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Heterodimerization of Ecdysone Receptor and Ultraspiracle on Symmetric and Asymmetric Response Elements

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Heterodimerization of nuclear receptors is facilitated by the interaction of two dimerization interfaces: one spanning the DNA-binding (C domain) region and the adjacent hinge (D domain) region, and the other in the ligand-binding (E domain) region. Ultraspiracle (USP) heterodimerizes with ecdysone receptor (EcR) and this complex participates in ecdysone signal transduction. The natural ecdysone response elements (EcREs) discovered so far are asymmetric elements composed of either imperfect palindromes or direct repeats. However, gel mobility shift assays have shown that both symmetric (perfect palindromes) and asymmetric (imperfect palindromes and direct repeats) elements can bind to the EcR/USP complex. Therefore, we analyzed EcR/USP domains involved in heterodimerization on different types of response elements (RE). Gel shift assays using full-length and truncated EcR and USP proteins showed that heterodimerization of these two proteins in the presence of asymmetric RE (DR4 and the natural EcRE hsp27) requires both dimerization interfaces present in CD and E domains of both proteins. In contrast, the dimerization interface present in the E domain of either EcR or USP was not essential for heterodimerization on symmetric RE such as PAL1 or IR1. We conclude that the use of heterodimerization interfaces present in CD and E domains of EcR/USP depends on the nature of response elements they bind to. Arch. Insect Biochem. Physiol. 60:55–70, 2005.

KEYWORDS: ecdysone receptor; EcR; ultraspiracle; USP; response elements; palindromes; direct repeats; heterodimerization; DNA binding

INTRODUCTION

The members of the nuclear receptor superfamily are ligand inducible structurally related transcription factors. These include receptors for steroid hormones as well as receptors for non-steroidal ligands such as thyroid hormones, bile acids, fatty acids, certain vitamins, and prostaglandins (Owen and Zelent, 2000; Robinson-Rechavi et al., 2003). This superfamily also includes a large number of receptors that do not have known ligands. Several proteins showing structural similarity to nuclear receptors have been identified in insects and other invertebrates (Riddiford et al., 2000).

The nuclear receptors activate gene transcription by binding to specific hormone response elements (RE) present in the promoters of target genes. They recognize derivatives of a hexameric core motif, arranged as direct repeats (DR), palindromes (PAL), inverted repeats (IR), or as everted repeats (ER) or inverted palindromes (IP) with variable spacing between the repeats. The steroid receptors bind pri-

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marily as homodimers to inverted repeats of the motif PuG(G/A)(T/A)CA separated by three nucleotides (Glass, 1994; Mangelsdorf et al., 1995). In contrast, the thyroid hormone receptor (TR), vitamin D receptor (VDR), retinoic acid receptor (RAR), peroxisome proliferator-activated receptor (PPAR), and some orphan receptors can form heterodimers with the retinoid X receptor (RXR), and bind preferentially to the PuG(G/T)TCA motif arranged as direct repeats with variable spacing. In addition to direct repeats, some RXR heterodimers can activate gene transcription by binding to palindromes and inverted palindromes (Chambon, 1996; Glass, 1994; Mangelsdorf et al., 1995).

All members of the nuclear receptor superfamily have a common, modular structure with an N-terminal A/B domain, a well-conserved DNA-binding domain containing two zinc-fingers (DBD or C domain), a hinge region D, and a C-terminal ligand-binding domain (LBD or E domain), and in some cases an F domain in the extreme C-terminus. Heterodimerization of RXR with other nuclear receptors is facilitated by two different dimerization interfaces. The DBD (C domain) and the hinge region together provide one of the dimerization interfaces in RXR heterodimers. A second, strong dimerization interface is present in the LBD (E domain). Several amino acids in the Zinc fingers of the DBD and in the T-box of the hinge region (also referred to as the C-terminal extension of the DBD), can form a dimerization interface for RXR/RAR, RXR/TR (Khorasanizadeh and Rastinejad 2001; Perlmann et al., 1993; Predki et al., 1994; Rastinejad 2001; Rastinejad et al., 1995; Zechel et al., 1994a,b), and RXR/PPAR heterodimers (IJpenberg et al., 1997; Juge-Aubry et al., 1997) for binding to direct repeats. The same region (DBD and the hinge region) was shown to be involved in heterodimer formation of RXR and VDR in solution, independent of DNA binding (Nishikawa et al., 1995).

The crystal structures of the LBDs of several nuclear receptors reveal the existence of a common fold, consisting of 12 α-helices and one β turn arranged as a three-layered antiparallel α-helical "sandwich" (reviewed in Egea et al., 2000; Moras and Gronemeyer, 1998). In RXR/RAR and RXR/PPAR heterodimers, the dimeric interface found in the LBD is contributed mainly by helices 9 and 10 (Bourguet et al., 2000a,b; Gampe et al., 2000). Similarly, residues in helices 10 and 11 were found to be involved in TR heterodimerization with RXR (Ribeiro et al., 2001). This dimerization region corresponds to the I-box, a 40-amino acid region identified in the LBDs of RAR, TR, and RXR, that was previously shown to be involved in heterodimerization and high affinity DNA binding (Perlmann et al., 1996). Aarnisalo et al. (2002) reported that a single amino acid substitution in the I-box region could completely abolish RXR heterodimerization with the orphan nuclear receptor Nurr1. Conserved amino acids present in helices 9 and10 of LBD are critical for heterodimerization of RXR and its partners (Au-Fliegner et al., 1993; Gorla-Bajszczak et al., 1999; Juge-Aubry et al., 1995; Lee et al., 2000; Zhang et al., 1994). Furthermore, mutating a single tyrosine residue (Y402) in helix 9 of RXR, can disrupt its heterodimerization with RAR, VDR, or TR (Vivat-Hannah et al., 2003).

In insects, the steroid hormone ecdysone regulates numerous developmental processes including molting and metamorphosis [ecdysone is used as a generic term to refer to α-ecdysone, its metabolite 20-hydroxyecdysone (20E) and other natural ecdysteroids with similar biological action (Riddiford et al., 2000)]. The primary receptor for ecdysone is a heterodimer of ecdysone receptor (EcR) and ultraspiracle (USP) (Thomas et al., 1993; Yao et al., 1992, 1993). A functional ecdysone response element (EcRE) was first identified as a 23-bp element in the *Drosophila* hsp27 promoter (Riddihough and Pelham, 1987). The hsp27 EcRE is an imperfect palindrome in which the two half-sites are separated by a single nucleotide. Natural ecdysone response elements with a similar structure have been found in several other *Drosophila* genes such as Fbp1 (Antoniewski et al., 1994), Sgs-4 (Lehmann and Korge 1995), Lsp-2 (Antoniewski et al., 1995), and Sgs-3 (Lehmann et al., 1997). Another EcRE element (ng-EcRE) has been identified in the coding region of *Drosophila* ng-1 and ng-2 genes

(Crispi et al., 1998; D'Avino et al., 1995). Unlike most other natural EcRE elements, which are composed of imperfect palindromes, the ng-EcRE is composed of two directly repeated half sites of the AGGTCA motif with 12 intervening nucleotides. In addition, the distal EcRE of the Eip 28/29 gene (Cherbas et al., 1991) contains a degenerate direct repeat with a 3-bp spacer (DR3) that can bind to the EcR/USP heterodimer (Zelhof et al., 1995). Hence, all the naturally occurring EcRE's have an asymmetric structure containing either imperfect palindromes or direct repeats. However, synthetic oligonucleotides composed of symmetric (perfect palindromes) as well as asymmetric (direct repeats and imperfect palindromes) elements have been shown to bind EcR/USP heterodimers in gel mobility shift assays (Antoniewski et al., 1996; Elke et al., 1997, 1999; Horner et al., 1995; Vogtli et al., 1998; Wang et al., 1998; Wu et al., 2004). In addition, reporter genes expressed under the control of some of these ecdysone response elements can be induced by ecdysone in the presence of EcR and USP (Antoniewski et al., 1996; Wang et al., 1998).

As mentioned above, there are several reports on DNA binding of EcR/USP to direct and inverted (palindromic) repeats, both in vitro and in vivo. However, the interaction of different domains of EcR and USP in the presence of specific response elements is yet to be determined. Previously, using several truncated versions of *Choristoneura fumiferana* EcR proteins and full-length USP, we found that the minimal EcR region required for binding to hsp27 includes the C, D, and a part of the E domains containing the 9th and 10th helices (Perera et al., 1999). In the present study, using truncated proteins of both EcR and USP, we examined heterodimer binding to symmetric and asymmetric response elements. Our results show that binding to asymmetric RE requires C, D, and E domains of both EcR and USP. These regions contain the two heterodimerization interfaces previously identified in vertebrate receptors. However, the second heterodimerization interface present in the LBD was not essential for binding to symmetric RE.

