on adhesion molecules and secreted proteins of *T. vaginalis* and discuss recent advances and limitations in this field.

Trichomonas adhesion

To survive as an extracellular parasite, *T. vaginalis* adheres to the epithelial lining or extracellular matrix components of the urogenital tract [5]. Attachment to cells, micro-organisms or other surfaces drives a transition of the ovoid free-swimming parasite into an amoeboid form [5,14] that may be highly adherent [5] (Figure 1). Independent isolates of *T. vaginalis* vary greatly in their ability to adhere to VECs (vaginal epithelial cells) in the laboratory [9], and adherent parasites often assume an amoeboid shape. It is unclear whether the greater surface area provided by the amoeboid form directly enhances adherence or

differences in protein expression between ovoid and amoeboid forms lead to the latter being more adherent. In addition to VECs, *T. vaginalis* can bind diverse cellular and non-cellular structures upon which a similar transition to an amoeboid form is observed. This suggests that the parasite utilizes either an adaptable non-specific or multiple specific binding mechanisms to mediate attachment and signal its morphological remodelling.

Multiple molecules have been postulated to be involved in attachment of *T. vaginalis* to target cells (Figure 1). These adhesion molecules can be separated into three categories: (i) a controversial set of metabolic proteins that appear to have secondary adhesive properties, (ii) a lipophosphoglycan and (iii) a collection of membrane proteins many of which have been recently identified through genomics and proteomics.

Adhesion molecules

Multifunctional metabolic proteins

The class of proteins designated as APs (adhesion proteins) consisting of AP23, AP33, AP51, AP65 and AP120 are the most controversial of the adhesion molecules [13]. With the exception of AP23, these are abundant metabolic enzymes primarily involved in carbohydrate metabolism and found in the hydrogenosome, a mitochondrion-like organelle found in *T. vaginalis* [15]. However, it has also been reported that these proteins are present on the surface of the parasite [16,17]. Finding proteins with multiple functions and localizations is not unreasonable, and these concepts are well reviewed by those who argue the dual function of these enzymes as metabolic proteins and adhesins [18]. Other research groups, however, have provided immunofluorescent microscopic evidence that these molecules are located exclusively in the hydrogenosome, challenging their dual localization [13]. Moreover, others have persuasively argued that these adhesin/hydrogenosomal proteins lack several characteristics of ‘true’ adhesion molecules (reviewed in [13]). On the basis of this it is argued that the observed binding of these proteins to host cells is promiscuous and lacks specificity. Indeed it has been shown that AP23, AP33, AP51 and AP65 adhere to several cell types and bind to these targets in the absence of membrane proteins [13,19]. These results suggest non-specific membrane binding, a property that would argue against a precise role in pathogenesis. Recent research shows that AP51 and AP65 bind to haem and haemoglobin, a property that paradoxically supports their promiscuous nature and lack of adherence specificity and implies a role in binding to a key nutrient [20].

Adding to this debate, it has recently been proposed that GAPDH (glyceraldehyde-3-phosphate dehydrogenase), a glycolytic enzyme, is involved in adherence of *T. vaginalis* to the host extracellular matrix protein fibronectin [21]. As with the other dual-functional adhesins/enzymes, additional evidence will be required before this protein is accepted as a true adhesin. Owing to their non-membrane localization and enzymatic function, dual-function adhesins are subject to a greater scrutiny, as is typically the case with any concept that counters generally accepted tenets. As previously recommended by Hirt et al. [13], definitive evidence that dual-functional proteins act as *T. vaginalis* adhesins will require finding specific binding partners in target cells and/or experimental demonstration of the trafficking of these proteins to the surface.

LPG (lipophosphoglycan)

T. vaginalis is coated by a complex structure of glycoconjugates known as a glycocalyx. Study of this glycocalyx began 30 years ago with lectin surveys of total parasite surface [22] and has recently narrowed to the investigation of a single surface glycan: LPG. The lectin studies demonstrated that parasites with virulent phenotypes, as defined by patient pathology and response of the mouse model, bound more strongly to soya bean agglutinin than less virulent parasites [22]. These results suggest that surface carbohydrates, specifically terminal *N*-acetylgalactosamine or galactose, are important in determining the virulence of *T. vaginalis.* Direct evidence supporting this hypothesis came through two observations. First, it was found that treating parasites with glycosylases caused a more than 20-fold reduction in binding of parasites to human epithelial cells [23]. In addition, exposure of *T. vaginalis* to periodate to oxidize the glycocalyx reduced parasite binding to laminin and adhesion to human vaginal epithelial cells [14,24]. Furthermore, periodate treatment of *Tritrichomonas foetus,* a related bovine pathogen, decreased binding of the parasite to bovine vaginal epithelial cells [25].

