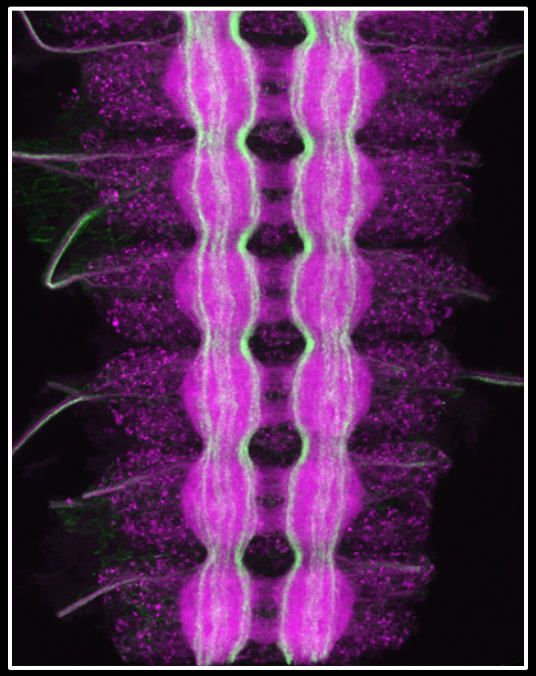
Techniques for examining ventral nerve cord development in *Tribolium* embryos



Tim Evans

