Behind the smile: cell biology and disease mechanisms of *Giardia* species

Johan Ankarklev, Jon Jerlström-Hultqvist, Emma Ringqvist, Karin Troell and Staffan G. Svärd

Abstract | The eukaryotic intestinal parasite *Giardia intestinalis* was first described in 1681, when Antonie van Leeuwenhoek undertook a microscopic examination of his own diarrhoeal stool. Nowadays, although *G. intestinalis* is recognized as a major worldwide contributor to diarrhoeal disease in humans and other mammals, the disease mechanisms are still poorly understood. Owing to its reduced complexity and proposed early evolutionary divergence, *G. intestinalis* is used as a model eukaryotic system for studying many basic cellular processes. In this Review we discuss recent discoveries in the molecular cell biology and pathogenesis of *G. intestinalis*.

Cyst
The resistant, transmissive form of the giardial parasite.

Relic organelle
A cellular organelle that has evolved into a reduced form with fewer or novel functions.

Department of Cell and Molecular Biology, Biomedical Center, BOX 596, Uppsala University, SE-751 24 Uppsala, Sweden
Correspondence to S.G.S.
e-mail: staffan.svard@icm.uu.se
doi:10.1038/nrmicro2317
Published online 19 April 2010

Giardia intestinalis (also known as *Giardia lamblia* and *Giardia duodenalis*) causes one of the most common parasitic infections worldwide. It contributes to an estimated 280 million symptomatic human infections (called giardiasis) per year[1] and has been included as part of the WHO Neglected Disease Initiative since 2004. *G. intestinalis* is a potential zoonotic pathogen, and infection in young farm animals can have an economic impact resulting from loss in productivity[2]. The parasite survives in the environment for prolonged periods, the infectious dose is low (10 cysts[3]) and the need to control waterborne transmission results in large economic losses for industry. Giardiasis is characterized by watery diarrhoea, epigastric pain, nausea, vomiting and weight loss. These symptoms appear 6–15 days after infection[4], and the clinical impact is stronger in young children and in undernourished or immunodeficient individuals; treatment is usually with metronidazole or other nitroimidazoles. Chronic infections are common but, conversely, about half of the infections during epidemics are asymptomatic and the infection frequently resolves spontaneously[4–6]. Recent data indicate that functional gastrointestinal disorders such as irritable bowel syndrome can be associated with a previous *G. intestinalis* infection[7]. Thus, the symptomatology is extremely variable. There is little insight into how *Giardia* spp. cause disease; they are not invasive and secrete no known toxin[4,5].

Eukaryotes have classically been divided into four main groups: animals, plants, fungi and protists. Animals, plants and fungi have been studied extensively, and our knowledge about eukaryotic organisms is mainly based on experimental data from organisms in these groups. Few protists have been studied in any detail, but these studies have identified several molecular mechanisms that were later shown to occur in most eukaryotes (for example, RNA editing and telomerase-mediated telomere maintenance[8]). The protist genus *Giardia* is a member of the diplomonads, which is a group of binucleated flagellates that are found in anaerobic or microaerophilic environments and that are now classified as part of the supergroup Excavata[9] (BOX1). *Giardia* spp. are some of the most divergent eukaryotes examined to date and provide unique opportunities for gaining basic insights into key pathways that characterize eukaryotic cells and also for identifying new molecular mechanisms. Owing to its unique and unusual ultrastructure (for example, it contains peripheral vesicles and lacks stacked Golgi), its bacterial-like metabolism, its simple *in vitro* differentiation and its sequenced genome, *G. intestinalis* provides a good model system for the investigation of relic organelles, cellular differentiation and minimal cellular mechanisms[8,10]. Reductive processes associated with a parasitic lifestyle are thought to be the main contributors to the minimal systems found in *Giardia* spp., but this simple organization may also reflect some evolutionarily basic characteristics.

This Review introduces the key characteristics of the cell differentiation, specific organelles and genome organization of *G. intestinalis* and then discusses the recent advances in our understanding of the host–parasite interactions, pathophysiology and disease mechanisms in giardiasis.

Box 1 | **Phylogeny of the genus *Giardia* and other diplomonads**

Diplomonads (for example, the genera *Giardia*, *Spironucleus*, *Trepomonas* and *Hexamita*) were thought to form one of the earliest diverging lineages of the eukaryotes[115]. They were described as 'biological fossils', as these eukaryotes have many peculiarities (including two nuclei, a different genetic code and a lack of aerobic mitochondria; they were also thought to lack nucleoli) and have retained properties of bacteria and archaea[23]. However, advances in genomics, molecular phylogenetics and cell biology during the past decade strongly suggest that this view is incorrect[116,117]. The current interpretation of the phylogeny of eukaryotes lends no support for diplomonads being the earliest eukaryotic branch[116,117]. Diplomonads have a sister group relationship to parabasalids, and these two groups are now classified in the eukaryotic supergroup Excavata[116]. Furthermore, diplomonads seem to have all of the features that were once thought to be lacking in these 'primitive' eukaryotes, including mitosomes[54] (which are organelles with mitochondrial ancestry), intron-containing genes[118] and nucleoli[68]. The bacterial properties of these organisms (for example, the metabolic pathways) are probably the result of lateral gene transfers[72,119]. Much genetic and biological variation also exists in diplomonads. For example, the genera *Spironucleus*, *Trepomonas* and *Hexamita* form a monophyletic clade that excludes the genus *Giardia* in phylogenetic trees, and species of these three genera use an alternative genetic code[119] in which TAA and TAG encode glutamine instead of being stop codons.

Cell differentiation

Cell differentiation in *Giardia* spp. involves two major developmental transitions: from the ingested, dormant cyst to the excyzoite to the trophozoite (a process known as excystation) and from the motile, replicating trophozoite back to the infective cyst (a process known as encystation) (FIG. 1). Differentiation in *G. intestinalis* is among the simplest eukaryotic developmental processes known[8], and it can be studied easily *in vitro*[10].

Excystation. The parasite uses a time-release capsule strategy for transmission. Outside the host the parasite is encapsulated in a hardy cyst wall (consisting of 60% carbohydrate and 40% protein) that protects it from hypotonic lysis in the environment (FIG. 1). The parasite in the cyst is dormant, and its metabolism is downregulated[11]. Following ingestion, the cyst becomes metabolically active and undergoes excystation. However, little is known about the molecular mechanisms regulating this rapid differentiation process, which takes place in only 15 minutes[12]. Excystation is initially triggered by host stomach acids, and the cyst then passes into the small intestine before rupturing. Flagella first appear through an opening in one of the poles of the cyst, followed by the excyzoite body[13] (FIG. 1). Cysteine proteases, released from the lysosome-like peripheral vesicles, are thought to have an important role in this process by degrading the cyst wall from the inside[14]. The liberated excyzoite undergoes cytokinesis twice without intervening S phases, finally producing four trophozoites[15] (FIG. 1). During this division process, the excyzoite increases its metabolism and gene expression, segregates organelles, upregulates proteins associated with motility and assembles the adhesive disc, an attachment organelle specific to *Giardia* spp.[11,16]. The adhesive disc functions as a suction cup and binds surfaces nonspecifically[16]. Ca^{2+} signalling and the phosphorylation and dephosphorylation of key proteins are important in the coordination of excystation[17–20], as inhibition of calmodulin[18] and protein kinase A (PKA)[20] block this developmental programme. Gene expression changes occur during excystation[12], but very few of these excystation-regulated genes have been identified. A study of gene expression changes during differentiation using serial analysis of gene expression (SAGE) has recently been completed (see Giardia DB) and has identified several new stage-specific genes expressed in various stages of the giardial life cycle, including excystation. Genes that change their expression during excystation regulate protein degradation (for example, proteasome subunits and cathepsins), the cytoskeleton (for example, α-tubulin, β-tubulin and median body protein) and mitosis (for example, kinesin 5, MAD2, Mob1 and cell division cycle 14A (CDC14A)). This fits with the fast reassembly of the cytoskeleton and fast cell division seen during early excystation. Many of the regulated genes are hypothetical genes specific for *Giardia* spp., and it will be of great interest to determine their functions.

The trophozoite is the disease-causing stage and has a shape resembling a pear bisected lengthwise (FIG. 2a). The flat side is the ventral surface and the convex side is the dorsal surface. The trophozoite of *G. intestinalis* is about 12–15 μm long and 5–9 μm wide. In comparison with other eukaryotes, *Giardia* spp. are unusual in that they have two nuclei (FIG. 2a) and lack mitochondria, peroxisomes and a typical Golgi apparatus. The cytoskeleton includes a median body, four pairs of flagella (the anterior, ventral, posterior/lateral and caudal flagella) and the adhesive disc (FIG. 2a). The median body has no known function, but its location gives the parasite the characteristic 'smile' that is seen after staining with Giemsa (FIG. 2a). The flagella and adhesive disc are composed of classical cytoskeleton proteins like α-tubulin and β-tubulin along with proteins from the giardin family (α-giardin, β-giardin, γ-giardin and δ-giardin), which is a family unique to *Giardia* spp.[16,21,22]. The parasite is dependent on high mobility and a strong attachment to the enterocytes in the upper small intestine to avoid peristaltic elimination, and this is accomplished by the adhesive disc and the flagella[23].

