Proc. Natl. Acad. Sci. USA
Vol. 94, pp. 13499–13503, December 1997
Biochemistry

Ultraspiracle: An invertebrate nuclear receptor for juvenile hormones

GRACE JONES* AND PHILLIP A. SHARP†‡

*School of Biological Sciences, Molecular and Cellular Biology Section, University of Kentucky, Lexington, KY 40506; and †Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Contributed by Philip A. Sharp, September 18, 1997

ABSTRACT **Juvenile hormones (JH), a sesquiterpenoid group of ligands that regulate developmental transitions in insects, bind to the nuclear receptor ultraspiracle (USP). In fluorescence-based binding assays, USP protein binds JH III and JH III acid with specificity, adopting for each ligand a different final conformational state. JH III treatment of *Saccharomyces cerevisiae* expressing a LexA-USP fusion protein stabilizes an oligomeric association containing this protein, as detected by formation of a protein–DNA complex, and induces USP-dependent transcription in a reporter assay. We propose that regulation of morphogenetic transitions in invertebrates involves binding of JH or JH-like structures to USP.**

Regulation of cellular and tissue commitment to form stage-specific morphological structures is controlled in insects and higher invertebrates by esterified sesquiterpene hormones generally referred to as "juvenile hormones" (JH) (1, 2). These JH molecules include methylepoxyfarnesoate (JH III) and its homologs and are chemically related to the vertebrate terpene group, represented by all-*trans* retinoic acid (RA), which regulates vertebrate differentiation and morphogenesis (3). In contrast to the vertebrate terpene group, some 60 years after the discovery of JH "the mode of action of JH at the molecular level remains an enigma" (4, 5), leaving a gaping hole in a field of transcriptional regulation that contains some of our most important invertebrate model systems (e.g., *Drosophila*, *Caenorhabditis elegans*). The looming world-wide resurgence of insect-vectored disease compels great urgency in understanding how these hormones and their transcriptional effectors regulate vector biology so that novel control strategies may be devised (6).

The active form of JH is considered to be the methyl ester because in a number of traditional bioassays the ester is significantly more active than is JH acid (1, 2). However, in several epidermal bioassays, the acid form of JH I is more active than is the ester JH III (7), and at a time during metamorphosis at which there is a high circulating titer of JH acid (8), there is observed release of JH acid (but not JH ester) by the JH-secreting glands (7). The similar terpenoid nature of JH, RA, and the plant hormones abscissic acid and gibberellin has urged propositions of a terpene receptor superfamily (9) and of similar functional actions (10). RA regulates gene expression by stabilizing heterodimerization between an RA receptor (RAR) that binds several isoforms of that ligand (all-*trans* RA, 9-*cis* RA) and another receptor that binds only 9-*cis* RA (RXR) (11, 12). Other natural ligands for RXR also have been proposed, all of which thus far are carboxylic acids (13, 14). In contrast, to date, no invertebrate nuclear receptor that binds JH (ester) or JH acid has been identified.

Although a relative of the vertebrate RXR, ultraspiracle (USP), which was isolated from insects (15–17), is not known to bind a ligand. Specifically, it has been reported not to bind to 20-OH ecdysone (18, 19), the ecdysone analog muristerone (19), or the terpenoid RA (15) or to either of the ester forms of the JH analogs methoprene and hydroprene (20). Furthermore, neither of the two carboxylic acids of methoprene nor hydroprene bound to USP under conditions in which they exhibited binding to RXR (20). We report here that the two active JH structures found in *Drosophila* bind with specificity to *Drosophila* USP, inducing conformational changes and homo-oligomerization activity.

MATERIALS AND METHODS

Hormones. JH III (a monoepoxide ester) and JH III bisepoxide were obtained from Sigma and Larry Gilbert (University of North Carolina, Chapel Hill), respectively, and are the two JHs reported thus far from *Drosophila* (21, 22) although four additional JH isoforms have been reported from various other insects (8). HPLC-purified, 97% pure JH III acid was provided by Michael Roe, North Carolina State University (Raleigh, NC), and HPLC-purified, 98% pure methyl farnesoate was provided by Hans Laufer (University of Connecticut, Storrs, CT). Although the known natural insect JHs are all understood to be esters, to further clarify discussions below on structure–activity relationships of the ester vs. acid forms, we sometimes expressly refer to JH III as JH III ester. 20-OH ecdysone, farnesol, and all-*trans* RA were obtained from Sigma.

