

Identification and Expression of TGF- β Smads in the ctenophore *Pleurobranchia bachei*

Jennifer Gardner^{1, 2}

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¹ University of Washington, Friday Harbor Laboratories, Friday Harbor, WA 98250

² School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98195

Contact Information:

Jennifer Gardner

School of Aquatic and Fishery Sciences

University of Washington

1122 NE Boat St

Seattle, WA 98105

jgardn92@uw.edu

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Abstract

The TGF- β superfamily of signaling pathways is key in regulating embryonic development (Heldin et al. 2009). It is known to be present in all metazoan lineages (Huminiecki 2009). Studying this pathway in the ctenophore *Pleurobrachia bachei* may allow insights into the recent placement of ctenophores as the most basal metazoan lineage (Dunn et al. 2008). Studying Smad4 expression in embryos will determine where the TGF- β pathway is active during development. Adult *P. bachei* were collected from Friday Harbor Laboratories in Friday Harbor, WA. They were spawned; embryos collected, fixed and stained using whole mount antibody staining. Expression patterns revealed possible maternal derivatives of Smad4 in early stage embryos as well as possible use of the TGF- β pathway as a neurotransmitter in larvae that have finished development.

Introduction

The transforming growth factor- β (TGF- β) signaling pathway is important in the regulation of metazoan embryonic development (Heldin et al. 2009, Holstein et al. 2011, Massague 1998, Massague and Wotton 2000, Moustakas and Heldin 2009). This pathway consists of many polypeptides including TGF- β receptors, Smad proteins, ligands, receptor antagonists, and pathway antagonists. Transcription factors turned on by the TGF- β pathway activate target genes in the nucleus of cells. These products can arrest cell division, induce cell death, or induce cell differentiation (Heldin et al. 2009, Massague 1998, Massague and Wotton 2000, Moustakas and Heldin 2009). First an extracellular ligand binds to a TGF- β Type II membrane-bound receptor. The Type II receptor then binds to a Type I receptor and activates it via phosphorylation. The phosphorylated Type I receptor then activates a Smad protein to be activated via phosphorylation. The Smad proteins are the intracellular components of the pathway that dimerize and act as transcription factors in the nucleus, where they regulate the target genes (Heldin et al. 2009, Holstein et al. 2011, Massague 1998, Massague and Wotton 2000, Moustakas and Heldin 2009). Different Smad proteins are used depending on the signal received by the receptor (Heldin et al. 2009, Massague 1998, Massague and Wotton 2000, Moustakas and Heldin 2009). The two major signaling classes within the TGF- β super family are TGF- β and bone morphogenetic protein (BMP). Smad 1 and 5 are associated with BMP-like signaling (Heldin et al. 2009, Massague 1998, Massague and Wotton 2000, Moustakas and Heldin 2009, Pang et al. 2011). Smad 2 and 3 are associated with TGF- β -like signaling (Heldin et al. 2009, Massague and Wotton 2000, Moustakas and Heldin 2009, Pang et al. 2011). Collectively these are known as receptor-

associated Smads (R-Smads). There is also a common-mediator Smad (Co-Smad), called Smad4. Once an R-Smad has been activated, the Co-Smad binds to it and this complex is what acts as a transcription factor in the nucleus. Additionally there are inhibitory Smads (I-Smads), like Smad 6, 7, which block R-Smads from binding to the Co-Smad and inhibit intracellular signal transduction (Heldin et al. 2009, Massague 1998, Massague and Wotton 2000, Moustakas and Heldin 2009, Pang et al. 2011).

Components of this pathway differ between metazoan taxa (Holstein et al. 2011, Pang et al. 2011). Among the bilaterians all the components are present (Holstein et al. 2011, Pang et al. 2011). However some basal metazoans, including the sponges, ctenophores, placozoans, and cnidarians, lack certain components (Holstein et al. 2011, Pang et al. 2011). It has recently been proposed that ctenophores may be the most basal metazoans rather than sponges (Dunn et al. 2008). Studying basic developmental signaling pathways in these two phyla may help resolve the root of the metazoan tree.