With several lines of evidence supporting the involvement of surface carbohydrates in virulence, researchers began to focus on isolating and defining specific molecules in the glycocalyx of *T. vaginalis*. Not surprisingly, the first, and only, surface glyconjugate isolated from *T. vaginalis* is the most abundant. With $2–3\times10^6$ molecules per cell, *T. vaginalis* LPG was thought to be a probable candidate to play a role in host–parasite interactions [26]. This complex molecule is composed of an inositol-phosphoceramide anchor [27] and a polysaccharide core composed of glucose, galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine, rhamnose and xylose at a consensus composition of 4.2%:14.6%: 35.8%:2.4%:27.1%:15.9% respectively [28,29]. Although, the entire structure remains unsolved, *T. vaginalis* LPG has been shown to contain poly-*N*-acetyllactosamine (Galβ1-4 GlcNAcβ1-3 Galβ1-4GlcNAc) repeats [30].

Even without a resolved structure, several important biological functions have been attributed to *T. vaginalis* LPG. Several lines of evidence demonstrate that *T. vaginalis* LPG is an adhesion molecule. First, exogenously added LPG inhibits binding of *Trichomonas* to epithelial cells in a species-specific manner [25,28,29]. Furthermore, mutated parasites expressing truncated LPGs have reduced adherence toward vaginal epithelial cells and their isolated LPGs do not inhibit binding of epithelial cells [28]. Finally, it has been demonstrated that LPG binds human galectin-1 and this interaction results in host cell attachment [31]. Although it is unclear whether galectin-1 binding causes alteration in host cell gene expression, LPG exposure to several epithelial cell types up-regulates interleukin-8 and macrophage inflammatory protein 3α expression [29]. Furthermore, LPG activates the pro-inflammatory transcription factor nuclear factor κB [30]. These activities support a role for LPG in parasite attachment, specificity of binding and manipulation of host cell gene expression.

Even though several functions of *T. vaginalis* LPG have been defined, important questions remain unanswered. First, it is unclear if galectin-1 is the only receptor for LPG, as several other galectins could function as receptors, particularly galectin-3, which is expressed by vaginal epithelial cells [32]. Furthermore, the available data do not demonstrate whether

galectin-1/LPG interaction leads to the observed changes in host gene expression or whether this interaction benefits the parasite or host [31,32]. The complete structure of LPG also remains unsolved, potentially obscuring several functions and novel drug targets. There is additionally an overall lack of knowledge about other molecules that make up the glycocalyx. The best solution to this problem is a large-scale glycomics analysis or systematic study of the entire surface glycosylation. The establishment of a glycome will allow for comparison of *Trichomonas* species to determine whether carbohydrates account for species preferences for certain niches and mammalian hosts. Furthermore, application of glycomics will allow us to expand on initial lectin studies and identify differences between strains of *T. vaginalis* that lead to virulence.

Membrane proteins

When attempting to identify proteins involved in attachment of a parasite to a host, an obvious starting place is proteins anchored to the parasite's surface. In fact, several observations suggest that *Trichomonas* surface proteins are involved in attachment to host cells and structures. Trypsinization of the surface of *T. vaginalis* decreases binding of the parasite to laminin, fibronectin and several human cell lines [14,33]. However, only recently has a membrane-localized protein been experimentally demonstrated to be involved in attachment of *T. vaginalis* [9]. Prior to this work, only the controversial moonlighting metabolic proteins discussed above had mixed experimental support for a surface association and involvement in attachment [18].

