Accelerated Publication

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 279, No. 51, Issue of December 17, pp. 52812–52815, 2004
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Printed in U.S.A.

Ring1b-mediated H2A Ubiquitination Associates with Inactive X Chromosomes and Is Involved in Initiation of X Inactivation*[S]

Received for publication, October 18, 2004
Published, JBC Papers in Press, October 26, 2004,
DOI 10.1074/jbc.C400493200

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Histone modifications are thought to serve as epigenetic markers that mediate dynamic changes in chromatin structure and regulation of gene expression. As a model system for understanding epigenetic silencing, X chromosome inactivation has been previously linked to a number of histone modifications including methylation and hypoacetylation. In this study, we provide evidence that supports H2A ubiquitination as a novel epigenetic marker for the inactive X chromosome (Xi) and links H2A ubiquitination to initiation of X inactivation. We found that the H2A-K119 ubiquitin E3 ligase Ring1b, a Polycomb group protein, is enriched on Xi in female trophoblast stem (TS) cells as well as differentiating embryonic stem (ES) cells. Consistent with Ring1b mediating H2A ubiquitination, ubiquitinated H2A (ubH2A) is also enriched on the Xi of both TS and ES cells. We demonstrate that the enrichment of Ring1b and ubH2A on Xi is transient during TS and ES cell differentiation, suggesting that the Ring1b and ubH2A are involved in the initiation of both imprinted and random X inactivation. Furthermore, we showed that the association of Ring1b and ubH2A with Xi is mitotically stable in non-differentiated TS cells.

Dynamic changes in chromatin structure play important roles in regulating DNA-templated processes such as transcription. Regulation of chromatin dynamics can be achieved through ATP-dependent chromatin remodeling and covalent modifications of histones, such as acetylation, methylation, phosphorylation, and ubiquitination (1–4). Studies in the past several years have established a fundamental role for histone acetylation and methylation in regulating gene activity (4–6). However, the role of histone ubiquitination is just beginning to be revealed (7).

Both histones H2A and H2B are subject to modification by ubiquitin. The identification of E2 and E3 involved in H2B ubiquitination (8–10) and the discovery of the "cross-talk" between histone methylation and ubiquitination (11, 12) have set the stage for functional analysis of histone H2B ubiquitination. Recent studies indicate that, unlike other reversible histone modifications in which addition or removal of a group from a histone molecule results in opposing transcriptional effects, sequential ubiquitination and deubiquitination are both involved in transcriptional activation (13, 14). Although histone H2A was the first protein identified to be ubiquitinated (15), its functional significance has been elusive until very recently when we biochemically purified the H2A-K119 ubiquitin E3 ligase and linked its function to Polycomb silencing (16).

Dosage compensation in mammals is achieved by transcriptional silencing of one of the two X chromosomes in female cells (17). X inactivation occurs during early embryonic development and is controlled by a large non-coding RNA, *Xist* (18, 19). After coating the inactive X chromosome (Xi), *Xist* RNA is believed to recruit silencing factors to mediate a series of epigenetic changes to the chromosome. The epigenetic changes include methylation and hypoacetylation of histones, incorporation of macroH2A, and CpG methylation (20).

Genetic and biochemical studies have revealed that Polycomb group (PcG)[1] proteins function in at least two distinct protein complexes including the Eed-Ezh2 complex and the PRC1 complex (21, 22). Previously, we as well as others (23–25), have demonstrated that the Eed-Ezh2 complex and its associated H3-K27 methyltransferase activity are recruited to Xi and participate in X inactivation. However, no link between components of the PRC1 complex and X inactivation has been established. Our recent finding that H2A ubiquitination is mediated by a Ring1b-containing PcG complex (16) prompted us to investigate a potential role of H2A ubiquitination in X inactivation. Our study revealed that both Ring1b and ubiquitinated H2A (ubH2A) are specifically enriched on the Xi of trophoblast stem (TS) cells as well as differentiating embryonic stem (ES) cells. In addition, analysis of the dynamic nature of the Xi enrichment suggests that Ring1b and ubH2A are likely involved in the initiation stage of X inactivation. Taken together, our study reveals a new epigenetic feature of Xi and suggests a role for Ring1b and ubH2A in the initiation stage of X inactivation in both extraembryonic and embryonic cell lineages.

