LLPS-directed ubiquitination is an interesting new example of protein quality control of phase-separated proteins. This work provides a possible mechanism for the recruitment of ubiquitination machinery to other membraneless compartments, including stress granules, in which ubiquitin-like post-translational modifications (e.g., SUMOylation and NEDDylation) have been shown to occur. Interestingly, disruption to phase separating components of stress granules could also be disease-causing, possibly leading to protein-containing ubiquitinated inclusions characteristic of neurological disorders such as ALS.

Recently, Schuster et al. (2018) show that LLPS systems can be robustly engineered to recruit and release cargo. Membraneless organelles can be programmed to compartmentalize proteins, much in the same way SPOP is compartmentalized to substrate-dependent membraneless bodies. These works provide a potential foundation for developing therapeutic avenues for SPOP-mediated cancers once we understand the molecular determinants and cellular signals for localization to different membraneless organelles.

REFERENCES

Barbieri, C.E., Baca, S.C., Lawrence, M.S., Demichelis, F., Blattner, M., Theurillat, J.-P., White, T.A., Stojanov, P., Van Allen, E., Stransky, N., et al. (2012). Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat. Genet. *44*, 685–689.

Boeynaems, S., Alberti, S., Fawzi, N.L., Mittag, T., Polymenidou, M., Rousseau, F., Schymkowitz, J., Shorter, J., Wolozin, B., Van Den Bosch, L., et al. (2018). Protein phase separation: a new phase in cell biology. Trends Cell Biol. *28*, 420–435.

Bouchard, J.J., Otero, J.H., Scott, D.C., Szulc, E., Martin, E.W., Sabri, N., Granata, D., Marzahn, M.R., Lindorff-Larsen, K., Salvatella, X., et al. (2018). Cancer mutations of the tumor suppressor SPOP disrupt the formation of active, phase-separated compartments. Mol. Cell *72*, this issue, 19–36.

Dao, T.P., Kolaitis, R.-M., Kim, H.J., O'Donovan, K., Martyniak, B., Colicino, E., Hehnly, H., Taylor, J.P., and Castañeda, C.A. (2018). Ubiquitin modulates liquid-liquid phase separation of UBQLN2 via disruption of multivalent interactions. Mol. Cell *69*, 965–978.e6.

Marzahn, M.R., Marada, S., Lee, J., Nourse, A., Kenrick, S., Zhao, H., Ben-Nissan, G., Kolaitis, R.-M., Peters, J.L., Pounds, S., et al. (2016). Higher-order oligomerization promotes localization of SPOP to liquid nuclear speckles. EMBO J. *35*, 1254–1275.

Mitrea, D.M., Cika, J.A., Stanley, C.B., Nourse, A., Onuchic, P.L., Banerjee, P.R., Phillips, A.H., Park, C.-G., Deniz, A.A., and Kriwacki, R.W. (2018). Self-interaction of NPM1 modulates multiple mechanisms of liquid-liquid phase separation. Nat. Commun. *9*, 842.

Mittag, T., and Parker, R. (2018). Multiple modes of protein-protein interactions promote RNP granule assembly. J. Mol. Biol. Published online August 9, 2018. https://doi.org/10.1016/j.jmb.2018.08.005.

Nagai, Y., Kojima, T., Muro, Y., Hachiya, T., Nishizawa, Y., Wakabayashi, T., and Hagiwara, M. (1997). Identification of a novel nuclear speckle-type protein, SPOP. FEBS Lett. *418*, 23–26.

Schuster, B.S., Reed, E.H., Parthasarathy, R., Jahnke, C.N., Caldwell, R.M., Bermudez, J.G., Ramage, H., Good, M.C., and Hammer, D.A. (2018). Controllable protein phase separation and modular recruitment to form responsive membraneless organelles. Nat. Commun. *9*, 2985.

Turakhiya, A., Meyer, S.R., Marincola, G., Böhm, S., Vanselow, J.T., Schlosser, A., Hofmann, K., and Buchberger, A. (2018). ZFAND1 recruits p97 and the 26S proteasome to promote the clearance of arsenite-induced stress granules. Mol. Cell *70*, 906–919.e7.

Entering the Pocket: Crystal Structure of a Prostaglandin D2 Receptor

Mithu Baidya,[1] Punita Kumari,[1] and Arun K. Shukla[1,*]
[1]Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur 2018016, India
*Correspondence: arshukla@iitk.ac.in
https://doi.org/10.1016/j.molcel.2018.09.024

In this issue of *Molecular Cell*, crystal structures of a prostaglandin D2 receptor determined by Wang et al. (2018) reveal novel insights into differential ligand recognition among the members of lipid-binding GPCRs, and provide a structural framework for the identification of novel therapeutics in inflammatory disorders.

