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Myoneurin, a Novel Member of the BTB/POZ–Zinc Finger Family Highly Expressed in Human Muscle

Patrick M. Alliel,*[,1] Nadia Seddiqi,†[,2] Danièle Goudou,* Carmen Cifuentes-Diaz,* Norma Romero,‡ Elena Velasco,§ François Rieger,* and Jean-Pierre Périn*

**Institut National de la Santé et de la Recherche Médicale, U488, Bâtiment Gregory Pincus, 80 rue du Général Leclerc, Le Kremlin Bîcêtre cedex, 94276, France; †Département de Biologie, Faculté des Sciences Semlalia, Université Cadi Ayyad, Avenue Moulay Abdellah, Marrakech, Morocco; ‡Institut de Myologie, Hôpital de la Salpétrière, Boulevard de l'Hôpital, Paris, France; and §Escuela de Medecina J.M. Vargas U.C.V., Caracas, Venezuela*

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Initially characterized as *Drosophila* developmental regulators, the BTB/POZ and zinc finger proteins (BTB/POZ-ZF) constitute a growing family of proteins with gene expression regulatory functions since they have been shown to be involved in both transcriptional activation and repression of various genes in a broad range of species, including mammals. Here we report the cloning of a novel human transcript, coding for a 68-kDa deduced BTB/POZ-ZF protein. This molecule, called myoneurin on the basis of its prevalent expression in the neuromuscular system, contains an amino-terminal BTB/POZ domain and eight tandemly repeated zinc-finger motifs of the C_2H_2 type. The murine myoneurin, identified in the mouse embryo, is highly homologous to the human protein.

Key Words: **myoneurin; BTB/POZ; zinc finger; muscle.**

A novel class of structurally related zinc finger proteins (ZF), has been implicated in the control of a wide range of developmental events in *Drosophila* and mammals. They are characterized by classical C_2H_2 zinc finger motifs, and a BTB/POZ protein–protein interaction domain (Zollman *et al.*, 1994; Bardwell and Treisman, 1994). Human BTB/POZ-ZF proteins can either activate or suppress transcription of distinct genes. BCL6/LAZ3 (Ye *et al.*, 1993; Kerckaert *et al.*, 1993) functions as a sequence specific transcriptional repressor (Chang *et al.*, 1996). As PLZF (Chen *et al.*, 1994), these molecules interact with a variety of corepressor proteins (Wong and Privalsky, 1998; Huynh and Bardwell, 1998). TRAX/RP58, a translin-associated protein (Aoki *et al.*, 1997), mediates a sequence specific transcriptional repression (Aoki *et al.*, 1998). ZID interacts with the CArG box region of the skeletal alpha-actin promoter (Bardwell and Treisman, 1994). Clone 18/HKR3 regulates MHCII genes (Sugawara *et al.*, 1994; Maris *et al.*, 1996). HcKrox (Widom *et al.*, 1997) is a transcriptional regulator of extracellular matrix genes. MIZ-1 has a potent growth arrest function related to its c-myc association ability (Peukert *et al.*, 1997). ZF5 (Sugiura *et al.*, 1997), another c-myc-binding protein (Numoto *et al.*, 1993) exerts a growth suppressive activity in mouse cell lines (Numoto *et al.*, 1995), and has been shown to be involved in both transcriptional activation and repression (Kaplan and Calame, 1997). Here we report a new transcript, conceptually coding for a protein with features of a BTB/POZ-ZF protein, and expressed in human skeletal muscle. The murine myoneurin has also been characterized by RT-PCR in 17-day embryo and in adult tissues and is highly homologous to the human counterpart.

METHODS

cDNA cloning, RT-PCR, and sequencing. Two clones (clones 1 and 2) were isolated following a classical procedure (Huynh *et al.*, 1985), during the screening of a human λgt11 testicular library (Clontech Laboratories, Palo Alto, CA) with a polyclonal antiserum directed against an ovine testicular 68 kDa heparin-binding protein (a gift from Dr F. Bonnet) using goat anti-rabbit IgG-alkaline phosphatase (1:7500 in TBS, 0.05% Tween 20) as a secondary antibody (Promega Corp., Madison, WI). Inserts were excised by *Eco*RI restriction digestion and subcloned in pGEM-4Z plasmid (Promega Corp.). Clone 3 was isolated during a subsequent screening of a λgt10 human testicular library with a digoxygenin-11 dUTP (Boehringer-Mannheim GmbH, Mannheim, Germany) randomly labeled *Eco*RI/*Xho*I restriction fragment covering the 5′-end of clone 1. A PCR amplification of the 5′-end region was carried out from the λgt11 human testis cDNA library using a λgt11 primer (Rgt11: 5′TTGACACCAGACCAACTGGTAATG3′) and antisense primers designed from the cDNA sequences from clones 1 and 3: HZ33, 5′CCACTTT-

The GenBank Accession Number for the human myoneurin sequence reported in this paper is AF148848.

