The regulation of abscission by multi-protein complexes

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Summary

The terminal stage of cytokinesis – a process termed abscission – is the severing of the thin intercellular bridge that connects the two daughter cells. Recent work provides new insight into the mechanism by which this microtubule-dense membrane bridge is resolved, and highlights important roles for multi-protein assemblies in different facets of abscission. These include the endosomal sorting complex required for transport (ESCRT), which appears to have a decisive role in the final scission event, and vesicle tethering complexes, which potentially act at an earlier stage, and might serve to prepare the abscission site. Here, we review recent studies of the structure, function and regulation of these complexes as related to abscission. We focus largely on studies of cytokinesis in mammalian cells. However, cell division in other systems, such as plants and Archae, is also considered, reflecting the mechanistic conservation of membrane-scission processes during cell division.

Key words: Abscission, ESCRT complex, Exocyst complex, Membrane trafficking

Introduction

After successful DNA replication during mitosis and separation of the cell components into two daughter cells, the membrane link between the two daughter cells must be cleaved. This occurs late in telophase in response to signals emanating from the mitotic-spindle microtubules and the spindle midzone. Mammalian cells assemble a contractile ring comprising the motor protein myosin II, actin filaments and other proteins. The interaction between myosin and actin drives constriction of the contractile ring, forming a cleavage furrow between the daughter nuclei, which demarcates the scission point (Fig. 1) (Barr and Greneberg, 2007; Glotzer, 2005; Guizetti and Gerlich, 2010).

After the completion of furrowing, the two daughter cells remain attached by a thin cytoplasmic bridge at the centre of which is the midbody (sometimes referred to as the midbody ring or Fleming body) (Fig. 1). This is a structure filled with a densely packed matrix of proteins that surround a tightly compressed bridge of two anti-parallel arrays of microtubules in which the plus ends of the microtubules interlock (Elad et al., 2010; Otegui et al., 2005; Steigemann and Gerlich, 2009). It is at, or near, this region that abscission takes place (Schiel and Prekeris, 2010). Accordingly, within the midbody, the cell is faced with the problems of (1) assembling the abscission machinery within the correct spatial-temporal coordinates, (2) severing the microtubule array, and (3) performing the terminal abscission event, i.e. cleaving the plasma membrane. Recent studies suggest that membrane trafficking or membrane-trafficking-associated complexes act in each of these processes.

Membrane trafficking involves several functionally separable steps: cargo selection, the movement of vesicles towards their correct cellular destination, the tethering of the vesicles and their subsequent fusion (Cai et al., 2007). These processes require the coordinated activity of large groups of proteins such as coat proteins, Rab GTPases, SNAREs [soluble NSF (N-ethylmaleimide-sensitive fusion protein) attachment protein receptors] and tethering factors, many of which function in multi-subunit complexes. This Commentary will focus initially on tethering complexes (Table 1), which we suggest act early in cytokinesis and might be involved in the thinning of the intercellular bridge and/or the formation of what we term an 'abscission zone' – an area of membrane that is specialised for subsequent remodelling (Fig. 1). We will then focus on recent studies of the endosomal sorting complex required for transport (ESCRT) complex (Table 2) and its role in the terminal events of abscission, i.e. microtubule severing and membrane scission. Collectively, these studies offer a detailed molecular view of the abscission process.

Membrane-tethering complexes in cytokinesis

The multi-subunit exocyst complex

First identified in yeast, the exocyst tethering complex comprises eight subunits: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84 (Table 1). It is proposed to mediate the interaction of post-Golgi vesicles with the plasma membrane prior to their fusion (Sztul and Lupashin, 2006). Early studies in yeast suggested that two components of the exocyst, Sec3 and Exo70, localise to the plasma membrane by interacting with Rac- or Rho-family GTPases. At the membrane, Sec3 and Exo70 act as a 'spatial landmark' for the remaining exocyst subunits. Sec10 and Sec15 are the most vesicle-proximal of the exocyst components. Sec10 binds directly to Sec15, which in turn binds Sec4, a Rab GTPase on the surface of transport vesicles en route to the plasma membrane (TerBush et al., 1996; Zhang et al., 2008). Sec10 then acts as a 'linker', by binding to the other exocyst components through Sec5 (Guo et al., 1999). Whether all components function in all trafficking events that involve the exocyst is not clear. Several studies suggest that sub-complexes of exocyst components have roles in distinct trafficking steps (discussed further below) (Jafar-Nejad et al., 2005; Mehta et al., 2005). Nonetheless, the exocyst complex might act

