

REVIEW

SUBJECT COLLECTION: CELL CYCLE

Delayed abscission in animal cells – from development to defects

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ABSTRACT

Cell division involves separating the genetic material and cytoplasm of a mother cell into two daughter cells. The last step of cell division, abscission, consists of cutting the cytoplasmic bridge, a microtubule-rich membranous tube connecting the two cells, which contains the midbody, a dense proteinaceous structure. Canonically, abscission occurs 1–3 h after anaphase. However, in certain cases, abscission can be severely delayed or incomplete. Abscission delays can be caused by mitotic defects that activate the abscission ‘NoCut’ checkpoint in tumor cells, as well as when cells exert abnormally strong pulling forces on the bridge. Delayed abscission can also occur during normal organism development. Here, we compare the mechanisms triggering delayed and incomplete abscission in healthy and disease scenarios. We propose that NoCut is not a bona fide cell cycle checkpoint, but a general mechanism that can control the dynamics of abscission in multiple contexts.

KEY WORDS: Aurora B, NoCut checkpoint, Abscission, Cytoplasmic bridges

Introduction

Cell division is a stepwise process during which the genomic and cytoplasmic contents of a cell are separated into two daughter cells. This requires the chromosomes first being segregated by microtubules emanating from the mitotic spindle (Fig. 1A). After chromosome segregation, the cytoplasm and membrane of the two cells also need to separate. This happens during the last stage of cell division, cytokinesis, which ends in abscission, a membrane scission event. Cytokinesis starts with the formation of a signalling hub at the centre of the spindle. This hub is called the central spindle and is formed during anaphase, just after the chromosomes start moving towards opposite poles of the cell. The central spindle triggers the formation of the cleavage furrow (Rappaport, 1985), culminating in the constriction of an acto-myosin ring that separates the cytoplasm of the two cells (Green et al., 2012; Srivastava et al., 2016). Signals from astral microtubules, as well as from the central spindle, are also important for the positioning of the furrow (Bringmann and Hyman, 2005; Canman et al., 2003; Glotzer, 2004; Inoue et al., 2004). Concomitantly, the central spindle is narrowed during furrow ingression and a thin intercellular bridge is formed. In the middle of the cytoplasmic (or intercellular) bridge is an electron-dense structure called the midbody, a protein-rich structure. The midbody then recruits the machinery that severs the microtubules and cuts and reseals the membranes of the bridges (Fig. 1) (Christ et al., 2016; Yang et al., 2008). Although cytokinesis is often viewed as a continuous process, there is a delay between the

apparent end of the constriction of the acto-myosin ring and abscission itself. Some of this delay is due to the time it takes for the cell to recruit the abscission machinery; however, there are several cases where abscission is further delayed owing to mitotic defects, such as strands of DNA trapped in the bridge (Norden et al., 2006; Steigemann et al., 2009), nuclear pore complex defects (Mackay et al., 2010) or strong forces on cytoplasmic bridges (Andrade et al., 2022; Lafaurie-Janvore et al., 2013). However, long-lived bridges can also be found during normal organism development at various developmental stages. For example, in germline cells in many animals, abscission sometimes never happens (de Cuevas et al., 1997; Gondos, 1973). In mouse embryos, abscission happens just before the subsequent cell division (Zenker et al., 2017), whereas in mouse embryonic stem cells, abscission takes upwards of 6 h (Chaigne et al., 2020). In this Review, after briefly describing the main players responsible for abscission, we summarize known molecular mechanisms underlying the regulation of the timing of abscission in physiological and pathological contexts of animal cells. In particular, we review recent advances in our understanding of the role of stable cytoplasmic bridges during development. We challenge the notion that the pathway controlling the dynamics of abscission in the case of mitotic defects, called ‘NoCut’, is a bona fide cell cycle checkpoint, and we hypothesize that it could control the dynamics of abscission in normal development (see Box 1).

Abscission – the last step of cell division

Formation of the central spindle

The partitioning of DNA in the two daughter cells during cell division involves aligning the chromosomes on the metaphase plate in the middle of the mitotic spindle and separating sister chromatids during anaphase. As chromosomes are pulled towards the poles, the mitotic spindle rearranges to form the central spindle (Douglas and Mishima, 2010). The central spindle is a complex multiprotein machinery that serves as an organizing centre for subsequent events. We will focus on three of the main cytokinesis signalling elements – the overlapping plus ends of the microtubules, the centralspindlin complex and the chromosomal passenger complex (CPC) (Fig. 1). Centralspindlin is a heterotetrameric complex consisting of KIF23 (also known as MKLP1) and the Rho-family GTPase-activating protein RacGAP1 (Mishima et al., 2002; Pavicic-Kaltenbrunner et al., 2007). The centralspindlin complex is activated at anaphase onset through removal of an inhibitory phosphorylation on KIF23 and subsequent phosphorylation of KIF23 by Aurora B kinase (Mishima et al., 2004), which is part of the CPC (Fig. 1). The CPC also contains INCENP, borealin and survivin (Carmena et al., 2012).

