Big Problems Faced in RNA Processing

The search for the secrets of splicing messenger RNA involves some steps forward and some back

Most RNA is synthesized as a precursor and must be processed before it can be active in the cell. A multiplicity of cleavage, ligation, addition, and chemical modification reactions have been recognized as essential steps in the maturation of RNA, the most notorious of which is the precise removal of noncoding, or intron, regions from the messenger RNA molecules of higher organisms. The discovery of split genes, announced 5 years ago this month, endowed the study of RNA processing with a glamor it previously lacked. It is now an intense field of activity, and one recently discussed at a Cold Spring Harbor meeting at the end of May.

Although, alas, the processing of introns from messenger RNA remains virtually as much a mystery as it was 5 years ago, the meeting was impressed with two firm and important messages, one of which has fascinating implications for the study of the origin of life.

First, while the primary sequence of RNA molecules is often essential for their function, it is now clear that the crucial dimension of activity is the tertiary structure. "For the first time many people appreciated higher order structure in RNA," says Norman Pace of the National Jewish Hospital, Denver, Colorado. "RNA is much more profound structurally than DNA."

Second, RNA is now known to be more than a simple passive molecular scaffold in some important instances. In combining with proteins, RNA might sometimes play a direct role in the mechanics of a catalyzed reaction. And in one case of RNA processing so far discovered, an RNA molecule displays enzymic activity in the total absence of protein. "This discovery helps simplify our thinking about the components required in the primordial replicating systems," says Pace. It also demands that the chemistry of other RNA molecules be examined more closely to see how general this phenomenon might be.

Beyond these broad conclusions, the Cold Spring Harbor meeting brought into sharp focus the key problems in RNA processing research. Specifically, these concern the physical nature of cellular context in which processing takes place and the detailed mechanics of the reactions involved. Ironically, details of some of the chemical reactions of certain processing steps are being elucidated while the cellular context remains largely obscure. Most frustrating of all, and contrary to all early expectations, no one has yet succeeded in developing an in vitro system that will reliably and with high efficiency remove introns from messenger RNA and ligate the remaining coding regions. More than two dozen laboratories are devoting at least some effort to this goal, and there is a desperate search for an inspired breakthrough.

RNA can be divided into two main groups. The first contains large RNA molecules, the principal targets of processing reactions: messenger RNA, ribosomal RNA, and transfer RNA, all of which are involved in protein synthesis. And the second is a rather enigmatic group of small RNA molecules, some of which are located in the nucleus, some in the cytoplasm, and others in both. With the exception of one, which was the subject of a surprise paper at the meeting, these dozen or so small RNA's, which fall into three major classes, have no known function. Virtually all RNA molecules, both large and small, are associated with specific ribonucleoproteins (RNP's).

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Cell biologists have for many years been trying to elucidate the internal structure of the cell nucleus by subjecting it to horrendous treatment, and then looking at the resultant bits and pieces, both under the electron microscope and in biochemical fractionation methods. The search has been particularly focused on the structure, or structures, responsible for processing the precursor to messenger RNA and transporting the product to the cytoplasm.

The messenger RNA precursors are commonly found associated with a conglomeration of protein molecules, in the form of heterogeneous ribonucleoprotein (hnRNP) particles. The precise number of protein molecules contained in these particles, their molecular weights, and the overall size of the particles varies according to the method used for their isolation. This fact encourages some people to believe that hnRNP particles are artifactual, to some degree at least. Others note that the potential association of precursor messenger RNA with protein particles is strikingly reminiscent of the beads-on-a-string structure of DNA in chromatin.

Ann Beyer, who collaborates with Oscar Miller at the University of Virginia, has produced some electron microscopy pictures of active transcription of DNA into RNA in *Drosophila* and in HeLa cells infected with adenovirus 2. She appears to have evidence that hnRNP-like particles are associated with newly growing transcripts of RNA in a nonrandom way. Specifically, she says that the distribution of protein particles might be determined by certain sequences in the RNA and that the resulting structure is important in cleaving complete transcripts from the growing chain and in further processing.

Just a few years ago a second set of RNA-containing particles became implicated in studies on messenger RNA processing. A set of small nuclear RNA's, with chain lengths varying between 107 and 189, are each associated with seven protein molecules which form small nuclear RNP (snRNP) particles. Some of the snRNP particles were found to sediment with the hnRNP's under centrifugation. Some were found to be hydrogen bonded with hnRNP's. Most intriguing of all, a short sequence at the beginning (the 5′ end) of one of the RNA's, U1, was shown to be complementary to the so-called consensus sequences at the ends of introns.

Michael Lerner and Joan Steitz of Yale University and John Rogers and Randolf Wall of the University of Cali-

0036-8075/82/0611-1208$01.00/0

fornia, Los Angeles, suggested that this complementarity might be the basis of a mechanism by which the two ends of the intron are brought together, thus making them available for cleavage and splicing. And then Jane Flint, now at Princeton, and Steitz and her colleagues showed that antibodies that precipitated the U1 snRNP particle appeared to block splicing of the messenger molecule.

Although only U1 RNA could be closely associated with a putative splicing function, its close relatives, U2 and U4-6, also acquired a special interest. What aspects of splicing might they be involved in? This was the apparent implication of the many similarities within this intriguing group of molecules.