Electrophoretic Mobility Shift Assay. At inoculation for overnight growth, the liquid yeast culture was made to 10 μM JH III (Sigma), or only ethanol carrier was added. Yeast containing a plasmid encoding either LexA alone or the LexA/USP fusion were grown to an OD_{600} of 0.7–1.0, and the extract was prepared as described (23). A double-stranded oligonucleotide probe (5′-AAAAGTA CTACTGTATATA-CATACAGTG ATATCCC-3′) containing the LexA operon, previously characterized for this purpose (23), was end-labeled with [γ^{32}P]-ATP and used in an electrophoretic mobility shift assay as follows: 5 μl of reaction buffer (10 mM Hepes, pH 7.9/0.5 mM EDTA/5 mM $MgCl_2$/50 mM KCl/5% glycerol/1 mM DTT), 1 μl of 1 μg/μl dIdC, 10–18 μl of water, 1 μl of 10 μM JH III or EtOH carrier, 1 μl of probe (10,000–15,000 cpm), and 1–8 μl of yeast extract. Anti-USP antibodies were provided by Fotis Kafatos (European Molecular Biology Laboratory, Heidelberg).

Preparation of Recombinant USP. Full length USP in expression vector pGEX-5X, provided by James Sutherland, European Molecular Biology Organization, Heidelberg, Germany, was expressed in bacteria as a fusion with glutathione

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EcR, ecdysone receptor; JH, juvenile hormone; RA, retinoic acid; RAR, RA receptor; RXR, retinoid X receptor; USP, ultraspiracle; GST, glutathione *S*-transferase.

‡To whom reprint requests should be addressed. e-mail: sharppa@mit.edu.

S-transferase (GST). After induction by isopropyl β-D-thiogalactoside, the bacteria were lysed and the USP–GST fusion protein was purified by passage over a glutathione–Sepharose resin (Pharmacia). USP was then cleaved from GST with Factor Xa (New England Biolabs) in cleavage buffer (10 mM Tris, pH 7.5/1 mM $CaCl_2$/10 mM NaCl). The cleavage reaction products were then concentrated in a Centricon-10 concentrator (Amicon) with several changes of cleavage buffer. For use in assay, aliquots then were diluted into cleavage buffer.

Fluorescence-Based Binding Assay. As previously used in a number of studies (24–29), changes were monitored in the intrinsic fluorescence of tryptophan residues in the subject protein that are caused by changes in protein conformation upon binding by a nonabsorbing ligand. Within each experiment, both the binding reaction volume (0.5–1.0 ml) containing bacterially expressed USP and the concentration of USP (0.1–0.4 μM) were held constant. Because only the two tryptophan residues in the USP contribute to the fluorescence signal, the concentration of USP cannot be lower than 0.1–0.4 μM. Ligands prepared as EtOH stocks were added to the binding reaction to keep the final EtOH concentration below 0.2%. After incubation for at least 6 h at 15°C, the binding reactions were excited at 290 nm, the fluorescence emission was measured at 336 nm (emission max for unliganded USP, prepared as described here), and/or the fluorescence spectrum was scanned between 305 and 400 nm. The protein concentration in each experiment was adjusted so that the fluorescence was linear with protein concentration (between 20 and 250 fluorescence units at 336 nm). The highest concentration of ligand used (10 μM) in assays testing individual ligands, and which was used for all ligands in the competitive binding studies, was selected to be near saturating with respect to USP, but lower than the concentration at which JH ester forms micelles (500 μM). The ligands used did not significantly fluoresce between 305 and 400 nm when excited at 290 nm, absorb at 305–400 nm, or quench USP excitation at this concentration (not shown). Preliminary tests established that GST alone and GST plus JH III ester (10 μM) gave essentially superimposable fluorescence curves, showing that JH does not interact with GST alone to suppress GST fluorescence. Trace Factor Xa in the preparation was present below the limit of fluorescent detectability.

Yeast Two-Hybrid Assay. *Drosophila* USP cDNA, kindly provided by Vince Henrich, University of North Carolina, was cloned from position 469 to 1686 (N-terminal A/B region deleted) into the vector pEG202 that provides a LexA fusion protein upstream of the USP sequence (23). The dimeric target of this bait protein was the same USP fragment fused to a B42 activation domain (30). These two expression constructs were transformed into yeast that also contained a reporter plasmid containing a LexA operon situated upstream of a β-galactosidase coding sequence. At inoculation for overnight growth, the liquid yeast cultures were brought to their respective ligand concentrations or inoculated with ethanol carrier only. When the yeast growth reached an OD_{600} of 0.5–1.0, the yeast were collected, lysed, and assayed for β-galactosidase activity using *o*-nitrophenyl-β-galactopyranoside substrate. Extracts yielding high readings were diluted so as to retain measurements in the linear region of product vs. absorbance. Preliminary tests confirmed that LexA/USP neither has a spurious protein–protein interaction in yeast with B42 nor activates the reporter promoter from a site other than the LexA operon. Nor does LexA/USP expressed alone in yeast respond to JH by stimulating production of the reporter β-galactosidase.

