presumably Fz endocytosis and signaling (Figure 1). Note that PIP_2 has a key role in recruiting and converting AP-2 from the inactive (closed) to the active (open) form at the plasma membrane (Jackson et al., 2010). It has been reported that Wnt stimulates the production of PIP_2 through Fz and Dvl, which activates lipid kinases PI4K and PIP5K (MacDonald et al., 2009; Qin et al., 2009). Although this Fz-Dvl induced PIP_2 production has only been implicated in Wnt/β-catein signaling thus far, the possibility that it also accounts for, together with direct Dvl-binding, AP-2 recruitment to the Fz complex during PCP signaling deserves consideration.

Have we arrived at the juncture where we can explain Dvl specificity in Wnt/β-catenin and PCP signaling? Unfortunately not. In fact, some recent studies added more weight to the argument that the simple notion that the DIX and DEP domains are specific for Wnt/β-catenin and PCP signaling, respectively, might be too simple after all. First, the DEP domain may have important contributions to Wnt/β-catenin signaling, for example, through the aforementioned positively charged surface (Simons et al., 2009) and binding/activating PI4K for PIP and PIP_2 production (Qin et al., 2009). Second, the DIX domain-mediated Dvl polymerization can be regulated by both canonical and noncanonical Wnt signaling, and intriguingly by the DEP domain (specifically the K to M mutation) during noncanonical signaling (Nishita et al., 2010). Therefore it appears that intramolecular interactions among different Dvl domains in Wnt pathways are elaborate and perhaps regulated, contributing to the activation of specific downstream events. The molecular insights on Dvl/AP-2 interaction (Yu et al., 2010) help to define a role of Dvl in PCP signaling, and in addition, as the authors pointed out, suggest that the bipartite/combinatory interaction may be a common theme in cargo/AP-2 coupling.

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Dynamics of Nuclear Receptors

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The dynamic properties of VDR LBD and full-length VDR/RXRα heterodimer in the presence and absence of ligands were investigated by hydrogen/deuterium exchange mass spectrometry (Zhang et al., 2010a). The results beautifully complement X-ray crystal structure data.

Nuclear receptors (NRs) exist in a cell interior and are responsible for sensing the presence of various molecules, including steroid and thyroid hormones (Olefsky and Saltiel, 2000). NRs are one of the largest classes of drug targets along with G protein-coupled receptors (GPCRs) and ion channels. Detailed studies of NRs are critical not only for understanding biology but also for the development of therapeutic agents.

Visualization of a protein structure enormously helps our understanding of the mechanism and function of the protein. A crystal structure of drug target protein is the basis of structure-based drug design. X-ray crystallography is one of the most powerful driving forces of modern biology and medicine, though there are limitations. One limitation is its applicability. Not all drug targets are crystallizable, let alone all the complexes involving drug targets. The other issue is the static nature of the information obtained. An X-ray crystal structure of a protein is a high-resolution snapshot of a dynamic entity. To fully describe the protein, it is desirable to obtain the dynamic characteristics of the protein in addition to the static structural information.

