

RNAi pathway components and function in *Paramecium bursaria*

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June 2, 2016

Overview

Motivation

RNAi in ciliates

Experimental RNAi induction in *P. bursaria*

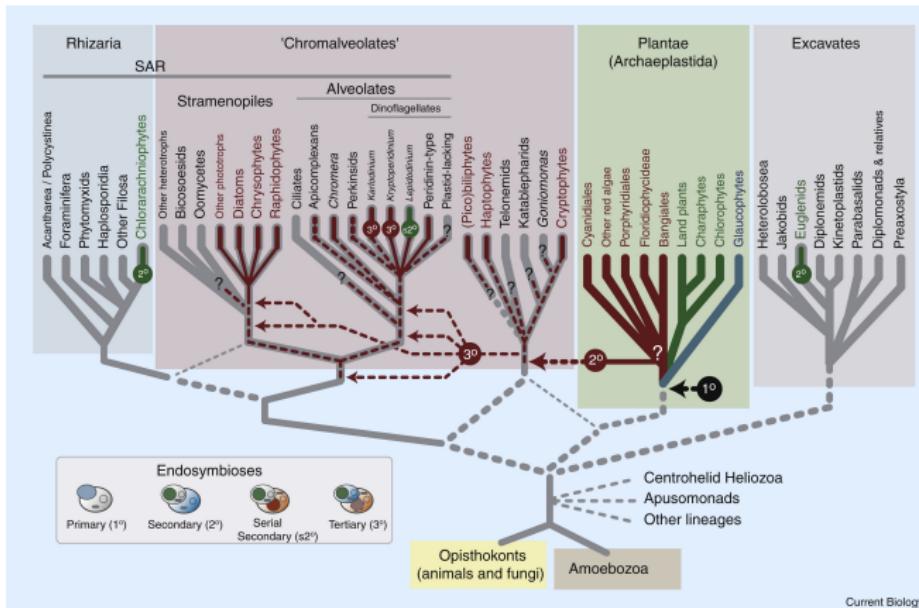
RNAi pathway components in active *P. bursaria* transcriptome(s)

In-silico analysis of potential endosymbiont 'cross-talk'

Conclusions

Why is *Paramecium bursaria*
potentially a good model for
(secondary photosynthetic)
endosymbiosis?

Broad diversity of second order plastid endosymbioses



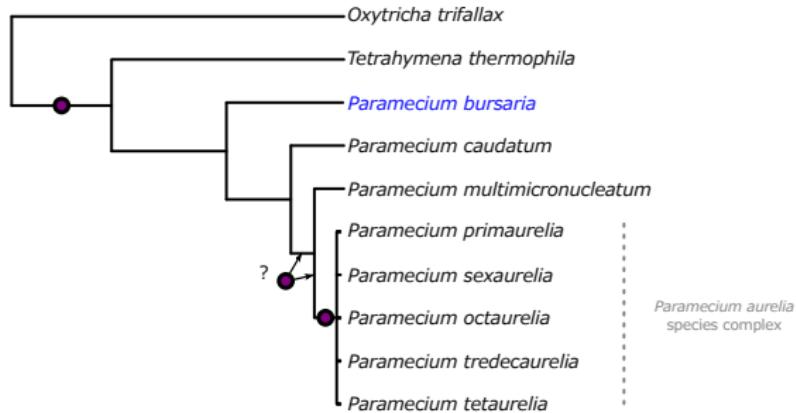
Reproduced from [Arc09]

Paramecium bursaria and its green algal endosymbionts



- ▶ 100 µm to 160 µm serial phagotrophic ciliate (nuclear dimorphism).
- ▶ ~ 300 endosymbiotic algae in stable heritable facultative(?) endosymbiosis.
- ▶ Multiple independent origins of these endosymbioses.
- ▶ Single cell transcriptome and genome of *P. bursaria-Micractinium reisseri* CCAP 1660/12.
- ▶ *P. bursaria* bulk transcriptome [KSD⁺14].

