The cDNA for the ubiquitin–52-amino-acid fusion protein from rat encodes a previously unidentified 60 S ribosomal subunit protein

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Rat cDNAs for a 52-amino-acid ribosomal protein (CEP52) that is typically formed as a ubiquitin fusion protein, were cloned following reverse transcription and PCR amplification. CEP52 sequence conservation is demonstrated by the similarity of the human and rat cDNA sequences and the identity of the predicted proteins. Amplification of rat cDNA with a primer specific for the 3′ non-coding region of the CEP52 gene, in combination with a consensus primer for the 5′ end of the ubiquitin coding sequence, provided evidence that the rat CEP52 gene is fused to a ubiquitin reading frame. Direct sequence analysis of this PCR product confirmed the in-frame fusion of a ubiquitin coding sequence to the rat CEP52 gene. Antibodies against a synthetic CEP52 peptide were used to show that expressed CEP52 is associated with the 60 S ribosomal subunit, and that it is not linked to ubiquitin. The quantity of CEP52 found in different tissues is quite variable, but appears to correspond to the amount of ribosomes present. Although the human, *Arabidopsis thaliana* and *Nicotiana tabacum* CEP52 genes contain introns within the CEP52 coding region, the rat CEP52 coding sequence appears to lack insertions.

INTRODUCTION

The characterized genes for two small eukaryotic ribosomal proteins are fused to ubiquitin coding sequences, or in one case to a ubiquitin-like protein [1]. The smaller polypeptide formed as a ubiquitin carboxyl-extension protein (CEP52) is typically 52 amino acids in length. CEP52 is conserved in sequence; for example, the predicted proteins from *Drosophila melanogaster* [2] and *Arabidopsis thaliana* [3] differ from human CEP52 [4] by only three and seven amino acids respectively. The fusion of genes for proteins involved in the opposing processes of protein catabolism (ubiquitin), and protein biosynthesis (ribosomal proteins) suggests that this unusual gene arrangement could have a regulatory purpose. Although, the fused gene arrangement is highly conserved, CEP52 can function in yeast even when not formed as a ubiquitin fusion protein [5]. However, the fusion protein is more effective and it has been proposed that ubiquitin serves as a chaperone for the ribosomal protein [5].

CEP52 gene deletions have shown that CEP52 is essential in yeast [5], but have not revealed its function. We sought to examine CEP52 expression in a mammal. Rat ribosomal components have been structurally characterized more thoroughly than those of any other vertebrate. As of 1991, 43 rat ribosomal proteins had been sequenced by biochemical or molecular methods [6]. The larger ubiquitin-fused ribosomal protein has been shown to correspond to rat ribosomal protein S27a [7], but no specific protein has been identified as the rat counterpart to CEP52. We have established that the rat produces a CEP52 polypeptide, determined its ribosomal subunit location and characterized its primary structure by cDNA sequence analysis.

MATERIALS AND METHODS

Biochemicals and dyes were obtained from Sigma Chemicals. Nitrocellulose (0.1 μm pore size) was manufactured by Schleicher & Schuell and Immobilon-P [poly(vinylidene difluoride) (PVDF)] membrane was purchased from Millipore Corp. Normal protein and prestained broad-range molecular mass markers were purchased from Bio-Rad while prestained Rainbow markers came from Amersham. RNasin and *Taq* DNA polymerase were obtained from Promega. MetaPhor agarose was obtained from FMC, and the DNA size markers were from Research Genetics. MetaPhor gels (3 %) were prepared in 1 × TBE (90 mM Tris base, 90 mM boric acid and 2 mM EDTA, pH 8.0).

Antisera

Anti-CEP52 antibodies were prepared against a synthetic peptide that corresponds to a portion of human CEP52 [8]. Ubiquitin antisera were a gift from M. Rechsteiner (University of Utah, Salt Lake City, UT, U.S.A.) and were prepared as reported [9]. Affinity-purified, secondary antibodies [peroxidase-conjugated goat anti-(rabbit IgG)] were purchased from Boehringer Mannheim or Jackson ImmunoResearch.

Primers

A partial mouse CEP52 cDNA sequence [10] was used to design CEP52-specific primers. The initial amplification utilized primer 52-1 which corresponds to the first 20 nucleotides of the mouse CEP52 coding sequence (ATCATTGAGCCATCCCTTCG). A nested primer, 52-2, matches bases 21 to 40 of the mouse CEP52 cDNA (TCAGCTTGCCCAGAAGTACA) and was used for second-round amplification in some studies. A forward primer that would amplify a ubiquitin gene was based on the consensus sequence of the first 21 nucleotides of each ubiquitin repeat of the cloned rat and mouse polyubiquitin genes [10,11] and some of the known ubiquitin–CEP52 gene sequences [3,4,10]. This forward primer, Ub-F, consisted of the sequence (ATGCAGATCTTCGTGAAGACC). A reverse primer (52-R) specific for the 3′ non-translated region of the rat CEP52 gene (GCCAGGTATGGCCCAGCTCTA), consisted of complementary sequence to the stop codon and 18 additional nucleotides of the 3′ non-coding region.

