SHORT COMMUNICATION

Smooth Muscle Myosin Heavy Chain Locus (*MYH11*) Maps to 16p13.13–p13.12 and Establishes a New Region of Conserved Synteny between Human 16p and Mouse 16

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The human smooth muscle myosin heavy chain locus (*MYH11*) was mapped by fluorescence *in situ* hybridization to the middle of the p arm of chromosome 16 using a genomic cosmid clone containing coding sequences of the gene as probe. Probe from coding sequence, when applied to Southern blots of a panel of hybrids containing different portions of human chromosome 16, localized the gene to 16p13.13–13.12. Coding sequence PCR primers, when used on the DNA from a CHO–mouse hybrid clone mapping panel informative for mouse chromosomes, showed that the gene was located on mouse chromosome 16. These results correct a recent assignment of *MYH11* from 16q12.2 to the region of the 16p-arm inversion breakpoint seen in acute myelomonocytic leukemia (AMML) M4Eo and demonstrate that the conflicting data do not result from the presence of additional MYH genes on the q arm of the chromosome. Also, a new region of conserved synteny between human 16p and mouse 16 is established.
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We have recently identified yeast artificial chromosomes (YACs) containing DNA from human chromosome 16 that cover the p-arm breakpoint of a pericentric inversion, inv(16)(p13;q22), associated with AMML M4Eo (3). These YACs were used to isolate clones from a chromosome 16 cosmid library (10) and one of these

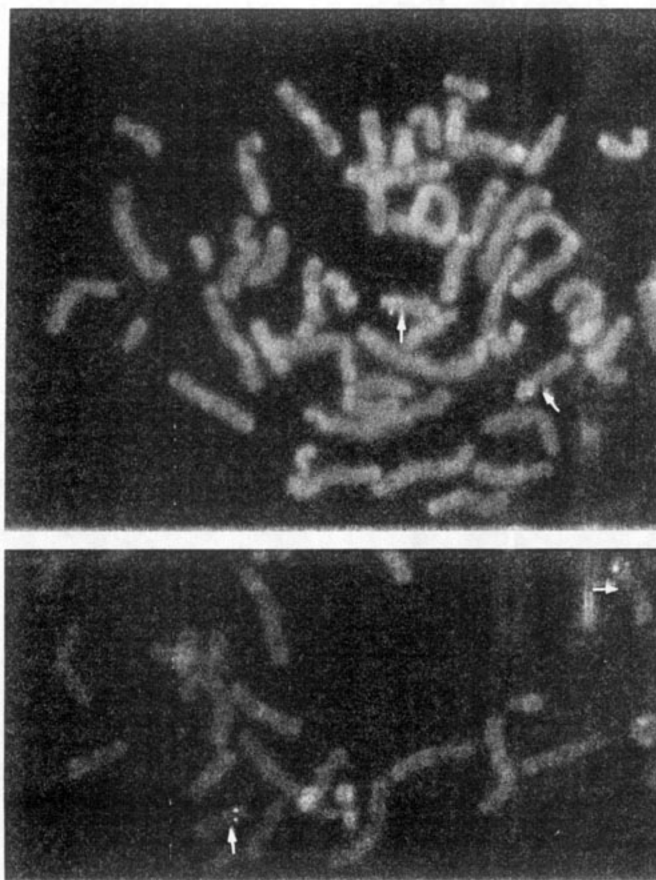

FIG. 1. FISH using cosmid 16C3 as probe conducted as described (3). In both the full metaphase (upper panel) and the partial metaphase (lower panel), the arrows indicate the positions of the centromeric constriction of chromosome 16. The presence of signal in the middle region of the short arm of both chromatids was a consistent observation.