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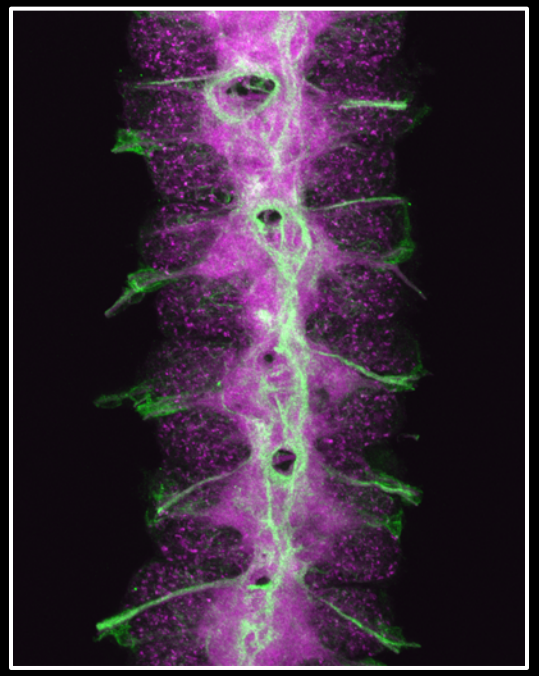
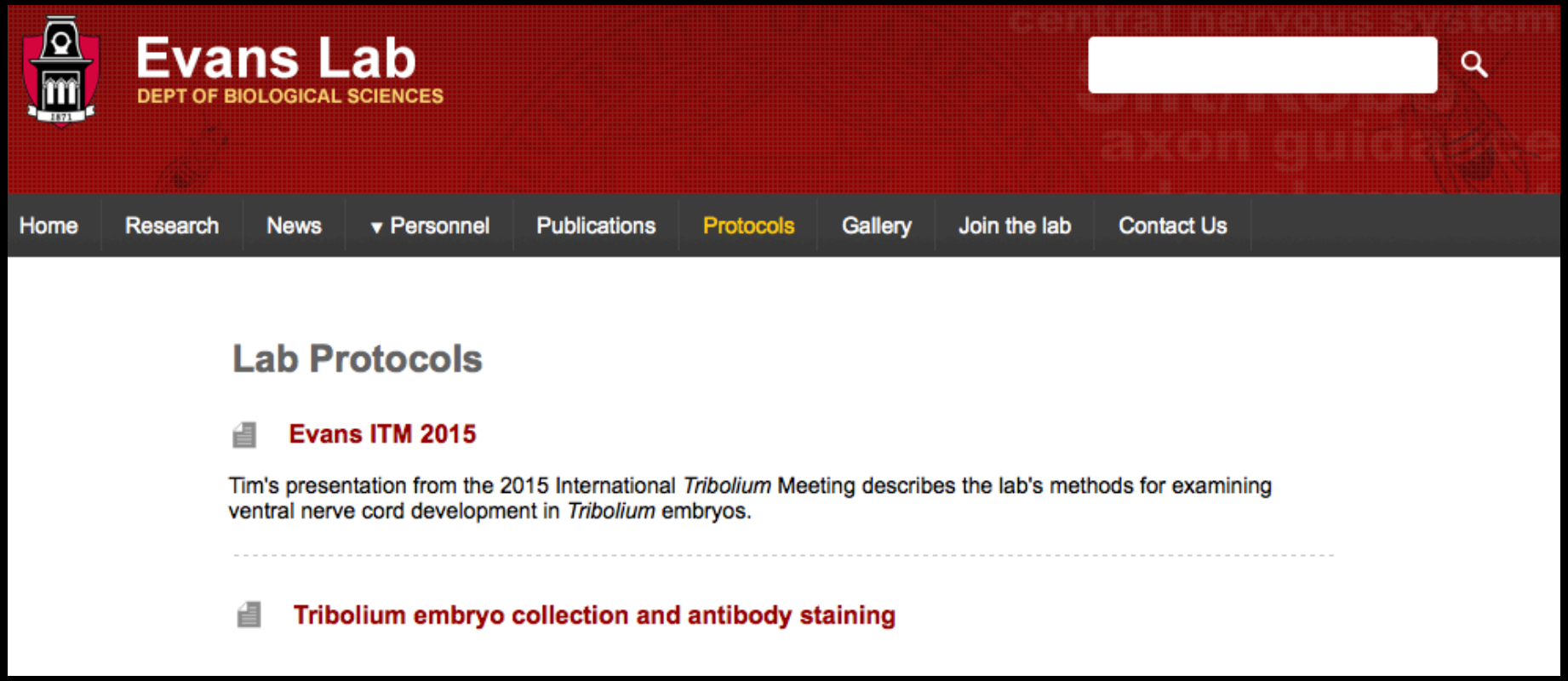
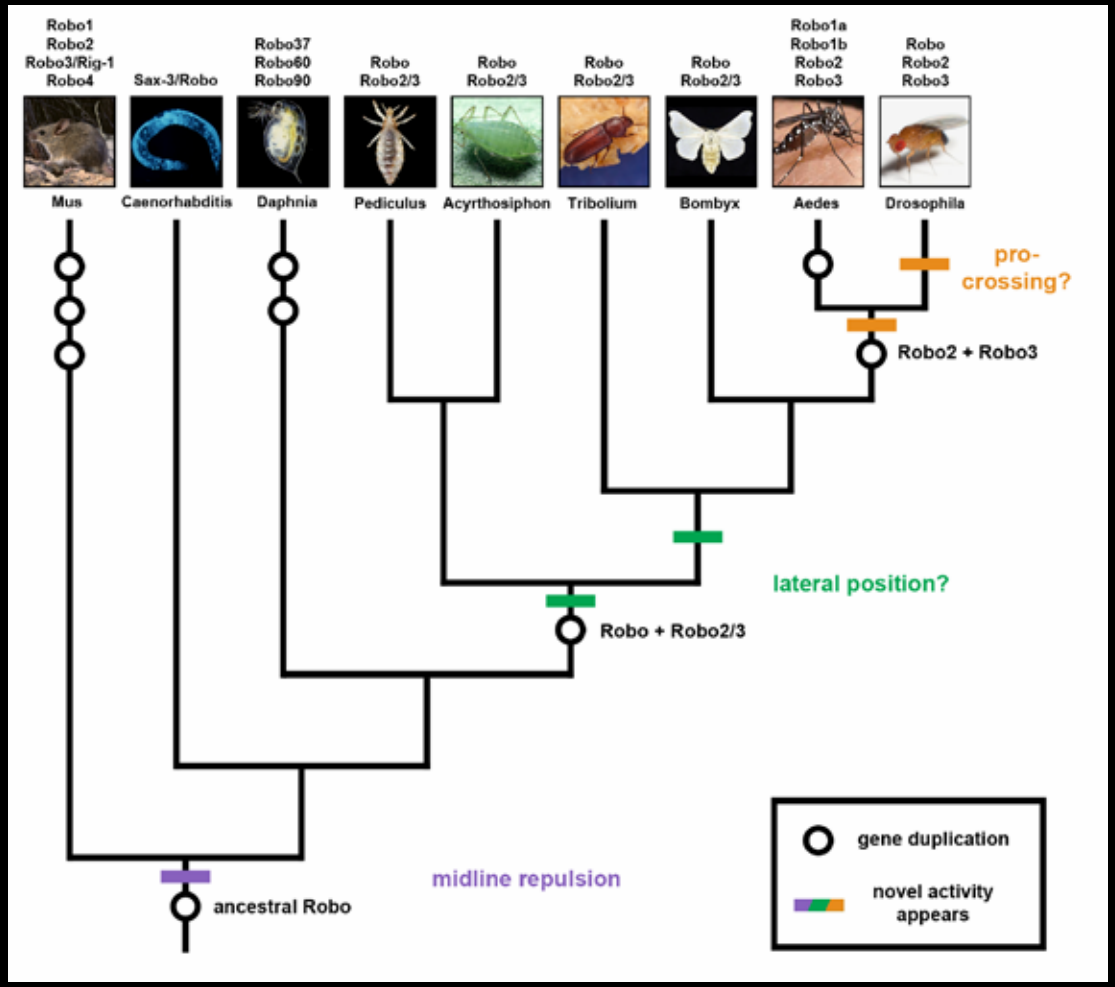