EXPERIMENTAL PROCEDURES

Cell Culture and Differentiation—TS cells were kindly provided by Gary Uy (26) and cultured as described (27). To induce TS cell differentiation, human fibroblast growth factor-4 (Sigma), heparin (Sigma), and embryonic mouse fibroblast-conditioned medium were removed from the culture medium. The female mouse ES cell line 129/cas16, derived from a female 129Sv/Jae-Castaneous hybrid blastocyst, was maintained and differentiated following a previously described procedure (28).

* This work was supported by National Institutes of Health Grants GM63076 and GM68804 (to Y. Z.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.

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[1] The abbreviations used are: PcG, Polycomb group; ubH2A, ubiquitinated H2A; TS, trophoblast stem; ES, embryonic stem; PBS, phosphate-buffered saline; PIPES, 1,4-piperazinediethanesulfonic acid; DAPI, 4,6-diamidino-2-phenylindole dihydrochloride.

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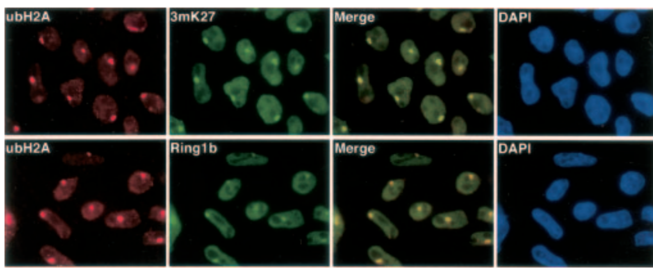

FIG. 1. **Colocalization of Ring1b and ubH2A on the inactive X chromosome in TS cells.** Female TS cells were stained with anti-ubH2A (*red*) and anti-H3–3mK27 (*green*), or anti-Ring1b (*green*) antibodies as indicated. DNA is counterstained with DAPI.

Immunostaining and Antibodies—For immunostaining, TS or ES cells were plated onto 0.2% gelatin (Sigma)-coated glass coverslips or a 6-well plate and cultured for 1 or 2 days. After washing with PBS and cold CSB buffer (0.1 M NaCl, 0.3 M sucrose, 3 mM $MgCl_2$ in 10 mM PIPES, pH 6.8), cells were permeabilized for 5 min with cold CSB buffer containing 0.5% Triton X-100. The cells were then washed twice with cold CSB buffer and fixed in 4% paraformaldehyde for 20 min. Fixed cells were then washed three times with blocking buffer (1% bovine serum albumin in PBS) and blocked for 30 min before incubating with primary antibodies for 1 h in a humidified chamber. After three consecutive 5-min washes in blocking buffer, cells were incubated with secondary antibodies for an additional hour. The cells were then washed with PBS and stained with 4,6-diamidino-2-phenylindole dihydrochloride (DAPI) in PBS. Cells were washed again twice with PBS and water and then mounted in fluorescent mounting medium (Dako). Antibodies against Ezh2, H3–3mK27, Ring1b, and Ring1a have been described previously (23, 29, 30). The anti-ubH2A antibody (E6C5), described previously (31), and anti-Bmi1 antibody were purchased from Upstate Biotechnology. Secondary antibodies for immunofluorescence were from Jackson ImmunoResearch Laboratories.

