Remodelling of membrane tubules by the actin cytoskeleton

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Inside living cells, the remodelling of membrane tubules by actomyosin networks is crucial for processes such as intracellular trafficking or organelle reshaping. In this review, we first present various in vivo situations in which actin affects membrane tubule remodelling, then we recall some results on force production by actin dynamics and on membrane tubules physics. Finally, we show that our knowledge of the underlying mechanisms by which actomyosin dynamics affect tubule morphology has recently been moved forward. This is thanks to in vitro experiments that mimic cellular membranes and actin dynamics and allow deciphering the physics of tubule remodelling in biochemically controlled conditions, and shed new light on tubule shape regulation.

In architecture and industrial design, form follows function (Sullivan, 1896). For biological objects, from proteins to organisms, shape and function are intimately related. Living cells modify their shape to achieve biological processes such as division or motility. These shape changes rely on the reorganisation of the actomyosin cytoskeleton, a dynamic network of biopolymers (actin filaments) and molecular motors of the myosin family, which remodel biological membranes (Blanchin et al., 2014). The actin cytoskeleton comprises at least two types of filaments assemblies: branched actin gels nucleated by the Arp2/3 complex and actin bundles nucleated by Formins. The Arp2/3 complex forms new actin filaments that emerge from a pre-existing filament with an angle of 70°. In the cell, Arp2/3 complex gels are found in the lamellipodium, the cortex and in endocytic patches (Mullins et al., 1998). Formins sit at the polymerising end of filaments and stimulate their parallel elongation forming structures in cells like filopodia or microvilli (Watanabe et al., 2014; Evangelista et al., 2003), among others. Changes in the actin cytoskeleton structure resulting from the type of filament nucleation results in different mechanisms of force production and therefore different types of membrane deformation. The actin cortex, an actin-rich region close to the plasma membrane, comprising myosin motors, and linked to the membrane through Ezrin-Radixin-Moesin (ERM) proteins (Maniti et al., 2013), is an important player for membrane remodelling. Its dynamics plays an important role in global cell shape changes, as the ones implied in cell motility, but also in local shape changes implied in endocytosis.

This review focuses on the actomyosin driven remodelling of a particular type of membrane structures, hereby called ‘membrane tubules’. They are cylinders made of a lipid bilayer, with a radius of tens of nanometres and a length up to micrometres. Tubular membrane structures are found all over the cell (Figure 1) and can be classified according to their lifetime (Roux, 2013). On the one hand, some are stable tubular structures, such as mitochondria or some regions of the endoplasmic reticulum (ER). If they can undergo dynamic rearrangements, they stay globally cylindrical over time. Hereafter, we call such cases ‘structural tubules’ (section Structural Tubules). On the other hand, some tubules are ‘transient’, like

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the ones extruded from the plasma membrane or the Golgi apparatus for intracellular trafficking, in which membranes intermittently deform into vesicular or tubular carriers (section Transient Tubules).

Last, some ‘intermediate’ cases exist: endosomes and melanosomes are examples of organelles with a permanent tubular region from which carriers are dynamically pulled out (section Intermediate Tubular Structure). In all of these cases, the evolution of a tubule follows the steps depicted in Figure 2: an initially flat membrane (i), undergoes budding (ii), then this bud is pulled out and elongated (iii), potentially constricted (iv) and finally scissioned (v).

In this review, we highlight the role of actin in the dynamics of membrane tubules with the eyes of physicists. Indeed, if the role of the microtubule cytoskeleton in the elongation of membrane tubules has been widely documented (Gurel et al., 2014; Terasaki et al., 1986; Egea et al., 2015; Delevoye et al., 2014; Du et al., 2016), we show here that actin dynamics also have a crucial role in membrane tubules formation, elongation and scission. It is crucial
to understand this interaction between actin and lipid tubules to fully describe the mechanisms of cell trafficking and remodelling. Indeed, this question is also of therapeutic importance, as it is clear that intracellular traffic deregulation, namely because of actin dynamics perturbation, is a hallmark of cancer progression (Mosesson et al., 2008). For instance, some human tumours exhibit an unusual expression of proteins (e.g., HIP1, HIP1R or cortactin) which coordinate endocytic sites remodelling by the actin cytoskeleton. Therefore, links between cancer and endocytosis, or more generally tubule remodelling, through the perturbation of the actin cytoskeleton, may be the subject of further therapeutic approaches and motivates this digest of the state of the art. In sections Structural Tubules, Transient Tubules and Intermediate Tubular Structure, we present in vivo experiments that clearly demonstrate actin-induced remodelling of structural, transient and intermediate tubules, respectively, showing the ubiquitous role of actin in tubule dynamics. In particular, we explore the mechanical role of actin in the steps depicted in Figure 2. Then, in section Physics of Membrane Tubule Remodelling, we describe the physical mechanisms that explain membrane tubule morphology changes by the action of cytoskeletal forces, by comparing the forces required to deform membranes with the ones generated by actin polymerisation. Finally, we show that biomimetic systems help to unravel the physical mechanisms underlying membrane tubule remodelling by actin. The advantage over in vivo situation is that physical and biochemical parameters can be tuned to test the validity of quantitative models and help to understand the experiments on living cells (section In Vitro Tubule Remodelling by Actin).

