# Illuminating the dark zone: insights into the evolving structure of the midbody

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## Abstract

During cell division, the DNA of the mother cell is duplicated and divided among the two daughter cells. After the sister chromatids have been separated, the interpolar microtubules form the fundaments of the central spindle. This central spindle is compacted by cleavage furrow ingression, resulting in the formation of an intercellular bridge with a protein dense structure, the midbody, in the center. The midbody consists of proteins, mRNA, and lipids. These originate from the central spindle, contractile ring, or are recruited from other parts of the cell. Recruitment of components is not the only change in the midbody. Other components are removed from the midbody or change in localization over time. Several midbody components play a role in abscission, the final step of cytokinesis that completely separates the daughter cells. After abscission, the midbody either remains connected to one daughter cell and is inherited by that cell, or it is shed to the extracellular environment from which other cells might take it up. This midbody remnant can be involved in signaling that changes the behavior and fate of the cell that receives it. The different components of the midbody and their function have been researched for over 40 years. Recently, mRNA was discovered as part of the midbody. Together with the increase in detected proteins at the midbody, this enables research into previously unknown functions or regulation of this organelle. This review aims to provide an overview of the components of the midbody, their function, and how they evolve over time.

## Plain language summary

During development, a single cell gives rise to a complete organism. In order to do so, many rounds of cell divisions are needed. Throughout life, cells keep dividing to enable growth, repair, or maintenance of tissues. Since a cell division starts with one cell and ends with two cells, the components of this one cell have to be duplicated. After duplication, the components are equally divided between the two daughter cells. As the final step of cell division, the two daughter cells are physically separated. This starts with pinching of the cell membrane, that forms the interface between the inside and outside of the cell. This pinching occurs in between the two prospective daughter cells. The position of the pinching is regulated by several proteins that cluster together in between these cells. After pinching, the two cells are connected through a thin tube of membrane. Pinching of the membrane does not only narrow the diameter of the membrane connection, but it also compresses the protein cluster that regulates the position of pinching. After compression, this protein cluster forms the foundation for a structure called the midbody. Apart from the proteins of this cluster, the midbody contains other components that come from different parts of the cell. The midbody has several functions that arise through the activity of its components. Firstly, many midbody components play a role in abscission. This is the process that eventually cuts the membrane tube and thereby separates the two new cells. The factors needed for abscission are recruited to the midbody after the membrane pinching. Abscission occurs on one or both sides of the midbody. So, it either remains connected to one daughter cell or it is released into the extracellular environment. Surrounding cells might take it up from this environment. After abscission, another function of the midbody can be carried out in a cell that the midbody remains attached to or that takes it up. This midbody function is to change the behavior of the cell that it ends up in. The midbody might, for example, make such a cell divide faster. Since the different functions of the midbody require different components to be at this organelle at different timepoints, the composition of the midbody changes over time. These changes include the recruitment of components that can be specifically delivered to the midbody, the removal of other midbody constituents, or the change in location of some elements. All these processes work together to ensure proper functioning of the midbody in regulating abscission. Although the midbody has been researched for over 40 years, new components and their function are still discovered. Knowledge on the components of the midbody and how they change over time can assist in our understanding of the regulation of cell division and the effect of the midbody in cells that take it up.

## Introduction

During mitosis, the duplicated DNA is separated by the mitotic spindle. This spindle also plays a role in the subsequent process of cytokinesis. It consists of astral, kinetochore, and interpolar microtubules that extend from either of the two centrosomes (Glotzer, 2009). In the spindle midzone, the plus ends of the interpolar microtubules from the two centrosomes interdigitate, forming a structure of antiparallel microtubules. Throughout anaphase, factors are recruited to these interpolar microtubules to form the central spindle (Glotzer, 2009). This central spindle signals to the cell cortex to initiate the formation of the actomyosin contractile ring. Contraction of the ring results in compaction of the central spindle and the region surrounding it (Mierzwa & Gerlich, 2014). Thereby, a protein dense structure called the midbody and a thin intercellular bridge (ICB) are formed respectively. This primary ingression by the contractile ring is not sufficient to separate the daughter cells. To complete the process, secondary ingression of the ICB followed by its abscission is required (Mierzwa & Gerlich, 2014; Peterman & Prekeris, 2019). This final abscission happens when the daughter cells have already started their next cell cycle and are in the G1 phase (Gershony et al., 2014).

The midbody has three regions (Addi et al., 2018; D'Avino & Capalbo, 2016; Peterman & Prekeris, 2019). The midbody core is the dense section at the center of the midbody. It is compacted to such an extent that it is impermeable to antibodies (D'Avino & Capalbo, 2016; Hu et al., 2012). Therefore, it has also been referred to as the midbody dark zone. The midbody ring surrounds the midbody core and is flanked by the midbody arms that extend into the ICB (D'Avino & Capalbo, 2016) (**Figure 1**). The midbody matures after its formation. During this process, the location of midbody proteins changes and proteins that execute or regulate abscission are recruited (D'Avino & Capalbo, 2016; Halcrow et al., 2022) (**Figure 2A**). Some cellular stresses can delay abscission by activating the NoCut pathway that inhibits the abscission machinery and thereby delays abscission (Addi et al., 2018; Kodba & Chaigne, 2023; Norden et al., 2006; Steigemann et al., 2009). Eventually, abscission takes place on one or both sides of the midbody, resulting in asymmetric or symmetric abscission respectively (Chen et al., 2013; Gromley et al., 2005; Peterman & Prekeris, 2019). After asymmetric abscission, the midbody is inherited by the daughter cell that it remains attached to (Peterman & Prekeris, 2019). Symmetric abscission leads to shedding of the midbody into the extracellular environment. At this stage, the midbody is referred to as the midbody remnant and is a specific class of extracellular vesicle (Rai et al., 2021; Suwakulsiri, Xu, Rai, Shafiq, et al., 2024). The midbody remnant can be taken up by surrounding cells through engulfment, a process that resembles phagocytosis and requires polymerization of F-actin (Crowell et al., 2014). After engulfment, the midbody remnant is probably degraded in lysosomes. However, it takes around 6 hours until the midbody remnant is completely degraded (Crowell et al., 2014). During this time, it might influence the behavior of the cell that engulfed it (Peterman et al., 2019; Rai et al., 2021).

To understand the functions of the midbody, it is important to know what the midbody is comprised of. The first protocols for isolation and characterization of midbodies were published around 1980 (Mullins & McIntosh, 1982). This is approximately 90 years after the midbody was first described by Walther Flemming (Addi et al., 2020; D'Avino & Capalbo, 2016; Mullins & McIntosh, 1982). At the time of the first characterization of the midbody, the protein content could only be determined through SDS-PAGE (Mullins & McIntosh, 1982). Later, the rise of mass spectrometry and RNA sequencing enabled more detailed investigation of

midbody components both before and after abscission. Through the use of these techniques, many proteins and mRNA transcripts have been detected in midbodies (Addi et al., 2020; Capalbo et al., 2019; Farmer et al., 2023; Park et al., 2023; Skop et al., 2004; Suwakulsiri, Xu, Rai, Chen, et al., 2024; Suwakulsiri, Xu, Rai, Shafiq, et al., 2024). Some of the components have been linked to cytokinesis (Addi et al., 2020; Skop et al., 2004). Others are predicted to play a role in signaling pathways, some of which might be involved in oncogenesis and regulation of stem cell like characteristics (Peterman et al., 2019; Peterman & Prekeris, 2019; Rai et al., 2021).

Although the midbody remnant might induce tumorigenesis through different signaling pathways, proper functioning of the midbody is required to reduce cancer risk since it regulates abscission to prevent multinucleated cells or aneuploidy (Capalbo et al., 2019; Gruneberg et al., 2006; Peterman & Prekeris, 2019). Knowledge on the components of the midbody and their regulation can increase the understanding of the roles of the midbody in cytokinesis and the signaling function of the midbody remnant. Therefore, this review focusses on the components of the midbody and how they change over time.

