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,

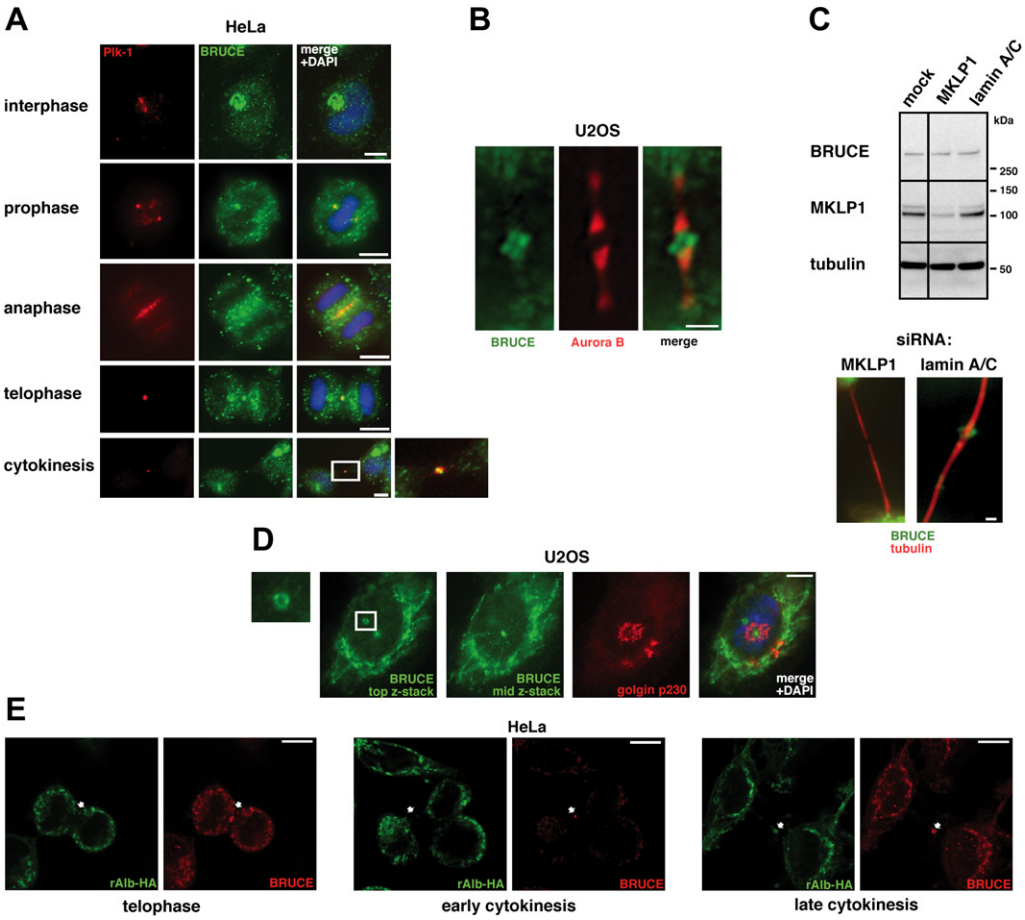

Figure 1. BRUCE Shows a Cell Cycle-Dependent Localization

(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,

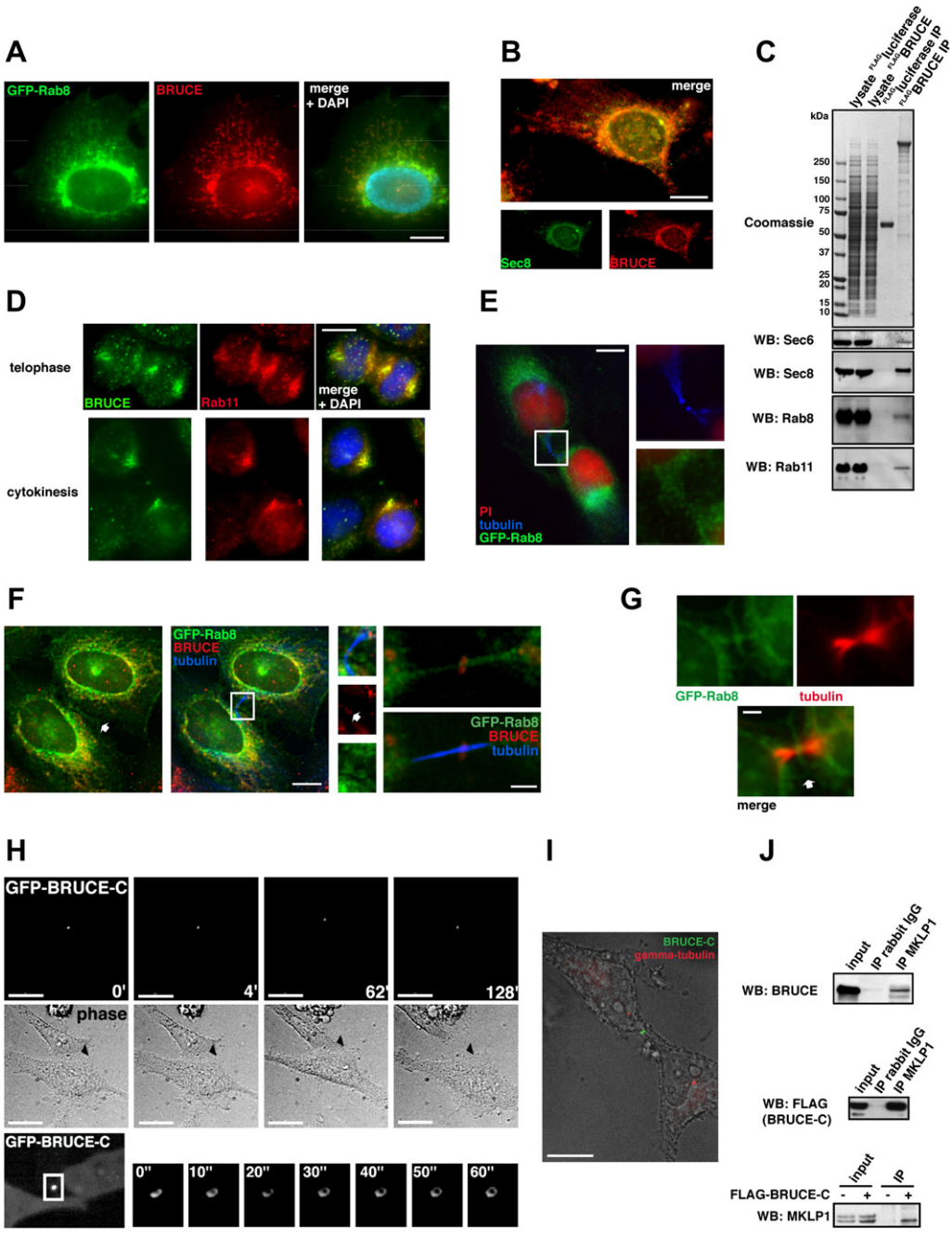

Figure 2. BRUCE Is a Component of Tubular Endosomes, Interacts with Membrane Targeting Factors, and Contains a Midbody Ring-Targeting Domain

(A) BRUCE localizes to Rab8 tubular endosomes in interphase cells. U2OS cells stably expressing GFP-Rab8 (green) were fixed and stained with anti-BRUCE (red) and DAPI (blue). The scale bar represents 10 μm.

(B) BRUCE colocalizes with the exocyst component Sec8 in a perinuclear compartment. Fixed U2OS cells; anti-BRUCE (red), anti-Sec8 (green). The scale bar represents 15 μm.

(C) BRUCE copurifies with exocyst components Sec6, Sec8, and endosomal GTPases Rab8, Rab11. HEK293T cells were transfected with either FLAG-epitope tagged luciferase or BRUCE. Cells were lysed and FLAG-fusion proteins purified. Bound material was eluted with FLAG peptide and analyzed by western blots (WB).

(D) Colocalization of BRUCE with Rab11 recycling endosomes in cytokinesis. Fixed HeLa cells; anti-Rab11 (red), anti-BRUCE (green), and DAPI (blue). The scale bar represents 15 μm.

(E) Rab8 tubular endosomes are found in the midbody region. Fixed U2OS cells; anti-tubulin (blue), and propidium iodide DNA staining (red). The scale bar represents 10 μm.

crucially coordinates several events that conclude cytokinesis, and additionally uncovers a previously unrecognized role of ubiquitin in this process.

