Ligand binding and heterodimerization with retinoid X receptor \( \alpha \) (RXR\( \alpha \)) induce farnesoid X receptor (FXR) conformational changes affecting coactivator binding

Received for publication, June 28, 2018, and in revised form, September 27, 2018. Published, Papers in Press, October 1, 2018, DOI 10.1074/jbc.RA118.004652

Na Wang\(^1\), Qiangan Zou\(^1\), Jinxin Xu\(^1\)*, Jiancun Zhang\(^1,2\), and @ Jinsong Liu\(^1\)

From the \(^4\)School of Life Sciences, University of Science and Technology of China, Hefei 230026, China, the \(^6\)State Key Laboratory of Respiratory Disease, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China, the \(^7\)Guangdong Provincial Key Laboratory of Biocomputing, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510530, China, and \(^8\)Guangzhou Henovcom Biosciences Inc., Guangzhou 510530, China

Edited by Dennis R. Voelker

Nuclear receptor farnesoid X receptor (FXR) functions as the major bile acid sensor coordinating cholesterol metabolism, lipid homeostasis, and absorption of dietary fats and vitamins. Because of its central role in metabolism, FXR represents an important drug target to manage metabolic and other diseases, such as primary biliary cirrhosis and nonalcoholic steatohepatitis. FXR and nuclear receptor retinoid X receptor \( \alpha \) (RXR\( \alpha \)) form a heterodimer that controls the expression of numerous downstream genes. To date, the structural basis and functional consequences of the FXR/RXR heterodimer interaction have remained unclear. Herein, we present the crystal structures of the heterodimeric complex formed between the ligand-binding domains of human FXR and RXR\( \alpha \). We show that both FXR and RXR bind to the transcriptional coregulator steroid receptor coactivator 1 with higher affinity when they are part of the heterodimer complex than when they are in their respective monomeric states. Furthermore, structural comparisons of the FXR/RXR\( \alpha \) heterodimers and the FXR monomers bound with different ligands indicated that both heterodimerization and ligand binding induce conformational changes in the C terminus of helix 11 in FXR that affect the stability of the coactivator binding surface and the coactivator binding in FXR. In summary, our findings shed light on the allosteric signal transduction in the FXR/RXR heterodimer, which may be utilized for future drug development targeting FXR.

The farnesoid X receptor (FXR, NR1H4)\(^2\) is a member of the nuclear receptor (NR) superfamily that plays a key role in the regulation of bile acids, lipid, and glucose metabolisms, as well as anti-inflammatory response and hepatocarcinogenesis inhibition (1, 2). FXR, which is abundantly expressed in liver, kidney, and intestine, has been identified as the main bile acid sensor (3). Over the years, FXR has been demonstrated to be an effective drug target to treat metabolic diseases, because FXR agonist obeticholic acid (OCA or INT747) has been successfully used in primary biliary cirrhosis and nonalcoholic steatohepatitis treatment (4).

FXR belongs to the structurally conserved family of nuclear receptors that function as ligand-regulated transcription factors. It consists of an N-terminal DNA-binding domain, which targets the receptor to specific genes, coupled by a flexible linker to a ligand-binding domain (LBD). The LBD can bind small lipophilic ligands and then serves as the transcriptional switch. Like most NRs, regulation of transcription by FXR is a complex process that relies on the release of corepressors and the ligand-dependent recruitment of coactivator proteins to a surface on the LBD. This surface, usually formed by helices 3, 4, 5, and 12 in FXR, is also called the activation function-2 (AF2) surface. In turn, those coregulatory proteins mediate the interactions with the basal transcriptional machinery, resulting in repression or activation of transcription (5).

FXR operates as a heterodimer with another nuclear receptor named retinoid X receptor-\( \alpha \) (RXR\( \alpha \), NR2B1) in vivo (6). RXR\( \alpha \) appears to respond to endogenous ligands, such as 9-cis-retinoic acid (9cRA) (7). FXR/RXR heterodimer binds to FXR response element (FXRE), which is mostly a single inverted repeat (IR1) in the promoter region of the target gene (8). RXRs also serve as obligate heterodimer partners for many of the subfamily 1 NRs, including receptors for peroxisome proliferator activator (PPARs), thyroid hormone (TR), retinoic acid (RAR), and vitamin D (9, 10). These different RXR heterodimers can be divided into three classes: permissive, conditional, and nonpermissive RXR heterodimers, based on their different response to RXR ligands (11, 12). Generally, RXR heterodimers that contain PPAR, LXR, and FXR, which can be...
activated by ligands for either partner in the dimer, are permissive RXR heterodimers. Heterodimer RXR/RAR demonstrates conditional permissiveness, because full response to RXR ligands occurs only in the presence of an RAR agonist. The nonpermissive RXR heterodimers (e.g., RXR/TR, RXR/vitamin D receptor) usually do not respond to RXR ligands. Dimerization renders the control of NR function intricate, because the signal across the heterodimer interface provides an exquisite degree of combinatorial control of transactivation. More structural information is needed to delineate the SCA network at the atomic level.

Table 1

| Complex                  | HNC143–FXR–LBD | HNC180–FXR–LBD | HNC143–FXR/9cRA–RXR | HNC180–FXR/9cRA–RXR | GW4064–FXR/9cRA–RXR |
|--------------------------|----------------|----------------|---------------------|---------------------|---------------------|
| Space group              | P2,2,2         | F23            | P4,2,2              | P2,2,2              | P4,2,2              |
| Cell dimensions          |                |                |                     |                     |                     |
| a (Å)                    | 79.38          | 160.35         | 83.38               | 98.33               | 102.85              |
| b (Å)                    | 98.77          | 160.35         | 83.38               | 96.13               | 102.85              |
| c (Å)                    | 119.27         | 160.35         | 161.63              | 114.24              | 109.46              |
| α = β = γ (°)            | 90             | 90             | 90                  | 90                  | 90                  |
| Resolution (Å)           | 2.88           | 2.60           | 2.10                | 2.95                | 3.05                |
| Rmin (Å)                 | 0.086 (0.507)* | 0.032 (0.267)  | 0.024 (0.247)       | 0.110 (0.338)       | 0.020 (0.274)       |
| Rfree (%)                | 6.9 (1.6)      | 17.2 (3.3)     | 18.9 (3.4)          | 5.4 (2.0)           | 19.4 (3.0)          |
| Completeness (%)         | 99.9 (100)     | 100 (100)      | 98 (98.8)           | 99.8 (99.7)         | 99.8 (99.7)         |
| Redundancy               | 6.2 (6.4)      | 10.3 (10.4)    | 13.1 (13.5)         | 3.5 (3.6)           | 13.8 (14.4)         |

