The Sca-1 antibody recognizes antigens encoded by members of the Ly-6 multigene family. These antigens are expressed on fetal and adult hematopoietic stem cells, progenitor cells, mature activated T cells, and some nonhematopoietic cells and are most likely encoded by the Ly-6E.1 and Ly-6A.2 genes. Characterization and isolation of regulatory elements of Ly-6E.1 and A.2 genes that govern tissue-specific and high levels of expression in the cells of the hematopoietic system (particularly stem cells) are of considerable interest. To characterize the control elements of this gene, we have cloned a 30-kb fragment encoding a fully functional Ly-6E.1 gene and 13 kb of 5' and 13 kb of 3' flanking sequence. Transfection studies in murine erythroleukemia (MEL) cells show that a 14-kb BamHI fragment from this clone is sufficient to confer Ly-6E.1 gene expression at levels equivalent to those of the endogenous gene. By mapping regions of chromatin sensitive to DNase I digestion, we have located hypersensitive sites in the 5' and 3' regions of the gene in FDCP-1 cells, MEL cells, and various T-cell lines. The appearance of two 5' hypersensitive sites in hematopoietic cells correlates with Ly-6E.1 expression after γ-interferon induction. We show that the presence of hypersensitive sites in the 5' and 3' regions corresponds to Sca-1 expression, and we also discuss the localization of putative regulatory control elements.

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pression in transfected murine erythroleukemia (MEL) cells. We discuss the role of such sites in Ly-6E.1 and A.2 gene expression during differentiation of the hematopoietic system.

MATERIALS AND METHODS

Cloning and characterization of genomic Ly-6E.1. High-molecular-weight DNA was prepared from BALB/c mouse liver and partially digested with MboI to produce fragments averaging between 30 kb to 50 kb in size, which were separated by NaCl gradient centrifugation as described by DiLeLLa and Wooton.26 The optimal sized fraction was ligated into the Bgl II site of cosmids pLTC, a modified pTCF cosmid27 with a Xho I site replacing the Hpa I site. Cosmid packaging was performed using Gigapack Gold II (Stratagene, La Jolla, CA). The library was plated on Genescreen Plus filters (DuPont, Wilmington, DE) and were screened28 with an oligolabeled Ly-6E.1 cDNA probe in the presence of 1 μg/mL competitive mouse and 5 μg/mL Escherichia coli and vector DNA. Positive clones were characterized by restriction digest and Southern blot analysis.

Polymerase chain reaction (PCR) primers specific for exon 4 (5' GTCCAGGTTGCTCGCTATT 3' and 5' GGAAGCTCTGGTTGTCCTCTGCG 3') were designed to specifically amplify a 214-bp fragment from the cosmid clone cA3.1. Reactions were performed as described by Innis et al.29 Sequence analysis was performed directly on the PCR products using a dyeoxynucleotide termination Sequenase kit (US Biochemical Corp., Cleveland, OH).

DNase I hypersensitive site mapping. A variety of hematopoietic cell lines; TM10G,29 BW5147, EL4, FDCP-1,30 and MEL cells were prepared for DNase I treatment as described by Pfeifer and Riggs.31 Approximately 1 × 10^6 cells were permeabilized with 250 μg of lysolethlin, and 100 μL aliquots of permeabilized cells were treated with increasing concentrations of DNase I (Boehringer Mannheim, Mannheim, Germany), from 0.8 to 120 U, for 10 minutes at 37°C. Reactions were stopped with an EDTA-containing buffer, and samples were treated with 50 μg RNase at 37°C for 1 hour and overnight at 55°C with 0.15 mg Proteinase K and were extracted twice with phenol/chloroform before ethanol precipitation. A total of 10 μg of purified DNA was digested with BamHI or Hpa I, Southern blotted, and probed with oligolabeled radioactive probes. Stringency conditions were increased to 2X SSC in the hybridization mix, with final washing of the filters performed at 0.1X SSC/0.1% sodium dodecyl sulfate at 65°C to reduce cross-hybridization of the probes to related genomic sequences. Quantitation of some hybridizing bands was performed on a Molecular Dynamics PhosphorImager (Kemsing, Seven Oaks, Kent, UK).

