

An Immunological Analysis of Ty1 Virus-like Particle Structure

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We present an immunological characterization of the Ty1 virus-like particle (VLP). A panel of monoclonal and polyclonal antibodies were raised against the TYA particle-forming protein. Using these antibodies in epitope availability assays two N-terminal regions of the TYA protein were mapped projecting from or at the surface of the proteinaceous shell of the VLP. Two different C-termini of the TYA protein, corresponding to the C-terminus of the full-length and truncated forms, were seen to be buried within the particle core and not available for antibody binding. RNase accessibility studies demonstrated a difference in the porosity of the protein shell surrounding the Ty1 nucleic acid between different particle types, suggesting differences in subunit organization. © 1995 Academic Press, Inc.

INTRODUCTION

The retrotransposon Ty1 is a 5.9-kb element found in nearly all laboratory strains of *Saccharomyces cerevisiae*. It produces a 5.7-kb primary transcript that acts as both the major message and transposition intermediate (Boeke *et al.*, 1985; Garfinkel *et al.*, 1985; Mellor *et al.*, 1985a). Within the 5.7-kb RNA there are two open reading frames, TYA and TYB, which are broadly analogous to the retroviral *gag* and *pol* genes, respectively (Kingsman and Kingsman, 1988; Curcio and Garfinkel, 1991). TYA encodes a 50-kDa protein, p1, which assembles into a virus-like core particle (virus-like particle: VLP; Adams *et al.*, 1987a) via specific p1-p1 interactions (Martin-Rendon *et al.*, unpublished observations). The TYB gene product is produced via a ribosomal frameshift event during translation resulting in a 190-kDa TYA:TYB fusion protein, p3. This protein contains the catalytic functions of the transposition unit and is also incorporated into VLPs via p1 interactions. The Ty1 enzymes show limited but significant homology to retroviral proteases, integrases, and reverse transcriptases and they fulfill the same functions as in the retrovirus life-cycle (see review by Sandmeyer, 1992). The Ty1 protease is required for the maturation of the VLP in that it mediates cleavage of p1 to a 48-kDa protein designated p2 and it cleaves the functional enzymes from the p3 precursor (Adams *et al.*, 1987a; Müller *et al.*, 1987).

Mature Ty1-VLPs can be regarded as the functional units of Ty retrotransposition and they are functionally

and structurally analogous to the cores of retroviruses. They have been shown to be capable of integrating Ty-related DNA into target DNA *in vitro* and their overproduction in yeast is correlated with increased transposition. As with retroviruses, very little is known about the structure of Ty particles. Given that the key events of reverse transcription and integration occur in the context of the particle, the molecular architecture of these structures is clearly important. The best definition of the Ty1-VLP, to date, was described by Burns *et al.* (1992), who used a combination of biophysical and biochemical techniques to show that the particle has a molecular weight of about 14 MDa, is composed of a monomolecular shell of about 300 TYA-encoded subunits, and is porous to small (~13 kDa) globular proteins. A curious feature of the particles is that though they show size variation across the population they also show clear symmetry. This suggests that the particles assemble by defined but flexible rules.

Although Burns *et al.* (1992) provided a useful working model for the VLP, they did not provide any information concerning the organization of protein subunits within the particle. We have now produced monoclonal and polyclonal antibodies against the p1 protein, determined their binding sites, and assayed the positioning of these epitopes within the assembled particle structure. The antibodies described map to the N- and C-terminal regions of the p1 protein and allow us to position the termini of the Ty1 structural protein within the assembled particle. The C-terminus is hidden from the surface of the particle and the N-terminal regions project from the surface. We also show that there may be a significant structural change associated with the maturation process.

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MATERIALS AND METHODS

Yeast strains

Strains of *Saccharomyces cerevisiae* used were based on the host strains BJ2168 and MC2. BJ2168 (*cir*⁺, *leu*2-3, 112, *ura*3-5, *trp*1, *pep*4-3, *prc*1-407, *prb*1-1122, *gal*2) has been described previously (Adams *et al.*, 1991); MC2 is a galactose revertant of BJ2168. The strains were made from transformation of either host with a 2 μ -based plasmid to overexpress the TYA gene product or truncations of the TYA gene product.

The pOGS40 and pMA91-14 constructs encode a truncated Ty1 TYA (Adams *et al.*, 1991) and the full-length TYA:TYB fusion protein, respectively. The pMA91-14 construct is essentially as pMA91-10 described by Mellor *et al.* (1985b). The full-length TYA construct (pSBF) was produced by PCR using the oligonucleotide primers 5'GCCCTAGGTTAGATGATGCTGAG3' and 5'CAAGGT-CGCATGCC3' with pMA91-14 vector DNA as a template. This yields an 188-nt-long fragment with *Bam*HI/*Sph*I termini which was then inserted into the pOGS40 vector (an inducible derivative of pMA5620 with a galactose-sensitive UAS sequence). The strain BJ2168/pMA91-14 expresses the Ty1 protein constitutively whereas the pOGS40 and pSBF transformed strains give galactose-inducible expression.

Other yeast strains were used in Western blotting screens to aid identification of the epitopes recognized by the different mAbs. These were MA5620-40 and OGS707 which overexpress the N- and C-terminally truncated p1 protein p41-381 and the C-terminally truncated p1 protein p1-360, respectively.

