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Synthesis of mumps virus nucleocapsid protein in yeast *Pichia pastoris*

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Abstract

The expression of mumps virus nucleocapsid protein in yeast *Pichia pastoris* was investigated. Viral nucleocapsid proteins usually elicit a strong long-term humoral immune response in patients and experimental animals. Therefore, the detection of antibodies specific to mumps virus nucleoprotein can play an important role in immunoassays for mumps diagnosis. For producing a high-level of recombinant mumps virus nucleoprotein the expression system of yeast *P. pastoris* was employed. The recombinant nucleocapsid protein was purified by cesium chloride ultracentrifugation of yeast lysates. Electron microscopy of the purified recombinant nucleocapsid protein revealed a herring-bone structure similar to the one discovered in mammalian cells infected with mumps virus. The yield of purified nucleocapsid-like particles from *P. pastoris* constituted 2.1 mg per 1 g of wet biomass and was considerably higher in comparison to the other expression systems.

Keywords: Mumps; Nucleocapsid-like particles; *Pichia pastoris*

1. Introduction

Mumps virus contains negative sense single stranded RNA and belongs to the subfamily *Paramyxoviridae*. The virus contains six major structural proteins. Nucleocapsid protein (NP), phosphoprotein (P), matrix (M), small hydrophobic (SH), two surface glycoproteins, hemagglutinin-neuraminidase (HN) and fusion (F) protein and a small amount of large (L) protein associated with NP are among them. NP appears to be in charge of viral genome packaging and formation of replication complexes along with other proteins. Viral nucleocapsid proteins usually elicit a strong long-term humoral immune response in patients

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and experimental animals. Therefore, mumps diagnosis can be based upon the detection of antibodies specific to mumps virus NP (Carbon and Wolinsky, 2001).

Due to the advance in gene expression technology, production of recombinant viral proteins in both eukaryotic and prokaryotic host systems has become easier and more efficient. The ease of genetic modification, the yield of recombinant proteins and the maintenance of post-translation modification often determine the choice of host systems. Yeast are unicellular eukaryotic microorganisms capable to maintain eukaryotic processing steps after the expression of polypeptides and are easy for gene manipulation. Since yeast are eukaryotes their intracellular environment is more suitable for the correct folding of eukaryotic virus proteins (Meyhack et al., 1989; Romanos et al., 1992; Faber et al., 1995). Data on homologous and heterologous virus-like particles formation in yeast continues to increase (Valenzuela et al., 1982; McAleer et al., 1984; Miyanohara et al., 1986; Adams et al., 1987; Cregg et al., 1987; Jacobs et al., 1989; Janowitz et al., 1991; Hofmann et al., 1996; Sasnauskas et al., 1999; Roth, 2000; Palkova et al., 2000; Gedvilaite et al., 2000; Samuel et al., 2002; Hale et al., 2002). Consequently, the yeast recombinant vaccine for human hepatitis B virus was licensed in 1986 and represented the world's first vaccine with an antigen prepared by genetic engineering (for review: McMahon and Wainwright, 1993). Our aim was to construct an efficient expression system in yeast for generating mumps virus NP as nucleocapsid-like particles (NLPs) for mumps serology. In this paper we describe the expression of mumps virus NP in *Pichia pastoris* and provide data on its structure and immunogenicity analysis.

2. Materials and methods

2.1. Strains, culture media and transformation

Bacterial recombinants were screened in *E. coli* DH5αF′ cells. The strain *P. pastoris* GS115 *his4* (Invitrogen, Groningen, Netherlands) was used in experiments. Transformation of *P. pastoris* GS115 *his4* was performed by electroporation (Bio Rad, Gene Pulser) according to Cregg and Russell (1998). *P. pastoris* His^+ transformants were selected on a minimal agar medium (1.34% YNB, 1% glycerol). High copy number transformants were selected on YEPD-agar plates containing 0.5–1.5 mg ml^{-1} antibiotics G418 (Amresco, Solon, USA). The copy number of inserted expression cassettes was determined according to Romanos et al. (1998).

