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-X2-Cys-X3-Cys-X13-19-Cys-X2-Cys-X19-25-Cys-X3-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 done ZNF262 (also known as KIAA0425) and expressed sequence tags (ESTs) W01125, N93066, R70433, AA280676, AA494557, AA280677, and AA001379.

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 A8007885 (for ZNF262).

1 To whom correspondence should be addressed at Institute of Cancer Research, Haddow Laboratories, 15 Cotswold Road, Belmont, Sutton, Surrey SM2 5NG, United Kingdom. Telephone: (44) 181-7724002. Fax: (44) 181-7770290. E-mail: damian@icr.ac.uk.

244–247 (1999)}
377 sequencer using a TaqFS 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 2^{nd} 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 MYM 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).
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

**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 ZNF 262 to 1p32–p34. (B) Expression of ZNF 258 and ZNF 262 on a multiple tissue Northern blot (Clontech).
proteins (5). The MYM motif could possibly have such a role in the function of this gene family. It also seems 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.

ACKNOWLEDGMENTS

This work included support from the Medical Research Council and Kay Kendall Leukaemia Fund.

REFERENCES


