Wnt signaling and head regeneration in *N. vectensis*

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What’s Wnt?

- From the Wnt homepage at Stanford: “Wnt proteins form a family of highly conserved secreted signaling molecules that regulate cell-to-cell interactions during embryogenesis.”
- Named after scientists realized that mouse int-1 was identical to drosophila wg.
- Seem to be conserved across most phyla, from cnidarians to vertebrates.

The canonical Wnt/Beta-Catenin pathway

Good job explaining Wnt genes and the Wnt pathway in sufficient but not slumber-inducing detail.

More specific? e.g., The effects of variable temperature and salinity on.....
A few examples of Wnt signaling in development

- "In zebrafish, slb/wnt11 is required for convergence and extension movements, but not cell fate specification during gastrulation...Ppt/Wnt5 regulates cell elongation and convergent extension movements in posterior regions of the gastrula, while its function in more anterior regions is largely redundant to that of Slb/Wnt11." - (Kilian, 2002)

- "Wnt signaling polarizes an early C. elegans blastomere to distinguish endoderm from mesoderm." - (Thorpe, 1997)

- "Wnt/β-catenin signaling is activated in the regenerating zebrafish tail fin and is required for formation and subsequent proliferation of the progenitor cells of the blastema" - (Stoick-Cooper, 2006)

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**Fig. 1.** Expression of wnt5 and wnt11 mRNA during gastrulation. (A) Maternally provided wnt5 RNA at the 16-cell stage; animal view. (B) wnt5 expression in the germ ring at shield stage; lateral view; dorsal to the left. (C, D) wnt5 expression in posterior regions of the gastrula (C) adjacent to the wnt11 expression domain in the anterior paraxial mesendoderm (D). (E) wnt5 expression in the posterior mesendoderm at the T-somite stage (E), close-up in (F); arrow in (F) points at the mesendodermal germ layer; lateral view, dorsal to the right. (from Kilian paper - see previous slide)

**Fig. 2.** E Adopts an MS-like identity in mom-2 embryos (A) Immunofluorescence and light micrographs of wild-type (left column), mom-2 (middle column), and mom-2/+ embryos (right column) from mom-2/mom-2 mothers. Embryos were allowed to develop 15 hr (a, b, e, and f), or 8 hr (c and d) at 20°C. (a, b, and c) Living embryos viewed with Nomarski optics. Pharyngeal tissue is surrounded by a prominent basement membrane (arrowheads), and contains a secreted cuticle (wide arrows). Visible in wild-type embryos, but not in mom-2 or mom-2/+ embryos, are intestinal cells with characteristically large nuclei containing a single large nucleolus (thin arrows in [a]). While homozygous mom-2 embryos fail to undergo any morphogenesis and inevitably arrest as unelongated clumps of differentiated tissue (b), mom-2/+ embryos, even those lacking intestine, often elongate into short, stubby worms (c and d). Paternal contribution of a wild-type copy of mom-2 does not rescue the intestine defect (Experimental Procedures). (d, e, and f) Intact embryos stained with J126, a monoclonal antibody (MAb) that recognizes intestinal cells. (g, h, and i) Intact embryos stained with 9.2.1, a MAb that recognizes pharyngeal muscle cells. (from Thorpe paper)

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**In Xenopus**

**Fig. 3.** Wnt posterior in Xenopus embryos. Activation of the Wnt/β-catenin pathway (+Wnt) leads to headless tadpoles (Wnt mRNA injected, lower panel); inhibition (-Wnt) results in enlargement of anterior and concurrent reduction of morph structures (mRNA injected, upper panel).
In *N. vectensis*…

Overlapping expression domains of Wnt genes in a *N. vectensis* planula.  

(Kusserow, 2005)

In *Hydra*, budding and regeneration don’t use exactly the same genes.  

(Lengfield, 2009)

Multiple developmental pathways make things more complicated

In-situ hybridization showing Wnt expression in budding / regenerating *Hydra*

(Lengfield, 2009)

So, I want to find out….

• Which genes are expressed in the region regenerating a head in *Nematostella vectensis*?

• Are they different from those known to build the head during embryogenesis?

• How are they similar to genes involved in regeneration in other species?

(Burton & Finnerty, 2009)
I suspect…

• NvWnt3 will be expressed in regenerating heads since it appears to relate to head and primary axis formation in *Hydra*, as well as in many other animals.
• NvWnt5 might be expressed since it has been linked to tentacle formation in both *Hydra* and *Nematostella*
• NvDkk protein may be expressed in regenerated phasas but not in regenerating heads. There is evidence that NvDkk appears along the primary body axis on an opposite concentration gradient as does Wnt3.
• WntA may appear in the head as it is involved in forming the blastopore during embryogenesis. This would indicate a reuse of embryogenesis genes during regeneration.

METHODS

Collection of specimens - Mud and silt were collected from the Great Sippewissett salt marsh in Cape Cod, Massachusetts. Back in the lab, *N. vectensis* specimens were found and isolated. All specimens came from pond 3 and ranged in size from 2 - 4mm (contracted).

Marsh water conditions:
• Average pH: 8.2
• Average temp: 71º

In-lab conditions:
• pH: 7.89
• Temp: 71.8º

EXPERIMENTAL SETUP

Four days after collection…

24x

12x 5mM DMSO control

12x 5μM Alsterpaullone

Experimental flow chart is very helpful in explaining protocol.

Minor point... you made “transverse” cuts or cuts “perpendicular to the primary body axis,” not “lateral” cuts.

