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Sp Family Members Stimulate Transcription of the *Hex* Gene *via* Interactions with GC Boxes[1]

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The 5′-flanking region of the mouse *Hex* gene was examined in order to identify transcription factors regulating its expression in hepatocytes and haematopoietic cells. We have identified two further GC boxes (GC boxes 3 and 4 at nucleotide positions −149 to −140 and −79 to −70, respectively), *i.e.* in addition to the two previously determined ones (GC boxes 1 and 2 at nucleotide positions −197 to −188 and −176 to −167, respectively). Luciferase reporter assays revealed that all four GC boxes are transcriptionally active in both MH_1C_1 rat hepatoma and K562 human chronic myelogenous leukemia cells. Electrophoretic mobility shift assays with specific competitors and antibodies showed that members of the Sp family, namely Sp1 and Sp3, bind to these GC boxes. Overexpression of Sp1 and Sp3 in *Drosophila* SL2 cells stimulated transcription of the *Hex* gene through interactions with GC boxes 1 to 4, Sp1 being a more potent activator than Sp3. Thus, we conclude that Sp1 and Sp3 stimulate transcription of the *Hex* gene in both MH_1C_1 and K562 cells.

Key words: differentiation, haematopoietic cell, hepatocyte, homeobox gene, transcription factor.

The haematopoietically expressed homeobox (*Hex*) gene, also known as the proline-rich homeobox (*Prh*), is a divergent homeobox gene, and its cDNA has been isolated from several animal species (*1–7*). We previously determined the genomic organization of *Hex* and showed that the Hex protein functions as a transcriptional repressor, its repressor domain being located in the N-terminal region (amino acids 45–136) from the homeodomain (amino acids 137–196) (*6, 8*). This nature has also been confirmed by others (*5, 9*). Recently, Guiral *et al.* (*10*) reported that the N-terminal region of *Hex* contains at least two repressor domains and, in addition, the homeodomain also has a repressor function. However, it has also been reported that the Hex protein also functions as a transcriptional activator (*11*). The molecular basis for this dual nature remains unknown.

Hex is expressed in a range of multipotent haematopoietic progenitor cells and restricted cell lineages, and its transcript is generally reduced during terminal cell differentiation (*12*). This expression pattern suggests that *Hex* is involved in the early stages of haematopoietic cell differentiation. In fact, over-expression of Hex in Myb-Ets transformed chicken blastoderm cells, which exhibit many characteristics of multipotent haematopoietic cells, inhibits its transformation and/or proliferation (*13*). Recently, *Hex* expression was also observed in the visceral endoderm in the distal region of the egg cylinder at embryonic age 5.5, developing thyroid, liver and lung during murine development (*14, 15*). Our gene targeting study has shown that mice null for the *Hex* gene exhibit embryonic lethality around embryonic age 10.5, due to no substantial liver formation, as shown by the absence of *albumin* expression (*16*). Haematopoiesis of monocytes is impaired in mutant embryos, while erythroid and granulocytic-macrophage lineages are unaffected. These results indicate that *Hex* plays an essential role in progenitor cells committed to the hepatic endoderm and also in the haematopoietic differentiation of the monocyte lineage. In addition, Martinez Barbera *et al.* (*17*) found in their gene targeting study that *Hex* is involved in forebrain formation and thyroid development.

Thus, identification of transcription factors regulating expression of the *Hex* gene should provide an insight into the events leading to the differentiation of cells such as hepatocytes and monocytes. We previously examined the 5′-flanking region of the *Hex* gene by means of transient reporter expression assays and identified two *cis*-acting elements involved in the regulation of *Hex* expression in hepatocytes, GC box 1 and GC box 2, comprising nucleotides −197 to −188 and −176 to −167, respectively (*8*). In the present study, we further examined these two *cis*-regulatory elements (GC boxes 1 and 2). Our results indicate that members of the Sp family, namely Sp1 and Sp3, bind to these GC boxes to stimulate transcription of the *Hex* gene in K562 human chronic myelogenous leukemia as well as MH_1C_1 rat hepatoma cells. In addition, two more *cis*-regu-

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Abbreviations: EMSA, electrophoretic mobility shift assay; SL2, Schneider line 2.

latory sites (GC box 3 and GC box 4) were identified, comprising nucleotides –149 to –140 and –79 to –70, respectively. Both Sp1 and Sp3 were also shown to bind to these GC boxes to transactivate *Hex* in K562 cells and MH_1C_1 cells.