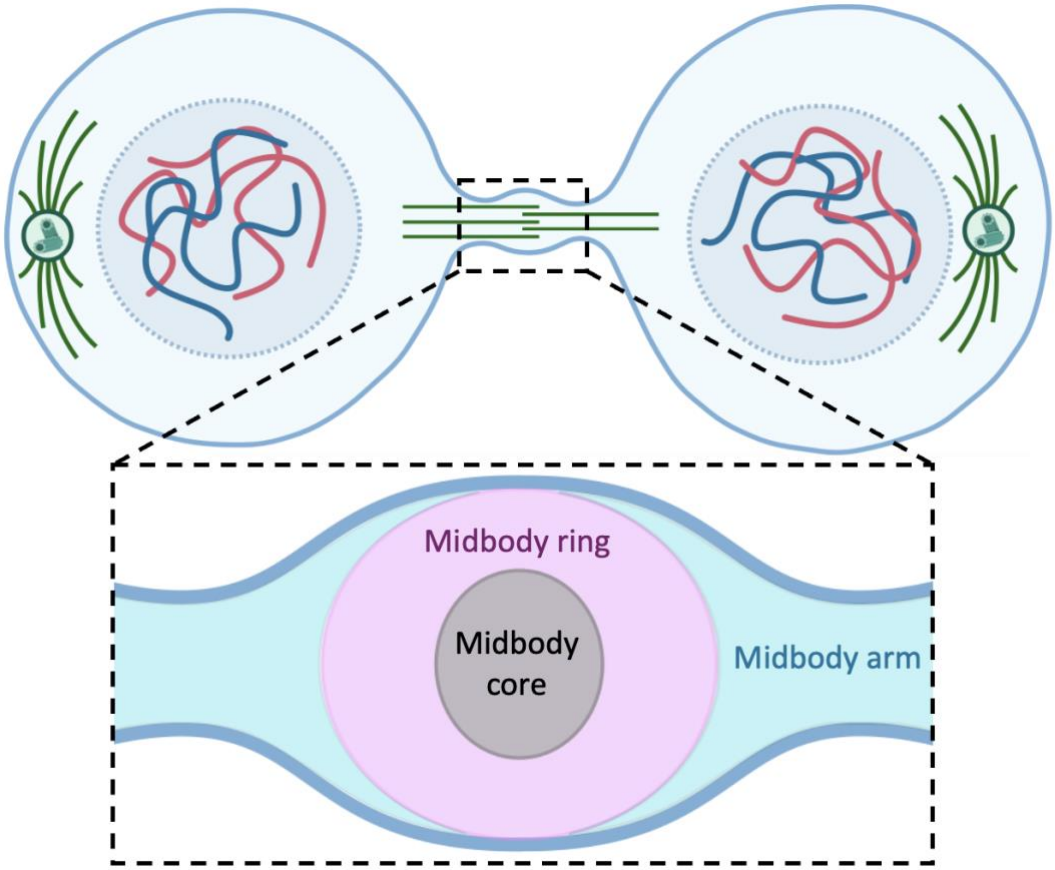


***Figure 1: Structure of the midbody***
*At the top, a dividing cell in telophase is shown. At this stage of the cell cycle, the two daughter cells are connected via an intercellular bridge. Within the bridge, the area with a dashed black outline is the midbody. This is enlarged at the bottom. The midbody has three regions. Firstly, the midbody core (grey) in the center. Secondly, the midbody ring (purple) that surrounds the midbody core. Thirdly, the midbody arms (blue) that flank the midbody ring and extend into the intercellular bridge. At the top, green lines indicate microtubules, the red and blue lines depict chromosomes. Figure created with BioRender.*

# 1. Components of the midbody

Since the midbody is formed through compression of the central spindle by the actomyosin ring, it contains components that originate from both of these structures (Mierzwa & Gerlich, 2014) (**Figure 2B and C**). These are however not the only components of the midbody as others are recruited later (**Figure 2**). The main components of the midbody are described next.

### 1.1 Components that the midbody inherits from the central spindle

As the interpolar microtubules serve as a platform for the recruitment of other components of the central spindle, they are an important part of this spindle (Glotzer, 2009). Microtubules consist of $\alpha$- and $\beta$-tubulin, both of these microtubule subunits are major components of the midbody (Mullins & McIntosh, 1982). Kinesin and dynein move along microtubules, some of these motor proteins have been observed at the midbody (Zhu et al., 2005). Kinesin superfamily protein (KIF) 23, KIF20A, KIF4A, and KIF4B are essential for cytokinesis in HeLa cells. Other kinesis that have been detected at the midbody are CENPE, KIF14, KIF1A, and KIF20B (Gruneberg et al., 2006; Hu et al., 2012; Janisch et al., 2018; Rai et al., 2021). Furthermore, a light intermediate chain of dynein is present in midbodies of an epidermal cancer cell line (Horgan et al., 2011).

The chromosomal passenger complex (CPC) is a crucial protein complex in both anaphase and cytokinesis (D'Avino & Capalbo, 2016). It is recruited to the central spindle by movement of the kinesin KIF20A along microtubules (**Figure 2B**). The CPC consists of the four proteins Survivin, Borealin, inner centromeric protein (INCENP), and Aurora B. These four components all have their own function at the midbody. Firstly, Aurora B is a kinase that has multiple functions throughout mitosis (Mathieu et al., 2013). In metaphase, it regulates chromosome attachment to the mitotic spindle. When the chromosomes are pulled to the opposite ends of the mitotic spindle in anaphase, Aurora B and the rest of the CPC remain at the central spindle. There, Aurora B regulates other processes such as furrow ingression and midbody formation (Mathieu et al., 2013; McKenzie et al., 2016). After contraction of the actomyosin ring, the CPC and Aurora B localize to the midbody arms where Aurora B can phosphorylate proteins (**Figure 2B**). Some of these Aurora B substrates are involved in abscission (McKenzie et al., 2016). Through phosphorylation of abscission related proteins, Aurora B governs the evolutionarily conserved NoCut pathway that delays abscission upon cellular stresses (Kodba & Chaigne, 2023). An example of such a cellular stress are chromosome bridges that activate Aurora B (Steigemann et al., 2009). Additionally, in yeast, defects in the intercellular bridge bring about activation of the Aurora B and INCENP homologs, resulting in inhibition of abscission (Norden et al., 2006). Aurora B has multiple substrates within this NoCut pathway (Carlton et al., 2012; Mathieu et al., 2013). In *Drosophila*, Aurora B phosphorylates the cell cycle protein Cdk1, causing delayed abscission (Mathieu et al., 2013). Moreover, Aurora B phosphorylates the abscission related protein charged multivesicular body protein (CHMP) 4C in HeLa cells (Carlton et al., 2012). This postpones abscission, possibly by sequestration of CHMP4C and other abscission regulators away from the abscission site (Carlton et al., 2012; Mierzwa & Gerlich, 2014). In addition to the NoCut pathway, the activity of Aurora B increases microtubule stability in stem cells, which also inhibits abscission (Kodba et al., 2024). The importance of the removal of microtubules for abscission will be described later. A second CPC protein, INCENP, interacts with the kinesin KIF20A to relocate the CPC from the kinetochores to the central spindle (D'Avino & Capalbo, 2016). Furthermore, INCENP is involved in the activation of Aurora B (McKenzie et al., 2016). Thirdly, Survivin plays a role in

the abscission process in *Drosophila* germ cells (Mathieu et al., 2013). Lastly, Borealin can engage with components of the midbody that regulate and execute abscission (D'Avino & Capalbo, 2016).

The CPC is present in the cell equator from early in mitosis, other central spindle proteins are recruited later. For example, KIF23 only localizes to the central spindle once it is phosphorylated by Aurora B (Douglas et al., 2010). Since Aurora B activity is highest in anaphase, KIF23 recruitment probably mainly happens in that phase of mitosis. Phosphorylation of KIF23 does not only result in its central spindle localization, but also its clustering. KIF23 localizes to the central spindle together with Rac GTPase activating protein 1 (RacGAP1) (**Figure 2B**), which forms the centralspindlin complex together with KIF23 (Capalbo et al., 2019; Hu et al., 2012). Centralspindlin has an important role in the organization and stabilization of microtubules at the central spindle. Furthermore, the complex connects the central spindle to the plasma membrane either by direct interaction of RacGAP1 with the membrane or through interaction of KIF23 with the membrane bound protein ARF6 (Lekomtsev et al., 2012; Makyio et al., 2012).

