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STRUCTURE AND FUNCTION OF NUCLEAR AND CYTOPLASMIC RIBONUCLEOPROTEIN PARTICLES

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INTRODUCTION

The pathway of expression of genetic information in animal cells, from DNA to protein via RNA intermediates, is highly complex and tightly regulated (Darnell 1982; Nevins 1983). Particularly intricate are the post-transcriptional processing events required to convert the primary gene transcripts into the functional intermediates designated messenger RNAs (mRNAs). The primary transcripts of RNA polymerase II, except for a

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distinct group of small RNAs, are referred to as heterogeneous nuclear RNAs (hnRNAs). In higher eukaryotes all hnRNAs have a 5′-cap structure (m^7Gppp-), and up to one-quarter of them also acquire a 3′-polyadenylate [poly(A)] tail. Only a subset of hnRNAs, about one-fifth to one-quarter of the hnRNAs in higher eukaryotes, are actually precursors to translatable cytoplasmic mRNA, and these are designated pre-mRNAs (Brandhorst & McConkey 1974; Herman & Penman 1977; Harpold et al 1979; Salditt-Georgieff et al 1981, 1982). Typically, pre-mRNAs contain polyadenylated tails, and the majority of them contain intervening sequences that are later spliced out (Abelson 1979; Green 1986; Padgett et al 1986). Little is known about the ensuing events except that the RNA is translocated through nuclear pores and that spliced mRNAs accumulate in the cytoplasm.

mRNAs conserve the 5′- (cap) and 3′- [typically a poly(A) tail; Brawerman 1981] ends of the respective pre-mRNAs and contain an uninterrupted reading frame for translation. At any given time, not all translatable mRNAs in the cell are actually translated. The actively translated mRNAs are engaged with ribosomes to form polyribosomes and can be readily separated from the untranslated mRNA by velocity sedimentation in sucrose gradients. The nonpolysomal-to-polysomal ratio is not the same for all mRNAs nor is it fixed. It can drastically change for specific mRNAs in response to specific signals and for some or all of the mRNAs under a variety of environmental (e.g. heat shock, virus infection) and developmental circumstances. Thus, although the relative abundance of specific mRNAs is typically the major factor that determines the relative amounts of various proteins synthesized, the translation repertoire of the eukaryotic cell can vary even for a given set of mRNAs due to differential selection of mRNAs for translation. This process is referred to as translational regulation or translational control. Unlike in prokaryotes, in eukaryotes the majority of mRNAs are quite stable, and many have a half-life of the order of the cell cycle time itself (Brandhorst & Humphries 1971; Singer & Penman 1972; Greenberg 1972; Brandhorst & McConkey 1974). Different mRNAs have different half-lives, and these also can be modulated for specific mRNAs in a given cell (e.g. globin mRNA, tubulin mRNA). Modulation of mRNA stability is an extremely important process because it can drastically affect the level of a specific mRNA; however, little is known about the elements that control this stability. Untranslated mRNAs are sometimes stabilized and stored for very long periods of time, as is the case in many oocytes. One of the ultimate goals of molecular and cell biology is to understand all of these processes in terms of both molecular detail and cellular topology.

Experimentally, hnRNAs can be distinguished from other RNAs on the

basis of their size, their subcellular compartmentation, and the characteristics (e.g. antibiotic sensitivity) of the RNA polymerase that transcribes them. With this operational definition, it was found that hnRNAs, like other polynucleotides in the nucleus, form complexes with specific proteins. The unique particles thus generated are termed hnRNP particles or hnRNP complexes. The hnRNP particles are one of the most abundant structures in the nucleus and are the sites of RNA processing. Similarly, mRNAs in the cytoplasm are associated with specific proteins to form mRNP complexes. The mRNP and hnRNP proteins are different, and an exchange of proteins therefore accompanies mRNA nucleocytoplasmic transport. The mRNP proteins are likely to be important in the translation, stability, and localization of mRNAs and perhaps also in mRNA nucleocytoplasmic transport. Interest in RNP complexes stems from the fact that they are the structural entities within which hnRNA and mRNA exist in the cell (rather than as naked polynucleotides). Therefore more needs to be learned about them to understand how the posttranscriptional portion of the pathway of expression of genetic information operates in the cell.

This review outlines major recent developments and significant earlier observations that led to current knowledge of the structure and function of the ribonucleoprotein complexes of hnRNA and mRNA. Space limitations preclude comprehensive citation of the numerous publications in the field, many of which are worthy of extensive discussion. Additional discussion and references are found in several recent reviews on these subjects (Martin et al 1980; Samarina & Krichevskaya 1981; Knowler 1983; Spirin & Ajtkhozhin 1985). The organization of hnRNP and mRNP particles in the nucleus and cytoplasm, respectively, and their possible association with underlying subcellular structures are issues of tremendous interest and potential significance but are beyond the scope of this review.

NUCLEAR RIBONUCLEOPROTEIN PARTICLES

Evidence for, and Isolation of, hnRNP Particles

MORPHOLOGICAL STUDIES The original ideas about the existence of nonribosomal nuclear RNA as a component of RNP complexes emanated from electron microscopic observations. In the 1950s Gall (1955, 1956) and Swift (1963) described ribonuclease-sensitive granules associated with or near chromosomes in several different cell types. Microscopic observations of RNA polymerase II transcripts on the large lampbrush chromosomes of amphibian oocytes suggested that the hnRNA becomes associated with proteins to form ribonucleoprotein structures (hnRNPs) immediately upon transcription (Gall & Callan 1962; Malcolm & Sommerville 1974). Using refined chromatin spreading techniques Miller and

colleagues (Miller & Bakken 1972; McKnight & Miller 1976) examined the morphology of nascent transcripts of specific genes. With these spreading techniques the chromatin is dispersed and the nascent RNP molecules, both extended and protein deficient, were visualized with remarkable clarity. They showed a linear array of beaded globular protein units, about 20 nm in diameter, connected by RNAase-sensitive strands (Foe et al 1976; Lamb & Daneholt 1979; Malcolm & Sommerville 1977; McKnight & Miller 1979; Sommerville 1981). More recent studies revealed an orderly arrangement of the protein particles on specific nascent hnRNAs in situ (Beyer et al 1980, 1981; Osheim et al 1985; Tsanev & Djondjurov 1982).

BIOCHEMICAL STUDIES Biochemical studies, beginning with the pioneering work of Samarina, Georgiev and their colleagues in the 1960s (Samarina et al 1966, 1968; reviewed in Samarina & Krichevskaya 1981), provided additional evidence for hnRNA-protein complexes and much information about the hnRNP particle. If protein denaturants (chaotropic reagents or ionic detergents) and high salt concentrations are avoided, most of the hnRNA can be released from nuclei with considerable amounts of protein associated with it. The most common methods of releasing hnRNA-protein complexes from nuclei are by mechanical disruption (e.g. sonication) or by leaching out after limited RNAase digestion (endogenous or exogenous RNAases) (Samarina et al 1968; Lukanidin et al 1972; Pederson 1974; Martin et al 1975; Beyer et al 1977; Karn et al 1977; Stevenin et al 1977; Maundrell & Scherrer 1979; Walker et al 1980). The bulk of the chromatin and nucleoli in mechanically ruptured nuclei are usually first removed by low-speed centrifugation, and the clarified fraction is defined as the nucleoplasm. The hnRNA in the nucleoplasm sediments in sucrose gradients (at moderate salt concentration of 50–100 mM NaCl) as a heterodispersed material between 30 and 250 S. It has a buoyant density (after fixation with formaldehyde or glutaraldehyde) of 1.3–1.4 g/ml, which indicates that it is composed of about 75–90% protein (Samarina & Krichevskaya 1981). In contrast, the protein-free hnRNA sediments much more slowly under the same conditions, at about 30 S or less, and has a buoyant density of 1.8 g/ml. All of the heterodispersed fast-sedimenting hnRNA in the nucleoplasm is converted by mild RNAase digestion to slower sedimenting material. Much of the latter forms relatively discrete homodispersed particles that sediment under the same conditions at 30–40 S (Pederson 1974; Beyer et al 1977; Karn et al 1977) (referred to hereafter as 30-S particles or monoparticles). The hnRNA-containing material that is released from nuclei by mild exogenous nuclease digestion or by prolonged incubation at 37°C (which presumably allows digestion with endogenous RNAase), also sediments as 30- to 40-S particles (Samarina et al 1968; Martin et al 1978; LeStourgeon et al 1978).

Each of the different cell fractionation methods has considerable limitations. When prepared as above, the nucleoplasm contains only about 50% of the total nuclear hnRNA and the hnRNP proteins; the rest are associated with the chromatin pellet and cannot be readily analyzed. The analysis of nucleoplasm therefore necessarily excludes a sizeable portion of the hnRNPs, and the sheer force may disrupt large hnRNP complexes. In the RNAase release method, the hnRNA and the hnRNPs are fragmented, and proteolysis and protein rearrangements (cf Stevenin et al 1979) can occur in the course of the prolonged incubations at high temperature. The specific portion of the total hnRNPs analyzed in these studies is not well documented and likely also represents only about 50% of the rapidly labeled nuclear RNA. It has, in fact, been suggested that in gently lysed nuclei all of the hnRNP particles are somehow associated with chromatin (Kimmel et al 1976).

An interesting approach that circumvents the need for any nuclear fractionation was utilized by Lahiri & Thomas (1985) to examine the hnRNPs in mitotic cells. In these cells gentle lysis of the plasma membrane is sufficient to release hnRNPs. The hnRNP particles from mitotic cells were found to be similar to those prepared from interphase cells by the above methods. Although the precise sedimentation properties of the released RNP complexes depend on the monovalent ion concentration and on the tissue of origin, complexes of similar general properties have been prepared from a wide and diverse range of cells in culture and in tissues. These cells include (in addition to those from mammals, avians, and amphibians) those from *Drosophila* (Risau et al 1983), *Artemia salina* (Marvil et al 1980; Nowak et al 1980), *Dictyostelium* (Firtel & Pederson 1975), and *Physarum* (Christensen et al 1977). There is little or no information in the literature about the RNP complexes in yeast, protozoa, or plants.

