Close Localization of the Genes for GM-CSF and IL3 in Human Genome

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Colony-stimulation factors (CSFs), a family of glycoprotein growth factors, have been shown to support clonal proliferation of hematopoietic progenitor cells in vitro [1]. Macrophage-CSF (M-CSF or CSF-1) [2] and granulocyte-CSF (G-CSF) [3] stimulate cells committed to the macrophage and granulocyte lineages respectively, whereas granulocyte-macrophage-CSF (GM-CSF) and interleukin-3 (IL3 or multi-CSF) are capable of stimulating proliferation and differentiation of progenitors along multiple pathways.

Successful cloning of cDNA and genomic copies of mouse and human genes for IL3 [3, 4] and GM-CSF [5, 6], as well as for M-CSF and G-CSF, have had a great impact on the analysis of biological properties of those molecules in vivo and in vitro [7, 8].

The GM-CSF and IL3 genes have been mapped to human chromosome 5 at bands q23-31 [9, 10], a region that is frequently deleted in patients with myeloid disorders [del(5q)] [11]. Several other growth factors and growth-factor receptors – in particular, the CSF-1 gene and proto-oncogene FMS, coding a protein possibly identical to the receptor for CSF-1 – are also located within this region of chromosome 5 [12]. There is a possibility that a family of genes responsible for regulation of cell growth during hematopoiesis is located within the limited segment of chromosome 5 [9]. Precise mapping of this region is essential to the understanding of functional relationships between the genes and could reveal the genes for other growth factors and their receptors that may be located within this region.

For isolation of genomic DNA clones containing genes for human GM-CSF and IL3 we prepared from human leukocyte DNA a genomic library of 1.5×10^6 clones. Using synthetic oligonucleotides, we identified eight individual clones that hybridized with the IL3 probe and five clones that hybridized with the GM-CSF probe; three of these hybridized with both probes. Southern blot hybridization analysis of DNA restriction fragments of individual phages revealed that three independently cloned 20-kb fragments of human genomic DNA contain sequences of both IL3 and GM-CSF genes. Physical maps of the isolated clones are shown in Fig. 1.

Next, we analyzed localization of the two genes in human placental DNA using Southern blot analysis (Fig. 2). The fragments generated by *Xba*I restriction endonuclease hybridized with both IL3 and GM-CSF probes.

The results strongly indicate a close genomic linkage of human IL3 and GM-CSF genes. The distance between the genes is 10 kb, and they are arranged in head-to-tail fashion, the gene for GM-CSF following the gene for IL3.

Close linkage of the two CSF genes may indicate either that they have coordinate regulation during T-lymphocyte gene expression, or that they have diverged from a common ancestral gene by duplication. The latter hypothesis is supported by the fact that both genes have similar exon-intron structures and com-

