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Ribosome-binding protein p34 is a member of the leucine-rich-repeat-protein superfamily

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Protein p34 is a non-glycosylated membrane protein characteristic of rough microsomes and is believed to play a role in the ribosome–membrane association. In the present study we isolated cDNA encoding p34 from a rat liver cDNA library and determined its complete amino acid sequence. p34 mRNA is 3.2 kb long and encodes a polypeptide of 307 amino acids with a molecular mass of about 34.9 kDa. Primary sequence analysis, coupled with biochemical studies on the topology, suggested that p34 is a type II signal–anchor protein; it is composed of a large cytoplasmic domain, a membrane-spanning segment and a 38-amino-acid-long luminally disposed C-terminus. The cytoplasmic domain of p34 has several noteworthy structural features, including a region of 4.5 tandem repeats of 23–24 amino acids. The repeated motif shows structural similarity to the leucine-rich repeat which is found in a variety of proteins widely distributed among eukaryotic cells and which potentially functions in mediating protein–protein interactions. The cytoplasmic domain also contains a characteristic hydrophilic region with abundant charged amino acids. These structural regions may be important for the observed ribosome-binding activity of the p34 protein.

INTRODUCTION

The rough-microsomal (RM) membranes of eukaryotic cells contain specific sites for binding with ribosomes synthesizing secretory or membrane proteins. These binding sites are postulated to be functionally associated with a putative protein translocation channel in the membrane which facilitates the import of the synthesized proteins into the lumen of the RM (Blobel and Dobberstein, 1975; Simon and Blobel, 1991). Previous studies indicated that the ribosome-binding activity is affected by mild treatment of RM with a proteinase and markedly decreases in the presence of increasing amounts of univalent ions, suggesting that the binding sites comprise proteinaceous factor(s) which can bind ribosomes through ionic bonds (Shires et al., 1971; Borgese et al., 1974; Hortsch et al., 1986). Two membrane proteins with relative molecular masses of 180 kDa and 34 kDa have so far been demonstrated to possess ribosome-binding activity, as determined with an *in vitro* binding assay system involving liposomes (Savitz and Meyer, 1990; Tazawa et al., 1991; Ichimura et al., 1992). The direct binding between these proteins and ribosomes was also demonstrated by experiments involving a bifunctional reagent (Collins and Gilmore, 1991; Tazawa et al., 1991). Recently, a mammalian counterpart of yeast Sec61p was identified and proposed to be a key component of the protein-translocation channel (Görlich et al., 1992). This protein was also shown to have affinity toward ribosomes (Görlich et al., 1992).

Several lines of evidence suggested the importance of the 34 kDa protein, which we termed p34, in the ribosome-binding ability of RM. First, the protein exhibits the major ribosome-binding activity in a detergent extract prepared from rat liver RM membranes (Tazawa et al., 1991). Secondly, its binding properties (e.g., proteinase- and salt-sensitivity) are very similar to those of intact RM (Tazawa et al., 1991; Ichimura et al., 1992). Thirdly, the protein is localized specifically in ribosome-attached organelles (RM and nuclear envelope) and fourthly, its counterparts were found, by immunochemical means, to be distributed widely in mammalian RM (Ichimura et al., 1992).

p34 is a non-glycosylated protein and, like the ribosome-binding activity, is highly sensitive to trypsin (Tazawa et al., 1991). The protein appears to be an integral membrane protein because it can only be released from the membrane on its solubilization with a detergent. Little is known, however, about the structure of protein p34.

Here we present the primary sequence of protein p34 and deduce its disposition in the RM membrane. This protein appears to be a type II signal–anchor protein with a large cytoplasmically disposed portion. Computer analysis of the sequence indicated that the protein has an internally repeated sequence that shows high similarity to the leucine-rich repeat (LRR).

EXPERIMENTAL

Screening of a rat liver cDNA library

A rat liver cDNA library constructed in phage λgt11 (Clontech, Palo Alto, CA, U.S.A.) was screened with rabbit polyclonal antiserum against p34 (Ichimura et al., 1992) as described by Young and Davis (1983). Alkaline phosphatase-conjugated goat anti-rabbit IgG and 5-bromo-4-chloroindol-3-yl phosphate/Nitroblue Tetrazolium were used to reveal the antigen–antibody complexes. Plaque hybridization was carried out by the method of Maniatis et al. (1989).

Northern-blot analysis

Northern-blot analysis was carried out essentially according to the procedure of Maniatis et al. (1989). Random-primed ^{32}P-labelled cDNA (1×10^9 c.p.m./μg) of pRAP-1 (*Pst*I fragment; see Figure 2a below) was used as the hybridization probe. The blot was hybridized with the cDNA in a solution comprising

Abbreviations used: RM, rough microsomes (microsomal); LRR, leucine-rich repeat; SRP, signal-recognition particle.
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50% (v/v) formamide, 5 × SSC (1 × SSC is 0.15 M NaCl/0.015 M trisodium citrate, pH 7), 1 × Denhardt's solution, 10% (w/v) dextran sulphate, 250 μg/ml heat-denatured salmon sperm DNA and 50 mM sodium phosphate, pH 6.5, at 50 °C overnight, and then washed twice in 0.2 × SSC/0.1% (w/v) SDS at 55 °C for 15 min.

