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A Surveillance Pathway Monitors the Fitness of the Endoplasmic Reticulum to Control Its Inheritance

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SUMMARY

The endoplasmic reticulum (ER) plays an essential role in the production of lipids and secretory proteins. Because the ER cannot be generated de novo, it must be faithfully transmitted or divided at each cell division. Little is known of how cells monitor the functionality of the ER during the cell cycle or how this regulates inheritance. We report here that ER stress in *S. cerevisiae* activates the MAP kinase Slt2 in a new ER stress surveillance (ERSU) pathway, independent of the unfolded protein response. Upon ER stress, ERSU alters the septin complex to delay ER inheritance and cytokinesis. In the absence of Slt2 kinase, the stressed ER is transmitted to the daughter cell, causing the death of both mother and daughter cells. Furthermore, Slt2 is activated via the cell surface receptor Wsc1 by a previously undescribed mechanism. We conclude that the ERSU pathway ensures inheritance of a functional ER.

INTRODUCTION

The endoplasmic reticulum (ER) functions as a gateway for newly synthesized secretory or membrane proteins. Upon synthesis, polypeptides coding for such proteins are targeted and translocated into the lumen of the ER as linear unmodified polypeptides. In the ER, the polypeptides undergo folding and modification processes to become their native functional structures. Only properly folded proteins can exit from the ER to their target sites (Bicknell and Niwa, 2009; Mori, 2000; Ron and Walter, 2007; Rutkowski and Kaufman, 2004). Misfolded proteins are toxic to the cell and become marked within the ER and targeted for degradation by a process termed ER-associated protein degradation (ERAD) (Hampton, 2002; Bukau et al., 2006; Vembar and Brodsky, 2008). Additionally, the ER is the primary site of lipid biosynthesis and thus influences the relative composition and overall abundance of lipids throughout the cell (McMaster, 2001). Essentially, the ER serves as a master regulator for the complex and error-prone process of protein maturation, quality control, and trafficking.

Morphologically, the ER is a continuous tubular-reticular network that is contiguous with the outer membrane of the nuclear envelope (Koning et al., 2002; Preuss et al., 1991). In yeast, the ER is comprised of two subdomains: the perinuclear ER/nuclear envelope and the reticulum of cortical ER (cER) tubules, which is found near the plasma membrane or the cortex of the cell. ER tubules, approximately 50–100 nm in diameter, connect the cER to the perinuclear ER (Voeltz et al., 2002). Most ER proteins analyzed to date can migrate freely between perinuclear ER and cER. The functional implication for the distinction between these ER domains is unclear.

Paradoxically, the mechanism of inheritance of perinuclear ER and cER appears to be different. The perinuclear ER remains closely associated with the nucleus and becomes segregated and partitioned between the two cells in a microtuble-dependent manner. In contrast, the inheritance of cER is actin-based and powered by a myosin motor (Du et al., 2001; Estrada et al., 2003; Prinz et al., 2000). Recent genetic studies have begun to elucidate the molecular events of cER inheritance. Very early in the cell cycle, ER cytoplasmic tubules align along the mother-bud axis and extend to the newly developed bud. These tubules become anchored at the bud tip (Du et al., 2001; Huffaker et al., 1988; Jacobs et al., 1988), followed by the tubules spreading along the periphery of the bud to form the cER of the daughter cell (Estrada et al., 2003, Estrada de Martin et al., 2005; Prinz et al., 2000, Wiederkehr et al., 2003). While interesting molecular components and mechanisms involved in ER transmission have been identified, the extent of regulation imposed on this process remains largely unexplored.

Proper segregation of cellular components is the essence of cell division and is critical to sustain life. In addition to genomic materials in the nucleus, cytoplasmic components must also be separated properly so that the newly generated daughter cells can autonomously carry out cellular events immediately after cell division (Peng and Weisman, 2008). Given the critical nature of ER function in the cell and as ER is not synthesized de novo but arises only from existing ER, we reasoned that a surveillance mechanism may exist to ensure that a minimal threshold of ER functional capacity is inherited by each daughter cell during the cell cycle.

In *S. cerevisiae*, we have previously shown that ER stress causes cell-cycle delay with high DNA content, large buds, and divided nuclei. Further analyses have revealed that ER stress

does not alter mitotic events, including the major cell-cycle regulator Clb2 production/degradation, mitotic phosphatase Cdc14 release into the cytoplasm, and mitotic spindle formation/depolymerization. ER stressed cells are unable to divide even after lyticase treatment, revealing that ER stress causes a cytokinesis defect rather than a problem in cell separation (Bicknell et al., 2007).

Here, we set out to investigate molecular events leading to the ER stress-induced cytokinesis delay. We find that the cytokinesis delay is part of a multifaceted cell-cycle response to ER stress, including septin alteration and ER inheritance delay. Furthermore, this ER surveillance response ensuring cER inheritance is independent of the canonical unfolded protein response (UPR) pathway, but rather coordinated by MAP kinase Slt2.

