Nucleic Acids Research, 1993, Vol. 21, No. 2 223–231

Structural and functional properties of ribosomal protein L7 from humans and rodents

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Received October 26, 1992; Revised and Accepted December 8, 1992

EMBL accession nos X56745 and X57958–X57961 (incl.)

ABSTRACT

By subtractive screening of a library made from mRNA of lipopolysaccharide (LPS)-stimulated mouse B lymphocytes we isolated cDNa-clones encoding the ribosomal protein L7. Human L7 mRNA was cloned from activated T-lymphocytes. Although no specific function of L7 in the translation apparatus is known as yet, it should be a critical one as indicated by its high degree of structural conservation during evolution and its regulated expression in lymphoid cells. Human and rodent L7 proteins carry sequences similar to the basic-region-leucine-zipper(BZIP)-motif of DNA-binding eucaryotic transcription factors. We show here that the region of L7 carrying the latter motif mediates L7-dimerization and stable binding to DNA and RNA. A preferential binding to RNA-structures is demonstrated.

INTRODUCTION

Activation of lymphocytes by antigen or mitogen induces activation of genes involved in the control of cell-growth and differentiation (1, 2, 3).

In an attempt to identify such genes we obtained from the mRNA of mitogen-activated mouse B-lymphocytes by subtractive screening cDNA-clones representing the ribosomal protein L7. The human L7 mRNA then was cloned from activated T-lymphocytes. cDNA clones encoding L7 have previously been isolated from regenerating liver of rats (4) and mice (5). A human L7 cDNA sequence is present in the Genbank (entry HSRPL7). Proteins homologous to L7 have been identified in yeast (6; 7) and dictyostelium (8) but there is apparently no bacterial homologue (4, 7, 9). No specific function in the translation apparatus has been demonstrated as yet for L7. In autoimmune arthritis diseases L7 is recognized as an autoantigen (10, and A.v Mikecz et al., in preparation).

Our attention was attracted to human and rodent L7 proteins when we found that their mRNA is induced upon activation of lymphocytes and when we realized that they carry a structural motif similar to the basic-region-leucine-zipper (BZIP)-motif (11) of many mammalian, fungal and plant transcription activation factors. The BZIP-motif comprises two clusters of basic amino acids separated by an aliphatic spacer. Adjacent to the basic region follows a repetitive leucine heptad with the potential to assume the conformation of an α-helix. The 'zipper' promotes dimerization of BZIP-regions by coiled-coil formation, and the basic regions of a BZIP-dimer are capable of interacting with DNA at specific recognition sites (reviewed in 12). To our best knowledge no involvement of BZIP-carrying proteins in the translation apparatus has been reported as yet, albeit leucine-rich hydrophobic repeats occur in yeast ribosomal proteins L44 and L45 (13).

MATERIALS AND METHODS

Animals

C57BL/6 and C57BL/6×BALB/c F_1 mice of both sexes came from the breeding colony at the Institute for Genetics, Cologne.

Cells from mice and humans

Murine B-lymphocytes were enriched from spleen cells by panning in anti-IgM coated culture flasks. Adherent cells were collected, washed and cultured in RPMI 1640 medium, supplemented with 10% fetal calf serum, 5×10^{-5} M mercaptoethanol and antibiotics in the presence of LPS (40 μg/ml, Sigma, or 1 μg/ml, gift of C.Galanos) as described (14).

Human mononuclear cells from peripheral blood (PBMC) were enriched on a Ficoll-Hyapaque density gradient (15). Human T-lymphocytes from peripheral blood were enriched from PBMC by passage through nylon-wool columns (15). For TPA-stimulation, human T-lymphocytes were cultured at a density of 10^6 cells/ml in RPMI 1640 medium, supplemented with 5% fetal calf serum and antibiotics, in the presence of 5 ng/ml 12-0-Tetradecanoylphorbol 13-acetate (TPA, Sigma).

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RNA preparation

Lymphocytes were depleted from erythrocytes and dead cells by a Ficoll/Hyapaque density gradient centrifugation. Living cells were washed twice in ice-cold PBS and cytoplasmic RNA was isolated according to standard methods (16).

RNA gel electrophoresis and Northern hybridisation analysis

Total cellular RNA from $3-5\times10^6$ cells was separated by electrophoresis in agarose gels, transfered to nylon membranes and hybridised to ^{32}P-labeled DNA-probes according to standard protocols (16). Hybridised filters were exposed to Kodak Xomat films at −70°C.

DNA probes

The 6.6 kb EcoRI fragment carrying the sequence of murine 28S rRNA is a gift of Dr. I.Grummt, Heidelberg. All mammalian 28S rRNAs are detected by this probe. L7-specific probes were used as described in the text.

Densitometry

RNAs on Northern blot filters were quantified by densitometric scanning of autoradiographs as described previously (14). The signal intensities of mRNAs were normalized to the intensity of the 28S rRNA signal. Relative amounts of mRNA are given as the mRNA/rRNA signal intensity ratio.

cDNA-cloning and substractive library screening

Total RNA was isolated from mouse lymphocytes which had been cultured with LPS for 3 days. PolyA$^+$ RNA was enriched in three sequential adsorption steps on oligodT cellulose. A cDNA library in λgt10 was prepared as described (17).

In order to enrich cDNA molecules representing LPS-induced mRNAs which are absent from plasma cells we hybridised a ^{32}P-labeled cDNA preparation made from the polyA$^+$ RNA of day 3 LPS-blasts to an excess of polyA$^+$ RNA obtained from hybridoma PC140.4.1.1.C11 until a r_0t value of ~2000 was achieved (18). Hybridoma PC140.4.1.1.C11 (19) is the fusion product of a PC140 myeloma cell (20) and a C57BL/6-derived plasma-cell. It secretes IgM,K and IgG3,K antibodies. Hydroxyapatite chromatography was employed to separate single-stranded cDNA molecules from the fraction of cDNA-RNA duplexes which finally contained 99.5% of the cDNA. The single-stranded cDNA was hybridised to the cDNA-library and the structurally related clones musL7-1 and CLS1 were isolated. Using musL7-1 as a probe we isolated clone musL7-2 from the same LPS-blast cDNA library, and related human cDNA-clones (humL7-1, -14) from libraries which were constructed with mRNA of the human lymphoma Jurkat in λgt10 (21), or with RNA from the IL2-stimulated human T-cell clone GWK1 (G.Wolff-Vorbeck, in preparation) in λ ZAP. The latter library was constructed using the Stratagene cDNA cloning kit. Inserts from lambda clones were analysed as subclones in plasmid vectors pGEM4, pT7T318U, or BluescriptIISK.

