
Recently molecular-biological techniques (especially monoclonal antibodies and recombinant DNA) are used in various fields of biological sciences. We prepared three monoclonal antibodies (Rh29, Rh112 and Rh311) against bovine rhodopsin purified by Con A-sepharose column chromatography [1]. Rh29 showed more specificity to opsin than rhodopsin, whereas Rh311 showed contrary specificity. Rh112 showed almost the same specificity to rhodopsin and opsin. Rh29 and Rh311 were specific to rhodopsin and did not react with cone visual pigments. Their binding sites were estimated. The results of ELISA and immunohistochemistry showed that Rh29 reacted with rhodopsin of octopus in addition of vertebrate rhodopsins, indicating that the antigenic determinants of bovine rhodopsin seem to be well conserved in the process of evolution. Opsin can be purified by Rh311-kinked Sepharose column chromatography.

In researches on vision, cDNAs of the proteins related with visual transduction have already been cloned and deduced the amino acid sequences of the proteins. We cloned genomic DNA of chicken rhodopsin and deduced the amino acid sequence of the rhodopsin [2]. The deduced amino acid sequence of chicken rhodopsin gives a similar profile of the hydrophathy plot to that of bovine or human rhodopsin. We arranged the primary sequence of chicken rhodopsin according to the proposed structural model for bovine rhodopsin and , we estimated the regions of rhodopsin, which interact with transducin, rhodopsin kinase and arrestin on absorbing light on the basis of the comparison of similarity in the amino acid sequences of chicken and other visual pigments. In the 5'-noncoding region upstream of TATA box, several separate segments of 8 to 13 nucleotides long are common in chicken and mammalian rhodopsin genes, suggesting that these sequences might be related to the cell-type-specific expression of the rhodopsin genes in these organisms.


Molecular Neurobiology of Drosophila
Teilichi TANIMURA, Department of Biology, Faculty of Science, Fukuoka University, Fukuoka 814-01 Japan

Due to the development of the modern molecular methodology, such as recombinant DNA and monoclonal antibodies production, molecular studies in various aspects of neurobiological problems in Drosophila melanogaster have remarkably advanced in recent years. Unfortunately these lines of studies are not represented in Japan. The genetic analysis of neurobiological problems in Drosophila mainly depend on whether we have mutants showing an abnormal phenotype with regard to a particular behavior or physiology of the fly. Over a hundred of such neurogenic mutants have been isolated so far. But the further isolation of mutants belonging to a new category is encouraging. We have been isolated and characterized mutants in taste receptor systems, muscle degenerations and the programmed cell death of muscles. I discussed the usefulness of the genetic approach to study these problems. In addition, referring to the recent molecular studies on the period gene affecting circadian rhythms, I reviewed the molecular approaches in analyzing neurobiological mutants of Drosophila to reveal their molecular defects involved.
The availability of molecular biological approaches to the developmental neurobiology of diffused nervous system in hydra.
Osamu KOIZUMI
Physiological Lab., Fukuoka Women's Univ., Fukuoka

The availability of molecular biological approaches to the developmental neurobiology of nervous system in hydra will be discussed. Freshwater coelenterate Hydra has the most primitive nervous system among animals, which has no ganglions and no brain. Due to immunocytochemistry using neuropeptide antisera and monoclonal antibodies, we can observe the hydra nerve net accurately and in details on whole mount samples. As a result, it is known that the hydra nervous system has many subsets of neurons and highly position-specific distribution patterns of neurons.

We have shown that neurons in adult hydra are plastic. We have demonstrated the position-dependent plasticity of neuropeptide expression using FMRFamide and vasopressin antisera. Moreover, the position-dependent conversion of ganglion cells to sensory cells using monoclonal antibodies. In these studies we will show the usefulness of various immunofluorescence labelling methods on whole mount samples.

Now we are studying the mechanisms of nerve net formation in the regenerating tissues. In these studies we use various kinds of developmental mutants and its chimeras. We will discuss the availability of these mutants to clarify the mechanisms of nerve net formation.
Cradle formation of attelabid weevils.

