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Coordinate regulation of *HOX* genes in human hematopoietic cells

(hematopoiesis/homeobox genes/lineage determination/transcription factors)

MARIA CRISTINA MAGLI*, PASQUALE BARBA, ANGELA CELETTI, GABRIELLA DE VITA, CLEMENTE CILLO, AND EDOARDO BONCINELLI

International Institute of Genetics and Biophysics, Consiglio Nazionale delle Ricerche, via Marconi 10, 80125 Naples, Italy

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ABSTRACT **Hematopoiesis is a continuous process in which precursor cells proliferate and differentiate throughout life. However, the molecular mechanisms that govern this process are not clearly defined. Homeobox-containing genes, encoding DNA-binding homeodomains, are a network of genes highly conserved throughout evolution. They are organized in clusters expressed in the developing embryo with a positional hierarchy. We have analyzed expression of the four human *HOX* loci in erythroleukemic, promyelocytic, and monocytic cell lines to investigate whether the physical organization of human *HOX* genes reflects a regulatory hierarchy involved in the differentiation process of hematopoietic cells. Our results demonstrate that cells representing various stages of hematopoietic differentiation display differential patterns of *HOX* gene expression and that *HOX* genes are coordinately switched on or off in blocks that may include entire loci. The entire *HOX4* locus is silent in all lines analyzed and almost all the *HOX2* genes are active in erythroleukemic cells and turned off in myeloid-restricted cells. Our observations provide information about the regulation of *HOX* genes and suggest that the coordinate regulation of these genes may play an important role in lineage determination during early steps of hematopoiesis.**

The hematopoietic system is organized in a developmental hierarchy in which mature blood cells have a limited life-span and must be constantly replaced by the proliferation and differentiation of bone marrow progenitor cells. The most primitive stem cells have an extensive self-renewal and proliferative capacity and can give rise to mature cells of all hematopoietic lineages (1). The molecular mechanisms that regulate this differentiation process are not yet clear. Homeobox-containing genes would appear to be strong candidate genes to regulate a number of developmental processes, including hematopoiesis. Genes of this family, although different from one another, contain a common sequence of 183 nucleotides that encodes a 61-amino acid domain, the homeodomain (2). The homeodomain is a DNA-binding domain capable of recognizing specific sequences by virtue of a helix–turn–helix structural motif. On the basis of structural similarities and of direct evidence that *Drosophila* homeodomain proteins are capable of binding DNA sequences and of modulating transcriptional activity, it is now generally accepted that homeodomain proteins are transcriptional regulators (3, 4). The homeobox was originally discovered in the homeotic genes responsible for segment identity in *Drosophila* development (5). Subsequently, homeobox-containing genes have been found in a number of evolutionarily distant organisms including nematodes and vertebrates (6, 7). In mice and humans, homeobox genes of the *HOX* family are organized in four clusters on different chromosomes that presumably evolved by duplication of a primordial gene cluster (8–10). Strikingly, the order of genes within each cluster is also highly conserved throughout evolution, suggesting that the physical organization of *HOX* genes may be essential for their expression (11).

HOX genes are expressed during embryogenesis in a tissue-specific and often stage-related fashion (12–14). Several reports have recently demonstrated that some homeobox genes are expressed within the hematopoietic system, although there has been no attempt to determine whether the organization of the *HOX* gene clusters is reflected in patterns of gene expression (15–19). Our aim has been to determine whether the physical organization of *HOX* genes reflects a regulatory network involved in the differentiation process of hematopoietic cells. As a first step we have studied expression of the four *HOX* gene clusters in human erythroleukemic, promyelocytic, and monocytic cell lines that represent various steps of hematopoietic differentiation. In these lines *HOX* genes appear to be switched on or off in blocks. These findings suggest that the hierarchical organization of precursor cells is reflected in the overall patterns of *HOX* gene expression and raise the possibility that these genes play a controlling role in precursor cell differentiation.

