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Introns within Ribosomal Protein Genes Regulate the Production and Function of Yeast Ribosomes

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SUMMARY

In budding yeast, the most abundantly spliced pre-mRNAs encode ribosomal proteins (RPs). To investigate the contribution of splicing to ribosome production and function, we systematically eliminated introns from all RP genes to evaluate their impact on RNA expression, pre-rRNA processing, cell growth, and response to stress. The majority of introns were required for optimal cell fitness or growth under stress. Most introns are found in duplicated RP genes, and surprisingly, in the majority of cases, deleting the intron from one gene copy affected the expression of the other in a nonreciprocal manner. Consistently, 70% of all duplicated genes were asymmetrically expressed, and both introns and gene deletions displayed copy-specific phenotypic effects. Together, our results indicate that splicing in yeast RP genes mediates intergene regulation and implicate the expression ratio of duplicated RP genes in modulating ribosome function.

INTRODUCTION

Splicing removes introns from nascent RNA transcripts to generate an uninterrupted protein-coding sequence suitable for translation. Although an increasing number of human diseases are associated with defects in the splicing of mRNA (Benz and Huang, 1997; Solis et al., 2008), the basic functions of introns remain unclear. Introns are kept through evolution, suggesting that they are not easily disposable junk sequences (Bulman et al., 2007; Roy and Penny, 2006; Russell et al., 2005). Introns are linked to many cellular functions including the regulation of gene expression and the generation of increased protein diversity via alternative splicing (Kriventseva et al., 2003; Stetefeld and Ruegg, 2005). However, why introns are preserved particularly in yeast, where alternative splicing is virtually absent, remains unclear.

The majority of yeast genes producing pre-mRNAs needing splicing carry a single intron located near the 5′ end of the ORF (Sakurai et al., 2002). The few introns in nonribosomal protein (non-RP) genes that affect cell growth under standard conditions exert their effect in a promoter-dependent manner, suggesting a link between splicing and transcription (Parenteau et al., 2008). However, the majority of introns are found in the most conserved and also most highly transcribed mRNAs, which code for RP (Ares et al., 1999; Spingola et al., 1999). Consequently, despite the relatively small number of intron-containing genes in yeast, nearly one-third of the total pre-mRNA population contains introns, and more than 70% of actively translating mRNAs originate from intron-encoding transcripts (Ares et al., 1999; Juneau et al., 2006, 2007, 2009). Whether the prevalence of introns in RP genes offers any growth advantage is currently unknown. Studying introns in the context of ribosomal genes, which are highly conserved, is particularly interesting because it may provide information relevant to all organisms including humans.

In baker's yeast, ribosome synthesis requires coordinated expression of ~150 ribosomal RNAs (rRNAs) and 137 RP genes (Warner, 1999). Interestingly, the majority of RP genes that contain introns are duplicated in this organism (Davidovich et al., 2009; Sugino and Innan, 2006). The rationale for maintaining duplicated intron-containing RP genes remains unclear. Initially, it was proposed that gene duplications permit adjusting the dose of RP to match that of rRNA synthesis, thereby ensuring optimal ribosome assembly (Arvas et al., 2007; Ihmels et al., 2007; Kafri et al., 2006; Ohta, 1988). Recently, it was shown that single-paralog deletions induced distinct phenotypic defects (Komili et al., 2007), arguing against an equal role for the duplicated genes. In this study we directly evaluated the impact of all RP-associated introns on RP expression, ribosome biogenesis (RB), and cell growth. Consistent with earlier studies, none of the intron deletions (ΔIs) affected cell growth under normal conditions, suggesting that yeast introns per se are not essential for life (Parenteau et al., 2008). However, most ΔIs drastically affected the expression of both of the cognate pair of duplicated RP genes, reduced fitness, or affected drug resistance in a paralog-specific manner. Together, our results reveal an intricate intron-dependent regulatory mechanism that regulates the intra- and interdependent expression of RP genes to increase the survival of yeast cells under stress.

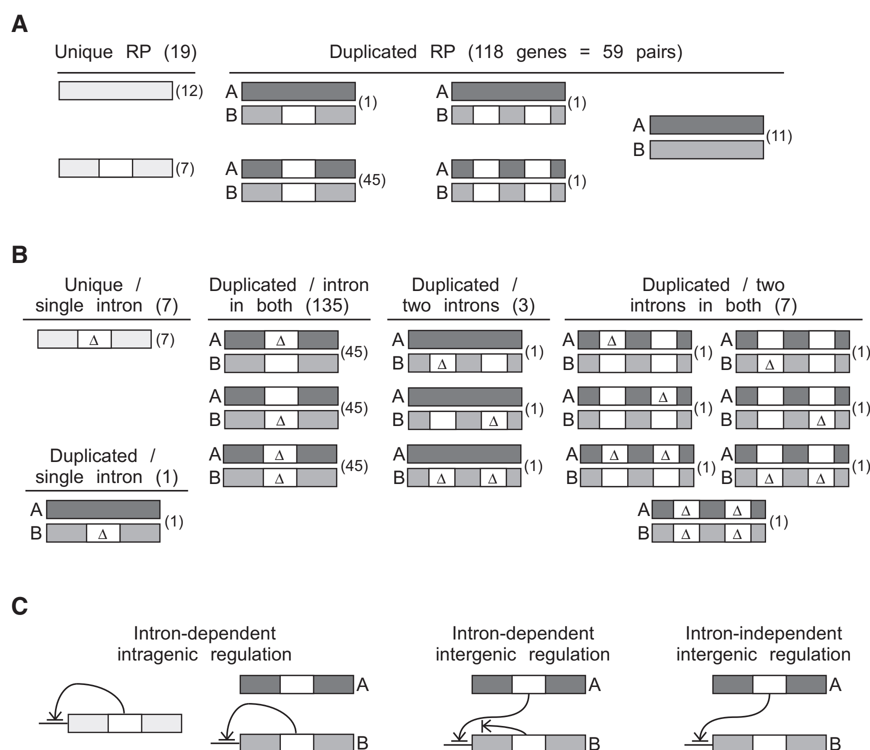

Figure 1. Schematic Representation of RP Genes in *S. cerevisiae*

(A) Unique and duplicated RP genes are schematically illustrated. The number in parentheses indicates the number of RP genes in each category. Introns and exons are represented in white and gray boxes, respectively. The A and B forms of the duplicated RP genes are indicated in different shades of gray (see list in Table S1).

(B) Graphical representation of the introns deleted in this study. The deleted introns are indicated by "Δ." The genes were grouped based on their duplication state as well as the number of introns in each gene and gene set. The numbers of cases in each group are indicated between parentheses. In each group of duplicated genes, the different deletion patterns created are presented for each pair of genes found in a single yeast strain, and the number of each deletion type is indicated on the right of each set.

(C) Illustration of the different mechanisms by which intron may impact gene expression. Up or positive regulation (removal of introns decreases expression) is indicated by "→," whereas down or negative regulation (removal of introns increases expression) is indicated by "⊣." For simplicity only a single allele-dependent regulation (i.e., A controlling B) is represented, but all different combinations of the model presented including bidirectional regulation of similar and different types were also considered.

RESULTS

Identification and Deletion of Introns Associated with RP Genes

Inspection of the *Saccharomyces cerevisiae* genome databases (e.g., SGDs) revealed 81 and 56 genes coding for mature large (LSU) and small ribosomal subunit (SSU) proteins, respectively. The position of introns within the RP genes was identified using the Ares Lab Yeast Intron Database (Grate and Ares, 2002) and the MIPS Yeast Genome Database (Weng et al., 2003). Overall, we identified 98 genes carrying single introns and 3 genes carrying 2 introns (Figure 1A, and see Table S1 available online). Introns were found in 70% of the LSU and 78% of the SSU RP genes, suggesting that intron distribution is not subunit specific and that introns may equally influence both subunits' functions. However, introns were found in 80% of the duplicated RP genes but only 37% of the unique RP genes, suggesting that introns are preferentially conserved in RP paralogs (Figure 1A). To evaluate those introns' functions, we deleted each intron using a standard 2-step (pop-in/pop-out) method (Parenteau et al., 2008) and tested the impact on growth, gene expression, and pre-rRNA processing. From genes carrying two introns, the deletions were performed sequentially, and the effect of the single and double deletion was assayed to identify any possible intron-specific functions. Introns were also removed from both copies of the duplicated RP genes in the same strain to score any cumulative effect on expression or function. All deletions are illustrated in Figure 1B and the results reported in Table S2. Surprisingly, none of the single or double ΔI strains showed any marked growth defect on rich media, indicating that RP gene introns are not required for growth under standard laboratory conditions (see Figure 5A, "Glucose" lane).

