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High Level Expression of Recombinant Mumps Nucleoprotein in *Saccharomyces Cerevisiae* and Its Evaluation In Mumps IgM Serology

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To develop improved reagents for mumps serology a high-level yeast expression system was employed to produce recombinant mumps nucleoprotein (rNP). The rNP was purified by CsCl gradient centrifugation and yielded approximately 15 mg/l of yeast culture. Electron microscopy of the rNP revealed characteristic herringbone structures. The electrophoretic mobility of rNP in yeast cells was similar to native NP in SDS-PAGE. Monoclonal antibodies to rNP reacted with native mumps virus nucleoprotein by immunofluorescence assay. A monoclonal antibody to native mumps virus NP reacted with rNP by Western blot assay. The rNP was investigated as antigen in an IgM capture enzyme immunoassay (EIA) using a horseradish peroxidase conjugate of monoclonal antibody to the rNP. Eighteen sera previously found to be positive by IgM capture radioimmunoassay (MACRIA) and 30 sera that were mumps IgM negative by MACRIA were tested by mumps IgM capture EIA. The results for the two test were concordant. In addition, 26 rheumatoid factor positive sera and 35 sera that were IgM positive for measles, rubella or parvovirus B19 were tested. Fifty-nine sera were negative by mumps IgM capture EIA but two sera collected from two infants 3 and 6 weeks after mumps, measles and rubella vaccination were positive. Mumps MACRIA confirmed these results. Compared to MACRIA the overall sensitivity was 100% (20/20) and specificity was 96.8% (30/31). The yeast expressed rNP was highly immunogenic and suitable for use in IgM capture EIA for the diagnosis of mumps. ***J. Med. Virol. 66:123–130, 2002.*** © 2002 Wiley-Liss, Inc.

KEY WORDS: mumps virus; recombinant nucleocapsid protein; electron microscopy; IgM serology

INTRODUCTION

Mumps virus is a member of the genus Rubulavirus in the family Paramyxoviridae. The virus contains six major structural proteins including nucleocapsid associated protein (NP), phosphoprotein (P), matrix (M), two surface glycoproteins namely, hemagglutinin-neuraminidase (HN) and fusion (F) protein, and a small amount of the large (L) protein associated with the NP [Wolinsky, 1996]. The presence of a membrane associated, non-structural small hydrophobic (SH) protein in mumps virus infected cells was demonstrated by Takeuchi et al. [1996].

Serological assays for mumps based on classical complement fixation tests (CFT) defined two mumps virus antigens, termed S and V [Henle et al., 1947]. Jensik and Silver [1976] demonstrated that the S and the V antigens corresponded to the NP and HN of mumps. Antibody responses to both NP and HN are generated soon after infection [Freeman and Hambling, 1980] but CFT antibody to S (NP) diminishes sooner and is somewhat a useful indication of recent infection; antibody to V (HN) usually persists for years. A complication of this is the reported cross-reactions of serum antibodies to mumps virus in subjects with a past infection with parainfluenza virus infections [Frankova et al., 1988]. Frankova et al. [1988] reported that cross-reacting antibodies were only observed using crude mumps virus antigens in an ELISA, but not when purified mumps virus NP was used. Furthermore, the use of highly purified mumps antigens allowed direct labelling with horseradish peroxidase [Gut et al., 1985; Grubhoffer et al., 1987; Frankova et al., 1988] that enabled development of a rapid IgM capture assay for mumps diagnosis. Mumps serology, however, remains

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problematic [Pipkin et al., 1999] and there is a need to make improved assays for diagnosis, surveillance, and vaccine efficacy studies.

The aim of the study was to investigate the use of a high-level yeast expression system to produce large quantities of recombinant mumps proteins for mumps serology. In this study we describe the cloning of mumps NP gene, production and characterisation of rNP expressed in *Saccharomyces cerevisiae* and present a preliminary report on its use in detecting mumps-specific IgM in human serum samples.

MATERIALS AND METHODS

Figure 1 illustrates the scheme employed for the cloning of the mumps NP gene into the yeast *S. cerevisiae* vector pFX7.

Cloning of Mumps Virus NP Gene

The mumps virus NP gene was amplified by PCR from cDNA prepared after extraction and reverse transcription of cell-culture grown wild-type mumps virus Gloucester strain, isolated in the UK [Wehner et al., 2000]. The primers used for amplification incorporated an XbaI site for subcloning into the yeast vector pFX7 and a single ATG initiation codon in the forward primer and a stop TAA codon in the reverse primer.

Primer sequences include: forward (5′–3′) ATA **TCT AGA** ATA ATG TCG TCC GTG CTC AAA G; reverse (5′–3′) ATA **TCT AGA** TTA CTC ATC CCA GTC GCC CA. The start and stop codons are underlined and the sites for restriction with XbaI are in bold.