DNA sequence analysis

The inserts derived form λgt11 clones were subcloned into the *Eco*RI site of pUC 18 or Bluescript SK(+) (Stratagene, La Jolla, CA, U.S.A.). The purified plasmid DNA was sequenced by the dideoxy-mediated chain-termination method (Sanger et al., 1977).

Purification and amino acid sequence analysis of p34

The p34 protein was isolated from salt-washed rat liver RM membranes by affinity chromatography on a concanavalin A–Sepharose column and then a chelating-Sepharose column (Tazawa et al., 1991) and purified by preparative PAGE followed by electroelution. The purified protein (100 μg) was digested with lysyl-endopeptidase or chymotrypsin, and the resultant fragments were separated on a reversed-phase Capsel Pak C18 column (0.46 cm × 15 cm; Shiseido, Tokyo, Japan) by elution with a linear gradient of acetonitrile (10–75%, v/v) in 0.1% trifluoroacetic acid. The amino acid sequence was determined with a model 470A automated sequencer (Applied Biosystems, Foster City, CA, U.S.A.) according to the protocol provided by the supplier.

Trypsin digestion and fractionation of membranes

Rat liver RM were treated with 0.5 mM puromycin/0.5 M KCl as described by Borgese et al. (1974). Stripped microsomes (100 μg of protein) were incubated with the indicated amount of trypsin (see below) at 0 °C for 60 min in 100 μl of 50 mM Tris/HCl buffer, pH 7.6, and then 30 units of Trasylol was added to stop the reaction. Where indicated, the trypsin-treated RM were washed in 0.1 M Na_2CO_3, pH 11.5, and then centrifuged in an Airfuge for 30 min at 100000 ***g***. Subsequent immunoblotting was carried out by the method of Tazawa et al. (1991).

RESULTS AND DISCUSSION

Isolation and analysis of p34 cDNA clones

Screening of a rat liver λgt11 expression library (2×10^5 transformants) with p34 antibodies (Ichimura et al., 1992) yielded six positive clones. From among them, the longest clone, termed pRAP-1, was selected for characterization in terms of the nucleotide sequence. The pRAP-1 clone contained about 2200 bases, including a short stretch of the poly(A) tail. However, since it still appeared to be shorter than the mRNA (3.2 kb) detected on Northern hybridization analysis (Figure 1, arrowhead), we rescreened the cDNA library by plaque hybridization using the *Nae*I(728)–*Sac*I(1169) fragment of the pRAP-1 5′ terminal side as the probe (see Figure 2a). Two longer clones, termed pRAP-2 and pRAP-3, were isolated. These cDNA clones were used for further sequence analysis.

Figure 2 shows the restriction map, the nucleotide sequence of the cDNA and the deduced amino acid sequence. The cDNA sequence determined contains a total of 2860 bases, including a single open reading frame starting with the first ATG codon at base 247 and ending with a TGA stop codon at base 1168. The sequence contains a 246-base GC-rich (72%) 5′ untranslated

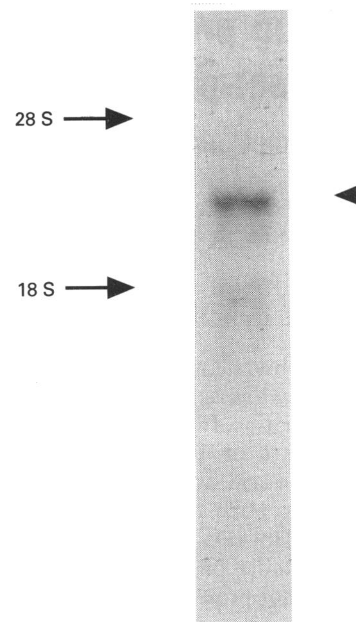

Figure 1 Northern-blot analysis

A Northern blot of total RNA (15 μg) from rat liver was probed with pRAP-1 cDNA (*Pst*I fragment). The positions of rat liver ribosomal RNAs (18 S = 1874 and 28 S = 4718 bases) are shown at the left. The arrowhead indicates the position of p34 mRNA.

region and a 1675-base 3′ untranslated region. In this 3′ untranslated region were found a polyadenylation signal (ATTAAA) and a part of the poly(A) tail (a stretch of 18 adenine residues).

The continuous open reading frame encodes a polypeptide of 307 amino acids with a molecular mass of 34.9 kDa (Figure 2b). The deduced sequence contained all the amino acid sequence (total 122 residues) determined on Edman degradation of the mature p34 as well as those of the fragments generated on digestion with lysyl-endopeptidase or chymotrypsin (underlined in Figure 2b). In addition, the amino acid composition and the molecular mass calculated from the deduced sequence were in close agreement with those of the p34 protein determined by amino acid analysis and SDS/PAGE respectively. We concluded, therefore, that the sequence of the cloned cDNA represents that of the p34 protein.

