DNA methylation is important for mammalian development and the control of gene expression. Recent data suggest that DNA methylation causes chromatin closure and gene silencing. During development, tissue specifically expressed gene loci become selectively demethylated in the appropriate cell types by poorly understood processes. Locus control regions (LCRs), which are cis-acting elements providing stable, tissue-specific expression to linked transgenes in chromatin, may play a role in tissue-specific DNA demethylation. We studied the methylation status of the LCR for the mouse T-cell receptor αβ locus using a novel assay for scanning large distances of DNA for methylation sites. Tissue-specific functions of this LCR depend largely on two DNase I-hypersensitive site clusters (HS1, HS1 (T-cell receptor α enhancer) and HS1*. We report that these HS induce lymphoid organ-specific DNA demethylation in a region located 3.8 kilobases away with little effect on intervening, methylated DNA. This demethylation is impaired in mice with a germline deletion of the HS1/HS1* clusters. Using 5′-deletion mutants of a transgenic LCR reporter gene construct, we show that HS1* can act in the absence of HS1 to direct this tissue-specific DNA demethylation event. Thus, elements of an LCR can control tissue-specific DNA methylation patterns both in transgenes and inside its native locus.

The process of reversible DNA methylation is highly active during mammalian embryogenesis and somatic cell differentiation. Studies of mice deficient in the DNA methylation machinery have shown that this process is required for successful mouse development (1). Mammalian DNA methyltransferase (2) catalyzes the addition of a methyl group at the 5 position of the cytosine ring in the CpG dinucleotide. The recently identified DNA demethylase catalyzes the reversal of this process (3). Differential methylation of genomic loci is implicated in cancerous transformation, genome stability, and tissue-specific gene expression. The effect of DNA hypermethylation has been implicated in the inactivation of tumor suppressor genes (4–7), and DNA undermethylation has been shown to lead to elevated mutation rates (8). During embryogenesis, a post-fertilization period of DNA demethylation removes the methyl groups from these C residues in the genome, thus erasing the germ cell DNA methylation patterns. Between uterine implantation and gastrulation, methyl groups are added back to the genome. This resets the overall methylation pattern to one that is characteristic of most somatic cells (9, 10). Later in development, DNA demethylation mechanisms remove the methyl groups at specific loci, creating tissue-specific DNA methylation patterns which are proposed to affect gene expression (10, 11).

DNA methylation is thought to regulate gene expression by three possible mechanisms. The first is direct interference with the binding of transcription factors by alteration of C nucleotides in their recognition sequences (12–14). The second mechanism involves the binding of specific factors to methylated DNA which then block the binding of other factors required for gene induction (15–17). The third mechanism proposes that DNA methylation indirectly regulates gene expression via alteration of chromatin structure (11, 18). Recent studies have indicated that the process of histone deacetylation may link the latter two mechanisms. Factors that specifically bind to methylated DNA have been reported to recruit histone deacetylases which act to close chromatin structure, rendering genes inaccessible to transcriptional machinery (19, 20). Thus, the processes late in development that allow the appropriate cell type-specific demethylation of tissue specifically expressed gene loci are important for preventing these genes from being silenced by global repression mechanisms (18, 21). However, only a small handful of tissue-specific demethylation phenomena have been studied (22, 23). Although this process is still poorly understood, evidence from these studies indicates that it may depend on the action of tissue-specific transcriptional control elements. We hypothesized that the action of the locus control region (LCR), which is thought to regulate chromatin structure (24–26), may be involved in establishing tissue-specific DNA methylation patterns. An LCR forms a tissue-specific open chromatin domain for a linked gene regardless of its position in the genome. These LCR induced chromatin changes could aid in directing DNA methylation and demethylation enzyme complexes to specific locations at specific stages in development.

