Molecular Cell
Article

Competition between Pre-mRNAs for the Splicing Machinery Drives Global Regulation of Splicing

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http://dx.doi.org/10.1016/j.molcel.2013.06.012

SUMMARY

During meiosis in yeast, global splicing efficiency increases and then decreases. Here we provide evidence that splicing improves due to reduced competition for the splicing machinery. The timing of this regulation corresponds to repression and reactivation of ribosomal protein genes (RPGs) during meiosis. In vegetative cells, RPG repression by rapamycin treatment also increases splicing efficiency. Downregulation of the RPG-dedicated transcription factor gene *IFH1* genetically suppresses two spliceosome mutations, *prp11-1* and *prp4-1*, and globally restores splicing efficiency in *prp4-1* cells. We conclude that the splicing apparatus is limiting and that pre-messenger RNAs compete. Splicing efficiency of a pre-mRNA therefore depends not just on its own concentration and affinity for limiting splicing factor(s), but also on those of competing pre-mRNAs. Competition between RNAs for limiting processing factors appears to be a general condition in eukaryotes for a variety of posttranscriptional control mechanisms including microRNA (miRNA) repression, polyadenylation, and splicing.

INTRODUCTION

Pre-mRNA splicing is a fundamental step of eukaryotic gene expression. It can vary in complexity from removal of a single intron to elaborate patterns of alternative splicing that create multiple distinct messenger RNAs (mRNAs). This complex set of mRNAs diversifies the functionalities of proteins that can be produced from a gene. Alternative splicing patterns arise from differences in key pre-mRNA features, such as splice site strength (Roca et al., 2005; Yeo and Burge, 2004), secondary structure (Hiller et al., 2007; Howe and Ares, 1997; Kreahling and Graveley, 2005; Plass et al., 2012; Shepard and Hertel, 2008), or transcription elongation rates (de la Mata et al., 2003; Howe et al., 2003; Kornblihtt, 2005; Roberts et al., 1998), as well as *trans*-acting splicing factors that bind pre-mRNA to differentially enhance or repress spliceosome recruitment (Black, 2003; Nilsen and Graveley, 2010). The regulation of alternative splicing is generally attributed to the changing activities of *trans*-acting splicing factors that control the likelihood of local spliceosome assembly.

Recent studies have attempted to capture the regulatory networks for individual splicing factors, usually by depleting or overexpressing a specific splicing factor and measuring changes in alternative splicing across the genome. Combining analyses of the global differences in tissue-specific alternative splicing (e.g., Barbosa-Morais et al., 2012; Merkin et al., 2012; Pan et al., 2004, 2008; Sugnet et al., 2006; Wang et al., 2008), tissue-specific splicing factor expression (e.g., Buckanovich et al., 1993; Calarco et al., 2009; Jin et al., 2003; Markovtsov et al., 2000; Underwood et al., 2005; Warzecha et al., 2009), and changes in splicing factor expression and splicing during differentiation (e.g., Boutz et al., 2007; Gabut et al., 2011; Kalsotra et al., 2008) reveals that alternative splicing is deeply integrated into the gene expression programs that define cell identity and state. To understand gene expression, splicing regulatory networks must be connected with transcriptional and posttranscriptional regulatory networks (reviewed in Kalsotra and Cooper, 2011), such as those of microRNAs (miRNAs), so that the contribution of splicing regulation to a change in cell identity or state can be understood. A largely ignored aspect of splicing regulation concerns systems-level accounting of substrate concentrations and availability of required factors. Recent reports suggest competition phenomena in splicing (Berg et al., 2012; Du et al., 2010; Kaida et al., 2010; Kanadia et al., 2003; Yin et al., 2012), indicating that splicing may also be regulated by changes in competition for a fixed level of factor activity.

In a previous study of meiosis in *Saccharomyces cerevisiae*, we identified relationships between two transcriptional regulatory networks and the Mer1 splicing regulatory network and examined the roles of the four target transcripts controlled by the Mer1 splicing factor (Munding et al., 2010). We also observed a general increase in splicing efficiency during meiosis (see also Juneau et al., 2007) that we could not assign to any particular *trans*-acting factor. Here, we identify the molecular basis for this improvement and provide evidence that the global increase in splicing is due to relief of competition for the splicing apparatus that occurs during the repression of ribosomal protein genes (RPGs) early in meiosis. This phenomenon is not restricted to meiosis, since blocking RPG transcription with rapamycin in vegetative cells also improves splicing. Downregulating

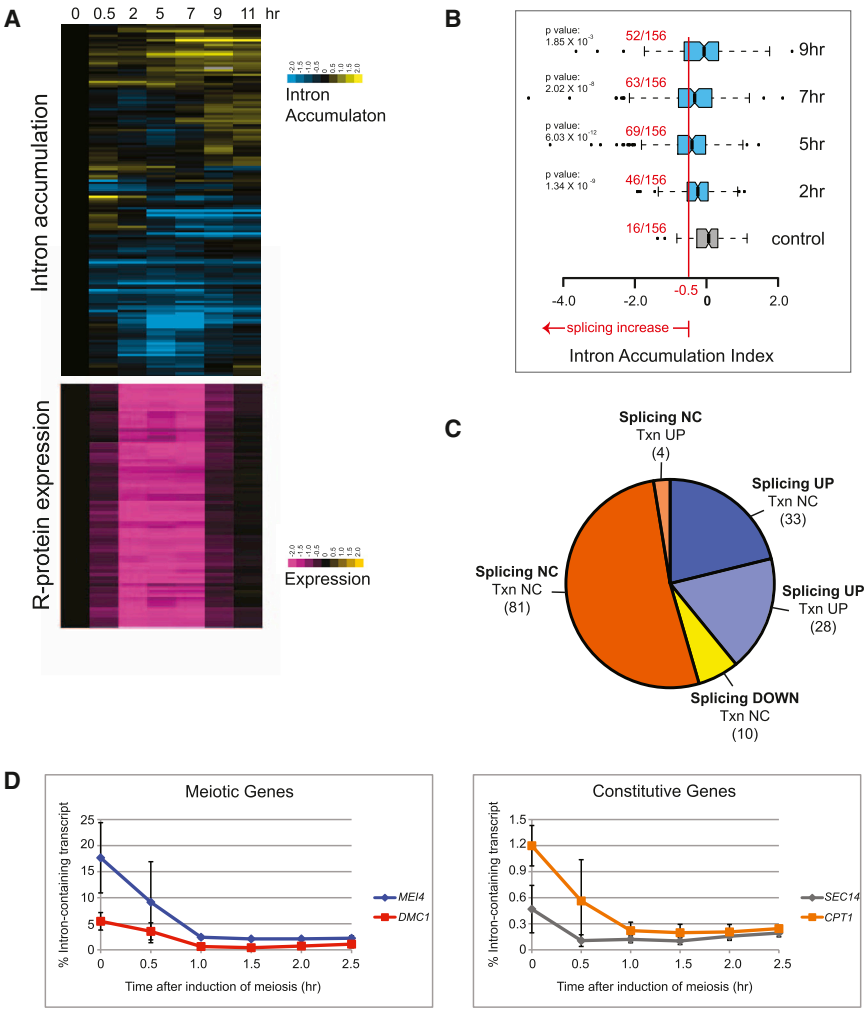

Figure 1. Splicing Improves Globally during Mid-Meiosis

(A) Top: changes in splicing during the meiotic time course as represented by intron accumulation indices. Increased intron accumulation (yellow) represents a decrease in splicing, while decreased intron accumulation (blue) indicates an increase in splicing. See Table S1 for data file. Bottom: changes in RPG expression during the meiotic time course. Purple represents a decrease in gene expression.

(B) Distribution of intron accumulation indices from the microarray data at 2, 5, 7, and 9 hr meiotic time points relative to the zero time point, and a control distribution from self comparison of replicates (see Experimental Procedures). Red line marks a 40% increase in splicing efficiency (IAI < -0.5) used as a threshold for significant splicing change. Numbers in red indicate the fraction of events in each distribution that exceeded the threshold. p values are derived from a one-tailed t test comparison of the individual 2, 5, 7, or 9 hr distributions to the control.

(C) Classification of splicing changes at mid-meiotic time points (2, 5, and 7 hr) for the 156 events whose expression does not decrease more than 2-fold during mid-meiosis. Bold letters indicate splicing change. NC indicates no change. Txn UP indicates genes that are transcriptionally induced $\geq$2-fold during mid-meiosis. Txn NC indicates genes whose expression changes $\leq$2-fold during mid-meiosis. Numbers in parentheses indicate the number of genes in each category.

(D) RT-qPCR measurement of percent of intron-containing transcript at the indicated time after induction of meiosis for two meiosis-specific genes (left panel) and two constitutively expressed genes (right panel). Error bars represent ± 1 SD. See also Table S1.

transcription of RPGs suppresses temperature-sensitive (ts) growth of the *prp4-1* and *prp11-1* spliceosome mutations and rescues splicing defects for nearly all intron-containing genes. These results imply that competition for limiting splicing machinery can be exploited to control splicing of less competitive substrates through transcriptional control of the overall substrate pool.

