




Lecture 11

Giardia and Antigenic Variation



Unique, science-based products designed to promote health awareness!

Wear with caution: only for the really serious science geeks!

Antigenic Variation Key Points

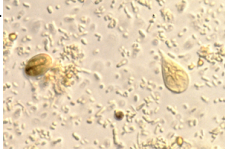

- General features of Antigenic Variation (non-viral)
 - Requires a family of variant surface antigen genes
 - Requires a mechanism to express only one gene at a time
 - Requires a mechanism to switch genes
- Trypanosomes - ~2000 VGS genes (variant surface glycoprotein)
- Expression occurs out of telomeric expression sites (ES) (tapes/ tape recorder or CDs/ CD player)
- Expression seems promoter independent
- To switch genes on, they are transposed into an active expression site by several mechanisms
- Expression seems to be controlled by a physical association of ES with a single RNA Pol I transcription particle (location) per nucleus

Learning Objectives


- Describe *Giardia*
- Describe life cycle and transmission
- Describe symptoms and treatments
- Describe unique *Giardia* features
- Describe RNA interference and relevance
- Describe VSP
- Describe role of RNAi in antigenic variation

Early Parasitologist

- My excrement being so thin, I was at diverse times persuaded to examine it; and each time I kept in mind what food I had eaten, and what I had drunk, and what I found afterwards. I have sometimes seen animalcules a-moving very prettily..

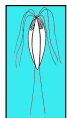




Most common flagellate of human intestinal tract

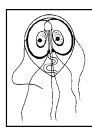



Order Diplomonadida

- **Family Hexamitidae**
 - parasitic
- *Giardia, Hexamita*
 - Bilateral symmetry
 - Defining family feature - 2 identical nuclei
 - Forms cysts
 - Binary fission - no sexual reproduction detected

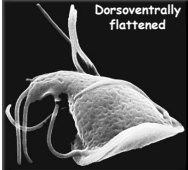
Hexamita meleagridis

Giardia lamblia

Giardia lamblia


- worldwide distribution (cosmopolitan)
 - higher prevalence in tropical or developing countries (20-30%)
 - 1-6% in temperate countries
- most common protozoa in stools
 - ~200 million cases/yr
- Giardiasis - beaver fever
 - often asymptomatic
 - acute or chronic diarrhea



Dorsoventrally flattened

Taxonomy

- one human species, aka:
 - *G. duodenalis*
 - *G. intestinalis*
- morphologically similar forms in other mammals



Giardia Life Cycle - Direct

- Fecal/Oral transmission
- **Cyst** - Infective stage
 - Resistant form
- **Trophozoite** - feeding, binary fission

Ingest cysts - pass through stomach, excystation in duodenum (small intestine).
 Inhabit lumen of small intestine
 As feces dehydrate in colon trophozoite encysts.

CDC
 http://www.cdc.gov/giardia

Giardia Life Forms

Cyst

Infective stage
Resistant

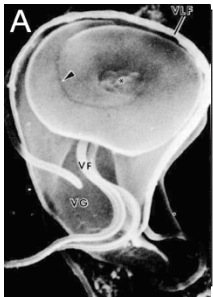
Trophozoite

Disease stage
Replicative form

Keys Features of Cysts

- oval shape
- 11-14 x 6-10 μm
- distinct cyst wall (CW) set apart from cytoplasm
- 4 nuclei at anterior end
- fibrils (axonemes) evident
- median bodies (MB)
- Resistant to chlorine

Key Features of Trophozoites



- pear shape
- 12-15 x 5-10 x 2-4 μm
- Dorsoventrally flattened
- 2 nuclei
 - Morphologically identical
 - Transcriptionally active
- fibrils (axonemes) evident
- bilateral symmetry
- pair of median bodies
- 4 pair flagella
 - motility like a falling leaf
- adhesive disk (not always evident)

Clinical Features and Symptoms

Range of Outcomes

- asymptomatic/latent
- acute short-lasting diarrhea
- chronic/nutritional disorders