We have studied the LCR for the T cell receptor (TCR) αβ locus. This locus contains the multiple gene segments that are rearranged by the V-D-J recombinase complex to create functional TCRα and TCRβ genes and proteins. These proteins are subunits of the receptors that the αβ and γδ T-cells, respectively, use to recognize foreign antigens (27). The rearrangement and expression of these genes are limited to T cells, which reside mainly in lymphoid organs such as thymus and spleen. Just downstream of these genes is the Dad1 gene, which in contrast to the TCR genes, is ubiquitously expressed (28).

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Thus, strict regulation of the accessibility of this locus in chromatin must be maintained to achieve the differential expression of these juxtaposed genes. The TCRα LCR is located downstream of the TCRα gene and is 3 kilobases (kb) upstream of the Dado1 exons (Fig. 1A). It contains nine DNase I-hypersensitive sites (HS) (29, 30). HS1 maps to the TCRα transcriptional enhancer (31). HS1’ (located less than 1 kb 3’ of HS1) is an important element involved in tissue-specific functions of the LCR (32). HS2–6 are downstream of HS1’ while HS7 and 8 lie 5’ of HS1. This LCR has been found to confer high-level, position-independent, copy number-dependent, and T-cell specific expression to linked transgenes in mice (29, 30). Furthermore, functional subdomains of this LCR have been identified which control its tissue specificity and chromatin opening functions (30). The major tissue-specific functions of this LCR have been mapped to HS1 and HS1’ (32).

We have investigated the DNA methylation status of this LCR using a novel method for scanning large regions of DNA to directly detect methylated CpG dinucleotides. Here we report the identification of a lymphoid organ-specific demethylation event in a localized region of the TCRα LCR. Studies in structural gene knockout animals indicate that this demethylation mechanism operates independently of TCR cell surface expression or gene rearrangement. In contrast, germline deletion of the HS1/HS1’ region of the LCR disrupts this demethylation event which occurs at a site 3.8 kb downstream of HS1’. The HS1’ element of the LCR has previously been shown to have a chromatin based (i.e. non-classical enhancer) activity affecting the tissue distribution of transgene expression (32). Experiments using transgenic mice containing various 5’ deletion mutants of an LCR reporter construct showed that HS1’ can act in the absence of HS1 to direct this novel demethylation mechanism. Thus, elements of an LCR can play a role in establishing tissue-specific DNA methylation patterns.

**EXPERIMENTAL PROCEDURES**

**Mice—**C57BL/6 mice, Rag1 deficient mice (33), and TCRα constant region deficient mice (34) (Jackson Laboratory, Bar Harbor, ME) were used in this study of the endogenous TCRα locus. TCRα enhancer knockout mice (Ea−/−) were described previously and kindly provided by B. P. Sleckman and F. W. Alt, Harvard (35). All immunocompromised mice were housed under strict microisolator conditions. LCR and isoamyl alcohol. After ethanol precipitation, genomic DNA was resuspended in TE (10 mM Tris, 1 mM EDTA) buffer.

**Cleavage with Methylation-sensitive Restriction Enzymes—**15 μg (for the endogenous locus) or 5 μg (for transgenic) genomic DNA were completely digested with MseI restriction enzyme to generate a 1.2-kb parent fragment containing the LCR HS4 region. The LCR HS2 region MseI parent fragment was 700 bp. Samples were ethanol precipitated and resuspended in 10 mM Tris (pH 8.0), and then digested with MspI, HpaII, or HII. Samples were run in 1.2% agarose (Life Technologies, Inc.), and analyzed by Southern blot. For analysis of the HS4 region, a 48-bp single-stranded synthetic oligonucleotide probe recognizing the 3’ end of the HS4 region MseI fragment was used (GCAGCCCAAGCACA- CACTGACAGTGGGAAACATTCTTCCCCAGGGAGAAG). This probe was labeled with [γ-32P]ATP using T4 polynucleotide kinase (New England Biolabs). These blots were washed to a final stringency of 0.1% SDS at 65 °C. For the analysis of HS2 methylation, the blot was probed with a 200-bp NcoI/AvrII fragment that recognized the 3’ end of the parent fragment. These blots were washed to a final stringency of 0.2% SSC, 0.1% SDS at 65 °C. The signals from the parent fragments and subfragments on these Southern blots were quantified by PhosphorImager analysis (Molecular Dynamics). Background signals were obtained for each lane. The background readings were then subtracted from the parent fragment and subfragment signals in the same lane. Percent demethylation was calculated by dividing the subfragment signals by the sum of the parent and subfragment signals. These percentages indicated the extent of demethylation.

