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Molecular Characterization of *trans*-Golgi p230

A HUMAN PERIPHERAL MEMBRANE PROTEIN ENCODED BY A GENE ON CHROMOSOME 6p12–22 CONTAINS EXTENSIVE COILED-COIL α-HELICAL DOMAINS AND A GRANIN MOTIF*

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Using autoantibodies from a Sjögren's syndrome patient, we have previously identified a 230-kDa peripheral membrane protein associated with the cytosolic face of the *trans*-Golgi (Kooy, J., Toh, B. H., Pettitt, J. M., Erlich, R. and Gleeson, P. A. (1992) *J. Biol. Chem.* 267, 20255–20263). Here we report the molecular cloning and sequence analysis of human p230 and the localization of its gene to chromosome 6p12–22. Partial cDNA clones, isolated from a HeLa cell cDNA library using autoantibodies, were used to obtain additional cDNAs, which together span 7695 base pairs (bp). The p230 mRNA is ~7.7 kilobases. Two alternatively spliced mRNAs for p230 were detected. These differed by 21- and 63-bp insertions in the 3′-sequence, resulting in differences in amino acid sequence at the carboxyl terminus. The predicted 261-kDa protein is highly hydrophilic with 17–20% homology with many proteins containing coiled-coil domains. Apart from two proline-rich regions (amino acids 1–117 and 239–270), p230 contains a very high frequency of heptad repeats, characteristic of α-helices that form dimeric coiled-coil structures. p230 also includes the sequence ESLALEELEL (amino acids 538–546), a motif found in the granin family of acidic proteins present in secretory granules of neuroendocrine cells. This is the first report of a cytosolic Golgi protein containing a granin motif. The structural characteristics of p230 indicate that it may play a role in vesicular transport from the *trans*-Golgi.

The Golgi apparatus is a highly complex and dynamic organelle organized into three functionally distinct regions: the *cis*, *medial*, and *trans* cisternae of the Golgi stack and two tubulovesicular networks, namely the *cis*-Golgi network and the *trans*-Golgi network (1, 2). Transport of newly synthesized proteins from the endoplasmic reticulum to Golgi cisternae, between adjacent cisternae, and from the cisternae to various destinations is mediated by vesicles shuttling between donor and recipient compartments (3). Numerous structural and regulatory proteins have been implicated in the budding, docking, and fusion of vesicles (3–5).

Soluble proteins involved in budding of vesicles include COPI and COPII coat proteins and the small GTP binding protein ARF-1. A *N*-ethylmaleimide-sensitive fusion protein, soluble *N*-ethylmaleimide-sensitive fusion protein attachment proteins (SNAPs)[1] and Rabs are involved in either vesicle docking or membrane fusion (3, 6–8). SNAP receptors (SNAREs), membrane proteins that form oligomeric complexes with SNAPs and *N*-ethylmaleimide-sensitive fusion proteins, are considered to promote fusion of vesicles with target membranes after specific docking mediated by SNAREs on vesicle and target membranes (3). However, many facets of the transport process remain unresolved. For example, the protein coat structures that mediate forward vesicle transport from the Golgi apparatus have not been fully characterized.

Additional peripheral membrane Golgi proteins have also been implicated in vesicular transport, for example three high molecular weight proteins in *cis* to *medial* Golgi transport (9). One of these, p115, contains coiled-coil domains and is related to Uso1p required for transport from endoplasmic reticulum to Golgi complex in *Saccharomyces cerevisiae* (10). Two peripheral membrane proteins have been implicated in budding of vesicles from the *trans*-Golgi network, namely p200, which associates with coated vesicles arising from the *trans*-Golgi network (11), and p62, which forms a complex with TGN38/41 and Rab6 (12).

Human autoantibodies are valuable reagents for identification of novel intracellular proteins. Using anti-Golgi autoantibodies from a patient with Sjögren's syndrome, we have previously identified a brefeldin A-sensitive peripheral membrane protein of 230 kDa (p230) localized to the cytosolic face of *trans*-Golgi (13). Other novel Golgi proteins have also been identified using autoantibodies, including golgins-95 and -160 (14), a protein of ~370 kDa (15, 16), which appears to be identical to giantin (17), and a *cis*-Golgi network p210 protein (18). For those Golgi proteins where sequences are known, a common structural feature is a high content of predicted coiled-coil domains. Here we have cloned and sequenced p230. The predicted protein consists of α-helical coiled-coil domains with abundant heptad repeats and contains a granin motif shared with proteins found in secretory vesicles. We propose that p230 has a role in membrane transport of proteins from the Golgi apparatus.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U41740.

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[1] The abbreviations used are: SNAPs, soluble *N*-ethylmaleimide-sensitive fusion protein attachment proteins; SNARE, SNAP receptors; PBS, phosphate-buffered saline; PCR, polymerase chain reaction.

EXPERIMENTAL PROCEDURES

Autoimmune Sera—Autoimmune sera were obtained from Gribbles Pathology (Melbourne, Australia). Sera were treated at 56 °C for 30 min to inactivate complement, and aliquots in 0.02% sodium azide were stored at −70 °C.

Immunofluorescence—Indirect immunofluorescence using human Hep2 cells (Kallstead) was performed as described previously (13).

Immunoblotting—Immunoblotting of HeLa cell extracts and recombinant p230 bacterial fusion protein was performed as described previously (13).

Isolation and Sequencing of cDNA Clones—10^5 recombinants of a λgt11 HeLa cell cDNA library (Clontech HL1022) were immunoscreened with autoimmune serum from a Sjögren's syndrome patient diluted 1:1000 in phosphate-buffered saline (PBS) as described previously (13). Positive plaques, detected with ^{125}I-labeled protein A, were rescreened and plaque-purified. These cDNA clones were subcloned into pBluescribe M13$^+$ (Stratagene) for sequencing. Additional cDNA clones were obtained by subsequently screening a λZAP Hepatoma cDNA library (Stratagene 935202) with ^{32}P-labeled cDNA probes. Positive plaques were purified, and plasmids were rescued according to the manufacturer's instructions. The ^{32}P-labeled cDNA probes were derived initially from the λgt11 clones and subsequently from λZAP clones. In addition, a ^{32}P-labeled 45-mer based on sequence from a λZAP cDNA clone was constructed from partially overlapping 30-mers, 5′-GAGCTAATCAACATTAGTAGTAGTAAAACT-3′ and 5′-AGAAAGAATGGCATTAGTTTTACTACTACT-3′ by a method described by Alderuccio *et al.* (19). Finally, a random-primed HeLa cell cDNA library in pUEX (20) was screened with a ^{32}P-labeled 240-bp fragment obtained by PCR using a λZAP clone as template. Nucleotide sequence of cDNA clones clone was determined by the dideoxy method, using a deaza reagent sequencing kit (Promega) in conjuction with T7 DNA polymerase (Pharmacia Biotech Inc.) or by automated sequencing using a Prism DyeDeoxy terminator cycle kit and a 373A DNA sequencer (Applied Biosystems).

Elution of Antibody from λ Plaques—To induce the expression of β-galactosidase fusion protein, nitrocellulose filters impregnated with 10 mM isopropyl-1-thio-β-D-galactopyranoside were overlaid on agar plates containing λgt11 plaques for 3 h at 42 °C. The filters were then washed in PBS, blocked in 3% casein, and incubated with autoimmune serum, diluted 1:1000 in PBS, for 1 h at room temperature. After washing in PBS, the bound antibodies were eluted from the filters by gently rocking in 3 M KSCN. The eluate was dialyzed overnight in PBS and concentrated approximately 20-fold using Centricon filters (Amicon).