RESULTS AND DISCUSSION

Ring1b and ubH2A Are Enriched on the Xi in Trophoblast Stem Cells—Blastocyst-derived female TS cells belong to the extraembryonic cell type (27). Since the paternal X chromosome is imprinted in TS cells, they provide one of the best *in vitro* models for analyzing imprinted X inactivation (32). Previous studies have demonstrated that the Eed-Ezh2 Polycomb complex and complex-mediated histone H3-K27 trimethylation are enriched on the Xi in TS cells (23, 24, 33). Our recent studies revealed that a Ring1b-containing PcG complex participates in histone H2A ubiquitination and is functionally linked to the Eed-Ezh2 complex in PcG silencing (16). To investigate whether Ring1b-mediated H2A ubiquitination participates in X inactivation in TS cells, we examined Ring1b and ubH2A distribution in female TS cells by immunofluorescence staining. Toward this end, TS cells were double-labeled with a monoclonal antibody E6C5 (31), which specifically recognizes histone H2A ubiquitinated on lysine 119 (H2A-ubK119), together with a polyclonal antibody against histone H3 trimethylated on lysine 27 (H3–3mK27), which marks the Xi (23). Results shown in Fig. 1 demonstrate a clear colocalization of ubH2A with H3–3mK27 on Xi (*top panels*). Consistent with our previous finding that Ring1b mediates H2A ubiquitination *in vitro* and *in vivo* (16), we also observed colocalization of Ring1b with ubH2A on Xi (*bottom panels*). In addition to Ring2, the human counterpart of the mouse Ring1b, the ubiquitin ligase complex purified from HeLa cells also include Ring1 (Ring1a), Bmi1, and HPH2 (16). However, similar staining using antibodies against Ring1a and Bmi1 did not reveal elevated levels of either at the Xi despite their specific nuclear staining (supplemental Fig. S1). This result reflects that Ring1b has alternate functional partners in different cell types. Collectively, the above data demonstrate that ubH2A and the relevant ubiquitin ligase Ring1b are enriched on the inactive X chromosome in TS cells.

Association of Ring1b and ubH2A with Xi in TS Cells Is Mitotically Stable—Previous studies in TS cells indicate that association of the Eed-Ezh2 complex with Xi is mitotically stable (32), consistent with a role of the complex in the maintenance of imprinted X inactivation in trophectoderm cells (25). To determine whether Ring1b and ubH2A are also stably associated with Xi during mitosis, we examined their staining patterns throughout mitosis in TS cells. Costaining of anti-ubH2A and anti-Ring1b antibodies was performed as above, and cells at different stages of mitosis were identified by DAPI staining. In parallel, co-staining of anti-H3–3mK27 and anti-Ezh2 antibodies was also performed as a control. Data shown in Fig. 2 indicate that both ubH2A and Ring1b antibodies stained a distinct chromosome in prophase and metaphase TS cells (*top two rows*). Interestingly, association of Ring1b protein with Xi is retained on individual sister chromatids at both anaphase and telophase, which colocalizes exactly with the ubH2A signals (*bottom two rows*). Similar results were also observed for the Eed-Ezh2 complex as well as for the H3–3mK27 marker (supplemental Fig. S2). One possible explanation of the result is that the Ring1b complex is retained through its interaction with the *Xist* RNA, similar to the retention of the Eed-Ezh2 complex (32). This result suggests that the Ring1b-mediated H2A ubiquitination could play a role in maintenance of X inactivation during the cell cycle.