MATERIALS AND METHODS

Cell Lines. The K562 cell line was derived from the pleural fluid of a patient with chronic myeloid leukemia in blast crisis (20). The OCIM2 cell line was derived from a patient with erythroleukemia, which represented the end stage of a previously identified myelodysplastic syndrome (21). The HL60 cell line was derived from the peripheral blood of a patient with acute promyelocytic leukemia (22). The U937 cell line was derived from a patient with a diffused hystiocytic lymphoma (23). Cells were maintained in Iscove's modified Dulbecco's medium supplemented with 5% fetal calf serum, α-thioglycerol at 46 μmol/liter and antibiotics.

RNA Isolation and Analysis. Total RNA was extracted by the guanidinium thiocyanate technique (24) and poly(A)$^+$ selected by one passage on oligo(dT)-cellulose columns. Poly(A)$^+$ RNA was electrophoresed on 1.25% agarose/formaldehyde gels, transferred to nylon (Schleicher & Schuell, 13 N) membranes by Northern capillary blotting, and hybridized to 10^7 cpm of DNA probe labeled by nick-translation to a specific activity of $3–8 \times 10^8$ dpm/μg. Prehybridization and hybridization were carried out as described elsewhere (25). After washing under stringent conditions (30 mM NaCl/3 mM sodium citrate/0.2% sodium dodecyl sulfate at 65°C), the blots were exposed for 1–7 days at −70°C to Kodak XR-5 films in an Xomatic intensifying screen cassette.

RESULTS

As a first step in determining whether the *HOX* genes might be involved in cell lineage determination, we analyzed expression of the *HOX* genes in erythroleukemic, promye-

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*To whom reprint requests should be addressed.

Table 1. *HOX* gene expression in K562, OCIM2, HL60, and U937 cells

	Hox1											*Hox2*									*Hox3*									*Hox4*								
	J	*I*	*H*	*G*	*A*	*B*	*C*	*D*	*E*	*K*	*F*	*E*	*D*	*C*	*B*	*A*	*F*	*G*	*H*	*I*	*G*	*F*	*H*	*I*	*B*	*A*	*C*	*D*	*E*	*I*	*H*	*F*	*D*	*C*	*E*	*B*	*A*	*G*
K562	−	−	−	−	−	−	−	−	±	−	+	+	+	+	+	±	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
OCIM2	±	−	−*	−	−	−	−	−	±	−	+	+	+	+	+	±	+	+	+	−	+	+	+	+	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−
HL60	+	+	+	−	±	±	±	±	±	−	+	−	−	−	−	−	−	−	−	−	±	±	+	+	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−
U937	+	+	+	+	±	±	±	±	±	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	+	+	+	−	−	−	−	−	−	−	−	−

Results are presented according to the presence (+) or absence (−) of transcripts detected by each probe. ±, Very weak expression.
*In this line the 2.4-kb band is absent, but the 2.0-kb band is detectable.

locytic, and monocytic cell lines representing cells of different lineages and degree of maturation. K562 and OCIM2 are erythroleukemic cells that bear surface markers of the erythroid, myeloid, and megakaryocytic lineages and differentiate along these pathways on induction with specific agents (20, 21, 26–28). Thus they may provide a model of pluripotent cells. A more mature stage of differentiation is represented by the cell line HL60, derived from a promyelocytic leukemia (22). Surface markers and the capacity to produce either granulocytic or monocytic cells after treatment with chemical agents suggest that these cells may correspond to hematopoietic progenitors restricted to the myeloid lineage (29, 30). U937 is a monocytic cell line derived from a hystiocytic lymphoma that, after induction with phorbol 12-myristate 13-acetate, generates elements of the monocytic lineage only (23, 31).

Poly(A)$^+$ RNA from each cell line was hybridized by Northern blotting with probes containing the 3′ untranslated region specific for each of 38 *HOX* genes (32–34), organized into four large clusters, *HOX1–HOX4* located on chromosomes 7, 17, 12, and 2, respectively (see Fig. 3).

