The economics of ribosome biosynthesis in yeast

Jonathan R. Warner

In a rapidly growing yeast cell, 60% of total transcription is devoted to ribosomal RNA, and 50% of RNA polymerase II transcription and 90% of mRNA splicing are devoted to ribosomal proteins (RPs). Coordinate regulation of the ~150 rRNA genes and 137 RP genes that make such prodigious use of resources is essential for the economy of the cell. This is entrusted to a number of signal transduction pathways that can abruptly induce or silence the ribosomal genes, leading to major implications for the expression of other genes as well.

EACH CELL MUST define a budget that balances conflicting demands for resources to maintain cell viability and cell function with demands for resources to support growth. As a major consumer of the cell's resources, ribosome biosynthesis plays a key role in the cell's budgeting process, especially because new ribosomes represent an investment in new plant, with the opportunity for faster growth – but only if other essentials will be available. Ribosome synthesis involves both macroregulation, in conjunction with other aspects of cell growth, and microregulation to ensure that the components of the ribosome are available in equimolar amounts (reviewed in Refs 1,2).

Recent results offer new insight into how the *Saccharomyces cerevisiae* cell uses the 'wisdom of evolution' to optimize this part of its economy and raise new questions about its implementation.

The cost of ribosome biosynthesis

The ratio of RNA to DNA in a rapidly growing cell of *S. cerevisiae* is 50:1 (indeed, the original name for RNA was 'yeast nucleic acid'). The approximate distribution of RNA is 80% rRNA, 15% tRNA and 5% mRNA. Comparison of the size of the genome (1.4×10^7 bp) with the RNA in a ribosome (5469 nucleotides) shows that there are nearly 200 000 ribosomes per cell (Fig. 1a). With a generation time of ~100 min, the cell must produce 2000 ribosomes per min. What are the implications of such high production for the economy of the cell?

The ribosomal RNA genes of *S. cerevisiae* make up 10% of the entire genome, in a single tandem array of ~150 identical repeats, although the number can vary because of unequal meiotic and mitotic recombination. As estimated from the synthesis of PolyA$^-$ RNA, as well as from calculations based on RNA abundance and stability, the transcription of rRNA by RNA polymerase I (Pol I) appears to represent nearly 60% of the total transcription in the cell[1].

The completion of the sequence of the *S. cerevisiae* genome coincided with the analysis of the entire set of mammalian ribosomal protein (RP) genes[3]. The evolutionary conservation of the RPs facilitated the complete classification of the yeast RP genes[4] (http://www.mips.biochem.mpg.de/proj/yeast/reviews/rib_nomencl.html; more detailed information on individual proteins is available at http://www.proteome.com/databases/YPD/index.html). A yeast ribosome has 78 RPs (Fig. 1b), one less than a mammalian ribosome, and they are generally small and highly basic (the smallest has only 25 amino acids, of which 16 are K or R). The 78 proteins are encoded by 137 genes: most RPs are encoded by two genes yielding nearly identical proteins, possibly a remnant of the postulated duplication of the yeast genome[5]. Although they represent only 2% of yeast genes, the RP genes contain 101 of the genome's 234 introns[6].

Determination of the *S. cerevisiae* transcriptome, the range of expressed mRNAs, has established the prominence of the RP genes: they are responsible for 20 of the 30 most abundant mRNAs (Ref. 7). The 132 RP genes on the Affymetrix™ chip account for 4437 of the cell's estimated 15 000 mRNAs (Ref. 8 and http://www.wi.mit.edu/young/expression.html). As the RP mRNAs are relatively short-lived compared with other mRNAs (Ref. 9), an estimated 50% of the RNA-polymerase-II-mediated transcription initiation events involve RP genes. The active transcription and the abundance of introns in RP genes means that, contrary to popular perception, about 40% of yeast mRNAs are spliced and that 90% of all mRNA splicing events occur on RP transcripts.

RPs are assembled into ribosomes in the nucleolus. Although small, they are imported into the nucleus using conventional nuclear localization signals[10,11], possibly utilizing a relatively specific importin β (Ref. 12). With approximately 150 pores per nucleus[13], each pore must import nearly 1000 RPs per min and export ~25 ribosomal subunits per min.

Ribosome synthesis and nutrition

In one sense the life of a yeast cell is clear: when there is food it grows; when there is none it does not. Yet, the simple growth curve of a culture masks a complex series of economic decisions made by each cell (reviewed in Ref. 14). Ribosome synthesis is the object of many of these decisions (Fig. 2).

