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Lineage-restricted expression of homeobox-containing genes in human hematopoietic cell lines

(DNA binding protein/leukemic cell line/transcription factor/erythropoiesis/embryogenesis)

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ABSTRACT **We investigated the role of homeobox-containing genes in human hematopoiesis because homeobox genes (*i*) control cell fate in the *Drosophila* embryo, (*ii*) are expressed in specific patterns in human embryos, and (*iii*) appear to function as transcription factors that control cell phenotype in other mammalian organs. Using four homeobox probes from the *HOX2* locus and a previously undescribed homeobox cDNA (PL1), we screened mRNAs from 18 human leukemic cell lines representing erythroid, myeloid, and T- and B-cell lineages. Complex patterns of lineage-restricted expression are observed: some are restricted to a single lineage, while others are expressed in multiple lineages. No single homeobox gene is expressed in all types of hematopoietic cells, but each cell type exhibits homeobox gene expression. *HOX2.2* and *-2.3* homeobox-containing cDNAs were cloned from an erythroleukemia cell (HEL) cDNA library, while the homeobox cDNA PL1 was isolated from a monocytic cell (U-937) library. Differentiation of HEL and K-562 cells with various inducers results in modulation of specific homeobox transcripts. In addition, *HOX2.2* is expressed in normal bone marrow cells. We have demonstrated (*i*) lineage-restricted expression of five homeobox genes in erythroid and monocytic cell lines; (*ii*) expression of additional homeobox genes in other cell lineages (HL-60 and lymphoid cells); (*iii*) expression of one homeobox gene in normal marrow cells; and (*iv*) modulation of expression during differentiation. These data suggest that these genes play a role in human hematopoietic development and lineage commitment.**

Although much information has emerged concerning hematopoietic growth factors and their receptors (reviewed in ref. 1), little is known about the nuclear events that orchestrate the differentiation process within hematopoietic stem cells and determine lineage commitment. We considered the family of homeobox-containing genes a strong candidate for a role in hematopoiesis. The homeobox is a highly conserved 180-nucleotide sequence discovered in several *Drosophila* genes (2, 3) that function as developmental "master switches" in determination of cell fate and tissue identity during embryogenesis (reviewed in ref. 4). The *Drosophila* homeobox-containing proteins are sequence-specific DNA binding proteins that appear to function as transcription factors (5, 6), and are presumed to regulate a variety of developmental genes.

Several homeodomains have been identified in the human genome and are highly homologous with the *Drosophila* homeoboxes (7). A cluster of at least six human homeobox-containing genes (the *HOX2* locus) has been localized to chromosome 17 (q11–q22) (8, 9). Other human homeobox-containing genes have been identified on chromosomes 2 (10), 7 (11), 12 (11), and 19 (12). Stage-related and tissue-specific expression of homeobox-containing genes in mouse (13–15) and human embryos (16, 17), as well as differentiation-related expression of these genes in teratocarcinoma cell lines *in vitro* (18, 19), suggested a role for these genes in the control of mammalian cell differentiation. To test the hypothesis that homeobox-containing genes are involved in hematopoietic differentiation, we screened a number of human leukemic cell lines representing a spectrum of hematopoietic phenotypes for expression of these genes, and we screened leukemic cell cDNA libraries for homeobox-containing clones.

MATERIALS AND METHODS

Northern Gel Analysis. RNA was isolated by the guanidinium thiocyanate method (20). mRNA was electrophoresed in 1.5% agarose/formaldehyde gels, transferred to GeneScreen, and cross-linked to the filters by UV irradiation (21). Initial probes included genomic homeobox-containing fragments from the human *HOX2* locus: a 1.7-kilobase (kb) *Hin*dIII fragment of *HOX2.1*; a 2.1-kb *Eco*RI fragment of *HOX2.2*; and a 5-kb *Eco*RI fragment of *HOX2.6* (19). Probes were labeled by the random-primer method and used for hybridization under stringent conditions (22). The actin probe was a 2.1-kb fragment of the human β-actin gene. Changes in homeobox-containing transcripts were established by densitometric scanning of autoradiographs.