The existence of hnRNA in hnRNA-protein complexes was easily accepted, but a clear and consistent picture of the composition and structure of hnRNP complexes did not readily emerge. Progress in unambiguously identifying the hnRNP proteins has been hampered, perhaps to the largest extent, by the limitations of the experimental methods (such as velocity sedimentation and isopycnic banding) commonly used to prepare hnRNP complexes. These methods have not resulted in the complete separation of intact and pure hnRNPs. They rely on copurification of proteins with RNA from fractionated cells as the criterion for the identification of RNP proteins and therefore have several serious shortcomings. First, because adventitious RNA-protein associations can occur, it is difficult to ascertain, in the absence of other data, that a protein was an authentic RNP protein in the cell. Second, authentic RNP proteins may

dissociate under the conditions used for the isolation of the RNA-protein complex. Third, contaminating structures of similar sedimentation and physical properties cannot be separated from actual hnRNPs. And fourth, the identification of RNPs is limited by the ability to unambiguously identify labeled hnRNA.

In spite of these inherent limitations, reproducible patterns on sucrose gradients did provide the basis for the consensus that gradually emerged that a group of proteins in the 30–43 kDa range is associated with hnRNA in 30-S monoparticles. These proteins, first described by Samarina et al (1968) as one or two proteins, were later, with improved electrophoretic techniques, shown to be considerably more complex (Martin et al 1974, 1978; Pederson 1974; Billings & Martin 1978; Beyer et al 1977; Karn et al 1977) and to consist of six bands by one-dimensional SDS-polyacrylamide gel electrophoresis (SDS-PAGE). These proteins, some or all of which are also occasionally referred to as "core proteins," are a distinct, nonchromatin subset of nuclear proteins. This specificity further argues for the authenticity of hnRNP particles. The A, B, C nomenclature (in HeLa cells A1 = 34 kDa; A2 = 36 kDa; B1 = 37 kDa; B2 = 38 kDa; C1 = 41 kDa; C2 = 43 kDa; Table 1) of Beyer et al (1977) is the most widely accepted and will be used here. Numerous other proteins of higher molecular weight were also reported, but their authenticity remained controversial; it was even suggested that there may be several different types of hnRNP monoparticles (Gattoni et al 1978; Stevenin et al 1977; Jacob et al 1981). The several-hour-long sedimentation could have affected the hnRNPs present via the effects of proteolysis, RNAases, centrifugal drag force, or protein rearrangements. Hence, at that time researchers understandably reasoned (e.g. Beyer et al 1977) that only proteins that tracked precisely with and only with hnRNA or hnRNA fragments could be classified as hnRNP proteins. Thus proteins that sedimented under the same conditions also outside of the hnRNA peak were not considered hnRNP proteins because it was assumed that hnRNP proteins must all be contained only in stable hnRNP particles. The criterion of cosedimentation is also subject to the limited resolution of one-dimensional SDS-PAGE, in which different proteins with similar mobilities can be erroneously regarded as the same protein.

PHOTOCHEMICAL CROSS-LINKING IN INTACT CELLS Decisive evidence for the existence of hnRNA in the nucleus in distinct hnRNA-protein complexes was recently obtained using UV-induced RNA-protein cross-linking in intact cells (Mayrand et al 1981; Mayrand & Pederson 1981; Van Eekelen et al 1981a,b; Dreyfuss et al 1984a,b; Bag 1984; Greenberg & Carroll 1985). This method of RNP identification overcomes the problems

encountered with the preparation methods described above, because it involves the identification of proteins that are in direct contact with RNA in vivo. The photochemical UV cross-linking method relies on the fact that UV light photoactivates RNA and converts it to an extremely reactive, short-lived molecule that reacts virtually indiscriminately with other molecules, including proteins, in direct contact with it. In effect this is photoaffinity labeling of the RNA binding protein in vivo. The cross-linked hnRNA-protein and mRNA-protein complexes can then be isolated from the nuclear and cytoplasmic fractions, respectively, after boiling in SDS and mercaptoethanol. The hnRNAs or mRNAs can be isolated by affinity chromatography on oligo(dT)-cellulose, to which they bind through their 3′-poly(A) tails. The method is general, simple and clean. The protein-denaturing conditions (boiling in SDS) ensure that only proteins covalently linked to the RNA are purified with it. This process eliminates proteins nonspecifically associated with the RNA and prevents the loss of genuine RNP proteins which may occur during cell fractionation. The proteins can be released from the RNA-protein cross-linked complexes by digestion with RNAases and analyzed by SDS-PAGE. This method is highly specific and very efficient; under proper conditions the yield of the reaction (in terms of both RNA recovery and protein cross-linking) is high, and RNA breakage is minimal (Adam et al 1986a). Thus one can examine the proteins that interact with essentially the entire polyadenylated RNA population, rather than a subset of it.

The major [^{35}S]methionine-labeled proteins that become cross-linked to polyadenylated hnRNA in the HeLa cells have molecular weights of 120, 68, 53, 43, 41, 38, and 36 kDa (Mayrand et al 1981; Van Eekelen et al 1981a; Economides & Pederson 1983; Dreyfuss et al 1984a,b; Choi & Dreyfuss 1984b). The cross-linked proteins at 36 and 38 kDa probably correspond to A and B proteins, and the bands at 41 and 43 kDa are the C1 and C2 of the 30-S hnRNP subparticles described by Beyer et al (1977). The cross-linking of hnRNA to a unique set of proteins in vivo indicates that the hnRNA is associated with a specific set of RNA binding proteins.

ISOLATION OF THE hnRNP COMPLEX BY SPECIFIC IMMUNOADSORPTION By immunizing mice with UV cross-linked RNA-protein complexes obtained in vivo, monoclonal antibodies to genuine RNA-contacting hnRNP proteins were recently generated (Dreyfuss et al 1984b; Choi & Dreyfuss 1984a). Several of these antibodies have been used as immunoaffinity reagents to isolate the hnRNP complex from vertebrate cell nucleoplasm employing rapid immunoadsorption (Choi & Dreyfuss 1984b). Immunoadsorptions with two different monoclonal antibodies (4F4 and 2B12) to the hnRNP C proteins both isolate a similar complex from Hela cells. The

complex contains proteins and large hnRNA of up to ~10 kilobases (kb in length (Choi & Dreyfuss 1984b). More than 50% of the rapidly labele nucleoplasmic RNA can be readily immunoprecipitated. By SDS-PAGE the major proteins of the isolated complexes labeled by [^{35}S]methionine t steady state are of 34, 36 (A1 and A2), 37, 38 (B1 and B2), 41, and 43 kD (C1 and C2); and doublets of 68 and 120 kDa (Figure 1). Additiona proteins of 45 kDa and much larger are also seen, but so far little is know about them. In SDS-PAGE the major proteins of the complex appea identical to those hnRNP proteins that become cross-linked to the hnRN upon UV-light exposure in vivo. Immunoprecipitation with different, non cross-reacting monoclonal antibodies to the 120-kDa protein (Choi & Dreyfuss 1984b) and to the A1 protein (Pinol-Roma et al, unpublishe results) isolates the same complex of proteins in a similar stoichiometry Similar hnRNP complexes were isolated from rodent and avian cells.

The advantages of the immunoaffinity procedure for the isolation of th hnRNP complex are that it is specific, rapid, and mild and that it is no dependent on radioactive labeling for detection of the hnRNA. It yield very large hnRNP particles that appear to be intact and pure. The co immunoprecipitation of the hnRNA and all of these proteins with anti

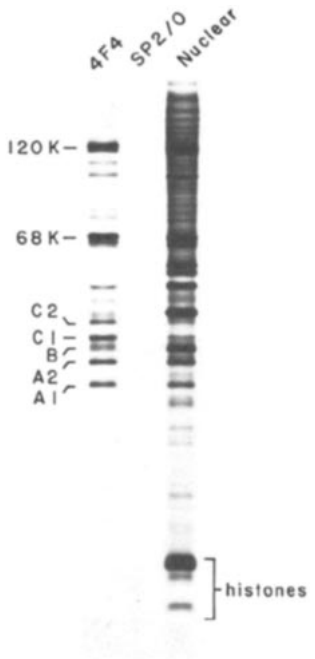

Figure 1 The proteins of human hnRN particles. hnRNP particles were isolate from growing HeLa cell nucleoplasm b immunoabsorption with a monoclon antibody (4F4) to the hnRNP C1 and C proteins. The proteins were labeled by cu turing the cells with [^{35}S]methionine ove night (Choi & Dreyfuss 1984b).

bodies to different genuine hnRNP proteins strongly suggests that the hnRNP complex is a unitary structure that comprises highly conserved protein components. The isolation of the hnRNP complex has further defined the components of this structure and will make it possible to study them in detail. Based on results from SDS-PAGE of [^{35}S]methionine-labeled proteins in isolated hnRNP and nuclear fractions, it has been estimated that the hnRNP complex is one of the most abundant structures in the nucleus, accounting for at least one-third of the protein in the nucleoplasm. The evidence accumulated so far indicates that there is only one type of hnRNP complex, but in principle, minor forms comprised of other proteins may also exist.

General Structural Features of hnRNP Particles

As described above, early sedimentation data and RNA and protein composition data indicated that large hnRNP complexes are heterodispersed polyparticles that are converted to monoparticles (30-S complexes) by mild nuclease digestion. A "beads-on-a-string" structure for the hnRNA and its associated proteinaceous particles (see Samarina & Krichevskaya 1981) was suggested by the sedimentation data and by the overall shape and dimensions visualized by the electron microscopy of sedimented polyparticles, 30-S particles (Samarina et al 1968; Beyer et al 1977; Karn et al 1977; Martin et al 1978), and of particles seen on nascent transcripts in spread chromatin preparations (Miller spreads; e.g. McKnight & Miller 1976; Beyer et al 1981; Tsanev & Djondjurov 1982). Each polyparticle appears to be a unit of one hnRNA chain with proteinaceous monoparticles. Although the hnRNA alone may by itself provide the link necessary to hold the 30-S particles together as a polyparticle, additional factors and protein-protein, protein-RNA, or RNA-RNA interactions may serve ancillary roles in holding the large hnRNP complex together. Most parts of the hnRNA, with the clear exception of the poly(A) tail, are engaged with or are between 30-S particles. The heterodispersed sedimentation observed for the bulk of nucleoplasmic hnRNA results from the differences in lengths and structure of specific hnRNAs, which determines the number of monoparticles associated with each. Specific hnRNAs may sediment as more discrete forms (Sperling et al 1985). Further RNAase digestion of monoparticles causes them to dissociate, which suggests that the RNA associated with monoparticles is essential for maintaining their integrity. RNA-protein interactions are therefore important in holding the monoparticles together, and protein-protein interactions are not sufficient to do so.

