Chromatin-remodeling SWI/SNF complex regulates coenzyme Q₆ synthesis and a metabolic shift to respiration in yeast

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Despite its relatively streamlined genome, there are many important examples of regulated RNA splicing in Saccharomyces cerevisiae. Here, we report a role for the chromatin remodeler SWI/SNF in respiration, partially via the regulation of splicing. We find that a nutrient-dependent decrease in Snf2 leads to an increase in splicing of the PTC7 transcript. The spliced PTC7 transcript encodes a mitochondrial phosphatase regulator of biosynthesis of coenzyme Q₆ (ubiquinone or CoQ₆) and a mitochondrial redox-active lipid essential for electron and proton transport in respiration. Increased splicing of PTC7 increases CoQ₆ levels. The increase in PTC7 splicing occurs at least in part due to down-regulation of ribosomal protein gene expression, leading to the redistribution of spliceosomes from this abundant class of intron-containing RNAs to otherwise poorly spliced transcripts. In contrast, a protein encoded by the non-spliced isoform of PTC7 represses CoQ₆ biosynthesis. Taken together, these findings uncover a link between Snf2 expression and the splicing of PTC7 and establish a previously unknown role for the SWI/SNF complex in the transition of yeast cells from fermentative to respiratory modes of metabolism.

Similar to other eukaryotic genomes, genes in Saccharomyces cerevisiae may be interrupted by non-coding sequences, called introns. Introns are removed from the pre-mRNA through the action of the spliceosome, a macromolecular machine composed of five small nuclear ribonucleoproteins. The spliceosome recognizes consensus sequence signals on the pre-mRNA, termed splice sites, by which it subsequently binds to the intron and catalyzes its removal via two transesterification reactions (1). Pre-mRNA splicing is critical for accurate gene expression in all eukaryotes, and there is significant evidence that alterations in microenvironments, such as changes in the chromatin state or chromatin-modifying factors, can affect splicing outcomes (1). However, the mechanisms for how chromatin and chromatin factors influence splicing are not completely understood.

Although the genome of S. cerevisiae contains a smaller number of introns than metazoan genomes, there are, nonetheless, numerous examples of intron-dependent gene regulation (2). The largest functional class of intron-containing genes (ICGs) in budding yeast is ribosomal protein genes (RPGs) that encode the protein components of the ribosome. Therefore, the energy-intensive process of translation is under the heavy regulatory control of the spliceosome, such that splicing of RPgs can be finely tuned to the cells’ environmental conditions and to nutrient availability (3).

Interestingly, this enrichment of introns within RPgs impacts the splicing of, as well as provides an opportunity for the regulation of, other ICGs within the yeast genome. About a third of yeast introns occur in RPgs, and the high transcription levels of these genes means that about 90% of the intron load encountered by the spliceosome is from this one functional class of genes (4). Indeed, the prevalence of RPG introns functions to titrate spliceosomes away from other introns, especially those containing suboptimal splice sites. Conversely, down-regulating RPG expression promotes the splicing of transcripts harboring suboptimal splice sites. This effect is perhaps best described during the process of yeast meiosis. Under conditions of vegetative growth, a number of meiosis-specific ICGs are expressed, but they possess suboptimal splice sites and are therefore poorly recognized by the spliceosome and suboptimally spliced. However, upon the down-regulation of RPgs during meiosis, increased availability of the previously limiting pool of spliceosomes leads to improved splicing efficiency of introns in meiosis-specific transcripts (5, 6).

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4 The abbreviations used are: ICG, intron-containing gene; RPG, ribosomal protein gene; ns, non-spliced; s, spliced; CoQ, coenzyme Q; DMQ₆, 5-demethoxy-Q₆; 4HB, 4-hydroxybenzoic acid; HHB, 3-hexaprenyl-4-hydroxybenzoic acid; qPCR, quantitative PCR; TOR, target of rapamycin.
SWI/SNF regulates CoQ₆ synthesis via PTC7 splicing

There are other important examples of intron-based regulation in *S. cerevisiae*, especially among ICGs with non-consensus splice sites (7, 8). One such gene is *PTC7*, which encodes a Mg²⁺/Mn²⁺-dependent, type 2C serine/threonine protein phosphatase (9). The intron within *PTC7* is particularly intriguing because it contains a non-consensus branch-point sequence, rendering its splicing relatively inefficient under logarithmic growth conditions. The *PTC7* intron lacks a premature termination codon and is translated in-frame. The longer, non-spliced (ns) form of the *PTC7* RNA encodes a longer protein (*Ptc7*ns) that contains a single trans-membrane helix located near the N terminus but is otherwise identical to the protein isoform derived from the spliced *PTC7* RNA (*Ptc7*s). The read-through nature of the *PTC7* intron is conserved across yeast species, indicating potential functionality for both protein isoforms having opposing effects on the CoQ₆ biosynthetic pathway in *S. cerevisiae*. First, we show that deletion of *Snf2* alters the relative levels of *Ptc7*s and *Ptc7*ns isoforms in yeast and increases both the rate of synthesis and steady-state levels of CoQ₆. This is due to down-regulation of RPG transcripts and an increase in the available pool of splicosomes. Moreover, we find that the Snf2 protein is down-regulated over time under batch growth conditions and nutrient depletion, and together with a concomitant increase in the splicing of *PTC7*, this leads to higher CoQ₆ levels in preparation for the transition from a fermentative mode of metabolism to a respiratory mode. Furthermore, we show that the two *Ptc7* isoforms have opposing effects on the CoQ₆ biosynthetic pathway, which may explain contradictory reports in the literature about the effects of *Ptc7* on CoQ₆ levels (11, 12). Importantly, although Snf2 is down-regulated in response to nutrient-depleted conditions, it is nonetheless required for growth on nonfermentable carbon sources, suggesting that dynamic control of Snf2 levels is crucial for the transition from fermentation to respiration.