MATERIALS AND METHODS

Construction of Truncated Clones

Truncated EcR clones were constructed as described in Perera et al. (1999). For USP truncations, PCR primers containing Hind III, BamH I, or EcoR I restriction sites, were designed based on the sequence of USP from *Choristoneura fumiferana* (Perera et al., 1998). An artificial start site containing 5′ ACC ACC ATG 3′ sequence was included in the primers used to produce N-terminal truncations of USP. For USP truncations containing the ABC and ABCD domains, a stop codon (TAA) was included in the reverse primers in addition to the restriction sites. The primers used for both EcR and USP are shown in Table 1. The PCR conditions used were the same as those described previously (Perera et al., 1999). After PCR amplification, the amplified fragments were cloned into pGEM-3Zf vector (Promega, Madison, WI).

Electrophoretic Mobility Shift Assays (EMSA)

In vitro translated proteins of full-length and truncated EcR and USP were used in gel mobility shift assays. The cDNA clones were translated using the Promega TNT-coupled reticulocyte lysate system (Promega, Madison, WI), according to the manufacturer's instructions. Native EcRE found in the promoter region of the hsp27 gene (Riddihough and Pelham, 1987), and a DR4 element, which has been shown to bind to the EcR/USP heterodimer with high affinity (Wang et al., 1998), were used as examples of asymmetric elements. The native hsp27 is an imperfect palindrome of the half-site GGTTCA with a single base pair spacer, whereas the DR4 element consists of directly repeated AGGTCA half-sites separated by four base pairs. The PAL1 (Vogtli et al., 1998) and IR1 (Wang et al., 1998) elements containing AGGTCA with a single A/T base pair spacer were used as examples of perfect palindromes. We also synthesized and used three mutant versions of hsp27 (hsp27a, b, and c) that showed an increased degree of symmetry than the native element; the most symmetri-

TABLE 1. The Primers Used for Constructing EcR and USP Truncations*

Clone	Forward primer	Reverse primer
EcR1	E20 - 5′ TAT AAG CTT ACC ACC ATG AAG AAG GGC CCT GC GAC CG 3′	E13 - 5′ TAT GGA TCC TCA GAG CAG CGC GGC CG 3′
EcR2	E21 - 5′ TAT AAG CTT ACC ACC ATG AGG CCT GAG TGC GTA GT 3′	E13
EcR3	E22 - 5′ TAT AAG CTT ACC ACC ATG CAG GAC GGG TAC GAG CA 3′	E13
EcR4	E17 - 5′ TAT GAA TTC AAG CCC GCG TAG GAT GTC 3′	E23 - 5′ TAT GGA TCC GTT CTT GAG CTT GAG GG 3′
EcR5	E17	B1694 - 5′ CGC GGA TCC GCG TAG CTC AGA GAG GAT TG 3′
EcR6	E17	B1626 - 5′ CGC GGA TCC GGT TCA GGA TAT AGA TGC GG 3′
EcR7	E17	B1591 - 5′ CGC GGA TCC ACC GCT GGA TTT CTT CCA CC 3′
EcR8[a]		
USP1	U20 - 5′ TAT AAG CTT ACC ACC ATG TGC TCT ATA TGC GGC GAC AG 3′	U13 - 5′ CGC GGA TCC CCA TAG TTC TTC TCA CAT CGG 3′
USP2	U21 - 5′ TAT AAG CTT ACC ACC ATG AAG CGA GAG GCG GTG CAA 3′	U13
USP3	U22 - 5′ TAT AAG CTT ACC ACC ATG TCG GTG CAG GTA AGC GAT G 3′	U13
USP4	U17 - 5′ TAT AAG CTT TTA GTG GAG TGC AGG GAT AG 3′	U26 - 5′ TAT GGA TCC TTA GCT ACT CGG GTG CGC ATC CTC 3′
USP5	U17	U27 - 5′ TAT GGA TCC TTA CAT ACC GCA AGC CAA ACA CTT 3′

*The EcR and USp primers are based on *C. fumiferana* EcR-b sequence (Kothapalli et al., 1995) and *C. fumiferana* USP sequence (Perera et al., 1998).
[a]The EcR8 truncation was constructed by digesting EcR-b with Kpn I, and re-ligating the fragment containing the pGEM-3Zf (Promega, Madison, WI) vector, the 5′ untranslated region and the coding region for the N-terminal 297 residues.

cal being hsp27b, which is a perfect palindrome of the half-site GGTTCA. Ecdysone response elements were end-labeled with [γ-^{32}P] ATP (6,000 Ci/mmol), and used for binding to EcR/USP complex. The conditions for DNA binding were the same as those described in Kothapalli et al. (1995). The components of the binding reaction mixture were separated on a 6% non-denaturing polyacrylamide gel. The gel was fixed in 7% acetic acid, dried, and exposed to X-ray film for autoradiography.

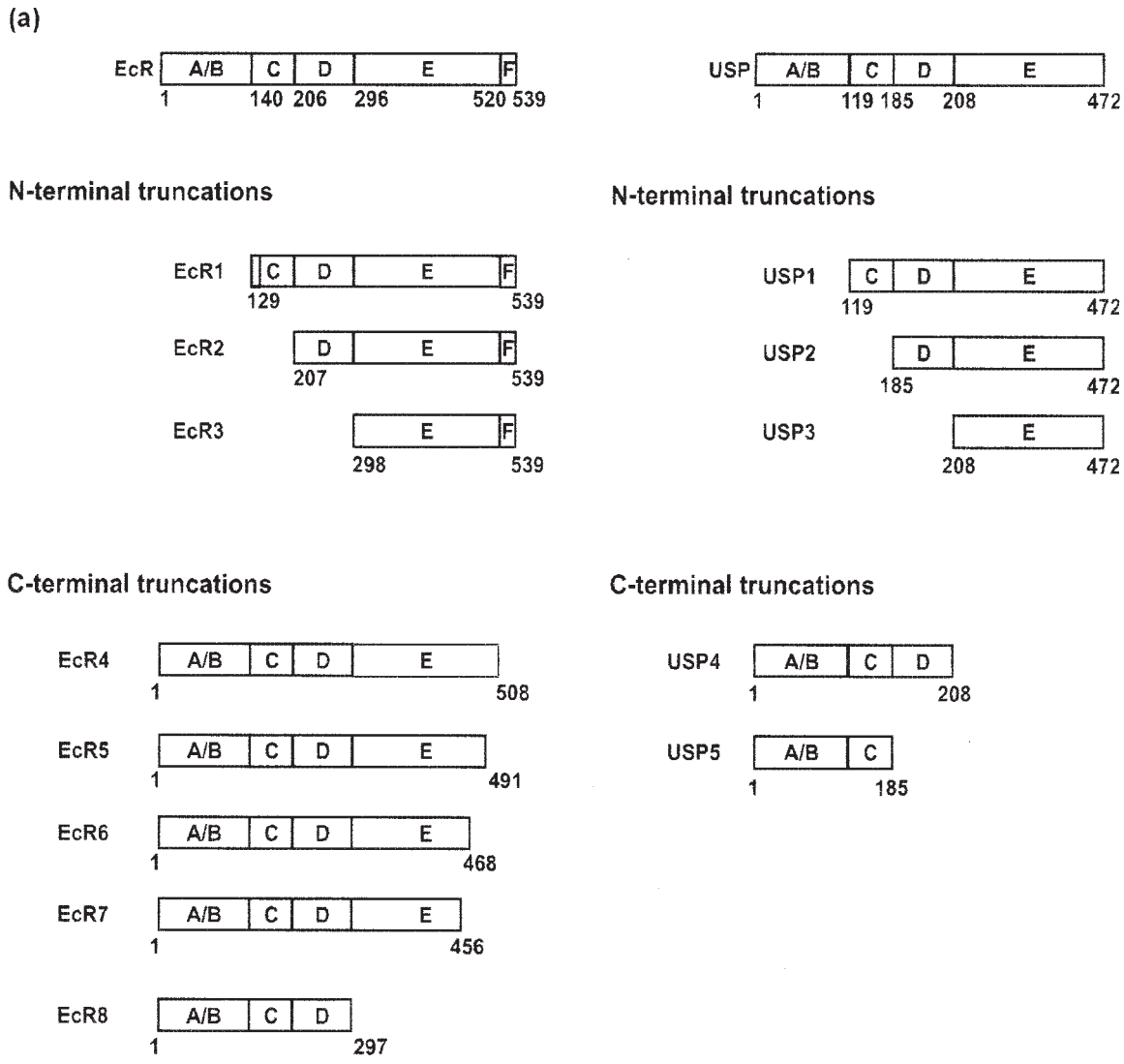
RESULTS

EcR and USP Clones, and Response Elements Used in EMSA

We were interested in determining the functional domains involved in heterodimerization of EcR and USP in the presence of symmetric vs. asymmetric response elements. Previously, we have prepared several truncated versions of EcR, and tested their binding to the asymmetric response element present in hsp27 gene, in the presence of full-length USP (Perera et al., 1999). In this study, we determined EcR/USP domains required for binding to several symmetric as well as asymmetric elements.

