Review

Molecules participating in insect immunity of *Sarcophaga peregrina*

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Abstract: Pricking the body wall of *Sarcophaga peregrina* (flesh fly) larvae with a needle activated the immune system of this insect and induced various immune molecules, including antibacterial proteins, in the hemolymph. In this review, I summarize and discuss the functions of these immune molecules, with particular emphasis on the dual roles of some of these molecules in defense and development.

Keywords: insect immunity, ontogeny, antibacterial proteins, dual roles of an immune protein

Introduction

I have been studying insect immunity for nearly 40 years. Originally, my major in graduate study was microbiology; however, during my stay at Yale University, I learned much about *Drosophila* development. At the end of December 1971, I was appointed as an assistant professor at the University of Tokyo, Faculty of Pharmaceutical Sciences, and returned from Yale University where I stayed for more than 3 years as a postdoctoral fellow. When I accepted the position of assistant professor, I decided to start a new project to study the molecular mechanism of the differentiation of *Drosophila* imaginal discs, with special reference to the function of the molting hormone 20-hydroxyecdysone. However, to my great disappointment, I soon realized that it was almost impossible to rear *Drosophila* in our Faculty, because of the deserted research environment produced during the University troubles.

At that time, I encountered *Sarcophaga peregrina*, a big flesh fly, at the National Institute of Health of Japan. This insect could be very easily handled and could be reared, even under poor experimental conditions, with dried milk, sugar cube and water (adults), or pork liver (larvae). Moreover, the amount of 20-hydroxyecdysone in *Sarcophaga* larvae could be controlled by using the dry-wet treatment, which is a great advantage in testing the effect of externally introduced 20-hydroxyecdysone.1) When *Sarcophaga* larvae are kept under wet conditions, secretion of 20-hydroxyecdysone from the ring gland stops, whereas it starts again when they are transferred to dry conditions.1) Therefore, I decided to use *Sarcophaga* instead of *Drosophila* and started investigating the effect of 20-hydroxyecdysone on imaginal disc differentiation by injecting the hormone in the larval body cavity under wet conditions. However, soon after starting the new project, a simple casual experiment motivated me to turn my attention to insect immunity.

In front of my working bench, one of my colleagues was injecting various chemical compounds to tumor-bearing mice to examine their chemotherapeutic effect. He was extremely nervous about sterilizing glass syringes in boiling water before their use because tumor-bearing mice are susceptible to bacterial infection. I used the same glass syringes to inject 20-hydroxyecdysone into *Sarcophaga* larvae. No infected or dead *Sarcophaga* appeared even when the larvae were injected using nonsterilized syringes, and almost 100% of the larvae pupated and metamorphosed into adults.

From this result, a serious question came to my mind: does larval hemolymph contain antibacterial activity? I promptly examined this possibility. I mixed the hemolymph from normal larvae with an *Escherichia coli* suspension. After incubation, I spread the mixture on agar plates, expecting that no *E. coli* colony will form. In contrast to my expectation, however, numerous bacterial colonies appeared on the plates, indicating that no appreci-

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able antibacterial activity was present in the hemolymph. Incidentally, I performed the same experiment using hemolymphs collected from larvae injected with 20-hydroxyecdysone or insect saline (control). Surprisingly, no bacterial colonies were detected in both of these hemolymph groups.\(^4\)

This observation clearly indicated that normal larvae do not have antibacterial activity, but injecting them with foreign substances promptly induced such activity. I was deeply impressed with this observation, and decided to participate in insect immunity studies, although I was quite unfamiliar with the field at that time. I was in my early 30s then.

In the past three decades, studies on self-defense mechanisms of insects have developed greatly and formed a special branch of insect study, called “insect immunity”.

