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Mechanism of suppression in Drosophila. VII. Correlation between disappearance of an isoacceptor of tyrosine tRNA and activation of the vermilion locus

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ABSTRACT

The possibility that tyrosine tRNA modifies the catalytic activity of tryptophan oxygenase that is produced by the vermilion mutant (v) in Drosophila melanogaster is reconsidered. Dietary conditions can modify the ratio of the two major isoacceptors of tyrosine tRNA: one condition allows 85–90% to exist as the second isoacceptor, and another condition allows <5% to exist in this form. The function lacking in the vermilion mutant is partially restored when the second isoacceptor of $tRNA^{Tyr}$ is reduced to low levels (<40%), but the function is greatly reduced when this isoacceptor is present as 50% or more of the total. These data support the hypothesis that $tRNA^{Tyr}$ may be associated with and regulate tryptophan oxygenase. The corresponding isoacceptor of $tRNA^{Tyr}$ found in a suppressor mutant, $su(s)^2$, should not have any effect on the function of the vermilion gene, and, indeed, it did not. The tRNAs for tyrosine, aspartic acid, and histidine all have one isoacceptor that contains nucleoside Q and all undergo parallel changes in flies raised on the various diets. It appears that these dietary changes affect the ability to synthesize or modify Q or to remove or insert it into tRNA.

INTRODUCTION

The vermilion locus in Drosophila is likely the structural locus for tryptophan oxygenase[1] (EC 1.13.11.11). In the absence of this enzyme activity, no synthesis of brown eye pigment occurs. The biosynthetic pathway involves the oxidation of tryptophan to produce formylkynurenine and then kynurenine; subsequent steps result in the production of xanthommatin, the brown eye pigment. Several alleles at the vermilion locus are known, and they may be assigned to two groups: v^s for alleles in which the synthesis of brown eye pigment is restored by a suppressor mutant $su(s)^2$ and v^u for those that are unsuppressible.[2] Also characteristic of these two groups is the fact that the v^s alleles synthesize brown eye pigment when grown on medium containing 1% dried brewer's yeast, whereas the v^u alleles do not produce brown eye pigment on this diet.[3-6] Both groups of vermilion are nonautonomous, which is shown by

transplanting the precursor of either the v^s or v^u eye (imaginal disc) into a v^+ host and obtaining synthesis of brown eye pigment because the host contains kynurenine that can diffuse into the developing eye tissue.[2,7] Since the v^u alleles do not respond to the yeast diet by producing brown eye pigment, the restoration of pigment production in the v^s alleles is not due to the presence of kynurenine in the yeast.

The mechanism by which the $su(s)^2$ mutant causes the restoration of tryptophan oxygenase is of interest. The possible involvement of tRNA was suggested by the report[8] that the second isoacceptor of tyrosine tRNA ($tRNA_B^{Tyr}$) was greatly reduced or absent in $su(s)^2$. Subsequent study[9] showed that $tRNA_B^{Tyr}$ was not missing but could appear to be so when the tyrosyl-tRNA synthetase was prepared in a certain manner; this enzyme could not charge $tRNA_B^{Tyr}$ but could charge $tRNA_A^{Tyr}$ and also the two isoacceptors from wild-type Drosophila, $tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$. The enzyme could be prepared by a different method so that it did charge $tRNA_B^{Tyr}$ and the other three tRNAs as well. These enzyme forms were called "discriminating" and "nondiscriminating," respectively. It was suggested that the $su(s)^+$ locus was responsible for the production of an enzyme that modified a nucleotide that would be found in $tRNA_2^{Tyr}$ but not in $tRNA_B^{Tyr}$ from the $su(s)^2$ fly.

