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p115 is a general vesicular transport factor related to the yeast endoplasmic reticulum to Golgi transport factor Uso1p

STEPHANIE K. SAPPERSTEIN*, DAVID M. WALTER*, ALEXANDRA R. GROSVENOR*, JOHN E. HEUSER†, AND M. GERARD WATERS*‡

*Department of Molecular Biology, Princeton University, Princeton, NJ 08544; and †Department of Cell Biology, Washington University School of Medicine, St. Louis, MO 63110

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ABSTRACT **A recently discovered vesicular transport factor, termed p115, is required along with *N*-ethylmaleimide-sensitive fusion protein (NSF) and soluble NSF attachment proteins for *in vitro* Golgi transport. p115 is a peripheral membrane protein found predominantly on the Golgi. Biochemical and electron microscopic analyses indicate that p115 is an elongated homodimer with two globular "heads" and an extended "tail" reminiscent of myosin II. We have cloned and sequenced cDNAs for bovine and rat p115. The predicted translation products are 90% identical, and each can be divided into three domains. The predicted 108-kDa bovine protein consists of an N-terminal 73-kDa globular domain followed by a 29-kDa coiled-coil dimerization domain, a linker segment of 4 kDa, and a highly acidic domain of 3 kDa. p115 is related to Uso1p, a protein required for endoplasmic reticulum to Golgi vesicular transport in *Saccharomyces cerevisiae*, which has a similar "head-coil-acid" domain structure. The p115 and Uso1p heads are similar in size, have ≈25% sequence identity, and possess two highly homologous regions (62% and 60% identity over 34 and 53 residues, respectively). There is a third region of homology (50% identity over 28 residues) between the coiled-coil and acidic domains. Although the acidic nature of the p115 and Uso1p C termini is conserved, the primary sequence is not. We discuss these results in light of the proposed function of p115 in membrane targeting and/or fusion.**

Eukaryotic cells contain many membrane-bound compartments with distinct sets of cellular functions. Although the compartments are discrete, they interact by a vigorous flow of vesicles (1). This membrane traffic, which is mediated by a number of soluble and membrane-associated proteins, requires vesicle formation, specific targeting to the next compartment, and membrane fusion.

Many of the components required for vesicular transport have been identified by biochemical methods in higher eukaryotic cells or by genetic analyses in the yeast *Saccharomyces cerevisiae* (2, 3). One well-studied mammalian *in vitro* system reconstitutes transport from the cis to the medial compartment of the Golgi apparatus (4). This system measures transfer of vesicular stomatitis virus G protein (VSV-G) from a Golgi stack deficient in GlcNAc transferase to one that contains the transferase by monitoring the addition of [^{3}H]GlcNAc to VSV-G. Biochemical fractionation of the soluble proteins required to drive this system led to the identification and purification of several factors—namely, the *N*-ethylmaleimide-sensitive fusion protein (NSF) (5), the soluble NSF attachment proteins (SNAPs) (6), and p115 (7). NSF and α-SNAP are the mammalian counterparts of the yeast proteins Sec18p (8, 9) and Sec17p (10), respectively. Both NSF/Sec18p and α-SNAP/Sec17p are involved in a late step of vesicular transport, either vesicle docking or membrane fusion (11–14). In addition to these late-acting factors, a number of proteins involved in vesicle budding—for example, coatomer and ARF (ADP-ribosylation factor)—are homologous in yeast and mammals (15–17). In general, it appears that the molecules and mechanisms used by mammals and yeast are quite similar (18–20).

Recently, a set of membrane proteins collectively called SNAREs (SNAP receptors) has been shown to form an oligomeric complex with NSF and SNAPs (21, 22). The SNAREs include VAMP (vesicle-associated membrane protein; also called synaptobrevin), syntaxin, and SNAP-25 (synaptosome-associated protein of 25 kDa). These proteins were originally described in neurons as components of synaptic vesicles [VAMP (23, 24)] or of the presynaptic membrane [syntaxin (25) and SNAP-25 (26)]. There are multiple VAMP (27), syntaxin (28), and SNAP-25 (29) isoforms, and in some cases they have been shown to reside on distinct intracellular membranes (28). Based on these observations, it has been proposed that NSF and SNAPs act to promote fusion of the vesicle with its target membrane after specific docking has been effected by the SNAREs (25, 30) that reside on the vesicle (v-SNARE) and target (t-SNARE) membranes (for review, see ref. 31).