The completion of the *T. vaginalis* genome [8] has launched theoretical investigations of protein families that contain transmembrane domains and domains shared with surface proteins implicated in pathogenesis in other organisms [13]. Four families encompassing 128 genes encoding serine-, cysteine- and metallo-proteases were identified. These include proteins similar to GP63, a metalloprotease implicated in *Leishmania* virulence, a subtilisin-like serine peptidase implicated in egress of apicomplexan parasites [34], and unidentified serine and calpain-like CPs (cysteine proteases). These proteases may play a role in degrading host proteins or extracellular matrix to clear attachment sites [13]. Alternatively, they may be involved in degrading parasite–host bonds allowing the parasite to be released and transmitted to the next host. Additionally, three gene families encoding 47 proteins similar to surface proteins expressed in other mucosal pathogens, chlamydial polymorphic membrane proteins, immunodominant variable surface antigens and giardial VSP (variant surface protein)-like proteins, were identified [13]. Whether these *T. vaginalis* proteins are capable of attaching to common mucosal sites encountered by mucosal organisms awaits direct testing. These bioinformatic analyses also revealed the presence of genes encoding 11 surface lectins that may bind to carbohydrates in the extracellular matrix or on the host cell [13]. This possibility is strengthened by a previous observation that treatment of host cells with periodate to oxidize carbohydrates reduced parasite binding [35].

Finally, the largest family identified through these studies was the BspA-like group named for their similarity to leucine-rich proteins of mucosal bacteria known to mediate adherence to host cells [13]. This gene family was initially found to encode 658 proteins, but further analysis revealed a total of 911 proteins with 191 containing predicted transmembrane

domains [8,36]. Furthermore, indirect immunofluorescence shows that at least one family member, TvBspA-625 (where TvBspA is *T. vaginalis* BspA), is expressed on the surface of the parasite [13,36]. As with all of the suspected membrane proteins, analysis of other TvBspA family members is needed to demonstrate whether localization signals translate into surface expression. The large size of this gene family has raised the question of whether these genes are differentially expressed and if expression patterns result from different environmental triggers. Future experiments are needed to both determine the expression patterns of predicted membrane proteins and to provide direct evidence for their involvement, if any, in the interaction of *T. vaginalis* with its host. Understanding both the function and expression patterns of these proteins will be critical to determine whether any may be valid targets for rational drug or vaccine design.

Proteomic approaches to address pathogenesis

The surface proteome of *T. vaginalis*

Theoretical investigations of the *T. vaginalis* genome [8] only reveal which proteins could be involved in the attachment of the parasite to host cells. Fortunately, completion of the genome also set the stage for several proteomic studies to be conducted (Table 1). Although the level of investigation lags behind other parasitic species, e.g. *Plasmodium* spp. and *Trypanosoma* spp., there have been major advances in our understanding of the parasite on the basis of these recent studies. The data derived from these large-scale analyses allow us to refine gene annotations and gene prediction algorithms while also providing important insights into the repertoire and level of protein expression. Moreover, these data have the potential to identify unique proteins and critical metabolic pathways that could be used as drug targets and vaccine candidates.

We have used a proteomic approach to better define the surface of *T. vaginalis* as a step towards further understanding mechanisms underlying the virulence of this organism [9]. Surface-specific proteins were isolated by labelling the surface of the parasite with membrane-impermeable biotin, followed by purification of the surface proteins by streptavidin-affinity chromatography [9]. Proteins from the surface of six strains of *T. vaginalis,* with different adherence capacities, were isolated and then compared using MudPIT (multidimensional protein identification technology) spectrometry, resulting in the identification of 411 proteins [9]. Using this approach, we identified proteins from 7 out of the 10 families discussed previously that were predicted to encode membrane proteins on the basis of genomic analyses [8,13]. These include TvBspA proteins, a GP63-like metalloproteinase, a subtilisin-like serine peptidase, calpain-like CPs, *Chlamydia* polymorphic membrane-like proteins, immunodominant variable surface antigen and the P270 surface immunogen [9].

