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Genetics

Enhancement in amount of P1 (hsp60) in mutants of Chinese hamster ovary (CHO-K1) cells exhibiting increases in the A system of amino acid transport

(transport/heat shock protein/plasma membrane/regulation/isoforms of hsp60)

MICHAEL JONES*†, RADHEY S. GUPTA‡, AND ELLIS ENGLESBERG*§

*Biochemistry and Molecular Biology, University of California, Santa Barbara, CA 93106; and ‡Department of Biochemistry, McMaster University, Hamilton, ON, Canada L8N 3Z5

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ABSTRACT **Mutants of CHO-K1 cells with varied levels of A system activity, probably the result of increases in absolute amount of the A system transporter, have corresponding increases in levels of peptides banding at 62–66 and 29 kDa. Mutant ala^{r}4-H3.9, showing the highest increase of A system activity and of 62- to 66- and 29-kDa peptides, was selected for this study. The N terminus 16-amino acid sequence of the 62- to 66-kDa peptide(s) of this mutant showed between 80% and 100% identity with the mammalian mitochondrial 60-kDa heat shock protein P1 (hsp60). Two-dimensional gel electrophoresis of the 62- to 66-kDa band showed two major, a minor, and several smaller spots (of same mass but different pI values) for both wild type (WT) and mutant, with the two major spots being of greater density in the mutant. Immunoblots with antibody to P1 identified the two major and minor peptides as P1 related. Two-dimensional gels of whole cell extracts of the WT and ala^{r}4-H3.9 confirmed these findings and indicated that the two major bands of the mutant were 2.4 times as abundant as that found for the WT. A plasma membrane fraction of the mutant, exhibiting 4.8 times more A system activity than the WT, contained 3.6 times as much P1 as the WT. Immunoblots with antibodies to P1, mitochondrial malate dehydrogenase, and to the mitochondrial F_1/F_0-ATPase demonstrated that the increased amount of P1 observed in the mutant was not the result of increases in amount of mitochondrial protein. Northern blot analysis demonstrated that the mutant had 2.5 times as much mRNA for P1 as the WT. The close analogy with the relationship between A system and Na^+,K^+-ATPase suggests that there is a coordinate regulation of the A system of amino acid transport, Na^+,K^+-ATPase, and P1 protein, probably as a result of mutation in a shared regulatory element. The possible role of P1 in A system function is discussed.**

Chinese hamster ovary cells (CHO-K1) require proline for growth. Proline, as well as small, polar, straight-chain amino acids such as alanine, are transported primarily through the A system (1, 2). The A system has been shown to be repressible and apparently under negative control (3). Mutants of CHO-K1 having increases in transport through the A system have been isolated by taking advantage of the proline auxotrophy of CHO-K1. The growth of this cell line is inhibited by amino acids, such as alanine, that competitively inhibit proline transport (4, 5). One such mutant, ala^{r}4, is constitutive for A system, having 5 times the velocity of A system transport over the wild type (WT) when grown under repressed conditions. This phenotype is thought to be the result of inactivation of a gene, R1, which has been postulated to encode an apo repressor inactivator (3, 6). ala^{r}4-H2.1 and ala^{r}4-H3.9 are mutants isolated sequentially, beginning with ala^{r}4, as resistant to increasing concentrations of alanine. These mutants were shown to have further increases in A system activity correlated with resistance to increasing concentrations of alanine. The increase in A system activity of ala^{r}4 and ala^{r}4-H3.9 was shown to be due to increases in the abundance of the A system transporter (7). ala^{r}4-H3.9 was also shown to overexpress two peptides of 29 and 62–66 kDa, which are associated with both the endoplasmic reticulum (ER) and the plasma membrane (PM) (8).

In this paper, we show that the 62- to 66-kDa peptide, which is upregulated in ala^{r}4-H3.9, has 80–100% amino acid sequence identity with the first (N terminus) 16 amino acids of a heat shock protein—namely, P1 (hsp60)—localized to the mitochondrial matrix (9–13). At least two major and one minor isoform of the P1 protein in CHO-K1 are also revealed. These isoforms were found to be present in increased abundance in the mutant as compared to the WT. This increase in abundance of P1 is correlated with a similar increase in P1 mRNA. The present report also demonstrates that the P1 protein(s) is associated with the PM.