RNAi pathways in the ciliates



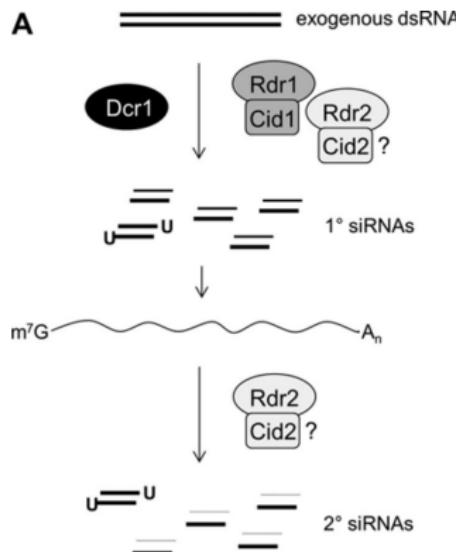
A good model needs a means to test hypotheses:

- ▶ Ciliate specific scnRNA system [MG04, CMM13].
- ▶ siRNA pathways present in *Paramecium tetaurelia* [GS01, GS02] (and *Tetrahymena thermophila* [CL06, YC05]):
 1. Transgene inducible pathway [GS01].
 2. Exogenous dsRNA inducible pathway (feeding or injection) [GS02].

Transgene pathway

- ▶ Microinjection and transformation of MAC with high-copy transgenes lacking 3' UTR [GS01]
 1. 23nt siRNA generated from transgene transcripts (Dcr1, Rdr2, Rdr) and Cid2) [LNS⁺⁰⁹, MCT⁺¹⁴].
 2. mRNA cleavage (Ptiwi13 and Ptiwi14)

Exogenous dsRNA pathway

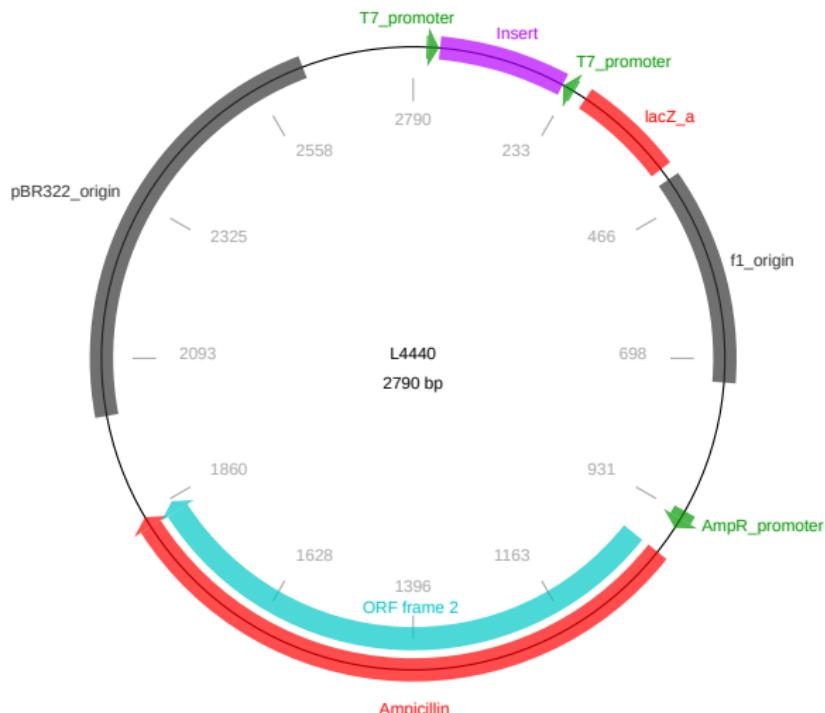


Reproduced from [CGA⁺15]

- ▶ Exogenous dsRNA via feeding (or microinjection) [GS02].
- ▶ 1° siRNA targeted cleavage (Ptwi13) [BGK⁺11].
- ▶ Undefined role in MAC for 2° siRNA (Ptwi12, Ptwi15) [MCT⁺14, CGA⁺15, BGK⁺11]
- ▶ Pds1 involved in uptake of dsRNA from vacuole? [CGA⁺15].
- ▶ Activated at low levels by ssRNA from normal food bacteria [CGA⁺15].

So, can we experimentally induce
RNAi in *P. bursaria*?

