The RNase Rny1p cleaves tRNAs and promotes cell death during oxidative stress in Saccharomyces cerevisiae

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The cellular response to stress conditions involves a decision between survival or cell death when damage is severe. A conserved stress response in eukaryotes involves endonucleolytic cleavage of transfer RNAs (tRNAs). The mechanism and significance of such tRNA cleavage is unknown. We show that in yeast, tRNAs are cleaved by the RNase T2 family member Rny1p, which is released from the vacuole into the cytosol during oxidative stress. Rny1p modulates yeast cell survival during oxidative stress independently of its catalytic ability. This suggests that upon release to the cytosol, Rny1p promotes cell death by direct interactions with downstream components. Thus, detection of Rny1p, and possibly its orthologues, in the cytosol may be a conserved mechanism for assessing cellular damage and determining cell survival, analogous to the role of cytochrome c as a marker for mitochondrial damage.

Results and discussion

The endonuclease Rny1p is responsible for tRNA cleavage

To identify the endonuclease responsible for tRNA cleavage, we screened several mutant yeast strains lacking predicted endonucleases for their effect on tRNA cleavage during H₂O₂ exposure or entry into stationary phase, when tRNA fragments are easily detected (Thompson et al., 2008). Only strains lacking RNY1, which is a member of the RNaseT2 family (MacIntosh et al., 2001),
Rny1p can also cleave ribosomal RNAs (rRNAs)

rRNA fragments are also detected in yeast, and increased rRNA fragment levels are observed during oxidative stress conditions (Thompson et al., 2008). Therefore, we asked whether loss of Rny1p also affects rRNA fragment levels during oxidative stress, using a probe against the 3′ end of the 25S rRNA that corresponds to a common rRNA cleavage product (Fig. 1, E and F). We observed that the rny1∆ strain did have fewer rRNA fragments overall, but rRNA fragments were still observed (Fig. 1, E and F). This demonstrates that Rny1p can affect rRNA fragment production, but that there

failed to produce tRNA fragments during oxidative stress, entry into stationary phase, or other stress conditions (Fig. 1, A and B; and not depicted). tRNA fragment production in the rny1∆ strain can be rescued by providing RNY1 on a plasmid (Fig. 1, C and D). Rny1p is likely to directly cleave tRNAs, as expression of a catalytically inactive version (rny1-ci), with two key histidine residues in the active site replaced by phenylalanines (H87F and H160F; Deshpande and Shankar, 2002; Acquati et al., 2005), fails to restore tRNA fragment production in an rny1∆ strain (Fig. 1, D). These observations argue that Rny1p is the nuclease cleaving tRNAs in yeast.

Figure 1. Rny1p is the endonuclease responsible for yeast tRNA cleavage. [A] 5′ tRNA-His(GTG) probe. tRNA cleavage in WT yeast during stationary phase entry. WT yeast were grown for 6 d. [B] 5′ tRNA-His(GTG) probe. tRNA cleavage does not occur in yeast lacking RNY1. rny1∆ yeast were grown as in A. The contrast in panels A and B were adjusted to similar levels to facilitate comparison. [C] 3′ tRNA-Met(CAT) probe. Expression of RNY1 from a plasmid restores tRNA cleavage. The pGAL-RNY1 vector (+) or the empty vector pRS426 (−) were transformed into WT and rny1∆ cells. Yeast were grown to mid-log phase. [D] 3′ tRNA-Met(CAT) probe. Overexpression of rny1-ci in rny1∆ cells does not rescue RNA cleavage; compare this with the overexpression of RNY1. Yeast were grown as in C. [E] 3′ 25S rRNA probe (arrow). rRNA cleavage in WT yeast during stationary phase entry. This is a reprobing of the blot shown in A. [F] 3′ 25S rRNA probe (arrow). rRNA cleavage in rny1∆ yeast during stationary phase entry. This is a reprobing of the blot shown in B. [G] 3′ tRNA-GluD probe. tRNA cleavage does not occur during postharvest cell lysis. WT (YRP840) and rny1∆ strains carrying a D. discoideum tRNA (tRNA-GluD) were cocultured or grown separately for 3 d in selective medium. [H] 5′ tRNA-His(GTG) probe. Overexpression of human RNASET2 in an rny1∆ strain rescues tRNA cleavage. Yeast were grown as in C. tRNA illustrations indicate full-length and fragment species. L = φX174/ Hind III ladder (sizes are indicated in nucleotides). Experiments were repeated at least three times; representative blots are shown.
are likely to be additional nucleases that can act on rRNA during oxidative stress. That Rny1p is able to cleave other RNAs in addition to tRNAs is consistent with work showing that T2 RNases have broad specificity (Deshpande and Shankar, 2002).

