ROLE OF AMINO ACIDS IN REGULATING RNA FORMATION

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Growing bacterial cells adjust their RNA level by a mechanism which affects the rate of synthesis of ribosomal RNA (Kjeldgaard, Maaløe, and Schaechter, 1958; Neidhardt and Magasanik, 1960; Kjeldgaard, 1961). This mechanism is not completely understood, but it apparently does not operate by varying the enzymatic capacity of the cell to make RNA (Fraenkel and Neidhardt, 1961).

Two recent communications have focused attention on the well-known dependency of bacterial RNA synthesis on amino acids. Kurland and Maaløe (1962) have extensively studied the effect of chloramphenicol on RNA synthesis and have proposed that RNA synthesis is regulated by the internal amino acid concentration in a repression-like mechanism in which transfer RNA acts as a repressor and amino acid adenylate as an inducer. Stent and Brenner (1962) have suggested essentially the same mechanism on the basis of their finding that a single genetic locus controls the dependency of RNA synthesis on amino acids. A cell altered at this locus (and termed "amino acid independent") will, if starved of any essential amino acid, continue to synthesize RNA in the absence of protein synthesis. This behavior is in contrast to that of auxotrophs derived from the wild type, which cease RNA production upon exhausting their amino acid supply.

Mutants of this kind, which synthesize RNA independently of amino acid supply, offer the possibility of assessing the

physiological significance of amino acid dependency as a regulatory device in RNA synthesis. A serious alteration in the regulation of RNA synthesis appearing simultaneously with the loss of the amino acid requirement for RNA synthesis would support the contention that this requirement is an essential part of the regulatory device.

We have examined two related strains of E. coli which Stent and Brenner (1962) found to possess an alteration freeing their RNA synthesis from dependency on a complete array of amino acids. One strain, K12W-6, is the methionine auxotroph described by Dr. Ernst Borek (1955) and obtained from him; the other, strain K-10, is the Cavalli Hfr strain, obtained from Dr. Luigi Gorini, and is a prototroph. A third E. coli, strain W, was chosen as a wild-type control. The three strains were found to behave as indicated, and were then used in the following experiments.

Strains K12W-6 and W were grown for many generations in media of different composition and their protoplasmic content of total RNA determined. The RNA:protein ratios were found to vary, in both strains, with the growth rate of the cells (Table I). This correlation exists in K12W-6 even when the growth rate of the cells is determined by the presence or absence of an amino acid supplement. K-10 behaved like K12W-6.

Another crucial test of the ability of a cell to regulate its rate of RNA synthesis is to transfer it from a medium which supports rapid growth to one which supports slower growth. Under these conditions, cells with a normal regulative mechanism can apportion all of their resources to protein and DNA synthesis by shutting off their synthesis of ribosomal RNA. Thus, as can be seen in Table II, strain W always enters a phase of markedly depressed RNA accumulation when transferred to a poorer medium.

TABLE I

Effect of Medium on RNA Content of an Amino Acid Independent and an Amino Acid Dependent Strain

Strain	K12W-6 (A.A. Independent)		W (A.A. Dependent)	
Media	Growth Rate	RNA:Prot	Growth Rate	RNA:Prot
glucose + NH_4^+ + tryptone	1.98	.570	2.00	.600
glucose + NH_4^+ + amino acids	1.43	.531	1.39	.550
glucose + NH_4^+	1.07	.500	1.04	.480
succinate + NH_4^+	0.78	.420	0.49	.320
maltose + NH_4^+	0.69	.410	-	-
glucose + L-tryptophan	0.15	.270	0.20	.240

The additions indicated were added to the basal salts solution (supplemented with L-methionine when necessary) described previously (Fraenkel and Neidhardt, 1961). The final concentration of each component was: glucose, maltose, and succinate, 0.4%; $(NH_4)_2SO_4$, L-tryptophan, and tryptone, 0.2%; amino acids, 10^{-4} M of each of 18 L-amino acids.

The growth rate is expressed as the value of k in the expression: $k = \ln 2/\text{mass doubling time in hours}$.

The RNA:Prot ratio was determined by the colorimetric procedures previously employed (Fraenkel and Neidhardt, 1961).

Strain K-10 behaves like W in a shift-down involving the carbon and energy source, or the nitrogen source, but exhibits an atypical behavior when transferred from a glucose medium containing amino acids to one devoid of them. In the latter case, accumulation of RNA continues, apparently pre-empting resources the wild type (strain W) can apportion to protein synthesis. It is of interest to note that failure to halt total RNA accumulation during such a shift-down is of enormous detriment to the mutant. Frequently lag periods of four and five hours occur before growth is resumed. The nature of this lag is currently being studied.

In summary, a mutant which seems to have lost its dependency on amino acids for RNA synthesis still exhibits a perfectly normal regulation of this synthesis under all instances of balanced growth examined, as well as during shifts-down involving the supply

TABLE II

Comparison of the Ability of an Amino Acid Independent and an Amino Acid Dependent Stain to Cease RNA Accumulation Following Transfers to Poorer Media

	K-10 (A.A. Independent)			W (A.A. Dependent)		
	Time after Transfer (Min)	Protein	RNA	Time after Transfer (Min)	Protein	RNA
Case A. Change of major carbon and energy source.	0	1.00	1.00	0	1.00	1.00
	30	1.18	1.00	90	1.09	0.94
	90	1.30	1.00	150	1.21	0.97
	145	1.41	0.95	225	1.55	0.94
Case B. Change of sole nitrogen source.	0	1.00	1.00	0	1.00	1.00
	30	1.08	0.95	30	1.08	1.05
	60	1.15	1.05	60	1.29	1.00
	120	1.27	1.05	120	1.46	1.05
	180	1.42	1.11	180	1.63	1.15
Case C. Change from complete amino acid to minimal medium.	0	1.00	1.00	0	1.00	1.00
	60	1.18	1.37	20	1.05	1.00
	120	1.39	1.85	40	1.31	1.10
	210	1.91	2.25	65	1.95	1.48
	240	2.67	2.52			

The cells were grown for at least three generations in the pre-shift medium, chilled and centrifuged at 0 time, then transferred to the post-shift medium and the incubation continued. Assays for protein and RNA were performed as previously described (Fraenkel and Neidhardt, 1961), and the results normalized to the value of the component present per ml of culture at 0 time. In Case A the pre-shift medium contained glucose + NH_4^+; the post-shift medium, succinate + NH_4^+. In Case B the pre-shift medium contained glucose + NH_4^+; the post-shift medium, glucose + L-tryptophan. In Case C the pre-shift medium contained glucose + NH_4^+ + 18 L-amino acids; the post-shift medium contained just glucose + NH_4^+.

of nitrogen or of carbon and energy. This mutant cannot, however, retard RNA accumulation when suddenly deprived of a rich exogenous supply of amino acids. If the mutant has truly lost amino acid dependency of its RNA synthesis, then such dependency seems essential for the regulation of RNA synthesis only during one kind of physiological stress.

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