Abbreviations used: RACE, rapid amplification of cDNA ends; PVDF, poly(vinylidene difluoride).

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The reported nucleotide sequence data will appear in the Genbank and EMBL Databases under accession number U25064.

cDNA cloning and sequencing

Total RNA was isolated from the liver of a female CD rat by the method of Chomczynski and Sacchi [12]. The synthesis of cDNA and its amplification was carried out using the 3′ rapid amplification of cDNA ends (RACE) kit from BRL, according to the manufacturer's instructions except for the addition of 40 units of RNasin to each reaction. Products from amplifications were blunt-end ligated into the *Srf*I site of the pCR-Script SK(+) plasmid obtained as a PCR cloning kit from Stratagene. Minipreps from white colonies were restriction-enzyme-digested to confirm the presence of inserts. Two clones were sequenced in both directions using the Silver Sequence kit from Promega. Sequencing of cDNA clones was done with the gene-specific 52-1 and 52-2 primers, the 3′ RACE primer, the M13 reverse primer (TCACACAGGAAACAGCTATGAC), and the M13 forward primer (CGCCAGGGTTTTCCCAGTCACGAC). The ubiquitin forward primer (UB-F) was used for direct PCR product sequencing. PCR products were purified using either Glass Max columns from BRL or Qiagen-spin 20 columns.

Isolation of ribosomes and ribosomal subunits

Ribosomes used for single-dimension gels were isolated essentially by the method of Sherton and Wool [13] except that Lubrol PX was substituted for Lubrol WX and the components of some buffers were modified as noted. Tissues were homogenized in medium B (50 mM Tris, pH 7.6, 250 mM KCl, 12.5 mM $MgCl_2$, 5 mM EGTA and 0.25 M sucrose) [13], but when liver and kidney extracts were prepared the EGTA was omitted. The final ribosomal pellet was collected by centrifugation at 90000 ***g*** (r_{av} 11.8 cm) through a 0.5 M sucrose shelf prepared in medium C (20 mM Tris, pH 7.8, 80 mM KCl and 12.5 mM $MgCl_2$) [13]. For electrophoresis of proteins, the ribosomal pellet was homogenized in medium C containing 5 mM 2-mercaptoethanol and boiled following the addition of SDS sample buffer.

For the preparation of subunits, ribosomes were not exposed to high salt concentrations until the dissociation step. Rat liver was homogenized in medium B lacking EGTA and with a reduced KCl concentration (80 mM). Ribosomes were pelleted through medium C containing 0.5 M sucrose. The resuspended ribosomes were then incubated at 37 °C for 15 min in medium D (20 mM Tris, pH 7.8, 880 mM KCl, 12.5 mM $MgCl_2$ and 20 mM 2-mercaptoethanol) [13] that contained 0.2 mM puromycin. The sample was then layered on to a 15–30 % linear sucrose gradient containing: 50 mM Tris, pH 7.6, 3 mM $MgCl_2$, 500 mM KCl and 5 mM 2-mercaptoethanol. Subunits were separated by centrifugation at 82600 ***g*** (r_{av} 11.8 cm) for 5 h 40 min at 26 °C in a Beckman SW-27 rotor. The absorbance was monitored using a UV flow cell as the gradient was displaced from the tube with a 50 % sucrose solution.

Protein samples for two-dimensional analysis of total ribosomal polypeptides were isolated from ribosomes prepared without the use of detergents as described [14] except that 30 μg/ml cycloheximide was included in the homogenization buffer to prevent polysome run off. The polysome pellet was resuspended in buffer containing 0.5 M salt (10 mM Tris, pH 7.6, 0.5 M KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, and 0.25 M sucrose) and the ribosomes pelleted through a 1 M sucrose shelf prepared in the same buffer by centrifugation at 123000 ***g*** (r_{av} 6.9 cm) for 8 h. Ribosomal proteins were then extracted with 66 % acetic acid and lyophilized as previously reported [14].

SDS/PAGE and protein blotting

SDS/PAGE was performed in 10–20 % polyacrylamide gradient gels followed by protein transfer to nitrocellulose or PVDF as described previously [15]. Two-dimensional gel analysis and chemiluminescent detection were conducted as described [8].