Acute Symptoms

- 1-2 week incubation
- sudden explosive, watery diarrhea
 - bulky, frothy, greasy, foul-smelling stools
 - no blood or mucus
- upper gastro-intestinal uneasiness, bloating, flatulence, belching, cramps, nausea, vomiting, anorexia
- usually clears spontaneously (undiagnosed), but can persist or become chronic
- Propensity for multiple re-infections

Clinical Features and Symptoms

Chronic Symptoms

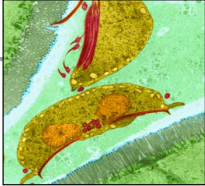
- Recurrent diarrheal episodes
- Sulfuric belching, malabsorption, steatorrhea, anorexia
- Cramps, nausea very common
- Can lead to weight loss and failure to thrive
- Deficiency in vitamins A, B12 and folate

Factors contributing to chronic giardiasis

- host factors
 - age, immune status, previous exposure, diet and concomitant intestinal microbiota
- parasite factors
 - genotype specific - rate of multiplication, variable surface proteins (VSP), resistance to drugs and ability to evade immune response.

Pathogenesis

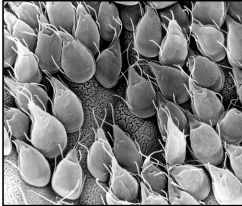
- Epithelial damage
 - Microvillus blunting
- Malabsorption
 - Large numbers of trophozoites



Possible Mechanisms

Table 2 | Proposed disease mechanisms in giardiasis

Disease mechanism	References
Apoptosis of enterocytes	5,103,104
Loss of epithelial-barrier function	5,101,109
Hyperscretion 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



Variable Symptomology

- Antigenic Variation - clonal phenotypic variation
 - Trophozoites express a single VSP covering the surface.

Table 1. Characteristics of antigenic variation in *Giardia lamblia*.

1. A property of all *Giardia lamblia* isolates
2. The varying-specific surface proteins (VSPs) are a family of related proteins
3. Occurs spontaneously in culture and *in vivo* in humans and laboratory animals
4. Rate of VSP change is isolate and VSP-dependent and ranges from one switch every 5–13 generations
5. Repertoires of VSPs may differ among isolates
6. Identical epitopes can be present in molecules of varying sizes
7. Monoclonal antibodies to VSPs are cytotoxic at lower dilutions and inhibit growth at higher dilutions
8. Switching during encystation–excystation occurs in some *Giardia*

Nash, T.E. Mol Micro (2002) 45: 585

Variable Symptomology

Table 2. Characteristics of variant-specific surface proteins of *Giardia lamblia*.

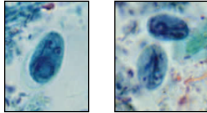
1. Vary in size from about 20 to 200kDa
2. Cysteine-rich with numerous CXXC motifs
3. Conserved hydrophilic tail
4. Hydrophilic carboxyl-terminal five amino acids are CRGKA
5. One or more conserved GGCCY motifs
6. Zn finger motif
7. Contains O-linked glucose and N-acetylglucosamine
8. Palmitate residue at carboxyl terminus
9. Amino terminus most peripheral at parasite–host interface

- VSPs are Cysteine-rich - 11-12% cysteine (disulfide linkages)
 - CXXC motifs usually associated with DNA binding proteins
 - VSP repertoire of 235 - 275 possible variants.
 - Clonal success depends on fighting host immunity and proteases.
 - Possibly all VSP genes are transcribed, but ONLY 1 protein is stable.
 - RNA interference regulates this expression!
 - If RNAi system is disturbed - parasite expresses all VSPs on the surface.