RESULTS

BRUCE Localizes to the Midbody Ring and Associates with Mitotic Regulators

BRUCE is a cytosolic protein, which is peripherally associated with endomembranes (Hauser et al., 1998). By using three different polyclonal antibodies directed against N- or C-terminal regions of BRUCE (Bartke et al., 2004; Hauser et al., 1998) (Figure S1 available online) we found in both HeLa and U2OS cells that BRUCE relocalizes considerably during the cell cycle. It concentrates in a pericentriolar compartment in interphase, moves partially to spindle poles in metaphase, and finally localizes to the spindle midzone and the midbody in telophase and during cytokinesis (Figure 1A and Figure S2). On the midbody, BRUCE localizes in a characteristic ring-like arrangement that is embraced by Aurora B and microtubules (Figures 1B and 1C), indicating that BRUCE is associated with the midbody ring (Gromley et al., 2005). By using siRNAs targeting MKLP1, a mitotic kinesin essential for the formation of the midbody matrix (Matuliene and Kuriyama, 2002), we observed in midbody forming cells a complete loss of BRUCE at the midbody (Figure 1C). In untreated cells, BRUCE localized to the midbody ring as soon as this structure first appeared in telophase, and BRUCE persisted on the ring even after its uptake by one of the daughter cells after completion of abscission (Figures 1D and 1E). To investigate membrane traffic and to trace vesicles containing constitutively secreted cargo, we constructed a HeLa cell line expressing HA-epitope tagged albumin. Localization of secretory vesicles on the midbody ring was visible only in late cytokinesis when the intercellular bridge had constricted to about 1 μm in diameter, whereas BRUCE, as an apparent firm resident of the midbody ring, localized to the midbody ring much earlier, concurrently when it had been formed (Figure 1E).

Given its highly dynamic localization pattern during cell division, we searched for binding partners and asked whether BRUCE might be a target for mitotic phosphorylation. Indeed, we found that BRUCE was particularly phosphorylated in S-phase extracts (data not shown) and that immunopurified BRUCE was also phosphorylated by copurified factors (Figure S3A). By immunopurification (IP) of FLAG-tagged BRUCE, or immunoprecipitation of endogenous BRUCE, we found BRUCE associated with the mitotic kinases Cdk1, MEK1, and Plk-1 (Figures S3B–S3G) and also α-tubulin (data not shown). Moreover, overexpressed BRUCE could immunoprecipitate endogenous survivin, and vice versa, indicating that these two BIRPs may cooperate at the midzone or the midbody, where both proteins were found (Figures S3H and S3I).

BRUCE Is a Component of Tubular and Recycling Endosomes and Associates with the Exocyst

To characterize BRUCE localization further, we assayed for colocalization with markers of the vesicular system. We found only partial colocalization with the TGN markers golgin p230 and TGN38 (Figure S2B and Hauser et al., 1998). However, we observed substantial colocalization with GFP-tagged versions of the endosomal GTPases Rab8 and Rab11, even with Rab8-positive, peripheral tubular endosomes (Figure 2A and Figure S4A) and with the exocyst subunit Sec8 (Figure 2B). Importantly, Rab8 and Rab11, as well as the exocyst subunits Sec6 and Sec8, physically associate with BRUCE, as demonstrated by immunoprecipitations (Figure 2C and Figures S4B and S4C). Similar to the mitotic kinases (Figure S3B), the binding site for the exocyst seems to lie within the N-terminal region of BRUCE (Figure S4D) and thus involves a different region in BRUCE than for midbody ring interaction (see below). Notably, similar to exocyst subunit Exo70 (Vega and Hsu, 2001), BRUCE also relocalizes to growth cones in neuronally differentiated cells (Figure S4E), strongly indicating that BRUCE is firmly connected to the exocyst and plays a more general role in targeted membrane delivery.

Rab11 and Rab8-Endosomes Are Sources of Membrane Material in Cytokinesis and the Midbody Ring Acts as a Barrier

Next, we asked whether vesicles containing BRUCE participate in cytokinesis. We could confirm previous observations (Fielding et al., 2005) that suggested a role for recycling Rab11-endosomes in cytokinesis. Two pools of Rab11-endosomes were found on both sides of the intercellular bridge during cytokinesis, and a small fraction also seemed to reach the midbody ring (Figure 2D). However, in contrast to a recent report on HeLa cells (Yu et al., 2007), we found in engineered U2OS cells also GFP-Rab8 staining at the midbody (Figures 2E and 2F) and also at the newly formed plasma membrane at the contact site of dividing cells (Figure 2G). Indeed, live-cell imaging suggests that Rab8-endosomes are delivered to the midbody ring and that this supplied material then diffuses at the plasma membrane laterally (data not shown). Remarkably, by using photobleaching experiments, we found that Rab8-endosomes are unable to move freely from one perspective daughter cell to the

(F and G) Rab8 is found at the midbody ring and at the newly formed plasma membrane between dividing cells. Fixed U2OS cells; arrowheads indicate the site of the newly formed plasma membrane. The scale bars represent, respectively, (F) 10 μm and (G) 2 μm. The smaller figures are enlargements of the framed areas. The position of the midbody ring is indicated by an arrow.

(H) The C terminus of BRUCE represents a midbody ring-targeting domain. GFP fused to the C terminus of BRUCE (amino acids 4711–4845, GFP-BRUCE-C) is localized to the midbody ring. The movie stills (of Movie S5) show GFP-BRUCE-C on the midbody ring of HeLa cells. The scale bar represents 15 μm.

(I) Localization of BRUCE-C relative to centrosomes. HeLa cells transfected with GFP-BRUCE-C (green) were stained with anti-γ-tubulin (red). The image shows a merge with the bright-field channel. The scale bar represents 15 μm.

(J) The midbody ring-targeting domain of BRUCE interacts with MKLP1. Top: lysates of HEK293T cells were immunoprecipitated (IP) with either rabbit IgGs or anti-MKLP1 antibodies and analyzed by anti-BRUCE WB. Middle: HEK293T cells transfected with GFP-FLAG-BRUCE-C were lysed and proteins immunoprecipitated with rabbit IgG (control) or anti-MKLP1 IgGs. Input and precipitates (IP) were analyzed by anti-FLAG blots (WB) detecting BRUCE-C. Bottom: HEK293T cells were either transfected with empty vector or GFP-FLAG-BRUCE-C. Lysates were subjected to anti-FLAG IP and analyzed by anti-MKLP1 WB.

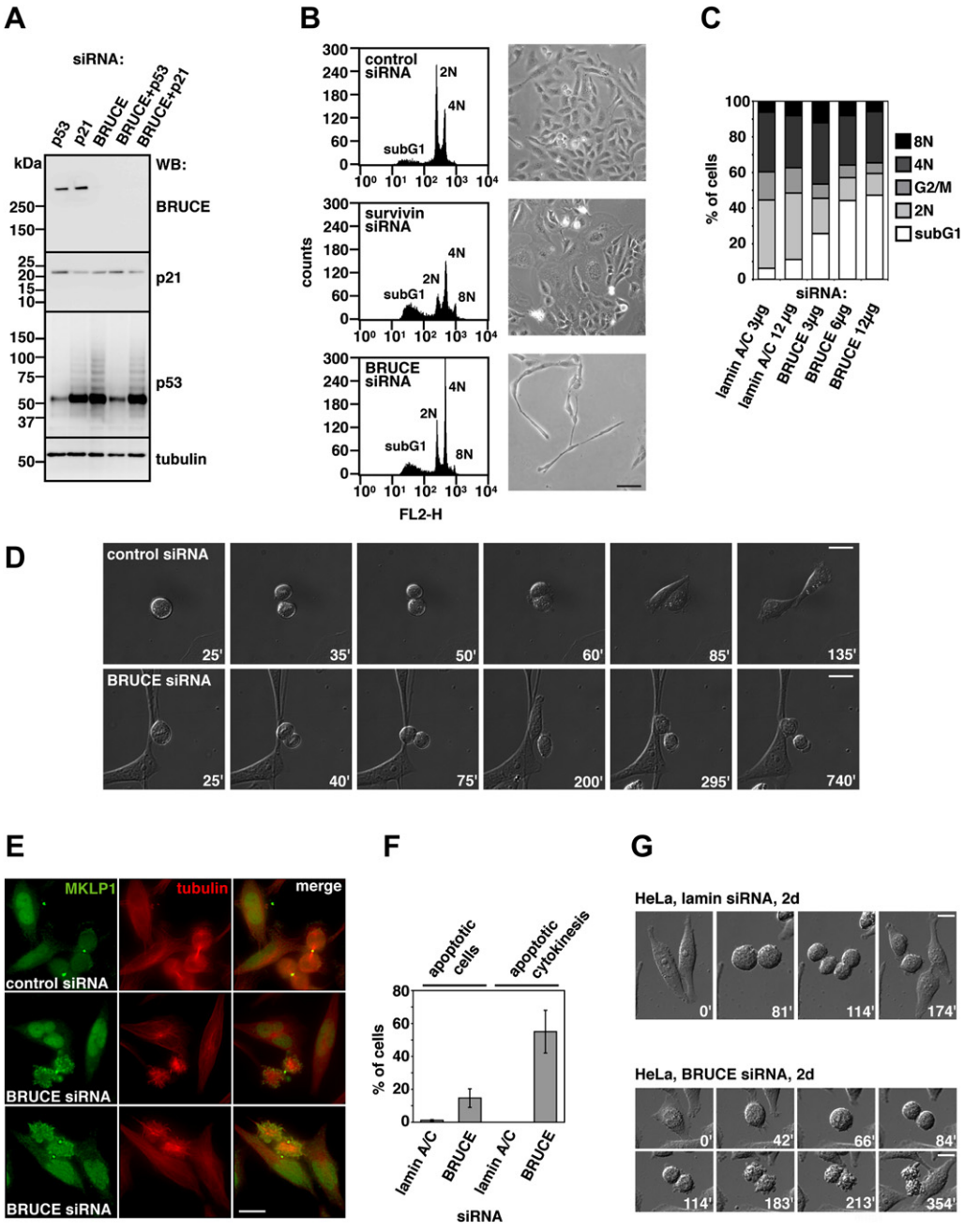

Figure 3. BRUCE Depletion Causes Cytokinesis Defects and Cytokinesis-Associated Apoptosis

(A) BRUCE depletion in U2OS cells has only minor consequences for p53 levels. Lysates of cells transfected with the indicated siRNAs were analyzed by western blots with the indicated antibodies.