[1] To whom correspondence and reprint requests should be addressed. Fax: (33) 1 45 21 19 40. E-mail: alliel@infobiogen.fr.

[2] N.S. must be considered as an equal first author.

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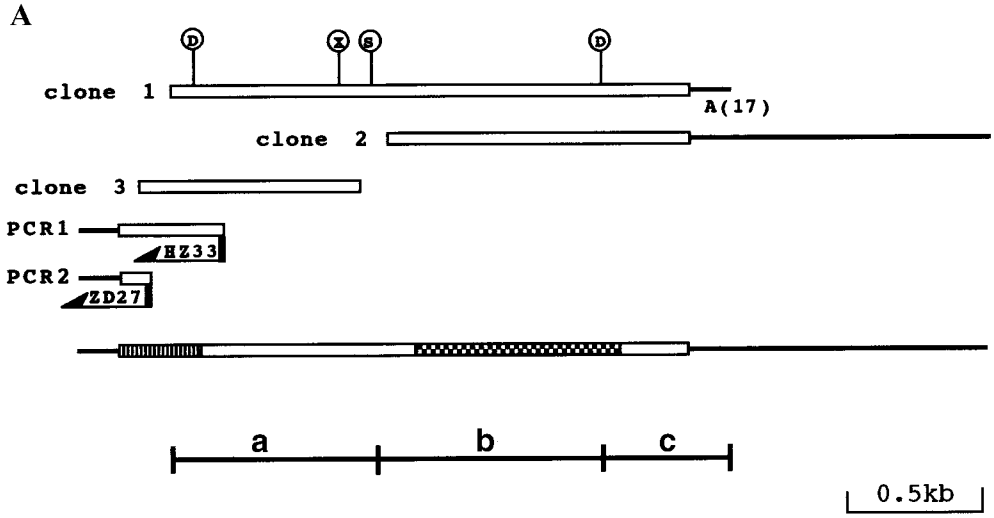

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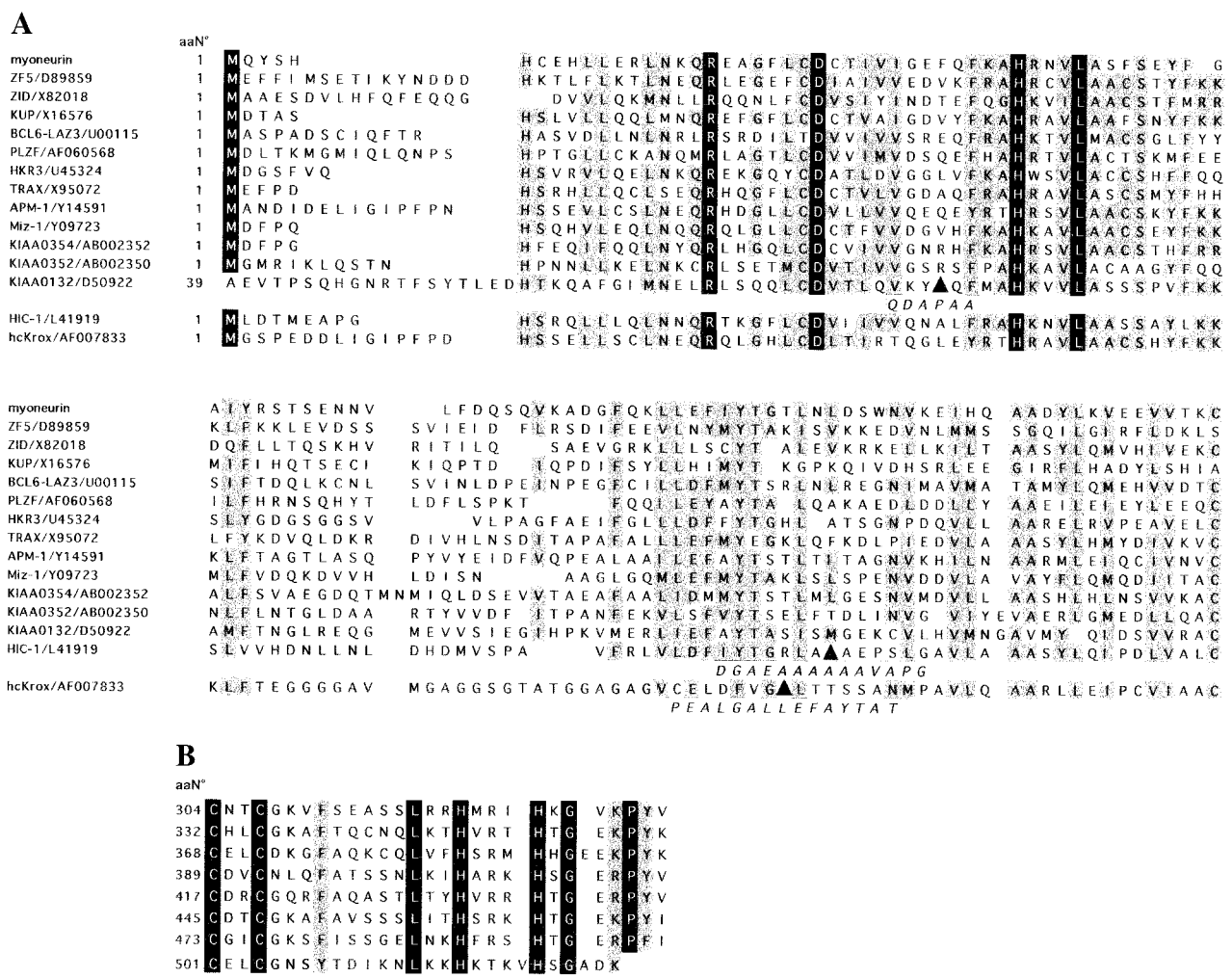

