

20.109
Laboratory Fundamentals in
Biological Engineering

Module 1
Nucleic Acids
Lecture 2

Primer Design - Additional Thoughts

- Hairpins
 - Particularly bad at 3' end
 - $\Delta G < -3$ kcal/mol may be tolerated
 - Self-dimers
 - Homologous to self, 3' end worse
 - $\Delta G < -5$ kcal/mol may be tolerated
 - Cross-dimers
 - Sense/antisense pair
- All reduce primer availability, annealing

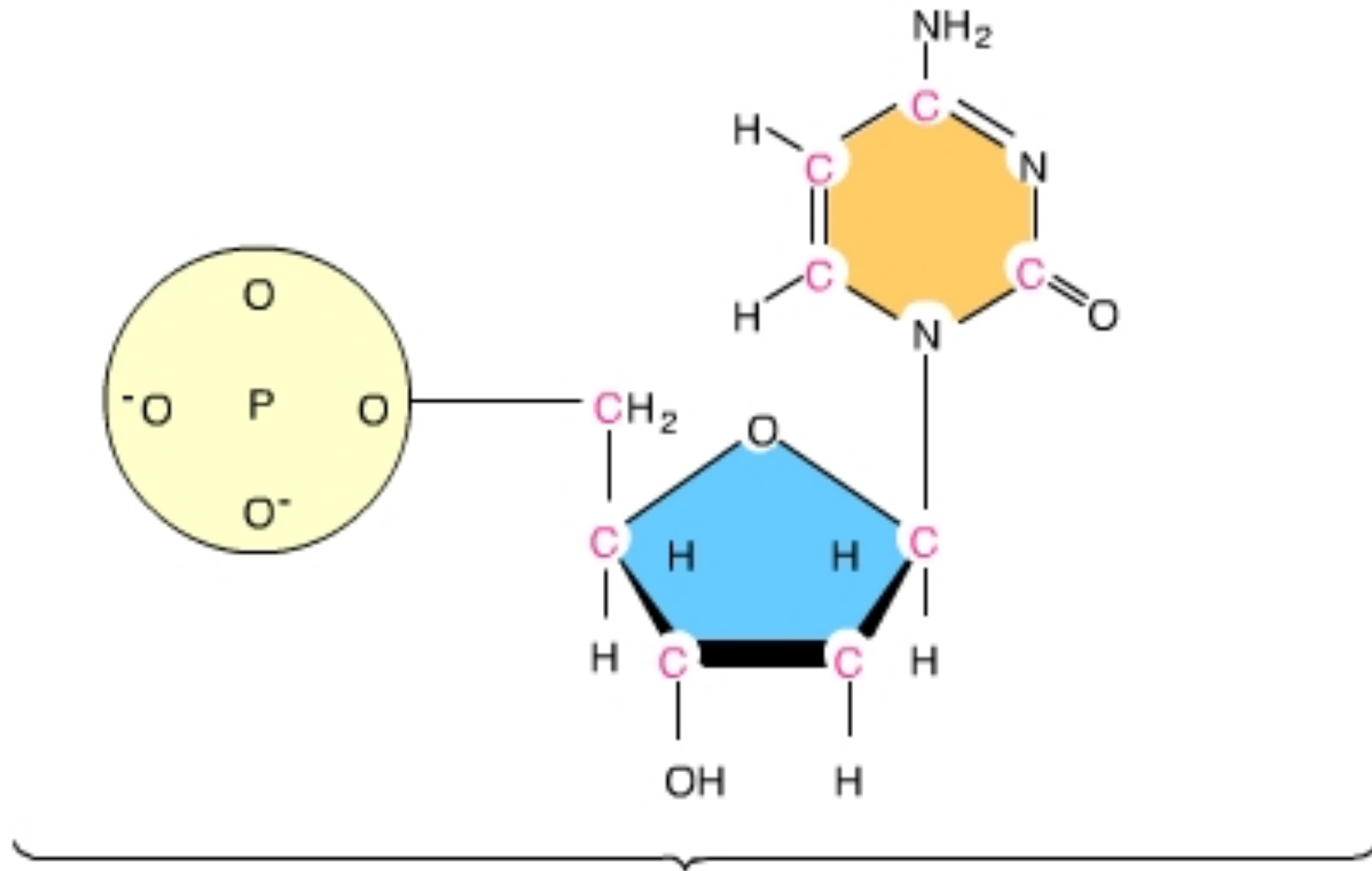
Primer Design - Additional thoughts