Several protein families that have yet to be examined in *T. vaginalis* and which may play an important role in parasite biology were also revealed in this membrane proteome [9]. These include tetraspanins [37], a rhomboid-like serine protease [38] and nicastrin precursors [39]. The known functions of these proteins in other organisms support a possible role for these surface molecules in *T. vaginalis* pathogenesis. For example, mammalian tetraspanins interact with specific receptors and signalling proteins and are predicted to act as molecular

facilitators regulating a variety of functions, including adhesion, migration, motility and tissue invasion [37], processes that are required for *T. vaginalis* to colonize its human host. Moreover, *Schistosoma mansoni* surface tetraspanins are protective antigens against schistosomiasis and are viewed as potential vaccine candidates [40]. Pathogenic roles for rhomboid proteases have been established in both apicomplexan parasites and *Entamoeba* [34,38,41]. These intramembrane serine proteases are known to cleave adhesins involved in invasion of apicomplexan parasites [38]. In *Entamoeba histolytica*, a rhomboid protease has been demonstrated to cleave the major surface Gal-GalGNc lectin involved in host cell attachment, phagocytosis and immune evasion [41]. Nicastrin is also a protease found in the gamma-secretase complex that is required for intramembrane cleavage of many type I membrane proteins [39]. Although an important role for intramembrane proteases is anticipated in *T. vaginalis*, biochemical analyses are necessary to define the function of these surface proteases.

The surface proteome was determined utilizing six strains of *T. vaginalis*: three highly adherent to VECs and three exhibiting low levels of adherence. Of the 411 proteins identified, 11 were found to be 5–42 times more abundant in at least two of the three adherent parasite strains [9]. These include two *Chlamydia* polymorphic membrane-like proteins, a serine/theronine protein phosphatase, an adenylate/guanylate cyclase, a subtilase-like serine protease and six hypothetical proteins. With the exception of the hypothetical proteins, the other proteins all have properties associated with pathogenesis. *Chlamydia* polymorphic membrane proteins exhibit antigenicity and phase-variability [42]. Serine peptidases are known to participate in invasion of malaria and *Toxoplasma* [34], and importantly, in *T. vaginalis*, antibodies to proteinases have been detected in vaginal washes of infected women [43]. Phosphatases and adenylate/guanylate cyclases play critical roles in cell signalling, suggesting that a signalling component is involved in *T. vaginalis* virulence. Further studies are necessary to establish whether any of these proteins are directly or indirectly involved in *T. vaginalis* pathogenesis.

The *T. vaginalis* genome has a multitude of unique hypothetical genes that encode proteins without any significant similarity to any found in other organisms. Over 25% of the proteins found in the surface proteomes of these six *T. vaginalis* strains fall into this category and, of these, nine were observed to be differentially expressed [9]. Two proteins, TVAG_244130 and TVAG_166850, were either 20 or 42 times more abundant in highly adherent parasites, and overexpression of either in a non-adherent strain increased attachment of transfected parasites to VECs ~2.2-fold [9]. These data reveal a role in adhesion for these novel abundant surface proteins. Furthermore, this work demonstrates the efficacy of directed proteomics to address facets of *Trichomonas* biology by identifying novel proteins that could not be found through genomic approaches. Revealing novel proteins on the surface of the parasite has provided a foundation for future studies with the potential of uncovering unprecedented host–parasite interactions that may be important for pathogenesis.

Analyses to identify soluble and released proteins potentially involved in pathogenesis

Although attachment to the host through molecules on the surface of *Trichomonas* is important for establishing infection and contacting nutrient sources, intracellular proteins are

likely to be critical to alter cell shape, process nutrients and signal other important cellular processes. Cuervo et al. [11] performed a comparative analysis of soluble proteins expressed in a laboratory-adapted, low virulence, *T. vaginalis* strain and in a fresh clinical isolate exhibiting a virulent phenotype, and found both quantitative and qualitative differences in protein profiles. Of the 29 protein spots found to be differentially expressed, 19 were overexpressed in the virulence strain. Most of these 19 are homologues of proteins involved in carbohydrate and energy metabolism, cytoskeletal structure or proteolysis. However, as only two strains were compared, the differences observed may result from non-virulence-related genetic changes or alterations in protein expression due to prolonged *in vitro* cultivation. To demonstrate that heterogeneity in protein expression is related to pathogenesis, an extended proteomic analysis involving a greater number of trichomonad isolates with differing degrees of virulence will be required.

