

---

# Hormonal Control of Metamorphosis

Caleb R. Baker

2003

kdawg@holly.colostate.edu

---

## Abstract

Metamorphosis of insects is controlled and regulated by effector hormones that are controlled by neurosecretory peptide hormones in the brain. Complete metamorphosis of an insect consists of an egg and the larvae emerges from the egg and usually molts 5 times, loses the cytoskeleton that it has out grown and produces a new one, and spins a cocoon and enters the cocoon as a pupa and emerges as an adult. The molting process starts with the release of prothoracicotropic hormone (PTTH) in response to neural, hormonal, or environmental factors. Prothoracicotropic hormone stimulates the production of ecdysone by the prothoracic glands. Neuropeptide prothoracicotropic hormone (PTTH) starts a transducing cascade in cells of the prothoracic gland, which increases the rate of ecdysteroid biosynthesis (up regulation). The levels of 20-hydroxyecdysone in the hemolymph feed back on the prothoracic gland causing a decrease in the rates of ecdysteroidogenesis (down regulation). The prothoracic gland cells convert<sup>the</sup> steroid cholesterol to the precursor of ecdysone and then 20-hydroxyecdysone. Ecdysone is a non-active prohormone and must be converted into an active hormone by a heme-containing oxidase in the mitochondria and microsomes of tissues such as the fat body. In the mitochondria and microsomes the ecdysone is changed to the active hormone 20-hydroxyecdysone. In the molting process there are one or more pulses of 20-hydroxyecdysone, this hydroxyecdysone stimulates the epidermal cells to produce enzymes that digest and recycle the components of the cuticle. Another major effector hormone in insect development is juvenile hormone (JH), which is secreted by the corpora allata. This hormone is responsible for preventing metamorphosis. Juvenile hormone is responsible for preventing metamorphosis, when JH is present; the hydroxyecdysone-stimulated molts result in a new larval instar. In the molt from the last larval instar to pupa juvenile hormone is inhibited and all juvenile hormone that is in the body is degraded. This drop in JH stimulates the release of PTTH from the brain and PTTH stimulates the prothoracic glands to secrete a small level of ecdysone. The resulting hydroxyecdysone, in the absence of JH, stimulates the cells to act in pupal development. The ecdysone level stimulates the formation of new pupal-specific gene products and the organism then shifts from larva to pupa. It was previously thought that a steady decline in juvenile hormone was directly correlated with metamorphosis but it is now thought to be a more complex picture. Generally, if JH is present during a JH-sensitive

period, the current developmental level will remain the same, but if JH is absent during that period, this tissue will progress to a more mature developmental state. The JH-sensitive period appears to be an autonomous state of the cell and is not controlled by hormones. When molting, if juvenile hormone is absent then the organism will progress from larva to pupa to adult. Juvenile hormone also allows imaginal discs to evert and differentiate. This paper will discuss the hormonal control of metamorphosis.

---

## **Introduction**

Metamorphosis allows insects to separate the resources they need for growth from those they need for reproduction. The earliest forms of metamorphosis seen in insect are ametabolous or direct development (Kukalova-Peck, J. 1978). The only difference between the adult stages and the juvenile stages is that in the juvenile stages the genitalia are not mature and the juveniles are smaller than the adults. Insects that exhibit incomplete metamorphosis, hemimetabolous insects, are polyphyletic while insects exhibiting complete metamorphosis, holometabolous insects, are monophyletic. The main developmental hormones in insects are hydroxylated steroid hormones and sesquiterpene hormones. In general ecdysone (hydroxylated steroid) is involved in molting and juvenile hormone (sesquiterpene) is involved in maintaining the insects current form. Ecdysone is produced by the prothoracic glands or the ventral glands. E-ecdysone is converted into 20-hydroxyecdysone in peripheral tissues, especially the midgut. The biochemical conversion of E-ecdysone to 20-hydroxyecdysone can be a rapid conversion or it can take a few days. The inactivation of 20-hydroxyecdysone occurs in two different pathways. One, the irreversible inactivation of 20-ecdysone occurs by further hydrolyation of 20-hydroxyecdysone to 20, 26 dihydroxyecdysone. Two, 20-hydroxyecdysone can be inactivated by sequestration in the form of polar conjugates. The polar conjugates are then

excreted or stored. During oogenesis 2-deoxyecdysone and ecdysone conjugates are put into the egg, though to be used in development before the embryo's own endocrine system becomes functional on its own. Juvenile hormone is an epoxyethyl farnesoate (JH-III) also a number of structures derived from homomevalonate. Epoxyethyl farnesoate appears to be the most common juvenile hormone among insects but there are exceptions. Juvenile hormone is released by the corpora allata. To be inactivated juvenile hormone must be attacked on the ester group or the epoxide group.