Kinesin KIF4A and protein regulating cytokinesis 1 (PRC1) together form another protein complex that is needed for the formation of the central spindle (Capalbo et al., 2019; Hu et al., 2012) (**Figure 2B**). Before anaphase, PRC1 is decorated with inhibitory phosphorylations placed by Cdk1 (Mierzwa & Gerlich, 2014). At the start of anaphase, these phosphorylations are removed so PRC1 can bundle the antiparallel microtubules at the central spindle (Neef et al., 2007). Furthermore, PRC1 interacts with Polo-like kinase 1 (Plk1) and several kinesins (Neef et al., 2007). The interaction of PRC1 with Plk1 is needed to recruit Plk1 to the central spindle of HeLa cells. At the central spindle, Plk1 interacts with other proteins to control their activity. For example, it can activate Rho-GTPase regulators such as RacGAP1 by phosphorylation (Bastos & Barr, 2010; Rai et al., 2021). Furthermore, Plk1 regulates the recruitment of centrosomal protein of 55 kD (CEP55) to the midbody (Bastos & Barr, 2010). CEP55 plays an important role in the recruitment of the abscission machinery that will be described later. Phosphorylation of CEP55 by Plk1 inhibits the association of CEP55 with KIF23 at the midbody (Bastos & Barr, 2010). This prevents the premature recruitment of CEP55. Degradation of Plk1 after anaphase is needed for the timely recruitment of CEP55. This is probably mediated by the anaphase-promoting complex/cyclosome when it is in complex with Cdh1 ($APC/C^{Cdh1}$) (Gershony et al., 2014; Pe'er et al., 2013). The $APC/C^{Cdh1}$ ubiquitinates Plk1 late in mitosis whereafter it is degraded via the proteasome.

Although CPC, centralspindlin, KIF4A, PRC1, and KIF20A all localize to the central spindle, they do not localize to the same parts of the midbody (Hu et al., 2012). Centralspindlin and interacting proteins, such as CEP55, localize to the midbody ring (**Figure 2A and B**). In contrast, KIF4A and PRC1 can be detected in the midbody core (**Figure 2B**). This localization of KIF4A and PRC1 might be explained since the antiparallel microtubules that are bundled by PRC1 are exclusively present in the midbody core. CPC and KIF20A do not localize to the central part of the midbody but can instead be found at the midbody arms (**Figure 2B**).

### 1.2 Components that the midbody inherits from the contractile ring

The central spindle is compacted through force exerted by the contractile ring (D'Avino & Capalbo, 2016). This ring is primarily made up of actin and myosin (Schiel et al., 2012). Myosin disappears from the cleavage furrow soon after primary ingression. Actin, however, remains at the ICB at least until late telophase (Dambournet et al., 2011; Schiel et al., 2012). Actin depolymerization is needed for abscission to take place, this can be mediated through several proteins that either decrease actin polymerization or increase actin depolymerization (Dambournet et al., 2011; Frémont et al., 2017; Schiel et al., 2012). Later, the mechanisms of actin removal are described more extensively.

Citron kinase (CIT-K) is another component of the contractile ring that localizes to the midbody (Capalbo et al., 2019) (**Figure 2C**). In HeLa cells, it interacts with components of both the contractile ring and the central spindle such as centralspindlin, CPC, and KIF20A (McKenzie et al., 2016). One domain of CIT-K is predicted to form a seven bladed β-propeller. Such a structure can serve as a scaffold for binding of multiple proteins. This could be needed for the function of CIT-K since this protein is required for the proper localization of multiple midbody components (Gai et al., 2011; McKenzie et al., 2016). Although CIT-K is necessary for the correct localization within the midbody, it is not needed for the initial recruitment of components to the midbody (McKenzie et al., 2016). The localization of CIT-K at the midbody ring and its interaction with central spindle components relies on the CPC and more specifically the activity of Aurora B (McKenzie et al., 2016). CIT-K itself is also a kinase, it phosphorylates INCENP at a residue that is important for Aurora B activation, implying crosstalk between these two kinases (McKenzie et al., 2016).

RhoA is one of the proteins that requires CIT-K for its proper localization at the midbody (Gai et al., 2011). RhoA is a small GTPase that controls the actin polymerization that drives furrow ingression. Its activity is regulated through guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) that in turn can be regulated by CIT-K (Gai et al., 2011). One of the GEFs, ECT2, localizes to the central spindle. At this location, it binds phosphorylated RacGAP1 and locally activates RhoA at the cortex surrounding the spindle (Mierzwa & Gerlich, 2014). This promotes contraction of the actomyosin ring. RhoA also regulates other proteins at the midbody, it can for example stabilize anillin (Gai et al., 2011) (**Figure 2C**). Anillin interacts with actin and microtubules and is important for the assembly and stability of the cleavage furrow. In addition, it recruits septins to the furrow (Gai et al., 2011; Renshaw et al., 2014). Early after midbody formation, RhoA, anillin, and septins are present at the midbody ring (Hu et al., 2012; Renshaw et al., 2014). Later, these proteins move towards the abscission sites. This localization of septins late in cytokinesis is needed to recruit the abscission related protein CHMP4B to the abscission site (Renshaw et al., 2014).

### 1.3 Components needed for abscission

The midbody is important in the regulation of abscission (D'Avino & Capalbo, 2016; Mierzwa & Gerlich, 2014). As a part of this role of the midbody, components that are required for abscission localize to the midbody or in its vicinity (**Figure 2D**). In contrast to components that are inherited from the central spindle or contractile ring, most of these components are recruited to the midbody after its formation.

Before abscission takes place, the ICB is constricted by proteins from the endosomal sorting complex required for transport III (ESCRT-III). These proteins form cortical filaments with a helical shape to constrict the bridge (Guizetti et al., 2011) (**Figure 2D**). VPS4 is required for the constriction of these ESCRT-III filaments and localizes to the midbody late in cytokinesis (Goliand et al., 2018; Morita et al., 2007) (**Figure 2D**). Apart from components that are needed for abscission, the abscission site contains negative regulators of abscission such as the microtubule and actin binding protein Gas2l3 (Pe'er et al., 2013). VPS4 and other ESCRT cofactors can be recruited through interactions between the microtubule interacting and trafficking (MIT) domains of the cofactors with MIT interacting motifs (MIM) of ESCRT-III (Wenzel et al., 2022). ESCRT-III itself is recruited by several other proteins. The most upstream of these is CEP55 which localizes to the midbody after its inhibitory phosphorylation by Plk1 is removed (Bastos & Barr, 2010). CEP55 interacts with two other ESCRT proteins, ALIX and tumor-susceptibility gene 101 (TSG101) (Morita et al., 2007) (**Figure 2D**). TSG101 and ALIX recruit the ESCRT-III subunit CHMP4B via different pathways. The pathway with TSG101 further relies on ESCRT-II subunits such as EAP20 that interact with CHMP6, another component of ESCRT-III (Christ et al., 2016). Recruitment of TSG101, ALIX, and the ESCRT-III subunit CHMP4B to the ICB can occur through vesicular transport that at least partly relies on Rab11 (Pust et al., 2023). Rab11 is one of the Rab GTPase proteins that are implicated in vesicular transport to the midbody. Other proteins that play a role in this are part of the SNARE and exocyst complexes (Gromley et al., 2005; Kouranti et al., 2006).

Vesicular transport might not only play a role in abscission through delivering ESCRT components or -interactors to the ICB. It can also deliver other proteins needed for abscission, such as secretory carrier membrane protein 2/3 (SCAMP2/3) and p50RhoGAP (Schiel et al., 2012). SCAMP2/3 and p50RhoGAP are transported in FIP3 positive endosomes. SCAMP2/3 could regulate the fusion of these endosomes with the plasma membrane in the ICB or recruit TSG101. Furthermore, SCAMP2/3 and p50RhoGAP can increase actin depolymerization. Apart from proteins, vesicles can deliver phospholipids that might be required for cytokinesis (Kouranti et al., 2006). The process of vesicle fusion with the plasma membrane in the ICB can also aid abscission by narrowing the ICB (Gromley et al., 2005; Schiel et al., 2012). The role of vesicles in cytokinesis is illustrated by the finding that in asymmetric abscission, a certain type of secretory vesicles was only derived from one daughter cell (Gromley et al., 2005). These vesicles exclusively fused to the plasma membrane on one side of the midbody. The side where the vesicles fused was also the side where abscission occurred.

