

## Mapping of the intermolecular association of the human T cell leukaemia/lymphotropic virus type I p12<sup>1</sup> and the vacuolar H<sup>+</sup>-ATPase 16 kDa subunit protein

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The p12<sup>1</sup> protein, a small hydrophobic protein encoded by the human T cell leukaemia/lymphotropic virus type I pX region, contains a proline-rich region located between two putative transmembrane (TM) domains. The p12<sup>1</sup> protein is associated with cellular endomembranes, and physically binds to the 16 kDa subunit of the vacuolar H<sup>+</sup>-ATPase proton pump. To investigate the nature of the 16 kDa and p12<sup>1</sup> interaction and to determine the oncogenic domain of p12<sup>1</sup>, we constructed p12<sup>1</sup> mutant proteins in which various portions of the TM domains were deleted, as well as a p12<sup>1</sup> mutant containing a single amino acid substitution. These mutants were tested for binding to the 16 kDa subunit of the vacuolar H<sup>+</sup>-ATPase in HeLa/Tat cells and for the capability to potentiate transformation by bovine papillomavirus type 1 E5 oncoprotein in mouse C127 cells. The results indicated that both TM domains of the p12<sup>1</sup> protein were dispensable for its interaction with the

16 kDa protein, whereas partial or complete deletion of the proline-rich region resulted in decreased or no binding of the p12<sup>1</sup> protein to the 16 kDa subunit. Immunofluorescence analysis of HeLa/Tat cells transfected with the p12<sup>1</sup> mutants showed that deletion of the proline-rich region did not alter the subcellular localization of these mutant p12<sup>1</sup> proteins, suggesting direct involvement of the proline-rich domain in binding rather than the failure of these p12<sup>1</sup> mutants to reach the appropriate cellular compartment. Mapping of 16 kDa subunit mutants in binding with the p12<sup>1</sup> protein suggested that molecular determinants located between the second and third TM domain of the 16 kDa protein might be involved in this interaction. Finally, most of the p12<sup>1</sup> mutants lost the ability to potentiate transformation of C127 cells indicating that binding of p12<sup>1</sup> to the 16 kDa subunit does not directly correlate with oncogenicity.

### Introduction

Human T cell leukaemia/lymphotropic virus type I (HTLV-I) (Poesz *et al.*, 1980; Miyoshi *et al.*, 1981; Gallo, 1986) induces T cell leukaemia (Hinuma *et al.*, 1981; Robert-Guroff *et al.*, 1982), most often after a long period of latency *in vivo*, and transforms T cells *in vitro* (Miyoshi *et al.*, 1981; Yamamoto *et al.*, 1982; Markham *et al.*, 1983; Popovic *et al.*, 1983). The mechanism of HTLV-I transformation *in vitro* and *in vivo* is poorly understood. The HTLV-I transactivator protein, p40<sup>tax</sup> (Cann *et al.*, 1985; Felber *et al.*, 1985; Sodroski *et al.*, 1985), has been shown to transcriptionally activate several cellular genes whose expression is relevant to T

cell activation and proliferation, including the interleukin 2 receptor alpha (IL-2R $\alpha$ ) chain, IL-2 and *c-fos* genes (Inoue *et al.*, 1986; Cross *et al.*, 1987; Siekevitz *et al.*, 1987; Fuji *et al.*, 1988; Grassman *et al.*, 1989; Nagata *et al.*, 1989). Furthermore, HTLV-I immortalization and transformation of T cells *in vitro* is associated with alteration in the expression of a cascade of specific cellular kinases (Maruyama *et al.*, 1987; Koga *et al.*, 1989; Yamanashi *et al.*, 1989; Mills *et al.*, 1992) including p56<sup>lck</sup> (Hatakeyama *et al.*, 1991) triggered by the IL-2/IL-2R interaction. The *tax* gene has also been shown to directly transform rat fibroblasts *in vitro* (Tanaka *et al.*, 1990) and, when expressed in the context of a herpesvirus saimiri-based vector, *tax* immortalizes T cells *in vitro* (Grassman *et al.*, 1989). However, *tax* appears to be insufficient to induce ligand (IL-2)-independent growth of human T cells (Akagi & Shimotohno, 1993) and the cooperation of other oncogenes of cellular or viral origin could be postulated.