The 'target of rapamycin' (TOR) pathway of protein kinases and phosphatases has been implicated in transducing the availability of nutrients or growth factors, or both, into growth (reviewed in Ref. 15). In mammalian cells, it does so, at least in part, by stimulating the translation of mRNAs encoding RPs. In *S. cerevisiae*, inhibition of the TOR pathway by addition of rapamycin leads to a rapid repression of transcription of both rRNA and RP genes and prevents the activation of ribosome synthesis in response to added growth stimuli[16,17].

The *ras*–cAMP–protein kinase A (PKA) pathway has also been implicated in the detection of changes in the source and availability of carbon and nitrogen. Constitutively active PKA leads to doubling of the amount of several RP mRNAs, whereas constitutively inactive PKA leads to reduced RP mRNA and to the cells' inability to induce RP transcription in response to a carbon source upshift[18]. Depletion of cAMP leads to cessation of growth and repression of RP mRNA levels[19]. It seems likely that this pathway is responsible for the ordered events of sporulation induced by deprivation of nitrogen and energy.

J. R. Warner is at the Dept of Cell Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, New York, Bronx, NY 10461, USA.
Email: warner@aecom.yu.edu

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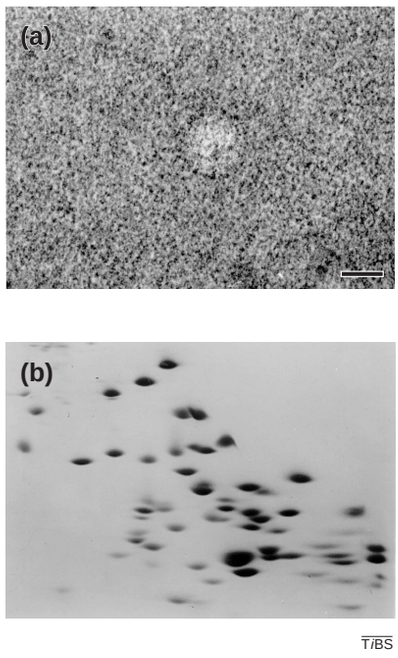

Figure 1

(a) A thin-section electron micrograph showing the density of ribosomes in the cytoplasm of *Saccharomyces cerevisiae* (courtesy of B. Byers, University of Washington). As much as 30–40% of the cytoplasmic volume is occupied by ribosomes (scale bar = 100 μm). **(b)** Most of the proteins of a yeast ribosome after two-dimensional electrophoresis. Each of the ribosomes pictured in (a) contains this array of proteins and to produce this number of proteins in precisely equimolar amounts is a challenge for the cell.

This includes a coordinate repression of the entire complement of RP genes, followed by resumption of transcription as the spores themselves develop[20]. Finally, the accumulation of a species of uncharged tRNA, due to deprivation of an amino acid, leads to the repression of both rRNA and RP transcription (i.e. the 'stringent response')[1,21].

The experiments described above were carried out under the extreme conditions of starvation or the constitutive (in)activation of a pathway. Yet, a cell ceases its ribosome synthesis when the culture is at <30% of its maximum density[22]. Subsequently, the cell starts to degrade its ribosomes; a cell in stationary phase has <25% of the ribosome complement of a cell in log phase. Clearly, the detection of and the response to the subtle changes in culture conditions that anticipate nutrient shortage or abundance form a key to selective survival. As they integrate this information, are the PKA and TOR pathways parallel, convergent, or intersecting? Are there other signalling pathways involved?

Ribosome synthesis and environmental insults

A mild heat shock, even between two temperatures at which the cell is viable, leads to a rapid but temporary repression of the RP genes[23,24]. The levels of RP mRNAs decline precipitously with a half life ($T_{1/2}$) of 5–7 min during the initial 15–20 min and then recover to nearly normal levels by about 60 min. Although the decline has been attributed to an accelerated turnover of RP mRNAs (Ref. 25), recent data suggest that their rate of turnover is normal[9]. Neither the effector nor the transcriptional basis for this repression is known. This is one case in which the effect of an environmental insult is far greater on RP transcription than on rRNA transcription[26].

Ribosome synthesis and intracellular insults

A defect anywhere in the secretory pathway – from early in the ER, through Golgi functions, to fusion of vesicles with the plasma membrane – leads to rapid repression of both rRNA and RP genes[27]. Inhibitors of the secretory pathway, such as tunicamycin and brefeldin A, have a similar effect. Initially, this seemed a surprising result, yet in retrospect, it should have been obvious that for a cell to maintain balanced growth there must be cross-talk between the major synthetic pathways of the cell, in this case between the secretory pathway and the ribosome biosynthesis pathway. It remains to be seen which other major pathways influence ribosome biosynthesis.