Western blot analysis of antibody binding

Yeast cell lysates were prepared by vortexing cell pellets with glass beads in TEN buffer with a cocktail of protease inhibitors. Samples were electrophoresed on 10% SDS-PAGE gels and blotted onto nitrocellulose using standard protocols. Ty1 proteins were visualized by incubation with monoclonal anti-Ty1 or polyclonal anti-Ty1 antibodies (described below) and subsequent detection with peroxidase-labeled rabbit anti-mouse or swine anti-rabbit antibodies (DAKOPatts, UK) using 4-chloro-1-naphthol and H₂O₂ as substrate.

Virus-like particle preparation

Preparation of the virus-like particles was essentially as described by Burns *et al.* (1991). All samples were filter-sterilized and then stored at either 4° or -80°.

Antibody production and purification

Monoclonal antibodies were produced against MA-5620 VLPs purified from yeast strain BJ2168/pOGS40/

pUG4IS. The VLPs were either denatured with SDS (1% SDS, 100°, 2') and then injected in suspension with Freund's adjuvant (TYG antibodies) or without pretreatment in Freund's adjuvant (BB antibodies) into Balb/C mice. Fusion of the immune spleens was performed according to standard protocols using SP2/0 cells (MacRae *et al.*, 1990). Antibodies were screened against whole cell lysates of BJ2168/pOGS40/pUG4IS \pm galactose induction by ELISA and Western blots (TYG antibodies) and by ELISA using purified particle preparations (BB antibodies). The resulting monoclonal antibodies were typed by immunodiffusion (ICN Immunobiologicals; TYG antibodies) or using the Amersham typing kit (Amersham, UK; BB antibodies). Antibodies were purified for use in some assays by affinity chromatography using protein G-Sepharose (Pharmacia, UK; for IgG antibodies) or Bakerbond AX (J. T. Baker, Reading, UK; for TYG5).

A rabbit polyclonal serum and mouse monoclonal antibodies were raised against a 16-mer peptide, CIQLNNK-HDLHLPETY, corresponding to amino acids 426-440 from the p1 protein, with an additional N-terminal cysteine residue to aid conjugation. The animals were injected with the peptide conjugated to keyhole limpet hemocyanin (Pierce) using the cross-linking agent *N*-succinimidyl-3-(2-pyridyldithio)propionate (Pierce) and antibodies were screened by ELISA against the peptide cross-linked using *M*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (Pierce) to BSA (Fraction V, Boehringer Mannheim) and by Western blot against BJ2168/pMA91-14 whole cell lysates.

PEPSCAN analysis

All monoclonal antibodies produced were tested on a TYA p1 protein PEPSCAN kit (Cambridge Research Biochemicals, Cheshire, UK) which consisted of a series of 10-mer peptides overlapping by eight amino acids along the entire sequence of p1. An ELISA assay was performed using the peptides as antigen to determine the precise epitope recognized by each monoclonal antibody.

Electron microscopy

Virus-like particles were visualized by negative staining using saturated uranyl acetate in water on freshly discharged carbon-coated formvar grids (Taab, UK). Grids were examined by TEM using a Phillips 400 microscope.

Immunological characterization of VLPs

The different particle types were mapped for the presence of the epitopes recognized by the range of anti-Ty1 antibodies produced. The following three assays were performed in an attempt to determine whether each anti-

body bound to a particle type, indicating an external location for that epitope, or not.

Immunogold electron microscopy

Immunogold labeling of the purified VLP preparations was performed by allowing ≈ 5 ng VLPs in 10 μ l TEN buffer (10 mM Tris-HCl, pH 7.4; 2 mM EDTA; 140 mM NaCl) to settle onto a freshly discharged carbon-coated formvar grid. The particles were then blocked with 1% PBSA (phosphate-buffered saline plus BSA; $3 \times 1'$) before incubating with the anti-Ty1 p1 monoclonal antibodies (culture supernatants diluted 1:50 in 0.1% PBSA; purified antibodies at 0.25 μ g/ml in 0.1% PBSA; 1 hr at 20°). The grids were then washed with 0.1% PBSA ($3 \times 5'$) and incubated with goat anti-mouse IgG antibody conjugated to 10 nm colloidal gold (BioCell, UK; 1 hr at 20°). After washing with 0.1% PBSA ($3 \times 5'$) and PBS ($3 \times 5'$) grids were negatively stained with uranyl acetate (saturated solution in H₂O) and examined by TEM using a Phillips 400 microscope.

For labeling with the IgA mAbs the method was modified slightly, a secondary rabbit anti-mouse antibody was used followed by a goat anti-rabbit antibody conjugated to 10 nm colloidal gold (BioCell). Labeling with the rabbit anti-peptide polyclonal antiserum was visualized using the same goat anti-rabbit gold-labeled antibody.

