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Participation by Drosophila transfer RNA in protein synthesis in an E.coli protein synthesizing system

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SUMMARY: Drosophila melanogaster tyrosyl-tRNA^{Tyr} is shown to participate directly and with high efficiency in an E. coli protein synthesizing system employing bacteriophage f2 RNA as messenger. This same tRNA from the Drosophila mutant suppressor of Hairy wing does not detectably suppress the amber mutation sus 3 in the f2 coat protein.

INTRODUCTION

E. coli protein synthesizing systems which can employ well defined messenger RNAs which code for proteins whose sequences are known are of potential usefulness in examining the coding properties of eucaryotic tRNA molecules. An efficient assay for the presence of tRNA capable of suppressing the amber (UAG) class of nonsense codons makes use of the RNA from the phage f2 and of its amber mutant sus 3 as messenger RNA in an E. coli cell-free protein synthesizing system (1). When f2 sus 3 RNA is used as messenger in a protein synthesizing system derived from a su^- strain, only the N-terminal fragment (fmet-ala-ser-asn-phe-thr) of the f2 coat protein is synthesized. This fragment is soluble in trichloroacetic acid. In the presence of tRNA from a su^+ strain, however, it is possible to detect as a trichloroacetic acid precipitable product the synthesis of the whole coat protein resulting from suppression of the amber codon which occurs at the position of the amino acid following threonine. This assay can detect suppression by both amber (UAG) and ochre (UAA) suppressors of E. coli (2). The purpose of this report is to demonstrate that Drosophila melanogaster tyrosyl-tRNA^{Tyr} can participate directly with E. coli ribosomes in the synthesis of f2 protein. This system is then employed to test the hypothesis that the D. melanogaster mutant, suppressor of Hairy wing, may produce a tRNA^{Tyr} capable of amber or ochre suppression.

The genetic properties of the allele, suppressor of Hairy wing-2 ($su(Hw)^2$), have been extensively studied (3,4). This mutation suppresses only certain alleles of nine scattered genes and has the additional property of suppressing three polar mutations in the bithorax gene complex. These

formal genetic properties are characteristic of nonsense suppressors in E. coli (5) and in yeast (6,7,8) prompting the suggestion that su(Hw)^2 may be a nonsense suppressor (9). Jacobson (personal communication) has evidence that su(Hw)^2 flies may contain an altered chromatographic species of tRNA^{Tyr}. The su(Hw)^2 locus, however, cannot be simply the structural gene for a species of tRNA because the mutant is completely recessive (4) and a deficiency for the locus (Df(3)red) mimics the mutation itself (Lewis, personal communication).

MATERIALS AND METHODS

Strains. D. melanogaster strains were obtained from Dr. E. B. Lewis. Since homozygous su(Hw)^2 females are sterile, hemizygous su(Hw)^2 flies were obtained as the F1 of a cross between 1(3)tr Df(3)red Sb/In(3L)P, Me', In(3R)P18, Ubx and su(Hw)^2 Ubx e^4/TM1. The only survivors of this cross are hemizygous su(Hw)^2 flies. Heterozygous su(Hw)^2 flies were obtained from the stock su(Hw)^2 Ubx e^4/TM1, and su(Hw)$^+$ flies from the stock 1(3)tr Sb/In(3L)P, Me', In(3R)P18, Ubx. Aminoacyl-tRNA synthetases were prepared from the stock sbd^2 su(Hw)^2/TM1. E. coli strains D24 (used to make S-30 extract and aminoacyl-tRNA synthetases) and K37 (host for f2 sus 3) were obtained from Dr. H. Lodish. f2 phage were purchased from Miles Laboratories. Purified tRNA from E. coli B was purchased from Schwartz/Mann.

Aminoacyl-tRNA. Transfer RNA was prepared by a modification of the method of Twardzik et al.(10). DEAE-cellulose chromatography was omitted, and instead polysaccharides were removed from solution by centrifugation in a Spinco 30 rotor at 30,000 rpm for 2 hours. The clear supernatant was treated with 2 volumes of cold ethanol to precipitate the RNA. The precipitate was extracted with 1 M NaCl, 10 mM Tris-HCl (pH 7.6), 1 mM EDTA to solubilize the tRNA. Transfer RNA preparations were deacylated by incubation in 2 M Tris-HCl (pH 9.1) at 37°C for 30 minutes. Aminoacyl-tRNA synthetases were prepared as described by Twardzik et al.(10). RNA was purified from the acylation mixture as described previously (2).