**Sarcophaga immune molecules**

We first intended to isolate an inducible antibacterial substance in the hemolymph of *Sarcophaga* larvae. For this purpose, at least 100 mL of hemolymph was needed as a starting material. It was possible to collect 30 µL of hemolymph per larva by decapitating each larva with fine scissors and then squeezing the body. Therefore, to collect 100 mL of hemolymph, more than 3,000 larvae had to be squeezed. Moreover, each larva had to be injected with 5 µL of insect saline before hemolymph collection. This was a very tedious and time-consuming process. However, we later found that, instead of injection of insect saline, mere pricking of larval body wall with a hypodermic needle was sufficient to induce antibacterial activity.\(^3\) This finding greatly accelerated the process of hemolymph collection, and allowed the collection of sufficient amounts of hemolymph for the characterization of antibacterial activity. Throughout this study, we learned that various proteins are induced in the hemolymph when the larval body wall was pricked. Many of these proteins were found to be involved in insect immunity.\(^4\)\(^,\)\(^5\)

We assume that contact with bacteria is needed to activate these immune protein genes. Since the experiments were conducted under nonsterile conditions, body pricking may have been sufficient to introduce bacteria into the larval body. Therefore, body pricking is equivalent to bacterial immunization. Subsequent studies revealed that the promoter region of these genes contains NF-κB binding motifs, suggesting that their expression is regulated by a family of transcription factors called the Rel proteins.\(^6\)\(^-\)\(^8\)

We have thus far purified and characterized 3 antibacterial proteins.\(^9\)\(^-\)\(^11\) 1 humoral lectin,\(^3\) 1 antifungal protein (AFP),\(^12\) and 1 small antibacterial compound (N-β-alanyl-L-S-glutathionyl-3,4-di-hydroxyphenylalanine [5-S-GAD])\(^13\) as immune molecules of *Sarcophaga* larvae. These molecules are not normally present in the insect but are promptly synthesized in response to bacterial immunization (body pricking), except for AFP which is constitutively present in the hemolymph of normal larvae.

A unique feature of these antibacterial proteins is the presence of structurally related multiple homologues. At least 3 homologues of sarcotoxin I,\(^14\) 3 homologues of sarcotoxin II,\(^15\)\(^,\)\(^16\) and 3 homologues of sapecin\(^17\)\(^,\)\(^18\) have been identified. Their genes form a tandem array in restricted genomic regions, are simultaneously expressed in response to bacterial infection, and prevent the spread of bacteria in the body cavity.\(^16\)\(^,\)\(^19\) *Sarcophaga* lectin, AFP, and 5-S-GAD were also found to be involved in insect immunity.\(^20\) In contrast to antibacterial proteins, both *Sarcophaga* lectin and AFP are single-gene proteins.

**Function of Sarcophaga immune molecules**

In this section, I will briefly explain how *Sarcophaga* immune molecules thus far identified are involved in insect immunity. We found that *Sarcophaga* synthesizes multiple antibacterial proteins having different bacterial specificity and kills various invading bacteria at once, on emergency. Furthermore, we found that it constructs a potent anti-fungal system utilizing an inducible antibacterial protein and basic AFP. These facts suggest that this insect developed a sophisticated self-defense system mobilizing a limited number of immune molecules effectively.

**a) Sarcotoxin I** The sarcotoxin I family is a group of antibacterial proteins each consisting of 39 amino acid residues. At least 3 sarcotoxin I congeners are present in immunized *Sarcophaga* larvae, and their primary structures were determined from their cDNA sequences (Fig. 1).\(^14\) We chemically synthesized sarcotoxin IA and studied its mode of action. Sarcotoxin IA is a bactericidal protein and its minimum inhibitory concentration values for various bacteria are shown in Table 1.\(^9\) Its bactericidal effect was detected both in growing and nongrowing conditions.\(^21\) Generally, gram-negative bacteria are more susceptible to sarcotoxin IA than gram-positive bacteria. The N-terminal half of this molecule is rich in positively charged amino acids and is hydrophilic,
whereas the C-terminal half is hydrophobic. Thus, this molecule is amphiphilic and readily interacts with the bacterial membrane.\(^{22)–25)}\)