MATERIALS AND METHODS

Cell Lines—MH_1C_1 (rat hepatoma) and K562 (human chronic myelogenous leukemia) cells were grown in Dulbecco's Modified Eagle Medium and RPMI1640 (Nissui Pharmaceuticals), respectively. Both media were supplemented with 10% fetal bovine serum (ICN), and cells were maintained at 37°C in a 5% CO_2 incubator. Schneider line 2 (SL2) cells, a *Drosophila* cell line, were grown in Schneider's *Drosophila* medium (Life Technologies GIBCO BRL) supplemented with 10% fetal bovine serum at 25°C. All cell lines were obtained from the American Type Culture Collection.

Plasmids—Construction of *Hex* promoter–luciferase plasmids, pHluc341, pHluc211, pHluc199, pHluc172, pHluc154, pHluc132, pHluc105, and pHluc67, which contain fragments of the mouse *Hex* gene, –341/+46, –211/+46, –199/+46, –172/+46, –154/+46, –132/+46, –105/+46, and –67/+46, respectively, was described previously (*8*). Plasmids $p(H\text{-}I)_2$ and $p(H\text{-}II)_2$, which contain two copies of H-I and H-II oligonucleotides ligated to pHluc67, respectively, were also described previously (*8*). Double-stranded oligonucleotides of H-III (–154/–133), H-IV (–105/–85), and H-IV (–84/–65) were synthesized and separately inserted into the *Nhe*I or *Xho*I site of pHluc67. The resultant plasmids were named $p(H\text{-}III)_7$, pH-IV $(-105/-85)_2$, and pH-IV $(-84/-65)_3$, which include seven, two, and three copies of the corresponding oligonucleotides, respectively. The nucleotide sequences of these oligonucleotides are shown in Table I. Plasmids used for SL2 cell DNA transfections: pPac-β-Gal was generously provided by Dr. Timothy F. Osborne (University of California, USA); and pPac, pPac-Sp1, and pPac-USp3 were generously provided by Dr. Guntram Suske (Philipps-Universitat Marburg, Germany).

Transfection and Enzyme Expression Assay—All plasmids used for transfection were prepared using a Qiagen plasmid Midi-kit. Transfection was performed using the calcium phosphate precipitation method (*18*) for MH_1C_1 cells and DMRIE-C Reagent (Life Technologies GIBCO BRL) for K562 cells, with 2 or 5 μg of various reporter plasmids and 40 or 100 ng of the pRL-SV40 plasmid as an internal control. The cells were incubated at 37°C in a 5% CO_2 incubator for 4–6 h, the medium was changed, and then the cells re-incubated. The cells were harvested after 42–44 h and lysed in 0.4 ml of passive lysis buffer (Promega). The cell extract was used to measure the firefly luciferase activity using a Promega Dual-Luciferase® Reporter Assay System. The results were normalized according to the *Renilla* luciferase activity of pRL-SV40. All transfections were repeated at least three times in duplicate.

DNA transfections for SL2 cells were carried out by a calcium phosphate method (*19*). Cells were plated at 1×10^6 cells/60 mm dish on day 0. On day 1, the cells were transfected with 2 μg of luciferase reporter plasmid, 0.1 μg of pPac-β-Gal, as an internal control, and 50 ng of pPac-derived expression vectors or pPac. The cells were harvested 42 h after transfection, and then luciferase and β-galactosidase activities were measured. β-Galactosidase assays were performed by means of a standard colorimetric procedure with 2-nitrophenyl-β-D-galactopyranoside as the substrate (*20*). The normalized luciferase activity was obtained by dividing the luciferase activity in relative light units by the β-galactosidase activity.

Site-Directed Mutagenesis—Site-directed mutagenesis was performed according to the Künkel method with some modifications (*21*). Site-directed mutation of H-I and H-II to produce pM-196/-191 and pM-175/-170, respectively, was previously described (*8*). Site-directed mutation of H-III

TABLE I. **Oligonucleotide sequences used for the construction of reporter plasmids.** Mutated bases are underlined.

Oligonucleotide (position)	Sequence (5′ to 3′)
H-I (–199 to –178)	CTAGCGGGGGCGGGAGCTGGGCCGGT
M-196/-191 (–208 to –182)	CGTGCGTCTCGGACTAGTGAGCTGGGC
H-II (–185 to –167)	TCGAGGGCCGGTGGGGGCGGATC
M-175/-170 (–187 to –158)	CTGGGCCGGTGGACTAGTATCGGCGGCGCGGGG
H-III (–154 to –133)	TCGAAGTGGGGGGCGGAGGCGAATCT
M-148/-143 (–154 to –133)	AGTGGGGAATTCAGGCGAATCT
H-IV (–105 to –85)	CTAGTGGCGGCAGGAAGGGGACCGA
(–84 to –65)	TCGAGCGCGGCCCCACCCCGCGGC
M-77/-72 (–84 to –65)	GCGCGGCACTAGTCCGCGGC

TABLE II. **Nucleotide sequences of oligonucleotides used for electrophoretic mobility shift assays (EMSAs).** Mutated bases are underlined.

