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Assignment of the human fast skeletal muscle myosin alkali light chains gene ($MLC1_F/MLC3_F$) to 2q 32.1–2qter

Odile Cohen-Haguenauer[1], Paul J. R. Barton[2], Nguyen Van Cong[1], Stéphane Serero[1], Marie-Sylvie Gross[1], Chantal Jegou-Foubert[1], Marie-France de Tand[1], Benoit Robert[2], Margaret Buckingham[2], and Jean Frézal[1]

[1]INSERM U12, Unité de Recherches de Génétique Médicale, Hôpital des Enfants-Malades, Clinique Maurice Lamy, 149, rue de Sèvres, F-75743 Paris Cedex 15, France
[2]Department of Molecular Biology, Institut Pasteur, 25, rue du Dr. Roux, F-75015 Paris, France

Summary. A DNA probe derived from a mouse intronless pseudogene including coding regions for the myosin fast skeletal muscle alkali light chains, $MLC1_F/MLC3_F$ (suggested HGM symbol, MYL1), was tested on a panel of 25 independent man-rodent somatic cell hybrids in order to assign the human $MLC1_F/MLC3_F$ gene to a human chromosome. A 3.7-kb TaqI human fragment was found to correlate with the presence of chromosome 2 in the hybrids, characterized both by cytogenetic analysis and reference enzyme markers. A regional assignment to 2q32.1-qter was possible using hybrids whose human parental strains bore a reciprocal translocation t(X;2)(p22;q32.1). The fact that IDH1 and the $MLC1_F/MLC3_F$ gene are closely linked on chromosome 1 in the mouse and map to the same region of human chromosome 2 in man indicates, that these chromosomes have a conserved region of homology between them and that the human 3.7-kb TaqI fragment corresponds indeed to a functional gene.

Introduction

In all eukaryotes, actin and myosin play a role in the maintenance of cell shape and in cellular movement. In striated muscles of multicellular organisms, myosin forms the major constituent of the sarcomere, the unit of muscle fibre.

In vertebrate striated muscle, myosin is composed of two heavy chains of about 200000 daltons each (MHC) and four light chains (MLC) of about 20000 daltons each, the latter belonging to two distinct functional types: the phosphorylatable, regulatory, or MLC2 type and the nonphosphorylatable, alkali, or MLC1 and MLC3 types (MYL1 according to the HGM symbols) (reviewed by Squire 1981). The role of the myosin alkali light chains in vertebrate skeletal muscle is unclear (see, e.g., Wagner and Giniger 1981; Sivaramakrishnan and Burke 1982), although in smooth muscle the alkali light chains have been shown to be involved in the active site of the myosin molecule (Okamoto et al. 1986).

Several isoforms of myosin alkali light chain have been identified. These isoforms are each associated with different muscle types and are encoded by a family of myosin light chain genes (for review see Barton and Buckingham 1985). In vertebrate fast skeletal muscle, two myosin alkali light chains are present: $MLC1_F$ and $MLC3_F$. These isoforms are functionally related and may be alternately associated with myosin heavy chains in homodimer or heterodimer combinations (Hoh 1978; d'Albis et al. 1979, Lowey et al. 1979). They are closely related in structure, having a common COOH terminal sequence of 141 amino acids. Analysis at the gene level has shown that these isoforms are in fact encoded by a single gene in mouse (Robert et al. 1984), rat (Periasamy et al. 1984), and chicken (Nabeshima et al. 1984). The structure of this gene is such that its expression involves both the use of two promoters and differential splicing of sequences at the 5′ end of the gene that encode NH_2 termini specific to $MLC1_F$ and $MLC3_F$. The shared amino acid sequence is encoded by four common coding exons followed by a shared 3′ noncoding exon.

In the mouse the gene encoding the $MLC1_F$ and $MLC3_F$ myosin alkali light chain isoforms ($MLC1_F/MLC3_F$ gene) has been shown to be located on chromosome 1 and is not linked to other myosin light chain genes (Robert et al. 1985); the gene encoding the ventricular muscle/slow skeletal muscle isoforms ($MLC1_V$ (Barton et al. 1985a) is on chromosome 9, and the atrial muscle gene ($MLC1_A$; equivalent to the fetal isoform gene $MLC1_{emb}$; Barton et al. 1985b) is on chromosome 11. These genes are also not linked to other contractile protein genes in the mouse (Robert et al. 1985), the following of which have been localized: $MLC2_F$ on chromosome 7 (Czosnek et al. 1982), the skeletal muscle myosin heavy chain genes on chromosome 11 (Leinwand et al. 1983; Robert et al 1985), the skeletal muscle actin gene on chromosome 3 (Czosnek et al. 1982), and a cardiac myosin heavy chain gene on chromosome 14 (Weydert et al. 1985), although there is a loose linkage between $MLC1_A$ and the skeletal muscle myosin heavy chain genes on chromosome 11.