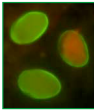
Prucsa C.G. et al. Nature (2008) 456: 750

Giardia Diagnosis

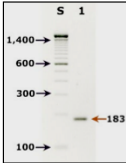
- Microscopic
 - Detection in stool samples
 - Multiple samples tested
 - Concentration of samples
 - Classical stains
 - Immunofluorescence
- Molecular
 - PCR-based methods
 - Detection from a single cyst



Iron-hematoxylin stain



Direct Fluorescence Assay

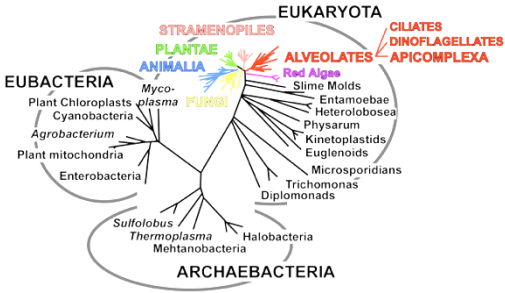


S 1
1,400 →
600 →
300 →
100 →
← 183

Control

- Highly contagious - cysts are quite resistant
- avoid fecal-oral transmission
- improve personal hygiene
 - especially institutions - day care centers, cafeterias
- treat asymptomatic carriers
 - eg, family members
- health education
 - hand-washing
 - sanitation
 - food handling
- protect water supply - breakdown in water treatment facilities
- treat water if questionable
 - boiling
 - iodine
 - not chlorine!

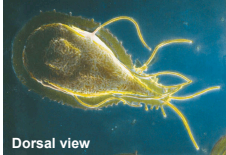
Tree of life - 3 kingdoms



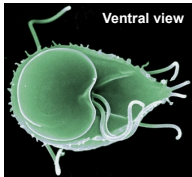
Mitch Sogin circa 1980s - 16S rRNA phylogenetic analysis

Giardia - Early branching eukaryote

- **Eukaryotic properties**
 - Genome sequenced 2007.
 - Nucleus - albeit 2!
 - Ribosomes
 - 60S/40S vs. 50S/30S
 - Multiple linear chromosomes
 - 11.7 MB genome
 - 5 chromosomes - 6470 ORFs
 - Lysosomal vesicles



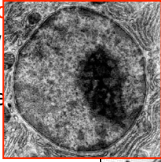
Dorsal view





Ventral view

Interesting Biological Features

- "Missing link" - eukaryotic evolution
- **Ventral adhesive disc**
- No nucleoli - now
- No peroxisomes
- No obvious stacks
- No mitochondria
 - Anaerobic metabolism
 - **Amitochondriate**







Giardia does have nucleoli

- Previous techniques - light, fluorescence, EM - no nucleoli
- Previously recognized anteronuclear zone
- Silver staining of nucleus organizing regions
- Distribution of markers, rRNA fibrillar, ribonuclear protein component (CBF5)

Available online at www.sciencedirect.com

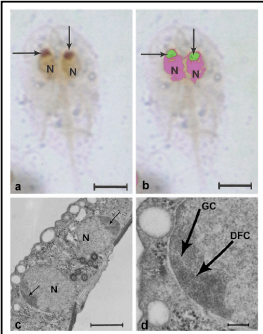
ScienceDirect

International Journal of Parasitology 34 (2004) 1201–1208

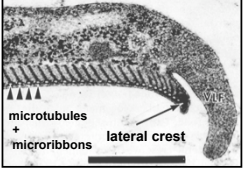
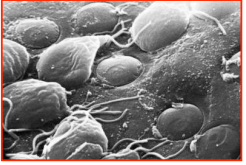
Rapid Communication

Identification of nucleoli in the early branching protist *Giardia duodenalis*

Leo Felipe Jimenez Garcia^a, Guadalupe Zecuba^b, Bibiana Chavez-Montano^a, Maria del Pilar Restrepo-Gonzalez^c, Gabriel Lopez-Villaseca^d, Maria de Lourdes Segura-Vakili^e, Cecilia Morones^f, Adrian H. Hodel^g, Raul Argente-Garcia^a, Guadalupe Ortega-Pimentel^a



Adhesive Disk Components

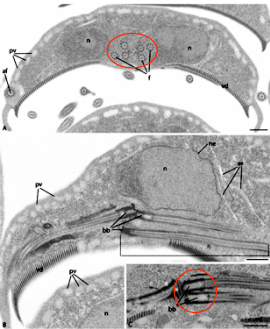
- Microtubules
 - Tubulins ✓
- Microribbons
 - Giardins ✓
- Lateral crest
 - Actin ✓
 - Myosin ???