Reverse Transcriptase Polymerase Chain Reaction—Total RNA was isolated from HeLa cells by guanidium thiocyanate extraction followed by ultracentrifugation in cesium chloride (21). After centrifugation, the RNA pellets were resuspended in formamide for storage and precipitated in four volumes of ethanol before use (22). HeLa cell RNA (2 μg) was heated to 70 °C for 2 min, and cDNA was synthesized using 5 μg/ml oligo(dT) (Promega) and 100 units of murine superscript reverse transriptase (Life Technologies, Inc.) in 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 4 mM $MgCl_2$, 0.01% gelatin, 1 mM of each of the four deoxynucleotide triphosphates (Pharmacia), and 20–40 units of RNAsin (Promega) at 37 °C for 2 h. Reactions were also carried out without reverse transcriptase or without RNA. Half of the volume of these reactions was used as templates for PCR. Oligonucleotide primers were used at 0.2 pmol/μl in the presence of 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.2 mM of each of the four deoxynucleotide triphosphates, 2.5 units of *Taq* polymerase (Life Technologies, Inc.), and 0.016 units of cloned Pfu DNA polymerase (Stratagene). The oligonucleotide primers (produced by Department of Microbiology, Monash University or by Bresatec, Adelaide, Australia) were as follows: P1 (5′-GCTCTAGATCAAGGAGGAGACGGCGA-3′), P2 (5′-CCTCAGTAAGACTTTCTA-3′), P3 (5′-CCCAAGTCTATGAGTCCA-3′), P4 (5′-GCGTCGACCACTGCCAACAATCACAG-3′), and P5 (5′-TTGGTCCAACCCAAATTG-3′). Cycle conditions for P1/P2 and P4/P5 were as follows: 1 cycle of 5 min at 95 °C, 1.5 min at 55 °C, and 3 min at 72 °C and 29 cycles of 1.5 min at 95 °C, 1.5 min at 55 °C, and 3 min at 72 °C. Cycle conditions for P3/P4 were as above except that the annealing temperature was 60 °C.

Southern Blot Analysis—PCR products were separated by agarose gel electrophoresis and then transfered to nylon membranes (Hybond-N$^+$, Amersham Corp.) in 0.4 M NaOH for 4 h. Membranes were then incubated with prehybridization buffer (0.9 M NaCl, 90 mM $Na_3C_6H_5O_7.2H_2O$, 10 mM EDTA, pH 7.0, 7% SDS, and 0.5% skim milk powder) for 30 min at 65 °C and hybridized overnight at 65 °C with ^{32}P-labeled DNA (specific activity, $>5 \times 10^8$ cpm/μg) in prehybridization buffer. Membranes were washed at 65 °C in 0.15 M NaCl, 0.015 M $Na_3C_6H_5O_7{\cdot}2H_2O$, pH 7.0, containing 0.1% SDS and exposed to x-ray film (Fuji, Ashigara, Japan).

Northern Blot Analysis—- Total RNA was prepared from HeLa cells as described above and enriched for poly(A)$^+$ RNA by oligo(dT)-cellulose chromatography using a poly(A) tract mRNA isolation system (Promega). Poly(A)$^+$ RNA was separated by electrophoresis on 1% agarose formaldehyde gel and transferred to nylon membranes (Hybond-N$^+$, Amersham Corp.) in 0.05 M NaOH for 2 h. After transfer, lanes containing RNA markers were cut into strips and stained in methylene blue (21). The remaining membrane was incubated with ^{32}P-labeled DNA probes as described for Southern analysis. The membranes were stripped for reprobing by the addition of 0.5% SDS at 100 °C followed by cooling to room temperature. The removal of the ^{32}P-labeled probe was confirmed by autoradiography.

Sequence Analysis—Analyses were carried out using the MacVector program (International Biotechnologies, Inc) or using on-line software provided by the Australian National Genome Information Service including the FASTA program, (23), the pattern library search program of Smith and Smith (24), and various programs contained in the University of Wisconsin GCG package (25). Coiled-coil structures were predicted using the method of Lupas *et al.* (26) using the STRIPE program for the Macintosh.[2]

Chromosome in Situ Hybridization—Human chromosome metaphase spreads were obtained from PHA-stimulated peripheral blood lymphocyte cultures synchronized with fluorodeoxyuridine (28) according to standard cytogenetic procedures. The method of *in situ* hybridization was modified from Harper and Saunders (29) and Choo *et al.* (30). The λg5 clone, labeled by random priming using [^{3}H]dATP, [^{3}H]dCTP, and [^{3}H]dTTP to a specific activity of 2.04×10^8 cpm/μg DNA was added to the hybridization solution and hybridized to chromosome preparations overnight at 40 °C. After washes in 0.15 M NaCl, 0.015 M $Na_3C_6H_50_7{\cdot}2H_20$, pH 7.0, at 42 °C, slides were dipped in EM-1 hypercoat nuclear emulsion (Amersham Corp.) and exposed for 8–33 days at 4 °C. Autoradiographs were developed, and chromosomes were G-banded in 2–5% Gurr's improved R66 Giemsa stain (Merck, Kilsyth, Australia) in 0.15 M Na_2HPO_4, pH 11, for 8 min (31). The distribution of silver grains over chromosomes was analyzed.

RESULTS

Isolation and Characterization of cDNA Clones—We have identified two autoimmune sera that show strong staining of the Golgi apparatus by immunofluorescence (Fig. 1) and that immunoblot a 230-kDa protein in HeLa cell extracts (Fig. 2). One of these sera (autoimmune serum 1), from a patient with Sjögren's syndrome (13), has high titre anti-Golgi autoantibodies by immunofluorescence (1:100,000) as well as lower titre (1:20,000) antinuclear antibodies. Immunoscreening a HeLa cell λgt11 cDNA expression library with diluted Sjögren's syndrome autoimmune serum yielded four clones designated λg2, λg5, λg7, and λg12 (Fig. 3). Autoantibodies eluted from nitrocellulose lifts of purified phage from each of the four clones showed immunofluorescence staining of the Golgi apparatus, whereas elution of bound antibodies from an irrelevant clone showed no staining (Fig. 1). Furthermore, the eluted autoantibodies showed no nuclear staining. These results indicate that all four clones encode Golgi-associated proteins. Clone λg5 was used to generate a bacterial fusion protein, using the expression vector pGEX (13). The two autoimmune sera specifically reacted with the 51-kDa glutathionine *S*-transferase fusion protein by immunoblotting (Fig. 2; see also Ref. 13). As reported previously (13), affinity-purified Sjögren's syndrome autoantibody eluted from the 51-kDa recombinant fusion protein not only reacted with the Golgi apparatus in HeLa cells by immunofluorescence but also immunoprecipitated and immunoblotted a 230-kDa protein; furthermore, a rabbit antibody raised to the 51-kDa fusion protein gave specific immunofluorescence of the Golgi apparatus and immunoblotted and immunoprecipitated the 230-kDa protein. These results confirm that

[2] A. C. E. Knight, unpublished results.

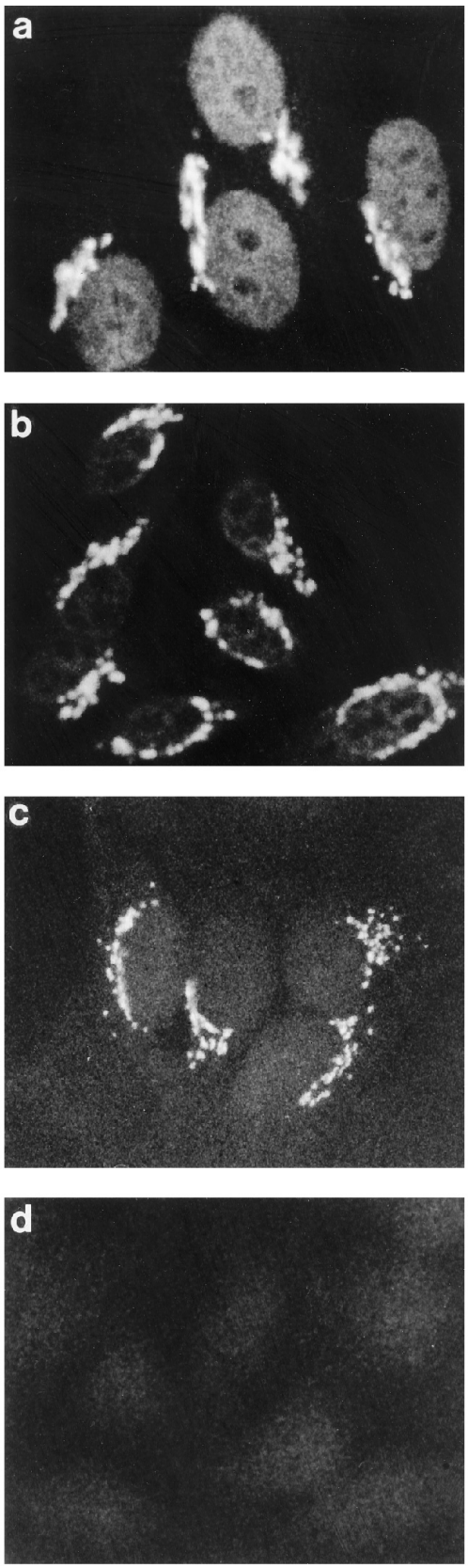

FIG. 1. **Intracellular localization of autoantigens by indirect immunofluorescence.** Human Hep2 cells were stained by indirect immunofluoresence with autoimmune serum 1 (*a*) and autoimmune serum 2 (*b*) or with autoantibodies from serum 1 eluted from clone λg5 (*c*) or from an irrelevant λgt11 clone (*d*). Magnifications: *a*, × 1000; *b*, × 400; *c*, × 800; *d*, × 500.

