Filamentous influenza viruses
Bernadeta Dadonaite,1 Swetha Vijayakrishnan,2 Ervin Fodor,1 David Bhella2 and Edward C. Hutchinson1,2

1Sir William Dunn School of Pathology, University of Oxford, South Parks Rd, Oxford OX1 3RE, UK
2MRC-University of Glasgow Centre for Virus Research, University of Glasgow, 464 Bearsden Rd, Bearsden, Glasgow, Lanarkshire G61 1QH, UK

Clinical isolates of influenza virus produce pleomorphic virus particles, including extremely long filamentous virions. In contrast, strains of influenza that have adapted to laboratory growth typically produce only spherical virions. As a result, the filamentous phenotype has been overlooked in most influenza virus research. Recent advances in imaging and improved animal models have highlighted the distinct structure and functional relevance of filamentous virions. In this review we summarize what is currently known about these strikingly elongated virus particles and discuss their possible roles in clinical infections.

Introduction
Influenza viruses are serious human and animal pathogens which have been intensively studied for decades. Despite such close attention, one of the most striking features of influenza infections has been typically overlooked. Although influenza viruses are often described as producing spherical virions, natural infections are characterized by the additional presence of filaments: extremely elongated virions, which can reach microns in length.

This oversight can be explained by the ease with which influenza viruses adapt to laboratory culture, a trait that has allowed so many other advances in their study. Passage in embryonated chicken eggs, which has been used to produce many commonly studied strains of influenza virus, rapidly selects against the production of filaments (Fig. 1) (Choppin, 1963; Hayase et al., 1995; Seladi-Schulman et al., 2013). Filaments are also less physically robust during laboratory purification methods than spherical virions, further complicating their characterization (Ada & Perry, 1958; Burnet & Lind, 1957; Roberts et al., 1998; Valentine & Isaacs, 1957; Vijayakrishnan et al., 2013). As a result, although influenza filaments have been recognized since 1946 (Mosley & Wyckoff, 1946), their study has, until recently, been sporadic.

It is now clear that mixtures of spherical and filamentous virions can be produced by influenza A, B and C viruses (Chu et al., 1949; Mosley & Wyckoff, 1946; Nishimura et al., 1990). Filament production has been repeatedly observed with low-passage clinical and veterinary influenza A virus isolates (Basu et al., 2012; Choppin et al., 1960; Chu et al., 1949; Elton et al., 2013; Hayase et al., 1995; Itoh et al., 2009; Kilbourne & Murphy, 1960; Lang et al., 1968; Seladi-Schulman et al., 2013; Shortridge et al., 1998) as well as in lung sections from a fatal human case (Nakajima et al., 2010). A similar mixture of filaments and spherical virions has been observed for other orthomyxoviruses: in a low-passage isolate from a fatal human thogotovirus infection (Kosoy et al., 2015) and in infectious salmon anaemia viruses in tissue cultures and the tissues of infected fish (Crane & Hyatt, 2011; Kibenge et al., 2001; Koren & Nylund, 1997). This suggests that the ability to produce filaments may be a general feature of the orthomyxovirus family.

Many observations of filament structure have been limited by the need to use electron microscopy (EM) methods such as negative staining, metal shadowing and ultrathin sectioning of resin embedded material. Although informative, these depend on heavy metal contrasting agents, and often chemical fixation, and are therefore prone to artefacts including sample deformation and shrinkage. Following the development of cryo EM, it has been possible to determine the structure of filaments to higher resolution in a close-to-native environment without sample preparation artefacts (Calder et al., 2010; Vijayakrishnan et al., 2013; Wasilewski et al., 2012). This has shown that filaments have a distinctive and highly ordered ultrastructure.