Introns Fine-Tune the Expression of RP Genes

It was previously proposed that introns regulate the level of gene expression (Ares et al., 1999; Juneau et al., 2006). Accordingly, we monitored the impact of ΔIs on the expression of the host genes using quantitative PCR (qPCR). Primers were designed to amplify fragments in the coding region of ΔI genes, and the expression level was calculated relative to the expression of housekeeping genes (e.g., *SPT15*). As shown in Figure 2, 84% of all ΔIs changed the expression of the host genes. Surprisingly, deletion of introns equally induced and inhibited gene expression (Figure 2), indicating that introns may either inhibit or induce gene expression (Figure 1C, "Intron-dependent intragenic regulation"). Gene expression was considered changed in an intron-dependent manner when reproducible variations in mRNA levels by 20% or more were detected in three biological PCR replicas conducted using three independent spores carrying a deletion of the same intron. The average decrease and increase in gene expression were 2- and 2.6-fold, respectively, with a standard deviation mean of 5.45%. Intron-dependent-negative regulation of gene expression (genes whose expression increases upon ΔIs; Figure 2A) was slightly more prevalent in genes coding for the LSU proteins, whereas positive regulation (genes whose expression decreases upon ΔIs; Figure 2B) was prevalent in the SSU proteins. However, both forms of regulation are used by both ribosomal subunits.

RPs may be divided into two groups: one includes proteins that bind early to the nascent pre-rRNA during ribosome

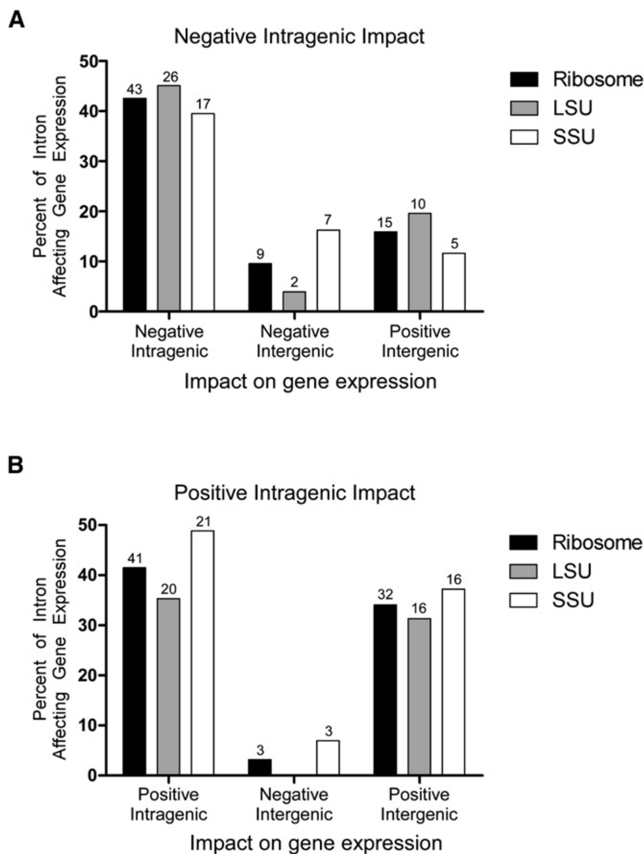

Figure 2. Introns May Function as Negative or Positive Regulators of RP Genes

Impact of ΔI on the expression of duplicated RP genes was measured by gene-specific qPCR in wild-type cells and cells carrying deletions in one or more introns. The percentage of introns that reduce (A) or increase (B) the expression of their host genes was determined by monitoring the impact of ΔIs on the expression of the host genes and its paralog. The values were illustrated in the form of bar graphs in percentages. The number of introns is also written above each bar. Negative and positive intragenic impacts indicate introns that normally repress and induce the expression of their host genes, respectively. Negative and positive intergenic impacts indicate introns that normally repress or induce the expression of the other copy of the duplicated genes set, respectively (see also Table S4). The effect on mRNA was paralog specific and not a general deregulation of gene expression (Table S3).

assembly, and they are important for proper RB; whereas the other includes late binders that mostly contribute to ribosome function (Fromont-Racine et al., 2003; Williamson, 2003). The majority of the genes affected by ΔIs were part of the first group, affecting RB (Table S2), suggesting possible functional specialization of intron-dependent regulation of gene expression. The highest change of gene expression (9.33 times) was detected after the deletion of the intron from the gene coding for SSU protein *RPS14B*, which is one of two copies of the *RPS14* genes that are required for 20S pre-rRNA processing (Antúnez de Mayolo and Woolford, 2003; Granneman et al., 2005). On the other hand, the most pronounced decrease in gene expression (14.29 times) was observed after the deletion of *RPL2A* intron. *RPL2A* is one of the two copies coding for the RNA binding RP L2 that was shown to contact most RNA domains of the large subunit and has been implicated in tRNA ribosome interaction (Meskauskas et al., 2008). We conclude that introns have a major regulatory contribution to the expression of RP genes and propose that this level of regulation is of particular importance for RB.

Duplicated RP Genes Are Asymmetrically Expressed and Regulated

Duplicated RP genes encode very similar proteins whose expression needs to be tightly coordinated with rRNA levels (Warner, 1999). Accordingly, a change in the expression of any single copy of the duplicated RP gene pair is expected to be compensated by a change in the expression of the other. To evaluate this hypothesis, we measured the impact of deleting the intron of one copy of the duplicated RP genes on the expression of the other using qPCR. The expression was evaluated as the amplification value of exonic sequence relative to housekeeping genes. As shown in Figure 3A, 63% of all ΔIs in duplicated RP genes that showed intron-dependent intragenic regulation also affected the expression of the other copy (either in the same or opposite way). However, and most surprisingly, in 80% of these cases, the intergenic regulation was not compensatory in nature (Figure 3B). To ascertain that the ΔI impact is not a result of generic perturbation of gene expression, we also tested the expression of a random sample of RP genes without introns, and non-RP and RP genes containing introns in ΔI strains exhibiting strong phenotypic effects. Those control experiments revealed no or very little changes in the expression of these latter genes (Table S3). Most ΔIs generated a simultaneous decrease or increase in the expression of both copies of the duplicated gene set (Figure 3A, "Same," and Table S5). Most compensatory changes ("Opposite," 19%) were observed when ΔI also increased the expression of the host genes ("Negative Intragenic Impact") and decreased the expression of the other copy ("Positive Intergenic Impact," Figure 2A, and Tables S4 and S5). In contrast the majority of ΔIs decreasing the expression of the host genes ("Positive Intragenic Impact") decreased the expression of its paralog ("Positive Intergenic Impact," Figure 2B, and Tables S4 and S5). The simultaneous decrease or increase in the expression of duplicated RP genes did not exhibit significant subunit bias (Figure 2). These results clearly refute the image of RP paralogs as redundant gene sets required for maintaining RP levels and, rather, suggest that copies of the RP genes may accomplish paralog-specific functions. Indeed, whereas the expression of the majority of the duplicated genes was interdependent (Figures 2 and 3A), each copy displayed a distinct intron-dependent regulatory mechanism. Interestingly, in most cases the paralog's introns did not exhibit similar amplitudes of effects (Table S2). Consistently, in wild-type cells ~70% of all duplicated RP genes are asymmetrically expressed. In the majority of the cases, one copy is expressed at least 30% more than the other copy, and the difference in the expression of the two gene copies may be as large as 9:1 (Figure 3C). We conclude that duplicated RP genes are asymmetrically expressed and regulated to maintain the expression pattern rather than strictly the dose of RP paralogs.