PCR reaction used 20 μL of cDNA, 5 μL of 10 × PCR buffer (Life Technologies, UK), 1.5 μL of 50 mM $MgCl_2$, 1 μL of 10 mM dNTP, 2.5 U of Taq DNA polymerase and made up to 50 μL with water. The cycling conditions were, 95°C for 2 min, followed by 25 cycles of 95°C for 1 min, 50°C for 1.5 min and 72°C for 2 min and a final extension at 72°C for 5 min. PCR products were separated on a 2% SeaKem Agarose gel. Bands corresponding to NP were excised, gel-purified and cloned into pCR® 2.1-TOPO®, as described by the manufacturer (Invitrogen, UK). The inserted mumps NP sequence in plasmids was confirmed by PCR and DNA sequencing.

Cloning of NP Gene Into Yeast *S. cerevisiae* Vector

The yeast vector pFX7 contains a *GAL10-PYK1* hybrid promoter that consists of a −138–511 nt fragment of *GAL10* with *GAL1-10* UAS sequences, a −4–271 nt fragment of *PYK1* and *PGK1* transcription terminator sequence (388 nt). Promoter and terminator sequences were separated by a recognition site for the restriction endonuclease *Xba*I and *Bgl*II. The vector also includes a fragment of 2 μ DNA and a dominant selective marker: the *FDH1* gene of *Candida maltosa*, conferring resistance to formaldehyde [Sasnauskas et al., 1999]. The mumps NP-encoding sequence isolated from the plasmid PCR2.1-TOPO-NP was inserted into the *Xba*I site

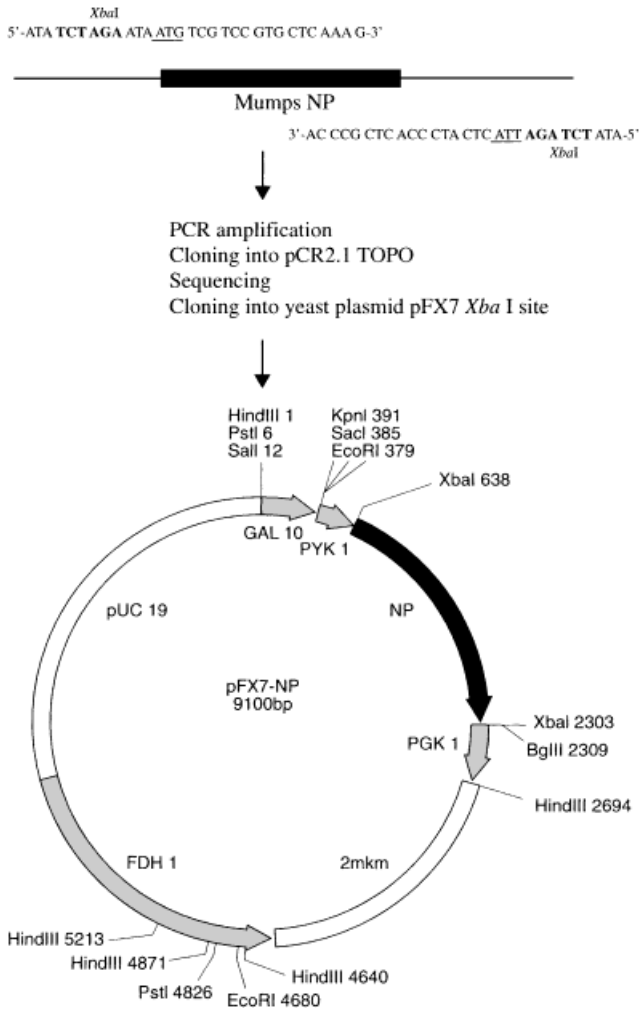

Fig. 1. Construction of the yeast expression plasmid pFX7-NP. Mumps nucleoprotein gene, NP; fragment of the *GAL 10* promoter (−511 nt to −138 nt), *GAL 10*; fragment of the *PYK1* promoter (−271 nt to −4 nt), *PYK1*; fragment of the *PGK1* transcription terminator sequence, *PGK1*; fragment of the yeast 2-μm DNA; 2 m; gene of *Candida maltosa*, conferring resistance to formaldehyde, *FDH1*; bacterial plasmid, pUC19.

of the yeast expression vector pFX7 under control of hybrid *GAL10-PYK1* promoter. The resulting plasmid pFX7-NP was used for transformation of yeast *S. cerevisiae* strain AH22 (*leu2 his4*). Yeast cells were grown in YEPD medium (yeast extract 1%, peptone 2%, and glucose 2%) supplemented with 5 mM formaldehyde or in induction medium YEPG (yeast extract 1%, peptone 2%, and galactose 3%).

