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Ribosomal Protein L7a Is Encoded by a Gene (*Surf-3*) within the Tightly Clustered Mouse Surfeit Locus

AGATA GIALLONGO,† JEFFREY YON, AND MIKE FRIED*

Department of Eukaryotic Gene Organization and Expression, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, England

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The mouse Surfeit locus, which contains a cluster of at least four genes (*Surf-1* to *Surf-4*), is unusual in that adjacent genes are separated by no more than 73 base pairs (bp). The heterogeneous 5′ ends of *Surf-1* and *Surf-2* are separated by only 15 to 73 bp, the 3′ ends of *Surf-1* and *Surf-3* are only 70 bp apart, and the 3′ ends of *Surf-2* and *Surf-4* overlap by 133 bp. This very tight clustering suggests a *cis* interaction between adjacent Surfeit genes. The *Surf-3* gene (which could code for a basic polypeptide of 266 amino acids) is a highly expressed member of a pseudogene-containing multigene family. By use of an anti-peptide serum (against the C-terminal nine amino acids of the putative *Surf-3* protein) for immunofluorescence and immunoblotting of mouse cell components and by in vitro translation of *Surf-3* cDNA hybrid-selected mRNA, the *Surf-3* gene product was identified as a 32-kilodalton ribosomal protein located in the 60S ribosomal subunit. From its subunit location, gel migration, and homology with a limited rat ribosomal peptide sequence, the *Surf-3* gene was shown to encode the mouse L7a ribosomal protein. The *Surf-3* gene is highly conserved through evolution and was detected by nucleic acid hybridization as existing in multiple copies (multigene families) in other mammals and as one or a few copies in birds, *Xenopus*, *Drosophila*, and *Schizosaccharomyces pombe*. The *Surf-3* C-terminal anti-peptide serum detects a 32-kilodalton protein in other mammals, birds, and *Xenopus* but not in *Drosophila* and *S. pombe*. The possible effect of interaction of the *Surf-3* ribosomal protein gene with adjacent genes in the Surfeit locus at the transcriptional or posttranscriptional level or both levels is discussed.

The mouse Surfeit locus contains at least four tightly clustered housekeeping genes (*Surf-1* to *Surf-4*) which have been characterized by sequence and transcriptional analyses (12, 46–48). The direction of transcription of each of the four Surfeit genes alternates in respect to that of its neighbor (Fig. 1). In contrast to the large distances (tens to hundreds of kilobases) usually found between most adjacent mammalian genes, a maximum of only 73 base pairs (bp) separates any two adjacent Surfeit genes (Fig. 1). The heterogeneous 5′ ends of the divergent *Surf-1* and *Surf-2* genes are separated by 15 to 73 bp (46), and the 3′ ends of the *Surf-1* and *Surf-3* genes are separated by only 70 bp (12). In addition, the 3′ ends of the processed transcripts of the *Surf-2* and *Surf-4* genes overlap by 133 bp (47). The *Surf-1* and *Surf-2* genes can specify more than one gene product as the result of differential splicing (46), and transcription of the *Surf-4* gene is differentially regulated (47). The *Surf-3* gene contains eight exons and seven introns spread over about 3.0 kilobases and, when transfected into monkey COS cells, is highly transcribed to produce a 1.0-kilobase processed mRNA which would encode a highly basic protein of 266 amino acids (12). The *Surf-3* gene is a member of a multigene family, but whereas the *Surf-3* gene is transcribed and contains introns, the other members of the family are predominantly, if not entirely, processed pseudogenes (12).

The unusual tight gene clustering within the Surfeit locus suggests some type of *cis* interaction between the Surfeit genes (12, 48). Thus, it is of importance to identify the functions of the genes in the locus. In this communication, we show that the *Surf-3* gene is highly conserved through evolution and codes for mouse ribosomal protein L7a.

* Corresponding author.
† On leave of absence from the Istituto di Biologia dello Sviluppo, Consiglio Nazionale delle Ricerche, 90123 Palermo, Italy.

MATERIALS AND METHODS

Production and assay of antisera to synthetic peptides. Synthetic peptides of the putative *Surf-3* N-terminal nine amino acids (PKGKKAKGK) and nine C-terminal amino acids (AKELATKLG) (12) were prepared as previously described (33) and coupled to either bovine serum albumin or thyroglobulin with succinimidyl 4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate as described by Rothbard et al. (32). New Zealand rabbits (two for each peptide) were injected subcutaneously at multiple sites with 1.0 mg of thyroglobulin-conjugated peptides emulsified with complete Freund adjuvant. The rabbits were injected with equivalent amounts of the thyroglobulin-conjugated peptides in incomplete Freund adjuvant at 15-day intervals. Blood was withdrawn after injection 4, and the serum was tested by dot immunobinding (13) with 1.0 μg of the corresponding bovine serum albumin-coupled peptide per single spot. The specificity of the antisera was tested by immunoblotting of mouse cell proteins after resolution by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and transfer to nitrocellulose (40).