Capture ELISA assay

An ELISA assay was performed using all the mAbs and their ability to "capture" the VLPs determined. Microtiter wells (Nunc Maxisorb; Nunc, UK) were coated with mAb (10 μ g/ml in 50 mM sodium carbonate buffer, pH 9.5), washed with PBS plus 0.1% Tween 20, blocked with 2% casein in PBS, incubated with MA620 VLPs (in trebling dilutions from 100 μ g/ml in PBS plus 2% casein and 0.1% Tween-20, 1 hr at 20°), washed as before, and the bound particles labeled using a rabbit polyclonal anti-MA5620 serum (1:1000 in buffer, 1 hr at 20°). Unbound rabbit serum was removed by washing (PBS + Tween 20, $3 \times 5'$), the plate was then incubated with a peroxidase-conjugated swine anti-rabbit Ab (DAKOPatts), washed ($3 \times 5'$), developed with substrate (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid/H₂O₂), and the A₄₁₀ determined. The rabbit polyclonal serum No. 271 was also tested against p1-440 particles by modifying the assay slightly to use BB2 as the antibody to detect bound particles and a peroxidase-conjugated rabbit anti-mouse antibody to label the BB2 antibody.

Measurement of VLP-antibody binding in solution

A photon correlation spectrophotometer (Malvern 4700c submicron particle analyzer) was used in an assay to determine whether the mAbs were binding to the particles. Diameter measurements were taken at 90° of a

solution of VLPs (12.5 μ g in 250 μ l TEN) after successive additions of antibody solution (2.5 μ g in 10 μ l TEN).

Ribonuclease protection assays. The susceptibility of the RNA within VLPs to degradation by ribonucleases was determined as follows. Particles were incubated with Ribonuclease A (EC 3.1.27.5; 25U in 0.5 ml TEN buffer; Boehringer), Benzonase (EC 3.1.30.1; 25U in 0.5 ml TEN buffer; Sigma), or Nuclease P1 (EC 3.1.30.1; 25U in 0.5 ml TEN buffer; Sigma) for 5' at 37° and then the particles removed by centrifugation (200 000 g; 1 hr at 4°). The A₂₆₀ of the supernatant was then determined and compared with the no enzyme control.

RESULTS

Particle types

Wild-type particles containing the enzyme activities are difficult to analyze because they are extremely polydisperse as a result of varying degrees of proteolytic maturation across the population (Burns *et al.*, 1992). We have worked, therefore, with two "model" particle types that have reasonably homogeneous populations, as assessed by sucrose gradient profiles and EM.

The first, designated 1-440, is composed of the full-length p1 protein expressed from plasmid pSBF. This plasmid contains the TYA gene alone expressed from a galactose inducible promoter (see discussion under Materials and Methods). The p1 protein assembles into VLPs but does not undergo maturation as the TYB coding sequence is missing. These 1-440 particles represent, therefore, the immature state. The second, designated 1-381, is composed of a derivative of p1 truncated at amino acid 381. Codon 381 is close to the p1-p2 cleavage site (Hurd *et al.*, unpublished) and so these particles represent the mature state. The truncated p1 protein is expressed from pOGS40 using the same promoter as the 1-440 version.

Antibodies

A series of antibodies were made to the TYA protein to enable construction of an immunological map of the assembled VLPs. We describe here five monoclonal antibodies raised against the 1-381 VLP, a polyclonal serum, No. 271, and a monoclonal antibody, pep3, raised against a peptide corresponding to the last 15 amino acids of the TYA p1 protein. This region is beyond the p1-p2 cleavage site and so these two antibodies are p1-specific antibodies.

The anti-1-381 mAbs, designated BB1, BB2, TYG2, TYG3, and TYG5, all recognize the 1-381 protein on Western blots (see Fig. 1) and in ELISAs using whole-cell lysates from MC2 transformed with pOGS40. These are IgG antibodies except for TYG5 which is an IgA (see Table 1). The epitopes recognized by each antibody were determined by an ELISA-

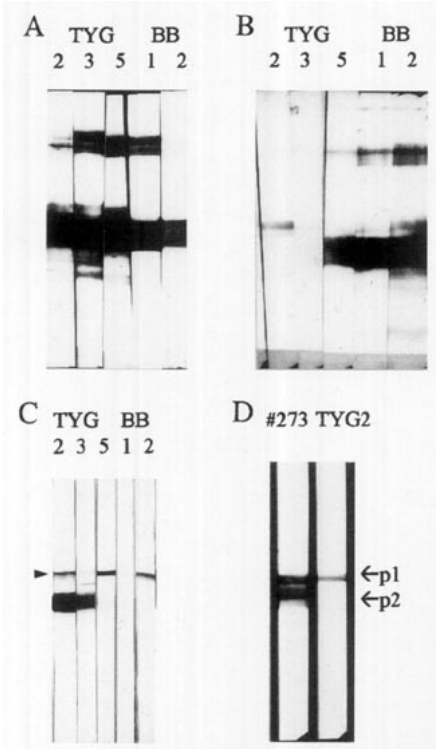


FIG. 1. Western blot analysis of antibody binding profiles using cell lysates from strains expressing (A) the MA5620 protein p1-381, (B) the C-terminally truncated TYA protein p1-363, and (C) the N- and C-terminally truncated protein p41-381. Note the higher-molecular-weight forms seen (A, B) and the endogenous Ty1 protein (arrows). Probing of wild-type MA91-14 VLP preparations with TYG2 and the polyclonal antisera No. 273 (D) shows it to label the full-length TYA p1 protein but not the processed p2 form.

based PEPSCAN analysis, using a series of 10-mer peptides overlapping by eight residues synthesized along the entire sequence of the p1 protein (see Table 1).