DNA analysis

DNA-sequencing was performed using the sequenase modification of the didesoxy chain-termination method (22). Oligonucleotides complementary to selected L7-sequences were used for priming. Sequence alignments and computer searches for sequence similarity were performed utilizing the software developed by the University of Wisconsin Genetics Computing Group (23). Sequences are available from the EMBL databank (acc. no. X57958−57961, X56745).

In vitro translation

Capped mRNAs were synthesized in a T3 RNA polymerase transcription system on plasmid humL7-14, and then were used to program translation in a reticulocyte lysate containing ^{35}S-Methionine.

L7-fusion proteins

Functional analyses were performed with the human L7 protein and with rat L7 protein as a representative of L7 from rodents. In order to generate a fusion protein comprising glutathione-S-transferase from Schistosama japonicum (GST) and the complete human L7-protein we cloned humL7-14 cDNA without its polyA-tail in frame as a BamH1-EcoR1 fragment into expression vector pGEX1. The segment of plasmid pL7-2 (4) encoding the N-terminal region of rat protein L7 (pos. 1−110, fig. 3) was amplified employing the polymerase chain reaction with appropriate primers. The resulting fragment was cloned in frame into expression plasmid pGEX3.1. Fusion proteins with GST were expressed in *E.coli* BL21 or BMH 7180 (24) and purified from bacterial lysates by affinity chromatography on glutathione agarose columns (25).

Dimerization studies

GST-L7 fusion protein dimers were denatured for 5 min at 80°C in 1% SDS and 12.5 mM dithiothreitol or 10% 2-mercaptoethanol, and analyzed by SDS-polyacrylamide gel-electrophoresis (SDS-PAGE). In vitro translated ^{35}S-labeled L7 proteins were cross-linked by adding ethyleneglycol-bis-succinimidylsuccinate (EGS) (26) to the 1 : 5 diluted reticulocyte lysate which had been dialyzed against PBS. Cross-linking was performed in 1 mM and 5 mM EGS for 0−30 min at 4°C in a volume of 20 μl, and stopped by the addition of lysine to a final concentration of 100 mM. After denaturation as described above proteins were analysed on reducing SDS-PAGE gels in a Phast-System (Pharmacia). Gels were then soaked in Amplify (Amersham), dried and exposed to X-ray films.

Ligand-blotting

Denatured proteins were transfered from SDS-PAGE-gels to nitrocellulose membranes in 25 mM TRIS-base, 192 mM glycine and 20% methanol using a semi-dry transfer system (BIORAD) at 3 mA/cm^2. Membranes were blocked for 12−16 hrs with 5% bovine serumalbumin (BSA) in 50 mM TRIS, 200 mM NaCl, pH 8.0 (TBS), and incubated with biotinylated (15) GST-ratL7-BZIP fusion protein (1 nM in 1% BSA) for another 16 hrs at room temperature. After extensive washing in TBS, 0.05% Tween20, the membranes were conventionally developed with streptavidine-coupled alkaline phosphatase and nitro-blue-tetrazolium.

DNA/RNA binding analyses

DNA-binding of L7 was studied by incubating protein electroblots with radiolabeled DNA (27) and by gel retardation assays (28). Nitrocellulose filters carrying GST-L7 fusion proteins and GST as a control were prepared by electrophoretic transfer of purified recombinant proteins resolved by SDS-PAGE on 12.5% gels. Filters were incubated at room temperature in DNA-binding buffer (10 mM TRIS/HCl, pH 7.0, 50 mM NaCl, 1 mM EDTA,

0,2% bovine serumalbumin, Ficoll 400, polyvinylpyrrolidone 150) and then for an additional hour in binding buffer containig 50 ng/ml ^{32}P-radiolabeled random cDNA. The latter was generated by PCR-amplification of a cDNA-library in λ ZAPII (see above), with ^{32}P-dCTP in the dNTP-mix and using the T3-primer in combination with the universal M13-primer. Finally, filters were washed three times and subjected to autoradiography.

As a ligand for L7-mediated gel retardation of DNA we amplified the polylinker region of plasmid BluescriptII employing PCR with the T3-primer and the universal M13-primer in the presence of ^{32}P-dCTP. 20 ng of labeled DNA were incubated with 0−5 μg L7-fusion proteins or GST in 50 μl binding buffer (100 mM TRIS/HCl, pH 7.0, 10 mM dithiothreitol, 10 mM EDTA, 50 mM NaCl). After 30 min at room temperature, samples were electrophoresed through a 4% native polyacrylamide gel in 0,5×TBE, and the position of DNA was visualized by autoradiography.

RNA-binding of L7 was analysed by gel retardation and filter binding. Radiolabeled capped mRNA was transcribed from the PCR-amplified multiple cloning site segment of plasmid BluescriptII for the gel retardation analysis. As a radiolabeled ligand in the filter binding analysis we used capped mRNA synthesized on plasmid humL7-14 in a T7 RNA-polymerase transcription system. Gel retardation was performed as described above. In the filter binding analysis to measure the dependence of RNA-binding on salt concentration and pH, 2 μg of GST-RatL7BZIP fusion protein or GST and 150 ng radiolabeled