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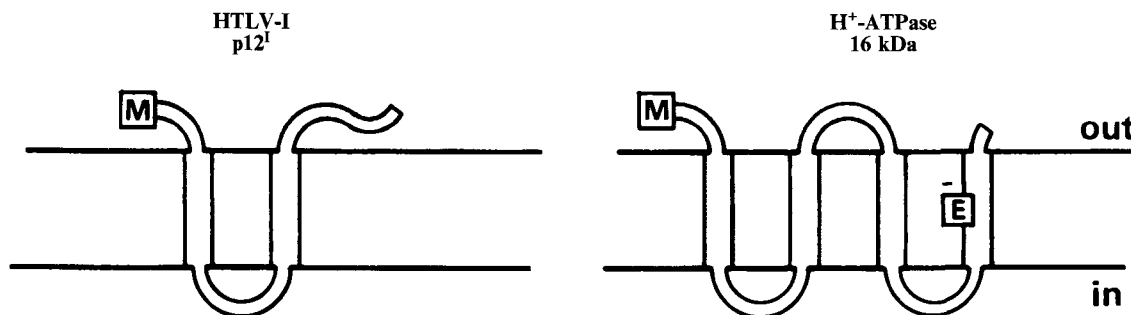


Fig. 1. Putative structure of the HTLV-I p12<sup>I</sup> and 16 kDa subunit of the vacuolar H<sup>+</sup>-ATPase. The schematic representation of the two TM domains of the HTLV-I p12<sup>I</sup> protein (left side of the figure) and of the 16 kDa protein (right side of the figure) shown was derived by computer analysis of their respective aa sequences as previously described (Franchini *et al.*, 1993). The two parallel lines represent the cellular membranes and the *out* and *in* refer to the lumen of organelles and the relative orientation of the 16 kDa protein. The HTLV-I p12<sup>I</sup> orientation in the cellular endomembranes is unknown. M, initiating methionine; E, glutamic acid.

Recently, a 12 kDa protein (p12<sup>I</sup>) encoded by the HTLV-I open reading frame I (orf I) (Koralnik *et al.*, 1992, 1993) has been shown to be weakly oncogenic as well (Franchini *et al.*, 1993). The p12<sup>I</sup> protein is a small, hydrophobic, membrane-associated protein which has some structural similarity to the bovine papillomavirus type 1 (BPV-1) E5 oncoprotein (Schlegel *et al.*, 1986) and potentiates the E5 transforming activity in C127 mouse cells (Franchini *et al.*, 1993). Both the p12<sup>I</sup> and E5 proteins physically bind to the 16 kDa subunit of the vacuolar H<sup>+</sup>-ATPase (Goldstein *et al.*, 1991; Franchini *et al.*, 1993), a proton pump ubiquitous in cellular organelles, which regulates their acidification (Mandel *et al.*, 1988; Nelson, 1989).

The biological significance of these protein interactions is presently unclear. In an attempt to unravel this complex protein interaction, we investigated the nature of the binding of the p12<sup>I</sup> and 16 kDa proteins by generating genetic mutants of both proteins. Furthermore, we studied the cellular localization of the HTLV-I p12<sup>I</sup> mutants and their transforming activity in cooperation with E5 in mouse C127 cells.

## Methods

**Generation of the HTLV-I p12<sup>I</sup> and the 16 kDa protein mutants.** Truncated p12<sup>I</sup> cDNAs were constructed by PCR amplification of previously described pX-orf I or pX-orf I AU1 cDNAs [carrying the five amino acid (aa) AU1 tag] (Koralnik *et al.*, 1992) using the primer sets whose DNA sequence is provided below. The primers M14/PX1AS were used to generate the p12<sup>I</sup> Δ14 mutant, which lacks the first 14 aa of the p12<sup>I</sup> protein. The M35/PX1AS primers were used for p12<sup>I</sup> Δ35, which lacks the first 35 aa, and primers M47/PX1AS for p12<sup>I</sup> Δ47, which lacks the first 47 aa. The primers set M35/T70 amplified a DNA fragment encoding aa 36–70 of p12<sup>I</sup> (mutant p12<sup>I</sup> 36–70), and the primers M47/T70 generated mutant p12<sup>I</sup> 48–70, which encompasses aa 48–70. The primers pair orf I/T43 amplified only the first 43 aa of p12<sup>I</sup>, (p12<sup>I</sup> 43) and primers orf I/T70 only the first 70 aa of the p12<sup>I</sup> protein (p12<sup>I</sup> 70). Finally, primers MO/PX1AS were used to eliminate

the initiation codon (ATG) of p12<sup>I</sup> which was mutated to a TTG, and to introduce a termination codon at the third codon position (p12<sup>I</sup> ΔATG). To generate the single aa change Q → G in the p12<sup>I</sup> Q → G mutant protein the codon CAA for the glutamine in position 47 was mutated to GGA which encodes a glycine, by using double PCR with separate primers orf I/MGAS and MGS/PX1AS. Amplified products (0.5 ng) were then annealed and amplified together using the primers orf I/PX1AS. For all the p12<sup>I</sup> mutants 30 cycles of PCR amplification were performed as follows: denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min with a 2 s increase of this incubation time per cycle, and extension of the final cycle at 72 °C for 7 min. All the amplified products were purified using the Magic PCR prep kit (Promega) and cloned at the *Xba*I–*Bam*HI sites of the expression vector pHCMV/HSPA. The DNA sequences of each plasmid containing the truncated or mutagenized form of the p12<sup>I</sup> gene were obtained using the Sanger method (Sanger *et al.*, 1977).

