Cytogenet Cell Genet 74:255–258 (1996)

Cytogenetics and Cell Genetics

Localization of the gene for the human MIG cytokine on chromosome 4q21 adjacent to INP10 reveals a chemokine "mini-cluster"

H.-H. Lee and J.M. Farber

Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, Bethesda MD (USA)

Abstract. MIG is a gamma interferon-inducible T cell chemoattractant that is a member of the chemokine family of cytokines. P1 clones containing the MIG gene were found also to contain the gene of a related chemokine, INP10. We localized MIG and INP10 to 4q21 by FISH, demonstrated by pulsed field gel electrophoresis that MIG and INP10 are distant from the tight cluster of other CXC chemokine genes at 4q12→q13, and determined that MIG and INP10 are oriented head to tail with their start codons separated by less than 16 kb.

MIG and INP10 are members of the chemokine family of small, inducible, secreted proteins. Although the best described activities of the chemokines are as chemotactic factors, chemokines also have effects on T cell activation, angiogenesis, and HIV infection. Most chemokines can be assigned to one of two subfamilies based on the arrangement of conserved cysteine residues found in their amino-terminal regions. CXC chemokines contain a single amino acid residue between the first and second invariant cysteines, while in the CC chemokines, these cysteines are adjacent. While most CXC chemokines are chemotactic for neutrophils, MIG and INP10 are unusual and similar in being CXC chemokines that are chemotactic for lymphocytes and inactive on neutrophils (Liao et al., 1995; Taub et al., 1993).

The CC chemokine genes are found as a cluster at 17q11→q21 (Rollins et al., 1991). With the exception of SDF1, a more distantly related member of the CXC subfamily that has been mapped to 10q11.1 (Shirozu et al., 1995), all the CXC chemokine genes for which data are available have been localized to 4q12→q21: platelet basic protein (PPBP) at 4q12→q13 (Wenger et al., 1991), GRO1/melanoma growth-stimulatory activity at 4q13→q21 (Richmond et al., 1988), platelet factor 4 (PF4) at 4q12→q21 (Griffin et al., 1987), ENA-78 (SCYB5) at 4q13→q21 (Chang et al., 1994) and INP10 at 4q21 (Luster et al., 1987).

Tunnacliffe et al. (1992) have shown that IL8, GRO1, GRO2, GRO3, PPBP and PF4 can all be found on a 700-kb *Sfi*1 fragment localized to 4q12→q13. Consistent with the studies placing INP10 at 4q21 (Luster et al., 1987) this gene could not be localized to the same tight cluster. As part of studies to discover new chemokine genes, we obtained P1 clones containing the MIG gene and we have shown that MIG and INP10 are adjacent at 4q21, supporting other data on the close relationship between these two genes and their proteins.

Supported by NIAID Intramural Research Program.

Received 27 February 1996; revision accepted 13 June 1996.

Request reprints from Dr. Joshua M. Farber, Bldg. 10 Rm. 11N-228 (U.S. Mail), Bldg. 10, Rm. 11C-104 (courier service), NIH, 9000 Rockville Pike, Bethesda MD 20892 (USA); telephone: 301-402-4910; fax: 301-496-7383; e-mail: joshua-farber@NIH.gov

Current address of H.-H.L.: Department of Microbiology and Immunology, Chonbuk National University Medical School, Institute for Medical Sciences, Chonju, Chonbuk 560-182 (Republic of Korea).

Materials and methods

P1-Clones

Clones in the P1-based vector p*AD*10*sac*BII (Pierce et al., 1992) were obtained from Genome Systems, St. Louis MO. Screening of the P1 human genomic library was done using PCR primers with sequences derived from the 3′-non-translated region of the MIG cDNA (5′ primer 5′-ATGTTCACC-CAACCACATCC-3′, bases 699–718; 3′-primer 5′-CACTGTGGAAGAAA-

KARGER

E-mail karger@karger.ch
Fax + 41 61 306 12 34
http://www.karger.ch

CAGGGA-3′, bases 1420–1439) and using an annealing temperature of 60 °C. For screening P1 clones for additional chemokine genes the primers were as follows:

