The unfolded protein response (UPR) pathway helps cells cope with endoplasmic reticulum (ER) stress by activating genes that increase the ER’s functional capabilities. We have identified a novel role for the UPR pathway in facilitating budding yeast cytokinesis. Although other cell cycle events are unaffected by conditions that disrupt ER function, cytokinesis is sensitive to these conditions. Moreover, efficient cytokinesis requires the UPR pathway even during unstressed growth conditions. UPR-deficient cells are defective in cytokinesis, and cytokinesis mutants activate the UPR. The UPR likely achieves its role in cytokinesis by sensing small changes in ER load and making according changes in ER capacity. We propose that cytokinesis is one of many cellular events that require a subtle increase in ER function and that the UPR pathway has a previously uncharacterized housekeeping role in maintaining ER plasticity during normal cell growth.

Introduction

The ER plays a crucial role in several important aspects of eukaryotic cell physiology. It assists in the folding and maturation of all nascent secretory proteins and initiates their distribution to the broader secretory pathway (Ellgaard et al., 1999). In addition, the ER influences the overall composition of the cellular proteome by mediating the ER-associated degradation (ERAD) pathway, a pathway that destroys misfolded proteins and also responds to specific degradation signals to regulate the levels of certain native proteins (Hampton, 2002). The ER also houses many lipid biosynthetic enzymes, which impact the relative composition and overall abundance of lipids throughout the cell (Daum et al., 1998).

Genes involved in protein folding, protein trafficking, ERAD, and lipid metabolism are all transcriptionally activated by a conserved ER-initiated signal transduction pathway called the unfolded protein response (UPR; Mori, 2000; Travers et al., 2000; Harding et al., 2001; Patil and Walter, 2001; Kaufman, 2002). In budding yeast, the UPR pathway begins with an ER transmembrane protein, Ire1p (Cox et al., 1993; Mori et al., 1993). The N terminus of Ire1p lies in the lumen of the ER, where it senses the ER’s condition. When Ire1p detects a need for increased ER function, it transmits a signal across the ER membrane to activate its own cytosolic kinase and endoribonuclease domains (Cox et al., 1993; Mori et al., 1993; Shamu and Walter, 1996; Sidrauski and Walter, 1997). Activated Ire1p then initiates the unconventional spliceosome-independent splicing of HAC1 mRNA (Cox and Walter, 1996; Sidrauski and Walter, 1997). Only the spliced form of HAC1 mRNA can be translated, making the splicing step a critical point of regulation (Chapman et al., 1998; Ruegsegger et al., 2001). Upon translation, Hac1p localizes to the nucleus, where it acts as a transcription factor to up-regulate a wide array of UPR target genes (Cox and Walter, 1996; Kawahara et al., 1997), thus increasing the ER’s capacity to serve its many functions (Travers et al., 2000).

Northern analysis, which measures the relative abundance of spliced HAC1 mRNA in the cell, is currently the most commonly used method of detecting UPR activation (Cox and Walter, 1996). Using this technique, previous studies have detected UPR activation only during extreme conditions of ER stress. For example, HAC1 mRNA splicing has been detected in cells treated with pharmacological agents that cause widespread protein misfolding (Cox and Walter, 1996; Kawahara et al., 1997) or in cells overexpressing mutant proteins that fold improperly (Spear and Ng, 2003). The inability to detect HAC1 mRNA splicing during normal growth has led to the designation of the UPR pathway as a stress response pathway. However, it is likely that cellular demand for ER function is dynamic even during unstressed growth conditions. This evokes the intriguing possibility that in addition to responding to conditions of extreme stress, the UPR pathway manages the everyday challenges of fluctuating ER demand. This housekeeping function for the UPR has been previously unnoticed, perhaps because it induces a level of Ire1p activity that is too subtle to be detected by conventional HAC1 Northern analysis.
Because progression through the cell cycle requires dramatic molecular and cellular changes, we hypothesized that cell cycle progression requires fluctuations in ER capacity. To isolate a cell cycle event that requires particularly high ER functionality, we used ER stress as a tool to disrupt ER function. We then asked whether any particular cell cycle event was sensitive to this reduction in ER capacity. Most cell cycle events that we examined did not require exceptionally high ER activity, as they occurred normally during ER stress. However, cells experiencing ER stress were specifically defective in cytokinesis, suggesting that elevated ER functionality is required for cells to carry out efficient cytokinesis.

Because cytokinesis required a greater ER capacity than other cell cycle events, we tested the possibility that the UPR plays a role in achieving an increased ER capacity during normal, unstressed cytokinesis. Indeed, we found that UPR-deficient cells were unable to carry out efficient cytokinesis even in the absence of external ER stress. This is the first time the UPR pathway has been shown to function in cells that are growing optimally, expressing no misfolded mutant proteins, exposed to any particular cell cycle event was sensitive to this reduction in ER capacity. Most cell cycle events that we examined did not require exceptionally high ER activity, as they occurred normally during ER stress. However, cells experiencing ER stress were specifically defective in cytokinesis, suggesting that elevated ER functionality is required for cells to carry out efficient cytokinesis.

