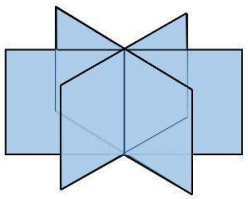
Process as Connectivity

Models of Interaction in Cellular Systems

Dr. Bradly Alicea (commentary from Dr. Eric Deeds)
Featuring contributions by Drs. Stephen Larson, Richard Gordon, and Tom Portegys, and Mr. Arnab Banerjee and Robert Stone



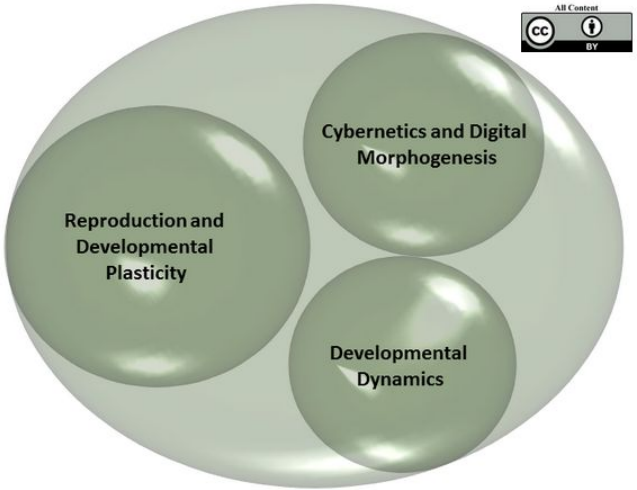
Orthogonal Research and Teaching Laboratory Champaign-Urbana

http://orthogonal-research.weebly.com

DevoWorm Group

http://devoworm.weebly.com

Developing the nematode (*C. elegans*) and other creatures using simulation, analysis, visualization.



Building the World's First Digital Organism

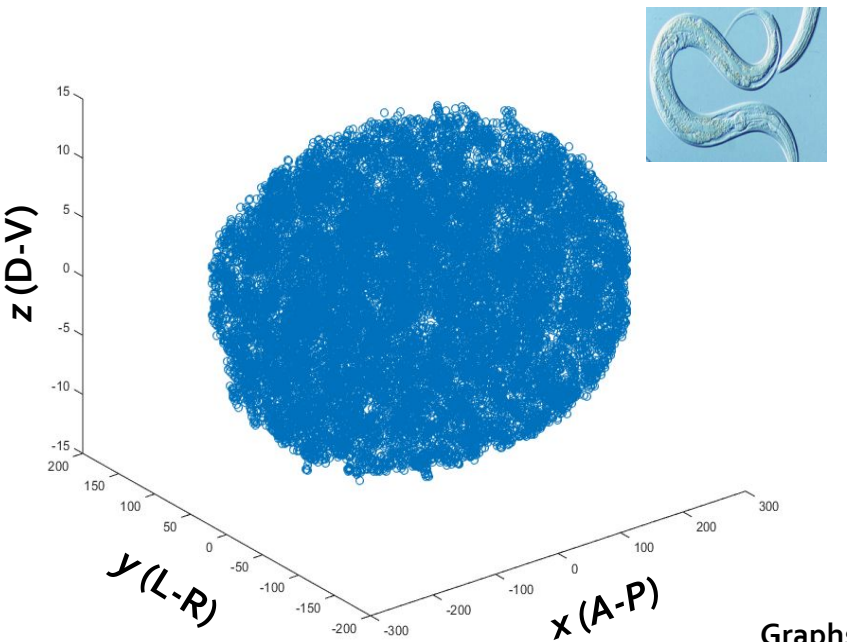
http://openworm.org

Openly-available Primary, Secondary, and Tertiary Datasets
devoworm.github.io/devozoo.htm

Segmented microscopy data, public repositories, literature mining.

Counterclockwise from bottom left (all images computational centroids):

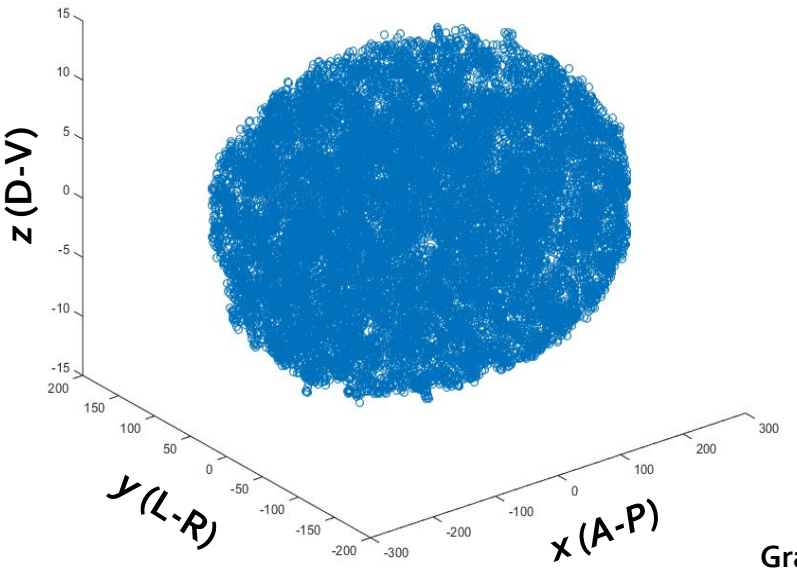
- *C. elegans*, cell nuclei pre-hatch (Bao et.al).

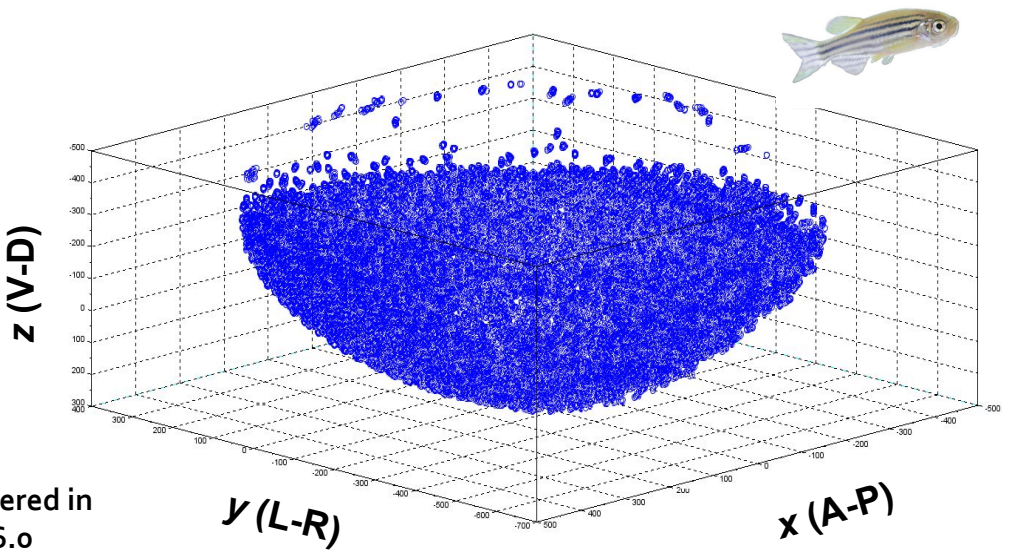


Graphs rendered in SciLab 6.0

Counterclockwise from bottom left (all images computational centroids):

- *C. elegans*, cell nuclei pre-hatch (Bao et.al).
- *Zebrafish*, nuclei during mid- to late-gastrula period (Keller et.al).





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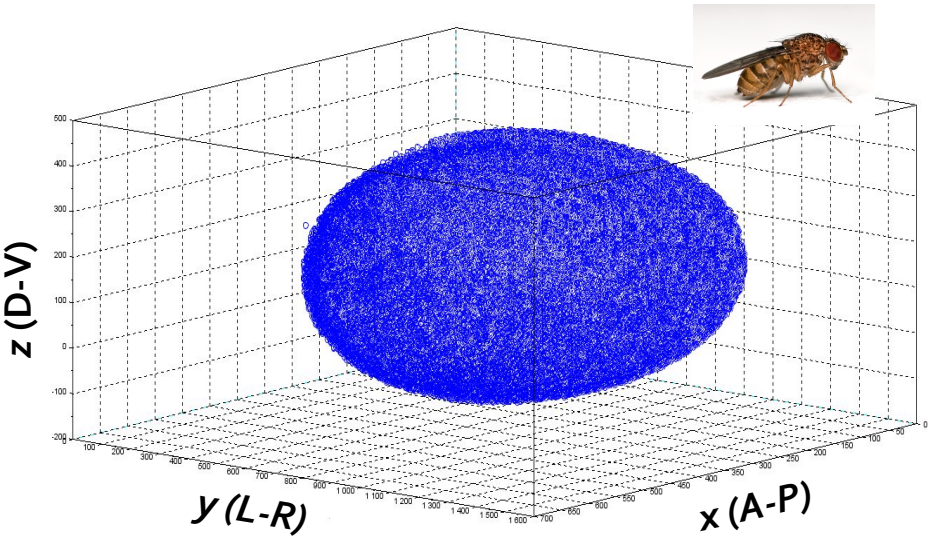
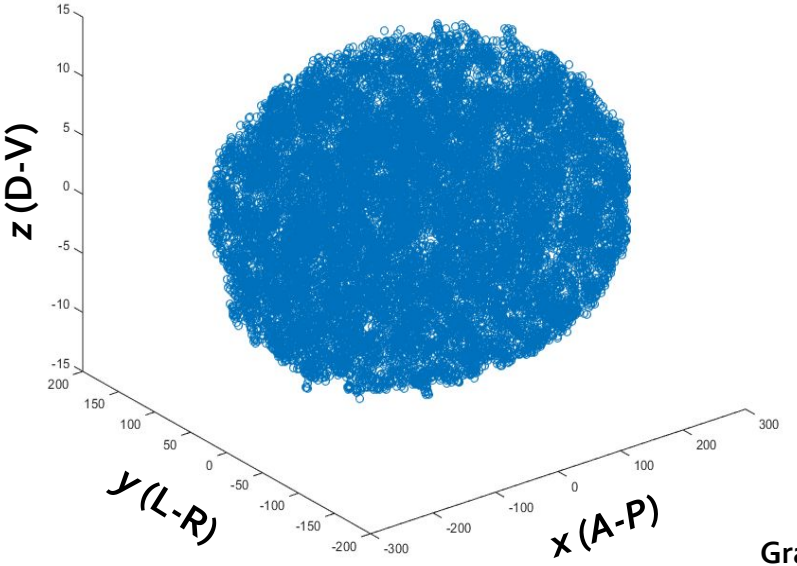
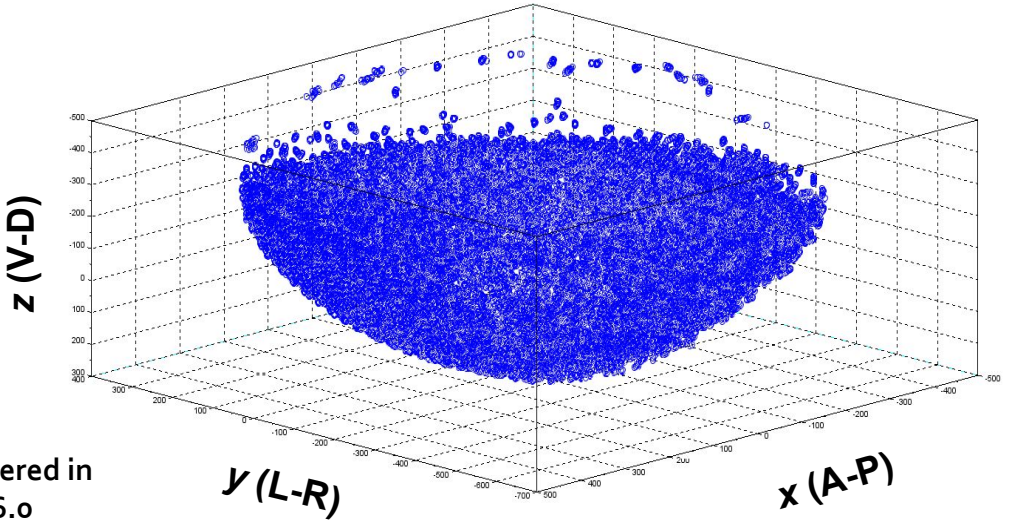
Counterclockwise from bottom left (all images computational centroids):

- *C. elegans*, cell nuclei pre-hatch (Bao et.al).
- *Zebrafish*, nuclei during mid- to late-gastrula period (Keller et.al).
- *Drosophila*, fused nuclei during cleavage (Keller et.al).