Encystation. Encystation is a long differentiation process that results in transformation of the motile trophozoite into the non-motile, infective cyst (FIG. 1). This process is induced in response to host-specific factors such as high levels of bile, low levels of cholesterol and a basic pH (reviewed in REF. 10). Early in encystation the trophozoite's flagella start to be internalized (FIG. 1). The parasite loses the ability to attach to the intestinal epithelium owing to fragmentation of the adhesive disc[16], and the differentiating parasite gradually rounds up and enters hypometabolic dormancy[11]. Ultrastructural observations of *G. intestinalis* cysts[24–26] revealed a thick cyst wall and an enclosed excyzoite with four nuclei, ribbon-like microtubule structures extending from the disassembled adhesive disc, and flagella in the centre (FIG. 2b). The *Giardia* spp. cyst wall is highly insoluble owing to strong interactions between the carbohydrate chains as well as between the cyst wall sugars and the cyst wall proteins

Excyzoite
A short-lived stage of the giardial parasite that initiates infection.

Trophozoite
The replicating, disease-causing form of the giardial parasite.

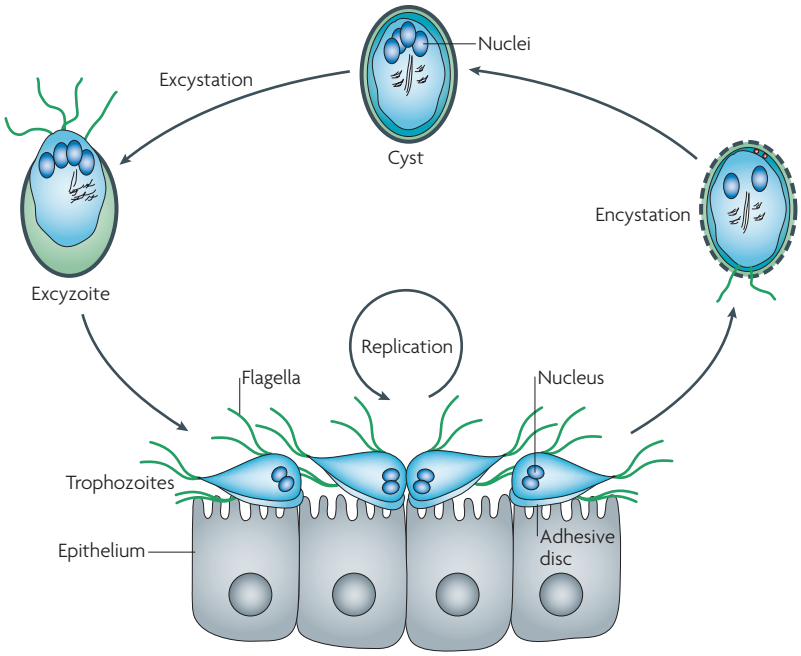

Figure 1 | **Life cycle of *Giardia intestinalis*.** Giardial cysts are exposed to gastric acid during their passage through the host's stomach, triggering excystation. This entails a rapid differentiation of cysts into vegetative trophozoites via the short-lived excyzoite stage. The excyzoite is different from the trophozoite in that it has not yet assembled the adhesive disc and it contains four tetraploid nuclei. The excyzoite divides twice, without DNA replication between the divisions, giving rise to four trophozoites containing two diploid nuclei each. Trophozoites attach to the intestinal epithelium with the adhesive disc and divide with a generation time of 6–12 hours *in vitro*. Trophozoites start to encyst *in vivo* when they migrate to the lower part of the small intestine. Encystation can be divided into an early phase and a late phase. In the early phase, trophozoites round up and encystation-specific vesicles become visible. These vesicles selectively transport the cyst wall proteins to the cyst wall. Furthermore, during encystation the adhesive disc disassembles into four crescent-shaped structures that are kept in the cytoplasm. The cell undergoes DNA replication but then exits the cell cycle at the G2 stage, to give a cell containing two tetraploid nuclei. During late encystation, these nuclei divide (giving four diploid nuclei) and the DNA is replicated once more, generating cysts with four nuclei and a ploidy of 16*n*. The cysts have a lower metabolic rate than the trophozoites and are highly resistant to environmental factors, being able to survive for several weeks in cold water outside the host.

(CWPs)[26]. To date, three CWPs have been identified (CWP1, CWP2 and CWP3), all of which contain leucine-rich repeats and have positionally conserved cysteine residues[27–29]. A fourth protein, high-cysteine non-variant cyst protein (HCNCp), has been shown to be associated with the cyst wall[30]. It resembles the variant-specific surface proteins (VSPs)[30,31] from *Giardia* spp., with its repeated CXXC motifs, and it localizes mainly to the surface of the excyzoite. The cyst wall is 0.3–0.5 μm thick and is lined by a double inner membrane[26] (FIG. 2b). The filamentous outer portion of the cyst wall is 60% carbohydrate, most of which is β(1-3)-*N*-acetyl-D-galactosamine[32]. The cyst wall sugars are synthesized *de novo* from endogenous glucose through an inducible pathway of five enzymes (namely, glucosamine-6-phosphate *N*-acetylase, phospho-*N*-acetylglucosamine mutase, uridine-diphospho-*N*-acetylglucosamine pyrophosphorylase, uridine-diphospho-*N*-acetylglucosamine 4-epimerase and glucosamine-6-phosphate isomerase, the rate-limiting enzyme), which are transcriptionally as well as allosterically upregulated during encystation[33,34]. Only 65 base pairs (bp) of upstream promoter sequence is needed for encystation-specific expression of each of the encystation-specific genes that have been studied to date[33,35], and these promoter regions contain binding sites for the transcription factor Myb2 (REFS 36,37). Furthermore, overexpression of Myb2 results in an increased expression of encystation-specific genes[37]. In addition, a GARP-like protein, a WRKY-like protein and an AT-rich interaction domain (ARID) protein have been shown to bind to the CWP promoters[38–40]. GARP, WRKY and ARID proteins regulate differentiation and dormancy responses in plants[38–40], and Myb proteins regulate differentiation of human stem cells[41]. Therefore, several different transcription factors, the homologues of which have roles in regulating development in animals and plants, bind and regulate encystation-specific genes in *Giardia* spp.

Early in encystation, the synthesis of CWPs leads to the formation of new, large encystation-specific vesicles (ESVs)[42]. It has been suggested that ESVs are unique, developmentally regulated Golgi-like organelles that are dedicated to the maturation and export of CWPs[43]. Homologues of proteins that are involved in the regulation of protein transport in higher eukaryotes (namely, the small GTPases Sar1, Rab1A and ADP-ribosylation factor (Arf1) (REF. 44), dynamin-like protein (DLP)[45], the clathrin heavy chains, YIP, β-COP, Rab11 (REF. 46) and different SNAP receptors (SNAREs)[47]) have been shown to be important in this maturation and export process. Several enzymes are needed for post-translational modifications of the CWPs; these are protein disulphide isomerases[48], cysteine proteinases[49,50] and a Ca^{2+}-binding granule-specific protein[51]. Proteasomes localize close to the ESVs early during encystation and have been suggested to be involved in the maturation of ESV cargo[52]. Recent morphological studies suggest that ESVs may be a common mechanism for the maturation, transport and deposition of cyst wall components in protozoan parasites[25], as similar structures have been seen in *Entamoeba invadens* and *Acanthamoeba castellani*. The importance of conserved protein transport factors in giardial ESV regulation implies that further studies in *Giardia* spp. will tell us much about the evolution of the protein transport system in eukaryotic cells.

The mitosome

For a long time, *G. intestinalis* was seen as a 'living fossil' (BOX 1), owing to its poorly developed membrane system, its lack of introns, its presumed lack of nucleoli and its bacterial-like metabolism, with key metabolic enzymes having been introduced into the giardial genome through lateral gene transfer from bacteria (reviewed in REF. 23). The apparent absence of mitochondria reinforced this view and suggested that *Giardia* spp. had diverged before the acquisition of this organelle. However, the discovery of a chaperonin 60 homologue (Cpn60) that is encoded in the *G. intestinalis*