FIG. 2. The BTB/POZ and ZF domains of the human myoneurin. (A) Sequence homologies of the BTB/POZ domain of myoneurin with human BTB/POZ-ZF. Identical amino acids in myoneurin and all previously identified human BTB/POZ-ZF proteins are in black boxes; conserved amino acids (I, L, V, M), (Y, F), (T, S), (R, K), (D, E), (N, Q), and (G, A), represented in more than 50% of the aligned sequences, are in gray boxes. ▲ denotes supernumerary amino acids found in three sequences. The gene identities and the GenBank accession numbers are listed in the first column (for references, see text except for KUP, Chardin *et al.*, 1991, and for HIC1, Wales *et al.*, 1995). (B) Alignment of the eight ZF motifs. Numbers indicate the amino acid position in the human deduced protein sequence. Black boxes designate invariant amino acids (C, L, H, G, and P). Gray boxes designate conserved amino acids (F/Y and K/R).

GAGATAGTCAGCAGCCT$^{3'}$; ZD27, $^{5'}$GGAGGCCAGCACATTCCTA-TGAGCTTT$^{3'}$ (35 cycles at an annealing temperature of 60°C for 1 min and extension and denaturation, 1 min each, at 72 and 94°C, respectively). Sequences from both strands of inserts or PCR products were determined by the dideoxynucleotide chain-termination method (Sanger *et al.*, 1977) with T7 DNA–polymerase Sequenase II (Amersham Pharmacia Biotech, Uppsala, Sweden) and (α-^{35}S) dATP, using SP6/T7 and internal specific primers. Sequencing compressions were resolved using dITP. The cloning strategy is summarized in Fig. 1.

FIG. 1. The human myoneurin. (A) cDNA cloning, PCR 5′ end extension, and probes localization. The open boxes correspond to the open reading frames, as determined from the nucleotide sequences from clones 1, 2, and 3 and the 5′-RACE PCR-amplified products (PCR1 and PCR2). Arrows indicate the position and the orientation of myoneurin HZ33- and ZD27-specific primers used for PCR amplification from the λgt11 library. Dashed and dotted boxes in the composite cDNA open reading frame designate the BTB/POZ and the ZF domains, respectively. $(A)_{17}$ indicates the poly(A) tail in clone 1 that was absent in clone 2. The restriction sites, indicated in clone 1, (D) for *Dra*I, (S) for *Sty*I, and (X) for *Xho*I, are shown to localize the cDNA regions used as probes for Northern blot analysis (probes a, b, and c). (B) Nucleotide and deduced protein sequences of myoneurin. The nucleotide sequence (regardless of the poly(A) tail in clone 1) is numbered from the 5′ upmost end of the PCR2 product to the 3′ end of clone2. The coding sequence is in block letters. The amino acid deduced protein sequence (one letter code) is shown below the nucleotide sequence. Position 1 denotes the methionine residue at the presumptive initiation codon. Three non-sense codons, located upstream and in frame with the initiation codon, and polyadenylation signals (effective and putative) are underlined, and * indicates the polyadenylation site in clone 1.

