Mapping of domains responsible for nucleocapsid protein–phosphoprotein interaction of henipaviruses

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Hendra virus (HeV) and Nipah virus (NiV) are members of a new genus, Henipavirus, in the family Paramyxoviridae. Each virus encodes a phosphoprotein (P) that is significantly larger than its counterparts in other known paramyxoviruses. The interaction of this unusually large P with its nucleocapsid protein (N) was investigated in this study by using recombinant full-length and truncated proteins expressed in bacteria and a modified protein-blotting protein-overlay assay. Results from our group demonstrated that the N and P of both viruses were able to form not only homologous, but also heterologous, N–P complexes, i.e. HeV N was able to interact with NiV P and vice versa. Deletion analysis of the N and P revealed that there were at least two independent N-binding sites on P and they resided at the N and C termini, respectively. Similarly, more than one P-binding site was present on N and one of these was mapped to a 29 amino acid (aa) C-terminal region, which on its own was sufficient to interact with the extreme C-terminal 165 aa region of P.

INTRODUCTION

Two newly emerged paramyxoviruses, now known as Hendra virus (HeV) and Nipah virus (NiV), were identified in Australia and Malaysia in 1994 and 1999, respectively. HeV first emerged in 1994 in Australia causing an explosive outbreak of respiratory disease in horses and claimed the lives of at least 14 horses and one human in Brisbane (Murray et al., 1995). NiV was responsible for an outbreak of fatal encephalitis in humans and respiratory illness in pigs. The NiV outbreak in 1998–1999 resulted in the death of over 100 people and the culling of more than 1 million pigs in Malaysia, causing extensive economic loss in the local swine industry (Chua et al., 1999, 2000). The complete genomes of both NiV and HeV have been determined (Gould, 1996; Wang et al., 1998, 2000; Yu et al., 1998a, b; Harcourt et al., 2000, 2001; Chan et al., 2001). NiV and HeV have been shown by phylogenetic analysis to be closely related and not members of existing genera. Instead, these two viruses have been classified in a separate genus, Henipavirus, in the subfamily Paramyxovirinae, family Paramyxoviridae (Mayo, 2002; Wang & Eaton, 2000).

Paramyxoviruses contain a non-segmented negative-stranded RNA genome that is encapsidated by the nucleocapsid protein (N) to form a helical nucleocapsid (NC) which functions as template for transcription and replication (for review, see Lamb & Kolakofsky, 2001). The large protein (L) interacts with the phosphoprotein (P) to form the polymerase complex (Horikami et al., 1992; Holmes & Moyer, 2002), which associates with the nucleocapsid via interaction between P and the assembled N in the NC (Curran et al., 1993; Buchholz et al., 1994).

The N–P interactions for members of the Paramyxoviridae have been demonstrated both in vivo and in vitro. In Sendai virus (SeV), complexes of N–P and P–L formed separately and subsequently mixed together are able to support the replication of the genome of defective interfering virus in vitro (Horikami et al., 1992). The SeV P has been found to complex with newly synthesized, unassembled N (N') during the nascent chain assembly step of genome replication. The N–N' complex prevents non-specific aggregation of N and P is consequently being viewed as a chaperone for N' (Curran et al., 1995; Errington & Emmerson, 1997; De et al., 2000). Domains involved in the N–P interaction of several paramyxoviruses have been determined. These viruses include SeV (Ryan & Kingsbury, 1988; Ryan & Portner, 1990; Homann et al., 1991; Ryan et al., 1991; Buchholz et al., 1994; Tuckis et al., 2002), Simian virus 5 (SV5) (Randall & Bermingham, 1996), Human parainfluenza virus-1 (HPIV1) and -3 (HPIV3) (Ryan et al., 1993; Zhao & Banerjee, 1995; De et al., 2000), Porcine rubulavirus (PoRV) (Svenda et al., 2002), Human parainfluenza virus-2 (HPIV2) (Nishio et al., 1996, 1999), Human respiratory syncytial virus (HRSV) (Garcia-Barreno et al., 1996; Slack & Easton, 1998; Lu et al., 2002), Bovine respiratory syncytial virus (BRSV) (Mallipeddi et al., 1996; Khattar et al., 2000, 2001a, b), Rinderpest virus (RPV) (Shaji et al., 2002).
Among the many molecular features unique to henipaviruses is the exceptionally large P of both HeV and NiV. With 707 amino acids (aa) for the P of HeV and 709 aa for NiV, the henipavirus Ps are approximately 100 to 400 aa larger than Ps of other known paramyxoviruses (Wang et al., 1998; Harcourt et al., 2000). Owing to this P size increase and the lack of significant sequence homology between henipavirus P and those of other paramyxoviruses, it was impossible to predict whether the N and P of henipaviruses would interact in a similar fashion as those reported previously. Hence, we conducted this study to explore the function of the henipavirus P in terms of forming the N–P interaction and to map the domains required for such interaction.

