Project Summary

I. Intellectual Merits

A. Background—NF-κB is one of the most intensively studied animal proteins—over 23,000 publications are indexed in PubMed under the keyword 'NF-kappaB'. NF-κB is of such great interest because it mediates cellular responses to a wide variety of biotic and abiotic stressors in a phylogenetically diverse range of taxa, from vertebrates to arthropods.

We have identified NF-κB in the sea anemone *Nematostella vectensis*, a member of the phylum Cnidaria, thereby pushing the origin of this protein back in evolutionary history, to a time before the origin of coelomate animals. In addition, we have identified the first naturally occurring polymorphism described for NF-κB. Interestingly, this single-nucleotide polymorphism (SNP) occurs in a residue that is known to have profound functional significance. The polymorphic residue (cysteine or serine) occurs in the DNA binding domain of the protein. Nearly all other animals that have been studied exhibit exclusively a cysteine at this position. The fruit fly *Drosophila* is unique in exhibiting exclusively a serine. Site directed mutagenesis has already demonstrated that a serine at this residue reduces oxidative and thiol induced inactivation of the protein while conferring greater binding affinity.

B. Specific Aims

The proposed research will address three main questions.

(1) Does *Nematostella* NF-κB mediate cellular stress responses?

(2) Is the polymorphism being maintained in the wild by natural selection?

(3) How does the Cys/Ser single nucleotide polymorphism affect fitness?

We hypothesize that *Nematostella* NF-κB is involved in mediating cellular stress responses, and that the 'Ser allele' confers a selective advantage on those individuals that inhabit oxidative environments. To test these hypotheses, we will accomplish the following four aims:

(1) characterize the population genetic structure of the Cys/Ser polymorphism in two natural populations (Humboldt, CA and Halifax, NS) and relate the spatial distribution of genotypes to potentially relevant environmental variables, including hydrogen peroxide concentrations.

(2) compare the regeneration rates among animals with all three genotypes under different degrees of oxidative stress (different levels of hydrogen peroxide)

(3) monitor the short-term evolutionary dynamics of the Cys and Ser alleles in laboratory mesocosms under different degrees of oxidative stress.

(4) determine whether *Nematostella* NF-κB is translocated to the nucleus in response to stress (as in coelomates) by monitoring the sub-cellular location of a GFP-NF-κB fusion protein.

II. Broader Impacts

(1) The proposed research will allow the co-PI to fully realize innovative new research initiatives that he developed. This will foster his development as an independent research scientist.

(2) The project represents a rare opportunity to investigate a functionally important SNP that occurs in nature using population genetic, developmental, and molecular approaches. The results of the study will contribute to the broader effort to understand the selective consequences of microevolutionary change. In addition, the results are likely to have future conservation applications.

(3) Both the PI and the co-PI have built resources for the scientific community to facilitate the use of *Nematostella vectensis* as a model system (http://nematostella.org; http://stellabase.org). These efforts would be directly supported by the funding of the current proposal.

(4) Both the PI and the co-PI have a history of training undergraduate researchers, including students from groups traditionally underrepresented among professional scientists. Funding of the current proposal would allow the PI and co-PI to mentor 1-2 additional undergraduates.

(5) Both the PI and the co-PI have closely integrated their research with the development of science curriculum. The proposal describes one concrete example of a curriculum unit on "Polymorphism and Natural Selection" that the co-PI will be developing as an outgrowth of the proposed research.

Project Description

I. Introduction

A. The NF-κB pathway—NF-κB and related proteins are a heavily studied group of intracellular signaling molecules—a query of the PubMed database for journal articles with the keyword "nf-kappaB" identifies over 23,000 hits. The NF-κB family is critically important for transducing information about a cell's environment to the nucleus. The NF-κB pathway mediates cellular responses to biotic and abiotic stress in animals as diverse as chordates and arthropods (www.nfkb.org, [1-5]; Fig.1). In addition, NF-κB-related proteins play important developmental roles. In *Drosophila*, the NF-κB protein *Dorsal* is required for proper dorsal-ventral patterning [6-8]. In vertebrates, NF-κB proteins affect cellular differentiation in diverse cell types, including liver cells and immune cells [9-11].

NF-κB belongs to a family of transcription factors that bind DNA through a domain known as the rel-homology domain (RHD). In the canonical pathway, the NF-κB protein is constitutively expressed and present in the cytoplasm, but it is maintained in an inactive state by the binding of a repressor protein (Fig. 1; [1]). The repressor protein consists of multiple Ankyrin repeats (ANK). In the case of NF-κB, the ANK repeats are encoded within the 3' portion of the transcript (self-inhibition). In the case of the vertebrate Rel proteins, the repressor protein is encoded by a separate inhibitor locus [1].

