ARGINYL-tRNA-PROTEIN TRANSFERASE IN EUCARYOTIC PROTISTS

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SUMMARY: Soluble extracts of Saccharomyces cerevisiae and Blastocladiella emersonii were found to catalyze the specific transfer of arginine from a mixture of [^{14}C] aminoacyl-tRNAs into protein. Arginine transfer was stimulated by bovine serum albumin. Glu-Ala, Asp-Ala and cystinyl-bis-Ala inhibited incorporation into protein, whereas dipeptides with other NH_2-terminal residues linked to alanine did not. These results indicate the presence of an enzyme in eucaryotic protists with the same donor and acceptor specificity as mammalian arginyl-tRNA-protein transferase.

Aminoacyl-tRNA-protein transferases are soluble enzymes which catalyze the transfer of certain aminoacyl residues from tRNA into peptide linkage with specific NH_2-terminal residues of protein or peptide acceptors (1). They account for the original observations of Kaji, Kaji and Novelli that extracts from rat liver and E. coli could, respectively, incorporate arginine (2) and leucine and phenylalanine (3) into protein in the absence of ribosomes. Arginyl-tRNA-protein transferase (EC 2.3.2.8) has been extensively purified from rabbit liver (4) and shown to recognize NH_2-terminal dicarboxylic amino acids as acceptor sites (5). Leucyl,phenylalanyl-tRNA-protein transferase (EC 2.3.2.6) purified from E. coli (6) utilizes basic amino acids as acceptor residues (7). The physiological function of these enzymes is not yet clear; however, studies of the bacterial enzyme have been greatly facilitated by the isolation of a mutant which lacks transferase activity (8). Analysis of this mutant has suggested that the enzyme plays a regulatory role in several metabolic pathways, one of which is concerned with the catabolism of proline (9). The importance of using a controlled genetic and physiologic system to establish cellular function has prompted us to seek a more satisfactory organism in which to examine the mammalian type transferase.

MATERIALS AND METHODS

L-[U-^{14}C] arginine (310 μCi per μmole, Schwarz/Mann) and stripped E. coli B tRNA (General Biochemicals) were used to prepare (4) [^{14}C]-arginyl-tRNA (1.1 nmoles per mg). A mixture of 15 uniformly labeled [^{14}C]-amino acids (New England Nuclear) and similar mixtures containing a 200-fold excess of either unlabeled arginine or unlabeled leucine and phenylalanine were used in the preparation of radioactive tRNA mixtures (6). Synthetic peptides were obtained from Cyclo Chemicals or Bachem.

Soluble extracts from rabbit lung and E. coli W4977-R18 (8) were prepared as described for rabbit liver (4) and E. coli B (6). Saccharomyces cerevisiae D273-10B (αwt) was grown in medium containing 1% yeast extract, 2% peptone and 2% glucose and harvested in mid-exponential phase. Cell pellets were suspended in 0.5 volumes of buffer (20 mM Tris-HCl (pH 7.8), 10 mM 2-mercaptoethanol) and disrupted by two passages through a hydraulic pressure cell at 10,000 psi. After centrifugation for 10 min at 12,000 x g and 90 min at 105,000 x g the supernatant fraction was concentrated by vacuum dialysis against buffer. Vegetative cells and zoospores from Blastocladiella emersonii grown in glucose-casamino acid medium (10) were broken by grinding with dry ice, suspended, respectively, in 1.0 and 2.5 volumes of buffer and centrifuged as described above.

Protein estimations were carried out by the method of Lowry et al. (11) using bovine serum albumin (Pentex) as a standard.

RESULTS

Soluble extracts from S. cerevisiae and from vegetative cells of B. emersonii were found to catalyze the transfer of radioactivity from [^{14}C]-arginyl-tRNA into protein (Fig. 1). The B. emersonii activity was extremely unstable perhaps because of the high content of proteases in this organism (13). Even with these crude supernatant fractions enzymatic transfer was markedly stimulated by the presence of bovine albumin, an acceptor protein in the arginine-transfer reaction (14). Puromycin (0.3 mM), which has no effect on the reaction catalyzed by mammalian arginyl-tRNA-protein transferase (14), did not inhibit incorporation. This concentration of puromycin has been found to inhibit both the bacterial transferase and ribosome-dependent protein synthesis (6).

A tRNA mixture enzymatically acylated with 15 radioactive amino acids and similar aminoacyl-tRNA mixtures containing either unlabeled arginyl-tRNA or unlabeled phenylalanyl- and leucyl-tRNAs were also examined as substrates (Table I). Extracts from S. cerevisiae and from both vegetative cells and zoospores of B. emersonii catalyzed the transfer of radioactivity from the

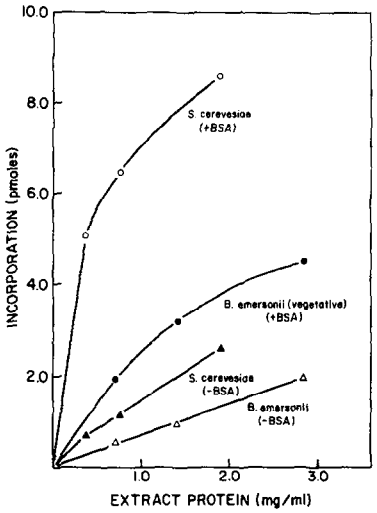

Fig. 1. Enzyme dose response and albumin dependence of arginine transfer from tRNA to protein. Reaction mixtures (75 µl) contained 50 mM Tris-HCl (pH 9.0), 50 mM 2-mercaptoethanol, 0.15 M KCl and [^{14}C] arginyl-tRNA (1.8 nmoles arginine per ml). Bovine serum albumin (BSA) was present at 75 µM where indicated. Incorporation of radioactivity into material insoluble in hot 5% trichloroacetic acid was determined by the filter paper disc technique (12) on 50 µl aliquots after incubation for 10 min at 37°.

