Cell Division Defects of Schizosaccharomyces pombe liz1\(^{-}\) Mutants Are Caused by Defects in Pantothenate Uptake

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The liz1\(^{+}\) gene of the fission yeast Schizosaccharomyces pombe was previously identified by complementation of a mutation that causes abnormal mitosis when ribonucleotide reductase is inhibited. Liz1 has similarity to transport proteins from Saccharomyces cerevisiae, but the potential substrate and its connection to the cell division cycle remain elusive. We report here that liz1\(^{+}\) encodes a plasma membrane-localized active transport protein for the vitamin pantothenate, the precursor of coenzyme A (CoA). Liz1 is required for pantothenate uptake at low extracellular concentrations. A lack of pantothenate uptake results in three phenotypes: (i) slow growth, (ii) delayed septation, and (iii) aberrant mitosis in the presence of hydroxyurea (HU). All three phenotypes are suppressed by high extracellular concentrations of pantothenate, where pantothenate uptake occurs by passive diffusion. liz1\(^{-}\) mutants are viable because they can synthesize pantothenate from uracil as an endogenous source. The use of uracil for both pantothenate biosynthesis and deoxyribonucleotide generation provides an explanation for the aberrant mitosis in the presence of HU. HU blocks ribonucleotide reductase, and we propose that the accumulation of ribonucleotides reduces uracil biosynthesis by feedback inhibition of aspartate transcarbamoylase. Thus, the addition of HU to liz1\(^{-}\) mutants results in a shortage of pantothenate. Because liz1\(^{-}\) mutants show striking similarities to mutants with defects in fatty acid biosynthesis, we propose that the shortage of pantothenate compromises fatty acid synthesis, resulting in slow growth and mitotic defects.

The cell division cycle is a tightly controlled cascade of independent events. Biochemical pathways, known as cell cycle checkpoints, impose dependency by ensuring that the initiation of later events does not occur before earlier events have been completed. Chromosome replication is monitored by the intra-S-phase checkpoint, which coordinates DNA replication, and by the S/M checkpoint, which delays entry into mitosis until DNA replication is complete (reviewed in reference 5). Both checkpoint pathways have been extensively studied in the fission yeast Schizosaccharomyces pombe (6, 34). The inhibition of ribonucleotide reductase (RNR), the enzyme that converts ribonucleotides to deoxyribonucleotides, by the addition of hydroxyurea (HU) to the growth medium blocks chromosome replication. In wild-type cells, this activates the intra-S-phase checkpoint to prevent further progression through S phase and activates the S-M checkpoint to prevent mitosis. Genetic screens for HU sensitivity have been exploited to identify mutants that inappropriately respond to incomplete DNA replication (11). These mutations not only identified checkpoint proteins but also defined proteins that are required to survive arrest within S phase (3, 4, 26).

One mutant obtained in such a screen, the liz1\(^{-}\) (lives if zapped 1) mutant, has an unusual phenotype for a HU-sensi-

tive mutant: like wild-type cells, most of the liz1\(^{-}\) cells appear to arrest mitosis following addition of HU. However, a proportion of liz1\(^{-}\) mutants undergo a highly aberrant mitosis (28). Intriguingly, when HU was added to a culture of liz1\(^{-}\) cells, passage through S phase was not a requirement for this defective mitosis to occur. In synchronized G2 cells, the first mitosis following HU addition was seen to be aberrant. In some of these cells, the new septum was seen to bisect an unseparated nucleus, a lethal event known as the “cut” phenotype (28). In less drastic cases, in the presence of HU, the liz1\(^{-}\) cells completed nuclear division and formed a septum but failed to decondense their chromosomes. Thus, liz1\(^{-}\) cells in G2 display a catastrophic mitosis when treated with HU. Conversely, when liz1\(^{-}\) cells in G1 are treated with HU, they arrest in S phase and activate a normal S-M checkpoint, and mitosis is prevented.

To rule out the possibility that HU acted to inhibit an alternative process in liz1\(^{-}\) cells, Moynihan and Enoch (28) introduced the cdc22-M45 mutation into a liz1 null mutant background. Cdc22 is the large subunit of RNR, and the cdc22-M45 mutation causes this to be inactive at the restrictive temperature. Inactivating RNR by a temperature shift resulted in phe-

nologies in liz1\(^{-}\) cells that are the same as those observed when cells are treated with HU (28). This confirms that RNR activity is required for a normal mitosis in liz1\(^{-}\) cells and that this phenotype is independent of passage through S phase.