During prometaphase and metaphase, the CPC is located at the inner centromeres of chromosomes. The CPC, including Aurora B translocates from the centromeres to the overlapping microtubules of the spindle midzone and cleavage furrow during early anaphase. The translocation is mediated by the kinesin protein KIF20A (also known as MKLP2) (Adriaans et al., 2020; Kitagawa et al., 2013; Serena et al., 2020). The plus ends of the microtubules meet midway

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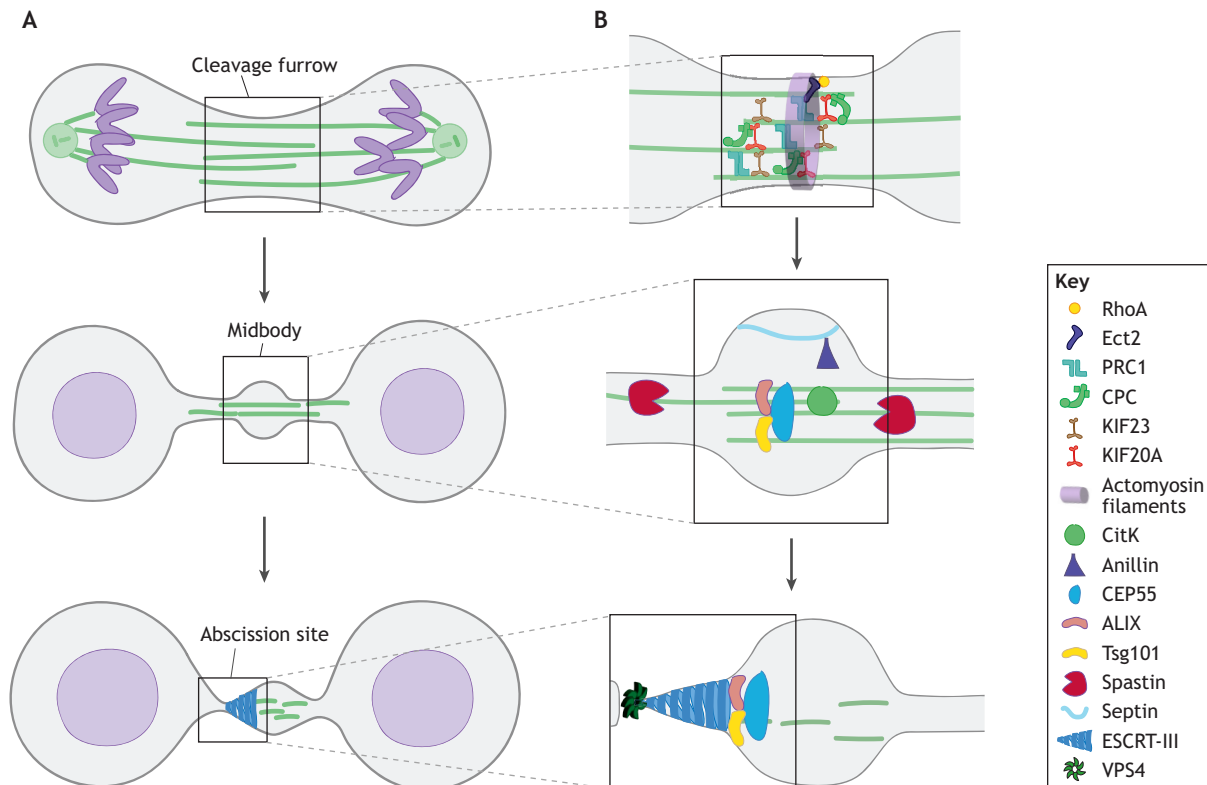


Fig. 1. Molecular mechanisms of membrane scission and cell separation during cytokinesis in animal cells. (A) The steps of cytokinesis. During early anaphase the chromosomes (purple) segregate and antiparallel microtubule (green) bundles form the spindle midzone. The cleavage furrow starts to narrow as cytokinesis progresses and an intercellular bridge forms with a dense protein structure, the midbody, in its centre. The midbody serves as a recruiter of protein complexes responsible for membrane scission, which is the final step of cytokinesis. (B) Magnification of A showing the molecular factors involved in cytokinesis. The central spindle forms through the bundling of antiparallel microtubules by PRC1 and the recruitment of the centralspindlin complex, including KIF23. KIF20A is recruited to the midbody and transfers the chromosomal passenger complex (CPC), which contains Aurora B, from the kinetochores to the microtubules and cleavage furrow. Signals from the central spindle microtubules activate RhoA at the cortex above it, while astral microtubules inhibit the localization of RhoA at the cell periphery. RhoA and its activator Ect2 are master regulators of the site of cleavage furrow formation. The cleavage furrow ingresses with the sliding of actin and myosin. With furrow ingression, an intercellular bridge is formed between the two daughter cells. CitK localizes to the midbody by binding to KIF23. CitK also physically and functionally interacts with anillin, which binds to septins. CEP55 is recruited to the midbody by binding to KIF23 and serves as a scaffold for ALIX and Tsg101. In turn, ALIX and Tsg101 recruit the ESCRT-III complex. ESCRT-III proteins form filaments around the midbody arms. Finally, VPS4 AAA-ATPases allow the bending of ESCRT-III filaments, which leads to the severing of the membrane between two daughter cells.

between the two spindle poles. Here, they are crosslinked and bundled by protein regulator of cytokinesis 1 (PRC1) (Bieling et al., 2010; Glotzer, 2009; Subramanian et al., 2010), which is recruited to the microtubules by the KIF4A motor (Bieling et al., 2010; Zhu and Jiang, 2005). In parallel, KIF23 is recruited at the plus ends of microtubules, which, together with the rest of the centralspindlin complex, promotes microtubule bundling and recruits other players of abscission (Mishima, 2016). Polo-like kinase 1 (PLK1) phosphorylates KIF4A and PRC1, which restricts the length of the microtubule overlap (Hu et al., 2011, 2012). Aurora B kinase, as well as Aurora A kinase, also participates in relaxing the polar cortical actomyosin network to facilitate cytokinesis (Longhini and Glotzer, 2022; Mangal et al., 2018; Ramkumar et al., 2021).

Furrow ingression and midbody formation

The position of the cleavage furrow is determined by two different pathways, one emanating from the central spindle and the other from the spindle asters (Bringmann and Hyman, 2005). The central spindle activates RhoA at the equatorial cortex (Bement et al., 2005), while astral microtubules inhibit its activation at the peripheral cortex (Dechant and Glotzer, 2003; Zanin et al., 2013).

