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Resurrecting remnants: The lives of post-mitotic midbodies

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Abstract

Around a century ago, the midbody was described as a structural assembly within the intercellular bridge during cytokinesis, which served to connect the two future daughter cells. The midbody has become the focus of intense investigation through the identification of a growing number of diverse cellular and molecular pathways that localize to the midbody and contribute to its cytokinetic functions ranging from selective vesicle trafficking, regulated microtubule, actin and ESCRT filament assembly and disassembly, and post-translational modification, such as ubiquitination. More recent studies revealed new and unexpected functions of midbodies that occur in post-mitotic cells. In this article, we provide a historical perspective, discuss exciting new roles for midbodies beyond their cytokinetic function and speculate on their potential contributions to pluripotency.

Keywords

cytokinesis; abscission; midbody; asymmetry; stem cells; cell fates

An overview of the midbody assembly

Cell division requires segregation of the duplicated genome and partitioning of cellular contents between daughter cells during cytokinesis. In animal cells, cytokinesis begins with ingression of the plasma membrane and culminates in a poorly understood event called abscission (Glossary), where daughter cells become physically separated from one another (for reviews, [1, 2]). Recent advances have uncovered several key features of abscission such as membrane trafficking ([3]; for reviews, [4, 5]), microtubule (MT) reorganization [6,

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7], helical filament formation driven by endosomal sorting complex required for transport (ESCRT; Glossary) [7–9], and regulatory mechanisms involving protein ubiquitination [10, 11]. Little is known about how these events are coordinated to achieve abscission other than they all occur in vicinity of the midbody (MB; Glossary). Disruption of any of these MB-associated events leads to abscission failure defining this organelle as a key player in abscission [3, 12, 13]. One result of abscission failure is polyploidy, a potential factor in cancer predisposition and progression [14] and in multicellular syncytium formation [15].

This review focuses on MB structure and function during cytokinesis, and the fate and function of post-mitotic MBs. It has been shown both *in vivo* and *in vitro* that post-mitotic MBs can be released from cells where they deteriorate with time [16–19]. They can also be retained by cells where they are either degraded or persist for extended periods of time [3, 19–23]. Their distinct fates in different cell types can dramatically impact cellular physiology and cell-fate determination [19, 22, 23]. These new functional roles for post-mitotic MBs have resurrected interest in MB form and function.

Midbody characteristics: a historical perspective

The biogenesis and architecture of the midbody revealed by electron microscopy

Walther Flemming first noted the presence of midbodies using histochemical methods [24], then others observed midbody dynamics by time-lapse light microscopy ([25, 26]; for detail, see Box 1). Electron microscopy (EM) later provided more detail of MBs and associated structures [16, 27–33], and again supported the early hypotheses of Flemming (see Box 1). EM studies confirmed that the overall structure of the developing MB was strikingly similar to the phragmoplast of plants (Glossary; [34]). Both MB and phragmoplast were composed of anti-parallel MTs with vesicles and amorphous electron-dense material centrally positioned (for review, [35]). Also among the MTs of the developing MB was a diversity of membranous organelles such as endoplasmic reticulum cisternae, Golgi complexes and electron-lucent and -dense vesicles. Double-membrane-bound electron dense bodies associated with multivesicular bodies (MVBs; Glossary) and reminiscent of autophagosomes (Glossary) were also found at these sites [16, 28, 30, 32]. After furrow ingression, membranous organelles gradually decreased at MT sites and concomitantly appeared at the junctions of the bridge and cell bodies (Figure 1b, middle panel; [16, 29, 30, 32]). MT bundles coalesced and the amorphous electron-dense material reorganized into, a continuous plaque-like structure between daughter cells, the Flemming body [16, 28, 30–32]. The Flemming body was thought to provide a diffusion barrier between daughter cells. However, more recent work suggests that the barrier might be selective (for detail, see Box 2 and [36–38]).

Text Box 1

The initial characterization of the midbodies

Early glimpses of the midbody

The midbody, or *Zwischenkörper* (“Zwischen” and “körper” mean “between” and “body”, respectively; also referred to as the Flemming body), was first described by

Walther Flemming in 1891 [24]. Using light microscopy (LM) and histochemical stains, he identified a chromophilic structure positioned between dividing daughter cells (Figure 1a) and speculated that it was derived from the spindle midzone between segregating chromosomes. He also suggested that it might be the animal counterpart of the phragmoplast (Glossary), a MT-enriched structure involved in plant cytokinesis (for review, [70]). In the 1960s and 70s, with the aid of electron microscopy (EM), most of Flemming's prescient theories were validated [27–29, 71].

Midbody dynamics revealed by time-lapse light microscopy

Microcinematographic light microscopy in the 1970s showed that the classical MB structure appeared within the intercellular bridge (Glossary) after furrow ingression (Figure 1b, left and middle panels; [25, 26]). In human cells, it formed a disc-like structure roughly 1–2 μm in diameter. As cytokinesis proceeded, the diameter of the intercellular bridge narrowed whereas MB size remained relatively constant. With time, the plasma membrane of the bridge wrapped tightly around the MB, creating a bulge in the bridge (Figure 1b, right panel). Despite this, the MB was able to slide within the bridge between the two connected daughter cells. Later, the intercellular bridge showed 'waving activity' that propagated away from both sides of the MB. The activity ceased initially on one side of the bridge. The other side continued this activity then narrowed to a thin thread of cytoplasm and was severed [25, 26]. This observation is also consistent with later studies that defined this and other asymmetric events prior to abscission (see below and [3, 7, 9, 21, 40]).

