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# PIV5 M protein interaction with host protein angiomotin-like 1

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ABSTRACT

Paramyxovirus matrix (M) proteins organize virus assembly, functioning as adapters that link together viral ribonucleoprotein complexes and viral glycoproteins at infected cell plasma membranes. M proteins may also function to recruit and manipulate host factors to assist virus budding, similar to retroviral Gag proteins. By yeast two-hybrid screening, angiomotin-like 1 (AmotL1) was identified as a host factor that interacts with the M protein of parainfluenza virus 5 (PIV5). AmotL1-M protein interaction was observed in yeast, in transfected mammalian cells, and in virus-infected cells. Binding was mapped to a 83-amino acid region derived from the C-terminal portion of AmotL1. Overexpression of M-binding AmotL1-derived polypeptides potently inhibited production of PIV5 VLPs and impaired virus budding. Expression of these polypeptides moderately inhibited production of mumps VLPs, but had no effect on production of Nipah VLPs. siRNA-mediated depletion of AmotL1 protein reduced PIV5 budding, suggesting that this interaction is beneficial to paramyxovirus infection.



## Introduction

Paramyxovirus particles, like those of other enveloped viruses, are formed by budding from cellular membranes. Budding occurs after viral structural components, including viral glycoproteins and viral ribonucleoprotein complexes (RNPs), have assembled together on infected cell plasma membranes (reviewed in (Schmitt and Lamb, 2004; Takimoto and Portner, 2004). Paramyxovirus assembly is coordinated by viral matrix (M) proteins, which occupy a position in virions that is between the glycoproteins and the RNPs. M proteins interact strongly with cellular membranes, and are known to self-oligomerize, forming dense layers along the inner surfaces of plasma membranes. During virus assembly, M proteins are thought to interact directly with the cytoplasmic tails of viral glycoproteins, and also with the nucleocapsid (NP) protein components of RNPs, effectively bridging these elements and concentrating them at locations from which virus particles will bud (reviewed in (Schmitt and Lamb, 2004; Takimoto and Portner, 2004).

For many paramyxoviruses, the processes of particle formation and release have been recapitulated in cells transfected to produce one or more viral proteins. The resulting virus-like particles (VLPs) are morphologically similar to authentic virions and have proven to be useful tools for dissecting steps involved in virus exit. Not surprisingly, paramyxovirus VLP production is critically dependent on the presence of the viral M proteins (Schmitt and Lamb, 2004). Nonetheless, substantial differences have been identified among paramyxoviruses in the requirements for efficient VLP production. For example, optimal production of Sendai VLPs requires expression of the viral C protein in addition to the M protein and viral glycoproteins (Sugahara et al., 2004). For the rubulaviruses, which do not encode C proteins, efficient VLP production requires co-expression of M proteins together with NP proteins and viral glycoproteins (fusion (F) protein for mumps virus; F protein or hemagglutinin-neuraminidase (HN) protein for parainfluenza virus 5 (PIV5)) (Li et al., 2009; Schmitt et al., 2002). Other paramyxoviruses, including Newcastle disease virus and Nipah virus (NiV), produce VLPs very efficiently even when M protein is expressed by itself (Ciancanelli and Basler, 2006; Pantua et al., 2006; Patch et al., 2008). Here, VLP production does not appear to increase upon co-expression of other viral proteins, although the co-expressed proteins are efficiently incorporated into the budding particles.

PIV5 (formerly SV5) is a prototype paramyxovirus. Like other paramyxoviruses, it possesses an RNA genome of negative-sense polarity that is encapsidated by the viral NP protein. The encapsidated genome is associated with the viral RNA-dependent RNA polymerase complex, composed of large (L) protein and phospho (P) protein subunits. Together, these components comprise the viral RNP core. The core is packaged into a viral envelope, which is obtained from the

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host cell plasma membrane during particle budding. The viral envelope contains densely-packed integral membrane spike proteins. The HN protein facilitates virion attachment to target cells by binding to sialic acid receptors and, in addition, possesses a sialidase activity that allows newly-formed virions to separate from infected cells. The F protein mediates fusion between viral and host cell membranes during virus entry. The small hydrophobic (SH) protein is a low-abundance membrane component that functions to block apoptosis of infected cells (He et al., 2001). The viral envelope is linked to the RNP core by the highly-abundant M protein.

