Mini-Review

The Nucleolus and the Four Ribonucleoproteins of Translation

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The classical view of the nucleolus as solely committed to ribosome biosynthesis has been modified by recent studies pointing to additional roles for this nuclear domain. These newly recognized features include the nucleolar presence of several nonribosomal RNAs transcribed by RNA polymerase III, as well as nucleolar roles in gene silencing, cell cycle progression, and cellular senescence. The signal recognition particle (SRP)[1] RNA, and several protein components of the SRP also recently have been detected in the nucleolus. Thus, the large and small ribosomal subunits, the 5S rRNA–ribonucleoprotein complex, and now the SRP, are known to be assembled in or pass through the nucleolus. These findings, together with the recent observations that some transfer RNA precursor molecules and the pretransfer RNA processing enzyme, RNase P, are also found in the nucleolus, raise the possibility that these translational components are congressed in the nucleolus in order to probatively interact with one another, perhaps as a test of proper conformational fit. We hypothesize that such interactions may be an important checkpoint during nucleolar assembly of the translational machinery at steps ranging from the regulation of nascent transcript processing to a possible transient preassembly of the entire translational apparatus.

Introduction

The nucleolus is a large and ultrastructurally complex intranuclear structure that typically has the most concentrated mass per unit volume of any region in the cell (Vincent, 1955; Goessens, 1984; Hadjiolov, 1985). Nucleoli arise from the transcriptional activity of the ribosomal RNA genes and contain a multitude of proteins and small RNAs that mediate processing and modification of rRNA and ribonucleoprotein assembly of nascent ribosomal subunits (Warner, 1990; Bachellerie et al., 1995; Shaw and Jordan, 1995; Maden and Hughes, 1997; Scheer and Hock, 1999; Weinstein and Steitz, 1999). Although the central role of the nucleolus in ribosome biosynthesis remains a

resoundingly confirmed principle of eukaryotic molecular cell biology, within two years the nucleolus has been implicated in aspects of eukaryotic cell biology beyond rRNA biosynthesis, i.e., gene silencing, cell cycle progression, and senescence (Pederson, 1998a; Cockell and Gasser, 1999; Garcia and Pillus, 1999). Moreover, it has been found that some RNAs unrelated to rRNA biosynthesis, primarily RNA polymerase III transcripts, also traffic through the nucleolus (Pederson, 1998b). In this article, we summarize recent findings that point to an additional important role for the nucleolus in the assembly, and perhaps quality control, of the multiple ribonucleoproteins involved in protein synthesis, rather than simply serving as a site for ribosome synthesis, per se.

The nucleolus was established as the site of ribosomal RNA synthesis in the 1960's (Perry, 1962; Birnstiel et al., 1963; Brown and Gurdon, 1964; Lerman et al., 1964; Ritossa and Spiegelman, 1965) and soon thereafter the presence of ribosomal proteins and the assembly of nascent ribosomes in the nucleolus was also revealed (Warner and Soeiro, 1967; Liau and Perry, 1969; Craig and Perry, 1970; Pederson and Kumar, 1971; Kumar and Warner, 1972). In addition to the high molecular weight RNAs of the large and small ribosomal subunits (28S and 18S rRNA, respectively, in vertebrate cells), two smaller ribosomal RNAs were discovered, 5S rRNA (Elson, 1961; Rosset and Monier, 1963; Galibert et al., 1965) and 5.8S rRNA (Pene et al., 1968). The genes for 5S rRNA lie outside the nucleolus in higher plant and animal cells, but 5.8S rRNA arises from processing of the pre-rRNA primary transcript and ends up base-paired with 28S rRNA in the nucleolus (Perry, 1976; Calvet and Pederson, 1981). In higher eukaryotes, newly synthesized 5S rRNA moves into the nucleolus from its extranucleolar transcription sites, and a ribonucleoprotein complex containing 5S rRNA and the ribosomal protein L5 has been implicated in both the 3′ end processing and nucleolar localization of 5S rRNA (Steitz et al., 1988; Michael and Dreyfuss, 1996). A cytoplasmic 5S rRNA–ribonucleoprotein complex has also been identified (Blobel, 1971). Finally, the SRP, the ribonucleoprotein machine that facilitates topologically correct protein synthesis into the ER, contains a small RNA and six bound proteins (Walter and Johnson, 1994). Thus, considered as ribonucleoproteins, the translational machinery may be regarded to be comprised of four particles: the large and small ribosomal subunits, the 5S rRNA–ribonucleoprotein complex, and the SRP.

