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BROMODOMAIN MOTIFS AND "SCAFFOLDING"?

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1. ABSTRACT

Bromodomain-containing multiprotein complexes share some of the properties of signal transduction scaffolds. Insights from MAP kinase signaling scaffolds, for example, may provide useful perspectives for future studies of bromodomain proteins. The regulatory processes of modification (phosphorylation, acetylation, ubiquitination), turnover, nuclear compartmentalization, feedback regulation and signaling pathway specificity are all likely to contribute to the mechanisms by which bromodomain-containing multiprotein complexes control transcription.

2. BROMODOMAIN "SCAFFOLDS"?

2.1. Two paradigms: Ste5 and CBP

Signal transduction scaffolds provide an interaction surface on which the participants in a signaling pathway may assemble. They are necessary because in their absence, simple, unfacilitated diffusion of the participants is insufficient to render a strong or rapid biological response, and they maintain pathway specificity (1). Well-studied scaffold systems include the Ste5 platform that undergirds MAP kinase signaling in *S. cerevisiae* (2-4), the JIP family that appears to be a mammalian functional equivalent of Ste5 (5-7), and the high-affinity SH2 and SH3 modules that recruit effectors for mammalian receptor tyrosine kinases (8).

Bromodomain-containing transcription complexes have features that are reminiscent of signal transduction scaffolds. Whereas mammalian receptor tyrosine kinase scaffolds are localized to the plasma membrane, as are yeast MAP kinase scaffolds upon activation, bromodomain proteins are localized to chromatin. In both cases this feature *limits* the diffusion of their associated factors and "anchors" their location (*see Dyson et al.; Frontiers in Bioscience, this issue*). Also like SH2/SH3 modules, which regulate signal transduction through their phosphorylation state (8), recent evidence suggests that histone tails may regulate transcription through a similar "code" of phosphorylation, acetylation, methylation and other modifications (9, 10). The analogy between bromodomains and SH2 modules is made explicit in the paper of Horn and Peterson (*Frontiers in Bioscience, this issue*). Both paradigms face the problem of pathway specificity: how can machinery that is common to multiple signaling pathways generate uniquely tailored transcriptional responses? With the possible exception of polybromo, polypeptide chains that contain bromodomain motifs are not simple skeletal frames. They often encode several additional functionalities, including zinc fingers (CBP, p300, MLL) or AT hooks (MLL), HAT activity (CBP, p/CAF, $TAF_{II}250$), binding sites for transcriptional co-activators like E1A or CREB (CBP), kinase activity ($TAF_{II}250$, RING3, BDF1/2), helicase activity (SWI/SNF), or plant homeodomains (KAP-1) (*see Dyson et al.; Frontiers in Bioscience, this issue*). How is transcriptional confusion avoided?

Upon exposure of *S. cerevisiae* to pheromone, starvation conditions or osmotic stress, the MAP kinase scaffold protein Ste5 associates with the G protein effectors Ste20 (at the MKKKK level), Ste11 (MKKK), Ste7 (MKK) and Fus3 or Kss1 (MAPK), to enable mating, invasive growth or cell wall changes, respectively, through Ste12-dependent transcription (11). Specific regions within Ste5 function as binding sites for the kinases (2), and Ste5 both

oligomerizes (12) and is phosphorylated, as are the kinase components (11). Despite the quite different signals that initiate this cascade, many of the components of the MAPK signaling cascade are shared in common. This pattern raises the question of how a specific transcriptional response is achieved in each case. Feedback regulation, phosphorylation, turnover and compartmentalization of the cascade components are now seen to contribute to pathway specificity, while the presence of the scaffold itself limits unwanted "cross-talk" between MAP kinases of unrelated signaling pathways (6, 8, 13, 14). These features of scaffold signaling in yeast may provide insight into the regulation of mammalian transcription complexes that contain bromodomain proteins.

One possible example of a mammalian bromodomain "scaffold" protein is CBP (CREB Binding Protein). This 265 kDa nuclear protein provides an interaction surface for several proteins such as the Ca^{+2}/cyclic AMP-Responsive Element Binding protein (CREB) that has been phosphorylated on serine 133 (15), as well as intrinsic histone acetyltransferase (HAT) activity and a bromodomain to tether the associated components to chromatin. CBP is considered to be a transcriptional adaptor or co-activator because it provides a bridge between CREB and the basal transcription apparatus, as demonstrated by the detection of CBP and RNA Polymerase II in co-immunoprecipitates (16). CBP also associates with p/CAF (17), which also contains a bromodomain and exhibits intrinsic HAT activity. The co-activator function of CBP can be readily appreciated upon consideration of the wide variety of activating transcription factors that it binds, which, apart from CREB, include but are not limited to: c-Jun, c-Myb and nuclear hormone receptors; and basal transcription factors including TBP, TFIIB and YY1 (reviewed in 18). Viral oncoproteins such as SV40 large T antigen and E1A also bind CBP, block certain bridging interactions, and thereby liberate potential transcriptional activators such as p/CAF (17) and pp90rsk (19).

