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A Life's Journey Through Insect Metamorphosis

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Abstract

This autobiographical article describes the research career of Lynn M. Riddiford from its early beginnings in a summer program for high school students at Jackson Laboratory to the present "retirement" at the Friday Harbor Laboratories. The emphasis is on her forays into many areas of insect endocrinology, supported by her graduate students and postdoctoral associates. The main theme is the hormonal regulation of metamorphosis, especially the roles of juvenile hormone (JH). The article describes the work of her laboratory first in the elucidation of the endocrinology of the tobacco hornworm, *Manduca sexta*, and later in the molecular aspects of the regulation of cuticular and pigment proteins and of the ecdysone-induced transcription factor cascade during molting and metamorphosis. Later studies utilized *Drosophila melanogaster* to answer further questions about the actions of JH.

EARLY YEARS

I grew up on a dairy farm in Illinois and always loved animals. My first introduction to science was in high school; I found it fascinating and challenging, particularly biology. In my junior year, I read in *Seventeen* magazine an article by a college student who had spent a summer at the Roscoe B. Jackson Memorial Laboratory in Bar Harbor, Maine, in which she mentioned that they also had a program for high school students. I applied and was accepted with 15 other students that summer. It was a fantastic experience. We did small pilot projects on mice, guinea pigs, and rabbits to learn various techniques, then wrote reports as mini–scientific papers. In addition, we went to lectures at the Laboratory, which that particular year included notable scientists in mouse genetics since it was the 25th anniversary of the Laboratory. I fell in love with research that summer and was fortunate to be one of four students asked back. The second summer, I did one project on cells found in ascites teratomas, which could differentiate into various tissues (later termed embryonic stem cells), with Dr. LeRoy Stevens at the main Laboratory, and one project with the director of the high school program, Mr. Frederick Avis, concerning the effect of hypoxia on mouse embryonic development.

Discussions with other students at Jackson Laboratory led me to apply to Eastern colleges noted for good science education. Radcliffe College awarded me a full tuition scholarship, allowing me to reap the benefits of Harvard University. Because of my summer experiences at the Jackson Laboratory, I was exempted from the beginning biology course, and so took comparative anatomy and animal physiology my freshman year. Starting the second semester of that year, I tried to find a place in a research laboratory but was told that I needed to have more coursework first. I got a job sifting and weighing sand samples for an oceanographic survey, and the next year, another job checking bibliographic references for Professor Alfred Romer for his book *Osteology of the Reptiles*.

In the fall semester of my junior year, I attended a meeting of the Radcliffe PreMed and Science Society at which Professor Carroll Williams spoke about his discovery of the source of the insect juvenile hormone (JH) and its action in preventing insect metamorphosis. I asked whether JH also acted to prevent tadpole metamorphosis. He said that he did not know but asked whether I would like to try to find out. I jumped at the chance and started working in his laboratory in the spring semester doing dishes for pay and research on the side. His graduate student Bill Harvey took me down to Cape Cod to collect bull frog tadpoles. In those days, the structure of JH was unknown, and we worked with the "golden oil" that Williams had extracted from the abdomens of the male *Hyalophora cecropia* moth (103). I injected this "golden oil" into tadpoles and also put it into the water. Neither treatment had any effect except to kill them at higher dosages. I then tested it on the brine shrimp *Artemia*, but the oily extract was incompatible with the high salt content of their culture medium despite the use of the detergent Tween-80. For my senior thesis in my Biochemical Sciences major the next year, I first chemically extracted day-old baby rats and powders of various bovine organs using the same procedure that Williams had used for the moth abdomens, then tested the extracts for JH activity on *Antheraea polyphemus* pupae. Another student, Jean Pulis, further purified the positive extracts the following year, and we reported in *Nature* that JH activity was found in mammalian thymus and adrenal cortex and human placenta (104).

In graduate school at Cornell University, I worked on two different projects, as my major professor Marcus Singer in Zoology felt that one should always be involved in two studies in case one did not work out. The first was a study on the role of the epidermis in formation of the blastema of the regenerating salamander limb. This culminated in a paper at the end of my second year (59). The second project was in my protein physical chemistry minor, working with Professor Harold Scheraga in Chemistry to elucidate the underlying basis of the stability of the α-helical rodlike molecule paramyosin from the clam adductor muscle. Since this project took longer, it became the subject of my Ph.D. dissertation (73, 74).