RESULTS

Characterization of recombinant proteins expressed in E. coli

The correct expression of each recombinant protein was examined by SDS-PAGE and Western blot. Recombinant proteins expressed from pRSET vectors were detected with the Anti-Xpress mAb (Invitrogen), which recognizes a common epitope fused at the N terminus of the pRSET-derived recombinant proteins (see Figs 3A, 4A and 5A). As observed previously for other paramyxovirus Ps (Wang et al., 1998), full-length and truncated Ps migrated slower in SDS-PAGE, thus giving rise to protein bands with higher molecular masses than calculated from their encoding sequences (Figs 2B, 3A and 4A). Biotinylated fusion proteins expressed from pDW363 or the PinPoint Xa vector were detected with AP–streptavidin conjugate (Fig. 6A). In the pDW363 vector, the insert was ligated in-frame with the malE and/or the birA gene (see Tsao et al., 1996); hence, all the pDW363-derived proteins are relatively large (see Fig. 6A). Extra bands were observed below the predicted size probably caused by degradation from the C terminus of the proteins. The level of expression for each protein was relatively similar as judged by the band intensity in the blots (Figs 3–6).

Homologous and heterologous N–P interaction

As described in Methods, the N–P interaction was analysed by PBPOA, and the bound N or P proteins were monitored by N- and P-specific mAbs, respectively. As shown in lanes 1 and 2 of Fig. 2(A) and 2(B), the N-specific mAb only reacted with N, and the same for the P-specific mAb. The results shown in Fig. 2(A) indicated that both NiV P and HeV P were able to bind to the immobilized NiV N, and the binding was concentration-dependent. Similarly, when NiV P

Plasmid construction. Three bacterial expression systems were used in this study. For expression of His tagged proteins, the pRSET vector (Invitrogen) was used, whereas for expression of biotinylated proteins, we used the pDWD36 vector (Tsao et al., 1996), kindly provided by Dr David S. Waugh (National Cancer Institute, Frederick, USA) or the PinPoint Xa vector (Promega). A general approach was used in plasmid construction: PCR primers (forward and reverse) with unique restriction sites incorporated in each primer were designed for each gene fragment to be expressed. After amplification using a high-fidelity DNA polymerase, the PCR fragments were gel-purified and cloned in-frame into appropriate expression vectors. The insert sequences were then confirmed by directly sequencing the recombinant plasmids. Owing to the large number of recombinant plasmids constructed in this study, details of the construction of each plasmid are not presented here, but will be provided on request. Instead, we have listed all the plasmids used in this study in Fig. 1, which summarizes the aa-residue number in each insert.

Expression of recombinant proteins in Escherichia coli.