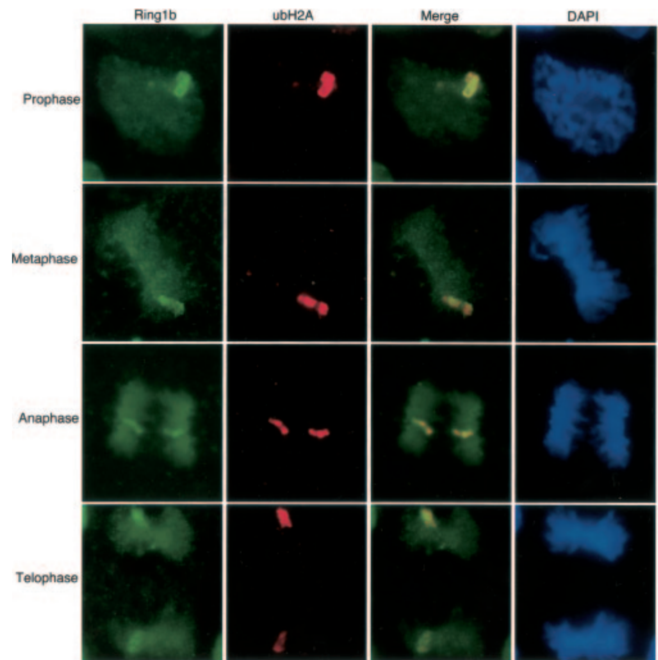

FIG. 2. **Ring1b and ubH2A remain on inactive X chromosome throughout mitosis in TS cells.** Female TS cells were double stained with anti-Ring1b (*green*) and anti-ubH_2A (*red*) antibodies at different stages of mitosis, as indicated. Merged images demonstrate that Ring1b and ubH2A colocalize and remain on Xi throughout the mitosis. DNA is counterstained with DAPI (*blue*).

Ring1b and ubH2A Dissociate from Xi during TS Cell Differentiation—Shortly after implantation, trophoblast cells differentiate into a number of extraembryonic cell types, including trophoblast giant cells. TS cells can also be differentiated into trophoblast giant cells *in vitro* after withdrawal of fibroblast growth factor-4 and heparin from the culture medium (27). To investigate whether Ring1b and Ring1b-mediated H2A ubiquitination participate in maintaining X inactivation in trophoblast giant cells, we asked whether Ring1b and ubH2A are present on the Xi in differentiated TS cells. After 2, 4, 8, and 12 days of differentiation, TS cells were costained with anti-Ring1b and anti-ubH2A antibodies or anti-Ezh2 and anti-H3–

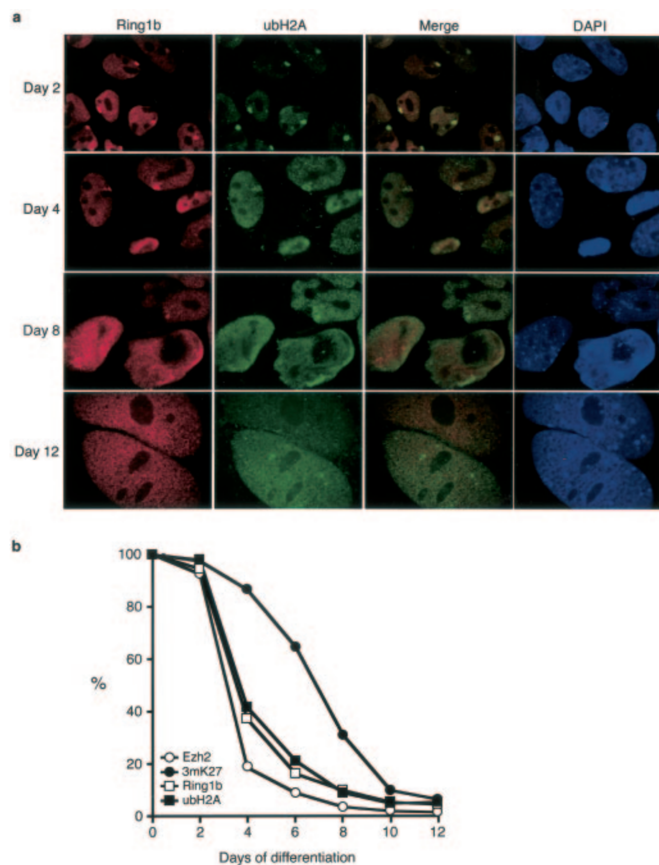

FIG. 3. **Enrichment of Ring1b and ubH2A on Xi is transient during female TS cell differentiation.** *a*, immunostaining of Ring1b (*red*) and ubH2A (*green*) in nuclei (*blue*) of female TS cells differentiated for 2, 4, 8, and 12 days. The merged images demonstrate that Ring1b and ubH2A colocalize and are enriched on the Xi in early, but not late, stages of differentiation. *b*, quantification of the data presented in *a* and parallel Ezh2/H3–3mK27 staining during TS cell differentiation. The graphs depict the percentage of cells with Xi accumulation for Ezh2 (*open circles*), H3–3mK27 (*solid circles*), Ring1b (*open squares*), and ubH2A (*solid squares*), respectively. More than 100 cells were counted for each time point.