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[1]*Abbreviations used in this paper:* GFP, green fluorescent protein; SRP, signal recognition particle.

Signal Recognition Particle Components in the Nucleolus

During the course of investigations on the traffic and localization of various species of RNA within the nucleus of living mammalian cells (Wang et al., 1991; Jacobson et al., 1995, 1997; Jacobson and Pederson, 1998a) it was found that microinjected SRP RNA rapidly became localized in nucleoli and subsequently appeared to depart from the nucleoli and enter the cytoplasm (Jacobson and Pederson, 1998b). These results were confirmed by in situ hybridization experiments (Politz et al., 1998, 2000) and biochemical fractionation studies (Chen et al., 1998; Mitchell et al., 1999), which showed that endogenous SRP RNA is also present in the nucleolus. Additional microinjection experiments showed that the specific domains in the SRP RNA molecule that were essential for nucleolar localization included known SRP protein binding sites (Jacobson and Pederson, 1998b). Each of the four SRP-specific proteins (Walter and Johnson, 1994) was tagged with the green fluorescent protein (GFP) and their intranuclear localization investigated after transfection into mammalian cells. Three of the four proteins, SRP19, SRP68, and SRP72, displayed nucleolar localization, as well as cytoplasmic localization as expected (Politz et al., 2000). In contrast, the fourth SRP-specific protein, SRP54, did not display nucleolar localization, nor did a human autoantibody specific for endogenous SRP54 stain nucleoli, although cytoplasmic SRP54 was detected as expected. In vitro SRP assembly studies had revealed that SRP54 does not bind SRP RNA until SRP19 has first bound (Walter and Johnson, 1994) and thus the in vivo studies suggested that SRP54 may bind to a partially assembled SRP particle outside the nucleolus. The finding that three SRP proteins and SRP RNA visit the nucleolus suggests that an essential step in the overall pathway of SRP assembly may occur there.

Genomic Organization of Loci for RNAs of the Translational Machinery

The extranucleolar transcription of 5S rRNA in higher eukaryotes, followed by its traffic to the nucleolus is intriguing since, at first thought, 5S rRNA could ostensibly be exported (perhaps as a ribonucleoprotein complex) from its nonnucleolar transcription sites directly to the cytoplasm and there join with ribosomes. Indeed, given the crowded and dynamic molecular landscape of rRNA processing and its multitude of attendant cofactors in the nucleolus (Bachellerie et al., 1995; Scheer and Hock, 1999; Weinstein and Steitz, 1999), it might seem more efficient for 5S rRNA to exit the nucleus and then assemble with finished ribosomes in the cytoplasm. Why then does 5S rRNA traffic to the nucleolus? Interestingly, in contrast to the case in higher eukaryotes, the 5S rRNA genes of *Dictyostelium* and fungi (and *Escherichia coli*) are interspersed with the large and small ribosomal subunit RNA genes (Maizels, 1976; Maxam et al., 1977; Nomura and Post, 1980), suggesting that the earliest nucleoli spatially coproduced and coassembled the two ribosomal subunits and the 5S ribonucleoprotein particle. It may be, then, that 5S rRNA moves to the nucleolus in higher eukaryotes not merely to interact with the nascent 60S ribosomal subunit (which, as

mentioned above, could seemingly take place just as well in the cytoplasm), but in fact to also participate in an obligatory step of the overall rRNA processing and/or assembly pathway. In support of this idea is the recent observation that the presence of *Saccharomyces cerevisiae* 5S rRNA in nucleoli is essential for the efficient completion of accurate processing of the large subunit rRNA (Dechampesme et al., 1999).

