

Responses to low dissolved oxygen in the bearded fireworm, *Hermodice carunculata* (Annelida: Amphinomidae)

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Background

Oxygen plays a key role in the metabolic processes of most organisms. In the marine environment, levels of dissolved oxygen (DO) can vary greatly over space and time and are a major factor in shaping biological communities. The bearded fireworm, *Hermodice carunculata*, is a common species of amphinomid polychaete throughout the Atlantic Ocean and its adjacent basins. *H. carunculata* inhabits shallow-water reef, rocky and coarse sediment habitats and reaches particularly high densities in areas where microbial decomposition depletes oxygen, such as coral-algae interfaces and fish farm outfalls. It is an omnivore which can cause damage to certain corals by direct feeding and as a vector to coral diseases. Because of its ecological importance, its common occurrence, wide geographic distribution, tolerance to low DO, and ease of collection and maintenance in culture, *H. carunculata* can serve as a model species to examine molecular and organismal responses to low DO.

Objectives

Identify genes responsible for sensing and responding to low DO in *H. carunculata*:

- Re-analysis of available transcriptome data to identify genes involved in oxygen sensing
- Differential gene expression analysis using field-collected specimens from normoxic and low DO sites
- Differential gene expression analysis under exposure to different DO levels in controlled lab experiments

Oxygen sensing overview

Some of the key players in oxygen sensing in animals are⁽¹⁾:

- Hypoxia Inducible Factor (HIFs): heterodimeric transcription factors with a basic helix-loop-helix structure which trigger downstream cellular responses to low DO.
 - HIF1- α : Under normoxic conditions the alpha subunit is hydroxylated at one of two proline residues and unstable. Under hypoxic conditions it dimerizes with the beta subunit to become a functional transcription factor
 - HIF1- β : stable subunit
- HIF Proline Hydroxylase (PHD): oxygen-dependent enzyme responsible for hydroxylation of HIF1- α under normoxic conditions
- Factor Inhibiting HIF (FIH): oxygen-dependent enzyme with a similar function as PHD, but remains functional under lower DO conditions than PHD

Analysis of existing data

Transcriptome re-assembly: a transcriptome of *H. carunculata* was sequenced by Mehr et al.⁽²⁾ and deposited in the NCBI SRA archive under Bioproject PRJNA177345. For the present study, Illumina read data were retrieved using the SRA Toolkit 2.5.7⁽³⁾ and re-assembled using Trinity v. 2.2.0^(4,5).

Assembly stats

Total assembled transcripts	348,690
Total Trinity "genes"	270,030
Total assembled bases	235,360,286
N50	1,080 bp
Average transcript length	675 bp
GC content	40.59 %

BLAST searches: the Open Reading Frames (ORFs) for the respective genes from other lophotrochozoan taxa (if possible) were used as "baits" to search the assembled *H. carunculata* transcriptome in CLC Genomics Workbench 8.0.1.

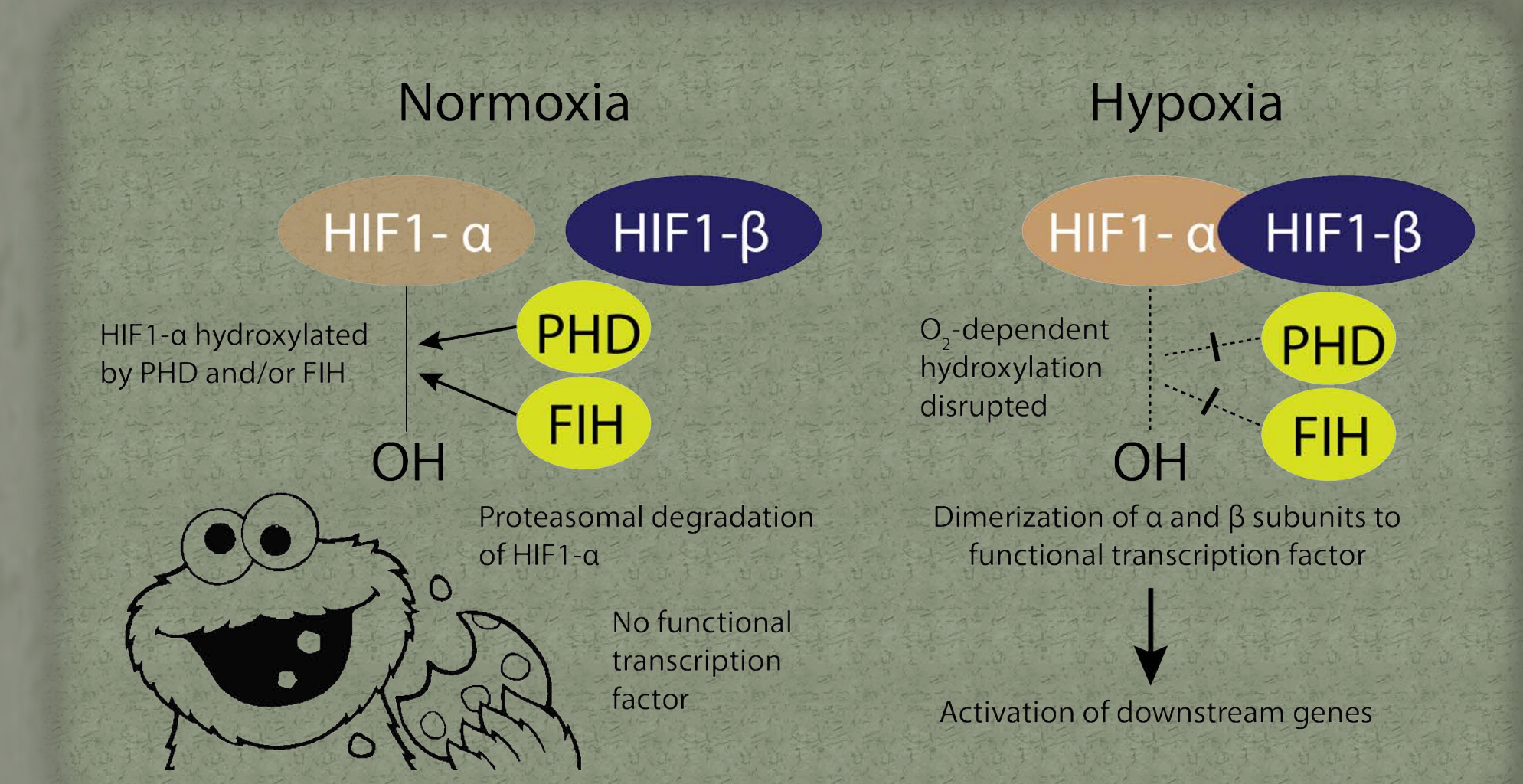
Conserved domain analysis: ORFs were extracted from transcripts with significant BLAST hits. Conserved domains were then identified using the NCBI Conserved Domain Database⁽⁶⁾ and plotted using ExpASy Prosite⁽⁷⁾.

