RIBOSOME BIOGENESIS AND NUCLEOLAR FUNCTION IN YEAST
Saccharomyces cerevisiae

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Abstract: This review provides an overview of the current knowledge of ribosome biogenesis, nucleolus structure and function and protein traffic into and out of the nucleus, with emphasis on the potential of yeast Saccharomyces cerevisiae as a model organism.

Key Words: Ribosome, Saccharomyces cerevisiae, Ribonucleoprotein, Nucleolus, Export-import, rRNA

INTRODUCTION

Ribosome biosynthesis plays a key role in cell metabolism. It takes place in the nucleolus. Nucleolar proteins can be divided into two categories: those that will make up the ribosomes, and those involved in pre-rRNA processing and ribosome assembly, which remain in the nucleolus when the mature ribosomes are exported into the cytoplasm. Despite the progress in our knowledge concerning ribosome structure, we are still ignorant about the molecular mechanisms regulating the ribosome biogenesis pathway. Many questions also remain concerning molecular events taking place in the nucleolus. Saccharomyces cerevisiae is an attractive system to study nucleolar structure and function thanks to the availability of genetic approaches. This non-differentiating, unicellular organism shares the cellular organisation and metabolic processes of higher eukaryotes. The development of genome sequencing has shown that many proteins exhibit evolutionary conservation, therefore the information on the function of yeast genes may be extended to similar genes in evolutionarily distant species.

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SYNTHESIS AND PROCESSING OF pre-rRNA

Ribosomal RNA accounts for approximately 85% of total cellular RNA. Cells must synthesise enough rRNA to assemble a very large number of ribosomes (1.7x10^5, 2x10^4, 10^6 per generation of growing bacterial, yeast and mammalian cell, respectively) which means that a yeast cell with a generation time of 100 min produces 2000 ribosomes per minute [1]. Adequate quantities of rRNA can be produced because the cell contains multiple copies of rDNA genes encoding rRNA. In most eukaryotes rDNA is organised as a tandem array and has the same arrangement: a small subunit rRNA gene (18S) and two large subunit rRNA genes (5.8S and 28S). The rRNA-coding regions are flanked by 5'- and 3'-external transcribed spacers (5'ETS and 3'ETS) and separated by internal transcribed spacers (ITS1 and ITS2). In all organisms, rRNAs are synthesised as precursors (pre-rRNAs) that undergo cleavage and nucleolytic elimination of the transcribed spacer regions. Transcription of a single rRNA precursor molecule ensures equimolar amounts of the three species of rRNA for assembly into ribosomes [reviewed in 1 and 2].

The S. cerevisiae genome contains, depending on the genetic background, 100-220 copies of rDNA on chromosome XII. A single rDNA copy is 9137 bp in length, and consists of two transcription units: the 35S rDNA precursor transcribed by RNA polymerase I and 5S rDNA transcribed in the opposite direction by RNA polymerase III. However, only approximately half of the rDNA genes are transcribed [3]. The newly synthesised 35S pre-rRNA is processed in a multistep pathway into three mature species: 18S, 25S and 5.8S rRNA (Fig.1). The precursor's life time is very short, approximately 10 seconds, and during this time all 2'-O-methyl modifications in pre-rRNA occur [4].

As shown in Fig.1 pre-rRNA processing involves a large number of trans-acting factors. An updated list of S. cerevisiae trans-acting factors involved in the maturation of pre-rRNA and assembly with ribosomal proteins is available under the WEB address http://www.expasy.ch/linder/proteins.html. Small nuclear RNAs (snRNAs), which mediate a spectrum of RNA processing events in eukaryotic cells, are not included in this list. Small nucleolar RNAs are present in all eukaryotic cells and reside in the nucleus. They range in size from about 77 to 600 nt and exist as complexes with nuclear proteins referred to as snRNPs (small nuclear ribonucleoproteins). The predicted total number of snoRNA species is from 75-100 in yeast and up to 200 in mammals [5]. There are two major classes of snoRNA characterised by highly conserved sequence motifs known as the C/D and H/ACA box. The C/D box snoRNAs mainly regulate the 2'-O-methylation of rRNA riboses. Most of the C/D box snoRNAs contain sequences complementary to rRNA required for direct base pairing of
Fig. 1. The steps of rRNA maturation. A scheme of processing of the S. cerevisiae 35S pre-rRNA molecule into mature rRNA molecules (according to Tollervey et al., 1991). 
ETS stands for external transcribed spacer and ITS for internal transcribed spacer. The
known trans-acting factors involved in successive steps of maturation are as follows:

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Factors Involved</th>
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<tbody>
<tr>
<td>2'-O-methylation</td>
<td>Box C+D snoRNP proteins-Nop1p, Nop56p, Nop5/58p</td>
</tr>
<tr>
<td>Pseudouridinc formation</td>
<td>Box H+ACA snoRNP proteins-Gar1p, Cbf5p, Nhp2p, Nop10p</td>
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<tr>
<td>A0</td>
<td>U3, Nop1p, Sof1p, Mpp10p, Nop58p, Imp2p, Imp3p</td>
</tr>
<tr>
<td>A1</td>
<td>3'-&gt;5' exonucleases-U3, U14, Nop1p, Sof1p, Mpp10p, Nop58p, Nop56p, Imp2, Imp3p</td>
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<tr>
<td></td>
<td>Box H+ACA snoRNP-snR30, snR10, Gar1p, Cbf5p, Nhp2p, Nop10p</td>
</tr>
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<td></td>
<td>RNA helicase-Rrp3p, Rok1p, Fal1p, nucleolar proteins-Rrp5p, Dim1p</td>
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<tr>
<td></td>
<td>U3 snoRNP-Lcp5p</td>
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<tr>
<td></td>
<td>endonuclease-Rnt1p</td>
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<tr>
<td></td>
<td>other-Rrp7p</td>
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<tr>
<td>A2</td>
<td>3'-&gt;5' exonucleases-U3, U14, Nop1p, Sof1p, Mpp10p, Nop58p, Nop56p, Imp2, Imp3p</td>
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<td></td>
<td>Box H+ACA snoRNP-snR30, snR10, Gar1p, Cbf5p, Nhp2p, Nop10p</td>
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<td>endonuclease-Rnt1p</td>
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<td></td>
<td>other-Rrp7p</td>
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<tr>
<td>A3</td>
<td>Endonuclease RNase MRP-Pop1p, Pop3p, Pop4p, Pop5p, Pop6p, Pop7p, Pop8p, Rpp1p, Smn1p, RNase MRP</td>
</tr>
<tr>
<td></td>
<td>nucleolar protein-Rrp5p</td>
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<tr>
<td>ExC2-&gt;E</td>
<td>3'-&gt;5' exonucleases-Rrp4p, Rrp40p, Rrp41p, Rrp42p, Rrp43p, Rrp44p, Rrp45p, Rrp46p, Mtr3p, Csl4p, Rrp6p</td>
</tr>
<tr>
<td></td>
<td>RNA helicase-Dob1p</td>
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The 35S pre-rRNA is cleaved at site A0 by the endonuclease Rnt1p generating the 33S pre-rRNA, subsequently processed at A1 and A2 sites. This gives products 20S and 27SA2 (precursors) resulting in the separation of the pre-rRNAs destined for the small and large ribosomal subunits. Early pre-rRNA cleavages A0 to A2 are carried out by a large snoRNP complex (Venema and Tollervey, 1995), assisted by the putative ATP-dependent RNA helicases Fal1p, Rok1p (Venema et al., 1997), and Rrp3p (O'Day et al., 1996). Interestingly the final maturation of the 20S precursor takes place in the cytoplasm, where an endonucleolytic cleavage at site D yields the mature 18S rRNA. The 27SA2 precursor is processed by two alternative pathways that both lead to the formation of mature 5.8S and 25S rRNAs. In the major pathway, the 27SA2 precursor is cleaved at the A3 site and then digested up to site B1S by 5' to 3' exonuclease activity to yield the 27SBS precursor. In the minor pathway the 27SA2 is cleaved at site B1L, producing the 27SBL pre-rRNA. While processing at site B1 is executed, the 3' end of mature 25S rRNA is generated at site B2. The late processing steps of 27SBS and 27SBL are identical. Cleavage at sites C1 and C2 releases the mature 25S rRNA plus the 7S pre-rRNAs, which undergo rapid 3'->5' exonuclease digestion up to the 3' end of the mature 5.8S rRNA (site E).

the snoRNA at the rRNA site to be modified. The H/ACA box snoRNAs are mainly involved in pseudouridylation of rRNA uridine bases. Several snoRNAs are required for the cleavage of pre-rRNA [reviewed in 6 and 7].
The availability of specific mutations in snoRNA and pre-rRNA enabled the
determination of the functions of some *S. cerevisiae* snoRNAs by *in vivo*
studies. The results of *in vivo* and *in vitro* studies in concert with a computer
analysis of the yeast genome allowed a "snoRNA Master Table" to be worked
out, available on the WEB under the address: http://www.bio.umass.edu/
biochem/rna-sequence/Yeast_snoRNA_Database/mastertable.html.