Structural tubules
Endoplasmic reticulum
The outer nuclear envelope and the ER enclose a single space called the ER lumen that takes 10% of the cell volume. The ER membrane accounts for half of the cellular membranes. Briefly, this organelle is essential for protein and lipid syntheses. In particular, it produces lipids and transmembrane proteins inserted inside lipid bilayers. The ER delivers proteins by producing transport vesicles addressed to the Golgi apparatus. It forms an interconnected membranous network made of flattened sheets (cisternae) and tubules spreading over the cytosol (Park and Blackstone, 2010). The diameter of these tubules lies in the range of 30–100 nm (Gurel et al., 2014), whereas their length is in the 0.5–2 μm range (Perkins et al., 2020).

The role of microtubules in the maintenance of the dynamic tubular structure of the ER in mammals has been widely documented (Terasaki et al., 1986; Gurel et al., 2014). Oppositely, in yeast, microtubules have little effect on ER tubule dynamics (Prinz et al., 2000). Generally, filamentous actin (or F-Actin) colocalises with the ER, and the ER dynamics are influenced by Arp2/3 complex nucleated actin networks (Prinz et al., 2000; Griffing, 2010; Lynch et al., 2011). The formation of tubules from the ER and the control of their length (elongation and retraction) is driven by the actomyosin cytoskeleton (Sparkes et al., 2009; Griffing, 2010) (Figure 3(i)). While these tubules do not undergo scission, the presence of actin maintains the ER sheet–tubule balance and affect their dynamics (Joensuu et al., 2014). Strikingly, the ER tubule network fluctuates less when actin is depolymerised (Prinz et al., 2000). This implies that actin polymerisation actively increases the fluctuations of such tubules. Altogether, these suggest that remodelling and maintenance of sheet–tubule balance in ER rely on branched actin networks that stabilise the morphology of ER sheets. Networks mediated by formins also associate with ER/mitochondrion and ER/endosome contacts (Figures 3(ii) and 3(iii)). While the role of such networks is debated (Chhabra et al., 2009), it has been reported that formin-mediated actin networks are involved for mitochondrial fission and endosome remodelling, as described later in sections Mitochondrion and Endosomes.

Mitochondrion
Mitochondria produce the energy (adenosine triphosphate) required to ensure cellular functions can occur. They are double-membrane-enclosed organelles that represent about a fifth of the cell volume and a third of its membrane surface (Alberts et al., 2002). Mitochondria have the shape of elongated cylinders, typically 1 μm long with a diameter of 150–300 nm. These dimensions may, however, vary between cell lines (Hatch et al., 2014).

Mitochondria undergo fusion or fission to meet the metabolic needs of the cell or to ensure their
proper sharing between daughter cells during cell division. Mitochondrial fission is an example of actin-dependent tubular remodelling, even if the physical mechanisms at play are unclear. The known elements of this process are depicted in Figure 3(ii). Fission occurs at ER/mitochondrion contact sites, where mitochondria are surrounded by tubules formed from the ER (Korobova et al., 2013, 2014). Arp2/3 complex and formin mediated actin networks, together with myosins, are recruited at these sites and induce mitochondrial constriction (Korobova et al., 2013; Ji et al., 2015; Moore et al., 2016; Schiavon et al., 2020). The role of actin polymerisation or myosin contraction forces on this constriction and its precise role on mitochondrial fission remains to be elucidated. Completion of mitochondrial scission requires an additional protein, dynamin-related protein 1 (Drp1). It has been reported in vitro that Drp1 had a high affinity with actin, suggesting that the presence of actin could induce its recruitment at mitochondrial fission site (Ji et al., 2015). Ultimately, Drp1 itself radially constricts and cuts the mitochondrion (Hatch et al., 2014).