The sequences of the primers are as follows: PXIAS, 5' TTT GAG GAT CCG CTG TGC TTG ACG GTT TGC 3'; ORF I, 5' CATAT TCT AGA CAC CTC GCC TTC CAA CTG 3'; M14, 5' CAT ATT CTA GAG CGA TCA TGG CGC TCC TGC TC 3'; M35, 5' CAT ATT CTA GAC CGA CTA TGG CGC CGT GCC TTC 3'; M47, 5' CAT ATT CTA GAC CTT TTG AAA TGC TCA GCA ATC 3'; T70, 5' TTG GAT CCT TAT ATG TAG CGG TAG GTG TCC GGG CTG AGG AGA AGA GGA AGC 3'; T43, 5' TTG GAT CCT TAT ATG TAG CGG TAG GTG TCG AAG AGG AGA AGG CAC GGC GC 3'; MO, 5' CAT ATT CTA GAC CTA GCA CTT TGC TGT AAC GCC TTC TCA GCC C 3'; MGS, 5' CCT TTT GGA ATA CTC AGC AAT C 3'; MGAS, 5' GAT TGC TGA GTA TTC CAA AAG G 3'.

pHCMV-16 kDa-HA1 was constructed by PCR amplification of the plasmid pTA9, containing the cDNA for the bovine 16 kDa protein tagged with the HA1 epitope (Mandel *et al.*, 1988) using the oligodeoxynucleotide primers ON#9 5' CTAGTA TCTAGA GCCACC ATG TAC CCA TAC GAT GTT CCA GAT 3' and ON#6 5' GAT CCA GGATCC CTA CTT TGT GGA 3'. The amplified fragment was cleaved with *Xba*I and *Bam*HI and cloned at the corresponding sites of the vector pHCMV/HSPA.

**Radioimmunoprecipitation of metabolically labelled proteins.** The cDNA of the 16 kDa protein (Mandel *et al.*, 1988), carrying the HA1 epitope at the amino terminus, was cotransfected with the p12<sup>I</sup> cDNA or the p12<sup>I</sup> mutants, all carrying the AU1 epitope, in HeLa/Tat cells. Twenty-four hours after transfection, cells were metabolically labelled with 50 μCi/ml each [<sup>35</sup>S]cysteine and [<sup>35</sup>S]methionine for 4 h. The cell lysate was then immunoprecipitated with the mouse monoclonal

antibodies 12CA5 or AU1, directed against the HA1 epitope or the AU1 epitope, respectively, as previously described (Koralnik *et al.*, 1992).

**Immunofluorescence.** Indirect immunofluorescence was performed on HeLa/Tat-transfected cells expressing the p12<sup>I</sup> mutants. Twenty-four hours after transfection cells were fixed on glass slides in 3.7% formaldehyde for 20 min at room temperature. The mouse monoclonal antibody AU1 was diluted 1:50 in phosphate-buffered saline (PBS) with 10% goat serum and 0.1% saponin. Cell and primary antibodies were incubated for 1 h at 37 °C, and the slides were washed several times in PBS and incubated for 45 min at 37 °C with goat anti-mouse fluoresceinated antibodies diluted 1:20 in PBS with 10% goat serum and 0.1% saponin. After several washes in PBS, cells were counterstained for 10 min at room temperature in Evans blue, mounted in glycerol, and examined with a Leitz Wetzlar microscope with a 50 × or 63 × objective.

**Focus forming assay.** Mouse C127 cells were plated at a density of  $5 \times 10^5$  per 60 mm dish. The next day cells were transfected in duplicate dishes over night using the calcium phosphate procedure, with 0.5 µg of the E5 DNA (pJS21) and 10 µg of the p12<sup>I</sup> AU1 or the p12<sup>I</sup> mutant cDNAs cloned in pHCMV/HSPA. Cells were then washed with medium, glycerol-shocked in 15% glycerol for 1 min, washed twice with PBS and allowed to recover for 24 h before trypsinization in 100 mm dishes. Cell monolayers were kept in culture for 3 weeks in DMEM–10% fetal calf serum, and the medium was changed twice a week. Foci were counted by direct visualization with a light microscope before and after staining and fixation with 1% methylene blue in ethanol for 30 min at room temperature.

