

Ribonucleoprotein Particles in Cellular Processes

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THE general way of thinking about the pathway of expression of genetic information in eukaryotes, from DNA to protein via RNA, instills a protein-centric point of view, as if the protein is the real goal and the RNA is just the intermediate. In fact, most of the RNA in the cell is not mRNA but rather part of modular structures, RNA-protein machines, that are termed ribonucleoprotein (RNP) particles or ribonucleoprotein complexes. The ribosome is one well-known example of such a complex. What is new and surprising is the discovery of the tremendous variety, complexity, and versatility of RNP particles which is reflected by the wide range of cellular processes in which they are involved.

RNPs are involved in each step along the pathway of gene expression in eukaryotes. mRNAs are formed from precursor transcripts (hnRNAs or pre-mRNAs) by splicing of introns, and transported to the cytoplasm where each needs to accumulate to the precise level that will produce the correct amount of the particular protein. What has emerged over the past few years is that key components involved in processing pre-mRNA are complexes of unique small RNAs (snRNAs) with specific proteins, small nuclear ribonucleoprotein particles (snRNPs). The nuclear precursor transcripts themselves are in fact also RNPs, being complexed with specific proteins, the hnRNP proteins, as are cytoplasmic mRNAs which are associated with the mRNP proteins. Parts of the protein synthetic machinery, particularly ribosomes, are also complexes of proteins with RNA. The continual and dynamic association of messenger RNAs and their precursor hnRNA molecules with specific sets of proteins in the cell underscores the importance of understanding the molecular nature of these hnRNP and mRNP complexes.

Recent work from numerous laboratories has led to the discovery of many additional RNP particles that are involved in an unexpected variety of cellular processes including protein targeting, priming of mitochondrial DNA synthesis, and transcription termination (see Table I). Here we review some of the most recent findings on the structure, composition, and function of RNP particles in the eukaryotic cell. Ribosomes, mRNPs, and viral RNPs are not discussed here. Also, since several reviews (1-6) have been published over the last two years that discuss pre-mRNA processing, we confine the discussion to only the most recent data in this rapidly advancing field.

Many of the unpublished observations in this minireview were first reported at a meeting on eukaryotic RNPs at the European Molecular Biology Laboratory, Heidelberg, Federal Republic of Germany on October 22-24, 1987.

Nuclear Events

hnRNPs

The hnRNAs, which are the substrates of pre-mRNA processing, have long been known to be associated with proteins, the hnRNP proteins, to form hnRNPs—one of the major structures in the nucleus (reviewed in reference 7). hnRNP particles have a more complex composition than previously thought. Purification of hnRNP particles with monoclonal antibodies revealed that they contain at least 20 different proteins (8). The previously described six abundant 30-40-kD polypeptides, A1, A2, B1, B2, C1, C2 (9), are a subset of this assortment. Additional, unexpected complexity of hnRNP proteins was further illustrated by the discovery of isoforms of the hnRNP protein A1 which differ from each other by only two amino acids (Riva, S., and K. Schafer, personal communications). hnRNP proteins, particularly the C proteins, have been previously shown to be involved in splicing (10) but the mechanism and specific details of their function have not yet been elucidated. Some clues to the role of hnRNP proteins in both splicing and hnRNA packaging may come from the surprising finding that at least some hnRNP proteins have RNA-binding specificity. The hnRNP C proteins, for example, bind preferentially to uridine-rich segments of RNA (10a) including the polypyrimidine segment near the 3' end of introns (Swanson, M. S., and G. Dreyfuss, manuscript submitted for publication). These observations raise the possibility that, far from being a static particle, the hnRNP proteins may assemble into a variety of functional complexes, flexible enough to shepherd nascent RNAs from chromatin to the cytoplasm according to the individual destiny of a specific RNA. The relationship between hnRNP and snRNP binding is an issue of major interest. Electron microscopy indicates an early interaction between snRNPs and nascent pre-mRNA (11, 12) and it has been suggested that U1 might influence the binding of hnRNP proteins to pre-mRNA (13). If hnRNP proteins are bound preferentially to introns, this could be important for both splicing and for the mechanism of removal of these proteins from the pre-mRNA that must accompany nucleocytoplasmic transport.