Refinement

- No. of unique reflections: 20,743, 10,107, 32,442, 19,737, 11,002
- Rfree/ Rfree (%)a: 21.1/26.2, 19.6/25.1, 19.8/24.6, 22.1/28.2, 22.5/28.8
- No. of atoms: Proteins 3954, 1982, 3860, 7384, 3638
- Ligand/ ion 76, 64, 76, 122, 58
- Water 4, 11, 81, 17, 1
- Average B-factors: Protein 61.8, 68, 43, 54.9, 126.3
- RMSD: Bond lengths (Å) 53.504, 42.99, 37.1, 49.5, 118.1
- Ramachandran plot: Favored (%) 96.4, 96.19, 96.67, 95.57, 94.01
- Allowed (%) 2.12, 2.97, 3.33, 3.52, 5.07

Notes:
- a Rfree is calculated from a randomly chosen 5% of reflections that have never been used in refinement, and Rfree (%) is calculated for the remaining 95% of the reflections.
- b RMSD is the root-mean-square deviation from ideal geometry.

Results

Structure determination

The hFXR–LBD was crystallized in the presence of FXR agonists (HNC143 and HNC180) and SRC1 (fragment 741–761) with one LXXLL motif. The purified hFXR/RXRα–LBD heterodimer was crystallized in complex with FXR agonists (HNC143, HNC180, or GW4064), RXR natural ligand 9cRA, and a synthetic peptide derived from the coactivator SRC1–2 (fragment 685–700, the second LXXLL motif in SRC1) containing a single LXXLL motif. Purification of the coexpressed hFXR/RXRα–LBD heterodimer (see “Experimental procedures”) and addition of compounds and SRC1 peptide were critical for the complex crystallization. FXR–LBD/ligand structures were solved by the molecular-replacement method with the FXR and RXRα monomer structures as the searching models in two steps. Electron density maps showed clear features for the respective ligands in the structures, the LXXLL motifs of the SRC1 peptides, and each LBD, except for helix 2 in RXRα and...
Allosteric signal transduction in FXR/RXR heterodimer

Figure 1. FXR/RXRα heterodimer structure complexed with HNC143, 9cRA, and SRC1 peptides. (a) ribbon diagram of the human FXR/RXRα heterodimer complex with two SRC1 peptides. The SRC1 peptides are in yellow. FXR is cyan and RXRα is colored magenta. HNC143 in FXR and 9cRA in RXRα are shown in space-filling representation colored by atom type: oxygen as red, nitrogen as blue, sulfur as yellow, chlorine as green, and carbon as pink. (b) heterodimer viewed from the bottom of H11, 90° rotation of a.

some regions in FXR. The statistics for data collection and structure refinement are summarized in Table 1.

Overall structure of the hFXR/RXRα heterodimer

The structures of the hFXR/RXRα LBD heterodimer complexes contain six components: two receptor LBDs, their respective ligands, and two SRC1 peptides (Fig. 1a). The heterodimer complex structure is similar to the previous published RXR LBD heterodimer complexes (15–20). Both LBDs adopted the canonical three-layered α helical sandwich fold, with FXR containing 12 α-helices (H1 to H12) and RXR containing 10 α-helices and 2 β-strands.

The FXR and RXRα ligands occupy their respective ligand-binding pockets, and both receptors adopt the active configuration, such that the H12 is folded against the main body of the LBD. This conformation generates a recognition surface (AF2 surface) constituted by mostly hydrophobic residues from H3, H4, and H12 of FXR and RXRα, which allow one SRC1 peptide to bind to each receptor. Two coactivator peptides fold as a two-turn α-helix in the heterodimer complex with the hydrophobic side chains (LXXLL) packed against the agonist-induced FXR and RXRα surfaces.

Binding characteristics of HNC compounds to hFXR

HNC compounds (HNC143 and HNC180) are GW4064 analogues (Fig. 2, a–c), sharing the similar hydrophobic head that can bind in the same position in FXR ligand-binding pocket. Compared with GW4064 and HNC143, HNC180 has a more potent EC50 in a coactivator recruitment assay (Fig. 2g) shows that there is no interaction of HNC143 and HNC180 with the FXR-RXR heterodimer structure (Fig. 2g) shows that there is a similar effect was found in RXRα–LBD where it is absent in the heterodimer structure because of a lack of electron density. These results suggest that FXR has considerable conformation dynamics upon binding with different ligands or forming heterodimer with RXR.

Heterodimerization and coactivator recruitment

Given the importance of heterodimerization in nuclear receptor signal pathway, we addressed the question whether association of FXR and RXRα modifies the intrinsic ability of the receptors to interact with coregulators. To this end, we purified FXR–LBD and RXRα–LBD with or without fused LXXLL motifs (see “Experimental procedures”) to examine whether nuclear receptor LBD dimerization can influence the coactivator binding. The fused SRC1 to one of the two receptors in the heterodimer was designed to assess the binding of biotin-labeled SRC1 to the nonfused receptor in coactivator binding assay. To validate this approach, FXR/RXR heterodimer fused with two SRC1 peptides was tested in the fluorescence anisotropy assay. The results show that there is no binding between the two–SRC1–fused heterodimer and fluorescence-labeled peptide (Fig. S1). We then examined the SRC1 binding affinity for FXR and RXR in the context of the heterodimer and in response to different ligands (Table S1). The affinity of SRC1676–700 LXXLL motifs binding to the FXR–LBD alone (Kd = 6.60 μM) is lower than binding to the FXR–LBD in the FXR/RXR–LBD heterodimer complex (Kd = 2.42 μM) (Fig. 3, a and c). A similar effect was found in RXRα–LBD (Fig. 3, a–c).
Therefore, dimerization enhances the binding affinity of FXR–LBD and RXR–LBD for LXLL motifs. In addition, contributing to the overall stability of the receptor, FXR agonists or RXR agonist (9cRA) can enhance the SRC1 binding, and the addition of 9cRA or FXR agonists will further increase the binding affinity in FXR/RXR H18528 SRC1 or FXR H18528 SRC1/RXR heterodimer (Fig. 3, e and f). We further tested the EC50 of FXR ligands in coactivator recruitment assay in the context of FXR monomer or FXR/RXR heterodimer, with or without RXR agonist 9cRA (Table 2). The results show that heterodimerization with RXR will improve the EC50 of all these ligands. However, when 9cRA is added, differentiated effects of the FXR compounds were observed. Ligands HNC180 and GW4064 have better EC50 in FXR/RXR–LBD dimer; on the other hand, EC50 of HNC143, INT747, and ivermectin decrease to FXR monomer level or even lower. This result suggested that the higher-order nuclear receptor complex structure improves coactivator binding, and the synergistic effect between the two receptors ligands may have ligand selectivity.