**Transient tubules**

Transient tubules are ubiquitously involved in intracellular transport, which encompasses all the cellular processes involving exchanges of material between the membrane-delimited compartments of the cell: cellular organelles and plasma membrane. Their formation follows the time sequence previously described, which ends with the release of a transport vesicle that will be carried through the cell. Such transporters gather proteins and lipids needed for the targeted region and vary in composition, size and shape (Ratamero and Royle, 2019). Most transporters are spherical but some can have a tubular shape (Martínez-Menarguez, 2013). At a given enclosed volume, a tube has a higher surface area than a sphere. Consequently, the tubular geometry favours lipid bilayer enrichment in transmembrane proteins, while spherical vesicles efficiently transport water soluble molecules.

**Endocytosis**

Endocytosis refers to the internalisation of molecules from outside of the cell by the formation of a membrane vesicle. The cell has several ways to invaginate the membrane for endocytosis. It either relies on clathrin, a protein that coats the membrane and initiates its curvature at the endocytic site (‘clathrin-mediated endocytosis’ or CME, sections Clathrin-Mediated Endocytosis in Mammalian Cells and Clathrin-Mediated Endocytosis in Yeast) or is clathrin-independent (‘clathrin-independent endocytosis’ or CIE) as in the case of Shiga toxin endocytosis (section Endocytosis of Shiga Toxin) or caveolin-mediated endocytosis (section Caveolae-Mediated Endocytosis). In the following, we describe the role of actin during these processes. In addition, one key player that controls
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**Figure 4 | Actin is involved during endocytosis**
Summary of the role of actin in several types of endocytosis: clathrin-mediated (mammals and yeast) and clathrin-independent (Shiga toxin and caveolae-mediated endocytosis). Endocytosis is based on budding, tubulation, constriction and fission. In the presence of clathrin, the timing indicates the time that precedes fission. A question mark indicates that the involvement of actin remains to be determined. Localisation of actin is indicative.

| Endocytosis Type            | Time   | Depolymerisation of Actin |
|-----------------------------|--------|---------------------------|
| **Clathrin-mediated**       | - 60 s | Arp2/3-complex mediated   |
| Mammalian cell              |        | Depolymerized actin       |
| Yeast                       | - 8 s  | Formin mediated           |
| Clathrin-independent        |        |                           |
| Shiga toxin                 | - 2 s  |                           |
| Caveolae-mediated           | 0 s    |                           |

Clathrin-mediated endocytosis in mammalian cells

It takes 1–5 min to go from invagination to fission, relying on the cooperation of numerous proteins (Yoshida et al., 2018) (Figure 4). The budding mainly results from the clathrin coat. However, electron microscopy images show that a dense branched actin network is present before tubulation and forms a collar-like actin patch that surrounds the bud (Collins et al., 2011). The orientation of the filaments suggests that the actin network produces compressive forces applied towards the bud. In a later time, during tubule maturation, depolymerisation of actin occurs until a new actin assembly is again required 1 min before completion of the fission (see first row, Figure 4). Therefore, actin polymerisation may be involved in producing compressive forces that enable scission. In addition, bent actin filaments have been observed close to the neck, this could be a way to store elastic energy that would later contribute to tubule constriction (Akamatsu et al., 2020).

Clathrin-mediated endocytosis in yeast

Several proteins cooperate to provide, in a short period of time (about 10 s), the necessary force to counteract the high hydrostatic pressure: pressure ∼10 atm = 1 MPa, membrane tension ∼0.5 mN/m and pulling force ∼1 μN for endocytosis (Minc et al.,...
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Endocytosis strictly relies on the assembly of a highly dynamic branched actin network (Gachet and Hyams, 2005; Goode et al., 2015) (Figure 4, second row) and both inhibition of actin polymerisation and stabilisation of filaments with jasplakinolide inhibits endocytosis (Ayscough, 2000; Kaksonen et al., 2003; Aghamo-hammadzadeh and Ayscough, 2009).

Endocytosis starts with the arrival of coat proteins, including clathrin and its adaptors with actin-binding domains (Skrzynz et al., 2012). This step prepares the site for the assembly of a branched actin network (Figure 4). At a late stage of the coating, about 8 s before fission, it is still unclear whether actin dynamics help the formation of the initial bud (Goode et al., 2015). Data indicates that inhibition of actin polymerisation with latrunculin A prevents membrane bending at the endocytic site (Kukulski et al., 2012; Picco et al., 2015), while immuno-EM images suggest that membrane bending precedes actin network assembly (Idrissi et al., 2012). It was suggested that membrane curvature appears only when the membrane and the actin polymerisation are coupled by clathrin adaptors at the tip of the invagination (Picco et al., 2018).

