ATP-dependent Remodeling by SWI/SNF and ISWI Proteins Stimulates V(D)J Cleavage of 5 S Arrays*

Received for publication, May 25, 2004, and in revised form, June 15, 2004
Published, JBC Papers in Press, June 16, 2004, DOI 10.1074/jbc.M405790200

Nadja Patenge‡§§, Sheryl K. Elkin‡§§, and Marjorie A. Oettinger‡§§

From the ‡Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114 and the §Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115

Control of V(D)J recombination is critical for the generation of a fully developed immune repertoire. The molecular mechanisms underlying the regulation of antigen receptor gene assembly are beginning to be revealed. Here we studied the influence of chromatin modifications on V(D)J cleavage of a polynucleosomale substrate, in which V(D)J cleavage is greatly reduced compared with naked DNA. ATP-dependent remodeling by human SWI/SNF (hSWI/SNF) in the presence of HMG1 led to a substantial increase of cleavage by the recombination activation gene (RAG) proteins. Either BRG1, the ATPase subunit of hSWI/SNF, or SNF2h, the ATPase of human ISWI complexes, was capable of stimulating V(D)J cleavage of the array, although these remodelers act by different mechanisms. No effect of histone hyperacetylation was detectable in this system. As is observed on naked DNA, in the presence of core RAG1, the full-length RAG2 protein proved to be more active than core RAG2 on these polynucleosomal arrays, reinforcing the importance of the RAG2 C-terminal domain for efficient recombination. Comparison of 5 S array cleavage by the RAG proteins or by the restriction enzyme HhaI after remodeling by hSWI/SNF suggested that RAG proteins and HhaI might have different requirements for maximal accessibility of the substrate.

Functional antigen receptor genes are assembled from germ line gene segments by V(D)J recombination (1). Each gene segment is flanked by recombination signal sequences (RSSs) that share conserved heptamer and nonamer sequences separated by a spacer region of relatively nonconserved sequence but conserved length of either 12 or 23 bp. Productive gene rearrangement requires one of each kind of RSS, a restriction known as the 12/23 rule (2). Initiation of V(D)J recombination is mediated by the lymphoid-specific gene products of the recombination activation genes (RAG) 1 and 2 (3, 4). The RAG proteins recognize the RSSs and introduce a double strand break at the coding-signal border (5). The intermediate reaction products are a hairpin coding end and a blunt signal end (5, 6). Subsequent joining of two corresponding coding ends and two signal ends is achieved by the ubiquitously expressed DNA double strand repair machinery of the nonhomologous end-joining pathway (7).

In order to express the correct antigen receptor gene in the appropriate cell and to ensure chromosomal integrity, tight control of V(D)J recombination is required (reviewed in Refs. 8 and 9). Antigen receptor assembly is thus restricted to developing lymphocytes and occurs in a lineage-specific manner, with T cell receptor genes being fully assembled only in T cells and Ig genes fully rearranged only in B cells. Temporal regulation is also observed. For example, at the Ig heavy chain (IgH) locus, D to J joining precedes V to DJ rearrangement, and IgH assembly precedes that of Ig light chain (IgL) loci. Furthermore, antigen receptor rearrangement at IgH and T cell receptor β loci is subjected to allelic exclusion, such that the expression of a productive assembled antigen receptor gene represses the rearrangement of the second allele (8). The same recombination machinery is used for all of these rearrangements, raising the question of how these different levels of regulation are achieved. Tissue-specific expression of RAG1 and RAG2 can explain the restriction of V(D)J recombination to developing lymphocytes. However, to explain lineage specific receptor gene assembly and the temporal order of rearrangement, other mechanisms to control the accessibility of the gene loci to the recombinase, probably requiring changes in chromatin structure, must be invoked (10).