The murine myoneurin sequence was established by a PCR-based method carried out on mouse 17-day embryo Marathon-Ready cDNA (Clontech). During a first round, using an adaptor primer AP1 (Clontech) and a human myoneurin antisense primer KZR4, $^{5'}$CATGAATCTTGAGATTGCTAGAA$^{3'}$ (40 cycles; 57°C for 1 min, 72°C for 3 min, and 94°C for 1 min), a PCR fragment was amplified whose 5′ terminal end sequence allowed the synthesis of a mouse specific sense primer MOZ-5, $^{5'}$GTTTCCCTTGGGCGGCTACG$^{3'}$. During a second round carried out under identical PCR conditions, and using a human myoneurin sense primer STY, $^{5'}$GGTATTCCAAGGCCAAGCCAAT$^{3'}$, and an antisense primer ZS119, $^{5'}$TCTGCGAGGACGGCCTAGCAA$^{3'}$, designated from a murine EST (GenBank Accession No. AA119157), a fragment was amplified whose sequence allowed the synthesis of a mouse antisense specific primer ZR2856, $^{5'}$GCATGAATCTTGAGGTTGCTAGAAG$^{3'}$ (a murine counterpart of KZR4). A last PCR using MOZ-5/ZR2856 (40 cycles, 62°C for 1 min, 72°C for 3 min, 94°C for 1 min) was performed. All the PCR carried out with DyNazyme II DNA–polymerase (Finnzymes OY, Finland) were duplicated with Appligene *Taq* DNA polymerase (Appligene–Oncor, Illkirch, France) and the products were subcloned in pGEM-T before sequencing.

Mouse RNAs were extracted and oligo(dT)-primed cDNAs were prepared following the classical methods (Maniatis *et al.*, 1982). PCR amplification was carried out with the STY/ZS119 primer set. Southern blot analysis was achieved with a digoxygenin-11 dUTP (Boehringer-Mannheim GmbH, Mannheim, Germany) randomly labeled human myoneurin probe (terminal washing conditions: 0.1× SSC, 0.1% SDS, 60°C).

Northern blot analysis. Normalized human Northern blots (Clontech) were hybridized with a myoneurin probe (Fig. 1) randomly labeled with [α-^{32}P]dATP/dCTP (2×10^6 cpm/ml) in a solution containing 5× SSPE, 2× Denhardt's solution, 0.5% SDS, 100 μg/ml salmon sperm DNA, for 18 h at 42°C. The blots were washed twice with a 2× SSC, 0.05% SDS solution at room temperature then four times (15 min each) with a 0.1× SSC, 0.1% SDS solution at 50°C, and exposed to Hyperfilm-MP (Amersham Pharmacia Biotech) for 72 h at −70°C.

RESULTS

Nucleotide and Deduced Amino Acid Sequence of the Human Myoneurin: Expression in Human Tissues

The cloning strategy is presented in Fig. 1A. The composite nucleotide sequence established by cDNA cloning and a RACE protocol, and the deduced amino acid sequence are shown in Fig. 1B. The accuracy of the 5′-end sequence was ascertained by its identification in a genomic clone (work in progress). The first ATG, encountered at position 137 in the longest open reading frame, is preceded by three in-frame non-sense codons (nt: 2–4, 41–43, 98–100), and fulfills the criteria of a eukaryotic initiation site (Kozak, 1991). In frame with this ATG, a TGA stop codon (nt: 1967), defines a 1830 bp open reading frame encoding a 610 amino acid long deduced protein. A poly(A) tail found in clone 1 indicates that an upstream polyadenylation signal, ATTAAA (nt: 2041), is not used in the transcript corresponding to clone 2. The 3′ untranslated region contains two additional putative polyadenylation sites (nt: 2430 and nt: 2820).

The 610 amino-acid long deduced protein has a calculated molecular weight of 68,682 and is basic (p*I* = 8.4). The 111 N-terminal amino acids define a BTB-POZ domain which shares specific features with BTB/POZ proteins of human origin, with two highly conserved motifs spaced by a small sized and poorly conserved sequence (Fig. 2A). The C-terminal region (Met^{304}-His^{522}), contains eight tandemly repeated zinc-finger motifs of C_2-H_2 type spaced by H-C links of the Krüppel type (Fig. 2B). The region from aa 112 to 302, located between the BTB/POZ domain and the zinc-finger region, includes two stretches of basic amino acids, ^{174}KKSSQTKKKKKATNSPK^{190} and ^{257}KRKRGK^{262}, reminiscent of nuclear localization signals. Six potential PEST proteolytic signals, usually found in regulatory proteins, are also identified (aa 114–148, 154–168, 217–252, 281–296, 493–512, 556–573).