**BIOGENESIS OF pre-RIBOSOMES**

In eukaryotes ribosome biogenesis is a complex process where approximately
80 ribosomal proteins (RP) and four rRNAs are assembled into mature
ribosomal subunits. Although ribosomes carry out their function in the
cytoplasm, they are assembled in the nucleolus. The assembly of 5S rRNA and
almost all of the ribosomal proteins begins while the pre-rRNA emerging from
the transcriptional machinery, and its association with proteins into
ribonucleoprotein complexes (RNP) persists during processing and throughout
the assembly of ribosomal subunits. Two-dimensional gel electrophoresis has
identified up to 45 different proteins from the 60S ribosomal subunit and 32
from the 40S ribosomal subunit [2, 3 and references therein]. The ribosomal
precursors also contain a number of snoRNAs and nonribosomal proteins
required for the proper maturation and assembly of the highly structured
pre-rRNAs with ribosomal proteins, which are then selectively dissociated to
yield mature ribosomal subunits. The completion of the sequence of the
*S. cerevisiae* genome and its global computational analysis brought to light a
number of novel proteins predicted to be involved in ribosome biogenesis,
making the picture of this process even more complicated. According to the
data available in the MIPS protein data base, 80 ORFs are assigned as encoding
proteins of the large cytoplasmic ribosome subunit and 56 as encoding proteins
of the small one. 39 ORFs encoding proteins involved in rRNA synthesis and
54 ORFs encoding proteins involved in rRNA processing have to be added to
these genes. It appears that most of the products of genes involved in rRNA
synthesis and processing are essential for cell life.

**STRUCTURE OF THE METAZOAN NUCLEOLUS**

Although the nucleolus was described in 1835 as a cell structure
distinguishable in phase contrast microscopy, it was only recognised as the site
of rRNA synthesis in the early sixties. At present the nucleolus is defined as an
unenclosed subdomain of the nucleus where ribosomal RNA (rRNA) is
synthesised and preribosomal particles are assembled [references 8 and 9]. The
combined methods of electron and immunoelectron microscopy led to the
identification of three specific nucleolar regions: the fibrillar center (FC), the dense fibrillar component (DFC), and the granular component (GC) surrounding the fibrillar center. These subnucleolar regions constitute the functional domains. The DNA coding for rRNA (rDNA) and its transcripts are associated with the fibrillar center. The FC consists largely of rDNA and proteins involved in its transcription, whereas the proteins involved in rRNA processing and preribosome formation are located in the DFC. Further assembly and maturation of preribosomes take place in the GC, which constitutes the bulk of the nucleolus. Nucleoli have a distinctive protein composition reflecting high concentrations of enzymes required for the synthesising and processing of precursor ribosomal RNA. Besides such obvious proteins as RNA polymerase I complex, a number of nucleolar proteins have been identified and some have been localised to particular subnucleolar compartments. The protein considered to be the pivotal component of the ribosome biogenesis machinery is nucleolin, found mainly in the DFC. This protein has a unique tripartite structure and each domain performs a specific function. It exhibits DNA helicase, RNA helicase and DNA-dependent ATPase activities. Nucleolin is involved in processes such as chromatin structure maintenance, rDNA transcription, rRNA processing, pre-ribosome assembly and transport into the cytoplasm. Proteins structurally related to nucleolin are found in all eukaryotic organisms studied so far [reviewed in 10]. Another generally conserved protein identified in unicellular eukaryotes and mammals is fibrillarin. It is an abundant constituent of RNP particles occurring in the DFC. The most highly conserved domain of fibrillarins is the N-terminal sequence rich in glycine and arginine residues (GAR domain). The human and Xenopus gene encoding this protein can complement deletion of the S. cerevisiae NOP1 gene [11]. Proteins involved in late processing identified as components of the GC in mammalian cells are B23, hPop1, Ki-67, Nop52 [12].

