SHORT COMMUNICATION

Assignment of the Human *TYRP* (*brown*) Locus to Chromosome Region 9p23 by Nonradioactive *in Situ* Hybridization

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Received August 15, 1991; revised November 15, 1991

The *TYRP* (*brown*) locus determines pigmentation and coat color in the mouse. The human homolog of the *TYRP* locus has been recently identified and shown to encode a 75-kDa transmembrane melanosomal glycoprotein called gp75. The gp75 glycoprotein is homologous to tyrosinase, an enzyme involved in the synthesis of melanin, forming a family of tyrosinase-related proteins. A genomic clone of human gp75 was used to map the human *TYRP* locus to chromosome 9, region 9p23, by nonradioactive fluorescent *in situ* hybridization. Specificity of hybridization was tested with a genomic fragment of human tyrosinase that mapped to a distinct site on 11q21. The 9p region has been reported to be nonrandomly altered in human melanoma, suggesting a role for the region near the *TYRP* locus in melanocyte transformation.

The *TYRP* (*brown*) locus alleles in the mouse affect coat color. The wildtype allele, *B*, determines a black coat, whereas recessive *b* alleles give rise to brown (cinnamon), cordovan, and white-based brown hues (16). It has been proposed that the *TYRP* locus product plays a role in the type of melanin synthesized and in the biogenesis of melanosomes (16). Jackson and co-workers recently mapped a cDNA (15) encoding tyrosinase-related protein-1 (TRP-1) to the mouse *TYRP* locus (7, 21). TRP-1 has 40% homology to the enzyme tyrosinase (the product of the *TYR*, *c*, or *albino* locus) at the amino acid sequence level, defining one of several products encoded by a tyrosinase-related family of genes. The mouse *TYRP* locus has been mapped to chromosome 4, a site that is distinct from the *TYR* locus that maps to chromosome 7.

The human homolog of the mouse *TYRP* locus has been recently identified (3, 18). The human *TYRP* product is a 75-kDa transmembrane melanosomal glycoprotein that appears to have little or no endogenous tyrosinase activity (18, 19). The sequences of human gp75 and mouse TRP-1 are conserved; analysis of cDNA encoding gp75 shows 90% homology between the derived amino acid sequences of the mouse and human *TYRP* products. The gp75 protein and human tyrosinase have several features in common, including similar mass, specific intracellular localization to melanosomal membranes, and homology at the amino acid (43.1%) and nucleotide sequence (55.3%) levels. The gene encoding human tyrosinase has recently been mapped to chromosome 11q14–q21 and 11p11.2 regions (2).

A 3-kb genomic clone of human gp75, encompassing the 5′ untranslated region and exons 1 and 2, was isolated from a genomic library constructed in the Lambda Fix vector (Stratagene) by using a cDNA encoding gp75 as a specific probe (18). To test specificity of hybridization, a 2-kb genomic DNA fragment encompassing 500 bases of the 5′ untranslated region and the first exon of human tyrosinase was isolated. We obtained highly specific signals for the gp75 and tyrosinase probes by *in situ* mapping, usually as symmetrical spots shown on both chromatids (Fig. 1). Chromosomal regions with hybridization signals were unequivocally identified using filter combinations for FITC and DAPI. The gp75 probe showed clustering of hybridization signals on chromosome region 9p23 (Figs 1A and 1B). Of 54 informative metaphase preparations studied, specific hybridization signals at 9p23 region were found in 72%; of these, 69% were on both chromatids, resulting in double fluorescent signals, and the remaining 31% were single spots. Double signals for the gp75 probe were not detected on any other chromosomal region. Recently, Abbott *et al.* (1) have also assigned the human homolog of the mouse *TYRP* gene to the short arm of chromosome 9. Of note, this region of human chromosome 9 contains regions of synteny with mouse chromosome 4, the site of the mouse *TYRP* locus. Tyrosinase mapped to a site in the 11q21 region. Of 68 informative metaphase preparations studied, 68% of the hybridization events were at 11q21. Among the hybridization events at 11q21, 65% were observed on both chromatids. These results confirm the previous localization of the human tyrosinase gene to 11q14–q21 (2).

