Chemistry & Biology, Vol. 9, 777–780, July, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S1074-5521(02)00171-0

Previews

Sno-Capped: 5′ Ends of Preribosomal RNAs Are Decorated with a U3 SnoRNP

Efficient ribosome biosynthesis is crucial for cell viability. Work recently published in *Nature* [1] demonstrates that a large ribonucleoprotein processes 18S rRNA in *S. cerevisiae*, indicating that macromolecular particles regulate ribosome maturation [1–3] and revealing the complex nature of this process.

Introduction

Eukaryotic ribosome biosynthesis is achieved through a series of concerted, ordered events. Although we do not fully understand how these events are orchestrated, a number of the important factors and stages have been identified over the last thirty years. Ribosome biosynthesis begins with the transcription of ribosomal RNAs (rRNAs) as one large pre-rRNA that must subsequently be processed into the mature rRNA molecules. Pre-rRNA processing, including RNA modification as well as cleavage of the pre-rRNA, and association of both nonribosomal and ribosomal proteins are all required for the production of mature ribosomal subunits that can function in translation.

In the late 1960s, experiments addressing events in the nucleolus revealed that large assemblages known as terminal knobs were associated with pre-rRNA transcripts [4]. Since the majority of steps required for ribosome maturation occur in the nucleolus, these results were likely the first clues that biosynthesis of functional ribosomes involves large macromolecular complexes. The discovery of small nucleolar RNAs (snoRNAs) and associated proteins, which function in ribosome biogenesis, have supported this idea. Now, more than three decades after Miller first observed the terminal knobs [4] and almost ten years after the first hints that these structures were involved in pre-rRNA processing [5], Dragon and colleagues have demonstrated that these knobs are related to massive U3 snoRNA-containing ribonucleoprotein particles (RNP) [1]. These findings and other recent work [2, 3] demonstrate that large RNP "factories" are required to assemble the prototypical RNP factory, the ribosome.

Small Nucleolar RNAs

SnoRNAs represent the largest population, 100–200 different molecules per nucleolus, depending on the species, of stable RNAs in eukaryotic cells [6–8 and references therein]. Two known functions of snoRNAs are cleavage of long pre-rRNA transcripts into mature rRNAs and site-specific rRNA modification. The vast majority of snoRNAs can be categorized one of two ways, either as box C/D- or box H/ACA-containing RNAs. These distinctions are based on the sequence, structure, and function of the snoRNA. Box C/D snoRNAs are involved in 2′-O-methylation of rRNA, and box H/ACA snoRNAs function in pseudouridinylation. All snoRNAs are generally found associated with proteins to form nucleolar RNPs, or snoRNPS. These snoRNPs perform critical roles in orchestrating ribosome biogenesis. While some proteins are found in all box C/D or H/ACA snoRNPs, it is clear that there are many unique proteins that function with specific snoRNAs to form functional complexes.

U3 snoRNA is one of only a few snoRNAs that are required for cleavage events during ribosome biogenesis [9, 10]. In addition to this function, it has been suggested that U3 snoRNA plays a role as a "chaperone" to guide the formation of a functional pseudoknot near the 5′ end of 18S rRNA [8, 11–13] . The two functions associated with U3 snoRNA may be orchestrated by a single snoRNP, or two distinct snoRNPs may be required, each participating in a single function. While a number of proteins have been shown to interact with U3 snoRNA [8 and references therein], no large U3 snoRNP had been identified until very recently, when a massive U3 snoRNA-containing complex was isolated [1] .

A U3 SnoRNP, a Small Ribosomal Subunit Processome

The recent work of Dragon et al. [1] differed from earlier work in a small but seemingly important way. Previous studies have relied on tagged U3 snoRNA for use in affinity chromatography to identify interacting components [14, 15] . Dragon et al. [1] relied on dual tagging of two proteins known to interact with U3 snoRNA [16, 17] . This approach allowed the purification and subsequent identification of a large complex containing not only these two proteins (Nop5p and Mpp10) and U3 snoRNA but also 26 additional proteins. This new macromolecular complex, with a sedimentation coefficient approaching 80S, has been termed the small subunit (SSU) processome. Disruption of two of its identified components in vivo results in a loss of terminal knob formation on pre-rRNA transcripts, suggesting that this complex exists in vivo and functions in pre-rRNA maturation. Therefore, over thirty years after they were first imaged, the molecular nature of terminal knob structures may have been revealed.

