Identification and characterization of the ecdysterone receptor in *Drosophila melanogaster* by photoaffinity labeling

(steroid hormones/blotting technique/irradiation)

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ABSTRACT Salivary glands of third-instar larvae of *Drosophila melanogaster* as well as *Drosophila* K_c tissue culture cells have been irradiated in the presence of ecdysterone. Irradiation covalently links ecdysterone to a single cellular protein, which is similar, if not identical, in salivary glands and in K_c cells. This protein has a molecular weight of 130,000 and it has the characteristics of a typical hormone-receptor molecule in terms of hormone-binding properties, translocation into the nucleus, and sedimentation characteristics. The yield of the photoinduced bonding of ecdysterone to receptor protein is around 15%. Ponasterone A competed with ecdysterone for the bonding. Also, ponasterone A itself reacted upon photoactivation with the β -ecdysterone receptor protein in *Drosophila* tissue culture cells. We have previously shown that ecdysterone can be bonded upon irradiation to specific hormone-controlled puffs of polytene chromosomes of *D. melanogaster* third-instar larvae [Gronemeyer, H. & Pongs, O. (1980) *Proc. Natl. Acad. Sci. USA* 77, 2108-2112]. Because we have now identified the molecular target of the ecdysterone photoreaction, these data show that a hormone-receptor complex translocates to the nucleus and directly binds to the genes, which are under hormonal control. A quantitative assay of hormone-receptor complex in K_c cells before and after hormone stimulation showed that ecdysterone does not regulate the synthesis and the available amount of its receptor. It was also observed that the translocated hormone-receptor complex resides in the nucleus as long as the hormone is present in the tissue culture medium.

Steroid hormones act directly on a few genes with a high degree of biological selectivity (1-3). The general understanding of steroid hormone action is that the hormone enters the target cell and binds to its cytoplasmic receptor protein. Thereupon the hormone-receptor complex translocates to the nucleus and elicits an alteration in the expression of particular genes. In *Drosophila melanogaster*, ecdysterone-dependent gene expression leads to a number of morphological and biochemical events as a prelude to molting and metamorphosis (2).

We have recently shown that the endogenous ecdysterone can be photoactivated in salivary glands of *D. melanogaster* larvae and thus can be covalently linked to the polytene chromosomes (3). The bonded ecdysterone was visualized by indirect immunofluorescence microscopy. Its binding sites could be correlated to the sequential induction of puffs, which takes place at defined chromosomal sites during the development of third-instar larvae (2). These data showed that ecdysterone binds directly and specifically to the genes that are under hormonal control (3). Because photoactivation of ecdysterone induced specific covalent binding of hormone to defined chromosomal sites, it was intriguing to investigate and to identify the nature of the molecule(s) that reacted with ecdysterone upon irradiation. We report in this paper that ecdysterone be-

comes covalently linked to a single protein, which has the properties of the ecdysteroid-receptor molecule (4, 5).

MATERIALS AND METHODS

The K_c cell line of *D. melanogaster* (6) was maintained as a suspension culture in D-22 medium (6) at 22°C.

Ecdysterone was obtained from Rotho Pharmaceutical (Osaka, Japan). [³H]Ecdysterone (40 Ci/mmol; 1 Ci = 3.7 × 10¹⁰ becquerels) was a generous gift from K. D. Spindler (Düsseldorf, Federal Republic of Germany) and [³H]ponasterone A (170 Ci/mmol) was a gift from C. Beckers (Darmstadt, Federal Republic of Germany). Antibodies against ecdysterone were prepared as described (3). 2-Acetylcysterone was kindly given to us by P. Welzel (Bochum, Federal Republic of Germany).

Irradiation of K_c Cells. Irradiation was performed in a buffer containing 60 mM KH₂PO₄, 30 mM K₂HPO₄, 10 mM Na₂HPO₄, 3 mM MgCl₂, and 0.25 M sucrose (pH 7.0). Before irradiation, approximately 10⁸ cells were harvested, incubated in 0.1 μ M ecdysterone in 2 ml of D-22 medium at room temperature for various times (see *Results*). Afterwards cells were pelleted, resuspended in 2 ml of buffer, and irradiated for 5 min as described (3). After irradiation, cells were pelleted (5 min, 1200 rpm in a Sigma 2K centrifuge), resuspended in 5 ml of buffer A (10 mM Tris-HCl/1 mM Na₂EDTA, pH 7.4) and then lysed by sonication for 5 min. The homogenate was centrifuged (20 min, 4600 rpm in a Sigma 2K centrifuge). Ammonium sulfate was added to the supernatant to a final concentration of 30% (wt/vol). The precipitate was collected by centrifugation for 20 min at 10,000 rpm in a Sorvall HB-4 rotor.