The maps of EcR and USP truncations used in EMSA are shown in Figure 1a. For both EcR and USP, three truncations were made from the N-terminus, by deleting the A/B (EcR1, USP1), A/BC (EcR2, USP2), and A/BCD (EcR3, USP3) domains. Two truncated USP clones were constructed with C-terminal deletions, one lacking the E domain (USP4), and the other lacking both D and E domains (USP5). For EcR, we used five clones carrying deletions in the C-terminus. One clone lacked

Fig. 1. **a:** Schematic representation of EcR, USP, and the truncated clones used in EMSA. The EcR and USP sequences are based on EcR-B (Kothapalli et al., 1995) and USP (Perera et al., 1998) sequences, respectively. In full-length EcR and USP, the numbers refer to the first amino acid of each domain and the extreme C-terminal amino acid. In the truncated EcR and USP clones, only the numbers corresponding to the N-terminal and C-terminal amino acids are shown. **b:** The response elements used in EMSA. The hexameric half-sites are underlined. The six base pairs in the left half-site are designated –6 to –1, and those in the right half-site are designated +1 to +6, as shown above hsp27. The elements hsp27a and hsp27c contain single base pair mutations (boxed) at positions +3 (C/G to A/T) and +6 (T/A to C/G), respectively. The hsp27b element is a perfect palindrome, which differs from wild type hsp27 at both +3 and +6 positions (boxed).



(b)

	-6 -1 +1 +6
hsp27	5' AGAGACAAGGGTTCAATGCACTTGTCCAAT 3'
hsp27a	5' AGAGACAAGGGTTCAATGAACTTGTCCAAT 3'
hsp27b	5' AGAGACAAGGGTTCAATGAACCTGTCCAAT 3'
hsp27c	5' AGAGACAAGGGTTCAATGAACCTGTCCAAT 3'
DR4	5' CGATGGACACAGGTCACAGGAGGTCACTTGAAGCT 3'
PAL1	5' GATCTAGAGAGGTCAATGACCTCGTCCAAG 3'
IR1	5' AGCTTCAAGAGGTCAATGACCTTGTCCATCG 3'

Figure 1.

the entire E and F domains (EcR8), while the others (EcR4, EcR5, EcR6, EcR7) were constructed by making a series of deletions in the E domain. The clones, EcR4 and EcR5, contain the entire heterodimerization interface in the E domain, while the highly conserved helix 10 was deleted in clone EcR6, and both helix10 and part of helix 9 were deleted in EcR7.

Figure 1b shows the symmetric (PAL1, IR1, hsp27b) and asymmetric (hsp27, hsp27a, hsp27c, DR4) response elements used in this study. We selected the REs hsp27, DR4, PAL1, and IR1 because they have been shown to bind to the EcR/USP heterodimer with high affinity (Elke et al., 1999; Wang et al., 1998). The hsp27 element was derived from the natural EcRE found in the *Drosophila* hsp27 gene (Riddihough and Pelham, 1987), and contains an imperfect palindrome of the half-site GGTTCA. The DR4 element used in this study consists of a direct repeat of the AGGTCA motif with a four-nucleotide spacer. The two palindromic response elements PAL1 and IR1, both contain an inverted repeat of the AGGTCA motif separated by a single nucleotide. However, PAL1 and IR1 sequences differ from each other in the regions adjacent to the half-sites. We also used three mutant versions of hsp27: hsp27a, hsp27b, and hsp27c. The hsp27b element contains mutated nucleotides at positions +3 (C/G to A/T) and +6 (T/A to C/G), thus converting it into a perfect palindrome. The hsp27a and hsp27c each contain a single nucleotide mutations at +3 and +6 positions, respectively.

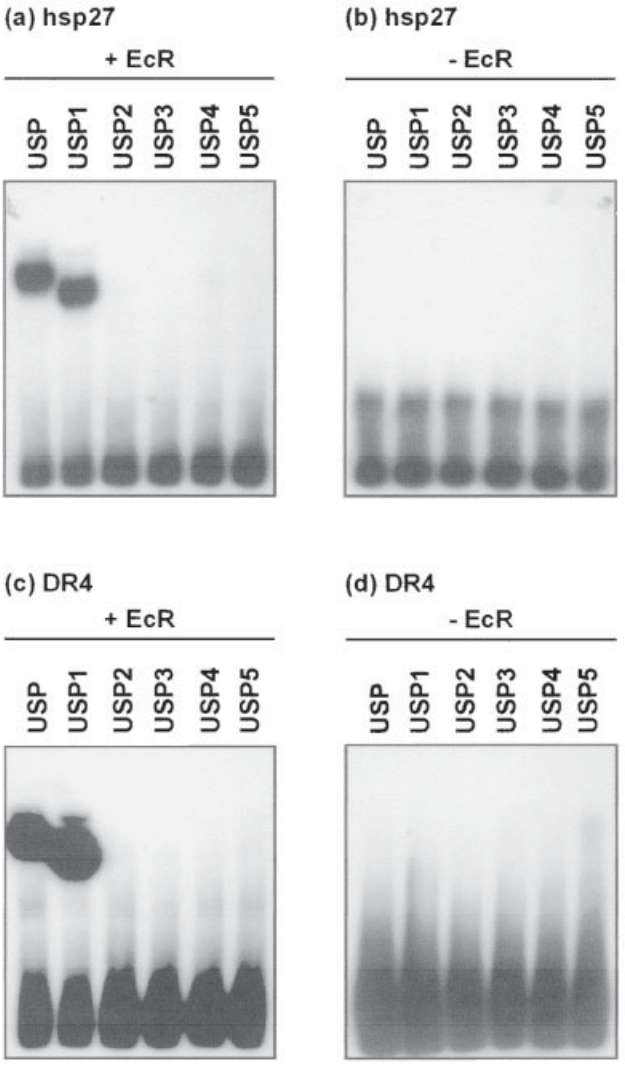


Fig. 2. Ultraspiracle or its truncated versions binding to hsp 27, in the presence (**a**) or absence (**b**) of EcR. Ultraspiracle or its truncated versions binding to DR4 in the presence (**c**) or absence (**d**) of EcR. In vitro translated EcR and USP (full-length and truncated) proteins were used for binding to [γ^{32}P]-labeled hsp27 or DR4 elements as described previously (Kothapalli et al., 1995). The components of the reaction were resolved in a 6% non-denaturing polyacrylamide gel and detected by autoradiography.

The DBD and LBD Are Involved in EcR/USP Heterodimerization on Asymmetric Response Elements, hsp27 and DR4

Full-length USP and USP containing CDE domains heterodimerize with full-length EcR and bind to hsp27 (Fig. 2a) and DR4 (Fig. 2c) response elements. In contrast, USP proteins that contained DE, E, A/BCD, or A/BC domains did not bind to either hsp 27 (Fig. 2a) or DR4 (Fig. 2c) response elements in the presence of full-length EcR. Therefore, only the A/B domain of USP can be deleted without affecting its binding to hsp27 and DR4 as a heterodimer with EcR. To determine if USP or any of its truncations can bind to hsp27 or DR4 elements as monomers or homodimers, we conducted EMSA using only the USP proteins. None of the USP proteins bound to hsp27 (Fig. 2b) or DR4 (Fig. 2d) elements, confirming that the bands

observed in Figure 2a and c are a result of EcR/USP heterodimer binding to the respective response elements.

Next, we examined binding of full-length USP and various truncations of EcR proteins to hsp27 and DR4 elements (Fig. 3). In the presence of full-length USP, the full-length EcR as well as three truncated EcR proteins (EcR1 lacking the A/B domain; and EcR4 and EcR5 lacking F domain and with C-terminal deletions in the E domain) showed binding to both hsp27 (Fig. 3a) and DR4 (Fig. 3c) elements. The two C-terminal truncations (EcR4 and EcR5) that bound to both response elements, contain the entire dimerization interface in the E domain. When the region corresponding to helices 9 and 10 were deleted from EcR (EcR 6–8), they did not bind to either hsp27 or DR4 element. Hence, the complete A/B And F domains can be deleted from EcR without affecting the heterodimerization with USP, and binding to hsp27 or DR4 elements. To determine if full-length EcR or any of its truncations can bind to hsp27 or DR4 elements as monomers or homodimers, we conducted EMSA using only EcR proteins. None of the EcR proteins bound to hsp27 (Fig. 3b) or DR4 (Fig. 3d) elements in the absence of USP confirming that the shifted bands observed in Figure 3a and c are a result of EcR/USP heterodimer binding to the respective response elements.