## Results

### General strategy in the construction of p12<sup>I</sup> and 16 kDa mutants

The p12<sup>I</sup> protein has been previously shown to be localized in the cellular endomembranes (Koralnik *et al.*, 1993). Computer analyses of the p12<sup>I</sup> protein revealed two putative transmembrane regions encompassing aa 12–32 and aa 48–68 (Franchini *et al.*, 1993) suggesting that the p12<sup>I</sup> protein transverse the cellular endomembranes twice as schematically represented in Fig. 1. The 16 kDa protein is very hydrophobic and is thought to transverse the cellular endomembranes of organelles (lysosome, endosome and Golgi) at least four times (Nelson, 1989) (Fig. 1). The orientation of the 16 kDa protein is known and indicated in Fig. 1, whereas for the p12<sup>I</sup> protein it is unknown. To investigate the nature of the interaction between these two hydrophobic proteins, mutants of p12<sup>I</sup> were constructed that lacked one or another transmembrane (TM) domain (TM-1 and TM-2), as well as various portions of the amino terminus or the carboxy terminus of the p12<sup>I</sup> protein. Similarly, mutants of the 16 kDa protein lacking TM-1 and TM-2, TM-3 and TM-4, or TM-4 were generated. Furthermore, the glutamine in p12<sup>I</sup> was changed to a glycine since the glutamine conserved between the BPV-1 E5 and HTLV-I p12<sup>I</sup> appears to be crucial in E5-induced transformation, as well as in the binding of E5 to the 16 kDa protein (Goldstein *et al.*, 1991, 1992*b*). In addition, the

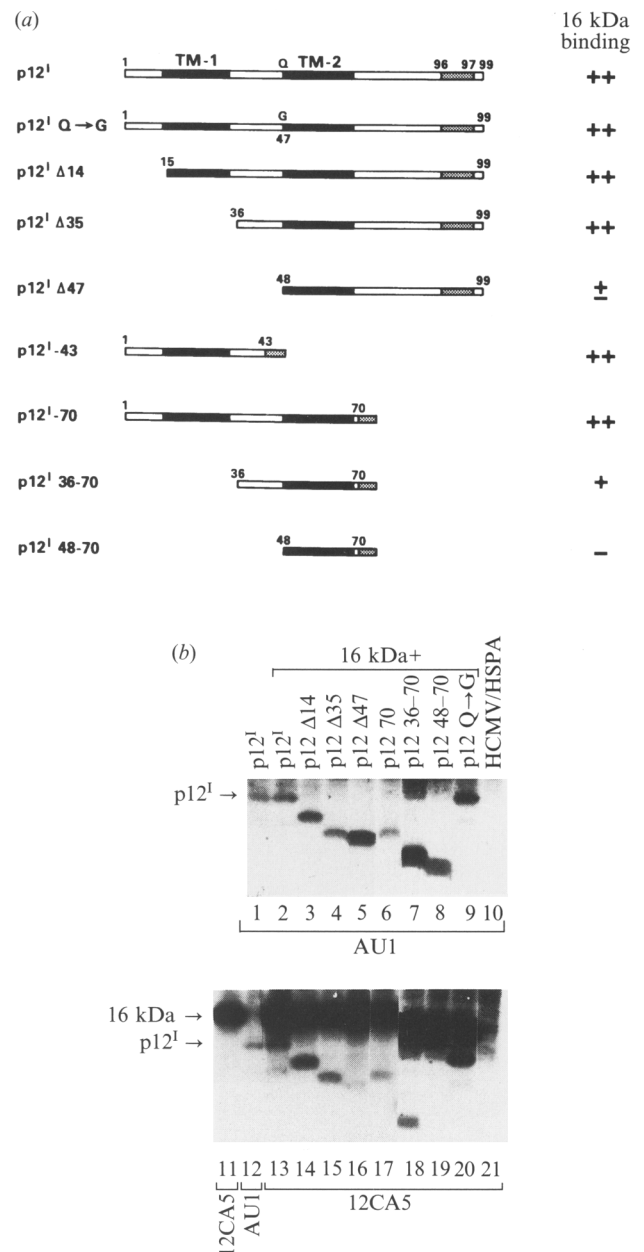


Fig. 2. Binding of the p12<sup>I</sup> mutants to the 16 kDa protein subunit. (a) Schematic representation of the p12<sup>I</sup> mutants is depicted. The two putative TM domains are indicated by solid bars. The shaded bars refer to the AU1 tag added to each protein (see Methods). The numbers refer solely to the aa number in the p12<sup>I</sup> protein excluding the tag. Q represents glutamine and G represents glycine. ++ etc. indicate the extent of binding between the wild-type p12<sup>I</sup> protein and the 16 kDa protein as evaluated on the intensity of the co-immunoprecipitated bands on acrylamide gels. (b) Radioimmunoprecipitation of metabolically labelled p12<sup>I</sup> and 16 kDa protein expressed in HeLa/Tat cells.

