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Synthesis of Ribosomes in *Saccharomyces cerevisiae*

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INTRODUCTION

The synthesis of ribosomes in a eucaryotic cell requires the coordinated expression of a large number of steps (Fig. 1). These include: (i) transcription of 5S ribonucleic acid (RNA) by RNA polymerase III; (ii) association of 5S RNA with specific ribosomal proteins and its assembly into the 60S subunit; (iii) transcription of the 35S precursor to ribosomal RNA (rRNA) by RNA polymerase I; (iv) processing of ribosomal precursor RNA by endonucleases, exonucleases, methylases, pseudouridylase, etc.; (v) transcription of genes for 75 or more ribosomal proteins by RNA polymerase II; (vi) processing of these transcripts by capping, splicing, and polyadenylation and subsequent migration of the transcripts to the cytoplasm; (vii) translation of the messenger RNAs (mRNAs) for the ribosomal proteins; (ix) transport of the newly formed ribosomal proteins to the nucleus; (x) assembly of these ribosomal proteins with the ribosomal precursor RNA and participation of the proteins in its processing; and (xi) export of the completed ribosomal subunit to the cytoplasm.

To carry out this complex process efficiently, there must be coordination of synthesis at three levels: among the ribosomal proteins, of the ribosomal proteins with the transcription of rRNA, and of RNA and proteins with the biosynthetic needs of the cell.

With imagination and ingenuity, the study of ribosome synthesis can lead to an understanding of many of the fundamental questions about the synthesis, regulation, and movement of macromolecules within a cell. The purpose of this review is to outline ways in which such fundamental problems have been approached and to point out additional areas that could be ripe for exploitation. It represents an updating of a previous review (183), to which the reader is referred for a summary of earlier studies; preparation of this review was facilitated by a number of recent reviews of the subject (38, 92, 130–132, 188, 184).

I regret that space does not permit inclusion of the synthesis of mitochondrial ribosomes, with their own unique features. Nor is there space to deal, except in passing, with the function of the yeast ribosome. The reader is referred in particular to reference 160 for a discussion of suppression by ribosomes.

rRNA

Structure of the rRNA Genes

The rRNA genes of the yeast *Saccharomyces cerevisiae* are organized in a single tandem array of 9.1-kilobase (kb) repeating units on chromosome XII (127). G. Carle and M. V. Olson (personal communication) have recently shown by size determination on pulsed-field gels that in certain strains there are approximately 220 copies of the ribosomal

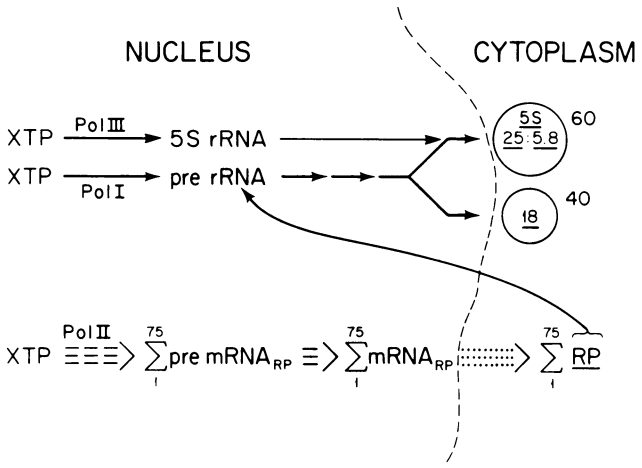


FIG. 1. Schematic representation of ribosome biosynthesis in eucaryotic cells. See text for details. Pol, RNA polymerase; RP, ribosomal protein.

deoxyribonucleic acid (rDNA) repeat, making up about two-thirds of chromosome XII. Other strains, however, have about 100 copies. Most interesting, however, is the substantial clonal variation within a strain. It is not uncommon to find a single colony in which the cells have fewer than 40 copies, without a substantial depression in growth rate.

The repeating unit is arranged as shown in Fig. 2. The arrangement of the rRNAs was established by Philippsen et al. (128). It is unusual in containing the genes both for the large rRNAs and for 5S RNA, which are transcribed in opposite directions. The complete sequence, as well as the endpoints for the mature RNAs, has been determined by the combined efforts of a number of groups (5, 7, 40, 94, 96, 141, 142, 155). These sequence data must be used with caution for any particular cloned sequence, because there are not only restriction sites polymorphisms between the repeats (126) but sometimes substantial deletions or insertions (62). The latter appear to be confined to certain regions of the nontranscribed spacer.

Analysis of the arrangement of the rDNA genes within the chromosome demonstrated that transcription occurs toward the centromere (97). At the telomere-proximal end of the array is a different kind of repeat, of 3.6 kb, that contains a modified 5S gene but none of the larger rRNAs (97). It is not known whether this 5S RNA is ever transcribed.

Replication of rDNA

The replication of yeast DNA initiates at a large number of autonomously replicating sequences (ARS) (reviewed in reference 110) in a region a few hundred nucleotides upstream of the initiation of transcription. In test plasmids, this ARS is weaker than several of the ARSs that are commonly used. During a given replication cycle in the genome, it appears that only 1 in 3 to 1 in 10 of the rDNA ARSs are used (13, 88, 144, 179). Several repeats can be found in a single replication bubble. Transcription continues during replication and appears to block replication proceeding in the opposite direction. Therefore, the replication bubble travels only about 1.5 kb in the upstream direction until it encounters the transcription apparatus (13, 88). Replication can apparently proceed in the direction of transcription until it reaches the next replicon. The transcription apparatus on the 5S gene does not seem to interfere.

Recombination within rDNA

One would expect the repetitive nature of rRNA genes to lead to unequal crossing over during recombination, yielding cells with too few or too many copies. Petes and co-workers (127, 198) showed that meiotic recombination between homologous chromosomes in the rDNA repeat occurs at a very low level compared with that in the bulk of the genome, although sister chromatid exchange occurs at a normal or even elevated rate. This may be due in part to the fact that the nucleolar organizer region does not form a tight synaptonemal complex (79). Mitotic recombination within the rDNA repeat appears also to be substantially lower than normal (163). Christman et al. (19) have recently shown that the suppression of mitotic recombination requires both topoisomerases I and II, which suggests that there is some interaction between the supercoiling of the rDNA and its susceptibility to recombination. Furthermore, Gottlieb and Esposito (43) have found that *SIR2*, identified as a part of the mating-type silencer apparatus, is necessary for suppression of both meiotic and mitotic recombination in rDNA. The groups of R. Esposito and of R. Keil have identified a number of genes that seem to be necessary for suppression of recombination in the rDNA, which suggests that the situation is more complex than originally imagined. Analysis of the special features used by the cell to suppress recombination specifically within the rDNA may shed light on the process of recombination itself.

