Production of polyomavirus structural protein VP1 in yeast cells and its interaction with cell structures

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Abstract The gene for mouse polyomavirus major structural protein VP1 was expressed in *Saccharomyces cerevisiae* from the inducible *GAL7* promoter. VP1 pseudocapsids were purified from cell lysates. Their subpopulation contained fragments of host DNA, which, in contrast to those of VP1 pseudocapsids produced in insect cells, did not assemble with cellular histones into pseudonucleocores. VP1 pseudocapsids accumulated in the yeast cell nuclei. A strong interaction of VP1 with tubulin fibres of the mitotic spindle was observed. The fibres of spindles were larger in diameter, apparently due to tight VP1 binding. Substantial growth inhibition of yeast cells producing VP1 was observed.

Key words: Polyomavirus VP1; Expression in *Saccharomyces cerevisiae*; Tubulin interaction; Mitotic spindle

1. Introduction

Mechanisms that viruses employ for the delivery of genetic information into the cell nuclei are inspiring for gene transfer techniques used in gene therapy. Many gene transfer methods have been developed using viruses or their parts. Proteins forming capsids of non-enveloped DNA viruses possess properties enabling them to internalise the viral nucleic acid, interact with cell receptors, facilitate penetration through the cell membrane, deliver information into the nucleus and mediate its release and expression.

Genomic double-stranded DNA of the non-enveloped, small mouse polyomavirus (Py) encodes, besides three early T antigens, three late structural proteins, VP1, VP2 and VP3. The late proteins, together with viral DNA and cellular histones (except H1), are assembled in the host cell nuclei into virions. VP1, a multifunctional major structural protein, is able to assemble itself into capsid-like structures (VP1 pseudocapsids), consisting of 72 VP1-pentameric capsomeres [1–3]. VP1 is also responsible for the attachment of the virions to the surface of host cells through the stereospecific recognition of sialic acid [4]. The identity of the receptor protein(s) remains to be elucidated. Another property of VP1 is the ability to bind DNA non-specifically through its N-terminal sequences [5,6]. The VP1 presence in the viral nucleoprotein core also suggests its role(s) in nucleocore formation. The functions of minor structural proteins VP2 and VP3 remain unclear. Their position in the polyoma virions was suggested based on X-ray and protein–protein interaction [7]. They form a bridge between the nucleocore and the VP1 capsid shell, interacting via their identical C-terminal parts (amino acids 140–181 of the VP3 sequence) with the axial cavities of the VP1 capsomeres [8].

We described previously that pseudocapsids, self-assembled from only the VP1 protein produced by recombinant baculoviruses in insect cells, can interact with heterologous DNA in vitro and deliver it to be expressed into the nuclei of mammalian cells in cell culture or into the cell nuclei of various tissues, when injected subcutaneously into nude mice [9,10].

Analysis of VP1 pseudocapsids produced in insect cells revealed that variable amounts of pseudocapsids contained a nucleoprotein core formed by VP1, cellular histones and host cell or baculovirus linear DNA fragments of a length comparable with polyomavirus genomic DNA [11,12]. The presence of host DNA in pseudocapsid preparations represents a handicap for the possible utilisation of the polyoma capsid-based system for the transfer of genetic information into cells. On the other hand, the finding that pseudocapsids lacking both minor antigens VP2 and VP3 can encapsidate inside the cell and protect heterologous DNA of the polyomavirus genome size (whereas only about 2.5 kb of 'naked' DNA can be packed in vitro [13]) is promising for further exploration of the DNA encapsidation system.

For the prospective development of a gene delivery system based on VP1 capsid-like particles, the mechanism of virion assembly, the interactions of virions and VP1 pseudocapsids with cellular structures, as well as the consequences of such interactions for both target cells and delivered viral particles, should be better understood. To get more information about the roles of viral VP1 and cellular structures in nucleocore assembly and about interactions of VP1 inside the cell, we decided to express the VP1 gene in yeast. Because the yeast genome has been completely sequenced and many well-defined mutants are available, we felt that the yeast cell might be a useful model system for this purpose. Here, we present the analysis of VP1 structures formed in yeast cells, their cellular localisation and effects of VP1 synthesis on yeast cells.

2. Materials and methods

2.1. Plasmids

The yeast shuttle vector pWYG7L with the *Saccharomyces cerevisiae GAL7* inducible promoter was kindly provided by M.A. Romanos [14].

Baculovirus transfer plasmid VL1393-VP1 for expression of the polyomavirus VP1 gene was constructed previously [15].

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2.2. Construction of the recombinant pWYG7L-VP1 plasmid

The recombinant pWYG7L-VP1 plasmid was constructed by ligation of the *Bam*HI–*Not*I fragment containing the VP1 gene, cut from the plasmid VL1393-VP1, and yeast pWYG7L shuttle vector cut with *Bam*HI and *Not*I.

2.3. Bacteria

Escherichia coli DH5α (*supE44*, Δ*lac U169 (80 lacZ* (M15)), *hsdR17, recA1, endA1, gyrA96, thi-1, relA1* [16]) was used for propagation of plasmids.

2.4. Yeast cells

S. cerevisiae GRF18 (*his3-11,15, leu2-3,112*) was obtained from the collection of yeast cultures of the Department of Genetics and Microbiology, Charles University, Prague.

Cultivation of the cells was performed in liquid minimal medium (0.1% KH_2PO_4, 0.5% $(NH_4)_2SO_4$, 0.05% $MgSO_4$, 0.1% Wickerham's vitamin solution [17]) supplemented with 50 mg/l histidine and 2% glucose (MMHGlc) on a reciprocal shaker at 28°C. For yeast growth on solid medium, agar was added into the medium to a final concentration of 2%.

Induction of expression from the *GAL7* promoter was achieved by the transfer of yeast cells into the MMH medium containing 2% galactose instead of glucose (MMHGal).

Transformation of intact yeast cells was performed using the lithium acetate method according to Gietz et al. [18].

2.5. Preparation of yeast cell lysates

The induced yeast cell culture (500 ml) was centrifuged, the cell sediment transferred into a stirring dish and the cells disrupted by stirring in liquid nitrogen. Then, buffer B (150 mM NaCl, 10 mM Tris–HCl pH 7.4, 0.01 mM $CaCl_2$) was added (approximately 3 ml) and the mixture was stirred again into a snow-like consistency. After subsequent thawing, the lysate was transferred into a tube and cell debris removed by centrifugation.

