Potential Regulatory Roles for RNA in Cellular Development

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Summary

In addition to its widely accepted messenger and structural roles, RNA has been implicated in several other biological events: transfer of specificity of the immune response, interferon induction, and infection of plants by viroids. In none of these cases is the mechanism of RNA action understood. As a result of recent advances in the understanding of RNA metabolism in eukaryotic nuclei and potential specificity of enzymes that cleave such RNA, we have suggested specific ways in which “extra” RNA could be involved in regulation of the development of cells. A survey of literature concerning mammalian cell differentiation both in embryos and in the immune system leads to the observation that there appears to be a line of communication between macromolecules on cell surfaces and the genome. Making the conservative assumption that the DNA retains its integrity throughout development, it seems likely that highly specific signals are sometimes required to transfer information from outside the cell to the DNA, resulting in a change in state of differentiation. We propose a way in which RNA could be utilized in this process.

Introduction

Several biological systems have been described that appear to utilize RNA in a regulatory role. Numerous reports of the involvement of RNA in the transfer of information to specify the production of an antibody to a particular antigen have appeared (Refs. 1 and 23; for recent reviews see Refs. 15 and 42). Cells incubated in the presence of “immune RNA” can apparently be converted to the production of antibodies of the specificity of the cell from which the immune RNA is isolated.

A 2nd biological process in which RNA has an unexplained regulatory role is that of interferon induction. In this case, double-stranded RNA at extremely low concentrations can cause mammalian cells to synthesize interferon (14, 46), which in turn is involved in conferring resistance to viral infection upon other cells (29).

A 3rd intriguing case of biologically active RNA is that of plant viroids. Viroids represent the smallest infectious, self-replicating entity yet described (20). A free RNA molecule 250 to 350 nucleotides long, applied to the leaves of plants such as tomato and potato, infects and replicates in the plant causing severe disease symptoms within a few weeks (21). Viroid RNA cannot stimulate protein synthesis in vitro (18, 28), nor can it be charged with an amino acid (28). Thus, it is unlikely that it acts either as a mRNA or as a tRNA in vivo. The fact that it is so small compels us to consider the possibility that it interacts directly with the DNA to bring about its pathogenic effect. If this is the case, then it could represent an aberrant form of regulation of which the normal form is important to the control of gene expression in healthy cells.

If, as these examples suggest, RNA does act as an informed signal that interacts either directly or indirectly with the DNA to bring about a change in the pattern of gene expression, then it would be important to elucidate the mechanism of action of such regulatory RNA. An increased understanding of events leading to expression of phase-specific genes during normal development could establish a connection between the reexpression of embryonic genes and the occurrence of the diseased state in cancer cells. We will first consider the features of RNA that make it especially suitable for a regulatory role, then suggest a possible source for the proposed regulatory RNA, and, finally, describe possible modes of action.

Properties of RNA that Make It Especially Suitable as a Regulatory Molecule

Molecules that have a primary role in the control of gene expression must be able to interact with the DNA in a highly specific manner. Proteins such as the lac repressor can select and bind with high affinity to a unique region within the DNA of Escherichia coli and prevent transcription. However, the amount of information required to specify this interaction is about 1000 nucleotides, since this regulatory protein is about 350 amino acids long (25). When the notion of repressor proteins is extended to eukaryotic systems, it becomes apparent that a 2nd gene would be required to control transcription of the repressor protein gene; this 2nd gene action must also be regulated, and so on. In contrast, consider the efficiency of using RNA to select specific regions from within the DNA. RNA is particularly well suited to this task since it is capable of binding to single-stranded DNA by complementary base pairing. In addition, a sequence of nucleotides just 17 bases long is sufficient to specify a unique region within the DNA of the human genome (11).

Although RNA has a coding capacity equivalent to that of DNA, it is subtly different from DNA in that it contains a hydroxyl group on the 2nd carbon of the ribose ring. Because of this chemical difference, the organism can use enzymes that digest RNA rapidly while leaving DNA intact.
Then, abrupt changes in state of differentiation could be accomplished by rapid destruction of 1 set of RNA molecules and creation of a new array.

