Title

Transcriptomic analysis of ribosome biogenesis and pre-rRNA processing during growth stress in *Entamoeba histolytica*

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Abstract:

Ribosome biogenesis, a multi-step process involving the transcription, modification, folding and processing of rRNA is the major consumer of cellular energy. It involves the sequential assembly of ribosomal proteins (RP)s via more than 200 ribogenesis factors. Unlike model organisms where transcription of rRNA and RP genes slows down during stress, in *Entamoeba histolytica*, pre-rRNA synthesis continues, and unprocessed pre-rRNA accumulates. To gain insight into the vast repertoire of ribosome biogenesis factors and understand the major components playing role during stress we computationally identified the ribosome biogenesis factors in *E. histolytica*. Of the total ~279 *S. cerevisiae* proteins, we could only find 188 proteins in *E. histolytica*. Some of the proteins missing in *E. histolytica* were also missing in humans. A number of proteins represented by multiple genes in *S. cerevisiae* had only a single copy in *E. histolytica*. It was interesting to note that *E. histolytica* lacked mitochondrial ribosome biogenesis factors and had far less RNase components as compared to *S. cerevisiae*. Northern hybridization using probes from different spacer regions depicted the accumulation of unprocessed intermediates during stress. Transcriptomic studies revealed the differential regulation of a number of ribosomal factors both in serum-starved and RRP6KD conditions. The ARB1 protein involved at multiple steps of ribosome biogenesis and NEP1 and TSR3 involved in chemical modification of 18S rRNA previously shown to accumulate pre-rRNA precursors upon downregulation in *S. cerevisiae* and humans were included. The data reveals the importance of some of the major factors required for regulating pre-rRNA processing during stress. This is the first report on the complete repertoire of ribosome biogenesis factors in *E. histolytica*.

Keywords: Ribosome biogenesis; *Entamoeba histolytica*; RNase; transcription; transcriptome.

Introduction:

The ribosome, in addition to being a conserved molecular factory for protein synthesis, is a complex multifaceted machinery engaged in the spatiotemporal control of gene expression (1). Due to the very large number of ribosomes in a typical cell their biosynthesis consumes a major part of cellular energy and nuclear space (2,3). Transcription of ribosomal RNA genes, and ribosome formation is thus highly regulated in response to general metabolism and specific environmental conditions (4,5). In eukaryotes, the small subunit of ribosome (40S) is composed of 18S rRNA assembled with 33 ribosomal proteins (RPs), while the large subunit (60S) has 5S, 5.8S, and 25S/28S rRNAs associated with 47 RPs (6). Ribosome biogenesis takes place in the nucleolus, in a multi-step process involving the transcription, modification, folding and processing of rRNA along with the assembly of RPs with the help of more than 200 ribogenesis factors. Much has been learnt about this complex process in model systems, especially *Saccharomyces cerevisiae*, and has been reviewed in various authoritative articles (7–13). A brief description of the ribosomal biogenesis process is as follows.

The primary rRNA transcript (35S in *S. cerevisiae*) undergoes multiple specific cleavages at the 5' and 3' external transcribed spacers (5'-ETS and 3'-ETS) and the internal transcribed spacers 1 (ITS1) and 2 (ITS2), to generate the mature 18S, 5.8S, and 25S/28S rRNAs (14). Pre-rRNA processing begins co-transcriptionally by the formation of 90S SSU processome, a ribonucleoprotein complex of ~70 assembly factors and several small nucleolar RNAs (snoRNAs). The protein components of the processome include RNA-binding proteins, endonucleases, RNA helicases, ATPases, GTPases, and kinases. The SSU processome proteins identified by co-purification with U3 snoRNA are termed the U three proteins (UTPs) (15). Both the RNA and protein components of the SSU processome are considered to function as a pre-

rRNA chaperone to assist in the correct folding of nascent pre-rRNA. This enables sequence-specific cleavages at sites A0, A1 and A2 to generate the pre-18S rRNA. Amongst the snoRNAs, U3 is a non-canonical C/D box-containing snoRNA that has the specialized function of assisting in pre-rRNA processing and maturation (16). It associates with the canonical C/D box-specific proteins, and the U3 snoRNA-specific RRP9/U3-55K protein (17) to form U3snoRNPs that base pair at specific sites within the 5'-ETS and the pre-18S rRNA to carry out processing at sites A0, A1 and A2 (18). The rRNA precursor is also chemically modified by the canonical C/D-box and the H/ACA-box containing snoRNAs required, respectively, for methylation and pseudouridylation of the rRNAs (19–22). After the modification, the snoRNAs that are base paired with their target sites in the pre-rRNA, are removed by the action of DExH/H-box RNA helicases. The correctly folded pre-rRNAs are assembled with RPs, which along with biogenesis factors are synthesized in the cytoplasm, and transported to the nucle(ol)us for assembly of pre-ribosomal particles (23).

The initial cleavages at sites A0 and A1 in the 5'-ETS lead to the 90S pre-ribosomal particle being separated into pre-40S and pre-60S particles via cleavage at site A2. It requires the release of 5'-ETS rRNA that is complexed with UTP-A, UTP-B, and U3snoRNPs (24). The endoribonucleases encoded by UTP24 (25), and RCL1 are likely involved in cleavage at A2 (26). The RNA helicase DHR1 also plays an important role in the dismantling of the 90S particle. After cleavage and trimming, the pre-40S particle is translocated to the cytoplasm through the nuclear pore complex. This process is facilitated by the GTPases GSP1/RAN and CRM1/XPO1 (27). The final stage of pre-40S maturation in the cytoplasm includes the NOB1 endoribonuclease-catalyzed cleavage at site D of the 20S pre-rRNA to form the mature 18S-rRNA (28).

Compared with 40S, the assembly of 60S particle appears to be much more complex. 5.8S rRNAs with different 5'-ends are produced by two alternative pathways (29,30). The maturation of 5'-end of 5.8S rRNA is coordinated with cleavage of the 3'-end of 25S/28S rRNA, which is a prerequisite for endonucleolytic cleavage of ITS2 at site C2 (31). Following this cleavage, the 7S precursor is released. The maturation of 5.8S 3'-end from the 7S precursor involves the nuclear exosome, assisted by the RNA helicase DOB1/MTR4, and 3'-5' exonucleases including RRP6 (32,33). The exonuclease RAT1 generates the mature 5'-end of the 25S rRNA from 26S precursor (34). Processing at both ends of ITS2 is catalyzed by the protein complex LAS1 (35). A number of export factors are required for final translocation of pre-60S particles to the cytoplasm. These factors interact with specific sites of the pre-60S ribosome. The export adaptor NMD3 binds to the large subunit and recruits the exportin CRM1/ XPO1 via its NES sequence (36). Other export factors include the MEX67-MTR2 heterodimer, and factors that shield the charged ribosomal surface against hydrophobic environment within the NPC channel (37,38).

Although many of the ribosomal biogenesis components described above are well conserved in yeast and human, a number of yeast-specific proteins have no known human homologs, pointing to important functional diversity amongst organisms. It will be interesting to explore the variations in this highly conserved function in evolutionarily distant organisms. We have been investigating the regulation of pre-rRNA processing and ribosome biogenesis under growth stress in the primitive parasitic protist, *Entamoeba histolytica*, which causes amoebiasis in humans (39). In this organism the rRNA genes are located exclusively on extrachromosomal circular molecules (40,41), and the nucleolus is organized at the nuclear periphery (42). Each rDNA circle may contain either one copy of the rDNA transcription unit, or two copies

organized as inverted repeats. In *E. histolytica* strain HM-1:IMSS, the 14 kb rDNA circle designated EhR2 contains one rDNA unit (43), for which the transcription start point has been mapped (44–46). We have earlier shown that pre-rRNA processing was inhibited in *E. histolytica* cells subjected to growth stress by serum starvation, leading to accumulation of unprocessed pre-rRNAs. There was strong accumulation of partially processed fragments of the 5'-ETS, which are otherwise rapidly degraded in unstressed cells (46). Anomalies in pre-rRNA processing during growth stress could result from downregulation of various components of the processing machinery, including endoribonucleases, exoribonucleases, and helicases. Our previous work has shown that the exosome-associated 3'-5' exoribonuclease EhRRP6 is down-regulated in serum-starved *E. histolytica*, and the enzyme is lost from the nuclei in starved cells (47). Since this enzyme is known to be involved in the removal of 5'-ETS sub fragments in model organisms (48,49), its down regulation during serum starvation could lead to the accumulation of partially processed fragments of 5'-ETS in *E. histolytica*.

