Altered nuclear tRNA metabolism in La-deleted Schizosaccharomyces pombe is accompanied by a nutritional stress response involving Atf1p and Pcr1p that is suppressible by Xpo-t/Los1p

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Abbreviations used: AAM, amino acid metabolism; EMM, Edinburgh minimal medium; pre-tRNA, precursor tRNA; TOR, target of rapamycin.

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INTRODUCTION

The protein La is a multifunctional RNA-binding protein (Maraia, 2001; Wolin and Cedervall, 2002; Bayfield et al., 2010) that serves as a chaperone for precursor-tRNAs (pre-tRNAs) during the intranuclear phase of their maturation, which includes folding, 5’ and 3’ RNA cleavages, multiple modifications, and CCA addition to the processed 3’ end (Maraia and Lamichhane, 2011). La is ubiquitous in eukaryotes and essential in mammals (Park et al., 2006) but nonessential in the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe, although its deletion causes aberrancies relative to the normal pattern of pre-tRNA intermediates (reviewed in Maraia and Lamichhane, 2011). Absence of S. cerevisiae La (Lhp1p) disrupts pre-tRNA 5’ processing by RNase P (Yoo and Wolin, 1997). S. pombe La (sla1) mutants that are defective in nuclear retention and export their pre-tRNA ligands to the cytoplasm cause premature splicing (Intine et al., 2002; Bayfield et al., 2007), whereas other sla1 mutants distinguish 3’ end protection from RNA chaperone-like activity for structurally impaired pre-tRNAs (Huang et al., 2006; Bayfield and Maraia, 2009). Thus La directs multiple aspects of pre-tRNA metabolism, and its absence from S. pombe causes imbalances in the
distribution of pre-tRNA intermediates that can be rescued by human La (Van Horn et al., 1997; Intine et al., 2000, 2002; Huang et al., 2006; Bayfield et al., 2007; Bayfield and Maria, 2009).

In addition to function in translation, tRNAs also serve widely as metabolic sensors (Soll, 1993; Banerjee et al., 2010; Phizicky and Hopper, 2010). Pathways for biogenesis and intracellular transport of tRNAs have been linked to growth, nutrition, and stress responses (Phizicky and Hopper, 2010). In S. cerevisiae, accumulation of aberrant pre-tRNAs that cannot be processed or defects in their nuclear export stimulate Gcn4p (Qi et al., 2000), a master transcription activator of amino acid metabolism (AAM) and other genes (Natarajan et al., 2001). However, whereas Gcn4p induction due to some stress pathways depends on the kinase Gcn2p (Hinnebusch, 2005), the GCN4-mediated response to aberrant pre-tRNA metabolism, termed nuclear surveillance, is independent of GCN2 (Qi et al., 2000). S. cerevisiae and S. pombe La proteins can offset the nuclear surveillance response and 3′ end-mediated decay of aberrant pre-tRNAs (Anderson et al., 1998; Huang et al., 2006; Copela et al., 2008; Ozanick et al., 2009; reviewed in Maria and Lamichhane, 2011).

The DNA damage response program also links pre-tRNA metabolism to Gcn4p (Weinert and Hopper, 2007). In this case, intron-containing pre-tRNAs accumulate in the nucleus due to altered shuffling of Los1p, the major nuclear exporter of tRNA (Ghavidel et al., 2007). This pathway is distinguished by the fact that, in contrast to tRNA splicing in vertebrates, which is nuclear and mating genes. Leucine auxotrophic S. pombe atf1Δ or + or pcrlΔ share stress phenotypes, they also show distinct deficiencies (Kanoh et al., 2000), reproducibly suppressed sla1Δ slow growth a bit more than hLa (data not shown). Because human La can suppress the pre-tRNA processing defects of sla1Δ in mammalian cells, slow growth in Edinburgh minimal media, and heat sensitivity.

RESULTS

Deletion of sla1Δ causes stress-response phenotypes: up-regulation of AAM genes, slow growth in Edinburgh minimal media, and heat sensitivity

Microarray analysis was done on RNA from our wild-type (WT) strain (yAS99, leu1-32 ura4Δ; Table 1) and its isogenic sla1Δ strain (yAS113, sla1Δ leu1-32 ura4Δ) grown in the standard rich media used for S. pombe—yeast extract with supplements (YES) media (Supplemental Figure S1). This revealed that sla1Δ cells have elevated levels of a set of mRNAs that significantly overlap (P = 3e−12) with genes in an AAM module previously defined (Tanay et al., 2005). Additional microarray analysis showed that most of the same AAM and other mRNAs were found significantly elevated in the sla1Δ cells when grown in EMM (Supplemental Figure S1). S. pombe AAM genes are enriched for an upstream DNA sequence, TGACT, which is similar to the binding sites for budding yeast Gcn4p (see supporting Figure 6 in Tanay et al., 2005).

We examined some of the S. pombe AAM mRNAs by Northern analysis: C132.04 (glutamate dehydrogenase, gdh2; involved in aspartate, proline, nitrogen, and glutamate metabolism), ppr1Δ (involved in oxidative stress response), C1105 (lysine biosynthesis), and C56E4.03 (amino acid aminotransferase). By comparing to rRNA, which provides a loading control, this confirmed the up-regulation in sla1Δ detected by microarray and showed that ectopic expression of sla1Δ from a plasmid reversed it (Figure 1A).

Considering up-regulation of AAM genes, it might be expected that sla1Δ cells may display a growth advantage in conditions that cause amino acid starvation, such as in 3-aminothiazole (3AT; Struhl and Davis, 1977). However, the slow growth of sla1Δ relative to wild type was unaffected by 3AT (data not shown). Moreover, although WT and sla1Δ cells grew comparably in rich (YES) media, sla1Δ exhibited slow growth in EMM, the standard minimal media for S. pombe, which was relieved by ectopic sla1Δ on a plasmid (10-fold dilutions; Figure 1B). EMM is defined media that contains dextrose, amino acids, vitamins, and other supplements that does not cause starvation-induced stress responses such as mating or sporulation (Forsburg, 2003). We also deleted sla1Δ in other genetic backgrounds, and they revealed slow growth in EMM (but not YES) relative to their isogenic sla1Δ parent strains (Figure 1D). We also tested sla1Δ− in other genetic backgrounds, and they revealed slow growth in EMM was worsened by sla1Δ deletion (Supplemental Figure S1).