Three antibodies recognize epitopes close to the N-terminus of the MA5620 protein; these are BB1, which binds to amino acids 9–14, and BB2 and TYG5, which recognize overlapping epitopes at amino acids 27–32 and 29–34, respectively.

TYG2 and TYG3 antibodies recognize C-terminal epitopes; the extreme C-terminus of the 1-381 protein (amino acids 372–380) is recognized by TYG2 whereas TYG3 binds to amino acids 359–364.

As expected the polyclonal serum No. 271, raised against the p1 specific peptide, is able to recognize p1 protein, but not the processed p2 form, on Western blots (Fig. 1) and by ELISA (data not shown). PEPSCAN analysis of the No. 271 polyclonal serum showed that it only binds to peptide 222 (amino acids 431–440) at the very C-terminus of the p1 protein (Fig. 2). The mouse monoclonal pep3 has similar properties to No. 271.

An immunological characterization of the VLPs

Using the panel of antibodies described above we have determined those TYA structural protein regions

that are present on the external surface of the particles and hence available to antibody, and those regions which appear to be buried and are thus unavailable for antibody binding. Three assays have been used: an immunogold EM labeling technique, a capture ELISA, and a light scattering assay. Examples of typical positive and negative reactions for each assay are given in Figs. 3–5. A summary of antibody binding in the three assays is shown in Table 2.

The N-terminal binding antibodies. The extreme N-terminal regions recognized by the antibodies BB1, BB2, and TYG5 are accessible in all three assays and are therefore likely to be external on both the 1-381 and 1-440 particles (Table 2 and Figs. 3–5).

The pattern of BB1 binding to the VLP samples throughout the three assays is broadly similar to BB2 (Table 2). The addition of BB1 to either model particle type in the light scattering assay, however, results in a lower increase in measured diameter than seen with BB2 (Figs. 5A and 5B). The BB1 epitope, amino acids 9–14, is perhaps more closely anchored into the body of the particle than the BB2 epitope, while still being available to antibody. The IgA antibody TYG5 shows a different binding pattern to the other closely related antibody BB2 in the light scattering assay (Fig. 5C). The large diameter increase seen in response to TYG5 addition is due to two separate but related events. Initially, the TYG5 binds to the 1-381 or 1-440 VLPs, increasing their diameter; subsequent additions of antibody result in cross-linking of the antibody-VLP sample as indicated by a large increase in the measured diameter at 20° compared with the 90° readings (data not shown). This was not observed after binding of either BB1 or BB2 to either VLP preparation and is presumably a function of the greater cross-linking capabilities of IgA.

TABLE 1

Epitopes of Anti-MA5620 VLP Monoclonal Antibodies^a

Antibody	Ig class	PEPSCAN ANALYSIS		
		Peptide numbers reacting	Epitope (aa) position	Sequence
N-terminal antibodies				
BB1	IgG2b	5–7	9–14	HSPISH
BB2	IgG1	14–16	27–32	HTNQDP
TYG5	IgA	15–17	29–34	NQDPLD
C-terminal antibodies				
TYG2	IgG1	190–191	372–380	SYTNTTP
TYG3	IgG1	182–184	359–364	NYRRNP

^a Summary of data from PEPSCAN peptide ELISA assay and Western blot screen using a range of different TYA protein constructs. (aa) Amino acid numbers in TYA protein sequence as reported by Mellor *et al.* (1985c).

