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.,

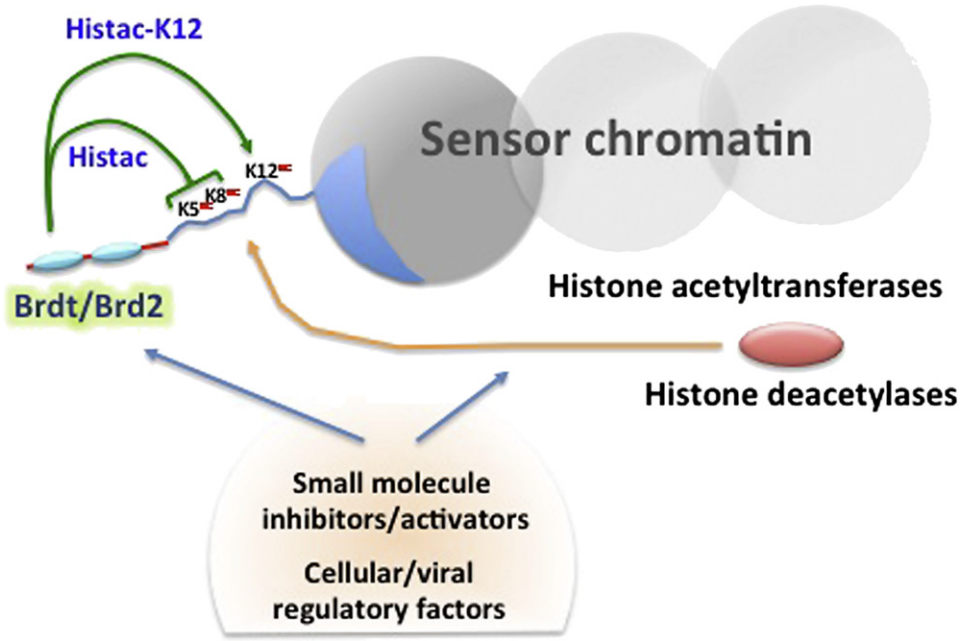

Figure 1. Generation of Chromatin Sensors Capable of Reporting the Occurrence of Site-Specific Histone H4 Acetylations
The expression of histone H4 bearing specific acetylated lysine-recognition modules, bromodomains of Brdt or Brd2, allows its assembly into chromatin. The resulting FRET probes not only allow demonstration of the dynamics of histone H4 acetylation at these specific sites as a function of different events, i.e., cell cycle, but also can serve as a reporter to measure the impact of particular HAT or HDAC on these acetylations. These probes also provide excellent readouts for the discovery of a wide range of small molecule effectors of enzymatic activities or bromodomains. Additionally, a panel of experiments could be designed to test the impact of particular cellular and viral regulatory factors on site-specific histone acetylation, using Histac/Histac-K12 as a reporter.

2009). Interestingly, a parallel structural work based on the crystal structure of Brdt's first bromodomain, BD1, uncovered the exact same property of double acetyl-lysine binding (Morinière et al., 2009) and therefore unquestionably supported the performance and the reliability of the designed probe. Reciprocally, this approach also elegantly showed that the crystal-based structural data are valid in a living cell, in the heart of a dynamic chromatin. The specificity of Brdt for H4K5ac-H4K8ac also made the probe a unique and therefore precious sensor of the occurrence of H4 hyperacetylation in living cells. Indeed, since simultaneous acetylation of H4 K5 and K8 is a signature of hyperacetylated H4 (Sasaki et al., 2009), Histac is a perfect probe to evaluate the efficacy of histone-deacetylase inhibitors (HDACi) by measuring the ability of these molecules to induce a full acetylation of histone H4. Hence, the measurement of HDAC inhibition by various types of inhibitors in living cell with an unprecedented sensitivity is the second outstanding application of Histac. Finally, Histac allowed a real-time visualization of histone hyperacetylation during mitosis revealing a dramatic loss of histone H4 hyperacetylation after the onset of chromosome compaction (Sasaki et al., 2009).

Following the same principle, Yoshida's group developed another related probe using Brd2 (Kanno et al., 2004) as the acetyl-reading module. This new probe revealed itself as being very complementary to Histac because it specifically signals the occurrence of acetylation on H4K12, hence named Histac-K12 (Ito et al., 2011). The two probes together therefore allow a real-time visualization of the occurrence of acetylation on different lysines of H4: K5-K8 and K12. The use of Histac-K12 showed that, in contrast to H4K5-8, acetylation of H4K12 is not affected by chromosome compaction during mitosis. Additionally, although Histac-K12 allowed evaluation of the acetylation response to HDACi treatments with the same sensitivity as Histac, this probe revealed an unexpected stability of H4K12 acetylation after the removal of several unrelated HDACis. The use of Histac to measure the stability of H4K5acK8ac under the same experimental conditions will tell if acetylation on these two residues is also unexpectedly stable. This may lead to the discovery of yet unknown mechanisms controlling bromodomain-dependent stability of specific acetylation marks or reveal an unexpected retention of some of the HDAC inhibitors in the treated cells.

In addition to HDACs, these probes also offer a highly sensitive readout for a new class of small inhibitory molecules: bromodomain inhibitors. Yoshida and colleagues used Histac-K12 to demonstrate the use of this probe in evaluating the efficacy of a new bromodomain inhibitor named BIC1 (Ito et al., 2011). Histac/Histac-K12 would of course now provide the community with excellent probes for the identification and characterization of new bromodomain inhibitors, which might constitute a new category of epigenetic drugs (Filippakopoulos et al., 2010). Finally, although not yet exploited, these probes open the way for evaluating the contribution of specific HDACs and histone acetyltransferases (HATs,) as well as their cellular and viral regulators, in site-specific histone acetylation in living cells by using either specific interfering small RNAs or specific small molecule inhibitors (Figure 1).

One of the remarkable aspects of the work by Yoshida's laboratory is the demonstration that an individual histone could be transformed into a sensor-probe without losing its ability to be incorporated into chromatin. The generation of Histac/Histac-K12 showed that a histone can in fact accommodate large nonhistone segments and still be recognized by the cellular chromatin assembly machinery. This demonstration is now paving the way for the generation of a multitude of histone-based probes bearing various histone PTM-recognizing modules. These probes are expected to tremendously accelerate our understanding of epigenetics and, among other things, provide highly sensitive readouts for the discovery of new epigenetic drugs.

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