Transcriptional enhancer regions within antigen receptor gene loci may serve as regulatory elements involved in V(D)J recombination (8, 9, 11). However, whereas sterile transcription has been observed to correlate with V(D)J recombination, it is not always required (12, 13). Hyperacetylation of lysines in the histone N-terminal tails, a modification generally correlated with active genes (14), has been observed at recombinationally active loci in vitro (15–22). A role for chromatin alterations achieved through remodeling by ATP-dependent enzymes of the SWI/SNF and ISWI protein families (23–26) can also be envisaged. Recently, BRG1, the ATPase subunit of human SWI/SNF, was found in vivo across loci that are poised to undergo V(D)J recombination (22).

A biochemical approach is complementary to the many in vivo observations linking chromatin modifications to V(D)J recombination and permits a more detailed analysis of how chromatin structure can affect V(D)J cleavage. Previous work has shown that RSSs reconstituted into mononucleosomes are refractory to V(D)J cleavage in vitro (27–29). The extent of inhibition is dependent on the position of the RSS in the mononucleosome and can be partially relieved by the addition of HMGI (27). Hyperacetylated histone proteins stimulate V(D)J cleavage of mononucleosomes (28, 30). ATP-dependent remodel-
eling by hSWI/SNF can restore V(D)J cleavage to a higher degree than histone acetylation, and the efficiency of its activity is enhanced in combination with hyperacetylation (30).

Although mononucleosomes allow some insight into structural and functional characteristics of chromatin and allow the generation of highly localized nucleosome positions, they do not represent the structure of chromatin at more complex levels. In addition, all of the experiments on mononucleosomes have been done using core RAG2 protein (RAG2C), lacking the C-terminal quarter of the protein, which has been implicated in the regulation of V to DJ joining at the IgH locus. Therefore, we have examined the effects of chromatin modifications on V(D)J cleavage of a polynucleosomal substrate and compared the cleavage activity of RAG2C with the more physiologically relevant full-length RAG2 (RAG2FL).

We find that ATP-dependent remodeling by BRG1, the ATPase subunit of hSWI/SNF, dramatically stimulates cleavage of the array substrate. Interestingly, SNF2h, the ATPase subunit of human ISWI complexes, enhances cleavage of the polynucleosomal substrate to the same extent as BRG1, although different remodeling mechanisms have been discussed for the two proteins (31–33). However, hyperacetylated histones did not relieve cleavage inhibition and were not able to increase the effect of hSWI/SNF remodeling activity. Whereas the overall cleavage activity of full-length RAG2 protein is greater than that of core RAG2 (both in the presence of core RAG1), no specific role for the C terminus in gaining access to chromatin substrates was observed.

EXPERIMENTAL PROCEDURES

Proteins—Recombinant core RAG1 (amino acids 384–1040) protein was purified from Escherichia coli carrying the plasmid pDR6533 as described previously (34). Core RAG2 (amino acids 1–383) and full-length RAG2 (amino acids 1–527) proteins were produced in HeLa cells using a vaccinia expression system (5, 35). Both proteins were Histag- and FLAG-tagged at the N terminus and HA-tagged at the C terminus. The DNA fragment was end-labeled with Klenow fragment and was subsequently purified by Sephadryl 1000 size exclusion chromatography. The DNA fragment was end-labeled with Klenow fragment. The DNA fragment was end-labeled with Klenow fragment. The DNA fragment was end-labeled with Klenow fragment. The DNA fragment was end-labeled with Klenow fragment. The DNA fragment was end-labeled with Klenow fragment. The DNA fragment was end-labeled with Klenow fragment.

Cleavage Assays—V(D)J cleavage reactions were performed in a 20-μl volume containing either 4 ng of naked DNA or 5 S array (1.5 nm mononucleosomes), 13 nm RAG1, 25 nm full-length RAG2 or 50 nm core RAG2, 25 nm HEPES (pH 7.5), 2 mM dithiothreitol, 40 mM potassium glutamate, 2 μg of bovine serum albumin, 2.5% glycerol, 3 mM MgCl2, 1 mM ATP, and 0.5 units of HhaI.

