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Putting the Pieces Together: Histone H2B Ubiquitylation Directly Stimulates Histone H3K79 Methylation

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Employing an in vitro reconstitution approach, McGinty et al. studied the mechanism of stimulation of the Dot1-catalysed histone H3 methylation at Lys79 by histone H2B ubiquitylation at Lys120. To generate nucleosome particles that carry the ubiquitylation at Lys120, they chemically connected three polypeptides—the main parts of histone H3 and ubiquitin expressed in bacteria and a branched synthetic peptide. Using the semisynthetically produced nucleosome substrates and purified Dot1 enzyme, they showed that Dot1 is directly stimulated by the ubiquitylation, thus ruling out the need for further protein factors to mediate the effect.

With very few exceptions, all cells of a multicellular organism contain the same genetic information. The various cell types are generated by differential expression of the genetic information in different cells, which is regulated by epigenetic control comprising (among others) the covalent modification of histone proteins and the DNA.[1,2] While DNA is only modified by methylation of cytosine bases, histone proteins are subject to many types of post-translational modifications, including acetylation of lysine residues, methylation of lysine and arginine residues, and phosphorylation of serine and threonine residues.[3–5] Interestingly, the histone proteins are also modified by attachment of the small proteins ubiquitin (comprising 76 amino acid residues) and SUMO (small ubiquitin-related modifier).[3,6] Ubiquitylation and sumoylation attach a much larger mark to the nucleosome than the other modifications mentioned. There are two sites of ubiquitylation on histone proteins, ubiquitylation of H2A at Lys119 has a repressive function, ubiquitylation of H2B at Lys120 is an activating mark, inducing two more modifications—H3K4 methylation and H3K79 methylation.[7] Such interdependence of different chromatin marks is not unique; other examples include an independent mechanism of regulating H3K4 methylation by H3R2 methylation[8,9] or the inhibition of H3K9 methylation by H3S10 phosphorylation.[10–12] The methylation of H3K79 by Dot1 is well conserved from yeast to man. It prevents chromatin condensation in yeast by interfering with the binding of Sir2 and 3 proteins, which are essential for telomeric and centromeric silencing.[3,6]

The mechanism of the connection of H3K79 methylation to H2B ubiquitylation was the subject of a recent publication by Muir, Roeder and colleagues.[13] Previously, it was not known if the stimulation of Dot1 was due to a direct interaction of the methyltransferase with the ubiquitylated nucleosome or if the effect was mediated by other proteins. Structural simulations suggested that a direct interaction was possible[14–16] (see also Figure 1). A direct interaction of chromatin marks with enzymes introducing other modifications has been observed in other cases as well, for example, several H3K9 histone lysine methyltransferases are inhibited by S10 phosphorylation, the mixed-lineage leukemia (MLL) H3K4 histone lysine methyltransferase is inhibited by H3R2 methylation and H3R2 methylation by protein arginine methyltransferase 6 (PRMT6) is inhibited by the presence of H3K4 methylation. However, for the stimulation of Dot1 by H2B ubiquitylation, other studies suggested the presence of mediating factors.[17–19] McGinty and colleagues have set up a very elegant in vitro-reconstituted system by using pure nucleosomes specifically ubiquitylated at K120 of H2B and recombinant Dot1 enzyme to study the stimulation of Dot1 by H2B ubiquitylation. This strategy provided full control of the experimental system, with respect to both the conditions and the protein factors involved. It enabled the authors to investigate the direct stimulation of Dot1 by ubiquitylation and to study mechanistic details of the stimulation of Dot1. One of the main challenges for this approach was to obtain a homogenous preparation of the ubiquitylated histone H2B protein, which could then be used for the reconstitution of full nu-

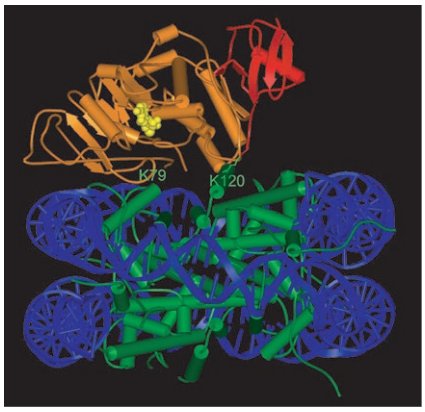

Figure 1. Model of the Dot1 enzyme (shown schematically in orange) bound to the loop containing H3K79. AdoMet is shown in space fill model in yellow, the histone proteins are coloured green and the DNA ribbon is blue. The positions of H3K79 and H2B K120 are labelled. Addition of a ubiquitin at H2B K120 (in red) illustrates that the size of the catalytic domain of the Dot1 enzyme does allow for a direct interaction between Dot1 and the ubiquitin. The model was generated by using WebLab viewer and the structures of Dot1 (1U2Z), ubiquitin (1UBQ) and the mononucleosome (1AOI).