RESULTS

JH Induces USP Association into a USP-Dependent Complex. Participation of USP in a JH-induced protein complex was detected by an electrophoretic mobility shift assay by using an oligomer probe containing a LexA operon and extracts from yeast containing a LexA/USP fusion protein, grown in the presence or absence of JH III ester (Fig. 1). LexA/USP in extracts from yeast grown in the absence of JH formed a single, specific complex with the probe (Fig. 1 *A* and *D*). When JH III ester was included in the liquid culture medium during growth, the resultant extracts (LexA/USP) yielded an additional spe-

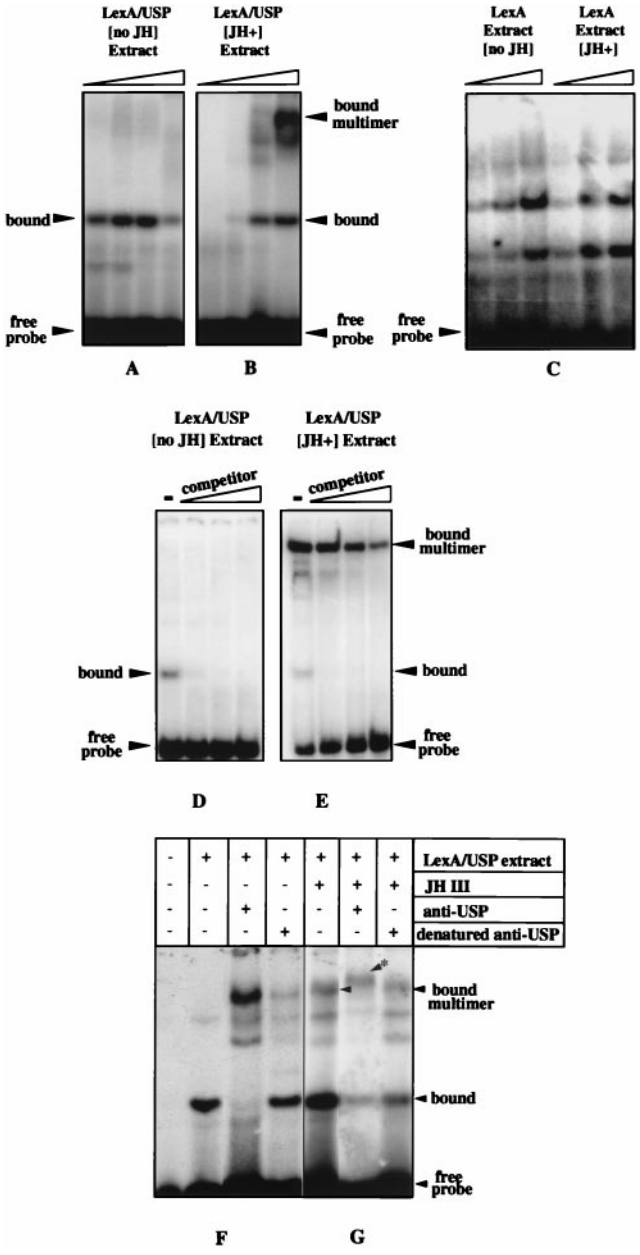

FIG. 1. JH III induces the formation of a USP-dependent, protein–DNA complex. (*A*) In the absence of JH, a single specific complex was formed between the LexA/USP fusion protein and the probe. (*B*) Inclusion of JH III ester in the liquid culture medium resulted in the formation of an additional major, slower migrating complex ("bound multimer"). (*C*) Addition of JH caused no differences in the migration of complexes formed by LexA alone, demonstrating that induction of the new protein–DNA complex by JH in *B* was dependent on USP. (*D* and *E*) The complexes formed with the probe using no-JH and JH+ extracts were specifically competed by cold probe reaching ×100 excess. The identity of the LexA/USP fusion as a component of the DNA–protein complex under nonhormone (*F*) and hormone-added (*G*) conditions was verified by its being supershifted after addition of a mAb against USP. Heat denaturation of the anti-USP antibody inactivated its effect on the mobilities of the complexes.

cific complex, migrating slower than the complex for extracts from cells not treated with JH (Fig. 1 *B* and *E*). This JH-induced change in migration of the protein–DNA complexes was mediated by USP because addition of JH had no effect on the nature of the complexes formed when USP was deleted from the fusion protein (Fig. 1*C*). The presence of the LexA/USP fusion protein in the specific complexes formed in no-JH extracts (Fig. 1*F*) and in JH+ extracts (Fig. 1*G*) was demonstrated directly by addition of an mAb against USP, which supershifted the migration of the complexes. These data suggest that the addition of JH resulted in the association of the expressed monomer LexA/USP fusion proteins into a USP-dependent, higher order protein–DNA complex.

