Supplemental File SI2. Calculating the costs of synthesis of ribosomes, mRNAs and tRNAs.

In principle, calculating the cost of synthesis of these components is relatively straightforward, since their molecular composition is known and the pure polymerisation costs are biochemically well characterised. However, for none of these components polymerisation is the only cost. In addition, there are various processing and modification steps, some of which consume energy. Since it is difficult to judge the importance of this additional cost relative to the pure polymerisation cost, we carefully examined the respective processes and estimated the total cost from the available literature data.

In the following analyses, the basic cost unit is the terminal phosphate bond of an ATP molecule (ie the hydrolysis of ATP to ADP consumes one cost unit). Hydrolysis of an ATP to AMP (such as occurs during aminoacylation and nucleotide polymerisation) equates to two cost units. Hydrolysis of any other nucleotide triphosphate is assumed to be equal to ATP hydrolysis.

Costs of synthesising the tRNA complement

Lengths of mature tRNA sequences were recovered from the tRNAdb database (1), and length of intron sequences for each tRNA from MIPS (http://mips.helmholtz-muenchen.de/proj/yeast/reviews/feldi/trnagen2.html). Primary tRNA transcripts are typically extended relative to the mature tRNAs by 5-15 nucleotides at both the 5'- and 3'-end (2). Since the recovered tRNA sequences do not contain these extensions, we added 20 nucleotides to each sequence length.

Similar to other eukaryotic RNA polymerases, yeast RNA polymerase III forms short abortive transcripts during transcription initiation. Currently available data indicate that on average 3 abortive transcripts of 5 nt length are formed for each productive one (2). For the short tRNA transcripts, the added cost of this is significant, and we include this as an additional 15 nucleotides = 30 ATP per transcript.

Following end trimming, three nucleotides are added to the 3'-end (3) and, in the case of Histidyl-tRNAs, another nucleotide is added to the 5'-end (4). Lastly, tRNA nucleotides are extensively modified, and some of these modifications are also energy-dependent including Ado-S-Met dependent methylation, threonylation (5) and acetylation (6). Sites of these modifications were recovered from the Modomics database (7) and the respective costs added.

Individual tRNA levels are calculated based on a total tRNA population of 3,000,000 and individual tRNA abundances proportional to tDNA gene numbers as described previously (8). tRNA turnover is assumed to be negligible in wild-type strains under fast growth conditions. The need for tRNA synthesis therefore only arises from the dilution through growth, which we approximate as dilution with a half-life of 90 minutes.

Based on these assumptions, the total cost of tRNA synthesis is 5 million ATP per minute.

Costs of synthesising the ribosome complement

Yeast ribosomal RNA is transcribed in two primary transcripts, a 6858 nt 35S transcript (9) and a 121 nt 5S transcript (10). Around 1% of nucleotides in rRNA are modified in energy-dependent methylation reactions (11), adding some minor modification cost.

In addition to the RNA component, yeast ribosomes contain 69 ribosomal proteins, formed via a total of 21733 amino acid bonds. In rich medium where amino acids do not need to be synthesised, the canonical cost of forming one amino acid bond is 5 NTP (two ATP for formation of the aminoacyl-tRNA, one GTP each for codon decoding and translocation, and a last ATP for E-site tRNA release). However, this is a minimal cost assuming no futile cycles. *In vitro*, aminoacylation reactions in the presence of other amino acids often require more than one ATP , either because cognate amino acids are falsely rejected or because near-cognate amino acids are only rejected after formation of an initial aminoacyl-bond. Similarly, cognate and near-cognate tRNAs can be rejected post-GTP hydrolysis during codon decoding. It is not known how many futile cycles occur on average for each amino acid bond *in vivo*. We arbitrarily add 2 ATP per amino acid bond in order to account for this extra cost. This brings the total per amino acid bond to 7 ATP, close to the value found experimentally for chicken (12).

In addition to the cost of synthesising ribosomal components, there is an energy cost associated with putting ribosomal subunits together. Many of the ribosome maturation factors are ATP-dependent RNA helicases, chaperones, or possess other types of NTPase activity (13). However, the quantitative relationship between energy use by these factors and the number of assembled ribosomes is not well established, and we do not consider the energy cost of these steps. We estimate that inclusion of these factors would increase the energy cost per ribosome by not more than 1%.

Yeast cells contain around 200,000 ribosomes per haploid cell (14). Ribosome turnover is assumed to be negligible in wild-type strains under fast growth conditions. The need for ribosome synthesis therefore only arises from the dilution through growth, which we approximate as dilution with a half-life of 90 minutes.

Based on these assumptions, the total cost of ribosome synthesis is 260 million ATP per minute.