2.6. Insect cells and baculovirus

Spodoptera frugiperda (Sf9) cells (ATCC CRL 1711) were grown as monolayer cultures at 27°C in TNF-FH medium containing 10% foetal calf serum (FCS) as described by Hink [19]. Recombinant baculovirus containing the polyomavirus VP1 gene was used for infection of Sf9 cells [15]. Infected cells were harvested 72 h post infection. Cell lysates were prepared as described previously [9].

2.7. Isolation of polyomavirus VP1 pseudocapsids from insect and yeast cell lysates

VP1 capsid-like particles were isolated from cell lysates by pelleting assembled VP1 structures through a 10% sucrose cushion (3 h, 35 000 rpm, Beckman, SW41 rotor). The pellets were resuspended in buffer B (150 mM NaCl, 10 mM Tris–HCl pH 7.4, 0.01 mM $CaCl_2$) and separated by CsCl and sucrose gradient centrifugations as described previously [9]. CsCl gradient fractions containing particles were concentrated by pelleting through a 10% sucrose cushion and resuspended in buffer B.

2.8. Isolation of DNA from the pseudocapsids

VP1 pseudocapsids were incubated (2 h at 37°C) with pronase E (1 mg/ml) under conditions in which the capsids disassemble into capsomeres and the viral nucleocore dissociates (100 mM EDTA pH 8.0, 20 mM DTT, 1% SDS). Released DNA was purified by phenol chloroform extraction, precipitated with three volumes of ethanol and dissolved in TE buffer (10 mM Tris pH 8.0, 1 mM EDTA).

2.9. Southern blot hybridisation

Restriction enzyme digests of total cellular DNA (10 μg) were separated on 0.8% agarose gel, blotted and hybridised with ^{32}P-labelled DNA isolated from 'heavy' VP1 particles by standard procedures [16].

2.10. Protein analysis

2.10.1. Electrophoresis. Proteins of isolated pseudocapsids were resolved on SDS–PAGE gel (linear gradient of polyacrylamide 5–20%) as described [16] and visualised by Coomassie blue.

2.10.2. Western blot analysis. Proteins resolved on SDS–PAGE were electrotransferred onto a nitrocellulose filter and immunoreaction was performed with αpyVP1-A monoclonal antibody, as described [15].

2.11. Indirect immunofluorescence and DAPI staining of yeast cells

The immunofluorescence technique was performed as described [20]. Briefly, *S. cerevisiae* GRF18/pWYG7L-VP1-induced cell suspension was incubated with 30 mM EGTA pH 7.0, and pepstatin (20 μg/ml) at 28°C for 5 min. Yeast cells were fixed with paraformaldehyde and spheroplasts were prepared by digestion with zymolyase (200 μg/ml) in KPC (33 mM citric acid, 0.13 M K_2HPO_4 pH 5.9) and pepstatin (20 μg/ml) for 60 min. Cells were washed twice in PEMI (0.1 M PIPES, 5 mM EGTA, 5 mM $MgCl_2$ pH 6.9, and pepstatin (10 μg/ml)). Cell membranes were permeabilised with 1% Triton X-100 in PEMI. After washing in PEMI and preincubation in 1% bovine serum albumin in PEMI for 10 min, mouse monoclonal antibody αPyVP1 IgG1 and/or rabbit polyclonal antibody Tu 206-1 in 1% bovine serum albumin in PEMI were applied as primary antibodies for 1 h at room temperature. Cells were washed twice in PEMI and the bound antibodies were detected by 45 min incubation with anti-mouse IgG conjugated with Cy3 and/or anti-rabbit IgG conjugated with FITC (fluorescein isothiocyanate) as secondary antibodies. After the final wash in PEMI, cells were resuspended in freshly prepared mounting medium (0.1% w/v *p*-phenylenediamine in 100 mM Tris–HCl, 100 mM NaCl, 5 mM $MgCl_2$ pH 9.5, and 0.5 μg/ml of DAPI (4,6-diamidino-2-phenylindole)).

2.12. Electron microscopy (EM)

Yeast cells were prepared for electron microscopy (EM) by the modified method of Byers and Goetsch [21]. Briefly, the water-washed and pretreated (0.2 M Tris pH 9.0, 20 mM EDTA, 0.1 M 2-mercaptoethanol) cells were prefixed in cacodylate-buffered 2% glutaraldehyde, 1 mM $MgCl_2$, 1 mM $CaCl_2$ for 30 min on ice. Removal of cell walls was done with 1500 units of lyticase (Sigma) in 50 mM Tris pH 7.5, 5 mM $MgCl_2$, 1.4 M sorbitol, 0.44% 2-mercaptoethanol for 10–15 min at room temperature, and postfixation was done by the osmium tetroxide–ferrocyanide mixture [22]. Cells were prestained by 1% aqueous uranyl acetate for 30–60 min at room temperature, dehydrated in a series of increasing ethanol concentrations and embedded in Agar 100 epon resin (Gröpl, Tulln, Austria). Ultrathin sections were stained in saturated aqueous uranyl acetate for 3 min and lead citrate for 3 min. Samples were cut on a Reichert–Jung Ultracut E.

Polyomavirus VP1 pseudocapsids were visualised by negative staining. Parlodion–carbon-coated grids, activated with glow discharge [22], were floated on the top of a 5 μl drop of the sample, washed in distilled water and transferred on a drop of 2% phosphotungstic acid (pH 7.0), stained for 1 min and dried.

Photomicrographs were taken using a JEOL JEM 1200EX electron microscope operating at 60 kV.