Immunoblotting analysis. Immunoblotting was performed as described previously (10), except that horseradish peroxidase-conjugated sheep anti-rabbit immunoglobulin G antibodies (Sera-lab) were used as second-layer antibodies. The peroxidase label was visualized by incubating the nitrocellulose strips for 2 min with a substrate containing 0.06% (vol/vol) 3′,3-diaminobenzidine and 0.02% (vol/vol) hydrogen peroxide in phosphate-buffered saline (PBS).

Cellular extracts and subcellular fractionation. Mammalian and chicken total cell extracts were prepared after cell lysis in Ripa buffer as previously described (10). Yeast extract was made by vigorous mixing of *Schizosaccharomyces pombe* cells in a small volume of hypotonic buffer (5 mM KCl, 1 mM $MgCl_2$, 20 mM HEPES [*N*-2-hydroxyethylpipe-

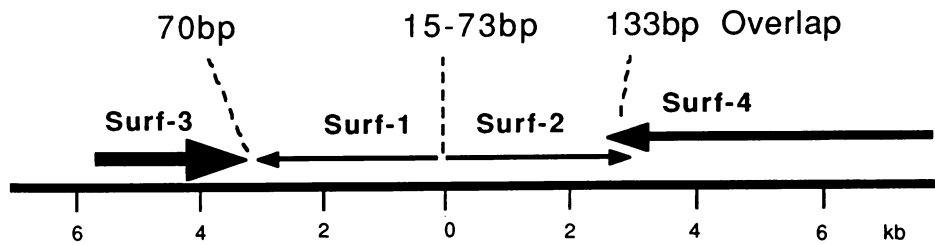

FIG. 1. Map of the mouse Surfeit locus. Genomic DNA is shown as a solid line, with distances in kilobase pairs (kb) indicated below. The direction and extent of the *Surf-1* to *Surf-4* transcription units are shown as solid arrows, with the different thicknesses representing the relative steady-state levels of the mRNAs. The 133-bp overlap of the 3′ ends of *Surf-2* and *Surf-4* (47), the 15 to 73 bp between the heterogeneous 5′ ends of *Surf-1* and *Surf-2* (46), and the 70 bp between the 3′ ends of *Surf-3* and *Surf-1* (12) are indicated.

razine-*N*′-2-ethanesulfonic acid, pH 7.1], 2 mM phenylmethylsulfonyl fluoride) in the presence of glass beads. *Xenopus* oocytes were lysed in homogenization buffer (0.1 M NaCl, 1% Triton X-100, 20 mM Tris hydrochloride [pH 7.6], 1 mM phenylmethylsulfonyl fluoride). After centrifugation at 10,000 × *g* for 10 min, the supernatant was removed and used for immunoblotting. *Drosophila* flies (wild type Oregon-R) were lysed directly in Laemmli sample buffer (16). The amount of *Drosophila* protein was estimated by running samples on SDS-PAGE. Protein concentrations in all of the other extracts were determined with the Bio-Rad protein assay kit. The crude nuclear fraction, particulate fraction (P100), and soluble cytoplasmic fraction (S100) from mouse cells were isolated after hypotonic lysis and differential centrifugation as previously described (10).

Immunofluorescence. Mouse 3T3 cells were fixed in 70% acetone–30% methanol for 20 min at −20°C 24 to 48 h after being seeded on cover slips. Fixed cells were incubated with either preimmune or immune serum diluted 1:20 to 1:40 with PBS for 60 min at room temperature. Cover slips were washed extensively in PBS before being incubated with a 1:50 dilution of fluorescein-conjugated goat anti-rabbit immunoglobulin G (Organon Teknika). After being washed in PBS, the cover slips containing the cells were mounted in 50% glycerol–PBS.

Ribosome purification. Ribosomes were prepared from mouse 3T3 cells as described by Siegmann and Thomas (34). Briefly, cells were lysed in hypotonic buffer containing 1% Triton X-100 and 1% deoxycholate. Nuclei were removed by centrifugation, and the supernatant was fractionated on a sucrose step gradient. The resulting ribosomal pellet is referred to as 80S ribosomes. Ribosomal subunits were prepared by the procedure of Ogata and Terao (26). After incubation with 0.2 mM puromycin at 37°C for 10 min, the ribosomal suspension was fractionated on a 15 to 30% sucrose linear gradient. The 60S subunit fraction was subjected to a second sucrose gradient to remove contaminating dimers of 40S subunits (26). Proteins from ribosomes and ribosomal subunits were solubilized directly in Laemmli sample buffer (16) before being run on SDS-PAGE.

Hybrid-selected translation assay. Poly(A)$^+$ RNA from mouse 3T3 cells was hybrid selected by the procedure of Parnes et al. (29) and translated in a rabbit reticulocyte lysate system (Promega Biotec). Translation products were analyzed by SDS-PAGE either directly or after immunoprecipitation (9) with preimmune or specific antibodies.