We found that sarcotoxin IA disrupts the electrochemical membrane potential, resulting in cessation of ATP synthesis and amino acid transport in \(\text{E. coli}\).\(^{22)}\) These amphiphilic molecules likely form channels in the bacterial membrane, causing its perturbation and loss of function. We found that the \(\text{E. coli uncA}\) mutant, which has a defect in oxidative phosphorylation, is much less sensitive to sarcotoxin IA than wild-type \(\text{E. coli}\). This may be because the \(\text{uncA}\) mutant does not require electrochemical gradient of protons for the synthesis of ATP, as ATP is supplied by substrate-level phosphorylation. These results suggest that the bactericidal effect of sarcotoxin IA is mainly due to its ability to disrupt electrochemical gradient of protons and stop ATP synthesis. In fact, the cellular ATP level of \(\text{E. coli}\) was shown to markedly decrease upon treatment with sarcotoxin IA (Fig. 2).

(b) **Sarcotoxin II.** We have purified 3 homologues of sarcotoxin II (IIA to IIC) from the...
hemolymph of immunized larvae. The molecular mass of these proteins is about 24,000, which is different from that of sarcotoxin I. We examined the mode of action of sarcotoxin IIA. A high concentration of sarcotoxin IIA (100 µg/mL) showed bactericidal effect when bacteria were in growing conditions, but it did not kill the bacteria under nongrowing conditions. This mode of action is different from that of sarcotoxin IA. At the concentration of 25 µg/mL in the medium, sarcotoxin IIA showed a bacteriostatic effect. The scanning electron microscopic images of E. coli under these conditions were very peculiar (Fig. 3). Sarcotoxin IIA-treated cells elongated and had unusual projections or bulges on their surface, which may have been due to intracellular osmotic pressure. These bulges may have been formed by the inhibition of peptidoglycan synthesis by sarcotoxin IIA, and their surface structure may be similar to that of spheroplasts obtained by treating E. coli with lysozyme.

The antibacterial spectrum of sarcotoxin IIA is very narrow; thus far, it has been shown to be effective only against a few gram-negative bacteria, including E. coli, and ineffective against gram-positive bacteria.

(c) Sapecin. Sapecin is another bactericidal protein of Sarcophaga, and at least 3 congeners (sapecin, sapecin B, and sapecin C) have been identified. Incidentally, we found that NIH-Sape-4 cells, an established embryonic cell line of Sarcophaga, synthesize and secrete sapecin into the culture medium. Therefore, it was possible to purify sapecin from the culture medium of NIH-Sape-4 cells. Sapecin is a bactericidal protein that consists of 40 amino acid residues, including 6 Cys residues that form 3 intramolecular disulfide bridges. In contrast to sarcotoxin IA, gram-positive bacteria are more sensitive to sapecin than gram-negative bacteria.

Similar to sarcotoxin IA, the primary target of sapecin was assumed to be the bacterial membrane. Therefore, we prepared 2 types of liposomes with similar phospholipid compositions to those of the membranes of Staphylococcus aureus and E. coli, respectively, each containing trapped glucose. We examined the effect of sapecin on these liposomes by measuring glucose release. An increase in sapecin concentration resulted in the release of glucose from liposomes resembling the phospholipid composition of the S. aureus membrane (i.e., formed from phosphatidylglycerol and cardiolipin in a molar ratio of 3:1). Liposomes prepared from phosphatidylinositol...
anolamine, phosphatidylglycerol, and cardiolipin in a molar ratio of 7:2:1, resembling the phospholipids composition of the *E. coli* membrane, were not affected, and no appreciable release of glucose was detected under these conditions (Fig. 4). These results explain why gram-positive *S. aureus* is more sensitive to sapecin than gram-negative *E. coli*.