(B) BRUCE depletion in U2OS cells induces syncytia-like structures. Cells were treated with siRNAs for 3 days as shown in Figure 1C targeting lamin A/C, survivin, or BRUCE. After 3 days of incubation, a representative area of cells was photographed (scale bar represents 20 μm), cells were harvested and prepared for propidium iodide staining, and the DNA content of cells was analyzed by flow cytometry. The 4N peak is marked by an arrow.

(C) Cell cycle distribution after treatment with increasing concentrations of siRNA. Cells were treated with siRNA as in (A). The graph represents the cell cycle distribution as obtained by flow cytometry of the DNA content.

(D) Defective cytokinesis after BRUCE depletion. siRNA-treated U2OS cells were observed by differential interference contrast (DIC) microscopy. The images represent stills of movies at representative time points. The scale bar represents 20 μm.

(E) HeLa cells depleted of BRUCE undergo cytokinesis-associated apoptosis. HeLa cells were treated with siRNAs for 2 days as in (A), fixed, and stained with anti-MKLP1 (green) and anti-α-tubulin (red). The scale bar represents 15 μm.

(F) Quantification of (E) with apoptotic cells relative to total cells and apoptotic cytokinesis versus total cells in cytokinesis (error bars represent ± SD of two independent experiments).

other once the midbody ring has been assembled in telophase (Movies S1 and S2), which differs from the otherwise mobile behavior of these vesicles during interphase. This finding considerably substantiates the idea that midbody rings, in addition to acting as the site for membrane delivery, may play a role as a diffusion barrier in-between the two prospective daughter cells (Schmidt and Nichols, 2004). Apparently, this barrier does not only prevent intercellular exchange of plasma membrane-associated material as shown before (Schmidt and Nichols, 2004), but also of endosomal vesicles.

BRUCE Harbors a Midbody Ring-Targeting Domain

The distinctive and dynamic localization of BRUCE suggested the presence of dedicated targeting domains. By using truncation constructs we in fact noticed that the typical localization of BRUCE at sorting compartments requires its C-terminal region (Figure S4F). Surprisingly, when we constructed a GFP-fusion of the C-terminal 145 amino acids of BRUCE (termed BRUCE-C), this construct localized exclusively to the midbody ring (Figures 2H and 2I and Movie S3 and S4). Time-lapse video microscopy revealed that the BRUCE-C-labeled ring-like structure showed a degree of mobility and tilting characteristic for the midbody ring, and that it was taken up by one of the daughter cells after abscission (Figure 2H and Movie S5). As the localization of endogenous BRUCE to the midbody ring depends on MKLP1 (Figure 1C), we speculated that BRUCE-C might contain a specific interaction domain with midbody ring components. Indeed, we found MKLP1 coprecipitating with full-length, endogenous BRUCE (Figure 2J, top panel) and also with BRUCE-C (Figure 2J, middle panel). Interestingly, BRUCE-C selectively bound the faster migrating variant, MKLP1, but not CHO1, a larger variant caused by alternative splicing that additionally possesses an actin-interacting tail (Figure 2J, lower panel). As both isoforms are present at the midbody (Kuriyama et al., 2002), this finding argues for different functions of these two variants during late stages of cytokinesis. Notably, the C-terminal tail of BRUCE (BRUCE-C) is highly conserved from flies to vertebrates, suggesting that the identified midbody ring-targeting domain (MTD) and the interaction with MKLP1 are features of all BRUCE family members.

BRUCE Depletion Causes Defective Abscission and Cytokinesis-Associated Apoptosis

Depleting BRUCE from cultured cells was previously shown to reduce cell viability (Ren et al., 2005). To identify the basis of this phenotype, we designed different siRNAs targeting BRUCE (see Supplemental Experimental Procedures and Figure S5). Delivery of BRUCE-targeted siRNA by electroporation in U2OS cells depleted BRUCE to undetectable levels 3 days after transfection (Figure 3A). These cells acquired a striking elongated appearance and built up interconnected cells, but in contrast to survivin depletion, no cells with giant nuclei developed (Figure 3B and Figure S1C). Moreover, reminiscent of centriolin depletion (Gromley et al., 2003), some apparently not completely detached cells fused again together or underwent mitosis (data not shown and see below). Higher siRNA concentrations and longer incubation times resulted in a decrease in the amount of normal G1 phase (2N) cells (Figure 3C and data not shown) but a strong rise of a sub-G1 population, indicative of syncytia-like cells, which finally undergo apoptosis. However, different from the results by Ren and coworkers (Ren et al., 2005), no rise in the levels of p53 or of p53 targets like p21 were observed (Figure 3A), suggesting that apoptosis in these cells is not an immediate response to BRUCE depletion but a later corollary. Live-cell video microscopy showed that BRUCE-depleted cells form a normal cleavage furrow, but do not complete cytokinesis even after hours, and that some cells even did not regain their normal flattened appearance (Figure 3D). When we depleted BRUCE in HeLa cells, cells underwent apoptosis, but, intriguingly, only during attempted cytokinesis (Figures 3E–3G). After the formation of the midbody ring, almost 60% of the cells were apoptotic (Figure 3F), and membrane blebbing occurred already 30 min after furrowing (Figure 3G). This suggests a mechanism by which apoptosis is triggered by cues that originate at the midbody in the absence of BRUCE.

BRUCE Is Involved in Membrane Delivery to the Midbody Ring

Because BRUCE-C binds to the midbody ring so well, we wondered whether we could utilize it as a dominant-negative tool specifically to monitor the function of BRUCE at this location. Indeed, overexpression of BRUCE-C could displace endogenous BRUCE from MKLP1 and the midbody ring (Figure 4A) and led to aberrant cytokinesis and binucleation (Figures 4B and 4C). Notably, after prolonged expression of BRUCE-C for 3 days, apoptosis occurred (Figure S6), indicating that the apoptotic signal is in fact triggered by the absence of BRUCE from the midbody ring. When we analyzed HeLa cells that stably express tagged albumin, we found secretory vesicles within the intercellular bridge. Intriguingly, however, cells overexpressing BRUCE-C showed single balloon-like accumulations of cargo-containing vesicles in close proximity to the intercellular bridge, but only on one of its sides (Figure 4B). From these findings we conclude that the presence of BRUCE at the midbody is needed for normal delivery of vesicles to the site of abscission and that prolonged absence of BRUCE interferes with cytokinesis.

To corroborate BRUCE's role in vesicle traffic, we next addressed in U2OS cells that stably express GFP-Rab11 whether also siRNA-directed BRUCE depletion interferes with normal membrane targeting. When we examined control cells (transfected with a control siRNA) that had just completed cytokinesis (Figure 4D, upper panel), Rab11 had already adopted typical interphase localization and phosphotyrosine was found on the midbody as reported (Kasahara et al., 2007). In striking contrast, BRUCE-depleted cells accumulated Rab11-endosomes, as well as phosphotyrosine-positive material, in close proximity to one side of the midbody (Figure 4D, lower panel). This finding confirms our conclusion that BRUCE is needed for the targeting of vesicles to the midbody, and further suggests that BRUCE is

(G) Stills with DIC live-cell microscopy of HeLa cells treated with lamin siRNA (top) or BRUCE siRNA (bottom) for 2 days. Note that cells depleted for BRUCE undergo apoptosis after attempted cytokinesis. The scale bar represents 15 μm.