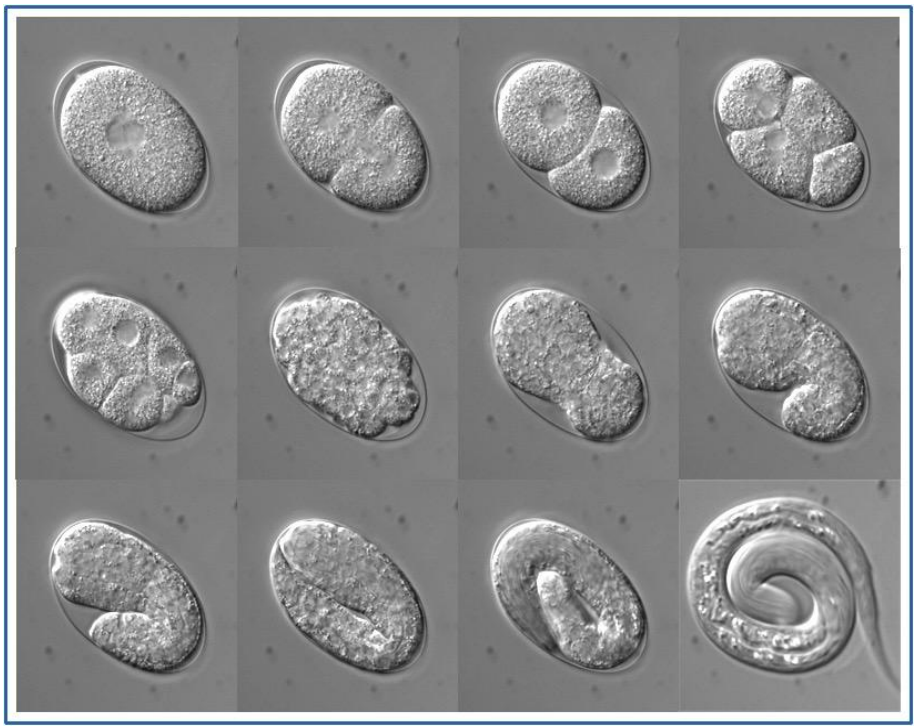






Graphs rendered in SciLab 6.0

Motivating Questions



COURTESY: Chin-Sang Lab, Queens University, Canada

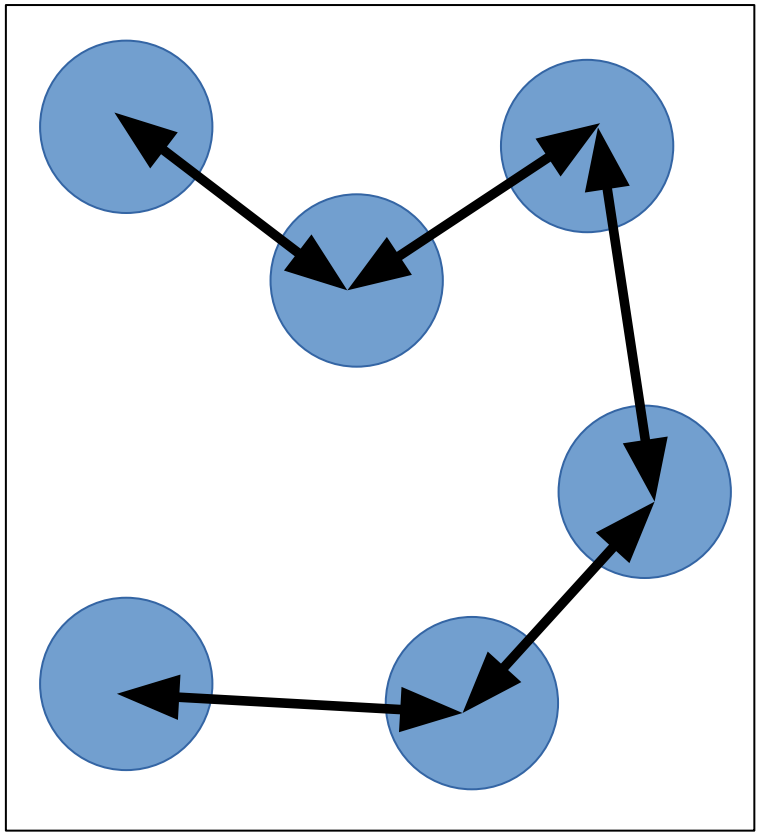
How does the embryo go from a spherical shape to an asymmetrical shape?

Are differentiation process a series of measurable transitions (e.g. symmetry-breaking)?

How can we characterize structural order over time (quantitatively)?

How does this relate to the connectome and adult phenotype?

Embryo Networks



x,y,z,t,θ

3-D space

time and context

x,y,z,t,θ

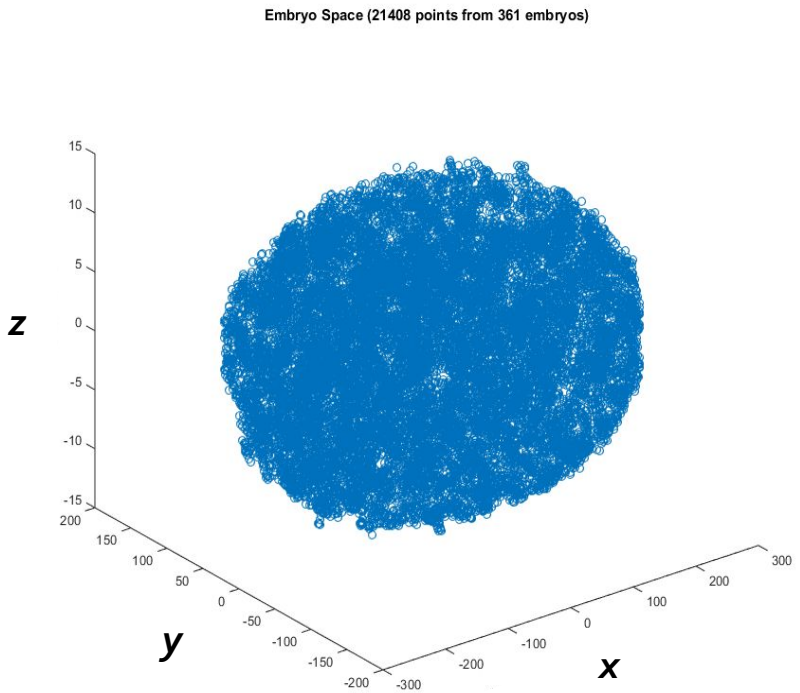
See our Jupyter Notebook for more information

GitHub

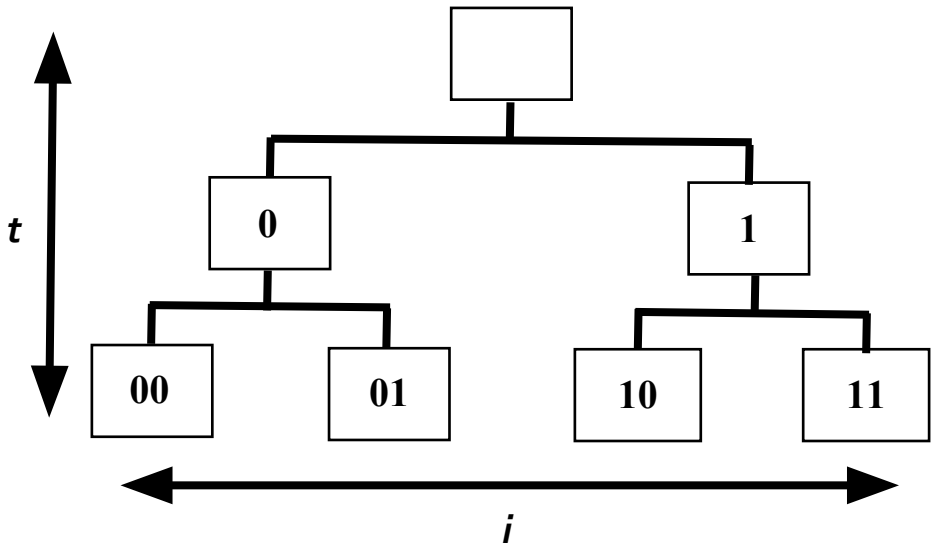
https://devoworm.github.io/

5-dimensional Data Structure

x, y, z, t, i

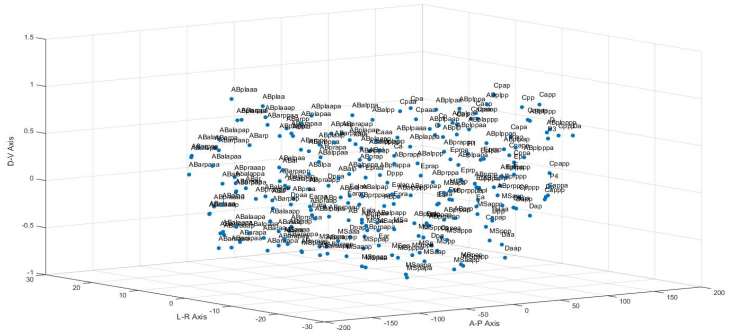


A generalized parameter space based on observations across *C. elegans* embryos (*x,y,z*)



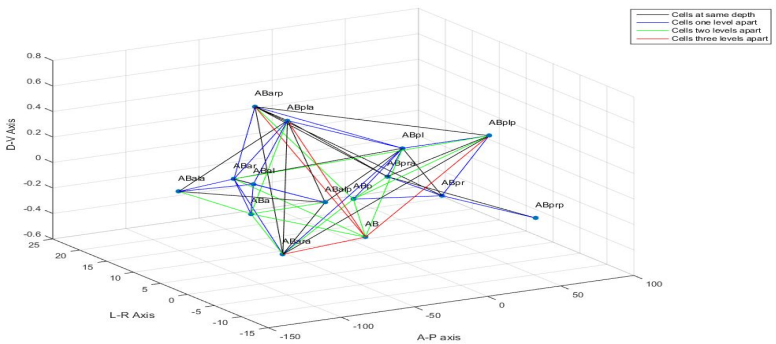
A spatially-independent parameter space ordered by A-P axial order (*i*) and lineage time (*t,i*)

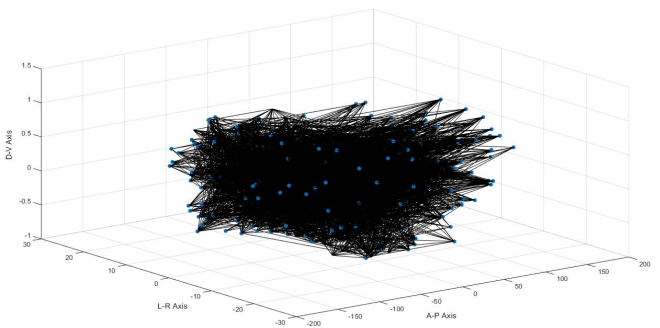
“The Worm and the Embryogenetic Hairball”