RESULTS

To obtain cDNA clones of the rat CEP52 gene, total rat liver RNA was reverse-transcribed using the 3′ RACE method and amplified with primer 52-1, which is specific for the beginning of the CEP52 coding region (Figure 1a). Due to possible mismatches between 52-1 and the rat gene, a low-stringency annealing temperature was used for five cycles (37 °C) followed by 30 additional cycles at 45 °C. PCR products, consisting of one major and three minor bands, were purified and reamplified using the nested 52-2 primer, resulting in the synthesis of a single product. Purified product from this second-round amplification, as well as unpurified DNA from an independent first-round amplification, were cloned into the pCR-Script SK(+) plasmid. Two clones containing inserts of similar size (pSK47 and pSK51) that could be reamplified with primer 52-2 and the RACE 3′ primer were used for sequence analysis.

Plasmid inserts were sequenced in both directions and found to be identical in all shared regions. A composite sequence of the clones is shown in Figure 1(b). In a single amplification clone (pSK47), the first 20 nucleotides of the CEP52 coding region automatically correspond to the 52-1 primer sequence and this region is not present in the clone (pSK51) derived from the nested amplification using primer 52-2. Therefore, the rat sequence of the region corresponding to primer 52-1 had to be determined independently (discussed below). The shared regions of the two clones were identical except for a minor difference at the 3′ ends, where pSK51 has ten additional nucleotides between the polyadenylation signal (AATAAA) and the beginning of the poly(A) sequence (Figure 1b).

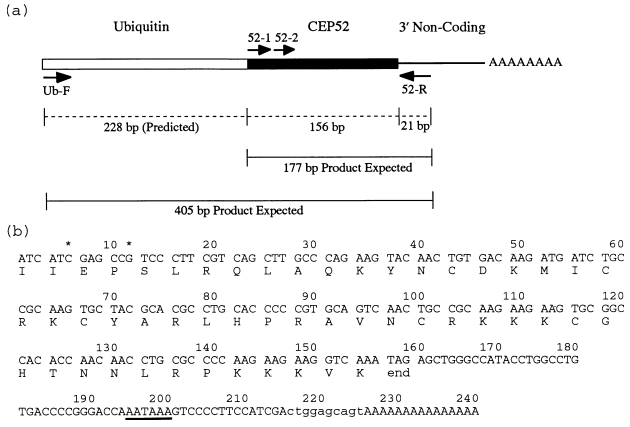

Figure 1 Schematic diagram and nucleotide sequence of the rat CEP52 cDNA

(**a**) The arrangement of ubiquitin and CEP52 coding sequences in the rat cDNA are shown. Arrows indicate the position and direction of gene-specific primers used in this study. The expected sizes for products generated from amplifications using either the 5′ forward (Ub-F) or 52-1 primer in combination with the gene-specific 3′ reverse primer (52-R) are indicated. (**b**) Nucleotide sequence determined for the rat CEP52 cDNA coding and 3′ non-coding regions. Nucleotides shown in lower-case type were present only in clone pSK51. The poly(A) signal is underlined and the amino acid sequence of rat CEP52 is indicated in the single letter format. Two nucleotides in the region of primer 52-1 that differ from the mouse sequence are indicated (*).

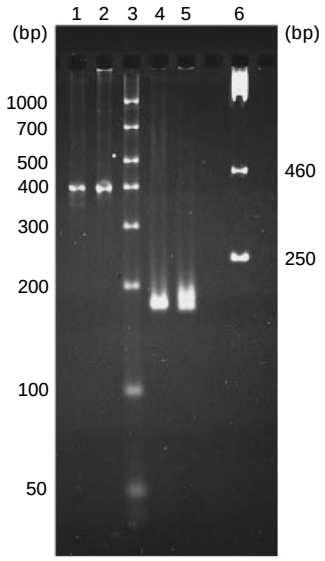

Figure 2 Size analysis of PCR products from cDNA and genomic templates using CEP52-specific primers

PCR products were examined by electrophoresis in a 3% MetaPhor gel prepared in 1 × TBE buffer. Rat cDNA was amplified with the ubiquitin-specific forward primer (Ub-F) and the CEP52-specific reverse primer (52-R). Crude products of the amplification are shown in lane 1. Lane 2 contains purified product from an independent amplification that was partially sequenced. Amplification products obtained using primer 52-1 and the reverse primer (52-R) are in lanes 4 and 5. The template was cDNA in lane 4 and genomic DNA in lane 5. Sizes of DNA markers in lanes 3 and 6 are indicated on the left or right respectively.