The importance of filaments in natural infections is highlighted by recent experimental studies with influenza A viruses in animal models. These showed that despite being selected against in egg passage, filament production is selected for during serial intranasal passage of a highly laboratory-adapted spherical strain in guinea pigs (Seladi-Schulman et al., 2013). Furthermore, filament formation correlates with transmissibility between co-housed guinea pigs and by respiratory droplets in ferrets (Campbell et al., 2014a; Lakdawala et al., 2011). Taken together with the many observations of recently-isolated strains producing
filaments, these data indicate that the filamentous phenotype is an important but neglected feature of natural influenza infections.

In this review we summarize seven decades of work on influenza virus filaments, with a particular emphasis on recent structural and molecular biology studies. We also discuss possible functions of this often-neglected trait in the virus life cycle.

**Filament structure**

Influenza infections do not produce virions of a single, well-defined size. However, the virions produced by laboratory-adapted ‘spherical’ influenza viruses have broadly consistent dimensions. The majority (typically 65–75%) are spherical (axial ratio <1.2), with a mean outer diameter of 120 nm (Harris et al., 2006; Yamaguchi et al., 2008). Irregularly-shaped virions are often observed (Almeida & Waterson, 1967a, b; Harris et al., 2006; Ruigrok et al., 1986; Stevenson & Biddle, 1966; Wrigley, 1979), but it appears that many of these result from damage during ultracentrifugation, storage and sample preparation for electron microscopy (Noda, 2011; Sugita et al., 2011). ‘Spherical’ strains also produce a minority of well-preserved but elongated virions, which for the most part are still less than 250 nm in length – too short to be described as filaments (Calder et al., 2010; Harris et al., 2006; Wasilewski et al., 2012; Yamaguchi et al., 2008). These intermediate-length virions, which often appear to be ellipsoidal, capsular or kidney-bean shaped, have been described as bacilliform (Vijayakrishnan et al., 2013).

Influenza viruses that retain their natural morphology produce not only spherical and bacilliform virions, but also a class of highly elongated virions, or filaments (Figs 1, 2) (Ada et al., 1958; Calder et al., 2010; Roberts et al., 1998; Vijayakrishnan et al., 2013). These striking structures are typically more than 250 nm in length and can reach many microns (Fig. 3). Filaments reaching or exceeding 30 µm in length have been reported (Cox et al., 1980; Roberts et al., 1998), though their exact range of size is hard to determine as they are fragile (Burnet & Lind, 1957; Valentine & Isaacs, 1957), comparatively hard to purify (Ada et al., 1958; Sugita et al., 2011), prone to aggregation (Cox et al., 1980) and are hard to capture completely when cutting thin sections for transmission EM. The proportion of filaments in a given sample varies widely and depends on the virus strain used, tissues infected and the handling of virions (Bourmakina & Garcia-Sastre, 2003; Rossman et al., 2012; Seladi-Schulman et al., 2013; Vijayakrishnan et al., 2013).

During the budding of filamentous influenza A and C viruses, cord-like associations of multiple filaments have often been observed (Beale et al., 2014; Bialas et al., 2014; Bruce et al., 2010; Elton et al., 2013; Morgan et al., 1956; Muraki et al., 2007; Muraki et al., 2004; Nishimura et al., 1990; Simpson-Holley et al., 2002). End-to-end association of filaments has also been reported (Calder et al., 2010; Vijayakrishnan et al., 2013), though it is unclear if this is due to separate filaments associating, concatenated filaments arising from incomplete budding or a single filament fragmenting.

Filaments can be distinguished from spherical virions not just by their great length, but because their width, which around 80 nm, is less than that of the spherical virions (Fig. 3a; Morgan et al., 1956; Vijayakrishnan et al., 2013). Infectious salmon anaemia virus, another orthomyxovirus, also produces narrower filamentous and wider spherical virions (Koren & Nylund, 1997). Bacilliform virions have an intermediate width of around 95 nm (Calder et al., 2010; Harris et al., 2006; Vijayakrishnan et al., 2013; Wasilewski et al., 2012; Yamaguchi et al., 2008), and show an inverse correlation between length and diameter (Fig. 3b) (Vijayakrishnan et al., 2013). Particles can therefore be categorized based on their axial ratio (<1.2 for spherical virions, >1.2 for bacilliform virions and filaments) and length (>250 nm for filaments; Fig. 3). Particle dimensions do not provide sharp distinctions between these categories but they are useful, as closer examination shows that each category of virion has a characteristic composition and structure.