INP10	5′-GCTGTGCAATCGCTGGTTTA-3′ and
	5′-CCCCAAAGCAGAAAGATTCC-3′
GRO1	5′-AAGCCCCTTTGTTCTAAGCC-3′ and
	5′-CTGCCCTTATAGGAACAGAA-3′
IL8	5′-GTGTGGGTCTGTTGTAGGGT-3′ and
	5′-ACAGCACTACCAACACAGCT-3′
PF4	5′-CACGCTGAAGAATGGAAGGA-3′ and
	5′-CACAGTTAGATTGAAACTGG-3′
PF4V1	5′-CTGAAGCTGAAGAAGATGGG-3′ and
	5′-CCAAGCCTTCCTTCATAATG-3′
PPBP	5′-GCCAAACTTCTTTAACTCCC-3′ and
	5′-CTTTTAACCAACCTTCTCAG-3′.

FISH

The MIG and INP10 probes are described in the text. The chromosome 4-specific probe was α-satellite probe D4Z1 obtained from Oncor Corp., Gaithersburg MD. FISH was performed as a service by the Laboratory of Cell and Molecular Structure, SAIC Frederick, NCI-Frederick Cancer Research and Development Center. FISH was done on metaphase chromosome spreads from human peripheral blood lymphocytes with digoxigenylated probes using standard techniques (Verma and Babu, 1995). Probes were digoxigenylated by nick translation and hybridizations were done using 15 ng per probe with 1,575 ng Cot-1 DNA (Life Technologies, Gaithersburg MD) per slide in 50% formamide, 2 × SSC at 37 °C for 16 h followed by washing with 2 × SSPE at 72 °C for 5 min. Probes were detected using anti-digoxigenin-fluorescein isothiocyanate. Chromosomes were counterstained with DAPI (MIG) or propidium iodide (INP10) and images were obtained and fused electronically using a Zeiss Axioscop and the Oncor Imaging System. Band assignments for MIG and INP10 were determined by measuring relative distances of the MIG and INP10 probes with respect to the border between the C band (centromere) and the first euchromatic band (4q11) and the telomere using published data (Francke, 1994).

Pulsed-field gel electrophoresis

Using standard techniques (Barlow, 1992), 5×10^6 human polymorphonuclear leukocytes were lysed in agarose plugs and digested with rare cutters *Mlu*I, *Not*I, *Sac*II and *Sfi*I (New England Biolabs, Beverly MA). One-half plug was loaded per lane on a 1% agarose gel that was run in a CHEF-DRII apparatus (Bio-Rad, Richmond CA) for 28 h at 200 V with a pulse time ramped linearly from 40 to 120 s. Following acid depurination, DNA was transferred to a Zeta-Probe nylon membrane (Bio-Rad), and sequential hybridizations and strippings were done according to the manufacturer's protocols. Probes were prepared using random nonamer primers with a kit and protocols from Amersham, Buckinghamshire, UK. MIG probe was a 2.5-kb cDNA, the INP10 probe a 1.2-kb cDNA, the IL8 probe a 1.75-kb cDNA (Matsushima et al., 1988) and the PF4 probe a 0.28-kb cDNA (Poncz et al., 1987). The IL8 cDNA was obtained from Kouji Matsushima and Joost Oppenheim, National Cancer Institute and the PF4 cDNA was obtained from Mortimer Poncz, Children's Hospital of Philadelphia. Exposures were done using an intensifying screen, and before reprobings, the filter was exposed to film to ensure complete removal of signal.

Results and discussion

A bacteriophage P1-based human genomic library was screened at Genome Systems, St. Louis MO using PCR primers with sequences derived from the 3′-non-translated region of the MIG cDNA. Positive clones P1-592 and P1-593 were identified. These clones contain human genomic fragments inserted into a *Bam*HI site in the P1 vector p*AD*10*sacB*II (Pierce et al., 1992). Digestion of P1-592 and P1-593 with *Bam*HI revealed extensive overlap between the two fragments of human DNA. P1-593 contained additional inserted DNA as compared to P1-592 and further restriction analysis revealed that P1-593 contained a total of approximately 85 kb of inserted DNA. Using appropriate primer pairs (see Materials and methods), P1-592 and P1-593 were screened by PCR for the genes for the additional CXC chemokines INP10, GRO1, IL8, PF4, PF4V1 and PPBP. Besides the gene for MIG, both P1-592 and P1-593 were positive for the INP10 gene, but not for the other CXC chemokine genes (not shown). Studies were done to map the MIG gene by FISH, to analyze the relationships of the MIG and INP10 genes to the genes for the other chemokines by pulsed-field gel electrophoresis, and to refine the map of the MIG and INP10 genes by analysis of the human genomic DNA in P1-593.

