A POSSIBLE ORIGIN OF RNA CATALYSIS IN MULTIENZYME COMPLEXES

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Abstract. Numerous attempts have recently been made to ascribe a preeminent role to RNA enzymes in primitive life systems. A model is proposed in which coenzyme-dependent RNA enzymes were initially organized in multienzyme complexes featuring (1) the continuous attachment of substrates to CoA-like carriers, as in fatty acid synthesis; and (2) the ordering of RNA enzymes via mRNA-like instructional strands. In this format, RNA enzymes would not have been required to recognize and specifically bind soluble substrates. The enzymes in this case may have required far less complexity than contemporary protein enzymes and thus less genetic information for their synthesis. An analogy is made between the proposed scheme and the protein translation mechanism, for which it may have been an evolutionary precursor.

1. Introduction

Recent discoveries that RNA molecules can function in some instances as enzyme-like catalysts have focused interest on the notion of primitive life systems based entirely on RNA (Orgel, 1986; Darnell and Doolittle, 1986; Watson *et al.*, 1987). The catalytic functions thus far recorded for RNA are associated with the processing of RNA itself, principally in the form of hydrolyses, phosphate-transfers and rearrangements (Cech and Bass, 1986; Zaug and Cech, 1986). These reactions may be incorporated in models of such critical systems as RNA replication and translation in what has come to be called the 'RNA World' (Gilbert, 1986).

Within this basic scenario, it would be desirable to extend the range of early RNA catalysis to include the vast domain of intermediary metabolism. Prior to the findings of RNA catalysis, White (1976, 1982) had proposed that primitive RNA enzymes may have employed organic coenzymes such as NADH and coenzyme A in metabolism. The nucleotide character of many such coenzymes was considered a possible evolutionary vestige of the RNA-coenzyme hybrids. Within RNA enzymes, organic coenzymes would have performed their characteristic function as the specific carriers of chemical units in group-transfer reactions. The ancestral analogue of pyridoxal phosphate, for example, would have donated amino groups in metabolism. As in protein enzymes, the functions of substrate binding and positioning would have fallen to the apoenzyme component. It is here, however, that RNA apoenzymes, which would most likely have contained a wide variety of nucleotides in poorly defined sequences, appear to be no match for contemporary protein apoenzymes composed of a strictly defined set of twenty amino acids linked in precise sequences. Without the remarkable capacity of protein enzymes to selectively bind extremely small substrate molecules in complex reaction mixtures, RNA

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enzymes would likely have reacted with a wide range of substrates in at best a metabolic chaos.

2. RNA Multienzyme Complexes

As one strategy for attaining metabolic order, it is proposed that RNA enzymes were initially organized in multienzyme complexes. In systems such as the fatty acid synthase of *E. coli* and the α-ketoacid dehydrogenases of the Krebs cycle, the substrates are fixed to the complexes as thioesters and sequentially carried to the various component enzymes. A hypothetical RNA complex employing a similar mechanism is shown in

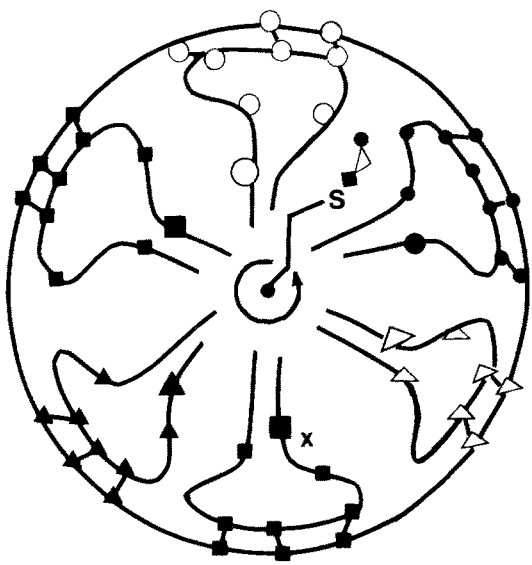

Fig. 1. RNA multienzyme complex with coenzyme A-like carrier. Starting from enzyme marked (×), three enzymatic steps have produced the thioester-linked metabolite shown. The carrier may be attached to another enzyme or possibly to a solid surface. Coding specificity arises here via coenzymic modifications of adjacent anticodonic bases, which generate ancestral analogues of the unusual tRNA bases. Representative interactions may include disulfide bridges, hydrophobic interactions and Watson-Crick pairing.

