Cell

Final Stages of Cytokinesis and Midbody Ring Formation Are Controlled by BRUCE

Christian Pohl[1] and Stefan Jentsch[1,*]
[1]Department of Molecular Cell Biology, Max Planck Institute of Biochemistry, Am Klopferspitz 18, 82152 Martinsried, Germany
*Correspondence: jentsch@biochem.mpg.de
DOI 10.1016/j.cell.2008.01.012

SUMMARY

Cytokinesis involves the formation of a cleavage furrow, followed by abscission, the cutting of the midbody channel, the final bridge between dividing cells. Recently, the midbody ring became known as central for abscission, but its regulation remains enigmatic. Here, we identify BRUCE, a 528 kDa multifunctional protein, which processes ubiquitin-conjugating activity, as a major regulator of abscission. During cytokinesis, BRUCE moves from the vesicular system to the midbody ring and serves as a platform for the membrane delivery machinery and mitotic regulators. Depletion of BRUCE in cell cultures causes defective abscission and cytokinesis-associated apoptosis, accompanied by a block of vesicular targeting and defective formation of the midbody and the midbody ring. Notably, ubiquitin relocalizes from midbody microtubules to the midbody ring during cytokinesis, and depletion of BRUCE disrupts this process. We propose that BRUCE coordinates multiple steps required for abscission and that ubiquitylation may be a crucial trigger.

INTRODUCTION

Cytokinesis is the concluding step of cell division by which the prospective daughter cells separate their cytoplasmic volumes. This process starts by contraction of a plasma-membrane-anchored actomyosin ring, leading to the formation of a cleavage furrow (Eggert et al., 2006). By the end of furrowing, the dividing cells are connected by a narrow, tubular intercellular bridge, which contains the midbody consisting of tightly bundled antiparallel microtubules, which embrace a phase-dense circular structure called midbody ring (or occasionally Flemming body). At the final stage of cytokinesis, in a process termed abscission, this bridge is cleaved, and two daughter cells are formed.

At the midbody, several cytokinesis-coupled events converge, including degradation of cell cycle regulators, cytoskeleton rearrangements, membrane traffic, and plasma membrane remodeling. Recent reports demonstrate a direct involvement of the traffic-regulating GTPases Arf1, Arf6 and Rab11 in cytokinesis (Albertson et al., 2005). Arf6 and Rab11 are coupled to the exocyst complex, which seems crucial for the proper targeting of vesicles to the site of abscission. Interestingly, some types of vesicles seem to arrive at the midbody ring chiefly from only one of the prospective daughter cells (Gromley et al., 2005), suggesting an intrinsic asymmetric element in cytokinesis. The factors that control targeting to the midbody and guide midbody ring assembly are largely unknown, but one protein required for exocyst targeting to the midbody ring was recently identified as centriolin, which also binds to the maternal centriole (Gromley et al., 2003, 2005).

Previous studies revealed an emerging role of ubiquitylation and proteasomal activity in regulating cytokinesis (Pines and Lindon, 2005). Notably, ubiquitin-activating enzyme E1 and the proteasome are concentrated on midbodies (Grenfell et al., 1994; Wojcik et al., 1995), and both proteolytic and non-proteolytic functions of ubiquitin seem to play a role. Chromosomal passenger proteins, the kinase Aurora B and the baculovirus inhibitor of apoptosis repeat (BIR)-containing protein survivin, as well as the Polo-like kinase Plk1 (Lindon and Pines, 2004) are degraded just before or during cytokinesis (Lindon and Pines, 2004; Sumara et al., 2007). Proteasome inhibition after anaphase onset results in incomplete cytokinesis (Straight et al., 2003), and, interestingly, combined inhibition of Cdk1 and proteasomes can revert late cytokinesis to an apparent preanaphase state (Potapova et al., 2006). A non-proteolytic role of ubiquitin in cytokinesis is suggested by the fact that the ubiquitin-controlled endosomal sorting complex required for transport (ESCRT) is necessary for abscission (Carlton and Martin-Serrano, 2007).

Proteins harboring a BIR domain (BIRPs) are primarily known for their function to protect cells against apoptosis by their activity to inhibit caspases and proapoptotic factors through binding and ubiquitin-dependent degradation (Verhagen et al., 2001). However, BIRPs like survivin and cIAP1 are also crucial for cell cycle events and cytokinesis (Li et al., 1999; Samuel et al., 2005). Here, we report that another conserved BIRP, the 528 kDa protein BRUCE (also known as Apollon or BIRC6), is a crucial regulator for the final stages of cytokinesis. BRUCE is a multifunctional protein owing to the presence of different functional domains and multiple binding partners (Bartke et al., 2004; Hauser et al., 1998). Close to its amino (N)-terminus, BRUCE harbors a single BIR domain, which most closely resembles the BIR of survivin. BRUCE can inhibit caspases through this domain,