Activation of the NF-κB pathway, which can be triggered by physical (*e.g.*, UV and oxidative stress) or biological stress (*e.g.,* lipopolysaccharides, dsRNA, CpG-DNA, 21 and other known classes of activators; [12]) leads to the proteolytic degradation of NF-κB's inhibitor. Degradation of the inhibitor results in the translocation of NF-κB to the nucleus, where it forms homodimers or heterodimers with other Rel-homology domain proteins (*e.g.,* RelA, RelB, and c-Rel in vertebrates) and activates transcription of target sequences. Downstream sequences regulated by NF-κB include suites of transcription factors, oxidative response elements, anti-microbial genes, interferon, and anti-apoptotic genes. The specific suite of target genes that is activated is context dependent.

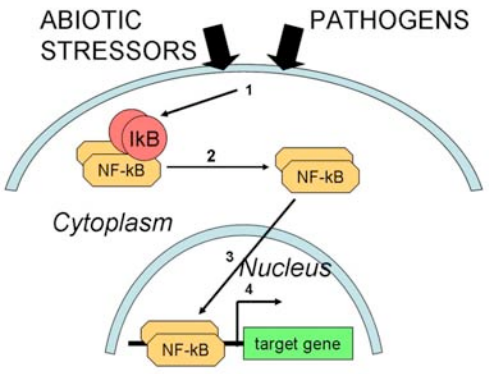

Figure 1. Diagrammatic representation of the canonical NF-κB pathway.

B. Evolution of NF-κB's role in stress response—Zheng *et al.* [13] have proposed that the NF-κB stress-response pathway is an invention of coelomate animals, reasoning that it could not have functioned properly prior to the evolutionary origin of the coelom, an internal body cavity enclosed by mesoderm. Zheng *et al.* argue that in an aquatic animal lacking such an internal body cavity (e.g., in diploblastic animals such as sea anemones), secreted signaling proteins utilized in the NF-κB stress-response pathway would become too diluted in the surrounding medium. However, the endoderm and ectoderm of diploblastic animals does enclose an internal space (typically filled with extracellular connective tissue known as mesoglea). Futhermore, diploblasts can create temporary internal cavities that enclose some or all of the main body cavity (the gastrovascular cavity or coelenteron).

Even if the NF-κB stress-response pathway evolved after the invention of the coelom, it would still be possible that the NF-κB protein itself could have evolved prior to this, and the protein could have been co-opted into a stress-reponse pathway later during coelomate evolution. Indeed, in coelomate animals, NF-κB homologs regulate both stress response and cellular differentiation pathways. It is currently unclear what the primitive role of the NF-κB protein was, stress-response or cell differentiation.

C. The starlet sea anemone *Nematostella vectensis*—To determine whether NF-κB and other RHD-containing proteins might have evolved prior to the origin of the coelom, we queried the recently sequenced genome of the starlet sea anemone, *Nematostella vectensis* (Joint Genome Insititute, Dept. of Energy; Dan Rokhsar, principal investigator) using a genomic database that we developed [14]. *Nematostella* belongs to the Phylum Cnidaria, an early emerging lineage of animals that diverged from

the ancestor of coelomate metazoans prior to the origin of the coelom (Fig. 2). Because the phylum Cnidaria is an outgroup to the superphylum Coelomata (also known as the Bilateria), those genes shared by *Nematostella* and coelomates must predate the origin of the Coelomata. *Nematostella* is a particularly valuable cnidarian model system for genomic, developmental, and environmental studies because it is easy to culture, it is easy to study in the field, and its entire genome has been sequenced [15].

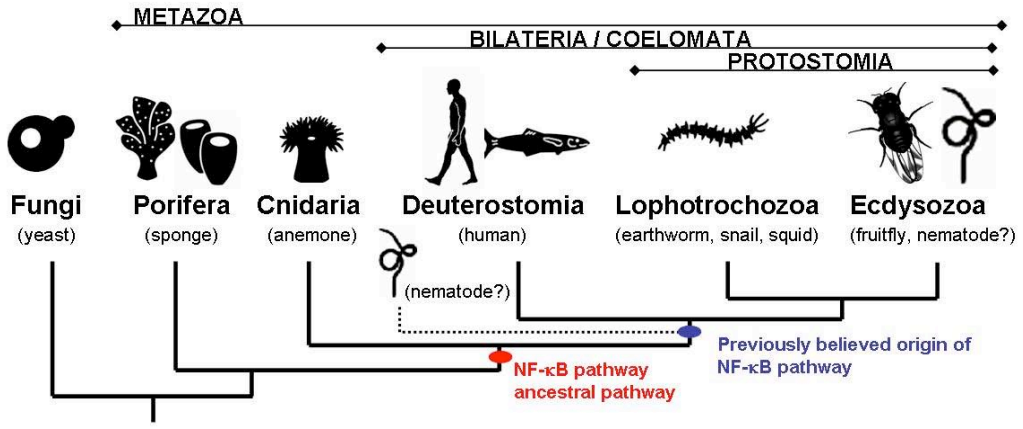

Figure 2. Phylogenetic relationships of cnidarians and coelomate animals.

