nucleates. Not only is the RNA, especially the portion insoluble in salt, more strongly held by histone, requiring, for dissociation, the more effective bromide rather than chloride, but the various fractions, although released characteristically over a wide molarity range, appear to differ less in composition than is observed with DNA. It will be seen in Table I that—in contrast to the DNA fractions exhibiting a gradual transition from the GC to the AT type[1,2]—only Fraction 1 of the insoluble RNA was significantly different in composition from that of the total in having a higher content of adenylic, uridylic and pseudouridylic acids. The 5 fractions yielded by the soluble RNA differed mainly with regard to the concentration of pseudouridylic acid, especially when Fractions 1 and 5 are compared. In all cases, the ratio 6-Am/6-K[10] was close to unity. It should be added that there is some evidence that the fractions, despite similarities in their composition, are distinguished by certain features of the nucleotide arrangement. This, as well as related investigations, will be discussed later in another context.

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Cell Chemistry Laboratory, Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y. (U.S.A.)

RAKOMA LIPSHITZ
ERWIN CHARGAFF

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Effect of magnesium on the amino acid-dependent pyrophosphate exchange catalysed by "RNA-low" systems

Recent evidence suggests that the stability of the leucine-activating enzyme present in rat-liver pH-5.0 fractions[2], is due to a special association between enzyme and "soluble" RNA, conferring protection upon labile thiol groups concerned both in pyrophosphate exchange, and in the transfer of [^{14}C]leucine to "soluble" RNA. The instability of activating enzymes found in "RNA-low fractions" obtained from protamine-treated rat-liver extracts[1,3], probably results from the interaction or

Abbreviations: RNA, ribonucleic acid; ATP, adenosine triphosphate; AMP, adenosine 5′-phosphate; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol.

removal of "soluble" RNA with protamine. Activating enzymes, precipitated with yeast RNA[3], are as unstable as those occurring in "RNA-low fractions".

It has now been observed that unstable activating enzymes are considerably stimulated by Mg^{++} in concentrations above 5 m*M*. At these levels the stable leucine-activating enzyme in the pH-5.0 fraction shows little stimulation (Table I), and often depression. This amino acid-dependent pyrophosphate exchange observed at high Mg^{++} levels is very readily and reproducibly depressed by AMP, in a manner suggesting that the AMP is involved in the reaction mechanism. At lower Mg^{++} concentrations the depressing effect of AMP is encountered less reproducibly, particularly with the leucine-activating enzyme of the pH-5.0 fraction. It is intended to re-examine this phenomenon using [^{14}C]AMP[4], to ensure that these inhibitory effects of AMP are not misleading.

TABLE I

THE EFFECT OF Mg^{++} AND AMP UPON AMINO ACID-DEPENDENT PYROPHOSPHATE EXCHANGE CATALYSED BY RAT-LIVER PREPARATIONS

Mg^{++}	*Pyrophosphate exchange (mμmoles/min/mg protein)*							
	"RNA-low" fraction				*pH-5.0 fraction*			
	Leucine		*Lysine*		*Leucine*		*Lysine*	
	−AMP	*+AMP*	*−AMP*	*+AMP*	*−AMP*	*+AMP*	*−AMP*	*+AMP*
1 m*M*	23	5	0	0	16	14	0	0
5 m*M*	77	66	15	7	52	56	14	4
15 m*M*	147	79	131	64	60	42	70	32
30 m*M*	82	43	246	116	46	16	104	58

The test system contained in a final volume of 0.55 ml: 3 μmoles sodium ATP, 1.5 μmoles sodium [^{32}P]pyrophosphate, 0.5 μmole amino acid, 50 μmoles Tris buffer (pH 7.2), 0.5 mg protein, and 8.7 μmoles sodium AMP (Sigma) or a compensating amount of NaCl in the tubes without AMP. Mg^{++} was added as indicated. Incubation was for 5 min at 37°. Separation of ATP from pyrophosphate in the presence of AMP by our method[3] required about 400 mg equivalent of dry charcoal. Each assay was accompanied by an amino acid-free blank, which remained constant throughout the series, and which has been subtracted from the data presented here.

