Environmental sensing and response genes in Cnidaria: the chemical defensome in the sea anemone *Nematostella vectensis*

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Keywords:
cytochrome P450; glutathione transferase; ABC transporter; aromatic hydrocarbon; nuclear receptor; metal; superoxide dismutase; oxidative stress

Abstract

The starlet sea anemone *Nematostella vectensis* has been recently established as a new model system for the study of the evolution of developmental processes, as cnidaria occupy a key evolutionary position at the base of the bilateria. Cnidaria play important roles in estuarine and reef communities, but are exposed to many environmental stressors.

Here I describe the genetic components of a 'chemical defensome' in the genome of *N. vectensis,* and review cnidarian molecular toxicology. Gene families that defend against chemical stressors and the transcription factors that regulate these genes have been termed a 'chemical defensome,' and include the cytochromes P450 and other oxidases, various conjugating enyzymes, the ATP-dependent efflux transporters, oxidative detoxification proteins, as well as various transcription factors. These genes account for about 1% (266/27200) of the predicted genes in the sea anemone genome, similar to the proportion observed in tunicates and humans, but lower than that observed in sea urchins. While there are comparable numbers of stress-response genes, the stress sensor genes appear to be reduced in *N. vectensis* relative to many model protostomes and deuterostomes. Cnidarian toxicology is understudied, especially given the important ecological roles of many cnidarian species. New genomic resources should stimulate the study of chemical stress sensing and response mechanisms in cnidaria, and allow us to further illuminate the evolution of chemical defense gene networks.

Cnidaria occupy a key basal evolutionary position within Metazoa (Dunn et al, 2008), with recent evidence suggesting that they are early-diverging bilaterians (de Jong et al, 2006; Matus et al, 2006). Cnidaria have important ecological roles as reef structure builders, and as predators and prey in planktonic and benthic ecosystems [e.g. (Harborne et al, 2006; Sebens, 1981)]. Cnidaria are sensitive to many environmental stressors, and have been used as indicators of water quality (Arkhipchuk et al, 2006; Davies and Freeman, 1995; Wiger and Stottum, 1985). With a better understanding of regulatory processes and development of appropriate endpoints [e.g. (Tarrant, 2007)], cnidaria will become valuable indicators of exposure to disruptive chemicals and other stressors.

The starlet sea anemone *Nematostella vectensis* has been recently established as a new model system for the study of the evolution of developmental processes (Darling et al, 2005; Putnam et al, 2007), and may act as a model for the basic molecular biology of anthozoans. The remarkable amenability of this species to laboratory manipulation has already made it a productive system for exploring cnidarian development and the origins of bilateral symmetry (Finnerty and Martindale, 1999; Finnerty et al, 2004; Fritzenwanker et al, 2004; Kusserow et al, 2005; Magie et al, 2005; Matus et al, 2006; Torras and Gonzalez-Crespo, 2005).

N. vectensis is a burrowing estuarine anemone, with populations in the eastern Pacific, northern English Channel, western North Sea, and western Atlantic (Hand and Uhlinger, 1994), although it is likely that all but the western Atlantic represent introduced populations (Reitzel et al, in press). It can tolerate a remarkably wide ranges of salinities (2-54 ppt; (Sheader et al, 1997)), temperatures (-1 to 28 °C; (Sheader et al, 1997)), and dissolved oxygen concentrations. The facility with which *Nematostella* populations can be investigated within their natural ecological context (Darling et al, 2005) suggests that this model may also be profitably expanded to address important questions in molecular and evolutionary ecology and toxicology. A mechanistic understanding of stress responses is essential to establishing this model system, as with all model systems.

An important question in biology is how cells and organisms maintain homeostasis in a variable environment. The need to deal with physical, chemical, and biological stressors has driven the evolution of an array of gene families and pathways (also known as

"environmental genes" (Ponting, 2008)) that afford protection from challenges. The immune system is one such protective mechanism, which responds to biotic stressors such as pathogens (Miller et al, 2007). Another set of genes comprises the "chemical defensome," encoding a network of defensive proteins that allows the organism to sense, transform, and eliminate potentially toxic chemicals (Goldstone et al, 2006).

The chemical defensome protects against chemically-mediated injury by environmental chemicals such as heavy metals, microbial products, and other natural exogenous compounds, as well as anthropogenically-derived compounds such as hydrocarbon derivatives and pesticides. These compounds are structurally diverse, requiring either non-specific enzymatic responses or a broad array of specific enzymatic actions. In addition, the maintenance of cellular homeostasis requires the inactivation and elimination of endogenous signaling molecules, such as eicosanoids, and defense against endogenously generated toxicants such as reactive oxygen species (ROS).

The chemical defensome is comprised of several classes of proteins that function coordinately to protect the cell (**Figure 1**). These proteins include enzymes that transform chemicals to less toxic and more readily excretable metabolites; efflux transporters that actively eliminate toxicants and transformed products; antioxidant enzymes protecting against externally and internally generated ROS or other radicals; and soluble receptors and ligand-activated transcription factors that act as sensors of toxicants or cellular damage.

Efflux transporter proteins such as the ATP-binding cassette (ABC) transporters can provide the first line of cellular defense (Dean et al, 2001). Once toxicants enter the cytoplasm, however, biotransformation is often required to inactivate or enhance the elimination of toxicants. Biotransformation enzymes include oxidative enzymes such as the cytochromes P450 (CYPs); reductive enzymes such as aldo-keto reductases (AKR), epoxide hydrolase (EH), and NAD(P)H-quinone oxidoreductase (NQO); and conjugative enzymes including glutathione-S-transferases (GST), sulfotransferases (SULT), UDP-glucuronosyl transferases (UGT), and N-acetyl transferases (NAT). Biotransformation generally results in detoxification, but oxidation, *N*-acetylation, sulfate, or glutathione conjugation can lead to toxic metabolites in a chemical and cell specific manner (Gamage et al, 2006; Guengerich et al, 2003; Surh, 1998).

Gene products that protect against injury from chemicals may be especially important in embryos given the complex chemical signaling pathways governing development (Davidson and Erwin, 2006; Hamdoun and Epel, 2007), as well as the need to protect the genome of the germ cells (Epel, 2003). In adults, some of these proteins also provide protection from environmental factors, such as oxidative stress, that can lead to senescence (Finkel and Holbrook, 2000). Many gene products in this network (e.g. CYPs) perform multiple roles, having important endogenous functions (including but not limited to development) as well as functioning in chemical defense.

Here I show that the major elements of the network of genes and pathways that allow an organism to mount a defense against toxic chemicals appear to be conserved in cnidaria , and review relevant aspects of cnidarian molecular toxicology. Almost all of the gene families or superfamilies that are characteristic of the chemical defensive network in deuterostomes (Goldstone et al, 2006) are also represented in the sea anemone (Table 1; see also (Reitzel et al, 2008)), indicating the presence of this system in the bilaterian ancestor and evolutionary conservation. However, while there is general conformity in the presence of higher order gene groups across taxa, in most cases gene orthology is more difficult to determine.

METHODS

Different types of evidence are available for the genes discussed in this paper. Predicted genes are derived from the US Department of Energy Joint Genome Institute (JGI) predictions of the whole genome shotgun assembly (www.jgi.doe.gov). Many of these predicted genes are supported by expression data from an extensive EST collection (Sullivan et al, 2008). Resources are available online at stellabase.org, cnidbase.bu.edu and nematostella.org. In this study, defensome genes were identified by Hidden Markov Model searches (Hmmer v2.3.2; (Eddy, 1998)) of the JGI gene predictions with conserved domains of known defense genes using the PFAM models. Gene homologies were confirmed by reciprocal BLAST of the predicted genes against Genbank. For this study the JGI "best models" were used without significant refinement. Alignments were constructed using Muscle v3.6b (Edgar, 2004), and are available upon request of the author.

The exact nomenclature of many genes presented in this paper is tentative because of the uncertainty in classifying genes to specific subfamilies within the major superfamilies represented in these analyses. I have attempted to follow the nomenclature guidelines for many of the defined gene superfamilies (Hyndman et al, 2003; Jez and Penning, 2001; Mackenzie et al, 2005; Nebert and Vasiliou, 2004; Nelson et al, 1993; Vasiliou and Nebert, 2005; Vasiliou et al, 2006) but due to evolutionary distances some of the subfamily assignments are tentative. Thus, new genes here are given names indicating my understanding of the homologous relationships, but that should not be taken as formal assignments. Formal assignments of new gene names are often reserved by specific nomenclature committees (e.g. the Cytochrome P450 Nomenclature Committee or the Aldo-Keto Reductase Nomenclature Committee). Based on evidence from our previous analysis of the sea urchin genome (Goldstone et al, 2006), gene orthologies may also not be predictive of function.

Phylogenetic trees were constructed by analyzing amino acid sequences using maximum likelihood (RAxML 7.0.3; (Stamatakis, 2006)). Regions of alignment uncertainty were excluded from phylogenetic analysis (Kreil and Ouzounis, 2003) by automatic masking using a custom-written script. The WAG-CAT model of amino acid substitution (Whelan and Goldman, 2001) with a gamma distribution of substitution rates was used in all likelihood analyses, based on likelihood tests using RAxML.

DEFENSOME GENE FAMILIES

Receptors and Signal Transduction

Homologs of most important stress receptors are present in the sea anemone genome, including the aryl hydrocarbon receptor (AHR), hypoxia-inducible factor 1 (HIF1α), and the aryl hydrocarbon nuclear translocator (ARNT), metal transcription factor 1 (MTF1), nuclear factor-kappa B (NFkB), and nuclear factor erythroid-derived 2 related 2 (NRF2), detailed below (**Figure 2**). Although some of the known components of the vertebrate and invertebrate xenobiotic receptor pathways are missing (e.g. pregnane X receptor, liver X receptor, farnesoid X receptor [PXR, LXR, and FXR]), receptors that are not clearly orthologous to known xenobiotic sensors may substitute, or there may be increased xenobiotic receptor promiscuity.

Aryl hydrocarbon receptor (AHR) and related bHLH-PAS proteins. Basic helix-loop-helix PER/ARNT/SIM (bHLH-PAS) family genes encode proteins involved in critical physiological and developmental signaling, including those that mediate responses to certain environmental pollutants (including polynuclear aromatic hydrocarbons) and low oxygen tension (Kewley et al, 2004). bHLH-PAS genes in chordates that have been shown to be important to physiological responses to environmental pollutants include the aryl hydrocarbon receptor (AHR), hypoxia-inducible factor 1 (HIF1α), and the aryl hydrocarbon nuclear translocator (ARNT).

The basic helix-loop-helix (bHLH) gene family has previously been examined in *N. vectensis* and other species (Simionato et al, 2007). Simionato et al. identified 68 bHLH genes, several of which also contained a PAS domain, including 1 or 2 ARNT genes and 0-2 HIF genes (the range depends on uncertainty in the phylogenetic clustering; (Simionato et al, 2007)), but could not identify an AHR in the *N. vectensis* genome. However, Reitzel et al (submitted) identify a gene (gi|156394392) as a putative AHR homolog, and note that its expression is confirmed through an EST. Both AHR and HIF1α form heterodimers with ARNT to regulate transcription of downstream targets through the recognition of specific DNA response elements. Transcriptional responses to potential activators of AHR and HIF have not been well studied in sea anemones, and no data is available to determine if these response elements are conserved in *N. vectensis*.