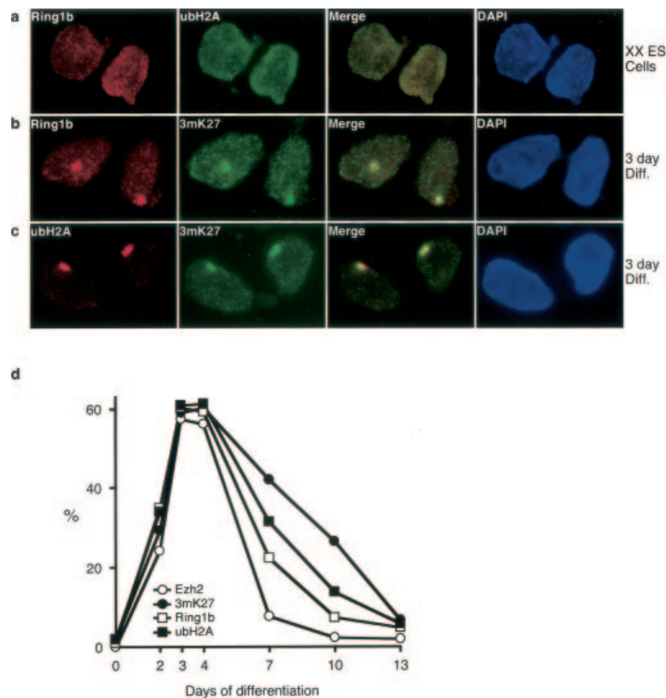

FIG. 4. **Enrichment of Ring1b and ubH2A on Xi is transient during female ES cell differentiation.** *a*, immunostaining of Ring1b (*red*) and ubH2A (*green*) in nuclei (*blue*) of undifferentiated female ES cells. *b* and *c*, after 3 days of differentiation, female ES cells were stained with anti-H3–3mK27 (*green*) and anti-Ring1b (*red*) or anti-ubH2A (*red*) antibodies as indicated. The merged images show that Ring1b and ubH2A colocalize and are enriched on the Xi. *d*, quantification of the immunostaining patterns during ES cell differentiation. The graphs depict the percentage of cells with Xi accumulation for Ezh2 (*open circles*), H3–3mK27 (*solid circles*), Ring1b (*open squares*), and ubH2A (*solid squares*), respectively. More than 100 cells were counted after 2, 3, 4, 7, 10, and 13 days of differentiation.

3mK27 antibodies. The immunostaining results indicate a progressive loss of enrichment of both Ring1b and ubH2A on Xi during TS cell differentiation (Fig. 3*a*). The Xi-enriched staining pattern for both Ring1b and ubH2A becomes diffused throughout the entire nucleoplasm at 4 days of differentiation, and most of the giant cells lost the Xi-enriched staining pattern after 8 days of differentiation (Fig. 3*a*). Quantitative analysis showed the dissociation of Ring1b and ubH2A from Xi occurs after the loss of Eed-Ezh2 and before the loss of 3mK27 (Fig. 3*b*). Given that *Xist* RNA continues to coat the Xi in trophoblast giant cells after 8 days of differentiation (23), these results suggest that the Ring1b complex and ubH2A are not required for the continued coating of Xi by *Xist* RNA, similar to that of Ezh2 and H3–3mK27 (23). These results also suggest that Ring1b and ubH2A may not be required for maintenance of imprinted X inactivation in differentiated trophoblast giant cells. Instead, they likely function during initiation of imprinted X inactivation.

