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Localization of the Gene Encoding the Ran-Binding Protein RanBP2 to Human Chromosome 2q11–q13 by Fluorescence *in Situ* Hybridization

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Ran (Ras-related small nuclear protein) is a member of the Ras superfamily of small GTP-binding proteins. It is involved in cell-cycle progression, nucleocytoplasmic transport, and pre-mRNA processing (7). Ran is predominantly located in the nucleus and cycles between the GTP-bound active and the GDP-bound inactive state (1). RanBP2 (Ran-binding protein 2) is the largest protein of the nuclear pore complex, localized to its cytoplasmic fibers (10–12). It contains four Ran-binding domains and a C-terminal cyclophilin-related region (11, 12) that has been found to act as chaperone for red/green opsin (3). A RanBP2-specific antibody inhibits nuclear protein import, indicating a functional role of RanBP2 in this process (12). The RanGTPase-activating protein RanGAP1 is posttranslationally modified by a small ubiquitin-related polypeptide and is thereby targeted to RanBP2 (6).

To assign the chromosomal locus of the RanBP2 gene, fluorescence *in situ* hybridization was performed according to the

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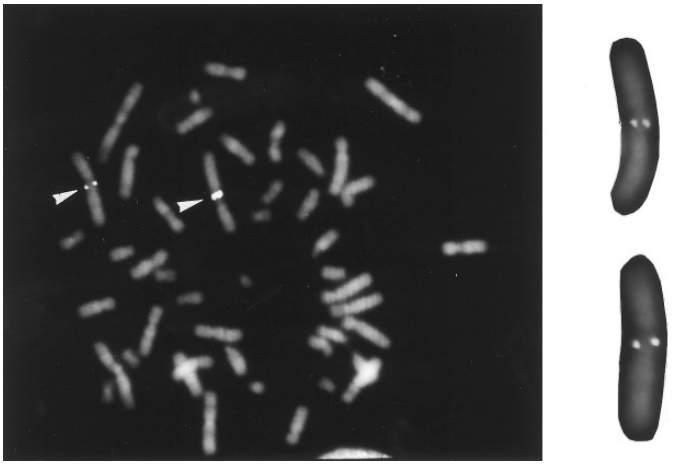

FIG. 1. Localization of the human RanBP2 gene on chromosome 2q11–q13 by fluorescence *in situ* hybridization. Normal male/female chromosomes were hybridized with biotinylated probes for RanBP2. The hybridization site on chromosome 2 is indicated by arrowheads.

protocol described (4). cDNA probes from a domain of RanBP2 (1) were used to identify yeast artificial chromosomes harboring the gene encoding RanBP2. The hybridization procedure was carried out as described (4). A YAC that hybridized with the RanBP2 probe (ICRF-Y-900C04133) was used as a template in amplification of human-specific DNA sequences by interspersed long-range PCR (9). The resulting probes were labeled by nick-translation using biotin-16–dUTP (Boehringer Mannheim). After *in situ* hybridization to human metaphase chromosomes fixed by methanol/acetic acid, signals were detected via avidin-conjugated fluorescein isothiocyanate (Vector Laboratories, Burlingame, CA). For identification and band assignment, chromosomes were counterstained by DAPI (4,6-diamidine-2-phenylindole dihydrochloride). Through the use of a cooled CCD camera (Photometrix, Tucson, AZ), digitized images were generated for each of the two fluorochromes and overlaid electronically after digital processing. This allowed the assignment of the RanBP2 gene to chromosome 2q11–q13 (Fig. 1).

To obtain evidence for the presence of introns in the gene, PCR was performed using DNA from a panel of hamster somatic cell hybrids (Coriell Institute for Medical Research, Camden, NJ) under the following conditions: 30 cycles of amplification, 1 min of denaturation at 96°C, 30 s of annealing at 55°C, and 1 min of extension at 72°C. Each reaction was carried out in 50 μl containing 10 m*M* Tris–HCl (pH 8.3), 2 m*M* $MgCl_2$, 50 m*M* KCl, 200 μM each dNTP, 0.25 m*M* each of two primers, 50 ng of DNA template, and 1 U *Taq* DNA polymerase. Using the sense primer 5′-GCAGAATTGCTTTGTAA-3′ and the antisense primer 5′-AGT TCTGACTGAATCAAG-3′, we obtained a specific 4-kb product from both the YAC and a hybrid cell line harboring human chromosome 2, whereas no product was obtained from hybrids containing other human chromosomes. This result suggests the presence of a 3.75-kb intron in this region. The PCR product was sequenced, and an exon/intron transition was identified at position +405 of the open reading frame (Genbank Accession No. D42063). Furthermore, we used an intron sequence primer, 5′-GACTAGTTGCTGAGGATTG-3′, to verify the presence of the intron. This confirms the localization of the gene for RanBP2 to chromosome 2q11–q13. Molecular genetic changes on 2q without identification of a specific gene have been described for human male germ cell tumors (5) and for neuroblastomas (8).

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