Oxidative and metal stress-response transcription factors Oxidative stress response factors in vertebrates include the CNC-bZIP family [nuclear factor erythroid-derived 2 (NFE2) and related factors (NRFs)], the BTB-bZIP proteins BACH1 and BACH2, and the small Maf proteins (MafF, MafG, and MafK in particular). Maf proteins in vertebrates are heterodimeric partners of NF-E2, NRFs, and Bach proteins (Igarashi and Sun, 2006). In addition to their roles as heterdimerization partners for various CNC proteins, small Maf proteins have critical roles in vertebrate stress signaling, oncogenesis, and may also have links to the inflammation response (Blank, 2008).

N. vectensis has 2 homologs of the small Maf proteins, an NRE2-like protein homologous to NRF2, and a KEAP1-like protein, which in the absence of oxidative stress in vertebrates encodes a protein that retains NRF2 in the cytoplasm and enhances its

proteasomal degradation (Nguyen et al, 2003). In vertebrates, the NRF2 signaling pathway provides a rapid response to electrophilic or oxidative compounds, and has been show to attenuate carcinogenesis and inflammation (Osburn and Kensler, 2008).

Other important oxidative stress-responding transcription factors with homologs in sea anemone include metal transcription factor 1 (MTF1), and NFkB. MTF1 is well known as a metal-responsive transcription factor (Laity and Andrews, 2007), but has also been proposed as a generalized sensor of oxidative stress (Murphy, 2004; Murphy et al, 1999; Murphy et al, 2005). MTF1 may also interact with HIF1α and contribute to HIF1α activation during hypoxia (Murphy et al, 2005).

Nuclear receptors Ligand-activated nuclear receptors (NRs) function as chemically-activated transcription factors, primarily with endogenous functions but also importantly in xenobiotic sensing. Of greatest interest with regards to the chemical defensome are those related to NRs in the NR1H and NR1I subfamilies which contain vertebrate FXR, LXR, PXR, constitutively active receptor (CAR), and the vitamin D receptor (VDR), as well as arthropod EcR (ecdysone receptor). Other NRs involved in xenobiotic response in vertebrates include estrogen receptor (ER; NR3A subfamily); the peroxisome proliferator receptors (PPARs; NR1C subfamily), which have target genes involved in lipid metabolism, energy homeostasis, and cell differentiation; and the retinoid X receptor (RXR; NR2B subfamily), which has many target genes involved in xenobiotic metabolism.

N. vectensis and other cnidaria appear to lack many nuclear receptors traditionally studied in response to toxicants (e.g. NR1s, ER; (Grasso et al, 2001; Reitzel et al, 2008)). *N. vectensis* appears to have a modest number of NRs (18), none of which are related to the NR1H (PPAR, LXR, FXR) or NR1I (VDR, PXR, CAR) families. However there are genes related to hepatocyte nuclear factor 4 (HNF4, NR2A) and to RXR, indicating the presence of ancestral NR2 subfamily members in this cnidarian. An RXR gene has been cloned from a cubazoan, *Tripedalia cystophora*, and the protein binds 9-*cis* retinoic acid with high affinity (Kostrouch et al, 1998).

Although there does not appear to be an ER in *N. vectensis*, the existence of a bilaterian ancestral steroid-binding receptor was inferred based on ancestral protein reconstruction (Thornton et al, 2003). Cnidaria appear to be susceptible to signal

disruption by exogenous estrogens (reviewed in (Tarrant, 2005, 2007)), although there appear to be differences between coral and hydra sensitivity (Pascoe et al, 2002; Tarrant et al, 2004). Estrogen signaling may still be important in corals, however, as estrogens have been found in and around spawning corals, and corals have the ability to metabolize estradiol and testosterone (Atkinson and Atkinson, 1992; Blomquist et al, 2006; Tarrant et al, 1999; Tarrant et al, 2003; Twan et al, 2003; Twan et al, 2006).

Efflux Transporter Proteins

Many toxic compounds are pumped against concentration gradients across membranes in an energy-dependent process. This first line of cellular defense, against amphipathic or slightly lipophilic compounds in particular, is mediated by efflux proteins known as ATP Binding Cassette (ABC) or multidrug efflux transporters, including the p-glycoproteins (PGP/ABCB), mitoxantrone resistance protein (MXR/ABCG2), and multidrug resistance proteins (MRP/ABCC) (Dean et al, 2001). Efflux transporters function to export both unmodified substrates and substrates modified by other defensome enzymes (Deeley et al, 2006). In embryos, efflux transporters may provide the primary defense against exogenous toxicants but also play important roles in developmental programs by establishing morphogen gradients (Hamdoun and Epel, 2007).

In chordates the ABC transporters are organized into 8 subfamilies designated ABC *A* through *H* (Annilo et al, 2006). A subset of these families includes proteins known to export toxicants: the ABC*B*, ABC*C* and ABC*G* transporters. These proteins are commonly called multidrug resistance (MDR) transporters after their ability to pump out multiple therapeutic drugs, a major obstacle to the efficacy of the treatment of several pathogens (Dean et al, 2005).

Genome searches revealed that sea anemones have 64 ABC genes organized into 6 subfamilies including the three multidrug transporter subfamilies (ABC B, C and G; Table 1, Supplemental Table S1). There is considerable variation in the total number of ABC genes within eukaryotic genomes, but the relative proportions have tended to stay constant (Annilo et al, 2006; Goldstone et al, 2006). The ABC genes clustered in the ABCA (5 genes), ABCD (6 genes), and ABCF (4 genes) families either do not have

known function, or do not have known roles in detoxification, and will not be considered further here.

N. vectensis has 7 ABCB genes, including two related to the ABCB1 (pgp) proteins. The pgp transporters are well known as multidrug resistance proteins involved in the efflux of toxic compounds. Additional genes related to known xenobiotic transporters include 6 ABCC4 (MRP4)-like genes, found in a sea anemone-specific cluster, 6 other ABCC-like genes including one ABCC5 (MRP5)-like sequence, and 24 other genes that cluster in a large anemone-specific clade within the ABCC family. Finally, analyses conducted in this study identified 6 ABCG sequences, including one sequence that clusters closely with the vertebrate ABCG2s. In vertebrates, ABCG2 proteins exhibit broad substrate specificity among xenobiotic compounds, and play critical roles in the clearance of certain drugs (Allikmets et al, 1998; Kusuhara and Sugiyama, 2007; Miyake et al, 1999).

Other potentially important anemone transporters include the organic anion polypeptides (OATP; solute carrier family 21, SLC21) and organic anion and cation transporters (OAT and OCT; solute carrier family 22, SLC22). Both SLC21 and SLC22 are part of the major facilitator superfamily. OAT substrates examined in vertebrates include estrone sulfate, urate, prostaglandins, heavy metals such as mercury and cadmium, and the herbicide 2,4-dichlorophenoxyacetic acid (Eraly et al, 2004; Kimura et al, 2002; Sweet, 2005). OATPs have partially overlapping substrate specificities for steroid conjugates, bile salts, anionic oligopeptides, and anionic xenobiotics including toxins and drugs (Hagenbuch and Meier, 2003; Jacobsson et al, 2007). The anemone genome contains 17 OATP genes, and 62 SLC22 (OCT and OAT) genes. However, orthology among non-vertebrate SLC families is difficult to assign, precluding any hypotheses regarding substrate specificity.

Oxidative or Reductive Biotransformation

Cytochromes P450

Oxidative modification of chemicals to more hydrophilic products is often the initial step leading to excretion. In bilaterians this is carried out by cytochrome P450 (CYP) and flavoprotein monooxygenase (FMO) enzymes, especially members of the CYP1, CYP2, CYP3, CYP6, CYP9, and CYP4 families. Toxicant oxidation can, however, also lead to

increased toxicity; for example, oxidation of benzo[a]pyrene by CYP1A leads to hepatotoxicity (Uno et al, 2001).

The sea anemone genome contains 82 CYP genes, which are in general not classifiable into established CYP families due to the low (<40%) identity with other known CYPs. A large scale reclassification of metazoan CYPs taking into account recent genomic data will be required to formally name the *N. vectensis* CYPs (Nelson, personal communication). However, broader classification into the CYP clan framework (Nelson, 1998, 2006) is possible: Clan 2, containing CYP families 1,2,17, 18, 21, 33, 34, and 35; Clan 3, containing primarily CYPs 3, 5, 6, 9, 28, 309, 310, and 317; Clan 4, containing CYPs 4, 311, 313, 316, and 318; and the mitochondrial clan, CYPs 11, 12, 24, 27, 44, 49, 302, 314, and 315.

Sea anemone CYPs are principally part of Clan 2 and Clan 3, with 39 and 20 genes in these two Clans, respectively, while there are only 3 Clan 4 genes (Table 2, Figure 3**,** Supplemental Figure S1**,** Supplemental Table S2). Many of the CYPs in these clans are involved in detoxification of exogenous and endogenous compounds (Lewis et al, 2004), although the functional information is primarily from vertebrates and insects. The anemone Clan2 CYPs are clustered more closely to the vertebrate CYP17s (and thus the important xenobiotic-detoxifying CYP1s, including aryl hydrocarbon hydroxylases) than to the vertebrate CYP2s. However, it is not clear that CYP1 genes exist outside the deuterostomes (Goldstone et al, 2007), and these sea anemone CYPs cannot be considered early CYP1-like genes. The Clan 3 genes are likewise less closely related to the vertebrate CYP3 or insect CYP6 detoxification genes than to other members of Clan 3, in this case the CYP5-like genes. CYP5 genes have unusual functionality in that they catalyze a rearrangement of a prostaglandin endoperoxide (Hecker and Ullrich, 1989), rather than a substrate oxidation. More generally, the sea anemone Clan 3 CYPs may oxidize prostaglandins, which are potent chemical defenses in marine systems (Paul and Puglisi, 2004; Paul et al, 2006).

N. vectensis does not have a CYP19 (aromatase), despite the fact that (low) aromatase activity has been demonstrated in a scleractinian coral, *Euphillia ancora* (Twan et al, 2003). CYP19 is not present in most invertebrates with a sequenced genome (Goldstone, Nelson, and Stegeman, unpublished data), although it is present in amphioxus (Castro et

al, 2005; Mizuta and Kubokawa, 2007). It is very possible that a different CYP, not CYP19, possesses aromatase activity.

Other redox enzymes

Other proteins that oxidize or reduce toxicants include the flavoprotein monooxygenases (FMO (Ziegler, 2002)), aldo-keto reductases (AKR (Jin and Penning, 2007)), aldehyde dehydrogenases (ALDH), NADPH-dependent quinone oxidoreductase (NQO), and epoxide hydrolase (EPHX). In contrast to the CYPs, much less is known about many of the substrates of these enzymes, even in humans (Krueger and Williams, 2005; Penning and Drury, 2007).

In addition to the 82 CYPs, analyses conducted in this paper identified genes for 6 FMO enzymes. Although both enzyme families are primarily monooxygenases, and have some overlapping substrate specificities(Krueger and Williams, 2005; Ziegler, 2002), FMOs are generally thought to oxidize soft nucleophiles, while CYPs often catalyze C-H abstraction (Cashman, 2005). FMOs are less stable enzymes than CYPs, which has contributed to the relative lack of functional knowledge. The sea anemone FMO enzymes are quite distinct from the known human FMOs, and, as with the anemone CYPs, specific functions cannot be easily guessed at.

