Using human ACS3-specific primers 5′AATTATTCTCTT-CTGGCATCA3′ [nt 2304–2324 of the (+) strand] and 5′TAGGTGGTAAAGTAAGTTGGT3′ [nt 2509–2529 of the (−) strand], the human ACS3 gene was detected only in a hybrid cell line (GM/NA10826B) containing 100% of human chromosome 2 (data not shown). No amplification was detected in other cell lines or in the parent cell line.

To define the location of the gene on chromosome 2 further, we used the cloned human ACS3 cDNA probe to localize the ACS3 gene on a chromosome spread by fluorescence *in situ* hybridization (FISH). FISH to BrdU-synchronized metaphase chromosomes was carried out as previously described (2). At least 50 metaphases were analyzed. A specific FITC signal generated from the biotin-labeled human ACS cDNA was consistently observed on the q34–q35 region of the long arm of chromosome 2 in the majority of the metaphase preparations examined (Fig. 1C).

ACKNOWLEDGMENTS

We thank Dr. Ian Gleadall for helpful comments on the manuscript and Kyoko Ogamo Karasawa and Nami Suzuki for secretarial assistance. This research was supported in part by research grants from the Ministry of Education, Science, Sports and Culture and the Ministry of Health and Welfare of Japan.

REFERENCES

1. Abe, T., Fujino, T., Fukuyama, R., Minoshima, S., Shimizu, N., Toh, H., Suzuki, H., and Yamamoto, T. (1992). Human long-chain acyl-CoA synthetase: Structure and chromosomal location. *J. Biochem. (Tokyo)* **111:** 123–128.
2. Endo, Y., Onogi, S., Umeki, K., Yamamoto, I., Kotani, T., Ohtaki, S., and Fujita, T. (1995). Regional localization of the gene for thyroid peroxidase to human chromosome 2p25 and mouse chromosome 12C. *Genomics* **25:** 760–761.
3. Fujino, T., and Yamamoto, T. (1992). Cloning and functional expression of a novel long-chain acyl-CoA synthetase expressed in brain. *J. Biochem. (Tokyo)* **111:** 197–203.
4. Fujino, T., Kang, M.-J., Suzuki, H., Iijima, H., and Yamamoto, T. (1996). Molecular characterization and expression of rat acyl-CoA synthetase 3. *J. Biol. Chem.* **271:** 16748–16752.
5. Suzuki, H., Kawarabayasi, Y., Kondo, J., Abe, T., Nishikawa, K., Kimura, S., Hashimoto, T., and Yamamoto, T. (1990). Structure and regulation of rat long-chain acyl-CoA synthetase. *J. Biol. Chem.* **265:** 8681–8685.

The entire metaphase is shown. Separate images of FITC hybridization signals and propidium iodine-stained chromosomes were merged using image analysis software, and photographs were taken from digital images without computer-assisted modification through a camera attached to a Zeiss LSM. Arrows indicate specific signals on the q34–q35 region of the long arm of chromosome 2.

Assignment of the Human Serine/Threonine Protein Phosphatase 4 Gene (*PPP4C*) to Chromosome 16p11–p12 by Fluorescence *in Situ* Hybridization

Holger Bastians,*[,1] Heike Krebber,*[,2] Jörg Hoheisel,† Sybille Ohl,‡ Peter Lichter,‡ Herwig Ponstingl,*[,3] and Stefan Joos‡

**Division of Molecular Biology of Mitosis, ‡Division of Organization of Complex Genomes, and †Division of Molecular-Genetic Genome Analysis, German Cancer Research Center, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany*