In light of the colinear arrangement of 5S rRNA genes and the large and small rRNA genes in fungi and mycetozoa (*Dictyostelium*), as well as the recent finding that SRP RNA and SRP proteins traffic through the nucleolus of mammalian cells, one might ask if, like the 5S rRNA genes, the SRP RNA gene(s) might also have once coresided with the large and small rRNA genes, in a primordial form of today's nucleolus. In at least two cases, the answer is yes. In the archaebacteria *Methanobacterium thermoautotrophicum* and *Methanothermus fervidus* the single SRP RNA gene resides together with a 5S rRNA gene and two tRNA genes within one of the organism's rRNA operons (Østergaard et al., 1987; Haas et al., 1990). Parenthetically, it is also interesting to recall that both tRNA and SRP RNA are associated with retroviral genomes. Indeed, the association of SRP RNA (then called 7S RNA) with retrovirus genomic RNA was the basis of its original discovery (Bishop et al., 1970; Walker et al., 1974). The tRNA molecule is now known to serve as a primer for reverse transcription of the RNA genome into proviral DNA, but the role of the SRP RNA bound to the retroviral genomic RNA remains unknown.

The Nucleolus as a Staging Site for Assembly of the Translational Ribonucleoproteins

These considerations raise the question of whether the nucleolus may stage some sort of a "preassembly" step during the production of the translational apparatus. According to this idea, a supramolecular assembly of the translational machinery would occur in the nucleolus, perhaps transiently, through the association of 5S rRNA and SRP with nascent ribosomal subunits. Such preassembly of the translational apparatus in the nucleolus could allow for a quality control step during the synthesis and processing of the various translational components. As mentioned, it already appears that this may be the case in yeast: the nucleolar presence of 5S rRNA is required for proper processing of the large subunit rRNA (Dechampesme et al., 1999). If a nucleolar preassembly were generally important as a checkpoint for potential functionality, other translation-related factors might also be expected to be present in the nucleolus to interact with this complex. One such potential factor is transfer RNA.

When the first radioisotopic studies of RNA biosynthesis in eukaryotic cells were being undertaken there were numerous indications that some labeled transfer RNA was present in nucleoli (Birnstiel et al., 1961; Perry, 1962; Comb and Katz, 1964; Birnstiel et al., 1965; Sirlin et al., 1966; Halkka and Halkka, 1968; Sirlin and Loening, 1968), even though the tRNA genes themselves were found to reside in the nonnucleolar chromatin (Woods and Zubay, 1965; Ritossa et al., 1966; Wimber and Steffensen, 1970). The notion that the biosynthesis of tRNA might involve a

nucleolar stage has recently been reactivated by the detection of several pre-tRNAs in the nucleolus by in situ hybridization (Bertrand et al., 1998). An apparently complementary finding is the presence in nucleoli of both the RNA and protein subunits of RNase P, the ribonucleoprotein enzyme that mediates 5' processing of pre-tRNAs (Jacobson et al., 1997; Bertrand et al., 1998; Jarrous et al., 1999). In addition, a *Saccharomyces cerevisiae* tRNA base modification enzyme has also been localized in nucleoli (Tolerico et al., 1999). Other potentially relevant observations are the findings that some tRNA aminoacylation occurs in the nucleus of frog oocytes (Arts et al., 1998; Lund and Dahlberg, 1998) and an intriguing preliminary report that an aminoacylated tRNA is found specifically in the nucleolus (Ko, Y.G., Y.-S. Kang, E.-K. Kim, W. Seol, J.E. Kim, and S. Kim. 1999. *Mol. Biol. Cell.* 10:438a).