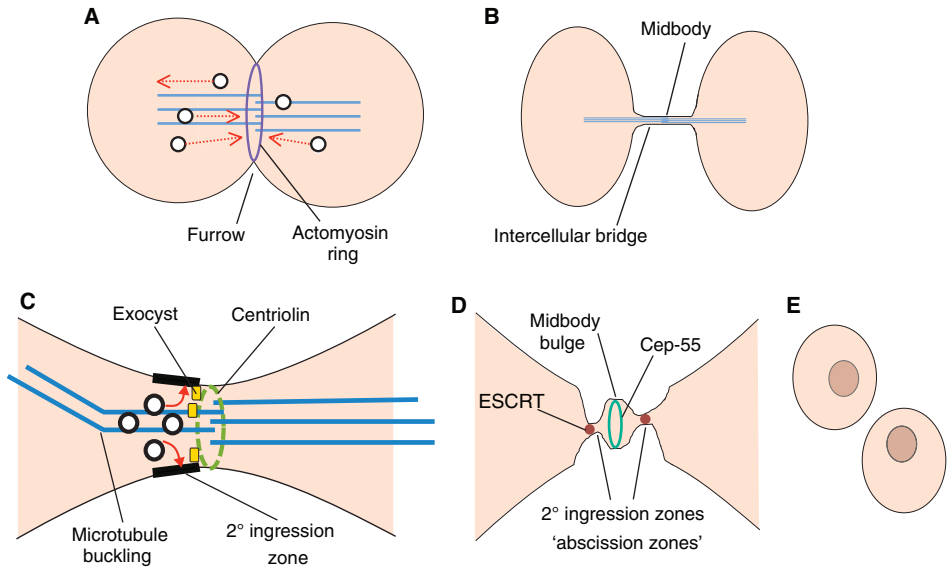

Fig. 1. Schematic of the stages of cytokinesis. (**A**) During telophase, endosomal vesicles (shown as circles) traffic bidirectionally into and out of the intercellular bridge, as indicated by red arrows. Interlocking microtubules are shown as blue lines, and the actomyosin ring in purple. Recycling endosomal vesicles, identified by the presence of Rab11 and its effector, Rab11–FIP3, interact with exocyst components into the intercellular bridge. (**B**) Furrowing proceeds until the daughter cells are connected by a thin intercellular bridge, at the centre of which is the dense midbody. Centriolin accumulates at the midbody and starts to orchestrate membrane traffic through interaction with components of the exocyst complex. Studies suggest that vesicles accumulate in the intercellular bridge during late telophase. (**C**) The establishment of the centriolin ring, and/or microtubule buckling, might then demarcate a specialised plasma membrane domain (the secondary ingression zones), to which the endosomal vesicles within the intercellular bridge are directed to fuse, thus resulting in (**D**) the thinning of the intercellular bridge and the formation of the midbody bulge (see text for details). (These zones can be thought of as 'abscission zones' – a functionally distinct region of the plasma membrane at which the terminal scission event occurs.) It is proposed that Cep-55 at this site begins the recruitment of the ESCRT complex (shown as red circles) which, in turn, mediates the final abscission event to yield two daughter cells (**E**) (see Fig. 2 and text for further details of the ESCRT complex). We suggest that during the establishment of the secondary ingression zones, the distribution of tethering complexes, such as exocyst components, between the plasma membrane and the endosomal vesicles assists in the directionality of exocytosis and thus the specialisation of the fusion machinery at these sites.

as a focus for the control of membrane traffic, integrating signals from an array of directions, including input from Ral, Rab and Arf GTPases.

A role for the exocyst in abscission is well established. In *Schizosaccharomyces pombe*, mutants in each subunit of the exocyst are capable of polarized growth, cell-surface expansion and division septum assembly, but are specifically defective in cleavage of the division septum and cell separation (Martin-Cuadrado et al., 2005; Wang et al., 2002). In plants, the exocyst is required for the formation of the cell plate (a partition formed during telophase in plants and some algae that separates the two newly formed daughter cells and is analogous to the cleavage furrow) (Fendrych et al., 2010; Hala et al., 2008). There is also evidence implicating the exocyst in mammalian cell cytokinesis (Barr and Greneberg, 2007; Prekeris and Gould, 2008). These data are further supported by functional interactions between exocyst components and proteins known to regulate abscission. For example, RalA and RalB interact with Sec5 and Exo84, respectively (Cascone et al., 2008; Chen et al., 2006). The interaction of RalB and Exo84 induces the assembly of a complex of beclin and the mammalian phosphatidylinositol 3-kinase Vps34 on nascent autophagosomes (Bodemann et al., 2011), which is potentially important for cytokinesis, because beclin and Vps34 are also implicated in this process (Sagona et al., 2010; Thoresen et al., 2010). Similarly, Sec15 and Sec10 interact with the endosomal GTPases Rab11 and Arf6, respectively (Prigent et al., 2003; Wu et al., 2005), both of which have an established role in abscission (Prekeris and Gould, 2008). These data prompt the question of the function of the exocyst in abscission.

The midbody contains both γ-tubulin and the coiled-coil protein centriolin (Fig. 1), which are arranged in a ring-like structure that surrounds the core of the midbody (Gromley et al., 2003; Gromley et al., 2005). Knockdown of *CNTRL* results in cells that remain connected by a long intercellular bridge, which is indicative of defective abscission (Gromley et al., 2003). Subsequently, centriolin was found to interact with exocyst components (Gromley et al., 2005), which, together with data implicating both endosomal and secretory traffic as essential for abscission, led us and others to the proposal that the exocyst-dependent docking of post-Golgi vesicles in the midbody is an essential step in abscission (Barr and Greneberg, 2007; Prekeris and Gould, 2008). Depletion of the exocyst results in the accumulation of intracellular vesicles in the midbody (Gromley et al., 2005), indicating that the function of this complex could be to promote the fusion of incoming post-Golgi vesicles with the intercellular-bridge plasma membrane. It has been suggested that the accumulation of secretory and/or endosomal vesicles within the intercellular bridge is a crucial facet of abscission (Fielding et al., 2005; Goss and Toomre, 2008; Gromley et al., 2005; Pohl and Jentsch, 2008). It is tempting to speculate that these accumulated vesicles fuse with the intercellular-bridge plasma membrane allowing a 'thinning' of the intercellular bridge (Fig. 1) – a point we shall return to below. Such observations led to the suggestion that a key function of the exocyst is the tethering of these vesicles in the intercellular bridge. Although studies of this complex in other systems indicate that this is a likely scenario, recent studies indicate that the exocyst might have further roles, including the formation of specialised membrane domains.

