Tudor hooks up with DNA repair

Lorenzo Corsini & Michael Sattler

Histone lysine methylation has a central role in transcriptional regulation and has recently been linked to DNA damage repair. Now it has been shown that the DNA damage repair factor 53BP1 is recruited to DNA double-strand breaks by its tandem tudor domain, which specifically recognizes histone H4 dimethylated at lysine 20.

The many types of post-translational modification in histones, which include acetylation, methylation, SUMOylation, ubiquitination, phosphorylation and more, are believed to act sequentially or in combination as a 'histone code' that extends the information stored in DNA[1]. Specific histone methylation and demethylation can both up- and downregulate the transcriptional activity of many genes[2–4].

An additional function for histone methylation was discovered in 2004, when two articles independently provided evidence that the mammalian DNA damage repair factor p53 binding protein-1 (53BP1) and its putative fission yeast ortholog Crb2 associate with DNA double-strand breaks (DSB) by binding methylated lysines in histones[5,6]. But whereas 53BP1 was shown to recognize Lys79 on histone H3 (H3-K79), its yeast ortholog was found to bind Lys20 on histone H4 (H4-K20). In a recent issue of *Cell*, Botuyan *et al.*[7] now demonstrate that 53BP1 is recruited to DSB sites *in vivo* exclusively by binding dimethylated H4-K20, in both yeast and mammals. The authors present the crystal structure of the 53BP1 tandem tudor domain in complex with a dimethylated H4-K20 peptide and show that this interaction depends on the methylation state of Lys20 *in vitro* and *in vivo*. This is the first time that the specific molecular recognition of dimethylated lysine over unmethylated or trimethylated lysine has been revealed at atomic resolution. The work of Botuyan *et al.*[7] also establishes the tudor domain alongside the chromodomains and plant homeodomain (PHD)-fingers as a reader of the histone lysine methylation code.

Botuyan *et al.*[7] screened H3-K79 and H4-K20 peptides in different methylation states for binding to the tandem tudor domain of 53BP1 by isothermal titration calorimetry and NMR. They found that 53BP1 binds mono- and dimethylated H4-K20 (H4-K20me1 and H4-K20me2) with dissociation constants (K_d) of 53 and 20 μM, respectively. Unmethylated and trimethylated H4-K20, as well as dimethylated H3-K79, have strongly reduced binding affinities. To understand the molecular details of methylation-state specificity, Botuyan *et al.*[7] solved the crystal structure of the 53BP1 tandem tudor domain in complex with an H4-K20me2 peptide at 1.7-Å resolution (**Fig. 1a**). They observed electron density for Arg19 and the dimethylated Lys20, which is caged by four aromatic residues (Trp1495, Tyr1502, Phe1519 and Tyr1523). The aromatic side chains of these residues coordinate the dimethylammonium ion of Lys20 via π-cation interactions. The side chain carboxylate of Asp1521 mediates specificity for di-over trimethylation by forming an ion pair with the dimethylammonium ion; according to theoretical calculations, such

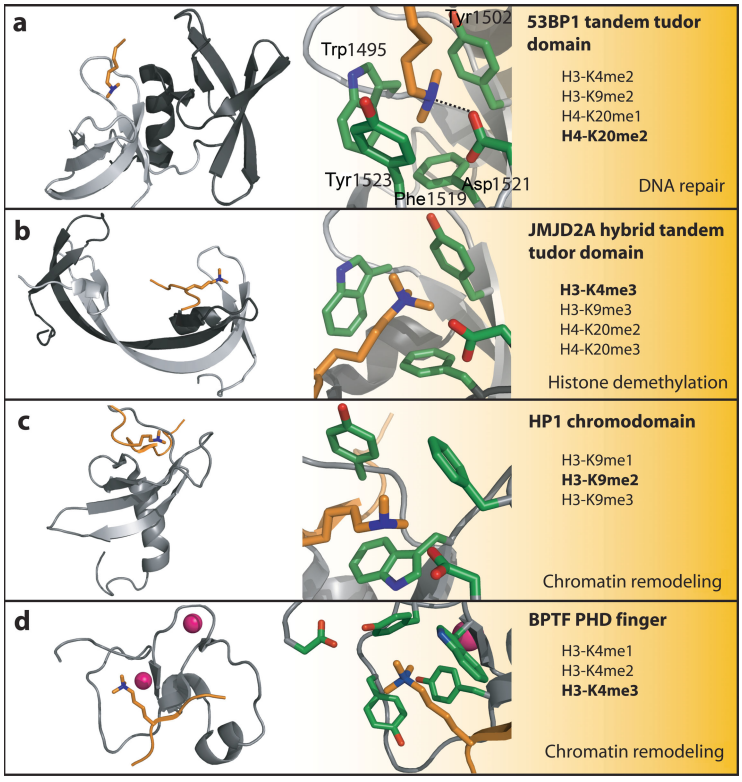

Figure 1 Methyllysine recognition by tudor domains, chromodomains and PHD fingers. (**a**–**d**) Three-dimensional structures of the tandem tudor domains of 53BP1 (ref. 7) (**a**), the interdigitated tudor domains of JMJD2A[8] (**b**), the HP1 chromodomain[9] (**c**) and the BPTF PHD finger[12] (**d**) bound to cognate methyllysine ligands (bold print on the right). The two tudor domains in **a** and **b** are shown as dark and light gray ribbons. Zinc atoms in the PHD finger are shown as pink spheres. Methyllysine ligand peptides and side chains of residues forming the aromatic cage are shown as orange and green sticks, respectively. Histone ligands identified as binding partners[14] and functional contexts of these interactions are indicated on the right. The tudor folds in **a** and **b** are shown in the same orientation; the related chromodomain fold in **c** is rotated by 90° about a vertical axis. Despite the structural homology of tudor domains and chromodomains, the residues coordinating the methyllysine are located on different secondary structure elements (compare **a** and **b** to **c**).