When lysine is used as substrate the results are clear-cut and reproducible, provided that freshly-made preparations are employed, since the enzyme concerned is very labile. When leucine is used as substrate, the results are more variable, and seem to alter with slight changes in those isolation procedures which might be expected to influence the association between "soluble" RNA and enzyme.

The transfer of [^{14}C]lysine to "soluble" RNA catalysed by the pH-5.0 fraction is only one half to one third that of the transfer obtained with [^{14}C]leucine. When, however, this transfer is examined in "recombined systems" it is found that the transfer of [^{14}C]lysine is equal to that of [^{14}C]leucine. This suggests that a faulty association exists between the enzyme in the pH-5.0 fraction and the "soluble" RNA, parallel with its instability, and type of response to Mg^{++} and AMP. Activating enzymes for valine and threonine, showing similar properties are found in these pH-5.0 fractions[3].

Recently, ALLEN *et al.*[5] have suggested that thiol groups may participate in the mechanism of amino acid activation. The results reported here indicate that an aminoacyl thioester may be involved as an intermediate, the mechanism of its formation being similar to that of acetyl-coenzyme A[4]. If this is so, then free AMP must appear

in the system before the transfer of the activated amino acid to the "soluble" RNA. This transfer is known to be reversed by free AMP (and pyrophosphate), suggesting that free AMP appears after the transfer has taken place (for discussion, see LIPMANN *et al.*[6]). The dilemma can be resolved if we assume that the amino-acyl thioester and amino-acyl ribose ester bonds are in equilibrium with each other, perhaps through an intermediary complex containing Mg^{++}. In the stable amino acid-activating systems this hypothetical complex may effect the "coupling" of thioester bond synthesis to the transfer of the activated amino acid to "soluble" RNA, the formation of thioester bonds being controlled through the response of the thioester-generating system to Mg^{++}. This controlling or "coupling" action may represent a highly interesting property of "soluble" RNA.

Medical Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, Ducane Road, London, W. 12 (Great Britain) PRISCILLA HELE

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The enzymic synthesis of thymidine diphosphate glucose and thymidine diphosphate rhamnose

Recently a large variety of thymidine-linked sugar nucleotides have been isolated from microorganisms[1–4]. The sugars include rhamnose[1,4], mannose[4] and a variety of unidentified sugars[2,3]. We wish to report evidence for the enzymic formation of thymidine diphosphate glucose and its conversion to thymidine diphosphate rhamnose in cell-free extracts of *Pseudomonas aeruginosa* (ATCC 7700). This organism has been shown to secrete a rhamnose-containing lipid into the culture medium[5,6].

Cells were grown in a 3% glycerol–mineral salts medium[6] for 100 h at 32°. The cells were harvested by centrifugation and washed with 0.05 *M* Tris–0.001 *M* EDTA, pH 8. The washed cells were suspended in 0.05 *M* Tris–0.01 *M* $MgCl_2$–0.001 *M* EDTA, pH 8, and ruptured by 18-min treatment in a 10 kc magnetostriction oscillator. Intact cells and large particles were removed by centrifugation at 20,000 × *g* for 20 min.

When the sonic extract was incubated with TTP and α-glucose 1-phosphate, followed by isolation of the nucleotides by adsorption on charcoal and chromatography in the neutral ethanol–ammonium acetate solvent[7], a new nucleotide spot

Abbreviations: Tris, tris(hydroxymethyl)aminomethane; TMP, thymidine 5′-phosphate; TDP, thymidine 5′-diphosphate; TTP, thymidine 5′-triphosphate; DPN, diphosphopyridine nucleotide; DPNH, reduced diphosphopyridine nucleotide; EDTA, ethylenediaminetetraacetate.