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A new yeast gene with a myosin-like heptad repeat structure

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Summary. We isolated a gene encoding a 218 kDa myosin-like protein from *Saccharomyces cerevisiae* using a monoclonal antibody directed against human platelet myosin as a probe. The protein sequence encoded by the *MLP1* gene (for myosin-like protein) contains extensive stretches of a heptad-repeat pattern suggesting that the protein can form coiled coils typical of myosins. Immunolocalization experiments using affinity-purified antibodies raised against a *TrpE-MLP1* fusion protein showed a dot-like structure adjacent to the nucleus in yeast cells bearing the *MLP1* gene on a multicopy plasmid. In mouse epithelial cells the yeast anti-*MLP1* antibodies stained the nucleus. Mutants bearing disruptions of the *MLP1* gene were viable, but more sensitive to ultraviolet light than wild-type strains, suggesting an involvement of *MLP1* in DNA repair. The *MLP1* gene was mapped to chromosome 11, 25 cM from *met1*.

Key words: Cytoskeleton – Nucleus – Coiled coil – DNA repair

Introduction

The yeast *Saccharomyces cerevisiae* contains at least two of the three major filament systems that form the basis of the eukaryotic cytoskeleton: namely actin microfilaments and microtubules (Gallwitz and Seidel 1980; Ng and Abelson 1980; Neff et al. 1983; Schatz et al. 1986). Despite their relative structural simplicity, microtubules and actin filaments each perform diverse functions. This functional diversity is apparently mediated by filament-associated proteins, several of which have been identified in yeast (Drubin et al. 1988; Magdolen et al. 1988; Liu and Bretscher 1989; Armatruda et al. 1990).

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We are interested in studying the role of yeast myosin. A myosin heavy chain gene (*MYO1*) had been characterized previously in *S. cerevisiae* (Watts et al. 1985). This myosin appears to be involved in cytokinesis (Watts et al. 1987). However, biochemical data had indicated the existence of more than one myosin in yeast (Drubin et al. 1988; D. Drubin and K. Wertman, personal communication). We therefore decided to look for additional myosin genes by screening a yeast genomic DNA library with a heterologous antibody probe. We describe here the properties of a yeast gene encoding a 218 kDa protein from yeast which resembles myosin but which shows no head-domain similarity to the skeletal myosins.

Materials and methods

Strains and media. The yeast strains used in this paper are listed in Table 1. Growth media and genetic manipulations were according to Sherman et al. (1974) and Rose et al. (1990).

Table 1. Yeast strains used in this study

Strain	Genotypes
DBY 1186	*MAT**a** ade6 arg4 aro7 asp5 met14 lys2 pet17 spo11 trp1 ura3*
DBY 1707	*MAT**a**/α leu2-3,112/leu2-3,112 lys2-801/+ ura3-52/ura3-52*
DBY 1827	*MATα his3-Δ200 leu2-3,112 ura3-52*
DBY 2063	*MAT**a** leu2-3,12 ura3-52*
DBY 5384	*MATα his3-Δ200 leu2-3,112 mlp1-Δ1::LEU2 ura3-52*
DBY 5385	*MATα leu2-3,112 ura3-52*
DBY 5386	*MAT**a** leu2-3,112 lys2-801 mlp1-Δ2::LEU2 ura3-52*
DBY 5387	*MAT**a** leu2-3,112 ura3-52*
DBY 5388	*MATα leu2-3,112 lys2-801 mlp1-Δ2::LEU2 ura3-52*
DBY 5289	*MAT**a** leu2-3,112 met1-1 ura3-52*
DBY 5390	*MATα ade2 mlp1::URA3 ura3-52*
DBY 5394	*MAT**a**/α leu2-3,112/leu2-3,112 lys2-801/+ mrp1-Δ1::LEU2/+ ura 3-52/ura3-52*

Plasmid constructions. Standard procedures were used for DNA manipulations (Maniatis et al. 1982). To generate an in-frame *TrpE-MLP1* fusion, plasmid pRK25 was constructed by inserting the 1.0 kb *Eco*RI *MLP1* fragment (Fig. 1, fragment no. 6) into the *Eco*RI site of pRB535 (pATH3, Dieckmann and Tzagoloff 1985). The 1.0 kb *Eco*RI fragment is located close to the 5′ end of the coding region. The *MLP1* overproducing plasmid pRK29 was obtained by insertion of a 9.5 kb *Bam*HI-*Sal*I fragment, containing the complete *MLP1* gene, into the 2 μm vector YEp420 (Broach 1983) cut with *Bam*HI and *Sal*I.

Gene disruption. To create a disruption of the chromosomal *MLP1* gene by a one-step gene replacement procedure, plasmid pRK19 was constructed. On this plasmid the 2.5 kb *Bam*HI-*Sal*I fragment from pRB684, containing the *LEU2* marker gene, is flanked by the 0.6 kb *Eco*RI-*Xho*I fragment, derived from the end of the *MLP1* gene and a 0.7 kb *Bst*EII-*Eco*RI fragment, which is from the beginning of the *MLP1* gene (Fig. 7A). *Bam*HI linkers had been attached to the *Xho*I site and *Sal*I linkers to the *Bst*EII site to create convenient restriction sites for cloning. A 3.8 kb fragment was isolated from pRK19 by *Hin*dIII and partial *Eco*RI digestion containing essentially the three fragments described above. This fragment was transformed into the diploid *leu2* strain DBY 1707 (Ito et al. 1983). *LEU2* transformants were analyzed for the presence of the *MLP1* disruption by Southern blotting (not shown). Following the same scheme a nearly perfect *MLP1* disruption was constructed, which removes the whole coding region except for ten amino acids at the N-terminus. This time the *LEU2* flanking fragments were generated by the polymerase chain reaction (PCR) using the Perkin-Elmer Cetus DNA amplification kit according to the instructions of the manufacturer. The upstream fragment extended from positions 1 to 552 and the downstream fragment from positions 6157 to 6925 in the *MLP1* sequence.

*Preparation of affinity-purified anti-*MLP1 *antibodies.* Antibodies were raised against a *TrpE-MLP1* fusion protein. The fusion protein was prepared from strain HB101 (Boyer and Roulland-Dussoix 1969) transformed with the plasmid pRK25. An overnight culture grown in M9/CAS plus 20 μg/ml tryptophan was diluted 1:25 in 500 ml M9/CAS and grown for 1 h at 37° C. Fusion protein synthesis was induced by the addition of indoleacrylic acid (final concentration 10 μg/ml, Sigma) and the culture was grown for a further 2 h. The fusion protein was recovered from the insoluble fraction, essentially as described by Kleid et al. (1981). The fusion protein was purified by two rounds of preparative SDS–PAGE. Bands were visualized by soaking the gel in cold 1 M KCl. The desired band was cut out of the gel and the fusion protein was electroeluted. The fusion protein was injected into rabbits (200 μg in complete Freund's adjuvant) and booster injections were given 3 and 6 weeks later (200 μg in incomplete Freund's adjuvant). The animals were bled 7 weeks after the first injection and the crude serum was analyzed for anti-*MLP1* reactivity. For further purification the antibodies were affinity-purified over columns containing bound fusion protein. The *TrpE-MLP1* fusion protein (14 mg) was bound to 2 ml AffiGel 15 matrix (Biorad) as described by the manufacturer, with the modification that 0.1% SDS was included in the coupling buffer. A parallel column was prepared with *TrpE* protein (15 mg) bound to the matrix, isolated essentially like the fusion protein but from strain HB101 bearing plasmid pRB535. The serum was pre-adsorbed to boiled *Escherichia coli* cells overproducing the *TrpE* protein to deplete the serum of anti-*TrpE* reactivities. The depleted serum was passed first over the *TrpE* column and then over the *TrpE-MLP1* column. Antibodies that bound to the fusion protein column were eluted with 0.1 M glycine pH 2.5. The affinity purification procedure was essentially as described by Peffer et al. (1983).

Immunofluorescence microscopy. Cells were grown in SD medium plus CAS to select for the presence of the 2 μm *MLP1* plasmid, which carries the *URA3* marker, at 26° C to a density of $1\text{–}2 \times 10^7$ cells/ml. They were fixed directly by the addition of 1 volume of 10% formaldehyde. The antibody staining was performed as described in Pringle et al. (1989). Methanol/acetone treatment was used routinely. Cells were photographed with Kodak 5053 TMY black and white film on a Zeiss standard microscope equipped for epifluorescence.