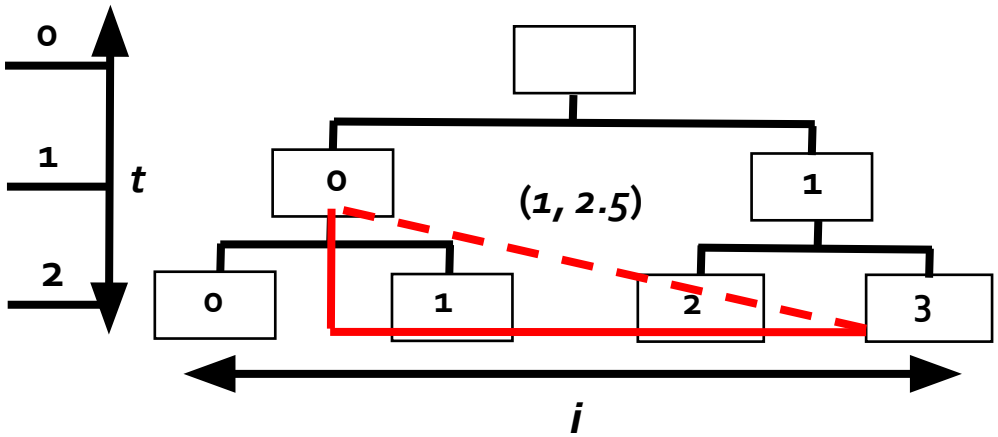
3-D spatial information can be translated to a series of Euclidean distances.

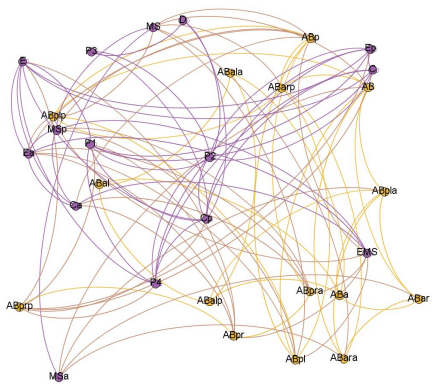
- distances can be used to establish “spheres of signaling influence” for a given cell.
- examine this interactome (analogous to paracrine signaling) at different spatial scales.





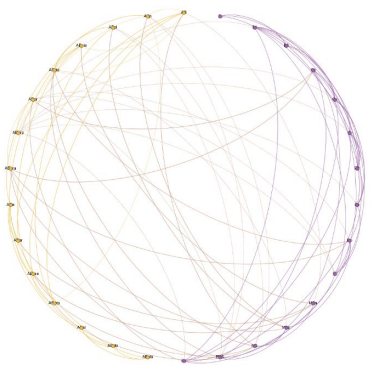
Correcting topology for effects of cell lineage (factor in a two-dimensional measure of distance). Factor of (*t,i*)



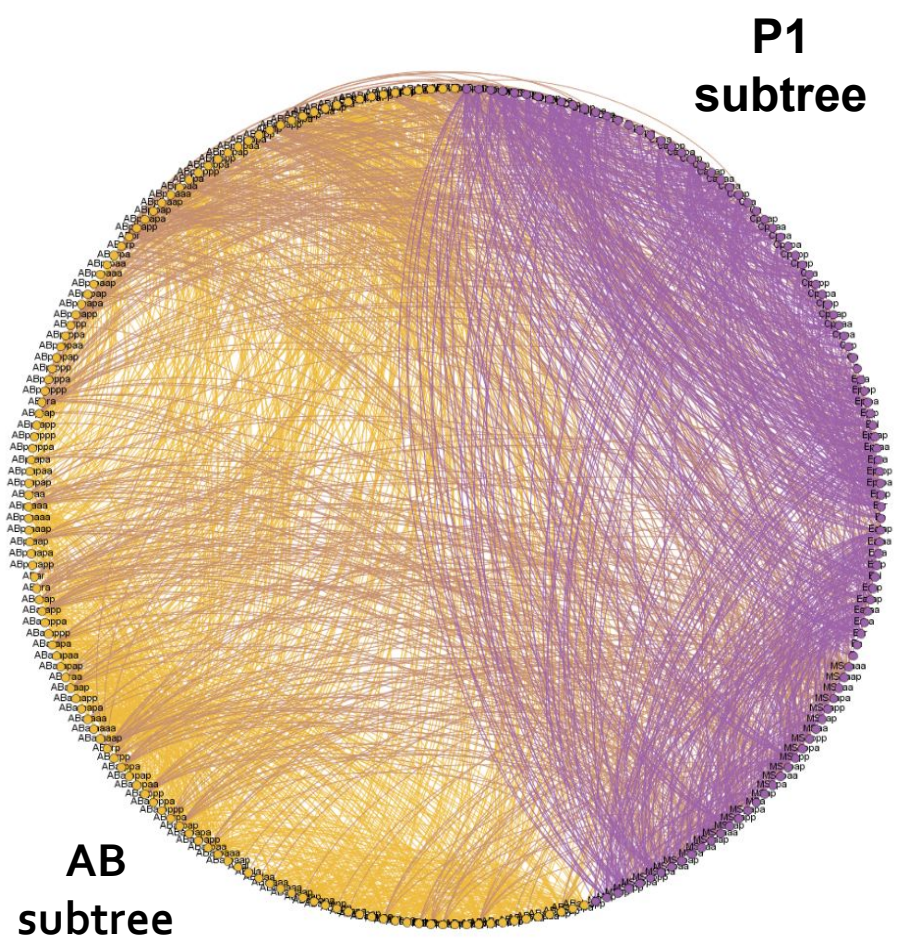


Uncorrected topology (n=30), threshold of 0.85

Lineage tree (*t,i* only; n=30), threshold of 0.85



Network based on *x,y,z,t,i* only (n=30), threshold of 0.85



Pairwise network that includes all cells from 3-layer tree to 8-layer tree (N = 224).

Distance threshold of 0.25 (all cells within 25% the maximum distance in embryo structure).

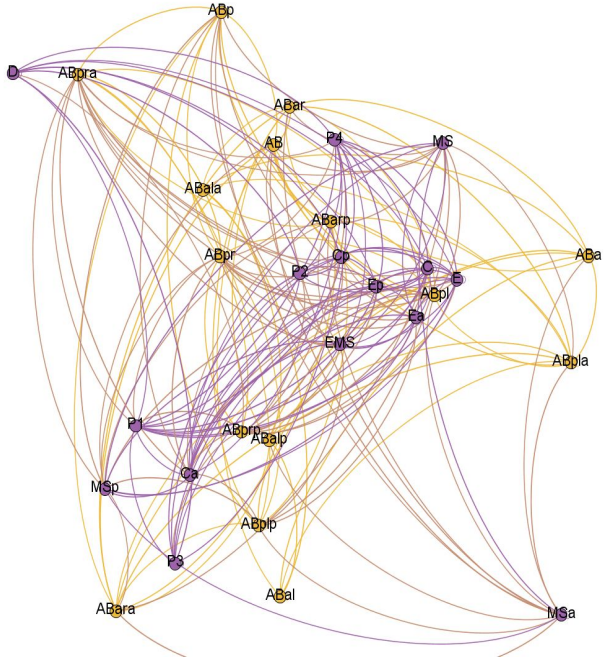
PeerJ Preprints

Alicea and Gordon,
doi:10.7287/peerj.preprints.26587

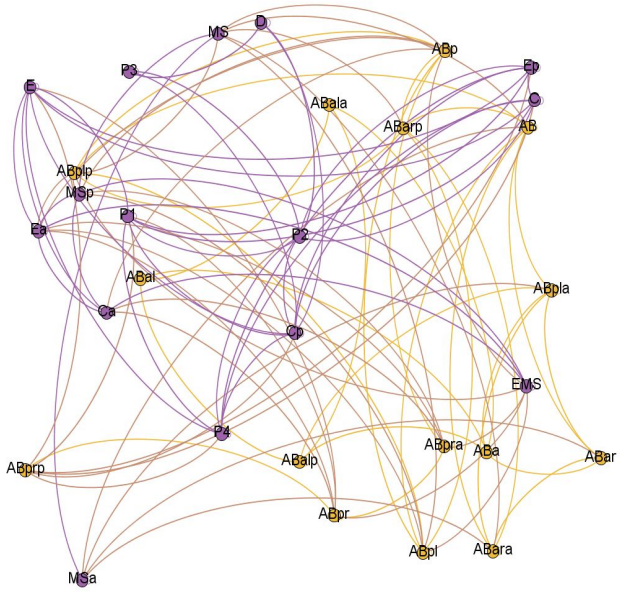
Open Science Framework

"Embryo Networks" repository,
https://osf.io/q9jvb/

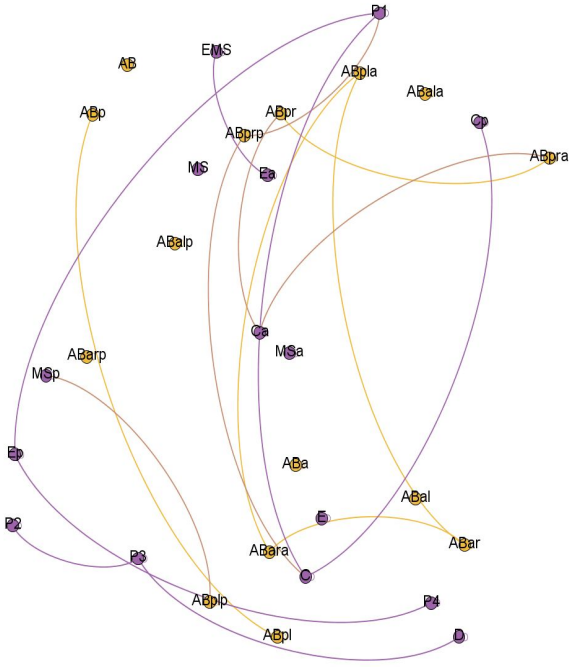
Cells in AB sublineage in yellow, cells in P1 sublineage in purple. Graphs rendered in Gephi v0.9.0



Hairball for 16 terminal-cell condition, threshold of 0.25.



Hairball for 16 terminal-cell condition, threshold of 0.15.

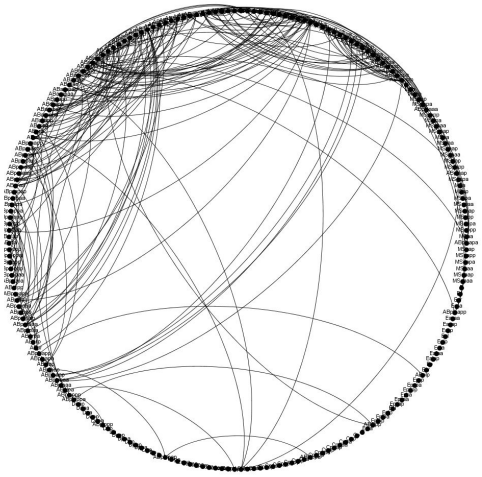


Hairball for 16 terminal-cell condition, threshold of 0.05.