Element	Sequence (5′ to 3′)	Position
H-I	CTAGCGGGGGCGGGAGCTGGGCCGGT	–199 to –178
H-I m	CTAGCGGACTAGTGAGCTGGGCCGGT	–199 to –178
H-II	TCGAGGGCCGGTGGGGGCGGATC	–185 to –167
H-II m	TCGAGGGCCGGTGGAAGCTTATC	–185 to –167
H-III	TCGAAGTGGGGGGCGGAGGCGAATCT	–154 to –133
H-III m	TCGAAGTGGGGAATTCAGGCGAATCT	–154 to –133
H-IV	TCGAGCGCGGCCCCACCCCGCGGC	–84 to –65
H-IV m	GCGCGGCACTAGTCCGCGGC	–84 to –65
Sp1	ATTCGATCGGGGCGGGGCGAGC	
MZF-1A	GATCTAAAAGTGGGGAGAAAA	
MZF-1B	GATCCGGCTGGTGAGGGGGAATCG	

and H-IV was performed by introducing *Eco*RI and *Spe*I sites into H-III and H-IV at regions –148/–143 and –77/–72, pM-148/-143 and pM-77/-72 being obtained, respectively. The oligonucleotides used for these mutageneses are described in Table I.

Electrophoretic Mobility Shift Assays (EMSAs)—Nuclear extracts of rat liver and K562 cells were prepared as described previously (*22, 23*). The nucleotide sequences of oligonucleotides used in EMSAs are listed in Table II. EMSAs were performed as described previously (*22*). For competition analysis, the indicated amount of competitor DNAs was added to the binding mixture. After completion of the binding reaction, the mixture was subjected to electrophoresis on a 4% polyacrylamide gel (19:1, acrylamide:bisacrylamide) in 45 mM Tris-borate, 1 mM EDTA (pH 8.0) at 200 V for 1 h. For supershift assays, antibodies for Sp1 or Sp3 (Santa Cruz Biotechnology) were first mixed with the nuclear extract on ice for 1 h, the ^{32}P-labeled probe was added to the mixture, and then another 30 min incubation was performed. The mixture was then subjected to electrophoresis on a 4% polyacrylamide gel (19:1, acrylamide:bisacrylamide), after which the gel was dried and exposed to X-ray film (Amersham).

RESULTS

Localization of Regulatory Elements in the 5′-Flanking Region—To elucidate the regulatory region of the mouse *Hex* gene in haematopoietic cells, we used the previously mentioned pHluc341, which contains a fragment comprising nucleotides –341 to +46 relative to the transcription start site of the *Hex* gene linked to the firefly luciferase gene, and series of 5′-deletion mutants of this fusion gene. These plasmids were then transfected into K562 cells and transient expression of the luciferase gene was examined. K562 cells, which express the *Hex* gene, are multipotential, haematopoietic malignant cells that spontaneously differentiate into recognizable progenitors of the erythrocytic, granulocytic and monocytic lineages (*24*). The pGL3-Control vector, which contains the simian virus 40 promoter and enhancer, was used as a positive control, and its activity was adjusted to 100%. As shown in Fig. 1, plasmid pHluc341 showed stronger activity than pGL3-Control in K562 cells. Deletion of –341 to –199 caused no significant change in activity. However, further deletion up to –172 caused a significant decrease in activity. Deletion of –154 to

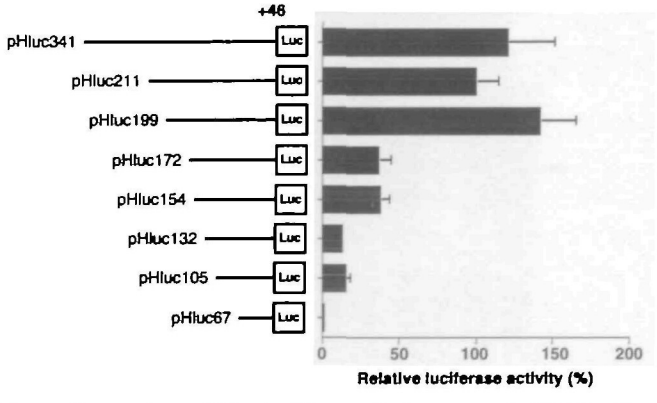

Fig. 1. **Expression of *Hex*-luciferase fusion genes in K562 cells.** The series of 5′-deletion mutants shown were transiently transfected into K562 cells with pRL-SV40. The relative luciferase activities of the deletion mutants are expressed as percentages, normalized as to pGL3-Control. Data shown are the means and standard error for at least three independent experiments. Luc, lucifer-ase gene.