Probes used to detect hypersensitive sites were derived from cosmid clone cA3.1. The 5' hypersensitive sites were detected using probes BA1 (BamHI-Asp718 300-bp fragment) and NB1 (a 813-bp Nco I-Bgl II intron 3 fragment; see Fig 3), with the 3' hypersensitive sites detected using probes 4.3 (300-bp Hpa I fragment) and Ex4 (Bgl II-EcoRI 595-bp fragment; see Fig 4) on BamHI- and Hpa I-restricted DNase I-treated DNA. These probes were found to contain no highly repetitive sequences.

Ly-6E.1 cell transfection and fluorescence-activated cell sorter (FACS) analysis. Plasmid pAB14neo, containing a 14-kb BamHI fragment (see Fig 1), encoding the Ly-6E.1 gene from cosmid clone cA3.1, was constructed in a modified pUC19 vector for transfection analysis. The plasmid contains a 1-kb Xho I and Sal I fragment from pMC1Neo (Stratagene), encoding the neomycin resistance gene under control of a thymidine kinase promoter, for the selection of transfected clones. A total of 50 μg of linearized pAB14neo was electroporated into MEL cells, and transfected cells were selected with 800 μg/mL G418 for 14 days. Resulting clones were expanded and induced with 250 U/mL recombinant γ-IFN (Genzyme, Boston, MA) for 48 hours and analyzed for transfected Ly-6E.1 gene expression by FACS analysis. Clones ranged from 1 to 26 copies of the transfected gene. Copy number was determined by probing 10 μg of EcoRI-digested genomic DNA with the Ly-6E.1 cDNA probe and quantitation on the Molecular Dynamics PhosphorImager using the Ly-6A.2 gene as an endogenous control.

MoAb Sca-1,32 which is specific for Ly-6A.2 and Ly-6E.1, was used to detect expression on FDCP-1, MEL, and T-cell lines. MoAbs from hybridomas SK70.9433 and S8.10634 were used to detect Ly-6E.1-transfected gene expression and endogenous Ly-6A.2 expression, respectively. Antimouse IgG-PE (Biogenesis, Bourne mouth, UK) was used as a second antibody for detection of specific antibody binding. Stained cells were analyzed by a FACScan cell sorter (Becton Dickinson, Mountain View, CA).

RESULTS

Cloning and characterization of the Ly-6E.1 gene. To identify control regions of the Ly-6E.1 and A.2 alleles important for expression in hematopoietic cells, we sought to isolate a large genomic fragment containing flanking regions that we could use as probes for DNase I-hypersensitive site mapping. We chose to isolate the Ly-6E.1 allele because most hematopoietic cell lines and useful mouse strains contain and express the Ly-6A.2 gene and allele-specific MoAbs can be used for the differential detection of Ly-6E.1 gene expression after transfection. Also, because expression patterns differ and restriction site polymorphisms exist between Ly-6A.2 and Ly-6E.1, we sought to investigate the effects of sequence variations between the alleles. Previously, no 3' sequence of the Ly-6E.1 has been cloned. Therefore, to isolate a full-length Ly-6E.1 genomic clone, a mouse library was constructed in the pLTC cosmid vector from BALB/c large molecular weight DNA. The Ly-6E.1 cDNA was used to screen 1 × 10^9 colonies and resulted in 30 positive clones. Clone cA3.1 was found to be the most strongly hybridizing cosmid. Southern blot analysis showed that this clone (Fig 1A) has a similar restriction map when compared with the restriction map of the previously cloned 5' portion of the Ly-6E.1 gene (Fig 1B) and the complete Ly-6A.2 gene (Fig 1C). Furthermore, PCR nucleotide-sequence analysis of a 214-bp fragment in exon 4 confirmed that clone cA3.1 contains the correct coding region for Ly-6E.1 and not for a pseudogene or related gene.

