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Ecdysone-inducible functions of larval fat bodies in *Drosophila*

(hormonal control of gene expression during development)

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ABSTRACT Late in the third instar larval stage of *Drosophila melanogaster*, the titer of the steroid hormone ecdysone increases sharply. This increase is blocked in the temperature-sensitive mutant *ecd*^{1} after a temperature shift from 20°C to 29°C. The mutant was used to prepare three samples of late third instar larvae with different titers of ecdysone; the titer was low in one sample because of an earlier temperature shift, high in a second sample because the larvae were subsequently transferred to ecdysone-supplemented food, and also high in a third sample that was kept at 20°C, providing a control for normal development. The effect of the high titer of ecdysone on proteins of the larval fat bodies was examined by comparing two-dimensional gel electrophoresis patterns of total proteins in stained gels. There were proteins at five positions in the gels for the high-ecdysone samples that were not detected at the corresponding positions in the gel for the low-ecdysone sample. The effect of ecdysone on these proteins was further studied by injecting [^{35}S]methionine into the larvae at both early and late third instar stages, in order to label proteins synthesized before and after the increase in ecdysone titer. The results indicate that ecdysone induces two major responses in the fat bodies; certain proteins that were synthesized earlier in the fat bodies and secreted into the hemolymph are incorporated back into the fat bodies, and other proteins are newly synthesized. Attempts to induce prematurely the synthesis of the new proteins by exposing early third instar larvae to exogenous ecdysone were unsuccessful, suggesting that development must proceed further before the fat bodies can respond to ecdysone.

By *in vitro* translation of RNA isolated from fat bodies of low- and high-ecdysone samples of larvae, it was shown that ecdysone greatly increases the amount of translatable messenger RNA for one of the newly synthesized proteins. A clone of DNA complementary to the induced messenger RNA has been isolated from a population of λ bacteriophage carrying segments of the *Drosophila* genome. Using the cloned DNA to measure amounts of complementary poly(A)-RNA in the fat bodies by DNA·RNA hybridization, we detected about 50 times more complementary poly(A)-RNA in the high-ecdysone sample of larvae than in the low-ecdysone sample. This finding provides direct evidence that ecdysone induces an increase in the amount of the messenger RNA. The ecdysone-induced appearance of a major messenger RNA in late third instar larval fat bodies represents a developmental response to ecdysone that appears to be gene-specific, tissue-specific, and stage-specific, and it has exceptionally favorable features for further molecular studies of the control of gene expression by a steroid hormone.

The recent isolation of a temperature-sensitive mutant of *Drosophila melanogaster*, called *ecd*^{1}, which becomes deficient in ecdysone after a temperature shift from 20°C to 29°C at various stages of development (1), provides a sensitive system for analyzing the effects of the steroid hormone on specific developmental processes. When the temperature shift is done early in the third instar, *ecd*^{1} larvae grow to the size of mature normal larvae, but the rapid increase in ecdysone titer that should occur later in the third instar is blocked. As a consequence of the ecdysone deficiency caused by the temperature shift, *ecd*^{1} larvae fail to pupariate, and instead remain viable larvae at 29°C for as long as 3 weeks. All of the late larval functions normally induced by the increase in ecdysone titer should be affected by the temperature shift. Such functions might be identified by an appropriate comparison of late third-instar *ecd*^{1} larvae that have an abnormally low titer of ecdysone because of an earlier temperature shift with *ecd*^{1} larvae that have a higher titer either because ecdysone was provided exogenously after the shift or the larvae were kept at 20°C until the ecdysone titer had increased in the course of normal development. In this report, functions of the larval fat bodies are examined in this way, including the formation of messenger RNA and protein and the incorporation into fat bodies of circulating proteins from the hemolymph (2, 3).

