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Timing is everything: Fine-tuned molecular machines orchestrate paramyxovirus entry

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ABSTRACT

The *Paramyxoviridae* include some of the great and ubiquitous disease-causing viruses of humans and animals. In most paramyxoviruses, two viral membrane glycoproteins, fusion protein (F) and receptor binding protein (HN, H or G) mediate a concerted process of recognition of host cell surface molecules followed by fusion of viral and cellular membranes, resulting in viral nucleocapsid entry into the cytoplasm. The interactions between the F and HN, H or G viral glycoproteins and host molecules are critical in determining host range, virulence and spread of these viruses. Recently, atomic structures, together with biochemical and biophysical studies, have provided major insights into how these two viral glycoproteins successfully interact with host receptors on cellular membranes and initiate the membrane fusion process to gain entry into cells. These studies highlight the conserved core mechanisms of paramyxovirus entry that provide the fundamental basis for rational anti-viral drug design and vaccine development.

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Introduction

Paramyxoviruses are a diverse family of viruses, which includes many human and animal pathogens that are of global importance to public health and economy. Highly infectious pathogens like respiratory syncytial virus (RSV), measles virus (MeV), mumps

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virus (MuV), parainfluenza viruses 1–5 (PIV1–5) and human metapneumovirus (hMPV) contribute significantly to the annual global disease burden in humans, infecting millions of individuals worldwide and leading to a large number of deaths in areas having inadequate health care resources. Many of these viruses are also re-emerging in previously immune populations due to a decrease in vaccination and corresponding breakdown of herd immunity (Gahr et al., 2014; Munoz-Alia et al., 2014; Rubin et al., 2012; Yang et al., 2014). Other paramyxoviruses are more sporadic in their outbreaks and viruses like the zoonotic Nipah virus (NiV) and Hendra virus (HeV) cause deadly localized outbreaks, resulting in high morbidity and mortality in human populations around the world. NiV and HeV are classified as Biosafety Level 4 (BSL-4) select agents. Cases of human-to-human transmission of NiV have become more prevalent in recent outbreaks in Bangladesh generating significant concern in terms of the epidemiology and transmission of these diseases (Daszak et al., 2012; Luby et al., 2009, 2006; Mahalingam et al., 2012). Animal viruses like the Newcastle disease virus (NDV) cause severe and sometimes fatal epidemics in poultry populations, leading to extensive economic losses. Canine distemper virus (CDV) is a fatal, highly contagious disease affecting canines. Many recent host reservoir sampling studies have indicated that a large number of paramyxoviruses remain undiscovered and uncharacterized, with no existing knowledge of the zoonotic potential, spread or host range of these viruses (Drexler et al., 2012; Lamb and Parks, 2013; Marsh et al., 2012). Both well-characterized and yet undiscovered paramyxoviruses highlight the considerable hazard posed by such emerging pathogens in an era of increasing global population, human-wildlife territorial conflicts and international travel.

Paramyxoviruses are enveloped viruses harboring a negative-sense RNA genome. Based on sequence homology and protein functions, paramyxoviruses are classified into two sub-families – *Pneumovirinae* and *Paramyxovirinae*, with the two sub-families further divided into multiple genera (Fig. 1). Like most viruses, paramyxoviruses utilize molecules present on cellular membranes, to identify host cells. Attachment via these viral 'receptors' leads to fusion of viral and cellular membranes and entry of the viral genome in the form of a nucleocapsid, into the host cell cytoplasm (Lamb and Parks, 2013). To infect host cells, most paramyxoviruses depend on the concerted actions of two major glycoproteins present on the viral membrane, namely the attachment protein (HN, H or G), and the fusion (F) protein (Heminway et al., 1994a; Horvath et al., 1992; Hu et al., 1992; Morrison and Portner, 1991; Yao et al., 1997). The membrane fusion event that mediates viral entry appears to occur at neutral pH on the plasma membrane for most paramyxoviruses. Unlike viruses of the subfamily *Paramyxovirinae*, in members of the subfamily *Pneumovirinae*, the F protein was found to be sufficient for viral propagation in cell culture (Biacchesi et al., 2005, 2004; Karron et al., 1997) and the cellular pathway of entry for this subfamily of viruses is yet unclear with membrane fusion at the cell membrane (Srinivasakumar et al., 1991), clathrin-mediated endocytosis (Kolokoltsov et al., 2007; Schowalter et al., 2009, 2006) or macropinocytosis (Krzyzaniak et al., 2013), suggested as entry routes for various members of this subfamily. Clathrin-mediated endocytosis (CME) was proposed as an entry pathway for RSV based on interactions with clathrin light chain proteins (Kolokoltsov et al., 2007) and association with cholesterol microdomains and membrane Rho-GTPases, (San-Juan-Vergara et al., 2012). Recently, Krzyzaniak and colleagues suggested macropinocytosis as the initial uptake step of RSV, based on the dependence of RSV infection on Rab5 and other macropinocytosis-associated proteins (Krzyzaniak et al., 2013). Thus *Pneumovirinae* appear to utilize one or more of these pathways to gain access to the host cell cytoplasm, while *Paramyxovirinae* primarily utilize the cellular surface entry route.