DNA blot analysis. A GeneScreen Plus membrane (New England Nuclear Corp.) containing *Eco*RI-cleaved genomic DNA from a number of different species was a kind gift of Paul Goodfellow. The filter was prehybridized overnight at 55°C in 1 M NaCl–10% dextran sulfate–1% SDS–denatured salmon sperm DNA at 250 μg/ml. Hybridization with a *Surf-3* cDNA probe was performed overnight in this solution, and the filter was washed at 55°C in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) before autoradiography.

RESULTS

Cellular location of the 32-kDa Surf-3 protein. Antisera were raised separately in rabbits against two synthetic peptides corresponding to the first nine amino acids at the N terminus and the last nine amino acids at the C terminus of the putative Surf-3 protein (12) (see Materials and Methods). Three of the four sera produced (two for each peptide) reacted specifically with a mouse cellular protein with an apparent molecular mass of 32 kilodaltons (kDa) by immunoblotting (see Fig. 2). None of the sera were able to immunoprecipitate the 32-kDa protein efficiently (see Fig. 5). One serum (AF3) against the C-terminal peptide reacted much more strongly than the other sera and was used in all of the subsequent experiments. The specificity of the antiserum reaction with the mouse 32-kDa protein was shown by both the absence of cross-reaction with preimmune serum (Fig. 2A, left lane) and the inhibition of binding after preincubation of the immune AF3 serum with an excess of the peptide used in the immunization (Fig. 2A, right lane). The apparent molecular mass of 32 kDa is consistent with that expected from the *Surf-3* open reading frame of 266 amino acids (12).

Initially, the intracellular location of the 32-kDa Surf-3 protein was investigated by crude fractionation of cellular components by differential centrifugation of mouse cells lysed in the absence of detergent. The proteins in the subcellular fractions were analyzed by immunoblotting after electrophoresis on SDS-polyacrylamide gels (Fig. 2B). Under these conditions, most of the 32-kDa protein was recovered in the particulate fraction (P100) containing membranes and microsomes (Fig. 2B, middle lane). A small amount of the 32-kDa protein was also found in the crude nuclear fraction (Fig. 2B, left lane), but none of the Surf-3 protein was detectable in the soluble fraction (Fig. 2B, right lane). The results from this subcellular fractionation were confirmed by immunofluorescence staining. Incubation with anti-peptide serum AF3 resulted in intense staining of the nucleolar region and cytoplasm of fixed mouse cells (Fig. 3).

Identification of the 32-kDa *Surf-3* gene product as a ribosomal protein. It had previously been noted that *Surf-3* had the properties of a ribosomal protein gene, being a highly expressed member of a pseudogene-containing multigene family and specifying a very basic polypeptide (12). The subcellular location (Fig. 2B) and the cytoplasmic and nucleolar staining pattern (Fig. 3) of the Surf-3 protein are expected for ribosomes found predominantly in the cyto-

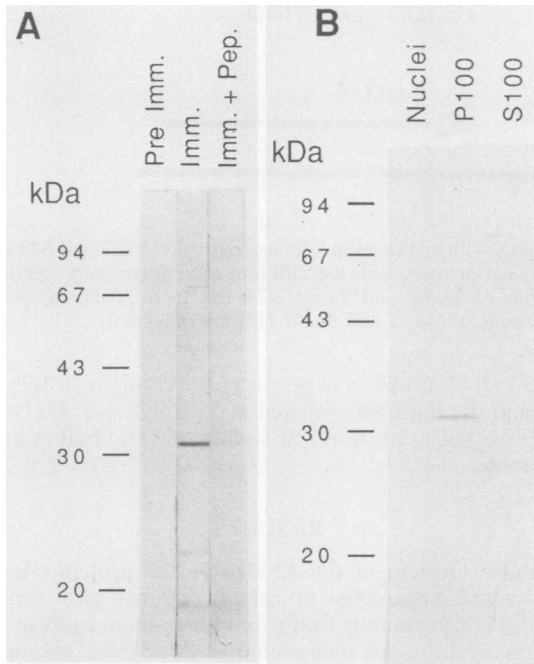

FIG. 2. Immunological detection of the Surf-3 32-kDa protein in total cell extract and subcellular fractions of mouse 3T3 cells. (A) Proteins from total mouse 3T3 cell extracts were resolved on SDS–12% PAGE, transferred to nitrocellulose, and reacted with rabbit preimmune serum (Pre Imm.), immune anti-peptide AF3 serum (Imm.), or AF3 serum preincubated for 30 min at 4°C with the C-terminal peptide (AKELATKLG) at a concentration of 1 mg/ml (Imm. + Pep.). A couple of faint bands, most likely due to the presence of a degradation product (small band) or weak and nonspecific cross-reactions often observed with anti-peptide sera, were competed out by the peptide, as well as the strong, specific 32-kDa band. (B) Detection of the Surf-3 protein by immunoblotting with AF3 serum in nuclear (Nuclei), particulate (P100), and cytoplasmic soluble (S100) fractions (for fractionation, see Materials and Methods). Equal cell equivalents were loaded in each lane. Molecular size markers were phosphorylase *b* (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), and trypsin inhibitor (20 kDa).