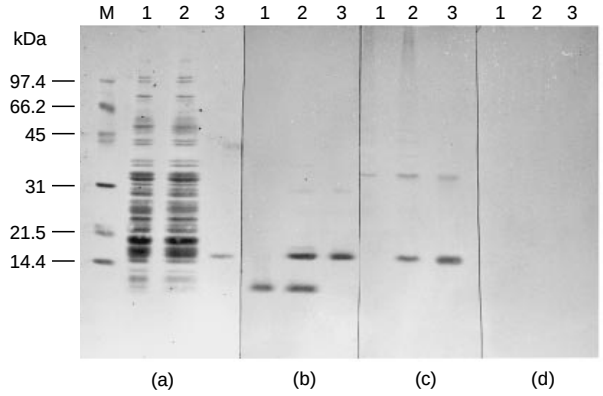

Figure 3 Expression of the rat CEP52 polypeptide

Proteins were resolved in a 10–20% polyacrylamide gel and transferred to nitrocellulose. Lanes contained rat ribosomal proteins (1), ribosomes plus the intact bacterially expressed ubiquitin-CEP52 fusion protein (Ub-52) (2), or the Ub-52 fusion protein alone (3). Proteins were detected using Amido Black stain (**a**), CEP52-specific antisera (**b**), or ubiquitin antisera (**c**). No immunoreactivity was seen with preimmune serum from the animal used to generate the CEP52-specific antiserum (**d**). The sizes of the Bio-Rad low-molecular-mass range markers included in lane (M) are indicated.

The rat CEP52 gene is fused to a ubiquitin gene

In order to specifically amplify CEP52 cDNAs, gene-specific primers for 3′ RACE corresponded to the CEP52 coding region. However, this approach made it impossible to determine from the cDNA clones whether the rat CEP52 gene is fused to a ubiquitin coding region. Two additional primers were synthesized in order to address this question. The forward primer (Ub-F) was designed to match the 5′ end of the highly conserved ubiquitin gene sequence as related in the Materials and methods section. A reverse primer (52-R) corresponded to the complementary sequence of the CEP52 3′ non-coding region. The positions of these primers and the size of the expected PCR product are indicated in Figure 1(a). Amplification of rat cDNA with this pair of primers produced a single product that was 400 bp in length (Figure 2, lanes 1 and 2). This result indicates that at least one ubiquitin gene is fused to the rat CEP52 coding sequence. The PCR product was purified and used as template in direct sequencing reactions. Sequence was obtained for approximately half of the ubiquitin coding region (over 100 bases) and it was nearly identical to known ubiquitin gene sequences (results not shown).

The 400 bp PCR product was also used to determine or confirm the sequence of the regions corresponding to primers 52-1 and 52-2. In the tract corresponding to primer 52-1 two nucleotide differences were identified between the mouse and rat sequences and these positions are indicated in Figure 1(b). The PCR product sequence corroborated the identity of the rat and mouse genes in the region of primer 52-2 as determined from the single amplification clone pSK47.

Comparison of the 156 bp coding regions for the rat and human CEP52 cDNA sequences revealed 89% identity. The 16 differences were all located in the third base of the affected codon and none of the changes altered the encoded amino acid. Therefore, the predicted amino acid sequence for rat (Figure 1b) and human CEP52 [4] are identical.

Ribosomal CEP52 is not ubiquitin fused

Synthesis of CEP52 in the rat was confirmed by Western blot analysis of rat ribosomal proteins using a CEP52-specific antiserum (Figure 3). The anti-CEP52 serum reacted strongly with a single ribosomal protein (Figure 3b, lane 1). The immunoreactive protein was considerably smaller than the intact human ubiquitin–CEP52 fusion protein which was isolated following expression of the fusion gene in bacteria [16]. No rat polypeptide that corresponds in size to the unprocessed fusion protein reacted with either CEP52 (Figure 3b) or ubiquitin antisera (Figure 3c), although both antisera readily detect the bacterially expressed fusion protein. Therefore, ribosomally associated CEP52 is not covalently linked to ubiquitin.

CEP52 is found in similar amounts to other ribosomal proteins

No rat ribosomal protein has been identified as the rat homologue of CEP52, but recent studies have shown that the high charge and small size of *Drosophila* CEP52 allowed its complete separation from other ribosomal proteins by two-dimensional electrophoresis, even when the sample consisted of total ribosomal protein [8]. The resolved *Drosophila* CEP52 was readily detected immunologically and by direct staining following the transfer of ribosomal proteins to a membrane support [8]. Application of the same system to total rat ribosomal protein (Figure 4) resulted in the identification of CEP52 as one of the smallest, most basic ribosomal proteins present on salt-washed rat ribosomes.

CEP52 is associated with the 60 S ribosomal subunit

To determine the ribosomal subunit location of rat CEP52, ribosomal subunits were separated by centrifugation through a sucrose gradient. Proteins present in every other fraction were subjected to electrophoresis and immunodetection. The analysis