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an ion pair is stronger than one formed with a trimethylammonium group. Furthermore, a hydrogen bond is formed with the dimethylammonium hydrogen, which would not be possible with a trimethylated lysine.

Next, Botuyan *et al.*[7] investigated whether H3-K79 methylation by the histone methyltransferase Dot1 is necessary for the recruitment of 53BP1 to DSB sites, as was reported by Huyen *et al.*[5]. Stable Δ-*dot1* knockdown in HeLa and A549 cell lines showed that down-regulation of Dot1 expression has virtually no effect on 53BP1 relocalization to DSBs induced by ionizing irradiation. Attempts to generate Dot1-knockout mice gave a late embryonic lethal phenotype. Therefore, Botuyan *et al.*[7] cultured *dot1*$^{-/-}$ mouse embryonic fibroblasts, which showed a lack of H3-K79 dimethylation and exhibited no defect in 53BP1 relocalization after ionizing irradiation, as was seen with the human cell lines. This led the authors to conclude that an interaction of 53BP1 with methylated H3-K79 is not required for its recruitment to DSB sites.

Instead, Botuyan *et al.*[7] suggest that 53BP1 is relocated to DSB sites via binding of its tandem tudor domain to dimethylated H4-K20. The authors generated cell lines deficient for the histone methyltransferase PR-Set7/Set8, which catalyzes H4K20 monomethylation, an obligatory step for the subsequent di- or trimethylation of this residue. HeLa cells triply transfected with short interfering RNA targeted against PR-Set7/Set8 showed severe impairment of 53BP1 recruitment to DSB sites upon ionizing irradiation treatment. The authors thus conclude that an interaction of the 53BP1 tandem tudor domain with dimethylated H4-K20, but not with methylated H3-K79, is required for its relocalization to DSB sites.

Several structures of methylated histone tails in complex with tudor domains[7,8], chromodomains[9–11] and PHD fingers[12,13] have been described in recent years. The folds and methyllysine binding modes of prototypical examples are shown in **Figure 1**. The mechanisms by which these proteins recognize methylated histone lysines are similar to that of 53BP1 in that they involve a cage of two to four aromatic amino acids and an acidic residue to balance the charge of the methylammonium ion. These proteins exploit differences in their aromatic binding cages, as well as in the amino acid sequences of the H3 and H4 histone tails flanking the methyllysines, to discern different histone lysine residues.

The ability to discriminate mono- or dimethyllysine (Kme1/2) from trimethyllysine (Kme3) has previously been demonstrated for malignant brain tumor (MBT) repeats[14,15]. The 53BP1 tandem tudor domain–Kme2 complex structure provides the first molecular insight into Kme2-specific recognition (**Fig. 1a**). Other methyllysine recognizing domains, such as the interdigitated tudor domain of JMJD2A, bind both di- and trimethyl lysine (Kme2/3)[8,14] (**Fig. 1b**). A preference for binding Kme2/3 has been observed for chromodomains and PHD fingers as well, even though these domains also bind monomethyllysine[4,14] (**Fig. 1c**,**d**). Finally, the WD40-repeat protein WDR5 has been found to bind histone tails independently of its methylation state (reviewed in ref. 4). Together, structural and biochemical studies indicate that there is a wide range of methyllysine binding specificities found in histone code–'reading' domains.

Interestingly, the tudor domain of the survival of motor neuron (SMN) protein has been shown to recognize symmetrically dimethylated arginine residues in spliceosomal Sm proteins[16,17]. Thus, even though the aromatic binding cages in the tudor domains of 53BP1, JMJD2A and SMN resemble one another, they can recognize rather different methylated ligands through variations in the binding site for methylated amino acids.

Botuyan *et al.*[7] show that H4-K20 methylation is a prerequisite for the recruitment of 53BP1 to DSB sites. However, the methylation state of H4-K20 is not altered upon ionizing irradiation–induced DNA damage. It is therefore not clear how 53BP1 is relocalized to DNA double-strand breaks *in vivo*. As one possible explanation, the authors suggest that a simultaneous interaction of histone γH2AX, which is phosphorylated upon DNA damage, and H4-K20me2 with 53BP1 may be required for the recruitment to DSBs. Consistent with this hypothesis, it has been shown that γH2AX can interact with 53BP1 (ref. 18).

In conclusion, Botuyan *et al.*[7] link the 53BP1–H4-K20me2 interaction to a DNA damage repair pathway. The growing number of known methyllysine recognition domains, their distinct binding preferences and the observation that a given methyllysine modification can be recognized by different domains suggest that the histone code is more complex than previously thought. Moreover, multiple simultaneous interactions of histone code–readers and cognate histone tails may signal a functional context *in vivo*. Further studies are required to decipher the network of interactions involved in the molecular recognition of histone tails and their role in the regulation of transcription and additional functions, such as DNA repair.

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

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