plasm but assembled in the nucleoli. Thus, a further analysis was undertaken to see whether the Surf-3 32-kDa protein was associated with ribosomes. Proteins from a total mouse cell extract (Tot.), purified mouse ribosomes (80S), and the small (40S) and large (60S) mouse ribosomal subunits separated by sucrose gradient centrifugation were assessed by immunoblotting for their reactivity with AF3 antibodies (Fig. 4). The Coomassie blue staining pattern of the mouse ribosomal proteins (Fig. 4A) was similar to the rat ribosomal pattern previously described (41). The Surf-3 32-kDa protein was found in the purified 80S ribosomes and was further localized to the 60S ribosomal subunit, showing that *Surf-3* codes for a ribosomal protein (Fig. 4B).

The *Surf-3* cDNA hybrid selects an mRNA that codes for a 32-kDa protein. The identification of the *Surf-3* gene product as a 32-kDa protein has so far been based entirely on its reaction with the anti-peptide sera (Fig. 2 to 4). To confirm this result by independent means, we used hybrid selection and subsequent in vitro translation of the selected mRNA. The in vitro translation product of the mouse mRNA selected with *Surf-3* cDNA (Fig. 5, lane 4) was a 32-kDa protein which reacted with AF3 anti-peptide antibodies (Fig. 5, lane 6). Thus, by using both an immunological technique and a technique based on nucleic acid homology, the *Surf-3* gene product was found to be a 32-kDa protein.

Conservation of the *Surf-3* DNA sequence and C-terminal protein sequence through evolution. To study the conservation of the *Surf-3* DNA sequence through evolution, *Eco*RI-digested genomic DNAs from different eucaryotes were probed with *Surf-3* DNA. The hybridization washing conditions used (2× SSC, 55°C) would detect a minimum of about 60 to 80% homology. Various degrees of cross-hybridization from *S. pombe* to humans were detected with the *Surf-3* cDNA probe (Fig. 6). Multiple cross-hybridizing *Eco*RI fragments were detected in the other mammals (rodents and primates). These presumably represent multigene families predominantly composed of pseudogenes, as found for a number of ribosomal protein genes in several mammalian species (8, 14, 28, 44, 45). In contrast to the multiple positive mammalian fragments, only one or a few cross-hybridizing *Eco*RI fragments were found in *S. pombe*, *Drosophila*, *Xenopus*, chicken, and turkey. A preliminary analysis of the cloned *S. pombe* 2.2-kilobase *Eco*RI fragment (Fig. 6) indicated the presence of a true conserved yeast *Surf-3* gene rather than that the positive hybridization was due to short runs of consecutive nucleotides which have homology to the mouse *Surf-3* probe (J. Yon, unpublished data). Thus, it appears that the *Surf-3* DNA sequence is highly conserved through evolution.

AF3 anti-peptide antibodies were used to probe Western blots (immunoblots) of cellular lysates from different eucaryotes to assess the conservation of the Surf-3 C-terminal protein sequence. A comigrating 32-kDa protein was detected in mouse, rat, human, monkey, chicken, and *Xenopus* extracts, whereas no cross-reacting protein was detected in *Drosophila* or *S. pombe* (Fig. 7). Thus, the extreme C terminus of the Surf-3 ribosomal protein is not highly conserved in lower eucaryotes.

DISCUSSION

The mouse Surfeit locus contains at least four tightly clustered genes (*Surf-1* to *Surf-4*) (Fig. 1). No more than 73 bp separate any two adjacent Surfeit genes, and *Surf-2* and *Surf-4* overlap by 133 bp at their 3′ ends (12, 46, 47). This tight clustering suggests some sort of *cis* interaction between neighboring Surfeit genes (12, 48). Thus, it would be of interest to know the function of the genes in the Surfeit locus. In the present work, we identified the *Surf-3* gene product as the L7a ribosomal protein.

Surf-3 is a highly expressed member of a mouse multigene family whose other members are predominantly pseudogenes which have a number of hallmarks of having been generated by reverse transcription (12). It had previously been postulated that Surf-3 is either a nuclear high-mobility group protein or a cytoplasmic ribosomal protein (12), since genes that code for these two sets of proteins are abundantly transcribed and are members of pseudogene-containing multigene families. To assess the nature of the *Surf-3* gene product and its cellular location, antisera were raised against peptides of the Surf-3 protein as deduced from the previously determined cDNA sequence (12). Rabbit polyclonal antibodies were produced separately against peptides consisting of the nine N-terminal amino acids and the nine C-terminal amino acids. Both sera detected a peptide-specific 32-kDa protein in immunoblots of total mouse cell proteins. The 32-kDa protein was found predominantly in the particulate cell fraction (Fig. 2B) and was further local-

