Reassignment¹ of *Mycs* gene to mouse chromosome XA1.2–2 by radiation hybrid mapping and fluorescence in situ hybridization

Z.H. Shan and N.C. Popescu

Laboratory of Experimental Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda MD (USA)

¹ This report is discordant with a previous assignment of this gene to chromosome 19B using FISH by Sugiyama et al. (1999).

Rationale and significance

The mouse *Mycs* gene is a member of the intronless *Myc* gene family. Both human and rat *Mycs* genes have been mapped to chromosome X (Moton et al., 1989; Serikawa et al., 1992), while the mouse *Mycs* was initially localized by fluorescence in situ hybridization (FISH) to chromosome 19B (Sugiyama et al., 1999). The apoptosis-inducing activity of intronless *Myc* genes is evolutionarily conserved in mammalian cells but is somewhat distinct from *c-myc* although both require activation of caspase-3 like proteases (Asai et al., 1994; Kagaya et al., 1997, Sugiyama et al., 1999). As a part of our attempt to identify the genes amplified from double minute chromosomes originating from mouse chromosome 19 identified in a hepatocellular carcinoma developed in *Myc* transgenic mice (Zimonjic et al., 2001), we found by FISH and radiation hybrid (RH) mapping that the mouse *Mycs* gene is located on the X chromosome. This localization confirms the recent correct assignment of *Mycs* gene on the X chromosome by RH only (Yamasaki et al., 2001) and places the *Mycs* gene locus at region XA1.2–2 (Fig. 1). Furthermore, this mapping provides evidence that *Mycs* genes are conserved in vertebrates, consistent with their similar biological function.

Materials and methods

Fluorescence in situ hybridization (FISH)

Four specific primers were designed from mouse *Mycs* sequence, *Mycs*-1 from 3' UTR (for: 5'-GTGGTGGGAGAAATGCTTTTCAGCTG-3'; rev: 5'-AGCTCAGTGGCATATCTAAGGAAGCAAG-3') and *Mycs*-2 from 5' UTR (for: 5'-TCCACATGCTCTGAGTTTGTTCCTCC-3'; rev: 5'-GTCTCCACCTGACAACGAAACACCT-3'). The primers were used to screen a mouse ES-129Sv/J BAC library (Genome Systems). A positive clone, 70C10, was identified and used for subsequent analyses based on the clone verification by PCR and sequence identity of the PCR product to *Mycs* sequence. BAC 70C10 DNA was labeled with digoxigenin-11-dUTP (Roche Molecular Biochemicals) by standard nick translation. Mouse X chromosome painting probe labeled with biotin-14-dCTP (Clontech) was co-hybridized with BAC 70C10 probe. FISH protocols were followed to map the probes to mouse metaphase chromosomes (Shan et al., 2000). Digital image acquisition was performed by using IPLab system (Scanalytics, Inc.) and image analysis was carried out as previously described (Shan et al., 2000).

Probe name: 70C10

Probe type: mouse genomic DNA

Vector: pBeloBAC

Insert size: 75–100 kb

Gene symbol: *Mycs*

Gene name: myc-like oncogene, s-myc protein

Proof of authenticity: PCR and partial DNA sequencing

Gene reference: GenBank accession number AB016289

Radiation Hybrid (RH) mapping

A T31 Mouse Radiation Hybrid Panel (Research Genetics) was used for physical mapping. A 347-bp fragment of the 3' UTR region of *Mycs* was amplified by PCR using the primers *Mycs*-1 for/rev. PCR cycling conditions were 40 s at 94 °C, 30 s at 60 °C, and 40 s at 72 °C for 30 cycles; the first denaturation was done for 3 min at 94 °C and the final extension for 3 min at 72 °C. The products were separated by electrophoresis on 2.5% agarose gel and visualized with ethidium bromide staining. The results were scored by the mouse band positive (1), negative (0). A score for each of the 100 cell lines in the RH panel (00001 01000 00110 00000 00110 11010 00001 00010 01000 00000 00001 11000 00000 00100 10000 00000 00010 00011 00011 00001) was submitted to The Jackson Laboratory RH mapping server (<http://www.jax.org/resources/documents/cmdata/rhmap/rhsubmit.html>, Rowe et al., 2000) for data analysis and chromosome location.