3. Results

3.1. Production of VP1 pseudocapsids in yeast cells

Construction of the recombinant pWYG7L-VP1 plasmid in which the polyomavirus VP1 gene was placed under the control of *GAL7* inducible promoter was performed as described in Section 2. *S. cerevisiae* cells carrying the construct were selected on the MMHGlc agar medium. The first colonies of transformants appeared 8 days after the transformation. VP1 gene expression was induced by the transfer of yeast cells from glucose- to galactose-containing MMH liquid medium. To find out whether the VP1 protein is able to form capsid-like structures within the yeast cells, cells harvested from the MMHGal medium 70 h post induction were disrupted, cell lysates centrifuged through a 10% sucrose cushion and the pellet analysed for the presence of the VP1 protein by SDS–PAGE and Western blot. The predominant band on Coomassie blue-stained gel migrated with the mobility corresponding to the VP1 protein and was recognised with the anti-VP1 monoclonal antibody on Western blot (not shown). VP1 structures were isolated by CsCl gradient centrifugations of

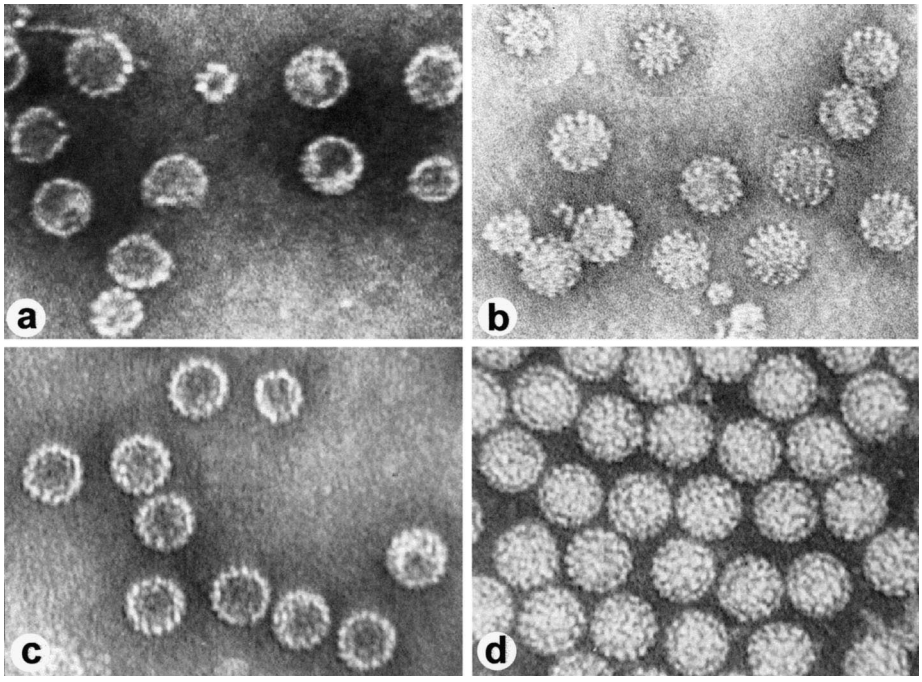

Fig. 1. VP1 pseudocapsids isolated from VP1-producing cells. EM of VP1 pseudocapsids isolated by CsCl gradient centrifugation from yeast cells (a, b) and insect cells (c, d). Particles visualised by negative staining with 2% phosphotungstic acid. Magnification: 200 000×.

the pellet obtained by centrifugation through a 10% sucrose cushion.

3.2. *Analysis of VP1 pseudocapsids isolated from yeast and comparison with those produced in insect cells*

VP1 particles isolated by CsCl gradient centrifugation from yeast cells are shown in Fig. 1a,b. VP1 pseudocapsids isolated from insect cells are given for comparison (Fig. 1c,d). Similar to VP1 particles from insect cells, at least two bands with slightly different buoyant densities, containing VP1 pseudocapsids, could be seen on the CsCl gradient. Lighter particles with $\rho = 1.290$ g/cm^3, when negatively stained for EM, appeared as circular structures with stained inner parts (Fig. 1a,c), while the heavier ones (Fig. 1b,d) were impenetrable, suggesting that this fraction of VP1 pseudocapsids might contain DNA. The nucleic acid contents of pseudocapsids were analysed on agarose gel. Full intact particles isolated from both yeast cells (Fig. 2a, lane 7) and insect cells (Fig. 2b, lanes 2, 3), when applied on the gel, gave a band strongly stained with ethidium bromide. After deproteinisation, the heaviest pseudocapsids produced by insect cells gave a band of linear DNA with mobility corresponding to the length of polyomavirus DNA, approximately 5 kb long (Fig. 2b, lane 4). The nucleic acid released from the particles produced by yeast cells migrated in distinct bands with mobilities corresponding to approximately 3, 2.3 and 1 kb (Fig. 2a, lane 2). The bands disappeared when samples were treated with DNase I (line 3) or Bal 31 exonuclease (line 5), but remained intact after RNase A treatment (line 4), as well as in the control samples incubated with digestion buffer containing Mg^{2+} cations only (line 6).

To establish the origin of DNA fragments encapsidated by pseudocapsids in the yeast cells, Southern blot analyses were performed (Fig. 3A). By hybridisation of in vitro radiolabelled DNA released from pseudocapsids with the electrophoretically separated restriction endonuclease digests of yeast chromosomal (Fig. 3A, lanes 1–5) and pWYG7L-VP1 plasmid DNAs (Fig. 3A, lanes 6–8), we observed efficient encapsidation of both chromosomal and plasmid DNAs. 'Heavy' pseudocapsids produced by insect cells (Fig. 3B) contained not only fragmented chromosomal DNA of insect cells as described by Gillock et al. [11], but also DNA of baculovirus origin (Fig. 3B, lanes 4–6). The comparison of ethidium bromide-stained electrophoregrams (Fig. 3B, a) and the hybridisation patterns (Fig. 3B, b) showed that the representation of fragments present in the pseudonucleocores of VP1 pseudocapsids produced in insect cells was not entirely random.