RESULTS

The first experiment is to compare the proteins in larval fat bodies before and after the ecdysone titer increases towards the end of the third instar (1, 4). Two samples of late third instar *ecd*^{1} larvae were prepared. One sample contained an abnormally low titer of ecdysone, about 20 pg per larva, because of an earlier temperature shift from 20°C to 29°C; the other sample was grown in the same way but afterwards was transferred to ecdysone-supplemented food, causing a rapid increase in the ecdysone titer sufficient to induce pupariation about 12 hr later. Some of the larvae in each sample were injected with [^{35}S]methionine, and 2 hr later the fat bodies were isolated by dissection from the labeled and unlabeled larvae. The proteins in the fat bodies were fractionated by two-dimensional gel electrophoresis (5); gels for the unlabeled samples were stained for total protein, and gels for the labeled samples were fluorographed (6) to detect proteins that incorporated the injected [^{35}S]methionine. Comparison of the stained gels (Fig. 1 *A* and *B*) shows that the fat bodies from the high-ecdysone sample contain all of the proteins found in the low-ecdysone sample and, in addition, several prominent new proteins not detected in the low-ecdysone sample (Fig. 1*C*). Three of the new proteins, at positions *1*, 3, and 5, appear to be single molecular species. There is a cluster of at least three new proteins at position *4* that differ slightly in isoelectric point or size. The proteins at position 2 are better resolved in a one-dimensional sodium dodecyl sulfate ($NaDodSO_4$) gel (Fig. 2, channels A and B); these proteins move to the same region of the gel as do the major proteins in third-instar larval hemolymph (Fig. 2, channel C), and probably correspond to the class of larval serum proteins that has been described in detail elsewhere (8). The new proteins at each of the five positions are identified in the text as *P1*, *P2*, *P3*, *P4*, and *P5*. The appearance of these proteins is a normal developmental response to the increase in ecdysone titer, as indicated by the identical results obtained with the fat bodies

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Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

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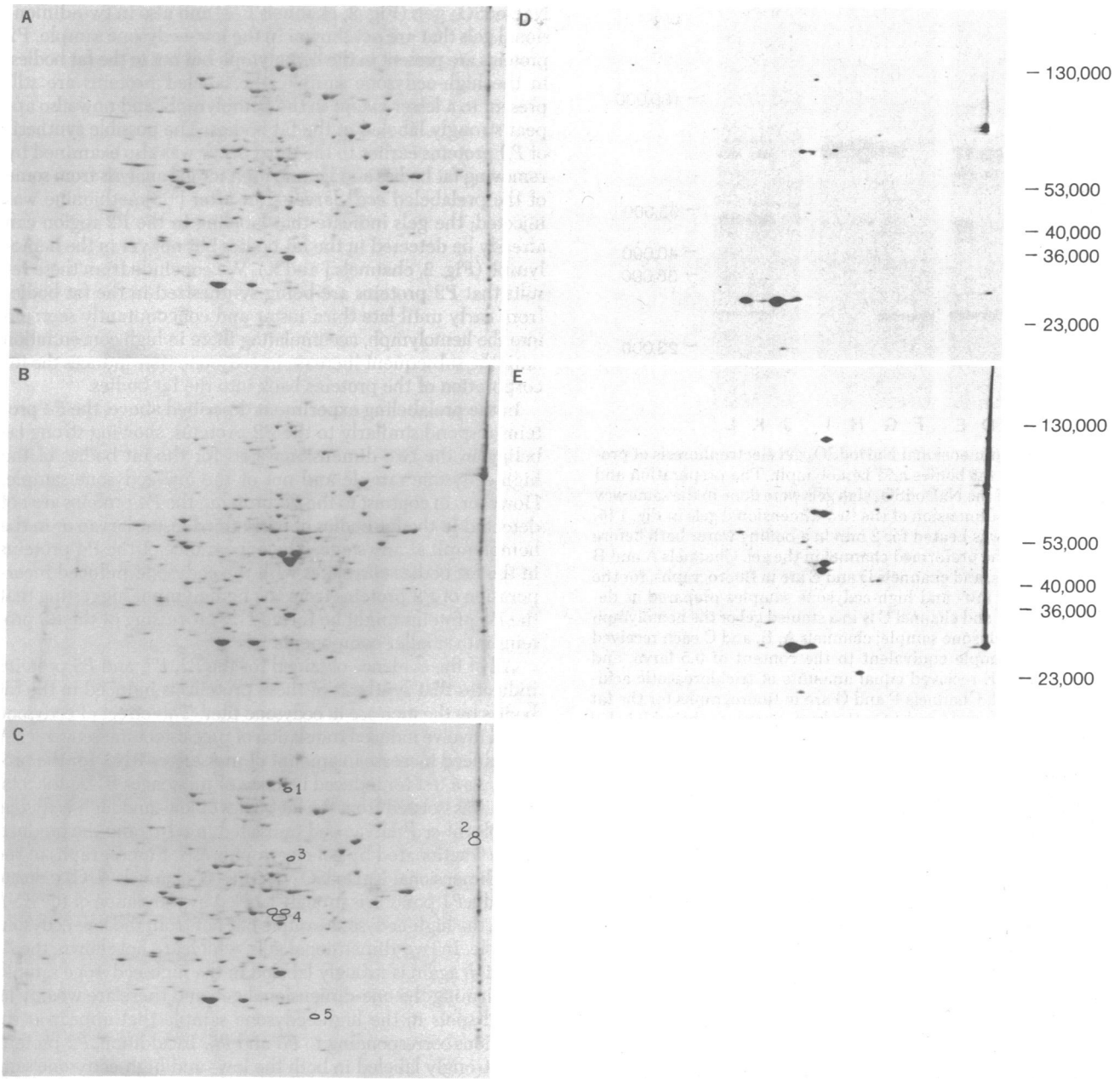

