Biochemistry

 Volume 41, Number 19 May 14, 2002

New Concepts

The Many Tales of a Tail: Carboxyl-Terminal Tail Heterogeneity Specializes Histone H2A Variants for Defined Chromatin Function

Juan Ausió* and D. Wade Abbott

Department of Biochemistry and Microbiology, University of Victoria, Victoria, British Columbia, Canada V8W 3P6

Received January 18, 2002; Revised Manuscript Received March 11, 2002

ABSTRACT: For many years, histones were considered to be passive structural components of eukaryotic chromatin. Experimental evidence that has accumulated during the past few years indicates that in addition to their structural role, histones play a very important functional role and that they can operate as epigenetic markers. This notion has rekindled the interest in histone variants and their participation in the processes of chromatin activation and inactivation. Recent papers have focused their attention on histone H2A variants. The variants of this overlooked histone participate in many biological processes ranging from transcriptional activation to DNA repair, meiosis, and apoptosis. A nucleosome containing at least one of these variants has been crystallized and biophysically characterized in solution. From all these results, a new concept has started to emerge, which supports the notion that the functional roles of H2A variants are exerted through alterations in chromatin stability and folding that result from the structural variation at the carboxyl-terminal end of this histone.

Significant steps have been made toward defining the role of histone H2A variants in the modulation of chromatin arrays. Interestingly, a general trend is emerging between C-terminal heterogeneity and the functional novelty of specialized chromatin environments. Indeed, the greatest region of variability between H2A isoforms maps to the prominent carboxyl tail, which has implications for the stability of nucleosome particles, and the higher-order structure of chromatin. This paper will highlight the structural and functional significance of H2A positioning and heterogeneity within the nucleosome, and the consequences of H2A.Z and H2AX[1] deposition and modification.

Nucleosome Dynamics

To package the informational polymer DNA within the nucleus, the cell utilizes a family of conserved proteins, termed histones, to fold the DNA and leave it accessible for regulatory events (*1*). This assembly contains several levels of structure, which organizes DNA from massive, singular chromatids during mitotic events down to the smallest unit

* To whom correspondence should be addressed: Department of Biochemistry and Microbiology, University of Victoria, Petch Building, 220, Victoria, BC, Canada V8W 3P6. Phone: (250) 721-8863. Fax: (250) 721-8855. E-mail: jausio@uvic.ca.

[1] The nomenclature for H2AX has changed in recent years. Originally, the protein was described with a period between H2A and X (H2A.X); however, this designation has disappeared in the majority of the literature since 1995.

10.1021/bi020059d CCC: $22.00
Published on Web 04/20/2002

of packaging known as the nucleosome that is present throughout the cell cycle. When observed as a native particle, the nucleosome primarily exists as a protein octamer that constrains DNA in 1.75 superhelical left-handed turns around its perimeter (*2*). This octamer consists of four core histones named H2A, H2B, H3, and H4 present in two copies each. The stability of this nucleoprotein complex arises from several forms of noncovalent interactions, including electrostatic linkages, hydrogen bonding, and hydrophobic interactions (*2*). Although nucleosomes represent a defined unit of chromatin structure and actually repress the activities of processive complexes, such as RNA polymerase (*3*, *4*), they are transient particles, which exist in equilibrium between free and complexed states (*5*−*8*). In ATP-dependent reactions, the nucleosome dissociation equilibrium can be collapsed by chromatin remodeling complexes, such as SWI/SNF, as histone-constrained DNA is relaxed and the template becomes poised for transcription (*9*). Such dynamic events are fundamental to transitions between active and silenced chromatin substrates and also facilitate novel deposition events during DNA synthesis.