We were interested in whether VP1 could form 'pseudonu-

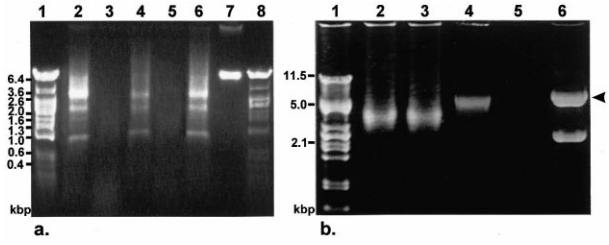

Fig. 2. Analysis of DNA fragments isolated from VP1 pseudocapsids. Agarose gel electrophoresis stained with ethidium bromide. a: VP1 pseudocapsids isolated from yeast cells: molecular weight markers (1, 8); CsCl gradient fraction of VP1 pseudocapsids with higher buoyant density ($\rho > 1300$ g/cm^3), stained with ethidium bromide (7); nucleic acids purified from VP1 pseudocapsids (2), treated with DNase I (3), with RNase A (4), with Bal 31 nuclease (5), incubated in the buffer with 10 mM Mg^{2+} for 1 h (6). b: VP1 pseudocapsids isolated from insect cells: molecular weight markers (1); 'heavy' pseudocapsids, stained with ethidium bromide (2), treated with DNase I (3); nucleic acids purified from VP1 pseudocapsids (4), treated with DNase I (5). pMJA2 plasmid, containing the complete polyomavirus genome, cut with *Eco*RI (6). The band of the linearised polyomavirus genome is indicated.

cleocores' with yeast DNA and yeast histones and/or other DNA binding proteins. Therefore, we analysed pseudocapsid-forming proteins on SDS–PAGE (Fig. 4a) and on Western blot (Fig. 4b). In contrast to the heaviest VP1 particles isolated from insect cells (Fig. 4, lanes 3), which contained, besides VP1, low molecular weight proteins migrating with mobilities similar to those of cellular histones, the yeast 'full' VP1 particles (lines 4) exhibited a protein pattern characteristic for the lighter forms of VP1 particles isolated from insect cells, without the presence of cellular histones (lines 5). From these results we can conclude that the majority of the analysed 'full' particles isolated from yeast cells contained linear DNA fragments, up to 3 kb long, apparently encapsidated as 'naked' DNA by empty VP1 particles.

3.3. Indirect immunofluorescence analysis of subcellular localisation of VP1 in yeast

S. cerevisiae GRF18/pWYG7L-VP1 cells were grown on the MMHGlc medium and VP1 gene expression was induced by transfer of cells into the MMHGal medium. Cells were harvested for immunofluorescence assays 16 h post induction. Mouse monoclonal antibody against VP1 (αPyVP1-A) and anti-mouse IgG conjugated with Cy3 labelled a linear structure resembling a mitotic spindle. Co-staining of cells with anti-tubulin rabbit polyclonal antibody Tu 206-1 (green), anti-VP1 antibody (red), and with DAPI (blue) which stains DNA, showed clear co-localisation of VP1 with tubulin (Fig. 5A). In some cells, new tubulin fibres arose when VP1 occupied the existing ones (Fig. 5B). In cells with a prolonged expression of VP1 (harvested 70 h post induction), accumulation of VP1 in the nuclei was observed (Fig. 5C). In addition, weak staining with the anti-VP1 antibody, spread in small inclusions in the cytoplasm, could be seen in some cells (not shown).

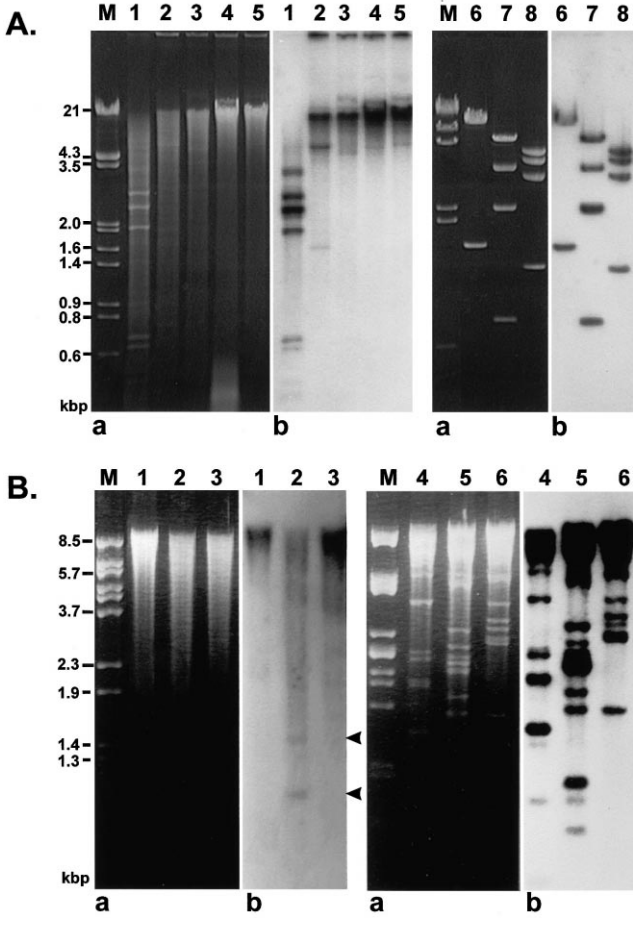

Fig. 3. Southern blot analysis of DNA fragments encapsidated by VP1 pseudocapsids in yeast (A) and insect (B) cells. a: Ethidium bromide-stained agarose gel; b: Southern blot. Hybridisation performed with in vitro ^{32}P-labelled DNA isolated from VP1 pseudocapsids produced in yeast (A) and insect (B) cells. A: Restriction fragments of yeast chromosomal DNA (1–4) and pWYGL7L-VP1 plasmid (6–8). Non-digested yeast chromosomal DNA (5); DNAs digested with *Eco*RI (2, 7), *Pst*I (3), *Xho*I (4), *Bam*HI, *Not*I (6) and *Hin*dIII (8). B: Restriction fragments of insect cell chromosomal DNA (1–3) and baculovirus DNA (4–6). DNAs digested with *Eco*RI (1, 4), *Hin*dIII (2, 5) and *Pst*I (3, 6).

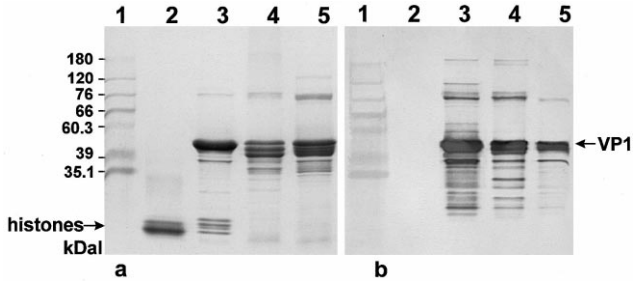

Fig. 4. The VP1 protein is not able to form 'heavy' particles containing histone-rich nucleocores in yeast cells. SDS gradient (5–20%) polyacrylamide protein electrophoresis, stained with Coomassie blue (a), and Western blot visualised with monoclonal anti-VP1 antibody (b). Molecular weight marker (1); calf thymus histones, type II-3, Sigma (2); 'heavy' VP1 particles isolated from insect cells (3); DNA containing yeast VP1 particles (4); 'empty ' VP1 particles isolated from insect cells (5).