Activated RhoA promotes actomyosin assembly at the equatorial cortex and subsequent ring contraction (Castrillon and Wasserman, 1994; Matsumura, 2005; Watanabe et al., 2008).

The sliding of actin and myosin drives constriction of the actomyosin ring at the spindle midzone (Maupin and Pollard, 1986). A positive feedback control between the ring myosin and the compression-driven flow of the cortex has also been proposed to drive actomyosin constriction (Khaliullin et al., 2018); the cortex flow increases the amount of myosin and therefore causes the ring to have a high constriction rate (Khaliullin et al., 2018). The contractile ring further contains actin crosslinking proteins (Ren et al., 2009), septin filaments (Joo et al., 2007), citron kinase (CitK) (Gai et al., 2011) and anillin, which links actin and myosin to the plasma membrane and the central spindle (Piekny and Maddox, 2010). CitK can interact with KIF14 and KIF23 to regulate PRC1 (Bassi et al., 2013; Gruneberg et al., 2006) and midbody formation, and is required for the recruitment of the CPC (McKenzie et al., 2016) and abscission completion (Giansanti et al., 2012; Watanabe et al., 2013).

The cleavage furrow ingression leads to the formation of an intercellular bridge connecting the two daughter cells (Fig. 1). The

Box 1. Is the NoCut abscission checkpoint a true cell cycle checkpoint?

The Aurora B-dependent control of abscission dynamics has been termed the NoCut abscission checkpoint. Checkpoints serve as surveillance mechanisms during the cell cycle; they control cell cycle events which include cell growth, DNA replication and chromosome segregation (Barnum and O'Connell, 2014). Failure of cells to properly satisfy a checkpoint leads to cell cycle arrest. However, the abscission checkpoint has not been shown to halt the cell cycle progression in any phase of the cell cycle, only to postpone abscission. Moreover, checkpoint satisfaction usually implies a surveillance and correction mechanism. To our knowledge, there is no direct evidence that, while abscission is stalled, mechanisms are at play to correct the errors leading to checkpoint abscission. It is only suggested that Aurora B kinase might resolve lagging chromosomes that have improper microtubule attachments through anaphase surveillance mechanisms (Orr et al., 2021). Furthermore, the known checkpoints act in all live cells and tissues, regardless of the cellular context. So far, the abscission checkpoint appears to be activated only if a cellular defect compromises proper cytokinesis. It is not known whether the abscission checkpoint is always active when cells progress from anaphase to the G1 phase of the new cell cycle. Therefore, it does not formally meet all criteria to be considered a true cell cycle checkpoint. Further research will demonstrate whether NoCut does stall the cell cycle to allow the cell time to correct the errors. Crucially, in the few cases where there is data on the mechanisms delaying abscission during normal development, features of NoCut, in particular the activity of Aurora B and the lack of recruitment of ESCRT-III, have been shown to play a role (Adar-Levor et al., 2021; Bai et al., 2019; Chaigne et al., 2020; Iwamori et al., 2010; Mathieu et al., 2013, 2022; Matias et al., 2015). Furthermore, inhibition of Aurora B triggers abscission even in the absence of chromatin bridges (Steigemann et al., 2009). Thus, NoCut is not a true checkpoint, but a universal mechanism controlling abscission duration, which allows the modulation of the dynamics of cytokinesis underlying cellular function.

intercellular bridge is a thin structure with an initial diameter of 1–2 μm that can persist for hours after the end of the mitotic phase of the cell cycle (Chaigne and Brunet, 2022; Gershony et al., 2014). In cultured cells, the intercellular bridge narrows before abscission (Chaigne et al., 2020; Guizetti et al., 2011), which requires membrane remodelling and removal of F-actin (Dambournet et al., 2011; Frémont et al., 2017; Schiel et al., 2012). In particular, changes in lipid composition mediated by the GTPase Rab35 (Kouranti et al., 2006) and its effector Rab11FIP1 (Iannantuono and Emery, 2021) are crucial for a successful abscission. Subsequent recruitment of abscission machinery to the intercellular bridge is then necessary to cleave the bridge and complete cytokinesis.

Recruitment of the ESCRT complex and abscission

During late cytokinesis, CEP55 binds to the centralspindlin complex by interacting with KIF23. This interaction is possible when the activity of PLK1 decreases and KIF23 is dephosphorylated (Bastos and Barr, 2010). When CEP55 is localized at the midbody, it recruits ALIX (also known as PDCD6IP) and TSG101, which belongs to the ESCRT-I family, to the midbody (Fig. 1B) (Carlton et al., 2008). Components of the ESCRT family were first identified as vacuolar protein sorting (VPS) genes in yeast (Bankaitis et al., 1986; Vida et al., 1990). ESCRT proteins participate in membrane remodelling during many cellular processes, including endosome maturation, virus budding and cell division (for a review on ESCRT proteins, see Vietri et al., 2020). The ESCRT family of proteins consists of three main protein complexes: ESCRT-I, ESCRT-II

and ESCRT-III. ESCRT-III proteins form filaments and can be recruited to the midbody and activated through interactions with ESCRT-I and -II, as well as other proteins such as CEP55. Mammalian ESCRT-III proteins include: CHMP1A, CHMP1B, CHMP2A, CHMP2B, CHMP3, CHMP4A, CHMP4B, CHMP4C, CHMP5, CHMP6, CHMP7 and CHMP8 (also known as IST1). ALIX can recruit CHMP4B by binding directly to its C-terminus, and can also recruit CHMP4C. CHMP4B can also be recruited via ESCRT-I cooperating with ESCRT-II and CHMP6 (Christ et al., 2016; Goliant et al., 2014). In *Drosophila*, there is no homologue of CEP55, and ALIX is directly recruited to the midbody by the homolog of KIF23, Pavarotti (Lie-Jensen et al., 2019).