Taken together, these various observations add up rather provocatively. Not only do all four translational ribonucleoproteins arise in or visit the nucleolus, some tRNAs, perhaps even aminoacylated tRNAs, are also localized there. Although this may simply be a chance spatial coincidence, it seems more likely that there is a functional significance to this congression of translational components. As mentioned above, a plausible explanation is that the four translational ribonucleoproteins interact with one another in some sort of quality control step during synthesis, processing, and/or assembly. The four ribonucleoproteins might undergo interparticle surface interactions to probatively eliminate misshaped partners arising from errors in ribonucleoprotein assembly. Such interactions might or might not be stoichiometric with respect to the four ribonucleoproteins; topological testing could be confined to transient dimeric heterotypic particle interactions or, at the other extreme, the entire tetrapartite ribonucleoprotein translational ensemble might form, with attendant binding of tRNA and other nucleolus-associated translation factors (e.g., Jiménez-García et al., 1993). Presumably any tRNA species in the nucleolus, including the aminoacylated form (vide supra), could probe the assembled 60S ribosomal subunit's tRNA entry site, but it is particularly interesting to note that the first (albeit preliminary) report of an aminoacylated tRNA in the nucleolus involves methioninyl tRNA (Ko, Y.G., Y.-S. Kang, E.-K. Kim, W. Seol, J.E. Kim, and S. Kim. 1999. *Mol. Biol. Cell.* 10:438a).

Although one might even expect mRNA to be involved in such a quality control step, there are few reports showing the presence of mRNA in the nucleolus (although, see Bond and Wold, 1993). However, detection of specific mRNAs in the nucleolus by in situ hybridization would be expected to be difficult, so the absence of such reports does not rule out the presence of some nucleolar mRNA. In this regard, it should be mentioned that, although considerable doubt has long existed as to whether protein synthesis occurs in isolated nuclei (Goldstein, 1970; Pederson, 1976), there does exist rather convincing evidence for amino acid incorporation into isolated nucleoli (Birnstiel and Hyde, 1963; Birnstiel and Flamm, 1964; Maggio, 1966). Whatever the level of possible cytoplasmic contamination of the initial nuclear preparations in these studies, what is now understood of the cell fractionation protocols employed would suggest that cytoplasmic contaminants of

the nuclei would have been significantly reduced in the subsequent nucleolar fraction (Maggio et al., 1963a,b; Bhorjee and Pederson, 1973), which nonetheless displayed a tenfold higher rate of amino acid incorporation than nuclei (Maggio, 1966). Although the significance of these observations is still unclear, they do not allow us to rule out the (unfashionable) possibility that some peptide bond formation is catalyzed by a translation preassembly complex in the nucleolus.

A final question is whether the putative interparticle associations within this preassembly complex persist during nucleocytoplasmic transport. Does there exist the possibility of coexport of two or more of the four translational ribonucleoproteins out of the nucleolus (and the nucleus)? Most of the available evidence suggests that the large and small ribosomal subunits are typically exported as separate particles, although there have been occasional suggestions of nuclear export of intact 76S ribosomes (e.g., Khanna-Gupta and Ware, 1989). In either case, it appears that 5S rRNA typically exits minimally as part of the 60S ribosomal subunit in somatic cells. At present, nothing is known about the nucleolar exit of SRP as regards piggybacking on ribosomal particles. As we have pointed out (Jacobson and Pederson, 1998b; Politz et al., 2000), it is conceivable that SRP is coexported with the large ribosomal subunit, since there is a known affinity of the SRP for nontranslating ribosomes (Ogg and Walter, 1995). However, coexport would not be expected to necessarily be stoichiometric with respect to SRP because SRP is typically present in cells at lower concentrations than ribosomes (Reddy and Busch, 1988).

Conclusion

It now appears that the eukaryotic cell stages the assembly of the two ribosomal subunits, the 5S rRNP and the SRP in the nucleolus, probably in the presence of other translational elements, such as tRNA. The biological rationale for this common intranuclear site of assembly is not clear at present, and indeed, each of the four translational ribonucleoproteins may simply independently assemble in the nucleolus. However, there exists the possibility that these four translational ribonucleoproteins interact with one another while congressed in the nucleolus. Effective interaction between these components could be required as an essential checkpoint during the production of the translational apparatus. In this way, the nucleolus may provide a preassembly site to verify the potential functionality of the machines of protein synthesis. This idea is a testable hypothesis and hopefully will help to catalyze future work on the full functional repertoire of the nucleolus.

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