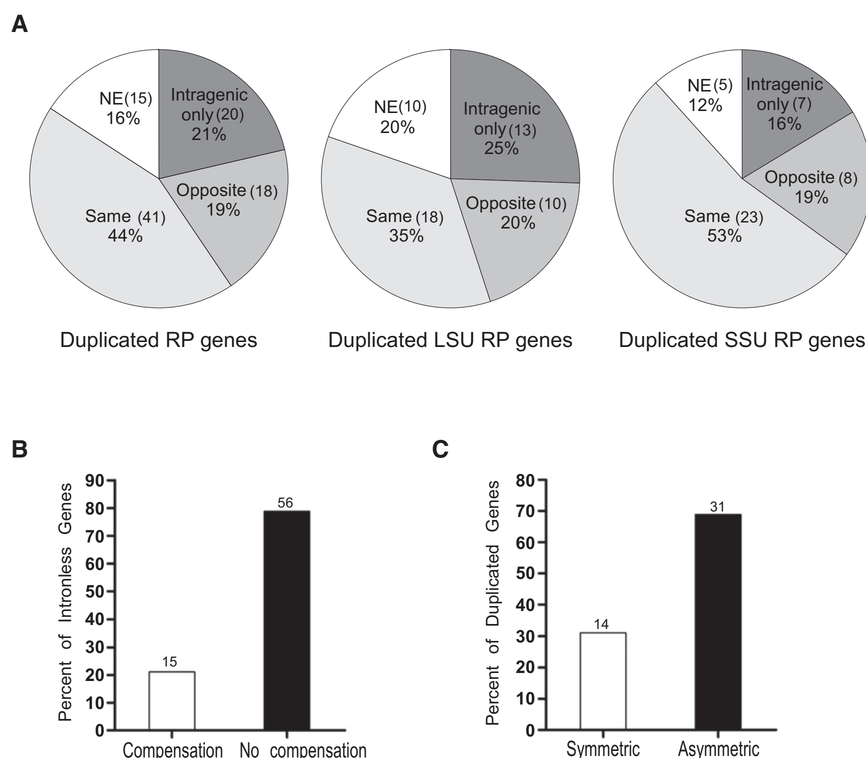

Figure 3. Asymmetric Expression and Post-transcriptional Regulation of Duplicated RP Genes

(A) Pie charts representing the impact of ΔIs on the expression of all duplicated RP genes or genes coding for either the LSU or SSU genes. ''NE,'' ''Intragenic only,'' ''Opposite,'' and ''Same'' indicate the percentage of ΔI strains (or in parentheses the number of ΔI strains) exhibiting no effect, only a change in the expression of the gene carrying the ΔI, increase in the expression of one copy of the paralogs and the decrease of the other, and either simultaneous decrease or increase of two paralogs, respectively (list in Tables S5 and S2).

(B) A histogram comparing the percentage (and the number) of ΔI strains where the change in the expression of one copy is compensated by reciprocal change in the other to those where no compensation was detected. Compensation (white) means that the sum of A and B mRNA levels in wild-type cells is equivalent to the one detected in the ΔI strains; no compensation (black) means that the sum of A and B mRNA levels in wild-type cells is lower or higher to the one detected in ΔI strains.

(C) Histogram illustrating the percentage (and the number) of duplicated RP genes showing a symmetric (white) or an asymmetric (black) mRNA expression level. Duplicated genes exhibiting 10% or more difference in the paralog expression level as determined by microarray are considered asymmetrically expressed (http://transcriptome.ens.fr/ymgv).

Mechanism of Intron-Dependent Gene Regulation

Isolated cases of RP genes were shown to be autoregulated in an intron-dependent manner via the binding of the RP to a specific structure in the intron of its own gene that impairs splicing (Vilardell and Warner, 1994, 1997). Therefore, we wished to examine whether or not the intergenic regulation of the duplicated RP paralogs required the intron of the responsive copy. Thus, we compared the impact of deleting the intron of one gene copy on the expression of the other copy either in the presence or the absence of its own intron. About 33% of the duplicated genes set responded to ΔI in one gene by changing the expression of the other copy only in the presence of its intron (Figure 1C, ''Intron-Dependent Intergenic Regulation,'' and Table S4), whereas in 30% of the cases, the introns were not required for the intergene response (Figure 1C, ''Intron-Independent Intergenic Regulation,'' and Table S4). In general, introns appeared to play a clear role in both intra- and intergene regulation. However, this is not the only means by which intergenic regulation is achieved because about 50% of the cases of intergenic regulation did not require introns. Thus, intergenic regulation required introns in both copies (like *RPL22*), in neither copy (like *RPL31*), or in one but not the other copy (like *RPL7*) (Table S4). The dependency of intergene regulation on introns was not linked to the intragenic effects of introns. Indeed, intron requirement was equally observed in both positive and negative intragenic-regulated genes (Table S4). Therefore, introns appear to mediate a variety of regulatory pathways designed to modulate intergenic regulation.

Introns of RP Genes Modulate the Processing of Pre-rRNA

To examine the contribution of introns to RB, we monitored the impact of the different ΔIs on pre-rRNA processing (Figure 4A). Processing intermediates were detected using qPCR (Figure 4B) in total RNA extracted from the different ΔIs or wild-type cells. Processing anomalies were scored when a processing intermediate increased or decreased by more than 20% when compared with that of wild-type cells. About 88% of all ΔIs increased or decreased at least one pre-rRNA processing intermediate (Table S2). Nearly half of the deletions resulted in the accumulation of one or more intermediates indicating a maturation defect, whereas the other half reduced the amount of precursors suggesting increased instability or accelerated processing. However, ΔIs do not significantly alter the ratio of the ribosomal subunits as is evident from the measurements of the ratio of rRNAs (Table S2). Maturation of the primary precursors 35S* that carry all rRNA transcripts and ends at the transcription termination site (T2) was perturbed by 15% of all ΔIs (Figure 4C). The majority of the ΔIs delaying the processing of 35S* was found in genes associated with the LSU, whereas the majority of those decreasing the precursor was found in SSU-associated genes, indicating that regulating the expression of LSU genes is more critical for the processing and stability of the primary rRNA transcript. In general the accumulation of the 3′ end and 5′ end-processed primary transcripts (33/35S, 32S, 32S*) was much less affected by ΔI, and the delay in these processing events was exclusive to deletions in the LSU genes, further confirming

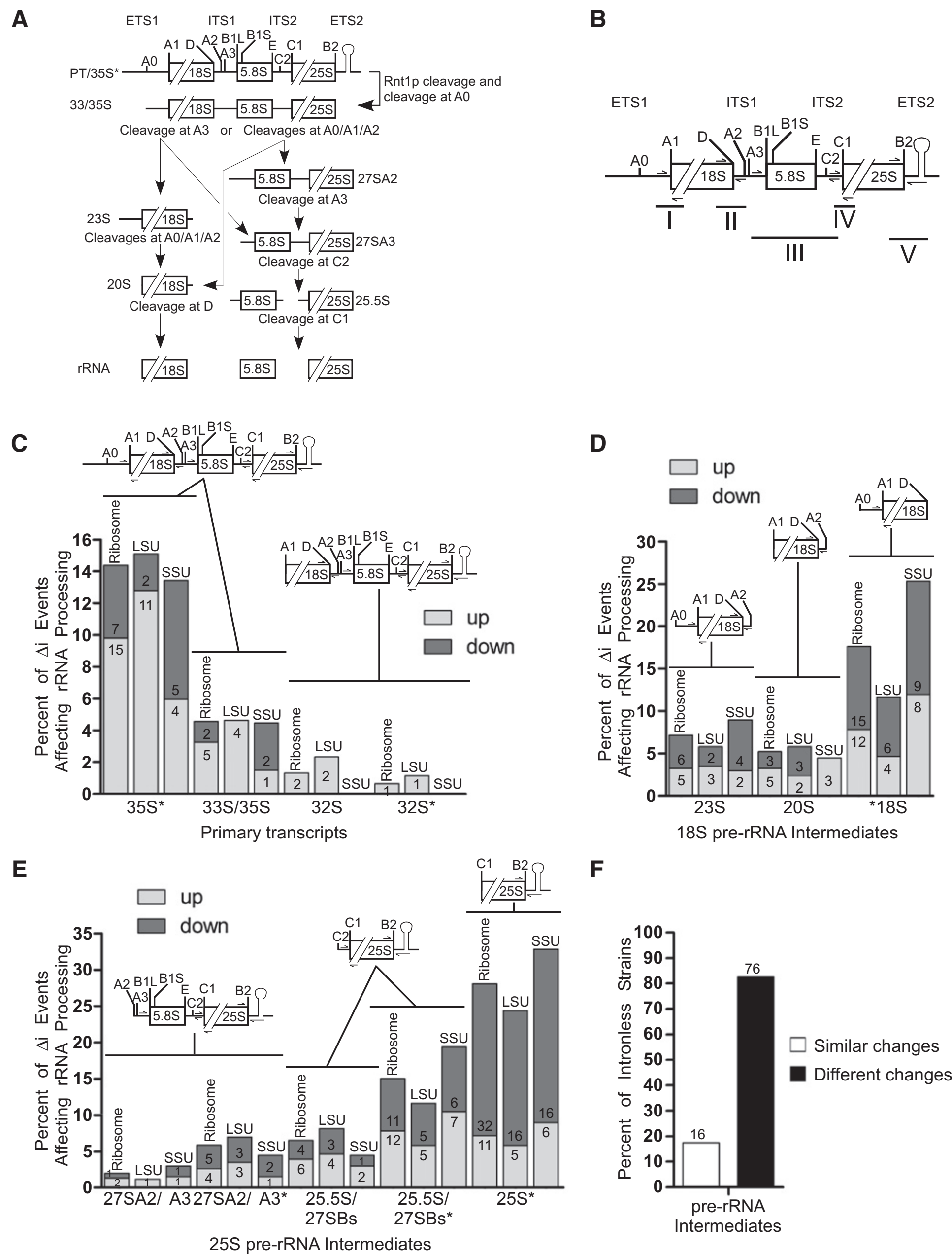

Figure 4. Introns in RP Genes Affect Pre-rRNA Processing in a Paralog-Specific Manner