The data presented in Figures 2 and 3 suggest

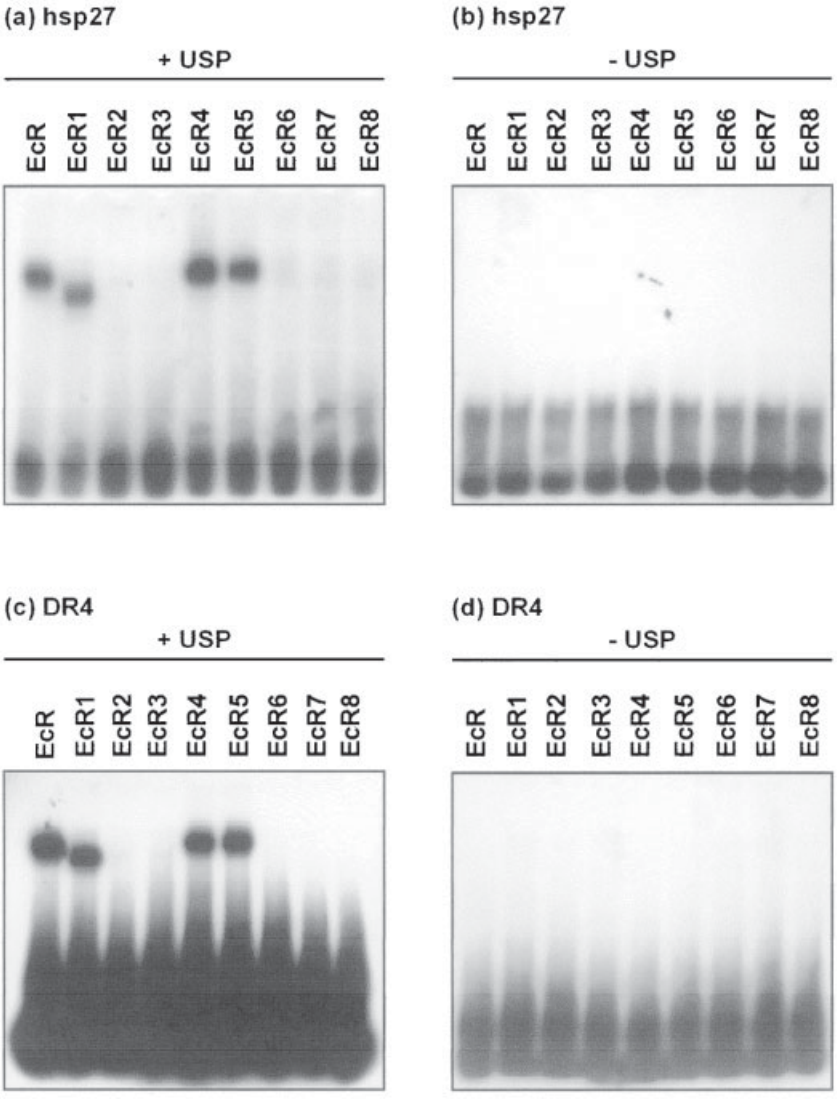


Fig. 3. Ecdysone receptor or its truncated versions binding to hsp27, in the presence (**a**) or absence (**b**) of USP. Ecdysone receptor or its truncated versions binding to DR4, in the presence (**c**) or absence (**d**) of USP. In vitro translated EcR and USP (full-length and truncated) proteins were used for binding to [γ^{32}P]-labeled hsp 27 or DR4 elements.

that hsp27 and DR4 act in a similar manner in promoting EcR/USP heterodimerization. They require the presence of CDE domains of both EcR and USP for heterodimer formation and binding to response elements. In EcR LBD, the region corresponding to helices 9 and 10 appear to play a crucial role in mediating heterodimerization in the presence of both hsp27 and DR4 elements.

The DBD and Hinge Regions Are Sufficient for EcR/USP Heterodimerization on Symmetric (Palindromic) Response Elements PAL1 and IR1

Figure 4 shows full-length or truncated USP proteins binding to two different palindromic response elements (inverted repeats), PAL1 and IR1, in the presence or absence of EcR. None of the USP proteins bound as monomers or homodimers to PAL1 (Fig. 4b) or IR1 (Fig. 4d). Full-length USP and two USP truncations (USP1 lacking the A/B domain and USP4 lacking the E domain) showed binding to PAL1 (Fig. 4a) and IR1 (Fig. 4c) as heterodimers with EcR. Hence, neither the A/B nor the E domain of USP is required for EcR/USP heterodimer binding to PAL1 or IR1. A similar observation was made when truncated EcR proteins were used in DNA binding assays (Fig. 5). EcR proteins lacking the A/B domain (EcR1) or the entire E and F regions (EcR8) formed heterodimers with full-length USP, on PAL1 (Fig. 5a) and IR1 (Fig. 5c). All the EcR proteins containing C-terminal deletions in the E domain (EcR4, EcR5, EcR6, EcR7) also bound the two response elements in the presence of USP (Fig. 5a and c) albeit at a lower level. Some of the truncated EcR proteins also showed binding to PAL1 and IR1, as monomers or homodimers, but only very weakly (Fig. 5b and d). These results suggested that removing the A/B or the E domain of either partner of the EcR/USP complex does not adversely affect its binding to perfect palindromes.

As mentioned above, we observed heterodimer formation between full-length EcR with USP4 (contains A/BCD domains), and full-length USP with EcR8 (contains A/BCD domains), on PAL1 and IR1 (Figs. 4 and 5) elements. Next, we tested EcR8 (contains A/BCD domains) in combination with

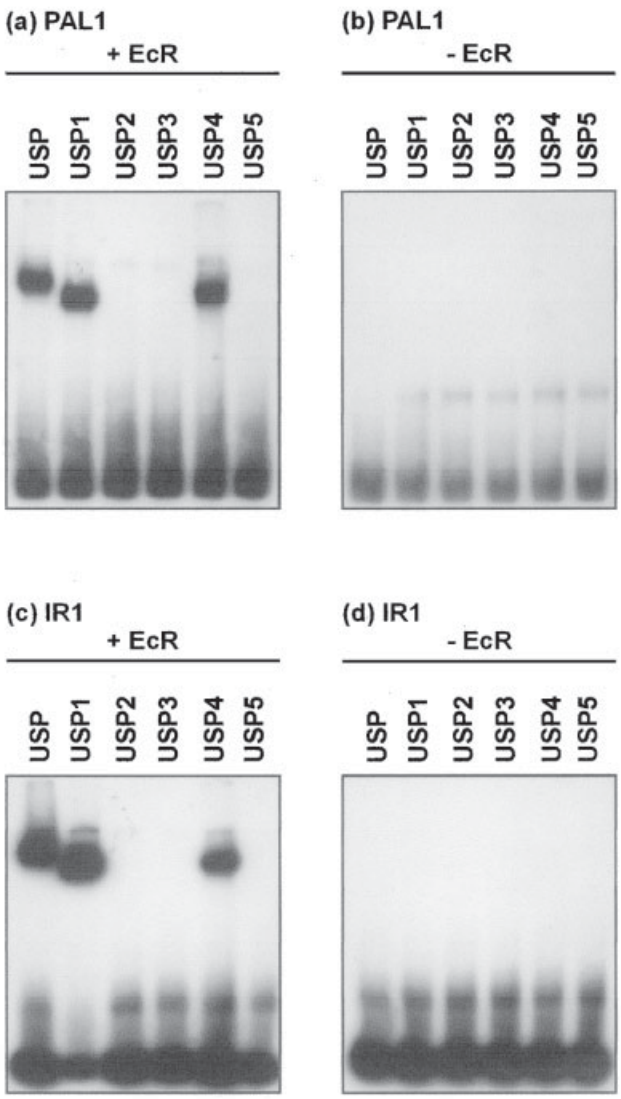


Fig. 4. Utlraspiracle or its truncated versions binding to PAL1, in the presence (**a**) or absence (**b**) of EcR. Ultraspiracle or its truncated versions binding to IR1, in the presence (**c**) or absence (**d**) of EcR. In vitro translated EcR and USP (full-length and truncated) proteins were used for binding to [γ^{32}P]-labeled PAL1 or IR1 elements.

all USP truncations for binding to PAL1 and IR1 elements (Fig. 6a and b). We also tested USP4 (contains only A/BCD domains) in combination with all EcR truncations for binding to the same response elements (Fig. 6c and d). EcR8/USP4 (EcR A/BCD /USP A/BCD) heterodimers bound to PAL1 and IR1. Since both proteins lacked the entire LBD (E domain) and were able to form heterodimers and bind to both PAL1 and IR1, it can be concluded