glutamic acid in the fourth TM domain of 16 kDa, also important in the binding to E5 (Andresson *et al.*, 1995), was changed to an arginine. All p12<sup>I</sup> and 16 kDa mutants were tagged with the AU1 or HA1 epitope as previously described.

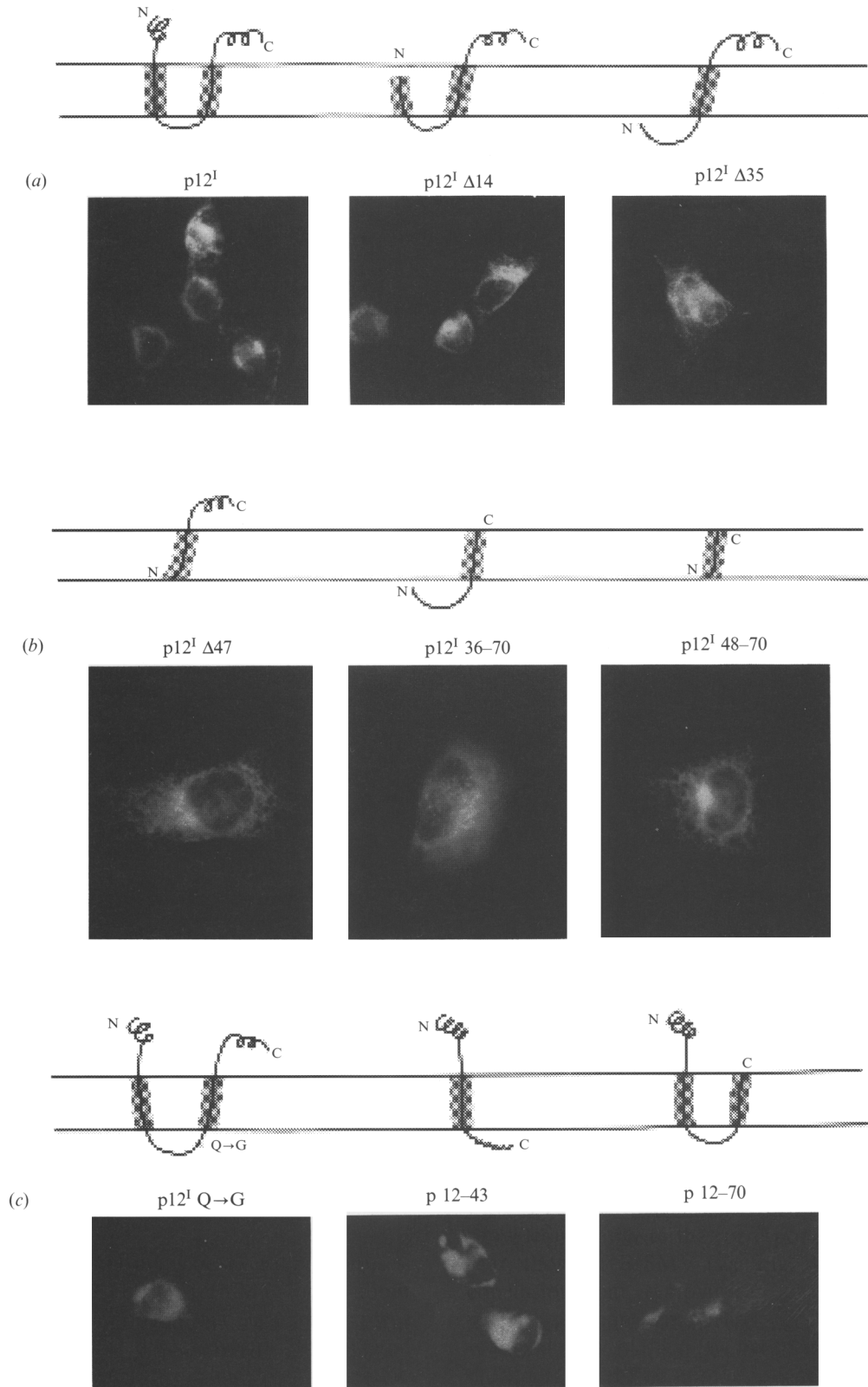


Fig. 3. (a, b, c) Cellular localization of the  $p12^I$  mutant proteins. On the top of each panel a schematic representation of each of the  $p12^I$  mutants is provided relative to their putative structure (the orientation and insertion of the  $p12^I$  mutants in the membrane is purely speculative and is used only for descriptive purposes) and on the bottom are the results of the indirect immunofluorescence assay using antibodies against the AU1 tag epitope.