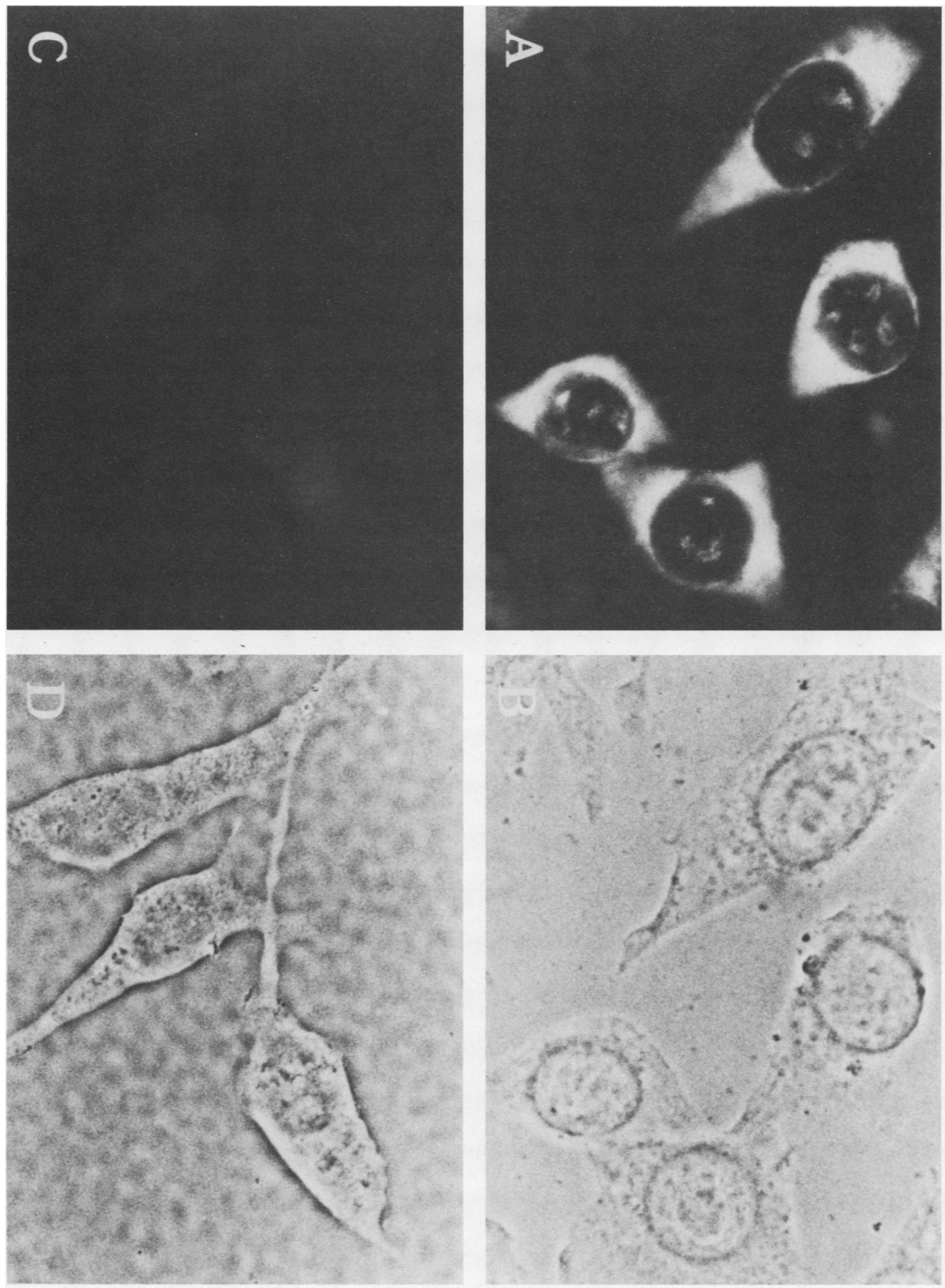

FIG. 3. Localization of the Surf-3 protein in mouse cells by indirect immunofluorescence. Panels: A, mouse 3T3 cells stained with AF3 serum; B, phase-contrast image of the cells in panel A; C, mouse 3T3 cells reacted with normal rabbit serum (preimmune); D, phase-contrast image of the cells in panel C. Magnification, ×500.