In man, the genes encoding the different isoforms of myosin alkali light chain have as yet not been localized, although other contractile protein genes have been given chromosomal assignments; the skeletal muscle myosin heavy chain genes (MYH1, MYH2, MYH3) are on chromosome 17 (Leinwand et al. 1983; Rappold and Vosberg 1983; Edwards et al. 1985), and the genes encoding the skeletal muscle (ACTA) and cardiac isoforms (ACTC) of alpha-actin are on chromosomes 1 and 15 respectively (Gunning et al. 1984).

Offprint requests to: O. Cohen-Haguenauer

We present here the chromosomal assignment of the $MLC1_F/MLC3_F$ (MYL1F) gene in man by means of a mouse DNA probe used to test Southern blots of DNA derived from a panel of man-mouse and man-hamster somatic cell hybrids. The presence of a human chromosome translocation within the somatic cell hybrid panel has allowed regional assignment of this gene.

Material and methods

Panel of somatic cell hybrids

Parental cells. The parental rodent lines grown with selective markers are mouse lines CL1D,L/TK$^-$ and CL1D,LA/TK$^-$, HPRT$^-$, and hamster lines V79/HPRT$^-$ and CH/HPRT$^-$.

Parental human cells are normal fibroblasts, strain 106 (46 XY), and fibroblasts possessing a balanced reciprocal translocation: strain 34, with 46,Y,t(X;2)(p22.3;q32.1) or, in short, Xp$^+$ and 2q$^-$; strain BL, with 46,X,t(X;2)(p22.3;q32.1) or, in short, Xp$^+$ and 2q$^-$; strain 56, with 46,X,t(X;5)(q21;q11) or, in short, Xp$^+$ and 5q$^-$; and strain 53, with 46,XX,t(2;17)(q14;q21) or, in short, 2q$^-$ and 17q$^+$.

Hybrid cells. Hybrid cells tested in this study come from the primary hybrid panel described by Nguyen Van Cong et al. (1986), where both hybrid production techniques and cytogenetic and enzyme analyses are reported.

This panel consists of seven man-mouse hybrids: two 53-CL1D (man-mouse hybrids nos. 1 and 2) and five 56-CL1DA (man-mouse hybrids nos. 3, 4, 5, 6, and 7), and eighteen man-hamster hybrids: one 106-V79 (man-hamster no. 1), one CH-106 (man-hamster no. 2), seven BL-CH (man-hamster hybrids nos. 3, 4, 5, 6, 7, 8, and 9), two 56-CH (man-hamster hybrids no. 10 and 11), and seven 34-CH (man-hamster hybrids nos. 12, 13, 14, 15, 16, 17, and 18).

Analysis of the panel

Cell extracts preparation for enzyme analysis. Human fibroblasts, mouse cells, hamster cells, and man-mouse and man-hamster hybrid cells were washed in PBS three times, then centrifuged at 2000 r/min for 5 min. The pellet was then homogenized in an equal volume of distilled water. The suspension was frozen and thawed three times.

Enzyme studies. The following enzymes were tested: malate dehydrogenase 1 (MDH1), isocitrate dehydrogenase 1 (IDH1) (Van Someren 1974); ribulose-5-phosphate-3-epimerase (RPE) (Spencer and Hopkinson 1970); glucose-6-phosphate dehydrogenase (G6PD), alpha-galactosidase A (GLA), and phosphoglycerate kinase (PGK1) (Meera Khan 1971).

Cytogenetic analysis. Chromosome preparations were stained according to Dutrillaux and Lejeune (1971) (R-banding). About 12 mitoses were analyzed for each hybrid.

$MLC1_F/MLC3_F$ gene probe

To detect the human $MLC1_F/MLC3_F$ gene, we have used a probe derived from a mouse $MLC1_F/MLC3_F$ pseudogene (Robert et al. 1984). Unlike the functional gene, this processed-type pseudogene contains no intron sequences and provides a convenient source of probe that shows 99% homology with the corresponding $MLC1_F/MLC3_F$ sequences.