Cloning and Sequencing of Homeobox-Containing cDNAs. cDNA libraries were constructed from HEL and U-937 cell poly(A)$^+$ RNA by the RNase H method (23) and λgt11 arms and Packagene mix (Promega Biotec). Moderate stringency screening (13) (using 43% formamide at 42°C) of the HEL cell library (1.1×10^6 independent plaques) with a *HOX2.2* genomic probe yielded five independent positive clones that corresponded to portions of the *HOX2.2* genomic sequence (W.-F.S., T.A.S., and C.L., unpublished observations), and a single clone that was shown to correspond to *HOX2.3* (see below). Screening of the U-937 cell library under similar moderate stringency (5×10^5 independent plaques) with the *HOX2.3* cDNA probe yielded five positive clones, two of which were isolated and shown to be identical portions of a previously undescribed homeobox-containing cDNA, which was named PL1. DNA sequencing was performed on single-stranded M13 subclones by the dideoxynucleotide chain-termination method with adenosine 5′-[α-[^{35}S]thio]triphosphate.

RNase Protection Experiments. A 304-base-pair DNA fragment consisting of the homeobox and 3′ nontranslated se-

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Table 1. Description of human leukemic cell lines studied

Cell line	Source	Predominant differentiation pathway
HEL	Erythroleukemia	Erythroid (26)
K-562	CML blast crisis	Erythroid (27)
MB-02	Megakaryoblastic leukemia	Erythroid*
HL-60	Promyelocytic leukemia	Granulocyte–macrophage (28)
PLB-985	Acute myeloblastic leukemia	Granulocyte–macrophage (29)
ML-1	Acute myeloblastic leukemia	Granulocyte–macrophage (30)
ML-2	Acute myeloblastic leukemia	Granulocyte–macrophage (30)
ML-3	Acute myeloblastic leukemia	Granulocyte–macrophage (30)
U-937	Histiocytic lymphoma	Macrophage (28)
KG-1	Acute myelogenous leukemia	Macrophage (27)
KG-1a	Acute myelogenous leukemia	None (31)
TMM	Acute myelogenous leukemia	None (32)
UCD-PC1	Multiple myeloma	B lymphocyte (33)
Su	Multiple myeloma	B lymphocyte (34)
CCRF CEM	Acute lymphoblastic leukemia	T lymphocyte (35)
MOLT-3	Acute lymphoblastic leukemia	T lymphocyte (36)
MOLT-4	Acute lymphoblastic leukemia	T lymphocyte (36)
Mo	Hairy cell leukemia	T lymphocyte (37)

CML, chronic myelogenous leukemia.
*Doris Morgan, personal communication.

quence of HOX2.2 was used to detect low-level transcripts in human bone marrow mRNA and K-562 cells. This fragment was subcloned into Bluescript for synthesis of an RNA probe using 5′-[α-^{32}P]UTP. The probe was purified by electrophoresis in 5% polyacrylamide/urea gels, hybridized to poly(A)$^+$ RNA in 80% formamide at 65°C, digested with RNases A and H, and the protected bands were electrophoresed in 5% polyacrylamide/urea gels.

Differentiation Experiments. Erythroid differentiation of HEL and K-562 cells was induced by treatment with either cytosine arabinofuranoside (0.1 μM) or hemin (40 μM) (24), and differentiation was indicated by positive benzidine staining after 5–6 days. Monocytic differentiation of HEL cells was induced by treatment with phorbol 12-myristate 13-acetate (0.1 μM) (25).

RESULTS

Eighteen human leukemia cell lines representing a spectrum of erythroid, monocyte–macrophage, and lymphocytic differentiation (Table 1) were screened for homeobox gene expression, using as probes homeobox-containing genomic fragments of *HOX2.1*, *HOX2.2*, and *HOX2.6*, from the human *HOX2* locus (8, 9, 19), and two homeobox-containing cDNAs (HOX2.3 and PL1) that we isolated from HEL and U-937 cell lines, respectively.

Homeobox Gene Expression in Erythroid Cell Lines. Fig. 1 shows a Northern blot which demonstrates that all four *HOX2* genes are expressed in one or both of two erythroid cell lines (HEL and K-562), but not in the three nonerythroid lines HL-60, KG-1, or U-937. Transcripts of 1.7, 1.4, and 2.1 kb are observed for HOX2.1, HOX2.2, and HOX2.6, respectively, while a set of closely spaced transcripts centered on 1.4 kb are observed for HOX2.3. To demonstrate that each transcript observed in Fig. 1 represented a homeobox-containing gene product, blots were reprobed with the respective homeobox portion of each gene (data not shown).