Enrichment of Ring1b and ubH2A on Xi during ES Cell Differentiation Correlates with Initiation of X Inactivation—ES cells are derived from the inner cell mass of blastocysts where X chromosomes undergo random X inactivation (17). Previous studies have established that X inactivation initiates shortly after ES cells are induced to differentiate (20, 34). To investigate a possible role for Ring1b-mediated H2A ubiquitination in random X inactivation, we performed immunostaining of mouse female ES cells during their differentiation using the Ring1b and ubH2A antibodies. Previously, we and others (23, 24) have shown that Ezh2-mediated H3-K27 trimethylation is transiently enriched on the Xi during ES cell differentiation. Therefore, parallel stainings using anti-Ezh2 and anti-H3-3mK27 antibodies were also performed. Consistent with the fact that X inactivation does not occur in undifferentiated ES cells, no enrichment of either Ring1b or ubH2A in Xi was detected (Fig. 4*a*). However, Ring1b and ubH2A start to accumulate on Xi as soon as ES cells were induced to differentiate. The percentage of cells with enriched Ring1b and ubH2A on Xi reached as high as 60% after 3–4 days of differentiation (Fig. 4, *b–d*), a result similar to that of two other PcG proteins Ezh2 and Eed (23, 24). Like that of Ezh2 and H3–3mK27, enrichment of Ring1b and ubH2A on Xi is transient as progressive loss of the enrichment occurs after 4 days of differentiation, and no enrichment was detected after 13 days of differentiation (Fig. 4*d*). The asynchronous nature of ES cell differentiation in culture likely accounts for the observation that enrichment of Ring1b and ubH2A on Xi does not occur in all cells at any given time point. Analysis of the association and disassociation dynamics of Ezh2/H3–3mK27 and Ring1b/ubH2A on Xi indicate that Ring1b/ubH2A appears to disassociate after Ezh2 but before H3–3mK27, although they have similar dynamics in their binding to Xi (Fig. 4*d*). This result, together with that shown in Fig. 3*b*, indicates that H3-K27 trimethylation is a relatively more stable marker than ubH2A. In addition to Ring1b and ubH2A, we also performed parallel immunostaining using the Ring1a and Bmi1 antibodies. Similar to our findings in TS cells (supplemental Fig. S1), neither

protein was found to be enriched on the Xi at any stage of ES cell differentiation (supplemental Fig. S3) suggesting that the Ring1b complex involved in X inactivation is likely different from that of the one purified from HeLa cells (16). Collectively, the above results also suggest that Ring1b-mediated H2A ubiquitination is involved in the initiation stage of X inactivation, since its enrichment on the Xi correlates with initiation of X inactivation.

Since the discovery of the *Xist* RNA (35, 36), a great deal of effort in the study of X inactivation has focused on identification of *Xist* RNA associated factors and characterization of Xi-linked epigenetic modifications (20). These efforts have resulted in identification of a number of Xi-linked chromatin features including histone hypoacetylation (37), H3-K9 (38–41), and H3-K27 (23, 24) methylation and incorporation of a variant H2A, macroH2A (42). In addition, Polycomb group proteins involved in H3-K27 methylation were also identified to associate with Xi in an *Xist* RNA-dependent manner (23, 24). The present study demonstrates that ubiquitinated H2A and its corresponding ligase Ring1b are epigenetic features of the Xi. Their dynamic association with Xi in TS and ES cells suggest a role in the initiation stage of both imprinted and random X inactivation.

The PcG of proteins were initially identified in *Drosophila* during genetic screens to identify factors involved in heritable silencing of homeobox genes and have since been found to be an evolutionarily conserved gene silencing system (43, 44). PcG proteins function in at least two distinctive protein complexes including the Eed-Ezh2 complex and the PRC1 complex (21, 22). The fact that the Eed-Ezh2 complex and its associated H3-K27 methyltransferase activity are recruited to Xi and participate in X inactivation (23–25), in combination with the fact that the two PcG complexes can function together to mediate Hox gene silencing, prompted us to investigate a possible role of Ring1b-mediated H2A ubiquitination in X inactivation. We demonstrated that, similar to Ezh2 and its associated H3-K27 methyltransferase activity, Ring1b and its associated H2A ubiquitination ligase activity are enriched in Xi and are mitotically stable in TS cells (Figs. 1 and 2). Interestingly, the enrichment of Ring1b and ubH2A on Xi does not persist during TS cell differentiation suggesting that it may not be required for maintenance of imprinted X inactivation during TS cell differentiation (Fig. 3). We also demonstrate that enrichment of Ring1b and ubH2A on Xi correlates with initiation of X inactivation during ES cell differentiation. Thus, our results identify a new epigenetic marker for Xi and link H2A ubiquitination to initiation of X inactivation. This study, coupled with our previous demonstration of the involvement of the Eed-Ezh2 complex in X inactivation (23), suggest that similar to Hox gene silencing, X inactivation may also involve coordinated action of the two PcG complexes and their associated enzymatic activities. Further studies should reveal the exact mechanism by which H2A ubiquitination participates in X inactivation.

