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A Case of Cross-Reactivity

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Studies using chemical inhibitors have suggested that p38 MAP kinase is a key regulator of Wnt/β-catenin signaling. In this issue, Verkaar et al. (2011) show that cross-reactivity of p38 inhibitors with casein kinase Iδ/ε is responsible for Wnt/β-catenin pathway inhibition.

Wnt proteins are members of an evolutionarily conserved family of signaling molecules that play a central role in embryonic development and adult tissue homeostasis (Logan and Nusse, 2004). Given this wide array of functions, it is not surprising that the deregulation of Wnt signaling is associated with a range of human diseases (Clevers, 2006); chief among these is cancer, providing a major incentive to define the molecular mechanism of Wnt signaling and to find inhibitors that can be used to target the Wnt pathway in cancer treatment.

In recent years, a detailed outline of the Wnt signaling mechanism has emerged. Wnt can signal through several distinct pathways, but the major pathway related to stem cell maintenance and cancer is the so-called canonical Wnt/β-catenin pathway (MacDonald et al., 2009). A central player in this pathway is β-catenin, which interacts with TCF transcription factors to co-activate the expression of Wnt target genes. In the absence of the Wnt ligand, β-catenin levels are downregulated by a destruction complex that consists of the scaffold protein Axin, the APC tumor suppressor protein, and the kinases casein kinase Iα (CKIα) and GSK3β, which phosphorylate β-catenin to target it for proteasome-mediated degradation. Binding of Wnt to its receptors Frizzled and LRP5/6 inhibits destruction complex function, leading to stabilization and translocation of β-catenin to the nucleus. Although the mechanism of destruction complex inhibition is still poorly understood, it is clear that phosphorylation of Disheveled (Dvl) by casein kinase Iδ/ε (CKIδ/ε) is an important step in this process (Bryja et al., 2007; Gao et al., 2002). Recently, the MAP kinase p38 has been implicated in Wnt/β-catenin signaling as well (Bikkavilli et al., 2008).

To identify low molecular weight compounds that inhibit the Wnt/β-catenin pathway, Verkaar et al. (2011) developed an elegant β-galactosidase complementation assay to detect translocation of β-catenin into the nucleus. In the study published in this issue of *Chemistry & Biology*, they applied this method to screen a relatively small collection of a little over 2000 compounds and found that two compounds, TAK-715 and AMG-548, potently inhibit the Wnt-dependent stabilization of β-catenin (Verkaar et al., 2011). TAK-715 and AMG-548 were previously characterized as inhibitors of p38 MAP kinase, which would be in agreement with the reported role of p38 in the Wnt/β-catenin pathway (Bikkavilli et al., 2008). However, further characterization of TAK-715 and AMG-548 using a panel of kinases revealed that these compounds also inhibit CKIδ/ε. This finding prompted the question of whether the two compounds might inhibit Wnt/β-catenin signaling through CKIδ/ε instead of p38. Consistent with this notion, it was found that selective p38 inhibitors that do not cross-react with CKIδ/ε have no effect on Wnt/β-catenin signaling, raising doubts about the role of p38 in this signaling pathway. Indeed, Verkaar et al. (2011) found that knockdown of p38 did not interfere with the Wnt-dependent phosphorylation of Dvl, and that p38 was not activated after Wnt stimulation.

This study has several important implications. First, it shows that results obtained with kinase inhibitors should be interpreted with caution and that potential cross-reactivity with other kinases should be rigorously tested. Second, the study provides compelling evidence that p38 MAP kinase is not involved in Wnt/β-catenin signaling. Finally, TAK-715 and AMG-548 are clinically evaluated compounds that can now be repositioned for the treatment of Wnt-dependent tumors. As TAK-715 and AMG-548 target the Wnt pathway at the level of CKIδ/ε, it is unlikely that they will be effective in tumors harboring mutations in downstream

components such as APC. It is becoming clear, however, that a wide range of cancers activate Wnt signaling by epigenetic downregulation of secreted Wnt inhibitors such as Dkk1, WIF1, and members of the secreted Frizzled-related family (Ying and Tao, 2009). Especially in these cases, CKIδ/ε inhibitors such as TAK-715 and AMG-548 may prove to be therapeutically important.

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In the Heart of a Dynamic Chromatin

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An audacious bet on transforming histone H4 into a real-time sensor probe has been won by the group of Minoru Yoshida, who designed the first FRET probes capable of signaling the occurrence of dynamic site-specific acetylations in live cells.

Analyzing and understanding the functional significance of genome signposting by covalent modifications of DNA and histones constitutes a real challenge in modern biology. An important feature of these signs is their dynamic nature, which correlates with their potential to act as a support for the so-called heritable epigenetic information. The precise vision of these dynamic alterations of DNA and histones is critical to fully understanding the epigenome and its regulatory circuits. The advent of live cell imaging and fluorescent probes is now allowing the design of experimental approaches to achieve this goal. Our increasing knowledge of cellular machineries capable of recognizing DNA methylation and specific histone posttranslational modifications (PTM) has opened the way for the design of fluorescent protein-based probes bearing specific binding domains of the corresponding factors and antibody fragments capable of recognizing specific modifications. Several FRET probes were also conceived using a combination of a histone PTM-binding element and a histone fragment capable of receiving the PTM (Kimura et al., 2010). These approaches had two major limitations. The first was that the probes needed to access their targets to generate meaningful signals, therefore creating a dependency on the state of chromatin and site accessibility. The second limitation was the inability of fusions comprising histone fragments to integrate chromatin, making them insensitive to chromatin-dependent events.

The design of a probe capable of being assembled into chromatin (in other words, a histone-sensor of PTMs) therefore appeared as an elegant way to circumvent these problems. However, feasibility issues hit the attractiveness of the idea and could have stopped many from trying. The group of Minoru Yoshida started the adventure by using a full-length histone H4 as a building basis for a histone-probe molecule. The aim was to develop a sensor of H4 acetylation by using an already characterized bromodomain-containing protein, fused to the N-terminal of the histone and separated from it by a flexible linker peptide. Two GFP-derived proteins (Venus and CFP) were also fused on either side of the resulting protein in order to generate a full-length probe susceptible of reporting a FRET signal after an acetylation-dependent conformation change (Sasaki et al., 2009; Ito et al., 2011).

Outstandingly, this fusion protein is recognized as a regular histone H4 by the cellular chromatin assembly machinery and is incorporated into chromatin, therefore giving the unprecedented power of sensing the occurrence of an acetylation event from the "inside" of a chromatin fiber. The first probe, named Histac, contained Brdt, a testis-specific double bromodomain protein (Pivot-Pajot et al., 2003) as the acetyl-recognition module. One of the first pieces of information revealed by the probe was the identity of the acetyl-acceptor sites, recognized by Brdt in living cells, as being lysine 5 and 8 (K5 and K8) on histone H4. The data also indicated the requirement of the simultaneous acetylation of H4K5 and H4K8 for Brdt to bind (Sasaki et al.,