Human La (hLa) also suppressed the growth deficiency of sla1Δ in EMM (Figure 1B). hLaΔSSM, which lacks a short basic motif that inhibits pre-tRNA processing in S. pombe (Intine et al., 2000), reproducibly suppressed sla1Δ slow growth a bit more than hLa (data not shown). Because human La can suppress the pre-tRNA processing defects of sla1Δ in mammalian cells and functionally reverse pre-tRNA processing and nuclear trafficking–related phenotypes (Intine et al., 2000, 2002; Huang et al., 2006; Bayfield et al., 2007; Bayfield and Maria, 2009), this suggested that the slow growth of sla1Δ is due to defective pre-tRNA metabolism.
Sla1p acts in part via atf1+ and pcr1+ to down-regulate expression of AAM genes and promote growth in EMM and at elevated temperature

As noted, S. pombe and S. cerevisiae AAM genes share similar up-stream DNA, and Atf1p and Pcr1p are candidate Gcn4p homologues in S. pombe. We deleted sla1− in existing atf1−Δ, pcr1−Δ, and parent strains (Jia et al., 2004) and examined AAM mRNA levels by Northern analysis (Figure 1C, each loaded at 1x and 2x amounts). Using rpl8− mRNA as a loading control with sequential probings of the same blot, we found that atf1+ or pcr1+ deletion in sla1−-replete cells decreased C132.04 and ppr1+ mRNA expression comparably relative to the WT parental strain (SPJ83, lanes 1–6), whereas deletion of both atf1+ and pcr1+ did not further decrease these mRNAs (lanes 7 and 8). Thus Atf1p/Pcr1p appears to drive expression of these genes in rich (YES) media (Figure 1C). Similar results were found in EMM, consistent with our microarray and Northern analyses (data not shown).

Although atf1+ or pcr1+ deletion also decreased C132.04 and ppr1+ mRNAs in sla1−Δ cells, the negative effects on these mRNAs were greater for atf1+ than pcr1+ (Figure 1C, lanes 9–14). Quantification (data not shown) revealed that deletion of atf1+ or pcr1+ in sla1− decreased these mRNAs ~1.7-fold more than their deletion in the WT (SPJ83). Whereas C1105 mRNA is negatively affected by sla1−, it appears to be unaffected by atf1+ or pcr1+ deletion (Figure 1C). isp6+ (induced during sexual differentiation or nitrogen starvation) was elevated in sla1−Δ cells relative to WT (Figure 1C; compare lanes 1 and 2 with 9 and 10), confirming the microarray data, and sensitive to atf1+ deletion in sla1− but less so to pcr1+ deletion (Figure 1C, lanes 9–14). We conclude that up-regulation of a subset of AAM genes in sla1− cells depends on Atf1p and Pcr1p, in some cases to different degrees, whereas others are up-regulated independent of Atf1p and Pcr1p, suggesting that other transcription factors are involved.

We next asked whether deletion of atf1+ or pcr1+ suppresses the slow growth of sla1− (Figure 1D). Deletion of atf1+ or pcr1+ from sla1−Δ or its isogenic WT strain improves growth in EMM (Figure 1D), consistent with roles for atf1+ and pcr1+ in general growth inhibition on EMM. Deletion of sla1− had little effect on atf1+ or pcr1+ growth, consistent with the idea that atf1+ and pcr1+ antagonize growth derepression by sla1+.

Whereas sla1−Δ cells grow normally in YES, they exhibit slow growth at 37°C, and this inhibition is suppressed by deletion of either atf1+ or pcr1+ (Figure 1D).

### sla1+ cells are defective in leucine uptake and hypersensitive to NH₄Cl and rapamycin

We analyzed different ingredients of YES and EMM for effects on sla1− cell growth (data not shown) and found that the NH₄Cl in EMM was inhibitory. Replacing NH₄⁺ with proline alleviated growth inhibition of sla1− cells (Figure 2A), which is intriguing since proline is believed to constitute a relatively poor nitrogen source (Weisman et al., 2005, 2007). Addition of NH₄⁺ to YES also resulted in very significant growth inhibition of sla1− (Figure 2A). Thus growth of sla1− cells is highly sensitive to NH₄Cl.

Further analysis suggested impaired leucine uptake by our sla1− cells, which carry metabolic markers leu1− and ura4−. Providing leu1+ (on pRep3X plasmid) suppressed sla1− slow growth in EMM (Figure 2B), whereas providing excess leucine in the media did not (data not shown). By contrast, providing ura4+ suppressed the growth phenotype to a far less extent than did leu1+ on the otherwise identical plasmid (Figure 2C).

The data suggested deficient leucine uptake by sla1− cells, worthy of more direct examination. Leucine uptake is regulated by the TOR pathway (Weisman et al., 2005). Therefore, as a control, we would like to further investigate the role of sla1− in leucine uptake.

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**TABLE 1: Yeast strains.**

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<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
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<td>Intine et al. (2000)</td>
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<tr>
<td>yAS113</td>
<td>h− ade6-704 leu1-32 ura4− sla1−</td>
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WT strains. Complementation of rapamycin sensitivity of sla1Δ by ectopic sla1Δ was partial but significant (Figure 3A, compare 2 and 3) and appeared to depend on the activity of the nmt1 promoter driving Sla1p expression, which is partially repressible by thiamine (Figure 3A). The nmt1 promoter remains significantly active in the presence of thiamine (Forsburg, 1993). It was previously found that pRep4X-sla1Δ in sla1Δ cells produces fourfold higher Sla1p levels than produced from the chromosomal sla1Δ locus (R. V. Intine and R. J. Maraia, unpublished results). When sla1Δ was grown in EMM lacking thiamine, minimal complementation of rapamycin sensitivity by ectopic sla1Δ was observed relative to WT (Figure 3A, 3). Thiamin significantly improved complementation of rapamycin sensitivity by ectopic sla1Δ (Figure 3A, 5). The data suggest that S. pombe growth inhibition by rapamycin is sensitive to Sla1p levels.

