

In this study, we examined whether these antigens exist in gametes and contribute to the self-incompatibility. We have found that the follicle cells overlying on the egg vitelline coat are positive to the antibody by immunocytochemical study and that the antigens in the follicle cells are proved to be three glycoproteins similar to those found in the coelomic cells.

These data show that these three proteins may mediate the self-defense and fertilization by a similar recognition system.

#### A SURVEY OF GENES EXPRESSED IN HEMOCYTES OF THE ASCIDIAN *HALOCYNTHIA RORETZI*

Takeshi Usami<sup>1</sup>, Daisuke Yamasaki<sup>1</sup>, Hideyoshi Yokosawa<sup>1</sup>, Minoru Tanaka<sup>2</sup>, Kaoru Azumi<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan and <sup>2</sup>Department of Molecular and Cellular Interactions, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

Hemocyte aggregation is an early inflammatory response to injury in the ascidian *Halocynthia roretzi*. We have already reported that *H. roretzi* hemocytes have immunoreceptor tyrosine-based activation motifs (ITAMs) containing protein (A74 protein), and are involved in signal transduction via tyrosine phosphorylation of the A74 protein. To investigate immune-related genes in *H. roretzi* hemocytes globally, we have begun performing expression sequence tag (EST) analysis of a hemocyte cDNA library of *H. roretzi*. We obtained 5000 randomly selected clones from the cDNA library, and determined 5' sequences of 1000. From the results of a homology search against DNA and protein databases, we found that *H. roretzi* hemocytes contained genes encoding proteins, such as Hsp 70, A74 protein, and phosphatidylinositol 3-kinase, involved in stress- and immune-responses, as well as in signal transduction.

#### COMPLEMENT GENES IN THE *CIONA* GENOME

Fumiko Yoshizaki, Masaru Nonaka

Department of Biological Sciences, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Tokyo 113-0033, Japan

Mammalian complement system consists of more than 30 proteins and plays an important role in host defense, recognizing and eliminating pathogens and immune complexes. Accumulating evidence indicates that the complement system was established by the emergence of deuterostomes, and developed by gene duplications in the vertebrate lineage. To elucidate the whole architecture of the apparently primitive complement system of basal chordate, we performed genome-wide search for complement genes of the solitary ascidian, *Ciona intestinalis*. Based on the sequence similarities and the domain structures, we identified a large number of possible complement genes comparable to, or more than those of mammals, including C1q-like and C6-like genes that had not been found in urochordates. Phylogenetic analysis indicated that gene duplication events occurred independently in urochordates and vertebrates to generate distinct set of homologous genes. Furthermore, EST clones proved expressions of most of the predicted genes. These results suggest that ascidians have a complicated, multi-component complement system with an important role in host defense where no adaptive immune system exists.

#### POSSIBLE IMMUNOLOGICAL CYTOLYTIC GENES OF ASCIDIANS.

Ayuko Kimura, Kazuyuki Endo, Fumiko Yoshizaki, Masaru Nonaka

Department of Biological Sciences, Graduate School of Science, the University of Tokyo, Hongo, Tokyo 113-0033, Japan

The terminal complement components and perforins assemble to form pores on the plasma membrane of the target cell and lyse it. MAC/PF domains (MP) have been found only in these proteins and are believed to play a key role in cytotoxicity. We isolated MP sequence-containing cDNAs (MP-cDNAs) from Urochordates, *Halocynthia roretzi* and *Ciona intestinalis* that are expected to have a primitive complement system. Using degenerate primers, partial sequences of the candidate genes were amplified by RT-PCR. To obtain the full-length cDNAs, the *Ciona* draft genome sequence was homology-searched using the partial sequences of *Ciona* as a query, and the *Halocynthia* cDNA library was screened by the partial *Halocynthia* cDNA probe. Consequently, 1 *Halocynthia* and 15 *Ciona* MP-cDNAs were obtained. Domain composition and copy number of MP-cDNAs showed a great variation among *Ciona*, *Halocynthia* and mammals, indicating that they have developed distinct sets of the MP-containing component. In the *Ciona* genome, highly homologous MP-containing genes tended to cluster in neighborhood, suggesting that these highly homologous genes arose by recent tandem gene duplication events.