In addition to NSF and SNAPs, three high molecular weight components are required to drive transport in an *in vitro* cis to medial Golgi transport reaction (7). One of these is a protein that migrates on SDS/polyacrylamide gels with an apparent molecular mass of 115 kDa. This protein, termed p115, was shown to be a homooligomeric protein that is peripherally associated with membranes. Immunofluorescence demonstrated that p115 is localized predominantly to the Golgi apparatus (7). In this report, we describe the amino acid sequence and structure of p115 and show that it is homologous to Uso1p, which is required for endoplasmic reticulum (ER) to Golgi transport in the yeast *S. cerevisiae* (32).§

MATERIALS AND METHODS

Electron Microscopy. Purified p115 (7) at 50 μg/ml in 25 mM Hepes·KOH, pH 7.4/200 mM KCl/1 mM dithiothreitol/10% (vol/vol) glycerol was visualized on mica by quick-freeze, deep-etch, rotary shadowing electron microscopy as described (33).

Cloning and Sequencing. To obtain the bovine p115 clone, 10^6 recombinants from an oligo(dT)-primed λ ZAP II kidney cDNA expression library (Stratagene) were screened with a pool of five monoclonal antibodies (mAbs) (mAb115-1 to mAb115-5) that had been raised against purified p115 (7). Screening was done according to the manufacturer's instructions except that blotting conditions were as used previously for these antibodies (7). Each antibody was used at a dilution

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Abbreviations: NSF, *N*-ethylmaleimide-sensitive fusion protein; SNAP, soluble NSF attachment protein; SNARE, SNAP receptor; SNAP-25, synaptosome-associated protein of 25 kDa; ER, endoplasmic reticulum; mAb, monoclonal antibody.

‡To whom reprint requests should be addressed.

§The sequences reported in this paper have been deposited in the GenBank data base [accession nos. U14186 (bovine) and U14192 (rat)].

of 1:20. A single clone was isolated, which extended from base 2087 to base 3990. A 326-bp *Eco*RI/*Hin*cII restriction fragment from the 5′ end of the insert was gel purified (GeneClean II; BIO101), labeled by the random-primer method with [^{32}P]dCTP (Prime-A-Gene kit; Amersham), and used to screen 10^6 recombinants from a random and oligo(dT)-primed λ ZAP II bovine liver cDNA library (Stratagene; from J. Wetterau, Bristol-Meyers Squibb) (34). Hybridizations were performed in Church and Gilbert buffer (35). Filters were washed once briefly in 0.1× standard saline citrate/0.5% SDS at room temperature and then three times for 15 min at 65°C. Four independent isolates were obtained. A 530-bp *Bgl* II/*Eco*RI fragment from the 5′ end of the clone containing new 5′ sequence was labeled as described above and used to screen a size-selected random-primed λ ZAP I bovine brain cDNA library (from R. Deihl and R. Dixon, Merck). Recombinants (1.7×10^5) of the >4.4-kb library (containing 5×10^4 independent recombinants) were screened as described above. Two clones were obtained. Both strands of the clone that contains the entire p115 open reading frame were sequenced with a Sequenase kit (United States Biochemical) and resolved using Sequagel rapid acrylamide (National Diagnostics).

For the rat clone, expression screening of an oligo(dT)-primed UniZap liver cDNA library (Stratagene) was done as described above except that only one mAb, mAb115-5 (7), was used at a dilution of 1:5. Twenty-one clones, with insert sizes ranging from 1.3 to 4.0 kb, were isolated. One of the clones contained all but approximately the first 65 bp of the open reading frame. Both strands of that clone were sequenced as described above. To obtain the 5′ end of the rat cDNA, the library was PCR amplified using a pair of primers nested in the 5′ end of the longest clone and one primer in the Bluescript polylinker (BS2) (36). In the first round of PCR, the outside primer and BS2 were used to amplify the above library, which had been lysed by heating for 5 min at 98°C in water. The second round of PCR was performed with the product from round one using BS2 and the inside primer (DW31), which has a *Cla* I restriction site engineered into the 5′ end to facilitate subcloning. The product of round two was subjected to restriction digestion with *Cla* I and *Xba* I and was subcloned into Bluescript II SK− linearized with the same enzymes (34). Plasmids containing the largest inserts were sequenced as described above. Of the nine that were sequenced, four contained the 5′ end of the rat cDNA clone.