Preparation of Nuclei. Cells were irradiated for 5 min in buffer A containing 1% Nonidet P-40. The cell lysate was then centrifuged through 2 ml of a 0.5 M sucrose cushion in buffer B (50 mM KCl/1.5 mM MgCl₂/10 mM Tris-HCl/0.1 mM phenylmethanesulfonyl fluoride, pH 7.0) for 10 min at 2000 × g. Nuclei were ground in a Dounce homogenizer in 2 ml of buffer B containing 1% Tween 80 and sodium deoxycholate. They were again centrifuged through 2 ml of 0.5 M sucrose cushion in buffer B. The combined supernatants of the first and second centrifugations were centrifuged at 100,000 × g for 1 hr. The resulting supernatant was used as the cytoplasmic fraction.

The nuclear pellet was resuspended in 2 ml of buffer A and centrifuged through 2 ml of 2.2 M sucrose in buffer B at 70,000 × g for 1 hr. The nuclear pellet was resuspended in 2 ml of buffer A and was sonicated for 2 min. The lysate was centrifuged for 10 min at 2000 × g. The chromatin pellet was washed for 15 min at 4°C in buffer A containing 0.5 M KCl. Proteins were precipitated from the supernatant of the nuclear fraction and from the cytoplasmic fraction by (NH₄)₂SO₄ addition.

Isolation of Salivary Glands and Irradiation. Larvae of *D. melanogaster*, Oregon R wild-type strain, were dissected in Grace's medium (7) containing 1 μ M ecdysterone. Mass isola-

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tion of salivary glands was performed according to the method of Cohen (8). Glands were transferred to 5 ml of irradiation buffer II [40 mM NaCl/40 mM KCl/2 mM KH_2PO_4 /1 mM Na_2HPO_4 /1.2 mM MgCl_2 /1.2 mM $\text{Mg}(\text{CH}_3\text{COO})_2$ /1 mM CaCl_2 /80 mM sucrose (pH 7.2)] containing 1 μM ecdysterone and incubated for 30 min. The salivary glands were then washed with 10 ml of irradiation buffer II without ecdysterone and irradiated as described (3).

After irradiation, the glands were centrifuged (1 min, 1000 rpm in a Sigma 2K centrifuge) and then resuspended in 10 ml of buffer A. They were disrupted by sonication for 5 min. The homogenate was cleared by centrifugation (20 min, 4600 rpm). Ammonium sulfate precipitation from the supernatant was carried out as described above for K_c cell lysates.

Radioimmunoassay for Hormone-Receptor Complexes. Aliquots of cell lysates or of sucrose gradient fractions (50 μl) were adsorbed on nitrocellulose sheets (2 $\times$ 2 cm) (Schleicher & Schüll). During this procedure, the sheets were repeatedly dried. They were incubated for 1 hr in 20 ml of a protein solution of 2% ovalbumin, then in 20 ml of anti-ecdysterone serum [half-maximal antibody binding capacity = 0.05, 1:10 dilution (3)] for 4 hr, and finally for 4 hr in ^{125}I -labeled protein A from *Staphylococcus aureus* (10^6 cpm/ml, 20 ml). After incubation in anti-ecdysterone-serum and ^{125}I -protein A, the sheets were washed for 30 min with several changes of phosphate-buffered saline (pH 7.2). Radioactivity on the dried sheets was determined in a gamma counter (Berthold, Federal Republic of Germany). Protein A from *S. aureus* was iodinated as described (9). The specific activity of the protein was 20 Ci/g.

Sucrose Gradient Analyses. Analyses of ecdysterone-labeled proteins were performed as described by Schrader (10). Ammonium sulfate-precipitated material was dissolved in 200 μl of buffer A and layered on top of the 5–20% sucrose gradients, which were centrifuged at 41,000 rpm for 18 hr at 4°C in a Beckman SW 41 rotor. Nuclear extracts of K_c cells were prepared according to Worcel and Babrubahan (11). Fractions (0.6 ml) of the gradients were collected after centrifugation. Bound hormone in the fractions was analyzed either by radioimmunoassay or by measuring the [^3H]ecdysterone radioactivity in trichloroacetic acid precipitates (Fig. 3).