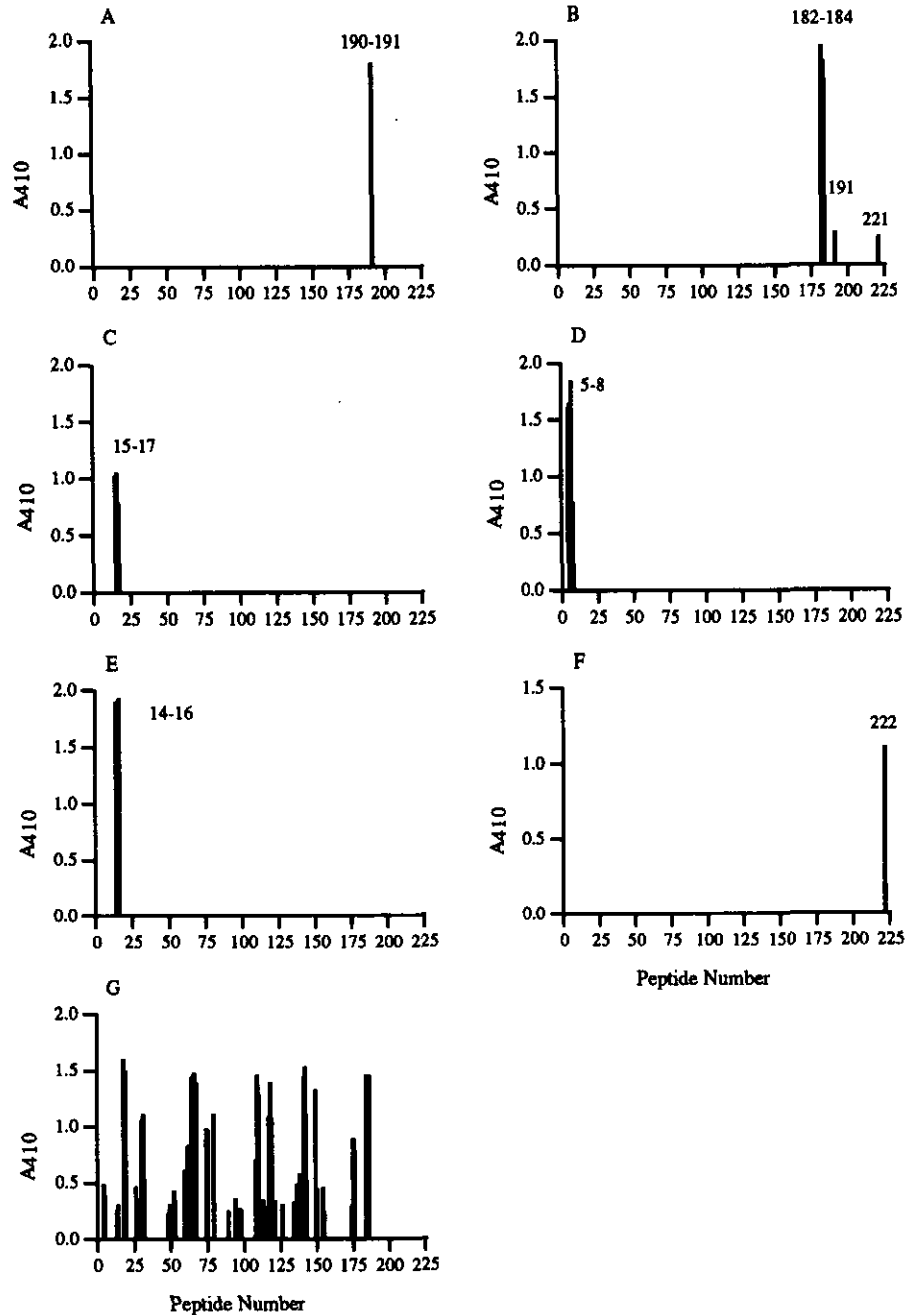


FIG. 2. PEPSCAN analysis of antibodies using an ELISA assay to detect binding to a series of overlapping Ty1 p1 peptides. The 10-mer peptides cover the entire p1 sequence (440 amino acids) as reported by Mellor *et al.* (1985c) beginning at peptide 3 and ending at peptide 222. Reactions below $0.2 A_{410}$ were not included.

The two particle types used in these assays were similar in their overall qualitative pattern of binding to the range of monoclonal antibodies throughout the tests but they showed different quantitative behavior, particularly in the light-scattering assay (Fig. 5). This is clearly illustrated by the binding of the BB2 and TYG5 antibodies, as measured by photon-correlation spectroscopy (PCS; Fig. 5). For example, addition of antibody BB2 to the 1-381 VLPs results in an increase in their diameter from

51 nm up to a saturation point of 75 nm (Fig. 5A) and further additions of antibody, up to $17.5 \mu\text{g}$ in total, gave no further increase in the particle diameter (data not shown). However, addition of BB2 to the 1-440 particles, at 60 nm in diameter, are slightly larger than the 1-381 VLPs, but subsequent addition of BB2 antibody gave an even greater than expected increase in the particle diameter up to 118 nm. Variation in the cross-linking