JH Binds to USP, Altering its Conformation. The binding of JHs to recombinant USP was assessed by a physical method that monitors changes in protein fluorescence to detect the differences in receptor conformation induced by different ligands. Binding of the two natural *Drosophila* JHs (JH III ester monoepoxide and bisepoxide, respectively) was observed to strongly suppress the fluorescence of USP (Fig. 2 *A* and *B*), to an extent within the range reported for a number of other protein–ligand interactions (31–35). The specificity of JH III ester binding to USP was indicated by failure of either farnesol or 20-OH ecdysone to suppress fluorescence (Fig. 2*C*, upper three curves). As shown in Fig. 3, JH-induced changes in fluorescence of USP were observed when initial concentrations of JH ester added were as low as 100 nM. The concentration of USP protein used in the fluorescence assay shown in Fig. 3 was 100 nM, and the change in fluorescence signal was not fully saturated at the maximal amount of ligand added. Thus, it is not possible to straightforwardly calculate a dissociation constant from the titration of the effect of ligand on the fluorescence of USP (25). We estimate that, under these conditions, the dissociation constant for this interaction is $<0.5\ \mu$M, the midpoint of the titration in Fig. 3. From the data presented in Figs. 2 and 3, we infer that both of the natural *Drosophila* JH ester ligands bind with specificity to the cognate *Drosophila* USP.

JH Acid Binds USP, Inducing a Different USP Conformation than Does JH Ester. Because the vertebrate estrogen receptor has been shown to adopt one conformation upon binding of its natural ligand but another conformation upon binding of the synthetic antagonist tamoxifen (36), we considered the possibility that hormones other than JH III and JH III bisepoxide might bind to USP and stabilize a different conformation that does not cause significant changes in fluorescence. Such binding could be detected by testing whether these other potential ligands compete with JH III ester for binding to USP. In such tests, we observed that, at concentrations as high as 10 μM, farnesol, 20-OH ecdysone, and methylfarnesoate (data not shown) did not competitively inhibit the JH III ester-induced suppression in USP fluorescence (Fig. 2*C*, lower three curves). However, inclusion of JH III acid in equimolar concentrations with JH III ester reproducibly yielded an intermediate level of fluorescence suppression (Fig. 2*A*) whereas the same concentration of JH III acid alone did not cause suppression of fluorescence (Figs. 2*A* and 3). These data indicate that JH acid does bind to USP but in a manner that results in a USP conformation that does not suppress USP fluorescence and thus is different from the conformation caused by JH III ester.

JH Induces Oligomerization of USP. As postulated above, one consequence of JH binding to USP might be to stabilize a homodimer or oligomer of the receptor. The action of JH III to induce USP oligomerization *in vivo* was demonstrated with the two-hybrid system. A target protein encoded by a fusion of USP with a transcriptional activation domain (B42) was expressed in cells along with the LexA/USP bait protein. If JH promotes oligomerization of USP, then transcription of a reporter gene containing a LexA binding site should be strongly induced by the hormone. In fact, JH III ester strongly induced transcription of the β-galactosidase reporter in cells containing this target–bait combination, JH III acid gave a weaker effect, and the structurally related compound farnesol was inactive (Table 1). This result strongly supports the inference that JH III promotes at least homodimerization of USP.