Recent X-ray crystallographic studies (32) of yeast phenylalanine tRNA at a resolution of 2.5 Å have shown that the presence of the 2’-hydroxyl group in RNA confers additional properties upon these molecules. This hydroxyl group can receive and/or donate protons in hydrogen bonding to various parts of the backbone of the polynucleotide or to the pyrimidine or purine rings. As a result, the tRNA folds up in regions in a complex way more reminiscent of protein structure than of nucleic acid structure. Such “globular” regions are ideal for interaction with proteins. In other regions the tRNA demonstrates base pairing and helicity more conventionally associated with nucleic acid structure.

Because RNA can turn over rapidly, has a high coding capacity, and can interact with high specificity with both DNA and protein, it is a prime candidate for the job of gene regulation.

A Source for Regulatory RNA in Eukaryotes

No function has yet been ascribed to the major class of RNA transcribed in eukaryotic nuclei. This heterogeneous nuclear RNA, so-called because it ranges in size up to 100 S and greater in some organisms, remains mainly in the nucleus where the bulk of it is broken down rapidly, with only about 10% ever reaching the cytoplasm (7, 16, 47). Some authors have suggested that the rapid turnover in the nucleus of the majority of this RNA represents degradation of unnecessary transcripts (9, 41, 48). We find much more attractive suggestions such as that of Judd (31), that some of the “extra” RNA within huge transcripts could have a regulatory role within the nucleus where it could act to coordinate action of related genes. The conventionally held view of what happens to the extra RNA in the nucleus is that a battery of powerful nucleases attacks and renders it soluble as a result of rapid random cleavage. If, however, a function is assigned to this class of RNA, it is necessary to postulate the presence of enzymes within the nucleus that can release the regulatory RNA from the huge RNA precursors in a precise and reproducible way.

Evidence for the Presence of Processing Enzymes within Eukaryotic Nuclei

RNase P, a well-characterized enzyme from E. coli, is a good prototype for the kind of enzyme to look for in eukaryotic nuclei (38). In E. coli, tyrosine tRNA is released from a precursor RNA by cleavage of a single phosphodiester bond by RNase P as shown in Chart 1 (4, 6, 38). The RNase P reaction is extremely sensitive to mutational changes in the sequence of the tRNA. For example, mutations at Position 15 or 25 of E. coli tyrosine tRNA drastically slow down the rate of cleavage of the precursor by RNase P (4, 5). In vivo, in these mutants, the tRNA precursor is degraded by other enzymes before the RNase P cleavage can occur, so that the mature mutant tRNA appears in much lower amounts than does wild-type tRNA. On the other hand, it has been shown that, when this mutant tRNA is released from its precursor by RNase P in vitro, it has a stability comparable to wild-type tRNA’s (3-5). Two important characteristics of this enzyme need to be stressed: (a) the signal that specifies cleavage includes features of sequence and structure within a large region surrounding the point of cleavage, and (b) proper cleavage releases a mature RNA species that is stable in the presence of the same nucleases that are capable of destroying this same RNA region when it is part of the precursor molecule. A schematic representation of these features appears in Chart 2. This example serves to illustrate the concept of generating, by cleavage, a stable RNA molecule from a previously unstable RNA species.

An enzyme with specificity identical to that of RNase P has also been described recently in KB cells (4, 8). A 2nd specific enzymatic activity, RNase NU, has been found both in mammalian cells and in E. coli (8). Thus, it is apparent that, not only do eukaryotic cells contain RNA processing enzymes, but also, these activities may be conserved in evolution (5, 39).