For localization of the MIG gene by FISH, 5.2-kb and 6.8-kb *Bam*HI fragments containing the MIG gene were isolated from P1-593 (see below) and cloned. For purposes of comparison and control, we also undertook to localize the INP10 gene. For INP10 we used as probes cloned fragments totaling 6.6 kb that had been obtained by *Bgl*II digestion of the 10.3-kb *Bam*HI fragment, containing INP10 sequences, that was isolated from P1-593 (see below). An α-satellite probe was included in each hybridization to identify the centromere of chromosome 4. As expected, and as shown in Fig. 1A, both MIG and INP10 hybridized to the long arm of chromosome 4. Band assignments were made as described in Materials and methods. Measurements for the MIG probe were made on nine chromosome spreads and these placed the signals between 4q21.1 and 4q21.3, with four of the measurements at 4q21.21. For the INP10 probes, measurements were made on three spreads and in each case the signal was placed at 4q21.21.These studies, consistent with the data from the P1 clones and with the published information on INP10, established the chromosome location and band position for MIG and confirmed that MIG and INP10 are found telomeric to the cluster of other CXC chemokine genes identified previously at 4q12→q13 (Tunnacliffe et al., 1992).

The relationship between the MIG and INP10 genes and CXC chemokine genes in the cluster at 4q12→q13 was investigated directly using pulse-field gel electrophoresis. As shown in Fig. 1B, the patterns for MIG and INP10 were identical. The fragments hybridizing to probes for IL8 and PF4 did not match the MIG and INP10 fragments. IL8 and PF4 hybridized to identical *Not*I and *Sfi*I fragments, consistent with published data placing IL8 and PF4 in the 4q12→q13 cluster.

The P1-593 DNA was used to establish more precisely the relationship between the INP10 and MIG genes. To verify that no artifactual changes had occurred during the cloning of the P1-593 genomic insert, P1-593 DNA and total human genomic DNA were digested with *Bam*HI and analyzed by Southern blot as shown in Fig. 2A. Probing with MIG and INP10 cDNA probes identified identical fragments in P1-593 and total genomic DNA, with the INP10 gene on a 10.3-kb fragment and the MIG gene present on fragments of 5.2 kb and 6.8 kb. These fragments can be identified in the ethidium bromide-stained gel of digested P1-593 DNA shown in Fig. 2A. Using *Nde*I and *Xba*I fragments of P1-593 DNA as probes, Southern analysis (not shown) enabled us to order six of the *Bam*HI fragments of P1-593 in the region of the INP10 and MIG genes, as shown in