RESULTS

Tandem repeats of Lytechinus variegatus 5 S rDNA constitutively in chromatin have been used widely as a model system to address questions of chromatin structure, mechanism of assembly, and nucleosome positioning (45–47). More recently, this system has been employed to study the mechanism of chromatin remodeling and the influence of chromatin structure on complex processes like transcription (41, 44, 48, 49). We used a similar template by introducing RSSs into 5 S arrays. All templates used in this study contained 10 repetitions of L. variegatus 5 S rDNA positioning sequences and a central region carrying five GAL4 binding sites, the E4 minimal promoter, and 12 or 23 RSSs (Fig. 1A). The RSSs were introduced downstream of the transcription start site, at the location of the second unpositioned nucleosome. Several unique restriction enzyme sites are also located on this fragment, of which the HhaI site is used here as an RSS-independent measure of substrate remodeling via restriction enzyme accessibility assays.

In order to establish that the template could serve as an efficient substrate for V(D)J cleavage, we subjected the naked substrate to cleavage by RAG1 and RAG2, comparing the different proteins and reaction conditions that would be used to assess cleavage on the assembled array. We compared core and full-length RAG2 (RAG2C and RAG2FL) and reactions with and without HMG1 on both the 12- and the 23-RSS templates (Fig. 1B). The samples were subjected to native agarose gel electrophoresis, and the extent of cleavage was monitored by phosphor imager analysis. Upper bands represent uncleaved substrate and nicked products, and lower bands correspond to hairpin products. In the presence of RAG1 and HMG1, RAG2FL showed about 2-fold higher cleavage of the 12- or 23-RSS substrate than RAG2C (Fig. 1B, compare lanes 2, 3, and 8 with lanes 4, 5, and 10). This corresponds to a 4-fold higher activity of RAG2FL preparations, because twice as much RAG2C as RAG2FL protein was used. The presence of HMG1 did not influence RAG cleavage activity of the 12-RSS substrate (Fig. 1B, lanes 2–5) but stimulated cleavage of the 23-RSS substrate 5-fold (Fig. 1B, lanes 7–10).

The 12- and 23-RSS templates were then reconstituted into a polynucleosomal array with histone octamers purified from

VDJ Cleavage of 5 S Arrays
HeLa cells. Typically, three different histone/DNA ratios were used for assembly, and the best one was chosen as a template for V(D)J cleavage. The extent of assembly was tested by several different methods, including analysis of DNA compaction on 1.3% low electroendosmosis-agarose gels (Fig. 2A), SDS-PAGE analysis of protein composition (Fig. 2B), and MNase digestion analysis (data not shown). The degree of nucleosomal assembly was also assessed by a restriction enzyme accessibility assay using HhaI and hSWI/SNF (Fig. 2C). Underloaded arrays were characterized by high HhaI cleavage levels in the absence of remodeling (Fig. 2C, lane 2). In contrast, an excessive histone/DNA ratio led to aggregation of the template and therefore reduced cleavage by HhaI, even in the presence of hSWI/SNF and ATP (Fig. 2C, lanes 6 and 7). In this study, we used templates that showed background HhaI cleavage of less than 25% and cleavage of about 90% in the presence of ATP, which represented properly assembled arrays (50) (Fig. 2C, lanes 4 and 5).

ATP-dependent Remodeling by hSWI/SNF Facilitates V(D)J Cleavage of 5 S Arrays—Because we found that V(D)J cleavage of chromatin substrates was much slower than that of naked DNA (see below), we could not employ end point assays, since some reactions were already completed whereas others were still proceeding at a linear rate (Fig. 3). To overcome this difficulty, we performed time course experiments and compared the speed of the reactions rather than the extent of cleavage at a particular time point. Reaction rate constants were obtained by first-order fits of the data sets (Fig. 3, B and C). For all of the experiments described below, the average from three independent experiments is shown, and the reaction rate constants are normalized to the cleavage of naked DNA unless stated otherwise.