### 1.4 Other components found at the midbody

The recruited midbody components described thus far are all related to abscission. However, components that do not seem to function in abscission are also recruited to the midbody. Several of these recruited components with distinct functions are described next.

Proteins that are involved in signaling transduction pathways, such as MAPK 1, 3, 4, and 6, are among these recruited components in a colorectal cancer cell line (Rai et al., 2021). Those signaling related proteins persist in midbody remnants, from which they might alter the behavior of cells that engulf them. These remnants do not only contain cytosolic signaling proteins. Membrane bound proteins such as EGFR and integrins have also been detected in midbody remnants of HeLa cells (Peterman et al., 2019). Downstream effectors of signaling pathways, for example the transcription factor YAP, were also observed in midbodies (Somma

et al., 2020). YAP is needed for cytokinesis, indicating that signaling proteins might also function during abscission (Konno et al., 2020).

In human epithelial cells, tight junction proteins are another protein class found in midbodies. These localize to the centrosome earlier in mitosis (Konno et al., 2020). The tight junction proteins are not necessary for cytokinesis. However, the role of these proteins could be to form new tight junctions close to the midbody during cytokinesis. The formation of these new tight junctions is needed to maintain the barrier function of an epithelial layer. Tight junction proteins are not the only proteins that are detected in the centrosome early in mitosis and are found at the midbody later in mitosis. The same is true for the abscission related proteins CEP55, TSG101, and ALIX (Morita et al., 2007). Another centrosomal protein, FYVE-CENT, is translocated to the midbody by KIF13A (Sagona et al., 2010). Other proteins that are at the centrosome at the start of mitosis and can later be detected in the midbody are CDKL5, CypA, centriolin, and BRUCE (Barbiero et al., 2017; Gorry et al., 2023; Pohl & Jentsch, 2008). The presence of these centrosomal proteins at the midbody could be linked to the behavior of the centrosome during cytokinesis. In HeLa cells, the mother centriole of at least one of the daughter cells gets close to the midbody before abscission (Piel et al., 2001). Later, this centriole moves away from the midbody. Just after the centriole retracts from the ICB, abscission occurs. Abscission preferentially takes place on the side of the ICB that was in close contact with the centriole. The daughter centrioles can also get close to the bridge. However, this movement is less correlated with abscission. Whether the presence of one or both mother centrioles at the ICB relates to asymmetric or symmetric abscission respectively, is yet unknown.

One of the centrosomal proteins, CDKL5 is associated with the kinase HIPK2 and indispensable for HIPK2 localization to the midbody (Barbiero et al., 2017). At the midbody, HIPK2 can phosphorylate spastin and histone 2B (H2B) (Rinaldo et al., 2012; Sardina et al., 2020). The phosphorylation of H2B is required for the regulation of cytokinesis. H2B is not the only nuclear protein found at the midbody since WD repeat-containing protein 5 (WDR5), a part of a histone methyltransferase complex, has also been detected at the midbody (Bailey et al., 2015).

The midbody does not only consist of proteins. Recently, RNA has also been identified as one of its components in mammalian cells (Farmer et al., 2023; Park et al., 2023) (**Figure 2A**). This encompasses mRNA, lncRNA, RNA from pseudogenes, and mitochondrial RNA (Farmer et al., 2023; Park et al., 2023; Suwakulsiri, Xu, Rai, Chen, et al., 2024). Among these are mRNA transcripts encoding proteins that are involved in regulation of cell fate, signal transduction, cell cycle, RNA binding, and RNA processing (Farmer et al., 2023; Park et al., 2023; Suwakulsiri, Xu, Rai, Chen, et al., 2024). Furthermore, Farmer *et al*. found mRNA that encodes proteins involved in microtubule dynamics and abscission. An example of the latter class are ESCRT-III components (Farmer et al., 2023). The mRNA detected at the midbody could be stabilized there by Ataxin-2, a protein that has been identified in proteomic analysis of midbodies in CHO cells (Skop et al., 2004; Yokoshi et al., 2014). mRNA might be further stabilized by the viral nucleocapsid protein ARC that is needed to localize mRNA to the midbody (Park et al., 2023). The mRNA at the midbody is actively translated (Farmer et al., 2023; Park et al., 2023). Since ribosomes are needed for the translation, it is not surprising that ribosomal proteins were detected in midbodies (Addi et al., 2020; Farmer et al., 2023; Park et al., 2023; Rai et al., 2021;

Skop et al., 2004). So, proteins are made at the midbody by translation of mRNA (Farmer et al., 2023; Park et al., 2023). On the other hand, proteins might also be degraded at the midbody since proteasomal proteins have been detected there (Addi et al., 2020; Rai et al., 2021).

A final class of midbody components are phospholipids. The phospholipid composition of the plasma membrane surrounding the midbody remnant differs from that of other cellular membranes of neural progenitor cells of mouse embryos (Arai et al., 2015). The most prominent changes are an increase in phosphatidylserine (PS) and phosphatidylethanolamine (PE). PE has a relatively wide 'head', resulting in an inverted cone shape that facilitates membrane curvature and thereby might aid abscission. PS can move to the outer leaflet of the midbody membrane after abscission (Arai et al., 2015; Peterman et al., 2019). The presence of this phospholipid on the outer leaflet also occurs during apoptosis, as a signal to phagocytes to engulf the apoptotic cell. It might have a similar function on the midbody membrane, especially since phagocytosis regulators are present in the midbody remnants of HeLa cells (Peterman et al., 2019). Whether the PS in midbodies indeed contributes to engulfment is still unclear since it is not consistently detected in the outer leaflet (Crowell et al., 2014; Peterman et al., 2019). Apart from more PS and PE, midbody remnants have more unsaturated phosphatidylcholine, indicating a less dynamic membrane (Arai et al., 2015).

In summary, the midbody has many protein, RNA, and lipid components. Some of these are inherited from the central spindle or contractile ring when the midbody is formed. Other components are recruited later and, for example, mediate abscission.

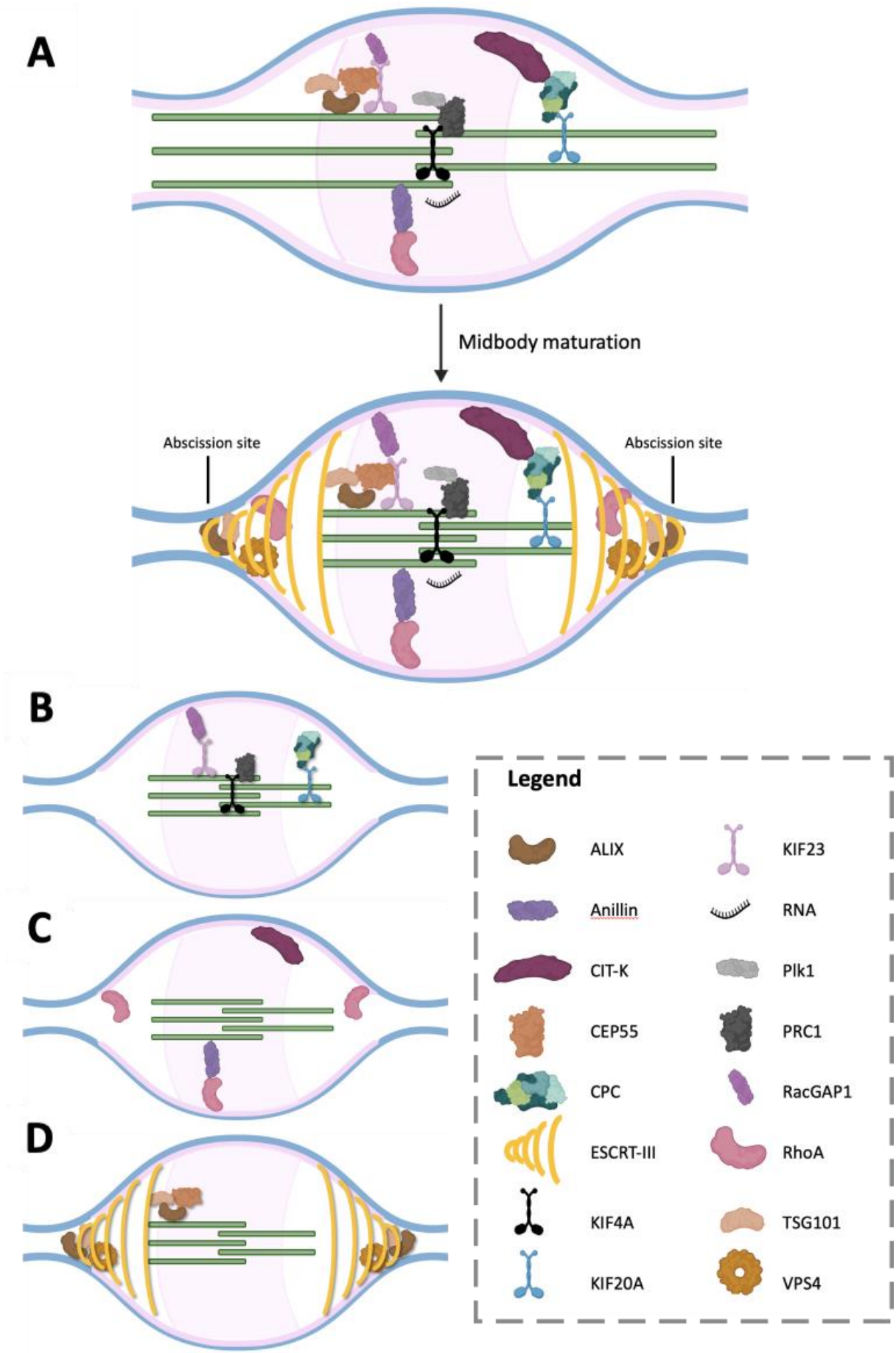
A
Midbody maturation
Abscission site
Abscission site
B
C
D
Legend
ALIX
Anillin
CIT-K
CEP55
CPC
ESCRT-III
KIF4A
KIF20A
KIF23
RNA
Plk1
PRC1
RacGAP1
RhoA
TSG101
VPS4