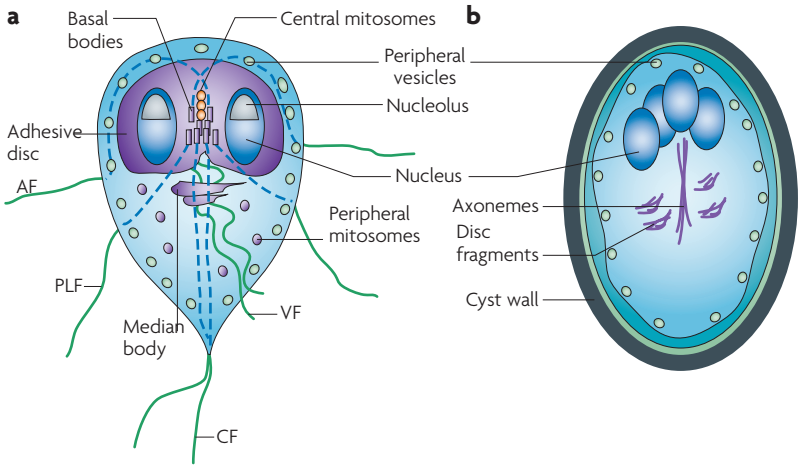

Figure 2 | **Key features of the giardial trophozoite and cyst. a** | The giardial trophozoite measures 12–15 µm in length and 5–9 µm in width. The trophozoite is shown here viewed dorsally. There are eight flagella organized in four pairs: the anterior flagella (AF), ventral flagella (VF), posterior/lateral flagella (PLF) and caudal flagella (CF); dashed lines indicate internal structures. The basal bodies are the sites from which the flagella originate. The median body is a microtubular structure of unknown function. The adhesive disc is a large, rigid attachment structure composed of microtubules. There are several central and peripheral mitosomes in the cell. Peripheral vesicles are lysosome-like vesicles that lie beneath the plasma membrane throughout the cell. **b** | In the cyst, the cyst wall and an inner layer consisting of two membranes protect the parasite. Giardial cysts are non-motile and oval shaped, and they measure 8–12 µm long by 7–10 µm wide. The outer cyst wall is 0.3–0.5 µm thick and is composed of a network of filaments ranging from 7 to 20 nm in diameter. This wall is mainly composed of *N*-acetylgalactosamine and three different cyst wall proteins (CWP1, CWP2 and CWP3). The adhesive disc and the flagella are disassembled and stored in the parasite. The cyst has four tetraploid nuclei.

nuclei[53] challenged the long-held view that *Giardia* spp. are primarily amitochondriate, and the organellar list of *Giardia* spp. was expanded in 2003 with the discovery of the mitosome[54]. It was shown that assembly of Fe–S clusters is compartmentalized in *Giardia* spp. by the localization of Fe–S centre synthesis subunit S (IscS), a cysteine desulphurase, and the metallochaperone IscU to elongated, double-membraned structures that are approximately 140 × 60 nm in size[54]. The number of these mitosomes varies from 25 to 100 per cell, with distinct accumulation at the basal bodies and at the bases of the flagellar axonemes. There seem to be two types of mitosomes in *Giardia* spp.[55,56]: the peripheral mitosomes, which are scattered throughout the cytoplasm, and the central mitosomes (FIG. 2a).

To date, ten proteins have been shown to localize to giardial mitosomes. These are involved in either the biosynthesis of Fe–S clusters (namely, IscU, IscS, IscA, glutaredoxin and ferredoxin) or the biogenesis of organelles (namely, Pam18, mitochondrial-type heat shock protein 70 (mtHSP70), Cpn60, α-mitochondrial protein-peptidase-like protein (GPP) and translocase of the mitochondrial outer membrane 40 (Tom40))[54,55,57–59]. In addition, the SNARE protein Qb3 (also known as putative Sec20) was localized to vesicular structures that are highly reminiscent of mitosomes[47], but the localization of Qb3 needs to be substantiated with verified mitosomal markers. In higher eukaryotes, proteins carrying a presequence are processed by a heterodimeric enzyme called mitochondrial processing peptidase (MPP) on their delivery to mitochondria[57]. GPP, the protease responsible for presequence removal after delivery to mitosomes in *Giardia* spp., cleaves only mitosomal presequences and is not capable of processing mitochondrial and hydrogenosomal presequences[57,58]. This probably reflects the co-evolution of the processing peptidases and presequences in the different organisms. However, mitosomal presequences can target proteins to hydrogenosomes in *Trichomonas vaginalis*[58] and even to human mitochondria[55], suggesting that the general mitochondrial targeting system existed in the last common ancestor of eukaryotes[60]. Progress has been made in elucidating the nature of the mitosomal import system in *Giardia* spp. The *G. intestinalis* homologue of the Pam18 subunit of translocase of the mitochondrial inner membrane 23 (Tim23) has been localized to mitosomes, together with mtHSP70. Recently, evidence for a mitosomal outer-membrane translocase was obtained[61]. This identification of a Tom40 homologue in *G. intestinalis* convincingly shows the mitochondrial ancestry of the mitosome, as this translocase component has no known homologues in bacteria or archaea[60]. Further components of the *Giardia* spp. mitosomal translocases remain elusive, as bioinformatic screens have failed to reconstruct the mitosomal import machinery in these species[57,60], and it remains to be seen how proteins are transported into and out of the giardial mitosome.

Giardia assemblages

Genetic studies of *G. intestinalis* isolates recovered from infected humans show that they fall into two main genetic groups or assemblages, called A and B[62], which have an average amino acid identity of only 78% in coding regions. Assemblage A isolates are the best studied, but recent epidemiological data suggest that assemblage B isolates can be more common in humans worldwide[62]. These two assemblages are found in other animals as well, such as livestock, cats, dogs and rats. Other *G. intestinalis* isolates that are genetically distinct from the human-associated assemblages A and B are found in a range of animals; these isolates are grouped into as assemblages C–G[62]. Parasites belonging to assemblages C and D have been identified in dogs, wolves, coyotes and cats; isolates from assemblage E have been found in cattle, sheep, pigs, goats and water buffaloes; assemblage F parasites have been identified in cats; and assemblage G isolates have been found in rats. There is an ongoing debate regarding a reclassification of the *G. intestinalis* assemblages into separate species[62,63]. Current genome-sequencing projects and phenotypic studies of new isolates will add substantial information to this debate.

The nuclei and genomes of *Giardia* spp.

Giardia spp., like all diplomonads, have two nuclei. These nuclei have been shown to be equivalent in size and in the amount of DNA that they contain, and both nuclei are transcriptionally active[64,65]. DNA replication is initiated almost simultaneously in each nucleus[65,66]. However, a few studies suggest that there are differences between the nuclei. Aneuploidy between the two nuclei

Fe–S cluster
An essential cofactor of proteins that are involved in catalysis and electron transport. A cluster contains a sulphide-linked di-, tri- or tetra-iron centre that can exist in one of several oxidation states..

(that is, differences in chromosome number) seems to occur in certain isolates[66]. Furthermore, in studies of the nuclear envelope of *G. intestinalis*, it was observed that the two nuclei are distinct in their number and distribution of nuclear pores[67]. The giardial nuclei were initially thought to lack nucleoli, but a nucleolar compartment was identified in each nucleus (FIG. 2a) by localizing several nucleolar markers[68]. Expression of the small nucleolar RNA (snoRNA) *GlsR17* was found to occur primarily in only one nucleolus[69]. The *GlsR17* RNA was shown to be a microRNA (miRNA) precursor, and this was the first report of miRNA production from a snoRNA. Having two nuclei with asymmetric gene expression gives *Giardia* spp. unique possibilities for the regulation of gene expression, and this could be one of the reasons for the presence of two nuclei in diplomonads. Further studies are likely to reveal other differences between the nuclei.

In the vegetative giardial trophozoite, each nucleus cycles between a diploid and a tetraploid state. Therefore, the whole cell cycles between a ploidy of $4n$ and $8n$[15]. In each cell cycle, both diploid nuclei divide to form four diploid nuclei, which are partitioned equationally in mitosis (that is, each daughter cell recieves one old and one new nucleus), with the left–right asymmetry being maintained during division[65]. Each progeny thus receives one copy of each parental nucleus. *Giardia* spp. have a semi-open mitosis, in which the microtubules from the mitotic spindles penetrate the nuclei through openings in the nuclear envelopes without complete nuclear disassembly. Another difference from mitosis in higher eukaryotes is the extended telophase that slows down cytokinesis[70]. After stimulation of encystation, the cell exits from the cell cycle in the G2 stage after a round of DNA replication[71]. During the end of encystation the cell replicates the DNA again, giving rise to a cyst with four tetraploid nuclei[15].

Pulsed-field separations of chromosomal DNA from different *G. intestinalis* isolates have identified five major chromosomes[23]. The genome of the assemblage A parasite *G. intestinalis* str. WB clone 6 was recently sequenced[72], and the structurally compact 11.7 Mb genome was found to contain 6,470 ORFs with a mean intergenic distance of 372 bp. Serial analysis of gene expression (SAGE) and cDNA sequences provided transcriptional evidence for 4,787 of these ORFs. Recently, 454 sequencing to 16 × coverage was carried out for the assemblage B isolate *G. intestinalis* str. GS clone H7[73], which is the only *G. intestinalis* isolate that has been used to experimentally infect both animals and humans successfully[74,75]. The two genomes have 78% nucleotide and amino acid identity in protein-coding regions. The main differences were seen in the large gene families encoding VSPs, NEK kinases and high-cysteine membrane proteins (HCMPs)[73], and the VSP repertoires seem to be completely different in the two isolates[73]. Comparative analysis identified 28 protein-coding genes unique to *G. intestinalis* str. GS clone H7 and 3 unique to *G. intestinalis* str. WB clone 6 that are not part of large gene families. Several of the unique genes have bacterial origins. It will be interesting to see what role these genetic differences have during infection and whether they can explain some of the phenotypic differences between assemblage A and assemblage B isolates. Although the haploid genome size and the total number of ORFs are similar in *G. intestinalis* and *Saccharomyces cerevisiae*, many eukaryotic processes seem to be less complex in the diplomonad than in the yeast[72,73]. Analyses of the genome indicate that *G. intestinalis* has genes for rudimentary forms of many cellular processes, with fewer subunits present in simplified cellular machineries, as well as a limited metabolic repertoire with many bacterial-like enzymes that were introduced by horizontal gene transfer[72,73]. Such simplified cellular machineries include those involved in DNA synthesis and transcription, RNA processing, cytoskeletal function and cell division[72,73]. The reduced complexity is useful when *G. intestinalis* is used as a model system, as proteins that are essential for a certain process can be identified easily using bioinfomatics. In addition, alternative proteins that substitute for missing proteins can be identified using biochemical approaches, and their functions can be studied. The unusual composition of these basic giardial machineries can be used in the development of new drugs to treat giardial infections.

