Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 7966–7970, August 1995
Developmental Biology

Drosophila hormone receptor 38: A second partner for *Drosophila* USP suggests an unexpected role for nuclear receptors of the nerve growth factor-induced protein B type

(ecdysone action/receptor interaction/retinoid X receptor)

JAMES D. SUTHERLAND*†, TATYANA KOZLOVA*†‡, GEORGE TZERTZINIS*†, AND FOTIS C. KAFATOS*†

*Department of Molecular and Cell Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138; †European Molecular Biology Laboratory, Postfach 10.2209, 69012 Heidelberg, Germany; and ‡Institute of Cytology and Genetics, Novosibirsk 630090, Russia

Contributed by Fotis C. Kafatos, May 11, 1995

ABSTRACT **In *Drosophila* the response to the hormone ecdysone is mediated in part by Ultraspiracle (USP) and ecdysone receptor (EcR), which are members of the nuclear receptor superfamily. Heterodimers of these proteins bind to ecdysone response elements (EcREs) and ecdysone to modulate transcription. Herein we describe *Drosophila* hormone receptor 38 (DHR38) and *Bombyx* hormone receptor 38 (BHR38), two insect homologues of rat nerve growth factor-induced protein B (NGFI-B). Although members of the NGFI-B family are thought to function exclusively as monomers, we show that DHR38 and BHR38 in fact interact strongly with USP and that this interaction is evolutionarily conserved. DHR38 can compete *in vitro* against EcR for dimerization with USP and consequently disrupt EcR–USP binding to an EcRE. Moreover, transfection experiments in Schneider cells show that DHR38 can affect ecdysone-dependent transcription. This suggests that DHR38 plays a role in the ecdysone response and that more generally NGFI-B type receptors may be able to function as heterodimers with retinoid X receptor type receptors in regulating transcription.**

The nuclear hormone receptor superfamily contains a large number of evolutionarily related transcription factors that mediate the action of small molecules such as steroid hormones. Members of this superfamily function by binding to short DNA sequences within gene promoters called hormone response elements, which usually consist of variably spaced repeats of 5 or 6 nt. Most receptors bind to the hormone response elements as homo- or heterodimers, reflecting the repetitive substructure of hormone response elements (1). Several members have been identified, however, that bind DNA as monomers (2).

Insect genomes include several genes that encode members of the nuclear receptor superfamily (3). Most of them are so-called orphan receptors, since no ligands have been identified that directly use them for signaling. Notable exceptions are the ecdysone receptor (EcR) and Ultraspiracle (USP) proteins, which mediate the action of the steroid hormone ecdysone. EcR and USP form functional heterodimers as a prerequisite for hormone and DNA binding (4–7). Ecdysone plays a key role in insect metamorphosis by triggering a cascade of gene expression that is thought to be modulated by changing hormone titers and regulated expression of receptors. Ecdysone titers show complex dynamics that are related to the developmental program (8). Moreover, there are several isoforms of EcR that are developmentally modulated and may have distinct functions (9–11). USP is expressed in many tissues throughout development with fluctuations in mRNA and protein levels (12). An important question in insect endocrinology is whether ecdysone action solely involves these three components (ecdysteroid, EcR, and USP) or whether other components, such as additional members of the hormone receptor superfamily, might be directly implicated. Thus far USP has only a single known partner, EcR (3). In contrast, vertebrate retinoid X receptors (RXRs), which are the USP homologues, function as heterodimers with multiple partners such as the retinoic acid, thyroid hormone, and vitamin D receptors (13).

We describe here two insect nuclear receptors§ *Drosophila* hormone receptor 38, DHR38, and *Bombyx* hormone receptor 38, BHR38, that are most closely related to a group of vertebrate orphan receptors typified by nerve growth factor-induced protein B (NGFI-B). The vertebrate members of this group are notable in being able to bind DNA as monomers and are also of interest because of their rapid and transient activation by serum, growth factors, and mitogens (14–16). DHR38 shows the unexpected property of interacting strongly with USP and altering its DNA binding properties. DHR38 can disrupt EcR–USP heterodimers *in vitro* and in cell culture, apparently because of its interaction with USP. This interaction is highly conserved in evolution. Therefore, we propose that NGFI-B type receptors can participate in and interfere with RXR-mediated signaling. In insects DHR38 may play an important role in modulating the ecdysone response.