Table 1. Multi-subunit tethering complexes

Complex	Components	Pathway
COG	Cog1, Cog2, Cog3, Cog4, Cog5, Cog6, Cog7, Cog8	Intra-Golgi-complex traffic; anterograde ER–Golgi traffic
Dsl1	Dsl1, Tip20, Sec20	ER–Golgi traffic
Exocyst	Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, Exo84	Plasma membrane exocytosis
GARP	Vps51, Vps52, Vps53, Vps54	Early-endosome–TGN traffic; lysosomal–TGN traffic
TRAPPI	Bet3, Bet5, Trs20, Trs 23, Trs31, Trs33	ER–Golgi traffic
TRAPPII	As TRAPPI plus Trs65, Trs120, Trs130	Intra-Golgi and endosome–Golgi traffic
TRAPPIII	As TRAPPI plus Trs85	Autophagy

The multi-subunit tethering complexes are shown, together with their components and the trafficking pathways within which they function. Only the TRAPPII complex has been identified in mammals. All three are present in yeast (Sztul and Lupashin, 2006).

Exocyst and the formation of specialised membrane domains

Several studies report an accumulation of membrane vesicles in the midbody, which appear to fuse with the plasma membrane before abscission (Goss and Toomre, 2008; Gromley et al., 2005; Pohl and Jentsch, 2008). So, what might the function of these vesicles be? We have suggested that the accumulated vesicles in the midbody act as a platform for the assembly of the abscission machine (Gould and Lippincott-Schwartz, 2009). Studies from a completely different system, in which the role of polarity complexes and vesicle trafficking during lumenogenesis in polarised cells was examined, offers a new view of exocyst regulation. These findings could be interpreted as supporting, and extending, this notion (Apodaca, 2010), and are described in more detail below.

The formation of a lumen in some cells can arise de novo by exocytosis of membrane vesicles at specific sites of cell–cell contact. Bryant and colleagues revealed a complex interplay between polarity proteins, recycling endosomes and exocyst components that resulted in the formation of a specialised membrane domain – termed the ‘apical membrane initiation site’ (AMIS) – at the zone of cell contact (Bryant et al., 2010). Apical proteins, such as the Crumbs polarity complex, are endocytosed into recycling endosomes, which in turn then accumulate at the AMIS. Interestingly, Sec10 is enriched at the AMIS, whereas Sec8 is localised to the periphery, suggesting that the differential accumulation of these exocyst proteins reflects a unique adaptation of the function of the exocyst in this specific polarised targeting event. The authors propose that polarisation continues at this site, with vesicle trafficking from recycling endosomes being directed to the AMIS in a Rab11- and Rab8-dependent manner. Importantly, Rab11 then recruits Sec15 to this site, which might potentiate exocytosis by binding to Sec10 (already localised to the AMIS), thereby recruiting other exocyst components and so facilitating further exocytosis and lumen formation (Bryant et al., 2010). This elegant study offers a new paradigm for the organisation of membrane domains, based around the specific accumulation of proteins within exocytic carriers (Crumbs in Rab11 vesicles), the selective localisation of key tethering factors (Sec10, Sec8) and then the facilitation of exocytosis at a specified domain, through the assembly of functional tethering complexes. Perhaps the role of the exocyst in cytokinesis is related: the exocyst could coordinate the exocytic delivery of specific vesicles to sites within the midbody in preparation for abscission (‘abscission zones’). Rather than acting in the abscission event directly, these vesicles could function as a precursor for the formation of the abscission zones (see below and Fig. 1). What such abscission zones represent remains to be determined, but we speculate here that such defined areas of the intercellular bridge plasma membrane function to recruit trafficking machinery involved in the pre-abscission ‘thinning’ stage and/or the subsequent recruitment of ESCRT machinery (see below).

The study described above in the context of lumenogenesis (Bryant et al., 2010), and results from Songer and Munson, which suggest a central role for Sec6 in the anchoring of the exocyst at the sites of secretion (Songer and Munson, 2009), imply that different exocyst components regulate complex assembly in different circumstances, and control distinct facets of trafficking. This view is even more clearly elaborated for other members of the CATCHR (complex associated with tethering containing helical rods) family, as described below (Table 1).

More than one tether: playing CATCHR-up?

As the name suggests, the primary function of tethering complexes is bringing different membrane compartments into close proximity so that membrane fusion can occur (Sztul and Lupashin, 2006). On the basis of their structural similarities, the exocyst, GARP (Golgi-associated retrograde protein), COG (conserved oligomeric Golgi) and Dsl1 were grouped as a subfamily of protein tethers, termed CATCHR (Table 1). All CATCHR proteins contain rod-like domains that are formed by linked helical bundles, created from between four to eight subunits (Vasan et al., 2010). Other multi-subunit tethering complexes include the transport protein particle (TRAPPI, TRAPPII and TRAPPIII in yeast) (Barrowman et al., 2010). Recent studies have begun to reveal roles for some of these tethers in cytokinesis. For example the *Drosophila melanogaster* homologue of COG5 was shown to be required for furrow ingression (Farkas et al., 2003), and mutation of *brunelleschi,* which encodes the *Drosophila* homologue of the yeast TRAPPII subunit Trs120p results in failure of furrow ingression during male meiosis (Robinett et al., 2009). *Brunelleschi* is required to localise Rab11 vesicles to the furrow, and a double knockout of *brunelleschi* and *Rab11* genes is synthetically lethal in *Drosophila*. Studies in plants support the hypothesis that TRAPPI and TRAPPII, as well as the GARP complex, have roles in the biogenesis of the cell plate, which demonstrates, once again, the importance of vesicle transport through the Golgi complex and trans-Golgi network (TGN) to the growing compartment between dividing cells (Thellmann et al., 2010).