While a cut cell phenotype is frequently observed in checkpoint mutants treated with HU, the phenotype of the liz1\(^{-}\) mutant is fundamentally different. For example, when hus1\(^{-}\) cells are treated with HU while they are in G2, they progress normally through the first mitosis and show no cuts. It is only
when they enter the subsequent S phase and are unable to activate the S-M checkpoint that they then proceed into the second mitosis with catastrophic and lethal results. This contrasts with the \textit{liz1} mutant, which can activate a normal S-M checkpoint, but in which a significant proportion of cells are unable to successfully complete the previous mitosis (28). Thus, the conundrum is why a cell with replicated chromosomes should require RNR activity to complete mitosis.

The \textit{liz1} gene was cloned and shown to encode a protein with similarity to the \textit{Saccharomyces cerevisiae} allantooate permease family of transport proteins (28, 30). Although the substate of Liz1 remained unidentified, the authors speculated that Liz1 might be involved in the transport of an intermediate in uracil biosynthesis (28). During our analysis of two \textit{S. cerevisiae} transport proteins, Vht1p and Fen2p, we identified Liz1 as a closely related protein by database searches. Vht1p and Fen2p facilitate the uptake of biotin and pantothenate, respectively (36, 37). This similarity suggested either biotin or pantothenate as a possible substrate for the Liz1-dependent transport. In this report, we provide evidence that Liz1 functions in the uptake of pantothenate, transporting it across the plasma membrane. Since pantothenate is essential for coenzyme A (CoA) biosynthesis, the failure of \textit{liz1} mutants to complete nuclear separation in the presence ofHU may be related to a defect in fatty acid synthesis. CoA is required for fatty acid synthesis, and it is well established that, in \textit{S. pombe}, mutants in the fatty acid biosynthesis pathway exhibit a cut phenotype (31). This presumably reflects a high demand for fatty acids during nuclear division.

We also present data demonstrating that uracil serves as a precursor for CoA biosynthesis in the absence of pantothenate uptake in \textit{liz1} mutants. This metabolic link provides an explanation for why the inhibition of RNR leads to defects in normal mitotic progression in \textit{liz1} mutants. HU blocks RNR activity, and this is likely to cause an increase in CTP and UTP. Since CTP and UTP allosterically inhibit aspartate transcarbamoylase, an essential enzyme in the uracil biosynthetic pathway, the cellular uracil pool would be expected to drop, reducing pantothenate synthesis in \textit{liz1} mutant cells. \textit{liz1} mutant cells may thus experience a depletion of CoA in the presence of HU, which in turn may compromise the supply of fatty acids that are required for a normal mitosis.

**Plasmid constructs and gene disruptions.** The open reading frame of the \textit{liz1} gene (SPBCG2.01c) was amplified by PCR and ligated into the EcoRI site of pUC19 (pUC19Liz1). \textit{liz1} does not contain introns. For overexpression of Liz1 in \textit{S. pombe}, the EcoRI fragment was introduced into pSAPE (38) to give plasmid pSAPLiz1. For expression in \textit{S. cerevisiae}, the EcoRI fragment was ligated into p424MET25 (29) to give plasmid pLiz1mc.

To create a disrupted allele of \textit{liz1}, the uracile gene was subcloned from pREP4X (12) into the HindIII site of pUC20R (25), liberated with BglII and PstI, and ligated into the BclI and NsiI sites of pUC19LIZ1. In the resulting plasmid, nucleotides 194 to 152 of the \textit{liz1} open reading frame were replaced by uracile, which was in antisense orientation relative to \textit{liz1}. The disrupted allele was liberated with EcoRI and transformed into \textit{S. pombe}, and the cells were spread on plates containing 100 \mu M pantothenate.