Just before abscission, ESCRT-III proteins move to one side of the midbody where they form rings, and then spirals (Fig. 1) (Goliant et al., 2018). *In vitro*, disassembly of ESCRT-III helices is then catalysed by the VPS4 AAA-ATPase (Pfitzner et al., 2020). In parallel, CHMP1B also recruits the AAA-ATPase spastin (Connell et al., 2009; Reid et al., 2005), which severs microtubules (Mierzwa et al., 2017). The microtubule-severing protein complex katanin also plays a role in abscission (Wenzel et al., 2022). In addition to filament disassembly *in vitro*, VPS4 was also shown to be involved in the exchange of ESCRT-III monomers in HeLa cells, possibly allowing the remodelling of the ESCRT-III rings into helices and allowing constriction (Mierzwa et al., 2017).

What constitutes the end point of abscission is not always straightforward. Indeed, abscission is usually considered finished when the membrane is cut on one side, although other definitions exist (for example, when the microtubules are cut, when the cytoplasm of the two cells are isolated, or when the midbody is released). Canonically, within these definitions, abscission lasts 1–3 h (Carlton et al., 2012; Chaigne et al., 2020; Gershony et al., 2017; Guizetti et al., 2011). However, abscission can be further delayed, leading to the maintenance of the connection between the two sister cells. As membrane scission closely follows microtubule severing (Guizetti et al., 2011), we consider abscission to be 'delayed' when it lasts more than 3 h from the end of cytokinesis and bridge formation to microtubule and/or membrane scission, as often observed in cells in culture.

Physiological functions of delayed abscission

Incomplete or delayed abscission can occur during the normal development of many organisms, leading to the maintenance of long-lived bridges. Here, we discuss what is known about the mechanisms of delayed abscission during different developmental stages of various multicellular animals.

In the germline

Gametogenesis is the process of gamete production from diploid precursor cells, a characteristic of which is the presence of delayed (in germ stem cells) (Matias et al., 2015; Morris and Spradling, 2011; Villa-Fombuena et al., 2021) or incomplete (in germ cells) (Pepling et al., 1999) abscission in many organisms, leading to the formation of stable cytoplasmic bridges. There are several hypotheses as to why incomplete abscission might be important for germline cells, including sharing of nutrients from the nurse cells to the oocyte (Pepling et al., 1999), transport of mRNA, proteins and organelles between cells (Ventelä et al., 2003), synchronization of the cell cycle (Pepling et al., 1999) and cell growth (Doherty et al., 2021). Mechanisms by which different species accomplish the delay in abscission are diverse, and can also vary between the male and female organisms within the same species (Haglund et al., 2011).

Female germline

Incomplete cytokinesis has best been described during the female gametogenesis in *Drosophila melanogaster*, where stable intercellular bridges connect cells in the female germline cysts (Robinson and Cooley, 1996). In *Drosophila*, these stable intercellular bridges are called ring canals and connect 16 cells in the egg chamber, the structural unit of the *Drosophila* ovary. During gametogenesis, 15 cells become nurse cells, with only one becoming an oocyte (Robinson and Cooley, 1997). The bridges allow the exchange of different molecules, including organelles, mRNA and proteins between nursing cells and the oocyte (Bohrmann and Biber, 1994; Haglund et al., 2011; Robinson and Cooley, 1997). The orientation of transport, from the nurse cells to the oocyte, is controlled by the polarity of F-actin and microtubules (Lu et al., 2021). Furthermore, syncytial organization, by allowing sharing of cellular content, could potentially be beneficial for the synchronization of mitotic division and entry into meiosis, as well as the coordination of programmed cell death (Gondos, 1973; Haglund et al., 2011; Zamboni and Gondos, 1968). Recently, it has also been shown that intercellular bridges regulate transition from pluripotency to meiotic divisions in mouse fetal oocytes, probably via dilution of the regulatory factors that can be found in the cytoplasm (Soygur et al., 2021).

In the *Drosophila* female germline, ring canals form because the constriction of the contractile ring is arrested (Greenbaum et al., 2011; Haglund et al., 2011; Robinson and Cooley, 1996). The ring canals contain two rims – an outer electron dense rim and a thicker (but less electron dense) inner rim, which participates in bridge stabilization (Tilney et al., 1996). The inner rim contains actin filaments, whereas the outer rim forms as a thickening of the plasma membrane (Robinson et al., 1997; Warn et al., 1985). Recent studies in *Drosophila* germlines and on mouse and *Hydra* spermatogenesis have shown that ring canals form from the midbody, which does not get discarded, but reorganizes to form an open-lumen ring. This depends on CitK (Sticky, FBgn0002466, in *Drosophila*), and for the first time shows that there might be a conserved mechanism to form ring canals (Price et al., 2023). Importantly, bridge stabilization requires a tight mechanical regulation between stiffening and contractility (Singh et al., 2022). Absence of ESCRT proteins is crucial for bridge stabilization; the maintenance of the ring canal in the cyst is controlled by the deubiquitylase USP8 through modification of CHMP4 (Shrub in *Drosophila*) and CHMP2B (Mathieu et al., 2022). Furthermore, Aurora B kinase inhibits abscission by phosphorylating cyclin B (Mathieu et al., 2013).

Ring canals in *Drosophila* ovaries contain a specific organelle, the fusome. The fusome is an endoplasmic reticulum (ER)-derived vesiculated organelle important for oocyte specification (de Cuevas and Spradling, 1998; Ong and Tan, 2010). The fusome has been shown to play different roles, including participating in the regulation and synchronization of the cell cycle (de Cuevas et al., 1996; Yue and Spradling, 1992). Furthermore, the fusome is involved in the establishment of the polarity of the oocyte through organizing the microtubule skeleton (Huynh, 2013).