We found that sapecin has a high affinity to cardiolipin, which is a major acidic phospholipid of the *S. aureus* membrane; however, its affinity to other acidic phospholipids was low. Thus, we assume that the interaction between sapecin and cardiolipin is a prerequisite for the bactericidal activity of sapecin. To examine the involvement of membrane cardiolipin in bacterial sensitivity to sapecin, we performed further studies. Although *E. coli* is less sensitive to sapecin than *S. aureus*, it becomes sensitive to sapecin at sufficiently high concentrations. Therefore, we examined the effect of sapecin on an *E. coli* mutant with a defect in cardiolipin synthesis. The cardiolipin content of this mutant was less than 10% of that of wild-type *E. coli*. This mutant was clearly more resistant to sapecin than the wild type, suggesting that cardiolipin content is a crucial factor for bacterial sensitivity to sapecin. However, membrane cardiolipin content alone cannot fully explain the preferential sensitivity of gram-positive bacteria to sapecin. We found that the lipopolysaccharide (LPS) of gram-negative bacteria is an effective barrier to sapecin. A rough mutant of *E. coli* that lacks the polysaccharide chains of LPS molecules was more sensitive to sapecin than the wild-type strain. Moreover, *E. coli* became more sensitive to sapecin when treated with EDTA (a condition under which *E. coli* is known to lose more than 60% of its LPS). Thus, LPS seems to be a barrier to sapecin and contributes to gram-negative bacteria being less sensitive to this bactericidal protein than gram-positive bacteria. Similar to sarcotoxin IA, sapecin is likely to disrupt membrane functions, although its precise mechanism is still unknown.

(d) **Sarcophaga lectin.** During studies on bactericidal proteins in the hemolymph of immunized *Sarcophaga* larvae, we detected hemagglutinating activity in the same hemolymph. This activity was specifically inhibited by galactose, indicating that it is due to a galactose-binding lectin, which we termed *Sarcophaga* lectin. We purified *Sarcophaga* lectin to homogeneity by affinity chromatography on galactose, and determined its primary structure by isolating its cDNA.33,34 *Sarcophaga* lectin is a large molecule with a molecular mass of 190,000, consisting of 2 subunits with molecular masses of 32,000 and 30,000, respectively, in a molar ratio of 4:2. These subunits were found to be essentially the same protein, and their difference in molecular mass can be explained by their difference in glycosylation.

We studied the physiological function of *Sarcophaga* lectin in insect immunity, and found that it plays a role in the elimination of foreign cells introduced in the larval body cavity.35–37 When sheep red cells were injected into larvae, a significant amount of *Sarcophaga* lectin was promptly induced. The red cells were gradually lysed, and eventually disappeared from the hemolymph. However, when an antibody against *Sarcophaga* lectin or galactose is injected simultaneously with the red cells, cell lysis was greatly inhibited (Fig. 5), indicating that *Sarcophaga* lectin is involved in the elimination of invading foreign cells. Although lysis of red cells occurred in the larval body cavity, *Sarcophaga* lectin itself had no hemolytic activity. Therefore, elimination of foreign cells is a complex process that implicates *Sarcophaga* lectin.

(e) **Antifungal protein.** We found that the hemolymph of *Sarcophaga* contains AFP.12 Unlike antibacterial proteins, AFP is a constitutive component of the larval hemolymph. We purified AFP, and found that it is a histidine-rich protein consisting of 67 amino acid residues. AFP is a fungicidal protein; it significantly inhibits the growth of *Candida albicans* but had no effect on bacterial growth.12
Interestingly, the antifungal activity of AFP was markedly enhanced in the presence of the antibacterial protein sarcotoxin IA. We found that when *C. albicans* was treated with a fixed amount of AFP (100 µg/mL), their viability decreased with the increase in the amount of sarcotoxin IA added, although sarcotoxin IA (10 µg/mL) alone had no fungicidal activity against *C. albicans* under these conditions (Fig. 6). This suggests that *Sarcophaga* develops an efficient antifungal system with constitutive AFP and inducible sarcotoxin IA when the larval body wall was injured to allow invasion of fungi.\(^{12}\) AFP and sarcotoxin IA possibly form a complex having much higher antifungal activity than AFP alone.