The plasticity of nucleosome structures is also influenced by several poorly understood epigenetic factors. For example, histones do not exist as chemically inert subunits within the octamer. Each of the subunits is susceptible to regulated and highly specific post-translational modifications such as acetylation, methylation, phosphorylation, and ubiquitination (*10*−*12*). The impacts of these events have proven to be very elusive in nature, as seldom is one chemical modification responsible for a single regulated event. This has led to recent theories which suggest that (a) modified histones present a "histone code" that is interpreted by nuclear modulators to provide intricately controlled levels of regulation (*11*, *13*) and (b) chemical signaling is coupled to the coordinated or synergistic effects of structural transitions to define specialized environments for chromatin function (*12*, *14*).

Histone Variants

Although post-translational modification, particularly acetylation, remains the most documented aspect of the epigenetic regulation of chromatin, the incorporation of histone variants into nucleosomes also has significant impacts for gene expression, repair cascades, and meiotic events (*12*). Histone variants are nonallelic, mRNA-polyadenylated isoforms of major histones, which display localized positioning and are synthesized at varying points throughout development and the cell cycle (*15*, *16*). Differential expression patterns of these proteins enable the deposition of specialized nucleosomes during cellular events outside the window of genomic duplication in the S phase, and suggest that the exchange of histone variants may be an active process throughout the cell cycle and quiescence. These subtypes generally are not essential, but provide specificity to chromatin domains by possibly influencing the stability of nucleosomes or interacting with trans-acting factors.

H2A has the largest macroheterogeneous family of described variants, which are active in distinct aspects of nucleosome dynamics and genomic function. This may be because H2A and H2B, which exist as a dimer within the octamer, are more labile and dissociate from the nucleosome under lower-ionic strength conditions (*17*−*19*). Therefore, the cell would gain a thermodynamic advantage utilizing a regulatory system dependent on H2A replacement, as opposed to either H3 or H4, each of which displays a greater affinity for the nucleosomal DNA. In this regard, the apparent lack of somatic macroheterogeneous H2B variants is intriguing. Suto and others (*20*) propose that this may result from the cognate interactions of self-associating histones within the octamer. Indeed, loop 1 displays a signature fold in each of three H2A isoforms (*2*, *20*), which may ensure the insertion of duplicate copies of a specific H2A. In support of this self-association theory, H3 (which interacts with H3′ through histone bundles) is replaced by the heteromorphous H3 variant Cenp-A in centromeric chromatin (*20*, *21*).

H2A Carboxyl-Terminal Tail Variability

A closer look at the primary sequence of H2A variants reveals that the region which provides the greatest chemical distinction to the nucleosome maps to the C-terminal region of the protein (*22*, *23*) (see Figure 1A). Previous experiments performed by Eickbush et al. (*24*) demonstrated that the carboxyl-terminal tail of H2A is essential for the stability of chromatin particles and that the H2A−H2B pair displays a significant decrease in affinity for the tetramer when the terminal 15 amino acids are removed by an endogenous protease. Interestingly, a new histone H2A variant has recently been described [H2A-Bbd[2] (*22*)] which exhibits a similarly truncated C-terminal domain. This histone is deficient in the inactive X chromosome and colocalizes with acetylated forms of histone H4 (*22*).

Across the family of macroheterogeneous isoforms, H2A retains the greatest degree of sequence homology at the histone domains, a structural motif essential for the formation of "handshake", "bundle", and DNA interactions within the nucleosome (*2*). In each subtype, the greatest degree of variability occurs near the carboxyl-terminal domain, in both tail length and amino acid composition (Figure 1A). Significantly, H2A contains the largest consensus C-terminal tail, when compared to the remainder of the core histone complement, which is exemplified in macroH2A (mH2A) by a massive non-histone fusion that represents two-thirds of the protein's molecular weight (*25*). The variability of H2A in this region may compensate for the modest degree of N-terminal post-translational modification of the histone (*12*), and explain the functional significance of its position within the nucleosome (Figure 1B).