RESULTS

A Global Increase in Splicing during Meiosis

Splicing of numerous meiosis-specific transcripts, including four that depend on the meiosis-specific splicing factor Mer1 (Cooper et al., 2000; Davis et al., 2000; Engebrecht et al., 1991; Munding et al., 2010; Nakagawa and Ogawa, 1999), improves early in meiosis (Juneau et al., 2007; Munding et al., 2010). In our previous study, strain SK1 was induced to enter a rapid, synchronous meiosis, and RNA was analyzed on splicing-sensitive microarrays (Munding et al., 2010). In addition to meiotic transcripts, we noticed that constitutively expressed transcripts also showed improved splicing. We detect improved splicing by a decrease in intron accumulation index (IAI; a measure of the change in ratios of intron signal to exon 2 signal between two samples; Clark et al., 2002). Measurement of splicing efficiency for genes undergoing transcriptional repression is confounded by the rapid loss of measurable pre-mRNA. For this reason, we examined the 156 intron-containing genes (ICGs) whose expression does not decrease more than 2-fold during mid-meiosis (55% of total ICGs; Figure 1). Splicing improved during mid-meiosis and then declined (Figure 1A, blue indicates reduced IAI, interpreted as improved splicing, data in Table S1, available online).

To determine a threshold for calling a change in splicing efficiency, we assessed noise in the data by estimating variation in the IAI distribution between replicate samples that should not show splicing changes (see Experimental Procedures; Figure 1B, control distribution; Table S1). We compared the distribution of IAI changes between time zero and the indicated time point for the set of 156 IGCs to this control (background) distribution (Figure 1B). Splicing globally increases in mid-meiosis, peaking at 5 hr. Of the 156 genes, 61 (39%) improve in splicing efficiency by at least 1.4-fold at 2 of 3 mid-meiotic time points (2 hr, 5 hr, or 7 hr; Figure 1C). Among the genes whose splicing improves during mid-meiosis, most (48/61) are constitutively

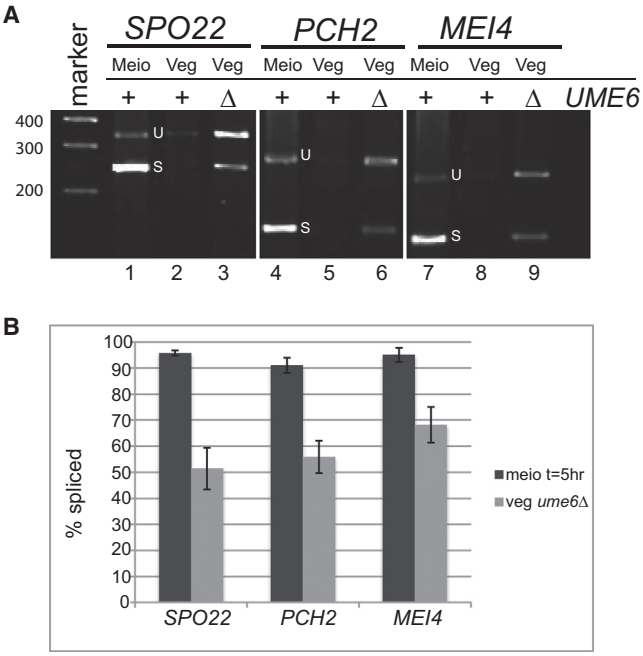

Figure 2. Splicing of Meiotic Transcripts Is More Efficient during Meiosis than during Vegetative Growth

(A) Expression and splicing of meiotic transcripts *SPO22*, *MEI4*, and *PCH2* in wild-type (+) meiotic (Meio) and vegetative cells (Veg) and in *ume6*Δ (Δ) vegetative cells. Marker sizes are in base pairs. PCR products representing spliced (S) and unspliced (U) are indicated.

(B) Quantification of splicing from at least three biological replicates. Dark gray bar indicates splicing efficiency at t = 5 hr after induction of meiosis; light gray bar indicates splicing efficiency in *ume6*Δ vegetative cells. Note that *ume6*Δ also derepresses *MER1*, which encodes a meiotic splicing factor necessary for *SPO22* pre-mRNA splicing (Munding et al., 2010). Error bars represent ± 1 SD.

expressed without known meiosis-specific functions (Figure 1C). Only a few genes (10/156, 6%) appear to decrease more than 1.4-fold in splicing efficiency, about as many as expected by chance given the control distribution (Figures 1B and 1C). We validated improved splicing for four genes by RT-qPCR (Figure 1D). We conclude that splicing for both meiotic and constitutively expressed ICGs globally increases during mid-meiosis. We hypothesize that a splicing regulatory mechanism not specifically restricted to meiotic transcripts is active during mid-meiosis to activate splicing globally.

Splicing Is Less Efficient when Ribosomal Protein Genes Are Expressed

Meiosis in yeast is triggered in part by nutrient signaling (Mitchell, 1994; Neiman, 2011), which also leads to transcriptional repression of RPGs (Chu et al., 1998; Gasch et al., 2000; Munding et al., 2010; Primig et al., 2000; Warner, 1999). RPGs represent the largest functional class of ICGs in *S. cerevisiae* (101 of 293 ICGs are RPGs). Given their high expression, RPG pre-mRNAs comprise ~90% of the splicing substrates in a vegetative cell (Ares et al., 1999; Lopez and Séraphin, 1999; Warner, 1999). After their repression early in meiosis, RPGs are induced in late meiosis (Chu et al., 1998; Munding et al., 2010; Primig et al., 2000), even though the starvation conditions continue. We wondered whether the increase in splicing during meiosis might be due to the reduction of RPG pre-mRNAs that normally occupy the spliceosome during vegetative growth. This idea is consistent with the timing of both improved splicing during RPG repression early in meiosis and loss of efficient splicing during RPG induction at about 9 hr (Figures 1A and 1B). Based on this, we tested the hypothesis that RPG expression reduces the splicing of other pre-mRNAs.

We first asked whether splicing of meiotic transcripts normally expressed only in the absence of RPG expression is less efficient during vegetative growth when RPGs are highly expressed. During vegetative growth, meiotic genes are repressed by Ume6 (Mitchell, 1994; Munding et al., 2010; Strich et al., 1994; Williams et al., 2002). Thus, we evaluated splicing in vegetative *ume6Δ* cells in which derepressed meiotic genes and RPGs are simultaneously expressed (Figure 2A). Transcripts from *SPO22*, *MEI4*, and *PCH2* are highly expressed and efficiently spliced during meiosis (Figure 2A, lanes 1, 4, and 7) and are not expressed in wild-type vegetative cells (Figure 2A, lanes 2, 5, and 8). Deletion of *UME6* in vegetative cells allows expression and some splicing of *SPO22*, *MEI4*, and *PCH2* (Figure 2A, lanes 3, 6, and 9). However, splicing is less efficient in vegetative cells in which RPGs are expressed. Quantification confirms that splicing is reduced by 25%–45% during vegetative growth as compared to mid-meiosis (Figure 2B).

Splicing Improves Globally when RPGs Are Repressed

If poor splicing of meiotic transcripts in vegetative *ume6Δ* cells (Figure 2) is due to RPG expression, then splicing should improve upon repression of RPGs. RPG transcription is promoted by nutrients through the conserved protein kinase target of rapamycin (TOR) (Cardenas et al., 1999; Hardwick et al., 1999; Powers and Walter, 1999). TOR is inactivated by rapamycin (Heitman et al., 1991), leading to rapid RPG repression (Hardwick et al., 1999; Powers and Walter, 1999). We treated vegetative *ume6*Δ cells with rapamycin (200 ng/ml) and monitored levels of pre-mRNA and mRNA for RPGs as well as non-RPGs. Upon rapamycin addition, steady-state levels of RPG pre-mRNA decay immediately, with a half-life of <7 min (Figure 3A), likely due to the combination of transcription inhibition and rapid splicing. RPG mRNAs decay more slowly than pre-mRNAs, with half-lives similar to those reported by others (Figure 3A, Holstege et al., 1998; Li et al., 1999; Wang et al., 2002). Splicing efficiency of non-RPG pre-mRNAs improves within 7 min of rapamycin addition (Figure 3B). This improvement is mediated through TOR because cells lacking Fpr1, a cofactor required for rapamycin binding to TOR (Heitman et al., 1991; Lorenz and Heitman, 1995), do not show improved splicing after rapamycin treatment (Figure S1A).

Most unspliced pre-mRNAs are decayed by nonsense-mediated mRNA decay (NMD) (Burckin et al., 2005; Sayani et al., 2008) after export to the cytoplasm (Kuperwasser et al., 2004). To exclude the possibility that rapamycin mimics improved splicing by increasing NMD, we tested cells lacking the essential NMD factor Upf1 (Leeds et al., 1991). In these cells, the steady-state levels of unspliced transcripts are much higher than in wild-type (Figure S1B); nonetheless, treatment with rapamycin results in dramatically increased splicing efficiency (Figure S1C).