Amide hydrogen/deuterium exchange, when coupled with proteolysis and mass

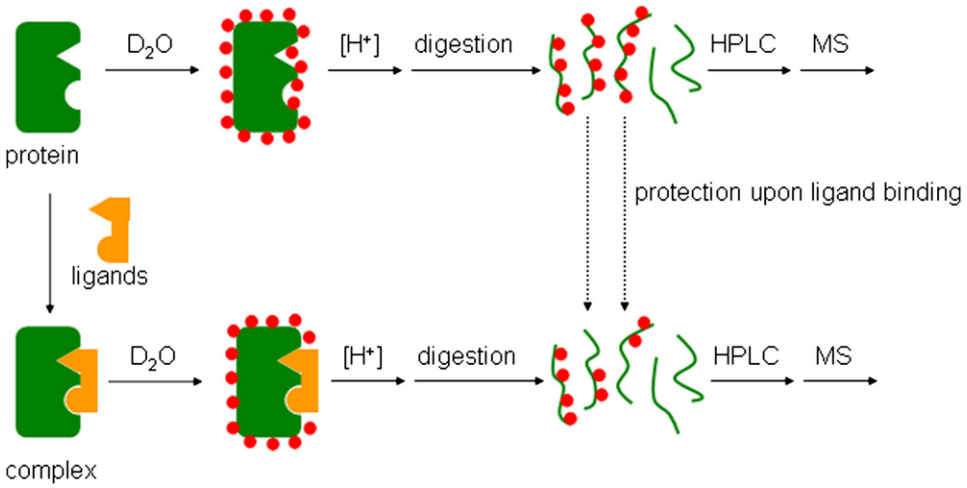

Figure 1. General Concept of HDX-MS for Protein-Ligand Interactions
Protein and peptide, green; ligand, orange; deuterium, red. A protein or a protein complex is first incubated in deuterated buffer. After HDX reactions are quenched by the addition of acid, the deuterated protein is next digested by pepsin. The level of deuterium incorporation in each digested fragment is then determined by LC-MS (Hamuro et al., 2003). The degree of deuterium incorporation in each peptic fragment can infer dynamic properties of the region of the protein (Englander and Kallenbach, 1983).

spectrometry (HDX-MS) (Figure 1), is a useful method to complement X-ray crystallography data (Chandra et al., 2008). First, HDX-MS is a highly applicable technology and enables scientists to investigate constructs and complexes whose structures are not available. Second, HDX-MS data describe the dynamic characteristics of a target protein at the submolecular level (Englander and Kallenbach, 1983).

HDX-MS is generally a medium-resolution, medium-throughput technique (Hamuro et al., 2003). The resolution is on average ten amino acids long, depending on the size of the peptic fragments generated by the proteolysis. Recent improvements in automation have increased the throughput, and one condition per day is easily accomplished (for instance, HDX-MS of the apo protein on one day and HDX-MS of the same protein with a ligand on the next day). This methodology recently became applicable to membrane proteins (Hebling et al., 2010; Zhang et al., 2010b).

In this issue of *Structure*, Griffin's group from Scripps Florida applied HDX-MS to investigate the dynamic characteristics of vitamin D receptor (VDR), ligand-binding domain (LBD), and full-length VDR/RXRα (retinoid X receptor α) heterodimer (Zhang et al., 2010a) (Figure 2). In this systematic study, the following HDX profiles were determined: (1) VDR LBD in the absence of ligands; (2) VDR LBD in the presence of three different ligands, 1,25D3, ED-71, and alfacalcidol; (3) full-length VDR/RXRα heterodimer in the absence of ligands; and (4) full length VDR/RXRα heterodimer in the presence of the aforementioned three ligands. Among eight VDR systems studied, X-ray crystal structures of two complexes are available; VDR LBD/1,25D3 (1DB1) and VDR LBD/ED-71 (2HAR).

The HDX profile of VDR LBD in the absence of ligands establishes the baseline for this study (Figure 2A). While the X-ray crystal structure of apo VDR LBD is not available, the similarity between overall HDX pattern of apo VDR LBD and that of VDR LBD with 1,25D3 suggests that the overall structure of apo VDR LBD is similar to that of VDR LBD with 1,25D3. It is important to note that the HDX profile of apo VDR LBD resembles those of other apo NR LBDs, such as PPARγ (Hamuro et al., 2006). All of them have a rigid upper subdomain and a dynamic lower subdomain containing a ligand binding pocket.

The HDX perturbation of VDR LBD upon binding to each of the three ligands sheds light on how each ligand interacts with VDR LBD. While detailed interpretation of the VDR LBD HDX-MS data is only possible by comparing with the X-ray structure data of VDR LBD/1,25D3 and VDR LBD/ED-71, HDX-MS can probe the interactions for which X-ray crystal structures are not available (e.g., VDR LBD/alfacalcidol).

The HDX perturbations by 1,25D3 and ED-71 are very similar, consistent with their similarly strong transcriptional potency (Figure 2C). However, a partial agonist, alfacalcidol, induces a different HDX perturbation (Figure 2B). The lack of protection near H11 and H12 is particularly intriguing and indicates that these helices remain dynamic when bound to alfacalcidol. Similar results were obtained for H11 and H12 of PPARγ LBD with full agonists and a partial agonist (Hamuro et al., 2006). HDX-MS results support the idea that the dynamic properties of H11 and H12 are critical for the transcriptional activity of NRs (Johnson et al., 2000; Bruning et al., 2007). If this is the case, HDX-MS can be used as a surrogate assay to differentiate a full agonist from a partial agonist. Also the strong protection (therefore rigidification) near H11 and H12 upon binding to a full agonist may be the reason why X-ray structures of NRs with full agonist complexes are more abundant than those of apo structures, antagonist complexes, or partial agonist complexes.