Homeobox Gene Expression in Myeloid and Monocytic Cell Lines. When the HOX2.3 cDNA was used as probe under high stringency in Northern gel analysis, transcripts were seen only in HEL and K-562 cell lines. However, when the same blots were probed with HOX2.3 under reduced stringency (50°C instead of 55°C), bands were visualized in all five cell lines (results not shown). As described below, we used the HOX2.3 probe to clone a previously undescribed homeobox cDNA from U-937 cells (PL1). When PL1 was used as a probe in Northern blot analysis, strong bands were seen only in U-937 and KG-1, cell lines with monocytic potential (Fig. 2). The two major transcripts were 2.2 and 3.0 kb. A fainter 2.1-kb band was also seen in HEL cells. Since the PL1 probe does not hybridize to HL-60 mRNA, we suggest there are other, uncharacterized homeobox-containing transcripts in HL-60 cells.

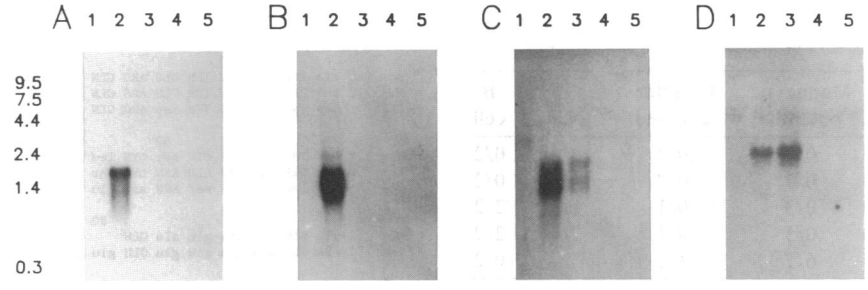

FIG. 1. Homeobox-containing gene expression in erythroleukemia cells. Northern gel analysis was performed on mRNA isolated from KG-1 cells (lane 1), HEL cells (lane 2), K-562 cells (lane 3), HL-60 cells (lane 4), and U-937 cells (lane 5). The Northern blot was hybridized with a *HOX2.1* genomic probe (*A*) under high-stringency conditions (50% formamide at 55°C). The filter was stripped and hybridized sequentially with a genomic *HOX2.2* probe (*B*), a cDNA *HOX2.3* probe (*C*), and a genomic *HOX2.6* probe (*D*). Markers are RNA ladders. *HOX2.1* and *HOX2.2* are restricted to HEL cells, while *HOX2.3* and *-2.6* are restricted to the erythroid cell lines HEL and K-562. None of these genes is expressed in the nonerythroid cell lines KG-1, HL-60, or U-937. An actin control for the filter used in all of these experiments is shown in Fig. 2 (*Lower*). Numbers on left are kb.

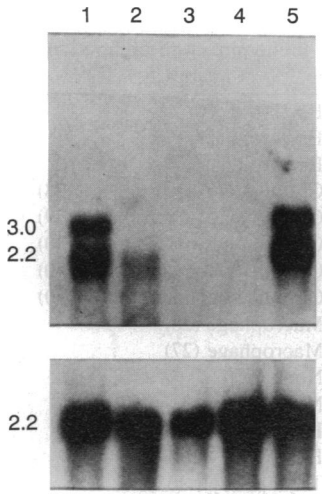

FIG. 2. Monocyte–macrophage restricted expression of homeobox genes in hematopoietic cells. Northern blot analysis was performed on mRNA from hematopoietic cell lines using a 2.3-kb PL1 probe isolated from a U-937 cDNA library. Lanes: 1, KG-1; 2, HEL; 3, K-562; 4, HL-60; 5, U-937. Size markers (kb) were estimated with RNA ladders. (*Lower*) Actin control.