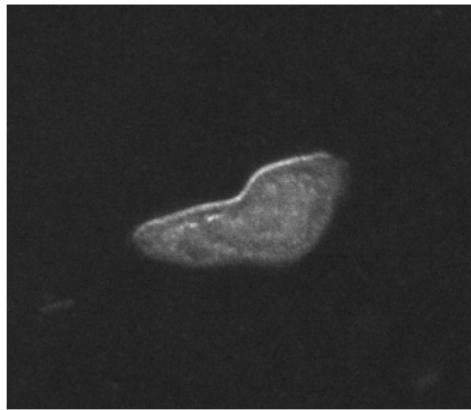
Experimental feeding vector



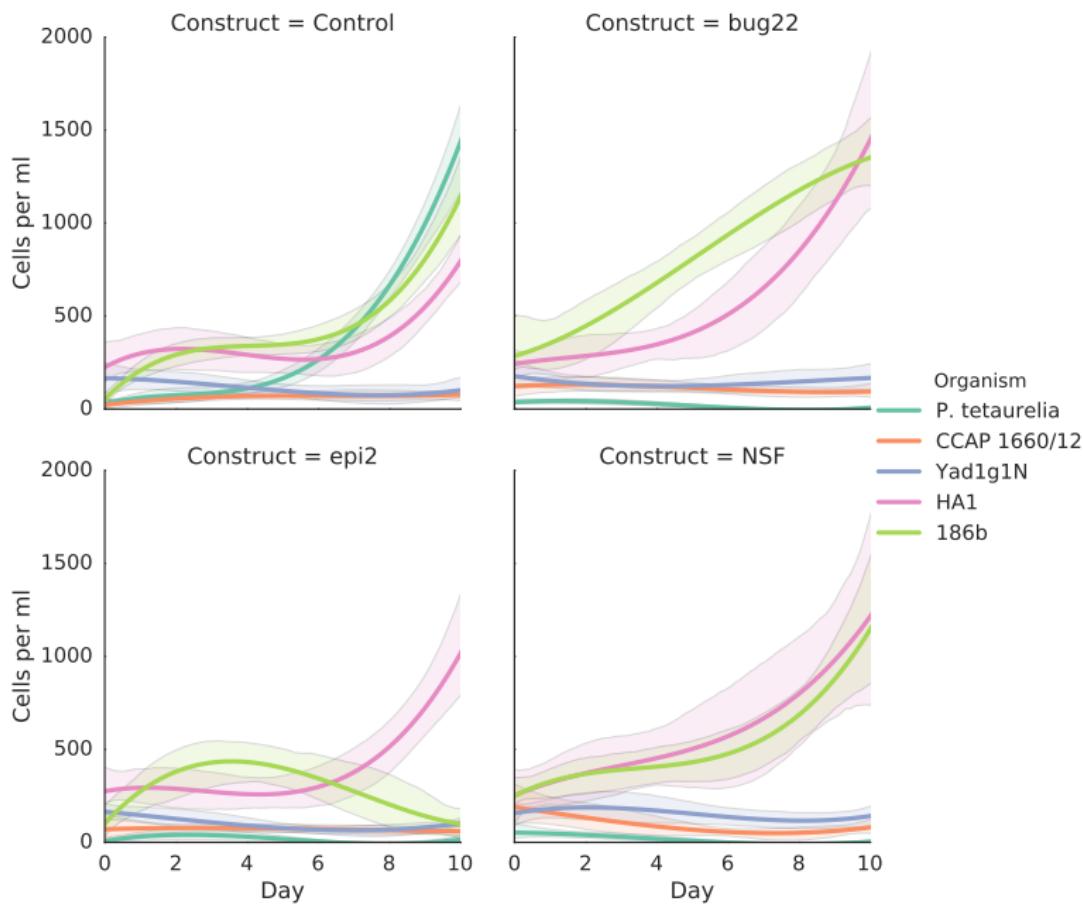
Transformed into *E. coli* with IPTG-inducible T7 polymerase and RNAse III deficiency.

Construct inserts

Gene	Function	RNAi phenotype in <i>P. tetraurelia</i>
<i>epi2</i>	Epiplasmin	"Monstrous" cells
NSF	Membrane fusion factor	Lethal
<i>bug22</i>	Basal body/ciliary protein	Slow swimming and death