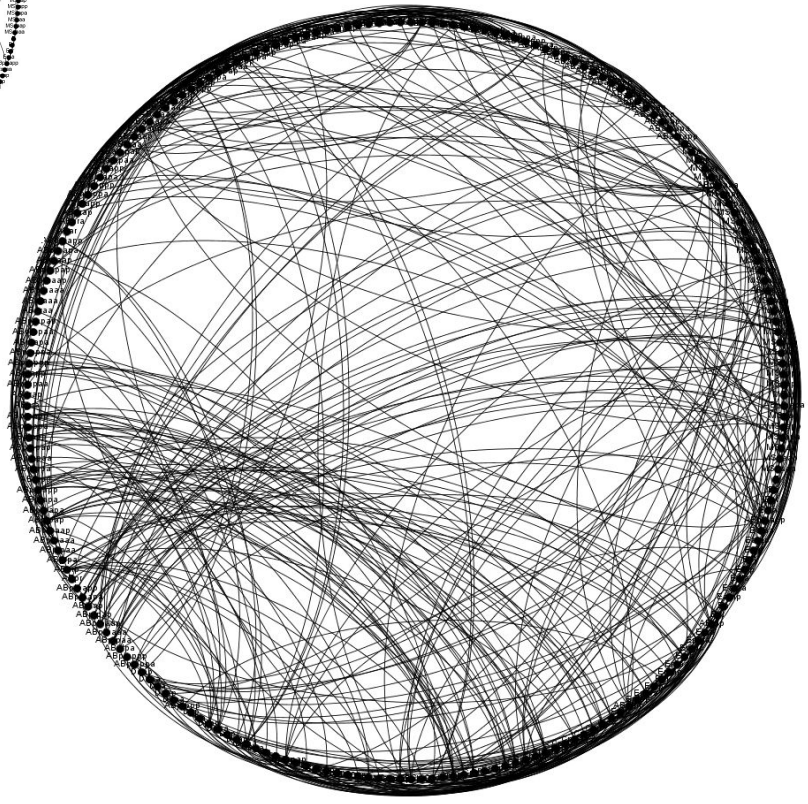
Increasing number of interconnections, AB-P1 bipartite modularity lost as distance threshold increases.

How does the global structure of an embryo network compare to random?

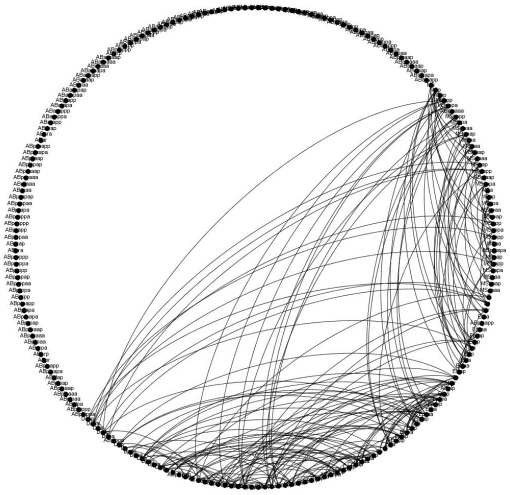
Stobb et.al, Graph Theoretical Model of a Sensorimotor Connectome in Zebrafish. PLoS One, 7(5), e37292.



Within AB sublineage



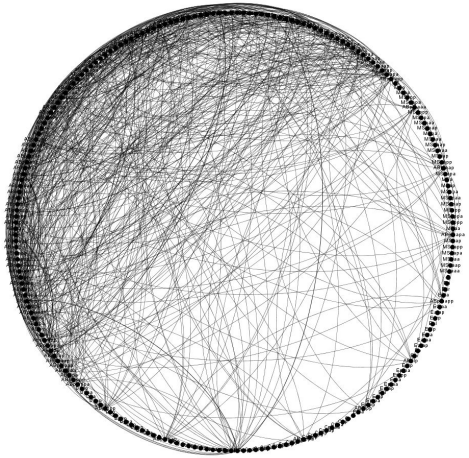
Within P1 sublineage



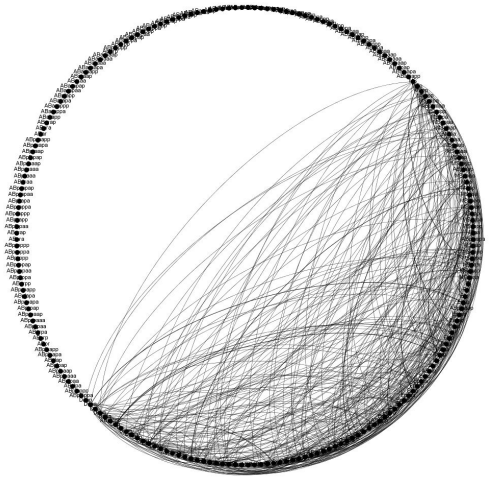
C. elegans

128-cell embryo,

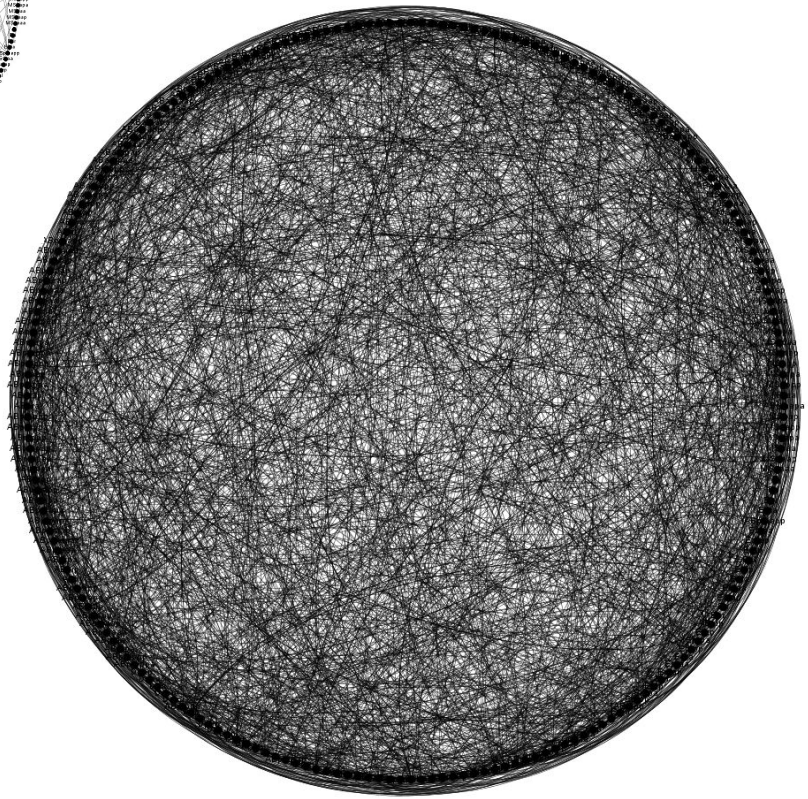
threshold = 0.05



Within AB sublineage



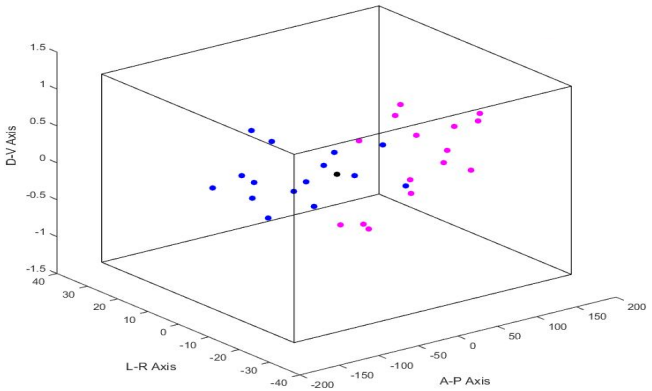
Within P1 sublineage



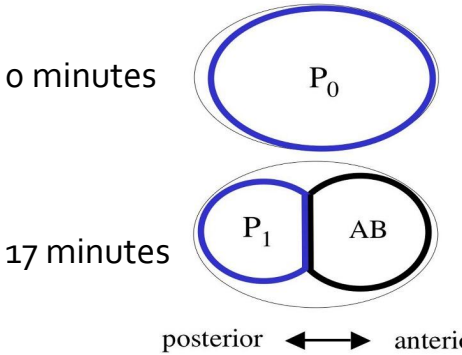
Random

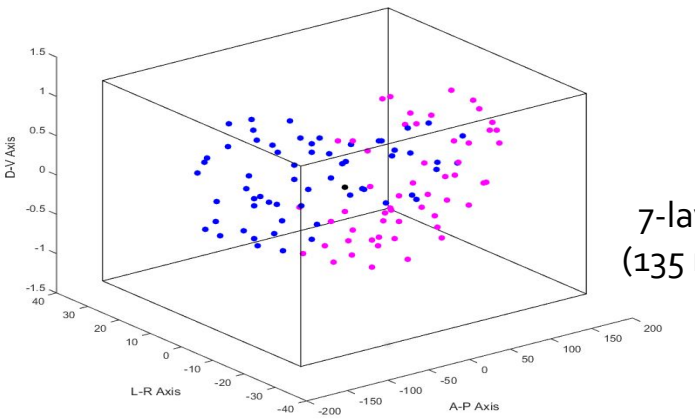
based on *C. elegans* 128-cell embryo,

threshold = 0.05

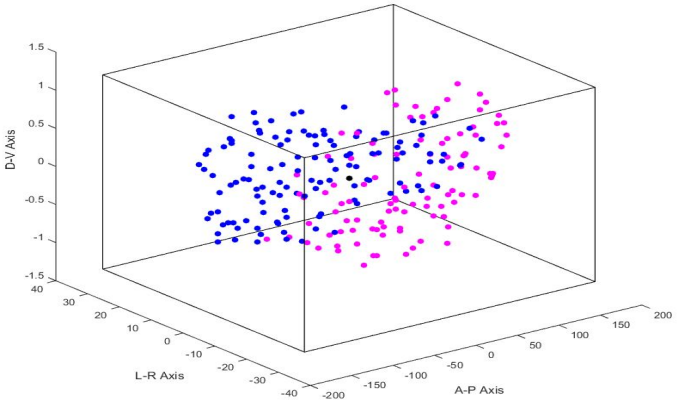


5-layer tree
(75 minutes)