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(16C3) was shown to span the p-arm inversion breakpoint. Screening a human large intestine cDNA library with a 1.2-kb *Eco*RI repeat-free fragment (16C3e) from this cosmid and sequencing an isolated clone resulted in the identification of the human smooth muscle myosin heavy chain gene (*MYH11*). This was part of the evidence indicating that *MYH11* was the p-arm gene interrupted by the inversion in AMML M4Eo (4). It was therefore with some concern that we noted a recent report by Matsuoka *et al.* (5) on the cloning and sequencing of *MYH11* followed by its assignment to the q arm of the chromosome at 16q12. Their assignment to chromosome 16 was by Southern blot hybridization with cDNA probe to a well-characterized hybrid clone panel. However, their methods of regional assignment were less conventional—fluorescence *in situ hybridization* (FISH) using a cDNA as probe and measurements of Southern blot hybridization intensity using DNA from human cell lines with partial deletion and trisomy involving chromosome 16. This disparity in the location of *MYH11* sequences has considerable biomedical significance and requires rapid resolution.

As a first step to that end, the cosmid containing *MYH11* coding regions (16C3) was biotin-labeled, had

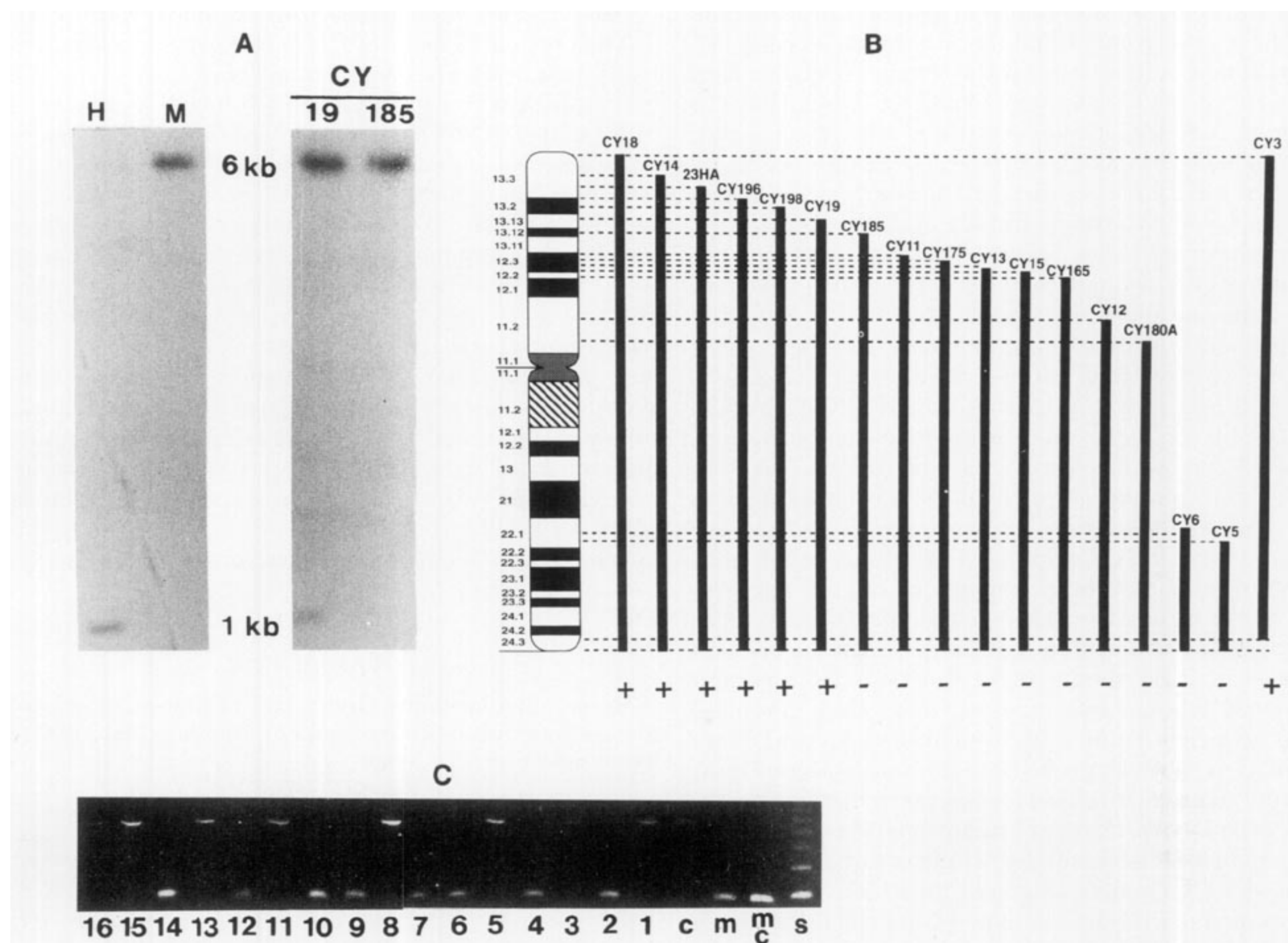

FIG. 2. (A) Results of Southern blot hybridization using a 100-bp probe generated by PCR using coding region primers conducted on human genomic DNA. PCR primers were those indicated in the text. Probe was prepared in a 50-μl PCR reaction containing 50 pmol of each primer, 200 μM of each dNTP, 100 ng of template DNA, and 1.5 U of AmpliTaq DNA polymerase from Perkin–Elmer–Cetus in 10 m*M* Tris-HCl (pH 9.0)/50 m*M* KCl/1.5 m*M* $MgCl_2$ buffer. Amplification incubation consisted of 4 min at 94°C followed by 35 cycles of 1 min at 92°C, 1 min at 60°C, 1 min at 72°C. A 10-min extension at 72°C concluded the reaction. The 100-bp product was gel-purified by squeeze–freeze and 25 ng labeled by random priming (Amersham Megaprime Kit). Hybridization was conducted at 65°C and washing was done at 25°C in 2× SSC, 0.1% SDS for 20 min followed by 5 min at 65°C in 0.2× SSC, 0.1% SDS. Channels contain *Eco*RI fragments from human (H), mouse (M), and the critical somatic cell hybrid DNAs of the