The recent publications of full genomes for some ctenophore and poriferan species have provided insight into this pathway in basal metazoans (Huminiecki et al., Moroz et al. 2012, Ryan et al. 2011, Srivastava et al. 2010). So far there is only one published ctenophore genome available, that of *Mnemiopsis leidyi* (Ryan et al. 2011). The genome of *Pleurobranchia bachei* has been sequenced but not yet published (Moroz et al. 2012). Most of the major components of the TGF- β pathway have homologues identified in the genome of *M. leidyi* (Pang et al. 2011). Genes for diffusible antagonists, including Chordin, Noggin, Follistatin and the CAN family members were not found (Pang et al. 2011). These genes were also not found in the sponges but are found in the cnidarians and bilaterians (Pang et al. 2011). Antagonists for the *Wnt* pathway are also

missing from the *M. leidy* genome (Pang et al. 2010), suggesting that extracellular antagonists for developmental signaling pathways evolved after the ctenophorans and poriferans diverged from the metazoans.

This study aimed to find patterns of expression of the activated Smad4 protein in the cydippid ctenophore *Pleurobrachia bachei*. Smad4 is ideal for studying the entire TGF- β super family as it dimerizes with all the R-Smads and functions in every type of signaling within the super family. Studying expression of the TGF- β pathway in two separate classes of ctenophore may offer insights into the evolution of developmental regulation in basal metazoans.

Materials and methods

Sampling and Handling:

Pleurobrachia bachei adults were collected off the dock at University of Washington Friday Harbor Laboratories, Friday Harbor, Washington. Adults were kept in sea water tables in containers with mesh sides to allow water flow. To spawn, no more than eight adults were placed in gallon spawning jars which were then covered to simulate night. Adults were left in spawning jars overnight and embryos were collected the following morning. If spawning did not occur adults were fed with ~5mL per jar of a five minute plankton tow and set up to spawn the following evening. Embryos were allowed to develop in glass dishes kept cool by sea water tables and were collected at appropriate developmental stages.

Whole Mount Antibody Staining:

Adult *P. bachei* were fixed in 4% paraformaldehyde in filtered seawater overnight. Embryos were fixed in 3.7% formaldehyde and 0.2% glutaraldehyde for five minutes then transferred into 3.7% formaldehyde for one hour. They were rinsed in phosphate buffered saline (PBS) then stored in methanol at -20°C. Embryos were rehydrated gradually in PBS and methanol and then stained using a 1:500 dilution of Anti-Smad4 antibody (Abcam ab137861) following general antibody staining protocol. Embryos were stained overnight in primary antibody and then again in secondary antibody. Mounting was done using Vectorshield glycerol based mounting medium. Control embryos were stained with a 1:500 dilution of Anti- β Catenin antibody (Abcam ab16051). Stained embryos were viewed using a Nikon E600 fluorescent microscope. Images were captured using a Q imaging micro publisher 5.0 Rtv and Q capture v2.7.3. Image colors were optimized using ImageJ 1.43u.

Phylogenetic analysis

Gene sequences were found using a BLAST search on NCBI GenBank using *Mnemiopsis leidyi* Smad4 sequence (Pang et al. 2011) as the query sequence. Additional taxa not found using the BLAST search were added from the supporting information protein alignment of Smad proteins from Pang et al. (2011). Protein sequences were compiled and aligned in MEGA 5.1 (Tamura et al. 2011). Sequences were aligned using Muscle and then aligned by eye for the MH1 and MH2 conserved domains. Maximum likelihood gene trees were constructed using MEGA 5.1. Gene trees followed the Jones-

Taylor-Thornton (JTT) model of amino acid substitution with Gamma distributed rates among sites with 500 bootstrap replicates.

