After the ribosome structures: How are the subunits assembled?

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ABSTRACT

The recent structures of the ribosome and the ribosomal subunits only heighten the intrigue of trying to understand how the ribosome is assembled. Biochemical and mechanistic studies have mapped out the basic series of protein binding events that occur, but we do not yet have a clear picture of the RNA conformational changes that must accompany the protein binding. Recent studies point to roles of protein folding chaperones and RNA helicases as facilitators of ribosome assembly, but the basic process of assembly seems to be encoded in the RNA sequences and can occur for the most part spontaneously in vitro, and quite possibly in vivo as well.

Viewing the three-dimensional structure of the bacterial ribosome (Cate et al. 1999; Ban et al. 2000; Schluenzen et al. 2000; Wimberly et al. 2000) makes a profound impact on the viewer that has a variety of manifestations. First, there is a level of detail in the structure that the brain is simply not equipped to grok. The soft and gentle curves from the models constructed by Jim Lake are now familiar and somehow pleasantly sufficient to represent the ribosome in our minds (Lake 1976). Second, there is a strangeness in seeing how all of the RNA that we knew in an abstract way was at the core of ribosome function, really is there at the core of the ribosome after all. It is as if the artist Christo was commissioned to drape the stately RNA core with a set of absurd protein festoons. Finally, as if the intricacy of the RNA fold in the ribosome was not overwhelming enough, we are left to grapple with the question "By Jove, how does this thing get put together?"

There is a vast amount of information concerning bacterial ribosome assembly, both in vitro and in vivo. We know the basic series of events that must occur to generate a functional ribosome as an overview, but the molecular picture to match our new molecular view of the final structure is still not well developed. Ribosome biogenesis in *Escherichia coli* begins with transcription of the ribosomal RNA operon, where the three ribosomal RNAs are synthesized as a single transcript. The subsequent steps surely begin before the entire transcript is completed. It is likely that extensive local secondary structure in the rRNA forms very quickly, and that ribosomal protein binding begins as the protein binding sites are completed. The rRNA transcript is chemically modified at a number of points, and it is processed by nucleolytic cleavage to ultimately generate the 16S, 23S, and 5S chains. The assembly process is a carefully choreographed series of RNA conformational changes, protein binding, ion binding, and processing events that occurs cotranscriptionally.

Much of our knowledge about this series of events comes from in vitro reconstitution procedures. Remarkably, both the 30S and the 50S subunits can be reconstructed by spontaneous assembly of purified components. In general, in vitro reconstitution is not as efficient as ribosome biogenesis, and it differs in two important ways. First, in vitro assembly lacks the inherent directionality of cotranscriptional assembly, because the entire RNA is used to initiate assembly. Second, reconstitution experiments are often done at equilibrium, or as single time-point or end-point assays. Nevertheless, this powerful biochemical approach has yielded a wealth of information concerning the mechanism of ribosome assembly.

The assembly map of the 30S subunit that outlines the order of assembly of ribosomal proteins onto 16S rRNA was worked out by Nomura (Held et al. 1974). A set of 30S proteins can bind to 16S rRNA independently as primary binding proteins, while secondary and tertiary binding proteins require prior binding of one or more other proteins.

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Article and publication are at http://www.rnajournal.org/cgi/doi/10.1261/rna.2164903.

An in vitro transcript of 16S rRNA can be used in reconstitution to give highly active 30S subunits, implying that any chemical modifications of 16S rRNA are not essential for either assembly or activity (Krzyzosiak et al. 1987). The 30S subunit has three clearly defined structural domains that can be independently assembled as discrete ribonucleoprotein complexes (Weitzmann et al. 1993; Samaha et al. 1994; Agalarov et al. 1998). The kinetics of the protein binding during in vitro assembly of the 30S subunit has been studied using chemical probes, and the assembly proceeds roughly from 5′ to 3′, as would be expected for cotransciptional folding (Powers et al. 1993). The assembly of the central domain has been shown to involve a hierarchical series of RNA conformational changes followed by protein binding, where successive regions of RNA structure are consolidated by protein binding (Agalarov et al. 2000).