Immunoblots. Yeast cell extracts were prepared by vortexing with glass beads. The equivalent of 5×10^6 cells/lane was loaded on a 7.5% SDS-polyacrylamide gel. Proteins were electroblotted to nitrocellulose membranes and the immunoreactive bands were detected using antisera and ^{125}I-protein A (from Amersham) as described by Burnette (1981).

RNA isolation. Cells were grown in 100 ml YEPD to a density of $1\text{–}2 \times 10^7$ cells/ml, harvested and resuspended in 1 ml extraction buffer (0.5 M NaCl, 0.2 M TRIS-Cl pH 7.6, 0.01 M EDTA, 1% SDS). About 2 g of glass beads and 1 ml phenol/chloroform were added and the samples were vigorously vortexed for 3 min. After the addition of 1.5 ml extraction buffer and 1.5 ml phenol/chloroform the samples were vortexed for a further 1 min. The aqueous phase was ethanol precipitated and the pellet was washed twice with 3 M sodium acetate pH 6 and once with 70% ethanol. The pellet was resuspended in 0.5 ml sterile water. Yield was about 100 μg/10^8 cells.

S1 nuclease mapping. The DNA probe was either 5′ end-labeled using T4 polynucleotide kinase or was 3′ end-labeled by filling-in recessed 3′ ends with Klenow polymerase (Maniatis et al. 1982). About 100 μg of total yeast RNA was ethanol precipitated with about $5\text{–}10 \times 10^4$ cpm of the labeled DNA probe (2–4 ng DNA). The precipitate was washed twice in 70% ethanol and was carefully resuspended in 50 ml denaturation buffer (40 mM PIPES-NaOH pH 6.3, 400 mM NaCl, 1 mM EDTA pH 6.5, 80% formamide). The samples were denatured at 85° C for 10 min and were then transferred to a 45° C

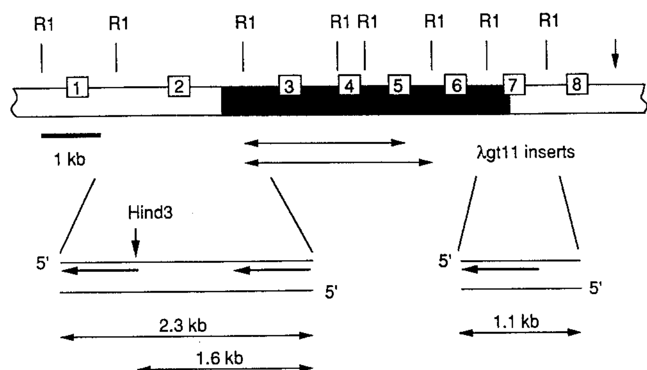

Fig. 1. *Eco*RI (RI) restriction map of the chromosomal *MLP1* region. The end of the chromosomal insert in pRK50-1 is marked by an *arrow*. The *dark box* represents the *MPL1* open reading frame. The results of the S1 nuclease mapping are summarized in the bottom part of the diagram, with *arrows* indicating the position of mapped transcripts. Also indicated are the positions of the original λgt11 inserts

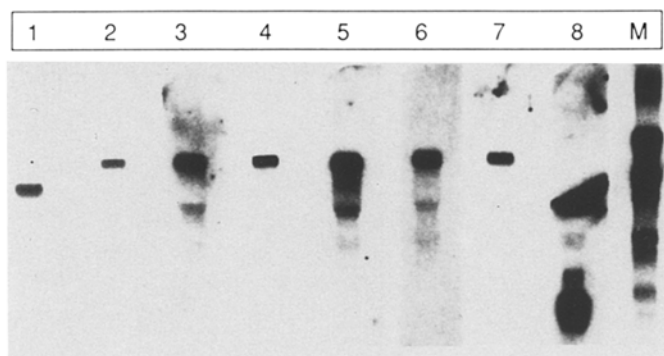

Fig. 2. Mapping of *MLP1* by Northern analysis. The lane numbers correspond to the numbers of the *Eco*RI fragments used as hybridization probes as defined in Fig. 1. M, λ*Eco*RI/*Hind*III molecular weight marker. Probes 2–7 hybridize to a transcript of about 6 kb

water bath for hybridization. The samples were totally submerged to avoid evaporation. After 3 h 0.5 ml of ice-cold S1 buffer were added (280 mM NaCl, 30 mM sodiumacetate pH 4.6, 4.5 mM zincacetate, 20 μg/ml calf thymus DNA). The samples were incubated at 37° C for 30 min with 200 units S1 nuclease (Biolabs). The reaction was terminated by the addition of 75 μl stop-mix (2.5 M ammoniumacetate, 50 mM EDTA pH 8). The samples were ethanol precipitated and resuspended in alkaline loading buffer and separated in an alkaline agarose gel (Maniatis et al. 1982).

Sequencing of the MLP1 *gene*. The *Eco*RI fragments 6 and 7 (Fig. 1) and a 5.7 kb *Xba*I fragment starting at position 1239 in the *MLP1* sequence were subcloned into M13 derivatives (Messing 1983) and sequenced by standard dideoxy sequencing methods (Sanger et al. 1977).

UV sensitivity measurements. A single colony was used to inoculate 5 ml YEPD medium and incubated for 48 h at 26° C. The cells were briefly sonicated and diluted to 1×10^5/ml in 5 ml phosphate buffered saline in a sterile glass petri dish. The cells were irradiated with 254 nm UV light (0.9 J/m^2 s) with constant swirling of the petri dish. After certain time intervals 100 μl aliquots were removed. For each time point the cells were plated at two different dilutions (2×10^2 and 2×10^3 cells/plate). Surviving colonies were counted after 3 days at 26° C.

Results and discussion

Cloning and mapping of the MLP1 *gene*

A monoclonal antibody directed against human platelet myosin heavy chains (PM-1; kindly provided by Dan Kiehart, Harvard University) was used to screen a λgt11 yeast genomic DNA library, essentially as described by Young and Davis (1983). Among 5×10^5 phage clones tested, three reacted with the anti-myosin antibody. The inserts of all three clones were derived from the same genomic region, as demonstrated by restriction mapping and Southern analysis (not shown). The gene for the cross-reacting protein was designated *MLP1* for myosin-like protein. Larger yeast DNA inserts from the *MLP1* region were isolated from a YCp50 plasmid library (Rose et al. 1987) using as probe a 1.7 kb *Eco*RI fragment present in all three positive phage clones. One plasmid clone, pRK50-1, which hybridized to the 1.7 kb *Eco*RI fragment, was characterized further. The approximate location of the *MLP1* gene on the plasmid insert was determined by Northern analysis (Fig. 2) of RNA isolated from the haploid yeast strain DBY 2063. Six consecutive *Eco*RI fragments (2–7 in Fig. 1) centered on the position of the original phage inserts hybridized to a 6.5 kb mRNA, while the flanking fragments (1 and 8) hybridized to transcripts of different sizes. The end points of the *MLP1* transcript are therefore likely to lie in fragments 2 and 7.

The 5′ and 3′ ends of the transcript were defined more precisely by S1 nuclease mapping (Fig. 3). With the 5′ end-labeled 2.3 kb *Eco*RI probe (fragment 2; see Fig. 1) a 740 bp fragment was protected from S1 nuclease degradation. However, no signal was detected with the shorter 1.6 kb *Eco*RI-*Hind*III fragment as probe. Therefore the 740 bp protected fragment must belong to a transcript which is oriented away from the *MLP1* gene and which starts around the *Hind*III site. No transcript running in the opposite direction, i.e. towards the main part of the *MLP1* gene, was detected. The 3′ end-labeled 2.3 kb *Eco*RI and 1.6 kb *Hind*III-*Eco*RI fragments both gave rise to a 760 bp signal. This signal derives from a transcript which terminates within the 2.3 kb *Eco*RI fragment 760 bp from the right boundary (as defined in Fig. 1) and which most probably corresponds to the *MLP1* transcript. *MLP1* transcription is therefore from right to left (see Fig. 1). The 5′ end was mapped using the 5′ end-labeled 1.1 kb *Eco*RI fragment as a probe (no. 7 in Fig. 1). A 780 bp DNA fragment was protected from S1 nuclease degradation. Since the beginning of the