Expression and Purification of Nucleocapsid-Like Particles From Yeast

YEPD medium (500 mL) supplemented with 5 mM formaldehyde was inoculated with *S. cerevisiae* cells harbouring pFX7-NP and grown with shaking at 28°C for 24 hr in 2.5 L flask. The cells were harvested and reinoculated into the same volume of YEPG induction medium and grown for 16 hr. The cells were harvested

and resuspended in 25 mL disruption buffer DB (0.01 M phosphate buffered saline, PBS, pH 7.2, 20 mM EDTA, 2 mM phenylmethylsulphonyl fluoride, PMSF, 0.05% Tween 20). The suspended cells were transferred to a chilled container for homogenisation in a blender. Glass beads (0.5 mm; Sigma, St. Louis, MO) equivalent to the total weight of the cells and disruption buffer were added to the re-suspended cells. The cells were then homogenised in a blender at 3,000 rpm for 30 min, ensuring the cells were kept chilled throughout this period. After homogenisation the suspension were centrifuged at $3{,}000 \times g$ for 10 min at 4°C. The supernatants were collected and loaded onto a chilled 30% sucrose cushion in centrifuge tubes and ultracentrifuged at $100{,}000 \times g$ for 3 hr at 4°C. The supernatant was discarded and the pellet once again re-suspended in a small volume (~12 mL) of chilled DB. The suspension was then loaded on top of a chilled CsCl gradient ranging from 1.23 g/mL to 1.38 g/mL and centrifuged at $100{,}000 \times g$ for 48 hr. Fractions containing proteins were identified by SDS-PAGE. Fractions containing protein corresponding to the molecular weight of NP (~60 kDa) were pooled, diluted with 1.31 g/mL CsCl and re-centrifuged on a second CsCl gradient. Fractions were collected and those containing rNP were pooled and dialysed against PBS for EM and Western blot analysis. The buoyant density of the fractions was determined with a refractometer.

SDS PAGE and Western Blotting

Samples were boiled in a reducing sample buffer, applied to a Novex™ 8–16% Tris-glycine polyacrylamide gel (Invitrogen, NL) and electrophoresed in SDS-Tris-glycine buffer [Laemmli, 1970]. Proteins were stained by the addition of Coomassie brilliant blue. After SDS PAGE proteins were transferred to nitro-cellulose membrane Hybond™ ECL (Amersham Life Science, Little Chalfont, Buckinghamshire) as described by Towbin et al. [1979]. The blots were incubated overnight with a protein solution (3% bovine serum albumin, BSA in PBS) to block residual protein binding sites. The blocking solution was removed and the blots incubated with either human anti-mumps positive and negative serum samples, anti-mumps monoclonal antibody, clone 1668 (a gift of Dr. Ferguson, NIBSC, London, UK) or monoclonal antibodies to rNP described below. For the detection of specific antibody-binding, horseradish peroxidase (HRP)-labelled anti-human IgG conjugate and anti-mouse IgG conjugates (Dako, UK) were used. The blots were stained with 3,3′,5,5′-tetramethybenzadine (TMB) substrate as described by Sheldon et al. [1986].

Electron Microscopy

Suspensions of rNP particles purified by ultracentrifugation were placed on 400-mesh carbon coated palladium grids. The samples were stained with 2% aqueous uranyl acetate solution and examined with a JEM-100S electron microscope.

Stability of Viral Particles

The stability of particles was tested by incubating with 20 mM EDTA, 10 mm EGTA or 5 mM DTT, 1 hr, at 37°C. After incubation electron microscopy analysis was carried out.

Monoclonal Antibodies

Monoclonal antibodies to rNP were produced essentially as described by Kohler and Milstein [1975]. Briefly, BALB/c mice were immunised subcutaneously with 50 μg of rNP emulsified in complete Freund's adjuvant. Mice were boosted twice with the same dose of NP in PBS on Day 30 and 60 after primary immunisation. Sera of the immunised mice were tested for the presence of specific antibodies by indirect, screening ELISA. Spleen cells of the best responder animal were fused with mouse myeloma NS0/1 cells using PEG 1500 as a fusion agent (PEG/DMSO solution, HybriMax, Sigma). Hybrid cells were selected in growth medium supplemented with hypoxanthine, aminopterin and thymidine (50× HAT media supplement, Sigma). Viable clones were screened by indirect ELISA using 96-well microtiter plates coated with rNP (5 μg/ml in 0.05 M Na-carbonate buffer, pH 9.5) and peroxidase-labelled secondary antibody (Amersham, NXA 931). Positive clones were stabilised by limiting dilution cloning on macrophage feeder layer using growth medium supplemented with recombinant huIL-6. The isotypes of the monoclonal antibodies were determined by ELISA using Monoclonal Antibody Isotyping Kit (Sigma, ISO-2).

Hybridoma cells were maintained in complete Dulbecco's modified Eagle's medium (DMEM) containing 15% foetal calf serum (Biochrom) and antibiotics.

Anti-Mumps HRP Conjugate

IgG antibody from one of the mumps clones (5H7) was purified on a protein A column by standard techniques and coupled to horseradish peroxidase, HRP by the method of Wilson and Nakane [1978].