After reconstitution of the template into 5 S arrays, both the 12- and 23-RSS substrates were refractory to V(D)J cleavage (Fig. 3A, lanes 3 and 9). The very low levels of cleavage that could be observed most likely represent cleavage at RSSs that are not assembled into nucleosomes. Because HMG1 had been shown to relieve cleavage repression on mononucleosomes (27), we compared 5 S array cleavage with and without HMG1. The addition of HMG1 alone to the reaction allowed a low level of cleavage to be detected (12 RSS, 2.5% of the rate constants of naked DNA cleavage; 23 RSS, less than 1% of the rate constants of naked DNA cleavage) (Fig. 3D). We next asked whether ATP-dependent remodeling by hSWI/SNF affected the accessibility of the substrate to the RAG1-RAG2 proteins. ATP-dependent remodeling by hSWI/SNF was able to stimulate the array cleavage rate constants to 14% of naked DNA cleavage (12 RSS) or to about 1% (23 RSS), respectively (Fig. 3D). In combination with HMG1, hSWI/SNF could enhance cleavage up to 18% (12 RSS) or up to 7% (23 RSS) (Fig. 3D). After 3 h, up to 20% of the substrate (Fig. 3A, lanes 6 and 12) and after incubation overnight up to 40% of the substrate was cleaved (data not shown), on par with cleavage of naked DNA substrates. Nicked products were not taken into account, because they are not detectable on a native gel. hSWI/SNF had no effect on RAG1-RAG2 cleavage of naked DNA, indicating that cleavage stimulation on 5 S arrays was due to its chromatin remodeling activity (Fig. 1C). Thus, ATP-dependent remodeling by hSWI/SNF had a substantial influence on the ability of the RAG proteins to perform V(D)J cleavage on a polynucleosomal substrate.

Comparison of Core RAG2 and Full-length RAG2 Activity on 5 S Arrays—Although RAG2C is able to perform all steps of V(D)J cleavage in vitro, the full-length protein is required for efficient V(D)J recombination of endogenous loci. Whereas DH to JH rearrangement is only mildly reduced in the presence of RAG2C, VH to DJH recombination is significantly impaired (52–54). It has been suggested that the C-terminal region of RAG2 folds into a plant homeodomain that may mediate protein-chromatin interactions (55). Additionally, recent work has
shown that plant homeodomains interact with nuclear phosphoinositides and that this interaction may serve to regulate nuclear processes, possibly including V(D)J recombination (56).

We asked whether RAG2C and RAG2FL differ in their ability to cleave chromatin substrates in the presence or absence of ATP-dependent remodeling. Since HMG1 was required for maximum levels of cleavage, especially for 23-RSS substrates, all experiments were performed in the presence of 100 ng of HMG1. Fig. 4A shows an agarose gel analysis of the last time point of a representative experiment. As with naked DNA, RAG1 plus RAG2FL was consistently 4-fold more active on array substrates than RAG1 plus RAG2C (Fig. 4A, compare lanes 2, 5, 9, and 12 with lanes 3, 7, 10, and 14). In the absence of ATP-dependent remodeling of the nucleosomal template, the cleavage rate constants of both RAG1-RAG2 complexes were less than 1% of their rate constants for naked DNA cleavage (Fig. 4B). Remodeling by hSWI/SNF stimulated the cleavage rate constants of the 12 RSS for both RAG1-RAG2C and RAG1-RAG2FL to about 10% of naked DNA cleavage (Fig. 4B). Similarly, cleavage of the 23-RSS array was stimulated to 10 or 7% of the naked DNA control for RAG1-RAG2C and RAG1-RAG2FL, respectively. Therefore, the C-terminal domain of RAG2 enhanced the efficiency of cleavage but did not show any chromatin-specific effect in these assays.

No Detectable Effect of Hyperacetylation on V(D)J Cleavage of 5 S Arrays

Acetylation of histone N-terminal tails is often correlated with active gene loci. In order to test whether the nucleosomal disruption caused by hyperacetylation is sufficient to improve accessibility of the polynucleosomal arrays to the recombinase, cleavage of arrays assembled with hyperacetyl-
ated histone octamers (AcH) (see “Experimental Procedures”) was compared with cleavage of the standard (CH) arrays described above. The acetylation state of the histones was confirmed by acid urea gel analysis (Fig. 5A, and the acetylation state of the assembled arrays was confirmed by Western hybridization (Fig. 5B).