(A) Illustration of the processing of rRNA. Total RNA was extracted from strains carrying the different ΔIs, reverse transcribed, and the different pre-rRNA precursors were amplified using a specific set of primers illustrated in (B) and on top of each panel. The percentage (and the number) of ΔIs causing more than 20% decrease or increase in primary transcripts (C), 18S processing intermediates (D), or 25S precursors (E) as determined by qPCR is illustrated in the form of bar graphs. The asterisk (*) indicates 5′ or 3′ extension to the rRNA intermediates. (F) Histogram showing the percentage (and the number) of ΔI strains that results in either copy-specific (black) pre-rRNA processing defect or results in similar pre-rRNA processing defect when introduced in either copy of the duplicated gene set (white).

the biased requirement of pre-rRNA processing on LSU proteins. As expected, the percentage of ΔIs in SSU proteins affecting 18S pre-rRNA processing was higher than those in LSU proteins. However, a number of the deletions in LSU proteins affected the processing at A1 and D sites near the 18S pre-rRNA (Figure 4D). This is consistent with previous reports suggesting interdependence between the processing of the 25S and 18S rRNA (Allmang et al., 1996; Allmang and Tollervey, 1998; Catala et al., 2008; Warner, 1999). Fewer than 9% of the deletions affected 27S pre-rRNA processing, and the majority of these deletions were in LSU genes with the exception of those affecting the A2/A3 processing separating the LSU and SSU

Table 1. Comparison between the Impact of Intron and Gene Deletions on RNA Expression, Cell Growth, and Pre-rRNA Processing

	Phenotypic Effect				mRNA Level					
	Drugs		Fitness		ΔI		Δ		Variation in the Accumulation of Pre-RNA Intermediates	
Gene	ΔI	Δ	ΔI	Δ	A	B	A	B	ΔI	Δ
RPS9A	S−	S−	0.93	1.52	6.35	0.18	0	1.11	D-35S*	D-20S
RPS9B	NE	S− R− H−	0.55	0.04	0.05	0.32	21.7	0.00	I-23S; D25S*	I-25.5S/27SBs*; I-23S
RPS17A	NE	S− H− C− Ch−	1.06	0.00	0.68	0.61	0.00	2.64	I-*18S; 25S*	I-25S*
RPS17B	S−	S−	1.32	1.71	0.66	1.33	1.49	0.00	D-25S*	NE
RPS29A	S−	S−	0.42	0.00	0.39	0.73	0.00	2.08	I-20S	I-25.5S/27SBs*; I-*18S
RPS29B	C− N−	S− H−	0.50	0.00	0.53	0.53	1.16	0.00	I-27SA2/A3*	NE
RPL7A	NE	S− R−	0.81	0.00	1.24	0.31	0.00	11.7	I-25S*	D-23S
RPL7B	NE	S−	0.95	1.63	0.66	7.21	0.89	0.00	D-25S*; D-20S	I-*18S
RPL33A	N−	ND[a]	1.09	0.00	0.55	0.53	0.00	1.50	I-35S*	D-*18S
RPL33B	NE	S+	1.24	0.32	1.27	1.69	1.32	0.00	I-32S	D-25.5S/27SBs*; D-23S
RPL34A	C− S−	S+	1.15	1.96	0.61	0.55	0.00	0.84	D-25S*	NE
RPL34B	S− M+	S+ R− H− C−	1.12	0.3	0.58	0.68	1.36	0.00	I-27SA2/A3; D-20S	D-35S*
RPL35A	S−	NE	0.76	0.00	1.36	0.64	0.00	2.15	I-35S*	I-*18S
RPL35B	S−	S+ R+	0.89	1.41	1.06	1.44	1.74	0.00	I-25.5S/27SBs; I-23S	NE

ΔI, intron deletion strain; Δ, gene deletion strain; C, caffeine; Ch, cycloheximide; H, hygromycin B; M, MMS; N, NaCl; R, rapamycin; S, staurosporine; −, reduced cell growth; +, increased cell growth; NE, no effect; ND, not determined; I and D, increase or decrease in a processing intermediate when compared to wild-type cells.

[a] Drug effect not determined due to severe growth defect in rich media.

rRNA (Figure 4E). Intriguingly, about 45% of the ΔIs perturbed the accumulation of transcripts extending at the 25S 3′ end beyond the B2 processing site (Figure 4E), suggesting that this processing site is particularly sensitive to variation in the expression of RP genes. We conclude that the intron-dependent regulation of RP gene expression modulates the maturation of rRNA.

Introns of Duplicated RP Genes Influence Pre-rRNA Processing in a Paralog-Specific Manner

In several cases, deletion of one or the other intron resulted in the same effect on pre-rRNA processing, as would be expected (Figure 4F). For example deleting introns from *RPL37* gene sets increased the 35S* amount regardless of the gene copy affected (Table S2). However, surprisingly, in the majority of cases, ΔI-dependent defects in pre-rRNA processing were not due to decrease in the expression of the RP genes. For example deletion of the *RPL13A* intron, which significantly increases the expression of the A and not the B copy, delayed processing of the 35S*, whereas deletion of the intron from *RPL13B* did not (Table S2). In this particular case it seems that pre-rRNA processing was much more sensitive to the intron-dependent expression of *RPL13B* because the deletion of the intron of this gene affected the accumulation of the 25.5S/27SBs*, regardless of the status of the *RPL13A* intron or its level of expression (Table S2). An example of intron-specific effect can be seen in the case of *RPL33* gene set. Deleting the intron from *RPL33A* or both *RPL33A* and *B* decreases the expression of both genes, leading to an increase in the 35S*, whereas deleting the intron of *RPL33B* increases the expression of both copies and inhibits the processing of 32S. Most interestingly, the complete deletion of the host genes failed in general to mimic the effect of ΔI. As listed in Table 1, deleting the intron of *RPL33A* inhibited the processing of the 35S*, whereas the deletion of the entire gene led to a decrease in unprocessed *18S pre-rRNA. Remarkably, whereas deleting the entire *RPS29B* had no effect on pre-rRNA processing, deleting the intron of this gene inhibited the processing of 27S pre-rRNA. This allele-specific effect on pre-rRNA processing was not observed only upon ΔIs but also with the complete gene because in most cases the complete deletion of one copy did not mimic the effect of the other (Table 1). This indicates that the duplicated RP genes do not contribute equally to pre-rRNA processing. We conclude that introns affect pre-rRNA processing in a paralog-specific manner suggesting a nonredundant function for each copy of the duplicated RP genes in pre-rRNA processing.

Introns Regulate Cell Fitness and Drug Response

To assess the biological impact of intron-dependent regulation of RP genes, we monitored the consequence of each single and double deletion on cell growth. Initially, all deletions were tested for growth under normal growth conditions, and none of the deletions displayed detectable growth defects (Figure 5A, "Glucose" lane). To determine the impact of introns on conditional growth, we performed a pilot study with strains carrying ΔIs in essential RP genes (Table S1). Thus, 9 ΔI strains were subjected to comprehensive functional assays that include growth on 8 different carbon sources, 16 different drugs affecting various cell functions, and growth at 3 different temperatures (Parenteau et al., 2008). Carbon sources, temperatures, and 11 of the 16 drugs tested had no effect on the growth of the ΔI strains (data not shown). Instead, five drugs (staurosporine, MMS, NaCl, caffeine, hygromycin B) related to protein synthesis induced a growth defect in strains carrying ΔIs in essential genes (Figure 5A). Based on these results, we tested all ΔI strains for

