The EMBO Journal vol.10 no.4 pp.757 - 766, 1991

Mbh1: a novel gelsolin/severin-related protein which binds actin *in vitro* and exhibits nuclear localization *in vivo*

George C.Prendergast and Edward B.Ziff

Howard Hughes Medical Institute, Department of Biochemistry and Kaplan Cancer Center, New York University Medical Center, 550 First Avenue, New York, NY 10016, USA

Communicated by C.H.Chothia

We describe the characterization of a novel cDNA, *mbh1* (*m*yc *b*asic motif *h*omolog-*1*), which was found during a search for candidate factors which might interact with the c-Myc oncoprotein. Embedded within the amino acid sequence encoded by *mbh1* is a region distantly related to the basic/helix−loop−helix (B/HLH) DNA-binding motif and a potential nuclear localization signal. Mbh1 encodes a polypeptide structurally similar to the actin-severing proteins gelsolin and severin. Translation of *mbh1* RNA in rabbit reticulocyte extracts produces an ~45 kd protein capable of binding actin-coupled agarose beads *in vitro* in a Ca^{2+}-dependent manner. Antiserum raised to a *trpE/mbh1* bacterial fusion protein recognizes an ~45 kb protein in murine 3T3 fibroblasts, suggesting that the cDNA encodes the complete Mbh1 protein. Examination of Mbh1 localization in 3T3 fibroblasts by indirect immunofluorescence reveals a larger cell population showing diffuse staining, and a smaller population exhibiting a distinct nuclear stain. Western analysis corroborates this intracellular localization and indicates that total cellular levels and localization of Mbh1 are not affectd by the cell growth state. The data suggest that Mbh1 may play a role in regulating cytoplasmic and/or nuclear architecture through potential interactions with actin.

Key words: actin-severing/cDNA/HLH proteins/Mbh1

Introduction

Regulation of nuclear architecture has been postulated to play an important role in DNA replication, transcription and RNA processing (Agutter, 1985; Jackson and Cook, 1985, 1986; Lawrence *et al.*, 1989; Spector, 1990). Study of nuclear structures which may be involved in these cellular processes has been in many cases problematic. However, a possible role for nuclear actin (Clark and Merriam, 1977; Jockusch *et al.*; 1978 LeStourgeon, 1978) in regulating transcription in some cells has been suggested by reports that anti-actin antibodies inhibit the transcription of RNA polymerase II-transcribed genes in amphibian oocyte injection experiments (Scheer *et al.*, 1984), and that a factor which strongly stimulates transcription by RNA polymerase II is actin (Egly *et al.*, 1984).

We have been interested in the molecular function of the c-*myc* oncogene, which is currently undefined. The C-terminal region of the c-Myc protein contains two structural motifs, the helix−loop−helix (HLH; Murre *et al.*, 1989a) and the 'leucine zipper' (Landschultz *et al.*, 1987). Both motifs are found across regions predicted to form amphipathic α helix, and are proposed to mediate protein dimerization. Immediately N-terminal to the c-Myc HLH motif is the c-Myc basic motif (Prendergast and Ziff, 1989), which when functionally dimerized can specifically interact with DNA (Blackwell *et al.*, 1990; Kerkhoff *et al.*, 1991; Prendergast and Ziff, 1991). We wished to screen for candidate proteins that could interact with the c-Myc HLH or 'leucine zipper' motifs, and hypothesized that proteins which could do so might have basic motif structures similar to c-Myc for DNA binding. Therefore, to screen for such proteins, we amplified and cloned 3T3 fibroblast cDNAs generated by an anchored polymerase chain reaction (PCR), using a degenerate primer to the c-Myc basic motif. Sequence from one of the initial cDNAs obtained, *mbh1* (*m*yc *b*asic motif *h*omolog-*1*), shows a distant relationship to the basic/helix−loop−helix (B/HLH) family of DNA-binding proteins. We present here an initial characterization of *mbh1* and show that the protein it encodes is both structurally and functionally related to a family of actin-severing proteins. We also present immunological evidence that Mbh1 is localized in 3T3 cell nuclei, suggesting that its ability to bind actin *in vitro* may correlate with a function in the cell nucleus *in vivo*.

Results

The predicted Mbh1 amino acid sequence contains motifs related to the helix−loop−helix and to a nuclear localization signal

We wished to screen for proteins that might interact with c-Myc through binding via HLH or 'leucine zipper' motifs to form heterodimers, as do the c-Fos and c-Jun proteins (e.g. Kouzarides and Ziff, 1988). Such proteins would likely contact either an HLH or 'leucine zipper' like that of c-Myc. The c-Fos and c-Jun proteins show high amino acid sequence similarity across a region of the basic motif thought to be important for DNA binding (for example, see Prendergast and Ziff, 1989). Therefore, to screen for structures that might interact with c-Myc, a degenerate PCR primer (Figure 1a) was made to a region of the c-Myc basic motif analogous to that which showed high similarity in c-Fos and c-Jun. We used this primer for anchored PCR from NIH 3T3 poly(A)$^+$ RNA as described in Materials and methods. Several candidate cDNAs generated by PCR amplification were cloned and their DNA sequence determined. Because we were screening for proteins that might interact with the c-Myc HLH and/or 'leucine zipper' dimerization motifs, sequences immediately downstream of the basic motif primer sequence were visually inspected for open reading frames encoding these motifs. One of the initial cDNAs obtained harbored an open reading frame encoding a region related to the HLH motif (see below). This cDNA was subsequently