Structure of p34

p34 has no signal sequence at its N-terminus, which is characteristic of many membrane and secretory proteins (Figure 2b). However, the initial methionine (residue 1, Figure 2b) was removed post-translationally, because the Edman degradation of the mature protein starts with Thr-2. The p34 protein would thus have 306 amino acids with a molecular mass of 34738 Da and a calculated pI of 10.0. The p34 protein contains five Ser/Thr residues that satisfy the consensus sequence for phosphorylation (Figure 2b, stippled), although at present there is no evidence for the phosphorylation of p34. In contrast, the protein does not contain a consensus sequence for an ATP- or GTP-binding site, which is characteristic of several nucleotide-binding proteins, including the 54 kDa subunit of the signal-recognition particle (SRP) and its cognate receptor (SRP receptor or docking protein).

Hydropathy analysis (Kyte and Doolittle, 1982) revealed one hydrophobic segment (residues 245–269) long enough to span

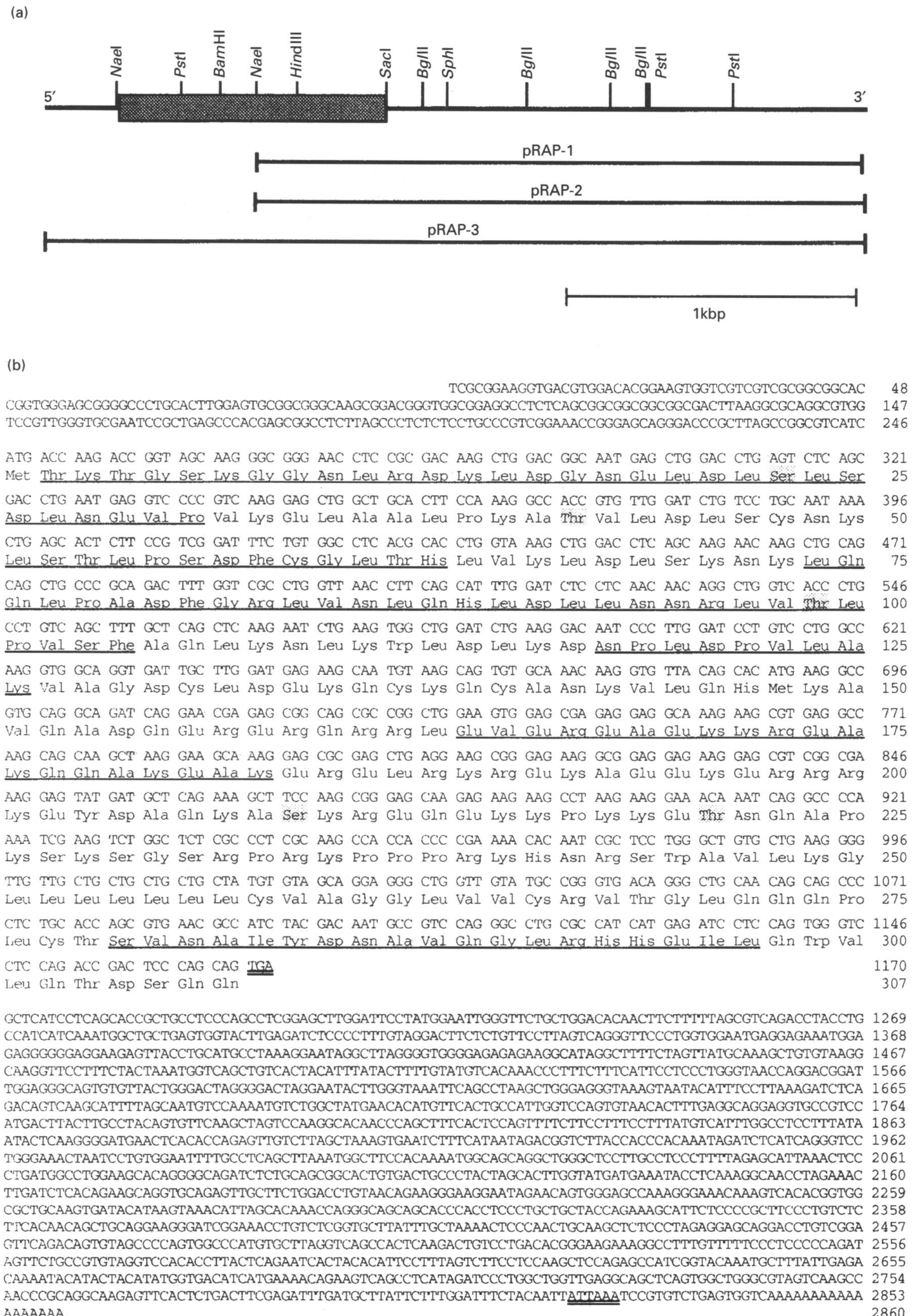

Figure 2 Restriction map, complete nucleotide sequence and deduced amino acid sequence of p34

The nucleotide and deduced amino acid sequences are numbers at the right of each line. Chemically determined amino acid sequences are underlined. The Ser/Thr residues that are potential sites of phosphorylation are stippled: these include Ser-23 and Thr-42 (for casein kinase II), Thr-99 (for Ca^{2+}/calmodulin-dependent protein kinase II), Ser-209 (for casein kinase II and protein kinase C) and Thr-221 (for cyclic AMP-dependent protein kinase). The termination codon (TGA) at positions 1168–1170 and the polyadenylation signal (ATTAAA) at positions 2820–2825 are indicated by double lines.