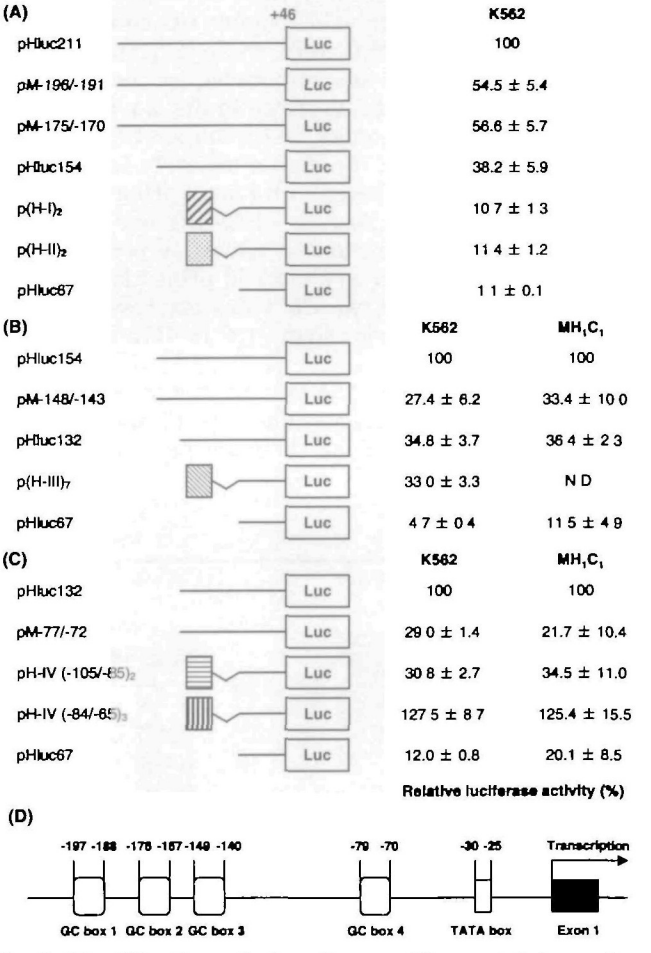

Fig. 2. **Identification of *cis*-acting positive regulatory elements.** (A) The lucifease activity of pHluc211 was adjusted to 100%. Plasmids containing two copies of H-I and H-II ligated to pHluc67 were individually transfected into K562 cells. Site-directed mutations of H-I and H-II were introduced into pHluc211 to produce pM-196/-191 and pM-175/-170, respectively, which were also individually transfected into K562 cells. (B) The luciferase activity of pHluc154 was adjusted to 100%. A plasmid containing seven copies of H-III ligated to pHluc67 was transfected into K562 cells. An *Eco*RI site was introduced into pHluc154 at –148/–143 to produce mutant construct pM-148/-143, which was transfected into both K562 and MH_1C_1 cells. N.D., not determined. (C) The luciferase activity of pHluc132 was adjusted to 100%. Plasmids containing two copies of H-IV (–105/–85) and three copies of H-IV (–84/–65) ligated to pHluc67 were individually transfected into K562 and MH_1C_1 cells. A *Spe*I site was introduced into pHluc132 at –77/–72 to produce mutant construct pM-77/-72, which was also transfected into both cell lines. (A to C) Luciferase activity was determined as described under "MATERIALS AND METHODS." Straight lines indicate *Hex* gene sequences and notches represent the polylinker site of pGL3-Basic. Data shown are the means and standard error for at least three independent experiments. Luc, luciferase gene. (D) Schematic representation of the regulatory regions of the mouse *Hex* gene. Nucleotide residues are numbered negatively from the transcription start site of the *Hex* gene.

–132 and –105 to –67 also caused significant decreases in activity. Plasmid pHluc67 showed essentially no activity in K562 cells, as observed previously for MH_1C_1 cells (*8*). These results suggest that regions spanning –199 to –173, –154 to –133, and –105 to –68 contain positive regulatory elements, and are similar to those observed previously in MH_1C_1 hepatoma cells (*8*).

Identification of cis-Acting Positive Elements—Since the region spanning –199 to –172 contains GC boxes 1 and 2, which are active in MH_1C_1 cells, we investigated whether or not these boxes are also responsible for the activity of this region in K562 cells. As shown in Fig. 2A, inclusion of H-I and H-II oligonucleotides, which contain GC box 1 and GC box 2, respectively, resulted in about 10-fold stimulation of the activity compared with that of pHluc67 in K562 cells. Conversely, mutations at –196/–191 and –175/–170 caused 45 and 43% reductions in activity, respectively, compared with that of wild-type plasmid pHluc211. These results indicate that the two GC boxes are responsible for most activity in the region from –199 to –172 in K562, as well as in MH_1C_1 cells.