Results

Phylogenetic analysis

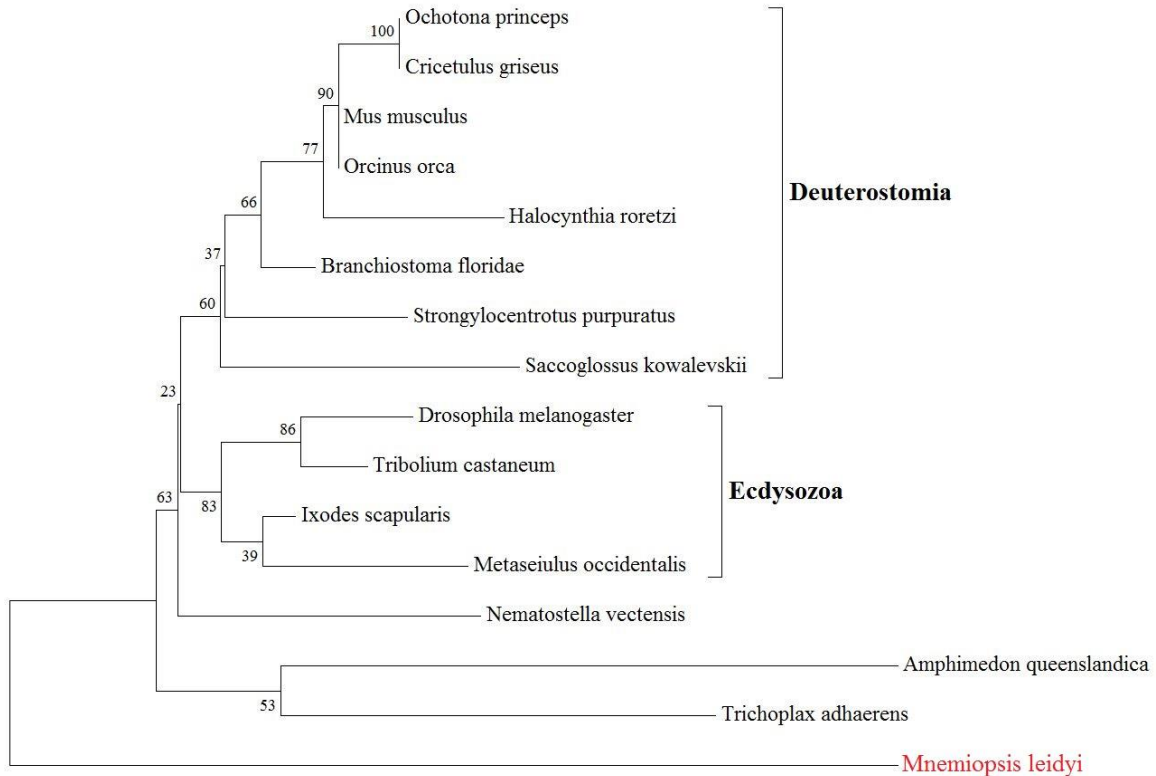


Figure 1. Phylogenetic gene tree of Smad4 across metazoan taxa.

Phylogenetic analysis of the Smad4 protein was able to accurately recover clades Bilateria, Deuterostomia, Ecdysozoa, and Mammalia. *M. leidyi* was placed as the root of the tree. Cnidaria comes out as sister to Bilateria. Branch lengths for *M. leidyi*, *A. queenslandica*, and *T. adhaerens* suggest that these sequences are divergent compared to the Cnidarian and Bilaterian sequences.