Immunofluorescence techniques for identification

Cross-reacting antibodies!

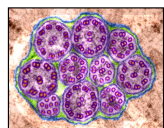
Cytoskeleton - rich in microtubules

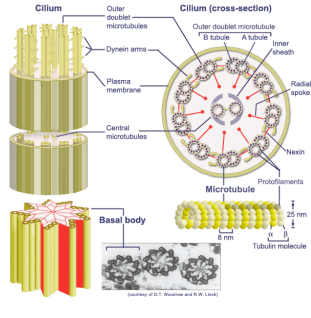
- Microtubule structures
 - Flagella
 - Basal body
 - Median body
 - Ventral disk
- Composed of Tubulin
 - α -tubulin - 2
 - β -tubulin - 3
 - γ -tubulin - 1



Microtubule structure

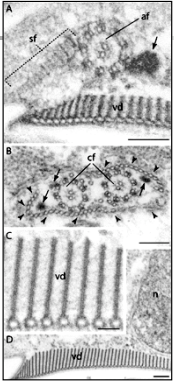
- Protofilaments
 - α -tubulin- β -tubulin dimer
- 9 + 2 arrangement
 - Central pair - singlets
 - Outer pair - doublets
- Basal body
 - Triplet arrangement
 - γ -tubulin





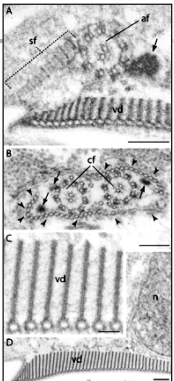
Specific localization

- Tubulin modifications
 - C-terminus
 - Polyglycylation
 - polyglutamylation
 - Phosphorylation
 - acetylation
- Monoclonal antibodies
 - α -tubulin - polyglut
 - Proximal Flagella
 - α -tubulin/ β -tubulin - polyglyc
 - Flagella, median body



Specific localization

- Giardins (~25-30 kDa)
 - Ventral disk
- Originally identified by 2D gel electrophoresis
 - α -giardin (19) - annexins
 - β -giardin (1) - SF-assemblin
 - γ -giardin (1) - no homology
 - δ -giardin (1) - no homology
- Classification is ongoing!



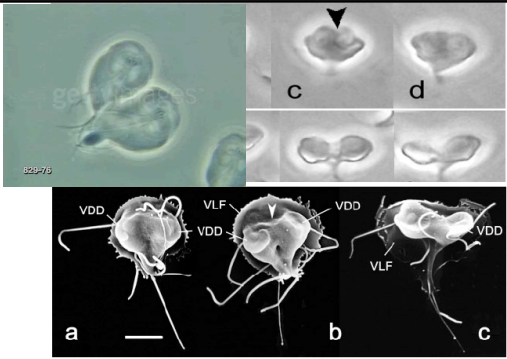


Fig. 7. Scanning electron microscopy showing development of two daughter adhesive discs (VDD1, VDD2) on the dorsal side of the attached, dividing parent cell. (b) Arrowhead indicates the plane of cytokinesis. (c) Parent ventrolateral flange (VLF) persists up to the end of the initial attachment phase of *Giardia* division. Bar: 3 μ m.



In vitro transformation

Excystation	Encystation
<ul style="list-style-type: none"> • brief exposure to acidic pH (~2) • flagellar activity within 5-10 min after return to neutral pH • breakdown of cyst wall (proteases) • trophozoite emerges from cyst • cytokinesis within 30 min 	<ul style="list-style-type: none"> • exposure to pH 7, no bile • exposure to pH 7.8, high bile • cyst wall secretion (appearance of vesicles) • loss of disk and flagella • nuclear division

Relatively Fast Changes

Current Opinion in Microbiology


Giardia lamblia encystation: from trophozoite to cyst. Images from left to right show a vegetative trophozoite, trophozoites after 21 and 42 h of encystation, and a water-resistant cyst. Encysting trophozoites gradually round up and develop numerous ESV (arrowheads) that export CW (arrow) components. F, flagella. Bar, 5 μm.