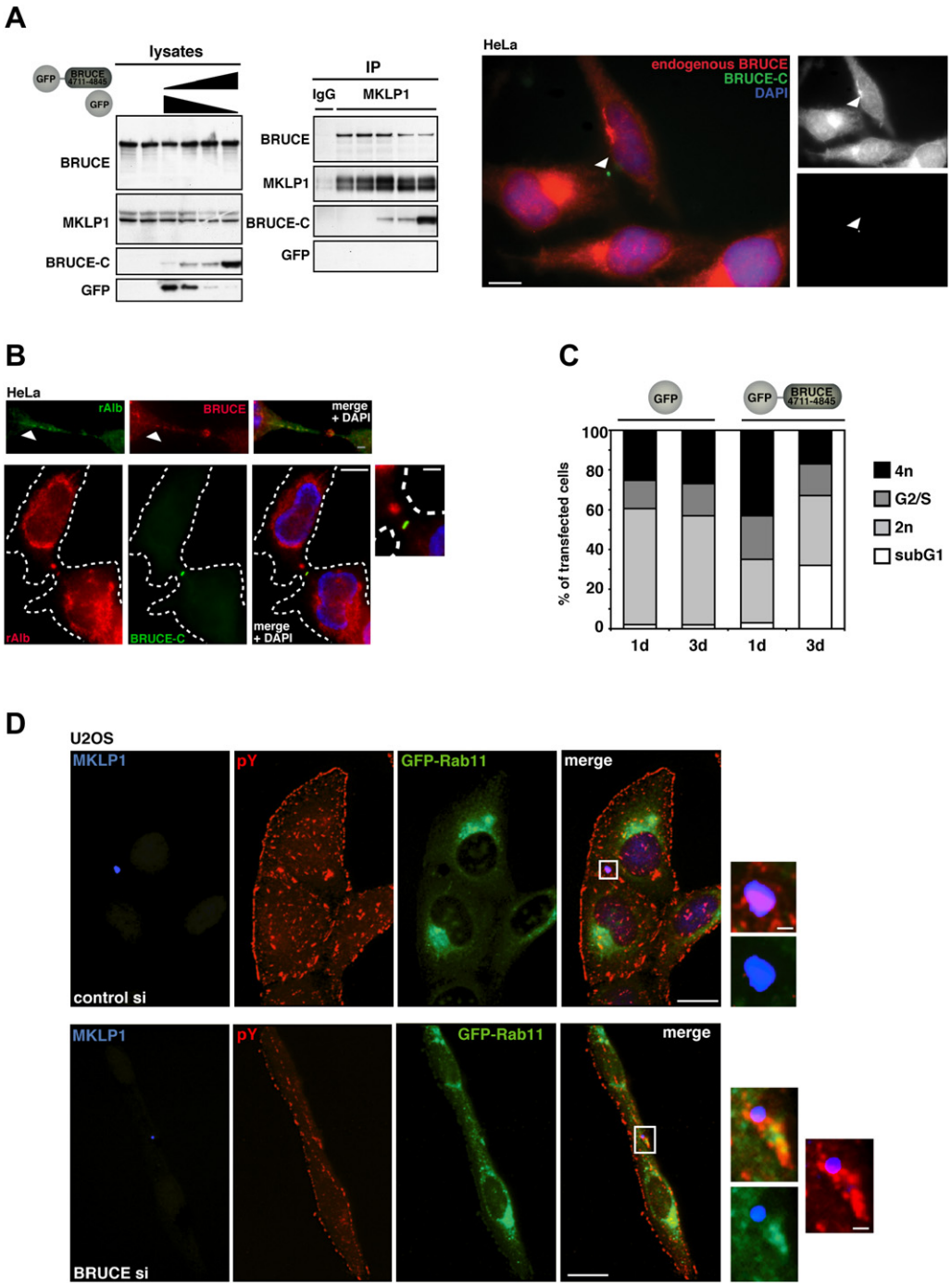

Figure 4. BRUCE Functions in Membrane Delivery to the Midbody Ring

(A) Exogenously expressed BRUCE fragment containing the midbody ring-targeting domain competes with endogenous BRUCE for midbody ring binding via MKLP1. HEK293T cells were transfected with increasing amounts of GFP-BRUCE-C and decreasing amounts of GFP. Lysates (inputs are shown in the left panel) were subjected to immunoprecipitation (IP) with rabbit IgGs (control) or anti-MKLP-1 antibodies, and precipitated proteins were analyzed by western blots. Competition on the single-cell level is shown for HeLa cells by immunofluorescence (endogenous, full-length BRUCE, red; GFP-BRUCE-C, green; DNA stained with DAPI). The arrowhead marks the midbody ring. The scale bar represents 10 μm.

(B) Expression of BRUCE-C leads to secretory vesicle accumulation in close vicinity to the midbody ring. Top: enlarged midbody region of a HeLa cell stably expressing albumin (green; cells as in Figure 1E) and stained for endogenous BRUCE (red). Bottom: similar to above, but cells were transfected with GFP-BRUCE-C (green), fixed, and stained. A representative cell in cytokinesis is shown with the enlarged midbody region on the right. The scale bar represents 10 μm; scale bar of clippings represents 2 μm.

crucial for the presence of tyrosine kinases at the midbody as well. Both functions might be linked, however, as sorting of Src and ERK to the midbody seems to depend on Rab11 (Kasahara et al., 2007).

Midbodies and Midbody Rings Are Platforms for Ubiquitylation

Since BRUCE possesses ubiquitin ligase activity (Bartke et al., 2004; Hauser et al., 1998), we next asked whether the midbody ring might be a site of ubiquitylation. Contrary to the current belief (Pines and Lindon, 2005), we could observe a dramatic relocalization of ubiquitin during cytokinesis (Figure 5A and Movie S6) using a cell line that stably expresses a GFP-ubiquitin fusion protein that can form conjugates in vivo (Dantuma et al., 2006). Parallel to the formation of the midbody ring in late telophase, ubiquitin is first concentrated on midzone microtubules but not on the midbody ring. However, once the microtubules start to constrict to form the midbody, the bulk of ubiquitin vanishes rapidly from this area, but after a brief period of absence, reappears concentrated on the midbody ring.

Motivated by this so far unrecognized behavior of ubiquitin, we performed fluorescent recovery after photobleaching (FRAP) experiments with U2OS cells to address the dynamics of this unusual process. In contrast to normal ubiquitin pools in the same cell in the cytosol or on midbody microtubules, ubiquitin on the midbody ring during cytokinesis showed a vastly reduced recovery (Figure 5B, left panels). Moreover, also midbody rings that had already been taken up by one daughter after completed cytokinesis showed virtually no recovery (Figure 5B, right panels). This suggests that ubiquitin has a high turnover at the midbody through active ubiquitylation and deubiquitylation of proteins near the midbody microtubules. In striking contrast, ubiquitin on midbody ring components, once this modification had occurred, can barely be replaced, suggesting that the local ubiquitylation activity is turned off at this stage. Notably, BRUCE and ubiquitin are closely associated during cell division and colocalize at the midbody ring during late cytokinesis (Figure 5C), suggesting that at least some of the detected ubiquitylation may be mediated by BRUCE (see Discussion).

Relocalization of ubiquitin during cytokinesis was similar in HeLa cells (Figure 5D, top panel), and cells of both cell lines often harbored besides the midbody ring engaged in cytokinesis one or rarely two additional rings, which most likely represent remnants from previous divisions. Intriguingly, only the putative active midbody ring in the intercellular bridge was decorated with ubiquitin whereas the midbody ring remnants had apparently gradually lost the modification (Figure 5D, lower panel). This finding is in agreement with our FRAP data (Figure 5B), showing that ubiquitylation of the midbody ring occurs only once during cytokinesis, before the onset of abscission.

BRUCE is known to homooligomerize (Bartke et al., 2004) and seems to associate in part with the deubiquitylating enzyme UBPY (alias USP8) and the RING-finger ubiquitin ligase Nrdp1 (alias FLRF) (Wu et al., 2004). We therefore asked whether deubiquitylating activity might influence midbody localized ubiquitin as well. By using a catalytically inactive form of UBPY ($UBPY^{C>A}$) that serves as a substrate trap (Alwan and van Leeuwen, 2007), we observed that it bound both BRUCE and MKLP1 (Figure 5E). Intriguingly, also $UBPY^{C>A}$ localizes to the midbody ring (Figure S7), suggesting that ubiquitylation and deubiquitylation activities may perhaps work as a switchboard at this site. BRUCE itself is ubiquitylated at its C-terminal domain (data not shown) and this modification is enhanced upon Nrdp1 overexpression, and conversely lost when UBPY is overexpressed (Figure 5F). Using a denaturing purification protocol that preserves ubiquitin modifications we also found that MKLP1 is modified by mono-/oligo- but not poly-ubiquitylation, and that this modification is strongly reduced if UBPY was overexpressed (Figure 5G). While BRUCE overexpression triggered no increase in MKLP1 ubiquitylation (data not shown), overexpression of the dominant-negative $UBPY^{C>A}$ version induced this modification (Figure 5G), suggesting that another ubiquitin ligase acts on MKLP1, or that deubiquitylation rather than ubiquitylation activity might be limiting and important for regulation. In fact, more than 30% of HeLa cells transfected with $UBPY^{C>A}$ exhibited cytokinesis defects linked with binucleation and mislocalized MKLP1 from its normal interphase localization in the nucleus to the cytoplasm (Figure 5H). This phenotype is reminiscent of an MKLP1 knockdown, suggesting that perhaps UBPY-mediated deubiquitylation of MKLP1 may trigger nuclear import, which appears to be needed for normal cytokinesis (Liu and Erikson, 2007).