Serum Specimens

These were from specimens tested previously by an "in-house" IgM capture radioimmunoassay (MACRIA) for mumps, measles, rubella [Perry et al., 1993], an "in-house" IgM enzyme immunoassay for parvovirus B19 [Brown et al., 1989] and latex test for rheumatoid factor (Behring, UK). The specimens comprised 18 mumps IgM positive and 30 mumps IgM negative specimens, 10 measles IgM positive specimens, five rubella IgM positive specimens, 20 parvovirus B19 IgM positive specimens and 26 rheumatoid factor positive specimens.

Immunofluorescence

Confluent cultures of Vero cells were grown on cover slips in 24-well tissue culture plates and inoculated with three wild-type mumps strains isolated in the

U.K. After 3–4 days post inoculation the cell were fixed with cold acetone/PBS (80:20). Indirect immunofluorescence was carried out using monoclonal antibodies to the rNP and a goat anti-mouse IgG-FITC conjugate (Chemicon, UK). The stained cover slips were mounted onto slides in a mounting medium (Chemicon) and the fluorescence observed using an epifluorescence microscope (Zeiss, Germany) with filter sets for the detection of FITC fluorescence.

Mumps Specific IgM Capture EIA

Rabbit anti-human IgM (Dako, UK) was absorbed to remove any residual anti-human IgG activity using a human IgG-Sepharose 4B immunosorbent using standard techniques [Harrison and Lachmann, 1986]. Nunc, Maxisorp microtitre plates (Life Technologies, UK) were coated with 100 μL/well of the absorbed antibody at 1 μg/mL in 0.05 M carbonate buffer, pH 9.6 and incubated for at least 18 hr at 2–8°C. The plates were then aspirated and washed twice with PBS containing 0.05% Tween-20 (PBS/Tw). The plates were then blocked using 300 μL/well of 5% (w/v) Solupro (Dynagel, US) in water for 2 hr at 37°C. The plates were then dried at 37°C and stored pouched with a desiccant at 2–8°C before use.

Test and control serum or plasma samples were diluted 1/200 in serum diluent (1% dried milk, Marvel (Cadbury, UK) in PBS/Tw. The diluted serum was added to anti-human IgM coated wells and incubated at 37°C for 30 min. The wells were aspirated and washed four times with PBS/Tw using an automatic plate washer. rNP was diluted to 0.1 μg/mL in PBS/Tw buffer containing 3% (w/v) BSA, 1 M NaCl and 0.05% Bronidox-L (Henkel, Germany) and added to the wells and incubated at 37°C for 30 min. The wells were washed as above and 100 μL of the diluted monoclonal anti-rNP-HRP conjugate was added to the wells and incubated for a further 30 min at 37°C. The wells were again washed and100 μL/well of TMB substrate (Microimmune, UK) was added for 10 min at room temperature. The reaction was stopped by adding 100 μL of 0.5M HCl to the wells. The optical densities at 450 nm and at a correction wavelength of 620 nm were read simultaneously within 5 min of stopping the reaction.

RESULTS

Generation of Mumps Nucleocapsid-Like Particles

SDS-PAGE of yeasts cells, after induction with galactose, revealed an additional band migrating with a molecular weight of approximately 66 kDa (Fig. 2a), consistent with the expected molecular weight reported in the literature [Wolinsky, 1996]. Immunostaining with monoclonal antibodies specific for nucleoprotein revealed a major band corresponding to the molecular size of NP (64–66 kDa) and a minor band at 52 kDa in the extract from galactose-induced plasmid pFX7-NP harbouring yeast (Fig. 2b, lane 3) and in the CsCl gradient purified preparation (Fig. 2b, lane 4). The integrity of the rNP was maintained using the optimised purification method described in the Materials and Methods section. In initial studies, the disruption buffer used for purification of rNP was 10 mM Tris buffer containing 150 mM NaCl and 1 mM $CaCl_2$, pH7.2 instead of PBS/EDTA. Under these conditions, the NP degraded and bands of lower molecular weight were detected (Fig. 2c, lane 4). The two monoclonal antibodies used (one produced using rNP as immunogen and the other using mumps virus preparation) gave slightly different staining patterns indicating that they recognised different epitopes. Both the antibodies detect a major protein of molecular weight ~52 kDa.