Whole Mount Antibody Staining

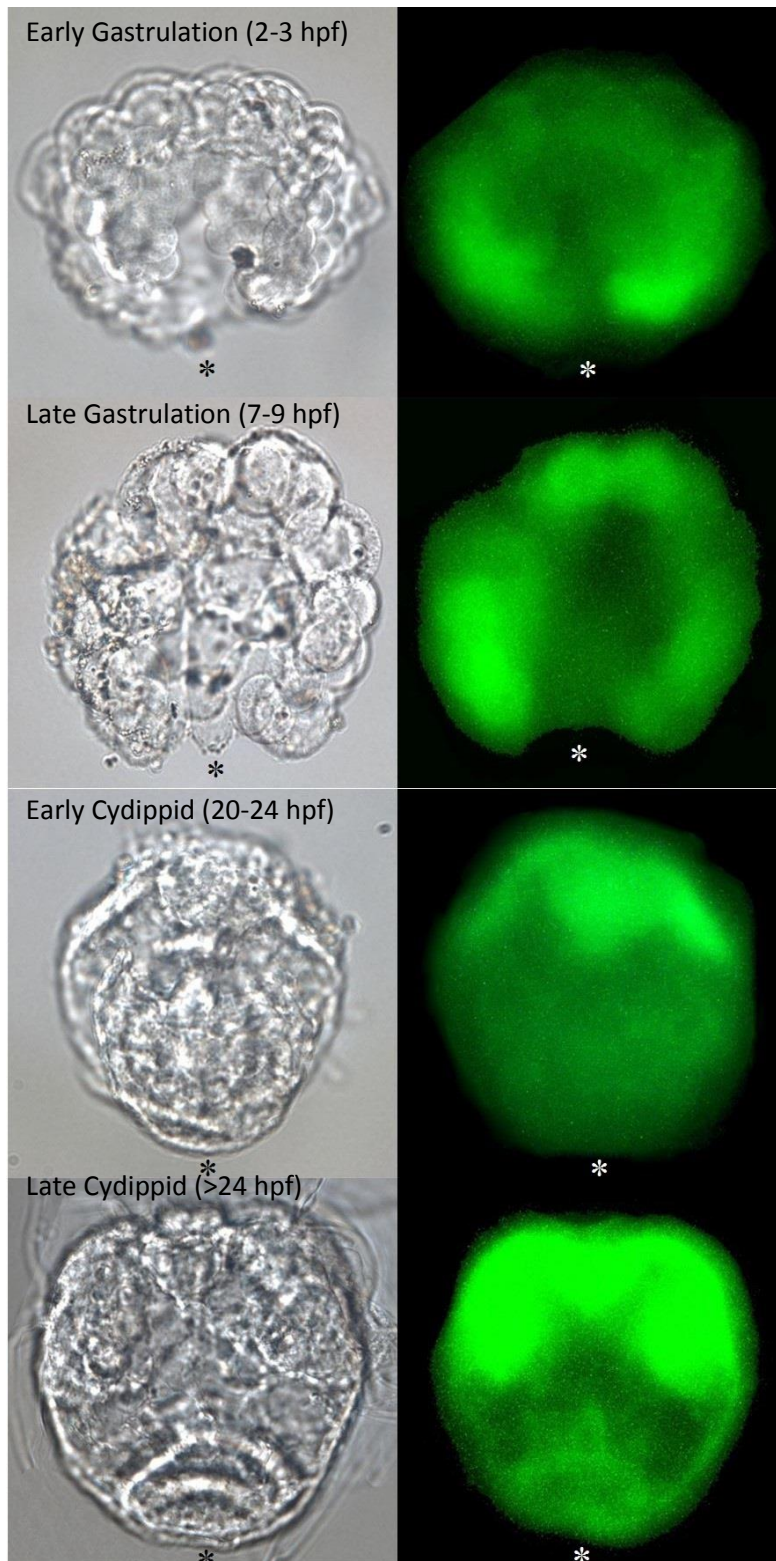


Figure 2. Immunofluorescent whole mount antibody staining for Smad4 in *Pleurobrachia bachei*. Left hand images are white light microscope images. Right hand images are the same embryo under fluorescent light. White light and fluorescent images were taken at the same plane of focus. * indicates the location of the blastopore or mouth. Stages labeled by name and time in hours post fertilization (hpf). All images taken at 20x magnification.

In early gastrulation Smad4 is expressed in the lips of the gastrulating embryo (Figure 2). Late gastrulation embryos show Smad4 expression near what will become the aboral organ (Figure 2). Additional expression was seen on the left side of the embryo that was not seen on the right (Figure 2). Early cydippid larvae show strong expression in the aboral organ (Figure 2). Late cydippid larvae show expression in the aboral organ and the tentacle bulbs (Figure 2).