MATERIALS AND METHODS

Cells and Cell Culture. Chinese hamster ovary cell line CHO-K1 (WT), mutant ala^{r}4, ala^{r}4-H2.1, and ala^{r}4-H3.9 have been described (3, 8). ala^{r}4-H3.9 grows very poorly under nonselective conditions as well as under selective conditions, and in the course of culturing this cell line the increase in A system activity previously reported was reduced to ≈12 and ≈3 times that of WT and ala^{r}4, respectively. A revertant of ala^{r}4-H3.9, ala^{r}4-H3.9r1, was isolated by cycling the former 50 times under nonselective conditions in medium MEMCHO-4. The WT and ala^{r}4-H3.9r1 were routinely grown as monolayers in MEMCHO-4 (5). ala^{r}4, ala^{r}4-H2.1, and ala^{r}4-H3.9 were grown in this medium containing 12.5 mM alanine and 0.05 mM proline, 50.0 mM alanine and 0.05 mM proline, and 125 mM alanine and 0.03 mM proline, respectively. Cells used in the preparation of membrane vesicles (MVs) were grown in roller bottles. For the growth of ala^{r}4-H3.9 under these conditions, the concentration of alanine was reduced to 50 mM.

Transport Studies with Whole Cells. Amino acid transport in whole cells was determined by using cells grown as a monolayer either in scintillation vials (5) or in 24-well Costar cluster trays (14).

MV Preparation and Transport Activity. The preparation of MVs, the assay of A system activity in these vesicles, the separation of PM from ER, and the cell fractionation proce-

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Abbreviations: WT, wild type; 2DGE, two-dimensional gel electrophoresis; PVDFM, poly(vinylidene difluoride) membrane; mMDH, mitochondrial malate dehydrogenase; mATPase, β subunit of the F_1 component of the mitochondrial F_1/F_0-ATPase; MV, membrane vesicle; ER, endoplasmic reticulum; PM, plasma membrane.

†Present address: Scripts Research Institute, La Jolla, CA 92037.
§To whom reprint requests should be addressed.

dure and their analysis using marker enzymes have been described (7).

Electrophoretic Analysis. Proteins were analyzed by SDS/PAGE, by a modification of the discontinuous gel procedure (8), and electroeluted (15) where indicated. Two-dimensional gel electrophoresis (2DGE) was performed essentially as described (16). Electroeluted proteins were concentrated by using a Centricon 30 microconcentrator (Amicon). Molecular size standards were run in the SDS/polyacrylamide gel to estimate the mass of the peptides.

Sequence Analysis. Protein sequencing was performed at the UCLA Protein Microsequencing Facility. The 62- to 66-kDa bands, transferred from an SDS/polyacrylamide gel onto a poly(vinylidene difluoride) membrane (PVDFM) (Immobilon-P transfer membrane; Millipore), were excised and placed directly into the cartridge of the sequenator. A sequence of 16 amino acids was obtained and this sequence was used to search a gene data bank using the University of Wisconsin Genetics Computer Group software package.

Immunoblot Analysis. Proteins were analyzed by a modification of a published method (17). After electrophoresis, the proteins were transferred onto a PVDFM. A blocking agent consisting of TBST buffer [0.01 M Tris·HCl (pH 8.0), 0.9% NaCl, 0.05% polyoxyethylenesorbitan monolaurate (Tween 20), and 3% (wt/vol) bovine serum albumin] was used. Rabbit antiserum, specific for protein P1, has been described (18). Rabbit antiserum, specific for the β subunit of the F_1 component of the mitochondrial F_1/F_0-ATPase (mATPase), which is exposed to the matrix side of the mitochondrial inner membrane, was a gift from Youssef Hatefi (Research Institute of Scripps Clinic, La Jolla, CA). Rabbit antiserum specific for mitochondrial malate dehydrogenase (mMDH), localized, as is P1 to the mitochondrial matrix, was a gift from George J. Markelonis (University of Maryland, Baltimore). Mouse monoclonal antibody specific for β-tubulin was that of Michael Klymkowsky (University of Colorado, Boulder) and was provided by Stuart C. Feinstein (University of California, Santa Barbara). Goat anti-rabbit IgG conjugated to alkaline phosphatase (Bio-Rad) was used as the secondary antibody. Antibody binding was visualized by an alkaline phosphatase-activated color reaction using 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Bio-Rad).