2.2. Integrity of the scaffold

Oncogenic fusion proteins that arise from reciprocal chromosomal translocations between CBP/p300 and MLL are linked to acute myeloid leukemias (*see Filetici et al. and Dyson et al., Frontiers in Bioscience, this issue*). These chimeric oncoproteins have traditionally been considered to possess intrinsic oncogenic activity within the fusion polypeptide, wherein its transforming ability derives from misdirection of the chimeric transcription factor to the wrong promoters. However, taking a scaffold view of these fusion proteins suggests that disrupted association with other transcription factors, histone-modifying activities, or viral oncoproteins could cause gain or loss of transcription functions at target promoters because the scaffold or platform itself is altered, and no longer recruits the proper activities. A "scrambled" scaffold may also no longer respond correctly to signal transduction information such as phosphorylation or acetylation, because important modification sites are missing or have been added through chromosomal translocation.

2.3. Modifications

Like Ste5 signaling in yeast, diverse transcriptional outcomes might in theory be achieved for bromodomain-containing multiprotein complexes with targeted degradation/inactivation of components, altered nuclear import/export or sequestration. Regulatory modification of bromodomain-containing proteins has received attention with the observation that phosphorylation of mammalian *brahma* and Brg-1 may ablate SWI/SNF activity at key points during the cell cycle (20, 21). Phosphorylation also inhibits Gcn5 histone acetyltransferase activity (22) and on some sites can repress the transcriptional activity of p300 (23) or on other sites increase the co-activation ability of CBP (18).

Many of the players in the chromatin-modifying machinery contain PEST sequences that are associated with phosphorylation and rapid ubiquitin-dependent degradation. p300 is ubiquitinated (24, 25), which precedes its proteasome-dependent destruction. This process could control the availability of a bromodomain scaffold during different stages of the cell cycle or during differentiation (25) and is likely to be relevant to other components of bromodomain-containing complexes as well. In an interesting twist to the story, $TAF_{II}250$ has recently been shown to ubiquitinate H1 histone and thereby promote transcription (26, 27). $TAF_{II}250$-directed ubiquitination of other proteins in transcription complexes naturally becomes a tantalizing possibility and suggests a hypothesis that p300 is a target of $TAF_{II}250$ ubiquitination activity. The ubiquitination status of p/CAF, mammalian *brahma*, Brg-1, Gcn5 and other bromodomain-containing components of the SAGA or SWI/SNF complexes has not yet been reported, although many transcription factors are known to exhibit regulated turnover by this mechanism (28). It is well established that E2Fs are ubiquitinated and degraded as a necessary step in cell cycle progression (reviewed most recently in 29). E2F probably associates with bromodomain proteins like $TAF_{II}250$ (30) and RING3 (31), which could regulate the ubiquitin-dependent turnover of E2F proteins, with important consequences for the cell cycle and cancer.

2.4. Localization

Scaffolds themselves are generally not mere skeletons, but frequently participate in signal transduction in an active way (11). For example, transit of Ste5 through the yeast nucleus is necessary for proper pheromone signaling (32). Several factors in bromodomain complexes have nuclear import signals that could permit a similar shuttling across the nuclear membrane. Nuclear localization of the SWI/SNF component human *brama*/SNF2-alpha (33) and of RING3 (34) has been reported; deletion of this *brahma* bromodomain leads to a loss of nuclear localization and protein destabilization (35). The possibility remains to be explored that other components of bromodomain-containing complexes exhibit regulated nuclear compartmentalization and that such compartmentalization might affect the availability of components that comprise the complex, or affect their transcriptional competence.

2.5. Overexpression

Finally, theoretical considerations of Ste5 and JIP scaffolds suggest that overexpression experiments with bromodomain scaffolds should be interpreted with caution (1). Overexpression of scaffolds could produce biological outcomes that are apparently inhibitory of a pathway. For instance, in cases where the concentration of the scaffold is

much greater than that of the components, these components could be sequestered from each other, resulting in a decline in pathway activity (36). Indeed JIP proteins received their name in this manner, as JNK Inhibitory Proteins (37). Similarly, overexpression of bromodomain proteins could negatively affect transcription or cell cycle progression, without being informative of any underlying biology.

3. SUMMARY

These considerations of modification, ubiquitination, turnover, localization and overexpression will be relevant for ongoing and future studies of bromodomain-containing scaffold proteins. This list is not comprehensive and many investigators were not cited due to lack of space. The identity and order of recruitment of chromatin-modifying activities to bromodomain scaffolds and the competence of the scaffold to marshal these activities may contribute to transcriptional outcomes. Studies of MAP kinase signaling cross-talk and transcriptional specificity may provide useful principles that will assist in the design of experiments and inform our understanding of chromatin remodeling complexes.

4. ACKNOWLEDGMENTS

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