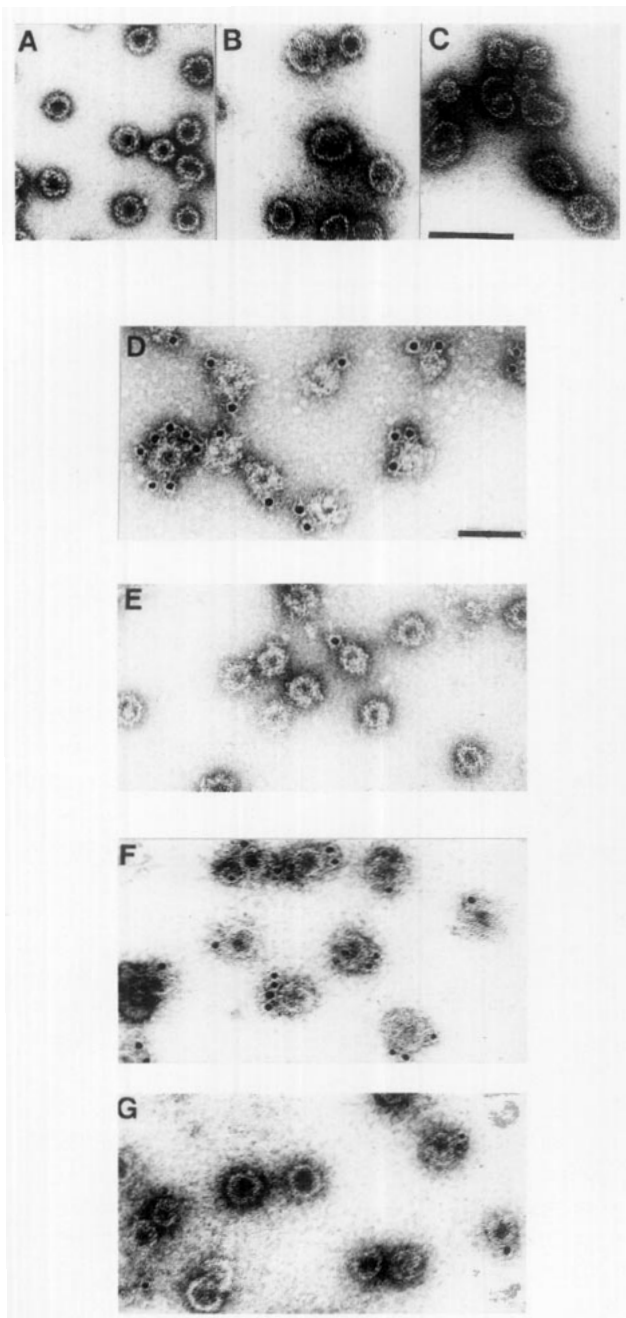


FIG. 3. Micrographs of negatively-stained and immunogold-labeled VLP preparations. The three particle types negatively stained using saturated uranyl acetate panels show (A) p1-381 VLPs, (B) p1-440 VLPs, and (C) overexpressed Ty1-15 wild-type MA91-14 VLPs. (D–F) Immunogold micrographs of p1-381 and p1-440 particles. The VLPs were incubated with an anti-MA5620 monoclonal antibody and bound antibody was then visualized using a 10-nm gold-conjugated second antibody. Representative reactions are shown here for both the model VLPs. The p1-381 (D and E) and the p1-440 VLPs (F and G) after labeling with (D and F) BB1 (+ve reaction) and (E and G) TYG2 (–ve reaction). Table 2 contains a summary of the immunogold binding data for all antibodies tested. Magnification bars = 100 nm.

of a proportion of the particles within the population could account for these results but there was no evidence of this as there was neither a change in the pattern

of distribution of the light scattering nor an increase in the measured diameter at 20° compared with the 90° readings (data not shown). The simplest interpretation of these data is that there is a structural difference between the 1-381 and 1-440 particles, although there is no obvious difference seen by electron microscopy of negatively stained samples (Fig. 3). It is conceivable that the diameter of the 1-440 particles, in the absence of antibody, is underestimated due to the presence of flexible spikes on the surface. If these spikes are rendered more rigid by binding antibody, then a greater increase in diameter would be obtained than if antibody was binding to a dense, rigid structure.

The C-terminal binding antibodies. Antibodies TYG2 and TYG3, which bind at amino acids 372–380 and 359–364, respectively, do not recognize the 1-381 or 1-440 VLPs in the immunogold EM and show much-reduced binding in the PCS assay and ELISA. Furthermore, antibodies against the C-terminus of p1, No. 271, and pep3 showed no binding to the 1-440 particles (Fig. 4B) implying that the extreme C-terminal region of the full-length p1 protein is not exposed at the surface of these particles. Taken together, these data would indicate that for both particle types the C-terminal regions are not ex-

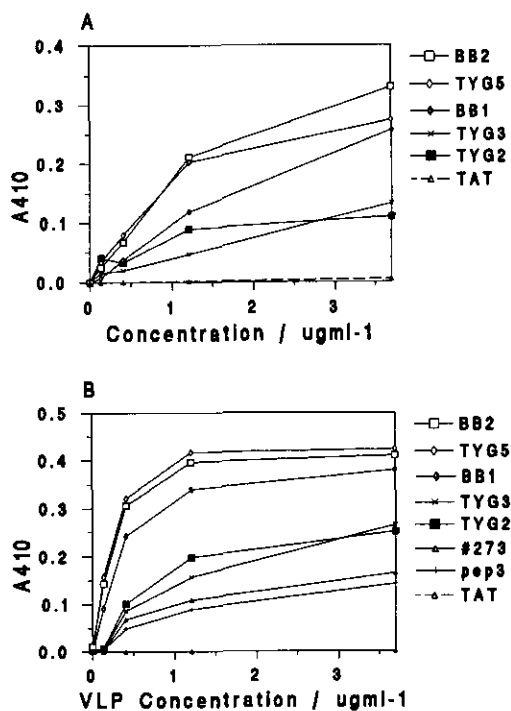


FIG. 4. Capture ELISA analysis of anti-MA5620 mAbs with the three VLP types. The p1-381 (A), p1-440 (B), and MA91-14 (C) particles were incubated with mAbs, and the amount of particles captured with increasing VLP concentration was determined by ELISA. Curves are shown for the binding of BB1, BB2, and TYG5 (defined as positive for binding to exterior of all particle types) and TYG2 and TYG3 (defined as negative for binding to exterior of all particle types) compared with the irrelevant control TAT, a monoclonal anti-tubulin antibody.

