ON THE INSTABILITY OF TRANSFER-RNA TERMINAL NUCLEOTIDE SEQUENCE IN YEAST

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Transfer-RNAs from various organisms have been shown to end with the 3'-hydroxyl terminal sequence ...pCpCpA which is required for the transfer function and can be restored in partially degraded transfer-RNA by a cytoplasmic enzyme system (Hecht et al.,1958 ; Canellakis and Herbert, 1960 ; Preiss et al.,1961 ; Furth et al.,1961). The properties of this system suggested that a turn-over of the terminal sequence could occur in vivo and such a turn-over has actually been demonstrated by Holt et al. (1962) in intact rabbit reticulocytes, in accordance with preliminary results obtained by one of us (Monier, 1962). Evidence for a similar turn-over in rat liver has also been recently reported by Scholtissek (1962).

With the exception of the experiments of Lacks and Gros (1959) on the kinetics of ^{14}C-adenine incorporation into Escherichia Coli transfer-RNA, no study had been made on rapidly growing microorganisms. Observations on the amounts of adenosine and cytidine liberated upon alkaline hydrolysis from transfer-RNA samples prepared at various stages of the growth of the yeast Saccharomyces Cerevisiae showed that, under certain conditions, the terminal AMP residue was lost in vivo without any further degradation of the polynucleotide chain (Yu and Zamecnik, 1960 ; Monier, 1962 ; Cantoni et al.,1962). We have now confirmed this finding by labelling

exponentially growing yeast cells with ^{14}C-adenine for a short time and transferring an aliquot of the labelled cells to a medium containing ^{12}C-adenine. As shown in Table I, ^{14}C-adenine incorporated in the terminal position was partially lost after this transfer The simultaneous rise in 2',3'-AMP radioactivity was due to the large size of the intracellular ^{14}C-adenine pool, which was not immediately diluted by ^{12}C-adenine (Cowie and Bolton, 1957).

Table I

^{14}C-Adenine incorporation into yeast transfer-RNA

	Radioactivity (CPM/mg transfer RNA) recovered after alkaline hydrolysis as	
	Adenosine	2',3'-AMP
I	1 720	1 820
II	420	18 300

S.Cerevisiae grown aerobically at 30°C on White's medium (generation time : 110 min.). I : Cells labelled in exponential phase (7 g/l, wet weight) during 15 min. by the addition of ^{14}C-Adenine (2 mC/mM) - 114 µC/l)- II : Cells labelled as in I transferred to fresh medium supplemented with ^{12}C-Adenine (0.004 M) and incubated for 90 min. Exponential growth resumed after 50 min. Transfer-RNA extracted and purified according to published methods (Monier, 1962). Adenosine and 2',3'-AMP were liberated and analysed according to conventional techniques.

In rabbit reticulocytes, the results of Holt et al.(1962) suggested that the terminal AMP turn-over was paralleled by a turn-over of the CMP residue(s) next to it. We have attempted to find out whether the same phenomenon occurs in exponential yeast using ^{32}P as a marker. The sequence ...pCpCpA of transfer-RNA is more easily hydrolyzed by snake venom phosphodiesterase than the rest of the chain, probably because of the secondary structure (Preiss et al.,1961). A short exposure of ^{32}P-transfer-RNA to a limited amount of enzyme results in the liberation of 5'-AM ^{32}P and 5'-CM ^{32}P exclusively from the 3'-OH terminal sequence. The

undegraded chains, recovered by acid precipitation, can then be completely freed of their terminal sequence by a second exposure to the enzyme. A third enzyme degradation liberates residues, which can be considered as truly "internal" to the chains. A comparison of the specific activities of "external" residues, liberated by the first enzyme degradation, with the specific activities of "internal" residues under various conditions of labelling or chasing is presented in Table II. After a 15 min. incorporation, external residues had higher specific activities than internal residues, and these differences were due to several factors including the sequential synthesis of the chains, the time necessary for equilibration of the nucleotide pools and, possibly, a turn-over of the terminal sequence. When part of the cells were transferred to an unlabelled medium, all nucleotides were essentially in equilibrium with each other after two generations and no significant differences

Table II

Specific radioactivities in external and internal nucleotide residues of ^{32}P-labelled yeast transfer-RNA

	Specific radioactivities (CPM/μmoles x 10^{-3})			
	external nucleotides		internal nucleotides	
	5'-AMP	5'-CMP	5'-AMP	5'-CMP
I	500	114	79	26.3
II	310	329	317	319
III	33.8	52.3	55.8	50.8

Growth conditions as in Table I. I: 15 min. incorporation in exponential phase (10 g/l, wet weight) in the presence of 1 mC/l of ^{32}P. II : Cells, labelled as in I, transferred to unlabelled medium. A lag occured after this transfer and the cells were harvested two generations after growth had resumed. III : Same as I, but the cells were harvested after six generations. The purified transfer-RNA was degraded three times with snake venom phosphodiesterase (Worthington) as described in the text. External nucleotides from the first, and internal nucleotides from the third enzyme degradation were isolated and counted according to conventional techniques.

were found between external and internal residues. Four generations later, however, a significant decrease of the specific activity of external AMP compared with internal AMP was observed, but no such difference was noticeable in the case of CMP. The specific activity of the ^{32}P-acidosoluble pool itself decreased by a factor of fifty during the same interval of time. These results show that a slow turn-over of AMP did occur but that no turn-over of CMP could be demonstrated in exponential yeast under our conditions. Calculations, to be reported in detail later, have been made and their results are consistent with a turn-over rate of less than 1 per cent per min. of the total transfer-RNA terminal AMP.

The hypothesis has been made (Scholtissek, 1962) that terminal AMP turn-over in transfer-RNA in vivo could be related in some way to the participation of transfer-RNA in protein biosynthesis. Our results suggest that this turn-over is not a direct consequence of the amino acid transfer, since its rate in growing yeast does not account for the total synthesis of cellular proteins. They support the conclusion drawn by Nathans and Lipmann (1961) from in vitro experiments that transfer-RNA does not lose its terminal AMP at the time of the amino acid transfer to ribosomal proteins.

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