The presence of hyperacetylation alone did not increase RSS accessibility; RAG1-RAG2FL-mediated V(D)J cleavage was inhibited to a similar extent on both AcH 5 S arrays and CH 5 S arrays (Fig. 5C, lanes 2, 4, 7, and 9). The relative rate constants of the reactions are shown in Fig. 5D. After remodeling by hSWI/SNF, cleavage inhibition was relieved, independent of the acetylation state of the arrays, to about 12–17% of the rate constants of naked DNA cleavage (12 RSS) or to 7–8% (23 RSS) (Fig. 5D). Similar results were found using RAG1-RAG2C, with remodeling by hSWI/SNF stimulating the cleavage rate constants to 10% of the level of naked DNA (data not shown). Thus, hyperacetylation did not detectably stimulate V(D)J cleavage of the polynucleosomal substrate and did not enhance the effect of hSWI/SNF on cleavage.

**The Restriction Enzyme HhaI and the RAG Proteins Have Different Requirements for Accessing the 5 S Array Substrate—** Although no difference between CH and AcH substrates could be detected under the conditions described above, it remained possible that hyperacetylation could influence the reaction if the remodeling enzyme activity became limiting. Under such a circumstance, the effect of histone acetylation through retention of the remodeler at the nucleosome carrying the RSS, for example, or in facilitating the remodeling reaction might be detectable.

We tested this possibility in V(D)J cleavage reactions that included HMGI and saturating amounts of RAG1 and RAG2FL and serial dilutions of hSWI/SNF from 4 to 0.06 nM. In the V(D)J cleavage experiments described above, hSWI/SNF was added to a concentration of 0.5 nM, which was determined empirically as the optimal concentration for our assays. As an
Remodeling Activities from the SWI/SNF and ISWI Families Can Facilitate V(D)J Cleavage of Polynucleosomal Arrays—Like members of the SWI/SNF family, ISWI complexes alter the structure of chromatin using the energy of ATP hydrolysis. It has been proposed that the two families act differently on chromatin (reviewed in Refs. 31—33). We were interested in whether the stimulation of V(D)J cleavage is restricted to remodeling by SWI/SNF or ISWI complexes. It is interesting to note that the two enzymes, HhaI and the recombinase, have different requirements for efficient cleavage of the same substrate.

Remodeling by either family altered the chromatin structure in favor of more accessibility to the RAG proteins.

**FIG. 7.** BRG1 and SNF2h can both facilitate V(D)J cleavage of 5 S arrays. A, agarose gel of a V(D)J cleavage experiment with 12 RSS substrates in the presence of hSWI/SNF, BRG1, or SNF2h as indicated at the 210-min time point. B, reaction rate constants from three independent time course experiments are combined and normalized to the level of naked DNA cleavage (relative rate constants).

**DISCUSSION**

**ATP-dependent Remodeling by Proteins of the SWI/SNF and ISWI Families Facilitate V(D)J Cleavage of Polynucleosomal Arrays**—The physiological relevance of the ability of hSWI/SNF to enhance V(D)J cleavage of chromatin substrates in vivo has been recently supported by in vitro observations. BRG1, the ATPase subunit of hSWI/SNF, has been detected in a stage- and lineage-dependent manner at antigen receptor loci that are poised to undergo V(D)J recombination (22). In addition, remodeling by hSWI/SNF was able to relieve V(D)J cleavage repression on mononucleosomes (30). We now show that hSWI/SNF has a profound effect on the accessibility of polynucleosomal arrays to the RAG proteins. Cleavage enhancement was seen, although, in the context of 5 S arrays, the nucleosome carrying the RSS was not flanked by free DNA ends.