We have cloned a 470-bp Sau3A fragment of this pseudogene which contains most of the sequences homologous to the common-coding exons of the $MLC1_F/MLC3_F$ gene and about 100 nucleotides of the 3′ noncoding sequence (see Robert et al. 1984). This fragment was cloned as four tandem repeats into the Bam HI site of pBR322 and is called pGLC450.4.

Southern blots

DNA preparation from somatic cell lines. Cell cultures in exponential growth phase were harvested in 10 m*M* Tris HCl pH 7.6, 10 m*M* EDTA pH 8, 50 m*M* NaCl, 0.02% SDS. The suspension was then incubated for 1 h at 42°C with ribonuclease A (Sigma) and then overnight at 42°C with 0.2 mg/ml proteinase K (Boehringer, Mannheim). High molecular weight DNA was recovered by ethanol precipitation following phenol and chloroform extractions.

Preparation of Southern blots. DNA samples derived from the 7 man-mouse hybrids, 18-man-hamster hybrids, and their human and rodent parental lines as well as from human cell lines containing one to four of each of the sex chromosomes (kindly provided by Marc Fellous) were digested with TaqI (Appligene, France), using 5 units of enzyme per microgram DNA.

The 20-µg samples were loaded with low-melting-point agarose onto 20-cm 0.7% agarose gels, and run for 40 h at 30 V and then transferred to nylon membranes (Hybond, Amersham) (Southern 1975).

Hybridization. Filters were prehybridized for 6 h at 37°C in 50% formamide, 5 × SSPE (1 × SSPE = 0.15 M NaCl, 10 m*M* Na phosphate, 1 m*M* EDTA pH 7.4), 0.1% SDS, 0.04% Ficoll, 0.04% polyvinylpyrrolidone, 0.04% bovine serum albumin, 5% dextran sulfate, and 250 µg/ml sonicated, denatured salmon sperm DNA.

Probe pGLC 450–4 was labeled by nick translation (Rigby et al. 1977) with ^{32}P deoxynucleotide triphosphate (Amersham) to a specific activity of 4×10^8 dpm/µg.

For each filter 500 ng (2×10^8 cpm) was added to the prehybridization solution, hybridized for 24 h at 37°C, and then washed under low stringency conditions: 2 × SSC, 0.1% SDS for 10 min at ambient temperature, followed by one wash at 65°C in 2 × SSC, 0.1% SDS for 1 min. Filters were then exposed to Kodak XAR-5 films with intensifying screens (Dupont Cronex Lightning Plus) at −70°C for 3 days.

Results

Using DNA cut with the restriction enzyme TaqI (Fig. 1), three fragments were observed in human parental strains: a 3.7-kb intense fragment and 4.5-kb and 3-kb faint fragments. These three fragments were distinguishable from the eight fragments observed in the mouse lines (CL1D and CL1DA) and from the two fragments detected in hamster lines (CH and V79/4) (Fig. 1). Since in the hybrid cells, the two faint human fragments are not detectable and since the exact identity of these bands is unclear (see "Discussion"), the presence (+) or absence (−) of $MLC1_F/MLC3_F$ sequence in the hybrids is based on the distribution of the intense 3.7-kb band.