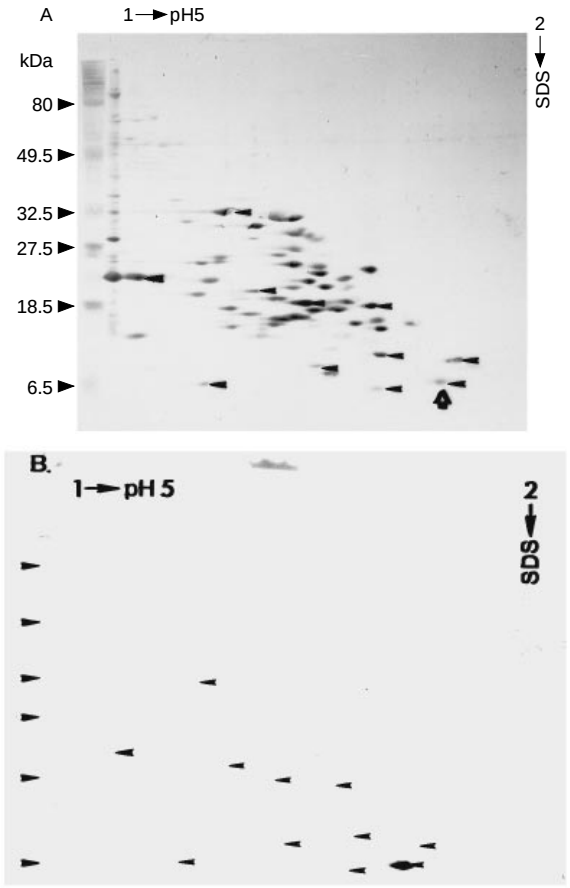

Figure 4 Identification of CEP52 in total ribosomal protein preparations

Ribosomal proteins prepared without the use of detergent as described in the Materials and methods section were suspended in acetic acid/urea sample buffer [8]. A 20 μl sample containing 300 μg of protein was applied to each gel and subjected to electrophoresis as reported [8]. (**A**) Amido Black-stained PVDF membrane transfer of a gel. (**B**) An identical gel was transferred to nitrocellulose and reversibly stained with Ponceau S to identify the positions of specific proteins (left-pointing arrows) and molecular-mass markers (right-pointing arrows) to serve as position and size markers. Following the reversible staining, CEP52 was detected immunologically. Detection utilized luminol chemiluminescence and a 2 s exposure to X-ray film. The arrow in panel (**A**) indicates the protein that reacts with anti-CEP52 antibodies. Protein sizes are based on Bio-Rad broad-range prestained molecular mass markers.

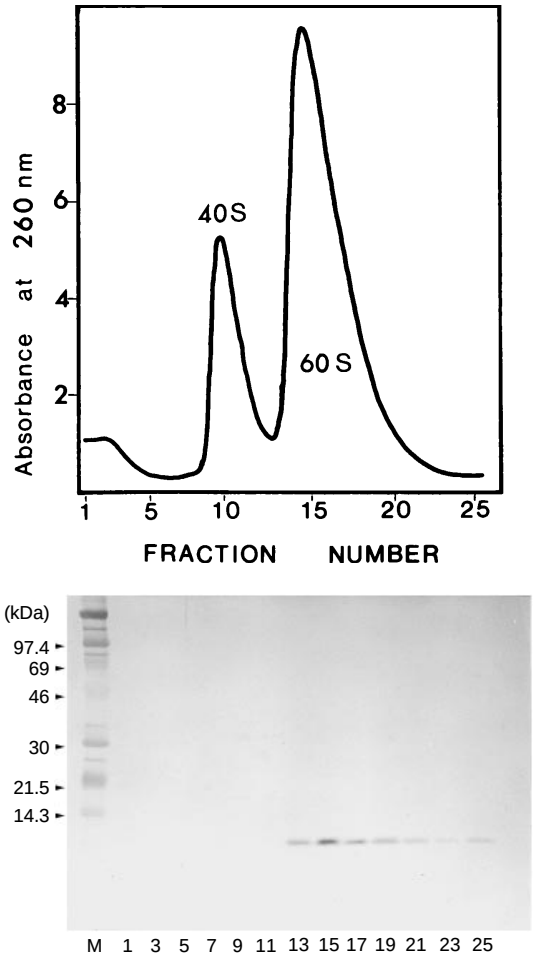

Figure 5 Ribosomal subunit location of rat CEP52

Rat ribosomal subunits were resolved by centrifugation through a 15–30% sucrose gradient. Fractions of 1.5 ml were collected following the measurement of the absorbance at 260 nm using a flow cell. Upper panel: Absorbance profile of the gradient in which sedimentation was from left to right. Lower panel: Proteins in odd-numbered fractions were resolved in 10–20% gradient gels. Following transfer to a nitrocellulose membrane, CEP52 was detected using CEP52-specific sera. Colorimetric detection of peroxidase-coupled secondary antibodies utilized 4-chloro-1-naphthol. The sizes of Rainbow molecular mass markers included in lane (M) are indicated.

revealed the presence of CEP52 in fractions corresponding to the large subunit and polysomes (Figure 5). This indicates that CEP52 is associated with the large ribosomal subunit, a finding that is consistent with observations made in other organisms [5,8].