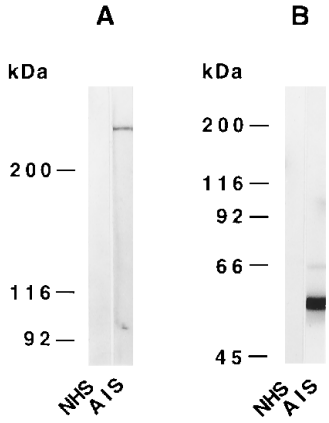

FIG. 2. **Immunoblot analysis of cell extracts and bacterial fusion protein with autoimmune serum 2.** HeLa cell proteins (*A*) and total proteins from isopropyl-1-thio-β-D-galactopyranoside-treated *E. coli* DH1 cells transformed with recombinant pGEX-clone λg5 autoantigen (*B*) were separated under reducing conditions on a 5 or 7.5% polyacrylamide gel, respectively, and transferred to nitrocellulose membranes. Membranes were incubated with autoimmune serum 2 (*AIS*) or normal human serum (*NHS*) followed by peroxidase-conjugated anti-human immunoglobulin. Bound immunoglobulin was detected by Enhanced Chemiluminesence.

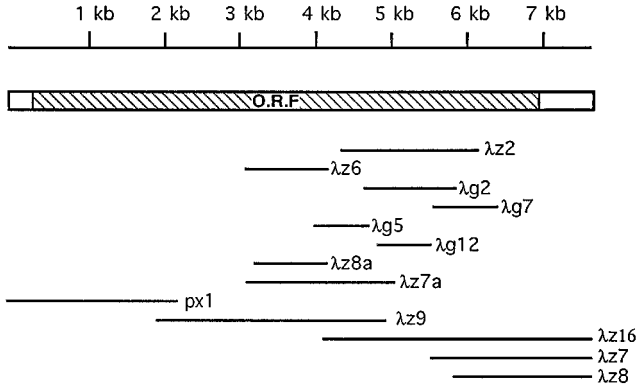

FIG. 3. **Map of p230 cDNA clones.** Clones λg2, λg5, λg7, and λg12 were isolated from a λgt11 HeLa cell cDNA library by immunoscreening with autoimmune serum 1. A λZAP hepatoma cDNA library was screened with λg5 yielding clones λz2 and λz6; screening the same library with λz6 resulted in clones λz7a and λz8a, which also showed positive hybridization with λg5. The λZAP Hepatoma cDNA library was also screened with a 300-bp fragment of λg7 resulting in λz7, λz8, and λz16, while a 45-bp segment of λz6 identified λz9. Finally, a PCR-generated fragment of the 5′ region of λz9 was used to screen a pUEX HeLa cell cDNA plasmid library, identifying clone px1.

clone λg5 encodes a polypeptide derived from p230.

The four clones (λg2, λg5, λg7, and λg12) showed an overlapping nucleotide sequence spanning approximately 2.0 kb (Fig. 3). To obtain additional sequence, we recreened a λZAP cDNA hepatoma library using clone λg5 as probe. Two further clones, designated λz2 and λz6, were isolated (Fig. 3). Further screening of the same library with λz6 resulted in clones λz7a and λz8a. These latter clones also gave positive hybridization signals with clone λg5, and DNA sequencing confirmed their overlapping sequence. Screening the λZAP hepatoma library with a 300-bp *Hin*cII fragment of λg7 identified clones λz7, λz8, and λz16, and screening with a 45-bp segment of λz6 identified λz9 (Fig. 3). The 5′-sequence was obtained from clone px1, isolated by screening a randomly primed HeLa cell plasmid cDNA library with a PCR-generated 240-bp fragment of the 5′-region of λz9. Together, all of the clones (Fig. 3) comprise 7.7 kb of cDNA, as determined by nucleotide sequencing. Three of the cDNA

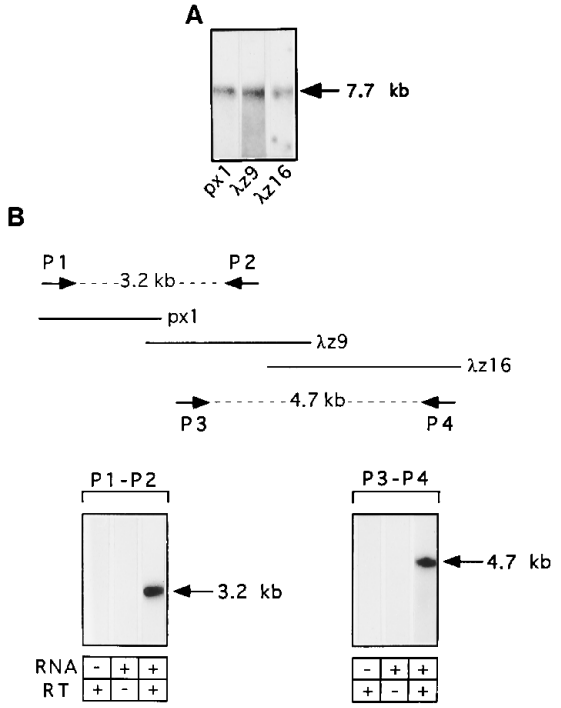

FIG. 4. *A*, Northern blot analysis. HeLa cell poly $(A)^+$ RNA was size fractionated by formaldehyde gel electrophoresis, transferred to Hybond N^+ membranes and hybridized with ^{32}P-labeled cDNA from clone px1. After washing the membrane and visualizing the signal by autoradiography, the ^{32}P-labeled DNA was stripped from the membrane, as described under "Experimental Procedures," and the membrane reprobed with ^{32}P-labeled DNA from clone λz9. A separate membrane was probed with ^{32}P-labeled DNA from clone λz16. *B*, reverse transcriptase PCR and Southern blot analysis. The three clones, px1, λz9, and λz16, which span the full length of the p230 cDNA overlap as indicated. To determine if the three clones are derived from the same mRNA, total RNA from HeLa cells was reverse transcribed, and the cDNA amplified using oligonucleotide primers P1 and P2 or P3 and P4. The expected sizes of the products are indicated. Incubations were also carried out in the absence of either RNA or reverse transcriptase. PCR products were analyzed by Southern blotting using independent internal cDNA probes, as described under "Experimental Procedures." The generation of PCR products of the expected size confirms the relationship of these overlapping clones.

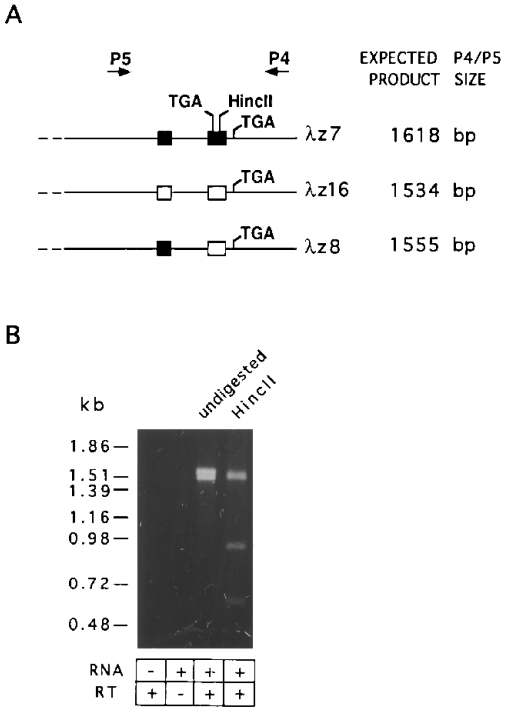

FIG. 5. **Identification of two p230 mRNA species.** *A*, nucleotides 6592–6612 are present (■) in clones λz7 and λz8, but absent (□) from clone λz16. Nucleotides 6950–7012 are present (■) in clone λz7, but absent (□) from clones λz16 and λz8. The sequence from 6950–7012 (■) contains an in-frame TGA stop codon and a *Hinc*II restriction site. P5 (18-mer) and P4 (24-mer) are oligonucleotides used for reverse transcriptase PCR to assess the presence of more than one p230 transcript. The expected sizes of the PCR products are indicated. *B*, total RNA from HeLa cells was reverse transcribed using oligo(dT), and the resulting cDNA were amplified using primers P4 and P5. The PCR product was divided into two, with one-half digested with *Hinc*II. Samples were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. Incubations were also carried out in the absence of either RNA or reverse transcriptase, as indicated.

clones, namely px1, λz9, and λz16, collectively span the entire 7.7-kb cDNA.