Text Box 2

Midbody and the associated membrane function as a diffusion barrier

With the ability to perform high resolution time-lapse imaging and the generation of a variety of fluorescence tags with different properties, researchers have now been able to test whether a diffusion barrier exists at the midbody and associated membrane in the intercellular bridge. It appears that this barrier is selective. Inner-leaflet plasma membrane proteins such as Lyn-GFP and transmembrane proteins such as LYFPGT46 were unable to transverse from one daughter cell to the other, as revealed by photorecovery assays. However, an outer-leaflet plasma membrane protein and a cytosolic protein appeared to pass through, as shown by photorecovery of YGP-GI-GPI and cytosolic GFP, and the microinjection of Lucifer Yellow dye [7, 36–38]. The selective passing of molecules could be modulated by channels in the MB that interconnect the cytoplasm of the two daughter cells [16, 38] or the space between the associated bridge membrane and the MB core. Only when MB structure and "permeability" are examined more thoroughly in different cell types and at carefully defined times during the process of cytokinesis, will the timing and functional significance for the MB as a barrier emerge.

By late cytokinesis, the MB is a prominent structure with a central core, flanking microtubules and associated organelles, and the surrounding plasma membrane (Figure 1b and 2a). The MB core has two components, the "MB matrix" and "MB ridges". The MB

matrix is mainly comprised of anti-parallel MT bundles and interspersed electron-dense material [16, 31, 33, 39]. MB ridges surrounding the matrix account for the bulges within the intercellular bridge (Figure 2a, bottom panel), and are comprised primarily of electron-dense material, a specialized thickening under the plasma membrane and a few MTs [16]. MB ridges likely correspond to MB rings (MRs, donut-shaped structures) revealed in more recent studies by phase contrast and immunofluorescence (IF) microscopy (Figure 2a, middle panel; [3]).

During early stages of abscission, the caliber of the bridge narrows and MT bundles within the bridge are reduced by severing and depolymerization [7, 16, 33, 39–41]. At a site adjacent to the MB (termed the ‘narrowing segment’ [16] or ‘constriction zone’ [7]), the bridge caliber narrows further and ‘ripple contours’ appear (Figure 2b, top panels; [7, 16]). Such ripples represent a series of membrane deformations along the bridge membrane, which are the helical filaments built by ESCRT components recently observed at this site (Figure 2b, bottom panel) [7]. In addition to reforming membranous compartments within cell bodies during late cytokinesis, vesicle clusters were frequently observed along MTs in the bridge with a concentration near the midbody [16, 30, 31]. Abscission occurred after vesicles trafficked to the MB where they presumably docked and fused [3, 21, 42], MT bundles were reduced and severed [6, 40, 41], and ESCRT-containing filaments were organized along the bridge membrane on one or both sides of the MBs [7, 9].

The fate of post-mitotic midbodies

Early EM studies suggested that post-mitotic MBs were transient structures that were released from cells where they deteriorated [16, 28, 39], leading to terms such as MB remnant and residual body. These post-mitotic MBs often retained recognizable MB matrix, ridges, anti-parallel MTs and plasma membrane [16]. However, these features were likely to be lost over time [17, 18]. Indeed, with the aid of immunoelectron microscopy to detect MB molecules, it was shown that the large and relatively homogeneously-sized electron-dense particles in brain ventricles were ‘aged’ post-mitotic MBs that had lost their typical morphological and biochemical signatures [18].

Closer inspection of changes in post-mitotic MB structure over time was enabled by time-lapse imaging, ordering of temporal events and biochemical analysis. These approaches showed that post-mitotic MBs associated with one of the two daughter cells after abscission [3, 18, 19, 21, 22]. Immediately after abscission on one side of the MB, the thin bridge on the other side of the MB retained its connection to the other daughter cell. This tether was reminiscent of the thin stalk described in studies using microcinematography and EM [16, 18, 25, 26]. The tether can retract, delivering the post-mitotic MB into the cytoplasm of one daughter cell for retention or degradation [3, 19, 22, 23]. Alternatively, the cell can sever the tether, releasing the post-mitotic MB into the extracellular space [7, 9, 18, 19] where it remains or is engulfed by a cell [16, 19, 22]. Retention, release or degradation of post-mitotic MBs appears to depend on cell type and status [19, 22, 23]. These findings indicate that fate determination of post-mitotic MBs involves multiple steps, is likely to be tightly regulated (see below and [19, 22, 23]), and is far different from their fate as remnants or residual bodies, as proposed decades ago [16].

The midbody as an important platform for abscission

Renewed interest in MB research over the last decade revealed the extraordinary complexity of this organelle through proteomic analysis [43], and led to the discovery of myriad molecules and pathways that contribute to abscission. Previously known functions of these pathways in other cellular processes helped generate hypotheses to test for mechanisms of abscission. These pathways include vesicle trafficking, membrane scission, MT-severing, and ubiquitination. As many recent reviews cover mechanisms of abscission, we focus on how these pathways may contribute to MB retention by or release from cells.