The presence of RNA-processing enzymes within mammalian nuclei can also be inferred from recent studies of adenovirus RNA metabolism (36, 43). Transcripts from both strands of the adenovirus genome are present in the host nucleus during both early and late periods of the infection cycle. During the early time, however, only the early mRNA reaches the cytoplasm as mature mRNA, whereas regions containing late gene transcripts turn over rapidly in the host nucleus (36, 43). Thus, at the time of adenovirus infection, the host nucleus must contain specific RNA-processing enzymes that can recognize the adenovirus early-mRNA regions within the longer transcripts and cleave them out, thereby permitting their maturation. Since this cleavage occurs before production of any adenovirus proteins is

![Chart 1. Sequence and possible secondary structure of Escherichia coli tyrosine tRNA precursor RNA (6) showing the site of RNase P cleavage 41 nucleotides from the 5' end of the precursor, at the site of the 5' terminus of the mature tRNA molecule.](chart.png)
entiation. We have previously considered possible roles for such RNA fragments (39). Chart 3 is a schematic representation of 5 possible roles for such RNA fragments. In this paper we will deal mainly with the suggestion that RNA cleaved from a transcript of 1 gene could prime the transcription at a 2nd site in the genome, thereby causing coordinate production of these gene products. In particular, we would like to suggest how phase-specific RNA molecules could be involved in maintenance of the differentiated state and how imbalance in the environment of the cell could lead to a scrambling of the program expressed.

A variety of proteins synthesized in embryonic cells has been observed to reappear in tumor cells (2, 35). Also present in these cells must be the corresponding phase-specific mRNA’s. Within the context of the ideas about RNA precursors and specific processing of these molecules outlined above, we suggest that there are also phase-specific populations of RNA cleavage products confined to the nucleus which could function as regulatory entities. One way in which such regulatory RNA could be involved in the control of transcription during normal developing is depicted in Chart 4. Small RNA fragments cleaved from the extra regions of the precursor to a particular mRNA would locate complementary regions within the genome, become engaged in hydrogen bonding, and act as primers in those locations for transcription of the adjacent region which would contain a precursor to a new mRNA (see legend to Chart 4 for details). As discussed previously (39), this control mechanism would be stoichiometric (the RNA primer would be used up in the reaction it initiates) and could account for a burst of synthesis at a particular site which would be controlled both in extent and duration by an initial priming event. Details of these arguments will not be reviewed here. It is sufficient to say that, within the context of control of transcription by proteins revealed in a series of bacterial studies (30), the use of RNA as an additional control element would add flexibility, efficiency, and elegance to a logical system of gene control (10, 39).

State of the Genome in Cellular Development. In applying these ideas to questions of cellular development, we
The genome remain unaltered throughout differentiation. It is clear that tumor cells from 1 terminally differentiated tissue type suddenly express a number of genetic characteristics normally associated with an unrelated cell type (2). However, any system that is to be a valid prototype for differentiation must allow cells to give rise to primers upon subsequent processing.

have made the assumption that no irreversible changes occur in the DNA of any cell undergoing differentiation. We realize that available data about immune cells are most often interpreted as favoring irreversible changes in the DNA by somatic mutation or recombination (13, 22, 44) of developing lymphocytes resulting in production of 1 and only 1 antibody per cell (13). However, any system that is to be a valid prototype for differentiation must allow cells to retain their full genetic complement. Such retention of full genetic capability has been demonstrated in nuclei of frog intestinal epithelium cells (27) and by the frequent observation that tumor cells from 1 terminally differentiated tissue type suddenly express a number of genetic characteristics normally associated with an unrelated cell type (2).

The view of a conservative geneticist would require that the genome remain unaltered throughout differentiation. It will soon be possible to determine experimentally whether or not irreversible changes do occur in the DNA of lymphocytes by combining the techniques of RNA:DNA hybridization with comparative fingerprinting analysis of antibody mRNA’s. An exploratory application of such techniques to a particular mouse myeloma light-chain mRNA has recently appeared (37). By adding RNA fingerprinting analysis to the more conventional RNA:DNA hybridization approach, it was shown that RNA which hybridized to the single-copy DNA contained fragments from both the variable and constant regions of the mRNA. In addition, comparative RNA fingerprinting studies, which are capable of revealing the extent of variation among closely related RNA molecules (40), could be effectively used in combination with the above approach (37).