2.2. Cloning of mumps virus NP gene into yeast vectors

All DNA manipulations were performed according to standard procedures (Sambrook and Russell, 2001). Enzymes and kits for DNA manipulations were purchased from Fermentas UAB (Vilnius, Lithuania). The mumps virus NP gene was amplified by RT-PCR from the wild-type mumps virus Gloucester strain isolated in the UK (GeneBank Accession No. AF280799). Primer sequences include: forward 5′-ATA **TCT AGA** ATA ATG TCG TCC GTG CTC AAA G-3′; reverse 5′- ATA **TCT AGA** TTA CTC ATC CCA GTC GCC CA-3′. The primers used for amplification incorporated the *Xba*I site (indicated in bold) for subcloning into the yeast vector. After amplification the mumps virus NP coding sequence was inserted into pCR2.1-TOPO vector (Invitrogen), sequenced and used for cloning into the *Avr*II site of *P. pastoris* vector pPIC3.5K (Invitrogen) under control of methanol inducible *AOX1* promoter. The resulting plasmid pPIC3.5-NP was linearized and used for electroporation into *P. pastoris* GS115 (Cregg and Russell, 1998).

2.3. Expression and purification of nucleocapsid-like particles from yeast

Selected *P. pastoris* GS115 [pPIC3.5-NP] transformants, containing approximately 10 integrated copies of mumps virus NP gene under control of *AOX1* promoter and transcription terminator, were inoculated into 250 ml of BMG medium (100 mM potassium phosphate, pH 6, 1.34% YNB, 4×10^{-5}% biotin, 1% glycerol) in 2.5 l flask and grown at 28 °C with shaking at 250 rpm. The

induction of *P. pastoris* was performed under Mut^+ conditions essentially as suggested by the manufacturers, except for the cells, that were resuspended in BMM medium (100 mM potassium phosphate, pH 6, 1.34% YNB, 4×10^{-5}% biotin, 1% methanol) to an absorbance ($A_{600\ nm}$) of 2 and the culture was supplemented with methanol at 6 h intervals. After the induction for 48 h the cells were harvested and stored at −70 °C (Cregg et al., 1987).

The NP was purified by successive ultracentrifugation of yeast lysates through 30% sucrose cushion (100 000 × *g*, 3 h) followed by ultracentrifugation in CsCl gradient ranging from 1.23 to 1.38 g ml^{-1} (36 h, 100 000 × *g*) as described (Samuel et al., 2002). Fractions containing proteins were identified by SDS-PAGE and Western blots. The buoyant density of the fractions was determined with a refractometer.

2.4. SDS-PAGE and Western blotting

Samples were boiled in a reducing sample buffer and gel electrophoresis was run in SDS–Tris–glycine buffer pH 8.8. Proteins were stained with Coomassie brilliant blue. After SDS-PAGE electrophoresis proteins were transferred to nitrocellulose membrane Hybond™ ECL (Amersham, UK) as described in Sambrook and Russell (2001). The blots were blocked with 5% nonfat milk in PBS for 2 h. The blocking solution was removed and the blots were incubated with anti-mumps NP monoclonal antibodies 5H7 (Samuel et al., 2002). For the detection of specific antibody-binding horseradish peroxidase (HRP)-labeled anti-mouse IgG conjugates (Dako, UK) were used. The blots were stained with TMB substrate (Fermentas UAB).

2.5. Electron microscopy

The suspension of NP particles was placed on 400-mesh carbon coated palladium grids. The samples were stained with 2% aqueous uranyl acetate solution and examined with JEM-100S electron microscope.