Sequence Analyses. The BLAST program (National Center for Biotechnology Information) was used to search the nonredundant peptide sequence and nucleotide data base (as of June 30, 1994). Further alignments were conducted using GAP, BESTFIT, and PILEUP (University of Wisconsin Genetics Computer Group sequence analysis package, version 7.3). Coiled-coil graphs were made from data generated by the COILS2 algorithm, which is an updated version of the COILS program (37). The MTDIK matrix and a window size of 21 were used. The COILS2 program and documentation are available from A. Lupas (Max-Planck-Institut für Biochemie, Munich).

RESULTS

Previous biochemical studies of purified p115 indicated that it is an elongated, oligomeric molecule (7). We have used quick-freeze, deep-etch, rotary shadowing electron microscopy (33) of purified p115 adsorbed to mica to examine the structure further. The micrographs (Fig. 1) indicate that p115 is a parallel homodimer, consisting of an extended rod-like domain of ≈45 nm, which we term the tail, and two globular heads, which are ≈10 nm in diameter. This structure is similar to that of several cytoplasmic motors, including the microtubule motor kinesin (38) and the actin motor myosin II (39). In contrast to the expectation for molecular motors, we have not been able to detect nucleotide binding by biochemical methods (data not shown), and the cDNA sequence does not encode a consensus nucleotide binding site.

To obtain primary sequence information, we isolated a full-length bovine p115 cDNA. The open reading frame is preceded by 159 bp of 5′ untranslated sequence, which includes four in-frame stop codons and the translation start is surrounded by stop codons in the other two reading frames. The context of the first ATG in the open reading frame is appropriate for translational initiation (40). The 3′ untranslated region is 948 nucleotides long and encodes two polyadenylylation signals (AAUAAA) (41) located 86 and 919 bp after the stop codon.

The cDNA encodes a protein with a predicted molecular mass of 107,514 Da and a pI of 4.66. The clone is authentic as it encodes seven peptides found by microsequence analysis of p115 tryptic fragments. Furthermore, p115 expressed in *Esch-*

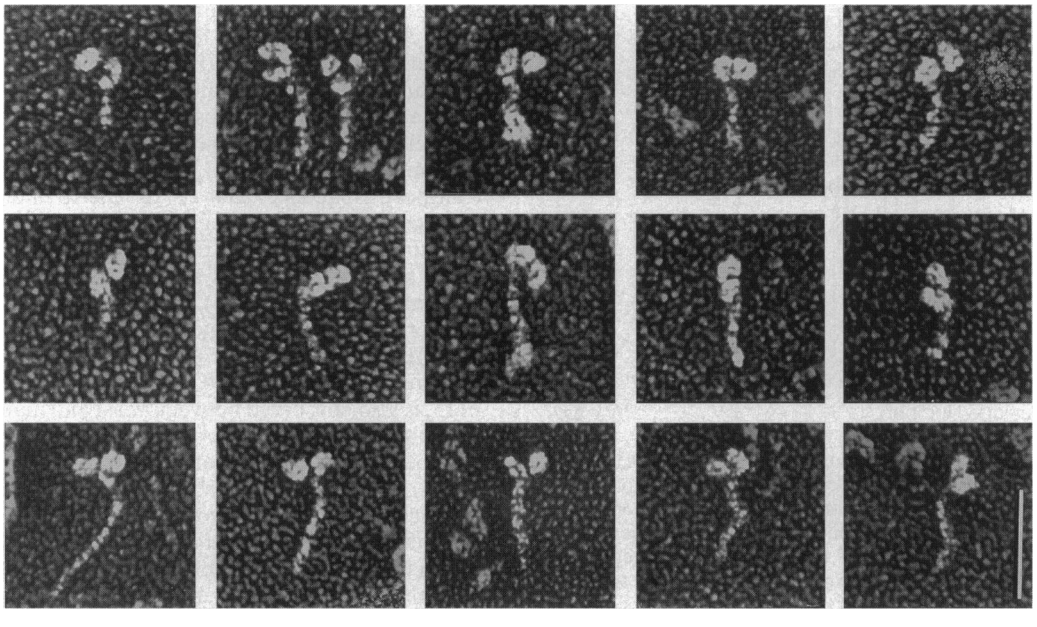

FIG. 1. Panel of electron micrographs of p115 adsorbed to mica and visualized by quick-freeze, deep-etch rotary shadow electron microscopy. (Bar = 50 nm; ×300,000.)