FUNCTIONAL COMPARTMENTALISATION OF THE NUCLEOLUS IN Saccharomyces cerevisiae

The S. cerevisiae nucleolus has been described as a dense crescent that occupies about one-third to one-half of the nuclear volume. Numerous data on immune mapping of RNA processing components indicate a high order of structural arrangement. However, the thick wall of the yeast cell makes it difficult to morphologically discriminate the nucleolar domains. The techniques of cryofixation and cryosubstitution electron microscopy combined with in situ detection of rDNA and rRNA allowed Nicole Gas and her collaborators [14] to identify the FC, DFC and GC subcompartments in the nucleolus of S. cerevisiae similar to those in the nucleoli of higher eukaryotes.
rDNA appeared to be a constituent of the FC domain whereas rRNA was
distributed along the nucleolar network including DFC and GC and
colocalised with RNA polymerase I and Nop1p and Gar1p, the two proteins
involved in early steps of pre rRNA processing [11, 13] detected in the DFC.
The authors also showed that export of pre-ribosomes takes place through all
the pores of the nuclear envelope.

NUCLEOLAR FUNCTIONS NOT RELATED TO RIBOSOME
BIOREGENESIS

There is a growing body of evidence that proteins with no suspected role in
rRNA synthesis and processing or in ribosome biogenesis reside in the
nucleolus. Garcia and Pillus [15] reviewed recent studies on cell cycle control
in S.cerevisiae indicating that some mitotic and meiotic regulatory proteins
reside permanently or transiently in the nucleolus. Four interacting proteins:
Net1, Cdc14, Sir2 and Nan1, were found within the nucleolus during
interphase. They have been named the RENT complex (Regulator of Nucleolar
Silencing and Telophase). However, in anaphase, Cdc14p and Sir2p are
dispersed in the nucleus whereas Net1p remains in the nucleolus throughout the
cell cycle. Cdc14p is a highly conserved protein phosphatase, and as an
activator of Clb cyclin degradation it is a member of a signalling cascade
critical for the exit from mitosis [16 and references therein]. Sir2p belongs to
the family of silencing proteins. It was known as a part of the repression
complex at subtelomeric regions. It appeared that Sir2p also plays a role in the
repression of rDNA repeats [17, 18]. Nan1p and Net1p are newly discovered
nucleolar components and their precise function is not yet known. Another cell
cycle regulatory protein found in the nucleolus is Pch2p, expressed in meiosis
and required for the pachytene check point. Guarantee and his colleagues
proposed the model in which the nucleolus is the critical site for cellular ageing
[19]. According to these authors uncontrolled rDNA recombination generates
hundreds of small circles accumulating in mother cells. The accumulated
extrachromosomal rDNA copies cause a decrease in the capacity to divide.
From studies on higher eukaryotes, views of expanded functions of the
nucleolus also emerged. This subcompartment has been implicated in the
processing and export of certain mRNAs (e.g. c-myc, N-myc, myoD oncogen
mRNAs), SRP RNA (Signal Recognition Particle), telomerase RNA, some	RNA precursors and U6 snRNA. HIV proteins: Rev and Tat; and HTLV-1 Rex
also accumulate in the nucleolus. These findings led to the plurifunctional
nucleolus hypothesis [reviewed in 20].
NOR - THE NUCLEOLAR ORGANISER REGION

The rDNA genes whose transcription and post-transcriptional events lead to the formation of a distinct subnuclear structure are known as the nucleolar organiser regions (NOR). However, the molecular mechanisms generating the highly dynamic nucleolar structure are far from understood. First of all, nucleoli are morphologically diverse, varying in appearance, number and size depending on cell type as well as its position in the cell cycle and the status of rDNA transcription. [21]. The most remarkable phenomenon is the disintegration of the nucleolus during mammalian cells mitosis [22]. Between prophase and telophase the repression of polymerase I occurs associated with the dispersion of nucleolar components as large aggregates distributed throughout the cell, termed nucleolus derived foci (NDF). The mechanism of reassembly of nucleoli at the end of mitosis remains obscure.