To obtain a more comprehensive picture of pre-rRNA processing, we have now computationally identified the *S. cerevisiae* homologs of pre-rRNA processing and ribosome biogenesis factors in *E. histolytica*. Using northern hybridization, we have shown the accumulation of unprocessed intermediates during serum starvation. Further, we have used transcriptomic analysis to study the regulation of these components during growth stress, and in EhRRP6 down-regulated cell lines. The data provide insights into the unique features of ribosome biogenesis components in *E. histolytica*.

Results and Discussion:

Computational identification of *E. histolytica* ribosome biogenesis and pre-rRNA processing proteins:

Ribosome biogenesis factors in eukaryotes affect diverse cellular pathways, and perturbations in ribosome biogenesis are linked to human disease (50). To understand the status of ribosome biogenesis in *E. histolytica*, we searched the *E. histolytica* sequence database for presence of all matches with proteins known to be involved in ribosome biogenesis. Since *S. cerevisiae* is the model organism giving the deepest insights in ribosome biogenesis, we enlisted all *S. cerevisiae* proteins involved in the process and looked for their counterparts in *E. histolytica* (as detailed in methods section). A list of the classes of proteins known to play a role in ribosome biogenesis is shown in Table 1. The complete list of proteins is given in Supplementary Table 1. Of the total ~279 *S. cerevisiae* proteins listed (51), we could find matches for 188 proteins in *E. histolytica* (Table 1). Their transcription, as determined from our RNA-Seq data (52,53) ranged from very high to low.

Of the 188 listed *E. histolytica* genes involved in pre-rRNA processing, transcripts of 47 genes were either absent or they belonged to the very low/ low-expression categories. The low-expressing genes mainly included helicases, some kinases, phospholipid transporting P-type ATPase, rRNA biogenesis protein RRP5, exosome complex exonuclease RRP44, tRNA splicing endonuclease and some HSP70 family genes. Around 44 genes were associated with high/ very high expression levels and included the U3snoRNA family, fibrillarin, 13kDa ribonucleoprotein-associated protein, centromere/microtubule binding protein, snoRNP protein GAR1, H/ACA ribonucleoprotein complex subunit 2-like protein. These protein sets are well established to play the pivotal roles in pre-rRNA processing. Other protein families included protein phosphatase,

elongation factor 2, snRNP Sm D2, LSM domain containing protein, enhancer binding protein 2 (EBP2), eukaryotic translation initiation factor 6 and 40S ribosomal proteins S2, S4, S6, S7, S9, S21. We could not detect 91 proteins in *E. histolytica*, some of which play an essential role in *S. cerevisiae*. Some of these were also missing in humans, suggesting their divergent nature (Table 2). The evolution of alternative pathways in *S. cerevisiae* or functional redundancy in *E. histolytica* may account for this loss. Alternatively, some of these proteins might have been missed due to poor sequence homology.

We further analyzed the repertoire of ribosome biogenesis proteins, to understand the conserved and divergent features of this essential function in *E. histolytica*. We observed that a number of different proteins were represented by a single gene in *E. histolytica* as opposed to multiple genes in *S. cerevisiae* (Table 3). These belonged to protein families with very similar sequences and might have evolved from the same gene by duplication and divergence. There were 42 such protein families and they mostly included helicases, casein kinases, exonucleases and methyltransferases. We also found that the factors required for mitochondrial ribosome biogenesis like MTF1, MTG, GEP3 and MRM1 (217, 218, 219 and 225 in Supplementary Table 1), were not present in *E. histolytica* which could be expected since *E. histolytica* lacks typical mitochondria (54,55). All of the Box C/D snoRNPs and Box H/ACA snoRNPs were present in *E. histolytica* except for a non-core component NAF1(56), and the expression level of snoRNPs was generally high (with a few showing medium expression) (Table 1), suggesting that the process of pre-rRNA modification (methylation and pseudo-uridylation) is well conserved and extensive in *E. histolytica*.

Amongst all the factors associated with 90S-, pre-60S- and pre-40S-particles, it was the RNases that were strikingly less conserved in *E. histolytica*, compared to the helicases, GTPases and Kinases. We failed to find any homologue of the RNases RNT1, REX4, NGL2, REX2 and REX1 (Supplementary Table 1.2.4; 1.4; Table 4). The RNases found in *E. histolytica* included a single copy of debranching enzyme DBR1, containing an N-terminal metallophos domain, required for its activity. There were two copies of RAT1 and a single copy of XRN1(57). RAT1 together with its counterpart XRN1 are highly conserved 5'-3' exoribonucleases. They play a crucial role in gene transcription, RNA processing and RNA surveillance. These are required specifically for regulating mRNA homeostasis by removing processing intermediates, aberrant molecules, and decapped mRNAs, by acting post transcriptionally and co-transcriptionally(58–60). Like *S. cerevisiae*, Humans also possess 8 different RNases (51) most of which were not found in *E. histolytica* (Table 4). It is possible that *E. histolytica* utilizes a limited number of RNases to target a variety of substrates.

The RNase MRP(RMRP) is another important ribonuclease associated with pre-60S particles (Supplementary Table 1.4). It is a ribonucleoprotein complex of 8-10 protein subunits which are bound to an RNA molecule required for catalytic activity (9). Our data showed very low sequence homology with two RMRP subunits (POP1and RPP1), while the other subunits were absent in *E. histolytica* (Supplementary Table 1.4.3.1). Amongst other protozoan parasites *Giardia lamblia* is reported to have five RMRP protein subunits and *Leishmania* and *Trypanosoma* showed homology with only one subunit (RPP25) (61). The presence of RMRP RNA component is reported in *E. histolytica* (62,63), while it is absent in *Leishmania*, *Trypanosoma* and *Giardia*. RMRP is known to cleave pre-rRNA at the A3 site in the ITS 1, in a

manner similar to RNase P (64,65), the RNA component of which acts as a ribozyme (66). Mutations in the RNA component of RMRP are reported to cause cartilage hair hypoplasia (67,68). However, the absence of RNA component in RMRP from a number of parasites, and poor conservation of protein subunits suggests significant evolutionary diversity of this enzyme.

Amongst the proteins associated with 90S particles are the U3 snoRNPs (Supplementary Table 1.2). Previous work has shown that *E. histolytica* has 5 copies of U3snoRNA (69) which associate with conserved U3snoRNPs for modification of pre-rRNA. Of the 30 U3snoRNPs in *S. cerevisiae*, 23 were present in *E. histolytica*. The seven missing components were RRP7, NOP14, UTP3, UTP8, UTP9, UTP16 and UTP30. Of these UTP8, UTP9 and UTP16 were also missing in human. Amongst other proteins in 90S particles we found that 8-10 DEAD/DEAH box helicases and a single GTPase BMS1 was present in *E. histolytica*. Of the twenty-two other 90S factors, eight were missing in *E. histolytica* of which CIC1, CMS1 and NOP6 were also missing in human and were non-essential in *S. cerevisiae*. The other five (KRI1, NOP9, PXR1, RRP36 and TMA23) were present in human while absent in *E. histolytica*.

In the pre-40S particles (Supplementary Table 1.3), of the 20 export and cytoplasmic maturation factors required during the formation of 40S, three factors LTV1, SXM1 and KAP114 were missing in *E. histolytica*, all being present in human. SLX9 and eukaryotic translation initiation factor 3 subunit J (HCR1) were also missing from pre-40S particles. The most important export factor CRM1 known to be responsible for the export of both 60S and 40S subunits to the cytoplasm was present in *E. histolytica* with moderate homology and sequence similarity; however, it is annotated as a hypothetical protein in the database. An important kinase of 60S

particle GRC3 (NOL9 in humans), which cooperates with the endoribonuclease LAS1 to process pre-rRNA at site C2 in ITS2 (70), was also missing in *E. histolytica*. However XRN2, which is also reported to play a role in the processing of ITS2, was present (Figure 1a) (71). Protein kinases HRR25 and RIO2 which are known to associate with pre-40S particle were present in *E. histolytica*. HRR25 phosphorylates ribosomal protein RPS3, dephosphorylation of which marks the maturation of 40S particle (72). A number of other accessory factors were missing in *E. histolytica*. Some of the unique features of *E. histolytica*, namely organization of rRNA genes as extrachromosomal circles (41), and location of nucleolus at the nuclear periphery (42), may explain the differences in ribosome biogenesis machinery between *E. histolytica* and *S. cerevisiae*. Our data highlighted that 67% of the 279 *S. cerevisiae* genes involved in ribosome biogenesis could be found in *E. histolytica* with a high degree of sequence conservation, implying the use of most of the conserved pathways found in *S. cerevisiae*. It will be interesting to see the impact of the missing 33% genes (some of which may have been missed due to poor homology) on alternative strategies of ribosome biogenesis employed by *E. histolytica*, and possibly by other protists.