Northern Blot Analysis. The details for this procedure have been described (19). The cDNA probe represents a 1.1-kb fragment from the 3′ end of the P1 transcript (11). P1 message was quantified by normalizing densitometry readings with the blot rehybridized with γ-^{32}P-end-labeled oligo(dT) (18-mer) (20).

RESULTS

A Comparison of the WT, ala^{r}4, ala^{r}4-H2.1, ala^{r}4-H3.9, and ala^{r}4-H3.9r1 with Regard to A System Activity and Abundance of the 62- to 66- and 29-kDa Proteins. Increases in the amount of a 62- to 66- and a 29-kDa peptide(s) in ala^{r}4-H3.9 have been shown to be associated with increases in A system activity and abundance of the A system transporter (7, 8). We extended this study with an analysis of A system activity of whole cells and MVs of ala^{r}4, ala^{r}4-H2.1, and the revertant ala^{r}4-H3.9r1, in addition to analysis of the WT and ala^{r}4-H3.9. Samples of the MV preparations were also analyzed by SDS/PAGE (Table 1 and Fig. 1). It will be noted that A system activity in both whole cells and MV extracts increases in the order ala^{r}4-H3.9 > ala^{r}4-H2.1 > ala^{r}4 > WT and that the abundance of 62- to 66- and 29-kDa peptides increases proportionally. The revertant ala^{r}4-H3.9r1 closely resembles ala^{r}4 in terms of both A system activity and concentration of both peptides. These results add further evidence to support the positive correlation between A system activity and abundance of these two peptides, as previously shown (8).

Table 1. Comparison of A system activity of CHO-K1 WT and various alanine-resistant mutants

Cell line	A system activity	
	Intact cells*	Mixed vesicles†
CHO-K1 (WT)	0.20 ± 0.06	1.21 ± 0.06
ala^{r}4	0.86 ± 0.06	2.87 ± 0.38
ala^{r}4-H2.1	2.16 ± 0.22	6.77 ± 0.51
ala^{r}4-H3.9	2.47 ± 0.17	7.03 ± 0.54
ala^{r}4-H3.9r1	0.96 ± 0.08	3.49 ± 0.23

*The A system activity was determined by measuring proline transport for 1 min using 24-well Costar cluster trays and is given as nmol of proline uptake per mg of protein per min.

†The A system activity was measured with 0.1 mM L-proline for 10 sec and is given as nmol of proline uptake per mg of protein per min. The A system activity values were normalized for 5′ nucleotidase activity of the individual mixed membrane vesicles (data not shown).

Amino Acid Sequence Analysis of the 62- to 66-kDa Peptide Associated with Increased A System Activity in the Mutant ala^{r}4-H3.9. For the remainder of this study, our analysis focuses on WT and mutant ala^{r}4-H3.9, henceforth referred to as H3.9 or mutant. The 62- to 66-kDa band from MV preparations of H3.9 was excised and sequenced. An N-terminal sequence of 16 amino acids was obtained and used to search a gene data bank. This analysis revealed that the 62- to 66-kDa peptide shared 80–100% identity with the first (N terminus) 16 amino acids of the mature heat shock protein P1, a mammalian homolog of the groEL protein of *Escherichia coli* (11).