In many attelabid weevils, females make a variety of leaf-roll cradles, in which single or multiple eggs are laid and on which the larva feeds. In this symposium, I referred to three aspects of this oviposition habit.

The angle between the axis of the roll and the main vein has been adopted as a criterion for the classification of the cradles (Prell, 1924; Kono, 1930; Morimoto, 1964). Two rhynchitinine species (one from Malaysia and the other from Japan) contradict those classification systems. Cradles are formed through a process in which female weevils perform a set of behavioural elements in a definite sequence. Therefore, cradles should be classified on the basis of the behaviour repertoire (folding, nibbling, cutting, rolling, etc.).

For female weevils, leaves of the host plant must be one of the most important objects in their environment. I carried out a series of ethological studies on leaf recognition (mainly of size) by three attelabid weevils (i.e., Chonostropheus chujoi Voss, Deporaus sp., and Apoderus baltetus Roelofs). In the three species, female weevils perform stereotype behaviour before cutting, and their course of walk before cutting corresponds with determinant of the cutting point suggested by numerical analysis of the cutting point. In addition to this evidence, results of a sliding leaf model show that female weevils perceive and evaluate leaf size by the walking.

In Attelabinae, cradles serve not only as food for larvae but also as chamber for pupation. Model leaf experiments with A. baltetus show that the insect measures both leaf length and leaf width to predict the shape and size of the cradle. Symbiotic relationship between weevils and some fungi is found in Euops splendidus Voss through a behavioural, morphological and histological study; the female weevil sows fungal spores on the cradle leaf and the larva feeds on cradle the leaf fermented by the fungus.


The hunting wasps display elaborate, relatively stereotyped behaviour pattern. Several ethologists have conveniently written the patterns with the following sequences of three essential elements; hunting(V), nest preparation(I), and oviposition(O). That is, VIO is typical in Pompilidae, IVO is typical in Sphecidae, and IOV is also typical in Eumenidae. There, most hymenopterists assume that VIO is primitive pattern and IOV is advanced one in evolutionary history of Hymenoptera. One reason why they think so, is that VIO seems to be post hoc chain and IOV seems to be foresighted. The IOV forms more complex "instinct". Indeed, such stereotyped behaviour that we know, must be constrained by some genetical base through natural selection. But, in certain species the behaviour pattern is frequently broken out, say when any cleptoparasitism occurs. The cleptoparasitism is not a property determined by individual genetics, but probably "subroutine" forced by ecology (economics). In next, particularly in hunting, the wasps must perform much flexibly and sophisticatedly to the responses of the prey spiders or insects. Unfortunately we do not know so much about the huntings. Also, since in the nesting stages various parasites (although the miltogrammine fly is serious, the most serious one is undoubtedly a cleptoparasite in the same species) visit to catch the chance, wasps are very busy to defend their prey, nests, and eggs from these parasites and need to work carefully and flexibly. In these context, we can newly interpret much more means of various traits in wasp's behaviour than what we have understood. From my field and armchair detections I try to read out "the program" of the nesting behaviour in addition of what conditions arise cleptoparasitism, and that of hunting, as an example of a spider wasp (Episyron arrogans) which hunts specifically the orb-weaving araneids. This trial may be a version of "software explanation" as Dawkins suggested. I hope that this program could "run" to some extent in natural "malevolent environments" with many parasites and predators having other programs.
Division of labor among workers of stingless bees --How is the life of a worker decided?-- Tamiji INOUE Lab. of Entomol., Fac. of Agric., Kyoto Univ.

In the stingless bee, *Trigona minangkabau*, division of labor among workers were studied. There were three distinguishable groups even in a cohort emerged at the same day; Core brood carers stayed in nest during almost all the life span, doing cell making and attending oviposition ritual; Peripheral and subsidiary brood carers skipped cell making and started pot making at younger age. Foragers skipped almost all the intranidal tasks and started foraging significantly earlier than the above two groups.