Received 14 March 2002; manuscript accepted 5 April 2002.

Corresponding author: Dr. Nicholas C. Popescu
Laboratory of Experimental Carcinogenesis
Center for Cancer Research, National Cancer Institute
Building 37, Room 3C05, 37 Convent Drive, MSC4255
Bethesda, MD 20892-4255 (USA); telephone: (301) 496-5688 ext 240
fax: (301) 496-0734; email: nicholas_popescu@nih.gov

Fig. 1. Chromosomal mapping of the mouse *Mycs* gene to region XA1.2–2 by FISH with chromosome X painting probe and BAC 70C10 probe.

Results

FISH mapping data

Most precise assignment: XA1.2–2

Number of cells examined: 60

Number of cells with specific signal: 55

Location of background signals (site with >2 signals): none observed.

RH mapping data:

The *Mycs* gene was mapped to the interval between DXMit101 18.1 cR proximal and DXMit203 33.3 cR distal of the X chromosome map. The RH mapping data show a LOD score of 13.4 to DXMit101 at the proximal end of the X map, and have LOD scores for over 6 to 20 data sets in this region that represent 18 loci (six anchor MIT markers and twelve EST markers, including AF082348, an EST for *Bmp15*), and there are no suggestions of any alternative map position.

References

- Asai A, Miyagi Y, Sugiyama A, Nagashima Y, Kanemitsu H, Obinata M, Mishima K, Kuchino Y: The s-Myc protein having the ability to induce apoptosis is selectively expressed in rat embryo chondrocytes. *Oncogene* 9:2345–2352 (1994).
- Kagaya S, Kitanaka C, Noguchi K, Mochizuki T, Sugiyama A, Asai A, Yasuhara N, Eguchi Y, Tsujimoto Y, Kuchino Y: A functional role for death proteases in s-Myc- and c-Myc-mediated apoptosis. *Mol cell Biol* 17:6736–6745 (1997).
- Morton CC, Nussenzweig MC, Sousa R, Sorenson GD, Pettengill OS, Shows TB: Mapping and characterization of an X-linked processed gene related to MYCL1. *Genomics* 4:367–375 (1989).
- Rowe LB, Barter ME, Eppig JT: Cross-referencing radiation hybrid data to the recombination map: lessons from mouse Chromosome 18. *Genomics* 69:27–36 (2000).
- Serikawa T, Kuramoto T, Hilbert P, Mori M, Yamada J, Dubay CJ, Lindpainter K, Ganten D, Guenet JL, Lathrop GM, Beckmann JS: Rat gene mapping using PCR-analyzed microsatellites. *Genetics* 131:701–721 (1992).
- Shan Z, Zabel B, Trautmann U, Hillig U, Ottolenghi C, Wan Y, Haaf T: FISH mapping of the sex-reversal region on human chromosome 9p in two XY females and in primates. *Eur J hum Genet* 8:167–173 (2000).
- Sugiyama A, Noguchi K, Kitanaka C, Katou N, Tashiro F, Ono T, Yoshida MC, Kuchino Y: Molecular cloning and chromosomal mapping of mouse intronless *myc* gene acting as a potent apoptosis inducer. *Gene* 226:273–283 (1999).
- Yamasaki Y, Helou K, Watanabe TK, Sjoling A, Suzuki M, Okuno S, Ono T, Takagi T, Nakamura Y, Stahl F, Tanigami A: Mouse chromosome 19 and distal rat chromosome 1: a chromosome segment conserved in evolution. *Hereditas* 134:23–34 (2001).
- Zimonjic DB, Zhang H, Shan Z, Factor V, Trent J, Thorgeirsson SS, Popescu NC: DNA amplification associated with double minutes originating from chromosome 19 in mouse hepatocellular carcinoma. *Cytogenet Cell Genet* 93:114–116 (2001).