The region spanning –154 to –133 was investigated for positive regulatory elements (Fig. 2B). Plasmid $p(H\text{-}III)_7$ contains seven copies of the oligonucleotide of the region called H-III in the sense orientation. When this plasmid was transfected into K562 cells, an about 7-fold increase in activity compared with that of pHluc67 was observed. The region from –154 to –133 contains one GC box named GC box 3, and an *Eco*RI site was introduced into pHluc154 at this box (–148/–143) to produce mutant plasmid pM-148/-143. This mutant was then transfected into K562 and MH_1C_1 cells. The luciferase activity of the mutant plasmid decreased to pHluc132 level in both K562 and MH_1C_1 cells. These results indicate that GC box 3 accounts for most of the activity of this region in both cell-types.

The region named H-IV spanning –105 to –65 was examined by dividing it into two sub-regions (Fig. 2C). Double-stranded oligonucleotides called H-IV (–105 to –85) and H-IV (–84 to –65) were ligated to pHluc67. Plasmids which contain two and three copies of the H-IV (–105 to –85) and H-IV (–84 to –65) oligonucleotides, respectively, were transfected into K562 and MH_1C_1 cells, and then luciferase activity was determined. No significant increase in activity was seen with H-IV (–105 to –85) in MH_1C_1 cells when compared to pHluc67, whereas this plasmid caused a 2.6-fold increase in activity in K562 cells. However, about 10-fold and 6-fold increases in activity in K562 and MH_1C_1 cells, respectively, were seen with the plasmid containing H-IV