During tubule elongation, nucleation and polymerisation of new branched filaments at the plasma membrane drive tubule elongation thanks to actin-linking proteins, such as epsin, which couples F-actin and the tip (Sirotkin et al., 2010; Kaksonen et al., 2003). Tubule elongation ends 2 s before fission, up to a tubule length of 140 nm and a tip radius of 7–40 nm (Picco et al., 2018). Then, the actin network reorganises, it extends its volume, F-actin severing increases and the polymerisation rate is reduced (Chen and Pollard, 2013; Picco et al., 2015; Sirotkin et al., 2010). This reorganisation drives fission and internalisation of a vesicle (Kaksonen et al., 2005, 2003).

**Endocytosis of Shiga toxin**
The endocytosis of the bacterial Shiga toxin is an example of clathrin-independent endocytosis (Figure 4, third row). The first step of this process is the induction of tubular membrane invaginations towards the cell interior (Romer et al., 2007). Actin networks are not required for the formation and elongation of these tubules, while their scission is actin dependent. Indeed, tubule formation is due to the imposition of a local negative curvature to the membrane by the binding of the toxin, without contribution of actin dynamics. Oppositely, tube scission is clearly actin dependent, as inhibition of actin polymerisation leads to the accumulation of stable and longer tubules. To explain this scission mechanism, it has been proposed that actin dynamics were triggering the formation of lipids nanodomains in the membrane, that could lead to tubule scission (Romer et al., 2010). This hypothesis emerges from two results: On one hand, actin affects the phase behaviour of lipid domains in model membranes (Liu and Fletcher, 2006), on the other hand, the presence of domains favours tubule scission because of the energetic cost of boundaries between domains (Roux et al., 2005; Allain et al., 2004). This suggests that actin polymerisation could favour the formation of lipid nanodomains in the membrane and thus indirectly induce membrane scission.

**Ultrafast clathrin-independent endocytosis**
While most evidence shows that tubule remodelling during endocytosis relies on the presence of a dense branched actin network able to produce substantial forces, some cells internalise cargoes thanks to formin-mediated actin bundles (Soykan et al., 2017; Shin et al., 2018). For instance, neurotransmission in mice is based on the fusion of synaptic vesicles followed by two types of endocytosis: slow CME of synaptic vesicle proteins with a time of about 1–10 s and ultrafast CIE that rapidly recycles synaptic vesicles within 100 ms (Soykan et al., 2017). In particular, tubule fission during CIE is associated with formin-mediated actin bundles, coupled with myosin II, rather than mediated by the Arp2/3 complex.

**Caveolae-mediated endocytosis**
Caveolae-mediated endocytosis involves invaginations formed by the transmembrane protein caveolin, that pinches off cholesterol-rich microdomains serving as reservoirs when the plasma membrane is stretched (Conner and Schmid, 2003; Bastiani and Parton, 2010; Sinha et al., 2011). This type of endocytosis leads to the internalisation of small vesicles with a diameter of about 50–60 nm (Conner and Schmid, 2003). Caveolae-mediated endocytosis is Arp2/3 complex independent and the endocytic sites associate with actin stress fibres (Rohlich
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Echarri et al., 2012; Echarri and Del Pozo, 2015) (Figure 4). For example, the virus SV40 hijacks the endocytic pathway to enter the cell. More specifically, this virus induces a breakdown of actin stress fibres to promote virus entry through caveolae-mediated endocytosis (Pelkmans et al., 2002). Last, an actin patch emerges to form an actin tail that propels the enclosed virus from the endocytic site. While actin is unquestionably present, its mechanical role during this form of endocytosis remains to be elucidated.

Transport from the Golgi apparatus

The morphology of the Golgi apparatus is highly variable among living systems, ranging from isolated tubules to stacked compartments (Egea et al., 2013). For eukaryotes, one cisterna faces the ER to trade material, the medial stacks sort proteins for correct addressing, while the last cisterna, the trans-Golgi network (TGN), faces the plasma membrane. The latter redirects vesicles towards different cell locations, such as the plasma membrane or endosomes. The study of the interaction between actin and the Golgi apparatus is challenging because of its proximity to the nucleus, a region abundant in proteins, including actin (Gurel et al., 2014). Besides, the microtubule cytoskeleton plays an important role in maintaining the shape and dynamics of the Golgi apparatus, as reviewed in (Egea et al., 2015). In particular, microtubules and microtubules-associated motors control the formation and fusion of tubular structures from the Golgi. Here, we restrict to the effect of actin dynamics on the fate of membrane tubules.