**RESULTS**

In addition to the study of the endogenous TCRα LCR, we use a transgenic model for TCRα LCR activity which has been previously described (30, 32). In this system, a human β-globin genomic fragment (24, 36–38) is linked as a reporter gene to the full-length (all 9 HS) LCR to generate the β1–8 transgene (previously described in Ref. 30). The position of the MfeI restriction sites and the probe (used in the following figure) are shown.

**Tissue Differential Methylation Patterns Exist in TCRα LCR DNA—**We initially wanted to determine the general DNA methylation patterns over the length of this LCR in organs that differentially express the genes of the locus. The enzyme

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**FIG. 1. The TCRα/Dad1 locus and transgenes. A, diagram of the endogenous mouse TCRα/Dad1 genomic locus. TCRα constant region and Dad1 exons are shown by dark boxes. Ea is the TCRα transcriptional enhancer. The DNase I-hypersensitive sites of the LCR are shown by the vertical arrows. Transcriptional orientations of the genes of the locus are shown by the horizontal arrows. B, diagram of the parent transgenic LCR reporter construct used in this study. The human β-globin reporter fragment is linked to the nine hypersensitive sites of the LCR to generate the β1–8 transgene (previously described in Ref. 30). The position of the MfeI restriction sites and the probe (used in the following figure) are shown.**
McrBC was used in a novel titration assay similar to the DNase I hypersensitivity assay (39). McrBC is a methylation-dependent restriction enzyme that recognizes a pair of methylated cytosine residues in the sequence 5'-Pu^mC(N_40–2000)Pu^mC-3', and cleaves within 30 bp from one of the methylated residues. Titration of this enzyme onto genomic DNA allows the scanning of large regions of DNA without regard to the presence of particular restriction enzyme recognition sites. The relatively loose sequence specificity allows, in principle, the detection of up to 50% of the CpG dinucleotides occurring in a given DNA sequence. McrBC treatment of isolated genomic DNA generates different fragment sizes depending on the location of methylated residues in the LCR fragment. These cleavage products can be detected by indirect end labeling of a parent restriction fragment of interest by Southern blot. Their positions can also be mapped relative to the fragment end recognized by the labeled probe. We analyzed the TCRα LCR in both endogenous and β1–8 transgene contexts. For our transgenic analysis, high-copy transgenic lines were used. Short exposure of Southern blots to film allows selective detection of the strong signals from transgene cleavage products when compared with the low signals from the endogenous locus. First, parent fragments were generated by restriction enzyme digestion of thymus and kidney genomic DNA from C57BL/6 wild-type mice and full-length LCR reporter construct (β1–8) transgenic mice. Fig. 2A shows the analysis of the transgenic LCR sequences. As increasing amounts of McrBC were added, the parent fragments became less evident, and smaller fragments progressively appeared (marked A-F). The detected McrBC cleavage indicated a high degree of DNA methylation in the TCRα LCR sequences. The cleavage patterns were roughly similar from kidney and thymus (sites A-C and E-F). The only major difference was the stark appearance of a 1.5-kb fragment in the kidney sample (site D). This indicated an area of hypermethylated CpG dinucleotides in kidney DNA which mapped close to the region of HS4 (Fig. 2B). This area appeared selectively unmethylated in thymus DNA. McrBC analysis of spleen and heart DNA from the same animals showed that the HS4 region was hypomethylated in heart DNA, but not in spleen DNA (data not shown). The results obtained from analysis of the endogenous TCRα LCR in the four organs were similar to those seen in the transgenic model (data not shown).