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Results

HAC1 mRNA splicing occurs during unstressed growth

Because previous HAC1 Northern analysis has not uncovered HAC1 mRNA splicing in unstressed cells (Cox and Walter, 1996), we performed HAC1 Northern analysis with 30 μg RNA rather than the 10 μg RNA that is traditionally assayed. Under these conditions, we could clearly detect the spliced form of HAC1 in unstressed optimally grown wild-type cells. This spliced form constituted 7.4 ± 0.6% of total HAC1 mRNA (Fig. 1). Basal splicing was IRE1 dependent, suggesting the presence of a bona fide UPR signal in unstressed cells. The results of our Northern analysis, which we confirmed by RT-PCR (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200702101/DC1), prompted us to seek a functional relevance for basal UPR induction.

ero1-1 cells are delayed in the cell cycle with high DNA content, large buds, and divided nuclei

To determine whether this low level of UPR activity has a role in cell cycle progression, we used the ero1-1 temperature-sensitive allele to identify cell cycle stages that are sensitive to ER perturbations. In the yeast ER, the essential proteins Ero1p (ER oxidoreductin 1) and Pdi1p (protein disulfide isomerase 1) work together to catalyze oxidative protein folding (Pollard et al., 1998; Frand and Kaiser, 1999; Tu et al., 2000). For cells carrying the ero1-1 temperature-sensitive allele, growth at the restrictive temperature rapidly induces ER stress (Frand and Kaiser, 1998).

In asynchronous cultures, the restrictive growth of ero1-1 cells caused an accumulation of cells with a 2C or greater DNA content (Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200702101/DC1). This suggests that ER stress delays cell cycle progression at a point subsequent to DNA replication. To specifically define this ER-sensitive stage of the cell cycle, we induced ER stress in α-factor synchronized ero1-1 cells (Fig. 2 A). When grown at the restrictive temperature, synchronized ero1-1 cells experienced severe ER stress, as measured by HAC1 splicing (Fig. 2, B and C). Compared with wild-type cells, these ER-stressed cells proceeded normally through the initial stages of the cell cycle. By 30 min after the temperature shift, both cell types completed DNA replication, thus adopting a 90–95% 2C DNA content (Fig. 2, D and quantitated in E). After 1 h of growth at 37°C, wild-type cells began to divide and reenter G1 phase. In contrast, only a small percentage of ero1-1 cells divided at 37°C. Instead, ER-stressed cells retained a 2C DNA content or began to acquire abnormally high amounts of DNA (Fig. 2 D).

Microscopic examination of synchronized wild-type and ero1-1 cells revealed that ER-stressed cells were delayed with large buds and divided nuclei. After 30 min of 37°C growth, 90% of cells of each cell type had initiated bud formation (Fig. 2, F and H). After 45 min, both cell types remained budded, and, by this time, 60–70% of both cell populations had divided nuclei (Fig. 2 G). After 1 h, wild-type cells began to divide and become newly divided unbudded cells with a single nucleus. In contrast, ero1-1 cells did not divide but remained budded with divided nuclei for the remainder of the time course (Fig. 2, F and G), suggesting that ER stress slows the cell cycle at a point after nuclear division, probably during late M phase or cytokinesis. In fact, many ero1-1 cells began to adopt a multibudded morphology after 1.5 h of 37°C growth (Fig. 2 H). This multi-budded morphology was never seen in wild-type cells. The appearance of extra buds coupled with the appearance of 3C/4C DNA peaks strongly suggests that ero1-1 cells initiate a new round of the cell cycle despite a block or delay in the previous cell division.

Tunicamycin-treated cells are delayed in the cell cycle with high DNA content, large buds, and divided nuclei

To confirm that ER stress is specifically responsible for delaying the cell cycle in ero1-1 cells, we examined the effects of another well-characterized ER stress inducer, tunicamycin (Tm), on cell
cycle progression. Tm inhibits N-linked glycosylation in the ER, which causes the accumulation of unfolded proteins. Consistent with previous studies (Arnold and Tanner, 1982; Vai et al., 1987), we found that Tm inhibits the budding process when added immediately after α-factor release (Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200702101/DC1). Budding inhibition is known to activate the morphogenesis checkpoint and induce a G2/M delay (McMillan et al., 1998), which would likely obscure a subsequent ER-induced delay. Therefore, we introduced Tm to synchronized cultures 30 min after G1 release, after cells had already initiated the budding process (Fig. S3).

Tm treatment recapitulated the cell cycle effects of the ero1-1 mutation. As expected, Tm-treated cells displayed 90% HAC1 mRNA splicing 1 h after α-factor release (Fig. 3, A and B) and retained maximal UPR induction for the entire 3-h time course. Both Tm-treated and untreated synchronized cultures contained ~90% 2C cells after 1 h, indicating that they had progressed through S phase and into G2/M phase (Fig. 3, C and D). After 1.25 h of growth, untreated cells began to divide, as indicated by the return to a 1C DNA content, and continued through the next cell cycle, ultimately losing synchronicity. Like ero1-1 cells, Tm-treated cells failed to divide and instead began to attain a 3C or 4C DNA content (Fig. 3 C).