Enveloped viruses typically do not encode all of the machinery necessary for proper transport, assembly, and budding of virus particles. Instead, host machinery is manipulated to allow efficient virus exit. For example, many retroviruses employ protein–protein interaction sequences (late domains) within their Gag polypeptides to recruit host factors to virus assembly sites (reviewed in (Bieniasz, 2005; Calistri et al., 2009; Chen and Lamb, 2008; Freed, 2002). Preventing this host factor recruitment significantly impairs retrovirus budding in many cases (Bieniasz, 2005; Freed, 2003; Fujii et al., 2007). The same late domains found in retroviruses are also present in the matrix proteins of some negative-strand RNA viruses such as Ebola virus (Harty et al., 2000) and Lassa fever virus (Perez et al., 2003), suggesting that the overall strategy of host protein manipulation to achieve efficient virus budding is highly conserved. Consequently, it is thought that paramyxovirus M proteins are also likely to bind host factors to facilitate virus exit. These M proteins lack the prototypical PPxY, P[T/S]AP, and YP$(x)_n$L late domains found in retroviral Gag proteins, suggesting that the details of host factor recruitment must be different for the paramyxoviruses. Alternative sequences have been identified in several paramyxovirus M proteins that have been proposed to direct host factor recruitment. These include the sequence FPIV within the PIV5 M protein (Schmitt et al., 2005), the sequence YLDL within the Sendai virus M protein (Irie et al., 2006), and the sequences YMYL (Ciancanelli and Basler, 2006) and YPLGVG (Patch et al., 2008) within the NiV M protein.

In this study, yeast two-hybrid screening was conducted in an attempt to identify host proteins that interact with the PIV5 M protein. Among the candidates identified was human angiomotin-like 1 (AmotL1), a tight junction-localized protein that contains PPxY motifs as well as a PDZ-binding motif. AmotL1 is thought to function in cells to allow normal endothelial migration and has been shown to colocalize with F-actin (Gagne et al., 2009; Zheng et al., 2009). Here, AmotL1 is shown to interact with PIV5 M protein in yeast, in transfected mammalian cells, and in virus-infected cells. Binding was mapped to a C-terminal region of the AmotL1 protein, and occurred independent of the FPIV sequence within PIV5 M protein. Overexpression of the C-terminal region of AmotL1 inhibited the budding of PIV5-like particles, and siRNA depletion of AmotL1 reduced the budding efficiency of PIV5. These results provide the first demonstration of physical and functional interaction between a host factor and a rubulavirus M protein.

## Results

### *Identification of PIV5 M-interacting host proteins by yeast two-hybrid screening*

To define cellular proteins that interact with PIV5 M protein, a yeast two-hybrid screening strategy was employed. To this end, two bait expression plasmids were constructed: one encodes full-length PIV5 M protein with LexA DNA binding domain fused to its N-terminus, and the other encodes full-length PIV5 M protein with LexA DNA binding domain fused to its C-terminus. Each of these could be expressed efficiently in yeast cells as judged by immunoblotting, and neither self-activated transcription in yeast (not shown). A premade HeLa cell-derived cDNA library containing $9.6\times10^6$ primary clones was screened using each of the two PIV5 M protein baits. The screens were performed in yeast strain L40, in which transcription of LexA-driven reporters results in the production of beta-galactosidase, and also allows growth of yeast cells on media lacking histidine. Approximately $5\times10^7$ clones were screened with each of the two baits. Library-derived cDNAs were PCR-amplified from $His^+$, beta-galactosidase$^+$ yeast colonies, and analyzed by DNA sequencing. Each of the two screens identified several candidate PIV5 M-interacting proteins, but only one of these candidates, angiomotin-like 1 (AmotL1), was identified in both screens.