### **Hormones during Embryogenesis**

The periodic peaks for ecdysone levels in embryogenesis correspond to production of the serosal cuticle and the three embryonic cuticles. Juvenile hormone involved in oogenesis is seen in the newly laid egg but is soon after degraded by esterases. It is thus absent in midembryogenesis and is seen again at the beginning of the nymphal molt and is associated with the final histodifferentiation of embryonic tissues. A consistent feature of juvenile hormone applied during midembryogenesis when juvenile hormone is normally absent is interference with the process of katatrepsis so that the embryo assumes abnormal positions within the egg. Depending on the doses of juvenile hormone mimic given during the normal absence results in small nymphs and a reduction in the number of abdominal segments (Nov'ak VJA 1969). Studies have been done blocking juvenile hormone and treating embryos with allatocidal compound, precocene producing a wide range of results (Bruning and Lanzrein 1985). The widely ranging results may relate to the fact that the chemical allatectomy requires that the corpora allata becomes synthetically active so that the prococene can be converted to its cytotoxic

metabolite. The advancement of the time of appearance of juvenile hormone might function in the transformation from a pronymphal stage to a functional larva.

### **Prothoracictropic Hormone**

Prothoracictropic hormone is secreted from the neurosecretory cells in the brain and regulates the levels of ecdysone through the prothoracic glands. Ecdysteroid levels rise in the hemolymph when stimulated by the secretion of prothoracictropic hormone. The period of ecdysteroid secretion can be rather prolonged even up to five days depending on the species and the point of their life cycle. The period of ecdysteroid secretion is longer during a molt between a change in stage rather than in a molt staying in the same stage, for example the period of ecdysteroid secretion during a molt from a pupa to an adult is longer than a molt from a larva to a larva. The period of ecdysteroid secretion is longer due to the fact that a change in tissue takes place. Changes that take place during an ecdysone surge are that cells prepare for mitosis, apoptosis, epicuticle deposition, endcuticle deposition, preecdysial pigmentation, and ecdysis. Ecdysis is the shedding of an outer integument or layer of skin. Ecdysone is a prohormone and it is not the hormone that is directly responsible for the molting, it is converted into 20-hydroxyecdysone by the fat body and epidermal cells. The final stage of the molt is ecdysis or eclosion. Eclosion is a term used for the emergence of an adult from its pupal cuticle. Ecdysis and eclosion are controlled by eclosion hormone, a neurosecretory hormone. Ecdysis and eclosion occur at specific times in the day/night presumably because of the insect's circadian rhythm or clock located in the brain (TRUMAN JW. 1971). After ecdysis the newly formed cuticle must harden to protect the insects. The hardening, sclerotization, of the cuticle is controlled by a neurosecretory hormone called

*bursicon*. In higher Diptera hardening of the larval cuticle during puparium is controlled by a set of neurosecretory hormones called the *pupariation factors*. The higher Diptera discard their whole thoracic and abdominal epidermis during metamorphosis and build a new adult body wall new from imaginal discs and abdominal histoblasts. To contain the almost total disintegration of the larval body wall the insects never ecdyses directly from the last larval cuticle. Rather, at the end of the larval stage the cuticle of the last larval instar contracts to a smooth bucket or barrel shape and this barrel hardens providing a safe place where pupation and adult development take place. This barrel like structure is called the puparium and is only seen in higher Diptera. Hardening or sclerotization of the puparium is controlled by a hormone called puparium tanning factor (PTF). Two additional hormone are involved in puparium formation they are anterior segment retraction factor (ARF) and puparium-immobilizing factor (PIF) (Zdarek 1985). Anterior segment retraction factor causes anterior segments of the larva to retract and form the larval integuments into the barrel-shaped puparium. Puparium-immobilizing factor causes larva to become immobile for a period of time. The three hormones seem to be secreted simultaneously to cause the hardened puparium.

### **Hormones During Postembryogenesis**

Nymphs and larvae are similar in that they both possess the same response to ecdysone acting in the presence of juvenile hormone. They differ in the pattern of endocrine secretion that directs metamorphosis. In hemimetabolous insects the juvenile hormone levels drop at the onset of the last nymphal stage and the ecdysone surge causes the formation of the adult stage. In holometabolous insects the juvenile hormone level declines during the last larval stage and there is a small surge of ecdysone that

follows that terminates larval feeding, stimulates cocoon spinning, and commits larval tissues to pupal development (Riddiford LM. 1978). There is a second large surge of ecdysone that produces cuticle. Juvenile hormone is then degraded before pupal ecdysis so that the following surge of ecdysone causes adult commitment and differentiation.