FIG. 1. Two-dimensional gel electrophoresis of proteins from larval fat bodies. Eggs were collected from a homozygous *ecd*^{1} stock (1) kept at 20°C, and development was synchronized subsequently by collecting the larvae that hatched at 20°C within a span of 2 hr. Larval development continued at 20°C for 108 hr, which was about 12 hr past the second molt, and afterwards at 29°C for an additional 24 hr. The larvae were divided into groups; one group, which was used as the high-ecdysone sample, was transferred to ecdysone-supplemented food (a thick paste of live yeast in 0.5% ethanol with β-ecdysone at 0.1 mg/ml) and the other group, which was used as the low-ecdysone sample, was transferred to the same food without ecdysone. After 6 hr at 29°C, some of the larvae in both groups were removed from the food, immobilized by exposure to ether, and injected with about 0.2 μl each of a solution of [^{35}S]methionine that also contained [^{35}S]cysteine (about 500 mCi/mmol and 10 μCi/ml) (1 Ci = 3.7×10^{10} becquerels). Two hours later the injected and uninjected larvae from both groups were dissected to isolate fat bodies, and the gonads (which are embedded in the tissue) were removed; samples of hemolymph were also collected with a micropipette as required for Fig. 2. The fat bodies from 10 larvae were combined for each sample, and the samples were stored at −50°C until used. Two-dimensional gel electrophoresis was done as previously described (5, 7), except that the isoelectric focusing gel contained only the pH 3–10 ampholyte mixture. The orientation for the first dimension isoelectric focusing gel is across the top of each panel, and for the second dimension $NaDodSO_4$ slab gel from the top to the bottom of each panel. For the first dimension, the sample was added to the low pH end of the isoelectric focusing gel. After the migration in the second dimension was completed, the unlabeled gels were stained with Coomassie blue, and the labeled gels were fluorographed (6). Molecular weights of five standards are shown on the right. (*A* and *B*) Stained gels for the low- and high-ecdysone samples, respectively; (*C*) diagram of the positions of the stained spots that are present only in the high-ecdysone sample superimposed on the gel for the low-ecdysone sample. (*D* and *E*) Fluorographs for the labeled low- and high-ecdysone samples, respectively. The fat bodies from the equivalent of three larvae were used for each of the stained gels, and the same amount of trichloroacetic acid-precipitable label was used for each of the labeled gels.

from a high-ecdysone control sample of *ecd*^{1} larvae grown at 20°C until late in the third instar.