Untreated and Tm-treated cells were ~90% budded after 1 h of synchronized growth (Fig. 3 E). After 1.5 h of growth, untreated cells divided and became unbudded before reentering the next cell cycle. Tm-treated cells remained 80–90% budded for the entire duration of the time course. Furthermore, after 1.75 h of growth, Tm-treated cells began to attain a multibudded morphology (Fig. 3 G).

We also examined the timing and integrity of nuclear division in Tm-treated cells. In addition to following the segregation of DAPI bodies in these cells, we expressed a GFP fusion protein that localized to both copies of chromosome IV (see Materials and methods). This allowed us to visualize sister chromatids segregating to separate nuclei during nuclear division (Biggins et al., 1999) to confirm that DNA segregation was immediately after α-factor release (Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200702101/DC1). Budding inhibition is known to activate the morphogenesis checkpoint and induce a G2/M delay (McMillan et al., 1998), which would likely obscure a subsequent ER-induced delay. Therefore, we introduced Tm to synchronized cultures 30 min after G1 release, after cells had already initiated the budding process (Fig. S3).

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sister chromatids, indicating that mitosis occurred properly in these Tm-treated cells (Fig. 3 G, white arrows denote GFP-marked chromosomes). Therefore, similar to ero1-1 cells grown at the restrictive temperature, cells experiencing ER stress as a result of Tm treatment were delayed with a budded morphology after nuclear division.

Tm treatment and ero1-1-restrictive growth had very similar effects on the cell cycle, strongly suggesting that these effects are the specific result of ER stress rather than ER-independent effects of Tm treatment or the ero1-1 allele. To verify that the cell cycle is sensitive specifically to ER stress, we examined the effects of Tm treatment on the cell cycle of synchronized hac1Δ cells. Because HAC1 is required for recovery from ER stress, hac1Δ cells should be unable to recover from any specific effect of ER stress but should respond normally to ER-independent stimuli. Indeed, the absence of HAC1 rendered cells incapable of recovering from the Tm-induced appearance of cells with a high DNA content. The percentage of 3C/4C cells in the wild-type Tm-treated populations peaked at 40% after 2 h of growth (see Fig. 5, A and B) and then began to decline, reaching 25% after 3 h of growth. In contrast, hac1Δ cells continued to be 40–45% 3C/4C for the entire 3-h time course.

ER stress induces cytokinesis delay
To distinguish between the possibilities of a late M-phase delay or a delay in cytokinesis, we examined the effect of ER stress on several mitotic events: Cdc2p production/degradation, Cdc14p release, and mitotic spindle formation/dedepolymerization. Cdc2p is a major regulator of cell cycle progression. Its levels increase as cells enter mitosis and decrease as cells exit mitosis. Cells delayed in mitotic exit typically display sustained high levels of Cdc2p (Mendenhall and Hodge, 1998). Directly after the temperature shift (0-h time point), both wild-type and ero1-1 cells contained very low levels of Cdc2p (Fig. 4 A), which is consistent with most cells being in G1 or S phase. In both cell types, Cdc2p levels began to increase 30 min after the temperature shift, marking mitotic entry 15 min before nuclear division (Figs. 3 F and 4 A). Similarly, Cdc2p degradation, marking mitotic exit, occurred at the same time (60 min) in wild-type and ero1-1 cells. In wild-type cells, this Cdc2p decrease correlated well with the onset of cytokinesis (Figs. 3 E and 4 A), but, in ero1-1 cells, cytokinesis did not occur.

The key events of mitotic exit are signaled by the phosphatase Cdc14p, which is only active during anaphase. During all other times in the cell cycle, Cdc14p is kept inactive by virtue of its nucleolar localization. After nuclear division, Cdc14p...
is released into the nucleus and cytoplasm, where it signals multiple key cell cycle events, including the completion of Cdc2 degradation, breakdown of the mitotic spindle, and cytokinesis (Shou et al., 1999; Stegmeier and Amon, 2004).

10 min after temperature shift, for both wild-type and *ero1-1* cells, Cdc14p-GFP colocalized with a portion of the nucleus, which is consistent with the expected nucleolar localization of Cdc14p (Fig. 4 B). After 55 min of 37°C growth, both cell types released Cdc14p-GFP into their nucleus and cytoplasm, demonstrating that these conditions of ER stress did not delay Cdc14p release. Wild-type cells divided and resumed the nucleolar localization of Cdc14p by 70 min. Mutant cells also reabsorbed Cdc14p into the nucleus at the 70-min time point but did not divide and eventually assumed a multibudded morphology (Figs. 3 G and 4 B).