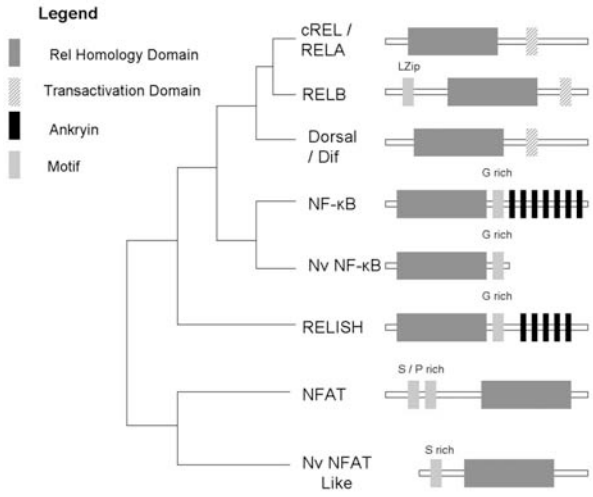

Figure 3. Structure of Nv-RHD proteins relative to RHD from other taxa.

II. Preliminary Results

A. Identification of NF-κB signaling components in the Cnidaria—We were able to identify two RHD-containing proteins in the *Nematostella* genome, one of which appears homologous to NF-κB (Fig. 3) [16]. Importantly, Nv-NF-κB (*Nematostella* NF-κB) is the first NF-κB identified which lacks a C-terminal ANK domain—this condition may reflect the ancestral state, and the genomic fusion of the NF-κB locus and its inhibitor could have occurred later in coelomate evolution. This inference is supported by the existence of a separate ANK-repeat inhibitor loci in the genome of coelomate animals, even though, in vertebrates, the NF-κB transcript encodes both the transcriptional activator portion of the protein and the inhibitory ankyrin-repeat region (Fig. 3). In addition to recovering NF-κB from *Nematostella*, we were able to identify homologs of many of the proteins that constitute the canonical NF-κB pathway (e.g., IκB, NFAT, Toll receptors, TNF) suggesting that the pathway may have evolved prior to the cnidarian-coelomate split.

B. A functionally significant polymorphism in *Nematostella* NF-κB We have discovered a single nucleotide polymorphism (SNP) within the DNA binding domain of NF-κB that, based upon functional analysis in other species, is certain to have functional significance at the molecular level. NF-κB is a redox-sensitive and redox-regulated transcription factor [2, 5, 17], and the presence of a cysteine at position 6 within the 17 amino acid NF-κB recognition loop imparts redox sensitivity. A Cys-to-Ser mutation at this position increases the DNA-binding affinity of the Rel/NF-κB protein complex [18-20] and renders the protein resistant to inhibition by thiol-reactive compounds [19-21] and insensitive to redox regulation [17, 22]. With the exception of *Drosophila* Relish, the Cys residue is ubiquitous at this

position, perhaps because the Cys version of the protein may be a more effective transcriptional regulator under typical physiological conditions. Surprisingly, we have identified the same Cys/Ser "polymorphism" that has been intensively investigated in site directed mutagenesis laboratory studies in wild populations of *Nematostella vectensis*—of the nine NF-κB ESTs which have been made publicly available through the *Nematostella* genome sequencing initiative five encode a serine at this residue and four encode a cysteine (http://www.ncbi.nlm.nih.gov/dbEST/; Fig. 5).

III. Specific Aims

The proposed research has three specific aims.

(1) To characterize the allele and genotype frequency of the Cys/Ser polymorphism in two wild populations of *Nematostella* (Humboldt Bay, CA and Halifax, Nova Scotia).

(2) To determine whether the genotype at this locus has consequences for the animals' response to oxidative stress by comparing the regenerative ability of heterozygotes and homozygotes (Cys/Cys and Ser/Ser) under varying concentrations of H_2O_2.

(3) To determine if Nv-NF-κB is translocated from the cytoplasm to the nucleus in response to stress using a GFP-Nv-NF-κB fusion construct and known triggers of the Nf-κB stress pathway.