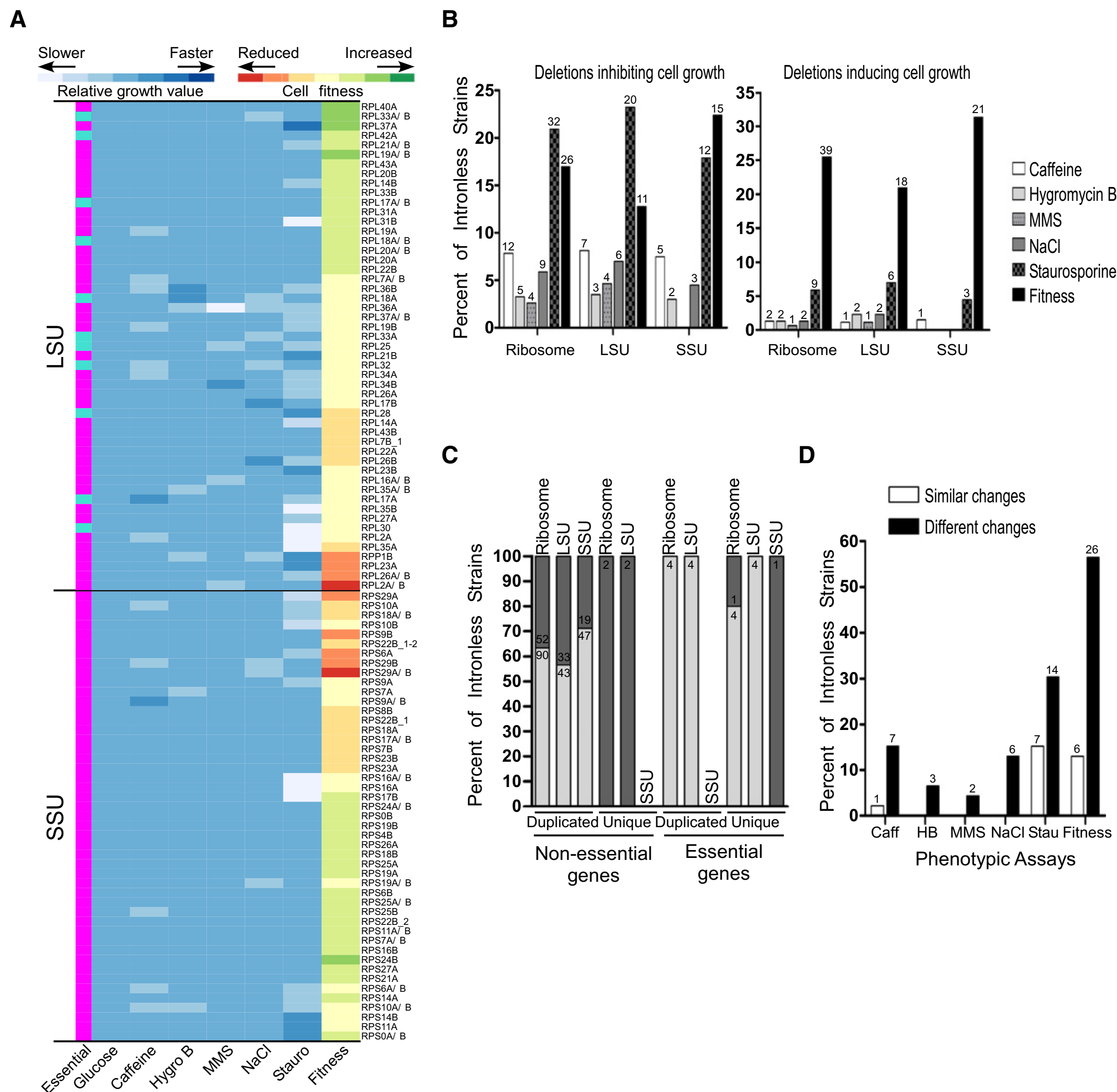

Figure 5. Introns of Duplicated RP Genes Modulate Cell Response to Stress and Competitive Growth in a Copy-Specific Manner

Wild-type cells and cells carrying different ΔIs were assayed for growth in five different growth conditions or in competition with wild-type cells to determine cell fitness. The maximum growth rate (μm) of the different strains was measured and compared to that of the wild-type strain. Cell fitness was determined by the ratio of wild-type to mutant cells observed after 50 generations of growth in mixed cultures. The values of these experiments are illustrated in the form of a heat map (A) that includes strains growing faster or slower than wild-type cells with ≤ 0.2 times and strains exhibiting 10% variation in fitness. The type of growth assay is indicated at the bottom. The genes were organized according to their subunit affiliation, indicated on the left. Essential RP genes are shown in cyan on the left.

(B) Histogram indicating the percentage (and the number) of ΔIs in RP genes, LSU genes, and SSU genes inhibiting (left panel) or inducing (right panel) growth under different conditions (see Table S2).

(C) Distribution of ΔIs in ribosome, LSU, and SSU genes that affect (light gray) or do not affect (dark gray) cell growth under certain conditions. The data were plotted in function of gene requirement for growth (e.g., essential or nonessential) and number of gene copy (e.g., unique or duplicated).

(D) Histogram showing the percentage (and the number) of ΔI strains in duplicated RP genes that results in either copy-specific phenotypic effects (black) or results in similar phenotypic effect (white) when introduced in either copy of the duplicated gene set. Caff, caffeine; HB, hygromycin B; Stau, staurosporine.

growth in the presence of these five drugs. In order to ensure that we did not miss a relevant phenotype, we included two more drugs that either inhibit amino acid synthesis (cycloheximide) or RB (rapamycin). These latter experiments did not identify any new phenotypic defects, confirming the accuracy of the initial drug selection criteria. The strongest effect was observed in the presence of the known apoptosis-inducing drug staurosporine (Heerdt et al., 2000; Nadano et al., 2001; Tuhácková, 1994). Inclusion of this drug in the media inhibited the growth of 21% of all strains carrying ΔIs with slight inhibitory bias toward ΔI in LSU RP genes (Figure 5B). Each of the other drugs inhibited growth of 3%–7% of the ΔI strains. Interestingly, ΔIs did not exclusively inhibit cell growth in the presence of drugs, but instead, 10% of the deletions actually enhanced growth

suggesting that introns may both negatively and positively regulate growth under stress. Overall, 37% of the ΔIs affected growth in the presence of one or more drugs (Table S2). As would be expected, ΔIs in essential genes showed a stronger effect on growth in the presence of drug than ΔIs in nonessential genes (Figure 5C). We conclude that introns play an important role in RP-associated drug response.

It was previously suggested that the presence of introns provides a growth advantage (Parenteau et al., 2008). Therefore, we grew ΔI strains in competition with wild-type cells to monitor cell fitness. As shown in Figures 5A and 5B, 17% of all ΔIs decreased cell fitness, and 25% of the deletions enhanced cell fitness in rich media. Surprisingly, unlike the effect of drugs, more deletions in genes associated with SSU affected cell fitness suggesting that intron impact on cell survival under stress or in competition is subunit specific. Indeed, 36% of the LSU genes affecting fitness were previously implicated in bridging the two subunits. Deleting the introns of one of these bridging protein genes *RPL2* reduced cell fitness to 14% of that of the wild-type cells, whereas deleting the introns of the SSU protein gene *RPS29* reduced fitness to 16% of the wild-type (Table S2). This suggests that even those LSU protein genes affecting fitness may do so by modifying or influencing SSU-related activity. In the majority of cases, the decreased fitness is associated with a decrease in the mRNA amount of one or both isoforms. However, in six cases (*RPL23A*, *RPL26B*, *RPL14A*, *RPL28*, *RPS22B*, and *RPS10A*), the fitness defect was observed upon a net increase in the expression. This clearly indicates that observed effect is not simply due to protein loss but in certain cases may be affected by the ratio of the RP expressed. We conclude that RP introns play an important role in modulating the competitive advantage of yeast cells.

Intron-Dependent Nonredundant Function of Duplicated RPs

If RP paralogs were redundant, deleting introns from them should generate an allele-independent phenotypic effect. As indicated in Figure 5D, the vast majority of ΔIs produced fitness and drug sensitivity effects that were specific to only one copy of the duplicated RP genes. For example deleting the intron of *RPL23A* reduced cell fitness to 20% of wild-type, whereas deleting the intron of *RPL23B* did not (Table S2). In this case it is notable that the effect on fitness was not due to a decrease in the mRNA expression of either of the gene copies, but instead, it stimulated the expression of only one of the two alleles. Only three gene sets displayed the same defects regardless of the copy targeted (e.g., *RPL14*, Table S2). In contrast, 13 gene sets showed a drug effect when the intron of either copy was targeted (e.g., *RPL18A*), whereas 5 gene sets exhibited copy-specific drug sensitivity (e.g., *RPS29A* and *B*, Table S2). These data further support the idea that introns of duplicated RP genes play a role in the manifestation of nonredundant functions for the RP paralogs in drug resistance and cell fitness. To confirm the nonredundant functions of the duplicated RP genes and better understand the role of introns in this phenomenon, we compared the phenotypes of strains carrying a ΔI to those displayed by strains carrying complete gene deletions. In almost all cases, the complete deletion of one or the other copy of the duplicated RP gene sets caused a different drug sensitivity pattern or cell fitness (Table 1). For example, whereas deleting *RPS9A* causes sensitivity to staurosporine, the deletion of *RPS9B* causes additional sensitivity to rapamycin and hygromycin B. The deletion of the intron or of the entire gene yielded similar drug sensitivity patterns in only three cases and with only one drug (i.e., staurosporine). The majority of the ΔI strains exhibited different and, in some cases, opposite effects compared to those carrying a gene deletion. For example, whereas deleting the intron of *RPL35B* reduces growth in the presence of staurosporine, the gene deletion enhances growth in the presence of staurosporine and rapamycin. In some cases, including *RPL35B*, this could be explained by the fact that the intron has a negative intragenic effect, i.e., the loss of it causes an increase in expression, whereas the gene deletion can only lead to loss of expression. We conclude that the duplicated RP genes play unique roles in drug resistance and competitive growth that are inter-regulated through splicing.