(f) 5-S-GAD. 5-S-GAD is a conjugate of glutathione and β-alanyl dehydroxyphenylalanine (Fig. 7).\(^{13}\) This compound has antibacterial activity, and, similar to various antibacterial proteins, is an inducible molecule.\(^{13}\) It seems to be rapidly synthesized by tyrosinase from glutathione and β-alanyl dehydroxyphenylalanine upon bacterial infection. The antibacterial effect of 5-S-GAD was found to be due to the hydrogen peroxide produced from this molecule. As hydrogen peroxide is an activator of Rel family transcription factors, such as NF-κB, 5-S-GAD may partly contribute to the activation of antibacterial protein genes by producing hydrogen peroxide. Besides its antibacterial activity, 5-S-GAD was found to have various pharmacological functions, including potent antioxidant and radical scavenger activities.\(^{38}–^{46}\) Thus, 5-S-GAD is likely to be also involved in wound healing and anti-inflammatory processes upon damage to larval tissues and the body wall.

**Dual roles of an insect immune protein in defense and development**

We were able to determine the primary structures of various *Sarcophaga* immune proteins by isolating their cDNAs. Using these cDNAs as probes, it was possible to examine the expression of these immune protein genes by northern blot hybridization. As expected, all of these genes were expressed in immunized larvae. However, some immune protein genes were found to be normally expressed at specific developmental stages of this insect.\(^{47}–^{49}\) The major tissue expressing these genes was the fat body, which is functionally equivalent to mammalian liver and/or kidney. These genes were also found to be expressed in some hemocyte species. Throughout these studies, we found a novel feature of these immune proteins for the first time. In *Sarcophaga*, some immune proteins seem to play 2 independent roles.\(^{50},51\) We found that *Sarcophaga* lectin and supecin are 2 convincing examples of these proteins.\(^{52},53\)

As described in the section on *Sarcophaga* lectin, it is clear that this lectin is essential for the elimination of foreign cells introduced in the larval body cavity. On the other hand, northern blot experiments revealed that the *Sarcophaga* lectin gene is naturally expressed at 2 stages in the life cycle of this insect, the embryonic stage and the early pupal stage.\(^{57}\) Thus, this lectin was also expected to play a role in these developmental stages.
Embryonic development is known to be accompanied by the elimination of apoptotic cells. At the pupal stage, most larval tissues disintegrate, and the resulting unnecessary cells are eliminated. The cells needed in ontogenetic processes are so-called “self” cells. Unnecessary cells, labeled “nonself,” are rapidly disintegrated. The mechanism of disintegration of nonself cells may be similar to the elimination of foreign cells, and Sarcophaga lectin may also play a crucial role in this process. I believe that innate immunity is required for the elimination of both invading foreign cells and self-derived nonself cells in insects, and Sarcophaga lectin is one of the key molecules of innate immunity.

Sarcophaga lectin seems to be indispensable not only for elimination of nonself cells but also for the development of adult tissues from imaginal discs at the pupal stage. When imaginal discs of Sarcophaga were cultured in vitro in the presence of 20-hydroxyecdysone, they differentiated and formed adult structures similar to Drosophila imaginal discs. The developmental stages of imaginal discs, such as eversion, elongation, apolysis, and terminal differentiation, were easily distinguishable under a binocular microscope. We found that an antibody against Sarcophaga lectin or galactose significantly interfered with the development of imaginal discs, whereas control IgG or glucose did not, which suggested that this lectin is indispensable for the development of imaginal discs.

As this culture system does not contain any component derived from Sarcophaga except imaginal discs, these results suggest that imaginal discs secreted Sarcophaga lectin during their differentiation. We found that a significant amount of Sarcophagi lectin is synthesized by the imaginal discs and secreted into the culture medium when they are cultured in the presence of 20-hydroxyecdysone (Fig. 8). Although imaginal disc cells were viable, they did not synthesize Sarcophaga lectin when cultured in the absence of the hormone. Therefore, it is clear that imaginal discs secrete Sarcophaga lectin in the presence of 20-hydroxyecdysone, and the resulting lectin promotes their further development in an autocrine manner.