Gel Electrophoresis. One- and two-dimensional sodium dodecyl sulfate/polyacrylamide gel electrophoreses were carried out as described (12, 13). Gels were stained with Coomassie brilliant blue (0.25% in 30% methanol/20% acetic acid), destained with 50% methanol/10% acetic acid, and stored in 7% acetic acid. Fluorography of polyacrylamide gels was performed according to Bonner and Laskey (14).

Blotting. Protein transfer from polyacrylamide gels to nitrocellulose filters (BA 85, Schleicher & Schüll) was carried out as described (15, 16). Blots were incubated with anti-ecdysterone serum and ^{125}I -protein A as described (16). They were exposed at -70°C to Kodak X-Omat R film for 30–60 min with an intensifier screen (Du Pont).

Charcoal Assay. As described in ref. 17, 0.5 g of charcoal in 20 ml of buffer A was coated with 0.1 g of dextran. Lysates of 10^8 K_c cells were diluted to 2 ml and incubated with 0.5 nM [^3H]ponasterone A for 30 min at room temperature. Then 500 μl of the charcoal suspension was added twice. Each time, the mixture was incubated for 5 min on ice and then centrifuged at 4000 rpm for 5 min in a Sigma 2K centrifuge. Bound hormone was determined by measuring radioactivity in the supernatant.

RESULTS

Ecdysterone is taken up by K_c cells. This results ultimately in an arrest of cell growth and a change of cell structure. Dose-response curves for the morphological response show that

maximal induction requires 0.1 μM ecdysterone (18, 19). Accordingly, K_c cells (10^8 cells per 3 ml of tissue culture medium) were incubated with 0.1 μM ecdysterone for 30 min at 22°C and then irradiated for a further 5 min in irradiation buffer as described (3). Similarly, 2000 salivary glands were dissected from midthird-instar larvae of *D. melanogaster*, transferred to irradiation buffer, and irradiated. After irradiation, cells and salivary glands (separately) were lysed and soluble cellular proteins were extracted. Proteins were separated on denaturing polyacrylamide gels. Proteins in these gels were blotted onto nitrocellulose membranes (15, 16). The blot was subsequently screened for proteins containing bonded ecdysterone by successively incubating with ecdysterone antiserum and with ^{125}I -protein A. Only one protein-hormone crosslink was detected in the resulting autoradiograms (Fig. 1). The denatured protein has an apparent molecular weight of 130,000, on the basis of electrophoretic mobilities in the gel. The protein to which ecdysterone became bonded is very similar, if not identical, in *Drosophila* larval salivary glands and in *Drosophila* tissue culture cells of the K_c line. This result encouraged us to carry out further photoaffinity-labeling experiments in tissue culture cells in order to characterize the ecdysterone-binding protein. The specificity of the photoaffinity labeling reaction was investigated (i) with an inactive ecdysterone analog and (ii) in the heat shock system. 2-Acetylcyclohexanone is inactive with respect to induction of puffs of polytene chromosomes of larval salivary glands. Also, it does not arrest growth of K_c cells. On the other hand, it is as immunoreactive as ecdysterone towards our anti-ecdysterone serum. K_c cells were incubated with 10 μM 2-acetylcyclohexanone and subsequently irradiated. Work-up of cellular proteins and transfer to nitrocellulose membrane, followed by incubation with anti-ecdysterone serum, did not exhibit hor-

FIG. 1. Identification of ecdysterone-binding proteins in salivary glands and in tissue culture cells of *D. melanogaster*. Protein extracts of salivary glands of third-instar larvae and of K_c cells containing covalently linked ecdysterone were prepared. After polyacrylamide gel electrophoresis according to Laemmli (12), proteins were blotted onto nitrocellulose membranes and incubated with ecdysterone antiserum and ^{125}I -protein A as described (16). Molecular weight standards (left lane) were the subunits of *Escherichia coli* RNA polymerase. The autoradiogram was obtained with proteins extracted from 5×10^8 irradiated K_c cells that had been incubated either with 0.1 μM ecdysterone (lane A) or with 10 μM 2-acetylcyclohexanone (lane B) for 15 min. Lane C shows the result with proteins that were extracted from 2000 irradiated salivary glands.

none covalently linked to protein (Fig. 1, lane B). Similarly, irradiation of heat-shocked cells abolished the photoinduced reaction of ecdysterone with its binding protein (data not shown). These data show that the photoreaction did not occur with an inactive hormone analog or under physiological conditions that were unfavorable for hormone action.

