Exogenous Pregnane X Receptor Does Not Undergo Liquid-liquid Phase Separation in Nucleus under Cell-based In Vitro Conditions

Pengfei Zhao¹#, Yue Gao¹#, Yanying Zhou¹, Min Huang¹, Shicheng Fan¹,²*, Huichang Bi¹,²*

¹ Guangdong Provincial Key Laboratory of New Drug Design and Evaluation, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, 510006, China

² NMPA Key Laboratory for Research and Evaluation of Drug Metabolism & Guangdong Provincial Key Laboratory of New Drug Screening, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou, 510515, China

# : These authors contributed equally to this work.
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*Correspondence to:* Dr. Huichang Bi and Dr. Shicheng Fan, School of Pharmaceutical Sciences, Southern Medical University, 1023# Shatai Nan Road, Baiyun District, Guangzhou 510515, China.

Email: bihchang@smu.edu.cn. fanshch3@smu.edu.cn.

Phone: +86-20-61648530.

Fax: +86-20-39943000

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Abbreviations

CYP: cytochrome P450; DAZAP1: DAZ-associated protein 1; FRAP: fluorescence recovery after photobleaching; IDRs: intrinsically disordered regions; NEAT: nuclear enriched abundant transcript 1; PEG: polyethylene glycol; PHR: photolyase homology region; PXR: pregnane X receptor; RIF: rifampicin; TAZ: transcriptional co-activator with PDZ binding motif; YAP: yes-associated protein;
Abstract

Pregnane X receptor (PXR) belongs to the nuclear receptor superfamily that plays a crucial role in hepatic physiological and pathological conditions. Phase separation is a process in which biomacromolecules aggregate and condense into a dense phase as liquid condensates and coexist with a dilute phase, contributing to various cellular and biological functions. Till now, whether PXR could undergo phase separation remains unclear. This study aimed to investigate whether PXR undergoes phase separation. Analysis of the intrinsically disordered regions (IDRs) using algorithms tools indicated a low propensity of PXR to undergo phase separation. Experimental assays such as hyperosmotic stress, agonist treatment, and optoDroplets assay demonstrated the absence of phase separation for PXR. OptoDroplets assay revealed the inability of the fusion protein of Cry2 with PXR to form condensates upon blue light stimulation. Moreover, phase separation of PXR did not occur even though the mRNA and protein expression levels of PXR target, CYP3A4, changed after sorbitol treatment. In conclusion, for the first time, these findings suggested that exogenous PXR does not undergo phase separation following activation or under hyperosmotic stress in nucleus of cells.

Keywords
Pregnane X receptor, Phase separation, Hyperosmotic stress

Significance statement

PXR plays a critical role in hepatic physiological and pathological processes. The present study clearly demonstrated that exogenous PXR does not undergo phase separation after activation by agonist or under hyperosmotic stress in nucleus. These findings may help understand PXR biology.
Introduction

Phase separation, also known as liquid-liquid phase separation, is a dynamic and reversible process in which biomacromolecules, including proteins and nucleic acids, aggregate and condense into a dense phase that coexists with a dilute phase (Alberti et al., 2019). This phenomenon is known to be the primary mechanism of the formation of membraneless organelles (Banani et al., 2017; Shin and Brangwynne, 2017), which are specific compartments formed by the aggregation of biomacromolecules (Banani et al., 2017). Recent studies have highlighted the physiological importance of phase separation and its involvement in human health and disease progression (Zhang et al., 2020). It has been reported that phase separation could activate or inactivate biological reactions, buffer protein concentration, mediate protein localization, etc. (Alberti et al., 2019). Furthermore, it plays a crucial role in diverse biological processes, including signal transduction, transcriptional regulation, protein homeostasis, DNA damage repair, immune response, genome organization, and neuronal synaptic signaling (Wang et al., 2021; Gu et al., 2022). Phase separation has also been implicated in neurodegenerative diseases and cancer (Zhang et al., 2020; Wang et al., 2021), indicating its significance in the context of these pathological conditions. Phase separation can be regulated by various factors, including biomacromolecule concentration, environmental conditions (such as pH, temperature, and salt concentration), post-translational modifications, and multivalent interactions (Li et al., 2012; Gu et al., 2022). Intrinsically disordered regions (IDRs) within proteins also contribute to phase separation (Alberti et al., 2019).