Sex in *Giardia* spp.

The allelic sequence heterozygosity (ASH) is surprisingly low (< 0.01%) in the tetraploid *G. intestinalis* str. WB clone 6 genome[72]. However, analyses of faecal cysts suggest that the levels of ASH vary in different assemblages, as heterogeneous sequencing profiles are much more common in isolates from assemblage B and assemblage E than in those from assemblage A[62,76,77]. This was verified after sequencing the genome of *G. intestinalis* str. GS clone H7 (REF. 73), which revealed a higher level of overall ASH (0.5%) than is seen in *G. intestinalis* str. WB clone 6. Furthermore, in assemblage A isolates it seems as if genotype A1 (which includes *G. intestinalis* str. WB clone 6) has much lower ASH than genotype A2 (REF. 76). This low level of ASH is unusual for an asexually reproducing organism with a polyploid genome and can indicate that some sort of genetic exchange takes place in and between trophozoites.

Recombination and DNA repair are means of maintaining a low level of ASH, and the question of whether *Giardia* spp. are capable of sexual reproduction has been raised. Sexual reproduction has never been observed in *Giardia* spp., but this could be explained by extremely infrequent, furtive or cryptic sexual reproduction. It has been suggested that differentiation in *Giardia* spp. resembles meiosis[15], and in other organisms ASH can be reduced during meiosis. Several studies have used a comparative genomics approach to survey the *G. intestinalis* str. WB clone 6 genome for genes that are known to be involved in meiosis in other eukaryotes[78,79]. These studies identified giardial homologues for 21 of the 29 eukaryotic genes that are known to have a role in meiotic recombination.

Allelic sequence heterozygosity
The sequence difference between different alleles of the same gene.

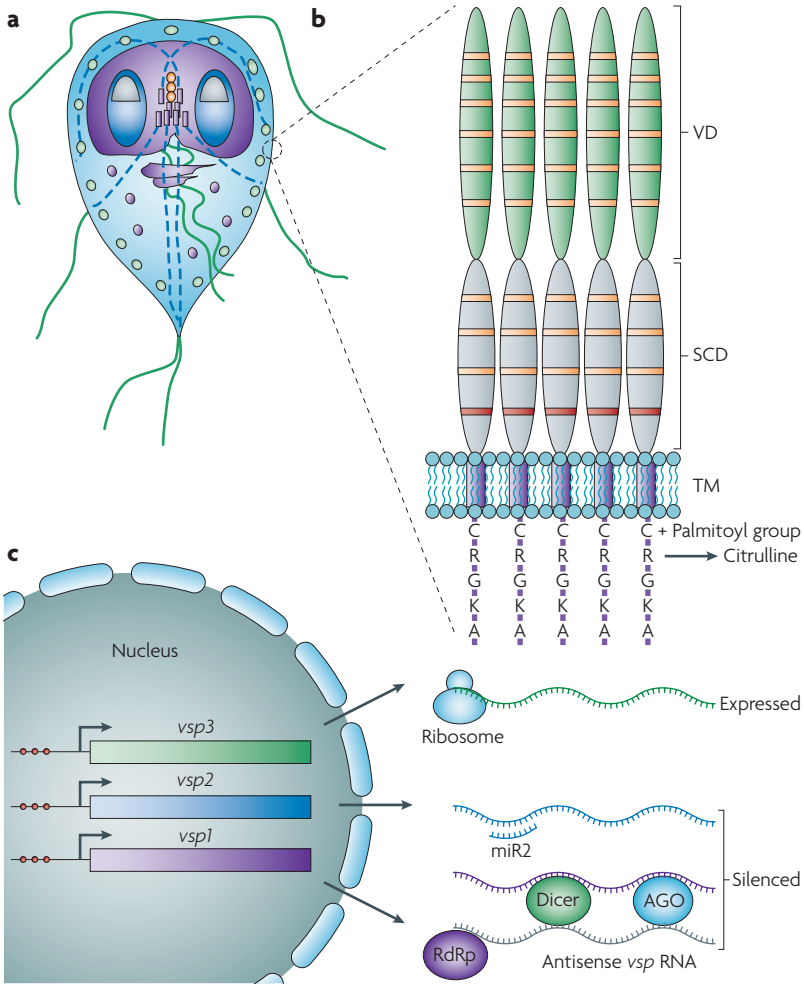

Figure 3 | **Antigenic variation in *Giardia* spp. a** | The giardial trophozoite. **b** | Variant-specific surface proteins (VSPs) cover the whole surface of the trophozoite, producing a dense coat. Normally, only one type of VSP is found on the trophozoite surface, except during differentiation and during the switching that occurs every 6–13 generations. In a population of parasites, one VSP usually dominates, but a few parasites express other VSPs. VSPs vary in size from 20 to 200 kDa, and the cysteine content is around 11–12%, most of which is found in numerous CXXC motifs (orange boxes) that build up disulphide bonds. The most variable portion of VSPs is the amino terminus (labelled as the variable domain (VD)), and this portion seems to be at the interface between the parasite and its environment. The extracellular domain closest to the membrane is a semi-conserved domain (SCD) and contains one or two GGCY motifs (red boxes). All VSPs have a conserved, cytoplasmatic carboxyl terminus with a hydrophobic tail that ends in the five hydrophilic amino acids CRGKA. The CRGKA motif can be modified by palmitoylation of the cysteine residue and by citrullination of the arginine residue. **c** | The level of histone modification (red circles) in the promoters of the *vsp* genes affects *vsp* transcription. *vsp* mRNAs can also be post-transcriptionally processed by the RNA interference machinery (including RNA-dependent RNA polymerase (RdRp), Dicer and Argonaute (AGO)) and microRNAs (such as miR2).

It has been claimed that this is evidence that sexual reproduction occurs in *Giardia* spp.[80], but this issue is complicated by the fact that many of the genes involved in meiotic recombination also participate in non-meiotic processes, and a few important meiotic proteins (such as MutS homologue 4 (MSH4), MutL homologue 3 (MLH3), meiotic recombination protein 8 (REC8) and sister chromatid cohesion protein (PDS5)) were not found[79]. The identification of recombination between assemblage A2 isolates[76] cannot be considered proof of giardial sexual reproduction, as it is not known how and when that recombination event occurred. Fluorescent *in situ* hybridization (FISH) with probes to an episomal plasmid was used to determine whether genetic exchange occurs between the different nuclei (a process termed 'diplomixis') during encystation[81]. This work showed that episomes that are present in only one of the two nuclei in the starting trophozoite can transfer between the nuclei during encystation. Nuclei with joined nuclear membranes were observed in encysting cells using electron microscopy[81], but it is not know whether these are the result of nuclear fusion or interrupted nuclear division during encystation. Proteins involved in meiotic recombination are present in the nuclei at this stage but not in trophozoite nuclei[81]. Further studies will show whether homologous chromosomes in different nuclei recombine during encystation and whether this can reduce ASH.

Antigenic variation

Both innate and adaptive immune responses are important for controlling giardial infections[82]. Specific antibody production correlates with clearance of the parasite, and there is a higher frequency of symptomatic infections in hypogammaglobulinaemic patients[82]. In mice, it has been shown that antibodies are needed for clearance late in infection and that secretory immunoglobulin A is the most important antibody isotype[82]. Several protozoan pathogens undergo antigenic variation, which is a mechanism for evasion of the humoral immune response of vertebrate hosts. In *Giardia* spp., this is achieved by the on–off switching of the expression of genes encoding VSPs, which are cysteine-rich surface proteins[83]. Antigenic variation was shown to occur in *G. intestinalis* in the late 1980s, both *in vitro*[84] and *in vivo*[85], and the first complete sequence of a VSP (TSA417) was obtained in 1990 (REF. 86). The VSP family comprises a repertoire of approximately 200 genes[72] in isolates of the two human-infecting assemblages (A and B), although no identical VSPs are found in the two assemblages[72,73]. The VSPs range in size from 20 to 200 kDa[72,83] and have variable amino termini and conserved carboxyl termini (FIG. 3b). The entire surface of a giardial cell is covered with VSPs, the expression of which is thought to be mutually exclusive, except during VSP switching and differentiation[83,87], when several VSPs are simultaneously expressed. Switching has been reported to occur spontaneously every 6 to 13 generations, although this is dependent on the isolate, the growth conditions and the specific VSPs being expressed[88]. Certain VSPs seem to be selected for and others are selected against in immunodeficient hosts[89] and when the pathogen is under antibiotic pressure[90], suggesting that factors other than the adaptive immune response also affect VSP expression.