BRUCE Is Crucial for Midbody Integrity, Midbody Ring Formation, and Midbody-Localized Ubiquitin

Prompted by the striking physical and functional connection of BRUCE with the midbody and the midbody ring, we next asked whether BRUCE itself is crucial for the organization of these structures. Indeed, we observed significantly reduced MKLP1-positive midbody material in BRUCE-depleted cells (Figure 6A and see also Figure 4D). Additionally, we detected a gradual effect on the midbody structure upon continued BRUCE depletion. Depletion for no more than three days led to moderately misorganized midbodies with unfocused Aurora B staining (Figure 6B, top panel) and unconstricted microtubules (Figure 6C, top panel). However, BRUCE depletion for five days resulted in syncytia-like cells, which contained severely malformed midbodies, as visualized by Aurora B or MKLP1 staining (Figures 6B and 6C lower panels). In the end, most cells failed to form their midbody rings altogether and no remnants persisted, and those few cells that retained their midbody rings (less than 10%), had hitherto not adopted a syncytia-like appearance (Figures 6D and 6E). We also explored whether BRUCE depletion affects the distribution of ubiquitin at the midbody.

(C) Prolonged expression of BRUCE-C leads to binucleation and apoptosis. HeLa cells were transfected either with GFP or GFP-BRUCE-C, stained with propidium iodide 1 and 3 days after transfection and DNA content was analyzed by flow cytometry gating for transfected cells.

(D) Recycling endosomes accumulate close to the midbody ring in BRUCE-depleted cells. U2OS cells stably expressing GFP-Rab11 (green) were treated with siRNAs as in Figure 1C fixed and stained for MKLP1 (blue) and phosphotyrosine (pY, red) with enlarged views of the midbody ring on the right. The scale bar represents 15 μm; scale bar of clippings represents 1 μm.

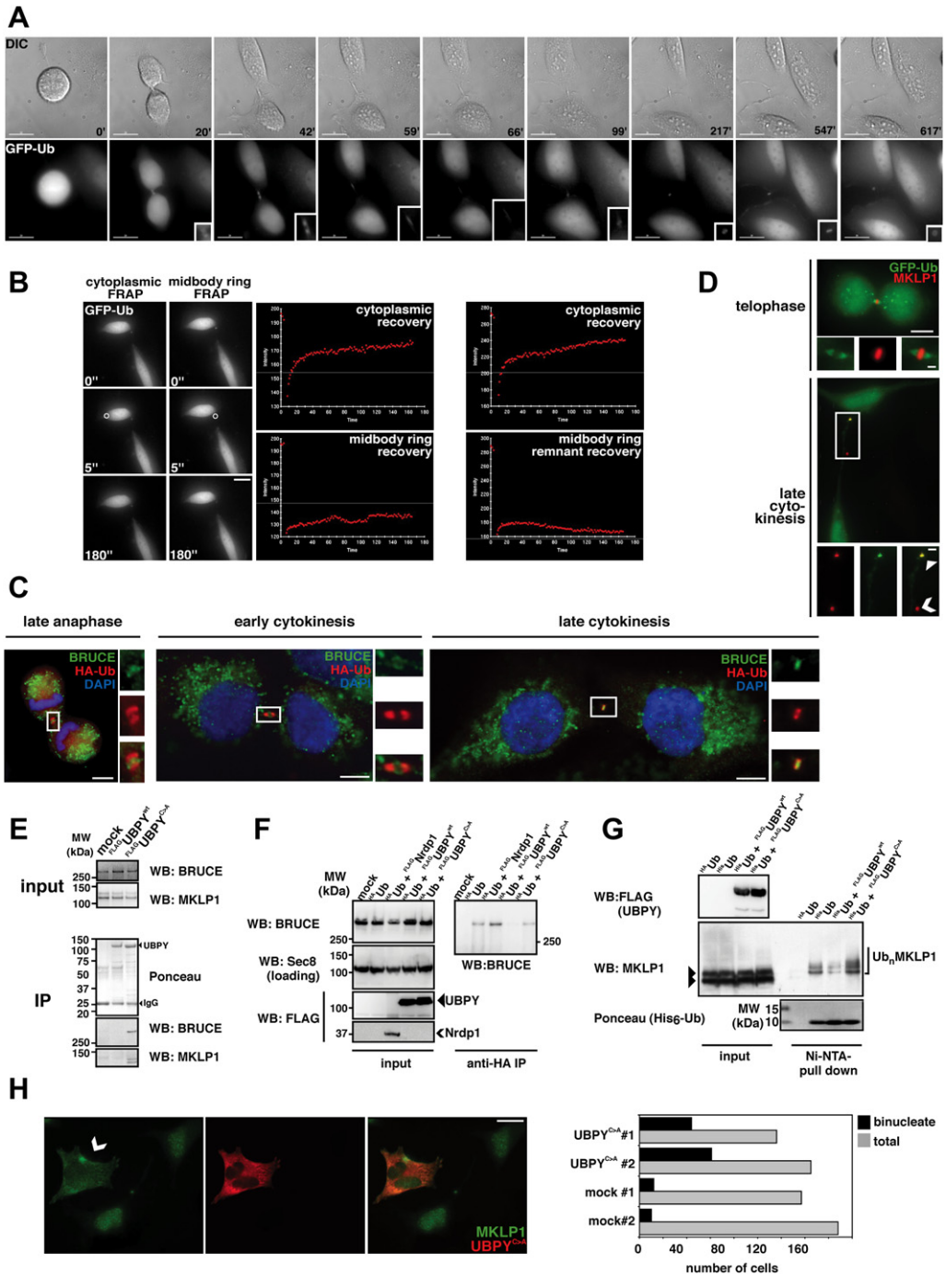

Figure 5. Ubiquitylation on the Midbody Ring

(A) Ubiquitin (Ub) is concentrated on midbody microtubules and reappears on the midbody ring after midbody constriction. U2OS cells stably expressing GFP-tagged ubiquitin (GFP-Ub) were analyzed by time-lapse video microscopy. A representative cell is shown with the movie stills (of Movie S6) starting in prometaphase and ending shortly before abscission. The scale bar represents 20 μm.

(B) Ubiquitin at the midbody ring shows almost no exchange with the cellular ubiquitin pool. U2OS cells as in (A) were used for FRAP experiments. Left: a cell with ubiquitin on the midbody ring was bleached in the cytoplasm or at the midbody ring. The recovery of the fluorescence signal is shown on the right. Right: a cell with a midbody ring remnant in the plane of the plasma membrane was either bleached in the cytoplasm or at the midbody ring remnant. Only fluorescence recovery curves are shown. The scale bar represents 20 μm.

(C) Association of BRUCE with ubiquitin at the midbody ring. U2OS cells were transiently transfected with HA-epitope tagged ubiquitin (HA-Ub, red). Cells were fixed and stained with anti-BRUCE (green) and DAPI (blue). The scale bar represents 10 μm. The framed areas are shown enlarged.

Indeed, in contrast to cells treated with a control siRNA (Figure 6F, upper panel, and Movie S7), cells depleted for BRUCE neither showed a distinct ubiquitin staining at midbody microtubules nor at the surviving midbody rings (Figure 6F, lower panel and Movie S8). These results suggest that BRUCE is not only crucial for normal vesicle targeting to the site of abscission, but also for the integrity of the midbody and the midbody ring, and its striking ubiquitin modification.

DISCUSSION

The two most perceptible processes of cytokinesis of animal cells, the contraction of the cleavage furrow and the physical separation of the daughter cells by abscission, are now recognized as two functionally and mechanistically different steps. Central to abscission is the midbody, which is the target site for membrane delivery and membrane fusion, the driving forces of abscission. In particular the unique midbody ring, located at the midpoint of the intercellular bridge, has drawn attention recently as it seems to be the key structural element that guides the events that lead to abscission. This rigid and unvaryingly sized structure, originally described by Flemming as "Zentralkörper" (Flemming, 1891), thus sometimes called "Flemming body," materializes in telophase and survives in one daughter cell often until the next division.

In this report, we identified BRUCE as a constant companion of the midbody ring and found that its normal assembly requires BRUCE. Moreover, we found that BRUCE is crucial for membrane delivery to the cleavage site and for normal abscission.

Membrane Dynamics

Recent studies have emphasized the decisive role of plasma membrane reorganization during abscission (Albertson et al., 2005). This process requires vesicle-mediated transport and the incorporation of additional membrane to the site of abscission by hetero- and homotypic membrane fusion events. As shown previously for (Rab11-positive) recycling endosomes, our data suggest that also (Rab8-positive) tubular endosomes significantly contribute to membrane dynamics during cytokinesis (Figure 2G). Previous studies suggested that membrane delivery to the midbody occurs largely asymmetric, i.e., only from one of the prospective daughter cells. However, when we monitored cargo delivery or endosomal sorting in unperturbed U2OS cells (which may require more membrane reshaping than HeLa cells) we failed to detect any marked asymmetry of membrane delivery to the intercellular bridge. However, upon interfering with BRUCE function by either BRUCE depletion or overexpression of the dominant-negative BRUCE-C variant (that does not bind the exocyst or Rab8/11 GTPases) cells strikingly accumulated large vesicular structures close to only one side of the midbody ring. Thus abscission has indeed an intrinsic asymmetric contribution, and BRUCE appears to be crucial for normal vesicle targeting. In fact, BRUCE seems well equipped to function as a midbody targeting factor for vesicles as it binds via its N-terminal region to the exocyst and endosomal components and via its C-terminal targeting domain to the midbody ring.