In light of the observed low level of recombination in rDNA, it is surprising that Roeder and co-workers (67, 176)

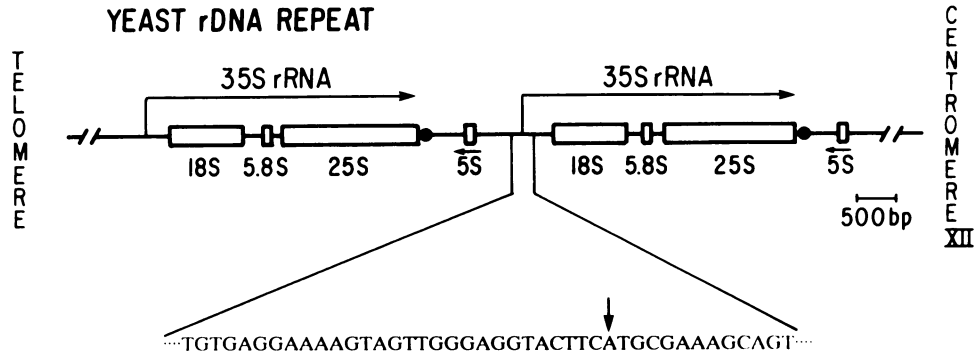


FIG. 2. rRNA genes of *S. cerevisiae*. Two repeats are shown. The regions corresponding to mature rRNA and to the transcripts are indicated; ●, location of the enhancer. Below is shown the sequence around the transcription initiation site (↓).

have identified a region of rDNA that promotes mitotic recombination when inserted in the vicinity of a test gene. This region, denoted *HOT1*, contains both the transcriptional enhancer (32) and the promoter region of the rDNA repeat. An rRNA terminator inserted between *HOT1* and the test gene blocks its action. Stimulation of recombination activity requires both the enhancer and the terminator, which suggests that transcription or something associated with transcription stimulates the recombination.

Transcription of the Large RNAs

The transcription of eucaryotic rRNA genes has recently been reviewed (156). In yeast cells, the major portion of the rDNA repeat consists of a single transcription unit leading to the synthesis of a 35S rRNA precursor that is processed to form the 18S RNA found in the 40S ribosomal subunit and the 25S and 5.8S RNAs found in the 60S subunit (Fig. 2). Despite considerable effort, there is still little understanding of the details of the transcription of this molecule, although it makes up more than 60% of the total transcription in a rapidly growing yeast cell. The initiation site (the origin in the numbering systems used below) has been identified because it can be found bearing a triphosphate terminus (5, 74, 75, 112) (Fig. 2). There is substantial homology at the initiation sites of several fungal rRNA genes (174).

A recent interesting observation is that inactivation of both topoisomerases I and II, but not either separately, leads to relatively specific inhibition of rRNA transcription in *S. cerevisiae* (14). This suggests that DNA conformation must play some, as yet unclear, role in rRNA transcription. A similar result was obtained in *Schizosaccharomyces pombe* (194).

Efforts to determine the sequences involved in the initiation of transcription have been carried out both in vitro and in vivo. By using a partially purified extract with truncated fragments of rDNA as a template, it has not been possible to obtain transcription by RNA polymerase I at nucleotide 1. Rather, transcription initiates at a site more than 2 kb upstream (161, 162) that has no apparent homology to the sequence shown in Fig. 2. Although this result suggested that initiation might occur at this site in vivo, there is no evidence to support substantial transcription of the rDNA spacer region. Furthermore, Lohr (89) has found that in the region of nucleotide 1 there is an abrupt change in the chromatin structure of rDNA, again suggesting that the transcription truly initiates at that site. While correct initiation of transcription of rDNA has been observed in nuclear extracts (69, 76, 90), these have not proved efficient or malleable enough to provide detailed information on just which adjacent sequences are essential for initiation of transcription.

As an alternative approach to determining the sequences essential for transcription, several groups have constructed minigenes carrying the initiation and termination sites of rRNA flanking a reporter sequence (31, 70, 134). Spacer sequences from upstream or downstream can then be added to test their effects on transcription. Such minigenes are transcribed effectively in vivo whether they are inserted within the rDNA repeat, within a normal gene on another chromosome, or on a circular plasmid. Thus, RNA polymerase I is not confined to a single location within the nucleolus but can find an rRNA gene wherever it is. Karpen et al. (65) have recently shown that in *Drosophila melanogaster* a full-sized transplaced rDNA gene is functional and forms an apparently complete mininucleolus.

A low level of transcription is found when the test gene carries 192 nucleotides (70) of upstream sequences but not when it carries only 105 nucleotides (68). When the test gene carries 212 nucleotides of upstream sequences, a substantial fraction of the transcripts initiate 10 to 30 nucleotides upstream of the normal site (31). The presence of 2,000 nucleotides of upstream sequences leads to a small increase in the level of transcripts, all of which initiate correctly. An additional 190 nucleotides of upstream sequences leads to a marked increase of transcription (31). It has become apparent that this 190-base-pair fragment, or portions thereof, acts as an enhancer element specific for RNA polymerase I, stimulating transcription by 10- to 50-fold. It is effective not only when it is in its normal position, 2 kb upstream of the initiation site, but also when placed only 200 nucleotides upstream, when inverted, and even when placed downstream of the gene (31, 32). More recent results (S. P. Johnson and J. R. Warner, submitted for publication) have shown that this enhancer is effective at a distance of as much as 6 kb from an initiation site even when downstream. This is the only enhancer in yeast cells known to work from a downstream position. Furthermore, it can work through (or around) another rDNA transcription unit.

Termination and Initiation in Tandem Genes

rRNA transcription differs from that of other genes in two major respects. First, it utilizes a specialized RNA polymerase and transcription factors. At least one of the factors is relatively species specific, which suggests that it can evolve far more rapidly than most of the participants in transcription (156). Second, the rRNA genes are tandemly arrayed, leading to the possibility that transcription of one gene is not independent of the transcription of adjacent genes (reviewed in reference 4). This has been evident from studies of *Xenopus laevis* (23, 80) and *Drosophila* sp. (107), in which under some conditions much of the spacer region is transcribed. Sequences that act as strong terminators occur a few hundred nucleotides upstream of the authentic initiation site. In *S. cerevisiae*, there does not seem to be transcription of the entire spacer region, possibly because of the presence of the 5S RNA gene (Fig. 2).

The terminus of the 35S rRNA transcription product is only 90 nucleotides upstream of the next enhancer element (69) (Fig. 2). This end is a processing site for an enzyme encoded by the *RNA82* gene. Mutations in this gene lead to RNA molecules extending into and perhaps beyond the enhancer element (69). In vitro processing at this site has recently been demonstrated (196). Furthermore, run-on transcription experiments show that transcription can continue into the enhancer region (S. P. Johnson and J. R. Warner, unpublished data). There may in fact be several termination sites, one or more within the enhancer fragment and others between it and the 5S RNA gene (69). The transcriptional barrier to rDNA replication is at $+300 \pm 80$ nucleotides from the end of 35S RNA (13), which is likely to be an upper limit for most transcriptional events. On the other hand, Saffer and Miller's micrographs appear to show occasional polymerases within the spacer region (144). As they have no RNA tails, one cannot tell whether these polymerases are simply sliding, are transcribing a very unstable RNA, or are an artifact of the preparation. Finally, there is also a strong termination site just upstream of the promoter (R. J. Planta, personal communication) that serves perhaps to derail any runaway RNA polymerase molecules.