Rny1p does not act in trans
Rny1p contains a putative N-terminal signal peptide for entry into the secretory pathway and it is thought to be a secreted protein (MacIntosh et al., 2001), but it also accumulates in yeast vacuoles (see the following paragraph). This partitioning of Rny1p away from the cytosol raised the possibilities that tRNA cleavage was either an artifact of cell lysis, with extracellular or vacuolar Rny1p interacting with tRNAs released from the cytoplasm during lysis, or that Rny1p could be transferred between cells. To test these possibilities, we cocultured two yeast strains: an rny1Δ strain expressing a Dictyostelium discoideum tRNA (referred to as tRNA-GluD; Shaheen and Hopper, 2005), and a wild-type (WT) strain lacking tRNA-GluD. We observed that tRNA-GluD is not cleaved by Rny1p provided in trans, although when tRNA-GluD is expressed in a WT strain, tRNA-GluD fragments are clearly visible (Fig. 1 G). This demonstrates that Rny1p-mediated tRNA cleavage only occurs within cells expressing Rny1p and is not an artifact of cell lysis.

Vacuolar release of Rny1p correlates with tRNA cleavage
An unresolved issue was how cytoplasmic tRNAs and secreted or vacuolar localized Rny1p might interact. One possibility was that tRNAs enter the vacuole during stress conditions, possibly via autophagy (Scherz-Shouval and Elazar, 2007). However, tRNA fragment production is identical in autophagy-deficient and WT yeast strains (Fig. S1). A second possibility was that oxidative stress induces release of Rny1p from the vacuole into the cytoplasm. To address this issue, we localized a GFP-tagged version of Rny1p before and during oxidative stress.

In the absence of oxidative stress, Rny1p was primarily concentrated in the vacuole, as assessed by colocalization with the vacuolar luminal dye 7-amino-4-chloromethylcoumarin (CMAC; Fig. 2 A). Smaller puncta of Rny1p are also observed, which may be vesicles involved in Rny1p biogenesis, as expected for a protein with a secretion peptide. After H2O2 exposure, the level of Rny1p in the vacuole is reduced, which suggests that Rny1p is delocalized from the vacuole during stress. Vacuolar exit does not appear to be the result of stress-induced lysis or breakdown of the vacuole, as the luminal CMAC dye is retained in vacuoles during oxidative stress (Fig. 2 A). Moreover, by Western blot analysis, the amount of Rny1p does not appreciably decline after exposure to oxidative stress, which argues that loss of the vacuolar signal is not caused by degradation of Rny1 protein (Fig. 2 B). The strongly fluorescent puncta observed after H2O2 treatment do not represent a novel cytosolic location for Rny1p, as they colocalize with mitochondria, and are present even in control strains without a GFP-tagged protein (Fig. S2 A), which suggests mitochondrial autofluorescence, possibly from oxidized flavoproteins (see Foster et al., 2006). Therefore, after oxidative stress, our results suggest that Rny1p is distributed in the cytosol, which provides a mechanism by which Rny1p could access tRNAs and rRNAs for cleavage.
To determine whether Rny1p is unique in being released from vacuoles during oxidative stress, we examined whether GFP-tagged versions of the vacuolar proteases Pep4 and Prc1, or the luminal protein Npc2 were also released into the cytosol after oxidative stress. We observed that all three of these vacuolar proteins are concentrated in vacuoles during log-phase growth (Fig. 2 C and Fig. S3, A and B). During oxidative stress conditions, these proteins, like Rny1p, decline in concentration in the vacuole and increase in the cytosol (as assessed away from the mitochondrial background), although their overall levels do not significantly decline during oxidative stress (Fig. 2 B and Fig. S3 C). These observations indicate that during oxidative stress, multiple proteins are released from the vacuole, presumably because of damage to, or increased permeability of, the vacuolar membrane. Moreover, this release of proteins from the vacuole is likely to be conserved, as previous studies have indicated that human lysosomal proteins are released during oxidative stress (for review see Guicciardi et al., 2004).