#### THE ORIGIN AND THE EVOLUTION OF THE THIOESTER-CONTAINING PROTEIN

Naoko Fujito<sup>1</sup>, Tadashi Takemoto<sup>2</sup>, Hiroshi Kimura<sup>2</sup>, Rei Ueshima<sup>1</sup>, Masaru Nonaka<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Graduate School of Science, University of Tokyo, Tokyo 113-0033, Japan and <sup>2</sup>Department of Experimental Radiology, Shiga University of Medical Science, Shiga 520-2192, Japan

In higher vertebrates, one of the major effector arms of the immune response is the complement system. The fact that multiple complement-like genes are present in the echinoderms, but not in *Drosophila* and *C. elegans* suggests that the complement system has been established in the deuterostome lineage after its divergence from protostomes. Although the central complement component C3 is believed to have diverged from a non-complement related protein,  $\alpha_2$ -macroglobulin ( $\alpha_2M$ ) by gene duplication, it is still not clear when this gene duplication occurred. Here, we have cloned the thioester containing proteins (TEPs) from molluscan snails and cnidarian hydras using degenerate primers constructed at the thioester region conserved between C3 and  $\alpha_2M$ . Both of them are  $\alpha_2M$ -like. Since the C3-like gene has been reported from coral recently, it is concluded that the gene duplication event of the common ancestor of C3/ $\alpha_2M$  preceded the appearance of Cnidaria. Though we could not find C3 from snails, the possibility of the presence of C3 in protostomes has not been denied completely. We discuss the remarkable way of evolution of the functional domains of TEP protein.

#### EXPRESSION PATTERNS OF GENES INVOLVED IN THE BLOW FLY CLOCK MECHANISM

Shinsuke Goto, Hideharu Numata

Department of Bio- and Geosciences, Graduate School of Science, Osaka City University, Osaka 558-8585, Japan

As a first step to approach the functional elements of the photoperiodic clock, we report here the sequences and expression patterns of the clock genes, *period*, *timeless*, *cycle*, *cryptochrome* in the blow fly, *Protophormia terraenovae*. A diel rhythmicity of *period* transcripts abundance was detected in the adult head (peak during scotophase) while its amplitude was less pronounced. The present study also revealed that *per* produced at least 2 transcripts. Photoperiods influenced the expression patterns of *per* transcripts: the peak of *per* expressions shifted in concert with onset of the scotophase.

#### ENTRAINABILITY OF TWO CIRCADIAN OSCILLATORS IN *DROSOPHILA CRY<sup>b</sup>* MUTANT FLIES TO LIGHT AND TEMPERATURE CYCLES

Taishi Yoshii, Kenji Tomioka

Department of Biology, Faculty of Science, Okayama University, Okayama 700-8530, Japan

*Drosophila* flies carrying *cry<sup>b</sup>* mutation exhibit two free-running rhythms, with a shorter (about 22.5 h) and a longer period (about 25 h), respectively, in constant light (LL). They are driven by two separate *period* gene-dependent oscillators. In this study, we investigated the entrainability of the two rhythms to light and temperature cycles. When exposed to temperature cycles with T = 22 h and 26 h under LL, both rhythms were entrained to these temperature cycles only when T was close to their free-running period. When exposed to a combination of temperature and light-dark cycles with different period lengths, the longer period component was exclusively entrained to light cycles, while the shorter component synchronized to a temperature cycle when T was close to its free-running period. These results suggest that, although two oscillators have entrainability to both temperature and light cycles, the oscillator driving the shorter free-running rhythm has a preference to the temperature cycle, whereas that for the longer rhythm to light cycle.

#### SEARCH FOR PHOTORECEPTORS INVOLVED IN THE CIRCADIAN LOCOMOTOR RHYTHM DISSOCIATION INDUCED BY CONSTANT LIGHT IN *DROSOPHILA CRY<sup>b</sup>* MUTANT FLIES

Kenji Tomioka<sup>1</sup>, Mari Murata<sup>2</sup>, Taishi Yoshii<sup>1</sup>

<sup>1</sup>Department of Biology, Faculty of Science, Okayama University, Okayama 700-8530, Japan and <sup>2</sup>Department of Physics, Biology and Informatics, Faculty of Science, Yamaguchi University, Yamaguchi 753-8512, Japan

CRYPTOCHROME (CRY) is a blue light photoreceptor involved in photic entrainment of the circadian clock in *Drosophila melanogaster*. Flies carrying *cry<sup>b</sup>* mutation that lack functional CRY show a rhythm dissociation into two components, running about 22.5 h and 25 h, respectively, in constant light (LL). In this study, we investigated the photoreceptor involved in the rhythm dissociation. We first examined the locomotor rhythms of *so:cry<sup>b</sup>* and *norpA:cry<sup>b</sup>* double mutant flies in LL. The former lacking the compound eyes and ocelli in addition to loss of functional CRY, showed severe reduction of flies with the rhythm dissociation. In the latter, without the photoreception in the compound eye and ocelli, the rhythm dissociation was totally suppressed, suggesting that the external photoreceptors are responsible for the rhythm dissociation. We then examined the effects of LL with different wavelengths on the rhythm dissociation in *cry<sup>b</sup>* flies, and found that green light was most effective and UV was least. Effect of blue light was intermediate between those of green and UV. The data might be interpreted that green light receptors play an important role in the rhythm dissociation.