Pregnane X receptor (PXR) (NR1I2) belongs to nuclear receptors superfamily (Zhao et al., 2022). PXR is predominantly expressed in liver and intestine (Moore and Kliewer, 2000; Petryszak et al., 2016; Xing et al., 2020), and plays significant roles in metabolism modulation, cell cycle arrest, inflammation and angiogenesis (Xing et al., 2020). PXR could regulate various metabolizing enzymes and transporters, especially cytochrome P450 2B6 (CYP2B6), CYP3A4, CYP2C8, CYP2C9, CYP2C19, and P-glycoprotein (Chai et al., 2013). Furthermore, PXR is implicated in various diseases, including liver fibrosis, cholestatic liver injury, liver regeneration, and liver cancer.
(Yao et al.; Zhao et al.; Jiang et al., 2019; Sayaf et al., 2021; Liang et al., 2022). These findings clarified the critical role of PXR in various physiological and pathological processes.

However, the potential of PXR to undergo phase separation and the role in mediating signal transduction through this process remained unknown. Therefore, in this study, we aimed to investigate whether PXR could undergo phase separation and condensates formation in response to hyperosmotic stress or activation. We also measured the downstream targets of PXR to investigate whether the expression changes under hyperosmotic stress. Overall, our findings provide evidence about that exogenous PXR could not undergo phase separation under hyperosmotic stress and after agonist activation in nucleus, which contribute to further investigating the regulatory mechanisms of PXR.
Materials and Methods

Chemicals and Reagents

Rifampicin (RIF, Cat. R3501) was acquired from Sigma (St. louis, MO, USA). DMSO (Cat. 196055) was obtained from MP Biomedicals (Santa Ana, CA, USA). Sorbitol (CAS. 50-70-4) was obtained from Xiya Chemistry (Linyi, Shandong, China). PEG-8000 (Cat. ST483) was obtained from Beyotime (Shanghai, China). Anti-CYP3A4 (Cat. A22229) was obtained from Abclonal (Wuhan, Hubei, China) and anti-β-actin (Cat. 4970) was obtained from Cell Signaling Technology (Danvers, USA). Anti-PXR (Cat. Sc-48340) was obtained from Santa Cruz Biotechnology (Dallas, TX, USA).

Cell culture

HEK293T and HepG2 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in high glucose (4.5 g/L) DMEM from Gibco (Carlsbad, CA, USA) supplemented with 10% FBS from Gibco (Carlsbad, CA, USA) and 1% streptomycin sulfate and penicillin sodium from Gibco (Carlsbad, CA, USA). The cells were maintained at 37°C in a humidified atmosphere with 5% CO₂.

Transfection of plasmids and live-cell imaging

Expression plasmids, including EGFP-PXR, PXR-mCherry-Cry2, EGFP-TAZ, EGFP-YAP, and YAP-mCherry-Cry2 were constructed by Bio-Transduction Lab Co.Ltd (Wuhan, Hubei, China). Transfection of cells was performed using Lipofectamine 2000 from Thermo Fisher Scientific (Waltham, MA, USA) and reduced serum medium Opti-MEM from Gibco (Carlsbad, CA, USA) according to the provided instructions. Glass bottom cell culture dishes were used for cell seeding, and imaging was conducted 48 h post-transfection using an Olympus FV3000 confocal microscope. Hoechst 33342 from Beyotime (Shanghai, China) was used to label the nuclei before confocal imaging. Cells were treated with sorbitol or polyethylene glycol (PEG)-8000 in the hyperosmotic solution treatment experiment.
transfected with EGFP-PXR were treated with DMSO or 20 μM RIF for 48 h in the agonist treatment experiment. Time-lapse images were taken to visualize the fusion behavior of the YAP and TAZ condensates.

**OptoDroplets assay**
HEK293T and HepG2 cells were seeded on glass bottom cell culture dishes and transfected with mCherry-Cry2, PXR-mCherry-Cry2, or YAP-mCherry-Cry2 plasmids for 48 h using Lipofectamine 2000 from Thermo Fisher Scientific (Waltham, MA, USA) and Opti-MEM reduced serum medium from Gibco (Carlsbad, CA, USA). Cells were stimulated with 488 nm blue light at 5% power for 30 s using the LSM stimulation module of an Olympus FV3000 confocal microscope. Time-lapse images were captured in the mCherry channel with a free interval, and one image was taken before stimulation.