3.4. EM of ultrathin cell sections

EM analysis of thin sections of yeast cells cultivated 70 h in the galactose medium proved predominant accumulation of VP1 pseudocapsids in the nuclei, often organised into clusters (Fig. 6). However, sporadic capsid-like particles occurred also in the cytoplasm (not shown). The linear fibre structure, resembling the mitotic spindle, was observed to be larger in diameter, measuring 45 nm instead of the reported 25 nm [23], which was very probably due to the tight VP1 binding (Figs. 7 and 8). The first assembled particles could be seen in the proximity of spindle fibres (Fig. 7). The majority of cells with the spindle structure occupied by VP1 contained only one nucleus, sometimes of a longer shape (Fig. 8c,d) and spindle structure with only one spindle pole body visible (Fig. 8a,b). This suggests, in agreement with immunofluorescence staining, that the majority of cells might be in the G1 phase. One rare example of a cell with the spindle occupied by VP1 and with a small bud is shown in Fig. 8e,f.

3.5. Growth inhibition of yeast cells expressing the VP1 protein

On the agar medium with galactose, the colonies of the strain expressing VP1 appeared with a delay of approximately 5 days in comparison with the colonies of the strain containing the plasmid without the VP1 gene. On the glucose-containing agar medium, both strains grew with similar efficiency (Fig. 9). This observation indicates that the expression of VP1 protein inhibits the growth of yeast cells. However, the fact that the cells of the colonies which appeared later on the induction medium contained the plasmid and produced VP1

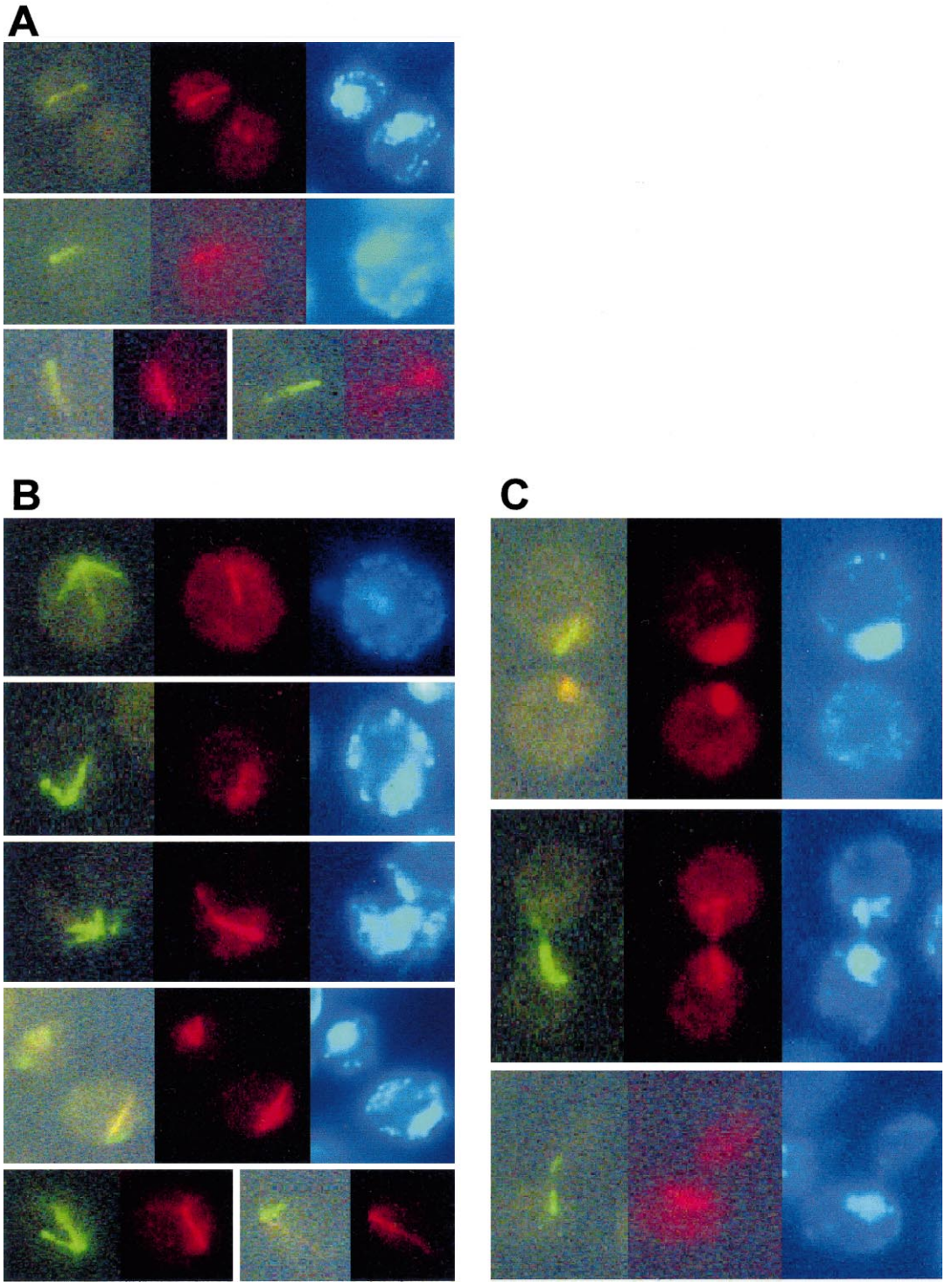

Fig. 5. VP1 co-localises in yeast cells with tubulin. Indirect immunofluorescence of yeast cells expressing VP1. Cells were co-stained with polyclonal rabbit anti-tubulin antibody and FITC-conjugated anti-rabbit IgG antibody (green), mouse monoclonal anti-VP1 antibody and Cy3-conjugated anti-mouse IgG antibody (red), and DAPI (blue). A: VP1 co-localises with tubulin and is bound to the mitotic spindle. B: After VP1 binding to the mitotic spindle, a new spindle is generated in some cells. C: VP1 accumulates in the nucleus.

implies that they are able to overcome the inhibition effect of VP1.