BRUCE and the Midbody Ring

Our studies suggest an intimate relationship between BRUCE and the midbody ring. In interphase cells, BRUCE localizes to the TGN and endosomes, but during cytokinesis, a fraction moves to the midzone where it specifically arrives at the midbody ring. Notably, BRUCE associates with the midbody ring concomitantly with its appearance in telophase, travels after completed abscission with the midbody ring into one daughter cell, and remains bound to the discarded midbody ring all along until a new ring is formed during the next cell division (Figure 1A and 5D). Most importantly, BRUCE depletion prevents normal midbody ring formation, suggesting that its activity may indeed be required for proper ring assembly.

The midbody ring appears to mediate at least two functions. First, it seems to play a role as the docking site for vesicles, which supply the plasma membrane with new material at the site of abscission. As detailed above, BRUCE is crucial for this process. Second, perhaps similar to the septin ring of yeast (Faty et al., 2002), the midbody ring seems to function as a diffusion barrier. Previous studies indicated that the midbody ring prevents plasma membrane diffusion across the intercellular bridge (Schmidt and Nichols, 2004). Our FRAP experiments extend these findings as they indicate that also Rab8-endosomes are unable to transverse the ring from one prospective daughter cell to the other. Therefore, the midbody ring may represent the first physical barrier that disunites the daughter cells. As observed before (Gromley et al., 2005), perhaps fueled by asymmetric membrane delivery, the midbody ring is pushed to one daughter cell where it is eventually dissolved. As BRUCE follows the same path, even the final fate of the midbody ring appears to be allied with BRUCE.

(D) Ubiquitin is lost from midbody ring remnants when cells acquired an active midbody ring. HeLa cells were transfected with GFP-Ub (green). Top panels show fixed cells in telophase with anti-MKLP1 staining (red). Lower panels show a cell in late cytokinesis with a putative active midbody ring and a remnant, both lying in the intercellular bridge. Note that the midbody ring remnant (open arrowhead) is not labeled with ubiquitin. The scale bar represents 10 μm and 2 μm (for enlarged areas).

(E) Both BRUCE and MKLP1 interact with UBPY. HEK293T cells were transfected with empty vector or FLAG-epitope tagged UBPY and its catalytically inactive mutant (C > A). Cells were lysed and proteins were immunoprecipitated (IP) with anti-FLAG and analyzed by western blots (WB).

(F) BRUCE is deubiquitylated by UBPY. HEK293T cells were transfected with empty vector and HA-tagged ubiquitin (^{HA}Ub) in combination with FLAG-tagged UBPY wild-type and mutant proteins (^{FLAG}UBPY, ^{FLAG}UBPY$^{(C > A)}$) and FLAG-tagged Nrdp1 (^{FLAG}Nrdp1). Lysates were prepared and subjected to anti-HA immunoprecipitation, and both lysates (Input) and immunoprecipitated material (IP) were analyzed by western blots (WB).

(G) MKLP1 is ubiquitylated in cells and is deubiquitylated by UBPY. HEK293T cells were transfected with ^{HA}Ub or His_6-epitope tagged ubiquitin (His6-Ub) in combination with ^{FLAG}UBPY and ^{FLAG}UBPY$^{(C > A)}$. Lysates were subjected to denaturing Ni-NTA pull down and analyzed by western blots (WB).

(H) Overexpression of catalytically inactive UBPY leads to MKLP1 mislocalization and binucleation of cells. HeLa cells were transfected with empty vector (mock) or ^{FLAG}UBPY$^{(C > A)}$. Left: cells were fixed and stained with anti-MKLP1 (green) and anti-FLAG (red) antibodies. The open arrowhead marks a transfected cell. The scale bar represents 15 μm. Right: quantification of binucleate versus total cells (two independent experiments labeled #1 and #2, respectively).

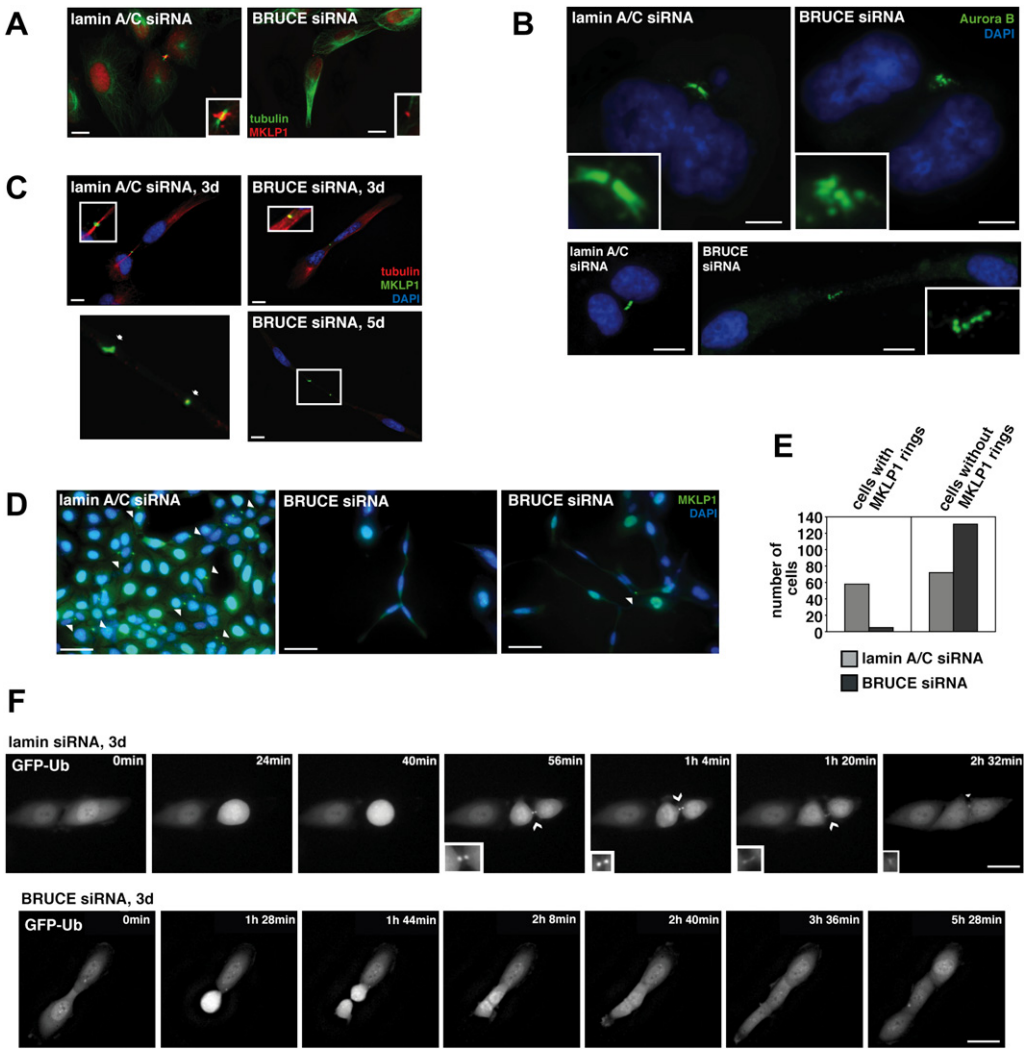

Figure 6. BRUCE Depletion Causes Structural Aberrations of the Midbody and Ubiquitin Mislocalization

(A) Reduced midbody material after BRUCE depletion as revealed by MKLP1 staining. U2OS cells were treated with siRNAs targeting lamin A/C or BRUCE as in Figure 1B. After 3 days, cells were fixed and stained with anti-α-tubulin (green) or anti-MKLP1 (red) antibodies. The scale bar represents 15 μm.

(B) Misorganization and rupture of the midbody after BRUCE depletion. U2OS cells were treated with siRNAs as in (A). Cells were fixed after 3 days and stained with anti-Aurora B (green) antibodies and DAPI. Top: BRUCE-depleted cells that have hitherto not acquired an elongated appearance exhibit aberrant midbodies (shown enlarged in insets) (scale bar ≅ 15 μm). Bottom: BRUCE-depleted cells that resemble syncytia contain fragmented Aurora B-positive material. The scale bar represents 20 μm.

(C) Midbody ring rupture in BRUCE-depleted cells as indicated by MKLP1 localization. The experiment was performed as in (B), and cells were fixed either after 3 or 5 days, stained for MKLP1 (green), α-tubulin (red), or DNA with DAPI (blue). After 5 days of siRNA treatment, syncytial cells arose with aberrant midbody rings (arrowheads). The scale bar represents 10 μm.

(D) Growth retardation and loss of midbody rings after BRUCE depletion. Experiment was performed as in (B); cells were fixed after 5 days and stained with anti-MKLP1 (green) and DAPI. Arrowheads point to MKLP1-stained midbody rings.

(E) Quantification of (D). The scale bar represents 30 μm.

(F) Concentration of ubiquitin at the midbody is lost in BRUCE-depleted cells. U2OS cells stably expressing GFP-Ub (see Figure 5A) were analyzed by time-lapse video microscopy. Stills are from Movie S8. The scale bar represents 15 μm.

