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LETTER TO THE EDITOR

A handful of intron-containing genes produces the lion's share of yeast mRNA

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Two studies have provided separate pieces of information that bear on the functional and evolutionary significance of introns in the budding yeast *Saccharomyces cerevisiae* (Holstege et al., 1998; Spingola et al., 1999). By the measure of the number of introns within its genes, budding yeast seems a disappointing eukaryote. Fewer than 250 of the more than 6,200 annotated genes are known to have introns, and fewer than 10 are known have more than one intron (Spingola et al., 1999). In contrast, metazoan genes are estimated to average nearly 10 introns, and the intronless gene is the exception rather than the rule. Although many essential yeast genes have introns, it would appear that introns are on the way out of the yeast genome, perhaps by a cDNA-directed homologous recombination mechanism proposed by Fink (1987).

A simple tally of the genomic presence of introns fails to measure their abundance in the RNA pool of an organism, arguably a more critical measure of their importance. To estimate the presence of introns in the pre-mRNA population of yeast, we obtained the expression data of Holstege et al. (1998), and compared it to our annotated database of genomic yeast introns (http://www.cse.ucsc.edu/research/compbio/yeast_introns.html). We separated yeast genes into intron-containing and intron-lacking classes and summed the estimated number of mRNAs synthesized each hour in each class (Table 1). Although the data is based on expression in a heat-treated RNA polymerase mutant that could introduce class-specific mRNA synthesis or turnover biases (Holstege et al., 1998), this analysis suggests that more than 10,000 of the nearly 38,000 mRNA molecules made each hour are derived from genes that have introns.

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The major functional class of intron-containing genes is the ribosomal protein genes class: of the 229 introns in the current annotation of the yeast genome, nearly 100 are in ribosomal protein genes. Table 1 shows that ribosomal protein mRNAs account for about 90% of all mRNA transcripts from intron-containing genes. Why should this be? One possibility is that there are intron-dependent regulatory events that help control global expression of ribosomal proteins. Another is that introns may improve expression of the genes that contain them in yeast, as apparently they do in mice (Choi et al., 1991; Palmiter et al., 1991). The proposal that reverse transcripts mediate intron loss in yeast (Fink, 1987) might predict that the most abundantly transcribed genes would be the first to lose their introns, but ribosomal protein genes have defied this expectation. For evolutionary reasons we have yet to divine, the few remaining intron-containing genes in yeast produce nearly a third of all mRNAs, so that at the level of RNA, introns are still very much the business of the yeast cell.

Table 1 also illuminates the extreme degree to which the work of the yeast splicing apparatus is devoted to ribosome biogenesis. Hartwell's original *rna* mutants were characterized as unable to make RNA at a restrictive temperature in a metabolic labeling experiment that measures primarily rRNA accumulation (Hartwell et al., 1970). Several years (as well as the discovery of splicing) ensued before it was noted that the *rna* mutations (since renamed *prp*) affect removal of introns (Rosbash et al., 1981), and that most inactivate components of the splicing apparatus as assayed in vitro (Lustig et al., 1986). In addition, many unusual trends in gene organization have been noted, including such curiosities as small nucleolar RNAs (involved in rRNA maturation) encoded within introns of ribosomal protein, translation factor, and ribosome assembly factor genes, or introns within snoRNA U3 genes (for review,

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TABLE 1. Comparison of estimated mRNA synthesis rates for intron-containing and non-intron-containing ribosomal protein and non-ribosomal protein genes.

Gene class	Number of genes	Estimated mRNA/h/cell
ribosomal protein, *with* introns	95	9,168
not ribosomal protein, *with* introns	134	1,009
ribosomal protein, *no* introns	37	3,414
not ribosomal protein, *no* introns	5,922	24,226

Totals: 6,179 genes and 37,749 mRNA/h/cell. For further details, see http://ribonode.ucsc.edu/IntronExprLet/table1.html.

see Spingola et al., 1999). Thus, although almost 30 years of traditional genetic and molecular studies have hinted that the spliceosome and the ribosome are in close regulatory communication, an expression-based splicing budget (Table 1) illustrates immediately why inhibition of splicing should lead to inhibition of rRNA accumulation. As genome-wide expression studies are performed on strains carrying mutations in the splicing machinery, it will be interesting to note the degree to which expression of genes involved in translation is secondarily affected, even if those genes do not themselves contain introns. In fact, the great absence of introns makes yeast possibly the only system in which to sort out primary from secondary effects of inhibiting splicing.

A final observation that can be made from this data concerns the activity of the splicing apparatus. Estimates of snRNA content of yeast using Northern blots indicate that there are 200–500 copies of the major snRNAs per haploid cell (Riedel et al., 1986). As seen in Table 1, at least 10,000 introns must be removed from pre-mRNA per yeast cell per hour using 500 molecules of U2 (an estimate based on internally controlled primer extensions; M.H. Pauling & M. Ares, unpubl.). With the assumption that every U2 molecule is part of the active pool of splicing factors, this means that each U2 snRNA molecule helps remove about 20 introns per hour, or 1 every 3 min. Splicing factors in greater or lesser abundance than U2 would act more slowly or more rapidly, respectively. If a pathway for snRNP reutilization exists in yeast as suggested for mammalian cells (Pellizzoni et al., 1998), it must be able to recycle a U2 snRNP in under 3 min.

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