During my graduate studies, I married Alan Riddiford, an Engineering Physics major; after I received my Ph.D. in 1961, we moved back to Harvard University, where Alan pursued his Ph.D. in Applied Physics. Alan and I divorced in 1966.

I was a postdoctoral fellow in Biology in Professor John Edsall's laboratory, where I worked on the physicochemical properties of human carbonic anhydrases (60, 61, 75) for two years. Then I began my academic career with an Instructorship in Zoology at Wellesley College in 1963, where I taught introductory biology and animal physiology for two years. My research at that time was still in protein physical chemistry, concerned primarily with further studies on the structure of paramyosin (62) and supported by the National Science Foundation (NSF), although one honors student studied the role of zinc in carbonic anhydrase structure.

My Instructorship at Wellesley was a two-year term appointment, so I had to make a decision as to what I wanted to do next. Although I enjoyed teaching, I found that the teaching load at Wellesley was too heavy to establish a good research program. At the time, the forefront of my area of protein physical chemistry was moving into analysis of 3D structures, which was not a viable area for me since I have a poor spatial sense. Therefore, I decided to go back to my biology roots and spent a year as a Research Fellow in Biology at Harvard in Carroll Williams's laboratory learning insect endocrinology.

HARVARD AND BECOMING AN INSECT ENDOCRINOLOGIST

The year in Williams's laboratory was an eye-opening year for me. I learned a tremendous amount about the development and reproductive physiology of saturniid moths, in addition to insect endocrinology. Williams had tea every afternoon from 5–6 p.m., when his graduate students, his research associates, Professor Fotis Kafatos, and often other Harvard faculty members as well as visitors came to discuss ongoing experiments, hot topics in biology, etc.

I started working with the saturniid moths and finding intriguing questions to ask. One derived from Williams's pilot experiments on the ovicidal effects of a JH mimic on *Hyalophora cecropia* (Cecropia) embryos after Sláma & Williams (86) showed that the Paper Factor (85) [later identified as juvabione (10)], which had JH activity specifically on the hemipteran family Pyrrhocoridae, acted as an ovicide for *Pyyrhocoris*. My studies on the effects of JH on Cecropia and other saturniid embryos showed that JH given early prevented the movements of blastokinesis but not later differentiation resulting in a larva that could not hatch (79). Since these experiments necessitated mating the various species in the laboratory, I discovered that *A. polyphemus* moths would only mate in the laboratory in the presence of oak leaves (their preferred food plant) (80). Later, I identified *trans*-2-hexenal as the leaf component that elicited the "calling" behavior (sex pheromone release) (63).

The next year (1966), I became an Assistant Professor of Biology at Harvard University, hired specifically to run the laboratories of the one-semester advanced introductory biology course Biology 2, which was headed by Professor Williams. I was the first woman to be on the faculty in the Department of Biology, and at the time, only one woman was a tenured professor in the entire Faculty of Arts and Sciences. Later in my term as an assistant professor, I developed my own advanced course in endocrinology. I was promoted to Associate Professor of Biology, a nontenured position, in 1971.

My research during the first years of my assistant professorship centered on the effects of JH on insect embryogenesis (65) and on saturniid larvae and the environmental and endocrine controls of mating behavior of the saturniid moths (66). During my years at Harvard, I had five graduate students who helped me expand my areas of expertise. Jim Truman discovered that a neurosecretory hormone, eclosion hormone, caused moth eclosion that happened at species-specific times of

day (92) and led me into the world of circadian rhythms. Eugenie Enslee showed that the disruption by JH on blastokinesis in *Pyrrhocoris* was dependent on its effects on the serosa (14). Alfred Ajami worked on the biochemistry of the ommochrome pigments in the eyes and nerve cord of Cecropia (1, 2). Margie Fain developed a culture system to study the control of metamorphosis of the crochets on the larval prolegs of the tobacco hornworm *Manduca sexta* (20), as well as the *black* larval bioassay for JH (18). Mary Nijhout studied the role of JH in egg maturation in *Manduca* (52).

MANDUCA LARVAL ENDOCRINOLOGY

My research world widened when, in 1969, Ray Hakim, a student of Fotis Kafatos, brought *Manduca* into the Harvard Biological Laboratories as an experimental animal on which to study labial gland development and metamorphosis (23). The ease of rearing *Manduca* on a synthetic diet, coupled with its large size and photoperiodically determined pupal diapause, made it ideal for the study of the larval endocrinology throughout the year, which was really not possible with the saturniid caterpillars.

