SHORT COMMUNICATION

Cloning and Mapping of Members of the MYM Family

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Tandem repeats of a novel, putative, zinc-binding motif (MYM) have been described within the products of two, highly homologous genes: *ZNF198*/*RAMP*/*FIM* and *ZNF261*/*DXS6673E*. *ZNF198*, mapping to 13q11–q12, was recently shown to fuse to the fibroblast growth factor receptor 1 gene in the t(8;13)(p11;q11–q12) rearrangement associated with a stem cell leukemia/lymphoma syndrome. *ZNF261* at Xq13.1 is disrupted by a t(X;13)(q13.1;q32) rearrangement in a mentally retarded patient and is a candidate gene for nonspecific X-linked mental retardation. Here we have cloned another member of this family, designated *ZNF258*, and mapped it to chromosome band 14q12. In addition, *ZNF262*/*KIAA0425* was identified as a further member of the family and mapped to 1p32–p34. The predicted protein products of *ZNF258* and *ZNF262* maintain the repeats of the MYM motif. Isolation of these new members will facilitate the functional characterization of the MYM family and motif.

The t(8;13)(p11;q11–q12) associated with a mixed leukemia/lymphoma syndrome has recently been demonstrated to fuse the fibroblast growth factor receptor 1 gene (*FGFR1*) at 8p11, to a novel gene at 13q11–q12, designated *ZNF198* (10, 16) [also known as *RAMP* (12) or *FIM* (9)]. Due to the similarity with other tumor-associated chromosomal rearrangements that involve receptor tyrosine kinases (2, 3, 6, 8), motifs in ZNF198 are proposed to oligomerize the fusion protein and lead to constitutive activation of FGFR1 tyrosine kinase activity. This is analogous to activation of the full-length receptor that follows ligand-dependent dimerization (5).

ZNF198 has substantial homology with *ZNF261* (also known as *DXS6673E*), which was shown to be disrupted in the 5′ untranslated region by a t(X;13)(q13.1;q32) in a patient with mental retardation and is hence a candidate gene for nonspecific X-linked mental retardation (15). In particular, ZNF198 and ZNF261 contain five tandem repeats of a Cys-X_2-Cys-X_{19-22}-Cys-X_3-Cys-X_{13-19}-Cys-X_2-Cys-X_{19-25}-Cys-X_3-Cys motif (designated the MYM motif for myeloproliferative and mental retardation motif), where X is any amino acid, although at certain positions there are distinct preferences for particular amino acids (Fig. 1). This motif closely resembles zinc-binding sequences, especially the RING, PHD/LAP, and LIM motifs (7, 11). However, the lack of a conserved histidine residue and the alternate spacing of two and then three residues between the pairs of cysteines distinguishes the MYM motif from known zinc-binding sequences, and BLAST searches with the ZNF261 and ZNF198 sequences failed to identify the motif in any other known protein sequences. Therefore ZNF198 and ZNF261 contain a novel, putative zinc-binding motif. The motif would be predicted to fold to bind two zinc atoms per motif, each zinc atom being bound in a tetrahedral coordination by four cysteines. Studies are being carried out on the MYM motif to establish whether the motif does truly have metal binding capabilities.

Although the motif was not seen in any other known proteins, database searches identified nucleotide sequences encoding the motif in the full-length cDNA clone *ZNF262* (also known as *KIAA0425*) and expressed sequence tags (ESTs) W01125, N93066, R70433, AA280676, AA494557, AA280677, and AA001379. To isolate the full cDNAs containing these EST sequences, 5′ and 3′ RACE reactions were carried out with the Marathon cDNA amplification kit (Clontech) and human thymus cDNA (Clontech) as described by the manufacturer with specific primers corresponding to the EST sequences. For sequence analysis, RACE products were subcloned into the TOPO TA Cloning kit (Invitrogen) following the manufacturer's instructions and sequenced using T7 (5′-TAATACGACTCACTATAGGG-3′) and M13 reverse (5′-CAGGAAACAGCTATGAC-3′) primers on an ABI

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Nos. AJ224901 (for *ZNF198*), AF055470 (for *ZNF258*), X95808 (for *ZNF261*), and AB007885 (for ZNF262).