Like Sarcophaga lectin, we found that the antibacterial protein sapecin also plays roles in both defense and development. Northern blot experiments showed that the sapecin gene is transiently expressed in the embryonic stage and early pupal stage without any outside stimulus. This expression pattern is similar to that of the Sarcophaga lectin gene.

We assume that sapecin acts as a growth factor in normal development. As shown in Fig. 9, DNA synthesis and cell number increased with the amount of sapecin added to the culture medium of NIH-Sape-4 cells, indicating that sapecin stimulates the
proliferation of *Sarcophaga* embryonic cells. As mentioned above, NIH-Sape-4 cells produce sapecin. Embryonic cells possibly produce sapecin during ontogenetic development, and sapecin stimulates cell proliferation in an autocrine manner. Furthermore, we also found that imaginal discs synthesize sapecin in the presence of 20-hydroxyecdysone, suggesting the participation of this protein in the differentiation of imaginal discs. This function seems to be quite similar to that of *Sarcophaga* lectin. Thus, sapecin may also play 2 independent roles—in defense and development (ontogeny)—like *Sarcophaga* lectin.

Insects possibly have the ability to mobilize a single protein for various purposes. This may be a new paradigm in insect biology, and may be extended to other animal species. *Drosophila* Toll is known as a receptor essential for the formation of the dorsal-ventral axis in embryonic development. Incidentally, the same receptor was found to induce drosomycin, an antifungal protein, in innate immunity. Although Toll is not an immune protein, this may be another example of 1 protein having dual functions in insects.

**Practical application of *Sarcophaga* immune molecules**

It became evident that some *Sarcophaga* immune molecules have potential practical applications. One is the creation of transgenic plants with sarcotoxin IA cDNA, which have acquired resistance to bacterial and fungal infection. For instance, transgenic tobacco plants that express sarcotoxin IA showed enhanced resistance to the causative pathogens of wild-fire disease (*Pseudomonas syringae* pv. *tobaci*) and bacterial soft rot disease (*Erwinia carotovora* subsp. *carotovora*). An example of infection with *Pseudomonas syringae* pv. *tobaci* is shown in Fig. 10. Clearly, transgenic plants have acquired resistance to this bacterium. Moreover, transgenic plants expressing higher levels of sarcotoxin IA were able to withstand fungal infection (*Rhizoctonia solani* and *Pythium aphanidermatum*) and remained healthy, whereas control plants died under the same conditions. Thus, the use of sarcotoxin IA cDNA made possible the breeding of transgenic crop plants that are resistant to various pathogenic bacteria and fungi. These transgenic plants have potential agricultural applications.

On the other hand, 5-S-GAD was found to have several pharmacological activities, based on its antioxidant property. Especially, we found that it significantly represses cataract progression. When rats were given diet containing 50% galactose, the onset of cataract was observed after 7 days, and lens opacities progressively worsened. After 28 days, all of the lenses had developed severe cataract. However, when galactose-fed rats received an instillation of 0.1–1.0% 5-S-GAD solution 4 times a day on a daily basis, cataract progression was significantly retarded after 14 days. No mature cataract was observed in the 5-S-GAD-treated groups even after 28 days (Fig. 11). A similar therapeutic effect of 5-S-GAD was detected in UV-B-induced cataract of rats. Thus, 5-S-GAD may have potential to be developed as an anticataractous ophthalmic solution. We assume that the radical scavenger activity of 5-S-GAD is implicated in the repression of cataract progression.