Jim Truman had become a Junior Fellow after he received his Ph.D. in 1970, and we got married that summer. He continued his studies on the ecdysis behavior in silkmoths and extended them to *Manduca*, from which he was eventually able to isolate and identify the eclosion hormone (42). We then isolated the gene for eclosion hormone and showed that it was synthesized by two pairs of neurosecretory cells in the ventromedial region of the brain (29). Jim's interest in circadian rhythms also led him to begin to study the endocrine events underlying larval molting in *Manduca*.

Jim, two graduate students (Mary Nijhout and Margie Fain), Williams' graduate student Fred Nijhout, and an undergraduate (Lou Safranek) all began working on various aspects of *Manduca* larval endocrinology in the early 1970s. Jim showed that larval ecdyses occurred at specific times of day due to photoperiodic "gating" of prothoracicotropic hormone (PTTH) release and that JH apparently was released shortly thereafter (90). Later studies by Fain & Riddiford (19) showed that the JH titer was high both before and after PTTH release and that the PTTH "critical period" defined by ligation marked the onset of PTTH release, whereas the JH "critical period" marked the end of PTTH release. Jim also showed that there was a later "critical period" during the molt for JH to prevent the melanization of the next instar larval cuticle (97). Serendipitously, at that time, a "black" mutant appeared in our *Manduca* colony (82) which we used for bioassay of the JH titer (18). We also determined the "critical periods" for PTTH and ecdysone release for the larval–pupal transformation (93), while Fred Nijhout and Williams (51) defined a "critical weight" above which the larva would undergo metamorphosis on schedule regardless of whether it continued to feed or was starved. After "critical weight," the JH titer declines, leading to PTTH release at the next photoperiodic gate after JH disappears (81). During this time, studies in Larry Gilbert's laboratory at Northwestern University were focused on the prothoracic glands and ecdysteroid titers (9) and the role of JH in ovarian maturation (87).

In 1973, we moved to the Department of Zoology at the University of Washington, where I became an Associate Professor and Jim became an Assistant Professor. I was promoted to Full Professor in 1975. During my long career there, I taught both animal and cell physiology, part of a graduate course in insect development, and various graduate courses on topics in endocrinology. Mary and Fred Nijhout came with us to help establish our new laboratories, as Mary was still finishing her thesis.

When we were defining the "critical periods" for molting and metamorphosis, we had found that the abdominal epidermis became committed to pupal differentiation during the transition from feeding to wandering (98). To explore this phenomenon further, I used Piepho's

integumental implantation method (56). These experiments showed that the integument (epidermis and overlying cuticle) from a feeding last instar larva implanted into a penultimate instar larva formed a new larval cuticle during the larval molt of the host (67). By contrast, the implanted integument from a wandering stage larva formed a pupal cuticle. Encouraged by the success that we had in inducing the larval crochet epidermis (crochets are the sclerotized hooks on the larval abdominal prolegs) to molt in response to 20-hydroxyecdysone (20E) in vitro (20), I began culturing the abdominal epidermis from feeding larvae with 20E or 20E and JH I, then assaying its commitment by the implantation assay. Not only did the epidermis respond to both hormones in vitro, but also both the induction of pupal commitment by 20E and its inhibition by JH I were caused by physiological concentrations of the hormones (67, 68). Thus, this was an excellent system in which to study the "status quo" effect of JH, which I did over the subsequent years, culminating with the finding that the transcription factor Broad appeared coincident with pupal commitment induced by in vitro treatment with 20E (108, 109). The presence of JH prevented this response to 20E. Two visiting Japanese scientists, Dr. Takashi Mitsui and Dr. Kenji Kiguchi, developed the epidermal culture system further so that it produced either larval or pupal cuticle in vitro (47, 48, 72).