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ZNF261	rpt 1 (307-389)	TKMTCAHCRTPLQKGQTAY--QRKGLPQLFCSSSCLTTFSKKP------SGKKTCTFCKKEIWNTKD-SVVAQTGSGGSFHEFCTSVCLSLY
ZNF198	rpt 1 (55-137)	LKFTCANCKKPLQKGQTAY--QRKGSAHLFCSTTCLSSFSHKP------APKKLCVMCKKDITTMKG-TIVAQVDSSESFQEFCSTSCLSLY
ZNF258	rpt 1 (96-173)	IKVFCSGCKKMLYKGQTAY--HKTGSTQLFCSTRCITRHSSPACLPPPP--KKTCTNCSKDILNPKD-VITTRFENSYPSKDFCSQSCLSSY
ZNF262	rpt 1 (13-100)	VRVSCSGCKKILQKGQTAY--QRKGSTQLFCSTLCLTGYTVPPARPPPPLTKKTCSSCSKDILNPKD-VISAQFENTTTSKDFCSQSCLSTY
ZNF261	rpt 2 (405-488)	DATRCSICQKTGEVLHEVS--NGS-VVHRLCSDSCFSKFRANKG-----LKTNCCDQCGAYIYTKTGSPGPELLFHEGQQKRFCNTTCLGAY
ZNF198	rpt 2 (149-230)	NKSRCTICGKLTEIRHEVS--FKN-MTHKLCSDHCFNRYRMANG-----LIMNCCEQCGEYLPSK-G-AGNNVLVIDGQQKRFCCQSCVSEY
ZNF258	rpt 2 (188-262)	ISTKCSMCQKNADTRFEVK--YQN-VVHGLCSDACFSKFHSTNN-----LTMNCCENCGSYCYSSSGPCQ--------SQKVFSSTSV-TAY
ZNF262	rpt 2 (115-196)	ISTKCSMCQKNAVIRHEVN--YQN-VVHKLCSDACFSKFRSANN-----LTMNCCENCGGYCYSGSGQCHMLQIEG--QSKKFCSSSCITAY
ZNF261	rpt 3 (494-578)	RVYPCVWCKTLCKNFEMLSHVDRNGKTSLFCSLCCTTSYKVKQA--GLTGPPRPCSFCRRSLSDP-----CYYNKVDRTVYQFCSPSCWTKF
ZNF198	rpt 3 (260-344)	KLTTCTGCRTQCRFFDMTQCIGPNGYMEPYCSTACMNSHKTKYA--KSQSLGIICHFCKRNSLPQ-----YQATMPDGKLYNFCNSSCVAKF
ZNF258	rpt 3 (268-352)	QIPPYALGKSLRPSAEMIETTNDSGKTELFCSINCLSAYRVKTV--TSSGVQVSCHSCKTSAIPQ-----YHLAMSNGTIYSFCSSSCVVAF
ZNF262	rpt 3 (211-295)	KITPCALCKSLRSSAEMIETTNSLGKTELFCSVNCLSAYRVKMV--TSAGVQVQCNSCKTSAIPQ-----YHLAMSDGSIRNFCSYSCVVAF
ZNF261	rpt 4 (587-664)	IHLSCHYCHSLFSGKPEVL--DWQDQVFQFCCRDCCEDFKRLR------GVVSQCEHCRQEKLLH------EKLRFSGVEKSFCSEGCVLLY
ZNF198	rpt 4 (363-440)	IQLKCNYCKNSFCSKPEIL--EWENKVHQFCSKTCSDDYKKLH------CIVTYCEYCQEEKTLH------ETVNFSGVKRPFCSEGCKLLY
ZNF258	rpt 4 (426-503)	VKLKCQHCNHLFATKPELL--FYKGKMFLFCGKNCSDEYKKKN------KVVAMCDYCKLQKIIK------ETVRFSGVDKPFCSEVCKFLS
ZNF262	rpt 4 (368-445)	VKLKCQHCNRLFATKPELL--DYKGKMFQFCGKNCSDEYKKIN------NVMAMCEYCKIEKIVK------ETVRFSGADKSFCSEGCKLLY
ZNF261	rpt 5 (675-751)	CCITCTYCSQTCQRGVTE---QLDGSTWDFCSEDCKSKYLLWY------CKAARCHACKRQGKLL------ETIHWRGQIRHFCNQQCLLRF
ZNF198	rpt 5 (451-527)	RCVTCNYCSQLCKKGATK---ELDGVVRDFCSEDCCKKFQDWY------YKAARCDCCKSQGTLK------ERVQWRGEMKHFCDQHCLLRF
ZNF258	rpt 5 (514-590)	YCKMCSYCSQTSPNLVEN---RLEGKLEEFCCEDCMSKFTVLF------YQMAKCDGCKRQGKLS------ESIKWRGNIKHFCNLFCVLEF
ZNF262	rpt 5 (456-532)	HCKMCSYCLQTSPKLVQN---NLGGKVEEFCCEECMSKYTVLF------YQMAKCDACKRQGKLS------ESLKWRGEMKHFCNLLCILMF

MYM MOTIF Cys-X_2-Cys-X_{19-22}-Cys-X_3-Cys-X_{13-19}-Cys-X_2-Cys-X_{19-25}-Cys-X_3-Cys

FIG. 1. Multiple sequence alignment of the five repeats of the novel, putative zinc-binding MYM motif seen in ZNF261, ZNF198, ZNF258, and ZNF262. The conserved cysteine residues with a potential role as zinc ligands are shown in boldface type, and where replaced in ZNF258 with other residues they are shown in boldface and underlined. The numbering in parentheses refers to the amino acid positions in the full-length protein sequences. The motif sequence is shown below the alignment where X refers to any amino acid, although there are distinct preferences at particular positions.