Besides the SWI/SNF family of chromatin-remodeling enzymes, proteins of the ISWI family of ATP-dependent remodeling enzymes are involved in a variety of different processes, including transcriptional activation, transcriptional repression, and chromatin assembly (26). The substrate requirements for members of the SWI/SNF and ISWI families are known to be different. Whereas histone tails are not required for nucleosome remodeling by SWI/SNF, ISWI activity depends on the integrity of the N-terminal domains (57, 58). Recent studies suggest that members of the two families act differently on chromatin (31—33). Here we demonstrate that proteins of both the ISWI family and SWI/SNF family are able to enhance RAG cleavage of chromatin substrates equally well. Thus, two dif-
different classes of remodeling enzymes, using different modes of operation, have the potential to open a locus to the RAG proteins. In a recent study, Baumann et al. (59) observed only a minimal amount of stimulation of RAG cleavage of RSSs in mononucleosomes after remodeling with the nucleosome remodeling factor (NURF) complex. Apparently, the nucleosome positioning activity of the RSS nonamer could not be overcome by the NURF complex. These findings suggest that a NURF-like remodeling complex is unlikely to be involved in mobilizing nucleosomes to facilitate V(D)J recombination but leave open the possibility that other ISWI-containing nucleosome-remodeling complexes could play a role. Here we demonstrate that, indeed, remodeling by SNF2H can facilitate cleavage on a nucleosome array, suggesting that the nucleosome positioning by the nonamer can be overcome by remodelers of the ISWI protein family. The question remains whether both types of complexes are actually involved in V(D)J recombination. Although BRG1 has been observed to be present at active loci, this does not exclude the possibility that other remodeling enzymes are involved at different stages of recombination or at different loci.

The Role of Hyperacetylation in the Regulation of V(D)J Recombination—The association of histone acetylation with the regulation of V(D)J recombination has become evident in recent years. Hyperacetylation of histone H3 correlates with active V(D)J recombination and is observed at loci that are poised to undergo D to J rearrangement (15, 16, 18–22). Furthermore, in tissue culture cells, treatment with histone deacetylase inhibitors increases V(D)J recombination (20, 28–30). In vitro data support the role of acetylation for V(D)J recombination. On the level of a single nucleosome, hyperacetylation can enhance V(D)J cleavage in vitro (30). However, we could not detect an influence of hyperacetylation on RAG cleavage of polynucleosomal arrays. What is the distinction between the two systems? There are two obvious differences. The first is that the RSSs were positioned on the mononucleosomes; the second is that the mononucleosomes were situated close to a free DNA end. In this regard, the greatest effect of AcH may be masked (29, 59). In a related study, the association of hyperacetylation with active loci strongly implies an involvement of histone acetylation in the regulation of V(D)J recombination. Although no major effect of hyperacetylation on the cleavage reaction rate constants could be observed in vitro, there remain a number of potential roles for acetylation. Hyperacetylation of histone tails is known to facilitate the disruption of the higher order chromatin structure (52). Accessibility to the RAG proteins might be achieved by opening the higher order structure through hyperacetylation followed by changes at single nucleosomes with the help of ATP-dependent remodeling. During activation of a locus, acetylation is often an important step for recruitment of regulatory factors, chromatin remodeling activities, or DNA demethylases (63–68). Histone acetylation at Ig or T cell receptor loci might allow binding of factors to regulatory elements that in turn initiate the recruitment of hSWI/SNF or other factors essential for opening of the locus to the recombinase. In two recent studies, it has been shown that acetylation mediates retention of hSWI/SNF at promoter nucleosomes in the absence of transcription factors (69, 70). We show here that unlike a restriction enzyme activity like HhaI, in vitro RAG cleavage is slower than ATP-dependent remodeling even at saturating concentrations. This observation suggests that if the RAG proteins act at a slow cleavage rate in vivo, the continuous presence of remodeling enzymes might be a prerequisite for ordered opening of antigen receptor loci. One could speculate that hyperacetylation of antigen receptor loci is necessary for the ordered opening of the loci and for the targeting and retention of required factors including ATP-dependent chromatin-remodeling enzymes.

Acknowledgments—We thank Nicole Francis and Geeta Narlikar for helpful discussions throughout this work and Geeta Narlikar for critical reading of the manuscript. We thank J. L. Workman for the gift of p208SS-G5E4. We are grateful to H. Y. Fan and G. Narlikar for providing SNF2h and BRG1 proteins.

