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STRUCTURAL AND FUNCTIONAL ANALYSIS OF SOME MOULTING HORMONE-RESPONSIVE GENES FROM *DROSOPHILA*

L. CHERBAS,[1,*] H. BENEŠ,[2,3] M. BOUROUIS,[4] K. BURTIS,[5] A. CHAO,[6] P. CHERBAS,[1,*] M. CROSBY,[7] M. GARFINKEL,[7] G. GUILD,[6] D. HOGNESS,[5] J. JAMI,[2] C. W. JONES,[5,†] M. KOEHLER,[1] J.-A. LEPESANT,[2] C. MARTIN,[2] F. MASCHAT,[2] P. MATHERS,[2] E. MEYEROWITZ,[7] R. MOSS,[1,‡] R. PICTET,[2] J. REBERS,[1,§] G. RICHARDS,[4] J. ROUX,[2] R. SCHULZ,[1,¶] W. SEGRAVES,[5] C. THUMMEL[5] and K. VIJAYRAGHAVAN[7]

[1]Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138, U.S.A., [2]Institut Jacques Monod, CNRS, INSERM et Université Paris 7, Paris Cédex 05, France, [3]Division of Gerontology Research, University of Arkansas for Medical Sciences, Little Rock, AK 72205, U.S.A., [4]LGME du CNRS, Faculté de Médecine, 67085 Strasbourg Cedex, France, [5]Department of Biochemistry, Stanford University Medical Center, Stanford, CA 94305, U.S.A., [6]Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, U.S.A. and [7]Division of Biology, California Institute of Technology, Pasadena, CA 91125, U.S.A.

In the course of the Seventh Ecdysone Workshop, molecular analyses of a number of ecdysteroid-responsive *Drosophila* genes were described. [The term "ecdysteroid" is used in this paper to denote compounds with moulting hormone activity. In other papers by many of us, the term "ecdysone" is used for the same purpose.] Our purpose in this communication is to summarize the results presented at the meeting, and to try to compare the various ecdysteroid-responsive genes with each other and with the vertebrate model systems currently available.

The genes with which we are concerned are listed in Table 1. They differ in the tissues in which they are known to be expressed and regulated and in the direction of ecdysone regulation. Another group of responsive genes, the small *Hsp* genes, and additional data concerning one of the genes we discuss here, 2B5, are the subjects of other papers in this volume (Morganelli and Berger, 1986; Galcerán *et al.*, 1986). All of these genes respond rapidly to ecdysteroids *in vitro* and all are likely therefore to be directly responsive loci. In no case has the response to the hormone been shown to be direct. All the experiments which we shall mention either identify the normal pattern of activity in these genes or are concerned with deletion analysis of the sequences required to give normal expression. In each case our working hypothesis is that activity is controlled directly by ecdysteroid activity but the reader should bear in mind that, until a correlation can be shown gene activity and the binding of a hormone-receptor complex, as has been shown for MMTV and chicken vitellogenin II (see below), the direct involvement of the hormone can only be hypothesized.

For comparison, we begin with a brief summary of the literature on steroid-regulated vertebrate genes.

THE VERTEBRATE MODELS

The most extensively studied steroid-responsive gene is the mouse mammary tumor virus (MMTV) provirus. In a variety of cell types, chromosomally inserted proviruses give rise to a single transcript, initiating within the left long terminal repeat (LTR). This transcript is present at very low levels until the cells are exposed to a glucocorticoid hormone. Within minutes following hormone treatment the titre of the MMTV transcript rises, reaching a level at least 50-fold above the basal level within 2 hr (Groner *et al.*, 1983; Ucker *et al.*, 1983).