**Fluorescence Recovery After Photobleaching (FRAP)**
HEK293T and HepG2 cells were transfected with EGFP-TAZ or EGFP-YAP. FRAP assay were conducted by using the LSM stimulation tool of Olympus FV3000 confocal microscope. After the region of condensate was selected, the region was bleached with 488 nm laser at 10%-100% power for 10 s. The images before and after bleach were captured in EGFP channel.

**Immunofluorescence**
HepG2 cells were seeded on glass bottom cell culture dishes for 24 h before 200 mM sorbitol treatment for 1 h, 3 h, and 6 h. Then cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 10% goat serum diluted with PBS. Then the cells were incubated with primary antibody diluted with 10% goat serum at 4°C overnight. Then cells were incubated with Alexa Fluor 488 conjugated antibody.

**Quantitative real-time PCR analysis**
Total RNA was isolated and cDNA was synthesized as described in our previous publication (Wang et al., 2018). RT-qPCR was performed using SYBR Green Pro Taq HS from Accurate Biotechnology (Changsha, Hunan, China) and a 7500 real-time PCR reaction system from Thermo Fisher Scientific (Waltham, MA, USA). The relative mRNA expression levels were determined using the ΔΔCt method.

**Western blot**

Western blot analysis was conducted as described in our previous publications (Wang et al., 2018). Total proteins were extracted from HepG2 cells transfected with EGFP-PXR, for 48 h and treated with 200 mM sorbitol. The cells were lysed and the protein sample was separated on a 10% SDS-PAGE gel and subsequently transferred to polyvinylidene fluoride membranes. After blocking, the membranes were incubated with the primary antibody overnight at 4°C. The secondary antibody was applied to the membranes and incubated at room temperature for 1 h. Chemiluminescence was detected using the ECL Detection Kit from Millipore (Darmstadt, Germany). Western blot analysis was quantified using Image J software.

**Statistical analysis**

All data were presented as means ± standard deviation (SD). Unpaired t-tests or one-way ANOVA were performed using GraphPad Prism 9.0 software (San Diego, USA) and SPSS 23.0 software from IBM Analytics (Armonk, USA) to determine significant differences between groups. A P-value < 0.05 was considered statistically significant.
Results

PXR lacks intrinsic ability to phase separate

PXR consists of domains which could interact with many other proteins (Watkins et al., 2001; Carnahan and Redinbo, 2005). PONDR and IUPred are tools used to predict IDRs within proteins, such as yes-associated protein (YAP) and transcriptional co-activator with PDZ binding motif (TAZ), which have been reported to undergo phase separation (Cai et al., 2019; Lu et al., 2020; Yu et al., 2021). The structural characteristics of PXR was analyzed to assess its potential for phase separation. The disordered score of amino acid sequence more than 0.5 indicated disordered. It has been reported that the length of IDRs could affect the ability of phase separation (Alberti et al., 2019). IDRs analysis of TAZ by PONDR (Fig. 1A) and IUPred (Fig. 1B) showed that TAZ contains a large proportion of IDRs, however, the analysis of PXR using PONDR (Fig. 1A) and IUPred (Fig. 1B) revealed a considerably lower proportion of IDRs which indicated a limited intrinsic propensity of PXR to undergo phase separation.

To validate these predictions in cells, EGFP-TAZ was expressed in HEK293T cells (Fig. S1A). The results indicated the formation of condensates of EGFP-TAZ in cells. Fusion behavior and fluorescence recovery after photobleaching (FRAP) assay of these condensates validated the phase-separated features of the condensates, which further confirmed that EGFP-TAZ undergoes phase separation in HEK293T cells (Fig. S1B). EGFP-PXR in both HEK293T cells (Fig. 1E) and HepG2 cells (Fig. 1F) was expressed, and we found that EGFP-PXR did not exhibit phase separation without any treatments.