The fluorographed gels for the samples labeled with [^{35}S]-methionine (Fig. 1 *D* and *E*, and Fig. 2, channels D and E) show that *P1*, *P3*, and *P5*, and two of the three components in the *P4* cluster, also occur as new or more intensely labeled proteins in the corresponding positions of the fluorographed gels for the high-ecdysone samples. (The labeling is somewhat more intense

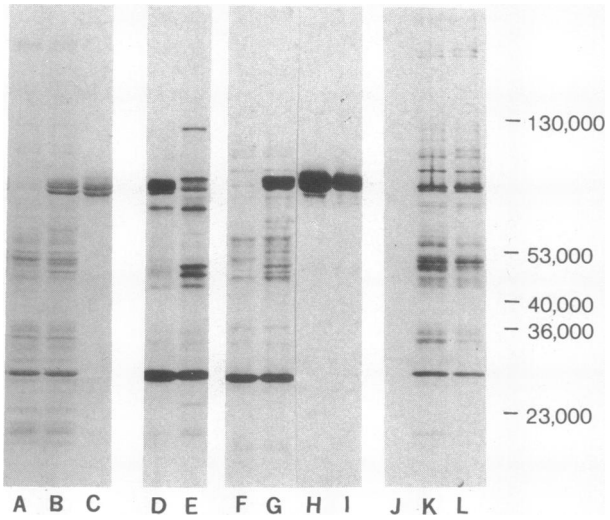

FIG. 2. One-dimensional $NaDodSO_4$ gel electrophoresis of proteins from larval fat bodies and hemolymph. The preparation and electrophoresis of the $NaDodSO_4$ slab gels were done in the same way as for the second dimension of the two-dimensional gels in Fig. 1 (5, 7). Each sample was heated for 2 min in a boiling water bath before it was added to the preformed channel in the gel. Channels A and B are in stained gels, and channels D and E are in fluorographs, for the fat bodies of the low- and high-ecdysone samples prepared as described for Fig. 1, and channel C is in a stained gel of the hemolymph from the high-ecdysone sample; channels A, B, and C each received an amount of sample equivalent to the content of 0.5 larva, and channels D and E received equal amounts of trichloroacetic acid-precipitable label. Channels F and G are in fluorographs for the fat bodies, and channels H and I for the hemolymph of the prelabeled low- and high-ecdysone samples, respectively, prepared as described in the text; channels F and G each received an amount of sample equivalent to the fat bodies of 0.5 larva, which also contained equal amounts of acid-precipitable label, and channels H and I each received the amount of hemolymph obtained from the equivalent of 0.5 larva. Channel J is in a fluorograph for the hemolymph, and channel K for the fat bodies, of the early third instar low-ecdysone sample prepared as described in the text, and channel L is in a fluorograph for the fat bodies of the early third instar high-ecdysone sample; channels K and L each received an amount of sample equivalent to fat bodies of 0.75 larva, which also contained equal amounts of acid-precipitable label, and channel J received the amount of hemolymph obtained from the equivalent of 0.5 larva.