2DGE of the 62- to 66-kDa Peptide Band and Immunoblot Analysis. The 62- to 66-kDa band was excised from SDS/polyacrylamide gels, as in sequencing (see above), electroeluted, and resolved by 2DGE. Coomassie blue staining of this gel indicated that the 62- to 66-kDa band in both WT and H3.9 is composed of at least two or three closely associated major peptides and several minor ones and that the major peptides were present in 5 times the amount in the mutant as compared to the WT (data not shown). Immunoblot analysis of a similar blot of the mutant with P1 antibody showed the

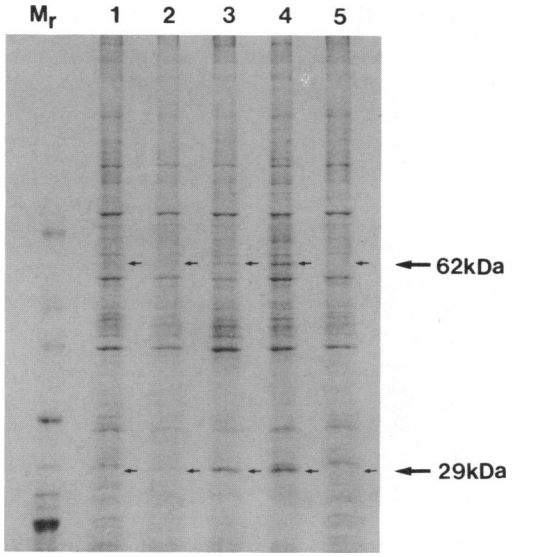

FIG. 1. SDS/PAGE analysis of the MV proteins of CHO-K1 WT and mutants. Each lane contained ≈50 μg of protein. The gel was stained with Coomassie blue. Arrows indicate bands at 62–66 and 29 kDa. The A system activity of the MV preparations, from which the proteins were analyzed in this gel, is given in Table 1. Size markers: lane 1, CHO-K1; lane 2, ala^{r}4; lane 3, ala^{r}4-H2.1; lane 4, ala^{r}4-H3.9; lane 5, ala^{r}4-H3.9r1.

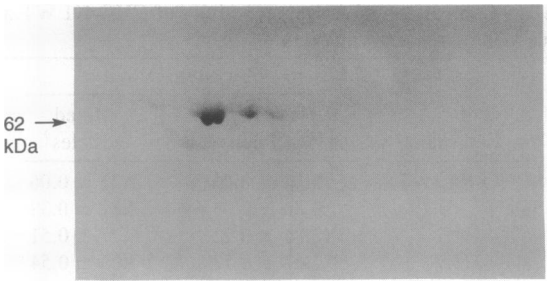

FIG. 2. Immunoblot analysis of a two-dimensional gel of partially purified 62- to 66-kDa peptide(s) of H3.9. Protein (30 μg) electroeluted from the 62- to 66-kDa band from a SDS/polyacrylamide gel of extract of H3.9 was fractionated by 2DGE. Isoelectric focusing was performed at a pH gradient of 4–7. Detection of anti-P1 antibody binding was performed as described.

two closely associated large spots and a minor spot to be P1-related proteins with isoelectric points of approximately pH 5.6, 5.3, and 4.7, respectively (Fig. 2).

Immunoblot Analysis of Whole Cell Extracts of WT CHO-K1 Cells and H3.9. To determine whether the differences between the WT and H3.9, and the number of P1 spots, were an artifact of our fractionation procedure, we analyzed directly whole cell extracts of the WT and H3.9 by 2DGE. In duplicate gels for the WT and the mutant, the P1 antibody recognized two major peptides at 62–66 kDa, which were also visualized by Coomassie blue staining (Fig. 3). In addition, the P1 antibody also identified a minor peptide in both WT and H3.9 at the same size. Densitometric analysis of the immunoblot revealed that both major peptides are present in an increased abundance of 2.4 times in extracts of H3.9 as compared to extracts of WT. Although the minor peptide spot appears more dense in the mutant, we did not attempt to quantify the difference.