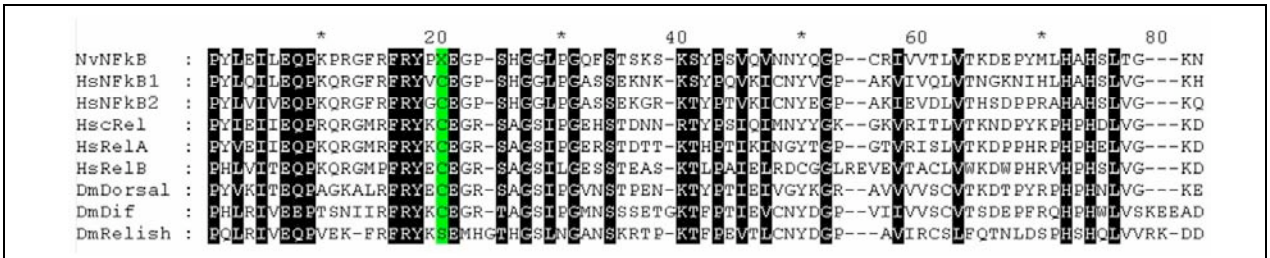

Figure 5. Alignment of the N-terminal portion of the RHD. The DNA recognition loop includes residues 15-30. Redox sensitivity is k imparted by residue 20 (highlighted in green) and indicated as 'X' for Nv-NF-κB, which encodes either a Cys or Ser. (Hs, *Homo sapiens*; Nv, *Nematostella vectensis*; Dm, *Drosophila melanogaster*)

A. Specific Aim 1

To characterize the allele and genotype frequency of the Cys/Ser polymorphism in two wild populations of *Nematostella* (Humboldt Bay, CA and Halifax, Nova Scotia).

1. Background and Significance

a. A rare opportunity to study a naturally occurring NF-κB polymorphism—*Nematostella* represents the only known naturally occurring Cys/Ser polymorphism in the DNA recognition loop of NF-κB. In *Drosophila melanogaster,* the *Relish* protein exhibits a serine residue at this position, while all other species that have been surveyed exhibit a cysteine (Fig. 5). Possession of a serine at this key position in the DNA recognition loop might impart a selective advantage in an oxidative environment because it would allow the protein to resist oxidative inactivation. Additionally, this mutation could allow individuals to withstand NF-κB inactivation caused by thiol-compounds.

b. Oxidative stress in *Nematostella's* natural habitat—*Nematostella* is found in estuarine environments, sometimes in tidal creeks but more typically in isolated pools in high marsh habitat. Hydrogen peroxide (H_2O_2) is generated by photochemical excitation of dissolved organic carbon (DOC), leading to free radical production and H_2O_2 formation [23-27]. Thiol-containing compounds, when present in *Nematostella*'s alkaline environment, become strongly nucleophilic thiolate ions.

Hydrogen peroxide concentration varies considerably on a regional scale (as a function of latitude/solar radiance) and on a *local* scale. Abele-Oeschger et al. [28] recorded differences in $[H_2O_2]$ spanning three orders of magnitude within a single marsh. Additionally, they noted that sediment, which is the habitat of the infaunal anemone *Nematostella*, acts as a sink for H_2O_2, with sediment and 'bottom water' having ~4x the hydrogen peroxide concentration of the water immediately above it. The concentration of hydrogen peroxide that they observed in the salt marsh (up to ~4.5 μM) is within the range that has been observed to inhibit algal growth [29] and reduce invertebrate metabolic rates [30, 31].

2. Hypotheses to be tested

(a) Heterozygotes or Ser/Ser homozygotes will have an advantage in oxidative environments because the protein encoded by that allele will function better.

(b) Cys/Cys homozygotes will have an advantage in less oxidative environments.

(c) Correlatively, in estuaries that are known to harbor both alleles, open water environments (*e.g.*, tidal creeks, tidal flats) which allow vertical and horizontal mixing of photochemically produced H_2O_2 will harbor mostly Cys/Cys homozygotes.

(d) Conversely, isolated pools, which experience sharply elevated peroxide levels each mid-day [31], will consist of heterozygotes and Ser/Ser homozygotes.

3. Preliminary Data

The Finnerty laboratory maintains an extensive collection of DNA samples from wild populations of *Nematostella* throughout the species' known range, including the Atlantic and Pacific coasts of North America and southern England. As part of a preliminary analysis, we have amplified a fragment of the RHD from 20 animals representing 13 wild populations. Eighteen animals were homozygous for the Cys allele. The remaining 2 animals—1 from Halifax, Nova Scotia and the other from Humboldt Bay, CA—were heterozygous (Figs. 6, 7).