We first analyzed expression of the *HOX* genes in K562 cells and observed that there was very low, if any, expression of *HOX1* and *HOX4* whereas *HOX2* and *HOX3* were present at much higher levels (see Fig. 1 and Table 1). Of the 11 *HOX1* genes only *1F*, at the extreme 3′ end of the locus, showed two appreciable transcripts of 1.7 and 2.2 kilobases (kb) (see Fig. 2). The four genes at the 5′ end of the locus, *1J, 1I, 1H*, and *1G*, were not expressed and the others (*1A–1K*) were barely detectable (Table 1). Conversely 8 of 9 genes of the *HOX2* locus were expressed although with different intensities. The expression pattern of *HOX2* and *HOX3* genes in K562 cells is illustrated in Fig. 1. *HOX2E, 2C, 2F*, and *2H* were the most highly expressed. With probes specific for *HOX2E* and *HOX2H* transcripts of 2.4 and 1.6 kb, respectively, were detected. *HOX2C* revealed the relatively abundant 1.6- and 1.4-kb mRNA species previously described in other tissues. *HOX2F* exhibited an intense 2.3-kb band with a minor 2.9-kb band. *HOX2G* was expressed at lower levels and with multiple transcripts of 3.4 kb and higher molecular weight. Single minor transcript classes were observed in *2A* and *2B*. Expression at low levels of *HOX2A* was confirmed by RNase