4. Discussion

In the present study, we addressed the following questions: (i) whether the polyomavirus VP1 molecules assemble themselves into capsid-like particles when produced in yeast cells, (ii) whether the milieu of the yeast cell gives rise to 'heavy' pseudocapsids with the pseudonucleocore composed of yeast DNA and proteins, (iii) where the VP1 structures are localised in yeast cells, and (iv) whether the presence of VP1 exhibits any effect on yeast cells.

The finding of 'yeast' VP1 pseudocapsid structures of differ-

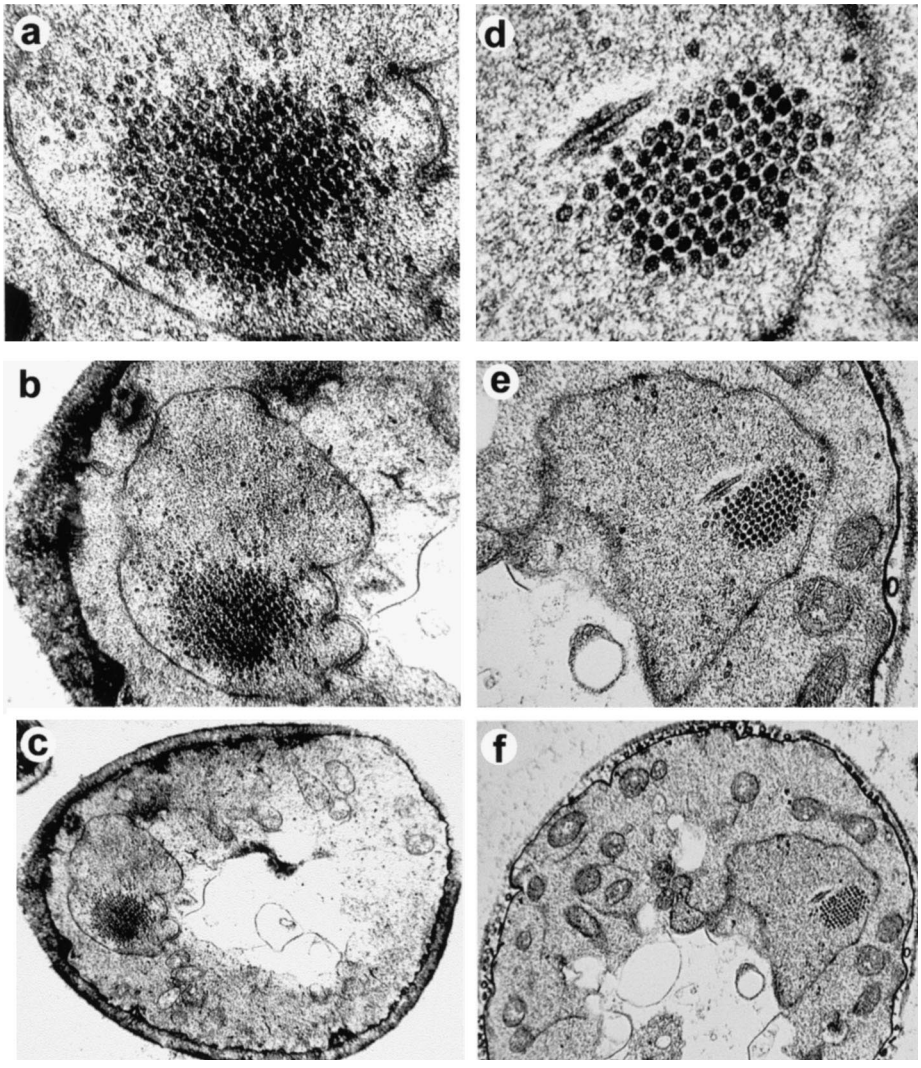

Fig. 6. VP1 pseudocapsids accumulate predominantly in the yeast cell nuclei. Electron micrographs of yeast cells producing VP1. Magnification: 41 400× (a), 24 300× (b), 11 500× (c), 60 800× (d), 25 500× (e), 13 740× (f).

ent buoyant densities and their EM analysis indicated that a subpopulation of pseudocapsids assembled in yeast cells may be packed with DNA. Gillock et al. suggested that the encapsidated DNA found in VP1 pseudocapsids produced in insect cells was generated from chromosomal DNA, degraded later post infection by baculovirus-encoded nuclease which cleaves the host cell (but not baculovirus) DNA [10]. We have found by Southern blot hybridisation (Fig. 4b) that the DNA encapsidated by pseudocapsids in insect cells can be both of baculovirus and insect cell origin. These results are in agreement with those obtained by Pawlita et al. for African green monkey B lymphotropic papovavirus [12]. The hybridisation experiment presented in Fig. 3 revealed a not entirely random choice of DNA fragments for the encapsidation. Oppenheim et al. reported that SV40 requires a specific DNA sequence for encapsidation, *ses* (SV40 encapsidation signal), including replication origin, repetitive GC boxes and part of the enhancer [24]. They proposed that encapsidation starts at the gap in nucleosomes near the replication origin and enhancer region, where viral structural proteins apparently displace cellular regulatory proteins. Cellular transcription factor Sp1 was found to interact with minor SV40 structural protein VP3, suggesting that Sp1 participates in recruiting VP3 to the SV40 minichromosome in SV40 assembly [25]. Agarose gel analysis revealed that the majority of DNA fragments isolated from 'yeast' pseudocapsids were shorter in comparison with the polyomavirus genome, forming distinct bands with mobilities corresponding to the DNA length up to 3 kb. Moreover, protein SDS electrophoresis of the pseudocapsids containing yeast DNA showed the absence of cellular histones (Fig. 3). Preparations of virions from mouse cells infected with polyomavirus (and preparations of VP1 pseudocapsids from insect cells) also contained subpopulations of particles that harboured fragments of host chromosomal DNA with the average length around 2.5 kb [26]. This length corresponds to the length of fragments which can be encapsidated in vitro as 'naked' DNA [13]. Yeast cells thus, in contrast to insect cells, do not enable the assembly of VP1 with host cell DNA and histones into nucleocore-containing pseudocapsids. The specificity of the yeast chromatin structures might represent a problem for nucleocore formation in yeast cells. It has been reported that SV40 VP1 is involved in spacing of nucleosomes in viral minichromosomes during nucleocore assembly. Nucleocore condensation is connected with removal of the H1 his-