International Tribolium Meeting 2015



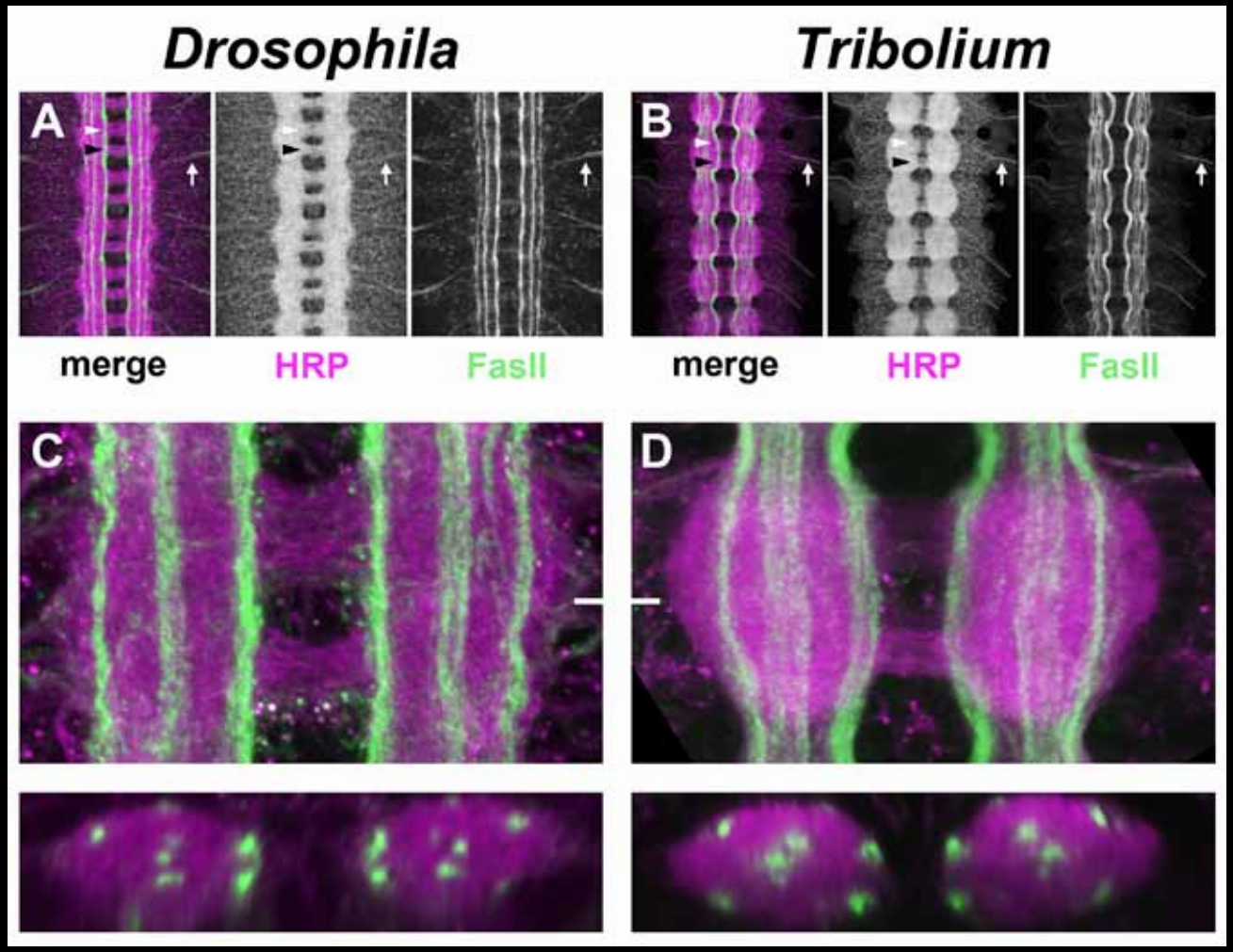
this powerpoint and other protocols available at:
www.evansflylab.com/protocols.php

My lab is interested in patterns of gene duplication and functional divergence in axon guidance genes



See my poster for more details:
P041 “Axon guidance roles of Robo receptors in *Drosophila* and *Tribolium*”

Using antibodies to examine axon pathways in the beetle embryonic ventral nerve cord



anti-HRP: labels all axons in the CNS

anti-FasII: labels pioneer axons early; longitudinal subset late, all motor axons

beetle cultures

Media: Unbleached supermarket flour plus 5% (by weight) active dry yeast

Vessels: One-pint glass mason jars or two-quart plastic gladware container (with vented lids)

150 g flour + 7.5 g yeast

500 g flour + 25 g yeast

beetle cultures

Percival Drosophila incubator
25°C, ~40% relative humidity

beetle wrangling

- 850 µm sieve removes adults, pupae, and late-stage larvae
- 300 µm sieve removes eggs from flour *

* when setting up egg collection cultures, use pre-sifted yeast granules <300 µm (grind yeast with coffee grinder, then sift with 250 µm sieve)

egg collection

- Remove eggs from flour/yeast with 300 µm sieve
- Transfer eggs to a collection basket (cut-off 50 ml conical tube with nylon mesh attached to one end)
- Remove chorion with 50% bleach solution (2-5 min), then rinse well with water

embryo fixation

- Using a wet paintbrush, transfer embryos to a glass scintillation vial containing heptane and fixative
- Shake vial vigorously for 30-45 min
- Remove formaldehyde phase and replace with 10 ml methanol

embryo sonication

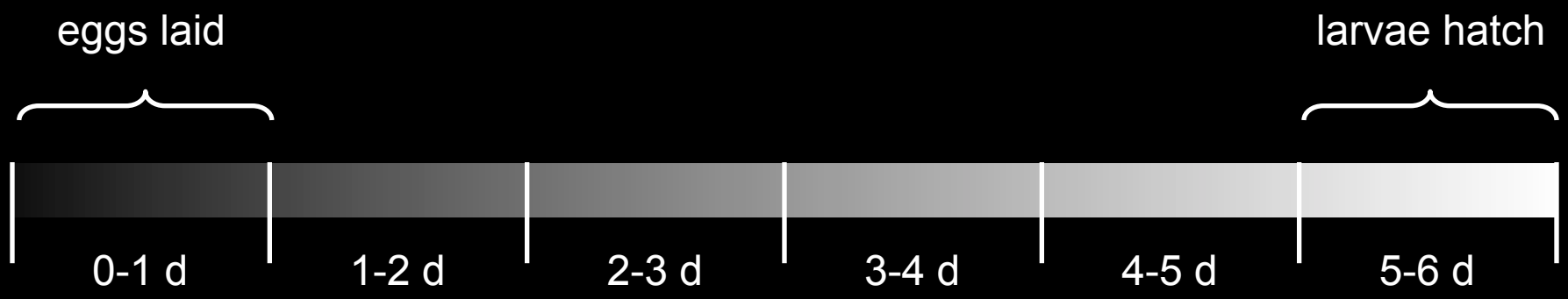
- 2-5 rounds of sonication (5 sec/round) to remove vitelline membrane

intact embryos and empty vitelline membranes remain at interface

de-vitellinized embryos and fragments fall to the bottom: recover these after each round of sonication