erichia coli from this cDNA comigrates with bovine p115 on SDS/polyacrylamide gel and crossreacts with p115 antibodies (data not shown).

In a similar manner, 21 rat p115 cDNA clones were isolated with an anti-bovine p115 mAb that crossreacts with the rat protein. The rat cDNA contains three potential polyadenylylation signals, two of which, the first and third, are in alignment with those in the bovine clone. All three polyadenylylation signals are used since clones were obtained with poly(A) tracts 24–26 bp downstream of each signal. The bovine and rat nucleotide sequences are 83% identical. The cDNA encodes a protein with a predicted molecular mass of 107,162 Da and a pI of 4.70. Overall, the predicted amino acid sequences of the bovine and rat proteins are 92% identical and 96% similar.

Many dimeric, as well as some trimeric, proteins interact via coiled-coils. These domains are composed of heptad repeats bearing hydrophobic residues in the first (a) and fourth (d) positions. The electron micrographs of p115 suggested that it might dimerize via a coiled-coil motif. Computer analysis of the predicted bovine p115 sequence indicates that the C terminus contains a 258-residue domain that has a high probability of forming a coiled-coil (Fig. 2*A*). Within this domain there are four separate blocks of residues predicted to form coiled-coils (Fig. 2*A*). These regions are displayed as heptad repeats in Fig. 2*B*. The preponderance of leucines rather than isoleucines in the d position of the heptad repeats suggests a two-stranded rather than a three-stranded coiled-coil (42).

The head domain of p115 is more highly conserved than the tail domain. The head domains (residues 1–651 for both bovine and rat) align perfectly and are 97% identical and 99% similar. Alignment of the tails, which are 79% identical and 89% similar, requires the introduction of three short gaps. This suggests that the coiled-coil nature of the tail, rather than the primary amino acid sequence, is important for the function of this region of the protein.

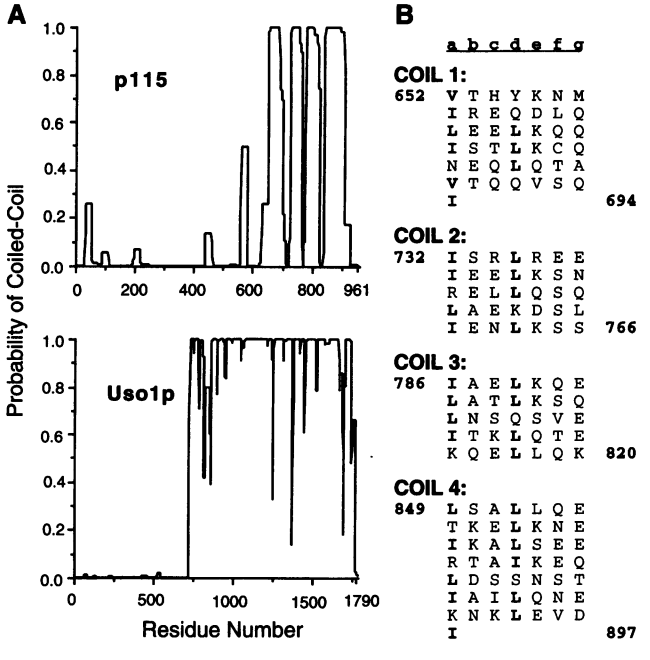

FIG. 2. p115 and Uso1p contain coiled-coil domains. (*A*) Probability of coiled-coil structure for p115 and Uso1p. p115 contains a predominantly coiled-coil domain in the C terminus between amino acids 652 and 909. Uso1p contains a coiled-coil domain between amino acids 729 and 1743 (32). (*B*) Heptad repeats in the four regions of bovine p115 that are predicted to form the coiled-coils. Letters a–g refer to positions in the heptad repeat. Hydrophobic residues in positions a and d are indicated in boldface. All four regions contain heptad repeats that, for the most part, maintain hydrophobicity at the first and fourth positions.