***Figure 2: Main components of the midbody***

*A. The main components of the midbody and their localization after its formation and the recruitment of the first components (top) or just before abscission (bottom) are shown. At the latter stage, the microtubules and cortical actin have been removed from the abscission sites to facilitate abscission (Addi et al., 2018). Subsets of the components and their localization at the stage just before abscission are shown in B, C, and D. B. Midbody components that originate from the central spindle. C. Components that the midbody inherits from the actomyosin ring. D. midbody components that are recruited after formation and play a role in abscission. Horizontal green lines indicate microtubules. Actin is shown in pink, the vertical pink band in the middle of each midbody shows the contractile ring. Components are colored based on their location at the midbody core (black or grey), midbody ring (dark pink or purple), or midbody arms (blue or green) or their function in abscission (orange or yellow). Figure created with BioRender.*

## 2. Changes in the midbody after its formation

The components of the midbody described previously are not all continuously present at the midbody and some change location within the midbody. Changes in components of the midbody often coincide with a change in its morphology (Halcrow et al., 2022). The most prominent morphological change in the midbody is due to the formation of the constriction sites. Since these are formed on either side of the midbody, it resembles a bow-tie at this stage (D'Avino & Capalbo, 2016). After further constriction and membrane scission, the midbody remnant is formed that can either remain connected to one of the daughter cells or is shed into the extracellular environment. Next, the mechanisms behind the changes in midbody components are described.

### 2.1 Recruitment of components to the midbody

Several of the RNA transcripts that localize to the midbody contain GA-rich regions (Farmer et al., 2023; Park et al., 2023). For some of the mRNA transcripts at the midbody, such as the one encoding CHMP4B, these regions are present in the 3'UTR (Farmer et al., 2023). Depletion of such regions disturbs localization of the mRNA to the midbody. Transport of mRNA with similar GA-rich regions to other cellular compartments depends on kinesin-1 proteins. Furthermore, there is overlap in the mRNA transcripts that are targeted to the midbody and that has previously been described to be transported with kinesin-1. Therefore, mRNA movement through kinesin-1 might be a mechanism of mRNA transport to midbodies (Farmer et al., 2023) (**Figure 3**). KIF23, a part of the centralspindlin complex, is not a member of the kinesin-1 family (Janisch et al., 2018). It is however needed for mRNA localization at the midbody (Park et al., 2023). KIF23 could function as a scaffold for the formation of an RNA granule. ARC is also needed for the localization of mRNA to the midbody but the mechanism behind its role is not fully known. Since ARC is a viral nucleocapsid protein, it might protect the mRNA from degradation (Park et al., 2023). Translation of the mRNA at the midbody can only start as soon as the daughter cells enter G1 (Park et al., 2023). Therefore, some proteins encoded by midbody targeted mRNA are only detectable after the start of G1.

Local translation of mRNA is not the only way to recruit a protein to a specific cellular location. Proteins and phospholipids can both be transported through the cell and to the midbody in vesicles (Kouranti et al., 2006; Pust et al., 2023) (**Figure 3**). The transport of some of these vesicles likely relies on kinesin-1 (Pust et al., 2023). Localization of proteins can also occur through specific protein-protein interactions (**Figure 3**). For example, ESCRT cofactors with a MIT domain can interact with ESCRT components with a MIM (Paine et al., 2023; Wenzel et al., 2022). Apart from the MIT-MIM interactions, additional protein domains might serve to retain other proteins at the midbody. For example, CIT-K and WDR5 both localize to the midbody and have a seven bladed β-propeller domain that might serve as a platform for interactions with other proteins (Bailey et al., 2015; McKenzie et al., 2016). Protein-protein interactions can change by post-translational modifications. In particular, changes in phosphorylation over time might change interaction partners and thereby the localization of proteins (Douglas et al., 2010; Neef et al., 2007). For example, CypA can only translocate from the centrosome to the midbody when it is phosphorylated (Gorry et al., 2023). Opposingly, CEP55 only gets to the central spindle once the Plk1 phosphorylation is removed (Bastos & Barr, 2010). CEP55 also relies on its ubiquitin binding domain and ubiquitin related proteins for its midbody localization (Boullé et al., 2022). This indicates that ubiquitin could play a role

in recruitment of components. Another possible role of ubiquitin is the removal of components that is described next.