PXR does not undergo phase separation under hyperosmotic stress

Hyperosmotic stress is one of the conditions that lead to phase separation. It has been reported that hyperosmotic stress could drive water outflow and reduce cell volume which further increases protein concentration in cells (Jalihal et al., 2021; Gao et al., 2022). It has been reported that YAP could undergo phase separation in response to hyperosmotic stress (Cai et al., 2019). To validate the reliability of the experiment, the
positive control, EGFP-YAP, was expressed in HEK293T cells (Fig. S2A), the results showed that YAP could not undergo phase separation without hyperosmotic stress condition. However, YAP condensates formation was induced after 200 mM sorbitol treatment in HEK293T cells (Fig. S2B). The fusion behavior and FRAP assay further demonstrated that YAP was undergoing phase separation in HEK293T cells (Fig. S2C). To investigate the effect of hyperosmotic stress on PXR, 200 mM sorbitol was used to treat HEK293T cells and HepG2 cells transfected with EGFP-PXR. The findings revealed that EGFP-PXR does not undergo phase separation upon 200 mM sorbitol treatment for approximately 5 minutes in both HEK293T cells (Fig. 2A) and HepG2 cells (Fig. 2B). The time-lapse images of EGFP-PXR after 200 mM sorbitol treatment within 4.5 min also showed the same result, there was no EGFP-PXR condensates after 200 mM sorbitol treatment in HEK293T cells (Fig. 2C) and HepG2 cells (Fig. 2D). The effect of sorbitol concentration on phase separation was examined by treating cells with 400 mM sorbitol. However, no phase-separated condensates were observed in cells expressing EGFP-PXR in HEK293T cells (Fig. 2I), suggesting that PXR did not undergo phase separation even when the concentration of sorbitol increased.

Since a previous study showed that the crowding agent such as non-ionic crowding agent PEG could promote phase separation (André and Spruijt, 2020), we further investigated the potential role of the crowding agent PEG-8000 in promoting phase separation. However, even upon treatment with 10% PEG-8000 for approximately 5 minutes, EGFP-PXR did not undergo phase separation in both HEK293T cells (Fig. 2E) and HepG2 cells (Fig. 2F). Time-lapse imaging confirmed the absence of condensates formed by EGFP-PXR following treatment with 10% PEG-8000 in both HEK293T cells (Fig. 2G) and HepG2 cells (Fig. 2H). Similarly, treatment with 25% PEG-8000 for approximately 5 minutes in HEK293T cells did not induce phase separation of EGFP-PXR either (Fig. 2J).

**PXR does not form condensate after activation**

Previous study has demonstrated that ligand-activated androgen receptor could
undergo phase separation in cells (Xie et al., 2022). Thus, we further explored the impact of agonist on phase separation of PXR. The effects of RIF, the most typical human PXR agonist (Zhao et al., 2022), on the phase separation of PXR were studied. The results showed that EGFP-PXR did not undergo phase separation after treated with 20 μM RIF for 48 h in HEK293T cells (Fig. 3A). Furthermore, the addition of 200 mM sorbitol or 10% PEG-8000 following RIF treatment did not induce phase separation of EGFP-PXR in HEK293T cells (Fig. 3A). Similarly, EGFP-PXR did not undergo phase separation after 48 h of agonist treatment, regardless of the presence or absence of 200 mM sorbitol or 10% PEG-8000 treatment in HepG2 cells (Fig. 3B).

**Fusion PXR to Cry2 does not lead to rapid light-dependent condensation**

OptoDroplets assay is an approach that utilizes light control to simulate and investigate protein phase separation within cells (Shin et al., 2017). In this study, we fused the photolyase homology region (PHR) of Cry2 with PXR, which were labelled with mCherry. Previous study revealed that YAP contains extended IDRs, and YAP could undergo phase separation (Cai et al., 2019). The YAP-mCherry-Cry2 was expressed in HEK293T cells as a positive control. The results showed that YAP-mCherry-Cry2 condensates were induced after 488 nm blue light stimulation for 30 s (Fig. S3A). mCherry-Cry2 was expressed to serve as the negative control in HEK293T cells (Fig. 4A) and HepG2 cells (Fig. 4B), and no condensates formation was observed after 30 s of 488 nm blue light stimulation. Similarly, the PXR-mCherry-Cry2 did not undergo condensates formation after 488 nm blue light stimulation for 30 s in HEK293T cells (Fig.4A) and HepG2 cells (Fig. 4B). These findings indicated that PXR may not possess the inherent ability to drive phase separation spontaneously.