**Filament composition**

**Viral components**

Haemagglutinin (HA) and neuraminidase (NA) are the two major viral glycoproteins present in the envelope of influenza virus. HA binds to sialic acid, the viral receptor, and is required for entry of the virus into the host cell. NA mediates the release of viral progeny from the cell by cleaving sialic acid from cell surface proteins. The glycoproteins have a characteristic fringe-like appearance in electron micrographs of virions (Fig. 2), and tomography shows that both spherical virions and filaments incorporate an abundance of...
HA along with smaller quantities of NA (Calder et al., 2010; Harris et al., 2006). NA forms clusters that, in bacilliform and filamentous virions, tend to be at the pole proximal to the budding site on the host cell surface, at the opposite end of the virion to the viral genome (Fig. 3a; Calder et al., 2010; Chlanda et al., 2015; Harris et al., 2006; Murti & Webster, 1986; Wasilewski et al., 2012). It is possible that this clustering of NA may play a role in the formation of cord-like bundles of budding filaments – with NA sequestered at the poles, sufficient sialic acid may remain attached to surface proteins along the length of the filaments to allow HA on adjacent filaments to bind. The viral glycoproteins appear to be more regularly distributed on filaments than on spherical virions, suggesting interactions with a more ordered matrix layer beneath (Wasilewski et al., 2012).

The matrix layer, which is bound to the internal surface of the viral membrane, is made of the M1 protein. M1 multimerizes to form a helical matrix, the organization of which appears to influence virion morphology (Calder et al., 2010). Multimerized M1 can form lattices with a range of curvatures: along the length of filaments it forms a rigid cylindrical helix, whereas in spherical particles and at the poles of filaments it appears to form a less-ordered spiral (Calder et al., 2010). The poles of filaments sometimes form enlarged oval structures. Where these enlarged structures have a diameter greater than 200 nm they are termed Archetti bodies (Fig. 2c) (Archetti, 1955; Vijayakrishnan et al., 2013). Archetti bodies retain a contiguous matrix layer and can sometimes contain coils of M1-like material that are not membrane-associated (Vijayakrishnan et al., 2013). Similar coils are observed within filaments as their structure transforms and becomes disorganized at low pH (Calder et al., 2010), a change that mimics the fragmentation of filaments in acidifying endosomes during viral entry (Rossman et al., 2012), suggesting that Archetti bodies may arise from a partial breakdown of filament structure.

The genome of influenza viruses consists of segments of viral RNA bound to the viral polymerase proteins (PB2, PB1 and PA/P3) and nucleoprotein (NP). It can be clearly visualized in spherical virions as a complex of rod-shaped segments, the longest spanning the internal diameter of the virion (Fig. 3a; Calder et al., 2010; Noda et al., 2006). Studies of mutant viruses suggest that genome packaging is not strictly necessary for virion assembly, although it can make assembly more efficient (Gavazzi et al., 2013; Hutchinson et al., 2008), and, in practice, most virions do not have a full complement of functionally active genome segments (Brooke et al., 2014; Brooke et al., 2013; Heldt et al., 2015). Images of the genome have been obtained in bacilliform virions (Fig. 2a) and, occasionally, in filaments, particularly shorter ones (Fig. 2b) (Calder et al., 2010; Noda et al., 2006; Vijayakrishnan et al., 2013; Wasilewski et al., 2012). In such cases, the viral genome appears to remain associated with one pole of the virion (Calder et al., 2010; Vijayakrishnan et al., 2013; Wasilewski et al., 2012) – the distal tip when virions bud from the cell membrane (Fig. 3a; Noda et al., 2006). The rod-shaped genome segments associate with the virion pole through their tips, and in filaments they appear to remain closely associated in a parallel array (Calder et al., 2010; Vijayakrishnan et al., 2013). In spherical virions, this ordered clustering of genome segments can also be observed, though disordered arrangements of the genome appear to be more common (Harris et al., 2006).