Although neither the precise site nor the mechanism of termination of transcription is clear, it is intriguing that

transcription continues into the enhancer. It seems likely that some sort of loop is formed to bring the enhancer-terminator together with either an upstream or a downstream promoter (69). However, the results with tandemly repeated test genes suggest that the enhancer can stimulate transcription of a downstream gene, through a proximal gene, indicating that it need not be transcribed itself (Johnson and Warner, submitted). These results suggest that looping must be considered in three dimensions, where many enhancers and many promoters may come together to form an array (on a nuclear matrix?) from which extend loops of transcribed rDNA and of the spacer, with its 5S RNA gene. It has not yet been possible to design experiments to test such a model. It is likely, however, that one or several proteins are involved. At least two and perhaps three candidates for such proteins have been identified (B. E. Morrow, S. P. Johnson, and J. R. Warner, J. Biol. Chem., in press). One, termed REB1, binds to a sequence within the enhancer that has been implicated in termination or processing. Interestingly, REB1 also binds to an almost identical sequence, with the opposite orientation, just 210 nucleotides upstream of the initiation of transcription.

Transcription of 5S RNA

The transcription of 5S RNA is carried out by RNA polymerase III (reviewed in reference 39). In yeast cells, as in other cells, this transcription is dependent on the prior association of the factors TFIIIA, TFIIIB, and TFIIIC with the 5S RNA gene (149). During oogenesis in *X. laevis*, the transcription of 5S RNA is regulated by the presence of transcription factor TFIIIA, which binds either to the 5S RNA gene or to the 5S RNA itself, leading to the proposal that there can be feedback regulation of 5S RNA synthesis (39). Brow and Geidushek (15) have demonstrated that such feedback can occur in an in vitro system derived from *S. cerevisiae*. 5S RNA inhibits its own synthesis, presumably by binding to TFIIIA, but the inhibition is suppressed by the presence of YL3, the ribosomal protein that is complexed to 5S RNA.

5S RNA is used in equimolar amounts with the other rRNAs; early evidence suggested that there is roughly equimolar synthesis of 5S RNA and the 5.8S RNA derived from the 35S transcript (168). Because the 5S gene not only is in the rDNA repeat but also is located between the enhancer element and the initiation of transcription of 35S RNA, it seemed possible that its synthesis would also be affected by the enhancer. However, a marked 5S gene introduced into the constructs designed to test enhancer function fails to respond to the enhancer (L. Neigeborn and J. R. Warner, unpublished data). Similarly, a strain carrying a temperature-sensitive RNA polymerase III, defective in 5S RNA transcription at the restrictive temperature, continues nevertheless to synthesize 35S RNA for many hours (46). Thus, although there is balance between the syntheses of 35S RNA and 5S RNA, there does not appear to be strong coupling between them.

RIBOSOMAL PROTEINS

Yeast Ribosomal Proteins

There are approximately 75 proteins in a yeast cytoplasmic ribosome. These have been studied mostly by two-dimensional polyacrylamide gel techniques (11, 100, 118, 187), although high-performance liquid chromatography is currently the method of choice for the purification of individual proteins (165). Unfortunately, both for the reader and for the workers in the field, there are three systems of nomenclature for the ribosomal proteins, which have been only partly correlated (119, 131, 183). When possible, the nomenclature based on the Kaltschmidt-Wittman gel system will be used; the most recent comparison of the systems is in reference 131. The molecular weights of the proteins are generally less than 35,000 (Table 1), with some being among the smallest proteins in yeasts, although two proteins of the 60S subunit are larger. With a few exceptions, the proteins range from basic to very basic. As determined by two-dimensional electrophoresis, roughly half of the ribosomal proteins are more basic than the core histones (187). Although the overall electrophoretic pattern suggests a 1:1 homology between species for most of the ribosomal proteins, there are simply too many proteins to make such a correlation by visual inspection. As the cloning and sequencing of ribosomal protein genes from a variety of organisms has progressed, however, it has been gratifying to see confirmation of the evolutionary conservation of ribosomal proteins (Table 1). For example, the phosphorylated ribosomal protein S10 of *S. cerevisiae* is 62% homologous to the mammalian S6 (85); yeast L2 is 58% homologous to *Xenopus* L1 (133); yeast L32 is 60% homologous to mouse L30 (21); and yeast Rp59 (81), mutations in which can provide resistance to cryptopleurine, is 81% homologous to mammalian S14, mutations in which can provide resistance to emetine (136). Furthermore, mouse protein L27′ can substitute for ribosomal protein L29 in *S. cerevisiae* (34). Indeed, of the 28 yeast ribosomal proteins fully sequenced, close homologs in other species have been identified for all but four (Table 1).

The acidic ribosomal proteins make up an interesting set, which has been confusing because of the similarity of the members of this gene family. Now that clones are available, it is clear that in *S. cerevisiae* there are at least four very similar genes, known as L44′ (135), L45 (135; previously sequenced as protein YPA1 [58]), A1 (103), and A2 (104). These proteins are from 106 to 110 amino acids long, are very acidic, and carry identical C termini for at least 13 amino acids. Another acidic protein, A0, is 312 amino acids in length but has the same C terminus (102). A similar set of proteins has been observed in human cells, where they are antigenic in certain autoimmune patients (137). The smaller proteins are thought to be the eucaryotic analogs of L7/L12 of *Escherichia coli*. A0 has no known function. Both large and small acidic proteins are phosphorylated (199), and they exchange on and off the ribosome (199).

Ribosomal Protein Genes

During the past few years, the genes for a large number of ribosomal proteins of *S. cerevisiae* have been cloned (10, 35–37, 191) and, in most cases, sequenced. The information available concerning ribosomal protein genes is summarized in Table 1.

Unlike the situation in *E. coli* (114), few of the ribosomal protein genes are closely linked. The exceptions are two copies of the head-to-tail pair RP28 and S16A (105) and the pairs S24 and L46 (83) and RP29 and L32 (21, 101), which are transcribed divergently.

Many but not all of the ribosomal protein genes in yeast cells contain a single intron (138), usually near the initiation codon, and in at least two cases upstream of it (101, 111). This feature is unusual because very few nonribosomal protein genes in yeast cells contain introns. In no case yet

TABLE 1. Characteristics of ribosomal proteins[a]

Ribosomal protein	Size (a.a.)	Copy no.	UASrpg	Intron	Homologies	Reference
L2 YL2	362	2	1	0	L1(X, D)	133
L3 (*TCM1*) YL1	387	1	0	0	L1(T, A)	147
L16 RP39 YL22	174	2	2	0	L5(E); L21(T)	164
L17a YL32	137	2	1	1	?	82
L25 YL25	137	1	2	1	L23(E)[b]	192
L29 YL24 (*CYH2*)	148	1	2	1	L29(N); L27a(M)	66
L32 RP73 YL38	105	1	2	1	L30(M, R)	21
L34 YL36	112	2	2	1	L31(R)	145
L41 YL27	103	?	?	?	L36a(H, R)	60
L43 YL35	88	?	?	?	L37(R)	115
L46 YL40	51	2	1	1	L39(R); L36(Sch)	83
L? (*UBI1*, *UBI2*)	52	2	2	1	—[c]	33a, 120
RP28	185	2	2	1	L18(R); L14(X)	105
RP29	155	2	2	1	?	101
AO	312	1	?	?	PO(H)	102
A1	106	1	?	?	—[d]	103
L44 (A2)	106	1	2	0	—[d]	104, 135
L44′	106	1	2	1	—[d]	135
L45	110	1	2	0	—[d]	135
S10 YS10 RP9	236	2	2	1	S6(H, M, R, Sch)	85
S16A YS16	143	2	2	1	S12(Hal)	105
S24 YS22	129	2	2	0	S16(Hal)	83
S26 YS25	87	?	?	?	S21(R); S28(Sch)	59
S31 YS23	108	2	2	1	?	111
S33 YL27	67	2	0	0	?	86
S37 YS24 (*UBI3*)	76	1	1	1	S27a(R)[c]	33a, 116, 120
RP51	136	2	2	1	S17(H, R, Ha, Ch)	164
RP59 *CRY1*	137	2	1	1	S14(H, Ha, D)	81