**Perspective and future directions**

It is believed that several millions of animal species are living on Earth. Of these organisms, more than 70% are insects and less than 4% are vertebrates. Therefore, insects are extremely successful
animals in terms of very high species abundance. Insects have developed a sophisticated self-defense mechanism, the so-called insect immunity, which is now considered an innate immunity. Insect immunity is activated only when insects are infected with microorganisms. As discussed in this article, many immune molecules, including antibacterial proteins, are induced when the body wall of Sarcophaga larvae was pricked to allow bacterial invasion. In this insect, the expression of these immune protein genes is regulated by Rel family transcription factors. These immune protein genes are simultaneously activated by a single-shot body pricking, which means that this insect is able to readily develop a potent self-defense system against various microorganisms.

Insect immunity is clearly indispensable for the elimination of invading microorganisms. However, throughout this study, I am more and more inclined to believe that the same immune system is participating in the clearance of unnecessary cells or tissue fragments produced during ontogenetic processes. We found that many immune protein genes are dormant throughout the life cycle of Sarcophaga, except in the early embryonic stage and early pupal stage in which these genes are transiently activated. Larval structures are formed throughout the embryonic development, whereas adult structures are formed in the metamorphic process, which takes place at the pupal stage. It is known that many unnecessary cells are produced both at the embryonic stage and the pupal stage. Especially at the pupal stage, many larval tissues disintegrate and new adult structures develop from imaginal discs. Since many immune proteins are synthesized at these stages, they must be functioning at these stages.

The function of these proteins may be 2-fold. One is elimination of self-derived nonself cells, and the other is stimulation of construction of new tissues from self cells. We showed that Sarcophaga lectin is indispensable for imaginal disc differentiation and sapecin stimulates proliferation of embryonic cells. These suggest that both proteins participate in the construction of new tissues from self cells. On the other hand, Sarcophaga lectin participates in the elimination of sheep red cells introduced into the larval body cavity, and sapecin has the ability to kill invading bacteria. Despite the absence of a direct evidence, we believe that these proteins participate in the elimination of self-derived nonself cells.

Studies on insect immunity have greatly progressed, and the molecular mechanisms of the activation of immune protein genes have been well understood. However, I think that 2 major issues remain unsolved. One is about the antiviral protein of insects. Do insects have a defense system against viruses? Although antibacterial and antifungal proteins have been extensively studied, very little information is available on antiviral proteins in insects. Thus, we should give much attention to the antiviral system of insects. The other issue is about the mechanism of immune tolerance against symbiotic microorganisms. About 60% of insect species are believed to contain symbiotic microorganisms, such as are bacteria or fungi. These symbiotic microorganisms are passed on from ancestors to descendants for many generations. Insects are usually very sensitive to bacterial infection. Namely, they develop an efficient immune system almost instantaneously upon exogenous introduction of bacteria. However, this immune system does not respond to symbiotic microorganisms. How symbiotic microorganisms in insects escape from host immune surveillance is a question that has to be solved in the future.

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Profile

Shunji Natori was born in 1938. He graduated from the Faculty of Pharmaceutical Sciences, University of Tokyo, in 1963, and started his research career in the field of microbiology, as a graduate student of the laboratory of Prof. Den-ichi Mizuno in the same University. After taking Ph.D. degree in 1968, he spent 3 years at Yale University as a postdoctoral fellow, under the supervision of Prof. Alan Garen. In 1971, he joined Prof. Mizuno’s laboratory as Assistant Professor, and mainly engaged in studies on the regulation of eukaryotic transcription. He discovered, purified, and characterized eukaryotic transcription elongation factor S-II/TFIIS for the first time, from Ehrlich ascites tumor cells. He was promoted to Associate Professor in 1976. When he was Assistant Professor, he incidentally found that insects have ability to induce antibacterial proteins when their body wall was injured. Based on this finding, he performed several pioneering studies on insect immunity. In 1980, he became Professor of the Faculty of Pharmaceutical Sciences, University of Tokyo, and continued these studies until his retirement in 1999. Then he moved to RIKEN as head of Natori Special Laboratory, and conducted studies on the development of new drugs from insect immune molecules. After closing Natori Special Laboratory in 2005, he moved to National Institute of Agrobiological Sciences as director. Now he is adviser of the same Institute.