The rapidly labeled RNA that cosediments with 30-S monomers can

range in length from about 100–1000 nucleotides (usually 500 ± 100) depending on the extent of RNAase digestion used in the preparation. This RNA includes pre-mRNA sequences, as determined by hybridization to total cytoplasmic polyribosomal cDNA (Kinniburgh & Martin 1976) and to specific mRNAs (Maundrell & Scherrer 1979; Pederson & Davis 1980; Munroe 1982; Stevenin et al 1982). Several reports (Sekeris & Niessing 1975; Deimel et al 1977; Gallinaro & Jacob 1979; Howard 1978; Zieve & Penman 1981) describe the detection of small nuclear RNPs (snRNPs) associated with hnRNP complexes. However, in most cases the specificity of the association is not certain. Evidence for interaction between snRNAs and hnRNAs in vivo has been presented (Calvet & Pederson 1981, 1982; Setyono & Pederson 1984).

Proteins of hnRNP Particles

The major steady-state [^{35}S]methionine-labeled proteins of HeLa hnRNP particles recognized so far are the A, B, and C proteins and doublets of 68 and 120 kDa (Figure 1 and Table 1). Except for some reduction in the 68K doublet, most of these proteins are also seen in the 30- to 40-S fraction after sedimentation in sucrose gradients. The major proteins cosediment with [^{3}H]hnRNA in the 30-S fraction in sucrose gradients after limited RNAase digestion. The protein complex is held together as long as it is associated with hnRNA fragments of about 125 ± 25 nucleotides or longer (Choi & Dreyfuss 1984b). These proteins are therefore part of the monomer particles, and they comprise almost the entire protein complement of large (polyparticle) hnRNP complexes. The proteins A1, A2, C1, 68-kDa, and 120-kDa are the most abundant by Coomassie blue staining and

Table 1 Proteins of human hnRNP monoparticles[a]

Protein	Molecular weight	Isoelectric point (±0.5)	Relative amount	Affinity for RNA[b]	Posttranslational modification[c]
A1	34,000	9.0	3–4	+	DMA
A2	36,000	8.0	3–4	+	DMA
B1	37,000	8.5	1	+	nd
B2	38,000	9.0	1	+	nd
C1	41,000	6.0	3–4	+ + +	Pi
C2	43,000	6.0	1–2	+ + +	Pi
$68K_{1,2}$	68,000	6–8	1–2	+ +	nd
$120K_{1,2}$	120,000	6.5	1–2	+ +	Pi

[a] In growing HeLa cell nucleoplasm.
[b] Determined by resistance to dissociation by salt and by binding to ssDNA (Y. D. Choi et al, unpublished).
[c] DMA = dimethylarginine; Pi = phosphorylation; nd = not determined.

[^{35}S]methionine steady-state labeling. Many, if not all, of these proteins are in contact with the hnRNA and can be cross-linked to it in intact cells by UV light (Choi & Dreyfuss 1984b). The picture derived from two-dimensional gel electrophoresis (Brunel & Lelay 1979; Suria & Liew 1979; Peters & Comings 1980; Knowler 1983; Wilk et al 1985) is considerably more complex: Many of the proteins resolve into several spots, and there is evidence for posttranslational modifications of many of them. It is quite certain (from examination of the electrophoretic patterns) that not all of the protein components of hnRNPs have been identified.

The hnRNP proteins are very abundant in the nucleus of growing cells, as abundant as histones. But unlike histones, the overall amount of hnRNP proteins can vary substantially depending on the growth or transcriptional state of the cell. With increased RNA polymerase II transcription the amount of hnRNP proteins increases. Indications of such correlations have been described in steroid-responsive tissues (Knowler 1976), and quiescent cells have been reported to contain less of some of the hnRNP proteins than do growing cells (Stunnenberg et al 1978; LeStourgeon et al 1978; Celis et al 1986). HeLa (human) hnRNP proteins are the best characterized, and most of the discussion here refers to them.

A AND B PROTEINS The A and B proteins are members of two related families of basic proteins that share common antigenic determinants (Leser et al 1984; Leser & Martin 1986). Proteins of this group are associated with the RNP fibers of transcriptionally active chromatin, which suggests that they become associated with nascent hnRNA (Martin & Okamura 1981). The A group proteins, A1 and A2, have isoelectric points of about 9.2 and 8.4, respectively (Beyer et al 1977; Wilk et al 1985). A1 and A2 have similar (but different) amino acid compositions containing a very high percentage of glycine (25%) and the rare modified amino acid N^G,N^G-dimethyl arginine (Boffa et al 1977; Christensen et al 1977; Beyer et al 1977; Karn et al 1977). The A proteins dissociate from the RNA in vitro in 0.13–0.15 M NaCl (Beyer et al 1977; LeStourgeon et al 1981). Results of mobility tests in SDS-PAGE after UV cross-linking in vivo indicate that A1 is efficiently cross-linked to hnRNA and that A2 is either not at all, or is much less, cross-linked (Choi & Dreyfuss 1984b). The ratio of A1 to A2 in hnRNP particles is about 1 : 1 in rapidly growing tissue culture cells; in total cell material, however, considerably more A2 is detected (Celis et al 1986). Much less A1 is found in cells that are stationary than in proliferating cells or tissues of adult animals (LeStourgeon et al 1978; Celis et al 1986). Similar proteins are found in all mammals (Beyer et al 1977; Karn et al 1977; Choi & Dreyfuss 1984b), but the size of A proteins in divergent vertebrates can vary (Leser et al 1984). Immunofluorescence

evidence suggests that the A proteins are confined to the nucleus (Leser et al 1984).

Recent immunological (Valentini et al 1985; Pandolfo et al 1985), peptide mapping, and cDNA sequencing data (Chase & Williams 1986; K. Williams, personal communication; S. Riva, personal communication) demonstrate that a 24-kDa single-stranded DNA binding protein from calf thymus (Herrick & Alberts 1976a), UP1, is a fragment of the A1 protein. The amino acid sequence of UP1 (Williams et al 1985) is identical to the first 195 amino acids of the amino acid sequence of the A1 predicted by the cDNA clone. Based on this DNA sequence, the A1 protein contains another 124 amino acids that constitute a very glycine-rich (about 40%) domain at the COOH terminus of the protein (K. Williams, personal communication). In addition to A1, polyclonal antibodies to UP1 recognize other hnRNP proteins, which are presumably A and B proteins (Valentini et al 1985). Thus antigenic determinants similar to A1 probably exist in these other hnRNP proteins. Previous studies on UP1 revealed that it binds single-stranded DNA (ssDNA) tightly but apparently without sequence specificity and that it is a very effective helix-destabilizing protein (Herrick & Alberts 1976b). Perhaps other proteins previously classified as ssDNA binding proteins in various cells and viruses are also hnRNP proteins.

A helix-destabilizing hnRNP protein related to the A proteins of mammals, HD40, was isolated from the brine shrimp *Artemia salina* (Marvil et al 1980; Nowak et al 1980). This 40-kDa protein binds to and disrupts residual secondary structure of single-stranded nucleic acids at a stoichiometry of about one protein per 12–15 nucleotides (Thomas et al 1981, 1983). A cDNA clone for HD40 was isolated, and genomic DNA cross-hybridizing with it was detected in divergent animals and plants (Cruz-Alvarez et al 1985).

The B proteins, B1 and B2 (37 and 38 kDa), have isoelectric points of about 8.3 and 9.2, respectively (Beyer et al 1977; Wilk et al 1985). Their prevalence in hnRNP particles is about one-third that of the A1, A2, and C1 proteins and about the same as that of C2 (Beyer et al 1977; LeStourgeon et al 1981; Choi & Dreyfuss 1984b). The B proteins dissociate from the RNA at moderate salt concentrations similar to those at which the A proteins dissociate (0.13–0.15 M) (Beyer et al 1977). Proteins of molecular weights that correspond to those of the B proteins are cross-linked to RNA by exposure to UV light in intact cells (Economides & Pederson 1983), but without specific antibody tests it is not certain that these are the B proteins. The B proteins are antigenically related to each other and to the A proteins, as shown by their reactions to monoclonal antibodies (Leser et al 1984). Immunofluorescence shows that they are confined to the

nucleus during interphase. Like the A proteins, the B proteins possess unusual amino acid compositions of 25% glycine and several moles each of the unusual residue dimethyl arginine (Beyer et al 1977; Wilk et al 1985). Two-dimensional gel electrophoresis has shown that the B proteins exist in several posttranslationally modified forms (Brunel & Lelay 1979; Peters & Comings 1980; Suria & Liew 1979; Wilk et al 1985), but the specific modifications have not been determined.