Specificity of the Increase in P1 in Mutant H3.9. Since the P1 protein had been identified as a mitochondrial protein, it was necessary to ascertain whether the increased abundance of the P1 peptides in whole cell extracts of the mutant was due to a general increase in mitochondrial proteins. To accomplish this, immunoblot analysis was performed with antibodies specific for two other mitochondrial proteins, mMDH and mATPase (see *Materials and Methods*). Whole cell extracts of the WT and H3.9 were fractionated by SDS/PAGE, and the proteins were transferred to PVDFM and processed as described in the legend to Fig. 4. As shown in Fig. 4*A* (lanes 4 and 5), the P1 antibody showed appreciably greater (2 times) binding to the whole cell extract of the H3.9 compared to that of the WT. No increase in the amount of binding to proteins of H3.9 over that of WT was observed for either the mMDH (lanes 6 and 7) or mATPase antibodies (lanes 8 and 9). Immunoblot analysis was repeated in which the blot containing extracts of both H3.9 and WT was incubated simultaneously with the antibodies specific for the P1 protein and mMDH (Fig. 4*B*). Densitometric analysis revealed a 2.8 times greater amount of antibody binding to the P1 protein of H3.9 compared to that of WT after normalization to the amount of mMDH antibody bound by the two extracts. These data indicate that the increased abundance of P1 is not due to a general increase in mitochondrial proteins in H3.9 cells.

Subcellular Localization of the P1-Related Peptides in WT and H3.9. Extracts of the mutant and the WT were fractionated into MVs, ER, and PM. The analysis for marker

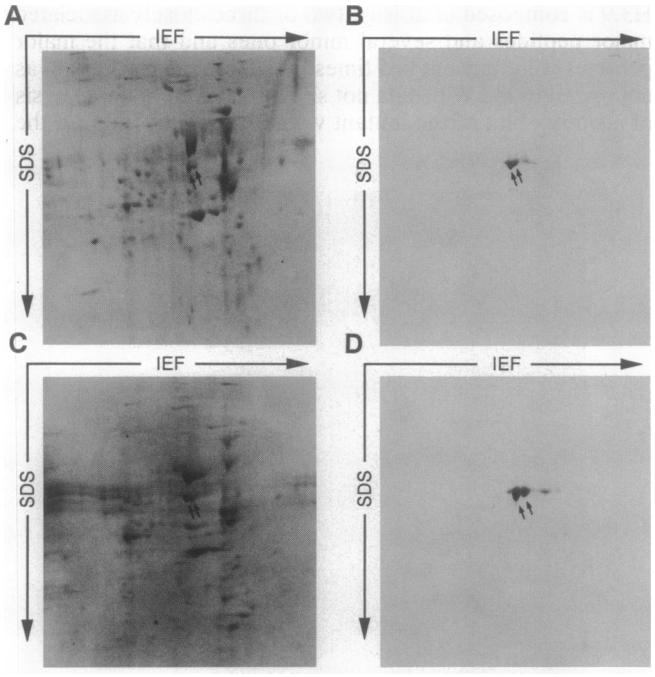

FIG. 3. Immunoblot analysis of whole cell extracts of WT and H3.9 fractionated by 2DGE. Protein (40 μg) was loaded on the gel and isoelectric focusing (IEF) was performed at a pH gradient of 4–7. After electrophoresis and blotting, one set of blots was stained with Coomassie blue as shown for WT (*A*) and for H3.9 (*C*). The other set was used for detection of anti-P1 antibody binding as shown for WT (*B*) and for H3.9 (*D*). Densitometric analysis was performed on the immunoblots. Arrows indicate the corresponding peptides in *A* and *B* and in *C* and *D*.

