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Theoretical aspects of the action of Juvenile Hormone

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The genetic construction manual

The construction manual for building an holometabolous insect is subdivided into three successive chapters. The first chapter gives instructions for transforming the egg into a larva. Days, weeks, or months later, the second chapter tells how to change the larva into an essentially new organism, the pupa. Then, within the closed system of the pupa, the third and final chapter prescribes the reworking of the cells and tissues to form the adult. This analogy serves to emphasize that the more advanced forms of metamorphosis involve the turning-on and acting-out of successive batches of genetic information. One may think of the genome as being subdivided into three different "gene-sets" corresponding to the successive chapters in the construction manual.

We do not underestimate the complexity of the mechanisms which operate within each of the three gene-sets to control the orderly playback and implementation of genetic information in specific cell types. These controlling mechanisms are presumably no less complicated in insects than in human beings. Be that as it may, we suspect that juvenile hormone (JH) has little to do with specifying detailed instructions to particular differentiated cells. Virtually everything we know about this hormone suggests that it is involved in gene-switching on a massive scale such, for example, as would be required for the turning-off of one gene-set and the turning-on of another — in short, that it controls sequential polymorphism (Wigglesworth, 1970; Ashburner, 1970).

Replication and recognition of the gene-sets

To provide a mechanism for this massive gene-switching, we suggest that the three gene-sets are distinguishable by information encoded in

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the genome and, moreover, that the members of each set are identifiable in terms of a certain nucleotide sequence which serves as their "promoters". According to this scheme, the promoters of the larval gene-set have template specificity for an RNA polymerase peculiar to the larva. In like manner, the promoters of the pupal gene-set will be recognized by a pupal RNA polymerase ; the promoters of the adult gene-set, by an adult RNA polymerase.

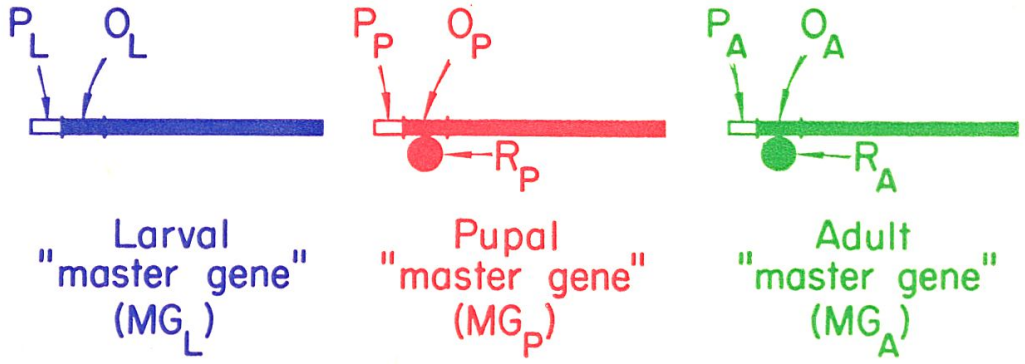
To account for those enzymes and structural proteins which are synthesized at all stages in development, we conjecture that the corresponding genes may be represented in all three gene-sets. Alternatively, it is possible that some or all of these constitutive genes have promoters recognized by a species of RNA polymerase present at all stages. Still another possibility is that these genes possess all three promoter sequences and may therefore be recognized by all three polymerases. Similarly, specific genes may participate in any two gene-sets ; the sets may be overlapping.

Since, according to these assumptions, the gene-sets and their corresponding promoters are encoded in the DNA, it follows that they are replicated in each mitotic cycle. Consequently, we presume that all three sets are defined in all cells throughout embryonic and post-embryonic development.