MATERIALS AND METHODS

Cloning of BHR38 and DHR38. The DNA binding domain of BHR38 was isolated by PCR by using degenerate oligonucleotides designed to amplify nuclear receptors (17). This fragment was used to isolate the BHR38 clone from an ovarian cDNA library. The same fragment was used to isolate DHR38 *Drosophila* genomic clones (unpublished data), which in turn were used to screen a cDNA library made from ecdysone- and cycloheximide-treated larval organs (a gift from C. Thummel, University of Utah). The predicted open reading frame from one of the isolated cDNAs, cTK11, is shown in Fig. 1*A*. The amino acid sequences of DHR38, BHR38, and NGFI-B were aligned by the CLUSTALW program (18) and manually adjusted. A multiple alignment of NGFI-B type sequences (Fig. 1*B*) and FTZ-F1 (GenBank accession no. M63711) was generated by using CLUSTALW. The A/B domains and residues 477–577 of FTZ-F1 were removed to avoid excessive gaps, and a neighbor-joining tree was calculated.

Abbreviations: USP, Ultraspiracle; EcR, ecdysone receptor; DHR38, *Drosophila* hormone receptor 38; BHR38, *Bombyx* hormone receptor 38; NGFI-B, nerve growth factor-induced protein B; RXR, retinoid X receptor; GST, glutathionine *S*-transferase; EMSA, electrophoretic mobility shift assay; CAT, chloramphenicol acetyltransferase.

§The sequences reported in this paper have been deposited in the GenBank data base [accession nos. X89246 (DHR38) and X89247 (BHR38)].

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.