Ten of the twenty-six identified proteins had previously been shown to interact with U3 snoRNA; some bind to U3 snoRNA specifically, while the remainder are general box C/D snoRNA binding proteins. However, not all previously identified U3 snoRNA binding proteins were found in this complex. This may suggest that these proteins were lost during the purification of this RNP. Alternatively, these proteins could be part of a separate U3-containing particle, and such a complex could be involved in the RNA chaperone function also attributed to U3 snoRNA.

All but one of the protein components of the SSU processome are essential for viability in *S. cerevisiae*. This suggests that the large number of components do

not merely represent functional redundancies, but each provides a necessary piece of the intricate processing machinery. All 17 of the novel U3-associating proteins (Utps, *U three proteins*) are localized to the nucleolus and all coimmunoprecipitate with Mpp10 as well as with U3 snoRNA. Lastly, depletion of any one of these 17 Utps results in a marked diminution in the detectable level of 18S rRNA, indicating that they do indeed function in 18S rRNA biosynthesis.

It is not surprising that many of the SSU processome proteins have well-defined RNA recognition/binding motifs. However, what is very striking is that none of the proteins reveal motifs consistent with any known RNA endonucleases. So while the SSU processome likely functions in pre-RNA cleavage, how this is achieved and the mechanism by which the precise cleavage site is selected still remain a mystery. It is possible that one of the SSU processome proteins performs this role, but by a novel mechanism that could not be identified by standard bioinformatics approaches. Alternatively, the endonuclease could be part of this complex in vivo that was lost as a consequence of the purification scheme. This could be due to a reduced affinity for this U3 complex in the absence of pre-RNA (substrate) or the loss of a proteinacous binding partner. In vivo, the endonuclease may only transiently interact with the processome and pre-RNA. It could bind, cleave, and then dissociate; this would make sense if the U3 complex remains bound and performs additional assembly/processing functions postcleavage. Given the high level of conservation of U3 snoRNA, a possible RNA-mediated cleavage event should not be completely overlooked. Identification of the endonuclease will begin to allow the mechanism of 18S rRNA maturation to be revealed.

Not included in the 28 proteins found associated with the small subunit processome are five small subunit ribosomal proteins. Likely these proteins are not counted since they are found in the mature 40S subunit and therefore are not part of the "modular" processing complex. However, these ribosomal proteins may be crucial for the function of the processome. Association of the ribosomal proteins with the pre-rRNA may aid in folding of the RNA into a conformation that is conducive to accurate cleavage. Also, these proteins may provide a scaffold on which the processome binds or assembles. Since little is known about the order that the yeast small subunit ribosomal proteins bind during 40S subunit biogenesis [18, 19], it is unclear if these proteins are poised to bind to pre-rRNA during the course of processing. These yeast small subunit ribosomal proteins reveal no homology to the well-studied 16S rRNA primary binding proteins involved in early stages of *E. coli* 30S subunit assembly. Thus, it is too early to conclude if the observed association of these proteins with the processome is indicative of the content of an early 40S subunit assembly intermediate (see below).

Many Large Complexes Involved in Ribosome Maturation

Two additional complexes that are involved in ribosome biogenesis have also been recently identified [2, 3]. These two complexes are involved in assembly of the large or 60S ribosomal subunit from *S. cerevisiae*. As with the work of Dragon et al. [1], the identified complexes contain many different proteins, and the pre-60S subunit complexes also contain rRNA as well. It is interesting to note that some of the small subunit ribosomal proteins as well as the majority of the large subunit ribosomal proteins copurify with one of these complexes [3]. Two of these small subunit proteins are identical to those found on the SSU processome. Thus, the appearance of these proteins in a variety of complexes may be an artifact. A more intriguing possibility is that these proteins may have extra-ribosomal functions and act as chaperones, aiding the assembly of a variety of RNPs. This would not be completely unprecedented, as *E. coli* ribosomal protein S12 has been shown to act as an RNA chaperone aiding intron folding [20]. The particles identified in the studies of 60S subunit maturation function in the 60S subunit assembly pathway at a later step than where the U3-containing particle acts in 40S subunit biogenesis. Thus, it is likely that large, multifaceted processing and assembly complexes act at many different stages of assembly of both of the subunits, and therefore many more of these processing, assembly machines are likely to be identified in the future.