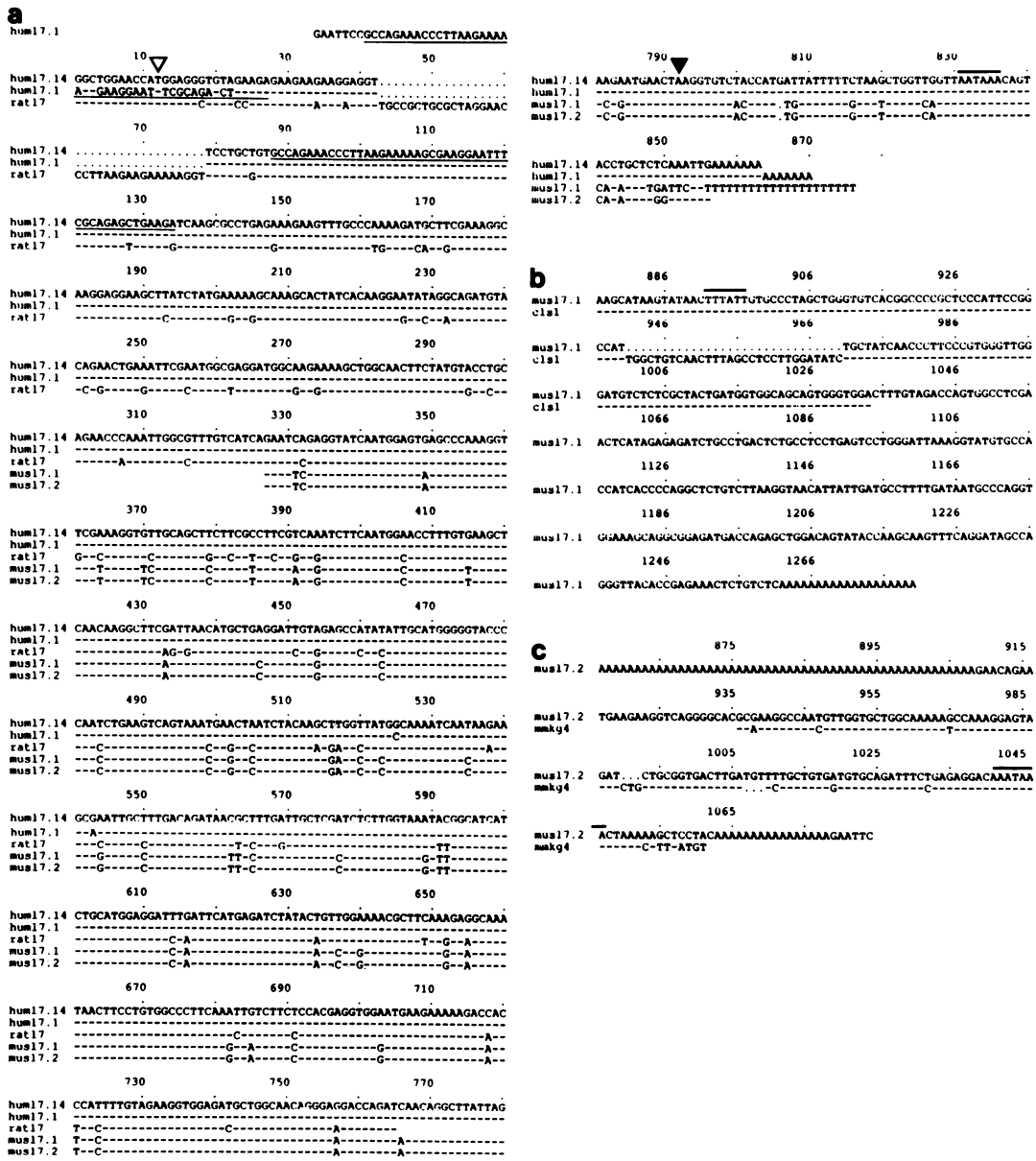

Figure 1. Nucleotide sequence of L7 cDNAs. **a)** Nucleotide sequence comparison of human, rat and mouse L7 cDNA clones. **b)** Comparison of musL7-1 to transcript Cls1. **c)** Comparison of musL7-2 to transcripts MMKG4. Direct repeats in humL7-1 are underlined. Start and stop signals of translation are marked, polyadenylation signals are overlined.

mRNA were incubated for 20 min at room temperature in 100 µl binding buffer (100 mM TRIS/HCl, pH 7.0, 10 mM dithiothreitol, 10 mM EDTA and increasing concentrations of NaCl, namely 50, 100, 200, 225, 250, 275, 300, 500 mM, or, 100 mM TRIS/HCl, pH 4.0, 4.5, 5.0, 6.0, 7.0, 7.5, 8.5, 10 mM dithiothreitol, 10 mM EDTA, 100 mM NaCl). The different samples were slowly sucked by means of a slot-blot device onto a nitrocellulose filter. Each slot had been equilibrated with the corresponding binding buffer. Slots then were washed with 2–4ml of the corresponding binding buffer and filter-bound radioactivity was quantified by means of a PhosphorImager.

Binding competition

90% binding of radiolabeled L7mRNA to GST-ratL7-BZIP fusion protein was inhibited by adding increasing amounts of cold competitor nucleic acids to the binding reaction in 100 mM TRIS/HCl, pH 7, 10 mM dithiothreitol, 10 mM EDTA, 100 mM NaCl. As competitors we used capped synthetic mRNA transcribed from the inserts of our above mentioned cDNA-library in lZAPII, double-stranded cDNA amplified from this library (see above), gel-purified human 28 S and 18 S rRNA from lymphocyte polyA$^-$RNA, yeast tRNA, and synthetic polyA (Boehringer, Mannheim). The binding analysis was performed and monitored as described above.

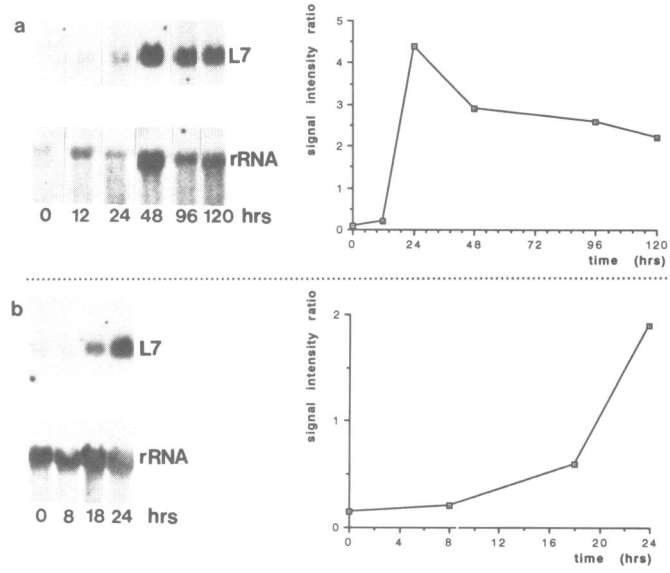

Figure 2. Expression of L7mRNA in activated lymphocytes. **a)** Expression of L7mRNA in surface Ig-positive C57BL/6xBALB/c F_1 splenic B-lymphocytes at various time-points of culture in the presence of LPS. **b)** Expression of L7mRNA in nylon-wool purified human blood T-lymphocytes at various time-points of culture in the presence of TPA. Northern blot filters were hybridised with the musL7 coding sequence and the rRNA probe. The mRNA/rRNA hybridisation signal intensity ratio is plotted against culture time.

RESULTS

L7-related cDNA clones

To identify in a cDNA library made from mRNA of LPS-activated B-lymphocytes the clones representing mRNAs induced by LPS, we used as a probe single-stranded cDNA from which molecules complementary to mRNAs existing in non-activated B-cells as well as in plasma cells had been subtracted.

Among the isolated clones cDNA clones musL7-1 and -2 showed strong homology to the nucleotide sequences encoding ribosomal protein L7 of rat and mouse (4; 5). Utilizing cDNA musL7-1 as a probe we then isolated clones humL7-1 and humL7-14 from cDNA-libraries representing the mRNAs of two activated human T-lymphocyte lines. A nucleotide sequence comparison of L7-related cDNA-clones from human, rat and mouse is shown in Fig. 1a.

cDNA clones musL7-1, -2 are truncated at the 5′ end of the protein coding region. This was caused by internal EcoRI cleavage during library preparation. Both clones differ at the C-terminus (RMN vs. QMN, see Fig. 3a) and in the 3′ UT sequence from the mouse L7 sequence musL7x (5). HumL7-14 seems to represent a functional mRNA and is identical to sequence HSRPL7 in the Genbank, whereas humL7-1 apparently is untranslatable because it does not have an in frame start codon. Upon comparison to humL7-14, rat and mouse L7 show insertions of 12 codons or, respectively, 10 codons at the 5′ end.