Different laboratory strains of S. pombe vary in genetic polymorphisms (Iben et al., 2011) and sensitivity to NH4+ versus proline (e.g., see Figure 1, B vs. D, and Supplemental Figure S2). To examine rapamycin sensitivity in another genetic background, we deleted sla1Δ from SPG17 (Table 1), a laboratory “wild-type” strain (Grewal and Klar, 1997; Irvine et al., 2009; Smith et al., 2010). Figure 3B shows that the sla1Δ-deleted SPG17, designated CY1604, is sensitive to rapamycin relative to its parent SPG17 and to CY1627, in which sla1Δ was reintroduced to its chromosomal locus.

It is remarkable that, whereas leu1+ rescues the slow growth phenotype of sla1Δ mutants in EMM with NH4+ (Figure 2B), leu1Δ does not rescue the rapamycin sensitivity (Figure 3C, 2 and 3). It is also notable that sla1Δ cells appear less sensitive to rapamycin in EMM with proline than with NH4+ (Figure 3C, 2–5), suggesting that NH4+ contributes to the sensitization of sla1Δ cells to rapamycin and that Sla1p promotes leucine uptake and rapamycin resistance via distinct mechanisms.

Because amino acid and tRNA metabolism are altered in sla1Δ cells we expected that slow growth may be accompanied by decreased protein synthesis. We therefore measured 35S-methionine (35S-met) incorporation into protein during log-phase growth in EMM with NH4+ or proline (Figure 3D). Quantitation revealed that 35S incorporation was lower in sla1Δ than in WT in EMM with NH4+ but not with proline (Figure 3D; see normalized values under lanes, left), consistent with sla1Δ growth in these media. We found no difference in 35S-met uptake between sla1Δ and WT measured under the same conditions as 35S-met incorporation (data not shown). Thus 35S-met incorporation in sla1Δ reflects decreased translation rather than limitation of methionine. Slow growth coupled with decreased translation in sla1Δ cells is consistent with involvement of TOR signaling.

created a tor1Δ mutant in the same genetic background that was expected to be deficient in leucine uptake (Weisman et al., 2005). We measured uptake in EMM containing either NH4+ or proline (Sychrova et al., 1989; Karagiannis et al., 1999; Matsumoto et al., 2002; Weisman et al., 2005). Figure 2D (top) shows that sla1Δ cells are quite defective in leucine uptake in EMM (containing NH4+), even relative to the tor1Δ mutant. The rate of leucine uptake by sla1Δ cells appeared to be less compromised in proline than in NH4+ (Figure 2D; compare top and bottom). Note that whereas increased leucine uptake characterizes sla1Δ cells, other limitations and/or parameters may contribute to their slow growth.

Rapamycin-mediated inhibition of TOR is manifested by growth inhibition of S. cerevisiae and mammalian cells, including of tumors, although some develop resistance (Choo and Blenis, 2009; Gibbons et al., 2009; Zhou et al., 2010). Whereas wild-type S. pombe is naturally resistant to rapamycin, regulation of leucine uptake is sensitive to rapamycin (Weisman et al., 2005). We found that sla1Δ mutants are sensitive to rapamycin relative to their isogenic

FIGURE 1: Stress-response phenotypes of sla1Δ cells, including AAM gene expression, involve genetic interactions with atf1Δ and pcr1Δ. (A) Ectopic sla1Δ suppresses up-regulation of AAM genes in the sla1Δ mutant. Northern blot of 10 μg of total RNA isolated from cells grown exponentially in EMM with essential supplements probed sequentially for the gene sequences indicated to the left. Bottom, ethidium bromide staining prior to transfer. Strains yAS99 (WT; leu1-32 ura4-Δ; Table 1) and yAS113 (sla1Δ leu1-32 ura4-Δ) were transformed with the ura4Δ-containing pRep4X plasmid vector (V) or pRep4X carrying sla1Δ as indicated above the lanes. (B) yAS99 (WT) and yAS113 (sla1Δ) cells grown in liquid EMM lacking uracil (EMM-ura) were spotted on agar plates as 10-fold serial dilutions and incubated at 32°C for 2–6 d. The strains were transformed with either the empty pRep4X (ura4Δ) plasmid vector (V) or pRep4X carrying sla1Δ or hLa as indicated to the left. (C) A Northern blot loaded with 5 and 10 μg of total RNA from each of the WT or mutants indicated above the lanes was probed, stripped, and reprobed sequentially for the gene sequences indicated to the left. Strains: SPJ83 (WT), SPJ193 (pcr1Δ), SPJ266 (atf1Δ), CY1391 (sla1Δ), CY1392 (sla1Δ pcr1Δ), CY1393 (sla1Δ atf1Δ), and CY1404 (atf1Δ pcr1Δ). Cells were grown in YES media; rpl8Δ is a loading control. (D) Deletion of atf1Δ or pcr1Δ suppresses slow growth in EMM and heat sensitivity of the sla1Δ mutant. Strains are as in C. The relative growth differences of the sla1Δ strains in B and D are attributed to differences in the strain backgrounds, in particular, the auxotrophic markers. Supplemental Figure S2 shows that S. pombe strains obtained from different labs exhibit different growth rates in EMM.
gests that during vegetative growth.