Rny1p promotes cell death
Because oxidative stress can promote apoptosis and also resulted in high levels of tRNA cleavage, we hypothesized that Rny1p and/or tRNA cleavage might contribute to cell death. To examine this possibility, we first determined the effects of overexpression or deletion of RNY1 on yeast undergoing oxidative stress, either from entry into stationary phase or from exogenously added H2O2. We observed that strains overexpressing RNY1 show reduced viability as cells enter stationary phase (Fig. 3 A). Additionally, yeast overexpressing RNY1 are hypersensitive to oxidative stress from H2O2 exposure (Fig. 3 B). This suggests that overexpression of RNY1 hypersensitizes cells to oxidative stress, thus promoting cell death during peroxide exposure and stationary phase onset.

Expression of human RNASET2 in yeast results in tRNA cleavage and a growth defect
Because tRNA cleavage during oxidative stress is conserved among eukaryotes (Thompson et al., 2008; Yamasaki et al., 2009), we hypothesized that orthologues of Rny1p might be responsible for tRNA cleavage in other organisms. To begin to address this issue, we examined the effects of expressing the human orthologue of RNY1, RNASET2, in an rny1Δ yeast strain. We observed that overexpression of RNASET2 restores tRNA fragment production in an rny1Δ yeast strain (Fig. 1 H) and confers a similar loss of viability in response to oxidative stress, as does overexpression of RNY1 (Fig. 3 C). siRNA knockdowns of RNASET2 levels in human cells do not reduce tRNA cleavage during stress conditions (unpublished data; Yamasaki et al., 2009), which is consistent with the angiogenin nuclease playing a predominant role in stress-induced tRNA cleavage in mammalian cells (Fu et al., 2009; Yamasaki et al., 2009). However, the ability of RNASET2 to complement an rny1Δ strain suggests that RNASET2 may have some role in tRNA cleavage in mammalian cells.

Figure 3. RNY1 promotes cell death during stationary phase and oxidative stress. (A) Overexpression of RNY1 affects viability. Yeast carrying the pGAL-RNY1 (OE RNY1) or an empty vector were grown for the indicated number of days. 10-fold serial dilutions of cells were plated. (B) Overexpression of RNY1 sensitizes cells to oxidative stress. Indicated strains were grown to mid-log phase, then exposed to 0 or 3 mM H2O2 for 1 h. 10-fold serial dilutions were plated. (C) Overexpression of human RNASET2 in an rny1Δ strain results in a growth defect. Yeast carrying the pGAL-RNY1 (OE RNY1), the pGAL-RNASET2 (OE RNASET2), or vector were grown as in A. 10-fold serial dilutions were plated. (D) Deletion of RNY1 rescues the bir1Δ growth defect. Indicated strains were grown for 3 d, then 10-fold serial dilutions were plated. (E) Overexpression of RNY1 exacerbates the bir1Δ growth defect. bir1Δ cells carrying either the pGAL-RNY1 plasmid (OE RNY1) or vector were grown for 3 d, then 10-fold serial dilutions were plated. (F) Overexpression of RNY1 exacerbates the yap1Δ growth defect in response to oxidative stress. yap1Δ cells carrying either the pGAL-RNY1 plasmid (OE RNY1) or vector were grown as in E. Experiments were repeated at least three times; representative experiments are shown. pRS426 was the empty vector control.
Rny1p and yeast apoptosis

WT and rny1Δ cells showed similar viability during entry into stationary phase and after H2O2 treatment (unpublished data), which suggests that the role of Rny1p in cell death is not limiting under these conditions. However, if Rny1p plays a significant, but nonlimiting, role in promoting cell death, then the loss of Rny1p might be expected to suppress mutations that predispose yeast cells to premature apoptosis. The yeast protein Bir1 is an apoptotic inhibitor, and the bir1Δ strain shows both increased cell death in response to stress and a growth defect due to premature cell death (Walter et al., 2006). Strikingly, we observed that deletion of RNY1 suppressed the growth defect of a bir1Δ mutant strain, resulting in increased viability (Fig. 3 D). Moreover, overexpression of RNY1 exacerbates the sensitivity of bir1Δ yeast to oxidative stress (Fig. 3 E). In similar fashion, overexpression of RNY1 also increases the sensitivity of yap1Δ yeast to oxidative stress (Fig. 3 F). Yap1p being a key factor in the response to oxidative stress in yeast (Ikner and Shiozaki, 2005). The fact that deletion of RNY1 suppresses the premature death of the bir1Δ strain, and that overexpression of RNY1 hypersensitizes yap1Δ and bir1Δ cells to oxidative stress, indicates that Rny1p contributes to the modulation of cell survival during oxidative stress.