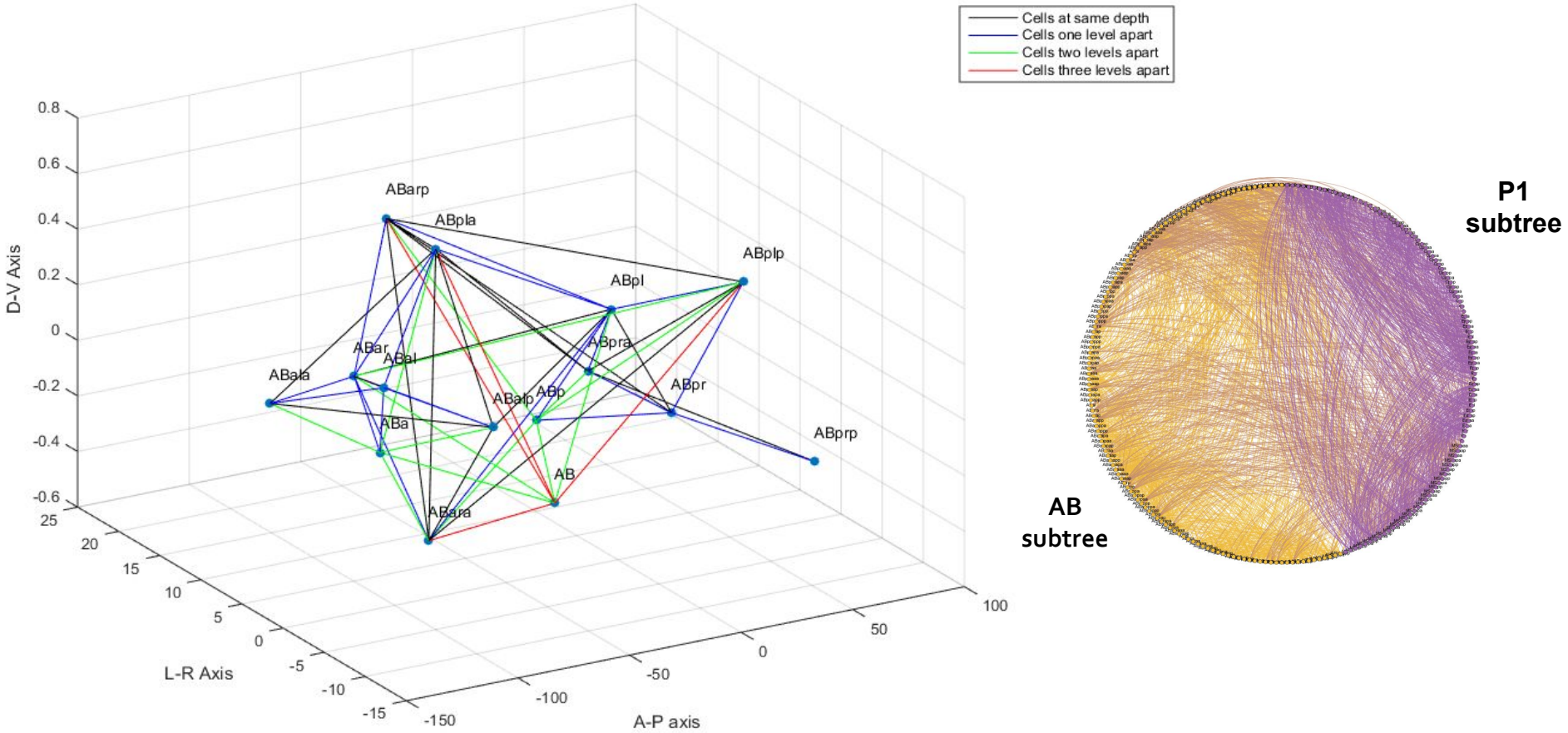
7-layer tree
(135 minutes)



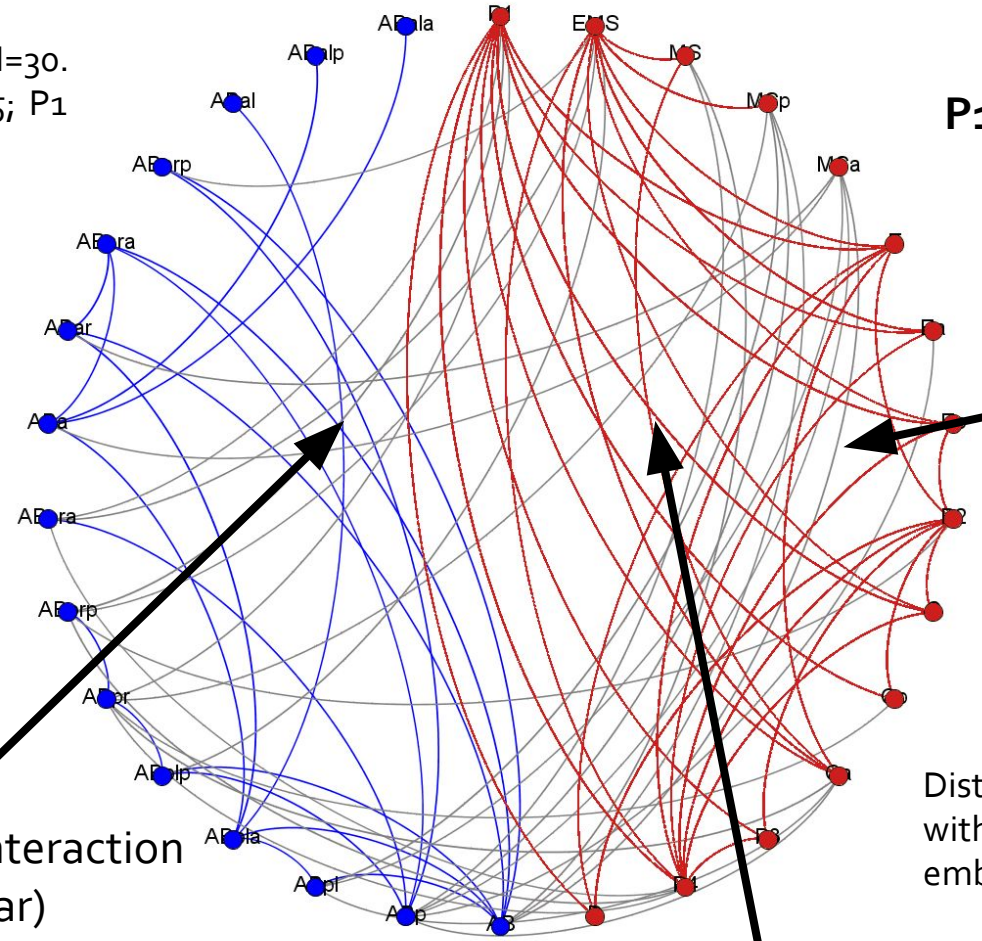
8-layer tree
(170 minutes)

Magenta points are AB sublineage, blue points are P1 sublineage. Degree of spatial separability greatest in 5-layer tree, most overlap seen in 8-layer tree.

[2] Data (N = 361) from Bao et.al, Developmental Biology, 318(1), 65-72 (2008); Murray et.al, Genome Research, 22(7), 1282-1294 (2012).


Cells at same depth
Cells one level apart
Cells two levels apart
Cells three levels apart
D-V Axis
L-R Axis
A-P axis
ABarp
ABpla
ABplp
ABpl
ABar
ABal
ABpra
ABala
ABalp
ABp
ABpr
ABa
ABprp
AB
ABara
P1
subtree
AB
subtree

4 level lineage tree: N=30. AB subtree (blue), *n*=15; P1 subtree (yellow), *n*=15.



P1 Subtree

Intra-subtree interaction (P_4, P_2)

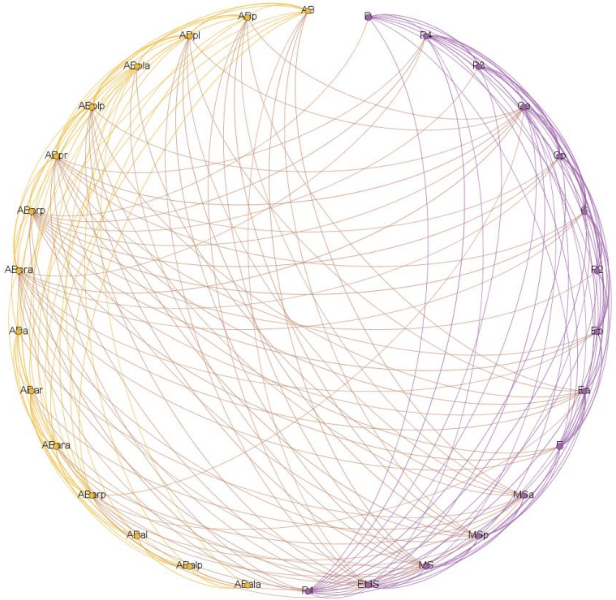
AB Subtree

Intra-subtree interaction (AB, ABar)

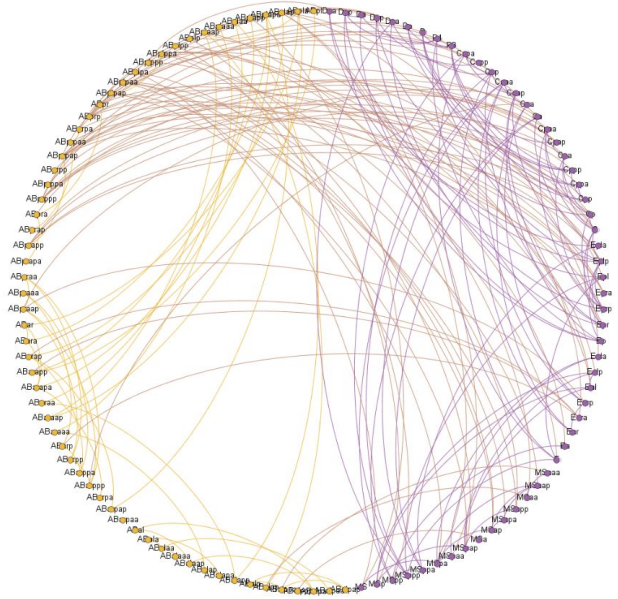
Distance threshold of 0.25 (all cells within 25% the maximum distance in embryo structure).

Inter-subtree interaction (MSa, ABa)

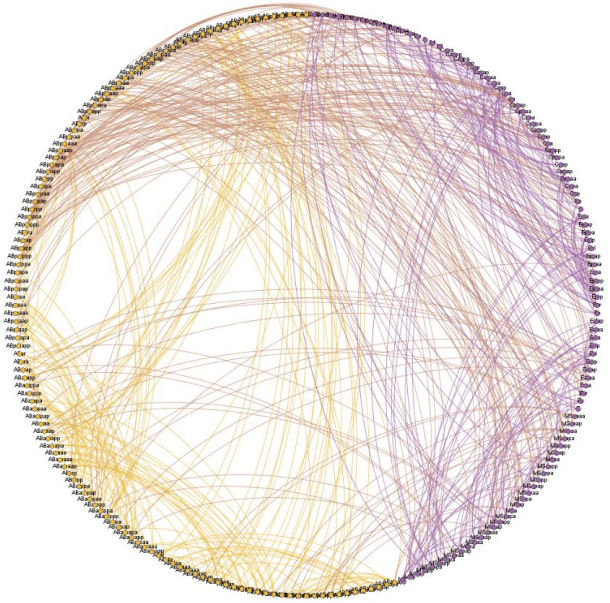
Cells in AB sublineage (left, yellow), cells in P1 sublineage (right, purple). Graphs rendered in Gephi v0.9.0



Circular plot for 16 terminal-cell condition, threshold of 0.25.

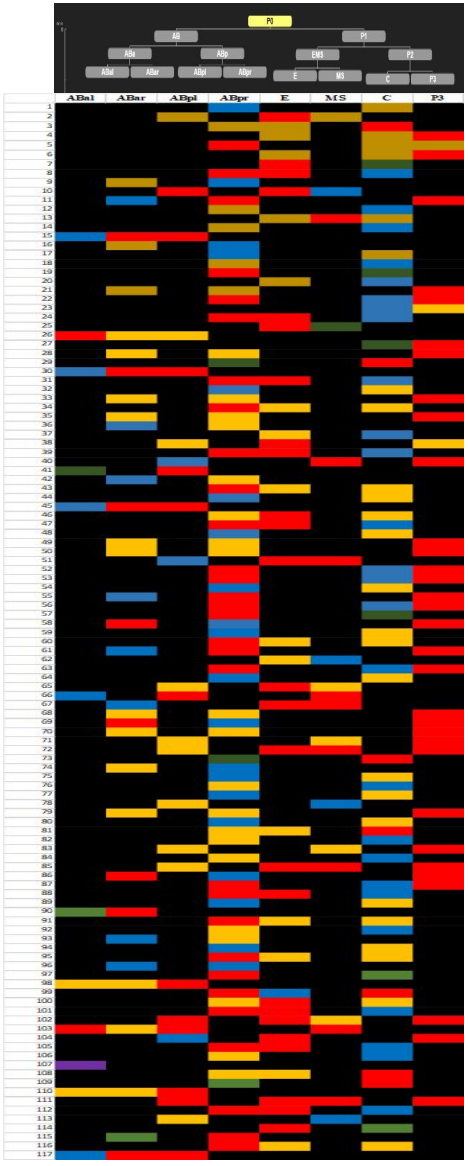


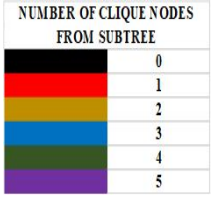
Circular plot for 64 terminal-cell condition, threshold of 0.05.



