III.12 Defining the Anti-Metamorphic Action of Juvenile Hormone

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1. Introduction

The morphological changes which accompany insect metamorphosis are frequently dramatic. Yet metamorphosis is controlled by the interaction of but two hormones. An ecdysteroid initiates all molts and promotes metamorphosis unless a juvenoid is also present. In the presence of both juvenoids and ecdysteroids insects molt without changing metamorphic stage.

What is the basis for this temporal polymorphism? Is it as Wigglesworth suggested [1] and most investigators since have accepted [2–5], that the genome is divided into distinct larval, pupal, and adult gene sets? If so, the evolution of metamorphosis must have been accompanied by the establishment of such stage specific gene sets. This could have occurred by acquisition of new genes (possibly via gene duplication and subsequent alteration) or by delegation of genes into metamorphic sets by providing them with new stage specific controlling elements. Certainly recent recognition of embryonic forms of enzymes and structural proteins [6], indeed the entire fetal antigen story [7], lends support to the concept that different genes are used in different developmental stages.

The goal of our laboratory has been to describe and understand the biochemical differences among the three metamorphic stages of the giant silkmoth, *Hyalophora cecropia*. The original intent was to identify molecules which would serve as indicators of each metamorphic stage so we could pursue the basis of juvenoid action. Surprisingly, our data have revealed that the generally accepted paradigm of hormonally regulated stage specific gene sets has no foundation. It is these data and their implications for the hormonal control of metamorphosis which will be the basis of this communication.

The molecules we have chosen to examine are cuticular proteins. As we showed several years ago, these are made by the epidermis [8]. The epidermis as demonstrated in tissue culture is a direct target for both juvenoids and ecdysteroids [9–11]. In Cecropia, the cuticle of each stage has distinct gross and microscopic anatomy, and a unique color and texture. Thus, cuticular proteins are obvious candidates for organization into stage specific gene sets. Furthermore, in Lepidoptera continuous populations of cells in the abdomen make, in turn, larval, pupal, and adult cuticles. This is unlike the situation in *Drosophila* where, with the exception of histoblasts, cell populations change

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between two of the three metamorphic stages. In Lepidoptera, then, we are asking whether a fixed population of cells makes its successive cuticles from a succession of stage specific gene sets.

Our work mirrors the history of developments in electrophoresis. We began with simple tube gels for DISC electrophoresis, moved to SDS gels, then into slabs, and isoelectric focusing and now 2D gels and immunoelectrophoresis. Along the way we purified a dozen bands sufficiently to determine their amino acid compositions and to be assured that different bands are, for the most part, distinct proteins [8]. Multiplicity of cuticular protein bands on gels reflects a diversity of primary sequence [12].

The presence on a gel of bands from cuticles of two different metamorphic stages with identical electrophoretic mobilities can mean one of two things:

1. The same gene is used in both stages.
2. Different genes are used, but have "indistinguishable" products.

If the first possibility is assumed, one must explain how the same gene comes to be activated in different hormonal environments. With the second situation, this is easier to explain, for one could postulate that gene duplication had occurred, with subsequent modification of controlling regions. (Of course, one would never engage in such speculation without first demonstrating more similarity than coincidence of mobility.)

If different proteins are found in cuticles taken from a single anatomical region at different metamorphic stages, then two interpretations are possible:

1. The same genes are active in both metamorphic stages, but different pre- and/or post-transcriptional modifications have occurred.
2. Different genes are expressed, reflecting stage specificity.

If modifications can be ruled out, then one has evidence for stage specific gene sets. Suppose, however, that examination of cuticular proteins from several anatomical regions revealed that proteins previously identified as belonging to a particular metamorphic stage were expressed in another stage. Perhaps then the proper interpretation would be that the cuticular properties (functions) of an anatomical region are more of a determinant of gene expression than metamorphic stage.

We will summarize recent work from our laboratory which shows that distinct sets of genes are not set aside for exclusive use in a single metamorphic stage. Rather, functional requirements appear to dictate which proteins will be secreted into the cuticle.

2. Methods and Guide to Interpretation

One needs variants as well as breeding tests for genetic analyses. Fortunately, we can meet these requirements. There are four species in the Hyalophora genus of giant North American silkworms, and we have used two of them, the standard Hyalophora cecropia and its more western and slightly smaller relative, H. gloveri. They will mate and form viable hybrids. Most important, species-specific variants in a limited number of cuticular proteins are seen when guanidine extracts are analyzed on isoelectric focusing slab gels.

Our analysis in this paper will emphasize a homologous set of proteins in the very acidic region of the gel, one from Cecropia and the other from Gloveri. Each species