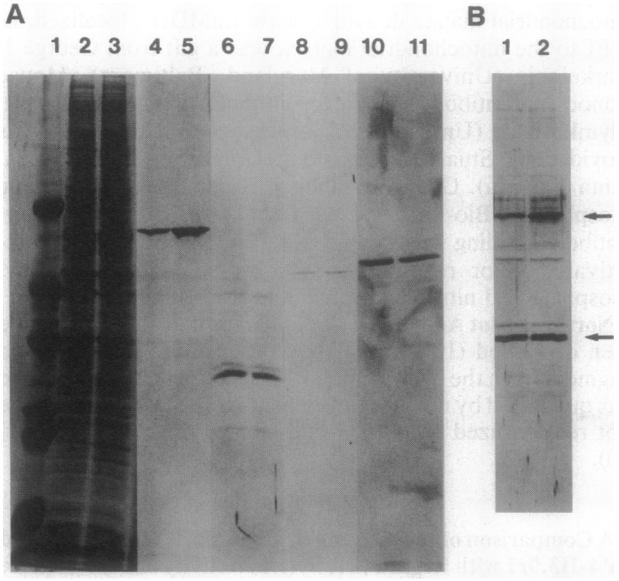

FIG. 4. Comparison of the mMDH and mATPase content with that of P1 in immunoblots of whole cell extracts of WT and H3.9 fractionated by SDS/PAGE. Protein (50 μg) was loaded onto each lane. After transfer of the peptides to a PVDFM, the membrane was cut into strips containing two lanes, one for the WT and the other for the mutant. (*A*) Lanes: 1, molecular size standards; 2, WT; 3, H3.9. Lanes 1–3 were stained with Coomassie blue. Lane pairs: 4/5, 6/7, 8/9, and 10/11 were incubated separately with antibody. Lanes: 4 and 5, binding with antibody to P1; 6 and 7, binding with antibody to mMDH; lanes 8 and 9, binding with antibody to mATPase; 10 and 11, binding with antibody to tubulin; 4, 6, 8, and 10, extracts of WT; 5, 7, 9, and 11, extracts of H3.9. Antibody binding was quantified by densitometry and normalized to the amount of binding by antibody for tubulin. (*B*) Blots of WT and H3.9 probed simultaneously with both antibody for P1 and for mMDH. Lane 1, WT; lane 2, H3.9. Antibody binding was quantified by densitometry and normalized to the amount of binding by the antibody to MDH. Upper arrow, antibody to P1; lower arrow, antibody to MDH.

enzymes indicated significant enrichments of the ER and PM fractions that were essentially the same for both the mutant and WT (data not shown). The PM fraction from H3.9 mutant showed a 4.8-fold increase in A system activity in comparison to the WT (Table 2). Equal amounts of protein from the MV, ER, and PM from both H3.9 and WT were fractionated by SDS/PAGE on a single gel and transferred onto a PVDFM. The membrane was then incubated simultaneously with the P1 and mMDH antibodies. The results (Fig. 5; Table 2) indicate that all fractions from H3.9, after normalizing for mMDH, have increased abundance of the P1-related peptides compared to WT. The highest increase in the P1-related peptides of H3.9 (3.9 times over WT) was observed in the PM fraction (Fig. 5, lanes 5 and 6). This correlates well with the increase in A system activity.

mRNA for P1 Protein in WT and H3.9. Northern blot analysis with P1 cDNA of WT and H3.9 (Fig. 6) demonstrated a 2.5-fold increase in P1-specific mRNA in H3.9 over that of WT. No such increase was detected with ala^{r}4 and the revertant ala^{r}4-H3.9r1. No decrease in the amount of mRNA for P1 was detected when H3.9 was grown under nonselective conditions, and there was no increase in P1 mRNA when the WT was incubated under conditions used in growing H3.9 (data not shown).

DISCUSSION

Results of our earlier studies (8) and those presented here show that mutants of CHO-K1, selected for resistance to increasing concentrations of alanine, have increases in abundance of two proteins of 29 and 62–66 kDa that are positively correlated with increases in the A system of amino acid transport (3, 8). In this paper, we have analyzed in detail the nature of the 62- to 66-kDa protein from the ala^{r}4-H3.9 (H3.9) mutant that expresses this phenotype to the greatest extent (3, 8).