Results

Deletion of Snf2 leads to enhanced splicing of PTC7 and a shift in the ratios of Ptc7 protein isoforms

Previously published RNA sequencing data for yeast lacking Snf2, the core ATPase component of the SWI/SNF complex (GEO accession number GSE94404), revealed an increase in splicing of a number of introns (6). Satisfyingly, the greatest improvement in splicing upon deletion of Snf2 is experienced by *RPL22B*, a previously described mechanism consistent with down-regulation of RPG expression (25). The next two largest improvements in splicing efficiency are experienced by *YBR062C* (an ORF of unknown function) and *PTC7*, a previously described type 2C serine-threonine mitochondrial phosphatase that contains all 11 canonical motifs of the PPM family (type 2C) protein phosphatases, previously reported to play a role in CoQ₆ biosynthesis in yeast (11) (Fig. 1A). This increase in splicing of *PTC7* RNA was verified by RT-PCR (Fig. 1B). In addition, the results from the RNA-seq and RT-PCR were also independently verified by qPCR (data not shown). It has previously been demonstrated that increased splicing of poorly recognized introns can be achieved by decreased expression of competing, highly expressed RPGs (5). Furthermore, we have shown that deletion of Snf2 causes *en masse* down-regulation of RPGs and consequent improvement in splicing of a large number of introns (6). RPG down-regulation in the absence of Snf2 was validated by RT-PCR analysis. For example, expression of *RPS16A* and *RPL34B*, two intron-containing RPGs, is down-regulated in *snf2Δ* yeast compared with WT (Fig. 1C).

The *PTC7* transcript makes two distinct protein isoforms, one from the nonspliced and one from the spliced RNA. The spliced isoform (*Ptc7*s) localizes to the mitochondria, whereas
the nonspliced isoform (Ptc7ns) has been reported to localize to the nuclear envelope (10). The PTC7 gene was endogenously HA-tagged, and Western blot analysis demonstrated that deletion of Snf2 leads to an increase in the levels of Ptc7s compared with Ptc7ns (Fig. 1D). It is noteworthy that the increase in the ratio of Ptc7s/Ptc7ns polypeptides in the WT and snf2Δ cells appears to be greater than the increased ratio of spliced/unspliced RNA.

It has previously been demonstrated that yeast strains lacking Snf2 fail to grow on non-fermentable carbon sources, such as glycerol or acetate (26). However, snf2Δ mutants frequently incur secondary mutations, and the growth of such strains can resemble WT. Therefore, growth on fermentable and non-fermentable carbon sources was used as a quality control for the assessment of the bona fide phenotype (24) of snf2Δ prior to each experiment (Fig. 1E).

Deletion of Snf2 leads to increased CoQ₆ synthesis in yeast and improves the flux from DMQ₆ to CoQ₆

Ptc7 has previously been described as playing a role in regulating CoQ₆ synthesis in S. cerevisiae (11). A schematic of the entire CoQ₆ biosynthetic pathway with 4-hydroxybenzoic acid as the ring precursor and the role of Ptc7 is detailed in Fig. 2A. Ptc7 is thought to enhance CoQ₆ biosynthesis via its activation of Coq7 and subsequent catalysis of the hydroxylation of DMQ₆, the penultimate step of CoQ₆ biosynthesis (Fig. 2B) (11, 27).

13C₆-Labeled 4-hydroxybenzoic acid (13C₆-4HB), a ring precursor for Q biosynthesis, was used to determine the levels of 13C₆-CoQ₆ biosynthesis in WT versus snf2Δ yeast grown to similar culture densities. The absence of Snf2 causes increased steady-state levels of CoQ₆ and increased de novo biogenesis of 13C₆-CoQ₆ (Fig. 3A). Additionally, there are significant changes in the levels of de novo synthesized DMQ₆, as well as 3-hexa-
prenyl-4-hydroxybenzoic acid (HHB), an early CoQ₆ biosynthetic intermediate (Fig. 3, B and D). Consistent with the increased synthesis of CoQ₆ being a consequence of Ptc7 action, the snf2/H9004 yeast show significantly lower ratios of ¹³C₆-DMQ₆ level to ¹³C₆-CoQ₆ content, indicating a significant increase in the efficiency of conversion of DMQ₆ to CoQ₆, namely the step catalyzed by Coq7, a target of Ptc7, (Fig. 3C) (11). Strikingly, we also observe that the levels of both steady-state and de novo synthesized HHB are significantly lower in snf2Δ than in the WT yeast (Fig. 3D). This suggests that the deletion of Snf2 not only causes higher CoQ₆ production by regulating catalysis from DMQ₆ but that it also funnels the early precursors more efficiently than WT, thus allowing a more streamlined conversion of intermediates of the pathway to the overall product of CoQ₆. This is reinforced by the observation that snf2Δ yeast show significantly lower ratios of ¹³C₆-HHB to ¹³C₆-CoQ₆ content (Fig. 3E).

**Depletion of Snf2 during batch growth is associated with increased PTC7 splicing and increased CoQ₆ production**

Because snf2Δ yeast have a significantly slower growth rate than WT, we considered the possibility that the increased CoQ₆...
SWI/SNF regulates CoQ₆ synthesis via PTC7 splicing

Figure 3. Deletion of SNF2 leads to increased steady state levels and de novo CoQ₆ biosynthesis in yeast and improves the flux from DMQ₆ to CoQ₆.

A. levels of steady-state CoQ₆ (¹³C-CoQ₆, blue bars) and de novo synthesized CoQ₆ (¹³Cᵣ-CoQ₆, orange bars) were determined in WT and snf2Δ yeast. ¹³C₆-4HB was added during midlog phase (A₄₉₀ = 0.5), and labeling was allowed to proceed until a cell density of A₄₉₀ = 1.75 was reached by both strains. ¹²C-CoQ₆ and ¹³C₆-CoQ₆ present in yeast cell pellets were quantified by HPLC-MS/MS, as described under “Experimental procedures.” Error bars, S.D. of n = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT, **, p < 0.005; ****, p < 0.0005). B. levels of steady-state (¹³C₆-DMQ₆, blue bars) and de novo synthesized DMQ₆ (¹³Cᵣ-DMQ₆, orange bars) were determined in WT and snf2Δ yeast. DMQ₆ was determined from the same cultures as in A. Error bars, S.D. of n = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT, *, p < 0.05). C. ratios of ¹³C₆-DMQ₆/¹³C₆-CoQ₆ in WT and snf2Δ yeast, depicting flux of conversion of ¹³C₆-DMQ₆ to ¹³C₆-CoQ₆. Error bars, S.D. of n = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT, ****, p < 0.00005). D. levels of steady-state HHB (¹³C₆-HHB, blue bars) and de novo synthesized HHB (¹³Cᵣ-HHB, orange bars) were determined in WT and snf2Δ yeast. HHB was determined from the same cultures as in A. Error bars, S.D. of n = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT, *, p < 0.05; **, p < 0.005). E. ratios of ¹³C₆-HHB/¹³C₆-CoQ₆ in WT and snf2Δ yeast, depicting flux of conversion of ¹³C₆-HHB to ¹³C₆-CoQ₆. Error bars, S.D. of n = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT, ****, p < 0.00005).