By virtue of the mysterious determinative events which begin in the early embryo and continue on a decreasing scale into the post-embryonic period, the cells are programmed for the specific roles which they and their progeny will play in the future larva, pupa, and adult. We offer no precise molecular explanation of these happenings which, in more ways than one, constitute the central problem of developmental biology (Geigy, 1931 ; Lüscher, 1944 ; Bodenstein, 1953 ; Goldman and Setlow, 1956 ; Williams, 1958 ; Gehring, 1970 ; Lawrence, 1970 ; Wigglesworth, 1970 ; Wildermuth, 1970). Thankfully, we are not obliged to do so since there is not the slightest evidence that JH plays any role in the spatial programming of cells or the prepatterning of the insect as a whole. For our present purpose the determinative events are nevertheless of great interest since they prescribe on a cell-by-cell basis those parts of each gene-set that can be read and those parts that cannot be read.

Juvenile hormone as a co-repressor of the master regulatory genes

We propose a model of JH action in which each of the three gene-sets is presumed to be under the control of a certain "master regulatory gene" which we have called MG_L, MG_P, and MG_A, respectively. As diagrammed in Figure 1, we suggest that the operator (O_P) of MG_P is subject to inhibition by a repressor (R_P) which is active only in the



R_P (the repressor of the "pupal master gene") is active only in the presence of a high titer of JH.

R_A (the repressor of the "adult master gene") remains active in the presence of a low titer of JH.

Both repressors are inactive in the total absence of JH.

Fig. 1

Figures 1–5. — Diagrammatic representation of a hypothetical scheme of gene control during insect growth and metamorphosis. The genome of each cell is viewed as subdivisible into three gene-sets, each controlled by its own master regulatory gene. Components of the larval gene-set are shown in blue, of the pupal gene-set in red, and of the adult gene-set in green. Juvenile hormone (JH) is depicted as a co-repressor which participates in the control of the master regulatory genes of the pupal and adult gene-sets. Abbreviations not defined in the diagrams are as follows: P_L, P_P, and P_A are the promoters of the corresponding master genes; O_L, O_P, and O_A are the operators. σ_C is the sigma factor of a constitutive RNA polymerase which recognizes the promoter sequences of all three master genes. mRNA is depicted as a messenger ribonucleic acid transcribed from a master gene and coding for the sigma factor(s) of the corresponding gene-set. r_L and r_P are repressors which bind to the operators of the larval and pupal master genes irrespective of the titer of JH. GP_L, GP_P, and GP_A are gene products which code for the proteins of the larval, pupal, and adult gene-sets, respectively. For further details, see text.