RNAi feeding had mixed results

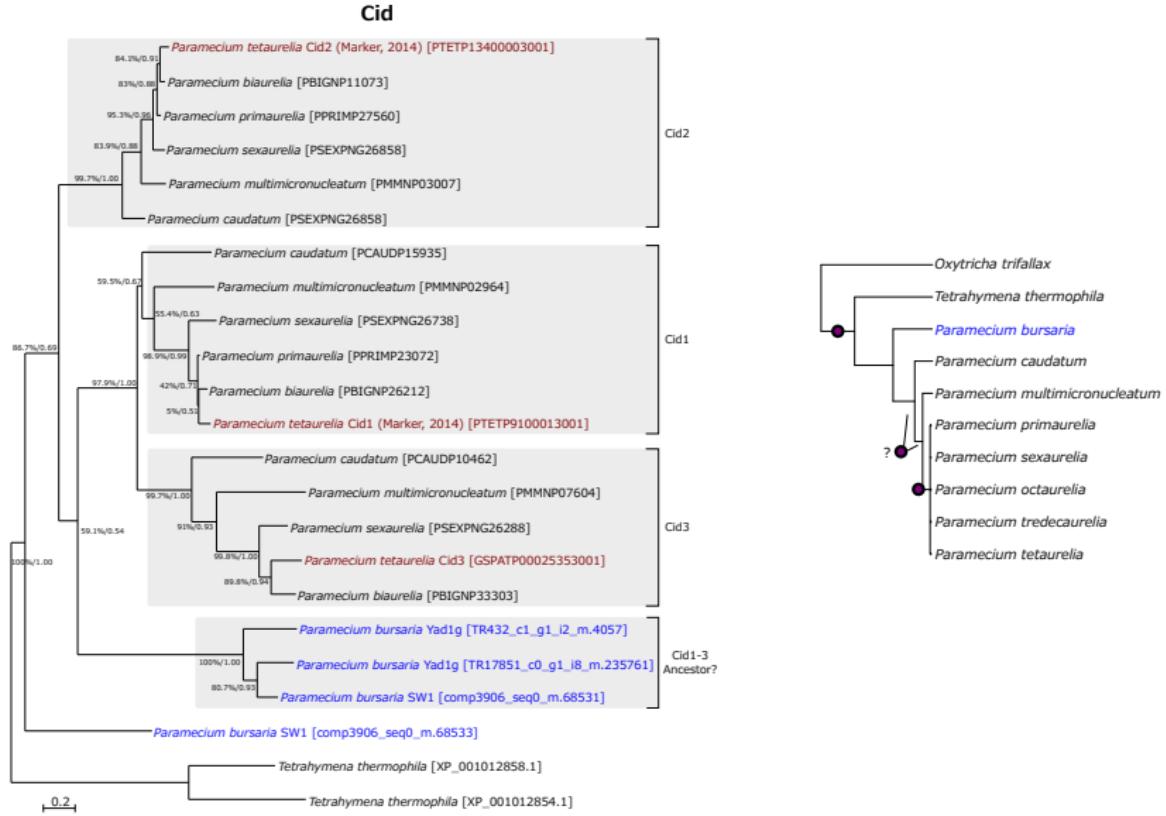


Are all the known RNAi pathway components present in the active transcriptome((s))?

Summary of known RNAi components

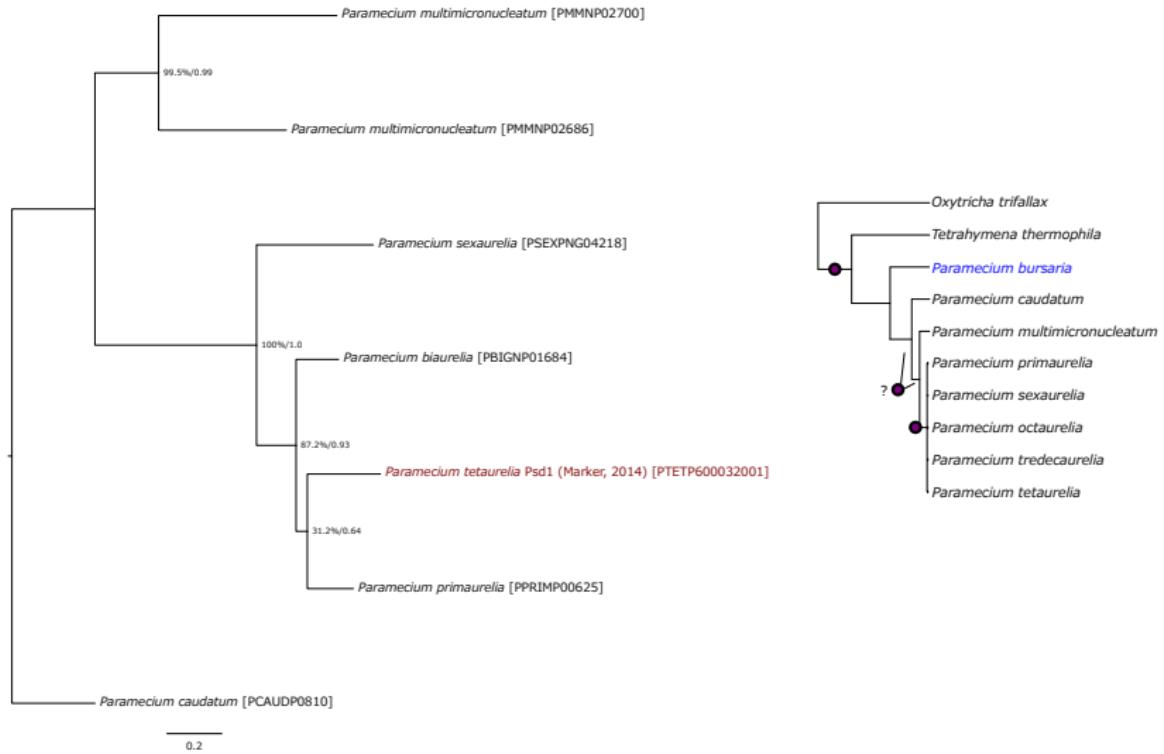
Pathway	Component	Function
transgene-induced siRNA	Rdr3 Ptwi14	RdRP Piwi
both pathways	Rdr2 Dcr1 Ptwi13 Cid2	RdRP Dicer Piwi Nucleotidyl transferase
exogenous dsRNA-induced siRNA	Rdr1 Cid1 Ptwi12 Ptwi15 Pds1	RdRP Nucleotidyl transferase Piwi Piwi Import of dsRNA?