The sea anemone genome has at least 12 AKR genes, 21 ALDH, and 1 EPHX gene. These numbers are comparable to deuterostome gene inventories. In vertebrates, EPHX contributes to the toxicity of benzo[a]pyrene by converting the benzo[a]pyrene epoxides produced by CYP1s to benzo[a]pyrene dihydrodiols (Shimada, 2006), which eventually can be oxidized to redox-cycling benzo[a]pyrene quinones by AKR (Palackal et al, 2001; Penning et al, 1999).

One of the most important ALDH reactions in vertebrate development is the irreversible oxidation of retinal to retinoic acid (Lee et al, 1991); retinoids play very important roles in vertebrate patterning and are also likely important in cnidarian development (Bouzaiene et al, 2007; Johnson and Chun, 1989; Kostrouch et al, 1998; Muller, 1984). ALDH enzymes may also help maintain the cellular redox balance via ROS scavenging and the production of reducing equivalents as NADPH or NADH.

NQO enzymes catalyze the two-electron reduction of quinones to hydroquinones, reducing the formation of semiquinones and the potential for reactive oxygen generation

(Vasiliou et al, 2006). Similar to sea urchins (Goldstone et al, 2006), analyses conducted for this paper revealed that sea anemones do not have NQO-like genes. This is in line with the observed lack of NQO genes in the worm, fly, sea squirt, or plants (Vasiliou et al, 2006).

Conjugative Biotransformation

Sea anemones possess relatively few proteins with direct homology to xenobiotic-conjugating enzymes, particularly in comparison to the purple sea urchin (Table 2; (Goldstone et al, 2006). Sea anemones have genes for 23 glutathione-S-transferases (GST) including 5 microsomal GSTs (MAPEG), 9 UDP-glucuronosyl transferase (UGT) genes, and 22 sulfotransferase (SULT) genes. No N-acetyl transferase (NAT) genes were found. These numbers are far lower than the large diversification of these gene families seen in sea urchins, but comparable to the numbers observed in mammalian genomes (the human genome contains 13 SULT, 13 UGT, and 21 GST genes; (Gamage et al, 2006; Mackenzie et al, 2005; Nebert and Vasiliou, 2004)).

Cytosolic GSTs are soluble proteins that catalyze the transfer of glutathione to an electrophilic substrate (Hayes et al, 2005). Microsomal, or membrane, GSTs (MAPEG) form an evolutionarily distinct class of enzymes that exhibit both glutathione transferase and lipid peroxidase activity (Bresell et al, 2005), thus detoxifying both xenobiotic compounds and ameliorating oxidative stress. The majority (15) of the 18 sea anemone GSTs are readily classifiable, including 3 mu-class, 3 omega-class, 6 sigma-class, 1 theta-class, 1 fungal-type, and 1 zeta-class. The 3 remaining GSTs appear homologous to the xenobiotic-metabolizing alpha/pi GSTs. This search also found a sequence homologous to the translation elongation factor 1g (EF1g), which contains a GST domain but does not have glutathione transferase activity. The *N. vectensis* genome also codes for a total of 5 microsomal GST (MAPEG) sequences, including 1 homologous to vertebrate MAPEG1, 1 sequence homologous to MAPEG3, and 3 sequences homologous to prostaglandin E synthase (PTGS). PTGS enzymes are MAPEG superfamily members important to eicosanoid synthesis and involved in the vertebrate inflammation response (Jakobsson et al, 1999). Prostaglandins in corals are very important in chemical defense [reviewed in (Paul and Puglisi, 2004; Paul et al, 2006)] and have been extensively studied in

gorgonians. A prostaglandin synthase with 50% identity to mammalian PTGS has been cloned from an Arctic soft coral (Koljak et al, 2001).

SULT and UGT enzymes catalyze the conjugation of sulfuryl groups donated by 3'-phosphoadenosine-5'-phosphosulfate (PAPS) or UDP-glucuronide, respectively, to a wide variety of substrates, including both xenobiotics and endogenous products (Bock and Kohle, 2004; Gamage et al, 2006; Runge-Morris and Kocarek, 2005). Cytosolic (soluble) SULTs are responsible for the metabolism of xenobiotic and small endogenous substrates (SULT1 and SULT2), while membrane-bound SULTs are involved in endogenous peptide, lipid, and aminosugar sulfonation (Gamage et al, 2006). I found 22 SULT genes in the sea anemone genome, all of which are more closely related to the SULT genes involved in energy metabolism rather than those SULT genes known from vertebrate studies to participate in detoxification reactions. The anemone genes are divided among the SULT3A family (8 genes), SULT3B (2 genes), SULT4 (4 genes), and carbohydrate keratan/chondroitin SULTs (8 genes). Chondroitin sulfation has been demonstrated in the nematocysts of *Hydra magnipapillata* (Yamada et al, 2007), and it is possible that the *N. vectensis* genes are involved in similar functions.

The sea anemone UGT genes are likewise not closely related to the UGT families with known xenobiotic metabolizing or detoxification roles. UGT1 genes in mammals consist of one gene with as many as 14 different first exons, complicating the assignment of UGT homology (Mackenzie et al, 2005). Based on our previous analysis of the large number of distinct genes in the sea urchin, exon duplication like that observed in the mammalian UGT families is not the only method of UGT diversification. However, the 9 anemone UGTs are not classifiable to any of the known vertebrate UGT families, and thus no function can even be hinted at. This finding is not unique to anemones, as other marine genomes contain what appear to be lineage-specific gene family expansions that are not readily assignable to known UGT functional classes (J. Goldstone, unpublished data).

Antioxidant proteins

Reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and hydroxyl radicals, are derived from a variety of cellular processes, including leakage from mitochondrial respiration. Reactive oxygen can also be produced by exposure to

toxicants and to ultraviolet radiation. ROS contribute to diseases and pathologies generally deriving from altered gene expression or damage to biomolecules, including proteins, lipids and DNA (Halliwell and Gutteridge, 1999; Lesser, 2006). General antioxidant defensive genes include superoxide dismutase (SOD), catalases (CAT), and peroxidases, including glutathione peroxidase (GPX), peroxiredoxin (PRDX) and thioredoxins (TXNs).

The sea anemone genome has a total of 6 superoxide dismutase (SOD) genes: 3 Cu/Zn SOD genes, 1 Mn SOD gene, 2 Fe SODs, as well as an SOD copper chaperone homolog (which contains an SOD domain but has no dismutase activity). Both EST and cDNA libraries support the expression of all 6 SOD forms under normal conditions (A. Reitzel, A. Tarrant, J. Goldstone unpublished data). In addition, there is one catalase (CAT), 12 glutathione peroxidase genes, and 6 other heme peroxidase genes. This abundance of antioxidant defense genes is complemented by a complete glutathione system (glutathione reductase and 4 gamma-glutamyl transferases), as well as thioredoxin (TXN) and thioredoxin reductase (TXNRD).

Metal detoxification

Heavy metals are important aquatic pollutants resulting from sewage, urban and agricultural runoff, and antifouling paint. Bioconcentration of heavy metals can lead to tissue concentrations that are 10 to 10 000-fold higher than environmental levels, resulting in a variety of toxic effects. Four phytochelatin synthase (PCS) homologs are present in the *N. vectensis* genome, and expressed under normal conditions (A. Reitzel, A. Tarrant, J. Goldstone unpublished data). Phytochelatins are metal-binding peptides composed primarily of glutathione groups that are important metal detoxifying genes in plants and fungi. Until phytochelatin synthase (PCS) was discovered in the nematode *C. elegans* (Clemens et al, 2001) it was believed that phytochelatins were present only in plants and fungi. Now it is clear that many other lineages contain PCS homologs (Clemens, 2006), including the sea urchin (Goldstone et al, 2006). Currently sequenced vertebrate genomes do not contain a gene homologous to PCS, nor do insect genomes, suggesting that phytochelatin synthesis ability was lost independently in some protostome and deuterostome lineages.

No metallothionein (MT) genes, neither plant- nor fungi- nor metazoan-related, were found in the sea anemone genome despite extensive searching, perhaps because of the presence of the alternative metal-complexing phytochelatin system. The absence of MT genes is apparently due to gene loss, as MT proteins are important metal detoxification proteins in plants (Cobbett and Goldsbrough, 2002), mollusks (Amiard et al, 2006), sea urchins (Nemer et al, 1985), and vertebrates, and are also present in sponges (Berthet et al, 2005; Philp, 1999).

Active efflux of toxic metals is another important route to detoxification. Both OAT and ABC efflux proteins (see above) export metals (Leslie et al, 2001; Sweet, 2005), and the anemone contains genes homologous to the transporters (within both the OAT and ABC families) known to facilitate metal export.

Heat Shock Proteins

Heat shock proteins (HSP) have been implicated in the response to various toxicants including cadmium, arsenic, and free radicals (Feder and Hofmann, 1999). The induction of HSP mRNA and protein by heat shock factor 1 (HSF1) appears to be part of generalized cellular stress response, and HSPs may not only act as chaperones but also assist in refolding of partially denatured proteins (Kim et al, 2006). Sea anemones have several families of heat shock proteins including HSP 90, 70, and small alpha crystalline HSPs (HSP20s). The largest family of heat shock proteins is the HSP20 family, containing at least 18 genes. Sea anemones also have at least 4 HSP90 genes and 9 HSP70s. Various coral and anemone HSP60, HSP70, and HSP90 proteins and cDNA sequences have been shown to be strongly induced not only by heat or cold shock (Choresh et al, 2007; Choresh et al, 2004; Choresh et al, 2001; Hashimoto et al, 2004; Robbart et al, 2004; Rossi and Snyder, 2001; Rossi et al, 2006; Sharp et al, 1997; Sharp et al, 1994; Snyder and Ross, 2004), but also by PCB118 (Wiens et al, 2000).

DISCUSSION

The chemical defensome is an integrated network of chemical sensing and response proteins that function as an organized defense against toxic chemicals, both endogenous and exogenous (Goldstone et al, 2006). Elucidation of the chemical stress-response repertoire of *N. vectensis* provides a framework for studies on a number of cnidarian-

specific questions as well as on broader evolutionary questions. Characterization of these stress response genes in *N. vectensis* facilitates the use of this and other anemones as sentinel species for changing environmental stressors. *N. vectensis* is a hardy species, tolerating extremes of temperatures unknown to other members of the family Edwardsiidae, which are restricted to temperate and polar coastal seas with less dramatic temperature and salinity variations (Daly, 2002). Identification of molecular responses to chemical stress will help us to develop markers that will allow *N. vectensis* and other anemones to act as sentinels of environmental contamination.

The major components of this defensive gene network are conserved in the sea anemone genome (Figure 4), indicating that they must have origins prior to the cnidarian-bilaterian split. Interphyla comparison of the components and linkages within the chemical defensome will help us understand the early evolution of the chemical stress response. Despite the fact that the individual genes within the defensome network may vary across organisms, this network may be comprised of evolutionarily conserved modules which are retained across evolution. Comparing the susceptibility of sea anemone embryos with that of deuterostome and protosome embryos, for a range of chemicals, could lead to fundamental insights into how these defensome "kernels" function to protect embryos from the myriad chemical challenges that could derail development. Predictions of defensome interactions (e.g. the roles of nuclear receptors in simultaneously modulating multiple parts of the defensome) are testable using microarray analysis of gene expression in combination with gene knockdown and protein overexpression.