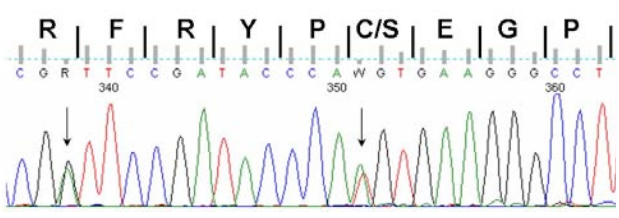

Figure 6. Representative chromatogram from a direct-sequencing read of the RHD amplified from a specimen (Humboldt Bay, CA). Polymorphic residues are marked by an arrow. The A/T transition at position 351 results in the critical Cys/Ser. Sequences from cloned amplicons reveals that the 'wobble' position polymorphism at residue 348 is associated with the polymorphism at position 351.

4. Experimental Methods

(a) Animal collection. We will collect ~300 *Nematostella* from a wide range of habitat types, including tidal creeks, tidal flats, and isolated pools at both of the sites where we know the rare serine allele is represented (Humboldt Bay and Halifax). Rhode River, Maryland is excluded from our study since the original collection site has been substantially degraded (A. Reitzel, personal communication). Collections will be conducted in August, when H_2O_2 levels are expected to be at their highest [28].

(b) Water and sediment sampling. Water samples will be collected at midday (peak [H_2O_2]) from multiple habitats within each site. Samples will be collected in triplicate, and the temporal sequence of sampling will be varied each day. Samples will be maintained in the dark on ice and transported as soon as possible for fluorometric or HPLC analysis to determine [H_2O_2] within the micro-molar range [28]. At each sampling site, temperature and oxygen saturation will be recorded, and sediment will be collected. The sediment will be dried at 60° C to determine water content then combusted at 480° C to determine organic content.

(c) Genotype determination. Animals will be transported to the Finnerty Lab at Boston University. A portion of the foot will be excised for DNA isolation. Each animal will be repeatedly cut and allowed to regenerate so that a living genetic stock may be preserved. Allele specific primers, which have already been designed and tested, will be utilized to determine the genotype of all individuals at the NF-κB locus. For a subset of samples (~20 representatives of each allele from each site), a 720 bp nucleotide fragment of genomic sequence that includes the first exon of the rel-homology domain will be amplified using a primer pair that has already been developed and tested (Fig. 6). These fragments will be sequenced so that we might determine their complete nucleotide sequence.

In addition, a subset of the animals from California and Nova Scotia will be subjected to AFLP fingerprinting so that we might characterize the genetic backgrounds of animals carrying the Cys and Ser alleles. The Finnerty lab has developed a set of robust AFLP procedures which produce well over 200

polymorphic loci. We have already successfully used these techniques to evaluate genetic structure at multiple scales (Fig. 7 [32, 33]).

(d) Statistical analyses. Wilk's λ test will be used determine if the observed allelic distribution is a function of peroxide concentration or one of the other measured environmental variables. Fisher's linear discriminate function will be used to model predictive relationships between significantly related variables [34].