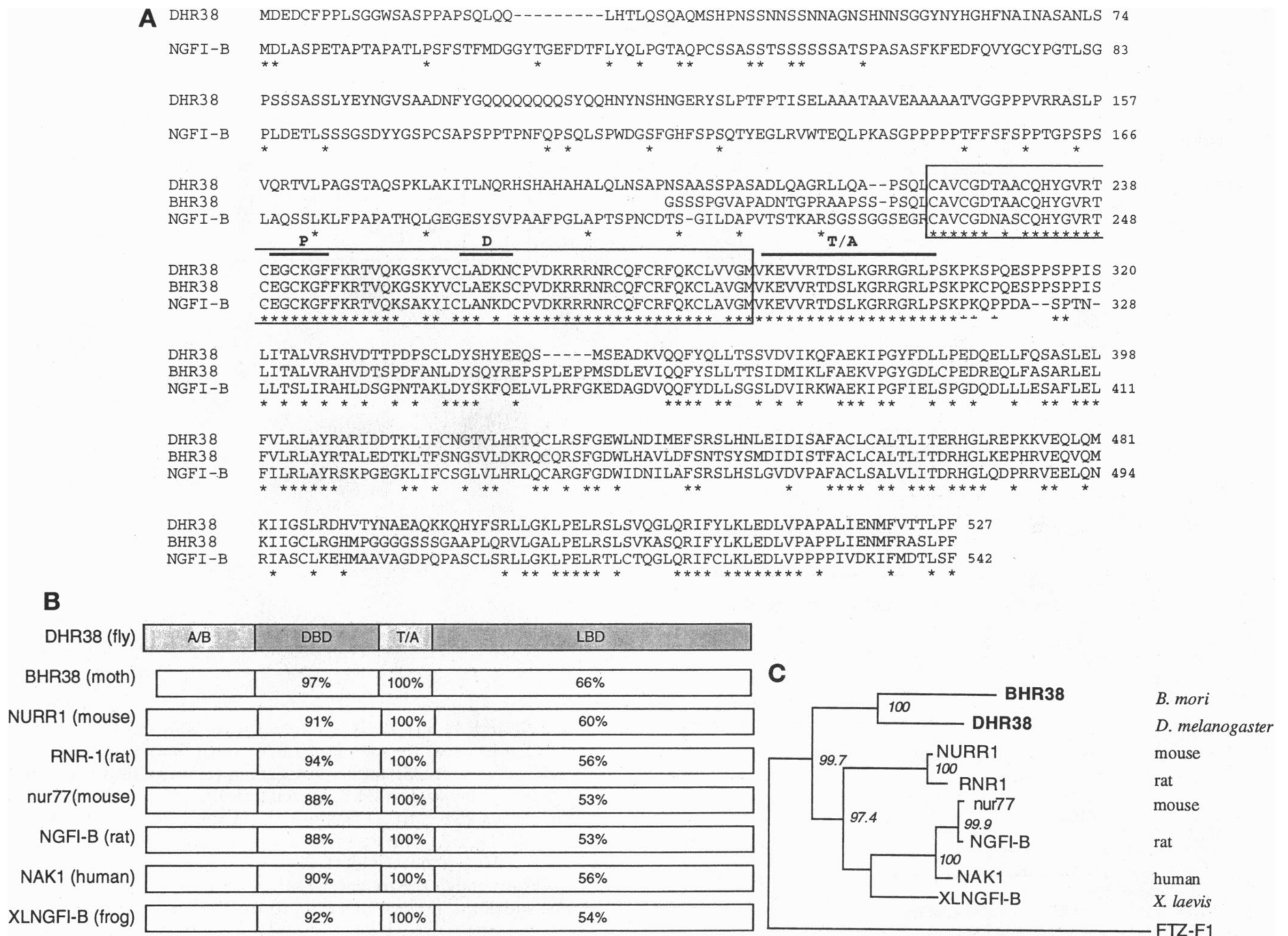

FIG. 1. DHR38 and BHR38 are members of the NGFI-B family. (*A*) Amino acid sequence comparison of the predicted amino acid sequences of the complete DHR38 and NGFI-B and partial BHR38 proteins. Asterisks indicate conserved residues and dashes represent gaps. The strongly conserved DNA binding domain is outlined and the P, D, and T/A boxes (19) are overlined. (*B*) Domain comparison of DHR38, BHR38, RNR-1 (GenBank accession no. L08595), XLNGFI-B (GenBank accession no. X70700), NURR1 (GenBank accession no. S53744), NAK1 (Swiss-Prot accession no. P22736), nur77 (GenBank accession no. J04113), and NGFI-B (Swiss-Prot accession no. P22829). Percentages indicate identity to DHR38 amino acid sequence. A/B, N-terminal domain; DBD, DNA binding domain; LBD, ligand binding domain. (*C*) Molecular phylogeny of the NGFI-B family. Amino acid sequences minus the A/B domain were aligned and used to generate a neighbor-joining tree. Bootstrap values of the nodes (above the 50% level) are shown in italic type as percentages of 1000 replicates. The tree was rooted with FTZ-F1 as the outgroup and topology was corroborated by maximum parsimony analysis (data not shown).

Protein Interactions in Yeast. LexA or B42 fusions were constructed from appropriate PCR fragments that were subcloned into vectors pEG202 or JG4-5, respectively (20). Reading frame and protein molecular weight were confirmed by DNA sequencing and Western blot analysis of yeast extracts, respectively. β-Galactosidase assays were performed as described (21).

Protein Production and Binding Assay. Schneider S2 or S3 cell nuclear extract and USP antibody were prepared as described (22, 23). Fragments used in constructing BHR38 and DHR38(214) (Fig. 2) were cloned into pGEX-1 to create the glutathione *S*-transferase (GST) fusions. Protein production and purification were performed as described (25). GST fusion protein (0.4 μg) immobilized on glutathione-Sepharose beads and 10 μg of S2 extracts were incubated in 50 mM Tris·HCl, pH 7.5/150 mM NaCl/0.5% Triton X-100 (TBST) in the presence of bovine serum albumin (0.1 μg/μl) at 4°C for 30 min, washed three times with TBST, and examined by electrophoresis and immunoblot analysis.

Electrophoretic Mobility Shift Assay (EMSA). EMSAs were performed by using 2 μg of S3 cell extract and purified GST or GST fusion protein (10 ng/μl) in 10 μl, as described (22). The hsp27EcRE (5) and B1A (19) probes were end-labeled with [γ-^{32}P]ATP. To resolve supershifted complexes, the free probe was allowed to migrate off the gel and only shifted complexes are shown. EcR monoclonal antibody (DDA2.7) was a gift of D. S. Hogness (Stanford University).

Transfection. hsp27EcRE–CAT contains one copy of the sequence (AGGGTTCAATGCACT) upstream of the thymidine kinase minimal promoter (tkCAT) (5). The hsp70–DHR38 contains the DHR38 cDNA in the plasmid pCaSpeR-hs. Schneider S3 cells were transfected by calcium phosphate precipitation. pPAc-β-galactosidase (0.3 μg) was added as a transfection control and DHR38 was induced at 37°C for 30 min (26). After a 90-min recovery at 25°C, 20-hydroxyecdysone (Sigma) was added at 2 μM. After 4 h of hormone treatment, the cells were harvested, chloramphenicol acetyltransferase (CAT) assays were performed (27), and the average of two experiments was calculated and plotted.

RESULTS AND DISCUSSION

Cloning of Insect Homologues of Vertebrate NGFI-B Type Receptors. While cloning the cDNA encoding the USP homologue from the silkmoth *Bombyx mori* (17), we recovered a