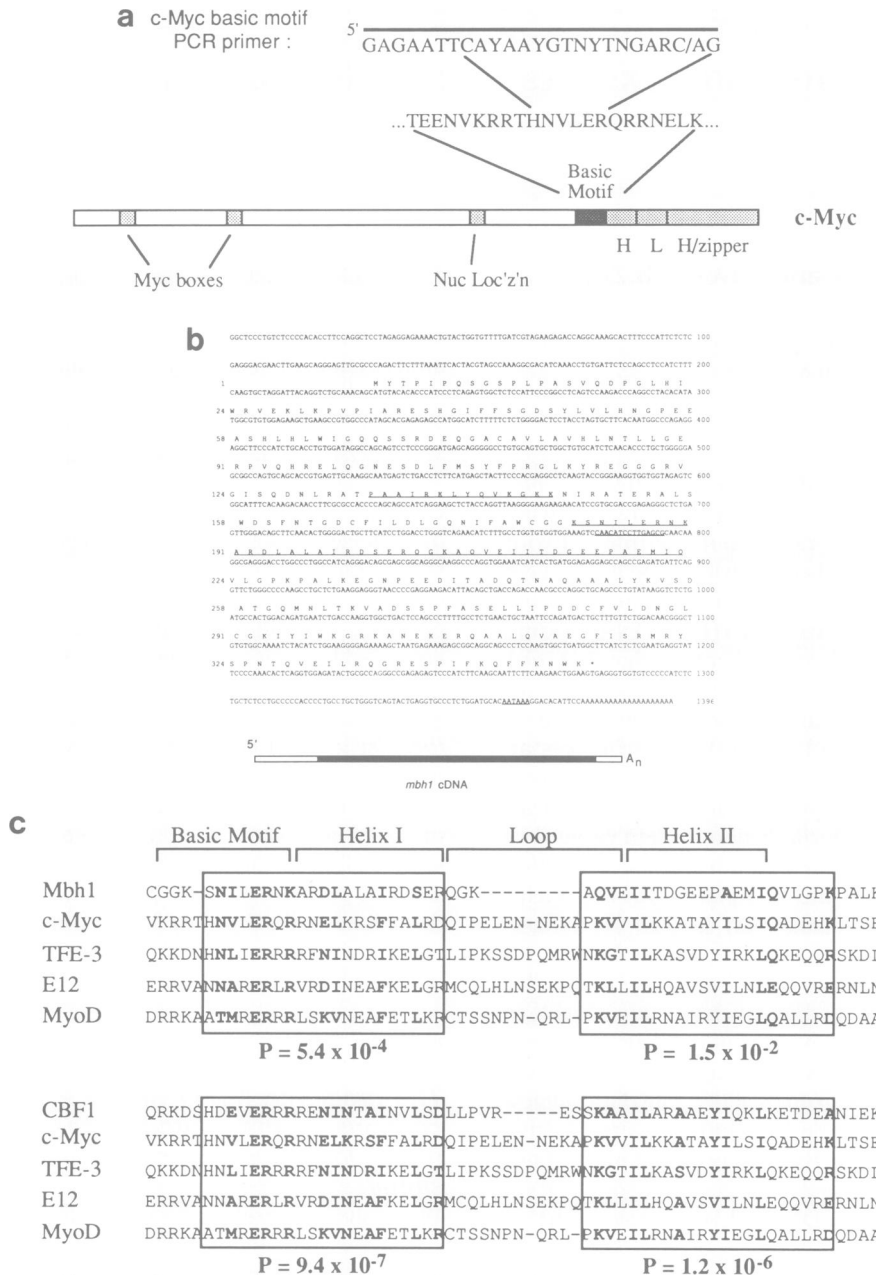

Fig. 1. Cloning of murine *mbh1* cDNA. (**a**) Degenerate c-Myc basic motif PCR primer used to amplify *mbh1* from NIH 3T3 poly(A)$^+$ RNA. (**b**) Complete cDNA and predicted amino acid sequence of pZ1.1/*mbh1* cDNA insert. A sequence similar to the PCR primer is underlined in the cDNA. Underlined in the predicted amino acid sequence is a potential nuclear localization signal (aa 134−147) and region related to the B/HLH motif (aa 182−223). (**c**) MACAW sequence alignments of Mbh1 with HLH proteins. Residues in bold were assigned relatively higher average score in alignment computations by MACAW. Amino acid sequences are as follows:. Mbh1 (aa 180−233), human c-Myc (aa 346−406; Battey *et al.*, 1983), TFE3 (aa 139−202; Beckmann *et al.*, 1990), E12 (aa 336−399; Murre *et al.*, 1989a), MyoD (aa 108−170; Davis *et al.*, 1987), CBF1 (aa 222−280; Cai and Davis, 1990).

used as a probe to isolate longer cDNAs from a λZAPII NIH 3T3 library by standard techniques. The complete DNA sequence of an ~1.4 kb clone, termed *mbh1*, is shown in Figure 1b. The *mbh1* sequence which was recognized by the c-Myc basic motif PCR primer in the amplification reaction is underlined. Shown above the *mbh1* cDNA sequence is the predicted amino acid sequence of a 349 aa open reading frame. In the sequence of the polypeptide, which we refer to below as Mbh1, the region related to the basic and HLH motifs (B/HLH; aa 182−223) and a potential nuclear localization signal (aa 134−147) are underlined (see below).

To assess the significance of the suggested similarity between Mbh1 and the HLH family, we used the Multiple Alignment and Construction Analysis Workbench (MACAW) program (Schuler *et al.*, 1990; see Materials and methods for a description). As a standard for relatedness, alignment of the yeast HLH protein CBF1 (Cai and Davis, 1990) with the same panel of HLH proteins produces significant results for basic motif/helix I ($P = 1.2 \times 10^{-6}$) and helix II ($P = 9.4 \times 10^{-7}$) regions (Figure 1c). Negative control alignments with randomly selected regions of Mbh1 all produced values $P = 1.0$ (data not shown), indicating that for $P < 1$, MACAW demands that all five

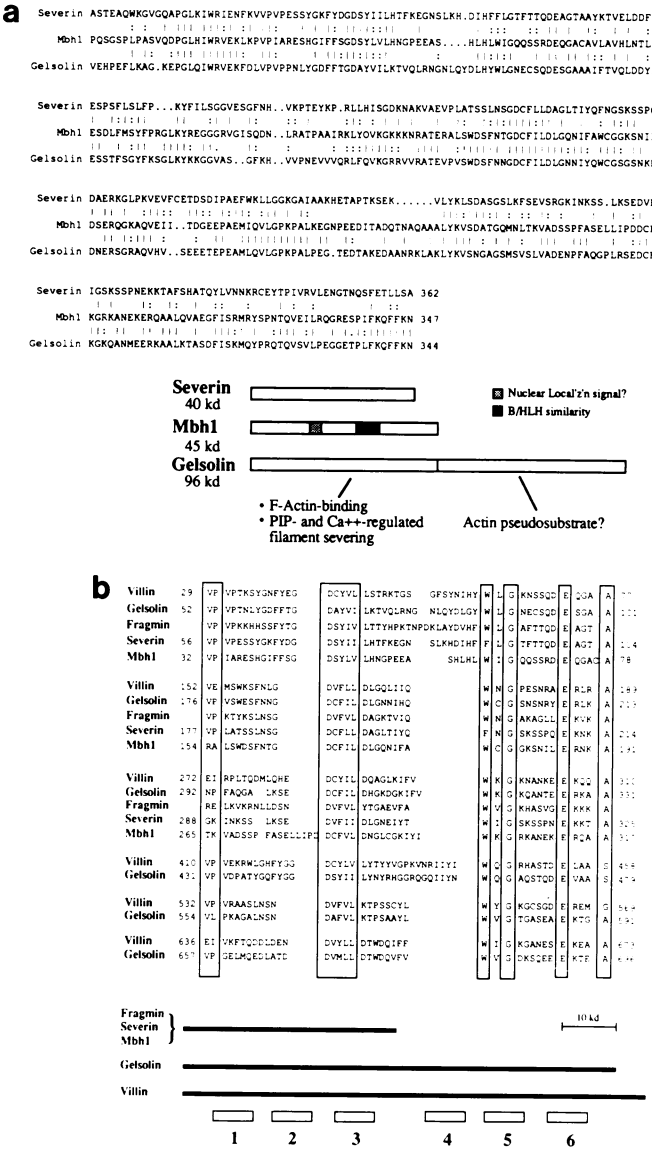

Fig. 2. Mbh1 sequence similarity with gelsolin and other actin-severing proteins. (**a**) Alignment of Mbh1 with murine gelsolin (Dieffenbach *et al.*, 1989) and *D.discoideum* severin (André *et al.*, 1988), using the BESTFIT algorithm in the Wisconsin sequence analysis programs (Devereaux *et al.*, 1984). Dashes indicate identities; colons show conservative substitutions. The potential nuclear localization signal and B/HLH-related motif are underlined in Mbh1. The identity of Mbh1 to the N-terminal region of gelsolin shown is 49.9%; with conservative substitutions, the overall similarity is 65.7%. The identity of Mbh1 to severin is 33.0%; with conservative substitutions, 49.2%. A graphical representation of the proteins is shown underneath; gelsolin characteristics are taken from Kwiatkowski *et al.* (1989). (**b**) Conservation of an ~50 aa repeat motif in Mbh1 and actin-severing proteins. Note that human gelsolin (Kwiatkowski *et al.*, 1986) and chicken villin (Bazari *et al.*, 1988) sequences are shown in this figure, which is adapted from Bazari *et al.* (1988).

regions in the alignment window show some relationship. Alignment of the proposed B/HLH region of Mbh1 with a panel of HLH proteins produces results significantly above random background ($P = 1.0$) for the basic/helix I ($P = 5.4 \times 10^{-4}$) and helix II ($P = 1.5 \times 10^{-2}$) regions (Figure 1c), but lower than the values for the B/HLH of CBF1. This suggests that the Mbh1 region of interest is related to the B/HLH motif, but less so than the analogous region of the HLH region CBF1. In addition to primary sequence similarity, a region containing a proposed HLH would be predicted to form two amphipathic α helices. Chou–Fasman and Garnier–Osguthorpe–Robson secondary structure calculations [carried out by the PEPTIDESTRUCTURE algorithm in the Wisconsin sequence analysis programs (Devereaux *et al.*, 1984)] predict a high degree of α-helical character across both proposed Mbh1 helices, despite the presence of a proline in the helix II (data not shown). Helical wheel analysis indicates that both helices have amphipathic character (data not shown). Thus is it reasonable to conclude that the aligned region of Mbh1 is distantly related to the B/HLH motif. Several caveats concerning this conclusion are considered below in the Discussion.

Visual inspection of the remaining Mbh1 sequence reveals a second region which is similar to a class of nuclear localization signals which contain helix-breaking amino acids followed by residues rich in arginine and lysine (Dang and Lee, 1989). Secondary structure predictions of this region of Mbh1 (carried out as before) are consistent with the formation of such a structure (data not shown). Along with

the presence of a B/HLH-related region, the presence of this motif suggests that Mbh1 might encode a nuclear protein.