ER–Golgi apparatus exchanges require activation of the Arp2/3 complex (Campellone et al., 2008). The induced branched actin network polymerises on the Golgi apparatus and is then involved in vesicle secretion from the TGN (Chen et al., 2004; Almeida et al., 2011). There is no evidence that actin is able to induce the budding while the Arp2/3 complex defect inhibits elongation of tubules (Almeida et al., 2011). A burst of actin, associated with several types of myosins near the Golgi, precedes fission events, suggesting the role of actomyosin dynamics during constriction and scission (Delestre-Delacour et al., 2017; Brownhill et al., 2009; Miserey-Lenkei et al., 2017; Almeida et al., 2011). Furthermore, it has been proposed that the association of actin might affect Golgi functions by spatially segregating its secretory activities to some given membrane regions, others being inhibited by the presence of actin that impairs membrane remodelling. The detailed physical mechanisms remain to be elucidated (Egea et al., 2015).

Intermediate tubular structures

Endosomes

The endosomes (from early to late) are the intermediates between organelles that send (Golgi, plasma membrane) and the ones that receive (Golgi, lysosome or plasma membrane) transport vesicles. An early endosome has a tubular-vesicular shape and is often located at the periphery of the cell to sort proteins coming from the plasma membrane. Some proteins are redirected back to the plasma membrane for recycling, while remaining early endosomes mature to form late endosomes. The late endosome is a hub that sorts proteins towards TGN or lysosomes (section Lysosomes). The nucleation of branched actin networks appears at the contact site between endosomal and ER tubules (Figure 3(iii)) (Rowland et al., 2014; Dong et al., 2016). Branched actin networks are involved in the maintenance of endosome morphology, as observed by knocking out the activation of the Arp2/3 complex (Gomez et al., 2012). However, its knockdown increases both endosomal membrane tubulation and fission defects (Derivery et al., 2009; Gomez and Bilodeau, 2009; Duleh and Welch, 2010). F-actin networks stabilise the morphology of endosomes, which might help initiate a membrane tubule, while microtubule associated-molecular motors further elongate these tubules (Delevoye et al., 2014) that eventually undergo fission thanks to the actin cytoskeleton.

Lysosomes

‘Lysosome’ is a term that includes numerous organelles, such as endolysosomes, phagolysosomes and autolysosomes, which all have different functions (Saffi and Botelho, 2019). In general, lysosomes degrade cargoes from endocytosis or autophagy. They are spherical organelles with an acidic lumen where molecules are digested. Transmembrane proteins ensure the passage of the digested products back towards the cytosol. The lysosomes also differ in shape: they can have either a vesicular or a 10 μm-long tubular shape (Chow et al., 2002). The lysosomes
are first remodelled by clathrin, which provides the initial membrane curvature required to form a bud that lately undergoes fission into a vesicle or is elongated into a tubule (Sridhar et al., 2013; Saffi and Botelho, 2019) (Figure 5A). Therefore, as for endosomal tubules, in vivo experiments show that extrusion, constriction and fission of lysosomal tubules might depend on branched actin dynamics, whereas microtubule-associated-molecular motors elongate these tubules. The physical mechanisms of actomyosin induced endosomal tubules remain to be explored.

Melanosomes

Melanosomes share common properties with endosomes and lysosomes (Marks et al., 2013). Similar to endosomes, they are hubs for endocytic cargoes, serve as a storage for melanin pigments and recycle proteins and lipids via tubule carriers. Tubulation itself might occur without the need of actin, while constriction and fission at the neck are mediated by myosin VI, a non-conventional myosin that walks on actin filaments towards their minus end, and rely on the presence of a branched actin network rather than formin-mediated actin filaments (Ripoll et al., 2018) (Figure 5B). Both depletion of the activator of the Arp2/3 complex and inhibition of the branched actin network assembly affect the neck constriction, and thus increase fission defects.