Female primordial germ cells in mice also form cysts of cells connected by stable intercellular bridges, also called ring canals, in a process very similar to *Drosophila* (Spradling et al., 2022). The initial cyst is formed from 30 cells but then breaks to form six smaller cysts containing five cells (four nurse cells and one oocyte). Nurse cells then transfer their organelles and cytoplasm to the cell that will become the oocyte (Lei and Spradling, 2016). Nurse cells shrink during cytoplasmic transfer and then die by a programmed cell death (Niu and Spradling, 2022).

Male germline

Cytokinesis is incomplete during development of sperm cells in a variety of animals. Sperm cells arise from male germline stem cells that divide asymmetrically and produce one germline stem cell and one spermatogonial cell. Spermatogonial cells usually undergo four rounds of mitosis and two rounds of meiosis before they produce the mature sperm (Brinster, 2007; Dym and Fawcett, 1971). During these cell divisions, in some species, such as *Drosophila*, abscission does not occur, and cells are interconnected via their intercellular bridges (Huckins, 1971). At the end of the second meiotic division in *Drosophila*, cells form a 64-cell haploid spermatid syncytium (Dym and Fawcett, 1971).

The stable intercellular bridges in *Drosophila* sperm contain various proteins, including anillin, Pav (the homolog of mammalian KIF23) (Adams et al., 1998) and various septins (Hime et al., 1996). Male intercellular bridges are also called ring canals; in contrast to what is seen with the female germline, they only contain an outer rim, but the significance of this discrepancy is unknown (Hime et al., 1996). The function of ring canals in male germline cells has been proposed to be similar to in the female germline, including sharing of the cellular content and synchronization of division (Pepling et al., 1999). Organelles, proteins and mRNA can travel between spermatids in the syncytia, rendering haploid cells phenotypically diploid (Morales et al., 2002; Ventelä et al., 2003; Braun et al., 1989).

Intercellular bridges in the male germline also participate in the fertility of mice. Studies performed in the adult mouse testis show that the TEX14 protein contributes to intercellular bridge stabilization and prevents cytokinesis (Greenbaum et al., 2006; Kim et al., 2015). TEX14 is also crucial for sperm cell viability, but it is unclear whether this role is connected to bridge stabilization (Greenbaum et al., 2006). At the intercellular bridges, TEX14 prevents abscission by binding CEP55, which prevents ALIX and TSG101 recruitment at the midbody (Iwamori et al., 2010). The exact mechanism by which TEX14 prevents abscission is still not known, given that CEP55 has been shown not to be crucial for the onset of abscission in many species (Little et al., 2021; Tedeschi et al., 2020). TEX14 also has roles in regulating the timing of abscission in ovaries of mice (Ikami et al., 2021; Niu and Spradling, 2022), pigs and humans (Fakhro et al., 2018; Gershoni et al., 2017; Sironen et al., 2011).

In embryos

Long-lived intercellular bridges have been observed in many different developing embryos, including human (Chatzimeletiou et al., 2005), mouse (Johnson and Maro, 1985; Zenker et al., 2017), zebrafish (Adar-Levor et al., 2021; Caneparo et al., 2011), *C. elegans* (Waddle et al., 1994), sea urchin (Sanger et al., 1985), sea anemone (Fritzenwanker et al., 2007) and squid embryos (Arnold, 1974) (for a review, see Chaigne and Brunet, 2022). Because most of these studies rely on fixed samples, it is not clear how long abscission is delayed. Live imaging of the mouse embryo has shown that abscission is delayed until shortly before the subsequent mitosis (Zenker et al., 2017). Although we do not know much about the mechanism of delayed abscission in embryos, a recent study showed that in the zebrafish embryo expression levels of the ESCRT gene *Chmp4bb* increase after the start of zygotic transcription, which correlates with complete abscission, suggesting that the expression of *Chmp4bb* gene could control abscission timing; consistent with this, *Chmp4bb* knockdowns showed delayed abscission (Adar-Levor et al., 2021).

In mouse embryos, intercellular bridges play an original role as a microtubule-organizing centre (MTOC). As early mouse embryos

do not have centrosomes to organize microtubules, interphase bridges serve as acentrosomal MTOCs and organize the trafficking of vesicles containing E-cadherin in the cell (Zenker et al., 2017).

In stem cells

Stable intercellular bridges have been proposed to play a crucial role in regulating cell fate transitions in mouse embryonic stem cells (ESCs) (Chaigne et al., 2020). Mouse ESCs are derived from peri-implantation embryos and can remain pluripotent or be triggered to exit naïve pluripotency and give rise to the three different germ layers – ectoderm, mesoderm and endoderm (Kalkan et al., 2017). Naïve mouse ESCs undergo a slower abscission (~6 h), than their differentiating counterparts (~2 h). Naïve cells do not recruit CHMP4B to the midbody, which might explain the delay in abscission and suggests a tight regulation between pluripotency and stable intercellular bridges (Chaigne et al., 2020). Accelerating abscission facilitates exit from naïve pluripotency, whereas artificial maintenance of the bridges prevents exit (Chaigne et al., 2020). Although it is still unclear how stable bridges sustain pluripotency, several lines of evidence suggest that the stem cell midbody can act as a pluripotency stabilizing organelle (Chaigne et al., 2020; Ettinger et al., 2011; Kuo et al., 2011). Interestingly, in stem cells in the female *Drosophila* germline, abscission, which also depends on ALIX and Shrub (Eikenes et al., 2015), is also delayed until the mid-G2 stage, and a complete loss of abscission maintains the stem cell fate (Matias et al., 2015). In mammalian neural stem cells, the duration of abscission is also differentially regulated between early and late differentiation stages (Little et al., 2021; McNeely and Dwyer, 2020).