In addition to being the main tools for immune evasion, VSPs are also involved in cellular signalling. Certain VSPs are specifically palmitoylated[91,92] on the cysteine residue and citrullinated[93] on the arginine residue in the conserved CRGKA carboxy-terminal motif, located in

Table 1 | **The major virulence factors of *Giardia* spp.**

Function	Virulence factor	References
Attachment	The ventral adhesive disc and surface lectins enable attachment to and colonization of the intestinal endothelium	16,120
Circumvention of the natural factors of the intestinal lumen	Flagellar motility enables re-localization to new endothelial cells during colonization, and VSPs potentially help to protect against luminal proteases, oxygen and free radicals	23,96
Antigenic variation	VSP on the trophozoite surface switches to avoid IgA-directed clearance	23,83
Alteration of host innate defences	Released arginine deiminase and other *Giardia* spp. products downregulate epithelial production of nitric oxide	82,105,106
Anti-inflammatory modifications	Unknown trophozoite products have anti-inflammatory roles	5,82
Survival in stomach acid and the external environment	Differentiation into cysts	10

IgA, immunoglobulin A; VSP, variant-specific surface protein.

the invariant cytoplasmic tail (FIG. 3b). Palmitoylation of VSPs helps to regulate the segregation of the proteins to domains on the plasma membrane that are detergent-resistant[92] (so-called 'lipid rafts'). Interference with the palmitoylation reaction excludes VSPs from lipid rafts and the parasite becomes less sensitive to cytotoxic VSP-specific antibodies[92]. Citrullination of the CRGKA arginine residue by arginine deiminase (ADI) is important for the VSP switching mechanism[93], as mutation of this residue affects the switching frequency.

Molecular mechanisms involved in the regulation of antigenic variation in *Giardia* spp. have been investigated intensively for the past two decades, but our knowledge about them is limited. There is no evidence of gene rearrangements, of sequence alterations to the DNA, of DNA modifications along with the presence of expression-linked copies, or of telomere-linked transcription requirements, all of which have been linked to antigenic switching in *Trypanosoma brucei* and *Plasmodium falciparum*[94]. However, a few recent reports have shed light on this topic.

It has been suggested that epigenetic mechanisms are involved in VSP regulation, on the basis of the fact that VSP expression is not associated with either gene movement or chromosome changes in the immediate upstream region[95]. Instead, it has been proposed that chromatin-mediated transcriptional silencing can be reversed by modification of histones and that there may be a direct dependence of VSP expression on histone acetylation (FIG. 3c).

Post-transcriptional silencing of *vsp* genes through a miRNA-mediated mechanism has been proposed to regulate VSP expression, as a miRNA from the *GlsR17* snoRNA (miR2) is complementary to the 3′ untranslated regions of several *vsp* mRNAs[69] (FIG. 3c). Post-transcriptional gene silencing of *vsp* transcripts through RNA interference (RNAi) has also been proposed[96] (FIG. 3c). In this study, simultaneous transcription of most VSPs in the genome was confirmed through nuclear run-on assays. Knockdowns of *G. intestinalis* Dicer[97] and RNA-dependent RNA polymerase led to the expression of multiple VSPs on the trophozoite surface. A successful downregulation of Argonaute was not achieved, so its effects on the regulation of VSP expression have not been evaluated *in vivo* as yet. However, the silencing machinery in *G. intestinalis* was shown to specifically process *vsp* RNAs *in vitro*[96]. It is possible that chromatin modification and post-transcriptional processes work in concert to regulate VSP expression. Parasites with deregulated expression of VSPs express most of the VSP repertoire, and it has been suggested that these proteins would be good vaccine targets[96]. It remains to be seen if this vaccination concept will work for *Giardia* spp. and other protozoans that undergo antigenic variation.

The molecular mechanisms of giardiasis

Efficient colonization of the upper intestinal tract by *Giardia* spp. is dependent on both the host and the strain of the infecting pathogen, as seen in experimental human infections[75]. Factors associated with disease outcome include the host's clinical and nutritional status, age and immune responses[82]. The immune response involves mast cells, B cells, T cells, dendritic cells, immunoglubulin A and nitric oxide (reviewed in REF. 82). Very few virulence factors have been identified in *Giardia* spp. (TABLE 1), but the main factors identified to date are the adhesive disc and the four flagella, together with differentiation and the VSP proteins. Several mechanisms have been proposed to be important for the induction of symptoms during a giardial infection, and the cause of giardiasis is probably multifactorial (TABLE 2). However, many different systems were used in the various studies of the disease, and it is difficult to compare the results. Early studies (reviewed in REF. 4) suggested several different disease mechanisms, including direct damage by the parasite, mucosal inflammation after infiltration of lymphocytes and mast cells, bile salt deconjugation and uptake, inhibition of trypsin and inhibition of brush-border enzymes. Furthermore, increased gastrointestinal transit and increased smooth-muscle contractility have been demonstrated[98,99]. Recent data have pointed towards a role for apoptosis[5,100–102]. Microarray analyses of the effects of *G. intestinalis* on differentiated Caco-2 cells indicated that apoptosis was induced in the host cells during interaction with the pathogen[100]. Lysates from

Intrinsic pathway
An apoptotic pathway in which the crucial step is the permeabilization of the outer mitochondrial membrane.

Extrinsic pathway
An apoptotic pathway that is mediated by the binding of an extracellular ligand to a transmembrane receptor.

different strains have been shown to have different abilities for inducing apoptosis, and proteolytic activities from the parasite are involved in this process[102,103]. Live *G. intestinalis* trophozoites induce apoptosis in host enterocytes by both the intrinsic pathway and the extrinsic pathway[104]. Two arginine-metabolizing enzymes from *G. intestinalis*, ADI and ornithine carbamoyl transferase (OCT), are released on interaction with host cells[105]. Cysteine proteinase activities also accumulate in the supernatant during co-incubation of *G. intestinalis* trophozoites with intestinal epithelial cells[106]. Proteases are known to induce apoptosis, and ADI is an apoptosis-inducing virulence factor in streptococci and mycoplasmas[5,105]. Investigation of patients with chronic giardiasis indeed confirmed the induction of apoptosis in intestinal epithelial cells during human giardiasis[101]. Thus, the diarrhoea that occurs during infection is likely to be the result of an epithelial-barrier dysfunction that causes Na^+ and glucose malabsorption and Cl^- hypersecretion[101]. The maintenance of the host epithelial barrier also varies in a pathogen-strain-dependent manner, and although some infections do not cause tight junction alterations, many cause rearrangements of F-actin and α-actinin together with tight junction protein ZO-1 disruption, lower levels of tight junction proteins and a resulting increased intestinal permeability[101,103,107–109]. Most chronic giardiasis patients do not show major signs of inflammation or villus shortening, but an increase in the crypt/villus ratio can be observed[110–112]. Diffuse shortening of microvilli or local or widespread depletion of microvilli in the infected region has been reported, depending on the infective giardial strain[101,113,114]. The effect of the pathogen on microvilli has been linked to $CD8^+$ T cells, but the effector mechanisms remain unknown[111]. Loss of microvilli reduces the overall mucosal surface by 75% in patients with giardiasis, according to human biopsy studies[101]. This results in the malabsorption of electrolytes, solutes and water and the inhibition of brush-border enzymes[4,5].

In conclusion, giardiasis is a multifactorial disease (the potential mechanisms of which are summarized in TABLE 2), and the mechanisms of disease comprise leak flux, malabsorptive and secretory components.

Table 2 | **Proposed disease mechanisms in giardiasis**

Disease mechanism	References
Apoptosis of enterocytes	5,103,104
Loss of epithelial-barrier function	5,101,109
Hypersecretion of Cl^-	101
Malabsorption of glucose, water and Na^+	5,101
Diffuse microvillus shortening	5,111
Immune reaction (involving mast cells, T cells, IgA and NO)	82,98,99
Inhibition of brush-border enzymes and trypsin	4
Interference with bile salt metabolism	4

IgA, immunoglobulin A.

However, we need to learn how different host–parasite factors interact in order to fully understand this disease.

Prospects for the future

Much information about the molecular cell biology and infection biology of *Giardia* spp. has accumulated during the past few years. However, this is only the start of an intensive research period that will see *Giardia* spp. being used as model systems to understand genome function and evolution, gene expression, cell biology and intestinal immune responses. The genomes of several *G. intestinalis* isolates from different assemblages are being sequenced currently, and this will make it possible to develop methods for transfection, gene knockouts, functional genomics and gene expression studies. We think that this will rapidly turn the diplomonads into a model system representing a branch of eukaryotic diversity that is currently very poorly studied. This is needed to complement the more established model systems such as yeast and *Caenorhabditis elegans*, so that we can gain an understanding of the basic principles of the eukaryotic cell and the mechanisms of reductive evolution and adaptation that have resulted from the parasitic lifestyle of these diplomonads. *Giardia* spp. will also be important model systems for other pathogenic protozoa and for studies of mucosal immunity.