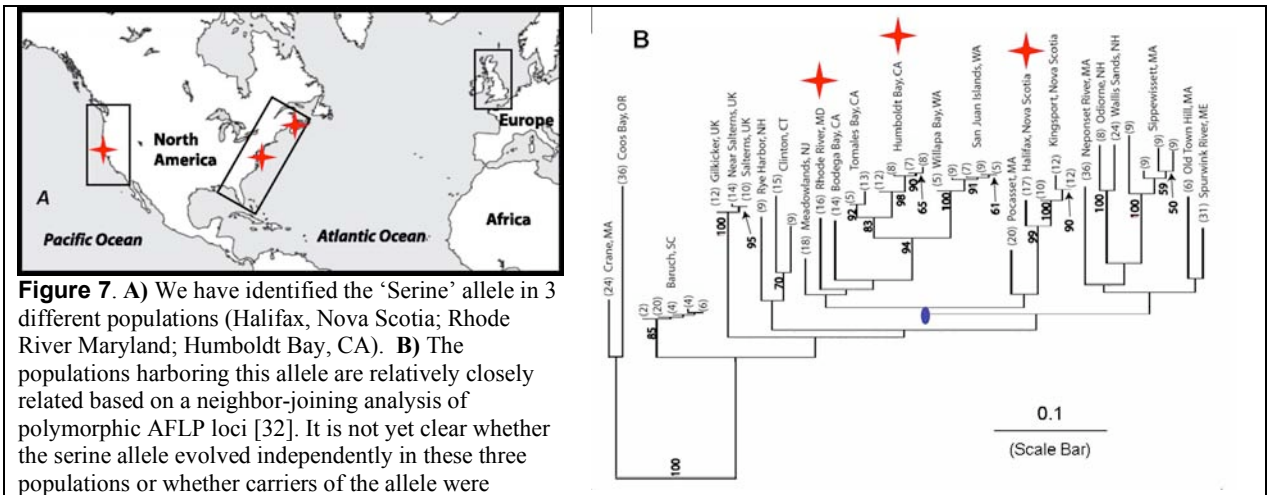

Figure 7. **A)** We have identified the 'Serine' allele in 3 different populations (Halifax, Nova Scotia; Rhode River Maryland; Humboldt Bay, CA). **B)** The populations harboring this allele are relatively closely related based on a neighbor-joining analysis of polymorphic AFLP loci [32]. It is not yet clear whether the serine allele evolved independently in these three populations or whether carriers of the allele were transported among estuaries, possibly via ship [32].

B. Specific Aim 2

To determine whether the genotype at the NF-κB locus has consequences for the animals' response to oxidative stress by comparing the regeneration rates of heterozygotes, Cys-Cys homozygotes, and Ser-Ser homozygotes under varying concentrations of H_2O_2.

1. Background and Significance

Specific aim 1 has the potential to reveal a statistical correlation between the allelic / genotypic frequencies and the measured environmental variables, but it does not *directly* test the hypothesis that the Cys/Ser polymorphism affects organismal performance or reproductive success. The genetic data could, in theory, reveal a spurious correlation between genotype and environment. Furthermore, because the data would represent a snapshot in time, they could fail to reveal the existence of a direct relationship that does exist. Fortunately, *Nematostella* is small and easy to culture over multiple generations, and clonal lines can be quickly generated, so the hypothesized relationship between genotype and organismal performance can be directly tested in the laboratory.

2. Hypotheses to be tested

(a) Ser/Ser and Ser/Cys individuals will regenerate more quickly and/or more successfully than Cys/Cys individuals in more oxidative environments.

(b) Cys/Cys individuals will regenerate more quickly and/or more successfully than Ser/Ser and Ser/Cys individuals in less oxidative environments.

(c) Over multiple generations, the allelic and genotypic composition of an artificially constructed population of *Nematostella* will evolve in response to hydrogen peroxide concentration.

i) At low levels of hydrogen peroxide, the Cys allele will increase

ii) At high levels of hydrogen peroxide, the Ser allele will increase.

3. Preliminary Data

The Finnerty lab has developed a staging scheme for *Nematostella* regeneration that can be used to detect environmental or genotypic causes of developmental variation. Several discrete ontogenetic transformations serve as developmental mileposts, including mouth formation, pharynx formation,

mesentery formation, and tentacle eruption. In addition, the mesenteries (typically eight) and the tentacles (typically 12-16) can serve as meristic characters whose counts may be monitored over time.

4. Experimental Methods

(a) Regeneration Assay. Clonal lines exhibiting each of the three genotypes (C/C, S/S, and S/C) will be bisected perpendicular to the primary body axis at the aboral margin of the mesenteries using a razor blade. The foot fragments will then be monitored as they regenerate missing head structures while being exposed to one of three different peroxide concentrations. The concentrations will be determined after an initial dose-response experiment. Nine treatments, each with thirty individuals, will be utilized (3 genotypes X 3 peroxide concentrations). This bivariate experiment will test for an interaction effect between genotype and peroxide concentration. We will monitor the regenerating animals daily and record the temporal progression of several standard markers of the regeneration process including time to (1) wound healing, (2) mouth formation, (3) pharynx formation, (4) appearance of first mesentery, and (5) appearance of the first tentacle. We will also monitor tentacle number versus time. Finally, we will score each individual for success or failure of regeneration, with success defined as the presence of multiple tentacles at 10 days post bisection. Backwards stepwise logistic regression will be used to determine if genotype, [H_2O_2], and an interaction term significantly affect probability of regeneration. To test for a genetic background effect, the experiment will be conducted on a total of 10 genotypes, which will be selected to maximize genetic distance as per AFLP analysis.