Membrane trafficking pathways are targeted to the midbody for abscission

It is no surprise that multiple membrane trafficking pathways are crucial for abscission (for reviews, [2, 4, 5]), because early EM studies identified many types of vesicles around the MB during early cytokinesis and prior to abscission. Recent studies showed that molecules involved in secretion and endocytosis were concentrated in the MB region during late cytokinesis [42, 44, 45]. Post-Golgi vesicles and proteins required for vesicle tethering (e.g., exocyst; Glossary) and fusion (e.g., SNAREs; Glossary) were recruited to the MB [3, 45–47] and this recruitment depended on centrosome/MB proteins (e.g., centriolin and CEP55; [3, 13]). Disturbing either the localization or function of these MB proteins or the recruitment of molecules required for secretion, caused abscission failure. This resulted in a dramatic collapse of the intercellular bridge creating binucleated cells [13, 46], long delays in abscission, or multicellular syncytia formation [3, 15]. These results clearly demonstrate the importance of selective secretion in abscission.

The endosomal pathway also contributes to abscission (for review, [4]). As shown previously, the endosome-associated Rab11-interacting proteins, FIP3 and FIP4, were targeted to the MB [42, 46] and interacted with the exocyst component, Exo70, and the MB protein, mgcRacGAP/Cyk4 [46, 48]. These interactions were essential for completing cytokinesis. These results indicate that the MB may serve as an anchoring scaffold for molecules and complexes that facilitate vesicle accumulation at or near this site and potentiate vesicle fusion during abscission. Alternatively, fusion of recruited secretory and endosomal vesicles may be required for elongating the intercellular bridge during late cytokinesis [25, 26], for narrowing the bridge caliber (see above), or for preparing the site in other ways for the final separation step of abscission.

As shown previously, vesicles appeared to be targeted asymmetrically to one side of the MB prior to abscission [3]. This observation was consistent with the reported asymmetric accumulation of Golgi-derived vesicles at the MB during late cytokinesis, following their initial symmetric accumulation at both sides of the MB [21]. With better temporal resolution, the recruited Golgi-derived vesicles appeared to dock at the MB and remain individually without fusing [21], suggesting that these tethered vesicles were awaiting a signal to initiate vesicle fusion and abscission [3]. Conceptually similar was the work showing that the Golgi complex was organized in two positions in each nascent daughter cell during abscission [49]. The two major Golgi complexes were far away from the bridge, behind the nuclei of both nascent daughter cells near the centrosomes. The two minor pools of Golgi-derived membranes were located at the juncture of the intercellular bridge. In one

of the two daughter cells, the minor Golgi-derived membrane pool was retracted back to the major site during late cytokinesis, leaving the minor pool of the other daughter cell in a position to deliver Golgi-derived membranes to one side of the MB to mediate abscission. Even though FIP3/4-positive endosomes trafficked symmetrically to the MB [42], more recent EM data suggests that they may not fuse until localized MT severing occurs asymmetrically on one side of the MB [40]. These new EM tomographic images leave room for the idea that fusion of endosomal vesicles may occur asymmetrically or sequentially although their recruitment is symmetric. This observation also suggests a potential link between vesicle fusion and MT severing during abscission.

Thus, broadly speaking, regardless of how vesicles are recruited (e.g., symmetrically, asymmetrically, or sequentially), fusion events may still be ‘asymmetric’ or ‘sequential’ presumably due to limiting factor(s) on one side of the MB or to sequential arrival at this site [21, 40]. The consequence of these asymmetric or sequential events would be inheritance of the post-mitotic MB by one of the two daughter cells [3, 21] or release after the bridge is severed again on other side of the MB (discussed below). How these asymmetric or sequential events might contribute to MB retention or release is postulated in Box 3.

Text Box 3

Proposed mechanisms for asymmetric inheritance of post-mitotic MBs

Asymmetric vesicle trafficking → asymmetric abscission → MB retention by one daughter cell

One model for asymmetric inheritance of post-mitotic MBs is that vesicles are recruited asymmetrically from one side of the MB for abscission and/or the ESCRT follows. The bridge is then severed resulting in MB inheritance to the opposing daughter cell. One example is the post-Golgi secretory vesicles labeled by luminal-GFP [3]. Given that other recycling endosomal vesicles have been observed to symmetrically traffic to the MB, we have compared the timing of luminal-GFP recruitment and the transport of FIP3- or Rab11-decorated endosomes to the MB. Luminal-GFP vesicles trafficked to the MB significantly later than FIP3 and Rab11 vesicles (Rosa and Doxsey, unpublished), suggesting that the asymmetric delivery of secretory vesicles is more likely to contribute to abscission, affecting where the post-mitotic MB ultimately resides. In another study, Golgi-derived membranes initially trafficked symmetrically to the MB but later became asymmetrically localized prior to abscission [21]. These observations suggest that different vesicles may function at different times and traffic in different ways during abscission. Therefore, experiments should be normalized to a given endpoint, such as times pre-abscission, to reliably compare contributions of different classes of vesicles and results from different investigators.

Symmetric vesicle trafficking → stochastic abscission → MB retention or release

If vesicle trafficking and membrane scission machinery concentrate at both sides of the MB and cut the bridge sequentially, rather than simultaneously, a post-mitotic MB can still be retained. Ultimately, the bridge on both sides of the MB could be severed by ESCRT, as recently suggested [7, 9], and then released [19]. This appears to be the case

for cells in which post-mitotic MBs are released extracellularly [17–19]. Time-lapse imaging and ultrastructural analysis revealed that these post-mitotic MBs remain connected to one daughter cell by a thin stalk for extended time before they are eventually severed [18, 19], suggesting that abscission machinery takes longer to cleave on one side of the MB than the other. These observations reinforce the idea that despite symmetric vesicle trafficking, severing capability (presumably the activity of abscission machinery) on the two sides of the MB may be different, leading to asymmetric MB inheritance and, potentially, later MB release.