Autolysosomes

The autophagy pathway allows degradation of intracellular contents—such as proteins, organelles or foreign bodies—first encapsulated in an autophago-some that ultimately fuses with lysosomes (Yu et al., 2018). The last step of autophagy consists in autophagic lysosome reformation, during which budding, pulling of micrometre-sized tubules and fission of their tip occur successively (Munson et al., 2015; Yu et al., 2010) (Figures 5C(i) and 5(ii)). The overall process is similar to CME: clathrin initiates budding (Rong et al., 2012), molecular motors walking on microtubule tracks pull the invagination (Du et al., 2016) while dynamin induces tubule fission (Schulze et al., 2013). The presence of a branched actin network localised at the base of the invagination is required for autolysosome tubulation and might facilitate its initiation (Dai et al., 2019). The role of actin during the remaining steps is still unexplored.

Physics of membrane tubule remodelling

To understand what could be the physical effect of actin dynamics in the examples of tubules remodelling presented before, let us start by estimating the maximal force generated by a single polymerising actin filament (Kovar and Pollard, 2004). The rigidity of an actin filament is the product of its persistence length $L_p$ by the thermal energy, $k_BT \times L_p \sim 6.10^{-26}$ N m$^2$ where $k_B$ is the Boltzmann constant, $T$ the temperature, and $L_p \sim 15 \mu m$ (Isambert et al., 1995). Then, the force to buckle a filament $F$ is proportional to this rigidity and inversely proportional to the filament length $L$: $F = \pi \frac{k_BT}{2L} \times L_p$. Buckling of a one-side attached filament has been experimentally observed for a filament of $L \sim 1 \mu m$ in length (Kovar and Pollard,
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Therefore, the maximal force generated by a single actin filament can be estimated to 1 pN.

The force at the tip of finger-like cell protrusions (filopodia) has been measured with micropipette suction and optical tweezers experiments at 10–50 pN (Peskin et al., 1993; Hochmuth et al., 1996; Shao and Hochmuth, 1996). The force at the protruding edge of the lamellipodia, measured by traction force microscopy, is much larger: 10–200 nN (Harris et al., 1980; Lee et al., 1994; Oliver et al., 1995). Altogether, this show that the assembly of various actin networks enable cells to exert forces in the range of 10–200 pN on cell membranes.

Besides, the physics of lipid bilayers can be characterised in our context by two parameters: their bending modulus \( k \) and tension \( \sigma \). In cells, the bending modulus lies in the range of 10–50 \( k_B T \), where \( k_B T \) is the thermal energy, therefore the membrane is subjected to intense thermal fluctuations, while the membrane tension varies from 10 to 300 \( \mu N/m \) (Gauthier et al., 2012; Lieber et al., 2013; Batchelder et al., 2011). In the context of tubule formation, membranes deform following the previously described sequence. The first step, tubule initiation, corresponds to the budding of a flat membrane to a curved bulge. The energy per unit area required to bend a flat membrane sheet is given by \( e_b = 2k/R^2 \) where \( R \) is the curvature radius of the bulge (Helfrich, 1973). Consequently, for \( R = 100 \text{ nm} \), the maximal energy per unit area associated to bending is \( e_b = 0.04 \text{ pN/nm} \). Then, the maximal force to bend the membrane over a height of \( R \) is \( F_b = e_b \times R = 4 \text{ pN} \). This force is thus comparable to the force generated by a single actin filament of 1 pN, Therefore, few polymerising filaments in a gel can generate sufficient forces to induce a membrane bulge.

After budding, tubulation occurs if a force \( f \) is further applied on the bulge. After an overshoot force for tubule initiation (Derényi et al., 2002), the free energy of the tubule reads:

\[
F_{\text{tubule}} = \frac{\pi k}{R_t} L + 2\pi R_L \sigma - fL
\]  

(1)

where \( L \) is the tubule length and \( R_t \) its radius. This free energy is composed of three terms that respectively describe the bending energy, the effect of tension and the mechanical work of the external force. This last expression allows calculating the equilibrium tubule radius \( R_0 \) and force \( f_0 \) of the tubule, that are related by (Waugh and Hochmuth, 1987) and (Derényi et al., 2002):

\[
f_0 = 2\pi \sqrt{2k\sigma} = \frac{2\pi k}{R_0}
\]

(2)

These relations link the tubule radius \( R_0 \) with its mechanical properties (bending modulus and membrane tension). Taking the values given above allows tubule radii in the range 8–160 nm and leads to forces in the 2–160 pN range. Therefore, several polymerising filaments are sufficient to elongate a membrane tubule by pulling at its tip, provided that its tension is not too large (Simon et al., 2019).