we use a Branson Sonifier 250 with microtip attachment, dialed to 10% output

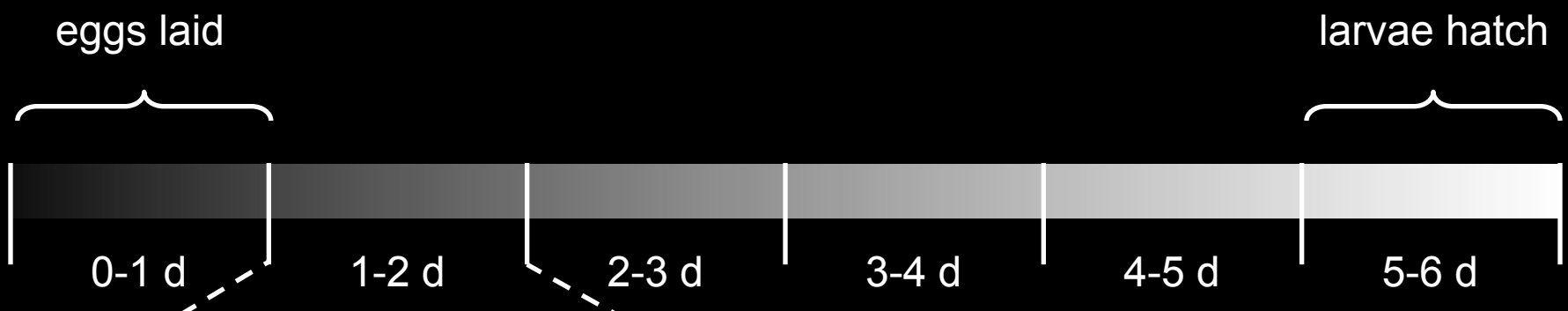
Timed egg collections

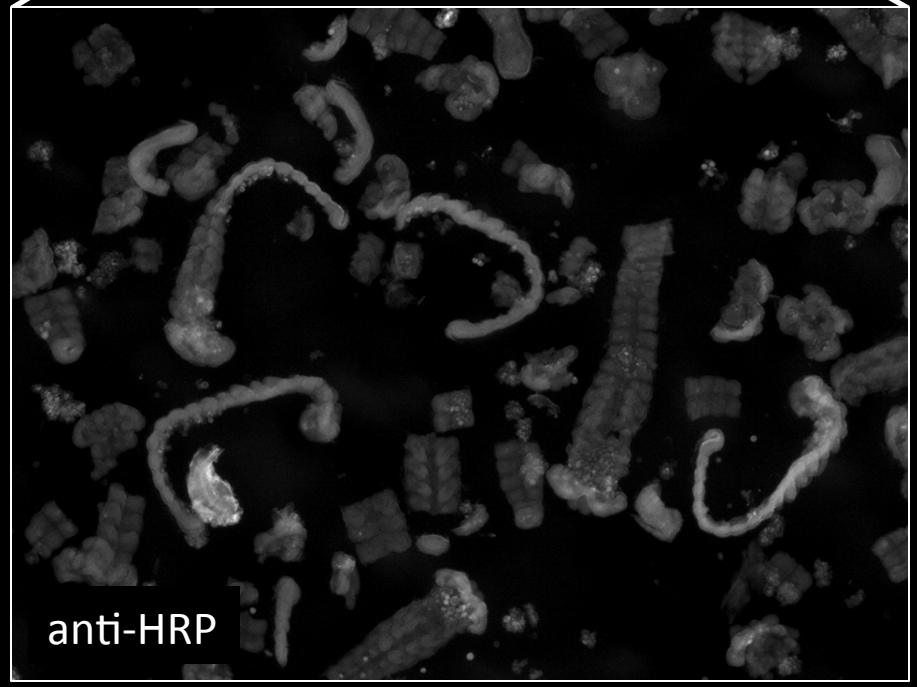


- Incubate adults in flour + sifted yeast (250 µm) for 24 hours
- Remove adults and fix immediately (for 0-1 d) or incubate eggs in flour for a further 1-5 days

To include all embryonic stages in the same collection, allow adults to lay eggs for 6 days, then collect all of the eggs (0-6 d)

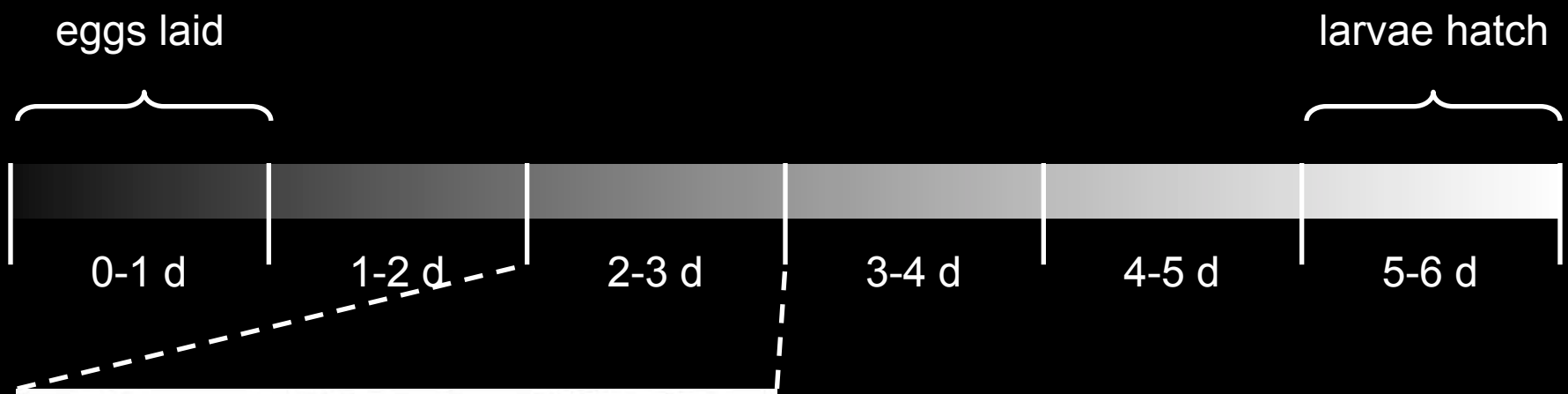
Timeline of embryonic nerve cord development (25°C)

