mutations in the homologous genes of the two organisms, and we are currently testing whether genes from budding yeast and *Drosophila* are functionally homologous. A number of circumstantial observations suggest that *polo*/*CDC5* may regulate microtubule behaviour. *cdc5* mutants show an unusual interaction with MBC, a drug that binds tubulin and depolymerizes microtubules[12]; and there is a strong interaction of *polo* with mutations in *asp* which appear to affect microtubule stability (C. Gonzalez, C. E. Sunkel and D.M.G., unpublished). The mitotic stage at which Polo kinase appears to be maximally active would be consistent with a role in orchestrating the changes in microtubule organization that have to occur late in anaphase and in telophase. The availability of mutations in the genes and antibodies to their proteins now offers the means of exploring some of these potential roles for the enzyme. □

Received 1 February; accepted 7 April 1993.

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ACKNOWLEDGEMENTS. We thank C. Gonzalez and H. Ohkura for comments on the manuscript, S. Llamazares for encouragement and for anti-*polo* antibodies, and Drs Kitada, Sugino, Johnston, Clay, Dunn, Golsteyn and Nigg for communicating results before publication. B.F. was supported by a postdoctoral Training Fellowship from the Medical Research Council. We acknowledge the support of the Cancer Research Campaign.

Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma

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HUMAN myxoid liposarcomas contain a characteristic chromosomal translocation, t(12;16)(q13;p11)[1,2], that is associated with a structural rearrangement of the gene encoding CHOP [3], a growth arrest and DNA-damage inducible member of the C/EBP family of transcription factors[4,5] residing on 12q13.1 [6]. Using a CHOP-specific complementary probe and antiserum we report here the presence of an abnormal CHOP transcript and protein in these tumours. Cloning of the translocation-associated *CHOP* gene product revealed a fusion between *CHOP* and a gene provisionally named *TLS* (translocated in liposarcoma). TLS is a novel nuclear RNA-binding protein with extensive sequence similarity to EWS[7], the product of a gene commonly translocated in Ewing's sarcoma. In TLS-CHOP the RNA-binding domain of TLS is replaced by the DNA-binding and leucine zipper dimerization domain of CHOP. Targeting of a conserved effector domain of RNA-binding proteins to DNA may play a role in tumour formation.

Northern blot analysis of RNA from myxoid liposarcoma (MLPS) tumour cells containing the t(12;16) rearrangement demonstrated the constitutive expression of an abnormally large *CHOP* transcript (Fig. 1*a*, lanes 5–9). A normal sized CHOP messenger RNA could also be induced in these cells by treatment with a DNA-damaging agent (lanes 6 and 8). Tumour cells that do not contain the t(12;16) rearrangement express only the normal sized CHOP transcript (lanes 2 and 4). To determine if the abnormal CHOP transcript was associated with the production of an abnormal protein, we immuneprecipitated the