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cleosomes by using the other histone proteins and DNA by following established techniques.[20]

Semisynthetic approaches in which an unmodified protein part expressed in bacteria is coupled to a chemically synthesized modified peptide are well suited to the synthesis of chemically modified proteins. In expressed protein ligation (also called native peptide ligation) a protein-α-thioester is generated by the thiolysis of a recombinant protein, which carries a C-terminal intein fusion part[21] that serves as a biologically built-in leaving group (Figure 2). This thioester can then be chemically ligated to a synthetic peptide carrying an N-terminal cysteine residue.[22,23] Native peptide ligation has been previously employed to produce histone H3 and histone H4 proteins with various modifications in their N-terminal tails.[24,25] However, in the case of H2B ubiquitylation, the modification is not located close to the N terminus, which required the ligation of three peptides to obtain the final substrate: the N-terminal fragment of histone H2B, the ubiquitin and a synthetic peptide providing the C-terminal part of H2B that connects other two protein parts (Figure 2). H2B and ubiquitin both were expressed in bacteria and purified as thioesters that were then connected to the synthetic C-terminal H2B peptide by employing two sulfhydryl groups. One thiol was linked to the ε-amino group of K120; this allowed ligation to the truncated ubiquitin to produce the endogenous ubiquitin connected to K120. The second sulfhydryl was provided by a cysteine positioned at the N terminus of the peptide at position A117 in the final H2B. Its side chain was protected during the first ligation step with a photoremovable *S*-(*o*-nitrobenzyl) group. After deprotection by photolysis, it was