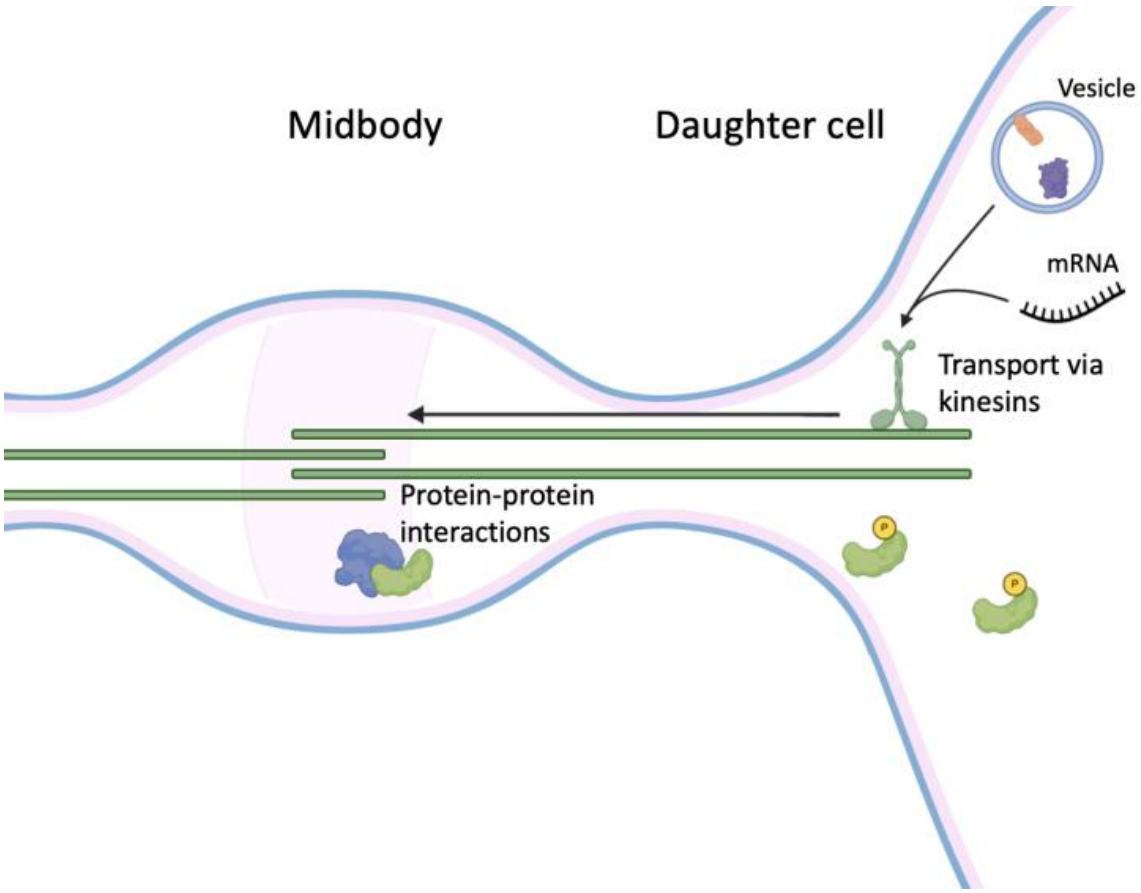


***Figure 3: Mechanisms of recruitment of components to the midbody***
*Shown are the midbody and a part of one of the daughter cells. Components are recruited to the midbody or retained there via two main mechanisms. The first are protein-protein interactions. Post-translational modifications can alter these interactions. Shown here is inhibition of the interaction by phosphorylation (Bastos & Barr, 2010). Furthermore, mRNA, proteins, and phospholipids can be recruited to the midbody via transport by kinesins (Farmer et al., 2023; Kouranti et al., 2006; Pust et al., 2023). For proteins and phospholipids, it is known that their transport can take place in vesicles (Kouranti et al., 2006; Pust et al., 2023). Horizontal green lines indicate microtubules. Actin is shown in pink, the vertical pink band in the middle shows the contractile ring. Figure created with BioRender.*

**2.2 Removal from the midbody**

After recruitment, proteins can be at the midbody stably or transiently (Halcrow et al., 2022). The difference between these two groups is likely mediated by a different regulation of ubiquitination, and possibly phosphorylation (**Figure 4**). There are differences in the interactions with ubiquitin related proteins between the stable and transient groups, which might provide an explanation for their different stability (Halcrow et al., 2022). Regulation of ubiquitination at the midbody could be through the action of E3 ubiquitin ligases and deubiquitinases (DUBs). AMSH and UBPY are two DUBs that are present in the midbody, both are recruited through MIT-MIM interactions with ESCRT components (Mukai et al., 2008; Wenzel et al., 2022). BRUCE is an E3 ubiquitin ligase found at the midbody. BRUCE is required for both targeting of vesicles to the midbody and the structure of the midbody (Pohl & Jentsch, 2008; Rai et al., 2021). Another E3 ubiquitin ligase that is active during cytokinesis is APC/C$^{Cdh1}$.

This ubiquitinates several midbody proteins such as Aurora B, Plk1, Anillin, and Gas2l3 (Pe'er et al., 2013). Ubiquitination by APC/$C^{Cdh1}$ directs the proteins for proteasomal degradation, the presence of proteasomal proteins at the midbody could indicate that the degradation happens locally (Addi et al., 2020; Rai et al., 2021). Some of the proteins ubiquitinated by APC/$C^{Cdh1}$ disappear after the 'bow-tie' stage of the midbody (D'Avino & Capalbo, 2016; Hu et al., 2012). KIF20A disappears from the midbody at a similar timepoint (D'Avino & Capalbo, 2016). KIF20A has not been described as substrate for APC/$C^{Cdh1}$, indicating that other E3 ubiquitin ligases or other mechanisms of removal also occur.

Possibly, midbody components can be removed from the midbody without degrading them. Dynein has been found at the midbody (Horgan et al., 2011). Since dynein goes to the minus-end of microtubules, a potential role for this protein in midbodies could be to move components away from the midbody.

Before abscission can take place, microtubules and actin have to be removed from the abscission site (Addi et al., 2018) (**Figure 4**). The microtubules can be severed by spastin or katanin (D'Avino & Capalbo, 2016; Guizetti et al., 2011). Both of these proteins have a MIT domain that can interact with a MIM of ESCRT components (Wenzel et al., 2022). This MIT domain is needed for the midbody localization of spastin and katanin. In stem cells, microtubules are stabilized by Aurora B earlier in cytokinesis (Kodba et al., 2024). The activity of Aurora B is reduced close to the abscission site, resulting in fewer stable microtubules. Another manner to destabilize microtubules is through SUMOylation of KIF4A which increases its affinity for the microtubule destabilizing protein stathmin 1 (Cuijpers et al., 2020). SUMOylation of proteins might play other roles in cytokinesis, since SUMO1 and SUMO3 have been detected in midbodies (Huang et al., 2015). Strikingly, the microtubules on both sides of the midbody are not severed simultaneously (Guizetti et al., 2011). How this difference in timing is regulated, is not yet known.

Removal of actin from the abscission site is mediated by the delivery of Rab35, or Rab8 and FIP3 positive vesicles. The Rab35 positive vesicles provides a phosphatidylinositol phosphatase, called OCRL (Dambournet et al., 2011) (**Figure 4**). The phospholipids that OCRL dephosphorylates, increase actin polymerization. Therefore, the action of OCRL inhibits actin polymerization. Rab35 also interacts with MICAL1 (Frémont et al., 2017) (**Figure 4**). This protein induces depolymerization of actin by oxidizing specific methionine residues. FIP3 positive endosomes transport many proteins, among which SCAMP2/3 and p50RhoGAP (Schiel et al., 2012). RNAi mediated depletion of these proteins increased F-actin levels, implying that they might have a role in the actin depolymerization too.