- Degenerate Oligos may be useful
 - When amino acid only known
 - When only reference is known
 - When variation is known
- Oligo synthesis is not perfect
 - Unless HPLC or PAGE purified, 98% efficient

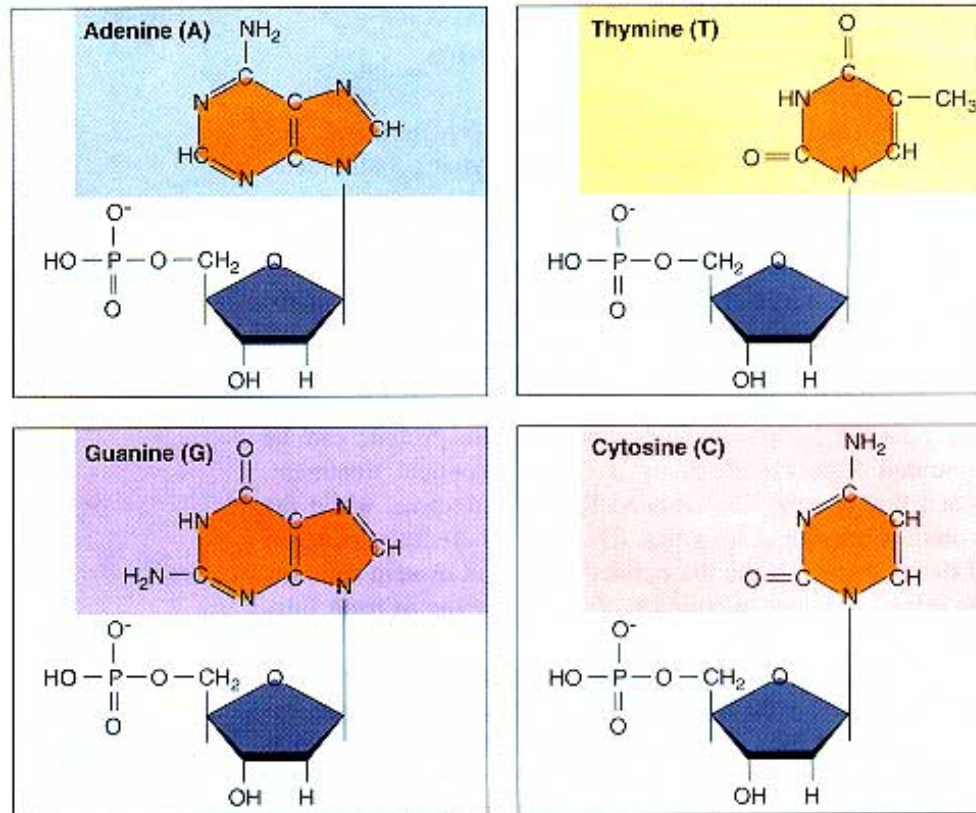
Primer Design - Additional thoughts

- Degenerate Oligos may be useful
 - When amino acid only known
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 - $(0.98)^{\text{oligolength}} = \% \text{ with correct sequence}$
 - 20mer = 66.7%

