
A Molecular Approach to Understanding Lobster Shell Disease

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Suppressive Subtractive Hybridization (SSH) and Polymerase Chain Reaction (PCR)

SSH or suppressive subtractive hybridization is a technique that relies on gene amplification using polymerase chain reaction (PCR) to quickly identify genes that are differentially expressed between two gene pools (i.e., mRNA isolated from healthy versus diseased lobsters). This method is very useful for "gene discovery."

This process will result in (cDNA) libraries enriched for genes that are differentially expressed in healthy (asymptomatic) lobsters versus diseased (symptomatic) lobsters.

PCR is a molecular technique that creates a large number of copies of a part of the DNA that you want to examine (called amplification). PCR is often used to identify new gene sequences in an organism, based on conservation in gene sequences between organisms. For example, the 16S rRNA gene is very well conserved among species and its sequence is commonly used to identify species of bacteria.

Real-time qPCR (quantitative PCR) uses fluorescence to quantify expression levels of a gene of interest (e.g., relative to a serially-diluted plasmid standard). In our studies, qPCR is used to measure expression levels of genes of interest in tissues from lobsters of different disease states.

Objectives of the Research:

1. Identify genes that are differentially regulated in healthy versus shell-diseased lobster and quantify expression of these genes in relation to disease state using two molecular techniques -suppressive subtractive hybridization (SSH) and quantitative real-time polymerase chain reaction (qPCR).
2. Establish associations between lobster shell disease and expression of genes associated with hormone and immune functions, molting, energy and xenobiotic metabolism, and shell formation.

Rationale:

The mechanisms behind stress-induced physiological disruption in crustaceans are largely unknown, hindering their prediction, prevention and remediation. We hypothesize that New England waters have become an increasingly stressful environment for lobsters over the past years. This has resulted in physiological disruption that has led to irreversible effects at the individual and population level, and may play an important role in lobster shell disease and lobster population declines. We further hypothesize that this physiological disruption will be reflected by changes in gene expression.

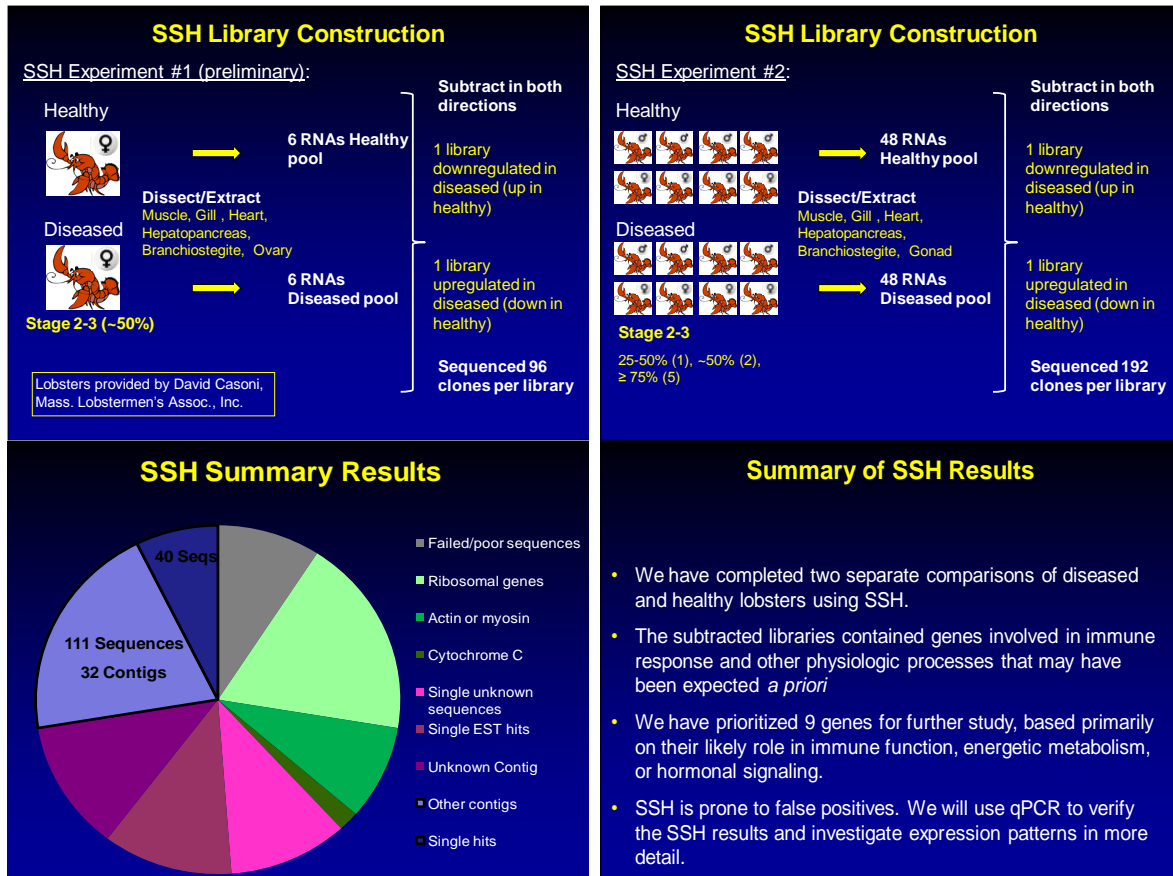
Progress to Date:

1. We have conducted two independent SSH experiments to identify genes that are differentially expressed in healthy and diseased lobsters, and began to describe tissue-specific expression patterns of nine of these genes.
2. We have cloned full length sequences for genes that are of particular interest (e.g., genes related to molting, hormone and immune function, shell formation) and designed qPCR primers for most of these.
3. We have focused the qPCR effort for now on the genes identified through SSH (because it tells a more complete story for publication) and have finished a first set of qPCR analyses.

Results to Date:

Objective 1. Identify factors that contribute to shell disease by comparing global gene expression between asymptomatic and symptomatic lobsters.

- RNA was isolated from muscle, hepatopancreas, heart, gonad, gills, and branchiostegite
- RNA obtained from several individuals were pooled to minimize variability
- RNA pools were generated for healthy (asymptomatic) and shell-diseased (symptomatic; moderately to severely) animals



Objective 2. Testing associations between lobster shell disease and gene expression shell

- Quantify the expression of approximately 15-30 genes in healthy (asymptomatic) and diseased (symptomatic; mild, moderate, severe) animals

SSH Genes for Further Analysis					
Gene	Up Disease	Down Disease	Role		
Crustin(s?)	3	2	2	Component of immune system.	
Mannose binding protein(s?)	1	6	4	6	Component of immune system. Related genes highly expressed in ovary.
Hemocyanin	1		2		Component of immune system; Oxygen binding and phenoloxidase activity. Skeletal repair.
Macroglobulin	1				Proteinase inhibitors, component of immune system.
Keratinocyte associated protein 2	2				Role unknown (immune?). Upregulated in shrimp gill during viral infection.
Arginine Kinase	1	1	3		Energetic metabolism (ATP homeostasis). Altered expression with shrimp disease.
Hydroxyacyl dehydrogenase			1		Energetic metabolism of fats, some forms essential to ovary/germ line development.
Mevalonate kinase		1			Isoprenoid synthesis (hormonal signaling).
GnRH-Receptor(?)			1		G-protein coupled receptor (hormonal signaling)

- Targeted genes

Planned genes for qPCR	
Nutrition/Energetic Metabolism	<ul style="list-style-type: none"> CHH A/B: sequenced from <i>Ha</i> Arginine Kinase, Hydroxyacyl dehydrogenase
Stress Response	<ul style="list-style-type: none"> HSP70, HSP90: sequenced from <i>Ha</i>
Defense/Immune System	<ul style="list-style-type: none"> Prophenoloxidase: sequenced from <i>Ha</i> Hemocyanin Crustin, Macroglobulin, Mannose-binding protein
Xenobiotic Metabolism	<ul style="list-style-type: none"> CYP45: sequenced from <i>Ha</i>
Molting/Hormonal Signaling	<ul style="list-style-type: none"> Shade-analog: <u>Cloned full sequence</u> EcR: <u>Cloned partial sequence</u> RXR/USP: <u>Cloned full sequence</u>, possible splice variants Mevalonate Kinase, "GnRH" Receptor
Shell Formation	<ul style="list-style-type: none"> Chitinase(s): two ESTs Chitinase: one EST
Housekeeping	<ul style="list-style-type: none"> 16S rRNA, β-actin, : <u>Cloned partial sequence</u>

Investigation of genes from SSH

Initial Objectives:

- To verify results from SSH (eliminate false positives)
- To identify the most appropriate tissues for each gene

Approach:

Pool RNA for each of 6 tissues from 8 healthy lobsters and from 8 diseased lobsters:

Healthy: SSH pool, Gill, Muscle, Heart, Hepatopancreas, Branchiostegite, Ovary, Testes	} 14 RNA samples
Disease: SSH pool, Gill, Muscle, Heart, Hepatopancreas, Branchiostegite, Ovary, Testes	

Conclusions:

- Most genes were primarily expressed in one to two tissues.
- Hemocyanin was expressed primarily in the Hepatopancreas, and Arginine Kinase was expressed primarily in muscle and ovaries.
- Some sequences similar to "mannose binding proteins" were annotated as highly expressed in ovary (These will be studied in more detail in ovaries of varying maturity and disease state).
- Macroglobulins were expressed in several other tissues at relatively high levels, including highly vascularized tissues such as muscle, gill and branchiostegite. We will continue to study expression of this gene in several tissues, including hemocytes.

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