Clones musL7-1 and -2 have 3′ untranslated regions which show similarity to the transcripts Cls1 (O.S., diploma thesis, Univ. Cologne, 1989) and MMKG4 (29) of which no functions are known as yet (Fig. 1b,c). The polyT or, respectively, polyA sequences within the 3′ UT region of musL7-1 and musL7-2 indicate that a processed mRNA integrated by retrotransposition into the L7-locus of the mouse.

The heterogeneity of murine and human L7 cDNAs indicates the presence of several L7 genes in the mammalian genome. Indeed, Southern hybridisation of mouse liver DNA and human placenta DNA utilizing a L7-probe show 5–8 bands which differ in signal intensity (not shown). This is in line with a previous analysis of mouse L7-genes (30).

Induction of L7-related mRNAs in small resting lymphocytes

The kinetics of L7mRNA induction in LPS-stimulated mouse spleen B-lymphocytes is shown in Fig. 2a. Levels of L7mRNA in a Northern blot lane are measured relative to the amount of 28S rRNA as the L7/rRNA hybridisation signal intensity ratio. The elevation of the L7mRNA/rRNA ratio in growth-stimulated lymphocytes is clearly the result of an mRNA increase and not due to the decrease of rRNA. In fact, an increase of rRNA indicating increased ribosome numbers is observed as already was reported by others (31). Mouse spleen cells which have been enriched for surface IgM bearing cells, i.e. small resting B-lymphocytes, do not express detectable amounts of L7mRNA until 12 hrs of cultivation in the presence of LPS. After 3–4 days the level of L7mRNA begins to decrease.

Control hybridisations routinely show that the immunoglobulin k-chain mRNA level begins to rise on day 2 when large B-cell blasts appear in the LPS-culture (not shown). The L7mRNA level in LPS-stimulated B-cells thus increases relative to rRNA but is apparently not correlated with blast formation. Mouse B-cell hybridoma PC140.4.1.1.C11 (19) of which we used the mRNA to 'subtract' cDNA derived from murine LPS-stimulated B-cells is negative in a Northern hybridisation analysis with a L7-probe (not shown).

L7mRNAs also can be induced by TPA in nylon wool purified human blood T-lymphocytes (Fig. 2b). In accordance with the similar kinetics of L7mRNA induction in mouse B-lymphocytes an increase of L7mRNA levels relative to rRNA is observed. Constant rRNA levels indicate that no synthesis of new ribosomes is induced.

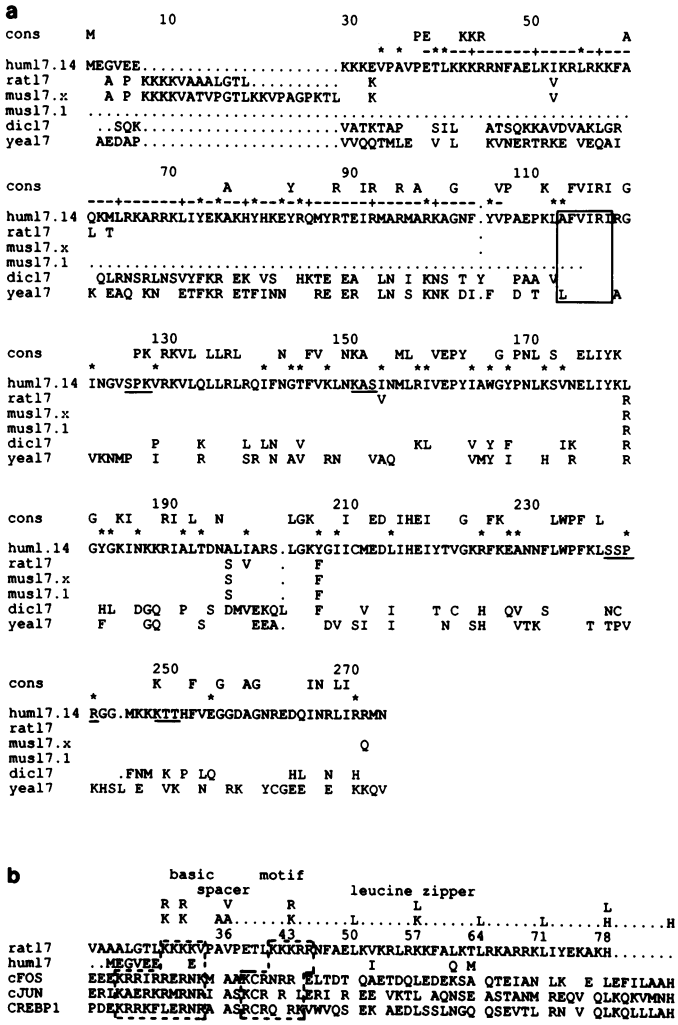

Figure 3. Amino acid sequences. **a)** Amino acid comparison of human, rat, mouse, dictyostelium and yeast L7. Deletions (.) have been introduced to maximize homology. The consensus sequence (cons) of L7 proteins is shown. (*) denotes conservative substitutions. Residues that align on a common face every two turns of an α-helix are marked (+ − − +). Potential Ser/Thre phosphorylation sites are underlined, Tyr-82 is a potential Tyr-phosphorylation site. The RNP2 motif is boxed. **b)** The basic-motif-leucine-ziper-like region of human mouse and ratL7 is compared to the BZIP region of cFOS, cJUN and CREBP1. The BZIP-like region is identical in mouse and ratL7. Conserved residues of the BZIP-like motif are shown. **c)** L7 genes encode part of an out-of-frame RNP1-motif. Nucleotide position numbering as in Fig. 1. ORF = open reading frame.