Once the tubule is formed, it can be affected by polymerisation of proteins, such as dynamin, leading to its constriction. Whether actin can induce the same polymerisation force remains to be explored. In the situation where this polymerisation applies a pressure \( P_a \) on the tubule surface, its free energy becomes (Roux et al., 2010):

\[
F_{\text{tubule}} = \frac{\pi k}{R_0} L + 2\pi R_0 \sigma + P_a \pi R^2 L
\]

(3)

In this equation, the bending modulus term tends to increase the tubule radius, while both membrane tension and positive actin polymerisation pressure will decrease it. There is, thus, a critical radius \( R_c = 2\sigma P_a \) at which these two compressive effects are equal. To estimate the polymerisation pressure \( P_a \) in the case of actin, we consider that each filament exerts a force of 1 pN on a surface \( \xi^2 = (50 \text{ nm})^2 \), which is the characteristic actin network mesh size (Pontani et al., 2009; Kawaska et al., 2012), yielding a pressure of \( P_a = 10^3 \text{ Pa} \). The actin pressure contribution will overtake the one of membrane tension for radii \( R_0 > R_c = 20–400 \text{ nm} \). Therefore, actin alone would be dominant for large membrane tubules, which are still in the range of few actin network mesh size. Potentially, the local compression of the tubule could help with its scission and, thus, the release of a transporter.

Besides its direct role in force generation, actin may also induce indirect forces, through friction (Allard et al., 2020a; Campillo et al., 2013; Guevorkian et al., 2015; Borghi and Brochard-Wyart, 2007). During the overall process of tubule formation, lipids flow from the flat membrane towards the tubule. Since the cell membrane is highly coupled with
abundant transmembrane proteins and connected with the underlying actin cortex, lipid mobility is hindered, which affect the dynamics of tubule extrusion. The presence of such a friction could ultimately lead to the scission of tubules under elongation (Simunovic et al., 2017). As previously described, there is experimental evidence of the role of actin in one or several steps of membrane tubule remodelling in vivo (sections Structural Tubules, Transient Tubules and Intermediate Tubular Structure). Due to the complexity of the processes involved in cells, deciphering the mechanical role of actin in tubule remodelling is difficult. The use of in vitro systems is thus an appealing alternative.

In vitro tubule remodelling by actin
Assessing the role of actin in vivo can be complex because of its numerous vital functions (division, migration, etc.). Thus, modifying the actin cytoskeleton to determine its effect on a particular process can induce perturbations on others. Therefore, the physical mechanisms by which actin affects tubule morphology are challenging to elucidate in the complex environment of the cell interior. Moreover, the nanometric size of tubules, comparable with the typical mesh size of actin networks (~30–50 nm; Kawaska et al., 2012), adds an additional difficulty. In order to reduce this complexity, in vitro systems present the advantage of a controlled biochemical environment and enable nano-imaging and mechanically probing the tubules, opening the way to decipher the mechanical role of actin in tubule remodelling.

Historically, biomimetic experiments based on actin polymerisation at the surface of micrometric objects could first reproduce actin-based motility on: hard beads (Loisel et al., 1999; Cameron et al., 1999; Noireaux et al., 2000; Bernheim-Gros-Fassler et al., 2002), liquid droplets (Boukellal et al., 2003; Trichet et al., 2008) or giant liposomes larger than 10 μm (Upadhyaya et al., 2003; Giardini et al., 2003). While new polymerisation occurs at the bead or droplet surface where Arp2/3 complex activators are located, elastic stresses build up in the actin network (Noireaux et al., 2000). These stresses induce the network rupture and actin forms a tail that propels the object forward.

In liposome experiments, polymerisation activators are coupled to lipid heads. These experiments recapitulate some steps of tubule evolution depicted in Figure 2. During the formation of an actin tail propelling liposomes bathed in cytoplasmic extract, a large tubule was first observed at the centre of the tail (Giardini et al., 2003).

Liposomes bathed in the cytosol of HEK cells exhibit many tubules (Anitei et al., 2017). To explain their extrusion and elongation, the authors propose the following mechanism: clathrin induces membrane budding and tubule elongation remains dependent on the mechanical action of the actin network. Recently, liposomes covered by a polymerisation activator, and bathed in a minimal cocktail of pure proteins exhibit two types of membrane protrusion (Simon et al., 2019): ‘spikes’ towards the liposome interior, reminiscent of filopodia, and tubules towards their exterior (Figures 6A–6C). These tubules are very similar to the ones observed by (Anitei et al., 2017) in cell extracts, showing that budding and tubule elongation can successfully happen in the absence of membrane-bending proteins, only because of actin polymerisation dynamics. Interestingly, membrane tension controls the type of protrusion: spikes form only at low tension, whereas tubules always appear. Altogether, these results highlight the crucial role of actin in tubule formation, and its ability to form tubules independently without the help of motors or membrane bending proteins. Inward and outward protrusions were also observed when actin polymerises at the inner surface of liposomes, and depend on the presence of capping proteins (Dürre et al., 2018). Furthermore, when non-muscle myosin II is added, fission of inward membrane deformations was observed.