377 sequencer using a *Taq*FS Dye Terminator Sequencing kit (ABI, Foster City, CA). All the ESTs were found to correspond to one new gene, which has been named *ZNF258*. The full cDNA sequence (GenBank Accession No. AF055470) encodes a predicted protein of 724 amino acids. Although a perfect Kozak consensus is not seen around the predicted initiation codon, the important adenine base at the −3 position was observed, and preceding stop codons were seen in all three frames. Probes corresponding to nucleotides 1–602 of *ZNF258* and 473–1383 of *ZNF262* were hybridized to a human genomic PAC library (constructed in-house by the Cancer Gene Cloning Laboratory, Institute of Cancer Research, Sutton, UK), and the positive clones were confirmed by Southern blotting. The positive PACs were mapped onto normal metaphase chromosomes by fluorescence *in situ* hybridization (FISH; Fig. 2A). These experiments demonstrated that *ZNF258* was located at chromosome band 14q12 and *ZNF262* mapped to 1p32–p34. The mapping of *ZNF262* was as predicted from database searches that reveal a match with STS marker WI-13474 from the 1p region. Labeling and hybridization for the FISH experiments were carried out as described previously (13).

Both ZNF262 and ZNF258 maintained the five tandem repeats of the putative, zinc-binding motif although interestingly ZNF258 failed to conserve the last two cysteines in the second repeat and the first two cysteines in the third repeat (Fig. 1). For each member of the MYM family, sequences on the N-terminal side of the repeats show little homology with the other members, while the regions to the C-terminal side of the MYM motifs are highly conserved. On the basis of homology, the family appears to be subdivided into (i) ZNF198 and ZNF261 and (ii) ZNF258 and ZNF262. Highly similar (>74% protein sequence identity) murine homologues of all the members of the family appear to exist from EST database searches. The conservation of these genes across species is supported by zoo blot experiments showing that *ZNF261* is highly conserved among vertebrates (15).

Previous Northern blot analyses have shown that *ZNF198* and *ZNF261* are expressed in nearly all human tissues tested (9, 10, 15, 16). Multiple tissue blots (Clontech) were hybridized according to the manufacturer's instructions with probes corresponding to nucleotides 1543–2395 of *ZNF258* and 12–493 of *ZNF262* (Fig. 2B). For *ZNF258* prominent transcripts of approximately 3 and 5 kb were observed with fainter bands of around 6 and 8 kb, except interestingly in the case of peripheral blood leukocytes where the 6- and 8-kb bands were the predominant forms. These bands were observed in each tissue upon prolonged exposure of the blot but the highest expression appeared to be in heart, skeletal muscle, kidney, and liver tissue. The large number of bands observed may represent alternative splice forms or could indicate cross-hybridization of the probe with the other highly homologous family members (the *ZNF198* transcript is known to have 5- and 8-kb forms, and *ZNF261* has several transcripts of approximately 6 kb). However, increasing the stringency of the 0.1× SSC/0.1% SDS wash from 50 to 65°C failed to remove any bands. Furthermore, reprobing the blot with a probe corresponding to nucleotides 1–602 of *ZNF258* gave the same pattern of bands apart from an extra 3-kb band that appeared to correspond to