**PXR target gene was altered under hyperosmotic stress without phase separation occurred**

One of the key biological functions of phase separation is driving transcriptional
regulation. As mentioned above, hyperosmotic stress was one of the conditions that induced phase separation, here the expression of PXR downstream target was measured under hyperosmotic stress to study the effect of hyperosmotic stress on transcriptional activation of PXR. HepG2 cells transfected with EGFP-PXR were treated with 200 mM sorbitol for varying durations of 1 h, 3 h or 6 h. The results revealed that the relative mRNA expression of CYP3A4, the typical target gene of PXR, was upregulated after 3 h and 6 h of 200 mM sorbitol treatment in HepG2 cells transfected with EGFP-PXR (Fig. 5A). Similarly, the western blot analysis demonstrated that the relative protein expression level of CYP3A4 was also upregulated (Fig. 5B).

To determine whether phase separation of PXR occurred simultaneously with changes of CYP3A4 expression, EGFP-PXR was expressed in both HEK293T cells and HepG2 cells. The results demonstrated that even with altered CYP3A4 expression, EGFP-PXR did not undergo phase separation after 3 h and 6 h of 200 mM sorbitol treatment in both HEK293T cells (Fig. 5C) and HepG2 cells (Fig. 5D). No endogenous condensates of PXR were observed either in the cytosol or nucleus of HepG2 cells after 200 mM sorbitol treatment for 1 h, 3 h, or 6 h (Fig. S4A).
Discussion

Phase separation is emerging as a crucial mechanism for transcriptional regulation and signal transduction. PXR plays an important role in many physiological and pathological processes, but whether PXR could undergo phase separation remains unclear. Therefore, in this study, we investigated the potential for PXR to undergo phase separation under various conditions, including hyperosmotic stress, crowded environments, activation by agonists, and fusion with Cry2 with blue light stimulation. The results showed that PXR did not form phase-separated condensates when subjected to sorbitol treatment, even when the expression of its target expression was altered. Consistent with the computational predictions that indicated a lack of IDRs and low propensity of PXR to undergo phase separation, optoDroplets assays demonstrated that PXR lacked the ability to spontaneously drive phase separation. Thus, our results indicate that exogenous PXR could not undergo phase separation under hyperosmotic stress or after activation in nucleus.

IDRs is one of the intrinsically driving force of protein to undergo phase separation (Alberti et al., 2019). The prediction of IDRs within proteins was performed by some computational tools, such as PONDR and IUPred. By using these tools, previous studies showed that YAP possess a high proportion of IDRs, which has great potential to undergo phase separation (Cai et al., 2019; Yu et al., 2021). The prediction of IDRs within PXR by using PONDR showed that PXR contains a small proportion of IDRs. However, it has been reported that the length of IDRs could affect the ability of phase separation (Alberti et al., 2019). And the IDRs analysis of PXR by IUPred showed a lack of IDRs with in PXR. Compared with the positive control, TAZ, which has been reported to undergo phase separation and contains a large proportion of IDRs (Lu et al., 2020), the proportion of IDRs of PXR is relatively lower than TAZ which indicated that only a small part of PXR might be disordered. These findings indicated that PXR might contains a short region of IDRs, and this region of PXR might could undergo phase separation. However, the intact PXR lacks the ability to undergo phase separation. And the following experimental assays further confirmed a limited intrinsic propensity of PXR to undergo phase separation. Previous studies
have shown that the hyperosmotic stress could drive proteins to reach saturation concentration and undergo condensation by reduce cell volume through exosmosis, which increase the effective protein concentration and induce intracellular crowding (Jalihal et al., 2021). It has been reported that YAP and amyotrophic lateral sclerosis-related proteins could undergo phase separation under hyperosmotic stress by treating with sorbitol (Cai et al., 2019; Gao et al., 2022). Earlier research demonstrated that YAP undergo phase separation in cells after 200 mM sorbitol treatment (Cai et al., 2019), and the formation of AMOT/KIBRA/SLMAP tri-scaffold condensates is associated with 400 mM sorbitol treatment (Wang et al., 2022). Based on these, we assessed the phase separation potential of PXR at these sorbitol concentrations. However, PXR did not undergo phase separation under 200 mM and 400 mM sorbitol treatment in our study. Previous literature has highlighted that upon ligand activation, the androgen receptor can transition into liquid-like condensates that are transcriptionally active (Xie et al., 2022). In addition, the activation of PXR may introduce new protein-protein interactions and post-translational modifications that could potentially drive phase separation. Thus, the agonist of PXR, RIF, was utilized to determine the potential of PXR phase separation in this study, and no condensates formation of PXR was observed after RIF treatment. The optoDroplets assay employs a fusion of the PHR of Arabidopsis thaliana Cry2 to proteins or IDRs, tagged with mCherry. When subjected to blue light stimulation, the fusion protein could form phase-separated condensates if the protein or IDRs possesses phase separation-driving capabilities (Shin et al., 2017). Our study demonstrated that the fusion protein of PXR and Cry2 did not form condensates after blue light stimulation. Interestingly, PXR downstream target CYP3A4 was upregulated under hyperosmotic stress regardless of EGFP-PXR transfection (Fig. 5A, S4B), but neither endogenous nor exogenous PXR undergo phase separation under hyperosmotic stress (Figure 5C, 5D, S4A), which suggested that the changes of CYP3A4 under hyperosmotic condition might be mediated by mechanisms related with hyperosmotic stress rather than PXR phase separation.