Learning Objectives

- Define RNA interference
- Define basic terminology
- Describe molecular mechanism
- Define VSP and relevance
- Describe role of RNAi in antigenic variation

A Nobel Way to Regulate Gene Expression


- Current number of reviews - 955 (2007)
 - 1383 (2008)
 - 1848 (2009 Feb)
 - 2144 (2009 Oct) -8684 DNA replication reviews
- 8 years following initial discovery in *C. elegans*:
the Nobel prize was awarded to Andy Fire and Craig Mello!!



Andy Fire
Stanford

Silence is Golden

"for their discovery of RNA interference:
gene silencing by double-stranded RNA"

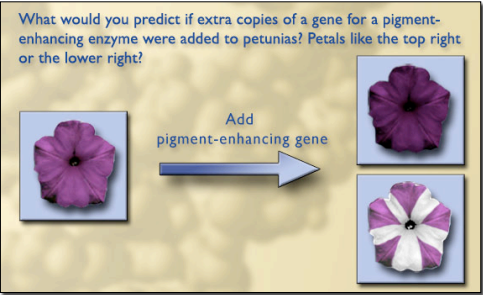


Craig Mello
UMass Med

2006 Nobel Prize in Physiology or Medicine

Experimental Expectations

What would you predict if extra copies of a gene for a pigment-enhancing enzyme were added to petunias? Petals like the top right or the lower right?



A Perplexing Result Loss of Pigment

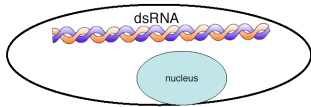
- If you guessed the top flower with the deeper purple, you're in good company. That's what the scientists doing the experiment expected.
- But the actual result looked like the lower flower—variegated petals with sections of no pigment.
- How could adding copies of a gene for enhancing pigment result in less pigment?



Result: Hypopigmentation

What is RNA interference?

- Way to silence gene expression - **post-transcriptional**
 - dsRNA does not produce global down regulation, but is sequence specific - leads to the specific degradation of a corresponding mRNA
- Gene silencing - no need to delete the gene
- Conserved pathway thought to be involved in retrotransposon and viral defense
- Now, gene regulatory mechanisms involving small RNAs



Discovery of RNAi

- First discovered in *C. elegans* by Andy Fire et al (1998).
Coined the term RNA interference
- Same year, initial observations of similar phenomenon in *T. brucei* by Elisabetta Ullu et al.
- Both sense and antisense RNAs were sufficient for silencing....How???
- Silencing can persist and is potent

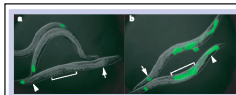


Figure 1 RNAi in *C. elegans*. Silencing of a green fluorescent protein (GFP) reporter in *C. elegans* occurs when animals are fed on bacteria expressing GFP dsRNA. (a) In an animal that was selective for RNAi. (b) Note that silencing occurs throughout the body of the animal, with the exception of a few cells in the tail that express some residual GFP. The signal is lost to interstitial cells near the tail (arrowhead) as well as near the head (arrow). The lack of GFP-positive entries in a bracketed region demonstrates the systemic spread and lateral bias of silencing.