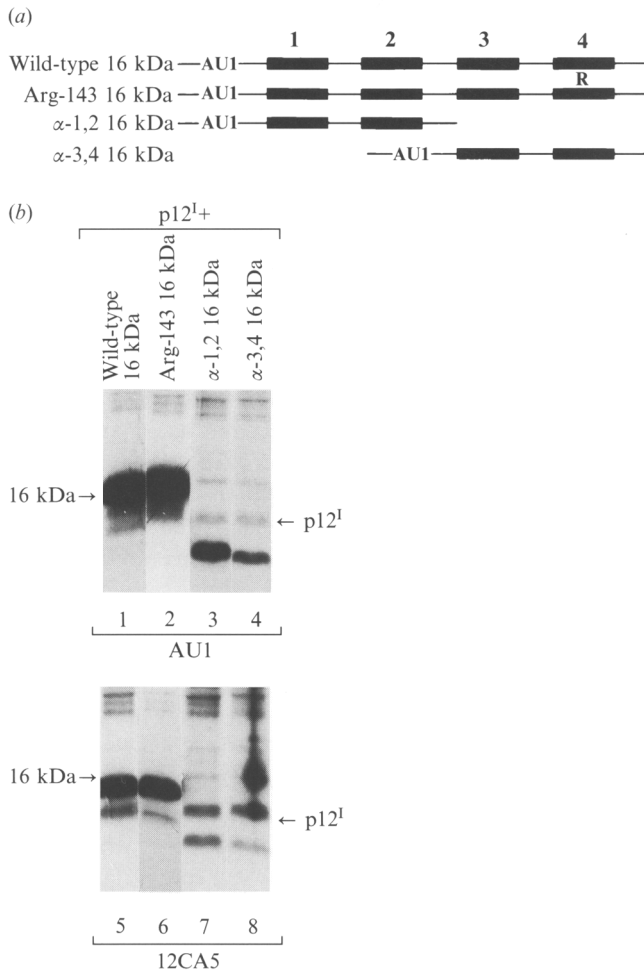


Fig. 4. Binding of the 16 kDa mutants to the wild-type p12<sup>I</sup> protein. (a) Depiction of the wild-type 16 kDa protein with its four TM domains (solid bars) with mutants lacking the third and fourth domains ( $\alpha$ 1,2 16 kDa mutant) and the first and second domains ( $\alpha$ 3,4 16 kDa mutant). In mutant Arg-143 16 kDa, the negatively charged glutamic acid at position 143 was mutated to a positively charged arginine residue. (b) Results of immunoprecipitation of the 16 kDa wild-type and mutant proteins carrying the AU1 epitope, and p12<sup>I</sup> carrying the HA1 epitope, coexpressed in HeLa/Tat cells.

#### Binding of HTLV-I p12<sup>I</sup> mutants to the 16 kDa subunit of the vacuolar H<sup>+</sup>-ATPase

The p12<sup>I</sup> and p12<sup>I</sup> mutant cDNAs, all carrying the AU1 epitope, were cotransfected separately with the 16 kDa cDNA, carrying the HA1 epitope at the amino terminus, in HeLa/Tat cells. The cell lysate from each cotransfection was then divided and immunoprecipitated with monoclonal antibody  $\alpha$ AU1 or the monoclonal antibody 12CA5. Both the p12<sup>I</sup> and 16 kDa proteins were promptly recognized by the antibodies against the respective tags when transfected alone as shown in Fig. 2 (lanes 1, 11 and 12). As previously demonstrated

(Franchini *et al.*, 1993), cotransfection of p12<sup>I</sup> and 16 kDa resulted in the co-immunoprecipitation of both proteins using the antibodies against the 16 kDa tag (Fig. 2, lane 13).