Asymmetric signaling → asymmetric abscission → MB retention by one daughter cell

The third model predicts that membrane fusion is a regulated process that occurs only on one side of the MB despite vesicle delivery to both sides. The regulation of membrane fusion/scission and abscission could be through asymmetric removal of limiting factors (e.g., MT bundles; [40]), the recruitment of fusion activators (e.g., kinase) or stimulation of a previously positioned activator by other means (e.g., Ca^{2+} release) as in regulated secretion, for example, insulin granule secretion (for review, [72]).

Combinations of these models are also possible. For example, triggers of membrane fusion/scission for abscission could be recruited asymmetrically to the MB or sequentially to either side of the MB, as proposed in the second possibility, or activated asymmetrically, as proposed in the third possibility.

ESCRT complexes build helical filaments during abscission

The recent finding that ESCRT is an evolutionarily-conserved pathway required for abscission provides insight into a late step in this process [7, 8, 50–53]. ESCRT has important roles in constriction of membrane during budding of viruses and vesicles (for reviews, [54–56]). The topology of an enveloped virus budding from a host cell is similar to the intercellular bridge connecting one daughter cell with her sister before abscission. Severing the bridge to disconnect daughter cells is analogous to virus release from the cell and may require identical or similar functions of ESCRT (for reviews, [5, 56]). As predicted by the model, multiple ESCRT components (e.g., Alix and Tsg101) are targeted to the MB for abscission [8, 50, 57]. However, more recent studies led to a different model [7, 9], where ESCRT-III components (e.g., CHMP2 and CHMP4A/B) appeared to localize to the MB ring initially, then, at a later time localized at a secondary site corresponding to the constriction zone [7, 9]. Depletion of CHMP2, the core component of ESCRT-III, led to the disappearance of the ripple contours and the spiral-shaped filaments [7], suggesting that ESCRT-III contributes to their assembly. However, it was not clear if ripples alone or both ripples and the constriction zone were lost upon CHMP2 depletion, and this could lead to a different interpretation of how ESCRT functions at these sites. Besides building filaments, ESCRT has been shown to interact with spastin [58], a AAA+ ATPase that severs MTs, providing a model where the ESCRT function is coordinated with MT severing during abscission.

Several issues with the ESCRT model require further clarification. Although the archaea ESCRT machinery spans the division site which is $\sim 1\text{-}\mu\text{m}$ in diameter at the beginning of cell fission (for review, [59]), ESCRT-deformed membranes identified in eukaryotic cells typically have diameters ranging from 50–100 nm [54–56]. These diameters are still significantly smaller than the diameter of the constriction zone at the intercellular bridge (200–500nm) [3, 7, 9, 16], so it is unclear how ESCRT forms filaments within the intercellular bridge and if they are functionally capable of inducing bridge severing. One potential model, as discussed above, is that vesicles and fusion machinery, which are organized topologically different from ESCRT, are recruited to the MB where they contribute to bridge remodeling and narrowing, thus allowing ESCRT to function in the later stages of abscission. Alternatively, ESCRT itself may extrude membrane and cytoplasm into the extracellular environment to narrow the bridge caliber through a mechanism similar to the viral budding before the final cut of the bridge, as observed in some EM studies [16, 18, 28]. Another model is that vesicle trafficking and ESCRT machinery work together to accomplish abscission, because both are increasingly recruited to the midbody and the constriction zone at least 10 minutes before bridge severing [3, 9, 21]. In short, it is still unclear how vesicles, fusion machinery and ESCRT remodel and/or sever the bridge membrane in a concordant manner.

More questions remain about the ESCRT model. How is ESCRT-III directed to the constriction zone? ESCRT-III spirals may grow from the MB where other ESCRT components reside, becoming increasingly smaller in diameter as they approach the constriction zone, approximately $1\text{ }\mu\text{m}$ distal to the MB. In a similar context, it is not known if recruitment of ESCRT-III and the appearance of ripple contours at the constriction zone require the prior presence of ESCRT-I or ESCRT-associated proteins at the MB, even though depletion of these ESCRT components results in abscission failure. It is also unclear if ESCRT functions together with regulated disassembly of cortical actin by F-actin modulators during abscission. Contradictory data suggests on one hand that ESCRT functions alone in abscission [7], or alternatively, that ESCRT acts only at certain stages of abscission prior to “secondary constriction”, where fusion of FIP3 endosomes and disassembly of F-actin contribute to the final separation [60]. These questions may be answered by live-imaging of F-actin and ESCRT-III components tagged with photo-distinguishable tags or by ultrastructural analysis of the constriction zone after selective depletion of ESCRT-I components and ESCRT-associated proteins. Despite these remaining questions, it is clear that the ESCRT pathway is crucial for abscission.