Hugely HOT topic
New information every month
Increasingly detailed knowledge
Large body of literature
Stick to the basics



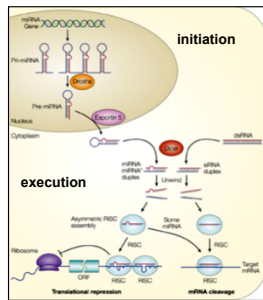
Terminology - RNA Players

- **dsRNA:** double stranded RNA longer than 30 nt
- **siRNA:** short interfering RNA, 21-25 nt
 - Mostly exogenous origin (synthetic RNAs)
 - dsRNA precursors (may be stemloop)
 - Target specific (1 siRNA = 1 target)
- **miRNA:** microRNA, 21-25 nt
 - Encoded by endogenous genes
 - Hairpin precursors
 - Recognize multiple targets (1 miRNA = ~5 targets)



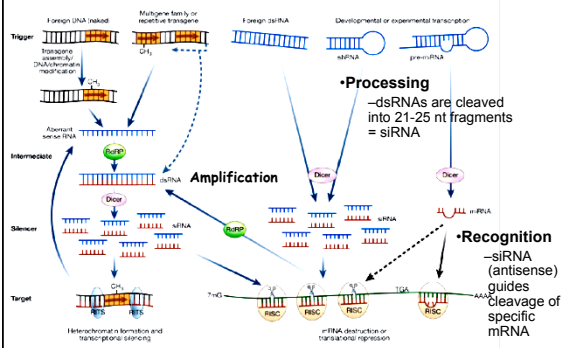
Regulation by small RNAs

- Exogenous vs. endogenous
- Share a common pathway
- End results:
 - Regulation via mRNA degradation (siRNA)
 - Regulation via translational repression (miRNA)





Models for RNAi



RNAi Players - simplified

- **Dicer**
 - ATP-dependent ribonuclease
 - 2 RNase III domains
- **siRNA**
- **RISC**
 - RNAi silencing complex
 - Contains siRNA
 - Directs complex to specific mRNA
 - Antisense strand mediated
 - Promotes cleavage of mRNA in the middle of siRNA

19 nt duplex
5' 3' 3' 5'
2 nt 3' overhangs

Mechanism of RNA interference

Zamore, P. D. Science 296, 1265-1269 (2002)

Key Points of *C. elegans* Experiments

- substoichiometric amounts of dsRNA relative to the targeted mRNA are required to completely eliminate the mRNA (i.e. the dsRNA is catalytic)
- dsRNA is 10-100X better than antisense or sense RNA
- doesn't work if introns or promoters are targeted by the dsRNA
- doesn't interfere with transcription initiation or elongation (it is possible to target a single gene in an operon) (i.e. RNAi is a post-transcriptional phenomena)
- the targeted mRNA is degraded (i.e. it can't be detected by probes)
- dsRNA can cross cellular boundaries (i.e. there is a transport mechanism)

RNAi as an experimental tool

- dsRNA (relative long dsRNA molecules)
 - Injected into cells - not heritable
 - *C. elegans* could be fed bacteria containing plasmids that synthesized dsRNA
 - DOES NOT work with mammalian cells
 - dsRNA (long) induces interferon system leading to non-specific cytotoxic effect
- siRNA
 - Injected into cells - not heritable - lose effect over cell generations
 - Vectors for cell specific situations
 - Long term steady expression
 - Therapeutic applications

Experimental induction of RNAi

The diagram illustrates four methods for inducing RNAi:

- C. elegans:** dsRNA is fed to worms, which then express the dsRNA in their tissues.
- Drosophila:** dsRNA is injected into embryos, and transgenic flies are created that express dsRNA in their germline.
- Cultured Drosophila cells:** dsRNA is added to a medium containing the cells.
- Cultured Mammalian cells:** dsRNA is transfected or injected into embryonic cells or early embryos. Alternatively, differentiated cells or whole organisms (in vivo) are transfected with synthetic siRNA.
- Plants:** dsRNA or a dsRNA hairpin transgene is overexpressed, or single-stranded RNA is introduced from a transgene or virus to trigger silencing.

Variable Symptomology

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Nash, T.E. Mol Micro (2002) 48: 585



Giardia RNAi Paper Discussion

Papers are loaded with huge amounts of information
What should you take away from the paper?

Identify a Hypothesis

List the Major Finding(s)

Learn some Techniques