Additionally, the mechanics and morphology of preformed membrane tubules are affected by the presence of branched actin networks (Figures 6D and 6E). Atomic force microscopy (AFM) nanomapping of tubules before and after actin polymerisation at their surface allows direct observation of a ~100 nm sheath of actin that surrounds the tubule and increases its effective rigidity (Lamour et al., 2020). Using optical tweezers, tubules surrounded by an actin sheath can mechanically be probed by mimicking tubule elongation in vivo under the action of molecular motors (Allard et al., 2020a). In this case, the fate of the tubule depends on the actin amount: A sleeve thicker than a few hundreds of nanometres is unable to deform and such a sheath of actin stabilises the entire tubule, in length and radius, while at smaller
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Figure 6 | In vitro systems to study the effect reconstituted actin networks on membrane nanotubes

(A–C) Confocal observations of inwards (spikes) and outwards (tubules) protrusions induced by the growth of a branched actin network on initially spherical vesicles. Membrane is observed in A, capping protein in B and merge in C, scale bar 5 μm. Reproduced from (Simon et al., 2019). (D and E) AFM imaging of bare preformed tubules on a glass substrate before (D) and after actin polymerisation (E). Reproduced from (Lamour et al., 2020). (F and G) Confocal images before (F) and after (G) pulling a preformed tubule held by an optical tweezer, revealing local changes in tubule radius due to lipid mobility hindrance by the presence of the actin cytoskeleton (arrows). See (Allard et al., 2020a) for more details.

sleeve thicknesses, pulling on the membrane tubule allows tearing the surrounding actin sleeve. Discontinuous tubule regions appear and smaller tubule radii are observed in portions where the actin sleeve is absent (Figures 6F and 6G). This heterogeneity stems from lipids flow hindrance under the actin sleeve. When maintained for several minutes, the tubule radius homogenises along its length. Inside the cell, actin may provide enough time and curvature geometries for the binding of remodelling proteins that act on tubule stability/instability (Morlot et al., 2012). Finally, the actin sheath damps the thermal fluctuations of the tubule (Allard et al., 2020b). In these studies, tubule scission by actin dynamics were never observed and may rely on other parameters such as the lipid composition or the presence of molecular motors.

**Conclusion**
The role of actin is crucial during all steps of tubule remodelling, namely membrane budding, tubule elongation, constriction and scission. Many in vivo examples presented in sections *Structural Tubules, Transient Tubules and Intermediate Tubular Structure* show that actin dynamics are implied in all stages of tubule formation and destabilisation, in a variety of physiological contexts. The general importance of the actin-microtubule crosstalk (Pimm and Henty-Ridilla, 2021) highlights the unelucidated question of the relative importance of actin and microtubule-induced tubulation. We have shown that microtubule-associated motors were mainly involved in the elongation of tubules, as in the case of the Golgi apparatus, or in the long-range transport of transport intermediates after tubule scission, in endocytosis for instance. Nevertheless, we have also highlighted that actin and microtubules were both necessary to maintain the dynamical shape of the ER notably, showing that they could have synergistic or redundant roles that are still unclear. In processes, as tubulation from the Golgi, actin and microtubules affect different steps of tubules formation, raising the question of how the timing of actin and microtubule-related
steps are orchestrated. Besides, we show in section Physics of Membrane Tubule Remodelling, based on simple considerations of membrane tubule and actin biophysics, that actin dynamics are theoretically able to bud, elongate and constrict membrane tubules. Finally, in section In Vitro Tubule Remodelling by Actin, we detail that in vitro experiments are powerful to decipher the mechanics of tubule remodelling. In particular, they clearly show that actin alone can initiate budding and elongation of membrane tubules (Simon et al., 2019). Actin polymerisation on formed tubules induces their stabilisation, whereas scission could have been expected from in vivo experiments. To go further in the biomimetic approach and closer to the cellular situation, the effect of other players has to be further investigated in vitro, in particular complex membrane compositions and motor proteins. A combination of top-down and bottom-up approaches promises exciting experiments for future years.

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**Conflict of interest statement**

The authors have declared no conflict of interest.

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