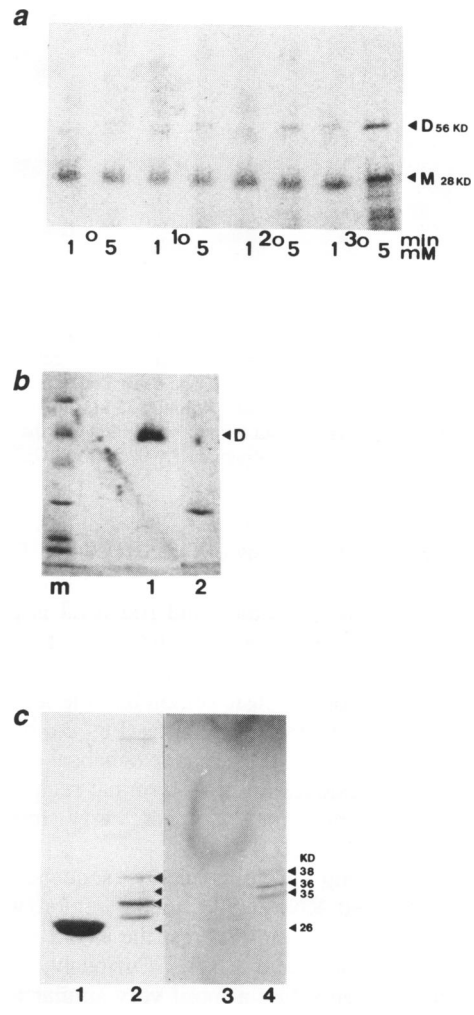

Figure 4. Homodimerization of L7. **a)** 'In vitro' translated ^{35}S-labeled human L7 proteins were cross-linked utilizing ethyleneglycol-bis-succinimidylsuccinate (EGS, 1 and 5 mM). Samples were taken and electrophoresed after 0, 10, 20, 30 min incubation time. M = monomers, D = dimers of L7. **b)** Spontaneous dimerization of GST-ratL7-BZIP (lane 1) and GST (lane 2). The preparation of fusion protein shown here is different from the ones used in the other analyses in that it does not contain truncated products. m = protein markers of 14, 21, 31, 45, 66, 98 kD. Proteins were stained with Coomassie blue. **c)** 10 μg GST (lanes 1,3) and 1 μg GST-ratL7BZIP (lanes 2,4) were electroblotted onto a nitrocellulose membrane and were either stained with Ponceau S (lanes 1,2) or developed with nitro-blue-tetrazolium after incubation with biotinylated GST-ratL7-BZIP and streptavidine-alkaline phosphatase conjugate (lanes 3, 4). Corresponding bands are marked.

Structure of L7-proteins

The amino acid sequences deduced from human, mouse and rat L7 cDNAs are compared to the related sequences from dictyostelium (8) and yeast (6) (Fig. 3a). Sequence similarity between mammalian and fungal L7-proteins is unevenly distributed. The C-terminal region comprising residues 107-end is more conserved (72% similarity) than the N-terminal region (43% similarity). In all known L7-proteins, a region comprising seventy positions between conserved prolines (37−106, Fig. 3a) seems to be capable of assuming 20 helical turns of an amphipathic α-helix. Oppositely charged amino acids occur in an arrangement three or four positions apart, thus stabilizing the α-helical conformation through intra-helical salt bridges. Structural predictions are supported by computer aided plots of hydropathy and α-helicity (not shown).

The humL7 and rodentL7 proteins carry a motif similar to the basic region-leucine zipper (BZIP) motif of mammalian, fungal and plant transcription activation factors. A comparison of L7

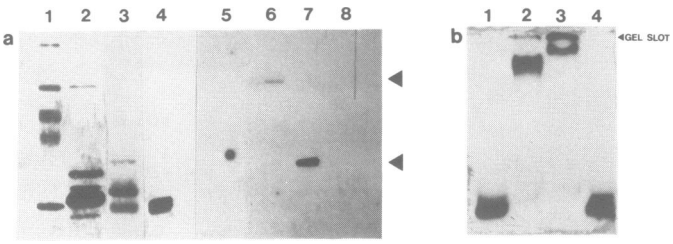

Figure 5. DNA-binding of L7. **a)** Southwestern analysis. 1 μg of GST-humL7 (lane 2,6), GST-ratL7-BZIP (lane 3,7), and GST (lane 4,8) were resolved by SDS-polyacrylamide gel electrophoresis (silver stained gel, lanes 1–4), electroblotted onto a nitrocellulose membrane, and hybridised to radiolabeled DNA (lanes 5–8). Positions of hybridising bands (58 kD, 38 kD) are marked. Protein markers (lanes 1,5) as in Fig. 4b, but without the 14 kD and the 21 kD band. **b)** Gel retardation of a 180 bp DNA-fragment by 0, 2, 4 mg GST-ratL7-BZIP (lanes 1–3) and 4 mg GST (lane 4).

to the prototypic BZIP-proteins cFOS, cJUN and CREBP1 is shown in Fig. 3b.

The BZIP-like motif of human and rodentL7 is part of the presumptive α-helical region at the N-terminus. In fact, the α-helix seems to start precisely at the 'spacer' region of the BZIP-motif. The right half of the basic region and the leucine repeat are in spatial register with the helical turns (Fig. 3a, b). Although fungal L7-proteins share the presumptive α-helical conformation and clustered basic residues in the N-terminal region with their mammalian equivalents, they do not carry motifes with pronounced similarity to BZIP.

Many RNA-binding proteins carry sequence motifes, designated RNP1 and RNP2 (32), in the region where they contact RNA (33). L7 proteins express the RNP2 motif (boxed in Fig. 3a) but do not have RNP1. Curiously, however, a nucleotide sequence encoding a motif very similar to RNP1 is present in L7 genes downstream from RNP2 but the motif is not in the L7 reading frame (Fig. 3c).

Dimerization of L7-proteins

The heptad repeat of leucines on the surface of a BZIP-region α-helix is thought to promote the dimerization of BZIP-proteins through coiled-coil formation. Since human and rodent L7-proteins carry a BZIP-like structural motif, we analysed whether they are capable of dimerization. To this end we expressed human L7 mRNA by 'in vitro' translation in a reticulocyte lysate. In addition, the N-terminal region of rat L7 (pos. 1–110, Fig. 3a) was expressed in bacteria as a fusion protein with glutathione-S-transferase (GST-ratL7-BZIP). It comprises the potentially α-helical region of L7 and carries the BZIP-like motif but not RNP2.

Dimerization of human L7-proteins is demonstrated by cross-linking them with ethyleneglycol-bis-succinimidylsuccinate (EGS) in a reticulocyte lysate (Fig. 4a). The concentration of L7-protein in the lysate is $\sim 5\times10^{-9}$ M whereas unrelated proteins are present at a concentration of 20 mg/ml. Molecules corresponding in size to L7-dimers (58 kD) are detected in dependence of reaction time and EGS-concentration, but there are no L7-multimers. Small amounts of denaturation-resistent L7 dimers are already detected at 0 min cross-linking.