Recent experiments have shown that glucocorticoid receptor binding sites are located within the MMTV LTR and that the same region of the LTR is both necessary and sufficient to confer glucocorticoid inducibility. Receptor binding sites are clustered between 80 and 300 base pairs upstream of the transcriptional initiation sites, and additional sites are present at several positions internal to the transcript (Geisse *et al.*, 1982; Govindan *et al.*, 1982; Pfahl, 1982; Hynes *et al.*, 1983; Payvar *et al.*, 1983; Pfahl *et al.*, 1983; Scheidereit *et al.*, 1983). A region required for hormone responsiveness has been shown, by deletion analysis, to lie between 105 and 204 base pairs upstream of the initiation site (Buetti and Diggelman, 1983; Majors and Varmus, 1983), and a fragment lying 109–449 base pairs upstream of the initiation site was found to confer corticosteroid inducibility upon a heterologous gene (Chandler *et al.*, 1983).

*Present address: Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A.
†Present address: Department of Biology, Bethel College, St Paul, MI 55112, U.S.A.
‡Present address: American Cancer Society, New York, NY 10016, U.S.A.
§Present address: Department of Zoology, University of Washington, Seattle, WA 98195, U.S.A.
¶Present address: Department of Biochemistry and Molecular Biology, University of Texas System Cancer Center, Houston, TX 77030, U.S.A.

Table 1. Genes described in this paper

Gene	Salivary gland polytene chromosome map position	Tissue in which regulation has been studied*	Direction of moulting hormone response
Sgs-3	68C	Salivary gland	Up/down
Sgs-7	68C	Salivary gland	Up/down
Sgs-8	68C	Salivary gland	Up/down
2B5	2B5	Salivary gland	Up
E74	74EF	Salivary gland	Up
E75	75B	Salivary gland	Up
EIP28/29	71C3,4-D1,2	Kc cells	Up
EIP40	55E3,4	Kc cells	Up
P1	70D1,2	Fat body	Up

*The gene may also be expressed and regulated in other tissues, see text.

Information is more fragmentary concerning the steroid regulation of other vertebrate genes which may be directly hormone-inducible. Table 2 summarizes results from six such genes. It would seem that the relatively simple picture, derived from MMTV, of a transcript regulated by a hormone-binding region upstream of its initiation site, may hold true for a number of cellular steroid-regulated vertebrate genes, although there seems to be a great deal of variation in the position of the hormone-binding region.

ECDYSTEROID-RESPONSIVE GENES FROM *DROSOPHILA*

Several hours before pupariation, the haemolymph ecdysteroid concentration increases (see e.g. Hodgetts *et al.*, 1977; Kraminsky *et al.*, 1980; Richards, 1981). This rise in titre results in dramatic changes in salivary gland gene expression, as monitored by polytene chromosome puffing. The resulting puffing patterns have been studied in detail and show that three multi-locus sets of ecdysteroid-regulated puffs (intermoult, early and late) are active in a precise sequence during the developmental period centred on puparium formation (Ashburner and Berendes, 1976). Intermoult and early puff sites both appear to respond more or less directly to the hormone (Ashburner *et al.*, 1974). Examples of both categories will be described below.

An additional category of ecdysteroid-responsive genes has been identified in *Drosophila* cell lines, especially the Kc line. These lines are of unknown histological type, but they all have properties suggesting that they represent an imaginal cell type which differentiates in response to ecdysteroid(s) (Cherbas and Cherbas, 1981). Thus, the ecdysteroid-induced genes in these cells are probably the analogs in some, as yet undefined, cell type of the salivary gland early puff sites. Indeed, at least one of the ecdysone-inducible genes in salivary glands is also induced in Kc cells (Jones, Burtis and Hogness, unpublished observations).

Finally, the P1 gene was isolated by virtue of the fact that its transcript appears rapidly in the fat body in response to ecdysteroid (Lepesant *et al.*, 1978, 1982; Nakanishi and Garen, 1983). Its activity appears to be restricted to the fat body, where it too may be analogous to the salivary gland early puff sites.

GLUE GENES: THE 68C COMPLEX

During the second half of the third larval instar of *Drosophila*, genes which encode the salivary gland secretion (*Sgs*) proteins are active in transcription. The sites of the *Sgs* genes correspond in general to the intermoult puff sites described by Ashburner and his colleagues. Two of these intermoult puff sites have been cloned and characterized in some detail. We shall describe in some detail. We shall describe here work from two of our laboratories (Pasadena and Strasbourg) on the *Sgs* genes at 68C; and we refer the reader to extensive published work from the laboratories of Hogness and Beckendorf on the *Sgs-4* gene at 3C11-12.