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complete mixture of aminoacyl-tRNAs but not from that containing [^{12}C]-arginyl-tRNA. This specificity with respect to the transferred aminoacyl residue is identical to that of the mammalian transferase exemplified here with a supernatant fraction from rabbit lung and completely different from that of the E. coli enzyme.

Substrate specificity with respect to acceptor residues was investigated using dipeptides possessing variable NH_2-terminal residues linked to alanine (Table II). Such peptides containing the appropriate NH_2-terminal amino acid have been shown to act as acceptors for the different transferases (5,7) and thereby competitively inhibit transfer into hot acid-insoluble material. The results were similar to those obtained with purified mammalian arginyl-tRNA-protein transferase (5) and suggest that a dicarboxylic NH_2-terminal aminoacyl residue is the important determinant of an acceptor molecule.

Table I

Specificity of Aminoacyl Transfer Catalyzed by Supernatant Fractions

Supernatant fraction	Incorporation (cpm)		
	[^{14}C] aminoacyl-tRNA mixture	Mixture including [^{12}C] leucyl and phenylalanyl-tRNA	Mixture including [^{12}C] arginyl-tRNA
E. coli	1171	36	1214
Rabbit lung	807	804	22
S. cerevisiae	1036	1254	24
B. emersonii (zoospore)	667	697	12
B. emersonii (vegetative)	920	1183	25

Reaction mixtures included 75 μM bovine albumin and were similar to those described in Fig. 1, except that they contained 2 x 10^6 cpm of the indicated mixture of aminoacyl-tRNAs per ml. Incorporation into protein was determined on 50 μl aliquots after incubation for 10 min.

DISCUSSION

The characteristics distinguishing mammalian and bacterial aminoacyl-tRNA-protein transferases are specificity for arginine rather than leucine or phenylalanine as the donated residue, specificity for a dicarboxylic rather than a basic NH_2-terminal amino acid as the acceptor residue, and resistance to puromycin. By these criteria the activities in *S. cerevisiae*, a budding ascomycete, and *B. emersonii*, an aquatic phycomycete, clearly resemble the mammalian enzyme. Arginyl-tRNA-protein transferase is thus a widely distributed, perhaps ubiquitous, eukaryotic enzyme. Conservation of this activity amongst such divergent species suggest that post-translational arginylation of certain proteins or peptides is required for one or more processes fundamental to eukaryotic cells.

The organisms studied in this work offer obvious advantages to the use

Table II

Inhibition of Arginine Transfer by Defined Peptides

Addition	Supernatant fraction	
	S. cerevisiae	B. emersonii
	(% inhibition)	
Glu-Ala	30	83
Asp-Ala	36	83
Cystinyl-bis-Ala	35	42
Lys-Ala, Arg-Ala, Trp-Ala, Ser-Ala, Pro-Ala, Gly-Ala, Ala-Ala, Val-Ala, Met-Ala, Ileu-Ala, Phe-Ala	<10	<10

Reaction mixtures were similar to those described in Fig. 1. They contained 75 μM bovine albumin and 1.9 and 2.8 mg per ml, respectively, of soluble protein from S. cerevisiae and vegetative cells of B. emersonii. The concentration of peptides was 17 mM. The complete systems for S. cerevisiae and B. emersonii incorporated 8.6 and 4.5 pmoles of arginine per 50 μl aliquot after 10 min incubation.

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of mammalian cells in understanding the cellular function of arginyl-tRNA-protein transferase. They grow rapidly on synthetic media and methods for obtaining and characterizing mutants of S. cerevisiae are well established (15). B. emersonii undergoes a clearly defined developmental cell cycle which has been useful as a model for understanding cellular differentiation (10) and techniques for mutant isolation have recently been developed (P. M. Silverman, unpublished data). The use of these organisms may establish whether the eukaryotic enzyme plays a physiological role similar to that of leucyl,phenylalanyl-tRNA-protein transferase.

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REFERENCES

1. Soffer, R. L. (1974) Adv. Enzymol. 40, 91-139.
2. Kaji, H., Novelli, G. D., and Kaji, A. (1963) Biochim. Biophys. Acta 76, 474-477.
3. Kaji, A., Kaji, H., and Novelli, G. D. (1965) J. Biol. Chem. 240, 1185-1191.
4. Soffer, R. L. (1970) J. Biol. Chem. 245, 731-737.
5. Soffer, R. L. (1973) J. Biol. Chem. 248, 2918-2921.
6. Leibowitz, M. J., and Soffer, R. L. (1970) J. Biol. Chem. 245, 2066-2073.
7. Soffer, R. L. (1973) J. Biol. Chem. 248, 8424-8428.
8. Soffer, R. L., and Savage, M. (1974) Proc. Nat. Acad. Sci. USA 71, 1004-1007.
9. Deutch, C. E., and Soffer, R. L. (1975) Proc. Nat. Acad. Sci. USA 72, 405-408.
10. Silverman, P. M., and Epstein, P. M. (1975) Proc. Nat. Acad. Sci. USA 72, 442-446.
11. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
12. Mans, R. J., and Novelli, G. D. (1960) Biochem. Biophys. Res. Commun. 3, 540-543.
13. Lodi, W. R., and Sonneborn, D. R. (1974) J. Bacteriol. 117, 1035-1042.
14. Soffer, R. L., and Horinishi, H. (1969) J. Mol. Biol. 43, 163-175.
15. Mortimer, R. K., and Hawthorne, D. C. (1969) in The Yeasts I (Rose, A. H., and Harrison, J. S., eds.), Academic Press, New York, pp. 385-460.