Received August 5, 1996; accepted February 12, 1997

Protein phosphatases together with protein kinases are involved in a multitude of cellular processes by determining the phosphorylation state of regulatory proteins. Serine/threonine-specific protein phosphatases can be subdivided into four distinct classes according to their use of *in vitro* substrates and their sensitivity to specific activators and inhibitors: PP1, PP2A, PP2B, and PP2C (5). In addition to isoforms of PP1, PP2A, and PP2B, cDNA cloning has now revealed an increasing number of novel catalytic subunits of serine/threonine protein phosphatases from various organisms. To date, in human cells three novel members have been identified, including the human protein phosphatase 4 (PP4, PPX) (2). The PP4 protein is localized to centrosomes in HeLa cells, suggesting a function in spindle assembly during mitosis (3). Here, we report the chromosomal localization of the gene for the human protein phosphatase 4 (*PPP4C*) by using fluorescence *in situ* hybridization (FISH) combined with a PCR-based somatic cell hybrid mapping.

We cloned a 985-bp cDNA from a HeLa λgt11 cDNA library containing the complete open reading frame of human PP4. The insert was labeled using the DecaPrime II random labeling kit (Ambion, Austin, TX) and was used to screen a YAC library spotted onto two nylon membranes (10; a gift from Hans Lehrach, Berlin). Hybridization was performed for 14 h at 65°C as described (4). Of 4×10^4 YACs screened, 1 showed a strong hybridization signal with the PP4 probe (ICRFy900N1440Q). To obtain a specific PP4 probe for FISH we performed interspersed repetitive sequence long-range PCR using YAC DNA as a template (9). As essentially described by Lichter *et al.* (7), the resulting DNA probes were labeled with biotin-16–dUTP (Boehringer, Mannheim) by nick-translation, hybridized *in situ* to methanol/acetic acid-

[1] Present address: Harvard Medical School, Boston, MA.

[2] Present address: Harvard Medical School and Dana-Farber Cancer Institute, Boston, MA.

[3] To whom correspondence should be addressed at the German Cancer Research Center, Division of Molecular Biology of Mitosis, 0230, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany. Telephone: ++49-6221-423408. Fax: ++49-6221-423460.

GENOMICS **42,** 181–182 (1997)
ARTICLE NO. GE974693
0888-7543/97 $25.00
Copyright © 1997 by Academic Press
All rights of reproduction in any form reserved.

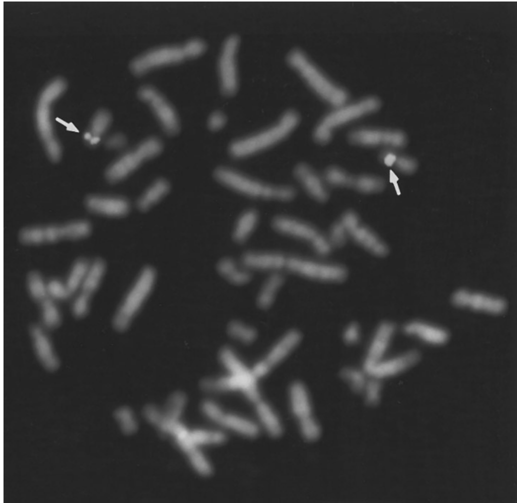

FIG. 1. Mapping of the human protein phosphatase 4 gene (*PPP4C*) to the short arm of chromosome 16 in band p11–p12 by fluorescence *in situ* hybridization.

fixed human metaphase chromosomes, and detected via avidin-conjugated fluorescein isothiocyanate (Vector, Burlingame, CA). For identification and band assignment, chromosomes were counterstained by 4,6-diamine-2-phenylindoledihydrochloride. A cooled CCD camera (Photometrix, Tucson, AZ) was utilized to generate digitized images for each of the two fluorochromes, which were overlayed electronically after digital processing. As shown in Fig. 1 this allowed the assignment of the human PPP4C gene to chromosome 16p11–p12 (Fig. 1).