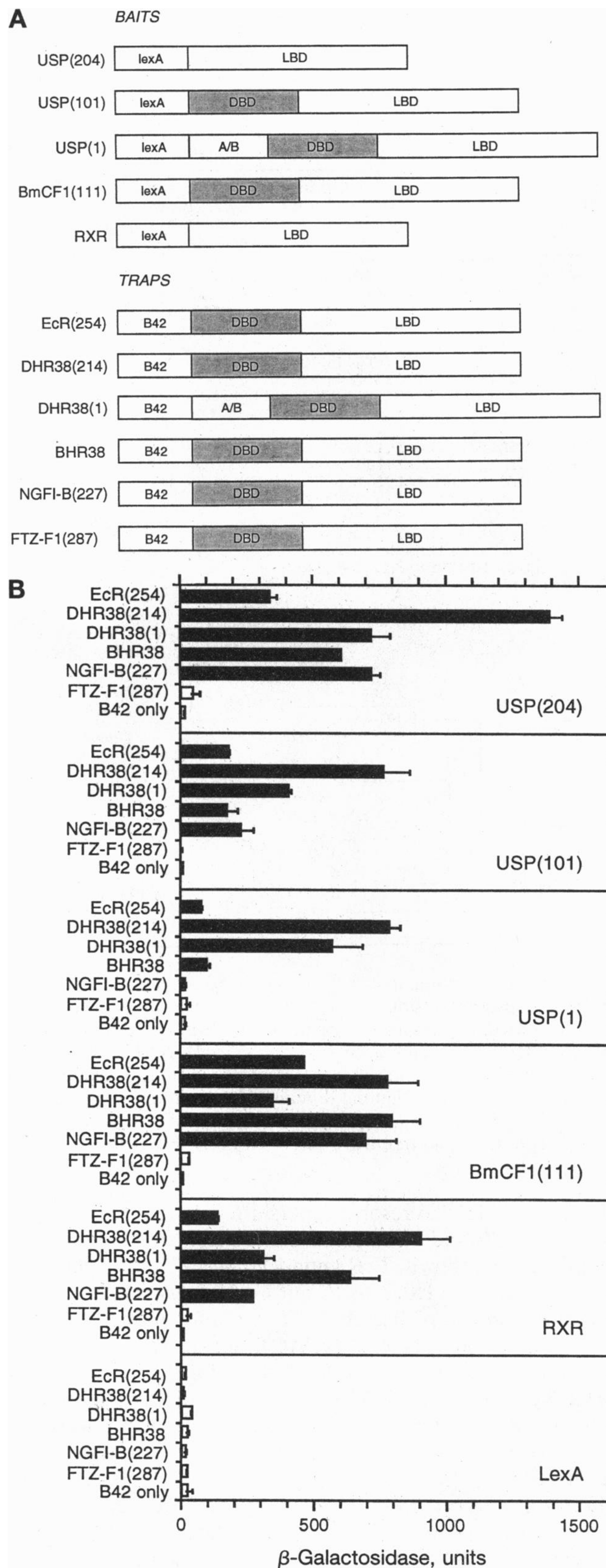

FIG. 2 Yeast two-hybrid assay reveals evolutionarily conserved interactions between nuclear hormone receptors. (*A*) Diagram of the fusion proteins used in this study. Baits consisted of domains of USP (*Drosophila*), BmCF1 (*Bombyx*), and RXR (human) fused to the LexA DNA binding domain (DBD). Traps consisted of domains of EcR and DHR38 (*Drosophila*), BHR38 (*Bombyx*), NGFI-B (rat), and FTZ-F1 (*Drosophila*) fused to the B42 acidic activation domain. Numbers in parentheses refer to the first amino acid of each receptor fragment. The RXR cônstruct has been described as LexARXR (24). The BHR38 construct is not numbered as it was constructed from an incomplete cDNA; it begins with the second serine N-terminal to the DNA binding domain. (*B*) USP/RXR type and NGFI-B type receptors can interact in yeast. Combinations of LexA and B42 fusions were cotransformed into yeast strain EGY48 containing a reporter plasmid (pSH18-34; eight LexA operators fused to the β-galactosidase gene; ref. 20). Cultures were harvested and enzyme activity was measured. Error bars represent 1 SD. EcR, which is known to interact with USP and other RXR receptors (4–7), was used as a positive control. FTZ-F1, LexA DNA binding domain alone, and B42 activation domain alone were used as negative controls (open bars). Each bar represents the average of at least two transformations.