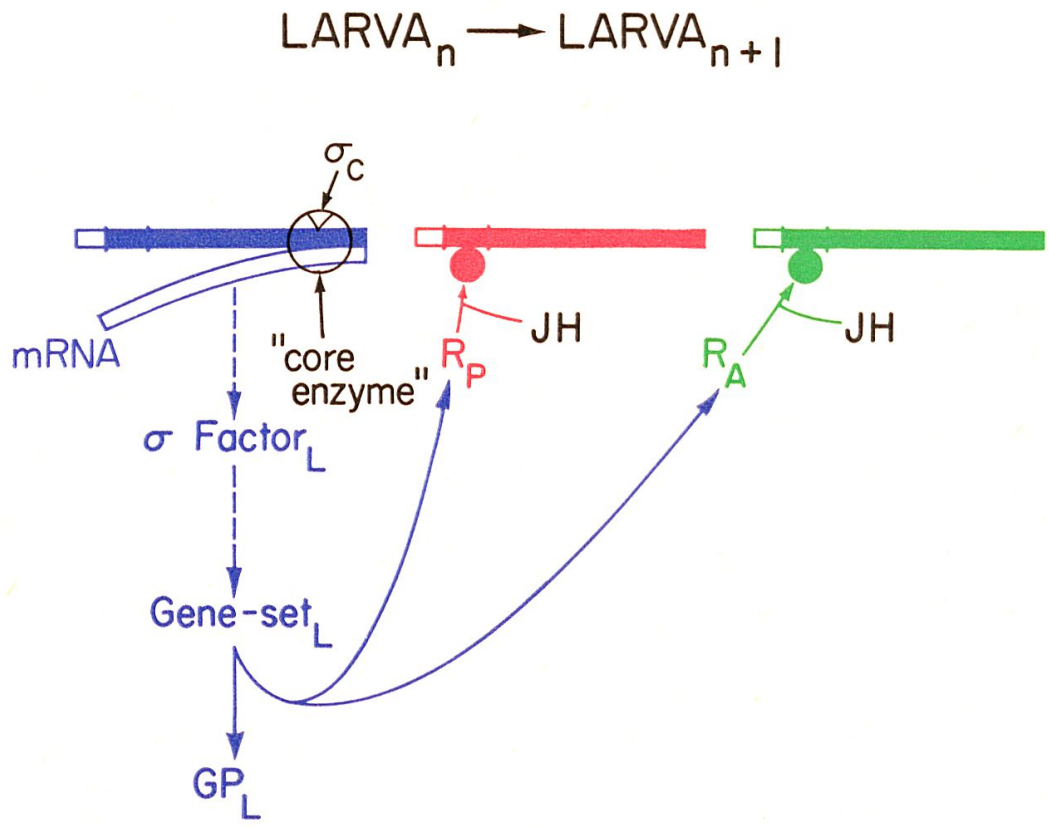

LARVA$_n$ → LARVA$_{n+1}$
σ_C
mRNA
"core enzyme"
R_P
JH
R_A
JH
σ Factor$_L$
Gene-set$_L$
GP$_L$

Fig. 2

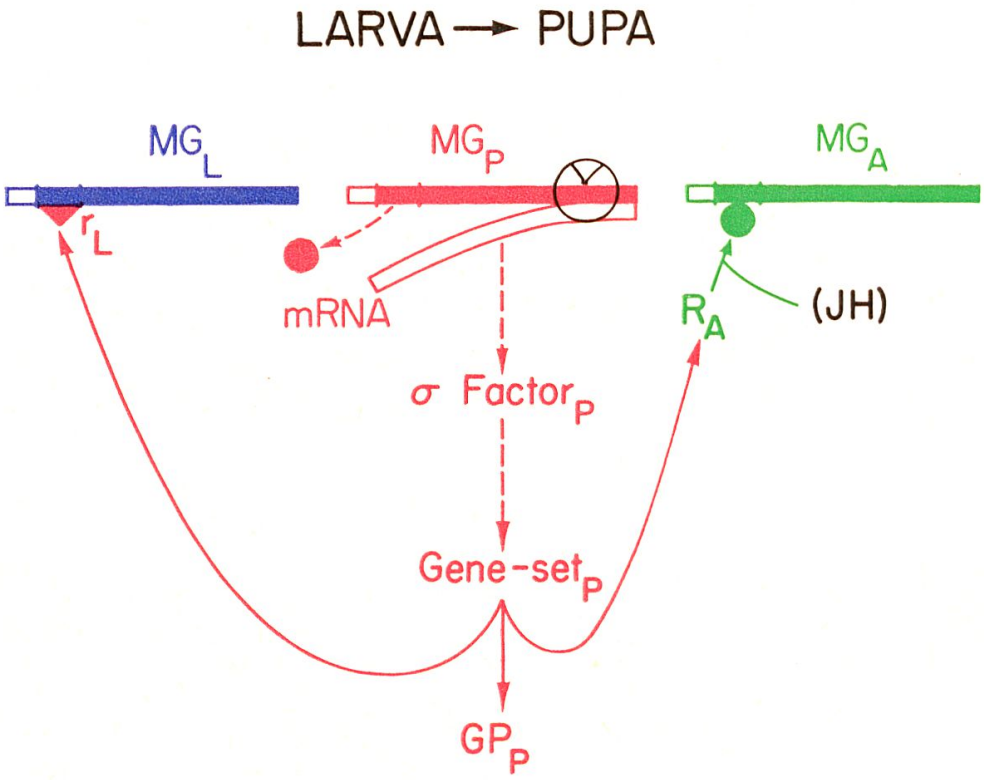

LARVA → PUPA
MG$_L$
MG$_P$
MG$_A$
r_L
mRNA
R_A
(JH)
σ Factor$_P$
Gene-set$_P$
GP$_P$