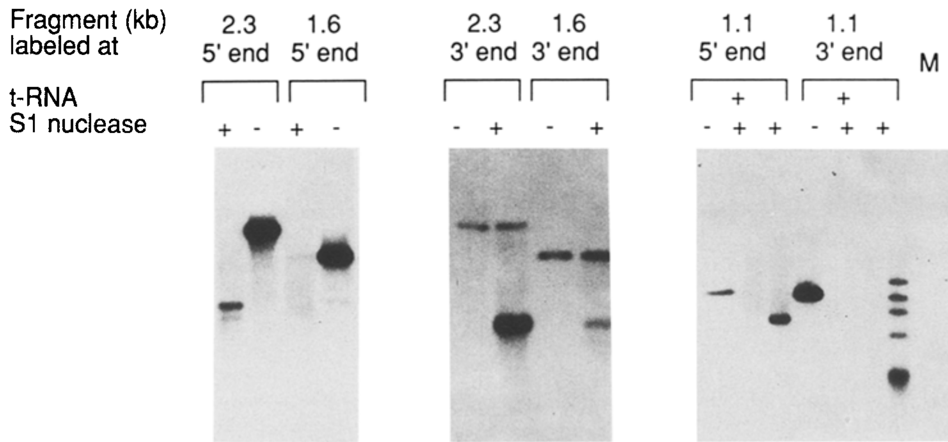

Fig. 3. S1 nuclease mapping of the *MLP1* transcript. In two reactions tRNA was used instead of total yeast RNA. M, φX174 *Hae*III molecular weight marker

MLP1 transcript is located within the 1.1 kb *Eco*RI fragment and since no other protected DNA fragments were observed, the 780 bp signal most probably defines the start of the *MLP1* transcript. No termination sites were detected using the 3′ end-labeled 1.1 kb *Eco*RI fragment as a probe.

Sequencing of the MLP1 *gene*

A 7.0 kb DNA fragment that includes the complete *MLP1* transcription unit as defined by S1 mapping was selected for sequencing. The sequence revealed a long open reading frame with the capacity to code for a 218 kDa protein (Fig. 4). The first ATG codon is located 200 bp downstream from the mapped mRNA start site and the reading frame ends 230 bp upstream from the mapped 3′ end of the transcript. From the fact that stop codons are located between the mapped 5′ end of the transcript and the first in-frame ATG codon we conclude that this 5′ end constitutes a true transcript start site and is not a 3′ splice junction. Therefore the *MLP1* gene does not appear to contain an intron.

We then compared the deduced *MLP1* protein sequence with proteins in the data bases using the program Fasta (Lipman and Pearson 1985). Most of the sequences similar to the *MLP1* protein sequence were either myosins or intermediate filament proteins. The degree of similarity was low, in the range of 15–20%, but the sequence stretches that showed similarity were extensive (up to 700 amino acids long). Since myosins and intermediate filament proteins are able to form coiled coil structures, we reasoned that this structural feature might be responsible for the similarities. Coiled coil-forming domains are α-helical and show a heptad repeat pattern on the amino acid level. Positions 1 and 4 of the heptads are preferentially occupied by hydrophobic amino acids which form a hydrophobic interface on one side of the α-helix. Two such helices can wrap around each other to form a coiled-coil. Other higher order periodicities and periodicities of acidic and basic amino acids are observed as well. A heptad repeat pattern is not always easily discernible. Non-hydrophobic amino acids may occur in positons 1 and 4 of the heptads and insertions and deletions introduce shifts in the pattern. To decide whether heptads are present in the sequence we devised a simple computer program that measures the distances between hydrophobic amino acids and counts how often these distances occur. If a protein contains a heptad repeat pattern one would expect to see peaks with a periodicity of seven in a graph where the frequency of the distances is plotted against the distance. Myosin was used as an example to test this prediction. The myosin heavy chain consists of a globular head domain which carries the enzymatic activies of myosin and the actin binding site(s) and of a long rod-like tail domain which forms a coiled-coil with another heavy chain molecule. Stretches of 200 amino acids from the head region and from the tail region were examined (Fig. 5) . No pattern was visible in the portion from the head region, while a pattern of evenly spaced peaks with a periodicity of seven was seen in the tail fragment.

The *MLP1* sequence was divided into 200 amino acid segments and analyzed in the same way. The results are summarized in Figs. 5 and 6. The *MLP1* protein contains two heptad repeat regions of about 400 and 600 amino acids. The two heptad domains are separated by a stretch of 200 amino acids that does not have repeats. The heptad repeats start immediately at the N-terminus of the protein while the C-terminus is formed by a presumably globular 700 amino acid domain without heptad repeats. This C-terminal domain is proline-rich while the heptad domains are virtually devoid of prolines. An unusual sequence of about 130 amino acids was noted in the C-terminal domain (positions 1675–1805) with a proline plus serine content of about 30%. This is a candidate for a so-called "PEST region" which has been implicated in protein turnover (Rogers et al. 1986).

Secondary structure analysis according to Chou and Fasman (1974) predicts an α-helix content of about 80% for the *MLP1* protein. High α-helix content and the presence of long stretches with heptad repeats make it very likely that *MLP1* is indeed a coiled coil-forming protein. Though the extent of coiled coil-forming regions in *MLP1* is comparable to myosin, the organization of the proteins, however, is different (Fig. 6). The myosin head region shows a high degree of sequence conservation between myosins from different species. The

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   1  GAATTCAAGT ATCCCTATGC AAGAATTTAA AAATTTCTCT CTCTATTTGA ACTGAACCAG CATTTTTTAT ATCTCTCCAC ATATCGCAGA

  91  AAGCTTTTCT ACTCTCAAGA AAAAATTGAG GGATGGGTCA ACCTTCCATA GATTCTATAT GGAATAATAA AATTTACTTC TTACTAACAT

 181  TATATCAGGG TGAATATTAC TGACAAAAAT AATAACTTAA GTCTTCTTTA TAATATGATG ATCGACGCGC GGGGTAACGC GCTCTTCCCA

 271  TCTTTGTTTC CTTTCATTCC TCTTGACATG ACAACAAGAG TATATGGGAA AAGAAAACTG GAAGGCAAAT TGAATACAGA CAGAGATATC

 361  AATAAAAGTG GTCCATTGAG TCTTACGGCG CCGGAGAGAT ACGCTCCATT TACAATATTT ACTGATAGAT ATATTGCTGC CTGTCTATTT

 451  TTCCATCTCT TGTGCTGACT AGGACTTAAC TGATACTCGC CGAAGCTACA CAAATAGTCA GTAACGCCAC GTTTTAGGAT AATGTCGGAT
   1                                                                                            M  S  D

 541  CATGATACTC CAATGGAAAG CATACAAAAT GGTGAAAATT CAGACGAAAG ATTGAACGCC ATTGCGTCTT TTTTCGGTTG CTCTTTAGAG
   4  H  D  T  P  M  E  S  I  Q  N  G  E  N  S  D  E  R  L  N  A  I  A  S  F  F  G  C  S  L  E

 631  CAGGTTAAAT CATTTGACGG TGATGTGGTA AAACACCTTA ACGATAAACT TTTACAGTTT AATGAACTTA AATCGGAGAA TCTAAAGGTT
  34  Q  V  K  S  F  D  G  D  V  V  K  H  L  N  D  K  L  L  Q  F  N  E  L  K  S  E  N  L  K  V

 721  ACCGTCTCAT TCGATGAATT GAAGGCTAGT TCTTTAAAGA AAATTGATGG TTTGAAGACA GAAATGGAAA ACGTTATAAG AGAAAATGAT
  64  T  V  S  F  D  E  L  K  A  S  S  L  K  K  I  D  G  L  K  T  E  M  E  N  V  I  R  E  N  D

 811  AAAATCCGAA AAGAGAGGAA TGATACTTTT GTTAAGTTCG AATCTGTAGA AAATGAAAAG ATGAAATTAT CAAGTGAGCT AGAGTTTGTG
  94  K  I  R  K  E  R  N  D  T  F  V  K  F  E  S  V  E  N  E  K  M  K  L  S  S  E  L  E  F  V

 901  AAAAGGAAGC TCGATGATTT AACTGAGGAG AAAAAGGAAA CCCAGAGTAA TCAACAGCGA ACCCTGAAAA TACTGGATGA AAGACTAAAA
 124  K  R  K  L  D  D  L  T  E  E  K  K  E  T  Q  S  N  Q  Q  R  T  L  K  I  L  D  E  R  L  K