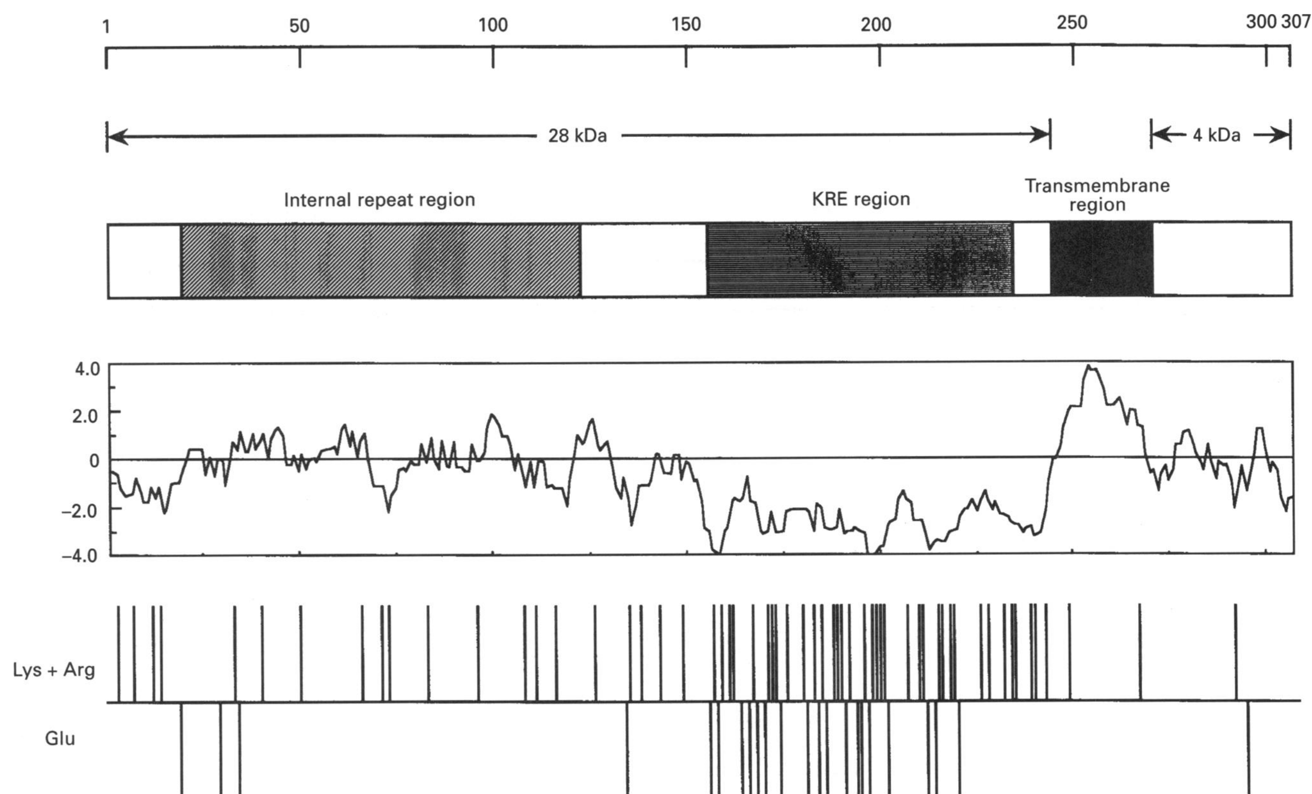

Figure 3 Linear model of the primary structure of p34

Top: The positions of the internal repeat, KRE and transmembrane regions (described in the text) in the sequence of 307 residues of p34, shown to scale. Middle: A hydropathy plot of the amino acid sequence, employing the method of Kyte and Doolittle (1982), using a window of seven residues. Bottom: The positions of Lys, Arg and Glu in the sequence, which are indicated by upward and downward vertical lines.

Residues	Sequence
20–31	LDLSLSDLNEV
32–55	PVKELAALPKATVLDLSCNKLSTL
56–78	P-SDFCGLTHLVKLDLSKNKLQQL
79–101	PA-DFGRLVNLQHLDLLNNRLVTL
102–123	PVS-FAQLKNLKWLDLKDNPLDPV
Consensus...	P---F--L--L--LDLS-N-L--L

Figure 4 Tandem repeats in the amino acid sequence of p34

The 4.5 tandem repeats found in the amino acid sequence of p34 are aligned with a minimum number of gaps. Highly conserved residues (identified in three or more repeats) are reversed out. The consensus sequence is also indicated at the bottom.

the hydrophobic core of the membrane (Figure 3). Since p34 appears to be an integral membrane protein (Ichimura et al., 1992), this region is most probably integrated into the membrane bilayer. It should also be noted that this hydrophobic segment is preceded by a cluster of positively charged residues (Arg-239, Lys-240 and Arg-243). It is known that such a positively charged cluster anchors the N-terminal portion of the transmembrane segment on the cytoplasmic surface and enhances the translocation of the following portion across the membrane during its biosynthesis (Parks and Lamb, 1991; Sakaguchi et al., 1992).

