AMINOACYL TRANSFER FROM PHENYLALANYL-tRNA MICROINJECTED INTO XENOPUS LAEVIS OOCYTES

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Summary. Xenopus laevis oocytes were injected with [^{14}C] phe-tRNA and the fate of the aminoacyl moiety was studied. The radioactive phenylalanine is gradually hydrolized off the tRNA once inside the cell. The rate of deacylation of the tRNA is not affected by inhibition of cellular protein synthesis by puromycin or cycloheximide. Part of the radioactive amino acid that leaves the tRNA (30 to 65%) is transferred directly into the oocyte nascent proteins as evidenced by the fact that its incorporation into proteins is not reduced by coinjection with a large excess of [^{12}C] phenylalanine. Aminoacyl transfer from injected phe-tRNA into proteins is inhibited by puromycin and cycloheximide.

The microinjection technique allows one to alter directly the macromolecular content of the cellular milieu. This technique has been used to introduce exogenous messenger RNAs into Xenopus laevis oocytes and to study their translation (1-4). In our laboratory we have investigated the aminoacylation and repair of tRNA injected into these cells (5,6). In the present work we describe experiments in which we have studied the fate of the radioactive aminoacyl moiety after the microinjection of [^{14}C] phenylalanyl-tRNA into Xenopus laevis. The results obtained show that inside the cell a fraction of the aminoacyl moiety is transferred from the tRNA directly to the nascent proteins of the oocyte while the rest of the amino acid leaves the tRNA to enter the cellular free amino acid pool.

Materials and Methods. Adult Xenopus laevis females were obtained from the South African Snake Farm, Fish Hoek, South Africa. Full-grown oocytes (1.0 to 1.1 mm in diameter) were dissected out manually as described earlier (7).

Microinjections were carried out essentially as described by Gurdon (8), injecting 20 to 50 nl volumes per oocyte. All materials microinjected were dissolved in sterile water and in all cases duplicate groups of five oocytes each were used.

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Oocytes were incubated in amphibian saline (63 mM NaCl, 1 mM KCl, 0.5 mM $CaCl_2$, 1 mM $MgCl_2$, 20 mM Tris-HCl pH 7.4 and 10 ug/ml each of penicillin and streptomycin sulfate) at 22°.

Phenylalanyl-tRNA was prepared using pure yeast $tRNA^{phe}$ purchased from Boeringer and a partially purified wheat germ aminoacyl-tRNA synthetase as described by Allende (9). [^{14}C]-phenylalanine (specific activity 270 μCi/μmol) was purchased from New England Nuclear.

Deacylation of injected [^{14}C] phe-tRNA was assayed as follows: Duplicate groups of five oocytes each were injected with 2-6 pmoles of [^{14}C] phe-tRNA per oocyte and incubated for the times indicated. The cells were subsequently homogenized and extracted with 4 ml of a 1:1 phenol-buffer mixture in which the buffer contained 0.2 M sodium acetate pH 5.0, 10 mM $MgCl_2$, 5 mM EDTA and 0.1% Triton X-100. The aqueous phase was separated by centrifugation at 10,000 x g for 10 minutes and duplicate aliquots of 0.5 ml were precipitated with 5 ml of cold 5% trichloroacetic acid containing 20 mM non-radioactive phenylalanine. The precipitate was collected on glass fiber discs and counted in a scintillation counter.

Amino acyl transfer from injected [^{14}C] phe-tRNA into oocyte proteins was assayed by incubating the group of injected oocytes under the same conditions as above but stopping the reaction by addition of 2 ml of 5% trichloroacetic acid to the oocytes. Subsequently the oocytes were homogenized in the acid with a glass rod and the suspension was heated at 90° for 15 minutes. The precipitate was filtered through glass fiber discs which were dried and counted.

Results and Discussion.

The results shown in figure 1 A indicate that phenylalanyl-tRNA becomes deacylated gradually after its microinjection into Xenopus laevis oocytes. This figure also shows that the presence of puromycin or cycloheximide, at concentrations known to inhibit over 90% of oocyte protein synthesis (7), do not significantly change the rate of the deacylation reaction. A similar experiment is shown in figure 1 B but in this case, concomitant with the dissapearance of radioactivity from the tRNA (dashed line) measurements were made for the transfer of the [^{14}C] phenylalanine into proteins as assayed by hot trichloroacetic acid precipitable radioactivity (solid line). In this experiment approximately 30% of the radioactivity that leaves the tRNA is incorporated into oocyte proteins. In experiments in which different amounts of [^{14}C] phe-tRNA are injected into the oocyte, varying proportions of the deacylated radioactive phenylalanine are transferred into proteins. Injecting low amounts of [^{14}C] phe-tRNA (2 pmol/oocyte), it has been possible