The extreme C terminus of p115 is very hydrophilic. Eighteen of the last 28 residues of the bovine protein are acidic (either aspartate or glutamate); in the rat protein 17 of the last 25 residues are acidic (Fig. 3*B*). The overall identity between these two proteins in the acidic domain is 54%.

Comparison of the predicted p115 protein sequence with current data bases using the BLAST program (43) indicates that the head domain of p115 is homologous to the *S. cerevisiae* ER to Golgi transport factor Uso1p (32). Like p115, Uso1p can be divided into an N-terminal head, a coiled-coil dimerization domain, and an acidic C terminus. Uso1p, which is 206 kDa, is significantly larger than p115. Most of the extra mass is present in the coiled-coil dimerization domain (Fig. 2*A*); the head domains are similar in size, being 651 residues for p115 and 728 residues for Uso1p. Overall, the head domains of bovine p115 and Uso1p are 25% identical and 50% similar; however, they share two regions of high homology (Fig. 3*A*). Homology region 1 (HR1) is 34 residues long and is 62% identical and 74% similar, while homology region 2 (HR2) is 60% identical and 77% similar over 53 residues (Fig. 3*A*). In addition, after the end of the predicted coiled-coil domains there is a third region of homology (Fig. 3*B*, HR3). Over the 28 amino acids in this region, bovine p115 and Uso1p are 50% identical and 75% similar (Fig. 3*B*). Finally, like the mammalian proteins, the yeast protein also contains a highly acidic domain at its C terminus, which consists of 15 contiguous aspartate or glutamate residues within the last 19 amino acids of the protein (Fig. 3*B*). However, since the degree of identity between the mammalian and yeast proteins is low, it appears that the overall negative charge, not a particular conserved sequence of amino acids, is the salient feature of the acidic domain.

DISCUSSION

p115 is required for cell-free intra-Golgi protein transport. It was identified and isolated by using an *in vitro* transport system, which was dependent on peripheral membrane proteins that had been removed from the Golgi membranes by salt extraction (7). Rotary shadowing electron microscopy and sequence analyses indicate that p115 is a dimeric molecule with two N-terminal heads and a C-terminal coiled-coil tail that terminates in an acidic domain (see Fig. 3*C*).

p115 is homologous, both structurally and at the primary sequence level, to a yeast protein termed Uso1p (32). Uso1p was identified in a screen for new secretion mutants that relies on the inability of the mutant cells to secrete sexual agglutinins (32). At the restrictive temperature, cells containing the temperature-sensitive *uso1-1* mutation accumulate ER and nuclear envelope membranes and they are unable to secrete invertase (32). The oligosaccharides on the intracellularly accumulated invertase have not been modified by Golgi enzymes, suggesting that Uso1p is required for ER to Golgi transport. Sequence analysis of Uso1p indicated that it is composed of a head, a long coiled-coil tail, and a highly acidic C-terminal domain (32). Furthermore, gel filtration and velocity sedimentation analyses of Uso1p revealed that it is a nonglobular oligomer (44), similar to p115 (7).

There are two homology regions in the head of p115, HR1 and HR2, that share >60% identity with Uso1p (Fig. 3*C*). A third region, HR3, located after the coiled-coils in the tail of p115, is also conserved between yeast and mammals (Fig. 3*C*). The high degree of conservation suggests that these regions may be critical to the protein's function. For example, they could represent surfaces that mediate specific protein–protein interactions. In contrast to the homology regions, the acidic domain is not conserved at the level of primary sequence. This is also the case between the bovine and rat proteins, suggesting that the large negative charge, not the sequence, is the important feature.