2.6. Immunogenicity test

For the immunogenicity test groups of inbred mice BALB/c (H-2d), CBA/6 (H-2k) and C57BL/2 (H-2b) were given a single subcutaneous injection of 50 μg NP either dissolved in PBS ($n = 5$), or emulsified in the complete Freund's adjuvant ($n = 2$). On the day 22nd after the first immunization mice were subcutaneously boosted with 30 μg NP in PBS. To evaluate specific antibody response in immunized mice blood samples from a tail vein were collected before immunization and on days 14, 22, 35 and 60 after primary immunization. ELISA test was performed in 96-well microtiter plates coated with recombinant mumps NP. Plates were developed using peroxidase-labeled secondary antibody against mouse IgG (Amersham) and *o*-phenylenediamine substrate.

3. Results and discussion

3.1. Synthesis of mumps virus NP protein in yeast P. pastoris

In our studies we demonstrated an efficient formation of mumps virus NP NLPs in yeast *P. pastoris*. We used Invitrogen expression system available commercially for NP expression. Mumps virus NP gene was inserted in the plasmid pPIC3.5K under control of methanol inducible *AOX1* gene promoter, resulting the plasmid pPIC3.5-NP. SDS-PAGE analysis of crude lysates of *P. pastoris* containing a high copy number of integrated pPIC3.5-NP (∼ 10 copies) after growth in methanol medium, revealed an additional protein band corresponding to the molecular weight of NP (66 kDa) (Fig. 1A, lane 2). No bands of such molecular mass were observed in crude lysates of *P. pastoris*, harboring integrated vector pPIC3.5K (Fig. 1A, lane 1).

Immunostaining with MAb 5H7 specific for mumps virus NP confirmed, that the additional protein band of 66 kDa present in yeast lysates after induction corresponded to NP protein (Fig. 1B, lane 2). The molecular mass of NP, which was estimated from the nucleic acid sequence (549 amino acids) appeared to be in the region of 61.3

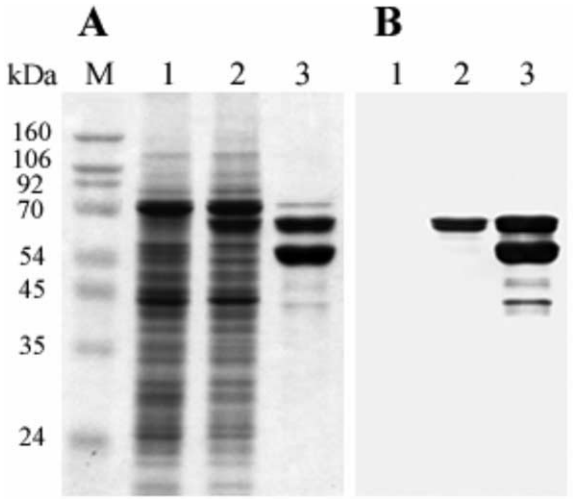

Fig. 1. SDS-PAGE (A) and Western blot (B) analysis of yeast lysates and CsCl-ultracentrifugation of purified yeast expressed mumps NP. 5 μg of purified NP from *P. pastoris* (Lane 3) or 20 μg of yeast lysates (Lanes 1, 2) were separated in a 12% SDS-PAGE and stained with Coomassie brilliant blue. Separated proteins were analyzed by Western blot using monoclonal antibodies 5H7 against mumps NP. Lanes 1, *P. pastoris* [pPIC3.5K] lysate; Lanes 2, *P. pastoris* [pPIC3.5-NP]; Lanes 3, CsCl purified mumps NP from *P. pastoris*; M, Prestained protein molecular mass markers (Fermentas UAB, Vilnius, Lithuania) 160, 106, 92, 70, 54, 45, 35 and 24 kDa.

kDa (Tanabayashi et al., 1990). Similar discrepancy between estimated and identified molecular weight was described for mumps virus NP derived from the other expression systems including the in vitro transcription systems and for NP protein isolated from the infected mammalian cells (McCarty and Johnson, 1980; Tanabayashi et al., 1990).