snRNPs

The snRNAs U1-U5 are transcribed by RNA polymerase II and snRNA U6 by RNA polymerase III (see reference 14 for review and references). Interestingly, U1-U5 and U6 share

Table I. Ribonucleoprotein Particles in Higher Eukaryotes

RNP	RNA*	Proteins	Localization	Function
Nucleus				
hnRNP	hnRNA	At least 20 (34–120 kD)	Nucleus	hnRNA packaging Pre-mRNA processing
U1, U2, U4/U6, U5 snRNP	U1, U2, U4, U5, U6 (107–215nt)	B, B', D, D', E, F, G (9–27 kD), and specific proteins	Nucleus	Pre-mRNA splicing
U3 snRNP	U3 (217nt)	34 kD + 5 proteins	Nucleolus	rRNA processing?
La	RNA pol III nascent transcripts, viral RNAs	55 kD	Nucleus and cytoplasm	Transcription termination of RNA pol III
Telomerase	At least two (75, 154nt) associated with enzyme	Not well characterized	Nucleus	Addition of telomeric repeats to chromosome ends
Rare species				
U7 snRNP	U7 (63nt)	Anti-Sm precipitable	Nucleus	Histone pre-mRNA 3'-end processing
U8 snRNP	U8 (140nt)	Anti-Sm precipitable	Nucleolus	Unknown
U9 snRNP	U9 (135nt)	Anti-Sm precipitable	?	Unknown
U10 snRNP	U10 (60nt)	Anti-Sm precipitable	?	Unknown
U11 snRNP	U11 (140nt)	Anti-Sm precipitable	Nucleus	Polyadenylation?
RNase P	Several RNAs (85–115nt) associated with enzyme	Not anti-Sm precipitable	Nucleus	Pre-tRNA cleavage
Cytoplasm				
mRNP	mRNA	Poly A binding protein (72 kD) + at least 4–10	Cytoplasm	mRNA translation?, stability?, storage?, localization?
Ribosomes	5.8, 18, and 28S	>50	Cytoplasm	Protein translation
SRP	7SL (300nt)	9, 14, 19, 54, 68, 72 kD	Cytoplasm	Targeting of proteins to ER
Ro scRNP	Y1–Y5 (80–110nt)	60 kD	Cytoplasm	Unknown
Ring bodies (prosome) (cylinder particles)	Small RNAs?	20–35 kD	Cytoplasm and nucleus	mRNP repression? multifunctional protease? tRNA processing?
Mitochondrial DNA primase	5.8S + others?	Not well characterized	Mitochondria	Priming mt DNA synthesis
RNase MRP	135nt	Not well characterized	Mitochondria	Cleaves RNA primer in DNA replication
Vaults	140nt	104 kD plus at least four others	Cytoplasm	Unknown

* The protein and RNA sizes given are, where possible, taken from mammalian RNPs.

at least one promoter element, the octamer motif ATGCAAAT (15, 16), and a common transcription factor appears to bind to this site in both genes (15). This is a striking finding because it implies that this factor may influence both RNA polymerase II and III. U6 requires a 3' run of uridines for termination but, in contrast to all previously characterized pol III genes, except 7SK RNA (17), U6 does not require gene internal promoter elements for transcription initiation (Reddy, R., personal communication). Indeed sequence inspection suggests that the 7SK and U6 promoters may be closely related. Plants also contain snRNAs but the transcription signals, at least for U2 and U5, are different from those in vertebrates. These two genes have similarities in their 5'-flanking regions, including TATA-like boxes at -30 (18). The snRNAs 3'-end formation (for U1–U5) is a complex process that involves recognition of promoter elements as well as a 13–16nt¹ element located downstream of the 3' end of the mature snRNA (19, 20). Mutational analysis of promoter sequences involved in U1–U5 3'-end formation has not

so far dissociated them from elements that are also required for initiation of transcription.