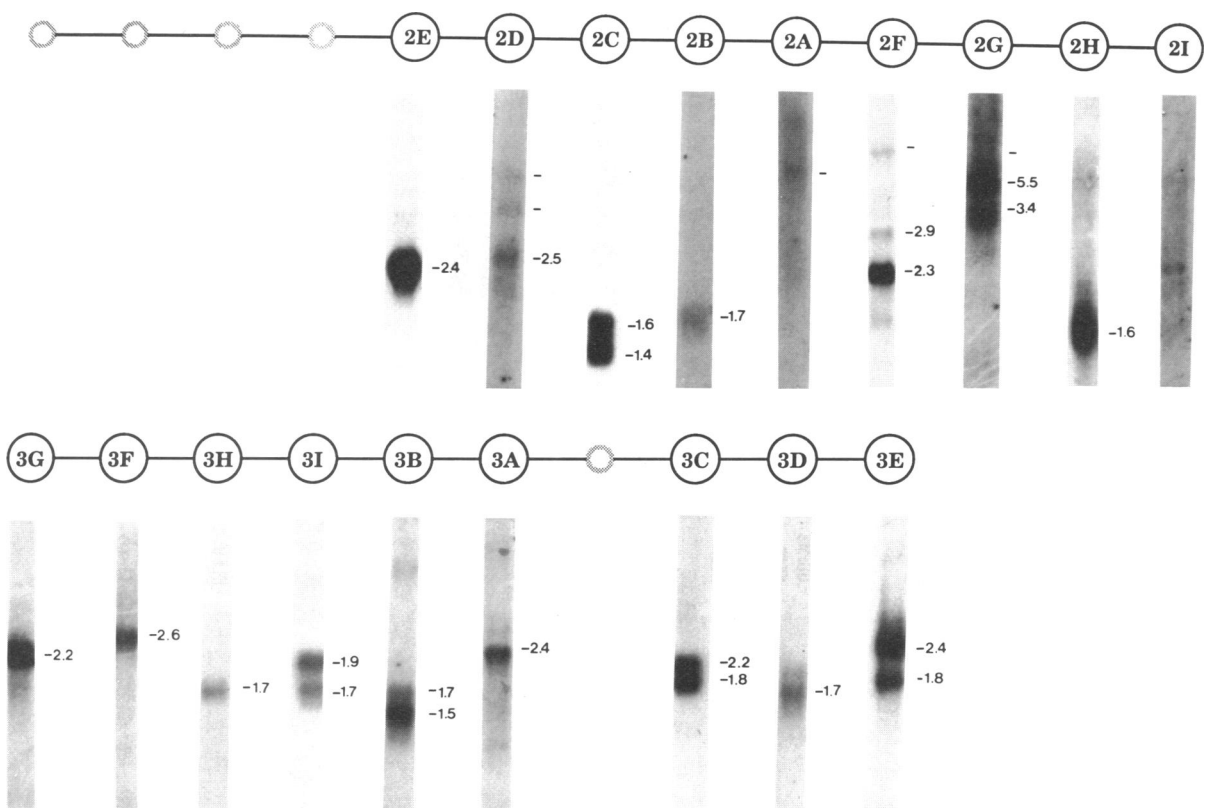

FIG. 1. Expression of *HOX2* and *HOX3* genes in K562 cells. *HOX* genes are aligned horizontally according to their physical position on the chromosomes and vertically on the basis of maximal sequence homology of the homeodomains. Small stippled circles indicate homeodomains predicted in the scheme but not yet found. Northern blots of 5 μg of poly(A)$^+$ RNA from K562 cells were hybridized to the probes indicated in the circles above each lane. Transcript sizes are given in kb.

protection analysis. Minor higher molecular weight bands were detected with *2D*, *2A*, *2F*, and *2G* probes.

The majority of *HOX3* genes were also expressed in K562 cells. *HOX3G* and *HOX3F* showed relatively abundant single-transcript classes of 2.2 and 2.6 kb, respectively. *HOX3H* was barely detectable while *HOX3I* exhibited two transcripts of 1.9 and 1.7 kb. Most transcripts of *HOX3* genes from *3B* through *3E* detected in these cells correspond to those observed in other tissues. Although four mRNA species of *HOX3E* have been described in human embryos (35), we detected only the 2.4- and 1.8-kb transcripts in K562 cells. Analysis of *HOX* gene expression in another erythroleukemic cell line, OCIM2, indicated that the general trend was the same as that in K562 cells and only minor differences were detected. In contrast, a significantly different pattern of *HOX* gene expression was observed in HL60 and U937 cells. Overall these myeloid-restricted cell lines expressed high levels of the 5′ genes of the *HOX1* and the 3′ genes of the *HOX3* loci, whereas virtually all the *HOX2* and *HOX4* genes were silent. Table 1 summarizes the expression of the 38 genes in K562, OCIM2, HL60, and U937 cells.

The expression of some relevant *HOX* genes in the four cell lines is compared in Fig. 2. *HOX1J*, *1I*, and *1H* were either not expressed or were barely detectable in erythroleukemic cell lines whereas they were expressed in HL60 cells and more intensively in U937 cells. *HOX1G* was not expressed in *HL60* cells and in the erythroleukemic lines; in contrast, it was intensively expressed with a 2.8-kb and a more abundant 1.9-kb transcript in the monocytic cells.

HOX1J exhibited multiple sized transcripts, the prevalent ones being 5.0, 3.2, and 2.3 kb in length while the minor ones were 8 and 2.8 kb long. Two transcripts of 2.3 and 2.0 kb were detected with the *HOX1I* probe, and the *HOX1H* probe revealed the presence of one 2.0-kb transcript class in OCIM2 cells and an additional 2.4-kb mRNA in HL60 and U937 cells. The higher molecular weight band seems to be at different intensity in the two myeloid cell lines.

As in K562 cells, expression of the *HOX1A–1K* genes was barely detectable in HL60 and U937 cells whereas *1F* showed two transcripts. All the *HOX2* genes appeared to be silent in the myeloid lines whereas they were highly expressed in the erythroleukemic cells. The four genes at the 3′ end of the *HOX3* locus, *3A*, *3C*, *3D*, and *3E*, are expressed in all the cell lines analyzed. However varying degrees in the expression of the 5′ *HOX3* genes were observed.