C PROTEINS The C proteins (C1, 41 kDa, and C2, 43 kDa) are major constituents of 30-S hnRNP monoparticles (Beyer et al 1977) and are the two most prominent proteins cross-linked to hnRNA by UV light treatment in vivo (Dreyfuss et al 1984b; Choi & Dreyfuss 1984b). The C proteins bind RNA more tightly than do the other major hnRNP proteins, as determined by resistance to dissociation from RNA in high salt concentrations (Beyer et al 1977; LeStourgeon et al 1981; Y. D. Choi & G. Dreyfuss, unpublished results) and by binding to ssDNA (S. Pinol-Roma & G. Dreyfuss, unpublished results). The two C proteins are highly similar to each other: They are both recognized by the same monoclonal antibodies, and antibodies raised against purified C1 also react with C2 (Dreyfuss et al 1984b; Choi & Dreyfuss 1984a). In humans the two proteins have different but related partial peptide maps and the same acidic isoelectric points (6.0 ± 0.5) (Dreyfuss et al 1984b). A monoclonal antibody raised against the human C proteins reacts with the C proteins in widely divergent species ranging from humans to reptiles (Choi & Dreyfuss 1984b). In all species examined there are two C proteins in the range from 39 to 42 kDa for C1 and from 40 to 45 kDa for C2. The C proteins are phosphorylated in vivo (Dreyfuss et al 1984b; Choi & Dreyfuss 1984a; Holcomb & Friedman 1984) and in vitro by a casein kinase type II (Holcomb & Friedman 1984). Like the other components of 30-S particles, they are associated with both poly(A)-containing and non-poly(A)-containing hnRNAs. Immunofluorescence microscopy demonstrated that the C proteins are segregated to the nucleus. Within the nucleus the C proteins are not found in nucleoli and are not associated with chromatin, as seen in cells in prophase (Choi & Dreyfuss 1984a). Nakagawa et al (1986) recently isolated cDNA clones for the human C proteins. These clones hybridize to genomic DNA sequences in divergent eukaryotes, including yeast, which suggests that C proteins are ubiquitous components of hnRNPs in eukaryotes. So far, the C proteins are the only hnRNP proteins shown to have a role in pre-mRNA splicing (Choi et al 1986). Van Eekelen & Van Venrooij (1981) suggested that the C proteins are associated with the nuclear matrix, but subsequent experiments with specific antibodies showed that only a very small fraction of these proteins remain with a

detergent-, nuclease-, and high salt–resistant nuclear substructure (Dreyfuss et al 1984b).

68- AND 120-kDa PROTEINS In one-dimensional SDS gel electrophoresis, doublets of proteins of 68 and 120 kDa are coimmunoprecipitated with other hnRNP proteins and are in contact with hnRNA as shown by photochemical UV cross-linking in intact cells (Dreyfuss et al 1984b; Choi & Dreyfuss 1984b). They are, therefore, authentic hnRNP components but are so far less well characterized than the A, B, and C proteins. From results on two-dimensional gels it is apparent that there are more than two proteins of these molecular weights (Y. D. Choi & G. Dreyfuss, unpublished results). The lower band of the 120-kDa doublet is recognized by a monoclonal antibody, 3G6 (Dreyfuss et al 1984b), and this antibody can immunoprecipitate the hnRNP complex (Choi & Dreyfuss 1984b). Like the C proteins, 120-kDa doublet is seen by immunofluorescence to be confined to the nucleus, is extensively phosphorylated, is conserved across vertebrates, and is associated with both polyadenylated and non-polyadenylated hnRNA (Dreyfuss et al 1984b).

3′-POLY(A) AND 5′-CAP BINDING PROTEINS All hnRNAs contain a 5′-m^{7}Gppp-cap structure. The protein(s) that are bound to the hnRNA cap in hnRNPs have not been identified. Using photoaffinity labeling with a cap analog, three proteins of about 120, 89, and 80 kDa were detected in the nuclear fraction (Patzelt et al 1983). The significance of these findings is uncertain; moreover, it is not clear whether or not the hnRNA cap is associated with 30-S monoparticles.

Unlike other parts of the hnRNA, the 3′-poly(A) tail is not associated with 30-S particles. Instead, it forms a distinct particle that sediments at 15 S. This indicates that the tail is associated with considerable amounts of protein, because the protein-free form sediments at 4 S (Quinlan et al 1974). The protein to RNA ratio is actually higher in these 15-S particles than in 30-S particles, as judged by their lower buoyant density (Quinlan et al 1977). However, these proteins do not confer any protection to the poly(A) tail from nucleases (Baer & Kornberg 1980; Tomcsanyi et al 1983). The protein composition of the hnRNA poly(A)-ribonucleoprotein complex isolated by sucrose gradients was investigated by several groups (Quinlan et al 1974, 1977; Samarina & Krichevskaya 1981; Firtel & Pederson 1975; Kish & Pederson 1975; Tomcsanyi et al 1983). These studies revealed several proteins of 60 and 70–90 kDa. Using UV cross-linking in nuclear extracts from HeLa cells, Setyono & Greenberg (1981) identified a 60-kDa protein that is in direct contact with the poly(A) sequence. However, additional proteins that do not become cross-linked to RNA may be found in the complex. Sachs & Kornberg (1985) recently

identified a 50–55 kDa poly(A) binding protein in nuclei of yeast. The physiological role of the hnRNA poly(A) binding proteins or the poly(A)-ribonucleoprotein complex is not known, but it is reasonable to assume that they may have a role in the formation of the poly(A) tail, such as in serving as a length-measuring system for the poly(A) polymerase.

Arrangement of Proteins in hnRNP Monomer Particles

The relative amounts of the major proteins of 30-S particles (see Table 1) of growing cells can be estimated from Coomassie blue staining and from steady-state [^{35}S]methionine labeling. A1 and A2 are present in similar amounts. The two B proteins are also present in equal amounts but at about one-third the level of the A proteins. C1 is present in the same amount as the A polypeptides; C2 (like the B proteins) is present at about one-third that amount. The 68 and 120-kDa proteins are about as abundant in mass (not necessarily stoichiometry) in isolated complexes as the A and C1 proteins are. Based on the estimated molecular mass of 30-S monoparticles isolated by sucrose gradient sedimentation, it has been suggested that these particles are composed of three or four repeating units each composed of 3A1, 3A2, 1B1, 1B2, 3C1, and 1C2 (Lothstein et al 1985). However, this suggestion does not take into account the high molecular weight proteins (e.g. 68 and 120 kDa) and other uncharacterized proteins that may be part of the structure.

Protease and RNAase digestion experiments and chemical and UV cross-linking experiments permit some conclusions about the relative positions of the RNA and proteins in hnRNP particles. In intact 30-S particles, proteins A2 and B1 occupy an internal, protease-protected position (Lothstein et al 1985), and they may exist as three or four tetramers of $(A2)_3(B1)$ or as pentamers of $(A2)_3(B1)(B2)$. After RNAase digestion of the hnRNP, A2, B1, and B2 remain associated in nuclease-resistant structures that readily aggregate. If digestion is performed in low Mg^{2+} concentration, twelve such residual tetramers assemble to form highly regular 20-nm 43-S particles.

The proteins A1, C1, and C2, on the other hand, are sensitive to mild proteolysis and dissociate from the internal A2, B1, and B2 complexes, which suggests that they are peripheral (LeStourgeon et al 1981). Consistent with this position are findings showing that A1, C1, and C2 are in direct contact with RNA, as determined by photochemical cross-linking, and that monoclonal antibodies against C1 and C2 and against A1 efficiently immunoprecipitate intact hnRNP particles and 30-S particles (Dreyfuss et al 1984b; Choi & Dreyfuss 1984b; Y. D. Choi et al, unpublished results). In these studies the 68-kDa proteins are also seen in contact

with the hnRNA. They probably occupy peripheral positions in the monoparticles because they are readily lost during sucrose gradient sedimentation (Y. D. Choi & G. Dreyfuss, unpublished results). The 120-kDa proteins also occupy peripheral positions: An antibody (3G6) that can bind them precipitates intact hnRNPs (Dreyfuss et al 1984b; Choi & Dreyfuss 1984b). From chemical cross-linking experiments with isolated 30-S particles (Lothstein et al 1985) it appears that A1, A2, and C1 each exist as homotypic trimers. These findings also indicate that the A1 and A2 proteins are next neighbors and that B proteins are in contact with A and C proteins.

The ratio of C1 to A1 in the immunoprecipitates can be used as an index of the intactness of the 30-S particle. When the hnRNA that is associated with monoparticles is degraded to segments of 125 ± 25 nucleotides, the monoparticles are still intact. However, when these segments of hnRNA are degraded further to 60–75 nucleotides, most monomers are no longer intact (Y. D. Choi et al, unpublished results). The C proteins, together with 68- and 120-kDa proteins, remain as a complex after digestion with a nuclease that yields intact RNA stretches shorter than 60–75 nucleotides. However, this residual structure dissociates upon further digestion, which suggests that it is held together by short stretches of RNA. By immunoprecipitation with the cognate monoclonal antibody, it appears that A1 is released as a single protein without the other major hnRNP proteins (Y. D. Choi et al, unpublished results). Because the 30-S monoparticle dissociates upon nuclease digestion, protein-protein interactions alone are clearly not sufficient to hold it together. Thus hnRNP proteins probably do not pre-exist as complexes of proteins only.

Arrangement of hnRNA in hnRNP Particles

Studies of protein composition, nuclease digestion, and sedimentation properties suggest that native large hnRNP complexes are composed mostly of multiple 30-S particles connected by highly nuclease-sensitive stretches of hnRNA. Data from nuclease digestion experiments and cross-linking of RNA to peripheral monoparticle proteins indicate that most, if not all, of the particle-associated hnRNA occupies a peripheral, nuclease-accessible position in intact 30-S particles. However, it is also possible that only parts of the hnRNA chain are initially exposed on the surface of hnRNP particles and that when these are cleaved, a structural change results that exposes the rest of the hnRNA. The fragments of hnRNA recovered with gradient-purified monoparticles average ~ 500–800 bases in length (Martin et al 1978; LeStourgeon et al 1981; Steitz & Kamen 1981). Studies on immunopurified hnRNP particles have shown that monoparticles are associated with $\sim 500 \pm 100$ nucleotides of hnRNA and

that this stretch of RNA can be further cleaved and trimmed to two or three stretches of 125 ± 25 nucleotides before the monoparticle dissociates (Choi & Dreyfuss 1984b; Y. D. Choi & G. Dreyfuss, unpublished results). The monoparticle proteins do not dissociate until the average RNA fragment is cleaved below 125 ± 25 bases in length (Choi & Dreyfuss 1984b). This could be the length of RNA in close contact with the proteins of the monoparticles, or it could be the length of RNA associated with protein subdomains within monoparticles. When the RNA is cleaved down to about 60–75 nucleotides, a residual particle containing C1, C2, and the 68- and 120-kDa proteins can be isolated with these short nucleotide segments. After cleavage of the hnRNA within the 30-S particle the hnRNA fragments and the proteins remain associated in a single complex, which suggests that the hnRNP monomer can serve as an "operating table" for hnRNA processing. From the amount of hnRNA lost upon nuclease conversion of large hnRNPs to 30-S monoparticles (Y. D. Choi & G. Dreyfuss, unpublished results) the average length of the intermonoparticle linker RNA is about 250 ± 50 nucleotides. As discussed above, the poly(A) segment of polyadenylated hnRNAs is not associated with 30-S particles but rather is bound to a 60-kDa protein and, probably together with several other proteins, forms a distinct 15-S particle.