Once formed, the monomethyl G-capped U snRNA is rapidly transported to the cytoplasm. There the modification of the cap occurs which converts it to a trimethyl G structure and the interaction with proteins to assemble the U snRNP takes place (reviewed in reference 21). Many of the U snRNPs have several common core proteins, B, B', D, D', E, F, and G (see reference 22 for a review of snRNP composition). The B, B', and D proteins are relatively frequent targets for human anti-Sm autoimmune antibodies often found in patients with connective tissue disease (23). Particular snRNPs also have specific proteins such as the U1 70 kD, A and C, and the U2 A' and B". One of the common and evolutionarily conserved features of several snRNAs (U1, U2, U4, and U5) is the sequence PuA(U)nGpu(n > 3) which is flanked by double-stranded stems. This region is the "Sm-binding site" for the anti-Sm-reactive snRNP proteins (see reference 21 for further references). The binding sites of the other snRNP proteins on snRNAs are also being determined and snRNPs that are functional by at least some criteria have been reconstituted in vitro (21, 24, 25; Green, M., personal

1. Abbreviations used in this paper: ER, endoplasmic reticulum; nt, nucleotides; SRP, signal recognition particle.

communication). The first glimpses of an snRNP, U1 snRNP, have been obtained by electron microscopy and it looks much like a head with two big ears (Kastner, B., and R. Lührmann, manuscript in preparation).

Systematic analysis of the snRNAs in the yeast *Saccharomyces cerevisiae* (see, for example, references 26–28) show that there is a direct one-to-one correspondence between the yeast and mammalian snRNAs involved in mRNA splicing. There are differences in size of most snRNAs; yeast U1 and U2 are three and six times larger, respectively, than their mammalian counterparts. A striking exception is the extraordinary conservation of U6 which is very similar in size and sequence to the mammalian counterpart (Brow, D., and C. Guthrie, personal communication).

Molecular cloning and sequence analysis of the cDNAs and the genes for several human snRNP proteins yielded much information about the primary structure of these proteins. These include the snRNP core proteins B, B', and D (Hoch, S., personal communication) and E (29), the U1 snRNP 70-kD protein (30–32), U1 snRNP A (33), the U2 snRNP A' (Fresco, L., and J. Keene, personal communication), and the U2 snRNP B'' protein (34). One generality that emerges so far is that several of the snRNP proteins (e.g., U1 70 kD and A, and the U2 B'') contain the RNP consensus sequence. This is a segment of eight amino acids, Lys (Arg) Gly Phe (Tyr) Gly (Ala) Phe Val × Phe (Tyr), which is the most highly conserved segment in a 90–100 amino acid RNA-binding domain. This larger domain also contains other less conserved elements, such as a segment of six aliphatic and aromatic residues ~35–40 amino acids amino terminal to the RNP consensus sequence (see reference 37 for discussion of this point). The RNP consensus sequence has also been found in other RNA-binding proteins including hnRNA-, mRNA-, and nucleolar pre-rRNA-binding proteins (35–37). Although experiments with the yeast poly (A)-binding protein suggest that the RNP consensus sequence itself may not be essential for the ability of the protein to associate with poly (A) (38), the RNP consensus sequence may be useful because of its potential predictive power; proteins that contain it are likely to be RNA-binding proteins. It also suggests that many RNA-binding proteins along the pathway of mRNA formation, those which contain the RNP consensus sequence, may have evolved from a common ancestral gene and that this motif may define a class of RNA-binding proteins. Of course, many RNA-binding proteins exist (ribosomal, virus nucleocapsid, etc.) that do not contain this consensus motif.