3.2. Purification of yeast derived mumps virus NP in CsCl gradient

To investigate the capacity of the expressed NP derivatives to self-assemble in yeast *P. pastoris*, the cells were disrupted with glass beads and analyzed after centrifugation through 30% sucrose cushion. The NP was found in pellets (data not shown), which confirmed its large multimeric organization. Further ultracentrifugation of the resolubilised pellet in CsCl gradient and fraction analysis revealed buoyant density of 1.29–1.31 g ml^{-1} common for most VLPs or NLPs (Thorne and Dermott, 1976; Robbins et al., 1980). Fractions of this buoyant density contained highly purified NP with minor contaminants as it was revealed by SDS-PAGE analysis. CsCl purified protein constituted the band of 66 kDa (Fig. 1A and B, lanes 3), which corresponded to the band identified in crude lysates (Fig. 1A and B, lanes 2). However, partial degradation of NP was observed during purification. The predominant protein band derived from crude lysates corresponded to 66 kDa alongside the minor band of 52 kDa (Fig. 1A and B, lanes 2). In contrast, the major band after purification corresponded to 52 kDa (Fig. 1A and B, lanes 3). Degradation of mumps virus NP during purification from mumps virus infected cells was described previously, and two additional polypeptides of 52 and 45 kDa, respectively, were observed (Orvel, 1978; McCarty and Johnson, 1980). Such bands of low molecular mass were clearly visible in Western blot also (Fig. 1B, lane 3).

Comparison of cell lysates after induction and CsCl purified preparation by SDS-PAGE and Western blot suggest, that purification of NP results in its degradation (Fig. 1, lane 3). However, this degradation did not influence self-assembly of NP in yeast cells. Furthermore, SDS-PAGE analysis showed evidence of a minute protein band (ca. 12 kDa), confirming proteolysis of NP after NLPs formation (data not shown). Possibly both fractions of NP exist within yeast derived NLPs and are maintained by intra- and inter-subunit interactions.

3.3. Electron microscopy

The EM examination of NP purified in CsCl gradient or even obtained after concentration through sucrose cushion revealed large quantities of tubular structures, which displayed some features common for mumps virus nucleocapsids (Fig. 2). They appeared as flexible rods of 18–20 nm in diameter with repeated serrations along edges and the central core approximately 5 nm in diameter. The average length of rods ranged in size and depended on purification procedures. Such structures were detected in mumps virus infected

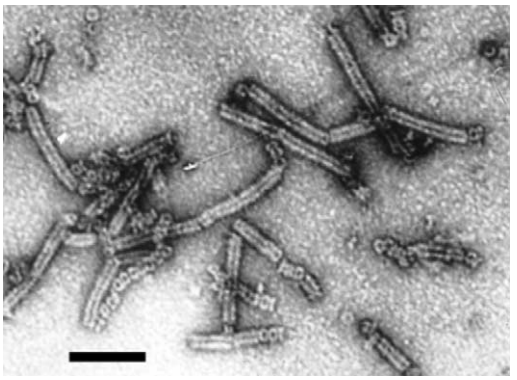

Fig. 2. Detection of yeast *P. pastoris* expressed NP-derived NLPs by negative stain electron microscopy. Bar 100 nm.

cell cultures, mammalian, insect and yeast *Saccharomyces cerevisiae* expression systems (Carbon and Wolinsky, 2001; Samuel et al., 2002). Formation of NLPs in yeast expression systems occurs in the absence of other viral proteins and the morphology of particles is similar to the one observed in mumps virus infected mammalian cells (Carbon and Wolinsky, 2001). If proteins in a host cell are involved in formation of NLPs, they could appear to be conserved both in yeast and mammalian cells. A wide distribution of length for the rods was found both in naturally occurring infection and recombinant yeast cells.

3.4. Yield and stability of NP particles

Several preparative CsCl gradient procedures yield approximately 2.1 mg per 1 g of wet biomass of purified NP from *P. pastoris* with a high copy number insertion of the NP gene and considerably surpass the yield of this protein obtained from the other expression systems (Samuel et al., 2002). NP particles were stable in solution of CsCl at 4 °C. Treatment with 10 mM EDTA, 10 mM EGTA or 5 mM DTT did not cause dissociation of NP particles, thus indicating that the assembled structure did not require the presence of divalent ions or disulfide bonds. Dialyzed samples were stored either at −20 °C in PBS containing 1 mM EDTA and 40% glycerol or lyophilized. The structure of recombinant mumps NLPs is retained after lyophilization.