Genetic interactions between sla1\(\textsuperscript{+}\) and TOR

\(S. \ pombe\) has two TOR kinases, Tor1p and Tor2p, and whereas Tor2p\(\textsuperscript{+}\) is essential for vegetative growth, Tor1p\(\textsuperscript{+}\) is nonessential but is required for normal responses to starvation and other stress (Kawai et al., 2001; Weisman and Choder, 2001). As noted earlier, leu1\(\textsuperscript{+}\) mutants are sensitive to rapamycin, dependent on inhibition of tor1\(\textsuperscript{+}\)-dependent amino acid uptake (Weisman et al., 2005). Our data that show loss of sla1\(\textsuperscript{+}\) causes leucine uptake deficiency, as well as NH\(_4\textsuperscript{+}\) and rapamycin sensitivity, strongly suggest genetic interactions between sla1\(\textsuperscript{+}\) and tor1\(\textsuperscript{+}\). Whereas the tor1\(\textsuperscript{+}\) mutant does not exhibit growth deficiency in NH\(_4\textsuperscript{+}\), this deletion has an additive effect on growth in combination with sla1\(\textsuperscript{+}\) mutation (Figure 4A). This suggests that tor1\(\textsuperscript{+}\) and sla1\(\textsuperscript{+}\) have overlapping yet distinct functions during vegetative growth.

\(S. \ pombe\) tsc1\(\textsuperscript{+}\) and tsc2\(\textsuperscript{+}\) are homologues of tuberous sclerosis genes, which have been linked to the TOR pathway, tumorigenesis, and nutrient availability (Serfontein et al., 2010). tsc1\(\textsuperscript{+}\) and tsc2\(\textsuperscript{+}\) negatively regulate tor2\(\textsuperscript{+}\), and their disruption leads to amino acid uptake deficiency (van Slegtenhorst et al., 2004; Weisman et al., 2005). We therefore deleted tsc1\(\textsuperscript{+}\) in our WT and sla1\(\textsuperscript{+}\) cells (Figure 4B). The tsc1\(\textsuperscript{+}\) mutant exhibited slow growth in NH\(_4\textsuperscript{+}\) but not proline, and this phenotype was exacerbated in the sla1\(\textsuperscript{+}\) cells (Figure 4B). This suggests that sla1\(\textsuperscript{+}\) and tsc1\(\textsuperscript{+}\) act in parallel to promote growth in EMM. The cumulative data support the existence of genetic interactions between sla1\(\textsuperscript{+}\) and the TOR pathway.

Ectopic expression of los1\(\textsuperscript{+}\) suppresses slow growth and up-regulation of AAM genes in sla1\(\textsuperscript{+}\) cells

Accumulation of aberrant pre-tRNA activates a process termed nuclear surveillance in \(S. \ cerevisiae\) via GCN4 derepression (Qiu et al., 2000). This GCN4-mediated response is reversed by ectopic expression of either RNase P, which processes pre-tRNAs at their 5’ ends, or LOS1, the major tRNA nuclear export factor, and further consistent with this, los1\(\Delta\) cells exhibit derepression of GCN4 (Qiu et al., 2000). Moreover, this response can be offset by overexpression of the \(S. \ cerevisiae\) La-homologous protein Lhp1 (Anderson et al., 1998; Calvo et al., 1999). Because sla1\(\textsuperscript{+}\) cells exhibit irregularities in pre-tRNA processing and our data suggest that los1\(\textsuperscript{+}\) activity is limiting in these cells (see later discussion), we asked whether overexpression of \(S. \ pombe\) los1\(\textsuperscript{+}\) would offset (suppress) their slow growth. Because LOS1 overexpression can be severely toxic (Hellmuth et al., 1998; Sopko et al., 2006), we titrated the activity of the nmt1 promoter driving its expression, with thiamine, including 0.05 \(\mu\)M thiamine, an intermediate level that partially represses nmt1 promoter activity (Javerzat et al., 1996). Figure 5A shows that at 0.05 \(\mu\)M thiamine, los1\(\textsuperscript{+}\) suppresses the slow growth of sla1\(\textsuperscript{+}\) cells. Because 15 \(\mu\)M thiamine is used widely in \(S. \ pombe\) with no reports of toxicity, the loss of suppression in Figure 5A, panel 4 versus panel 3, appears to be due to loss of los1\(\textsuperscript{+}\) expression as a result of more efficacious repression of the nmt1 promoter. No suppression is seen without thiamine (Figure 5A, 2), likely due to toxicity of high-level los1\(\textsuperscript{+}\) overexpression (Hellmuth et al., 1998; Sopko et al., 2006), since under these conditions, los1\(\textsuperscript{+}\) expression is indeed much higher (Figure 5B).

We used Northern analysis to confirm that 0.05 \(\mu\)M thiamine partially repressed expression of los1\(\textsuperscript{+}\) from the ectopic nmt1 promoter (Figure 5B). As expected in no thiamine, los1\(\textsuperscript{+}\) mRNA was expressed at high levels from nmt1-los1\(\textsuperscript{+}\) in the WT and sla1\(\textsuperscript{+}\) cells (Figure 5B, lanes 5–8 vs. 1–4). Using rpl8\(\textsuperscript{+}\) mRNA as a loading control in Figure 5B, middle, we see that lanes 9–16 show that at 0.05 \(\mu\)M thiamine, los1\(\textsuperscript{+}\) mRNA levels were higher in nmt1-los1\(\textsuperscript{+}\) cells (lanes 13–16) than with empty vector (+V, lanes 9–12). Quantification of the los1\(\textsuperscript{+}\):rpl8\(\textsuperscript{+}\) mRNA ratios in lanes 11/12 and 15/16 confirmed this (Figure 5B, numbers under lanes). The sla1\(\textsuperscript{+}\) cells with nmt1-los1\(\textsuperscript{+}\) expressed los1\(\textsuperscript{+}\) mRNA at 1.9-fold higher levels than in the same cells with empty vector (1.3/0.7 = 1.9-fold; see lanes 15 and 16 vs. 11 and 12, quantitation under lanes). Thus a near-twofold increase in los1\(\textsuperscript{+}\) expression appears to be sufficient to suppress slow growth of sla1\(\textsuperscript{+}\) cells in EMM.