Costs of synthesising the mRNA complement

In order to calculate the length of all individual transcripts, we retrieved coordinates of start and stop codons for each gene from SGD (15). In addition, we retrieved the lengths of 5'- and 3'-untranslated regions from two published studies (16, 17). During transcription termination, DNA polymerase II transcribes ~100 nt past the polyadenylation site (18). Following transcription, yeast transcripts are polyadenylated to an average poly(A) tail length of 70 nt (19). 5'-UTR length, distance between start and stop, 3'-UTR length, an extra 100 nucleotides for transcription and 70 nucleotides for polyadenylation were therefore added to give the total transcribed RNA length.

We considered that for RNA polymerase II, abortive transcript formation during transcription initiation would only consume minor resources compared to those needed for transcription of the long transcripts and did not consider this in our calculations.

mRNA transcription is required because of decay and dilution through growth. We used decay constants for individual mRNAs during growth in YPD from (20), steady state levels averaged from a compilation of studies (14), and growth dilution with a half-life of 90 minutes to calculate transcription frequencies for each gene.

Based on these assumptions, the total cost of mRNA synthesis is 1.7 million ATP per minute.

References

1. Juhling F et al. (2009) tRNAdb 2009: compilation of tRNA sequences and tRNA genes. *Nucleic Acids Research* 37:D159-D162.

2. Bhargava P, Kassavetis GA (1999) Abortive initiation by Saccharomyces cerevisiae RNA polymerase III. *The Journal of Biological Chemistry* 274:26550-26556.

3. Aebi M et al. (1990) Isolation of a temperature-sensitive mutant with an altered tRNA nucleotidyltransferase and cloning of the gene encoding tRNA nucleotidyltransferase in the yeast Saccharomyces cerevisiae. *The Journal of Biological Chemistry* 265:16216-16220.

4. Gu W, Jackman JE, Lohan AJ, Gray MW, Phizicky EMC-289149 (2003) tRNAHis maturation: an essential yeast protein catalyzes addition of a guanine nucleotide to the 5' end of tRNAHis. *Genes & Development* 17:2889-2901.

5. Qian Q, Curran JF, Bjork GRC-107094 (1998) The methyl group of the N6-methyl-N6-threonylcarbamoyladenosine in tRNA of Escherichia coli modestly improves the efficiency of the tRNA. *Journal of Bacteriology* 180:1808-1813.

6. Ikeuchi Y, Kitahara K, Suzuki TC-2500205 (2008) The RNA acetyltransferase driven by ATP hydrolysis synthesizes N4-acetylcytidine of tRNA anticodon. *The EMBO Journal* 27:2194-2203.

7. Czerwoniec A et al. (2009) MODOMICS: a database of RNA modification pathways. 2008 update. *Nucleic Acids Research* 37:D118-D121.

8. Chu D, Barnes DJ, von der Haar T (2011) The role of tRNA and ribosome competition in coupling the expression of different mRNAs in Saccharomyces cerevisiae. *Nucleic Acids Research* 39:6705-14.

9. Fatica A, Tollervey D (2002) Making ribosomes. *Current Opinion in Cell Biology* 14:313-318.

10. Ciganda M, Williams NC-3278907 (2011) Eukaryotic 5S rRNA biogenesis. *Wires RNA* 2:523-533.

11. Piekna-Przybylska D, Decatur WA, Fournier MJC-1800513 (2007) New bioinformatic tools for analysis of nucleotide modifications in eukaryotic rRNA. *RNA* 13:305-312.

12. Aoyagi Y, Tasaki I, Okumura J, Muramatsu T (1988) Energy cost of whole-body protein synthesis measured in vivo in chicks. *Comparative biochemistry and physiology. A, Comparative physiology* 91:765-768.

13. Hage AE, Tollervey D (2004) A surfeit of factors: why is ribosome assembly so much more complicated in eukaryotes than bacteria? *RNA Biology* 1:10-15.

14. von der Haar T (2008) A quantitative estimation of the global translational activity in logarithmically growing yeast cells. *BMC Systems Biology* 2:87.

15. Cherry JM et al. (2012) Saccharomyces Genome Database: the genomics resource of budding yeast. *Nucleic Acids Research* 40:D700-D705.

16. Nagalakshmi U et al. (2008) The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science* 320:1344-1349.

17. Miura F et al. (2006) A large-scale full-length cDNA analysis to explore the budding yeast transcriptome. *Proceedings of the National Academy of Sciences of the United States of America* 103:17846-17851.

18. Birse CE, Minvielle-Sebastia L, Lee BA, Keller W, Proudfoot NJ (1998) Coupling termination of transcription to messenger RNA maturation in yeast. *Science* 280:298-301.

19. Dheur S, Nykamp KR, Viphakone N, Swanson MS, Minvielle-Sebastia L (2005) Yeast mRNA Poly(A) tail length control can be reconstituted in vitro in the absence of Pab1p-dependent Poly(A) nuclease activity. *The Journal of Biological Chemistry* 280:24532-24538.

20. Wang Y et al. (2002) Precision and functional specificity in mRNA decay. *Proceedings of the National Academy of Sciences of the United States of America* 99:5860-5865.