 991  GAAATAGAAT TGGTCAGGGT TGAGAATAAT CGCTCCAATA GTGAATGTAA GAAACTACGC TCTACAATAA TGGATTTAGA AACAAAACAA
 154  E  I  E  L  V  R  V  E  N  N  R  S  N  S  E  C  K  K  L  R  S  T  I  M  D  L  E  T  K  Q

1081  CAGGGCTATA TTACTAATGA CCTGAATTCT AGAACTGAAC TGGAAAGAAA AACACAAGAA TTGACTTTAT TGCAGTCAAA TAATGATTGG
 184  Q  G  Y  I  T  N  D  L  N  S  R  T  E  L  E  R  K  T  Q  E  L  T  L  L  Q  S  N  N  D  W

1171  CTAGAAAAGG AGCTACGTTC AAAAAATGAA CAGTATCTCT CCTACAGACA AAAAACCGAC AAAGTAATTC TAGATATCAG GAATGAATTA
 214  L  E  K  E  L  R  S  K  N  E  Q  Y  L  S  Y  R  Q  K  T  D  K  V  I  L  D  I  R  N  E  L

1261  AATCGTTTAA GGAATGATTT TCAAATGGAA AGAACGAATA ATGATGTTTT GAAGCAAAAA AATAACGAAT TGTCAAAATC TTTACAAGAA
 244  N  R  L  R  N  D  F  Q  M  E  R  T  N  N  D  V  L  K  Q  K  N  N  E  L  S  K  S  L  Q  E

1351  AAACTACTGG AAATCAAGGG TCTATCCGAC TCCCTAAATT CCGAAAAGCA AGAATTTTCT GCAGAAATGT CCCTAAAGCA AGCTTTAGTG
 274  K  L  L  E  I  K  G  L  S  D  S  L  N  S  E  K  Q  E  F  S  A  E  M  S  L  K  Q  A  L  V

1441  GACCTTTTAG AATCACAATT GAACGCTGTA AAAGAAGAAT TGAACAGTAT AAGAGAGTTG AACACTGCAA AGGTAATAGC AGATGATTCA
 304  D  L  L  E  S  Q  L  N  A  V  K  E  E  L  N  S  I  R  E  L  N  T  A  K  V  I  A  D  D  S

1531  AAAAAACAAA CTCCCGAAAA TGAAGACTTA CTTAAGGAAT TGCAGTTAAC GAAAGAAAAA TTAGCACAAT GCGAAAAAGA ATGTCTACGT
 334  K  K  Q  T  P  E  N  E  D  L  L  K  E  L  Q  L  T  K  E  K  L  A  Q  C  E  K  E  C  L  R

1621  TTATCCTCTA TAACTGACGA AGCAGATGAA GATAATGAAA ATTTATCTGC AAAATCTAGT TCTGATTTTA TATTCCTGAA GAAACAATTA
 364  L  S  S  I  T  D  E  A  D  E  D  N  E  N  L  S  A  K  S  S  S  D  F  I  F  L  K  K  Q  L

1711  ATTAAAGAAA GGCGTACCAA GGAACATCTT CAAAATCAAA TTGAAACATT CATCGTAGAG TTGGAACATA AAGTGCCCAT TATAAACTCT
 394  I  K  E  R  R  T  K  E  H  L  Q  N  Q  I  E  T  F  I  V  E  L  E  H  K  V  P  I  I  N  S

1801  TTCAAAGAAA GAACTGACAT GTTGGAAAAC GAATTGAATA ACGCTGCATT GTTACTAGAG CATACATCGA ACGAGAAGAA TGCAAAGGTT
 424  F  K  E  R  T  D  M  L  E  N  E  L  N  N  A  A  L  L  L  E  H  T  S  N  E  K  N  A  K  V

1891  AAGGAATTAA ATGCCAAAAA CCAAAAGCTA GTGGAATGTG AAAATGATCT TCAAACTTTA ACTAAACAAC GTCTCGATCT ATGCCGTCAA
 454  K  E  L  N  A  K  N  Q  K  L  V  E  C  E  N  D  L  Q  T  L  T  K  Q  R  L  D  L  C  R  Q

1981  ATACAATACC TTTTAATTAC CAATTCTGTT TCTAATGACT CGAAGGGACC CTTACGTAAG GAAGAAATTC AATTTATTCA AAACATTATG
 484  I  Q  Y  L  L  I  T  N  S  V  S  N  D  S  K  G  P  L  R  K  E  E  I  Q  F  I  Q  N  I  M

2071  CAGGAAGACG ATAGTACTAT CACAGAATCT GACTCTCAAA AAGTCGTAAC TGAAAGACTA GTTGAATTCA AAAACATTAT TCAATTACAA
 514  Q  E  D  D  S  T  I  T  E  S  D  S  Q  K  V  V  T  E  R  L  V  E  F  K  N  I  I  Q  L  Q

2161  GAAAAAAATG CAGAACTTTT GAAAGTAGTA AGAAACTTAG CCGATAAGTT GGAATCGAAA GAAAAGAAAT CTAAACAAAG TCTTCAGAAA
 544  E  K  N  A  E  L  L  K  V  V  R  N  L  A  D  K  L  E  S  K  E  K  K  S  K  Q  S  L  Q  K

2251  ATCGAAAGTG AAACAGTAAA TGAGGCTAAA GAGGCTATAA TAACTTTAAA GAGTGAAAAA ATGGATCTAG AATCAAGAAT TGAGGAACTA
 574  I  E  S  E  T  V  N  E  A  K  E  A  I  I  T  L  K  S  E  K  M  D  L  E  S  R  I  E  E  L

2341  CAGAAAGAGC TTGAAGAATT GAAAACTTCT GTTCCCAACG AAGATGCGTC ATACAGCAAT GTAACTATAA AACAGTTAAC CGAAACTAAG
 604  Q  K  E  L  E  E  L  K  T  S  V  P  N  E  D  A  S  Y  S  N  V  T  I  K  Q  L  T  E  T  K

2431  AGAGACCTCG AATCTCAAGT ACAAGACTTG CAAACTCGTA TCTCGCAAAT TACTAGGGAG TCTACTGAAA ATATGTCACT TTTAAACAAG
 634  R  D  L  E  S  Q  V  Q  D  L  Q  T  R  I  S  Q  I  T  R  E  S  T  E  N  M  S  L  L  N  K

2521  GAGATACAGG ACCTGTATGA CAGCAAGAGC GACATATCCA TTAAGCTTGG AAAGGAAAAA TCATCGAGAA TATTGGCAGA GGAACGATTT
 664  E  I  Q  D  L  Y  D  S  K  S  D  I  S  I  K  L  G  K  E  K  S  S  R  I  L  A  E  E  R  F

2611  AAACTACTTT CGAATACGTT AGATCTAACT AAAGCTGAGA ACGACCAACT GCGCAAAAGG TTTGATTATT TACAGAATAC TATTTTAAAA
 694  K  L  L  S  N  T  L  D  L  T  K  A  E  N  D  Q  L  R  K  R  F  D  Y  L  Q  N  T  I  L  K

2701  CAAGATTCCA AAACACACGA GACACTTAAT GAATACGTTT CCTGTAAATC TAAGTTAAGC ATTGTTGAAA CAGAATTATT GAACCTGAAA
 724  Q  D  S  K  T  H  E  T  L  N  E  Y  V  S  C  K  S  K  L  S  I  V  E  T  E  L  L  N  L  K

2791  GAAGAACAGA AATTAAGAGT TCATTTAGAA AAGAACTTGA AACAAGAACT GAATAAACTC TCCCCTGAAA AGGACAGTTT ACGCATCATG
 754  E  E  Q  K  L  R  V  H  L  E  K  N  L  K  Q  E  L  N  K  L  S  P  E  K  D  S  L  R  I  M

2881  GTAACTCAAT TACAAACTTT ACAAAAGGAG CGTGAAGATC TATTGGAAGA GACTAGGAAA TCATGTCAAA AGAAAATAGA TGAACTTGAA
 784  V  T  Q  L  Q  T  L  Q  K  E  R  E  D  L  L  E  E  T  R  K  S  C  Q  K  K  I  D  E  L  E
```

Fig. 4. *MLP1* sequence

```
2971 GATGCTCTCA GCGAACTTAA AAAGGAAACT TCTCAAAAAG ACCATCATAT CAAACAGCTG GAAGAAGACA ACAATTCAAA TATAGAATGG
 814 D  A  L  S   E  L  K   K  E  T   S  Q  K  D   H  H  I   K  Q  L   E  E  D  N   N  S  N   I  E  W