The position of hnRNA intron and exon sequences in hnRNP particles and the specific arrangement of monoparticles and monoparticle proteins on specific hnRNAs have been the subjects of intense investigation, but as yet no conclusions can be drawn regarding these important issues. To understand the assembly and function of hnRNP particles we must answer two important questions: Are the positions of 30-S particles and of individual 30-S proteins specific and fixed on the primary transcript? If so, is this the result of sequence-specific features or of an assembly process that also acts as a measuring device? Electron microscopic studies of specific *Drosophila* transcripts (Beyer et al 1981; Osheim et al 1985) in highly dispersed preparations using Miller's chromatin spreading techniques revealed an orderly arrangement of protein particles of about 20 nm in diameter (about the size of isolated 30-S particles) along the hnRNA. The particles are neither randomly nor uniformly distributed; rather, their location correlates with nascent transcript cleavage. A class of RNPs stable under the preparation conditions are associated specifically with splice junctions. The significance of these findings rests on the identity of the protein particles seen in these preparations. This is not yet known, but it seems doubtful that typical 30-S hnRNP particles could survive the specific detergent, pH, and ionic conditions used for the preparation of the specimens. In contrast, examination of less dispersed (less deproteinized) preparations (Osheim et al 1985) showed that the transcripts are completely

covered with protein in a roughly particulate form (average particle diameter ~22.5 nm). This suggests little or no specificity in the packaging of hnRNA with protein in particles, presumably 30-S particles. This is consistent with the findings obtained by nuclease digestions (Steitz & Kamen 1981; Munroe 1982). Differential sensitivity to nuclease, which suggests nonrandom distribution of proteins on specific pre-mRNAs, has been reported in the case of polyoma (Steitz & Kamen 1981) and β-globin (Patton et al 1985; Patton & Chae 1985). The usefulness of nuclease digestion mapping is limited because it is not certain which proteins generate the observed pattern and because the hnRNP structures are very labile and could vary depending on the isolation conditions. Using UV cross-linking, proteins associated with specific regions of adenovirus pre-mRNAs have been detected (Van Eekelen et al 1982; Ohlsson et al 1982). The significance of these patterns is not obvious from these or other studies (Stevenin et al 1982; Huang & Chae 1983; Munroe & Pederson 1981), thus the question remains unanswered. Clearly, more mapping of specific proteins on specific hnRNAs is needed.

Also of considerable interest are the precise location of specific hnRNA sequence features [such as double-stranded regions and oligo(A) and oligo(U) stretches] within hnRNP particles and the contribution of such features to the particle structure. The presence of such sequences has been documented, but their significance is not yet apparent. Internal oligo(A) sequences of pre-mRNA, 20–40 nucleotides long, were found in 30-S particles (Kinniburgh & Martin 1976; Martin et al 1978). Kish & Pederson (1977) found oligo(U) sequences of ~15–50 nucleotides in HeLa hnRNP particles. These sequences may be complexed with oligo(A) or poly(A) sequences. They are resistant to nuclease and may be covered with proteins. Small amounts of double-stranded RNA (dsRNA) were detected in 30-S particles (Ryskov et al 1973) by several methods (see Samarina & Krichevskaya 1981), including nuclease digestion (Calvet & Pederson 1978). These sequences do not seem to bind 30-S particle proteins or other proteins; they may protrude from the surface of 30-S monoparticles as well as being located between monoparticles (Martin et al 1978).

Assembly and Disassembly of hnRNP Particles

The assembly of hnRNP particles apparently occurs as the hnRNA is still a nascent transcript. This assumption is based primarily on the above-cited microscopic observations showing that chromatin-associated transcripts are bound with proteins and on analysis of the rapidly labeled RNA in this fraction (Augenlicht & Lipkin 1976). Martin & Okamura (1981) used immunocytochemical procedures to show that the A and B groups of the hnRNP proteins are localized to regions containing actively transcribed

RNA, and they suggested that these proteins become associated with nascent transcripts. Economides & Pederson (1983) showed by UV cross-linking that proteins in the 30–40 kDa range, which presumably correspond to the A, B, and C proteins, become cross-linked to rapidly labeled hnRNA soon after transcription. The 30-S monoparticle hnRNP proteins interact with RNA even if it is not polyadenylated (Dreyfuss et al 1984b; Pullman & Martin 1983; Wilk et al 1983). These findings further suggest that assembly of hnRNP particles is an early posttranscriptional event that precedes polyadenylation and splicing of pre-mRNA.

hnRNP proteins must be reutilized since protein synthesis is not immediately required for incorporation of hnRNA into hnRNP particles and because the hnRNP proteins examined are very stable relative to the half-life of the hnRNAs (Martin & McCarthy 1972). hnRNA turnover and the turnover of the major hnRNP proteins are therefore not tightly coupled. There does not seem to be (at least when analyzed in nucleoplasm by sedimentation) a very large pool of free (not hnRNA-bound) hnRNP proteins. Therefore, disassembly of hnRNPs prior to transport of mRNA to the cytoplasm is likely to be a major source of hnRNP proteins for reassembly on nascent hnRNAs.

What are the signals for hnRNP assembly? Possible determinants include the RNA polymerase II complex itself, the cap structure (m^7Gppp in hnRNAs) or other posttranscriptional modifications, the size of the RNA, and possibly the subnuclear localization of the transcripts. Clearly, small nuclear RNAs (snRNAs) are not associated with hnRNP proteins but rather with distinct snRNP proteins. This, however, does not mean that it is not the polymerase complex itself (some of the snRNAs are also transcribed by RNA polymerase II) that determines the specificity because the snRNP proteins and the trimethyl cap of the snRNAs are acquired in the cytoplasm, and it is therefore possible that the snRNAs initially are bound with hnRNP proteins. The contribution, if any, of introns and factors that interact with intron- or intron/exon-junction sequences is not yet known. It has been suggested (Pederson 1983) that intronless transcripts may not be assembled into hnRNP complexes. This conclusion is based on observations in heat-shocked *Drosophila* cells (Mayrand & Pederson 1983), but these findings have recently been questioned (Kloetzel & Schuldt 1986). The order of assembly of hnRNP proteins on the hnRNA is not known.

Protein-RNA reconstitution experiments so far have demonstrated that proteins from the 30–40 S nucleoplasmic region of sucrose gradients bind RNA and form complexes similar to hnRNP particles in sedimentation properties and general appearance by electron microscopy (Kulguskin et al 1980; Wilk et al 1983; Pullman & Martin 1983). While this is a very

promising approach, the criteria used so far to assess reconstitution are not sufficient because there are no specific functional assays for hnRNP monoparticles. It will be necessary to show that the composition, stoichiometry, and specific arrangement of the individual proteins in these reconstituted particles are similar to those found in hnRNP particles isolated from cells.

Little is known about the process of disassembly of hnRNP particles. It must occur in the nucleus prior to or coincident with mRNA transport and is likely to be the source of most of the hnRNP proteins which are probably recycled to form new particles with newly synthesized hnRNA. Allosteric effectors or covalent posttranslational modification of hnRNP proteins by specific enzymes in the vicinity of the nuclear-pore complexes may be involved in decreasing the normally high affinity of hnRNP proteins for the RNA. Specific conditions within the nuclear pore complexes may favor dissociation of the hnRNP particle. In vitro reconstituted systems should be very useful tools for exploring these possibilities.

Functions of hnRNP Particles and hnRNP Proteins

Two extreme functions for hnRNP complexes are likely and not mutually exclusive: hnRNPs may have a predominantly structural role, namely, they may be involved in the packaging of hnRNA. The packaging could serve several functions, such as preventing tangling of transcripts, compacting the hnRNA, facilitating RNA strand displacement and release from template DNA, and protecting the hnRNA from degradation by endogenous nucleases. Alternatively, hnRNPs may be more directly and actively involved in the posttranscriptional processing of hnRNA and in mRNA production, including splicing of pre-mRNA and transport to the cytoplasm. hnRNPs may provide correct substrate presentation and possibly process enzymatic activities. Although a complete account of the physiological significance of hnRNP particles is well beyond our present knowledge and may take a long time to obtain, it has recently become possible to experimentally address specific questions about the role of hnRNP particle proteins in the biogenesis of mRNA.

The idea that pre-mRNA processing occurs in the nucleus in hnRNP particles and that the hnRNP complex itself is a critical element in the formation of mRNA has been a major theme in this field of research since the earliest observations of RNP complexes. The hnRNP monoparticle appears to have the necessary properties to serve as an "operating table" for RNA splicing, and hnRNPs are indeed associated with pre-mRNA and spliced mRNA in the nucleus (Y. D. Choi & G. Dreyfuss, unpublished results). Until recently, however, it was difficult to test experimentally the functional significance of hnRNPs because there were no definitive probes

for hnRNP proteins and no in vitro assay for pre-mRNA splicing. With the development of in vitro cell-free systems that faithfully splice mRNA precursors (Hernandez & Keller 1983; Padgett et al 1983, 1984; Krainer et al 1984; reviewed in Green 1986; Padget et al 1986) and with the availability of specific antibodies to proteins of hnRNP complexes it has become possible to examine the question of whether hnRNP proteins play a role in the splicing of pre-mRNA.