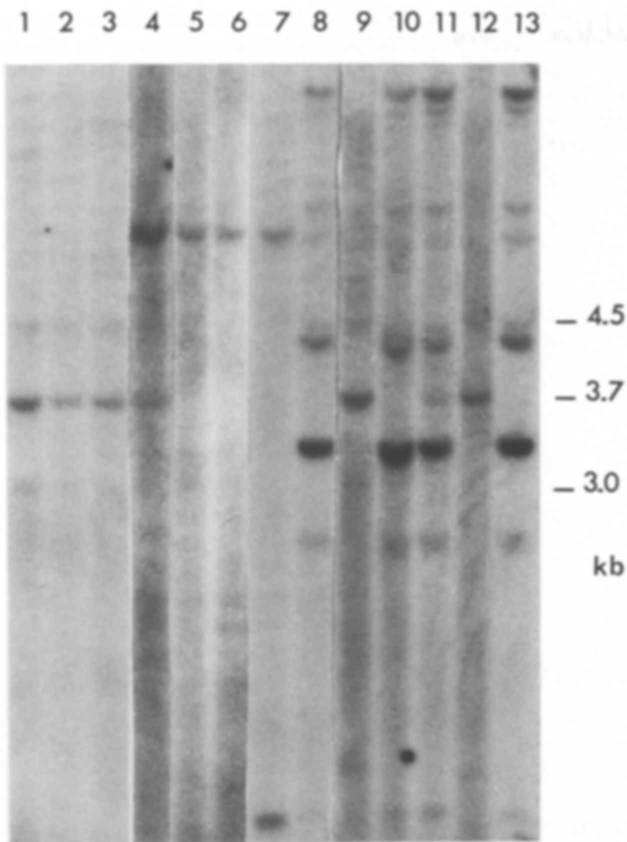

Fig. 1. Autoradiograms of the $MLC1_F/MLC3_F$ pGLC450.4 mouse probe hybridized to both human or rodent parental strain and hybrid cell DNAs. *Lanes 1, 2,* and *3,* lymphoblastoid human cell lines; *4,* man-hamster hybrid $(MLC1_F/MLC3_F)^+$; *5* and *6,* man-hamster hybrid $(MLC1_F/MLC3_F)^-$; *7,* hamster CH; *8, 13,* mouse Cl1DA and Cl1D; *9, 12,* human parental fibroblast strains; *10,* man-mouse hybrid $(MLC1_F/MLC3_F)^-$; *11,* man-mouse hybrid $(MLC1_F/MLC3_F)^+$. Molecular weights of the hybridizing TaqI restriction fragments are indicated on the right

A panel of 25 man-rodent hybrids was tested for the presence of the $MLC1_F/MLC3_F$ 3.7-kb fragment, and the distribution of the signal was compared to both the cytogenetic characteristics of the panel and to the signal observed with several chromosome 2 enzyme markers (IDH1, MDH1) and with several X chromosome markers (PGK, GLA, G6PD). The following data were obtained:

Comparison of $MLC1_F/MLC3_F$ signal and enzyme markers (Table 1)

A strong positive correlation was observed with the presence of 3.7-kb fragment and the well-characterized chromosome 2q markers IDH1 and RPE. Among the 25 hybrids analyzed, 19 were positive for $MLC1_F/MLC3_F$, IDH1, and RPE, and 6 were negative for all three markers.

In contrast to this, no positive correlation was observed between $MLC1_F/MLC3_F$ and MDH1 (a well-known marker for the short arm of chromosome 2: 2p23): ten hybrids were positive for both $MLC1_F/MLC3_F$ and MDH1; nine hybrids were positive for $MLC1_F/MLC3_F$, but MDH1 negative; five hybrids were negative for both markers; and one hybrid was negative for $MLC1_F/MLC3_F$ and positive for MDH1.

These results indicate that the $MLC1_F/MLC3_F$ 3.7-kb TaqI fragment correlated with IDH1, and not with MDH1.

Comparison between $MLC1_F/MLC3_F$ and cytogenetic characterization of the panel (Table 1)

Among the six hybrids that were negative for the 3.7-kb $MLC1_F/MLC3_F$ fragment, all human chromosomes except chromosomes 2, 18, and Y were represented in at least 30% of the mitoses analyzed in at least one hybrid. Consequently only these three chromosomes could not be excluded for the localization of the $MLC1_F/MLC3_F$ gene. Furthermore, chromosomes 18 and Y were observed at low frequency (less than 30% of cells) in one of these $(MLC1_F/MLC3_F)$ negative hybrids, whereas intact chromosome 2 was never observed in any of the cells analyzed from these six hybrids.

No positive correlation could be established with chromosome 18; among 25 hybrids analyzed, nine were positive for both $MLC1_F/MLC3_F$ and chromosome 18; three were positive for $MLC1_F/MLC3_F$, but chromosome 18 was present in less than 30% of the mitoses; seven were positive for $MLC_F1/MLC3_F$ and negative for chromosome 18; five were negative for both; and one was negative for $MLC1_F/MLC3_F$ and positive for chromosome 18.

Similarly, no positive correlation could be observed with chromosome Y: chromosome Y is detected in two hybrids only, man-hamster nos. 1 and 17, while $MLC1_F/MLC3_F$ signal was positive in 19 hybrids. Furthermore, $MLC1_F/MLC3_F$ was equally present in male and female fibroblast strains, and no gene-dosage effect could be revealed in the human lymphoblastoid cell line containing no to four chromosomes Y. These data are against $MLC1_F/MLC3_F$ gene localization to chromosomes 18 and Y.