3.5. Immunogenicity of NP particles

Immunogenicity of recombinant mumps NP was investigated by evaluation of humoral immune response according to the long-term immunization scheme of different mice strains. Mice were immunized twice with or without the adjuvant stimulation. Specific antibody response to recombinant mumps NP was determined by ELISA in blood samples collected from the immunized mice at regular intervals. A significant level of specific anti-NP antibodies in blood samples was observed after a single injection of recombinant mumps NP. Boost immunization on day 22 resulted in an increase of NP-specific IgG response (Fig. 3). This indicates the occurrence of affinity maturation of specific antibodies and the switch of IgM to IgG. Although the adjuvant usage induced higher titers of NP-specific antibodies immunization with NP without the adjuvant also provided long-term humoral immune response. The comparison of capability to produce antibodies to NP in different mice strains revealed no significant differences.

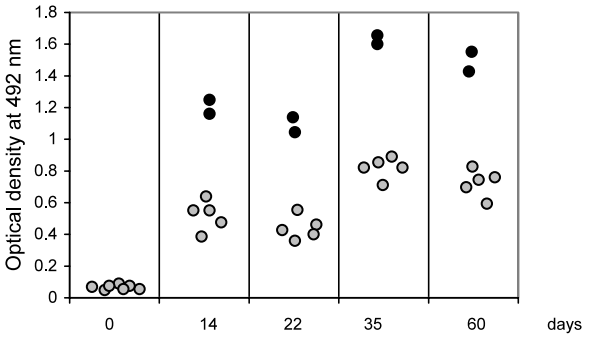

Fig. 3. Development of specific antibody response in mice BALB/c immunized with yeast-derived mumps NP. Mice were immunized subcutaneously with 50 μg NP either in PBS or emulsified in the complete Freund's adjuvant (FCA) and boosted on day 22 with 30 μg of NP in PBS. Blood samples were collected before immunization and on day 14, 22, 35 and 60 after primary immunization. The level of NP-specific IgG antibodies was determined by ELISA indirectly in blood samples diluted 1:400. Black circles represent mice immunized with NP/FCA ($n = 2$); grey circles represent mice immunized with NP without the adjuvant ($n = 5$).

3.6. Conclusions

Expression of virus surface and nucleocapsid proteins in the absence of other viral gene products in animal host maintains the intrinsic capacity to self-assemble into VLPs (Ulrich et al., 1998; Pumpens and Grens, 1999). We aimed to develop an efficient expression system suitable for generation of mumps virus NLPs and verify the formation of NLPs in yeast from mumps virus NP without the involvement of other gene products of mumps virus.

The yeast system offers many advantages, which should be of relevance for synthesis of NLPs. The majority of recombinant proteins produced in yeast are expressed via *S. cerevisiae* as a host system (Romanos et al., 1992). Nowadays molecular biologists are familiar with this organism, its genetics and physiology. However, there are certain limitations for heterologous protein expression in yeast *S. cerevisiae* as a host. For example, the yield of product usually is not high with the exception for some products. Methylotrophic yeast are very attractive as host for expression because of the strong, tightly regulated promoter elements. Methylotrophic yeast expression systems involving *AOX* gene regulatory elements have been characterized as the most efficient among yeast. Moreover, the advanced fermentation technology has been developed for the production of yeast on methanol media. The methylotrophic yeast are now commercially exploited for the production of foreign proteins (Buckholz and Gleeson, 1991). However, only few examples exist relating VLPs formation in methylotrophic yeast (Cregg et al., 1987; Janowitz et al., 1991; Gellissen et al., 1995).