Protein Synthesis. The protein synthesizing system has been described by Salser et al.(11) and Young (12). The concentration of f2 RNA in the incubation mixtures was 5.2 A_{260}/ml and that of f2 sus 3 RNA was 6.8 A_{260}/ml. Incubation mixtures (0.10 ml) always contained 0.058 A_{260} of [^{35}S]Met-tRNA^{Met} (unfractionated) from E. coli B as a source of label to measure f2 protein synthesizing activity. The amount of exogenous [^{3}H]Tyr-tRNA^{Tyr} (unfractionated) added to each incubation mixture was calculated from the specific activity of the [^{3}H]tyrosine used in the acylation. All amino acids were

present in the incubation mixture (13,000 picomoles/0.10 ml) except cysteine. Following incubation at 37°C for 15 minutes, 0.5 ml bovine serum albumin (0.5 mg/ml) and 0.10 ml 1 M NaOH were added to each assay and incubation continued for 10 minutes more to hydrolyze the remaining labeled aminoacyl-tRNA. Radioactively labeled protein was precipitated by the addition of 0.5 ml cold 10% trichloroacetic acid, and the precipitate was collected on glass fiber filters (Whatman GF/A), washed with cold acid, dried and counted in a toluene based fluor.

RESULTS AND DISCUSSION

Participation of Drosophila Tyrosyl-tRNA^{Tyr} in Peptide Bond Synthesis. Table 1 shows the result of the addition of [^{3}H]Tyr-tRNA^{Tyr} from either Drosophila or E. coli to the protein synthesizing system utilizing f2 RNA as messenger. Each assay also contains [^{35}S]Met-tRNA^{Met} from E. coli B to act as an independent measure of protein synthetic activity. Incorporation of [^{3}H]tyrosine occurs with high efficiency. It will be noted that increasing amounts of exogenous [^{3}H]Tyr-tRNA^{Tyr} decrease the activity of the system as measured by either [^{3}H]tyrosine or [^{35}S]methionine incorporation. This effect is independent of the source of added tRNA. Increasing the Mg^{++} concentration of the incubation mixture does not reverse the inhibitory effect (data not shown). Comparison of the data in Table 1 for Drosophila with that for E. coli indicates that exogenous Drosophila [^{3}H]Tyr-tRNA^{Tyr} donates tyrosine as readily as exogenous E. coli [^{3}H]Tyr-tRNA^{Tyr}, in competition with the endogenous tRNA and added E. coli B tRNA, to f2 protein synthesis. Indeed, two of the Drosophila preparations compete with a greater efficiency than the E. coli preparation.

Incorporation of [^{3}H]tyrosine into f2 protein may occur either by direct participation of the added [^{3}H]Tyr-tRNA^{Tyr} in peptide bond formation or by transacylation of the [^{3}H]tyrosine (through a tyrosyladenylate-synthetase complex) to E. coli tRNA^{Tyr} prior to peptide bond formation. The incubation mixture contains a 13,000 fold molar excess of non-radioactive tyrosine, and thus the observed incorporation of [^{3}H]tyrosine cannot be due to hydrolysis of the [^{3}H]Tyr-tRNA^{Tyr} or pyrophosphorolysis of a [^{3}H]tyrosyladenylate-synthetase complex and subsequent reincorporation of the [^{3}H]tyrosine into [^{3}H]Tyr-tRNA^{Tyr} of E. coli. In order for transacylation to be possible, the proper E. coli aminoacyl-tRNA synthetase must be able to recognize Drosophila Tyr-tRNA^{Tyr}. Such recognition also implies an ability to recognize the unacylated tRNA^{Tyr}.