Once more S. cerevisiae proved to be an excellent tool in studies on the role of genes coding for rRNA as the centre assembling the nucleolar components into a defined structure. Warner and his collaborators [23] characterised rRNA transcription and processing in a wild type strain and its derivative in which the rDNA was completely deleted and rRNA was transcribed from plasmid-borne genes. Although the efficiency of gene transcription carried on plasmids was low, the processing of 35S pre-rRNA seemed not to be affected and instead of a single crescent-shaped nucleolus multiple “mininucleoli” were observed. These results imply that each rRNA gene can serve as a nucleolar organiser and are in agreement with the results of an experiment showing that insertion of a Drosophila rRNA gene into the euchromatic region of a chromosome leads to the formation of a nucleolus at that site [24]. On the other hand, rdn mutants carrying multicopy plasmids with rDNA genes transcribed by polymerase II (from GAL7 promoter) contained a compact nucleolus. However, no continuity with the nuclear envelope was observed. This experiment suggests that the type of polymerase and/or transcription factors play a role in the organisation of the nucleolus [25]. As a result the main hypothesis concerning nucleolar organisation is that proper nucleolar function does not require a specific organellar morphology as much as a specific set of molecular associations of the nucleolar components. On the other hand Carotenuto et al., [26], who investigated the presence and location of spectrin in the nuclei of Discoglossus pictus and Xenopus laevis oocytes, recognised a spectrin-like molecule as a part of the outer shell of nucleoli. The authors hypothesised that spectrin, together with actin, might be instrumental in keeping nucleoli attached to the inner nuclear membrane.
NUCLEAR TRANSPORT

The synthesis of ribosomes involves multiple crossings of the nuclear envelope. The nuclear envelope consists of an inner and an outer nuclear membrane. The outer membrane is continuous with the ER membrane, and the space between it and the inner membrane is continuous with the ER lumen. The extensive traffic of materials between the nucleus and cytosol occurs through nuclear pore complexes (NPCs) and is mediated by the interaction of transport factors with nucleoporins at the NPCs. The nuclear pore complexes display remarkable structural conservation among diverse organisms. They are highly organized structures, with symmetries in both the axial and vertical planes. The ~60MDa NPC is composed of at least 50 different proteins named nucleoporins - Nup's. Although vertebrate cell-free systems provided excellent models to study NPC assembly, direct genetic screens for NPC biogenesis are only possible in *S. cerevisiae*. Bucci and Wente [27] undertook the challenge of global genetic analysis of NPC components. The authors developed a method based on the tagging of nucleoporins with GFP, assuming that a defect in NPC assembly would provoke a change in fluorescence signals compared with wild type cells. Preliminary results led to the identification of a number of Nup's which validated this strategy. The interactions between nucleoporins have been highlighted by genetic studies characterising the synthetic lethal interactions between different *nup* mutants [28].

The guiding of proteins to the nucleus is an example of sorting that operates independently of the mechanisms sorting proteins destined for the Golgi, vacuole, plasma membrane or secretion. The exchange of macromolecules between the nuclear and cytoplasmic compartments is mediated by karyopherins (importins or exportins). The particles to be transported (cargo) contain nuclear localisation signal (NLS) or nuclear export signal (NES) sequences that are recognised by importins or exportins, respectively. The signals are short amino acid sequences not limited to a particular site within proteins and not removed from the proteins during or after nuclear entry. Because these signals are not removed, nuclear proteins can be imported repeatedly [29]. The examples of known cargo and signal sequences for nuclear import and/or export are listed in Table 1.

Nuclear import of proteins containing classical NLSs is mediated by a heterodimeric protein complex, composed of karyopherin α and β1, that the NLS-protein to the NPC docks via β1. Distinct nuclear import and export pathways mediated by members of the karyopherin β family include the following examples: karyopherin β2 mediates import of mRNA binding proteins; karyopherin β3 and β4 mediate import of ribosomal proteins; CRM1 mediates export of proteins containing leucine-rich NES; CAS mediates
Tab.1. Cargo and signals for nuclear import and export