(b) Selection Assay. We will populate fifteen mesocosms (glass bowls) with 20 animals each, ten Ser/Ser homozygotes and ten Cys/Cys homozygotes, split evenly among males and females. All of the animals will derive from four AFLP-fingerprinted clones (2 male clones and 2 female clones; all clonal lines will be of known AFLP 'fingerprints'). Each mesocosm will be maintained at one of three different peroxide concentrations (the same peroxide concentrations used in the regeneration assay) for a period of 100 days. As the adult females can lay eggs every 4-7 days, and the offspring require 50 days to reach sexual maturity, the time allotment will allow for the possibility that the F2 generation will have developed into sexually mature polyps. Every other day, the culture medium will be replaced with fresh artificial seawater and peroxide, and the animals will be fed (freshly hatched *Artemia* nauplii). A census of the polyps will be taken at each feeding/water change, and the egg masses will be counted. At the end of the 100 days, all polyps in the mesocosms will be (1) genotyped at the NF-κB locus using the allele specific primers and (2) AFLP fingerprinted. These genetic data will allow us to monitor changes in the frequency of each allele and infer the relative contributions of sexual versus asexual reproduction to population growth [33]. One-two undergraduates will be recruited to participate in this experiment.

C. Specific Aim 3

To determine if Nv-NF-κB is translocated from the cytoplasm to the nucleus in response to stress using a GFP-Nv-NF-κB fusion construct and exposing transgenic animals to known triggers of the NF-κB stress response pathway.

1. Background and Significance

(a) Does Nv-NF-κB plays a role in stress response—*Nematostella* is known to possess the key proteins that compose the NF-κB-mediated stress-response pathway of coelomate animals [16]. Preliminary experiments demonstrate that a protein in *Nematsotella* lysates that is recognized by an anti-NF-κB antibody does bind canonical κB binding sites (Figure 8). However, it is not yet known whether the Nv-NF-κB pathway is mobilized in response to biotic and abiotic stressors as in coelomate animals. Even if our regeneration assays reveal an affect of the Cys/Ser polymorphism under different peroxide treatments, this won't demonstrate that Nv-NF-κB is playing a role in mediating oxidative stress. Oxidative inactivation of Nv-NF-κB could also have a detrimental effect on regeneration if Nv-NF-κB is playing a role in directing cellular differentiation.

A critical event in NF-κB-mediated stress response of coelomates is the translocation of the NF-κB protein from the cytoplasm to the nucleus in response to an appropriate stressor. Recently, Wikranamayake et al. used a GFP fusion protein to study a similar intracellular translocation of the beta

catenin protein in *Nematostella* [35]. To determine if those stimuli that activate NF-κB in coelomates also activate NF-κB in *Nematostella*, we will pursue a similar approach–we will introduce an NF-κB/GFP fusion construct into *Nematostella* and expose these transgenic animals to known activators.

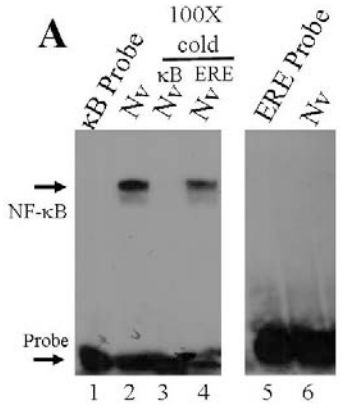

Figure 8. A) Gel shift assays provide preliminary evidence that Nv-NF-κB functions like its homolog in coelomates. A protein present in *Nematostella* extracts that is detected by an anti-NF-κB antibody binds to a canonical κB binding site (lane 1). This binding is out-competed by a 100-fold excess of unlabeled probe (lane 3), yet it persists in the presence of an unlabeled estrogen-response-element (ERE; lane 4). B) Variation between the intensity and migration of κB probe and 50 mg of lysate from individual *Nematostella* (top) suggests a functional difference between NF-κB from different specimen, potentially resultling from the identified SNP. Note that the use of extracts pooled from multiple specimen (bottom) in the same EMSA assays abolishes this variability. (T. Gilmore; See Letter of Collaboration, Appendix C2)

2. Hypotheses to be tested

(a) If NF-κB mediates organismal stress response in *Nematostella* in a manner that is homologous to that of coelomate animals, then an NF-κB/GFP fusion protein expressed in *Nematostella*

i) will be localized to the cytoplasm in non-stressed animals, and

ii) will be translocated to the nucleus in response to stress.

3. Preliminary Data

The Martindale lab (University of Hawaii) is adept at the injection of GFP-labeled synthetic mRNAs into *Nematostella* fertilized eggs. The labeled mRNAs are translated in all cells of the embryo, and their sub-cellular localization can be tracked in real time using fluorescence microscopy. Professor Martindale and colleagues used a similar approach to monitor an analogous situation— the translocation of GFP tagged Beta-catenin protein from the cytoplasm to the nucleus during axial patterning and germ layer specification [35]. This rapid, though transient assay for cellular stress can be performed upon individual living embryos. Dr. Martindale has agreed to train the co-PI in this technique at his laboratory in Hawaii (see Letter of Collaboration, Appendix C1).