snRNPs in Splicing

At least five snRNPs, U1, U2, U4, U5, and U6, are involved in pre-mRNA splicing (see references 1–5 for reviews). U1 binds to the 5' intron junction and U2 binds to the branch site. Results from various laboratories suggest that the U2 binding requires not only ATP but also at least one other factor. Two candidates, designated U2AF (39) and IBP (which has been found in two forms possibly related by proteolysis [40, 41]), have been identified. Both of these bind to the 3' part of introns independently of snRNPs, although IBP may associate with an snRNP, probably U5. These factors are therefore proposed to recognize the 3' intron junction and to promote U2 snRNP binding to the branch point. U2AF and IBP are

not identical. Unfortunately, the fractionation conditions used in the most extensive purification of splicing activities to date (42) are not similar enough to those used in the study of U2AF or IBP to identify them with one of the splicing factor (SF1–SF4) activities. Two of these activities, SF1 and SF3, which appear to be proteins, are both required for U2 snRNP binding to the branchpoint (Krämer, A., personal communication).

SnRNPs U4 and U6, which are also required for splicing, exist as a base-paired complex-U4/U6, (43–45). They probably do not interact with the pre-mRNA directly (46). Work from several groups indicates that the snRNPs exist in complexes in both mammalian cells and yeast and that their interactions are ordered, dynamic, and ATP dependent (46–51) (see Fig. 1). U4, U5, and U6 form a major complex independent of other snRNPs (47, 50, 51) which may react differently to exogenous ATP in yeast and mammalian extracts, although results obtained using different methods in yeast disagree on the effect of ATP on the U4–U5–U6 complex (50, 51). Analysis of splicing complexes following native gel electrophoresis or affinity purification showed that U4 dissociates from the spliceosome at the same time as, or before, cleavage at the 5' splice site and that U2, U5, and U6 remain in a complex that is released with the intron lariat (47, 49, 50, 52). The snRNPs, therefore, have to dissociate and assemble in the nucleus with every splicing cycle, reminiscent of the cycling of the translational machinery.

The impressive power of genetic analysis in yeast combined with the yeast *in vitro*-splicing system has been further used and continues to turn up new information. Several of the *rna* mutants (53), many of which turned out to be splicing mutants (54, 55), have now been characterized. The gene product of RNA 8, for example, is essential for viability and encodes a large 260-kD protein which is stably associated with U5 and is detected in the spliceosome (51). A new collection of additional splicing mutants has been generated (Vijayraghavan, U., and J. Abelson, personal communication) and it can be anticipated that after analysis much more will be learned about nuclear RNA metabolism.

Substantial progress is also being made in the biochemical fractionation of both HeLa (42) and yeast (56) nuclear extract for splicing activities. Fractionation is greatly facilitated by the use of high resolution gel electrophoretic separations of intermediate complexes and analysis of specific pre-mRNA mutants; for example, to resolve which accessory factors, if any, are required for UsnRNP binding to the intron, as discussed above. A new column chromatography preparative fractionation method of functional and probably highly purified spliceosomes has been developed and used for the observation of the complexes by electron microscopy (Reed, R., and T. Maniatis, personal communication). It can be expected that important information will be obtained by this approach, particularly once specific antibodies are used for immunoelectron microscopy.

The combination of biochemical and genetic approaches will no doubt yield a detailed picture of RNA splicing. But it is already apparent that the multicomponent splicing complex is an efficient and precise splicing machine that is made up of modular parts, snRNPs, and other components, such as hnRNP proteins, that assemble in an ordered manner to perform the reaction and dissociate once it is done. In the end, it may well turn out that the RNA constituents of the