3061 TACCAAAATA AAATCGAAGC TTTGAAGAAA GATTATGAAT CAGTAATAAC TTCTGTAGAT AGTAAGCAAA CTGACATTGA GAAATTACAA
 844 Y  Q  N  K   I  E  A   L  K  K   D  Y  E  S   V  I  T   S  V  D   S  K  Q  T   D  I  E   K  L  Q

3151 TATAAAGTCA AATCACTAGA AAAGGAAATC GAGGAGGACA AGATTCGTTT ACATACTTAT AATGTTATGG ATGAAACAAT TAACGATGAT
 874 Y  K  V  K   S  L  E   K  E  I   E  E  D  K   I  R  L   H  T  Y   N  V  M  D   E  T  I   N  D  D

3241 TCCCTACGCA AGGAGTTGGA AAAATCCAAG ATTAACTTAA CTGATGCTTA TTCACAAATC AAAGAATACA AGGATCTCTA CGAGACTACC
 904 S  L  R  K   E  L  E   K  S  K   I  N  L  T   D  A  Y   S  Q  I   K  E  Y  K   D  L  Y   E  T  T

3331 TCTCAGTCTT TGCAGCAAAC GAATTCTAAA TTGGATGAAT CTTTCAAGGA CTTTACTAAC CAGATTAAAA ACCTAACTGA TGAAAAAACT
 934 S  Q  S  L   Q  Q  T   N  S  K   L  D  E  S   F  K  D   F  T  N   Q  I  K  N   L  T  D   E  K  T

3421 AGTTTGGAGG ATAAGATTTC GCTTCTAAAA GAGCAAATGT TTAATTTGAA TAATGAGCTA GATTTGCAGA AAAAAGGGAT GGAAAAAGAA
 964 S  L  E  D   K  I  S   L  L  K   E  Q  M  F   N  L  N   N  E  L   D  L  Q  K   K  G  M   E  K  E

3511 AAAGCTGACT TTAAGAAAAG GATATCAATT TTACAGAACA ATAATAAAGA AGTCGAAGCT GTTAAGTCCG AATATGAATC GAAGTTATCA
 994 K  A  D  F   K  K  R   I  S  I   L  Q  N  N   N  K  E   V  E  A   V  K  S  E   Y  E  S   K  L  S

3601 AAAATCCAAA ACGACCTTGA TCAACAAACT ATATATGCTA ATACTGCGCA AAACAACTAT GAACAAGAAC TACAGAAACA TGCAGATGTT
1024 K  I  Q  N   D  L  D   Q  Q  T   I  Y  A  N   T  A  Q   N  N  Y   E  Q  E  L   Q  K  H   A  D  V

3691 TCTAAGACGA TTAGTGAATT AAGAGAGCAA TTACATACGT ACAAAGGTCA AGTTAAGACC CTGAACTTAT CGCGTGATCA ACTGGAGAAT
1054 S  K  T  I   S  E  L   R  E  Q   L  H  T  Y   K  G  Q   V  K  T   L  N  L  S   R  D  Q   L  E  N

3781 GCTCTGAAAG AAAACGAAAA GAGTTGGTCC TCCCAGAAGG AATCTTTATT AGAACAGCTA GATTTATCGA ATTCTCGTAT TGAGGATTTA
1084 A  L  K  E   N  E  K   S  W  S   S  Q  K  E   S  L  L   E  Q  L   D  L  S  N   S  R  I   E  D  L

3871 TCCTCCCAAA ATAAACTATT GTATGATCAA ATACAAATCT ACACAGCTGC GGACAAAGAA GTCAATAATT CGACAAACGG ACCTGGTTTG
1114 S  S  Q  N   K  L  L   Y  D  Q   I  Q  I  Y   T  A  A   D  K  E   V  N  N  S   T  N  G   P  G  L

3961 AATAATATTT TAATTACACT ACGTCGCGAA AGGGATATTC TTGATACAAA AGTGACGGTG GCTGAAAGAG ATGCAAAAAT GTTGAGACAA
1144 N  N  I  L   I  T  L   R  R  E   R  D  I  L   D  T  K   V  T  V   A  E  R  D   A  K  M   L  R  Q

4051 AAAATTTCTT TGATGGATGT TGAATTACAA GATGCTCGTA CTAAGCTAGA TAATTCAAGA GTTGAAAAGG AAAATCATTC TTCCATTATT
1174 K  I  S  L   M  D  V   E  L  Q   D  A  R  T   K  L  D   N  S  R   V  E  K  E   N  H  S   S  I  I

4141 CAACAGCATG ACGACATTAT GGAGAAATTA AATCAATTAA ATCTATTAAG AGAAAGTAAC ATAACATTGC GGAATGAGCT GGAAAACAAC
1204 Q  Q  H  D   D  I  M   E  K  L   N  Q  L  N   L  L  R   E  S  N   I  T  L  R   N  E  L   E  N  N

4231 AATAACAAGA AGAAGGAACT GCAATCTGAA TTAGATAAAT TGAAGCAAAA TGTTGCGCCT ATCGAGTCCG AATTGACAGC CTTGAAATAT
1234 N  N  K  K   K  E  L   Q  S  E   L  D  K  L   K  Q  N   V  A  P   I  E  S  E   L  T  A   L  K  Y

4321 TCTATGCAAG AAAAAGAGCA AGAGCTCAAA TTAGCTAAAG AAGAGGTTCA TCGTTGGAAA AAGCGCTCAC AAGACATATT GGAGAAACAT
1264 S  M  Q  E   K  E  Q   E  L  K   L  A  K  E   E  V  H   R  W  K   K  R  S  Q   D  I  L   E  K  H

4411 GAACAATTGA GCTCAAGCGA TTATGAGAAG CTAGAAAGCG AGATAGAAAA TTTGAAGGAG GAACTAGAAA ATAAGGAGCG TCAAGGAGCG
1294 E  Q  L  S   S  S  D   Y  E  K   L  E  S  E   I  E  N   L  K  E   E  L  E  N   K  E  R   Q  G  A

4501 GAAGCCGAGG AAAAATTTAA CAGGCTGAGA AGACAAGCGC AAGAGAGATT AAAAACATCA AAACTCTCAC AGGACTCATT GACTGAACAA
1324 E  A  E  E   K  F  N   R  L  R   R  Q  A  Q   E  R  L   K  T  S   K  L  S  Q   D  S  L   T  E  Q

4591 GTAAATAGTC TAAGGGATGC AAAGAACGTG TTGGAAAATT CCTTGAGTGA GGCAAACGCG AGAATCGAAG AGTTACAAAA TGCAAAAGTA
1354 V  N  S  L   R  D  A   K  N  V   L  E  N  S   L  S  E   A  N  A   R  I  E  E   L  Q  N   A  K  V

4681 GCACAAGGTA ACAACCAGTT AGAAGCAATA AGAAAATTAC AAGAAGACGC AGAAAAGGCT TCAAGAGAGC TTCAAGCCAA GTTAGAAGAA
1384 A  Q  G  N   N  Q  L   E  A  I   R  K  L  Q   E  D  A   E  K  A   S  R  E  L   Q  A  K   L  E  E

4771 AGTACGACTT CTTACGAATC TACGATAAAC GGCTTAAATG AAGAGATTAC AACATTAAAA GAAGAAATTG AAAAACAAAG GCAAATCCAG
1414 S  T  T  S   Y  E  S   T  I  N   G  L  N  E   E  I  T   T  L  K   E  E  I  E   K  Q  R   Q  I  Q

4861 CAACAGTTAC AAGCTACATC TGCAAATGAA CAAAATGACT TATCTAACAT AGTTGAGTCT ATGAAAAAGT CTTTTGAAGA AGATAAAATC
1444 Q  Q  L  Q   A  T  S   A  N  E   Q  N  D  L   S  N  I   V  E  S   M  K  K  S   F  E  E   D  K  I

4951 AAATTCATCA AAGAAAAAAC CCAAGAAGTT AATGAAAAAA TACTCGAGGC CCAAGAAAGG CTAAATCAAC CTTCCAATAT CAATATGGAG
1474 K  F  I  K   E  K  T   Q  E  V   N  E  K  I   L  E  A   Q  E  R   L  N  Q  P   S  N  I   N  M  E

5041 GAGATTAAAA AAAAATGGGA ATCTGAGCAC GAACAGGAAG TATCTCAAAA GATTCGCGAA GCTGAGGAAG CCCTCAAAAA GCGAATCAGA
1504 E  I  K  K   K  W  E   S  E  H   E  Q  E  V   S  Q  K   I  R  E   A  E  E  A   L  K  K   R  I  R