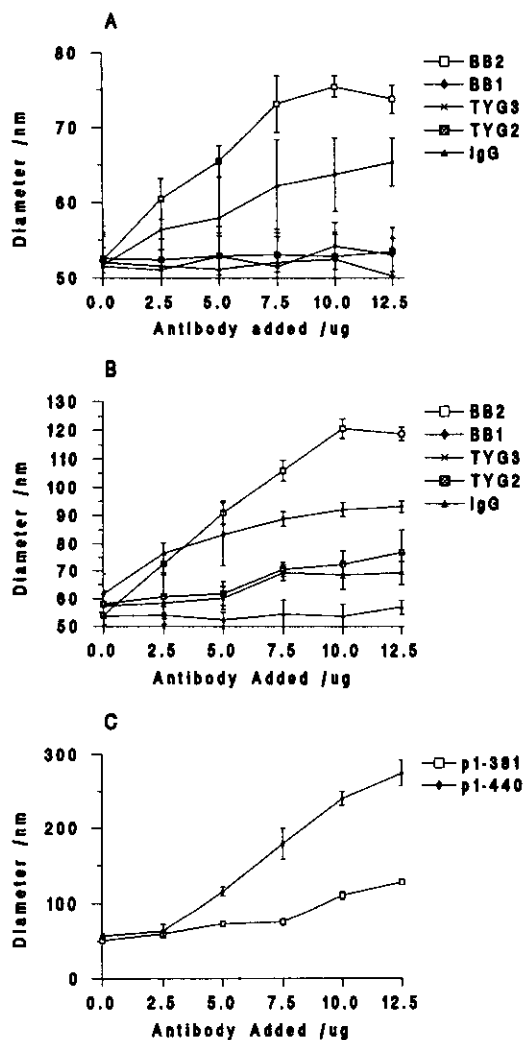


FIG. 5. Binding of anti-MA5620 mAbs to p1-381 VLPs and p1-440 VLPs as determined by light scattering (PCS). The change in particle diameter measured after addition of antibody to the particle solution is shown for both the p1-381 VLP (A and C) and the p1-440 VLP (B and C). Curves are shown for the binding of BB1 and BB2 (defined as positive for binding to exterior of both particle types) and TYG2 and TYG3 (defined as negative for binding to exterior of particles) compared with the control IgG binding profile (A and B). (C) shows the different binding profile obtained with the IgA antibody TYG5 when incubated with either p1-381 or p1-440 particles. Note the differences in y-axis scale between the three plots.

posed and that the unprocessed C-terminus of the p1 protein is buried within the structure.

Nuclease susceptibility assays

The notion that the quantitative differences in antibody binding between the 1-381 and 1-440 VLPs in the PCS assay is due to structural differences between the two particles prompted us to ask whether accessibility of nucleases through the protein shell to the RNA within the VLP may also differ. Burns *et al.* (1992) observed

that the 1-381 particle was porous, allowing immediate access to the 13.7-kDa RNase A but not to the larger, 30-kDa benzonase molecule. Therefore, the susceptibility of RNA, within the two types of particle, to any of three nucleases, RNase A, benzonase, and nuclease P1, was tested (Table 3). The RNA within both 1-440 and 1-381 particles was susceptible to the small RNase A (13.2 kDa) but neither particle allowed immediate access to the 30-kDa benzonase nuclease. The 1-440 particles, however, did differ from the 1-381 VLPs in their susceptibility to RNA degradation by the 24-kDa nuclease P1. The 1-381 VLP structure allowed access of the nuclease to the RNA, whereas the 1-440 particle did not (Table 3). This is consistent with there being a small structural difference between the particles, and that this difference changes the size of the "holes" in the protein shell.

DISCUSSION

The N-terminus of the TYA protein is common to both the 1-381 and 1-440. This study has shown that sections of the N-terminal region of these proteins are available to antibody. The spacing of the antibody binding sites along this N-terminal region suggest that two areas centered around amino acids 9–14 and 27–34 are present at or projecting from the surface of the VLP.

The 1-381 and 1-440 particles are composed of proteins with differing C-termini. However, none of the antibodies that recognize the C-terminal regions access their epitopes in solution. This applies equally to the anti-peptide antibodies that recognize the C-terminal 15 amino acids of p1 as it does to the antibodies that recognize amino acids 372–380 and 359–364. These data suggest that the C-terminus is buried within the particle.

This overall orientation of N-terminus out and C-terminus in is, of course, the same as the orientation of the GAG precursor in the immature core of a retrovirus (Gelderbloom *et al.*, 1987; Linial and Miller, 1990). Clearly, this is compatible with ensuring that the enzyme activities of p3, the TYA:TYB fusion protein, are packaged. The observation that the N-terminus is on the surface is interesting in the context of the differing *modi operandi* of retroviruses and retrotransposons. In a retrovirus the amino terminus of the GAG precursor protein is myristoylated and the protein is targeted to the plasma membrane where the myristic acid penetrates the bilayer. The N-terminus of the retrovirus is therefore, highly specialized and "needs" to be on the surface. No similar need exists for the Ty1 p1 protein.