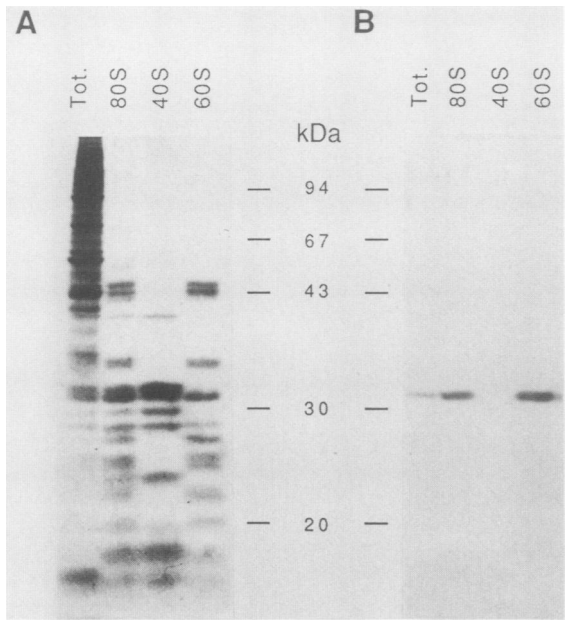

FIG. 4. Identification of the 32-kDa Surf-3 protein as a ribosomal protein present in the 60S subunit. Ribosomes (80S) and ribosomal subunits (40 and 60S) were isolated as described in Materials and Methods. Equal A_{260} units were loaded in each lane; 50 μg of total mouse cell extract (Tot.) was run in a parallel lane as a control. Panels: A, Coomassie blue staining; B, immunoblotting with AF3 serum of the fractions shown in panel A. The size markers were as in Fig. 2.

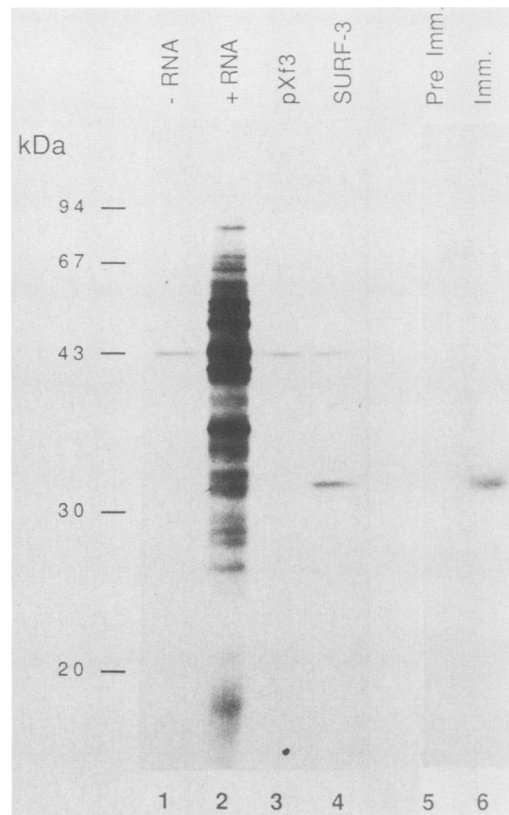

FIG. 5. Identification of the 32-kDa *Surf-3* gene product by hybrid-selected translation. Poly(A)$^+$ RNA from mouse 3T3 cells was hybridized to *Surf-3* cDNA (12) or plasmid pXf3 that had been bound to nitrocellulose filters. The hybridized RNA was eluted and translated in a reticulocyte lysate system in the presence of [^{35}S]methionine. The products were either immunoprecipitated or directly analyzed by SDS-PAGE followed by fluorography. Lanes: 1, in vitro translation without added poly(A)$^+$ RNA; 2, translation products of 0.015 μg of unselected poly(A)$^+$ RNA; 3, products of poly(A)$^+$ RNA hybrid selected with pXf3; 4, products of poly(A)$^+$ RNA hybrid selected with *Surf-3* cDNA; 5, immunoprecipitation with preimmune serum of products of poly(A)$^+$ RNA hybrid selected with *Surf-3* cDNA; 6, immunoprecipitation with anti-peptide AF3 serum of products of poly(A)$^+$ RNA hybrid selected with *Surf-3* cDNA. Lanes 5 and 6 were exposed four times longer than the other lanes because of inefficient immunoprecipitation with the AF3 serum (see the text). The size markers were as in Fig. 2.

ized to the cytoplasm and nucleolus by immunofluorescence staining (Fig. 3). Such staining is consistent with the cellular location of ribosomal proteins and not high-mobility group proteins. The Surf-3 32-kDa protein was found in purified 80S ribosomes and could be further localized to the large 60S ribosomal subunit (Fig. 4). The immunological detection of a Surf-3 32-kDa protein was confirmed by production of a 32-kDa protein after in vitro translation of mouse cell poly(A)$^+$ mRNA selected with the *Surf-3* cDNA clone (Fig. 5).