From the changes in ratio of $tRNA_1^{Tyr}$ to $tRNA_2^{Tyr}$ during development in Drosophila and from minor nucleoside analysis, White et al.[10] suggested that $tRNA_1^{Tyr}$ differed from $tRNA_2^{Tyr}$ in only one nucleoside: $tRNA_1^{Tyr}$ contained nucleoside Q and $tRNA_2^{Tyr}$ did not. The developmental study included wild-type and $su(s)^2$ flies, and the minor nucleoside analysis was performed on tRNA from wild-type flies. Since both isoacceptors of $tRNA^{Tyr}$ of $su(s)^2$ co-chromatograph with those of wild-type $tRNA^{Tyr}$, it is reasonable to conclude that the first and second isoacceptors of $su(s)^2$ also differ by the presence or absence of nucleoside Q. Thus neither the wild-type or $su(s)^2$ second isoacceptor of $tRNA^{Tyr}$ has nucleoside Q. Therefore nucleoside Q cannot be involved in determining whether the "discriminating" tyrosyl-tRNA synthetase aminoacylates $tRNA_B^{Tyr}$; some other nucleoside modification probably determines this.[9]

When we considered how tryptophan oxygenase could be produced in $su(s)^2$ v flies as a result of what was thought then to be the absence of the second isoacceptor of $tRNA^{Tyr}$, we hypothesized that this tRNA was an inhibitor.[11] In its absence trypto-phan oxygenase from the vermilion mutant could again function. This hypothesis was modified later[9] to suggest that both tryptophan oxygenase and the "discriminating" form of tyrosyl-tRNA synthetase were unable to react with the $tRNA_B^{Tyr}$ produced by

su(s)^2. It is possible that both enzymes depend on a single modified nucleoside to form a stable complex with this isoacceptor.

The experiment to support this hypothesis was to treat the vermilion tryptophan oxygenase with RNase.[11] The tryptophan oxygenase activity was partially restored and could in turn be inhibited by tRNA from a region of chromatogram that contained mainly the $tRNA_2^{Tyr}$. Subsequently, we were unable to perform the activation by RNase,[9] as were Mischke et al.[12] Wosnick and White[13] approached the problem differently, altering the relative amounts of the two isoacceptors of $tRNA^{Tyr}$ in wild-type and su(s)^2 v; bw flies by varying the diet and temperature for rearing the flies. They observed no changes in eye color in the latter when the amount of second isoacceptor of $tRNA^{Tyr}$ was diminished to quite low levels or raised to rather high levels. From this experiment they concluded that it is unlikely that $tRNA_B^{Tyr}$ is involved in regulating the vermilion form of tryptophan oxygenase.

From the observation that the $tRNA_B^{Tyr}$ from su(s)^2 did not inhibit vermilion tryptophan oxygenase (unpublished) and the correlation, mentioned above, between the isoacceptor specificity of vermilion tryptophan oxygenase and of "discriminating" tyrosyl-tRNA synthetase, we would have predicted that no change in eye color of su(s)^2 v; bw should occur as the second isoacceptor of $tRNA^{Tyr}$ waxes and wanes. However, if such changes were to occur in v; bw, where the second isoacceptor of $tRNA^{Tyr}$ is normally modified, then we would predict that brown eye pigment synthesis might be restored by reducing the amount of this isoacceptor. The following experiments demonstrate that restoration of brown eye pigment is indeed accompanied by decreased levels of $tRNA_2^{Tyr}$; when $tRNA_2^{Tyr}$ is kept at high concentrations there is little or no brown pigment made in the eye. Thus the prediction is supported.

MATERIALS AND METHODS

Drosophila melanogaster, Oregon-R and Samarkand are wild type. The double mutant v; bw and the mutant w can produce neither the brown eye pigment, xanthommatin, nor the red eye pigments of the drosopterin class; in su(s)^2 v; bw the production of xanthommatin is restored.[14] The mutant bw cannot produce the drosopterins[14]; v is deficient in tryptophan oxygenase and in xanthommatin.[15]

The diets for rearing Drosophila were (1) the normal diet consisting of 8% cornmeal, 5.8% glucose, 2.9% sucrose, 2.9% dried brewer's yeast, and 0.6% agar; (2) the yeast diet consisting of the stated percent (w/v) of dried brewer's yeast and