Mbh1 is structurally related to gelsolin, severin and other actin-binding proteins

Comparison of Mbh1 to the sequences in the GenBank database revealed high similarity to gelsolin and severin (Figure 2a), cytoplasmic proteins which cap and sever actin cables (for reviews, see Stossel *et al.*, 1985; Pollard and Cooper, 1986; Matsudaira and Janmey, 1988). Alignment of Mbh1 with these proteins shows ~50% identity to the N-terminal half of gelsolin and ~33% identity to severin (Figure 2a). Gelsolin and severin belong to a large family of non-muscle actin-binding proteins, and are structurally related to the actin-severing proteins villin and fragmin (Ampe and Vandekerckhove, 1987; André *et al.*, 1988; Bazari *et al.*, 1988). Gelsolin (87 kd) and villin (95 kd) each contain a tandem array of sequences similar to severin (40 kd) and fragmin (42 kd).Partial proteolysis of gelsolin and villin generates two ~44 kd fragments, roughly the size of severin/fragmin monomers, supporting this structural prediction. Current models for the conformation of gelsolin in the absence of actin propose that an N-terminal actin-binding domain interacts with the COOH terminus (Way *et al.*, 1989), which may serve as a pseudosubstrate (Kwiatkowski *et al.*, 1989). That Mbh1 may be more closely related to severin and fragmin than to gelsolin is suggested by the similar sizes of the Mbh1 and severin polypeptides. A close sequence comparison of human gelsolin, chicken villin, severin and fragmin reveals the presence of a 35–50 aa unit, repeated six times in the larger proteins and three times in the smaller proteins (Bazari *et al.*, 1988; Matsudaira and Janmey, 1988; Way and Weeds, 1988). We observe that Mbh1 shares this structural organization (see Figure 2b; adapted from Bazari *et al.*, 1988). The B/HLH-related motif in Mbh1 appears at the end of the second repeat, in a region which shows somewhat less sequence relatedness to these proteins.

Expression and evolutionary conservation of mbh1 sequences

To determine whether the *mbh1* cDNA was full-length, we performed Northern analysis of total cytoplasmic NIH 3T3 RNA using the *mbh1* cDNA as a probe. We observed a single ~1.4 kb band (Figure 3a), suggesting that the *mbh1* cDNA encodes almost the entire message expressed in murine 3T3 cells. Because mRNAs which encode some B/HLH proteins are regulated by serum growth factors, we examined RNA from NIH 3T3 cells that were subconfluent, quiescent or serum-stimulated for changes in *mbh1* RNA abundance. No observable change in levels of the *mbh1* message was noted (data not shown). To obtain initial information concerning the extent of *mbh1* expression in the rodent, we examined total cellular RNA from a variety of rat tissues by Northern blotting (Figure 3b). When normalized to L32 RNA, which encodes a universally expressed large ribosomal subunit protein (Rajchel *et al.*, 1988), we observed low levels of *mbh1* RNA in lung, heart and kidney, and barely detectable levels in other tissues.

We also used the *mbh1* cDNA for high- and low-stringency Southern analysis using human, mouse, bovine, chicken and yeast genomic DNAs (Figure 3c). Under low stringency hybridization conditions (48°C/2 × SSC), we observed low copy number abundance bands in all genomic DNAs, including yeast. Under more stringent conditions (55°C/0.2 × SSC), only bovine sequences can be observed in addition to mouse. Taken together, the sequence similarity and hybridization data indicated that *mbh1* represents an evolutionarily conserved gene which may encode an actin-severing or actin-capping function.

Mbh1 displays actin-binding activity in vitro

The above data predicted that Mbh1 binds actin. To test this, we performed an *in vitro* binding experiment using F-actin-coupled Affigel–agarose beads and ^{35}S-labeled Mbh1 protein, produced by *in vitro* transcription/translation of the *mbh1* cDNA as described in Materials and methods. We also

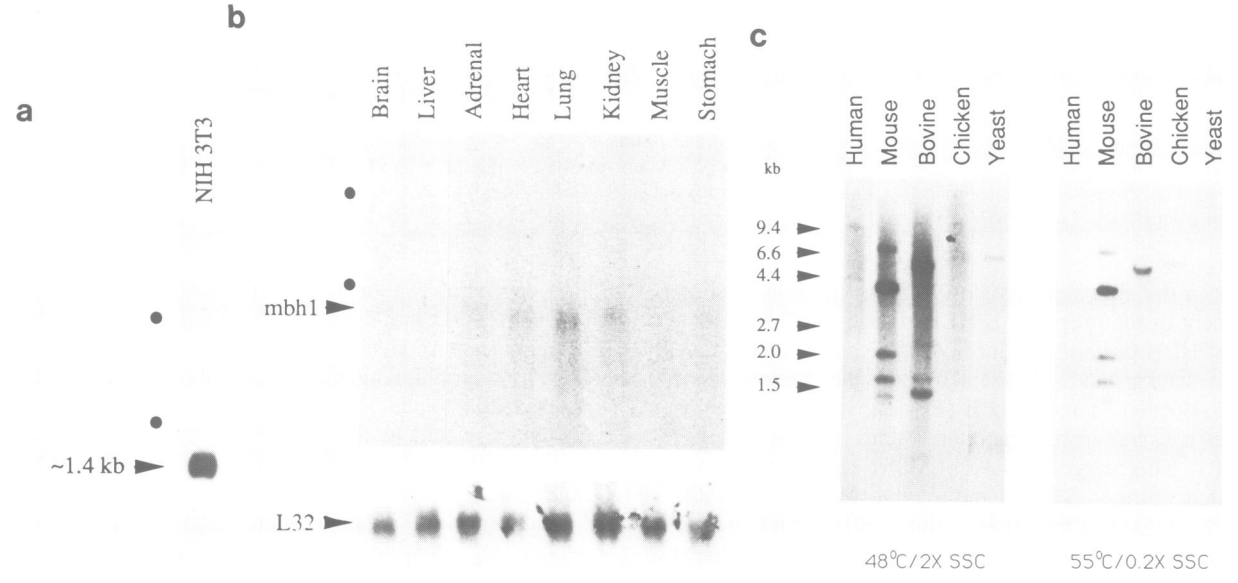

Fig. 3. Expression and evolutionary conservation of *mbh1* sequences. (**a**) Northern analysis of total cytoplasmic NIH 3T3 RNA. (**b**) Northern analysis of total cellular RNA from selected adult rat tissues. To control for RNA loading, the blot was stripped and hybridized to a probe encoding L32 large ribosomal protein (courtesy of S.Tilghman). 8 μg RNA per lane was analyzed. Dots indicate the mobility of the 28S and 18S ribosomal RNAs. (**c**) Southern analysis of selected cytoplasmic DNAs under high and low stringency conditions. The pZ1.1/*mbh1* cDNA insert was used as probe in all hybridizations shown.

examined the ability of an internal deletion mutant of Mbh1, Mbh1ΔStu, to bind to F-actin. Mbh1ΔStu lacks amino acids 21–113 of the predicted Mbh1 polypeptide. Within this region is the first of the three repeated motifs present in gelsolin-related proteins (domain 1 in Figure 2b). As negative controls, the HLH factors E12 (Murre *et al.*, 1989) and MyoD1 (Davis *et al.*, 1987) were generated by *in vitro* transcription/translation and tested for actin binding. The binding assay was performed in the presence of either 1 mM EGTA or 0.2 mM $CaCl_2$ since Ca^{2+} is observed to control the ability of gelsolin to bind actin *in vitro* (Stossel *et al.*, 1986). Briefly, ^{35}S-labeled proteins were incubated with agarose beads coupled to rabbit muscle F-actin and bound proteins were pelleted, washed several times, and analyzed by SDS–PAGE and fluorography. The results are shown in Figure 4. Labeled polypeptides whose size is consistent with the predicted sizes of the Mbh1 and Mbh1ΔStu proteins were observed following transcription/translation *in vitro* (the upper band in each lane is an endogenous translation extract product). Mbh1 was found to be retained on the F-actin–Affigel beads following incubation and washing in the presence of Ca^{2+} but not EGTA. Approximately 50% of the input Mbh1 protein was bound when Ca^{2+} was present (data not shown). The Mbh1ΔStu deletion mutant was not efficiently bound under either condition, as was also the case for the negative control MyoD1 and E12 proteins (data not shown). This suggests that all three repeats of the gelsolin-related motif present in the Mbh1 protein (Figure 2b) are required for F-actin binding in the presence of Ca^{2+}. We conclude that the *mbh1* cDNA encodes an ~45 kd polypeptide which is capable of binding F-actin *in vitro* in a Ca^{2+}-dependent manner.

Anti-Mbh1 antisera detect an ~45 kd cellular protein

To examine the expression of Mbh1, we prepared rabbit polypeptide antisera to a bacterially produced trpE/Mbh1 fusion protein using methods previously described (Spindler *et al.*, 1984). The trpE/Mbh1 protein was constructed by fusing in-frame an *Sma*I–*Eco*RI fragment from the *mbh1* cDNA to the *trpE* expression vector pATH 11, producing pATH/*mbh1* (Figure 5a). The fusion protein was induced in a bacterial host with indoleacrylic acid, then isolated and purified as described (Spindler *et al.*, 1984; Gorham *et al.*, 1990) for rabbit inoculations. Antisera obtained to the pATH/Mbh1 fusion protein was affinity-purified as described in Materials and methods.