Cid ancestor

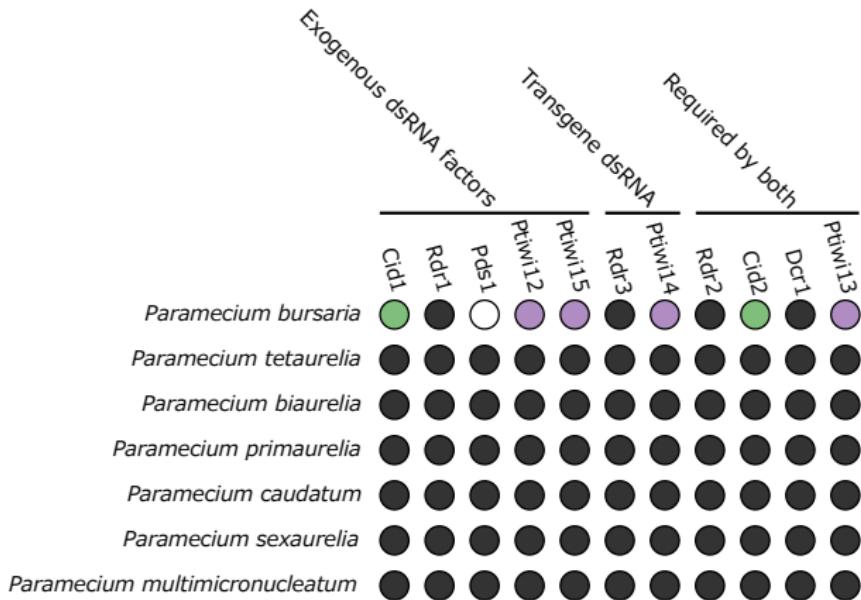


Pds1 absent

Pds1

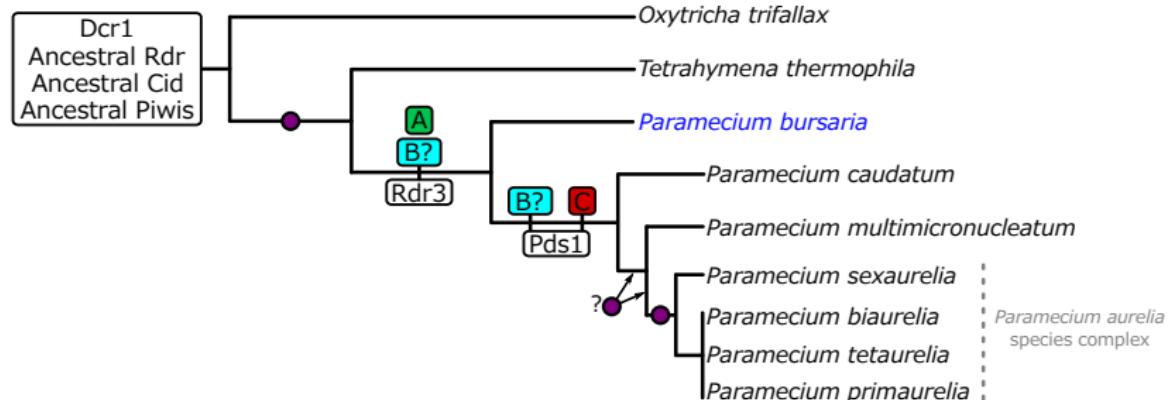


Presence/absence of known pathway components



- Absence of homologue
- Presence of homologue
- Unresolved
- Putative unduplicated ancestral orthologue

