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Giardia duodenalis: Genetic recombination and its implications for taxonomy and molecular epidemiology

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ABSTRACT

Traditionally, species within the *Giardia* genus have been considered as eukaryotic organisms that show an absence of sexual reproduction in their simple life cycles. This apparent lack of sex has been challenged by a number of studies that have demonstrated (i) the presence in the *Giardia duodenalis* genome of true homologs of genes specifically involved in meiosis in other eukaryotes, and their stage-specific expression; (ii) the exchange of genetic material in different chromosomal regions among human isolates of the parasite; (iii) the fusion between cyst nuclei (karyogamy) and the transfer of genetic material (episomal plasmids) between them. These results are pivotal for the existence of sexual recombination. However, many details of the process remain elusive, and experimental data are still scarce. This review summarizes the experimental approaches and the results obtained, and discusses the implications of recombination from the standpoint of the taxonomy and molecular epidemiology of this widespread pathogen.

1. Introduction

Giardia duodenalis (syn. *Giardia intestinalis*, *Giardia lamblia*) is a flagellated protozoan parasite that causes giardiasis in humans, pets, livestock, and wildlife. This peculiar organism has attracted much interest not only because of its medical and veterinary importance (Thompson and Monis, 2004), but also because of its presumed "primitive" nature, to the point that it has been described as a 'biological fossil', namely a true eukaryote with many peculiarities that retained some ancestral prokaryotic properties (Upcroft and Upcroft, 1998). Even if this view has been largely disproved by more recent studies (Embley and Martin, 2006), *Giardia* remains an interesting model organism for the study of many cellular processes (for example cell differentiation and protein trafficking), also thanks to the possibility to reproduce its simple life cycle, which comprises the vegetative trophozoite and the cyst, under axenic culture conditions.

Like all diplomonads, *Giardia* has two diploid nuclei that are morphologically indistinguishable, replicate at approximately the same time, and are both transcriptionally active (Adam, 2000). In each cell cycle, both nuclei in a trophozoite divide, giving rise to a total of four daughter nuclei. It has been shown that the two daughters of a single nucleus segregate to different trophozoites, namely that segregation is equational (Yu et al., 2002; Sagolla et al., 2006). An important prediction results from equational segregation: differences between the nuclei would be expected to accumulate over time. If the two nuclei contain the same complement of genes and chromosomes, these differences would be demonstrated in the form of heterogeneity of homologous chromosomes and allelic sequence heterozygosity (ASH). Chromosome size heterogeneity is well documented, and ASH of repeat copy number for the *vsp* genes is common (Adam, 2000).

However, ASH at the sequence level is quite uncommon in *G. duodenalis* and estimates from the WB strain genome project suggest this level to be 0.01% (Morrison et al., 2007). This finding is considered unusual for polyploid organisms like *Giardia* spp., which have been generally assumed to be asexual organisms of ancient origin, and has puzzled researchers for many years. One mechanism that can explain the maintenance of a low level of ASH is genetic recombination, but direct evidence for this was lacking.

In the following sections, we will first review the recent experimental evidence in favor and against the occurrence of recombination in *Giardia* and then discuss the implications for taxonomy and epidemiology.

2. Evidence for meiotic genes in *Giardia*

The question of whether *Giardia* is potentially capable of sexual reproduction was first addressed by Ramesh et al. (2005), who

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surveyed the *G. duodenalis* genome sequence (of the strain WB, assemblage A) for a common set of genes required for meiotic recombination (and thus, sex) in other eukaryotes (animals, plants and fungi). This meiotic gene inventory showed that true homologs of genes specifically required for meiosis in model eukaryotic species are widely distributed among diverse eukaryotes. In particular, five genes (*Dmc1*, *Spo11*, *Mnd1*, *Hop1*, and *Hop2*) known to function specifically during meiosis in other eukaryotes, are present in *Giardia*, as further confirmed by cloning and sequencing of PCR products obtained from genomic deoxyribonucleic acid (DNA). Furthermore, Ramesh et al. (2005) provided evidence for the *bona fide* nature of the identified homologs by constructing phylogenies from the proteins encoded by each of these genes, and showing that the *Giardia* proteins group unequivocally with other eukaryotic homologs, usually as a deep branch. A deep branch can be explained in two ways.

1. Phylogenetic analyses places *Giardia* as a primitive early-branching eukaryote and a pivotal 'missing link' between prokaryotes and eukaryotes. Thus, future studies of meiosis in *Giardia* will provide exciting new insights in the "origin of meiosis". It should, however, be remember that these genes may have a non-meiotic function in the last common ancestor of *Giardia* and other eukaryotes; indeed, many researchers believe that the eukaryotic meiosis machinery originally evolved from genes involved in DNA damage repair (Vielleneuve and Hillers, 2001).
2. Recent developments in evolutionary biology has led to believe that amitochondriate organisms, such as *Giardia*, are not primitive, but instead highly evolved and specialized for their specific environments (Dacks et al., 2008). *Giardia* might therefore utilize the "core meiosis machinery" for a process, which might be deviated from meiosis (and sex) found in animals, plants and fungi (Haig, 1993). The core meiosis machinery of *Giardia* might function as a DNA repair machinery – between the two nuclei, thereby causing lower ASH than expected.