### **Hormone Control of Imaginal Growth**

In the derived imaginal discs found in higher Diptera the rates of cell addition are constant without regard to the periodic surges of hormones that cause molting. The way in which juvenile hormone is repressed in imaginal disc formation is comparable to the repression of morphogenetic growth in embryogenesis (Truman and Riddiford 1999). The presence of juvenile hormone throughout larval growth keeps repression of the imaginal discs, but in the last instar juvenile hormone disappears and disc formation takes place.

### **Endocrinology of the move from larva to pupa**

Hormone controls are different between holometabolous insects and hemimetabolous insects. One hormone control that is not seen in hemimetabolous insects is the peak of ecdysteroid that stimulates the termination of larval feeding, starts premetamorphic behaviors, and commits larval tissues to form pupa tissues (Riddiford 1995, Nijhout 1994). Another hormone control that is seen in holometabolous insects and not hemimetabolous insects is the reappearance of juvenile hormone at the prepupal surge of ecdysone (Baker et al. 1987). It seems that juvenile hormone is important in the larval to pupal transition in the cells that have determined their final fates before formation of the cuticle in pupa.

### **Hormone Control of Butterfly Wing Pattern Development**

The pattern of colors on the wings of butterflies is determined during late larval and early pupal development. The ecdysone receptor (EcR) from the butterflies *Precis coenia* and *Bicyclus anynana* was found and sequenced. Ecdysone receptor was stained during color pattern formation in wing. It was observed that EcR is expressed in the cell nuclei corresponding to wing lacunae and future veins. EcR is seen in "focal" cells which are thought to secrete determining signals in a process that leads to eyespot formation. Cells that form first are the scale forming cells and they show EcR in most of the wing including eyespot foci, but not in future eyespots. The EcR in the eyespots seem to play a role in formation of scale rows occurring before the later expression in scale-forming cells. It demonstrates that EcR is locally expressed in all major events of wing development and color pattern formation (Koch et al. 2002).

### **Ecdysone and Ultraspiracle in Metamorphosis**

Metamorphosis is controlled through the ecdysone receptor complex which is a heterodimeric nuclear receptor that is made up of the ecdysone receptor (EcR) and ultraspiracle (USP). Differentiation in *Drosophila* ovary at metamorphosis is related to USP and EcR-A isoform being present locally in all but one mesoderm derived somatic cell type. The one derived somatic cell type is the larval terminal filament (TF) where only USP is present during differentiation. In cells that are determined to form the basal stalks and the anterior oviduct USP is present with what seems to be EcR-B2 isoform. Deletion in the EcR gene induces several defects. Flies with a strong USP allele show accelerated terminal filament differentiation. Flies that are heterozygous for both EcR and USP show additional phenotypes including many heterochronic shifts, delayed start and finish of terminal filament morphogenesis, and delayed ovarian differentiation.

Studies show that proper expression of the ecdysone receptor complex is required in order to maintain proper progression and timing of events involved in ovarian differentiation in *Drosophila* (Hodin and Riddiford 1998).

### **Methoprene and Parasitism**

The parasitism of *Manduca sexta* (tobacco hornworm) by *Cotesia congregata* (wasp) stimulates developmental arrest of the host in the larval stage. In the final instar stage the host's juvenile hormone levels are elevated preventing host metamorphosis. Wasps emerge when there is a second surge in ecdysone, but when the analogue to juvenile hormone, methoprene, is applied to the hosts' the wasps are delayed in their emergence or they don't emerge at all. Application of methoprene also disrupted metamorphosis of parasitoids (Beckage et al. 2002). The inhibitory effects of methoprene seem to be mediated by the synthesis or release of ecdysis triggering hormone (ETH) in the parasitoid (Park et al. 1999. 2002).

### **Fenoxycarb in Molting**

Fenoxycarb, a juvenile hormone analog, has been shown to stimulate another molt when applied to the silkworm *Bombyx mori* 3<sup>th</sup> or 4<sup>th</sup> instar. The 5<sup>th</sup> instar period was shortened and the additional 6<sup>th</sup> instar period lasted about eight to twenty days depending on the amount of fenoxycarb applied. When the silkworms were starved before the application of fenoxycarb the extra molt occurred 100% of the time. When larva underwent extra molting the level of ecdysone was much lower than the level of 20-hydroxyecdysone in the 4<sup>th</sup> and the 5<sup>th</sup> instars (Kamimura and Kiuchi 2002).