The ecdysterone receptor in K_c -tissue culture cells can be titrated with the ecdysteroid [3 H]ponasterone A (4, 5). A large excess of unlabeled ecdysterone competes with binding of [3 H]ponasterone A. Therefore, we tested whether ecdysterone competed with the photoinduced bonding of [3 H]ponasterone A. Cytoplasmic extracts of K_c cells were incubated with [3 H]ponasterone A for 30 min at room temperature. Dextran-coated charcoal was added to remove unbound hormone. Bound hormone was determined by measuring the radioactivity in the supernatant after removal of charcoal. Bonded hormone was measured after irradiation of the charcoal-treated incubation mixture followed by trichloroacetic acid precipitation. The data of Table 1 show that we found about 1300 receptor molecules per K_c cell, which is in good agreement with previously published figures (4, 5). Twenty-five percent of the bound hormone became covalently bonded upon photoactivation. This yield of the photoreaction is comparable to that of a similar photoreaction of androgen with androgen receptor (20).

When [3 H]ponasterone A was incubated together with a 2000-fold excess of cold ecdysterone, we could not detect, after irradiation, trichloroacetic acid-precipitable radioactivity above background, indicating that photoinduced [3 H]ponasterone A bonding to K_c cell protein can be competed with by an excess of unlabeled ecdysterone. This suggested to us that the ecdysterone-binding protein that was detected in salivary gland or K_c cell lysates after irradiation (Fig. 1) was the ecdysterone receptor. In order to determine in which cell compartment (cytoplasm or nucleus) the photoreaction took place at various times after ecdysterone had been added to K_c cells, tissue culture cells were incubated with ecdysterone for different times before irradiation. Subsequently, cells were divided into a cytoplasmic and a nuclear protein fraction. Aliquots of these fractions were transferred to nitrocellulose sheets and tested for presence of bonded hormone with ecdysterone antiserum (for details see *Materials and Methods*). Nuclei were extensively washed with detergent and centrifuged through 2.2 M sucrose in order to free the nuclei of adhering cytoplasm as much as possible. Fig. 2 shows that 70% of the ecdysterone-binding protein migrated from the cytoplasm into the nucleus within 1 hr. The total cellular amount of detectable ecdysterone-binding

Table 1. Reversible and irreversible binding of [3 H]ponasterone A to cytosol of *Drosophila* tissue culture cells

[3 H]Ponasterone A	dpm
Bound to cytosol after charcoal treatment	79,374
Precipitated with trichloroacetic acid:	
Before irradiation	164
After irradiation	19,408
Precipitated with trichloroacetic acid after irradiation in the presence of a 2000-fold excess of ecdysterone	300

For each experiment, cytosol of 10^6 cells was incubated with 0.5 nM [3 H]ponasterone A for 30 min at room temperature. Incubation mixtures were then treated with 1% charcoal (17). Then radioactivity of bound [3 H]ponasterone A in the supernatant of the charcoal-treated incubation mixtures was determined. Where indicated, samples were treated with 5% trichloroacetic acid. Precipitates were collected on glass-fiber filters and assayed for [3 H]ponasterone A content by measuring the radioactivity retained on the filters. Irradiation times were 5 min at room temperature.

FIG. 2. Distribution of ecdysterone binding protein in cytoplasm and nuclei of K_c cells. Cells were incubated with 0.1 μ M ecdysterone and irradiated at the times indicated. The amount of covalently linked ecdysterone in protein extracts of cytoplasmic (■) or nuclear (●) fractions was determined immunologically with ecdysterone antiserum and 125 I-protein A. In a second experiment cells were irradiated immediately after 0.1 μ M ecdysterone had been added. Cytoplasmic (□) and nuclear (○) protein fractions were prepared after continuing the incubation for the times indicated. Error bars indicate SD.

protein, however, remained constant over the 16-hr time span investigated.