My first graduate student at the University of Washington, Nancy Beckage, explored the effects of the endocrine interactions between *Manduca* and its parasitoid *Apanteles congregatus* (now *Cotesia congregatus*) on the development of both the parasitoid and its host (6). In this system, the parasitoid wasp normally lays eggs in the first instar *Manduca* larva, then the third instar wasp larvae emerge, spin cocoons, and metamorphose on the surface of the host. She showed that parasitism caused a large increase in the JH titer, leading to an extension of the feeding period that allowed the parasitoid larvae sufficient time to feed and mature (7). The subsequent decline of the JH titer allowed the appearance of a small peak of ecdysteroid, which was necessary for the emergence of the parasitoids and their subsequent spinning behavior but, importantly, did not elicit wandering behavior of the host, thus ensuring the undisturbed metamorphosis of the parasitoids. Clearly, the host's physiology is altered by the parasitoids so as to maximize their own developmental success. These and Beckage's subsequent studies were pioneering for the field of host–parasitoid interactions (78).

MOLECULAR ASPECTS OF *MANDUCA* ENDOCRINOLOGY

In 1979–1980, I took a sabbatical year to spend time in Professor Robert Schimke's laboratory at Stanford University to learn molecular biology. Fortunately, Zoecon, a company working on JH mimics as Insect Growth Regulators, was nearby and had a *Manduca* colony, so I was able to use *Manduca* epidermis for my studies. I focused on the changing pattern of translatable mRNAs during the final larval instar and the onset of metamorphosis, using antisera to larval and pharate pupal cuticle proteins that we had made and an antibody to the epidermal pigment protein insecticyanin from Peter Cherbas to identify the mRNAs for these proteins (69). After I worked out the RNA extraction technique and made an initial developmental survey, my technicians back home cultured the epidermis under various hormonal regimens and extracted the RNA, and then sent it to me for translation and analysis. I showed not only that the mRNAs for insecticyanin and the larval cuticular proteins shut off at the time of pupal commitment, with most of the mRNAs for pharate pupal cuticular proteins not appearing until later during the prepupal period, but also that a group of mRNAs for five major larval cuticular proteins appeared on day 3 of the final instar, the day before wandering. In vitro studies showed that the disappearance of the mRNAs for the early larval cuticular proteins and insecticyanin was dependent on 20E acting in the absence of JH to cause pupal commitment, whereas the appearance of the new cuticular proteins on day 3 was independent of the hormonal milieu. Later studies showed that a smaller rise in ecdysteroid on

day 2 activated the appearance of these mRNAs in the absence of JH (28, 106). Concurrent studies on the regulation of synthesis of these proteins were done by graduate students Mary Kiely (34, 35) and Bill Wolfgang (106). Later, the cDNAs for insecticyanin and several cuticular proteins were characterized by graduate student Wan-cheng Li and postdoctoral associates John Rebers and Frank Horodyski (reviewed in Reference 70). Bruce Webb, a graduate student, studied the regulation of the expression and synthesis of the two major storage proteins made in the fat body during the final larval instar (101, 102).

Sequencing of one of the early fifth instar larval cuticular protein genes LCP14 showed that there was a sequence in the carboxy terminus region that was similar to a sequence found in other proteins found in flexible cuticles (57). This sequence was later termed the Rebers-Riddiford consensus sequence (3) and found to bind to chitin (58). The new cuticular proteins that appear on day 3, coupled with the loss of synthesis of most of the cuticular proteins synthesized earlier in the instar, were found to correlate with a change in the lamellar structure of the endocuticle being deposited (106). This tenfold thinning of the cuticular lamellae, followed by cessation of lamellar deposition the following day (when wandering behavior begins), was correlated with a greatly decreased extensibility and increased flexural stiffness of the wandering stage cuticle (107). These changes in the mechanical properties of the cuticle are thought to facilitate its serving as a rigid mold inside of which the pupa can more readily form.

In the early 1980s, Dr. Kiyoshi Hiruma came to my laboratory initially as a postdoctoral fellow, then stayed on as a research associate for over 20 years. His main focus was on the hormonal regulation of melanization of *Manduca* larval cuticle (summarized in Reference 24). The absence of JH at the time of head capsule slippage during the molt to the final instar larva was found to cause the deposition of premelanin granules containing an inactive prophenoloxidase into the newly forming cuticle. When the ecdysteroid titer declined at the end of the molt, the prophenoloxidase was activated and oxidized the incoming dopamine to melanin. The decline of the ecdysteroid titer also was found to be necessary for the increase of dopa decarboxylase (DDC) at this time, which caused an increase in dopamine synthesis. Although DDC mRNA always increased at this time, its level was found to be higher in larvae lacking JH during the critical period. Kiyoshi's expertise in allatectomy (surgical removal of the corpora allata, the source of JH) and in tissue culture made him an invaluable collaborator on many other projects in the laboratory over the years.