Lineage-Restricted Expression. We extended our survey to 18 leukemia cell lines including 3 erythroid lines, 9 myeloid lines (7 with monocytic potential and 2 uninducible), and 6 lymphoid lines (4 T- and 2 B-cell lines). The results of Northern gel analysis using the five homeobox probes described above are summarized in Table 2. *HOX2.1* and *-2.2* were expressed almost exclusively in erythroid cell lines. In contrast, PL1 was seen in 6 of 7 myeloid cell lines with monocytic potential, in HEL, which has monocytic as well as erythroid potential (25), and only weakly in 1 of 4 T-cell lines. *HOX2.3* and *-2.6* were both seen in erythroid and lymphoid cell lines. Low-stringency screening of lymphoid mRNA samples with HOX2.1 revealed an apparent 2.1-kb band in all samples. Rescreening with a HOX2.1 flanking region probe demonstrated that these bands represented different homeobox-containing genes, which did not contain HOX2.1-specific sequences but must share homology with the HOX2.1 homeobox (data not shown).

Cloning Homeobox-Containing cDNAs. An HEL cell cDNA library was constructed from HEL cell poly(A)$^+$ RNA and screened with a 2.1-kb *HOX2.2* genomic probe, yielding five positive clones. Sequence analysis revealed that four clones contained homeobox sequences identical to those in the previously reported *HOX2.2* genomic sequence (7), except for changes in the nucleotide region 1–16 (Fig. 3), which altered the first five amino acids. The identity of these cDNA clones as derivatives of the *HOX2.2* gene was established by comparison of ≈800 bp of *HOX2.2* genomic flanking region sequence to the cDNA clones (W.-F.S., T.A.S., and C.L., unpublished observations). One clone was isolated whose nucleic acid sequence, with the exception of two A for G changes (positions 54 and 109), is identical in both the box and the flanking regions (W.-F.S., T.A.S., and C.L., unpublished observations) to HOX2.3, previously isolated from simian virus 40-transformed human fibroblasts (38).

Since the hybridization of HOX2.3 to myeloid cell lines under reduced stringency suggested the presence of additional homeobox gene transcripts in these cells, a U-937 cell cDNA library was constructed and screened with HOX2.3 at moderate stringency. A different homeobox-containing cDNA (PL1) was isolated and partially sequenced (Fig. 3). As expected from the cross-hybridization of HOX2.3 with HOX2.2 during the library screening, these two cDNAs share extensive nucleotide homology (97% over two extended

Table 2. Lineage-restricted homeobox gene expression

	Erythroid lines	Myeloid lines		Lymphoid lines	
		Monocytic potential	Undifferentiated	T cell	B cell
HOX2.1	2/3	0/7	0/2	0/4	0/2
HOX2.2	3/3*	0/7	0/2	0/4	0/2
HOX2.3	3/3	0/3	0/1	1/4†	2/2
HOX2.6	3/3	0/3	0/1	2/4†	2/2
PL1	1 (weak)/3‡	6/7	1/2	(1)/4†	0/2

*Although HOX2.2 transcripts are not observed in K-562 cells by Northern analysis, RNase protection experiments reveal that homeobox-containing transcripts are expressed in K-562 cells at levels approximately 1/20th those in HEL cells.

†HOX2.3, -2.6, and PL1 are all weakly expressed in the MOLT-3 cell line. PL1 is ≈1/20th of U-937 cells. HOX2.6 is also weakly expressed in CCRF CEM cells.

‡A faint band is seen in HEL cells (see text).

