Investigating the roles of the canonical Wnt and Notch signaling pathways in establishment of the tardigrade anteroposterior axis

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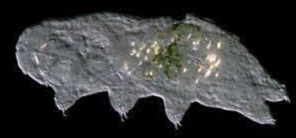
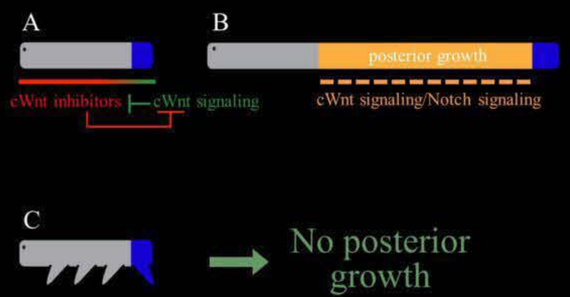


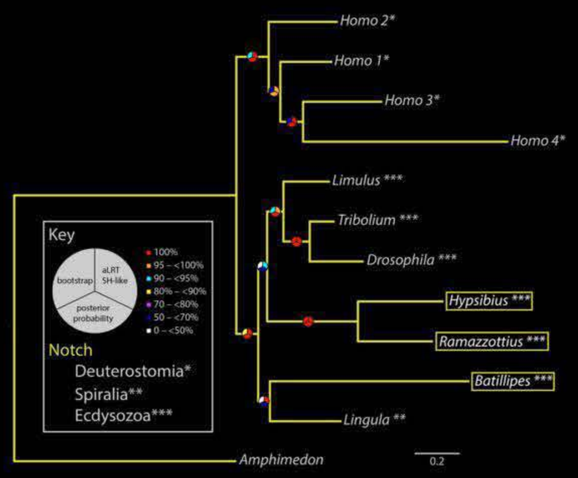
Introduction: Canonical Wnt (cWnt) signaling establishes the A/P axis in many animals (A). Later, the cWnt pathway interacts with the Notch signaling pathway to regulate posterior growth (B). Tardigrades lack posterior growth (C) but retain posterior identity.



Aim 1: Determine whether cWnt signaling establishes the highly compact A/P axis in tardigrades.

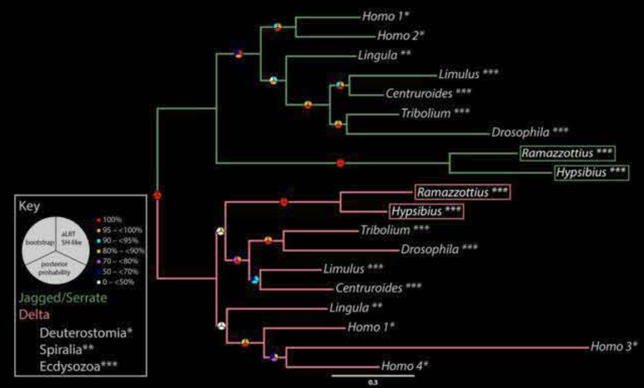
Aim 2: Determine the role of the Notch signaling pathway during establishment of the A/P axis in tardigrades.

Part I: Tardigrades retain the receptor component of the canonical Notch signaling pathway.



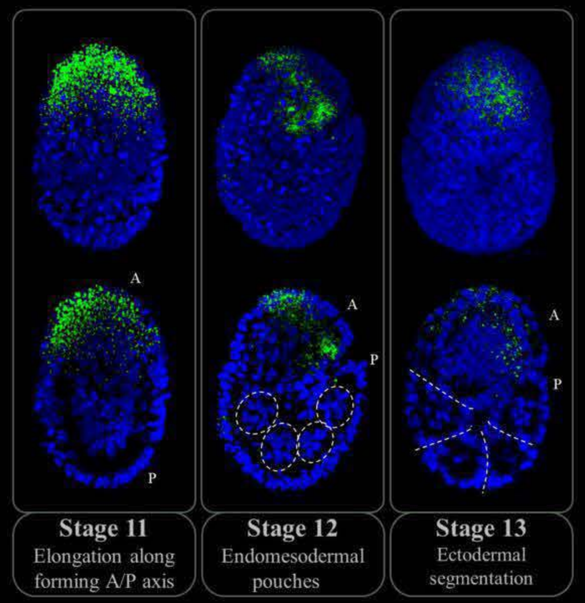
Tardigrade genes are boxed.
Spiralian, ecdysozoan, and deuterostome genes are included.
Bootstrap support out of 500 replicates.

Part II: Tardigrades have both DSL class ligands.



