Structure
Preview

A New RING Tossed into an Old HAT

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p300 and CBP are multi-domain histone acetyltransferases (HATs) that regulate gene expression and are mutated in human diseases including cancer. Delvecchio and colleagues report the structure of the p300 catalytic core, revealing the presence of a previously unknown RING domain that regulates the enzyme's activity.

Reversible acetylation of lysine residues on histones, transcription factors, and transcriptional coactivators plays a central role in activating eukaryotic transcription (Eberharter and Becker, 2002). Histone acetyltransferase (HAT) enzymes catalyze lysine acetylation and are grouped into three main families based on sequence homology within their catalytic domains: the Gcn5 *N*-acetyltransferase (GNAT) family, the Morf, Ybf2, Sas2, and Tip60 (MYST) family, and the p300/CBP family (Lee and Workman, 2007). Although all three classes of HATs use acetyl-CoA as the acetyl donor and catalyze the same reaction, they differ substantially in structure and use different reaction mechanisms (Berndsen and Denu, 2008). A variety of mechanisms have evolved to regulate acetyltransferase activity and prevent inappropriate gene activation. These mechanisms include incorporation of the HAT into larger complexes that potentiate its enzymatic activity and the presence of one or more chromatin reader domains, which each recognize a specific type of histone modification (Lee and Workman, 2007). By binding to individual histone modifications, such as acetylated or methylated lysine, or to combinations of modifications, chromatin reader domains restrict HAT activity to the appropriate chromosomal context (Lee and Workman, 2007). Misregulation of enzymatic activity has been associated with cancer for all three HAT families, and many aggressive tumors are characterized by differences in HAT expression levels (Cohen et al., 2011).

CBP and p300 are large, highly similar HATs of over 2,400 amino acid residues with overlapping cellular functions. The two proteins are ~64% identical in primary sequence, with even greater levels of conservation across their catalytic cores (Kalkhoven, 2004). p300/CBP contains at least nine annotated domains, in addition to the HAT domain, including a bromodomain, which binds acetylated lysines (Kalkhoven, 2004), and a predicted PHD domain, which typically binds methylated lysines (Lee and Workman, 2007). In addition to associating with transcription factors such as TATA-binding protein and TFIIB, p300/CBP also interacts with tumor suppressor proteins, like p53 and BRCA1, as well as oncoproteins such as fos and myb (Kalkhoven, 2004). Among HAT enzymes, p300/CBP is particularly interesting because it activates itself by autoacetylating a basic loop in the HAT domain (Thompson et al., 2004) and also acetylates at least 70 non-histone proteins, including p53 (Wang et al., 2008). Mutations in CBP give rise to the congenital development disorder, Rubinstein-Taybi Syndrome (RTS) (Kalkhoven, 2004), and multiple human cancers arise from mutations and translocations of p300/CBP (Figure 1). While an earlier

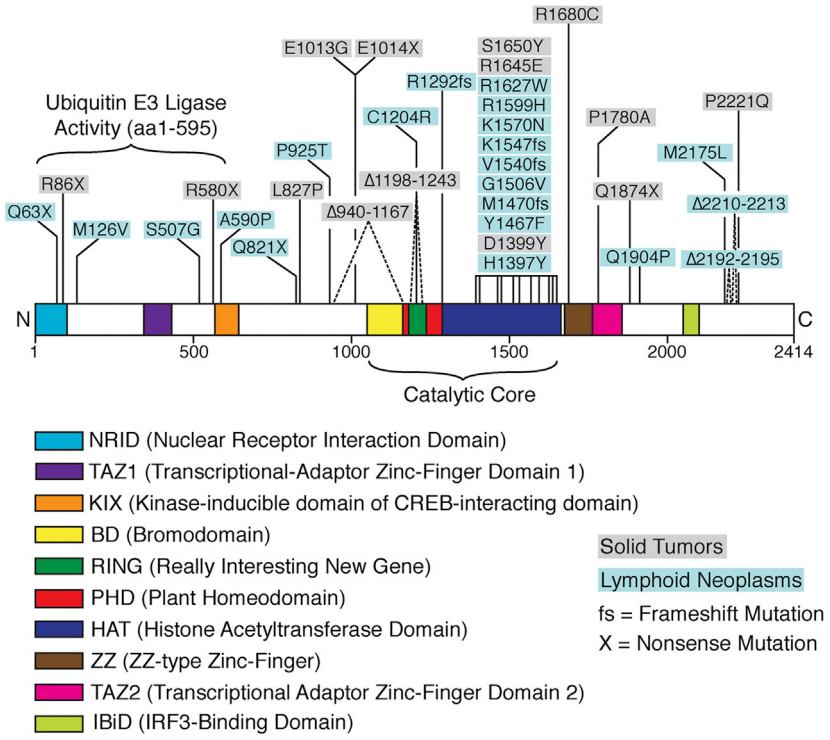

Figure 1. p300 Domains and Oncogenic Mutations
Domain structure of p300 overlaid with mutations and truncations found in solid tumors and lymphoid neoplasms (Iyer et al., 2004; Pasqualucci et al., 2011). The catalytic core of p300 is a hotspot for cancer mutations.

structure of the isolated p300 HAT domain showed how certain oncogenic mutations disrupted catalytic activity (Liu et al., 2008), most known mutations and deletions either map outside of this domain or have no obvious impact on catalytic function (Cohen et al., 2011; Kalkhoven, 2004). Given our current understanding of p300/CBP-catalyzed acetylation, the mechanism by which these core mutations dysregulate the acetyltransferase activity of p300/CBP and contribute to carcinogenesis has remained elusive.