Restriction-site mapping showed that the cA3.1 clone contains a 30-kb insert, with 13 kb of 5' and 13 kb of 3' sequence flanking the Ly-6E.1 gene. This mapping also showed previously unreported restriction fragment-length polymorphisms between the Ly-6A.2 and E.1 alleles. A comparison of clone cA3.1 with the Ly-6E.1 and A.2 fusion-gene construct shows considerable HindIII polymorphisms between these clones in the 3' region, whereas none are found with the Ly-6A.2 gene. In addition to the EcoRI polymorphism in the first intron of the Ly-6A.2 gene,16 we have found BamHI-site polymorphisms in the 5' and 3' regions of the Ly-6E.1 gene (Fig 1). A Ly-6-related sequence was found to be present 10 kb downstream of the Ly-6E.1 gene by hybridization with the Ly-6E.1 cDNA. We have mapped this homologous sequence to a region further downstream
of the Ly-6E.1 gene, when compared with the Ly-6A.2 gene. This gene is in the same transcriptional orientation compared with the Ly-6E.1 gene; however, it is not known if it is transcriptionally active.

To confirm that clone cA3.1 encodes a functional gene, we analyzed Ly-6E.1 expression in transfected MEL cells. MEL cells contain an endogenous Ly-6A.2 allele and are normally negative for Sca-1 expression. However, Sca-1 expression is inducible in addition of γ-IFN. A 14-kb BamHI fragment of the Ly-6E.1 gene was cotransfected with a neo expression cassette into MEL cells. G418-resistant cells were isolated and examined for Ly-6E.1 and endogenous Ly-6A.2 expression by FACS using the S8.106 (A-specific) and SK70.94 (E-specific) antibodies before and after induction with γ-IFN. The expression pattern was analyzed in a population of transfected cells and in 8 clones. Low levels of basal expression of the transfected gene were observed with anti-Ly-6E.1 and Sca-1 antibodies. On γ-IFN induction, expression of the transfected Ly-6E.1 gene was comparable or higher than endogenous Ly-6A.2 gene expression. As shown in Fig 2, a representative clone, containing 5 copies of the transfected gene, expressed Ly-6E.1 at low basal levels and high levels after γ-IFN induction. Expression of the Ly-6E.1-transfected gene was found to slightly exceed that of the endogenous Ly-6A.2 gene and is probably the result of multiple copy expression. Thus, regulatory elements necessary for γ-IFN-induced and high-level expression in hematopoietic cells are present in the transfected 14-kb BamHI Ly-6E.1 gene fragment.

DNase I hypersensitive sites are present in the 5' and 3' regions of the endogenous Ly-6A.2 gene. To determine which regions of the Ly-6A.2 gene may be necessary for expression in hematopoietic cells, we mapped DNase I hypersensitive sites in the flanking regions of the endogenous gene using probes from the Ly-6E.1 genomic clone. Because the multigene family consists of at least 18 cross-hybridizing members, we isolated probes from the genomic clone that were most specific for the detection of the Ly-6A.2 gene. Also, to ensure that we could unequivocally identify specific DNase I hypersensitive fragments of the gene, we mapped each site with 2 independent probes and 2 different restriction digests. BA1 and NBI probes were used to detect hypersensitive sites in the 5' end of the gene (Fig 3), and probes Ex4 and 4.3 were used to detect hypersensitive sites in the 3' region of the gene (Fig 4).