synthesis was a consequence of the increased time in culture required to achieve equal cell density. To address this, rates of CoQ₆ biosynthesis in WT and snf2Δ yeast were determined at timed intervals of culture. First, measurements of steady-state levels of CoQ₆ plateau within 4–6 h of labeling (Fig. 4A). We also observe decreasing levels of Snf2 as the time course progresses and nutrients are depleted (Fig. 4B). Consistent with the role of Snf2 in RPG transcription, RPG levels decrease with time in batch cultures of yeast, in a manner that tracks well with decreasing levels of Snf2 (Fig. 4C). This decrease also coincides with a concomitant increase in the splicing of PTC7 (Fig. 4, D and E). Notably, splicing of the PTC7 transcript in snf2Δ yeast starts off higher than in WT yeast, but as Snf2 is depleted from the WT strain, splicing of the PTC7 transcript approaches the levels of splicing in the snf2Δ strain (Fig. 4F).

To better understand the kinetics of CoQ₆ synthesis, a shorter time course was performed to capture points preceding the plateau, between 0 and 5 h of labeling. Within 4 h after labeling with ¹³C₆-4HB precursor, significant down-regulation in the levels of Snf2 protein is evident (Fig. 5A). The decrease in the level of Snf2 protein is mirrored in the increase in splicing efficiency of PTC7 transcript in the WT strain (Fig. 5, B–E). It is interesting to note that the PTC7 transcript is initially better spliced in the snf2Δ strain than in WT, but as the levels of Snf2 in the WT yeast decrease, splicing improves to a degree comparable with the snf2Δ strain (Fig. 5, D and compare C and F).

Additionally, there is a striking increase in the overall CoQ₆ product and its de novo biosynthesis in the snf2Δ yeast within 0–5 h of labeling, as compared with CoQ₆ levels of the WT.
SWI/SNF regulates CoQ₆ synthesis via PTC7 splicing

Figure 4. Snf2 levels decrease during batch growth, coinciding with increased PTC7 splicing and increased CoQ₆ synthesis. A, levels of steady-state CoQ₆ (13C₆-CoQ₆, blue bars) and de novo synthesized CoQ₆ (13C₆-CoQ₆, orange bars) in WT and snf2Δ yeast were determined at the designated hours after labeling with 13C₆-4HB. Error bars, S.D. of n = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT; *, p < 0.05; **, p < 0.005). B, steady-state levels of Snf2 protein in WT cells corresponding to samples from A were determined by immunoblot. Pgk1 served as a loading control. C, steady-state and de novo synthesized levels of DMQ₆, HHB, and HMB were also measured in the 5-6 time course of WT and snf2Δ yeast (Fig. 6, B, C, G, and H). Strikingly, the conversion of de novo DMQ₆ to CoQ₆ increases (as shown by the decreased ratio of 13C₆-DMQ₆ to 13C₆-CoQ₆) in a manner concurrent with the decrease in Snf2 levels and increase in PTC7 splicing in WT (compare Fig. 6D with Fig. 5A). The steady-state and de novo synthesized levels of DMQ₆ and HHB were also measured in the 5-6 time course of WT and snf2Δ yeast (Fig. 6, B, C, G, and H). The role of Ptc7 in the increased synthesis of CoQ₆ in the absence of Snf2 can be inferred from the observation that whereas the conversion efficiency from DMQ₆ to CoQ₆ is higher in the absence of Snf2, the level of DMQ₆ itself does not change appreciably between WT and snf2Δ yeast over the 5-6 time course (Fig. 6, compare B and G). However, the snf2Δ cells also show significantly lower rates of HHB synthesis (Fig. 6, compare C and H), as well as lower ratios of 13C₆-HHB to 13C₆-CoQ₆ content (Fig. 6, compare E and J), consistent with the observation that deletion of Snf2 markedly accelerates the synthesis of CoQ₆, presumably by expediting the conversion of these intermediates to the final product.

RPG down-regulation in general leads to increased PTC7 splicing

Our previous work showed that Snf2-dependent down-regulation of ribosomal protein genes enhances splicing, particularly of genes with nonconsensus splice sites. To determine whether the observed increase in PTC7 splicing is a consequence of RPG down-regulation per se, rapamycin was used to inhibit target of rapamycin (TOR)-dependent RPG transcription in a Snf2-independent manner (28) (Fig. 7A). It has also been previously published that rapamycin mitigates certain mitochondrial disorders in Drosophila and improves lifespan in response to TOR inhibition, purportedly by modulating carbon metabolism (29). In our work, rapamycin treatment led to a significant increase in the splicing of the PTC7 transcript (Fig. 7, B and C). As previously observed, the change in the ratio of Ptc7/Wt/ptc7NH protein (Fig. 1D) is greater than the change in the ratio of spliced to nonspliced transcript upon the deletion of Snf2 (Fig. 1B). This suggests that whereas Snf2-dependent RPG down-regulation changes the splicing of the PTC7 transcript,
SWI/SNF regulates CoQ₆ synthesis via PTC7 splicing

Figure 5. The decrease in Snf2 levels over time in batch cultures of WT yeast correlates with enhanced splicing of PTC7 RNA. A, steady-state levels of Snf2 protein in WT cells corresponding to samples from indicated time points were determined by immunoblot. Pgk1 (phosphoglycerate kinase 1) served as a loading control. B, expression and splicing of PTC7 in WT yeast cells corresponding to samples from A; PCR products represent the spliced and nonspliced forms, as indicated. C, quantification of splicing of PTC7 transcript (black line) in WT yeast cells corresponding to samples from B. Shown is the mean of three biological replicates. Error bars, S.D. D, the Snf2 protein is absent in snf2Δ cells corresponding to samples from the indicated time points as determined by immunoblot. Pgk1 served as a loading control. E, expression and splicing of PTC7 in snf2Δ yeast cells corresponding to samples from D; PCR products representing the spliced and nonspliced forms are indicated. F, quantification of splicing of PTC7 transcript (red line) in snf2Δ yeast cells corresponding to samples from E. Shown is the mean of three biological replicates. Error bars, S.D. (unpaired Student’s t test between corresponding bars for snf2Δ and WT in C, *, p < 0.05; **, p < 0.005).

there are probably additional layers of gene regulation that control the relative levels of the Ptc7ₐ and Ptc7ₐₙ proteins. Experiments probing these mechanisms are currently ongoing. Nonetheless, these results are consistent with a model whereby down-regulation of RPG expression redirects spliceosomes from these abundant transcripts to otherwise poorly spliced transcripts, such as PTC7 (5, 6). In light of the role of Snf2 in RPG expression, changes in Snf2 levels allow fine-tuning of splicing in response to the cell’s metabolic needs.