Signaling Network. Ligand activated transcription factors form a significant component of the defensome, integrating the stress response and potentially activating many different pathways simultaneously. The evolutionary history of both the bZIP (Amoutzias et al, 2007) and bHLH-PAS (Simionato et al, 2007) receptor superfamilies is complicated, but most major clades of these receptors are present in cnidaria. Although some of the known components of the vertebrate and invertebrate xenobiotic receptor pathways are missing, homologs of most important stress receptors are present, including AHR, ARNT, HIF1α, MTF, and NRF2. The deuterostome xenobiotic-responsive NR1H and NR1I subfamilies are missing, however, and it is not currently known whether other

NRs are functioning as xenobiotic receptors. Cnidaria have been shown to have a retinoid response, including a functional homolog of the RXR (Bouzaiene et al, 2007; Johnson and Chun, 1989; Kostrouch et al, 1998; Muller, 1984).

Gene Family Diversification. Many defense gene families have undergone diversification and expansion in marine invertebrates in comparison to vertebrate genomes. In general, analysis of entire genomes is required to determine that a specific family or superfamily has undergone diversification, and thus current examples of such events is scattered. Class or order level diversification may be presumed, based on the model organisms with sequenced genomes, but caution should be exercised when extrapolating. Although there is general conformity in the presence of higher order gene groups, in many cases gene orthology is more difficult to determine.

For example, the sea anemone contains 82 CYP genes, and those related to CYP gene families 1-4 constitute a large proportion (76%) of the total, suggesting evolutionary pressure to maintain broad functionality in these important defense gene families. Multiple gene duplications in the toxicologically important CYP families appear to have taken place in many different lineages, leading to taxon-specific gene clades that are related to known CYP families yet distinct enough to preclude definitive assignment of names based on current CYP nomenclature guidelines. The extensive birth-death process of CYP diversification is not solely represented by invertebrates – within the vertebrates there is significant evidence for extensive gene duplication and loss within xenobiotic-metabolizing CYP families (Thomas, 2007).

In *N. vectensis*, particular examples of family diversification relative to known sequences are the CYPs and the ABC transporters. Other defensome gene families do not appear significantly expanded, nor do they have genes distributed into completely novel subfamilies. This observation is interesting in light of the fact that *N. vectensis* lives in the challenging environment of a temperate estuary, and ranges from subtropical to subarctic estuaries (Hand and Uhlinger, 1994).

Symbiosis.

An important consideration for the study of cnidarian chemical defense genes is that many species are host to photosynthetic endosymbionts (zooxanthellae or zoochorellae). There are unique aspects of both normal physiology and toxicological responses that are

related to the presence of endosymbionts. Notably, photosynthesis produces oxygen, and surrounding host tissues require additional protection against ROS to withstand hyperoxygenation, such as additional superoxide dismutase genes. Indeed, Allemand and coworkers have characterized multiple SOD forms in the Mediterranean sea anemone *Anemone viridis*. They found up to seven SOD activity bands in various tissues, and detected several forms of CuZnSOD, MnSOD, and FeSOD in the various compartments (Richier et al, 2003). Two of the CuZnSODs were cloned, and encode both extracellular and intracellular CuZnSODs with different putative transcription binding sites (Plantivaux et al, 2004).

Although *N. vectensis* is an apparently asymbiotic anemone, the genome has genes for 6 different SODs, all of which are expressed under normal conditions. This abundance of SOD genes may be a general pattern, particularly in anthozoans. Interestingly, greater diversity in SOD activities was found in the symbiotic anemone species (*A. viridis*) than in an asymbiotic species (*Actinia schmidti*), and the asymbiotic anemone experienced significantly greater oxidative protein damage upon exposure to hyperoxia (Richier et al, 2005). Thus, the presence of photosynthetic endosymbionts and the concomitant possibility of hyperoxia may have driven the evolution of multiple additional SOD forms in cnidaria. Several different catalase forms were also characterized in *A. viridis*, with tissue-specific distributions and activities (Merle et al, 2007). Inhibition of the host anemone catalase led to symbiont expulsion, suggesting an active response to increased oxidative stress.

A second effect of algal symbiosis is the sensitivity of symbiotic cnidarian species to herbicidal contamination (Jones, 2005; Jones and Kerswell, 2003). Notably, there is increasing distribution of herbicides such as the s-triazine Irgarol 1051 that have been incorporated into marine anti-fouling paints along with copper (Carbery et al, 2006; Gardinali et al, 2004), and there is significant runoff of herbicide-containing waste from agricultural regions. Irgarol 1051 is a photosystem II binding agent that inhibits photosynthetic electron transport, resulting in a shortage of NADPH and the formation of singlet oxygen (Fufezan et al, 2002). Acute exposure of coral to Irgarol 1051 resulted in induction of SOD and MXR (ABCG) proteins and decreases of GPX, CAT, and certain CYP proteins (Downs and Downs, 2007). While other herbicides have been investigated

(e.g diuron (Harrington et al, 2005; Negri et al, 2005; Raberg et al, 2003)), there have been relatively few investigations of the molecular mechanisms of herbicide toxicity in cnidaria, and it is generally thought that damage is primarily a result of the disruption of host-algal symbiosis (Jones, 2005).

Reactive oxygen and UV. ROS production can also be an important consequence of UV exposure (Lesser, 2006; Mopper and Kieber, 2000). While UV responses have been studied in a number of coral species, many coral physiological responses to UV appear to be related to the physiological responses of their algal symbionts (Baruch et al, 2005; Torres et al, 2007; Verde and McCloskey, 2002). UV has been shown to interfere with pattern formation in regenerating hydra and promote budding of intact hydra, possibly in response to tissue damage (Ghaskadbi et al, 2005; Znidaric et al, 1992). Both exogenous hydrogen peroxide and UV treatments have been shown to increase DNA strand breaks in cnidaria, demonstrating the potential for genotoxic ROS effects (Baruch et al, 2005; Mitchelmore and Hyatt, 2004)

A very important protective mechanism in corals, as well as in diverse other marine organisms, is the accumulation of sunscreening compounds known as mycosporine-like amino acids (MAAs; (Shick and Dunlap, 2002)). MAAs may facilitate larval survival (Wellington and Fitt, 2003) as well as adult UV tolerance (Ferrier-Pages et al, 2007; Torres et al, 2007), and may also contribute to antioxidant capacity (Dunlap and Yamamoto, 1995; Yakovleva et al, 2004). In contrast to other animals, *N. vectensis* apparently possesses the shikimic acid pathway thought to be necessary for MAA production (Starcevic et al, 2008), presumably obtained via lateral gene transfer from bacteria. Many cnidaria have been thought to accumulate MAAs from their symbionts (Shick and Dunlap, 2002), or from their diet, as is the case for sea urchins (Carroll and Shick, 1996). Given the presence of the MAA biosynthetic pathway in the *N. vectensis* genome, and the clustering of sea anemone MAA complement by anemone phylogenetic distribution rather than endosymbiont identity, presence, or other environmental factors (Shick et al, 2002), it is likely that the ability of cnidaria to biosynthesize MAAs is not restricted to *N. vectensis*.

In contrast to tropical corals and many littoral anemones, *N. vectensis* is a burrowing anemone, and it is possible that *N. vectensis* adult may be able to avoid UV damage

despite living in shallow ponds. However, larval *N. vectensis* may require more protection from ROS than adults, leading to the apparent diversification of ROS defenses. Examination of the UV-stress response of *N. vectensis* will aid in understanding the different roles that antioxidant enzymes and sunscreening compounds may play in protecting sea anemones. Comparisons of *N. vectensis*, an apparently asymbiotic anemone, with symbiotic anemones (e.g. *A. viridis*) or symbiotic reef-building corals could elucidate the protective mechanisms required by symbiotic cnidaria, and shed light on the role of antioxidant enzymes in thermotolerance and bleaching (Downs et al, 2002; Merle et al, 2007; Richier et al, 2005; Richier et al, 2003).

Molecular Toxicology. Many toxicological studies of cnidaria involve metals, particularly copper, cadmium, and zinc. In particular, the acute and structural effects of copper, cadmium, and zinc have been investigated in various hydrozoan species, including both freshwater and marine hydra (Holdway et al, 2001; Karntanut and Pascoe, 2000, 2002, 2005) and a variety of anthozoa, including scleractinian corals (Mitchelmore et al, 2007). Other biological responses to heavy metals in cnidaria include coral bleaching (Jones, 1997), and effects on coral metabolism (Alutoin et al, 2001; Nystrom et al, 2001), larval mortality, and inhibition of reproduction including settlement, motility and fertilization of larvae (Negri and Heyward, 2000, 2001; Reichelt-Brushett and Harrison, 2000, 2005). Few studies have examined molecular biomarkers or molecular mechanisms of metal stress (Mitchelmore et al, 2002; Morgan et al, 2001).

The availability of the *N. vectensis* genome will make many mechanistic studies possible, and should spur the development of metal stress biomarkers in various species. In particular, the presence of multiple genes for phytochelatin synthase provides obvious markers for metal stress, despite the lack of metalothionein genes.

As with metal contamination, there are few studies of either molecular markers or mechanisms of exposure to organic contaminants other than herbicides in cnidaria (Rougee et al, 2006). A number of biochemical studies of cnidarian CYP biochemistry have been carried out, however. CYP carbon monoxide difference spectra have been observed in six different species of sea anemone (Heffernan and Winston, 1998, 2000; Sole and Livingstone, 2005) and three different species of scleractinian corals [*Favia fragum*, *Siderastrea sidea*, and *Montastraea faveolata*; (Garcia et al, 2005; Gassman and

Kennedy, 1992; Ramos and Garcia, 2007)]. Furthermore, benzo[a]pyrene hydroxylase activities have been observed in sea anemones, likely due to the action of CYP mixed-function oxygenases (Heffernan et al, 1996; Winston et al, 1998). The presence of inducible (versus constituent) CYP content in corals has also been demonstrated in coral due to benzo[a]pyrene or fuel oil exposure (Ramos and Garcia, 2007; Rougee et al, 2006). The same PAH exposures induced components of the reactive oxygen defense systems, including CAT, SOD, and GST. Finally, an important molecular marker of genotoxic damage, the Comet assay of DNA damage, has been assessed in cnidaria in response to benzo[a]pyrene exposures (Mitchelmore and Hyatt, 2004). Benzo[a]pyrene was found to increase DNA strand breaks in the temperate anemone *Anthopleura elegantissima*, suggesting also that cnidaria, like vertebrates, are capable of bioactivating benzo[a]pyrene to genotoxic metabolites, likely by CYPs.

More subtle effects of organic contamination might include in particular disruption of endogenous signaling pathways by exogenous hormones or hormone mimetics. Recent research indicates that cnidaria are susceptible to this sort of signal disruption, although the precise mechanisms are unknown (Fukuhori et al, 2005; Pachura-Bouchet et al, 2006; Pascoe et al, 2002; Tarrant, 2005, 2007; Tarrant et al, 2004). In particular, Tarrant et al (2004) observed that spawning and growth rates were reduced in corals exposed to exogenous steroidal estrogens. As noted above, cnidaria do not posses a homolog of the vertebrate estrogen receptor, although there may be other nuclear receptors that function as a steroid receptors (Reitzel et al, 2008; Tarrant, 2007). Cnidaria are steroid-rich organisms (Withers et al, 1982), but the roles these steroids play in normal physiology are not clear (Tarrant, 2005). Steroids and secosteroids from gorgonian and soft corals have been show to have antimicrobial and antifouling activity (Qi et al, 2008; Sica and Musumeci, 2004), suggesting that many of these compounds may be produced for chemical defense. CYP enzymes often participate in steroid synthesis and modification, and the diversity of *N. vectensis* CYPs may relate to the diversity of cnidarian steroids, although the steroid content of *N. vectensis* has not been investigated.