The asymmetric hFXR–RXRa heterodimer interface

Consistent with other RXR heterodimer structures, FXR and RXRa interact via the conserved asymmetric dimer interface, largely comprised of H11 in each monomer, with additional contacts from H7 and H9, loops L8–9 and L9–10. The interactions consist of an intricate network of hydrophobic and polar contacts (Table S2), with H11 of RXRa/H9251 and FXR forming a coiled coil structure as shown in Fig. 4a. During the protein purification, we found that the isolated FXR–LBD is mostly monomeric, whereas mixing equivalent FXR and RXR LBDs yields essentially heterodimer (data not shown).

Comparing the structurally available RXR heterodimers with RXR homodimer, RXR partners differ in the degree of bending and orientation of H11, even though they share similar electrostatic and polar contacts in the heterodimer interface. In the nonpermissive TR/RXR heterodimer, TRβ H11 has a marked shift, resulting in a rotation of H11 and H5 in RXRα and then disruption of the active conformation of RXRα (20). Although the backbone of the N terminus of H11 in the permissive RXR
partners superimpose well with the RXR homodimer, noticeable shifts exist in the C terminus of H11 (Fig. 4b). The differences in the heterodimer interface (especially H11) between the permissive and nonpermissive RXR heterodimer suggest that receptor/ligand-specific bending of H11 also contributes to the cross-dimer signaling.

Role of helix 11 in cross-dimer signaling

Previous studies have shown that an electrostatic tethering across the dimer interface exists in most RXR heterodimers in the plane of ligand, suggesting a conduit for structural information from the ligand to the partner receptor (22). In our FXR/RXR heterodimer structures, residue Glu434 in RXR can form a hydrogen bond across the dimer interface with His445 in FXR (Fig. 5d). We then tested several RXR–LBD Glu434 mutations (E434N, E434Q, E434K, and E434A) with WT FXR–LBD in the coactivator binding assay, using RXR agonist 9cRA and FXR agonist GW4064. The results showed that RXR mutants could affect the signal transduction between the heterodimer (Fig. 5a). When Glu434 in RXR was mutated to Ala, the FXR agonist could hardly increase the ability of RXR to recruit SRC1. Using FXR and RXR mutants, cell-based transfection assay demonstrated that both full-length FXR H445A and RXR E434A mutations decreased the synergistic effect of 9cRA and GW4064 (Fig. 5b). These results indicated that the interaction between the RXR Glu434 and FXR His445 in the heterodimer

**Table 2**

EC_{50} of ligands in the coactivator recruitment assay with monomeric or heterodimeric FXR–LBD

The data are shown as the mean values ± S.D. (n = 3).

| Receptor     | HNC143 | HNC180 | GW4064 | INT747 | Ivermectin |
|--------------|--------|--------|--------|--------|------------|
| FXR–LBD      | 0.228 ± 0.015 | 0.043 ± 0.002 | 0.131 ± 0.033 | 0.521 ± 0.027 | 0.468 ± 0.049 |
| FXR/RXR–LBD  | 0.086 ± 0.005 | 0.032 ± 0.001 | 0.036 ± 0.012 | 0.359 ± 0.008 | 0.396 ± 0.036 |
| FXR/RXR–LBD+9cRA | 0.211 ± 0.030 | 0.049 ± 0.021 | 0.050 ± 0.004 | 0.843 ± 0.159 | 0.726 ± 0.035 |
interface is essential for the synergistic effect. We then looked at whether His^{445} in FXR is conserved in other RXR partners. Structure-based sequence alignment showed that the presence of basic amino acid (His) at this particular position is unique for FXR. In other nuclear receptors, the residue that forms the electrostatic tethering with Glu^{434} in RXR corresponds to the position one turn following the His^{445} in H11 of FXR (Fig. S3).

Using SCA, Ranganathan and co-workers (11) identified a network of 27 coevolved amino acids that are energetically coupled and mediate allosteric signaling in the RXR heterodimers. The mutagenesis study showed that FXR E326A and the corresponding mutations in other permissive partners LXR, PPAR, and Nurr1 exhibit different transcriptional activity in RXR heterodimer. In our GW4064–FXR/9cRA–RXR structure, residue Glu^{326} in FXR forms salt bridges with and stabilizes Arg^{441} and Arg^{436}, which participate in the formation of Allosteric signal transduction interface (Fig. 5d). The E326A mutation would disrupt the interaction network and hinder the allosteric signal transduction from RXR, thereby decreasing the transcriptional activity of FXR. Furthermore, we generated other FXR mutations (R441A and R455S) that belong to the SCA network; each of them decreased the synergistic effect of the two receptor agonists in the cell transactivation assay (Fig. 5c). Taken together, these findings are directly in line with previous findings found in other RXR heterodimers: there are two patches of complementary clustered residues for signal transduction through H11 in FXR–RXR heterodimer. One is through the physically connected AF2 hydrophobic core of the receptor across the dimer interface in the SCA network. The other one connects the dimer interface via an electrostatic tethering across H11 at the plane of the ligands (Fig. S4).