Among the 19 hybrids positive for the 3.7-kb $MLC1_F/MLC3_F$ fragment, the q32.1-qter fragment of chromosome 2 was always present either as the intact chromosome 2 or as the Xp^+ or $17q^-$ translocations carrying this region of chromosome 2. Among 6 hybrids negative for the 3.7-kb fragment, chromosomes 2, $17q^+$, and Xp^+ were never observed. These data allow the regional mapping of $MLC1_F/MLC2_F$ on the 2q32–2qter fragment of human chromosome 2.

Comparison of $MLC1_F/MLC3_F$ signal with chromosome Xp^+, and enzyme markers for chromosome 2 and the X chromosome (Fig. 2)

Among 14 man-hamster hybrids arising from X-2 reciprocal translocation (Xp^+, $2q^-$), two were negative for Xp+, IDH1, RPE, and $MLC1_F/MLC3_F$ and positive for PGK, GALA, and G6PD, and twelve were positive for Xp^+ and the set of markers just mentioned. These results indicate that $MLC1_F/MLC3_F$ IDH1, and RPE are on chromosome Xp^+. The two dissociated results IDH1−, RPE−, $(MLC1_F/MLC3_F)$−, PGK+, GALA+, G6PD+ (man-hamster nos. 9 and 12, see Table 1) can be explained by secondary breakage of Xp^+ with loss of the fragment bearing IDH1, RPE, and $MLC1_F/MLC3_F$ coming from chromosome 2 and retention of the fragment bearing PGK, GALA, and G6PD coming from the X chromosome. This fragment of the X chromosome bearing the HPRT gene is retained by the HAT selective medium (hamster parental cell lines being HPRT−).

As eight hybrids are Xp^+ and MDHI−, MDHI is not translocated to the X chromosome (Fig. 2).

Table 1. Analysis of human chromosomes and enzymes in negative and positive man-rodent hybrids for MCL1$_F$/MLC3$_F$. On the left, the 25 independent man-rodent somatic cell hybrids (Nguyen Van Cong et al. 1986) are arranged according to the presence or the absence of the human MLC1$_F$/MLC3$_F$ TaqI 3.7-kb fragment. The human chromosome content of each hybrid is indicated in the second column: chromosomes detected in less than 30% hybrid cells are given in brackets; X, active X; Xi, inactive X. The third column gives data concerning enzyme marker analysis; IDH1 (2q32-qter), RPE (2q32-qter), MDHI (2p23), G6PD (Xq28), PGK1 (Xq13), and GLA (Xq21-q22): +, human enzyme is detected in the hybrid; −, human enzyme not detected; ·, not tested. This illustrates the strong correlation between MLC1$_F$/MLC3$_F$ and both IDHI and RPE