The puff at 68C contains the structural genes for three glue polypeptides, *Sgs-3*, *Sgs-7* and *Sgs-8*, clustered in a DNA region 5 kilobases in length (Meyerowitz and Hogness, 1982). Accumulation of

Table 2. Regulatory regions in some vertebrate steroid-responsive genes

Gene	Hormone	Region (0 = initiation site)	Type of assay*	Reference†
Chicken vitellogenin II	Oestrogen	−725 – −450	R, T	1, 2
Chicken lysozyme	Glucocorticoid	−164 – 0	D, R	3, 4
	Progesterone	−164 – 0	D, R	3, 4
Chicken ovalbumin	Progesterone	−222 – −95	D	5
		−247 – −135	R	6
Human somatotropin	Glucocorticoid	−500 – 0	D	7
Human metallothionein II	Glucocorticoid	−265 – −245	R	8
Rabbit uteroglobin	Glucocorticoid	~ −2700	R	9

*R = receptor binding; T = hormone-stimulated transcription *in vitro*; D = deletion analysis. †References: (1) Jost *et al.*, 1984; (2) Jost *et al.*, 1985; (3) Renkawitz *et al.*, 1984; (4) Ahe *et al.*, 1985; (5) Dean *et al.*, 1983; (6) Compton *et al.*, 1983; (7) Robins *et al.*, 1982; (8) Karin *et al.*, 1984; (9) Cato *et al.*, 1984..

all three transcripts requires a low level of ecdysteroid and the presence of a high level of the hormone causes the repression of their transcription. In addition, ecdysteroid activity causes the regression of the 68C puff. These conclusions are derived from experiments with the temperature-sensitive "ecdysoneless" mutation *l(1)su(f)*^{ts67g} (Hansson and Lambertsson, 1984) and from incubations of salivary glands *in vitro* (Crowley and Meyerowitz, 1984).

Several approaches have been used to define the DNA regions required for normal levels of stage- and tissue-specific transcription. Chromosomal rearrangements which have breakpoints within the 68C region, *In(3L)HR15* and *Df(3L)vin*^{3}, have shown that both normal puffing and proper transcription of *Sgs-3* require no more than a 20 kilobase region (Crosby and Meyerowitz, unpublished observations). P element transformation experiments (Spradling and Rubin, 1982) have been used to define the region more precisely.

Slightly different strategies for fly transformation have been used by the Pasadena and Strasbourg groups. In both cases the transforming gene is distinguished from the endogenous gene by virtue of the fact that they represent different alleles, whose RNA and protein products are easily separable. The Pasadena laboratory transformed Hikone-R and Formosa flies (*Sgs-3* transcript 1000 and 800 N, respectively) with an Oregon R gene (*Sgs-3* transcript 1100 N; Crosby and Meyerowitz, unpublished observations). The Strasbourg group transformed Oregon R flies with a Formosa gene (Richards *et al.*, 1983; Bourouis and Richards, 1985). The selectable marker present in the transforming plasmid was *Adh* (Pasadena) or *ry* (Strasbourg). These differences in technique may account for the slightly different results obtained by the two laboratories.

Both groups found that, after transformation, fragments containing the structural gene plus 2.3 kb or more of 5′ flanking sequence gave normal levels and proper developmental control of the *Sgs-3* transcript, but not in general a normal puffing pattern. Bourouis and Richards found that deletion of sequences more than 1.4 kb upstream of the transcript gave a marked decrease in the level of transcript, and deletion of sequences more than 127 bp upstream of the transcript gave at least a 100-fold reduction in transcript. They showed also that inversion of the region from 0.13 to 2.3 kb upstream of the transcript did not diminish the level of transcript. Crosby and Meyerowitz found that deletion of sequences more than 127 bp upstream gave only a 10-fold decrease in transcript. Both groups found that fragments giving reduced levels of transcript retained the temporal- and tissue-specificity of the intact gene. Despite the quantitative differences in their results, the two groups agree that the controlling elements of the *Sgs-3* gene can be divided into a region near the transcriptional initiation site and/or internal to the gene which confers temporal and tissue specificity, and an upstream region, whose orientation is apparently not crucial, which is required for normal levels of expression.