Dimerization of L7 is mediated by residues in the N-terminal region as demonstrated by the spontaneous dimerization of GST-ratL7-BZIP fusion proteins which had been left at a concentration of 5×10^{-5} M in phosphate-buffered saline (Fig. 4b). The dimers in Fig. 4b, lane 1, are resistant to treatment with 1% SDS and reducing agents for 5 min. at 80°C but disintegrate upon boiling (not shown). Multimers of the fusion protein are not detected. Nearly complete dimerization in this analysis as compared to little dimerization in the previous one (Fig. 4a) may be attributed to a much higher concentration of reactants. GST as a control protein does not dimerize (Fig. 4b, lane 2).

The interaction between L7 N-terminal regions is also demonstrated in a ligand-blotting analysis (Fig. 4c). Fig. 4c, lane 2, shows 1 μg GST-ratL7-BZIP fusion protein transferred from a SDS-gel to a nitrocellulose membrane. The 38 kD band corresponds to the complete fusion protein. Smaller bands of 36, 35, 32 kD represent truncated proteins which presumably resulted from premature stops of transcription or translation in the bacterial producers. Because of this the proteins should be truncated at the C-terminus, and also because affinity purification on glutathione-agarose selects for the presence of an intact N-terminal GST. The 38, 36 and 35 kD bands of the immobilized fusion protein but not the 32 kD band react with biotinylated GST-ratL7-BZIP as a ligand (Fig. 4c, lane 4). This suggests that a complete 'zipper'-like motif (which ends 39 residues away from the C-terminus of the fusion protein) is required for the interaction. The N-terminal regions of L7-molecules seem to interact with high affinity since immmobilized proteins are bound at a ligand-concentration of 10^{-9} M. As a control we show that 10 μg immobilized GST do not interact with biotinylated GST-ratL7-BZIP (Fig. 4c, lanes 1,3).

The N-terminal region of L7 can bind to DNA and RNA

All known BZIP-region carrying proteins are transcription factors and recognize distinct DNA-sequence motifes. Human and rodent L7 proteins carry a BZIP-like motif but very likely are involved in translation rather than in transcription. In a first approach to characterize recognition of DNA, and also of RNA by L7-proteins we performed Southwestern binding-, gel retardation-,and filter-binding analyses either with a fusion protein containing the complete human L7-polypeptide (GST-humL7) or the GST-ratL7-BZIP fusion protein described above.

DNA-binding to L7 proteins is presented in Fig. 5. A Southwestern binding analysis (Fig. 5a) shows that the immobilized complete fusion proteins GST-humL7 (58 kD) and GST-ratL7-BZIP (38 kD) bind radiolabeled cDNA (compare lanes 2,3 with lanes 6,7), whereas gel-markers and immobilized GST do not bind (compare lanes 1,4 with lanes 5,8). One should not draw from this finding the conclusion that protein L7 binds DNA as a monomer. Denatured monomeric L7 molecules may dimerize during the re-naturation step that preceeds electro-transfer to the nitrocellulose membrane. There may be dimers on the membrane at the position that corresponds to the gel-position of monomers. Most interestingly, all truncated fusion proteins cannot bind DNA (compare lanes 2,3 with lanes 6,7). As their size is 33 kD and smaller they should not carry a complete BZIP-like motif. The presence of this motif thus seems to be associated with the DNA-binding capability of L7.

Binding of GST-ratL7-BZIP to DNA also is demonstrated in a gel-retardation analysis (Fig. 5b). 2 μg fusion protein are sufficient to form stable complexes with 20 ng radiolabeled cDNA (Fig. 5b, lanes 2,3).

RNA-binding by the N-terminal region of L7 proteins is shown in Fig. 6. 1 μg of GST-ratL7-BZIP fusion protein are sufficient to form stable complexes with 50 ng radiolabeld mRNA in a gel-retardation analysis (Fig. 6a, lanes 2,3). This amount of GST-

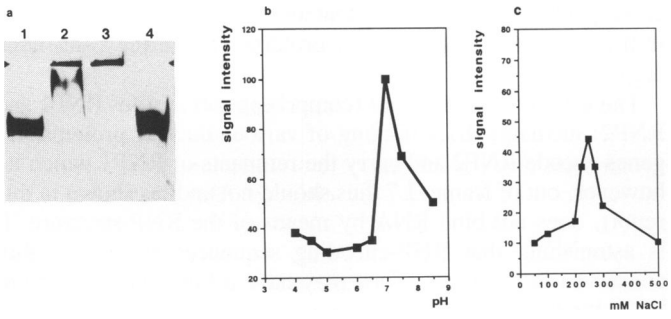

Figure 6. RNA-binding of L7. **a)** Gel-retardation of mRNA by 0, 1, 2 mg GST-ratL7-BZIP (lanes 1–3) and 2 mg GST (lane 4). The position of gel slots is marked. **b)** pH-dependence of RNA-binding to GST-ratL7-BZIP. **c)** Salt concentration dependence of RNA-binding to GST-ratL7-BZIP. Background binding to GST is subtracted. Signal intensity is given in arbitrary units.

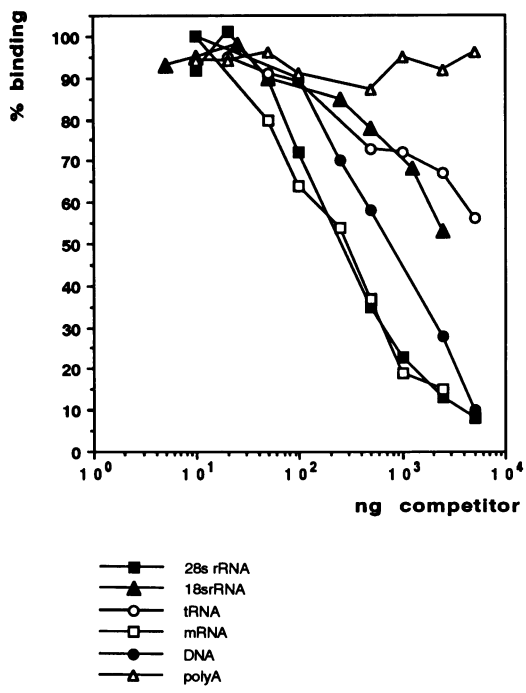

Figure 7. Binding competition. Binding of radiolabeld L7mRNA to GST-ratL7-BZIP fusion protein is inhibited by cold competitors, namely synthetic mRNA (-□-), 28 S rRNA (-■-), 18 S rRNA (-▲-), double-stranded newcDNA (-●-), tRNA (-○-), and polyA (-Δ-).

rat-L7-BZIP then was used to analyse the dependence of RNA-binding on pH and salt concentration in a filter-binding analysis. Optimal mRNA-binding is observed at pH 7 (Fig. 6b) and 250 mM NaCl (Fig. 6c). The same mRNA binding-optima are observed with the fusion protein carrying the complete human L7 polypeptide (not shown).