The data in Table 2 demonstrate the relative ability of an E. coli

TABLE 1

f2 MESSENGER DIRECTED PROTEIN SYNTHESIS

Content of Protein Synthesizing System	A [^{35}S]Met Incorporation (CPM)	B Added [^{3}H]Tyr-tRNA^{Tyr} (picomoles)*	C [^{3}H]Tyr Incorporation (picomoles)	Percent Incorporation C/B x 100
no messenger	3,417†			
f2+ messenger	9,634			
f2+ messenger and Drosophila [^{3}H]Tyr-tRNA^{Tyr}				
su(Hw)$^{+}$	7,034	1.20	0.61	51
	8,653	0.60	0.35	58
	8,893	0.30	0.17	57
hemizygous su(Hw)^{2}	5,378	1.40	0.70	50
	8,889	0.70	0.37	53
	9,245	0.35	0.22	63
heterozygous su(Hw)^{2}	7,579	1.42	0.36	25
	8,718	0.71	0.22	31
	8,962	0.35	0.13	37
f2+ messenger and E. coli [^{3}H]Tyr-tRNA^{Tyr}				
CA265 su$^{+}_{III}$ (amber)	8,352	0.26	0.10	38

* The specific activity of the [^{3}H]tyrosine was 2,000 CPM/picomole.

† This figure represents incorporation directed by endogenous messenger in the absence of added tRNA. Exogenous tRNA decreases this incorporation. This background was determined for each concentration of exogenous tRNA and is subtracted from the appropriate figures below.

TABLE 2

ACTIVITY OF THE E. COLI AMINOACYL-tRNA SYNTHETASE PREPARATION

tRNA (A_{260}/0.1 ml)	Tyrosyl-tRNA Formed (picomoles/0.1 ml/10 minutes)
None	0.16
E. coli	
1.1	7.4
2.2	15.6
4.4	39.2
Drosophila	
1.3	0.21
2.6	0.24
5.1	0.34

synthetase preparation to acylate E. coli and Drosophila tRNA with [^{3}H]tyrosine. As virtually no activity is seen with the Drosophila tRNA, the data strongly suggest that the E. coli tyrosyl-tRNA synthetase does not form a functional complex with Drosophila tRNA^{Tyr}. This same Drosophila tRNA preparation readily accepts [^{3}H]tyrosine when a Drosophila synthetase preparation is used. The ability of Drosophila [^{3}H]Tyr-tRNA^{Tyr} to form any complex with the E. coli synthetase has been tested using the membrane filter assay of Yarus and Berg (13) with negative results. Nor does Drosophila tRNA compete with the binding of E. coli [^{3}H]Tyr-tRNA^{Tyr} to the synthetase using this assay (data not shown).

The relative stabilities of E. coli and Drosophila [^{3}H]Tyr-tRNA^{Tyr} in the S-30 incubation mixture under deacylation conditions are shown in Figure 1. While both species of [^{3}H]Tyr-tRNA^{Tyr} are unstable, only the E. coli [^{3}H]Tyr-tRNA^{Tyr} shows a decreased stability in the presence of AMP and pyrophosphate. In contrast, the Drosophila [^{3}H]Tyr-tRNA^{Tyr} shows a greater stability in the presence of AMP and pyrophosphate. Quantitatively similar results have been found using a purified E. coli aminoacyl-tRNA synthetase preparation instead of the S-30 preparation used in the experiment shown in Figure 1. The loss of [^{3}H]Tyr-tRNA^{Tyr} in the absence of added AMP and pyrophosphate is due to hydrolysis. This experiment indicates that the tyrosyl-tRNA synthetase present in the S-30 preparation is active in deacylating the [^{3}H]Tyr-tRNA^{Tyr} of E. coli but not of Drosophila.

I conclude from the above experiments that Drosophila [^{3}H]Tyr-tRNA^{Tyr} participates directly in peptide bond formation and does not participate in the transacylation of [^{3}H]tyrosine to endogenous E. coli tRNA^{Tyr} while exogenous E. coli [^{3}H]Tyr-tRNA^{Tyr} may do so. This may explain why Drosophila preparations donate [^{3}H]tyrosine to f2 protein synthesis with a greater efficiency than does an E. coli preparation (Table 1).