BRUCE Is Crucial for Faithful Cell Division

A further consequence of BRUCE depletion is the appearance of syncytial cells and sometimes a reunion followed by mitosis of apparently incompletely separated cells (Movie S8). Syncytial cells also form upon centriolin depletion (Gromley et al., 2005), suggesting that perhaps BRUCE and centriolin, a protein of unknown biochemical activity, fulfill related or coupled functions in cytokinesis. Although *BRUCE*$^{-/-}$ embryos survive embryogenesis and usually die only shortly before birth, we also noticed a delay in cell cycle progression of mouse embryonic fibroblasts (MEFs) isolated from *BRUCE*$^{-/-}$ embryos (Lotz et al., 2004) and aberrant divisions characterized by multipolar spindles

and tripolar cytokinesis in spontaneously immortalized *BRUCE*$^{-/-}$ MEFs (unpublished data). This suggests that cells may adapt to a loss of BRUCE activity, however, with the predisposition (perhaps depending on the cell type) of erroneous cell divisions. Intriguingly, BRUCE depletion in HeLa cells induces apoptosis precisely at the time of attempted abscission (Figure 3G). Since we also observed apoptosis upon prolonged expression of BRUCE-C, which displaces endogenous BRUCE from the midbody ring (Figure S6D), the apoptotic cue seems to materialize specifically when BRUCE is absent from this structure. As BRUCE possesses antiapoptotic activity and binds caspases (Bartke et al., 2004), it seems reasonable to assume that the observed cell death may be a direct consequence of the lack of BRUCE activity at the midbody ring. Notably, BRUCE also physically associates with survivin, which also localizes to the midbody, and BRUCE is able to ubiquitylate this BIRP in vitro (Figure S3 and data not shown). It thus seems attractive to speculate that perhaps BRUCE and survivin normally collaborate, and that BRUCE depletion alters the local antiapoptotic activity with possible consequences for caspase activation (O'Connor et al., 2000).

Ubiquitin Dynamics During Cytokinesis

In this study we discovered a unique and highly dynamic localization pattern of ubiquitin conjugates at the midbody during cytokinesis. At the time when the actomyosin ring has maximally constricted, ubiquitin is unusually concentrated in two large foci symmetrically localized near both plus-end tips of midbody microtubules (Figure 5A). This almost compartmentalized compaction may be indicative of a concentration of ubiquitylation/deubiquitylation activities and extensive protein turnover at these sites. It seems attractive to speculate that substrates may include mitotic regulators or components of the vesicular sorting machinery. Strikingly, in the succeeding 15–30 min interval, ubiquitin disappeared from the midbody to undetectable levels, but reappears firmly associated with the midbody ring. Ubiquitylation of the midbody ring seems to occur only once during cytokinesis and appears to be stable until abscission is completed, as indicated by our photobleaching experiments. Furthermore, this modification appears to be largely mono-ubiquitin as indicated by the fact that conjugates of GFP-ubiquitin, in which the ubiquitin domain lacks lysine residues (GFP-ubiquitin-K_0), enriched normally at the midbody ring but not at the midbody flanks (Figure S8). Together these data suggest that midbody ring (mono) ubiquitylation may serve structural or regulatory functions rather than promoting proteasomal proteolysis.

As BRUCE is tightly connected to the midbody ring and required for its integrity, it seems reasonable to speculate that its ubiquitin ligase activity may contribute to these ubiquitylation events. However, ubiquitylation is also required for protein sorting via the ESCRT pathway, which plays a role in cytokinesis (Carlton and Martin-Serrano, 2007; Morita et al., 2007). In fact, the BRUCE-associated deubiquitylating enzyme UBPY harbors a so-called MIT-domain that mediates association with proteins of the ESCRTIII complex (Row et al., 2007). Moreover, disassembly of the ESCRTIII complex is thought to be catalyzed by Vps4, an AAA-ATPase that seems to play a crucial role at late stages of cytokinesis (Morita et al., 2007). Whatever the crucial ubiquitylation targets are, the unique combination of multiple activities in a single large protein suggests that BRUCE may coordinate cytokinesis events perhaps in a stepwise fashion. Strikingly, after completed cytokinesis, midbody ring-localized BRUCE is discarded into only one of the two daughter cells as well, possibly representing the final manifestation of the apparent built-in asymmetry of cytokinesis.

EXPERIMENTAL PROCEDURES

Plasmids and siRNA

Cloning of *BRUCE* and engineering of mutants and truncations was described previously (Bartke et al., 2004; Hauser et al., 1998). *BRUCE* cDNAs were expressed from pCI (Promega) or *pCMV2* vectors (Stratagene) as was FLAG-tagged cDNA of luciferase. GFP-BRUCE-C (amino acids 4711–4845) was cloned into pEGFP-CI (Clontech) using an internal *Xho*I site (bp 14135). Nrdp1 was amplified from HeLa mRNA using primers that generate an N-terminal FLAG-epitope tag and cloned into pCI. GFP-Rab8 was a gift of J. Peränen, GFP-Rab11 was obtained from M. Zerial, GFP-ubiquitin and GFP-ubiquitin-K_0 were gifts of N. Dantuma, HA-epitope-tagged rat albumin was a gift of D. LeBel and FLAG-epitope-tagged UBPY and its mutant were from H. Alwan.

Antibodies

A detailed list of antibodies can be found in the Supplemental Experimental Procedures.

Cell Culture and Transfections

U2OS, HeLa, and HEK293T cells were maintained at 37°C, 7.5% CO_2 in DMEM (SIGMA) supplemented with 10% FCS. Transient transfections were performed in 10 cm dishes (293T) or 6-well plates (HeLa) using calcium phosphate or lipofection protocols, as described previously (Bartke et al., 2004). U2OS cells were transfected by electroporation using AMAXA Nucleofector kit according to the manufacturer's recommendations. siRNA duplexes were obtained from MWG and were transfected at a final concentration of 100 nM. siRNA sequences are listed in supplemental material and methods. HeLa cells stably expressing HA-rAlb and U2OS cells stably expressing GFP-Rab8, GFP-Rab11 and GFP-ubiquitin were generated by selecting cells with 750 μg/ml G418 (SIGMA) after lipofection for 3–4 weeks.

Flow Cytometry

DNA histograms were obtained by flow cytometry of PI-stained ethanol-fixed cells using standard protocols, a FACSCalibur system and CELLQuest analysis software (Becton Dickinson).

Coimmunoprecipitations and Immunoblot Analysis

Immunoprecipitations, purification of FLAG-fusion proteins, and immunoblot analysis were carried out as described previously (Bartke et al., 2004).

Denaturating Ni-NTA Pull-Down

Transiently transfected HEK293T cells were harvested and pellets were washed once in PBS. Cells were lysed in 6 M guanidine-HCl, 0.1M NaH_2PO_4, 0.1% Tween-20, 0.01M Tris (pH 8.0). Lysates were briefly sonicated to shear DNA. An aliquot of the lysate was kept for TCA precipitation. The remaining lysate was incubated with Ni-NTA agarose beads (QIAGEN) for 3–5 hr. Beads were washed 5 times with 8 M urea, 0.1 M NaH_2PO_4, 0,1% Tween-20, 0.01M Tris (pH 8.0), and twice with PBS, 0.01% Tween-20. TCA precipitates and beads were boiled in loading buffer and subjected to SDS-PAGE and immunoblotting.

Immunofluorescence and Live-Cell Microscopy

Immunofluorescence was performed according to standard protocols after fixation in methanol (−20°C, 3 min). Images were acquired on a Leica DM RXA microscope equipped with a Hamatsu ORCA-ER camera. Image acquisition and deconvolution of images was carried out using Openlab software. For fluorescence live-cell video microscopy and photobleaching experiments,

U2OS cells were cultured in 35 mm μ-dishes with No. 1.5 bottom (Integrated BioDiagnostics). Confocal laser scanning microscopy was performed with a Leica TCS SP2 confocal scanning microscope. Live-cell video microscopy and FRAP were carried out on an AppliedPrecision DeltaVision RT system equipped with a quantifiable laser module. After photobleaching of the GFP fluorescence with 25%–50% laser intensity, images were obtained every second during a time frame of 3 min. Fluorescence intensities were measured and quantified and images processed using SoftWorx software (AppliedPrecision). For live-cell video microscopy, DIC or phase and fluorescence images were taken every 1–5 min over a time frame of 10–48 hr.

SUPPLEMENTAL DATA

Supplemental Data include Supplemental Experimental Procedures, Supplemental References, eight figures, and eight movies and can be found with this article online at http://www.cell.com/cgi/content/full/132/5/832/DC1/.

ACKNOWLEDGMENTS

We thank U. Cramer for technical assistance; T. Bartke, S. Mishra, and O. Stemmann for advice and comments on the manuscript; H. Alwan, N. Dantuma, D. LeBel, J. Peränen, and M. Zerial for materials. This work is supported (to S.J.) by the Max Planck Society, Munich Center of Integrated Protein Science, Rubicon EU Network of Excellence, and Fonds der Chemischen Industrie.