Fig. 3

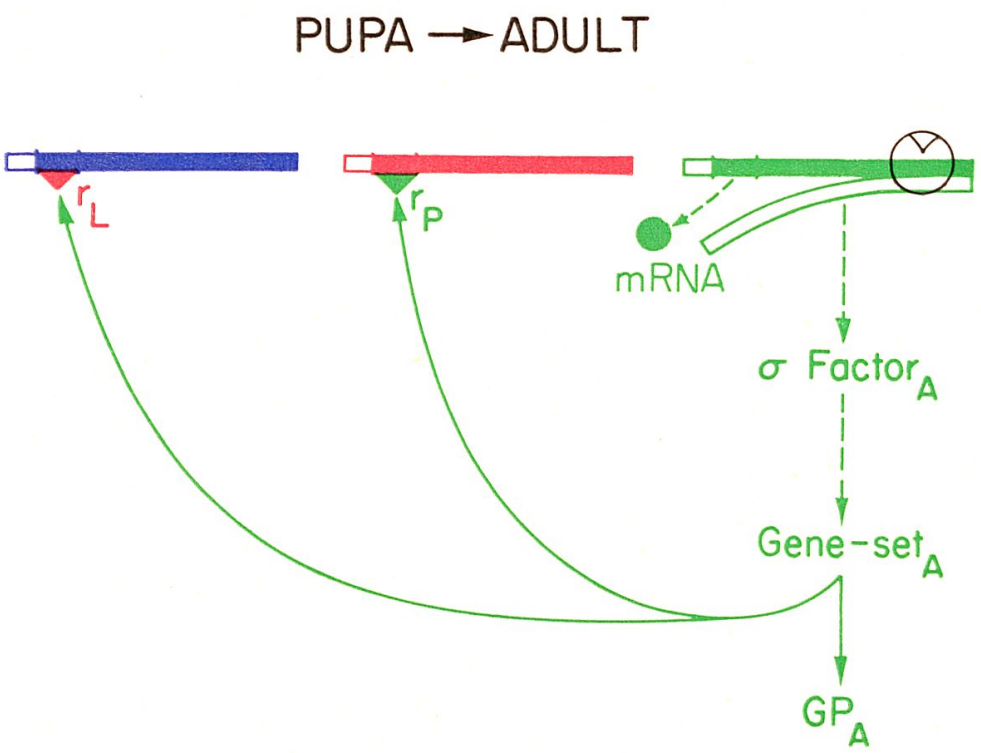

PUPA → ADULT
r_L
r_P
mRNA
σ Factor$_A$
Gene-set$_A$
GP$_A$

Fig. 4

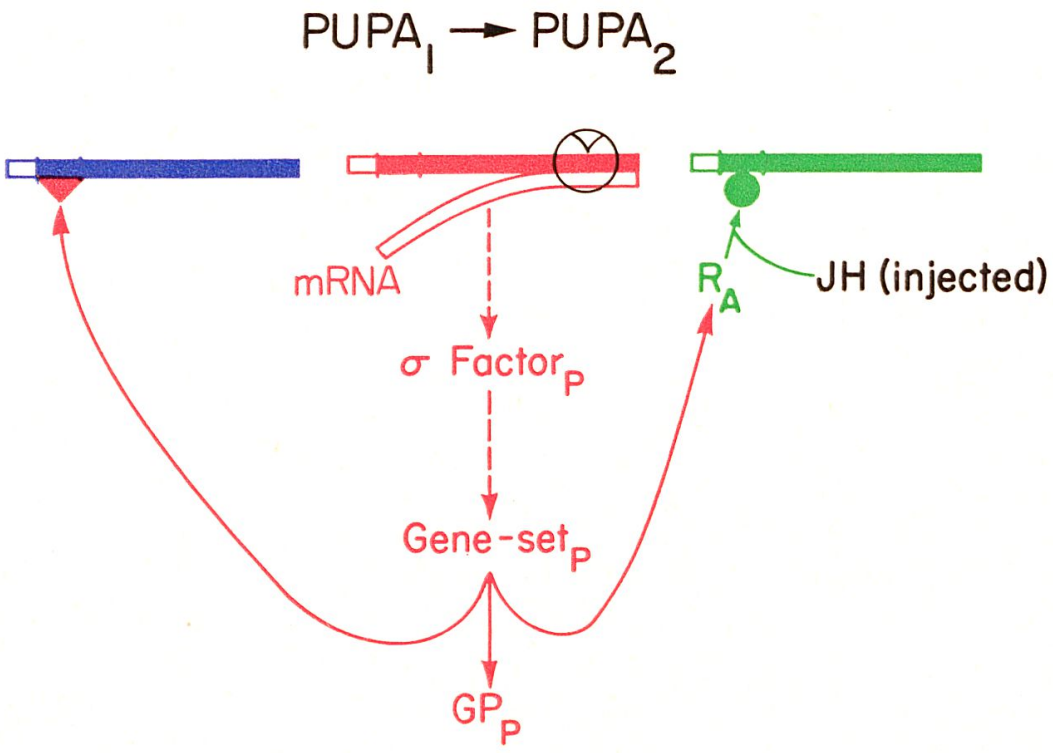

PUPA$_1$ → PUPA$_2$
mRNA
σ Factor$_P$
Gene-set$_P$
GP$_P$
R_A
JH (injected)

Fig. 5

presence of a high titer [1] of JH. So, also, the operator (O_A) of MG_A is subject to inhibition by another repressor (R_A), the important difference being that this repressor remains active in the presence of even a low titer of JH. Both repressors become inactive in the absence of JH. Thus, according to this model, JH is involved in the "negative control" of transcription of the master regulatory genes.

The most economical hypothesis is to regard JH as a co-repressor which activates R_P and R_A by binding directly to them. Under this point-of-view, R_P would have a lower affinity for JH than does R_A. Alternatively, JH might affect the repressors in an indirect manner — for example, by binding to JH receptors elsewhere in the cells and thereby bringing into play one or more "second messengers" which react with the repressors.

Gene-switching in unicellular metamorphosis

Manifestly, the model up to this point is a simple adaptation of Jacob-Monod principles as derived from studies of gene-switching in bacteria. Further aspects of the model lean heavily upon recent findings of positive control, which have likewise been derived from investigations of microbial systems. We refer in particular to the pioneering studies which Losick and his collaborators have carried out on sporulation in *B. subtilis* (Losick and Sonenshein, 1969 ; Sonenshein and Losick, 1970 ; Losick *et al.*, 1970a ; Losick *et al.*, 1970b).

The broad implications of these studies have already been lucidly set forth by Watson (1970). In brief, the environmentally induced transformation of the vegetative bacillus into a spore proves to be a *bona fide* metamorphosis involving the shut-down of the gene-set responsible for vegetative growth and, simultaneously, the activation of a second gene-set which directs the new synthetic operations required for the formation of the spore. Losick and his co-workers have evidence that the unicellular metamorphosis implicit in sporulation involves the synthesis of a new sigma (σ) factor which differs from that used by the vegetative cell (Losick, personal communication). The new σ combines with the "core enzyme" of RNA polymerase to form a new polymerase with specific template affinity for the promoter sites of the gene-set for making the spore. Simultaneously, the core enzyme is altered so that it loses its affinity for the old σ of the vegetative cell. In this manner the old gene-set is turned-off and the new gene-set is turned-on.

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[1] There is substantial evidence that hormones produce covert effects which are cumulative within the cells and tissues (Ohtaki *et al.*, 1968 ; Zdarek and Fraenkel, 1970). Therefore, here and elsewhere in this manuscript, the term "titer" does not necessarily imply the presence of any particular concentration of the hormone itself.