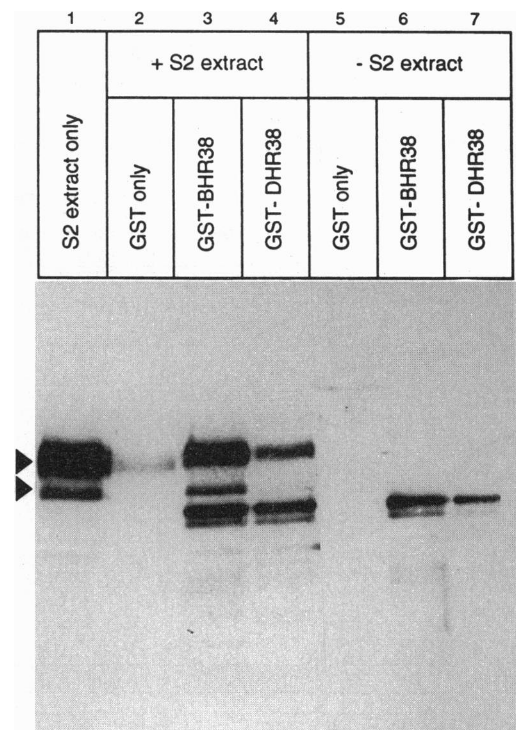

FIG. 3. DHR38 and BHR38 interact with USP in solution. USP protein (arrowheads) was detected on Western blots with USP antibody AB11 (or other USP antibodies, data not shown); the upper band corresponds to the known USP molecular weight (23). Schneider cell nuclear extract (S2 extract) was used as a source of USP (lane 1). GST–BHR38 or GST–DHR38 fusion proteins are able to interact and "pull-down" USP (lanes 3 and 4). GST alone cannot interact (lane 2). The USP antibody cross-reacts weakly with GST–DHR38 or GST–BHR38, which are present in excess (dots; lanes 5 and 6).

partial cDNA clone that encodes a nuclear receptor related to rat NGFI-B. A *Drosophila* homologue was then isolated by cross-hybridization and mapped by *in situ* hybridization to division 38 of salivary gland polytene chromosomes (data not shown). According to convention, the isolated fly receptor was named *Drosophila* hormone receptor 38 (DHR38) and its *Bombyx* homologue was named BHR38.

The full-length DHR38 and partial BHR38 sequences belong to the NGFI-B family of nuclear receptors (Fig. 1 *A* and *B*), which have the unusual property of binding to DNA as monomers (28). Although DHR38 and BHR38 are most closely related to each other, they bear striking similarity to the vertebrate members of this family in the putative DNA binding domain (88–94% identity); the adjacent T/A box, which is thought to modulate DNA binding (29), is 100% identical across the family. In contrast, as is typical of the nuclear receptor superfamily, the N-terminal (A/B) domain is highly variable. The C-terminal ligand binding domain is intermediate, with 53–66% overall identity across the family and with