Since differential methylation seemed to be specific to the HS4 region of the LCR, we focused on this area to confirm the results of the McrBC assay with a more widely used method. We utilized restriction enzymes whose cleavage activities were differentially sensitive to DNA methylation. An HS4 region-specific probe was used to detect enzyme cleavage by Southern blot analysis. MspI and HpaII restriction enzymes recognize the same sequence 5'-CCGG-3'. MspI cuts DNA regardless of its methylation status, but HpaII only cuts the sequence if the internal cytosine residue is unmethylated. We also used HhaI, which recognizes the sequence 5'-CCGC-3' and only cuts unmethylated DNA. C57BL/6 genomic DNA from thymus, spleen, kidney, and heart were digested with the MseI restriction enzyme to generate a 1.2-kb parent fragment spanning the HS4 region, and then further digested with MspI, HpaII, or HhaI to assess DNA methylation in the region (Fig. 3A). As expected, treatment with MspI resulted in cleavage of the parent fragment in DNA from all organs (Fig. 3B, top). Treatment with HpaII or HhaI resulted in extensive parent band cleavage in thymus and spleen but not in kidney or heart. The signals present in the parent fragment and enzyme cleavage subfragments (in the HhaI cleavage samples) were quantified by PhosphorImager analysis. After subtracting the lane background signal, the percent demethylation (subfragment signal divided by the sum of the subfragment and parent signals) was calculated and graphed (Fig. 3C). A high percentage indicates relative demethylation in the region, while a low percentage implies methylated DNA. Such PhosphorImager analysis of the C57BL/6 DNA Southern blot showed nearly 100% demethylation in the thymus and spleen and below 30% demethylation in kidney and heart. These same results were reproduced in the 1.5–8 transgenic genomic DNA subjected to the same restriction enzyme treatment (data not shown). Since HpaII and HhaI only cleave unmethylated DNA, this result confirmed that the HS4 region of the TCRα LCR was hypermethylated in non-lymphoid organs, and undermethylated in lymphoid organs.

The HS1/HS1' Region of the LCR Is Involved in Lymphoid-
specific Demethylation—Germline deletion of TCRα enhancer region (HS1 and HS1′) results in a deficiency in TCRα gene rearrangement and transcription (35). To assess the DNA methylation status of this impaired TCRα locus, we obtained the TCRα enhancer knock-out mice and performed the restriction enzyme cleavage assay. Fig. 3B (middle) shows that this mutation resulted in the disruption of the lymphoid organ-specific DNA demethylation mechanism operating on the HS4 region of the endogenous locus. This is evidenced by the persistence of some HS4 region parent fragment equivalently in all organs upon treatment with HpaII and significantly reduced parent fragment cleavage by HhaI. This indicates equivalent partial DNA methylation of this region in all organs rather than the clear tissue-specific patterns seen in the wild-type locus. PhosphorImager analysis confirmed this result (Fig. 3C). These HS1/HS1′ knockout mice also have a defect in thymic development due to the absence of cell surface TCR (34). To determine if the DNA demethylation defect was secondary to impaired thymic maturation, the TCRα constant region knock-out mice were also analyzed. These mice have the identical block in thymic development present in the regulatory region mutant, but have an intact HS1/HS1′ region. The lymphoid organ-specific DNA demethylation activity in these mice was similar to that of the wild type counterpart (Fig. 3B, bottom, and C). This indicated that the mutation of the HS1/HS1′ region was indeed directly involved in the lymphoid organ-specific demethylation of HS4 DNA.