We do not know whether this scheme of positive control of transcription applies to eucaryotic cells. However, Roeder and Rutter (1969; 1970a; 1970b) have demonstrated two RNA polymerase activities in rat liver nuclei (apparently differing in subunit structure; Rutter, personal communication) and a shifting balance of three distinct RNA polymerase activities in developing sea urchin embryos. No studies have thus far been reported as to the presence of σ factors in insect cells, but specificity factors are attractive candidates for controlling the template specificities of the successive RNA polymerases which we postulate. In any case, the essence of the model is positive control of gene-sets by master regulatory genes, themselves under hormonally-influenced negative control. Although we specify that each master regulatory gene codes for one (or more) specific σ factors, we would be equally happy if evidence were found that it codes for entirely new RNA polymerases (as in the case of T_7 infection; Chamberlin *et al.*, 1970).

Larval growth without metamorphosis

The recurrent molting of immature larval insects is known to take place when ecdysone is secreted in the presence of a high titer of JH. As we suggest in Figure 2, JH acts in conjunction with the repressors, R_P and R_A, to repress the master regulatory genes, MG_P and MG_A. By contrast, MG_L exists in the active state and is therefore subject to transcription by RNA polymerase. As indicated in Figure 2, we suggest that this polymerase makes use of a "constitutive σ factor" (σ_C) with template specificity for the promoters of all three master regulatory genes.

According to the scheme diagrammed in Figure 2, MG_L continues to be transcribed as a messenger RNA which codes for the synthesis of σ $Factor_L$. The latter combines with pre-existing core enzyme to form an RNA polymerase with template specificity for the promoters of genes in the larval gene-set. Consequently, $Gene\text{-}set_L$ remains operational and continues to be transcribed as gene products (GP_L) which code for the enzymes and other proteins of the larva. Among these proteins are the repressors, R_P and R_A. So, as long as a high titer of JH is present, $Gene\text{-}set_L$ remains turned-on and $Gene\text{-}set_P$ and $Gene\text{-}set_A$ remain locked-off.

The larval-pupal transformation

In the mature larva the corpora allata curtail their activity so that pupation takes place in the presence of a high titer of ecdysone and a low but finite titer of JH. That being so, the repressor, R_P, loses its affinity for MG_P and falls off (Fig. 3). The net result is that the constitutive RNA polymerase can proceed to transcribe MG_P as a mRNA coding for the new σ $Factor_P$ whose template specificity is for the

promoters of Gene-set$_P$. The latter is thereby turned-on, provoking the transcription of gene products (GP_P) which code for synthetic operations of the pupal type. These operations include the continued synthesis of the repressor, R_A, and the synthesis of a new repressor (r_L). The latter differs from pre-existing repressors in that its affinity for the operator of MG_L does not depend on the presence of JH.

In summary, the declining titer of JH within the mature larva allows Gene-set$_P$ to be turned-on and MG_L and MG_A to be turned-off.

The pupal-adult transformation

The corpora allata are known to be inactive in the pupa as well as throughout the first two-thirds of adult development. The latter therefore takes place when ecdysone acts in the absence of JH. The consequences are diagrammed in Figure 4.

In the absence of JH, the repressor, R_A, loses its affinity for MG_A and falls off. This leads to the transcription of MG_A, the synthesis of σ Factor$_A$, and the activation of Gene-set$_A$. The latter codes for gene products of the adult type (GP_A), including templates of repressors r_L and r_P whose activity does not depend on JH. Thus, Gene-set$_A$ is turned-on and Gene-set$_L$ and Gene-set$_P$ are locked-off.

Tests of the model

The model can account for nearly all of the developmental aberrations which one can induce by the administration of JH. For example, it is well known that by the injection of JH one can block the pupal-adult transformation and provoke the formation of a second pupa. Figure 5 illustrates how the model can explain this result. The injected hormone activates the repressor, R_A, thereby blocking the transcription of MG_A. Since MG_L is repressed by a JH-independent repressor, the presumably unstable pupal repressor, R_P, cannot be replenished, and MG_P remains active regardless of how much JH is supplied. In analogous manner, injected JH could block the larval-pupal transformation by preventing the dissociation of the repressor, R_P, from MG_P. In this case the observed result is the retention of larval characters due to the sustained activity of Gene-set$_L$ as illustrated in Figure 2.