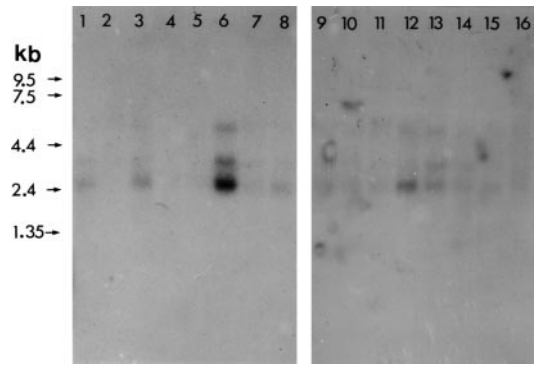

FIG. 3. Northern blot analysis of myoneurin mRNA in human tissues. Poly(A^+) mRNA (2 μg/lane) from heart (1), brain (2), placenta (3), lung (4), liver (5), skeletal muscle (6), kidney (7), pancreas (8), spleen (9), thymus (10), prostate (11), testis (12), ovary (13), small intestine (14), colon (15), and peripheral blood leukocyte (16) were hybridized with the human myoneurin probe a (see Fig. 1). The positions of the size markers, 1.35, 2.4, 4.4, 7.5, and 9.5 kb, are indicated on the left side.

As revealed by Northern blot analysis, a major 2.5 kb transcript was mainly identified in muscle and more weakly in other tissues, including testis, ovary and placenta (Fig. 3). Larger but less abundant transcripts (3.3 and >5.0 kb), clearly identified in muscle were not equally distributed in all the analyzed tissues. The 3.3 kb mRNA appeared to be under-expressed in testis and a larger transcript was overexpressed in thymus. The specificity of the observed signals corresponding to the 2.5, 3.3 and 5.0 kb muscle transcripts was ascertained by using probes b and c depicted in Fig. 1 (not shown).

Mouse and Human Myoneurins Are Highly Homologous

The whole mouse myoneurin coding sequence was established by a PCR approach. The mouse and human coding sequences are 88% homologous and the deduced amino acid sequences are 95% identical, but the differences are not random (Fig. 4). The BTB/POZ and the ZF domains are highly conserved (a single I/V change over the 111 N-terminal amino acids and two differ-