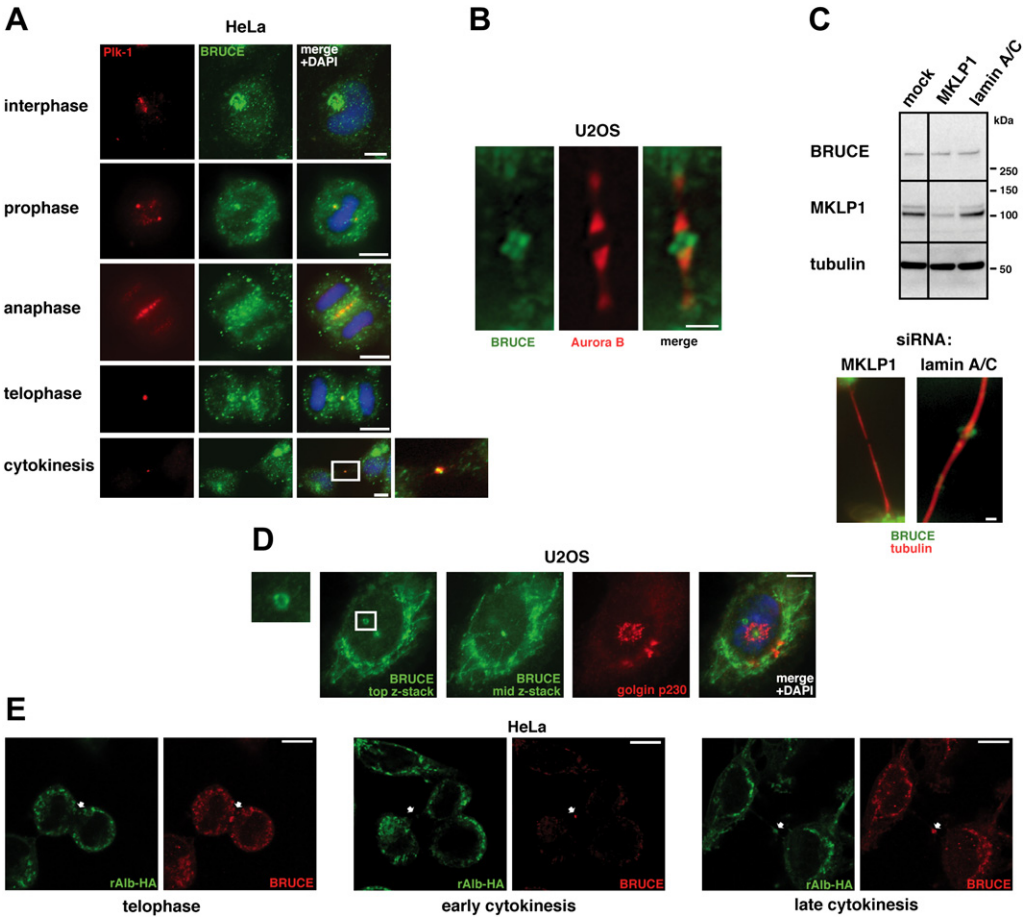

Figure 1. BRUCE Shows a Cell Cycle-Dependent Localization

(A) BRUCE localizes to mitotic structures. Immunofluorescence (IF) of HeLa cells stained with anti-Plk-1 (red) and anti-BRUCE (green) and DAPI to visualize DNA. The box outlined in yellow shows the enlarged midbody region of the cytokinesis merge. The scale bar represents 10 μm.

(B) BRUCE localizes to the midbody ring of U2OS cells. The midbody region is shown with anti-Aurora B (red) and anti-BRUCE (green) staining. The scale bar represents 1 μm.

(C) Localization of BRUCE to the midbody ring depends on MKLP1. Top: lysates from siRNA-transfected HeLa cells were analyzed by BRUCE, MKLP1, and α-tubulin immunoblotting. Bottom: midbodies of siRNA-treated cells are shown stained with anti-α-tubulin (red) and anti-BRUCE (green). The scale bar represents 1 μm.

(D) BRUCE is found on midbody ring remnants. U2OS cells were stained with anti-BRUCE (green) and anti-golgin p230 (red), and DAPI (blue). The scale bar represents 10 μm. The top z-stack on the left shows a midbody ring remnant in the plane of the plasma membrane (shown enlarged on the left).

(E) Relationship between BRUCE and constitutive secretory cargo. HeLa cells stably expressing HA-albumin are shown in different cell cycle stages with anti-HA (green) and anti-BRUCE (red) staining. The scale bar represents 10 μm. The arrow shows the position of the midbody ring.

and has antiapoptotic potential (Bartke et al., 2004). However, its antiapoptotic function may be particularly relevant for the trans-Golgi network (TGN) and vesicular structures where it mainly localizes (Hauser et al., 1998). Near its carboxy (C)-terminal end, BRUCE carries a ubiquitin-conjugating (UBC) domain, which endows the protein with a hybrid E2/E3 ubiquitin ligase activity (Bartke et al., 2004; Hauser et al., 1998). In vitro, BRUCE primarily mono- or oligo-ubiquitylates proteins, suggesting that its main role is non-proteolytic (Bartke et al., 2004). BRUCE-knockout mice usually die perinatally due to impaired placental development that can be attributed to insufficient differentiation (Lotz et al., 2004), and depletion of BRUCE in cultured cells sensitizes against apoptotic stimuli and finally leads to cell death (Hao et al., 2004; Ren et al., 2005).

In this report we show that BRUCE is an important novel player of cytokinesis and important for abscission. We demonstrate that BRUCE localizes to the midbody ring during cytokinesis, where it binds mitotic regulators and components of the vesicle targeting machinery. Microscopic studies and live-cell imaging with wild-type and BRUCE-depleted cells, and of cells that express a dominant-negative version, revealed that BRUCE is involved in the correct delivery of membrane vesicles to the site of abscission and for the integrity of the midbody, in particular the midbody ring. We further discovered a remarkable dynamic relocalization of ubiquitin from the midbody to the midbody ring, show that both BRUCE and MKLP1 are ubiquitylated and that UBPY serves as their deubiquitylating enzyme. Our work suggests that this giant protein, through its multiple activities,