Circular plot for 128 terminal-cell condition, threshold of 0.05.

Connection Type	**Intra-sublineage AB**	**Intra-sublineage P1**	**Inter-sublineage**	**TOTAL**
16-terminal cell	0.31	0.37	0.32	1.00
64-terminal cell	0.33	0.46	0.21	1.00
128-terminal cell	0.39	0.35	0.26	1.00





Clique Analysis

A clique analysis was conducted to find subsets of vertices where every node is fully connected with the other nodes in that subset.

Clique analysis conducted on a network of 224 cells, distance threshold of 0.95 (0.05 total length).

The optimal clique size was determined by balancing the maximum number of cliques found with the largest possible clique size itself.

117 cliques (out of 1530 total cell pairs) of **size five (5)** generated.

Generated cliques most often included more than one cells from sublineages ABpr and C, least often included cells from sublineage ABal.

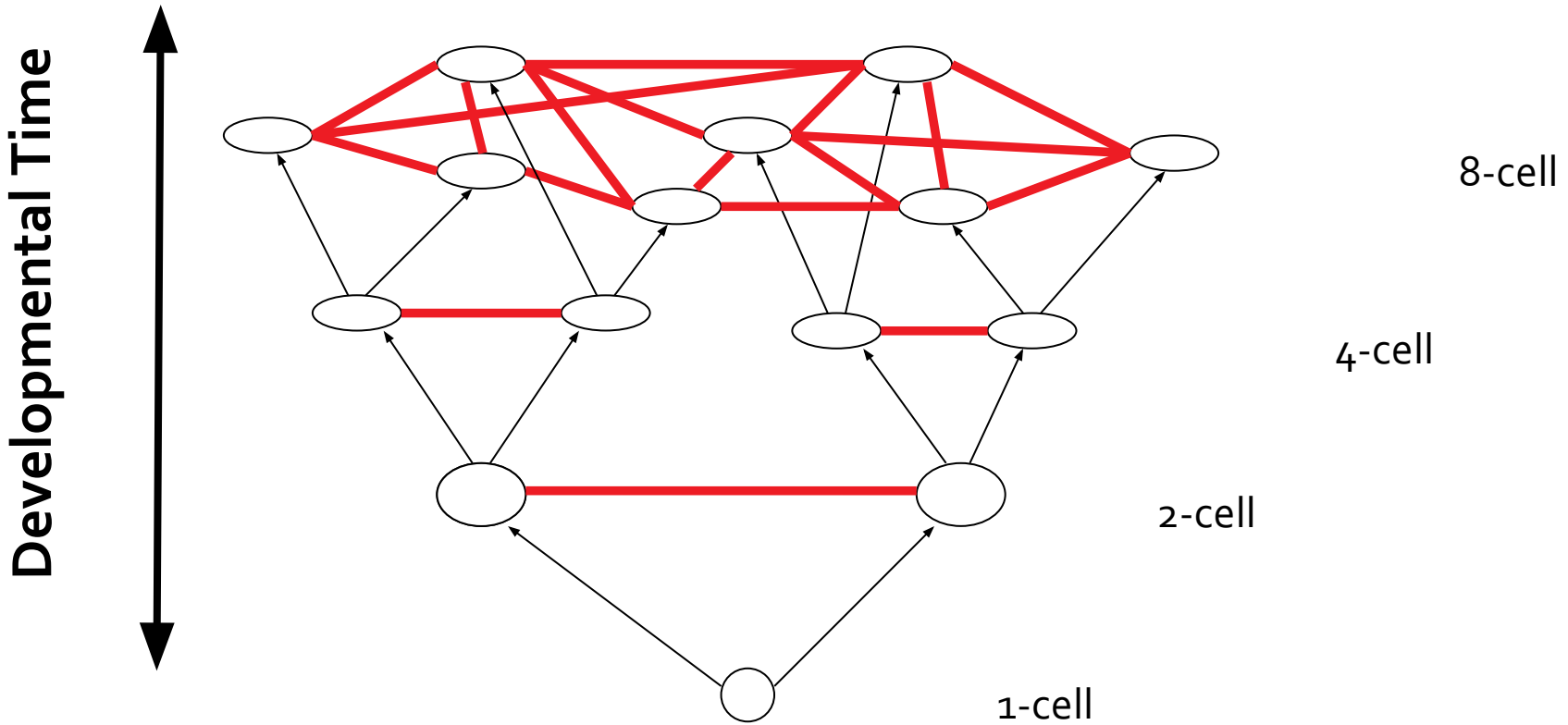
Differentiation Tree Sublineages as Network Clique Members

Clique analysis also shows that cells both within and between sublineages can be members of a clique.

- conducted on the 128-terminal cell condition, yielded 117 cliques of size 5 (optimal clique number-size tradeoff).

	Sublineage							
	ABpl	**ABpr**	**ABar**	**ABal**	**MS**	**E**	**C**	**P3**
Number of members of sublineage across all cliques generated	42	151	68	34	35	58	159	38
	AB				**P1**			
Number of cliques with overlap	75.2%							
Number of cliques without overlap	15.4%				9.4%			

Multiplex Networks

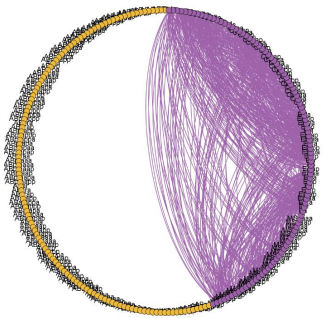


Observe different patterns of connectivity within subtrees, sort by lineage depth of cell:

Do the "intra-" patterns of connectivity have any biological significance?

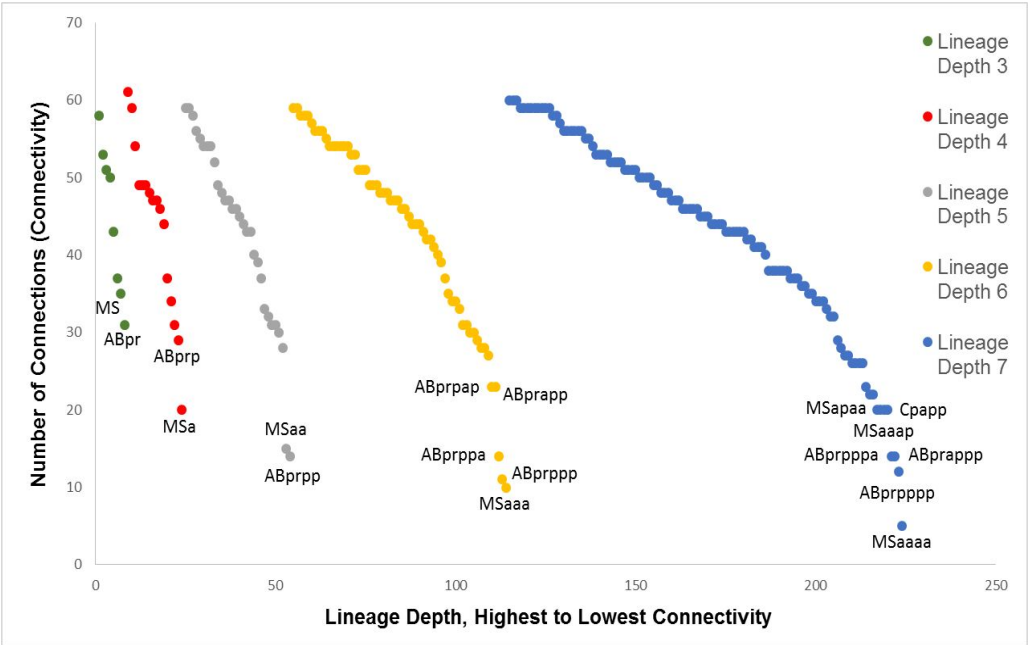
For all in subtree [AB]

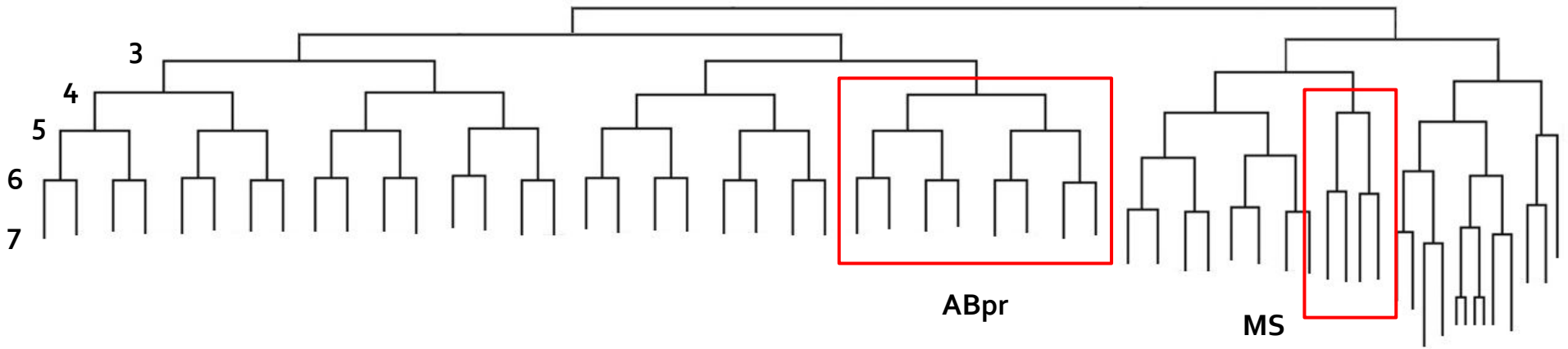
For all in subtree [P1]