In differentiated cells

Although stable intercellular bridges are less common in differentiated tissues, they have been observed in several species; however, it is often not clear whether abscission is delayed or incomplete. For example, intercellular bridges have been observed in *Drosophila* larval brain and larval wing imaginal disc epithelia (Kramerova and Kramerov, 1999). In the wing disc, the bridges are stabilized by the cooperation of the midbody components Cindr and anillin (also known as Scraps in flies) (Haglund et al., 2010). In the *Drosophila* brain, cortex glia cells use various strategies leading to formation of a syncytium, including endomitosis (division without cytokinesis), and stabilization of cytoplasmic bridges (Rujano et al., 2022). Some cells in organoid models of the liver also go through endomitosis via destabilization of the contacts between the midbody and the membrane, and regression of the cleavage furrow (Darmasaputra et al., 2023 preprint). Bridges can also be found connecting somatic follicular cells in some species of insects (Airolidi et al., 2011; McLean and Cooley, 2013; Meola et al., 1977). Proliferating intestinal cells in *Hydra* form clusters of cnidoblasts, which are connected by intercellular bridges (Fawcett et al., 1959). In mice, erythroblasts (precursors of red blood cells) form by incomplete abscission (Asano et al., 1991).

Overall, long-lasting bridges appear at multiple stages of organismal development. The molecular mechanisms of bridge maintenance are not always clear, although a recurrent theme is the mislocalization of ESCRT proteins (Adar-Levor et al., 2021; Chaigne et al., 2020; Iwamori et al., 2010; Mathieu et al., 2013, 2022) and the activity of Aurora B (Bai et al., 2019; Mathieu et al., 2013). Physiological bridges have been shown to be important for intracellular transport (Zenker et al., 2017), cell fate (Chaigne et al., 2020) and cell–cell communication (Adar-Levor et al., 2021; Doherty et al., 2021; Pepling et al., 1999; Ventelä et al., 2003), but

more work is required to understand how bridge structures support their multiple functions in different animal cell types.

Unexpected delays – defects that cause incomplete abscission

Long-lasting intercellular bridges have been well described in tumour cell lines as a consequence of stalled abscission, potentially to allow for correction of an error (Amaral et al., 2016; Mendoza et al., 2009; Steigemann et al., 2009). For example, abscission delay can lead to binucleation, which in turn can lead to the formation of cancer cells (Was et al., 2022). Stalled abscission can also occur in the eye and is associated with cataract formation (Gulluni et al., 2021). In tumour cell lines, stalled abscission can depend on the activation of the so-called NoCut abscission checkpoint, which relies on the continued activity of the master regulator Aurora B kinase. In this section, we discuss examples of defects that can trigger Aurora B-dependent abscission delays, which we broadly regroup under the term NoCut, although it is not clear whether all defects trigger the exact same molecular pathway delaying abscission (Fig. 2). As we will argue that the NoCut abscission checkpoint is not a bona fide cell cycle checkpoint (see Box 1), we avoid referring to NoCut as a checkpoint hereafter.

Defects triggering NoCut

Chromatin bridges

To maintain the integrity of the genome, DNA must be partitioned equally between daughter cells during division. Chromatin bridges are long stretches of DNA between two segregating chromosome masses (Finardi et al., 2020). Persistence of chromatin bridges until cytokinesis and abscission can cause DNA strand breakage, which mostly results in micronucleus formation (Crasta et al., 2012). Therefore, it is important to resolve chromatin bridges before abscission occurs (Petsalaki and Zachos, 2021). NoCut was first shown to be activated by chromatin bridges in *Saccharomyces cerevisiae* through the activity of Ipl1 kinase, a yeast homologue of Aurora B kinase (Mendoza et al., 2009; Norden et al., 2006). Intercellular bridges with chromatin bridges contain actin patches (Steigemann et al., 2009), which are thought to play a role in stabilizing the intercellular bridge (Dambournet et al., 2011; Frémont et al., 2017).

Nuclear pore complex defects

Defects in assembly of nuclear pore complexes (NPCs) also enable the activation of NoCut (Mackay et al., 2010). After anaphase onset, nucleoporins (Nups) are recruited to the surface of decondensing chromatin, where together with lipids, they form the two nuclear membranes and the NPCs. Downregulation of the levels of the NPC protein Nup153 leads to mistargeting of other NPC proteins, such as Tpr and Nup50 (Mackay et al., 2010). When the NPC basket is not correctly reformed after cell division, cells delay abscission via activation of NoCut (Mackay et al., 2010) and the formation of abscission checkpoint bodies (see below) (Strohacker et al., 2021). Delayed abscission has been proposed to prevent the separation of sister cells before the nuclear membrane is correctly formed (Mackay et al., 2010).