BLAST results

Gene	Bait sequences	BLAST hit in <i>H. carunculata</i> (AA)	% identity	Bit score	E value
HIF1- α	<i>Haliotis diversicolor</i> (KC149963)	TRINITY_DN102595 (804)	67	194.2	2E-47
	<i>Crassostrea gigas</i> (AB289857)		65	169	6E-40
	<i>Crassostrea virginica</i> (HM441046)		69	178	1E-42
HIF1- β	<i>Haliotis diversicolor</i> (KC256820)	TRINITY_DN128350 (1103)	76	823.6	0E-00
	<i>Crassostrea gigas</i> (KUY127505)		74	675.7	0E-00
PHD	<i>Octopus bimaculoides</i> (XM_014920396)	TRINITY_DN127990 (434) TRINITY_DN109650 (401)	70/64	140.1/55.4	2E-31/6E-06
	<i>Aplysia californica</i> (XM_005090729)		71/66	131.1/82.4	1E-28/5E-14
	<i>Crassostrea gigas</i> (XM_011418954)		70/69	120.3/116.7	2E-25/2E-24
FIH	<i>Macrobrachium nipponense</i> (KP965882)	TRINITY_DN119862 (289)	67	143.8	1E-32



Hermodice carunculata (Photo: Tiffany Rudek)



Interactions among some of the key players in oxygen sensing in animals under normoxic and hypoxic conditions

Experimental set-up (in progress)

- 25 specimens of *Hermodice carunculata* were collected by SCUBA divers off the coast of Panama City, FL
- The fireworms will be maintained in two experimental tanks and one control tank
- Oxygen levels will be controlled by bubbling nitrogen into the experimental tanks, while the control tank will remain normoxic
 - Experimental tank 1: ~3 ml O₂/l
 - Experimental tank 2: ~5 ml O₂/l
 - Control tank: ~8 ml O₂/l
- Specimens will be sacrificed for RNA-Seq analyses at 1 week, 2 weeks and 4 weeks after the start of the experiment

Summary

- The re-analysis of the *H. carunculata* transcriptome has revealed transcripts for four major players in oxygen sensing
- The domain structure of HIF1- α corresponds to that found in a wide range of other animal taxa
- At least two different isoforms exist for PHD
- The data strongly suggest that *H. carunculata* utilizes the same conserved oxygen sensing mechanism as other metazoans

Future directions

- Identify oxygen thresholds at which the HIF cascade is activated
- Identify downstream genes activated by the HIF cascade

BIG THANKS to:

- Jacque Cresswell, Joe Bosquez and the students of the Scientific Diving class at TAMU-Galveston for collecting *H. carunculata*
- Tiffany Rudek and Jesus Vega for taking great care of the worms
- Katie St. Claire and the staff of the TAMUG Sea Life Facility for logistics support
- TAMU-CAPES Collaborative Grant Program (grant 2015-16)

References

- (1) Kaelin Jr WG and Ratcliffe PJ (2008). Mol. Cell 30: 393-402
- (2) Mehr S et al. (2015). BMC Genomics 16: 1-13
- (3) Leinonen R et al. (2010). Nucl. Acids Res. 2010: 1-3
- (4) Grabherr MG et al. (2011). Nat. biotech. 29: 644-652
- (5) Haas BJ et al. (2013). Nat. Protoc. 8: 1494-1512
- (6) Marchler-Bauer A et al. (2011). Nucl. Acids Res. 39: D225-D229
- (7) De Castro E et al. (2006). Nucl. Acids Res. 34: W362-365

Conserved domains of HIF1- α