Role of de/ubiquitination in modifying the MB upon abscission

In line with the ESCRT model of abscission, it was shown that two ESCRT-modulating deubiquitinating enzymes (DUBs), UBPY/USP8 and AMSH, were recruited to the MB during cytokinesis [11]. Depletion of either DUB led to cytokinesis failure. AMSH depletion induced binucleated cells and cells interconnected by long bridges, and UBPY depletion induced binucleated cells. This phenotypic difference may reflect different specificities of the two DUBs toward different MB protein substrates and ubiquitin conjugates [11, 61, 62]. The spatial distribution of UBPY and AMSH were different from anaphase to cytokinesis, suggesting selective interactions with different ESCRT and non-ESCRT molecules, which

could be crucial for ordered abscission. The role of ubiquitin-mediated modifications in abscission is supported by work showing that BRUCE, a giant protein possessing E2/E3 ubiquitin ligase activity, moved to the MB, interacted with MB components, such as mitotic kinesin-like protein 1 (Mklp1), and blocked abscission when depleted [10]. The MB fraction of both BRUCE and Mklp1 were heavily mono- or oligo-ubiquitinated upon abscission, and both proteins appeared to be targeted by UBPY [10]. Taken together, ubiquitinating enzyme(s) and ESCRT-interacting DUBs modulate the ubiquitination status of MB proteins, which may play a role in abscission and fate determination of post-abscission MB (see below).

Multiple pathways control the fate of post-mitotic midbodies

Intracellular degradation via macroautophagy

One of the intriguing discoveries regarding the fate of post-mitotic MBs is the role of macroautophagy (hereafter referred to as autophagy; [19, 22, 23]). As reviewed previously, autophagy contributes to the recycling of amino acids, embryonic development and disease pathogenesis [63, 64]. Autophagy can be selective and mediated by autophagic receptors. Receptor recognition is followed by autophagosome formation, autophagosome fusion with lysosomes and degradation of encapsulated organelles or proteins [65]. The NBR1 autophagic receptor was shown to play a dominant role in degradation of post-mitotic MBs through its recognition of CEP55, a core MB component crucial for abscission; another receptor, p62, was also implicated in this process [22, 23]. Besides receptor recognition, the level of autolysosomal activity can affect the degradation efficiency of post-mitotic MBs [22]. Taken together, autophagy is a major contributor to MB clearance.

Midbody release from cells

As shown by early EM studies, an alternative fate of the post-mitotic MB was release into the extracellular space [16]. Using bone marrow-derived cells *in vitro*, extracellular, and partially deteriorated, MBs were observed [16]. The first evidence for MB release *in vivo* came from the observation that electron-dense particles were found inside the lumen of the neural tube of mouse embryos, although these particles were not initially recognized as released MBs [17]. In subsequent studies, time-lapse imaging and EM demonstrated that MBs were released from neural precursor cells *in vivo* not only in mice, but also in chicken [18] and from mouse neural stem cells *in vitro* [19]. Interestingly, released MBs collected in mouse ventricular fluid increased significantly at early stages of neurogenesis (E10.5–12.5; [17]), coincident with the time when differentiating (neurogenic) divisions become prominent [17, 66]. This suggested that MB release might depend on cell type/status and could contribute to these states. Depletion of ESCRT-associated protein Alix reduced the amount of free MBs in the medium of neural stem cells (NS-5) and neuroblastoma cells (Neuro-2a), suggesting that MB release was likely to involve the secondary scission event at the tether between the MB and the daughter cell [19]. It is also possible that Alix depletion might affect the first scission event between the two nascent daughters, leading to binucleated cell formation. However, this was not observed in the aforementioned cells [19] maybe because the role of Alix in abscission was minor or slightly different in these two cell types. Collectively, MB release is clearly a dominant feature of certain cell types and

appears to be dependent on culture conditions *in vitro* or the developmental or differentiating state of cells *in vivo* (discussed below; [19, 22]).

Alternative routes that lead to MB clearance

MB release and autophagic degradation of MBs most likely represent parallel pathways for MB clearance [16, 19, 22, 23]. HeLa cells and mouse neuroblastoma cells (Neruo-2a) can degrade MBs by autophagy and release them into the extracellular space [19, 22, 23], demonstrating that both pathways can be used in a single cell and suggesting interplay between these pathways. Because MB fate has not been characterized in great detail until recently, it is reasonable to envision additional routes for MB clearance that ultimately lead to intracellular degradation or release from cells. In fact, recent ultrastructural analysis of post-mitotic MBs revealed characteristics different from mitotic MBs (e.g., fewer or no MTs), presumably due to MB aging [18, 19]. These morphological changes could represent the products of different resident times in autolysosomes or products of pre-autophagic events that change post-mitotic MB integrity and composition. It is also possible that long after release from cells, post-mitotic MBs are internalized by endocytosis/phagocytosis followed by heterolysosomal or autophagic degradation [19]. These different, yet not mutually exclusive, routes may all exist but they are difficult to distinguish. It is also difficult to know if the same autophagy machinery is required as a final step for all these routes. Nevertheless, the temporal analysis of MB degradation [22] and the ultrastructural analysis of post-mitotic MBs [19] sheds light on when and how MBs are destined for intracellular degradation. Additional studies are required to more closely track the degradation process and test how different degradation pathways are utilized by different cells.