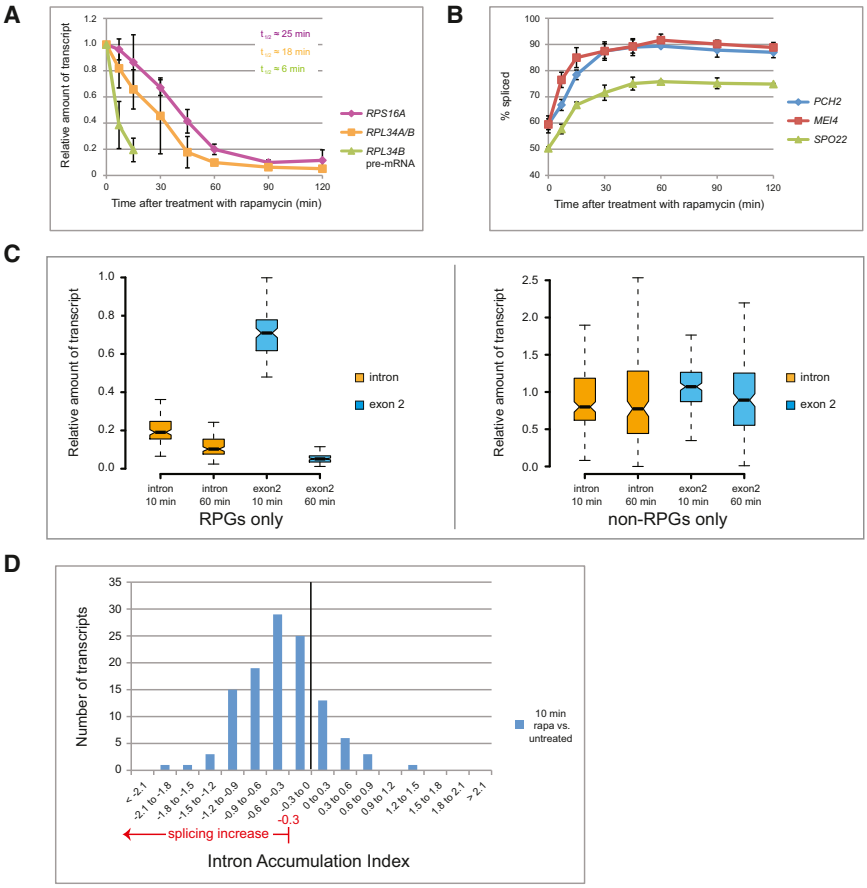

Figure 3. Splicing Increases after Treatment with Rapamycin

(A) Quantification of total (exon 2) transcript levels for *RPS16A* and *RPL34A/RPL34B* and for unspliced *RPL34B* pre-mRNA by RT-qPCR relative to *SEC65* and normalized to t = 0 in *ume6*Δ vegetative cells at indicated times after treatment with rapamycin. Transcript half-lives ($t_{1/2}$) are indicated in the inset.

(B) Quantification of splicing efficiency of meiotic transcripts *SPO22*, *MEI4*, and *PCH2* by semi-quantitative RT-PCR in *ume6*Δ vegetative cells at indicated times after treatment with rapamycin.

(C) RNA-seq measurement of global expression after rapamycin treatment. Box plot representing change in RPG (n = 107 events) (left panel) and non-RPG (n = 165 events) (right panel) intron reads versus exon 2 reads after 10 or 60 min of treatment with rapamycin, normalized to untreated wild-type cells.

(D) Global changes in splicing of genes whose expression does not change greater than 2-fold after 10 min of rapamycin treatment relative to untreated wild-type cells represented by intron accumulation indices (IAI). Black bar indicates IAI = 0 or no change in splicing efficiency. Red arrow indicates splicing changes above the threshold. Error bars represent ± 1 SD. See also Figure S1.

To explore the transcriptome-wide effect on splicing after RPG repression, we performed RNA sequencing (RNA-seq). We evaluated expression of intron-containing RNA (measured by intronic reads) and total RNA (measured by exon 2 reads) of both RPGs and non-RPGs in cells treated with rapamycin for 10 and 60 min (Figure 3C). RPG pre-mRNAs decreased to ~20% of initial levels within 10 min of rapamycin treatment, whereas total RPG RNA (mostly mRNA) only fell substantially after 60 min (Figure 3C, left panel). In comparison, non-RPG expression remained relatively unchanged (Figure 3C, right panel). We evaluated splicing in cells treated with rapamycin for 10 min relative to untreated cells, using a cutoff of a 1.25-fold change in splicing ($|IAI| \geq 0.3$), a threshold established using a control distribution (see Experimental Procedures and Figure S1D). Of the 116 ICGs whose expression changed less than 2-fold upon rapamycin treatment, 68 improved in splicing efficiency by at least 25% (Figures 3D and S1D). Thus, in both vegetative and meiotic cells, RPG expression is associated with inefficient splicing of other transcripts.

Downregulation of an RPG-Dedicated Transcription Factor Suppresses Spliceosomal Defects

While searching for a way to manipulate RPG expression without rapamycin, we found a report from John Woolford's lab regarding extragenic "supersuppressors" that rescued multiple spliceosomal mutations (Maddock et al., 1994). One class of suppressors was found in the *SPP42* gene, now also known as *FHL1* and since shown to encode one of several transcription factors dedicated primarily to RPG transcription (Martin et al., 2004; Rudra et al., 2005; Schawalder et al., 2004; Wade et al., 2004; Zhao et al., 2006). Our hypothesis that pre-mRNAs compete for a limiting splicing apparatus prompted a new interpretation of their suppressor results. If RPG pre-mRNAs compete with essential pre-mRNAs, then competition might be exacerbated in a strain with a compromised spliceosome, for example the ts *prp4-1* and *prp11-1* strains (Galisson and Legrain, 1993; Hartwell, 1967). Furthermore, if ts growth is a consequence of failure to splice growth-rate-limiting pre-mRNAs, this defect might be suppressed by relieving the competition for the compromised splicing machinery. The ability of *spp42-1* to suppress multiple different splicing mutations (Maddock et al., 1994) and its subsequent identification as a dedicated RPG transcription factor suggested that it reduced RPG expression and relieved competition.

To test the idea that downregulation of an RPG-dedicated transcription factor might suppress different ts spliceosome mutations, we constructed strains carrying either the ts *prp4-1* or *prp11-1* alleles and a glucose-repressible promoter controlling expression of the dedicated RPG transcription factor encoded by *IFH1*, a protein required by *FHL1*/*SPP42* to promote RPG transcription (Rudra et al., 2005; Schawalder et al., 2004). *PRP4* encodes a protein in the U4/U6 small nuclear ribonucleoprotein particle (snRNP), which enters the spliceosome as part of the U4/U6-U5 tri-snRNP, whereas *PRP11* encodes a subunit of the U2-associated SF3a complex that establishes U2 snRNP

association with the intron branchpoint at an early step (see Will and Lührmann, 2011 for review). These two proteins contribute to very different steps in the splicing pathway. The *prp4-1; GAL-IFH1* and the *prp11-1; GAL-IFH1* strains grow similarly to their corresponding *IFH1* strains at permissive temperature (26°C) on glucose medium. But at the nonpermissive temperature (30°C for *prp4-1; IFH1* and 33°C for *prp11-1; IFH1*), both ts mutations are suppressed by downregulation of *IFH1*, as signified by improved growth on glucose-containing media (Figure 4A). Using qPCR, we find that at 26°C on glucose, *prp4-1; GAL-IFH1* cells express reduced levels of *IFH1* and RPG mRNAs (Figure 4B). These genetic observations suggest that a modest decrease in the RPG pre-mRNA pool rescues growth defects of the *prp4-1* strain by improving splicing of other essential transcripts.

To confirm this, we performed RNA-seq and examined the global effect of *IFH1* downregulation on splicing of other transcripts. We compared splicing for genes whose expression does not change more than 2-fold in *prp4-1; GAL-IFH1* cells to *prp4-1; IFH1* cells. Of 225 ICGs, 209 (93%) improve in splicing by at least 1.25-fold in *prp4-1; GAL-IFH1* cells (Figure 4C). This includes most RPG (88/93) as well as non-RPG (121/132) splicing events. Validation for four genes by RT-qPCR shows that splicing is restored by downregulation of *IFH1* (Figure 4D). We conclude that subtle downregulation of a dedicated RPG transcription factor can rescue spliceosomal defects through an unusual suppression mechanism. We infer that by reducing the overall load of RPG pre-mRNAs, the demand on the compromised spliceosome is sufficiently relieved to allow splicing of inefficiently spliced essential transcripts. The RNA-seq data incidentally revealed that the mutant Prp4-1 protein has the substitution F320S in a WD repeat domain (data not shown).