At present, the efficiency with which filaments package the viral genome is unclear. Early observations suggested that filaments might incorporate more viral genome, and be more infectious, than spherical virions (Ada & Perry, 1958;
Ada et al., 1958, 1957; Burleigh et al., 2005; Roberts et al., 1998), a hypothesis consistent with the greater resistance of filament-containing stocks to ultraviolet inactivation (Smirnov et al., 1991), and recalling the polyploid filamentous virions of Ebola virus (Beniac et al., 2012). However, these observations could also be explained by the tendency of multiple filaments to form cord-like bundles. Other negative data do not support the hypothesis that filaments can package multiple copies of the genome: clear images of the genome have only been obtained in a minority of longer filaments, multiple copies of the genome have not been clearly visualized within a single virion (Morgan et al., 1956; Vijayakrishnan et al., 2013) and fragmentation of filaments using a number of methods does not increase the infectious titre (Ada & Perry, 1958; Burnet & Lind, 1957; Donald & Isaacs, 1954; Valentine & Isaacs, 1957).

The ion channel M2 has not been detected by immunofluorescence in filaments, suggesting that it is not an abundant component (Rossman et al., 2010a). However, its presence can be inferred as an M2-binding antibody causes filaments to fragment, while an M2 inhibitor allows filaments to resist fragmentation at low pH (Rossman et al., 2010a, 2012). NS1 and NEP are known to be present at low levels in spherical virions (Hutchinson et al., 2014), but their presence in filaments has not been assessed.

**Host components**

All influenza virions incorporate membrane from the host cell. As with spherical virions, the envelopes of filaments are resistant to low-temperature non-ionic detergent extraction and contain material with a low buoyant density, implying the incorporation of lipid rafts (Simpson-Holley et al., 2002). Cholesterol also appears to be important for filament stability (Rossman et al., 2010a). Spherical virions incorporate a substantial quantity of host-encoded proteins, resembling those incorporated into exosomes (Hutchinson et al., 2014; Shaw et al., 2008). It is reasonable to assume that filaments also incorporate such host proteins – quite possibly more than spherical virions, due to their larger membrane area and internal volume – but this has not been assessed in detail. Fibrillar material, which does not have the appearance of the viral genome, has been observed inside filamentous particles but its identity is still unclear (Vijayakrishnan et al., 2013).

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**Fig. 3.** Dimensions of influenza virions. The dimensions of influenza virions, shown (a) as a schematic of budding and released virions, with typical sizes indicated, and (b) as measurements of purified influenza A/Udorn/72 virions. For (a) it should be noted that the incorporation of NS1 and NEP has so far only been examined in spherical virions, and their general incorporation is inferred from this. For (b) measurements of 96 virions were taken by cryoelectron microscopy (data replotted from Vijayakrishnan et al., 2013). Open circles indicate filaments that extended beyond the field of view and so are longer than measured. Spherical virions (s) are distinguished from bacilliform virions (b) by having an axial ratio less than 1.2 (dashed line); filaments (f) are distinguished from bacilliform virions by having a length greater than 250 nm (solid line).
Filamentous influenza viruses