Finally, we used a *TUB1*-GFP fusion gene (Straight et al., 1997) to examine the formation and breakdown of the mitotic spindle during ER stress. By 45 min after the temperature shift, both wild-type and *ero1-1* cells exhibited fully formed mitotic spindles between their two spindle pole bodies, indicating that ER stress did not delay spindle formation. Spindle breakdown also occurred at the same time (75 min) in both cell types. Again, *ero1-1* cells did not divide. In the absence of cell division, some *ero1-1* cells rereplicated their spindle pole bodies, rebudded, and reformed a mitotic spindle, thus forming the unusual cells depicted in Fig. 4 C (150′ panel).

We also examined Cdc2 fluctuations, Cdc14p release, and mitotic spindle formation and breakdown in synchronized untreated and Tm-treated cells. We found that like *ero1-1*, Tm had no effect on these mitotic markers (Fig. S4, available at http://www.jcb.org/cgi/content/full/jcb.200702101/DC1). Therefore, ER stress delays cell division but does not affect mitotic entry, mitosis, or mitotic exit, suggesting that ER stress specifically inhibits cytokinesis or cell separation.
Cytokinesis creates a membrane barrier between mother and daughter cells. After cytokinesis, the septum continues to hold the two cells together; the septum must be degraded for cell separation to occur (Yeong, 2005). Experimentally, lyticase can be used to degrade the septum of delayed cells, thus differentiating between a cytokinesis defect and a defect in cell separation.

Lyticase treatment demonstrated that ER-stressed cells fail to divide because of incomplete cytokinesis rather than incomplete cell separation. We collected ero1-1 cells 2.5 h after temperature shift as described in Fig. 2A except that α factor was added back to the medium 45 min after G1 release to prevent the initiation of a second cell cycle. As before, most cells were delayed with a budded morphology at this time point. Their delay was clearly caused by a cytokinesis defect, as 79% of these budded cells were resistant to cell separation by lyticase treatment (Fig. 4E). Confirming that lyticase treatment only separated cells that had completed cytokinesis, wild-type cells in M phase (collected 1 h after α-factor release) remained 96% budded after lyticase treatment. In addition, cts1Δ cells, which are known to be defective in cell separation (Kuranda and Robbins, 1991), were 43% budded 1.5 h after α-factor release (unpublished data). Of the budded cts1Δ cells, 86% were separated by lyticase (Fig. 4E), confirming that the experimental conditions used here were sufficient to dissociate the majority of separation-defective cells.

Successful cytokinesis requires that cortical actin patches become polarized to either side of the bud neck late in the cell cycle (Kilmartin and Adams, 1984; Novick and Botstein, 1985; Mulholland et al., 1994; Doyle and Botstein, 1996; Waddle et al., 1996). We followed actin patch localization in synchronized cells and found that wild-type and ero1-1 cells displayed bud-localized cortical actin patches throughout S, G2, and most of M phase (Fig. 4D). Just before cytokinesis, the actin patches of ero1-1 cells redistributed to the bud neck in a manner indistinguishable from wild-type cells (Fig. 4D). Therefore, the ER stress–induced cytokinesis defect is not caused by a delay or alteration in actin patch redistribution.

**Figure 5.** UPR signaling facilitates cytokinesis during normal cell growth. (A) Wild-type (MNY1002) and hac1Δ (MNY1010) cells were treated with Tm 30 min after α-factor release. Cells were fixed, stained with Sytox green, and analyzed by flow cytometry. Bars define a subpopulation of high DNA content cells, and the given numbers indicate percentages of the total live cell population that has a high DNA content. (B) Quantitation of A. (C) Wild type (WT; MNY1002), hac1Δ (MNY1010), and ire1Δ (MNY1011) were released from α-factor arrest for 3 h before fixation and microscopic examination. (D) Wild type (RHY2724), hrd1Δ (RHY5088), hof1Δ (RHY5954), chs2Δ (RHY5955), cyk3Δ (RHY5956), mlc2Δ (RHY5957), doa10Δ (RHY5958), and bni1Δ (RHY5959) cells, which expressed a 4× UPRE-GFP reporter construct, were analyzed by flow cytometry to measure UPR activity in the absence of externally induced ER stress. Graphs represent fold induction compared with wild-type cells. All error bars represent the SD of three repeats.
UPR signaling facilitates cytokinesis during normal cell growth

The induction of ER stress in synchronized cell populations revealed that cytokinesis is highly sensitive to the state of the ER. This suggests that ER capacity increases during cell division, a process that might be facilitated by UPR signaling. To determine whether UPR signaling affects cytokinesis during normal cell growth, we examined cytokinesis in hac1Δ strains. In the absence of any external ER stressor, wild-type cell populations never exhibited cells with a >2C DNA content. In contrast, after 1.5 h of normal synchronized growth, 15% of untreated hac1Δ cells were >2C. This number increased to 20% after 2 h of growth and remained ~20% until the end of the 3-h time course (Fig. 5A). Untreated hac1Δ cells were almost as cytokinesis deficient as wild-type cells treated with Tm (Fig. 5A, compare wild-type + Tm to hac1Δ − Tm). Furthermore, we examined hac1Δ and ire1Δ strains for the multibudded morphology that is indicative of cells with a cytokinesis defect. We found that a small percentage of cells (<1%) did display this multibudded morphology, whereas we never observed multibudded cells in wild-type populations (Fig. 5C). A complete cytokinesis block should cause a much higher percentage of cells to attain multiple buds. Therefore, UPR mutants are delayed in cytokinesis rather than blocked.