To exclude the possibility that the increase in splicing observed in these three conditions (meiosis, rapamycin treatment, and *IFH1* downregulation) is associated with improved expression of the splicing machinery, we evaluated expression of the 5 snRNAs and 110 genes encoding splicing proteins in all 3 treatments (Table S2). Although expression differs across conditions, no global upregulation of the splicing apparatus is observed under any condition. Furthermore, there is no single gene whose expression is correlated with splicing improvement in all conditions (Table S2). Late in meiosis, RPGs are induced and splicing efficiency goes down (Figures 1A and 1B). In a preliminary attempt to increase competition in vegetative cells, we overexpressed the actin intron from a strong promoter and observed reduced splicing for several weakly competitive pre-mRNAs (data not shown). We conclude that pre-mRNAs compete with each other for a limiting splicing apparatus and that increased splicing is associated with relief of competition by reduced RPG expression.

Pre-mRNA Substrates Compete at an Early Step of Spliceosome Recruitment

Inspection of the splice sites in pre-mRNAs that compete poorly revealed many with canonical splice-site and branchpoint sequences, without convincing enrichment for any single feature that might identify a poorly competitive pre-mRNA. To explore whether substrates with suboptimal splicing signals vary in their competitive ability, we used ACT1-CUP1 reporters (Lesser and Guthrie, 1993) containing mutations in the 5′ splice site (5′ss), branchpoint (bp), and 3′ splice site (3′ss; Figure 5A). We tested the effect of rapamycin treatment on reporter splicing in vegetative cells, expecting that a substrate altered in a feature required for competition would show the most improvement in response to RPG repression. Of the seven different mutants tested, only two branchpoint mutants (C256A and A259C) improved in splicing after treatment with rapamycin (Figure 5B). We separately evaluated first and second steps of splicing and found that rapamycin significantly improves the first step for both C256A and A259C mutant pre-mRNAs (Figure 5C). Other substrates with defects in the first step, such as the 5′ss mutant U2A, did not significantly improve (Figure 5B). While A259C also shows improvement in the second step, this effect is likely a consequence of the 2-fold improvement in the first step. The 3′ss mutant U301G (defective in the second step of catalysis) showed no significant improvement (Figure 5B). Attempts to identify the limiting component by overexpressing individual factors known to act at the branchpoint failed to improve splicing (data not shown). Taken together, these data indicate that competition is likely to involve factors acting with the intron branchpoint to commit the pre-mRNA to splicing.

DISCUSSION

These results provide strong evidence that pre-mRNAs compete for the splicing apparatus. For this reason, changes in the composition of the pre-mRNA pool in the nucleus have a significant impact on splicing regulation. By manipulating the composition of the pool of competing pre-mRNAs through transcription (Figures 3 and 4), we show that the balance of splicing competition is important for cell function. The ability of competing RNAs to influence splicing by a transcompetition control mechanism appears related to a larger group of phenomena described in vertebrate cells in which competition between RNAs for a limiting regulatory factor leads to global changes in gene expression. This mechanism is established for miRNA regulation, whereby repression of an mRNA by a miRNA is affected by the level of other competing RNAs (called competitive endogenous RNAs, ceRNAs; Salmena et al., 2011). This process, first described in plants and called target mimicry (Franco-Zorrilla et al., 2007), also regulates muscle development (Cesana et al., 2011) and affects cancer progression (Poliseno et al., 2010) in animals. Our results indicate that a parallel mechanism is at work in splicing regulation, whereby pre-mRNAs compete for limiting splicing machinery, and splicing of many introns is influenced by changes in the composition of the transcript pool. In the case of splicing, the competing RNAs are also substrates, rather than inert decoys.

Evidence that splicing regulation is subject to the composition of a pool of endogenous competing RNAs is not limited to yeast. In models of the human disease myotonic dystrophy, abnormal expression of a CUG repeat expansion RNA acts as a ceRNA for the MBNL1 splicing factor, mimicking a loss of MBNL1 function in splicing (Du et al., 2010; Kanadia et al., 2003; Miller et al.,

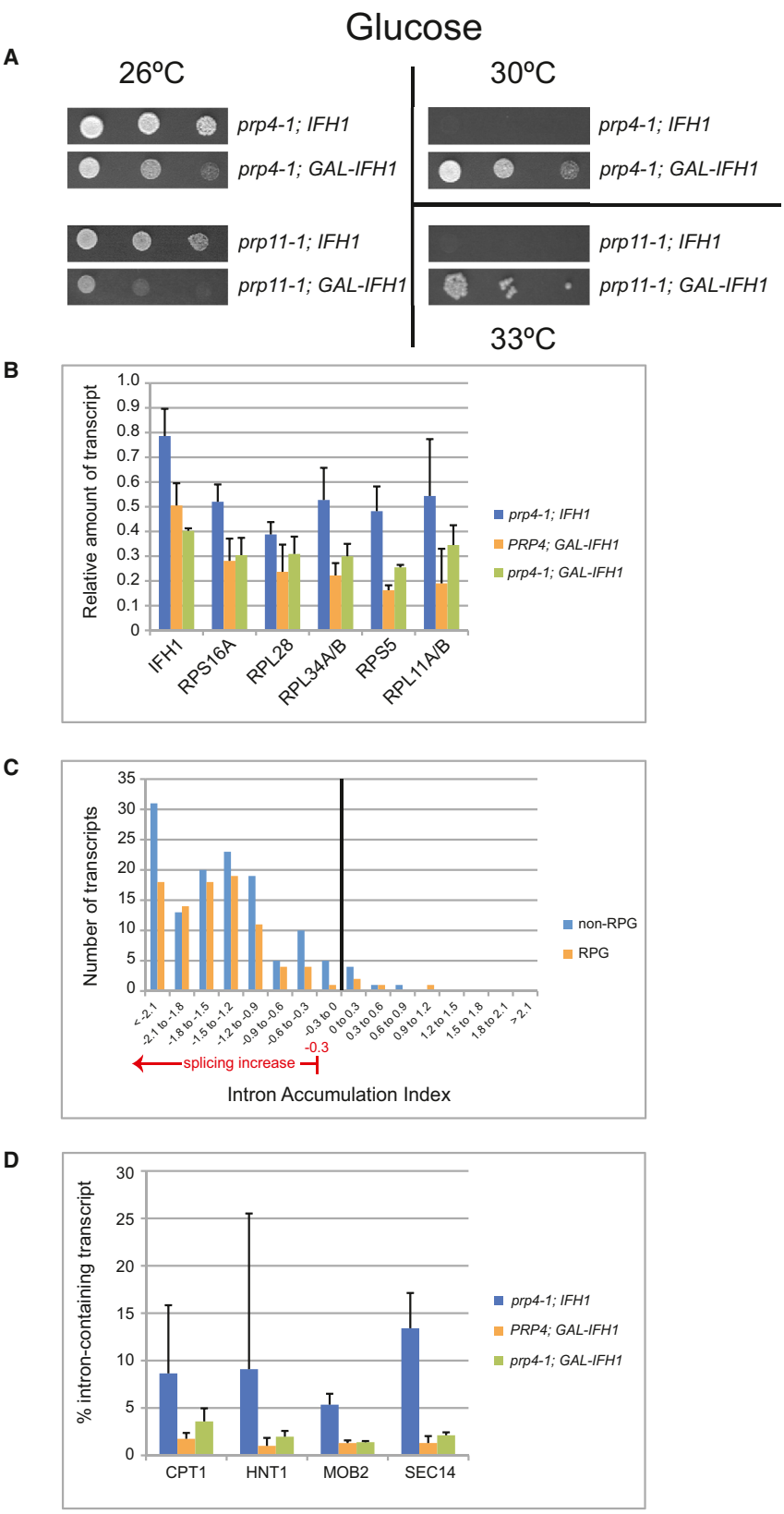

Figure 4. Splicing Defects Are Suppressed by Downregulation of RPG Transcription

(A) Growth of *IFH1* and *GAL-IFH1* strains carrying temperature-sensitive splicing mutations *prp4-1* or *prp11-1* on glucose (*IFH1* downregulated) at 26°C (permissive temperature) and 30°C (nonpermissive temperature for *prp4-1*) or 33°C (nonpermissive for *prp11-1*).

(B) RT-qPCR measurement of *IFH1* and RPG expression relative to *SEC65* in YPD at 26°C in *prp4-1*; *IFH1*, *PRP4; GAL-IFH1*, and *prp4-1; GAL-IFH1* yeast normalized to WT (*PRP4; IFH1*).

(C) Genome-wide changes in splicing of RPG and non-RPG transcripts in *prp4-1; GAL-IFH1* cells relative to *prp4-1; IFH1* cells. Black bar indicates IAI = 0 or no change in splicing efficiency. Red arrow indicates splicing changes above the threshold.