Crystallographic structural analysis of the nucleosome has illustrated the strategic placement of H2A within the core particle. The carboxyl α-helix disposes the C-terminal domain to dock with the tetramer, and extend to the nucleosome surface near the entry and exit sites of DNA (*2*). Therefore, exchange of H2A variants within the nucleosome offers molecular heterogeneity to both the tetramer and linker DNA interface, which has implications for the association of chromatin remodeling machinery, H1 histones, and, consequently, the higher-order structure of chromatin. For example, the enrichment of mH2A in the inactivated X chromosome suggests that the large C-terminal fusion of the variant may specialize these nucleosomes to repress and condense chromatin within the Barr body during interphase

[2] H2A-Bbd (Barr body deficient) (*22*).

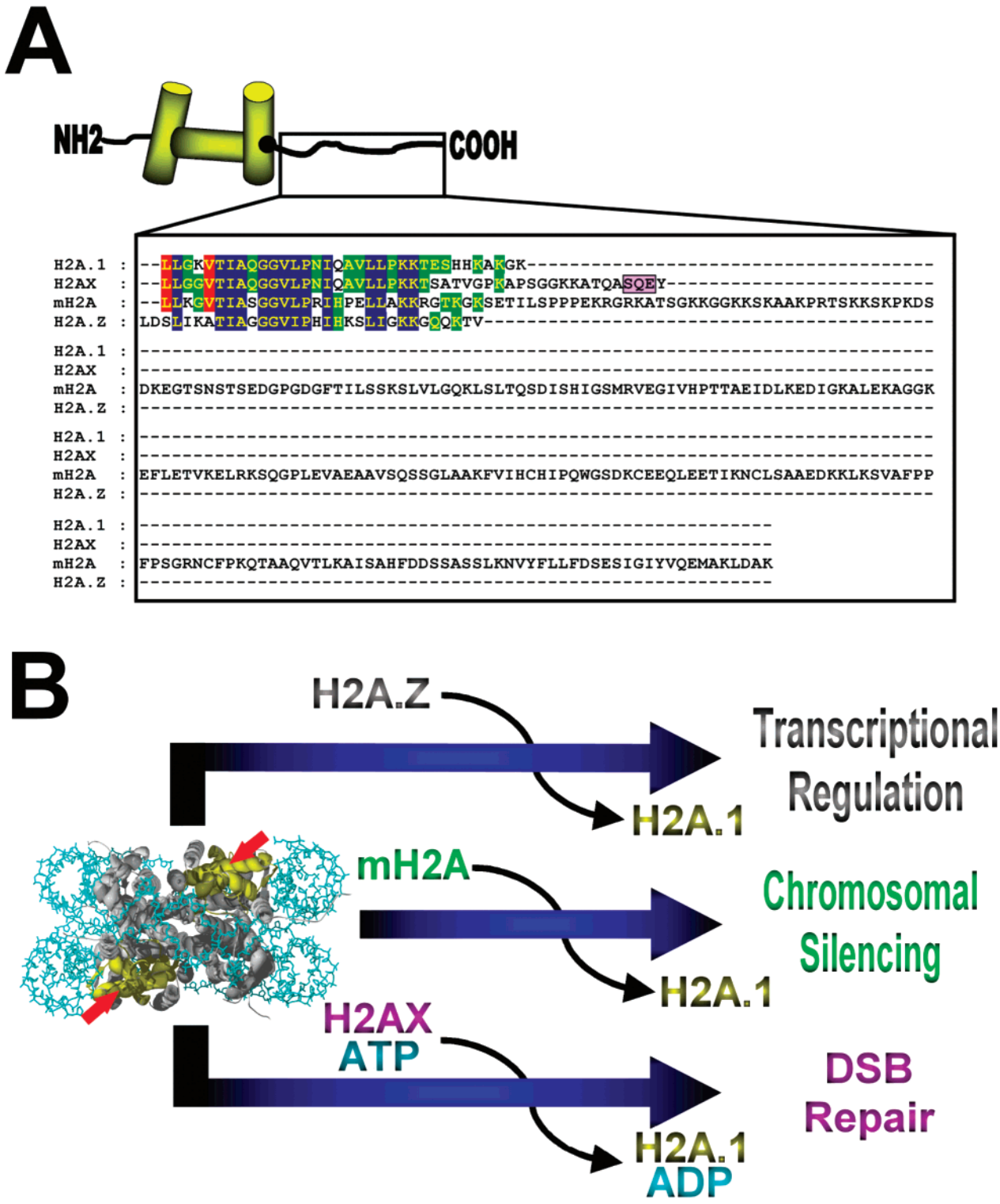

FIGURE 1: (A) Schematic representation of a consensus histone H2A structure, highlighting the variable carboxyl domain. C-Terminal amino acid sequences of H2A.1, H2AX, mH2A, and H2A.Z are inset and aligned to display the similarity and heterogeneity between isoforms. The color scheme depicts the degree of similarity between proteins: blue background, common to all four subtypes; red background, common to three subtypes; and green background, common to two subtypes. The functional SQE phosphorylation domain of H2AX is denoted with light purple. (B) Model for epigenetic regulation of chromatin function by histone H2A variant insertion into the nucleosome. The H2A position within the core particle is important for chromatin transitions during many cellular activities. H2A variants may play critical roles in generating these novel structural assemblies or interacting with downstream elements and propagating chemical signals through heterogeneity in their carboxyl tails. H2A.1 (yellow) is replaced by H2A.Z (gray) during transcriptional activation and silencing events. mH2A (green) is enriched in the inactivated X chromosome, possibly to compensate for RNA synthesis dosages. H2AX (purple) is phosphorylated during DNA fragmentation events such as DSB repair. The red arrows point to the sites where the carboxyl-terminal tails of histone H2A exit the nucleosome.

(*26*). Such events describe a defined regulatory system of chromatin domains by the interaction of novel H2A carboxyl-terminal tails with other modulating proteins. In the cases of H2A.Z and H2AX, this carboxyl variability may specialize

nucleosomes for transcriptionally active (*23*) or double-strand repair specific domains (*27*).

H2A.Z Nucleosome Structure and Transcriptional Activation