Table 1. Influenza A virus mutations known to influence filament production

<table>
<thead>
<tr>
<th>Segment (gene)</th>
<th>Residues</th>
<th>Phenotype</th>
<th>Context</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 (M1)</td>
<td>R95K</td>
<td>Reduces filament formation</td>
<td>WSN with Udorn M1 gene; residues changed to match WSN</td>
<td>Bourmakina &amp; Garcia-Sastre (2003)</td>
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<td></td>
<td>E204D</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>A41V</td>
<td>Reduces filament formation</td>
<td>Udorn, selected for resistance to an anti-M2 antibody</td>
<td>Roberts et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>P41A</td>
<td>Reduces filament length</td>
<td>SNP04 and PR8:SNP04 reassortants</td>
<td>Campbell et al. (2014b)</td>
</tr>
<tr>
<td></td>
<td>S85N</td>
<td>Confers filamentous morphology</td>
<td>PR8 with Miami M1 gene; residues changed to match Nkt</td>
<td>Elton et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>N231D</td>
<td>Confers filamentous morphology</td>
<td>WSN</td>
<td>Burleigh et al. (2005)</td>
</tr>
<tr>
<td>7 (M2)</td>
<td>S71A+M72A+R73A</td>
<td>Reduces filament formation</td>
<td>Udorn</td>
<td>Rossman et al. (2012)</td>
</tr>
<tr>
<td>5 (NP)</td>
<td>R214K and I217S</td>
<td>Reduces filament formation</td>
<td>WSN with Aichi M1 gene; NP residues changed to match Aichi</td>
<td>Bialas et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>F253I</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Morphology (filamentous or spherical) is as originally defined in the cited studies. Abbreviations: Aichi, A/Aichi/2/68; Miami, A/equine/Miami/63; Nkt, A/equine/Newmarket/11/03; PR8, A/PR8/34; SPN04, A/swine/Spain/53207/2004; Udorn, A/Udorn/72; Vic, A/Victoria/3/75; WSN, A/WSN/33.

General requirements for virion formation

All influenza virions are formed at the cell surface in a concerted process that requires both viral and host components (Fig. 3a; Hutchinson & Fodor, 2013; Noda et al., 2006; Rossman & Lamb, 2011). Viral glycoproteins accumulate at the apical plasma membrane and are individually sufficient to cause budding when over-expressed (Chen et al., 2007; Chlanda et al., 2015). M1 is neither necessary nor sufficient for the production of virus-like particles, but it does appear to be required for the production of infectious virions (Chen et al., 2007). This may be due to its interactions with genome segments: genome packaging increases the efficiency of budding, though the importance of this appears to depend on cell type (Hutchinson et al., 2008). Finally, abscission of the budded virion is mediated by M2 in an ESCRT (endosomal sorting complexes required for transport)-independent process (Rossman et al., 2010b). While defects in normal virion assembly can produce irregular virions that superficially resemble filaments – for example, the elongated and distended virions resulting from mutations in the cytoplasmic tails of NA or HA (Jin et al., 1997; Mitnaul et al., 1996) or the beads-on-string structures due to M2 scission mutants (Rossman et al., 2010b) – ‘well-formed’ filaments appear to result from a process which involves a number of host and viral determinants.

Host determinants of filament formation

Virions require host processes to assemble, and interfering with these processes can impair filament formation. Drugs targeting the actin cytoskeleton, depletion of Rab11-family interacting protein 3 (FIP3, which regulates actin dynamics and membrane trafficking) and cholesterol depletion have all been shown to specifically reduce filament formation, while depletion of or mutation of Rab11 (a GTPase involved in endocytic recycling) affects both spherical and filamentous particle production (Bruce et al., 2010; Roberts et al., 1998; Rossman et al., 2010a; Simpson-Holley et al., 2002). It has been posited that all of these processes affect the supply of lipid-raft enriched membrane required to form the extensive surfaces of filaments. In addition, mutation of an LC3-interacting region (LIR) in the viral M2 protein, a motif which allows interaction with autophagosomal membranes via LC3, or depletion of ATG16L1, which is required for LC3 activation, reduces filament formation and decreases the stability of filamentous virions. This suggests that autophagosomal membranes are recruited to support filament formation (Beale et al., 2014).