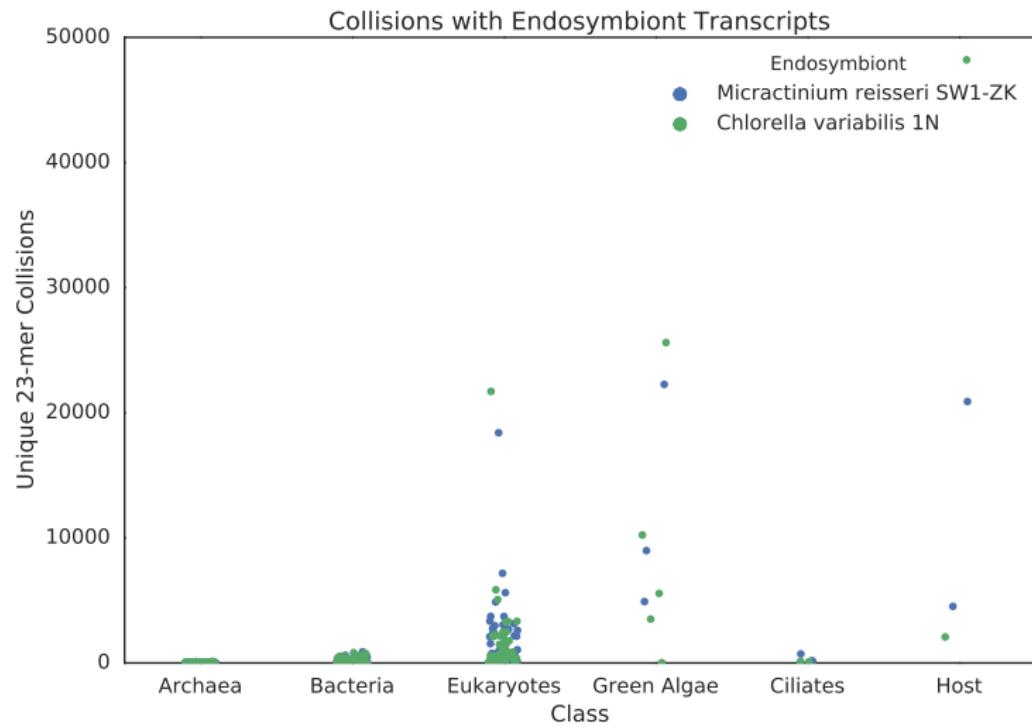
Putative RNAi component evolution scenario



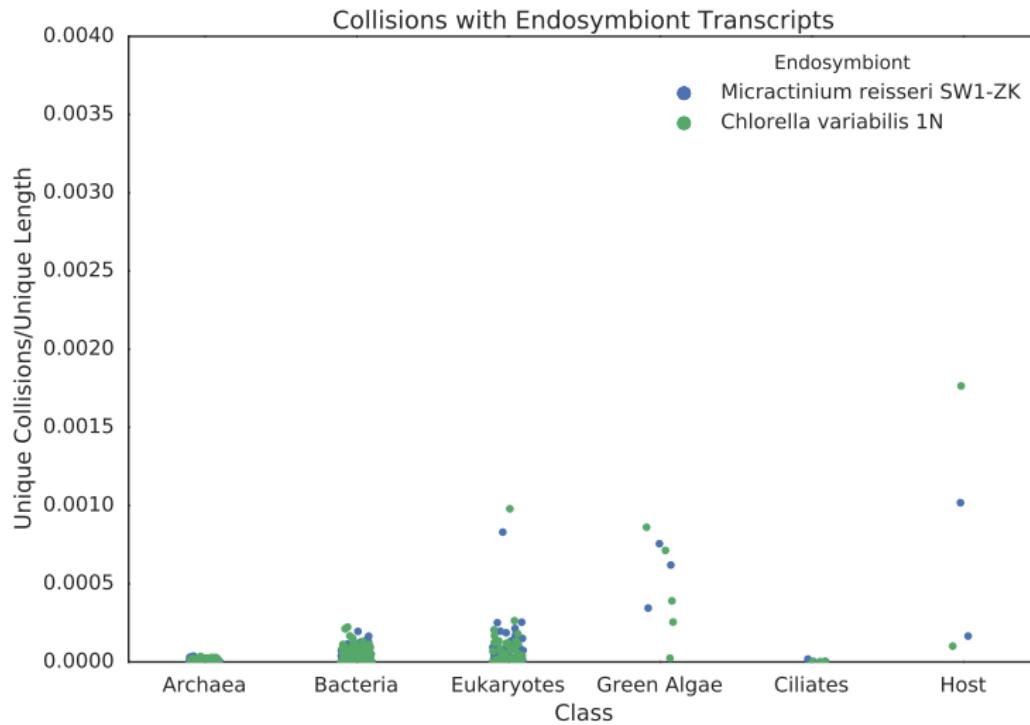
- [A] Duplication of ancestral Rdr into Rdr1 and Rdr2
- [B?] Duplication of ancestral Cid into Cid2 and Cid1-Cid3 ancestor
- [C] Duplication of Cid1-Cid3 ancestor into Cid1 and Cid3
- [●] Whole genome duplication