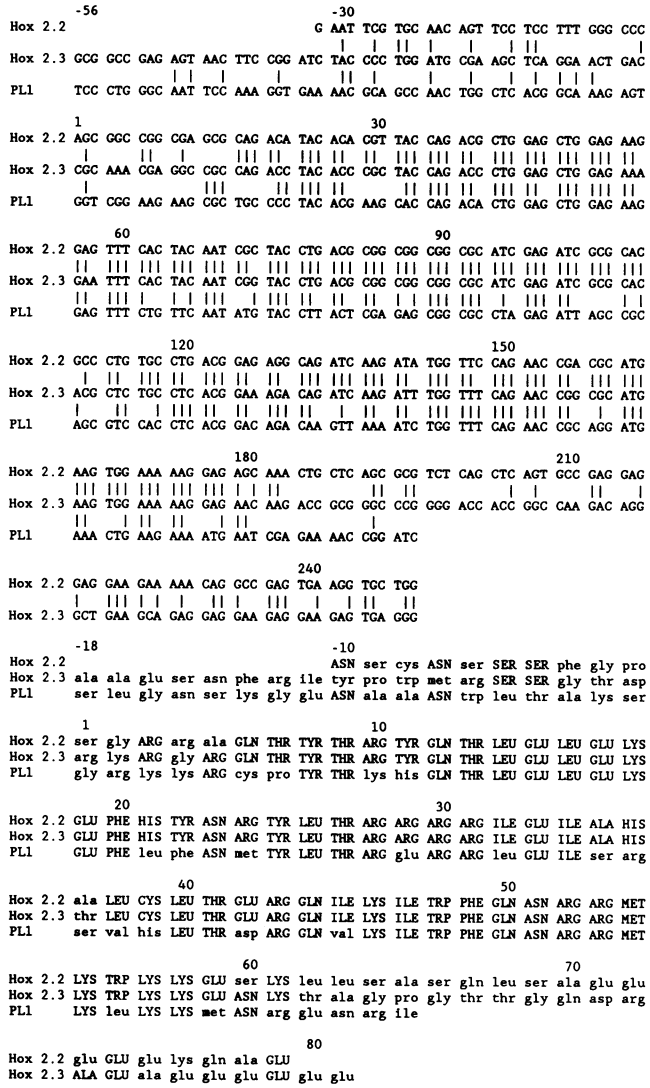

FIG. 3. Homeobox-containing sequences of cDNAs cloned from human hematopoietic cell libraries. HOX2.2 and Hel-8 (HOX2.3) were isolated from an HEL cell library. PL1 was isolated from a U-937 cell library. Partial cDNA sequences (*Upper*) and derived amino acid sequences (*Lower*) for HOX2.2, Hel-8, and PL1 cDNAs around the homeobox region are shown. Nucleotides numbered 1–180 and the corresponding amino acids (1–60) comprise the homeobox.

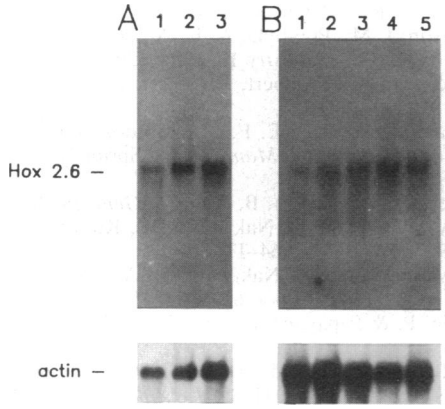

FIG. 4. Increased HOX2.6 mRNA after hemin-induced erythroid differentiation of HEL or K-562 cells. Northern gel analysis of *HOX2.6* gene transcripts after hemin-induced erythroid differentiation of K-562 cells and HEL cells. (*A*) HEL cells at day 0 (lane 1), and 1 and 5 days after hemin treatment (lanes 2 and 3, respectively). (*B*) K-562 cells at day 0 (lane 1), and 6 hr (lane 2), 1 day (lane 3), 3 days (lane 4), and 5 days (lane 5) after hemin treatment.

regions of 69 and 30 base pairs and 84% overall). In contrast, PL1 is only 57% identical to HOX2.2 and 59% identical to the HOX2.3 sequence.

Fig. 3 also shows the amino acid sequences for the three homeobox-containing cDNAs. All three proteins diverge sharply in the regions flanking the homeobox. Within the homeobox, HOX2.2 and HOX2.3 are highly conserved (90% identical), while PL1 is only 57% and 60% identical to HOX2.2 and HOX2.3, respectively. The amino acid differences between PL1 and the other two homeobox sequences are clustered into three regions: box residues 1–11, 21–24, and 32–42. The remaining 34 residues are 88% identical and include the highly conserved region from residues 42–50 observed for other homeobox cDNAs and genes, which has been proposed to form a recognition helix for DNA binding (4).