We wanted to determine whether nmt1-los1\(\textsuperscript{+}\)-mediated suppression of slow growth was accompanied by suppression of AAM mRNA levels. We examined AAM mRNAs from cells transformed with empty vector or ectopic nmt1-los1\(\textsuperscript{+}\) (Figure 5C), grown in 0.05 \(\mu\)M thiamine and under the same conditions as for Figure 5, A and B. Lanes 1–4 of Figure 5C, top, show that ectopic nmt1-los1\(\textsuperscript{+}\) in WT cells does not affect C132.04 mRNA expression. In striking contrast, the highly elevated C132.04 mRNA in sla1\(\textsuperscript{+}\) cells (Figure 5C, lanes 5 and 6) was completely repressed by ectopic nmt1-los1\(\textsuperscript{+}\) (lanes 7 and 8). Ectopic nmt1-los1\(\textsuperscript{+}\) also
Ectopic expression of los⁺ increases low pre-tRNA levels in sla⁻ cells and suppresses imbalance of pre-tRNA intermediates

Given the foregoing findings that reveal a relationship between los⁺ and sla⁻, it might be expected that ectopic los⁺ would affect the pattern of pre-tRNAs in sla⁻ cells. We assessed this using the same RNA samples in Figure 6B as used for Figure 5C. We examined the intron-containing pre-tRNA⁺, which is a standard to follow pre-tRNA metabolism in sla⁻ cells (Van Horn et al., 1997; Intine et al., 2000; 2002; Huang et al., 2006; Bayfield et al., 2007). An intron probe detects pre-tRNA⁺ intermediates that differ by whether or not their 5′ leaders and/or 3′ trailers have been removed (Van Horn et al., 1997; Huang et al., 2006; Bayfield and Marai, 2009). The upper band represents nascent pre-tRNA that contains an intact 5′ leader and 3′ trailer. The lowest band has lost both the 5′ leader and the 3′ trailer. The middle band can be a mix of species that lack either an intact 5′ leader or the 3′ trailer, as indicated to the right of Figure 6B, including those that have been nibbled by 3′ exonucleases (Maraia and Lamichhane, 2011). The uppermost band does not accumulate as an intact species in sla⁻ cells due to instability (Van Horn et al., 1997; Intine et al., 2000; 2002; Huang et al., 2006; Bayfield et al., 2007; reviewed in Maraia and Lamichhane, 2011). Subtle mobility differences of upper and middle bands can be best appreciated in the Figure 6B, top, by comparing lanes 4/5 and 8/9.

Los1p is a major nuclear exporter of tRNA in yeast; its vertebrate homologue is exportin-1 (Xpo-t), and intron-containing pre-tRNAs are substrates for nuclear export by Los1p/Xpo-t (reviewed in Hopper, 2006). Deficiencies in this export pathway are reflected by alteration of the pattern of pre-tRNA intermediates because Los1p/Xpo-t prefers to bind end-processed tRNA species, that is, the intron-containing lower band (L) in Figure 6B. Accumulation of the unsliced L band in los⁻ mutants (Hopper et al., 1980; Hurt et al., 1987) reflects that tRNA splicing occurs in the cytoplasm of S. cerevisiae (Yoshihisa et al., 2003; Hopper, 2006). Thus a distinctive pattern of intron-containing pre-tRNAs is observed in cells lacking Los1p because its favored ligand, pre-tRNA with matured 5′ and 3′ ends, specifically accumulates (Arts et al., 1998; Lipowsky et al., 1999; also see Sarkar and Hopper, 1998; Grosshans et al., 2000; Hopper and Shaheen, 2008). We quantified the ratio of the bottom to top or middle bands in Figure 6B (top, ratios given under the lanes). Although this ratio is ~0.3 in wild-type cells, set as the control value of 1.0 and 0.93 in lanes 1 and 2 respectively, it increases 2.64-fold in our los⁻ cells (compare lanes 1 and 2 with 9 and 10). Moreover, the L band is depleted in lanes

FIGURE 3: sla⁻ cells are sensitive to rapamycin and exhibit decreased protein synthesis. (A, B) Cells were grown in liquid EMM medium with essential supplements, and 10-fold serial dilutions were spotted on the plates containing different media as indicated and incubated at 32°C for 2–6 d. (A) yAS99 (WT) and yAS113 (sla⁻) cells transformed with empty pRep3X vector (V) or pRep3X carrying sla⁻ as indicated to the left were spotted as 10-fold serial dilutions. (B) Rapamycin sensitivity of an independent sla⁻ strain derived from SPG17 is complemented by introduction of chromosomal copy of sla⁻. (C) Rapamycin sensitivity of sla⁻ cells is independent of leucine auxotrophy. WT and sla⁻ strains were transformed with pRep3X (leu⁺) and grown in EMM containing either NH₄⁺ or proline as indicated. (D) General translation is reduced in the sla⁻ mutant. Cells were grown in EMM with NH₄⁺ or 10 mM proline as the nitrogen source in the presence of [35S]methionine. [35S] Incorporation was quantitated in each lane of the autoradiograph per unit of total protein based on scanning the Coomassie-stained gel and normalized to 1.0 as reflected below the lanes. The WT and sla⁻ strains used are as in A and B.

suppressed the up-regulation of C1105 and ppr⁺ mRNA in sla⁻ (Figure 5C). Therefore overexpression of los⁺ suppresses both slow growth and up-regulation of AAM genes in the sla⁻ mutant. As data to be presented suggest, ectopic nmt1-los⁺ also complements a tRNA export deficiency in these same sla⁻ cells in which it suppresses the AAM mRNA up-regulation. nmt1-los⁺ also suppresses up-regulation of AAM genes in the sla⁻ los⁻ double mutant (Figure 5C, lanes 13–16). Figure 5 strengthens the idea that the stress-related growth inhibition and AAM gene up-regulation phenotypes of sla⁻ are caused by defects in nuclear pre-tRNA metabolism. Consistent with this, deletion of sla⁻ and los⁻ have additive effects on growth (Figure 6A), verifying genetic interaction.
FIGURE 4: Genetic interactions between sla1+ and the TOR pathway. Cells were grown in liquid EMM with essential supplements, and 10-fold serial dilutions were spotted onto the indicated media and incubated at 32°C for 2–6 d. (A) Deletions of sla1+ and tor1+ have additive effects on slow growth in EMM. Strains: yAS99 (WT), yAS113 (sla1–), CY1472 (tor1–), and CY1473 (sla1– tor1–). (B) Deletions of sla1+ and tsc1+ have additive effects on slow growth in EMM. Strains: yAS99 (WT), yAS113 (sla1–), CY1472 (tor1–), CY1473 (sla1– tor1–), CY1474 (tsc1+), and CY1475 (sla1– tsc1–).

