SHORT COMMUNICATION

Mybl2 (*Bmyb*) Maps to Mouse Chromosome 2 and Human Chromosome 20q13.1

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Mybl2 encodes a transcription factor that is thought to play an important role in cell cycle progression. Here we report the chromosomal localization of *Mybl2* in mouse and human. Using mouse *Mybl2* cDNA clones as probes, we assigned *Mybl2* in an interspecific backcross panel to distal Chromosome 2. Using human cDNA probes in combination with FISH analysis, we localized *MYBL2* to chromosome 20q13.1, a region that is commonly deleted in myeloid disorders. Both chromosomal regions are highly homologous, and the map positions, therefore, confirm each other. However, our findings are in contrast to a previous report by Barletta *et al.* (*Cancer Res.* 51: 3821–3824, 1991) that placed the *MYBL2* gene on human chromosome Xq13.

The *Bmyb* gene (here referred to as myeloblastosis oncogene-like 2, *Mybl2*) belongs to the *Myb* family of transcription factor genes and plays an essential role during cell cycle progression. *Mybl2* transcripts are detectable in a wide variety of dividing cell types (9). MYBL2 activates cdc2 and cyclin D1 gene expression in proliferating fibroblasts, and antisense oligonucleotides specific to *MYBL2* inhibit proliferation of human hematopoietic cell lines (1, 17). *Mybl2* expression is also regulated at the G1/S phase transition, and its transcription relies on E2F activity in a cell cycle-dependent manner (10, 11, 15). Thus, unlike c-*myb* and *Amyb* (here referred to as *Mybl1*) whose transcriptional activity is mainly restricted to hematopoietic, spermatogenic, and neuronal progenitor cells, *Mybl2* appears to possess a broader function during cell proliferation (7, 12, 20). In addition to its distinct cellular function and expression pattern, MYBL2 also lacks the transactivation domain that is otherwise conserved between MYBL1 and C-MYB. Hence, the mechanism by which MYBL2 regulates transcription differs from that of MYBL1 and C-MYB, and as such chicken and mouse MYBL2 fail to transactivate c-*myb* responsive promot-

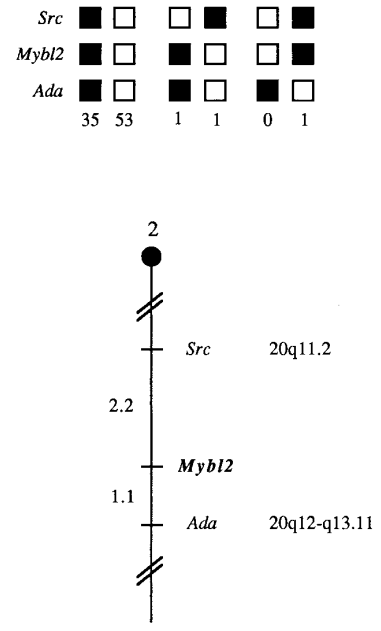

FIG. 1. *Mybl2* maps to the distal region of mouse Chromosome 2. *Mybl2* was placed on the mouse Chromosome 2 by interspecific backcross analysis. The segregation pattern of *Mybl2* and flanking genes in 91 backcross animals that were typed for all loci are shown at the top of the figure. Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*)F_1 parent. The shaded boxes represent the presence of a C57BL/6J allele, and white boxes represent the presence of a *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial Chromosome 2 linkage map showing the location of *Mybl2* in relation to linked genes is shown at the bottom of the figure. Recombination distances between loci in centimorgans are shown to the left of the chromosome, and the position of loci in human chromosomes, where known, are shown to the right (14). Recombination distances were calculated as described using the computer program SPRETUS MADNESS (6).

Mapping data from this article have been deposited in Mouse Genome Database under Accession No. MGD-CREX-485.