1.5% agar (Bacto-agar, Difco); and (3) formula 4-24 (obtained from Carolina Biological Supply) seeded with live yeast. In the yeast diet the two ingredients were mixed with deionized water and autoclaved for 20 min at 254° and 16 psi; after the mixture was cooled to 55° it was made to 0.38% propionic acid and 0.056% H_3PO_4 to inhibit growth of mold, and 50 ml was poured into 1/2-pint bottles. The next day, immediately prior to inoculation, 0.5 ml of 1% sucrose–1% acetic acid–1% ethanol was added to each bottle; inoculation was with 30–40 adult flies that were 1–7 days old. Four days later all the adult flies were removed; usually about half of them were dead at this time. Emergence occurred at 10–18 days after inoculation, and the offspring were collected every 2 days, scored for eye color, and stored at -80° to await further analysis, usually within 2 weeks. Incubator temperature was 25 ± 1°, and the lights were employed at irregular intervals.

The tRNA and aminoacyl-tRNA synthetase were prepared as described previously.[8] In all cases the "nondiscriminating" form of tyrosyl-tRNA synthetase was employed for aminoacylation so that $tRNA_B^{Tyr}$ would react.[9] Sample preparation and chromatography on RPC-5 followed previously described procedures.[9]

Xanthommatin analysis followed the procedure of Butenandt et al.[16] 100 mg of adult flies was homogenized in 2.0 ml 2 N HCl, and 1.5 ml of the homogenate was added to a glass-stoppered 25-ml flask containing 2.0 ml n-butanol. The mixture was gassed with SO_2 for 60 sec, stoppered, and shaken for 30 min at room temperature. After the mixture was centrifuged at 5° for 60 min at 1500 rpm (International), the absorbance at 492 nm was determined for the material in the butanol phase. In the absence of a standard xanthommatin solution, the scale limits were determined by the absorbance of the mutants bw, which contains a full complement of xanthommatin, and w, which contains none.

RESULTS

Relationship of Diet to Production of Brown Eye Color by v; bw

When v; bw was grown on a normal diet, the eyes were nearly white (the color is more accurately described as ivory). Flies of different ages, from 1 to 35 days old, showed no darkening of the eye color; some of the older ones actually appeared whiter. Likewise, on the "4-24" diet flies of ages up to 20 days showed no darkening of the eye color. However, when raised on a diet consisting of autoclaved brewer's yeast, many flies exhibited various degrees of brown pigmentation of the eyes. Many had eyes that

appeared as fully pigmented as those seen in $su(s)^2$ v; bw. Various concentrations of yeast were tested, ranging from 0.4 to 2% in the diet. The brown pigmented eyes were apparent at all concentrations; no adults or pupae appeared at 0.4%, but at 0.6% and above the number of offspring increased with the yeast concentration. The survival of the egg-producing parents was poor at all yeast concentrations; they were removed at 4 days but would not have survived more than 5.

The newly emerged adults survived for 3–4 days; they were routinely collected every 2 days. The flies were divided into two groups: one had eyes that appeared to be nearly as brown as $su(s)^2$ v; bw, and the other had eyes that ranged from ivory through various shades of tan. Usually four collections at 2-day intervals were possible. The first collection, 2 days after the appearance of the first adult flies, was invariably composed of a larger proportion of ivory and tan-colored eyes than any of the later collections. Table I shows how the eye color groups were distributed in successive collections. In several such experiments the proportion of brown-eyed flies ranged from 0.73 to 0.89 for the 1% yeast diet and from 0.72 to 0.97 for the 2% yeast diet. Rearing $su(s)^2$v; bw, Oregon R or Samarkand strains on the yeast diet had no visible effect on the eye color.