	Human chromosomes detected	Enzymes					
		IDH1	RPE	MDH1	G6PD	PGK1	GLA
Hybrids (MLC1$_F$/MLC3$_F$)$^-$							
Man-mouse 2	3, 6, 7, (8), 12, (13), (14), 17, 19, 20, 21	−	−	−	−	·	·
Man-hamster 1	10, 15, (19), 20, 21, X, (Y)	−	−	−	+	·	·
Man-hamster 2	6, 8, 11, 12, 20, (21), (22), X	−	−	−	+	·	·
Man-hamster 9	1, 4, 5, 6, 7, 9, 11, 16, (18), 19, 20, 21, 22, Xi	−	−	−	+	+	+
Man-hamster 10	8, 14, 15, 16, 19, 21, Xi, (5q$^-$)	−	−	−	+	·	·
Man-hamster 12	3, 5, 8, 9, 11, 12, 13, 14, 16, 19, 20, (21)	−	−	+	+	+	+
Hybrids (MCL1$_F$/MLC3$_F$)$^+$							
Man-mouse 1	(1), (2), 3, 4, 5, (6), 7, 8, 11, 13 (14), 17, (20), (X), 2q$^-$, 17q$^+$	+	+	+	+	·	·
Man-mouse 3	(2), 3, (4), 5, 6, 7, 11, (15), (16), 17, (19), 20, 21, 22, (Xi)	+	+	+	+	·	·
Man-mouse 4	(1), 2, 3, 4, 5, 6, 7, 8, 10, (11), 12, 13, 16, 17, 18, (19), 20, 21, 22, Xi	+	+	+	+	·	·
Man-mouse 5	2, 3, (4), 5, 7, 8, 12, 13, (14), 16, 17, 18, 20, 21, 22	+	+	+	+	·	·
Man-mouse 6	2, 3, 6, 7, 11, 12, 17, 21, Xi	+	+	+	+	·	·
Man-mouse 7	1, 2, 4, 5, 7, 8, (10), 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, (Xi)	+	+	+	+	·	·
Man-hamster 3	3, (4), 6, (8), (9), (10), (13), 14, 15, 16, (18), 19, Xp$^+$	+	+	−	+	+	+
Man-hamster 4	4, 5, 6, (8), 10, 11, 13, 14, (16), 19, 21, (Xi), Xp$^+$	+	+	−	+	+	+
Man-hamster 5	1, 3, 4, 6, 9, (10), 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, Xi, Xp$^+$	+	+	+	+	+	+
Man-hamster 6	4, (8), 11, 12, 18, 19, Xi, Xp$^+$	+	+	−	+	+	+
Man-hamster 7	4, 5, 6, 7, (8), 11, 12, 13, 14, 15, 16, 18, 20, (21), 22, (Xi), Xp$^+$	+	+	+	+	+	+
Man-hamster 8	3, 5, 6, (7), (10), 11, 13, 15, (18), (20), 21, 22, Xi, Xp$^+$	+	+	−	+	+	+
Man-hamster 11	1, 2, 3, (4), 5, 6, 8, 9, 12, 18, 19, 22, Xq$^+$	+	+	+	+	·	·
Man-hamster 13	(17), 21, Xp$^+$	+	+	−	+	+	+
Man-hamster 14	6, (7), (8), 10, 11, 12, 14, (16), 20, 21, 22, Xp$^+$	+	+	+	+	+	+
Man-hamster 15	1, 3, 4, 5, (6), 8, 9, 11, 12, 13, 14, (15), 18, 19, 20, Xp$^+$	+	+	−	+	+	+
Man-hamster 16	1, (3), 6, 8, 9, (10), 11, 12, 13, 15, 16, 18, 21, Xp$^+$	+	+	−	+	+	+
Man-hamster 17	3, 4, 8, 14, (16), (19), 20, Y, Xp$^+$	+	+	−	+	+	+
Man-hamster 18	4, 6, 8, (9), 10, 12, 13, (14), (18), (20), 21, Xp$^+$	+	+	−	+	+	+

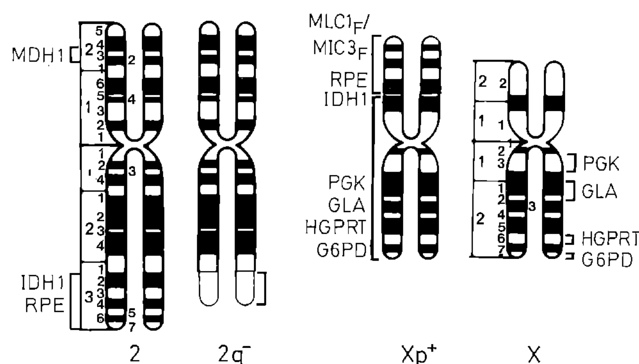

Fig. 2. Diagram representing the reciprocal translocation between chromosomes 2 and X of the human parental strains BL, 46,X,t(X;2)(p22;q32.1), and 34, 46,Y,t(X;2)(p22;q32.1). In the female BL strain, the intact X chromosome is inactive. In the 34 male strain, the intact X chromosome is lost. In the cell hybrids arising from BL and 34, 2q$^-$ is never observed. Regional assignment of MDH1 (2p23) (Hamerton 1975), IDH1 (2q32-qter) (Weil et al. 1977), RPE (2q32-qter) (Gross et al. 1982), PGK1 (Xq13) (Hors-Cayla et al. 1981), GLA (Xq21-q22) (Fox et al. 1984), G6PD (Xq28) (Pai et al. 1980), HPRT (Xq26-q27.3) (Pai et al. 1980), and MLC1$_F$/MLC3$_F$ (this work) are indicated. Chromosomes 2q$^-$ and Xp$^+$ show regional assignment of the previous markers according to our data

Discussion

In order to map the gene encoding the fast skeletal muscle alkali light chains MLC1$_F$ and MLC3$_F$ in man, a probe corresponding to most of the coding sequence of the mouse MCL1$_F$/MLC3$_F$ gene and about 100 nucleotides of the 3′noncoding region was hybridized to a panel of 25 man-rodent somatic cell hybrids.