The hydrophobic segment divides the protein into two domains: a large N-terminal domain (molecular mass 28 kDa) and a small C-terminal domain (molecular mass 4 kDa). The N-terminal domain, comprising 244 residues, contains two distinct structural regions. The first region begins with residue 20 and ends at residue 123. It is composed of 4.5 successive repeats of

Table 1 LRR protein superfamily

Consensus sequences are aligned according to the consensus sequence originally proposed by Takahashi et al. (1985). Hyphens and Greek alphas (α) indicate non-conserved positions and conserved aliphatic positions respectively. Lower-case letters indicate relatively less conserved position, as indicated in the original reports of these sequences. References for consensus sequences: p34, the present paper; leucine-rich α_2-glycoprotein, Takahashi et al. (1985); human acid-labile subunit, Leong et al. (1992); rat acid-labile subunit, Dai and Baxter (1992); carboxypeptidase N 83 kDa subunit, Tan et al. (1990); human proteoglycan I (biglycan), Fisher et al. (1989); bovine proteoglycan I (biglycan), Neame et al. (1989); human proteoglycan II (decorin), Krusius and Ruoslahti (1986), Patthy (1987); chick proteoglycan II (decorin), Li et al. (1992); fibromodulin, Oldberg et al. (1989); chick keratan sulphate proteoglycan (lumican), Blochberger et al. (1992); proteoglycan-Lb, Shimomura and Kimata (1992); slit, Rothberg et al. (1990); human oligodendrocyte-myelin glycoprotein, Mikol et al. (1990); bovine oligodendrocyte-myelin glycoprotein, Tan and Lim (1992); platelet GPIbα, Titani et al. (1987), Lopez et al. (1987); platelet GPIbβ, Lopez et al. (1988); platelet GPV, Shimomura et al. (1990), Roth et al. (1990); platelet GPIX, Hickey et al. (1989); LH/CG receptor, McFarland et al. (1989); connection, Nose et al. (1992); chaoptin, Reinke et al. (1988); Toll, Hashimoto et al. (1988); *Saccharomyces cerevisiae* adenylate cyclase, Kataoka et al. (1985); *Schizosaccharomyces pombe* adenylate cyclase, Yamawaki-Kataoka et al. (1989); GRR1, Flick and Johnston (1991); human RNAase inhibitor, Lee et al. (1988); porcine RNAase inhibitor, Hofsteenge et al. (1988); U2 snRNP A′ protein, Sillekens et al. (1989), Fresco et al. (1991); sds22+, Ohkura and Yanagida (1991); Drt100 (*Arabidopsis thaliana*), Pang et al. (1992); internalin and *inlB*-gene product (*Listeria monocytogenes*), Gaillard et al. (1991); ESAG8 (*Trypanosoma brucei*), Revelard et al. (1990); eESAG8c, Ross et al. (1991); RAD1 and RAD7, Schneider and Schweiger (1991).

Protein	Species	Extra/Intracellular distribution	Repeats	Consensus sequence
p34	Rat	RM membrane	4.5	P---F--L--L--LDLS-N-L--L
Leucine-rich α_2-glycoprotein	Human	Serum	8	P--LL-----L--L-L--N-L--L
Acid-labile subunit	Human	Serum		
	Rat	Serum	19–20	
Carboxypeptidase N 83 kDa subunit	Human	Plasma	12	P--αF--L--L--L-L--N-L--L
Proteoglycan I (biglycan)	Human	Extracellular matrix	12	Pk--fs----L--L-L-nNkI--V
	Bovine	Extracellular matrix	10	L--L-L--N-LS-L
Proteoglycan II (decorin)	Human	Extracellular matrix	10	----------L--L-L--N-IS-V
	Chick	Extracellular matrix	9	L--L-L--N-L/I
Fibromodulin	Bovine	Extracellular matrix	10	P------------L--L-L-HN-I---
Keratan sulphate proteoglycan (lumican)	Chick	Extracellular matrix	9	L--L-L--N-L/I
Proteoglycan-Lb	Chick	Extracellular matrix	8	----F--L--L--L-L--N-I--I
slit	*Drosophila*	(secretory protein)	18	----F--L--L--L-L--N-I--L
Oligodendrocyte-myelin glycoprotein	Human	Plasma membrane*	7.5	p---l----nL--LdLSnN-Lt-L
	Bovine	Plasma membrane*	3	P-GLα--L--LL-L--N-L--L
Platelet GPIbα	Human	Plasma membrane†	7	P-GLL--LP-L--L-LS-N-LTTL
Platelet GPIbβ	Human	Plasma membrane†	1	
Platelet GPV	Human	Plasma membrane†	(≥ 10)	P---F--L--L--L-L--N-L--L
Platelet GPIX	Human	Plasma membrane†	1	
LH/CG receptor	Rat	Plasma membrane	14	
Connectin	*Drosophila*	Plasma membrane	10	---AFA-L--L--LNL-NN-I/L--I/L
Chaoptin	*Drosophila*	Plasma membrane	41	P---F--L--L--LDLS-N-L--I
Toll	*Drosophila*	Plasma membrane	15	P--LF-H--NL--L-L--N-L--L
Adenylate cyclase	*S. cerevisiae*	Plasma membrane	26	P--α--L--L--L-L--N-α--α
	S. pombe	Plasma membrane		P--α--L--L--L-L--N-α--α
GRR1	*S. cerevisiae*	Cytoplasm	12	Lα--CP-L--V-L--C-NITD--L--
RNAase inhibitor	Human	Cytoplasm	7	L-S-LR----L-EL-LS-N-LGDA
	Porcine	Cytoplasm	8	---L--P---LE-L-L--C-LT---C--L
			7	--αL-----L-EL-L--N-LGD-Gα--L
U2 snRNP A′ protein	Human	Nuclei	5.5	---ll--L--L--L-α--N-α--L
sds22+	*S. pombe*	Nuclei, Cytoplasm	11	-I--L--L--L--L-L--N-I-
Drt100	*A. thaliana*	Chloroplast	4	
Internalin	*L. monocytogenes*	Cell wall	15	PL--LTNL--L-L/I--NQI-DI-
InlB-gene product	*L. monocytogenes*		8	I/L--L--L--L--L-L--N-I-D
ESAG8	*T. brucei*		9	--α-Dα-Gα--L-nLEeLs/yLsGC
			10	--α--α--α--L-NL-v/eLdαSgC
eESAG8c	*T. equiperdum*		9	nl--lkvL-αs-C--α-dLsGL--L--LE-L-L-gC--αt----α
RAD1	*S. cerevisiae*		9	
RAD7	*S. cerevisiae*		12	