The brown eye color of Drosophila is caused by a single pigment, xanthommatin, that is a product of tryptophan metabolism.[16] The mutant bw is incapable of synthesizing the red eye pigment but does produce xanthommatin; the mutant w can make neither red nor brown pigment; and v is unable to produce xanthommatin, but in the presence of $su(s)^2$ its ability to produce this pigment is restored. The amount of xanthommatin in each of these mutants was measured (Table II). Similarly, for flies that had been raised on 1% and 2% yeast, the amount was measured in the group with ivory-to-tan eyes and in the group with brown eyes. These values were compared with the amount found in the mutants grown on a normal diet. The brown-eyed group contained nearly 80% of the xanthommatin found in the genetically suppressed vermilion [$su(s)^2$ v; bw], which in turn contained half the pigment of bw.

When any of the other components of the normal diet were added to the 2% yeast medium, the brown pigment was greatly reduced.[3,4] Indeed, in the case of glucose the eyes were noticeably whiter than the ivory color of v; bw. This was reflected in the xanthommatin assay (Table II).

Chromatographic Characterization of Tyrosine tRNA from v; bw and Other Strains Grown on Yeast Diets

TABLE I. Eye color of v; bw raised on 1% and 2% yeast diet

Diet	Collection	No. of flies[a] with: Ivory-to-tan eyes	Brown eyes	Percent with brown eyes
1% Yeast	1	83	2	2
	2	361	459	56
	3	54	602	92
	4	3	270	99
2% Yeast	1	505	416	45
	2	152	729	83
	3	50	405	89
	4	3	268	99

[a] 50 bottles of 1% and 50 bottles of 2% yeast diet were inoculated with 30–40 adult v; bw per bottle. The number of flies listed is from all 50 bottles.

[^{3}H]tyrosyl-tRNA or [^{14}C]tyrosyl-tRNA was examined by RPC-5 chromatography. Flies grown on a normal diet and collected at 0–2 days of age had the majority of the tyrosyl-tRNA as the second isoacceptor (Fig. 1 O-R N). The tyrosyl-tRNA of flies grown on 1% yeast had much less of the second isoacceptor; Oregon-R had 21% and su(s)^2 v; bw had 18%. Of particular interest was the difference between the two groups of v; bw: the group with ivory-to-tan eyes had 59% whereas the group with brown eyes had 38%. Both of these are lower than the 85% obtained from v; bw grown on the normal medium.

Comparison of the tyrosyl-tRNA in the group with ivory-to-tan eyes with the group with brown eyes was repeated several times. Table III shows that the amount of the second isoacceptor was greatly decreased regularly in the brown-eyed v; bw. The white-eyed flies that were reared on 2% yeast–6% glucose regularly had most of the tyrosine tRNA in the second isoacceptor form but in a smaller amount than those reared on normal medium. The group with ivory-to-tan eyes was rather heterogeneous in eye color and, of course, contained the flies from all four collections; the relative amount of $tRNA_2^{Tyr}$ was always intermediate between the brown-eyed and white-eyed groups in that experiment. Also, the shift toward $tRNA_1^{Tyr}$ was more pronounced at the higher

TABLE II. Effect of diet on xanthommatin in v; bw

Genotype	Diet[a]	Eye Color	Xanthommatin[b]	
			A_{492} X 10^3	Percent of bw
w	N	white	16.5 ± 2	0
bw	N	brown	147 ± 7	100
su(s)^{2}v; bw	N	brown	84.5 ± 3.5	52
v; bw	N	ivory	25.5 ± 5	7
v; bw	1%	tan	38 ± 15	16
v; bw	1%	brown	67.5 ± 2	39
v; bw	2%	tan	38.5 ± 2	17
v; bw	2%	brown	68.5 ± 0.7	40
v; bw	YG	white	19.5 ± 0.7	2

[a] Diet designations are N = normal diet, 1% = 1% dried brewer's yeast, 2% = 2% dried brewer's yeast, YG = 2% yeast + 6% glucose.

[b] The absorbances at 492 nm were determined relative to an n-butanol blank and are shown with the standard deviation for two experiments, Exp. 3 and Exp. 4 of Table III. The absorbance of w was subtracted from all other values prior to calculating "percent of bw."

yeast concentration.