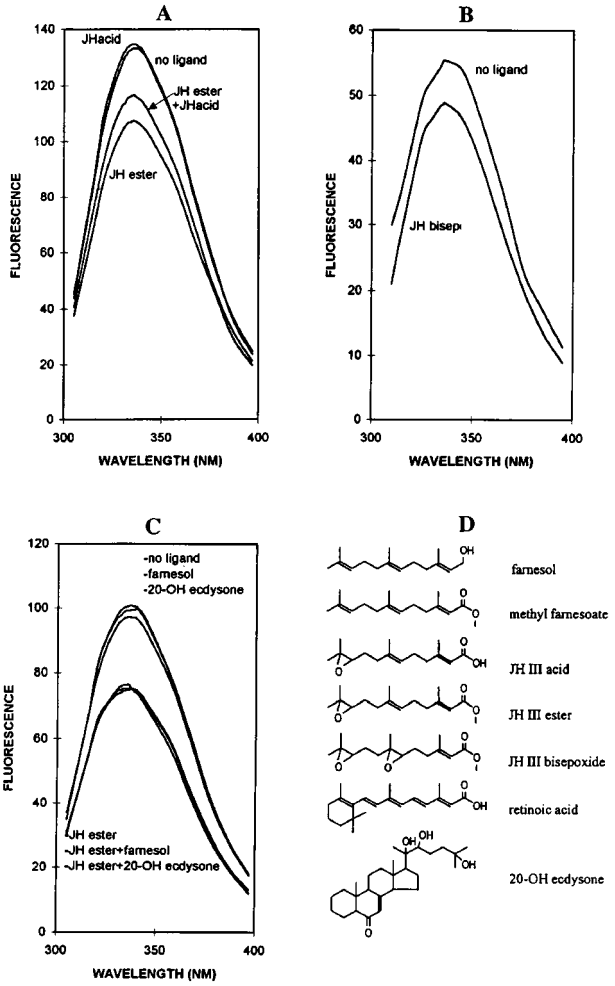

FIG. 2. Competitive binding assays show specific binding of biologically active *Drosophila* JHs to *Drosophila* USP. (*A*) The natural *Drosophila* hormone JH III ester distinctly suppressed the fluorescence of USP whereas addition of JH III acid had little effect. When incubated in combination, addition of JH acid interfered with the suppression of fluorescence by JH ester, to yield an intermediate level of suppression. This result, reproduced with independent USP preparations, indicates that JH acid competitively binds to USP, but in a manner that causes a different conformational change than caused by JH III ester. (*B*) The other natural *Drosophila* JH, JH III bisepoxide, also bound to USP in a manner that caused a distinct suppression of USP fluorescence. (*C*) Higher (20-OH ecdysone) and lower (farnesol) products in the terpene biosynthesis pathway than JH did not significantly bind USP, as shown by the lack of suppression of fluorescence when present alone (upper three curves) and by their lack of competitive inhibition of the suppression induced by JH III ester (lower three curves) used in the competitive binding studies. (*D*) Ligands used in the competitive binding studies and the related compound all-*trans* RA.

DISCUSSION

Specificity of Binding Biologically Active JH Structures. The above results demonstrate that USP can bind specifically to ligands in the JH family of structures (Fig. 2*D*). This conclusion is supported by USP's similar interaction with the

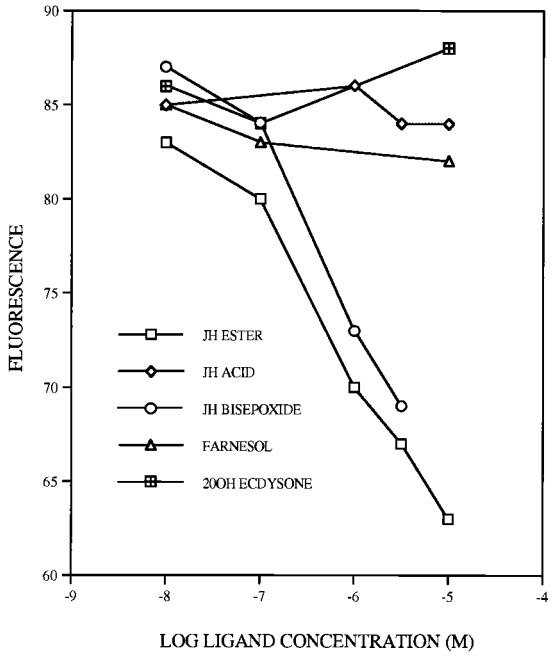

FIG. 3. Fluorescence detection of ligand binding to USP. Similar affinity of USP for both natural JH esters of *Drosophila*. Both JH III ester and JH III ester bisepoxide induced similar detected patterns of suppression in the fluorescence of USP at 100 nM and higher concentrations whereas farnesol, JH acid, and 20-OH ecdysone did not have a suppressive effect, even at 10 μM. The two tryptophan residues in USP impart a level of fluorescence such that the concentration of USP in the binding experiments must be at least 100 nM, which in some reactions is similar to or greater than the concentration of free ligand added. Thus, the binding affinity of USP for JH III ester and JH III ester bisexpoxide may be even stronger than that shown by the binding curves here, plotted with initial concentrations of added ligand.

two known natural JH esters in *Drosophila*, by its differential binding to the immediate nonepoxidized JH precursor (methyl farnesoate) and the immediate JH ester metabolite JH acid, by the high discrimination made by the receptor for epoxidized JH structures vs. lower farnesoid and higher steroid products of the terpene biosynthesis pathway, by the concordancy of the activities observed for the ligands in yeast *in vivo* and in fluorescence and in DNA binding assays *in vitro*, and by the evolutionary relationship between USP and RXR, the latter of which binds the chemically related structure RA.

