SHORT COMMUNICATION

The Gene for *PAX7*, a Member of the Paired-Box-Containing Genes, Is Localized on Human Chromosome Arm 1p36

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The murine *Pax-7* gene and the cognate human gene, formerly designated HuP1, are members of the multigene paired-box-containing class of developmental regulatory genes first identified in *Drosophila*. By analysis of somatic cell hybrids segregating human chromosomes, the gene encoding *PAX7* was localized to human chromosome 1. Fluorescence *in situ* hybridization confirmed this assignment and allowed mapping of the gene to the terminal region of the short arm (1p36) of the chromosome. Additionally, these results confirm the extensive homology between human chromosome 1p and the distal segment of mouse chromosome 4, extending from bands C5 through E2.

In vertebrates, several putative developmental control genes have been identified by taking advantage of the conserved sequence motifs of *Drosophila* segmentation and homeotic genes (3, 6, 14). One such DNA-binding domain is encoded by the paired box, which was first described in the paired (*prd*) class of *Drosophila* segmentation genes and subsequently found to constitute a multigene family in the mouse, including at least eight paired-box genes (19). The murine *Pax* genes have been shown to encode DNA-binding transcription factors whose expression is temporally and spatially restricted to discrete regions of the developing mouse embryo (2, 7, 10, 13). Based upon sequence comparison and genomic organization, the eight murine *Pax* genes can be grouped into four distinct classes (11). Further, the presence or absence of conserved regions coding for a signature octapeptide motif or for a paired-type homeobox, as well as sequence similarities in the remaining coding region, serves to distinguish different classes (3, 4).

The murine *Pax-7* gene encodes a sequence-specific DNA-binding protein containing not only a paired box, but also the octapeptide and a paired-type homeobox, and shows extensive structural and sequence homology with *Pax-3*. This homology at the protein level extends from the paired domain through the homeodomain of both proteins. The paired domains of *Pax-3* and *Pax-7* are very similar, with only a few conservative changes restricted to the 54-amino-acid carboxyl region of the paired domain (10, 13). Similarly, their expression patterns during mouse embryogenesis indicate that both genes may play an important role in neurogenesis. However, unlike *Pax-3*, *Pax-7* expression can also be specifically followed during myogenesis from the somitic dermomyotome to skeletal muscle-containing tissues (13).

Strikingly, the paired boxes and the octapeptides of *Pax-7* and the cognate human gene, originally identified by reduced stringency screening using a paired-box probe from the *Drosophila gsb* gene, are 100% identical at the amino acid level (4). Moreover, the highly charged sequence of alternating clusters of basic and acidic amino acids extending between these two domains shows only four conservative and one nonconservative substitution. In addition, the human and murine genes exhibit the same intron/exon structure in the known sequences. Although no downstream sequence extending 3′ from the octapeptide where a homeobox would be predicted is available for the human *PAX7* gene, the extensive homologies between murine *Pax-7* and the cognate human sequence strongly suggest that they represent equivalent genes in mouse and man.

The *Pax-7* gene has been mapped by linkage analysis to the distal segment of mouse chromosome 4 between the *JUN* and *D4Pas-1* loci (19). Thus the *Pax-7* locus resides within a segment of mouse chromosome 4 that exhibits extensive homology to the human chromosome region 1p31–pter, a subregion (1p36) of which has been implicated in the development or progression of a number of human tumors, including neuroblastoma, melanoma, medullary thyroid carcinoma, and pheochromocytoma (8, 9, 15). Our efforts to identify additional probes within this region to better characterize chromosomal abnormalities in these tumors led us to determine the location for the human homologue of the murine *Pax-7* gene.