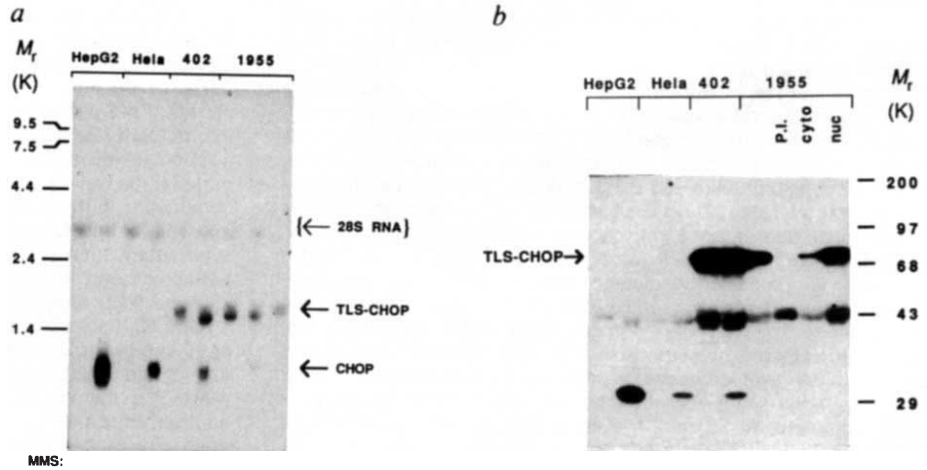


FIG. 1 Myxoid lipsarcoma cells contain an abnormal CHOP transcript and protein. *a*, Northern blot RNA from hepatoblastoma (HepG2), cervical carcinoma (HeLa) and myxoid liposarcoma (402/91 and 1955/91) cell lines, hybridized at high stringency with a CHOP probe. Where indicated, the normal CHOP gene was induced by treatment of the cultured cells with the DNA-damaging agent methylmethanesulphonate (MMS). Position of the normal CHOP transcript and abnormal TLS-CHOP transcript is indicated, as are the 28S ribosomal bands that react weakly with the CHOP probe. Lanes 1–8 contain total cellular RNA and lane 9 the poly(A)$^+$ fraction. Longer exposure shows the induction of CHOP by MMS also in the 1955/91 cell line. *b*, C/EBPβ 'zipper blot' of proteins immune precipitated from cellular extracts with a CHOP-specific antiserum. Preimmune serum was used as control in lane 8. Whole-cell extracts were used in lanes 1–8, lane 9 contains cytosol and lane 10 nuclear extract. The position of normal (CHOP) and abnormal (TLS-CHOP) proteins is indicated, as are the molecular mass markers. The asterisks to the left of the autoradiogram marks the superposition of a nonspecific zipper-reactive protein and proteins that specifically coprecipitate with TLS-CHOP in the myxoid liposarcoma cells.
METHODS. *a*, Freshly fed cultured cells (1955/91 and 402/91 are MLPS cell lines established by immortalizing cells from two different tumours with SV40 large-T antigen) were treated for 4 h with MMS (100μg ml^{-1}). RNA preparation and northern blot hybridization with the CHOP probe were as previously described[5]. *b*, Cellular proteins were precipitated with a CHOP-specific antibody, resolved by 10% SDS–PAGE, transferred to a nitrocellulose filter and the filter was reacted with a 'zipper-probe' consisting of the bacterially expressed ^{32}P-labelled dimerization domain of C/EBPβ[5].

a

TLS-CHOP

```
   1  ATGCTCAGTCCTCCAGGCGTCGGTGCTCAGCGGTGTTGGAACTTCGTTGCTTGCTTGCCT

  61  GTGCGCGCGTGCGCGGACATGGCCTCAAACGATTATACCCAACAAGCAACCCAAAGCTAT
                    M  A  S  N  D  Y  T  Q  Q  A  T  Q  S  Y

 121  GGGGCCTACCCCACCCAGCCCGGGCAGGGCTATTCCCAGCAGAGCAGTCAGCCCTACGGA
  15  G  A  Y  P  T  Q  P  G  Q  G  Y  S  Q  Q  S  S  Q  P  Y  G

 181  CAGCAGAGTTACAGTGGTTATAGCCAGTCCACGGACACTTCAGGCTATGGCCAGAGCAGC
  35  Q  Q  S  Y  S  G  Y  S  Q  S  T  D  T  S  G  Y  G  Q  S  S

 241  TATTCTTCTTATGGCCAGAGCCAGAACACAGGCTATGGAACTCAGTCAACTCCCCAGGGA
  55  Y  S  S  Y  G  Q  S  Q  N  T  G  Y  G  T  Q  S  T  P  Q  G

 301  TATGGCTCGACTGGCGGCTATGGCAGTAGCCAGAGCTCCCAATCGTCTTACGGGCAGCAG
  75  Y  G  S  T  G  G  Y  G  S  S  Q  S  S  Q  S  S  Y  G  Q  Q

 361  TCCTCCTACCCTGGCTATGGCCAGCAGCCAGCTCCCAGCAGCACCTCGGGAAGTTACGGT
  95  S  S  Y  P  G  Y  G  Q  Q  P  A  P  S  S  T  S  G  S  Y  G

 421  AGCAGTTCTCAGAGCAGCAGCTATGGGCAGCCCCAGAGTGGGAGCTACAGCCAGCAGCCT
 115  S  S  S  Q  S  S  S  Y  G  Q  P  Q  S  G  S  Y  S  Q  Q  P

 481  AGCTATGGTGGACAGCAGCAAAGCTATGGACAGCAGCAAAGCTATAATCCCCCTCAGGGC
 135  S  Y  G  G  Q  Q  Q  S  Y  G  Q  Q  Q  S  Y  N  P  P  Q  G

 541  TATGGACAGCAGAACCAGTACAACAGCAGCAGTGGTGGTGGAGGTGGAGGTGGAGGTGGA
 155  Y  G  Q  Q  N  Q  Y  N  S  S  S  G  G  G  G  G  G  G  G  G

 601  GGTAACTATGGCCAAGATCAATCCTCCATGAGTAGTGGTGGTGGCAGTGGTGGCGGTTAT
 175  G  N  Y  G  Q  D  Q  S  S  M  S  S  G  G  G  S  G  G  G  Y

 661  GGCAATCAAGACCAGAGTGGTGGAGGTGGCAGCGGTGGCTATGGACAGCAGGACCGTGGA
 195  G  N  Q  D  Q  S  G  G  G  G  S  G  G  Y  G  Q  Q  D  R  G

 721  GGCCGCGGCAGGGGTGGCAGTGGTGGCGGCGGCGGCGGCGGCGGTGGTGGTTACAACCGC
 215  G  R  G  R  G  G  S  G  G  G  G  G  G  G  G  G  Y  N  R

 781  AGCAGTGGTGGCTATGAACCCAGAGGTCGTGGAGGTGGCCGTGGAGGCAGAGGTGGCATG
 235  S  S  G  G  Y  E  P  R  G  R  G  G  G  R  G  G  R  G  G  M
                                <-- TLS ┬ CHOP -->
 841  GGCGGAAGTGACCGTGGTGGCTTCAATAAATTTGGTGTGTTCAAGAAGGAAGTGTATCTT
 255  G  G  S  D  R  G  G  F  N  K  F  G  V  F  K  K  E  V  Y  L

 901  CATACATCACCACACCTGAAAGCAGATGTGCTTTTCCAGACTGATCCAACTGCAGAGATG
 275  H  T  S  P  H  L  K  A  D  V  L  F  Q  T  D  P  T  A  E  M

 961  GCAGCTGAGTCATTGCCTTTCTCCTTCGGGACACTGTCCAGCTGGGAGCTGGAAGCCTGG
 295  A  A  E  S  L  P  F  S  F  G  T  L  S  S  W  E  L  E  A  W

1021  TATGAGGACCTGCAAGAGGTCCTGTCTTCAGATGAAAATGGGGGTACCTATGTTTCACCT
 315  Y  E  D  L  Q  E  V  L  S  S  D  E  N  G  G  T  Y  V  S  P

1081  CCTGGAAATGAAGAGGAAGAATCAAAAATCTTCACCACTCTTGACCCTGCTTCTCTGGCT
 335  P  G  N  E  E  E  E  S  K  I  F  T  T  L  D  P  A  S  L  A

1141  TGGCTGACTGAGGAGGAGCCAGAACCAGCAGAGGTCACAAGCACCTCCCAGAGCCCTCAC
 355  W  L  T  E  E  E  P  E  P  A  E  V  T  S  T  S  Q  S  P  H

1201  TCTCCAGATTCCAGTCAGAGCTCCCTGGCTCAGGAGGAAGAGGAGGAAGACCAAGGGAGA
 375  S  P  D  S  S  Q  S  S  L  A  Q  E  E  E  E  E  D  Q  G  R

1261  ACCAGGAAACGGAAACAGAGTGGTCATTCCCCAGCCCGGGCTGGAAAGCAGCGCATGAAG
 395  T  R  K  R  K  Q  S  G  H  S  P  A  R  A  G  K  Q  R  M  K

1321  GAGAAAGAACAGGAGAATGAAAGGAAAGTGGCACAGCTAGCTGAAGAGAATGAACGGCTC
 415  E  K  E  Q  E  N  E  R  K  V  A  Q  L  A  E  E  N  E  R  L

1381  AAGCAGGAAATCGAGCGCCTGACCAGGGAAGTAGAGGCGACTCGCCGAGCTCTGATTGAC
 435  K  Q  E  I  E  R  L  T  R  E  V  E  A  T  R  R  A  L  I  D

1441  CGAATGGTGAATCTGCACCAAGCATGAACAATTGGGAGCATCAGTCCCCCACTTGGGCCA
 455  R  M  V  N  L  H  Q  A  *  462

1501  CACTACCCACCTTTCCCAGAAGTGGCTACTGACTACCCTCTCACTAGTGCCAATGATGTG

1561  ACCCTCAATCCCACATACGCAGGGGGAAGGCTTGGAGTAGACAAAAGGAAAGGTCTCAGC

1621  TTGTATATAGAGATTGTACATTTATTTATTACTGTCCCTATCTATTAAAGTGACTTTCTA

1681  TG(A)n  1682
```

