A transcription factor that enables metamorphosis

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Animals with complex life cycles undergo an obligatory transition from one discrete phenotype to another in a process we call metamorphosis. Such life histories are a special form of polyphenism (1). Metamorphosis is among the most successful evolutionary innovations and was probably a critical adaptive trait in early animal evolution (2). As noted by Darwin (3), the possession of distinct forms allows the metamorphosing animal to adapt independently to more than one ecological niche during its life. Higher insects (Holometabola) display metamorphosis in its most dramatic form in a process involving transformation of a morphologically simplified larval form first into a pupa, then into an adult. Sometimes termed “indirect development” (as opposed to hemimetabolous “direct development”), this is “complete metamorphosis,” in which the holometabolous larval form does not resemble the adult at all (4, 5). In the larva, cells destined to produce most of the adult body are wholly or partially set aside early in development as imaginal discs and adult structures are not formed until the pupal stage. The ensuing transition is inevitably complex, often taking as long as all the larval stages put together. Complete metamorphosis evolved ~400 Ma and today insects undergoing this form of metamorphosis are the most speciose of all animals, probably accounting for >50% of all animal biodiversity (6).

In PNAS, a new paper by Truman and Riddiford (7) identifies a Drosophila gene that plays a key role in enabling complete metamorphosis by specifying the larval condition. The gene’s expression pattern supports the proposal that the larval stages of holometabolous insects represent a prolongation of embryonic development, an idea that can be traced back more than 2,300 y to Aristotle (8).

Because blueprints for all the successive body plans are encoded within the same genome, the radically different form and function of the three different holometabolous life stages is the outcome of the separately regulated expression of largely overlapping larval, pupal, and adult gene sets (9). This has evolved through “adaptive decoupling” (4, 10), in which the existence of separate developmental controls on traits expressed in the alternative phenotypes allows them to influence one life stage without exerting pleiotropic effects on others. The new paper (7) goes some way toward explaining how this is accomplished.

The decision of an insect to initiate metamorphosis has long been known to depend on a mixture of circulating hormones, the most important of which is juvenile hormone (JH), the “status quo” hormone in both hemimetabolous and holometabolous insects (5, 11–13). But because JH acts on almost every tissue of the insect at all life stages, if adaptive decoupling between different phenotypes is to be achieved, JH action must be channeled by the existence of stage-specific bottlenecks in the hormone’s signaling pathways, at which switching takes place between strongly differentiated downstream target gene expression patterns.

It is just over 50 y since a speculative discussion paper by Williams and Kafatos (14) proposed a theoretical mechanism for such developmental channeling of JH action. The component genes of the three (larval/pupal/adult) gene sets, they suggested, might each be regulated by a single stage-specific transcription factor; the three “master genes” would together constitute what would now be called a gene regulatory network (GRN) switching kernel (15) with the specific function of overseeing the transformation from one stable transcriptional state to the next. This metamorphic decision-making module would sit hierarchically above the subsidiary GRNs that actually direct the formation of the larval, pupal, or adult body (Fig. 1).

Some details of the original Williams–Kafatos model do not fit current paradigms for eukaryotic gene regulation (e.g., it was wrongly supposed that insects might possess bacteria-like sigma factors specific to larval, pupal, and adult stages), but the basic idea of switchable master genes affecting transcription of downstream targets has proved remarkably prescient. Importantly, the model recognized that the decision to progress from larva to pupa or from pupa to adult must in each case be decisive and irreversible; to achieve only a partial metamorphosis or to allow a backward step would probably be lethal. Thus, it was postulated that each of the three metamorphic master genes must strongly inhibit expression of the other two. The role of the hormonal control system would then be first to “tip” the balance from the larval to the pupal master gene, and then later to “tip” it again, this time from the pupal to the adult master gene. Once the decision to allow metamorphosis to progress to the next stage had been taken, it would be hard to reverse.

Two of the three master genes postulated in the Williams–Kafatos scheme have previously been identified as broad and ecysdione-inducible protein 93F (E93) (12, 16). These genes are not “merely” transcription factors; they also exert their effects by altering the chromatin landscape around genes known to be involved in metamorphosis (17). Satisfyingly, Broad and E93 have already been shown to be mutually inhibitory, exactly as predicted. But the factor responsible for supervising the formation of the larva, or indeed whether such a factor exists, has until now remained unknown.