N. vectensis is a physical stress-tolerant organism, tolerating a wide range of environmental conditions (Sheader et al, 1997). With this robustness to physical stress, *N. vectensis* is in a prime position to act as a sentinel species towards chemical stress in

estuaries. *N. vectensis* is also an excellent laboratory model, with simple maintenance needs, and the generation of clonal stocks by forced regeneration allows great scope for genetic manipulation. Furthermore, this sea anemone is an excellent model for the study of embryonic development (Matus et al, 2006). With the description of these chemical defense genes, we can study of the evolution of cellular defense during embryonic development. Development inherently is a robust process; which parts of the process are more susceptible to disruption, and from which stressors, is not clear (Hamdoun and Epel, 2007). The description of this defense gene set will allow us to examine the evolution of generalized cellular stress responses in bilaterian embryos and to understand how these stress responses function in adult cnidaria.

Acknowledgements

Funding was provided by the WHOI Ocean Life Institute and NIH R01-ES015912. This manuscript benefitted by discussion with Ann Tarrant, Adam Reitzel, and Cécile Sabourault, and from the comments of several anonymous reviewers.

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Figures

Figure 1. Conceptual organization of the cellular defensome. Organic and inorganic toxicants are actively exported, and also subjected to a variety of biotransformative reactions. Modified from (Goldstone et al, 2006).

Figure 2. Some of the stress response transcription factor pathways with homologs in *N. vectensis*. Hypoxia activates both the HIF1α and MTF1 pathway, metal stress activates MTF1, oxidative stress activates the NRF2 pathway (as well as others, not shown), and organic xenobiotics activate AHR or various NRs. These transcription factors have specific response elements (REs) in the regulatory regions of responsive genes, including hypoxida RE (HRE; HIF1α/ARNT), metal RE (MRE; MTF1), antioxidant RE (ARE; NRF2), xenobiotic RE (XRE; AHR/ARNT), and specific NR-REs (e.g. estrogen response elements).

Figure 3. Distribution of genes in CYP Clans in human, tunicate, sea urchin, and sea anemone. Data for the tunicate and sea urchin assignments was taken from (Goldstone et al, 2006).

Figure 4. Gene count comparisons for various classes of defensome genes. The area of each circle is proportional to the total number of genes classified into the defensome. Receptors include bHLH-ZIP, NR, and CNC receptors. Transporters are ABC and OAT transporters; oxidative and reductive modification genes include CYP, FMO, ALDH, EH, and AKR; conjugative genes are GST, MAPEG, UGT, and SULT; antioxidant genes are SOD, CAT, PXR, and GPX. Other genes include PCS, HSP20, HSP70, and HSP90.

Table 1. Gene counts of xenobiotic transporter genes. Data for human is taken from (Dean and Annilo, 2005) and for urchin from (Goldstone et al, 2006).

Superfamily	Gene Family	Human	Urchin	Anemone
ABC Superfamily	ABCB	11	12	7
	ABCC	12	30	36
	ABCG	5	9	6
	Other	20	14	16
	Total	48	65	65
Major Facilitator	SLC21A	11	30	17
	SLC22	5	46	62

Table 2. Gene counts of biotransformative genes in the humans, sea urchin, and sea anemone genomes.

Classification	Gene	Human	Urchin	Anemone
Oxidative	CYP Clan 2	21	85	39
	CYP Clan 3	5	13	20
	CYP Clan 4	12	10	3
	FMO	6	16	6
	ALDH	19	20	21
Conjugative	GST	21	38	18
	MGST	3	12	5
	SULT	13	73	22
	UGT	13[a]	50	9
	NAT	2	1	0
Reductive	AKR-like	8	10	12
	EPHX	2	5	1
	NOQ1	2	0	0

[a] Not applicable – see text. [b] Not including multiple first exon expression in UGT1.