Drugs Affecting Protein Synthesis Differentially Affect the Expression of RP Paralogs

In order to further confirm the allele-specific nature of RP genes' contribution to drug resistance, we directly tested the impact of drug exposure to the expression of a selected set of RP genes. The RNA was extracted from cells at a drug concentration that reduces the growth of wild-type by 50%, and the expression of the different RP genes was compared to that of housekeeping genes using qPCR. As shown in Figure 6, in most cases the drugs unequally affected the expression of the duplicated genes. For example mRNA of *RPS9A* extracted from wild-type cells was not sensitive to caffeine and NaCl, whereas the expression of the B paralog was increased by caffeine and decreased by NaCl. Exposure to MMS, hygromycin B, and staurosporine reduced the expression of both alleles of *RPS9*, but in all cases except for hygromycin B, the drugs altered the ratio of the two RP copies relative to that of wild-type cells grown without drugs (Figure 6, top-left panel). Similar behavior was also observed with the other genes. The drug-induced changes in the expression of RP genes were dependent on the presence of introns (Figure 6). For example, whereas exposure to caffeine drastically increases the expression of wild-type *RPS29A*, it did not alter the expression of the intron-less version (Figure 6, middle-left panel). Therefore, the presence of intron confers differential responsiveness to drugs that target protein synthesis, further strengthening the link between introns, RB and function.

DISCUSSION

The relatively small number of introns and the virtual absence of alternative splicing in budding yeast raise questions about the function of introns and their requirement for cell growth. In this study we show that whereas introns are not essential for cell growth under laboratory conditions, they influence cell survival under stress and competition for limited resources. Deletion of introns in RP genes affected cell fitness and growth in the presence of drugs regardless of the requirement of the gene for growth and its ribosomal subunit association, suggesting a general and independent function for introns of RP genes

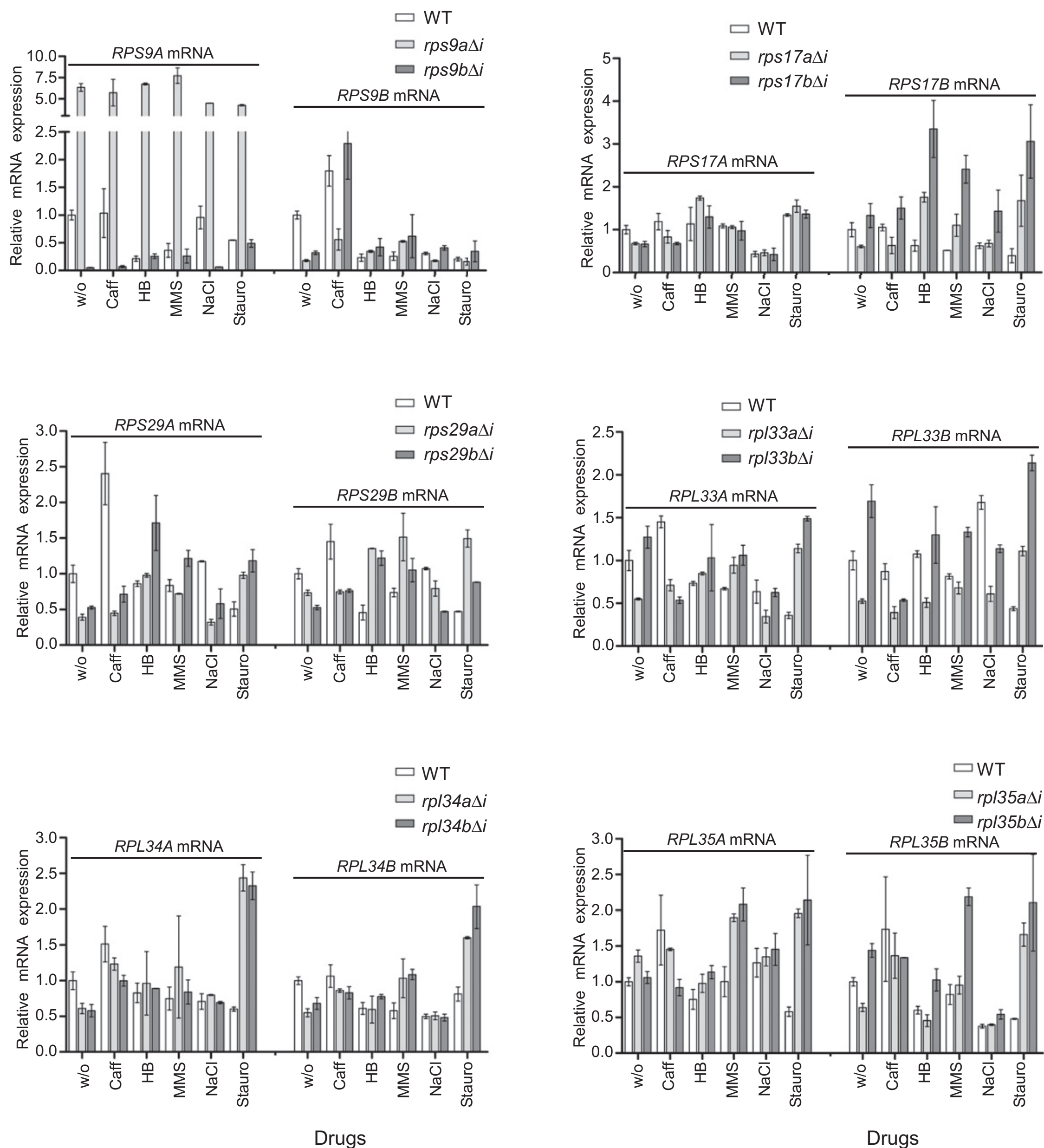

Figure 6. The Drugs Unequally Affect the Expression of the Duplicated RP Genes

Impact of drugs on the expression of duplicated RP genes was measured by gene-specific qPCR in wild-type cells (white) and cells carrying ΔIs (aΔi, light gray; bΔi, dark gray). The levels of A (left) and B (right) mRNA were normalized to *SPT15* and relative to the expression of wild-type mRNA extracted from cells grown in the absence of drugs. The strains grown in the presence of drugs were done with two biological replicates, whereas the strains grown without (w/o) drugs were done with at least three biological replicates; the error bars indicate the standard deviation. The bar graphs illustrate the data of six sets of duplicated RP genes: *RPS9*, *RPS17*, *RPS29*, *RPL33*, *RPL34*, and *RPL35*. Caff, caffeine; HB, hygromycin B; Stauro, staurosporine, WT, wild-type.

(Figure 5). However, the most perceptible effect of ΔIs was the deregulation of the expression of duplicated RP genes (Figure 2). Surprisingly, the presence of introns does not necessarily inhibit gene expression, as one might expect due to the obligate delay in RNA maturation, but can also stimulate gene expression. Furthermore, introns affected the expression of the duplicated RP genes in an allele-specific manner, causing a phenotypic effect that was mostly different from the complete gene deletion (Table 1). Therefore, introns appear to provide an additional layer of regulation allowing yeast cells to vary the relative expression of duplicated genes. Accordingly, we propose that introns are required for fine-tuning the expression of duplicated RP genes, thereby offering means to exploit their functional difference and to alter ribosome homeostasis in response to changes in growth conditions. However, although it was previously shown that ΔI affects both RNA and protein levels (Juneau et al.,

2006) and that ΔI of RP genes may affect protein synthesis, we cannot exclude the possibility that the effects observed are only due to variation in RNA levels or changes in the timing of protein expression.