[a] This compilation includes for the most part ribosomal proteins whose genes have been cloned and sequenced. In the case of L41, L43, and S26, only the protein sequence is known. The ribosomal proteins that have been sequenced are named as in ref. 131. The alternate names with the prefix Y are from reference 119; those with the prefix RP are from reference 183; the relevant genes are also given. The size is that predicted from the open reading frame identified in the gene. The homologies have in some cases been identified in the original papers. For this compilation, I have used a collection of ribosomal protein sequences assembled by Tatsuo Tanaka, Department of Biochemistry, Yamagata University School of Medicine, Yamagata, 990-23, Japan, to whom I am very grateful. His collection also includes a number of N-terminal sequences determined from purified proteins by Otaka and co-workers (115–117). Dr. Tanaka has agreed to maintain an up-to-date collection of ribosomal protein sequences that he will make available on request. He asks that new sequences be sent to him. Abbreviations: A, *Arabidopsis thaliana*; Ch, chicken; D, *Drosophila melanogaster*; E, *E. coli*; H, *Homo*; Ha, *Cricetulus* (hamster); Hal, *Halobacterium morismortui*; M, *Mus*; R, *Rattus*; Sch, *Schizosaccharomyces pombe*; T, *Tetrahymena thermophila*; X, *Xenopus laevis*.

[b] Not unequivocal from sequence comparison but clear from RNA-binding studies (28).

[c] The tails on the ubiquitin genes are highly homologous across a large number of organisms. These two ribosomal proteins now serve as the most dramatic examples of the evolutionary conservation of ribosomal proteins.

[d] There is clear homology of the acidic proteins of *S. cerevisiae* to those of all other organisms that have been identified (see text).

reported does a ribosomal protein gene contain more than one intron.

Many but not all of the ribosomal protein genes are duplicated (36). In those cases where this characteristic has been examined, both copies are functional, although usually not at the same level (1, 85, 139; see below). No pseudogenes have been detected. This is in contrast to the situation in mammalian cells, where for each protein so far examined there is only a single functional gene in a sea of pseudogenes (26). The two copies have presumably been formed by gene duplication; this is particularly evident in the case of *RP28* and *RPS16A*, which are linked head to tail in an identical arrangement on chromosomes XIV and XV (122). For another pair of genes linked head to head, *RP29* and *RPL32*, only *RP29* has been duplicated (101). The open reading frames of the duplicate genes are highly conserved; the proteins differ by no more than a few conservative replacements. Sequences within the introns, however, and especially in the 5′ and 3′ leaders, have diverged substantially. (See references in Table 1).

Remarkably, genes for two of the ribosomal proteins, one of which is S37, are found fused behind functional ubiquitin moieties (33a, 134a). The ubiquitin cleaved from the fusion protein after translation is the main source of the cell's ubiquitin during log-phase growth. This arrangement appears to be common to a wide variety of organisms, from the genus *Saccharomyces* to *Trypanosoma* to *Homo* (146).

Ribosomal proteins are abundant, each representing 0.1 to 0.5% of the total cellular protein, depending on size. Their mRNAs are correspondingly abundant. The codon bias of the translated parts of the ribosomal protein mRNAs matches that found for other abundant proteins (9).

Transcription of Ribosomal Protein Genes

The concentration of mRNA for several of the ribosomal proteins is approximately equimolar because of equal levels of transcription and similar half-lives (72). Transcription of the ribosomal protein genes appears to be coordinately regulated. During starvation for an amino acid, the inhibition

of transcription of rRNA (153) is accompanied by a decrease in the amount of translatable mRNA for many if not all ribosomal proteins (185). Addition of glucose to a culture growing on ethanol leads to a rapid increase in transcription of both rRNA (71) and mRNA for ribosomal protein (25). Mild temperature shock leads to an inhibition of transcription of ribosomal protein genes (73). These results led to a search of the ribosomal protein genes for common upstream sequences that might be responsible for the regulation of transcription. Several potential sequences were identified from the first few genes examined (164). By detailed deletion and linker-scanning analysis, Rotenberg and Woolford demonstrated that three sequence elements are involved in transcription (140). One is a T-rich region that is commonly found upstream of many active yeast genes, acting as a general promoter region (159). The other two sequences, designated HOMOL1 (164) and RPG (84), had been observed upstream of many ribosomal protein genes.

Two lines of research have come together to demonstrate the relationship and importance of these two sequences. On the one hand, deletion experiments with the genes for ribosomal proteins L29 (148), L25 (193), and RP59 (140) have shown that both HOMOL1 and RPG sequences can contribute to the promoter strength of each gene. On the other hand, Huet et al. (55) have identified a protein, dubbed TUF because it was originally found to bind upstream of the genes for elongation factor 1, that binds to both HOMOL1 and RPG. From competition experiments (175), it is now clear that the two represent a common, but rather degenerate, upstream activating sequence designated UAS_{rpg} (reviewed in 92): $^{A}_{T}ACA^{T}_{C}CC^{A}_{G}T^{A}_{G}CAT^{TT}_{CA}$. The locations of UAS_{rpg}, upstream of a number of ribosomal protein genes, are summarized in references 175 and 140. The sequence ACACCCATACAT appears to promote the strongest binding (175), but TUF will generally protect from nuclease at least a 14-nucleotide stretch with substantial deviations from the consensus (55).

Many but not all ribosomal protein genes have two adjacent copies of UAS_{rpg} (131; Table 1), which act more or less additively. Consistent with this is the observation that there is no cooperativity of the binding of TUF to adjacent sites (175).

At least two ribosomal protein genes, *RPS33* (86) and *TCM1* (48), have no UAS_{rpg}. Detailed analysis of *TCM1*, the gene for ribosomal protein L3, has identified a different sequence, UAS_T, which is essential for transcription of this gene (48). UAS_T binds a protein termed TAF, which is likely to be a transcription factor, since mutations in UAS_T that decrease binding also decrease transcription (48). UAS_{rpg} is distinct from UAS_T; TUF is distinct from TAF. These results suggest that coordinate regulation of the transcription of ribosomal protein genes is not due to the modification of a single transcription factor.