To obtain evidence for the presence of intron sequences in the gene on chromosome 16, we performed a PCR-based mapping approach using specific PP4 primers and DNA isolated from the NIGMS Human Mutant Cell Repository panel of monochromosomal human–Chinese hamster somatic cell hybrids as a template. Through the use of two sequence-specific primers (5′-CTTCCTGTGACCCCGC-3′ and 5′-CTTTGAAGTACCAAGTTCA CC-3′), a 216-bp PCR product corresponding to the 3′ untranslated region of PP4 was amplified from HeLa genomic DNA and from NA10567, a hybrid containing human chromosome 16. Furthermore, a second set of primers (5′-GCCATCATCGATGGCAAG-3′, nt 460–477, and 5′-CCACAGAAGACCCAGTCTGGTC-3′, nt 588–609) was used to obtain a specific 1.45-kb product, using the same DNAs as a template, indicating an intron of 1.3-kb between nt 477 and nt 588 (amino acid residues 160–196). No amplification products were obtained with DNAs from hamster or from several other monochromosomal human–Chinese hamster hybrids that contained human chromosomes 2, 6, and X.

Since PP4 could be involved in the regulation of mitosis and is inhibited by the potent tumor promotor okadaic acid (3), the assignment of *PPP4C* to 16p11–p12 is of interest. A number of tumor types show a high incidence of loss of heterozygosity (LOH) on chromosome 16q (6), whereas no LOH of 16p has been observed for cancers. Yet several translocations involving 16p13 (1) and 16p11 (8) are associated with acute leukemias. PP4 is expressed in a wide variety of human tissues, with the highest level of expression in testis (2). Therefore it is of relevance to examine whether alterations in the *PPP4C* gene may predispose to cancer, particularly in this tissue.

ACKNOWLEDGMENT

This work was supported by a grant from the DFG to H.P.

REFERENCES

1. Borrow, J., Stanton, V. P., Andresen, J. M., Becher, R., Behm, F. G., Chaganti, R. S. K., Civin, C. I., Disteche, C., Dube, I., Frischauf, A. M., Horsman, D., Mitelman, F., Volinia, S., Watmore, A. E., and Housman, D. E. (1996). The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. *Nature Genet.* **14:** 33–41.
2. Brewis, N. D., and Cohen, P. T. W. (1992). Protein phosphatase X has been highly conserved during mammalian evolution. *Biochim. Biophys. Acta* **1171:** 231–233.
3. Brewis, N. D., Street, A. J., Prescott, A. R., and Cohen, P. T. W. (1993). PPX, a novel protein serine threonine phosphatase localized to centrosomes. *EMBO J.* **12:** 987–996.
4. Church, G. M., and Gilbert, W. (1984). Genomic sequencing. *Proc. Natl. Acad. Sci. USA* **81:** 991–1995.
5. Cohen, P. (1989). The structure and regulation of protein phosphatases. *Annu. Rev. Biochem.* **58:** 453–508.
6. Doggett, N. A., Breuning, M. H., and Callen, D. F. (1996). Report of the Fourth International Workshop on Human Chromosome 16 Mapping 1995. *Cytogenet. Cell Genet.* **72:** 271–293.
7. Lichter, P., Tang, C. C., Call, K., Hermanson, G., Evans, G. A., Housman, D., and Ward, D. C. (1990). High resolution mapping of human chromosome 11 by *in situ* hybridization with cosmid clones. *Science* **247:** 64–69.
8. Rabbitts, T. H., Forster, A., Larson, R., and Nathan, P. (1993). Fusion of the dominant negative transcription regulator *CHOP* with a novel gene *FUS* by translocation t(12;16) in malignant liposarcomas. *Nature Genet.* **3:** 175–180.
9. Wilgenbus, K. K., Mincheva, A., Korn, B., Lichter, P., and Poustka, A. (1995). IRS-long range (LR) PCR: A simple method for efficient amplification of human genomic DNA from complex sources. *Methods Mol. Cell. Biol.* **5:** 214–221.
10. Zehetner, G., and Lehrach, H. (1994). The reference library system—Sharing biological material and experimental data. *Nature* **367:** 489–491.