Northern analysis showed that the three clones, px1, λz9, and λz16 all hybridized with a similar sized transcript from HeLa cell poly$(A)^+$ RNA of about 7.7 kb (Fig. 4*a*). To confirm that the overlapping clones shown in Fig. 3 were derived from the same transcript, reverse transcriptase PCR was carried out using total RNA isolated from HeLa cells and oligonucleotide primers as indicated in Fig. 4*b*. A 3.2-kb product was obtained using primers P1 and P2, and a 4.7-kb product was generated using primers P3 and P4; the sizes of these PCR products were in accordance with that expected from the nucleotide sequence of the clones. The identity of the PCR products was confirmed by Southern blot analysis using internal probes. The P1/P2 product was probed with a 1.3-kb fragment from clone px1 and the P3/P4 product with the above mentioned 45-mer (Fig. 4*b*). Taken together, our data imply that we have isolated a full-length cDNA encoding p230.

The clones shown in Fig. 3 gave identical sequences in the overlap regions. The nucleotide sequence reported here was verified by either sequence of both strands of a cDNA clone, identical sequences from at least one other independent clone, or from reverse transcriptase PCR products. However, two small differences were detected in the three 3′ clones. These differences comprised a 21-bp stretch (nucleotide 6592–6612) present in clones λz7 and λz8, but absent in λz16 and a 63-bp stretch (nucleotide 6950–7012) present in λz7 but absent from clones λz16 and λz8 (Fig. 5). The absence of the 21 nucleotides does not disrupt the open reading frame but results in the loss of the amino acids VTIMELQ, while the absence of the 63 nucleotides results in insertion of an alternative stop codon and the amino acid sequence of p230 ending in SWLRSSS rather than FTSPRSGIF. Reverse transcriptase PCR using oligonucleotide primers P4 and P5 resulted in two products of 1.5 and 1.6 kb, sizes consistent with the 63- and 21-bp insertion and deletion (Fig. 6). There is a unique *Hinc*II site in the 63-bp insertion, and as expected, the 1.6-kb PCR product was susceptible to *Hinc*II digestion, giving the expected fragments of 0.98 and 0.64 kb. These results indicate the presence of alternatively spliced mRNAs derived from the same gene. Whether these different mRNAs represent regulated splicing events or products from inaccurate splicing is not known.

Sequence Analysis—The nucleotide sequence of p230 cDNA comprises 7695 bp and contains an open reading frame of 6690 bp (Fig. 6). The open reading frame spans an in-frame ATG initiation codon at nucleotide position 286 and a TGA termination codon at position 6976. A second potential ATG initiation codon is located at nucleotide 388. Both initiation codons have a purine residue 3 bases 5′ upstream of the ATG, representing favorable translation initiation sites (32). Based on the linear scanning model of translation initiation (33), the first AUG

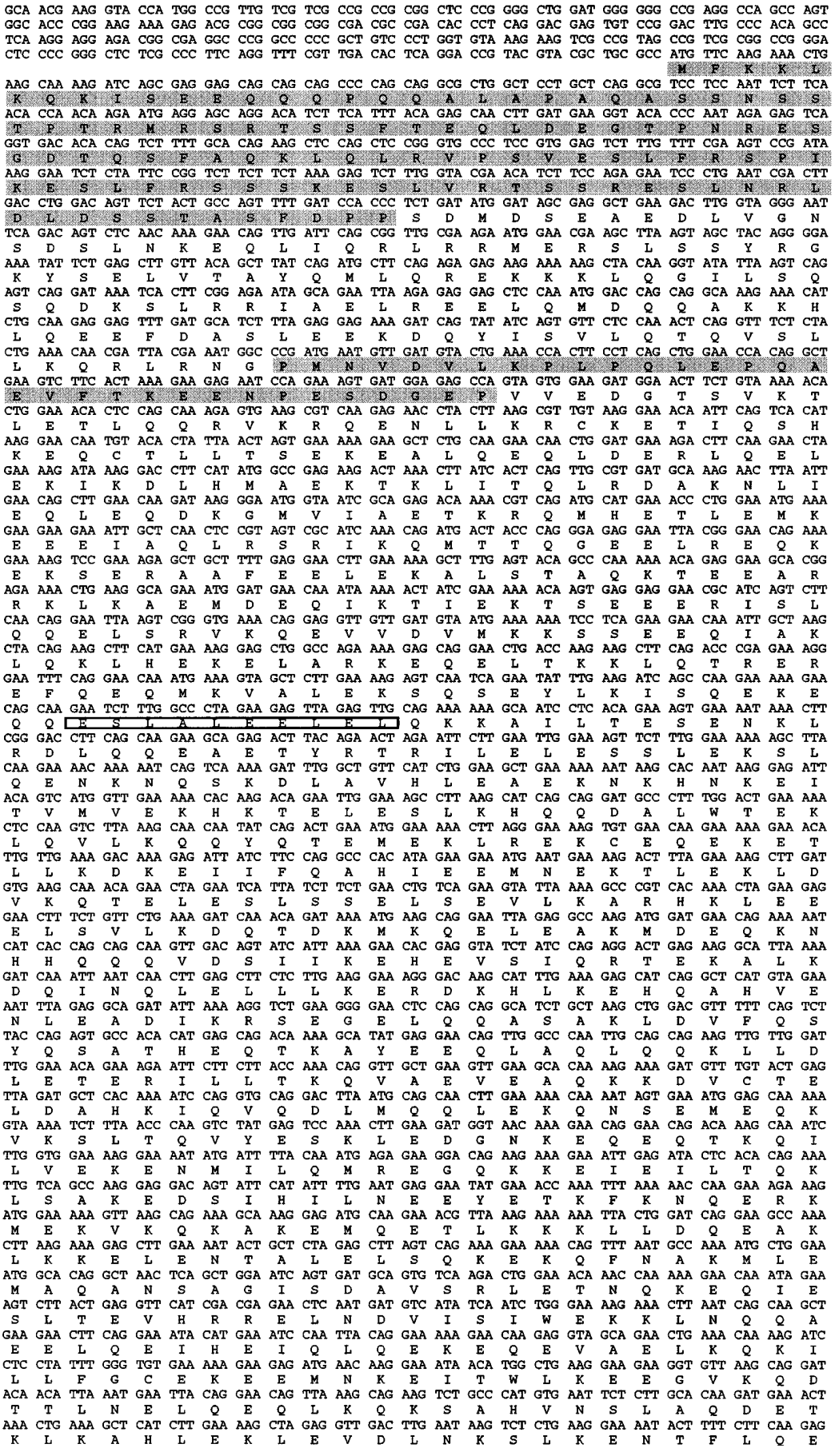

FIG. 6. **cDNA and predicted amino acid sequence of p230.** Nucleotide sequence of p230 is shown, together with the predicted sequence of the encoded polypeptide. Proline-rich regions of the predicted polypeptide (see text; domains 1 and 3) are *shaded* and the granin signature is *boxed*.