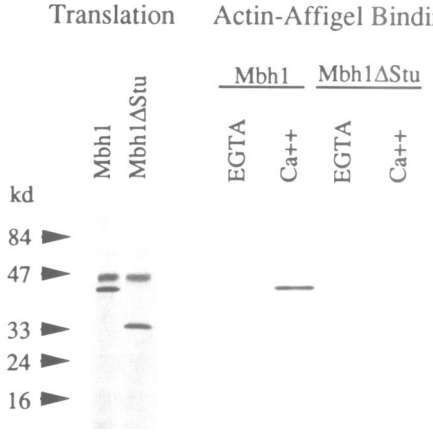

Fig. 4. Binding of Mbh1 to actin–agarose. The first panel shows expression of the Mbh1 and Mbh1ΔStu polypeptides following translation of capped RNAs in rabbit reticulocyte extracts (~50% the amount of protein analyzed for F-actin-binding was examined). Mbh1ΔStu is an internal deletion mutant which lacks amino acids 21–113 of the predicted Mbh1 polypeptide, a region containing the first of three repeats of a gelsolin-related motif (domain 1 in Figure 2b). The second panel shows the results of a binding assay to F-actin-coupled Affigel–agarose beads, as described in Materials and methods, carried out in the presence of either 0.2 mM $CaCl_2$ or 1 mM EGTA.

Immunoprecipitation was carried out from whole cell lysates of NIH 3T3 cells labeled with [^{35}S]methionine, using as a positive control Mbh1 protein generated by *in vitro* transcription/translation as above (Figure 5b). We observed an ~45 kd polypeptide precipitated with anti-Mbh1 antisera from 3T3 cell lysates which is indistinguishable in size from the control Mbh1 protein. The protein is not detected in immunoprecipitations with either preimmune sera or anti-Mbh1 antisera previously blocked with the trpE/Mbh1 bacterial fusion protein.

To determine whether steady-state levels of Mbh1 were affected by the cell growth state, we examined equivalent amounts of whole cell protein lysates from subconfluent, quiescent and serum-stimulated cells. BALB/c 3T3 cells were chosen for this analysis since they are tightly regulated by serum growth factors, and can be made quiescent quite readily by decreasing serum levels in the growth media. As a positive control for detection, the trpE/Mbh1 fusion protein was included in the Western analysis. The results are shown in Figure 5c. We observed an ~45 kd band, similar to that observed in immunoprecipitations from NIH 3T3 cells, whose level does not vary detectably between growing, quiescent and serum-stimulated cells. The intensity of the bands observed is ~1–10% that observed for trpE/Mbh1, of which 10 ng was loaded. Since 10 μg protein lysate/lane was examined, one can calculate that steady-state Mbh1 levels contribute ~0.001–0.01% of total BALB/c 3T3 protein. We conclude that the affinity-purified anti-Mbh1 antisera recognizes an ~45 kd murine fibroblast protein of low to middle abundance which appears to be fully encoded by the *mbh1* cDNA.

Two cell populations are distinguished by the appearance of distinct nuclear Mbh1 immunofluorescence

The presence of amino acid sequences in Mbh1 related to nuclear localization signals and the HLH DNA-binding motif suggested that Mbh1 might be localized in the cell nucleus. To test this, we performed Western analysis on fractionated nuclear and cytoplasmic proteins, and directly examined the localization of Mbh1 in BALB/c 3T2 cells by indirect immunofluorescence. To determine any possible effects of the cell growth state on localization, we examined cells under different growth conditions as before.

Nuclei and cytosol from BALB/c 3T3 cells were fractionated as described in Materials and methods, and equivalent amounts of nuclear and cytoplasmic protein from subconfluent, quiescent or serum-stimulated cells were analyzed by Western blotting (Figure 6). We observed the ~45 kd Mbh1 protein to be present on both the nuclear and cytoplasmic protein fractions. Neither the general level nor the ratio of nuclear to cytoplasmic Mbh1 is detectably affected by the cell growth state (it is not possible to compare directly nuclear and cytoplasmic levels since the ratio of protein analyzed in this experiment does not reflect the ratio

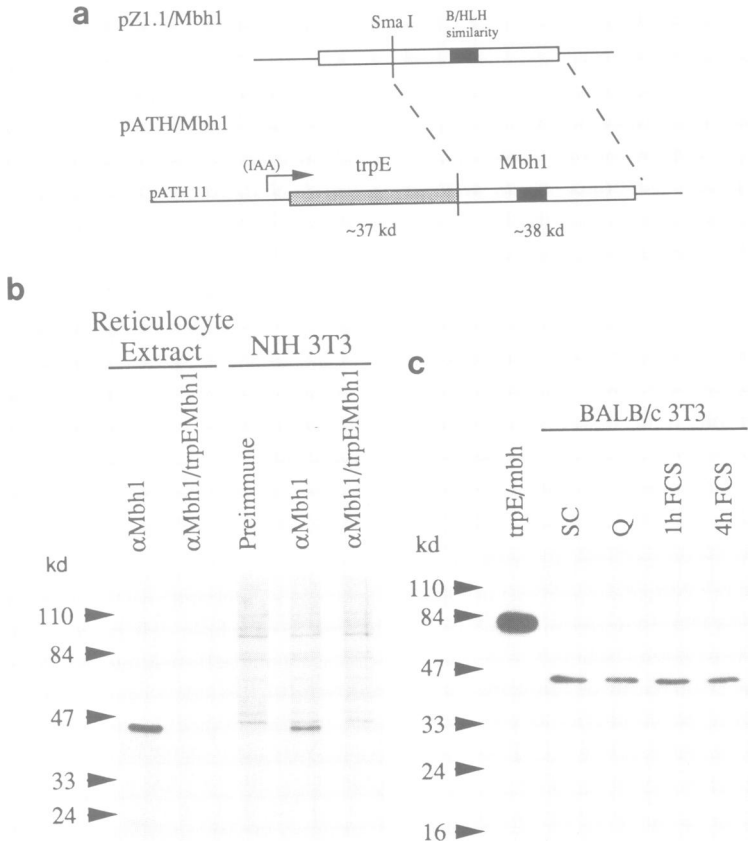

Fig. 5. Detection of a 45 kd protein *in vivo* with anti-trpE/Mbh1 antisera. (**a**) Construction of the pATH/Mbh1 vector used to generate the trpE/Mbh1 fusion protein in *E.coli*. (**b**) Immunoprecipitation of an ~45 kd 3T3 protein. As a positive control, Mbh1 protein from reticulocyte extracts programmed with *mbh1* RNA was also precipitated. For NIH 3T3 precipitations, ^{35}S-labeled lysate was mixed with unpurified preimmune sera, affinity-purified anti-Mbh1, or the same blocked with an ~10-fold molar excess of trpE/Mbh1 bacterial protein and processed as described in Materials and methods. (**c**) Western analysis of BALB/c 3T3 protein. As a positive control, 10 ng of trpE/Mbh1 protein was included on the Western blot. Ten micrograms of total 3T3 protein was analyzed from cells that were subconfluent, quiescent or serum-stimulated for 1 or 4 h with 20% FBS as described in Materials and methods.

of nuclear to cytoplasmic protein found in the total cellular fraction). There are at least three possible interpretations of the observed result. The first is that Mbh1 may be localized in all cells in the population examined in both the cytoplasm and nucleus. The second is that different populations of cells which localize Mbh1 in either the nucleus or the cytoplasm exist. Lastly, there may be some more complex situation which is a combination of the interpretations above.