Using SCA, Ranganathan and co-workers (11) identified a network of 27 coevolved amino acids that are energetically coupled and mediate allosteric signaling in the RXR heterodimers. The mutagenesis study showed that FXR E326A and the corresponding mutations in other permissive partners LXR, PPAR, and Nurr1 exhibit different transcriptional activity in RXR heterodimer. In our GW4064–FXR/9cRA–RXR structure, residue Glu^{326} in FXR forms salt bridges with and stabilizes Arg^{441} and Arg^{436}, which participate in the formation of Allosteric signal transduction interface (Fig. 5d). The E326A mutation would disrupt the interaction network and hinder the allosteric signal transduction from RXR, thereby decreasing the transcriptional activity of FXR. Furthermore, we generated other FXR mutations (R441A and R455S) that belong to the SCA network; each of them decreased the synergistic effect of the two receptor agonists in the cell transactivation assay (Fig. 5c). Taken together, these findings are directly in line with previous findings found in other RXR heterodimers: there are two patches of complementary clustered residues for signal transduction through H11 in FXR–RXR heterodimer. One is through the physically connected AF2 hydrophobic core of the receptor across the dimer interface in the SCA network. The other one connects the dimer interface via an electrostatic tethering across H11 at the plane of the ligands (Fig. S4).

Using SCA, Ranganathan and co-workers (11) identified a network of 27 coevolved amino acids that are energetically coupled and mediate allosteric signaling in the RXR heterodimers. The mutagenesis study showed that FXR E326A and the corresponding mutations in other permissive partners LXR, PPAR, and Nurr1 exhibit different transcriptional activity in RXR heterodimer. In our GW4064–FXR/9cRA–RXR structure, residue Glu^{326} in FXR forms salt bridges with and stabilizes Arg^{441} and Arg^{436}, which participate in the formation of Allosteric signal transduction interface (Fig. 5d). The E326A mutation would disrupt the interaction network and hinder the allosteric signal transduction from RXR, thereby decreasing the transcriptional activity of FXR. Furthermore, we generated other FXR mutations (R441A and R455S) that belong to the SCA network; each of them decreased the synergistic effect of the two receptor agonists in the cell transactivation assay (Fig. 5c). Taken together, these findings are directly in line with previous findings found in other RXR heterodimers: there are two patches of complementary clustered residues for signal transduction through H11 in FXR–RXR heterodimer. One is through the physically connected AF2 hydrophobic core of the receptor across the dimer interface in the SCA network. The other one connects the dimer interface via an electrostatic tethering across H11 at the plane of the ligands (Fig. S4).

**Ligand effect in the heterodimer interface and helix 11 conformation**

We then addressed the question of whether the dimerization interface could be altered depending on the type of bound ligands, thereby providing a structure basis for the allosteric communication between RXR heterodimers and diverse gene activation profiles (23). In our three FXR–RXR heterodimer structures, all RXR{\alpha} agonists are 9cRA (Fig. S5). Structure superposition of these complexes showed that the RXR parts superpose well (Fig. 6a), with root-mean-square deviations being 0.460 Å (GW4064–FXR/9cRA–RXR versus HNC143–FXR/9cRA–RXR) and 0.457 Å (HNC180–FXR/9cRA–RXR versus HNC143–FXR/9cRA–RXR) over RXR{\alpha}/H9251 atoms. One significant difference is observed in the C terminus of H11 of RXR in HNC143–FXR/9cRA–RXR heterodimer, among which movement of residues (Cys^{432}, His^{435}, Leu^{436}, and Phe^{439}) induce positional change of the ligand 9cRA correspondingly (Fig. 6b). The most significant difference is located in the C terminus of H11 of FXR, with the notable structural change starting from Asn^{444} and His^{445}. Compared with HNC143 bound heterodimer, the main chain hydrogen bonds formed by Asn^{444}–Glu^{448} and His^{445}–Glu^{449} are lost in HNC180- and GW4064-bound heterodimers (Fig. S6). These two pairs of hydrogen bonds are necessary for maintaining a stable helix structure; thus, losing those bonds will trigger the C terminus of helix 11 to turn into a loop. Furthermore, residues His^{447}, Leu^{451}, Trp^{454}, Leu^{465}, and Trp^{469} shift toward the bound compounds HNC180 or GW4064 (Fig. 6c), leading to a closer ligand/receptor interaction. The change from helix conformation to loop also orientates residue Leu^{451} to the hydrophobic core, and hydrophilic residue His^{459} becomes solvent-exposed.
It is also worth mentioning that the position of Arg455 changed significantly in the C terminus of H11 in these heterodimeric structures with different FXR agonists bound. As shown in Fig. 6d, Arg455 in HNC143-bound FXR pushed against the C terminus of H12, potentially rendering the H12 unstable in HNC143–FXR/9cRA–RXR heterodimer. Thus, it may explain why the coactivator recruitment ability of HNC143 is lower than that of HNC180, because a stable AF2 surface is necessary for coactivator recruitment. In addition, five more residues of coactivator SRC1 were identified in HNC180–FXR/9cRA–RXR than in HNC143- or GW4064-bound heterodimer, resulting in more interactions between FXR (His313 in H4 and Asp470 in H12) and SRC1 in HNC180–FXR/9cRA–RXR complex (Fig. 6e).

Interestingly, H11 is almost identical in the monomeric FXR structures with different ligands (HNC143, HNC180, and GW4064) bound. Compared with the monomer structures, the C terminus of H11 in FXR in the heterodimer changed significantly in the HNC180– FXR/9cRA–RXR heterodimers, but not in HNC143-bound heterodimer (Fig. 7, a–d, Fig. S7). Further analysis of these six structures revealed changes in a network of residues. In HNC180–FXR monomer structure, His445 forms a hydrogen bond with Glu449, but when heterodimerized with RXR, this interaction within FXR is replaced by a hydrogen bond between FXR His445 and RXR Glu434, and at the same time FXR Arg441 forms a hydrogen bond with RXR Ser427. These changes induce significant movement of FXR residues Arg441, Asn444, and His445, disrupting the hydrogen-bond network (Asn444–Glu449 and His445–Glu449) within FXR. Similar scenario is also presented in the GW4064–FXR/9cRA–RXR structure. However, in the HNC143–FXR monomer structure, His445 does not interact with Glu449, whereas in heterodimeric structure with RXR, no significant changes are observed for residues Asn444 and His445 in FXR and overall H11. Interestingly, the superposition of these three ligand-bound FXR–LBD monomer structures reveals only subtle changes induced by the different ligands, with the most notable change occurred in the side chain of His445 (Fig. 7e).