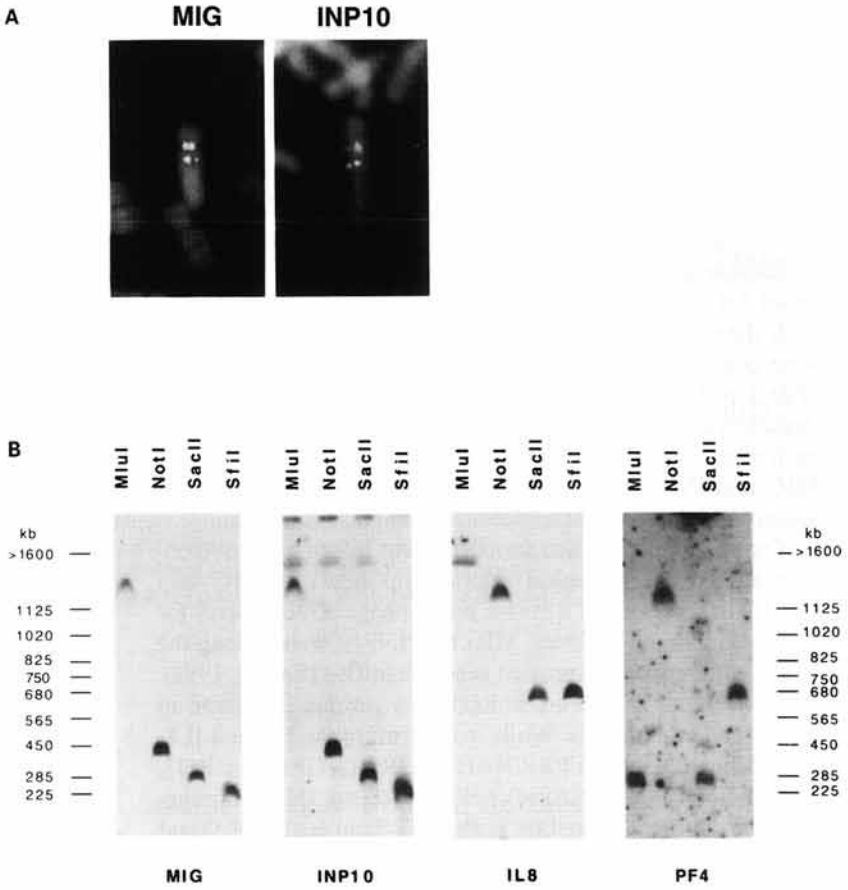

Fig. 1. (**A**). Localization of the MIG and INP10 genes by FISH on human chromosome 4. The α-satellite probe D4Z1 was used to identify the centromere of chromosome 4. The MIG and INP10 signals are at 4q21. (**B**). Analysis of genes for CXC chemokines by pulsed-field gel electrophoresis. Human polymorphonuclear leukocytes were lysed in agarose plugs and the DNA was digested with rare cutters *Mlu*I, *Not*I, *Sac*II and *Sfi*I as indicated. Chromosomes of *S. cerevisiae* were run as size markers. For the sake of clarity, only the positions of selected chromosomal markers are shown. Sequential hybridizations and strippings of a single blot were done using the cDNA probes as indicated.