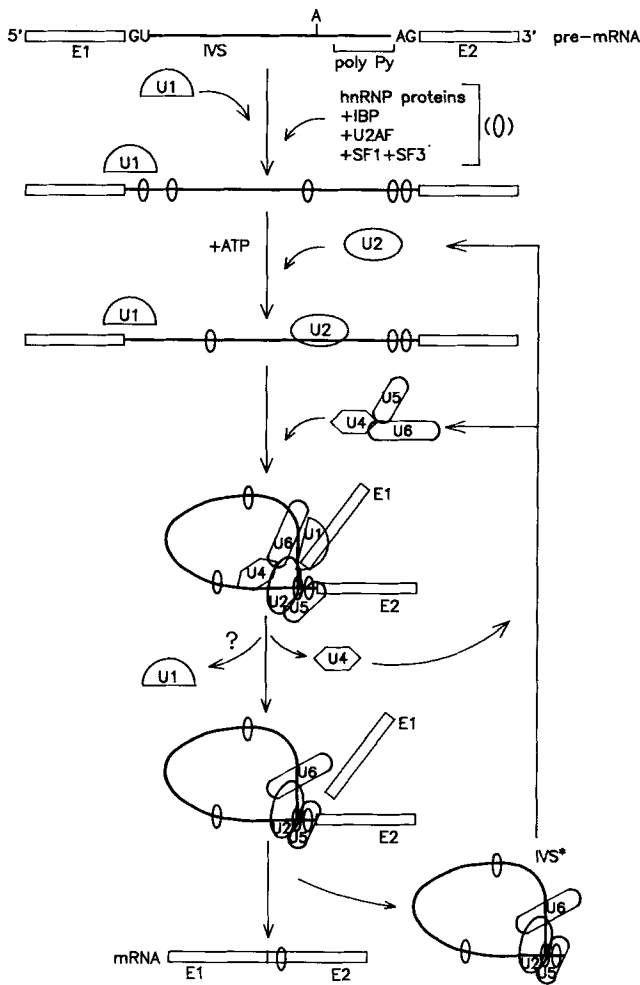


Figure 1. The splicing pathway of pre-mRNA in mammalian cells. *IVS*, intervening sequence (intron); *IVS**, intervening sequence in lariat form; *poly Py*, polypyrimidine stretch; *E*, exons; *IBP*, intron-binding protein(s); *U2AF*, U2 snRNP auxiliary factor; *SF1*, *SF3*, splicing factors 1 and 3. The question mark at the exit point of U1 snRNP indicates the uncertainty as to the stage at which U1 snRNP dissociates.

snRNPs catalyze the cleavage and ligation reactions, without protein enzymes, and that the function of the snRNPs is to bring the junctions together to form the reaction center.

snRNPs in 3'-end Processing, Polyadenylation, and Transcription Termination

Analysis of 3' cleavage and polyadenylation *in vitro* has led to the discovery that this process, like intron splicing, takes place in large complexes. Formation of these complexes is inhibited by either mutation within, or chemical modification of, the AAUAAA polyadenylation signal (57-60).

Various autoimmune antisera containing antibodies directed against snRNPs have been shown to inhibit polyadenylation reactions *in vitro* (61, 62) and sequences including the AAUAAA signal are protected from RNase digestion by nuclear extract components, some of which have determinants recognized by anti-Sm antisera (63). These observations have led to the expectation that at least one U snRNP

would be involved in polyadenylation. However, cleavage and polyadenylation *in vitro* are not inhibited specifically by micrococcal nuclease digestion, although there is a requirement for bulk RNA in the extract (64). This might simply mean that any snRNA required is resistant to nuclease digestion, and one of the fractions required for polyadenylation contains snRNP(s), predominantly an snRNA-designated U11 (65). The complete nucleotide sequence of U11 has now been determined (Montzka, K., and J. Steitz, personal communication) and it does not contain any complementarity to the polyadenylation motif AAUAAA. It therefore remains unclear whether U11 snRNP provides for specific recognition of the polyadenylation signal. A 64-kD nuclear protein that binds to RNA segments that contain the AAUAAA sequence has been detected (66) and may be a better candidate.

