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JEB Classics

THE CONTROL OF METAMORPHOSIS IN THE KISSING BUG

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THE DETERMINATION OF CHARACTERS AT METAMORPHOSIS IN *RHODNIUS PROLIXUS* (HEMIPTERA)

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(With One Plate and Twelve Text-figures)

Two factors have been recognized as regulating moulting and metamorphosis in *Rhodnius prolixus*: a "moulting hormone" and a so-called "inhibitory hormone" (Wigglesworth, 1934, 1936).

(i) *Moulting hormone*. The secretion of this factor is brought about by the stretching of the abdomen by a large meal of blood. Such distension apparently provides a nervous stimulus that is conveyed to the brain. The hormone seems to be secreted in the head, and it was suggested that the corpus allatum is the source of it. But no satisfactory experimental evidence could be produced for this view, which will be refuted in the present paper. The moulting hormone circulates freely in the blood, and after a "critical period" (some 5 days after feeding in the 4th stage, some 7 days in the 5th stage), if the blood is transfused through a capillary tube into other nymphs deprived of the source of hormone (by decapitation while unfed or shortly after feeding), these will be induced to moult. The moulting hormone is present in the blood of each of the five nymphal stages; it initiates cell division, which is followed by the deposition of a new cuticle. In the presence of this factor alone, the structural characters of this cuticle, no matter which instar has been caused to moult, are those of the adult insect.

(ii) *Inhibitory hormone*. This factor becomes operative only when growth and moulting have already been initiated by the moulting hormone. It is present in the blood of the first four nymphal stages, and has the effect of preventing the development of the imaginal characters. (It is not inhibitory in the sense of opposing the action of the moulting hormone, as some authors have assumed). In the presence of this hormone (in addition to the moulting hormone) the insect develops nymphal characters once more when it moults. The inhibitory hormone is secreted by the corpus allatum in the first four nymphal stages. This gland appears to produce it when exposed to the action of the moulting hormone; nervous stimulation is not required. Further, the inhibitory action of the corpus allatum is greater in the earlier nymphal stages than in the later: the gland in the 3rd stage will prevent a

Lynn Riddiford writes about Sir Vincent B. Wigglesworth's classic paper on the control of metamorphosis entitled 'The determination of characters at metamorphosis in Rhodnius prolixus (Hemiptera)'. A copy of the paper can be obtained at http://jeb.biologists.org/cgi/reprint/17/2/201.

Sir Vincent B. Wigglesworth (1899–1994) was the father of insect physiology and more particularly of insect endocrinology. He published some 264 papers (26 of them in the *Journal of Experimental Biology*) on many different aspects of insect physiology, and all but 18 of his papers were sole-authored. His work on *Rhodnius prolixus*, the kissing bug, beginning in 1933, was seminal to the field of insect endocrinology as he was the first to discover the hormone that prevented metamorphosis, juvenile hormone (JH), before going on to elucidate some of its roles.

This JEB Classic paper 'The determination of characters at metamorphosis in *Rhodnius prolixus* (Hemiptera)' (Wigglesworth, 1940) represents a major contribution to insect endocrinology. It renames his previously described 'inhibitory hormone' as 'juvenile hormone' since he found that it could cause reversal of metamorphosis of parts of the adult epidermis. Moreover, the paper revises and extends some of his earlier ideas about the control of molting in *Rhodnius* and defines the role of JH in the cellular response to molting in very modern terms, despite the lack of knowledge at the time of how developmental switches occur.

Rhodnius was in many ways the ideal insect in which to study the physiology of molting and metamorphosis because molting only occurs when the nymph is fed a blood meal. Metamorphosis normally occurs at the end of the fifth nymphal instar, or stage. Therefore, the onset of the processes leading to the molt can be easily manipulated and utilized in a systematic fashion to define the processes occurring during the molt leading to the formation of the new cuticle and the subsequent ecdysis, or shedding of the old cuticle. Moreover, Wigglesworth used this information to his advantage in determining the hormonal regulation of both molting and metamorphosis by the use of a technique called parabiosis, in which two or more insects were surgically joined together so that they had a common hemolymph (blood) supply. Thus, a circulating hormone(s) produced by one at a certain stage could affect the tissues of the second, when the hormone was not normally present.

In 1934 Wigglesworth had concluded, based on a histological analysis, that the corpora allata, a pair of glands in the head behind the brain, secreted the molting hormone, which appeared in the hemolymph after feeding (Wigglesworth, 1934). In the first section of this 1940 paper, Wigglesworth tests that hypothesis by implanting the corpora allata, the brain, and/or the sympathetic and the subesophageal ganglia from fed, molting animals into nymphs decapitated just after feeding. Only those receiving brain implants molted, and preliminary experiments showed that the dorsal half of the brain was implicated in this process. Because Hanstrom in 1938 had described some large, apparently secretory neurons in this region of the brain (Hanström, 1938), Wigglesworth asked if these cells showed any histochemical changes upon feeding, but found none. Despite this evidence, and findings in Diptera by Fraenkel (Fraenkel, 1935) and others that the molting hormone was secreted by the ring gland in the thorax, Wigglesworth concluded that the *Rhodnius* brain secreted the molting hormone. This conclusion was wrong. Much later he showed that the ventral glands (also known as the prothoracic glands) in the thorax released the molting hormone (Wigglesworth, 1952) following on from studies in Lepidoptera, which had found that the brain activated the prothoracic glands to cause the molt (Fukuda, 1944; Williams, 1947).