For comparison, we note that a region about 500 bp upstream of the *Sgs-4* transcriptional initiation site appears to be essential for proper expression of the *Sgs-4* gene and contains DNase I-hypersensitive sites (Muskavitch and Hogness, 1982; Shermoen and Beckendorf, 1982).

Transformation with DNA flanking the *Sgs-8* gene suggests that sequences governing correct developmental regulation of *Sgs-3* and *Sgs-8* are not shared, despite their close proximity and coordinate expression (Meyerowitz, unpublished observations).

Regulation of the *Sgs-3* gene is not simply the result of direct interactions with an ecdysteroid-receptor complex. Belyaeva *et al.* (1981) studied the effect of *l(1)npr-1*, a mutation of the early puff site 2B5 (see below), on puffing at 68C; Crowley *et al.* (1984) extended this work with molecular studies of expression of the *Sgs* genes. In animals carrying the mutation, the intermoult puff at 68C forms normally but fails to regress *in vivo* or after ecdysteroid treatment *in vitro*. Nevertheless, accumulation of RNAs from the *Sgs-3*, *Sgs-7* and *Sgs-8* genes in such mutants was reduced to undetectable levels. These results suggest that the product of 2B5 may be involved directly or indirectly in two aspects of regulatory control at 68C, namely, transcription of the *Sgs* genes, and regression of the puff. Transformation studies showed that the interaction of the 2B5 product with 68C requires no more than a 6 kb region from 68C.

It is intriguing to note that these experiments, as well as the transformation experiments described above, show that puffing and transcription are separable. These results contrast with the apparent identity of elements required for induction and puffing of the heat-shock genes *Hsp70* and *Hsp26* (Simon *et al.*, 1985).

SALIVARY GLAND "EARLY" GENES

Ecdysteroid activity rapidly induces a set of about six large polytene chromosome puffs in the third larval instar salivary gland chromosomes of *Drosophila*. Within a few hours, these early ecdysteroid-responsive puffs begin to regress and are replaced by a large number of late ecdysteroid-inducible puffs. While the induction of the early puffs is insensitive to inhibition of protein synthesis by cycloheximide, both their regression and the induction of the late puffs is blocked by such treatment. This and other observations support the model proposed by Ashburner and co-workers more than 10 years ago, whereby an ecdysteroid-receptor complex interacts with the "early" gene loci, causing the production of protein products which act both to repress early gene activity and to induce "late" gene activity (Ashburner *et al.*, 1974). We discuss here the molecular analysis of three of the salivary gland early puffs, 2B5, 74EF and 75B.

Puff site 2B5

Analysis of the salivary gland puffing pattern, using light microscopic, electron microscopic and transcription autoradiographic data, in combination with chromosomal breakpoints, has localized one of the "early" puffs to the 2B5 band (Belyaeva *et al.*, 1980). Additional genetic and cytogenetic experiments indicate that this region is organized into an "overlapping complementation complex" (*occ*) containing four complementation groups [*br*, broad

wings; *rbp*, reduced bristle number on the palpus; *l(1)pp-1*; *l(1)pp-2*]. Several observations (Belyaeva *et al.*, 1980, 1981) suggest that the *occ* contains a 2B5 "early" gene. Firstly, all four complementation groups contain alleles which are lethal during the late third larval instar-to-pupal interval, indicating that these functions are necessary for the continued development of the animal. This is consistent with the idea that this complex has a regulatory role in subsequent gene expression. Secondly, experiments using well-mapped deficiency chromosomes indicate that the *occ* maps precisely to the 2B5 band and its flanking interband regions. Thirdly, animals made deficient for only the 2B5 region are defective in their ecdysteroid-response: the "late" salivary gland puffing sequence fails to take place, which again suggests a regulatory role for the 2B5 region. This defective puffing response is not a result of an ecdysteroid-production deficiency, since the ring glands of these animals do produce the hormone, and hormone added exogenously fails to rescue the phenotype (Kiss *et al.*, 1978). The regulatory role of the 2B5 product(s) is apparent at other levels (Belyaeva *et al.*, 1981; Crowley *et al.*, 1984) since its absence prevents regression of one of the "intermoult" puffs (see above), inhibits the puffing response (60–70%) of the 74EF and 75B "early" puffs, and prevents puffing of another "early" puff (63F). An additional, autoregulatory, role is suggested by the observation (Belyaeva *et al.*, 1981) that, although the 2B5 early puff is fully induced by ecdysone, its regression takes much longer in glands from homozygous *occ* mutants.