(D) RT-qPCR validation of splicing improvement as measured by percent intron-containing transcript for *CPT1*, *HNT1*, *MOB2*, and *SEC14* in YPD at 26°C in *prp4-1*; *IFH*, *PRP4; GAL-IFH1*, and *prp4-1; GAL-IFH1* yeast normalized to WT. Error bars represent ± 1 SD. See also Table S2.

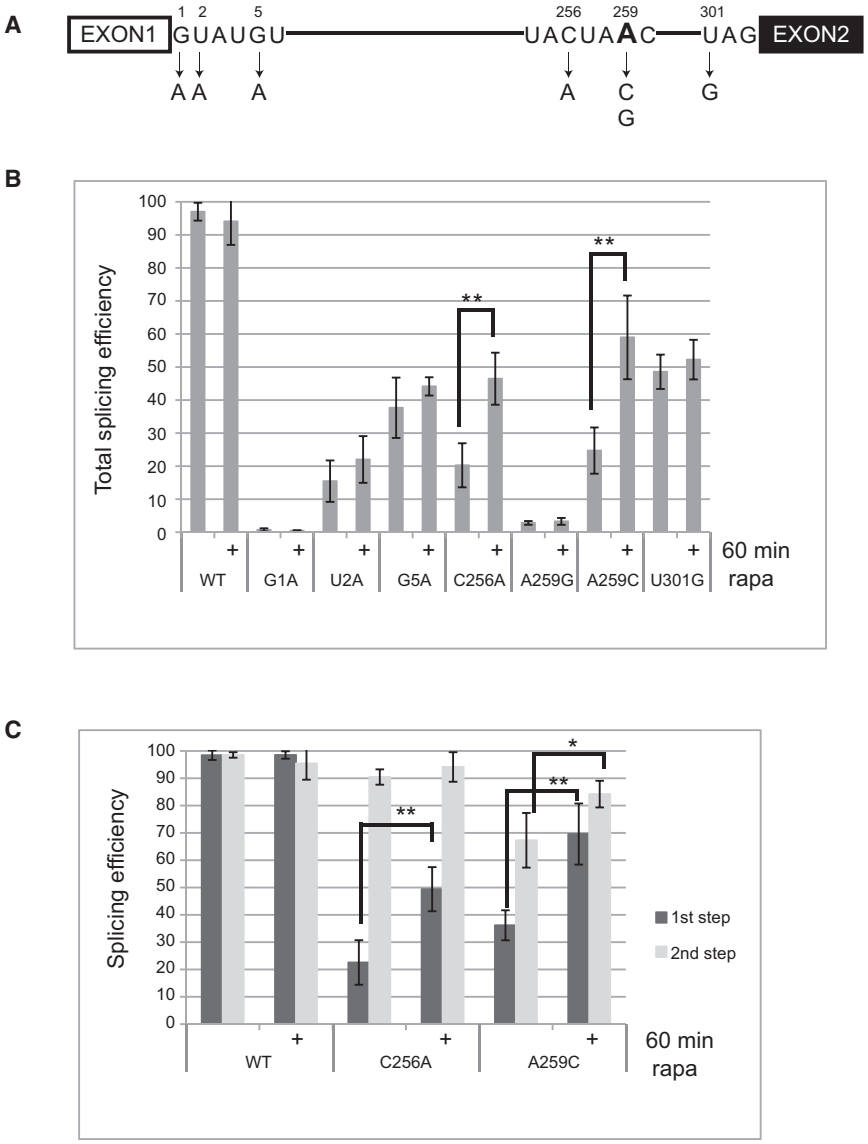

Figure 5. Competition Is Imposed at Early Steps of Spliceosome Assembly

(A) ACT1-CUP1 reporter pre-mRNA schematic indicating 5′ splice site, branchpoint, and 3′ splice site mutations used in this study.

(B) Quantification of total splicing efficiency as measured by primer extension of wild-type and the indicated mutant ACT1-CUP1 reporters before and after (+) treatment for 60 min with rapamycin (60 min rapa). **$p < 0.01$ in a one-tailed t test.

(C) Quantification of splicing efficiency in the first step (dark gray bars) and second step (light gray bars) as measured by primer extension of WT, C256A, and A259C ACT1-CUP1 reporters before and after (+) treatment for 60 min with rapamycin (60 min rapa). *$p < 0.05$, **$p < 0.01$ in a one-tailed t test. Error bars represent ± 1 SD.

2000), indicating that pre-mRNAs compete for MBNL1. Similarly, small nucleolar long noncoding RNAs (sno-lncRNAs) have been identified as a kind of ceRNA for pre-mRNAs dependent on the splicing factor RBFOX2 (Yeo et al., 2009; Yin et al., 2012). Under conditions in which sno-lncRNAs are depleted (such as in Prader-Willi syndrome; Yin et al., 2012), competition for RBFOX2 is relieved. A third example involves the U1 snRNP, which appears limiting for an activity that influences polyadenylation site selection (Berg et al., 2012; Kaida et al., 2010). When the levels of pre-mRNA increase, the spectrum of poly(A) sites utilized in the cell changes, creating mRNAs with alternative 3′ UTRs, with each pre-mRNA presumably acting as a ceRNA for all the others. Thus, understanding posttranscriptional gene regulation requires accounting of changes in the levels of the limiting regulatory factor as well as changes in composition of the larger transcript pool that affects competition for that limiting factor.

What Conditions Are Required for Transcompetition Control?

Splicing can be regulated by changes in physical levels, specific activity, or localization of splicing factors that control the rate-limiting step of splicing in a transcript-specific fashion (Black, 2003; Nilsen and Graveley, 2010). Transcompetition control accounts for changes in splicing factor activity observed by altering the effective load of pre-mRNAs that also employ the limiting factor or other RNAs that occupy the factor. Thus, splicing regulation may be achieved by either changing the abundance of a limiting factor (or exchanging one limiting factor for another) or by altering the dynamics of competition by changing the composition of the RNA pool (Figure 6A). These systems-level considerations argue that understanding the demand for the splicing machinery and how pre-mRNA competition changes during development will be required to integrate regulatory networks into their gene expression programs. In mammalian systems, induction of gene expression programs can result in large changes in the composition of the transcript pool (Berg et al., 2012), altering competition for the splicing machinery. Under such conditions, the competitive advantage of alternative exons for the splicing machinery may be decreased, resulting in a shift of mRNA isoforms.

The principles of transcompetition control can be explained using a modification of the general Michaelis-Menten model for competitive inhibition in which two different substrates (S_1 and S_2) compete (Figure 6B). In this case, when the spliceosome is limiting, the amount of mRNA product (P_1) depends on both the concentration of pre-mRNA S_1 ($[S_1]$) and its splicing rate (k_1) as well as the concentration ($[S_2]$) and splicing rate (k_2) of the competing pre-mRNA substrate (Figures 6B and S2). This simple model shows that splicing regulation can be achieved

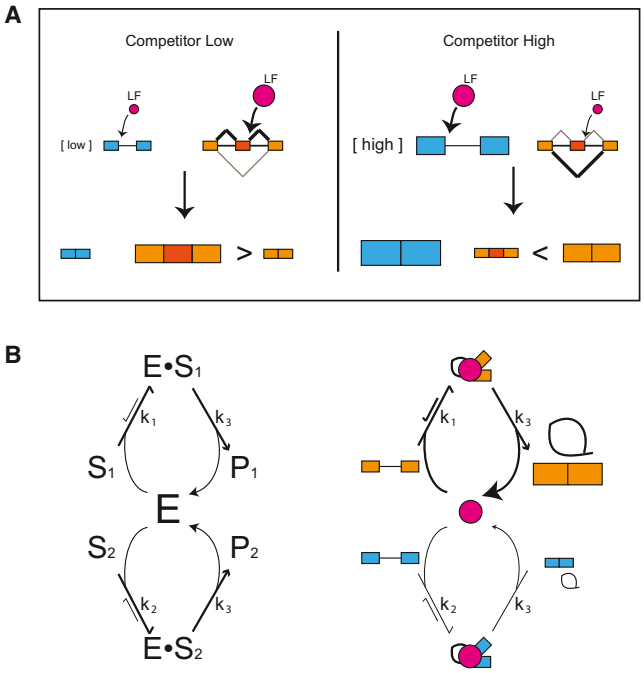

Figure 6. Transcompetition Control of Splicing

(A) Transcompetition control of alternative splicing. When competitor pre-mRNA levels are low, demand for the limiting factor (LF) is low, resulting in efficient inclusion of the weakly competitive cassette exon. When competitor pre-mRNA levels are high, competitor pre-mRNAs titrate increased amounts of the limiting factor, resulting in much less efficient inclusion of the weakly competitive cassette exon.