FIGURE 6: Analyses of pre-tRNALysCUU levels. (A) WT levels upon expression of nmt1-los1+ in 0.05 μM thiamine (lanes 7 and 8). The ratio and abundance of the pre-tRNALysCUU intermediates differ in sla1+ and WT cells (Figure 6B, lanes 1/2 and 5/6). The top band is diminished due to lack of the stabilizing effects of La protein in sla1− cells. The L band is relatively prominent in sla1− cells (Figure 6B, compare lanes 6 and 2). The high ratio of the L/M bands in lanes 5 and 6 relative to lanes 7 and 8 provides evidence that nuclear export of the L species is limited in sla1− cells. Furthermore, ectopic los1+ unexpectedly increased the amount of the M species pre-tRNA in sla1− cells to a level that more resembles that in the WT cells (Figure 6B, lanes 1/2 and 7/8). The unexpected increase of the M band by ectopic los1+ in lanes 7 and 8 relative to 5 and 6 suggests that Los1p has a stabilizing effect on 5′ leader–containing, 3′ end–processed pre-tRNALysCUU in sla1− cells. The cumulative data argue that ectopic los1+ helps alleviate response to aberrant nuclear pre-tRNA metabolism in sla1− cells.

We stripped the blot in Figure 6B, top (data not shown), and rehybridized with a probe specific for the 5′ leader of pre-tRNALysCUU (Figure 6B, middle). This revealed that the 5′ leader–containing species is at relatively low levels in sla1− cells (lanes 5 and 6) but more prominent in the sla1− los1Δ cells (Figure 6B, middle, lanes 7 and 8; see quantitation normalized for loading by US small nuclear RNA [snRNA] levels under the bottom lanes). These data suggest that overexpression of los1+ stabilizes pre-tRNA in the absence of Sla1p, potentially compensating, at least in part, for the lack of Sla1p. Based on gel migration and binding properties of Los1p/Xpo-t (see Discussion), we suspect that the los1Δ-stabilized pre-tRNA in lanes 7 and 8 contains 3′ CCA, consistent with 3′ exonucleases mediating CCA turnover in S. cerevisiae nuclei (Copela et al., 2008).

DISCUSSION

Here we report consequences of disrupting the gene encoding the S. pombe La protein on genome-wide mRNA expression and associated metabolic parameters. The S. pombe response to sla1Δ deletion involves a network of genetic outputs that affects growth and metabolism. Altered pre-tRNA metabolism is a principal effect of sla1Δ deletion, and this appears to be a signal for the response, similar to but distinct from the nuclear surveillance system previously described for S. cerevisiae (Qiu et al., 2000). Thus the conclusion that emerges is that in S. pombe sla1Δ regulates AAM mRNA production through its effects on nuclear tRNA processing and maybe nuclear export.

La proteins associate with, stabilize, and promote the nuclear retention, proper order of 5′ and 3′ processing, and folding of pre-tRNAs, affording opportunity for processing, nucleotide modifications, and proper folding in an orderly manner (Yoo and Wolin, 1997; Intine et al., 2002; Chakshusmathi et al., 2003; Copela et al., 2006; Huang et al., 2006; Bayfield et al., 2007; Bayfield and Maraia, 2009; Maria and Lamichhane, 2011).

Despite involvement of La with specific mRNAs (Cardinali et al., 2003; Intine et al., 2003; Trottta et al., 2003; Costa-Mattioli et al., 2004; Brenet et al., 2009), our results indicate loss of its nuclear function in pre-tRNA metabolism as the cause of the sla1Δ phenotypes. Evidence for this is that the altered pattern of pre-tRNA intermediates in sla1Δ cells was accompanied by apparent decrease in los1Δ-mediated tRNA nuclear export activity and that overexpression of los1+ reversed these effects, as well as AAM gene up-regulation and slow growth of sla1Δ cells. Limitation of Los1 has also been observed in S. cerevisiae strains that exhibit stress (DNA damage) response (Ghavidel et al., 2007) and perturbations of pre-tRNA biogenesis (Karkusiewicz et al., 2011).

Aberrant tRNA processing in sla1Δ cells and a nuclear surveillance–like response

Defects in tRNA processing or nuclear export in S. cerevisiae lead to a stress response termed nuclear surveillance that induces AAM expression via GCN4 (Qiu et al., 2000). S. pombe AAM genes with promoters similar to Gcn4p-binding sites (Tanay et al., 2005) are activated by sla1Δ deletion. Suppression by los1Δ is consistent with the idea that a sensing component of the S. pombe response is via nuclear pre-tRNA. We note that some effects of sla1Δ deletion may reflect low levels of mature tRNA or increases in uncharged tRNA, as initially considered and later dismissed for the GCN4 response (Vazquez de Aldana et al., 1994; Qiu et al., 2000), and we cannot exclude this possibility.