Pathways for MB clearance in different cell types: consensus and dissensus

In the grand scheme, increasing the activities of either or both autophagy and MB release can promote MB clearance when MBs generated during proliferation need to be removed. One such scenario is differentiation [19, 22]. In this context, cells derived from different developmental lineages or with different pluripotency status may employ one or both pathways for MB clearance, depending on the environment and what is available in the toolbox. Therefore, differentiating cells may promote MB clearance either by elevating autophagic activity as observed in fibroblasts derived from human embryonic stem cells (hESCs; [19, 22, 23]), or by enhancing MB release (NS-5 and Neuro-2a; [19]) to achieve the same goal, the elimination of MBs.

The aforementioned model does not necessarily imply low MB release in undifferentiated cells, such as multi/oligopotent stem cells. In fact, significant basal levels of MB release have been observed in neural progenitors *in vivo* and neural stem cells *in vitro*, although the levels increase further during the early stages of neurogenesis (differentiation) *in vivo* and during induced neuronal differentiation [17–19]. However, we currently do not know if constitutively high MB release is also a characteristic of hESCs (e.g., H1, H9) and induced pluripotent stem cells (iPSCs), and if pluripotency status (e.g., pluripotency *versus* oligo/unipotency) or developmental lineage influence MB clearance. Nevertheless, it was agreed in the two recent studies that autophagy plays little to no role in fate-determination of post-

mitotic MBs in embryonic and neural stem cells [19, 22]; although it is unclear if this is due to the low autophagic activity of this cell type or high MB release.

Another question arising from these studies is how to interpret the variability of MB retention and release across multiple cancer-derived cell lines. *A priori*, the genomic instability in such cells makes it difficult to evaluate results between different cell lines. It was shown that neural stem cells had a high basal level of MB release (90% of the MBs after abscission) whereas neuroblastoma cells and other cancer cells had only a medium to low level of release (10–50%; [19]). It was proposed that cancer cells exploit autophagy for MB clearance, which is consistent with what was shown recently [22]. One problem with the interpretation of the data from these two studies is that each was focused on a different cellular process and for the most part on different cell types. What is needed is a study designed to examine both degradation and release of MBs in the same panel of cell types *in vivo* and *in vitro*. Under the same experimental conditions, one could better understand the contributions of autophagic degradation, MB release and possibly other pathways to MB fate determination in cell types with low to high pluripotency.

Emerging non-cytokinetic roles of midbodies

Not until recently have researchers begun to study the fate of midbodies after cytokinesis [18, 19, 22, 23]. The few studies completed thus far suggest that MBs may have non-cytokinetic functions, such as polarity specification [67, 68], intercellular communication [17, 18] and cell fate determination [19, 22]. This is intriguingly analogous to studies showing multifunctionality of other structural assemblies generated during cytokinesis, such as bud scars in yeast and cytokinesis- or cell-division-remnants in nematode. In addition to the cytokinetic roles of these assemblies, they also define polarity for the upcoming cell division (for detail, see Box 4).

Text Box 4

Bud scars, cytokinesis remnants, cell-division remnants, and post-mitotic midbodies

Midbodies (MBs) are formed between dividing cells and appear to have additional non-cytokinetic roles as recently revealed (this review; [19, 22, 67, 68]). Similar structural assemblies formed during cytokinesis are also observed in unicellular organisms, such as yeast (for review, [73]). Although the molecular composition of these structural assemblies, namely bud scars, is different from MBs, their ring-like morphology is surprisingly similar [74, 75]. In addition to their cytokinetic roles, it is important to note that bud scars appear to send prohibitory signals to avoid repetitive budding at the same site in the next division cycle, but allow budding at abutted sites [75–77]. This function involves: 1) timely recruitment of multiple molecules to the bud neck region before cytokinesis is completed and 2) restrictive regulation of GTPase activity around the bud ([75, 76]; for review, [73]). These events contribute to cell polarity and defining the division axis for the next cell division. Thus, a post-mitotic scar in yeast also has additional non-cytokinetic role(s).

In *C. elegans*, a “cytokinesis remnant” is formed when cytokinesis is completed [78–80]. This structure was first identified as a membrane invagination enriched with F-actin and actin-capping proteins that remains at the anterior cortex of the posterior cell in 2-cell embryos but does not seem to accumulate after multiple divisions [79, 80]. In the work where different fixation conditions were used, multiple F-actin-enriched remnants were observed, and the total number was one less than the number of divisions (e.g., 7 remnants if 8 divisions in total; [79]). These accumulating structures were thus named “cell-division remnants” because it was unclear if they are the same structures as “cytokinesis remnants”. Moreover, it is unclear if these structures are MBs, as most metazoans’ MBs are not F-actin-enriched, and the remnants have not been shown to contain *bone fide* MB components.

As shown previously, upon completion of cytokinesis, a ring-like cytokinesis remnant surrounding the post-mitotic midbody starts to form [78–80]. The spindle in the cell with cytokinesis remnant rotates and aligns toward the cytokinesis remnant, where actin, actin-capping proteins and dynein are enriched [78–82]. The alignment seems to require microtubules, motors and actin [78, 81, 83]. This newly-formed cytokinesis remnant is the one that directs spindle alignment in the nematode embryo, just as the newest bud scar defines the cell division axis in yeast. Thus, the *C. elegans* cytokinesis remnant, the membrane domain surrounding the remnant or both could have additional roles beyond cytokinesis.