Even without active intervention, some cell lines are less permissive to filament formation than others. The same strain of virus can have different morphologies in different cell lines (Al-Mubarak et al., 2015; Bialas et al., 2012; Itoh et al., 2009; Lakdawala et al., 2011), though whether a particular cell line is permissive for filaments can vary between different strains of the virus (Al-Mubarak et al., 2015).

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Generally, polarized cell types are more permissive to filament formation compared with non-polarized cells, consistent with a role for the cytoskeleton in determining viral morphology (Roberts et al., 1998).

**Viral determinants of filament formation**

Filament formation is a heritable trait. Spherical virions purified from filament-forming stocks can form filamentous progeny (Chu et al., 1949) and the selection against filament formation during passage in embryonated chicken eggs can be slowed by passing only the minimal amount of virus necessary for an infection, thereby excluding low-frequency mutants from the stock (Burnet & Lind, 1957). Although viral genes clearly contribute to filament formation, the trait is complex. There appear to be multiple pathways to filament formation, with some loci relevant only in particular genetic backgrounds, while others suppress filament formation in genotypes that would normally support it. A summary of loci in influenza A virus genes known to affect filament formation is given in Table 1.

Unsurprisingly, considering its role in maintaining virion structure (Calder et al., 2010), the majority of mutations affecting filament formation have been mapped to M1. Reverse-genetic studies using reasortant viruses showed that the M1 gene of influenza A/Udorn/301/72 virus (Udorn), one of the few strains to retain filament-forming ability after laboratory passage (Roberts et al., 1998), can confer filament-forming ability on spherical strains such as the influenza A/WSN/33 (WSN) or A/Puerto Rico/8/1934 (PR8) viruses (Bourmakina & Garcia-Sastre, 2003; Noton et al., 2007). Reciprocally, the M1 gene from the spherical WSN strain abrogates filament production by the normally filament-forming influenza A/Victoria/3/75 virus (Elleman & Barclay, 2004). While most studies of filament formation have considered influenza A viruses, a key role for M1 has also been identified in an influenza C virus (Muraki et al., 2007).

To date, mapping filament determinants in M1 has not produced a clear mechanistic model of filament formation. Difficulties in interpretation arise from the multiple structural roles of M1, which include membrane binding, homo-oligomerization and interactions with other viral proteins (Burleigh et al., 2005), as well as from the overlap of the M1 gene with other viral genes, notably for the ion channel M2. For example, M1 residue 41 was one of the first residues to be experimentally associated with filament formation (Campbell et al., 2014b; Roberts et al., 1998; Zebedee & Lamb, 1989); it is also known to have mutated during adaptation of the WSN strain, which is now spherical, to mouse brain passage (Ward, 1995). However, mutations affecting this position have also been shown to create a splice-variant form of the M2 protein, whose role in morphology remains to be determined (Wise et al., 2012).

One mechanism underlying filament formation can be inferred from studies of helix six, a basic alpha helix in M1. Scanning alanine mutagenesis shows that a number of residues in this region are required for the production of regularly-shaped virions and one mutation, M1 K102A, confers filament-forming abilities on spherical viruses (Burleigh et al., 2005). This mutation, which is adjacent to a proposed M1–M1 interaction site (Harris et al., 2001), causes M1 in virions to form a helix with symmetry similar to that observed in the filamentous Udorn strain, emphasizing the importance of an ordered M1 helix in maintaining filament structure (Calder et al., 2010).

Other loci influencing filament formation have been mapped to the HA, NP, NA and M2 proteins (Table 1). Most strikingly, an appropriate NA can enhance filament formation in a laboratory-adapted virus and even induce limited filament formation when overexpressed alone (Campbell et al., 2014a; Chlanda et al., 2015). The influence of these proteins on filament formation has generally been attributed to altered interactions with M1 (Bialas et al., 2014; Chen et al., 2008; Liu et al., 2002) or by their influence on processes upstream of virion assembly. For example, it has been suggested that mutations in M2 and NA influence filament production by altering the recruitment of lipid rafts required for the virion membrane (Enami & Enami, 1996; Jin et al., 1997; Mitnau et al., 1996; Rossman et al., 2010a; Zhang et al., 2000).