You should at least provide the full name of DMSO and mention why it serves as a control. Important point: is it present in the Alsterpaullone trials?

Make clear on diagram that adding alsterpaullone would mimic effect of Wnt signaling by repressing GSK3.

(Philipp, 2009)
Control (DMSO) group, specimen 9

Day 1

Day 3

Day 9

Magnification varies - grid squares are 1mm²

Higher magnification view of foot would have been compelling here.

Alsterpaullone Group, specimen 6

Day 1

Day 3 - aboral end

Day 9

Magnification varies - grid squares are 1mm²

Tentacle buds?

Higher magnification view of foot would have been compelling here also.

- After 2 days (and then after 7 days) 4 specimens from each group were cut laterally and aboral ends were collected (separated into control/experimental pools).
- Phenol-Chloroform RNA extraction followed, nanodrop used to verify purity of RNA
- cDNA cloning: New England BioLabs ProtoScript M-MuLV First Strand cDNA Synthesis Kit using the Oligo dT primers at 42° for 1 hour
- Custom mRNA primers were designed using Perl Primer and ordered from Eurofins MWG|Operon:

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- PCR: primers from Eurofins, dNTPs from Qiagen, buffer / Taq from New England Bio Labs
- Gel Electrophoresis was performed on the products.

Gel Results

PCR: primers from Eurofins, dNTPs from Qiagen, buffer / Taq from New England Bio Labs

Gel Electrophoresis was performed on the products.

How many cycles of PCR? Why the difference between controls?

It's good to remind audience what you expect to be expressed in alsterpaullone treated regenerating physae.

It would help to indicate expected band sizes for each (e.g., with arrows to the side of the gel).

Make clearer the difference between the two gels--e.g., was the same template used in both cases?
The second set up

12 N. vectensis specimens from Sippewissett were cut in half laterally. 6 were placed in DMSO, 6 into alsterpaullone as before. However, after 2 days, 2 specimens from the alsterpaullone group were moved to a petri dish containing sea water while the rest remained in their chemical baths.

Results, Summarized

• NvWnt3 expressed in generating head, appears to be slightly expressed in physa
• NvDkk, NvWnt5, NvWntA neither expressed in physa or regenerating head

So What? (Discussion/Conclusions):

Wnt3 expression in regenerating heads: Expected result. In many organisms in which the canonical Wnt pathway has been shown to be responsible for parts of embryogenesis and regeneration, Wnt3 is the specific protein involved in organizing head production (Lewis 2008, Lan 2006).
• More evidence for how highly conserved Wnt functions are, encourages searching for more homologs with similar functions in greatly different species.
• Shows that some genes are used in both embryogenesis and regeneration for the same function (in Nematostella, at least)

Wnt5 - expected around tentacle buds
WntA - implicated in oral-end morphogenesis

So What? (Discussion/Conclusions):

The absence of NvDkk is curious:
• In Hydra, HyDkk is the ‘ouch’ signaling molecule. It is expressed strongly during the first hour after injury and begins the process of regeneration, but once the budding stage is reached, it’s silenced (Guder, 2005). If NvDkk, the Nematostella ortholog, was going to be expressed, it would be shortly after injury and well before the physa or head organizer has formed. This really calls for a more time controlled study of gene expression.
• Other possibility: Hydra’s cell maintenance pathway is greatly different from that of Nematostella.
Fun Fact: Head formation is actually INHIBITED as long as *Nematostella* is in the alsterpaullone bath. Wnt3 has roles as an inhibitor as well as initiator. This agrees with research on vertebrates ([Niehrs p. 131](#)) which shows Wnt signaling inhibiting head formation once the head organizer is established. These results show that the (ectopic) head organizer can be established within 2 days (of alsterpaullone treatment).

• Has implications for Wnts being self-regulating

Be careful not to imply that the "head" of a cnidarian is homologous to the "head" of a vertebrate

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**Day 2**

(A) *Nematostella* specimen regenerating in 5mM DMSO control solution
(B) *Nematostella* specimen regenerating in 5μM alsterpaullone solution
(C) A different *Nematostella* specimen in same experimental soln.

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**Day 3**

(A) Same specimen from prev. slide
(B) Same specimen, same soln.
(C) Same specimen moved to 1/3 strength sea water

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**Day 6**

(A) Same specimen, note complete physa
(B) Same specimen, note NO tent. growth
(C) Same specimen moved to 1/3 strength sea water, tentacles have appeared.

This one instance seems compelling. How many replicates have you done?
References

- The Wnt Homepage - [http://www.stanford.edu/~rnusse/wntwindow.html]
- Killian, et al. 2003 The role of Ppt/Wnt5 in regulating cell shape and movement during zebrafish gastrulation
- Thorpe et al., 1997 Wnt Signaling Polarizes an Early C. elegans Blastomere to Distinguish Endoderm from Mesoderm
- Stoick-Cooper 2006 Distinct Wnt signaling pathways have opposing roles in appendage regeneration
- Christoph Niehrs in *The Vertebrate Organizer*
- Kusserow, 2005 Unexpected complexity of the Wnt gene family in a sea anemone
- Lengfeld et al., 2009 Multiple Wnts are involved in Hydra organizer formation and regeneration
- Burton and Finnerty - Conserved and novel gene expression between regeneration and asexual fission in *Nematostella vectensis*
- Philipp et al. 2009 - Wnt/β-Catenin and noncanonical Wnt signaling interact in tissue evagination in the simple eumetazoan Hydra