When superposing the three FXR/RXR heterodimers via RXR, we observed that different orientations of the ligand tails in FXR ligand-binding pocket induce a shift in H2, H3, and H11 and then affect the location of AF2, potentially influencing the affinity for the coactivator binding to the heterodimer (Fig. 7f). Therefore, the change of side chain His445 in these compound-bound complexes, combined with distinct conformation of FXR ligands, results in different conformations for H11 of

Figure 5. The role of helix 11 in modulating the allosteric signal. a, FXR–LBD–SRC1/RXR–LBD (WT RXR–LBD and Glu434 mutants) proteins were used in coactivator recruitment assay, and FXR agonist GW4064 (1 μM) and RXR agonist 9cRA (1 μM) were added. RLU, relative light units. b and c, 293T cells were cotransfected with full-length WT FXR or FXR mutant expression plasmids, WT RXR or RXR mutant expression plasmids, FXR-responsive luciferase reporter (pGL3-IR1), and Renilla reporter plasmids. The cells were treated with DMSO, FXR agonist 9cRA (1 μM), and FXR agonist GW4064 (1 μM) for 24 h. Relative activity was defined as pGL3-luciferase activity/Renilla activity. The values are means ± S.D. of three independent experiments. **, p < 0.001; ***, p < 0.0001 (Student’s t test). d, signal transduction pathway from the FXR/RXR heterodimer interface. H11 of FXR and RXR in GW4064-bound heterodimer are shown in cartoon; interacting residues on the heterodimer interface are shown in sticks.
FXR in the heterodimer. Taken together, these structural evidences demonstrated that different FXR ligands can exert unique influence on H11 and then affect the stability of H11 and H12 of FXR when dimerized with RXR, thereby modulating the recruitment of coactivator and transactivation of target genes.

**Discussion**

The liver is a vital organ with critical functions in metabolism. FXR is an important transcriptional regulator in liver. Except for regulating bile acid cholesterol and lipid and glucose metabolism, recent studies found that FXR has much broader role in liver regeneration, energy balance, and postnatal liver maturation (24–26). These functions indicate that FXR is an attractive therapeutic target for liver diseases. Structural information of FXR will greatly contribute to the rational drug design.

In this study, we provide a structural and biochemical characterization of farnesoid X receptor in complex with its obligate heterodimer partner RXR. In agreement with previous studies, especially a recent FXR/RXR heterodimer structure with known FXR ligand WAY-362450 (27), we observed that FXR/RXR heterodimer has the similar heterodimer interface with other RXR heterodimers. Our structural data provide an allosteric mechanism in which communications across the dimer interface, together with the two respective agonists, enhance the affinity of FXR and RXR for binding to individual LXXLL motifs. The increase in binding affinity for coactivators in nuclear receptor complexes versus individual receptors has also previously been noted for RAR, CAR (constitutive androstane receptor), and PXR (19, 28, 29).

It has previously been predicted that receptors whose C termini lie at the same position as PPARγ should be permissive for activation by RXR ligands, because Lys431 in RXR will form a salt bridge with the C terminus of the AF2 in the partner receptor (15). FXR, having one residue shorter than PPARγ at the C terminus, is a RXR permissive partner. However, in our structures, the RXR did not shift to accommodate this one residue difference as was predicted. Therefore, the permissiveness for activation by RXR ligands is not solely determined by the position of partner’s C terminus. There should be other mechanisms to determine the permissiveness or nonpermissiveness. When superposing RXR heterodimer structures using the conserved RXR H11 as the reference, we found that the orientation of H11 in heterodimer partner varies by receptors, and the permissive partner have more bending than nonpermissive partners. This can allow permissive heterodimers to sense both receptor ligands, whereas nonpermissive heterodimers only respond to partner ligands.

Previous studies have shown that the C terminus of helix 11 could be differentially positioned by distinct ligands, thereby controlling the packing of helix 12 in the agonist conformer in NR monomer, especially in PPARγ (30). In our structures, we first identified that the C terminus of the H11 in FXR can have different conformation when dimerized with RXR, and the change is ligand-specific. The C terminus of H11 of FXR in the HNC180/GW4064 heterodimer becomes a loop, instead of α-helix, and brings a closer interaction with ligand. This change has not been found in other RXR heterodimers. We speculate that this is a new regulation mechanism in RXR heterodimers. Through the conformational change in H11 of FXR, the synergistic effect of the two receptor ligands can be modulated.

Here, we also demonstrated that heterodimerization can improve the EC_{50} of FXR agonists in the coactivator recruitment assay. However, the synergic effect only exists in specific

---

**Figure 6. The ligand-induced structural change in the C terminus of helix 11.**

- **a**, alignment of the three FXR/RXR heterodimers. Cyan, heterodimer with HNC143; magenta, heterodimer with HNC180; slate, heterodimer with GW4064; pale cyan, SRC1 in HNC143 complex; red, SRC1 in HNC180 complex; yellow, SRC1 in GW4064 complex.
- **b**, shift in C terminus of H11 in RXR induced by heterodimerization with FXR and corresponding change in the position of RXR ligands 9cRA.
- **c** and **d**, the details of the difference on H11 and H12 with different ligands in FXR.
- **e**, coactivator binding interface (AF2 surface) of FXR in three heterodimers.
ligand pairs. This is due to the diverse effect different ligands could exert on the heterodimer interface. Drug screening efforts have identified small-molecule receptor modulators using only individual LBDs, usually not considering that allosterically acting ligands could be identified if the entire receptor complex is used in the screening. Although the structure for the ligand–receptor complexes are well studied, it is still impossible to precisely predict the correlation between ligand structure and physiological function. Recently, Gabler et al. (31) have developed imatinib and its optimized derivatives as first-in-class allosteric FXR modulators without RXR activity. This allosteric FXR modulation could open a new avenue to gene-selective FXR modulators. Our study presents a very intricate regulation mechanism in ligand-induced FXR/RXR heterodimer signaling.

