ARTEMIA TRACHLEALESS GENE:

POSSIBLE GENETIC CONTROL IN CRUSTACEAN HEMOPOIESIS AND IONOCYTOPOIESIS

- In organisms that contain a closed circulatory system, it has been found that the organism needs to control the fluid volume/ionic composition of its blood or hemolymph to sustain its life. Physiological studies have shown that both renal and extra-renal organs regulate these processes.
- Similarly, respiration mechanisms are needed to provide oxygen to the tissues of the organism in order to maintain production of energy for cellular metabolism. Physiological studies have shown that aquatic gills or gill leaflets and terrestrial lung organs are the major sites where gas transport systems provide the exchange system between oxygen and carbon dioxide to take place. Hence, the sustenance of life for the organism is maintained.

Embryological Development

- In the anostracan family of Crustaceans,(A. species), it has been shown in past physiological and developmental studies that the embryological development from the fertilized egg to the cyst stage of life, the organisms genetic tools are preparing the embryo for life outside of the protective shield of the female brood sac or the shell coating the dormant cyst. These mechanisms have been uncovered and have shown to be unique system of molecular compounds. In dormant cysts, which are also anhydrous, have stored guanine molecular structures for energy usage. These guanine compounds are high energy analogs and, therefore, can substitute for the required cellular energy compound ATP and in doing so can keep the cysts alive. In addition, the cyst being in a anhydrous state can not utilize water for cellular growth until it is hatched from shell coating covering the cyst.. Therefore, the dormant cyst has no method of making a circulating system that can furnishes nutrients to all the bodily tissues. So, there is a lack of a respiratory delivery mechanism. It must depend upon physical diffusion of gaseous environmental oxygen to meet its aerobic needs.. Upon hatching of the anhydrous cyst it has been shown that hypoxia inducible factors are known to become active which permits the cyst to overcome its anhydrous state. Its proteins are active in the manufacturing of respiratory pigments that bind to oxygen, such as hemoglobin or hemocyanin. This system accomplishes the physiological task of building a respiratory system needed for the continuing embryological development.

- However, at hatching the embryo must begin development of special structural systems to be able to live and grow to adulthood in its new aquatic habitat. In the case of brine shrimp, Artemia, this habitat is an aquatic solution that is extremely high in salinity with sodium chloride as the predominate ionic specie. Physiological and biochemical studies have shown that in the larval stage of development, the organism initiates the differentiation of specialized cells that can regulate ionic composition of intracellular fluid by creating polar domains in epithelial cells in a special organ called the salt gland. The cells contain proteomic pumps, such as Na-K-ATPase and CFTR transport proteins that create on intracellular route for eliminating excess sodium chloride from blood hemolymph to the external environment. This permits the nauplius to drink seawater and allow the hemolymph to remain at a stable salinity. Therefore, in the naupliar stage of development, genetic pathways must exist to form these new protein pumps.

Larval Salt Gland vs Adult Leg Segment

- Additionally, since it has been shown that the anhydrous condition is being overcome by the formation of hemolymph, the naupliar stage can continue to become transformed into the juvenile stage and then into the adult stage. In the molting cycle, the salt gland of the naupliar stage undergoes sequential destruction until it is completely destroyed. At that time the organism has formed a complete abdominal system. The adult state has all of the segments which can provide the adult stage with completely functional respiration, excretion, digestion, movement by swimming apparatus In the adult leg segments, the differentiation of the segment into leg leaflet type epithelium contains the proteomic pumps like those found in the ionocytes of the salt gland. Therefore, they become to new extra renal organ which regulates sodium chloride secretion into the environment. However, it has been shown that the epipodites and fat cells of the identical leg segment contains the respiratory pigments of hemoglobin II andIII. This poses the question."Do these cells synthesize these pigments and provide the organism with normal circulatory system that handles the oxygen/carbon dioxide exchange mechanism and provides the necessary acid-base balance?"

- Lastly, Is the differentiation of naupliar salt gland and the adult leg segments be controlled by the Trachealess gene family in that they posses the genetic pathways that provide a cross-linkage in the evolution of crustacean hemopoiesis and ionocytopoises. The evidence to support this hypothesis is found in the following pathways provided by the paper by B. Mitchell and S. Crews in Evolution and Development 4:5 pp 344-353 (2002). J. Wang et. al. in Molecular Biolgy Rep. (2011) Molecular cloning and its expression of trachealisss gene (AsTrh) during development in brine shrimp, Artemia sinica