This repression is not due to the 'unfolded protein response', does not depend on PKA and is not related to the 'stringent response'[28]. The secretory pathway in *S. cerevisiae* is largely devoted to the synthesis of cellular membranes and the secretion of proteins involved in the cell wall. The integrity of the cell surface is monitored by the PKC pathway[29]. We have now found that Pkc1 and Wsc1, its membrane-bound upstream effector[30], are essential for the repression of rRNA and RP genes in response to a defect in the secretory pathway[28], which suggests that, in a cell that can no longer synthesize either the plasma membrane or the cell wall, the continued synthesis of proteins leads to osmotic stress. The cell responds by repressing ribosome synthesis.

Ribosome synthesis and the cell cycle

In a recent study of the cell-cycle dependence of more than 6200 genes, using α-factor arrest, a *cdc15* mutant or elutriation (based on cell size), about 800 genes showed consistent cell-cycle dependence[31]. None of the 137 RP genes reached the threshold defined in this analysis.

Nevertheless, this approach does not address the physiological question of whether the decision of a non-cycling G0 cell to initiate a cell cycle is independent of its decision to initiate ribosome biosynthesis. The observation that disruption of the PKA or the TOR pathway leads cells to accumulate in a G0 state suggests that, for G0 cells, initiation of ribosome synthesis might be needed for the initiation of a cell cycle. This could be a key difference between the G0 and G1 states, as cells that have been cycling can initiate a cell cycle even when repressing ribosome synthesis (e.g. during the approach of stationary phase).

Why transcription?

It is intriguing that *S. cerevisiae* utilizes transcription as its primary means of regulating RP synthesis, whereas both eubacteria[32] and vertebrates[33] utilize translation, albeit in very different ways. Why is the $T_{1/2}$ of RP mRNAs so short? To replace RP mRNAs at frequent intervals seems an unnecessary use of resources. The $T_{1/2}$ of the mRNAs that encode the abundant glycolytic enzymes are much longer. One possible explanation is that there is selective pressure to maintain a short $T_{1/2}$ for RP mRNAs in order to control the relative production of the many RPs more closely.

Transcription of rRNA genes

The mechanisms of Pol I transcription have been recently reviewed[34]. Compared with the plethora of factors employed in Pol II transcription, far fewer have been reported to be necessary for Pol I transcription in metazoans. However, Keys and colleagues[35] have identified genetically a number of additional proteins (encoded by the *RRN* genes) that participate in yeast rRNA transcription. Current genetic and biochemical results suggest that the minimum requirements for active Pol I transcription include TBP, Rrn3 and two complexes: CF (consisting of Rrn6, -7, -11) and UAF (comprising Rrn5, -9, -10, histones H3 and H4, and protein p30)[36]. Is rRNA transcription far more complex in yeast? Or have the available genetic methods permitted identification of components that are as yet invisible to the biochemical approaches applied to metazoans?

Major transcriptional signals for rRNA transcription in *S. cerevisiae* lie within ~200 bp upstream of the transcriptional initiation site[37]. In addition, an enhancer element lies just downstream of the rRNA transcription unit[38] that encompasses the termination site[39]. In contrast to most activation elements in *S. cerevisiae*, this enhancer can work either upstream or downstream of the transcription unit[38].

It is remarkable how little is understood about the vigorous, highly regulated transcription by Pol I. Whereas activated forms of Pol I, associated with Rrn3 (Ref. 40), have been reported, the knowledge of what regulates this activation is limited. Chromatin analysis suggests that only 50% of the rRNA genes are active, even in rapidly growing cells. Approaching stationary phase, this proportion drops by less than half[41], whereas transcription declines by >90% (Ref. 22), which suggests that the 'opening' or 'closing' of rRNA genes is not the basis of the regulation of rRNA transcription. What then is the basis? Is the tandem arrangement of the rRNA genes important for their regulation or is each an independent entity?