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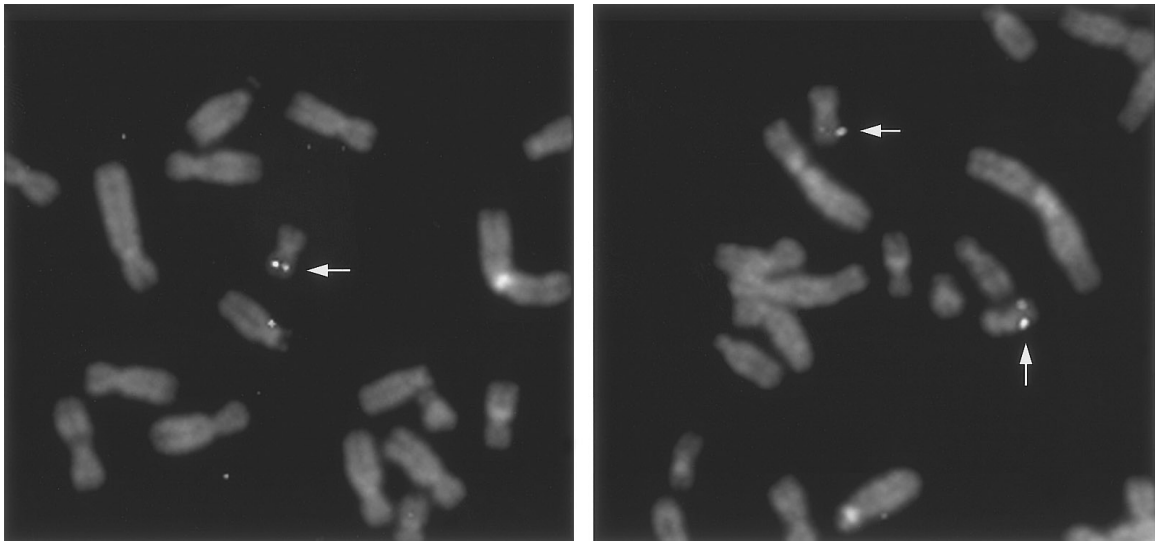

FIG. 2. *MYBL2* maps to human chromosome 20q13.1. Human lymphocytes from a normal male donor were cultured for 72 h at 37°C in RPMI 1640 medium containing phytohemagglutinin and 10% fetal bovine serum. The cells were synchronized by treatment with 5-bromdeoxyuridine (0.18 mg/ml, Sigma) for 16 h, followed by release from the block by incubation for 6 h in fresh medium containing 2.5 mg/ml thymidine (4). Metaphase cells were harvested and chromosome spreads were prepared according to standard procedures. Metaphase spreads were observed using a Zeiss Axiophot microscope. Images were captured by a cooled CCD camera connected to a computer workstation. Digitized images of DAPI staining and fluorescein signals were merged as described (19). Specific hybridization is observed at or beside chromosome 20q13.1 (arrows). The original images exhibited adjacent green (FITC, Bmyb30 probe) and red (rhodamine; Bmyb40 probe) signals or overlapping yellow signals. The photographs represent computer-generated images of FITC-signals, rhodamine signals, and DAPI-stained chromosomes.

ers. Instead, MYBL2 acts as an inhibitor of v-MYB- and c-MYB-induced transcription (5, 21). *MYBL2* was previously localized to human chromosome Xq13, a region that is involved in chromosomal abnormalities and myeloid neoplasias, suggesting that an altered *MYBL2* function accounts for the malignant transformation (2).