Figure 1

Click here to download line figure: stella_defensome_F1.ppt

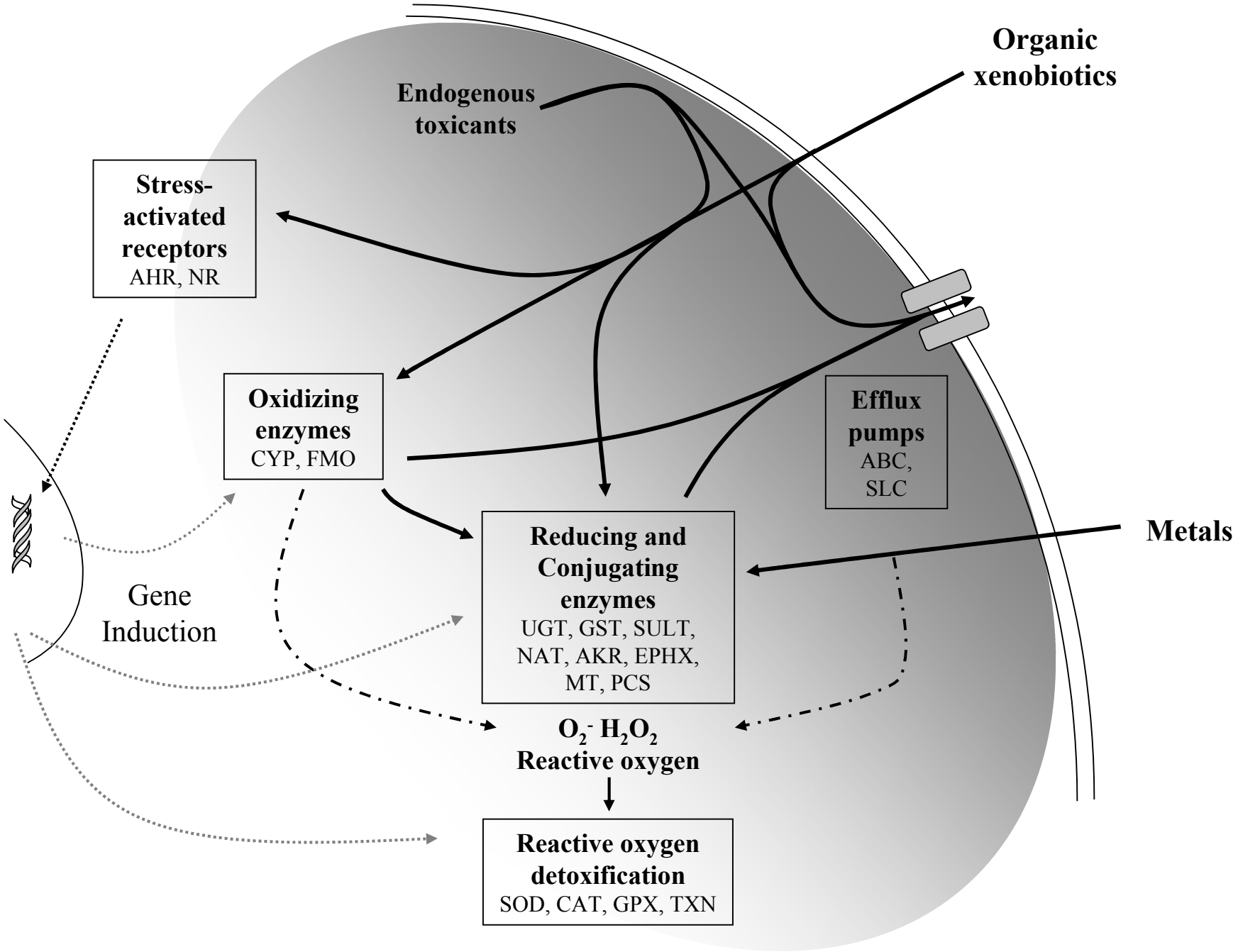

Figure 2

Click here to download line figure: stella_defensome_F2_revised.ppt

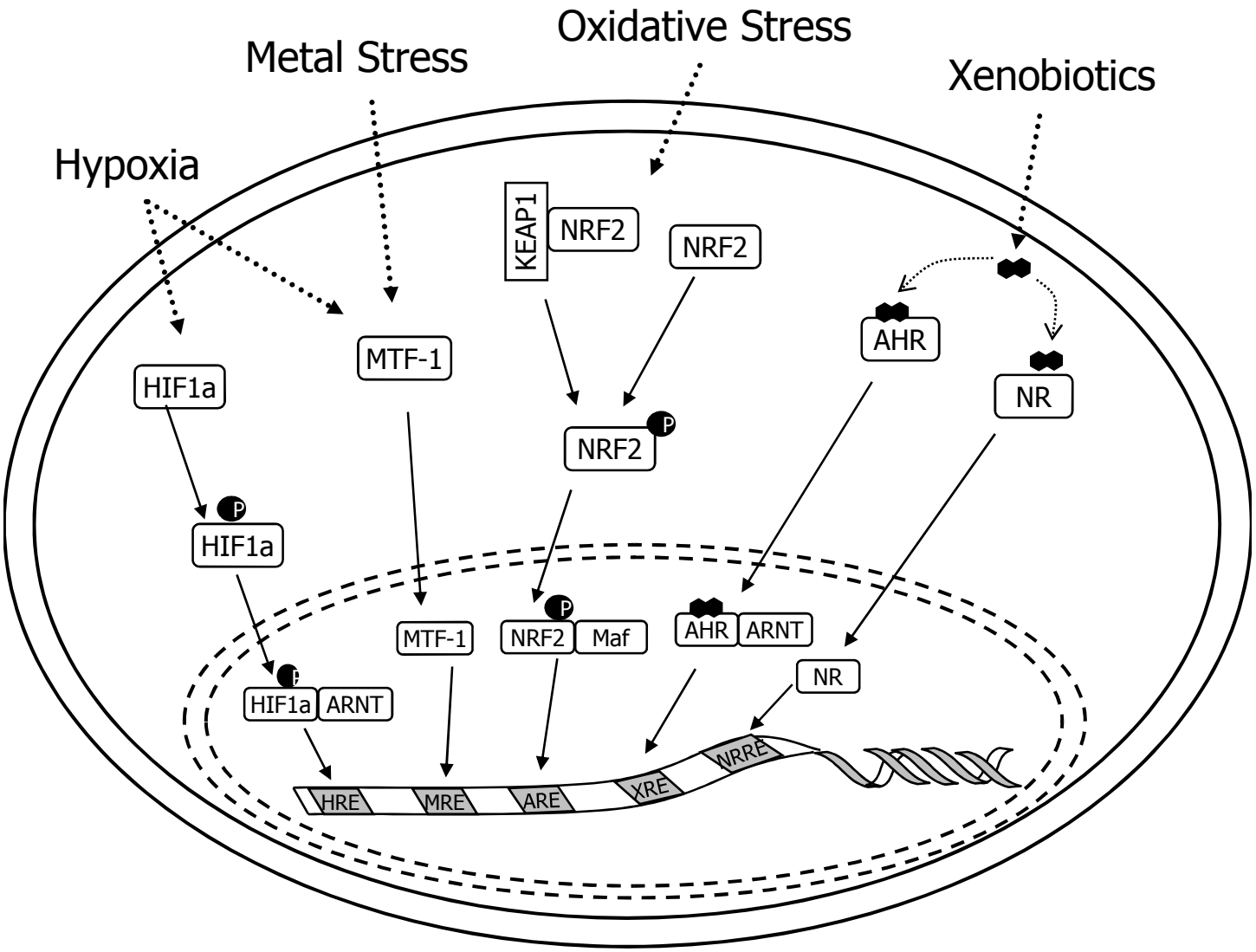

Figure 3

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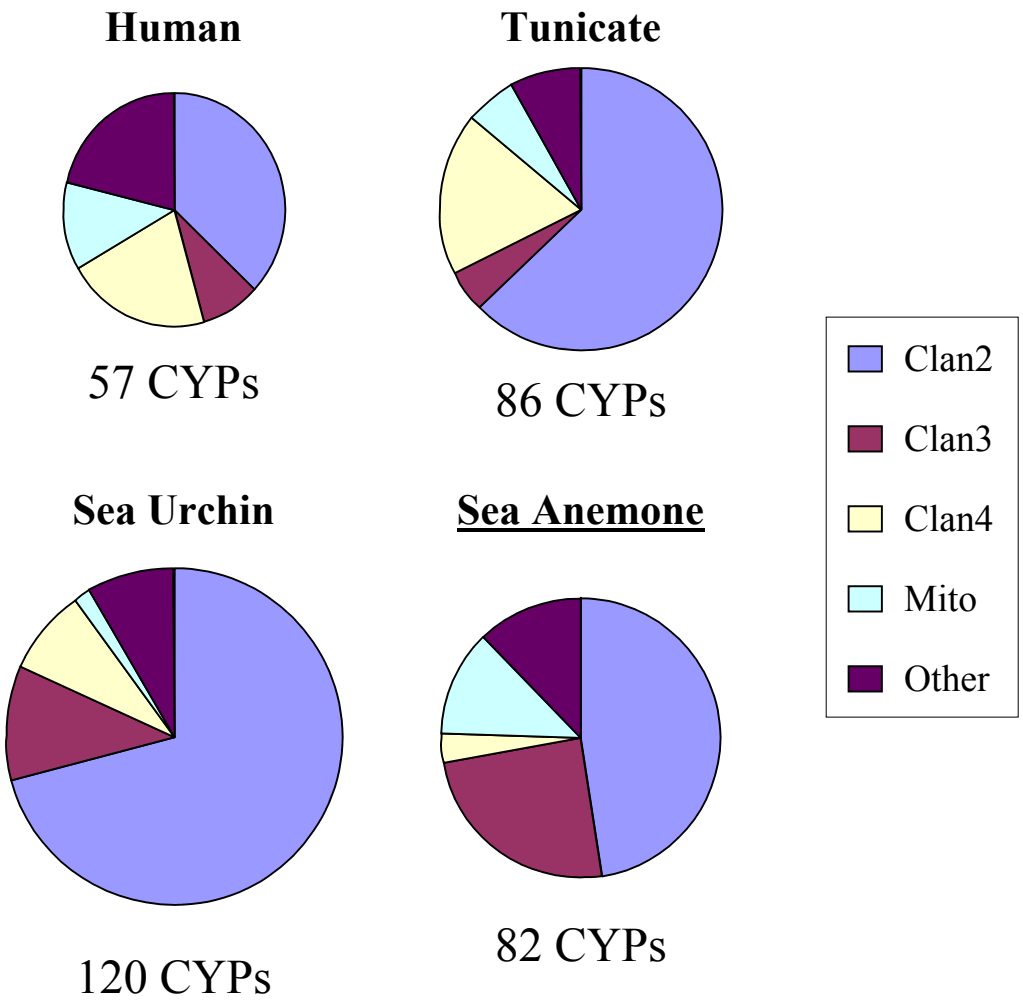

Figure 4

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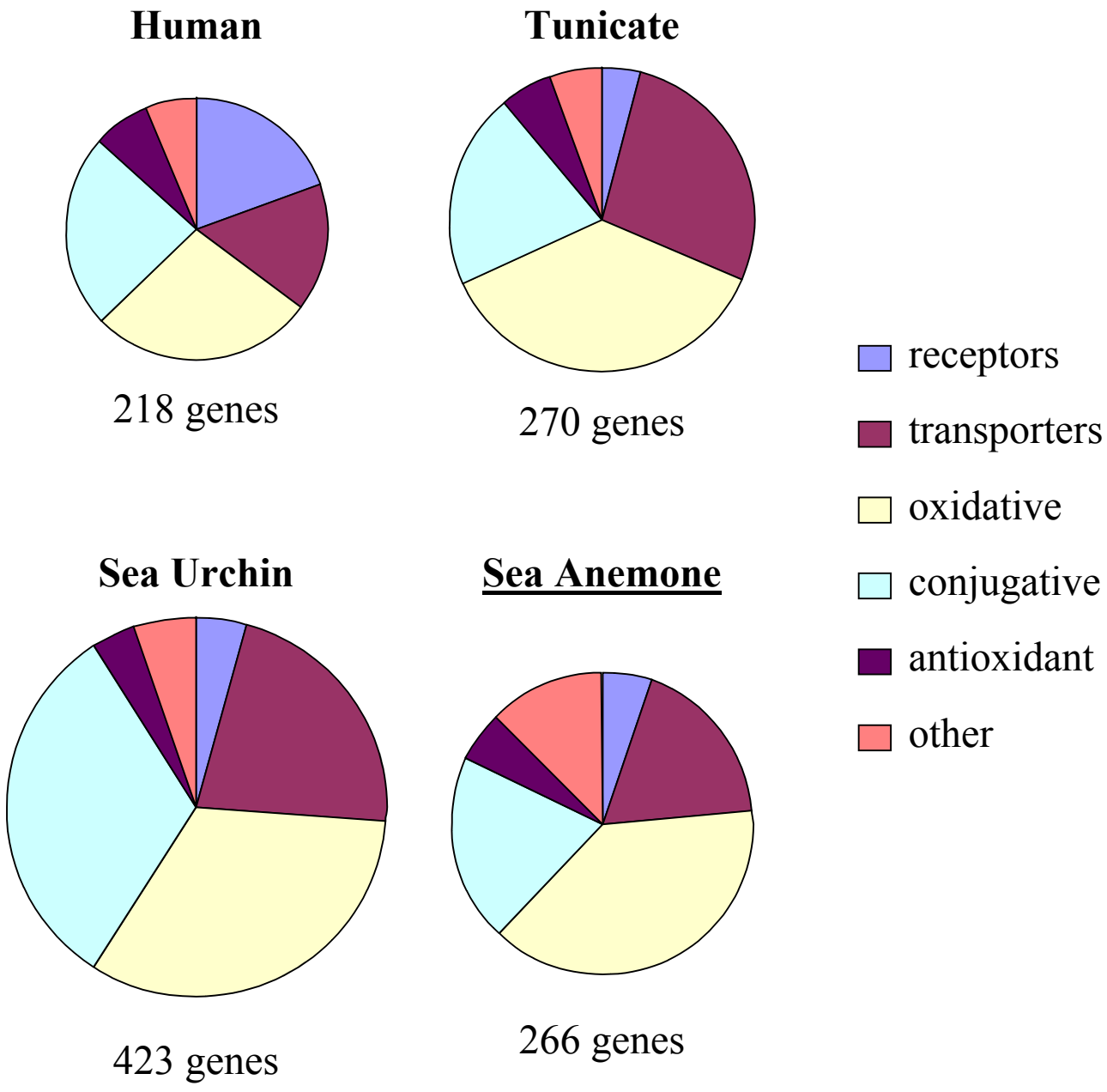


Goldstone, JV. Environmental sensing and response genes in Cnidaria: the chemical defensome in the sea anemone *Nematostella vectensis*

Supplemental Data

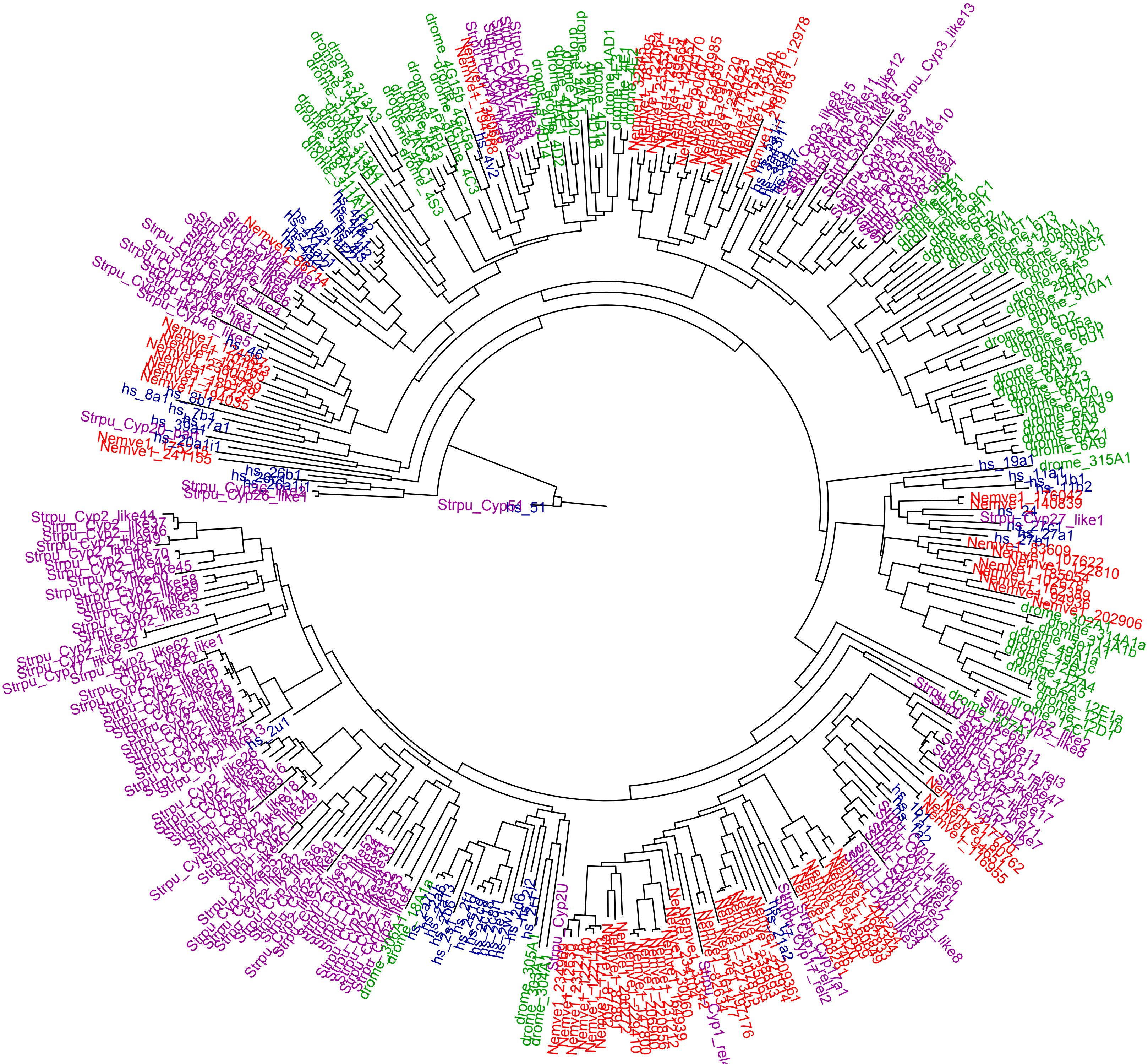
Figure S1. Maximum likelihood phylogenetic tree of cytochrome P450 (CYP) predicted and known protein sequences from *Homo sapiens* (hs, blue), *Strongylocentrotus purpuratus* (Strpu, purple), *Drosophila melanogaster* (drome, green) and *Nematostella vectensis* (Nemve1, red). Sequences were aligned with Muscle (v3.6) and automatically masked using the alignment quality score. Maximum likelihood analysis was perfomed using the PROTMIXWAGF model in RAxML (v7.0.3).