(B) Left: Michaelis-Menten scheme showing two substrates with different affinities (S_1 and S_2) competing for the same enzyme, E. Formation of products P_1 and P_2 is determined by the concentration of each substrate and the substrate's K_M when the enzyme is limiting. Right: splicing scheme of two substrates competing for a limiting splicing machinery (pink circle). In this example, both substrates are present at the same initial concentration, but the orange substrate outcompetes the blue substrate due to its higher affinity ($k_1 >> k_2$). Note that rates of enzyme-substrate complex (ES) formation will also change between pre-mRNAs of equal affinity when one is at a higher concentration. See also Figure S2.

by altering the competitive status of a target pre-mRNA through modulation of the levels of other RNAs that compete for a limiting factor. In a cell, there are thousands of competing introns, each with its own affinity for the spliceosome; as the concentration of any one of them changes, the splicing efficiency of all the others must then change as well. Similar to the queuing theory (Cookson et al., 2011), in which degradation of unrelated proteins dependent on a common enzyme become coupled due to competition for the enzyme, change in the demand for the spliceosome leads to coregulation of pre-mRNAs with similar competitive abilities.

Functional Importance of Transcompetition Control

The striking relationship between RPG expression and the change in splicing efficiency during meiosis suggests a role for transcompetition control in maintaining separation between the meiotic and vegetative gene expression states. Weakly competitive introns reduce the chances that meiotic genes would be expressed during vegetative growth. Repression of RPGs may have become necessary to allow sufficient splicing during meiosis. However, it is not known whether meiosis can proceed in the absence of RPG repression. Thus, there is no direct evidence that transcompetition control is required for meiosis.

Strong evidence for the functional importance of balanced competition comes from the suppression of splicing defects upon downregulation of RPGs (Figure 4). Rescue of the ts phenotype of *prp4-1* and *prp11-1* arises from poor splicing of essential pre-mRNAs because they are outcompeted by RPG pre-mRNAs. Restoring the competitive balance decreases the demand on the splicing machinery by reducing the load represented by intron-containing RPGs and allows improved splicing of essential non-RPG pre-mRNAs that then increases viability of the *prp4-1* and *prp11-1* strains.

A number of human diseases are associated with missense mutations in core spliceosome components (reviewed in Padgett, 2012), such as Prp8 and Prp31 (retinitis pigmentosa) and SF3B1 (myelodysplastic syndrome and chronic lymphocytic leukemia). These cases may mirror the subtle loss of splicing capacity observed for the *prp4-1* and *prp11-1* mutations and alter the competitive landscape for splicing, contributing to disease. Different pre-mRNAs clearly have distinct dependencies on conserved components of the splicing machinery (Burckin et al., 2005; Clark et al., 2002; Park et al., 2004; Pleiss et al., 2007), suggesting that transcripts may compete for different limiting factors depending on the context. Thus, the key to understanding why certain mutations in conserved splicing factor genes lead to specific diseases may lie in the nature of the composition of the transcript pool in the specific cell type affected and the specific functions of the proteins encoded by the pre-mRNAs that do not compete well for splicing in that context.

EXPERIMENTAL PROCEDURES

Strains and Plasmids

Strains are listed in Table S3. *GAL-IFH1* strains were constructed (Longtine et al., 1998; Wach et al., 1997) and verified by PCR so that the *GAL1* promoter (marked by the *Saccharomyces kluyveri HIS3* gene) was placed upstream of *IFH1*. Strains carrying the *prp4-1* or *prp11-1* mutations were provided by S. Ruby (Ruby et al., 1993). The *prp4-1; GAL-IFH1* and *prp11-1; GAL-IFH1* strains were constructed by crossing to the *GAL-IFH1* strain. ACT1-CUP1 reporter plasmids (Figure 5) are from Lesser and Guthrie (1993).

Media and Culture Conditions

Standard methods for yeast culture conditions were used (Sherman, 1991). Rapamycin was added to cells grown to optical density 600 (OD_{600}) ≈ 0.5 at 200 ng/ml for the indicated time. All yeast strains were grown at 30°C unless otherwise indicated.

RNA Isolation

RNA was isolated as described in Ares (2012). Total meiotic RNA was extracted according to Method 2 to ensure uniform RNA extraction from late spore stages. Total vegetative RNA was prepared from cells grown to OD_{600} = 0.5 according to Method 1.

Transcriptome Profiling

Microarray data (Munding et al., 2010) is from the Gene Expression Omnibus under accession number GSE24686. RNA-seq data in Figure 3 is from two

independent rapamycin time courses. RNA-seq data in Figure 4 represents one culture from each strain (grown to $OD_{600} \approx 0.5$ in YEPD medium at 26°C). Details on the methodology and analysis of the microarray and RNA-seq data are included in the Supplemental Information.

RT-PCR and qPCR

Reverse transcribed RNA (complementary DNA, cDNA) was amplified using the primers in Table S4. Semiquantitative RT-PCR was carried out by limiting cycle numbers to 21 and using cDNA derived from 300 ng of total RNA. Estimates of splicing efficiency used the Agilent 2100 Bioanalyzer. qPCR was performed using a master mix (Fermentas). Additional experimental details are included in the Supplemental Information.

Primer Extension

At least three colonies of BY4741 transformed with each ACT1-CUP1 reporter plasmid were grown to OD = 0.5 in synthetic complete dextrose (SCD) medium lacking leucine. Next, 5 μg of total RNA was annealed to 0.1 ng of PE1 primer (5′-CCTTCATTTTGGAAGTTA-3′), and the primer was extended as previously described (Perriman and Ares, 2007). Extension products were analyzed on a Typhoon imaging system (GE Healthcare). First-step splicing efficiency was calculated as (M + L) / (M + L + P); second-step splicing efficiency was calculated as M / (M + L); and total splicing efficiency was calculated as M / (M + L + P), where M is mRNA, L is lariat intermediate, and P is pre-mRNA.

ACCESSION NUMBERS

RNA-seq data have been deposited in the Gene Expression Omnibus under accession number GSE44219.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, two figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.molcel.2013.06.012.

ACKNOWLEDGMENTS

We would like to thank the UCSC Genomics Core for sequencing, Jon Warner for generosity with suggestions and reagents, and Rhonda Perriman for encouragement and critical reading of the manuscript. Thanks also to Hinrich Boeger, Ted Powers, Grant Hartzog, and Alex Hoffmann for comments and suggestions. This work was primarily supported by GM040478 from the National Institutes of Health to M.A. L.S. and J.P.D. were supported by GM084317. E.M.M. was partially supported by National Institutes of Health Training Grant T32 GM008646.

Received: February 24, 2013
Revised: May 18, 2013
Accepted: June 18, 2013
Published: July 25, 2013

REFERENCES

Ares, M. (2012). Isolation of total RNA from yeast cell cultures. Cold Spring Harb Protoc *2012*, 1082–1086.

Ares, M., Jr., Grate, L., and Pauling, M.H. (1999). A handful of intron-containing genes produces the lion's share of yeast mRNA. RNA *5*, 1138–1139.

Barbosa-Morais, N.L., Irimia, M., Pan, Q., Xiong, H.Y., Gueroussov, S., Lee, L.J., Slobodeniuc, V., Kutter, C., Watt, S., Colak, R., et al. (2012). The evolutionary landscape of alternative splicing in vertebrate species. Science *338*, 1587–1593.

Berg, M.G., Singh, L.N., Younis, I., Liu, Q., Pinto, A.M., Kaida, D., Zhang, Z., Cho, S., Sherrill-Mix, S., Wan, L., and Dreyfuss, G. (2012). U1 snRNP determines mRNA length and regulates isoform expression. Cell *150*, 53–64.

Black, D.L. (2003). Mechanisms of alternative pre-messenger RNA splicing. Annu. Rev. Biochem. *72*, 291–336.

Boutz, P.L., Stoilov, P., Li, Q., Lin, C.H., Chawla, G., Ostrow, K., Shiue, L., Ares, M., Jr., and Black, D.L. (2007). A post-transcriptional regulatory switch in polypyrimidine tract-binding proteins reprograms alternative splicing in developing neurons. Genes Dev. *21*, 1636–1652.

Buckanovich, R.J., Posner, J.B., and Darnell, R.B. (1993). Nova, the paraneoplastic Ri antigen, is homologous to an RNA-binding protein and is specifically expressed in the developing motor system. Neuron *11*, 657–672.

Burckin, T., Nagel, R., Mandel-Gutfreund, Y., Shiue, L., Clark, T.A., Chong, J.L., Chang, T.H., Squazzo, S., Hartzog, G., and Ares, M., Jr. (2005). Exploring functional relationships between components of the gene expression machinery. Nat. Struct. Mol. Biol. *12*, 175–182.

Calarco, J.A., Superina, S., O'Hanlon, D., Gabut, M., Raj, B., Pan, Q., Skalska, U., Clarke, L., Gelinas, D., van der Kooy, D., et al. (2009). Regulation of vertebrate nervous system alternative splicing and development by an SR-related protein. Cell *138*, 898–910.

Cardenas, M.E., Cutler, N.S., Lorenz, M.C., Di Como, C.J., and Heitman, J. (1999). The TOR signaling cascade regulates gene expression in response to nutrients. Genes Dev. *13*, 3271–3279.