5131 TTACCCACTG AGGAGAAAAT TAATAAGATA ATCGAACGAA AGAAGGAGGA ATTGGAAAAA GAGTTTGAAG AAAAGGTTGA GGAGAGAATA
1534 L  P  T  E   E  K  I   N  K  I   I  E  R  K   K  E  E   L  E  K   E  F  E  E   K  V  E   E  R  I

5221 AAATCAATGG AACAATCTGG AGAAATAGAC GTGGTGCTTC GAAAACAGCT AGAAGCTAAG GTTCAAGAGA AACAAAAGGA ATTGGAAAAC
1564 K  S  M  E   Q  S  G   E  I  D   V  V  L  R   K  Q  L   E  A  K   V  Q  E  K   Q  K  E   L  E  N

5311 GAGTATAACA AAAAATTACA AGAAGAACTC AAAGATGTAC CACACTCAAG TCATATCTCA GATGATGAAA GGGACAAATT ACGAGCAGAA
1594 E  Y  N  K   K  L  Q   E  E  L   K  D  V  P   H  S  S   H  I  S   D  D  E  R   D  K  L   R  A  E

5401 ATCGAAAGCA GGTTGAGGGA GGAGTTCAAC AATGAACTGC AAGCCATAAA GAAGAAATCC TTCGACGAAG GAAAGCAACA AGCAATGATG
1624 I  E  S  R   L  R  E   E  F  N   N  E  L  Q   A  I  K   K  K  S   F  D  E  G   K  Q  Q   A  M  M

5491 AAAACTACCC TTTTGGAAAG AAAACTTGCC AAGATGGAAT CTCAATTGTC AGAAACAAAA CAAAGTGCCG AGAGTCCTCC GAAATCTGTT
1654 K  T  T  L   L  E  R   K  L  A   K  M  E  S   Q  L  S   E  T  K   Q  S  A  E   S  P  P   K  S  V

5581 AACAATGTAC AAAATCCATT ACTAGGATTA CCTAGGAAAA TCGAAGAGAA TTCAAATTCA CCATTCAATC CGTTACTTTC CGGTGAAAAA
1684 N  N  V  Q   N  P  L   L  G  L   P  R  K  I   E  E  N   S  N  S   P  F  N  P   L  L  S   G  E  K

5671 CTCTTAAAGC TAAATTCTAA GTCTTCATCA GGTGGATTTA ACCCTTTTAC CTCGCCATCC CCAAATAAGC ACTTACAAAA TGATAATGAC
1714 L  L  K  L   N  S  K   S  S  S   G  G  F  N   P  F  T   S  P  S   P  N  K  H   L  Q  N   D  N  D
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Fig. 4 (continued)

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5761  AAAAGGGAGT CGTTGGCTAA CAAGACAGAT CCACCAACTC ATTTGGAACC CAGCTTCAAC ATTCCCGCCT CAAGGGGTCT AATATCTTCA
1744  K  R  E  S   L  A  N   K  T  D   P  P  T  H   L  E  P   S  F  N   I  P  A  S   R  G  L   I  S  S

5851  TCTTCCACTT TGTCAACTGA TACAAATGAT GAAGAACTTA CTAGCAACAA TCCTGCCCAA AAGGATTCAT CGAACAGAAA TGTTCAATCG
1774  S  S  T  L   S  T  D   T  N  D   E  E  L  T   S  N  N   P  A  Q   K  D  S  S   N  R  N   V  Q  S

5941  GAAGAGGATA CAGAAAAAAA GAAAGAGGGA GAACCTGTTA AAAGAGGAGA GGCAATAGAA GAGCAGACGA AATCCAACAA GCGACCTATT
1804  E  E  D  T   E  K  K   K  E  G   E  P  V  K   R  G  E   A  I  E   E  Q  T  K   S  N  K   R  P  I

6031  GATGAGGTAG GAGAGCTGAA AAATGATGAA GACGACACTA CAGAAAACAT TAATGAGTCA AAAAAGATCA AGACTGAAGA TGAGGAAGAA
1834  D  E  V  G   E  L  K   N  D  E   D  D  T  T   E  N  I   N  E  S   K  K  I  K   T  E  D   E  E  E

6121  AAAGAAACCG ATAAGGTGAA TGACGAGAAC AGTATATAAA GGAGATAGTA AAAACAAGGG ATCAATACAA ACTAAACCTT TTTCAATGTG
1864  K  E  T  D   K  V  N   D  E  N   S  I