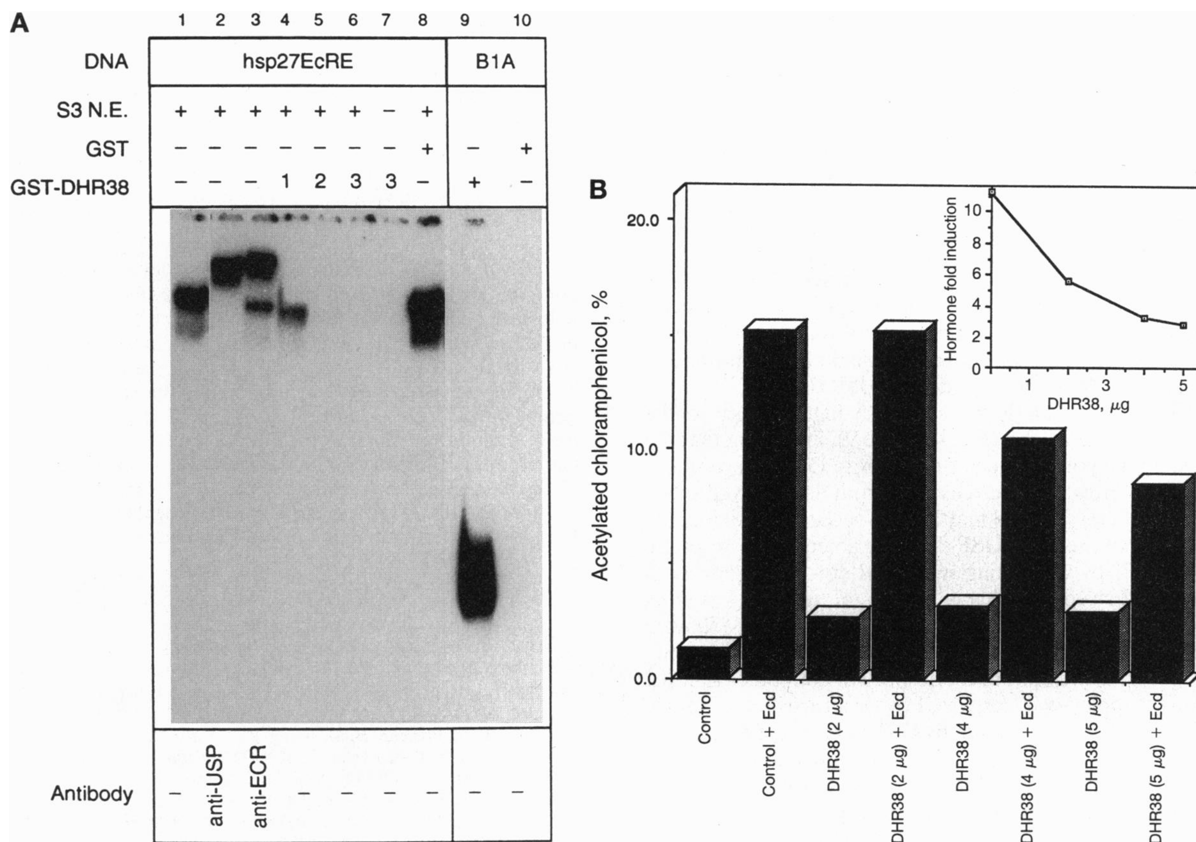

FIG. 4. DHR38 disrupts the USP–EcR heterodimer *in vitro* and represses ecdysone-induced transcription of a reporter gene in Schneider cells. (*A*) EMSAs were performed by using Schneider cell nuclear extract (S3 N.E.) and the hsp27 EcRE target site (5). One complex is observed (lane 1) that contains the USP–EcR heterodimer, as shown by antibody supershifts (lanes 2 and 3). Adding increasing amounts (1–3 μl; 10 ng/μl) of GST–DHR38 protein (described in Fig. 3) disrupts this complex and does not create a new one (lanes 4–6). GST–DHR38 alone does not bind to the EcRE (lane 7), and GST alone does not disrupt USP–EcR (lane 8). By using purified GST–DHR38 and the B1A site of NGFI-B (19) a single complex is observed (lane 9) that does not form in the presence of GST alone (lane 10). (*B*) Induction of CAT activity in Schneider S3 cells transfected with 0.3 μg of hsp27EcRE–CAT and the indicated amounts of the hsp70–DHR38 plasmid. DHR38 causes a decrease in the ecdysone-stimulated transcriptional activation and an increase of the level of transcription in the absence of hormone. Effectively, this results in a steep drop in hormone fold induction (*Inset*). Ecd, Ecdysone.

several subsegments that are more highly conserved, possibly reflecting the diverse functions of this domain (30, 31).

Phylogenetic analysis of the amino acid sequences (excluding the A/B domain) by the neighbor-joining method (Fig. 1*C*) confirms that DHR38 and BHR38 are more similar to vertebrate NGFI-B type receptors than to *Drosophila* FTZ-F1 (32) (another monomeric receptor) or dimerizing receptors such as USP and EcR (data not shown). The identification of insect members of the NGFI-B family establishes that this family, like USP/RXR receptors, existed prior to separation of the arthropod and vertebrate lineages. The family further diverged within the vertebrates, with an apparent gene duplication giving rise to two clades: NGFI-B/nur 77 and NURR1/RNR1. The latter branch and the insect receptors appear to share numerous ancestral features, namely 70% (DHR38) or 51% (BHR38) of the residues at the 43-amino acid positions that distinguish between the vertebrate clades. The NGFI-B/nur 77 branch shows greater divergence, matching DHR38 and BHR38 in only 30% and 35%, respectively, of the distinguishing positions. It remains to be established whether this pattern of conservation and divergence is functionally significant.

Heteromerization Properties of BHR38 and DHR38. We have been using the yeast two-hybrid system (20) to screen known vertebrate and invertebrate receptors for interaction with members of the RXR/USP family. Surprisingly, we discovered a strong, specific, and evolutionarily conserved interaction between insect or vertebrate NGFI-B type receptors and members of the RXR family. The fusion protein constructs we made included "baits" (Fig. 2*A*) that combine the lexA binding domain with USP, *Bombyx mori* USP (BmCF1), or human RXRα and "traps" that combine the acidic activation domain B42 with DHR38, BHR38, or NGFI-B. When cotransformed into a yeast strain that carried a *lacZ* reporter downstream of lexA binding sites, pairwise combinations of these RXR and NGFI-B type fusions led to strong β-galactosidase expression (Fig. 2*B*). Similarly, the EcR positive control interacted with all RXRs, as expected (4–7), whereas the FTZ-F1 additional control failed to interact with RXRs, consistent with its known monomeric nature (32). Without fusions, the lexA or B42 fragments were incapable of interactions. In addition, a fusion containing a USP C-terminal deletion (missing the last 52 amino acids) that disrupts the ability of USP to interact with EcR also disrupts its interaction with DHR38 (data not shown).

In these experiments, different fusion proteins were expressed in comparable amounts, as estimated by Western blot analysis (data not shown). Thus, β-galactosidase activities reflected the strength of interaction between bait and trap, although more accurate measures of affinity (e.g., dissociation constants) were not determined. The interactions of DHR38 with RXR type receptors were invariably enhanced in the absence of the DHR38 A/B domain. Similarly, the strongest interactions involving USP were observed by using its ligand binding domain alone. It is interesting that inclusion of the A/B domain makes the USP interactions more species specific: full-length USP failed to interact significantly in the cross-species combination with NGFI-B, but the conspecific com-

bination of full-length USP and full-length DHR38 showed strong interaction. In general, of the 20 tested combinations between NGFI-B type and RXR type constructs, 19 showed significant interactions that were usually stronger than those shown between EcR and the same RXR type constructs.

