Solution structure of the equine infectious anemia virus p9 protein: a rationalization of its different ALIX binding requirements compared to the analogous HIV-p6 protein

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Background
The equine infection anemia virus (EIAV) p9 Gag protein contains the late (L-) domain required for efficient virus release of nascent virions from the cell membrane of infected cells. The genomic position of p9 is analogous to that of the HIV-1 p6 protein and other similar proteins from different lentiviruses. Compared to HIV-1 p6, EIAV p9 has only minimal amino acid sequence homology and a considerable variation in the predicted secondary structure. Besides the function of p9 in viral DNA production and processing of the provirus [1], p9 plays, like p6 of HIV-1, an essential role in virus release, which is governed by late assembly domains (L-domains) [2-4].

Results
In the present study the p9 protein and N- and C-terminal fragments (residues 1-21 and 22-51, respectively) were chemically synthesized and used for structural analyses. CD and ¹H NMR spectroscopy provide the first molecular insight into the secondary structure and folding of this 51-amino acid protein under different solution conditions. Qualitative ¹H chemical shift and NOE data indicate that in a pure aqueous environment p9 favors an unstructured state. In its most structured state under hydrophobic conditions, p9 adopts a stable helical structure within the C-terminus. Quantitative NOE data further revealed that this α-helix extends from Ser-27 to Ser-48, while the N-terminal residues remain unstructured. The structural elements identified for p9 differ substantially from that of the functional homologous HIV-1 p6 protein.

Conclusion
These structural differences are discussed in the context of the different types of L-domains regulating distinct cellular pathways in virus budding. EIAV p9 mediates virus release by recruiting the ALG2-interacting protein X (ALIX) via the YPDL-motif to the site of virus budding, the counterpart of the YPXnL-motif found in p6 [4,5]. However, p6 contains an additional PTAP L-domain that promotes HIV-1 release by binding to the tumor susceptibility gene 101 (Tsg101) [2]. The notion that structures found in p9 differ from that of p6 further support the idea that different mechanisms regulate binding of ALIX to primary versus secondary L-domains types.

References
1. Jin S, Chen C, Montelaro RC: Equine infectious anemia virus Gag p9 function in early steps of virus infection and provirus production. J Virol 2005, 79:8793-8801.
2. Demirov DG, Freed EO: Retrovirus budding. Virus Res 2004, 106:87-102.
3. Bieniasz PD: Late budding domains and host proteins in enveloped virus release. Virology 2006, 344:55-63.
4. Martín-Serrano J: The Role of Ubiquitin in Retroviral Egress. Traffic 2007, 8:1297-1303.
5. Williams RL, Urbe S: The emerging shape of the ESCRT machinery. Nat Rev Mol Cell Biol 2007, 8:355-368.