The TUF protein appears to have widespread use, far beyond the ribosomal protein genes. There is a TUF binding site upstream of the gene for a common subunit of RNA polymerases I and III (95). Buchman et al. (16, 17) have found a protein that binds to a sequence essentially identical to UAS_{rpg} upstream of several genes, including the carbon-metabolizing genes *ADH1*, *ENO1*, and *PYK1*, the protein kinase regulatory subunit *BCY1*, and the phosphate-metabolizing gene *PHO5*. They term this protein general regulatory factor 1 (GRF1). GRF1 also binds to sequences in the telomeric region. Shore et al. (151) have identified a protein, which they term RAP1, that binds to a similar sequence in the region of the genome responsible for silencing the silent copies of the mating-type genes. They have cloned and sequenced the gene for RAP1 (150). It seems clear that TUF, RAP1, and GRF1 are the same protein, which binds to many sequences in the yeast genome, regulating transcription, and perhaps other events, as a function of neighboring sequences. The sensitivity of the transcription of ribosomal protein genes to the carbon source (see below) may reflect a generalized response of the cell, mediated through this factor. It is particularly interesting that this protein acts in a positive fashion on the ribosomal protein genes and in a negative fashion on the silent mating-type genes. Presumably, it acts through a second factor in each case. One can speculate that in the evolution of the silencing apparatus, the cell adopted a ubiquitous and constitutive DNA-binding protein for a new purpose.

REGULATION OF RIBOSOME SYNTHESIS

Duplicate Copies of Ribosomal Protein Genes

The ribosomal proteins are needed in equimolar amounts. There is equimolar synthesis of mRNA for ribosomal proteins (72). Why does *S. cerevisiae* have two copies of many of the ribosomal protein genes? Does each make a full complement of mRNA? Is each active only under certain conditions? Do they function to make ribosomes of different specificity? The answer to each of these questions seems to be no.

Abovich et al. disrupted individual copies of the *RP51* gene and found that mutants carrying either functional gene grew but at a slower rate than did the parental strain (1). Spores without an intact *RP51* gene do not germinate. Probing with oligonucleotides specific for each gene revealed that *RP51A* mRNA accounts for 60 to 70% of the total and that deletion of *RP51A* leads to a loss of 60 to 70% of the total mRNA for RP51. In that case, the growth rate decreases about 35%. The growth rate can be restored to normal by additional copies of the *RP51B* gene. These results indicate that the protein encoded by either *RP51A* or *RP51B* can form a fully functional ribosome and that neither copy of the gene is essential. They also show that there is no increase in the transcription of one gene to compensate for the loss of the other, even if that loss leads to a severe deficiency for the encoded protein. Presumably, there has been evolution of the promoter strength of the two copies so that their sum is equivalent to the promoter strength of the ribosomal protein genes present in a single copy.

Similar results have been found for a number of other pairs of genes: *RPL16* (139) and *RP28* and *RPS16A* (N. J. Pearson, M. P. Remington, D. A. Stewart, J. C. Crouse, T. J. McQuade, M. P. Neeper, and D. M. Donovan, in press). There is not necessarily a linear relationship between the proportion of mRNA available and the growth rate. In the case of L16 (139), cells with two-thirds the normal amount of L16 mRNA grow normally; cells with only one-third grow significantly more slowly. It is difficult to know, however, what factor is limiting growth of a cell under any particular set of conditions.

One interesting result of the deficiency for a single ribosomal protein is the imbalance that develops between the concentrations of the 60S and 40S subunits. L16 is a protein of the 60S subunit; cells in which one of its genes, *RPL16A*, is disrupted accumulate large amounts of free 40S subunits (139). The lack of 60S subunits also leads to the appearance on polysomes of half-mers, where initiating 40S complexes queue up, waiting for 60S partners.

The duplication of most of the ribosomal protein genes seems not to result from the need for large amounts of the protein, since many of the proteins of the cell are made in even larger amounts from a single-copy gene, nor does it seem to be a way of making ribosomes with different functional properties, since each of the pair of genes can provide normal growth if present in enough copies. Only a few growth conditions have been examined, however. Perhaps the duplication is a safety measure to ensure the production of these essential proteins. Whereas a number of the essential housekeeping proteins, such as histones, ubiquitin, and tubulin, share the property of having duplicated genes, many do not.

Mild Heat Shock

In the course of studying temperature-sensitive mutants, it became apparent that shifting wild-type cells from 23 to 36°C led to a substantial decrease in the synthesis of ribosomal proteins, although total protein synthesis actually increased (41, 190). There is continued synthesis of rRNA, but its processing is slowed, presumably because of the deficiency of new ribosomal protein (41). The effect appears to be due to a temperature change itself, since a similar effect, though smaller in magnitude, is observed after shifts from 23 to 30°C or from 30 to 36°C.

The decrease in ribosomal protein synthesis is due to a decrease in concentration of mRNA for ribosomal proteins. The rate of transcription of ribosomal protein genes is substantially, but temporarily, decreased after a temperature shift (73). Although one might tend to implicate the factor binding to the RPG box, attempts to identify the *cis*-acting sequences responsible for the decrease have not been successful (49, 140). Recent results suggest that an increased rate of turnover of the mRNAs for ribosomal proteins may also occur after heat shock (49), although that is not the case for most mRNAs (87).

Carbon Source Upshift

In the classic studies of *E. coli*, the content of ribosomes was found to be proportional to the growth rate (91). The same is true in *S. cerevisiae*, although perhaps to a lesser extent (177, 178). Cells utilizing ethanol in minimal medium grow at one-third the rate and have less than 40% the ribosome content of cells growing on glucose (71). When glucose is added to such a culture, within 30 min there is a 2.5- to 4-fold increase both in the transcription of rRNA and in the synthesis of ribosomal proteins (71). This is due to an increase in the transcription of the mRNA for ribosomal proteins (25). Sequences upstream of the genes, in particular the UAS_{rpg}, mediate the response to glucose addition (50). The factors affecting the transcription of rRNA are less clearly defined. The enhancer element appears to be involved; it is substantially more effective in cells growing on glucose than in cells growing on ethanol (Q. Ju and J. R. Warner, unpublished results).

Parikh et al. (123) have recently found that in [*rho*^{0}] cells, there is substantial transcription of much of the rDNA spacer, initiating a few hundred nucleotides downstream of the enhancer and proceeding through the 5S gene (Fig. 2). This transcription is repressed by glucose. The significance of these observations remains to be seen, but the authors speculate that the transcription may reflect a regulatory interaction of the nuclear and mitochondrial genomes.

Amino Acid or Nitrogen Deprivation

S. cerevisiae responds to the deprivation of amino acids by specifically shutting down the transcription of rRNA (153). The overall synthesis of transfer (tRNA) and mRNA is relatively unaffected, although a substantial depletion of mRNA for ribosomal proteins soon becomes apparent (186). The parallels with the stringent response of *E. coli* (114) have led to numerous searches for the nucleotide ppGpp or similar compounds, as yet unsuccessful except for the detection of a small amount, probably of mitochondrial origin (121). One relaxed mutant of a yeast strain, apparently due to mutation in a single gene (157), has been reported (180). Unfortunately, because of technical difficulties with the strain and its genetics, this mutant has not yet revealed much about the mechanism of the regulation of ribosome synthesis during amino acid deprivation.

