Molecular & Biochemical Parasitology 198 (2014) 92–99

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Review

The biology of *Trichomonas vaginalis* in the light of urogenital tract infection

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ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 29 January 2015
Accepted 30 January 2015
Available online 9 February 2015

Keywords:
Trichomonas vaginalis
Host–parasite interaction
Adhesion
Surface proteins
Secreted molecules
Morphogenesis

ABSTRACT

The human pathogen *Trichomonas vaginalis* is a parasitic protist. It is a representative of the eukaryotic supergroup Excavata that includes a few other protist parasites such as *Leishmania*, *Trypanosoma* and *Giardia*. *T. vaginalis* is the agent of trichomoniasis and in the US alone, one in 30 women tests positive for this parasite. The disease is easily treated with metronidazole in most cases, but resistant strains are on the rise. The biology of *Trichomonas* is remarkable: it includes for example the biggest protist genome currently sequenced, the expression of about 30,000 protein-encoding genes (and thousands of lncRNAs and pseudogenes), anaerobic hydrogenosomes, rapid morphogenesis during infection, the secretion of exosomes, the manipulation of the vaginal microbiota through phagocytosis and a rich strain-dependent diversity. Here we provide an overview of *Trichomonas* biology with a focus on its relevance for pathogenicity and summarise the most recent advances. With some respect this parasite offers the opportunity to serve as a model system to study certain aspects of cell and genome biology, but tackling the complex biology of *T. vaginalis* is also important to better understand the effects that accompany infection and direct symptoms.

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1. Introduction

Food tasters of lords and kings were once referred to as *Parasitos*; their only duty was to savour whether food was putrid or poisoned. *Parasitos* were served without having to do any other labour and jealously among other slaves quickly translated into their profession being negatively perceived. In the most simple sense, parasites scavenge nutrition at the expense of their, in most cases much bigger, host. The host is usually not taxonomically related to its parasite and the most efficient and problematic parasites are strictly host-specific. Parasitism features obvious advantages, but parasites must also adapt to their hosts' internal physiology, need to overcome the host's immune defences and sometimes evolve sophisticated modes of transmission; good examples include the malaria agent *Plasmodium falciparum* (vector: anopheles mosquitoes) and *Trypanosoma brucei* (vector: tsetse flies) that causes sleeping sickness. The consequence of such adaptions and specialisation in most cases translates into parasites

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http://dx.doi.org/10.1016/j.molbiopara.2015.01.004
0166-6851/

that can no longer thrive outside their ecological niche and which fully depend on their hosts to survive.

Many human pathogens, such as *Plasmodium* and *Trypanosomes*, are single-celled eukaryotes (protists). A selection of some of the most commonly studied parasitic protists in humans are shown in Table 1, but in the mini-review at hand we will focus on the less-well studied excavate *Trichomonas vaginalis* that infects the human urogenital tract. Each of these protist parasites employs a specific set of molecules that aid in the initial attachment to, and in the case of some species in the subsequent invasion of, host cells. The extracellular matrix (ECM) of human cells presents one of the initial barriers parasites such as *Trichomonas*, *Plasmodium* or *Dientamoeba* encounter. The ECM consists of collagen-producing fibroblasts, polysaccharides and fibres such as fibronectins, elastins and laminin that together generate a complex network, which connects adjacent cells and builds up a basal lamina. Each tissue has its unique ECM's composition that furthermore exposes a specific series of receptors including integrins, discoidin domain receptors and syndecans [1]. The manipulation and degradation of the ECM and associated tissue is an essential part of pathogenicity. The secretion of lytic enzymes enables parasites to migrate to other locations within the host or to make direct contact and fuse with the host cells' plasma membrane. Therefore, one branch of host–parasite research focuses on the characterisation of parasite-derived proteases [2]. Piña-Vázquez and colleagues reviewed the current status of identified proteases – discussed in the further course of the text – for nine different species, and which includes the extracellular parasite *T. vaginalis*.

2. *Trichomonas vaginalis* at a glance

T. vaginalis is the causative agent of the sexually transmitted disease trichomoniasis. The parabasalian parasite is estimated to infect about 276 million new hosts annually worldwide [3], and in the US about 1 in 30 women tests positive for *Trichomonas* [4]. The parasite mainly affects the urogenital tract of both men and women, but it has also been isolated from the respiratory tract of infants [5] and adults [6] and a few *in vitro* studies have demonstrated that *Trichomonas* can attach to fibroblasts, muscle- and MDCK-cells [7,8]. Within the urogenital tract the parasite invades the squamous epithelium, which can not only lead to vaginitis/prostatitis, but also increase the risk of HIV transmission and the risk of low birth weights [9]. Treatment of trichomoniasis is based on 5-nitroimidazole derivatives such as metronidazole and tinidazole, which affect both anaerobic bacteria and anaerobic protists.