Independent confirmation of interaction between USP and DHR38 was obtained by *in vitro* experiments based on purified fusion proteins containing the DNA binding and ligand binding domains of DHR38 and BHR38 fused with GST and by using previously characterized antibodies (23) to monitor USP (Fig. 3). When mixed with Schneider cell nuclear extracts, GST–DHR38 and GST–BHR38 (immobilized on glutathione-Sepharose beads) interacted with the endogenous USP (lane 1), which was recovered with the fusion proteins upon sedimentation (lanes 3 and 4). GST alone did not "pull down" USP (lane 2).

Like NGFI-B (19), DHR38 binds with high affinity to the single half-site element B1A (Fig. 4*A*, lane 9), indicating that the monomeric binding of this type of receptor to DNA is evolutionarily conserved. However, we reasoned from its observed interaction with USP that DHR38 may act in a second mode: it could prevent binding of the EcR–USP dimer to an ecdysone response element (EcRE) by competing with EcR for interaction with USP. EMSAs showed that this is indeed the case (Fig. 4*A*). A nuclear extract of S3 cells mixed with the hsp27 EcRE (5) showed a retarded complex (lane 1), which contained both EcR and USP, as shown by supershifting with the respective antibodies (lanes 2 and 3). This complex was destroyed by progressive addition of GST–DHR38 (lanes 4–6) but not by GST alone (lane 8). No new complex was observed in lanes 4–6, and thus the presumed USP–DHR38 heterodimer did not bind to the EcRE. Whether the heterodimer can bind to different DNA sequences remains to be determined.

Possible Implications for the *in Vivo* Functions of DHR38. We studied the effect of DHR38 on cultured Schneider cells, by cotransfection of variable amounts of DHR38 with a constant amount of an EcRE-driven CAT reporter plasmid. In the absence of hormone, DHR38 progressively raises CAT expression, whereas in the presence of hormone it lowers CAT expression (Fig. 4*B*). Significantly, DHR38 causes a drastic drop in the ecdysone-induced stimulation of expression (Fig. 4*B Inset*). These results are consistent with the interpretation that DHR38 can compete *in vivo* with EcR for USP heterodimerization, thereby compromising the ability of the USP–EcR complex to bind DNA and act either as a repressor (without hormone; ref. 27) or as an activator (with hormone; refs. 4–6). Interestingly, heat-shock-induced overexpression of DHR38 in transgenic third instar larvae carrying DHR38 under the control of the hsp70 promoter causes a high degree of lethality during pupation, a stage governed by ecdysone control (T.K and F.C.K., unpublished data).

The results presented here have several interesting implications for the field of nuclear receptors. (*i*) They identify a second partner for USP that until now was only known to interact with EcR. (*ii*) They suggest a possible mechanism of fine-tuning ecdysone action. Because of effective competition with EcR, *Drosophila* DHR38 (or its *Bombyx* homologue BHR38) may modulate the ecdysone response in tissues or at developmental stages when USP is limiting (12). (*iii*) It is plausible that USP–DHR38 heterodimers may have a new DNA-binding specificity. This may explain USP phenotypes not directly related to the ecdysone response (3, 33) and imply the involvement of USP in several regulatory pathways, analogous to RXR. By using *Drosophila* genetics, the significance of these findings can be tested rigorously when DHR38 mutants become available.

Finally, our results also have general implications beyond the field of insect endocrinology. They suggest that NGFI-B type receptors may act *via* at least two mechanisms. In addition to binding monomerically to DNA, they are capable of strongly interacting with RXR type receptors and preventing their interactions with other partners; in so doing, they can influence RXR-mediated processes. It is interesting to speculate how many orphan receptors might ultimately prove to be implicated in the regulation of ligand signaling through their protein–protein interactions, rather than through direct ligand binding.

Note Added in Proof. Two recent reports (34, 35) support our observations by showing similar interactions between vertebrate RXR and NGFI-B type receptors.

This paper is dedicated to the memory of Carroll M. Williams honoring his pioneering contributions to the study of insect endocrinology and development. J.D.S., T.K., and G.T. contributed equally to this work. We thank R. Brent and members of his laboratory for the yeast strains and reagents, R. Finley for helpful discussions, C. Thummel for his cDNA library and communicating results, J. Milbrandt for the NGFI-B clone, D. Moore for lexARXR, F. A. Stewart for reagents, D. S. Hogness for EcR antibody and suggestions on the manuscript, I. Zhimulev for help in cytological localization of DHR38, H. Stunnenberg for helpful discussions, and S. Lønstrup for secretarial assistance. This work was supported by the National Institutes of Health, and the Commission of the European Community.