Replication stress

Replication stress can also activate NoCut. During S phase, DNA in the nucleus duplicates, preparing the genome for the next cell division. However, certain regions of the genome are more prone to incomplete replication than others (Gaboriaud and Wu, 2019). Under-replicated regions of DNA can accumulate the DNA damage response 1 (DDR1) protein 53BP1, which protects these under-

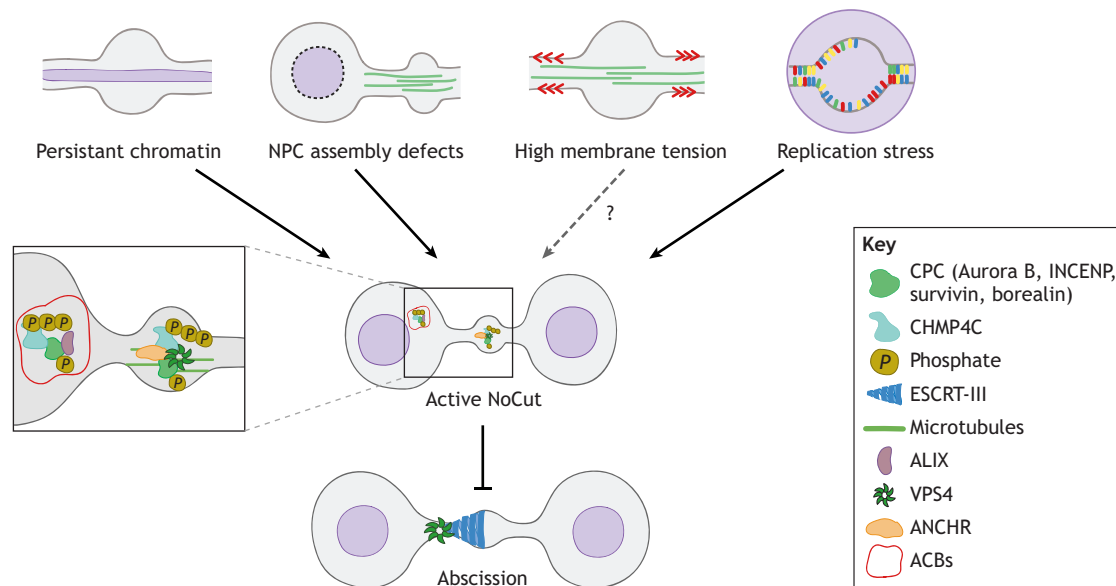


Fig. 2. Cellular defects triggering NoCut. Different events can cause the activation of the abscission checkpoint and therefore delay abscission. Delayed abscission can be a result of persistent chromatin trapped in the intercellular bridge, defects in nuclear pore complex (NPC) assembly, high membrane tension, or replication stress. Chromatin bridges, replication stress and defects in NPC assembly lead to the activation of NoCut, which inhibits abscission. It is still not clear whether high membrane tension delays abscission through the same mechanisms. When NoCut is active, the CPC phosphorylates its targets, including the abscission regulator CHMP4C. Evidence suggests that CHMP4C phosphorylation acts through several pathways. Phosphorylated CHMP4C can recruit ANCHR, and in turn sequester VPS4 at the midbody central region where it cannot reach CHMP4B filaments and induce abscission. Triple phosphorylated CHMP4C (CHMP4CPPP) can also form cytoplasmic bodies with Aurora B kinase and ALIX and prevent ALIX recruitment at the midbody which inhibits abscission. Aurora B can also delay abscission through several pathways including KIF20A and ULK3 (not depicted).

replicated regions until the repair occurs. Accumulation of 53BP1 foci at the DNA can cause an increase in the number of cells connected by intercellular bridges, suggesting a delayed abscission (Mackay and Ullman, 2015). In yeast, replication stress can cause ultrafine chromatin bridges to appear, leading to Aurora B-dependent intercellular bridge maintenance (Amaral et al., 2016).

Mechanical forces and delayed abscission

Mechanical forces, in particular the pulling forces that the sister cells exert on the intercellular bridge, can also control abscission dynamics. When forces are released, for example through laser ablation, the abscission machinery cuts the membrane (Lafaurie-Janvore et al., 2013). However, it is not clear whether high membrane tension prevents timely abscission through the activation of NoCut. Indeed, ULK3, a kinase proposed to be part of NoCut, has been shown to participate in regulating abscission dynamics downstream of tension changes (Caballe et al., 2015). However, recent data has shown that caveolae, cup-shaped invaginations of the plasma membrane, can buffer high membrane tension on the intercellular bridge and therefore control abscission without the involvement of Aurora B (Andrade et al., 2022). In dividing cells, caveolae have been found to localize at the midbody, the abscission site and the interface between the intercellular bridge and connected cells (Andrade et al., 2022).

Adhesion-dependent tension can also regulate abscission. Indeed, trafficking of integrins, which mediate cell–extracellular matrix (ECM) interactions, is essential for successful abscission in adherent cells (Pellinen et al., 2008). For example, integrin-dependent cell–substrate adhesion is crucial to allow correct cytokinesis in cells with impaired contractility (Dix et al., 2018). Adherent cells plated on non-adherent substrates display a delay in the recruitment of the ESCRT machinery (Kamranvar et al., 2016). Conversely, in the absence of the midbody-dependent recruitment of ESCRT machinery, an adhesion-dependent

pathway can take over and allow abscission to occur in human fibroblasts (Gupta et al., 2018). Finally, cells that become more migratory upregulate KIF23, anillin and septin 6 on stiffer substrates and fail to complete abscission, a hallmark of cancer (Rabie et al., 2021; Simi et al., 2018).

Overall, multiple cellular defects can lead to a delay in abscission. Differences in response to these defects, for example linked to allelic polymorphism of CHMP4C (Sadler et al., 2018), or to different expression levels of Aurora B (Chieffi, 2018), can lead to increased cancer susceptibility, highlighting the importance of understanding the molecular regulation of abscission (Petsalaki and Zachos, 2021; Sagona and Stenmark, 2010). In the next section, we review in more detail the molecular mechanisms of Aurora B activation and its consequences.