It is noteworthy that the predicted coiled-coil domain of p115 is broken into four blocks, each of which contains five to

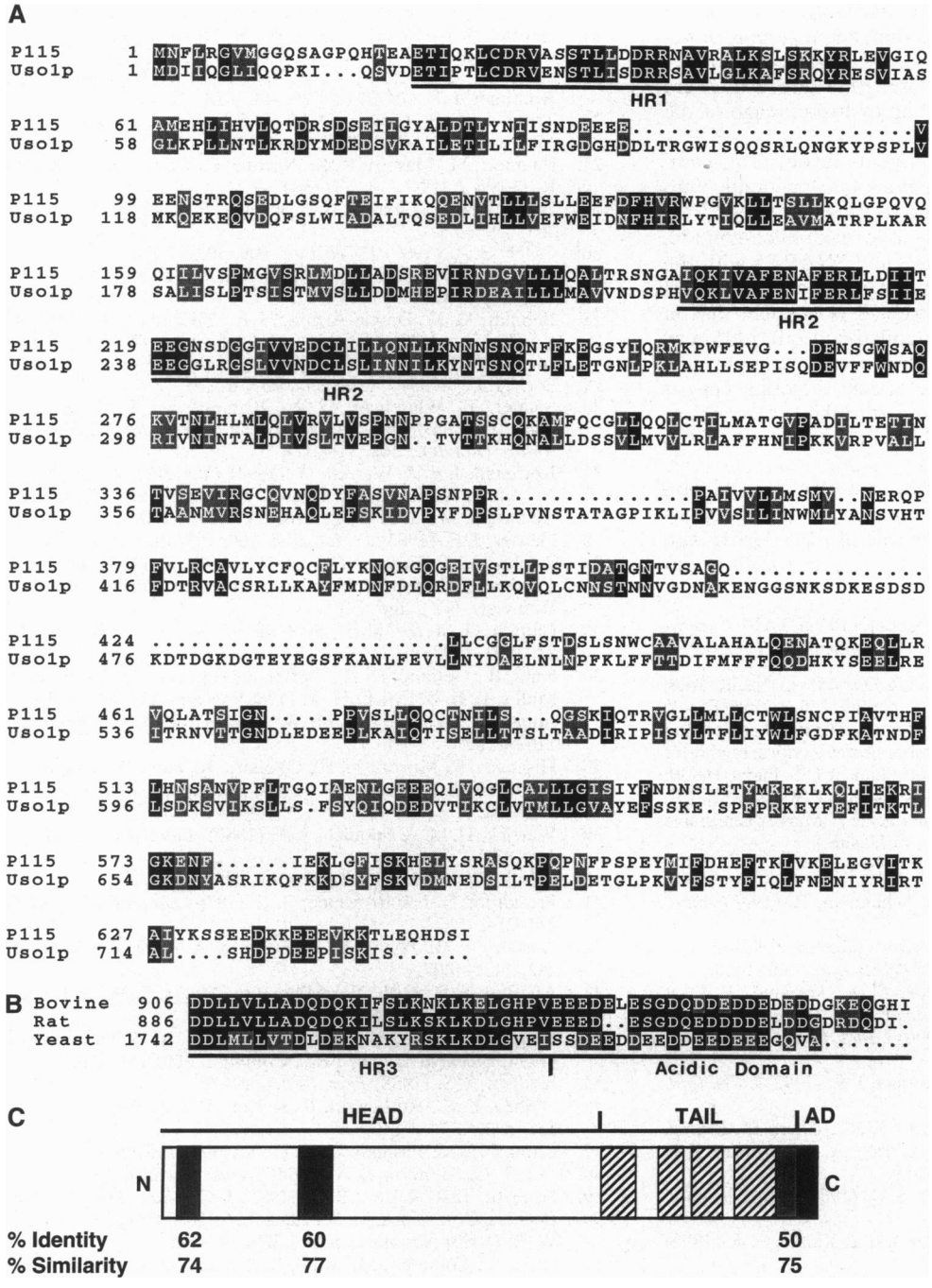

FIG. 3. Comparison of bovine p115 and Uso1p amino acid sequences. Identical residues are on a black background and similar residues are shaded. (*A*) Comparison of head domains of p115 and Uso1p. There are two domains of homology in the head. Homology region 1 (HR1) is 34 residues long (aa 21–54 in p115) and is 62% identical and 74% similar between bovine p115 and Uso1p. Homology region 2 (HR2) is 53 residues long (aa 200–252 in p115) and is 60% identical and 77% similar between the two proteins. (*B*) Comparison of the C termini of bovine and rat p115 with Uso1p. This region, which begins at the end of the predicted coiled-coil domain, contains two regions of homology between p115 and Uso1p. The first 28 amino acids comprise a third homology region (HR3) over which the two proteins are 50% identical and 75% similar. Immediately after this region, beginning with residue 934 of p115, is the acidic domain. (*C*) Schematic representation of the structure of p115. p115 is divided into three domains: a head, a tail, and an acidic domain. The solid box represents the acidic domain (AD). The three shaded boxes represent the two regions in the head (HR1 and HR2) and the short region between the end of the coiled-coils and the acidic domain (HR3) that contain high degrees of homology with the *S. cerevisiae* protein Uso1p. Below each box is the degree of identity and similarity with Uso1p. Hatched boxes represent blocks of coiled-coil secondary structure in the tail. All alignments were made using the PILEUP program, which introduces gaps to optimize alignment.

seven heptad repeats (Fig. 2*B*). Structural studies with synthetic peptides (45, 46) suggest that blocks of this size are likely to form stable coiled-coils. In p115, the regions separating the blocks of coiled-coil contain proline and glycine residues, which disrupt α-helices (47) and are therefore unlikely to enter into coiled-coil interactions. It has been suggested that discontinuities such as these impart flexibility to coiled-coil domains and may be the structural basis for the "hinge" region in the tail of myosin I (37, 48). The coiled-coil domain of Uso1p does not contain regularly spaced breaks like p115, but it does contain regions that have lower probabilities of forming coiled-coils (Fig. 2*A*).