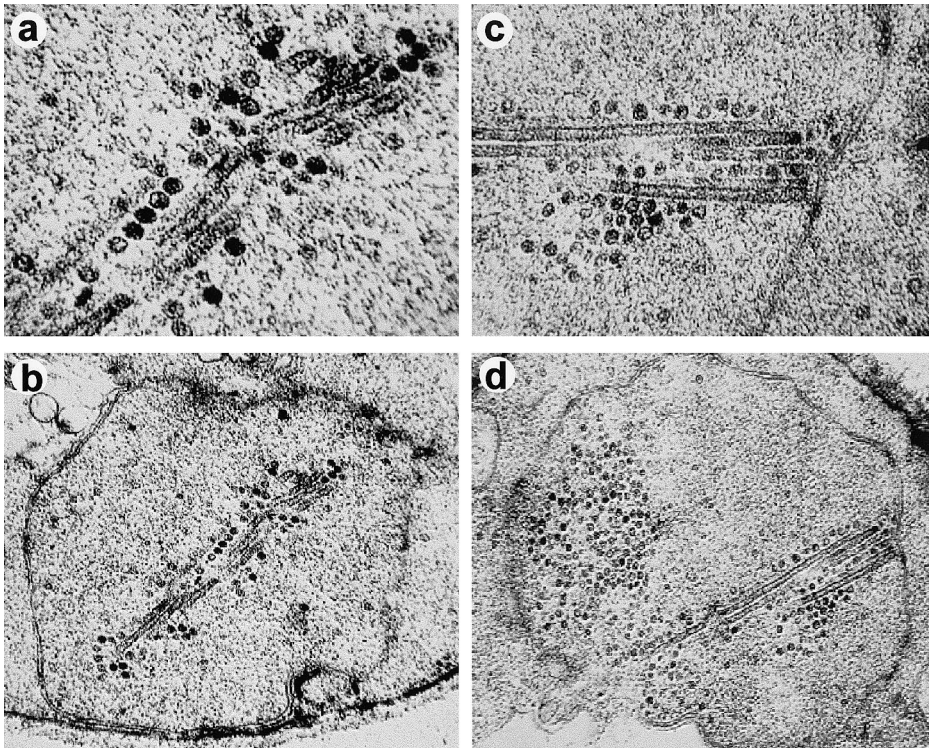

Fig. 7. Assembled particles appear first around the linear fibre structure of the mitotic spindle. Electron micrographs of yeast cells producing VP1. Magnification: 70 500× (a), 30 400× (b), 51 700× (c), 28 000× (d).

tone from an intracellular intermediate of viral chromatin [27]. While in higher eukaryotic cells the H1 histone associates with the linker region of chromatin and seems to be involved in the determination of nucleosome spacing, it does not play a role in nucleosome positioning in the yeast chromatin [28]. Recently, it has been found that VP1 interacts through its DE loop with the mouse cellular transcription factor and nuclear matrix binding protein YY1 [29]. This multifunctional cellular regulatory protein has been found previously to have several binding sites in polyomavirus genomic DNA [30]. YY1, which might be a good candidate for cooperation with VP1 in nucleocore assembly and virion formation, does not have significant sequence homology with any protein of *S. cerevisiae*. The assembly of VP1 pseudocapsids containing the nucleocore might also require some helper function(s) of the baculovirus. So far, we do not know whether the nucleocore-containing particles can be made in mouse cells expressing only the VP1 gene.

As we expected, VP1 pseudocapsids accumulated in high quantities in the yeast cell nuclei at later times (70 h) post induction (Fig. 6). Sporadic capsid-like particles were observed also in the cytoplasm. Although their cytoplasmic appearance in yeast cells producing VP1 was higher than in control parental cells, it cannot be excluded that the particles observed in the cytoplasm were of yeast double-stranded RNA virus origin. Virions of totiviruses, isometric particles of 39–42 nm in diameter [31], are 'hardly' distinguishable from polyomavirus pseudocapsids on ultrathin sections. Interestingly, Sasnauskas et al. described recently that capsid-like particles of hamster polyomavirus (HaPV), formed in *S. cerevisiae* by the complete VP1 protein, were located almost exclusively in the yeast cytoplasm. They observed a nuclear localisation of some capsid-like particles when they expressed N-terminally extended VP1 gene of HaPV with an improved nuclear localisation signal [32].

Our results also revealed that VP1 bound tightly to the mitotic spindle in the yeast cells and that VP1-producing cells were temporarily inhibited in growth. The cells containing a fibre structure that can be stained with both anti-VP1 and anti-tubulin antibodies (Fig. 5) were very probably arrested in the G1 phase of the cell cycle, as only one (non-dividing) nucleus and no bud formation could be seen in such cells. We found only a few cells with a stained mitotic spindle oriented between two dividing cells. One explanation might be that, in spite of abundant VP1 binding to the mitotic spindle (or to mitotic spindle-binding protein(s)), such cells passed through mitosis and finished cytokinesis. It is known that various defects in different spindle components activate the spindle checkpoint that arrests the mitotic cycle. This arrest can be transient; the yeast cells are able to overcome a spindle damage and to finish mitosis [33]. The appearance of new tubulin fibres originating from one pole body of the blocked spindle (or perhaps from a duplicated pole formed in the proximity of the old one) could represent the effort of the cells to overcome the inhibition by VP1. It corresponds to our observation that VP1-producing cells started to grow after prolonged cultivation on inducing agar medium. The cell with the spindle occupied by VP1 and with a small bud (Fig. 8e,f) could be another document of the cell escape from the growth arrest. The question remains why the newly synthesised spindle is not immediately occupied by VP1 and blocked again. One possibility might be that at the time when a new spindle is initiated, the cells contain most of the VP1 already assembled into pseudocapsids, which can be less interactive. The observation