Several lines of evidence presented here indicate that the 62- to 66-kDa protein overexpressed in H3.9 is related to the mammalian P1 or hsp60 chaperonin family of proteins (11, 13, 21). First, the sequence of the N-terminal 16 amino acids of the 62- to 66-kDa protein shows between 80% and 100% identity to the first 16 amino acids of mature mammalian P1 protein from CHO cells (11). Second, we observed that when the gel-eluted 62- to 66-kDa band is analyzed by 2DGE, it shows the presence of two major and a minor spot of size similar to P1, plus several other smaller spots. One of these major spots moves in the same position as P1 in two-dimensional gels (18, 22), while the other major and minor spots differ in their isoelectric points. In immunoblots of the two-dimensional gels, the two major and one minor spot noted above all show reactivity with an antibody specific for the mammalian P1 or hsp60 protein (19). Third, two-dimensional immunoblots of whole cell extracts of H3.9 cells showed a 2.4-fold increase in the amount of protein in the two major spots that reacted with the P1-specific antibody. This increase in the amount of P1 antibody reactive spots and bands in the mutant cells is not caused by a general increase in mitochondria, since no increase in reactivity was observed for two other mitochondrial proteins (malate dehydrogenase and β subunit of F_0/F_1-ATPase) that were examined. Lastly, the Northern blot analysis provides evidence that, in comparison to WT cells, the amount of P1 mRNA in the H3.9 cells is increased 2.5 times. This increase was independent of the medium used for culturing the cells. It should be mentioned that in earlier studies with CHO cells, only a single spot or species corresponding to the P1 protein, which showed reactivity with the P1 antibody, was observed in two-dimensional gels (or immunoblots) of the WT cells (18, 23). In contrast, in the present studies with CHO-K1 cells, P1 antibody has shown reactivity with two or three protein spots of similar size but differing in their pI values. The fact that similar patterns are observed in both WT and H3.9 cells indicates that the two or three spots are not the result of

Table 2. A system activity and P1 abundance of MV and purified ER and PM from H3.9 and WT

Fraction	A system activity*		P1†	
	WT	H3.9	WT	H3.9
MV	0.69 ± 0.25	4.44 ± 0.36 (6.4)	0.9	1.6 (1.8)
ER	0.11 ± 0.09	1.05 ± 0.34 (9)	1.2	2.3 (2.0)
PM	1.78 ± 0.34	8.47 ± 2.57 (4.8)	2.5	9.0 (3.6)

Membrane fractions correspond to those in Fig. 5. Values in parentheses are mutant data divided by WT.
*The A system activity is as described in Table 1.
†Densitometry scans were made of the bands corresponding to binding by anti-P1 and anti-mMDH antibodies. Data represent -fold increase of the anti-P1 band as compared to that of anti-mMDH.

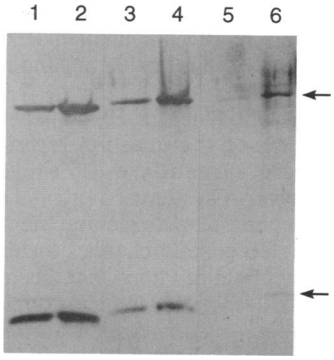

FIG. 5. Immunoblot analysis of the MV, ER, and PM fractions of the WT and H3.9. Proteins (50 μg) from the MV, ER, and PM fractions of the WT and H3.9 were fractionated by SDS/PAGE and transferred to PVDFM. The membrane was simultaneously treated with antibody to P1 and mMDH. After incubation with secondary antibody, the membrane strips containing particular fractions from both WT and the mutant were separately developed until the specific peptide bands were visualized. Lanes: 1 and 2, MV; 3 and 4, ER; lanes 5 and 6, PM; 1, 3, and 5, WT fractions; 2, 4, and 6, H3.9 fractions. Quantitation of the protein bound by the P1 antibody (upper arrow) was determined for, and normalized to, the amount of mMDH (lower arrow) as described in Fig. 4*B*. Increases (-fold) in anti-P1 antibody binding in H3.9 over the corresponding fractions of WT are shown in Table 2.

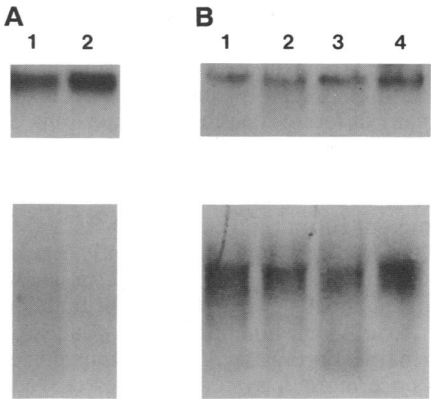

FIG. 6. Northern blot analysis for mRNA for P1 in WT and H3.9. (*A*) (*Upper*) Lanes: 1, WT; 2, H3.9. Blot was first hybridized with ^{32}P-labeled cDNA for P1. (*Lower*) After autoradiography, the blot was stripped and hybridized with a ^{32}P-labeled oligo(dT) probe. (*B*) (*Upper*) Hybridized with ^{32}P-labeled cDNA for P1 as in *A*. Lanes: 1, WT; 2, WT incubated for 16 hr in medium containing 50 mM alanine and 0.03 mM proline; 3, ala^{r}4; 4, ala^{r}4-H3.9r1. After autoradiography, the blot was hybridized with ^{32}P-labeled oligo(dT) as described above. The amount of P1 mRNA was determined by densitometry and normalized against the corresponding oligo(dT) reading.