To further investigate the link between UPR signaling and the cytokinesis process, we measured basal UPR activity in various cytokinesis mutants using a 4× UPR-GFP reporter construct (Pollard et al., 1998). MLC2, CHS2, HOF1, CYK3, and BN11 all participate in cytokinesis (see Discussion). Of the cytokinesis mutants tested, mle2Δ and chs2Δ strains did not exhibit basal UPR activity (Fig. 5D). However, in the absence of any external ER stress induction, hof1Δ, cyk3Δ, and bn1Δ strains exhibited three- to sixfold UPR reporter gene expression compared with wild-type cells. This level of reporter activity reflects a true link between the UPR and cytokinesis, as hrd1Δ and daa10Δ mutants, which are ERAD deficient and are known to induce functionally relevant levels of UPR activity (Travers et al., 2000; Swanson et al., 2001), exhibited similar levels of reporter gene expression. The finding that some cytokinesis mutants exhibit UPR activation is quite novel: the detection of basal UPR activity has been previously limited to mutants with specific ER defects.

Discussion

A housekeeping function for the UPR: how the ER adapts to normal fluctuations in cellular demand

In eukaryotic cells, critical cellular functions are organized and performed by functionally specialized organelles. This compartmentalization of function eases the maintenance of cellular homeostasis, as each organelle can separately control its own function in accordance with the complex requirements of the cell. The ER, for example, has a vital role in the production of lipids and proteins that make up the secretory pathway, plasma membrane, and, in yeast, the cell wall. Even during normal, unstressed growth, different internal cellular conditions, such as different stages of the cell division cycle, probably require different levels of ER functionality. However, the precise mechanism of adapting ER function to suit physiological fluctuations in internal cellular conditions is unknown. Because such a mechanism would be capable of sensing the condition of the ER and adjusting the ER’s capacity, the UPR pathway is an excellent candidate for a mechanism of ER adaptation.

In our study, we have shown that cytokinesis requires higher levels of ER functionality than other cell cycle events. This finding implies that ER functionality increases during cytokinesis and allowed us to examine the UPR’s role in achieving this functional increase. We found that UPR-deficient strains were cytokinesis defective. In addition, several cytokinesis-defective strains displayed elevated basal UPR activity. Collectively, our data establish a function for the UPR pathway in facilitating cell division during normal cell growth. The UPR presumably achieves this function by adapting ER capacity.

The UPR’s role in cytokinesis, which is revealed in this study, represents a novel type of UPR activity, as it can be detected during optimal unstressed growth conditions. All previous studies of UPR mutants describe their inability to respond to unusually stressful growth conditions such as inositol starvation (Nikawa and Yamashita, 1992; Cox et al., 1993), drug treatments that induce widespread protein misfolding (Cox et al., 1993; Mori et al., 1993), overexpression of a misfolded mutant protein (Casagrande et al., 2000; Friedlander et al., 2000; Spear and Ng, 2003), or development into a specialized secretory cell (Reimold et al., 2001; Gass et al., 2002; Ikawashi et al., 2003; van Anken et al., 2003). Each of these known UPR-reaching conditions imposes a massive load on the ER. The newly discovered importance of UPR signaling during normal cell growth uncovers a novel housekeeping function for the UPR pathway. In addition to responding to stressful growth conditions, the UPR must monitor and manage the cell’s fluctuating ER requirements.

The UPR’s ability to serve a housekeeping function sheds new light on the mode of UPR activation. In theory, the UPR pathway might operate according to one of two modes of activation. It could activate in a manner similar to an on/off switch. In this case, the pathway remains “off” until a threshold level of stress is experienced, at which point the pathway turns “on” and becomes highly active. Alternatively, the UPR pathway might operate as a dimmer switch in which the off state and on state actually represent two extremes on a continuum. Previous studies have investigated the UPR pathway by inducing crisis levels of ER stress (Cox et al., 1993; Mori et al., 1993; Casagrande et al., 2000; Spear and Ng, 2003). If the UPR pathway could fine tune the level of ER function, this could actually prevent such an ER crisis by allowing the gradual adaptation of ER capacity.

Data from previous studies provide support for both modes of activation. In support of the on/off switch mode of activation, HAC1 mRNA remains unspliced during normal cell growth but becomes rapidly and efficiently spliced upon treatment with DTT or Tm or upon the overexpression of misfolded proteins (Cox and Walter, 1996). In addition, certain modest amounts of ER stress have been shown to not activate the UPR pathway at all. For example, expression of the misfolded mutant protein CPY* from its genomic locus does not activate UPR
signaling, and ERAD of genomic CPY* does not require UPR components (Friedlander et al., 2000). However, data are also accumulating to support the dimmer switch mode of UPR activation. For example, certain mutations in the ERAD pathway have been shown to induce intermediate levels of UPR activity (Cox and Walter, 1996; Friedlander et al., 2000; Travers et al., 2000). Our study further supports the dimmer switch mode of UPR activation, as we have shown that subtle activation of the UPR pathway contributes to efficient cytokinesis.