Chromatographic Characterization of Tyrosine tRNA of Strains of Drosophila on Different Diets and at Various Temperatures

Wosnick and White[13] found that flies raised on the "4-24" medium had smaller amounts of $tRNA_2^{Tyr}$ than did flies raised on the normal diet. They employed flies that were aged 1–2 weeks after eclosion. The younger flies, 0–2 days, used in the present study were examined. Samarkands raised on "4-24" had 0.31 of the tyrosine tRNA as $tRNA_2^{Tyr}$ compared with 0.77 when raised on normal medium. The su(s)^2 v; bw had 0.43 as $tRNA_2^{Tyr}$ when raised on "4-24" as compared with 0.52 and 0.56 (two determinations) when raised on normal medium. The v; bw raised on "4-24" had 0.84 and 0.92 as $tRNA_2^{Tyr}$ as compared with 0.85, 0.87, and 0.88 when raised on normal medium. For Samarkands and su(s)^2 v; bw these changes are not as extreme as those reported by

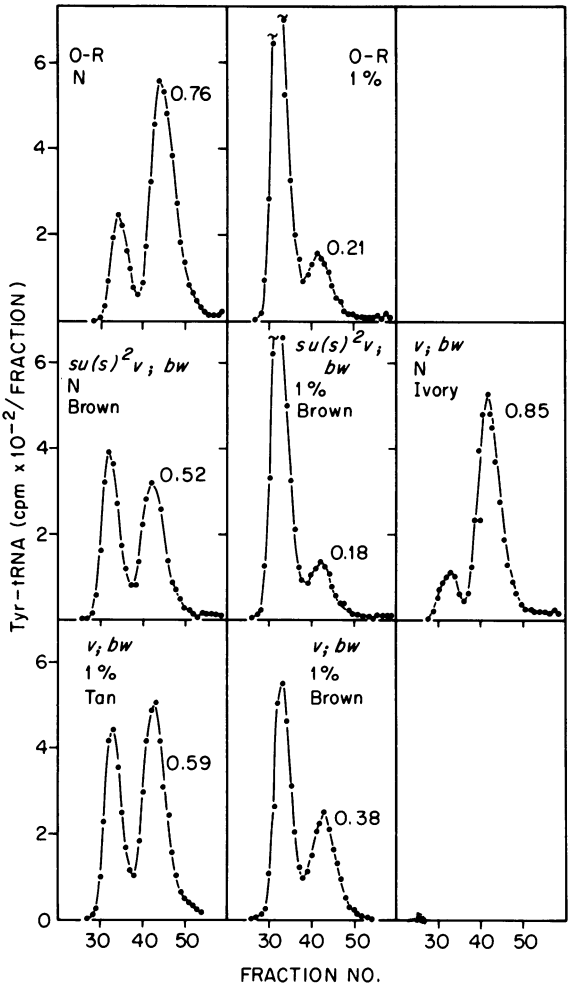

Fig. 1. Effect of diet on tyrosyl-tRNA. The tRNA was charged with either [^{14}C]tyrosine or [^{3}H]tyrosine, washed on DEAE-cellulose to remove unbound tyrosine and other ingredients of the reaction mixture, and eluted in 1 M NaCl-10 mM acetate (pH 4.6)-10 mM $MgCl_2$. The sample was diluted to 0.35 M NaCl and applied to the RPC-5 column (0.6 x 15 cm) at 36°. The elution of tyrosyl-tRNA employed a linear gradient of NaCl consisting of 100 ml 0.5 M NaCl in the mixing chamber and 100 ml 0.6 M NaCl in the reservoir. Fraction volumes were constant for each chromatogram and were approximately 1.0 ml (0.92-1.05 depending on the chromatogram). The radioactivity was determined by adding 10 ml of counting mixture (0.27% 2,5-bis-[2(5-tert-butylbenzoxazolyl)]-thiophene-67% toluene-33% Triton X-100) and counting in a scintillation spectrometer. The diets on which the flies were raised are designated "N" for the normal diet and "1%" for the diet consisting of 1% dried brewer's yeast. The Oregon-R and su(s)^{2}v; bw tRNA was from Exp. 3 and the v; bw was from Exp. 1 of Table III. The relative amount of the second peak is designated on each chromatogram. The eye color of the flies is shown on each panel.