A more definite role for an snRNP has been reported for histone pre-mRNA 3'-end processing. Work in sea urchins led to the discovery of a snRNA, U7, which is required for 3'-end processing of sea urchin histone pre-mRNA (6, 67). In the mammalian system it appears that a cell cycle-dependent heat labile factor is also involved and this is now being purified (6, 68). Two mammalian U7 homologues from human (69) and mouse (70) have also been identified and sequenced and contain segments that can base pair with the downstream element of the histone pre-mRNA that is required for its processing. U11 and U7 are much lower in abundance (~1:10-1:100) than U1 snRNA and they can be precipitated with anti-Sm antibodies. Many more anti-Sm precipitable low abundance snRNAs can be detected on two-dimensional gels and remain to be characterized (Steitz, J., personal communication). It will be interesting to see if they, like the high abundance U snRNAs, also correspond to some of the many identified yeast snRNAs (71).

A subset of seven tri-methyl capped snRNAs, including the yeast U3 equivalent (72), have recently been shown to be found associated with ribosomal RNA precursor species in the *S. cerevisiae* nucle(ol)us (73). Deletion of one of them, snR10, results in impaired cleavage of the 35S precursor RNA, although evidence for direct participation of any of these RNAs in particular pre-rRNA processing steps will probably have to await the development of an *in vitro* processing system.

The autoantigen RNP protein La, which is associated with RNA polymerase III products, has also been cloned and sequenced from human (74; partial sequence) and *Xenopus* (complete sequence; Scherly, D., and S. Clarkson, manuscript in preparation) and it also contains a region which resembles the RNP consensus sequence. La binds to the U-rich end of all newly synthesized RNA polymerase III transcripts. An extremely exciting finding about the function of La is that it appears to be a transcription termination factor, required for accurate and efficient dissociation of RNA polymerase III from nascent transcripts (Gottlieb, E., and J. Steitz, manuscript in preparation).

snRNPs in Other Nuclear Functions

The latest addition to the family of nuclear functions involving RNPs is telomerase (75). This enzyme, which adds telomeric repeats to the ends of chromosomes, has so far only been characterized in *Tetrahymena*. The similarity in telo-

mere structure exhibited by various organisms, however, suggests that the telomerase activity may also be conserved. The enzyme is sensitive to both RNase and protease digestion and highly purified enzyme is associated with at least two short RNAs. This example adds to the diversity of functions involving RNPs, and serves as a warning against the assumptions that newly characterized activities are not RNPs or that newly characterized RNPs are necessarily involved in RNA metabolism.

Cytoplasmic Events

Apart from ribosomes the most studied cytoplasmic RNP is the signal recognition particle (SRP).¹ SRP is an abundant small RNP that mediates the targeting to the endoplasmic reticulum (ER) of secretory, membrane, and lysosomal proteins (76). SRP, at least in some in vitro systems, recognizes and interacts with the signal peptide in these proteins while they are ribosome associated and causes pausing in the elongation (arrest) of translation. The SRP-arrested ribosome complex is then targeted to and docked at the ER through binding to a SRP receptor, which has also been called docking protein (77, 78). With this interaction, a translation-translocation complex is established, and the translation that now resumes is coupled to trans-membrane insertion of the nascent polypeptide into the lumen of the ER. The SRP is composed of one molecule of RNA, 7SL RNA, (of ~300 nucleotides), and six proteins (79). It has been purified to homogeneity and reconstituted (80) and its structure, functions, and interactions with other components of the translational apparatus and the ER have been studied in detail. The 7SL RNA appears to serve a structural role; it is an elongated scaffold for the organized binding of the proteins (81, 82). The four largest proteins bind at one end to form a domain which promotes translocation into the ER lumen, while the proteins bound at the other end are thought to cause translational arrest (83, 84). Consistent with this structural role for the RNA is the finding that the nucleotide sequence of 7SL RNA from the yeast *Schizosaccharomyces pombe* bears very little similarity to mammalian 7SL RNA, but both RNAs can be folded into similar predicted secondary structures (85, 86, 87). The same holds true for 7SL RNA from the yeast *Yarrowia lipolytica*. In spite of this lack of sequence conservation, mammalian SRP proteins bind specifically to these yeast RNAs, as determined by RNA footprinting (87). 7SL thus provides an interesting contrast to other small RNAs (e.g., sn RNAs U1, U2, and U7), which function in nucleic acid (pre-mRNA) recognition through specific, conserved, base pairing.