To corroborate this result and to try to resolve its interpretation, we next determined the cell staining pattern of Mbh1 in BALB/c 3T3 cells by indirect immunofluorescence as described in Materials and methods. The results of these experiments are shown in Figure 7. We observed two patterns of fluorescence in subconfluent cells: a diffuse cellular stain including cytoplasmic and nuclear compartments (Figure 7a and b), or a distinct nuclear stain (Figure 7c and d). Cells treated with either preimmune sera or anti-Mbh1 previously blocked with trpE/Mbh1 protein showed no detectable fluorescence under similar conditions (data not shown). We have also observed these staining patterns in human foreskin fibroblasts, under conditions in which gelsolin shows a cytoplasmic localization (data not shown). A close examination of the nuclear staining pattern revealed that Mbh1-specific immunofluorescence is excluded from unknown circular or spherical structures, which by their size, shape and number do not appear to represent nucleoli (Figure 7c and d). Nuclear-staining cells tend to be observed in clusters, though fields containing both populations are by no means uncommon (see Figure 7d). The staining pattern differs from that observed using anti-gelsolin antibodies, which produce a slightly filamentous, diffuse cytoplasmic staining pattern lacking a nuclear component (Cooper *et al.*, 1988; Carron *et al.*, 1986). We estimate that the population of cells showing cytoplasmic staining is at least 10-fold larger than the nuclear population. However, the relative ratio appears to vary between trials, and we do not yet understand the basis for this. It is clear, though, that the proportion of cytoplasmic- to nuclear-staining cells is not reproducibly altered in quiescent or serum-stimulated populations; nor does cell density appear to correlate with staining patterns (data not shown). In summary, the observations from immunofluorescence experiments corroborate the Western analysis of nuclear and cytoplasmic protein fractions, and are consistent with the interpretation that there exist two populations of 3T3 fibroblasts which differentially localize Mbh1.

Discussion

Actin-severing proteins comprise a structurally related subset of a large family of non-muscle actin-binding proteins (for reviews see Stossel *et al.*, 1985; Pollard and Cooper, 1986;

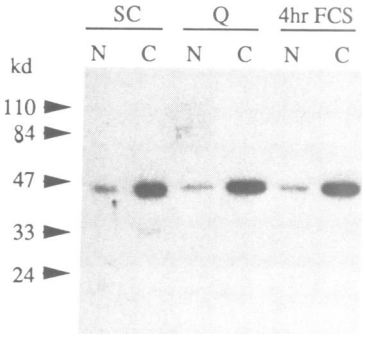

Fig. 6. Western analysis of nuclear and cytoplasmic protein fractions from BALB/c 3T3 cells. Protein fractionated as described in Materials and methods was collected and analyzed from cells that were subconfluent, quiescent or serum-stimulated for 4 h, as in Figure 5c.

Matsudaira and Janmey, 1988). Much of what is known about actin-severing proteins has come from studies of gelsolin, the first such protein characterized (Yin and Stossel, 1979). Gelsolin is found in the cytoplasm and, in a variant secreted form, in blood plasma. It can sever microfilaments by interrupting actin−actin interactions, block the 'barbed' (+) end of microfilaments, and nucleate microfilament assembly *in vitro* through its ability to bind two monomers per molecule. Severing by gelsolin is activated by Ca^{2+} and inhibited by phosphatidyl inositol 4-monophosphate (PIP) and phosphatidyl inositol 4,5-bisphosphate (PIP_2) *in vitro*, suggesting that gelsolin may play a role in reorganization of cytoskeletal actin filaments following cell stimulation by these agonists. We have observed that Mbh1 is ~50% identical to the N-terminal domain of gelsolin and ~33% identical to severin. The size of Mbh1 suggests, however, a close relationship to severin, an actin-severing protein characterized from the slime mold *Dictyostelium discoideum* (André *et al.*, 1988). Severin binds and severs actin in a Ca^{2+}-dependent manner but, unlike gelsolin, binds only one actin monomer per molecule and nucleates microfilament assembly weakly relative to gelsolin (Yin *et al.*, 1990). The role of the repeated ~50 aa sequences conserved in Mbh1 and the actin-severing proteins is unknown. They are not a general feature of other types of actin-binding proteins (Way and Weeds, 1988). The possibility that they encode actin-binding sites seems unlikely since they lie in protease-resistant regions which cannot be chemically modified in the undenatured proteins (Bazari *et al.*, 1988).

The high degree of amino acid sequence similarity and organization suggests functional homology between Mbh1 and actin-severing proteins. Consistent with this possibility is our observation that Mbh1 can interact with F-actin *in vitro* in the presence of Ca^{2+}. However, there is at least one caveat to this suggestion. Deletion mutagenesis of human gelsolin has revealed a 10−20 aa region (aa 122−142 in murine gelsolin, Figure 2a) in the N-terminal domain which appears to be required for PIP_2-regulated actin severing (Kwiatkowski *et al.*, 1989). Intriguingly, this region is quite poorly conserved in Mbh1 (aa 116−138 in Figure 2a). The lack of conservation at this region suggests that Mbh1 may not function in actin severing. Therefore, the structural relatedness of Mbh1 to actin-severing proteins may indicate functional homologies other than severing.

On the basis of statistical calculations, Mbh1 contains a region which appears to be related to the B/HLH motif, which is proposed to provide DNA-binding and protein oligomerization activities (Murre *et al.*, 1989a). Because gelsolin structure appears to contain two copies in tandem array of an Mbh1-like sequence, the notion of HLH-mediated Mbh1 dimerization to form a 'gelsolin-like' oligomer is intriguing. Does the Mbh1 B/HLH-related motif encode an oligomerization and/or DNA-binding function? In considering the motif a close inspection reveals two deviations of the Mbh1 region from a canonical B/HLH motif. First, the C terminus of the proposed Mbh1 helix I varies by substituting a serine for a canonical leucine in the final hydrophobic position of the 3−4 amphipathic helix repeat. This results in a shortened hydrophobic face on helix I. Second, the putative Mbh1 helix II contains a proline in place of a canonical tyrosine. Though still encoding a hydrophobic amino acid, proline is commonly considered a helix breaker. However, in a study of amino acid preferences in crystallographically defined α-helices, proline was observed in ~8% of 215 helices from 42 proteins in the Brookhaven protein structure database (Richardson and Richardson, 1988). Proline-containing helices are typically kinked at ~30°, due to backbone constraint at the ϕ torsion angle in proline (Richardson and Richardson, 1989). Therefore, if formed, the hydrophobic face of the proposed helix II cannot make a classical coiled-coil interaction. However, other hydrophobic interactions between helices may be possible since the proposed helix II retains a hydrophobic face, as shown by helical wheel analysis (data not shown). As a possible DNA-binding motif, the Mbh1 basic motif appears comparable to those of other HLH proteins. In the first of the two functionally important (Davies *et al.*, 1990) clusters of basic residues in the basic motif, one to three arginines or lysines may be sufficient for DNA-binding (see Benezra *et al.*, 1990 for a list of B/HLH proteins). Gaps between the two basic clusters, as observed in Mbh1, also appear to be functionally tolerated in DNA-binding complexes [e.g. in the proteins encoded by *achaete-scute Drosophila* locus (Villares and Cabrera, 1987; Murre *et al.*, 1989b)]. The arrangement of basic residues in the second basic cluster of the Mbh1 basic motif is more similar to the HLH enhancer-binding factor E12 (Murre *et al.*, 1989a) than to c-Myc. Thus its DNA binding specificity, if any, may not resemble c-Myc despite the similarity between the two clusters of basic residues in their motifs.

Consistent with the possibility that Mbh1 contacts DNA is the observation of a population of Mbh1 in the cell nucleus. We have presented extensive similarity between Mbh1 and actin-severing proteins, and have shown that Mbh1 can associate with actin *in vitro*. This raises the possibility that Mbh1 may contact nuclear actin, if it exists, and perhaps provide a means for altering nuclear structures in cells. The differential localization of Mbh1 by two 3T3 populations may be a result of some unknown regulatory phenomenon which controls access of Mbh1 to the nucleus (or cytoplasm). Ca^{2+}, which appears to accentuate the ability of Mbh1 to associate with actin *in vitro*, might effect changes in Mbh1 localization. Other actin-binding proteins which localize to the nucleus have been observed. One of these, cofilin, is a 20 kd protein which lacks actin-severing activity and is nuclear localized in cells following exposure to DMSO or heat-shock (Matsuzaki *et al.*, 1988). Two 34 kd actin-binding proteins, possibly related, have recently been purified from the nuclei of *Acanthamoeba* cells (Rimm and Pollard, 1989)