Wosnick and White, but they are similar since the change is always to show a decrease in $tRNA_2^{Tyr}$. Wosnick and White did not report any results for v; bw. These results

TABLE III. Variation with dietary conditions of tyrosine tRNA in 0- to 2-day-old *Drosophila melanogaster*

Experiment	Source of tRNA	Diet[a]	No. of flies[b]	Eye color[c]	Relative amount[d] of $tRNA_2^{Tyr}$
Exp. 1	v; bw	N	>4000†	ivory	0.85
		1%	942*	tan	0.59
		1%	2638*	brown	0.38
Exp. 2	v; bw	2%	583*	tan	NM
		2%	1836*	brown	<0.05
		YG	NC	white	0.69
Exp. 3	Oregon-R	N	>7000‡	dark red	0.76
		1%	NC	dark red	0.21
	su(s)^2 v; bw	N	3800†	brown	0.52
		1%	NC	brown	0.18
	v; bw	N	>4000†	ivory	0.85
		1%	615*	tan	NM
		1%	2390*	brown	0.10
		2%	1489*	tan	0.13
		2%	4054*	brown	<0.05
		YG	1996‡	white	0.55
Exp. 4	v; bw	N	>4000‡	ivory	0.88
		1%	501*	tan	0.61
		1%	1333*	brown	0.31
		2%	710*	tan	0.30
		2%	1818*	brown	0.17
		YG	1295**	white	0.73

[a] Diet designations are N = normal diet, 1% = 1% dried brewer's yeast, 2% = 2% dried brewer's yeast, YG = 2% yeast + 6% glucose.

[b] Total number of 0- to 2-day-old flies obtained from the indicated number of bottles of media: *50, †8, ‡10, **9. NC = not counted.

[c] Eye color observed under dissecting microscope.

[d] Amount of $tRNA_2^{Tyr}$ relative to the total amount of $tRNA_1^{Tyr}$ plus $tRNA_2^{Tyr}$ obtained after chromatography on RPC-5. NM = not measured.

are presented to confirm the finding of Wosnick and White that diet has a major effect on the isoacceptors of $tRNA^{Tyr}$.

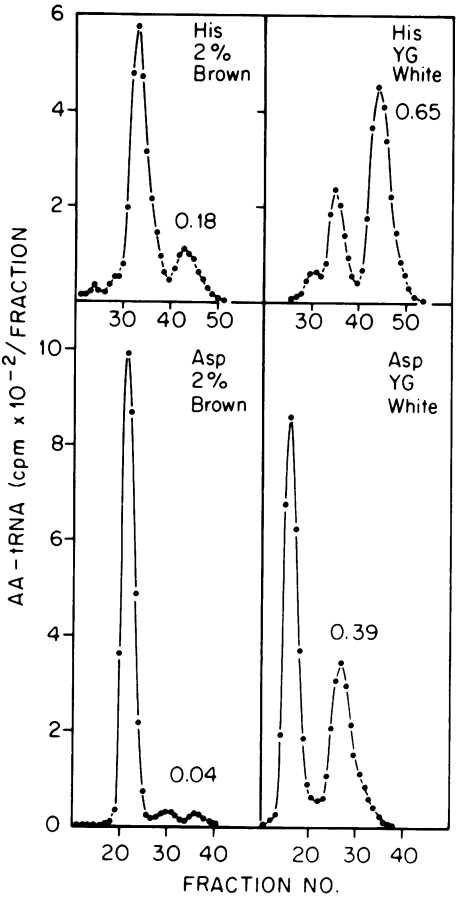

Fig. 2. Effect of diet on histidyl- and aspartyl-tRNA from v; bw. The tRNA was charged with [^{3}H]histidine or [^{3}H]aspartic acid and processed as described in Fig. 1, except the NaCl gradient was from 0.50 to 0.75 M NaCl. The diets on which the flies were raised are designated "2%" for the diet consisting of 2% yeast and "YG" for the diet consisting of 2% yeast + 6% glucose; eye color is also shown. This tRNA was from Exp. 4 of Table III.