Differentiation-Related Changes in Homeobox Gene Expression. Changes in the levels of homeobox-containing mRNA were examined during differentiation of HEL and K-562 cells. Hemin-induced erythroid differentiation of HEL cells resulted in increased HOX2.6 mRNA levels (2- to 3-fold) (Fig. 4*A*). A greater increase in HOX2.6 (5.5-fold) was observed when K-562 cells were induced with the same agent (Fig. 4*B*). Treatment of K-562 cells with cytosine arabinofuranoside, which also induces erythroid differentiation (24), results in a specific diminution of the upper of two HOX2.3 transcripts (Fig. 5), while remaining HOX2.3 and the HOX2.6 transcripts do not change. Monocytic differentiation of HEL cells resulted in a transient decrease in all four homeobox mRNA levels at 6 hr: HOX2.1 and HOX2.3 were decreased ≈8-fold, while HOX2.2 and -2.6 were only reduced 70% and 40%, respectively (data not shown).

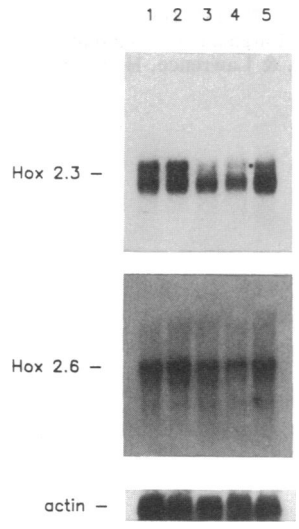

FIG. 5. Changes in HOX2.3 mRNA levels after cytosine arabinofuranoside-induced erythroid differentiation of K-562 cells. Northern gel analysis of mRNA isolated from K-562 cells at 0 (lane 1), 1 day (lane 2), 3 days (lane 3), 5 days (lane 4), and 7 days (lane 5) after erythroid differentiation with cytosine arabinofuranoside. The filter was sequentially probed with HOX2.3, HOX2.6, and actin.

Expression in Normal Hematopoietic Cells. Northern gel analysis of mRNA from peripheral monocytes, granulocytes, and lymphocytes and from unfractionated normal bone marrow cells failed to reveal detectable transcripts for any of the five homeobox genes studied. However, using the more sensitive technique of RNase protection, we have demonstrated *HOX2.2* expression in normal bone marrow (results not shown).

DISCUSSION

Evidence is growing for involvement of homeobox-containing genes in human development. Human homeobox genes are expressed in the embryo in stage-specific and tissue-specific patterns (16, 17, 38). In the human NT2 teratocarcinoma cell line, homeobox expression is seen after induction of differentiation (19). In a human colon cancer cell line, levels of homeobox-containing transcripts increased during differentiation (39). Recent data show that two other homeobox-containing proteins function as tissue-specific transcription factors in mammalian cells. The homeobox protein (GHF-1, Pit-1), regulates growth hormone transcription in the pituitary (40, 41). The human B-lymphocyte-specific transcription factor (Oct-2) binds to the immunoglobulin octamer DNA motif and is thought to regulate immunoglobulin synthesis (12).

We have demonstrated here a broad and complex pattern of homeobox gene expression in human leukemic cell lines. We show that at least four homeobox-containing genes from the *HOX2* locus are transcriptionally active in erythroid cell lines in a restricted pattern—all four are active in HEL and MB-02 cells, but only two are transcribed in K-562 cells. One homeobox-containing gene is specifically expressed in six of seven cell lines with monocytic potential. We have also obtained data suggesting that other homeobox-containing transcripts are present in HL-60 and lymphoid cell lines. At least one homeobox gene, *HOX2.2*, is expressed in normal bone marrow, indicating that activation of these genes is not simply a phenomenon of leukemic cells. Finally, treatment of HEL and K-562 cells with various inducers results in the modulation of homeobox mRNA levels.

Analysis of 21 published human and mouse homeobox DNA sequences revealed that the PL1 gene expressed in the monocytic cell lines is not identical to any previously described homeobox. The lineage-restricted expression of homeobox-containing genes in three human erythroleukemia cell lines and six of seven monocytic lines and the modulation of that expression during differentiation suggests that the products of these genes play a role in regulation of blood cell development. In this regard, while this manuscript was in review, Kongsuwan *et al.* (42) reported expression of multiple homeobox-containing transcripts in a range of mouse hematopoietic cell lines and in human K-562 cells. These authors have also presented data that support our hypothesis of lineage-specific expression of homeobox genes in hematopoietic cells (43). We note, however, that the expression pattern of homeobox genes we observed is correlative and

that a causal relationship with either cellular differentiation or cell lineage remains to be established.

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