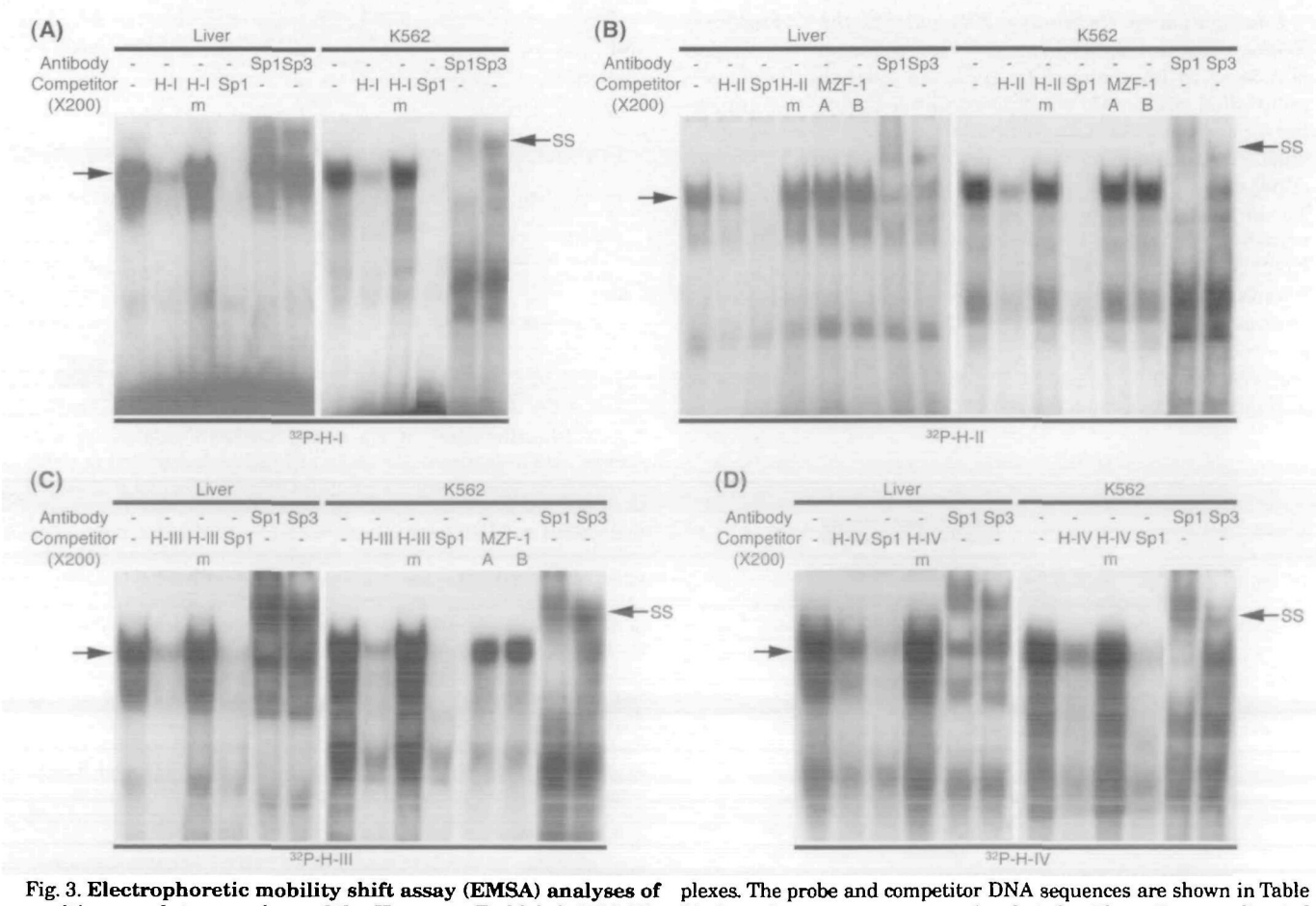

Fig. 3. **Electrophoretic mobility shift assay (EMSA) analyses of positive regulatory regions of the *Hex* gene.** End-labeled H-I (A), H-II (B), H-III (C) or H-IV (D) was incubated with 5 μg of either a rat liver or K562 nuclear extract. Probe DNAs are shown at the bottom. The competitor DNAs shown were used at a 200-fold molar excess. The arrows on the left indicate the positions of protein-DNA complexes. The probe and competitor DNA sequences are shown in Table II. A nuclear extract was preincubated with antiserum directed against Sp1 or Sp3 for 30 min prior to the addition of the probe. The Sp1 and Sp3 antisera used are shown at the top. The arrows on the right indicate a supershifted complex (SS) with antibodies.

(–84 to –65) when compared to pHluc67. Since the H-IV region also contains one GC box (GC box 4), a *Spe*I site was introduced into pHluc132 at this box (–77/–72) to produce mutant construct pM-77/–72. The luciferase activity of this mutant was reduced to 22% in MH_1C_1 cells compared with that of wild-type plasmid pHluc132 and similar to the level caused by pHluc67. In K562 cells, the activity of pM-77/-72 was still 2.4-fold higher than that of pHluc67, although its value was 29% of that of pHluc132 (Fig. 2C). Therefore, the region from –105 to –85 may also contain another positive regulatory element, which is slightly active only in K562 cells. Nevertheless, GC box 4 accounts for most of the activity of the region from –105 to –65 in both cell types.

Therefore, we have further identified two other GC boxes in the 5′-flanking region of the mouse *Hex* gene, GC box 3 at nucleotides –149 to –140 and GC box 4 at nucleotides –79 to –70, in addition to the existing GC boxes 1 and 2, at nucleotides –197 to –188 and –176 to –167, respectively (Fig. 2D).

Sp Family Members Bind to Multiple GC Boxes—EMSAs were performed to identify potential transcription factors that bind to multiple regulatory sites of the 5′-flanking region of the *Hex* gene promoter. End-labeled H-I, H-II, H-III, and H-IV oligonucleotides were incubated with either rat liver or K562 nuclear extracts in the presence or absence of a 200-fold molar excess of competitor DNAs. We used oligonucleotides containing a binding site for Sp family members (Sp1) or MZF-1 (MZF-1A and MZF-1B) as competitors. Oligonucleotides containing mutated sequences at GC boxes 1 to 4 were also used as competitors. As shown in Fig. 3A to D, all DNA/protein complexes produced with the two extracts were efficiently competed by a 200-fold excess of the Sp1 oligonucleotide as well as the corresponding unlabeled oligonucleotide. All oligonucleotides containing mutated sequences at GC boxes failed to inhibit the formation of protein complexes with the corresponding labeled probes. When Sp1- and Sp3-specific antibodies were added to the reaction mixture containing a rat liver or K562 nuclear extract, supershifted bands were observed for all labeled probes. The addition of non-immune serum did not produce any supershifted band (data not shown). Potential binding sites for MZF-1, which is expressed almost exclusively in early myeloid progenitor cells (*24*), are present in the H-II and H-III regions, but MZF-1A and MZF-1B oligonucleotides did not efficiently compete for binding of the K562 extract to the labeled probes (Fig. 3, B and C). These results indicate that members of the Sp family such as Sp1 and Sp3 are responsible for all DNA/protein complexes produced on incubation with rat liver and K562 nuclear extracts.