Figure 1. Here a series of RNA enzymes surround a central rotating carrier analogous to the coenzyme A-like prosthetic group of fatty acid synthase. Each enzyme possesses a coenzyme (large shape) which transfers specific chemical units (similar small shapes) to the thioester-linked substrate. The sequence of RNA enzymes in this case is determined via specific base-pairing with a circular instructional RNA strand. An analogy with the protein translation mechanism is apparent with the instructional strand functioning as mRNA, the RNA enzymes serving as tRNA-like adaptors, and the coenzyme-borne units (*e.g.*, methyl) replacing amino acids as the transferrable entities. In this configuration, it is noted that the RNA enzymes may not be required to bind with their substrates at all. Specific binding between the 'anticodons' of the enzymes and the 'codons' of the

instructional strand instead suffices for the alignment of pathway intermediates with specific coenzymes.

The metabolic efficiency of the proposed scheme may be appreciated by comparing its informational requirements with those of a corresponding system of protein enzymes. In contemporary metabolism, a protein enzyme may require for its own synthesis 100 codons to specify 100 amino acids, for example. In contrast, the same metabolic step catalyzed by this enzyme may be achieved in Figure 1 by an RNA enzyme reading just one 'metabolic codon'. The direct translation of genetic information into metabolic pathways via RNA enzymes would thus have made feasible extremely compact genomes requiring not only fewer constituent nucleotides, but also fewer replicational copies, as early RNA replicases could have more faithfully copied the shorter sequences. The combined effect may have been a substantial reduction in the metabolic load on early biological systems.

A detailed scenario of primitive metabolism based on the above scheme might feature banks of RNA multienzyme complexes organized in large grid-like arrays. Within such supercomplexes, metabolites formed on the thiol carrier of one cycle may have been easily transferred to the carriers of adjacent cycles to serve as starting substrates there. In this way, the problem of binding with soluble substrates would have again been avoided as *all* substrates would have been fixed to the supercomplex as thioesters. Metabolites such as succinyl-CoA and malonyl-CoA may perhaps be viewed as metabolic 'fossils' of such an ancient system.

3. Emergence of Biological Translation

The emergence of protein translation may perhaps be visualized in RNA complexes which are engaged in amino acid synthesis. Within such complexes, the 3′-termini of the RNA enzymes might be suitably positioned to readily accept amino acids formed on the thiol carrier, as indicated in Figure 1. In this case, the resultant aminoacyl-tRNA analogues must necessarily possess unique amino acid-anticodon pairings in each sector of the amino acid-forming cycle. In conjunction with a 'true' mRNA, these species might then combine to form coded polypeptides. This development of one mode of translation from a precursor mode may in turn be suggestive of an evolutionary progression in which adaptors donate units of increasing size in translation to make ever larger metabolic products. In this scenario, the earliest and smallest units to be transferred may have been electrons in processes of energy transduction. Following the stages of metabolic translation, featuring larger coenzyme-specified units (*e.g.*, methyl groups), and protein translation, characterized by still larger amino acids, subsequent stages of translation may have evolved within the eukaryotic nucleus, where entire gene sequences may be mobilized in still unelucidated processes of 'developmental translation'.

4. Validification

Difficulties with the hypothesis of coenzyme-dependent RNA enzymes have been

discussed by White (1982) and apply equally to the current proposals. To a possibly even greater degree than White's model, the notion of RNA multienzyme complexes may be largely insusceptible to experimental testing. The main recourse in this case may be to further refine the model to such an extent that the earliest 'triggering' events of the scenario might be postulated and tested in the laboratory. Such events may possibly have revolved around the key processes of energy transduction and the formation of organic coenzymes, especially the ancestral forms of coenzyme A and lipoic acid, which have not been considered here.

5. Summary and Conclusions

In summary, primitive RNA enzymes may have attained substrate specificity indirectly through the agency of multienzyme complexes rather than via direct binding with soluble substrates. Fixed to ancestral analogues of coenzyme A or lipoic acid, the substrates in these early complexes could have been carried through enzyme sequences just as fatty acyl substrates are processed in fatty acid synthesis today. Unlike contemporary organisms, which require both a set of tRNA adaptors for translation and a set of protein enzymes for metabolism, the proposed ancestral format features a single set of RNA adaptor/enzymes which performed the combined process of 'metabolic translation'. In this simple scheme, the informational requirements of the earliest genomes may have been much lower than hitherto believed.

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