panel (CY19 and CY185) in this analysis. Note that CY19 has both the mouse 6-kb as well as the human 1-kb fragments while CY185 has only the mouse form. (**B**) Idiogram of human chromosome 16 and diagrammatic representation of the portions of human chromosome 16 retained in hybrids CY18 through CY3. Along the bottom is indicated the presence (+) or the absence (−) of the human 1-kb *Eco*RI fragment scored for each hybrid. (**C**) Ethidium bromide-stained gel indicating the PCR fragments produced from Chinese hamster (c), mouse (m), and mouse Chinese hamster hybrid DNAs (lanes **1–16**). Lane **s** contains size markers (123-bp ladder). Primers and description of PCR methods are as indicated above. Lane m/c contains DNA from a mixture of Chinese hamster and mouse DNA. When both mouse and Chinese hamster copies of the gene are in the template, the weak Chinese hamster fragment (550 bp) is not visible by this procedure. In reconstruction experiments (data not shown), mouse-only fragments are seen when the amount of mouse vs Chinese hamster template is as little as 10%. Therefore, hybrids are scored + for the presence of the mouse *MYH11* gene if they have the 100-bp band; those hybrids are 2, 4, 6, 7, 9, 10, 12, and 14.

repetitive sequences blocked with unlabeled low C_0t human DNA, and was used for FISH on normal human lymphoblast metaphases. As shown in Fig. 1, the signal was specific to the p arm of chromosome 16, supporting our contention on its location. Multiple MYH genes have been identified, and there are examples of more than one being assigned to the same chromosome. Genes for skeletal muscle forms map to chromosome 17 (*MYH1, MYH3, MYH4* at 17pter–p11 with *MYH2* at 17p13.1 and *MYH8* at 17pter–p12), and genes for two cardiac muscle forms (*MYH6, MYH7*) have been assigned to 14q11.2–q13 (6). We therefore considered the possibility of more than one gene encoding smooth muscle isoforms on chromosome 16 to explain the difference between our assignment and that of Matsuoka *et al.* (5). It is possible that the basis for the strong FISH signal on the p arm of the chromosome could be due entirely to noncoding, nonrepetitive sequences in the cosmid. These sequences would be different from noncoding sequence of a putative second gene located on the q arm and thus specific to the p arm where it mapped.