Ly-6E.1 and A.2 are known to be expressed in activated T cells and on early double-negative thymocytes. Therefore, three T-cell lines containing an endogenous Ly-6A.2 gene were tested for expression using the Sca-1 antibody and were mapped for DNase I hypersensitive sites within the Ly-6A.2 chromatin; TM10G, a CD4 and CD8 double-positive early thymocyte was Sca-1 negative; BW5147, a mature T cell was Sca-1 positive and; EL4, a CD4 single-positive mature T cell was Sca-1 low-expressing. Figure 3 shows the mapping of the 5' DNase I hypersensitive sites in these cells. Probe NBI detected the parental band at 4.2 kb after digestion with Hpa I. On DNase I digestion, a 3.4-kb subfragment (HS-1.2) was observed only in the expressing cell lines (BW5147 and EL4), and a band at 2.3 kb (HS-0.1) in the three T-cell lines (Fig 3A). The intensity of these bands is in inverse correlation with hybridization to the parental band. Because of the cross-hybridization of the probe to related sequences within the Ly-6 multigene family (16, 12, 10, 8, 3.8, 2.1, 1.8, and 1.2 kb), it was necessary to confirm that the
bands observed after DNase I digestion were Ly-6A.2-related and were not subfragments from another family member. Therefore, the same DNA was digested with BamHI and hybridized with the more specific BA1 probe (Fig 3B). BA1 was found to hybridize with a subfragment of 2.3 kb (HS-1.2) only in Sca-1-expressing cell lines (BW5147 and EL4) and with a 3.4-kb (HS-0.1) band in all three T-cell lines (Fig 3B). Again, the appearance of these bands is in inverse correlation with the parental 14-kb Ly-6A.2 BamHI fragment and not with the single cross-hybridizing family member at 7.2 kb. We also observed an 8.4-kb fragment (indicative of a 3' hypersensitive site, HS + 4.9) in all three T-cell lines. As shown in Fig 3, hypersensitive sites mapped from opposite directions give rise to fragments that colocalize at -1.2 kb and approximately at -0.1 kb 5' to the transcriptional start site. Also, the 8.4-kb fragment was localized to a site at +4.9 kb downstream of the transcriptional start.

Because hypersensitive sites have been found in the 3' regions of other genes expressed in the hematopoietic cells (eg, CD2), we sought to confirm the HS + 4.9 site and to
identify other hypersensitive sites in the 3' flanking sequence of the Ly-6A.2 gene. Treated DNA was digested with Hpa I and probed with Ex4. This probe hybridized most strongly to the parental fragment at 6.4 kb and very strongly to a 2.6-kb subfragment (HS + 4.9) in all three T-cell lines, more weakly to a 1.7-kb subfragment (HS + 4.0) close to the 3' end of the gene only in high expressing BW5147 cells and weakly to a 1-kb fragment (HS + 3.3) in TM10G only (Fig 4A). Cross-hybridizing bands from family members were also detected at 16, 12, 10, 8, 2.3, and 1.8 kb. When the same DNA was hybridized with a more specific probe, 4.3, from the opposite direction after BamHI digestion, a 5.7-kb band (HS + 4.9) appeared in all three T-cell lines (Fig 4B). However additional 5-kb (HS + 5.6), 3.9-kb (HS + 6.7), 1.9-kb (HS + 8.7), and 1.7-kb (HS + 8.9) bands were seen only in Sca-1 expressing BW5147 and EL4 cells. The site of intense DNase I hypersensitivity colocalizes to a sequence at HS + 4.9. Although some sites were mapped in only one direction, they are derived from the Ly-6A.2 gene because the subfragments only appear in in-
HSS MAPPING OF THE LY-6A.2 AND E.1 GENES

Fig 4. Mapping of 3’ DNase I hypersensitive sites of Ly-6A.2. The same DNA from DNase I-treated TM10G (Sca-1 negative), BW5147 (Sca-1 positive), and EL4 (Sca-1 low) as shown in Fig 3, was digested with restriction enzymes, blotted, and hybridized with probes from the Ly-6E.1 gene. Hypersensitive sites were again mapped in both directions to confirm the localization of these sites to the Ly-6A.2 gene. The exons of Ly-6A.2 are represented by shaded boxes, and the hypersensitive sites seen are depicted with arrows. Sites are numbered with respect to their location in kilobases from the transcriptional start site. (A) Probe Ex4 hybridized to Hpa I-restricted DNA is shown. The map depicts the parental fragment size after Hpa I (H) digestion, the site of probe Ex4, and the resulting 3’ subfragments observed after DNase I digestion. (B) Probe 4.3 hybridized to BamHI (B)-restricted DNA confirms the regions of 3’ DNase I hypersensitivity and identifies further 3’ hypersensitive sites. The map shows the parental fragment size and the resulting sizes of the subfragments after DNase I digestion.

verse correlation to the parental fragment (14.0 kb) and because the 4.3 probe cross-hybridizes weakly to only one other fragment (7.0 kb) from a related family member.