Mouse ribosomal proteins have not been studied as well as those of the rat. By analogy with one-dimensional gel separation of rat large-subunit ribosomal proteins (41) (very similar to that of the mouse large-subunit ribosomal proteins presented in Fig. 4A), the mouse *Surf-3* gene would code for either of the large-subunit ribosomal proteins L7a and L8, both of which comigrated in the gel system used. The amino acid compositions of purified rat L7a and L8 ribosomal proteins have previously been determined (41), and the amino acid composition (16 amino acids measured) of the rat L7a protein, but not that of the L8 protein, is in perfect agreement with the amino acid composition of the putative Surf-3 mouse protein (12). Although neither the DNA sequence nor the protein sequence of either of these rat ribosomal proteins is presently in the EMBL, GenBank, or PIR data base (12), 39 amino acids at the N terminus of rat ribosomal protein L7a have previously been determined (50). The N-terminal region of the mouse *Surf-3* translation product (12) shows 95% homology with the previously sequenced N-terminal peptide of the rat L7a protein (50). Thus, on the basis of gel mobility (Fig. 4), overall amino acid composition (41), and partial amino acid sequence analysis (50), the *Surf-3* gene product was identified as mouse ribosomal protein L7a. Because of its trypsin sensitivity, the L7a ribosomal protein is thought to be on the external surface of the 60S ribosomal subunit (22) and has been found to be capable of being cross-linked to a number of other ribosomal proteins (42), as well as the 28S rRNA (25).

By amino acid sequencing, footprinting, and cDNA hybridization to mRNAs in Northern (RNA) blots, some but not all ribosomal protein genes have been found to be conserved to various degrees through evolution (6, 23, 49, 50). By DNA hybridization to the genomic DNAs of a number of species, the *Surf-3* ribosomal gene appears to be highly conserved through evolution. *Surf-3*-related sequences have been detected in the genomic DNAs of other rodents, primates, fowl, amphibians, insects, and yeasts (Fig. 6). It is of interest that *Surf-3* multigene families appear to be present in Old World and New World monkeys and humans (Fig. 6). If these families, as in mice, are also predominantly composed of pseudogenes, then the primate

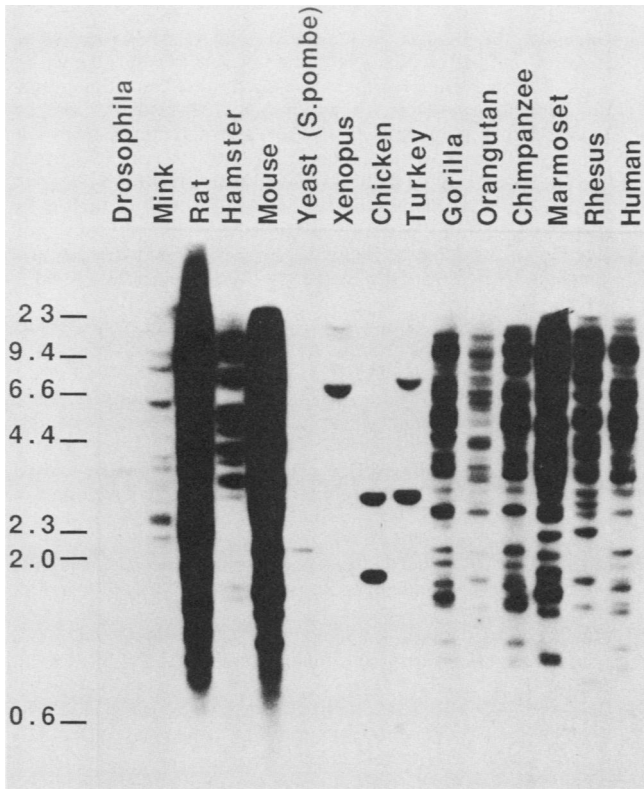

FIG. 6. Conservation of *Surf-3* DNA sequences in eucaryotes. A GeneScreen Plus filter containing *Eco*RI-cleaved genomic DNA from a number of different eucaryotes (indicated at the top) was hybridized with a *Surf-3* cDNA probe and washed under reduced-stringency conditions (see Materials and Methods). The numbers on the left indicate the positions, in kilobases, of *Hin*dIII-cleaved lambda DNA size markers.

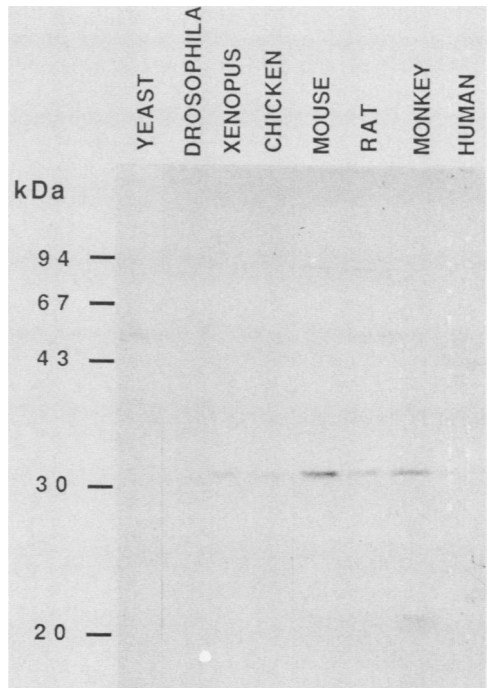

FIG. 7. Detection of the Surf-3 protein in cellular extracts from different eucaryotes. Extracts were prepared from cells of different eucaryotes (indicated at the top), as described in Materials and Methods. Lanes: YEAST, *S. pombe*; DROSOPHILA, Oregon-R wild-type flies; XENOPUS, *Xenopus* oocytes; CHICKEN, chicken embryo primary fibroblasts; MOUSE, 3T3 cell line; RAT, Rat-1 cell line; MONKEY, Cos-1 cell line; HUMAN, PAF cell line. Immunoblotting was performed with AF3 serum. The size markers were as in Fig. 2.