Cell Survival Promotes Intron Conservation

It was suggested that yeast introns are being phased out of the yeast genome through reverse transcription/recombination mechanisms (Fink, 1987). However, RP genes constitute the most highly expressed group of intron-containing genes in *S. cerevisiae*, arguing in favor of intron function. Introns may increase gene expression or provide an important regulatory mechanism for calibrating the levels of RP (Ares et al., 1999). However, it remains unclear whether introns are important in yeast, or given sufficient time, all introns would be lost. We previously showed that none of the approximate one-third of all yeast introns tested was required for growth under permissive laboratory conditions and that the combined deletion of all introns in one metabolic pathway did not slow growth (Parenteau et al., 2008). This result argued that yeast might survive without the majority of its introns. Here, we demonstrate that none of the introns in RP genes individually is essential for growth under normal laboratory conditions. Strikingly, loss of RP introns did not affect growth in alternative carbon sources or temperatures, even when the introns were from unique essential RP genes or both copies of duplicated genes. This clearly supports the previous conclusion that introns are not essential for viability. However, this does not mean that yeast introns do not carry out important functions and that they are dispensable for yeast living under natural conditions. Although we found no correlation between the expression level or the extent of the ΔI effect on expression and the phenotypic effects, we have established that the vast majority of introns are required for regulating the expression of RP genes, modulating cell growth under stress, and/or growth in competition with wild-type cells (Figure 5 and Table S2). Therefore, cells lacking RP introns are unlikely to compete with other yeast cells and may be hypersensitive to antibiotic-producing microbes in their environment. Accordingly, we propose that introns are a conserved feature of the yeast genome that is maintained by evolutionary pressure promoting cell survival.

Introns Impact on Gene Expression

Introns were found to either negatively or positively regulate the expression of RP genes (Figures 1C and 2). Intron-dependent-negative regulation may occur through inefficient splicing (Juneau et al., 2007) or defects in export of non-RP genes (Preker and Guthrie, 2006). Negative regulation was also suggested for *RPL30* (L32), a gene coding for one of the LSUs implicated in pre-rRNA processing. In this case the protein was shown to bind to a specific structure in the intron leading to inefficient splicing, and *rpl30* mutants delayed processing of the 27S pre-rRNA (Vilardell and Warner, 1997). We have also found that the ΔI of *RPL30* delays the processing of 27S pre-rRNA. However, *RPL30*ΔI did not, as expected, increase the expression of the gene but, rather, promoted a decrease in RNA level (Table S2). Therefore, introns may enhance the general expression of genes while providing a target for autoregulation or conditional expression. Indeed, it was proposed that the splicing of these specific genes is required for inclusion of the protein into ribosomes, underlining the positive contribution of introns to protein function (Vilardell and Warner, 1997). In higher eukaryotes, intron-dependent-positive regulation of gene expression was proposed to enhance every step of gene expression, from transcription to RNA stability (Wang et al., 2007). In yeast, intron-containing genes produce more RNA and proteins than those without introns, and the deletion of introns in non-RP genes reduces expression (Juneau et al., 2006). However, the mechanism by which introns stimulate expression remains unclear. It is important to note that defects in cell survival were observed when introns displayed negative and positive regulation, and both types of introns similarly impaired pre-rRNA processing (Figures 4 and 5, and Table S2). Therefore, a similar impact of introns in gene expression ultimately may lead to very different phenotypic impact.

Introns of Duplicated Genes Regulate Gene Expression and Functional Diversity

In metazoan cells, alternative splicing increases the functional diversity of genes to support cellular and organism complexity (Benz and Huang, 1997; Zhuo et al., 2007). A pre-mRNA generated by a single gene may be alternatively spliced to generate tens and, in some cases, thousands of proteins with different functions (Lee et al., 2010). In yeast, alternative splicing is restricted to a handful of genes, and in these cases the change in splicing often leads to a change in mRNA maturation (Davis et al., 2000) and localization (Juneau et al., 2009). The vast majority of the genes containing introns in yeast are constitutively spliced (Davis et al., 2000; Parenteau et al., 2008; Spingola et al., 1999), which implies that regulation is not required. If this was the case, one would expect that the deletion of most introns in yeast would either have no effect on gene expression or would increase gene expression by shortening the production of mature mRNA. In contrast we have observed that the deletion of the majority of the so-called constitutively spliced introns affects gene expression and function (Figures 2, 3, and 5). The fact that ΔI reduced gene expression in about 40% of the cases strongly argues against the idea that introns are always constitutively removed (Figure 2). Indeed, here, we show that the majority of RP introns establish a pattern of expression responsive to variation in growth conditions (Figure 6). However, perhaps the most startling discovery of this study is the fact that the deletion of constitutively spliced introns of duplicated RP genes affects not only the expression of the host gene (intragenic regulation) but also the expression of the paralog (Figures 2 and 3). Thus, the interplay between two splicing events in duplicated RP genes may set the specific ratio of similar but not identical mRNAs providing a substitute to alternative splicing. Therefore, interdependent gene regulation of duplicated genes might represent a rudimentary analog of the highly sophisticated mechanism of alternative splicing.

Posttranscriptional Regulation of the Ribosome

Duplicated RP genes were thought to function primarily as a homeostatic mechanism that ensures the constant supply of RPs. This notion was based on the fact that in most cases

individual copies of the duplicated gene sets are not essential and exhibit a tendency to cocluster in the same protein complexes (Li et al., 2010). Indeed, products from duplicated RP genes are found in the same ribosomal subunit, and each form at least partially rescues the deletions of both (Babenko and Krylov, 2004). More recently, a survey of RPs affecting the expression of the mating-type switch suppressor *ASH1* revealed a copy-specific localization and function for duplicated RP genes, suggesting the existence of a ribosome code (Komili et al., 2007). However, the mechanism that introduces and regulates the variation in ribosome function and the exact nature of these variations remained unclear. Here, we provide evidence that ribosome function is influenced by an intron-dependent regulatory mechanism capable of providing the flexibility needed for regulating a single copy, coregulation of both copies, or opposite regulation of the paralogs. Furthermore, interfering with this regulatory mechanism impacted cell fitness and responsiveness to drug-induced stress (Figure 5), although the genetic networks behind the function impact of ΔIs remain unclear. However, perhaps the most revealing finding in this context is the clear difference between the impact of gene loss and intron loss. Moreover, the fact that changes in the expression of RP genes can by itself modulate RB and function strongly supports the existence of a ribosome code. Indeed, this code appears to be dynamic and responsive because changes in the environment induced copy-specific change in the expression of duplicated RP genes. It is now essential to examine the presence and the contribution of ribosome code to the biology of organisms other than yeast. In mammals regulation of ribosomal gene expression might be governed by analogous mechanism like alternative splicing to accomplish the function of the duplicated genes in yeast.

EXPERIMENTAL PROCEDURES

Strains

Two independent colonies of the strain JPY10I were used in parallel for ΔIs (Parenteau et al., 2008). To remove intron, we used the same direct intron-displacement strategy described in Parenteau et al. (2008). The multiple intergene deletion set was made by mating two ΔI strains. The resulting diploid strain was sporulated to produce cells containing two ΔIs. Yeast cells were grown in standard yeast media (Rose et al., 1990; Zakian and Scott, 1982).

RNA Extraction and qPCR Analysis

Total RNA was isolated from cells grown 5 hr with or without drugs using a commercial kit (Omega Bio-Tek). DNase treatment (QIAGEN) was performed. The integrity of the treated RNA was examined using Agilent 2100 Bioanalyzer that calculates the rRNA ratio, and 50 ng was reverse transcribed using Transcriptor Reverse Transcriptase (Roche). PCRs were performed in a realplex (Eppendorf) as previously described (Brosseau et al., 2010). In general the assays included three biological and two technical replicates. RNA levels were normalized to unrelated RNAs (e.g., Spt15, Act1). The list of the primers used for real-time PCR can be supplied upon request.

Growth Assays and Fitness Test

Growth assays were performed in triplicate as described in Parenteau et al. (2008). Growth curve analysis was performed as described (Parenteau et al., 2008; Toussaint et al., 2006). Competitive growth assays or fitness test was performed in duplicate as described (Parenteau et al., 2008).

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures and five tables and can be found with this article online at doi:10.1016/j.cell.2011.08.044.

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REFERENCES

Allmang, C., and Tollervey, D. (1998). The role of the 3′ external transcribed spacer in yeast pre-rRNA processing. J. Mol. Biol. *278*, 67–78.

Allmang, C., Henry, Y., Morrissey, J.P., Wood, H., Petfalski, E., and Tollervey, D. (1996). Processing of the yeast pre-rRNA at sites A(2) and A(3) is linked. RNA *2*, 63–73.

Antúnez de Mayolo, P., and Woolford, J.L., Jr. (2003). Interactions of yeast ribosomal protein rpS14 with RNA. J. Mol. Biol. *333*, 697–709.

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.

Arvas, M., Kivioja, T., Mitchell, A., Saloheimo, M., Ussery, D., Penttila, M., and Oliver, S. (2007). Comparison of protein coding gene contents of the fungal phyla Pezizomycotina and Saccharomycotina. BMC Genomics *8*, 325.

Babenko, V.N., and Krylov, D.M. (2004). Comparative analysis of complete genomes reveals gene loss, acquisition and acceleration of evolutionary rates in Metazoa, suggests a prevalence of evolution via gene acquisition and indicates that the evolutionary rates in animals tend to be conserved. Nucleic Acids Res. *32*, 5029–5035.