To estimate the strength of hypersensitivity, we reprobed one of the filters with a probe specific for Thy-1. Hypersensitive sites for the Thy-1.2 locus have been previously mapped.25 Using a Thy-1 probe, we confirmed the reported site within intron 3. We found that the intensity and appearance of hypersensitive sites in the Ly-6A.2 gene correspond well to those of the thymocyte-specific site in intron 3 of the Thy-1 gene (data not shown).

DNase I hypersensitive sites are found in other hematopoietic cells. In addition to certain T-cell subsets, Sca-1 has been found to be expressed on the surface of hematopoietic stem cells and erythroid progenitors. Therefore, to determine if differential sensitivity exists between hematopoietic lineages, we performed DNase I mapping on Sca-1-positive FDCP-1 cells (multipotential cell line) and on Sca-1-negative...
tive MEL cells (erythroblast cell line). Both cell lines contain an endogenous Ly-6A.2 gene. As shown in Fig 5, both 5' hypersensitive sites (HS-0.1 and HS-1.2) were found in the FDCP-1 cell line when probed with the NBI fragment. Mapping with the BA1 probe verified these sites (not shown). In the region 3' to the gene, we found the strong HS + 4.9 site in the FDCP-1 cells, as we did in the T-cell lines with the Ex4 probe. In addition, weak HS + 4.0 and HS + 3.3 sites were found (very faint in Fig 5). The 4.3 probe verified the HS + 4.9 site and showed the presence of further 3' sites; HS + 8.7 and HS + 8.9 (not shown).

When mapping was performed on MEL-cell DNA (not shown), neither the HS-1.2 or HS-0.1 hypersensitive sites were found in the 5' flanking regions using the NBI and BA1 probes. The 3' probes showed only the strong HS + 4.9 site and weak site at HS + 3.3. Thus, the appearance of certain hypersensitive sites in hematopoietic cells strongly correlates with Sca-1 expression as observed by FACS analysis when the DNase I maps of various cell lines are compared.

**DNase I hypersensitive site mapping in transfected MEL cells.** To firmly establish that we were mapping hypersensitive sites specific to the Ly-6A.2 and E.1 alleles and not to other homologous Ly-6 genes, we analyzed DNase I hypersensitivity in MEL cells transfected with the 14-kb BamHI Ly-6E.1-gene fragment. DNA was isolated from γ-IFN induced and uninduced transfected MEL-cell clones containing 5 (Fig 6) and 20 (data not shown) copies of the transfected gene and was tested for 5' and 3' DNase I hypersensitive sites. Figure 6A shows the enhanced appearance of the HS-0.1 site in the induced cells after Hpa I digestion and hybridization with probe NB1. The HS-1.2 site, which is within the region previously thought to harbor a γ-IFN-responsive element, is absent in uninduced cells and cells induced for 48 hours. However, the HS-1.2 site was found to be present transiently 12 hours after induction (data not shown). The 4.2-kb parental band is the strongest hybridizing band, thus confirming that the Ly-6E.1 gene and not related family members are responsible for the production of DNase I-sensitive subfragments. The mapping of these sites was confirmed using BamHI-digested DNA probed with BA1 (not shown). These sites correspond to the 5' hypersensitive sites mapped in the Ly-6A.2 gene in untransfected cells (Figs 3 and 5).