Perhaps significantly, the 9p chromosome region is nonrandomly involved in human melanoma (9, 12). Cytogenetic data suggest that abnormalities involving breakpoints of 9p could be an early event in melanoma progression (11). Cowan *et al.* (4) found monosomy of 9 or the loss of short arm region 9pter–p22 in melanomas and in dysplastic nevi, a putative precursor lesion of melanoma, suggesting that mutations of a gene on 9p are

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GENOMICS 13, 227–229 (1992)
0888-7543/92 $3.00

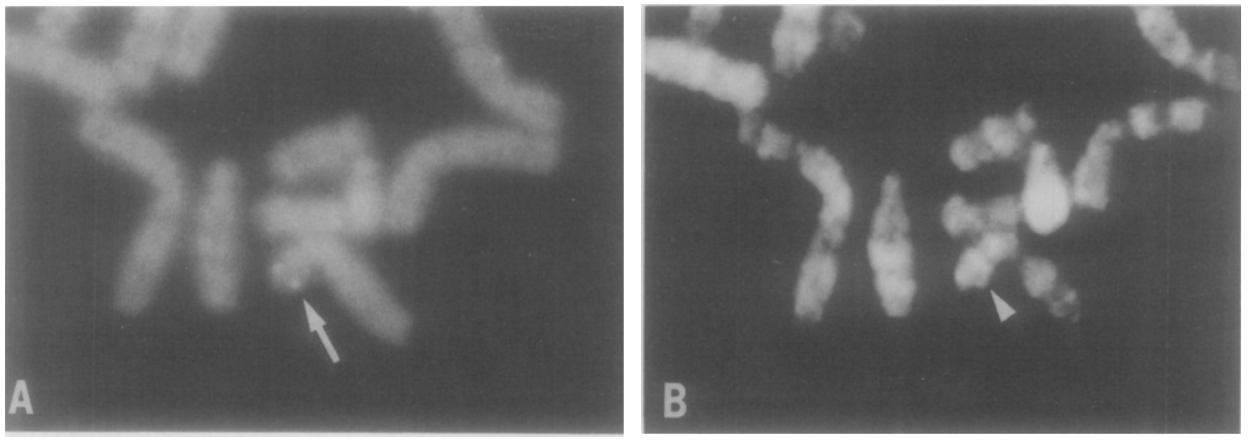

FIG. 1. Fluorescence *in situ* hybridization mapping of the human *TYRP* (*brown*) locus to 9p23. Chromosome preparations from phytohemagglutinin-stimulated and 5-bromodeoxyuridine-synchronized lymphocyte cultures were hybridized with biotin–11-dUTP-labeled probes with modifications of described methods (8, 10, 20). Posthybridization washes were done at 45°C in 50% formamide/2× SSC for 20 min, followed by 2× SSC washes for 1 hr. The hybridization signal was detected by indirect immunofluorescence using biotinylated anti-avidin-conjugated fluorescein isothiocyanate (FITC–avidin) and biotinylated anti-avidin as described (6, 14). Slides were mounted in antifade (*p*-phenylenediamine) containing propidium iodide (**A**) and 4′,6-diamidino-2-phenylindole (DAPI) (**B**). Slides were screened with filter combinations B2A for FITC and UV-2A for DAPI. (**A**) FITC signal (arrow) and propidium iodide staining; (**B**) pattern of G-bands after DAPI stain (arrowhead shows 9p23 band).

common primary events in malignant transformation of melanocytes. Two reports studying the genetic changes during the progression of metastatic melanoma have suggested that 9p changes are early events. Dracopoli *et al.* (5) found losses of an alleleic fragment in six metastatic lesions derived from the same patient using D9S3 probe that maps to 9pter–p24 (17). Subsequently, Pedersen and Wang (13) also showed that del(9)(:p11→q32:) was one of three common markers shared by eight tumors derived from the same patient. Other possibly relevant genes in this region are interferon-α on 9p13–p22 and interferon-β on 9p22–pter (17). Genetic alterations in this region of 9p appear to represent early events in tumor progression of melanoma.

ACKNOWLEDGMENTS

We thank Dr. Raju Chaganti for guidance and valuable discussions. This work was supported by the Louis and Anne Abrons Foundation and grants from the National Cancer Institute.

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