Wosnick and White[13] also observed somewhat less $tRNA_2^{Tyr}$ when the flies were grown at 25° than when they were grown at 22° or 29°. In this study we saw a decrease at 18° compared with 25° in the case of Samarkands (0.31 vs 0.77) and a slight decrease at 28° compared with 25° in the case of v; bw (0.81 vs 0.92). Although the changes with temperature were not characterized in detail, these results are also consistent with those of Wosnick and White. No change in eye color of v; bw accompanied the slight decrease of $tRNA_2^{Tyr}$ at 28°.

Chromatographic Characterization of Histidyl-tRNA and Aspartyl-tRNA from v; bw Grown on a Yeast Diet

Four tRNAs contain nucleoside Q (namely, tyrosine, histidine, aspartic acid, and asparagine tRNAs),[17] and these four undergo nearly simultaneous changes in the relative amounts of the second isoacceptor as the Drosophila proceeds through the larval, pupal, and adult stages of development.[10] The tRNA from flies grown on 2% yeast ± 6% glucose (Exp. 4 of Table III) was charged with histidine and aspartic acid and chromatographed. As shown in Fig. 2, the proportion of the tRNA in the second isoacceptor of histidyl-tRNA changed from 65% for 2% yeast–6% glucose medium to 18% for flies on 2% yeast; similarly, for aspartyl-tRNA the 2% yeast–6% glucose medium gave 39%, and the 2% yeast gave 4%. Wosnick and White[13] observed that the proportion of the second isoacceptor of $tRNA^{Asp}$ changed from 64 to 5% as they changed the diet and temperature.

DISCUSSION

Several studies on the biochemical changes in v and $su(s)^2$ v are of interest. Shappard[18] demonstrated that nonprotein tryptophan levels in v were elevated more than 3-fold over those in the wild type and that the presence of the $su(s)^2$ allele caused reduction to one-half the level in v. She commented that further studies would benefit from measurements of tryptophan oxygenase. Baglioni[15] then reported measurements of tryptophan oxygenase in Drosophila; the enzyme was reduced in v to less than 10% of wild-type levels and was increased in $su(s)^2$ v to 20–30% of normal. Marzluf[19] could find no difference in pH, optimum K_m, or temperature stability between the tryptophan oxygenases of wild-type flies and $su(s)^2$ v; bw. However, although he favored another explanation, he did consider the possibility that suppression involved the removal of a tightly bound, irreversible inhibitor from the enzyme produced by v. Tartof[1] observed that the pH optimum for tryptophan pyrrolase from $su(s)^2$ v was the same as from wild type (pH 7.4), but the enzyme from $su(s)^2$ v^k had an optimum at pH 8.0, the amount of activity in v and v^k was <1% of wild type and that of $su(s)^2$ v was 50% of wild type. He postulated that the subunits for tryptophan oxygenase formed a multimer but that the subunit produced by v could not associate unless $su(s)^2$ changed the intracellular environment to a state more favorable for aggregation of subunits. In 1971 the activation of tryptophan oxygenase in vermilion extracts by RNase was reported; the active mutant enzyme was inhibited by $tRNA_2^{Tyr}$.[11] Subsequently the inability to repeat the RNase activation was reported both from this lab and others.[9,12] Recently Wosnick and White[13] demonstrated that diet and temperature caused the alteration of