To generate a knockout construct for \textit{pam6} (SPAC5H10.08c), the gene was amplified by PCR and ligated into the single NolI site of a modified pUC19 vector. Next, the kanMX gene from pFA6a-kanMX4 (39) was introduced as a SafI-EcoRI restriction fragment into the Sall-Mel sites within pam6, eliminating 202 bp of the coding region. The final disruption construct was liberated with NolI and used to transform \textit{S. pombe} cells. All gene disruptions were verified by PCR.

**Transport assays and FACS and microscopic analyses.** The transport of pantothenate was determined at an initial outside concentration of 1.75 \mu M at pH 6.0 in 18 mM citric acid, 64 mM NaHPO4, and 1% \textit{v}-glucose, as previously described (37). DAPI (4′,6-diamidino-2-phenylindole) was used to stain the DNA for scoring cut phenotypes, and calcofluor was used to stain the septa of dividing cells. At least 200 cells were counted for each time point. Fluorescence-activated cell sorter (FACS) analysis of \textit{S. pombe} was performed as previously described (10) by using propidium iodide to stain the DNA.

**RESULTS**

\textit{liz1} complements mutations in the \textit{S. cerevisiae} \textit{FEN2} gene.

The genes encoding the \textit{S. cerevisiae} plasma membrane transporters for pantothenate (\textit{FEN2}) and biotin (\textit{VHT1}) were recently identified (36, 37). The protein encoded by the \textit{S. pombe} \textit{liz1} gene is a homolog of both vitamin transporters. Based on direct protein comparisons and phylogenetic analyses of proteins from the allantooate transporter families of \textit{S. cerevisiae} and \textit{S. pombe} (35), Liz1 is most similar to the \textit{S. cerevisiae} pantothenate transporter Fen2p.

To test whether Liz1 functions as a pantothenate transporter, we expressed \textit{liz1} in \textit{S. cerevisiae} \textit{fen2Δ} mutants (Fig. 1A). \textit{fen2Δ} mutants were unable to grow on plates containing 1 \mu M pantothenate, whereas this concentration was sufficient for growth of \textit{S. cerevisiae} wild-type cells (Fig. 1A). Heterologous expression of \textit{liz1} restored the growth of \textit{fen2Δ} cells in the presence of low pantothenate concentrations, strongly indicating that Liz1 acts as a pantothenate transporter. Consistent with this view, expression of \textit{liz1} failed to sustain the growth of \textit{S. cerevisiae} \textit{vht1Δ} mutants defective in biotin transport (data not shown). Together with the recent cloning of the \textit{S. pombe} biotin transporter gene \textit{vht1} (35), these data identify pantothenate as the substrate for Liz1.

\textit{liz1} encodes a high-affinity proton pantothenate symporter.

The pantothenate transport activity of Liz1 was characterized in detail in \textit{S. cerevisiae} \textit{fen2Δ} mutants. Whereas the transport of [14C]pantothenate across the plasma membrane was negligible in control cells, \textit{liz1}–expressing cells showed a rapid uptake of pantothenate (Fig. 1B). Consistent with a proton symport mechanism, pantothenate uptake by Liz1 was stimulated by \textit{v}-glucose and was strongly inhibited by protonophores (80% inhibition by 50 \mu M carbonyl cyanide m-chlorophenyl hydrazone; data not shown). Liz1-mediated pantothenate transport was maximal at an external pH of 6.0, at which pantothenate is negatively charged. Pantothenate transport

**MATERIALS AND METHODS**

**Yeast strains and media.** The \textit{S. pombe} leu1-32 strain (17) served as a donor of genomic DNA for the amplification of \textit{liz1} and \textit{pam6} genes. \textit{S. pombe} FY254 (ade6-M210 can1-1 leu1-32 ura4-d18 D18h-1) (21) was used for all other experiments. The genotype of the bas1Δ mutant was bas1Δ-LEU2 ura4-d18 leu1-32 h-1 (19).

Where indicated, cells were made ura4-positive by transformation with pREP4X (12). The pantothenate transport-deficient \textit{S. cerevisiae} strain 711/1c (MATa ura3-52 triplΔ1 fen2Δ-URA3) (1, 37) was used for the expression of the \textit{liz1} gene.