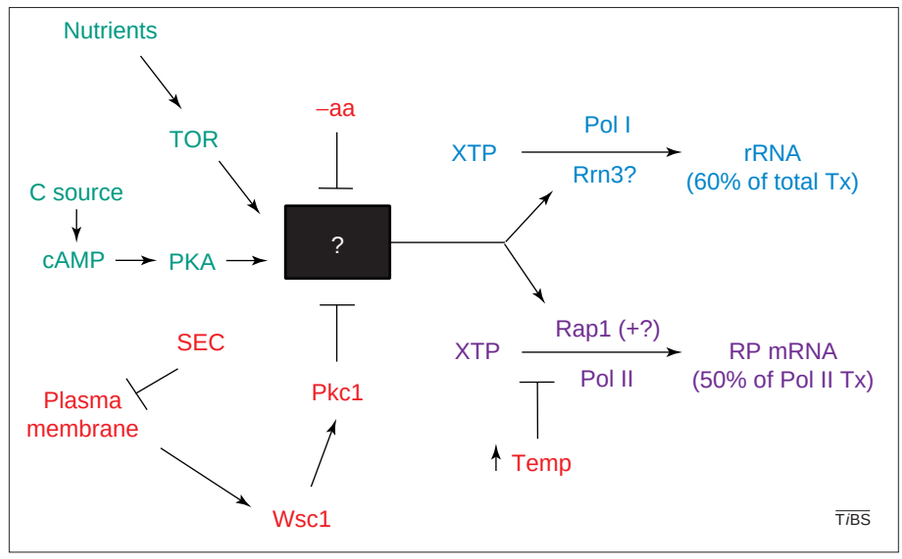

Figure 2

Some of the elements that regulate the production of ribosomes in *Saccharomyces cerevisiae*. This figure is a simplified summary of many observations. Is there a single 'black box' that transduces the signals from these numerous sources into the coordinate transcription of the ~150 rRNA genes and the 137 RP genes? It is currently not known whether there is communication between the Pol I and Pol II systems, although, under certain experimental conditions, rRNA transcription occurs in the absence of RP synthesis[26] and *vice versa*[49]. Abbreviations: aa, amino acids; PKA, protein kinase A; Pkc1, protein kinase C 1; SEC, any defect in the secretory pathway; TOR, 'target of rapamycin' pathway; XTP, nucleoside triphosphates; Wsc1, a plasma membrane-bound protein that detects stress, upstream of Pkc1 (also known as Hcs77).

Transcription of RP genes

The upstream activator sequence (UAS)/promoter regions of most RP genes have a similar architecture (Fig. 3; reviewed in Refs 1,2). Two Rap1 binding sites provide most of the activation, followed by one or two T-rich elements that will support a low level of transcription. A few RP genes have a single Abf1 binding site in place of the two Rap1 sites. Computer analysis suggests that all but a handful of the 137 RP genes fall into one of these categories[42].

The transcriptome of cells in which a variety of components of the transcriptional apparatus have been deleted or mutated, including subunits of Pol II, general transcription factors, histone acetylation factors, suppressor of RNA polymerase B (SRB) and TATA-box-binding-protein-associated factor (TAF) proteins, failed to identify any that appeared to be specific for the RP genes[8]. Nevertheless, given the coordinate, high level of transcriptional activity of the RP genes, it seems not unlikely that some factor involved in the transcriptional apparatus is utilized specifically for RP genes.

Rap1, the superfactor of *S. cerevisiae*

Rap1 plays an extraordinary role in the economy of the cell[43]. As an activator of the RP genes it accounts for ~50% of Pol II transcription. As an activator of other genes related to translation (e.g. EF1α), and of the abundantly transcribed glycolytic genes, it accounts for even more, *in toto* possibly 60–75% of all Pol II transcription. As the protein that coats the telomeres it is also responsible for maintaining proper telomere length. In cooperation with Sir3 and Sir4, Rap1 acts to silence genes (e.g. the *MATa* and MATα loci), thus permitting the sexual behavior of *S. cerevisiae*.

Because of its prominent regulatory role, Rap1 is an obvious target for the activation and repression of transcription of the RP genes. Indeed, the Rap1 sites of a RP gene can confer sensitivity to amino acid starvation[21], the *ras*–PKA pathway[18,19] and a secretory defect[9]. This could be repression, in which Rap1 is prevented from activating transcription, or silencing, in which Rap1 actively prevents transcription. In cells carrying *rap1-17*, from which the silencing domain has been deleted, RP transcription is far less sensitive to a defect in the secretory pathway[44]. This is true even for RP genes with an Abf1 binding site instead of Rap1 sites. These observations suggest that Rap1 both activates and silences the RP genes. Yet, the normal silencing cofactors, Sir2, Sir3 and Sir4, are not required for this effect[9].

Surprisingly, other cis-acting elements of an RP gene can also lead to repression (Fig. 3). Replacement of the Rap1 binding sites in *RPL30* by Gal4 binding sites produces a gene that is dependent on galactose and repressed by glucose. Nevertheless, it is repressed by at least 75% in a *sec* mutant, but not in a *rap1-17* strain[9]. A similar observation has been reported for nitrogen starvation[19].