Choi et al (1986) investigated the effect of several monoclonal antibodies to hnRNP proteins on pre-mRNA splicing. It was found that splicing in vitro in HeLa nuclear extract of a mRNA precursor was inhibited by a monoclonal antibody to the hnRNP C proteins. The inhibition with the anti-C antibody, 4F4, is at an early step of the reaction—cleavage at the 3′-end of the upstream exon and the formation of the intron lariat. In contrast, preboiled 4F4, or a different anti-C monoclonal antibody (designated 2B12), or antibodies to other hnRNP proteins (the 120- and 68-kDa proteins), and nonimmune mouse antibodies have no inhibitory effect. Sedimentation experiments showed that the 4F4 antibody diminishes, but does not prevent, the ATP-dependent formation of a 60-S splicing complex (spliceosome) that contains pre-mRNA, proteins, and snRNAs. This complex is probably necessary for the progression of the splicing reaction (Brody & Abelson 1985; Grabowski et al 1985). Furthermore, the 60-S splicing complex contains C proteins, and it can be immunoprecipitated with the antibody to the C proteins. Moreover, depletion of C proteins from the splicing extract by immunoadsorption with 4F4 or 2B12 results in the loss of splicing activity, whereas mock depletion with nonimmune mouse antibodies has no effect. A 60-S splicing complex does not form in a C protein–depleted nuclear extract. These results indicate an essential role for the proteins of the hnRNP complex in the splicing of mRNA precursors. The splicing complex therefore appears to be a modified hnRNP monoparticle or monoparticle subdomain that contains, in addition to hnRNP proteins, other components specifically necessary for splicing, including the snRNPs U1 and U2 (Black et al 1985; Krainer & Maniatis 1985) and possibly also U5 (Chabot et al 1986), U4, and U6 (D. L. Black & J. A. Steitz, personal communication) (see also Green 1986 and Padgett et al 1986). The stabilized particles seen by Osheim et al (1985) may correspond to these complexes. Because very short pre-mRNAs can be spliced in vitro, it may be that a spliceosome is composed not of a complete monoparticle but only of one of its subdomains containing a C-protein. Since hnRNP formation precedes polyadenylation, hnRNP proteins may also be important in this process. Tentative schematic presentations of the structure of hnRNP particles and their involvement in mRNA biogenesis are shown in Figure 2.

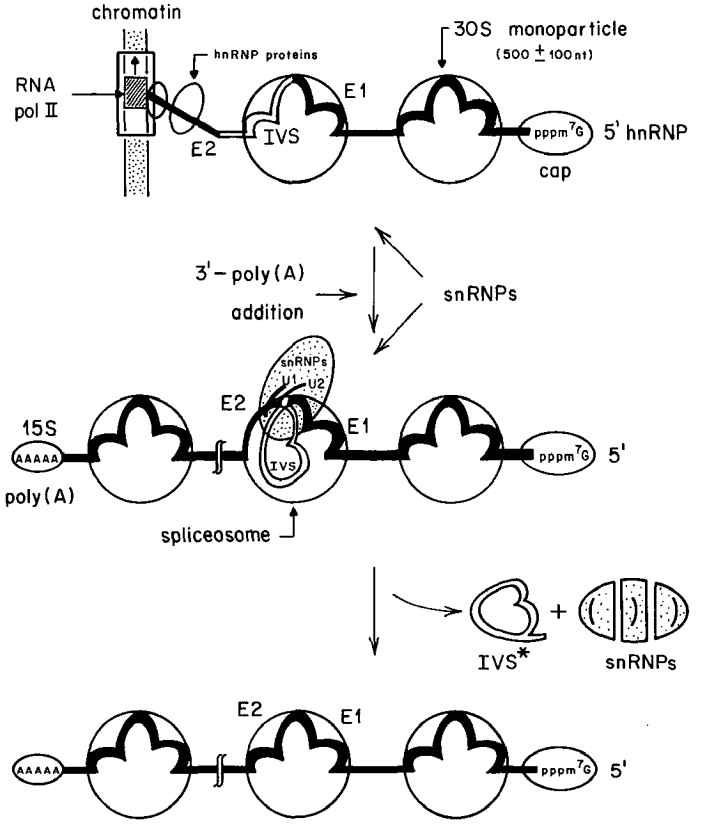

Figure 2 Schematic presentation of a tentative generalized model of hnRNP particle structure and its involvement in pre-mRNA splicing. E = exon coding sequences. IVS = intervening sequence (intron). The figure depicts splicing of a small intron of a size that can be accommodated in a 30-S particle. For much larger introns two general possibilities can be envisioned: the intron loops out of the surface of the same monoparticle (possibly associated with hnRNP proteins) and splicing occurs within the context of that monoparticle. Alternatively, the donor (5′) and acceptor (3′) exon/intron junctions could be positioned on two different 30-S monoparticles, and splicing could occur by bringing the two together. The latter would require considerably more complicated spatial maneuvering of monoparticles. At present it is not known if snRNPs interact with the hnRNA (pre-mRNA) during or after hnRNP particle assembly or how they affect the packing of the hnRNA.

CYTOPLASMIC RIBONUCLEOPROTEIN PARTICLES

Evidence for, and Isolation of, mRNP Complexes

As noted earlier, not all mRNAs are translated at the same time or with the same efficiency. Actively translated cytoplasmic mRNAs are engaged with ribosomes (polyribosomal mRNAs) and untranslated mRNAs (nonpolysomal mRNAs) are not. In addition to defining the translational state of mRNA, association with polyribosomes is the basis for the physical separation of these two functional classes of mRNAs by fractionation of cytoplasmic extracts on sucrose density gradients.

Spirin and collaborators provided the first evidence that mRNA can exist apart from polyribosomes in the form of a nucleoprotein complex. They demonstrated that in early stages of development in fish and sea urchin embryos mRNA was found in RNP particles that sedimented more slowly (20–75 S) than did ribosomes (80 S). The nonribosomal nature of the particles was demonstrated by their distinct buoyant density in CsCl gradients after fixation with formaldehyde (Spirin et al 1965): 1.42–1.45 g/ml versus 1.52 g/ml for ribosomal subunits. Evidence that the RNA in these particles, which the authors termed informosomes, contained mRNA was drawn from their ability to direct protein synthesis in vitro (Spirin & Nemer 1965; Spirin 1969). The buoyant density of fixed particles became an important criterion for defining and characterizing ribonucleoprotein complexes (Spirin et al 1965). This method was subsequently used to demonstrate that nonpolysomal mRNAs exist in diverse eukaryotic cells (Perry & Kelley 1968; Henshaw & Loebenstein 1970; Spohr et al 1972; Gander et al 1973), and its application to polysomal mRNAs helped establish the generality of the concept that mRNA in the cytoplasm is always found in the form of mRNP complexes. mRNAs released from polyribosomes by a variety of treatments (Lebleu et al 1971; Schochetman & Perry 1972; Lee et al 1971; Blobel 1972; Henshaw 1968; Perry & Kelley 1968) were also shown to be complexed with protein and to have a buoyant density similar to that of mRNPs in CsCl gradients.

Following the detection of poly(A) segments on mRNAs (reviewed in Brawerman 1983), it became possible to rapidly resolve mRNA with its associated proteins from ribosomal material by oligo(dT) or poly(U) chromatography without prior fixation and without CsCl gradients (Lindberg & Sundquist 1974). This important advance spawned numerous observations of mRNPs in a wide variety of organisms. Unlike deproteinized mRNA, which can be eluted from an oligo(dT) column at a low salt concentration, the column-bound mRNPs can only be eluted with formamide or at elevated temperatures (Lindberg & Sundquist 1974;

Jain et al 1979). Even when prepared by these methods, mRNPs are not completely free of ribosomal and other cytoplasmic proteins. Thus the assignment of these proteins to the mRNP fraction remains uncertain in the absence of other data because it cannot be ascertained that the eluted proteins were retained by the column strictly through specific association with the poly(A)$^+$ mRNA.

Early on, the physiological significance of mRNP particles was called into question (Baltimore & Huang 1970) because it was found that RNA incubated with cytoplasmic extract forms RNA-protein complexes of similar sedimentation properties and buoyant density. However, these artificial complexes, unlike mRNPs isolated from cells, do not form at NaCl concentrations of 0.15 M or higher. This finding influenced subsequent investigators to isolate mRNP complexes at 0.5 M NaCl. This procedure, though necessary, only decreases the likelihood of nonspecific associations, it does not eliminate them. In addition, it carries the risk of promoting the dissociation of genuine mRNP proteins from the complex.

The first reports of the protein composition of mRNPs came from work done on reticulocytes from duck and rabbit (Morel et al 1971; Blobel 1972). Both reports found two similar major proteins of $M_r \sim 52{,}000$ and 78,000. Although many different proteins have been identified in various studies, proteins of similar molecular weights (the larger being the poly(A) binding protein, see below) seem to be consistent mRNP components and have been detected in many different cell types, including HeLa cells (Kumar & Pederson 1975), KB cells (Lindberg & Sundquist 1974; Van der Marel et al 1975; Sundquist et al 1977), Ehrlich ascites cells (Jeffrey 1977; Van Venrooij et al 1977), kidney cells (Irwin et al 1975), and muscle cells (Bag & Sarkar 1975, 1976; Heywood et al 1975). Because a polyadenylate sequence, unlike other RNA stretches, is not degraded by some ribonucleases (pancreatic and Tl RNAases), poly(A) tails can be prepared and purified by chromatography on oligo(dT). Kwan & Brawerman (1972) first suggested that the poly(A) segment of mRNA is associated with protein on the basis of sedimentation experiments and binding to nitrocellulose. Blobel (1973) analyzed the composition of the poly(A)-protein RNP complex and found a single protein of 78 kDa. This protein is the most highly conserved and most extensively studied RNP protein. A protein of similar molecular weight (72,000–78,000, referred to hereafter as 72,000) is found to be a consistent and major component of mRNP complexes in almost all reports. The tight association of this protein with the poly(A) tail of mRNA has been reported in diverse cells (e.g. Barrieux et al 1975; Schwartz & Darnell 1976; Kish & Pederson 1975, 1976; Gaedigk et al 1985; Jain et al 1979; Jeffrey 1978; Vincent et al 1981).