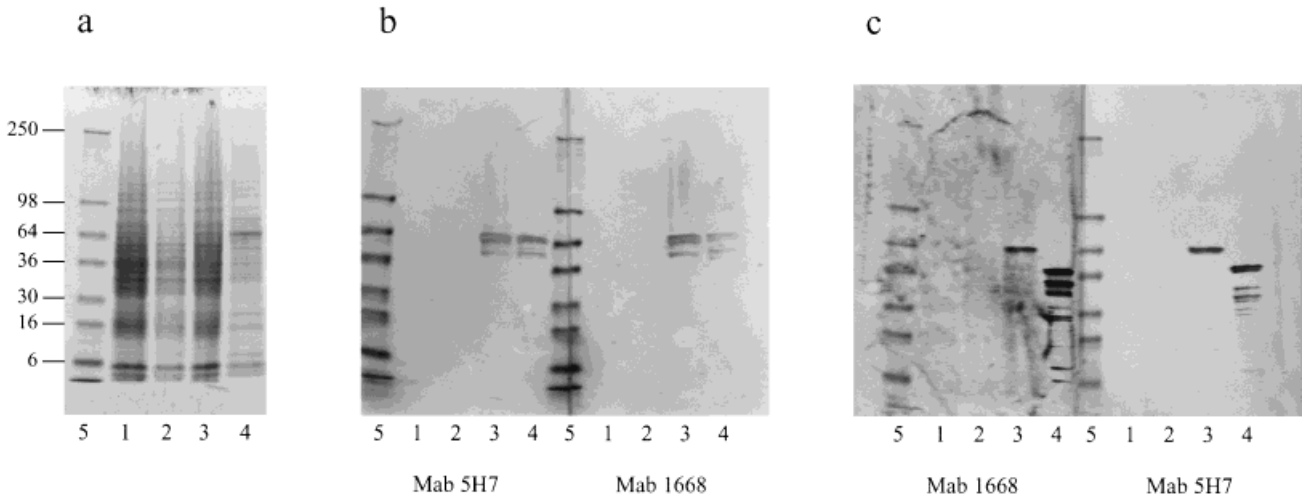

Fig. 2. SDS PAGE analysis of yeast lysates and CsCl centrifugation purified mumps rNP on an 8–20% Tris-glycine gradient gel (Novex, Invitrogen, UK). **a:** Coomassie blue stained gel. **b:** Immunoblot using MAbs 5H7 and 1668 –rNP purified from yeast cells disrupted in PBS/EDTA buffer. **c:** Immunoblot using MAbs 5H7 and 1668 –rNP purified from yeast cells disrupted in Tris/NaCl/$CaCl_2$ buffer. Preparations loaded onto gels were as follows: **Lane 1**, *S. cerevisiae* [pFX7] in YEPG medium (induction medium); **Lane 2**, *S. cerevisiae* [pFX7-NP] in YEPD medium (growth medium); **Lane 3**, *S. cerevisiae* [pFX7-NP] in YEPG medium (induction medium); **Lane 4**, CsCl purified mumps NP from *S. cerevisiae* [pFX7-NP] and **Lane 5**, protein molecular weight markers, SeeBlue (Novex) 250, 98, 64, 36, 30, 16, and 6 kDa.

Immunoblots with mumps IgG positive human sera gave similar banding patterns to that of the monoclonal antibodies, i.e., a single major band was observed in the induced plasmid pFX7-NP harbouring yeast (data not shown). The electrophoretic mobility of this immunostained band was similar to that of the NP in mumps virus.

Electron Microscopy

Electron microscopy of the purified rNP revealed characteristic herring-bone structure (Fig. 3). Rods of ~18–20 nm with repeated serration along the edges and a central core of ~5 nm were observed.

Yield and Stability of rNP Particles

In several preparative procedures, the yield of CsCl gradient purified rNP was between 10–15 mg/L of induced yeast cells or 30 g w/w of cells. The rNP particles were stable in CsCl solution at 4°C. Treatment with 20 mM EDTA, 10 mM EGTA or 5 mM DTT did not cause dissociation of rNP particles indicating the assembled structure does not require divalent ions. The particles were most stable in PBS. In 50 mM Tris-HCl pH 7.5 and 1 M NaCl the nucleocapsid like particles aggregate and incomplete structures were revealed. Therefore for yeast disruption, particles purification and storage PBS containing 10 mM EDTA was used. Dialysed samples were stored at −20°C in PBS containing 10 mM EDTA and 40% glycerol.

Characterization of Monoclonal Antibodies

Several hybridomas reacting with NP were generated and seven of these were cloned by limiting dilution. All of these antibodies reacted with mumps NP in an indirect ELISA. Six antibodies were of isotype IgG2a and one was of isotype IgG2b. All seven monoclonal antibodies reacted with Vero cells infected with mumps virus in immunofluorescence tests (Fig. 4). The immunofluorescence staining was localised in the cytoplasm.

Mumps Specific IgM Capture Enzyme Immunoassay

The distribution of optical densities ($OD_{450/620\ nm}$) in the mumps IgM enzyme immunoassay given by sera from different categories is shown in Figure 5. Eighteen sera positive for mumps IgM by MACRIA gave high $OD_{450/620\ nm}$ and the results were well separated from those given by 30 sera negative for mumps IgM by MACRIA. Based on a positive cut-off value of 0.5 $OD_{450/620\ nm}$, the results for this panel of 48 sera showed complete agreement with those of MACRIA.