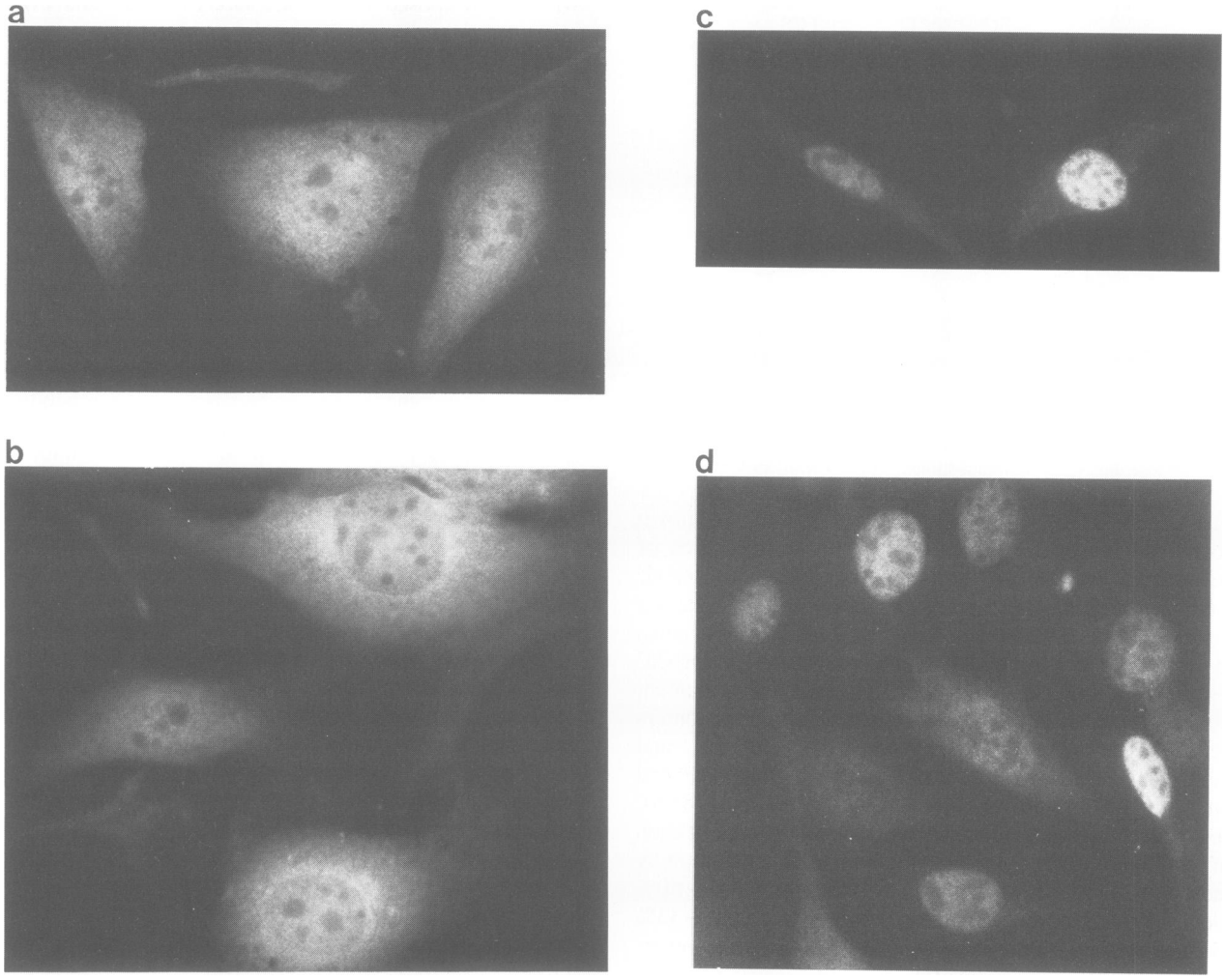

Fig. 7. BALB/c 3T3 cells stained with affinity-purified anti-Mbh1 antisera. Micrographs of cells showing general cellular staining (**a**,**b**) and nuclear staining (**c**,**d**) of 3T3 cells examined by immunofluorescence as described in Materials and methods.

and *Xenopus* ocoytes (Ankernbauer *et al.*, 1989). One of these proteins is also reported to nonspecifically associate with DNA (Rimm and Pollard, 1989). Other nuclear constituents reported to contact both actin and nucleic acid are histone (Magri *et al.*, 1978) and poly(A) polymerase–endoribonuclease IV (Schroder *et al.*, 1982). In the latter case, association with actin filaments regulates endonuclease activity. It will be interesting to determine whether potential contacts between Mbh1 and actin modulate the interactions of Mbh1 with other cellular elements.

Materials and methods

Anchored PCR cloning of an mbh1 cDNA

Poly(A)$^+$ RNA from NIH 3T3 cells was used as a target for anchored PCR, using the '(dT_{17})-adaptor' and 'adaptor' 3′ anchor primers as described (Frohman *et al.*, 1988). The 5′ sequence-specific primer was AGAATTCCAYAAYRTNYTNGARC/AG, encoding the amino acid sequence HNVLER from the putative c-Myc DNA-binding motif (Prendergast and Ziff, 1989, 1991). First strand cDNA was prepared as described (Frohman *et al.*, 1988) from 1 μg NIH 3T3 fibroblast poly(A)$^+$ RNA in a volume of 50 μl, using 0.5 μg (dT_{17})-adaptor to prime synthesis by reverse transcriptase. One microliter of the first strand product was used for PCR in a 100 μl reaction containing 10 mM Tris–Cl, pH 8.3/50 mM KCl/3 mM $MgCl_2$/0.01% gelatin/0.25 mM dNTPs and 2 U Taq polymerase (Perkin-Elmer Cetus), using 25 pmol of the 3′ 'adaptor' primer (Frohman *et al.*, 1988) and 100 pmol of the degenerate HNVLER sequence-specific primer. The first strand reaction product was denatured 90 s at 94°C, primers annealed 90 s at 42°C, and second strand cDNA was extended 40 min at 70°C. The double-stranded cDNA product was then subjected to 30 cycles of amplification in a PCR thermocycler (Perkin-Elmer Cetus) using a step program (94°C, 45 s; 50°C, 1 min; 72°C, 2 min). Amplified DNA was fractionated by agarose electrophoresis and fragments were separately cloned and sequenced by standard methodology. Clones of interest were identified by visual inspection of the predicted amino acid sequence immediately downstream of the sequence-specific primer for helix–loop–helix-related motifs. An 0.7 kb *mbh1* cDNA was identified in this manner and used as a probe for subsequent cloning.

cDNA cloning, sequencing and expression

Using standard techniques, a full-length *mbh1* cDNA was isolated from an NIH 3T3 λZXAPII cDNA library (Strategene). Approximately 3 × 10^5 recombinant phage were screened using the PCR-generated 0.7 kb *mbh1* fragment as a probe. An ~1.4 kb cDNA, Z1.1/*mbh1*, was subcloned and the complete cDNA sequence on both strands determined using the Sequenase kit and plasmid sequencing protocol (US Biochem). For the actin-binding experiment, an Mbh1 internal deletion mutant, Mbh1ΔStu, was constructed by excising an *Stu*I fragment from the pZ1.1/*mbh1* cDNA. This creates an in-frame deletion of amino acids 21–113 from the Mbh1 protein (removing the first of the three repeated domains in Mbh1). To express cDNAs *in vitro*, linearized plasmids were transcribed with T7 polymerase (Promega) in the presence of ^{7}meGpppG cap (Pharmacia) and processed as recommended by the vendor. Approximately one-tenth of the resulting RNA generated was translated in rabbit reticulocyte extracts containing 30 μCi [^{35}S]methionine, as recommended by the vendor (Promega).

Sequence comparisons

Sequence manipulations and GenBank database searches were performed using the Wisconsin Genetics Group Sequence Analysis programs for the VAX computer (Devereaux *et al.*, 1984). WORDSEARCH was used to compare Mbh1 sequences to the GenBank database; BESTFIT was employed for analyzing the relationship between Mbh1, gelsolin and severin polypeptide sequences; PEPTIDESTRUCTURE was used to predict Mbh1 secondary structures, calculated according to Chou–Fasman and Garnier–Osguthorpe–Robson routines. Amino acid similarity between HLH proteins and the predicted Mbh1 polypeptide which was recognized by visual inspection was analyzed using a beta version of the Multiple Alignment and Construction Analysis Workbench (MACAW) program (Schuler *et al.*, 1990). MACAW uses a statistical algorithm (Karlin and Lipman, 1990) to assess the probability that a given alignment of a set of amino acid sequences is significant. Probability scores (*P* values) range between zero and one. Scores near zero imply that alignments are likely to be significant; alignments considered no better than chance are assigned scores of $P = 1.0$. In our tests we aligned the suggested basic motif/helix I and the helix II regions from Mbh1 with the HLH proteins c-Myc, TFE-3, E12 and MyoD (Battey *et al.*, 1983; Davis *et al.*, 1987; Murre *et al.*, 1989a; Beckmann *et al.*, 1990) (Figure 1c). The *P* values for a given aligment were optimized by varying the breadth of the alignment window. As a positive control for relatedness, the HLH protein CBF1 (Cai and Davis, 1990) was aligned in place of Mbh1 with the same set of proteins shown above; as a negative control, randomly selected regions of Mbh1 were manually aligned.