The emergence of powerful biochemical techniques, in particular, chromatin immunoprecipitation (CHIP), has enabled researchers to take a reductionalistic approach in helping to define the relationships between histone variability and specific DNA loci. This has proven to be an invaluable technique for attempting to connect localized nucleosome modifications to transition events, such as transcription. In one such experiment, Santisteban and colleagues (*28*) identified H2A.Z at promoter sites under repressive conditions. Interestingly, upon induction the intergenic DNA became depleted of H2A.Z, which suggests that the variant may be involved in poising the template for expression, but is dispensable for maintaining an active state. A similar event was also observed in *Drosophila melanogaster*. CHIP assays indicated that the inducible *hsp70* gene was enriched with H2A.Z when compared with its constitutively expressed hsp83 counterpart, and subsequent immunolocalization showed an inverse relationship between RNA polymerase II (RNAP II) hyperactivity and H2A.Z deposition (*29*). Supporting these results, an association between H2A.Z and transcription machinery has been shown using antibodies directed at RNAP II and TBP (*30*). It was demonstrated that recruitment of RNAP II and TBP to promoters was dependent on the carboxyl-terminal region of H2A.Z, as H2A chimeras fused with the C-terminal 37 residues of H2A.Z could successfully associate with the activation factors and initiate transcription.

To attempt to define the structural role of H2A.Z in chromatin function, we recently characterized the stability and folding of H2A.Z containing nucleosome core particles and oligonucleosome arrays under increasingly higher ionic strength conditions (*23*). This biophysical study provided the first clues into the transitional dynamics of H2A.Z chromatin particles. Interestingly, it appears that H2A.Z has a bifunctional role in modulating chromatin states, both of which are in agreement with a direct role in transcriptional activation. First, analysis of chromatin fibers described a complex that resists condensation, when compared to its major H2A counterpart. By increasing the ionic strength of the solution, synthetic electrostatic interactions mimic the endogenous effects of protein−protein interactions in vivo (*23*). Therefore, the relaxed conformations of H2A.Z fibers at higher salt concentrations suggest that deposition of the variant nucleosomes may poise chromatin for transcriptional initiation by providing a mechanism for decondensation (*28*, *31*). This may be facilitated by the extension of an acidic patch of the carboxyl-terminal end of H2A.Z, generating charge repulsion between neighboring nucleosomes or DNA−phosphate backbones, and inducing a thermodynamically more favorable extended conformation. Furthermore, higher-order structures may also be influenced by the novel carboxyl-terminal divalent cation coordination site displayed on the particle surface (*20*). Histidine 112 is conserved in yeast (*30*) and every other identified H2A.Z homologue, and a stabilizing imidazole ring is present at His114 in eukaryotic organisms. This feature may enable variant nucleosomes to interact with chromatin remodeling machinery or H1 proteins (*20*).