Two-dimensional gel analysis of 60 S subunit proteins

Since CEP52 is one of the most rapidly migrating proteins in a first-dimension gel of total ribosomal protein, it must be one of the most mobile proteins in the large subunit as well. Extracted 60 S subunit proteins were resolved on two-dimensional gels as carried out for total ribosomal protein. The stained pattern of proteins is shown in Figure 6(a). Immune detection identified the second fastest migrating protein as CEP52. Our two-dimensional gel system compares closely to system I of Madjar et al. [14]. In their analysis of rat 60 S subunit proteins, only L37a, L38 or L39 appear to be small and basic enough potentially to correspond to CEP52. However, the sequences of all three proteins have been determined and they do not coincide with CEP52 [17–19]. Therefore, we conclude that rat CEP52 is a previously uncharacterized 60 S subunit protein.

Tissue levels of CEP52 vary

The CEP52 polypeptide has not been directly characterized in a mammalian system. Since the mRNA level for CEP52 drops dramatically in stationary yeast [20] and *Drosophila* cells [2] we sought to determine whether CEP52 expression varies between tissues that have dividing cells and those where little cell division is taking place. CEP52, which is 30% lysine and arginine, was acid-extracted along with other basic proteins. Acid-soluble proteins were then resolved by SDS/PAGE and blotted to a membrane that was probed with anti-CEP52 sera. The immunoblot demonstrated remarkable differences in the quantity of CEP52 between tissues (Figure 7). Tissue weight to volume ratios were maintained during extraction; therefore certain tissues such

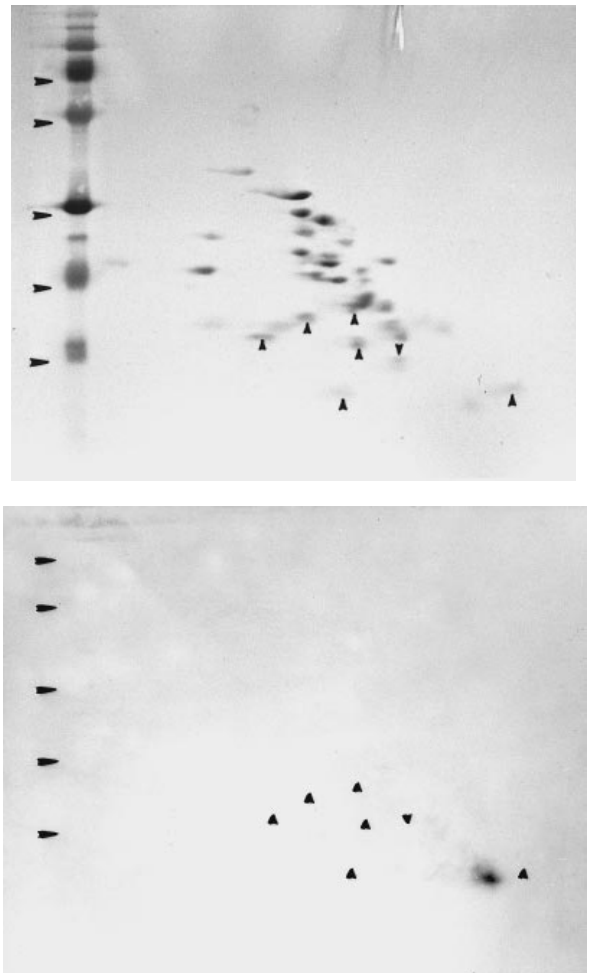

Figure 6 Two-dimensional electrophoresis of 60 S subunit proteins

Proteins extracted from isolated 60 S subunits were resolved as described for total ribosomal protein (Figure 4). Upper panel: Amido Black-stained nitrocellulose transfer. Lower panel: An identical gel was reversibly stained with Ponceau S, then immunodetection was carried out using anti-CEP52 serum. The immunoreactive protein was detected using luminol chemiluminescence and X-ray film [8]. Right-pointing arrows indicate the positions of the five smaller protein standards (Rainbow Markers) as shown in Figure 5.

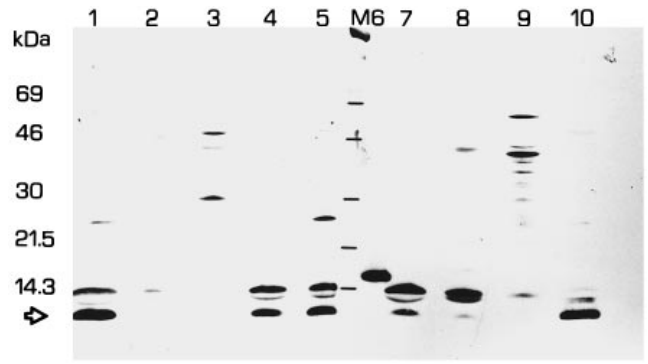

Figure 7 Tissue-specific levels of CEP52 expression

Acid extracts of rat tissues were prepared by the method of Bonner et al. [24], except that the extract was neutralized with NH_4OH following the addition of one-third volume of 4 × SDS sample buffer. Sample volume was held constant relative to tissue weight and equal volumes of extract were resolved on a 10–20% gradient gel. Proteins reactive with the anti-CEP52 serum were detected using luminol-based chemiluminescence [8]. Extracts were prepared from spleen, 1; brain, 2; skeletal muscle, 3; kidney, 4; liver, 5; lung, 7; heart, 8; intestine, 9; and salivary gland, 10. Intact ubiquitin–CEP52 was loaded into lane 6. The sizes of Rainbow molecular-mass markers present in lane M are shown on the left. The arrow indicates the position the CEP52 polypeptide.