the isoacceptor pattern of the tRNAs that contain the Q nucleoside, including $tRNA^{Tyr}$. They stressed that the alteration of the relative amount of $tRNA_B^{Tyr}$ in $su(s)^2$ v; bw did not result in any change of brown eye pigment. As argued in the Introduction to this paper, it is reasonable to consider that the second isoacceptor of $tRNA^{Tyr}$ in the $su(s)^2$ mutant is not equivalent to that in a fly containing $su(s)^+$. If this is so and if, indeed, that isoacceptor in $su(s)^2$ is incapable of inhibiting the form of tryptophan oxygenase produced by the vermilion mutant, then brown eye pigment synthesis would not be expected to be restored in $su(s)^2$ v; bw by reducing the amount of $tRNA_2^{Tyr}$. On the other hand, $su(s)^+$ is present in v; bw, and therefore $tRNA^{Tyr}$ is of the wild-type form. In that case the presence of $tRNA_2^{Tyr}$ would prevent activity of tryptophan oxygenase, and the reduction of the amount of that isoacceptor by dietary means would be expected to relieve the inhibition and allow production of the brown eye pigment. The data presented seem to be consistent with this hypothesis. To reiterate, the inhibition of the vermilion form of tryptophan oxygenase may be relieved in two ways (1) by replacing $su(s)^+$ with $su(s)^2$, which results in the partial or complete loss of a modified nucleoside that is essential for interaction with tryptophan oxygenase and with the discriminating form of tyrosyl-tRNA synthetase; or (2) by rearing the flies on a diet composed of autoclaved brewer's yeast, which results in a marked increase of the Q nucleoside and a marked decrease in the second isoacceptor of $tRNA^{Tyr}$. The modified nucleoside that is the presumed product of $su(s)^+$ may not be formed or its inhibition of tryptophan oxygenase may not occur when the first isoacceptor is predominant.

The yeast diet produces a marked change in the isoacceptors of the Q-containing tRNAs but is incapable of producing many flies as compared with the normal diet or the "4-24" diet. We find that "4-24" produces fewer flies than the normal diet but many more than the yeast diet. The 1% or 2% yeast media cannot keep flies alive for more than 4–5 days, whereas "4-24" will maintain adult flies for many weeks. Wosnick and White[13] showed that "4-24" produced flies that at 1–2 weeks of age had reduced amounts of $tRNA_2^{Tyr}$; this is likely to be caused not only by the diet and temperature but by the age of the flies as well. Addition of 6% glucose to the yeast diet used in this study greatly increased viability of the parents and production of offspring, in addition to restoring the $tRNA_2^{Tyr}$ levels.

The Q nucleoside is present in the first isoacceptor of $tRNA^{Asp}$ and $tRNA^{Tyr}$ of Drosophila tRNA and presumably in $tRNA^{His}$ and $tRNA^{Asn}$ as well.[10] Two different dietary regimens affect the amount of the Q-containing isoacceptor: "4-24" and yeast

alone both favor the production of this form. In the latter case the addition of glucose largely prevents the disproportional production of the Q form. It appears that the yeast diet alters the ability of the fly (1) to synthesize Q, (2) to modify it as Q*,[17] or (3) to remove Q from or insert Q into tRNA.[21,22]

The su(s)$^+$ locus presumably is responsible for causing the production of a modified nucleoside in $tRNA_2^{Tyr}$ but at some site other than Q. The presumed modified nucleotide would then be largely responsible for the ability of this isoacceptor to inhibit the mutant tryptophan oxygenase in v and to react with the discriminating form of tyrosyl-tRNA synthetase. When su(s)^2 is present, the modification would be either absent or incomplete, and the $tRNA_B^{Tyr}$ would then be unable to inhibit tryptophan oxygenase. Jacobson et al.[9] suggested that su(s)^2 is not responsible for the presence or absence of Q but of some other modified nucleoside. Further studies on this tRNA seem warranted.

It must be remembered that not only do the data presented show a correlation between diminished amounts of $tRNA_2^{Tyr}$ and the synthesis of xanthommatin, but they also show that the second isoacceptors of $tRNA^{His}$ and $tRNA^{Asp}$ also decrease along with $tRNA_2^{Tyr}$. The correlation with phenotype changes is not unique to $tRNA_2^{Tyr}$. We have also begun examining the levels of tryptophan oxygenase in the flies produced on the yeast diet and find that the brown-eyed v; bw have significant levels of this enzyme, whereas the flies with ivory-colored eyes have no detectable tryptophan oxygenase (unpublished).

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