While hyperosmotic stress and agonist activation are commonly employed to
induce phase separation, factors such as specific post-translational modifications, protein-protein interactions, and long noncoding RNAs might also mediate the phase separation. Post-translational modifications have been known to modulate phase separation (Luo et al., 2021; Li et al., 2022). Previous research has also illustrated the role of long noncoding RNAs in phase separation, for instance, small nucleolar RNA host gene 9 has been implicated in driving the phase separation of large tumor suppressor kinase 1 (Li et al., 2021). It has been reported that long noncoding RNA nuclear enriched abundant transcript 1 (NEAT1) plays an important role in triggering phase separation (Maharana et al., 2018). NEAT1_2 and DAZ-associated protein 1 (DAZAP1) are important components of paraspeckles which is the membraneless nuclear body formed via LLPS (Naganuma et al., 2012; Li et al., 2017; Mitamura et al., 2023). It has been reported that PXR could interact with NEAT1_2 and DAZAP1 which are the components of the paraspeckles and ligand activation of PXR by RIF could induce the disassociation of PXR from paraspeckles in ShP51 cells, which is established by overexpressing human PXR in HepG2 cells (Mitamura et al., 2023). Moreover, the knockdown of NEAT1_2 and DAZAP1 could significantly enhance the induction of the PXR target, CYP3A4, by RIF through increasing the transactivation of PXR. (Mitamura et al., 2023). These findings suggest that the paraspeckles formed by liquid-liquid phase separation could potentially affects drug metabolism by trapping PXR and negatively regulate PXR function in the absence of ligands. The previous study mainly focused on the relationship between the paraspeckle components, NEAT1_2 and DAZAP1, and PXR function. As mentioned above, membraneless organelles such as paraspeckles are formed via phase separation, however, it is not clear whether PXR itself could undergo phase separation. Our current study aimed to explore the potential occurrence of phase separation in PXR. Due to the dynamic property of the phase separation, exogenous EGFP tagged PXR was transfected to live-cells to observe the dynamic process of PXR in current study. And the discrepancy between the previous study and current study might due to the difference of the endogenous over-expressed PXR and the EGFP tagged PXR. In addition, our study did not investigate the specific post-translational modifications or...
protein-protein interactions that might drive phase separation of PXR. Furthermore, it remains to be determined whether PXR can act as client proteins and be recruited into condensates by scaffold proteins. It has been reported that PXR promoted liver regeneration by interacting and activating YAP (Jiang et al., 2019), and YAP has been shown to undergo phase separation under sorbitol treatment (Cai et al., 2019). The potential recruitment of PXR to YAP or other protein condensates during phase separation remains to be investigated. In addition, apart from the cell-based experiments, the purified protein of PXR in vitro still need be further investigated. Recent studies focusing on phase separation mainly rely on in vitro purified protein and cell-based in vitro experiments. It is difficult to directly investigate the characteristics of phase separation in vivo due to technical limitations. Exogenous tagged protein which could undergo phase separation always exhibit spherical condensates in cells, but it cannot accurately reflect the true situation of protein physiological concentration levels in cells. Although the dynamic changes of proteins in cells could be observed through artificially overexpressing tagged-protein or light-induced phase separation technology (Shin et al., 2017; Bracha et al., 2019), how the in vivo result may mimic the in vitro results still needs further investigation. Therefore, more accurate experimental techniques need to be developed to study phase separation in vivo.

In summary, our research indicates that under various conditions, such as agonist administration and hyperosmotic stress, exogenous PXR did not undergo phase separation in nucleus under cell-based in vitro conditions. This work contributes to a better understanding of the underlying mechanisms involving PXR and provides valuable insights for continued exploration within the realm of phase separation.