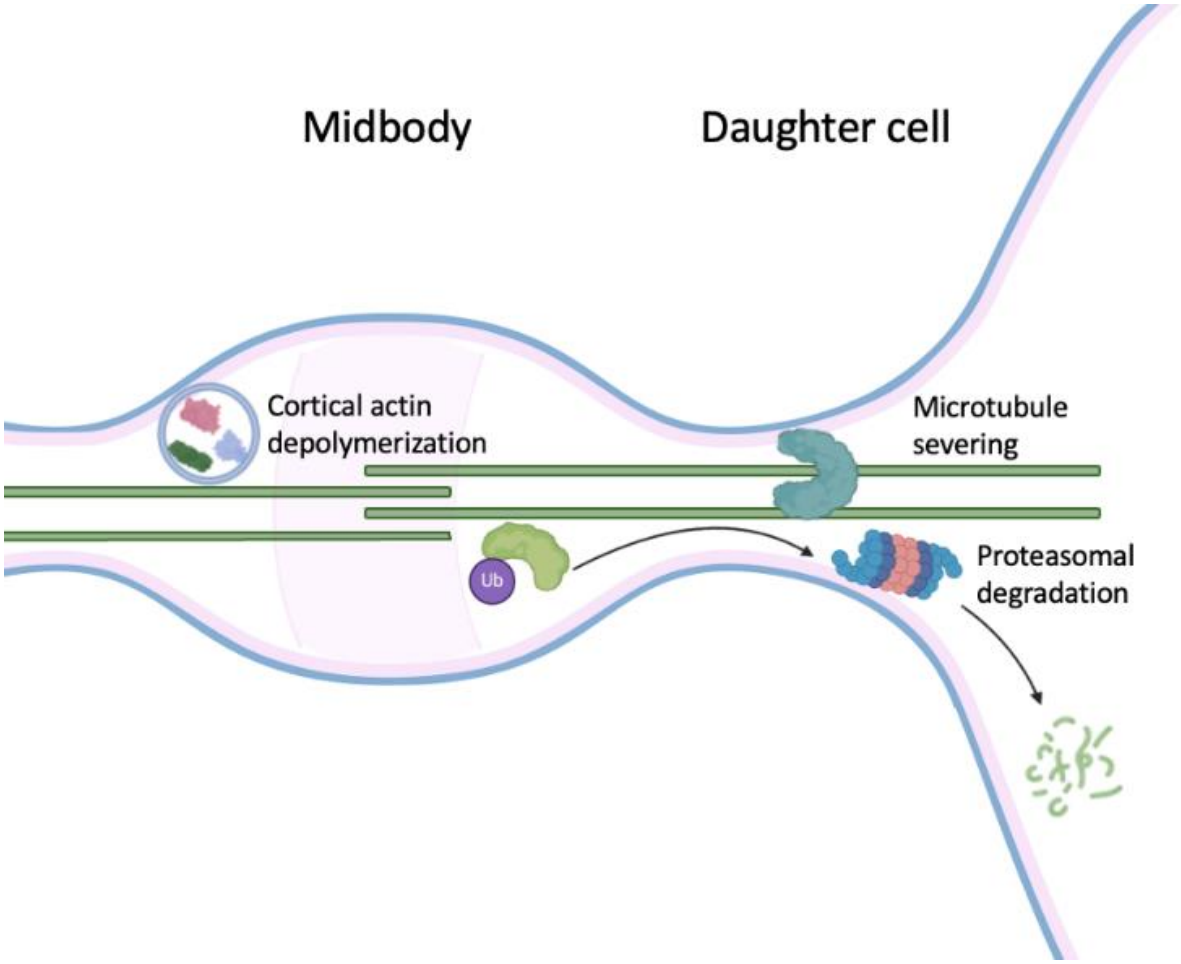


***Figure 4: Mechanisms of removal of components from the midbody***
*Shown are the midbody and a part of one of the daughter cells. Midbody components can be removed via multiple mechanisms. Actin and microtubules are removed by specific enzymes. For actin, the enzymes OCRL and MICAL1 can be delivered to the midbody in Rab35 positive vesicles* (Dambournet et al., 2011; Frémont et al., 2017)*. Microtubule removal is mediated by the microtubule severing enzymes spastin and katanin* (D'Avino & Capalbo, 2016; Guizetti et al., 2011). *A mechanism of removal that is used for more different proteins is ubiquitin mediated proteasomal degradation* (Halcrow et al., 2022; Pe'er et al., 2013)*. Horizontal green lines indicate microtubules. Actin is shown in pink, the vertical pink band in the middle shows the contractile ring. Figure created with BioRender.*

### 2.3 Change in localization at the midbody over time

Many components that are needed for abscission are first recruited to the midbody ring and later relocate to the abscission sites. Some of the most prominent examples of proteins that undergo such a change in localization are ESCRT components ALIX and TSG101 (Addi et al., 2020; Hu et al., 2012). ESCRT-III undergoes a similar change in localization, it relies on ALIX and TSG101 for this (Addi et al., 2020). ESCRT-III components IST1 and CHMP4B show an initial localization in two rings flanking the midbody (Adar-Levor et al., 2019; Goliand et al., 2018) (**Figure 5**). These can each form a spiral-like helical filament that elongates and constricts over time (Goliand et al., 2018) (**Figure 5**). Constriction of the ESCRT-spiral results in narrowing of the ICB since the spiral is anchored to the plasma membrane. Membrane anchorage can occur, for example, through interactions of ALIX with syntenin and syndecan-4 (Addi et al., 2020). The spiral is not only connected to the membrane, it also remains connected to the midbody (Goliand et al., 2018). Since the filaments keep constricting at the tip, the anchorage at the midbody results in mechanical stress in the spiral. The combination of this stress and VPS4

activity might cause the spiral to break (Goliand et al., 2018). After breaking of the spiral, there is a pool of ESCRT-III at the midbody and another pool at the abscission site (**Figure 5**). The part at the abscission site could form a dome shaped end-cap, possibly resulting in the scission of the plasma membrane (Goliand et al., 2018). The growth of the ESCRT-III spiral is halted at the future abscission site through local polymerization of F-actin (Advedissian et al., 2024). This regulation is also required for correct localization of the microtubule severing enzyme spastin. The local increase in actin is removed before abscission via the actin severing protein Cofillin-1 (Advedissian et al., 2024). This protein also moves from the midbody to the abscission site. Both RhoA and Anillin undergo the same change in localization (Hu et al., 2012; Renshaw et al., 2014). However, anillin is removed from the abscission site before ESCRT-III is recruited there (Renshaw et al., 2014).

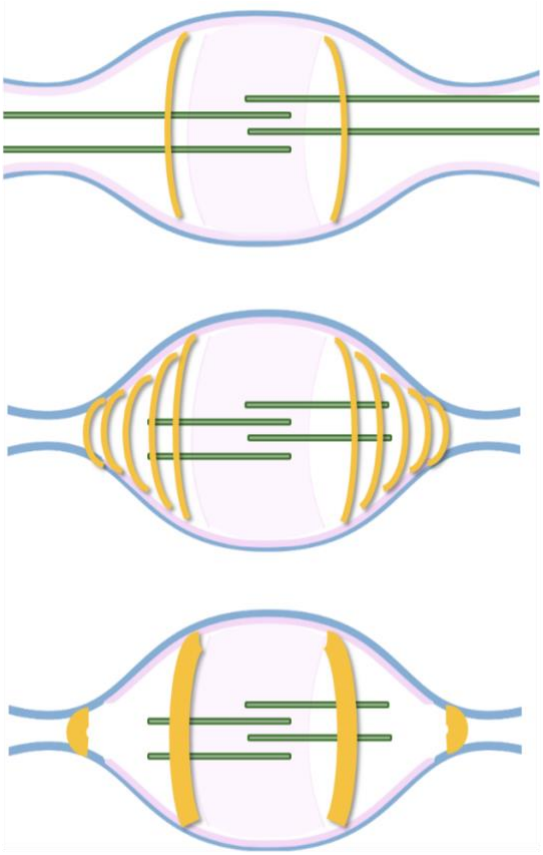

***Figure 5: Change of localization of ESCRT-III at the midbody over time***
*ESCRT-III (yellow) is recruited to the midbody ring (top) (Goliand et al., 2018). From there, it forms helical filaments that elongate into the abscission site (middle). Actin and microtubules are removed from the abscission site to facilitate abscission (Addi et al., 2018). Due to mechanical stress in the filaments and VPS4 activity, the helical ESCRT-III filaments can break (Goliand et al., 2018). This results in a pool of ESCRT-III at the midbody and a pool at the abscission site (bottom). Horizontal green lines indicate microtubules. Actin is shown in pink, the vertical pink band in the middle of each midbody shows the contractile ring.*

Overall, the components of the midbody change over time. They can be recruited, removed, or relocated as the midbody matures. The recruitment of all types of components might rely on kinesins. Proteins can specifically be recruited through local translation or protein-protein interactions. Removal of protein components could be mediated by ubiquitin-dependent degradation. In the case of microtubules and actin, the removal is performed by specific severing enzymes.

## 3. Functional aspects of midbody components

As described before, the midbody contains different types of components. These have distinct roles at the midbody. The next section aims to provide an overview of possible functional aspects of the components of the midbody.

### 3.1 Sequestering abscission components until G1

Both proteins and RNA have been found at the midbody. Recruiting a component as a protein or as mRNA transcript might have a consequence for the timing of the activity of the component. The mRNA cannot be translated until the daughter cells are in G1, resulting in the encoded protein only being at the midbody after that time (Park et al., 2023). Therefore, components that are recruited as protein might be needed for processes earlier in cytokinesis or the maturation of the midbody. On the contrary, components recruited as mRNA might function later, for example during abscission.

At the midbody, mRNA translation is probably not the only RNA-dependent process since different splicing factors have been found there (Suwakulsiri, Xu, Rai, Shafiq, et al., 2024). More research into the activity of splicing factors at the midbody can for example give information on whether certain splice variants might be favored. If this would be the case, it can be a way to specifically regulate protein isoforms at the midbody.

