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DUALITY IN BROMODOMAIN-CONTAINING PROTEIN COMPLEXES

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TABLE OF CONTENTS

1. ABSTRACT

Proteins that contain a motif called a bromodomain are implicated in both transcriptional activation and repression. The bromodomain of p/CAF, the only solution structure of a bromodomain that has been solved to date, reveals that the motif binds N-acetyl-lysine groups, presumably to anchor enzymatic functions to histones and by extension to chromatin. The enzymatic activities can either be encoded within the same polypeptide as the bromodomain motif, or associated with a multiprotein complex. Thus, a wide variety of chromatin-directed functions, including but not limited to phosphorylation, acetylation, methylation, transcriptional co-activation or recruitment, characterize the complexes that contain bromodomain motifs. Their versatility and ubiquity ensures diverse, rapid and flexible transcriptional responses.

2. TRANSCRIPTIONAL ACTIVATION AND REPRESSION

The field of chromatin structure has experienced an explosion of interest recently, with almost 2000 Medline citations on the subject in the last two years. Many contemporary reports have focused attention on the causal links between specific modifications of histones, such as N-lysyl-acetylation or deacetylation, and the resultant loosening or tightening of nucleosome structure and consequent transcriptional activation or repression of genes. These local shifts are connected to global changes in cell physiology, such as growth and mitosis. The field has been extensively reviewed of late (1-7). Moreover, the relationship between different types of histone modification has garnered notice, particularly the observation that phosphorylation of serine-10 and acetylation of lysine-14 on histone H3 are coupled processes with important biological consequences (8, 9). In mammalian cells, histone H3 phosphorylation has been identified as a key step both in rapid responses to growth factor stimulation (10,11), resulting in transactivation of "immediate-early" type genes such as *c-fos* and *c-myc* (12), as well as much later in chromatin condensation during mitosis (13). An expansive view of the chromatin landscape has come from investigation of the genetics and biochemistry of chromatin remodeling complexes: large, multisubunit catalytic entities perform the work of histone modification that leads either to transcriptional activation or repression of target genes. Here, promoter selectivity for sequence-specific DNA binding proteins must guide the assembly of these big chromatin-modifying machines, yet the genetic regulatory elements must also be able to respond rapidly to changing transcriptional requirements. Active investigation of chromatin remodeling continues in many laboratories, from the level of sequence-specific modification of specific histones to the level of multiprotein complex assembly.

A particular protein motif called a "bromodomain" has been noticed in many of the proteins that compose the chromatin modifying machinery. It was first identified in 1992 as a 61 - 63 amino acid signature (14). Although it lacked a known function at the time, it has subsequently been identified in transcription factors, co-activators and other proteins that are important in transcription or chromatin remodeling and its boundaries have been expanded to about 110 amino acids. The number of such proteins was about forty at last report (15, 16) and several important additions to the family have been made since then. The first described bromodomain protein, yeast Gcn5 (17), was shown to be necessary for amino acid metabolism and was characterized as a transcriptional co-activator (18). It provides a histone acetylation (19) component of the ADA (Adapter) and SAGA (Spt-Ada-Gcn5 acetyltransferase) transcription complexes (20), which is fundamental and essential for viability (21). Gcn5 is also structurally related to the mammalian proteins CBP, p300 and Hat1 (22). In mammals, CBP and p300 also have intrinsic HAT activity (23, 24) and interact with many important transcription factors as co-activators of transcription. Virtually all of the nuclear histone acetyltransferases (HATs) contain bromodomains (16), but not all bromodomain proteins are HATs. For example, other classes of bromodomain proteins include MLL, a putative transcription factor (25, 26) that interacts with the SWI/SNF chromatin remodeling complex (27); Spt7, an acidic transcriptional activator and component of the SAGA complex (28); and a helicase superfamily that includes Snf2, Rsc1/Rsc2 and Sth1, components of the

SWI/SNF (29) and RSC complexes (30); Brg1, which binds RB (31, 32); and *brahma*, which also contacts RB, is related to Swi2/Snf2 (33, 34) and has homeotic functions in *Drosophila* (35 - 37). The role of bromodomains in transcription complexes has been controversial because their deletion has widely different consequences: in yeast, bromodomain deletion of Spt7 has no phenotype, of Snf2 causes slow growth, but of Sth1, Rsc1 and Rsc2 causes lethality (16). Much of the apparent significance of bromodomain proteins lies in their either having intrinsic HAT activity, or being associated with promoter-bound complexes that contain HAT or histone deacetylase (HDAC) activity. Bromodomain proteins are thereby potentially important players in the transcriptional control of a wide variety of eukaryotic genes, including those that control growth.