Kiyoshi's most extensive collaborations were on the hormonal regulation of the transcription factor cascade in the epidermis initiated by 20E during the larval and pupal molts, as well as at the time of pupal commitment (summarized in Reference 25). These studies, begun in the mid-1990s, involved the cloning of cDNAs for the ecdysone receptor EcR and Ultraspiracle USP, as well as the transcription factors themselves, then analysis of their mRNA expression in response to hormones both in vivo and in vitro, as well as immunocytochemical studies in selected cases. Thus, many postdoctoral associates (Haru Fujiwara, Bela Keshan, Marek Jindra, Que Lan, Rosalie Langelan, Reddy Palli, Tetsu Shinoda, Geoff Stilwell, Xiaofeng Zhou) and graduate students Baohua Zhou and Masako Asahina contributed to this effort. Although the *Manduca* transcription factor cascade in the abdominal epidermis was found to be very similar to that seen in *Drosophila melanogaster* at the time of metamorphosis (89), it provided a unique insight into how JH regulated this cascade. The most important finding was that the Broad transcription factor only came up at the time of pupal commitment in response to 20E and that its appearance could be prevented by the presence of JH (108). Broad was expressed in the epidermis from the time of pupal commitment through the first three days of pupal life, then disappeared (108, 110). Yet when JH was given to the pharate pupa to cause the formation of a "second pupa" rather than an adult, Broad was expressed in the epidermis during the ecdysteroid peak for the adult molt (110). These findings led us to switch to *Drosophila*, where we found that misexpression of Broad during the adult molt caused the

formation of a *Drosophila* "second pupa" with suppression of adult cuticle genes and re-expression of pupal cuticle genes (110). Moreover, Broad misexpression during the second (penultimate) instar caused suppression of a larval cuticle gene and premature expression of a pupal cuticle gene during the final larval molt. Thus, Broad acts as a pupal-specifying transcription factor. This last study was a true collaboration with my postdoctoral fellow Dr. Xiaofeng Zhou, as he did the molecular work in my laboratory, while I analyzed the pharate adults in which Broad was misexpressed when on sabbatical in Professor Michael Akam's laboratory in Cambridge University in 2000–2001.

MANDUCA IMAGINAL DISC AND PRIMORDIA GROWTH AND METAMORPHOSIS

In the early 2000s, we began exploring the hormonal regulation of the metamorphosis of *Manduca* wing discs and eye and leg primordia. A collaboration with David Champlin, Jim's former postdoctoral fellow at the University of Southern Maine, showed that feeding final (fifth) instar larvae on a sucrose diet initiated *broad* expression (a sign of its pupal commitment) in the eye, but protein was required to initiate the proliferation needed to form the eye disc (41). Later studies with Jim found that the decline of JH that occurred upon feeding was critical for this metamorphic development of the imaginal primordia and the wing discs (91). Allatectomy during the molt to the fifth instar caused the onset of their morphogenetic growth even when the larvae were starved and in the absence of ecdysone. Thus, JH has an important role during the larval intermolt period to suppress morphogenetic growth of the imaginal primordia. This role is independent of ecdysteroid action. In the final instar, a nutrition-dependent factor called Metamorphosis Initiating Factor (91) overcomes this JH suppression. Later experiments indicated that an insulinlike factor (or factors) is likely involved (37).

Further studies with the allatectomized fifth instar larvae revealed that they no longer had to reach a critical weight checkpoint before beginning metamorphosis (88). Instead, they began wandering four days after the onset of feeding. This occurred even when they had been starved for up to five days before feeding. Protein feeding for 12–24 h was sufficient to initiate this metamorphic timer, but neither lipid nor sugar sufficed. Although wandering occurred at very small sizes, normal pupae were not formed unless the larvae fed longer and attained weights of approximately one-half to two-thirds of their normal weight at wandering. Thus, JH enforces the "critical weight" checkpoint on this metamorphic timer so that, under adverse conditions, a sufficient size can be attained to ensure successful metamorphosis.