The *HOX3G–3B* genes, well expressed in the erythroleukemic lines, were expressed at low levels in HL60 cells and were barely detectable or undetectable in the monocytic cell lines. The entire *HOX4* locus was everywhere silent with the exception of the *HOX4I* gene, which was selectively expressed in OCIM2 and HL60 lines. Further investigation will be necessary to understand this observation.

The expression patterns of the 38 homeobox genes in the cell lines analyzed are summarized in Fig. 3. It appears that during myeloid differentiation the 4 most 5′ *HOX1* genes are switched on whereas 8 genes belonging to *HOX2* and the five genes located at the 5′ end of *HOX3* are switched off. No variation is observed with regard to 3′ *HOX3* genes, which are active in all the cell lines, and to the *HOX1A–K* and the *HOX4* genes, which are everywhere either silent or barely detectable. The three genes *1F*, *2I*, and *4I* located at the extreme 3′ end of *HOX1* and *HOX2* and at the far 5′ end of *HOX4* represent somehow special cases.

DISCUSSION

Our results demonstrate that cells representing various steps of hematopoietic differentiation display differential patterns of *HOX* gene expression and highlight that *HOX* genes are switched on or off in blocks. These blocks contain a variable

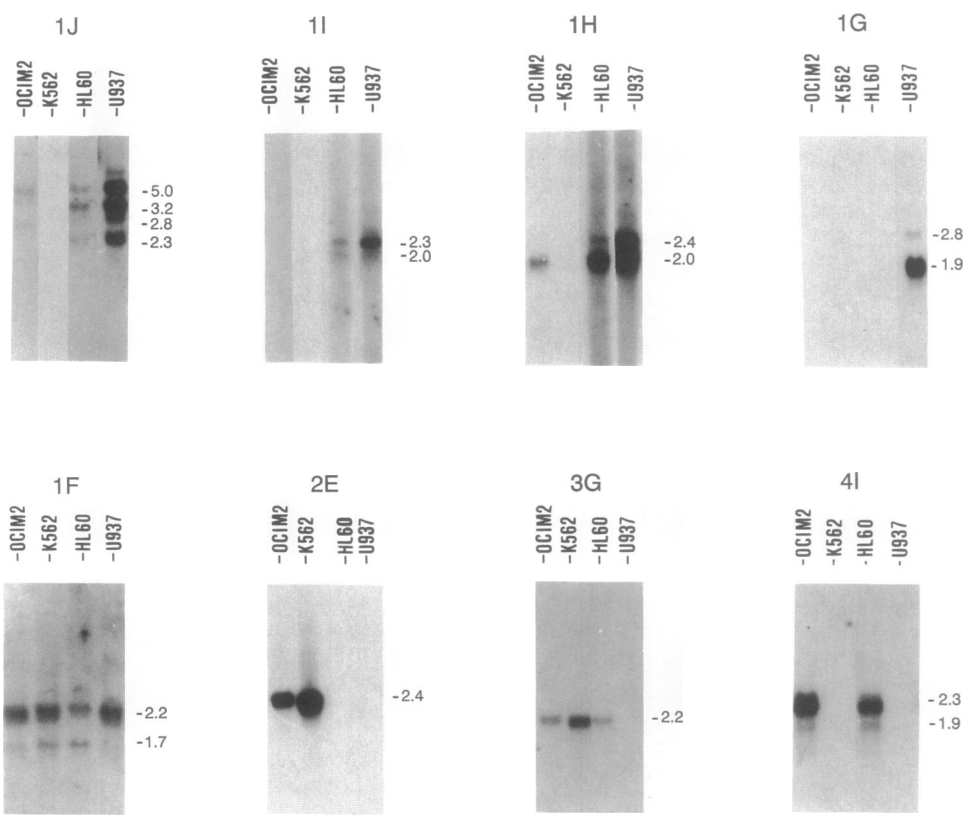

FIG. 2. Comparison of *HOX* gene expression patterns in OCIM2, K562, HL60, and U937 cells. Northern blots of 5 μg of poly(A)$^+$ RNA from the various cell lines were hybridized with probes representing the 3′ untranslated region of *HOX1J*, *1I*, *1H*, *1G*, *1F*, *2E*, *3G*, and *4I*. Transcript sizes are given in kb.