Discussion

Phylogenetic analyses of the Smad4 protein closely followed the current hypothesis of the metazoan tree (Figure 1) (Dunn et al. 2008). The ctenophore *Mnemiopsis leidyi* was placed as the root of the tree as was found in the analysis of whole genomes by Dunn et al. (2008). The clade Ecdysozoa was recovered with high support and Deuterostomia was recovered with low support (Figure 1). There was no out group included in the analysis as an appropriate out group does not exist due to the fact that this signaling pathway is found only in metazoans (Holstein 2011, Swalla, personal communication). The choanoflagellate *Monosiga brevicollis* contains a Smad-like protein sequence (Pang et al. 2011). This sequence works well as an out group for analyses of the entire family of Smad proteins but is not an orthologue to any single Smad and thus is inappropriate as an out group for an analysis of only Smad4 sequences. Additionally, Lophotrochozoans are not represented in the tree due to poor taxon sampling and poor sequence quality of the sampled taxa.

Whole mount antibody staining of *P. bachei* embryos revealed some interesting trends in four different stage embryos (Figure 2). Expression of Smad4 was seen in the

lips surrounding the blastopore of an embryo that was undergoing early gastrulation (Figure 2). Pang et al. (2011) performed *in situ* hybridization in embryos of *M. leidy* and found no mRNA expression in embryos this early in development. Finding protein expression but not mRNA expression in this early stage suggests that the protein is of maternal origin. In order to confirm this repeat staining and *in situ* hybridization would need to be done on embryos of the same species.

Late stage gastrulating embryos showed Smad4 expression in cells destined to become part of the aboral organ (Figure 2). This stage of embryo showed the same expression pattern in *M. leidy* *in situ* hybridization (Pang et al. 2011). Expression was also seen on the left side of the embryo but not on the right (Figure 2). This is suggestive of left-right asymmetry that could be attributed to Nodal signaling. To determine which type of signaling is being expressed here it would be necessary to repeat the experiment with a Nodal or BMP inhibitor added.

Cydidippid larvae showed expression in the aboral organ and at later stages in the tentacle bulbs (Figure 2). *In situ* hybridization showed some expression in the aboral organ but little expression in the tentacle bulbs (Pang et al. 2011). At this stage development is more or less complete so it is unlikely that the TGF- β pathway is still being used to regulate development. Recent work in ctenophores has suggested that developmental pathways such as *Wnt* and TGF- β may be used post-development as neurotransmitters (BJ Swalla, personal communication). The expression pattern seen in the cydidippid larvae is suggestive of the use of the TGF- β pathway as a neurotransmitter (Figure 2).

There is much further research that could be done with this project. First to ensure that Smad4 was actually being stained for a Smad inhibitor could be added and staining would be repeated. If the same expression was not seen in the presence of an inhibitor this would indicate that original staining was working. Cloning the Smad genes from *P. bachei* and creating probes to perform *in situ* hybridization would offer a direct comparison of mRNA to protein expression in embryos of the same species. Additionally, sequencing these genes would allow further phylogenetic analysis to be done and offer interesting insights in the comparison of *P. bachei* to *M. leidyi*. There is much that could be done to explore the use of developmental signaling pathways as neurotransmitters post-development such as adding recombinant TGF- β or BMP and monitoring for behavioral changes.