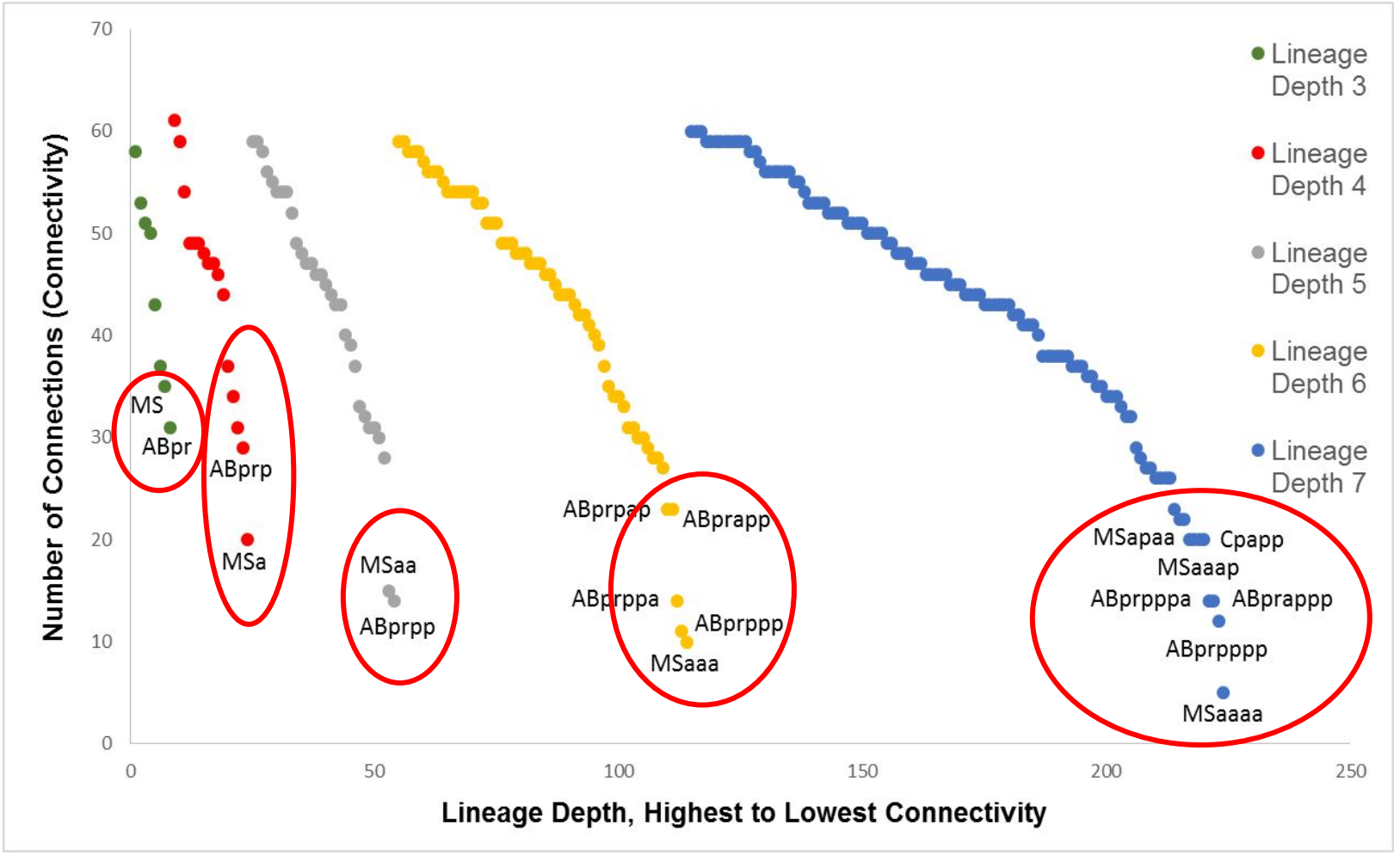
No preferential hubs (as measured by connectivity distribution, network statistics).

- scale-free network topology, with some influence of ancestral cells.
- sublineages such as MS, ABpr yield outliers.

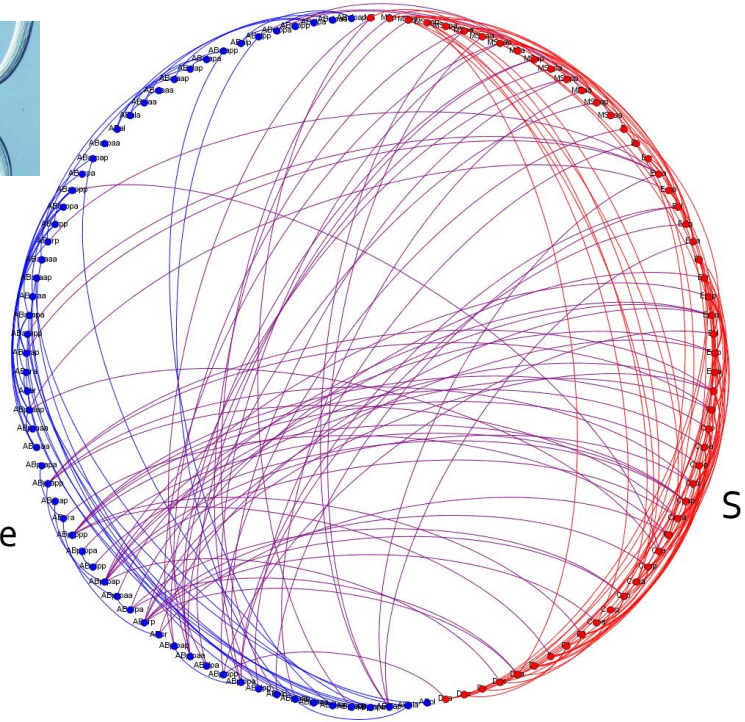




Sublineages (MS, ABpr) yield outliers



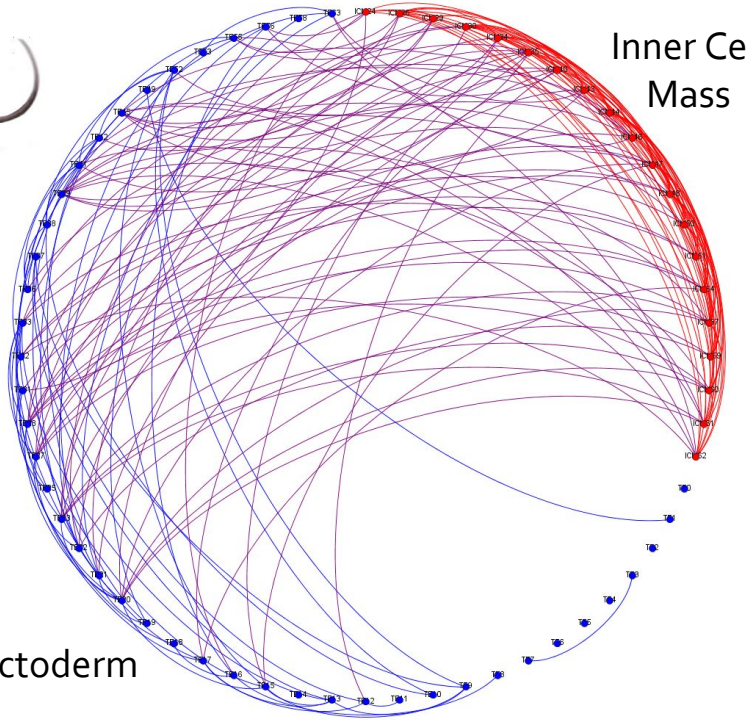
Does this method of inquiry extend to other embryos? *C. elegans* versus Mouse*



Sublineage AB

Sublineage P1

Inner Cell Mass

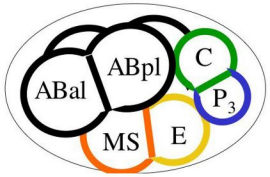


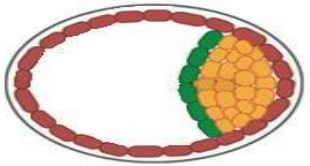
Trophectoderm

C. elegans 64-terminal cell embryo (mosaic development, colored sublineages defined by cell size and type)

M. Musculus 64-terminal cell embryo (regulative development, colored sublineages defined by tissue type)

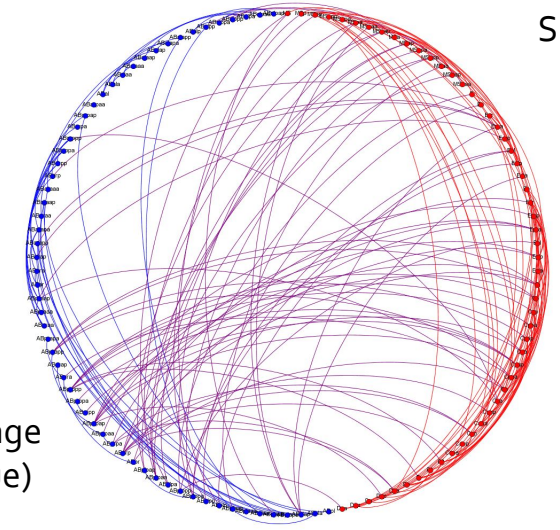
* Nature Methods, 13, 139–142 (2016).





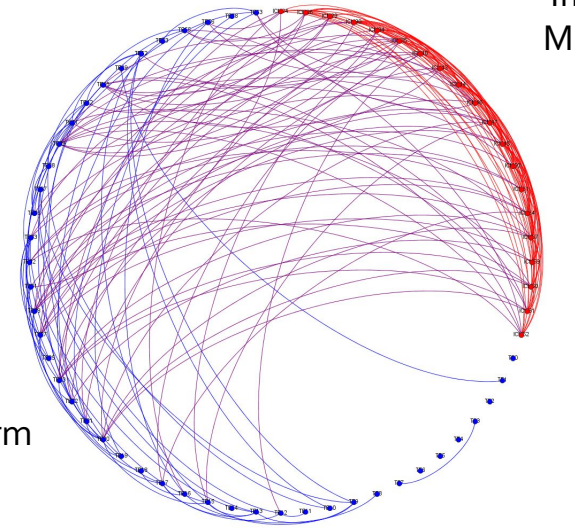
Sublineage (P1, red)

Sublineage (AB, blue)



Inner Cell Mass (red)

Trophectoderm (blue)



Model picks up geometry of axial segregation of AB vs. non-AB cells, cells on boundary.

Model picks up geometry of blastocoel, inner vs. outer trophectoderm.

Developmental Connectome

Using connectome data* based on cell-cell connections, can the embryonic assembly of a connectome be observed?

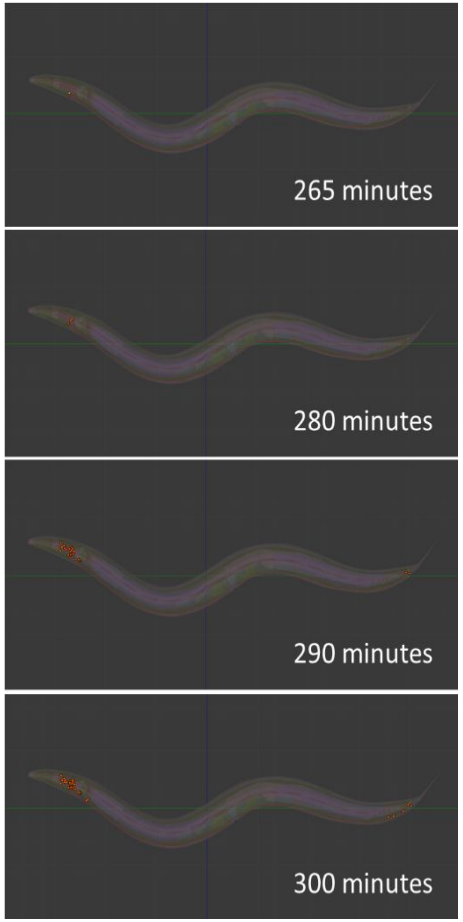
* PLoS Computational Biology, 7(2), e1001066.

Developmental Connectome

Using connectome data based on cell-cell connections (Varshney et.al, 2011), can the embryonic assembly of a connectome be observed?