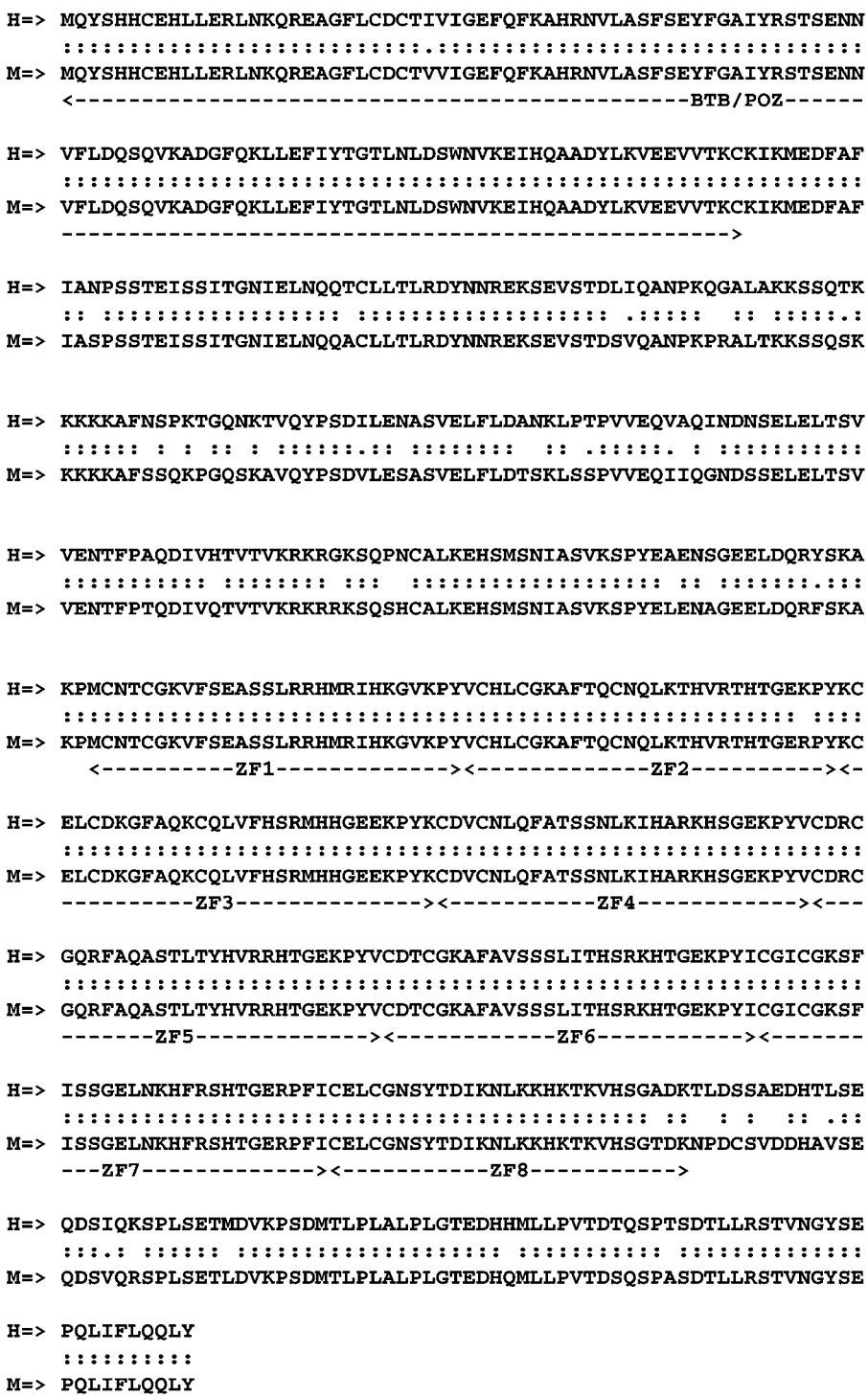

FIG. 4. Comparison of the human and murine myoneurins. The human myoneurin amino acid sequence (H) and its murine counterpart (M) are aligned. Identical amino acids and conserved amino acids are indicated by double and single dots, respectively. The BTB/POZ domain and the eight ZF motifs are underlined.

ences, K/R and A/T, within the eight ZF motifs, respectively). A PCR analysis carried out on murine cDNAs with the STY/ZS119 primers allowed the identification by a human probe (probe b in Fig. 1) of amplified products of the expected size (984 bp) in cerebellum, skeletal muscle, testis, hearth, brain, and liver (Fig. 5). A faint minor band was additionally identified below the major amplified product.

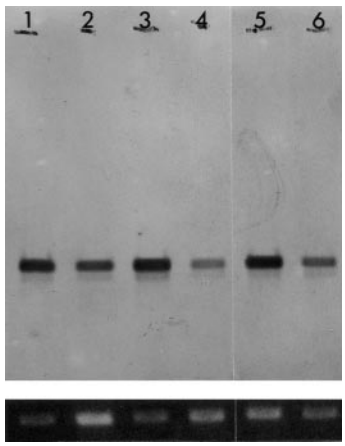

FIG. 5. Myoneurin expression in murine tissues. PCR were performed with STY/ZS119 primers and oligo (dT)-primed cDNAs: cerebellum (1), muscle (2), testis (3), heart (4), brain (5), and liver (6). Western blot of the amplified products (upper panel) was hybridized with the human myoneurin probe b depicted in Fig. 1. Lower panel corresponds to the amplification of β-actin.

DISCUSSION

Myoneurin shows similarities with factors belonging to the family of eukaryotic BTB/POZ and ZF proteins involved the control of gene expression. In the so far identified members of this family, the ZF motifs are centrally located or distributed in the vicinity of the C-terminus and the BTB/POZ domain is found in the amino-terminal region. The myoneurin protein sequence is typical of this organization. The eight tandemly repeated zinc-finger motifs of the Krüppel type may be of importance to nucleic acid-binding and also suggest various combinatorials to regulate different genes. Two potential nuclear localization signals, located between the BTB/POZ and ZF domains, could contribute to the nuclear import. The BTB/POZ domain, which encompasses the 111 N-terminal amino acids in myoneurin, is homologous to those encountered in genes involved in developmental control (Albagli *et al.,* 1995). This domain is assumed to act as a protein–protein interaction motif, promoting either homomerization or heteromerization (Bardwell and Treisman, 1994; Dong *et al.*, 1996). Several members of this family are thought to contribute to the expression of muscle or neuron specific genes. Broad-Complex is implicated in the control of the development of thoracic myotendinous junctions (Sandstrom *et al.,* 1997). Tramtrack (Harrison and Travers, 1990), is essential for the control of cellular competence for neural differentiation (Lai *et al.,* 1997). Lola controls axon pathfinding and is involved in developmental defects of axon growth and guidance (Giniger *et al.,* 1994; Cavarec *et al.,* 1997). Abrupt, is expressed in muscle nuclei in *Drosophila,* and is involved in the control of the specificity of neuromuscular connections (Hu *et al.,* 1995). The overall structure and the prevalent expression of myoneurin in human muscle suggest a possible contribution of the BTB/POZ zinc finger protein myoneurin in the regulation of the expression of muscle genes. Preliminary results are indicative of myoneurin expression at the neuromuscular junction (unpublished observation). Human ESTs homologous to myoneurin have been found in neurons, brain, bladder, uterus, ovary, kidney and testis (GenBank Accession Nos. AA362928, AA227448, AA345806, AA337411, H13408, H29122, AA191075, AA857432, R45310, AA228040, W20232, H29021, AA292098, AA748172, AA227869, AI913410, AL133070). We also detected significant level of myoneurin transcripts in human testis, ovary and placenta suggesting that this novel protein could also fulfill regulatory functions in the genital tract. Mouse myoneurin, highly related to the human protein in its BTB/POZ and ZF motifs, has been identified in embryo and in adult tissues, suggesting its implication in gene expression regulatory mechanisms throughout development and adulthood.