1. Lane, S. & Lloyd, D. Current trends in research into the waterborne parasite *Giardia*. *Crit. Rev. Microbiol.* **28**, 123–147 (2002).
2. O'Handley, R. M., Buret, A. G., McAllister, T. A., Jelinski, M. & Olson, M. E. Giardiasis in dairy calves: effects of fenbendazole treatment on intestinal structure and function. *Int. J. Parasitol.* **31**, 73–79 (2001).
3. Rendtorff, R. C. The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. *Am. J. Hyg.* **59**, 209–220 (1954).
4. Farthing, M. J. The molecular pathogenesis of giardiasis. *J. Pediatr. Gastroenterol. Nutr.* **24**, 79–88 (1997).
5. Buret, A. G. Mechanisms of epithelial dysfunction in giardiasis. *Gut* **56**, 316–317 (2007).
6. Ortega, Y. R. & Adam, R. D. *Giardia*: overview and update. *Clin. Infect. Dis.* **25**, 545–549 (1997).
7. Hanevik, K., Dizdar, V., Langeland, N. & Hausken, T. Development of functional gastrointestinal disorders after *Giardia lamblia* infection. *BMC Gastroenterol.* **9**, 27 (2009).
8. Svard, S. G., Hagblom, P. & Palm, J. E. *Giardia lamblia* – a model organism for eukaryotic cell differentiation. *FEMS Microbiol. Lett.* **218**, 3–7 (2003).
9. Simpson, A. G. Cytoskeletal organization, phylogenetic affinities and systematics in the contentious taxon Excavata (Eukaryota). *Int. J. Syst. Evol. Microbiol.* **53**, 1759–1777 (2003).
10. Lauwaet, T., Davids, B. J., Reiner, D. S. & Gillin, F. D. Encystation of *Giardia lamblia*: a model for other parasites. *Curr. Opin. Microbiol.* **10**, 554–559 (2007).
11. Paget, T. A., Macechko, P. T. & Jarroll, E. L. Metabolic changes in *Giardia intestinalis* during differentiation. *J. Parasitol.* **84**, 222–226 (1998).
12. Hetsko, M. L. *et al.* Cellular and transcriptional changes during excystation of *Giardia lamblia in vitro*. *Exp. Parasitol.* **88**, 172–183 (1998).
13. Buchel, L. A., Gorenflot, A., Chochillon, C., Savel, J. & Gobert, J. G. *In vitro* excystation of *Giardia* from humans: a scanning electron microscopy study. *J. Parasitol.* **73**, 487–493 (1987).
14. Ward, W. *et al.* A primitive enzyme for a primitive cell: the protease required for excystation of *Giardia*. *Cell* **89**, 437–444 (1997).
15. Bernander, R., Palm, J. E. & Svard, S. G. Genome ploidy in different stages of the *Giardia lamblia* life cycle. *Cell. Microbiol.* **3**, 55–62 (2001).
The first demonstration of ploidy changes during the compete giardial life cycle.
16. Palm, D. *et al.* Developmental changes in the adhesive disk during *Giardia* differentiation. *Mol. Biochem. Parasitol.* **141**, 199–207 (2005).
17. Slavin, I. *et al.* Dephosphorylation of cyst wall proteins by a secreted lysosomal acid phosphatase is essential for excystation of *Giardia lamblia*. *Mol. Biochem. Parasitol.* **122**, 95–98 (2002).