Aurora B kinase localization at the midbody and regulation of its kinase activity

The main regulator of NoCut is Aurora B kinase, which plays many important roles during cell division to ensure faithful chromosome segregation (Liang et al., 2020). As described earlier, Aurora B is a part of the CPC. At anaphase onset, the CPC is transferred from the kinetochores of the chromosomes to the microtubules of the central spindle by the kinesin KIF20A (Gruneberg et al., 2004). As cytokinesis progresses, Aurora B localizes at the midbody (Fig. 2) (Halcrow et al., 2022; Serena et al., 2020). Aurora B needs to bind INCENP through its IN-box domain for proper localization (Carmena et al., 2012). During the early stages of mitosis, cyclin-dependent kinase 1 (CDK1) phosphorylates INCENP at threonine (Thr)59, which allows the CPC to bind to the centromeres of mitotic chromosomes. When Cdk1 activity decreases at anaphase onset, the dephosphorylation of INCENP-Thr59 allows the association of INCENP with KIF20A, permitting binding to the microtubules and displacement of the CPC to the spindle midzone (Sessa et al., 2005).

The activity of Aurora B kinase in promoting abscission delay depends on the activation of its kinase domain; this relies on autophosphorylation within its activation loop at Thr232. Moreover, Aurora B needs to phosphorylate INCENP at two consecutive serine (Ser) residues in a conserved Thr-Ser-Ser motif (Bishop and Schumacher, 2002). For Aurora B activation to be complete, the kinase must also be phosphorylated at its C-terminal tail on Ser331. During prometaphase and metaphase, DNA checkpoint kinase 1 (Chk1; also known as CHEK1) phosphorylates Ser331, while at the midbody, CDC-like kinases CLK1, CLK2 and CLK4 complete the activation of Aurora B kinase (Mackay and Ullman, 2015; Petsalaki and Zachos, 2016; Petsalaki et al., 2011). Protein kinase C ϵ (PKC ϵ) also plays an important role in NoCut by phosphorylating Aurora B at Ser227, which in turn facilitates Aurora B-mediated phosphorylation of borealin and other targets (Pike et al., 2016). Borealin is an essential part of the CPC complex; it forms a three-helix bundle with INCENP and survivin, and is required for CPC localization (Jeyaprakash et al., 2007). Apart from its role in the localization of the CPC, Borealin can bind the ESCRT-III protein CHMP4C, which delays abscission (Carmena et al., 2012). Once Aurora B is activated, it can trigger a downstream signalling cascade both at the midbody and in the cytoplasm (Carlton et al., 2012; Strohacker et al., 2021). However, how the defects described in the previous section lead to the activation of Aurora B remains unknown.

Downstream Aurora B kinase signalling

At the midbody

Aurora B phosphorylates a component of the ESCRT-III complex, CHMP4C, which in turn delays abscission (Carlton et al., 2012). CHMP4C localizes initially to the midbody, then to the abscission site, and its depletion accelerates abscission, suggesting that CHMP4C acts like a brake (Carlton et al., 2012). Borealin can also interact with CHMP4C, and this might regulate the timing of abscission (Capalbo et al., 2012). Borealin interacts with the N-terminal domain of CHMP4C, whereas Aurora B kinase phosphorylates CHMP4C at Ser210, Ser214 and Ser215 inside the C-terminal domain of CHMP4C (Capalbo et al., 2012). In *Drosophila*, the protein Shrub is not phosphorylated by Aurora B, and it has been proposed that Shrub interactions with borealin directly activate NoCut (Capalbo et al., 2012).

Aurora B can also delay abscission indirectly through several pathways. The protein abscission checkpoint regulator (ANCHR; also known as ZFYVE19) can be activated by Aurora B kinase and prevent abscission (Fig. 2) (Thoresen et al., 2014). ANCHR localizes at the midbody centre during late cytokinesis and binds the AAA-ATPase VPS4, preventing its proper localization at the secondary ingression sites. It has been proposed that ANCHR forms a ternary complex with CHMP4C and VPS4 inside the midbody centre and prevents abscission in an Aurora B-dependent manner (Thoresen et al., 2014). Aurora B also phosphorylates KIF20A, which blocks abscission; the phosphatase B56-PP2A counteracts Aurora B-dependent KIF20A phosphorylation (Fung et al., 2017).

Furthermore, another potential downstream target of Aurora B kinase, ULK3, localizes at the midbody centre and can delay abscission (Caballe et al., 2015). ULK3 can phosphorylate the ESCRT-III subunit IST1, which prevents its binding to VPS4 (Caballe et al., 2015).

Outside the midbody

Aside from its role at the midbody, Aurora B has also been proposed to play a role in the cytoplasm of the sister cells. Aurora B kinase can form abscission checkpoint bodies (ACBs), which contain

phosphorylated Aurora B, tri-phosphorylated CHMP4C and ALIX (Fig. 2). ACBs are located in the cytoplasm of both sister cells and are able to delay abscission by preventing the recruitment of the ESCRT proteins at the midbody (Strohacker et al., 2021), suggesting that the localization of Aurora B is tightly controlled to allow its role in abscission dynamics. Finally, other pathways probably remain to be discovered; indeed, the microtubule-severing protein spastin has also been shown to influence abscission dynamics when NoCut is active, although the mechanism at play is completely unknown (Wenzel et al., 2022).

Future perspectives

We have known for some time that abscission can in some cases be severely delayed. Delayed abscission can be a feature of abnormal cell division, which involves the NoCut pathway and in particular the master regulator Aurora B, but delayed and incomplete abscission sometimes also happens during normal organism development. Although we now have a broad mechanistic insight of the action of Aurora B, it is not always clear what molecular mechanisms contribute to its activation. Furthermore, it is yet to be discovered whether the mechanisms at play during delayed abscission in cells with mitotic defects also play a role in normal development. Recent research has shed some light on the mechanisms of delayed abscission in the germline, embryos and stem cells. Model systems such as stem cells, which can modulate their abscission dynamics according to their developmental state, will allow us to delve further into the molecular mechanisms of cell division regulation.

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