6211  GAGGAGCTTC ATTCTGCCCT ATGTCAAGAC CATTTGTAAT ATACGTACAA CAGCATTCTT GTTTCATAAA GTTTATATAT GTATAAGCTA

6301  AGATGAAGTC ACGTAAATAG CTCAAATGCC GATTTTTTAA TACTCCAAAC ACAGAATCCT CTATATATGG AAATGGAATC ATGAATCTTA

6391  CGGTTTGTGT TTTGAATGTT CCAAACAGAT TTTCTTAGTG TTCCGATCTC TGTTCCACCC ACCAAACGAG AAAAAAGTAG GTCTTCGCGA

6481  AAACTCCAGC GACACCGCTG GTCCTTTAAG TTAAAATAAT ATGAATGGTG GAAGAACCCA GATCAACTTG ACTGAAACAA GTCTGCCAAC

6571  ATAGACAGCT AAGAAAATTG ATGACAAGCA AACGGGAAAA GTCACTGGAT CACACATTGT AAGTAGAAGC AGTTTTTCAA TGGGAAGACC

6661  GCACTGCATA GTTTACTAAC TATTTAAACT TTCCAAACAT TAGAGAACTA AAGATACCGT TTGAAACGGA GCGACAAGCG ACCATAGCAA

6751  CCAAAGTCCT ATCTCCGGAC CCGATTTTGA AGCCACAAGA TTTTCAAGTA GACTACAGTT CCGAGAAAAA TGTCATGCTA GTCCAGTTCA

6841  GAAGCATTGA TGATAGGGTG CTTCGAGTGG GAGTTAGCAG TATCATAGAC AGTATCAAAA CCATTGTGGA AGCCATGGAC GTTCTATCAT

6931  AAATAATTAG AACACCGTAT TAGTGTACAT ATAGTATAAG TATCTAGA
```

Fig. 4 (continued)

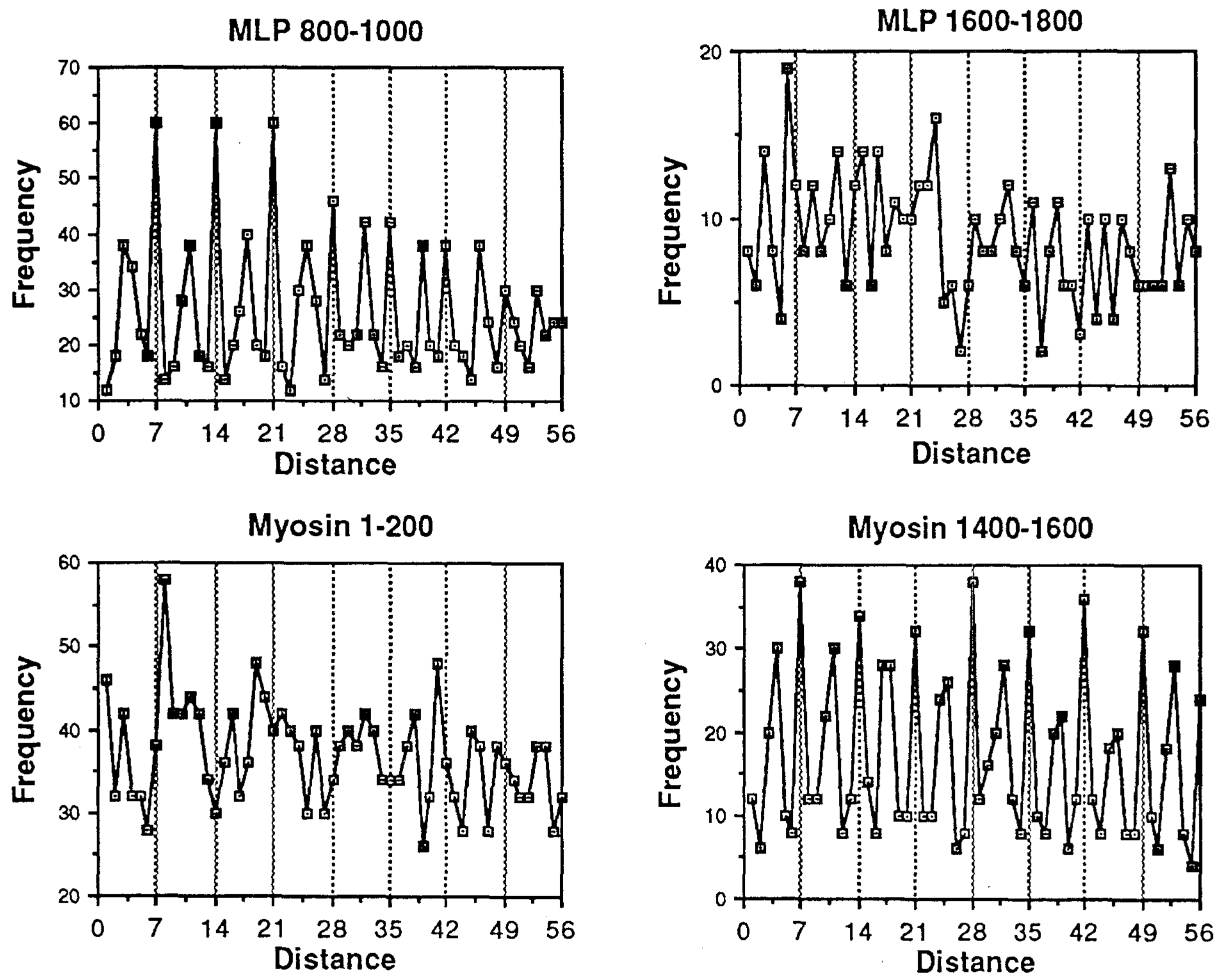

Fig. 5. Segments of the *MLP1* and a myosin heavy chain protein sequence were analyzed for the presence of heptad repeats. The analyzed segments were 200 amino acids long (their position within the protein sequence is indicated on top of each diagram). For each protein, two representative segments, which contain or do not contain heptad repeats, are shown

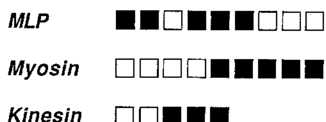

Fig. 6. Heptad-repeat distribution. The proteins shown were analyzed according to Fig. 5. Each box represents a 200 amino acid segment. *Black box,* heptad repeat domain; *white box,* domains without a discernible heptad repeat pattern

S. cerevisiae MYO1 head region for instance is 40% homologous to nematode myosin (Watts et al. 1987). No such homologies were found in the *MLP1* protein.

When the *MLP1* protein sequence was compared with those in the databases the sequence with the highest degree of similarity was a protein fragment that was fused to part of the *raf* protooncogene. This protein fragment belongs to an unknown rat protein, termed *tpr* for translocated (tumor) promoter region (Ishikawa et al. 1987). *tpr* sequences are also found in a fusion with the human *met* oncogene (King et al. 1988). These *tpr* fusions have transforming activity. The similarity to *MLP1* extends over the whole length of the unknown sequence (215 amino acids) and ends exactly at the junction to *raf*. The similarity between *MLP1* and *tpr* is only 22% but there are indications that it reflects a true homology. First, the *tpr* is predicted to be α-helical and has a heptad repeat pattern throughout the whole length of the sequence. Despite the presence of many sequences in the databases with heptad-repeat patterns, the match between the short *tpr* fragment and *MLP* is better than with any myosin and intermediate filament protein sequence. The match is achieved with the addition of only two single-amino acid gaps whereas with the other sequences more or larger gaps had been introduced for optimal alignment. Second, *MLP1* and TPR are unusual in that both proteins have coiled-coil domains immediately at the N-terminus of the proteins.

Disruptions of the MLP1 *gene*

We disrupted the chromosomal copy of the *MLP1* gene as described in Materials and methods and shown in Fig. 7A. About 2.7 kb of *MLP1* were deleted and replaced by the *LEU2* marker gene. The gene disruption was verified by Southern analysis (not shown). The diploid strain DBY 5394 containing one wild-type and one disrupted copy of the *MLP1* gene was sporulated and dissected. All spores turned out to be viable. The *MLP1* gene is therefore not essential for growth. Pairs of strains carrying the disruption were able to mate and strains homozygous for the disruption were able to sporulate.

The haploid disruption strain DBY 5384 was compared to a wild-type strain (DBY 1827) and to a derivative of this strain that had been transformed with a multicopy (2 μm) plasmid carrying the entire *MLP1* gene. Immunoblotting (Western analysis) is shown in Fig. 7B and Northern analysis in Fig. 7C. On immunoblots with anti-*MLP1* antibodies against yeast cell extracts from strain DBY 1827 the antibodies reacted with a species with an apparent molecular size of 200 kDa and with two other major forms with approximate sizes of 105 and 35 kDa. Several cyles of affinity purification against TrpE-MLP1 fusion protein resulted in no change in the pattern. The 200 kDa band is missing in extracts of the haploid disruption strain and is much stronger in the 2 μm-*MLP1* strain; these results confirm the identify of the 200 kDa band as a product of the *MLP1* gene. The other two bands were still present in the disruption strain, demonstrating that these bands are not *MLP1* breakdown products.

A different pattern was observed with the monoclonal PM-1 antibody that had been used originally to screen the λgt11 library. A doublet of bands around 220 kDa and 130 and 35 kDa bands were seen (not shown). This pattern, however, was identical in wild-type and the *MLP1* disruption strain. Thus, the sensitivity of the PM-1 antibody is not sufficient to visualize a *MLP1* band from yeast cell extracts. Instead, the antibody reacts with two other high molecular weight proteins that remain candidates for additional myosin-like proteins in yeast.

The Northern analysis showed that *MLP1* sequences downstream from the inserted *LEU2* marker were transcribed in the disruption strain giving rise to a 1.5 kb mRNA (Fig. 7C). Such transcription could, in theory,

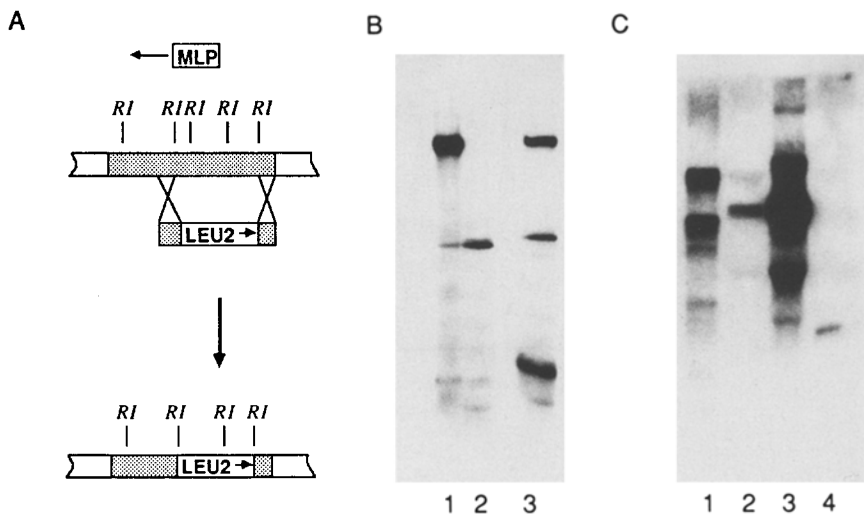

Fig. 7A–C. Disruption of the *MLP1* gene. **A** The *MLP1* open reading frame is indicated by a *hatched box,* the *arrows* point into the direction of transcription of *MLP1* and *LEU2*. **B** Western blot, whole cell extract from the following strains: 1, 2 μm *MLP1*; 2, *mrp1-Δ1::LEU2*; 3, wild-type *MLP1*. **C** Northern blot, RNA prepared from the following strains, 2, wild-type *MLP1*; 3, 2 μm *MLP1*; 4, *mrp1-Δ1::LEU2*, 1, λ*Eco*RI/*Hind*III marker