The model can also account for the precocious metamorphosis observed in immature larvae after the excision of corpora allata. Precocious pupation, as illustrated in Figure 3, would take place if the succeeding molt occurred in the presence of a low but finite residual titer of JH. This is the typical result observed, for example, in *Bombyx mori* (Fukuda, 1944). But if the succeeding larval molt takes place in the absence of residual JH, one would anticipate a bizarre state-of-affairs — namely, the simultaneous activation of both pupal and adult gene-sets followed by the shut-down of MG_P. This could account for

the faulty metamorphosis of mature Cecropia silkworms after the excision of corpora allata (Williams, 1961). Thus, as illustrated in Figure 6, the typical result is the formation of pupal-adult intermediates in which the abdomen shows pupal characters, but numerous organs, including the eyes, antennae, legs, wings, genitalia, and gonads have differentiated adult characters.

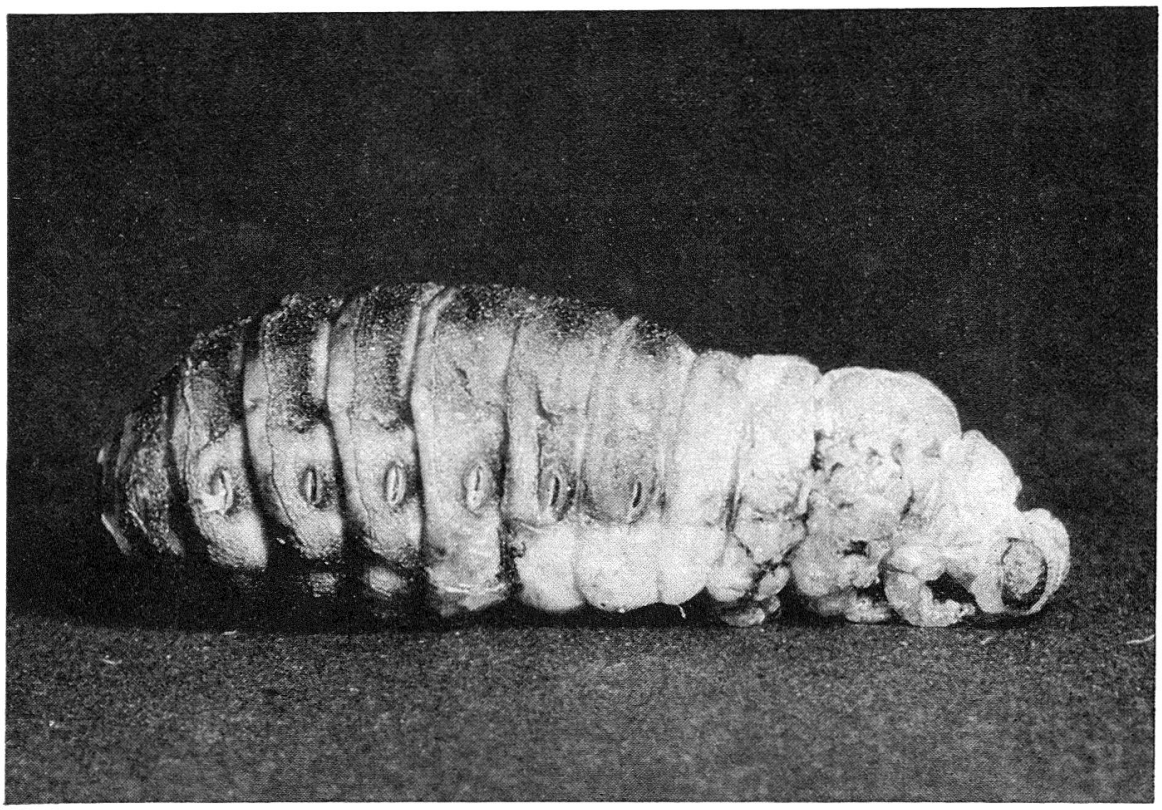

Figure 6. — By excision of the corpora allata during the final larval instar, this *Cecropia* silkworm was caused to undergo pupation in the absence of JH. A large number of larval tissues and organs have undergone precocious adult differentiation without traversing the pupal stage. (From Williams, 1961).