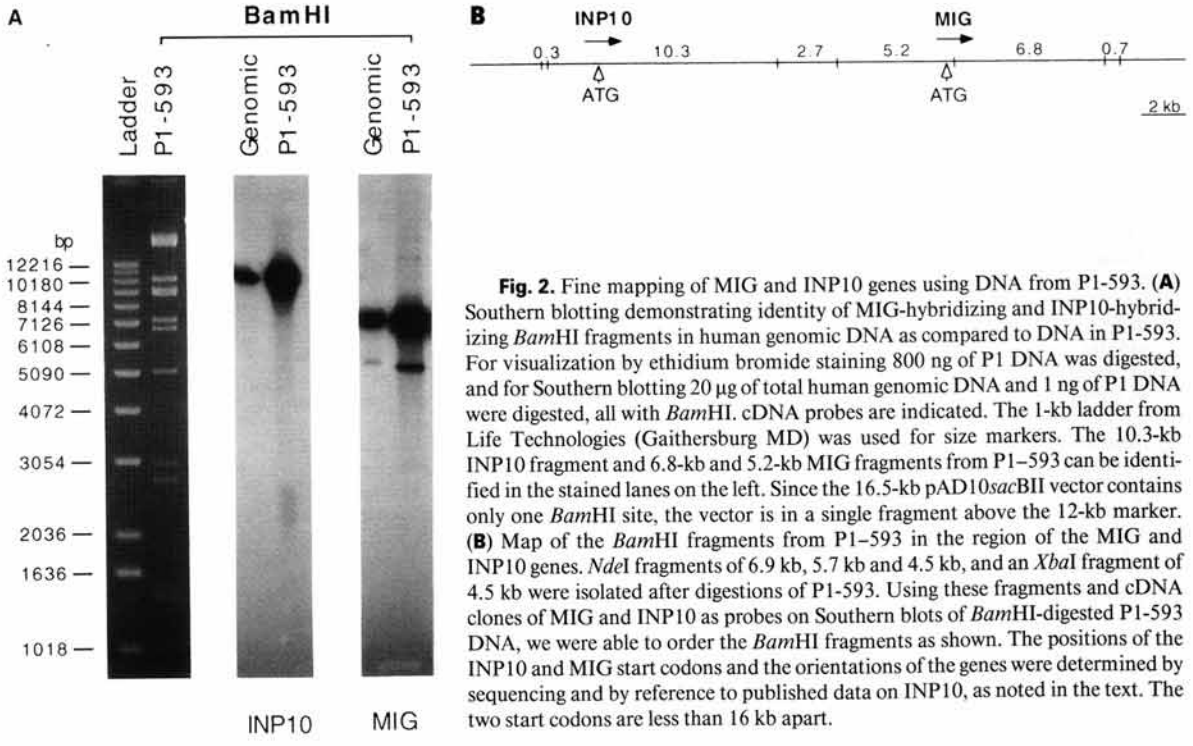

Fig. 2. Fine mapping of MIG and INP10 genes using DNA from P1-593. (**A**) Southern blotting demonstrating identity of MIG-hybridizing and INP10-hybridizing *Bam*HI fragments in human genomic DNA as compared to DNA in P1-593. For visualization by ethidium bromide staining 800 ng of P1 DNA was digested, and for Southern blotting 20 μg of total human genomic DNA and 1 ng of P1 DNA were digested, all with *Bam*HI. cDNA probes are indicated. The 1-kb ladder from Life Technologies (Gaithersburg MD) was used for size markers. The 10.3-kb INP10 fragment and 6.8-kb and 5.2-kb MIG fragments from P1–593 can be identified in the stained lanes on the left. Since the 16.5-kb pAD10*sac*BII vector contains only one *Bam*HI site, the vector is in a single fragment above the 12-kb marker. (**B**) Map of the *Bam*HI fragments from P1–593 in the region of the MIG and INP10 genes. *Nde*I fragments of 6.9 kb, 5.7 kb and 4.5 kb, and an *Xba*I fragment of 4.5 kb were isolated after digestions of P1-593. Using these fragments and cDNA clones of MIG and INP10 as probes on Southern blots of *Bam*HI-digested P1-593 DNA, we were able to order the *Bam*HI fragments as shown. The positions of the INP10 and MIG start codons and the orientations of the genes were determined by sequencing and by reference to published data on INP10, as noted in the text. The two start codons are less than 16 kb apart.

the map in Fig. 2B. The position for the start codon and the orientation of the MIG gene were determined by sequencing the ends of the 5.2-kb and 6.8-kb fragments (not shown), while these data for the INP10 gene were obtained by combining our mapping results and published data (Luster et al., 1987). The MIG and INP10 genes are oriented head to tail, and their start codons are less than 16 kb apart.

The positioning of the MIG and INP10 genes adjacent to each other and at some distance from the cluster containing genes for PF4 and the IL8-related chemokines is consistent with the relationships revealed by a computer-generated pairwise comparison of the CXC chemokine protein sequences (Pile Up, Genetics Computer Group, Madison, WI), which demonstrates that MIG and INP10 are more closely related to each other than they are to other chemokines (data not shown). MIG and INP10 show 37% amino acid identity over the 76 amino acids of the mature proteins that are comparable.

Our mapping data also support recent information demonstrating a close biological relationship between MIG and INP10. In screening of a mouse macrophage cDNA library for lymphokine-induced genes, MIG and INP10 were among the eleven differentially-expressed genes identified (Farber, 1992). Both MIG and INP10 are inducible by gamma interferon in mouse and human cells. While in contrast to the IL8 and IL8-related chemokines (PPBP/NAP-2, GRO1, GRO2, GRO3, GCP-2, ENA-78, and NAP-4), neither MIG nor INP10 is a chemoattractant for neutrophils, both MIG (Liao et al., 1995) and INP10 (Taub et al., 1993) can act *in vitro* as chemotactic factors for activated T cells. Moreover, MIG and INP10 each desensitize T cells to the effects of the other in a calcium flux assay, suggesting that MIG and INP10 share a receptor (Liao et al., 1995).

The proximity of the MIG and INP10 genes suggests that they arose from a common ancestor. Moreover, MIG/INP10 gene duplication was not a very recent event, since both genes have mouse homologues (Farber, 1992; Vanguri and Farber, 1990) and we have obtained a P1 clone from a mouse genomic library that contains genes for the murine homologues of both MIG and INP10 (D. Amichay and J.M Farber, unpublished). Recent work has suggested that the range of activities for the chemokines may be broader than appreciated initially, including functions beyond leukocyte trafficking especially with regard to lymphocyte physiology. It will be of interest to discover if the MIG/INP10 "mini-cluster" contains additional interferon-inducible genes and/or related CXC chemokines that specifically target lymphocytes.