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Fig. 1. Role of the molecular trinity GRN switching kernel in the metamorphosis of the fruit fly *D. melanogaster* as suggested by ref. 7. JH modulates the Chinmo-Broad-E93 GRN switching kernel, which tips the balance among the three master transcription factors to select larval, pupal, or adult sub-GRNs and thus the dependent gene sets. How JH acts to do this is unknown. Negative cross-talk between the three trinity genes ensures stability and smooth switching between alternative phenotypes. Dotted lines indicate inhibitory interactions still to be confirmed.

The missing piece of the puzzle has now been found (7). The larval master gene in *Drosophila melanogaster* has been identified as *chronologically inappropriate morphogenesis* (*chinmo*), which encodes a putative BTB-zinc finger nuclear DNA-binding transcription factor. The set of three master genes for specifying metamorphosis has been dubbed the “molecular trinity.” *chinmo* is not a newly discovered gene; it had previously been identified as essential for patterned neuronal development in *Drosophila* (18) as well playing a role in eye development, tumor formation, and male gametogenesis (19).

The evidence that *chinmo* is the long-sought larval master gene is convincing; Chinmo protein is expressed throughout late embryonic and larval stages in the cells of most *Drosophila* body tissues and disappears at exactly the expected time (i.e., when the insect becomes committed to form a pupa). This is true not only in larval tissues like salivary glands and epidermis but also in cells destined for an adult fate that are set aside early in development. Thus, if Chinmo’s function is to preside over the larval state, then this involves not only promoting a typically larval pattern of growth and development but also inhibiting it in imaginal discs. It is significant that the Chinmo decline occurs at the time of the weight threshold coinciding with an irreversible switch from typically larval renewal growth to pupal/adult differentiative growth, just when *broad* expression is first seen.

The original Williams-Kafatos hypothesis supposed that each of the three “master genes” acts to inhibit expression of the other two. Thus, experimentallyprovoked absence of Chinmo in embryos leads to premature differentiation. This causes drastic disruption of normal development and *chinmo*-negative mutants are embryonic lethals, dying toward the end of embryogenesis. Because of this, it is difficult to use knockout mutations to examine the gene’s function; instead, an RNA interference mosaic approach was used to remove Chinmo from the posterior halves of larval segments and imaginal discs, allowing comparison of cells that lacked Chinmo with their normal neighbors (7). Viable larval development continued for longer, enabling a more complete view of what *chinmo* does. The basic finding of this elegant analysis is that *chinmo* expression is absolutely required for normal development in both larval-destined tissues such as salivary gland and epidermis and also in adult-destined cells of imaginal disks.

The discovery of a larval “master gene” has implications for the evolutionary origin of complete metamorphosis. One hypothesis is that hemimetabolous nymphal stages were laterally transformed by developmental simplification into equivalent larval stages, the pupa being a specially modified terminal nymphal stage; this is the “Hinton 2” model (5, 20). The opposing view (“Berlese”) is that the key event was prolongation of typically embryonic development into postembryonic molting stages to produce the simplified body form of the holometabolous larva, while the original nymphal stages were lost with the exception of the terminal one, which was transformed into the pupa (11, 12). If Chinmo and Broad negatively regulate each other as in Fig. 1, then we would expect that Broad could not be expressed until Chinmo has disappeared. This is indeed what happens in both hemimetabolous and holometabolous insects. Whereas *chinmo* continues to be expressed in both late embryos and all larval stages of *Drosophila* (7), in the cockroach *Blattella germanica* Chinmo disappears early in embryogenesis, at about the time of embryonic germ-band formation (21). By contrast, Broad is not found in larval tissues in the fly and appears only as pupation approaches. The situation in the cockroach is more complicated; although multiple transcripts of *broad* are present throughout embryogenesis (some coincident with *chinmo*), some of these splice variants appear only at about the time of germ-band formation then remain high throughout the nymphal stages (22). This highlights the urgent need to understand the roles of Chinmo’s protein isoforms (there are two in *Drosophila*).

These observations unambiguously support the Berlese model for how complete metamorphosis evolved by indicating that the larval condition of the fly is the result of continuing to express Chinmo after hatching, thus suppressing Broad and thereby prolonging developmental processes originally associated with embryogenesis. Based as it is on only two species, our understanding of how the molecular trinity GRN was modified to allow the evolution of holometabolous metamorphosis is obviously incomplete. Comparative and experimental work on other insects is needed; it is clear that discovery of *chinmo*’s role in the GRN that regulates metamorphosis will give new impetus to research in insect development.
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