# I have my notochord but where did I leave my tail?

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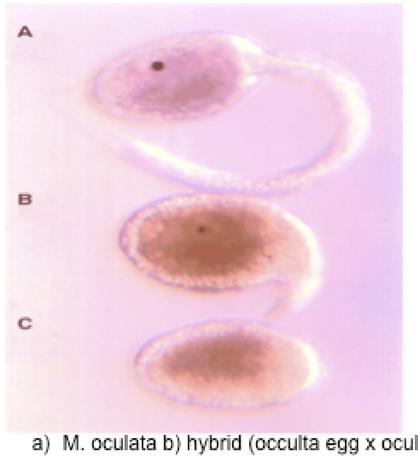
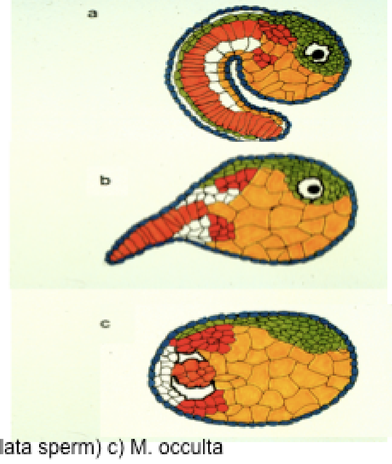
## Abstract

In the molgulid clade of ascidians several species have individually evolved tail loss during their larval stage of development. The notochord, one of the key features of chordates, is found in the center of the tail in most ascidian larvae. In almost all solitary ascidians, including *M. oculata*, 40 notochord cells have been found extended in the tail. However, in a closely related species, *M. occulta* (tailless), only 20 notochord cells were found, and these express *brachyury*. These two species are only 3% divergent and are able to cross hybridize. Some of the hybrid also have 20 notochord cells, however the notochord cells extend and converge in a shortened version of *M. oculata* tail. Through the use of high throughput sequencing technologies and subsequent allelotyping analysis, we are able to quantitatively investigate the express of genes associated with notochord development in the parent species and in the hybrid. Our transcriptome assemblies are freely available for download; contact ctb@msu.edu. We thank the BEACON Center and USDA NIFA for funding.

## Molgula species studied



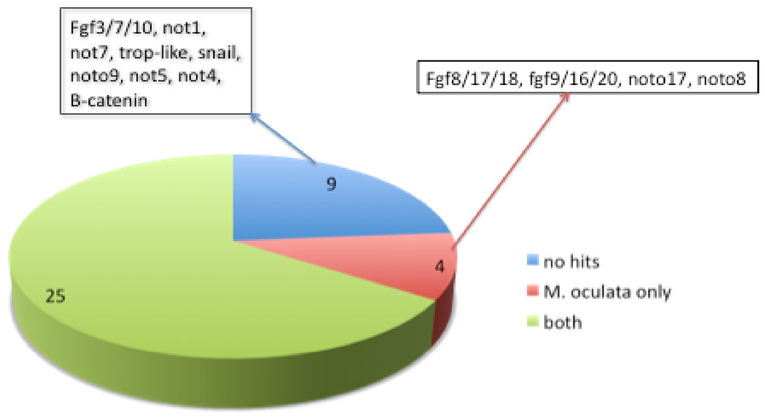

a) M. oculata b) hybrid (occulta egg x oculata sperm) c) M. occulta
Notochord cells in orange