Trichomonas secreted proteins are likely to interact with host cells or the extracellular matrix. Contact-independent cytolytic mechanisms have been proposed and major cytolytic effects have been attributed to substances released by the parasite in the culture medium [44,45]. Characterization of these proteins is necessary to determine the role secreted factors play in pathogenesis and modulating the host response to trichomonosis. Towards this end, proteome analyses have been performed on proteins released by *Trichomonas* in the presence of VECs using two-dimensional electrophoresis and MALDI–TOF-MS (matrix-assisted laser-desorption ionization–time-of-flight MS). Nineteen proteins were identified, with the most abundant being several enzymes involved in carbohydrate metabolism, CPs, heat-shock proteins, thioredoxin reductase and coronins [46]. Enzymes involved in carbohydrate metabolism such as GAPDH, enolase and malate dehydrogenase have also been identified in the ‘secreted’ proteomes of *Plasmodium falciparum* and *S. mansoni*. Two of the abundant proteins, GAPDH and malic enzyme, found to be released by *T. vaginalis* are among the controversial dual-functional proteins discussed earlier. Should it be possible to demonstrate that these highly abundant cellular proteins are actively secreted as opposed to released passively upon cell damage or lysis, detailed molecular studies and identification of host binding partners should be performed to confirm their function in attachment and/or secretion.

In strong support of a role for CPs in infection is the presence of numerous trichomonad proteinases [43], including CPs, in vaginal washes of infected women. Similarly, two yet to be identified proteases of 39 and 65 kDa found in released fractions from *T. vaginalis* have proposed roles in cytotoxicity [47,48]. Several different CPs have also been identified in other studies [45]. A fraction referred to as CP30 that contains at least four extracellular CPs (CP1, CP3, CP4 and CPT) was shown to induce apoptosis in a primary human VEC cell line *in vitro* [44,45]. An immunoproteomic analysis of the serum of a trichomoniasis patient identified at least five CPs of ~30 kDa, including the most reactive, CP2, CP4 and CPT [12]. Furthermore, CP4 and CPT were identified as two of the six CPs found to be expressed at higher levels in whole cell extracts of a virulent strain [10]. The observations that CP4 and CPT are present in a soluble fraction capable of inducing apoptosis in VECs [45], are immunogenic [22] and more highly expressed in a virulent strain of the parasite [10] suggests that these two CPs have a central role in pathogenesis and warrant further investigation to assess their mechanism of action. These data also lend support to the

suggestion that parasite CPs may be useful targets for the development of novel chemotherapies [49]. Thus further research on CPs will not only provide new insights into *T. vaginalis* pathogenesis, but may also open up new therapeutic avenues.

Conclusions

As an obligate extracellular parasite that must bind to the epithelial lining of the urogenital tract to survive, *T. vaginalis* appears to utilize multiple membrane molecules to adhere to its human host. Compelling evidence exists that the LPG coating the surface of the parasite specifically binds to a host cell receptor(s) in a carbohydrate-mediated fashion [28,31,32]. Additionally, multiple genomic, proteomic and biochemical analyses suggest that a variety of parasite membrane proteins are involved in adhesion. Soluble metabolic proteins have also been reported to play a role in adherence although these studies are controversial. Several CPs that are released from the parasite are also implicated in pathogenesis; however, their specific functions and targets remain unknown. Although recent analyses of the *T. vaginalis* genome have provided clues to possible surface proteins with potential roles in pathogenesis, such data are limited as localization or proposed activity on the basis of sequence similarity to proteins of known function does not necessarily confer these properties. The application of proteomics to uncover proteins that are differentially expressed on the surface of parasite strains with varying pathogenic properties has similar limitations and requires biochemical analyses of identified proteins to reveal their functions. Cleverly designed proteomics and glycomic analyses in *T. vaginalis* should be useful for selecting candidate molecules for further investigation. Although many challenges lie ahead, a more efficient genetic and biochemical manipulation of this important human pathogen provides promise that, in conjunction with proteomics and classical biochemical methods, the intriguing biology of this parasite and its complex interaction with the host will be unravelled.