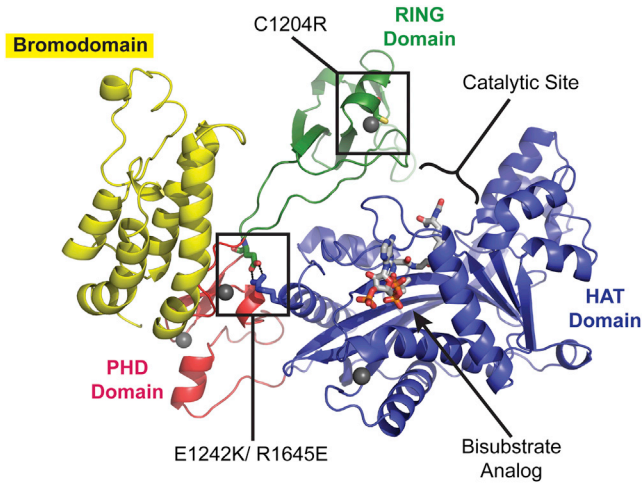

Figure 2. Structure of p300 Core Fragment
Individual domains colored as in Figure 1.Certain p300 mutations found in cancer map to the newly discovered autoinhibitory RING domain. C1204R coordinates a zinc atom in the RING domain itself, whereas E1242 and R1645 form a salt bridge between the RING and HAT domains. (PDB ID 4BHW).

The structure reported by Delvecchio et al. (2013) of a larger p300 catalytic core fragment contains an unanticipated feature that is key to understanding p300/CBP HAT regulation. In addition to the HAT domain bound to a lysine-CoA bisubstrate analog, the fragment also contains a bromodomain and a sequence known as the CH2 region, which contains a PHD domain. The structure quite unexpectedly revealed that the CH2 region also contains a structurally unusual RING domain, which is inserted within the PHD domain. RING domains are found in a subset of ubiquitin E3 ligases, where they mediate interactions with both E2 ubiquitin conjugating enzymes and ubiquitin (Deshaies and Joazeiro, 2009). In the new p300 structure, the RING domain contacts the active site of the HAT domain and blocks the substrate-binding cleft, thus suggesting that the RING domain might regulate HAT activity. Indeed, the authors showed that mutating key residues that perturb binding of the RING domain to the HAT domain results in a hyperactive form of the enzyme, pointing to an autoinhibitory role for the RING domain in p300/CBP catalysis. A number of mutations and deletions that give rise to cancer and RTS also map to the RING-HAT interface, illuminating the likely mechanism by which previously uncharacterized mutations misregulate p300/CBP activity and cause disease. For example, the C1204R mutation found in some B cell lymphomas disrupts the integrity of the RING domain by removing a zinc coordination site (Figure 2). Other curated mutations affect the RING domain as well; mutations that disturb the interaction between the RING and active site, such as R1645E and E1242K, are found in malignant melanoma and RTS, respectively (Figure 2), while the RING is entirely deleted in some forms of breast cancer. Accordingly, cells transfected with any of these mutant enzymes display hyperacetylation of p53 relative to a wild-type control.

Why was the presence of the RING domain in p300/CBP overlooked? First, the RING is embedded in a PHD domain, whose histone-interacting residues have been rearranged as a result. Second, while canonical RING domains contain two structural zinc atoms in separate coordination sites (Deshaies and Joazeiro, 2009), the p300 RING contains a single bound zinc, with tightly packed hydrophobic interactions replacing the second metal-binding site. There is also an insertion in the p300 RING loop L2 that, in other RINGs, interacts with E2 enzymes (Deshaies and Joazeiro, 2009). Based on its established role in the ubiquitin conjugation pathway, Delvecchio et al. (2013) tested the p300 RING for E3 ligase activity in vitro but could detect none, at least with the panel of E2 enzymes tested. Although an as-yet undiscovered role in ubiquitination cannot be ruled out, the authors' findings indicate that the RING domain in p300 has been adapted to autoregulate HAT activity.

The discovery of a previously unknown RING domain that gates the activity of the HAT domain is a key step forward in understanding how p300 is regulated. It remains to be seen to what degree the RING domain may coregulate HAT activity in concert with the autoregulatory loop in the HAT domain, which was deleted in both the present and previous (Liu et al., 2008) structural studies of p300 because it interfered with crystallization. The role of the PHD domain also remains to be determined, because it lacks features that enable other PHD domain to bind methylated lysines on histones. Perhaps this PHD domain evolved to perform a structural role, anchoring the inserted RING domain near the catalytic site and forming a compact core structure that bridges the bromodomain and catalytic domain. Although the newly identified RING domain does not appear to be an active member of the ubiquitination machinery, previous work has shown that the first ~600 residues of p300/CBP have E3 ligase activity and mediate ubiquitination of the tumor suppressor protein p53 (Grossman et al., 2003). Could it be that this portion of p300/CBP also contains a divergent RING that is not detectable by primary sequence analysis? More broadly, the discovery of an unanticipated domain that plays a critical role in regulating HAT activity is a reminder that other such domains likely await discovery. The inventory of regulatory mechanisms applied to HATs will surely continue to grow as structural biologists successfully tackle larger and larger protein fragments and complexes.

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