Two distinct activities, both associated with mitochondrial DNA replication, have been recently shown to have RNP components. The mitochondrial DNA primase activity is closely associated with cytosolic 5.8S rRNA, and this RNA is essential for its activity. Other possible RNA and protein components of this enzyme are as yet not well characterized (88). The other enzyme, an endoribonuclease involved in primer RNA cleavage, contains a nuclear encoded 135 nt RNA and protein components that have not yet been identified (89). The intriguing problem of how nuclear RNAs enter the mitochondrion has not as yet been tackled, but it may be instructive to compare this process with the biogenesis of

snRNPs, whose assembly in the cytoplasm is required before nuclear transport of the assembled RNP (21; see reference 89 for a discussion of the point).

Other small cytoplasmic RNPs are still elusive in their function or have their membership cards to the RNP club hotly contested. The latter include the ringlike bodies, cylinder particles, and prosomes which are found both in the nucleus and cytoplasm, at least in some cell types. The debate (90–92) centers on whether all of the different “ring body” preparations are in fact the same, and whether they indeed contain stoichiometric, or even significant, amounts of RNAs. Careful analysis of the particles from *Xenopus* oocytes failed to reveal evidence for a specific RNA component (90), however, these particles, defined by their ringlike morphology, size, and a characteristic set of proteins, are apparently ubiquitous from plants to man and it may be that the particles show either species or cell-type differences in composition. Their “legitimacy” as RNPs notwithstanding, there is now the interesting possibility that at least some of these ring bodies are, or at least copurify with, large multifunctional proteases (93, 94) and as such may be important elements in protein turnover. Another small cytoplasmic RNP has been recently isolated and termed vaults to indicate its distinct morphology which resembles the multiple arches of cathedral vaults (95). Vaults are composed predominantly of a 104-kD protein and a small RNA but their function is not yet known.

It has also been reported (96) that RNase P activity copurifies with ring particles from *Xenopus* oocytes although whether RNase P and ring particles are identical, or even associated with one another, is disputed. RNase P cleaves tRNA precursors to generate the mature 5' end and is, in prokaryotes, an RNP whose RNA moiety, M1 RNA, is capable alone of catalysing the cleavage reaction in vitro, but whose protein component is also essential in vivo (see reference 97 for a review). Eukaryotic RNase P has not been purified to homogeneity, but there is evidence that it is also an RNP. The enzyme activity from *S. pombe* is associated with two RNAs, both of which are transcribed from one gene, whose predicted secondary structures are similar to that of the M1 RNA. The *S. pombe* activity is sensitive to digestion with micrococcal nuclease (98). HeLa cell nuclear RNase P can also be dissociated into RNA and protein components. Interestingly, activity can be reconstituted from these fractions by adding back the complementary protein or RNA fraction from *E. coli* RNase P (99).

Perspectives

Altogether, it is already clear that the complexity of the structure and function of the RNAs and of the RNP proteins is much greater than previously envisioned. It is almost certain that RNPs will be found to possess wide-ranging activities in RNA and protein formation, stability, and function. The versatility of RNA-protein machineries is reflected in the ability of RNAs to recognize other nucleic acids by base pairing and to serve as scaffolds upon which proteins can be assembled to form modular RNA-protein units. The RNA-protein interaction may also influence their catalytic activity. In addition, the potential of RNA itself to function in catalysis (100) lends yet another novel perspective to the topic of

RNPs and their evolution. Since the ways to peek inside—the systems and tools of research—continue to develop, the excitement and pace of discoveries in this field are not likely to abate soon.

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