The role of Rny1p in cell death is independent of its catalytic activity

The similarity of tRNA cleavage in yeast and mammalian cells, and the ability of the human orthologue RNASET2 to complement an rny1Δ yeast strain, suggested that the biological response to oxidative stress involving these nucleases is a conserved process. Previous work has shown that additional expression of RNASET2, or of recombinant RNASET2 protein, inhibits colony formation and metastasis of tumor cell lines, though in a manner independent of the catalytic activity of RNASET2 (Acquati et al., 2001, 2005; Smirnoff et al., 2006). This suggested two possible overlapping mechanisms by which Rny1p could influence cell fate. In one, release of Rny1p from the vacuole increases tRNA cleavage, resulting in an inhibition of cellular function, either by the reduction of critical RNA levels or by the tRNA fragments themselves having an inhibitory function. Alternatively, in a manner similar to release of cytochrome c from the mitochondria triggering apoptosis (for review see Eisenberg et al., 2007), movement of Rny1p out of the vacuole could inhibit cellular function in a manner independent of its nuclease activity, possibly as part of a mechanism to sense vacuolar damage.

To distinguish between these possibilities, we generated a catalytically inactive form of Rny1p (rny1-ci) by replacing two highly conserved residues in the catalytic core. Similar mutations in other RNase T2 family members inactivate the enzyme (Deshpande and Shankar, 2002; Acquati et al., 2005). Overexpression of rny1-ci does not rescue tRNA fragment production in an rny1Δ mutant strain (Fig. 1 D), which demonstrates that these mutations inhibit the nuclease activity of Rny1p. However, rny1-ci protein is expressed at levels similar to WT Rny1p (both expressed from the GAL promoter), localizes to the vacuole during mid-log phase growth, and is released during oxidative stress (Fig. S2, B and C). Thus, mylp-ci behaves similarly to WT Rny1p but is unable to cleave RNAs.

Examination of the phenotype of cells expressing rny1-ci led to two important observations. First, like WT RNY1, overexpression of rny1-ci reduces cell viability in response to stationary-phase entry or peroxide stress, or in a yap1Δ or bir1Δ strain (Fig. 4 and unpublished data). Second, expression of RNY1 or rny1-ci at endogenous levels in the bir1Δ rny1Δ strain suppressed the enhanced viability conferred by deletion of RNY1 (compare Fig. 4 D to Fig. 3 E). These results demonstrate that the effects of Rny1p on growth are independent of its nuclease activity, similar to the role of RNASET2 in tumor suppression in mammalian cells.

A conserved control circuit controlling cytosolic RNA cleavage and cell fate

Our results describe a control circuit that modulates the response to oxidative stress, affecting both cytosolic RNA cleavage and, separately, cell fate. This circuit has the following key features (Fig. 5). First, oxidative stress prompts relocation of Rny1p, as well as other vacuolar proteins (Mason et al., 2005), from the vacuole to the cytosol (Fig. 2). Second, the presence of Rny1p in the cytosol allows tRNA cleavage, as well as...
Vacuole/lysosome homeostasis appears to affect cells and tumor progression in a manner consistent with our observations. For example, we have observed that in a yeast strain defective in vacuolar pH maintenance, Rny1p-GFP is not concentrated in vacuoles, and tRNA cleavage is increased (unpublished data). Moreover, lysosomal proton pumps are up-regulated in several human cancers, which contributes to the tendency of tumor microenvironments to acidity (see Fais et al., 2007). Finally, proton pump inhibitors have proven effective in inducing apoptosis in human tumor cells both in vitro and ex vivo by a mechanism involving increased permeability of the lysosomal membrane (De Milito et al., 2007). These observations, and the effect of RNASET2 on tumor progression, suggest that release of Rny1p or its orthologues in response to oxidative stress is not only a conserved process, but one that may modulate tumor progression in humans.