Table S1. JGI model numbers for ABC, SLC21, and SLC22 (transporter) genes in *N. vectensis* genome assembly version 1.

Table S2. JGI model numbers for oxidative and reducing genes in *N. vectensis* genome assembly version 1.

Table S3. JGI model numbers for conjugating genes in *N. vectensis* genome assembly version 1.

Table S4. JGI model numbers for antioxidant and metal conjugating genes in *N. vectensis* genome assembly version 1.


Strpu_Cyp51
hs_51
Strpu_Cyp3_like13
Nemve1_12978
drome_4AD1
drome_310A1
hs_19a1
drome_315A1
Nemve1_140839
Strpu_Cyp27_like1
Nemve1_202906
hs_8a1
Nemve1_241155
Strpu_Cyp26_like4
Strpu_Cyp2_like44
Strpu_Cyp1_rel4
Nemve1_116655
drome_305A1

Supplemental Table S1

JGI Protein Model	Gene family assignment
Nemve1|102	ABC transporter, ABCC
Nemve1|4776	ABC transporter, ABCC
Nemve1|10039	ABC transporter, ABCC1
Nemve1|10108	ABC transporter, ABCC1
Nemve1|10208	ABC transporter, ABCC
Nemve1|10332	ABC transporter, ABCC
Nemve1|20036	ABC transporter, ABCC4
Nemve1|20166	ABC transporter, ABCC4
Nemve1|23523	ABC transporter, ABCG4
Nemve1|30195	ABC transporter, ABCA3
Nemve1|30362	ABC transporter, ABCC4
Nemve1|31495	ABC transporter, ABCB9
Nemve1|50359	ABC transporter, ABCG
Nemve1|82183	ABC transporter, ABCB1
Nemve1|82333	ABC transporter, ABCA3
Nemve1|84633	ABC transporter, ABCC
Nemve1|87084	ABC transporter, ABCC
Nemve1|87844	ABC transporter, ABCA2
Nemve1|88546	ABC transporter, ABCC10
Nemve1|94692	ABC transporter, ABCC
Nemve1|97844	ABC transporter, ABCC1
Nemve1|99374	ABC transporter, ABCG2
Nemve1|99533	ABC transporter, ABCC
Nemve1|103967	ABC transporter, ABCG
Nemve1|104944	ABC transporter, ABCC
Nemve1|113115	ABC transporter, ABCC
Nemve1|113931	ABC transporter, ABCC4
Nemve1|115547	ABC transporter, ABCC
Nemve1|116645	ABC transporter, ABCG4
Nemve1|116678	ABC transporter, ABCC
Nemve1|116684	ABC transporter, ABCC
Nemve1|118015	ABC transporter, ABCC4
Nemve1|124699	ABC transporter, ABCC
Nemve1|130091	ABC transporter, ABCC
Nemve1|134819	ABC transporter, ABCA
Nemve1|134820	ABC transporter, ABCA
Nemve1|136552	ABC transporter, ABCC11
Nemve1|139647	ABC transporter, ABCC
Nemve1|139660	ABC transporter, ABCC
Nemve1|139662	ABC transporter, ABCC
Nemve1|139684	ABC transporter, ABCC
Nemve1|139853	ABC transporter, ABCC
Nemve1|146653	ABC transporter, ABCC
Nemve1|159164	ABC transporter, ABCC
Nemve1|166963	ABC transporter, ABCC
Nemve1|168119	ABC transporter, ABCD4
Nemve1|168315	ABC transporter, ABCG4
Nemve1|168501	ABC transporter, ABCB10
Nemve1|168693	ABC transporter, ABCC
Nemve1|172449	ABC transporter, ABCF1
Nemve1|180295	ABC transporter, ABCC4
Nemve1|190171	ABC transporter, ABCC1
Nemve1|190285	ABC transporter, ABCF3
Nemve1|195521	ABC transporter, ABCD4
Nemve1|206043	ABC transporter, ABCB10

JGI Protein Model	Gene family assignment
Nemve1|216377	ABC transporter, ABCA
Nemve1|224771	ABC transporter, ABCF
Nemve1|234370	ABC transporter, ABCC
Nemve1|234439	ABC transporter, ABCC
Nemve1|237874	ABC transporter, ABCB1
Nemve1|243828	ABC transporter, ABCD4
Nemve1|244883	ABC transporter, ABCF
Nemve1|245176	ABC transporter, ABCD1
Nemve1|246933	ABC transporter, ABCD3
Nemve1|247032	ABC transporter, ABCC
Nemve1|11296	Organic Anion Transporter Polypeptide
Nemve1|20640	Organic Anion Transporter Polypeptide
Nemve1|31122	Organic Anion Transporter Polypeptide
Nemve1|51341	Organic Anion Transporter Polypeptide
Nemve1|52639	Organic Anion Transporter Polypeptide
Nemve1|104948	Organic Anion Transporter Polypeptide
Nemve1|105114	Organic Anion Transporter Polypeptide
Nemve1|119016	Organic Anion Transporter Polypeptide
Nemve1|125243	Organic Anion Transporter Polypeptide
Nemve1|136329	Organic Anion Transporter Polypeptide
Nemve1|205781	Organic Anion Transporter Polypeptide
Nemve1|210013	Organic Anion Transporter Polypeptide
Nemve1|211340	Organic Anion Transporter Polypeptide
Nemve1|215081	Organic Anion Transporter Polypeptide
Nemve1|218525	Organic Anion Transporter Polypeptide
Nemve1|222752	Organic Anion Transporter Polypeptide
Nemve1|247942	Organic Anion Transporter Polypeptide
Nemve1|1130	SLC22 Family
Nemve1|8463	SLC22 Family
Nemve1|10639	SLC22 Family
Nemve1|14487	SLC22 Family
Nemve1|21316	SLC22 Family
Nemve1|40646	SLC22 Family
Nemve1|40753	SLC22 Family
Nemve1|47059	SLC22 Family
Nemve1|50785	SLC22 Family
Nemve1|60630	SLC22 Family
Nemve1|61471	SLC22 Family
Nemve1|63188	SLC22 Family
Nemve1|85329	SLC22 Family
Nemve1|86395	SLC22 Family
Nemve1|89058	SLC22 Family
Nemve1|90692	SLC22 Family
Nemve1|91052	SLC22 Family
Nemve1|94638	SLC22 Family
Nemve1|101713	SLC22 Family
Nemve1|101715	SLC22 Family
Nemve1|104560	SLC22 Family
Nemve1|109102	SLC22 Family
Nemve1|109190	SLC22 Family
Nemve1|111000	SLC22 Family
Nemve1|115717	SLC22 Family
Nemve1|118993	SLC22 Family
Nemve1|119271	SLC22 Family
Nemve1|122742	SLC22 Family
Nemve1|123813	SLC22 Family
Nemve1|125326	SLC22 Family

JGI Protein Model	Gene family assignment
Nemve1|128943	SLC22 Family
Nemve1|128952	SLC22 Family
Nemve1|131775	SLC22 Family
Nemve1|138108	SLC22 Family
Nemve1|145688	SLC22 Family
Nemve1|146442	SLC22 Family
Nemve1|149554	SLC22 Family
Nemve1|160661	SLC22 Family
Nemve1|165851	SLC22 Family
Nemve1|165915	SLC22 Family
Nemve1|170798	SLC22 Family
Nemve1|172071	SLC22 Family
Nemve1|173562	SLC22 Family
Nemve1|174480	SLC22 Family
Nemve1|200404	SLC22 Family
Nemve1|202226	SLC22 Family
Nemve1|205215	SLC22 Family
Nemve1|205600	SLC22 Family
Nemve1|206126	SLC22 Family
Nemve1|207968	SLC22 Family
Nemve1|207969	SLC22 Family
Nemve1|208384	SLC22 Family
Nemve1|211167	SLC22 Family
Nemve1|219492	SLC22 Family
Nemve1|220670	SLC22 Family
Nemve1|223668	SLC22 Family
Nemve1|224087	SLC22 Family
Nemve1|224161	SLC22 Family
Nemve1|225531	SLC22 Family
Nemve1|240064	SLC22 Family
Nemve1|241870	SLC22 Family
Nemve1|241972	SLC22 Family
Nemve1|244617	SLC22 Family

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JGI Protein Model	Gene family assignment
Nemve1|181421	aldehyde dehydrogenase
Nemve1|179476	aldehyde dehydrogenase
Nemve1|200699	aldehyde dehydrogenase
Nemve1|192075	aldehyde dehydrogenase
Nemve1|245626	aldehyde dehydrogenase
Nemve1|162370	aldehyde dehydrogenase
Nemve1|89632	aldehyde dehydrogenase
Nemve1|235302	aldehyde dehydrogenase
Nemve1|231413	aldehyde dehydrogenase
Nemve1|150665	aldehyde dehydrogenase
Nemve1|177644	aldehyde dehydrogenase
Nemve1|149662	aldehyde dehydrogenase
Nemve1|102986	aldehyde dehydrogenase
Nemve1|175287	aldehyde dehydrogenase
Nemve1|186547	aldehyde dehydrogenase
Nemve1|154509	aldehyde dehydrogenase
Nemve1|170078	aldehyde dehydrogenase
Nemve1|156946	aldehyde dehydrogenase
Nemve1|224399	aldehyde dehydrogenase
Nemve1|96970	aldehyde dehydrogenase
Nemve1|3850	aldehyde dehydrogenase
Nemve1|181888	Aldo/keto reductase family
Nemve1|224560	Aldo/keto reductase family
Nemve1|190239	Aldo/keto reductase family
Nemve1|90844	Aldo/keto reductase family
Nemve1|208820	Aldo/keto reductase family
Nemve1|117787	Aldo/keto reductase family
Nemve1|228986	Aldo/keto reductase family
Nemve1|71477	Aldo/keto reductase family
Nemve1|220742	Aldo/keto reductase family
Nemve1|166341	Aldo/keto reductase family
Nemve1|163935	Aldo/keto reductase family
Nemve1|70665	Aldo/keto reductase family
Nemve1|107875	Cytochrome P450, CYP Clan 2
Nemve1|116955	Cytochrome P450, CYP Clan 2
Nemve1|122119	Cytochrome P450, CYP Clan 2
Nemve1|122140	Cytochrome P450, CYP Clan 2
Nemve1|12630	Cytochrome P450, CYP Clan 2
Nemve1|13146	Cytochrome P450, CYP Clan 2
Nemve1|143569	Cytochrome P450, CYP Clan 2
Nemve1|147800	Cytochrome P450, CYP Clan 2
Nemve1|161477	Cytochrome P450, CYP Clan 2
Nemve1|164344	Cytochrome P450, CYP Clan 2
Nemve1|164939	Cytochrome P450, CYP Clan 2
Nemve1|171703	Cytochrome P450, CYP Clan 2
Nemve1|182833	Cytochrome P450, CYP Clan 2
Nemve1|197176	Cytochrome P450, CYP Clan 2
Nemve1|200272	Cytochrome P450, CYP Clan 2
Nemve1|204234	Cytochrome P450, CYP Clan 2
Nemve1|206900	Cytochrome P450, CYP Clan 2
Nemve1|208281	Cytochrome P450, CYP Clan 2
Nemve1|209361	Cytochrome P450, CYP Clan 2
Nemve1|217770	Cytochrome P450, CYP Clan 2
Nemve1|220856	Cytochrome P450, CYP Clan 2