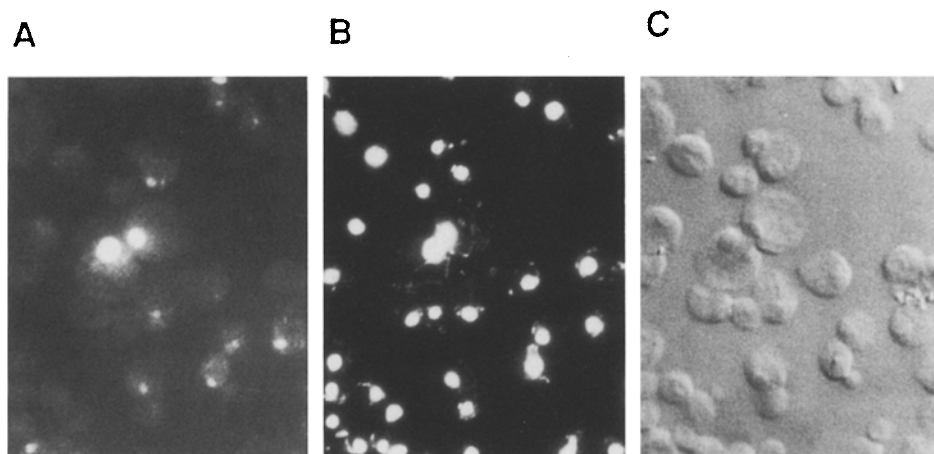

Fig. 8A–C. Localization of *MLP1* in the yeast cell by immunofluorescence microscopy. **A** Staining with yeast anti-*MLP1*p antibody. **B** Staining of nuclei with 4,6-diamidino-2-phenylindole (DAPI) . **C** Phase contrast

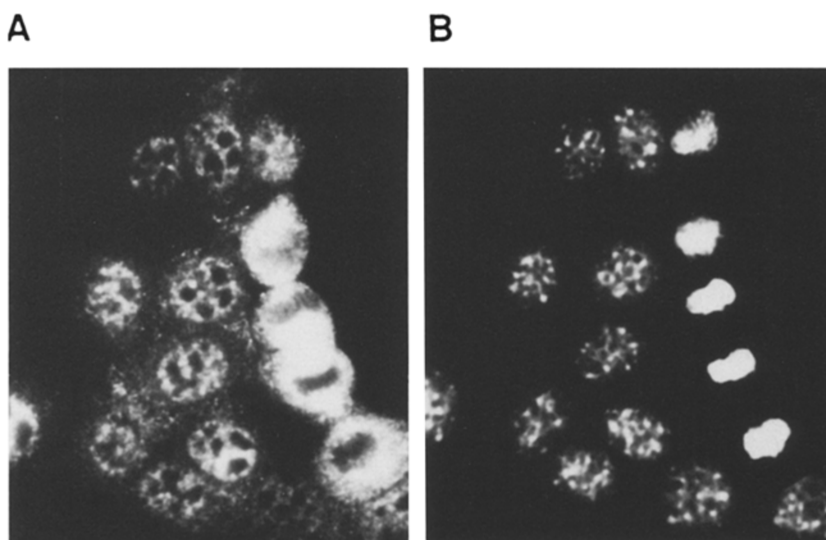

Fig. 9A, B. Immunofluorescence microscopy with mouse epithelial cells using yeast anti-*MLP1* antibodies. **A** Anti-*MLP1*p antibodies. **B** Staining of nuclei with DAPI

code for a truncated, but partially active *MLP1* protein, offering an explanation why the disruption strains are viable. To exlude this possible we created a nearly perfect disruption which removes the whole *MLP1* coding region except for ten amino acids at the N-terminus using flanking fragments generated by PCR. Haploid strains carrying this disruption were still viable and were indistinguishable from strains with the original disruption.

Immunolocalization of MLP

We performed immunofluorescence microscopy localization experiments using affinity-purified anti-*MLP1*p antibodies raised against the *TrpE-MLP1* fusion protein as described in Materials and methods. We also stained with 4,6-diamidino-2-phenylindole (DAPI) to visualize the nuclear DNA. The structures seen with DBY 1827 cells containing the *MLP1* gene on a 2 μm plasmid (pRK29) are shown in Fig. 8. Intensely staining dots and sometimes rings were seen which were always located adjacent to the nucleus, as judged by DAPI staining. Wild-type cells containing only a single copy of the *MLP1* gene (DBY 1827) did not stain with the antibody; only occasionally did they show very faint staining of the nucleus (not shown).

Immunofluorescence experiments with mouse epithelial cells suggested that there might be a mouse analogue of MLP1. In mouse cells the nucleus was stained by anti-MLP1 antibodies (Fig. 9). The nucleoli were not stained. In mitotic cells the cytoplasm was stained more intensely than in interphase cells while the chromosomes appeared dark. We cannot exclude that this nuclear staining results from the reaction with an unrelated protein. On immunoblots with mouse cells extracts about ten bands were detected, including three bands with mobilities similar to the bands seen with yeast cell extracts.

Since the immunofluorescence experiments suggested an association of *MLP1* with the nucleus, we tested the *MLP1* disruptions for phenotypes related to nuclear functions. Neither chromosome stability nor the mitotic recombination frequency was altered in the disruption strain. Meiotic recombination seemed to be unaffected. However, one difference was noted. *MLP1* disruption strains were slightly UV-sensitive. This was true for both types of disruptions described above. To show that the UV-sensitivity was linked to the *MLP1* disruption, two tetrads derived from diploid strains heterozygous for the

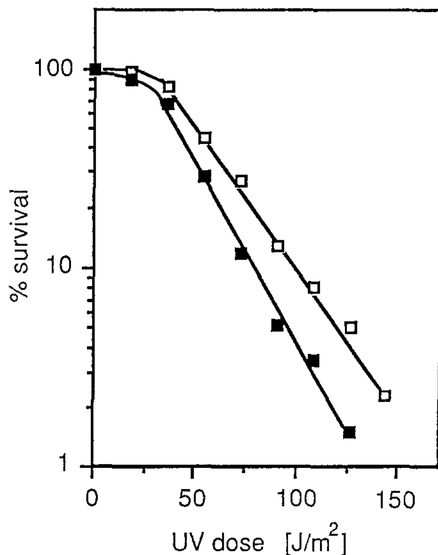

Fig. 10. Comparison of the UV-sensitivity of wild-type and *mlp1* disruption strains. Cells derived from one tetrad were examined (DBY 5385–5388): *open squares*, average of the measurements from the two wild-type strains; *closed squares*, average of the measurements from the two *mlp1* disruption strains

perfect disruption were examined in detail. In both tetrads UV-sensitivity segregated 2:2 and was linked to the *MLP1* disruption, which could be followed by the integrated *LEU2* marker in an otherwise *leu2* background. The results obtained with the spores from one tetrad (DBY 5385–5388) are shown in Fig. 10. These results indicate that the UV sensitivity is indeed caused by the *MLP1* disruption.

In this connection it is important to note that the product of the yeast *RAD50* gene appears also to have a myosin-like heptad repeat structure that suggests a coiled-coil structure (Alani et al. 1989). Disruption of the *RAD50* gene are viable, but they show considerably more sensitivity to radiation than do the disruptions of the *MLP1* gene.

Genetic mapping of the MLP1 *gene*

The *MLP1* gene was mapped to chromosome 11 by chromosome blotting. Through tetrad analysis, linkage was established between the *LEU2* marker inserted into the *MLP1* locus and the *met1* marker on chromosome 11 (cross: DBY 5389 × DBY 5384). The calculated map distance was 25 cM (PD:NPD:T was 29:1:17). No linkage was detected between *mlp1::LEU2* and *met14*, which is located 48 cM from *met1* between *met1* and the centromere (cross: DBY 1186 × DBY 5390; PD:NPD:T was 7:4:25). The *MLP1* gene is therefore located distal to *met1* and should be close to the *bls2* marker.

Conclusions

We have identified a new yeast gene, *MLP1*, that encodes a protein that has a myosin-like heptad repeat structure. Immunolocalization in both yeast and higher cells suggests a nuclear function, and this is supported by the only phenotype thus far found for disruptions of the gene, namely a modest increase in sensitivity to ultraviolet irradiation. The suggestion of a nuclear function is also supported by the observation that the most closely related yeast protein whose sequence is in the databases is the *RAD50* protein. The protein that seems to have the most extensive similarity in overall structure to the *MLP1* protein is a protein about which little is known except that it is implicated in oncogenesis: it has been found fused to the *raf* and *met* oncogenes in tumors (Ishikawa et al. 1987; King et al. 1988). Further understanding of the function of the *MLP1* gene product will require the discovery, by genetic or biochemical means, of other proteins with which it must interact.

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Note added in proof

The *MLP1* sequence has been deposited in the genbank database, accession number: LO1992