Stress response analogy may extend further. LOS1 and GCN4 are involved in DNA damage response that leads to a decrease in the G1 cyclin, Cin2p (Ghavidel et al., 2007). sla1Δ cells are hypersensitive to the DNA-damaging agent ethyl methanesulfonate...
EMM, a standard growth medium that normally does not induce with amino acid starvation, our levels was evident in some of our experiments. For example, Figure S1). Indeed, unexpected sensitivity to alterations of Los1p mRNA relative to WT cells in EMM but not YES (Supplemental Data), including pcr1+ mRNAs using strains as in B plus CY1569 (los1–) and CY1570 (sla1– los1–) transformed with empty pRep4X (V) or los1+ in pRep4X as in A, grown in EMM containing 0 (lanes 1–8) or 0.05 μM thiamine (lanes 9–16) as indicated. (C) Ectopic los1+ mRNA suppresses up-regulation of AAM genes in sla1– cells. Northern blot of C132.04, C1105, and ppr1+ mRNAs using strains as in B plus CY1569 (los1–) and CY1570 (sla1– los1–) transformed with empty pRep4X (V) or los1+ in pRep4X and grown in EMM with 0.05 μM thiamine. Bottom, rRNA.

S. pombe mating program is partially derepressed in sla1– cells
Microarray and Northern analysis of sla1– cells in EMM revealed partial derepression of mRNAs that are up-regulated during mating (Supplemental Data), including pcr1+ mRNA, pas1+ (mating-specific cyclin), isp6+ (transcribed during sexual differentiation and induced by nitrogen starvation; Figure 1C), and fpb1+ (data not shown). Atf1p and Pcr1p up-regulate cyclin), spc1+ (data not shown). In addition, deleting scp1–/sty1+ did not restore growth of sla1– cells in EMM (data not shown). Therefore a Sta1p-independent function of Atf1/Pcr1 (Lawrence et al., 2007) is likely involved in AAM gene induction, as well as other TFs. 

Gcn4p-like function in S. pombe is likely performed by multiple AP-1–related TFs
Our data show that up-regulation of only a subset of the AAM genes tested in sla1– cells is dependent, at least in part, on Atf1p and/or Pcr1p. Deletions of sla1+ and atf1+ or pcr1+ show additive effects on some mRNAs for which atf1+ and pcr1+ would appear to act independently of each other. Because Pcr1p and Atf1p perform overlapping and distinct functions (Sanso et al., 2008), Sla1p may antagonize AAM gene transcription either independently or as a Atf1/Pcr1p heterodimer. Further, since some mRNAs up-regulated in sla1– are not affected by atf1+ or pcr1+, these may be controlled by other TFs. In either case this appears to be different from the situation in S. cerevisiae, in which a single TF, Gcn4p, induces all of the target genes (Natarajan et al., 2001).

Another distinction is with regard to Atf1/ Pcr1 TF activity, controlled by MAP kinase Spc1/Sty1 under conditions of extreme stress, such as oxidative stress during starvation (Nemoto et al., 2010). There is no apparent involvement of Spc1/Sty1 in our system, based on Sty1p phosphorylation (data not shown). In addition, deleting scp1–/sty1+ did not restore growth of sla1– cells in EMM (data not shown). Therefore a Sta1p-independent function of Atf1/Pcr1 (Lawrence et al., 2007) is likely involved in AAM gene induction, as well as other TFs.

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Overlap between Sla1p and TOR
Growth of wild-type S. pombe is not inhibited by rapamycin, but that of leu1–deficient cells is, due to inhibition of tor1+–mediated amino acid uptake (Weisman et al., 2005). Ectopic leu1+ suppressed slow growth of sla1–Δ , whereas excess leucine in the media did not, consistent with a defect in leucine uptake. Indeed, the sla1– mutant is hypersensitive to rapamycin. When sla1– cells were grown in EMM with NH4+ , the translation rate was only ~30% relative to isogenic WT cells, consistent with TOR involvement. Genetic analysis further suggested that Sla1p acts in parallel with Tsc1p and Tor1p to promote growth in EMM.

We examined mRNA levels for the putative permeases 7G5.06 and isp5+, orthologous to S. cerevisiae GAP1, and C869.10,  

(data not shown) and have approximately twofold less cyc17+/cig2+ mRNA relative to WT cells in EMM but not YES (Supplemental Figure S1). Indeed, unexpected sensitivity to alterations of Los1p levels was evident in some of our experiments. For example, whereas pre-tRNA distribution was distorted in los1–Δ cells relative to wild type, ectopic nmt1–los1+ in 0.05 μM thiamine did not fully complement this (Figure 6B), presumably because Los1p levels produced by nmt1–los1+ do not match that in wild-type los1+ cells. Consistent with this assumption, los1+ mRNA levels were significantly lower in the los1–Δ nmt1–los1+ than in the wild-type los1+ cells (data not shown). These findings support the idea that regulation of Los1p homeostasis is critical to normal S. pombe metabolism.

Although our data reflect likeness to S. cerevisiae nuclear surveillance, there are distinctions. The GCN4 response is triggered by excess unprocessed pre-tRNA, whereas sla1– cells have a deficiency, suggesting that imbalance of pre-tRNA levels or processing is a commonality. Second, no genome-wide mRNA profiling of S. cerevisiae lhp1–Δ or analysis of sensitivity to NH4+ has been reported. Third, the two yeasts would appear to differ in response to La deletion since no growth deficiency was observed for S. cerevisiae lhp1–Δ. In addition, whereas AAM induction in S. cerevisiae occurs with amino acid starvation, our sla1–Δ cells exhibit AAM induction in EMM, a standard growth medium that normally does not induce starvation-related responses. Finally, we tested the S. cerevisiae lhp1–Δ mutant, and it did not show growth deficiency under amino acid starvation or in minimal medium (data not shown).
prehensive view of cellular protein synthesis and degradation. Growth of fission yeast, which is a key model organism for understanding human diseases, is significantly affected by the expression of the denosine triphosphate synthetase (DPS1) gene. DPS1 encodes a protein that is essential for cell growth and viability. The study presented here aimed to investigate the role of DPS1 in the regulation of cellular processes, particularly in response to environmental stress.

The key findings were as follows:

1. The expression level of DPS1 was found to be significantly correlated with the growth rate and viability of fission yeast cells.
2. Cells with reduced DPS1 expression exhibited decreased growth and increased sensitivity to environmental stress.
3. Interestingly, the expression of DPS1 was found to be regulated by the DNA damage response pathway.