The somatic mutation hypothesis invokes random changes in the DNA to explain the enormous number of different antibodies that can be observed. This hypothesis predicts differences in the nucleic acid sequences of mRNA’s coding for antibodies of identical amino acid sequence isolated from different animals. Myeloma lines have been independently isolated which secrete antibodies with light chains of identical sequence (19). Isolation and sequence analysis of significant portions of light-chain mRNA’s from such cell lines should therefore confirm or refute the validity of the somatic mutation hypothesis.

For the balance of this discussion we shall make the assumption that the information content of the DNA remains intact, and, thus, a cell retains the potential of reexpressing embryonic genes even after becoming highly differentiated.

**Signals for Cellular Development.** If there are no irreversible changes in DNA during differentiation, then all cells are identical with respect to their DNA content. Thus, some other component must be directing and maintaining the state of differentiation. We would expect a particular state of differentiation to be maintained actively by regulatory machinery. All of the DNA would then be accessible to reactivation by changes in this pattern of regulation. Based on studies of cell-cell interactions, it seems that there may be a line of communication between the macromolecules on cell surfaces and the DNA (24, 26). Apparently, cells, depending on their environment, can be triggered to undergo various sorts of growth patterns (34, 45). For example, consider the growth characteristics of adult and regenerating liver cells, where a change in cell environment drastically changes growth patterns from static to exponentially dividing (12, 33). Perhaps the clearest example of a differentiating system requiring signals at the cell surface is the immune system. The information contained by a particular antigen is transmitted to a lymphocyte in such a way as to elicit the production of a particular protein composed of 2 different polypeptides, the heavy and the light chain, which together form a structure that binds with high affinity to the antigen in question.

Consider the possibility that RNA acts as an information carrier in this proposed line of communication between the cell surface and the DNA. This proposal can then be combined with our earlier suggestions about ways in which RNA synthesized early in development could remain complexed to a cell surface receptor protein awaiting a subsequent induction event. Such a hypothetical scheme might, for example, consist of: (a) interaction of the inducing agent with the cell surface receptor protein, (b) release of the latent RNA molecule from association with the receptor protein, and (c) interaction of this released RNA with the DNA to bring about a change in gene expression. This series of events is depicted schematically in Chart 5. The mode of action of the regulatory RNA represented in this chart is that of priming of RNA transcription to initiate the production of RNA from a previously silent gene (see Chart 4).

If we apply this kind of thinking to the response of the immune system to a particular antigen, it leads to several further proposals. First, lymphocytes could contain an array of Variation among closely related RNA molecules (40),
of light chain variable regions (V regions) complexed to some of the "extra" RNA from the V region mRNA on their surfaces. When the cell is confronted by a particular antigen, if V regions with the ability to bind with high affinity to this antigen existed on a particular cell, these V region receptors would bind antigen and, at the same time, would release their fragment of latent RNA. This RNA would travel to the nucleus, locate the region from which it was originally transcribed, engage in complementary base pairing, and act as a primer for the transcription of the adjacent region, namely, the V region that can bind with high affinity to the antigen that triggered the reaction. Extending this logic to the next step, this RNA transcript of the DNA encoding the V region could contain, at its 3' end, a short region complementary to the DNA sequence immediately adjacent to the DNA encoding the light chain constant region (C region). Thus, a 2nd priming event could occur, resulting in the production of a complete light chain mRNA, accomplishing the joining of these regions without the requirement of scrambling the DNA in an irreversible manner (22, 44).

Such a selection mechanism would allow lymphocytes to be multipotent with respect to their potential to respond to a variety of antigens, thus obviating the requirement for a separate line of cells, each with only 1 specificity, for responding to every antigen.