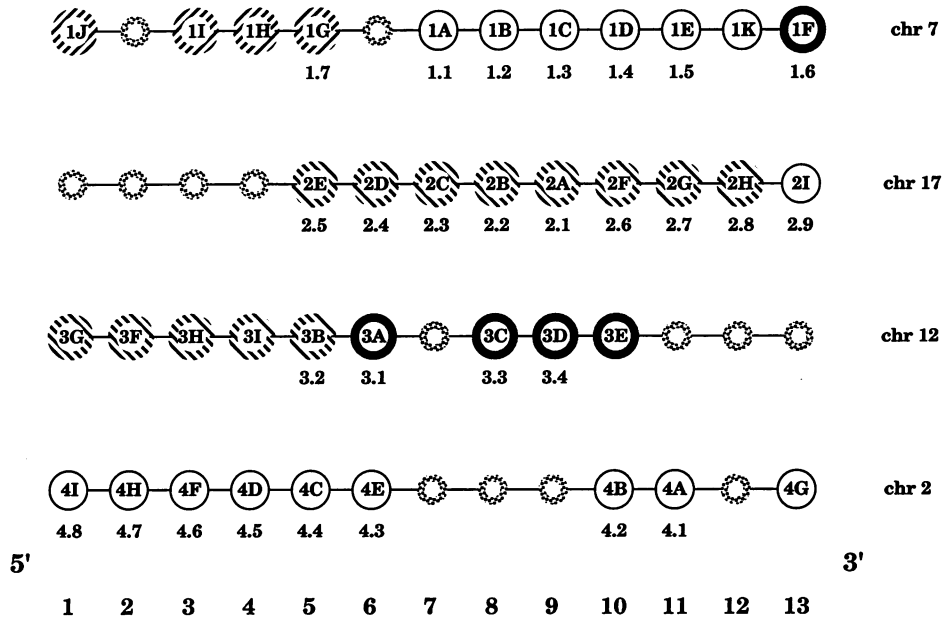

FIG. 3. Schematic representation of *HOX* gene expression in erythroleukemic, HL60, and U937 cells. The horizontal alignment of *HOX* genes represents their physical position on each chromosome. The vertical alignment identifies 13 groups with maximal sequence homology of the homeodomains. The designations of the known murine *Hox* homologs are shown below the circles. Stippled circles indicate homeodomains predicted in the scheme but not yet identified. ○, *HOX* genes not expressed in all the cell lines; ●, *HOX* genes expressed in all the cell lines; ◌, *HOX* genes inactive in erythroleukemic cells and switched on in myeloid-restricted cells; ◌, *HOX* genes active in erythroleukemic cells and switched off in myeloid-restricted cells.