We now know that the lateral neurosecretory cells in this dorsal region of the brain secrete the prothoracicotropic hormone that stimulates the prothoracic (also known as ventral) glands to secrete the molting hormone ecdysone (Steel and Vafopoulou, 2006). The medial

neurosecretory cells in *Drosophila* (Rulison et al., 2002) and *Bombyx* (Masumara et al., 2000) secrete insulin-like peptide(s) in response to sugar feeding. Likely these medial cells in *Rhodnius* also contain insulin-like peptides that are important for the molting following feeding (Sevala et al., 1992). The histochemical methods used by Hanstrom and Wigglesworth for the cell bodies in the brain would likely not show depletion since release is actually from the axon tips in the corpora cardiaca, a gland found behind the brain, and synthesis in the cell bodies continues unabated.

Most of the paper is devoted to a thorough analysis of the cellular changes that occur during a nymphal molt in the presence of the 'inhibitory hormone' *versus* changes that occur during the adult molt in its absence. Wigglesworth was particularly interested in the distinctly different patterns of mitoses in the epidermis in the two molts and the timing of this pattern change during the molt. Therefore, he parabiosed fed final instar nymphs each day after feeding to penultimate-stage nymphs that were undergoing the nymphal molt to the final instar under control of high levels of inhibitory hormone, to determine when this hormone could no longer affect the type of cuticle produced. He found that the switch-over is gradual within and between segments and variable in rate in different areas. Importantly, he also found that within a single cell some processes were determined as adult before others so that, for instance, a cell could no longer secrete the special nymphal plaque cuticle but was still able to secrete the nymphal unpigmented, stellate-folded epicuticle. He showed that the commitment to adult differentiation was reversible to some extent during the initial cellular changes, but once the epidermis began secreting cuticle, the type of cuticle could no longer be altered.

These studies were influential in my laboratory's studies of the regulation of molting and pupal commitment of the epidermal cells of the tobacco hornworm *Manduca sexta* by ecdysone and juvenile hormone, where we found a similar change in patterning of the ability of the epidermis to molt depending on whether it was a larval or the pupal molt (Truman et al., 1974). We now know that this patterning during a larval molt reflects the response of the cells in different regions to ecdysone, as signaled by the appearance of one of the ecdysone-induced transcription factors, MHR3 (Langelan et al., 2000). When the cells respond to ecdysone in the absence of JH, a new pupal-specific transcription factor Broad is induced in the pattern that mirrors the loss of the capacity to make a larval cuticle and the acquisition of the ability to make a pupal cuticle in the presence of JH (Zhou and Riddiford, 2001). How these patterns of appearance of ecdysone-induced transcription factors are controlled by the underlying patterning genes is unknown. In the milkweed bug *Oncopeltus fasciatus*, Broad appears during the embryonic formation of the nymph and is necessary for progressive nymphal morphogenesis, including changes in pigmentation, then disappears at metamorphosis to the adult (Erezyilmaz et al., 2006). Could it be that the switching off of Broad in a patterned manner is the key to the loss of the ability to make nymphal cuticle in *Rhodnius*?

An important but less remembered part of this paper shows that not only can adults molt when given sufficient ecdysone, but also that in *Rhodnius* one can obtain partial reversion to nymphal cuticle characteristics during an induced adult molt if there is sufficient JH present. This latter finding is quite surprising in terms of what we know about the irreversibility of metamorphosis in most holometabolous insects (those with complete metamorphosis). A rare exception to this irreversibility is some of the pupal epidermal cells of the waxmoth *Galleria*, which revert to their larval form when implanted into young larvae (Piepho, 1942). This conundrum deserves re-examination with modern cellular and molecular tools.

These findings led Wigglesworth to formulate a model for the control of metamorphosis whereby the molting hormone initiates cuticle formation. The presence of the inhibitory hormone was thought to activate the 'nymphal system of enzymes, acting 'perhaps as a "co-enzyme" ', and to suppress the production of adult structures since molting occurred more rapidly. Adult cuticle formed due to the activation of the imaginal enzyme system in the absence of the inhibitory hormone. Although many more cellular and molecular details of these processes are known today, the basic idea that JH is guiding the molecular action of ecdysone by preventing switching-on of metamorphic-specific genes still holds. Whether it activates nymphal- or larval-specific genes for the molts during the immature growth phase is still not known, however, and is the focus of further research.

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