Effect of growth stress on transcription of pre-rRNA processing and ribosome biogenesis-genes in *E. histolytica*

Stress signals cease the transcription of rRNA genes in most model organisms. The demand for ribosomes is reduced in response to various environmental stresses e.g. serum starvation (73), inhibition of protein synthesis by cycloheximide treatment, and oxidative stress (74–79). This growth-dependent regulation of rRNA synthesis is conserved from bacteria to vertebrates (73).

Growth stress induced by serum starvation in *E. histolytica* leads to the accumulation of unprocessed pre-rRNA, and 5'-ETS intermediates, which are otherwise rapidly degraded during normal growth conditions (46). We have earlier shown that the exosomal subunit RRP6 is lost from the nucleus during serum starvation, which could lead to the accumulation of 5'-ETS sub fragments (47). To obtain a more comprehensive picture of the defects in pre-rRNA processing during growth stress in *E. histolytica* we looked for the accumulation of other intermediates during serum starvation, using probes from each segment of pre-rRNA, in a northern hybridization analysis. We observed an accumulation of different intermediates in *E. histolytica* with all the probes. The 5'-ETS probe, showed accumulation of 0.7 -0.9 kb fragment while the ITS1, 5.8S and ITS2 probes showed accumulation of a ~2 kb intermediate (Figure 1b). These data imply a more generalized defect in pre-rRNA processing during serum starvation. This could be due to the cumulative effect of loss of activity of a number of proteins, including endo- or exonucleases, required for the cleavage and trimming of pre-rRNA, and RNA helicases, the role of which in accumulation of pre-rRNA intermediates is well known (80).

A number of check points prevent entry of immature ribosomes into the translating pool (8,11). The discovery of ribosomopathies, due to mutations in genes encoding ribosome biogenesis factors, has shed new light on the link between ribosome biogenesis and cell fate (81). Defects in pre-rRNA processing, nucleolar organization and ribosomal subunit accumulation have been detected in the cells from patients suffering from ribosomopathies. To explore the possible factors responsible for the defects in pre-rRNA processing during growth stress in *E. histolytica*, we analyzed the transcriptome of trophozoites grown in normal conditions and after 24hrs of serum starvation. Differential RNA expression analysis of all the ribosome biogenesis proteins

present in *E. histolytica* led us to identify a number of possible factors that could interfere in pre-rRNA processing. The expression values of all the listed genes in normal and serum starved growth conditions are represented as heat map in Figure 2a. Of the 253 genes involved in pre-rRNA processing in *E. histolytica*, 40 genes showed differential expression (>1.5-fold change) of which 22 were upregulated and 18 were downregulated (Table 5, 6 respectively). The upregulated genes included three HSPs, four helicases, casein kinase, mRNA decay protein, and cytoplasmic export protein, NMD3. Casein Kinase showed the highest fold change of 3.56. NMD3 upregulation indicates the initiation of mRNA decay pathway during growth stress. RNA helicases function at many steps, including snoRNA unwinding after pre-rRNA modification (82) (Table 5, 6). The downregulated transcripts included H/ACA snoRNP complex, NEP1 methylase, NMD4 and the exosome component 10 (RRP6) which has a 3'-5' exonuclease activity. It is involved in 5'-ETS degradation and 5.8S rRNA processing, and our earlier work has shown that it is down regulated and is lost from nuclei during serum starvation (47,69). We also demonstrated that EhRRP6 acts as a stress sensor, as upregulating EhRRP6 protected the cells against stress while its downregulation hampered cell growth. Casein kinase in yeast is reported to regulate a switch between productive and non-productive pre-rRNA processing pathways post-transcriptionally during stress (83). Downregulation of NEP1 methylase has been shown to block pre-rRNA processing at sites A0, A1 and A2 (84). Thus, incomplete pre-rRNA modification, non-productive pre-rRNA processing, and the inefficient process of RNA intermediate degradation, along with other above-mentioned factors, could together or individually lead to the accumulation of unprocessed intermediates.

Effect of EhRRP6 downregulation on expression of ribosome biogenesis factors in *E. histolytica*:

The downregulation of exonuclease RRP6 and its loss from the nucleus during serum starvation (47) led us to investigate the global effect of RRP6 silencing on *E. histolytica* transcriptome compared to the changes observed in serum starvation. The heat map in Figure 2a represents the expression values of all the ribosomal genes in TOC, RRP6 downregulation, normal and SS growth conditions. Of the ribosome biogenesis factors present in *E. histolytica*, 102 genes showed >1.5fold change in EhRRP6 silenced cells as compared to 40 genes in serum starved conditions.

78 genes were upregulated in RRP6-silenced cells, of which 63 were significant (p-value < 0.05), 14 of these 78 were also upregulated in serum starved cells, of which only two were significant in both datasets (Figure 2b, Table 7). These two proteins were protein phosphatase (PTC2) and non-transported Abc protein ARB1. Along with the snoRNPs the other upregulated factors included helicases, GTPase, elongation factors, HSPs, cell cycle proteins, other components of exosome complex and RNase MRP. Interestingly ARB1 associates with ribosomes and has been proposed to serve as a mechanochemical ATPase, stimulating multiple steps in 40S and 60S ribosome biogenesis. Its depletion from *S. cerevisiae* led to slower cleavage at A0, A1 and A2 sites in pre-rRNA (85). Its transcriptional up regulation under conditions where pre-rRNA processing has slowed down could indicate its alternate roles in *E. histolytica*, or that the gene may be post-transcriptionally regulated, as is the case with RPs in *E. histolytica* (86).

34 genes were downregulated in RRP6-silenced cells, with 14 genes having significant p-values (Table 8). These included helicases, HSPs, casein kinases, Box H/ACA snoRNPs and U3 snoRNP subunits, TSR4 (a programmed cell death protein) and LSM-domain containing proteins. Four of these genes were downregulated in serum starved cells as well, with two having significant p-values. These genes, downregulated in both datasets, were NEP1 and TSR3. The latter is annotated as a hypothetical protein in *E. histolytica*. Interestingly, both of these genes are involved in chemical modification of the hypermodified nucleotide N1-methyl-N3-aminocarboxypropyl(acp) pseudouridine located on 18S rRNA next to the P-site tRNA (87). This highly conserved base modification in eukaryotes is completed in three steps. In the first step uridine is converted to pseudouridine guided by a snoRNP. In the second step NEP1 methyltransferase adds a methyl group donated by S-adenosylmethionine (SAM). In the third step, which takes place in the cytoplasm, TSR3 adds the APC group from SAM to this nucleotide. This is a crucial step in 18S rRNA maturation. TSR3 downregulation results in accumulation of 20S rRNA precursor, along with accumulation of full-length pre-rRNA (35S) in yeast and (47S) in human. NEP1 also has a crucial role, and its downregulation results in blockage of pre-rRNA processing at sites A0, A1 and A2 (84). Our data suggest that these proteins are crucial in *E. histolytica* as well, and their expression could be regulated in a common pathway that is responsive both to serum stress and RRP6 downregulation.

In conclusion, our data reveal the multiple factors that simultaneously lead to the inhibition of pre-rRNA processing and accumulation of intermediates during growth stress (Figure 3), thus effectively inhibiting ribosome biogenesis by the non-availability of mature rRNAs. Of special interest are the genes affected in a similar manner by both serum starvation and RRP6 down

regulation. This work opens up further avenues for detailed molecular investigation of ribosome biogenesis in *E. histolytica*.