b

TLS, COOH-terminus

```
                                (<--TLS | CHOP -->)
                                      GCCCTCGGGACCAAGGATCACGT
 266                                G  P  R  D  Q  G  S  R

 901  CATGACTCCGAACAGGATAATTCAGACAACAACACCATCTTTGTGCAAGGCCTGGGTGAG
 275  H  D  S  E  Q  D  N  S  D  N  N  T  I  F  V  Q  G  L  G  E

 961  AATGTTACAATTGAGTCTGTGGCTGATTACTTCAAGCAGATTGGTATTATTAAGACAAAC
 295  N  V  T  I  E  S  V  A  D  Y  F  K  Q  I  G  I  I  K  T  N

1021  AAGAAAACGGGACAGCCCATGATTAATTTGTACACAGACAGGGAAACTGGCAAGCTGAAG
 315  K  K  T  G  Q  P  M  I  N  L  Y  T  D  R  E  T  G  K  L  K

1081  GGAGAGGCAACGGTCTCTTTTGATGACCCACCTTCAGCTAAAGCAGCTATTGACTGGTTT
 335  G  E  A  T  V  S  F  D  D  P  P  S  A  K  A  A  I  D  W  F

1141  GATGGTAAAGAATTCTCCGGAAATCCTATCAAGGTCTCATTTGCTACTCGCCGGGCAGAC
 355  D  G  K  E  F  S  G  N  P  I  K  V  S  F  A  T  R  R  A  D

1201  TTTAATCGGGGTGGTGGCAATGGTCGTGGAGGCCGAGGGCGAGGAGGACCCATGGGCCGT
 375  F  N  R  G  G  G  N  G  R  G  G  R  G  R  G  G  P  M  G  R

1261  GGAGGCTATGGAGGTGGTGGCAGTGGTGGTGGTGGCCGAGGAGGATTTCCCAGTGGAGGT
 395  G  G  Y  G  G  G  G  S  G  G  G  G  R  G  G  F  P  S  G  G

1321  GGTGGCGGTGGAGGACAGCAGCGAGCTGGTGACTGGAAGTGTCCTAATCCCACCTGTGAG
 415  G  G  G  G  Q  Q  R  A  G  D  W  K  C  P  N  P  T  C  E

1381  AATATGAACTTCTCTTGGAGGAATGAATGCAACCAGTGTAAGGCCCCTAAACCAGATGGC
 435  N  M  N  F  S  W  R  N  E  C  N  Q  C  K  A  P  K  P  D  G

1441  CCAGGAGGGGGACCAGGTGGCTCTCACATGGGGGGTAACTACGGGGATGATCGTCGTGGT
 455  P  G  G  G  P  G  G  S  H  M  G  G  N  Y  G  D  D  R  R  G

1501  GGCAGAGGAGGCTATGATCGAGGCGGCTACCGGGGCCGCGGCGGGGACCGTGGAGGCTTC
 475  G  R  G  G  Y  D  R  G  G  Y  R  G  R  G  G  D  R  G  G  F

1561  CGAGGGGGCCGGGGTGGTGGGGACAGAGGTGGCTTTGGCCCTGGCAAGATGGATTCCAGG
 495  R  G  G  R  G  G  G  D  R  G  G  F  G  P  G  K  M  D  S  R

1621  GGTGAGCACAGACAGGATCGCAGGGAGAGGCCGTATTAATTAGCCTGGCTCCCCAGGTTC
 515  G  E  H  R  Q  D  R  R  E  R  P  Y  *  526

1681  TGGAACAGCTTTTTGTCCTGTACCCAGTGTTACCCTCGTTATTTTGTAACCTTCCAATTC

1741  CTGATCACCCAAGGGTTTTTTTTGTGTCGGACTATGTAATTGTAACTATACCTCTGGTTC

1801  CCATTAAAAGTGACCATTTTAGTT(A)n  1824
```