The transcription of rRNA does not decline substantially in response to the inhibition specifically of the synthesis of ribosomal proteins (152). Therefore, the effect of amino acid deprivation must involve some deeper level of regulation. Indeed, the synthesis of ribosomes is in some way very responsive to the environmental conditions in which the cell finds itself. This is manifest not only in the variation of ribosome content with growth rate (177, 178) but also in the slowing of ribosome synthesis relatively early in the growth cycle. The transcription of rRNA has almost ceased by the time the culture has reached half its maximum density. Indeed, there is a net loss of ribosomes during the last stages of the growth cycle (Ju and Warner, unpublished data). It will be interesting to learn whether the complex system involving protein kinases that regulates cell growth and the cell cycle (18) is central to the regulation of ribosome biosynthesis as well.

The synthesis of ribosomes is substantially repressed in cells deprived of nitrogen; on readdition of a nitrogen supply, ribosome synthesis rapidly recovers (125). The regulation of ribosomal protein synthesis under these conditions occurs primarily through the transcription of the ribosomal protein genes (77).

Extra Copies of Ribosomal Protein Genes

The characteristic of ribosome synthesis that has been most intriguing is the balance that must be maintained in the production of all components of a ribosome. In an effort to understand the basis of that balance, a number of groups have introduced extra copies of the gene for a ribosomal protein into the cell (30, 51, 124, 167, 184). The overall result is clear. There is little if any regulation of the transcription of an individual ribosomal protein gene, no matter how many copies are present in the cell. The regulation of transcription as described in the sections above is designed for the ribosomal protein genes collectively, not for individual genes. Most of the compensation for the extra copies of a ribosomal protein gene takes the form of rapid degradation of the excess protein that is synthesized. The overexpression of one gene does not affect the synthesis of other ribosomal proteins, in contrast to the situation in *E. coli*, in which whole operons can be controlled by overexpression of a single protein (114).

However, other features of the control of ribosomal proteins are also revealed in experiments using multiple copies of a ribosomal protein gene. In some cases, the copy number of the plasmid carrying the ribosomal protein gene is far smaller than that of the vector alone (124), suggesting a

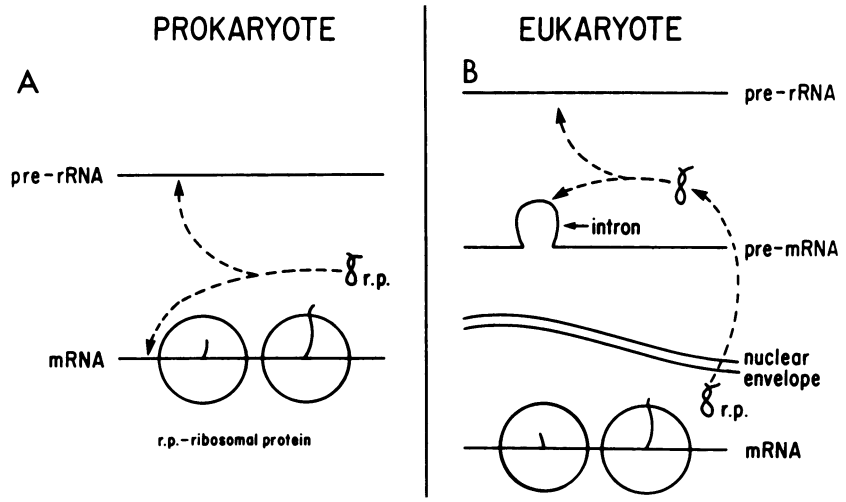


FIG. 3. Autogenous regulation of the translation of ribosomal proteins in *E. coli* (114) (A) and of splicing of the transcript of *RPL32* in *S. cerevisiae* (20) (B). Reprinted from reference 189 with permission.

selection against excess copies of the ribosomal protein gene. In some cases, the amount of mRNA is less than expected from the number of copies of the gene (30, 124). For example, in a cell carrying five extra copies of *TCM1*, encoding ribosomal protein L3, the rate of transcription of the mRNA for L3 was increased about 5-fold but the concentration of the mRNA was increased only about 2.5-fold, suggesting that there is more rapid turnover of this mRNA (124). Initial experiments suggested that there was some regulation at the level of translation of the mRNAs for L3, L29 (124, 184), and RP59 (51). However, reexamination of the question by using pulse-labels of less than 1 min and more detailed analysis of the distribution of the mRNA on polyribosomes (30, 93, 167) led to the conclusion that there is little if any regulation at the level of translation. The earlier results had underestimated the rate of ribosomal protein turnover (see below). Independent evidence that there is no regulation of the translation either of L3 or of L29 comes from the experiments of Nam and Fried (109), who put the gene for L3 or L29 under the control of the *GAL1-GAL10* UAS. The cells grow well in galactose but cannot grow in the presence of glucose, which represses transcription that is dependent on the UAS_{gal}. By comparing carefully the concentration of mRNA and the rate of translation of those two proteins after the addition of glucose, the authors concluded that there was no compensatory increase in the efficiency of translation as the level of mRNA became limiting. There is no current well-documented case for the regulation of yeast ribosomal protein synthesis at the level of translation. However, such regulation would be difficult to detect because of the extremely short lifetime of any ribosomal protein not assembled into a ribosome.

Many other organisms do regulate the synthesis of their ribosomal proteins at the level of translation, usually at the initiation step (reviewed in reference 61). In *E. coli*, the balance of proteins is maintained by the autogenous regulation of translation whereby one protein of an operon can bind to the 5′ leader of a mRNA to prevent its translation (114) (Fig. 3). In mammalian cells deprived of growth factors, only a small proportion of the mRNA for ribosomal proteins is being translated. The rest is in some form of ribonucleoprotein in the cytoplasm. On addition of growth factors, this latent mRNA is activated and translated effectively (reviewed in reference 98). Addition of glucocorticoids to growing lymphosarcoma cells selectively inhibits the translation of ribosomal proteins (99). In *Dictyostelium discoideum*, when cells enter the differentiation cycle, the mRNAs for ribosomal proteins are rapidly removed from polyribosomes (158). For several hours, however, they remain available for use should the cells be refed. During the maturation of *Xenopus* eggs, mRNA for ribosomal proteins is translationally inactivated (57). In none of these cases is the mechanism of translational regulation known. In none is there differential regulation of one or a few ribosomal proteins. The contrast between yeasts and other organisms in the lack of regulation at the level of translation is reminiscent of the situation after heat shock, where most organisms actively turn off translation of existing mRNA but *S. cerevisiae* merely turns off transcription (87). The rapid turnover of mRNA in yeast cells soon takes care of the problem.

Splicing

On the introduction into yeast cells of multicopy plasmids carrying either *RPL29* or *RPL32*, there is a substantial accumulation of unspliced transcript of the gene (189). We suggested that this accumulation might represent an autogenous regulation of splicing, as a eucaryotic analog of the autogenous regulation of translation observed in procaryotes (Fig. 3). Further analysis demonstrated that while the accumulation of unspliced transcript of *RPL29* is due simply to inefficient splicing of the transcript, the accumulation of unspliced transcript of *RPL32* is dependent on overproduction of L32 itself (20). Confirmation of the hypothesis is provided by the observation that depletion of RNA polymerase I, with the concomitant inhibition of rRNA synthesis, leads to accumulation of unspliced transcripts of *RPL32* (M. Wittekind and M. Nomura, personal communication). Recent results suggest that the regulation is effected by sequences near the 5′ splice site (F. Eng, S. P. Johnson, and J. R. Warner, submitted for publication). The splicing of the mRNA for ribosomal protein L1 of *X. laevis* is similarly regulated (12).