Received: October 9, 2007
Revised: December 17, 2007
Accepted: January 8, 2008
Published: March 6, 2008

REFERENCES

Albertson, R., Riggs, B., and Sullivan, W. (2005). Membrane traffic: a driving force in cytokinesis. Trends Cell Biol. *15*, 92–101.

Alwan, H.A., and van Leeuwen, J.E. (2007). UBPY-mediated epidermal growth factor receptor (EGFR) de-ubiquitination promotes EGFR degradation. J. Biol. Chem. *282*, 1658–1669.

Bartke, T., Pohl, C., Pyrowolakis, G., and Jentsch, S. (2004). Dual Role of BRUCE as an Antiapoptotic IAP and a Chimeric E2/E3 Ubiquitin Ligase. Mol. Cell *14*, 801–811.

Carlton, J.G., and Martin-Serrano, J. (2007). Parallels between cytokinesis and retroviral budding: a role for the ESCRT machinery. Science *316*, 1908–1912.

Dantuma, N.P., Groothuis, T.A., Salomons, F.A., and Neefjes, J. (2006). A dynamic ubiquitin equilibrium couples proteasomal activity to chromatin remodeling. J. Cell Biol. *173*, 19–26.

Eggert, U.S., Mitchison, T.J., and Field, C.M. (2006). Animal cytokinesis: from parts list to mechanisms. Annu. Rev. Biochem. *75*, 543–566.

Faty, M., Fink, M., and Barral, Y. (2002). Septins: a ring to part mother and daughter. Curr. Genet. *41*, 123–131.

Fielding, A.B., Schonteich, E., Matheson, J., Wilson, G., Yu, X., Hickson, G.R., Srivastava, S., Baldwin, S.A., Prekeris, R., and Gould, G.W. (2005). Rab11–FIP3 and FIP4 interact with Arf6 and the exocyst to control membrane traffic in cytokinesis. EMBO J. *24*, 3389–3399.

Flemming, W. (1891). Neue Beiträgezur Kenntnis der Zelle. Arc. Mikr. Anat. *37*, 685–751.

Grenfell, S.J., Trausch-Azar, J.S., Handley-Gearhart, P.M., Ciechanover, A., and Schwartz, A.L. (1994). Nuclear localization of the ubiquitin-activating enzyme, E1, is cell-cycle-dependent. Biochem. J. *300*, 701–708.

Gromley, A., Jurczyk, A., Sillibourne, J., Halilovic, E., Mogensen, M., Groisman, I., Blomberg, M., and Doxsey, S. (2003). A novel human protein of the maternal centriole is required for the final stages of cytokinesis and entry into S phase. J. Cell Biol. *161*, 535–545.

Gromley, A., Yeaman, C., Rosa, J., Redick, S., Chen, C.T., Mirabelle, S., Guha, M., Sillibourne, J., and Doxsey, S.J. (2005). Centriolin anchoring of exocyst and SNARE complexes at the midbody is required for secretory-vesicle-mediated abscission. Cell *123*, 75–87.

Hao, Y., Sekine, K., Kawabata, A., Nakamura, H., Ishioka, T., Ohata, H., Katayama, R., Hashimoto, C., Zhang, X., Noda, T., et al. (2004). Apollon ubiquitinates SMAC and caspase-9, and has an essential cytoprotection function. Nat. Cell Biol. *6*, 849–860.

Hauser, H.P., Bardroff, M., Pyrowolakis, G., and Jentsch, S. (1998). A giant ubiquitin-conjugating enzyme related to IAP apoptosis inhibitors. J. Cell Biol. *141*, 1415–1422.

Kasahara, K., Nakayama, Y., Nakazato, Y., Ikeda, K., Kuga, T., and Yamaguchi, N. (2007). Src signaling regulates completion of abscission in cytokinesis through ERK/MAPK activation at the midbody. J. Biol. Chem. *282*, 5327–5339.

Kuriyama, R., Gustus, C., Terada, Y., Uetake, Y., and Matuliene, J. (2002). CHO1, a mammalian kinesin-like protein, interacts with F-actin and is involved in the terminal phase of cytokinesis. J. Cell Biol. *156*, 783–790.

Li, F., Ackermann, E.J., Bennett, C.F., Rothermel, A.L., Plescia, J., Tognin, S., Villa, A., Marchisio, P.C., and Altieri, D.C. (1999). Pleiotropic cell-division defects and apoptosis induced by interference with survivin function. Nat. Cell Biol. *1*, 461–466.

Lindon, C., and Pines, J. (2004). Ordered proteolysis in anaphase inactivates Plk1 to contribute to proper mitotic exit in human cells. J. Cell Biol. *164*, 233–241.

Liu, X., and Erikson, R.L. (2007). The nuclear localization signal of mitotic kinesin-like protein Mklp-1: effect on Mklp-1 function during cytokinesis. Biochem. Biophys. Res. Commun. *353*, 960–964.

Lotz, K., Pyrowolakis, G., and Jentsch, S. (2004). BRUCE, a giant E2/E3 ubiquitin ligase and inhibitor of apoptosis protein of the trans-Golgi network, is required for normal placenta development and mouse survival. Mol. Cell. Biol. *24*, 9339–9350.

Matuliene, J., and Kuriyama, R. (2002). Kinesin-like protein CHO1 is required for the formation of midbody matrix and the completion of cytokinesis in mammalian cells. Mol. Biol. Cell *13*, 1832–1845.

Morita, E., Sandrin, V., Chung, H.Y., Morham, S.G., Gygi, S.P., Rodesch, C.K., and Sundquist, W.I. (2007). Human ESCRT and ALIX proteins interact with proteins of the midbody and function in cytokinesis. EMBO J. *26*, 4215–4227.

O'Connor, D.S., Grossman, D., Plescia, J., Li, F., Zhang, H., Villa, A., Tognin, S., Marchisio, P.C., and Altieri, D.C. (2000). Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. Proc. Natl. Acad. Sci. USA *97*, 13103–13107.

Pines, J., and Lindon, C. (2005). Proteolysis: anytime, any place, anywhere? Nat. Cell Biol. *7*, 731–735.

Potapova, T.A., Daum, J.R., Pittman, B.D., Hudson, J.R., Jones, T.N., Satinover, D.L., Stukenberg, P.T., and Gorbsky, G.J. (2006). The reversibility of mitotic exit in vertebrate cells. Nature *440*, 954–958.

Ren, J., Shi, M., Liu, R., Yang, Q.H., Johnson, T., Skarnes, W.C., and Du, C. (2005). The Birc6 (Bruce) gene regulates p53 and the mitochondrial pathway of apoptosis and is essential for mouse embryonic development. Proc. Natl. Acad. Sci. USA *102*, 565–570.

Row, P.E., Liu, H., Hayes, S., Welchman, R., Charalabous, P., Hofmann, K., Clague, M.J., Sanderson, C.M., and Urbe, S. (2007). The MIT domain of UBPY constitutes a CHMP binding and endosomal localisation signal required for efficient EGF receptor degradation. J. Biol. Chem. *282*, 30929–30937.

Samuel, T., Okada, K., Hyer, M., Welsh, K., Zapata, J.M., and Reed, J.C. (2005). cIAP1 Localizes to the nuclear compartment and modulates the cell cycle. Cancer Res. *65*, 210–218.

Schmidt, K., and Nichols, B.J. (2004). A barrier to lateral diffusion in the cleavage furrow of dividing mammalian cells. Curr. Biol. *14*, 1002–1006.

Straight, A.F., Cheung, A., Limouze, J., Chen, I., Westwood, N.J., Sellers, J.R., and Mitchison, T.J. (2003). Dissecting temporal and spatial control of cytokinesis with a myosin II Inhibitor. Science *299*, 1743–1747.

Sumara, I., Quadroni, M., Frei, C., Olma, M.H., Sumara, G., Ricci, R., and Peter, M. (2007). A Cul3-based E3 ligase removes Aurora B from mitotic chromosomes, regulating mitotic progression and completion of cytokinesis in human cells. Dev. Cell *12*, 887–900.

Vega, I.E., and Hsu, S.C. (2001). The exocyst complex associates with microtubules to mediate vesicle targeting and neurite outgrowth. J. Neurosci. *21*, 3839–3848.

Verhagen, A.M., Coulson, E.J., and Vaux, D.L. (2001). Inhibitor of apoptosis proteins and their relatives: IAPs and other BIRPs. Genome Biol. *2*, reviews3009.1–reviews3009.

Wojcik, C., Paweletz, N., and Schroeter, D. (1995). Localization of proteasomal antigens during different phases of the cell cycle in HeLa cells. Eur. J. Cell Biol. *68*, 191–198.

Wu, X., Yen, L., Irwin, L., Sweeney, C., and Carraway, K.L., 3rd. (2004). Stabilization of the E3 ubiquitin ligase Nrdp1 by the deubiquitinating enzyme USP8. Mol. Cell. Biol. *24*, 7748–7757.

Yu, X., Prekeris, R., and Gould, G.W. (2007). Role of endosomal Rab GTPases in cytokinesis. Eur. J. Cell Biol. *86*, 25–35.