\textit{S. cerevisiae} and \textit{S. pombe} cells were grown in synthetic minimal medium (0.67% yeast nitrogen base without amino acids [Difco], 2% \textit{v}-glucose) that contained a fixed concentration of pantothenate (1.68 \mu M). Media for growth assays were prepared from an autoclaved solution containing 2% \textit{v}-glucose and 2% Difco Bacto agar. Pantothenate-free yeast nitrogen base (final concentration, 0.67%), 50 mg of adenine/liter, and 50 mg of leucine/liter, as well as other compounds, were added from filter-sterilized stocks before pouring the plates. For growth assays, cells were washed and diluted in water to an \textit{A}600 of 0.8, and four serial 10-fold dilutions were performed in a 96-well plate. Aliquots of the cells were transferred to plates by using a metal replicating device.
of the ura4 cells, we disrupted the open reading frame by the integration of liz1/H11002. Reduced pantothenate transport from 60 pmol of pantothenate was previously shown to cause partial uracil auxotrophy (28), we were able to disrupt the liz1 gene. Although disruption of liz1 was previously shown to cause partial uracil auxotrophy (28), we were able to recover ura4 mutants that had the disruption correctly integrated. In agreement with a role in pantothenate uptake, disruption of the liz1 gene in S. pombe cells strongly reduced pantothenate transport from 60 pmol of pantothenate h⁻¹ × mg cell⁻¹ in wild-type cells to 1.8 pmol of pantothenate h⁻¹ × mg cell⁻¹ for liz1 mutants (Fig. 1C). Overexpression of liz1 increased pantothenate transport 430-fold over wild-type levels (26 mmol of pantothenate h⁻¹ × mg cell⁻¹ [Fig. 1D]). These data confirm that the physiological role of Liz1 is the uptake of pantothenate. Since pantothenate transport is almost completely lost upon disruption of liz1 (Fig. 1C), Liz1 seems to be the only functional plasma membrane pantothenate transporter in S. pombe. Our data are consistent with the localization of green fluorescent protein-tagged Liz1 to the S. pombe plasma membrane (28).

FIG. 1. Liz1 is a plasma membrane transport protein for pantothenate. (A) Multicopy expression of liz1 rescues the growth defect of S. cerevisiae fen2Δ mutants. Cells of the S. cerevisiae fen2Δ mutant strain 711/lc were transformed with an empty plasmid or with pLIZ1mc for multicopy expression of liz1°. The cells were resuspended in water (A₆₀₀° = 0.1), and 10 μl of the suspension was streaked on a plate containing 1 μM pantothenate. A S. cerevisiae wild-type strain is shown for comparison. The plate was photographed after a 3-day incubation at 30°C. (B) Expression of liz1° confers pantothenate uptake to S. cerevisiae fen2Δ cells. Transport of [¹⁴C]pantothenate was determined with fen2Δ cells harboring a control plasmid (●) or with fen2Δ cells carrying liz1° on a multicopy plasmid (□). (C) Pantothenate uptake into S. pombe cells after deletion of liz1° (●). A S. pombe wild-type strain carrying a control plasmid (□) is shown for comparison. (D) Pantothenate uptake in S. pombe cells after overexpression of liz1° (■) and in wild-type cells carrying a control plasmid (□).

To confirm the existence of such a pathway, we analyzed S. pombe cells lacking the pan6° gene. The protein encoded by pan6° is 60% identical to the Escherichia coli pantopte β-alanine ligase, an enzyme involved in pantothenate biosynthesis from β-alanine (27). As shown in Fig. 2, the deletion of pan6° in the liz1Δ strain abolished all background growth at low pantothenate concentrations. This suggests that pantothenate is synthesized from β-alanine in the absence of exogenous pantothenate supplies. As expected, the addition of high pantothenate concentrations to the medium suppressed the growth defect of pan6Δ liz1Δ double mutants. Thus, S. pombe is capable of synthesizing pantothenate from a intracellular source, and this pathway is essential for the survival of liz1Δ mutants.

The cell cycle phenotypes of liz1Δ mutants are rescued by high extracellular concentrations of pantothenate. Our data and that of Moynihan and Enoch (28) indicate that the HU sensitivity of liz1Δ mutants is caused by the absence of pantothenate uptake. To confirm this, we compared liz1Δ, hus1Δ, and wild-type cells for their responses to HU. The experiments were performed in medium containing a low concentration of pantothenate (left panels of Fig. 3A and B) or upon the addi-
tion of 1 mM pantothenate, which supplies the vitamin in the absence of Liz1 (right panels of Fig. 3A and B).