Reach out and touch…

Some striking new data offers a fascinating insight into the mechanism by which tethers capture vesicles (Ren et al., 2009). The Dsl1 complex is the simplest member of the CATCHRs (it has only three subunits) and is involved in traffic from the Golgi complex to the endoplasmic reticulum (ER). The structure of this complex was

modelled using overlapping crystal structures of the individual components (Ren et al., 2009) and revealed a 'tower' of approximately 200 Å in length with a flexible 'lasso' of about 110 amino acids at the top. At the membrane-proximal end of the tower, the complex appears to interact with SNARE proteins in the ER membrane. The lasso domain is proposed to then capture or tether the vesicle, which is due in part to the observation that this domain binds to subunits of the coat protein I (COPI) complex, which coats vesicles that are transporting proteins from the cis-Golgi complex back to the ER. But, in addition to a potential role in vesicle tethering, the complex binds to individual SNARE proteins and assembled SNARE complexes, and might also accelerate SNARE complex assembly (Hughson and Reinisch, 2010; Ren et al., 2009). Dsl1 has also been proposed to accelerate uncoating of COPI vesicles, suggesting that, at least in the case of this complex, functionality is not restricted to tethering, but encompasses vesicle capture and uncoating and the control of SNARE-dependent fusion (Hughson and Reinisch, 2010). Consistent with this multiplicity of function, the Dsl1 complex contains several 'hinge' regions, which imply that the protein complex is capable of adopting different conformations, perhaps at different stages of its functional cycle (Hughson and Reinisch, 2010).

A similar story is emerging from structural studies of the COG complex, which have been analysed in reconstituted systems using single-particle electron microscopy (Lees et al., 2010). This study revealed a Y-shaped structure comprising three flexible extended towers that are formed from the four essential COG subunits (Cog1, Cog2, Cog3 and Cog4). The C-termini of three of these subunits lie at the ends of the legs in regions close to those that are known to interact with other trafficking machinery (notably syntaxins and members of the Sec1/Munc18 family of proteins). These exciting observations prompt the notion that all members of the CATCHR family might operate on similar structural lines. However, besides this canonical function, tethers also fulfil other roles: acting as regulators of SNARE proteins, exhibiting nucleotide-exchange functions and interacting with certain lipids or Rab GTPases (e.g. Barrowman et al., 2010; Lees et al., 2010; Sztul and Lupashin, 2006). Thus, tethering complexes do not mediate a strictly conserved process in vesicular transport but are diverse regulators that act after vesicle budding and before membrane fusion.

Implications for cytokinesis

Before abscission, the intercellular bridge undergoes a secondary ingression, which decreases its diameter from 1–2 μm to ~100 nm (Fig. 1). The membrane cleavage event during abscission is thought to occur at these points, which are symmetrically arranged either side of the dense midbody (Elia et al., 2011; Guizetti and Gerlich, 2010; Guizetti et al., 2011). Electron microscopy images of mammalian cells undergoing cytokinesis have revealed that these constriction zones arise late in cytokinesis, substantially thinning the intercellular bridge, and forming a 'midbody bulge'. Abscission is characterised by the narrowing of one side of this bulge, followed by microtubule severing and ultimately membrane cleavage. As we will discuss below, these regions are active foci for the functional recruitment of ESCRT complex members, but how might this 'thinning' arise?

One idea is that thinned regions of the intercellular bridge arise in part from the fusion of secretory and/or endosomal vesicles, coupled to membrane-remodelling events that are perhaps similar to the formation of specialised zones of exocytosis (such as those involved in lumenogenesis). Several studies have reported the accumulation of recycling endosomal vesicles in the intercellular bridge late in telophase (Goss and Toomre, 2008; Gromley et al., 2005; Pohl and Jentsch, 2008; Wilson et al., 2005). These vesicles do not appear to contain sufficient membrane to create a 'separating membrane' that could encompass the entire intercellular bridge (Boucrot and Kirchhausen, 2007; Boucrot and Kirchhausen, 2008). However, it is interesting to speculate that the tethering and subsequent fusion of recycling endosomal vesicles at these secondary ingression sites could be important for the thinning of the bridge. This model has been eloquently supported by studies from the Prekeris group, who coupled high-resolution tomography to study the architecture of the midbody, with real-time imaging of endosome dynamics. This provided compelling evidence that the initiation and expansion of the secondary ingression sites that are observed in late telophase involve the trafficking of endosomes that are positive for the Rab11 effector FIP3 (also known as Rab11–FIP3) and the vesicle-associated membrane protein VAMP8 to the sites of secondary ingression, where they fuse with the plasma membrane (Schiel et al., 2011). Consistent with this model, some studies have reported that fusion of secretory vesicles (Gromley et al., 2005) and endosomal vesicles (Goss and Toomre, 2005; Wilson et al., 2005) with the plasma membrane occurs just before abscission. By contrast, others observed a gradual disappearance of Golgi-derived vesicles from the bridge well before abscission, and did not detect any vesicles in the constriction zones (Guizetti et al., 2011).

Whether these differences reflect imaging of different classes of post-Golgi vesicles (e.g. Guizetti and colleagues imaged Rab8, others imaged Rab11), different imaging methodologies or distinctions of mechanism will require further analysis. It is of note that a detailed study of vesicle trafficking into the intercellular bridge revealed a considerable level of heterogeneity: studies of vesicular somatitis virus glycoprotein tagged with yellow fluorescent protein (VSVG-YFP) revealed the accumulation of this marker in puncta at the cleavage furrow in approximately 60% of cells, with these puncta localised either on only one side of the midbody or on both (Goss and Toomre, 2008). Such variability coupled to the differential trafficking of different classes of vesicles might underlie these discrepancies. Regardless of these differences, it is clear that components of recycling endosomal vesicles are required for abscission (e.g. Rab11, Rab35, FIP3), and we postulate that the clearly established requirement for the exocyst complex in cytokinesis reflects the importance of coordination of trafficking at specific regions within the intercellular bridge. The precise role and timing of these trafficking steps awaits further clarification. We shall now turn our attention to the terminal cleavage event.