Materials and methods

Yeast strains and growth conditions

Strains used in this study are listed in Table S1. BY4741 (Thermo Fisher Scientific) was the WT strain used unless indicated. The bir1Δ strain was obtained from F. Madeo (Karl-Franzens University, Graz, Austria; Walter et al., 2006). Yeast were cultured at 30°C in yeast extract-peptone (YEP) medium with 2% glucose (YEPD) or in synthetic complete medium (SCM) supplemented with 2% glucose. As all plasmids used in this study carried a URA3 yeast auxotrophic marker, “selective SCM” indicates SC medium lacking uracil. For galactose induction of gene expression, strains were grown overnight in selective SCM containing 2% sucrose, then diluted to OD600 = 0.1 in fresh medium containing 2% galactose. “Mid-log phase” indicates growth to OD600 = 0.3–0.5. Coculturing of strains (Fig. 1 G) involved mixing equal amounts of cells (based on OD600) from overnight cultures for a total starting OD600 of 0.1. Cultures were grown for 2 d, then harvested for RNA.

Evidence suggests that a related pathway is conserved in mammalian cells. First, endonucleolytic cleavage of tRNAs in response to oxidative stress is observed in mammalian cells (Thompson et al., 2008; Yamasaki et al., 2009). Second, overexpression of the human RNY1 orthologue RNASET2 both complements an rny1Δ strain with regard to tRNA cleavage and also reduces yeast viability after stress (Figs. 1 H and 3 C). Third, expression of RNASET2 has been shown to affect cell growth in mammalian cells and to reduce the metastatic potential of tumor cell lines both in vivo and in vitro, independent of its nuclease activity (Acquati et al., 2001, 2005; Smirnoff et al., 2006). Finally, release of lysosomal proteins into the cytosol during oxidative stress has been observed in mammalian cells and is thought to promote entry of cells into apoptosis (for review see Guicciardi et al., 2004). However, the observations that angiogenin cleaves tRNAs during oxidative stress (Fu et al., 2009) and that tRNA fragments may have an additional role in inhibiting translation (Yamasaki et al., 2009) suggest that the mammalian process may be more complex. One possibility is that mammalian cells have evolved separate mechanisms to inhibit cell growth, using RNASET2, and to cleave tRNAs and alter translation, performed by angiogenin.
To delete *rny1* in *yRP840* (*yRP2449*), the *rny1::kanMX* deletion cassette was amplified from strain Y02129 using *arp1381* and *arp1382*, and transformed into *yRP840*. To replace RNY1 with a URA3 cassette in the *bir1* strain (which already has a kanMX marker), we generated a *myc::URA3* cassette by amplifying the URA3 gene from pRS426 (Christianson et al., 1992) using *oRP1383* and *arp1384*, and transformed the PCR product into *bir1* yeast. Strain *yRP2496* was generated by plating *yRP2446* yeast on 5-fluoroorotic acid–containing plates to select for loss of the URA3 marker.

**Apoptosis and stationary phase**

To assess the viability of cells after oxidative stress, or to collect protein extracts from stressed yeast, strains were grown in selective SCM with 2% galactose to mid-log phase, and treated with 0 or 3 mM H$_2$O$_2$ for 1 h; then, either serial dilutions (10-fold, starting at OD$_{600}$ = 0.3) were plated on the media, or cells were collected and frozen for protein extraction.

For stationary phase growth experiments, cultures were diluted into fresh medium and grown for 3–6 d at 30°C. WT and *rny1* strains were grown in SCW with 2% glucose; yeast carrying GAL overexpression vectors or the empty vector pRS426 were grown in selective SCM with 2% galactose, and yeast carrying the single-copy *RNY1* vectors were grown in selective SCM with 2% glucose. Aliquots were taken from the culture each day for analysis, and serial dilutions (10-fold, starting at OD$_{600}$ = 2) were plated on the same media to assess viability.