It is unclear why different organisms, from unicellular to multicellular and from yeast to man, have evolved or conserved a similar structural assembly that is used for cytokinesis then reused for other post-mitotic purposes, such as defining polarity *in vivo*. Of note, these structure assemblies appear to be compositionally quite different. What is the selective pressure driving formation of structurally similar but molecularly diverse organelles? For example, bud scars are mainly chitinous (for review, [73]). Cytokinesis remnants are membrane invaginations enriched in dynein, actin and actin-capping proteins. Metazoan midbody derivatives are protein structures composed of multiple MB proteins, membranes and microtubules, but do not appear to contain actin. Additional comparative analysis of the midbody and its counterparts in other organisms is required to understand the roles of these related structures and to determine if they have common functions beyond cytokinesis.

Post-mitotic MBs have also been implicated in cell polarity. In the fly notum and chick spinal cord, post-mitotic MBs of neurons were found at the polarized/apical domain where the future neurite or apical process sprouted [67, 68]. This led to the model that post-mitotic MBs might specify neuronal polarity. Post-mitotic MBs have also been implicated in intercellular communication, presumably to maintain the balance between differentiating cells and progenitors [17]. This idea is based on the fact that MB release into the ventricle lumen increases significantly after neurogenesis [17, 18]. One of the candidates for delivering such intercellular signals is Prominin-1 (CD133), a (cancer) stem cell marker and pentaspan membrane protein concentrated at the MB of neural progenitors (for review, [69]). As neurogenesis proceeds, small Prominin-1-positive vesicles arise from decomposing released MBs and may participate in intercellular communication. These intriguing results

nicely link post-mitotic MBs to polarity specification and intercellular signaling in the nervous system; however, more work is required to confirm these ideas and determine what molecule(s) are involved.

In addition to these potential roles of post-mitotic MBs, it was recently shown that experimental manipulation of MB clearance can sensitize cells for cell fate conversion, including differentiation and reprogramming programs [19, 22]. For example, inhibiting MB clearance/release could sensitize oligopotent neural progenitors to differentiate toward differentiated neurons. The opposite cell fate conversion, from terminal-differentiated fibroblasts to iPSCs may be sensitized by inducing MB retention within cells [19, 22]. This correlation between MB-low cells and differentiation status and MB-high cells and pluripotency status is an intriguing relationship that needs to be further analyzed to identify the role of MBs in these processes. While the potential of MBs to drive cells toward both differentiation and pluripotency pathways may seem contradictory, it is plausible if the modulator, presumably the MB, is itself modified in different ways. The reciprocal conversion between stem cells and differentiated cells could potentially result from different signaling molecules harbored in post-mitotic MBs or more simply, through the overall level of MBs in cells with different pluripotency status, from high (oligopotent) to low/none (differentiated cells). One must also consider as a contributing factor in cell fate conversion the different experimental manipulation used to modulate potency in the two studies (e.g., inhibiting MB degradation *versus* MB release). Despite these open questions, the role of post-mitotic MBs in cell fate conversion is clearly emerging. For example, increased MB retention in neural stem cells achieved by inhibiting MB release promotes cell fate conversion toward differentiation [19]. Increased MB retention in differentiated cells achieved by depleting the NBR1 autophagic receptor enhances cell fate conversion toward pluripotency [22]. Therefore, together with the other studies reviewed above, the idea that MBs possess multiple non-cytokinetic roles, unrelated to cell division, is becoming apparent. These new findings certainly require further investigation to tease out the molecular mechanisms, and will in turn move our understanding on (asymmetric) cell divisions in stem cell biology forward.

Concluding remarks

For almost a century, the MB has been perceived simply as a link between two dividing daughter cells that was discarded after division. More recently, its role as an organizing site for abscission was proposed [3, 8, 12, 13, 21, 42]. Moreover, based on the diversity of proteins associated with MBs identified in numerous studies and the ‘MB parts list’ obtained from mass spectrometry[43], additional functions of MBs and post-mitotic MBs will surely be uncovered for years to come.

As discussed above, data from many studies as well as suggestions from the MB proteome indicate that multiple pathways converge in vicinity of the MB during abscission [3, 6–9, 21, 42–45]. Although further clarification is needed on how these pathways mediate abscission, one must consider cooperative functioning of fusion and fission machinery either consecutively or concordantly, as an essential part of this process. For example, vesicle fusion along the bridge to remodel the bridge membrane might set the stage allowing

ESCRT to function, or work concordantly with ESCRT to accomplish the ultimate membrane fission for abscission. We favor the latter model, given the similar timing of elevated vesicle trafficking to the bridge and ESCRT-III translocation to the constriction zone [3, 9, 21]. ESCRT may coordinate MT disassembly in the bridge by timed interaction with MT-severing proteins [58]. This coordination could be a critical factor during abscission. However, complete MT loss prior to abscission may not be required given the considerable number of MT bundles that are retained by post-mitotic MBs following abscission [3, 7].

The inheritance and retention of the post-mitotic MB by one daughter cell is quite remarkable and makes the two genetically identical daughter cells no longer equal. One inherits a large organelle comprised of hundreds of proteins; the other receives no post-mitotic MBs. In parallel, post-mitotic MBs can be released presumably to remove critical molecules from daughter cells. Although questions remain about the details, intriguing new evidence has begun to uncover the functional significance of post-mitotic MBs (see above). We are beginning to get glimpses of MBs as structures whose retention and loss can influence stem cells, cancer cells and differentiated cells. The potential roles of MBs in influencing cell fate determination are unanticipated and exciting. Further investigation is required to understand precisely how these organelles impact cellular function. In any case, it is clear that the current perception of post-mitotic MBs has risen from remnants jettisoned from cells to post-mitotic organelles serving unexpected functions.