The ER’s role in cytokinesis

Although DNA replication, mitotic entry, spindle formation, nuclear segregation, Cdc14p release, mitotic exit, spindle disassembly, and actin patch repolarization all occur normally during ER stress, cytokinesis does not (summarized in Fig. 6). Therefore, we have found that ER stress specifically disrupts cytokinesis, and we have ruled out the possibility that this disruption is caused by a defect in actin patch repolarization. This disruption could be caused by a stress-induced attenuation of any of the ER’s many functions, including secretion, ERAD, or phospholipid metabolism.

Despite the ER’s well-characterized role in initiating protein secretion, it remains unknown whether ER stress inhibits the entire secretory pathway. If it does, there are several reasons that this may impact cytokinesis. Cytokinesis begins with the assembly and contraction of an actomyosin ring. In animal cells, it has been shown that membrane deposition at the cleavage furrow must accompany actomyosin ring contraction for proper cytokinesis to occur (Skop et al., 2001; Shuster and Burgess, 2002). The extra membrane, which is delivered in the form of secretory vesicles, presumably relieves the tension created by membrane constriction. Perhaps, as in animal cells, the yeast secretory pathway assists in cytokinesis by providing membrane to the site of ring contraction, and it is the lack of membrane at the bud neck that prevents cytokinesis under conditions of ER stress.

Regardless of whether membrane addition itself is required for yeast cytokinesis, it is clear that Golgi-derived vesicles are targeted to the yeast bud neck at the end of the cell cycle and that these vesicles assist in the process of cytokinesis. First, vesicles carry cargo that is necessary for actomyosin ring contraction. Cells that are defective in vesicle fusion assemble an actomyosin ring normally, but the assembled ring is unstable and does not properly contract (VerPlank and Li, 2005). Second, during cytokinesis, secretory vesicles provide the yeast bud neck with the enzymes responsible for septum formation, a process that is essential for yeast cytokinesis (Shaw et al., 1991; Valdivia and Schekman, 2003; VerPlank and Li, 2005). Therefore, if ER stress disrupts vesicle trafficking, this could slow membrane deposition, ring contraction, and/or septation and, thereby, delay cytokinesis, thus explaining the results of our study. This explanation implies that during normal cytokinesis, the UPR manifests its housekeeping function by increasing the cell’s secretory capacity, thus fulfilling the enhanced secretory requirements of cytokinesis.

Despite expectations that ER stress would broadly inhibit secretion, some studies find that ER stress has a minimal impact, if any, on the overall secretory pathway (Casagrande et al., 2000; Spear and Ng, 2003). This suggests that the ER might play a role in cytokinesis through one of its cellular functions besides protein folding and trafficking. This possibility is especially intriguing, as it implies that the UPR pathway can detect ER functional cues other than the simple accumulation of unfolded proteins in the ER. Although previous studies have not tested this prospect directly, UPR target genes represent the entire spectrum of ER functions (Travers et al., 2000).

In addition to functioning in protein folding and secretion, the ER has the task of regulating phospholipid metabolism. Because cytokinesis entails a membrane fusion event and the creation of a membrane barrier between mother cell and daughter cell, it is not surprising that certain phospholipids are necessary for its proper completion. Phosphatidylethanolamine and phosphatidylinositol 4,5-bisphosphate become locally concentrated to the cleavage furrow during cytokinesis in various eukaryotic cell types. Interfering with the production of either of these two phospholipids results in a cytokinesis defect (Brill et al., 2000; Emoto and Umeda, 2001; Emoto et al., 2005; Janetopoulos and Devreotes, 2006). Therefore, the disruption of cytokinesis by ER stress may be caused by the effects of ER stress on phospholipid metabolism. If this is the case, the UPR’s role during normal cytokinesis may be to up-regulate genes involved in phospholipid metabolism.

Three cytokinesis mutants, bni1Δ, hof1Δ, and cyk3Δ, exhibit constitutive UPR activity. Strains deleted for MLC2...
or CHS2, which are involved in the cytokinesis processes of actomyosin ring disassembly and septum formation, respectively (Shaw et al., 1991; Luo et al., 2004), did not activate the UPR. During yeast cytokinesis, BNI1 promotes actomyosin ring assembly (Tolliday et al., 2002), HOFL coordinates ring contraction with septum formation (Lippincott and Li, 1998; Luo et al., 2004), and CYK3 mediates septum formation (Korinek et al., 2000). There is no indication that any of these mutants are defective in protein secretion or any other aspect of ER function. This is the first instance of UPR activity in mutants that are not directly defective in an ER-associated function. Furthermore, unlike previous cases of basal UPR activity in mutants, none of these three genes is a UPR target gene (Travers et al., 2000). Therefore, the UPR induction in these mutants does not represent the cell’s attempt to transcriptionally activate the specific gene that is absent. Increased UPR activity in hof1Δ, cyk3Δ, and bni1Δ strains probably helps these cells partially overcome their cytokinesis defect. This implies that the UPR pathway can directly or indirectly sense and modify the cell’s cytokinesis efficiency.