Methods:

Computational identification of *E. histolytica* ribosome biogenesis and pre-rRNA processing proteins

S. cerevisiae was used as a reference organism and homologous proteins involved in ribosome biogenesis and pre-rRNA processing in *E. histolytica* were searched. A list of proteins already known to be the part of ribosome biogenesis was compiled using *Saccharomyces* Genome Database (SGD) by applying Gene Ontology Term: ‘rRNA processing’ and ‘Ribosome biogenesis’. The enlisted proteins were further filtered using ‘UniProt’ as Source. Proteins were cross checked in KEGG (Kyoto Encyclopaedia of Genes and Genomes) PATHWAY Database using Keyword: ‘Ribosome biogenesis in eukaryotes’ and filtered keeping ‘*S. cerevisiae*’ as the concerned organism. Proteins were annotated using ‘Function’ from UniProt or ‘Description’ from SGD. Homologous proteins in *E. histolytica* were obtained using PANTHER (Protein Analysis THrough Evolutionary Relationships), AmoebaDB and EggNOG (evolutionary genealogy of genes: Non-supervised Orthologous Groups) databases. BLASTP was performed against respective protein sequence of *E. histolytica* obtained from AmoebaDB restricting the search only to “*Entamoeba histolytica* HM-1:IMSS (taxid:294381)” to obtain an E-value, score, coverage, maximum identity and domains present. The proteins were functionally classified using the KEGG classification. The accession number and annotation were obtained from NCBI,

protein database. The expression level of respective genes was extracted from the transcriptome data.

Cell culture and growth conditions

Trophozoites of *E. histolytica* strain HM-1:IMSS were axenically maintained in TYI-S-33 medium supplemented with 15% adult bovine serum (Biological industries, Israel), Diamond's Vitamin mix, Tween 80 solution (Sigma–Aldrich) and antibiotics (0.3 units/ml penicillin and 0.25 mg/ml streptomycin) at 35.5°C (88). For serum starvation, medium from early to mid-log phase grown trophozoites (48 hrs) was replaced with TYI-S-33 medium containing 0.5% adult bovine serum and incubation continued for 24hrs.

RNA isolation and Transcriptome analysis

Cells were harvested from normal, serum starvation, TOC and Rrp6-depleted conditions (47). Total RNA from $\sim 5 \times 10^6$ cells were purified using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Total RNA, from two biological replicates of each sample was used for selection of polyA plus RNA and library preparation was done after oligo (dT) selection. RNA-Seq libraries were generated and subjected to paired-end sequencing on the Illumina HiSeq2500 (v3 Chemistry) platform (52). The pre-processed reads were aligned to the *E. histolytica* (HM1:IMSS) genome for which the gene model was downloaded from AmoebaDB (<http://amoebadb.org/common/downloads/release-27/EhistolyticaHM1IMSS/gff/data/>). The alignment was performed using Tophat program (version 2.0.11) with default parameters. The

RSEM program (version 1.3.0) was used for estimating expression of the genes and transcripts(89). The differential gene expression analysis was performed using cuffdiff program of cufflinks package with default settings to analyze the difference between normal and serum starved cells. Log₂ fold change was set as a cut-off for differential expression. Real time qRT PCR was done to validate the data (52). Heatmap was prepared using ggplot2 in R Studio. The Venn diagram was plotted using Venny^{2.1} (<https://bioinfogp.cnb.csic.es/tools/venny/>).

Northern blotting

For Northern blot analysis 10 µg of total RNA was resolved on a 1.2% formaldehyde-agarose gel in gel running buffer (0.1 M MOPS (pH 7.0), 40 mM sodium acetate, 5 mM EDTA (pH 8.0)) and 37% formaldehyde at 4 V/cm. The RNA was transferred on to GeneScreen plus R membrane (PerkinElmer). [α -³²P]dATP-labeled probe was prepared by random priming method using the DecaLabel DNA labeling kit (Thermo Scientific). Hybridization and washing conditions for RNA blots were as per the manufacturer's instructions.

Figure legends:

Figure 1: (a) Schematic linear view of rDNA transcription unit indicating Sc processing sites. The dotted arrows indicate the sites present in *E. histolytica*. The size of the 5'-ETS (2.672 kb), 18S (1.921 kb), ITS1 (149 bp), 5.8S (150 bp), ITS2 (123 bp), 28S (3.544 kb) and IGS (5.183 kb) accounts for the 14 kb rDNA of *E. histolytica*. (b) Northern hybridization of total RNA from *E. histolytica* cells grown under normal (N) and serum starved (SS) conditions. The probes used are indicated. The lanes with 18S and 28S probes were exposed very briefly and show the position

of the mature rRNAs. 28S rRNA in *E. histolytica* has an internal nick and two probe sets were used as indicated. The bands accumulated in SS cells are marked in circle.

Figure 2: (a) Heat map showing the log₂ expression of all ribosome biogenesis factors in *E. histolytica* during TOC, Rrp6KD, normal and serum starved growth conditions. Red color depicts high expressing genes and blue color depicts low expressing genes. (b) Venn diagram shows an overlap of the differentially expressed genes in all the four conditions. Down_SS-Downregulated in SS; Up_SS-Upregulated in SS; Down_RRP6KD-Downregulated in RRP6KD; Up_RRP6KD- Upregulated in RRP6KD.

Figure 3: Model depicting factors regulating pre-rRNA processing and accumulation of intermediates during growth stress

Competing interests

The authors declare no competing interests.

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Authors' contributions

SN and SB were involved in the study design. SN, SSS, DK, YP, AM conducted the study. SN and SB analyzed and interpreted the data. SN and SB drafted the manuscript. All authors read and approved the final manuscript.

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Table 1: Ribosome biogenesis factors and their expression status in *E. histolytica*

Classes of proteins involved in various steps of ribosome biogenesis		No of genes in Sc	No of genes in Eh	Expression level (VH,H>M>VL,L>A)
SnoRNPs	Box C/D	4	5	03>2>0>0
	Box H/ACA	6	8	07>1>0>0
90s particles	U3 snoRNA associated proteins	30	27	0>24>2>1
	Helicase	9	18	1>13>03>1
	RNase	1	1	0>0>0>1
	GTPase	3	1	0>1>0>0
	Kinase	4	6	0>5>0>1
	Other 90S particle	22	20	0>13>5>2
Pre-40S particles	Kinases	3	7	0>6>1>0
	Export and cytoplasmic maturation factors	20	22	1>17>4>0
	Other pre-40S particles	6	13	2>7>3>1
Pre-60S particles	Helicase	11	13	0>11>2>0
	RNase	15	5	0>5>0>0
	GTPase	5	7	3>4>0>0
	Kinase	2	--	
	Export and cytoplasmic maturation factors	22	23	3>11>4>5
	5-FMC complex	3	2	0>2>0>0
	Other pre-60S particle	52	39	2>34>3>0
Other ribosome biogenesis factors		13	6	0>6>0>0
Miscellaneous proteins		54	41	15>22>0>4>0

*VH, very high; H, high; M, medium; L, low; A, absent.

Table 2: *S. cerevisiae* ribosome biogenesis proteins with no homologues in *E. histolytica*

	Gene family	Gene name	Accession Number	Human	Functions in <i>S. cerevisiae</i>
SnoRNPs	Box H>ACA	NAF1	KZV08498	Q96HR8	RNA-binding protein required for the assembly of box H>ACA snoRNPs.