In contrast to the genome and associated coding-capacity of many other parasites, the one of *Trichomonas* is overall not experiencing reduction. For the majority of gene families the contrary appears to be true. The original genome sequencing of *T. vaginalis* estimated the parasite encodes up to 60,000 proteins on six haploid chromosomes that together comprise approximately 160 Mbp [10]. After clustering identical sequences only 46,000 genes remained, but at the same time the genome was now estimated to reach up to 175 Mbp in length [11]. These different estimations of genome size and number of coding genes, but also the high amount of repeats, are largely due to the repetitive nature of the genome – up to 65% of the genome is thought to consist of repetitive sequences – and an unknown amount of genome duplication events that has also caused some gene families to massively expand. While the parasite has lost many genes associated with mitochondrial functions due to the reduction of the organelle to a hydrogenosome (see below), protein families relevant to infection and pathogenicity appear specifically expanded [10]. It is not known whether the repetitive sequences are the results or in parts also the cause of the duplication events, but it appears a rather common phenomenon among *Trichomonas* and phylogenetically associated *Tritrichomonas* genera [12]. These repetitive genome sequences, in combination with a large array of transposable elements, hinder a more complete assembly of the genome as a whole. The genome assembly of *T. vaginalis* still consists of thousands of individual scaffolds listed at TrichDB [13] today.

The transcriptome data currently available for the parasite reflects the complexity of the genome. Although only about half of the encoded genes appear expressed [14], 93% of the encoded gene families are expressed together with many hundred pseudogenes and long non-coding RNAs (lncRNAs) whose expression is driven independently from neighbouring genes [15]. Transcriptome analyses have further demonstrated that individual members of gene families are differently regulated upon environmental changes [14,16,17], and in particular the exposure to oxygen results in an expression change of hundreds of genes within minutes [14]. The latter reflects the adaption of the parasite to its natural habitat and the evolution of a sophisticated mechanism that buffers oxygen stress.

Trichomonadida thrive in anaerobic habitats. Minimally elevated levels of O_2 can however boost growth [17] and as long as the partial pressure of CO_2 is high enough [18], the duplication rate of *T. vaginalis* is not significantly impaired (Fig. 1). In any case, as a consequence of their anaerobic lifestyle, their once aerobic mitochondria have evolved into anaerobic hydrogenosomes [19]. *Trichomonas* research focused on the biochemistry of these organelles for many years. They import pyruvate and malate to generate ATP through substrate-level phosphorylation without the use of oxygen as the terminal electron acceptor, releasing hydrogen and acetate as end-products [20,21]. Understanding the organelle's biochemistry is an important part of *Trichomonas* research, because the main set of drugs we use to treat trichomoniasis target metabolic pathways of the hydrogenosome. Metronidazole is incorporated by *T. vaginalis* through passive diffusion and subsequently activated by a non-enzymatic reduction inside the hydrogenosomes through low potential electrons stemming from hydrogenosomal metabolism [22,23]. This activation generates nitro-radicals that are locally toxic to the *Trichomonas* cells by interfering with proteins and protein trafficking. 2.5–10% of *Trichomonas* strains currently tested do not respond to metronidazole treatment [24]. Resistant strains are on the rise, which highlights the importance to commence work on finding alternative treatments.

Lacking a genome and in consequence a 70S translation machinery, all hydrogenosomal proteins need to be imported from the cytosol. This is achieved by a streamlined TOM/TIM complex (translocase of the outer and inner mitochondrial membrane) found in all mitochondria and organelles derived thereof [25]. Protein targeting to the organelles appears to depend on more canonical N-terminal motifs similar to those of mitochondria, as well as cryptic internal motifs [26] and the identification of hydrogenosomal proteins is ongoing [25,27]. Core enzymes of the hydrogenosomal metabolism include pyruvate:ferredoxin oxidoreductase and Fe–Fe hydrogenases that are highly oxygen-sensitive due to the presence of iron–sulphur clusters, whose damage is irreversible [28]. The parasite has evolved sophisticated mechanisms to avoid inactivation of these enzymes and remove reactive oxygen species at times when the parasite experiences elevated levels of O_2, for example during transmission or with fluctuating vaginal oxygen levels during the menstruation cycle [14,29]. Enzymes including NADH-, NADPH-oxidases, superoxide dismutases and peroxiredoxins are massively upregulated during oxygen stress [14]; essential to ensure that the only way hydrogenosomes can generate ATP (through substrate-level phosphorylation) remains functional.

Free-swimming *Trichomonas* cells are pyriform. They have four anterior flagella and a fifth recurrent flagella that is associated with