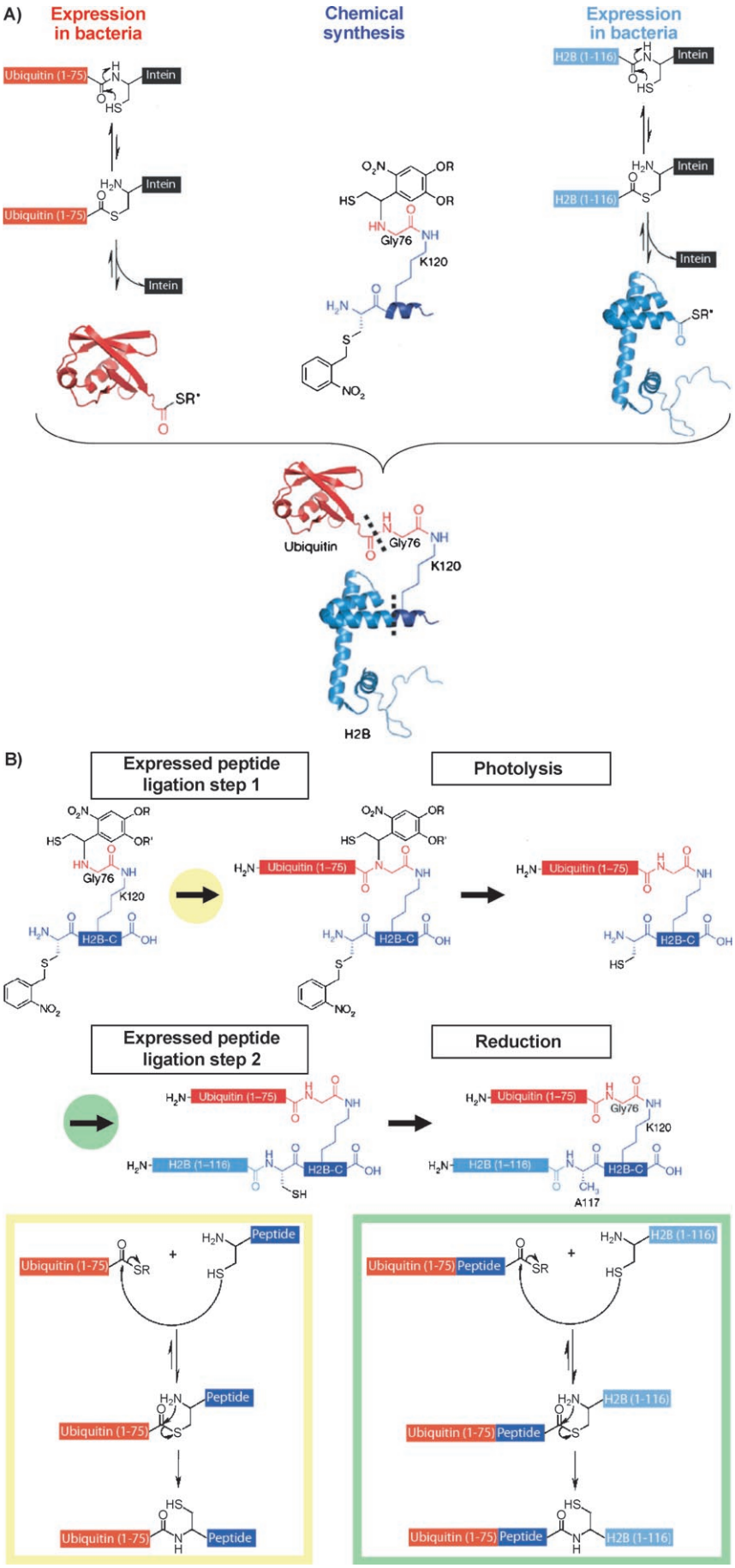

Figure 2. Semisynthetic strategy of producing histone H2B ubiquitylated at K120. A) General strategy of preparing H2B ubiquitylated at K120 by connection of a ubiquitin thioester, an H2B (1–116) thioester and a H2B 117–129 peptide. The proteins were produced in *E. coli* connected to a C-terminal intein tag that allows the purification as thioester. B) Details of the semisynthetic strategy involving two steps of native peptide ligation. Part of Figure 2 was reproduced from ref. [13] with permission. Copyright Nature Publishing Group, 2008

ligated to the N-terminal part of the histone H2B protein. After this step, the unnatural cysteine residue at H2B position 117 was chemically reduced to alanine leading to a traceless connection of the three polypeptides.

The study of McGinty et al. shows robust stimulation of Dot1 activity by ubiquitylation of H2B, thus indicating that the histone methyltransferase directly senses the presence of the ubiquitin. To study whether ubiquitylation in one nucleosome could stimulate H3K79 methylation also in adjacent nucleosomes, they prepared dinucleosomes with different ubiquitylation states and introduced H3K79R mutations (that prevent Dot1 methylation) to rapidly assign the sites of modification (Figure 3). The data nicely show that ubiquitylation does not stimulate the methylation of adjacent nucleosomes (although some interesting additional combinations of dinucleosomes were not tested); this further supports a direct interaction model of Dot1 with the ubiquitin. Concerning the mechanism of stimulation, they demonstrate that ubiquitylation leads to a general increase in Dot1 activity that causes an elevated level of H3K79me1 and H3K79me2; a result that is in agreement with a recent study in yeast cells.[26] The activation of Dot1 could be due to improved binding and longer residence time at the target site or an allosteric activation of Dot1 by the interaction with the ubiquitin. Since McGinty et al. did not observe any effect of the nucleosome ubiquitylation on binding of Dot1, and high concentrations of free ubiquitin did not interfere strongly with the stimulation of Dot1, they prefer the latter model to explain their data. Future studies will address the identification of the putative Dot1–ubiquitin interface. The uncovering of the detailed mechanism of stimulation of Dot1 will probably await the availability of structures of Dot1 bound to unmodified and ubiquitylated nucleosomes.

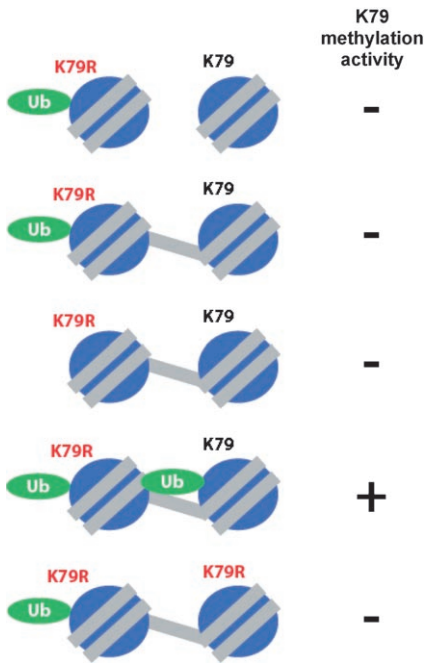

Figure 3. Investigation of the intra- or internucleosomal stimulation of H3K79 methylation by H2B ubiquitylation functions. To this end, dinucleosomes were prepared that contained different combinations of H2B ubiquitylation and H3K79R mutations (which prevent Dot1 activity at this site).

Keywords: enzymes · histone · methylation · nucleosomes · ubiquitylation

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