HS4 Region Demethylation Is Local and Occurs Independently of VDJ Recombination—The results from the above studies of mice with mutant TCRα loci raised the possibility that demethylation of the HS4 region DNA may precede the onset of VDJ recombination or gene expression. To confirm this, recombinase-activating gene Rag1 deficient mice were analyzed to determine the requirement of VDJ recombination for the lymphoid organ-specific demethylation activity. The Rag1 gene product is required early in T cell development for TCR gene rearrangement. Thymocytes in these mutant mice are arrested at a very early stage of development (33). Using the same methylation sensitive restriction enzymes used previously, we found that HS4 demethylation occurs normally in these mice (Fig. 3C). PhosphorImager analysis of Southern blots (HhaI samples) of Rag1−/−, in comparison to those experiments shown in Fig. 3B, confirmed that Rag1−/− and wild-type DNA are similar with respect to the methylation status of the HS4 region of the LCR. The only mutation that affects the wild-type DNA demethylation pattern is the Ea−/− deletion. This shows that unrearranged TCRα alleles can undergo demethylation in the lymphoid system. This also suggests that the demethylation mechanism acts early in thymocyte development prior to gene rearrangement.

The results of the McrBC assay strongly suggested that the DNA demethylation event was strictly localized to the HS4 region of the LCR. To confirm this, we analyzed intervening regions of the LCR in wild-type and mutant mice, with MspI, HpaII, and HhaI enzymes (Fig. 4). We found that the HS2 region is heavily methylated in lymphoid organ DNA despite the demethylated state of the neighboring HS4 region. In nonlymphoid organs, HS2 region DNA appears to be partially methylated. In contrast to the strong effect of the Ea−/− mutation on the tissue-specific methylation status of HS4 region DNA, this mutation has only a very minor effect on the wild-

![Fig. 3. Demethylation of the HS4 region is impaired by the Ea−/− mutation.](image-url)
type HS2 DNA methylation pattern in these organs (Fig. 4, B and C). DNA derived from lymphoid organs is still heavily methylated and non-lymphoid organ DNA remains partially methylated.

**HS1** Induces Lymphoid Organ-specific DNA Demethylation in the Absence of HS1—As previously mentioned, two HS clusters are missing from the LCR in HS1/HS1 knockout mice. We employed our transgenic model to determine the contribution of each HS cluster to the control of HS4 region DNA demethylation in lymphoid organs (Fig. 5A). Genomic DNA from mice transgenic for the β1:1–6, β1:1–6, and β2:2–6 constructs were analyzed. As mentioned before, these constructs are the previously described deletion mutants of the parent β1:8 transgene (Fig. 1A) (30, 32). Once again, we used the methylation sensitive restriction enzymes to determine DNA methylation status of the transgenes. As shown in Fig. 5B, the cleavage pattern of the β1:1–6 and β1:1–6 transgenes were generally similar to those of the β1:8 transgene (data not shown) and the endogenous LCR (Fig. 3B, top). These data show that HS7, HS8, and HS1 were not critical for lymphoid organ-specific HS4 demethylation. In contrast, analysis of β2:2–6 transgenic mice showed that the HS4 region parent fragment of this construct was resistant to cleavage by HhaI and HpaII in all four organs analyzed. This indicated that the ability to demethylate this DNA segment in lymphoid organs is lost upon deletion of the HS1 region. This result was confirmed in other independent lines transgenic for the β1:1–6 and β2:2–6 constructs (data not shown). Therefore, the difference in DNA methylation status between these two transgenes is not due to the position of transgene integration. PhosphorImager analysis confirmed these results (Fig. 5c).