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                    |---------------basic-helix-loop-helix--------------|
Af-Trh     (1)    MRKEKSRDAARSRRGKENYEFYELAKMLPLPPQITSQLDKASIIRLTIAFLKLKEFTSNG
D-Trh      (77)   L••••••••••••••••••••••••••••••AA•••••••••••••••SY•••RD•SGH•
Bm-Trh     (71)   L••••••••••••••••••••••••••••••AA•••••••••••••••SY•••RD•SGH•
H-NPAS1    (45)   Q••••••N••••••••••L•••••••L••••GA•SI•••••••V••SVTY•R•RR•AAL•
H-NPAS3    (21)   L•••••••••••••••••F•••••••L••••AA•••••••••••••••SY••MRD•ANQ•

                  ---------------------PAS-1--------------------|
Af-Trh     (219)  RGCHMKSS------------------------------GYRVILIVGRMRAQQTGATSSDG
D-Trh      (308)  ••••F•••GYRASDATSNCNNGNNASNNAKNVKNPGSN•S•V•LLCKL•P•Y•FSH•RKS
Bm-Trh     (290)  ••••F•••------------------------------••••V•MLC•L•P•YSFSH•RKS
H-NPAS1    (258)  ••L•V•A•------------------------------••K••HVT••L••--------HA
H-NPAS3    (244)  ••V•I•••------------------------------••K••H•T••L•LRVSLSHGRTV

Af-Trh     (429)  TVEMRPQFNQVQDLDSVEVPTEVARIGSSENEETYAARLDSARESEIVRSPSGVSRRGR
D-Trh      (545)  LSAGDMKL•SPKTDSEGHSHRGRG•SAAASHGSSMNSLTMIKDSPTPLGVEIDSGVLPT
Bm-Trh     (499)  RHPAERTEPPTNSETASSAPVNASDVTHLTRLNDAQPLPLHTTPERTSPM•ARRLKKRK
H-NPAS1    (454)  EAPQTQGKRIKVEPGPRETKGSEDSGDEDPSSHPATP•PEFTSVIRAGVLKQDPV•PWG
H-NPAS3    (452)  KSDEKGNQSENSEDPEPDRKKSGNACDNDM•CNDDGHSSSNPDSRDSDD•FEHSDFENP
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Protein Sequence of Artemia Trh

Gene	% Identity to Af-Trh		
	bHLH	PAS-1	PAS-2
Bombyx Trh	91	55	60
Drosophila Trh	91	58	56
Human NPAS3	85	50	42
Human NPAS1	72	48	44
Drosophila Sim	70	29	37
Human HIF-1α	66	24	43
Drosophila Sima	62	25	30
Drosophila Ss	37	22	22
Human Ahr	35	23	19
Human ARNT	26	18	23
Human Clock	24	18	29

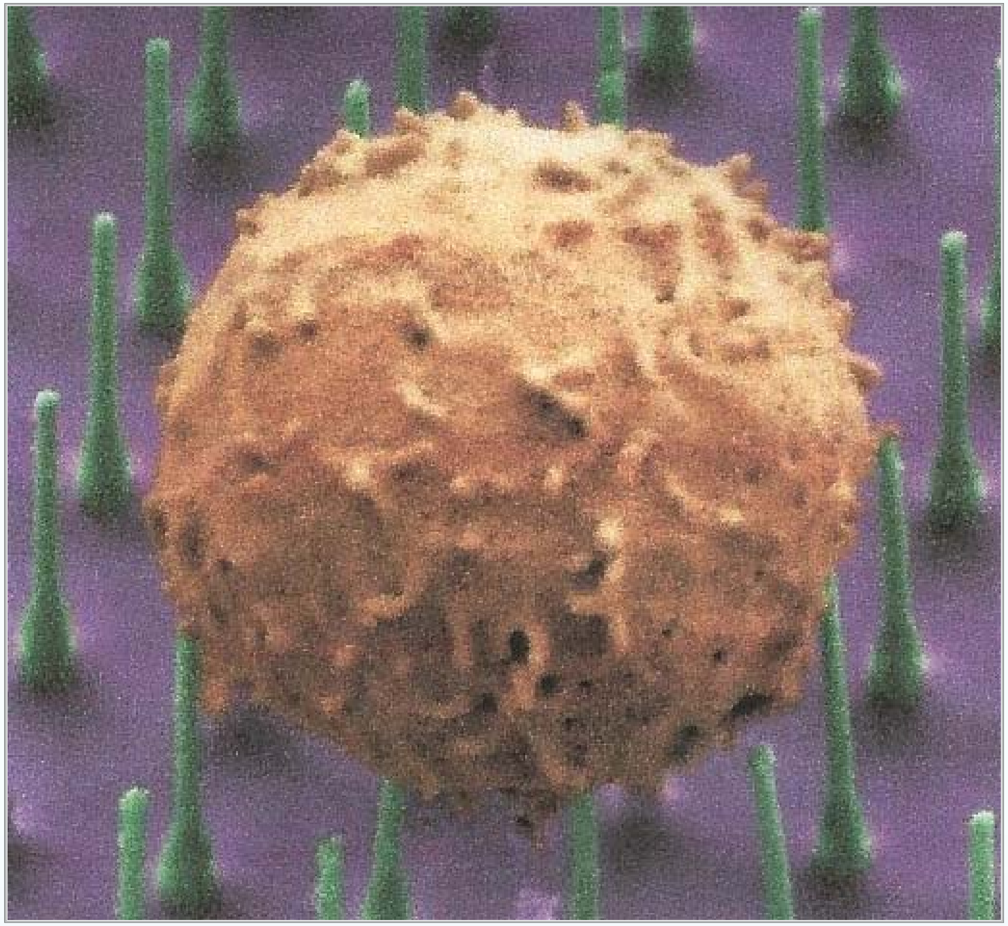
Percent Sequence Identity: Af-Trh and other insect/vertebrate sequences