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CAG CTA GTT GAA CTG AAG ATG CTG GCA GAA GAA GAT AAG CGG AAG GTT TCT GAG TTG ACT AGC AAG TTG AAA ACC   3825
 Q   L   V   E   L   K   M   L   A   E   E   D   K   R   K   V   S   E   L   T   S   K   L   K   T   1180
ACA GAT GAA GAA TTC CAG AGT TTG AAA TCT TCA CAT GAA AAA AGT AAC AAA AGC CTA GAG GAC AAG AGC TTG GAA   3900
 T   D   E   E   F   Q   S   L   K   S   S   H   E   K   S   N   K   S   L   E   D   K   S   L   E   1205
TTT AAA AAA CTG TCT GAG GAA CTA GCG ATT CAG CTA GAT ATT TGC TGT AAG AAA ACC GAA GCC TTA TTA GAA GCT   3975
 F   K   K   L   S   E   E   L   A   I   Q   L   D   I   C   C   K   K   T   E   A   L   L   E   A   1230
AAA ACA AAT GAG CTA ATC AAC ATT AGT AGT AGT AAA ACT AAT GCC ATT CTT TCT AGG ATT TCT CAT TGT CAG CAC   4050
 K   T   N   E   L   I   N   I   S   S   S   K   T   N   A   I   L   S   R   I   S   H   C   Q   H   1255
CGT ACA ACT AAA GTT AAG GAG GCA CTG TTA ATT AAA ACT TGC ACA GTT TCT GAA TTA GAA GCA CAA CTT AGA CAG   4125
 R   T   T   K   V   K   E   A   L   L   I   K   T   C   T   V   S   E   L   E   A   Q   L   R   Q   1280
TTG ACA GAG GAG CAA AAT ACA CTA AAT ATT TCT TTT CAA CAG GCT ACT CAT CAG TTA GAA GAA AAA GAA AAT CAA   4200
 L   T   E   E   Q   N   T   L   N   I   S   F   Q   Q   A   T   H   Q   L   E   E   K   E   N   Q   1305
ATT AAG AGC ATG AAG GCT GAT ATT GAA AGT CTT GTA ACA GAA AAA GAA GCC TTA CAG AAG GAA GGA GGC AAT CAG   4275
 I   K   S   M   K   A   D   I   E   S   L   V   T   E   K   E   A   L   Q   K   E   G   G   N   Q   1330
CAA CAG GCT GCT TCT GAA AAG GAG TCT TGT ATA ACA CAG TTG AAG AAA GAG TTA TCT GAA AAC ATC AAT GCT GTC   4350
 Q   Q   A   A   S   E   K   E   S   C   I   T   Q   L   K   K   E   L   S   E   N   I   N   A   V   1355
ACA TTG ATG AAA GAA GAG CTT AAA GAA AAA AAA GTT GAG ATT AGC AGT CTT AGT AAA CAA CTA ACT GAT TTG AAT   4425
 T   L   M   K   E   E   L   K   E   K   K   V   E   I   S   S   L   S   K   Q   L   T   D   L   N   1380
GTT CAG CTT CAA AAT AGC ATC AGC CTA TCC GAA AAA GAA GCA GCC ATT TCA TCA CTA AGA AAG CAG TAT GAT GAA   4500
 V   Q   L   Q   N   S   I   S   L   S   E   K   E   A   A   I   S   S   L   R   K   Q   Y   D   E   1405
GAA AAA TGT GAA TTG CTG GAT CAG GTG CAA GAT TTA TCT TTT AAA GTT GAC ACT CTG AGT AAA GAG AAA ATT TCT   4575
 E   K   C   E   L   L   D   Q   V   Q   D   L   S   F   K   V   D   T   L   S   K   E   K   I   S   1430
GCT CTT GAG CAG GTA GAT GAC TGG TCC AAT AAA TTC TCA GAA TGG AAG AAG AAA GCA CAG TCA AGA TTT ACA CAG   4650
 A   L   E   Q   V   D   D   W   S   N   K   F   S   E   W   K   K   K   A   Q   S   R   F   T   Q   1455
CAT CAA AAC ACT GTT AAA GAA TTG CAG ATC CAG CTT GAG TTA AAA TCA AAG GAA GCT TAT GAA AAG GAT GAG CAG   4725
 H   Q   N   T   V   K   E   L   Q   I   Q   L   E   L   K   S   K   E   A   Y   E   K   D   E   Q   1480
ATA AAT TTA TTG AAG GAA GAG CTT GAT CAG CAA AAT AAA AGA TTT GAT TGT TTA AAG GGT GAA ATG GAA GAC GAC   4800
 I   N   L   L   K   E   E   L   D   Q   Q   N   K   R   F   D   C   L   K   G   E   M   E   D   D   1505
AAG AGC AAG ATG GAG AAA AAG GAG TCT AAT TTA GAA ACA GAG TTA AAG TCT CAA ACA GCA AGA ATT ATG GAA TTA   4875
 K   S   K   M   E   K   K   E   S   N   L   E   T   E   L   K   S   Q   T   A   R   I   M   E   L   1530
GAG GAC CAT ATT ACC CAG AAA ACT ATT GAA ATA GAG TCC TTA AAT GAA GTT CTT AAA AAT TAC AAT CAA CAA AAG   4950
 E   D   H   I   T   Q   K   T   I   E   I   E   S   L   N   E   V   L   K   N   Y   N   Q   Q   K   1555
GAT ATT GAA CAC AAA GAA TTG GTT CAG AAA CTT CAA CAT TTT CAA GAG TTA GGA GAA GAA AAG GAC AAC AGG GTT   5025
 D   I   E   H   K   E   L   V   Q   K   L   Q   H   F   Q   E   L   G   E   E   K   D   N   R   V   1580
AAA GAA GCT GAA GAA AAA ATC TTA ACA CTT GAA AAC CAA GTT TAT TCC ATG AAA GCT GAA CTT GAA ACT AAG AAG   5100
 K   E   A   E   E   K   I   L   T   L   E   N   Q   V   Y   S   M   K   A   E   L   E   T   K   K   1605
AAA GAA TTA GAA CAT GTG AAT TTA AGT GTG AAA AGC AAA GAG GAG GAG TTA AAG GCA TTG GAA GAT AGG CTT GAG   5175
 K   E   L   E   H   V   N   L   S   V   K   S   K   E   E   E   L   K   A   L   E   D   R   L   E   1630
TCA GAA AGT GCT GCA AAA TTA GCA GAG TTG AAG AGA AAA GCT GAA CAA AAA ATT GCT GCC ATT AAG AAG CAG TTG   5250
 S   E   S   A   A   K   L   A   E   L   K   R   K   A   E   Q   K   I   A   A   I   K   K   Q   L   1655
TTA TCT CAA ATG GAA GAG AAA GAA GAA CAG TAT AAA AAA GGT ACA GAA AGC CAT TTG AGT GAG CTA AAT ACA AAA   5325
 L   S   Q   M   E   E   K   E   E   Q   Y   K   K   G   T   E   S   H   L   S   E   L   N   T   K   1680
TTG CAG GAA AGA GAA AGG GAA GTT CAC ATC TTG GAA GAA AAA CTT AAG TCA GTG GAA AGT TCA CAG TCA GAA ACA   5400
 L   Q   E   R   E   R   E   V   H   I   L   E   E   K   L   K   S   V   E   S   S   Q   S   E   T   1705
TTA ATT GTA CCC AGA TCA GCA AAA AAT GTG GCA GCA TAT ACT GAA CAA GAA GAA GCA GAT TCC CAA GGC TGT GTG   5475
 L   I   V   P   R   S   A   K   N   V   A   A   Y   T   E   Q   E   E   A   D   S   Q   G   C   V   1730
CAG AAG ACA TAT GAA GAA AAA ATC AGT GTT TTA CAA AGA AAC TTA ACT GAA AAA GAA AAG CTA TTG CAG AGG GTA   5550
 Q   K   T   Y   E   E   K   I   S   V   L   Q   R   N   L   T   E   K   E   K   L   L   Q   R   V   1755
GGG CAG GAA AAA GAA GAG ACA GTT TCT TCT CAT TTT GAA ATG CGA TGC CAA TAC CAG GAG CGC TTA ATA AAG CTA   5625
 G   Q   E   K   E   E   T   V   S   S   H   F   E   M   R   C   Q   Y   Q   E   R   L   I   K   L   1780
GAA CAT GCT GAG GCA AAG CAA CAT GAA GAT CAA AGT ATG ATA GGT CAT CTT CAA GAG GAG CTT GAA GAA AAA AAC   5700
 E   H   A   E   A   K   Q   H   E   D   Q   S   M   I   G   H   L   Q   E   E   L   E   E   K   N   1805
AAG AAA TAT TCC TTG ATA GTA GCC CAG CAT GTG GAA AAA GAA GGA GGT AAA AAT AAC ATA CAG GCA AAG CAA AAC   5775
 K   K   Y   S   L   I   V   A   Q   H   V   E   K   E   G   G   K   N   N   I   Q   A   K   Q   N   1830
TTG GAA AAT GTG TTT GAC GAC GTC CAG AAA ACC CTC CAG GAG AAG GAA CTA ACC TGT CAG ATT TTG GAG CAA AAG   5850
 L   E   N   V   F   D   D   V   Q   K   T   L   Q   E   K   E   L   T   C   Q   I   L   E   Q   K   1855
ATA AAA GAG CTG GAT TCC TGC TTA GTA AGA CAG AAA GAA GTA CAT AGA GTT GAA ATG GAA GAG TTG ACC TCA AAA   5925
 I   K   E   L   D   S   C   L   V   R   Q   K   E   V   H   R   V   E   M   E   E   L   T   S   K   1880
TAT GAA AAA TTA CAG GCT TTA CAA CAG ATG GAT GGA AGA AAT AAA CCC ACA GAA CTT TTG GAA GAA AAC ACT GAA   6000