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REFERENCES

Albagli, O., Dhordain, P., Deweindt, C., Lecoq, G., and Leprince, D. (1995) The BTB/POZ domain: A new protein–protein interaction motif common to DNA- and actin-binding proteins. *Cell. Growth Differ.* **6,** 1193–1198.

Aoki, K., Ishida, R., and Kasai, M. (1997) Isolation and characterisation of a cDNA encoding a Translin-like associated protein, TRAX. *FEBS Lett.* **40,** 109–112.

Aoki, K., Meng, G., Suzuki, K., Takashi, T., Kameoka, Y., Nakahara, K., Ishida, R., and Kasai, M. (1998) RP58 associates with condensed chromatin and mediates a sequence-specific transcriptional repression. *J. Biol. Chem.* **273,** 26698–26704.

Bardwell, V. J., and Treisman, R. (1994) The POZ domain: A conserved protein–protein interaction motif. *Genes Dev.* **8,** 1664–1677.

Cavarec, L., Jensen, S., Casella J. F., Cristescu, S. A., and Heidmann, T. (1997) Molecular cloning and characterization of a transcription factor for the copia retrotransposon with homology to the BTB-containing lola neurogenic factor. *Mol. Cell. Biol.* **17,** 482–494.

Chang, C. C., Ye, B. H., Chaganti, R. S., and Dalla-Favera, R. (1996) BCL-6, a POZ/zinc finger protein, is a sequence-specific transcriptional repressor. *Proc. Natl. Acad. Sci. USA* **93,** 6947–6952.

Chardin, P., Courtois, G., Mattei, M. G., and Gisselbrecht, S. (1991) The KUP gene, located on human chromosome 14, encodes a protein with two distant zinc fingers. *Nucleic Acid Res.* **1,** 1431–1436.

Chen, Z., Guidez, F., Rousselot, P., Agadir, A., Chen, S. J., Wang, Z. Y., Degos, L., Zelent, A., Waxmann, S., and Chomienne, C. (1994)

PLZF-RAR alpha fusion proteins generated from the variant t(11;17) (q23;q21) translocation in acute promyelocytic leukemia inhibit ligand-dependent transactivation of wild-type retinoic acid receptors. *Proc. Natl. Acad. Sci. USA* **91,** 1178–1182.

Dennis, P., and Prody, C. A. (1997) Multiple nuclear proteins bind a novel *cis*-acting element that regulates the muscle-specific expression of the mouse nicotinic acetylcholine receptor alpha-subunit gene. *DNA Cell. Biol.* **16,** 1099–1110.

Dong, S., Zhu, J., Reid, A., Strutt, P., Guidez, F., Zhong, H. J., Wang, Z. Y., Licht, J., Waxman, S., Chomienne, C., Chen, Z., Zelent, A., and Chen, S. J. (1996) Amino-terminal protein–protein interaction motif (POZ-domain) is responsible for activities of the promyelocytic leukemia zinc finger–retinoic acid receptor-α fusion protein. *Proc. Natl. Acad. Sci. USA* **93,** 3624–3629.

Giniger, E., Tietje, K., Jan, L. Y., and Jan, Y. N. (1994) *Lola* encodes a putative transcription factor required for axon growth and guidance in *Drosophila*. *Development* **120,** 1385–1398.

Harrison, S. D., and Travers, A. A. (1990) The *tramtrack* gene encodes a *Drosophila* finger protein that interacts with the ftz transcriptional regulatory region and shows a novel embryonic expression pattern. *EMBO J.* **9,** 207–216.

Hu, S., Fambrough, D., Atashi, J. R., Goodman, C. S., and Crews, S. T. (1995) The *Drosophila* abrupt gene encodes a BTB-zinc finger regulatory protein that controls the specificity of neuromuscular connections. *Genes Dev.* **9,** 2936–2948.