Acknowledgments—We thank Gary Uy for TS cells and Miguel Vidal for Ring1b and Ring1a antibodies.

REFERENCES

1. Narlikar, G. J., Fan, H. Y., and Kingston, R. E. (2002) *Cell* **108,** 475–487
2. Jenuwein, T., and Allis, C. D. (2001) *Science* **293,** 1074–1080
3. Strahl, B. D., and Allis, C. D. (2000) *Nature* **403,** 41–45
4. Zhang, Y., and Reinberg, D. (2001) *Genes Dev.* **15,** 2343–2360
5. Kouzarides, T. (2002) *Curr. Opin. Genet. Dev.* **12,** 198–209
6. Roth, S. Y., Denu, J. M., and Allis, C. D. (2001) *Annu. Rev. Biochem.* **70,** 81–120
7. Zhang, Y. (2003) *Genes Dev.* **17,** 2733–2740
8. Robzyk, K., Recht, J., and Osley, M. A. (2000) *Science* **287,** 501–504
9. Hwang, W. W., Venkatasubrahmanyam, S., Ianculescu, A. G., Tong, A., Boone, C., and Madhani, H. D. (2003) *Mol. Cell* **11,** 261–266
10. Wood, A., Krogan, N. J., Dover, J., Schneider, J., Heidt, J., Boateng, M. A., Dean, K., Golshani, A., Zhang, Y., Greenblatt, J. F., Johnston, M., and Shilatifard, A. (2003) *Mol. Cell* **11,** 267–274
11. Sun, Z. W., and Allis, C. D. (2002) *Nature* **418,** 104–108
12. Dover, J., Schneider, J., Tawiah-Boateng, M. A., Wood, A., Dean, K., Johnston, M., and Shilatifard, A. (2002) *J. Biol. Chem.* **277,** 28368–28371
13. Kao, C. F., Hillyer, C., Tsukuda, T., Henry, K., Berger, S., and Osley, M. A. (2004) *Genes Dev.* **18,** 184–195
14. Henry, K. W., Wyce, A., Lo, W. S., Duggan, L. J., Emre, N. C., Kao, C. F., Pillus, L., Shilatifard, A., Osley, M. A., and Berger, S. L. (2003) *Genes Dev.* **17,** 2648–2663
15. Goldknopf, I. L., Taylor, C. W., Baum, R. M., Yeoman, L. C., Olson, M. O., Prestayko, A. W., and Busch, H. (1975) *J. Biol. Chem.* **250,** 7182–7187
16. Wang, H., Wang, L., Erdjument-Bromage, H., Vidal, M., Tempst, P., Jones, R. S., and Zhang, Y. (2004) *Nature* **431,** 873–878
17. Avner, P., and Heard, E. (2001) *Nat. Rev. Genet.* **2,** 59–67
18. Cohen, D. E., and Lee, J. T. (2002) *Curr. Opin. Genet. Dev.* **12,** 219–224
19. Plath, K., Mlynarczyk-Evans, S., Nusinow, D. A., and Panning, B. (2002) *Annu. Rev. Genet.* **36,** 233–278
20. Brockdorff, N. (2002) *Trends Genet.* **18,** 352–358
21. Zhang, Y., Cao, R., Wang, L., and Jones, R. S. (2004) *Cold Spring Harbor Symp. Quant. Biol.* **49,** in press
22. Lund, A. H., and van Lohuizen, M. (2004) *Curr. Opin. Cell Biol.* **16,** 239–246
23. Plath, K., Fang, J., Mlynarczyk-Evans, S. K., Cao, R., Worringer, K. A., Wang, H., de la Cruz, C. C., Otte, A. P., Panning, B., and Zhang, Y. (2003) *Science* **300,** 131–135