It is interesting to note that the rate of hydrolysis of the Drosophila [^{3}H]Tyr-tRNA^{Tyr} is greater than that of E. coli (Figure 1). This is so whether the S-30 preparation or a purified aminoacyl-tRNA synthetase preparation is used and raises the possibility that an enzyme is present which specifically recognizes the Drosophila Tyr-tRNA^{Tyr} as foreign or as an apparently "non-compatible" association of amino acid and tRNA. Yarus (14) has described a "verification" reaction in which phenylalanyl-tRNA synthetase carries out a specific hydrolysis of misacylated Ile-tRNA^{Phe}. This type of hydrolytic reaction is not confined to phenylalanyl-tRNA synthetase (15). Hydrolysis of cognate aminoacyl-tRNA by aminoacyl-tRNA synthetase has also been observed

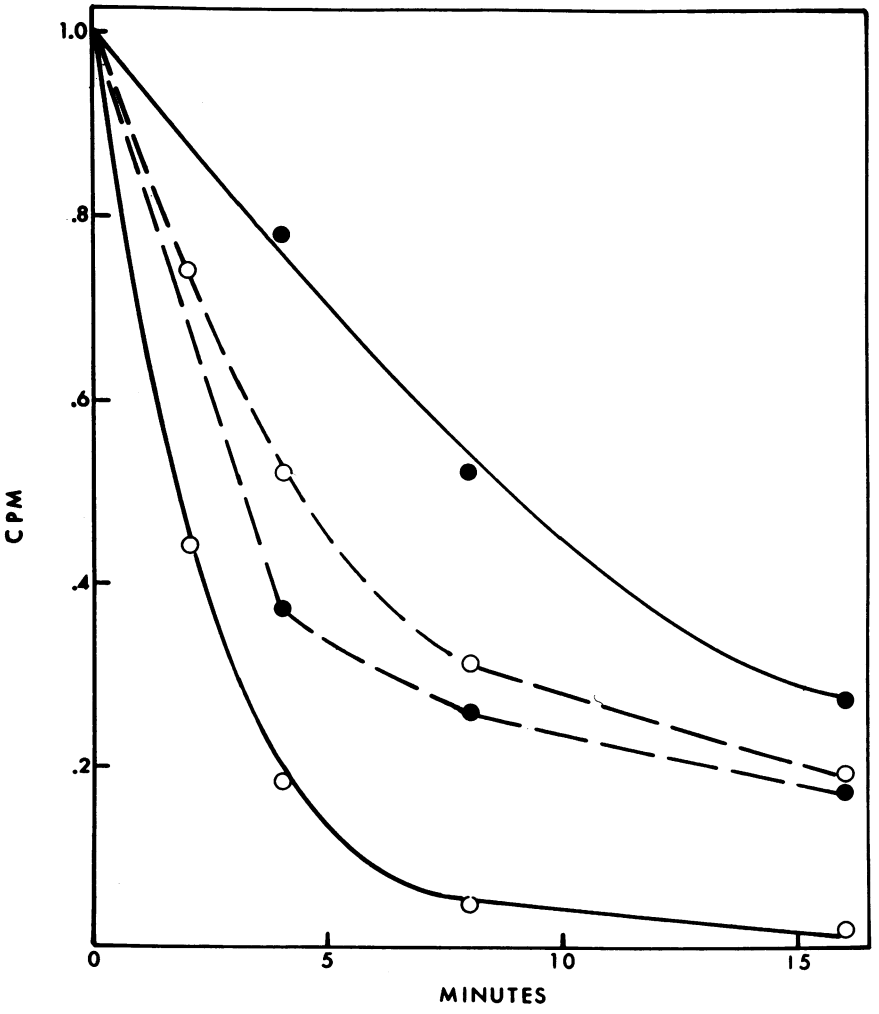

Figure 1. The Stability of E. coli and Drosophila [^{3}H]Tyr-tRNA^{Tyr} in the S-30 Extract Used for Protein Synthesis. The concentration of E. coli aminoacyl-tRNA is 4.3 picomoles/ml and that of Drosophila is 4.2 picomoles/ml. The buffer is 63 mM Tris-HCl (pH 7.8), 50 mM NH_4Cl, 8 mM Mg acetate (the same as used in protein synthesis, except for the omission of energy sources). 0.4 mM tyrosine is present, as are 1 mM Na pyrophosphate and 10 mM AMP where indicated. The charged tRNA was measured by trichloroacetic acid precipitation. ●———●, E. coli, and ●– – –● Drosophila; O———O, E. coli, and O– – –O, Drosophila in the presence of AMP and pyrophosphate.