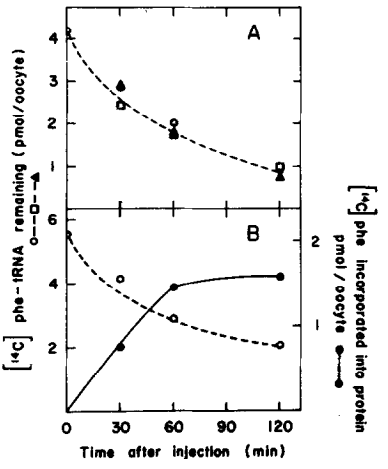

Figure 1

Deacylation and aminoacyl transfer from [^{14}C] phe-tRNA injected in oocytes. Duplicate groups of 5 oocytes each were injected with 4.5 (in A) and 5.5 (in B) pmol of [^{14}C] phe-tRNA^{phe} per oocyte and incubated for the times indicated. Deacylation of injected [^{14}C] phe-tRNA and incorporation of [^{14}C] phe into oocyte proteins was measured as detailed in Methods. In A, the deacylation of [^{14}C] phe-tRNA was measured in oocytes incubated in amphibian saline (control, 0) and in cells that were pre-incubated for 1 hour prior to injection in the presence of 0.1 mM cycloheximide (▲) or 0.5 mM puromycin (□) and subsequently incubated in the continued presence of the protein synthesis inhibitors. In B, parallel groups of the same batch of injected oocytes were used to measure deacylation (o--o) and incorporation into oocyte proteins (●-●).

to obtain as high as 64% of the label transferred into protein after 1 hour of incubation (result not shown). In experiment 1 of table I, it can be observed that puromycin and cycloheximide are also potent inhibitors of the transfer of radioactivity from injected [^{14}C] phe-tRNA into oocyte proteins. The second experiment reported in table I attempts to answer the question whether the [^{14}C] phenylalanyl moiety injected as [^{14}C] phe-tRNA is transferred directly into the nascent proteins or whether it goes through the free amino acid pool before becoming incorporated into the cellular proteins. In this experiment [^{12}C] phenylalanine at a molar concentration 1000 times higher than that of [^{14}C] phe-tRNA was co-injected with the radioactive compound. If the [^{14}C] phenylalanine were deacylated from the

Table I

CONDITIONS AFFECTING THE TRANSFER OF AMINO ACID FROM tRNA TO PROTEIN IN MICROINJECTED OOCYTES

Experiment	[^{14}C]Phe-tRNA microinjected	Treatment	[^{14}C]Phe incorp. into protein —	
	pmol		pmol 1 hr	2 hr
1.	12	None	-	4.7
	"	+ Cycloheximide	-	0.6
	"	+ Puromycin	-	0.3
2.	3.7	None	0.9	1.4
	3.8	Coinjection of [^{12}C] Phe	0.7	0.8

Duplicate groups of 5 oocytes each were injected with [^{14}C] phe-tRNA as indicated and after 1 or 2 hours incubation the amount of [^{14}C] phenylalanine transferred into oocyte protein was determined as detailed in Methods. Where indicated oocytes were preincubated for 1 hour in the presence of 0.1 mM cycloheximide or 0.5 mM puromycin in amphibian saline prior to the injection and subsequently the cells were again incubated in the presence of the protein synthesis inhibitors. In experiment 2, as specified, some oocytes were coinjected with 3.8 pmoles of [^{14}C] phe-tRNA and 4000 pmoles of [^{12}C] phenylalanine.

tRNA and entered the free amino acid pool before becoming incorporated into protein under these conditions, it would suffer such a large dilution of the label that no incorporation of the radioactivity should be observed. This is clearly not the case since most of the radioactivity still labels the proteins in the oocytes co-injected with the unlabeled amino acid. At longer incubation periods the inhibition caused by the co-injected [^{12}C] phenylalanine increases as would be expected since deacylation is also continuing.

The experiments described above demostrate that tRNAs introduced into amphibian oocytes are not only functional in their capacity to be aminoacylated (4) but also are operative in the endogenous protein synthesis machinery of these cells since they can transfer directly their amino acid into nascent

proteins. It is interesting to note that the amino acid esterified to the tRNA apparently exchanges very slowly with the free amino acid in the cell. This finding may be explained by the presence in the living cell of factors like protein synthesis elongation factor 1 which specifically bind aminoacyl-tRNA and inhibit the deacylation reaction catalyzed by aminoacyl-tRNA synthetases (10). This technique opens the possibility of studying the relative utilization of different tRNA isospecies in oocyte protein synthesis.

Acknowledgements

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