The bromodomain proteins that interact with RB highlight an important duality in transcriptional control: the need also to turn promoters off. In particular, the transcriptional control of E2F-regulated mammalian cell cycle genes is essential for proper progression through each stage of the cell cycle. Whereas transcriptional activation of one set of genes is necessary to enter a stage of the cell cycle, repression of certain other genes associated with the previous stage is necessary to exit from that stage. RB (and its family members p107 and p130) bind to E2F proteins and block their transcription activation function (38, 39).

Recent evidence has revealed that in addition to this direct repression, RB also recruits a histone deacetylase (40, 41), as do p107 and p130 (42), through cooperation with mammalian *brahma* and other proteins in the SWI/SNF complex (31, 32). Coordinated transcriptional activation and repression of the key E2F-regulated mammalian cell cycle genes *cyclin E*, *cyclin A* and *cdc2* permit proper transitions between G_1 and S phases, and S and G_2 phases (43). This dual nature of chromatin remodeling complexes was first suspected in yeast, where SWI/SNF complexes, initially associated with transcriptional activation (44), were later linked to repression as well: more genes are activated than repressed by SWI/SNF mutations (45). It now appears that SWI/SNF function may establish a widely applicable paradigm in chromatin remodeling complexes, whereby transcriptionally active euchromatin can be converted to inactive heterochromatin and *vice versa* in part through the exchange of HAT and HDAC enzymes in the complex (46). This model has been refined lately with the observation in yeast that several inducible genes active during interphase can recruit HAT activity independently of SWI/SNF, whereas mitotic genes require SWI/SNF to recruit HAT activity (47). This observation emphasizes the importance of coordinated complex formation for proper transit of the cell cycle.

A central development in the field of bromodomain-containing proteins came with a report of Zhou and colleagues (48), who used nuclear Overhauser enhancements to solve the solution structure of the bromodomain of p/CAF in association with N-acetylated lysine. The highly conserved structure of bromodomain proteins suggests a hypothesis that many of them will bind N-acetyl-lysine in histones, however by no means will this necessarily be true for all. The presence of bromodomains in many proteins that are known independently to possess HAT activity strongly supports the Zhou hypothesis. A looser notion that this motif is present in proteins that are involved in chromatin modification and transcription regulation is the best guide to their classification at the moment. The future discovery of bromodomains in proteins that are uninvolved in chromatin restructuring will be a test of the utility of such a classification.

In this special issue, several authors have been invited to contribute their perspectives on the developing field of bromodomain proteins and associated chromatin-modifying activities. Major questions that they address continue to provoke the development of the field, and include:

A. What are the number and type of histone modifications, including phosphorylation, acetylation, methylation, ADP-ribosylation and ubiquitination, that could regulate the recruitment of different classes of chromatin-modifying enzymes and might these represent a kind of combinatorial "histone code"? How do modifications of bromodomain-containing proteins reciprocally affect histone modification activities?

B. Should bromodomain-containing proteins be thought of as a kind of bridge or platform that recruits diverse enzymatic activities, such as HATs, HDACs, kinases or helicases, to chromatin? Why are these activities present in some bromodomain-containing proteins as independently-folding domains of a single polypeptide chain and in other cases as separate proteins? Does the weak affinity constant for a single bromodomain binding to N-acetyl-lysine (~0.1 mM) imply that bromodomains can function only in multiprotein complexes with multiple interaction sites?

C. Do different bromodomains have different functions, including those that are present more than once in a single protein? For example, double bromodomains, such as those in $TAF_{II}250$ might provide mutual cooperativity for protein binding to chromatin or might interfere with binding instead; or they might confer differential promoter specificity.

D. Why are some bromodomains essential for enzymatic function or cell viability whereas deletion of others has no apparent phenotype? Does this behavior reflect redundancy within bromodomain-containing complexes, so that for example SWI/SNF activities on some promoters can partially substitute for HAT-containing complexes such as SAGA?

E. What is the significance of the time order of recruitment of SWI/SNF activities and HAT activities to certain promoters? Why does SWI/SNF recruitment of HAT activity impact yeast transcriptional activation during late mitosis (47), whereas many inducible promoters recruit HATs independently of SWI/SNF earlier in the cell cycle, and how widespread is this behavior in eukaryotes?

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