Cesana, M., Cacchiarelli, D., Legnini, I., Santini, T., Sthandier, O., Chinappi, M., Tramontano, A., and Bozzoni, I. (2011). A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. Cell *147*, 358–369.

Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P.O., and Herskowitz, I. (1998). The transcriptional program of sporulation in budding yeast. Science *282*, 699–705.

Clark, T.A., Sugnet, C.W., and Ares, M., Jr. (2002). Genomewide analysis of mRNA processing in yeast using splicing-specific microarrays. Science *296*, 907–910.

Cookson, N.A., Mather, W.H., Danino, T., Mondragón-Palomino, O., Williams, R.J., Tsimring, L.S., and Hasty, J. (2011). Queueing up for enzymatic processing: correlated signaling through coupled degradation. Mol. Syst. Biol. *7*, 561.

Cooper, K.F., Mallory, M.J., Egeland, D.B., Jarnik, M., and Strich, R. (2000). Ama1p is a meiosis-specific regulator of the anaphase promoting complex/cyclosome in yeast. Proc. Natl. Acad. Sci. USA *97*, 14548–14553.

Davis, C.A., Grate, L., Spingola, M., and Ares, M., Jr. (2000). Test of intron predictions reveals novel splice sites, alternatively spliced mRNAs and new introns in meiotically regulated genes of yeast. Nucleic Acids Res. *28*, 1700–1706.

de la Mata, M., Alonso, C.R., Kadener, S., Fededa, J.P., Blaustein, M., Pelisch, F., Cramer, P., Bentley, D., and Kornblihtt, A.R. (2003). A slow RNA polymerase II affects alternative splicing in vivo. Mol. Cell *12*, 525–532.

Du, H., Cline, M.S., Osborne, R.J., Tuttle, D.L., Clark, T.A., Donohue, J.P., Hall, M.P., Shiue, L., Swanson, M.S., Thornton, C.A., and Ares, M., Jr. (2010). Aberrant alternative splicing and extracellular matrix gene expression in mouse models of myotonic dystrophy. Nat. Struct. Mol. Biol. *17*, 187–193.

Engebrecht, J.A., Voelkel-Meiman, K., and Roeder, G.S. (1991). Meiosis-specific RNA splicing in yeast. Cell *66*, 1257–1268.

Franco-Zorrilla, J.M., Valli, A., Todesco, M., Mateos, I., Puga, M.I., Rubio-Somoza, I., Leyva, A., Weigel, D., García, J.A., and Paz-Ares, J. (2007). Target mimicry provides a new mechanism for regulation of microRNA activity. Nat. Genet. *39*, 1033–1037.

Gabut, M., Samavarchi-Tehrani, P., Wang, X., Slobodeniuc, V., O'Hanlon, D., Sung, H.K., Alvarez, M., Talukder, S., Pan, Q., Mazzoni, E.O., et al. (2011). An alternative splicing switch regulates embryonic stem cell pluripotency and reprogramming. Cell *147*, 132–146.

Galisson, F., and Legrain, P. (1993). The biochemical defects of prp4-1 and prp6-1 yeast splicing mutants reveal that the PRP6 protein is required for the accumulation of the [U4/U6.U5] tri-snRNP. Nucleic Acids Res. *21*, 1555–1562.

Gasch, A.P., Spellman, P.T., Kao, C.M., Carmel-Harel, O., Eisen, M.B., Storz, G., Botstein, D., and Brown, P.O. (2000). Genomic expression programs in the response of yeast cells to environmental changes. Mol. Biol. Cell *11*, 4241–4257.

Hardwick, J.S., Kuruvilla, F.G., Tong, J.K., Shamji, A.F., and Schreiber, S.L. (1999). Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the Tor proteins. Proc. Natl. Acad. Sci. USA *96*, 14866–14870.

Hartwell, L.H. (1967). Macromolecule synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. *93*, 1662–1670.

Heitman, J., Movva, N.R., and Hall, M.N. (1991). Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. Science *253*, 905–909.

Hiller, M., Zhang, Z., Backofen, R., and Stamm, S. (2007). Pre-mRNA secondary structures influence exon recognition. PLoS Genet. *3*, e204.

Holstege, F.C., Jennings, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.R., Lander, E.S., and Young, R.A. (1998). Dissecting the regulatory circuitry of a eukaryotic genome. Cell *95*, 717–728.

Howe, K.J., and Ares, M., Jr. (1997). Intron self-complementarity enforces exon inclusion in a yeast pre-mRNA. Proc. Natl. Acad. Sci. USA *94*, 12467–12472.

Howe, K.J., Kane, C.M., and Ares, M., Jr. (2003). Perturbation of transcription elongation influences the fidelity of internal exon inclusion in Saccharomyces cerevisiae. RNA *9*, 993–1006.

Jin, Y., Suzuki, H., Maegawa, S., Endo, H., Sugano, S., Hashimoto, K., Yasuda, K., and Inoue, K. (2003). A vertebrate RNA-binding protein Fox-1 regulates tissue-specific splicing via the pentanucleotide GCAUG. EMBO J. *22*, 905–912.

Juneau, K., Palm, C., Miranda, M., and Davis, R.W. (2007). High-density yeast-tiling array reveals previously undiscovered introns and extensive regulation of meiotic splicing. Proc. Natl. Acad. Sci. USA *104*, 1522–1527.

Kaida, D., Berg, M.G., Younis, I., Kasim, M., Singh, L.N., Wan, L., and Dreyfuss, G. (2010). U1 snRNP protects pre-mRNAs from premature cleavage and polyadenylation. Nature *468*, 664–668.

Kalsotra, A., and Cooper, T.A. (2011). Functional consequences of developmentally regulated alternative splicing. Nat. Rev. Genet. *12*, 715–729.

Kalsotra, A., Xiao, X., Ward, A.J., Castle, J.C., Johnson, J.M., Burge, C.B., and Cooper, T.A. (2008). A postnatal switch of CELF and MBNL proteins reprograms alternative splicing in the developing heart. Proc. Natl. Acad. Sci. USA *105*, 20333–20338.

Kanadia, R.N., Johnstone, K.A., Mankodi, A., Lungu, C., Thornton, C.A., Esson, D., Timmers, A.M., Hauswirth, W.W., and Swanson, M.S. (2003). A muscleblind knockout model for myotonic dystrophy. Science *302*, 1978–1980.

Kornblihtt, A.R. (2005). Promoter usage and alternative splicing. Curr. Opin. Cell Biol. *17*, 262–268.

Kreahling, J.M., and Graveley, B.R. (2005). The iStem, a long-range RNA secondary structure element required for efficient exon inclusion in the Drosophila Dscam pre-mRNA. Mol. Cell. Biol. *25*, 10251–10260.

Kuperwasser, N., Brogna, S., Dower, K., and Rosbash, M. (2004). Nonsense-mediated decay does not occur within the yeast nucleus. RNA *10*, 1907–1915.

Leeds, P., Peltz, S.W., Jacobson, A., and Culbertson, M.R. (1991). The product of the yeast UPF1 gene is required for rapid turnover of mRNAs containing a premature translational termination codon. Genes Dev. *5*(12A), 2303–2314.

Lesser, C.F., and Guthrie, C. (1993). Mutational analysis of pre-mRNA splicing in Saccharomyces cerevisiae using a sensitive new reporter gene, CUP1. Genetics *133*, 851–863.

Li, B., Nierras, C.R., and Warner, J.R. (1999). Transcriptional elements involved in the repression of ribosomal protein synthesis. Mol. Cell. Biol. *19*, 5393–5404.

Longtine, M.S., McKenzie, A., 3rd, Demarini, D.J., Shah, N.G., Wach, A., Brachat, A., Philippsen, P., and Pringle, J.R. (1998). Additional modules for versatile and economical PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast *14*, 953–961.

Lopez, P.J., and Séraphin, B. (1999). Genomic-scale quantitative analysis of yeast pre-mRNA splicing: implications for splice-site recognition. RNA *5*, 1135–1137.

Lorenz, M.C., and Heitman, J. (1995). TOR mutations confer rapamycin resistance by preventing interaction with FKBP12-rapamycin. J. Biol. Chem. *270*, 27531–27537.

Maddock, J.R., Weidenhammer, E.M., Adams, C.C., Lunz, R.L., and Woolford, J.L., Jr. (1994). Extragenic suppressors of Saccharomyces cerevisiae prp4 mutations identify a negative regulator of PRP genes. Genetics *136*, 833–847.

Markovtsov, V., Nikolic, J.M., Goldman, J.A., Turck, C.W., Chou, M.Y., and Black, D.L. (2000). Cooperative assembly of an hnRNP complex induced by a tissue-specific homolog of polypyrimidine tract binding protein. Mol. Cell. Biol. *20*, 7463–7479.