When the 3' hypersensitive sites were examined with probe Ex4 after Hpa I restriction, the HS + 3.3 and HS + 4.9 sites were present in both uninduced and induced MEL cells (Fig 6B). However, whereas the sensitivity of the HS + 4.9 site remained constant, the sensitivity of the HS + 3.3 site increased by a factor of 6 in γ-IFN-induced cells, as determined by phosphorimaging. Also, site HS + 5.6 appeared after γ-IFN induction and may be faintly present in uninduced cells. The intensity of the parental 6.4-kb band of the transfected gene confirms the origins of the DNase I-generated subfragments. These data were confirmed with probe 4.3 on DNA after BamHI digestion (data not shown). With probe 4.3, sites at HS + 5.6, HS + 6.7, HS + 8.7, and HS + 8.9 were found in uninduced cells. Only the HS + 5.6, HS + 8.7, and HS + 8.9 sites increased in hypersensitivity in the induced MEL clones. Therefore, the sites at HS-0.1, HS-1.2, HS + 3.3, HS + 5.6, HS + 8.7, and HS + 8.9 appear to be affected and/or enhanced by γ-IFN induction of the Ly-6E.1 gene in the transfected MEL clones. This enhancement correlates well with the high levels of transfected Ly-6E.1 gene expression and confirms the mapping of these sites in the Ly-6A.2 gene (Figs 4 and 5) in untransfected cell lines. The intensity of site HS + 4.9 is unchanged in induced and uninduced cells and suggests that this site may be im-
important in transcriptional competence of the gene rather than dependent on active transcription.

DISCUSSION

We have reported here the cloning of the complete Ly-6E.1 gene and have compared the cosmid map with a chimeric Ly-6E.1 and Ly-6A.2 fusion gene and a genomic Ly-6A.2 fragment (Fig 1). We find considerably more polymorphisms between the fusion gene and Ly-6E.1 than between the complete Ly-6A.2 and Ly-6E.1 alleles. These differences appear to represent deletions that may have occurred during the construction of the fusion gene. Our mapping of the 3' region of the Ly-6E.1 genomic fragment further verifies the allelic relationship of Ly-6E.1 and Ly-6A.2 genes.

More importantly, we have shown high level, γ-IFN-induced expression of a 14-kb BamHI Ly-6E.1--gene fragment. We have identified putative hematopoietic cell and


γ-IFN–specific regulatory elements in the 5' and 3' flanking regions of both the Ly-6E.1 and A.2 genes by extensive DNase I hypersensitive site mapping in the chromatin of a variety of Ly-6E.1 and A.2 expressing and nonexpressing cell lines. A summary of our hypersensitive site mapping data performed in two directions with specific probes from the Ly-6E.1 clone is shown in Fig 7.

Analysis of the promoter region for susceptibility to DNase I digestion has identified sites HS-1.2 and HS-0.1, which appear in all expressing cell lines (FDCP-1, BW5147, and EL4) and in γ-IFN-induced MEL clones (12-hour induction). Neither site is found in nonexpressing MEL cells, with site HS-0.1 only weakly observed in TM10G. Interestingly, there are no complete consensus IFN-responsive elements in the Ly-6E.1 promoter. However, three CCAAT boxes are located in the region between −1.7 kb and −0.9 kb with two located at site HS-1.2. Because CCAAT box-binding proteins have been reported to be involved in the IFN inducibility of murine class-II major histocompatibility complex (MHC) Eα-chain gene, it may be possible that these CCAAT boxes are vital to the IFN inducibility of the Ly-6E.1 gene. Also, it is interesting to note that a CCAAT-box motif was found within the region of the HS-0.1 hypersensitive site. This CCAAT box and a purine-rich element within a region of 138 bp that has been shown to be within the minimal sequence necessary for constitutive expression in fibroblasts. Therefore, the appearance of the hypersensitive sites in Ly-6E.1- and A.2-expressing cells within regions containing CCAAT boxes and of the γ-IFN-inducible sites in MEL clones suggest that CCAAT box-binding proteins may be important transcription factors involved in Ly-6E.1 and A.2 gene regulation within the hematopoietic system. In addition, the consensus AAGTGA motif which binds IFN regulatory factor 1, a partial conserved regulatory element (CRE) consensus sequence and a partial IFN consensus sequence of MHC class-I genes have been found at HS-1.2. These putative factor-binding sites may also be important in the transcriptional regulation of Ly-6E.1 and A.2 genes within lymphocytes and hematopoietic progenitor cells.

