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Cracking the Histone Code: One, Two, Three Methyls, You're Out!

In this issue of *Molecular Cell*, Zhang et al. report the structure of a ternary complex between the SET domain histone methyltransferase DIM-5, its cofactor, and a histone H3 peptide. The insight gained from analysis of a key amino acid provides an exciting opportunity to dissect the possible functional meaning of mono-, di-, and trimethylation of histone lysine residues *in vivo* that will complement existing approaches in the quest to crack the histone methylation code.

Although the nucleosome hypothesis was proposed nearly 30 years ago (see Kornberg and Lorch, 1999), only gradually has it been appreciated that chromatin structure plays a vital role in regulating gene expression, DNA replication, recombination, and repair. This awakening has been accompanied by steady movement of chromatin structure into pole position among studies of DNA-templated mechanisms that control cellular differentiation and responses to external stimuli (Felsenfeld and Groudine, 2003). Furthermore, chromatin and the histones that make up the structural core of the nucleosome represent the crux of epigenetic mechanisms for the inheritance of phenotypic traits (Jenuwein and Allis, 2001). Unraveling the physical basis for control of these various processes through alterations in chromatin structure will ultimately determine the depth to which we can understand the development and behavior of complex eukaryotic organisms.

Histones are subject to a bewildering array of post-translational modifications—acetylation, methylation, phosphorylation, ubiquitination, and ADP-ribosylation—that have been closely linked to the control of many cellular activities (recently reviewed by Ausio et al., 2001). Decades of work on histone modification and regulation of gene expression have coalesced into a “histone code” hypothesis that encapsulates the function of histone modifications in chromatin structure and in the regulation of nuclear functions (Strahl and Allis, 2000; Turner, 2000). In the language of the histone code, specific histone modification patterns control the association of proteins with chromatin through their effect on the strength and specificity of direct physical interaction with histones. The association of histone binding pro-

teins with chromatin leads directly or indirectly to changes in the functional state of the underlying DNA (for example, transcriptionally active or inactive) through changes either in the higher order structure of chromatin or in the ability to subsequently recruit specialized cellular machinery such as the RNA polymerase transcription complex. The histone code hypothesis relies on there being two groups of biochemical activities: (1) enzymes to write the code, which can add or remove modifications at specific target sites in the histones, and (2) proteins to interpret the code, which can specifically interact with histones displaying appropriate modification patterns and with other factors to mediate subsequent functional effects. There is now considerable evidence documenting the existence of both classes of activities, strongly supporting the notion of a histone code. This has stimulated intense interest in cracking the complete histone code so that we might perhaps “read” the functional status of any region of the genome through its histone modifications as easily as we can translate DNA sequence into protein sequence using the genetic code. While progress on this quest to date has been significant, early results indicate that the histone code is exquisitely subtle and complex (see, for example, Fischle et al., 2003), and complete decryption of the code will require a sustained effort combining biochemistry, genetics, and structural biology. A practical application of this knowledge lies in the treatment of numerous human developmental disorders and diseases caused by defects in the machinery that writes and reads the histone code.

Out of the many histone modifications, attention has recently focused on lysine methylation because of its close correlation with transcriptional activity (Kouzarides, 2002). Many histone methyltransferase enzymes have been identified, followed in quick succession by several crystal structures (Zhang et al., 2003). Methylation of lysine differs from acetylation in several important aspects. It does not remove the charge of the lysine, and uniquely, the ϵ -amino group of a targeted lysine residue can accept one, two, or three methyl groups to form mono-, di-, or trimethylated products. Recent findings suggest that di- and trimethylation have different functional connotations within a transcriptional context, although the significance and exact nature of any difference in meaning remains unknown (reviewed by Turner, 2003). However, given that we know all three methylation products are observed *in vivo* and that histone methyltransferase enzymes differ with respect to the level of methylation they can achieve (Zhang et al., 2003), it is, at the very least, an extremely provocative

suggestion that lysine mono-, di-, or trimethylation adds another layer of complexity to the histone code, and one which surely demands further investigation. Antibodies have until now been the mainstay of methods for measuring histone modifications, allowing us to correlate histone modification patterns with functional states, but what if we could deliberately tinker with the enzyme machinery that writes the histone code to accurately change the level of methylation at specific sites (molecular graffiti in vivo?)—what outcome would we observe?

That such an approach is feasible in vivo is a conclusion we can now make based on the results of the study by Zhang et al. (2003 [this issue of *Molecular Cell*]). They determined the crystal structure of DIM-5, a histone H3-specific methyltransferase, in complex with cofactor product *S*-adenosyl-*L*-homocysteine, and histone H3 peptide. The ternary structure shows how the target for DIM-5 methylation, lysine 9 (K9), is positioned in a narrow channel that leads to the cofactor methyl donor and reveals a wealth of details concerning substrate binding and catalytic mechanism. Importantly, Zhang et al. compared the DIM-5 ternary complex structure with the recently determined structure of a ternary complex of the histone H3 K4 specific methyltransferase, SET7/9 (Xiao et al., 2003), to see if they could locate structural differences that account for their different product specificities. Starting from unmodified lysine, DIM-5 can rapidly trimethylate H3 K9, while SET7/9 can only monomethylate H3 K4 even after prolonged reaction (Xiao et al., 2003; Zhang et al., 2003). Structural comparison indicates that the principal factor governing product specificity is the ability to accommodate the increasing bulk of the lysine ϵ -amino group in the confines of the channel leading to the methyl donor cofactor as subsequent methyl groups are added. DIM-5 has a more spacious channel, permitting it to accommodate unmodified, monomethyl or dimethyl lysine, while in SET7/9 the channel is less spacious so that it can accommodate only unmodified lysine without conformational change (Xiao et al., 2003; Zhang et al., 2003). In essence, the difference boils down to a single amino acid that occupies a structurally similar position in both enzymes (F281 of DIM-5 and Y305 of SET7/9). Zhang et al. therefore created DIM-5 and SET7/9 mutants that swapped these residues and remarkably show that this almost com-

pletely switches methylation product specificity; importantly, however, neither the lysine target specificity (K9 versus K4) nor the overall reaction rate for each enzyme was changed. Thus, DIM-5 is switched from a K9 trimethylase to a K9 monomethylase, while SET7/9 now generates dimethylated instead of monomethylated K4. This is the first time such a result has been achieved for any histone modification enzyme.

It will be interesting to see whether this molecular tinkering can be continued to complete a tool kit of histone H3 K4 and K9 methylation enzymes. Will it be possible to generate a DIM-5 enzyme that only dimethylates K9 or a SET7/9 that trimethylates K4? Work has probably already begun to put these or additional similar switch-hitter enzyme mutants to bat in vivo so that the functional output of specific lysine methylation states can be determined. We await the outcome of a new ball game that is surely worth the ticket.

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