Therefore, we have approached the question of whether coding sequences for *MYH11* are located in more than one place on chromosome 16 by designing PCR primers within a 100-bp segment of coding region identified (4) within 16C3e—5′-GTACTGCTCGGCCATCTTG-3′ and 5′-AGAGAAACAGGCGGCCAC-3′. This 100-bp coding segment is identical to a portion of the cDNA reported by Matsuoka *et al.* (bp 2847–2946 in Ref. 5; GenBank Accession No. D10667). These primers were used to investigate the presence of human sequences in a human–mouse hybrid clone panel well-characterized for different regions of human chromosome 16 (1) to identify the specific chromosomal location of the *MYH11* coding sequences. Since the primers produced PCR fragments of the same size from human and mouse DNA (not surprising since they amplified coding sequence from a highly conserved gene), they were used to generate probe from human genomic DNA. This 100-bp probe was used for Southern blot hybridizations of the DNA from the chromosome 16 region-informative hybrid clone panel. *Eco*RI digests of panel DNAs produced a 1-kb human fragment identified by the PCR probe that was well-distinguished from the 6-kb mouse form (Fig. 2A). This fragment was present in all 7 hybrids containing the p13.13–p13.12 region of human chromosome 16 and not present in the 10 hybrids lacking that region, 8 of which had been characterized as retaining the 16q12 region (Fig. 2B). These data confirm and localize the p-arm position of *MYH11* and exclude the presence of such sequences from the q arm.

Since different portions of the p arm of human chromosome 16 are known to be homologous with regions of mouse chromosomes 11, 17, 16, and 7 (from distal to proximal), while the q arm has been shown to have homology only with mouse chromosome 8 (2), identifying the chromosomal location of *MYH11* in the mouse could be useful not only in helping to support the chromosomal location of the human gene but also in resolving the boundaries of homology. To do this, the same PCR primers were used directly on the DNAs of a 16-member mouse–Chinese hamster hybrid clone panel that was informative for every mouse chromosome. Mouse chromosomes present in each hybrid, as determined by biochemical and molecular marker analysis (9), are indicated in Table 1. Fortuitously, mouse and hamster PCR fragments generated were of different sizes, making this analysis possible. These data are shown in Fig. 2C. The presence or absence of mouse fragments for *MYH11* among the hybrids was perfectly concordant (0% discordancy) with mouse chromosome 16, while discordancies for all other mouse chromosomes ranged from 31 to 56% (% discordancy with mouse chromosome 8 was 56%).

The protamine locus (*PRM1*) was previously the only locus assigned to human chromosome 16 that has also been assigned to the mouse 16 (8), identifying what is referred to as a homologous segment (7) between the two chromosomes. The human protamine was shown (1) to be present in hybrids CY18–CY198 and CY3 while absent from CY19–CY5 of the human chromosome 16 regional mapping panel (see Fig. 2B defining those region). Since no genes between the *PRM1* location (16p13.2) and the more proximal location of *MYH11* have been mapped in the mouse, the assignments of *MYH11* in man and mouse now define a new region of conserved synteny (7) between those genomes. It will be of interest to assign additional genes of the region in the mouse to determine the extent and boundaries of the conserved synteny. Genetic linkage studies of the loci in the mouse will then enable us to determine if it is a region of true linkage conservation—i.e., maintenance of gene order (7).

TABLE 1

Mouse Chromosomes Retained in Chinese Hamster × Mouse Hybrids as Determined by Biochemical and Molecular Marker Analysis