Expression of the pRSET constructs in E. coli BL21(DE3) pLysS cells (Invitrogen) was carried out as described in the manufacturer’s instruction manual. Expression of the pDWD363 constructs in E. coli HB101 cells was performed following the protocol described by Tsao et al. (1996). Biotinylated proteins from the PinPoint Xa system were expressed in E. coli HB101 cells following the protocol in Promega’s instruction manual. In all cases, expression of the recombinant protein was induced by adding IPTG to a final concentration of 1 mM. For direct analysis of the expressed proteins by SDS-PAGE, cell pellets were resuspended in SDS-PAGE sample buffer [50 mM Tris/HCl pH 6–5, 2 % (w/v) SDS, 0–1 % (w/v) bromophenol blue, 10 % (v/v) glycerol and 1 % (w/v) DTT] and denatured for 2 min at 100 °C before electrophoresis. For production of soluble cell lysates, cell pellets were resuspended in MTPBS (150 mM NaCl, 16 mM Na2HPO4, 4 mM NaH2PO4, pH 7–3) at one-tenth of the original culture volume. The cell suspension was sonicated on ice 5–10 times, each for 30 s with 30 s intervals, by using a Dynatech Sonic Dismembrator at the high intensity setting. After sonication, Triton X-100 was added to a final concentration of 1 % (v/v). Cell lysates were used directly or frozen at −20 °C for future use.

Protein-blotting protein-overlay assay (PBPOA). The method described by Homann et al. (1991) was followed with minor modifications. Briefly, cells lysed in SDS-PAGE sample buffer were separated on 10–13 % polyacrylamide gels and the separated proteins were transferred onto PVDF membranes. The membrane was then blocked by incubating overnight at 4 °C in blotto containing 5 % (w/v) skimmed milk in TBST [10 mM Tris/HCl pH 8–0, 150 mM NaCl and 0–1 % (v/v) Tween 20]. All subsequent incubations were carried out in blotto containing antibody or conjugate solution at pre-determined dilutions, and washings were carried out with TBST. After blocking, the intact membrane or 4–5 mm membrane strips were incubated for 2–3 h at room temperature with a chosen cell lysate. After three washings, the membrane was incubated for 1 h at room temperature either with a mAb or with the AP–streptavidin conjugate. After washing three times, the membrane incubated with mAb was further processed by 1 h incubation with AP-conjugated anti-mouse antibody, followed by three washings. For colour development, both types of membranes (probed either with AP–streptavidin conjugate alone or with mAb+AP-conjugated anti-mouse antibody) were incubated with BCIP/NBT substrate mixture (Promega).

METHODS

Antibodies. Monoclonal antibodies (mAb) against HeV N and P proteins were kindly provided by Dr John White (CSIRO Australian Animal Health Laboratory, Geelong, Australia). Alkaline phosphatase (AP)-conjugated streptavidin was purchased from Promega. Anti-Xpress mAb was purchased from Invitrogen. AP-conjugated goat anti-mouse IgG and IgM were purchased from Chemicon.

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Fig. 1. Diagrammatic summary of all the constructs used in this study for P (A) and N (B) proteins. The shaded box represents the region expressed in each clone, with the name of construct shown on the left and aa-residue position and length of each insert on the right. NN and HN represent NiV N and HeV N fragments, respectively. Also shown on the extreme right are the abilities of the truncated proteins to interact with full-length N or P of either NiV or HeV in the PBPOA. The degree of interaction is illustrated by plus or minus signs from strong binding (+ +) to no binding (−).
was immobilized, both NiV N and HeV N were captured in a concentration-dependent fashion (Fig. 2B). Similar results were obtained when HeV N or HeV P were used as the immobilized proteins (Fig. 3B and 3C, lanes 1 and 10; Fig. 5B, lanes 1 and 2). These data demonstrated that N and P of HeV and NiV could interact not only homologously, i.e. NiV N with NiV P, but also heterologously, i.e. NiV N with HeV P and vice versa. It is also important to note that the N–P interaction can be detected with either full-length N or P immobilized on the membrane, which was not always true when truncated N or P fragments were used in similar studies (see below). When the viral N and P proteins of Bovine parainfluenza virus-3 and a recombinant V protein of Salem virus (Renshaw et al., 2000) were used in the same assay system, no specific interaction with any of the henipavirus N or P was detected (data not shown).