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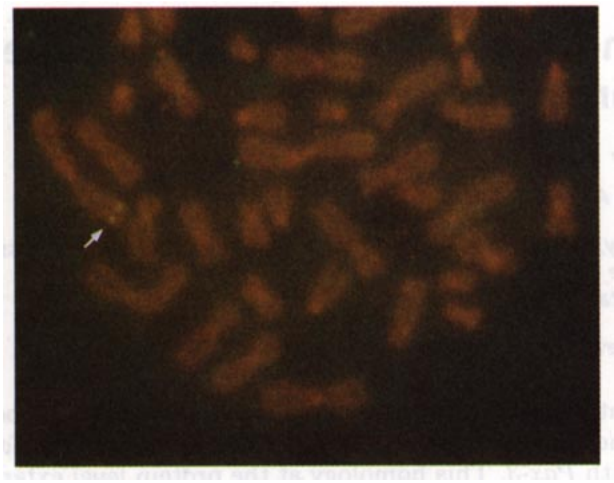

FIG. 1. Fluorescence *in situ* hybridization of normal metaphase chromosomes showing the hybridization signal for *PAX7* at the distal end of chromosome 1 in band 1p36 (arrow). *In situ* hybridization and detection were performed as previously described and resulted in specific labeling of 80% of chromosome 1 chromatids in this region (16).

To assign *PAX7* to a specific human chromosome, a panel of human × rodent hybrids that segregate human chromosomes was used (16). Analysis of genomic DNA from these cell hybrids was performed by amplifying a 456-bp product using primers derived from intronic sequence flanking the 5′-most exon of *PAX7* (4). DNA from a total of 25 hybrids (19 independent fusions) was analyzed (data reviewed but not shown). At least two discordant clones (*i.e.*, gene present and chromosome absent or vice versa) were observed for each chromosome except chromosome 1, which showed no discordancy. There was in particular 24% discordancy for chromosome 2, to which the closely related *PAX3* gene has been localized, as well as 16% discordancy for chromosome 20, to which the *PAX1* gene has recently been assigned (17, 18). From these data we concluded that *PAX7* resides on chromosome 1. To confirm and more precisely refine the localization of *PAX7*, we next used fluorescence *in situ* hybridization. Analysis of 20 metaphases with a *PAX7* genomic phage clone revealed the presence of positive hybridization signals in the distal part of the short arm of chromosome 1 (*Fig. 1*). This observation corroborates the results of the PCR analysis of the somatic cell hybrids and further maps the *PAX7* gene to chromosome band 1p36.

Mapping of *PAX7* to human chromosome 1p36 extends the information about the extensive homology between human chromosome 1 and the distal segment of mouse chromosome 4, spanning now at least 30 cM on the genetic map of the latter between *JUN* and *PND* (1). Unlike the *PAX3* and *PAX6* genes, for which both murine and human dominant acting mutations have been described, and the murine *Pax-1* gene, whose mutation results in the *undulated (un)* phenotype, no disorder has yet been linked to the *PAX7* loci (5, 11, 12). However, as more refined comparative map data are developed within these respective chromosomal segments, e.g., between *JUN* and *PND* on both chromosomes, further information about candidate mutations for these genes may emerge.