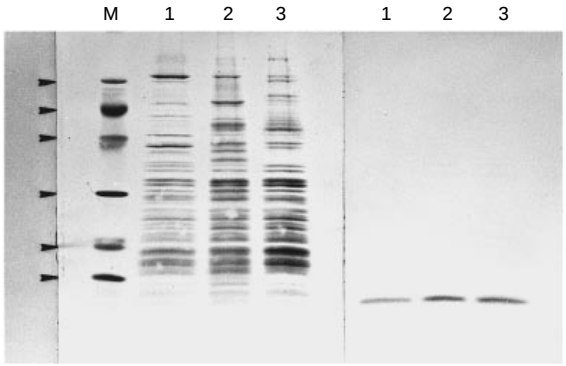

Figure 8 Ribosomes from different tissues contain similar amounts of CEP52

Proteins from equal quantities of ribosomes (0.7 A_{260} units) from rat liver (1), kidney (2), or skeletal muscle (3) were separated in 10–20% gradient gels. (**a**) Amido Black-stained transfer of resolved proteins. (**b**) Immunoblot of an identical gel reacted with anti-CEP52 serum. Immune detection utilized the colorimetric reaction of peroxidase with 4-chloro-1-naphthol. Molecular mass markers (lane M) were the same as those shown in Figure 3.

as spleen, liver and salivary gland contain higher amounts of CEP52 per gram (wet weight) than other tissues. CEP52 could be detected in all rat tissues except intestine; however, detection in skeletal muscle and brain required extended exposure of the blot (results not shown).

Using the bacterially expressed Ub-52 fusion protein for comparison (Figure 7, lane 6), no more than a minor amount of unprocessed Ub-52 fusion protein appears to be present in any tissue. However, a pair of 14 kDa proteins intermediate in size between CEP52 and the intact ubiquitin–CEP52 fusion protein were readily detected in a number of tissues. These proteins have been detected in multiple acid extract preparations, using three independent antibody preparations (results not shown). Although these proteins are too small to be the intact Ub-52 fusion protein, it is possible that a ubiquitin–CEP52 conjugate might migrate on gels differently from the linear fusion protein. In an attempt to gain evidence for the conjugate hypothesis, extracts were reacted with anti-ubiquitin sera. However, the 14 kDa proteins failed to react with anti-ubiquitin sera (results not shown). We also considered one other possibility, that these proteins result from altered CEP52 mRNA splicing (see below).

The amount of CEP52 per ribosome is constant

From the previous analysis (Figure 7) it is not clear whether the levels of CEP52 detected in rat tissues represent the amount of ribosomes present within that tissue, or if some tissues, such as muscle and brain, contain ribosomes that generally lack this small protein. To examine this question, ribosomes were prepared from tissues that express low amounts (skeletal muscle) or higher amounts (liver and kidney) of CEP52. Equal quantities of ribosomes from these sources were analysed by SDS/PAGE (Figure 8). The intensity of the stained proteins confirmed that essentially equivalent amounts of ribosomes had been loaded on the gels (Figure 8a). Immunoblot analysis of an identical set of

samples revealed a comparable amount of CEP52 in ribosomes from all three tissues (Figure 8b). This result indicates that the CEP52 content of ribosomes is independent of their tissue source.

The rat CEP52 coding region lacks introns

The CEP52 genes from man [4] and *Nicotiana tabacum* [21] each contain one intron with an in-phase open reading frame that could be translated if not spliced out (intron 4 in the human gene). For these genes, the insertion would not alter the reading frame of the region following the intron. In fact, the presence of an open reading frame in the tobacco insertion [21] probably delayed recognition of the sequence as an intron [22]. For these particular introns, translation of unspliced mRNA could result in the generation of larger forms of CEP52 that would still react with antibodies prepared against the C-terminus of CEP52. Although the genomic sequence of the rat CEP52 gene is not yet known, we sought to determine whether translation of unspliced CEP52 mRNA could potentially explain the larger proteins observed in acid extracts of rat tissues that react with the anti-CEP52 serum (Figure 7). Amplifications of the CEP52 region using the 52-1 primer and the CEP52-specific reverse primer (52-R) were carried out using cDNA and genomic DNA as templates. A 177 bp product is expected from the cDNA and the size calculated for the actual cDNA-derived PCR product was 180 bp. The product generated from genomic DNA formed a broader band of similar size. Even though the gel in Figure 2 suggests the potential for two bands in the genomic PCR product, a duplicate gel failed to give any indication for two bands in the sample (results not shown). To account for even a minimally sized intron (approx. 35 bp), the products from genomic DNA would be over 200 bp in length. However, as seen in Figure 2, the products from both genomic DNA and cDNA are clearly less than 200 bp. Therefore, we conclude that the rat CEP52 coding region lacks introns.