At low pantothenate concentrations, the inhibition of RNR by HU triggered an increase in the number of liz1−/H11002 mutant cells undergoing a catastrophic mitosis (cut phenotype). This increase was completely suppressed by the presence of high pantothenate concentrations, suggesting that pantothenate uptake is indeed required for normal mitotic progression in the presence of HU. Unlike liz1−/H9004 cells, hus1−/H9004 cells entered a catastrophic mitosis independently of the pantothenate concentration in the medium (Fig. 3A). The different behavior of hus1Δ and liz1Δ cells is consistent with the function of Hus1 in the replication checkpoint pathway and supports the previous observation that the replication checkpoint functions normally in liz1− cells (28).

A careful analysis of the kinetics of cell cycle progression in these experiments suggested to us that septation proceeded more slowly in liz1− cells. When HU was added to asynchronous wild-type cell cultures, the septation index dropped from about 15 to 0%, reflecting the fact that the cells have stopped cell cycle progression. In contrast, when HU was added to an asynchronous culture of checkpoint-deficient hus1− cells, they failed to arrest the cell cycle, and septated cells accumulated from approximately 15 to over 70%, because the septum cuts through the nucleus leading to cell death (the terminal phenotype). Interestingly, in media containing a low pantothenate concentration, logarithmically growing cultures of liz1− cells possess a twofold higher content of septated cells prior to treatment with HU, indicating that septation proceeds more slowly in these mutants. Consistent with the interpretation that liz1− cells spend an increased length of time septating, the septation index of liz1Δ mutants decreased more slowly in the presence of HU than in wild-type cells and leveled off at around 20% at later time points (Fig. 3B), reflecting the terminal phenotype. All the septation-related phenotypes were suppressed to wild-type levels by the addition of high pantothenate concentrations to the growth medium, demonstrating a direct link to pantothenate import.

We next performed FACS analysis to determine the DNA content of wild-type cells and liz1Δ mutants (Fig. 3C). In the absence of HU, wild-type cells possessed a 2C DNA content. This is expected for an asynchronous S. pombe culture. In contrast to wild-type cells, the liz1Δ culture contained cells with a 4C DNA content (Fig. 3C). This 4C peak is consistent with the idea that the lack of pantothenate slows septation. Because septation is slow, the daughter cells remain attached for longer. During this time they each undergo S phase and reach a 2C DNA content. The fact that two 2C cells remain attached thus explains the appearance of a 4C peak in FACS analysis. As predicted, we also observed that high pantothenate supplementation increased the 2C peak of liz1Δ cultures at the expense of the 4C peak (Fig. 3C). Upon the addition of HU, liz1Δ cells

FIG. 3. Supplementation of pantothenate corrects the cell cycle phenotypes of S. pombe liz1Δ mutants. S. pombe cells were grown in media containing a low (1.68 μM) or high (1 mM) concentration of pantothenate (PAN), freshly diluted into the same media, and 10 mM HU was added. Cells were stained with calciofluor to visualize septa and with DAPI to visualize nuclei. At least 200 cells were examined for each time point. All strains analyzed were prototrophic for uracil and were grown in media lacking uracil, wild type: □, liz1Δ: ◻, hus1Δ. In panel A, the percentages of cells showing the cut phenotype are given, and in panel B, the percentages of cells showing septa (i.e., cells in S phase) are given. In panel C, the DNA content of asynchronous wild-type or liz1Δ cells was determined by FACS analysis. Samples were obtained after growth in media with low (L) or high (H) concentrations of pantothenate, as described above. Where indicated, the cells were treated for 2 h with 10 mM HU. Approximately 2,000 cells were fixed, stained with propidium iodide, and analyzed.
Growth was recorded after 3 days at 30°C indicating compounds were present at a concentration of 1 mM containing no pantothenate. All cells tested here were ura-positive. The indicated compounds were present at a concentration of 1 mM. Growth was recorded after 3 days at 30°C.

started to arrest in G1, with a 1C DNA content consistent with a functional checkpoint pathway (Fig. 3C). In contrast to wild-type cells, liz1Δ mutant cultures took longer to exhibit a peak of cells with 1C DNA content by FACS analysis. This is again consistent with a delay in cell cycle progression during septation, since two 1C cells joined by a septum will migrate as a 2C particle in this analysis. High concentrations of pantothenate restored normal arrest kinetics, supporting the model that pantothenate is required not only for a normal mitosis in the presence of HU but also for normal progression of septation (Fig. 3C).