### Mapping of P domains involved in N–P interaction

While the NiV N and HeV N share an overall 92% sequence identity, the NiV P and HeV P are less conserved, sharing only 68% sequence identity. However, the two terminal regions of P are more conserved than the middle region (Wang et al., 1998; Harcourt et al., 2000). The N–P interaction studies above indicated that the NiV P and HeV P contained one or more conserved domains, which are involved in binding the Ns of both viruses. To map these

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**Fig. 2.** Study of N–P interaction by using the PBPOA. Full-length NiV N (A) or NiV P (B) was subjected to a preparative SDS-PAGE followed by Western blotting. The blotted membrane was then sliced into strips and each strip was separately incubated with different dilutions of P (A) or N (B) of both HeV and NiV. The bound proteins were detected by mAb specific to P (A) or N (B). Both panels contain three common strips: L, ladder of low-range pre-stained molecular mass markers, from Bio-Rad (kDa values given on the left); lane 1, anti-N mAb alone; lane 2, anti-P mAb alone. For each protein tested, five strips were incubated with different dilutions of the same protein lysate with the first strip containing 10 µl of cell lysate, next strip containing 5 µl, followed by 1 in 5 dilutions for the subsequent three strips. The triangle indicates the direction of dilution with the left lane having the highest concentration, and the name of the protein used in each incubation is given above the triangle.

**Fig. 3.** Binding assay of different P fragments with full-length NiV N and HeV N. Full-length and truncated P expressed from the pRSET system were separated by SDS-PAGE, followed by Western blot. The size and intensity of each expressed product was monitored by the Anti-Xpress mAb (A). Two additional blotted membranes were incubated with NiV N (B) and HeV N (C), respectively. Ns bound to the immobilized Ps (in B and C) were detected by anti-N mAb. Lanes 1 and 10 are full-length NiV P and HeV P, respectively; lane 2, P4–550; lane 3, P1–251; lane 4, P3–220; lane 5, P200–372; lane 6, P1–107; lane 7, P62–251; lane 8, P253–709; lane 9, cell lysates from vector alone control; L, ladder of low-range pre-stained molecular mass markers from Bio-Rad (kDa values given on the left).
putative N-binding domains, various constructs were made to express N-terminal (P4–550, P1–251, P3–220, P1–107 and P62–251), C-terminal (P253–709, P549–709, P549–637 and P636–709) and the less conserved middle region (P200–372) of NiV P (see Fig. 1A for the location of insert in each construct). Various protein fragments were immobilized onto a single membrane, and probed with full-length NiV N and HeV N, respectively. The results presented in Fig. 3 showed that both the N- and C-terminal fragments, but not the middle fragment, of the NiV P were able to interact with the Ns of both viruses. There were no significant differences in binding pattern between NiV N and HeV N with the exception of clone P62–251, which interacted weakly with NiV N but failed to interact with HeV N (Fig. 3B, C). P3–220 represents the smallest N-terminal fragment which was able to bind N. Neither the 107 aa N-terminal fragment (P1–107) nor the 173 aa middle fragment (P200–372) was able to bind NiV N or HeV N (Fig. 3B, C, lanes 5 and 6). This suggests that residues between 107 and 220 are important for binding to P. However, these residues alone are not sufficient to support the binding as demonstrated by clone P62–251. On the other hand, further deletion analysis of the C-terminal constructs revealed that an extreme C-terminal 74 aa region of NiV P, in clone P636–709, was able to bind both NiV N and HeV N (Fig. 4B, lane 4). Taking all the results together as summarized in Fig. 1, it was clear that there were at least two independent N-binding sites present in NiV P, one located at the N terminus (aa 3–220) and the other in the C-terminal region (aa 636–709).