### 3.2 Role of trafficking at the midbody

Not all midbody proteins are translated in this organelle. Proteins that are associated with other organelles, such as the ER and Golgi, have been found in midbodies (Suwakulsiri, Xu, Rai, Shafiq, et al., 2024). These proteins might have ended up in the midbody by the transport of vesicles that originated from these organelles. Vesicles could also be a way in which the specific phospholipids are transported to the midbody (Vance, 2015). In addition, proteins and RNA from mitochondria were found at the midbody, their function at the midbody is not yet known (Skop et al., 2004; Suwakulsiri, Xu, Rai, Chen, et al., 2024; Suwakulsiri, Xu, Rai, Shafiq, et al., 2024). There is a possibility that the mitochondrial components are a contamination (Suwakulsiri, Xu, Rai, Shafiq, et al., 2024). Therefore, further research into the presence and possible function of mitochondrial components at the midbody is needed. ER, Golgi, and mitochondria are not the only organelles of which components end up in midbodies. Several proteins go from the centrosome to the midbody ring during mitosis (Barbiero et al., 2017; Pohl & Jentsch, 2008). Their recruitment to the midbody might be through vesicles since two of those proteins, BRUCE and centriolin, interact with exocyst (Gromley et al., 2005; Pohl & Jentsch, 2008). Furthermore, kinesins might be involved in the transport of centrosomal proteins to the midbody (Sagona et al., 2010). Since the movement of the mother centriole to the midbody relies on microtubules, kinesins might play a role in the transport of centrosomal proteins through moving the mother centriole (Piel et al., 2001).

### 3.3 Cell-cell junctions and polarity specification after cell division

A set of proteins found at the centrosome and midbody of epithelial cells are best-known for their presence at tight junctions (Konno et al., 2020). Their role at the midbody could be to mediate the formation of new tight junctions after cytokinesis. Furthermore, tight junction proteins can interact with proteins regulating polarity (Konno et al., 2020). Tight junction proteins are probably not the only midbody components that can influence polarity. In polarized epithelia, the midbody is located at the apical membrane after abscission (Bai et al.,

2020; Lujan et al., 2017). This can be mediated by asymmetric furrow ingression as observed during the intestinal morphogenesis of *C. elegans* embryos (Bai et al., 2020). Delivery of the midbody to the basal instead of the apical side can partially reverse polarity of MDCK cells (Lujan et al., 2017). This could be due to the presence of midbody components that induce apical specification. Furthermore, the midbody remnant is needed for the formation of a primary cilium on the apical side of these MDCK cells (Bernabé-Rubio et al., 2016). The midbody remnant mediates this process by delivering components of the machinery that is needed to form the primary cilium. Early in the development of the *C. elegans* embryo, the inheritance of the midbody relies on the polarity of the daughter cells (Bai et al., 2020). So, the midbody does not only influence polarity, but polarity can also determine the fate of the midbody.

**3.4 Role of signaling at the midbody**

Polarity specification might not be the only signaling that the midbody is involved in, as other signaling proteins are present at the midbody (Lujan et al., 2017; Peterman et al., 2019; Skop et al., 2004). CIT-K and WDR5 are both found at the midbody, and possess a seven bladed β-propeller (Bailey et al., 2015; McKenzie et al., 2016). Other proteins with β-propellers have been associated with signaling since they can interact with signaling proteins (Bailey et al., 2015). Therefore, CIT-K and WDR5 could be interacting with the signaling proteins from the midbody. These signaling proteins can alter the behavior of cells that engulf or inherit the midbody remnant after cytokinesis (Peterman et al., 2019; Peterman & Prekeris, 2019; Skop et al., 2004). After asymmetric division of stem cells, the midbody is predominantly taken up by the daughter cell with the oldest centrosome (Kuo et al., 2011). This is usually the stem cell. In mouse embryonic stem cells, inducing premature midbody remnant release results in faster exit of naïve pluripotency (Chaigne et al., 2020). This indicates that the midbody could be involved in retaining an undifferentiated state. The role of the midbody remnant in differentiation is further exemplified by the finding that differentiating stem cells or differentiated cells shed more midbody remnants than undifferentiated cancer cells (Ettinger et al., 2011). In cell types that have fewer midbody remnants, the remnants are in membrane-enclosed compartments that are likely lysosomes (Kuo et al., 2011). The remnants probably localize there after autophagy. On the other hand, cells that have more midbody remnants usually store them in the cytosol. The midbody remnant does not only have a role in differentiation of stem cells as midbody remnants taken up by HeLa cells increased their colony forming potential (Kuo et al., 2011). Furthermore, midbody remnants from a colorectal cancer cell line could increase the colony forming potential and invasiveness of a fibroblast line (Rai et al., 2021). The components of the midbody that are responsible for the effects on differentiation and proliferation are unknown.

In short, the different components of the midbody can all have different functions. For some components, the consequence of the form in which they are recruited or the role they have in abscission is not fully known. However, their function during other stages of the cell cycle could provide clues on their purpose at the midbody. This role at the midbody is not always associated with abscission as the midbody is also involved in polarity, differentiation, and proliferation.

## Conclusion and Discussion

In conclusion, the midbody consists of many different proteins, RNA transcripts and phospholipids with different functions. These components are inherited from the central spindle or contractile ring, or they are recruited to the midbody after it is formed (D'Avino & Capalbo, 2016; Mierzwa & Gerlich, 2014). The recruited components often have a role in processes such as abscission, translation, or signaling (Addi et al., 2020; Farmer et al., 2023; Park et al., 2023; Peterman et al., 2019). The composition of the midbody changes over time through the recruitment, removal, and change in localization of components. These changes are, among other mechanisms, mediated by translation of mRNA and post-translational modifications such as phosphorylation and ubiquitination (Bastos & Barr, 2010; Capalbo et al., 2019; Douglas et al., 2010; Gershony et al., 2014; Gorry et al., 2023; Halcrow et al., 2022; McKenzie et al., 2016; Park et al., 2023; Rinaldo et al., 2012).

Unknown midbody components can be discovered through mass spectrometry or RNA sequencing of midbodies or midbody remnants (Addi et al., 2020; Farmer et al., 2023; Park et al., 2023; Peterman et al., 2019; Skop et al., 2004). The methods used to acquire midbodies for these analyses differ between studies and timepoints in which the midbody is harvested. This greatly limits the possibility to compare the identified components from one timepoint to another. Therefore, it is difficult to understand how the midbody changes over time. Determining the localization of midbody components is another challenge since the midbody core is so dense that it does not allow for antibody staining (Hu et al., 2012; Mullins & McIntosh, 1982). Consequently, studying this requires fluorescently tagged proteins. For many components, it is difficult to determine whether these tags might influence protein activity or localization.

Despite these technical limitations, the localization, function and activity of many midbody components have been identified. Nevertheless, there are still outstanding questions on aspects such as the regulation of their localization. For example, the relocation of ESCRT-III from the midbody to the abscission site happens through the formation of helical filaments (Goliand et al., 2018). However, the trigger of the extension of these filaments is not known. It has been proposed to be linked to membrane tension, possibly through actin (Frémont et al., 2017). Furthermore, other midbody components such as RhoA and Anillin also change localization, the regulation of this is yet unknown (Hu et al., 2012).

Knowledge on the components of the midbody and how they change over time can improve our understanding in the regulation of cell division, specifically abscission. Regulation of abscission, for example by the NoCut pathway, is needed to prevent premature abscission and damage to potential chromosome bridges (Carlton et al., 2012; Steigemann et al., 2009). The midbody remnant can provide insights on processes beyond cell division. An engulfed midbody remnant can change the behavior of the recipient cell (Kuo et al., 2011; Peterman & Prekeris, 2019; Rai et al., 2021). It can promote oncogenesis or influence the differentiation of stem cells. The mechanisms and midbody components that are responsible for this are unknown. This knowledge could be valuable in our understanding of both tumorigenesis and regulation of stem cell differentiation.

Overall, this review provides an overview of the components of the midbody and their regulation. The research on the midbody and its components has contributed to the illumination of the composition and function of this structure and its dark zone. However, there is still a lot to discover about the midbody components and their function. One interesting avenue of research is the influence of the midbody on differentiation, proliferation, and invasiveness of cells. Knowing which midbody components regulate these functions and whether these might differ between cell types and differentiation states would give further insights into the regulation of these mechanisms. The use of organoids that contain stem- and differentiated cells in a 3D context could be valuable for this. Another way to further understand the midbody, requires the development of techniques to harvest midbodies at different stages while using similar protocols. This would give a better overview of how the midbody evolves over time.

## Generative AI statement

No generative AI has been used in the writing of this writing assignment.


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