Effects of Sp1 and Sp3 on the Hex Promoter Activity in Drosophila SL2 Cells—The effects of Sp family members on the transcriptional activity of the *Hex* gene promoter were examined using *Drosophila* SL2 cells, as this cell line is devoid of any endogenous Sp family members (*26*). Deletion mutants of the 5′-flanking region were co-transfected with either the Sp1 or Sp3 expression plasmid. As shown in Fig. 4A, deletion of GC boxes 1 and 2 decreased the promoter activity by about 80%. Further 15 and 5% decreases in activity were observed with deletion of GC boxes 3 and 4, respectively, and pHluc67 showed essentially no activity. Sp1 showed much higher transcriptional activating capacity than Sp3. Mutation analyses of individual GC boxes

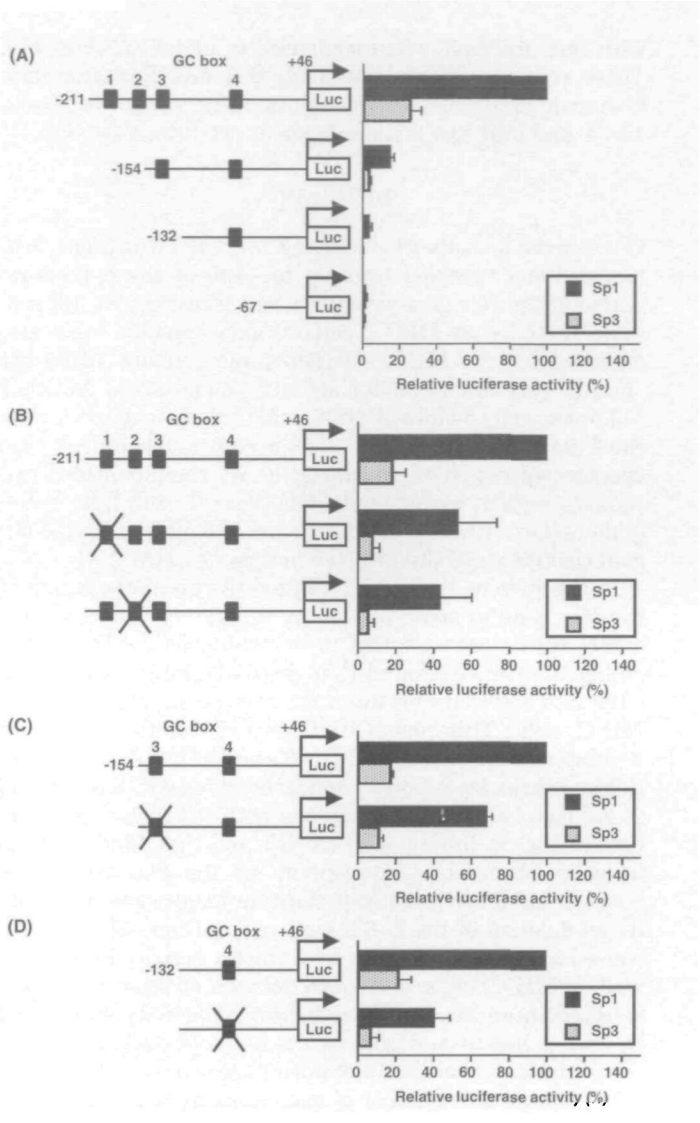

Fig. 4. **Effects of Sp1 and Sp3 on the GC boxes of the *Hex* gene promoter.** The effects of Sp family members on the transcriptional activity of the *Hex* gene promoter were examined in *Drosophila* SL2 cells. (A) Deletion mutants of the 5′-flanking region were co-transfected with either the Sp1 or Sp3 expression plasmid into SL2 cells. (B) Plasmids with site-directed mutation of pHluc211 to individually disrupt GC boxes 1 and 2 were co-transfected with either the Sp1 or Sp3 expression plasmid into SL2 cells. (C) A plasmid with a site-directed mutation of pHluc154 at GC box 3 was co-transfected with either the Sp1 or Sp3 expression plasmid into SL2 cells. (D) A plasmid with a site-directed mutation of pHluc132 at GC box 4 was co-transfected with either the Sp1 or Sp3 expression plasmid into SL2 cells. (A to D) Luciferase activity was determined as described under "MATERIALS AND METHODS." Straight lines indicate *Hex* gene sequences and filled boxes represent GC boxes. Data shown are the means and standard error for at least three independent experiments. Luc, luciferase gene.

were carried out to confirm the results of the deletion analysis. The separate mutations of pHluc211 at GC boxes 1 and 2 resulted in 47 to 55% and 56 to 67% reductions in transcriptional activity in SL2 cells by Sp1 and Sp3, respectively (Fig. 4B). Mutation of GC box 3 caused an about 30% reduction of the activity elicited by Sp1 and Sp3 when compared to wild type plasmid pHluc154 (Fig. 4C). GC box 4 mutation resulted in about 40% transcriptional activity

with Sp1 and Sp3, when compared to pHluc132 (Fig. 4D). These results indicate that both Sp1 and Sp3 stimulate transcription of the *Hex* gene by interacting with GC boxes 1 to 4, and that Sp1 is a more potent activator than Sp3.