RESULTS

ER Stress Alters the Localization and Morphology of the Septin Ring

Previously, we showed that cytokinesis block in cells experiencing ER stress is not due to delayed or altered actin patch distribution. To probe the molecular basis of blocked cytokinesis in ER-stressed cells, we examined the localization dynamics of the septin complex during the cell cycle after treating cells with tunicamycin (Tm). Tm is a well-characterized inducer of ER stress that inhibits N-linked glycosylation, leading to an accumulation of unfolded protein in the ER. As currently understood, assembly of the five septin complex subunits, Cdc3, Cdc10, Cdc11, Cdc12, and Shs1, at the bud neck establishes a septation site for cytokinesis and is thought to act at one of the most upstream levels of the yeast cytokinesis pathway (Bertin et al., 2008; Gasper et al., 2009; Gladfelter et al., 2001; Iwase et al., 2006; Kim et al., 1991; McMurray and Thorner, 2009). We monitored the localization of the septin subunit Cdc10-GFP (Cid et al., 1998) in synchronized cells after treatment with Tm. In unstressed cells, we observed septin ring formation at the bud neck of each mother cell during bud formation with conversion to hourglass structures over time. (Caviston et al., 2003; Dobbelaere et al., 2003; McMurray and Thorner, 2009) (Figure 1A). ER stress did not affect septin subunit targeting or ring formation, which were similar in normal and stressed cells. In unstressed cells, the septin ring went on to disperse toward the end of the cell cycle as cytokinesis progressed (Figure 1A; 60 min after release from mating pheromone). In contrast, in stressed cells, the septin ring did not disperse, and cytokinesis was not observed even after 90 min (Figure 1A; compare −Tm and +Tm at 60 and 90 min). Ultimately, the septin fluorescence was observed distal from the bud neck in stressed cells, a localization that was never seen in unstressed cells. The aberrant behavior of the septin complex in Tm-treated cells was a general consequence of ER stress. This behavior was also observed when ER stress was induced by two other well-characterized means: DTT treatment, which disrupts disulfide bonds, or inactivation of the Ero1 protein (endoplasmic reticulum oxidoreductin I) through expression of the *ero1-1* temperature sensitive allele by shifting from permissive temperature (at 24°C) to nonpermissive temperature (at 37°C) (Frand and Kaiser, 1998; Pollard et al., 1998) (Figure 1B). This effect of ER stress that we observed on septin was not specific to the Cdc10-GFP reporter, as we observed similar changes in strains expressing Cdc11-GFP and Shs1-GFP fusion proteins.

The morphology of and choreographed changes in the septin ring that are observed in normal cells as the cell cycle progresses are known to be regulated by posttranslational modifications that affect the stability of interactions between septin subunits (Dobbelaere et al., 2003). To test the possibility that ER stress stabilizes the septin complex, giving rise to the persistent septin ring appearance observed (Figure 1A), we examined cells bearing the *cdc12-6* mutation. This temperature-sensitive mutation of the septin subunit *CDC12* is known to cause septin ring disassembly at the restrictive temperature (at 30°C), presumably by destabilizing interactions between septin subunits (Dobbelaere et al., 2003). Thus, we reasoned that ER stress might stabilize the septin ring sufficiently to rescue *cdc12-6* cell growth at the restrictive temperature. Growth of the *cdc12-6* mutant at the restrictive temperature is known to result in the formation of elongated cells that fail to undergo cytokinesis (Figure 1C, 30°C −Tm) (Dobbelaere et al., 2003; Kim et al., 1991). Remarkably, addition of the ER stress inducer Tm to *cdc12-6* cells at 30°C resulted in cells with a normal septin ring morphology and, ultimately, normal cell shape and cytokinesis (Figure 1C, 30°C +Tm), resulting in the rescue of overall cell growth (Figure 1D, 30°C +Tm).

Thus, ER stress suppressed the cytokinesis defect due to the *cdc12-6* mutation. Similarly, we found that Tm treatment also rescued aberrant septin localization and morphology, and elongated shape and growth of cells deleted for *SHS1* (Figures S1A and S1B available online), which encodes a subunit of the septin ring. These observations suggest that ER stress stabilizes the abnormal septin rings of *cdc12-6* and *shs1Δ* cells sufficiently to allow normal septin behavior and cytokinesis to occur. Furthermore, this observation suggests that in WT cells ER stress delays cytokinesis by stabilizing the septin ring.

ER Stress Induces an Inhibition of Cortical ER Inheritance

Since ER stress delays cell-cycle progression, we asked whether ER stress also affects ER inheritance. Using the ER marker GFP-HMG CoA reductase (Hmg1-GFP) (Du et al., 2001; Hampton et al., 1996), we examined the distribution of both cortical and perinuclear ER in mother and daughter cells in the presence and absence of ER stress. In the absence of stress, cortical ER (cER) was delivered to the daughter cell very early in the cell cycle, consistent with previous reports (Estrada de Martin et al., 2005). As soon as a bud was visible, 96% of buds contained some cER (Figure 2A; yellow arrows, no stress or *ero1-1* at 24°C, class I). As the bud grew, the cER began to spread along the cortex of the bud (Figure 2A; no stress or *ero1-1* at 24°C, class II and III). Perinuclear ER was inherited later in the cell cycle, during mitosis, along with the nucleus (Figure 2A; red arrows, no stress or *ero1-1* at 24°C, class III).

When ER stress was induced, whether with Tm, DTT, or the *ero1-1* allele grown at 37°C, cER entry into the daughter cell was significantly inhibited (Figure 2A; quantified for DTT- and Tm-induced ER stress in Figure 2B and Figure S2A, respectively). Early in the cell cycle, prior to nuclear division, only