**Filament function**

Decades of observational work, and more recent experimental studies in animal transmission models (Campbell et al., 2014a; Lakdawala et al., 2011; Seladi-Schulman et al., 2013), clearly show that filament-forming viruses have a selective advantage in natural influenza infections. While it is possible that filament production itself is a ‘spandrel’ – a conspicuous by-product of some underlying trait (Gould & Lewontin, 1979) – the more obvious explanation is that filaments act directly to increase viral fitness in their natural hosts. However, the particular functions of filaments have been difficult to determine due to the difficulty in completely separating spherical and bacilliform virions from filaments during analysis, and as filaments do not typically provide an advantage in embryonated eggs or in the tissue culture systems most suited to detailed functional studies. Despite these difficulties, a number of properties of filaments have been identified which suggest functional roles.

**The costs of making filaments**

Filamentous strains have a clear selective disadvantage in embryonated chicken eggs (Seladi-Schulman et al., 2013). This may be due to the specific constraints of egg passage. For example, virions grown in eggs incorporate a different profile of host proteins to those grown in mammalian cells, notably by utilizing different members of the tetraspanin family of membrane proteins (Hutchinson et al., 2014). It is possible that these egg-specific proteins, which are relatively abundant in virions, may increase the costs of filament production.
Alternatively, it may be that filaments have intrinsic costs that are not compensated for during laboratory passage. This also applies to passage in tissue cultures, in which the rate at which filamentous strains replicate varies, but does not typically exceed that of spherical strains. Filaments require a greater amount of membrane and viral proteins to form each virion, and the route of filament entry is different from that of spherical virions. Although filaments have receptor binding activity (Ada et al., 1958; Burnet & Lind, 1957; Chu et al., 1949; Donald & Isaacs, 1954; Seladi-Schulman et al., 2014), they are too large to enter cells through the canonical clathrin-mediated endocytic pathway that can take up spherical and bacilliform virions. Instead, filaments undergo delayed uptake through macropinocytosis, and break apart into smaller fragments as endosomal acidification triggers conformational changes in the virion (Rossman et al., 2012; Sieczkarski & Whitaker, 2005). The comparative efficiency of this process is unclear. While these issues may account for some of the fitness cost of filaments in laboratory culture, the structure of filaments suggests two advantages that may overcome these costs during a natural infection.

**Filaments may be more robustly infectious**

Firstly, some studies suggest that filaments may have higher specific infectivities than spherical virions (Ada & Perry, 1958; Ada et al., 1958, 1957; Burleigh et al., 2005; Roberts et al., 1998). As discussed above, the implication that this is due to packaging multiple genomes is contentious, and the same effect could also be achieved by the frequently observed association of individual filaments into higher-order cord-like structures (Beale et al., 2014; Bialas et al., 2014; Bruce et al., 2010; Elton et al., 2013; Morgan et al., 1956; Muraki et al., 2007, 2004; Nishimura et al., 1990; Simpson-Holley et al., 2002). Physically associating multiple genomes, in a single particle or a cluster of particles, is unlikely to be advantageous in the high-multiplicity infections that characterize laboratory growth and (presumably) foci of infection within the host. However, it would be expected to increase the efficiency of low-multiplicity infections during the spread of viruses within and between hosts, given that virions typically lack at least one functional gene segment (Brooke et al., 2013; Heldt et al., 2015). Redundant copies of the genome may also provide some resistance to ultraviolet inactivation during between-host passage (Smirnov et al., 1991). Packaging additional genomes which lack a full complement of functional segments even raises the intriguing possibility of variable gene dosage for each segment within the context of a high-multiplicity infection (Brooke et al., 2014).