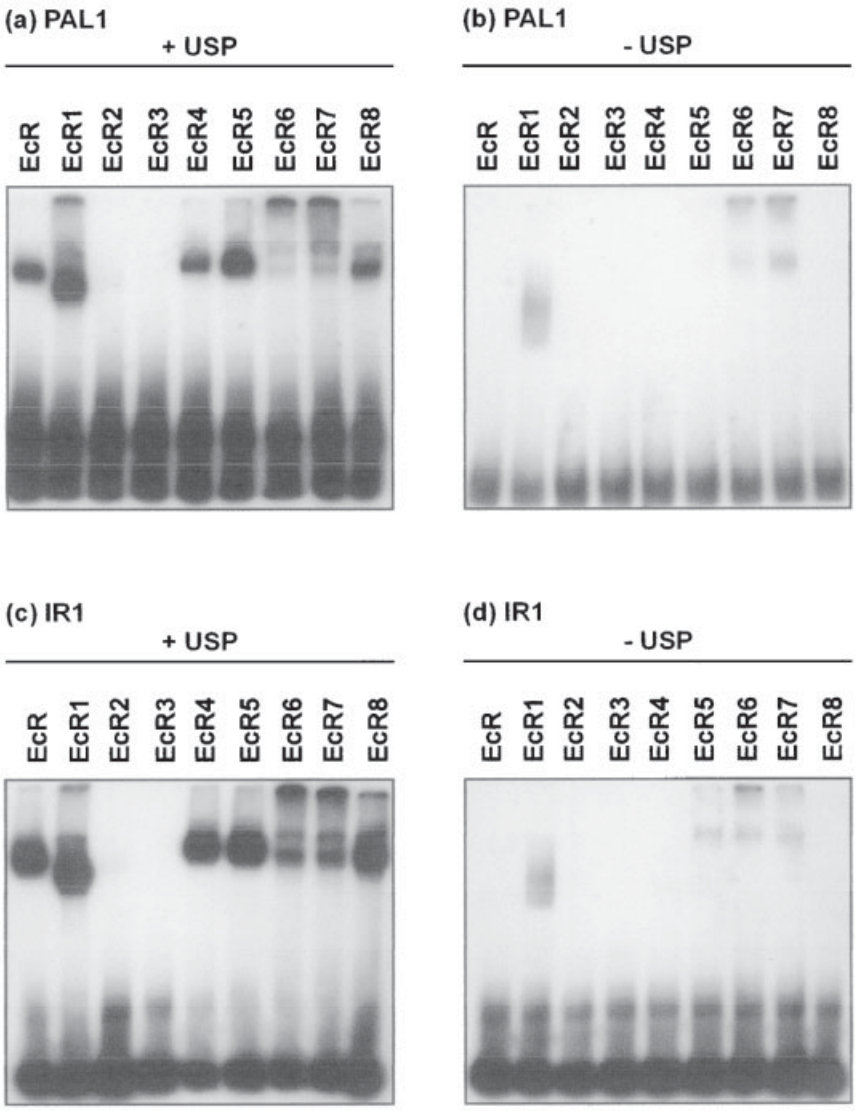


Fig. 5. Ecdysone receptor or its truncated versions binding to PAL1, in the presence (**a**) or absence (**b**) of USP. Ecdysone receptor or its truncated versions binding to IR1, in the presence (**c**) or absence (**d**) of USP. In vitro translated EcR and USP (full-length and truncated) proteins were used for binding to [γ^{32}P]-labeled PAL1 or IR1 elements.

that the heterodimerization interface present in the E domain is not essential for EcR/USP heterodimerization in the presence of perfect palindromes.

Surprisingly, deleting the A/B domain from USP (USP1) abolished binding to PAL1 and IR1 in the presence of truncated EcR8 (Fig. 6a and b). However, when the A/B domain of EcR was deleted, the truncated EcR protein (EcR1) was still able to bind to PAL1 and IR1 in the presence of both the full-length USP (Fig. 5a and c) and the truncated USP4 (Fig. 6c and d). It is possible that the removal of the A/B domain in USP affected the stability of the heterodimer complex, when a truncated EcR protein was used as its partner for dimerization.

The data on EcR/USP binding to PAL1 and IR1 suggest that the dimerization interface in the LBD does not play a role in heterodimerization on these two response elements. However, the dimerization interface in the DBD and hinge region was essential for the formation of the heterodimer complex.

Converting the Asymmetric hsp27 Into a Perfect Palindrome Enables the Mutated Response Element to Bind EcR/USP Complexes Lacking the LBD

Our EMSA data showed that EcR/USP complexes could bind to perfect palindromes (PAL1 and IR1) even in the absence of an intact LBD for both pro-

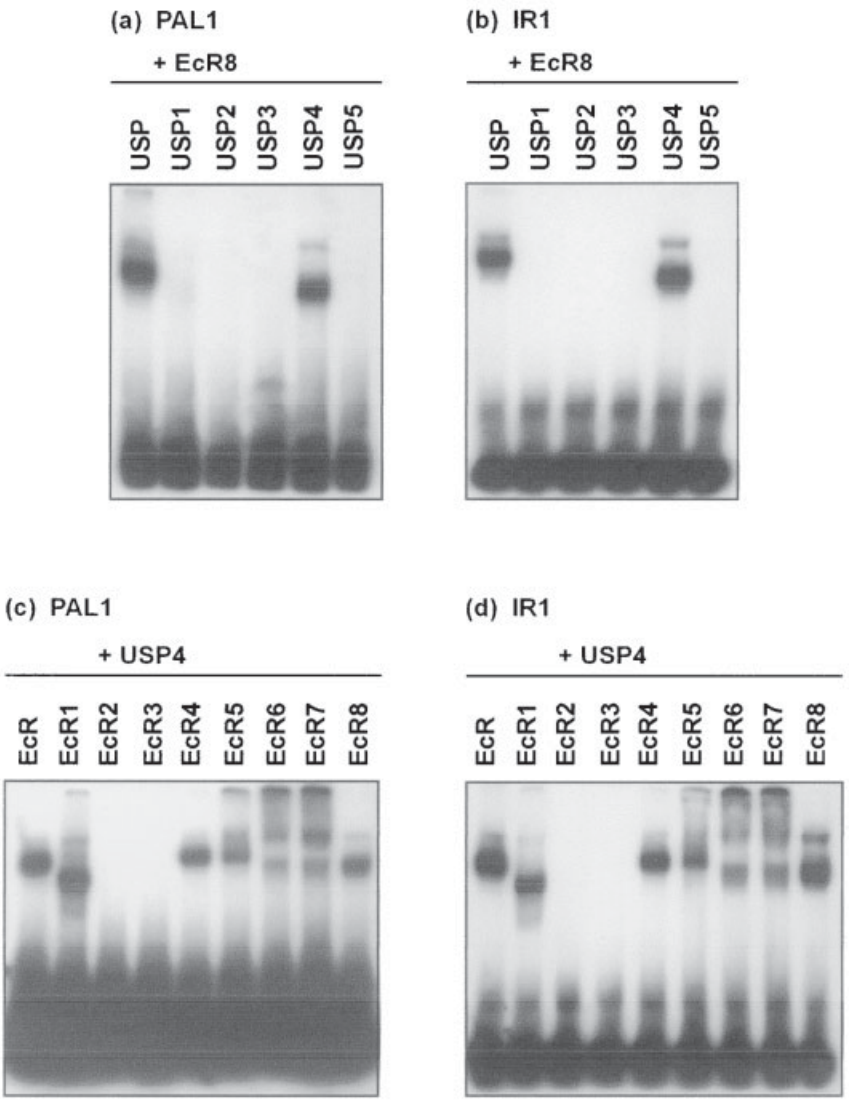


Fig. 6. Ultraspiracle and its truncated versions (**a,b**) or EcR and its truncated versions (**c,d**) binding to PAL1 (a,c) and IR1 (b,d) in the presence of EcR8 (a truncated EcR containing only the A/BCD domains) or USP4 (a truncated USP containing only the A/BCD domains). In vitro translated EcR and USP (full-length and truncated) proteins were used for binding to [γ^{32}P]-labeled PAL1 or IR1 elements.

teins (Figs. 4–6). In contrast, EcR and USP lacking LBD would not bind to asymmetric elements such as direct repeats (DR4) or the natural EcRE hsp27 (Figs. 2 and 3). These observations led us to explore the interaction of EcR and USP dimerization interfaces, while binding to mutant hsp27 elements. To this end, we synthesized oligonucleotides for three variations of hsp27 element by altering the +3 (C/G to A/T) and/or +6 (T/A to C/G) positions (Fig. 1b). Single nucleotide mutations at +3 (hsp27a) and +6 (hsp27c) conferred an increased degree of symmetry to hsp27, whereas altering nucleotides at both positions (hsp27b) converted it into a perfect palindrome. Figure 7 shows EcR and USP heterodimer binding to wild type and mutant hsp27 elements. All four elements were able to bind to complexes containing full-length EcR and USP (Fig. 7a). EcR and USP proteins that lack E domain were able to bind to hsp27a and hsp27b but not wild type or hsp27c elements (Figs. 7b–d). These data show that converting the hsp27 element into a perfect palindrome (hsp27b) increases its affinity for binding to EcR and USP even in the absence of their LBDs.