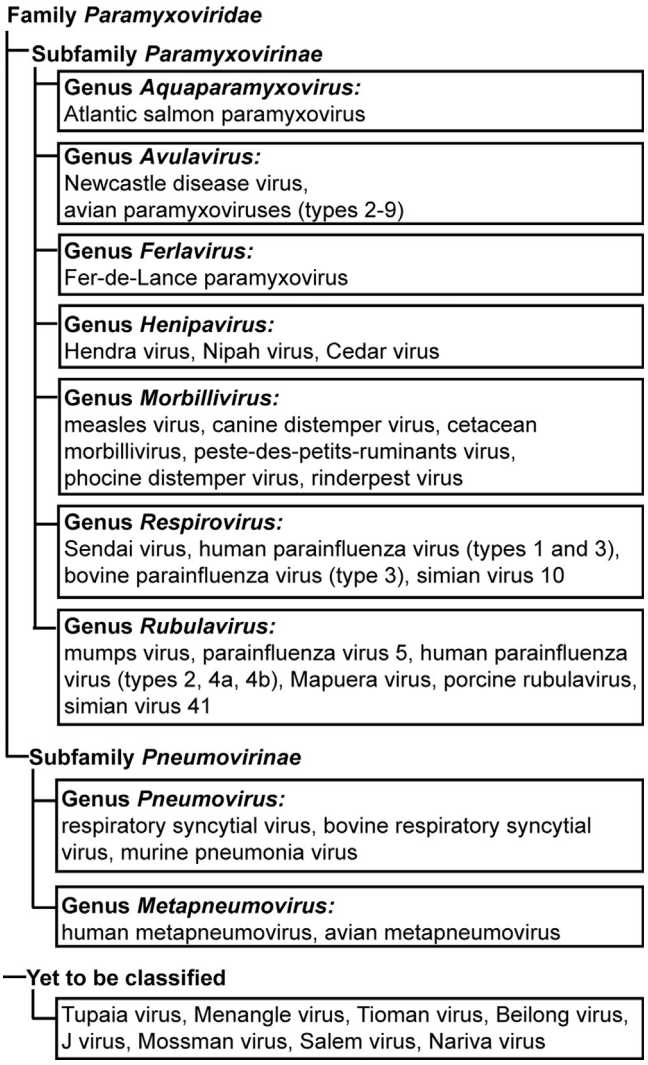

Fig. 1. Family *Paramyxoviridae*. Classification of viruses in the family *Paramyxoviridae*, showing subfamilies – *Paramyxovirinae* and *Pneumovirinae*, along with the various genera and representative examples of each genus.

Gaining access to the cytoplasm: viral membrane fusion proteins

Paramyxovirus glycoproteins F and HN, H or G are important for the initial infection step, as well as subsequent cell–cell spread. The latter mode of transmission has being suggested as the major clinical route of spread within tissues of a living host (Duprex et al., 1999; Ehrengruber et al., 2002; Sattentau, 2008). F and HN, H or G transiently expressed in cells are able to cause cell–cell fusion, potentially creating a transmission route for the viral nucleocapsid between adjacent cells (McChesney et al., 1997). Additionally, a recent report shows a secondary route for cell–cell spread of PIV5 using actin-associated intercellular connections that may bypass membrane fusion requirements between some cells of a tissue (Roberts et al., 2015).

Paramyxovirus F proteins are Class I viral membrane fusion proteins which are structurally and functionally similar to other Class I viral membrane fusion proteins from viruses that include Ebola virus, human immunodeficiency virus (HIV), influenza virus and severe acute respiratory virus-coronavirus SARS-CoV among many others (Bartesaghi et al., 2013; Caffrey et al., 1999; Chan et al., 1997; Julien et al., 2013; Lee et al., 2008a; Li et al., 2005; Malashkevich et al., 1999; McLellan et al., 2013, 2011; Pancera et al., 2014; Swanson et al., 2010; Varghese and Colman, 1991; Weissenhorn et al., 1998; Wiley

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