TABLE 3

f2sus3 MESSENGER DIRECTED PROTEIN SYNTHESIS

Content of Protein Synthesizing System	A [^{35}S]Met Incorporation (CPM)	B Added [^{3}H]Tyr-tRNA^{Tyr} (picomoles)*	C [^{3}H]Tyr Incorporation (picomoles)	Percent Incorporation C/B x 100
no messenger	3,800†			
f2sus3 messenger	-338			
f2+ messenger	9,721			
f2sus3 messenger and Drosophila [^{3}H]Tyr-tRNA^{Tyr}				
su(Hw)$^{+}$	0‡	1.50	0.05	3
	0	0.75	0.08	11
	0	0.38	0.05	13
hemizygous su(Hw)^{2}	-19	0.93	0.10	11
	53	0.47	0.04	9
	-95	0.23	0.04	17
heterozygous su(Hw)^{2}	-134	1.42	0.13	9
	58	0.71	0.07	10
	-95	0.35	0.05	14
f2sus3 messenger and E. coli [^{3}H]Tyr-tRNA^{Tyr}				
CA244(su^{-})	0‡	1.00	0.04	4
	0	0.50	0.07	14
	0	0.25	0.05	20
CA265 su$^{+}_{III}$ (amber)	794	1.02	0.32	30
	1,052	0.51	0.12	24
	814	0.26	0.03	12

* See Footnote to Table 1

† See Footnote to Table 1

‡ f2sus3 messenger added to the protein synthesizing system containing exogenous tRNA is observed to slightly decrease the incorporation of [^{35}S]methionine. To take this factor into account the data for tRNA from suppressor strains have been normalized with respect to the pertinent nonsuppressor strain. This has resulted in negative values for some assays.

but not always commented upon (16,17).

Failure to Detect Amber Suppression by Drosophila Tyrosyl-tRNA^{Tyr}. The results obtained when f2 sus 3 messenger is used are shown in Table 3. The incorporation of [^{35}S]methionine into f2 coat protein is a measure of amber suppression and is apparent only in the case where the exogenous [^{3}H]Tyr-tRNA^{Tyr} is from the amber suppressing strain CA 265 su$^{+}_{III}$. The data in Table 3 show that some incorporation of [^{3}H]tyrosine does occur even in the absence of suppression. This incorporation is not correlated with either the strain or the species from which the exogenous [^{3}H]Tyr-tRNA^{Tyr} was derived and may be due to some degradation of the f2 sus 3 messenger, yielding fragments which escape the polar effect of the amber mutation (18). The failure to observe suppression is not due to some general failure of Drosophila tRNA to participate in protein synthesis directed by f2 sus 3 messenger as Drosophila [^{3}H]Phe-tRNA^{Phe} readily participates in the synthesis of the N-terminal fragment of the f2 coat protein induced by the sus 3 mutation (data not shown).

The level of suppression demonstrated by CA 265 su$^{+}_{III}$ is due to the action of only 15% of the indicated amount of this tRNA added to the assay since this is the estimated fraction of tRNA^{Tyr} in this strain capable of recognizing the amber codon (19). Despite the relatively high background caused by endogenous messenger, the quantitation of amber suppression by tRNA from the E. coli amber suppressor and from the less efficient E. coli ochre suppressor (2) is quite reproducible. With these facts in mind, I conclude that, were only 5% of the su(Hw)^{2} [^{3}H]Tyr-tRNA^{Tyr} capable of amber suppression with an efficiency comparable to that of the E. coli amber suppressor, this assay would have detected suppression.

The above experiment lends no support to the hypothesis that the su(Hw)^{2} mutation leads to the production of a species of tRNA^{Tyr} which can suppress amber or ochre codons. It should be noted, however, that certain known, tyrosine inserting, amber and ochre suppressor strains of yeast do not contain tRNA^{Tyr} which can suppress the f2 sus 3 mutation (2). If, like the UGA suppressor of E. coli (20), a eucaryotic suppressor species of tRNA is altered outside of the anticodon, E. coli ribosomes may not be capable of recognizing such an altered species of tRNA as a suppressor even though it may participate in protein synthesis through the translation of sense codons.

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