**Mapping of N domains involved in N–P interaction**

To map the P-binding domains of N, we first divided the NiV N into two overlapping halves and demonstrated that only the C-terminal half was involved in interaction with P (Fig. 5B). Various C-terminal fragments of NiV N were then produced as shown in Fig. 1(B). Using the same strategy as that for the mapping of the N-binding domains of P, we immobilized different pRSET-derived NiV N fragments onto a single PVDF membrane, which was subsequently incubated with full-length NiV P or HeV P. Protein binding assay of these truncated N fragments showed that all but the NN1–135 and NN378–469 fragments...
were able to bind both NiV P and HeV P (Fig. 5). The smallest clone still capable of binding to P is NN432–532, which suggests that the region between aa 470–532 is important for P-binding (Fig. 5). Amino acid sequence alignment of NiV N and HeV N covering this region showed very high sequence identity (Fig. 7C). We then produced smaller biotinylated N fragments to further narrow down the P-binding site (Fig. 1B). Owing to the high sequence identity in this region between the two Ns and the existence of constructs already made for other studies, the HeV N gene was used instead of the NiV N gene. Using the same strategy as above, we immobilized different N fragments onto the same membrane, followed by incubation with NiV P or HeV P. The bound P was detected using the Anti-Xpress mAb. Fig. 6(B) shows that the 29 aa fragment HN468–496 was the smallest fragment that maintained the binding activity to both NiV P and HeV P (Fig. 6B, lane 9).

**Direct interaction between the C-terminal regions of N and P proteins**

In the mapping studies described above, the binding abilities of different truncated protein fragments were tested against the full-length protein of their interacting partner, i.e. truncated P fragments with full-length N and vice versa. We extended this mapping study by investigating the direct binding of truncated fragments of both interacting partners. Firstly, we examined whether the N- and C-terminal fragments of NiV P (P3–220 and P636–709, respectively) were able to bind any of the truncated biotinylated HeV N fragments. The assay was carried out essentially as described in Fig. 6(B) with one modification, i.e. replacing the full-length pRSET-derived P with pRSET-derived P3–220 and P636–709 fragments, which represented the two minimum N- and C-terminal N-binding domains.

No interaction was detected in either assay for any of the biotinylated proteins, including the full-length biotinylated NiV N (data not shown). We then repeated the experiment using other pRSET-derived truncated P fragments and showed that the C-terminal fragments P253–709 and P549–709 were able to bind the immobilized biotinylated HeV N fragments in the same way as the full-length P (Fig. 6B). However, none of the N-terminal fragments (P1–251, P3–220 and P62–251) was able to bind any of the biotinylated proteins including the full-length NiV N (data not shown). These results suggested that the minimum protein sequences required for direct N–P interaction lie within aa 549–709 of NiV P and aa 468–496 of NiV N or HeV N as these two fragments alone (P549–709 and HN468–496) were able to support the interaction in this particular assay system. It is also interesting to note that the immobilized P3–220 fragment was able to bind full-length Ns in the mobile phase (Fig. 3B, C, lanes 4), but the reverse was not true.

**DISCUSSION**

It has been well established that the N–P interaction is essential for genome replication and transcription of paramyxoviruses (Horikami et al., 1992; Curran et al., 1993, 1995; Curran, 1998; Tuckis et al., 2002) and that P associates with L to carry out RNA synthesis (Horikami et al., 1992; Holmes & Moyer, 2002). Thus, P has been viewed as the central component of the virus replication machinery in forming complexes with L, N⁺ and NC.