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Supplement 1

CLUSTAL 2.1 multiple sequence alignment of Smad4 protein sequences

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Ochotona_princeps      GGESETFAKRAIESLVKKLKEKKDELDSLITAITTNGAHPSKCVTIQRTL
Cricetulus_griseus     GGESETFAKRAIESLVKKLKEKKDELDSLITAITTNGAHPSKCVTIQRTL
Mus_musculus           GGESETFAKRAIESLVKKLKEKKDELDSLITAITTNGAHPSKCVTIQRTL
Orcinus_orca           GGESETFAKRAIESLVKKLKEKKDELDSLITAITTNGAHPSKCVTIQRTL
Halocynthia_oretzi     GGESETFAKRAIESLVKKLKEKKDELEGLIAAITTNGAHPTTCVTIQRTL
Branchiostoma_floridae GGETETFAKRAIESLVKKLKEKKDELDSLITAITTNGAHPSKCVTIQRTL
Strongylocentrotus_purpuratus GGESESAKRAIESLVKKLKEKRDELDSLITAITTNGAHPSKCVTIQRTL
Saccoglossus_kowalevskii GGESESAKRAIESLVKKLKEKRDELDSLITAITTNGAHPSKCVTIQRTL
Drosophila_melanogaster GGESESAKRAIESLVKKLKEKRDELDSLITAITTNGAHPSKCVTIQRTL
Tribolium_castaneum    GGESESAKRAIESLVKKLKEKRDELDSLITAITTNGAHPSKCVTIQRTL
Ixodes_scapularis      GGESESAKRAIESLVKKLKEKRDELDSLITAITTNGAHPSKCVTIQRTL
Metaseiulus_occidentalis GGESESAKRAIESLVKKLKEKKDELDSLITAITTNGAHPSKCVTIQRTL
Nematostella_vectensis GGESESAKRAIESLVKKLKEKKDELDSLITAITTNGAHPSKCVTIQRTL
Amphimedon_queenslandica GGESESAKRAIESLVKKLKEKKDELDSLITAITTNGAHPSKCVTIQRTL
Trichoplax_adhaerens   GGESESAKRAIESLVKKLKEKKDELDSLITAITTNGAHPSKCVTIQRTL
Mnemiopsis_leidy       GGEDETFAKRAIESLVKKLKEKRDELDAIIAVTMSGRRPSKCVTIQRTL
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Ochotona_princeps      DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Cricetulus_griseus     DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Mus_musculus           DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Orcinus_orca           DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Halocynthia_oretzi     DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Branchiostoma_floridae DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Strongylocentrotus_purpuratus DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Saccoglossus_kowalevskii DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Drosophila_melanogaster DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Tribolium_castaneum    DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Ixodes_scapularis      DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Metaseiulus_occidentalis DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Nematostella_vectensis DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Amphimedon_queenslandica DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Trichoplax_adhaerens   DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
Mnemiopsis_leidy       DGRLQVAGRKGFPFHVYARLWRWPD LHKNELKHVKYCQYAFDLKCDSDVCV
*****:*****:*.***:.*: *.* * : :.*** **

Ochotona_princeps      NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Cricetulus_griseus     NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Mus_musculus           NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Orcinus_orca           NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Halocynthia_oretzi     NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Branchiostoma_floridae NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Strongylocentrotus_purpuratus NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Saccoglossus_kowalevskii NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Drosophila_melanogaster NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Tribolium_castaneum    NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Ixodes_scapularis      NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Metaseiulus_occidentalis NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Nematostella_vectensis NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Amphimedon_queenslandica NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Trichoplax_adhaerens   NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
Mnemiopsis_leidy       NPYHYERVVSPGIDL-SGLTLPQSMVLKDPNIPVASTTPEYWCSIAFYEM
*****:*****:*.***:.*: *.* * : :.*** **

Ochotona_princeps      DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL
Cricetulus_griseus     DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL
Mus_musculus           DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL
Orcinus_orca           DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL
Halocynthia_oretzi     DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL
Branchiostoma_floridae DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL
Strongylocentrotus_purpuratus DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL
Saccoglossus_kowalevskii DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL
Drosophila_melanogaster DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL
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Ixodes_scapularis      DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL
Metaseiulus_occidentalis DVQVGETFKVPSSCPVTVTDGYVDPSSGDRFCLGQLSNVHRTAIERARL