It is a common theme that components identified based on activity in intra-Golgi transport (NSF, SNAPs, p115) have homologs required for ER to Golgi transport in yeast (Sec18p, Sec17p, Uso1p). The fact that Sec18p/NSF is required for multiple stages of transport through the secretory pathway indicates that these are general, not stage-specific factors (11, 49–52). By extension, since SNAPs act to bind NSF to the membrane (53, 54), they are likely to be required for all steps known to require NSF. Based on this precedent and the fact that p115 is required for intra-Golgi transport in mammalian cells and that the homologous protein Uso1p is required for ER to Golgi traffic in yeast, we propose that p115 is also likely to be a general, not a stage-specific transport factor. Alternatively, there could be a similar although distinct p115-related factor for each stage of transport.

Recently, Elazar *et al.* (55) showed that the *in vitro* cis to medial Golgi transport system used to isolate p115 (7), which is a modified version of the standard assay system, measures direct Golgi–Golgi fusion and not a complete round of budding, targeting, and fusion. This is due to the low levels of coatomer (55), which is thought to both drive vesicle budding (56, 57) and prevent homotypic (Golgi–Golgi) fusion. Additionally, Taylor *et al.* (58) have recently shown that transport in the absence of ARF, which is required for coatomer–

membrane binding, proceeds by a similar Golgi–Golgi fusion mechanism. Nevertheless, this p115-dependent system represents a physiologically relevant partial reaction since it requires both NSF and SNAPs (7). Therefore, p115 and Uso1p are likely to be required for the targeting or fusion stage of the vesicular transport cycle (55).

Interestingly, several other components involved in membrane targeting and fusion have regions that may enter into coiled-coil interactions. Syntaxin and its yeast homolog SED5 contain domains that are very likely to form a coiled-coil (30, 59). Analysis of the α- and β-SNAPs and SNAP-25 with the COILS2 algorithm reveals that they also contain coiled-coil domains (data not shown). Therefore, it is possible that in addition to homodimerizing via its coiled-coil tail, p115 may also interact with one or more of these components through coiled-coil interactions to promote vesicle targeting and/or fusion. Alternatively, it may interact with other not yet identified factors, which are involved in the later steps of the vesicular transport cycle.

Development of reagents that specifically block the function of p115 *in vitro* and further analysis of mutants of *USO1* in yeast should further elucidate the cellular role of p115/Uso1p and yield insights into the mechanism of vesicular traffic in eukaryotic cells.

Note. The sequences of rat p115 (this paper) and rat TAP (transcytosis-associated protein; ref. 60) are 99.8% identical.

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