Our data did not differentiate between the binding of P to N⁺ or NC but provided direct in vitro evidence for N–P interaction. The formation of the N–P complex by using recombinant N and P produced in bacteria would suggest...
that the N–P interaction could occur in the absence of post-translational modification of either protein and independent of host cellular proteins. We also demonstrated that heterologous N and P proteins of NiV and HeV could form an N–P complex, but such interaction was not detected when N, P or V proteins of other paramyxoviruses were used. To the best of our knowledge, this was the first demonstration of heterologous N–P complex formation for any two paramyxoviruses by using bacterial recombinant proteins. This observation further supported the close phylogenetic relationship between these two viruses revealed by sequence analysis (Wang et al., 1998; Ye et al., 1998b; Harcourt et al., 2000) and is also consistent with our recent findings on the functional heterologous interaction between the fusion and attachment proteins of HeV and NiV (Bossart et al., 2001, 2002). It has been shown for several paramyxoviruses that the co-expression of N, P and L proteins was sufficient to support replication of naked virus genome RNA (Horikami et al., 1992; Grosfeld et al., 1995; Ye et al., 1995). Halpin et al. (2004) have recently demonstrated that the same was true for NiV. Moreover, it was shown that the NiV N, P and L proteins were also able to support the replication and transcription of HeV mini-genome, but not MeV. We are currently investigating the

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<tr>
<td>NiV</td>
<td>1 MDKLELVDGLNIIIDFIQKNEIQTGRRSSIQQPSKID</td>
<td>540 VKEPIKINKLESIDRVLAKNTALSTIEGHLVSMIMIP</td>
<td>423 GSQDDEGEEIPEIQGRQVSFTKREMSTSSLANSVPSS</td>
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| HeV | 1 D...D..................T..  | 538 M................ID | 423 GE........E.........H........M....D.  
| NiV | 41 QTAWEDFLQCTSGESEQVEGGSMDGWVENRNLDAE | 580 GKGKGERKGNPPELPVQVRDILEQQSLFSFDNKNFRD | 463 SVSTGGRTLNSLLLRLRSAKAAKEAASSNATDPAI |
| HeV | 41 R...S........H...A...F.N...G.T..V..V..  | 578 T................N...E...L...  | 463 .T...GVAR...S...S...L...A  
| NiV | 81 TSTTGTIGKAVSRMDAQDQQVTDVYHDG | 620 GSLTNPQAVQREDLILPELFNTNASQPFVMAADD | 660 SRDVKTLRTHIKDELRSELIGYILKAIENDEIEIQIAN |
| HeV | 81 VTTSS......Q...S...P.D...M.  | 618 D....GVARID........S.....L...A | 658 .K...VR.M...........MD...R...T...V...V..  
| NiV | 121 GECTGYCDTSPEWSDTSGANGNVLCLVSDAKMLSYA | 161 PEAVSKCEDRTDLHLENKLTSTGLNPTAVFPTLNLSD | 700 TVNDIIDGNI |
| HeV | 121 H...P..S........YHM....THD...RA.P.T.V.PN. | 161 .KTT.PE.VE.I...IG...D.FASA.....A....VF.PKQ.T | 698 |

Fig. 7. Amino acid (aa) sequence alignment of NiV and HeV proteins. (A) Alignment of N-terminal regions of Ps. (B) Alignment of C-terminal regions of Ps. (C) Alignment of C-terminal regions of Ns. Numbers given on the left are aa-residue positions and the symbol * marks every tenth aa residue. Identical aa residues in HeV proteins are indicated by dots whereas conservative aa changes are shown in bold.
possibility of functionally mixing the N, P or L proteins in such studies for HeV and NiV.