Other methods for preparing mRNPs have also been introduced but have not become as widely used. These include centrifugation in Cs_2SO_4 without prior fixation (Greenberg 1977) and electrophoresis in non-denaturing agarose gels (Tasseron-De-Jong et al 1979). It is reassuring that they revealed a similar pattern of prominent mRNP proteins. The same proteins were more recently shown to be tightly associated with mRNA by photochemical cross-linking in fractionated cells (Greenberg 1980, 1981; Setyono & Greenberg 1981). The detection of a simple and consistent pattern of tightly associated proteins in diverse mRNAs (Bryan & Hayashi 1973) from widely divergent organisms and cell types, using different methodologies, gives considerable credence to the authenticity and biological significance of mRNPs. Blobel's (1973) observation that the poly(A) segment of mRNA is associated with the 72,000 poly(A) binding protein was of particular significance because it was the first indication of sequence specificity.

PHOTOCHEMICAL CROSS-LINKING IN INTACT CELLS The copurification of proteins with RNA does not prove that they interact with one another in the intact cell. The proteins bound to mRNA in intact vertebrate cells were identified using UV cross-linking (Wagenmakers et al 1980; Van Eekelen et al 1981c; Van Venrooij et al 1982; Dreyfuss et al 1984a; Bag 1984; Greenberg & Carroll 1985; Adam et al 1986a). The predominant [^{35}S]-methionine-labeled polypeptides have approximate molecular weights of 72,000, 68,000, 53,000, and 50,000. A considerable number of less abundant proteins were also detected. By label transfer from [^{3}H]nucleotide-labeled RNA the major proteins have molecular weights of 72,000, 68,000, and 53,000 (Wagenmakers et al 1980; Van Eekelen et al 1981b; Adam et al 1986a; Dreyfuss et al 1986). The 68-kDa protein, unlike the 72- and 53-kDa proteins, may dissociate from mRNA in uncross-linked complexes at 0.5 M KCl, which would explain why it was not previously considered a major mRNP protein. By Coomassie blue or silver staining, the 72-kDa protein is the most abundant cross-linked mRNP. The poly(A) tail of mRNA is selectively cross-linked to the 72-kDa protein (Greenberg & Carroll 1985; Adam et al 1986a). The cross-linked proteins are not ribosomal, and their cross-linking is strictly dependent on UV irradiation of intact cells. The sequence selectivity and the lack of random cross-linking to abundant cytoplasmic proteins underscore the specificity of the photochemical cross-linking procedure. Under typical irradiation conditions (15 W germicidal lamp, 4.5 cm distance, 3 min) about 87% of the poly(A)$^+$ sequences are recovered and very little RNA chain breakage occurs (Adam et al 1986a).

Structure and Function of mRNP Complexes

The major proteins shown to interact with the mRNA both in vivo and in vitro have molecular weights of 72,000, 68,000, 53,000, and 50,000. The 72-kDa protein is bound to the poly(A) tail. The binding sites of the other proteins have not been determined. Several proteins that bind specifically, but probably transiently, to the 5′-cap structure of the mRNA have been found. These have molecular weights of 24,000, 50,000, and 80,000 (Sonnenberg 1981; Griffo et al 1982; Pelletier & Sonnenberg 1985). The 24-kDa protein, the best-characterized mRNA cap binding protein, is not detected by UV cross-linking in vivo, but it can be readily detected by UV cross-linking in vitro if the label is placed in the cap itself (S. A. Adam et al, unpublished results). The cap binding activity is part of a cap binding complex; it contains eIF4b proteins, and it functions in the initiation of protein synthesis. The cap binding proteins were recently reviewed by Shatkin (1985) and will not be discussed further here. To date there is no direct evidence that sequences other than the poly(A) tail and the cap are bound to proteins. Specific functions for other mRNP proteins have not been directly shown. In addition to the major mRNP proteins, some of which are almost consistently found in all studies, a multitude of other proteins have been described. At this point, enumeration of these proteins is fruitless because practically no two papers record the same patterns. Thus it is not possible to evaluate the authenticity or significance of any of them. In addition to the major proteins common to all mRNAs, there probably are proteins that bind specific mRNAs and also proteins that bind the same mRNA in different subcellular compartments (e.g. polyribosomal and nonpolyribosomal). This may explain the numerous minor proteins that become cross-linked with the mRNA upon exposure to UV light or other agents. What is needed to sort out this complexity is definitive evidence for the authenticity of the mRNP protein (such as proof that they interact specifically with mRNA in the cell), demonstration of a function for these proteins, or evidence for a specific binding site on the mRNA. Specific probes for the proteins, including antibodies and cDNA clones, will be essential in addressing these questions.

Little is known about the structure of the mRNP complex other than about the 3′-poly(A) segment and the 5′-cap. The poly(A) tail appears to form a particle of unique periodicity with the specific poly(A) binding protein (Baer & Kornberg 1980). There are as yet no indications that the proteins of mRNPs interact with other proteins to form higher order structures analogous to the monoparticles of hnRNPs. Altogether the structure of the mRNP complex appears to be much simpler than that of hnRNP. Indeed, the function of mRNA in translation suggests that the

mRNA must be exposed, accessible, and able to thread through ribosomes. In principle it is also possible that the translational machinery may be able to move through some loosely associated binding proteins. However, mRNA packaging may be different in special circumstances such as in the storage of mRNAs in early embryogenesis, when packing, sequestering, and protecting the RNA (rather than its immediate translation) may be important. There are also reasons to believe that in other special circumstances (e.g. heat shock) additional specific mRNA segments, e.g. 5′ and 3′ untranslated sequences are bound by specific proteins.

POLY(A)-RIBONUCLEOPROTEIN COMPLEX The poly(A) segment of mRNA was found to cross-link to the 72-kDa poly(A) binding protein upon exposure to UV light in vitro (Setyono & Greenberg 1981; Greenberg 1981; Greenberg & Carroll 1985) and in vivo (Greenberg & Carroll 1985; Adam et al 1986a). Recently, Sachs & Kornberg (1985) also identified cytoplasmic poly(A) binding activity in yeast in the molecular weight region between 66,000 and 79,000. Moreover, Adam et al (1986b) identified a 72-kDa protein that is cross-linked to the poly(A) tail of yeast mRNA in vivo and has poly(A)-specific binding activity in vitro. The fact that such a protein was found in diverse cells suggests that it is common to many, and perhaps all, mRNAs. In fact, the 72-kDa protein is also cross-linked to the poly(A) tail of vesicular stomatitis virus mRNAs in infected cells (Adam et al 1986a). This protein may also be associated with other parts of the mRNA. There are conflicting reports as to whether the poly(A) tail is associated with the 72-kDa protein only in polyribosomal mRNAs or also in nonpolyribosomal mRNAs (e.g. Van Venrooij et al 1977; Vincent et al 1981; Butcher & Arenstein 1983; Greenberg & Carroll 1985).

Baer & Kornberg (1980), using digestion with a non-base-specific RNAase (T2), detected a repeating structure in the cytoplasmic poly(A)-ribonucleoprotein complex. The repeating structure consists of multiples of about 25–27 adenosine residues bound to protein and can form spontaneously by incubation of poly(A) with cytoplasmic but not with nuclear extract. The protein responsible for this repeating structure was subsequently isolated from rat liver and found to be of molecular weight 75,000 (Baer & Kornberg 1983). It is probably the same protein that binds poly(A) in vivo. The absence of similar poly(A) organizing activity in the nuclear extract (Baer & Kornberg 1980) and the lack of cross-linking of the 72-kDa protein to nuclear RNA (Setyono & Greenberg 1981; Dreyfuss et al 1984a,b) suggest that this protein is not present in the nucleus, in contrast to the finding of Kumar & Pederson (1975). Kelly & Cox (1982) observed that the size distribution of the poly(A) tail of globin mRNA in

vivo shows peaks at intervals of ~25 residues, which agrees with the expected periodicity. This appears to reflect the fashion in which the poly(A) binds to the proteins, and these interactions may control mRNA degradation. The ability of the poly(A) binding protein to protect the poly(A) tail from nuclease suggests a role for the protein in mRNA stability.

Although the poly(A) binding protein is the most abundant and was the first mRNA binding protein described, little is known about its structure or function. The intimate association of the poly(A) binding protein with the poly(A) tail probably indicates that their functions are interrelated. It has been suggested that the poly(A) tail-protein complex is involved in various key aspects of mRNA metabolism, including nucleocytoplasmic transport (Schwartz & Darnell 1976), mRNA stability (Zeevi et al 1982), and translation (Van Venrooij et al 1977; Vincent et al 1981; Schmid et al 1983; Jacobson & Favreau 1983), and that it may be related to a poly(A) polymerase (Rose et al 1979). It has been difficult to directly address these questions because antibody and gene probes for the protein were not available. The mRNA poly(A) binding protein from vertebrates is poorly immunogenic in mice and rabbits, and previous attempts by a number of laboratories to produce antibodies to it were not successful. Adam et al (1986b) recently identified, and produced antibodies to, the major proteins that interact with polyadenylated RNAs in the yeast *Saccharomyces cerevisiae*. The poly(A) segment of the mRNA in yeast is also selectively cross-linked to a 72-kDa protein. Mice immunized with purified, UV cross-linked RNA-protein complexes produced antibodies to the major yeast mRNP proteins, including the poly(A) binding proteins. A yeast genomic DNA library constructed in an expression vector was screened immunologically, and a recombinant phage producing a large β-galactosidase-RNP fusion protein bearing the gene for the poly(A) binding protein was isolated. The expressed fusion protein had specific poly(A) binding activity. DNA blot analysis suggested a single gene for the poly(A) binding protein, and mRNA blot analysis detected an mRNA of 2.1 kb in length (Adam et al 1986b). A. Sachs & R. Kornberg (personal communication) also produced antibodies to the yeast protein and isolated the gene. These findings open the way for molecular and genetic characterization of the mRNA poly(A) binding protein.

mRNP PROTEINS OF SPECIFIC mRNAs The studies discussed so far isolated and characterized the proteins associated with all or with a large number of different mRNAs. Important insights can be obtained from analysis of the proteins that interact with specific mRNAs. This approach presents the considerable difficulty of selecting specific mRNAs with bound

proteins. For some mRNAs, e.g. globin and protamine, partial purification can be accomplished by velocity sedimentation alone because of their distinct size and abundance in a specific cell type. Morel et al (1971), Blobel (1972), Burns & Williamson (1975), and Vincent et al (1981) prepared partially purified globin mRNPs from chicken and mouse erythroblasts, and Gedamu et al (1977) isolated protamine mRNP particles from trout testis. Several proteins were detected, and the 72-kDa poly(A) binding protein was common. Several recent studies used immobilized cDNA for hybrid selection of specific mRNAs after UV irradiation of intact cells to purify cross-linked mRNP complexes. Van Venrooij et al (1982) selected adenovirus 2 mRNAs and found that they are bound in infected cells to typical major host mRNP proteins. Ruzdijic et al (1984) used the same method to isolate the nonpolyadenylated histone H4 mRNA from HeLa cells. They found that in the polyribosomal fraction it is cross-linked to 49- and 52.5-kDa proteins, whereas the nonpolyribosomal H4 mRNA is cross-linked to 43- and 57-kDa proteins.