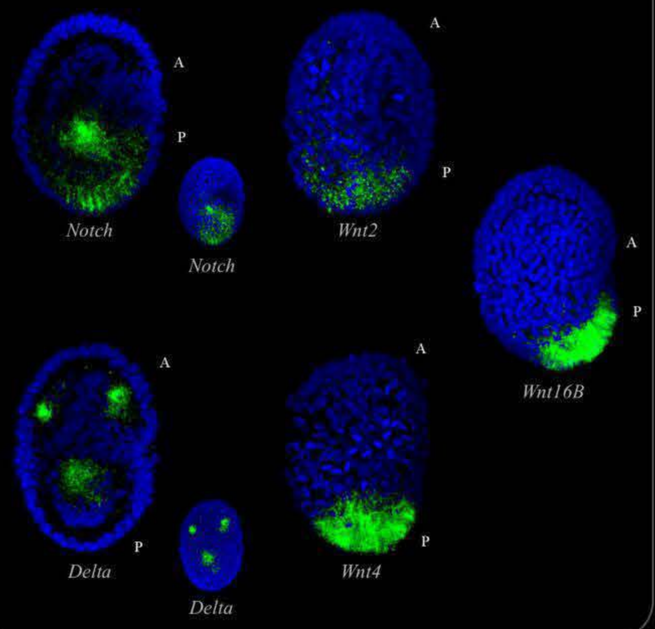
Tardigrade genes are boxed.
Spiralian, ecdysozoan, and deuterostome genes are included.
Bootstrap support out of 500 replicates.

Part III: Establishment of anteroposterior axis during early tardigrade development.



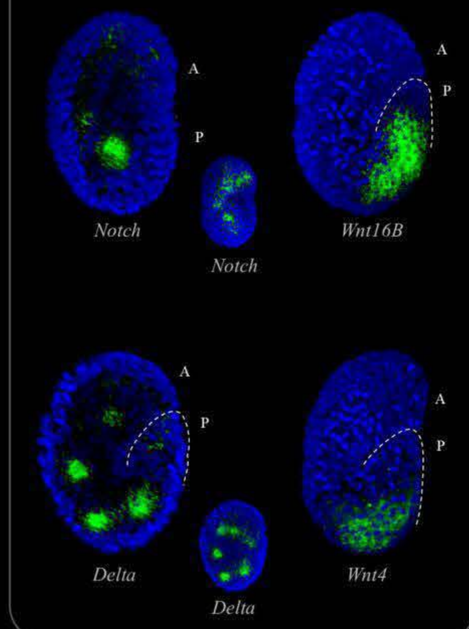
Six3 (green) as a genetic marker for anterior identity. DAPI (blue). Stage 11 embryos are dorsoventrally mounted. Stage 12 and stage 13 embryos are laterally mounted and facing right. Top row is whole mount; bottom row is longitudinal section of same embryo. A, anterior; P, posterior.

Part IV: Stage 11 Elongation.



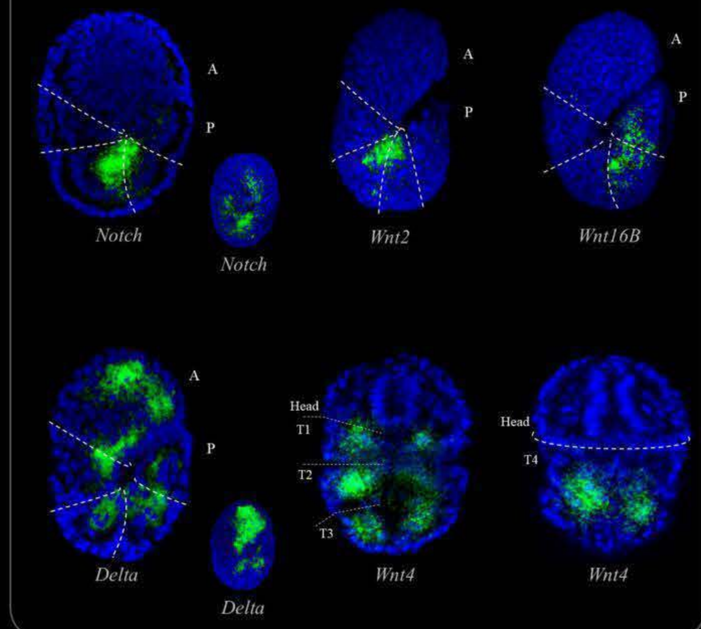
Notch and *Wnt16B* embryos are laterally mounted and facing right; the rest are dorsoventrally mounted. Whole mount embryo for *Notch* and *Delta* are shown as smaller images. In situ stain (green). DAPI (blue). A, anterior; P, posterior.

Part V: Stage 12 Endomesodermal Pouches.



Embryos are laterally mounted and facing right. Posterior tip is outlined. No *Wnt2* expression at this stage. In situ stain (green). DAPI (blue). A, anterior; P, posterior.

Part VI: Stage 13 Ectodermal Segmentation.



Wnt4 embryo is dorsoventrally mounted; the rest are laterally mounted and facing right. Segment boundaries are outlined. In situ stain (green). DAPI (blue). A, anterior; P, posterior; T1-T4, trunk segment 1-4.

Conclusions

- Tardigrades retain core components of the canonical Notch signaling pathway.
- *Delta*/*Notch* expression patterns are predominantly around an endomesodermal region during establishment of A/P axis.
- *Wnt2*, *Wnt4*, and *Wnt16B* are restricted to posterior regions during the earliest stage of A/P axis establishment.
- This suggests that the compact body plan of tardigrades evolved by conservation of the A/P axis establishment function of the cWnt signaling pathway, with loss of the later acting posterior growth functions of the cWnt and Notch signaling pathways.

Future Directions

- Perform gene knockdown experiments on the cWnt and Notch signaling pathways to identify their functional roles in Tardigrada.

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