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REFERENCES

1. Abbot, C. M., Blank, R., Eppig, J. T., Friedman, J. M., Huppi, K. E., Jackson, F., Mock, B. A., Stoye, J., and Wiseman, R. (1992). Mouse chromosome 4. *Mamm. Genome* **3:** S55–S64.
2. Balling, R., Deutsch, U., and Gruss, P. (1988). undulated, a mutation affecting the development of the mouse skeleton, has a point mutation in the paired box of Pax-1. *Cell* **55:** 531–535.
3. Bopp, D., Burri, M., Baumgartner, S., Frigerio, G., and Noll, M. (1986). Conservation of a large protein domain in the segmentation gene paired and in functionally related genes of Drosophila. *Cell* **47:** 1033–1040.
4. Burri, M., Tromvoukis, Y., Bopp, D., Frigerio, G., and Noll, M. (1989). Conservation of the paired domain in metazoans and its structure in three isolated human genes. *EMBO J.* **8:** 1183–1190.
5. Chalepakis, G., Fritsch, R., Fickenscher, H., Deutsch, U., Goulding, M., and Gruss, P. (1991). The molecular basis of the undulated/Pax-1 mutation. *Cell* **66:** 873–884.
6. Dressler, G. R., Deutsch, U., Balling, R., Simon, D., Guenet, J.-L., and Gruss, P. (1988). Murine genes with homology to Drosophila segmentation genes. *Development* **104**(Suppl.): 181–186.
7. Dressler, G. R., Deutsch, U., Chowdbury, K., Nornes, H. O., and Gruss, P. (1990). Pax2, a new murine paired box containing gene and its expression in the developing excretory system. *Development* **109:** 787–795.
8. Fong, C. T., Dracopoli, N., White, P. S., Merrill, P., Griffith, R., Housman, D., and Brodeur, G. M. (1989). Loss of heterozygosity for the short arm of chromosome 1 in human neuroblastoma: Correlation with N-*myc* amplification. *Proc. Natl. Acad. Sci. USA* **86:** 3753–3757.
9. Goldstein, A. M., Dracopoli, N. C., Ho, E. C., Fraser, M. C., Kearns, K. S., Bale, S. J., McBride, O. W., Clark, W. H., Jr., and Tucker, M. A. (1993). Further evidence for a locus for cutaneous malignant melanoma-dysplastic nevus (CMM/DN) on chromosome 1p, and evidence for genetic heterogeneity. *Am. J. Hum. Genet.* **52:** 537–550.
10. Goulding, M. D., Chalepakis, G., Deutsch, U., Erselius, J. R., and Gruss, P. (1991). Pax-3, a novel murine DNA binding protein expressed during early neurogenesis. *EMBO J.* **10:** 1135–1147.
11. Gruss, P., and Walther, C. (1992). Pax in development. *Cell* **69:** 719–722.
12. Hill, R. E., Favor, J., Hogan, B. L. M., Ton, C. C. T., Saunders, G. F., Hanson, I. M., Prosser, J., Jordan, T., Hastie, N. D., and Van Heyningen, V. (1991). Mouse small eye results from mutations in a paired-like homeobox-containing gene. *Nature* **354:** 522–525.
13. Jostes, B., Walther, C., and Gruss, P. (1990). The murine paired box gene, Pax7, is expressed specifically during the development of the nervous and muscular system. *Mech. Dev.* **33:** 27–38.
14. Kessel, M., and Gruss, P. (1990). Murine developmental control genes. *Science* **249:** 374–379.
15. Mathew, C. G. P., Smith, B. A., Thorpe, K., Wong, Z., Royle,

N. J., Jeffreys, A. J., and Ponder, B. A. J. (1987). Deletion of genes on chromosome 1 in endocrine neoplasia. *Nature* **328:** 524–526.

16. Morris, S. W., Nelson, N., Valentine, M. B., Shapiro, D. N., Look, A. T., Kozlosky, C. J., Beckmann, P., and Cerretti, D. P. (1992). Assignment of the genes encoding human interleukin-8 receptor types 1 and 2 and an interleukin-8 receptor pseudogene to chromosome 2q35. *Genomics* **14:** 685–691.
17. Schnittger, S., Gopal Rao, V. V. N., Deutsch, U., Gruss, P., Balling, R., and Hansmann, I. (1992). PAX1, a member of the paired box-containing class of developmental control genes, is mapped to human chromosome 20p11.2 by *in situ* hybridization (ISH and FISH). *Genomics* **14:** 740–744.
18. Tsukamoto, K., Tohma, T., Ohta, T., Yamakawa, K., Fukushima, Y., Nakamura, Y., and Niikawa, N. (1992). Cloning and characterization of the inversion breakpoint at chromosome 2q35 in a patient with Waardenburg syndrome type I. *Hum. Mol. Genet.* **1:** 315–317.
19. Walther, C., Guenet, J-L., Simon, D., Deutsch, U., Jostes, B., Goulding, M. D., Plachov, D., Balling, R., and Gruss, P. (1991). Pax: A murine multigene family of paired box-containing genes. *Genomics* **11:** 424–434.