DISCUSSION

The rat CEP52 coding sequence

The similarity between the 5′ end of a partial mouse CEP52 cDNA [10] and the rat coding sequence allowed rapid cloning of the rat CEP52 cDNA. Although multiple products were observed in first-round amplifications that included five cycles at a low annealing temperature (37 °C), only one product was observed when all first-round cycles were annealed at 45 °C or when first-round products were reamplified with a nested primer. Two independently amplified clones were found to be identical in their shared regions, indicating the probable lack of *Taq* polymerase-generated sequence errors. The only difference in the clones, the length of the region between the poly(A) signal and the actual poly(A) sequence, would appear to be the result of 3′-end processing variability. The sequence of the PCR product resulting from amplification of cDNA with the Ub-F and 52-R primers revealed that the cloned CEP52 coding sequence is fused in-frame to the 3′ end of a ubiquitin gene. The lack of any PCR products larger than 400 bp in amplifications with primers Ub-F and 52-R suggests that a single ubiquitin gene is fused to the CEP52 coding sequence, but this must be confirmed by genomic analysis due to the bias of PCR toward smaller products.

CEP52 genes from multicellular organisms often contain introns [3,4,21], but not in all cases [2]. The possibility for uninterrupted translation of unspliced CEP52 mRNA exists for the fourth intron of the human gene [4] and for the only intron in a *Nicotiana tabacum* CEP52 gene [21]. The larger immunoreactive protein detected in Western blots of rat acid extracts caused us to consider the possibility that the rat CEP52 region might contain a translatable intron. Ribosomal protein genes from many organisms contain introns, but alternative splicing has only recently been reported for a ribosomal protein mRNA [23]. The comparison of PCR products from rat cDNA and genomic DNA showed no significant difference in size, when analysed in a high-resolution 3 % MetaPhor agarose gel, where a difference of 10 bases in length would have been detectable. Since the shortest known introns are just over 30 bases in length, and all known mammalian introns are considerably longer, we conclude that there is no evidence for the presence of introns within the CEP52 coding region of the rat ubiquitin–CEP52 gene. Preliminary experiments do indicate the presence of introns in the ubiquitin coding portion of the rat CEP52 gene (K. L.Redman, unpublished work).

The rat CEP52 polypeptide

Antibodies generated against the C-terminal end of the human CEP52 [8] readily detected rat CEP52 due to the total sequence identity between these proteins. The small size of the protein detected by anti-CEP52 sera (Figures 3 and 4) indicates that CEP52 found on ribosomes is not fused to ubiquitin. Free CEP52 probably results from proteolytic processing of the initial translation product, since the rat CEP52 cDNA characterized here and all other previously cloned CEP52 genes are ubiquitin fused. However, genomic analysis will be required to demonstrate that the rat has only ubiquitin-fused CEP52 genes.

CEP52 is not among the rat ribosomal proteins identified in studies of ribosomal proteins using two-dimensional gels. Previous studies used stained gels, but factors such as small size and acid solubility (resulting in loss from stained gels) may make transfer to a membrane an essential part of CEP52 detection. CEP52 is quite stable in 80 S ribosomes, but it can become susceptible to proteolysis following subunit separation (K. L. Redman, unpublished work). This can result in the absence of CEP52 from isolated 60 S subunits. Even in this study where resolved proteins were transferred to membranes, CEP52 was not detected as readily in two-dimensional gels of large subunit proteins as in gels of total ribosomal protein. Since CEP52 does appear to be present in quantities comparable with other small ribosomal proteins (Figure 4) we suggest that it be added to the ribosomal protein nomenclature as rat large ribosomal subunit protein L40.

Rat tissues were shown to contain significantly different amounts of CEP52. However, when isolated ribosomes from tissues with different levels of CEP52 were compared, equal amounts of CEP52 were found. Therefore, it appears that the variability in CEP52 quantity seen in acid extracts of tissues (Figure 7) represents the differences in ribosome abundance, rather than tissue-specific expression of CEP52. Intestine was the only tissue where CEP52 was not detected. It is not clear whether intestine does not synthesize CEP52, the protein abundance was below the level of detection, or the CEP52 was degraded during extraction. Further analysis will be required to clarify these possibilities as well as to establish the identity of the 14 kDa cross-reactive proteins found in most tissues.

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