FIG. 2 Nucleotide and predicted amino-acid sequence of TLS-CHOP and TLS. *a*, Sequence of the full-length TLS-CHOP cDNA isolated from the myxoid liposarcoma library. The junction between TLS and CHOP is indicated. The initiator methionine, normally used in the translation of CHOP, is in bold. *b*, The normal TLS sequence from the junction point with CHOP. The Arg-Gly-Gly tripeptide repeats in the C terminus of TLS are underlined. The numbering is continuous with that of TLS-CHOP,

METHODS. An unamplified λ-Zap (Stratagene) cDNA library constructed using mRNA from the myxoid liposarcoma cell line 1955/91 was hybridized to the murine CHOP cDNA. Over 40 independent clones encoding TLS-CHOP were isolated. The TLS portion of the TLS-CHOP cDNA was used as a probe to isolate clones encoding the normal TLS protein. Rapid amplification of cDNA ends (RACE[15]), with primers specific for TLS and TLS-CHOP was used to isolate the 5′ ends of both transcripts and demonstrated that they are identical. The TLS-CHOP clone was shown to contain the full-length coding region by demonstrating that the cDNA could direct the expression, in COS-1 cells, of a protein indistinguishable in size from tumour TLS-CHOP (Fig. 3*a*). A variant form of TLS-CHOP, differing from the sequence shown here in 15 N-terminal residues was also isolated from the 1955/91 library. But this cDNA, when expressed in COS-1 cells, gave rise to a protein different in size from that found in the tumour cells, suggesting that it is a minor variant.

CHOP-reactive proteins from extracts of MLPS cells with a CHOP-specific antiserum and analysed them with the labelled dimerization domain of C/EBPβ in a zipper blot assay[5]. The zipper probe readily recognizes the MMS-induced normal CHOP protein (29K) in both MLPS and control cells (Fig. 1*b*, lanes 2, 4 and 6). The MLPS cells contain, in addition to CHOP, a constitutive, larger protein (75K), that is immunoprecipitated by the CHOP antiserum and also reacts with the zipper probe (lanes 5–9). The 75K protein is nuclear (lane 10) and associates with other proteins (40–45K) that are also recognized by the C/EBPβ zipper probe. This co-precipitating activity consists predominantly of C/EBPβ which is the major dimerisation partner of CHOP (ref. 5, and data not shown). In conjunction with the gene mapping information, localizing the breakpoints in *CHOP* to the 5′ region of the gene[3] these data indicate that MLPS cells contain a fusion protein, the C terminus of which is encloded by *CHOP* and includes its leucine zipper dimerization domain. The tumour-specific N-terminal extension is derived from another gene, provisionally named *TLS*.

We isolated clones encoding TLS-CHOP from an MLPS cDNA library. The 3′ ends of all clones contained human CHOP sequence; however, upstream of the normal junction between CHOP exons 1 and 2, the sequence abruptly diverged from CHOP, confirming the presence of a tumour-specific fusion transcript (Fig. 2*a*). Reverse transcription of MLPS tumour RNA and PCR amplification of the junction between TLS and