ESCRT complexes and cytokinesis

A remarkable advance in our understanding of abscission has come from the identification of a role of ESCRT proteins in this event, as has been reviewed elsewhere (Guizetti and Gerlich, 2010; McDonald and Martin-Serrano, 2009). ESCRT proteins are involved in the formation of multi-vesicular bodies (MVBs) and the process of enveloped-virus egress, events which both use a topologically similar membrane-scission event to that which occurs at abscission (Hurley and Hanson, 2010). Interestingly, both MVBs and enveloped viruses initially recruit the ESCRT-I component tumour-susceptibility gene-101 (Tsg101) (Table 2) to the site of scission. Current models then suggest that the sequential action of other ESCRT components (Table 2) culminate in the recruitment

of ESCRT-III to these sites (Hurley and Hanson, 2010; Wollert and Hurley, 2010; Wollert et al., 2009a); ESCRT-III then provides the membrane-scission mechanism (see below).

Both Tsg101 and the ESCRT-related protein ALIX (apoptosis-linked gene 2-interacting protein X) localise to the midbody during cytokinesis through an interaction with the midbody resident Cep-55 protein (Carlton and Martin-Serrano, 2007; Lee et al., 2008; Morita et al., 2007). Recent studies have revealed that Cep-55 relocates into the midbody in response to phosphorylation and/or dephosphorylation by cyclin-dependent kinase-1 and Polo-like kinase (Plk) (see below), where it interacts with the centralspindlin component mitotic-kinesin-like protein 1 (MKLP1). The non-canonical coiled-coil domain of Cep-55 interacts with the $GPPx_3Y$ motifs of Tsg101 and ALIX, thus resulting in the assembly of the ESCRT-I machinery at the site of membrane scission (Hurley and Hanson, 2010). Present models then propose that ESCRT-III is recruited to this site to drive the terminal scission step (Hurley and Hanson, 2010; McDonald and Martin-Serrano, 2009). The fact that MVB formation, viral egress and abscission use a common set of proteins to mediate a common underlying event (membrane scission) lends credence to this model.

In yeast there are four 'core' ESCRT-III subunits and three regulatory subunits; there are up to 12 ESCRT-III complex proteins in humans (it is not presently clear whether these have redundant or distinct functions) (Table 2). The elegant work of Hurley and others has revealed that the four core ESCRT-III subunits, together with the AAA-ATPase Vps4, are capable of driving scission in vitro (Wollert et al., 2009a). Present models for scission suggest that charged multivesicular body protein 6 (CHMP6) initiates the assembly of ESCRT-III, followed by multiple molecules of CHMP4 which polymerise into a spiral, with CHMP3 and CHMP2 'closing' the spiral (Hurley and Hanson, 2010; Wollert et al., 2009a). Such a complex assembles at the neck of the membrane bud (in the context of MVB formation or virion egress) and the energy for scission is provided by the strong interactions between the proteins themselves and the protein–lipid boundaries, as discussed further below (Hurley and Hanson, 2010). The AAA-ATPase Vps4 then disassembles the ESCRT-III polymers. Interestingly Vps4 is essential for all ESCRT-dependent processes (Morita et al., 2010; Stuchell-Brereton et al., 2007).

How does the ESCRT complex work at the midbody?

Various ESCRT-III subunits (CHMP2A, CHMP4A, CHMP5) and Vps4 have all been observed in the intercellular bridges of mammalian cells during cytokinesis (Carlton and Martin-Serrano, 2008; Carlton and Martin-Serrano, 2007; Dukes et al., 2008; Guizetti and Gerlich, 2010; McDonald and Martin-Serrano, 2009), which is consistent with the notion that ESCRT-III catalyses the final abscission step. However, until recently, some observations did not entirely support this model. For example, ALIX and Tsg101 were observed in the central region of the midbody (Carlton and Martin-Serrano, 2007), whereas the ESCRT-III components noted above were observed to populate distinct 'rings' on either side of the midbody structure (Schiel and Prekeris, 2010; Steigemann and Gerlich, 2009). Furthermore, analysis of ESCRT-III fission events in MVB formation (vesicles smaller than 50 nm) or in viral egress (less than 100 nm) do not reveal whether ESCRT-III could drive scission of a much larger intercellular bridge of around 1 μm or more. Finally, how can the ESCRT-III complex deal with the dense array of microtubules in the spindle midzone at the heart of the intercellular bridge? Some answers to these questions appear to be at hand.

Rings of ESCRT-III drive membrane scission

As noted above, membrane cleavage during abscission occurs at points in the intercellular bridge that are observed to 'thin' just prior to abscission (Fig. 1). During abscission, one side of the midbody bulge narrows, the microtubules are severed and the membrane is finally cleaved. So how does the distribution of ESCRT proteins map onto this architecture? Two recent studies offer striking new insight into this question (Elia et al., 2011; Guizetti et al., 2011).