In summary, our results provide structure insights into the FXR/RXR heterodimer with different combination of ligands. Particularly, the modulation through the conformational change in helix 11 of FXR in the heterodimer will greatly extend our understanding on the RXR heterodimer signal transduction. Importantly, further analysis of allosteric effects on different gene transcription profiles, combined with structural information of nuclear receptor/ligand interactions, will provide a unique opportunity for rational drug design.

**Experimental procedures**

**Compounds**

HNC143 and HNC180 were synthesized based on the published patent WO2017118294. H NMR spectra and mass spectra data (Fig. S8) confirm the structure correctness and purity of the compounds. GW4064 was purchased from Selleck. INT747 was purchased from MedChem Express. Ivermectin and 9cRA were purchased from sigma.

**Protein expression and purification**

The RXR–LBD (residues 225–462) was expressed from the expression vector pET21a (Novagen) without a His tag or from vector pET28a (Novagen) with an N-terminal His6 tag, and the FXR–LBD (225–472) was expressed as a N-terminal His6 fusion protein from the expression vector pET24a (Novagen). For FXR–RXRα–LBD heterodimer protein production, Rosetta 2 cells transformed with two expression plasmids (RXR-pET21a and FXR-pET24a) were grown in LB broth at 37 °C to an A600 of ~1.0 and induced with 0.1 mM isopropyl β-D-thiogalactopyranoside for 16 h when cooled down at 16 °C. The cells were harvested, resuspended in 80 ml of extraction buffer A (20 mM Tris, pH 8.0, 150 mM NaCl, 10% glycerol, 1 mM tris-(2-carboxyethyl) phosphine hydrochloride) per 1 liter of
Allosteric signal transduction in FXR/RXR heterodimer

The FXR monomer structures were determined by molecular replacement with the MOLREP software from CCP4 package (35), using the apo FXR structure (PDB code 3DCT) as the initial search model. For HNC143–FXR–LBD structure, the molecular replacement solution was obtained with a correlation coefficient (CC) of 0.44 and an R factor of 0.81, with the second unrelated peak having a CC of 0.31 and an R factor of 0.87. For HNC180–FXR–LBD, the solution was obtained with a CC of 0.78 and an R factor of 0.36, with the second unrelated peak having a CC of 0.42 and an R factor of 0.58. The FXR/RXR heterodimer structures were determined by molecular replacement with MOLREP in two steps. The apo FXR structure (PDB code 3DCT) was used as the search model in the first step. After obtaining the correct solution for FXR, the apo FXR structure (PDB code 1FBY) was used to locate the position of RXR, whereas FXR was fixed. For HNC143–FXR/9cRA–RXR, in the first step, we obtained top solution with a CC of 0.46 and an R factor of 0.55 in the searching for FXR, with the second unrelated peak having a CC of 0.37 and an R factor of 0.60. In the second step searching for RXR, the top solution has a CC of 0.65 and an R factor of 0.45, with the second unrelated peak having a CC of 0.39 and an R factor of 0.58. The other two heterodimer structures were determined by molecular replacement using the HNC143–FXR/9cRA–RXR as search model. For HNC180–FXR/9cRA–RXR, the solution was obtained with a CC of 0.68 and an R factor of 0.38, and the second unrelated peak had a CC of 0.36 and an R factor of 0.56. For GW4064–FXR/9cRA–RXR, the solution was obtained with a CC of 0.65 and an R factor of 0.50, and the second unrelated peak had a CC of 0.41 and an R factor of 0.60. Model building and refinement was progressed with PHENIX (36) and COOT (37). For HNC143 heterodimer complex structure, the model refinement was optimized using PDB-REDO (38). The statistics of the structure refinement are summarized in Table 1.

Fluorescence anisotropy assay

A fluorescence anisotropy assay was conducted based on fluorescence signal differences between free and protein-bound fluorescein-labeled peptide. A fluorescein-labeled SRCI peptide (5-FAM-CPSSHSLTERHKILHRLQEGSPS), residues 678–700, was synthesized. The reaction system was composed of 50 mM FAM-SRCI peptide, assay buffer (25 mM HEPES, pH 7.5, 100 mM NaCl, 5% glycerol, and 1 mM DTT), and various concentrations of protein. Ligands were added at the concentrations 3–6-fold of the receptors. The mixture was incubated for 1 h at room temperature and then examined by 2104 Envision multilabel reader (PerkinElmer Life Sciences) using FITC FP dual module with an excitation filter of FITC FP 480 and an emission filter of FITC FP P-pol535 and S-pol535. Curve fitting was carried out using Prism 5.0 (GraphPad). Dissociation constants ($K_d$) were calculated by fitting to a nonlinear regression, one-site saturation binding model.

Coactivator recruitment assay

The binding affinity of the compound to FXR–LBD was determined by AlphaScreen assays using a hexahistidine detec-
tion kit from PerkinElmer Life Sciences. The experiments were conducted with 100 nM receptor LBD and 50–200 nM biotinylated SRC1–2 peptides in the presence of 10 μg/ml donor and acceptor beads in a buffer containing 50 mM MOPS, 50 mM NaF, 0.05 mM CHAPS, and 0.1 mg/ml BSA, all adjusted to pH 7.4. Protein, peptide, ligand, and beads were added together in the buffer, followed by incubation for 2 h in the dark. The signals were measured on a plate reader (PerkinElmer Life Sciences). Statistical analyses were performed with GraphPad Prism.

The peptide with an N-terminal biotinylation is listed below

SRC1–2 (SRC1 residue 676–699; SPSSHSLTERHKIL-HRLLLQEGSP), FXR, and RXR mutant expression plasmids were generated by site-mutation PCR (from Guangzhou IGE Biotech) and verified by DNA sequencing. Statistical analyses were generated by site-mutation PCR (from Guangzhou IGE Biotech) and verified by DNA sequencing. Statistical analyses were performed with GraphPad Prism.

Cell culture and Luciferase reporter assay

HEK 293T cell lines were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen), supplemented with 10% fetal bovine serum (Gibco), and cultured at 37 °C in 5% CO₂ atmosphere. The cells were plated in 96-well plates at 1.5 × 10⁴/well, 25 ng/well pCMV–GAL4–DBD–hFXR–LBD expression vector, 25 ng/well PGL5 luciferase, and 5 ng/well Renilla luciferase reporter plasmids were transiently transfected into cells using 0.17 μl/well Lipofectamine 2000 (Invitrogen). 8 h after transfection, the cells were treated with FXR agonists in fresh Dulbecco’s modified Eagle’s medium. Compounds were dissolved in DMSO and added in the concentrations indicated. Luciferase activities were measured after additional 24 h using a Dual-Luciferase reporter assay system (Promega). Relative activity was defined as pGL5–luciferase activity/Renilla luciferase activity. All experiments were repeated at least three times. Statistical analyses were performed with GraphPad Prism.