	snoRNPs				
90S particles	U3 small nucleolar RNA-associated proteins	RRP7	KZV12820	----	Along with Utp22 conducts early processing of 18S rRNA, binds at the central domain of 18S rRNA.
		NOP14	KZV12078	P78316	Interacts with EMG1 protein for 18S rRNA maturation and 40S ribosome production.
		SAS10	KZV12073	Q9NQZ2	Dock other proteins to the nucleic acid substrate, binds Mpp10 protein.
		UTP8	KZV11358	----	Forms complex with amino acyl tRNA synthetase and translocate it from nucleolus to nuclear export receptors, helps processing of 35S pre-rRNA to 18S pre-rRNA.
		UTP9	KZV10940	----	Bind the ssu processosome directly without utp8.
		BUD21	KZV07967	----	Component of ssu processosome complex, involved in nucleolar processing of 18S pre-rRNA.
		UTP30	KZV10001	----	Putative subunit of U3-containing 90S pre-ribosome complex, involved in production of 18S rRNA and assembly of small ribosomal subunit.
	RNase	RNT1	KZV09092	----	Ribonuclease (RNase III), involved in rRNA processing and U2 snRNA 3' end formation, cleaves pre-rRNA at A0 site in the 5'-ETS and 3'-ETS.
		REX4	KZV07802	----	Putative RNA exonuclease; possibly involved in pre-rRNA processing and ribosome assembly.
Other 90S particles		CIC1	KZV10776	----	Required for synthesis and nuclear export of 60S ribosomal subunits.
		CMS1 YLR003C	KZV09250	----	Putative subunit of the 90S pre-ribosome processosome complex.
		KRI1	KZV08317	Q8N9T8	Essential nucleolar protein required for 40S ribosome biogenesis, associate with snR30 and Krr1p.
		NOP6	KZV12009	----	rRNA binding protein required for 40S ribosomal subunit biogenesis, contains an RRM.
		NOP9	KZV10262	Q86U38	Essential subunit of U3-containing 90S pre-ribosome, involved in production of 18S rRNA and assembly of small ribosomal subunit, part of pre-40S ribosome.
		PXR1	KZV11514	P50542	Involved in rRNA-processing at A0, A1 and A2 sites through its action in U18 and U24 snoRNA 3'-end trimming.
		RRP36	KZV08174	Q96EU6	Component of 90S pre-ribosomes, involved in early cleavages of the 35S pre-rRNA and in production of the 40S ribosomal subunit.
		TMA23	KZV09123	----	Nucleolar protein implicated in ribosome biogenesis.
		Pre-40S particles	Export and cytoplasmic maturation	LTV1	KZV09794
SXM1	KZV12639			----	Nuclear transport factor (karyopherin).

	factor	KAP114	KZV10987	----	Karyopherin, responsible for nuclear import of specific proteins.
Other pre-40S particle		HCR1	KZV09436	----	eIF3j component of translation initiation factor 3 (eIF3), required for 20S pre-rRNA processing; binds eIF3 subunits Rpg1p, Prt1p and 18S rRNA
		SLX9	KZV11307	----	Associated with 90S pre-ribosome and 43S small ribosomal subunit, interacts with U3 snoRNA.
Pre-60S particles	RNase	NGL2	KZV09140	----	Involved in 5.8S rRNA processing, required for correct 3'-end formation of 5.8S rRNA at site E.
		REX2	KZV09305	----	3'-5' RNA exonuclease, involved in 3'-end processing of U4>U5 snRNAs, 5S and 5.8S rRNAs, and RNase P and RNase MRP RNA.
		RNH70	KZV11510	----	3' exoribonuclease required for 5S rRNA maturation, along with REX2 helps maturation of the 5.8S rRNA
	Rnase MRP	POP3	KZV08343	Q9HBV1	Required for processing of 5.8S rRNA at site A3 and for 5' and 3' processing of pre-tRNA.
		POP4	KZV13345	O95707	Subunit of RNase MRP and nuclear RNase P, RNase MRP cleaves pre-rRNA, while nuclear RNase P cleaves tRNA precursors to generate mature 5' ends.
		POP5	KZV13434	Q969H6	Component of RNase MRP, which cleaves pre-rRNA sequences.
		POP6	KZV11255	----	Subunit of RNase MRP, nuclear RNase P and telomerase; forms a soluble heterodimer with Pop7p that binds P3 domain of RNase MRP and RNase P RNAs; RNase MRP cleaves pre-rRNA
		POP7	KZV13248	O75817	Subunit of RNase MRP, nuclear RNase P and telomerase; forms a soluble heterodimer with Pop6p that binds P3 domain of RNase MRP and RNase P RNAs.
		POP8	KZV13053	----	Component of RNase MRP, which cleaves pre-rRNA sequences.
		RMP1	KZV09389	----	Subunit of ribonuclease MRP, which is involved in rRNA processing in mitochondria.
		SNM1 YDR478W	KZV12719	----	Ribonuclease MRP complex subunit.
	Kinase	GRC3	KZV09214	----	Polynucleotide kinase present on rDNA, required for efficient transcription termination by RNA polymerase I, functions with Las1p to modulate ribosome biogenesis.
	Export and cytoplasmic maturation factors	ARX1	KZV12343	----	Nuclear export factor for the ribosomal pre-60S subunit, facilitates translocation through the nuclear pore complex
		ALB1	KZV10155	----	Shuttling pre-60S factor; involved in the biogenesis of ribosomal large subunit, interacts directly with Arx1p.
		SSA1	KZV13464	P19474	ATPase involved in protein folding and NLS-directed nuclear transport,

					member of HSP70 family.
		SSA4	KZV11871	----	highly induced upon stress, plays a role in SRP-dependent co-translational protein-membrane targeting and translocation.
		SSA3	KZV12991	----	ATPase involved in protein folding and the response to stress, plays a role in SRP-dependent co-translational protein-membrane targeting and translocation.
		SSB2	KZV08414	Q99619	Cytoplasmic ATPase that is a ribosome-associated molecular chaperone, functions with J-protein partner Zuo1p, may be involved in the folding of newly-synthesized polypeptide chains.
		MTR2	KZV09748	Q8IYT2	mRNA transport regulator, Mex67p and Mtr2p form a mRNA export complex.
		MEX67	KZV07345	----	Poly(A)RNA binding protein involved in nuclear mRNA export; component of the nuclear pore
		LOC1	KZV11600	----	Required for efficient assembly and nuclear export of the 60S ribosomal subunit.
		SQT1	KZV10631	----	May be involved in the late step of 60S ribosomal subunit assembly or modification in the cytoplasm.
5-FMC Complex		IPI1	KZV10820	----	Component of the Rix1 complex and possibly pre-replicative complexes, required for processing of ITS2, component of the pre-60S ribosomal particle with the dynein-related AAA-type ATPase Mdn1p.
Other pre-60S particles		BUD20	KZV09320	----	C2H2-type zinc finger protein required for ribosome assembly, shuttling factor which associates with pre-60S particles in the nucleus.
		CIC1	KZV10776	----	Required for synthesis and nuclear export of 60S ribosomal subunits.
		CGR1	KZV11196	----	Protein involved in nucleolar integrity and processing of pre-rRNA, transcript is induced in response to cytotoxic stress.
		MAK11	P20484	----	Protein involved in an early step of 60S ribosomal subunit biogenesis.
		NOP8	Q08287	----	Required for 60S ribosomal subunit synthesis.
		NOP16	KZV11750	Q9Y3C1	Constituent of 66S pre-ribosomal particles, involved in 60S ribosomal subunit biogenesis.
		NOP53	KZV07369	Q9NZM5	Nucleolar protein, involved in biogenesis of the 60S subunit of the ribosome, interacts with rRNA processing factors Cbf5p and Nop2p and with the nucleolar proteins Nop17p and Nip7p.
		NSA1	KZV11117	Q6RFH5	Constituent of 66S pre-ribosomal particles, involved in 60S ribosomal subunit biogenesis.
		RAI1	KZV10982	Q7Z5J4	Targets mRNAs with unmethylated 7-methylguanosine cap structures and 5'-triphosphates, binds and stabilizes the exoribonuclease Rat1p.