Table 2. Subunits of the ESCRT complex

	Complex function	Subunit	Subunit role
ESCRT-0	MVB formation and autophagy	HRS	Binds to PtdIns(3)*P*, ESCRT-I, ubiquitin and clathrin
		STAM1, STAM2	Binds to ubiquitin and deubiquitylating enzymes
ESCRT-I	MVB formation, autophagy and cytokinesis	Tsg101	Binds to ubiquitin and ESCRT-0, binds to viral Gag proteins, binds Cep-55
		Vps28	Binds ESCRT-11
		Vps37a–Vps37d	Mammalian Vps37 binds Ist1; membrane binding components
		MVB12a	Stabilises ESCRT-I oligomeric structure
ESCRT-II	MVB formation and autophagy	Vps22	Membrane binding protein
		Vps25	Binds to Vps20 to recruit ESCRT-III to the bud neck
		Vps36	Binds to phosphatidylinositol-enriched membranes, ESCRT-I and ubiquitin
ESCRT-III	MVB formation, autophagy and cytokinesis	CHMP6	Initiates ESCRT-III assembly
		CHMP4A–CHMPC	Drives membrane scission
		CHMP3	Completes scission
		CHMP2A, CHMP2B	Recruits Vps4, thus initiating ESCRT-III disassembly
ESCRT-III-related	MVB formation, autophagy and cytokinesis	CHMP1A, CHMP1B	Recruits spastin to midbody and Vps4
		CHMP5	Unknown function
		Ist1	Binds Vps4 and ESCRT-I
Others		Vps4A,B	AAA-ATPase
		ALIX	Recruits Snf7 for scission; binds to Cep55 in cytokinesis; binds viral YPxL motifs

Metazoan protein names are shown. The main function of each protein is summarised. The interested reader is referred to the more extensive lists found elsewhere (Hurley and Hanson, 2010; Wollert et al., 2009b).

Using structured-illumination microscopy and high-sensitivity temporal imaging approaches, the dynamics of fluorescently labelled ESCRT-III components was studied in relation to these bulges and constriction zones. Tsg101 and the ESCRT-III subunit CHMP4B are sequentially recruited into the centre of the intercellular bridge where they form a series of cortical rings (Elia et al., 2011). Later in cytokinesis, CHMP4B concentrates at the narrow constriction zones noted above; Vps4 then follows CHMP4B to this site and abscission occurs almost immediately (Fig. 2) (Elia et al., 2011). Such data support the notion that ESCRT-III acts to sever the membrane-bound intercellular bridge late in cytokinesis, and appears to resolve the dichotomy of the distinct localisations of ESCRT-III components to distinct rings or domains within the intercellular bridge, by establishing the temporal order of their arrival.

In a separate study, Guizetti et al. also applied high-resolution imaging to study abscission (Guizetti et al., 2011). At the sites of microtubule disassembly in HeLa cells, the cortex of the intercellular bridge ingresses to a thin stalk (presumably analogous to the thinned secondary ingression or constriction zones described above), and is deformed by regularly spaced electron-dense 'ripples' at either side of the midbody (Fig. 2) (Guizetti et al., 2011) [similar ripples were also indentified by others (Schiel et al., 2011)]. CHMP4B is also present in the intercellular bridge at low levels early in cytokinesis, but its levels increase markedly at later stages, forming two narrow cortical rings adjacent to the midbody before disassembly of the microtubule (Elia et al., 2011). Strikingly, ESCRT-III subunits (including CHMP4B) extend towards these sites of cortical constriction (Fig. 2), suggesting that polymerisation of ESCRT-III mediates the formation of these constriction zones, a conclusion that is supported by the observation that the late-stage

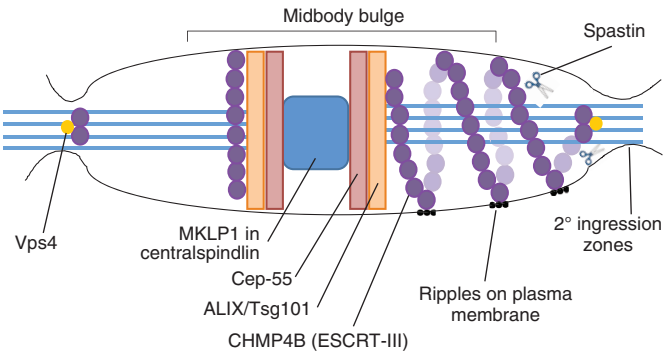

Fig. 2. The ESCRT complex in abscission. The centralspindlin component recruits Cep-55 to the midbody. Cep-55 interacts with the ESCRT-I protein Tsg101 or the adaptor protein ALIX, which are in turn proposed to recruit ESCRT-III components. Tsg101 and the ESCRT-III subunit CHMP4B (shown here as purple spheres) are sequentially recruited into the centre of the intercellular bridge where they form a series of cortical rings, shown here in the left half of this schematic midbody (Elia et al., 2011). Later in cytokinesis, CHMP4B concentrates at the narrow secondary abscission zones, closely followed by Vps4 (yellow circles), which leads to abscission at these sites. CHMP4B forms two narrow cortical rings adjacent to the midbody prior to disassembly of the microtubule (Guizetti et al., 2011). ESCRT-III subunits (such as CHMP4B) extend towards these sites of cortical constriction forming a series of intertwined, regularly spaced, filaments, which extend towards the site of secondary ingression, shown here on the right of this schematic midbody. These rings also give rise to the appearance of 'ripples' on the plasma membrane. ESCRT-III can then recruit the microtubule-severing enzyme spastin (shown here as scissors).

intercellular bridges in cells that are depleted of *CHMP2A* by RNAi are devoid of these 'rippled constriction zones' (Guizetti et al., 2011). Importantly, Guizetti and co-workers show that addition of a microtubule-depolymerising drug after furrow ingression in *CHMP2A*-depleted cells does not restore abscission. This suggests that microtubule disassembly alone cannot drive membrane scission.