The model does not account for two developmental phenomena which have generally been attributed to JH. For example, it cannot explain the ability of JH to trigger the deposition of yolk in the eggs of certain species of adult insects. This so-called gonadotropic function of JH appears to be peculiar to species in which the adults are long-lived. Non-feeding, short-lived adults such, for example, as the Cecropia silkmoth, can mature their eggs in the absence of JH (Williams, 1959). For these several reasons we suspect that during evolution JH was "captured" for the ancillary gonadotropic function in certain adult insects and that its mode of action in adults may be substantially different from its primary role in controlling metamorphosis.

The model, at least in its present form, also fails to account for the "reversal of metamorphosis" — the occasional reappearance of traces of juvenile characters in pupae or adults which are caused to molt in the presence of JH. We do not doubt the validity of the experimental observations as reported in the literature (for summary, see Wigglesworth, 1970). However, it is clear that reversal of metamorphosis is exceedingly difficult to accomplish ; in silkmoths we have not substantiated any trace of this phenomenon to date. In fact, under most circumstances undirectionality is one of the most striking aspects of metamorphosis. An attractive novel feature of the present model is that it explains unidirectionality.

Prospects

So, in summary, we have proposed what we believe to be the most parsimonious model of JH action which can account for all, or nearly all, of the firmly established experimental findings. The worth of the model is to be measured, not so much by its certain inadequacies, as by its ability to suggest new avenues of approach to the comprehension of insect metamorphosis.

One such approach is to search among the hundreds of presently known lethal strains of *Drosophila* (Hadorn, 1961) for aberrations in development which may qualify as mutations of one of the hypothetical master regulatory genes. A mutation of this sort would probably be recessive and, when homozygous, would presumably inactivate one of the postulated σ factors, thereby preventing the transcription of an entire gene-set.

By attention to the diagrams which we have presented in the several figures, one may predict the consequences of such mutations. Thus, a mutation of MG_L would permit the proliferation of embryonic cells while blocking any trace of the formation of the first instar larva. In like manner, a mutation of MG_P would block pupation ; one might anticipate a prolongation of larval life including one or more extra larval molts or, indeed, the precocious appearance of adult characters.

A mutation of MG_A would probably be most easily detected. MG_P would continue to be transcribed to produce one or more extra pupal instars without any trace of the appearance of adult characters.

On the assumption that such mutants already exist or can be produced by appropriate tactics, the task of screening for them remains formidable. Yet this obstacle can undoubtedly be surmounted. Once presumptive mutant tissues and organs are available, it will be necessary to show that they cannot be "rescued" by implanting them into non-mutant hosts.

The model also encourages a search for the site of action of JH. This line of inquiry has now become feasible thanks to the synthesis of hotly labeled JH. If injected hormone reacts directly with the

hypothetical repressors of the master regulatory genes, one would expect to recover a significant portion of the label in the nuclear fraction of homogenates. Most importantly, nuclear label should be bound relatively tightly to a protein fraction, and the JH-protein complex, should be specifically retained on insect-DNA columns. By contrast, a preferential binding to the non-nuclear fraction would argue in favor of an indirect action of JH.

Finally, we direct attention to a central requirement of the model — namely, that multiple RNA polymerases or σ factors be demonstrated in insects. Moreover, these polymerases or sigmas must be shown to undergo systematic change during successive phases of metamorphosis. This aspect of the model appears to be immediately accessible to direct experimental attack by methods worked out on previously-mentioned systems. That being so, a detailed study of insect RNA polymerases and a search for σ factors have already been initiated at the Harvard laboratory.

* * *

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