Nucleotide structure



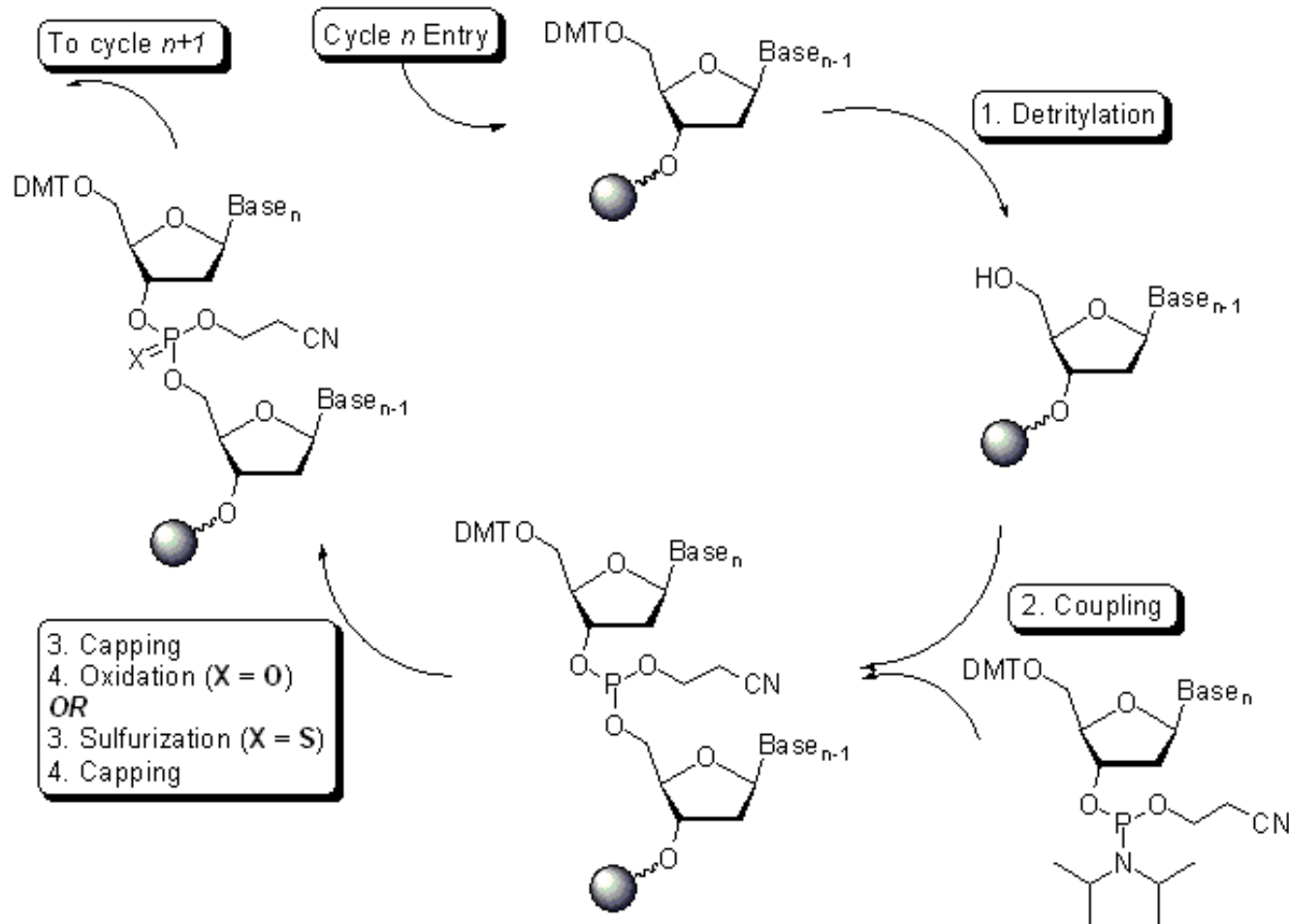
Nucleotide structure



The four nucleotide subunits that make up DNA

The nucleotide subunits of DNA are composed of three elements: a central five-carbon sugar, a phosphate group, and an organic, nitrogen-containing base.