24. Silva, J., Mak, W., Zvetkova, I., Appanah, R., Nesterova, T. B., Webster, Z., Peters, A. H., Jenuwein, T., Otte, A. P., and Brockdorff, N. (2003) *Dev. Cell* **4,** 481–495
25. Wang, J., Mager, J., Chen, Y., Schneider, E., Cross, J. C., Nagy, A., and Magnuson, T. (2001) *Nat. Genet.* **28,** 371–375
26. Uy, G. D., Downs, K. M., and Gardner, R. L. (2002) *Development* (*Camb.*) **129,** 3913–3924
27. Tanaka, S., Kunath, T., Hadjantonakis, A. K., Nagy, A., and Rossant, J. (1998) *Science* **282,** 2072–2075
28. Rougeulle, C., Chaumeil, J., Sarma, K., Allis, C. D., Reinberg, D., Avner, P., and Heard, E. (2004) *Mol. Cell. Biol.* **24,** 5475–5484
29. Cao, R., Wang, L., Wang, H., Xia, L., Erdjument-Bromage, H., Tempst, P., Jones, R. S., and Zhang, Y. (2002) *Science* **298,** 1039–1043
30. Schoorlemmer, J., Marcos-Gutierrez, C., Were, F., Martinez, R., Garcia, E., Satijn, D. P., Otte, A. P., and Vidal, M. (1997) *EMBO J.* **16,** 5930–5942
31. Vassilev, A. P., Rasmussen, H. H., Christensen, E. I., Nielsen, S., and Celis, J. E. (1995) *J. Cell Sci.* **108,** 1205–1215
32. Mak, W., Baxter, J., Silva, J., Newall, A. E., Otte, A. P., and Brockdorff, N. (2002) *Curr. Biol.* **12,** 1016–1020
33. Erhardt, S., Su, I. H., Schneider, R., Barton, S., Bannister, A. J., Perez-Burgos, L., Jenuwein, T., Kouzarides, T., Tarakhovsky, A., and Surani, M. A. (2003) *Development* (*Camb.*) **130,** 4235–4248
34. Keohane, A. M., O'Neill L, P., Belyaev, N. D., Lavender, J. S., and Turner, B. M. (1996) *Dev. Biol.* **180,** 618–630
35. Brockdorff, N., Ashworth, A., Kay, G. F., McCabe, V. M., Norris, D. P., Cooper, P. J., Swift, S., and Rastan, S. (1992) *Cell* **71,** 515–526
36. Brown, C. J., Hendrich, B. D., Rupert, J. L., Lafreniere, R. G., Xing, Y., Lawrence, J., and Willard, H. F. (1992) *Cell* **71,** 527–542
37. Jeppesen, P., and Turner, B. M. (1993) *Cell* **74,** 281–289
38. Heard, E., Rougeulle, C., Arnaud, D., Avner, P., Allis, C. D., and Spector, D. L. (2001) *Cell* **107,** 727–738
39. Mermoud, J. E., Popova, B., Peters, A. H., Jenuwein, T., and Brockdorff, N. (2002) *Curr. Biol.* **12,** 247–251
40. Peters, A. H., Mermoud, J. E., O'Carroll, D., Pagani, M., Schweizer, D., Brockdorff, N., and Jenuwein, T. (2002) *Nat. Genet.* **30,** 77–80
41. Boggs, B. A., Cheung, P., Heard, E., Spector, D. L., Chinault, A. C., and Allis, C. D. (2002) *Nat. Genet.* **30,** 73–76
42. Costanzi, C., and Pehrson, J. R. (1998) *Nature* **393,** 599–601
43. Francis, N. J., and Kingston, R. E. (2001) *Nat. Rev. Mol. Cell. Biol.* **2,** 409–421
44. Cao, R., and Zhang, Y. (2004) *Curr. Opin. Genet. Dev.* **14,** 155–164