### **Evolution of the Ecdysone Receptor Complex**

Ecdysone receptor complex consists of the ligand binding site on the ecdysone receptor and the heterodimeric ultraspiracle. Ecdysone receptor complex has been found to have two to three isoforms. The isoforms have been found in holometabolous insects including *Drosophila*, *Tenebrio*, *Manuca*, and *Bombyx* (Riddiford LM et al. 2001). There are shifts in the ultraspiracle isoforms during molting in certain species including *Aedes* during vitellogenesis (Wang et al. 2000). There seem to be special usage of the ecdysone receptor complex isoforms involved in metamorphosis. The three heterodimeric isoforms are ecdysone receptor A (EcR-A), ecdysone receptor B1 (EcR-B1), and ecdysone receptor B2 (EcR-B2). These three ecdysone receptor isoforms are encoded for on the EcR gene and activated by the orphan nuclear receptor USP (Bender M et al. 1997). In the reorganization of the insect central nervous system during metamorphosis 20-hydroxyecdysone stimulates a wide range of cellular responses including neuronal cell proliferation, maturation, cell death, and the rearrangement of the neurons in the larval stage to their adult form. In neuron reorganization during metamorphosis motor-terminal retraction and dendritic regression can be experimentally uncoupled, meaning that central actions of ecdysteroids stimulates dendritic regression while peripheral actions stimulates terminal retraction (Knittel LM, Kent KS. 2002). In *Drosophila*, expression of specific ecdysone receptor (EcR) isoforms has been correlated with responses; this suggests that different ecdysone receptor isoforms may govern distinct steroid stimulated responses in these cells. *Drosophila* with deleted ecdysone receptor B promoter, eliminating the expression of ecdysone receptor B1 and ecdysone receptor B2 but maintaining ecdysone receptor A, have been observed to have defective larval molts arresting at the boundaries in between the three larval instars. The reorganization of the neurons during

metamorphosis occurs when cells are expressing high levels of ecdysone receptor B1 and low levels of ecdysone receptor A (Schubiger M 1998).

### **Juvenile Hormone**

Juvenile hormone is secreted by the corpora allata. The natural juvenile hormone is highly unstable. They are degraded easily by esterases in the hemolymph and by sunlight. After secretion juvenile hormone attaches to a specialized hemolymph protein called a juvenile hormone binding protein. The protein stabilizes the hormone and protects the hormone from the esterases in the hemolymph. The synthesis of juvenile hormone is done in the corpora allata. The corpora allata is controlled by the nervous system in so species and by neurosecretory cells in other species. There is no storage of juvenile hormone in these structures so the secretion of juvenile hormone is limited by the rate of synthesis.

### **Spatial Patterns in Molting and Metamorphosis**

Ecdysteroids and juvenile hormone circulate in the hemolymph throughout the insect's body, so as to expose all the tissues to the same concentrations of ecdysteroid and juvenile hormone at the same time. The epidermal cells do not respond equally or simultaneously to the hormones. Epidermal cells require different amounts of exposure to ecdysteroid depending on species. Pupal commitment originates in different species and spreads throughout the insect's body.

### **Juvenile Hormone Receptor**

Juvenile hormone and the way tissues respond to it have been an important part of the evolution of insect life histories. The product of the *Methoprenetolerant* gene in *Drosophila* is thought to maybe be the juvenile hormone receptor (Wilson et al. 1986).

Loss-of-function *Methoprenetolerant* mutants a reduced sensitivity to the effects of juvenile hormone and a reduced intracellular juvenile hormone binding activity (Shemshedini L, Wilson TG 1990). Null *Methoprenetolerant* mutants show no defects in embryogenesis or larval development or metamorphosis, but reduced vitellogenesis (Wilson TG, Ashok M. 1998).

### **Juvenile Hormone and Developmental Pathways**

Juvenile hormone or juvenile hormone agonists in *Drosophila* applied during the beginning of metamorphosis are lethal. It was thought that the juvenile hormone agonist, methoprene, might be interfering with the Broad Complex (BRC). It has been determined that feeding of applying methoprene topically disrupted the metamorphic reorganization of the nervous system, salivary glands, and muscle development depending on the dose applied. Methoprene phenocopies a subset of Broad Complex (BRC) defects and phenocopies Deformed produces abnormalities not associated with known mutations. Methoprene disrupts metamorphic developments involving Broad Complex and combinations of all forms of the Broad Complex isoforms and not disrupting those events that involve Broad Complex isoform subsets. Mutants of *Methoprenetolerant* are resistant to all the features of this “methoprene syndrome” (Restifo LL, Wilson TG 1998).