	RIX1	KZV10941	----	Component of the Rix1 complex required for processing of ITS2 sequences from 35S pre-rRNA.
	RRP1	KZV12324	P05386	Necessary for biogenesis of 60S ribosomal subunits and for processing of pre-rRNAs to mature rRNA, associated with several distinct 66S pre-ribosomal particles.
	RRP17	KZV12656	Q96S79	Component of pre-60S ribosomal particle, exonuclease required for 5' end processing of pre-60S rRNA.
	RSA1	KZV07319	----	Protein involved in the assembly of 60S ribosomal subunits, functionally interacts with Dbp6p.
	RSA3	KZV09463	----	Required for accumulation of wild-type levels of large (60S) ribosomal subunits, binds to the helicase Dbp6p in pre-60S ribosomal particles in the nucleolus.
	SRD1	KZV12864	----	Protein involved in the processing of pre-rRNA to mature rRNA, contains a C2>C2 zinc finger motif.
	TMA16	KZV08139	Q96EY4	Protein of unknown function that associates with ribosomes.
	URB1	KZV09928	O60287	Associated with the 27SA2 pre-ribosomal particle, proposed to be involved in the biogenesis of the 60S ribosomal subunit
	URB2	KZV10308	Q14146	Protein required for normal metabolism of the rRNA primary transcript, nucleolar protein.
Other ribosome biogenesis factors	BCP1	KZV12601	----	Involved in nuclear export of the lipid kinase MSS4 and of the 60S ribosomal subunit.
	MTF1	KZV09080	Q14872	Although strongly related to dimethyladenosine transferase proteins, it lacks the methyltransferase activity.
	MRM1	KZV08091	Q6IN84	Catalyzes the formation of 2'-O-methylguanosine in mitochondrial ribosomal RNA.
	BMT2	KZV13221	Q1RMZ1	Methylation of the N1 position of adenine 2142 in 25S rRNA.
	BMT5	KZV10521	----	Methylation of the 25S rRNA, associates with precursors of the 60S ribosomal subunit.
	BMT6	KZV09309	----	Methylation of the 25S rRNA.
	MTG1	KZV08938	Q9BT17	Mitochondrial GTPase involved in assembly of the large ribosomal subunit.
Miscellaneous Proteins	GEP3	KZV08094	----	Required for mitochondrial ribosome small subunit biogenesis.
	RSC9	KZV08711	----	Component of RSC chromatin remodeling complex, involved in the synthesis of rRNA.
	CBT1	KZV09728	----	Protein involved in 5' RNA end processing, substrates include mitochondrial COB.
	CSL4	KZV08391	Q9Y3B2	Exosome non-catalytic core component, involved in 3'-5' RNA processing

				and degradation in both the nucleus and the cytoplasm.
	RRP42	KZV12118	Q15024	Exosome non-catalytic core component, involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm.
	RRP43	KZV12884	Q96B26	Exosome non-catalytic core component, involved in 3'-5' RNA processing and degradation in both the nucleus and the cytoplasm.
	LRP1	KZV10816	Q07954	Nuclear exosome-associated nucleic acid binding protein, involved in RNA processing, surveillance, degradation, tethering, and export, forms a stable heterodimer with Rrp6p.
	MPP6	KZV08651	Q9NZW5	Nuclear exosome-associated RNA binding protein, involved in surveillance of pre-rRNAs and pre-mRNAs, and the degradation of cryptic non-coding RNAs (ncRNA).
	NPL3	KZV12675	----	RNA-binding protein
	FHL1	KZV07616	Q13642	Regulator of ribosomal protein (RP) transcription, recruits coactivator Ifh1p or corepressor Crf1p to RP gene promoters.
	FAF1	KZV10599	Q9UNN5	Protein required for pre-rRNA processing, and 40S ribosomal subunit assembly.
	IFH1	KZV09465	----	Coactivator, regulates transcription of ribosomal protein (RP) genes, recruited to RP gene promoters during optimal growth conditions via Fhl1p, subunit of CURI.
	PIH1	KZV10756	----	Component of the conserved R2TP complex, interacts with Hsp90 to mediate assembly large protein complexes such as box C>D snoRNPs and RNA polymerase II.
	RTC3	KZV10823	----	May play a role in RNA metabolism, rRNA-processing, and in a process influencing telomere capping.
	RRP15	Q06511	Q9Y3B9	Required for processing of the 27S pre-rRNA at A3 and B1 sites to yield mature 5.8S and 25S rRNAs.
	REX3	KZV09354	----	RNA exonuclease, required for maturation of the RNA component of RNase MRP.
	EFG1	KZV11506	----	Essential protein required for maturation of 18S rRNA.
	FCF2	KZV09297	----	Nucleolar protein involved in the early steps of 35S rRNA processing, interacts with Faf1p.
	FYV7	KZV09314	----	Essential protein required for maturation of 18S rRNA.
	SBP1	KZV10682	Q13228	Binds eIF4G and has a role in repression of translation, binds to mRNAs under glucose starvation stress, associates with snoRNAs snR10 and snR11.
	LSM6	KZV12620	P62312	Involved in mRNA decay.

Table 3: *E. histolytica* genes which match multiple *S. cerevisiae* genes

SnoRNPs	Box C>D snoRNPs	EHI_053440	SNU13, YEL026W	13 kDa ribonucleoprotein-associated protein
			NHP2, YDL208W	
90S particles	U3 snoRNA- associated proteins		UTP23, YOR004W	Hypothetical protein
			FCF1, UTP24, YDR339C	
	Kinase	EHI_075700	CKB1, YGL019W	Casein kinase II regulatory subunit family protein
			CKB2, YOR039W, OR26.33	
		EHI_152350	CKB1, YGL019W	
			CKB2, YOR039W, OR26.32	
		EHI_030840	CKB1, YGL019W	
			CKB2, YOR039W, OR26.34	
		EHI_035250	CKB1, YGL019W	
			CKB2, YOR039W, OR26.35	
		EHI_006800	CKA1, YIL035C	Casein kinase
			CKA2, YOR061W, YOR29-12	
		EHI_038670	CKA1, YIL035C	Protein kinase domain containing protein
			CKA2, YOR061W, YOR29-13	
	Helicases	EHI_077640	PRP43, YGL120C	ATP-dependent helicase
			ECM16, DHR1, YMR128W	
		EHI_096230	PRP43, YGL120C	
			DHR2, YKL408, YKL078W	
		EHI_096390	ROK1, YGL171W, G1652	DEAD>DEAH box helicase
			DBP2, YNL112W, N1945	
			DRS1, YLL008W	
			DBP3, YGL078C	
		EHI_122790	PRP43, YGL120C	
			DHR2, YKL408, YKL078W	
		EHI_148930	PRP43, YGL120C	
			DHR2, YKL408, YKL078W	
		EHI_184530	PRP43, YGL120C	
			DHR2, YKL408, YKL078W	

		EHI_014080	PRP43, YGL120C DHR2, YKL408,YKL078W					
		EHI_033720	PRP43, YGL120C DHR2, YKL408,YKL078W					
Other 90S particles		EHI_014120	MRD1, YPR112C NOP15, YNL110C, N1954	RNA recognition motif domain containing protein				
Pre-60S particles	Export and cytoplasmic maturation factors	EHI_154230	REI1, YBR267W, YBR1736 REH1, YLR387C	Zinc finger protein 622				
		EHI_045120	AFG2, YLR397C, RIX7, YLL034C	Cell division cycle protein 48				
		GTPase	EHI_164370	NUG1, YER006W NOG2, YNR053C, N3484,NUG2	GTPase			
			Helicases	EHI_165110	DBP7, YKR024C DBP6, YNR038W, N3303	DEAD>DEAH box helicase		
	EHI_169630	HAS1, YMR290C DRS1, YLL008W DBP6, YNR038W, N3306						
	EHI_175030	ROK1, YGL171W, G1651 DRS1, YLL008W DBP3, YGL078C						
	EHI_069410	DRS1, YLL008W DBP10, YDL031W, D2770						
	EHI_111040	RRP3, YHR065C DBP8, YHR169W						
		DRS1, YLL008W DBP6, YNR038W, N3304						
	EHI_119620	HCA4, DBP4, J1250, ECM24, YJL033W, DRS1, YLL008W DBP6, YNR038W, N3305						
	RNase	EHI_133330		XRN1,DST2,KEM1, RAR5,SEP1,SKI1 RAT1, HKE1, TAP1, YOR048C	5'-3' exonuclease domain containing protein			
		Other pre-60S particles		EHI_004900	IMP4, YNL075W, N2353 RPF1, YHR088W		U3 snRNP IMP4	
		EHI_012190		SSF1, YHR066W SSF2, YDR312W	Brix domain containing protein			