Electron tomography revealed the presence of 17-nm-wide perpendicularly disposed cortical filaments at the HeLa cell constriction sites, regularly spaced every ~35 nm (Guizetti et al., 2011). These filaments are absent in HeLa cells depleted of *CHMP2A*, supporting the contention that these filaments are either composed of ESCRT-III complex proteins or that CHMP2A is required for their formation. The filaments sometimes intertwined around the site of constriction (Fig. 2), thereby offering the view that it is these filaments that generate the contractile force required for membrane scission, a result that is supported by recent studies in Archae (see below). These compelling studies, coupled to the detailed in vitro biochemical analysis of ESCRT biology, strongly suggest that ESCRT-III drives the terminal abscission event (Elia et al., 2011; Guizetti et al., 2011; Hurley and Hanson, 2010).

However, it is important to note that we await biochemical confirmation that these filamentous rings are composed of ESCRT components. It is possible that these rings or filaments comprise other structural proteins, and a possible candidate is septin-9 (Estey et al., 2010; Spiliotis et al., 2005). Septins are GTPases that are known to have crucial roles in cell division (Joo et al., 2005), and might orchestrate the localisation of the exocyst. Recent studies have provided compelling evidence that septin-9 is an essential component of abscission (Estey et al., 2010); it is possible that the rings observed by Guizetti and colleagues might, in fact, be septin based.

Microtubule severing is a rate-limiting step for abscission

A further question addressed by these studies concerns the cleavage of the microtubules, which is required for completion of cytokinesis (Elia et al., 2011; Guizetti et al., 2011). It is reasonable to propose that cleavage of the midbody microtubules and membrane scission would require precise temporal control. Consistent with this, recent results have revealed that further components of the ESCRT-III complex, CHMP1B and Ist1, bind the microtubule-severing protein spastin (Connell et al., 2009; Renvoise et al., 2011; Yang et al., 2008). Such data collectively offer the cogent model that ESCRT-III, functioning at defined sites within the intercellular bridge that are perhaps prepared for abscission by membrane trafficking, can couple microtubule severing with membrane scission and so resolve the intercellular bridge. So how does the distribution of spastin correlate with the membrane ripples or the constriction zones? Guizetti and co-workers (Guizetti et al., 2011) report that depletion of spastin delays abscission, consistent with other published results; these spastin-depleted cells, however, still exhibit constriction zones with electron-dense ripples. This delay in abscission could be overcome by the addition of the microtubule-depolymerising drug T138067. Such data prompted the authors to suggest that it is the spastin-mediated scission of microtubules that is rate limiting for abscission, and moreover that once the intercellular bridge has formed, the microtubules are not required for abscission (Guizetti et al., 2011).

An alternative view of microtubule severing has been offered by Scheil and colleagues (Schiel et al., 2011) who suggest that reorganisation of central-spindle microtubules is driven instead by

highly restricted zones of microtubule buckling and breaking, revealed by the presence of visible 'kinks' or 'buckles' in tomograms of microtubules in late telophase (Fig. 1). In late telophase, over 80% of central spindle microtubules were kinked, compared with less than 10% in early telophase. These microtubule-buckling points might be coupled to the accumulation of endosomal vesicles at the secondary ingression site. So where does spastin fit into this model? Although at first sight quite distinct, these studies might not be as divergent as they appear. Microtubule-severing enzymes prefer bent or buckled microtubules (Baas et al., 2005), suggesting that the buckling of microtubules increases the efficiency with which they are severed by molecules such as spastin. Schiel and co-workers comment that spastin might increase the efficiency of buckled microtubule severing, noting that localised microtubule severing and depolymerisation coincide with microtubule buckling (Schiel et al., 2011). Further studies are required to define the inter-relationship of spastin levels and microtubule buckling, and whether spastin can accumulate at the sites of buckling.

Lessons from Archae

The importance of ESCRT-III in cell division is given yet further credence by startling observations that Archae of the genus *Sulpholobus* express genes encoding ESCRT-III and Vps4 homologues (Lindas et al., 2008; Samson et al., 2008). The mRNA for these proteins exhibits pronounced cell-cycle-dependent regulation, with the highest levels in dividing cells. These ESCRT-III and Vps4 homologues localise between segregating nucleoids, where they exhibit ring-like structures with a diameter that decreases in parallel with membrane ingression (Ettema and Bernander, 2009). Such data strongly support the notion that the mechanism of abscission described above for mammalian cells is widely conserved through evolution (Lindas et al., 2008).

Archae lack other ESCRT proteins, such as ALIX or Tsg101, raising the question of how the ESCRT-III homologues are recruited to their sites of action. The answer lies with the identification of an additional open reading frame termed CdvA, which also localises to the mid-cell structure between separating nucleoids (Samson et al., 2011). This protein interacts both with the *Sulphobolus* ESCRT-III homologue and with archael lipids, suggesting that in this case, the lipid itself has more than a simple passive role in abscission. Is there evidence that this is also true in mammalian systems?