For the full-length receptor cotransfection assay, 293T cells were transfected with an IR1 (pGL3–FXRE–luc) luciferase reporter plasmid, expression plasmids for FXR, RXRα (pCDNA3.1–hFXR and pCDNA3.1–hRXRα), and Renilla using Lipofectamine 2000. The other procedures were the same as mentioned above.

Author contributions—N. W. and J. L. conceptualization; N. W., Q. Z., J. X., and J. Z. data curation; N. W. formal analysis; N. W. writing-original draft; N. W. and J. L. writing-review and editing; J. Z. and J. L. supervision; J. L. funding acquisition; J. L. project administration.

Acknowledgments—We thank the staff at Beamlines 17U1, 18U1, and 19U1 of the Shanghai Synchrotron Radiation Facility of China for support in the diffraction data collection. We also gratefully thank the Guangzhou Branch of the Supercomputing Center of the Chinese Academy of Sciences for support.

References
1. Lee, F. Y., Lee, H., Hubbert, M. L., Edwards, P. A., and Zhang, Y. (2006) FXR, a multipurpose nuclear receptor. Trends Biochem. Sci. 31, 572–580 CrossRef Medline
2. Wang, Y. D., Chen, W. D., Moore, D. D., and Huang, W. (2008) FXR: a metabolic regulator and cell protector. Cell Res. 18, 1087–1095 CrossRef Medline
3. Wang, H., Chen, J., Hollister, K., Sowers, L. C., and Forman, B. M. (1999) Endogenous bile acids are ligands for the nuclear receptor FXR BAR. Mol. Cell 3, 543–553 CrossRef Medline
4. Fiorucci, S., Cipriani, S., Mencarelli, A., Baldelli, F., Bisfulco, G., and Zampella, A. (2011) Farnesoid X receptor agonist for the treatment of liver and metabolic disorders: focus on 6-ethyl-CDCA. Mini. Rev. Med. Chem. 11, 753–762 CrossRef Medline
5. Darimont, B. D., Wagner, R. L., Apriletti, J. W., Stallcup, M. R., Kushner, P. J., Baxter, J. D., Fletterick, R. J., and Yamamoto, K. R. (1998) Structure and specificity of nuclear receptor-activator interactions. Genes Dev. 12, 3343–3356 CrossRef Medline
6. Forman, B. M., Goode, E., Chen, J., Oro, A. E., Bradley, D. J., Perlmann, T., Noonan, D. J., Burk, L. T., Mendenhall, T., Lamph, W. W., Evans, R. M., and Weinberger, C. (1995) Identification of a nuclear receptor that is activated by farnesol metabolites. Cell 81, 687–693 CrossRef Medline
7. Champon, P. (1996) A decade of molecular biology of retinoid acid receptors.FASEB J. 10, 940–954 CrossRef Medline
8. Schuetz, E. G., Strom, S., Yasuda, K., LeCureur, V., Asseman, M., Brimer, C., Lamba, J., Kim, R. B., Ramachandran, V., Komoroski, B. J., Venkataraman, R., Cai, H., Sinal, C. J., Gonzalez, F. I., and Schuetz, J. D. (2001) Disrupted bile acid homeostasis reveals an unexpected interaction among nuclear hormone receptors, transporters, and cytochrome P450. J. Biol. Chem. 276, 39411–39418 CrossRef Medline
9. Mangelsdorf, D. J., and Evans, R. M. (1995) The RXR heterodimers and orphan receptors. Cell 83, 841–850 CrossRef Medline
10. Klierew, S. A., Umesono, K., Noonan, D. J., Heyman, R. A., and Evans, R. M. (1992) Convergence of 9-cis retinoic acid and peroxisome proliferator signaling pathways through heterodimer formation of their receptors. Nature 358, 771–774 CrossRef Medline
11. Shulman, A. I., Larson, C., Mangelsdorf, D. J., Ranganathan, R. (2004) Structural determinants of allosteric ligand activation in RXR heterodimers. Cell 116, 417–429 CrossRef Medline
12. Forman, B. M., Umesono, K., Chen, J., and Evans, R. M. (1995) Unique response pathways are established by allosteric interactions among nuclear hormone receptors. Cell 81, 541–550 CrossRef Medline
13. Leblanc, B. P., and Stunnenberg, H. G. (1995) 9-cis Retinoic acid signaling: changing partners causes some excitement. Genes Dev. 9, 1811–1816 CrossRef Medline
14. Fernandez, E. J. (2018) Allosteric pathways in nuclear receptors: potential targets for drug design. Pharmacol. Ther. 183, 152–159 CrossRef Medline
15. Gampe, R. T., Jr., Montana, V. G., Lambert, M. H., Miller, A. B., Bledsoe, R. K., Milburn, M. V., Klierew, S. A., Willson, T. M., and XU, H. E. (2000) Asymmetry in the PPAR γ ligand-binding domains. Mol. Cell 5, 543–553 CrossRef Medline
16. Bourguet, W., Vivat, V., Wurtz, J. M., Chambon, P., Gronemeyer, H., and Moras, D. (2000) Crystal structure of a heterodimeric complex of RAR and RXR ligand-binding domains. Mol. Cell 5, 289–298 CrossRef Medline
17. Svensson, S., Ostberg, T., Jacobsson, M., Norström, C., Stefansson, K., Hallén, D., Johansson, I. C., Zachrisson, K., Ogg, D., and Jendeberg, L. (2003) Crystal structure of the heterodimeric complex of the LXRα and RXRβ ligand binding domains in a fully agonistic conformation. EMBO J. 22, 4625–4633 CrossRef Medline
18. Suino, K., Peng, L., Reynolds, R., Li, Y., Cha, J. Y., Repa, J. J., Klierew, S. A., and Xu, H. E. (2004) The nuclear xenobiotic receptor CAR: structural determinants of constitutive activation and heterodimerization among nuclear receptors. Mol. Cell 5, 545–555 CrossRef Medline
19. Chukwu, J. C., Cavett, V., Nowak, J., Chalmers, M. J., Marciano, D. P., Noonan, D. J., Burka, L. T., Mcmorris, T., Lamph, W. W., Evans, R. M., and Weinberger, C. (1995) Identification of a nuclear receptor that is activated by farnesol metabolites. Cell 81, 687–693 CrossRef Medline
20. Kojetin, D. I., Matta-Camacho, E., Hughes, T. S., Srinivasan, S., Nwachukwu, J. C., Cavett, V., Nowak, J., Chalmers, M. J., Marciano, D. P., Kamenecka, T. M., Shulman, A. I., Rance, M., Griffin, P. R., Bruning, J. B., and Nettles, K. W. (2015) Structural mechanism for signal transduction in
RXR nuclear receptor heterodimers. Nat. Commun. 6, 8013 CrossRef Medline