Silicon Nanometric Technology

- **The development of a silicon nanometric-wire platforn, whose structural spicules have dimensions as the thinnest needle of a syringe, can be inserted into the cells of the isolated salt**

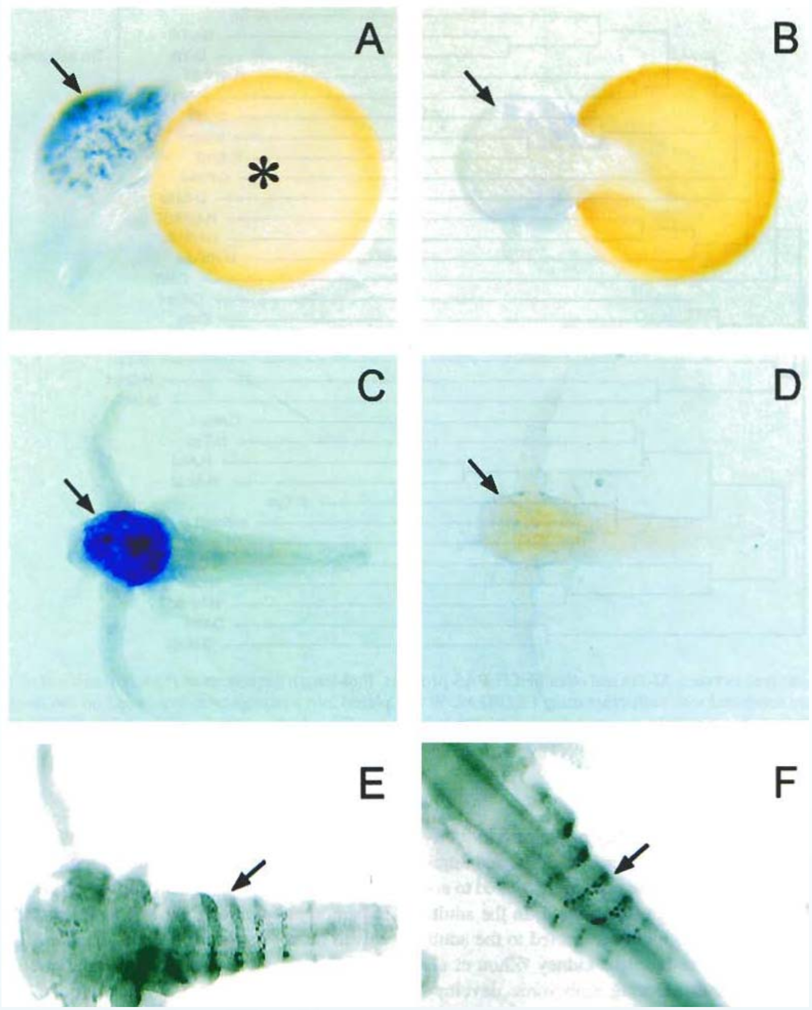
- **gland and/or fat cells and epipodite cells of the adult leg segments without causing disruption of genetic pathways in cellular development. Using this new technology, it is possible for researchers to tease apart the cross-linkage of genetic pathways that are common to both**

- **hemoglobin synthesis and proteomic pump (Na,K-ATPASe) and CFTR pump channels.**



Just as the thin needle of a syringe can be inserted into the skin and cause no more than a small pinching sensation, silicon nanowires can be inserted into Th17 cells (shown here), causing minimal disruption. Using this new technology, researchers teased apart this immune cell's wiring.

Image courtesy of Sigrid Kneymeyer, Broad Communications



Af-trh genes in Salt Gland Adult Leg segments