 Y   E   K   L   Q   A   L   Q   Q   M   D   G   R   N   K   P   T   E   L   L   E   E   N   T   E   1905
GAA AAG TCC AAA TCA CAT TTG GTC CAA CCC AAA TTG CTT AGT AAC ATG GAA GCC CAG CAC AAT GAT CTG GAG TTT   6075
 E   K   S   K   S   H   L   V   Q   P   K   L   L   S   N   M   E   A   Q   H   N   D   L   E   F   1930
AAA TTA GCC GGG GCA GAA CGG GAG AAA CAG AAA CTG GGC AAG GAG ATT GTT AGA TTG CAG AAA GAC CTT CGA ATG   6150
 K   L   A   G   A   E   R   E   K   Q   K   L   G   K   E   I   V   R   L   Q   K   D   L   R   M   1955
TTG AGA AAG GAG CAT CAG CAA GAA TTG GAA ATA CTA AAG AAA GAA TAT GAT CAA GAA AGG GAA GAG AAA ATC AAA   6225
 L   R   K   E   H   Q   Q   E   L   E   I   L   K   K   E   Y   D   Q   E   R   E   E   K   I   K   1980
CAG GAG CAG GAA GAT CTT GAA CTG AAG CAC AAT TCC ACA TTA AAA CAG CTG ATG AGG GAG TTT AAT ACA CAG CTG   6300
 Q   E   Q   E   D   L   E   L   K   H   N   S   T   L   K   Q   L   M   R   E   F   N   T   Q   L   2005
GCA CAA AAG GAA CAA GAG CTG GAA ATG ACC ATA AAA GAA ACT ATC AAT AAG GCC CAG GAG GTG GAG GCT GAA CTT   6375
 A   Q   K   E   Q   E   L   E   M   T   I   K   E   T   I   N   K   A   Q   E   V   E   A   E   L   2030
TTA GAA AGC CAT CAA GAA GAG ACA AAT CAG TTA CTT AAA AAA ATT GCT GAG AAA GAT GAT GAT CTA AAA CGA ACA   6450
 L   E   S   H   Q   E   E   T   N   Q   L   L   K   K   I   A   E   K   D   D   D   L   K   R   T   2055
GCC AAA AGA TAT GAA GAA ATC CTT GAT GCT CGT GAA GAA GAA ATG ACT GCA AAA GTA AGG GAC CTG CAG ACT CAA   6525
 A   K   R   Y   E   E   I   L   D   A   R   E   E   E   M   T   A   K   V   R   D   L   Q   T   Q   2080
CTT GAG GAG CTG CAG AAG AAA TAC CAG CAA AAG CTA GAG CAG GAG GAG AAC CCT GGC AAT GAT AAT GTA ACA ATT   6600
 L   E   E   L   Q   K   K   Y   Q   Q   K   L   E   Q   E   E   N   P   G   N   D   N   V   T   I   2105
ATG GAG CTA CAG ACA CAG CTA GCA CAG AAG ACG ACT TTA ATC AGT GAT TCG AAA TTG AAA GAG CAA GAG TTC AGA   6675
 M   E   L   Q   T   Q   L   A   Q   K   T   T   L   I   S   D   S   K   L   K   E   Q   E   F   R   2130
GAA CAG ATT CAC AAT TTA GAA GAC CGT TTG AAG AAA TAT GAA AAG AAT GTA TAT GCA ACA ACT GTG GGG ACA CCT   6750
 E   Q   I   H   N   L   E   D   R   L   K   K   Y   E   K   N   V   Y   A   T   T   V   G   T   P   2155
TAC AAA GGT GGC AAT TTG TAC CAT ACG GAT GTC TCA CTC TTT GGA GAA CCT ACC GAA TTT GAG TAT TTG CGA AAA   6825
 Y   K   G   G   N   L   Y   H   T   D   V   S   L   F   G   E   P   T   E   F   E   Y   L   R   K   2180
GTG CTT TTT GAG TAT ATG ATG GGT CGT GAG ACT AAG ACC ATG GCA AAA GTT ATA ACC ACC GTA CTG AAG TTC CCT   6900
 V   L   F   E   Y   M   M   G   R   E   T   K   T   M   A   K   V   I   T   T   V   L   K   F   P   2205
GAT GAT CAG ACT CAG AAA ATT TTG GAA AGA GAA GAT GCT CGG CTG ATG TTT ACT TCA CCT CGC AGT GGT ATC TTC   6975
 D   D   Q   T   Q   K   I   L   E   R   E   D   A   R   L   M   F   T   S   P   R   S   G   I   F   2230
TGA GTA AAC CAT CAG TCT GTG CTT AGT TAA CAT GTG TCA TGG CTC CGA TCT TCA TCT TGA AGA AGA GTG ACA TTG   7050
GGT GAC TGC TGC TTG GAA AAC TGT CCA CAC TTG CTA CTC TTT GAG AAT GAA GTT GTC ATT CAG GGC CCC TCA TGT   7125
AGC CAA AAG ACC AAG AAA AAT CTG GCC CAC AGA TAA GTT GCA GAC TGC CTT TAA AAT AGA TTT TAT CAG TGG AGA   7200
AAT GGT GAT AGT TTT TTC TTC AGT TTT CTC TTG GGA AGG AGT TTT ATG TTG TTT AAA AGA TAT TTT GAT AAC TTA   7275
ACC TGC TTT ATG GGC TTA CAT AAT ATT CCT TTC ATC CAT TCT TTT TAA AGA ACG GCT TAC CTT TCC TAT TTA TTT   7350
TTA GGG TGA TTT TTT AAA AAG ACT TGT GCA ATA CAT TTT GAG GTG AAA CTT AGT GGA TTT TTT CTG ATA AAT TAG   7425
AGC ATT TAA TTG ACT ATT TTA TTC AGG TTG ATC TGT TGA ATA TTT GCT AAA GAC CAG TTC TTT AAG CTA AGA CAT   7500
GTA AAA AAT CCC AAA TGG CAG TAC CTC ATT GTT TAC TTA GCT TTT GTA CTT ATA TTT TTC AGA GGA AAA AAC ACT   7575
ACT GTA AAT TGT GAA TAG CCA ATA CAT AAC TGT ATT GTA TGC AAA TCT GTG ATT GTT GGC AGT GTC ATC TCT GAG   7650
AAA CAG ATA AAT AAA GTT TAT TTA CTA TAA AAA AAA AAA AAG                                               7695
```

FIG. 6—*continued*

TABLE I

Sequences present in GenBank™ that are homologous to p230

The FASTA program (23) was used to search the GenBank™ database for loci that are homologous to p230. The table lists the GenBank™ loci that were identical or nearly identical to p230, together with the region of p230 to which homology was shared and the length of the region of homology. The percentage identity of each locus with the homologous segment of p230 is also shown.

Locus name	Region of identity in p230	Size of homologous region	% Identity
		bp	
R32028	Nucleotides 8–376	369	91.3
R22854	Nucleotides 14–372	359	89.4
B21241	Nucleotides 14–375	362	92.3
R63927	Nucleotides 15–279	265	97.4
T91725	Nucleotides 26–376	351	98.6
M85542	Nucleotides 37–248	212	96.7
HS721PMR	Nucleotides 1853–2434	582	99.8
T10747	Nucleotides 4455–4658	204	96.6
T50957	Nucleotides 4982–5294	313	95.6
HHEA86R	Nucleotides 5915–6306	392	100
R32081	Nucleotides 7058–7413	356	95.1
R21240	Nucleotides 7140–7452	313	95.0
R64018	Nucleotides 7140–7515	376	98.2
R22853	Nucleotides 7170–7470	301	92.5
HUMGS01825	Nucleotides 7455–7681	227	100

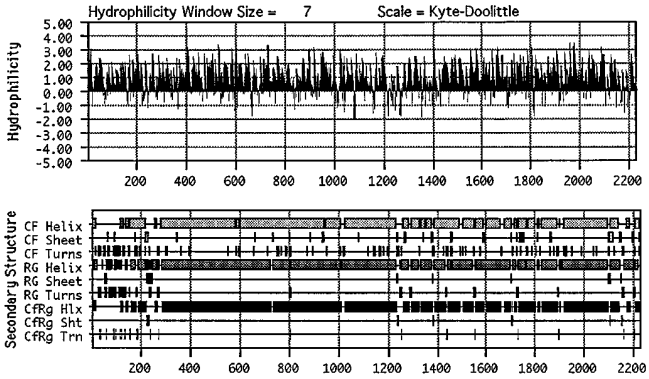

FIG. 7. **Secondary structure predictions for p230.** *Top panel,* hydrophilicity profile of p230 using Kyte-Doolittle hydropathy scale with a window of 7 (49). Positive values denote hydrophilic regions that may be exposed on the outside of p230. *Bottom panel,* a summary of secondary structure predictions using the methods of Chou-Fasman (50–53) (*CF, light shaded bars*) and Robson-Garnier (54, 55) (*RG, dark shaded bars*) with a hydrophilicity window size of 11 is shown, together with a composite where both Chou-Fasman and Robson-Garnier predictions are in agreement (*CfRg, black shaded bars*). The presence of a bar indicates regions of the protein predicted to form α-helices, β-pleated sheet, or reverse turns by each of the methods used. These plots were generated using the MacVector program (International Biotechnologies Inc.).

initiation codon would be expected to be the dominant signal. The TGA codon is followed by a polyadenylation signal AATAAA (34) at position 7660 and a poly(A) tail from position 7679 to 7694.