* Localized at myelin.
† Localized at platelet.

23–24 amino acids. The repeat motif has regularly spaced leucine residues at positions 8, 11, 14, 16, 21 and 24 and shares conservative proline, phenylalanine, aspartic acid and asparagine residues at positions 1, 5, 15 and 19 respectively (Figure 4). The second region begins with residue 156 and ends at residue 235. This region is rich in charged amino acids, especially lysine (K), arginine (R) and glutamic acid (E) (64 % of the total number of amino acids), thus we termed it the 'KRE region'. We also noted that these amino acids are highly concentrated in this KRE region; it contains 48.6, 68.2 and 78.3 % of the total numbers of lysine, arginine and glutamic acid residues respectively (Figure 3, lower panel). The C-terminal domain of approx. 38 residues, on the other hand, has few charged amino acids and no repeating unit.

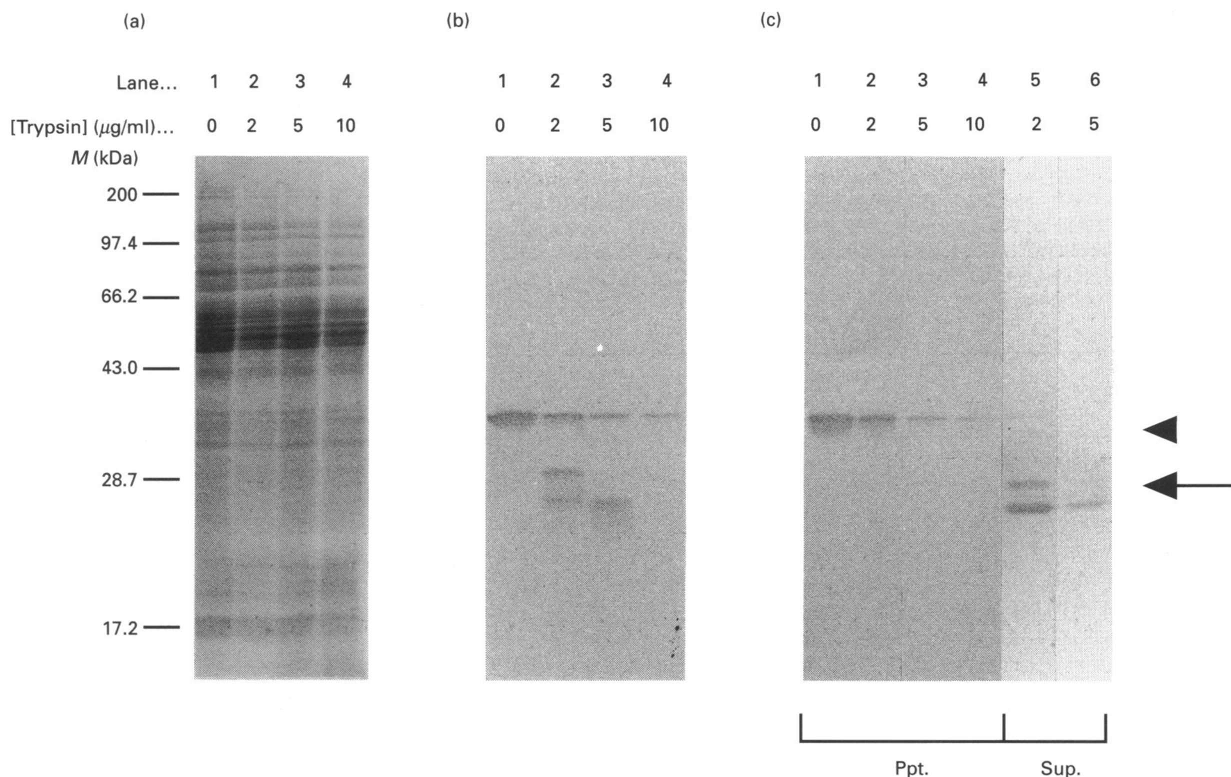

Figure 5 Immunoblotting with anti-p34 antibody of trypsin-treated stripped RM membranes