Huynh, T. V., Young, R. A., and Davis, R. W. (1985) *in* DNA Cloning. A Practical Approach (Glover, D. M., Ed.), Vol. 1, pp. 49–78, IRL Press, Oxford.

Huynh, K. D., and Bardwell, V. J. (1998) The BCL-6 POZ domain and other POZ domains interact with the co-repressor N-CoR and SMRT. *Oncogene* **17,** 2476–2483.

Kaplan, J., and Calame, K. (1997) The ZiN/POZ domain of ZF5 is required for both transcriptional activation and repression. *Nucleic Acids Res.* **25,** 1108–1116.

Kerckaert, J.-P., Deweindt, C., Tilly, H., Quief, S., Lecocq, G., and Bastard, C. (1993) LAZ3, a novel zinc-finger encoding gene, is disrupted by recurring chromosome 3q27 translocations in human lymphomas. *Nature Genet.* **5,** 66–70.

Kozak, M. (1991) Structural features in eukaryotic mRNAs that modulate the initiation of translation. *J. Biol. Chem.* **266,** 19867–19870.

Lai, Z. C., Fetchko, M., and Li, Y. (1997) Repression of *Drosophila* photoreceptor cell fate through cooperative action of two transcriptional repressors Yan and Tramtrack. *Genetics* **147,** 1131–1137.

Maniatis, T., Fritsch, E. F., and Sandbrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Maris, J. M., Jensen, S. J.,. Sulman, E. P, Beltinger, C. P., Gates, K., Allen, C., Biegel, J. A., Brodeur, G. M., and White, P. S. (1996) Cloning, chromosomal localization, physical mapping, and genomic characterization of HKR3. *Genomics* **35,** 289–298.

Numoto, M., Yokor, O. K., Yanagihara, K., Kamiya, K., and Niwa, O. (1995) Over-expressed ZF5 gene product, a c-myc-binding protein related to GL1-Kruppel protein, has a growth-suppressive activity in mouse cell lines. *Jpn. J. Cancer Res.* **86,** 277–283.

Peukert, K., Staller, P., Schneider, A., Carmichael, G., Hänel, F., and Eilers, M. (1997) An alternative pathway for gene regulation by Myc. *EMBO J.* **16,** 5672–5686.

Sandstrom, D. J., Bayer, C. A., Fristrom, J. W., and Restifo, L. L. (1997) Broad-Complex transcription factors regulate thoracic muscle attachment in *Drosophila*. *Dev. Biol.* **181,** 168–185.

Sanger, F., Nicklen, R., and Coulson, A. R. (1977) DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.

Sugawara, M., Scholl, T., Ponath, P. D., and Strominger, J. L. (1994) A factor that regulates the class II major histocompatibility complex gene DPA is a member of a subfamily of zinc finger proteins that includes a *Drosophila* developmental control protein. *Mol. Cell. Biol.* **14,** 8438–8450.

Sugiura, K., Muro, Y., Nagai, Y., Kamimoto, T., Wakabayashi, T., Ohashi, M., and Hagiwara, M. (1997) Expression cloning and intracellular localization of a human ZF5 homologue. *Biochim. Biophys. Acta* **1352,** 23–26.

Wales, M. M., Biel, M. A., El Deiry, W., Nelkin, B. D., Issa, J.-P., Cavenee, W. K., Kuerbitz, S. J., and Baylin, S. B. (1995) p53 activates expression of HIC-1, a new candidate tumor suppressor gene on 17p13.3. *Nature Med.* **1,** 570–577.

Widom, R. L., Culic, I., Lee, J. Y., and Korn, J. H. (1997) Cloning and characterization of hcKrox, a transcriptional regulator of extracellular matrix gene expression. *Gene* **198,** 407–420.

Wong, C. W., and Privalsky, M. L. (1998) Components of the SMRT corepressor complex exhibit distinctive interactions with the POZ domain oncoproteins PLZF, PLZF-RAR alpha, and BCL-6. *J. Biol. Chem.* **273,** 27695–27702.

Ye, B. H., Lista, F., Lo Coco, F., Knowless, D. M., Offit, K., Chaganti, R. S., and Dalla-Favera, R. (1993) Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large-cell lymphoma. *Science* **262,** 747–750.

Zollman, S., Godt, D., Privé, G. G., Couderc, J.-L., and Laski, F. A. (1994) The BTB domain, found primarily in zinc finger proteins, defines an evolutionarily conserved family that includes several developmentally regulated genes in *Drosophila*. *Proc. Natl. Acad. Sci. USA* **91,** 10717–10721.