Ptc7 isoforms have differing and opposing effects on CoQ₆ synthesis

The predicted structures of the two isoforms of Ptc7, Ptc7ₐ and Ptc7ₐₙ, have been modeled (Fig. 8, A and B). In fact, the Ptc7ₐₙ contains a transmembrane helix, encoded for by the PTC7 intron, which is capable of spanning the nuclear membrane. Overall, the presence of this transmembrane helix is not predicted to influence the folding of the rest of the protein, thus potentially retaining its phosphatase activity (Fig. 8).

To determine the effect of each Ptc7 isoform on CoQ₆ synthesis, we assayed CoQ₆ levels in cells expressing both forms of Ptc7, Ptc7ₐ only, Ptc7ₐₙ only, or neither (ptc7Δ). As reported previously, there is no significant change in CoQ₆ synthesis levels in the ptc7Δ mutant (12, 30). However, exclusive expression of Ptc7ₐ leads to an increase in CoQ₆ synthesis, whereas exclusive expression of Ptc7ₐₙ leads to a decrease in CoQ₆ synthesis (Fig. 9A). The relative RNA levels from each strain are shown (Fig. 9B). Moreover, there are no significant changes observed in the protein levels of Snf2 or Coq7, the target of Ptc7 activity (Fig. 9C), in these strains. Whereas each of these isoforms was expressed within the endogenous context and from the endogenous PTC7 promoter, protein levels of the Ptc7ₐₙ appeared to be increased relative to the other isoforms (Fig. 9, B and C), perhaps due to a cellular feedback mechanism that increases expression or enhances stability of Ptc7ₐₙ.

The steady-state and de novo synthesized levels of CoQ₆ were also measured in a 5-h time course with the yeast strains expressing either Ptc7ₐₙ or Ptc7ₐ. Both steady-state and de novo CoQ₆ biosynthesis are significantly lower in Ptc7ₐₙ strain than in the Ptc7ₐ and in fact appear to be actively repressed, suggesting that the two isoforms of Ptc7 have differing and opposing effects on CoQ₆ biosynthesis (Fig. 9D). In addition, the exclusive presence of Ptc7ₐₙ causes increased de novo biosynthesis of ¹³C-CoQ₆ as compared with the exclusive presence Ptc7ₐₙ (Fig. 9D). Whereas the positive effect of Ptc7ₐₙ on CoQ₆ biosynthesis is consistent with the mechanisms of Ptc7 action described previously, it is clear that Ptc7ₐₙ has a repressive effect on CoQ₆ biosynthesis (compare Ptc7ₐₙ and ptc7Δ in Fig. 9A). To begin to elucidate the mechanism of this repression, we assayed the mRNA transcript levels of genes encoding components of the CoQ₆ biosynthetic complex (viz. COQ1−11 and PTC7). On average, there is little change in the expression of the complex upon deletion of Snf2 (Fig. 9, G and H) or with the exclusive expression of Ptc7ₐₙ or ptc7Δ. However, exclusive expression of Ptc7ₐₙ is associated with pronounced down-regulation of every
SWI/SNF regulates CoQ₆ synthesis via PTC7 splicing
member of the CoQ-synthome (Fig. 9, E and F). Although the mechanism by which these components are down-regulated is unclear, it is interesting that Ptc7\textsubscript{ns} has previously been localized to the nuclear membrane (10), hinting at a novel role for this isoform in expression of the RNAs encoding the CoQ-synthome. Two possible mechanisms by which nucleus-localized Ptc7\textsubscript{ns} may affect synthesis of the CoQ-synthome are via direct action on nucleus-localized Coq7 or via indirect effects on gene expression. It is important to mention here that to the best of our knowledge, no reports have demonstrated nuclear localization of, or a nuclear role for, Coq7 in \textit{S. cerevisiae}.

Interestingly, yeast strains engineered to express either Ptc7\textsubscript{s} or Ptc7\textsubscript{ns} still retain the ability to grow on medium containing a non-fermentable carbon source, as do ptc7Δ null mutants (data not shown). This is consistent with our prior observations that \textasciitilde1–10\% of CoQ\textsubscript{6} levels are sufficient for comparable growth on medium containing a nonfermentable carbon source. It has been postulated that residual CoQ\textsubscript{6} levels are observed due to the overlapping activities of Ptc5 and/or Ptc6, and in fact the ptc5Δ ptc7Δ double null mutant has impaired growth under conditions of temperature stress (11, 31). It is also worth noting that unlike the deletion of SNF2, the conversion efficiencies or ratios between the early components of the pathway (DMQ\textsubscript{6} or HHB) and CoQ\textsubscript{6} do not vary between strains exclusively expressing either Ptc7 isoform (Fig. 10, C and D). This is because although there are significant changes in the levels of \textit{de novo} synthesized DMQ\textsubscript{6}, as well as HHB when comparing Ptc7\textsubscript{s} to Ptc7\textsubscript{ns} (Fig. 10, A and B), Ptc7\textsubscript{s} is synthesizing higher levels of \textit{de novo} CoQ\textsubscript{6}, DMQ\textsubscript{6}, and HHB, compared with overall lower levels of these same lipids in Ptc7\textsubscript{ns} (Fig. 10, C and D). Thus, the overall conversion efficiencies (ratios) between both isoforms are comparable (Fig. 10, C and D). This is consistent with our interpretation that the absence of Snf2 contributes to the metabolic state of the cell in other ways in addition to its role in regulation of the Ptc7 isoforms.