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Nematostella_vectensis	DQQVGEIFKVTSNCPSVTVDGYVDPSPGGNRFCLGQLSNVHRTEASERARL
Amphimedon_queenslandica	DAPVGECKFKVPASLTSVSDGFDVDPSPGGDRFCLGRLSNVHRTEASERARL
Trichoplax_adhaerens	DLQVGESFKVPSQFHTVSVSDGFDVDPSPGGNRFCLGQLSNVHRTEASERARL
Mnemiopsis_leidy	DHKVGETFKVIAQYREVKIDGYVNPSEPNRFCLGQLSNVHRTEASEKARL
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Ochotona_princeps	HIGKGVQLECKEGEDVWVRCLSDHAVFVQSYLLDREAGRPGDAVHKIYP
Cricetulus_griseus	HIGKGVQLECKEGEDVWVRCLSDHAVFVQSYLLDREAGRPGDAVHKIYP
Mus_musculus	HIGKGVQLECKEGEDVWVRCLSDHAVFVQSYLLDREAGRPGDAVHKIYP
Orcinus_orca	HIGKGVQLECKEGEDVWVRCLSDHAVFVQSYLLDREAGRPGDAVHKIYP
Halocynthia_oretzi	HIGKGVQLVCHGEDVWVKCLSDHAVFVQSYLLDREAGRPGDAVHKIYP
Branchiostoma_floridae	HIGKGVQLDLRGEEDVWVRCLSDHAVFVQSYLLDREAGRPGDAVHKIYP
Strongylocentrotus_purpuratus	HIGKGVQLELKGEDVWVRCLSDHAVFVQSYLLDREAGRPGDAVHKIYP
Saccoglossus_kowalevskii	HIGKGVQLDLRGEEDVWVRCLSDHAVFVQSYLLDREAGRPGDAVHKIYP
Drosophila_melanogaster	HIGKGVQLDLRGEEDVWVRCLSDNSVVFQSYLLDREAGRTPGDAVHKIYP
Tribolium_castaneum	HIGKGVQLDLRGEEDVWVRCLSDHSVVFQSYLLDREAGRQPGDAVHKIYP
Ixodes_scapularis	HIGKGVQLDLRGEEDVWVRCLSDHSVVFQSYLLDREAGRPGDAVHKIYP
Metaseiulus_occidentalis	HIGKGVQLDVKGEDVWVRCLSDHSVVFQSYLLDREAGRQPGDAVHKIYP
Nematostella_vectensis	HIGKGVQLDVRGEEDVWVRCLSEHSVVFQSYLLDREAGRCPGDAVHKIYP
Amphimedon_queenslandica	HIGKGVQLDVRGEEDVWVRCLSEHSVVFQSYLLDREAGRCPGDAVHKIYP
Trichoplax_adhaerens	HIGKGVRLCHGEDVWVLSCLSEHSVVFQSYLLDREAGRPFDYVHKIYP
Mnemiopsis_leidy	HVGKGVKLTLSEGEDVWVLECSQHPVFQSYLLDKEAKRPGDAVHKIYP
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Ochotona_princeps	SAYIKVFDLRQCHRMQQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Cricetulus_griseus	SAYIKVFDLRQCHRMQQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Mus_musculus	SAYIKVFDLRQCHRMQQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Orcinus_orca	SAYIKVFDLRQCHRMQQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Halocynthia_oretzi	NAYIKVFDLRQCYRQMQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Branchiostoma_floridae	SAYIKVFDLRQCHRMQQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Strongylocentrotus_purpuratus	TAYIKVFDLRQCYRQMQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Saccoglossus_kowalevskii	NAYIKVFDLRQCHRMQQAATAQAGAS-----SVGGIAPAVSK
Drosophila_melanogaster	AACIKVFDLRQCHQMHLATNAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Tribolium_castaneum	SAYIKVFDLRQCHQMHTQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Ixodes_scapularis	SAYIKVFDLRQCHQMQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Metaseiulus_occidentalis	YAYIKVFDLRQCHQMQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Nematostella_vectensis	SAYIKVFDLRQCHQMQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Amphimedon_queenslandica	KAYIKVFDLRQCHQMQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Trichoplax_adhaerens	KAYIKVFDLRQCHQMQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
Mnemiopsis_leidy	GTHLKVFDLRQCHQMQAATAQAGVDDLRLRLCILRMSFVKGWGPDIYPR
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Ochotona_princeps	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Cricetulus_griseus	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Mus_musculus	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Orcinus_orca	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Halocynthia_oretzi	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Branchiostoma_floridae	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Strongylocentrotus_purpuratus	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Saccoglossus_kowalevskii	L-----LLSLKHATYMYNNMEMQ
Drosophila_melanogaster	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Tribolium_castaneum	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Ixodes_scapularis	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Metaseiulus_occidentalis	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Nematostella_vectensis	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Amphimedon_queenslandica	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Trichoplax_adhaerens	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
Mnemiopsis_leidy	QSIKETPCWIEIHLHRLQLLDEVLTMPADIADPQPLD
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