Our data highlight a new role for the UPR pathway in cytokinesis. Cytokinesis probably represents only one of many normal cellular functions that invoke a moderate level of UPR induction. Although difficult to detect, these instances of moderate UPR induction could help the ER constantly maintain an appropriate capacity in a fluctuating cellular environment.

### Table I. Yeast strains used in this study

| Strain | Relevant genotype | Source |
|--------|------------------|--------|
| MNY1000 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15 | Cox et al., 1993 |
| MNY1001 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, ero1-1::HIS3 | Frand and Kaiser, 1998 |
| MNY1002 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, HIS3, bar1::LEU2 | This study |
| MNY1003 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, ero1-1::HIS3, bar1::LEU2 | This study |
| MNY1004 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15, UPRE: lacZ: HIS3 | Cox et al., 1993 |
| MNY1005 | MATa, leu2-3,112, trp1-1, lacO:TRP1, can1-100, ura3-1, ade2-1, his3-11,15: p-CUP1-GFP: lacO: HIS3, bar1::LEU2, CDC14-GFP: KanMX | Biggins et al., 1999 |
| MNY1006 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15::HIS3, bar1::LEU2, CDC14-GFP:: KanMX | This study |
| MNY1007 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15::HIS3, bar1::LEU2, ero1-1::HIS3, CDC14-GFP:: KanMX | This study |
| MNY1008 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15::HIS3, bar1::LEU2, ero1-1::HIS3, CDC14-GFP:: KanMX | This study |
| MNY1009 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15::HIS3, bar1::LEU2, ero1-1::HIS3 | This study |
| MNY1010 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15::HIS3, bar1::LEU2, hac1Δ:: KanMX | This study |
| MNY1011 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15::HIS3, bar1::LEU2, ire1Δ:: KanMX | This study |
| MNY1012 | MATa, leu2-3,112, trp1-1, can1-100, ura3-1, ade2-1, his3-11,15::HIS3, bar1::LEU2, chs2Δ:: KanMX | This study |
| RHY2724 | MATa, me2, lys2-801, ura3-52::4xUPRE-GFP::URA3, ade2-101, his3Δ200 | This study |
| RHY5088 | MATa, me2, lys2-801, ura3-52::4xUPRE-GFP::URA3, ade2-101, his3Δ200, hrd1Δ:: KanMX | This study |
| RHY5954 | MATa, me2, lys2-801, ura3-52::4xUPRE-GFP::URA3, ade2-101, his3Δ200, hof1Δ:: KanMX | This study |
| RHY5955 | MATa, me2, lys2-801, ura3-52::4xUPRE-GFP::URA3, ade2-101, his3Δ200, chs2Δ:: KanMX | This study |
| RHY5956 | MATa, me2, lys2-801, ura3-52::4xUPRE-GFP::URA3, ade2-101, his3Δ200, cyk3Δ:: KanMX | This study |
| RHY5957 | MATa, me2, lys2-801, ura3-52::4xUPRE-GFP::URA3, ade2-101, his3Δ200, mlc2Δ:: KanMX | This study |
| RHY5958 | MATa, me2, lys2-801, ura3-52::4xUPRE-GFP::URA3, ade2-101, his3Δ200, doa10Δ:: NatMX | This study |
| RHY5959 | MATa, me2, lys2-801, ura3-52::4xUPRE-GFP::URA3, ade2-101, his3Δ200, bni1Δ:: NatMX | This study |

### Materials and methods

#### Strains, media, growth conditions, and synchronization

Yeast strains containing MNY numbers were in the W303 strain background, and strains containing RHY numbers were derived from the S288C strain background. All strains were generated using standard genetic methods and are listed in Table I. MNY1008 and MNY1009 were constructed by integrating StuI-linearized pAFS125 (Straight et al., 1997) at the URA3 locus. All strains carrying the UPRE-GFP reporter were constructed by integrating StuI-linearized pCS82-GFP (Pollard et al., 1998) at the URA3 locus. Wild-type and ero1-1 CDC14-GFP strains were constructed using a one-step PCR-mediated technique (Longtine et al., 1998). All deletion strains were constructed by amplification of the Research Genetics heterozygous diploid collection followed by G418 selection and verification by PCR.

Cells were grown in YPD medium (1% yeast extract, 2% bactopeptone, and 2% glucose) at 30°C unless otherwise noted. All strains carrying the 4× UPRE-GFP reporter construct were grown in synthetic complete –URA medium at 30°C. For synchronization, a factor (stored as 1-mg/ml stock in PBS at –20°C) was added to early logphase cultures to a final concentration of 50 mg/ml for 2.5 h (30°C growth conditions) or 3 h (25°C growth conditions). To release cells from α-factor arrest, cells were collected by centrifugation, washed twice with an equal volume of medium, and resuspended in fresh medium to an OD of 0.25. Tm was stored as 10-mg/ml stock in DMSO and added to cells at a final concentration of 1 μg/ml. During Tm experiments, 0.1% DMSO was added to untreated cells to control for effects of the vehicle.