THE SEARCH FOR THE ELUSIVE JUVENILE HORMONE RECEPTOR

In the mid-1980s, I teamed up with the chemist Professor Glenn Prestwich at State University of New York at Stony Brook and insect biochemist Professor Bruce Hammock at the University of California at Davis under the auspices of a collaborative NSF grant to identify and isolate the JH receptor from *Manduca*. Glenn synthesized photoaffinity analogs of JH that were active in the *black Manduca* larval assay. My postdoctoral associates, Reddy Palli and Ellie Osir, then incubated these with nuclear extracts and isolated the photo-cross-linked proteins. One of these was present in both larval and early pupal epidermis, as expected for a JH receptor (54). Isolation of this protein and subsequent cloning of its cDNA followed by its production in a baculovirus yielded a purified 29-kD protein that specifically bound JH (55). However, further studies showed that the apparent specific binding was an artifact due to the copurification of esterases that stripped off the tritium label that was on the methyl ester group of JH I during the JH-binding assay (12). When these

esterases were removed by further purification, the 29-kD protein was found to have only low affinity for JH. Its presence both in the nucleus and in the cytosol associated with insecticyanin granules and its developmental expression dependent on JH in the larva but not in the pupa (84) suggested that it might serve to prolong the presence of JH within the cell. Further studies have not been done, so its precise role remains obscure.

Even though we were unsuccessful in isolating the JH receptor, I am proud to say that two of my former postdoctoral associates, Dr. Marek Jindra and Dr. Jean-Philippe Charles, later in their own laboratories were responsible for conclusively proving that Methoprene-tolerant (Met) is the JH receptor. The *Met* gene, which encodes a basic helix-loop-helix, Pas-domain transcription factor, was first identified by Wilson & Fabian (105) as the possible JH receptor, since its loss in *D. melanogaster* greatly decreases the animal's sensitivity to treatment with JH. Konopova & Jindra (36) showed that the suppression of *Met* expression in the flour beetle *Tribolium castaneum* caused precocious metamorphosis. Moreover, Charles et al. (11) showed that *Tribolium* Met specifically binds JH with high affinity, and later, Jindra et al. (31) showed that Germ cells-expressed (Gce), the Met paralog in *Drosophila* (4), also specifically bound JH. Chieka Minakuchi and Tetsu Shinoda, also former postdoctoral associates, showed that loss of the JH-regulated Krüppel homolog 1 (Kr-h1) caused precocious metamorphosis in *Tribolium* (43). Subsequent studies have shown that Kr-h1 is the transcription factor maintaining larval status in insects (30).

INSECT OLFACTION

I had first become interested in insect olfaction when studying the mating behavior of saturniid moths at Harvard and had done some inconclusive studies on the water-soluble proteins in the moth antenna (64). Later, my graduate student Dick Vogt found two soluble proteins specifically in the sensillar lymph of antennae of male Polyphemus moths—an abundant pheromone-binding protein and an esterase that degrades the pheromone (99). The binding protein was thought to serve as a carrier for the hydrophobic pheromone through the sensillar lymph to its membrane receptor in the sensory dendrite and also to slow pheromone degradation by the esterase (100). Although not the olfactory receptors, these were the first proteins from an insect with specific functions in olfactory sensing to be isolated and characterized. Odorant binding proteins similar to the pheromone-binding protein have now been found throughout the insects (39). A recent study in *Drosophila*, however, found that deletion of a specific odorant binding protein did not decrease the physiological responses of the mutant sensillum, as would be expected if this protein were essential for transport to the receptor (38). The modifications seen suggested that, instead, it may function in gain control.

STUDIES ON *DROSOPHILA*

I first began working on *Drosophila* during a sabbatical at Cambridge University with Professor Michael Ashburner in the Department of Genetics in 1986. At the time, I was curious about the role of JH in the *Drosophila* larva and found that exogenous JH had little effect on larval development except to prolong the final (third) instar (71). The role of JH in *Drosophila* metamorphosis did not become clear until much later, when I was able to genetically allatectomize the larvae using a GAL4 construct to drive the cell death gene *grim* in the corpus allatum to cause the death of these cells by the third instar. These allatectomized larvae pupariated, then died at head eversion to the pupa (76). Thus, JH was essential at pupariation for regulation of prepupal development, as has been found in many holometabolous insects.