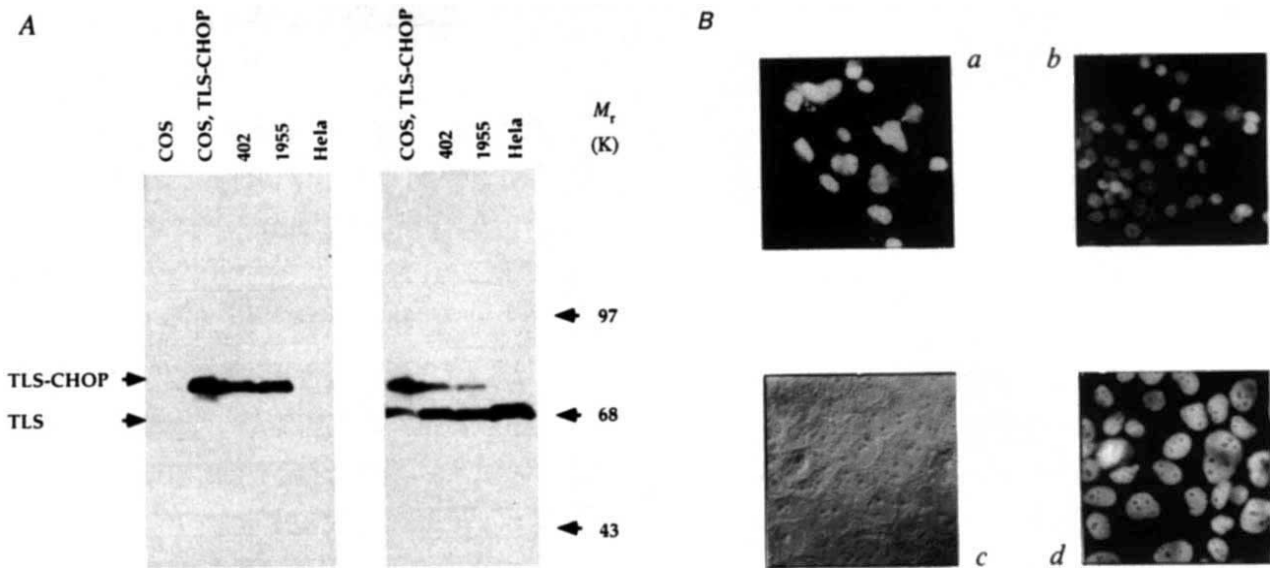


FIG. 3 TLS-CHOP and TLS are nuclear proteins. *A*, Western immunoblot of nuclear extracts from nontransfected COS-1 cells, COS-1 cells expressing the cloned TLS-CHOP, the myxoid liposarcoma cell lines 402/91 and 1955/91 and HeLa cells, probed with CHOP (left panel) and TLS (right panel) antisera. Both antisera recognize a 75K protein in the nuclear extracts of TLS-CHOP-containing cells. The TLS antiserum also recognizes a 68K protein, present in all cell lines. The position of the two proteins is indicated. The relatively faster migration on SDS–PAGE of the larger TLS protein, compared with the smaller TLS-CHOP is compatible with the previously recognized anomalous slow migration of CHOP[5]. *B*, Immunocytochemical localization of CHOP and TLS-CHOP. CHOP and TLS antisera stain the nucleus of 402/91 MLPS cells (*a* and *b*, respectively). In JEG-3 human choriocarcinoma cells that contain TLS but not TLS-CHOP, the TLS antiserum stains the nucleus and spares the nucleoli, which appear as dark holes (*c*, Nomarski image and *d*, anti-TLS immune fluorescence). JEG-3 cells were chosen because of the good definition of nuclear morphology.

METHODS. A full-length expression plasmid for TLS-CHOP constructed in pCDNA-1 was transfected into COS-1 cells. Nuclear extracts and western immunoblots were as previously described[5]. Antisera were raised in rabbit against a full-length bacterially expressed CHOP-GST (glutathione *S*-transferase) fusion protein and a similar fusion protein containing the N-terminal portion of TLS (amino acids 78–244). The anti-CHOP antiserum and anti-TLS antiserum were used at a dilution of 1:1,000 as previously described[5]. Cells are shown at a magnification X400.

CHOP demonstrated the presence of a TLS-CHOP fusion transcript in all three cell lines and seven MLPS tumours tested and in none of the other liposarcomas tested (data not shown). The full-length fusion TLS-CHOP cDNA is similar in size to the abnormal CHOP transcript observed in the MLPS cells (Fig. 1*a*). It contains an uninterrupted open reading frame of 462 amino acids. The protein encoded by the fusion transcript consists of an N-terminal portion contributed by *TLS* and the full-length *CHOP* open reading frame in the C terminus. Linking them is a segment, translated only in the fusion mRNA, of the normal CHOP 5′ untranslated region, between the beginning of CHOP exon 2 and its normal initiator methionine.

The cDNA library was rescreened for the full-length TLS cDNA. Several clones were isolated, the largest of which (Fig. 2*b*) is similar in size to the 1.9–2.0 kilobase (kb) mRNA present in variable amounts in all cell lines and tissues examined (data not shown) and contains an uninterrupted open reading frame of 526 amino acids. Antiserum against bacterially expressed TLS was used to probe western immunoblots containing nuclear extracts from MLPS cells. Both the CHOP antiserum and the TLS antiserum react with an indistinguishable 75K protein that is present in two different MLPS cell lines (Fig. 3*A*). In addition to the 75K TLS-CHOP the TLS antiserum also recognizes the normal *TLS* gene product, a 68K protein present in all cell lines tested. Immune fluorescent microscopy demonstrated that both TLS-CHOP, in MLPS cells and TLS, in normal cells, are nuclear proteins (Fig. 3*B*), as has been previously shown for CHOP[5].

