Development of RNA interference in *Hymenolepis*

Natalia Pouchkina-Stantcheva and Peter D. Olson

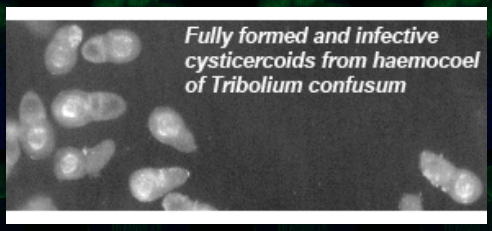
Department of Zoology, The Natural History Museum, London SW7 5BD, UK

e-mail:natap2@nhm.ac.uk

RNAi in tapeworms was first demonstrated in *Monezia* by Pierson et al (2009, IJP) and has been also successful in *Echinococcus* (K. Brehm, pers. comm.). We have recently begun work to develop RNAi in the model tapeworm *Hymenolepis microstoma* aimed at characterizing loss-of-function phenotypes of Hox and other genes. In particular we are interested in arbitrating between cause and consequence for genes exhibiting segmental expression patterns such as *Post-2* (a homolog of *abdominal-B*). So far, we have tested the RNAi pathway and demonstrated knock down of Hox *Post-2* transcripts by soaking of cysticercoids (an encysted larval form of the worm, derived from the haemocoel of intermediate host *Tribolium confusum*) in Post-2 dsRNA. Real-time PCR analysis revealed RNAi induced knock down up to 60%. We are currently working on culturing dsRNA treated and non-treated larvae in vitro in order to monitor associated changes in morphology. At present we have successfully cultured larvae from encysted cysticercoids to non-strobilate juvenile worms for ~7-10 days. We are working towards full development in vitro.

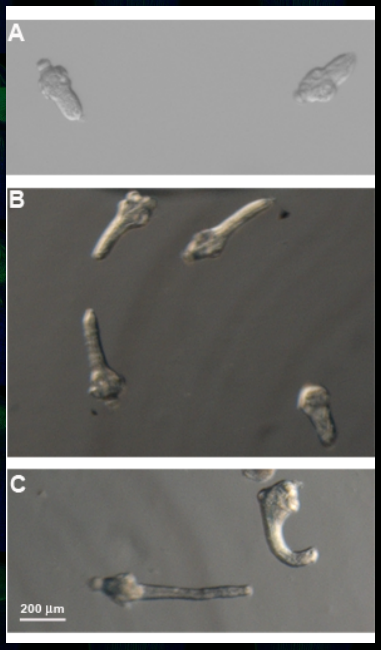
In vitro Cultivation

- Isolation of patent cysticercoids from beetles (from day 7 post-infection onwards)



- Excystment of cysticercods in 1% pepsin and 1% HCl solution
- Trypsin pretreatment, series of washes
- Cultivation in diphasic culture media (blood agar/CMRL supplemented with antibiotics, bile salts, FBS and hemin)

Monitoring Development *in vitro*



18 hrs in culture
Rostellum exposed
Good motility

48 hrs in culture
Increased motility
Increase in length (up to 1.5x compared to 18hr culture)
Excretory ducts developed

144 hrs in culture
Increase in size up to 2 times compared to 18 hrs culture

Development of RNAi

Cultivation of *H. microstoma* excysted cysticeroids in diphasic culture allows their development to non-strobilate juvenile worms in 7 days, thus already providing us with opportunity to test RNAi in vitro

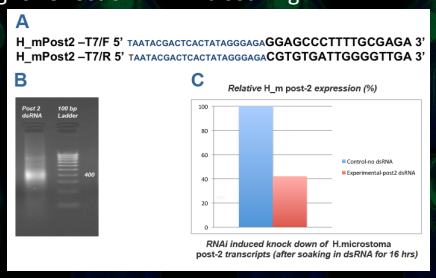
Preliminary transcriptome analysis demonstrated presence of RNAi machinery in *H. microstoma*:

Putative RNAi pathway sequences in *Hymenolepis microstoma* (*Hymenolepis* transcriptome data in collaboration with Matt Berriman et al, Sanger Institute, Hinxton, Cambridge, UK)

RNAi pathway	*H. microstoma* putative orthologs (unpublished)	Function	Identity with *Schistosoma mansoni* (%)
Endoribonuclease			
Dicer	>aby 176464	Processing of miRNA precursor	60% | EF204544.1| S.mansoni dicer mRNA
Drosha	>aby 14668	Processing of primary miRNA transcripts	71% | Smp_142510.2 (SmDrosha2)
Component of RISC			
Argonaute like	>aby 67247	Short RNA binding	73% | Smp_140010 (SmAgo1)

Current Work

- Generation of dsRNA populations for genes of interest (eg. Hox genes, Wnt pathway genes, Pax6, Piwi, etc)
- Testing for effect of RNAi via soaking



A. Design of gene specific primers with added T7 promoter sequences, PCR, cloning, plasmid template preparation
B. Preparation of double-stranded RNA (MEGAscript RNAi Kit, Ambion)
C. Measuring gene expression levels via Real-time PCR after soaking of excysted cysticercoids in culture media with or without addition of dsRNA

Future Work

- Observing RNAi associated changes in morphology
- Whole mount in situ hybridization on control and experimental samples with gene specific riboprobes

Acknowledgements

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