The corresponding assembly map of the 50S ribosomal subunit has been worked out primarily by Nierhaus (Herold and Nierhaus 1987). This assembly map is much more complex than that of the 30S subunit for at least three apparent reasons. First, there are nearly twice as many proteins involved and the RNA is over twice as large. Second, it is now clear from the structure of the 50S subunit that the five classic "domains" on the secondary structure diagram do not correspond well to independent structural domains. This implies that the folding of the RNA is likely to be extremely cooperative, and the binding sites for some proteins will certainly require assembly of most of the subunit. In *E. coli,* reconstitution of the 50S subunit with an in vitro transcript of 23S rRNA is extremely inefficient (Green and Noller 1996), although in other organisms it has been shown that 23S rRNA modifications are not essential for assembly or function of the 50S subunit (Green and Noller 1999; Khaitovich et al. 1999). The domain dissection (Weitzmann et al. 1993; Samaha et al. 1994; Agalarov et al. 1998) and sequential deletion strategy (Agalarov and Williamson 2000) that was successful for the 30S subunit is not likely to be as generally applicable for the 50S subunit. Third, some of the large subunit proteins are devoid of regular folds or secondary structures, appearing to serve as caulking compound to weatherproof the intricately folding 23S rRNA (Ban et al. 2000).

Although it is clear that the ribosomal subunits can be reconstituted in vitro without exogenous cofactors, it is likely that there are some cellular factors involved in facilitating ribosome assembly. The two types of activities that have been implicated in this role are protein folding chaperones and RNA helicases. It has been demonstrated that the protein folding chaperone DnaK reduces the need for a heating step during the in vitro reconstitution of *E. coli* 30S subunits (Maki et al. 2002). Protein chaperones typically function by unfolding the protein, at least partially, and then releasing it to refold. It may be that DnaK can facilitate RNA folding by unfolding proteins to cause them to release the bound RNA, thereby allowing the RNA the opportunity to refold as well. The RNA helicase DbpA has been found to interact with specific structures in 23S rRNA (Diges and Uhlenbeck 2001). Disruption of RNA structure by an ATP-driven helicase is another way that RNA structure can be unfolded and then allowed to refold.

The implications of protein chaperone and RNA helicase activities important for ribosome assembly are clear. As pointed out by Noller and Nomura, ". . . long, stable helices could create kinetic 'traps,' interfering with correction of nonproductive folding errors during assembly" (Noller and Nomura 1987). Studies on the in vitro folding of other large RNA molecules such as the Tetrahymena ribozyme and RNAse P have clearly shown that when misfolded structures do occur, they are often extremely stable. The stabilization of misfolded structures by native interactions does, in fact, result in kinetic traps on their folding pathway. The rate-limiting step for in vitro reconstitution is likely to be such a kinetic trap, because a heating step is required to complete the assembly. Another hallmark of kinetic traps is that addition of mild amounts of denaturants can accelerate folding, and in fact, inclusion of an osmolyte greatly increases the efficiency of 50S reconstitution (Semrad and Green 2002). As the size of an RNA increases, the ruggedness of the folding landscape should increase due to the many stable native interactions that exist in the later folding intermediates. The same features that stabilize native interactions, such as RNA tertiary interactions, ion binding, and protein binding will also stabilize any misfolded structures. The chaperone and helicase activities are ways for the cell to deal with the seemingly inevitable kinetically trapped intermediates that are populated during ribosome assembly.

Clearly, many challenges remain for a complete understanding of ribosome assembly. The recent structures of the ribosome do not obviate the need for continued study of assembly, but rather provide a valuable framework from which testable hypotheses can be generated. The accumulated man-millenia of work on ribosome assembly will serve as the foundation for new experimental approaches made possible by technology advances in molecular biology and biophysics. In the coming years, a more detailed picture of the protein binding events and RNA conformational changes that occur during assembly will emerge. It remains an open question to see if the assembly process itself can be targeted with small molecules as an antimicrobial strategy. Finally, it is clear that understanding bacterial ribosome assembly is a simple pilot project for understanding the Byzantine ribosome biogenesis program that involves a significant fraction of the genome in eukaryotes. The impression is that a government defense contractor was given a fully functional working prototype bacterial ribosome that was subsequently "reengineered" to do the same job, and was ultimately delivered at a cost of one third of the nation's gross domestic product.

How much more do we need to know about ribosome assembly? One can draw an analogy to that of an automo-

bile assembly line. At one end, all of the known parts to assemble a ribosome are fed in, and at the other end out pops a 2002 Cherry Red Ribosome GT Coupe. For some people, this is enough to know, and they are content to be a consumer of manufactured ribosomes. For others, the Encyclopedia Brittanica view of the assembly line is satisfactory, where a series of general steps is outlined: Build the chassis, add the engine, install the seats, affix the body panels, bolt on the wheels, fill-er-up, and drive. For some, myself included, nothing less than the knowledge of how and when each bolt, cotter pin, and gasket is installed is satisfactory. The most intriguing problem to me in view of the recent structure of the ribosome is "What is the detailed mechanism of ribosome assembly?"

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