		EHI_050420	ERP2, YAL007C,FUN54	Hypothetical protein
			ERP4, YOR016C	
		EHI_088070	SPB1, YCL054W, YCL431, YCL54W	Ribosomal RNA methyl transferase
			MRM2, YGL136C, G2830	
		EHI_188010	FPR4, YLR449W	Hypothetical protein
	FPR3, NPI46,YML074C			
Pre-40S particles	Export and cytoplasmic maturation factor	EHI_148190	GSP1, YLR293C, CNR1, CST17	Ran family GTPase
			GSP2, YOR185C,CNR2	
Other pre-40S particle		EHI_110320	PTC3, YBL056W	Protein phosphatase
			PTC2, YER089C	
Miscellaneous Proteins		EHI_012360	RPS14A, CRY1, YCR031C, YCR31C	Ribosomal protein S14
			RPS14B, CRY2, YJL191W, J0354	
		EHI_179000	RPS9A, RPS13A, YS11A, YPL081W	40S ribosomal protein S9
			RPS9B, RPS13B, YBR189W, YBR1317	
		EHI_125780	RPS9A, RPS13A, YS11A, YPL081W	
			RPS9B, RPS13B, YBR189W, YBR1318	
		EHI_126870	RPS21A, RPS25, RPS25A, YKR057W	40S ribosomal protein S21
			RPS21B, RPS25B, RPS26B, YJL136C	
		EHI_091070	RVB1, TIH1, TIP49A, YDR190C	ruvB-like DNA helicase
			RVB2, TIH2, TIP49B, YPL235W, P1060	
		EHI_040320	MTR3, YGR158C	3' exonuclease family protein
			RRP46, YGR095C	
			SKI6, ECM20, RRP41, YGR195W, G7587	
		EHI_040360	RVB1, TIH1, TIP49A, YDR190C	ruvB-like DNA helicase
			RVB2, TIH2, TIP49B, YPL235W, P1061	
		EHI_081410	RPS0A, NAB1, YST1, NAB1A, YGR214W	40S ribosomal protein SA
			RPS0B, NAB1B, NAB4, YST2, YLR048W,	
		EHI_068580	LSM5, SAD1, At5g48870, K24G6.22	U6 snRNA-associated Sm-like protein LSm2
			LSM2, SMX5, SNP3, YBL026W, YBL0425	

Table 4: RNases in *S. cerevisiae* and homologs in human and *E.histolytica*

Gene Name (Sc)	Accession Number	Uniprot (Humans)	Gene Name (Humans)	Gene symbol (E. histolytica)	Transcription Status	Domains
RNT1, YMR239C	KZV09092	Q9NRR4	DROSHA, RNASE3L	--	--	--
REX4, YOL080C	KZV07802	Q9GZR2	REXO4, PMC2	--	--	--
DBR1, YKL149C,	KZV09788	Q9UK59	DBR1	EHI_062730	Low	RNA lariat debranching enzyme
NGL2, YMR285C,	KZV09140	Q6L8Q7	PDE12	--	--	--
REX2, YLR059C	KZV09305	Q9Y3B8	REXO2, SFN	--	--	--
REX1, YGR276C	KZV11510	Q8IX06	REXO1L1P, GOR	--	--	--
XRN1	KZV11055	Q8IZH2	XRN1, SEP1	EHI_133330	Medium	5'-3' exonuclease domain containing protein
RAT1, YOR048C	CAA99240	Q9H0D6	XRN2	EHI_128220 EHI_133330	Medium Medium	5'-3' exonuclease domain containing protein
RNase MRP subunits						
POP1, YNL221C	KZV08402	Q99575	POP1 KIAA0061	EHI_127090	Medium	Hypothetical protein
POP3, YNL282W	KZV08343	--		--	--	--
POP4, YBR257W	KZV13345	--		--	--	--
POP5, YAL033W	KZV13434	Q969H6	POP5, AD-008	--	--	--
POP6, YGR030C	KZV11255	--		--	--	--
POP7, YBR167C	KZV13248	--		--	--	--
POP8, YBL018C	KZV13053	--		--	--	--
RPP1, YHR062C	KZV10792	P78346	RPP30 RNASEP2	EHI_086240 EHI_179350	Medium Medium	Ribonuclease P protein subunit p30 Ribonuclease P protein subunit p30
RMP1, YLR145W	KZV09389	--		--	--	--
SNM1 YDR478W,	KZV12719	--		--	--	--

Table 5: Up regulated (>1.5-fold change) genes during serum starvation as compared to normal

	Gene Family	Gene Description	Log ₂ Fold Change	P-value
90S particles	U3 snoRNA-associated proteins	EHI_138370 Hypothetical protein	0.73	0.073
	Helicase	EHI_033720 DEAD>DEAH box helicase	0.60	0.338
		EHI_122790 Helicase	0.77	0.394
90S particles>pre-60S particle	Helicase	EHI_169630 DEAD>DEAH box helicase	0.86	0.111
Pre-40S particles	Kinase	EHI_050990 RIO1 family protein	0.64	0.406
		EHI_156390 Casein kinase	3.56	0.078
	Export and cytoplasmic maturation factor	EHI_175470 Hypothetical protein	1.00	0.008
Other pre-40S particle		EHI_056420 Protein phosphatase	0.92	0.012
		EHI_174280 Phospholipid transporting P-type ATPase	0.67	0.680
Pre-60S particles	Helicase	EHI_125170 DEAD>DEAH box helicase	0.96	0.121
	Rnase MRP	EHI_179350 Ribonuclease P protein subunit p31	0.92	0.382
		EHI_086240 Ribonuclease P protein subunit p30	0.73	0.304
		GTPase	EHI_166810 Elongation factor 2	1.13
	Export and cytoplasmic maturation factors	EHI_113410 Heat shock protein 70	1.49	0.138
		EHI_175440 Non transporter ABC protein	0.62	0.331
		EHI_083120 Nonsense mediated mRNA decay protein 3	0.83	0.329
		EHI_192440 Heat shock protein 70, hsp70A2	0.94	0.063
		EHI_199590 Heat shock protein 70	1.25	0.098
Other pre-60S particles		EHI_050420 Hypothetical protein	1.43	0.082
		EHI_038310 60S ribosome subunit biogenesis protein NIP11	0.70	0.185
		EHI_059540 Brix domain containing protein	0.95	0.709
Miscellaneous Proteins		EHI_187180 Ubiquitin-protein ligase	0.62	0.451

The genes with a P-value < 0.5 are highlighted in yellow.

Table 6: Down regulated (>1.5-fold change) genes during serum starvation as compared to normal

	Gene family	Gene Description	Log ₂ Fold Change	P-value
SnoRNPs	Box H>ACA snoRNPs	EHI_001850 H>ACA ribonucleoprotein complex subunit 2-like protein	-0.60	0.022
		EHI_102280 H>ACA ribonucleoprotein complex subunit 2-like protein	-0.60	0.022
90S particles	U3 snoRNA associated proteins	EHI_179100 Hypothetical protein	-1.13	0.195
		EHI_012960 Hypothetical protein	-0.63	0.574
		EHI_198890 Hypothetical protein	-0.61	0.469
Other 90S particles		EHI_033750 Hypothetical protein	-0.61	0.168
		EHI_044610 Ribosome biogenesis protein NEP1	-1.56	0.025
Pre-40S particles	Export and cytoplasmic maturation factor	EHI_164410 Hypothetical protein	-0.60	0.524
		EHI_001120 Hypothetical protein	-0.80	0.066
		EHI_100490 Hypothetical protein	-0.63	0.050
Pre-60S particles	Helicase	EHI_134610 DEAD>DEAH box helicase	-0.79	0.209
	GTPase	EHI_164370 GTPase	-1.03	0.111
	Export and cytoplasmic maturation factors	EHI_052860 Heat shock protein 70	-0.62	0.136
		EHI_179200 Hypothetical protein	-0.65	0.475
		EHI_104460 Nonsense mediated mRNA decay protein 4	-1.23	0.476
Other pre-60S particles		EHI_105790 Nucleolar complex protein 2 homolog	-0.65	0.572
Miscellaneous Proteins		EHI_021400 Exosome component 10	-0.77	0.444
		EHI_104570 Ubiquitin ligase	-1.75	0.171

Table 7: Up-regulated genes (>1.5-fold change) in Rrp6KO as compared to TOC

	Gene family	Gene Description	Log ₂ Fold Change	P-value
SnoRNPs	Box C>D snoRNPs	EHI_183900 snoRNA binding protein putative	0.95	0.003
		EHI_104600 13kDa ribonucleoprotein-associated protein	0.81	0.008