at a few other positions in the two-dimensional gel for the high-ecdysone sample, but these are weak and variable responses of questionable significance.) The effect of ecdysone on the *P2* proteins is more complex. In the low-ecdysone sample, bands in the *P2* region are strongly labeled, although these do not appear in the stained gel (compare channels A and D in Fig. 2). In the high-ecdysone sample the pattern of labeling in the *P2* region is altered. An explanation for some of these results is suggested by two additional observations. One is that *P2* proteins are present in the hemolymph (Fig. 2, channel C), and the other is that ecdysone can induce the incorporation of proteins from the hemolymph into the fat bodies (2, 3). Therefore, the *P2* proteins detected in the fat bodies of the stained gels for the high-ecdysone sample might not be newly synthesized, but instead might be *P2* proteins from the hemolymph that were synthesized earlier and incorporated into the fat bodies after the increase in ecdysone titer. In order to test the validity of this explanation, ecd^1 larvae were prelabeled by injecting [^{35}S]methionine at the time of the temperature shift early in the third instar, and 24 hr later some of the larvae were transferred to ecdysone-supplemented food. Fat bodies and hemolymph were collected from the low- and high-ecdysone samples 6 hr later, and were fractionated in one-dimensional $NaDodSO_4$ gels (Fig. 2, channels F–I) and also in two-dimensional gels that are not shown. In the low-ecdysone sample, *P2* proteins are present in the hemolymph but not in the fat bodies; in the high-ecdysone sample, the labeled proteins are still present to a lesser extent in the hemolymph, and now also appear strongly labeled in the fat bodies. The possible synthesis of *P2* proteins earlier in the third instar was also examined by removing fat bodies and hemolymph for gel analysis from some of the prelabeled ecd^1 larvae 2 hr after [^{35}S]methionine was injected; the gels indicate that labeling in the *P2* region can already be detected in the fat bodies but not yet in the hemolymph (Fig. 2, channels J and K). We conclude from these results that *P2* proteins are being synthesized in the fat bodies from early until late third instar and concomitantly secreted into the hemolymph, accumulating there in high concentration until the subsequent increase in ecdysone titer induces the incorporation of the proteins back into the fat bodies.

In the prelabeling experiment described above, the *P4* proteins respond similarly to the *P2* proteins, showing strong labeling in the two-dimensional gels for the fat bodies of the high-ecdysone sample and not of the low-ecdysone sample. However, in contrast to the *P2* proteins, the *P4* proteins are not detected in the fat bodies of early third instar larvae or in the hemolymph at any stage. The appearance of the *P4* proteins in the fat bodies correlates with the ecdysone-induced incorporation of *P2* proteins from the hemolymph, suggesting that the *P4* proteins might be formed by processing of the *P2* proteins into smaller components.

All of the evidence obtained for the *P1*, *P3*, and *P5* proteins indicates that synthesis of these proteins is induced in the fat bodies by the increase in ecdysone titer. This effect of ecdysone could involve induced translation of preexistent messenger RNA or induced increase in amount of messenger RNA for the proteins. As a test for induced increase of messenger RNA, the total RNA was isolated from the fat bodies of low- and high-ecdysone samples of ecd^1 larvae and translated *in vitro*, and the products were fractionated by gel electrophoresis. Fluorographs of the one-dimensional $NaDodSO_4$ gel (Fig. 3, channels A–C) indicate that the *P1* position is strongly labeled by translation of the RNA from the high-ecdysone sample but not from the low-ecdysone sample. In two-dimensional gels, which are not shown, the *P1* position again is strongly labeled in the high-ecdysone sample, confirming the one-dimensional gel, and there are weakly labeled spots in the high-ecdysone sample that appear at the positions corresponding to *P3* and *P5*. In addition, *P2* proteins are strongly labeled in both the low- and high-ecdysone samples, although in the *in vivo* labeling experiments certain *P2* proteins that are strongly labeled in the low-ecdysone sample are unlabeled in the high-ecdysone sample (Fig. 2, channels D and E), suggesting either that the messenger RNA for these proteins is still present but not translated *in vivo* or that the proteins are modified *in vivo* and move to other positions in the gel that are strongly labeled in the high-ecdysone sample. The *P4* proteins are unlabeled *in vitro* in both samples, consistent with the previous evidence that these proteins might be derived from processing of the *P2* proteins, a reaction that should not occur in the *in vitro* system. The principal conclusion from the *in vitro* translation test is that one major new species of messenger RNA, coding for the *P1* protein, appears in the fat bodies as a result of the increase in ecdysone titer.