The second possible structural link between H2A.Z particles and transcriptional activation is that H2A.Z core particle stability is significantly more labile than that of its H2A counterpart. It appears that the noncovalent protein−protein interactions between H2A.Z−H2B dimers and the H3−H4 tetramer are weakened within variant nucleosomes. This result was previously hypothesized (*20*, *31*) and may be explained by the substitution of C-terminal Gln104 in major H2A with Gly106 in H2A.Z, which eliminates hydrogen bonding between the glutamine amide group and neighboring atoms in H3 at the docking site (*2*, *20*). By compromising the stability of specialized nucleosomes, the cell may lower the energy of activation for polymerase migration through chromatin structures (*19*, *32*).

H2AX, a Unique Phosphorylation Substrate

In a variety of cellular processes, the fragmentation of DNA has been observed to induce the post-translational phosphorylation of terminally localized H2AX (termed γ-H2AX) nucleosomes. These regulated events include double-strand break repair (*27*, *33*), meiotic recombination preceding synaptic crossover (*34*), apoptotic digestion following caspase-activated DNase activity (*27*), and V(D)J splicing (*35*) and class switch recombination (*36*) during the development of immunoglobulin variability. The modification of this histone variant occurs at a well-conserved serine residue (Ser129 in yeast and Ser139 in mammals) that is a component of the phosphatidylinositol 3-kinase consensus sequence (Ser-Gln-Glu). Recent evidence demonstrates that the players in this reaction are not monogamous as three distinct kinases have been shown to phosphorylate H2AX. Significantly, the crosstalk between these related proteins may turn out to be a redundant theme as in a complementary system DNA-PK, ATM and ATR compete for Ser15 on the tumor suppressor p53 in the presence of DNA damage (*37*, *38*). In the case of H2AX phosphorylation, DNA-PK appears to catalyze initial and background signaling events (*33*); on the other hand, ATM may be the dominant constitutive modulator following double-stranded injury (*39*, *40*), and ATR displays replication arrest dependence (*41*). The participation of multiple kinases suggests that the generation of γ-H2AX foci at double-strand breaks requires sensitive control, which may help to coordinate downstream events in a structural or informational fashion. Consequently, it is tempting to speculate that DNA-PK may specifically phosphorylate H2AX during constant region recombination (*36*) and possibly V(D)J crossover events, due to the fact that the kinase associates with both DSB repair and class switching machinery (*42*).

The addition of a bulky phosphate group, with a double-negative charge, has structural implications for chromatin, and may induce localized transitional dynamics between a compact and permissive substrate. In theory, the generation of decondensed domains would allow greater accessibility of modulating enzymes, and facilitate the assembly of repair complexes at lesion sites. In this regard, genomic nuclease hypersensitivity was observed in yeast S129E mutants, which mimic the charge state of γ-H2AX (*43*).

Another possible implication for H2AX phosphorylation is that γ-H2AX serves as a chemical signal to facilitate downstream events and stimulate novel protein−protein

interactions. Following double-strand DNA damage, Rad50/51 and BRCA1 are recruited to γ-H2AX foci in human cells, and the formation of the repairosome is inhibited in the absence of phosphorylation (*33*). Similarly, γ-H2AX generation precedes the localization of DMC1 recombinases during meiosis (*34*). Therefore, the functional novelty of the H2AX C-terminal tail may confer both structural and functional properties to nucleoprotein complexes that operate in an independent or coordinated fashion. Possibly, the repair of double-stranded breaks may require both the unfolding of chromatin domains to allow accessibility of modulating enzymes and serine phosphosignaling to recruit downstream proteins to the site of DNA breakage.

