Tail loss: investigating the Molgula

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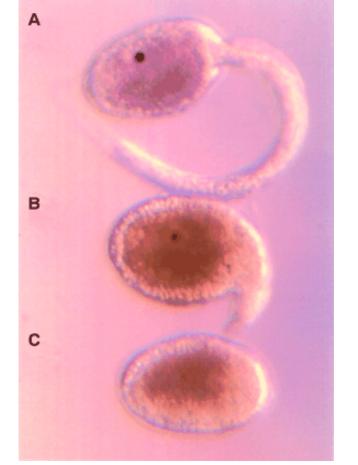
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Arend Hintze Jason Pell Rosangela Canino-Koning



National Institute of Food and

Two Species of Closely Related Ascidians and a Hybrid Larva



Molgula oculata has a head, tail, and otolith - chordate embryo.

M. occulta egg X M. oculata sperm has a head, short tail, and otolith.

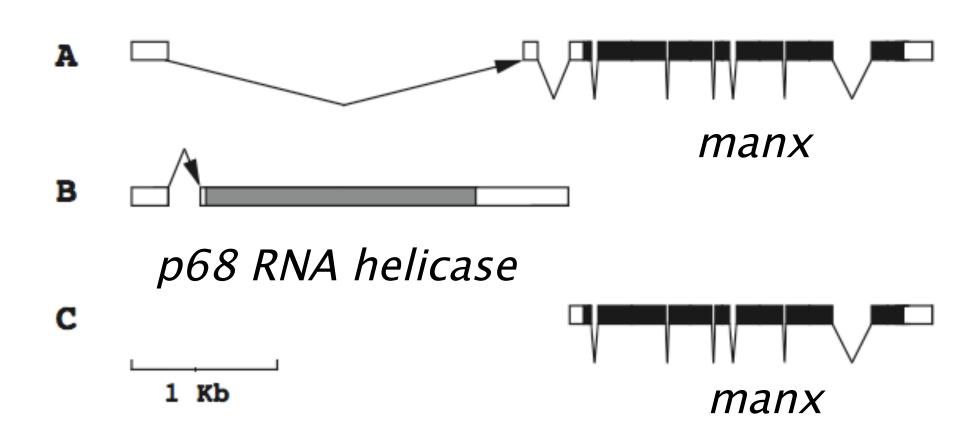
Molgula occulta lacks chordate features - head, tail, and otolith. Is the transcriptome assembly any good?

- No reference genome! ...
- ▶ 96/98 Sanger-sequenced Molgulid genes found in assembly of individual lanes.

MDSSNRSHPNAHLQYHTDYNYPPFRRVMLAAVKEGLYHPRLPSLRRMDMDTATHKLPDEH 60 MDSSNRSHPNAHLQYHTDYNYPPFR+VMLAAVKEGLYHPRLPSLRRMDMDTATHKLPDEH Sbjct: 50 MDSSNRSHPNAHLQYHTDYNYPPFRKVMLAAVKEGLYHPRLPSLRRMDMDTATHKLPDEH 229

Can we distinguish & count parent transcripts in the hybrid?

manx and p68/bobcat probable isoform variation

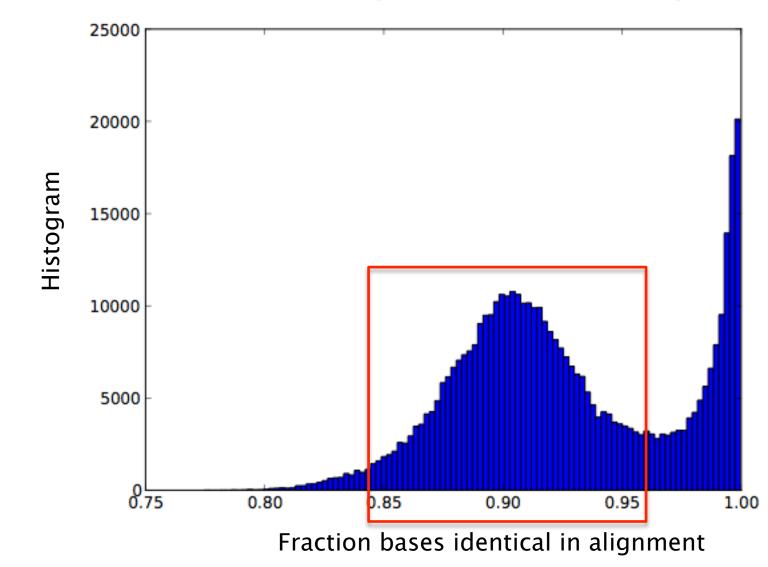


Swalla et al. (1999) *Development* 126:1643-1653

→occ/gastrula

---hyb/gastrula

A large subset of transcripts have variation ~10% (at DNA level)



Hybrids express *only* M. occulta (tailless) versions??

Sample Name	M. oculata manx	M. occult manx		
M. oculata (gastrula)	9	0	11	0
Hybrid (gastrula)	0	28	0	255
M. occulta (gastrula)	0	41	0	76
M. oculata (neurula)	5	0	4	1
Hybrid (neurula)	0	38	0	223
M. oculata (tailbud)	6	0	6	0
Hybrid (tailbud)	0	8	0	121

M. occulta (tailless) *bobcat* gene – differential expression by exon

position in locus

Expression analysis - how?

Preliminary round of sequencing

Molgulids have many anural embryos - why??

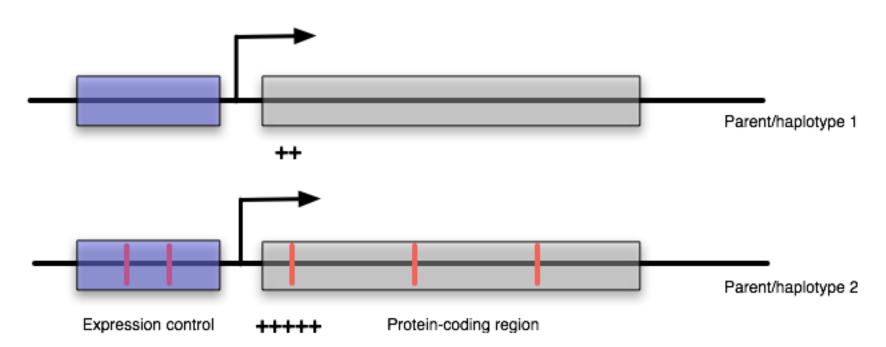
(Ascidians generally: ~1% of 3,000 are tailless)

M. occulta and M. oculata genes assemble together as splice variants

Sample Name	Total reads after trim+filter	Loci (total genes, > 500)	Total incl splice variants (> 500)
M. oculata	25 252 225	10.170	10.000
(gastrula) Hybrid	35,252,607	13,172	16,269
(gastrula)	38,690,601	14,148	24,209
M. occulta			
(gastrula) M. oculata	22,548,831	8,046	10,802
(neurula)	38,030,938	10,365	11,043
Hybrid (neurula)	38,699,913	14,400	29,189
M. oculata			
(tailbud)	38,073,640	14,204	17,835
Hybrid (tailbud)	34,307,907	15,399	26,594

Allele specific expression

Detect haplotype-specific changes in gene expression as indicator of functional cisvariation.



Decompose sequence into k-mers (k \sim 32); count; take median k-mer Counts: 0,0,0,0,0,1,1,1,1,2,2,3,3,3,5,5,6,6,7,8,8,9,10,100,10000,100000

median count: 5