JGI Protein Model	Gene family assignment
Nemve1|221914	Cytochrome P450, CYP Clan 2
Nemve1|22420	Cytochrome P450, CYP Clan 2
Nemve1|227345	Cytochrome P450, CYP Clan 2
Nemve1|229410	Cytochrome P450, CYP Clan 2
Nemve1|230060	Cytochrome P450, CYP Clan 2
Nemve1|231212	Cytochrome P450, CYP Clan 2
Nemve1|231218	Cytochrome P450, CYP Clan 2
Nemve1|234104	Cytochrome P450, CYP Clan 2
Nemve1|234969	Cytochrome P450, CYP Clan 2
Nemve1|235065	Cytochrome P450, CYP Clan 2
Nemve1|238893	Cytochrome P450, CYP Clan 2
Nemve1|33342	Cytochrome P450, CYP Clan 2
Nemve1|60849	Cytochrome P450, CYP Clan 2
Nemve1|80762	Cytochrome P450, CYP Clan 2
Nemve1|82634	Cytochrome P450, CYP Clan 2
Nemve1|87502	Cytochrome P450, CYP Clan 2
Nemve1|87891	Cytochrome P450, CYP Clan 2
Nemve1|94651	Cytochrome P450, CYP Clan 2
Nemve1|114170	Cytochrome P450, CYP Clan 3
Nemve1|120897	Cytochrome P450, CYP Clan 3
Nemve1|120985	Cytochrome P450, CYP Clan 3
Nemve1|122064	Cytochrome P450, CYP Clan 3
Nemve1|129215	Cytochrome P450, CYP Clan 3
Nemve1|12978	Cytochrome P450, CYP Clan 3
Nemve1|132216	Cytochrome P450, CYP Clan 3
Nemve1|142057	Cytochrome P450, CYP Clan 3
Nemve1|175140	Cytochrome P450, CYP Clan 3
Nemve1|176140	Cytochrome P450, CYP Clan 3
Nemve1|176175	Cytochrome P450, CYP Clan 3
Nemve1|181495	Cytochrome P450, CYP Clan 3
Nemve1|190607	Cytochrome P450, CYP Clan 3
Nemve1|219163	Cytochrome P450, CYP Clan 3
Nemve1|220823	Cytochrome P450, CYP Clan 3
Nemve1|233531	Cytochrome P450, CYP Clan 3
Nemve1|32720	Cytochrome P450, CYP Clan 3
Nemve1|32845	Cytochrome P450, CYP Clan 3
Nemve1|890	Cytochrome P450, CYP Clan 3
Nemve1|89564	Cytochrome P450, CYP Clan 3
Nemve1|86714	Cytochrome P450, CYP Clan 4F
Nemve1|136068	Cytochrome P450, CYP Clan 4V
Nemve1|194368	Cytochrome P450, CYP Clan 4V
Nemve1|102678	Cytochrome P450, CYP Clan Mito
Nemve1|107622	Cytochrome P450, CYP Clan Mito
Nemve1|122810	Cytochrome P450, CYP Clan Mito
Nemve1|140839	Cytochrome P450, CYP Clan Mito
Nemve1|162389	Cytochrome P450, CYP Clan Mito
Nemve1|176042	Cytochrome P450, CYP Clan Mito
Nemve1|185054	Cytochrome P450, CYP Clan Mito
Nemve1|202906	Cytochrome P450, CYP Clan Mito
Nemve1|83609	Cytochrome P450, CYP Clan Mito
Nemve1|94936	Cytochrome P450, CYP Clan Mito
Nemve1|101023	Cytochrome P450, CYP unassigned
Nemve1|101105	Cytochrome P450, CYP unassigned
Nemve1|113799	Cytochrome P450, CYP unassigned
Nemve1|11772	Cytochrome P450, CYP unassigned
Nemve1|124567	Cytochrome P450, CYP unassigned
Nemve1|175215	Cytochrome P450, CYP unassigned

JGI Protein Model	Gene family assignment
Nemve1|180149	Cytochrome P450, CYP unassigned
Nemve1|194035	Cytochrome P450, CYP unassigned
Nemve1|236002	Cytochrome P450, CYP unassigned
Nemve1|241155	Cytochrome P450, CYP unassigned
Nemve1|141685	Epoxide Hydrolase
Nemve1|231029	Flavin-binding monooxygenase-like
Nemve1|185021	Flavin-binding monooxygenase-like
Nemve1|110766	Flavin-binding monooxygenase-like
Nemve1|235461	Flavin-binding monooxygenase-like
Nemve1|33129	Flavin-binding monooxygenase-like
Nemve1|55039	Flavin-binding monooxygenase-like

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JGI Protein Model	Gene family assignment
Nemve1|178546	eEF1-gamma
Nemve1|237669	Glutathione S-transferase, GSTtheta
Nemve1|186779	Glutathione S-transferase, GSTzeta
Nemve1|83876	Glutathione S-transferase, fungal-type
Nemve1|184426	Glutathione S-transferase, GSTmu
Nemve1|86756	Glutathione S-transferase, GSTmu
Nemve1|226731	Glutathione S-transferase, GSTmu
Nemve1|210927	Glutathione S-transferase, GSTomega
Nemve1|80855	Glutathione S-transferase, GSTomega
Nemve1|202412	Glutathione S-transferase, GSTomega
Nemve1|198281	Glutathione S-transferase, GSTpi
Nemve1|188039	Glutathione S-transferase, GSTpi
Nemve1|192646	Glutathione S-transferase, GSTsigma
Nemve1|162659	Glutathione S-transferase, GSTsigma
Nemve1|236609	Glutathione S-transferase, GSTsigma
Nemve1|216187	Glutathione S-transferase, GSTsigma
Nemve1|179696	Glutathione S-transferase, GSTsigma
Nemve1|113255	Glutathione S-transferase, GSTsigma
Nemve1|195944	MAPEG family
Nemve1|238552	MAPEG family
Nemve1|240887	MAPEG family
Nemve1|248820	MAPEG family
Nemve1|82570	MAPEG family
Nemve1|78952	Sulfotransferase SULT3A
Nemve1|79079	Sulfotransferase SULT3A
Nemve1|106039	Sulfotransferase SULT3A
Nemve1|106239	Sulfotransferase SULT3A
Nemve1|86774	Sulfotransferase SULT3A
Nemve1|111868	Sulfotransferase SULT3A
Nemve1|199956	Sulfotransferase SULT3A
Nemve1|149898	Sulfotransferase SULT4
Nemve1|113472	Sulfotransferase SULT3B
Nemve1|111938	Sulfotransferase SULT3A
Nemve1|247365	Sulfotransferase SULT4
Nemve1|231277	Sulfotransferase SULT3B
Nemve1|2578	Sulfotransferase SULT4
Nemve1|207647	Sulfotransferase SULT4
Nemve1|34192	Sulfotransferase keratan/chondroitin sulfatase
Nemve1|202521	Sulfotransferase keratan/chondroitin sulfatase
Nemve1|20906	Sulfotransferase SULT4
Nemve1|207646	Sulfotransferase keratan/chondroitin sulfatase
Nemve1|207648	Sulfotransferase keratan/chondroitin sulfatase
Nemve1|182889	Sulfotransferase keratan/chondroitin sulfatase
Nemve1|122621	Sulfotransferase keratan/chondroitin sulfatase
Nemve1|107994	Sulfotransferase keratan/chondroitin sulfatase
Nemve1|122091	UDP-glucoronosyl and UDP-glucosyl transferase
Nemve1|116699	UDP-glucoronosyl and UDP-glucosyl transferase
Nemve1|240864	UDP-glucoronosyl and UDP-glucosyl transferase
Nemve1|170378	UDP-glucoronosyl and UDP-glucosyl transferase
Nemve1|111143	UDP-glucoronosyl and UDP-glucosyl transferase
Nemve1|90373	UDP-glucoronosyl and UDP-glucosyl transferase
Nemve1|11044	UDP-glucoronosyl and UDP-glucosyl transferase
Nemve1|140893	UDP-glucoronosyl and UDP-glucosyl transferase
Nemve1|122110	UDP-glucoronosyl and UDP-glucosyl transferase

Supplemental Table S4

JGI Protein Model	Gene family assignment
Nemve1|103289	catalase
Nemve1|236349	manganese superoxide dismutase
Nemve1|231554	Iron/manganese superoxide dismutase
Nemve1|94316	Iron/manganese superoxide dismutase
Nemve1|165732	Copper/zinc superoxide dismutase
Nemve1|234825	Copper/zinc superoxide dismutase
Nemve1|227361	Copper chaperone for copper/zinc superoxide dismutase
Nemve1|3582	Extracellular Copper/zinc superoxide dismutase
Nemve1|110690	Phytochelatin synthase
Nemve1|136743	Phytochelatin synthase
Nemve1|202690	Phytochelatin synthase
Nemve1|96744	Phytochelatin synthase
Nemve1|90698	Glutathione Peroxidase
Nemve1|93209	Glutathione Peroxidase
Nemve1|55851	Glutathione Peroxidase
Nemve1|140021	Glutathione Peroxidase
Nemve1|225874	Glutathione Peroxidase
Nemve1|131086	Glutathione Peroxidase
Nemve1|63846	Glutathione Peroxidase
Nemve1|238222	Glutathione Peroxidase
Nemve1|81508	Glutathione Peroxidase
Nemve1|81388	Glutathione Peroxidase
Nemve1|114609	Glutathione Peroxidase
Nemve1|9969	Glutathione Peroxidase
Nemve1|180776	Glutamate-cysteine ligase
Nemve1|75832	Glutamate-cysteine ligase
Nemve1|130991	Eukaryotic glutathione synthase
Nemve1|105627	Hsp20 protein
Nemve1|105643	Hsp20 protein
Nemve1|127462	Hsp20 protein
Nemve1|127468	Hsp20 protein
Nemve1|127482	Hsp20 protein
Nemve1|127489	Hsp20 protein
Nemve1|127508	Hsp20 protein
Nemve1|127544	Hsp20 protein
Nemve1|129111	Hsp20 protein
Nemve1|129113	Hsp20 protein
Nemve1|142100	Hsp20 protein
Nemve1|143593	Hsp20 protein
Nemve1|143610	Hsp20 protein
Nemve1|156025	Hsp20 protein
Nemve1|160645	Hsp20 protein
Nemve1|239200	Hsp20 protein
Nemve1|46373	Hsp20 protein
Nemve1|86804	Hsp20 protein
Nemve1|86017	Hsp70 protein
Nemve1|216823	Hsp70 protein
Nemve1|189485	Hsp70 protein
Nemve1|195315	Hsp70 protein
Nemve1|234533	Hsp70 protein
Nemve1|233881	Hsp70 protein

JGI Protein Model	Gene family assignment
Nemve1|172038	Hsp70 protein
Nemve1|243437	Hsp70 protein
Nemve1|79267	Hsp70 protein
Nemve1|178640	Hsp90 protein
Nemve1|181671	Hsp90 protein
Nemve1|162107	Hsp90 protein
Nemve1|153358	Hsp90 protein