G protein-coupled receptors (GPCRs) bind an incredibly diverse spectrum of ligands including small molecules, peptides, hormones, proteins, and lipids. There is a large number of GPCRs that specifically recognize various types of lipids such as phospholipids, lysophospholipids, fatty acids, and eicosanoids as their cognate ligands, and initiate a wide range of downstream signaling responses (van Jaarsveld et al., 2016). Considering the widespread role of lipids in different aspects of cellular signaling and physiology, structural understanding of lipid-binding GPCRs has emerged as a major focus area in recent years. Indeed, in the past few years alone crystal structures of sphingosine-1-phosphate receptor (S1P1R), lysophosphatidic acid receptor (LPA1R), free fatty acid receptor (FFAR1), leukotriene B4 receptor (BLT1R), and cannabinoid receptor (CB1R) have all been determined. In this issue, Wang et al. now present the crystal structures of a prostaglandin D2 receptor, referred to as CRTH2 (chemoattractant receptor-homologous molecule expressed on

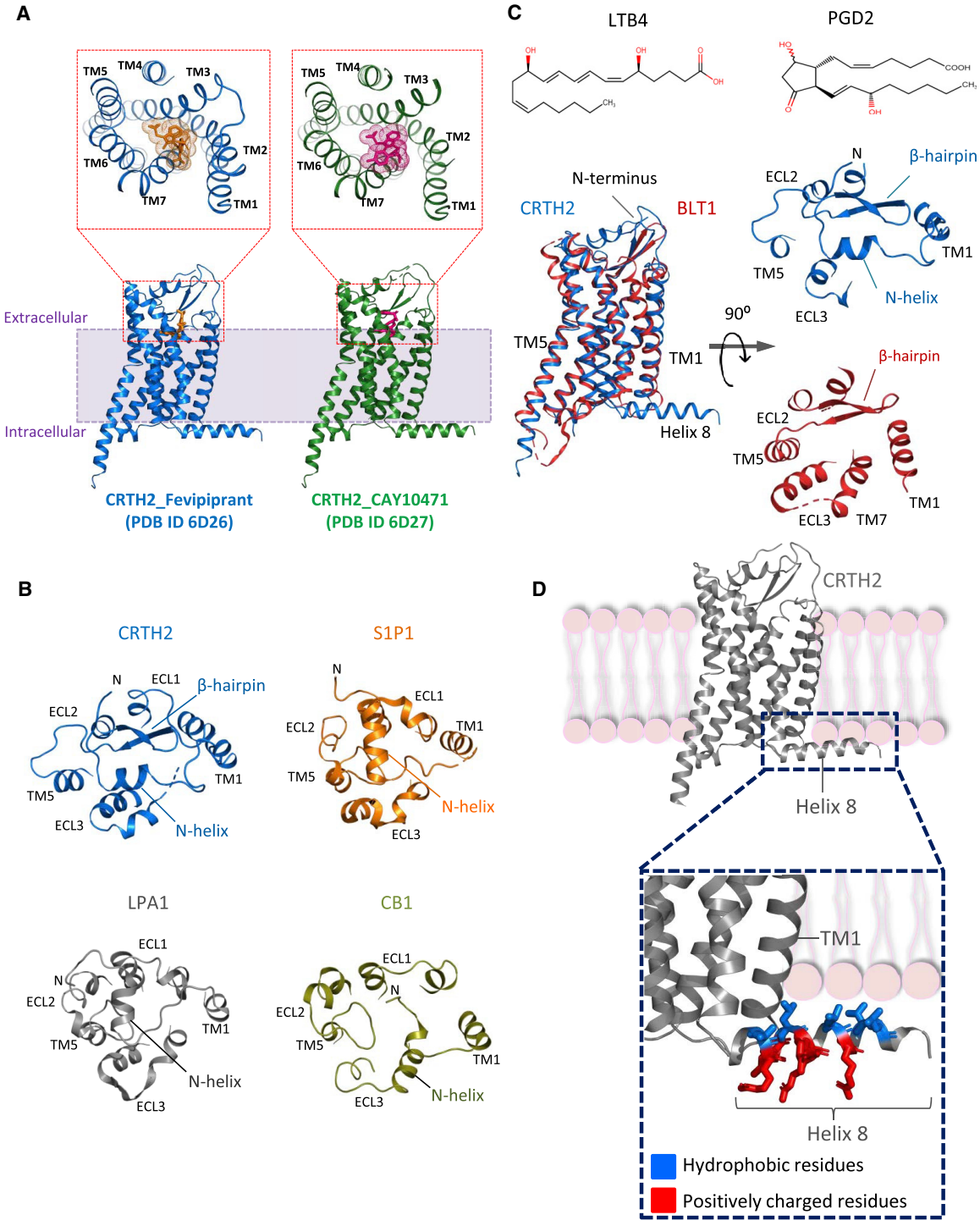

Figure 1. Crystal Structures of the CRTH2 Receptor Reveal Diversity in Ligand Recognition by Lipid-Activated GPCRs

(A) Overall structures of CRTH2 receptor in complex with fevipiprant (left) and CAY10471 (right). The panels above show the top view to highlight the overall ligand-binding poses.