Oligonucleotide synthesis

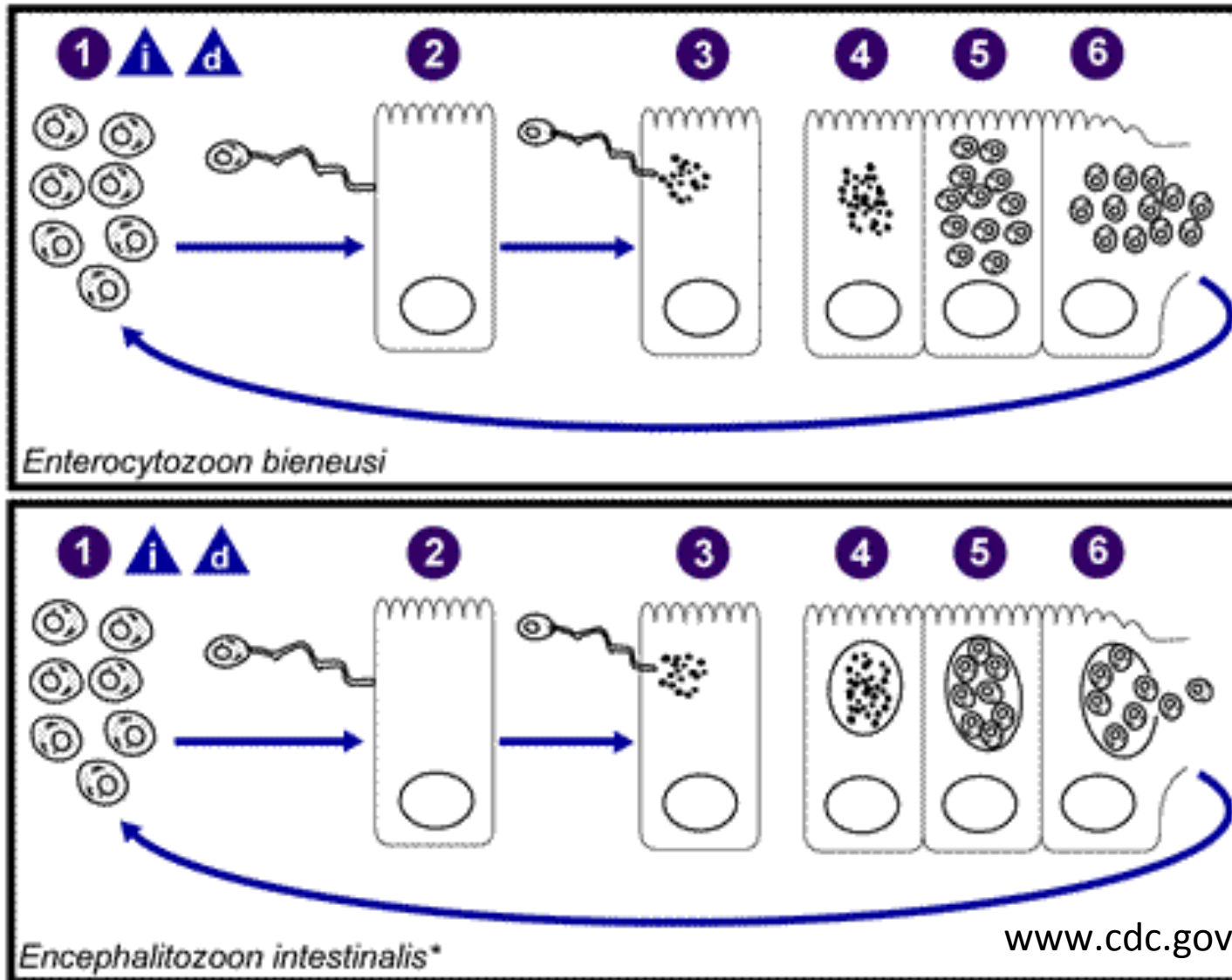


Microsporidia are a new disease

- Obligate intracellular
- Fungi
- Diverse taxonomic distribution (insects, fish, mammals, other?)
- Unknown impact overall
- 15-45% of AIDS with unknown diarrheal etiology
- 90% are *E. bienersi*, other mostly *E. intestinalis*
- Varied infection strategies



Microsporidia life cycle

Intracellular development of *E. bienersi* and *E. intestinalis* spores.