Fifty-six of the 61 other sera tested gave $OD_{450/620\ nm}$ values of < 0.5. There were, however, five sera that gave OD values as high as those obtained for two low positive mumps IgM sera (Fig. 5). Further investigation revealed that two of these sera, one measles and one parvovirus B19 IgM positive, were obtained from infants who had received MMR vaccine respectively 3 and 7 weeks earlier. It was noticeable that the

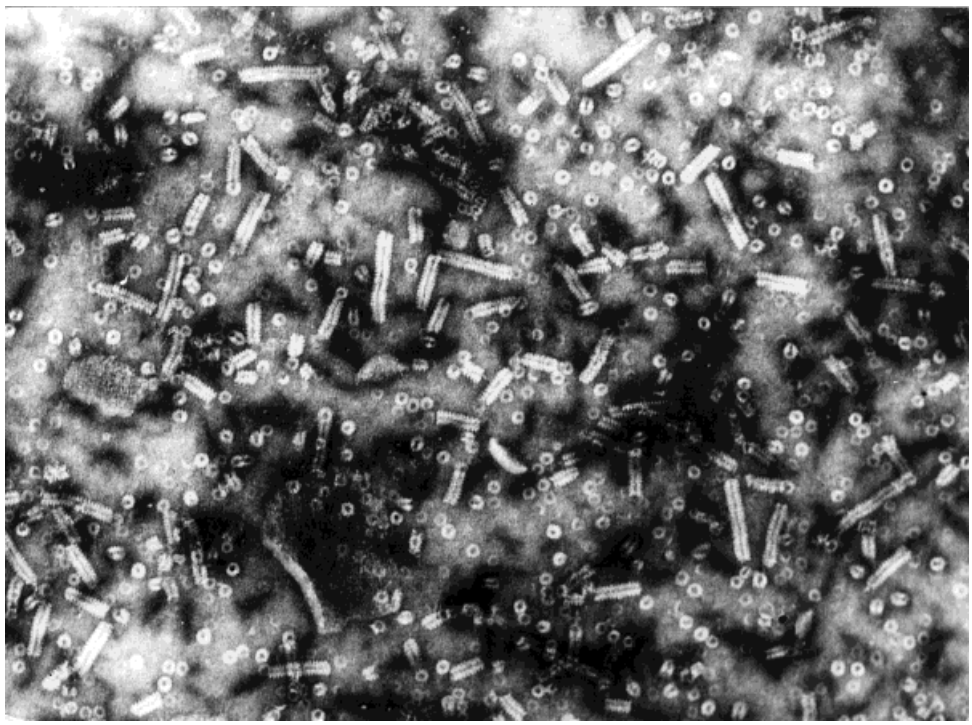

Fig. 3. Electron micrograph of rNP purified by CsCl gradient centrifugation. The yeast cells were disrupted in PBS/EDTA before purification. Scale bar = 100 nm.

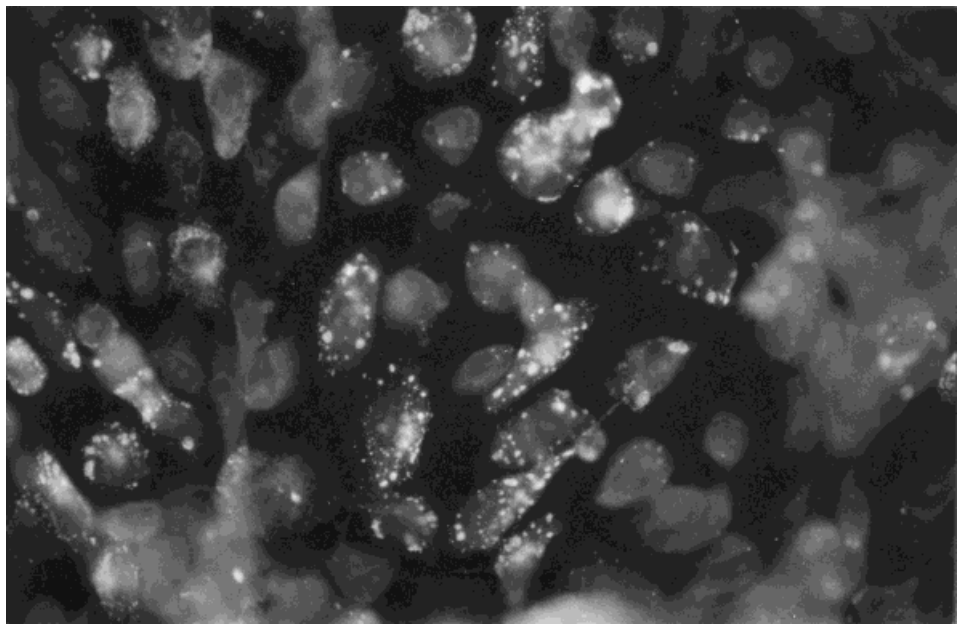

Fig. 4. Indirect immunofluorescence staining of mumps virus infected Vero cells using monoclonal antibody 5H7.