The growth defects of S. pombe in the absence of pantothenate can be overcome with uracil, ureidopropionate, and β-alanine. To this point, we have demonstrated that a lack of pantothenate uptake correlates with a slow septation rate and an increase in the number of cut cells upon inhibition of RNR.

Lack of pantothenate uptake affects the cell division cycle. The characterization of Liz1 as a pantothenate transporter was surprising because a connection between pantothenate transport and specific cell cycle events has not been revealed by previous studies. Here we demonstrate that the lack of pantothenate uptake is indeed responsible for all of the reported phenotypes of liz1Δ mutants and provide a rational framework to explain how this occurs.

The phenotypes of liz1Δ cells include a high percentage of seceded cells or cells with 1C DNA content by FACS analysis. This is again consistent with a delay in cell cycle progression during septation, since two 1C cells joined by a septum will migrate as a 2C particle in this analysis. High concentrations of pantothenate restored normal arrest kinetics, supporting the model that pantothenate is required not only for a normal mitosis in the presence of HU but also for normal progression of septation (Fig. 3C).

The phenotypes of liz1Δ cells include a high percentage of seceded cells in the absence of HU (Fig. 3B) and the inability to separate the nucleus (cut phenotype) in the presence of HU (Fig. 3A and reference 27). Both of these phenotypes are reversed when pantothenate is supplemented in concentrations that allow uptake in the absence of Liz1 activity. This demonstrates that the lack of pantothenate transport is responsible for these cell cycle perturbations.

This raises the question of how pantothenate influences septation and mitosis. Pantothenate is an essential metabolite and its functions as a building block for the synthesis of CoA, CoA and metabolites derived from it have multiple cellular roles. Fatty acid biosynthesis and elongation depend on malonyl-CoA, which is produced from acetyl-CoA in a reaction catalyzed by acetyl-CoA carboxylase and the α subunit of fatty acid synthase, respectively (31).

Cut6Δ and lsd1Δ mutants are temperature sensitive and, at the restrictive temperature, produce daughter nuclei of unequal size accompanied by a high degree of chromatin condensation in the smaller daughter nucleus (31). Moreover, lsd1Δ mutants were shown to complete cytokinesis (septation) in these circumstances, despite the fact that nuclear division has not been completed. This resulted in cut cells, similar to the phenotype described for liz1Δ mutants. These data strongly suggest that fatty acid metabolism plays a central role in nuclear division and chromatin condensation and that fatty acid
metabolism is compromised in liz1Δ cells. Fatty acids are structural components of phospholipids. Thus, cut6Δ, lsd1Δ, and liz1Δ mutants may be defective in the biosynthesis of phospholipids. In S. cerevisiae, the activities of acetyl-CoA carboxylase (encoded by ACC1) and of other proteins involved in lipid metabolism are increased in G1 as a prelude to DNA synthesis (7). Moreover, net phospholipid accumulation occurs specifically during S phase (reviewed in reference 18). Thus, mutations in cut6Δ, lsd1Δ, or liz1Δ may be deleterious because they interfere with the increased demand for phospholipids, which are required for nuclear division and septation.

Alternatively, mutations in cut6Δ, lsd1Δ, or liz1Δ may affect the biosynthesis of a specific class of lipids. In S. cerevisiae, fatty acid biosynthesis has a 17-fold higher affinity for malonyl-CoA than does fatty acid elongation (9). Thus, a shortage of malonyl-CoA may predominantly affect the elongation of fatty acids. Evidence in favor of this model comes from conditional S. cerevisiae acc1 mutants. These mutants possess highly aberrant nuclear envelopes, which display expansions of the intermembrane space and accumulation of vesicles between the two membranes (32). Moreover, the acc1 mutants develop large nuclei that do not enter the daughter cells during mitosis (2). The defect in these mutants has been assigned to a reduced abundance of C26 fatty acids on sphingolipids (2, 32). The fact that supplementation of C16 fatty acids does not rescue the growth defect of liz1Δ mutants (data not shown) may indicate that it is indeed the lack of very-long-chain fatty acids that slows the growth in liz1Δ mutants. This model, however, cannot be tested directly because exogenous very-long-chain fatty acids are not readily taken up or activated (33).