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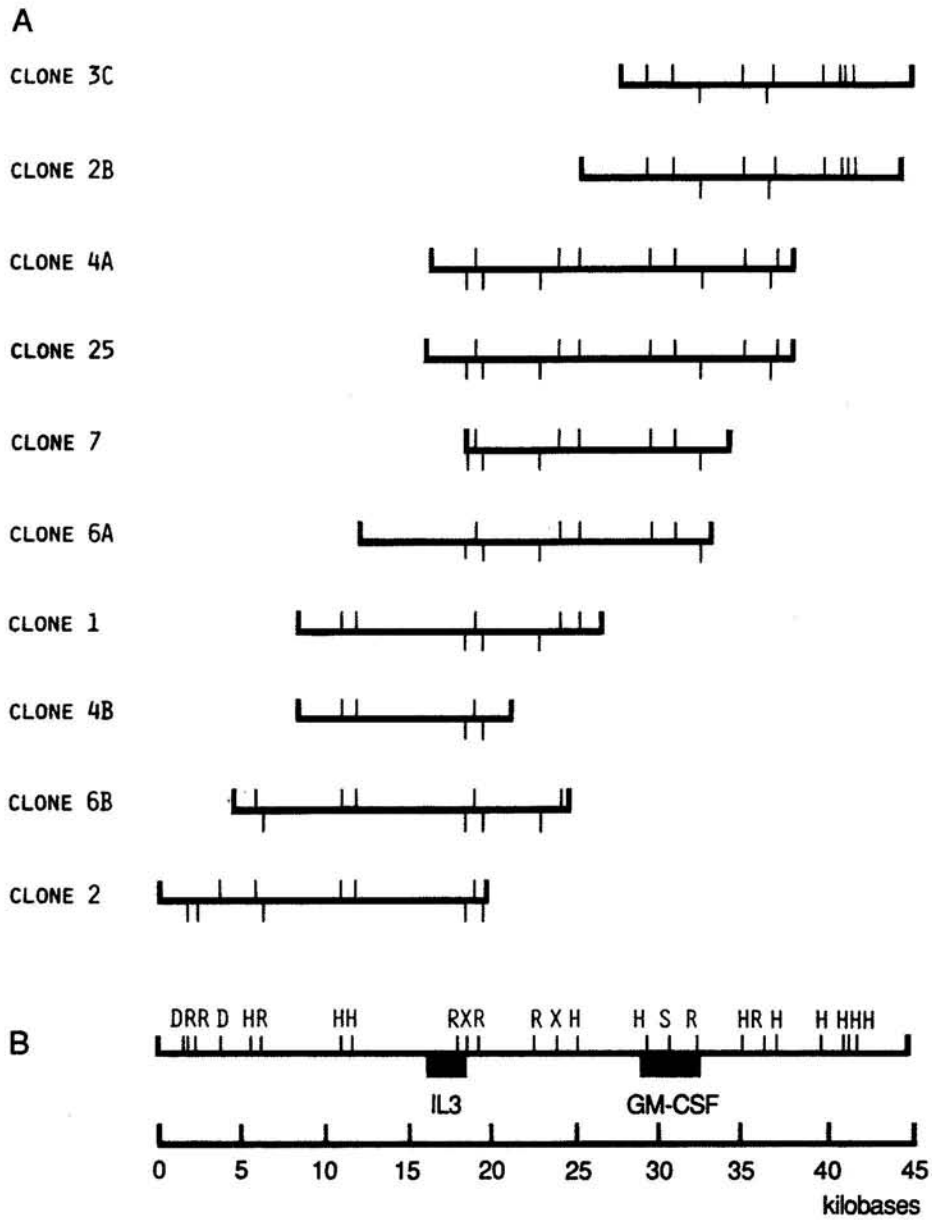

Fig. 1. A Physical maps of the individual phage clones containing genes for human GM-CSF and IL3. The positions of the recognition sites for restriction endonucleases *Eco*RI (*R*), *Hind*III (*H*), *Xho*I (*X*), *Sal*I (*S*), and *Xba*I (*D*) are indicated. **B** Physical map of the whole region of human genome containing the genes

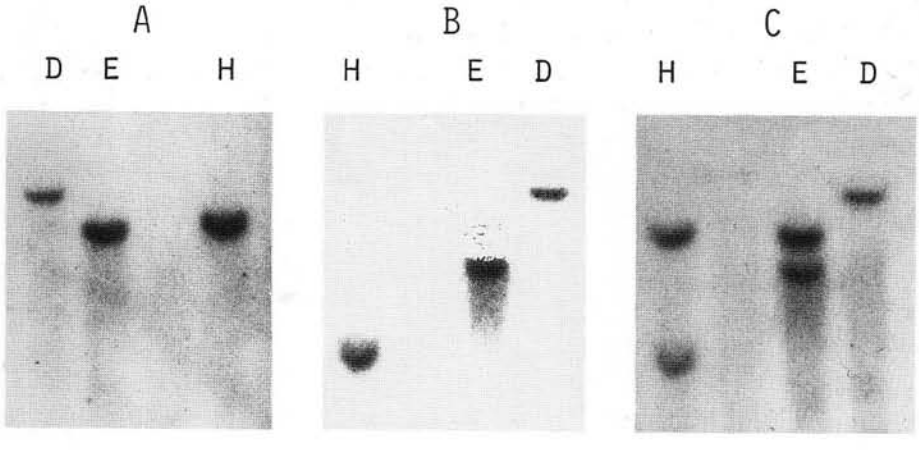

Fig. 2A–C. Southern blot analysis of human DNA. The DNA from human placenta was cleaved with *Eco*RI (*E*), *Hind*III (*H*), and *Xba*I (*D*) and hybridized with the probes for IL3 **(A)**, GM-CSF **(B)**, and with both probes together **(C)**

mon features in the secondary structure of the two polypeptides displayed in distribution of alfa-helical regions.

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