ribosomal pseudogenes must have arisen independently of those in mice, because on the basis of sequence differences (between *Surf-3* and its pseudogenes) the mouse *Surf-3* pseudogenes must have arisen 10 to 25 million years ago (12), while primates and rodents diverged about 100 million years ago. Another point of interest is that in the nonmammalian species there seems to be only one copy or a few copies of cross-hybridizing *Surf-3* sequences (Fig. 6). It had been noted previously that genes which are members of pseudogene-containing multigene families in mammals are usually single-copy genes in birds (7, 43). This suggests differences in reverse transcription in the germ line between birds and mammals. In contrast to the DNA homology, the *Surf-3* C-terminal anti-peptide antibody detected a 32-kDa protein only in chicken, *Xenopus*, and other mammalian species and did not appear to react with any *Drosophila* or yeast protein (Fig. 7). These results indicate that the extreme C-terminal region of the protein is not conserved in lower eucaryotes but does not exclude conservation in these species of other regions of the L7a ribosomal protein.

Although numerous mammalian ribosomal protein mRNAs have been isolated as cDNAs (1–5, 8, 11, 14, 15, 17–20, 24, 27, 28, 30, 31, 35–39, 44, 45), only a few active intron-containing genomic mammalian ribosomal protein genes have been cloned (8, 14, 30, 44, 45). This is because of the difficulty of isolating the one (or few) active intron-containing gene(s) in the presence of the multiple copies of the pseudogenes which exist in many ribosomal protein multigene families. The *Surf-3* multigene family contains 15 to 30 pseudogenes (12). The location of the active *Surf-3* L7a ribosomal protein gene containing introns, as opposed to one of the many *Surf-3* pseudogenes, within the tightly clustered Surfeit locus reinforces the notion that the Surfeit genes may interact with one another at the transcriptional or posttranscriptional level (12, 48). Ribosomal proteins account for up to 15% of cellular proteins, and their expression is up regulated or down regulated, depending on the protein synthetic requirements of the cell, for instance, during growth and differentiation (for a review, see reference 21). Differences in the amount of transcription of the *Surf-3* ribosomal protein gene correlated with such changes may well have an effect on the expression of the neighboring *Surf-1* gene, whose 3′ end is only 70 bp away from the 3′ end of *Surf-3* (Fig. 1) (12), or changes in *Surf-1* transcription could have an effect on the expression of the neighboring *Surf-3* gene. This could occur at the transcriptional level via inhibition of the promoter of one of the Surfeit genes as the result of transversal by the RNA polymerase involved in the active transcription of an adjacent Surfeit gene (polymerase interference) (12, 48). Alternatively, gene expression could be inhibited at the posttranscriptional level by antisense control as the result of the formation of double-stranded RNA derived from the transcripts of the two closely spaced adjacent Surfeit genes. The Surfeit transcripts in double-stranded RNA might not be available for translation into proteins because of RNA instability, inability to be processed, or lack of transport to the cytoplasm from the nucleus. Further studies on the expression of the different

Surfeit genes under different conditions or in the absence of an actively expressed neighboring gene (or both) should indicate the effect of one Surfeit gene on the expression of another in the locus.

Although *Surf-3* has been identified as coding for a ribosomal protein, there is no reason to believe that the other genes in the Surfeit locus also code for ribosomal proteins. The other Surfeit genes do not have the characteristics of ribosomal protein genes, since they are not very highly expressed and are single-copy genes rather than members of multigene families (48). However, we cannot rule out the possibility that the other Surfeit genes code for proteins involved in translation that are associated with ribosomes. We are currently making antibodies to peptides and fusion proteins from the other Surfeit genes to be able to determine their functions and cellular locations.

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ADDENDUM IN PROOF

It has recently come to our attention that a human cDNA has been isolated (S. C. Kozma, M. S. Redmond, F. Xiao-Chang, S. M. Saurer, B. Groner, and N. E. Hynes, EMBO J. 7:147–154, 1988) whose translational gene product differs by only one amino acid from that of the mouse Surf-3 L7a ribosomal protein described by Huxley et al. (12). In addition, we have recently mapped the human Surfeit gene locus (48) containing the Surf-3 L7a ribosomal protein gene to human chromosome 9q33-34 (J. Yon, R. W. Palmer, D. Sheer, and M. Fried, Ann. Hum. Genet., in press).

LITERATURE CITED

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