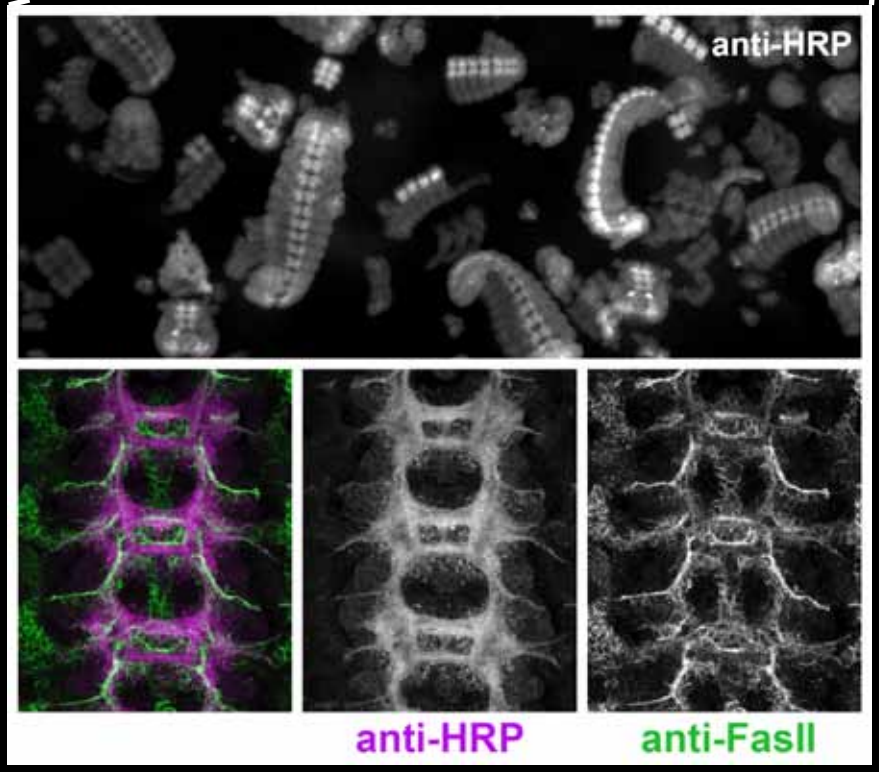




- germband extended
- neural specification is occurring
- no neuronal staining yet with anti-HRP or anti-FasII
- similar to stage 10-11 fly embryos

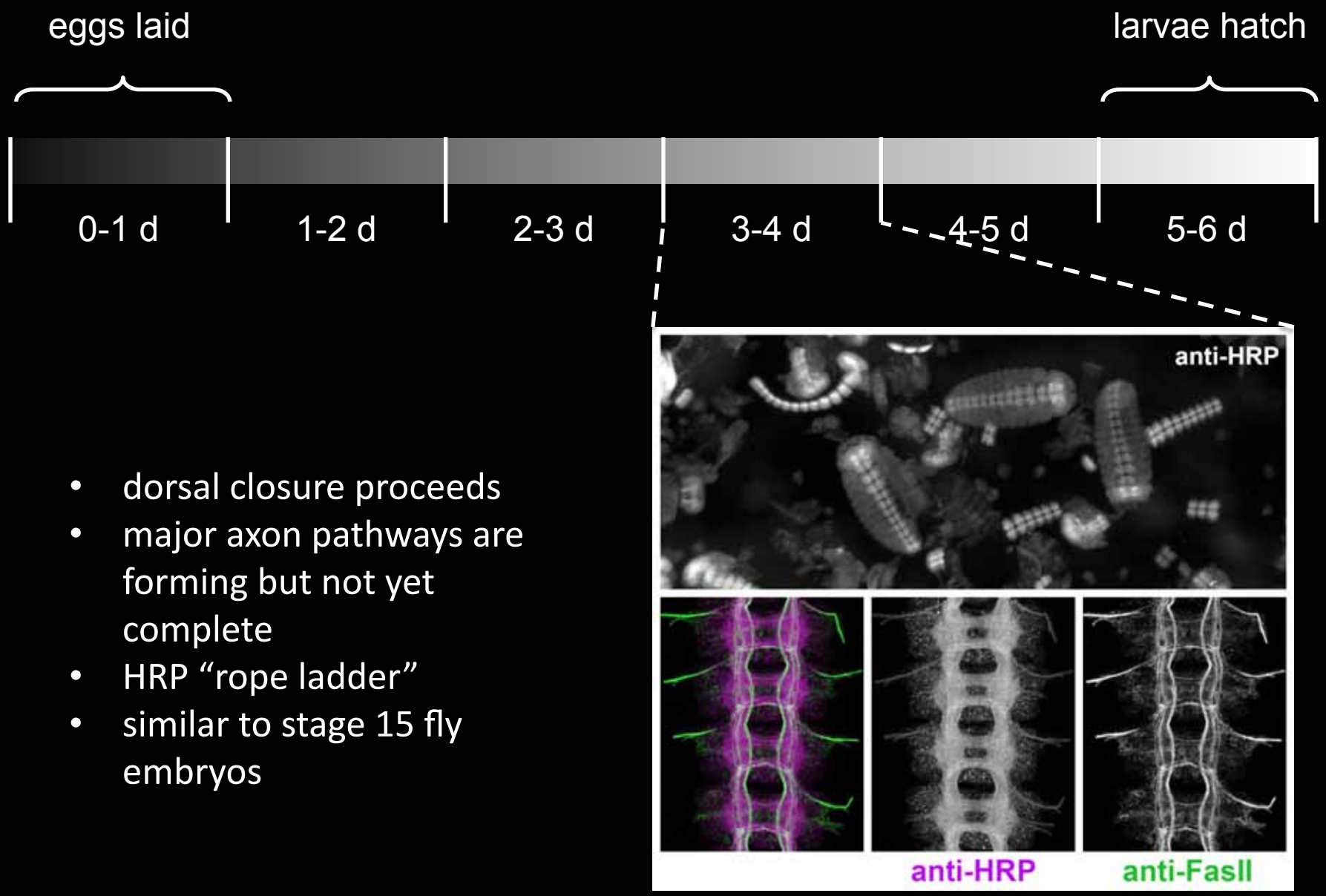
Timeline of embryonic nerve cord development (25°C)