As shown in Fig. 2, photoinduced bonding of ecdysterone to protein has no recognizable effect on the migration of hormone-protein complex into the nucleus. When tissue culture cells irradiated immediately after addition of ecdysterone were incubated further for up to 2 hr; hormone-protein complex migrated into the nucleus with kinetics similar to those obtained with irradiation of incubation mixtures at various times after hormone addition. In another experiment, K_c cells were incubated with 0.1 μ M ecdysterone for 2 hr, irradiated for 5 min, and then incubated in hormone-containing tissue culture medium for a further 4 hr. Cells were then lysed and fractionated into nuclei and cytoplasm as described for Fig. 2. They were found to be virtually unchanged. This indicated that the protein to which hormone is covalently bound migrates into the nucleus but does not readily move back into the cytoplasm. Cytoplasmic, or nuclear, fractions containing bonded [3 H]ecdysterone were concentrated by $(\text{NH}_4)_2\text{SO}_4$ precipitation and were centrifuged on linear sucrose gradients. Fig. 3 shows that, in the cytoplasmic protein fraction prepared after a 15-min incubation with hormone, there are three radioactive peaks sedimenting at approximately 4, 6, and 9 S. On the other hand, the nuclear fraction prepared by extracting chromatin with 0.5 M KCl after a 60-min incubation with hormone exhibited only one major radioactive peak, sedimenting at approximately 9 S. This 9S material apparently represents the chromatin-binding form of the ecdysterone-binding protein. When we incubated cells for 30 min with hormone, the 4S peak was no longer observed. Very similar data were obtained with salivary glands of third-instar larvae of *D. melanogaster*. Extracts of irradiated salivary glands were prepared by sonication of the tissue. No attempt was made to separate cytoplasm and nuclei of the glands. In this case, the sucrose gradient was analyzed by immunological methods as

FIG. 3. Analysis of ecdysterone-protein complexes in K_c cell extracts by sucrose gradient centrifugation. K_c cells (5×10^6) were incubated with $0.1 \mu\text{M}$ [^3H]ecdysterone for 15 or 60 min and subsequently irradiated. Cytoplasmic (Lower) and nuclear (Upper) protein fractions were prepared and layered on top of 5–20% sucrose gradients. Centrifugation was at 41,000 rpm for 18 hr at 4°C in a Beckman SW 41 rotor. Marker proteins were rabbit immunoglobulin and ovalbumin. Aliquots of the fractions of the gradients were assayed for radioactivity after filtration through glass-fiber filters.

described for Fig. 2 in order to detect bonded ecdysterone. Fig. 4 shows a typical result that was thus obtained. The majority of bonded hormone sedimented around 5 and 9 S. In light of the data obtained with tissue culture cells, the 6S peak apparently contains the cytoplasmic and the 9S peak the nuclear form of the ecdysterone-protein complex.

We then studied whether the same protein became labeled with [^3H]ecdysterone in the cytoplasm and the nucleus of K_c cells. The cells were incubated for 15 or 60 min with [^3H]ecdysterone and then irradiated. Cytoplasmic and nuclear protein fractions were prepared as described above. Ammonium sulfate-precipitated proteins were separated on a denaturing polyacrylamide gel. The stained gel together with the corresponding autoradiogram is shown in Fig. 5. It can be seen that the proteins that reacted with [^3H]ecdysterone in the cytoplasm and in the nucleus ran at identical positions on the gel, corresponding to a protein with a molecular weight of 130,000. Similarly, a cytoplasmic protein extract of K_c cells incubated with [^3H]ponasterone A and treated with charcoal before irradiation, as described for Table 1, was separated on a denaturing polyacrylamide gel. Again, only one protein band carried label of [^3H]ponasterone A, and it migrated at the identical position as the [^3H]ecdysterone-labeled protein (Fig. 5, lane C). A cytoplasmic extract of K_c cells, which was obtained after incubation with [^3H]ecdysterone for 15 min and subsequent irradiation (18), was also analyzed by two-dimensional gel electrophoresis

FIG. 4. Analysis of ecdysterone-protein complexes in salivary glands of third-instar *D. melanogaster* larvae by sucrose gradient centrifugation. Irradiation of salivary glands and extraction of protein were as described in the text. Conditions of centrifugation were as for Fig. 3. The fractions of the gradient were absorbed on nitrocellulose membranes, which were subsequently incubated with ecdysterone antiserum followed by ^{125}I -protein A. $\times$, Radioactivity retained on the nitrocellulose membranes.