These results taken together suggest that the 2B5 region contains a genetically complex "early" puff encoding an important transcriptional regulatory factor which plays roles in the repression of "intermoult" puffing, the autoregulation of its own puff, and the induction of members of both the "early" and "late" gene sets. If these interpretations are correct, they suggest that, in addition to the postulated hierarchy of gene regulation which exists among the genes representing intermoult, early and late puffs (Ashburner *et al.*, 1973), another regulatory hierarchy may exist, among members of the early puff set.

DNA representing the 2B5 early gene(s) has been cloned by two groups. Chao and Guild (unpublished observations) used transposon-tagging and chromosomal walking techniques to collect a set of cloned DNA segments which represent approx. 230 kb in the 2B cytogenetic region. *In situ* hybridization and genomic DNA blotting experiments using well-characterized chromosomal breakpoints (Belyaeva *et al.*, 1980) demonstrated that this stretch of DNA corresponds to the cytogenetic divisions 2B1,2–2B5. Galcerán *et al.* (1986) have used polytene chromsome microdissection and microcloning techniques to recover DNA from the 2B5 band—their studies are described elsewhere in this volume.

Chao and Guild carried out transcriptional characterization of the 2B1,2–2B5 area by probing the cloned DNA segments with random oligonucleotide-primed cDNAs derived from poly(A) + RNA populations from control and ecdysteroid-induced third instar larvae, salivary glands and imaginal discs. These experiments show that: (1) long stretches of the DNA in the 2B3,4-2B5 region are active at a low level in control tissues and are induced to a higher level by ecdysteroid activity; (2) several DNA segments in this region show tissue-specific transcripts which are ecdysteroid-responsive; and (3) ecdysteroid-inducible transcripts synthesized in imaginal discs are derived from both strands. However, it is not yet known whether any anti-parallel transcripts overlap. Additional cDNA experiments with a portion of the 2B5 region (Galcerán *et al.*, 1986) indicate that RNA from this region may also be maternally inherited and synthesized late during embryogenesis.

Analysis of cDNA clones and salivary gland RNA species derived from the 2B5 region shows that at least one transcription unit covers in excess of 20 kb of DNA to yield a 4.6 kb RNA, and alternative splicing may play a role in transcript diversity.

Puff sites 74EF and 75B

One prediction of the model proposed by Ashburner and co-workers is that altering the dosage of a regulatory "early" gene should affect the response kinetics of genes subservient to it (Walker and Ashburner, 1981). Walker and Ashburner tested this hypothesis by analysing puffing in glands from flies monosomic or trisomic for a 59 band region containing both the large 74EF and 75B "early" puffs. The results were consistent with the proposed regulatory role of one or more genes in this region; both the rate of regression of the "early" puffs and the rate of induction of some of the "late" puffs were seen to be sensitive to the dosage of this region. The early genes at 74EF and 75B are thus implicated in an ecdysteroid-triggered regulatory hierarchy. In order to study their roles, Hogness and co-workers have sought to isolate the ecdysteroid-responsive genes within these early puffs.

Using chromosomal walking techniques, several hundred kilobases of DNA each have been isolated from the 74EF puff region (Burtis, Jones, Thummel and Hogness, unpublished observations) and the 75B puff region (Segraves and Hogness, unpublished observations). By differential screening of the isolated genomic clones with cDNA prepared from ecdysteroid-induced and uninduced RNAs, by S1 nuclease and cDNA primer extension analysis, and by sequence analysis of cloned genomic and cDNAs, these workers have identified within each region a very long transcription unit which is inducible by ecdysteroid treatment of isolated third instar tissues *in vitro*.