mutation leading to the H3.9 phenotype. Two different electrophoretic isoforms of the mammalian P1/hsp60 proteins have also been reported in rat cells (12) and human lymphocytes (24, 25).

The increase in abundance of P1 or hsp60 in H3.9 cells compared to WT raised the question regarding its genetic origin. In this regard, our analyses of a revertant of H3.9, which has both a lower A system activity and smaller amount of 62- to 66-kDa peptide(s), provide arguments against independent, unassociated genetic changes leading to this phenotype. Gene amplification does not seem likely as an explanation of this association, since double minutes and homogeneous staining regions, which are characteristic of this type of event, have not been detected in H3.9 (8). Further arguments supporting this conclusion come indirectly from an analysis of the association between A system activity and the Na^+,K^+-ATPase (14, 26). It has been previously shown that, similar to the overexpression of P1 as observed in the present studies, the various ala^r mutants show a proportional increase in the absolute amounts of the α subunit of the Na^+,K^+-ATPase. Southern analysis using a cDNA probe for the α subunit of the Na^+,K^+-ATPase failed to detect any sign of gene amplification in H3.9. In addition, cell–cell hybridization experiments indicated that the increased levels of Na^+,K^+-ATPase are recessive to the WT phenotype (Nan-Xin Qian and E.E., unpublished data). The available evidence therefore suggests that in H3.9 cells there is a joint or coordinate increase in the activities of the A system of amino acid transport, Na^+,K^+-ATPase, and P1 protein, probably as a result of a mutation in a shared regulatory element that controls the transcription of these genes.

The P1 or hsp60 family of proteins are known to act as molecular chaperones in the proper folding of other newly synthesized polypeptide chains as well as in their transport and secretion (13, 21, 27–29). In the past, P1 homologs have generally been assumed to be localized and function only within organelles such as mitochondria and chloroplasts (13, 21, 27, 28, 30, 31). We have observed in the present studies that one or more of the P1-related proteins are associated with the PM and possibly the ER of CHO-K1 cells. Also there are several recent reports indicating that P1 or a related protein is present in association with PM and other membrane-enclosed vesicles in different types of cells (32–35). In a human Burkitt lymphoma cell line (Daudi) the presence of hsp60 on the cell surface has been demonstrated by several lines of evidence (33). In a study of mouse pancreatic B cells, using immunoelectron microscopy, P1 antibody was shown to bind to the mature insulin secretory granules as well as to mitochondria (34). Recently, immunoelectron microscopic studies of many different types of cells, including CHO, have revealed low but specific binding of P1 antibody to sides outside of the mitochondria (B. J. Soltys and R.S.G., unpublished results).

The location of P1 on the PM in CHO-K1 and the finding that P1 and the A system appear to be jointly regulated, as we have indicated in this paper, raises the question of whether P1 may be involved directly or indirectly in the functioning of the A system. An obvious function of P1 would be that of a chaperonin in transporting the A system transporter complex to the PM since more A system transporter would require more P1 peptide(s). The finding of P1 on the PM may indicate that P1 peptide(s) may be structurally associated with the A system transporter. While amino acid composition and sequence data for P1 are not indicative of proteins having extensive membrane-spanning domains, it is possible that the peptide(s) associates in the manner of peripheral membrane protein with one or more transmembrane proteins, and it is this complex that may be required for amino acid transport by the A system in CHO-K1 cells. Still another possible role of P1, pertaining to the A system, is that of a regulator. hsp60 has been shown to modulate the activities of src tyrosine kinases (35) and by interacting with $p21^{ras}$, possible at the cell surface, may assist $p21^{ras}$ in associating with its effectors (36).

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