4. Experimental Methods

(a) Production of transient transgenic embryos. NF-κB/GFP-fusion proteins will be produced using established protocols [35]. The GFP message will be appended to either the 5' or 3' end of the NF-κB message because its location could sterically hinder some necessary function. That is, we will use more than one strategy since NF-κB binding to IκB is responsible for retention in the cytoplasm under non-stressful conditions, and up-regulation of target genes in the nucleus requires protein dimerization.

(b) Stress assays. Transgenic animals will be exposed to a series of abiotic and biotic stressors that are known to mobilize the NF-κB pathway in coelomate animals. Abiotic stressors will include peroxide exposure and ultraviolet light. Biotic stressors will include *E. coli*, lipopolysaccharides, and double-stranded RNA. The sub-cellular localization of NF-κB/GFP will be monitored using fluorescent microscopy. The effect of each stressor will be compared to animals maintained under standard culture conditions to determine if these stressors are capable of effecting translocation of NF-κB to the nucleus.

IV. Broader Impacts

(1) The co-PI is currently a fourth-year student in the Ph. D. program at BU. He has developed the research initiatives described here, which lie outside the funded research program of the PI. The

proposed research will allow the co-PI to fully realize these innovative new research initiatives. This will foster his development as an independent research scientist.

(2) The proposed research represents a rare opportunity to investigate a functionally important SNP that occurs in nature using population genetic, developmental, and molecular approaches. The results of the study will contribute to the broader effort to understand the selective consequences of microevolutionary change. In addition, the results are likely to have future conservation applications. *Nematostella* is closely related to corals (Class Anthozoa), a group of extremely important species from ecological, economic, and recreational perspectives. Discovery of the functionality of the Ser allele, combined with additional support for an ancient origin of this allele with the Anthozoa, suggests that the Ser allele may be present in coral species. This is very significant given that oxidative stress is commonly implicated in coral reef degradation.

(3) Both the PI and the co-PI have built resources for the scientific community to facilitate the use of *Nematostella vectensis* as a model system (http://nematostella.org; http://stellabase.org; [14]). These efforts would be directly supported by the funding of the current proposal. The genomic database developed by the co-PI has already greatly benefited the burgeoning *Nematostella* research community, and the data generated by the proposed research (functional SNP assays, AFLP loci) would be very appropriate additions to the relational database.

(4) Both the PI and the co-PI have a history of training undergraduate researchers, including students from groups traditionally underrepresented among professional scientists. The current proposal would allow the PI and co-PI to mentor 1-2 additional undergraduates.

(5) The co-PI has extensive experience in teaching and curriculum development (Associate Chemistry Professor, North Shore Community College, 2002-03; NSF GK12 Fellow, Grant # DGE-0231909, 2004-2005; Boston University Teaching Fellow, 20005-2006; Boston University Summer Challenge Instructor, 2005-present), and he has endeavored to integrate research with science pedagogy education whenever possible. He has developed numerous curriculum pieces at the high school and college level, many of which are based on current research ([36]; see http://www.urbanhabitats.org/v03n01/classroom_full.html and http://www.nematostella.org/Resources_Classroom.html).

If this proposal is funded, Mr. Sullivan will develop a two-week curriculum piece that will utilize the allelic variation present in *Nematostella* to illustrate natural selection to high school students in an engaging inquiry-based approach**.** The curriculum piece will be piloted this summer (2007) in three separate sessions of a college immersion program for talented high school students, for which Mr. Sullivan is responsible for the development and deployment of science curriculum (Boston University Summer Challenge Program, http://www.bu.edu/summer/high_school/index.html). The unit will be demonstrated to faculty participants of Boston University's NSF-funded Project Stamp GK-12 program (see Letter of Collaboration, Cynthia Brossman, Appendix B1), which may facilitate its implementation next year in a number of NSF DGE funded classrooms. Finally, the lesson will be implemented in high school classrooms in the Boston Public School systems during the 2007-2008 academic year (see Letter of Support, Theresa O'Neill). The lessons will be implemented in a public school system with a majority of students from socio-economic groups which are underrepresented in the STEM disciplines.

The unit will be published freely, in a peer-reviewed science education journal and/or at http://www.nematostella.org/Resources_Classroom.html. A series of Power Point lectures, with notes for teachers, will accompany the published lesson plan. Kits, which will include a video instruction, animals of each of the three genotypes, Instant Ocean® (or equivalent) for salt water preparation, DNA samples from each genotype, and allele specific primers, will be developed.

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