During normal development, the ecdysone titer does not reach the level required to induce synthesis of the *P1* protein in the larval fat bodies until a few hours before pupariation. The next experiment is to test whether the synthesis of *P1* can be induced prematurely in early third instar larvae by providing ecdysone exogenously. The ecd^1 larvae were prepared by shifting to 29°C early in the third instar and immediately

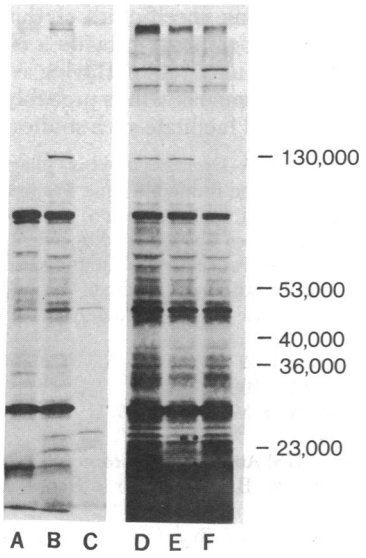

FIG. 3. *In vitro* translation of messenger RNA from larval fat bodies. The fat bodies were isolated by dissection and immediately frozen and stored at −50°C. The frozen tissue was homogenized for 30 sec at 60°C in a solution of 0.1 M Tris·HCl buffer at pH 9 and 0.5% $NaDodSO_4$, and afterwards an equal volume of water-saturated phenol was added and homogenization was continued for 10 min at 60°C. The solution was chilled and spun for 15 min at 1600 × *g*, and the aqueous and phenol phases were separated. The phenol phase was equilibrated again with an equal volume of the buffer solution and centrifuged. The first and second aqueous phases were combined and equilibrated again with phenol. The final aqueous phase was adjusted to 0.2 M sodium acetate at pH 5.2, and the RNA was precipitated by adding 1½ vol of 95% ethanol, keeping overnight at −20°C, and collecting the precipitate by spinning at 4000 × *g* for 10 min. The precipitate was dissolved in a solution of 0.05 M Tris·HCl at pH 7.5 and 0.15 M NaCl, and the RNA was put through two additional cycles of precipitation in ethanol. This total RNA preparation was used for the *in vitro* translations shown in channels A and B. The poly(A)-RNA preparation used for the *in vitro* translations shown in channels D, E, and F was obtained by passing the total RNA through a column of oligo(dT)-cellulose in a solution of 0.01 M Tris·HCl at pH 7.5 and eluting the bound RNA with a solution of the same buffer and 0.5 M KCl; the eluate was bound to, and eluted from, the column twice more, and the final eluate was precipitated in ethanol. The *in vitro* translation was done with a rabbit reticulocyte extract (9) in a final volume of 70 μl, and the mixture was incubated for 60 min at 37°C; the reaction was stopped by freezing and the samples were stored at −50°C until used. One-dimensional electrophoresis was done as described for Fig. 2, and afterwards the gels were fluorographed. Channels A and B show the products of translation using as templates 25 μg of the total RNA from the fat bodies of low- and high-ecdysone samples, respectively, of late third instar larvae prepared as described for Fig. 1, and channel C shows the control without added RNA; equal amounts of trichloroacetic acid-precipitable label were added to channels A and B, and the same volume of the incubation mixture was added to channel C. The template used for channels D, E, and F was 0.2 μg of purified poly(A)-RNA from the high-ecdysone sample of larval fat bodies, which was pretreated before translation as follows: for channel D, the poly(A)-RNA was equilibrated at 65°C for 60 min in a solution of 0.01 M Tris·HCl at pH 7.5/0.001 M EDTA/0.1 M KCl; for channel E, 2 μg of denatured DNA from a λ bacteriophage stock without *Drosophila* DNA inserts was added to the solution; for channel F, 2 μg of denatured DNA from a λ–*Drosophila* hybrid clone† was added to the solution. After pretreatment of the poly(A)-RNA, the solutions were lyophilized and subsequently translated in the rabbit reticulocyte system as described above for channels A and B. The pretreatment used for channels D, E, and F provides conditions for DNA·RNA hybridization that block translation of hybridizable messenger RNA, as described for liver messenger RNA (10).