In summary, GST-fusion proteins with the complete human L7-polypeptide or the N-terminal fragment of rat L7 can bind DNA and RNA as demonstrated by various methods. Free GST never showed more than background binding in the corresponding control reactions (Fig. 5a, lane 8; Fig. 5b, lane 4; Fig. 6a, lane 4, not shown in Fig. 6b,c). It is thus the L7-part of the respective fusion proteins, and in particular its N-terminal region carrying the BZIP-like motif, which mediates DNA- and RNA-binding.

Binding specificity

In order to analyse the binding-specificity of L7 we inhibited the binding of radiolabeled L7mRNA to GST-ratL7-BZIP fusion protein with various nucleic acids. A typical competition analysis is shown in Fig. 7. The best competitors for RNA-binding are the mixture of capped synthetic mRNAs, and 28 S rRNA. They inhibit binding about 3fold better than double-stranded cDNA and at least 10fold better than 18 S rRNA and tRNA. PolyA does not compete at all. Unfractionated rRNA (polyA$^-$RNA) and L7mRNA show competition curve profiles (not shown) identical to the ones of 28 S rRNA and the mRNA mixture. There seems to be preferential binding of L7 to as yet undefined structures in mRNA and 28 S rRNA.

DISCUSSION

L7-genes and their expression

The genome of man and mouse contains 5–8 copies of L7-like genes. It has been shown, that some of the murine genes are non-functional (5; 30). As suggested by the polyA/T sequences in their 3′ untranslated region transcripts musL7-1 and -2 seem to stem from processed genes. Although sequences musL7-1 and -2 do not show crippling mutations we cannot decide whether the corresponding transcripts are functional because they are represented by incomplete cDNAs. The human L7-gene family contains at least one apparently non-functional gene from which mRNA humL7-1 is transcribed.

L7-mRNAs are induced in resting B- and T-lymphocytes by TPA or by mitogen which activate the metabolism of phosphatidylinositol, indicating an involvement of enzymes belonging to the PKC enzyme family. In the complex cascade of gene activation events initiated by a mitogen L7-mRNAs enter the stage several hours after the onset of the synthesis of new ribosomal RNA. TPA induces mRNA but not the synthesis of new rRNA. L7mRNA induction and rRNA synthesis are apparently not correlated here. This is unexpected of the mRNA of a ribosomal protein. mRNAs for ribosomal proteins L18, L32, S16, for instance, in resting and TPA- or hormone-stimulated cells behave differently in that the respective mRNA levels and rRNA levels remain correlated (34, and references therein).

Structure and function of L7 proteins

There are 5 tandem repeats of 12 amino acids at the N-terminus of rat L7 (4). Mouse L7 shows 6 of these repeats and human L7-14 has 4 of them. At the 5′ end of humL7-1 mRNA a repeat of 48 bp is separated by 24 bp of unrelated sequence, altogether representing 2 blocks of 12 amino acids (Fig. 1a). Such repeats are unlikely to encode functional domains in the L7-molecule, as has been suggested (4), because it is hard to conceive that human, rat and mouse L7-molecules should functionally differ from each other. Moreover, 5′-terminal repeats with a 36 bp-periodicity are present in the non-translatable transcript humL7-1. The various forms of duplications at the 5′ end of L7-genes presumably have been generated by unequal crossovers within the L7 multigene family and behaved neutrally in evolution.

The N-terminal region of L7 proteins (Fig. 3a, pos. 1–106) is less conserved than the C-terminus (Fig. 3a, pos. 107–end). However, all known L7 proteins apparently share the capability of forming an α-helix which progresses from proline 27 over

about twenty helical turns to proline 106. This part of the molecule is hydrophilic as predicted in a hydropathy plot (not shown) and is solvent-exposed, as shown by Lin (35).

Human and rodent L7 proteins carry in this region a BZIP-like sequence motif (10). Leucines 2 and 5 in the 'zipper region' are substituted by lysine and histidine, respectively. However, an imperfect leucine repeat is not untypical of BZIP-proteins (36–39). At the position seven residues downstream of the last 'zipper'-leucine, that is in α-helical register with the leucine repeat, a conserved histidine is found in human and rodent L7-proteins (pos. 77) and in most members of the CREB, FOS and JUN BZIP-protein families. This residue has been shown to stabilize JUN-FOS heterodimers (40).

The N-terminal region of mammalian L7-proteins is capable of forming highly stable homodimers. This could be mediated by the 'zipper'-like region through formation of a coiled-coil stabilized by the interaction between hydrophobic side-chains and by hydrogen bonds between the polar side chains of one α-helix and the peptide carbonyls of the opposite chain.The 'zipper'-like motif in L7 meets all structural requirements of the molecular model for 'zipper'-dimerization proposed by Tropsha et al. (41). This may explain the high stability of L7 dimers.

Whilst the 'zipper' is believed to promote dimerization of BZIP-regions by forming a coiled-coil, positively charged residues oppose each other in the basic region and intertwined α-helices should be forced to bifurcate. The 'scissors-grip' model of eucaryotic transcriptional activation factors postulates a Y-shaped BZIP-dimer whose arms contact DNA. In order to continue tracking in the major groove of DNA the hypothetical α-helix of the basic region must be broken at a point near the spacer. All BZIP-proteins reported so far have an invariant asparagine three positions left of the 'spacer' (see Fig. 3b) thus terminating the continuity of hydrogen bonds necessary for an α-helix by 'N-capping' (42). This asparagine is not found in human and rodent L7, but the α-helical structure of the basic motif should be terminated by two prolines flanking the 'spacer'.

We demonstrate in this study stable binding of the N-terminal region of rat L7-proteins to DNA and RNA. Distinct optima at physiological pH and high salt concentrations suggest specific RNA-binding. In addition, preferential binding to as yet undefined structures in 28 S rRNA and mRNA is indicated by the finding that the latter RNAs are the relatively most effective competitors in a binding inhibition analysis. These data support the idea that 28 S rRNA- and/or mRNA binding is required for the biological function of L7. 28 S rRNA-binding is expected of a protein believed to be an integral structural component of the large ribosomal subunit, mRNA-binding rather not.

We cannot decide from the binding analyses presented in this study whether protein L7 interacts with nucleic acids as a monomer or as a dimer. A definitive decision requires binding studies involving mutant L7 molecules with impaired capability of dimerization. Nevertheless, L7 molecules mainly seem to exist in solution as dimers (see Fig. 4b). This at least suggests that it is the dimer rather than the monomer which interacts with DNA and RNA in gel-retardation and filter-binding analyses.

The known fungal and mammalian L7 proteins share the potential α-helical conformation in the N-terminal region and a similar pattern of basic repeats and hydrophobic residues but only mammalian L7 shows a pronounced similarity to BZIP. L7 proteins from vertebrates may have acquired this motif during evolution, or fungal L7-like proteins may have lost it. This idea is compatible with the finding that there is much more divergence in the N-terminal region of L7-proteins than in the C-terminal region.