The position of the C-terminus of the 1-381 particle is also of interest for the production of hybrid VLPs. The use of C-terminal fusions of foreign proteins at codon 381 to

TABLE 2
Summary of Data from Antibody Binding Assays

Antibody	Epitope recognized (amino acid numbers)	p1-381 VLPs			p1-440 VLPs		
		Immunogold EM	PCS	Capture ELISA	Immunogold EM	PCS	Capture ELISA
N-terminal antibodies							
BB1	9-14	+	+	+	+	+	+
BB2	27-32	+	+	+	+	+	+
TYG5	29-34	+	+	+	+	+	+
C-terminal antibodies							
TYG2	372-380	-	-	-	-	-	-
TYG3	359-364	-	-	-	-	-	-
p1-specific antibodies							
No. 271	431-440	n/a	n/a	n/a	-	n.d.	-
pep3	-	n/a	n/a	n/a	-	n.d.	-

Note. (+) Epitope accessible for antibody binding; (-) epitope inaccessible; (n/a) assay not applicable, as No. 271 and pep3 antibodies were raised against a sequence not present in the p1-381 protein; (n.d.) experiment not done.

generate hybrid particles as antigen carriers has been described by Adams *et al.* (1987b). These antigen carrier particles have been used to elicit an immune response with VLPs containing both large and small antigens. With some antigens such as HIV p24 (24 kDa) immunogold and various biophysical analyses show that, in contrast to our data here, the added antigen is on the surface of the particles (Adams *et al.*, 1994). Accordingly, humoral responses to the antigen are high. However, p24 is exceptional in that it has its own assembly determinants which may influence its position in the particle. Other antigens, such as the HIV envelope region V3 give poor humoral responses and are not detectable on the surface of the particles. This is consistent with our data here. Furthermore, when the V3 loop is moved to an internal site close to the N-terminus the humoral response is dramatically increased (Richardson *et al.*, unpublished data), again consistent with the N-terminus being surface exposed.

Amino acid 381 is close to the p1/p2 processing site

TABLE 3
Nuclease Protection Assays^a

Enzyme added	ΔA_{260} p1-381 VLPs	ΔA_{260} p1-440 VLPs
RNase A	0.119 ± 0.003	0.104 ± 0.004
Nuclease P1	≤ 0.002	0.060 ± 0.004
Benzonase	≤ 0.003	≤ 0.003
Benzonase + SDS	0.156 ± 0.008	0.126 ± 0.006

^a The ability of various ribonuclease activities to degrade RNA encapsidated by the two model particle types, p1-381 VLP and p1-440 VLP, was determined by the liberation of RNA into the surrounding buffer, as measured by a change in A_{260} , after incubation with enzyme. Values given are mean \pm sd, $n = 3$. Particles were also disrupted by 0.075% SDS and the available RNA digested with benzonase.

(Hurd *et al.*, unpublished). The two particle types used in this study represent, therefore, unprocessed and processed p1. Two items of data suggest that the structures of these two particles are subtly different. First, the quantitative responses to BB2 and TYG5 in the light-scattering experiments (Fig. 5) and second, the differential susceptibility of RNA to RNase P1 in the two types of particles (Table 3). This might suggest that the C-terminal processing event that is mediated by the Ty1 protease may induce a structural change as a result of a change in the relationships of the particle subunits. It remains to be seen whether that change is a key step in activating transposition. Changes in subunit interactions leading to changes in porosity of viral protein shells have been reported previously. The tomato bushy stunt virus undergoes a Ca^{2+} - and pH-dependent expansion in structure upon entry into its host, which is due to changes in the conformation of individual protein subunits. This expansion is thought to be an initial step in the disassembly reaction triggered by entry into a cellular environment (Kruse *et al.*, 1982). The togaviruses appear to have a similar expanded structure, but they do not appear to have a corresponding compact structure; however, unlike the tomato bushy stunt virus core the togavirus cores are usually protected from the extracellular environment by the presence of an envelope (Harrison, 1990).

Overall, this study takes us one step further toward an understanding of the structure of the Ty1 retrotransposon particle (Fig. 6). The orientation of the subunit protein is consistent with its function and although the processing of Ty1 p1 protein is less extensive than the analogous GAG proteins of retroviruses, like retroviruses it appears that the processing event may lead to structural and perhaps functional changes in the particle.

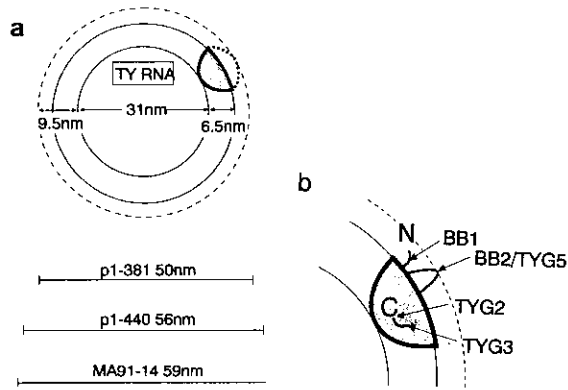


FIG. 6. A model for the Ty1 virus-like particle. (a) A cross section of a MA5620 p1-381 VLP with a hollow central core containing Ty1 RNA surrounded by a 9.5 nm proteinaceous shell, with the bulk of the protein found in a central 6.5-nm-wide band (Burns *et al.* 1992). The relative dimensions of the three particle types studied are represented below for comparison. (b) A diagrammatic illustration of a typical p1-381 monomer within the VLPs protein shell showing external N-terminal regions and a buried C-terminus. The position of the monoclonal antibody binding epitopes are represented by arrows.

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