The open reading frame encodes a putative polypeptide of 2230 amino acids with a predicted molecular weight of 261,126 Da and a pI of 5.16. The amino acid composition of this putative polypeptide is rich in glutamic acid (16.6%), lysine (12.7%), leucine (12.5%), and glutamine (10.1%) residues. Comparison with the GenBank™ data base reveals regions of p230, which are identical or nearly identical to 15 previously isolated partial cDNA clones of no known function. A list of these loci together with the corresponding regions of identity in p230 is given in Table I.

Comparison of the translated amino acid sequence of p230 with the translated GenBank™ data base reveals significant homology (17–27% identity) with many proteins known or predicted to encode coiled-coil domains. These include various conventional and nonconventional myosins, tropomyosins, cytokeratins, vimentin, neurofilaments, laminin, hemeolytic streptococcal M proteins, dystrophin, the Golgi proteins golgin-95, golgin-160, and giantin, the Uso1 protein of *S. cerevisiae* implicated in endoplasmic reticulum to Golgi transport and its mammalian homologue p115 or TAP, and the early endosome-associated protein EEA-1 (35). In addition, this homology was shared with other structural and motor proteins of the cytoskeleton including human cytoplasmic linker protein-170, kinetocore protein CENP-E, lamins, dynein, and kinesins. In general, this pattern of identity was scattered throughout the length of p230, although similar comparisons with translated GenBank™ sequences using a series of overlapping segments of p230 revealed that such homology was less pronounced in the amino-terminal 130 amino acids of p230.

There are two proline-rich domains at the amino terminus of the putative polypeptide; amino acids 1–117 contains 6.8% proline residues, while the segment from amino acids 239–270 contains 18.8% proline residues (Fig. 6), suggesting a compact structure for these domains. The remainder of the protein has scant proline residues, most of which are clustered at the extreme carboxyl terminus. Thus, p230 can be divided into four putative domains on the basis of its proline distribution, *i.e.* domain 1 (amino acids 1–117), domain 2 (amino acids 118–238), domain 3 (amino acids 239–270), and domain 4 (amino acids 271-2230).

These analyses are consistent with the two proline-rich domains having a high probability of globular structures and an elongated structure for the intervening domains 2 and 4 (36). These features are supported by results of secondary structure predictions (Fig. 7), which suggest a predominantly α-helical structure for p230 with the exception of the proline-rich domains 1 and 3. Analyses of hydrophilicity (Fig. 7) suggest a predominantly hydrophilic structure, with no evidence for a hydrophobic transmembrane domain, consistent with biochemical data reported by Kooy *et al.* (13). Charge plots (not shown) show no evidence for discrete acidic or basic domains.

The above features raised the possibility that p230 adopts a coiled-coil structure, stabilized by heptad repeats. A search for these structures was performed using the method of Lupas *et al.* (26). The results of this analysis (Fig. 8*a*) reveal an extraordinarily high level of heptad repeats in domains 2 and 4, which predict a coiled-coiled structure with a high degree of confidence. Detailed sequence analysis of the longest of these regions is shown in Fig. 8*b*, which shows a run of 31 heptad repeats extending over 245 amino acids with four heptad frame-shifts. In common with various fibrous coiled-coil proteins (37, 38), this region shows a high frequency of apolar residues in positions *a* (75%) and *d* (52%) of the heptads and the absence of acidic residues in these positions. The preference for leucine over isoleucine at the *d* position suggests that the protein has a dimeric rather than a trimeric quaternary structure (39, 40). This region also shares with the fibrous proteins a high frequency (54.9%) of charged residues in the outer positions (*b, c, e, f,* and *g*), with only 14.3% of residues in these positions being apolar. However, in contrast to the fibrous proteins, this region has a relatively high number of lysine and arginine residues in the *d* position, which, together with the concomitant reduction in apolar residues at this position and the presence of other discontinuities including frameshifts and "stutter residues," would be expected to confer marginal stability on the coiled-coil (36).

p230 has multiple consensus motifs for tyrosine phosphorylation, protein kinase C phosphorylation, casein kinase II phosphorylation, cAMP/cGMP phosphorylation, *N*-myristoylation,

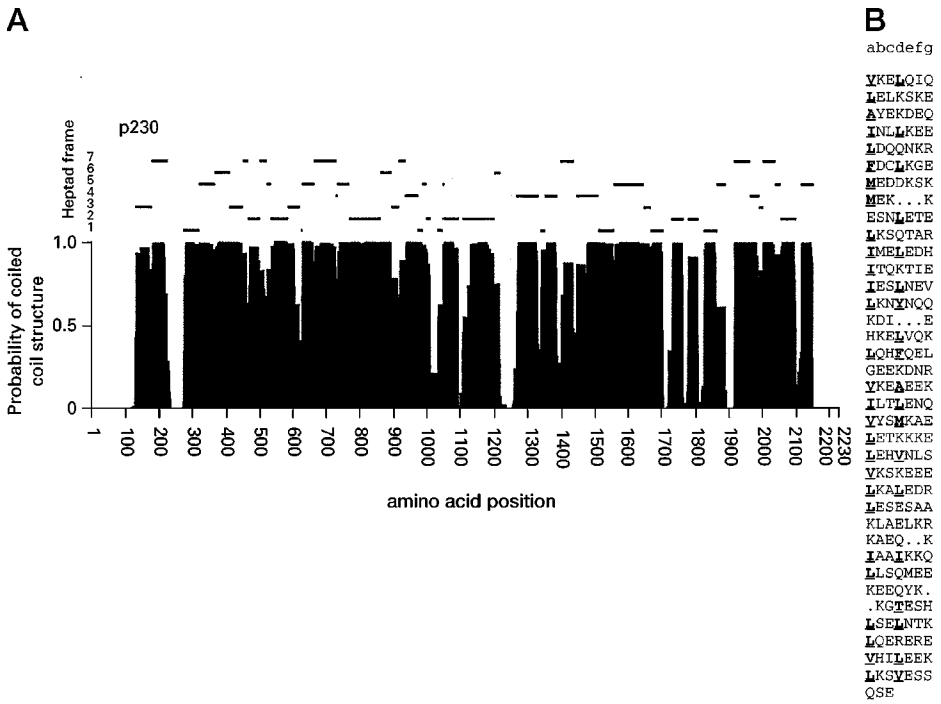

FIG. 8. *A*, prediction of coiled-coil segments of p230. The *lower panel* is a histogram of the probability of forming a coiled-coil structure according to the method of Lupas *et al.* (26). $p > 0.9$ are significant. *Bars* above the histogram indicate which of the possible seven "frames" the heptad repeats follow for each region of the protein with P>0.5 for formation of a coiled-coil structure. The presence of numerous "frameshifts" provides evidence of discontinuities in the coiled coil structure (see text). *B*, primary sequence of the longest uninterrupted region of heptad repeats from p230. The amino acid sequence of p230 from position 1460 to 1704 is plotted to show the position of each residue within a heptad repeat of the form abcdefg (38). Apolar residues at positions *a* and *d* are shown in *boldface*.

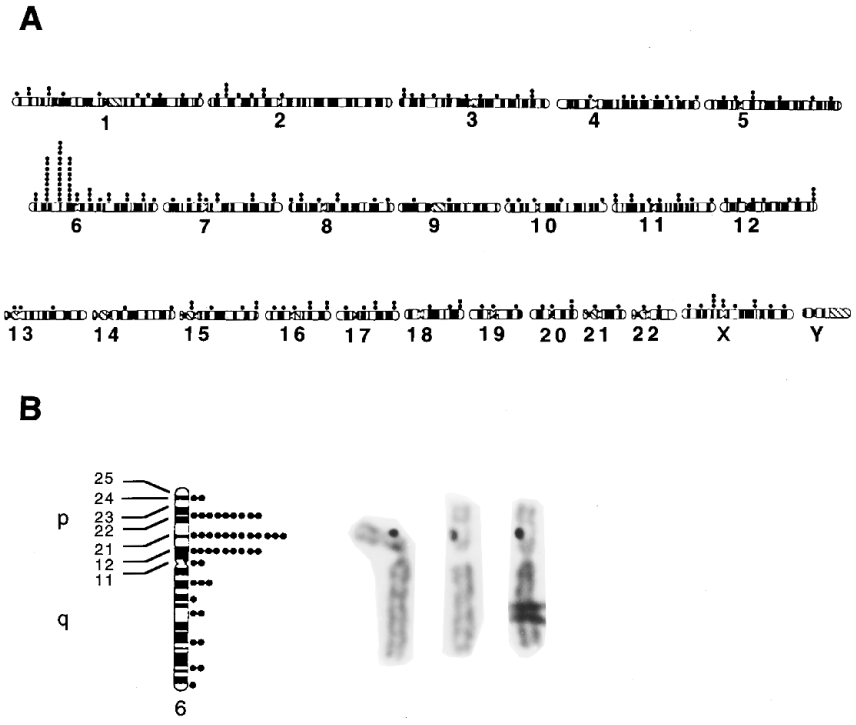

FIG. 9. **Chromosomal localization of human p230 gene.** *A*, diagram showing the grain distribution in 70 metaphase spreads on one slide following hybridization with ^{3}H-labeled DNA from clone λg5; *B*, an enlargement of grains recorded on chromosome 6 together with photographs showing silver grains on G-banded chromosome 6.