<table>
<thead>
<tr>
<th>(a) Import cargo</th>
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<tbody>
<tr>
<td>Protein</td>
<td>Nuclear localisation signal (NLS)*</td>
</tr>
<tr>
<td>SV40 T antigen</td>
<td>PKKKRKVK (monopartite NLS)</td>
</tr>
<tr>
<td>nucleoplasmin</td>
<td>KRPAATKKAGQAKKKK (bipartite NLS)</td>
</tr>
<tr>
<td>c-myc</td>
<td>PAAKRVKLD</td>
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<tr>
<th>(b) Export cargo</th>
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<tbody>
<tr>
<td>Protein</td>
<td>Nuclear export signal (NES)</td>
</tr>
<tr>
<td>PKI (Rat)</td>
<td>LALKLAGLDI</td>
</tr>
<tr>
<td>Rev (HIV-1)</td>
<td>LPPLERLTLTD</td>
</tr>
<tr>
<td>TFIIIA (Xenopus)</td>
<td>SLVLDKLTI</td>
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<th>(c) Shuttling proteins</th>
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</thead>
<tbody>
<tr>
<td>hnRNP protein A1</td>
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<tr>
<td>import signal=export signal</td>
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</table>

*Bold letters mark residues that have been shown to be particularly important for signal function.

re-export of karyopherin α. All members of the karyopherin β family are able to bind RanGTP and interact directly with nucleoporins at the NPC. The data on the components of active inward and outward transport through the nuclear pores in *S. cerevisiae*, converted into schematic drawings, are shown in Figure 2.

The interaction of cargo with its transporter, modulated by the small GTPase Ran, is presented in Figure 3. For importins, the binding of cargo and Ran-GTP is antagonistic and for exportins it is co-operative. As shown in Figure 3 NLS-containing cargo binds to the importin α/β complex in the cytosol and enters the nucleus. In the nucleus presence of Ran•GTP induces dissociation of the importin α/β complex. Importin β in complex with Ran•GTP return to the cytoplasm. Importin α is recycled into the cytoplasm by interaction with the cap-binding complex required for U snRNA export (Fig.2) [30].

Although the identification of nucleoporins in *S. cerevisiae* is believed to be close to completion the dynamic structure of NPC does not allow the stable, structural NPC components to be positively distinguished from the reversibly associated shuttling proteins [31].
Fig. 2. The components of active transport into and out of the nucleus in *S. cerevisiae*. 
Fig. 3. The recycling of RanGTP.
REGULATION OF RIBOSOME SYNTHESIS

Although studies on all aspects of ribosome biogenesis have advanced rapidly, the understanding of the mechanisms regulating the synthesis of equimolar quantities of ribosomal proteins and rRNA for the construction and supply of ribosomes adequate, for cell demands is far from complete. *Saccharomyces cerevisiae* is a particularly suitable organism for the study of the kinetics of rRNA and RP synthesis in eukaryotes since its growth rate can be varied over a wide range by changing the composition of the growth medium. Furthermore, many mutants with altered rRNA or RP synthesis have been isolated. Sebastian et al., [32] studied the effect of growth rate on the total amount of RNA per cell in *S. cerevisiae*, and found a linear relationship between growth rate, rRNA content and the level of RNA polymerase I. The genetic and biochemical data indicate that for the fully activated level of rDNA transcription the polymerase I complex must contain Rrn3p, core factor (CF consisting of Rrn6p, Rrn7p and Rrn11p), upstream activation factor (UAF consisting of Rrn5p, Rrn9p, Rrn10p, histones H3 and H4 and p30) and TBP (TATA-binding protein) [33]. The mechanism of regulation of rDNA transcription is unknown. Even less is known about the regulation of ribosomal protein synthesis. The changes in rRNA synthesis correlate with the changes in RP level. However, a fivefold difference found between the most and the least abundant RP mRNAs indicates that transcription is not the only level of regulation of RP synthesis [34].

In nutritional shift-up experiments the increased synthesis of rRNA and ribosomal proteins in parallel with increased growth rate was observed [35-38]. Ju and Warner [39] followed the rate of ribosome synthesis during yeast culture growth and demonstrated that transcription of rRNA begins to decline at an early stage in the growth phase reaching 50% of its initial value and is accompanied by a decrease in the ribosomal protein mRNAs to about 25% of the maximum. The changes in the rate and efficiency of ribosome synthesis in response to changes in availability of carbon and nitrogen seems to be at least partially mediated by the TOR (target of rapamycin) and PKA (ras-cAMP-protein kinaseA) signaling pathways. Inhibition of the TOR pathway by rapamycin exerts drastic repression of transcription RP genes as well as synthesis and processing of 35S pre-rRNA [40, 41]. Inhibition of PKA pathway also leads to repression of RP mRNA synthesis and an inability to induce transcription in response to nutrient upshift [42].

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