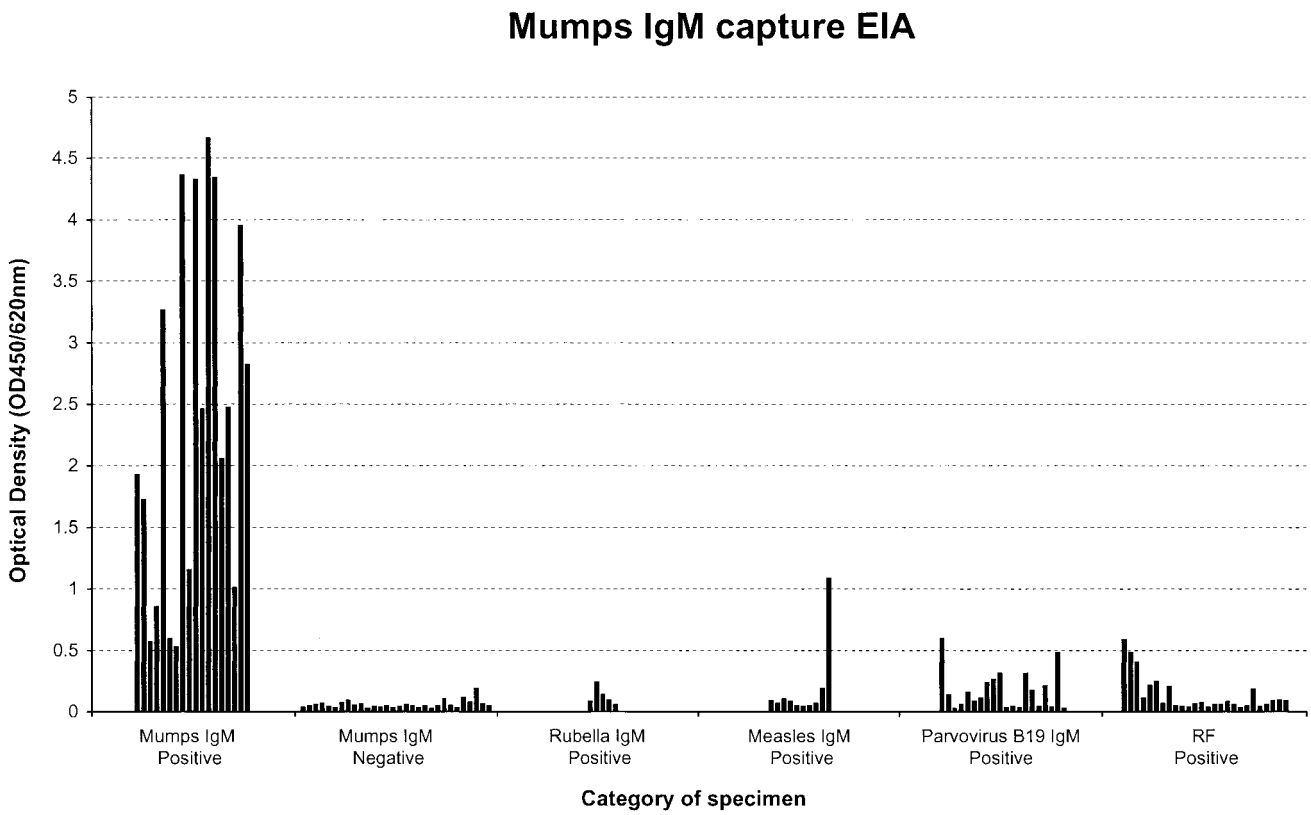

Fig. 5. Distribution of optical densities obtained for the indicated category of specimens in the mumps IgM capture EIA.

$OD_{450/620\ nm}$ for the serum collected 3 weeks post-vaccine was higher than for the serum collected 7 weeks post vaccine. Both of these samples were confirmed subsequently to be mumps IgM positive by MACRIA.

The three other sera giving $OD_{450/620\ nm} \geq 0.5$ were also tested by MACRIA. Two gave equivocal MACRIA results (one parvovirus B19 IgM serum and one of the RF positive serum) and one serum (RF positive) gave a negative MACRIA result.

The high OD values with some rheumatoid factor, RF sera was investigated by carrying out the IgM capture assay on all the specimens and omitting the rNP antigen to determine if interference was due to the rheumatoid factor binding non-specifically to the conjugate. All sera including rheumatoid factor positive serum samples gave background OD values of < 0.06.

Overall, of the 53 sera tested by both EIA and MACRIA, 20 gave concordant positive results (sensitivity of EIA = 100%). Thirty sera gave concordant negative results. Excluding the two sera that gave equivocal MACRIA results, the specificity of the test was 96.8% (30/31).