REFERENCES

1. Tenega, S. (1983) Nature 302, 575–581.
2. Lewis, S. M. (1994) Adv. Immunol. 54, 127–150.
3. Oettinger, M. A., Schatz, D. G., Gorka, C., and Baltimore, D. (1990) Science 248, 1517–1523.
4. Schatz, D. G., Oettinger, M. A., and Baltimore, D. (1989) Cell 59, 1035–1048.
5. McBlane, J. F., van Gent, D. C., Ramsden, D. A., Romeoc, C. A., Gellert, M., and Oettinger, M. A. (1995) Cell 83, 387–395.
6. van Gent, D. C., McBlane, J. F., Ramsden, D. A., Sadowsky, M. J., Hesse, J. E., and Oettinger, M. (1996) Curr. Top. Microbiol. Immunol. 217, 1–10.
7. Lieber, M. R. (1996) Genes Cells 1, 77–85.
8. Bassing, C. H., Swat, W., and Alt, F. W. (2002) Cell 109, (suppl.) 45–55.
9. Hessel, D. G., and Schatz, D. G. (2001) Adv. Immunol. 78, 169–232.
10. Yancopoulos, G. D., and Alt, F. W. (1986) Annu. Rev. Immunol. 4, 339–368.
11. Sleekman, S. P., Gorman, J. R., and Alt, F. W. (1996) Annu. Rev. Immunol. 14, 459–481.
12. Angelin-Dulos, C., and Calame, K. (1998) Mol. Cell. Biol. 18, 6253–6264.
13. Stanhope-Baker, P., Hudson, K. M., Shaffer, A. L., Constantinescu, A., and Schlissel, M. S. (1996) Cell 85, 887–897.
14. Maes, J., O’Neill, L. P., Cavelier, P., Turner, B. M., Rougeon, F., and Goodhardt, M. (2001) J. Immunol. 167, 866–874.
15. Chowdhury, D., and Sen, R. (2001) EMBO J. 20, 6394–6403.
16. Agata, Y., Katakai, T., Ye, S. K., Sugai, M., Gonda, H., Honjo, T., Ikuta, K., and Shimizu, A. (2001) J. Exp. Med. 193, 873–880.
17. McCormurty, M. T., and Krangel, M. S. (2000) Science 287, 485–498.
18. Muenke, N., Hempel, W. M., Spicuglia, S., Verhoy, C., and Ferrier, P. (2000) J. Exp. Med. 192, 625–636.
19. Tripathi, R., Jackson, A., and Krangel, M. S. (2002) J. Immunol. 168, 2321–2324.
20. Morschhead, K. B., Ciccone, D. N., Taverna, S. D., Allis, C. D., and Oettinger, M. A. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 11577–11582.
21. Kingston, R. E., and Narlikar, G. J. (1999) Mol. Cell. Biol. 13, 2323–2352.
22. Vignali, M., Hassan, A. H., Neely, K. E., and Workman, J. L. (2000) Mol. Biol. Cell 10, 1899–1910.
23. Peterson, C. L., and Workman, J. L. (2000) Curr. Opin. Genet. Dev. 10, 187–192.
24. Langst, G., and Becker, P. B. (2001) J. Cell Sci. 114, 2561–2568.
25. Kwon, J., Imbalzano, A. N., Matthews, A., and Oettinger, M. A. (1998) Mol. Cell 2, 829–839.
26. McBlane, F., and Boyes, J. (2000) Curr. Biol. 10, 483–486.
27. McBlane, J., and Boyes, J. (2000) Curr. Biol. 10, 483–486.
28. McBlane, J., and Boyes, J. (2000) Curr. Biol. 10, 483–486.
29. McBlane, J., and Boyes, J. (2000) Curr. Biol. 10, 483–486.
30. McBlane, J., and Boyes, J. (2000) Curr. Biol. 10, 483–486.
31. McBlane, J., and Boyes, J. (2000) Curr. Biol. 10, 483–486.
32. McBlane, J., and Boyes, J. (2000) Curr. Biol. 10, 483–486.
VDJ Cleavage of 5 S Arrays