The autoradiogram of this gel showed one spot of molecular weight 130,000 and an isoelectric point of approximately 5.8 (Fig. 6). When the data of Figs. 2, 5, and 6 are combined with those of Table 1, they demonstrate that one protein becomes preferentially labeled with ecdysteroid hormone. Ecdysterone and ponasterone A react with the same protein. In this reaction one ecdysteroid competed with the other. The reaction takes place in cytoplasm as well as in nucleus, depending on the incubation conditions. All these data, therefore, show that ecdysterone becomes bonded to its receptor upon photoactivation. The receptor protein in K_c tissue culture cells and that in salivary glands are apparently similar, if not identical.

DISCUSSION

The recent availability of ponasterone A of high specific radioactivity made it possible to demonstrate a saturable, specific,

FIG. 5. Identification of [^3H]ecdysteroid-binding protein in cytoplasmic and nuclear protein fractions of K_c cells by denaturing polyacrylamide gel electrophoresis. Lane A of the 7.5% polyacrylamide gel contains the cytoplasmic and lane B the nuclear proteins, which were labeled with [^3H]ecdysterone and prepared as described for Fig. 3. Lane C contains a cytoplasmic extract that was incubated with 0.5 nM [^3H]ponasterone A, treated with charcoal, and subsequently irradiated. Protein was stained on the left; the autoradiogram is on the right. BSA, bovine serum albumin.

FIG. 6. Identification of the ecdysterone-binding protein in tissue culture cells by two-dimensional gel electrophoresis. Cells (5×10^6) were incubated with $0.1 \mu\text{M}$ [^3H]ecdysterone for 15 min and subsequently irradiated. Cytoplasmic proteins prepared as described for Fig. 3 were separated by two-dimensional gel electrophoresis according to O'Farrell (13). The first dimension was an isoelectric focusing gel, the second dimension, a 7.5–15% polyacrylamide gradient. Marker proteins were the subunits of *E. coli* RNA polymerase, bovine serum albumin, and lysozyme. After staining, the gel was fluorographed according to ref. 14 for 14 days.

high-affinity protein receptor for ecdysteroids in *Drosophila* imaginal discs (21) and in *Drosophila* K_c cells. O'Connor and colleagues (4, 5) have reported that this receptor rapidly translocates upon hormone addition to K_c cells into the nucleus. It takes about 30 min to translocate approximately 70% of the ponasterone A-receptor complex from the cytoplasm into the nucleus. Our irradiation experiments (Fig. 2) showed a similar behavior for the ecdysterone-protein complex.

The cytoplasmic form of ponasterone A-receptor of *Drosophila* tissue culture cells sedimented at 4.2 S and the nuclear form at 6.3 S (5). These sedimentation values were determined with receptor preparations to which the ecdysteroid was added. Our data, on the other hand, were obtained by covalently linking ecdysterone to its binding protein *in situ*. This yielded material that sedimented predominantly at 6 and 9 S on sucrose gradients. A comparison of the data of Figs. 3 and 4 to the above ponasterone A data shows that we have found one more component, which sediments at 9 S. Otherwise, our photocrosslinking data are quite comparable to the hormone-binding data. The discrepancy with regard to the 9S peak between the previous ponasterone A data and ours is not clear. The sedimentation behavior of reversible hormone-receptor complexes might be more sensitive to preparative artefacts. For example, progesterone receptor sediments in low ionic strength sucrose gradients at 6 and 8 S, whereas it does so at 4 S in high ionic strength sucrose gradients (22). This receptor exhibits three different values of sedimentation depending on the isolation procedure. Electrophoretic analyses of cytoplasmic and of nuclear receptor protein yielded identical results. However, the cytoplasmic and nuclear receptor might still differ in terms of minor modifications not detected in the present experiments.

The comparison of the hormone-binding protein in larval salivary glands to the K_c ecdysteroid receptor (Fig. 1) showed that the two hormone receptors appear to be identical. Because ec-

dysterone has so many different target tissues in the fly, the question, of course, is whether, they all contain the same receptor.

The result, that our irradiation experiments covalently linked ecdysterone to its receptor, has important consequences with respect to our previously reported immunofluorescence work (3). It actually shows that the fluorescence, which we have observed in puffed sites of polytene chromosomes, represents hormone-receptor complex, not only hormone. This observation strongly suggests that steroid hormone-receptor complexes modulate transcriptional activity directly at the level of the gene. They do so by binding to a cytoplasmic receptor, which upon hormone binding translocates to the nucleus. The receptor then binds directly to the genes, which are under its control as shown by the data in this paper and by our previous immunofluorescence work (3). This observation should stimulate future work in search of the chromosome signals involved in hormone-receptor action.

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