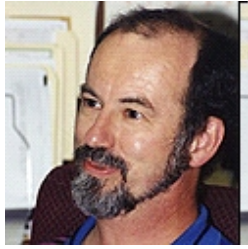


GK-12 Graduate Fellows Program

Funded by National Science Foundation under Grant No. 0139171

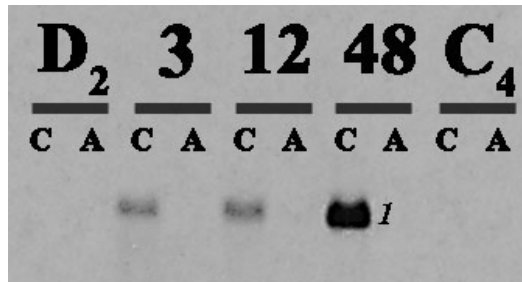
Dr. Tom Shafer: the molecular role



I am a developmental/molecular biologist interested in changes in gene expression as marine organisms progress through key phases of their life cycle. Most of the activity in my laboratory centers around analyzing proteins and glycoproteins (proteins with attached sugars) extracted from the cuticles of blue crabs (*Callinectes sapidus*). This typically involves the cloning and sequencing of the genes that code for these proteins.

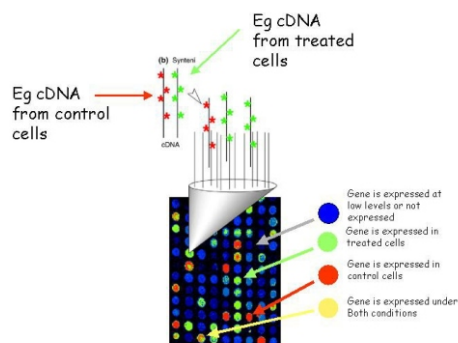


A blue crab in the act of molting. The soft-shelled (i.e. as yet unmineralized) crab is beneath the old mineralized carapace from which it is emerging.



A “Northern” blot showing the presence of a specific mRNA in cuticle that will mineralize (C) and joint membrane that will remain flexible (A) at different times in the molt cycle. D₂ is premolt; 3, 12, 48 are hours postmolt; and C₄ is an intermolt (or hard) crab. This gene is “on” only in the hardening cuticle and only during postmolt. This is consistent with it coding for a protein that promotes nucleation in the cuticle layers that are deposited after the molt.

regulators of crystal nucleation, the initial step in mineral deposition.



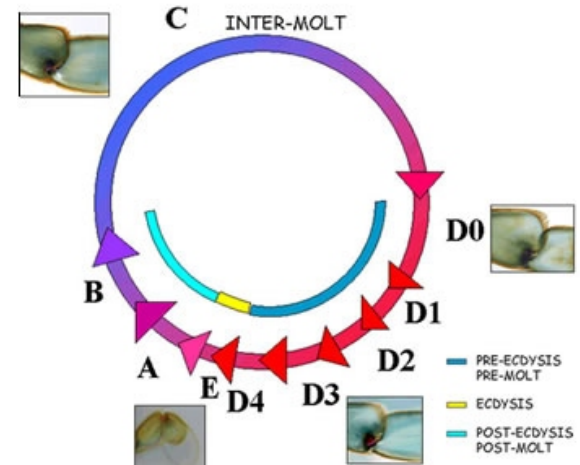
A cartoon explanation of how microarrays are used to measure

The goal of the mineralization project is to understand when and how it occurs from a molecular perspective. I collaborate with Dr. Richard Dillaman and Dr. Robert Roer, who study the morphology and physiology, respectively, of crab mineralization. We chose to study the crab because it is a model system, uniquely producing and then mineralizing a new skeleton over and over as it molts throughout its life time.

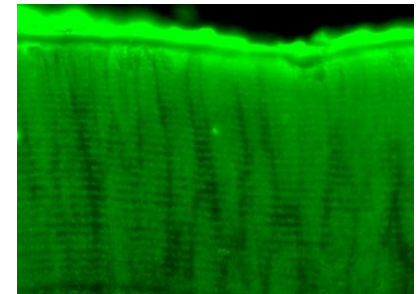
The most interesting results come from proteins that change during the molt cycle, or are differentially produced in cuticle that calcifies versus cuticle that does not (imagine the flexible cuticle at the joints of the crab).

Changes related to molting and/or differences between cuticle types indicate that these are likely to be regulators. These are proteins which control the deposition of calcium carbonate in the cuticle at the right time and place. We have found several proteins, or analyzed the genes for proteins, that we hypothesize to be both positive and negative

Another current project in my laboratory involves large-scale sequencing of expressed sequence tags (ESTs) from cDNA libraries made from two different blue crab tissues. This has produced a very useful database of the transcribed genes in this crustacean, and will ultimately lead to production of a crab DNA microarray. Microarrays are important tools to assess genome-wide changes in gene expression related to developmental, physiological, or toxicological effects.



Molt cycle of a crab. Adjacent pictures show external changes of the swimmeret at various stages of molting.



Immunostaining of the cuticle of a crab 3 hours after molting. The antibody (green color) is staining a protein that we believe to be an inhibitor of mineralization. The staining pattern indicates that the protein is not present in exactly the places where mineralization will first begin in the next few hours.



Dr. Shafer carefully holding a live blue crab.