DISCUSSION

The morphology and the electrophoretic mobility of the rNP was similar to the nucleocapsid in native mumps virus infected cells. Antigenic similarity between native mumps virus NP and the rNP was demonstrated by monoclonal antibodies. Antibodies to the rNP reacted with native mumps virus NP and antibodies to native NP reacted with rNP. To the best of our knowledge this is the first report of expression of mumps nucleoprotein as nucleocapsid like particles. Moreover, this is the first time mumps NP has been expressed in a yeast expression system. Expression and secretion of the extracellular domain of mumps virus fusion protein in *Pichia pastoris* [McAleer and Rima, 2000] and the expression of mumps hemagglutinin-neuraminidase and fusion protein in mammalian COS-7 cells [Tanabayashi et al., 1992] have been described previously. The experiments reported above suggest that mumps nucleocapsids assemble without the assistance of other mumps gene products and if cellular proteins are involved, they are found in both human and yeast cells. The buoyant density of mumps rNP particles in CsCl (1.29–1.30 g/mL) corresponds to values observed for empty nucleocapsid-like particles of other viruses [Spehner et al., 1991]. This is consistent with the hypothesis that they do not contain RNA and that mumps NP can assemble into nucleocapsid-like structures. Although phenol deproteinization and electrophoretic analysis of purified particles failed to detect RNA (data not shown), it is possible that nucleocapsid formation depends on a nucleation event that uses small amounts of RNA of unspecified sequence.

Three forms of rNP were observed in immunoblots. In galactose-induced *S. cerevisiae* [pFX7-NP] cells, before purification, a major protein with an apparent molecular weight of ~66–64 kDa and a minor band at ~52 kDa were observed. Two dominant bands (66–64 kDa) in immunoblots may reflect post-translational modification that took place in yeast cells, or limited proteolysis. When the *S. cerevisiae* [pFX7-NP] cells were disrupted in Tris buffer containing NaCl and $CaCl_2$ and the rNP isolated by ultracentrifugation, several immunostained bands of lower molecular weight were observed in the purified preparation (Fig. 2c, lane 4). In contrast, disruption of *S. cerevisiae* [pFX7-NP] cells in PBS/EDTA gave similar immunostaining pattern for the purified NP as for the intact yeast cells. The multiple bands observed may be as a result of proteolytic degradation in the Tris/NaCl/$CaCl_2$ buffer. McCarthy and Lazzarini [1982] also observed multiple bands of MW 54–56 kDa, 45 kDa and 39 kDa in CsCl gradient purified mumps NP from mumps virus infected Vero cells and suggested that the virion NP protein contains protease-sensitive sites. Similar degradation attributed to protease activity was observed by Warnes et al. [1994] for measles rNP produced in three different expression systems and in measles virus infected cells and by Hummel et al. [1992] for the measles rNP expressed in insect cells using recombinant baculovirus at high multiplicity of infection. Despite the degradation of purified rNP observed in immunoblots, the cleavage of rNP protein does not necessarily affect its ability to form or remain in nucleocapsid-like structure suggesting that strong intra or inter molecular, non-covalent bonds within the structure maintain the conformation of the protein. The limited fragmentation of rNP observed in PBS/EDTA buffer (Fig. 2b, lane 4) indicates that metaloproteinases may be responsible for degradation.

The rNP was evaluated as a diagnostic reagent for mumps IgM serology. A preliminary IgM capture enzyme immunoassay with monoclonal antibody to rNP indicated that these reagents are likely to prove useful for the diagnosis of acute mumps infections. Although the evaluation was small, we found high sensitivity (100%) and specificity (96.8%) compared to mumps MACRIA. Further evaluation using a larger panel of serum samples is necessary to establish more precisely the performance characteristics of the IgM capture EIA. Non-specific signals were generated with some rheumatoid factor positive serum. Rheumatoid factor is known to cause problems in IgM tests [Marmot and Ziola, 1978; Saonen et al. 1980] and these are due to the captured immobilised rheumatoid factor of IgM isotype binding the conjugate [Kurtz and Malic, 1981] or due to the IgM-rheumatoid factor binding antigen-specific IgG, if present in high concentration [Duermeyer et al., 1979]. In our test, the rheumatoid factor did not bind conjugate as evidenced by tests in which the rNP was omitted. The presence of high levels of circulating mumps-specific IgG in some rheumatoid factor positive serum samples is probably the reason for high optical density signals in the EIA with these sera.

The use of purified rNP and enzyme-labelled monoclonal antibody in the mumps IgM EIA overcomes the disadvantages of MACRIA using radioisotopes and

culturing potentially infectious virus for antigen preparation. Furthermore the use of purified rNP and specific monoclonal antibody offers the potential for assay improvement. In this context, we intend to examine whether the reported cross-reactions of mumps NP in sera from subjects with other paramyxoviruses infections can be overcome using the rNP and specific monoclonal antibodies. Our preliminary investigations indicate that rNP is also useful for detecting specific IgG in sera from all age-groups (data not shown). Further work is needed to establish whether these antibodies are due to re-stimulation of immune response by circulating mumps virus or whether antibodies to NP persist when detected using more sensitive assays. This information is necessary to establish if IgG tests based on rNP would be useful for epidemiological sero-surveillance studies and for monitoring responses to vaccine

In summary, a recombinant mumps nucleoprotein was expressed in a yeast system that will be a valuable resource for studies of viral morphogenesis, diagnosis, and surveillance.

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