† J. A. Lepesant, J. Kejzlarova-Lepesant, and A. Garen, unpublished data.

Table 1. Hybridization of poly(A)-RNA from fat bodies of low- and high-ecdysone samples to nitrocellulose membranes containing an excess of the cloned *Drosophila* DNA

	Hybridized poly(A)-RNA			
	cpm		% of total	
DNA source	Low ecdysone	High ecdysone	Low ecdysone	High ecdysone
No DNA	220	230		
Control DNA (λ only)	230	240	0	0
Cloned DNA (λ + *Drosophila*)	590	18,300	0.017	0.87

The DNA was extracted from purified λ bacteriophage by using either a standard λ stock without *Drosophila* DNA inserts, or the λ–*Drosophila* hybrid stock analyzed in Fig. 3.† The membranes containing DNA were prepared by transferring 2.5 μg of the DNA in a small volume of 0.01 M Tris·HCl buffer at pH 7.5 to a 1-cm diameter circle of Schleicher and Schuell BA85 nitrocellulose membrane paper, drying the paper in air, and denaturing the DNA by immersion for 20 sec in a solution of 0.1 M sodium hydroxide and 1.5 M sodium chloride. The membranes were neutralized with a solution of 0.2 M Tris·HCl at pH 7.5/0.3 M sodium chloride/0.03 M sodium citrate/0.01 M EDTA and dried for 8 hr at 70°C. The membranes were first incubated with a solution containing 500 μg of *Drosophila* ribosomal and 5S RNA, 50% (vol/vol) formamide, 0.75 M sodium chloride, and 0.075 M sodium citrate for 12 hr at 42°C, and afterwards transferred for an additional 24 hr to a fresh aliquot of the same solution with 0.5 μg of labeled poly(A)-RNA added; the poly(A)-RNA was labeled by incubating it with [γ-^{32}P]ATP and polynucleotide kinase, which yielded 4 × 10^6 cpm per μg of poly(A)-RNA.† The membranes were washed successively in the same solution without RNA, in 0.3 M sodium chloride and 0.03 M sodium citrate, and finally in 0.15 M sodium chloride and 0.015 M sodium citrate, and were dried and assayed for radioactivity in a scintillation counter.

transferring one group to ecdysone-supplemented food; 2 hr later the larvae in both groups were injected with [^{35}S]methionine, and 2 hr after the injections the fat bodies were isolated and the labeled proteins were examined in one-dimensional $NaDodSO_4$ gels (Fig. 2, channels K and L) and also in two-dimensional gels that are not shown. No induced labeling could be detected at the position of the *P1* protein, indicating that development must proceed until later in the third instar before synthesis of *P1* can be induced in the fat bodies.

Poly(A)-RNA preparations from the fat bodies of low- and high-ecdysone samples of *ecd*^{1} larvae were used as probes to isolate, from a population of λ bacteriophage carrying inserted segments of the *Drosophila* genome (11), a clone with a DNA sequence complementary to the messenger RNA for the *P1* protein (9).† Hybridization of the cloned DNA to the poly(A)-RNA from the high-ecdysone sample causes specific inhibition of the *in vitro* coding activity for the *P1* protein (Fig. 3, channels D–F), indicating that the DNA hybridizes specifically to the messenger RNA for the *P1* protein. Therefore, the amount of the messenger RNA in a poly(A)-RNA preparation can be estimated from the extent of hybridization to an excess of the cloned DNA. The results of such measurements (Table 1) show that there is about 50-fold increase in the amount of messenger RNA for the *P1* protein in the high-ecdysone sample as compared to the low-ecdysone sample, providing direct quantitative confirmation of the evidence obtained by *in vitro* translation (Fig. 3, channels A–C).