18. Reiner, D. S. *et al.* Calcium signaling in excystation of the early diverging eukaryote, *Giardia lamblia*. *J. Biol. Chem.* **278**, 2533–2540 (2003).
19. Lauwaet, T. *et al.* Protein phosphatase 2A plays a crucial role in *Giardia lamblia* differentiation. *Mol. Biochem. Parasitol.* **152**, 80–89 (2007).
20. Abel, E. S. *et al.* Possible roles of protein kinase A in cell motility and excystation of the early diverging eukaryote *Giardia lamblia*. *J. Biol. Chem.* **276**, 10320–10329 (2001).
21. Elmendorf, H. G., Dawson, S. C. & McCaffery, J. M. The cytoskeleton of *Giardia lamblia*. *Int. J. Parasitol.* **33**, 3–28 (2003).
22. Weiland, M. E., McArthur, A. G., Morrison, H. G., Sogin, M. L. & Svard, S. G. Annexin-like alpha giardins: a new cytoskeletal gene family in *Giardia lamblia*. *Int. J. Parasitol.* **35**, 617–626 (2005).
23. Adam, R. D. Biology of *Giardia lamblia*. *Clin. Microbiol. Rev.* **14**, 447–475 (2001).
24. Sheffield, H. G. & Bjorvatn, B. Ultrastructure of the cyst of *Giardia lamblia*. *Am. J. Trop. Med. Hyg.* **26**, 23–30 (1977).
25. Chavez-Munguia, B. *et al.* Ultrastructure of cyst differentiation in parasitic protozoa. *Parasitol. Res.* **100**, 1169–1175 (2007).
26. Erlandsen, S. L., Macechko, P. T., van Keulen, H. & Jarroll, E. L. Formation of the *Giardia* cyst wall: studies on extracellular assembly using immunogold labeling and high resolution field emission SEM. *J. Eukaryot. Microbiol.* **43**, 416–429 (1996).
27. Mowatt, M. R. *et al.* Developmentally regulated expression of a *Giardia lamblia* cyst wall protein gene. *Mol. Microbiol.* **15**, 955–963 (1995).
The first cloning and characterization of an encystation-specific gene.
28. Lujan, H. D., Mowatt, M. R., Conrad, J. T., Bowers, B. & Nash, T. E. Identification of a novel *Giardia lamblia* cyst wall protein with leucine-rich repeats. Implications for secretory granule formation and protein assembly into the cyst wall. *J. Biol. Chem.* **270**, 29307–29313 (1995).
29. Sun, C. H., McCaffery, J. M., Reiner, D. S. & Gillin, F. D. Mining the *Giardia lamblia* genome for new cyst wall proteins. *J. Biol. Chem.* **278**, 21701–21708 (2003).
30. Davids, B. J. *et al.* A new family of giardial cysteine-rich non-VSP protein genes and a novel cyst protein. *PLoS ONE* **1**, e44 (2006).
31. Nash, T. E. Surface antigenic variation in *Giardia lamblia*. *Mol. Microbiol.* **45**, 585–590 (2002).
32. Gerwig, G. J. *et al.* The *Giardia intestinalis* filamentous cyst wall contains a novel β(1–3)-*N*-acetyl-D-galactosamine polymer: a structural and conformational study. *Glycobiology* **12**, 499–505 (2002).
33. Knodler, L. A., Svard, S. G., Silberman, J. D., Davids, B. J. & Gillin, F. D. Developmental gene regulation in *Giardia lamblia*: first evidence for an encystation-specific promoter and differential 5′ mRNA processing. *Mol. Microbiol.* **34**, 327–340 (1999).
A description of the first cloning of an enzyme involved in the cyst wall sugar synthesis pathway and the characterization of an encystation-specific promoter.
34. Karr, C. D. & Jarroll, E. L. Cyst wall synthase: *N*-acetylgalactosaminyltransferase activity is induced to form the novel *N*-acetylgalactosamine polysaccharide in the *Giardia* cyst wall. *Microbiology* **150**, 1237–1243 (2004).
35. Davis-Hayman, S. R., Hayman, J. R. & Nash, T. E. Encystation-specific regulation of the cyst wall protein 2 gene in *Giardia lamblia* by multiple cis-acting elements. *Int. J. Parasitol.* **33**, 1005–1012 (2003).
36. Sun, C. H., Palm, D., McArthur, A. G., Svard, S. G. & Gillin, F. D. A novel Myb-related protein involved in transcriptional activation of encystation genes in *Giardia lamblia*. *Mol. Microbiol.* **46**, 971–984 (2002).
37. Huang, Y. C. *et al.* Regulation of cyst wall protein promoters by Myb2 in *Giardia lamblia*. *J. Biol. Chem.* **283**, 31021–31029 (2008).
38. Sun, C. H., Su, L. H. & Gillin, F. D. Novel plant-GARP-like transcription factors in *Giardia lamblia*. *Mol. Biochem. Parasitol.* **146**, 45–57 (2006).
39. Wang, C. H., Su, L. H. & Sun, C. H. A novel ARID/Bright-like protein involved in transcriptional activation of cyst wall protein 1 gene in *Giardia lamblia*. *J. Biol. Chem.* **282**, 8905–8914 (2007).
40. Pan, Y. J., Cho, C. C., Kao, Y. Y. & Sun, C. H. A novel WRKY-like protein involved in transcriptional activation of cyst wall protein genes in *Giardia lamblia*. *J. Biol. Chem.* **284**, 17975–17988 (2009).
41. Boheler, K. R. Stem cell pluripotency: a cellular trait that depends on transcription factors, chromatin state and a checkpoint deficient cell cycle. *J. Cell. Physiol.* **221**, 10–17 (2009).
42. Reiner, D. S., Douglas, H. & Gillin, F. D. Identification and localization of cyst-specific antigens of *Giardia lamblia*. *Infect. Immun.* **57**, 963–968 (1989).
43. Marti, M. & Hehl, A. B. Encystation-specific vesicles in *Giardia*: a primordial Golgi or just another secretory compartment? *Trends Parasitol.* **19**, 440–446 (2003).
44. Stefanic, S. *et al.* Neogenesis and maturation of transient Golgi-like cisternae in a simple eukaryote. *J. Cell Sci.* **122**, 2846–2856 (2009).
45. Gaechter, V., Schraner, E., Wild, P. & Hehl, A. B. The single dynamin family protein in the primitive protozoan *Giardia lamblia* is essential for stage conversion and endocytic transport. *Traffic* **9**, 57–71 (2008).
46. Marti, M. *et al.* An ancestral secretory apparatus in the protozoan parasite *Giardia intestinalis*. *J. Biol. Chem.* **278**, 24837–24848 (2003).
47. Elias, E. V. *et al.* Characterization of SNAREs determines the absence of a typical Golgi apparatus in the ancient eukaryote *Giardia lamblia*. *J. Biol. Chem.* **283**, 35996–36010 (2008).
48. Knodler, L. A. *et al.* Novel protein-disulfide isomerases from the early-diverging protist *Giardia lamblia*. *J. Biol. Chem.* **274**, 29805–29811 (1999).
49. DuBois, K. N. *et al.* Identification of the major cysteine protease of *Giardia* and its role in encystation. *J. Biol. Chem.* **283**, 18024–18031 (2008).
50. Touz, M. C. *et al.* The activity of a developmentally regulated cysteine proteinase is required for cyst wall formation in the primitive eukaryote *Giardia lamblia*. *J. Biol. Chem.* **277**, 8474–8481 (2002).
51. Touz, M. C., Gottig, N., Nash, T. E. & Lujan, H. D. Identification and characterization of a novel secretory granule calcium-binding protein from the early branching eukaryote *Giardia lamblia*. *J. Biol. Chem.* **277**, 50557–50563 (2002).
52. Stefanic, S., Palm, D., Svard, S. G. & Hehl, A. B. Organelle proteomics reveals cargo maturation mechanisms associated with Golgi-like encystation vesicles in the early-diverged protozoan *Giardia lamblia*. *J. Biol. Chem.* **281**, 7595–7604 (2006).
53. Roger, A. J. *et al.* A mitochondrial-like chaperonin 60 gene in *Giardia lamblia*: evidence that diplomonads once harbored an endosymbiont related to the progenitor of mitochondria. *Proc. Natl Acad. Sci. USA* **95**, 229–234 (1998).
This article describes the identification of the first mitochondrial gene in a *Giardia* species.
54. Tovar, J. *et al.* Mitochondrial remnant organelles of *Giardia* function in iron-sulphur protein maturation. *Nature* **426**, 172–176 (2003).
This study identifies the mitosome, which is a reduced mitochondrion lacking DNA and many typical mitochondrial functions.
55. Regoes, A. *et al.* Protein import, replication, and inheritance of a vestigial mitochondrion. *J. Biol. Chem.* **280**, 30557–30563 (2005).
56. Hehl, A. B., Regos, A., Schraner, E. & Schneider, A. Bax function in the absence of mitochondria in the primitive protozoan *Giardia lamblia*. *PLoS ONE* **2**, e488 (2007).
57. Smid, O. *et al.* Reductive evolution of the mitochondrial processing peptidases of the unicellular parasites *Trichomonas vaginalis* and *Giardia intestinalis*. *PLoS Pathog.* **4**, e1000243 (2008).
58. Dolezal, P. *et al. Giardia* mitosomes and trichomonad hydrogenosomes share a common mode of protein targeting. *Proc. Natl Acad. Sci. USA* **102**, 10924–10929 (2005).
59. Rada, P. *et al.* The monothiol single-domain glutaredoxin is conserved in the highly reduced mitochondria of *Giardia intestinalis*. *Eukaryot. Cell* **8**, 1584–1591 (2009).
60. Dolezal, P., Likic, V., Tachezy, J. & Lithgow, T. Evolution of the molecular machines for protein import into mitochondria. *Science* **313**, 314–318 (2006).
61. Dagley, M. J. *et al.* The protein import channel in the outer mitosomal membrane of *Giardia intestinalis*. *Mol. Biol. Evol.* **26**, 1941–1947 (2009).
62. Caccio, S. M. & Ryan, U. Molecular epidemiology of giardiasis. *Mol. Biochem. Parasitol.* **160**, 75–80 (2008).
63. Monis, P. T., Caccio, S. M. & Thompson, R. C. Variation in *Giardia*: towards a taxonomic revision of the genus. *Trends Parasitol.* **25**, 93–100 (2009).
64. Kabnick, K. S. & Peattie, D. A. *In situ* analyses reveal that the two nuclei of *Giardia lamblia* are equivalent. *J. Cell. Sci.* **95**, 353–360 (1990).
65. Yu, L. Z., Birky, C. W. Jr & Adam, R. D. The two nuclei of *Giardia* each have complete copies of the genome and are partitioned equationally at cytokinesis. *Eukaryot. Cell* **1**, 191–199 (2002).
66. Tumova, P., Hofstetrova, K., Nohynkova, E., Hovorka, O. & Kral, J. Cytogenetic evidence for diversity of two nuclei within a single diplomonad cell of *Giardia*. *Chromosoma* **116**, 65–78 (2007).
67. Benchimol, M. *Giardia lamblia*: behavior of the nuclear envelope. *Parasitol. Res.* **94**, 254–264 (2004).
68. Jimenez-Garcia, L. F. *et al.* Identification of nucleoli in the early branching protist *Giardia duodenalis*. *Int. J. Parasitol.* **38**, 1297–1304 (2008).
69. Saraiya, A. A. & Wang, C. C. snoRNA, a novel precursor of microRNA in *Giardia lamblia*. *PLoS Pathog.* **4**, e1000224 (2008).
The first publication showing that snoRNAs can be miRNA precursors.
70. Sagolla, M. S., Dawson, S. C., Mancuso, J. J. & Cande, W. Z. Three-dimensional analysis of mitosis and cytokinesis in the binucleate parasite *Giardia intestinalis*. *J. Cell Sci.* **119**, 4889–4900 (2006).
71. Reiner, D. S. *et al.* Synchronisation of *Giardia lamblia*: identification of cell cycle stage-specific genes and a differentiation restriction point. *Int. J. Parasitol.* **38**, 935–944 (2008).
72. Morrison, H. G. *et al.* Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*. *Science* **317**, 1921–1926 (2007).
Sequencing of the first giardial genome, showing that *G. intestinalis* has reduced cellular complexes.
73. Franzen, O. *et al.* Draft genome sequencing of *Giardia intestinalis* assemblage B isolate GS: is human giardiasis caused by two different species? *PLoS Pathog.* **5**, e1000560 (2009).
The first example of *de novo* 454 sequencing of a protozoan parasite, identifying genomic differences between giardial assemblage A and assemblage B isolates that suggest that they are different species.
74. Byrd, L. G., Conrad, J. T. & Nash, T. E. *Giardia lamblia* infections in adult mice. *Infect. Immun.* **62**, 3583–3585 (1994).
75. Nash, T. E., Herrington, D. A., Losonsky, G. A. & Levine, M. M. Experimental human infections with *Giardia lamblia*. *J. Infect. Dis.* **156**, 974–984 (1987).
A classic paper that uses Koch's postulate to show that *G. intestinalis* is a true pathogen.
76. Cooper, M. A., Adam, R. D., Worobey, M. & Sterling, C. R. Population genetics provides evidence for recombination in *Giardia*. *Curr. Biol.* **17**, 1984–1988 (2007).
77. Lebbad, M. *et al.* Dominance of *Giardia* assemblage B in Leon, Nicaragua. *Acta Trop.* **106**, 44–53 (2008).
78. Ramesh, M. A., Malik, S. B. & Logsdon, J. M. Jr. A phylogenomic inventory of meiotic genes; evidence for sex in *Giardia* and an early eukaryotic origin of meiosis. *Curr. Biol.* **15**, 185–191 (2005).
79. Malik, S. B., Pightling, A. W., Stefaniak, L. M., Schurko, A. M. & Logsdon, J. M. Jr. An expanded inventory of conserved meiotic genes provides evidence for sex in *Trichomonas vaginalis*. *PLoS ONE* **3**, e2879 (2008).
80. Logsdon, J. M. Jr. Evolutionary genetics: sex happens in *Giardia*. *Curr. Biol.* **18**, R66–R68 (2008).
81. Poxleitner, M. K. *et al.* Evidence for karyogamy and exchange of genetic material in the binucleate intestinal parasite *Giardia intestinalis*. *Science* **319**, 1530–1533 (2008).
A paper introducing the concept of diplomixis: the transfer of DNA between the two nuclei in *Giardia* spp.
82. Roxstrom-Lindquist, K., Palm, D., Reiner, D., Ringqvist, E. & Svard, S. G. *Giardia* immunity — an update. *Trends Parasitol.* **22**, 26–31 (2006).
83. Prucca, C. G. & Lujan, H. D. Antigenic variation in *Giardia lamblia*. *Cell. Microbiol.* **11**, 1706–1715 (2009).
84. Adam, R. D. *et al.* Antigenic variation of a cysteine-rich protein in *Giardia lamblia*. *J. Exp. Med.* **167**, 109–118 (1988).
85. Aggarwal, A. & Nash, T. E. Antigenic variation of *Giardia lamblia in vivo*. *Infect. Immun.* **56**, 1420–1423 (1988).
86. Gillin, F. D. *et al.* Isolation and expression of the gene for a major surface protein of *Giardia lamblia*. *Proc. Natl Acad. Sci. USA* **87**, 4463–4467 (1990).
87. Svard, S. G., Meng, T. C., Hetsko, M. L., McCaffery, J. M. & Gillin, F. D. Differentiation-associated surface antigen variation in the ancient eukaryote *Giardia lamblia*. *Mol. Microbiol.* **30**, 979–989 (1998).