TLS has extensive sequence similarity (55.6% identity; Fig. 4*a*) to the recently described *EWS* gene, involved in a reciprocal balanced translocation, t(11;22)(q24;q12), that occurs in Ewing's sarcoma and other primitive neuro-ectodermal tumours[7]. In Ewing's sarcoma the N terminus of EWS is fused to the DNA-binding domain of FLI-1, a member of the ETS family of transcription factors. The N terminus of TLS and EWS is extremely rich in the amino acids glutamine, threonine, serine, proline, tyrosine and glycine and contains multiple copies of the repeated hexapeptide: Ser/Gly-Tyr-Ser/Gly-Gln-Gln/Ser-Ser/Gln/Pro. In the C terminus, absent in the tumour-specific fusion proteins, both TLS and EWS contain a region of similarity to a conserved 80 amino-acid domain, the RNP-CS (ref. 8), found in several RNA-binding proteins, from species as distant as hamster and yeast (Fig. 4*b*). TLS and EWS both contain similar substitutions (Phe→Asp/Glu and Gly→Ala) in the highly conserved RNP-1 box and have an unusually large predicted loop structure between the α-helix-1 and β-sheet-2 of the RNP-CS. TLS and EWS share sequence similarity (52% identity) with the *Drosophila* protein Dpen p19 (ref. 9) and all three proteins appear to be members of a new sub-class of RNP-CS-containing proteins. In addition to the highly conserved RNP-CS motif in the C-terminus, both TLS and EWS contains multiple repeats of the Arg-Gly-Gly tripeptide, a sequence motif that has been implicated in RNA binding[10].

Bacterially expressed full-length TLS and its C terminus both bind labelled RNA in a 'northwestern' ligand blot assay (Fig. 4*c*). Binding was enriched in the poly $(A)^+$ fraction of the RNA, suggesting that the targets for TLS are in the messenger RNA fraction. The localization of TLS to nuclear regions outside the nucleolus (Fig. 3*B, d*) is also consistent with TLS binding a non-ribosomal RNA, perhaps to products of RNA polymerase II. Binding to poly$(A)^+$ RNA may, however, simply reflect an increased affinity for homo-polymeric tracts of single-stranded nucleic acids. RNA binding by TLS maps to its region of similarity with other RNA-binding proteins. But the C-terminal fragment of TLS that retains RNA-binding activity is missing