Figure 6. Overall conversion efficiency of the CoQ\textsubscript{6} biosynthetic pathway increases upon depletion of Snf2, with increased conversions of both DMQ\textsubscript{6} to Q\textsubscript{6} and HHB to Q\textsubscript{6}. A, levels of steady-state CoQ\textsubscript{6} (\textit{13}C\textsubscript{6}-CoQ\textsubscript{6}, blue bars) and \textit{de novo} synthesized CoQ\textsubscript{6} (\textit{13}C\textsubscript{6}-CoQ\textsubscript{6}, orange bars) in WT yeast cells were determined at the designated hours after labeling with \textit{13}C\textsubscript{6}-4HB. Error bars, S.D. of \textit{n} = 3 biological replicates. B, levels of steady-state DMQ\textsubscript{6} (\textit{13}C\textsubscript{6}-DMQ\textsubscript{6}, blue bars) and \textit{de novo} synthesized DMQ\textsubscript{6} (\textit{13}C\textsubscript{6}-DMQ\textsubscript{6}, orange bars) in WT yeast were determined at the designated hours after labeling with \textit{13}C\textsubscript{6}-4HB. Error bars, S.D. of \textit{n} = 3 biological replicates. C, levels of steady-state HHB (\textit{13}C\textsubscript{6}-HHB, blue bars) and \textit{de novo} synthesized (\textit{13}C\textsubscript{6}-HHB, orange bars) in WT and snf2Δ yeast were determined at the designated hours after labeling with \textit{13}C\textsubscript{6}-4HB. Error bars, S.D. of \textit{n} = 3 biological replicates. D, the ratio of \textit{13}C\textsubscript{6}-DMQ\textsubscript{6}/\textit{13}C\textsubscript{6}-CoQ\textsubscript{6} in WT yeast was determined at the designated hours after labeling with \textit{13}C\textsubscript{6}-4HB. Error bars, S.D. of \textit{n} = 3 biological replicates. E, the ratio of \textit{13}C\textsubscript{6}-HHB/\textit{13}C\textsubscript{6}-CoQ\textsubscript{6} in WT yeast was determined at the designated hours after labeling with \textit{13}C\textsubscript{6}-4HB. Error bars, S.D. of \textit{n} = 3 biological replicates. F, levels of steady-state CoQ\textsubscript{6} (\textit{13}C\textsubscript{6}-CoQ\textsubscript{6}, blue bars) and \textit{de novo} synthesized (\textit{13}C\textsubscript{6}-CoQ\textsubscript{6}, orange bars) in snf2Δ yeast cells were determined at the designated hours after labeling with \textit{13}C\textsubscript{6}-4HB. Error bars, S.D. of \textit{n} = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT in A; *, \textit{p} < 0.05; **, \textit{p} < 0.005; ***, \textit{p} < 0.0005). G, levels of steady-state DMQ\textsubscript{6} (\textit{13}C\textsubscript{6}-DMQ\textsubscript{6}, blue bars) and \textit{de novo} synthesized DMQ\textsubscript{6} (\textit{13}C\textsubscript{6}-DMQ\textsubscript{6}, orange bars) in snf2Δ yeast were determined at the designated hours after labeling with \textit{13}C\textsubscript{6}-4HB. Error bars, S.D. of \textit{n} = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT in B; *, \textit{p} < 0.05). H, levels of steady-state HHB (\textit{13}C\textsubscript{6}-HHB, blue bars) and \textit{de novo} synthesized HHB (\textit{13}C\textsubscript{6}-HHB, orange bars) in snf2Δ yeast were determined at the designated hours after labeling with \textit{13}C\textsubscript{6}-4HB. Error bars, S.D. of \textit{n} = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT in C; *, \textit{p} < 0.05; **, \textit{p} < 0.005; ***, \textit{p} < 0.0005). I, the ratio of \textit{13}C\textsubscript{6}-DMQ\textsubscript{6}/\textit{13}C\textsubscript{6}-CoQ\textsubscript{6} in snf2Δ yeast cells was determined at the designated hours after labeling with \textit{13}C\textsubscript{6}-4HB. Error bars, S.D. of \textit{n} = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT in D; *, \textit{p} < 0.05; **, \textit{p} < 0.005; ***, \textit{p} < 0.0005). The 0-h time point is excluded, because the ratio is not indicative of pathway conversion. J, the ratio of \textit{13}C\textsubscript{6}-HHB/\textit{13}C\textsubscript{6}-CoQ\textsubscript{6} in snf2Δ yeast cells was determined at the designated hours after labeling with \textit{13}C\textsubscript{6}-4HB. Error bars, S.D. of \textit{n} = 3 biological replicates (unpaired Student’s t test between corresponding bars for snf2Δ and WT in E; *, \textit{p} < 0.05; **, \textit{p} < 0.005). The 0-h time point is excluded, because the ratio is not indicative of pathway conversion.
SWI/SNF regulates CoQ₆ synthesis via PTC7 splicing

Figure 8. Structural predictions of mitochondrial Ptc7s and nuclear membrane traversing Ptc7ₙₙs. A, PHYRE2 homology modeling of mature mitochondrial Ptc7s, which is experimentally determined to start at amino acid Gly39 (46). 85% of residues modeled at >90% confidence (15% of residues modeled ab initio). The N terminus and C terminus of the protein are shown. B, PHYRE2 homology modeling of nuclear membrane Ptc7ₙₙs. The predicted trans-membrane helix encoded by the intron is shown in cyan. 86% of residues modeled at >90% confidence (14% of residues modeled ab initio). To show the interaction with the nuclear membrane, the N-terminal loop residing on the one side of the nuclear membrane is proposed to be linked to the modeled transmembrane helix, which is then proposed to be linked to the rest of the Ptc7 protein that is predicted to reside on the alternate side of the nuclear membrane. The nine black dashes connecting the helix to the larger portion of the protein represent nine amino acids in the intron that were in an unmodeled region.

These data reveal a novel role for Snf2 in respiration and specifically in the transition from a largely fermentative mode of metabolism to a largely respiratory one in *S. cerevisiae*, as shown by the model in Fig. 11. Under conditions of high nutrient availability, Snf2-dependent transcription of intron-rich RPGs sequesters spliceosomes away from transcripts with weak splice sites, such as *PTC7*. As a consequence, both isoforms of the Ptc7 protein are expressed at appreciable levels, and their opposing effects on CoQ₆ synthesis ensure that CoQ₆ is maintained at a relatively low level. As the nutrients in the medium are depleted, the levels of Snf2 and, consequently, RPG transcripts, decrease concurrently, freeing up spliceosomes to act on *PTC7*. This leads to better splicing of *PTC7* and a shift in the relative abundances of the two protein isoforms, which eventually leads to an increase in CoQ₆ synthesis.