Adam et al (1986a) employed a different approach to examine the proteins that are associated in vivo with a small and unique set of mRNAs, the mRNAs of vesicular stomatis virus (VSV). This method does not require isolation of these mRNAs from the total mRNA as the mRNAs of VSV can be selectively labeled in vivo because the polymerase of the virus, unlike host RNA polymerase II, is not inhibited by actinomycin D (at 5 μg/ml). The proteins that were cross-linked in vivo specifically to the five mRNAs of VSV were labeled by incorporating radioactive nucleotides into VSV mRNAs only. The same major proteins that become cross-linked to host mRNAs also became cross-linked to VSV mRNAs (Adam et al 1986a). The poly(A) segment of VSV mRNAs, like that of host mRNAs, was also associated with the 72-kDa poly(A) binding protein. The major mRNPs are therefore ubiquitous and are common to different mRNAs in the same cell. Furthermore, that the VSV mRNAs are transcribed in, and are entirely confined to, the cytoplasm argues that mRNAs can acquire the major mRNP proteins in the cytoplasm, presumably without nuclear processes.

Reconstitution experiments with specific mRNAs represent another useful and promising avenue for studying mRNP structure and for identifying sequence-specific mRNA binding proteins. Gaedigk et al (1985) and Greenberg & Carroll (1985) examined the proteins that bind purified globin mRNA in mouse erythroleukemia cells and rabbit reticulocyte lysate, respectively. The major proteins that bound the globin mRNA were similar to those normally detected when the entire mRNA population is analyzed.

One conclusion from all of these studies is that the major mRNP proteins

are common to different mRNAs and therefore must recognize common mRNA features. These include the poly(A) tail, the cap, the polyadenylation signal (AAUAAA), and non-sequence-specific ssRNA or dsRNA binding proteins. However, it is very likely that there are also specific proteins for specific mRNAs. These could be of two general classes: (*a*) sequence-specific proteins that would bind only to mRNAs containing a particular sequence (e.g. the leader sequence of heat-shock mRNAs), and (*b*) compartment-specific mRNA binding proteins that would bind all mRNAs in a particular subcellular topological or metabolic compartment or in a special physiological state (e.g. translated, untranslated, membrane-bound, or putative cytoskeleton-associated mRNAs). These sequence-specific and compartment-specific proteins superimposed on the major mRNP proteins that are common to all mRNAs could explain the multiplicity of components of mRNPs seen in different studies even for specific mRNAs.

mRNP COMPLEXES IN VIRUS-INFECTED CELLS Virus-infected animal cells are unique systems in which to study mRNP and hnRNP proteins. During lytic infection, copious amounts of a small number of well-defined viral genes are transcribed, and drastic changes in mRNA formation and translation in the host usually occur. These changes in host mRNA metabolism are a consequence of the expression of virus proteins. Furthermore, because the abundant mRNAs of the virus are structurally similar to those of the host, they can be considered prototypes of host mRNA.

A tight association of a virus-encoded protein with mRNA was first observed by Lindberg & Sundquist (1974) in HeLa cells infected with adenovirus (Ad) 2. mRNP complexes isolated from these cells at high salt concentrations contained large amounts of the 100-kDa late nonstructural viral protein (Van der Marel et al 1975; Tasseron-De-Jong et al 1979). The 100-kDa protein could not, however, be detected by nucleotide label transfer after UV cross-linking (Van Venrooij et al 1982). The significance of these observations is not yet clear.

A complex of a viral protein with mRNA was also found in VSV-infected cells. Grubman & Shafritz (1977) showed that mRNP particles from VSV-infected cells contain the viral N protein. Adam et al (1986a), using UV cross-linking in intact VSV-infected cells, also detected the N protein in mRNP complexes and found that VSV mRNAs are associated with host mRNPs, including the poly(A) binding protein. Rosen et al (1982) isolated from VSV-infected cells a unique mRNP particle that contained the five VSV mRNAs and almost exclusively the N protein. Although the function of the N-VSV mRNA interaction is not known, it is probably functionally relevant: Rosen et al (1984) demonstrated that

N-VSV mRNA particles inhibit protein synthesis in rabbit reticulocyte lysate and wheat germ extracts. The inhibition is at an early step of initiation of protein synthesis, i.e. the formation of the ternary complex eIF-2.GTP.Met-tRNA. The N protein–mRNA complex may therefore be involved in the shutting off of total protein synthesis that occurs in VSV-infected cells. In general, viral protein-mRNA complexes are potentially of great importance because they may give rise to new RNP forms that may modify elements of normal host pathways and facilitate viral functions.

TRANSLATED AND UNTRANSLATED mRNAs The search for differences in the proteins that bind translated and untranslated mRNAs is motivated by the interest in identifying the factors that control the state of translation of mRNA. It has received much attention, and over the years numerous studies have compared polyribosome-bound and "free," non-polyribosomal mRNPs. Although some differences were detected (e.g. Butcher & Arenstein 1983; Schmid et al 1983; Ruzdijic et al 1984; Jeffrey 1977; Blobel 1973; Liautard et al 1976; Bag 1984; Vincent et al 1981; Van Venrooij et al 1977), no consistent differences that can account for the functional state of the mRNA were found, and a coherent picture did not emerge. This task is complicated by the very fine and blurred line of distinction between mRNP proteins and translational factors that may cofractionate with mRNPs. Specific functional assays will be necessary to establish a direct role for mRNP proteins in translation.

Stored mRNAs can be considered to be a special class of untranslated mRNPs. The early work of Spirin and colleagues demonstrated that these mRNAs in sea urchin and fish embryos are found in mRNP complexes. Several proteins that are associated with stored mRNPs have been identified.

DYNAMIC STRUCTURE OF mRNP COMPLEXES In addition to the changes found in mRNP complexes after viral infections, structural changes in these complexes have been observed in cells treated with inhibitors of mRNA synthesis. VSV infection, actinomycin D (5 μg/ml), camptothecin, and DRB (5,6-dichloro-1-β-ribofuranosyl benzimidazole), all of which inhibit transcription by RNA polymerase II, cause a prominent protein of $\sim$38 kDa that cannot be normally cross-linked to mRNAs to become cross-linkable to mRNAs in vivo (Dreyfuss et al 1984a). The onset of the effect is rapid, and it is completely and rapidly reversible. Inhibitors of protein synthesis, rRNA synthesis, and polyadenylation do not affect the cross-linking of the 38-kDa protein to mRNA. These agents that promote the cross-linkable interaction of the 38-kDa protein with mRNA do not affect proteins in contact with poly(A)$^+$ hnRNA and do not markedly

affect protein synthesis. Although the significance of the interaction of the 38-kDa protein with mRNA is not known, these observations demonstrate that commonly used inhibitors of transcription bring about a structural change in mRNA-ribonucleoprotein complexes in vivo.

Greenberg's work (1980) indicates that the mRNP complex is a dynamic structure in which bound proteins can exchange with an unbound pool. In vitro the same proteins became cross-linked to mRNAs (upon UV exposure) in cytoplasmic extracts from cells treated with actinomycin D as in control cytoplasms. It was therefore suggested that mRNA-associated proteins can exchange with a free pool of proteins because the cytoplasm of the actinomycin-D-treated cells contained no newly made mRNAs and ongoing mRNA synthesis was not required for mRNP formation. However, this may not happen in the intact cell. The cross-linking of mRNA and a protein that may correspond to the 38-kDa protein discussed above was also detected in Greenberg's (1980) study after treatment with actinomycin D. The exchange of mRNP proteins, which does not occur with proteins in other RNA-containing structures such as ribosomes, may be important in converting mRNAs from one functional state to another, as for instance, in the activation of stored maternal mRNAs upon egg fertilization.

RIBONUCLEOPROTEINS AND NUCLEOCYTOPLASMIC TRANSPORT OF mRNA

The transport of mRNA from the nucleus to the cytoplasm is a critical process in eukaryotes. Little is known about this process, and it is still somewhat difficult to design useful experiments because the important mechanistic questions are not clear. However, it has now been shown by UV cross-linking and through the use of specific antibodies that the major hnRNP proteins are confined to the nucleus in vivo (Jones & Martin 1980; Dreyfuss et al 1984a,b; Choi & Dreyfuss 1984a; Martin & Okamura 1981; Leser et al 1984) and that the mRNA in the cytoplasm is associated with a different set of proteins. These findings are consistent with data previously obtained from fractionated cells (Kumar & Pederson 1975; Liautard et al 1976) and suggest that mRNAs must exchange the proteins with which they are associated in the nucleus upon transport to the cytoplasm. The translocation of the mRNA across the nuclear envelope through nuclear pore complexes is, therefore, accompanied by a protein exchange process. The nuclear proteins must dissociate prior to, or at the time of, mRNA translocation. This suggests that the dissociation of hnRNP proteins from the mRNA must be an early event in the transport

pathway, and it is thus a distinct process that can now be addressed experimentally, both in vivo and in vitro.

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