Swalla, B. et al. Science, Vol 274, Issue 5290, 1205-1208 , 15 November 1996

## Expression profiles

| Gene | Locus | M. oculata (gast.) | Hybrid (gast.) | M. occulta (neur.) | M. oculata (neur.) | Hybrid (neur.) | M. oculata (tailbud) | Hybrid (tailbud) |
|---|---|---|---|---|---|---|---|---|
| **ATP sulfurylase/APS kinase [Ciona intestinalis]** | **occulta** | **0** | **0** | **7** | **0** | **0** | **0** | **0** |
| ATP sulfurylase/APS kinase [Ciona intestinalis] | oculata | 17 | 0 | 0 | 13 | 0 | 9 | 0 |
| ATP sulfurylase/APS kinase [Ciona intestinalis] | oculata | 117 | 0 | 0 | 103 | 0 | 225 | 104 |
| leprecan [Ciona intestinalis] | oculata | 55 | 0 | 0 | 75 | 0 | 73 | 64 |
| leprecan [Ciona intestinalis] | oculata | 33 | 0 | 0 | 40 | 0 | 67 | 29 |
| **leprecan [Ciona intestinalis]** | **occulta** | **0** | **23** | **23** | **0** | **28** | **0** | **36** |
| transcription factor protein foxa-a [Ciona intestinalis] | oculata | 37 | 34 | 0 | 43 | 17 | 199 | 141 |
| **transcription factor protein foxa-a [Ciona intestinalis]** | **occulta** | **0** | **3** | **110** | **0** | **63** | **0** | **0** |
| **merlin [Molgula tectiformis]** | **occulta** | **0** | **5** | **16** | **0** | **20** | **0** | **10** |
| **merlin [Molgula tectiformis]** | **occulta** | **0** | **23** | **29** | **0** | **6** | **0** | **18** |
| merlin [Molgula tectiformis] | oculata | 9 | 0 | 0 | 7 | 0 | 13 | 0 |
| merlin [Molgula tectiformis] | oculata | 19 | 0 | 0 | 86 | 0 | 41 | 0 |
| fibroblast growth factor 8/17/18 [Ciona intestinalis] | oculata | 4 | 0 | 0 | 1 | 0 | 20 | 0 |
| fibroblast growth factor 9/16/20 [Ciona intestinalis] | oculata | 12 | 0 | 0 | 12 | 0 | 49 | 0 |
| noto17 [Molgula tectiformis] | oculata | 24 | 0 | 0 | 34 | 0 | 127 | 0 |
| noto17 [Molgula tectiformis] | oculata | 5 | 0 | 0 | 8 | 0 | 39 | 0 |
| **noto6 [Molgula tectiformis]** | **occulta** | **0** | **11** | **11** | **0** | **30** | **0** | **29** |
| noto6 [Molgula tectiformis] | oculata | 10 | 0 | 0 | 18 | 0 | 3 | 0 |
| noto6 [Molgula tectiformis] | oculata | 8 | 0 | 0 | 15 | 0 | 12 | 0 |
| prickle 1 [Ciona intestinalis] | **occulta** | 0 | 193 | 88 | 0 | 90 | 0 | 57 |
| prickle 1 [Ciona intestinalis] | oculata | 56 | 0 | 0 | 50 | 0 | 126 | 0 |
| prickle 1 [Ciona intestinalis] | oculata | 19 | 0 | 0 | 23 | 0 | 17 | 0 |

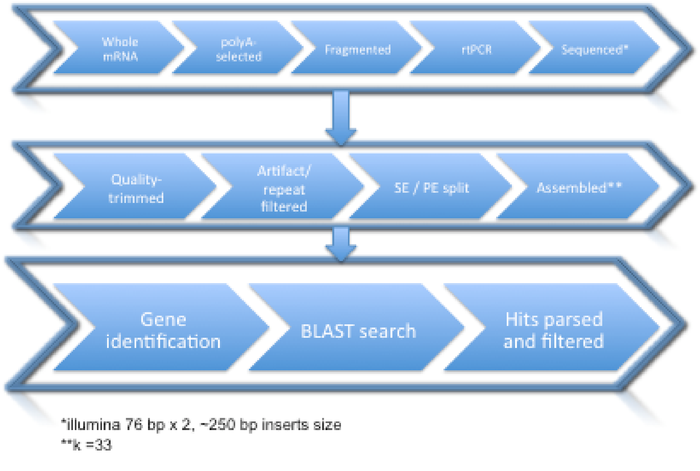
## BLAST hits




## Discussion

The notochord is important for tail formation. We examined 38 genes associated with notochord development. Of 38 selected, all but 9 were found in either *M. oculata* or *M. occulta*. 25 of the remaining 29 were found in both species, while 4 were found only in *M. oculata*. In the future we plan to confirm gene presence by in situ, increase our query gene set, and examine more time points in *M. occulta.*

## Method




*illumina 76 bp x 2, ~250 bp inserts size
**k =33

## Gene list

- 706e(ki-3)
- ATPcitrate-lyase
- ATPsulfurylase.APSkinase
- Notch
- Mt-merlin
- Mt-noto17
- Not1
- Not2
- Not3
- Not4
- Not5
- Not6
- Not7
- Notch
- beta-catenin
- beta4galactosyltransferase
- Brachyury
- col1
- netrin
- cdc45
- ezrin.radixin.moesin(ERM)-like protein
- fibrinogen-like protein
- Fibroblastgrowthfactor3/7/10/22
- Fibroblastgrowthfactor8/17/18
- Fibroblastgrowthfactor9/16/20
- leprecan
- Mt-noto6
- noto8
- noto9
- pellino
- prickle1
- rel1
- snail
- Suppressor of hairless homolog
- foxa-a
- foxd
- tropomyosin-like protein
- tyrosinephosphatase

## Key work cited

- Kugler et al. 2011. Evolutionary changes in the notochord genetic toolkit: a comparative analysis of notochord genes in the ascidian Ciona and the larvacean Oikopleura. BMC Evolutionary Biology 11:21
- Imai et at. 2004. Gene expression profiles of transcription factors and signaling molecules in the ascidian embryo: towards a comprehensive understanding of gene networks. Development 131, 4047-4058
- Hotta et al. 2008. Brachyury-downstream gen sets in a chordate, Ciona intestinalis: integrating notochord specification, morphogenesis and chordate evolution. Evolution and Development 2008, 10:1, 37-51

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