**DISCUSSION**

Recent literature suggests that DNA methylation can precede, and indeed may guide the modification of chromatin structure and transcription. Thus, it is important to understand the control elements regulating the differential DNA methylation machinery and its targets. This is especially true for the tissue-specific DNA demethylation events that prevent particular genes from being silenced by global mechanisms in the specific cells in which they should be active. Here we introduce a novel assay for the detection and mapping of sites of DNA methylation. The major existing technique detects methylation indirectly and usually employs frequent cutting restriction enzymes (e.g. HpaII and HhaI). This makes them difficult to use in the analysis of large regions of DNA. We have used an McrBC titration assay to detect methylated CpG dinucleotides over longer stretches of DNA without regard to the presence of particular restriction sites. This assay also enables the detection and mapping of regions of tissue-differential DNA methylation without specific prior knowledge of their likely location. Once a region of difference is found, more traditional assays can be used to examine that particular, and perhaps unexpected region. We used this combination of assays to identify a novel, lymphoid organ-specific DNA demethylation activity that operates in a region of the TCRα/Dad1 locus. Furthermore, we demonstrate that a distant cis-acting element of the LCR is involved in the control of this tissue-specific demethylation both in transgenic constructs as well as in its endogenous locus.

**Elements Controlling Specific DNA Methylation Status**—Other cis-acting elements affecting DNA methylation have been previously identified in only a few gene loci. Transgenic models have been used to detect an exogenous DNA element targeting a transgene locus for methylation by the mouse strain-specific methylation-modifier gene product, Ssm1 (40). Using transient reporter gene transfection assays and cultured cell lines, cis-acting elements controlling DNA demethylation events have been described in the rat α-actin promoter (22) and in the immunoglobulin κ light chain enhancer (Iκκ) (23, 41).
The Igκ demethylation was recently confirmed in vivo in B-cells of transgenic mice. It appears to occur in a mono-allelic fashion prior to VDJ recombination of the κ locus early in B-cell development (42). The TCRα HS4 region demethylation described here also seems to occur prior to VDJ recombination of the TCRα/δ locus. Rag1−/− mice are not able to carry out VDJ recombination and only contain immature lymphocytes in their lymphoid organs. Yet, HS4 is still demethylated in these immature cells. The HS4 demethylation seen in thymus and spleen DNA from wild-type mice is complete and, therefore, is most likely not mono-allelic. Unlike the Igκ locus, VDJ recombination at the TCRα/δ locus does not display allelic exclusion (43, 44). As the Igκ demethylation is postulated to have a role in allelic exclusion, mono-allelic demethylation would not be expected in the TCRα/δ locus. Furthermore, the complete demethylation of HS4 seen in spleen DNA indicates that this process occurs in both B-cells (70–75% of splenocytes) and T-cells (25–30% of splenocytes). Similar complete demethylation of this region occurs in Rag1−/deficient spleen. These data further suggest that HS4 demethylation may also occur in non-lymphoid hematopoietic cell types (e.g. monocytes and granulocytes). These blood cell populations can, variably, make up as much as 50% of a Rag1−/ or Rag2-deficient spleen (33, 45). Further study of these populations in wild-type mice is necessary to confirm this possibility. Nevertheless, demethylation of HS4 DNA seems to occur at an early stage either in lymphoid precursors, or possibly even earlier in hematopoietic development.

HS1′ can control the lymphoid-specific demethylation of HS4 region DNA, yet it is present in the nuclei of all organs examined (28, 30, 32). This is consistent with our previous data demonstrating an important role for HS1′ in tissue-specific functions of the LCR. The data we present here add strength to the hypothesis that distinct sets of nuclear factors act via functions of the LCR. The data we present here add strength to the hypothesis that distinct sets of nuclear factors act via LCR hypersensitive sites linked to the human β-globin reporter gene. B, methylation status analysis of HS4 region of transgenic constructs β1–6 (22 copies, top), β1′–6 (42 copies-middle), and β2–6 (28 copies, bottom) in mice using methylation-sensitive restriction enzymes. Positions of the 1.2-kb parent fragments and 0.2–0.3-kb subfragments are indicated. β1–6 and β1′–6 methylation patterns are similar to that of wild type C57BL/6 (Fig. 3, top) and that of β1–8 transgenic mice, while the pattern of β2–6 transgenic mice is not. C, PhosphorImager analysis of the HhaI lanes of the above Southern blots. Percent demethylation is calculated as described under “Experimental Procedures.” T, thymus (stippled bars); S, spleen (striped bars); K, kidney (solid bars); H, heart (open bars).
It is important to point out that the impaired demethylation of HS4 DNA that we observe in HS1/HS1’ knockout animals is unlikely to be the indirect result of DNA methylation which follows the inactivation of a locus. This mutant locus is not transcriptionally silent. Reduced transcription still occurs at both TCRα and TCRδ genes and Dad1 transcription is unaffected by the mutation (35). Similarly, the removal of HS1 and HS1’ from transgenic constructs (to create β2–6) does not render the transgene inactive (30), but prevents its demethylation nonetheless.

