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Assignment[1] of the Bog gene (RBBP9) to syntenic regions of mouse chromosome 2G1–H1 and human chromosome 20p11.2 by fluorescence in situ hybridization

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[1] To our knowledge this is the first time this gene has been mapped.

Rationale and significance

We have isolated a rat cDNA encoding a novel protein called Bog from a rat liver epithelial cell line. Bog is a retinoblastoma (pRb) binding protein which can bind all of the retinoblastoma family members. Overexpression of Bog can displace E2F-1 from pRb rendering the cell insensitive to the growth inhibitory effects of TGF-β1. Further, overexpression of Bog can lead to and is associated with a transformed phenotype (Woitach et al., 1998). Since overexpression of Bog is associated with transformation, the Bog gene was mapped to mouse chromosome 2G1–H1 and to human chromosome 20p11.2 to gain insight into the possible relationship with the transformed phenotype in other cell types. These are syntenic in mouse and human genomes. In the human, the region of 20p11.2 has been reported to be a recurrent region of rearrangement, demonstrating both amplification in several cancers (Knuutila et al., 1998) and deletion in Wilm's tumors (Altura et al., 1996).

Materials and methods

Probe

A full length cDNA clone was isolated by screening a mouse kidney cDNA library with a probe from the rat cDNA sequence. The mouse 1.9-kb cDNA fragment was used to isolate a bacterial artificial chromosome (BAC) clone containing the mouse Bog gene and the human Bog gene from their respective genomic libraries. The identification of the mouse and the human BAC clones was verified by partial DNA sequencing.

Fluorescence in situ hybridization (FISH) analysis

Purified BAC DNA was labeled with biotin (Random-Prime DNA labeling kit; Boehringer Mannheim Corp., Indianapolis, IN) and used for in situ hybridization of chromosomes derived from normal methotrexate-synchronized peripheral leukocyte cultures and normal mouse spleen cultures. The conditions of hybridization, processing, analysis and direct fluorescent signal localization and banded chromosomes were performed as previously described in detail (Zimonjic et al., 1994)

Probe name: 60i3 (mouse); 281k20 (human)
Probe type: genomic DNA from mouse strain 129/Sv and genomic DNA from human male
Vector: pBeloBACII
Proof of authenticity: DNA sequencing
Gene reference: Woitach et al. (1998)

Results

Mapping data: mouse
Location: 2G1–H1
Number of cells examined: 50
Number of cells with specific signal: 47; 1 (2) 2 (1) 3 (0) 4 (44) chromatids per cell
Most precise localization: 2G1–H1
Location of background signals (sites with >2 signals): none observed

Mapping data: human
Location: 20p11.2
Number of cells examined: 25
Number of cells with specific signal: 23; 1 (1) 2 (2) 3 (0) 4 (20) chromatids per cell
Most precise localization: 20p11.2
Location of background signals (sites with >2 signals): none observed

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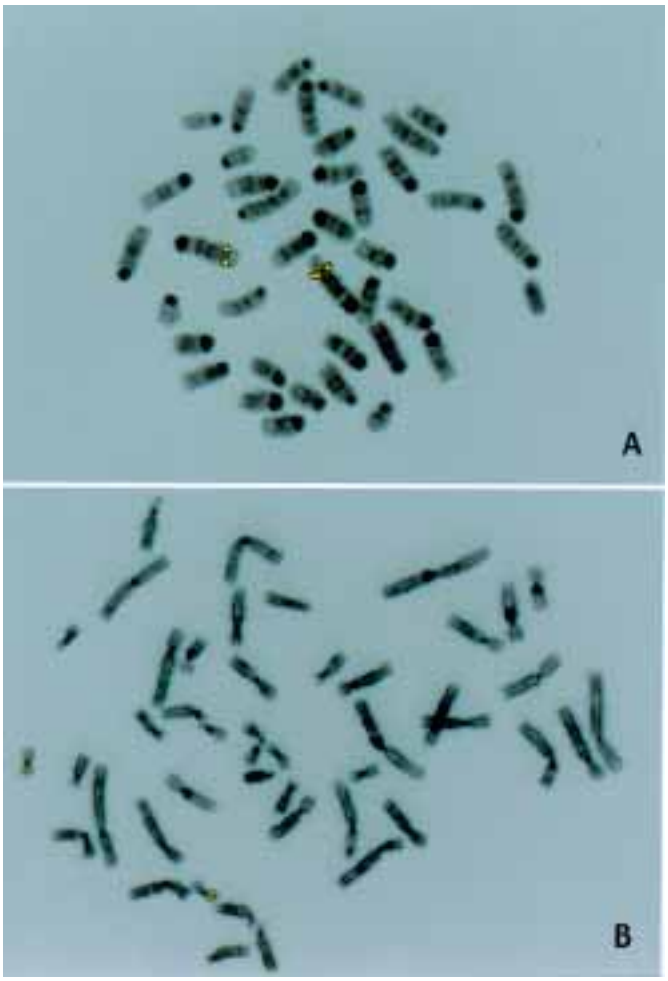

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Fig. 1. FISH localization of the mouse and human Bog gene RBBP9. Metaphase chromosome spread after hybridization with a biotinylated probe. Symmetrical flourescent signals located on (**A**) mouse chromosome 2G1–H1 and (**B**) human 20p11.2 as identified by inverted DAPI G-like banding and confirmed by chromosome specific paint.