A metabolic link between uracil catabolism and pantothenate biosynthesis. Whereas mutations in cut6Δ or lsd1Δ have severe effects on the nuclear morphology by themselves, liz1Δ mutants display their most drastic phenotypes only when RNR is inhibited (Fig. 3 and reference 28). This indicates that there are metabolic links between the two unrelated processes, pantothenate uptake and reduction of ribonucleotides.

Our experiments led to the discovery that S. pombe cells are capable of deriving pantothenate from the breakdown of uracil (Fig. 2 and 4). This pathway of pantothenate biosynthesis depends on the presence of the pan6Δ gene (Fig. 2 and 4), and thus proceeds via β-alanine. We find that uracil, ureidopropionate, and β-alanine are able to substitute for pantothenate in S. pombe (Fig. 4). Ureidopropionate and β-alanine are part of a catabolic pathway in S. kluveri that provides β-alanine (15). Two enzymes of this pathway have been identified: 5,6-dihydrodropyrrimidine amidohydrolase (encoded by PYD2 [14]) and β-alanine synthase (encoded by PYD3 [16]). S. pombe lacks an obvious ortholog of PYD2, consistent with our finding that dihydrouracil is not able to substitute for pantothenate (Fig. 4). S. pombe also has no ortholog of PYD3, but our experiments showed that the cells were able to grow in the presence of ureidopropionate (Fig. 4). It is not clear if ureidopropionate is converted to β-alanine by spontaneous hydrolysis (8) or if the identification of the S. pombe β-alanine synthase is prevented by the sequence diversity known to occur in eukaryotic β-alanine synthases (16, 24).

Nonetheless, our data support the existence of a novel metabolic pathway in S. pombe that leads from uracil to β-alanine and does not have dihydrouracil as an intermediate. This pathway may not be unique to S. pombe. When LaRue and Spencer (20) analyzed 123 yeast species for their ability to utilize different pyrimidines as their sole source of nitrogen, they found that 69 strains were able to grow on uracil. Of these, 22 were unable to utilize dihydrouracil, demonstrating that in these strains dihydrouracil was not an intermediate of uracil catabolism.

Although the precise pathway of uracil degradation in S. pombe remains to be established, our data clearly show that uracil is a precursor of pantothenate. Thus, when the plasma membrane pantothenate transporter Llz1 is defective, uracil biosynthesis has to satisfy the demand of two essential processes: CoA synthesis and deoxyribonucleotide production via RNR (Fig. 5). S. pombe cells are unable to synthesize sufficient uracil to support both pathways (Fig. 2 and 4). We speculate that the concentrations of CTP and UTP increase in the presence of HU, which blocks RNR activity. This may lead to a feedback inhibition of aspartate transcarbamoylase, the committed step in the synthesis of pyrimidines which is known to be allosterically regulated by CTP and UTP (13, 23, 41). Thus, HU may lead to a reduction of de novo production of uracil (Fig. 5) and a further drop in the CoA concentrations in liz1Δ cells.

Summary. All three phenotypes of liz1Δ cells, slow growth, delayed septation, and aberrant mitosis in the presence of HU,
are reversed by the addition of high pantothenate concentrations to the growth medium. Since pantothenate is required for CoA synthesis, the lack of Liz1 is expected to reduce the cellular CoA pool, rendering cells dependent on CoA synthesis from endogenous sources, a pathway requiring uracil degradation. The use of uracil for both CoA generation and deoxyribonucleotide synthesis by RNR provides the explanation for the appearance of aberrant mitosis in presence of HU. HU blocks RNR activity, and the increase in RNR substrates slows uracil production. As a result, the CoA pool will drop below a critical threshold, resulting in a shortage of fatty acids during mitosis. The pantothenate transporter Liz1 thus provides a surprising example that shows how substrate transport across the plasma membrane can influence cellular events in many as yet unexpected ways.

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