To evaluate *Mybl2* as a candidate gene for a mouse mutation we first isolated *Mybl2* clones from mouse cDNA libraries using the human *MYBL2* gene as probe (Bmyb30 and Bmyb40; kindly provided by Dr. N. Nomura (13)). The isolated cDNA clones were sequenced and proven to be identical with the published *Mybl2* cDNA sequence (pMBmyb1, (9, 10)). The mouse chromosomal location of *Mybl2* was then determined by interspecific backcross analysis using progeny derived from matings of (C57BL/6J × *Mus spretus*)F_1 × C57BL/6J mice (3). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer, and hybridization were performed essentially as described (8). The probe, an ~2-kb *Eco*RI fragment of the mouse cDNA clone pMBmyb1, was labeled with [α-^{32}P]dCTP using a nick-translation labeling kit (Boehringer Mannheim); washing was performed to a final stringency of 1.0× SSCP, 0.1% SDS, 65°C. The 3.1- and 1.4-kb *Pst*I *M. spretus*-specific RFLPs were used to follow the segregation of the *Mybl2* locus in backcross mice. The mapping results indicated that *Mybl2* is located in the distal region of mouse Chromosome 2 linked to *Src* and *Ada*. Ninety-one mice were analyzed for *Mybl2* and are shown in the segregation analysis (Fig. 1). The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are centromere–*Src*–2/91–*Mybl2*–1/91–*Ada*. The recombination frequencies expressed as genetic distances in centimorgans (cM) and the upper and lower 95% confidence limits are –*Src*–2.2 (5.14–0.74)–*Mybl2*–1.1 (3.26–1.06)–*Ada*. Comparison of our interspecific map with a composite mouse linkage map revealed that *Mybl2* maps to a region that lacks mouse mutations that may be associated with alterations in this locus (data not shown).

The distal region of mouse Chromosome 2 shares a region of homology with human chromosome 20 (summarized in Fig. 1). To determine whether *MYBL2* resides on chromosome 20 as predicted by the mouse mapping results, we applied fluorescence *in situ* hybridization (FISH) analysis on metaphase spreads. As probes we used the human cDNA clones, Bmyb30 (0.86 kb) and Bmyb40 (1.4 kb), which together span the entire coding region of *MYBL2* (13). Nick-translation was used to label Bmyb30 DNA with biotin 16–dUTP and Bmyb40 DNA with digoxigenin-11–dUTP (Boehringer Mannheim). The two probes were cohybridized to metaphase spreads. Hybridization of the biotin-labeled Bmyb30 probe was detected with avidin-FITC, and hybridization of digoxigenin-labeled Bmyb40 DNA was detected with anti-digoxigenin rhodamine. Chromosomes were counterstained with diamidino-2-phenylindole (DAPI, Oncor). As predicted from the position of *Mybl2* on the mouse linkage map, hybridization of the Bmyb30 and Bmyb40 probes to metaphase spreads revealed specific labeling on human chromosome 20 (Fig. 2). Bmyb30 FITC signals were detected on chromosome

20 in 27 of 30 metaphases examined. Among the 27 labeled spreads, signals were distributed as follows: 2 chromatids (17 cells), 3 chromatids (7 cells). Sixty-seven of 97 FITC signals (69.1%) were located at 20q and were concentrated at band q13.1. Bmyb40 rhodamine signals were detected on chromosome 20 in 21 of these same 30 metaphases, with the following distribution of signals: 1 chromatid (2 cells), 2 chromatids (10 cells), 3 chromatids (4 cells), 4 chromatids (5 cells). Altogether, 54 of 94 signals (57.4%) were localized on or beside band 20q13.1 (Fig. 2). A deletion of chromosome 20q is often associated with hematological disorders such as myeloid leukemia and myeloplastic syndrome (16, 18). Recently, the commonly deleted region of 20q, which is associated with myeloid leukemias, was confined to a small chromosomal region containing the *SRC* and *ADA* genes (16). Given the chromosomal location and its function during the cell cycle, *MYBL2* can be considered a possible candidate gene for this myeloid malignancy.

Our studies strongly suggest that *MYBL2* resides on human chromosome 20q13.1 and not on Xq13 as previously reported by Barletta *et al.* (2). Although both studies used the same reagent (Bmyb40), the reason for the discrepancy is not known.

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