We have shown that the 3' regions of the Ly-6E.1 and A.2 genes also contain hypersensitive sites. These sites are located at HS + 3.3, HS + 4.9, HS + 5.6, HS + 6.7, HS + 8.7, and HS + 8.9 (Figs 4 through 7). In addition, another site at HS + 4.0 was observed in the chromatin of the mature high-expressing T-cell line, BW5147. The appearance of the sites at HS + 5.6, HS + 6.7, HS + 8.7, and HS + 8.9 specifically in Ly-6E.1 and A.2-expressing cells suggests that these downstream elements are involved in gene regulation. Furthermore, γ-IFN–induced expression of Ly-6E.1 in transfected MEL cells corresponds with the appearance and/or enhancement of sensitivity at sites HS + 3.3, HS + 5.6, HS + 8.7, and HS + 8.9. The appearance of DNase I-hypersensitive sites in the chromatin of MEL clones containing multiple copies of the Ly-6E.1 gene confirms our endogenous-gene hypersensitive-site mapping. Several investigators have reported 3' enhancer elements and other distally located strong transcriptional-control elements (LCRs) in the

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**Fig 7.** Diagram shows 5' and 3' hypersensitive sites of the Ly-6E.1 and Ly-6A.2 genes in hematopoietic cells. Arrows mark the regions that show hypersensitivity to DNase I digestion and are numbered according to their location from the transcriptional start site. Exons are represented by shaded boxes, and restriction sites HpaI (H) and BamHI (B) are indicated. The degree of hypersensitivity is represented by the boldness of the arrow, with dashed arrows indicating weak sites. All cell lines are untransfected and uninduced. *Indicates the MEL clone–transfected and γ-IFN–induced in Figs 2 and 6. $Indicates the transient HS-1.2 site after 12 hours γ-IFN induction of the transfected MEL clone. The sites for T-cell line EL4 are not shown but correspond to those seen in BW5147, except for the absence of site HS + 4.0.
chromatin of genes of the hematopoietic system. These elements are absolutely required for high levels of tissue-specific expression. It will be interesting to determine whether the 3' hypersensitive sites in the Ly-6E.1 and A.2 genes are indicative of such elements, because the promoter region of Ly-6E.1 appears to be insufficient to drive expression in vivo.

The transfection analysis of a 14-kb BamHI fragment encoding the Ly-6E.1 gene in MEL cells has verified the mapping of most hypersensitive sites in the endogenous gene. Taken together, these data strongly suggest that sequences around HS-1.2, HS-0.1, HS + 5.6, HS + 6.7, HS + 8.7, and HS + 8.9 are involved in producing high levels of Ly-6E.1 and A.2 expression (Fig 7) and that HS-0.1, HS-1.2, HS + 3.3, HS + 5.6, HS + 8.7, and HS + 8.9 sites play a role in γ-IFN inducibility. Although the strong HS + 4.9 site is unaffected by Ly-6E.1 and A.2 expression, it is possible that this site plays a role in transcriptional competence of this gene. This has been observed with some hypersensitive sites in the chromatin of human β-globin gene locus. Sequence determination and in vitro and in vivo deletion analysis of the regions containing the hypersensitive sites are currently underway and could show factor-binding sites and whether enhancer or LCR elements are contained within the Ly-6E.1 and A.2 genes. Such studies will help identify regions specific for stem cell expression and sequences required for the later hematopoietic expression patterns and, in addition, may suggest signals required for the differentiation of pluripotent hematopoietic stem cells.

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REFERENCES

27. Grosveld FG, Lund T, Murray EJ, Mellor AL, Dahl HHM, Flavell RA: The construction of cosmid libraries which can be used to transform eukaryotic cells. Nucleic Acids Res 10:6715, 1982
Cloning of the complete Ly-6E.1 gene and identification of DNase I hypersensitive sites corresponding to expression in hematopoietic cells

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