Recently, Melo et al. (2008) have analysed the expression the transcription of some of the genes potentially involved in meiosis. They have identified two homologs of the *Dcm1* gene (that were named *Dcm1a* and *Dcm1b*) and one homolog of *Spo11* and *Hop1* genes. Using semi-quantitative RT-PCR, the authors have shown that *Dcm1b*, which has the same sequence of *Rad51*, a gene whose product is involved in DNA repair during mitosis, is transcribed almost constitutively during the life cycle of *Giardia*, and suggested that it may indeed have a role similar to *Rad51*. On the other hand, *Dcm1a* is strongly induced during early encystation and early excystation, and its expression remains high while *Spo11* and *Hop1* are transcribed. The authors concluded that transcription of these three genes may facilitate the exchange of genetic material between and within the two nuclei during encystation and excystation.

3. Indirect evidence from molecular genotyping studies

Giardia duodenalis is considered as a species complex, whose members show little variation in their morphology, yet can be assigned to seven distinct genetic groups (assemblages A–G) based on protein and DNA polymorphisms (Monis et al., 2003; Cacciò and Ryan, 2008). Among the seven assemblages, only assemblages A and B have been found in humans and in a wide range of other mammalian hosts, whereas assemblages C–G seem to have a more restricted host range (Table 1).

The direct characterization of *Giardia* cysts at the molecular level by the use of PCR techniques is widely used in many laboratories to study the epidemiology of the infection. The vast majority of studies have relied on the analysis of the small subunit ribosomal RNA (*ssu-rRNA*), the β-giardin (*bg*), the glutamate dehydrogenase (*gdh*), the elongation factor 1-alpha (*ef*-1), the triose phosphate isomerase (*tpi*), the GLORF-C4 (*C4*) genes and recently, the inter-genomic rRNA spacer region (Cacciò and Ryan, 2008). In early studies, there was a strong bias towards the use of ssu-rRNA that, due to its multicopy nature and high degree of sequence homology, represented the locus of choice for the genotyping of *Giardia* isolates. As a consequence, the reliability of different genetic loci in the assignment of isolates to specific *G. duodenalis* assemblages was not assessed, the assumption being that these loci will only differ in terms of their polymorphism. Recent studies based on a multilocus approach have shown that a number of isolates, of both human and animal origin, cannot be unequivocally assigned at the assemblage level, because the genotyping data from different loci were not consistent (Traub et al., 2004; Gelanew et al., 2007; Cacciò et al., 2008).

To obtain a more informative picture, we performed an analysis of a dedicated database which combines epidemiological data from field isolates (country, year of isolation, source, symptoms, etc.) and sequence data of the *ssu-rRNA*, *bg*, *gdh* and *tpi* genes. This database has been developed in the context of the ZOOnotic Protozoa NETwork (ZOOPNET), a European network of veterinary and public health Institutions working on *Cryptosporidium* and *Giardia*, and currently includes over 2400 *Giardia* sequences (including those from Genbank). From ~30% of the *Giardia* isolates in the ZOOPNET database, two or more markers are known (August 2008). The analysis of these isolates for the presence of inter-assemblage "mixing" (in other words, inconsistent typing between two markers) showed that this phenomenon occurred in ~15% of the isolates, and was predominantly observed in humans and dogs (Table 2). Intra-assemblage mixing (e.g. AI plus AII) was also observed for assemblages A–E (not shown).

Taken at face value, these results are compatible with recombination events occurring between different assemblages and in different hosts. However, as genotyping was performed directly on DNA extracted from stool samples, inter-assemblage "mixing" can also be explained by PCR bias in the presence of mixed infec-

Table 1
Giardia duodenalis assemblages and their distribution in mammalian hosts.

Assemblages	Host (s)
A	Human, non-human primates, livestock, horses, dogs, cats, guinea pigs, fallow deer, white-tailed deer, moose, ferrets
B	Human, non-human primates, livestock, horses, dogs, coyotes, muskrats, beavers
C, D	Dogs, cats, coyotes, wolves
E	Cattle, sheep, goat, water buffaloes, muflons
F	Cats
G	Rats

Table 2
Unreliable assignment of individual isolates to specific *G. duodenalis* assemblages. Data were taken from the ZOOPNET database (August 2008).

Mixed assemblages	Occurrence (*n*)	Host
A and B	39	Human, dog, cat, monkey
A and C	2	Dog
A and D	2	Dog
A and E	3	Cattle
B and C	4	Dog
B and D	2	Dog
B and E	1	Sheep
C and D	10	Dog
C and E	0	
D and E	1	Sheep