site of class II MHC molecules is the homologous loop — residues 137–143 of the class II β-chain — to that of class I which interacts with CD8. König *et al.*[10] establish this point by mutagenesis of the β-chain; Cammarota *et al.*[11], in a complementary study, show that peptides derived from this region bind to CD4. Exactly how these loops interact with CD4 and CD8, and whether they provide the only sites of contact, are questions awaiting the cocrystallization of MHC molecules and their coreceptors. □

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GENE REGULATION

Ecdysone and the onion

Greg Guild and Geoff Richards

THE axiom that more than half of the secret of finding something is knowing where to look was well illustrated by two meetings* devoted to the molecular analysis of steroid response in insects. By exploiting the combined genetic and *in vivo* approaches available with *Drosophila*, levels of detail in the steroid regulation of gene networks are emerging that are rarely seen elsewhere.

Although they are structurally related to vertebrate steroids, the insect ecdysones seem to be harmless to mammals even at their relatively high physiological levels of 10^{-6} M. That stimulated many insect physiologists to characterize the steroid response with the aim of producing ecdysone derivatives which might prove to be specific regulators of insect populations. Their ingenuity in devising both *in vivo* and *in vitro* test systems using ligatures, microsurgery and organ culture has resulted in a detailed understanding of the ecdysone response. But the small size of insects proved to be a liability in the biochemical isolation of the ecdysone receptors, and the molecular breakthrough finally came from a long-term *Drosophila* project.

Molecular analysis of the ecdysone response in *D. melanogaster* was stimulated by the chromosome 'puffing' model of Ashburner *et al.*[1], and started with the isolation of genes from defined puffs of the giant polytene chromosomes. Following studies which revealed that three of the primary ecdysone responsive puffs encode different families of transcription factors[2,3], the ecdysone receptor itself has now been shown to be present in at least three isoforms (M. Koelle, W. Talbot and D. Hogness, Stanford Univ.). The combinatorial possibilities of isoforms of both the receptor and the primary responsive genes are much larger than those suggested by the formal model, and the present aim is to understand the stage- and tissue-specific response to a single hormonal stimulus (ecdysone). If the available molecular complexity proves insufficient to explain that response, there are further potential players in the receptor-related molecules in *Drosophila* now under study (V. Henrich, Univ. North Carolina, Greensboro), and in the new primary responsive genes that have been isolated (P. Hurban and A. Andres, Univ. Utah).

Comparative studies in other insects such as *Manduca sexta* (L. Riddiford, Univ. Washington, Seattle; W. Segraves, Salk Inst.) and *Galleria mellonella* (M. Jindra, Caske Budovice, Czechoslovakia) show that the *D. melanogaster* organization of these alternatively spliced genes is not unique. This bears on the interpretation of certain novel isoforms (for example one-fingered receptors)[4] and opens the possibility of extending the results to insect species of medical or economic importance.

Every good nuclear receptor needs a DNA-binding site, and a clearer picture of the elusive EcRE (ecdysone response element) is now emerging. Although closely related to the model hormone response elements of vertebrates, the insect elements are not necessarily defined solely by consensus sequences (P. and L. Cherbas, Indiana Univ.; C. Antoniewski and M. Laval, Inst. Jacques Monod) and most probably require interactions between receptors and other transcription factors for their specific activity *in vivo*.

One striking feature of the meetings was the return to the *in vivo* analysis of mutants, necessarily left to one side during the difficult molecular analyses of the monster primary response genes whose isoform transcripts extend over 100 kilobases. Particularly notable was the report of the rapid molecular characterization of fly strains bearing receptor mutants induced by classical mutagenesis (M. Bender and D. Hogness). Equally, mutants in two of the early genes, the *Broad Complex* (J. Deutsch, Inst. Jacques Monod; G. G.; R. Hodgetts, Univ. Alberta; L. Von Kalm, Univ. California, Berkeley; F. Karim, Univ. Utah) and E74 (J. Fletcher, Univ. Utah) have been used to show their importance for the transcription of a number of downstream genes.

Unsuspected detail in the *Drosophila* ecdysone response *in vivo* is becoming apparent from the use of techniques, such as immunocytology, with isoform-specific antibodies for ecdysone receptors (J. Truman, Univ. Washington, Seattle, with W. Talbot and D. Hogness), or transcript analyses using finely staged material by both classical northern analyses (F. Karim; C. Bayer, Univ. California, Berkeley) or simultaneous RTase-PCR analysis of isoforms of different primary response genes in tissues of individual *Drosophila* larvae (G.R.). These studies show that adjacent cells in the same tissue may express different receptor isoforms; that isoform switching in the primary response genes is a rapid, but sometimes transient, response to hormone; and that there are clear differences in the regulation of the primary response in different tissues of individuals. Similar developmental studies in a number of tissues have revealed an ecdysone response in the mid-third instar of *Drosophila*, which occurs before the dramatic hormone responses that initiate pupariation and metamorphosis. Molecular characterization of this response will be necessary to understand the reprogramming of cells for the later ecdysone responses. Products of the *Broad Complex* locus are also involved in many of the mid-third-instar responses — but not all of them, as shown for the ecdysone-dependent modulation of the diptericin promoter's response to bacterial infection (M. Meister, Univ. Louis Pasteur), a first tentative link between the endocrine and immune systems in insects.

For old hands all this showed that we have penetrated another layer of the onion and, as best expressed by Jim Truman, we are in for a period of intense data collection before we can hope to explain the remarkably rich biological complexity, both in time and space, that characterizes the regulation of these gene networks *in vivo*. It may well be that the vertebrate laboratories, which have dominated the last decade of steroid research, will have something to learn from these studies. □

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