Could having a eukaryotic endosymbiont and RNAi activated by dsRNA in vacuoles be deleterious?

Higher level of collisions with eukaryotes



Collisions are a function of transcriptome size



Conclusions

- ▶ RNAi phenotypes not inducible in most *P. bursaria* strains via feeding.
- ▶ *P. bursaria* lacks Pds1 (in active transcriptome) thus may be unable to take up RNA from digestive vacuoles.
- ▶ High levels of 23-mer collisions between *P. bursaria* and eukaryotic endosymbiont transcriptomes may lead to deactivation of dsRNA uptake from vacuoles.
- ▶ Presence of other factors in active transcriptomes of *P. bursaria* indicate transgene and microinjected exogenous dsRNA pathways may function.

Acknowledgements

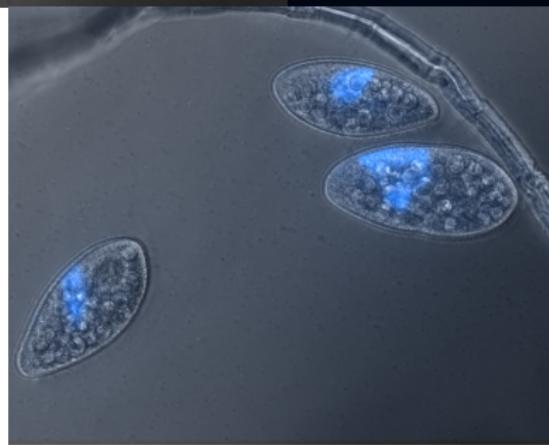
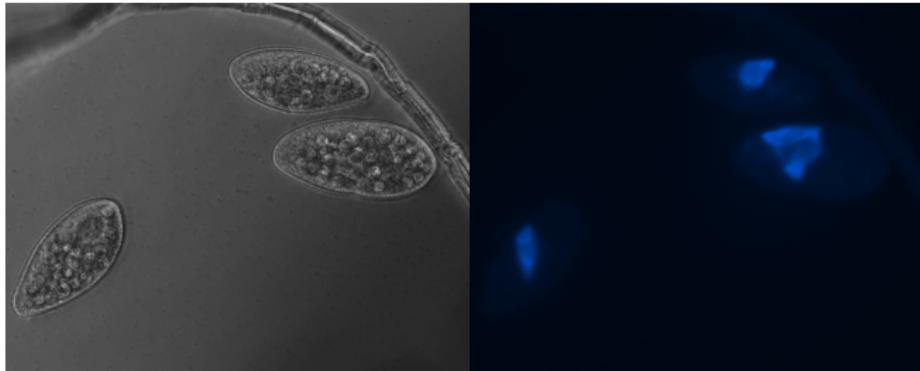
- ▶ Ben Jenkins (feeding experiments)
- ▶ David Milner (labwork)
- ▶ Tom Richards (PI)
- ▶ NHM-UCL PhD Studentship (main funding)