DISCUSSION

Most of the naturally occurring ecdysone response elements identified to date are asymmetric

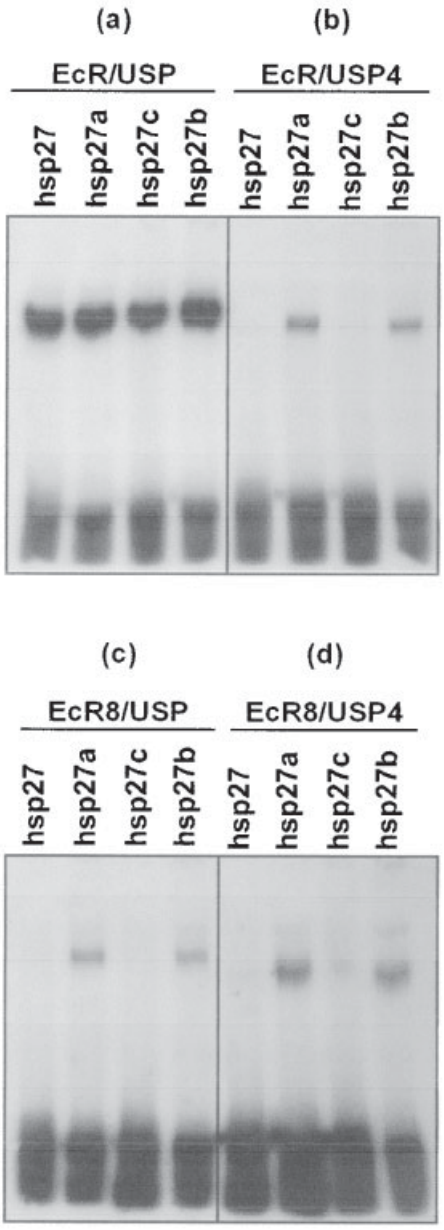


Fig. 7. Ecdysone receptor and USP (**a**), EcR and USP4 (**b**), EcR8 and USP (**c**), or EcR8 and USP4 (**d**) binding to hsp27, hsp27a, hsp27b and hsp27c elements. EcR8 and USP4 are truncated clones containing only the A/BCD domains.

elements composed of either imperfect palindromes or direct repeats containing the half-site (Pu)G(G/T)TCA (Antoniewski et al., 1994, 1995; Cherbas et al., 1991; Crispi et al., 1998; D'Avino et al., 1995; Lehmann and Korge 1995; Lehmann et al., 1997; Riddihough and Pelham 1987; Zelhof et al., 1995). However, EcR/USP heterodimers showed a higher binding affinity to more symmetric elements such as perfect palindromes. The most preferred response element for EcR/USP heterodimer was found to be a PAL1 (IR1) element, which is a perfect palindrome of the AGGTCA half-site separated by a single nucleotide (Vogtli et al., 1998; Wang et al., 1998). In this study, we set out to determine if there are differences in the heterodimerization and binding of EcR and USP to these two types of response elements (symmetric vs. asymmetric).

Dimerization Interfaces in EcR and USP

The results of EMSA are summarized in Table 2. Our data indicate the presence of two distinct regions in both EcR and USP that can interact to form the heterodimers. As in other RXR-type receptors, these two domains are present in the C and D domains (DBD and hinge region), and in the E domain (LBD). In the presence of full-length EcR (EcR A/BCDEF) or USP (USP A/BCDE), only the A/B domain of its partner could be deleted without affecting binding to any of the four response elements. A further N-terminal deletion into the C domain completely abolished DNA binding, indicating that this domain is involved in EcR/USP heterodimer binding to DNA. The hinge (D domain) region of USP also played a role in heterodimer formation, as evidenced by the fact that in the presence of full-length EcR, USP4 (containing A/BCD) but not USP5 (containing A/BC) was able to bind to PAL1 and IR1 elements. We have shown that deleting the hinge region of EcR can affect dimerization with USP (Perera et al., 1999). The role of C and D domains in heterodimerization of nuclear receptors is well documented for vertebrate RXR-type receptors. Specific amino acids in the zinc fingers of DBD and in the T-box of the hinge region (the region adjacent to the DBD) are involved in forming the protein-protein and protein-DNA contacts and are necessary for heterodimer binding to response elements. Consequently, RXR heterodimers are formed on a DNA-supported interface, primarily through amino acid interactions between their DBDs and hinge

TABLE 2. Summary of EcR/USp Binding to Symmetric and Asymmetric Response Elements Used in This Study*

	USP (ABCDE)				USP4 (ABCD)	
EcR	hsp27	DR4	PAL1*[a]	IR1[a]	PAL1[a]	IR1[a]
EcR (ABCDEF)	+	+	+	+	+	+
EcR1 (CDEF)	+	+	+	+	+	+
EcR2 (DEF)	–	–	–	–	–	–
EcR3 (EF)	–	–	–	–	–	–
EcR4 (ABCDE)	+	+	+	+	+	+
EcR5 (ABCDE)	+	+	+	+	+	+
EcR6 (ABCDE)	–	–	+	+	+	+
EcR7 (ABCDE)	–	–	+	+	+	+
EcR8 (ABCD)	–	–	+	+	+	+

	EcR (ABCDEF)				EcR8 (ABCD)	
USP	hsp27	DR4	PAL1*[a]	IR1[a]	PAL1[a]	IR1[a]
USP (ABCDE)	+	+	+	+	+	+
USP1 (CDE)	+	+	+	+	–	–
USP2 (DE)	–	–	–	–	–	–
USP3 (E)	–	–	–	–	–	–
USP4 (ABCD)	–	–	+	+	+	+
USP5 (ABC)	–	–	–	–	–	–

	USP (ABCDE)				USP4 (ABCD)			
EcR	hsp27	hsp27a	hsp27c	hsp27b*	hsp27	hsp27a	hsp27c	hsp27b*
EcR (ABCDEF)	+	+	+	+	–	+	–	+
EcR8 (ABCD)	–	+	–	+	–	+	–	+

*The different domains found in each protein are shown in parentheses.
[a]Symmetric elements.

regions (Nishikawa et al., 1995; Perlmann et al., 1993; Predki et al., 1994; Rastinejad, 2001; Rastinejad et al., 1995; Wilson et al., 1992; Zechel et al., 1994a,b).

As mentioned above, we found a second heterodimerization interface in the LBD of EcR and USP. EcR A/BCD (EcR8) or USP A/BCD (USP4) failed to bind to hsp27 or DR4 element as heterodimers with full-length USP or EcR, respectively. Therefore, removing the entire E domain prevented EcR/USP heterodimerization on these two response elements. Removing only the C-terminal 48 amino acids in EcR (EcR5) did not have any effect on DNA binding; whereas a further truncation of an additional 23 amino acids in the E domain (EcR6), which removed the 10th helix, impaired binding to hsp27 or DR4 elements. In RXR/RAR and RXR/PPAR heterodimers, the dimeric interface found in the LBD is contributed mainly by helices 9 and 10 (Bourguet et al., 2000a,b; Gampe et al., 2000). Therefore, as in the case of RXR-type vertebrate nuclear receptors, the highly conserved 10th helix of EcR is also involved in heterodimer formation with USP.

Differential Utilization of Dimerization Interfaces for Binding to Symmetric and Asymmetric Response Elements

The RXR-heterodimers assume a defined polarity upon binding to direct repeats, imposed by the asymmetric nature of these response elements (Rastinejad, 2001). In RXR/TR and RXR/VDR heterodimers bound to direct repeats, RXR occupies the 5′ half-site (Mader et al., 1993; Perlmann et al., 1993; Rastinejad et al., 1995), whereas it is located in the 3′ half-site in the PPAR/RXR heterodimer (IJpenberg et al., 1997). In the case of RXR/

RAR heterodimers, the polarity of the complex is determined by the spacing between the half-sites, with RXR occupying the 5′ half-site in DR2 and DR5 elements and the 3′ half-site in DR1 elements (Mader et al., 1993; Perlmann et al., 1993; Predki et al., 1994; Rastinejad et al., 2000; Zechel et al., 1994b). Devarakonda et al. (2003) found the dimerization and DNA-binding interfaces to be the same for EcR–USP and EcR-RXR complexes. The binding polarity of the heterodimer is reflected by the dimerization interface contributed by each partner. Structural and biochemical analyses showed that the two zinc fingers in the DBD and the T-box of the hinge region are involved in the formation of dimer contacts (IJpenberg et al., 1997; Niedziela-Majka et al., 2000; Perlmann et al., 1993; Rastinejad et al., 1995; Zechel et al., 1994a,b). Niedziela-Majka et al. (2000) have reported that EcR/USP heterodimers assume a defined orientation upon binding to the imperfect palindrome hsp27. In vitro DNA binding assays using truncated EcR and USP proteins showed that USP is located in the 5′ half-site of the response element. These observations led to the authors' suggestion that hsp27 acts as a functionally asymmetric element, similar to directly repeated elements, in their ability to differentially orient the EcR/USP heterodimer complex (Niedziela-Majka et al., 2000). In contrast, response elements composed of perfect palindromes confer no polarity on the EcR/USP heterodimer (Vogtli et al., 1998).