Conclusion

The importance of the prominent and heterogeneous carboxyl-terminal tail in H2A isoforms is a subject of emerging interest that has received little historical attention in the chromatin literature. Its existence has been shown to be essential for the stability of the nucleosome (*24*), and variation in this protein domain modulates chromatin stability (*20*, *23*). Also, as discussed above, the variability between nonallelic subtypes has been shown to add functional specialization to chromatin domains. On the basis of all this, we propose a model by which H2A variants exert their multiple functions through chemical variation of the carboxyl-terminal tails (see Figure 1B). Accordingly, replacement of H2A.1 with different H2A variants would alter chromatin structure in a way that would make it possible to impart epigenetic regulation of chromatin function (Figure 1B). In the case of H2A.Z, the carboxyl-terminal region associates with transcriptional machinery (*30*) and destabilizes the interaction with the tetramer (*20*, *23*). MacroH2A is specialized to silence the female vertebrate X chromosome through its massive carboxyl-terminal non-histone fusion (*25*, *26*). Likewise, the unique C-terminal tail of H2AX is a phosphorylation substrate during double-stranded fragmentation of DNA in many cellular processes (*27*, *34*−*36*). The structural variability of H2A and its multifaceted functional implications enhance the notion that histones are not mere passive structural elements of an otherwise highly dynamic chromatin complex. Further exploration into the elusive nature of H2A variants using powerful techniques such as CHIP assays and biophysical methods should help to clarify the spatial distribution of these isoforms and define their structural and functional roles in specialized chromatin environments.