	Box H>ACA snoRNPs	EHI_115300 Centromere>microtubule binding protein	0.81	0.002
90S particles	U3 small nucleolar RNA-associated proteins	EHI_142200 U3 snRNP subunit	1.21	0.051
		EHI_048860 U3 snRNP MPP10	1.28	0.065
		EHI_198890 Hypothetical protein	0.81	0.133
		EHI_067830 WD domain containing protein	0.65	0.046
		EHI_140750 WD domain containing protein	0.70	0.014
		EHI_138370 Hypothetical protein	0.90	0.043
		EHI_148770 Hypothetical protein	1.04	0.043
	Helicases	EHI_119620 DEAD>DEAH box helicase	1.10	0.023
		EHI_169630 DEAD>DEAH box helicase	3.50	0.003
		EHI_175030 DEAD>DEAH box helicase	0.61	0.010
		EHI_096390 DEAD>DEAH box helicase	1.01	0.005
		EHI_013960 DEAD>DEAH box helicase	1.08	0.019
		EHI_122790 Helicase	0.62	0.279
		EHI_014080 DEAD>DEAH box helicase	0.86	0.001
		EHI_033720 DEAD>DEAH box helicase	1.80	0.000
	GTPase	EHI_196410 Ribosome biogenesis protein BMS1	0.69	0.021
Other 90S particles		EHI_160440 rRNA biogenesis protein RRP5	1.90	0.048
		EHI_142050 Hypothetical protein	1.01	0.022
		EHI_124060 rRNA biogenesis protein RRP5	1.77	0.147
		EHI_092660 Hypothetical protein	1.45	0.025
		EHI_014120 RNA recognition motif domain containing protein	0.79	0.053
Pre-40S particles	Kinase	EHI_151950 Casein kinase	0.70	0.021
		EHI_156390 Casein kinase	4.97	0.004
	Export and cytoplasmic maturation factor	EHI_175470 Hypothetical protein	0.78	0.177
		EHI_171760 Hypothetical protein	0.65	0.020
		EHI_013870 Dimethyladenosine transferase	2.51	0.001
		EHI_008390 Hypothetical protein	0.79	0.002

		EHI_004960 Bystin	1.12	0.008
	Other pre-40S particle	EHI_096620 Phospholipid transporting P-type ATPase	1.01	0.006
		EHI_024120 Phospholipid transporting P-type ATPase	1.16	0.000
		EHI_056420 Protein phosphatase	1.21	0.007
Pre-60S particles	Helicases	EHI_069410 DEAD>DEAH box helicase	0.82	0.013
		EHI_078560 DEAD>DEAH box helicase	1.03	0.024
		EHI_125170 DEAD>DEAH box helicase	1.18	0.010
		EHI_165110 DEAD>DEAH box helicase	1.07	0.022
	GTPase	EHI_164370 GTPase	1.15	0.024
		EHI_174940 Nucleolar GTP-binding protein	1.41	0.007
		EHI_118800 Hypothetical protein	1.03	0.027
		EHI_155660 Elongation factor 2	1.21	0.005
		EHI_164510 Elongation factor 2	0.76	0.001
		EHI_166810 Elongation factor 2	1.49	0.024
	RNase MRP	EHI_127090 Hypothetical protein	1.48	0.027
		EHI_086240 Ribonuclease P protein subunit p30	1.28	0.007
	Export and cytoplasmic maturation factors	EHI_175440 Non transporter ABC protein	0.97	0.011
		EHI_188830 DnaJ domain containing protein	1.14	0.027
		EHI_006170 Eukaryotic translation initiation factor 6	1.54	0.003
		EHI_113410 Heat shock protein 70	0.87	0.108
		EHI_199590 Heat shock protein 70	1.75	0.003
		EHI_013760 Heat shock protein 70, mitochondrial	0.64	0.361
		EHI_007150 Heat shock protein 70, mitochondrial	1.11	0.026
		EHI_154230 Zinc finger protein 622	0.90	0.015
		EHI_104460 Nonsense mediated mRNA decay protein 3	0.97	0.437
		EHI_081250 Importin beta-3 family protein	0.95	0.056
Other pre-60S particles	EHI_009860 Hypothetical protein	1.59	0.016	
	EHI_104410 Pumilio family RNA binding protein	1.64	0.042	
	EHI_045120 Cell division cycle protein 48	1.09	0.002	

	EHI_176970 Cdc48 like protein	1.01	0.002
	EHI_006010 Hypothetical protein	0.65	0.011
	EHI_009540 Hypothetical protein	1.37	0.010
	EHI_088070 Ribosomal RNA methyl transferase	0.87	0.085
	EHI_012190 Brix domain containing protein	1.19	0.014
	EHI_118030 WD repeat protein	0.93	0.002
	EHI_014120 RNA recognition motif domain containing protein	0.79	0.053
	EHI_198880 Proliferating cell nucleolar antigen p120	1.12	0.002
	EHI_153780 Hypothetical protein	1.27	0.056
Miscellaneous Proteins	EHI_187180 Ubiquitin-protein ligase	1.54	0.003
	EHI_163510 Exosome complex exonuclease Rrp4	1.36	0.006
	EHI_000580 Exosome complex exonuclease	1.03	0.055
	EHI_160720 Exosome complex exonuclease	0.78	0.043
	EHI_111220 tRNA splicing endonuclease	1.37	0.044
	EHI_053830 La ribonucleoprotein	1.53	0.032
	EHI_199620 Hypothetical protein	2.21	0.002
	EHI_097980 Hypothetical protein	0.86	0.010
	EHI_040360 ruvB-like DNA helicase	0.72	0.008
	EHI_104570 Ubiquitin ligase	1.65	0.046
	EHI_011530 Ubiquitin-protein ligase	0.62	0.164

Table 8: Down regulated genes (>1.5-fold change) in Rrp6KO as compared to TOC

	Gene Name (Sc)	Gene Description	Log ₂ Fold Change	P-value
SnoRNPs	Box H>ACA snoRNPs	EHI_053440 13kDa ribonucleoprotein-associated protein	-0.85	0.070
90S particles	U3 small nucleolar RNA-associated proteins	EHI_035170 U3 snRNP subunit	-0.62	0.050
		EHI_146820 U3 snRNP subunit	-0.62	0.050
		EHI_184000 U3 snRNP subunit	-0.62	0.050
		EHI_012960 Hypothetical protein	-0.68	0.110
		EHI_199160 Hypothetical protein	-1.47	0.037
	Helicases	EHI_184530 Helicase	-2.62	0.098
		EHI_077640 Helicase, ATP-dependent helicase	-0.67	0.155
		EHI_093900 DEAD>DEAH box helicase	-0.95	0.019
	Kinase	EHI_006800 Casein kinase	-0.63	0.060
		EHI_038670 Protein kinase domain containing protein	-1.85	0.170
EHI_152350 Casein kinase II regulatory subunit family protein		-2.25	0.016	
Other 90S particles		EHI_044610 Ribosome biogenesis protein NEP1	-2.19	0.042
		EHI_085680 WD repeat protein	-1.06	0.205
		EHI_083380 Hypothetical protein	-1.28	0.145
Pre-40S particles	Kinase	EHI_049390 Casein kinase	-0.84	0.034
		EHI_170330 RIO1 family protein	-0.62	0.056
	Export and cytoplasmic maturation factor	EHI_001120 Hypothetical protein	-1.70	0.016
		EHI_169830 Hypothetical protein	-0.91	0.116
		EHI_100490 Hypothetical protein	-0.87	0.030
		EHI_062760 Programmed cell death protein 2	-0.87	0.012
Pre-60S particles	Helicases	EHI_052790 DEAD>DEAH box helicase	-0.87	0.159
	Export and cytoplasmic maturation factors	EHI_192440 Heat shock protein 70, hsp70A2	-0.74	0.592
		EHI_102940 60S acidic ribosomal protein PO	-1.20	0.003
Other pre-60S particles		EHI_050420 Hypothetical protein	-1.31	0.014

	EHI_188010 Hypothetical protein	-2.39	0.004
	EHI_038310 60S ribosome subunit biogenesis protein NIP7	-1.23	0.054
Other ribosome biogenesis factors	EHI_118860 Putative methyltransferase NSUN5	-0.62	0.014
	EHI_152400 Hypothetical protein	-0.69	0.083
Miscellaneous Proteins	EHI_020280 40S ribosomal protein S2	-0.81	0.042
	EHI_068580 U6 snRNA-associated Sm-like protein LSm2	-0.69	0.232
	EHI_151310 U6 snRNA-associated Sm-like protein LSm3	-1.02	0.077
	EHI_049370 LSM domain containing protein	-1.18	0.231
	EHI_025840 LSM domain containing protein	-0.63	0.027