*Development inside parasitophorous vacuole also occurs in *E. hellem* and *E. cuniculi*.

Microsporidia - human infection

 = Infective Stage
 = Diagnostic Stage

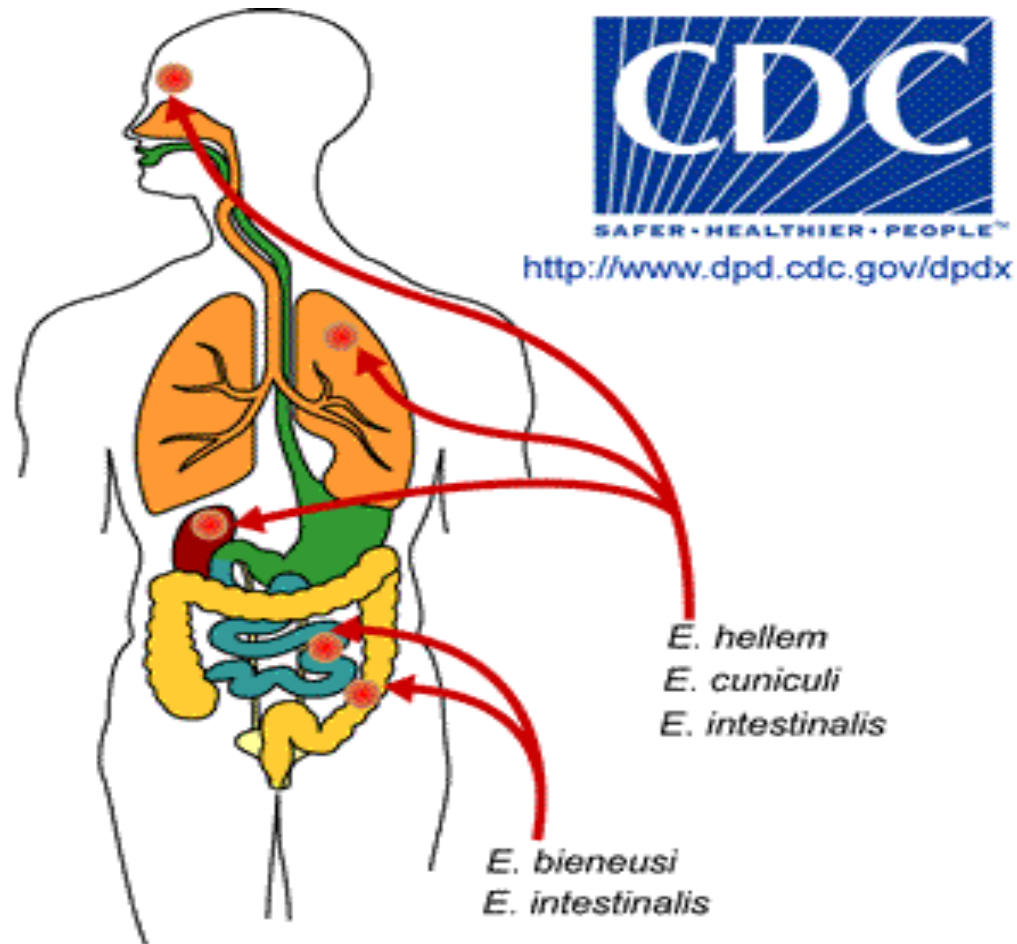
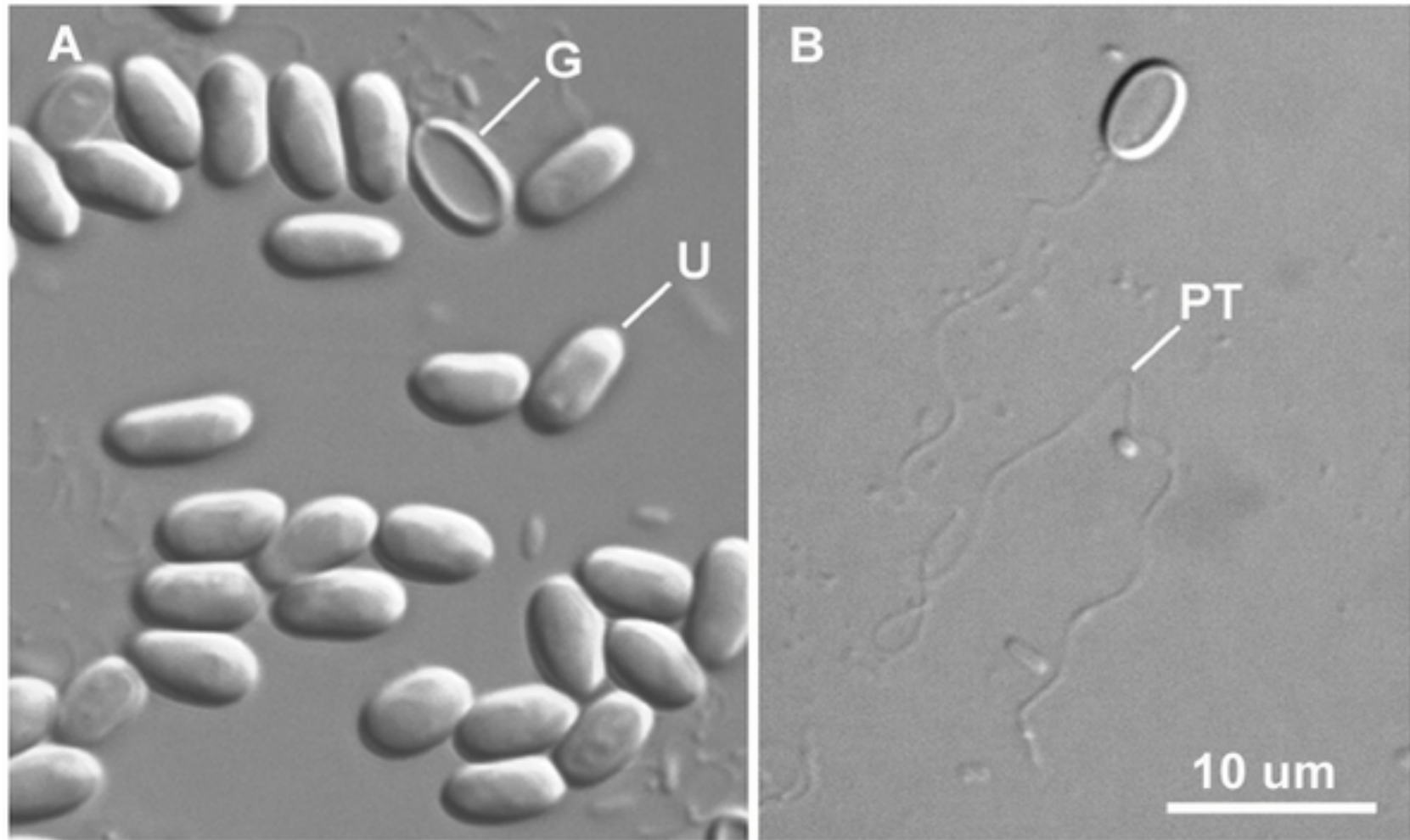
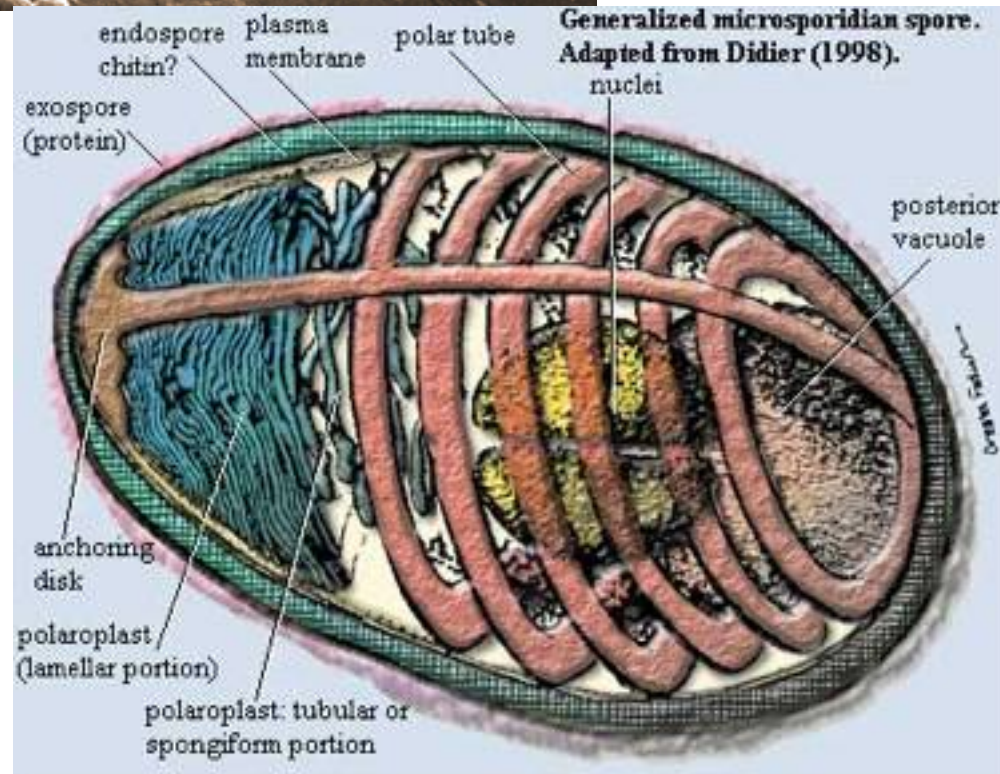
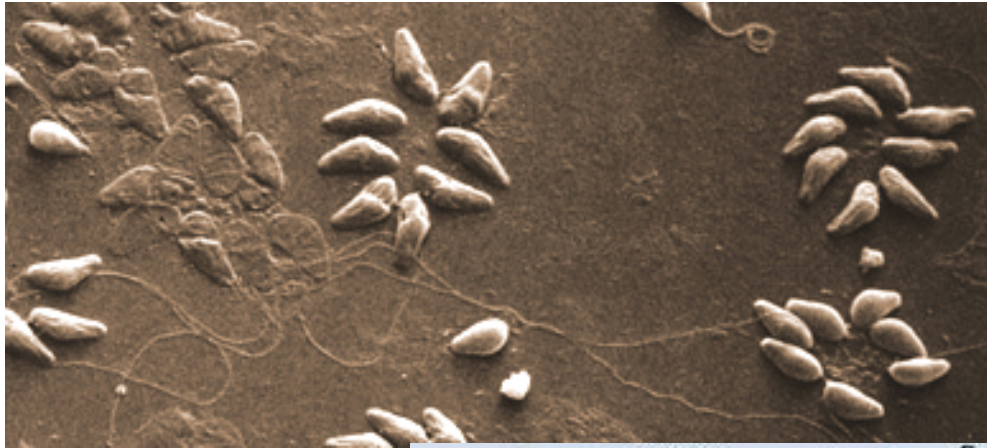


Figure 1. Light micrograph of *Antonospora locustae* with pressure-induced polar tube eversion.



Keeling P (2009) Five Questions about Microsporidia. PLoS Pathog 5(9): e1000489. doi:10.1371/journal.ppat.1000489
<http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1000489>



Clinical Signs of Microsporidiosis

- Chronic diarrhea and wasting but different species invade different sites (the cornea, urinary track and muscles).
- Disseminated - cholecystitis (inflammation of the gallbladder), renal failure, respiratory infection, headache, nasal congestion, ocular pain and sinus involvement.
- Infections of the brain or other nervous tissue - seizures, headache and other symptoms.