The gene found at the 74EF locus, which we have called *E74*, responds to ecdysteroid in isolated salivary glands in much the same way as do the puffs. Induction of the *E74* transcript is rapid and peaks by 4–6 hr, after which the level of the transcript decreases. Cycloheximide does not affect the induction of the *E74* gene but does block its regression. Ecdysteroid-induced transcription of the *E74* gene results in the accumulation of an approx. 6 kb RNA size class, not only in salivary glands but also in several other late third instar larval tissues. This RNA also rises and falls during late embryogenesis, first and second larval instars, and in the pupal and adult phases, corresponding roughly to the developmental changes in the ecdysteroid titre. At least

one member of this size class consists of eight exons spread over the approx. 60 kb of the transcription unit. Transcription mapping and DNA sequence analysis of the *E74* region indicate that the spliced RNA product has an unusually long 5′ untranslated region, containing several upstream AUGs, punctuated by multiple stop codons. Following this is a large open reading frame capable of encoding a protein of approx. 90 kd. The identification of the nucleotide sequences required for the developmentally specific spatial and temporal control of *E74* expression is underway, as are experiments directed toward the identification and function of its protein products.

The ecdysteroid-inducible transcription unit found at 75B, which we call *E75*, is transcribed in many larval tissues to yield two RNA size classes, each of which appears to be composed of six or more exons which span the length of the unit (approx. 50 kb). These transcripts respond to ecdysteroid in a fashion similar to that observed for *E74* and for the 75B puff. Transcript mapping and DNA sequencing indicate the presence, at the shared 5′ end of these messages, of almost 400 bases of untranslated DNA preceding the start of a long open reading frame in the mRNAs. In the case of one *E74* cDNA clone, this open reading frame encodes a protein in excess of 100 kd.

Like *E74*, the *E75* gene is expressed many times during development. However, in this case, different combinations of at least four different RNA size classes are produced. In the late third larval instar the relative abundance of the two major size classes in third instar tissues appears to vary in a tissue-specific manner.

Of the "early" salivary gland puffs only the 2B puff has been the subject of detailed genetic analysis (see above). In order to undertake a genetic analysis of *E74* and *E75*, the Hogness group has been using F2 screens to isolate lethal complementation groups within the 74EF and 75B puff regions. Three members of a five-member complementation group have been mapped to the genomic DNA of the *E75* locus, leading us to believe that we have identified mutants in this locus. Homozygous or heteroallelic mutant individuals die, either as early larvae or as pupae depending on the combination of alleles. In conjunction with molecular data indicating a multiplicity of *E75* RNA products, this suggests that genetically separable functions of the *E75* locus are required in different tissues and/or at different times during development.

ECDYSTEROID-RESPONSIVE GENES FROM Kc CELLS: THE *EIP* GENES

Kc cells show a dramatic morphological, enzymatic and proliferative response to ecdysteroids (Cherbas and Cherbas, 1981). Within the first 30 min of the response, increased synthesis of a few polypeptides is detectable. Their rate of synthesis is maximal, at about 10 times the basal level, after about 4 hr of hormone treatment (Savakis *et al.*, 1980, 1984). These polypeptides are designated "ecdysteroid-inducible polypeptides" (EIPs) 28, 29, 35 and 40, according to their apparent mol. wt in kilodaltons. The induction kinetics and hormone-dependence of the EIP RNAs are similar to those for salivary gland "early" puff sites (Savakis *et al.*, 1980; Bieber and Cherbas, unpublished observations; Rebers, 1984). cDNA clones containing sequences encoding the EIPs 28, 29 and 40 were isolated (Savakis *et al.*, 1984). They were found to correspond to two genes which we shall now describe individually.