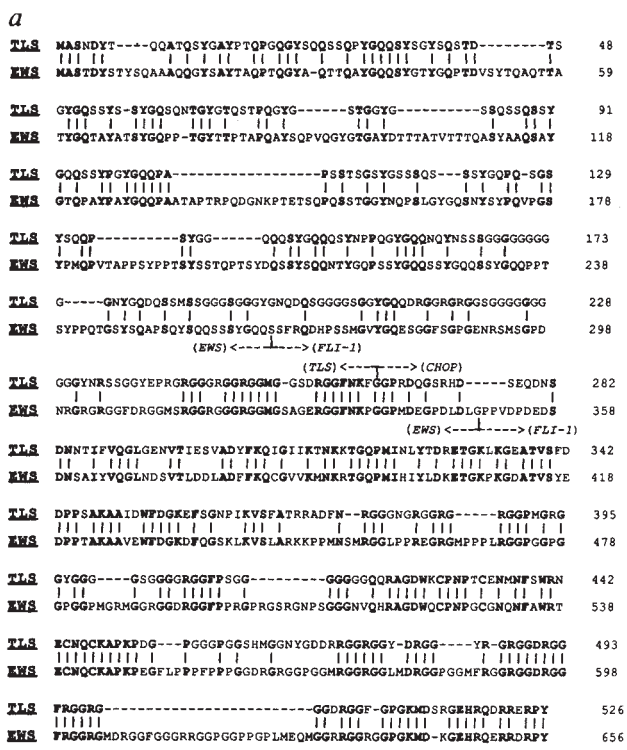
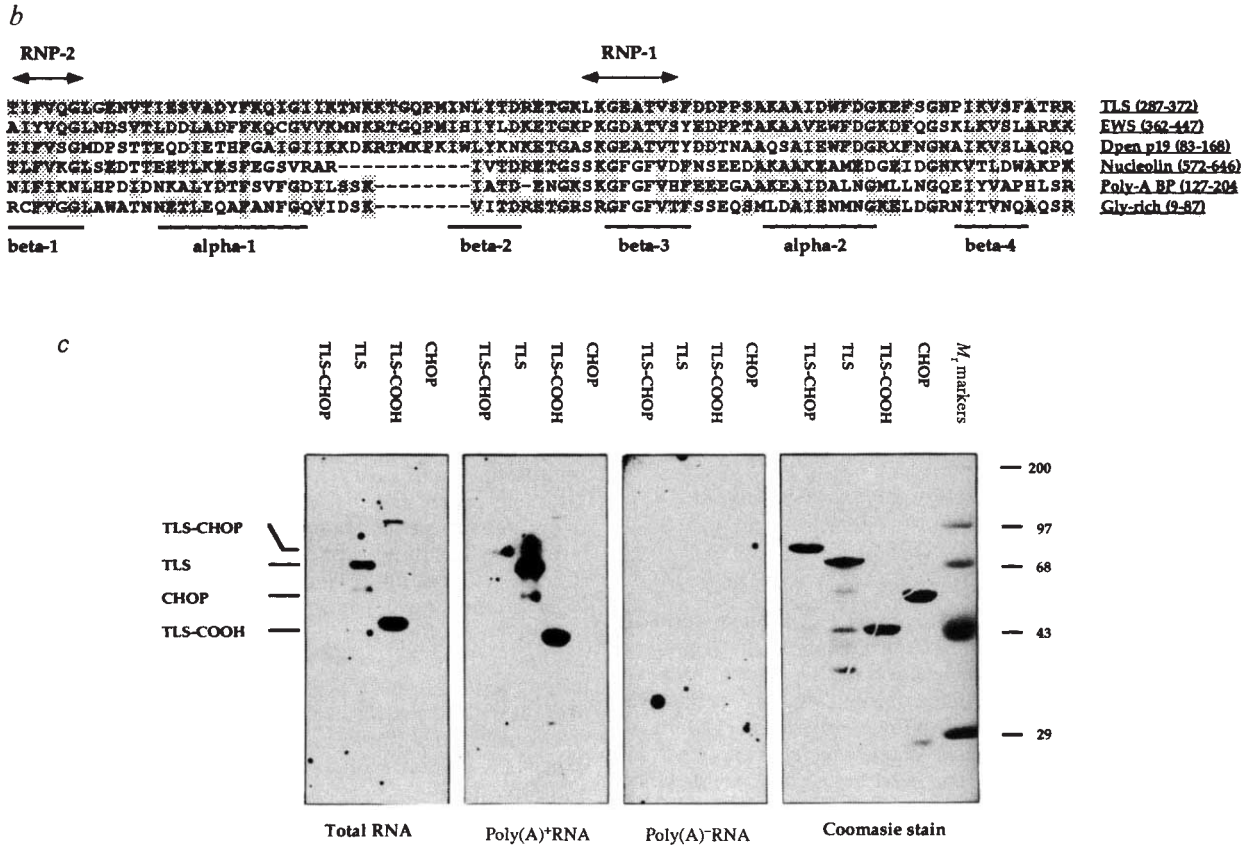


FIG. 4 *a*, Alignment of the amino-acid sequence of TLS and EWS. Vertical bars indicate identical residues. The position of the tumour-specific fusions with CHOP and FLI-1 are indicated. *b*, Alignment of the amino-acid sequences of TLS and other RNA-binding proteins in the region corresponding to the conserved RNP-CS domain[8] in the human *EWS* gene product[7], *Drosophila melanogaster* Dpen p19[9], hamster nucleolin[16], poly(A)$^+$ binding protein from *Saccharomyces cerevisiae*[17] and glycine-rich protein from sorghum[18]. The subdomains of the RNP-CS are indicated[8]. *c*, Left panel: northwestern blot of bacterially expressed proteins probed with either native, total cellular RNA, end labelled with ^{32}P or the similarly labelled poly(A)$^+$ or poly(A)$^-$ fractions. Right panel: photograph of the Coomassie-stained proteins. Arrows on the left and right point to the location of the proteins and molecular mass markers, respectively.

METHODS. TLS-CHOP and TLS were expressed in *Escherichia coli* fusion proteins with poly-histidine tag in the N terminus and purified by nickel-chelate chromatography. 'TLS-COOH' is a GST fusion protein contgaining amino acids 294–526 of TLS and 'CHOP' is a GST fusion containing the full-length murine CHOP sequence[5]. The purified proteins were resolved by 8% SDS–PAGE and transferred to nitrocellulose. The membrane was blocked for 1 h in 20mM Tris–HCl, pH 7.9, 50 mM NaCl, 0.2mM EDTA, 1mM DTT, 1mM $MgCl_2$ containing 1% BSA and 20 μg ml^{-1} yeast transfer RNA, and reacted, in the same buffer, with ~0.4μg ml^{-1} RNA that had been dephosphorylated with calf intestinal alkaline phosphatase and end-labelled with [^{32}P]γ–ATP, using T4 kinase (left panel). To demonstrate expression of all proteins an identical gel was stained with Coomassie blue (right panel).



several amino acids of the conserved RNP-2 portion of RNP-CS motif. It therefore appears likely that RNA-binding, measured in the northwestern blot assay, reflects also the activity of the dense cluster of Arg-Gly-Gly repeats in the C terminus of the molecule, perhaps working in conjunction with the RNP-CS motif. The presence of several similar tripeptide repeats in the N-terminal portion of TLS may account for the weak RNA-binding activity of TLS-CHOP (visible on longer exposure of the blot shown in Fig. 4*c*).