There is good evidence that ESCRT-O binds phosphatidylinositol 3-phosphate through the HRS FYVE domain, which suggests that this phosphoinositide has a key role in the assembly and/or regulation of the ESCRT complex in MVB formation by assembling the ESCRT complex at the site that becomes the bud neck (reviewed by Guizetti and Gerlich, 2010; Hurley and Hanson, 2010). Moreover, retroviral budding has been proposed to take place from cholesterol-enriched lipid-raft domains. For example, HIV-1 Gag protein and the envelope glycoprotein (Env) localise to lipid-raft domains, suggesting that the sites of assembly of the ESCRT complexes in viral egress are mechanistically linked to the lipid composition of the plasma membrane (Carlton and Martin-Serrano, 2009; Hurley et al., 2010). This is also indirectly supported by the observation that viruses, such as influenza, which do not use ESCRT-dependent budding mechanisms, instead use an amphipathic helix that is localised within the M2 viral protein to deform membranes in a cholesterol-dependent manner. Importantly, this M2 helix can drive budding in vitro and localises to the neck of budding vesicles that also coincide with phase-separation boundaries (Rossman et al., 2010). Does this relate to cytokinesis in mammalian cells? Cholesterol-rich membrane domains accumulate in the furrow of dividing cells, and vesicles enriched in phosphatidylinositol 3-phosphate accumulate in the midbody of dividing cells (reviewed by Neto et al., 2011). Such data offer the tantalising hint that perhaps the mechanisms of membrane scission in these diverse systems use more than a common set of proteins, but rather integrate commonality of mechanism from lipid species through to the assembly of the scission machine.

Where will we be ESCRT-ed next?

Although these data offer a level of insight that represents a step-change in our understanding, as one would expect from such studies, they open up further important questions: how is ESCRT-III polymerisation achieved in the absence of ESCRT-II? How is the re-distribution from the midbody to the constriction zone achieved or regulated? How is the ESCRT machinery anchored to the membrane tightly enough to facilitate scission? What are the function(s) of all the ESCRT-III complexes in cytokinesis? The tools and approaches are in place for these mysteries to be quickly addressed.

Spatial control of ESCRT-mediated abscission by kinases

One area of recent progress has been the identification of a mechanism that initiates abscission. The ability to coordinate these complex membrane-trafficking and membrane-remodelling systems to a precise set of temporal (and indeed spatial) coordinates is an essential part of the cell cycle. Recent work from the Barr laboratory offers a new insight into how abscission timing is controlled (Bastos and Barr, 2010). As noted above, recruitment of Cep-55 to the midbody is an important step in abscission, because Cep-55 interacts with Tsg101 and ALIX, which in turn then recruit ESCRT-III (Fig. 2). Bastos and Barr determined that, during anaphase, Cep-55 is phosphorylated by Plk1 on residue Ser436, thereby preventing the interaction of Cep-55 with MKLP1 and precluding the accumulation of Cep-55 in the midbody (Fig. 3) (Bastos and Barr, 2010). During mitotic progression towards mitotic

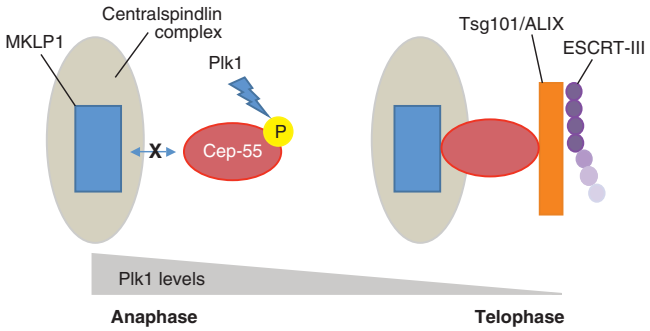

Fig. 3. Polo-like kinase is a trigger for initiation of abscission by temporal control of ESCRT recruitment. Cep-55 recruitment to the midbody is an important step in abscission, as Cep-55 interacts with Tsg101 and ALIX, which subsequently recruit ESCRT-III. During anaphase, Cep-55 is phosphorylated by Plk1 on residue Ser436, which prevents the interaction of Cep-55 with MKLP1 in the centralspindlin complex, and so precludes the accumulation of Cep-55 in the midbody (Bastos and Barr, 2010). During progression towards mitotic exit, Plk1 is degraded, leading to dephosphorylation of Cep-55, which then interacts with MKLP1 and accumulates in the midbody. Only after Plk1 is degraded does Cep-55 accumulate in the midbody and thus promote the final stages of abscission by recruiting Tsg101 and ALIX.

exit, Plk1 is degraded, which leads to dephosphorylation of Cep-55 and its subsequent accumulation in the midbody through interactions with MKLP1. Only after Plk1 is degraded does Cep-55 accumulate in the midbody and thus promote the final stages of abscission. This is a remarkably elegant means of initiating the final abscission event, which ensures that only after levels of a key kinase are sufficiently reduced does this process begin (Fig. 3) (Bastos and Barr, 2010).

Interestingly, other facets of membrane traffic in cytokinesis might also be regulated by phosphorylation. The localisation of Rab11 endosomes in flies and human cells was recently shown to be controlled by phosphorylation of FIP3 (humans) and Nuclear Fallout (flies) by IKK (inhibitor of nuclear factor κB kinase)-related kinases during development (Gould, 2011; Otani et al., 2011). Furthermore, the recent identification of the exocyst component Sec5 as a phospho-regulated protein offers another example of a potential regulatory mechanism (Chen et al., 2011). Collectively, such observations indicate that other facets of membrane trafficking during cytokinesis might similarly be controlled by cell-cycle-regulated kinases. Unravelling these control mechanisms represents a challenge for the field.

Conclusions

Multi-subunit protein complexes, such as the exocyst and ESCRT complexes, have been implicated in cytokinesis for some time. Key themes emerging from these recent studies include the realisation that these complexes might function by mechanisms distinct from – or at least in addition to – the classical function of these complexes, and that during cytokinesis the function or regulation of these complexes might be hijacked to perform a specific role (e.g. the apparent lack of a requirement of ESCRT-II in cytokinesis). These studies reveal a high level of molecular detail of the abscission event and, coupled with our increasing understanding of how these processes are coordinated with the cell cycle, offer a unique insight into this fundamental cellular process.

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