EIP28/29

The *EIP28/29* gene is a single-copy structural gene located at 71C3,4-D1,2 on the polytene chromosome map (Savakis *et al.*, 1984; Cherbas, Schulz, Koehler, Savakis and Cherbas, unpublished observations). It gives rise to a 2 kb transcript which is processed by the excision of 3 introns to yield a 1 kb mRNA. A 12 nucleotide variation in the position of one of the splice junctions is responsible for the generation of two polypeptide products, EIP28 and EIP29 (Schulz, Cherbas and Cherbas, unpublished observations). The effect of ecdysteroid is simply to elevate the titres of these mRNAs by about 10-fold without altering their ratio or any detail of their structure.

The *EIP28/29* gene is part of a region of complex transcriptional activity (Schultz, Shlomchik and Cherbas, unpublished observations). The *EIP28/29* transcript itself is found in a number of tissues, and it is not yet known in which tissues it may be regulated by ecdysteroid activity. In addition, the following transcripts, at least, arise from the same region of DNA: (1) two major, substantially overlapping, testis-specific RNAs, transcribed in the same direction as *EIP28/29*—one species terminates about 200 nucleotides upstream of the *EIP28/29* initiation site, and the other terminates within the *EIP28/29* protein-coding region; (2) two substantially overlapping transcripts found at moderate abundance in ovaries and at lower levels in Kc cells—these transcripts share the 3′ ends of the testis-specific transcripts just described; and in Kc cells the level of these transcripts is not affected by ecdysteroids; and (3) one or more alternate forms of the *EIP28/29* transcript, differing in their most 5′ exon—such variant form(s) are found in a number of fly tissues, and since the protein coding region begins in the 5′ exon of *EIP28/29* (Cherbas, Schulz, Koehler, Savakis and Cherbas, unpublished observations), these variants must encode modified polypeptides. [This situation contrasts with that found in *Drosophila Adh* (Benyajati *et al.*, 1983) and mouse alpha-amylase (Hagenbüchle *et al.*, 1981), where alternate promoters are used to generate alternative RNAs encoding a conserved protein.]

A functional analysis of this region has begun, using the stable transformation of *Drosophila* cell lines as described by Bourouis and Jarry (1983) and modified by Moss, Cherbas, Koehler and Cherbas (unpublished observations). Preliminary results suggest that a 6 kb fragment, including 2 kb upstream of the *EIP28/29* initiation site, and 2 kb downstream of the *EIP28/29* polyadenylation site, includes the entire ovarian transcription unit and all the sequences required for expression and induction of the *EIP28/29* gene. However, 650 bases of the *EIP28/29* promoter permits expression but does not confer ecdysteroid

inducibility upon a heterologous gene to which it is fused (Cherbas, Rosenthal and Koehler, unpublished observations). More detailed mapping of the controlling region(s) is in progress.

EIP40

The *EIP40* gene is a single-copy gene located at polytene position 55E3,4 (Rebers, 1984; Savakis *et al.*, 1984; Karp, pers. commun.). It gives rise to a single unspliced transcript, whose titre increases 5–6-fold after ecdysteroid treatment. Transcripts from the *EIP40* region have not been studied in fly tissues, but the transcriptional pattern in Kc cells is quite complex (Rebers, 1984). Within a few hundred nucleotides on one side of the *EIP40* transcript is another transcript, and on the other side is a pair of overlapping transcripts. These flanking transcripts are induced little if at all by ecdysteroids. A fifth transcript substantially overlaps the *EIP40* transcript, but is made from the opposite strand of DNA. This "antisense" transcript is present at a much lower level than the *EIP40* transcript, but it is induced approx. 25-fold by ecdysteroid activity. Its function in the cell is not known, nor is it clear whether the two antiparallel transcripts share a hormone-regulatory region.