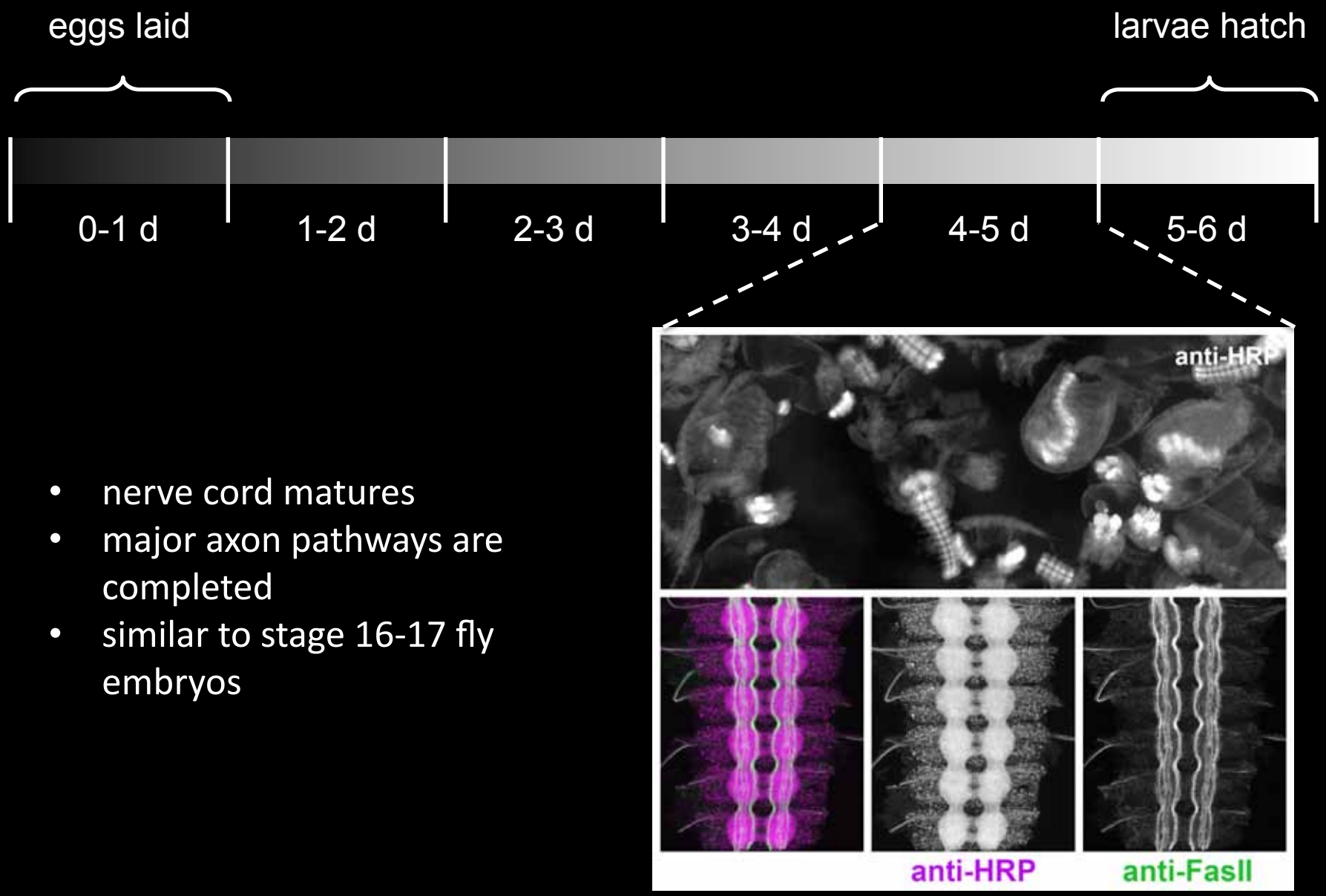
- germband retraction
- initial axon pathways are forming
- pioneer axons labeled by anti-FasII in older embryos
- HRP “bow tie”
- similar to stage 12-14 fly embryos

Timeline of embryonic nerve cord development (25°C)



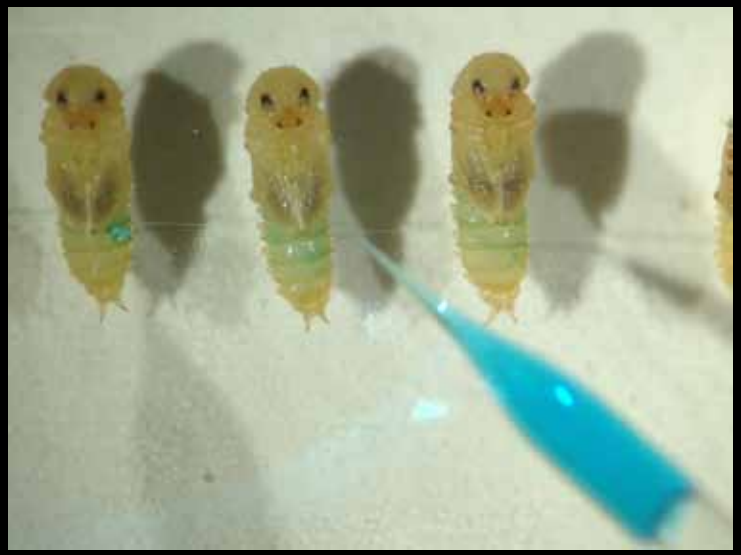
- dorsal closure proceeds
- major axon pathways are forming but not yet complete
- HRP “rope ladder”
- similar to stage 15 fly embryos