(B) Comparison of the extracellular surface of the CRTH2 receptor with other selected lipid-binding GPCRs (S1P1R, PDB: 3V2W; LAP1R, PDB: 4Z35; CB1, PDB: 5U09). Top views are presented to highlight the alpha helix in the N terminus (referred to as N helix) and the β-hairpin structure in ECL2.

(C) Superimposition of CRTH2 receptor structure with another eicosanoid lipid-binding GPCR, i.e., leukotriene B4 receptor (BLT1; PDB: 5X33), reveals the lack of structured N terminus in BLT1 but presence of the β-hairpin structure. Chemical structures of Leukotriene B4 (LTB4) and prostaglandin D2 (PGD2) are presented to highlight their structural similarity.

(D) Schematic depiction of amino acid distribution in the helix 8 of CRTH2 receptor, suggesting a potential role in specific orientation through membrane association.

T helper type 2 cells), in complex with two different antagonists, namely fevipiprant and CAY10471 (Wang et al., 2018). These crystal structures not only reveal the overall structural features of CRTH2 and high-resolution details of ligand-receptor interaction, but they also allow a direct comparison to other lipid-binding GPCRs, which in turn allow us to appreciate an interesting diversity in ligand recognition modes among these receptors.

Prostaglandin D2 (PGD2), an eicosanoid lipid metabolite derived from arachidonic acid, is produced primarily by the mast cells, and it exerts its cellular actions via two GPCRs, namely the DP1 receptor (DP1R) and the DP2 receptor (DP2R; also referred to as CRTH2) (Alexander et al., 2017). Of these, CRTH2 couples primarily to Gαi subtype of heterotrimeric G proteins and plays a central role in the physiology and pathophysiology of allergy and inflammation, making it a potential drug target in various inflammatory disorders including asthma and allergic rhinitis (Jandl and Heinemann, 2017). In fact, several CRTH2 antagonists including fevipiprant have been developed and tested in clinical trials for asthma showing reasonably promising outcomes (Singh et al., 2017). The high-resolution CRTH2 X-ray crystal structures reported in Wang et al. (2018) may facilitate the identification of additional CRTH2 ligands with therapeutic potential. For structure determination of CRTH2, a carboxyl terminus truncated construct with a fusion of modified T4 lysozyme in the 3rd intracellular loop is utilized and crystals are obtained using the lipidic cubic phase (LCP)-based method, a strategy that has now yielded crystal structures of several GPCRs (Ghosh et al., 2015; Thorsen et al., 2014). The two crystal structures of CRTH2 in complex with fevipiprant and CAY10471 are overall structurally very similar to each other, although there are some key differences between the interaction patterns of the two ligands with the receptor (Figure 1A).

On the extracellular surface, there are two interesting features present in the CRTH2 structures: the first is a short alpha helix (termed N helix) in the N terminus of the receptor, and the second is a β-hairpin structure in the second extracellular loop (ECL2). Wang et al. propose that the combined structural positioning of the N helix and β-hairpin might play a crucial role in regulating and guiding the ligand entry and orientation on the receptor. Typically, the N terminus of GPCRs is considered structurally flexible and, hence, often truncated to generate crystallizable constructs. Even if it is present in the crystallized protein, it is not well resolved in the crystal structure. Therefore, the presence of a structured N terminus in CRTH2 is noteworthy and may indeed have a specific role in ligand entry and recognition, as suggested by the authors. Interestingly, an alpha helix, as present in the N terminus of CRTH2, is also observed in the crystal structures of several other lipid-binding GPCRs such as the S1P1R, LPA1R, and CB1R (Figure 1B), and it is proposed earlier to serve as a lid to restrict and/or fine-tune the ligand access port for these receptors. However, these other lipid-binding GPCRs mentioned above lack the β-hairpin structure present in the ECL2 of CRTH2. On the other hand, the leukotriene B4 receptor (BLT1R), which also binds an eicosanoid lipid (i.e. leukotriene B4) as an agonist, harbors the β-hairpin structure in ECL2 similar to CRTH2 but lacks a well-structured N terminus (Figure 1C). Thus, it is tempting to speculate that these fine structural differences and similarities among the lipid-binding GPCRs are pivotal in determining ligand specificity and selectivity for these closely related receptors, although a systematic structure-function study remains to be performed to test this notion.