Transcriptional Implications of JH-Induced Changes in Tertiary/Quaternary Structure. USP will form a heterodimer with the ecdysone receptor (EcR), and this heterodimerization stabilizes the binding of the EcR to DNA (37, 38). Binding of the ligand 20-OH ecdysone to the EcR in the EcR/USP complex appears necessary to further stabilize binding of the heterodimer to its response element, from which the ecdysteroid-activated EcR/USP complex induces ecdysone-dependent transcription (38, 39). Other candidates for natural heterodimeric partners for USP have been proposed, including orphan receptors (40, 41). It has been shown that heterodimeric binding of USP monomer with another steroid superfamily nuclear receptor DHR38, which is distinct from EcR, sequesters USP from availability to heterodimerize with EcR. As anticipated, this physical sequestration of USP suppresses transcription of a EcR/USP-dependent promoter (41). The physical and functional data presented here using eukaryotic yeast cells indicate that esters of JH modulate oligomerization of USP and that esters and acids induce different conformational states of USP. Such oligomerization of USP also could suppress activation of transcription by heterodimers of USP and other receptors in a manner similar to DHR38. There are other possibilities in which the ester and acid of JH could regulate metamorphic transitions by stabilizing a homodimer that activates transcription or by acting synergistically with another hormone in modulating the activity of a heterodimer or by simply inducing a unique conformation in the USP monomer. Our data demonstrating that JH ester vs. JH acid each induce different conformational states to USP raise the possibility that these two different conformational states may confer different transcriptional activities to USP. Several receptors probably adopt different conformations upon binding different ligands. The example of estrogen receptors bound to estrogen or tamoxifen was mentioned previously (36). Recently, Kersten *et al.* presented evidence of two conformational states of RXR, one with a high affinity for the ligand 9–*cis* RA and one with a lower affinity (27).

Table 1. JH induction of interaction between LexA/USP bait and USP/B42 target in yeast two-hybrid system

Ligand	Ligand concentration, M	β-Gal reporter activity, mean ± SE	Fold > over, no ligand
JH III ester	10^{-5}	1.74 ± 0.25	8.3
	10^{-6}	0.34 ± 0.13	1.6
	10^{-7}	0.10 ± 0.03	0.5
JH III acid	10^{-5}	0.50 ± 0.12	2.4
	10^{-6}	0.18 ± 0.04	0.9
Farnesol	10^{-5}	0.17 ± 0.17	0.9
None	—	0.21 ± 0.16	1.0

Assays were done as described in *Materials and Methods*. Values given are the resultant absorbancy readings at 336 nm. $n = 3$ for each treatment. β-Gal, β-galactosidase.

Biological Relevance of Local Hormone Concentrations. The estimated dissociation constant for binding of JH ester for USP receptor is ≈0.5 μM. Although this is not a high affinity for most ligand/receptor binding, it is not unprecedented. For example, the affinity of methoprene acid for RXR is 1–2 orders of magnitude lower than that of JHIII for USP (20). The 0.5 μM dissociation constant is significantly higher than the estimated concentration of JH ester typically found in either whole body or serum concentrations of 0.01–0.1 μM (8). However, this is not inconsistent with the proposal that JH is a physiological regulator of USP. First, USP in a heterodimer with other receptors could have a higher affinity for JH (9). As an example, the EcR has a higher affinity for ecdysone as a heterodimer with USP than as a homodimer (38). Second, research on retinoid–receptor interactions has placed increasing importance on the local biological concentration of the hormone that the receptor is exposed to in the nucleus of the particular tissue (26, 28). Recent studies have emphasized that, when the RXR receptor is present at a near 1-μM concentration, as occurs in the nucleus (42), RXR forms tetrameric RXR aggregates with unique positively cooperative ligand-binding properties (29). Formation of these aggregates is highly sensitive to changes in ligand concentration in the region of 1 μM (26), which may occur locally in the nucleus. It is possible, and in fact likely, that JH is present locally at higher concentrations than the average whole body or serum concentrations of 0.01–0.1 μM. A dramatic example is the male accessory gland, in which JH accumulates to such a high concentration (≈10 μM; G. Bhaskaran, personal communication) that for a number of years the gland was used as a source of the natural hormone (43).