Hybrid number	Hybrid name	Mouse chromosomes present
1	0379	5, 9, 12, X
2	MSRJK16	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, X
3	MSAA16	1, 2, 6, 7, 8, 10, 11, 12, 13, 14, 15, 17, 19, X
4	MSAA22	1, 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, X
5	TM20	1, 2, 3, 4, 6, 7, 8, 9, 12, 13, 15, X
6	TM29	7, 9, 10, 12, 13, 14, 15, 16, 17, X
7	TM30	2, 6, 7, 9, 10, 13, 15, 16, 18, X
8	TM101	2, 3, 6, 7, 12, 14, 15, 18, X
9	TM107	6, 8, 9, 14, 15, 16, 18, X
10	TM117	1, 2, 3, 6, 8, 9, 12, 13, 16, 17, 18, X
11	TM118	1, 2, 4, 6, 7, 8, 9, 12, 13, 14, 15, 17, 19, X
12	TM120	4, 13, 14, 16, X
13	TM122	3, 12, X
14	TM137	1, 3, 6, 7, 12, 14, 15, 16, 19, X
15	TM25	6, 7, 8, X
16	TM50	1, 2, 7, 8, 13, 15, 18, 19, X

In summary, the conserved synteny data, combined with the direct mapping of the *MYH11* coding sequences, provide clear evidence for the 16p13.13–p13.12 location of *MYH11*.

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REFERENCES

1. Callen, D. F., Doggett, N. A., Stallings, R. L., Chen, L. Z., Whitmore, S. A., Lane, S. A., Nancarrow, J. K., Apostolou, S., Thompson, A. D., Lapsys, N. M., Eyre, H. J., Baker, E. G., Shen, Y., Holman, K., Phillips, H., Richards, R. I., and Sutherland, G. R. (1992). High-resolution cytogenetic-based physical map of human chromosome 16. *Genomics* **13:** 1178–1185.
2. Davisson, M. T., Lalley, P. A., Peters, J., Doolittle, D. P., Hillyard, A. L., and Searle, A. G. (1991). Report of the comparative committee for human, mouse and other rodents. *Cytogenet. Cell Genet.* **58:** 1152–1189.
3. Liu, P., Claxton, D. F., Marlton, P., Hajra, A., Siciliano, J., Freedman, M., Chandrasekharappa, S. C., Yanagisawa, K., Stallings, R. L., Collins, F. S., and Siciliano, M. J. Identification of yeast artificial chromosomes containing the inversion 16 p-arm breakpoint associated with acute myelomonocytic leukemia. *Blood* **82:** 716–721.
4. Liu, P., Tarle, S. A., Hajra, A., Claxton, D. F., Marlton, P., Freedman, M., Siciliano, M. J., and Collins, F. S. Fusion between transcription factor CBFβ/PEBP2β and a myosin heavy chain in acute myeloid leukemia. *Science* **261:** 1041–1044.
5. Matsuoka, R., Yoshida, M. C., Furutani, Y., Imamura, S.-I., Kanda, N., Yanagisawa, M., Masaki, T., and Takao, A. (1993). Human smooth muscle myosin heavy chain gene mapped to chromosomal region 16q12. *Am. J. Med. Genet.* **46:** 61–67.
6. McAlpine, P. J., Shows, T. B., Boucheix, C., Huebner, M., and Anderson, W. A. (1991). The 1991 catalog of mapped genes and report of the nomenclature committee. *Cytogenet. Cell Genet.* **58:** 5–102.
7. Nadeau, J. H. (1989). Maps of linkage and synteny homologies between mouse and man. *Trends Genet.* **5:** 82–86.
8. Reeves, R. H., Gearhart, J. D., Hecht, N. B., Yelick, P. C., Johnson, P., O'Brien, S. J. (1989). Mapping of *PRM1* to human chromosome 16 and tight linkage of *Prm-1* and *Prm-2* on mouse chromosome 16. *J. Hered.* **80:** 442–446.
9. Shawlot, W., Siciliano, M. J., Stallings, R. L., and Overbeek, P. A. (1989). Insertional inactivation of the downless gene in a family of transgenic mice. *Mol. Biol. Med.* **6:** 299–307.
10. Stallings, R. L., Doggett, N. A., Callen, D., Apostolou, S., Chen, L. Z., Nancarrow, J. K., Whitmore, S. A., Harris, P., Michison, H., Breuning, M., Saris, J. J., Fickett, J., Cinkosky, M., Torney, D. C., Hildebrand, C. E., and Moyzis, R. K. (1992). Evaluation of a cosmid contig physical map of human chromosome 16. *Genomics* **13:** 1031–1039.