The overall arrangement of the transmembrane domain of CRTH2 is similar to that of other related GPCRs crystallized in inactive conformation receptors. On the intracellular side, however, CRTH2 displays an interesting feature in the form of an extended helix 8, which exhibits a peculiar pattern of amino acid distribution (Figure 1D). While the membrane proximal side is lined with a number of hydrophobic residues, the cytoplasmic face exhibits an enrichment of positively charged amino acids. Although purely speculative, such a pattern of amino acid distribution might have a functional contribution in membrane anchorage of helix 8, either in lieu of or in addition to receptor palmitoylation. It is also intriguing that a previous study has reported that CRTH2 helix 8 may harbor structural determinants for surface expression and G protein coupling as perturbation of helix 8 by partial truncation inhibits surface levels but enhances the levels of inositol phosphate and inhibition of cAMP (Schröder et al., 2009). Now, a direct structural visualization of helix 8 boundary may facilitate a systematic mutagenesis study to identify the existence and the nature of potential determinants of surface trafficking and G protein coupling more precisely. Furthermore, investigating the possible contribution of this extended helix 8 in the recruitment pattern and conformational signatures of β-arrestins may also be an attractive avenue to understand CRTH2 signaling, trafficking, and regulation (Ranjan et al., 2017).

Overall, the crystal structure of CRTH2 reveals the intricate details of antagonist binding for the first time, and it provides a previously lacking structural template for identification and optimization of novel CRTH2 ligands with therapeutic potential. Going forward, crystal structures of the other subtype of PGD2 receptor (i.e. DP1R) may illuminate the mechanism of subtype selectivity of ligands at these two related receptors, especially considering that previous studies have reported differential signaling and trafficking of DP1 and CRTH2 receptors (Gallant et al., 2007). More interestingly, structure determination of PGD2-bound CRTH2, in conjunction with corresponding structures of other lipid-binding GPCRs, should provide a better understanding of receptor activation by lipid ligands and ensuing downstream signaling outcomes.

REFERENCES

Alexander, S.P., Christopoulos, A., Davenport, A.P., Kelly, E., Marrion, N.V., Peters, J.A., Faccenda, E., Harding, S.D., Pawson, A.J., Sharman, J.L., et al.; CGTP Collaborators (2017). The concise guide to pharmacology 2017/18: G protein-coupled receptors. Br. J. Pharmacol. *174* (*Suppl 1*), S17–S129.

Gallant, M.A., Slipetz, D., Hamelin, E., Rochdi, M.D., Talbot, S., de Brum-Fernandes, A.J., and Parent, J.L. (2007). Differential regulation of the signaling and trafficking of the two prostaglandin D2 receptors, prostanoid DP receptor and CRTH2. Eur. J. Pharmacol. *557*, 115–123.

Ghosh, E., Kumari, P., Jaiman, D., and Shukla, A.K. (2015). Methodological advances: the unsung heroes of the GPCR structural revolution. Nat. Rev. Mol. Cell Biol. *16*, 69–81.

Jandl, K., and Heinemann, A. (2017). The therapeutic potential of CRTH2/DP2 beyond allergy and

asthma. Prostaglandins Other Lipid Mediat. *133*, 42–48.

Ranjan, R., Dwivedi, H., Baidya, M., Kumar, M., and Shukla, A.K. (2017). Novel structural insights into GPCR-β-arrestin interaction and signaling. Trends Cell Biol. *27*, 851–862.

Schröder, R., Merten, N., Mathiesen, J.M., Martini, L., Kruljac-Letunic, A., Krop, F., Blaukat, A., Fang, Y., Tran, E., Ulven, T., et al. (2009). The C-terminal tail of CRTH2 is a key molecular determinant that constrains Galphai and downstream signaling cascade activation. J. Biol. Chem. *284*, 1324–1336.

Singh, D., Ravi, A., and Southworth, T. (2017). CRTH2 antagonists in asthma: current perspectives. Clin. Pharmacol. *9*, 165–173.

Thorsen, T.S., Matt, R., Weis, W.I., and Kobilka, B.K. (2014). Modified T4 lysozyme fusion proteins facilitate G protein-coupled receptor crystallogenesis. Structure *22*, 1657–1664.

van Jaarsveld, M.T., Houthuijzen, J.M., and Voest, E.E. (2016). Molecular mechanisms of target recognition by lipid GPCRs: relevance for cancer. Oncogene *35*, 4021–4035.

Wang, L., Yao, D., Deepak, R.N.V.K., Liu, H., Xiao, Q., Fan, H., Gong, W., Wei, Z., and Zhang, C. (2018). Structures of the human PGD_2 receptor CRTH2 reveal novel mechanisms for ligand recognition. Mol. Cell *72*, this issue, 48–59.