Dr. Christen Mirth came to the University of Washington in 2003 to work with Jim and me on the problem of size assessment for metamorphosis in *Drosophila*. She first found that the "critical weight" (the size at which further feeding was not necessary for the normal timing of the onset of metamorphosis) and the "minimal viable weight" (the size at which normal metamorphosis can occur) were very similar in *Drosophila* larvae (45). Manipulation of the growth of the prothoracic gland portion of the ring gland using members of the insulin signaling pathway led to an alteration in size at metamorphosis: Smaller prothoracic glands in the final instar led to larger larvae at metamorphosis, whereas larger glands led to smaller larvae. This negative relationship between prothoracic gland size and larval size at metamorphosis is only seen when the gland size is manipulated by nutrient-dependent insulin signaling and not when it is manipulated by other means, such as using *myc* to change cell size (13). Later, Christen found that the differentiative patterning that normally begins in the wing discs after the larvae attain the critical weight for metamorphosis is dependent on the small rise of ecdysteroid at that time, which causes derepression of the patterning genes (46).

My serious studies on *Drosophila* began when Jim and I retired from the University of Washington in 2007 and went to the Janelia Farm campus of the Howard Hughes Medical Institute (HHMI) in Ashburn, Virginia, as Group Leader and Senior Fellow, respectively. This position allowed me to do research full time, which was a joy. It was here that the genetic allatectomy scheme was perfected (76). The allatectomized larvae grew more slowly due to suppressed insulin signaling but pupariated on time, forming smaller pupae (44). Although the allatectomized larvae pupariated normally, they died at pupal head eversion (76). One of the most striking anomalies in these animals during the prepupal period was the precocious adult development of the optic lobe. In the absence of JH, EcR-B1 appeared precociously in the prepupal optic lobe and was accompanied by proliferative and differentiative changes usually found later in early adult development. Further studies in collaboration with Jim and Aljoscha Nern, who has made enhancer lines specific to most neuron cell types in the adult optic lobe, showed that normal adult development is dependent on a pulse of JH early in the prepupal period; it must then be absent for later development to occur normally (77). When JH was given during the late prepupal or early pupal periods, differentiative events such as the growth of the dendritic arbors of the lamina neurons and the appearance of specific enhancers that appear in the second half of adult development were prevented, whereas those occurring in the first half were unaffected. The suppression of the normal appearance of EcR-B1 during early adult development by the JH was only partially responsible for its adverse effects.

As a Senior Fellow, I could have two people in my laboratory, which at first included Christen Mirth and my last graduate student from the University of Washington, Hans Kelstrup. Hans continued his studies on the endocrinology of wasp behavior in Brazil, working with two species of swarm-founding wasps, *Polybia micans* and *Synoeca surinama*. The two species showed significant differences in their dependence on JH for the regulation of both ovarian maturation and social behaviors (32, 33). My two later postdoctoral fellows, Julide Bilen and Aaron Baumann, worked on *Drosophila*. By eliminating the corpora allata only during adult development, we showed that JH in the adult female was necessary for both the maturation of mating behavior and the production of the major sex pheromones (8). Interestingly, these effects were found to be mediated by the Met rather than the Gce receptor. Although we tried and failed to make a reliable antibody to distinguish immunocytochemically between the two JH receptors in *Drosophila*, we were able to explore their tissue-specific expression using Bacterial Artificial Chromosome recombineering along with a transgenic knock-in construct for Gce (5). Most tissues contained both receptors, although within the nervous system, there were some cell types that only contained either Met or Gce. Most notable was the finding that the larval imaginal discs contained only Met.

Overexpression of Gce in these discs, however, did not render them sensitive to JH. Moreover, the discs were shown to express Taiman, the cofactor for the DNA binding of the JH receptor (11, 40). Thus, the mystery of why JH cannot prevent the metamorphosis of the imaginal discs in *Drosophila* remains.