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Summary

- Adhesion of *T. vaginalis* to host cells is a process that appears to involve multiple protein and carbohydrate factors. The parasite's surface *LPG* mediates carbohydrate-dependent adherence. Proteins have also been implicated in attachment, including hypothetical membrane proteins, multiple families of predicted membrane proteins and dual-functional metabolic proteins.
- The parasite's surface *LPG* has been shown to mediate carbohydrate-dependent adherence by directly binding the host receptor galectin-1.
- Genome analyses have revealed the presence of several large protein families containing both transmembrane domains and motifs shared with surface proteins implicated in pathogenesis in other organisms.
- Two recently described hypothetical membrane proteins identified in the surface proteome that are unique to *T. vaginalis* increase the adherence to VECs when these proteins are overexpressed individually in the parasite.
- Proteomics provides a powerful tool for identifying novel proteins with no known homologues in other organisms that may play a role in pathogenesis. Such analyses are also helpful in parsing out which of the many proteins uncovered through genomic analyses are expressed and involved in host–pathogen interactions.
- Preliminary analyses of proteins released from the parasite implicate several cysteine proteases as potential *T. vaginalis* virulence factors.

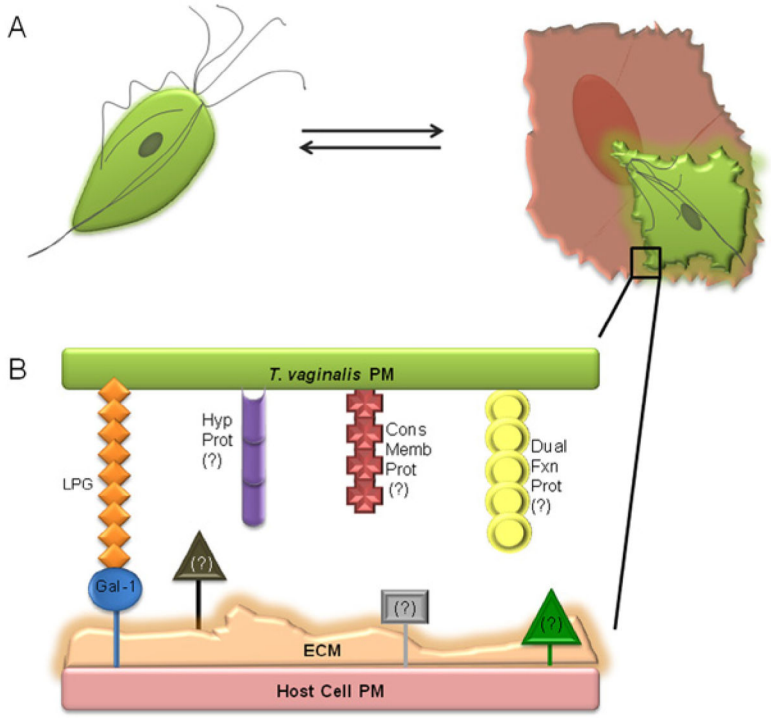

Figure 1. *T. vaginalis* interaction with extracellular membrane (ECM) and host cell plasma membrane (PM)

The ovoid free-swimming form of *Trichomonas* (upper left-hand side) transitions to an amoeboid form upon binding to a host epithelial cell (upper right-hand side). Lower panel: various *T. vaginalis* membrane molecules: LPG, hypothetical proteins (Hyp Prot), membrane proteins with conserved domains (Cons Memb Prot) and dual-functional proteins (Dual Fxn Prot) implicated in the interaction with ECM and host cell PM receptors. Galectin-1 (Gal-1) is the only reported host cell receptor.

Table 1

Summary of *T. vaginalis* proteomic studies

Sample proteins	Separation method	Total number of proteins identified	References
Surface-labelled proteins	Subcellular fractionation and MudPIT	411	[9]
Soluble proteins	2D SDS/PAGE followed by MALDI–TOF	164	[36c]
Soluble proteins	2D SDS/PAGE followed by MALDI–TOF	67	[36b]
Soluble proteins in iron-rich and iron-depleted medium	2D SDS/PAGE followed by MALDI–TOF	45	[36a]
Soluble proteins between isolates	2D SDS/PAGE followed by MALDI–TOF	29	[11]
Secreted fraction	2D SDS/PAGE followed by MALDI–TOF	19	[46]
Active degradome/immunoproteome	2D electrophoresis, 2D zymograms, 2D Western blotting, and MS	13	[12]
CP profiles	2D SDS/PAGE followed by MALDI–TOF	8	[10]
Hydrogenosome proteins	2D SDS/PAGE followed by MS	61	[36d]

2D, two dimensional.