It is remarkable that the DNA demethylation we describe is strictly localized to the HS4 region of the LCR. The tissue-specific elements of the LCR seem to be able to demethylate this particular region 3.8 kb downstream with little effect on the methylation status of the intervening DNA. This is different from the more global demethylation of transfected constructs that was described in the Igκ gene system. These data point both to a unique mechanism of targeted demethylation and a potential role of HS4 and its demethylation in LCR activity. Our previous data have shown that the fragment of the locus.

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REFERENCES

1. Li, E., Bestor, T. H., and Jaenisch, R. (1992) Cell 69, 915–926
2. Bestor, T., Laudano, A., Mattaliano, R., and Ingram, V. (1988) J. Mol. Biol. 203, 971–983
3. Bhattacharya, S. K., Ramechandani, S., Cervoni, N., and Szyf, M. (1999) Nature Genet. 23, 579–583
4. Gonzalez-Zulueta, M., Bender, C. M., Yang, A. S., Nguyen, T., Beart, R. W., Turnout, J. M. V., and Jones, P. A. (1995) Cancer Res. 55, 4531–4535
5. Foster, S. A., Wong, D. J., Barrett, M. T., and Galloway, D. A. (1998) Mol. Cell. Biol. 18, 1793–1801
6. Herman, J. G., Jen, J., Merlo, A., and Baylin, S. B. (1996) Cancer Res. 56, 722–727
7. Robertson, K. D., and Jones, P. A. (1998) Mol. Cell. Biol. 18, 6457–6473
8. Chen, R. Z., Pettersson, U., Beard, C., Jackson-Grusby, L., and Jaenisch, R. (1998) Nature 395, 89–93
9. Kafri, T., Ariel, M., Brundell, M., Shemer, R., Urven, L., McCarrey, J., Cedar, H., and Razin, A. (1992) Genes Dev. 6, 705–714
10. Jaenisch, R. (1997) Trends Genet. 13, 323–329
11. Eden, S., and Cedar, H. (1994) Curr. Opin. Genet. Dev. 4, 255–259
12. Bednarik, D. P., Ductet, C., Kim, S. U., Perez, V. L., Griffis, K., Guenther, P. C., and Fulks, T. M. (1991) New Biol. 3, 969–976
13. Comb, M., and Goodman, H. M. (1990) Nucleic Acids Res. 18, 3975–3982
14. Watt, F., and Molloy, P. L. (1988) Genes Dev. 2, 1136–1143
15. Kudo, S. (1998) Mol. Cell. Biol. 18, 5492–5499
16. Boys, J., and Bird, A. (1991) Cell 64, 1123–1134
17. Antequera, F., Macleod, D., and Bird, A. P. (1989) Cell 56, 509–517
18. Razin, A. (1996) EMBO J. 17, 4955–4968
19. Nan, X., Ng, H.-H., Johnson, C. A., Laherty, C. D., Turner, B. M., Eisenman, R. N., and Bird, A. (1998) Nature 393, 386–389
20. Jones, P. L., Veenstra, G. J. C., Wade, P. A., Vermaak, D., Kass, S. U., Landsberger, N., Strubbe, J. L., and Wolfe, A. P. (1998) Nat. Genet. 19, 187–191
21. Siegfried, Z., and Cedar, H. (1997) Curr. Op. Biol. 7, R205–R207