EVOLUTION OF METAMORPHOSIS

Jim and I became interested in the evolution of metamorphosis while on a one-quarter leave in Dr. Eldon Ball's laboratory in the School of Biological Sciences at Australian National University in Canberra. We found that JH given to *Schistocerca gregaria* embryos just before the pronymphal molt inside the egg (which normally occurs in the absence of JH) caused precocious nymphal development (94), as had been previously described (53, 83). Review of the literature combined with the suite of effects that we saw led us to the hypothesis that the hemimetabolous embryonic pronymph evolved into the larva of holometabolous insects by first becoming a protolarva that could feed on yolk within the egg, leading later to a free-living larva and reduction of the nymphal stages to one, the pupa (94, 95). In this scenario, the ancestral role of JH was to regulate embryogenesis, which then evolved into the regulation of postembryonic metamorphosis in the Holometabola. Deniz Erezymilaz, a graduate student in Jim's laboratory, later showed in the cricket (*Acheta domesticus*) embryo that JH not only caused precocious differentiation but also adversely affected proximo-distal growth of the leg, although not its initial patterning (15). She later found in the milkweed bug *Oncopeltus fasciatus* that Broad appeared in the embryo (17), was dependent on JH, and was necessary during nymphal molting to promote stage-specific changes in pigmentation and anisometric growth of the wing pads (16). The necessary presence of this pupal-specifying factor of holometabolous insects in hemimetabolous nymphs for normal nymphal development supported the idea that the nymphal stages of the hemimetabolous insects had been condensed into the pupa of the holometabolous insects. We recently refined our hypothesis based on new developmental and molecular information that has appeared over the past 20 years (96).

SABBATICALS: REJUVENATION AND REDIRECTION

Sabbaticals were my own personal lifeline to learn new skills and approaches that would subsequently change the direction of the research in my lab. My first full sabbatical in Bob Schimke's laboratory at Stanford (1979–1980) introduced me to molecular biology; the second in Michael Ashburner's laboratory at Cambridge University (1986–1987) introduced me to *Drosophila* genetics. That year was split so that the second half was spent at the International Center of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya, where both Jim and I worked on tsetse flies. The third sabbatical year (1993–1994) was spent in Australia at the Commonwealth Scientific and Industrial Organization (CSIRO) headquarters in Canberra, where I worked with Dr. Marion Healy in Dr. John Oakeshott's laboratory on the role of the JP29 protein in *Drosophila*, a project I later discarded when we found that JP29 was not the JH receptor. We returned three years later for three months in Eldon Ball's laboratory at Australian National University to begin our studies on locust embryos and the evolution of metamorphosis. Further studies on JH in both locust and firebrat embryos in Michael Akam's laboratory at Cambridge University on our next sabbatical in 2000–2001 deepened our insights into this evolution.

In addition, these sabbaticals and two months in Kenji Kiguchi's laboratory in Japan studying *Bombyx* embryos provided new insights into different cultures, both scientific and social. Explorations of the surrounding countryside, especially in Kenya and Australia, introduced us to new natural worlds.

"RETIREMENT" AT FRIDAY HARBOR LABORATORIES

Now we have retired back to the Northwest, working at the Friday Harbor Laboratories on both *Drosophila* and Crustacea with our equipment from Janelia and a one-time generous gift from HHMI. After finishing my study on the effects of JH on *Drosophila* optic lobe development discussed above, Jim and I have been investigating the role of the adult specifying transcription factor E93 in adult development of the nervous system and the effect of JH. Also, I have made initial forays into the problem of whether methyl farnesoate (the crustacean JH) plays a role in the embryonic and/or naupliar development of mysid shrimp using a JH mimic. It is a great time to explore and think.

CONCLUDING REMARKS

I have been fortunate in my career to have excellent mentors early on who encouraged exploration of the unknown with rigor and in detail, as well as timely and clear communication of the outcomes of my research. These principles have guided my training of graduate students and postdoctoral associates. I have always encouraged my graduate students to define their own problem in an area that most interests them, then to run with it wherever it may take them. Postdoctoral associates were similarly encouraged, although their projects may have been somewhat more restricted depending on the source of funding. This approach brought in many new techniques and ideas. For instance, studies of heat shock on protein synthesis in *Manduca* epidermis (21, 22), expression of the homeotic gene *Abdominal A* in *Manduca* embryos (49) and morphogenesis in early *Bombyx* embryos (50), and the roles of EcR and USP in both *Drosophila* (26) and paedogenetic gall midge (27) ovarian maturation by three Ph.D. students, Cathy Fittinghoff, Lisa Nagy, and Jason Hodin, respectively, were outside of the main themes of my laboratory but enriched our lab meetings and thinking.

My career in insect research has been greatly enhanced by my husband and colleague, Jim Truman, who is a true entomologist continually asking fascinating questions. Without him I would not have ventured into so many intriguing areas or had so many exciting intellectual challenges and adventures.

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