Glossary Box

Abscission	The final stage of cytokinesis that culminates in severing the intercellular bridge to generate two physically separate daughter cells. This process requires several membrane trafficking pathways, the ESCRT complex and the machinery for microtubule severing
Autophagosome	An intracellular double-membrane vacuole/vesicle formed <i>de novo</i> that engulfs molecules or organelles from the cytoplasm and degrades them by fusing with lysosomes. Autophagosome formation is known to require the autophagy-related gene (Atg) 6, or Beclin-1, and two ubiquitin-like conjugation systems (e.g., LC3/Atg8)
Endosomal sorting complex required for transport (ESCRT)	A protein complex containing four subcomplexes (ESCRT-0, -I, -II and -III) and associated proteins (e.g., Alix, Ist1 and Vps4), that sorts and transports target molecules to endosomes. It is required for viral budding and has recently been shown to play a role in abscission
Exocyst	A protein complex required for the tethering and targeting of post-Golgi vesicles to the plasma membrane for secretion and abscission

Intercellular bridge	The cytoplasmic connection between a dividing cell in late cytokinesis that contains the midbody and is the ultimate site of cell separation
Midbody (MB)	A prominent organelle located within the intercellular bridge. It is comprised of a central proteinaceous disc or ring-like core flanked by microtubules that overlap within the ring region as anti-parallel bundles. This core is also referred to as Flemming body or midbody ring (MR). The MB undergoes many morphological and molecular changes prior to abscission, and is essential for the completion of cell division
Multivesicular body (MVB)	A specialized membranous compartment in the endosomal pathway characterized by internalized vesicles within its lumen. After fusing with lysosomes, the molecular contents within MVBs can be degraded
Phragmoplast	A spindle-derived structure formed during plant cytokinesis. It is comprised of microtubules, actin and a variety of membranous components, and required for completion of cytokinesis
SNAREs	An acronym of Soluble NSF Attachment Protein RE ceptors. This is a large protein superfamily whose major function is to facilitate fusion between vesicles and the membrane of target compartments. SNAREs can thus further be divided to vesicle SNAREs (v-SNAREs) such as endobrevin/VAMP8, and target SNAREs (t-SNAREs) such as syntaxin-2

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Figure 1.

Schematic representation of cytokinesis progression, abscission and fate of post-mitotic midbody. (a) A representative drawing of Zwischenkörper by Walther Flemming. The black dot between the two reforming nuclei (N) represents the midbody (MB; white arrow). (b) Cytokinesis progression from early to late cytokinesis is shown from left to right and can be generally dissected into three stages. Left: Anti-parallel microtubules (MTs) and electron-dense material initially form a patchwork at the midzone across the forming furrow where vesicles are concentrated. Middle: After furrow ingression, the patchwork of midzone MTs transforms into one major bundle between two daughter cells and vesicles concentrate at the end of the bundle. Right: As cytokinesis proceeds, the intercellular bridge connecting the

two daughter cells is gradually remodeled and narrowed, making the MB a prominent bulge in the bridge. (c) Enlargement of inset in (b), showing the process of abscission and the fate of the post-mitotic midbody. From left: As the cell approaches the final stage of abscission, the density of MTs decreases and different vesicle types again appear in vicinity of the MB. Meanwhile, ESCRT complexes are recruited to the constriction zone, about 1 μm from the MB, and interact with the MT-severing protein (e.g. spastin). Presumably, abscission requires the orchestration of these pathways (e.g. vesicle trafficking and fusion, ESCRT machinery and MT severing). After abscission, the post-mitotic MB is either released after a secondary bridge-severing event (bottom left panel) or inherited by one of the two daughter cells (bottom middle and right panels). The inherited MBs may be retained freely in the cytoplasm (bottom middle panel) or degraded in the autolysosome after recognized by autophagic receptors (grey ovals) and engulfed by autophagosomes (bottom right panel). Thus, two major mechanisms for MB clearance are autophagy and MB release. The drawing of Zwischenkörper was reproduced from [24].

Figure 2.

The architecture of the midbody during late cytokinesis and abscission. (a) Electron micrograph of a midbody (MB) is shown in the top panel. An immunostained MB at a comparable stage with corresponding differential-interference contrast (DIC) microscopic image are in the middle and bottom panel, respectively. At the center is the electron-dense MB core (top), which is comprised of the “MB ridges” and “MB matrix”. The MB ridges (arrowheads; top) correspond to the bulge by DIC microscopy (arrows; bottom) and the MB ring by immunofluorescence (arrowheads; middle). The MB matrix, in contrast, contains many anti-parallel microtubules and electron-dense material. α -tubulin, intercellular bridge (green); Mklp1, MB (red). (b) Electron micrograph of a MB residing in an intercellular bridge prior to abscission. Top left: About 1 μ m to the right of the MB is the constriction zone. Top right: Enlargement of inset in the top left panel, showing “ripple contours” prior to abscission. Bottom: the right four images are from serial sections, showing the helical

filaments that account for the appearance of ripple contours and that disappear upon depletion of ESCRT-III components. Images of immunostained MB (a) are provided courtesy of C.-T. Chen and S.D. Electron micrographs (a, b) were reproduced with permission from [7, 16].