In TLS-CHOP the RNA-binding portion of TLS is replaced by CHOP DNA-binding sequence. *CHOP* is transcriptionally silent in continuously dividing cells[4], it is induced, however, in most cells in response to DNA-damaging agents[4] and in fibroblasts during their differentiation *in vitro* to adipocytes[5].

The coincidental induction of CHOP in a variety of situations associated with growth arrest suggests a role for the protein in control of cellular proliferation. The portion of TLS that is present in the TLS-CHOP fusion protein contains a glutamine-rich region similar to that found in transcriptional activation domains of regulatory proteins such as SP-1 (ref. 11) and a glycine-rich region, similar to that found in other RNA-binding proteins[12]. We speculate that the portion of TLS that is present in TLS-CHOP serves an important effector function in the normal RNA-bound TLS, perhaps playing a role in transcriptional regulation. In TLS-CHOP, addition of this portion of TLS to the full-length CHOP protein may serve to convert a transcription factor involved in growth arrest to one associated with abnormal cellular proliferation. Inappropriate targeting of this domain of TLS to regulatory elements of specific genes by the DNA-binding and dimerization domain of CHOP may be important in the formation of myxoid liposarcoma. The regulation of adipocyte-specific genes by C/EBP proteins[13] and the association of CHOP with both adipogenesis[5] and the response to nutritional alterations in pre-adipocytes[14], suggests that TLS-CHOP may regulate genes involved in adipose tissue-specific tumorigenesis. Fusion of the homologous N terminus of EWS to the DNA-binding domain of FLI-1 (ref. 7) suggests a conservation of function in the transforming activity present in both tumours. Ewing's sarcoma and MLPS are the first human solid tumours containing well defined chromosomal translocations in which the involved genes have been identified. The discovery of structurally related proteins in both cases suggests that juxtaposition of an effector domain from this new sub-class of RNA-binding proteins with the DNA-binding domain of other transcription factors may represent a novel molecular mechanism for tumour formation common to several solid tumours. □

Received 6 January; accepted 5 April 1993.

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ACKNOWLEDGEMENTS. This work was supported by a grant from the Arthritis Foundation (D.R.) and the Swedish Cancer Society (P.A and N.M.).

A TCP1-related molecular chaperone from plants refolds phytochrome to its photoreversible form

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FOLDING of the major cytoskeletal components in the cytosol of mammalian cells is mediated by interactions with t-complex polypeptide-1 (TCP1) molecular chaperones[1–6], a situation analogous to the chaperonin 60-aided folding of polypeptides in bacteria[7,8], chloroplasts[9,10] and mitochondria[11–13]. We have purified a TCP1-related molecular chaperone from etiolated oat seedlings that has a unique structure. Although immunologically related to TCP1, and having amino-acid sequence similarity, its quaternary structure is different from animal TCP1 proteins[5,6,14]. Electron microscopy and image analysis reveals that the chaperone has two stacked rings of six subunits each, and is distinct in size and configuration. The chaperone copurifies with the soluble cytosolic photoreceptor phytochrome[15], and can stimulate refolding of denatured phytochrome to a photoactive form in the presence of Mg–ATP. We propose that this protein is the cytosolic chaperone involved in phytochrome biogenesis in plant cells.

Phytochrome is a cytosolic plant photoreceptor that exists in two forms that are interconvertible by light[15]. The physiologically inactive P_r form (λ_{max} 666 nm) can be transformed to the functional P_{fr} form (λ_{max} 730 nm) by red light, resulting in the specific activation and repression of many light-dependent genes, and concomitant morphogenesis[15,16]. During the isolation of phytochrome from etiolated oat (*Avena sativa*) seedlings, an oligomeric protein containing 60K polypeptides copurified with the photoreceptor (Fig. 1*a*). This polypeptide has limited crossreaction with antisera raised against pea (*Pisum sativum*) chloroplast chaperonin 60 (cpn60) (Fig. 1*b*), and microsequencing gave the amino-terminal sequence Ala-Leu-Glu-Ser, which has some homology to the chloroplast cpn60 α-subunit of *Ricinus communis*[9].

We purified the protein to homogeneity as judged by silver staining of sodium dodecyl sulphate (SDS) gels (Fig. 1*c*). Nondenaturating gel electrophoresis of the purified protein revealed a predominant oligomer of about 600K, and traces of a second protein of about 800K (Fig. 1*d*). Immunoblotting of non-denaturating gels revealed an interaction of the minor 800K protein with antisera raised against *Escherichia coli* GroEL or chloroplast cpn60, but only a weak interaction of the predominant 600K protein with anti-chloroplast cpn60, and none with anti-GroEL (Fig. 1*e*, *f*). In contrast, if the immunoblots were prepared using monoclonal antibodies raised against mouse TCP1, the major 600K protein crossreacted strongly, but the minor 800K species and the bacterial GroEL marker did not interact (Fig. 1*g*). There were similar patterns of cross reactivity with various antisera when the protein bands were excised from nondenaturating gels, resolved on SDS gels and immunoblotted (data not shown). The 600K protein is, therefore, immunologically related to TCP1. The 800K protein probably originates from contaminating mitochondria or plastids. To confirm this, chloropast, etioplast and mitochondrial fractions were prepared

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