## References

- Baker FC, Tsai LW, Reuter CC, Schooley DA. 1987. *In vivo* fluctuation of JH, JH acid, and ecdysteroid titer, and JH esterase activity during development of the fifth stadium *Manduca sexta*. *Insect Biochem.* 17:989-96
- Bender M, Imam FB, Talbot WS, Ganetzky B, Hogness DS. Drosophila ecdysone receptor mutations reveal functional differences among receptor isoforms *CELL* 91 (6): 777-788 DEC 12 1997
- Bruning E, Saxer A, Lanzerein B. 1985. Methyl farnesoate and juvenile hormone III in normal and precocene-treated embryos of the ovoviviparous cockroach *Nauphoeta cinerea*. *Int. J. Invertebr. Reprod. Dev.* 8:269-78
- Kamimura M, Kiuchi M Applying fenoxycarb at the penultimate instar triggers an additional ecdysteroid surge and induces perfect extra larval molting in the silkworm *GENERAL AND COMPARATIVE ENDOCRINOLOGY* 128 (3): 231-237 OCT 1 2002
- Knittel LM, Kent KS. 2002. Remodeling of an identified motoneuron during metamorphosis: Central and peripheral actions of ecdysteroids during regression of dendrites and motor terminals *JOURNAL OF NEUROBIOLOGY* 52 (2): 99-116 AUG 2002
- Kukalova-Peck, J. 1978. Origin and evolution of insect wings and their relation to metamorphosis, as documented by the fossil record. *J. Morphol.* **156**, 53-126
- Nijhout HF. 1994 *Insect Hormones*. Princeton, NJ: Princeton Univ. Press
- Nov'ak VJA. 1969. Morphological analysis of the effects of juvenile hormone analogues and other morphologically active substances on embryos of *Schistocerca gregaria* Forsk. *J. Embryol. Exp. Morphol.* 21:1-21
- Restifo LL, Wilson TG. A juvenile hormone agonist reveals distinct developmental pathways mediated by ecdysone-inducible Broad Complex transcription factors *DEVELOPMENTAL GENETICS* 22 (2): 141-159 1998
- Riddiford LM, Cherbas P, Truman JW. 2001. Ecdysone receptors and their biological actions. *Vitam. Horm.* 60:1-73
- Riddiford LM. 1978. Ecdysone-induced change in cellular commitment of the epidermis of the tobacco hornworm, *Manduca sexta*, at the initiation of metamorphosis. *Gen. Comp. Endocrinol.* 34:438-46
- Riddiford LM. 1995. Hormonal regulation of gene expression during lepidopteran development. In *Molecular Model Systems in the Lepidoptera*, ed.

- Schubiger M, Wade AA, Carney GE, Truman JW, Bender M. *Drosophila* EcR-B ecdysone receptor isoforms are required for larval molting and for neuron remodeling during metamorphosis. *DEVELOPMENT* 125 (11): 2053-2062 JUN 1998
- Shemshedini L, Wilson TG. 1990. Resistance to juvenile hormone and an insect growth regulator in *Drosophila* is associated with an altered cytosolic juvenile hormone-binding protein. *Proc. Natl. Acad. Sci. USA* 87:2002-76
- Truman JW. 1971. Hour-Glass Behavior Of Circadian Clock Controlling Ecdysis Of Silkworm *Antheraea-pernyi*. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 68 (3): 595-& 1971
- Truman JW, Riddiford LM. 1999. The origins of insect metamorphosis. *Nature* 410:447-52
- Wang S-F, Li C, Zhu J, Miura K, Miksicek RJ, Raikhel AS. 2000. Differential expression and regulation by 20-hydroxyecdysone of mosquito ultraspiracle isoforms. *Dev. Biol.* 218:99-113
- Wilson TG, Ashok M. 1998. Insecticide resistance resulting from an absence of target-site gene product. *Proc. Natl. Acad. Sci. USA* 95:140-44
- Wilson TG, Fabian J. 1986. A *Drosophila melanogaster* mutant resistant to a chemical analog of juvenile hormone. *Dev. Biol.* 118:190-201
- Zdarek, J. 1985. Regulation of pupariation in flies. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, ed. G.A. Kerkut and L.I. Gilbert vol 8:301-333. Pergamon, New York
-