DISCUSSION

The fat bodies respond in two distinct ways to the increase in ecdysone titer that occurs late in the third instar. One response is an incorporation of proteins from the hemolymph into the fat bodies; the other response is a major increase in the amount

of both the messenger RNA and encoded protein for one of the fat body proteins and a lesser increase for two other proteins. The latter response could involve ecdysone-induced transcription of the genes coding for the proteins, in accord with a model proposed for the action of mammalian steroid hormones (12) on ecdysone-induced stabilization of selected messenger RNA species. There is no information about the mechanism by which ecdysone induces the incorporation of hemolymph proteins into the fat bodies; ecdysone might exert either a direct effect on the permeability of the cells to exogenous proteins or an indirect effect as a consequence of the induced synthesis of one or more proteins that cause a change in permeability.

Most of the proteins incorporated into the fat bodies belong to the class of larval serum proteins that are the major components of third instar larval hemolymph (8) (see Fig. 2). Our findings indicate that these proteins are synthesized in the larval fat bodies during the third instar, secreted into the hemolymph, and then incorporated back into the fat bodies when the ecdysone titer increases. These results support other evidence of ecdysone-induced incorporation of hemolymph proteins into larval fat bodies (2, 3); the incorporated proteins appear to be sequestered in cytoplasmic granules and stored there, possibly for subsequent use as a nutrient source during pupal development (13).

There are three aspects of the ecdysone-induced appearance of new messenger RNA and protein in late third instar larval fat bodies that should be noted. First, only one major species of induced messenger RNA could be detected by *in vitro* translation, suggesting that the response is mainly gene-specific; second, the protein specified by the major induced messenger RNA could not be detected at the same stage in four other larval and imaginal tissues examined, suggesting that the response is also tissue-specific; third, exposing early third instar larvae to a high titer of ecdysone does not induce the synthesis of the protein in the fat bodies, in contrast to the induction that occurs in late third instar larvae, suggesting that the response is also stage-specific. The striking specificities of this hormonally regulated developmental response provide a clear focus for further genetic and molecular studies. Having available clones of the responding gene,† some of which probably include regulatory sequences, should facilitate such studies.

The precious "library" of λ-*Drosophila* hybrid phages, from which a clone carrying the ecdysone-inducible gene was isolated, was prepared by J. Lauer and T. Maniatis and generously made available to us. Expert advice and assistance was provided by two of our colleagues, Drs. Ching-Hung Kuo and Paul Farrell. The project is supported by grants from the National Institutes of Health of the U.S. Public Health Service and from the American Cancer Society.

1. Garen, A., Kauvar, L. & Lepesant, J. A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5099–5103.
2. Thomasson, W. A. & Mitchell, H. K. (1972) *J. Insect. Physiol.* **18**, 1885–1899.
3. Keeley, L. L. (1978) *Annu. Rev. Entomol.* **23**, 329–352.
4. Hodgetts, R. B., Sage, B. & O'Connor, J. D. (1977) *Dev. Biol.* **60**, 310–317.
5. O'Farrell, P. H. (1975) *J. Biol. Chem.*, **250**, 4407–4421.
6. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341.
7. Kuo, C. H. & Garen, A. (1978) *Dev. Biol.*, in press.
8. Roberts, D. B., Wolfe, J. & Akam, M. E. (1977) *J. Insect Physiol.* **23**, 871–878.
9. Pelham, H. R. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256.
10. Hastie, N. D. & Held, W. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1217–1221.
11. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K. & Efstratiadis, A. (1978) *Cell*, in press.
12. O'Malley, B. W., Coty, W. A., Schwartz, R. J. & Schraeder, W. T. (1977) *Cancer Biology*, eds. Borek, C., Fenoglio, C. M. & King, D. W. (Stratton Intercontinental Medical Book Corp., New York), Vol. 4, pp. 79–96.
13. Tysell, B. & Butterworth, F. M. (1978) *J. Insect Physiol.* **24**, 201–206.