Interestingly, our results indicate that the type of response element can dictate the interaction of specific dimerization interfaces in the heterodimer formation. In directing EcR/USP heterodimerization, the imperfect palindrome hsp27 behaved in a manner similar to the asymmetric DR4 element, rather than to the perfect palindromes PAL1 and IR1. In the presence of hsp27 and DR4, both dimerization interfaces in CD and E domains were involved in heterodimerization of EcR and USP. However, converting hsp27 into a perfect palindrome (hsp27b) enabled it to bind to truncated EcR/USP heterodimers lacking the entire E domain. Similarly, the entire E domain of both EcR and USP could be deleted without affecting binding to PAL1 and IR1, which indicates that the dimerization interface in the E domain was not essential for EcR/USP heterodimerization on perfect palindromes. In RXR heterodimers, the major factor that determines the specificity of DNA binding has been found to be the dimer contacts within the C and D domains. It has been suggested that the strong dimerization function in the E domain has no selective power for response element recognition, but serves only to stabilize the heterodimer complex (Rastinejad et al., 1995; Zechel et al., 1994a). Our results also indicate that the dimerization function in the C and D domain is essential for the formation of EcR/USP heterodimer. Nevertheless, the dimerization function in the E domain was also required for EcR/USP heterodimerization on functionally asymmetric elements like hsp27 and DR4, but not on perfect palindromes. It is possible that the formation of strong dimer contacts between the LBDs of EcR and USP favors the positioning of the heterodimer in a preferred orientation (USP on the 5′ half-site and EcR on the 3′ half-site; Niedziela-Majka et al., 2000) for binding to asymmetric response elements. Although the dimerization interface in LBD is not directly involved in response element recognition, the EcR/USP heterodimer may not be able to achieve the correct polarity on functionally asymmetric elements without the additional stability offered by the dimerization interface in the E domain. Since the heterodimer complex does not have a preferred orientation on symmetric elements such as PAL1 and IR1 (Vogtli et al., 1998), the dimerization function in the LBD would be dispensable for EcR/USP heterodimerization on these response elements.

As mentioned above, a perfectly symmetrical form of hsp27 (hsp27b) facilitated the binding of a truncated EcR/USP complex lacking the E domain. This symmetrical element differed from the native hsp27 at nucleotide positions +3 (C/G converted to A/T) and +6 (T/A converted to C/G). In hsp27 elements containing a single nucleotide mutation at either +3 (hsp27a) or +6 (hsp27c) position, only the latter seems to have an effect on the binding of truncated EcR/USP complex. These results concur with a previous study by Ozyhar and

Pongs (1993), which showed that the +3 position but not the +6 position of hsp27 can be altered without affecting its binding affinity to a partially purified ecdysone receptor complex.

To determine the polarity of EcR/USP complex on hsp27 element, Niedziela-Majka et al. (2000) used truncated EcR and USP proteins from *Drosophila melanogaster*. Using only the EcR and USP proteins containing C and D domains, they observed heterodimer binding to hsp27, as well as monomer and homodimer binding. In our DNA binding assays, we failed to detect any heterodimer binding to hsp27 when the E domain was deleted from either EcR or USP proteins. Moreover, we did not observe monomer or homodimer binding of EcR or USP to the hsp27 element. Therefore, the heterodimerization and DNA binding properties of *C. fumiferana* ecdysone receptor appear to be slightly different from those of *D. melanogaster*. In contrast to our data with *C. fumiferana* EcR/USP, the LBD of *D. melanogaster* EcR and USP does not seem to play a role in stabilizing the heterodimer in its preferred orientation on asymmetric response elements.

In conclusion, we found that there are two surfaces involved in DNA-dependent heterodimerization of the *C. fumiferana* EcR and USP complex. The requirement for these surfaces is influenced by the nature of the response element (symmetric vs. asymmetric) used for DNA binding. The dimerization interface present in the C and D domains was proven to be essential for heterodimer formation on direct repeats as well as on perfect and imperfect palindromes. The second dimerization interface present in the E domain is required only for EcR/USP heterodimerization on functionally asymmetric elements like hsp27 and DR4.

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LITERATURE CITED

Aarnisalo P, Kim CH, Lee JW, Perlmann T. 2002. Defining requirements for heterodimerization between the retinoid X receptor and the orphan nuclear receptor Nurr1. J Biol Chem 277:35118–35123.

Antoniewski C, Laval M, Dahan A, Lepesant JA. 1994. The ecdysone response enhancer of the Fbp1 gene of *Drosophila melanogaster* is a direct target for the EcR/USP nuclear receptor. Mol Cell Biol 14:4465–4474.

Antoniewski C, O'Grady MS, Edmondson RG, Lassieur SM, Benes H. 1995. Characterization of an EcR/USP heterodimer target site that mediates ecdysone responsiveness of the *Drosophila* Lsp-2 gene. Mol Gen Genet 249:545–556.

Antoniewski C, Mugat B, Delbac F, Lepesant JA. 1996. Direct repeats bind the EcR/USP receptor and mediate ecdysteroid responses in *Drosophila melanogaster*. Mol Cell Biol 16:2977–2986.

Au-Fliegner M, Helmer E, Casanova J, Raaka BM, Samuels HH. 1993. The conserved ninth C-terminal heptad in thyroid hormone and retinoic acid receptors mediates diverse responses by affecting heterodimer but not homodimer formation. Mol Cell Biol 13:5725–5737.

Bourguet W, Andry V, Iltis C, Klaholz B, Potier N, Van Dorsselaer A, Chambon P, Gronemeyer H, Moras D. 2000a. Heterodimeric complex of RAR and RXR nuclear receptor ligand-binding domains: purification, crystallization, and preliminary X-ray diffraction analysis. Protein Exp Purif 19:284–288.

Bourguet W, Vivat V, Wurtz JM, Chambon P, Gronemeyer H, Moras D. 2000b. Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains. Mol Cell 5:289–298.

Chambon P. 1996. A decade of molecular biology of retinoic acid receptors. Faseb J 10:940–954.

Cherbas L, Lee K, Cherbas P. 1991. Identification of ecdysone response elements by analysis of the Drosophila Eip28/29 gene. Genes Dev 5:120–131.

Crispi S, Giordano E, D'Avino PP, Furia M. 1998. Cross-talking among Drosophila nuclear receptors at the promiscuous response element of the ng-1 and ng-2 intermolt genes. J Mol Biol 275:561–574.

D'Avino PP, Crispi S, Cherbas L, Cherbas P, Furia M. 1995. The moulting hormone ecdysone is able to recognize target elements composed of direct repeats. Mol Cell Endocrinol 113:1–9.

Devarakonda S, Harp JM, Kim Y, Ozyhar A, Rastinejad F. 2003.

Structure of the heterodimeric ecdysone receptor DNA-binding complex. Embo J 22:5827-5840.

Egea PF, Klaholz BP, Moras D. 2000. Ligand-protein interactions in nuclear receptors of hormones. FEBS Lett 476:62–67.

Elke C, Vogtli M, Rauch P, Spindler-Barth M, Lezzi M. 1997. Expression of EcR and USP in Escherichia coli: purification and functional studies. Arch Insect Biochem Physiol 35:59–69.

Elke C, Rauch P, Spindler-Barth M, Spindler KD. 1999. DNA-binding properties of the ecdysteroid receptor-complex (EcR/USP) of the epithelial cell line from *Chironomus tentans*. Arch Insect Biochem Physiol 41:124–133.

Gampe RT, Jr., Montana VG, Lambert MH, Miller AB, Bledsoe RK, Milburn MV, Kliewer SA, Willson TM, Xu HE. 2000. Asymmetry in the PPARgamma/RXRalpha crystal structure reveals the molecular basis of heterodimerization among nuclear receptors. Mol Cell 5:545–555.

Glass CK. 1994. Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. Endocr Rev 15:391–407.