Benz, E.J., Jr., and Huang, S.C. (1997). Role of tissue specific alternative pre-mRNA splicing in the differentiation of the erythrocyte membrane. Trans. Am. Clin. Climatol. Assoc. *108*, 78–95.

Brosseau, J.P., Lucier, J.F., Lapointe, E., Durand, M., Gendron, D., Gervais-Bird, J., Tremblay, K., Perreault, J.P., and Elela, S.A. (2010). High-throughput quantification of splicing isoforms. RNA *16*, 442–449.

Bulman, S., Ridgway, H.J., Eady, C., and Conner, A.J. (2007). Intron-rich gene structure in the intracellular plant parasite *Plasmodiophora brassicae*. Protist *158*, 423–433.

Catala, M., Tremblay, M., Samson, E., Conconi, A., and Abou Elela, S. (2008). Deletion of Rnt1p alters the proportion of open versus closed rRNA gene repeats in yeast. Mol. Cell. Biol. *28*, 619–629.

Davidovich, C., Belousoff, M., Bashan, A., and Yonath, A. (2009). The evolving ribosome: from non-coded peptide bond formation to sophisticated translation machinery. Res. Microbiol. *160*, 487–492.

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.

Fink, G.R. (1987). Pseudogenes in yeast? Cell *49*, 5–6.

Fromont-Racine, M., Senger, B., Saveanu, C., and Fasiolo, F. (2003). Ribosome assembly in eukaryotes. Gene *313*, 17–42.

Granneman, S., Nandineni, M.R., and Baserga, S.J. (2005). The putative NTPase Fap7 mediates cytoplasmic 20S pre-rRNA processing through a direct interaction with Rps14. Mol. Cell. Biol. *25*, 10352–10364.

Grate, L., and Ares, M., Jr. (2002). Searching yeast intron data at Ares lab Web site. Methods Enzymol. *350*, 380–392.

Heerdt, B.G., Houston, M.A., Mariadason, J.M., and Augenlicht, L.H. (2000). Dissociation of staurosporine-induced apoptosis from G2-M arrest in SW620 human colonic carcinoma cells: initiation of the apoptotic cascade is associated with elevation of the mitochondrial membrane potential (deltapsim). Cancer Res. *60*, 6704–6713.

Ihmels, J., Collins, S.R., Schuldiner, M., Krogan, N.J., and Weissman, J.S. (2007). Backup without redundancy: genetic interactions reveal the cost of duplicate gene loss. Mol. Syst. Biol. *3*, 86.

Juneau, K., Nislow, C., and Davis, R.W. (2009). Alternative splicing of PTC7 in *Saccharomyces cerevisiae* determines protein localization. Genetics *183*, 185–194.

Juneau, K., Miranda, M., Hillenmeyer, M.E., Nislow, C., and Davis, R.W. (2006). Introns regulate RNA and protein abundance in yeast. Genetics *174*, 511–518.

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.

Kafri, R., Levy, M., and Pilpel, Y. (2006). The regulatory utilization of genetic redundancy through responsive backup circuits. Proc. Natl. Acad. Sci. USA *103*, 11653–11658.

Komili, S., Farny, N.G., Roth, F.P., and Silver, P.A. (2007). Functional specificity among ribosomal proteins regulates gene expression. Cell *131*, 557–571.

Kriventseva, E.V., Koch, I., Apweiler, R., Vingron, M., Bork, P., Gelfand, M.S., and Sunyaev, S. (2003). Increase of functional diversity by alternative splicing. Trends Genet. *19*, 124–128.

Lee, C., Kim, N., Roy, M., and Graveley, B.R. (2010). Massive expansions of Dscam splicing diversity via staggered homologous recombination during arthropod evolution. RNA *16*, 91–105.

Li, J., Yuan, Z., and Zhang, Z. (2010). The cellular robustness by genetic redundancy in budding yeast. PLoS Genet. *6*, e1001187.

Meskauskas, A., Russ, J.R., and Dinman, J.D. (2008). Structure/function analysis of yeast ribosomal protein L2. Nucleic Acids Res. *36*, 1826–1835.

Nadano, D., Aoki, C., Yoshinaka, T., Irie, S., and Sato, T.A. (2001). Electrophoretic characterization of ribosomal subunits and proteins in apoptosis: specific downregulation of S11 in staurosporine-treated human breast carcinoma cells. Biochemistry *40*, 15184–15193.

Ohta, T. (1988). Evolution by gene duplication and compensatory advantageous mutations. Genetics *120*, 841–847.

Parenteau, J., Durand, M., Véronneau, S., Lacombe, A.A., Morin, G., Guérin, V., Cecez, B., Gervais-Bird, J., Koh, C.S., Brunelle, D., et al. (2008). Deletion of many yeast introns reveals a minority of genes that require splicing for function. Mol. Biol. Cell *19*, 1932–1941.

Preker, P.J., and Guthrie, C. (2006). Autoregulation of the mRNA export factor Yra1p requires inefficient splicing of its pre-mRNA. RNA *12*, 994–1006.

Rose, M.D., Winston, F., and Hieter, P. (1990). Methods in Yeast Genetics: A Laboratory Course Manual (Cold Spring Harbor, N.Y: Cold Spring Harbor Laboratory Press).

Roy, S.W., and Penny, D. (2006). Large-scale intron conservation and order-of-magnitude variation in intron loss/gain rates in apicomplexan evolution. Genome Res. *16*, 1270–1275.

Russell, A.G., Shutt, T.E., Watkins, R.F., and Gray, M.W. (2005). An ancient spliceosomal intron in the ribosomal protein L7a gene (Rpl7a) of *Giardia lamblia*. BMC Evol. Biol. *5*, 45.

Sakurai, A., Fujimori, S., Kochiwa, H., Kitamura-Abe, S., Washio, T., Saito, R., Carninci, P., Hayashizaki, Y., and Tomita, M. (2002). On biased distribution of introns in various eukaryotes. Gene *300*, 89–95.

Solis, A.S., Shariat, N., and Patton, J.G. (2008). Splicing fidelity, enhancers, and disease. Front. Biosci. *13*, 1926–1942.

Spingola, M., Grate, L., Haussler, D., and Ares, M., Jr. (1999). Genome-wide bioinformatic and molecular analysis of introns in *Saccharomyces cerevisiae*. RNA *5*, 221–234.

Stetefeld, J., and Ruegg, M.A. (2005). Structural and functional diversity generated by alternative mRNA splicing. Trends Biochem. Sci. *30*, 515–521.

Sugino, R.P., and Innan, H. (2006). Selection for more of the same product as a force to enhance concerted evolution of duplicated genes. Trends Genet. *22*, 642–644.

Toussaint, M., Levasseur, G., Gervais-Bird, J., Wellinger, R.J., Elela, S.A., and Conconi, A. (2006). A high-throughput method to measure the sensitivity of yeast cells to genotoxic agents in liquid cultures. Mutat. Res. *606*, 92–105.

Tuhácková, Z. (1994). Staurosporine and gossypol are inhibitors of the function of peptide elongation factor 1 alpha from rabbit reticulocytes. Biochem. Mol. Biol. Int. *32*, 545–554.

Vilardell, J., and Warner, J.R. (1994). Regulation of splicing at an intermediate step in the formation of the spliceosome. Genes Dev. *8*, 211–220.

Vilardell, J., and Warner, J.R. (1997). Ribosomal protein L32 of *Saccharomyces cerevisiae* influences both the splicing of its own transcript and the processing of rRNA. Mol. Cell. Biol. *17*, 1959–1965.

Wang, H.F., Feng, L., and Niu, D.K. (2007). Relationship between mRNA stability and intron presence. Biochem. Biophys. Res. Commun. *354*, 203–208.

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

Weng, S., Dong, Q., Balakrishnan, R., Christie, K., Costanzo, M., Dolinski, K., Dwight, S.S., Engel, S., Fisk, D.G., Hong, E., et al. (2003). Saccharomyces Genome Database (SGD) provides biochemical and structural information for budding yeast proteins. Nucleic Acids Res. *31*, 216–218.

Williamson, J.R. (2003). After the ribosome structures: how are the subunits assembled? RNA *9*, 165–167.

Zakian, V.A., and Scott, J.F. (1982). Construction, replication, and chromatin structure of TRP1 RI circle, a multiple-copy synthetic plasmid derived from *Saccharomyces cerevisiae* chromosomal DNA. Mol. Cell. Biol. *2*, 221–232.

Zhuo, D., Madden, R., Elela, S.A., and Chabot, B. (2007). Modern origin of numerous alternatively spliced human introns from tandem arrays. Proc. Natl. Acad. Sci. USA *104*, 882–886.