**Discussion**

Whereas it has been broadly acknowledged that chromatin states and chromatin factors influence splicing outcomes in various organisms, identifying the functional importance of such regulation under biologically relevant conditions remains a challenge. We have shown previously that down-regulation of Snf2, the core ATPase component of the SWI/SNF chromatin-remodeling complex, in response to nutrient depletion leads to a change in cellular splicing outcomes due to down-regulation of RPGs and subsequent redistribution of spliceosomes (5, 6). We show here that Snf2-dependent changes in splicing of *PTC7* during yeast growth, combined with the general conditions in the cell in the absence of Snf2, causes a shift in the ratio of two distinct isoforms of the Ptc7 protein that have distinct and opposing effects on CoQ₆ biosynthesis. This change in the ratio of the isoforms is concomitant with an increase in CoQ₆ levels in the cell, preparing for the transition from a largely fermentative to a respiratory mode of metabolism.

Previous studies have presented contradictory evidence regarding the involvement of *PTC7* in CoQ₆ biosynthesis. Ptc7 is required for the dephosphorylation of Coq7, thus transitioning Coq7 to its “active” form, which is able to catalyze the penultimate step of the CoQ₆ pathway. This led to the prediction that the *ptc7Δ* strain would demonstrate decreased CoQ₆ synthesis, as assayed by quantification of lipids from purified mitochondria (11). Surprisingly, although Ptc7 supports general respiratory function, the absence of *PTC7* does not lead to a deficiency in CoQ₆ levels, as assayed in lipid extracts of whole cells (12) (Fig. 9A). The studies here help to resolve this apparent contradiction. Studies with the *ptc7Δ* cells fail to address the opposing roles that the two Ptc7 isoforms have in the cell under WT conditions. Only cells with the capacity to express both Ptc7s and Ptc7ₙₙs can accurately reflect the full extent of Ptc7 function. We demonstrate that exclusive expression of Ptc7ₙₙs has a significant repressive effect on CoQ₆ biosynthesis (Fig. 9, A and D). Notably, the rates of conversions from precursors in the pathway to the final product remained unchanged, indicating down-regulation of the entire pathway (Fig. 10, C and D). Consistent with this, we observe down-regulation of almost all of the components of the CoQ₆ biosynthetic complex upon exclusive expression of Ptc7ₙₙs (Fig. 9F). The mechanism by which Ptc7ₙₙs affects RNA expression is as yet unknown, and investigations to understand the same are ongoing.

*PTC7* is not the only known example of a gene in *S. cerevisiae* encoding functional proteins from both the nonspaced pre-mRNA as well as the “mature” spliced mRNA (10). We recently reported translation of unspliced GCR1 pre-mRNA leading to a functional Gcr1 protein, although in this case, translation starts from within the retained intron (7). Whereas the read-through nature of the intron is conserved across most Saccharomyces species, the intron is excised in the same species (analysis of publicly available RNA-seq data sets; data not shown), rendering it likely that both forms of the protein are necessary and functional. This is illustrated in the case of Tetrapisipora blattae, which, like *S. cerevisiae*, underwent a whole genome duplication event; but unlike *S. cerevisiae*, which lost the duplications of most of its genes, *T. blattae* retains two copies of the *PTC7* gene. Interestingly, the two *PTC7* genes in *T. blattae* subfunctionalized into a gene that encodes a mitochondrial PP2C (Ptc7ₙₙs) from a spliced transcript of *PTC7b* con-
taining a stop codon within its intron) and a second gene encoding a PP2C predicted to localize to the nuclear envelope (Ptc7, from an nonspliced transcript of PTC7a (32). This conservation further suggests that both protein isoforms derived from the PTC7 transcript in S. cerevisiae are functional. Cells lacking Ptc7ns show increased sensitivity to latrunculin A treatment, compared with strains expressing both isoforms of Ptc7 or lacking Ptc7s (10). Such sensitivity might suggest a distinct role for Ptc7ns in actin filament formation.

It is noteworthy that nuclear roles for numerous metabolic enzymes have been described previously. The ability of metabolic enzymes to “moonlight” in the nucleus, affecting gene regulation at various steps, appears to be crucial for the ability of cells to sense and adapt to their potentially distinct nutrient environments (33). Numerous mitochondrial enzymes, such as succinate dehydrogenase, fumarase, aconitase, and malate dehydrogenase (all components of the Krebs cycle), have been shown to have significant nuclear roles in the regulation of gene expression (34–38). In some of these cases, enzymatic activity of these enzymes has been shown to be crucial to their nuclear roles (39). This precedence, combined with the evolutionarily conserved presence of an isoform of Ptc7 in the nuclear membrane, raises the possibility that a nucleus-localized phosphatase is crucial to regulation of components of the CoQ6 biosyn-
SWI/SNF regulates CoQ₆ synthesis via PTC7 splicing

Figure 10. Exclusive expression of Ptc7 isoforms dramatically alters levels of CoQ₆ biosynthetic pathway intermediates DMQ₆ and HHH, yet overall conversion efficiency between both isoforms is comparable. A, levels of steady-state DMQ₆ (1³C₆-DMQ₆, blue bars) and de novo synthesized DMQ₆ (1³C₆-DMQ₆, orange bars) in PTC7,HA and PTC7sHA yeast cells were determined at the designated hours after labeling with 1³C₆-4HB. Error bars, S.D. of n = 3 biological replicates (unpaired Student's t test between corresponding bars for PTC7,HA and PTC7sHA; *, p < 0.05; **, p < 0.005; ***, p < 0.0005). B, levels of steady-state HHH (1³C₆-HHH, blue bars) and de novo synthesized HHH (1³C₆-HHH, orange bars) in PTC7,HA and PTC7sHA yeast cells were determined at the designated hours after labeling with 1³C₆-4HB. Error bars, S.D. of n = 3 biological replicates (unpaired Student's t test between corresponding bars for PTC7,HA and PTC7sHA; *, p < 0.05; **, p < 0.005). C, ratio of 1³C₆-DMQ₆/1³C₆-CoQ₆ in PTC7,HA and PTC7sHA yeast cells were determined at the designated hours after labeling with 1³C₆-4HB. Ratios were derived from levels of 1³C₆-CoQ₆, as shown in Fig. 7D. Error bars, S.D. of n = 3 biological replicates. The 0-h time point is excluded, because the ratio is not indicative of pathway conversion. D, ratio of 1³C₆-HHH/1³C₆-CoQ₆ in PTC7,HA and PTC7sHA yeast cells were determined at the designated hours after labeling with 1³C₆-4HB. Ratios were derived from levels of 1³C₆-CoQ₆, as shown in Fig. 7D. Error bars, S.D. of n = 3 biological replicates. The 0-h time point is excluded, because the ratio is not indicative of pathway conversion.