In this study we have demonstrated, that yeast *P. pastoris* is an excellent host for a high-level production of mumps virus NLPs. Independently of co-stimulation with the adjuvant yeast-derived mumps NLPs possess high immunogenicity and elicit long-term immune response in experimental animals. Mumps virus NLPs described above represent useful tools for the development of new virus detection systems and exemplify the effectiveness of yeast *P. pastoris* as a host for generation of recombinant proteins organized in such complex structures like human virus NLPs.

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References

Adams, S.E., Mellor, J., Gull, K., Sim, R.B., Tuite, M.F., Kingsman, S.M., Kingsman, A.J., 1987. The functions and relationships of Ty-VLP proteins in yeast reflect those of mammalian retrovirus proteins. Cell 49, 111–119.

Buckholz, R.G., Gleeson, M.A.G., 1991. Yeast systems for the commercial production of heterologous proteins. Biotechnology (New York) 9, 1067–1072.

Carbon, K.M., Wolinsky, S., 2001. Mumps virus. In: Knipe, D.M., Howley, P.M. (Eds.), Fields Virology, fourth ed. Lippincott, Williams & Wilkins, Philadelphia, pp. 1381–1400.

Cregg, J.M., Russell, K.A., 1998. Transformation. In: Higgins, D.R., Cregg, J.M. (Eds.), Pichia Protocols. Methods in Molecular Biology, vol. 103. Humana Press, Totowa, NJ, pp. 27–39.

Cregg, J.M., Tschop, J.F., Sillman, C., Siegel, R., Akong, M., Craig, W.S., Buckholtz, R.G., Madden, K.R., Kellaris, P.A., Davis, G.R., Smiley, B.L., Cruze, J., Torregrossa, R., Velicelebi, G., Thill, G.P., 1987. High-level expression and efficient assembly of hepatitis B surface antigen in the methylotrophic yeast, *Pichia pastoris*. Biotechnology (New York) 5, 479–485.

Gedvilaite, A., Frömmel, C., Sasnauskas, K., Micheel, B., Özel, M., Behrsing, O., Staniulis, J., Jandrig, B., Scherneck, S., Ulrich, R., 2000. Formation of immunogenic virus-like particles by inserting epitopes into surface-exposed regions of hamster polyomavirus major capsid protein. Virology 273, 21–35.

Gellissen, G., Hollenberg, C.P., Janowicz, Z.A., 1995. Gene expression in methylotrophic yeast. Bioprocess Technol. 22, 195–239.

Faber, K.N., Harder, W., Geert, A.B., Veenhuis, M., 1995. Review: methylotrophic yeast as factories for the production of foreign proteins. Yeast 11, 1331–1344.

Hale, A.D., Bartkeviciute, D., Dargeviciute, A., Jin, L., Knowles, W., Staniulis, J., Brown, D.W.G., Sasnauskas, K., 2002. Expression and antigenic characterization of the major capsid proteins of human polyomaviruses BK and JC in *Saccharomyces cerevisiae*. J. Virol. Methods 104, 93–98.

Hofmann, K.J., Neeper, M.P., Marcus, H.Z., Brown, D.R., Muller, M., Jansen, K.U., 1996. Sequence conservation within the major capsid protein of human papillomavirus (HPV) type 18 and formation of HPV-18 virus-like particles in *Saccharomyces cerevisiae*. J. Gen. Virol. 77, 465–468.

Jacobs, E., Rutgers, T., Voet, O.P., Dewerchin, M., Cabezon, T., de Wilde, M., 1989. Simultaneous synthesis and assembly of various hepatitis B surface proteins in *Saccharomyces cerevisiae*. Gene 80, 279–291.

Janowitz, A.Z., Melber, K., Merckelbach, A., Jacobs, E., Harford, N., Comberbach, M., Hollenberg, C.P., 1991. Simultaneous expression of the S and L surface antigens of hepatitis B, and formation of mixed particles in the methylotrophic yeast *Hansenula polymorpha*. Yeast 7, 431–443.