Southern analysis

Three micrograms of human, mouse, bovine, chicken (Clontech) and 0.4 μg yeast genomic DNAs were digested to completion with *Eco*RI, electrophoresed in 0.6% agarose gels, and transferred to Duralon membranes by vacuum blotting and UV crosslinking (Stratagene). For hybridization, blots were incubated as described (Church and Gilbert, 1984) with ~10^7 c.p.m. of ^{32}P-labeled *mbh1* cDNA insert prepared by the random oligo-labeling method (Feinberg and Vogelstein, 1984). Low stringency washes were performed 2 × 10 min with 2 × SSC/0.1% SDS at 48°C; high stringency washes were performed once with the above buffer and twice for 10 min with 0.2 × SSC/0.1% SDS at 55°C. Autoradiography was for 2–4 days.

Northern analysis

Total cytoplasmic RNA was prepared from NIH 3T3 cells; total cellular RNA from rat tissues was obtained as a gift from D.Leonard. RNAs were prepared and analyzed as described (Prendergast and Cole, 1989).

In vitro actin binding assays

Actin-coupled agarose beads were generated by mixing 1 ml of a 0.5 mg/ml solution of rabbit muscle F-actin (a gift from J.Pardee) in 0.1 × MOPS, pH 7.0, with 0.5 ml activated Affigel 10 (BioRad). Following coupling for 1 h at 4°C, beads were washed once with 0.1 × MOPS, pH 7.0, and then incubated for 15 min at 4°C with 1 M ethanolamine, pH 8.5. After an additional wash with 0.1 × MOPS, pH 7.0, the actin-coupled Affigel beads were stored at 4°C until use. Typical Mbh1 binding experiments proceeded as follows. Twenty-five microliters of actin–Affigel 10 slurry per binding reaction was gently mixed for 30 min at 25°C with 1 ml 17 mM Tris–Cl, pH 7.5/50 mM NaCl/0.07% Tween 20/0.1% BSA/50 μM ATP/1 mM $MgCl_2$, then washed twice with 10 mM Tris–Cl, pH 7.5/50 mM NaCl/50 μM ATP/1 mM $MgCl_2$/1 mM DTT (binding buffer). Concurrently, 4 μl of a programmed ^{35}S-labeled reticulocyte translation mixture was mixed with 0.1 ml binding buffer containing 1 mM EGTA or 0.2 mM $CaCl_2$ and incubated 15 min at 25°C. The washed actin–agarose beads were then resuspended in the diluted reticulocyte extract mixture and shaken for 1 h at 4°C. [^{35}S]polypeptides bound to the actin–Affigel were vigorously washed twice with binding buffer, resuspended and boiled in 1 × SDS–PAGE loading buffer, and analyzed by SDS–PAGE and fluorography.

Preparation of affinity-purified rabbit polyclonal anti-Mbh1 antisera

The *Escherichia coli trpE* fusion vector pATH11 was used to produce an *mbh1/trpE* bacterial fusion protein for rabbit immunization, essentially as described (Spindler *et al.*, 1984). An ~0.95 kb *Sma*I–*Eco*RI fragment from pZ1.1/*mbh1* encoding ~34 kd of the COOH-terminus of the Mbh1 polypeptide was ligated in-frame to the pATH11 expression vector. A recombinant HB101 clone harboring the pATH/*mbh1* vector was cultured and induced with indoleacrylic acid. The fusion protein was isolated and purified as described (Gorham *et al.*, 1990) and used for production of rabbit antisera (Pocono Rabbit Farm, Canadensis, PA, USA). Anti-Mbh1 antiserum was titered by quantitative immunoprecipitation of ^{35}S-labeled Mbh1 polypeptide generated by transcription/translation *in vitro*. Affinity purification of crude sera was performed essentially as described (Gorham *et al.*, 1990). Briefly, IgG was isolated from 15–20 ml of crude rabbit sera using a commercial purification kit (BioRad) according to the manufacturer's instructions. trpE-specific antibodies were removed from the IgG fraction by one passage over a 2 ml column containing Affigel coupled to a nonspecific *trpE* fusion protein. The flowthrough from the nonspecific column was then bound to a similar column containing Affigel–*trpE/mbh* resin. Affinity purified anti-Mbh1 antibodies were eluted as described (Gorham *et al.*, 1990).

Immunoprecipitation

NIH 3T3 cells were grown in DMEM containing 10% calf serum. For ^{35}S-labeling, subconfluent cells were labeled for 1 h with 0.5 mCi TransLabel (ICN) in DMEM–methionine (Gibco) containing 2% FCS. Monolayers were washed twice with ice-cold PBS and cells were harvested and stored at −80°C. Total cell lysates were made by vigorously resuspending pellets in an appropriate volume of RIPA buffer containing 100 μg/ml PMSF and then pelleting insoluble material for 10 min in a microfuge. For immunoprecipitation, 5 × 10^6 c.p.m. of lysate in 0.3 ml RIPA buffer was precleared with 25 μl protein A–Sepharose (Pharmacia), and then mixed and incubated 1 h at 4°C with 150 ng affinity-purified anti-Mbh1 antisera. Immune complexes were precipitated by additional protein A–Sepharose, washed three times with 50 mM Tris–Cl, pH 7.5, 5 mM EDTA/500 mM NaCl/5% sucrose and once with 10 mM Tris–Cl, pH 7.5/1 mM EDTA/50 mM NaCl, and analyzed by SDS–PAGE and fluorography.

Western analysis

BALB/c 3T3 (clone A31) cells were grown in DMEM containing 10% fetal bovine serum (FBS). Subconfluent cells were actively proliferating and harvested within 24 h of feeding. Quiescent cells were generated by incubating confluent monolayers for 3 days in DMEM containing 0.5% FBS. For serum stimulations, DMEM containing 20% FBS was added to quiescent cells for various times before harvest. Total cell lysates were made by lysing monolayers washed in cold PBS with RIPA buffer containing 100 μg/ml PMSF. For nuclear and cytoplasmic protein fractionation, cells were lysed in NP-40 buffer and nuclei were pelleted in a microfuge for 2 min at 4°C. The lysate supernatant containing cytoplasmic proteins was withdrawn and brought to RIPA buffer conditions (50 mM Tris–Cl, pH 7.5/150 mM NaCl/0.5% SDS/10 mM KCl/5 mM EDTA). The pelleted nuclei were lysed in RIPA buffer. Equivalent amounts of protein as judged by Lowry assay were fractionated by SDS–PAGE; parallel gels were stained with Coomassie blue to ensure that equivalent amounts of protein were loaded per lane. Western blots were generated and processed as described (Gizang-Ginsberg and Ziff, 1990), using affinity-purified anti-Mbh1 antisera at a 1:100 dilution.

Cellular immunofluorescence

Murine BALB/c 3T3 or human foreskin fibroblasts were seeded in DMEM containing 10% FBS onto glass coverslips. For some experiments, 3T3 cells were made quiescent or serum-stimulated as described above. For immunofluorescence, cells were washed three times in PBS, fixed for 10 min in 3.7% paraformaldehyde PBS, lysed by incubation for 10 min in 0.3% Triton X-100 PBS, and then washed twice in PBS and once in 50 mM Tris–Cl/pH 7.5, 0.3% BSA/150 mM NaCl/0.2% Tween 20 (TBST). Coverslips were then incubated 1 h with a 1:100 dilution in TBST of pre-immune or affinity-purified anti-Mbh1 sera, or affinity-purified anti-Mbh1 antisera blocked for 1 h with a 10-fold molar excess of the trpE/Mbh1 bacterial fusion protein. Following three washes in PBS and one in TBST, cells were treated for an additional hour with a 1:500 dilution of affinity-purified FITC-conjugated goat anti-rabbit antisera (Boehringer Mannheim). The coverslips were then washed again three times in PBS and processed for fluorescence microscopy.

Acknowledgements

G.P. is grateful to Nick Cowan, Jim Gorham, Rick Metz and Paul Walden for criticism and technical advice, and to Greg Schuler for a beta version of the MACAW sequence analysis program. We thank Bernard Goldschmidt for oligonucleotide synthesis, Robert Davis for the *myo*D1 cDNA (pV2C11α), and Cornelis Murre for the E12 cDNA (pE12R). VAX computing was supported by NSF grant no. DIR-8908095. G.P. was supported by a postdoctoral fellowship from the ACS, and this work by the Howard Hughes Medical Institute and grants to E.Z. from the NIH and ACS.