**Plasmids**

All plasmids used in this study carried a URA3 yeast auxotrophic marker. The *GAL4-RNY1* plasmid (pRP1584) was obtained from Thermos Fisher Scientific, and pRS426 (Christianson et al., 1992) was used as an empty vector. *GAL-rny1-ci* (pRP1587) was generated using QuikChange (Agilent Technologies) and pRP1584, changing both residue 87 and 160 from histidine to phenylalanine.

pRP1618 was generated by PCR-amplifying RNY1 coding, promoter, and 3′ end sequences using *arp1381* and *arp1382*. The PCR product was TA cloned (pGEM TEasy; Promega), and the cassette was digested with NotI and cloned into the single-copy plasmid pRS416 (Christianson et al., 1992). pRP1618 was QuikChange mutated as with pRP1587 to generate pRP1619.

pRP1621 expresses human RNASET2 using the GAL promoter, the RNY1 3′ end, and the RNY1 secretion sequence instead of the RNASET2 secretion sequence (aminio acids 1–24). It was constructed by yeast in vivo recombination using RsrI-linearized pRP1620 and a recombination cassette made by PCR-amplifying human RNASET2 with primers *arp1387* and *arp1388*. For pRP1620, RNY1 coding and 3′ end sequences were amplified with *arp1404* and *arp1382*, and the PCR product was TA cloned, then digested with NotI and cloned downstream of the GAL promoter in pRP1623 (*pRS426* with the GAL promoter cloned between the Xhol and BamHI sites). An in-frame *RIR1* II site was added immediately after the signal sequence using QuikChange and *arp1385* and *arp1386*.

Two GFP-RNY1 vectors with GAL promoters were constructed, with GFP in-frame either at the C terminus (shown in Fig. 2) or immediately downstream of the secretion signal. Localization of both proteins was identical. pRP1622 (internal GFP) was constructed by in vivo recombination using RsrI-linearized pRP1620 and the GFP coding sequence amplified from pRP1391. pRP1547 was generated from pRP1622 using QuikChange as with pRP1587. pRP1729 (C-terminal GFP) was constructed by yeast in vivo recombination using KpnI-linearized pRP1584 and a GFP cassette amplified using pRP1428 and pRP1429.

**RNA analysis**

RNA extraction and blotting were performed essentially as described previously (Caponigro et al., 1993). Probe sequences are listed in Table S2.

**Protein analysis**

For Western blot analysis, 5–20 μg of total protein was separated on 10% (wt/vol) SDS-PAGE gels and blotted onto Protran membrane (GE Healthcare). GFP-tagged proteins were detected using an anti-GFP antibody (1:1,000; Covance), followed by HRP-conjugated goat anti–mouse secondary antibody (1:5,000; Sigma-Aldrich). Proteins were detected using SuperSignal DuraWest Extended Duration Substrate (Thermo Fisher Scientific), followed by exposure to film. Films were digitized on a scanner (Scanjet 4890; HewlettPackard).

**Microscopy**

MitoTracker Red chloromethyl X-rosamine (CMXRos) and CellTracker blue CMAC were obtained from Invitrogen. Yeast were routinely grown to mid-log phase in selective SCM with 2% galactose; WT yeast were grown in SCM with 2% galactose; and those carrying GFP-tagged Npc2p, Pep4p, and Prc1p were grown in SCM with 2% glucose. CMAC was added at a final concentration of 10 nM, 30 min before the initial viewing. MitoTracker was added at a final concentration of 50 nM, 15 min before the initial viewing. H$_2$O$_2$ was added to a final concentration of 3 mM for 20 min to induce oxidative stress.

Cells were harvested and spotted onto slides for immediate microscopic examination. All images were acquired using a Deltavision RT microscope system running softWoRx 3.5.1 software [Applied Precision, LLC], using a 100×, oil immersion, 1.4 NA objective lens (Olympus). Images were acquired as z-series stacks of 10–15 images, and collected as 512 × 512 pixel files with a CoolSnapHQ camera (Photometrics) using 2 × 2 binning. All images except those in Fig. S3 B, which were not deconvolved but otherwise treated identically were deconvolved using standard softWoRx deconvolution algorithms (enhanced ratio, low noise filtering). Imadjel [National Institutes of Health] was used to collapse stacks to a single image and to adjust images to equal contrast ranges for each fluorochrome within individual experiments.

**Online supplemental material**

Fig. S1 shows that apoptosis defects do not appear to affect RNA fragmentation or production. Fig. S2 documents mitochondrial autofluorescence during oxidative stress and shows that myc-l-pci also localizes to vacuoles. Fig. S3 provides additional examples of vacuolar protein release during stress. Table S1 contains yeast strain information. Table S2 lists the sequences of oligos used in this study. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200811119/DC1.

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**References**