These findings suggest that DPS1 plays a crucial role in the regulation of cellular processes, particularly in response to environmental stress. The study provides new insights into the mechanism by which DPS1 regulates cellular function and opens up new avenues for research on the role of this gene in human diseases.

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**MATERIALS AND METHODS**

**CDNA microarray analysis**

Total RNA for microarray analysis was obtained from early-log-phase (OD600 = 0.2–0.4) cells grown either in YES or EMM media with NH4Cl or proline as the nitrogen source and prepared as described.

**Analysis**

The analysis of the microarray data revealed significant differences in gene expression between the two conditions. The genes whose expression was significantly altered were identified and their functions were analyzed in the context of cellular metabolic pathways. The data was further validated using qRT-PCR and Western blotting.

**Conclusion**

The study provides a comprehensive view of the changes in gene expression associated with the growth and viability of fission yeast cells. The findings suggest that DPS1 plays a crucial role in the regulation of cellular processes, particularly in response to environmental stress. The study provides new insights into the mechanism by which DPS1 regulates cellular function and opens up new avenues for research on the role of this gene in human diseases.
RNA labeling, microarray hybridization, data processing, and normalization were carried out as previously described (Lyne et al., 2003).

**Yeast strains and growth media**

Yeast strains are listed in Table 1. CY1391, CY1392, and CY1393 were constructed by replacing sla1+ with sla1::NAT (Sato et al., 2005) in SPJ83, SPJ193, and SPJ266 respectively. CY1404 was made by replacing pcr1+ with pcr1::NAT in SPJ266. CY1407 and CY1408 were generated from ya599 and ya5113, respectively, by replacing pub1+ with pub1::kan MX6. Similarly, CY1472 and CY1473 were generated by replacing tor1+ with tor1::kan MX6, and CY1474 and CY1475 by replacing tsc1+ with tsc1::kan MX6 in ya599 and ya5113, respectively. CY1569 and CY1570 were made by replacing los1+ with los1::urad4+ (Bahir et al., 1998) in ya599 and ya5113, respectively. The obtained strains were selected on 5-fluoroorotic acid medium to counterselect for urad4+. All gene disruptions were confirmed by PCR.

Media were prepared according to standard recipes. For some applications, the NH4Cl in EMM was replaced with 10 mM proline. Recipe for EMM: potassium phthalate (3 g/l), Na2SO4 (0.04 g/l), ZnSO4 (0.4 mg/l), Na2HPO4 (2.2 g/l), pantothenic acid (1 mg/l), CuSO4 (40 μg/l), CaCl2 (14.7 mg/l), boric acid (0.5 mg/l), citric acid (40 μg/l), dextrose (20 g/l), inositol (10 mg/l), potassium iodide (0.1 mg/l), MgCl2 (anhydrous) (0.492 g/l), D-biotin (0.01 mg/l), nicotinic acid (10 mg/l), molybdic acid (40 μg/l), dextrose (20 g/l), inositol (10 mg/l), potassium iodide (0.1 mg/l), MgCl2 (anhydrous) (0.492 g/l), D-biotin (0.01 mg/l), CuSO4 (40 μg/l), CaCl2 (14.7 mg/l), boric acid (0.5 mg/l), citric acid (1 mg/l), KCl (1 g/l), and MnSO4 (0.4 mg/l). When supplemented, EMM also contained leucine, adenine, and uracil, each at 225 mg/l. Rapamycin was used at 100 ng/ml. Thiamine was used at 0.05 μM (intermediate repression) or 15 μM (full repression).

**Plasmids**

pRep4X hLa and pRep hLaA328-344 in Rep4X were described previously (Intine et al., 2000; Fairley et al., 2005). The isps5+ open reading frame was PCR amplified using genomic DNA as template with the primers GTCGACATGTCGGCTTAATGCTTG (reverse), digested with BamHI, and cloned into Rep4X. los1+ open reading frame was PCR amplified with the primers GTCGACATGAATAATTAC- GGGTCTCTTCC (forward) and GGATCCTCATACATTACCTTTT(GG)IACGACGACGAGAAGATGAGGACG (reverse), digested with SalI and BamHI, and cloned into Rep4X. los1+ open reading frame was PCR amplified with the primers GTCGACATGTCGGCACCCAGGATGTC (forward) and GGATCCTCATACATTACCTTTT(GG)IACGACGACGAGAAGATGAGGACG (reverse), digested with SalI and BamHI, and cloned into Rep4X.

**RNA purification and Northern blotting**

Total RNA was purified as described (Lyne et al., 2003). For mRNA Northern analysis 5 and 10 μg of total RNA were separated in 1% denaturing agarose gel, transferred to a nylon membrane (GeneScreen Plus; PerkinElmer, Waltham, MA), UV cross-linked, baked, and subjected to hybridization at 42°C overnight with random primed 32P-DNA fragments of genes of interest. Hybridization solution was 5x Denhardt’s, 5x saline–sodium citrate, 50% formamide, 0.2% SDS, 5 mM EDTA, and 100 μg/ml total yeast RNA. Northern blotting of small RNAs was done essentially as described (Intine et al., 2000). Quantitation was done using a PhosphorImager FLA-3000 (Fujifilm, Tokyo, Japan).

**35S-methionine incorporation**

Ten milliliters of cells was grown exponentially to an OD600 = 0.2 and transferred to medium containing 1 mCi of 35S-methionine (PerkinElmer) and grown for 3.5 h at 32°C. Washed cells were harvested, and 25 and 50 μg of the whole-cell extract were separated on 10% PAGE. Gels were stained with SimplyBlue (Invitrogen, Carlsbad, CA), fixed, and dried, and 35S was quantified with a PhosphorImager FLA3000.

**3H-leucine uptake**

This was performed as described, in EMM containing 225 mg/ml leucine (~1.6 mM) and trace amounts of 3H-leucine (Weisman et al., 2005).

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La deficiency causes growth defects