Timeline of embryonic nerve cord development (25°C)



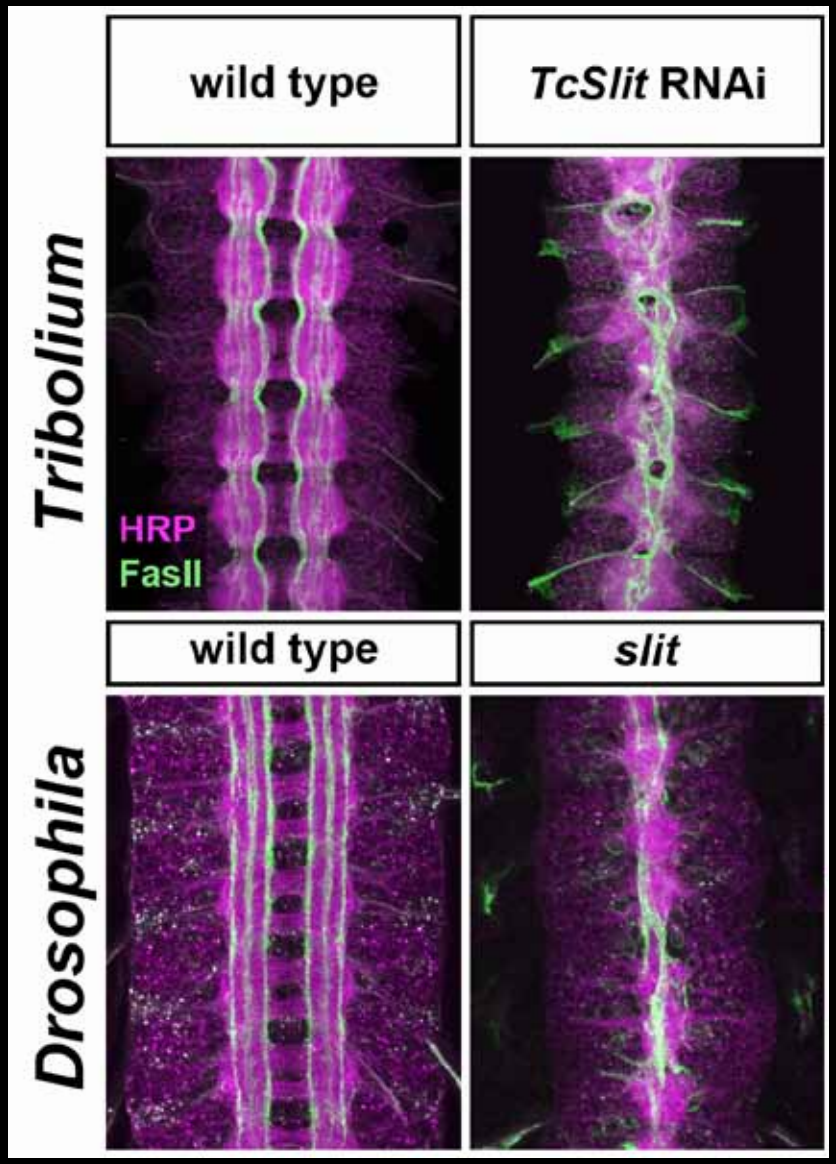
- nerve cord matures
- major axon pathways are completed
- similar to stage 16-17 fly embryos

knocking down embryonic genes via parental RNAi



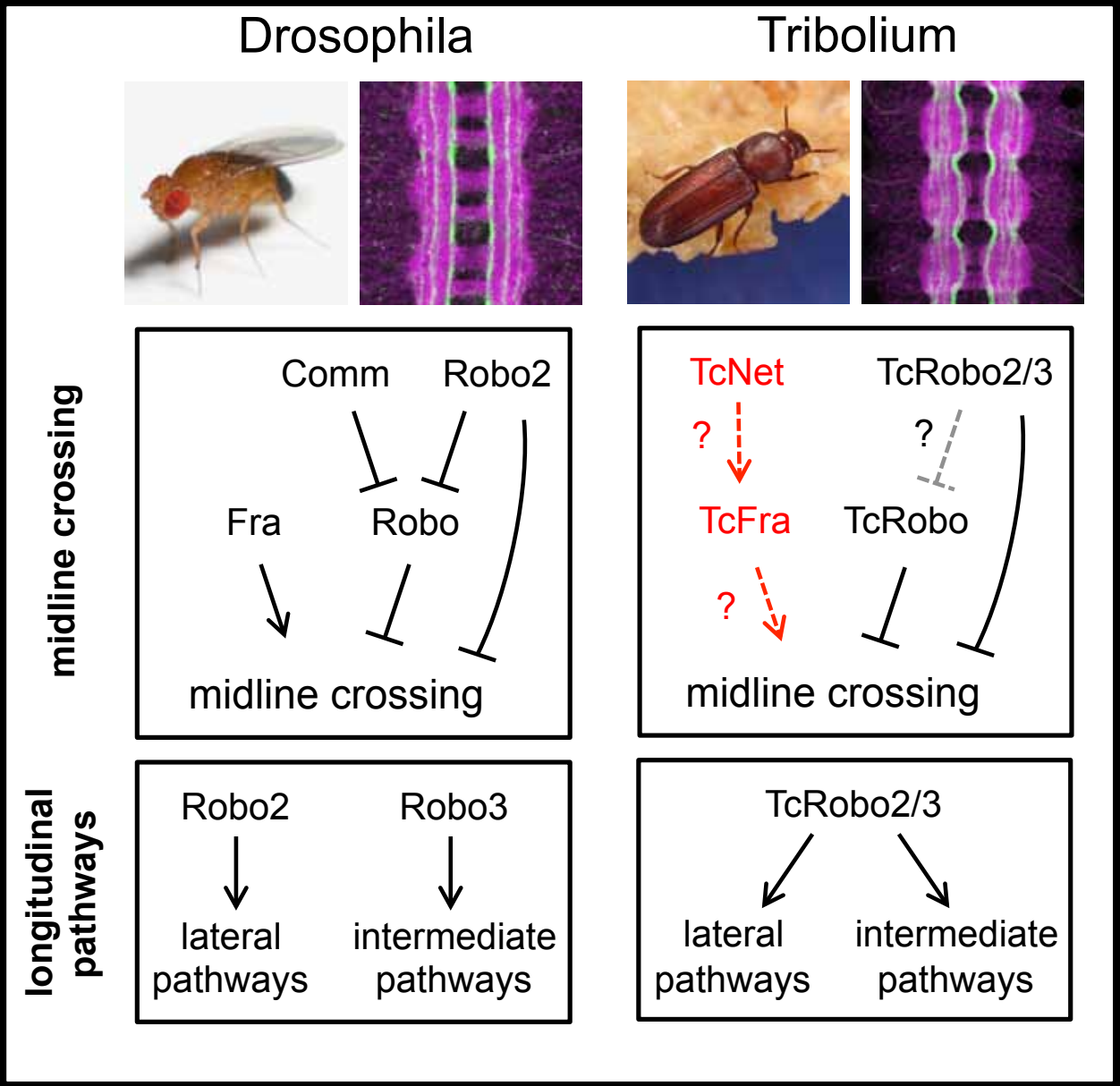
- dsRNA synthesized in vitro (500-800 bp)
- dsRNA injected into female pupae or ether-anesthetized adult females (concentration 0.25-1.0 µg/µL)
- mated with untreated males; egg collections begin one week after injection