Deletion of the amino-terminal 14 and 35 aa of p12<sup>I</sup> did not alter the binding of the two proteins (see mutant p12<sup>I</sup>  $\Delta$ 14 and p12<sup>I</sup>  $\Delta$ 35, Fig. 2, lanes 3, 4, 14 and 15), whereas the amount of the p12<sup>I</sup>  $\Delta$ 47 mutant protein co-immunoprecipitated with 16 kDa was greatly decreased (Fig. 2, lanes 5 and 16). These data suggest that the region in p12<sup>I</sup> included between aa 36 and aa 48 greatly influences the interaction between these proteins. This interpretation is supported by the lack of binding of the p12<sup>I</sup> 48–70 as well as by the reduced binding of the p12<sup>I</sup> 36–70 mutants to the 16 kDa protein (Fig. 2, lanes 7, 8, 18 and 19). Consistent with this interpretation is the association of both p12<sup>I</sup> 70 (Fig. 2, lanes 6 and 17) and p12<sup>I</sup> 43 (data not shown) mutants, which contain the proline-rich region, to the 16 kDa protein. Finally, the change of a glutamine to a glycine in the p12<sup>I</sup> Q  $\rightarrow$  G mutant did not alter binding to the 16 kDa protein (Fig. 2, lanes 9 and 20).

#### Cellular localization of the p12<sup>I</sup> mutants

Indirect immunofluorescence was performed in HeLa/Tat cells transfected with each of the p12<sup>I</sup> mutants. The p12<sup>I</sup>  $\Delta$ 14 and p12<sup>I</sup>  $\Delta$ 35 mutants localized to the cellular endomembranes, as previously demonstrated for the p12<sup>I</sup> protein (Koralnik *et al.*, 1993) (Fig. 3a). Similarly, the cells transfected with mutants p12<sup>I</sup>  $\Delta$ 47 and p12<sup>I</sup> 48–70 displayed an immunofluorescence pattern, consistent with the localization of the mutant proteins to the cellular endomembrane (Fig. 3b). Similarly, the changes of a glutamine to glycine did not alter the cellular localization of p12<sup>I</sup> Q  $\rightarrow$  G (Fig. 3c).

#### Mapping of the 16 kDa interacting region(s) to the p12<sup>I</sup> protein

Three 16 kDa mutant proteins carrying the AU1 epitope (T. Andresson and others, unpublished observations) were used in the cotransfection assay with the wild-type p12<sup>I</sup> protein (Fig. 4a). Mutant Arg-143 16 kDa had the leucine in position 143 substituted by arginine. The  $\alpha$ 1,2 16 kDa lacks both third and fourth TM domains and the  $\alpha$ 3,4 16 kDa consists of the third and fourth TM domains only.

Cotransfection experiments with the 16 kDa and the p12<sup>I</sup> tagged cDNAs revealed upon immunoprecipitation, binding of the p12<sup>I</sup> protein to the wild-type 16 kDa and the Arg-143 16 kDa, as well as to the  $\alpha$ 1,2 and  $\alpha$ 3,4 16 kDa mutants (Fig. 4b, lanes 1–8). Together, these data

Table 1. *Biological activity of HTLV-I p12<sup>I</sup> mutants cotransfected with E5 in C127 mouse cells*

	Exp. 1		Exp. 2		Average fold increase
	Foci number*	Fold increase	Foci number*	Fold increase	
E5	54		44		
E5+p12 <sup>I</sup>	472	8.7	250	5.7	7.2
E5+Δ14	76	1.4	142	3.2	2.3
E5+Δ35	86	1.6	70	1.6	1.6
E5+Δ47	74	1.4	76	1.7	1.5
E5+36-70	164	3.0	76	1.7	2.3
E5+48-70	58	1.0	72	1.6	1.3
E5+43	12	0.2	50	1.1	0.6
E5+70	82	1.5	90	2.0	1.7
E5+Q → G	184	3.4	150	3.4	3.4
E5+ΔATG	62	1.1	64	1.4	1.2
Vector	0		0		

\* The foci number was derived from the average count of duplicate plates.

suggest that the p12<sup>I</sup> protein interacts with more than one domain of the 16 kDa protein. Alternatively, the p12<sup>I</sup> could bind to the region of 16 kDa included between the second and third TM domain since this region is present in all the 16 kDa mutants tested.

The intermolecular association between p12<sup>I</sup> and 16 kDa differs from that observed with the BPV-1 E5. E5 appears to bind mainly to the 16 kDa fourth TM domain and the binding is greatly decreased when a polar charged aa, such as arginine, is introduced in the fourth TM domain (T. Andresson and others, unpublished observations). As we have demonstrated, such a change did not interfere with the p12<sup>I</sup> and 16 kDa interaction. Although our data do not enable us to definitively delineate the regions of the 16 kDa protein which interact with p12<sup>I</sup>, they clearly demonstrate differences between the E5 and p12<sup>I</sup> binding to the 16 kDa protein.