21. Akwabi-Ameyaw, A., Bass, J. Y., Caldwell, R. D., Caravella, J. A., Chen, L., Creech, K. L., Deaton, D. N., Jones, S. A., Kaldor, I., Liu, Y., Madaus, K. P., Marr, H. B., McFadyen, R. B., Miller, A. B., Navas, F., III, et al. (2008) Conformationally constrained farnesoid X receptor (FXR) agonists: naphthoic acid-based analogs of GW 4064. Bioorg. Med. Chem. Lett. 18, 4339–4343 CrossRef Medline

22. Nettles, K. W., and Greene, G. L. (2005) Ligand control of coregulator recruitment to nuclear receptors. Annu. Rev. Physiol. 67, 309–333 CrossRef Medline

23. Downes, M., Verdecia, M. A., Roecker, A. J., Hughes, R., Hogenesch, J. B., Kast-Woelbern, H. R., Bowman, M. E., Ferrer, J. L., Anisfeld, A. M., Edwards, P. A., Rosenfeld, J. M., Alvarez, J. G., Noel, J. P., Nicolaou, K. C., and Evans, R. M. (2003) A chemical, genetic, and structural analysis of the nuclear bile acid receptor FXR. Mol. Cell 11, 1079–1092 CrossRef Medline

24. Kim, K. H., and Moore, D. D. (2017) Regulation of liver energy balance by the nuclear receptors farnesoid X receptor and peroxisome proliferator activated receptor α. Dig. Dis. 35, 203–209 CrossRef Medline

25. Li, G., and Guo, L. G. (2015) Farnesoid X receptor, the bile acid sensing nuclear receptor, in liver regeneration. Acta Pharm. Sin. B 5, 93–98 CrossRef Medline

26. Peng, L., Piekos, S. C., Guo, G. L., and Zhong, X. B. (2017) Role of farnesoid X receptor in the determination of liver transcriptome during postnatal maturation in mice. Nucl. Receptor. Res. 4, 101308 Medline

27. Zheng, W., Lu, Y., Tian, S., Ma, F., Wei, Y., Xu, S., and Li, Y. (2018) Structural insights into the heterodimeric complex of the nuclear receptors FXR and RXR. J. Biol. Chem. 280, 1625–1633 CrossRef Medline

28. Pogenberg, V., Guichou, J. F., Vivat-Hannah, V., Kammerer, S., Pérez, E., Germain, P., de Lera, A. R., Gromemeyer, H., Royer, C. A., and Bourguet, W. (2005) Characterization of the interaction between retinoic acid receptor/retinoid X receptor (RAR/RXR) heterodimers and transcriptional coactivators through structural and fluorescence anisotropy studies. J. Biol. Chem. 280, 1625–1633 CrossRef Medline

29. Wright, E., Vincent, J., Fernandez, E. J. (2007) Thermodynamic characterization of the interaction between CAR/RXR and SRC-1 peptide by isothermal titration calorimetry. Biochemistry 46, 862–870 CrossRef Medline

30. Bruning, J. B., Parent, A. A., Gil, G., Zhao, M., Nowak, J., Pace, M. C., Smith, C. L., Afonine, P. V., Adams, P. D., Katzenellenbogen, J. A., and Nettles, K. W. (2010) Coupling of receptor conformation and ligand orientation determine graded activity. Nat. Chem. Biol. 6, 837–843 CrossRef Medline

31. Gabler, M., Kramer, J., Schmidt, J., Pollinger, J., Weber, J., Kaiser, A., Lühr, F., Proschak, E., Schubert-Zsilavecz, M., and Merk, D. (2018) Allosteric modulation of the farnesoid X receptor by a small molecule. Sci. Rep. 8, 6846 CrossRef Medline

32. Battye, T. G., Kontogiannis, L., Johnson, O., Powell, H. R., and Leslie, A. G. (2011) iMOSFLM: a new graphical interface for diffraction-image processing with MOSFLM. Acta Crystallogr. D Biol. Crystallogr. 67, 271–281 CrossRef Medline

33. Evans, P. (2006) Scaling and assessment of data quality. Acta Crystallogr. D Biol. Crystallogr. 62, 72–82 CrossRef Medline

34. Evans, P. R. (2011) An introduction to data reduction: space-group determination, scaling and intensity statistics. Acta Crystallogr. D Biol. Crystallogr. 67, 282–292 CrossRef Medline

35. Collaborative Computational Project, Number 4 (1994) The CCP4 suite: programs for protein crystallography. Acta Crystallogr. D Biol. Crystallogr. 50, 760–763 CrossRef Medline

36. Murshudov, G. N., Skubák, P., Lebedev, A. A., Pannu, N. S., Steiner, R. A., Nicholls, R. A., Winn, M. D., Long, F., and Vagin, A. A. (2011) REFMAC5 for the refinement of macromolecular crystal structures. Acta Crystallogr. D Biol. Crystallogr. 67, 355–367 CrossRef Medline

37. Emsley, P., and Cowtan, K. (2004) Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126–2132 CrossRef Medline

38. Joosten, R. P., Long, F., Murshudov, G. N., and Perrakis, A. (2014) The PDB_REDO server for macromolecular structure model optimization. IUCrJ 1, 213–220 CrossRef Medline