#### Cell extracts, Northern blotting, and immunoblotting

For Western blot analysis, ~3 × 10⁶ cells were harvested by centrifugation at 4°C, washed with 1 ml H₂O, frozen with liquid N₂, and stored at –80°C. Pellets were resuspended in 100 μl of lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1% NP-40, 1 mM sodium pyrophosphate, 1 mM PMSE, 1 mM sodium orthovanadate, 2 μg/ml pepstatin A,
2 μg/ml leupeptin, 20 mM NaF, 5 μg/ml aprotinin, and 1.75 mM β-glycerophosphate). 100 μl of acid-washed glass beads were added, and cells were vortexed at 4°C for 5 min. Lyssates were centrifuged at 13,000 g for 8 min at 4°C, and the supernatant was collected. Protein concentration was determined using a BCA protein assay kit (Pierce Chemical Co.). 30 μg of protein was denatured at 95°C in 2× loading buffer (125 mM Tris-Cl, pH 6.8, 2% SDS, 5% glycerol, 12% β-mercaptoethanol, and 0.02% bromophenol blue) and was loaded on a 10% SDS-polyacrylamide gel (Invitrogen). Cib2p was detected with a 1:1,000 dilution of anti-Cib2 antibody (Santa Cruz Biotechnology, Inc.) followed by anti-rabbit secondary antibody at a dilution of 1:10,000 (GE Healthcare) and ECL detection (GE Healthcare). RNA isolation and Northern blotting were performed essentially as described previously (Cox and Walter, 1996) and were quantified using a phosphorimager (Typhoon; GE Healthcare).

DNA staining and flow cytometry
Approximately 107 cells were collected by centrifugation at 4°C, washed with 1 ml of ice-cold H2O, and resuspended in 400 μl of cold H2O. 1 ml of ice-cold EtOH was added slowly, and cells were fixed at 4°C overnight or longer. After fixation, cells were collected by centrifugation, washed with 1 ml PBS, and treated with 1 mg/ml RNase A in 100 μl PBS at 37°C for 2–12 h. Cells were then washed with 5 mg/ml pepsin in 200 μl H2O, pH 2.0, at 37°C for 20 min followed by washing and resuspension in 1 ml PBS. Cells were sonicated for 15 s at 150. 100 μl of cells (106 cells) were stained with 1 μM Sytox green (Invitrogen) in PBS. Data were collected using a flow cytometer (FACSCalibur; BD Biosciences) and analyzed using FlowJo software (Tree Star).

Strains carrying the 4× UPRE-GFP reporter construct were analyzed for UPR induction by measuring GFP fluorescence in live log-phase cells with a FACSCalibur flow cytometer. The mean fluorescence for each strain was divided by the mean fluorescence of an isogenic wild-type strain to calculate fold induction.

Microscopy
Cells were fixed in 4% PFA and sonicated briefly before analysis. Budding index was calculated as the number of cells with an obvious bud divided by the total number of cells counted. For visualization of nuclei, DAPI was added to a concentration of 0.04 μg/ml. Nuclear division was scored as positive when two separate DAPI bodies were present in a single cell. To visualize sister chromatid segregation, MNY1005 cells expressed a Lac12-GFP fusion protein and contained a Lac operon at the TRP1 locus. This caused both copies of chromosome IV to be GFP marked (Biggins et al., 1999). For the visualization of actin, cells were fixed in 4% PFA/PBS, washed with PBS, and incubated with 6.6 μM AlexaFluor546-phalloidin (Invitrogen). All cells were visualized using a microscope (Axiovert 200M; Carl Zeiss MicroImaging, Inc.) and analyzed using Axiovision software (Carl Zeiss MicroImaging, Inc.).

Lyticase treatment
Cells were fixed in YPD/4% formaldehyde for 10 min followed by 1 h in 400 mM KHPO4, pH 6.5, 500 μM MgCl2, and 4% formaldehyde. Cells were then washed in 400 mM KHPO4, pH 6.5, and 500 μM MgCl2 and resuspended in 400 mM KHPO4, pH 6.5, 500 μM MgCl2, and 1 M sorbitol. Fixed cells were sonicated (15% for 15 s) and treated with 80 U/ml lyticase at 37°C for 1 h.

Online supplemental material
Fig. S1 shows by RTPCR that spliced HAC1 mRNA is present in unstressed wild-type cells but not in erα1Δ cells. Fig. S2 shows that asynchronous erα1-1 cells accumulate with a 2C or greater DNA content when shifted to restrictive growth. Fig. S3 demonstrates that Tm inhibits budding when added directly after α-factor release but has no effect on DNA replication. This budding inhibition is bypassed when Tm is added 30 min after α-factor release. Fig. S4 shows that Tm treatment does not affect cell growth/proliferation/diagenesis, Cdc14 release, mitotic spindle formation/depolymerization, or actin patch relocalization but still inhibits cytokinesis. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200702101/DC1.

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