In summary, Rap1 acts not only as an activator of most RP genes, for which it must bind to the upstream elements, but also as a silencer, possibly of all RP genes, for which it need not bind to the upstream elements. Perhaps this dual function of Rap1 originally evolved to bring about rapid, global changes in transcription of RP genes, which allows the cell to compete effectively in a rapidly changing environment. One could suggest that, as the Mat **a**/α form of sexuality evolved, the silencing aspect of Rap1 was adapted, through the development of Sir3 and Sir4, to silence the silent MAT loci.

Microregulation of RP synthesis

The challenge of regulating the production of an RP differs from that for most proteins because the cell needs equimolar amounts of the individual components of the ribosome. However, the transcriptome data reveal that there is a fivefold difference between the most and the least abundant RP mRNAs (Ref. 8). Presumably, there has been a coordinated

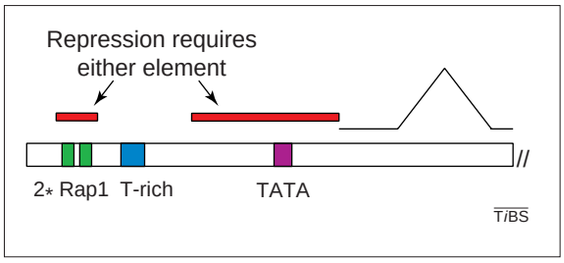

Figure 3

A typical ribosomal protein (RP) promoter. For most (~90%) of RP genes two Rap1 binding sites, one T-rich stretch and a TATA box drive the transcription of a pre-mRNA containing an intron near the 5′ end. The red bars indicate two regions, either of which can confer RP-type silencing on other genes, which suggests that important regulatory elements remain to be discovered.

evolution, so that for each RP the product of its transcription (from its one or two genes), its mRNA half-life, and its translation leads to roughly equimolar output. The relevant measurements have never been made. RPs, being small, highly charged, nucleic-acid-binding proteins, could be a danger to the cell unless safely assembled within a ribosome. Indeed, excess RPs are degraded with a $T_{1/2}$ of 0.5–3.0 min (reviewed in Ref. 1). How does a cell know, within 30 seconds, that a protein synthesized in the cytoplasm is not needed for assembly into a ribosome in the nucleolus?

In only two known cases does an RP control the level of its own mRNA. The transcript of *RPL30* (formerly *RPL32*) is normally spliced efficiently but in the presence of excess L30, unspliced precursor accumulates. L30 that is not assembled into ribosomes binds to its own transcript near the 5′ splice site, which prevents the complete assembly of the spliceosome[45]. The ability to regulate the level of L30 mRNA contributes substantially to the biological fitness of the cell[46], which suggests that even a minor excess of this RP has some deleterious effects.

Another case concerns S14, a protein that is encoded by two genes, *CRY1* and *CRY2*, whose mRNAs are found in a ratio of about 10:1, even though the two genes are transcribed approximately to an equal extent[47]. Excess S14 can bind to the *CRY2* transcript, and might inhibit its splicing, thus leading to its rapid degradation[48]. The selective preservation of introns in RP genes leads to the suspicion that many more could play a role in feedback inhibition. It is possible that this inhibition leads to such rapid RNA degradation that it is not yet detectable, as is the case for *CRY2* in wild-type cells.

Conclusions and prospects

The importance of ribosome synthesis to the economy of the *S. cerevisiae* cell has driven the evolution of unique and sometimes unexpected regulatory systems. Whereas some insight has been developed into these systems, many questions remain. How is transcription by Pol I and Pol II balanced, especially when the number of rRNA genes varies? Is there crosstalk between them? What is the mechanism of silencing, and of activating, the 137 scattered RP genes? When RP transcription is silenced, what is the impact on other genes of the sudden release of 50% of the Pol II transcriptional potential of the cell? Is there a system to buffer the transcriptome from such a shock?

The magnitude of ribosome synthesis in mammalian cells does not match that of yeast and the economy of the cell is more complex, being based largely on homeostasis. Yet, the basic biological problem remains the same: (i) selecting a rate of ribosome production that matches the needs of the cell; for example, a liver cell that needs only to compensate for molecular turnover, or a lymphocyte that must be prepared to churn out masses of immunoglobulins, and (ii) providing the rRNA and RPs needed to accomplish that rate of production. Identification of the signal(s) that bring this about would reveal an ideal target for therapy against tumor growth.

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