22. Paroush, Z., Keshek, I., Yanai, J., and Cedar, H. (1996) Cell 63, 1229–1237
23. Lichtenstein, M., Keini, U., Cedar, H., and Bergman, Y. (1994) Cell 76, 913–923
24. Grosveld, F., van Assendelft, G. B. G., Greaves, D. R., and Kollia, G. (1987) Cell 51, 975–985
25. Fraser, P., and Grosveld, F. (1998) Curr. Opin. Cell Biol. 10, 361–365
26. Kioussis, D., and Festenstein, R. (1997) Curr. Opin. Genet. Dev. 7, 614–619
27. Janzen, C. A., and Transvers, P. (1994) Immunobiology: The Immune System in Health and Disease, Current Biology Ltd/Garland Publishing Inc., London
28. Hong, N. A., Cado, D., Mitchell, J., Ortiz, B. D., Hsieh, S. H., and Winoto, A. (1997) Mol. Cell. Biol. 17, 2151–2157
29. Diaz, P. C., and Winoto, A. (1994) Immunity 1, 207–217
30. Ortiz, B. D., Cado, D., Chen, Y., Diaz, P. W., and Winoto, A. (1997) EMBO J. 16, 5037–5045
31. Winoto, A., and Baltimore, D. (1989) EMBO J. 8, 729–734
32. Ortiz, B. D., Cado, D., and Winoto, A. (1999) Mol. Cell. Biol. 19, 1901–1909
33. Mombaerts, P., Iacomini, J., Johnson, R. S., Herrup, K., Tonegawa, S., and Papaiannou, V. E. (1992) Cell 68, 869–877
34. Mombaerts, P., Clarke, A. R., Rudnicki, M. A., Iacomini, J., Itahara, S., Lafaille, J. J., Wang, L., Ichikawa, Y., Jaenisch, R., Hooper, M. L., and Tonegawa, S. (1992) Nature 360, 225–231
35. Sleckman, B. P., Borden, C. G., Ferrini, R., Davidson, L., and Alt, F. W. (1997) Immunity 7, 505–515
36. Chada, K., Magram, J., Raphael, K., Radice, G., Lucy, E., and Costantini, F. (1985) Nature 314, 377–380
37. Townes, T. M., Lingrel, J. B., Chen, H. Y., Brinster, R. L., and Palmer, R. D. (1985) EMBO J. 4, 1715–1723
38. Greaves, D. R., Wilson, F. D., Lang, G., and Kioussis, D. (1989) Cell 56, 979–986
39. Wu, C. (1989) Methods Enzymol. 176, 269–289
40. Engler, P., Doglio, L. T., Bozek, G., and Storb, U. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 10763–10768
41. Kirillov, A., Kistler, B., Mostoslavsky, R., Cedar, H., Wirth, T., and Bergman, Y. (1998) Genes Dev. 12, 1801–1811
42. Sleckman, B. P., Khor, B., Monroe, R., and Alt, F. W. (1998) J. Exp. Med. 188, 1465–1471
43. Padevan, E., Casorati, G., Dellabona, P., Meyer, S., Brookhaus, M., and Lanuzzaeacchia, A. (1995) Science 262, 422–424
44. Shinaki, Y., Rathbun, G., Lam, K.-P., Oltz, E. M., Stewart, V., Mendlosh, M., Charron, J., Datta, M., Young, F., Stall, A. M., and Alt, F. W. (1992) Cell 68, 855–867
45. Weng, A., Engler, P., and Storb, U. (1995) Mol. Cell. Biol. 15, 572–579
46. Bestor, T. H. (1998) Nature 393, 311–312
47. Selker, E. U. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9430–9435