88. Nash, T. E., Banks, S. M., Alling, D. W., Merritt, J. W. Jr & Conrad, J. T. Frequency of variant antigens in *Giardia lamblia*. *Exp. Parasitol.* **71**, 415–421 (1990).
89. Singer, S. M., Elmendorf, H. G., Conrad, J. T. & Nash, T. E. Biological selection of variant-specific surface proteins in *Giardia lamblia*. *J. Infect. Dis.* **183**, 119–124 (2001).
90. Muller, J., Sterk, M., Hemphill, A. & Muller, N. Characterization of *Giardia lamblia* WB C6 clones resistant to nitazoxanide and to metronidazole. *J. Antimicrob. Chemother.* **60**, 280–287 (2007).
91. Hiltpold, A., Frey, M., Hulsmeier, A. & Kohler, P. Glycosylation and palmitoylation are common modifications of *Giardia* variant surface proteins. *Mol. Biochem. Parasitol.* **109**, 61–65 (2000).
92. Touz, M. C., Conrad, J. T. & Nash, T. E. A novel palmitoyl acyl transferase controls surface protein palmitoylation and cytotoxicity in *Giardia lamblia*. *Mol. Microbiol.* **58**, 999–1011 (2005).
93. Touz, M. C. *et al.* Arginine deiminase has multiple regulatory roles in the biology of *Giardia lamblia*. *J. Cell Sci.* **121**, 2930–2938 (2008).
94. Lopez-Rubio, J. J., Riviere, L. & Scherf, A. Shared epigenetic mechanisms control virulence factors in protozoan parasites. *Curr. Opin. Microbiol.* **10**, 560–568 (2007).
95. Kulakova, L., Singer, S. M., Conrad, J. & Nash, T. E. Epigenetic mechanisms are involved in the control of *Giardia lamblia* antigenic variation. *Mol. Microbiol.* **61**, 1533–1542 (2006).
96. Prucca, C. G. *et al.* Antigenic variation in *Giardia lamblia* is regulated by RNA interference. *Nature* **456**, 750–754 (2008).
This article shows the importance of RNAi in the regulation of antigenic variation.
97. Macrae, I. J. *et al.* Structural basis for double-stranded RNA processing by Dicer. *Science* **311**, 195–198 (2006).
The determination of the protein structure of a Dicer enzyme.
98. Andersen, Y. S., Gillin, F. D. & Eckmann, L. Adaptive immunity-dependent intestinal hypermotility contributes to host defense against *Giardia* spp. *Infect. Immun.* **74**, 2473–2476 (2006).
99. Li, E., Zhao, A., Shea-Donohue, T. & Singer, S. M. Mast cell-mediated changes in smooth muscle contractility during mouse giardiasis. *Infect. Immun.* **75**, 4514–4518 (2007).
100. Roxstrom-Lindquist, K., Ringqvist, E., Palm, D. & Svard, S. *Giardia lamblia*-induced changes in gene expression in differentiated Caco-2 human intestinal epithelial cells. *Infect. Immun.* **73**, 8204–8208 (2005).
101. Troeger, H. *et al.* Effect of chronic *Giardia lamblia* infection on epithelial transport and barrier function in human duodenum. *Gut* **56**, 328–335 (2007).
A study of human patients with chronic *G. intestinalis* infection, revealing the importance of apoptosis and a disrupted epithelial barrier in the induction of disease.
102. Yu, L. C. *et al.* SGLT-1-mediated glucose uptake protects human intestinal epithelial cells against *Giardia duodenalis*-induced apoptosis. *Int. J. Parasitol.* **38**, 923–934 (2008).
103. Chin, A. C. *et al.* Strain-dependent induction of enterocyte apoptosis by *Giardia lamblia* disrupts epithelial barrier function in a caspase-3-dependent manner. *Infect. Immun.* **70**, 3673–3680 (2002).
104. Panaro, M. A. *et al.* Caspase-dependent apoptosis of the HCT-8 epithelial cell line induced by the parasite *Giardia intestinalis*. *FEMS Immunol. Med. Microbiol.* **51**, 302–309 (2007).
105. Ringqvist, E. *et al.* Release of metabolic enzymes by *Giardia* in response to interaction with intestinal epithelial cells. *Mol. Biochem. Parasitol.* **159**, 85–91 (2008).
106. Rodriguez-Fuentes, G. B. *et al*. *Giardia duodenalis*: analysis of secreted proteases upon trophozoite-epithelial cell interaction in vitro. *Mem. Inst. Oswaldo Cruz* **101**, 693–696 (2006).
107. Teoh, D. A., Kamieniecki, D., Pang, G. & Buret, A. G. *Giardia lamblia* rearranges F-actin and α-actinin in human colonic and duodenal monolayers and reduces transepithelial electrical resistance. *J. Parasitol.* **86**, 800–806 (2000).
108. Buret, A. G., Mitchell, K., Muench, D. G. & Scott, K. G. *Giardia lamblia* disrupts tight junctional ZO-1 and increases permeability in non-transformed human small intestinal epithelial monolayers: effects of epidermal growth factor. *Parasitology* **125**, 11–19 (2002).
109. Scott, K. G., Meddings, J. B., Kirk, D. R., Lees-Miller, S. P. & Buret, A. G. Intestinal infection with *Giardia* spp. reduces epithelial barrier function in a myosin light chain kinase-dependent fashion. *Gastroenterology* **123**, 1179–1190 (2002).
110. Oberhuber, G., Kastner, N. & Stolte, M. Giardiasis: a histologic analysis of 567 cases. *Scand. J. Gastroenterol.* **32**, 48–51 (1997).
111. Scott, K. G., Yu, L. C. & Buret, A. G. Role of $CD8^+$ and $CD4^+$ T lymphocytes in jejunal mucosal injury during murine giardiasis. *Infect. Immun.* **72**, 3536–3542 (2004).
112. Cevallos, A., Carnaby, S., James, M. & Farthing, J. G. Small intestinal injury in a neonatal rat model of giardiasis is strain dependent. *Gastroenterology* **109**, 766–773 (1995).
113. Chavez, B., Knaippe, F., Gonzalez-Mariscal, L. & Martinez-Palomo, A. *Giardia lamblia*: electrophysiology and ultrastructure of cytopathology in cultured epithelial cells. *Exp. Parasitol.* **61**, 379–389 (1986).
114. Buret, A., Hardin, J. A., Olson, M. E. & Gall, D. G. Pathophysiology of small intestinal malabsorption in gerbils infected with *Giardia lamblia*. *Gastroenterology* **103**, 506–513 (1992).
115. Sogin, M. L., Gunderson, J. H., Elwood, H. J., Alonso, R. A. & Peattie, D. A. Phylogenetic meaning of the kingdom concept: an unusual ribosomal RNA from *Giardia lamblia*. *Science* **243**, 75–77 (1989).
An early molecular study suggesting an early divergence of the genus *Giardia* and sparking basic research in *Giardia* spp.
116. Adl, S. M. *et al.* The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J. Eukaryot. Microbiol.* **52**, 399–451 (2005).
117. Simpson, A. G., Inagaki, Y. & Roger, A. J. Comprehensive multigene phylogenies of excavate protists reveal the evolutionary positions of "primitive" eukaryotes. *Mol. Biol. Evol.* **23**, 615–625 (2006).
118. Nixon, J. E. *et al.* A spliceosomal intron in *Giardia lamblia*. *Proc. Natl Acad. Sci. USA* **99**, 3701–3705 (2002).
119. Andersson, J. O. *et al.* A genomic survey of the fish parasite *Spironucleus salmonicida* indicates genomic plasticity among diplomonads and significant lateral gene transfer in eukaryote genome evolution. *BMC Genomics* **8**, 51 (2007).
120. Weiland, M. E., Palm, J. E., Griffiths, W. J., McCaffery, J. M. & Svard, S. G. Characterisation of alpha-1 giardin: an immunodominant *Giardia lamblia* annexin with glycosaminoglycan-binding activity. *Int. J. Parasitol.* **33**, 1341–1351 (2003).

Acknowledgements
S.G.S. is supported by the Swedish Research Council VR–M, the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas) and the Swedish International Development Cooperation Agency (SIDA).

Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/genomeprj
Acanthamoeba castellani | *Entamoeba invadens* | *Giardia intestinalis* | *G. intestinalis* str. GS clone H7 | *G. intestinalis* str. WB clone 6 | *Plasmodium falciparum* | *Saccharomyces cerevisiae* | *Trichomonas vaginalis* | *Trypanosoma brucei*
UniProtKB: http://www.uniprot.org
ADI | Arf1 | CDC14A | β-COP | Cpn60 | Dicer | DLP | glutaredoxin | GPP | IscS | IscU | MAD2 | median body protein | MLH3 | MSH4 | mtHSP70 | Qb3 | Rab1A | Rab11 | REC8 | Sar1 | TSA417 | β-tubulin | YIP

FURTHER INFORMATION
Staffan G. Svärd's homepage: http://www.icm.uu.se/
Giardia DB: http://www.giardiadb.org

ALL LINKS ARE ACTIVE IN THE ONLINE PDF