DISCUSSION

Our previous study (*8*) involving a similar transient luciferase assay revealed that up to –199 of the 5′-flanking region of the *Hex* gene promoter was necessary for full promoter activity in MH_1C_1 cells. Similar results were also observed for the H4IIE rat hepatoma cell line (data not shown). Very low promoter activity was observed for other cell lines such as HeLa, COS-7, and Caco-2, suggesting that the 5′-flanking region up to –199 is responsible for cell-type specific expression of the *Hex* gene. We then identified two positive regulatory elements, GC boxes 1 and 2, in nucleotide region –197 to –167, by means of double-stranded oligonucleotides and site-directed mutagenesis (*8*).

In the current study, we analyzed the promoter region of the *Hex* gene in order to identify regulatory elements necessary for its transcription in haematopoietic cells as well as hepatocytes. We found that the 5′-flanking region up to –199 was necessary for full activity in K562 cells as well as MH_1C_1 cells. This region contains two additional positive regulatory sites, named GC box 3 and GC box 4, *i.e.* in addition to previously reported regulatory sites (GC boxes 1 and 2). All four GC boxes are active in both cell types and members of the Sp family, such as Sp1 and Sp3, bind to these boxes to stimulate transcription. Of the four GC boxes, boxes 1 and 2 are more important for transcriptional activity as deletion of the 5′-flanking region from –199 to –172 caused a marked reduction in promoter activity in K562 as well as MH_1C_1 cells, and since deletion of these two boxes resulted in an about 80% reduction in activity stimulated by Sp1 or Sp3 in SL2 cells. Sp1 is a ubiquitously expressed transcription factor that is known to play major roles in the transcription of a number of gene promoters (*27*). Recently, other closely related proteins of the Sp family, such as Sp2, Sp3, and Sp4, have been identified (*28–30*). Sp1 and Sp3 are ubiquitously expressed in mammalian cells and are known to compete for common target sequences with similar binding affinities, including the GC box and GT box, respectively (*27–29, 31–33*). However, Sp1 is a transcriptional activator (*27*), whereas Sp3 functions both as a transcriptional activator and repressor (*30, 31*). The functional diversity of Sp3 depends on the promoter context as well as the cellular background (*34*). Here we show that Sp3 functions as a transcriptional activator for the *Hex* gene, although its activity is much weaker than that of Sp1. Very recently, Denson *et al.* (*35*) also reported that Sp1 but not Sp3 can stimulate transcription of the reporter gene containing nucleotides –235 to +22 of the *Hex* gene in SL2 cells. The reason for the discrepancy between the two results is not known.

As *Hex* is expressed in other organs besides the liver and haematopoietic cells such as thyroid and lung (*15*), it is speculated that both tissue-specific and ubiquitous transcription factors are responsible for transcription of the *Hex* gene. Therefore, it seems quite plausible that members of the ubiquitous Sp transcription family would contribute to the liver- and haematopoietic cell–specific activation of the *Hex* gene *via* GC boxes, as shown by our data. In fact, the Sp sites contribute significantly to the hepatic and intestinal expression of the *apolipoprotein A-1* gene (*36*). Denson *et al.* (*35*) have reported that HNF3β and GATA-4 transactivate the *Hex* gene *via* response elements located within nucleotide regions –44 to –17 and –62 to –41, respectively, and are responsible for liver-enriched activation of the *Hex* gene in HepG2 cells. However, our previous data showed that the 5′-flanking *Hex* promoter region from –67 to +46 exhibited only minimal basal activity in MH_1C_1 cells (*8*). The present results indicate that this is also the case for K562 cells, although these cells express members of the GATA transcription factor family, namely GATA-1 and 2, which bind to the same site as GATA-4 (*37, 38*). Thus, MH_1C_1 and K562 cells may express reduced levels of tissue-enriched transcription factors such as HNF3β, and GATA-1, 2, and 4. Nevertheless, our results strongly suggest that members of the Sp family contribute greatly to *Hex* gene expression in both hepatocytes and haematopoietic cells. Sp1 and Sp3 may interact with members of the GATA family and HNF3β for tissue-specific transcription of the *Hex* gene. More studies addressing this issue are required. In addition, the region from –105 to –85 may contain a weak positive regulatory element, which is active in K562 but not MH_1C_1 cells. However, this element remains to be determined. Our findings indicate that the 5′-flanking region of the *Hex* gene promoter up to –199 is sufficient for cell-type specific expression in both hepatocyte and certain haematopoietic cells. Sp1 and Sp3 of the Sp transcription factor family bind to multiple GC boxes and greatly contribute to the transcription of *Hex* in these cell types. Sp1 has also been shown to have higher transcriptional activity than Sp3 in SL2 cells.

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