Secondly, it has recently been noted that influenza genomes can pass directly between cells though an actin-dependent, NA-independent process without packaging into virions (Mori et al., 2011; Roberts et al., 2015). Although this has not been demonstrated for filamentous influenza viruses, it is plausible that filaments could enhance cell-associated spread and provide an advantage in natural infections, for example, by evading mucociliary clearance. However, direct transmission of a filamentous influenza virus has not been demonstrated.

**Filaments may be an adaptation to spread through mucus**

Influenza virions bind to sialic acid, which is cleaved by the viral NA. This NA activity is required during the initiation and within-host spread of an infection, to prevent virions being sequestered by sialic acid moieties on the mucins in respiratory mucus, as well as on the surfaces of infected cells when new virions are produced (Chlanda et al., 2015; Cohen et al., 2013; Matrosovich et al., 2004; Yang et al., 2014). Filaments appear to have an elevated NA activity, which would potentially enhance infectivity in natural hosts (Campbell et al., 2014a, b; Seladi-Schulman et al., 2014). Surprisingly, this activity is increased by mutations in M1 that allow filament formation, even when NA itself is unaltered (Campbell et al., 2014a, b; Seladi-Schulman et al., 2014). It is unclear whether this is due to the clustering of NA at the tips of filaments (Calder et al., 2010, 2015) or to the abundance of NA in filaments, which has not been determined.

There are only limited data available on the relative stability of filaments and spherical virions within mucus. Filaments do appear to be fragile during laboratory manipulations, but despite this they appear to be no more susceptible to heat inactivation than spherical strains (Beale et al., 2014; Seladi-Schulman et al., 2014). They can be bound by antibodies (Chu et al., 1949), but they appear to be no more susceptible to neutralization than spherical virions in *in vitro* assays (Seladi-Schulman et al., 2014). Conversely, it has been suggested that non-infectious filaments may serve as an immune decoy by sequestering IgA antibodies away from smaller particles during infection (Vijayakrishnan et al., 2013). Finally, as well as being a barrier to infection and within-host spread, mucus is ultimately the vehicle for between-host spread. Longer filaments would be able to extend through the low-viscosity periciliary layer, which coats the airway epithelium to a depth of around 7 µm (Button et al., 2012; Fahy & Dickey, 2010), potentially providing more efficient access to the gel-like airway mucus beyond and increasing the likelihood of their respiratory transmission. Respiratory transmission of influenza genomes in ferrets occurs most efficiently in large (>4 µm) droplets of mucus. While the same distribution between droplet sizes is observed for both filamentous and spherical strains, it is notable that these droplets are large enough to contain intact filaments (Lakdawala et al., 2011).

**Conclusion**

Influenza viruses naturally exhibit a range of morphologies from small spherical particles to extremely long filamentous structures. It appears that filament production may be a
common feature of orthomyxoviruses as it has been observed in influenza A, B and C viruses as well as in thogotovirus and infectious salmon anemia virus. It is also notable that a number of unrelated respiratory viruses can form filamentous virions, including respiratory syncytial virus and certain paramyxoviruses (Compans et al., 1966; Liljeroos et al., 2013; Shaikh et al., 2012; Yao & Compans, 2000), although other respiratory viruses form exclusively spherical virions.

For influenza viruses, filament formation is a heritable trait that is selected in natural transmission. Despite this, long filaments have often been neglected in laboratory studies, and the reason for their production remains unclear. Recent technical advances have allowed the structure of filaments to be analysed in detail and the conditions which select them to be studied in a laboratory setting. This has strengthened the case for filaments as a class of well-formed viral structures which provide functional benefits during natural influenza infections. What these benefits are though remains uncertain, and in clarifying them we will need to fundamentally revise our models of how influenza virus genomes are transmitted outside of a laboratory setting.

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