In the absence of a suitable cDNA clone we have derived a probe for MCL1$_F$/MLC3$_F$ sequences from a mouse MLC1$_F$/MLC3$_F$ processed pseudogene. With other genomic clones containing flanking and intron sequences, hybridization to the panel using the same conditions would have revealed rodent sequences only.

The use of TaqI for the DNA digestion of the panel was helpful in differentiating rodent and human fragments with all the cDNA sequences so far tested on the panel. Hybridization was performed using low stringency conditions (37°C in 50% formamide) to allow hybridization of the mouse probe to human DNA (much diluted among the DNA of the hybrids).

The assignment of MLC1$_F$/MLC3$_F$ to chromosome 2 was founded on both cytogenetic characterization of the panel and

comparison of the DNA probe signal to assigned enzyme markers.

The chromosomal composition of the hybrids was analyzed on a dozen mitoses for each hybrid. As the results based on karyotype analysis is instrumental, the notation + was adopted only when the presence of a chromosome was observed in more than 30% of the mitoses. Whenever (in a given hybrid negative for the 3.7-kb TaqI band) a chromosome was present in less than 30% of mitoses – it could not be eliminated for the localization of $MLC1_F/MLC3_F$. According to the exclusion criterion, i.e., presence of the chromosome and absence of a marker, three chromosomes – 2, 18, and Y – could not be excluded for the localization of $MLC1_F/MLC3_F$.

We observed a perfect cosegregation between the 3.7-kb TaqI $MLC1_F/MLC3_F$ band and both IDH1 and RPE signals; but no positive correlation could be observed with MDH1, hinting at a regional localization closer to IDH1 and RPE on chromosome 2. The study of a translocation t(X;2)(p22.3; q32.1) in two human parental strains confirms the regional assignment of the $MLC1_F/MLC3_F$ gene to the 2q32.1-qter segment. These data are in keeping with previously published results: IDH1 assigned to 2q32.1-qter (Weil et al. 1977); RPE, to 2q32.1-qter (Gross et al. 1982); and MDH1, to 2p23 (Hamerton 1975).

The assignment of $MLC1_F/MLC3_F$ was based on the presence of a 3.7-kb TaqI human fragment. The two fainter 4.5-kb and 3-kb bands in the human DNA samples may correspond to either (i) other fragments derived from the human $MLC1_F/MLC3_F$ gene or (ii) fragments containing other myosin light chain genes. We were unable to follow the segregation of these bands as their signal was too weak and are therefore unable to determine whether they come from the same of different loci. A myosin light chain pseudogene is present in a limited number of mouse species (Robert et al. 1984) and maps to another chromosome than the functional gene (Robert et al. 1985). No myosin light chain pseudogenes have been identified in other species (rat: Periasamy et al. 1984; chicken: Nabeshima et al. 1984).

In mouse the functional gene is linked to IDH1 with a percentage of recombination (θ) of 2 centimorgans (Robert et al. 1985). Thus, the 3.7-kb TaqI $MLC1_F/MLC3_F$ fragment, shown to be on the same fragment as IDH1 in the present study, might well correspond to the human $MLC1_F/MLC3_F$ functional gene.

These observations are most interesting for comparative mapping. As observed for 6-phosphogluconate dehydrogenase (PGD) and enolase-1 (ENO1), which are closely linked in man, with $\theta = 2$ centimorgans (HGM8 1985), and found to be syntenic in many different species analyzed, the $MLC1_F/MLC3_F$, IDH1 syntenic group may well be conserved among mammals. Furthermore, recent evidence has shown that the gene encoding villin (Pringault et al. 1986), a Ca^{++}-dependent actin-binding protein (see Mooseker 1985), is also tightly linked to the $MLC1_F/MLC3_F$ and IDH1 genes in mouse (Guenet, personal communication) and is regionally localized at position 2q35–36 (Rousseau-Merck et al., personal communication). These genes therefore appear to identify a homologous region between mouse chromosome 1 and human chromosome 2.

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