Data Availability
The authors declare that all the data supporting the findings of this study are contained within the paper.

Author contribution
Participated in research design: Bi, Fan, Gao, Huang

Conducted experiments: Zhao, Gao, Fan, Zhou

Performed data analysis: Zhao, Gao, Fan

Wrote or contributed to the writing of the manuscript: Zhao, Gao, Fan, Bi
Reference


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**Footnotes**

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Figure Legend

Figure 1. Disorder analysis showed low possibilities of phase separation for PXR. (A) Disorder analysis of TAZ and PXR by PONDR. The predictor used was VSL2. The disordered score of amino acid sequence more than 0.5 indicated disordered. (B) Disorder analysis of TAZ and PXR by IUPred. The disordered scores of amino acid sequence higher than 0.5 were regarded as disordered. (C, D) EGFP-PXR (green) in HEK293T cells (C) and HepG2 cells (D) did not undergo phase separation without treatment. Nuclei were stained with Hoechst 33342 (blue). Scale bars, 20 μm.

Figure 2. PXR did not undergo phase separation after sorbitol or PEG-8000 treatment. (A, B) EGFP-PXR did not undergo phase separation after 200 mM sorbitol treatment in HEK293T cells (A) and HepG2 cells (B). Nuclei were stained with Hoechst 33342 (blue). (C, D) Time-lapse imaging showed that EGFP-PXR did not undergo phase separation after 200 mM sorbitol treatment in HEK293T cells (C) and HepG2 cells (D). (E, F) EGFP-PXR did not undergo phase separation after 10% PEG-8000 treatment in HEK293T cells (E) and HepG2 cells (F). Nuclei were stained with Hoechst 33342 (blue). (G, H) Time-lapse imaging showed that EGFP-PXR did not undergo phase separation after 10% PEG-8000 treatment in HEK293T cells (G) and HepG2 cells (H). (I, J) EGFP-PXR in HEK293T cells did not form condensates in the presence of 400 mM sorbitol (I) or 25% PEG-8000 (J). Scale bars, 20 μm.

Figure 3. PXR did not undergo phase separation after RIF treatment. (A) EGFP-PXR did not undergo phase separation in the absence or presence of 20 μM RIF before and after 200 mM sorbitol or 10% PEG-8000 treatment in HEK293T cells. (B) EGFP-PXR did not undergo phase separation in the absence or presence of 20 μM RIF before and after 200 mM sorbitol or 10% PEG-8000 treatment in HepG2 cells. Scale bars, 20 μm.

Figure 4. OptoDroplets assay showed that PXR did not exhibit condensates formation after blue light stimulation. (A) Time-lapse imaging shows that mCherry-Cry2, PXR-mCherry-Cry2 did not undergo phase separation before and after 488 nm laser blue light stimulation in HEK293T cells. (B) Time-lapse imaging showed that mCherry-Cry2, PXR-mCherry-Cry2 did not undergo phase separation before and after 488 nm laser blue light stimulation in HepG2 cells. Scale bars, 20 μm.

Figure 5. The target expression of PXR altered under hyperosmotic stress without phase separation occurred. (A, B) RT-qPCR analysis (A) and western blot analysis (B) results showed that the relative mRNA levels and protein expression levels of PXR target gene, CYP3A4, was changed after 200 mM sorbitol treatment for 3 h and 6 h in HepG2 cells expressed EGFP-PXR (n=3). Data are presented as mean ± S.D., **P < 0.01, ****P < 0.0001, compared with the control group. (C, D) EGFP-PXR did not undergo phase separation after 200 mM sorbitol treatment for 3 h or 6 h in HEK293T cells (C) and HepG2 cells (D). Scale bars, 20 μm.
Figure 3

**A**

- EGFP-PXR
  - Control
  - 200 mM Sorbitol
  - 10% PEG-8000

- DMSO
  - Control
  - 200 mM Sorbitol
  - 10% PEG-8000

- 20 µM RIF
  - EGFP-PXR
  - Control
  - 200 mM Sorbitol
  - 10% PEG-8000

**B**

- EGFP-PXR
  - Control
  - 200 mM Sorbitol
  - 10% PEG-8000

- DMSO
  - Control
  - 200 mM Sorbitol
  - 10% PEG-8000

- 20 µM RIF
  - EGFP-PXR
  - Control
  - 200 mM Sorbitol
  - 10% PEG-8000