TcSlit parental RNAi phenocopies *Drosophila slit* mutants

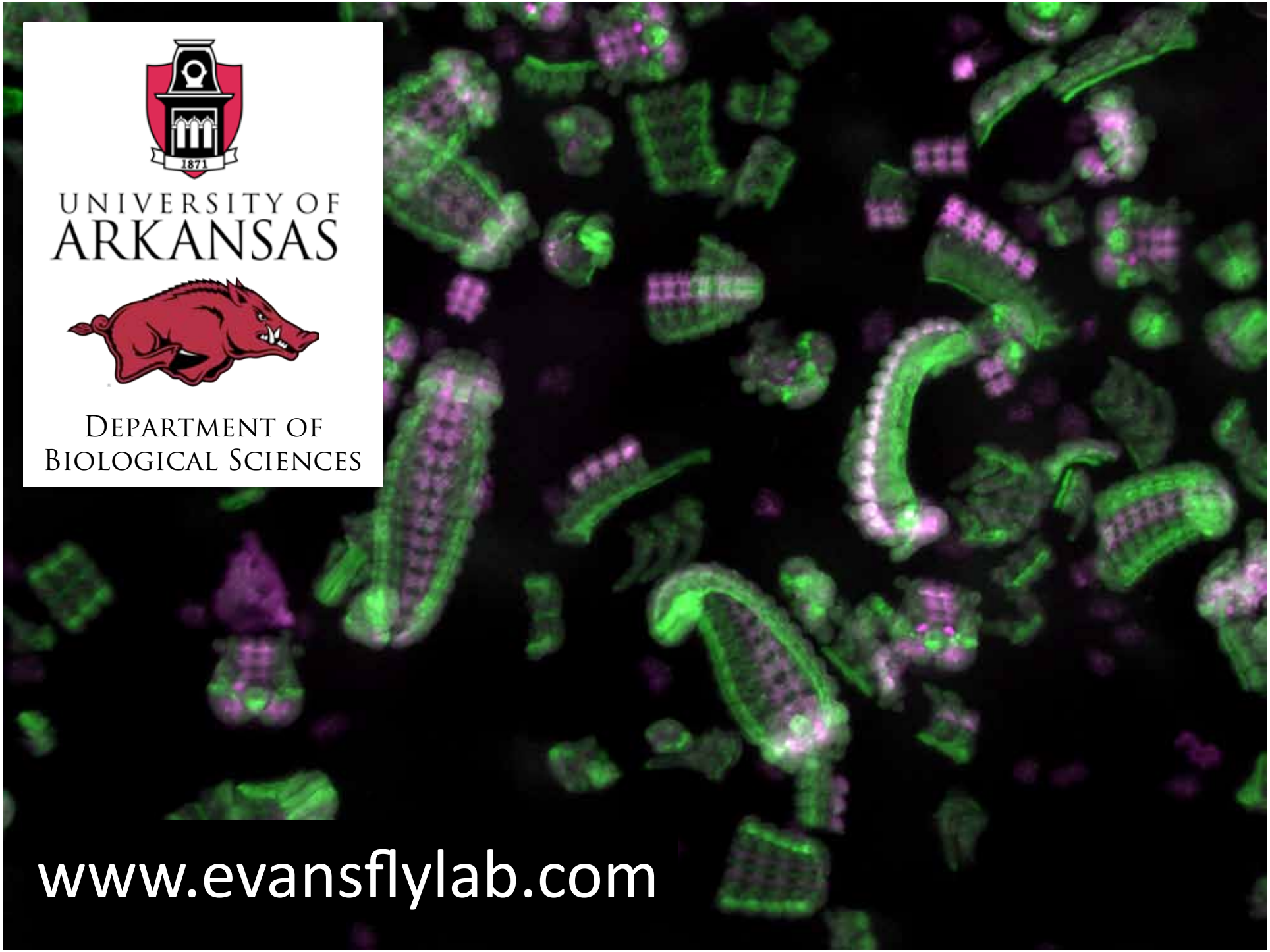


Evans and Bashaw, 2012

Logan Terry: midline attraction via Netrin-Frazzled/DCC in *Tribolium*



Logan Terry
(CEMB PhD student)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