The RNP-consensus motif (comprising sub-motifes RNP1 and RNP2) mediates RNA-binding of various nuclear proteins. L7 genes encode RNP2 and carry the remnants of RNP1 which is, however, out of frame. L7 thus should not and, as shown in this report, does not bind RNA by means of the RNP-structure. It is astonishing that RNP-encoding sequences are still highly conserved in L7-genes. They may have a function unrelated to RNA-binding.

The C-terminal region of L7-proteins (pos. 107–end) is highly conserved in evolution (Fig. 3). It seems to provide an invariant framework of the L7-molecule. As hydrophobic residues are abundant, most of this framework should not be exposed to the solvent.

ACKNOWLEDGEMENTS

We thank R.Rzepka, S.Klein and S.Irlenbusch for providing cells and for help with tissue culture. C.Esser donated a Northern blot filter, T.Mak the Jurkat cDNA-library, I.Grummt and I.G.Wool gave DNA-probes. LPS is from C.Galanos. R.Knippers and V.Erdmann critically read the manuscript which was prepared by C.Hoffmann. Financial support was provided by the Deutsche Forschungsgemeinschaft through grant Pe 151/10 and Sonderforschungsbereiche 74 and 156.

REFERENCES

1. Berridge,M.J. (1987) *Ann. Rev. Biochem.*, **56**, 159–193.
2. Cambier,J.C. and Ranson,J.T. (1987) *Ann. Rev. Immunol.*, **5**, 175–199.
3. Veillette,A. and Davidson,D. (1992) *Trends Gen.*, **8**, 61–66.
4. Lin,A., Chan,Y.L., McNally,J., Peleg,D., Meyuhas,O. and Wool,I.G. (1987) *J. Biol. Chem.*, **262**, 12665–12671.
5. Meyuhas,O. and Klein,A. (1990) *J. Biol. Chem.*, **265**, 11465–11473.
6. Murray,J.M. and Watts,F.Z. (1990) *Nucl. Acids Res.*, **18**, 4590.
7. Mizuta,K., Hashimoto,T. and Otaka,E. (1991) *Nucl. Acids Res.*, **20**, 1011–1016.
8. Szymkovski,D.E., Kelly,B. and Deering,R.A. (1989) *Nucl. Acids Res.*, **17**, 5393.
9. Wool, I. (1979) *Ann. Rev. Biochem.*, **48**, 719–754.
10. Absi,M., LaVergne,J.P., Marzouki,A., Girand,F., Rigal,D., Reboud,A.M., Reboud,J.P. and Monier,J.C. (1989) *Immunol. Letters*, **23**, 35–42.
11. Landschulz,W.H., Johnson,P.F. and McKnight,S.L. (1988) *Science*, **240**, 1759–1764.
12. Busch,S.J. and Sassone-Corsi,P. (1990) *Trends Gen.*, **6**, 36–40.
13. Tsurugi,K. and Mitsui,T. (1991) *Biochem. Biophys. Res. Comm.*, **174**, 1318–1323.
14. Esser,C. and Radbruch,A. (1989) *EMBO J.*, **8**, 483–488.
15. Hudson,L. and Hay,F.C. (1989) Practical Immunology, 3rd edition, Blackwell Scientific Publications, Oxford.
16. Sambrock,J., Fritsch,E.F. and Maniatis,T. (1989) Molecular Cloning, 2nd ed., Cold Spring Harbor Laboratory Press, New York.
17. Krawinkel,U. and Zoebelein,R. (1986) *Nucl. Acids Res.*, **14**, 1913.
18. Davis,M.M., Cohen,D.I., Nielsen,E.A., Steinmetz,M., Paul,W.E. and Hood,L. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2194–2198.
19. Klein,S.C. (1987) Ph.D. Thesis, University of Cologne, Cologne, FRG.
20. Thammana,P. and Scharff,M.D. (1983) *Eur. J. Immunol.*, **13**, 614–.
21. Yanagi,Y., Chan,A., Chin,A., Minden,M. and Mak,T.M. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 3430–3434.
22. Tabor,S. and Richardson,C.C. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 4767–4771.
23. Devereux,J., Haeberli,P. and Smithies,O. (1984) *Nucl. Acids Res.*, **12**, 387–395.
24. Studier,F.W. and Moffat,B.A. (1986) *J. Mol. Biol.*, **189**, 113–120.
25. Smith,D.B. and Johnson,K.S. (1988) *Gene*, **67**, 31–36.

26. Abdella,P.M., Smith,P.K. and Royer,G.P. (1979) *Biochem. Biophys. Res. Comm.*, **87**, 734–738.
27. Bowen,B., Steinberg,J., Laemmli,U.K. and Weintraub, (1980) *Nucl. Acids Res.*, **8**, 1–21.
28. Sapp,M., Knippers,R. and Richter,A. (1986) *Nucl. Acids Res.*, **14**, 6803–6821.
29. Filion,M., Lussier,M., Royal,A., Gervais,C. and Suh,M. (1988) *Biochem. Biophys. Acta*, **950**, 255–262.
30. Klein,A. and Meyuhas,O. (1984) *Nucl. Acids Res.*, **12**, 3763–3776.
31. Cooper, H.L., and Braverman, R. (1977) *J. Cell. Physiol.*, **93**, 213–226.
32. Dreyfuss,G., Swanson,M.S. and Pinol-Roma,S. (1988) *Trends Biol. Sci.*, **13**, 86–91.
33. Nagai,K., Oubridge,C., Jessen,T.H., Li,J. and Evans,P.R. (1990) *Nature*, **348**, 515–520.
34. Hershey,J.W.B. (1991) *Ann. Rev. Biochem.*, **60**, 717–755.
35. Lin,A. (1991) *FEBS Lett.*, **287**, 121–124.
36. Hai,T., Lin,F., Coukos,W.J. and Green,M.R. (1989) *Genes Dev.*, **3**, 2083–2090.
37. Paluh,J.L., Orbach,M.J., Legerton,T.L. and Yanofsky,C. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 3728–3732.
38. Montminy,M.R. and Bilezikijan,L.M. (1987) *Nature*, **328**, 175–178.
39. Yoshimura,T., Fujisawa,J.I. and Yoshida,M. 1 (1990) *EMBO J.*, **9**, 2537–2542.
40. Cohen,D.R. and Curran,T. (1990) *Oncogene*, **5**, 929–939.
41. Tropsha,A., Bowen,J.P., Brown,F.K. and Kizer,J.S. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 9488–9492.
42. Vinson,C.R., Sigler,P.B. and McKnight,S.M. (1989) *Science*, **246**, 911–916.