Stripped (ribosome-depleted) RM membranes were treated with the indicated amounts of trypsin for 60 min at 0 °C. The samples (30 µg each) were then analysed by SDS/PAGE (Coomassie Blue staining, **a**) and immunoblotting (**b**). The samples were also washed in 0.1 M Na_2CO_3 (pH 11.5), separated into pellet (Ppt.) and supernatant (Sup.) fractions by centrifugation (30 min, 100000 ***g***), and then analysed by SDS/PAGE followed by immunoblotting (**c**). The positions of p34 and 28 kDa fragment were indicated by an arrowhead and an arrow respectively. The molecular-mass (*M*) markers were myosin (200 kDa), phosphorylase *b* (97.4 kDa), BSA (66.2 kDa), ovalbumin (43.0 kDa), carbonic anhydrase (28.7 kDa) and myoglobin (17.2 kDa). Note that the trypsin treatments do not affect most RM proteins.

Secondary-structure prediction using the Chou and Fasman program indicated the presence of α-helices (42%), β-sheets (37%), and seven potential β-turns in the p34 molecule. The predicted secondary structures of the internal repeats did not show clear repetitive patterns. The predicted structures of the KRE region and the C-terminal domain, on the other hand, showed mainly α-helical and β-sheet structures respectively.

Sequence similarity to other proteins

We searched several protein/nucleotide sequence databases using both the entire sequence of p34 and various segments of the sequence. No striking sequence similarity was found, using the entire sequence or the C-terminal domain sequence (residues 270–307), with any other proteins present in the databases, including known RM proteins. We found, however, that the repeated sequence showed significant similarity to the tandem repeat motif called LRR (Table 1). LRRs have been identified in a variety of proteins from a wide spectrum of eukaryotes (see Table 1). These repeats are, on average, 24 amino acids in length and are characterized by a periodic distribution of hydrophobic amino acids, especially leucine, separated by hydrophilic amino acids. The motif repeats can adopt an amphipathic sequence (Takahashi et al., 1985) and potentially function in mediating protein–protein (Wicki and Clemetson, 1985; Handa et al., 1986; Krantz and Zipursky, 1990; Suzuki et al., 1990) or protein–membrane (Takahashi et al., 1985; Kataoka et al., 1985) interactions. On the other hand, the LRRs of p34 are unique in the residues between the conservative residues mentioned above, as well as the number of repeating units (4.5 times) (shown in Table 1). In addition, p34 does not contain sequences extending to either N- or C-terminal LRR-flanking sequences, which are known to show amino acid similarity in some of the members of this protein family.

We also found a striking resemblance of the KRE region of p34 with a series of nucleotide-binding proteins, including sea-urchin histone H1 (residues 128–206; 28% identity), *Drosophila* 70 K U1 snRNP (residues 285–362; 25% identity), *Xenopus* EF-1 γ-chain (residues 204–283; 21% identity) and bovine ribonucleoprotein La (residues 301–380; 25% identity). In addition, the C-terminal half of this region (residues 192–235) exhibits, when two gaps are inserted, 40% amino acid identity with the V region of the SRP receptor (Lauffer et al., 1985). The similarities between the KRE region and these regions of the described proteins are primarily due to the frequent occurrence of positively charged amino acids. We propose that this region and/or the LRR region may be important for the observed ribosome-binding activity of the p34 protein, as described below.

Membrane topology of p34

Protein p34 was not extracted from the RM membranes on treatment with Na_2CO_3 (Ichimura et al., 1992; see also Figure 5). This, together with its high sensitivity to trypsin (Tazawa et al.,

1991; Ichimura et al., 1992), suggested that protein p34 might be a membrane-embedded RM surface protein with a large cytoplasmically disposed portion.

To study the disposition of p34 in the RM membrane, we incubated stripped (ribosome-depleted) RM with various concentrations of trypsin, and the resultant tryptic fragments of p34 were analysed by immunoblotting with polyclonal anti-p34 antibodies (Figure 5). We found that a partially proteolysed form with a molecular mass of about 28 kDa, which is almost the same as the size of the large N-terminal domain of p34, was generated during the digestion with the lowest concentration of trypsin (Figure 5b, lane 2; indicated by an arrow). The 28 kDa fragment was released from the RM membrane by washing the membrane with 0.1 M Na_2CO_3 (Figure 5c, lane 5), indicating that it is disposed in the cytoplasmic face.

The amino acid sequence of the p34 protein contains only one hydrophobic segment long enough to span the membrane bilayer, but does not contain a cleavable signal sequence at the N-terminus. On the basis of these and the newly found topological features, we propose the structural model for p34 illustrated in Figure 6. p34 resides in the membrane with a single spanning segment between residues 245 and 269, its N-terminal side (244 residues) being disposed in the cytoplasm and its C-terminal side (38 residues) being luminal in orientation (type II orientation). This topology places the positively charged side chains (Arg-239, Lys-240 and Arg-243) located on the N-terminal side of the postulated membrane-spanning domain on the cytoplasmic surface, which is consistent with the fact that positive charges are found preceding the membrane-spanning sequences of many eukaryotic type II membrane proteins.