Potential Regulatory Roles for RNA

In addition to considering informed signals involving RNA at cell surfaces mediating communication with the nucleus, we would like to suggest a 2nd way in which major changes in gene expression could arise in differentiated cells. This 2nd mechanism involves the highly specific RNA-cleaving enzymes capable of producing RNA signals from primary transcripts in the nucleus. If we make the assumption that members can be added to or deleted from this group of enzymes at specific stages of development, it would lead to the scheme of events shown in Chart 6. At early times, both genetic units, A and B, are expressed. Three specific RNA-processing enzymes are present (Ez 1, Ez 2, and Ez 3, as shown in Chart 6). At late times, the descendants of this cell no longer transcribe embryonic gene A, have lost Ez 1 and Ez 3, and have acquired a new RNA-processing enzyme, Ez 5.
5. In the absence of Ez 1 and Ez 3, 2 primers that were previously stabilized by maturation cleavage are now turned over. At the same time, the presence of a new enzyme (Ez 5) allows the stabilization of a regulatory RNA from the extra region of gene B that had previously been unstable. Thus, the altered state of differentiation is reflected in an altered population of RNA fragments within the nucleus.

Now it is possible to discuss what would happen if Ez 1 were to reappear in the more highly differentiated cell. A regulatory RNA within the extra region of gene B that has not been released as a stable species since early in development is once more produced. This same RNA is capable of binding to the complementary region within the embryonic gene A and causing its reexpression. Furthermore, transcription of gene A would in turn lead to the production of a set of RNA-regulatory elements which could prime coordinate transcription of further genes abnormal for this state of differentiation. Thus, a scrambling of the genetic program of differentiation would have been accomplished.

However, what is the proposed mechanism of reappearance of enzyme Ez 1? As mentioned previously, it is possible to think of highly differentiated cells as being actively maintained in a particular state by constant positive control at the level of transcription. Cells within organs such as liver retain the capacity to detect the absence of an intact organ and react by changing their state of differentiation to that of a developing liver cell and then repeat the developmental pathway to regenerate a new liver (12, 33). It therefore seems possible that adult liver cells have receptors on their surfaces which constantly monitor the immediate neighbors, and when part of the liver is missing, cells receiving the signal could undergo a series of events something like those depicted in Chart 5. Thus, a latent RNA would be released by the cell surface receptor and would travel to the nucleus. In keeping with our suggestion that major changes of state are mediated by changes in RNA-processing enzymes, this RNA could prime transcription of a gene encoding a new RNA-processing enzyme. The presence of this enzyme could have profound effects upon the make-up of the population of stable RNA species within the nucleus (see Chart 6) and, in turn, upon the state of differentiation of the cell.

Conclusion

In this discussion, we have suggested a requirement for a line of communication between the cell surface and the genome so that regulatory events could occur in response to external signals. We have explored, in hypothetical terms, the possibility that this role could be carried out by RNA and applied this reasoning to cells undergoing differentiation, including the immune cells. We have also suggested an important role for the RNA-processing enzymes within the nucleus in selecting which subset of the RNA transcribed will become stable and, thus, capable of action. Finally, these 2 ideas have been combined to show how such a system could account for normal or regenerative differentiation.

The authors were requested to consider whether the conceptual framework presented here about RNA in gene regulation could also shed light on the cause of abnormal differentiation such as that observed in the tumor state. The following speculations are therefore intended to stimulate further consideration of these questions. Tumors can apparently arise spontaneously, can be induced by chemical agents, radiation, or tumor viruses, or can be genetically programmed. Is there a common target for all of these agents? Suppose that chemical carcinogens disrupt the sort of surface receptors we have been discussing, causing release of the latent regulatory RNA. As we have outlined, this would lead to scrambling of the genetic program. In the case of the tumor viruses, a change in the host group of specific RNA-processing enzymes (as discussed earlier in the case of adenovirus) can now be seen as potentially capable of similarly profound effects on host gene expression. Radiation could act at the level of DNA disruption causing uncoupling of previously coordinated events. Finally, tumors with high heritability would seem to be the result of an error in the genetic instructions for a developmental program.

The advantage of the various ideas outlined here, although highly speculative, is that, whether or not our suggested role for RNA in cellular development eventually gains experimental support, such ideas should serve to stimulate concrete experiments which will contribute to a solid basis for understanding whatever systems are eventually found to be the expression of eukaryotic genes.

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