number of contiguous genes within each locus. Four genes 5′ of *HOX1*, silent in erythroleukemic cells, become active in cells already committed to myeloid differentiation. Similarly, although in the opposite direction, genes 5′ of *HOX3*, well expressed in more primitive cells, are switched off in the maturation process of myeloid cells. Furthermore, almost the entire *HOX2* locus is expressed at significant levels in erythroleukemic cells whereas it is silent in myelomonocytic elements. Finally, features common to all the lines analyzed are the undetectable expression of *HOX4* and, conversely, the ubiquitous expression of the genes located at the 3′ end of *HOX3*. It appears that the expression of blocks of genes or even of entire *HOX* loci is coordinately regulated in hematopoietic cells. There have been previous reports that *HOX* genes are expressed in hematopoietic tissue (15, 17). The expression of a few individual genes has been analyzed in numerous human cell lines representing erythroid, myeloid, and T- and B-cell lineages. In these studies, lineage-restricted expression of certain genes has been found and modulation of expression of some *HOX* genes was observed on terminal differentiation; thus, a correlation between the expression of individual genes and cell phenotype was suggested. Our study, aimed to analyze the entire organization of *HOX* gene clusters, indicates that it is the activation and/or the inactivation of sets of genes that correlates with cell differentiation rather than the expression of specific *HOX* genes or specific alternative transcripts. It has been observed previously that the highly conserved organization of *HOX* genes in clusters seems to reflect a regulatory hierarchy within this gene family. For example, in *Drosophila*, the physical order of the genes within the clusters correlates with the order in which they are expressed along the anteroposterior axis of the embryo (36). This colinearity has been observed also in mammals (6). Our results provide evidence that in hematopoietic cells the positional hierarchy of *HOX* genes reflects a regulatory hierarchy. These observations are consistent with the idea that one or more upstream promoter elements account for the concerted expression of *HOX* genes in specific hematopoietic cell lineages. Experimental evidence for a major promoter upstream of several HD-containing exons of the *HOX3* locus has been reported (35). It is conceivable therefore that other loci may have a similar transcriptional organization.

It is interesting to note that homeobox genes within the four *HOX* loci can be vertically aligned on the basis of the maximal sequence homology of their homeodomains. This alignment defines 13 homology groups (33). In some instances, for example in the mouse embryonic central nervous system and prevertebral system, it has been found that corresponding genes within the groups are expressed in the same domains (37). This does not seem to be the case in the hematopoietic system; here *HOX* gene expression appears rather to be concerted within the individual loci. However, we observed that vertical group 5 seems to represent a boundary that defines genes 3′ and 5′ displaying different expression patterns. It is also noteworthy that, in other systems, group 5 seems to mark a distinction between upstream and downstream genes. For example, in teratocarcinoma cells induced to differentiate with retinoic acid, genes 5′ of group 5 remain silent as in undifferentiated cells whereas genes 3′ of group 5 are activated (32).

The almost identical expression pattern observed in similar cell lines together with the profound differences detected in lines representing cells with different phenotypes suggest that the pattern of *HOX* genes is not associated with the establishment of cell lines in culture but rather is correlated with cell type. Our results seem to indicate that commitment to the myelomonocytic lineage involves the inactivation of eight of nine genes of the *HOX2* locus. This result is consistent with an interesting observation recently reported by Perkins *et al.* (19). They showed that normal bone marrow cells infected with a retroviral vector containing both *HOX2.4* cDNA and the gene encoding interleukin 3 (IL-3) develop a transplantable myeloid leukemia *in vivo* with elevated levels of immature cells. However, constitutive expression of the IL-3 gene alone caused a nontransplantable myeloproliferative syndrome with a great excess of mature cells. Perkins *et al.* hypothesize that *HOX2.4* expression impedes the programmed terminal differentiation of myeloid cells. Our observations raise the possibility that the down regulation of the entire *HOX2* locus might be required for cell maturation.

In addition, our data suggest that commitment to the myelomonocytic lineage involves the switch on of a set of genes 5′ of *HOX1*. This conclusion is also supported by the observation that primary cells of acute myeloid leukemias show relatively high expression of 5′ *HOX1* genes (unpublished work). Further investigation will be required to assess to what extent myeloid differentiation and 5′ *HOX1* gene expression are correlated events.

It is interesting to note that virtually the entire *HOX4* locus is silent in all the cell lines analyzed. The undetectable expression of *HOX4* genes suggests that this locus either is

not involved in hematopoiesis or that the *HOX4* cluster is expressed only in a very restricted group of cells not represented in our cell lines. In summary, the results presented here suggest that the *HOX* gene clusters display characteristic patterns of expression in different hematopoietic cell lineages and provide an indication that the coordinate regulation of *HOX* genes may play an important role in lineage determination during early steps of hematopoiesis. Therefore, it will be of interest to examine the expression of the *HOX* gene clusters among purified populations of progenitor cells within the stem cell hierarchy.

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