#### *Phenotype of the p12<sup>I</sup> mutants cotransfected with E5 in transformation of C127 mouse cells*

We have previously demonstrated that although p12<sup>I</sup> alone does not transform C127 mouse cells, it does enhance the E5-mediated transforming activity (Franchini *et al.*, 1993). To investigate the contribution of different portions of the p12<sup>I</sup> protein to this biological activity, each p12<sup>I</sup> mutant was cotransfected with E5 DNA (pJS21) and the number of foci induced in the absence and in the presence of any given p12<sup>I</sup> mutant, as well as p12<sup>I</sup> wild-type, was evaluated. Results of two experiments performed on duplicate plates are presented in Table 1. With the exception of the p12<sup>I</sup> Q → G mutant which showed decreased but consistent enhancement effect, none of the other p12<sup>I</sup> mutants had a significant biological effect on E5 transformation, suggesting that

all the various deletions introduced in this small protein greatly affect its biological activity.

## Discussion

The data reported here describe the expression, the cellular localization, and the binding to the 16 kDa subunit of the vacuolar H<sup>+</sup>-ATPase of the HTLV-I p12<sup>I</sup> mutant proteins. Furthermore, the ability of these HTLV-I p12<sup>I</sup> mutants to potentiate cell transformation in cooperation with the BPV-1 E5 oncoprotein was investigated. The binding studies of the p12<sup>I</sup> mutants to the 16 kDa protein showed the importance of the p12<sup>I</sup> proline-rich domain, included between aa 36 and 48 of the p12<sup>I</sup> protein, in the interactions of these proteins. The correlation between binding to the 16 kDa protein and the biological activity of p12<sup>I</sup> needs to be re-evaluated using single or few aa p12<sup>I</sup> mutants. However, our present data already suggest that a clear correlation will not be found since mutants binding efficiently to 16 kDa failed to show biological activity at least in this cell system. Similarly, a lack of clear correlation was shown with E5 oncogenicity and binding to the 16 kDa protein (Goldstein *et al.*, 1992*b*).

The binding of 16 kDa mutants with the wild-type p12<sup>I</sup> indicated that possibly more than one domain of 16 kDa is mediating the interaction of the proton pump subunit with p12<sup>I</sup>. This contrasts with previous experiments performed with the BPV-1 E5 protein which indicated that E5 binds to the fourth TM domain of the 16 kDa protein. The E5 polar glutamine residue appears to be important in this interaction. Recently, the E5 proteins of human papillomavirus (HPV)-6 and -16 have been shown to interact with 16 kDa (Conrad *et al.*, 1993) indicating that this association is maintained in the human strains as well.

The 16 kDa protein is a membrane component of the vacuolar H<sup>+</sup>-ATPase, which is also found in clathrin-coated vesicles, lysosomes, endosomes, Golgi vesicles and synaptic vesicles; this proton pump is responsible for the acidification of these intracellular vesicles and might have a crucial role in dissociating ligand-receptor complexes (Nelson, 1989; Finbow *et al.*, 1991). Interference with the normal function of the proton pump through binding of viral proteins such as HTLV-I p12<sup>I</sup>, BPV E5 or HPV E5 proteins might result in dysregulation of growth factor/receptor interaction and lead to increased recycling of receptors to the cell surface (Finbow *et al.*, 1991). This concept is supported by the finding that epidermal growth factor receptor down-regulation is observed in keratinocytes transformed by the HPV 16 E5 (Straight *et al.*, 1993); additionally, the 16 kDa protein appears to be the mediator of E5 binding to the platelet-derived growth factor receptor in the

transformed C127 cells (Kulke & DiMaio, 1991; Petti *et al.*, 1991; Goldstein *et al.*, 1992a; Petti & DiMaio, 1992). The p12<sup>I</sup> protein also has been shown to interact specifically with the IL-2R  $\beta$  and  $\gamma$  but not  $\alpha$  chains (J. C. Mulloy and G. Franchini, unpublished observations) suggesting its possible involvement in the mechanism of IL-2 signalling and ultimately T cell transformation. A possible role for p12<sup>I</sup> in signalling is further supported by the finding that the p12<sup>I</sup> protein interacts specifically with the acidic region of the IL-2R  $\beta$  chain which has been shown to be the target of p56<sup>lck</sup> (Hatakeyama *et al.*, 1991), a major tyrosine protein-kinase of T cells. Thus, the understanding of the 16 kDa and p12<sup>I</sup> interactions might help in elucidating the role of HTLV-I p12<sup>I</sup> protein in immortalization and/or transformation of human T cells and at the same time provide insights into the physiology of the IL-2R signalling.

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