1. Tsai, M.-J. & O'Malley, B. W. (1994) *Annu. Rev. Biochem.* **63**, 451–486.
2. Laudet, V. & Adelmant, G. (1995) *Curr. Biol.* **5**, 124–127.
3. Segraves, W. A. (1994) *Semin. Cell Biol.* **5**, 105–113.
4. Yao, T.-P. Segraves, W. A., Oro, A. E., McKeown, M. & Evans, R. M. (1992) *Cell* **71**, 63–72.
5. Thomas, H. E., Stunnenberg, H. G. & Stewart, A. F. (1993) *Nature (London)* **362**, 471–475.
6. 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.
7. Koelle, M. R. (1992) Ph.D. thesis (Stanford Univ., Stanford, CA).
8. Riddiford, L. M. (1993) in *The Development of Drosophila melanogaster*, eds. Bate, M. & Martinez-Arias, A. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 899–939.
9. Koelle, M. R., Talbot, W. S., Segraves, W. A., Bender, M. T., Cherbas, P. & Hogness, D. S. (1991) *Cell* **67**, 59–77.
10. Robinow, S., Talbot, W. S., Hogness, D. S. & Truman, J. W. (1993) *Development (Cambridge U.K.)* **119**, 1251–1259.
11. Talbot, W. S., Swyryd, E. A. & Hogness, D. S. (1993) *Cell* **73**, 1323–1337.
12. Henrich, V. C., Szekely, A. A., Kim, S. J., Brown, N. E., Antoniewski, C., Hayden, M., Lepésant, J.-A. & Gilbert, L. I. (1994) *Dev. Biol.* **165**, 38–52.
13. Laudet, V. & Stehelin, D. (1992) *Curr. Biol.* **2**, 293–295.
14. Milbrandt, J. (1988) *Neuron* **1**, 183–188.
15. Hazel, T. G., Nathans, D. & Lau, L. F. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8444–8448.
16. Ryseck, R.-P., Macdonald-Bravo, H., Mattéi, M.-G., Ruppert, S. & Bravo, R. (1989) *EMBO J.* **8**, 3327–3335.
17. Tzertzinis, G., Malecki, A. & Kafatos, F. C. (1994) *J. Mol. Biol.* **238**, 479–486.
18. Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) *Nucleic Acids Res.* **22**, 4673–4680.
19. Wilson, T. E., Fahrner T. J., Johnston M. & Milbrandt, J. (1991) *Science* **252**, 1296–1300.
20. Gyuris, J., Golemis, E., Chertkov, H. & Brent, R. (1993) *Cell* **75**, 791–803.
21. Bartel, P. L., Chien, C.-T., Sternglanz, R. & Fields, S. (1993) in *Cellular Interactions in Development*, ed. Hartley, D. A. (IRL, Oxford), pp. 153–179.
22. Shea, M. G., King, D. L., Conboy, M. J., Mariani, B. D. & Kafatos, F. C. (1990) *Genes Dev.* **4**, 1128–1140.
23. Khoury-Christianson, A. M., King, D. L., Hatzivassiliou, E., Casas, J. E., Hallenbeck, P. L., Nikodem, V. M., Mitsialis, A. S. & Kafatos, F. C. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 11503–11507.
24. Lee, J. W., Moore, D. D. & Heyman, R. A. (1994) *Mol. Endocrinol.* **8**, 1245–1252 (1994).
25. Smith, D. B. & Johnson, K. S. (1988) *Gene* **67**, 31–40.
26. DiNocera, P. P. & Dawid, I. B. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 7095–7098.
27. Dobens, L., Rudolph, K. & Berger, E. M. (1991) *Mol. Cell Biol.* **11**, 1846–1853.
28. Wilson, T. E., Fahrner, T. J. & Milbrandt, J. (1993) *Mol. Cell. Biol.* **13**, 5794–5804.
29. Wilson, T. E., Paulsen, R. E., Padgett, K. A. & Milbrandt, J. (1992) *Science* **256**, 107–110.
30. Leid, M., Kastner, P. & Chambon, P. (1992) *Trends Biochem. Sci.* **17**, 427–433.
31. Forman, B. M. & Samuels, H. H. (1990) *Mol. Endocrinol.* **4**, 1293–1301.
32. Lavorgna, G., Ueda, H., Clos, J. & Wu, C. (1991) *Science* **252**, 848–851.
33. Oro, A. E., McKeown, M. & Evans, R. M. (1992) *Development* (Cambridge, U.K.) **115**, 449–462.
34. Perlmann, T. & Jansson, L. (1995) *Genes Dev.* **9**, 769–782.
35. Forman, B. M., Umesono, K., Chen, J. & Evans, R. M. (1995) *Cell* **81**, 541–550.