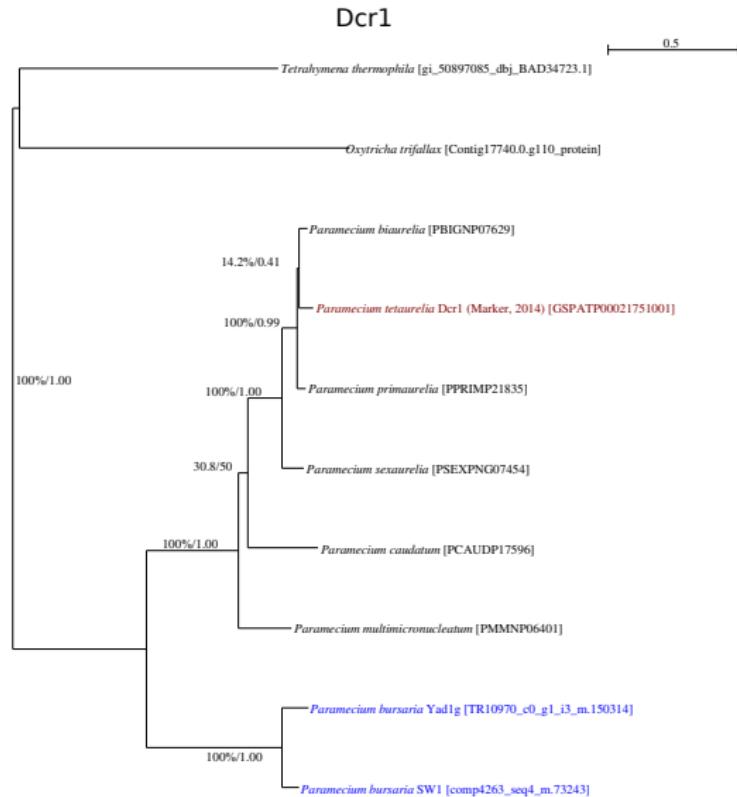
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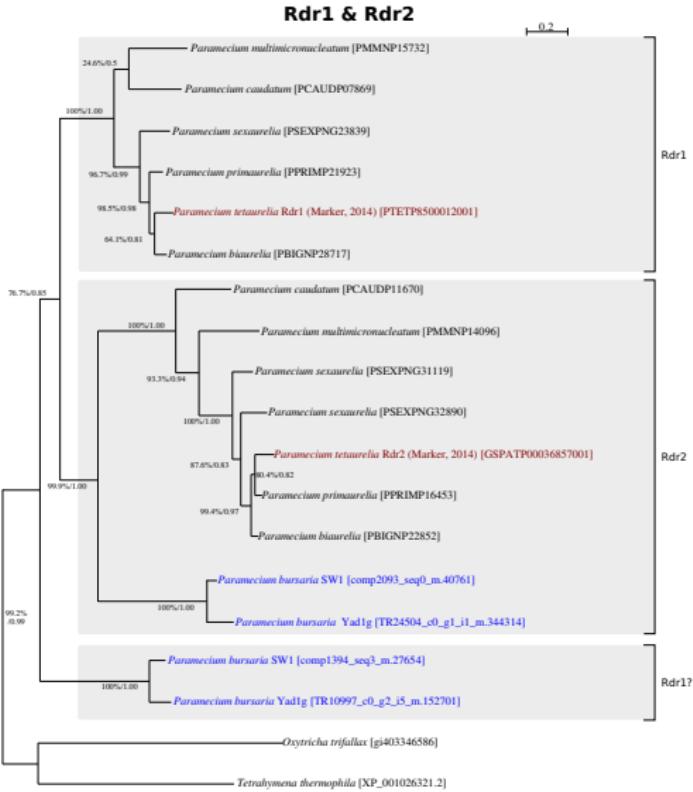
Microinjection proved difficult



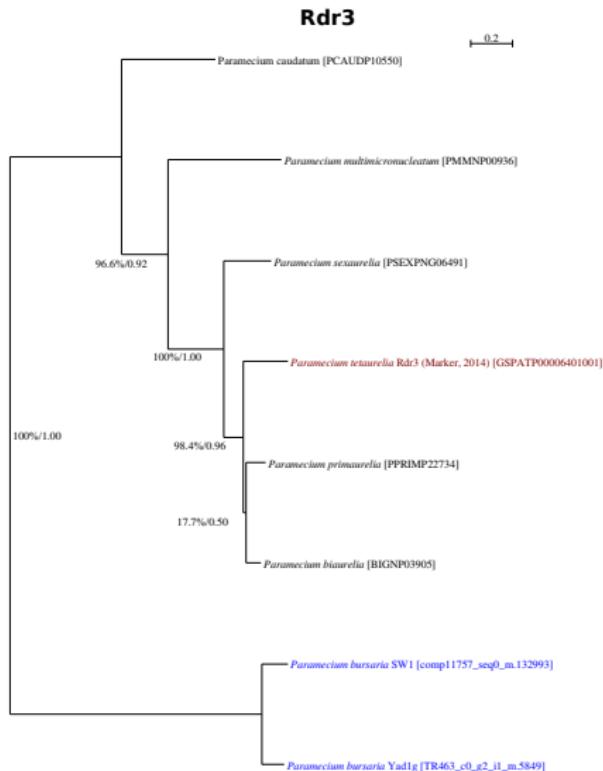
Dcr1



Rdr1 Rdr2



Rdr3



Psd1 Structure