Theoretic pathway. Intriguingly, CLK-1 and COQ7, the C. elegans and human homologs of Coq7, which is a target for Ptc7 in S. cerevisiae (11), have been demonstrated to localize to the nucleus and are postulated to have roles independent of CoQ biosynthesis (40). COQ7 has also been shown to associate with chromatin in HeLa cells (40), although recently this has been attributed to a transformed cell phenomenon (41). Whereas nuclear localization of Coq7 in S. cerevisiae has not been demonstrated, we suggest a potential role in nuclear gene regulation by the increased conversion of early precursors. Whereas deletion of Snf2 does not, on average, change the expression of the components of the CoQ₆-synthome (Fig. 9, G and H), it is possible the Snf2 may have other effects of CoQ₆ flux. We are exploring these possibilities.

Intriguingly, although the absence of Snf2 enhances levels of CoQ₆, yeast strains lacking Snf2 have a severe growth defect on non-fermentable carbon sources, such as glycerol or acetate (26) (Fig. 1E). However, Snf2 protein is undetectable by immunoblotting during growth in medium containing glycerol or acetate as the only carbon source (data not shown). This leads us to hypothesize that before Snf2 protein is down-regulated in response to glucose depletion, it is required for the transition from a fermentative metabolic state to one that is predominantly respiratory in nature. The molecular details of the requirement for Snf2 in this transition are the subject of ongoing investigation. However, it is probably at least in part due to its reported role in the activation (de-repression) of genes whose expression had previously been subject to glucose-mediated catabolite repression.
We postulate that once the gene expression profile required for adaptation to the new nutrient environment has been initiated and/or established, the requirement for Snf2 is relieved, and in fact, the down-regulation of Snf2 enhances splicing of $PTC7$. This transient requirement for Snf2 bears striking similarities to a previous report detailing the role of Snf2 in reversing Ume6-mediated repression at certain meiotic genes early in meiosis, before it is itself down-regulated to enable splicing of meiotic transcripts (6).

This work reveals a mechanism by which SWI/SNF acts as a nexus point in the fermentation–respiratory transition in $S.\ ceriseiia$. We also demonstrate opposing effects of isoforms of a single gene, $PTC7$, on the process of CoQ biosynthesis, via distinct mechanisms. Numerous aspects of these mechanisms remain to be studied, as well as their potential roles in the gene regulation response to other physiological conditions that yeast might experience.

Figure 11. Model for a novel role for Snf2 in respiration, and in the transition from a primarily fermentative mode of metabolism to a primarily respiratory mode of metabolism. A, during $S.\ ceriseiia$ batch growth, the abundance of Snf2 decreases in conjunction with depletion of nutrients in the medium. RPGs under the control of Snf2 are down-regulated, allowing redistribution of spliceosomes to other poorly spliced transcripts. Splicing of the $PTC7$ transcript increases, enhancing the ratio of $Ptc7_s$/Ptc7$_m$ and overall levels of Ptc7.. These changes in Ptc7 isoform levels lead to increased conversion of DMQ$_6$ and increased synthesis of CoQ$_6$. The darker arrow represents a greater effect or reaction conversion, whereas a lighter arrow represents a smaller effect or reaction conversion. B, Ptc7$_{ns}$ has a repressive effect on CoQ$_6$ biosynthesis. CoQ$_6$ levels are low or high, depending on the levels of the Ptc7$_m$ isoform relative to Ptc7.. Similar to A, darker arrows and bars denote a larger effect, whereas lighter arrows and bars denote a smaller effect.

Experimental procedures

Yeast strains and culture conditions

The yeast strains used in this study are listed in Table 1. All strains except W303Δcoq2 are derived from the BY background. Yeast strains were grown in YPD (1% yeast extract, 2% peptone, 2% dextrose) medium at 30 °C. Snf2 and Ptc7 null strains were maintained with a backup expression plasmid (pRS316 backbone harboring either $SNF2$ or $PTC7$). The plasmid was shuffled out by growth on 5-fluoroorotic acid before using the strains in experiments. Strains with tagged isoforms of Ptc7 were a kind gift from Dr. Ron Davis (10). These strains were back-crossed against WT or snf2Δ strains, and daughter strains used for this study are listed in Table 1. The snf2Δ strain was observed to spontaneously mutate if grown on YPD for longer than 7–8 days, acquiring suppressor mutations that made it difficult to distinguish from WT. Hence, for all experiments with snf2Δ, the plasmid contain-
SNF2 was shuffled out prior to each experiment, allowing a fresh snf2Δ strain with each experiment, to avoid these suppressor mutants. We found that this was absolutely instrumental to observe the proper phenotype and behavior of the snf2Δ strain.

**RNA-sequencing analyses**

The RNA-sequencing data reported in this study were generated previously (6). Briefly, RNA sequencing libraries were prepared using the Illumina Truseq® V3 kit and ribosomal RNA depletion (Ribo-Zero, Illumina). Single-end, 50-nucleotide sequence reads (HiSeq 2000) were aligned to SacCer3 and spliced transcripts from the Ares Lab Yeast Intron Database version 3 (42) in a single step using STAR (43). Only the highest scoring alignments for each read were kept, allowing for at most a single tie. Reads/kb/million were computed for each gene by dividing the total number of reads that aligned entirely within the gene’s exon boundaries by the gene’s total exon length in kilobase pairs per million mapped reads. Reads within ICGs were categorized as exonic, spliced, or unspliced. Exonic reads map entirely within an exon, as defined by the Ares Lab Yeast Intron Database. Introns with annotated small nucleolar RNAs within the defined intron boundaries were disregarded in this analysis. Spliced reads are those that align with a gap that corresponds to an annotated intron, and unspliced reads map partially within an exon and partially within an intron with no gap. Spliced and unspliced read counts were normalized by dividing total spliced counts by the number of potential unique alignment positions that contribute to the total. For spliced reads, this is read length minus one for every intron. For unspliced read counts, this is the length of the intron plus the read length minus one. Splicing efficiency for each intron was calculated as normalized spliced divided by the sum of the normalized spliced and normalized unspliced counts. Changes in splicing efficiency were calculated as percentage difference over WT efficiency and plotted against expression levels (reads/kb/million) in WT. Data are available under GEO accession number GSE94404, and detailed methods were described previously (6).