Effects of colony conditions on systems of division of labor were analyzed by adding observations in the other 4 colonies at different colony developmental stages. The order of tasks performed by every worker takes a definite general course from the nest interior to extranidal activities. Some tasks might be skipped. Whether a given task is skipped or not is determined basically by the relative necessity of the task concerned to the colony at the time concerned. Once skipped, return to the task concerned does not happen even by the rise of the necessity to the task. Skip of one task has after-effect on the performance of other tasks. The prospective duration of the performance of a given task (except foraging at the end of the task sequence) is determined by the relative abundance of younger cohort. The elder workers did more risky tasks. There are some individual differences even among workers emerged under the same colony condition.

The above results are compared with those in honey bees to discuss factors and mechanisms which determine the task performance of individual workers, e.g. colony conditions, food at larval stage and genetic variations of the queen and sperm.

We are interested in elucidating the molecular events underlying photoreceptor excitation using Drosophila as a model system. The main pathway of converting a light signal to the closing of Na⁺ channels in vertebrate photoreceptor cells involves the activation of cGMP phosphodiesterase through the photoreceptor-specific GTP-binding protein, transducin. Vertebrate S-antigen, which is also called the 48K protein, is believed to bind to photoexcited and phosphorylated rhodopsin, thereby quenching the transducin cycle. Both transducin-like and S-antigen-like entities have been reported in invertebrate photoreceptors, including those of flies. Furthermore, molecular cloning studies indicate that the amino acid sequences and the predicted secondary structures of rhodopsins are conserved between Drosophila and mammals. These results imply that similar, but not necessarily identical, transduction mechanisms function in vertebrate and invertebrate photoreceptor cells.

At least 7 classes of photoreceptor-specific proteins of Drosophila separated on two-dimensional gels and the additional 5 proteins on one-dimensional SDS-PAGE have been found by the author. Efforts have been made to clone some of these photoreceptor-specific proteins, i.e., the ninaC gene products and the 49K protein to elucidate the physiological function of these proteins.

Phototransduction in photoreceptors. Kei NAKATANI, Howard Hughes Medical Institute and Dept. of Neuroscience, Johns Hopkins University, School of Medicine.

One mechanism proposed for the phototransduction is that light increases internal free Ca²⁺, which then blocks the conductance. Another proposed mechanism is that light triggers the hydrolysis of cGMP, a substance keeping the conductance open in darkness. We have studied the movement of Ca²⁺ across the plasma membrane of the rod outer segment and found that the rate of Ca efflux from a rod outer segment decreased rapidly in the light; this suggested that Ca²⁺ in the outer segment decreased rather than increased during illumination. In other experiments we also found that internal Ca²⁺ did not appear to directly shut down the conductance. These results indicate that intracellular Ca²⁺ does not mediate phototransduction in rods. Using excised, inside-out membrane patches we found, as did Fesenko et al., that cGMP activated an ionic conductance in rod outer segment membrane. The action of cGMP seemed to a direct agonistic effect rather than via a protein kinase. In the presence of divalent cations, single-channel activity was not resolvable. In the absence of divalent cations (Ca²⁺ and Mg²⁺), single-channel activity could be observed at low cGMP concentration. We have observed a cGMP induced current in a truncated rod outer segment, indicating that the cGMP-activated conductance was indeed present in the plasma membrane. Furthermore, in the presence of GTP this cGMP-activated conductance could be suppressed by light. We therefore conclude that cGMP is indeed the internal transmitter for phototransduction in rods. We have also done the same experiment in cones, and found a very similar phototransduction mechanism in these cells. Recently, we have studied the role of Ca²⁺ on light adaptation in rods and cones. In normal Ringer, the response to a light step rose transiently to a peak but rapidly relaxed to a lower level, indicative of light adaptation. In the absence of Ca²⁺ feedback, the response relaxation was absent, and the steady response levels at different light step intensities could be well predicted by a statistical superposition of invariant single photon responses. We therefore conclude that the Ca²⁺ feedback underlies all light adaptation in rods and cones.