Remaining Questions

Along with identification of additional processomes, future experiments will try to understand the assembly of the complex particles that are in turn required to assemble other complex particles. Can the preformed SSU processome bind to pre-rRNA or does it assemble on the substrate? Some clues to this question could be uncovered by looking at what parts of U3 snoRNA are "available" in the processome. Are the nucleotides in U3 snoRNA necessary for interacting with pre-rRNA available in the complex? If not, then is there a large conformational change upon binding, or is this suggestive of assembly of the processome on the pre-rRNA substrate? What actually catalyzes the cleavage reaction and how does this component identify the appropriate cleavage site? Questions like these will keep researchers in this field busy for some time. Lastly, we must also ask why the complexity? The answer to this question is not clear, nor is it clear when we will truly understand the limits of biological complexity as it relates to ribonucleoprotein particle assembly and function.

Gloria M. Culver
Department of Biochemistry, Biophysics and Molecular Biology
Iowa State University
Ames, Iowa 50011

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Chemistry & Biology, Vol. 9, July, 2002, PII S1074-5521(02)00170-9

Stochastic Sensing of IP_3 Has Far-Reaching Consequences

Hagan Bayley's group at Texas A&M University has devised a stochastic sensing methodology for the quantitation of the second messenger inositol 1,4,5-trisphosphate. The unique sensing scheme is very selective and has the potential to measure cytosolic concentrations of IP_3.

Inositol 1,4,5-trisphosphate (IP_3) is a second messenger used in a variety of signal transduction events [1]. The binding of extracellular signals, such as hormones acting as agonists, can elicit a cascade of biochemical processes, leading to the production of *sn*-1,2-diacylglycerol (DAG), which remains associated with the inner leaflet of the plasma membrane, and IP_3, a diffusable cytosolic messenger capable of releasing intracellular stores of Ca(II). This release can have various effects on cellular metabolism, including the activation of protein kinase C (PKC), which has a heightened sensitivity to Ca(II) levels in the presence of DAG. PKC catalyzes the phosphorylation of various serine and threonine residues, leading to the modulation of activity for additional enzymes and proteins. Known phosphorylation targets for PKC include the insulin receptor, β-adrenergic receptor, cytochrome P-450, and tyrosine hydroxylase. Clearly, IP_3 is a crucial cellular player, serving as a key control point for a wide range of cellular processes.

The ability to quantitatively monitor the intracellular levels of various messengers would be useful for developing accurate models of diverse cell functions, development, growth, and responses to stimuli. Although there are now outstanding methods for following Ca(II) using fluorescent probes [2], progress in monitoring IP_3 has been much slower. In one interesting approach, Allbritton and coworkers at the University of California, Irvine, have demonstrated the capability to use cultured cells as detectors for IP_3 sampled from oocytes [3]. In Allbritton's approach, the effluent from a sampling/electrophoresis capillary is directed onto a partially permeabilized cell, allowing IP_3 to release Ca(II) from intracellular stores; the resultant spike in [Ca(II)] can then be measured using fluorescence imaging. A second existing strategy for sensing IP_3 uses a synthetic receptor that binds IP_3 strongly in water and methanol mixtures [4] and is currently being used to analyze IP_3 with capillary electrophoresis (E.V.A. and J.B.S., unpublished results).

In this issue of *Chemistry & Biology*, an article from Stephen Cheley and Li-Qun Gu in Hagan Bayley's group at Texas A&M University reports sensing of IP_3 [5]. In this case, the sensing approach utilized a transmembrane pore, αHL, that was engineered to have affinity and selectivity for IP_3. The pore allows ions to flow through the membrane, and a planar bilayer device measures a current modulation that is indicative of the activity of the pore. When the ion of interest binds the interior of the pore, the channel is effectively blocked, reducing the flow of current. The frequency with which the analyte binds to the pore is indicative of the concentration of that analyte, while the amplitude and duration of the current modulation steps reveals the identity of the analyte.

Cheley and coworkers engineered αHL to have affinity for phosphate and IP_3 by placing guanidinium groups into the lumen of the pore. This was inspired by biological phosphate receptors, which are known to often contain the amino acid arginine. A variety of amino acids in the lumen and on the mouth of the pore were modified in an incremental fashion as a means to change the behavior of this channel and tune it toward IP_3 binding. The final design consisted of 14 arginines near the *cis* end of the barrel-shaped pore. The refined pore was unaffected by simple anions, such as chloride and nitrate, and remarkably was also unresponsive to cAMP and only slightly affected by ADP and inositol-2-monophosphate. However, when the pore was presented with IP_3, a dramatic change in conductivity was detected. In an attempt to mimic intracellular conditions, salts, ATP, Mg(II), and buffer were used at cellular levels, while IP_3