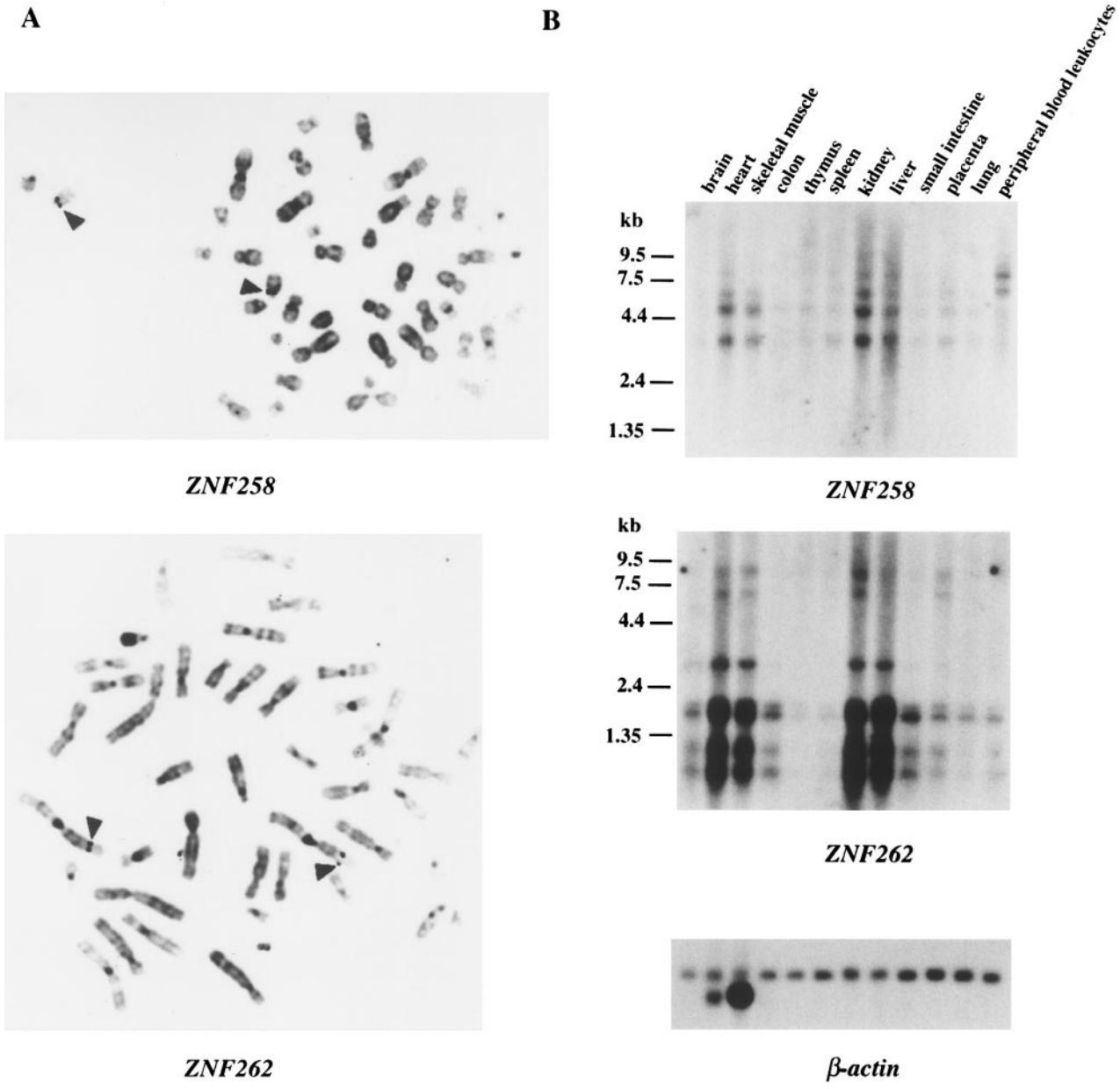

FIG. 2. (**A**) Physical mapping of *ZNF258* and *ZNF262* by FISH. The DAPI-stained images have been adjusted to show banding, and arrowheads indicate the position of any fluorescence signals. *ZNF258* consistently mapped to 14q12 and *ZNF262* to 1p32–p34. (**B**) Expression of *ZNF258* and *ZNF262* on a multiple tissue Northern blot (Clontech).

the 3-kb band seen in the *ZNF262* probing (see below). From the sizes of the *ZNF258* transcripts and predicted open reading frames, the untranslated regions appear to encompass at least 800 bp. For the *ZNF262* probe, bands at 3 kb, a doublet at 1.5 kb, and another two bands below 1 kb were observed, as were fainter bands of around 7 and 9 kb. As for *ZNF258,* these bands were observed in each tissue upon prolonged exposure of the blot with highest expression in heart, skeletal muscle, kidney, and liver tissue. The bands observed do not match the sizes reported for other family members, indicating that *ZNF262* may have a number of alternative splice forms or that the probe cross-hybridizes to unrelated products. However, increasing the stringency of the 0.1× SSC/0.1% SDS wash from 50 to 60°C failed to remove any bands while further increasing the wash to 65°C resulted in complete removal of the probe. Finally, all the family members have many matching ESTs, isolated from a wide variety of tissues, which confirms this widespread pattern of expression.

A potential, uncloned tumor suppressor gene in ovarian carcinoma maps to the location of *ZNF258* (14q12–q13; Ref. 1), making *ZNF258* a possible candidate gene. Similarly, the uncloned muscle–eye–brain disease gene (4) and a meningioma-associated tumor suppressor gene (14) map to 1p32–p34, the site of *ZNF262.* Recent evidence has shown that zinc-binding motifs are often involved in protein–protein interactions via their ability to self-associate and interact with other

proteins (5). The MYM motif could possibly have such a role in the function of this gene family. It also seem likely that the repeated MYM motifs in ZNF198 will be responsible for the oligomerization and activation of the t(8;13) fusion product. Future work will focus on the role this motif plays in the function of the ZNF198–FGFR1 fusion protein and in the MYM family in general.

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