Martin, D.E., Soulard, A., and Hall, M.N. (2004). TOR regulates ribosomal protein gene expression via PKA and the Forkhead transcription factor FHL1. Cell *119*, 969–979.

Merkin, J., Russell, C., Chen, P., and Burge, C.B. (2012). Evolutionary dynamics of gene and isoform regulation in Mammalian tissues. Science *338*, 1593–1599.

Miller, J.W., Urbinati, C.R., Teng-Umnuay, P., Stenberg, M.G., Byrne, B.J., Thornton, C.A., and Swanson, M.S. (2000). Recruitment of human muscleblind proteins to (CUG)(n) expansions associated with myotonic dystrophy. EMBO J. *19*, 4439–4448.

Mitchell, A.P. (1994). Control of meiotic gene expression in Saccharomyces cerevisiae. Microbiol. Rev. *58*, 56–70.

Munding, E.M., Igel, A.H., Shiue, L., Dorighi, K.M., Treviño, L.R., and Ares, M., Jr. (2010). Integration of a splicing regulatory network within the meiotic gene expression program of Saccharomyces cerevisiae. Genes Dev. *24*, 2693–2704.

Nakagawa, T., and Ogawa, H. (1999). The Saccharomyces cerevisiae MER3 gene, encoding a novel helicase-like protein, is required for crossover control in meiosis. EMBO J. *18*, 5714–5723.

Neiman, A.M. (2011). Sporulation in the budding yeast Saccharomyces cerevisiae. Genetics *189*, 737–765.

Nilsen, T.W., and Graveley, B.R. (2010). Expansion of the eukaryotic proteome by alternative splicing. Nature *463*, 457–463.

Padgett, R.A. (2012). New connections between splicing and human disease. Trends Genet. *28*, 147–154.

Pan, Q., Shai, O., Misquitta, C., Zhang, W., Saltzman, A.L., Mohammad, N., Babak, T., Siu, H., Hughes, T.R., Morris, Q.D., et al. (2004). Revealing global regulatory features of mammalian alternative splicing using a quantitative microarray platform. Mol. Cell *16*, 929–941.

Pan, Q., Shai, O., Lee, L.J., Frey, B.J., and Blencowe, B.J. (2008). Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nat. Genet. *40*, 1413–1415.

Park, J.W., Parisky, K., Celotto, A.M., Reenan, R.A., and Graveley, B.R. (2004). Identification of alternative splicing regulators by RNA interference in Drosophila. Proc. Natl. Acad. Sci. USA *101*, 15974–15979.

Perriman, R.J., and Ares, M., Jr. (2007). Rearrangement of competing U2 RNA helices within the spliceosome promotes multiple steps in splicing. Genes Dev. *21*, 811–820.

Plass, M., Codony-Servat, C., Ferreira, P.G., Vilardell, J., and Eyras, E. (2012). RNA secondary structure mediates alternative 3'ss selection in Saccharomyces cerevisiae. RNA *18*, 1103–1115.

Pleiss, J.A., Whitworth, G.B., Bergkessel, M., and Guthrie, C. (2007). Transcript specificity in yeast pre-mRNA splicing revealed by mutations in core spliceosomal components. PLoS Biol. *5*, e90.

Poliseno, L., Salmena, L., Zhang, J., Carver, B., Haveman, W.J., and Pandolfi, P.P. (2010). A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature *465*, 1033–1038.

Powers, T., and Walter, P. (1999). Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signaling pathway in Saccharomyces cerevisiae. Mol. Biol. Cell *10*, 987–1000.

Primig, M., Williams, R.M., Winzeler, E.A., Tevzadze, G.G., Conway, A.R., Hwang, S.Y., Davis, R.W., and Esposito, R.E. (2000). The core meiotic transcriptome in budding yeasts. Nat. Genet. *26*, 415–423.

Roberts, G.C., Gooding, C., Mak, H.Y., Proudfoot, N.J., and Smith, C.W. (1998). Co-transcriptional commitment to alternative splice site selection. Nucleic Acids Res. *26*, 5568–5572.

Roca, X., Sachidanandam, R., and Krainer, A.R. (2005). Determinants of the inherent strength of human 5′ splice sites. RNA *11*, 683–698.

Ruby, S.W., Chang, T.H., and Abelson, J. (1993). Four yeast spliceosomal proteins (PRP5, PRP9, PRP11, and PRP21) interact to promote U2 snRNP binding to pre-mRNA. Genes Dev. *7*, 1909–1925.

Rudra, D., Zhao, Y., and Warner, J.R. (2005). Central role of Ifh1p-Fhl1p interaction in the synthesis of yeast ribosomal proteins. EMBO J. *24*, 533–542.

Salmena, L., Poliseno, L., Tay, Y., Kats, L., and Pandolfi, P.P. (2011). A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell *146*, 353–358.

Sayani, S., Janis, M., Lee, C.Y., Toesca, I., and Chanfreau, G.F. (2008). Widespread impact of nonsense-mediated mRNA decay on the yeast intronome. Mol. Cell *31*, 360–370.

Schawalder, S.B., Kabani, M., Howald, I., Choudhury, U., Werner, M., and Shore, D. (2004). Growth-regulated recruitment of the essential yeast ribosomal protein gene activator Ifh1. Nature *432*, 1058–1061.

Shepard, P.J., and Hertel, K.J. (2008). Conserved RNA secondary structures promote alternative splicing. RNA *14*, 1463–1469.

Sherman, F. (1991). Getting started with yeast. Methods Enzymol. *194*, 3–21.

Strich, R., Surosky, R.T., Steber, C., Dubois, E., Messenguy, F., and Esposito, R.E. (1994). UME6 is a key regulator of nitrogen repression and meiotic development. Genes Dev. *8*, 796–810.

Sugnet, C.W., Srinivasan, K., Clark, T.A., O'Brien, G., Cline, M.S., Wang, H., Williams, A., Kulp, D., Blume, J.E., Haussler, D., and Ares, M., Jr. (2006). Unusual intron conservation near tissue-regulated exons found by splicing microarrays. PLoS Comput. Biol. *2*, e4.

Underwood, J.G., Boutz, P.L., Dougherty, J.D., Stoilov, P., and Black, D.L. (2005). Homologues of the Caenorhabditis elegans Fox-1 protein are neuronal splicing regulators in mammals. Mol. Cell. Biol. *25*, 10005–10016.

Wach, A., Brachat, A., Alberti-Segui, C., Rebischung, C., and Philippsen, P. (1997). Heterologous HIS3 marker and GFP reporter modules for PCR-targeting in Saccharomyces cerevisiae. Yeast *13*, 1065–1075.

Wade, J.T., Hall, D.B., and Struhl, K. (2004). The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes. Nature *432*, 1054–1058.

Wang, Y., Liu, C.L., Storey, J.D., Tibshirani, R.J., Herschlag, D., and Brown, P.O. (2002). Precision and functional specificity in mRNA decay. Proc. Natl. Acad. Sci. USA *99*, 5860–5865.

Wang, E.T., Sandberg, R., Luo, S., Khrebtukova, I., Zhang, L., Mayr, C., Kingsmore, S.F., Schroth, G.P., and Burge, C.B. (2008). Alternative isoform regulation in human tissue transcriptomes. Nature *456*, 470–476.

Warner, J.R. (1999). The economics of ribosome biosynthesis in yeast. Trends Biochem. Sci. *24*, 437–440.

Warzecha, C.C., Shen, S., Xing, Y., and Carstens, R.P. (2009). The epithelial splicing factors ESRP1 and ESRP2 positively and negatively regulate diverse types of alternative splicing events. RNA Biol. *6*, 546–562.

Will, C.L., and Lührmann, R. (2011). Spliceosome structure and function. Cold Spring Harb. Perspect. Biol. *3*, a003707.

Williams, R.M., Primig, M., Washburn, B.K., Winzeler, E.A., Bellis, M., Sarrauste de Menthiere, C., Davis, R.W., and Esposito, R.E. (2002). The Ume6 regulon coordinates metabolic and meiotic gene expression in yeast. Proc. Natl. Acad. Sci. USA *99*, 13431–13436.

Yeo, G., and Burge, C.B. (2004). Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals. J. Comput. Biol. *11*, 377–394.

Yeo, G.W., Coufal, N.G., Liang, T.Y., Peng, G.E., Fu, X.D., and Gage, F.H. (2009). An RNA code for the FOX2 splicing regulator revealed by mapping RNA-protein interactions in stem cells. Nat. Struct. Mol. Biol. *16*, 130–137.

Yin, Q.F., Yang, L., Zhang, Y., Xiang, J.F., Wu, Y.W., Carmichael, G.G., and Chen, L.L. (2012). Long noncoding RNAs with snoRNA ends. Mol. Cell *48*, 219–230.

Zhao, Y., McIntosh, K.B., Rudra, D., Schawalder, S., Shore, D., and Warner, J.R. (2006). Fine-structure analysis of ribosomal protein gene transcription. Mol. Cell. Biol. *26*, 4853–4862.