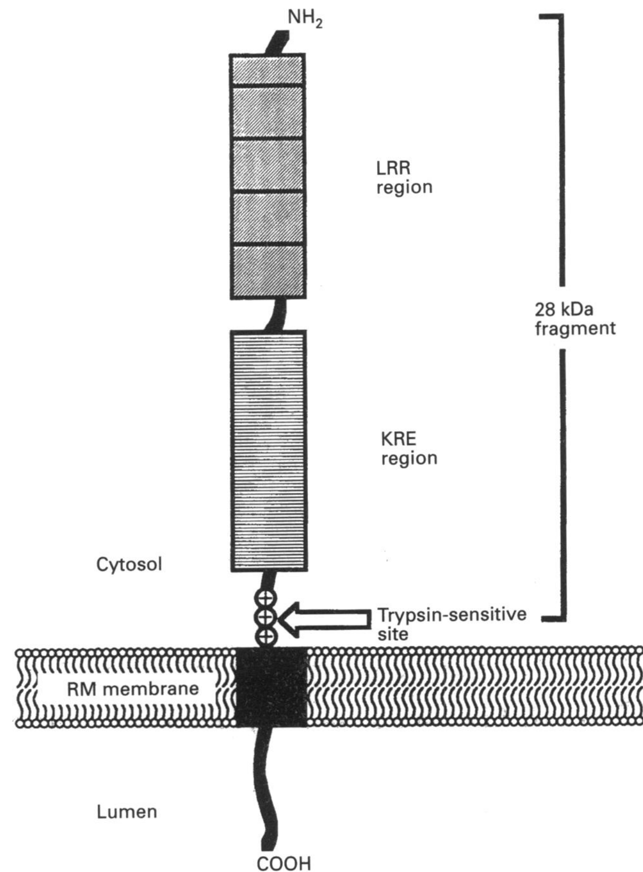

Figure 6 Model for the topology of p34 in the RM membrane

The LRR, KRE and transmembrane regions are shown by diagonally hatched, horizontally hatched and closed bars respectively. The positively charged residues located at the N-terminus of the membrane-spanning domain are depicted as small circles each containing a plus sign. The putative trypsin-sensitive site is indicated by the arrow.

Functional implications

The signal hypothesis predicts that the signal peptides of secretory proteins allow the targeting of ribosomes and nascent polypeptides to the RM membrane, and that the attachment to the RM membrane is mediated by the ribosomes (Blobel and Dobberstein, 1975). The ribosome receptor is believed to be involved in this attachment process and regulates the turning on/off of ribosomes (ribosomal cycle) in the course of protein translocation. It appears likely that the ribosome receptor is not single polypeptide chain but a site comprising many or all of the RM membrane factors required for translocation [see, for a review, Sanders and Schekman (1992)]. Little is known, however, about the molecular mechanism that regulates the protein-translocation-coupled ribosomal cycle. Recently, Simon and Blobel (1991) demonstrated that the attached ribosome keeps the protein translocation channel in an open conformation.

Sequence analysis of p34 revealed two potential sites that could serve for the attachment of ribosomes to the RM membrane. The p34 protein contains 4.5 LRRs (Figure 4) in the postulated cytoplasmic domain (Figure 6). There is direct evidence that LRRs are involved in protein–protein interactions. For instance, the LRRs of human glycoprotein Ibα bind to von Willebrand factor (Wicki and Clemetson, 1985; Handa et al., 1986), and those of yeast adenylate cyclase appear to interact with *ras* protein and are necessary for adenylate cyclase activation (Suzuki et al., 1990). By analogy, the LRRs of p34 may provide a site of interaction with ribosomes or may associate with proteins to form translocation sites through which nascent polypeptides are transferred across the membrane bilayer of the RM.

Another site is the hydrophilic region (KRE region). Since the interaction between p34 and ribosomes appears to largely result from an electrostatic interaction (Ichimura et al., 1992), the charged residues in this region might be responsible for the ribosome-binding activity of p34. The KRE region is similar to regions of a series of nucleotide-binding proteins. The corresponding region of histone H1 is known to bind with DNA, and the region of 70K U1 snRNP might contact RNA (Query et al., 1989). The V region of the SRP receptor is proposed to be a site of interaction with 7SL RNA in SRP, together with regions III and IV (Lauffer et al., 1985). The similarities between the KRE region and the above suggest the possibility that this region could interact with the RNA part of a ribosome, although there is no evidence for such an interaction at present. There has been no report on the involvement of RNA molecules in the ribosomal cycle, with the exceptions of 7SL RNA in SRP and ribosomal RNA in ribosomes. It appears unlikely that p34 binds with 7SL RNA, since no detectable 34 kDa-range polypeptides were eluted from an affinity column coupled with SRP (Tajima et al., 1986).

It is thus possible that either LRRs or KRE regions, or both, contribute to the ribosome binding on the rough endoplasmic reticulum. Clearly, however, further detailed examination of the ribosome-binding properties of these regions is needed to test this or other hypothesis.

To our knowledge, the p34 protein is the first member of the LRR family identified in the RM membrane. Knowledge of its amino acid sequence and the availability of its cDNA should aid

elucidation of its potential biological function in the ribosome-binding process. This study should also facilitate studies concerning the roles of the LRR protein superfamily.

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