A year ago prospects looked promising for work on the small nuclear RNA's and their involvement in splicing, and as a result one-third of the Cold Spring Harbor meeting was devoted to them. Times and favors change, and there is now less optimism about the certainty of this relationship. "Enough people have been working on this for enough time to reveal a simple splicing function if one existed," comments Hugh Robertson of Rockefeller University. Indeed, some of the earlier results on antibody-blocking of splicing now look less secure than they did. The gloss is off the small nuclear RNA's, temporarily at least.

Peter Walter of Rockefeller University described in an unscheduled talk convincing evidence that a cytoplasmic small RNA, 7*S* RNA, functions in the transport of proteins that are exported from the cell. Six proteins are associated with the 7*S* RNA, and this particle interacts with the signal sequence attached to the front of every exported protein. The 7*S* particle binds with a membrane receptor and helps guide the protein out of the cell as it is being synthesized. Continued translation of exported proteins depends on the presence of the 7*S* particle on the ribosome. The particle is therefore in some ways an extension of the ribosome. Walter does not yet know whether the interaction between the 7*S* particle and the signal sequence involves protein or RNA binding.

Another hint of a role in translation for some small RNA's comes from Thomas Shenk of the State University of New York, Stony Brook. He finds that if certain small RNA's associated with adenovirus 2 infection are defective through mutation, initiation of translation is prevented. Undoubtedly the small RNA's will all eventually be assigned roles, and, says James Dahlberg of the University of Wisconsin, "they may be involved in a wide range of functions."

The hnRNP particles, meanwhile, are on even less secure ground. They might be real and important, but perhaps as part of a larger structure. Roger Kornberg and his colleagues of Stanford University provoked enthusiasm and skepticism in about equal proportions with the suggestion that premessenger processing might take place in a large complex of proteins and RNA, a 500 to 700 Å in diameter virus-like arrangement with the nucleic acid protected in the interior.

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The evidence indicating the existence of such a structure, which might be the equivalent of a conglomeration of 10 to 15 hnRNP particles, is still highly tentative. James Darnell of the Rockefeller University describes the work as "an important preliminary finding." And Robertson and Steitz agree that the idea is very appealing. Premessenger molecules from which several introns must be removed and the coding regions precisely linked surely require some kind of superstructure on which ordered folding can take place. A complex such as the one Kornberg proposes might provide this, they speculate. Others consider the idea and the results to be fanciful.

"If splicing does depend on some kind of complex structure, this might explain why it is proving so difficult to reproduce the reaction in vitro," says James Manley of Columbia University, who 3 years ago developed the widely used in vitro DNA transcription system.

Manley points out that in the in vitro splicing of transfer RNA in yeast, a system that has been aggressively developed by John Abelson and his colleagues at the University of California, San Diego, the cleavage enzyme apparently resides in a membrane. The ligation step is separate and is carried out by a soluble enzyme. "There might be lessons here for messenger RNA splicing."

The Cold Spring Harbor meeting heard Walter Keller of the German Cancer Research Center, Heidelberg, explain that his in vitro splicing system was not working as well as it apparently did a year ago. Meanwhile, Selina Chen-Kiang of the Sloan Kettering Institute and Ryszard Kole from Sherman Weissman's laboratory at Yale both report in vitro splicing activity, albeit at low levels. "There are hints that it is beginning to work," acknowledges Manley. The development of an efficient system is vital to a successful dissection of the elements involved in splicing in vivo.

The impressive characteristic of many aspects of RNA processing is the accuracy of cleavage and ligation reactions. A central goal of research here is to discover how this accuracy is achieved. The detailed chemical interaction between individual bases is certain to be important, as are sequences of stretches of the RNA molecules in many cases. But when Harry Noller of the University of California, Santa Cruz, conducted a guided tour through planetarium-like pictures of the 16*S* RNA fragment of the ribosome, the rich three-dimensional quality of the molecule strongly impressed the audience. This conclusion is reinforced by the apparent maintenance of tertiary structure between species, even though the primary structure varies. Darnell describes Noller's work as "most significant progress, if true."

The problems of reconstructing tertiary structure from a knowledge of the primary structure of RNA are, however, daunting, especially given the size of many messenger RNA molecules. The audience was chastened, although also enlightened, when Ignacio Tinoco of the University of California, Berkeley, explained the current limitations of his "rules" for estimating higher structures of RNA. "Nevertheless, it is the only thing we have at present," says Pace, "and higher order structures clearly demand our greater attention. The ordered stems, loops, and folds of the molecule are probably important means by which specific sections of the molecule can be presented accurately to enzyme action."

In the precursor to ribosomal RNA in *Tetrahymena thermophila*, the RNA appears not only to fold into a three-dimensional structure that specifies the sites of excision of a 413 base–long intervening sequence, but also to possess the catalytic activity to carry out the splicing function. Moreover, the excised section is cyclized, again in the absence of protein. The processing is initiated by the presence of a guanosine nucleoside which, says Thomas Cech and his colleagues at the University of Colorado, slots into a specific binding pocket in the precursor.

The discovery of autocatalytic activity in RNA is perhaps the most tantalizing aspect of the rapidly expanding study of RNA processing. It certainly underlines the conclusion that RNA is by no means a less interesting molecule than its usual progenitor, DNA.—ROGER LEWIN