**RT-PCR and real-time PCR analysis**

RNA was isolated from a 5-ml aliquot of cell culture corresponding to time points described in each experiment. After DNase treatment (Roche Applied Science), equal quantities of total RNA from each sample were used to make cDNA using a cDNA synthesis kit (Fermentas). To detect PTC7 splicing isoforms, primers flanking the intronic sequences were used for PCR using 1 μl of cDNA diluted 1:20. PCR products were then separated on a 2% agarose gel and imaged. RT-qPCR was done in a 10-μl reaction volume with gene-specific primers using 1 μl of cDNA diluted 1:20 using Perfecta SYBR Green Fastmix (Quanta Biosciences) and a CFX96 Touch System (Bio-Rad). All samples were analyzed in triplicate for each independent experiment. RT-qPCR was also performed for the scRI (cytoplasmic signal recognition particle RNA subunit) RNA from each cDNA sample. Gene expression analysis was done by 2^−ΔΔCt methods using scRI as a reference. -Fold expression of mRNA was measured compared with WT by 2^−ΔΔCt methods (44).

**ImmunobLOTS**

Protein was isolated from cell pellets with FA-1 lysis buffer (50 mm HEPES, pH 7.5, 150 mm NaCl, 1 mm EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 1 mm PMSF, and protease inhibitors) with bead beating. The buffer was supplemented with protease inhibitor mixture tablet (Roche Applied Science). Total protein was resolved by SDS-PAGE. The gel was transferred to PVDF membrane, and proteins were detected with the following antibodies at the stated dilutions: anti-SNF2 antibody (yN-20, Santa Cruz Biotechnology) at a 1:200 dilution in 2% milk, anti-HA antibody (901514, BioLegend) at a 1:2000 dilution in 5% milk, anti-Pgk1 antibody (459250, Invitrogen) at a 1:3000 dilution in 5% milk, or anti-Coq7 antibody (described previously (16) at a 1:2000 dilution in 3% milk. Signal was detected with enhanced chemiluminescence (Thermo Scientific) as described by the manufacturer.

**Metabolic labeling of CoQ6 with 13C6-labeled precursors**

Yeast strains were grown overnight in 25 ml of YPD in a shaking incubator (30 °C, 250 rpm) and diluted to an A600 of 0.1 in 60 ml of fresh YPD the next morning. The cultures were incubated as before to an A600 of 0.5 (midlog phase) and subsequently treated with 13C6-4HB at 10 μg/ml (600 μg total) or ethanol vehicle control (0.015%, v/v). At designated time periods, cells were harvested by centrifugation at 3000 × g for 5 min, from 50-ml aliquots (used for lipid extraction) or 10-ml aliquots (used for RNA and protein analysis). Cell pellets were stored at −20 °C.

**Analysis of CoQ6 and CoQ6 intermediates**

Lipid extraction of cell pellets was conducted as described (18) with methanol and petroleum ether and CoQ6 as the internal standard. Lipid measurements were performed by HPLC-
MS/MS and normalized to total OD. Prior to mass spectrometry analysis, all samples were treated with 1.0 mg/ml benzoquinone to oxidize hydroquinones to quinones. Mass spectrometry analyses utilized a 4000 QTRAP linear MS/MS spectrometer (Applied Biosystems), and data were acquired and analyzed using Analyst version 1.4.2 and 1.5.2 software (Applied Biosystems). Separation of lipid quinones was performed with a binary HPLC delivery system and a Luna 5μm phenyl-hexyl column (100 x 4.6 mm, 5 μm; Phenomenex). The mobile phase consisted of a 95:5 methanol/isopropyl alcohol solution with 2.5 mM ammonium formate as solution A and a 100% isopropyl alcohol solution with 2.5 mM ammonium formate as solution B. The percentage of solution B was increased linearly from 0 to 5% over 6 min, whereby the flow rate was increased from 600 to 800 μl. Initial flow rate and mobile phase conditions were changed back to initial phase conditions linearly over 3.5 min. Each sample was analyzed using multiple-reaction monitoring mode. The following precursor-to-product ion transitions were detected as well as the +17 m/z ammoniated adducts for each of the metabolic products: 13C6-HHB m/z 553.4/157.0 (ammoniated: 570.4/157.0), 12C-HHB m/z 547.4/151.0 (ammoniated: 564.4/151.0), 13C6-DMQ m/z 567.6/173.0 (ammoniated: 584.6/173.0), 12C-DMQ m/z 561.6/167.0 (ammoniated: 578.6/167.0), 13C6-CoQ m/z 597.4/203.1 (ammoniated: 614.4/203.1), 12C-CoQ m/z 591.4/197.1 (ammoniated: 608.4/197.1), and 12C-CoQ m/z 455.4/197.0 (ammoniated: 472.4/197.0).

Plate dilution assays

Strains were grown overnight in 5 ml of YPD and diluted to an A600 of 0.2 in sterile PBS. A 5-fold serial dilution in PBS was performed, after which 2 μl of each dilution (1×, 5×, 25×, 125×, and 625×) were spotted onto the designated carbon sources. The final A600 of the aforementioned dilution series are 0.2, 0.04, 0.008, 0.0016, and 0.00032, respectively.

PHYRE homology modeling

PHyRE2 is a modeling program designed to analyze protein structure, function, and mutations (45). It is used to analyze the primary sequence of a protein and, with homology detection methods, constructs a structure that compares the protein of primary sequence of a protein and, with homology detection, structure, function, and mutations (45). It is used to analyze the structure and alignment coverage contains 86% of residues modeled at >90% confidence, with 14% of residues modeled ab initio. Additionally, the spliced isoform of Ptc7 (Ptc7sh), which is localized and processed in the mitochondria, comprised of 305 amino acids, resulting from the removal of the 31-amino acid intron and the excision of the predicted mitochondrial targeting sequence (the 38 N-terminal amino acids of Ptc7, 46), was also modeled using the PHYRE2 intensive modeling mode. The resulting structure and alignment coverage contains 85% of residues modeled at >90% confidence, with 15% of residues modeled ab initio.

Author contributions—A. M. A. and S. V. contributed equally to this work (both conducted the majority of the experiments, analyzed the results, and wrote the paper together). A. N. and M. C. B. assisted A. M. A. in conducting experiments; A. N. assisted A. M. A. in analyzing mass spectrometry results. A. R. G. and L. N. assisted S. V. in experiments, with A. R. G. also helping in the background research relating to PTC7 differential splicing in the snf2Δ strain shown in Fig. 1A. S. D. aligned the RNA-sequencing data and calculated splicing efficiencies. M. C. B. and L. N. thoroughly read and edited the working draft of the paper. C. F. C. and T. L. J. oversaw all details related to the project and provided guidance on experiments, data analysis, and the writing of this paper.

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