McAleer, W.J., Buynak, E.B., Maigetter, R.Z., Wampler, D.E., Miller, W.J., Hilleman, M.R., 1984. Human hepatitis B vaccine from recombinant yeast. Nature 307, 178–180.

McCarty, M., Johnson, R.T., 1980. A comparison of the structural polypeptides of five strains of mumps virus. J. Gen. Virol. 46, 15–27.

McMahon, B., Wainwright, R., 1993. Protective efficacy of hepatitis B vaccines in infants, children and adults. In: Ellis, R. (Ed.), Hepatitis B Vaccine in Clinical Practice. Dekker, New York, pp. 243–261.

Meyhack, B., Hinnen, A., Heim, J., 1989. Heterologous gene expression in *Saccharomyces cerevisiae*. In: Hershberger, C.L., Queener, S.W., Hageman, G. (Eds.), Genetics and Molecular Biology of Industrial Organisms. American Society for Microbiology, Washington, pp. 311–321.

Miyanohara, A., Imamura, T., Araki, M., Sugawara, K., Otomo, N., Matsubara, K., 1986. Expression of hepatitis B virus core antigen gene in *Saccharomyces cerevisiae*: synthesis of two polypeptides translated from different initiation codons. J. Virol. 59, 176–180.

Orvel, C., 1978. Structural polypeptides of mumps virus. J. Gen. Virol. 41, 527–539.

Palkova, Z., Adamec, T., Liebl, D., Stokrova, J., Forstova, J., 2000. Production of polyomavirus structural protein in yeast cells and its interaction with cell structures. FEBS Lett. 478, 281–289.

Pumpens, P., Grens, E., 1999. Hepatitis B core particles as a universal display model: a structure-function basis for development. FEBS Lett. 442, 1–6.

Robbins, S.J., Bussel, R.H., Rap, F., 1980. Isolation and partial characterization of two forms of cytoplasmic nucleocapsids from measles virus-infected cells. J. Gen. Virol. 47, 301–310.

Romanos, M.A., Scorer, C.A., Clare, J.J., 1992. Foreign gene expression in yeast: a review. Yeast 8, 423–488.

Romanos, M., Scorer, K., Sreekrishna, K., Clare, J., 1998. The generation of multicopy recombinant strains. In: Higgins, D.R., Cregg, J.M. (Eds.), Pichia Protocols. Methods in Molecular Biology, vol. 103. Humana Press, Totowa, NJ, pp. 55–72.

Roth, J.-F., 2000. The yeast Ty virus-like particles. Yeast 16, 785–795.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning. A Laboratory Manual, third ed. CSHL Press, Cold Spring Harbor, New York.

Samuel, D., Sasnauskas, K., Jin, L., Beard, S., Zvirbliene, A., Gedvilaite, A., Cohen, B., 2002. High level expression of recombinant mumps nucleoprotein in *Saccharomyces cerevisiae* and its evaluation in mumps IgM serology. J. Med. Virol. 66, 123–130.

Sasnauskas, K., Buzaite, O., Vogel, F., Jandrig, B., Razanskas, R., Staniulis, J., Scherneck, S., Krüger, D.H., Ulrich, R., 1999. Yeast cells allow the high-level expression and formation of polyomavirus-virus like particles. Biol. Chem. 380, 381–386.

Tanabayashi, K., Takeuchi, K., Hishiyama, M., Yamada, A., Segiura, A., 1990. Nucleotide sequence of the leader and nucleocapsid protein gene of mumps virus and epitope mapping with the in vitro expressed nucleocapsid protein. Virology 177, 124–130.

Thorne, H.V., Dermott, E., 1976. Circular and elongated linear forms of measles virus nucleocapsid. Nature (London) 264, 473–474.

Ulrich, R., Nassal, M., Meisel, H., Kruger, D.H., 1998. Core particles of hepatitis B virus as carrier for foreign epitopes. Adv. Virus. Res. 50, 141–182.

Valenzuela, P., Medina, A., Rutter, W.J., Ammerer, G., Hall, B.D., 1982. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. Nature 298, 347–350.