HDX-MS allows one to investigate physicochemical properties of full-length VDR/RXRα heterodimer at the submolecular level (Figure 2D). Crystallization of a full-length protein with dynamic regions is

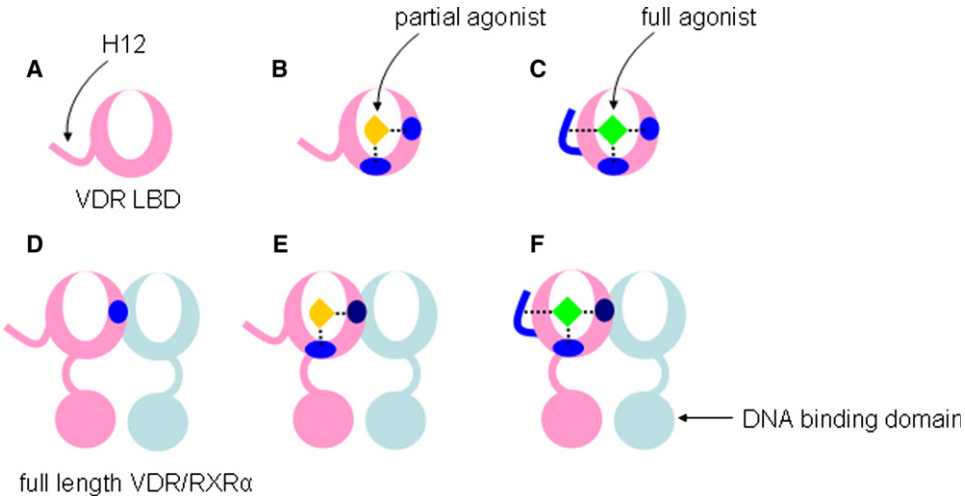

Figure 2. Schematic of Dynamic Properties of VDR LBD in VDR LBD and Full-Length VDR/RXRα Heterodimer
Top row is VDR LBD and bottom row is full-length VDR/RXRα heterodimer. Left column is in the absence of ligand; middle is in the presence of a partial agonist, alfacalcidol, and right column is in the presence of a full agonist, 1,25D3 or DE-71. Pink is VDR and light blue is RXRα. Gold is a partial agonist and light green is a full agonist. Blue and dark blue indicate protected regions. Dotted lines indicate an interaction.
(A) The standard state, VDR LBD, in the absence of ligand.
(B) VDR LBD in the presence of a partial agonist, alfacalcidol. A few regions are less dynamic than the standard state (blue).
(C) VDR LBD in the presence of a full agonist, 1,25D3 or DE-71. In addition to the regions protected by the partial agonist (blue), H12 is also protected (blue).
(D) Full-length VDR/RXRα heterodimer in the absence of ligand. A region near H10 that is expected to interact with RXRα exchanges slower than VDR LBD (blue).
(E) Full-length VDR/RXRα heterodimer in the presence of a partial agonist, alfacalcidol. Similar protection as in VDR LBD (blue) was observed with extra protection at RXRα interface (dark blue).
(F) Full-length VDR/RXRα heterodimer in the presence of a full agonist, 1,25D3 or DE-71. Similar protection as in VDR LBD (blue) was observed with extra protection at RXRα interface (dark blue).

usually more difficult than that of a rigid domain, and NRs are no exception. However, HDX-MS can be applicable to a full-length protein with dynamic regions that may prevent it from crystallizing. Nearly identical HDX patterns of VDR LBD and full-length VDR/RXRα heterodimer in the absence of ligands imply that the structural integrity of full-length VDR is conserved in the short construct of VDR LBD.

The resemblance between the HDX protection of VDR LBD and that of full-length VDR/RXRα heterodimer upon binding to the ligands also suggests that each ligand interacts with VRD LBD and the heterodimer similarly (Figures 2E and 2F). This observation in a way supports the validity of rational drug design using VDR LBD crystal structures.

A natural extension of this study includes HDX-MS analysis of the RXRα moiety of the heterodimer and HDX-MS analysis of the effects of various peptide cofactors on the same complexes employed here. HDX-MS is a relatively new and widely applicable technique that gives unique information on protein dynamics and complements X-ray structure data very nicely. Continual growth in this field is expected.

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