This is an intriguing method of regulation that also appears to be used in *Drosophila* cells, particularly in several genes in the sex determination pathway (8). The widespread use of such regulation for the ribosomal proteins of *S. cerevisiae*

could explain the atypical presence of introns in the ribosomal protein genes. However, *RPL32* is the only one of about six intron-containing ribosomal protein genes so far tested that shows this type of regulation. Perhaps it was more common in the recent evolutionary history of *S. cerevisiae* and at that time provided selection for the presence of an intron.

Turnover of Ribosomal Proteins

It now seems clear that although the overall balance of ribosomal proteins is established by the transcription of similar amounts of mRNA of similar half-lives, the ultimate equimolarity of the ribosomal proteins is maintained by an efficient garbage service, which scavenges and destroys any that are unassembled into ribosomes. The intriguing feature is the speed and efficiency with which this is carried out.

In yeast cells, the half-life of unused ribosomal proteins is 2 to 3 min or even less (30, 93, 167, 189). In mammalian cells, unused ribosomal proteins turn over with a half life of 30 to 40 min (181). It seems likely that the cell has developed such a system to avoid accumulation in the nucleus of proteins with a high affinity for RNA. The mechanism by which the turnover of ribosomal proteins takes place is unclear. It is not carried out by the vacuolar proteases (167; J. R. Warner and E. Jones, unpublished data). Could the cotranslation of ubiquitin with two of the ribosomal proteins (33a, 120) be a hint? In any case, it is remarkable that the cell requires only a few seconds to decide that a protein synthesized in the cytoplasm is superfluous to participate in a process that takes place in the nucleolus. Although one might suggest that there is some aspect of the transport of ribosomal proteins to the nucleus which detects the relative numbers of the different proteins, our understanding of that transport, although limited, argues against such a notion (see below).

ASSEMBLY OF YEAST RIBOSOMES

The assembly of ribosomes involves a number of steps: import of the ribosomal proteins into the nucleus; assembly of proteins onto the RNA, probably in several stages; processing of the rRNA, which depends on the presence of the ribosomal proteins; and finally, transport of completed ribosomal subunits to the cytoplasm.

Nuclear Localization of Ribosomal Proteins

Ribosomal proteins, like all other proteins of eucaryotic cells, are synthesized in the cytoplasm. To be assembled onto rRNA in the formation of a ribosome, they must enter the nucleolus by way of the nucleus. There have been suggestions that the ribosomal proteins, since they are relatively small, need no nuclear localization signal, reaching their destination by passive diffusion through the nuclear pores. This seems unlikely, both for esthetic reasons and because in mammalian cells the ribosomal proteins concentrate 50-fold in the nucleolus within 3 to 5 min after their synthesis (182).

Studies of DNA-binding proteins, e.g., simian virus 40 large T antigen, suggest that the localization of proteins to the nucleus is due to short sequences rich in basic amino acids (reviewed in reference 24). Analysis of the nuclear localization signals within yeast ribosomal proteins has only begun. The first 21 amino acids of L3 are sufficient to cause an L3–β-galactosidase fusion protein to accumulate in the nucleus (106). Protein L29 contains two sequences, each of which is capable of carrying a fusion protein into the nucleus (M. R. Underwood and H. M. Fried, personal communication). The two sequences are amino acids 7 to 13 (KTRKHRG), upstream of the intron, and amino acids 22 to 30 (IGKHRKHPG), downstream of the intron. For ribosomal protein L25, the first 17 amino acids, but not the first 10, are sufficient to cause nuclear localization of a fusion protein (H. A. Raué, personal communication). The sequence between amino acids 10 and 17 is KKAVVKG. In all ribosomal proteins examined so far, the signals are rather basic but do not bear a great deal of resemblance to the simian virus 40 large-T-antigen nuclear localization sequence PKKKRKV (64).

L25 is one of the tight RNA-binding proteins (see below), and its binding region has been localized to the carboxy portion of the protein (Raué, personal communication). In this case, therefore, the nuclear localization signal is distinct from the RNA-binding signal, which demonstrates that the nuclear localization of L25 is not due simply to differential binding of a freely diffusible protein.

The large number of ribosomal proteins that must make their way to the nucleus will provide a fertile ground for understanding the basis of nuclear localization, all the more so because they are clearly a different class of proteins from the DNA-binding proteins that have been the subject of most studies (24).

The Yeast Nucleolus

The yeast nucleolus is less well defined morphologically than are the nucleoli of mammalian cells. What is apparently the nucleolus, known as the gray crescent (3, 154), occupies more than one-third of the nucleus, as might be expected for an organism in which ribosomes make up 15% or more of the cell mass. No one has yet reported a convincing method for the purification of yeast nucleoli.

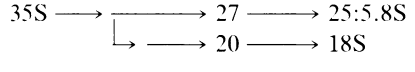
The evidence for the gray crescent actually being the nucleolus has not been very strong, being based mainly on size and homogeneity and the fact that there have been reports of its arising out of a chromosome, as do other nucleolar organizers. However, the attribution is probably correct, since two proteins of the gray crescent have recently been identified immunologically as nucleolar proteins: a highly antigenic 38-kilodalton protein immunologically related to mammalian fibrillarin, itself a nucleolar protein (3), and a 33-kilodalton protein (63), originally identified as a single-stranded-DNA-binding protein but whose function in vivo is unknown.

Indeed, the concept of the nucleolus as an independent entity is now open to some question. Results for *Drosophila* sp. suggest that it may be nothing more than the rRNA transcription apparatus, the transcripts themselves, and the associated proteins and factors that gather around to process the RNA and to assemble the ribosomes (65).

rRNA Processing

As in most other eucaryotic cells, the primary rRNA transcription product is processed into three molecules: the 18S RNA found in the 40S subunit, and the 5.8S and 25S RNAs found hydrogen-bonded together in the 60S subunit. The sequences of the rRNAs can be readily folded into the two-dimensional structure characteristic of all rRNAs (52, 53, 172).

The sequence of rRNA processing steps is:



(168, 173). Although early reports (168) concluded that methylation occurred only after completion of the transcript, it has recently been suggested that the first step of processing takes place on nascent molecules (170). The discrepancy may be due to strain differences or to subtle differences in the conditions of growth, to which the rates of the processing steps are very sensitive (168). In any case less than, perhaps much less than, 1% of the rRNA is found as 35S molecules.