Human AmotL1 (GenBank NM_130847; largest potential isoform) is a 956 amino acid long protein. AmotL1 (also known as JEAP) belongs to a family of proteins called the motins, together with angiomotin (Amot) and angiomotin-like 2 (AmotL2) (Bratt et al., 2002). These proteins are known to have cellular functions related to endothelial cell migration, angiogenesis, embryonic cell movements, and maintenance of cell polarity (Bratt et al., 2005; Gagne et al., 2009; Huang et al., 2007; Levchenko et al., 2003; Shimono and Behringer, 2003; Troyanovsky et al., 2001; Zheng et al., 2009). The N-terminal region of AmotL1 protein contains a glutamine-rich domain, and two PPPEY sequences. The central region of the protein contains a coiled-coil domain, and at the C-terminal end of the AmotL1 protein is a PDZ-binding motif (Fig. 1). None of the AmotL1 prey sequences identified by yeast two-hybrid screening corresponded to the full-length protein. Rather, all of these sequences corresponded to one of three AmotL1 subfragments designated here as AmotL1-a, AmotL1-b, and AmotL1-c (Fig. 1). Each of the three prey fragments was identified multiple times and with both baits. A short region of overlap is common to all three fragments. The overlap region spans amino acid residues 667–749 of the full-length protein, and corresponds to the C-terminal portion of the coiled-coil region.

Pairwise yeast two-hybrid assays were used to confirm the screening results. Prey plasmid DNAs were isolated from $His^+$, beta-

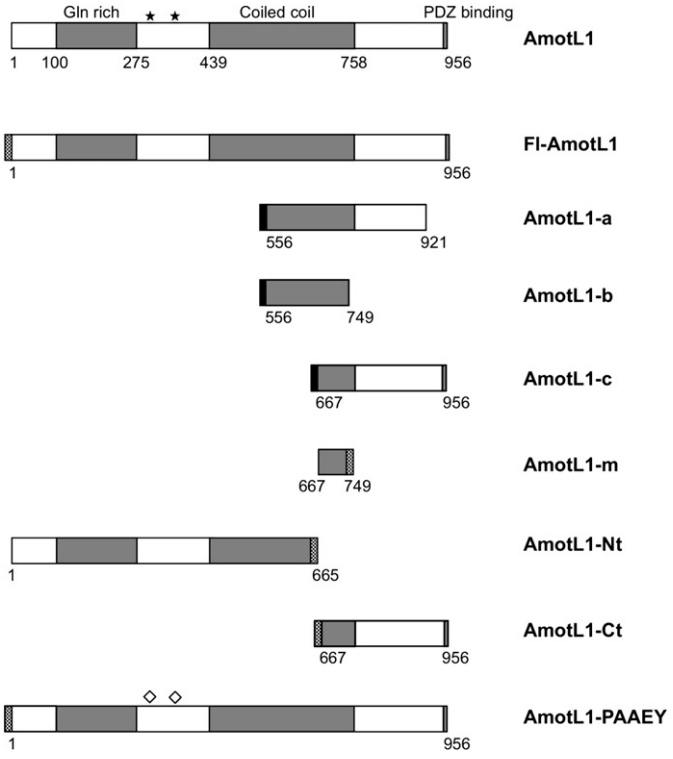


**Fig. 1.** Schematic illustration of AmotL1 protein and AmotL1-derived polypeptides. Numbers indicate the span of amino acid residues derived from AmotL1 that is contained within each construct. Checkered regions denote Flag tags, and solid black regions denote HA tags. Stars mark the positions of the two PPPEY sequences, and diamonds are used to indicate replacement of PPPEY with PAAEY.