P1: AN ECDYSTEROID-RESPONSIVE GENE FROM FAT BODY

The P1 gene is located in the region 70D1-2 of chromosome 3 (Levine *et al.*, 1981; Lepesant *et al.*, 1982; Moreau *et al.*, 1985). It is transcribed specifically in the fat body during the second half of the third larval instar into a unique, abundant 3.4 kb mRNA encoding a 100 kd polypeptide. The function of the P1 protein remains unknown (Lepesant *et al.*, 1978; Levine *et al.*, 1981; Lepesant *et al.*, 1982). A detailed structural analysis showed that this gene is not interrupted by introns and allowed the mRNA transcription start site to be mapped (Maschat, Roux, Beneš, Pictet, Jami and Lepesant, unpublished observations). Transcription of the P1 gene is under the positive control of ecdysteroid activity (Lepesant *et al.*, 1978, 1982; Nakanishi and Garen, 1983).

P element-mediated transformation (Spradling and Rubin, 1982) has been used to identify the *cis*-active sequences required for proper developmentally and hormonally regulated expression of this gene. Maschat and her coworkers substituted for the P1 coding sequences those of the *E. coli* xanthine guanine phosphoribosyl transferase gene (XGPRT, also called *Ecogpt*), which had previously been used to transform eukaryotic cell lines (Mulligan and Berg, 1980). The initial hybrid gene contained a P1 promoter sequence including the P1 mRNA start site and 1.4 kb upstream. This P1 sequence was fused to a DNA fragment isolated from the pSV2gpt plasmid constructed by Mulligan and Berg. This fragment contains the *Ecogpt* coding sequence itself fused at its 3′ end to the SV40 *t* antigen splicing and polyadenylation sequences.

Five transformed lines of *Drosophila* containing only one copy of the P1-*Ecogpt* hybrid gene were obtained. In each of these lines a specific pattern of transcription of the *Ecogpt* sequence is observed with the same spatial, temporal and hormonal specificities as those of the endogenous P1 gene. These observations provide evidence that control elements essential for the hormonally and developmentally regulated expression of P1 are located within a 1.5 kb region 5′ to this gene.

CONCLUSIONS

The genes which we have described seem to fall into two distinct categories:

(1) The 68C glue genes and P1 are simple, self-contained transcription units. So far as we can tell, each is active in a single tissue at a single developmental stage. In each of these four genes it is clear that ecdysteroid activity causes an alteration in the rate of transcription (Crowley and Meyerowitz, 1984; Maschat, Roux, Beneš, Pictet, Jami and Lepesant, unpublished observations). Their small size has made them amenable to P element-mediated transformation studies. Such studies have shown that their regulatory regions are contained in about 1.5 kb of 5′ flanking region (P1) or, possibly, within the transcribed region (*Sgs-3*). Progress in the mapping of regulatory elements for these genes should be rapid in the next few years. It is possible that their regulation may turn out to be very similar to that of MMTV, differing only in details such as the position of the regulatory region.

(2) The salivary gland "early" puff sites contain extremely long ecdysteroid-inducible transcription units. These regions are transcribed in many tissues, at a variety of stages, and possibly always under ecdysteroid control. 2B5 and *E75* yield multiple mature RNAs by means of alternative splicing patterns and/or alternative promoters, and the pattern of RNAs is tissue-specific. The *EIP28/29* and *EIP40* transcription units are relatively short, but they are found in regions of extremely complex transcriptional activity. The *EIP40* region is transcribed symmetrically, the complementary transcripts both being induced by ecdysteroid activity in Kc cells. The *EIP28/29* gene has at least two tissue-specific promoters, and is contained in a region of tissue-specific overlapping transcription units, most of which appear not to be controlled by ecdysteroids. Genetic and cytogenetic studies suggest that the 2B5 region, *E74*, and *E75* encode regulatory proteins; the proteins have not yet been identified. Protein products of *EIP28/29* and *EIP40* are known, but their functions are not. So far, it is not known whether ecdysteroid activity regulates transcription at any of these loci, and mapping of functional regions has been slowed both by the extreme length of the salivary gland "early" genes and by the transcriptional complexity of all five genes.

The glue genes, the vertebrate genes listed in Table 2, and, probably, P1 all encode major tissue-specific products. But the salivary gland "early" genes and (perhaps) the EIPs appear to be both transcriptionally complex and hormone-responsive in a number of tissues. It is possible that they represent elements of an early response which is both general and in some ways tissue-specific. Their developmental significance will be interesting to explore.

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