Acknowledgements

The authors are grateful to Steven Rivard, Danuta Laskowska, and Matthew Gonda of SAIC Frederick for performing FISH, to Mortimer Poncz for providing the PF4 probe, to Kouji Matsushima, Joost Oppenheim, and Howard Young for providing the IL8 probe, to Brian Moldover for help with multiple sequence comparisons, and to Keith Peden for helpful discussions.

References

Barlow DP: Preparation, restriction, and hybridization analysis of mammalian genomic DNA for pulsed-field gel electrophoresis, in Burmeister M, Ulanovsky L (eds): Methods in Molecular Biology, pp 107–128 (The Humana Press Inc., Totowa 1992).

Chang M, McNinch J, Basu R, Simonet S: Cloning and characterization of the human neutrophil-activating peptide (ENA-78) gene. J biol Chem 269:25277–25282 (1994).

Farber JM: A collection of mRNA species that are inducible in the RAW 264.7 mouse macrophage cell line by gamma interferon and other agents. Mol cell Biol 12:1535–1545 (1992).

Francke U: Digitized and differentially shaded human chromosome ideograms for genomic applications. Cytogenet Cell Genet 65:206–219 (1994).

Griffin CA, Emanuel B, Larocco P, Schwartz E, Poncz M: Human platelet factor 4 gene is mapped to 4q12→q21. Cytogenet Cell Genet 45:67–69 (1987).

Liao F, Rabin RL, Yannnelli JR, Koniaris LG, Vanguri P, Farber JM: Human Mig chemokine: biochemical and functional characterization. J exp Med 182:1301–1314 (1995).

Luster AD, Jhanwar SC, Chaganti RSK, Kersey JH, Ravetch JV: Interferon-inducible gene maps to a chromosomal band associated with a (4;11) translocation in acute leukemia cells. Proc natl Acad Sci, USA 84:2868–2871 (1987).

Matsushima K, Morishita K, Yoshimura T, Lava S, Kobayashi Y, Lew W, Appella E, Kung HF, Leonard EJ, Oppenheim JJ: Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. J exp Med 167:1883–1893 (1988).

Pierce JC, Sauer B, Sternberg N: A positive selection vector for cloning high molecular weight DNA by the bacteriophage P1 system: improved cloning efficacy. Proc natl Acad Sci, USA 89:2056–2060 (1992).

Poncz M, Surrey S, Larocco P, Weiss MJ, Rappaport EF, Conway TM, Schwartz E: Cloning and characterization of platelet factor 4 cDNA derived from a human erythroleukemic cell line. Blood 69:219–223 (1987).

Richmond A, Balentien E, Thomas HG, Flaggs G, Barton DE, Spiess J, Bordoni R, Francke U, Derynk R: Molecular characterization and chromosomal mapping of melanoma growth stimulatory activity, a growth factor structually related to β-thromboglobulin. EMBO J 7:2025–2033 (1988).

Rollins B, Morton CC, Ledbetter DH., Eddy RLJ, Shows TB: Assignment of the human small inducible cytokine A_2 gene, $SCYA_2$ (encoding JE or MCP-1), to 17q11.2→12: Evolutionary relatedness of cytokines clustered at the same locus. Genomics 10:489–492 (1991).

Shirozu M, Nakano T, Inazawa J, Tashiro K, Toda H, Shinohara T, Honjo T: Structure and chromosomal localization of the human stromal cell-derived factor 1 (SDF1) gene. Genomics 28:495–500 (1995).

Taub DD, Lloyd AR, Conlon K, Wang JM, Ortaldo JR, Harada A, Matsushima K, Kelvin DJ, Oppenheim JJ: Recombinant human interferon-inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. J exp Med 177:1809–1814 (1993).

Tunnacliffe A, Majumdar S, Yan B, Poncz M: Genes for β-thromboglbulin and platelet factor 4 are closely linked and form part of a cluster of related genes on chromosome 4. Blood 79:2896–2900 (1992).

Vanguri P, Farber J: Identification of CRG-2: An interferon-inducible mRNA predicted to encode a murine monokine. J biol Chem 265:15049–15057 (1990).

Verma RS, Babu A: Human Chromosomes, Principles and Techniques, Second Edition (McGraw-Hill, New York 1995).

Wenger RH, Hameister H, Clemetson KJ: Human platelet basic protein/connective tissue activating peptide-III maps in a gene cluster on chromosome 4q12→q13 along with other genes of the β-thromboglobulin superfamily. Hum Genet 87:367–368 (1991).