and *N*-glycosylation. It is not known at present how many of these motifs are utilized; however, p230 appears to be devoid of *N*-glycans (13), consistent with location of the protein on the cytoplasmic face of Golgi membranes. p230 also contains between amino acids 538 and 546 the sequence ESLALEELEL, a motif found in otherwise diverse members of the granin (chromogranin/secretogranin) family of acidic proteins found in the secretory granules of neuroendocrine cells. A search of the translated GenBank™ data base revealed that in addition to chromogranins, this region and its immediate flanking sequence showed homology with a number of proteins involved in subcellular compartmentalization and motor functions, including flagellin, calnexin, the Golgi protein giantin, β8-tubulin, neurofilament L, caldesmon, chromokinesin, and with the human microtubule-associated protein E-MAP-115. No known microtubule binding motifs were found in p230.

Chromosomal Localization—Using λg5 clone as a probe, individual grains recorded on a cumulative idiogram revealed a significant accumulation of signal on a region of the short arm of human chromosome 6, assigning the probe to 6p12–22. Fig. 9*a* shows a diagrammatic presentation of the genomic distribution of grains observed in 70 metaphase spreads on one slide. An enlarged diagram of the grains recorded on chromosome 6, showing a peak at 6p12–22, together with photographs of a silver grain located at the specific site of hybridization, are shown in Fig. 9*b*.

DISCUSSION

The cDNA clones we have isolated encode the full-length p230 Golgi protein for the following reasons. First, autoantibodies, affinity-purified from clones λg2, λg5, λg7, and λg12, gave immunofluorescence staining of the Golgi apparatus of Hep2 cells. Second, rabbit antibodies raised against a bacterial fusion protein incorporating clone λg5 not only stained the Golgi apparatus by immunofluorescence and immunoelectron microscopy, but also immunoblotted and immunoprecipitated a 230-kDa protein from HeLa cells (13). Third, cDNAs of clones px1, λz9, and λz16 together span 7.7 kb, in agreement with the size of the mRNA obtained from Northern blots; and fourth, reverse transcripase PCR demonstrated that these three clones were derived from the same transcript.

The deduced amino acid sequence of p230 suggests a hydrophilic, modestly acidic protein capable of forming a dimeric coiled coil structure. While most (>90%) of the protein is predicted to form this structure, the extreme amino-terminal 130 amino acids and a segment between amino acids 239 and 270 are predicted to form compact structures consistent with globular regions. While it is well established that stable static coiled coils can serve as multimerization motifs in structural proteins, dynamic coiled-coil formation can play a central role in generation of conformational changes resulting in dramatic movements of one part of a protein relative to another. Such coiled-coil regions have been implicated in the function of the nonclaret disjunctional kinesin-related microtubule motor protein, which translocates on microtubules toward their minus ends and is required for proper chromosome segregation in *Drosophila* oocytes (41). This protein has a central stalk region consisting of heptad repeats predicted to form coiled-coils. A mutant that lacks the amino-terminal third of the coiled-coil stalk exhibits partial loss of function, having a translocation velocity and torque generation similar to wild-type protein, but only partially rescues a null mutant for chromosome missegregation. A similar effect has been reported for a cytoplasmic myosin II protein partially deleted for its coiled-coil tail (42). In this case, the mutant protein is expressed at a level comparable with the wild-type protein and translocates on actin filaments *in vitro* with the same velocity as wild-type protein, but in spite of this, exhibits frequent failure of cytokinesis *in vivo*. Of particular interest is the loss of function reported for partial deletions of the coiled-coil domain of the yeast Uso1 protein (43), a protein involved in vesicular transport, where such mutations are temperature-sensitive lethal, resulting in a severe defect of endoplasmic reticulum to Golgi protein transport at the nonpermissive temperature. It has been suggested that a key structural feature of coiled-coils that participate in conformational changes is the presence of regions of marginal stability caused by discontinuities in heptad repeats of the coiled coil as a result of deletions, insertions, or out-of-frame residues (44). The presence of extensive regions of this type in p230 raises the possibility of dynamic coiled-coil formation, which could play a role in regulation of multimerization or in induction of conformational changes in the protein. Dramatic conformational changes have been found to occur in other coiled-coil proteins involved in membrane fusion events (for review, see Ref. 44).

p230 also has a short region of homology to the conserved carboxyl-terminal domain of the granin (chromogranin/secretogranin) family of proteins, a diverse group of acidic proteins present in secretory granules of endocrine and neuroendocrine cells (for review, see Ref. 45). Granins are suggested to be precursors of several peptide hormones and to regulate proteolytic processing and selective aggregation of secretory proteins in the *trans*-Golgi network of neuroendocrine cells (46). Only one short region at the carboxyl terminus is shared among the granins. This region bears the consensus sequence E(N/S)L*X*(A/D)*X*(D/E)*X*EL which is closely related to a sequence found in p230 (Fig. 6). A short region of the carboxyl terminus of chromogranin A, containing the granin motif, may be responsible for pH-regulated multimerization of the protein (47) and, together with the pH-dependent association of chromogranin A with integral membrane proteins of the secretory vesicle, suggests a role for chromogranins A in the sorting of these membrane proteins during vesicle biogenesis in the *trans*-Golgi network (48). However, the relevance of this motif in p230 is unclear since p230 is orientated on the cytosolic side of Golgi membranes.

There is an increasing number of proteins implicated in vesicular transport that have extensive coiled-coil domains. These domains have potential for dynamic interactions associated with this highly complex process. The potential dynamic coiled-coil structure of p230, its localization to the *trans*-Golgi network,[3] and sensitivity to brefeldin A (13) suggest that p230 may have a key role in vesicular transport from this distal Golgi compartment.

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