REFERENCES

1. Kornberg, R. D., and Lorch, Y. (1999) *Cell 98*, 285−294.
2. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) *Nature 389*, 251−260.
3. Knezetic, J. A., and Luse, D. S. (1986) *Cell 11*, 95−104.
4. Matsui, T. (1987) *Mol. Biol. Cell 7*, 1401−1408.
5. Annunziato, A. T., Schindler, R. K., Riggs, M. G., and Seale, R. L. (1982) *J. Biol. Chem. 257*, 8507−8515.
6. Ausió, J., Seger, D., and Eisenberg, H. (1984) *J. Mol. Biol. 176*, 77−104.
7. Ausió, J., and van Holde, K. E. (1986) *J. Biol. Chem. 25*, 1421−1428.
8. Dong, F., Nelson, C., and Ausió, J. (1990) *Biochemistry 29*, 10710−10716.
9. Flaus, A., and Owen-Hughes, T. (2001) *Curr. Opin. Genet. Dev. 11*, 148−154.
10. Wolffe, A. P., and Guschin, D. (2000) *J. Struct. Biol. 129*, 102−122.
11. Jenuwein, T., and Allis, C. D. (2001) *Science 293*, 1074−1080.
12. Ausió, J., Abbott, D. W., Moore, S., and Wang, X. (2001) *Biochem. Cell Biol. 79*, 681−692.
13. Turner, B. M. (2000) *BioEssays 22*, 836−845.
14. Ren, Q., and Gorovsky, M. A. (2001) *Mol. Cell 7*, 1329−1335.
15. Wolffe, A. P., and Dimitrov, S. (1993) *Crit. Rev. Eukaryotic Gene Expression 3*, 167−191.
16. Alvelo-Ceron, D., Niu, L., and Collart, D. G. (2000) *Mol. Biol. Rep. 27*, 61−71.
17. Svaren, J., and Chalkey, R. (1990) *Trends Genet. 6*, 52−56.
18. Hendzel, M. J., and Davie, J. R. (1990) *Biochem. J. 271*, 67−73.
19. van Holde, K. E., Lohr, D. E., and Robert, C. (1992) *J. Biol. Chem. 267*, 2837−2840.
20. Suto, R. K., Clarkson, M. J., Tremethick, D. J., and Luger, K. (2000) *Nat. Struct. Biol. 7*, 1121−1124.
21. Basrai, M. A., and Hieter, P. (1995) *BioEssays 17*, 669−672.
22. Chadwick, B. P., and Willard, H. F. (2001) *J. Cell Biol. 152*, 375−384.
23. Abbott, D. W., Wang, X., Isanova, V., Bonner, W. M., and Ausió, J. (2001) *J. Biol. Chem. 276*, 41945−41949.
24. Eickbush, T. H., Godfrey, J. E., Elia, M. C., and Moudrianakis, E. N. (1988) *J. Biol. Chem. 263*, 18972−18978.
25. Pehrson, J. R., and Fried, V. A. (1992) *Science 257*, 1398−1400.
26. Costanzi, C., and Pehrson, J. R. (1998) *Nature 393*, 599−601.
27. Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., and Bonner, W. M. (1998) *J. Biol. Chem. 273*, 5858−5868.
28. Santisteban, M. S., Kalashnikova, T., and Smith, M. M. (2000) *Cell 103*, 411−422.
29. Leach, T. J., Mazzeo, M., Chotkowski, H. L., Madigan, J. P., Wotring, M. G., and Glaser, R. L. (2000) *J. Biol. Chem. 275*, 23267−23272.
30. Adam, M., Robert, F., Larochelle, M., and Gaudreau, L. (2001) *Mol. Cell. Biol. 21*, 6270−6279.
31. Clarkson, M. J., Wells, J. R. E., Gibson, F., Saint, R., and Tremethick, D. J. (1999) *Nature 399*, 694−697.
32. Jackson, V. (1990) *Biochemistry 29*, 719−731.
33. Paull, T. T., Rogakou, E. P., Yamazaki, V., Kirchgessner, C. U., Gellert, M., and Bonner, W. M. (2000) *Curr. Biol. 10*, 886−895.
34. Mahadevaiah, S. K., Turner, J. M. A., Baudat, F., Rogakou, E. P., de Boer, P., Blanco-Rodriguez, J., Jasin, M., Keeney, S., Bonner, W. M., and Burgoyne, P. S. (2001) *Nat. Genet. 27*, 271−276.
35. Chen, H. T., Bhandoola, A., Difilippantonio, M. J., Zhu, J., Brown, M. J., Tai, X., Rogakou, E. P., Brotz, T. M., Bonner, W. M., Ried, T., and Nussenzweig, A. (2000) *Science 290*, 1962−1965.
36. Petersen, S., Casellas, R., Reina-San-Martin, B., Chen, H. T., Difilippantonio, M. J., Wilson, P. C., Hanitsch, L., Celeste, A., Muramatsu, M., Pilch, D. R., Redon, C., Ried, T., Bonner, W. M., Honjo, T., Nussenzweig, M. C., and Nussenzweig, A. (2001) *Nature 414*, 660−665.
37. Smith, G. C. M., and Jackson, S. P. (1999) *Genes Dev. 133*, 916−934.
38. Drocher, D., and Jacson, S. P. (2001) *Curr. Opin. Cell Biol. 13*, 225−231.
39. Andegeko, Y., Moyal, L., Mittelman, L., Tsarfatyy, I., Shiloh, Y., and Rotman, G. (2001) *J. Biol. Chem. 276*, 38224−38230.
40. Burma, S., Chen, B. P., Murphy, M., Kurimasa, A., and Chen, D. J. (2001) *J. Biol. Chem. 276*, 42462−42467.
41. Ward, I. M., and Chen, J. (2001) *J. Biol. Chem. 276*, 47759−47762.
42. Jackson, S. P., and Jeggo, P. A. (1995) *Trends Biochem. Sci. 20*, 412−415.
43. Downs, J. A., Lowndes, N. F., and Jackson, S. P. (2000) *Nature 408*, 1001−1004.

BI020059D