Gorla-Bajszczak A, Juge-Aubry C, Pernin A, Burger AG, Meier CA. 1999. Conserved amino acids in the ligand-binding and tau(i) domains of the peroxisome proliferator-activated receptor alpha are necessary for heterodimerization with RXR. Mol Cell Endocrinol 147:37–47.

Horner MA, Chen T, Thummel CS. 1995. Ecdysteroid regulation and DNA binding properties of Drosophila nuclear hormone receptor superfamily members. Dev Biol 168:490–502.

IJpenberg A, Jeannin E, Wahli W, Desvergne B. 1997. Polarity and specific sequence requirements of peroxisome proliferator-activated receptor (PPAR)/retinoid X receptor heterodimer binding to DNA. A functional analysis of the malic enzyme gene PPAR response element. J Biol Chem 272:20108–20117.

Juge-Aubry CE, Gorla-Bajszczak A, Pernin A, Lemberger T, Wahli W, Burger AG, Meier CA. 1995. Peroxisome proliferator-activated receptor mediates cross-talk with thyroid hormone receptor by competition for retinoid X receptor. Possible role of a leucine zipper-like heptad repeat. J Biol Chem 270:18117–18122.

Juge-Aubry C, Pernin A, Favez T, Burger AG, Wahli W, Meier CA, Desvergne B. 1997. DNA binding properties of peroxisome proliferator-activated receptor subtypes on various natural peroxisome proliferator response elements. Importance of the 5′-flanking region. J Biol Chem 272:25252–25259.

Khorasanizadeh S, Rastinejad F. 2001. Nuclear-receptor interactions on DNA-response elements. Trends Biochem Sci 26:384–390.

Kothapalli R, Palli SR, Ladd TR, Sohi SS, Cress D, Dhadialla TS, Tzertzinis G, Retnakaran A. 1995. Cloning and developmental expression of the ecdysone receptor gene from the spruce budworm, *Choristoneura fumiferana*. Dev Genet 17:319–330.

Lee SK, Lee B, Lee JW. 2000. Mutations in retinoid X receptor that impair heterodimerization with specific nuclear hormone receptor. J Biol Chem 275:33522–33526.

Lehmann M, Korge G. 1995. Ecdysone regulation of the Drosophila Sgs-4 gene is mediated by the synergistic action of ecdysone receptor and SEBP 3. Embo J 14:716–726.

Lehmann M, Wattler F, Korge G. 1997. Two new regulatory elements controlling the Drosophila Sgs-3 gene are potential ecdysone receptor and fork head binding sites. Mech Dev 62:15–27.

Mader S, Chen JY, Chen Z, White J, Chambon P, Gronemeyer H. 1993. The patterns of binding of RAR, RXR and TR homo- and heterodimers to direct repeats are dictated by the binding specificites of the DNA binding domains. Embo J 12:5029–5041.

Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans R. 1995. The nuclear receptor superfamily: the second decade. Cell 83:835–839.

Moras D, Gronemeyer H. 1998. The nuclear receptor ligand-binding domain: structure and function. Curr Opin Cell Biol 10:384–391.

Niedziela-Majka A, Kochman M, Ozyhar A. 2000. Polarity of the ecdysone receptor complex interaction with the palindromic response element from the hsp27 gene promoter. Eur J Biochem 267:507–519.

Nishikawa J, Kitaura M, Imagawa M, Nishihara T. 1995. Vitamin D receptor contains multiple dimerization interfaces that are functionally different. Nucleic Acids Res 23:606–611.

Owen GI, Zelent A. 2000. Origins and evolutionary diversification of the nuclear receptor superfamily. Cell Mol Life Sci 57:809–827.

Ozyhar A, Pongs O. 1993. Mutational analysis of the interaction between ecdysteroid receptor and its response element. J Steroid Biochem Mol Biol 46:135–145.

Perera SC, Palli SR, Ladd TR, Krell PJ, Retnakaran A. 1998. The ultraspiracle gene of the spruce budworm, *Choristoneura fumiferana*: cloning of cDNA and developmental expression of mRNA. Dev Genet 22:169–179.

Perera SC, Sundaram M, Krell P, Retnakaran A, Dhadialla TS, Palli SR. 1999. An analysis of ecdysone receptor domains required for heterodimerization with ultraspiracle. Arch Insect Biochem Physiol 41:61–70.

Perlmann T, Rangarajan PN, Umesono K, Evans RM. 1993. Determinants for selective RAR and TR recognition of direct repeat HREs. Genes Dev 7:1411–1422.

Perlmann T, Umesono K, Rangarajan PN, Forman BM, Evans RM. 1996. Two distinct dimerization interfaces differentially modulate target gene specificity of nuclear hormone receptors. Mol Endocrinol 10:958–966.

Predki PF, Zamble D, Sarkar B, Giguere V. 1994. Ordered binding of retinoic acid and retinoid-X receptors to asymmetric response elements involves determinants adjacent to the DNA-binding domain. Mol Endocrinol 8:31–39.

Rastinejad F. 2001. Retinoid X receptor and its partners in the nuclear receptor family. Curr Opin Struct Biol 11:33–38.

Rastinejad F, Perlmann T, Evans RM, Sigler PB. 1995. Structural determinants of nuclear receptor assembly on DNA direct repeats. Nature 375:203–211.

Rastinejad F, Wagner T, Zhao Q, Khorasanizadeh S. 2000. Structure of the RXR-RAR DNA-binding complex on the retinoic acid response element DR1. Embo J 19:1045–1054.

Ribeiro RC, Feng W, Wagner RL, Costa CH, Pereira AC, Apriletti JW, Fletterick RJ, Baxter JD. 2001. Definition of the surface in the thyroid hormone receptor ligand binding domain for association as homodimers and heterodimers with retinoid X receptor. J Biol Chem 276:14987–14995.

Riddiford LM, Cherbas P, Truman JW. 2000. Ecdysone receptors and their biological actions. Vitam Horm 60:1–73.

Riddihough G, Pelham HRB. 1987. An ecdysone response element in the Drosophila hsp27 promoter. EMBO J 6:3729–3734.

Robinson-Rechavi M, Escriva Garcia H, Laudet V. 2003. The nuclear receptor superfamily. J Cell Sci 116:585–586.

Thomas HE, Stunnenberg HG, Stewart AF. 1993. Heterodimerization of the *Drosophila* ecdysone receptor with retinoid X receptor and ultraspiracle. Nature 362:471–475.

Vivat-Hannah V, Bourguet W, Gottardis M, Gronemeyer H. 2003. Separation of retinoid X receptor homo- and heterodimerization functions. Mol Cell Biol 23:7678–7688.

Vogtli M, Elke C, Imhof MO, Lezzi M. 1998. High level transactivation by the ecdysone receptor complex at the core recognition motif. Nucleic Acids Res 26:2407–2414.

Wang SF, Miura K, Miksicek RJ, Segraves WA, Raikhel AS. 1998. DNA binding and transactivation characteristics of the mosquito ecdysone receptor-Ultraspiracle complex. J Biol Chem 273:27531–27540.

Wilson TE, Paulsen RE, Padgett KA, Milbrandt J. 1992. Participation of non-zinc finger residues in DNA binding by two nuclear orphan receptors. Science 256:107–110.

Wu X, Hopkins PM, Palli SR, Durica DS. 2004. Crustacean retinoid-X receptor isoforms: distinctive DNA binding and receptor-receptor interaction with a cognate ecdysteroid receptor. Mol Cell Endocrinol 218:21–38.

Yao TP, Segraves WA, Oro AE, McKeown M, Evans RM. 1992. *Drosophila* ultraspiracle modulates ecdysone receptor function via heterodimer formation. Cell 71:63–72.

Yao TP, Forman BM, Jiang Z, Cherbas L, Chen JD, McKeown M, Cherbas P, Evans RM. 1993. Functional ecdysone receptor is the product of EcR and Ultraspiracle genes. Nature 366:476–479.

Zechel C, Shen XQ, Chambon P, Gronemeyer H. 1994a. Dimerization interfaces formed between the DNA binding domains determine the cooperative binding of RXR/RAR and RXR/TR heterodimers to DR5 and DR4 elements. Embo J 13:1414–1424.

Zechel C, Shen XQ, Chen JY, Chen ZP, Chambon P, Gronemeyer H. 1994b. The dimerization interfaces formed between the DNA binding domains of RXR, RAR and TR determine the binding specificity and polarity of the full-length receptors to direct repeats. Embo J 13:1425–1433.

Zelhof AC, Yao TP, Chen JD, Evans RM, McKeown M. 1995. Seven-up inhibits ultraspiracle-based signaling pathways in vitro and in vivo. Mol Cell Biol 15:6736–6745.

Zhang XK, Salbert G, Lee MO, Pfahl M. 1994. Mutations that alter ligand-induced switches and dimerization activities in the retinoid X receptor. Mol Cell Biol 14:4311–4323.