References

Aguter,P.S. (1985) in Smuckler,E.A. and Clawson,G.A. (eds) *Nuclear Envelope Structure and RNA Maturation.* Alan R.Liss, New York, pp. 539–559.

Ampe,C. and Vandekerckhove,J. (1987) *EMBO J.*, **6**, 4149–4157.

André,E., Lottspeich,F., Schleicher,M. and Noegle,A. (1988) *J. Biol. Chem.*, **263**, 722–727.

Ankernbauer,T., Kleinschmidt,J.A., Walsh,M.J., Weiner,O.H. and Franke,W.W. (1989) *Nature*, **342**, 825.

Battey,J., Moulding,C., Taub,R., Murphy,W., Stewart,T., Potter,H., Lenoir,G. and Leder,P. (1983) *Cell*, **34**, 779–787.

Bazari,W.L., Matsudaira,P., Wallek,M., Smeal,T., Jakes,R. and Ahmed,Y. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 4986–4990.

Beckmann,H., Su,L.-K. and Kadesch,T. (1990) *Genes Dev.*, **4**, 167–179.

Benezra,R., Davis,R.L., Lockshun,D., Turner,D.L. and Weintraub,H. (1990) *Cell*, **61**, 49–59.

Blackwell,K., Kretzner,L., Blackwood,E.M., Eisenman,R.N. and Weintraub,H. (1991) *Science*, **250**, 1149–1152.

Cai,M. and Davis,R.W. (1990) *Cell*, **61**, 437–446.

Carron,C.P., Hwo,S., Dingus,J., Benson,D.M., Meza,I. and Bryan,J. (1986) *J. Cell Biol.*, **102**, 237–245.

Church,G.M. and Gilbert,W. (1984) *Proc. Natl Acad. Sci. USA*, **81**, 1991–1995.

Clark,T.G. and Merriam,R.W. (1977) *Cell*, **12**, 883–891.

Cooper,J.A., Loftus,D.J., Frieden,C., Bryan,J. and Elson,E.L. (1988) *J. Cell Biol.*, **106**, 1229–1240.

Dang,C.V. and Lee,W.M.F. (1989) *J. Biol Chem.*, **264**, 18019–18023.

Davis,R.L., Weintraub,H. and Lassar,A.B. (1987) *Cell*, **51**, 987–1000.

Davis,R.L., Cheng,P.-F., Lassar,A.B. and Weintraub,H. (1990) *Cell*, **60**, 733–746.

Devereaux,J., Haeberli,P. and Smithies,O. (1984) *Nucleic Acids Res.*, **12**, 387–395.

Dieffenbach,C.W., SenGupta,D.N., Krause,D., Sawzak,D. and Silverman,R.H. (1989) *J. Biol. Chem.*, **264**, 13281–13288.

Egly,J.M., Miyamoto,N.G., Moncollin,V. and Chambon,P. (1984) *EMBO J.*, **3**, 2363–2371.

Feinberg,A. and Vogelstein,B. (1984) *Anal. Biochem.*, **32**, 6–13.

Frohman,M.A., Dush,M.K. and Martin,G.R. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 8998–9002.

Gizang-Ginsberg,E. and Ziff,E.B. (1990) *Genes Dev.*, **4**, 477–491.

Gorham,J.D., Baker,H., Kegler,D. and Ziff,E.B. (1990) *Dev. Brain Res.*, **57**, 235–248.

Jackson,D.A. and Cook,P.R. (1985) *EMBO J.*, **4**, 919–925.

Jackson,D.A. and Cook,P.R. (1986) *EMBO J.*, **5**, 1403–1410.

Jockusch,B.M., Becker,M., Hindennach,I. and Jockusch,H. (1974) *Exp. Cell Res.*, **89**, 241–246.

Karlin,S. and Altschul,S.F. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 2264–2268.

Kerkhoff,E., Bister,K. and Kliempnauer,K.-H. (1991) *Proc. Natl Acad. Sci. USA*, in press.

Kouzarides,T. and Ziff,E. (1988) *Nature*, **336**, 646–650.

Kwiatkowski,D.J., Stossel,T.P., Orkin,S.H., Mole,J.E., Colten,H.R. and Yin,H.L. (1986) *Nature*, **323**, 455–458.

Kwiatkowski,D.J., Janmey,P.A. and Yin,H.L. (1989) *J. Cell Biol.*, **108**, 1717–1726.

Landschultz,W.H., Johnson,P.F. and McKnight,S.L. (1987) *Science*, **240**, 1759–1764.

Lawrence,J.B., Singer,R.H. and Marselle,L.M. (1989) *Cell*, **57**, 493–502.

LeStourgeon,W.M. (1978) In Busch,H. (ed.), *The Cell Nucleus.* Academic Press, New York. Vol. 6, pp. 305–326.

Magri,E., Zaccarini,M. and Grazi,E. (1978) *Biochem. Biophys. Res. Commun.*, **82**, 1207–1210.

Matsudaira,P. and Janmey,P. (1988) *Cell*, **54**, 139–140.

Matsuzaki,F., Matsumoto,S., Yahar,I., Yonezawa,N., Nishida,E. and Sakai,H. (1988) *J. Biol. Chem.*, **263**, 11564–11568.

Murre,C., McCaw,P.S. and Baltimore,D. (1989a) *Cell*, **56**, 777–783.

Murre,C., McCaw,P.S., Vaessin,H., Caudy,M., Jan,L.Y., Jan,Y.N., Cabrera,C.V., Buskin,J.N., Hauschka,S.D., Lassar,A.B., Weintraub,H. and Baltimore,D. (1989b) *Cell*, **58**, 537–544.

Pollard,T.D. and Cooper,J.A. (1986) *Annu. Rev. Biochem.*, **55**, 987–1035.

Prendergast,G.C. and Cole,M.D. (1989) *Mol. Cell. Biol.*, **9**, 124–134.

Prendergast,G.C. and Ziff,E.B. (1989) *Nature*, **341**, 392.

Prendergast,G.C. and Ziff,E.B. (1991) *Science*, **251**, 186–189.

Rajchel,A., Chan,Y.L. and Wool,I.G. (1987) *Nucleic Acids Res.*, **16**, 2347.

Richardson,J.S. and Richardson,D.L. (1988) *Science*, **240**, 1648–1652.

Richardson,J.S. and Richardson,D.L. (1989) In Fasman,G. (ed.), *Prediction of Protein Structure and the Principles of Protein Conformation.* Plenum Press, New York, pp. 1–98.

Rimm,D.L. and Pollard,T.D. (1989) *J. Cell Biol.*, **109**, 585–591.

Scheer,V., Hinssen,H., Franke,W.W. and Jockusch,B.M. (1984) *Cell*, **39**, 111–122.

Schroder,H.C., Zahn,R.K. and Muller,W.E.G. (1982) *J. Biol. Chem.*, **257**, 2305–2309.

Schuler,G.D., Altschul,S.F. and Lipman,D.J. (1991) *Proteins*, **9**, 180–190.

Spector,D. (1990) *Proc. Natl Acad. Sci. USA*, **87**, 147–151.

Spindler,K.R., Rosser,D.S.E. and Beck,A.J. (1984) *J. Virol.*, **49**, 132–141.

Stossel,T.P., Chaponnier,C., Ezzell,R.M., Hartwig,J.H., Janmey,P.A., Kwiatkowski,D.J., Lind,S.E., Smith,D.B., Southwick,F.S., Yin,H.L. and Zaner,K.S. (1985) *Annu. Rev. Cell Biol.*, **1**, 353–402.

Villares,R. and Cabrera,C.V. (1987) *Cell*, **50**, 415–424.

Way,M. and Weeds,A. (1988) *J. Mol. Biol.*, **203**, 1127–1133.

Way,M., Gooch,J., Pope,B. and Weeds,A.G. (1989) *J. Cell Biol.*, **109**, 593–605.

Yin,H.L. and Stossel,T.P. (1979) *Nature*, **281**, 583–586.

Ying,H.L., Janmey,P.A. and Schleicher,M. (1990) *FEBS Lett.*, **264**, 78–80.

Received on October 23, 1990; revised on December 28, 1990

Note added in proof

After submission of this work, Yin and co-workers reported the isolation and cloning of gCap39, a mouse actin-capping protein (Yu,F.X., Johnston,P.A., Südhof,T.C. and Yin,H.L. (1990) *Science*, **250**, 1413–1415). A comparison of the amino acid sequences of gCap39 and Mbh1 reveals ~94% identity between the two proteins, suggesting that they are encoded by highly related or identical mouse genes.