Our results showed that there are at least two independent N-binding regions in NiV P, located at the N-terminal (aa 3–220) and the C-terminal (aa 636–709) regions. The protein fragment containing aa 1–107 failed to bind N and the truncated fragment containing aa 62–251 had weak binding to NiV N, but no binding to HeV N. Together, these mapping data suggested that the first 220 aa and the last 74 aa of P represent two essential and independent binding regions for interaction with N. Although no direct analysis was done to map the N-binding domain for HeV P, it is tempting to say that the homologous regions of the two N-binding points of NiV P in HeV P are the contact points with HeV N because the two N-binding regions of NiV P were able to interact with HeV N as well. In addition, aa sequence alignment of NiV P and HeV P within the two N- and C-terminal N-binding regions showed high similarity between the two viruses (Fig. 7A and B). This is largely consistent with results obtained so far for other paramyxoviruses. There are many reports on the mapping of N–P-interacting domains for Ps in the family Paramyxoviridae. One of the best studied viruses is SeV, the type species of the genus Respirovirus. Two non-continuous regions at the SeV P C terminus are required to form the complex with N (Ryan & Kingsbury, 1988; Ryan & Portner, 1990; Ryan et al., 1991). In addition, the N-terminal region of P is able to form a stable complex with N' (Curran et al., 1995). In the genus Morbillivirus, the MeV P (Harty & Palese, 1995; Liston et al., 1997) has also been demonstrated to contain two separate N-binding domains located at N and C termini. For viruses in the genus Rubulavirus, the N-binding domains of HPIV2 P (Nishio et al., 1996) and SV5 P (Randall & Bermingham, 1996) have also been mapped to contain both the N- and C-terminal regions. As for pneumoviruses, which are more distantly related to members of the subfamily Paramyxovirinae, two separate N-binding domains also exist in HRSV P (Hengst & Kiefer, 2000). It can therefore be concluded that the two independent and non-continuous N-binding domain structures are well conserved in all paramyxovirus Ps, including those of henipaviruses. Such conservations have been extended to other negative-stranded RNA viruses including Rabies virus (RABV) and Vesicular stomatitis virus in the family Rhabdoviridae. In both viruses, the P N- and C-terminal regions are involved in binding with N (Takacs et al., 1993; Chenik et al., 1994). Like SeV, the N terminus of RABV P is involved in N–P complex binding, whereas the C-terminal domain is required for NC–P binding (Fu et al., 1994). This is consistent with the observation that among different paramyxoviruses the sequences of the N- and C-terminal regions of P are more conserved than those in the middle region and that the significant size increase in the exceptionally large henipavirus Ps seems to be due to the expansion of the middle region. It will be interesting to see whether deletion of this middle region in henipavirus Ps will have any functional effect.

Interestingly, previous mapping studies indicated that most paramyxovirus Ns contain at least one P-interacting domain located at the least conserved C terminus. For SeV N, the P-binding sites are located on the C-terminal and the middle regions (Homan et al., 1991; Buchholz et al., 1994). For HPIV2 N, two separate C-terminal domains are involved in binding to P (Nishio et al., 1999). Murray et al. (2001) reported that the C-terminal region of N is responsible for binding to HRSV P. Liston et al. (1997) demonstrated that there are two separate independent P-binding sites on the MeV N and they reside at the hypervariable C terminal region and the conserved middle region. In this study, we have demonstrated that the 29 aa C-terminal region (aa 468–496) of henipavirus N alone was sufficient to bind both the full-length P and the C-terminal fragment of P (aa 549–709). Although we could not directly map any other P-binding sites in this study, we did show that the N-terminal region of P (aa 3–220) was able to independently interact with the full-length N, but not the C-terminal region of N. This would suggest that it is highly possible that additional P-interacting site(s) exists in the henipaviruses N, but was not revealed in this study, probably because this site(s) is more conformation dependent, and the current approach of bacterial expression and PBPOA is not suitable for such mapping studies.

In this study, we have successfully demonstrated N–P in henipaviruses interaction by using bacterially expressed recombinant proteins. The PBPOA described here provided a simple and rapid method not only allowing the detection of the N–P interaction but also mapping of domains in such interaction. The N–P interaction detected here was specific and sensitive, as direct analysis of crude bacteria cell lysate was carried out without involving any purification of recombinant proteins.

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