Ligand Binding Site of Retinoid Receptors. USP is similar to the superfamily of hormone receptors for steroids in that it contains a conserved motif, the activating function-2 region. A number of orphan receptors that are not thought to bind a ligand do not contain this conserved motif (44). Recent crystallographic evidence from studies on RAR and RXR indicate that binding of ligand causes a conformational change in the position of the activating function-2 region, bringing

residues in it into positions favorable for interaction with transcriptional regulators (45, 46). Especially intriguing here is that USP can bind either JH ester or JH acid, in contrast to RXR, which does not bind RA analogs missing a free carboxyl group. Examination of the primary structure of USP (15) suggests that it conserves the two basic residues (Lys 287 and Arg 355) in positions near those corresponding to Lys 274 and Arg 316 of RAR that interact with the two carboxylate oxygens of RA (45, 46). USP also binds JH esters, which do not have free carboxyl groups, with distinctive effects on USP conformation. The motifs at the binding site of nuclear receptors that specify binding with ester ligands are not known, but the data presented here indicate that the ester and acid ligands interact with different conformational determinants of USP.

We are grateful to D. Jones for providing indispensable contributions to the fluorescence studies; to M. Roe for providing the HPLC-purified, 97% pure JH III acid; to L. Gilbert for providing JH III bisepoxide; to J. Sutherland and F. Kafatos for the *Drosophila* USP cDNA clone in a bacterial expression vector and anti-USP mAb; to H. Laufer for providing the HPLC-purified, 98% pure all-*trans* methyl-farnesoate; to V. Henrich for the original *Drosophila* cDNA clone for USP; to R. Brent and E. Golemis for vectors and helpful advice in the two-hybrid experiments; to S. Cahill for ready access to a fluorescence spectrometer; and to D. Sawchuk for pivotal assistance. This work was supported in part by grants from the National Institutes of Health (DK 39197 to G.J.; GM34277 and AI32486 to P.A.) and the National Science Foundation (DCB 9005184 to G.J.) and partially supported by a National Cancer Institute Center Core Grant (CA14051).