The 3′ end of 35S rRNA is generated by an endonucleolytic cleavage, followed by the exonucleolytic removal of approximately seven nucleotides (171). An interesting mutant allele of *RNA82* is defective in this exonucleolytic activity (69) as well as in a similar activity directed towards 5S RNA (129). The mutant appears, however, to have no impairment of growth. The 20S RNA, as isolated, has the 5′ end of mature 18S RNA and a 3′ end 207 nucleotides downstream from the 3′ end of 18S RNA (22). A 7S RNA intermediate in the formation of 5.8S RNA has also been identified (173). Although these observations represent a promising start, ultimately determination of the precise nucleotides at which endonucleolytic cleavage takes place and the relative role of endo- and exonucleases must await the isolation of appropriate mutants and the establishment of an in vitro system for the processing of ribosomal precursor RNA. It is clear, however, that ribosomal proteins are necessary for these processing steps to be carried out (190). Interestingly, the processing of 20S to 18S RNA appears to take place in the cytoplasm (169). This step is essential before the 40S subunits can participate in protein synthesis.

A new approach to understanding the features of rRNA necessary for processing has been developed by Musters et al. (108), who have constructed a plasmid containing an entire rDNA gene whose 25S RNA sequence has been marked by the introduction of a few extra nucleotides. When introduced into the cell, this gene is transcribed and the RNA is properly processed, as shown by the appearance of the marked 25S RNA in polysomes. Remarkably, transcription and proper processing of the 25S RNA occur even when two-thirds of the 18S RNA sequences have been deleted from the construct.

Despite a considerable amount of searching, only one mutation has yet been found that blocks specifically one of the steps of rRNA processing: a temperature-sensitive mutation in a gene designated *RRP1* that prevents the step 27S→25S RNA (2). The unprocessed 27S RNA as well as all the newly formed 60S subunit proteins are rapidly degraded, most with half-lives of less than 7.5 min (42). The 40S subunits are made normally. The *RRP1* gene has recently been cloned, and it appears to code for a product too large to be a ribosomal protein (33). It is unclear how it acts in the processing of RNA. A variety of other mutations (references 6 and 45, inter alia) appear to affect both sensitivity to antibiotics and the processing of rRNA, but little inquiry into the nature of the lesions has been carried out.

A promising new aspect of rRNA processing lies in the analysis of nucleolus-associated small RNA molecules, of which there seem to be at least seven in *S. cerevisiae* (166). snR190 associates with 20S RNA; snR128 associates with 27S RNA (197). Of the two, only snR128 is essential for cell growth. Nevertheless, with our new appreciation of the role of small RNA molecules in mRNA and tRNA processing (47), it seems a safe prediction that they will be found to play a role in rRNA processing as well.

Assembly with Ribosomal Proteins

Whereas the reconstitution in vitro of *E. coli* ribosomes has been an important tool in understanding both the structure and the assembly of the ribosome (114), no such in vitro system has been established for any eucaryotic cell despite substantial efforts. Our understanding of ribosome assembly, therefore, must be largely inferred from in vivo labeling data and from disassembly data obtained in studies in which proteins were stripped off the ribosome by increasing concentrations of ions. In that way, El-Baradi et al. (27) found that the most tightly bound proteins of the large subunit are L25 > L4 and L8 > L10 > L12 and L16. These proteins also seem to be among the first assembled onto the rRNA in vivo (78), although such analyses are subject to substantial uncertainties. A small number of ribosomal proteins can reassociate specifically with 25S RNA. In nuclease protection studies of such reassociation, the site of L25 binding has been localized to nucleotides 1465 to 1632 and 1811 to 1861 (27). This is a highly conserved region of the RNA; L25 of yeast cells will bind to the analogous region of *E. coli* 23S RNA; L23 of *E. coli*, an apparently homologous protein, will bind to the yeast sequence (28). L15 has a similar relationship to L11 of *E. coli* (29). Furthermore, L15 interacts with the acidic ribosomal proteins (143) just as L11 does in *E. coli*. These are gratifying results, because although there is unassailable evidence for the conservation of the basic structure of the RNA throughout ribosomes of all organisms (113), it has rarely been possible to cross-identify homologous ribosomal proteins between eucaryotes and procaryotes. Presumably, our inability to recognize homologous structures from sequence alone has masked the underlying evolutionary conservation through evolution of the proteins of the ribosome.

A surprising result has come from attempts to study the nuclear localization signals of ribosomal proteins. In cells carrying *lacZ* fused near the carboxy end of ribosomal protein L3 (106) or RP51 (44), the β-galactosidase activity was found associated with functional ribosomes. Thus, these fusion proteins can associate with the rRNA to form a functional ribosome even with the long tail of procaryotic sequences. In the case of RP51, this occurred only when the fusion protein was the sole source of RP51. The fusion protein could not compete with authentic RP51 in the assembly process. In the case of L3, the fusion protein was functional only if a hinge region was introduced between the ribosomal protein and the LacZ peptide.

In the long run, further analysis of the basic structure of yeast ribosomes, such as the cross-linking begun by Yeh and co-workers (195), will be essential for understanding ribosome assembly. The topography to yeast ribosomes has been reviewed by J. C. Lee *in* A. Rose, ed., *The Yeasts*, 2nd ed., vol. 4, in press).

Export to the Cytoplasm

Assembled ribosomal subunits must be exported from the nucleus to the cytoplasm, presumably through the nuclear pores. Essentially no experiments dealing with this issue have been published. An intriguing mutation in the gene *RNA1* appears to block such export (56), but it also blocks mRNA export and tRNA splicing (54). Such pleiotropy has precluded its use for a clear experimental approach to nuclear export.

CONCLUDING REMARKS

It is unlikely that we will soon reach as thorough an understanding of the structure and the assembly of the yeast ribosome as we have for the *E. coli* ribosome. Yet the isolation of the genes for rRNA and for many of the ribosomal proteins makes available the tools of genetics, mutation and suppression, that can lead to more insight into the interactions among the ribosomal proteins and between the proteins and rRNA.

Perhaps more important will be the exploitation of these genes to study ribosome biosynthesis, with the ultimate aim of attacking questions fundamental to the nature and function of the eucaryotic cell. Thus, a clear identification of the nuclear localization signals of a number of ribosomal proteins may provide insight into the essential common features. The strong selection available for the nuclear localization of ribosomal proteins provides an opportunity to identify mutations in the localization machinery that will suppress a defective localization signal. The major limitation at present is the lack of rapid and quantitative procedures for isolating nuclei and the lack of any procedures for preparing pure nucleoli. A longer-range goal will be the isolation of mutants leading to an understanding of the nuclear export machinery, about which we know practically nothing.

It is clear from the data presented above that there are some unique features to the transcription of rRNA and, in all likelihood, to the transcription of mRNA for ribosomal proteins. While a responsive in vitro transcription system will ultimately be needed to define these features precisely, the genetic and reverse genetic approaches should soon lead to insights that are not available in other systems. Similar claims can be made for questions on the processing of rRNA, the role of small nuclear ribonucleoproteins, the existence and function of nucleolar scaffolding proteins to help assemble the ribosome, etc.

Finally, one of the most promising conclusions that has come from recent work is the confirmation at a molecular level of our suspicions, and hopes, that eucaryotic ribosomes would be highly conserved through evolution. This is clearly the case for structure (Table 1; 52, 53). Thus, studies on one eucaryotic organism are likely to be useful for all. On the other hand, organisms have developed markedly different mechanisms to regulate the synthesis of ribosomes to meet their particular needs. Studies of these systems may provide some insight into the evolution of the regulatory processes themselves.

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