Modeled in an adult phenotype, we can observe:

- addition of cells to connectome network between 265 and 300 minutes of embryogenesis.



bioRxiv
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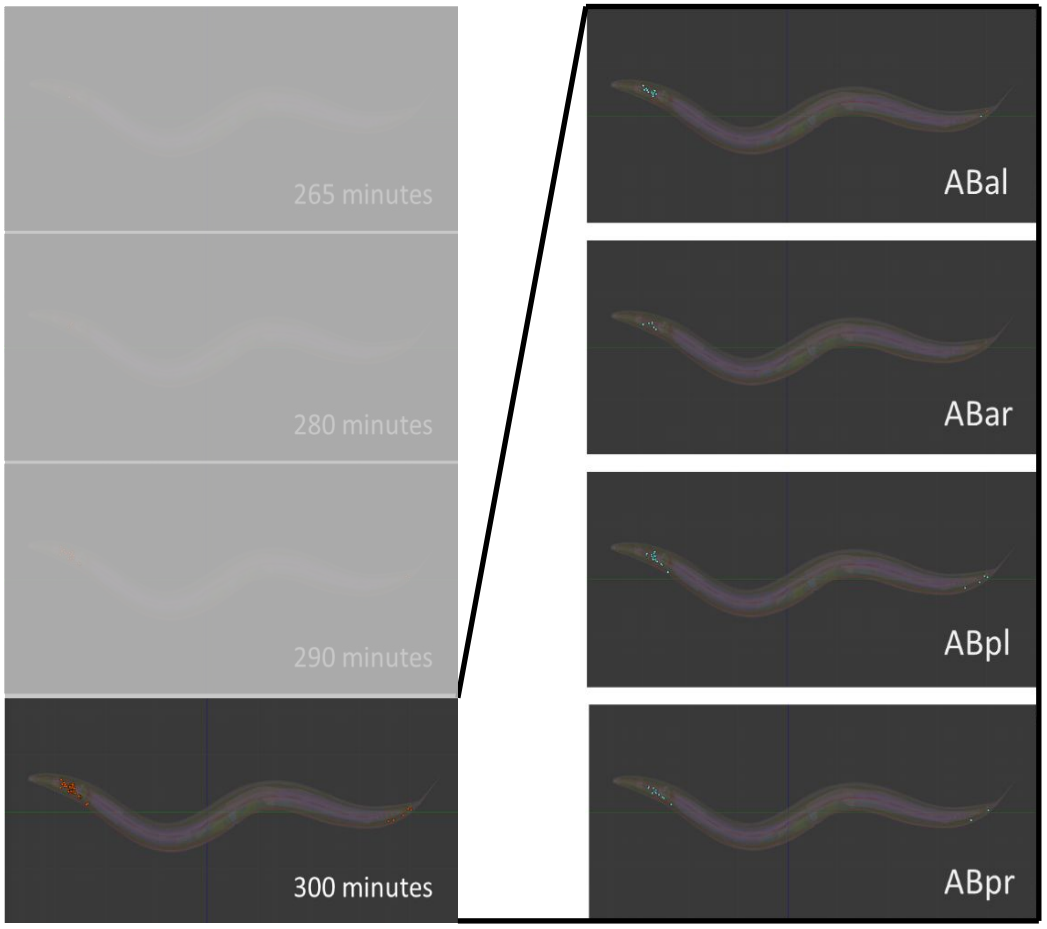
DevoWorm group,
doi:10.1101/146035

Developmental Connectome



Using connectome data based on cell-cell connections, can the embryonic assembly of a connectome be observed?

Modeled in an adult phenotype, we can observe:

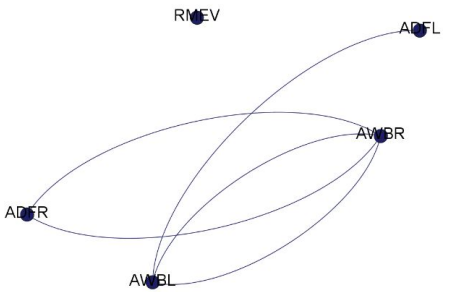
- addition of cells between 265 and 300 minutes of embryogenesis.
- the terminal-differentiation of pharyngeal neurons by developmental subtree.

Developmental Connectome

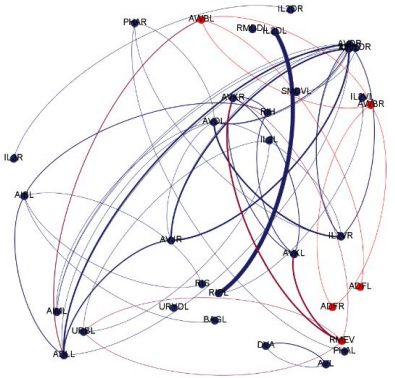
Using connectome data based on cell-cell connections, can the embryonic assembly of a connectome be observed?

Modeled in an adult phenotype, we can observe:

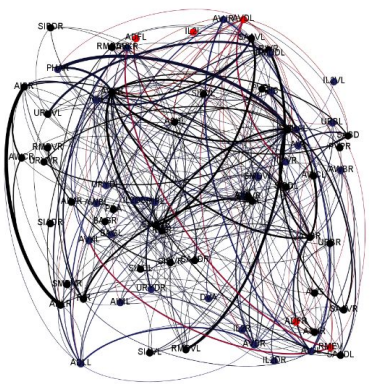
- addition of cells between 265 and 300 minutes of embryogenesis.
- the terminal-differentiation of pharyngeal neurons by developmental subtree.
- connectome can be compared to the developmental cell networks (embryos prior to 200 minutes).



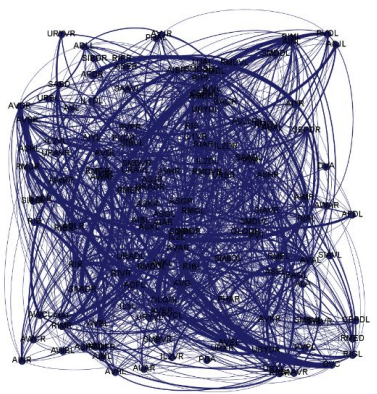
280 Minutes



290 Minutes



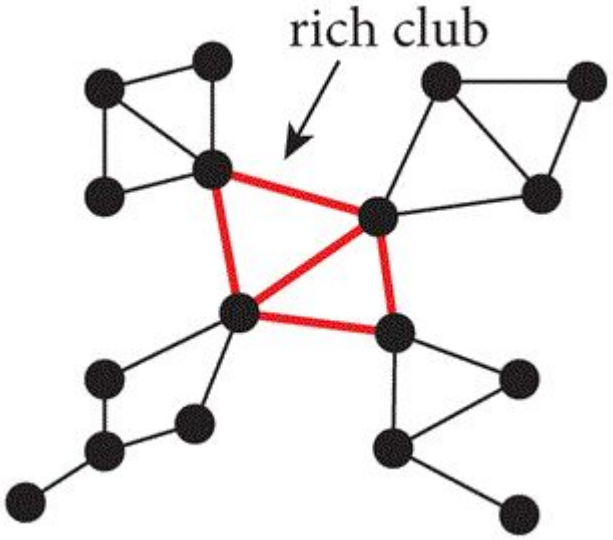
300 Minutes



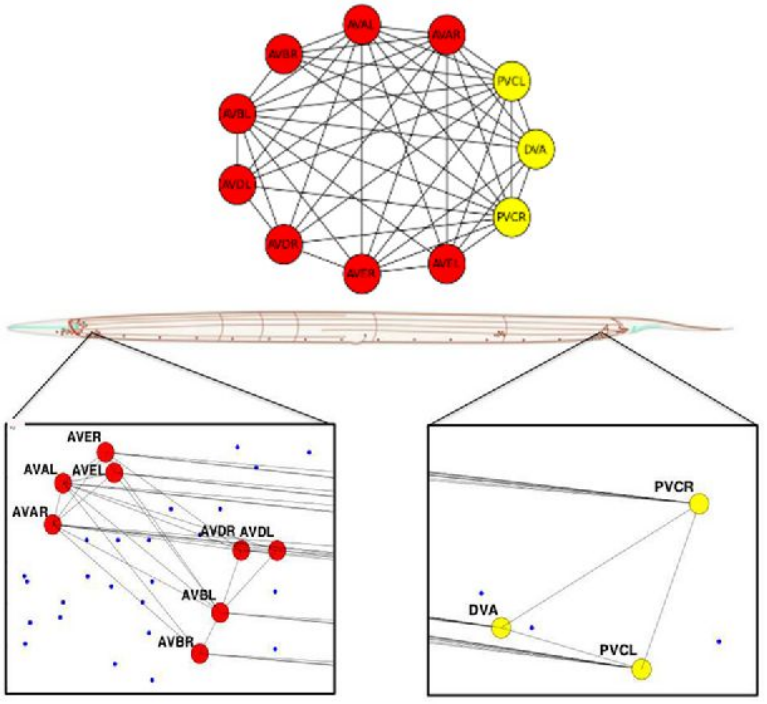
400 Minutes

Graphs rendered in Gephi v0.9.0

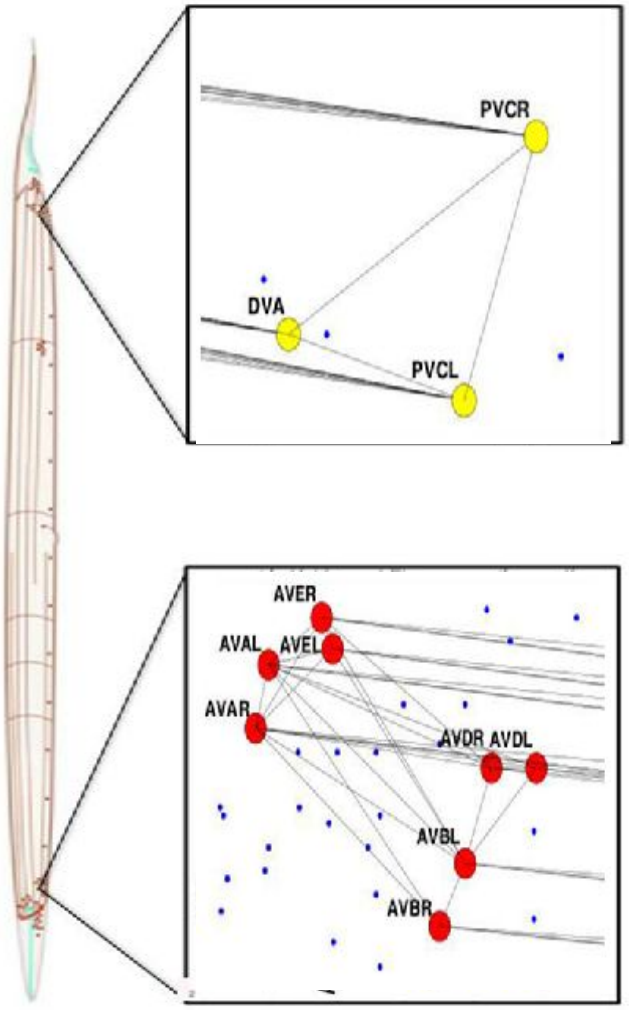
How does the emergence of terminally differentiated cells in developmental time compare with the "rich club" of neruons in *C. elegans* connectome as defined by Towlson et.al, 2013?



Adapted from Figure 1 in Walker, Chew, and Schafer, *Genetics of Behavior in C. elegans*. In "*The Oxford Handbook of Invertebrate Neurobiology*".



Adapted from Figure 1 in Towlson, *Journal of Neuroscience*, 33, 6380–6387 (2013).



Cell Name	Neuron ID	Birth Time (min)	Lineage	Annotation
AVAR	AS9 (P9.apa)	295	ABal	Ventral Cord Motorneuron
AVAL	AS8 (P8.apa)	295	ABal	Ventral Cord Motorneuron
AVBR	ASER	300	ABpr	Ventral Cord Interneuron
AVBL	ASEL	350	ABpl	Ventral Cord Interneuron
AVDR	ASGR	290	ABal	Ventral Cord Interneuron
AVDL	ASGL	290	ABal	Ventral Cord Interneuron
AVER	ASHR	350	ABpr	Ventral Cord Interneuron
AVEL	ASHL	350	ABal	Ventral Cord Interneuron
PVCR	---	350	ABpr	Ventral Cord Interneuron
PVCL	---	350	ABpl	Ventral Cord Interneuron
DVA	CP9 (P11.aapp)	280	ABpr	Ring Interneuron

Thanks for your Attention!