1. Riddiford, L. M. (1991) in *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, eds. Kerkut, G. A. & Gilbert L. I. (Pergamon, Oxford), Vol. 8, pp. 37–84.
2. Riddiford, L. M. (1993) in *The Development of Drosophila melanogaster*, eds. Bate, M. & Martinez-Arias, A. (Cold Spring Harbor Lab. Press, Plainview, NY), Vol. 2, pp. 899–939.
3. Gudas, L., Sporn, M. & Roberts, A. (1994) in *The Retinoids: Biology, Chemistry and Medicine*, eds. Sporn, M., Roberts, A. & Goodman, D. (Raven, New York).
4. Jones, G. (1995) *Annu. Rev. Entomol.* **40,** 147–169.
5. Riddiford, L. M. (1996) *Arch. Insect Biochem. Physiol.* **32,** 271–286.
6. Bruno, J. M., Feacheun, R., Godal, T., Nchinda, T., Ogilvie, B., Mons, B., Mshana, R., Radda, G., Samba, E., Schwartz, M., Varmus, H., Diallo, S., Doumbo, O., Greenwood, B., Kilama, W., Miller, L. H. & da Silva, L. P. (1997) *Nature (London)* **386**, 541.
7. Bhaskaran, G., Sparagana, S. P., Barrera P. & Dahm, K. H. (1986) *Arch. Insect Biochem. Physiol.* **3,** 321–338.
8. Baker, F. C., Tsai, L. W., Reuter, C. C. & Schooley, D. A. (1987) *Insect Biochem.* **12,** 989–996.
9. Moore, D. D. (1990) *New Biol.* **2,** 100–105.
10. Laufer, H. & D. Borst, D. (1983) in *Endocrinology of Insects*, eds. Downer, G. H. & Laufer, H. (Liss, New York), pp. 203–216.
11. Heyman, R. A., Mangelsdorf, D. J., Dyck, J. A., Stein, R. B., Eichele, G., Evans, R. M. & Thaller, C. (1992) *Cell* **68,** 397–406.
12. Levin, A. A., Sturzenbecker, L. J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeisen, C., Rosenberger, M., Lovey, A. & Grippo, J. F. (1992) *Nature (London)* **355,** 359–361.
13. Allenby, G., Bocquel, M.-T., Saunders, M., Kazmer, S., Speck, J., Rosenberger, M., Lovey, A., Kastner, P., Grippo, J. F., Chambon, P. & Levin, A. A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 30–34.
14. Lemotte, P. K., Keidel, S. & Apfel, C. M. (1996) *Eur. J. Biochem.* **236,** 328–333.
15. Oro, A. E., McKeown, E. & Evans, R. M., (1990) *Nature(London)* **347,** 298–301.
16. Henrich, V. C., Szekely, A. A., Kim, S. J., Brown, N. E., Antoniewski, C., Hayden, M., Lespesant, J.-A. & Gilbert, L. I. (1994) *Dev. Biol.* **165,** 38–52.
17. Shea, M. J., King, D. L., Conboy, M. J., Mariani, B. D. & Kafatos, F. C. (1990) *Genes Dev.* **4,** 1128–1140.
18. Yao, T. P., Segraves, W. A., Oro, A. E., McKeown, M. & Evans, R. M. (1992) *Cell* **71,** 63–72.
19. Yao, T. P., Forman, B. M., Jiang, Z., Cherbas, L., Chen, J. D., McKeown, M., Cherbas, P. & Evans, R. M. (1993) *Nature (London)* **366,** 476–479.
20. Harmon, M. A., Boehm, M. F., Heyman, R. A. & Mangelsdorf, D. J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 6157–6160.
21. Bownes, M. & Rembold, H. (1987) *Eur. J. Biochem.* **164,** 709–712.
22. Richard, D. S., Applebaum, S. W., Sliter, J. J., Baker, F. C., Schooley, D. A., Reuter, C. C., Henrich, V. C. & Gilbert, L. I. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1421–1425.
23. Golemis, E. A. & Brent, R. (1992) *Mol. Cell Biol.* **12,** 3006–3014.
24. Chen, Z. P., Shemshedini, L., Durand, B., Noy, N., Chambon, P. & Gronemeyer, H. (1994) *J. Biol. Chem.* **269,** 25770–25776.
25. Kersten, S., Pan, L., Chambon, P., Gronemeyer, H. & Noy, N. (1995) *Biochemistry* **34,** 13717–13721.
26. Kersten, S., Pan, L. & Noy, N. (1995) *Biochemistry* **34,** 14263–14269.
27. Kersten, S., Dawson, M. I., Lewis, B. A. & Noy, N. (1996) *Biochemistry* **35,** 3816–3824.
28. Kersten, S., Gronemeyer, H. & Noy, N. (1997) *J. Biol. Chem.* **272,** 12771–12777.
29. Kersten, S., Kelleher, D., Chambon, P., Gronemeyer, H. & Noy, N. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 8645–8649.
30. Estojak, J., Brent, R. & Golemis, E. A. (1995) *Mol. Cell. Biol.* **15,** 5820–5829.
31. Xu, Y., Nenortas, E. & Beckett, D. (1995) *Biochemistry* **34,** 16624–16631.
32. Csermely, P., Kajtar, J., Hollosi, M., Jalsovszky, G., Holly, S., Kahn, C. R., Gergely, P., Jr., Soti, C., Mihaly, K. & Somogyi, J. (1993) *J. Biol. Chem.* **268,** 1901–1907.
33. Chang, W. I. & Matthews, K. S. (1995) *Biochemistry* **34,** 9227–9234.
34. House-Pompeo, K., Xu, Y., Joh, D., Speziali, P. & Hook, M. (1996) *J. Biol. Chem.* **271,** 1379–1384.
35. Collins, R. A., Kelly, S. M., Price, N. C., Fothergill-Gilmore, L. A. & Muirhead, H. (1996) *Protein Eng.* **9,** 1203–1210.
36. Berry, M., Metzger, D. & Chambon, P. (1990) *EMBO J.* **9,** 2811–2818.
37. Thomas, H. E., Stunnenberg, H. G. & Stewart, A. F. (1993) *Nature (London)* **362,** 471–475.
38. Yao, T.-P., Forman, B. M., Jiang, Z., Cherbas, L., Chen, J.-D., McKeown, M., Cherbas, P. & Evans, R. M. (1993) *Nature (London)* **366,** 476–479.
39. Antoniewski, C., Laval, M., Dahan, A. & Lepesant, J. A. (1994) *Mol. Cell. Biol.* **14,** 4465–4474.
40. Zelhof, A., Yao, T.-P., Chen, J. D., Evans, R. M. & McKeown, M. (1995) *Mol. Cell. Biol.* **15,** 6736–6745.
41. Sutherland, J. D., Kozlova, T., Tzertinis, G. & Kafatos, F. C. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7966–7970.
42. Nervi, C., Grippo, J. F., Sherman, M. I., George, M. D. & Jetten, A. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5854–5858.
43. Shirk, P. D., Dahm, K. H. & Roller, H. (1976) *Z. Naturforsch., C* **31,** 199–200.
44. Durand, B., Saunders, M., Gaudon, C., Roy, B., Losson, R. & Chambon, P. (1994) *EMBO J.* **13,** 5370–5382.
45. Renaud, J. P., Rochel, N., Ruff, M., Vivat, V., Chambon, P., Gronemeyer, H. & Moras, D. (1995) *Nature (London)* **378,** 681–689.
46. Bourguet, W., Ruff, M., Chambon, P., Gronemeyer, H. & Moras, D. (1995) *Nature (London)* **375,** 377–382.