44. Logie, C., and Peterson, C. L. (1997) *EMBO J.* 16, 6772–6782
45. Simpson, R. T., Thoma, F., and Brubaker, J. M. (1985) *Cell* 42, 799–808
46. Meersseman, G., Pennings, S., and Bradbury, E. M. (1991) *J. Mol. Biol.* 220, 89–100
47. Hansen, J. C., van Holde, K. E., and Lohr, D. (1991) *J. Mol. Biol.* 220, 89–100
48. Owen-Hughes, T., and Workman, J. L. (1996) *EMBO J.* 15, 4702–4712
49. Neely, K. E., Hassan, A. H., Wallberg, A. E., Steger, D. J., Cairns, B. R., Wright, A. P., and Workman, J. L. (1999) *Mol. Cell* 4, 649–655
50. Steger, D. J., and Workman, J. L. (1997) *EMBO J.* 16, 2463–2472
51. Narlikar, G. J., Phelan, M. L., and Kingston, R. E. (2001) *Mol. Cell* 8, 1219–1239
52. Akamatsu, Y., Monroe, R., Dudley, D. D., Elkin, S. K., Gartner, F., Talukder, S. R., Takahama, Y., Alt, F. W., Bassing, C. H., and Oettinger, M. A. (2003) *Proc. Natl. Acad. Sci. U. S. A.* 100, 1209–1214
53. Kirch, S. A., Rathbun, G. A., and Oettinger, M. A. (1998) *EMBO J.* 17, 4881–4886
54. Liang, H. E., Hsu, L. Y., Cado, D., Cowell, L. G., Kelsee, G., and Schlissel, M. S. (2002) *Immunity* 17, 639–651
55. Callebaut, I., and Moron, J. P. (1998) *Cell Mol. Life Sci.* 54, 880–891
56. Gozani, O., Karuman, P., Jones, D. R., Ivanov, D., Cha, J., Lugovskoy, A. A., Baird, C. L., Zhu, H., Field, S. J., Lessnick, S. L., Villasenor, J., Mehrotra, B., Chen, J., Rao, V. R., Brugge, J. S., Ferguson, C. G., Payrastre, B., Myszka, D. G., Cantley, L. C., Wagner, G., Divecha, N., Prestwich, G. D., and Yuan, J. (2003) *Cell* 114, 99–111
57. Georgel, P. T., Tsukiyama, T., and Wu, C. (1997) *EMBO J.* 16, 4717–4726
58. Guyon, J. R., Narlikar, G. J., Sif, S., and Kingston, R. E. (1999) *Mol. Cell. Biol.* 19, 2688–2697
59. Baumann, M., Mamaia, A., McBlane, F., Xiao, H., and Boyes, J. (2003) *EMBO J.* 22, 5197–5207
60. Cherry, S. R., and Baltimore, D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 10784–10793
61. Anderson, J. D., Lowary, P. T., and Widom, J. (2001) *J. Mol. Biol.* 307, 977–985
62. Tse, C., Sern, T., Wolfe, A. P., and Hansen, J. C. (1998) *Mol. Cell. Biol.* 18, 4629–4638
63. Strahl, B. D., and Allis, C. D. (2000) *Nature* 403, 41–45
64. Jenuwein, T., and Allis, C. D. (2001) *Science* 293, 1074–1080
65. Agalioti, T., Lomvardas, S., Parekh, B., Vie, J., Maniatis, T., and Thanas, D. (2000) *Cell* 103, 667–678
66. Dilworth, F. J., Fremont-Ramain, C., Yamamoto, K., and Chambon, P. (2000) *Mol. Cell* 6, 1049–1058
67. Merika, M., and Thanas, D. (2001) *Curr. Opin. Genet. Dev.* 11, 205–208
68. Agalioti, T., Chen, G., and Thanas, D. (2002) *Cell* 111, 381–392
69. Hassan, A. H., Prochasson, P., Neely, K. E., Galasinski, S. C., Chandy, M., Carrozza, M. J., and Workman, J. L. (2002) *Cell* 111, 369–379
70. Hassan, A. H., Neely, K. E., and Workman, J. L. (2001) *Cell* 104, 817–827