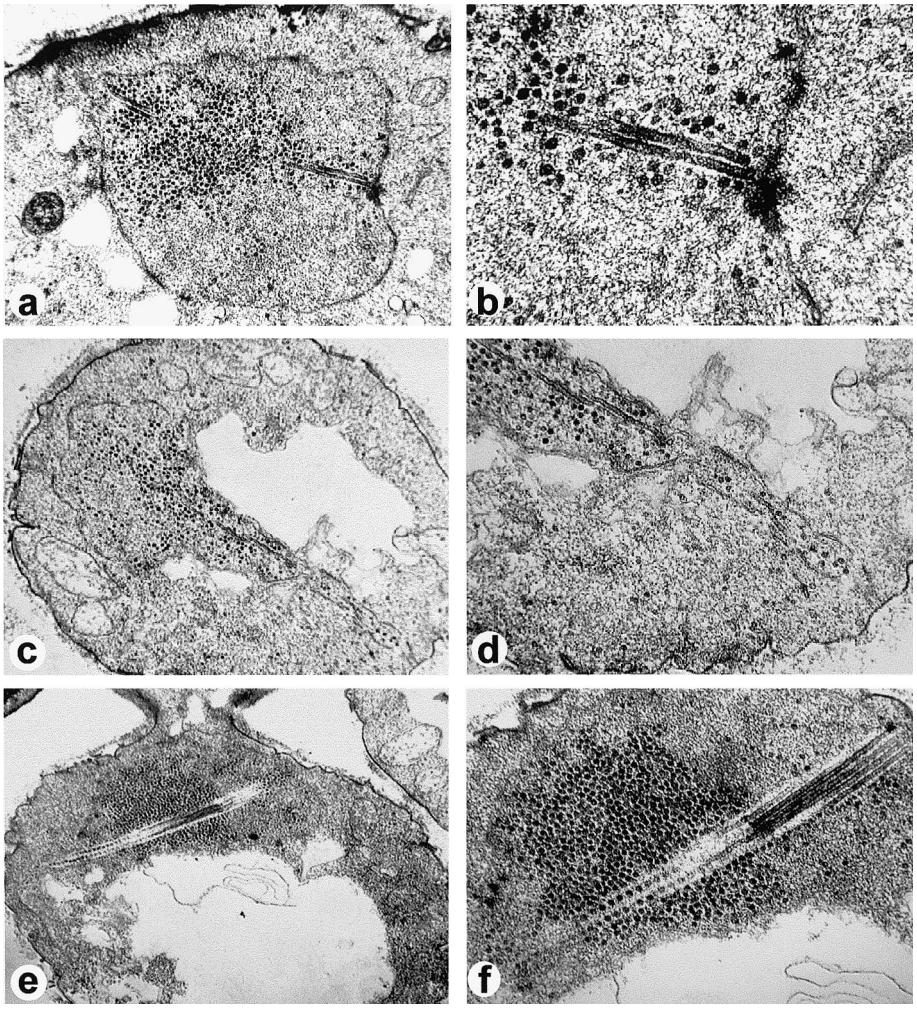

Fig. 8. Various morphologies of the yeast cells producing VP1. Cells with nuclei of longer shapes (a–d), a cell with the spindle structure and one spindle pole body (a, b), a bud with an atypical localisation with regard to the spindle (e, f). Electron micrographs. Magnification: 17 600× (a), 450 880× (b), 13 200× (c), 21 100× (d), 10 100× (e), 24 900× (f).

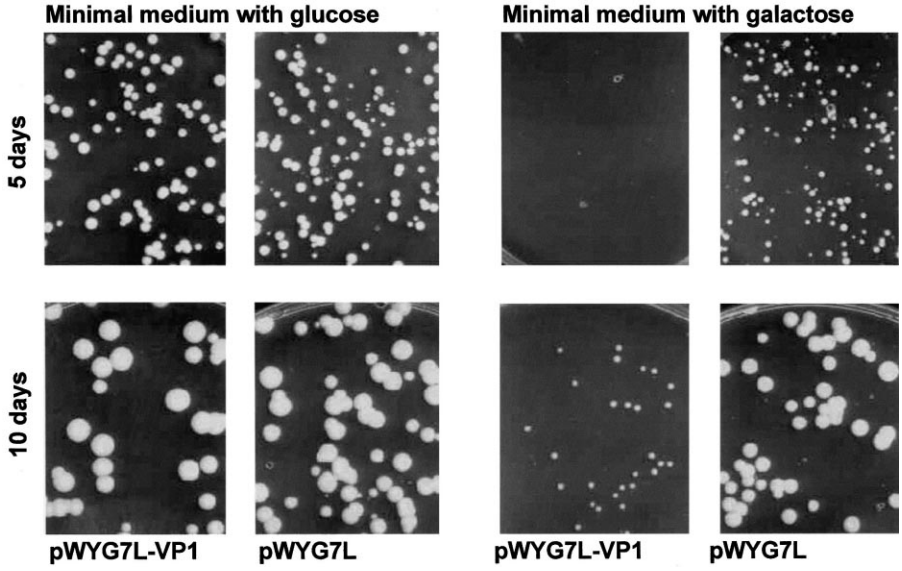

Fig. 9. Expression of the VP1 gene inhibits growth of yeast cells. Cells were plated on the agar medium with the same efficiency.

that the fibre structures found with anti-VP1 and anti-tubulin antibodies were quite long suggests that the spindle block occurs in later phases of the cell cycle, perhaps in early mitosis when 'long' spindles are formed. The binding of VP1 to the mitotic spindle may not be direct; it can be mediated by some other proteins which are present only in the M phase of the cell cycle. Another possibility is that VP1 binds only to specifically modified forms of tubulin.

The VP1 spindle binding seems not to be an artefact of the yeast cell system. Talmage et al. [34] found an association of VP1 protein with the mitotic apparatus in subpopulations of mouse cells of polyomavirus-induced tumours. Some metaphase cells were stained with anti-VP1 antibody in centrosomal areas and some cells were stained both in centrosomal areas and along the spindle. In late anaphase or early telophase, VP1 co-localised with spindle fibres in the areas between the two sets of chromosomes and also between the chromosomes and centrosomes. It was suggested that such VP1-producing cells may be important in the late development of aneuploidy in tumours [34]. Our results suggest that newly synthesised, non-assembled VP1, rather than capsid-like particles, interacts with spindle fibre structures and affects yeast cell growth. Studies on the mechanisms of growth inhibition of yeast and mammalian cells by VP1 as well as analyses of the affection of target mammalian cells by incoming VP1 pseudocapsids and by disassembled VP1 after the uncoating process are under way.

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