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Developmental transcriptomes from penaeid shrimp

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Demand for genetically improved lines is strong in Southeast Asia

Since elite genotype shrimp (right) reflect much higher performance than wild-caught shrimp (left), breeders hope to develop a mechanism for genetic copyright.

Australian shrimp farms primarily grow black tiger shrimp, Penaeus monodon, for which elite genetic lines have been successfully produced by selective breeding. As reported by Brett Glencross and co-authors in *Aquaculture Nutrition* in 2013, these elite lines provide harvest yields more than double that of unselected lines and have improved survival and growth performance, and lowered metabolic rates and energy requirements.

The demand to access these genetically improved lines is strong from the countries of Southeast Asia. However, the Australian shrimp-farming industry is highly motivated to prevent unlicensed breeding. As there are currently no mechanisms to confer failproof reproductive sterility and thus genetic copyright on a commercial scale in penaeid shrimp, this has precluded the sale of elite stocks beyond the individual farm enterprises that breed them. Remarkably little is known about the underlying biochemical and genetic processes that control fertility, sex and germ line determination, or other aspects of shrimp development.

To develop an alternative mechanism for genetic copyright, the authors used a bioinformatics approach to identify germ line genes that could be potentially targeted to ablate the germ line. This approach has also resulted in the production of developmental transcriptomes for penaeid shrimp.

Preparing embryos for genetic sequencing

The kuruma shrimp, *Penaeus japonicas*, is readily spawned at one of the research stations of Australia's Commonwealth Science and Industrial Research Organisation, so this species was used as a model. It was first demonstrated by Takao Kajishima in 1951 that when P. japonicus embryos were separated by a sharp needle at the two-cell stage, the "animal" half only developed into a hollow ball of cells, while the "vegetal" half continued developing.

By utilizing this technique, researchers had the opportunity to look for embryonic genes differentially expressed in animal or vegetal half-embryos. They predicted that germ line genes might be enriched in the vegetal half, since the primordial germ cell was hypothesized to arise from vegetal determinants.

P. japonicus embryos were separated at the two-cell stage and allowed to develop until the animal and vegetal half-embryos could be distinguished. The animal and vegetal half-embryos were pooled separately, total ribonucleic acid was isolated and reverse transcribed to complementary DNA, and the resulting transcriptomes were sequenced.

Reads from each library were assembled, annotated and screened for known germ line and mesoderm genes. Pre-existing *P. monodon* ovary and nauplius transcriptome libraries were also screened for developmental genes of interest.

Germ line genes

The germ line genes vasa and nanos were previously found in shrimp by cloning methods, but neither vasa nor nanos appeared in the embryo transcriptomes. The authors found other candidate germ line genes, including *pumilio, germ cell-less, staufen* and *tudor*, in both animal and vegetal transcriptomes. All four of these were more highly expressed in ovary and/or testes than in other adult tissues and could be detected during embryonic development. Next, the authors looked for where the selected germ line genes were expressed in the developing embryos.

Custom monoclonal antibodies were generated to examine the protein expression of shrimp *vasa, nanos, pumilio*, and *germ cell-less genes* by immunoblotting and immunolocalization. The vasa and nanos antibodies labeled a structure in embryos previously hypothesized to be a germ granule. This structure has been detected is several shrimp species and is inherited by embryonic cells hypothesized to give rise to the primordial germ cell. The *pumilio* and *germ cell-less* antibodies are still being characterized.

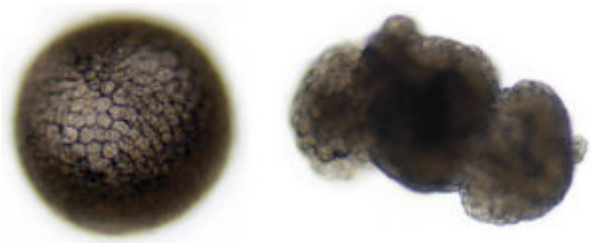
Mesoderm genes

The mesoderm genes t*wist, snail, mef-2* and *brachyury* were found in the P. japonicus half-embryo transcriptomes. These all function as transcription factors, which activate or repress other genes in mesoderm, muscle or related tissues. The *twist* and *brachyury* genes were found only in the vegetal transcriptomes, while snail and mef-2 were found in both animal and vegetal transcriptomes.

Recently, five developmental transcriptomes – embryo, nauplius, zoea, mysis and postlarva – from the Pacific white shrimp, *Litopenaeus vannamei*, were published in the gene databases by Jianhai Xiang's laboratory. The *L. vannamei* versions of these mesoderm genes were identified, and primers were developed in collaboration with the Xiang lab to study their expression by quantitative polymerase chain reaction in this species.

The expression of the mesoderm genes is consistent with their known functions as transcription factors in other organisms. For *twist* and *snail*, expression was not detected until later embryonic stages and continued into the larval and postlarval stages. The *mef-2* expression was detected at all stages of development from zygote to postlarva. For *brachyury*, expression was highest during gastrulation – consistent with its known role in promoting cell movements during embryogenesis.

The next step will be to study the expression patterns of these genes in embryos and larvae, and antibodies to shrimp twist, *mef2* and *brachyury* are in production. In the model amphipod crustacean *Parhyale hawaiensis*, work by Nipam Patel's laboratory has shown that *twist* and *snail* proteins are expressed in the posterior mesoderm, while *mef-2* protein is expressed in posterior mesoderm and persists in developing muscles.



After five hours of development, shrimp "animal" half-embryos (left) form a hollow ball of cells, while "vegetal" half-embryos (right) undergo gastrulation and abnormal segmentation.

Application: germ line knockdown

The availability of germ line gene sequences and antibodies to detect their protein products allows for experiments to ablate the shrimp germ line by targeted gene knockdown.

RNA interference is a powerful and highly accurate natural biological pathway in shrimp that can be utilized for such studies. This can be performed by administration of double-stranded RNA via tail-muscle injection or oral delivery.

The mesoderm/muscle gene sequences and molecular tools developed in parallel should be useful in further understanding the basic biology of mesoderm and muscle development in shrimp, for example in detecting cellular changes in faster-growing genetic lines.

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