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Multiple gene control of the tRNA aminoacylating system in *Drosophila melanogaster**

By RAYMOND W. ROSE† AND RALPH HILLMAN
Biology Department, Temple University, Philadelphia, Pa., U.S.A.

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SUMMARY

The stimulation of *in vitro* tRNA aminoacylation by post-microsomal supernatant enzymes isolated from *Abnormal Abdomen* (A^{53g}) adult flies is a function of the residual genome of these flies rather than of the major mutant gene, A^{53g}. Genes controlling this stimulation are located on the *X* chromosome as well as on the autosomes. These observations are discussed in terms of the phenotypic effect of the major mutant gene in response to changes in protein synthesis which are under the control of modifier genes responsible for aberrant genetic translation mechanisms.

1. INTRODUCTION

In *Drosophila melanogaster* the mutation *Abnormal*^{53g} (A^{53g}), which has been described by Hillman (1973), is responsible for a developmental abnormality of the abdominal histoblasts which results in an inability of the adult imaginal hypoderm to form segmental folds. This is in turn followed by abnormal tergite formation. Penetrance and expressivity are under the influence of environmental factors (e.g. temperature, humidity, crowding, and the age of the culture) and are also controlled by a series of modifier genes located throughout the genome.

Investigations of the A^{53g} genotype at the molecular level (Rose & Hillman, 1969) have shown that subcellular constituents of A^{53g} adult flies are capable of stimulating the *in vitro* incorporation of amino acids into protein at a rate higher than that recorded when comparable wild-type constituents are tested. Also, the post-microsomal supernatant enzymes prepared from A^{53g} adults are capable of amino-acylating tRNA *in vitro* at a higher rate than comparable wild-type enzymes. The stimulation of tRNA aminoacylation is due to increased aminoacylation of iso-accepting tRNA molecules, rather than a general, non-specific mischarging (Rose & Hillman, in preparation). The present work is an investigation of the relationship between the complex genotype responsible for the morphological changes in the adult abdomen and the increased aminoacylation of tRNA which is observed in the *in vitro* tRNA charging system.

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† Present address: Department of Biology, Beaver College, Glenside, Pa.

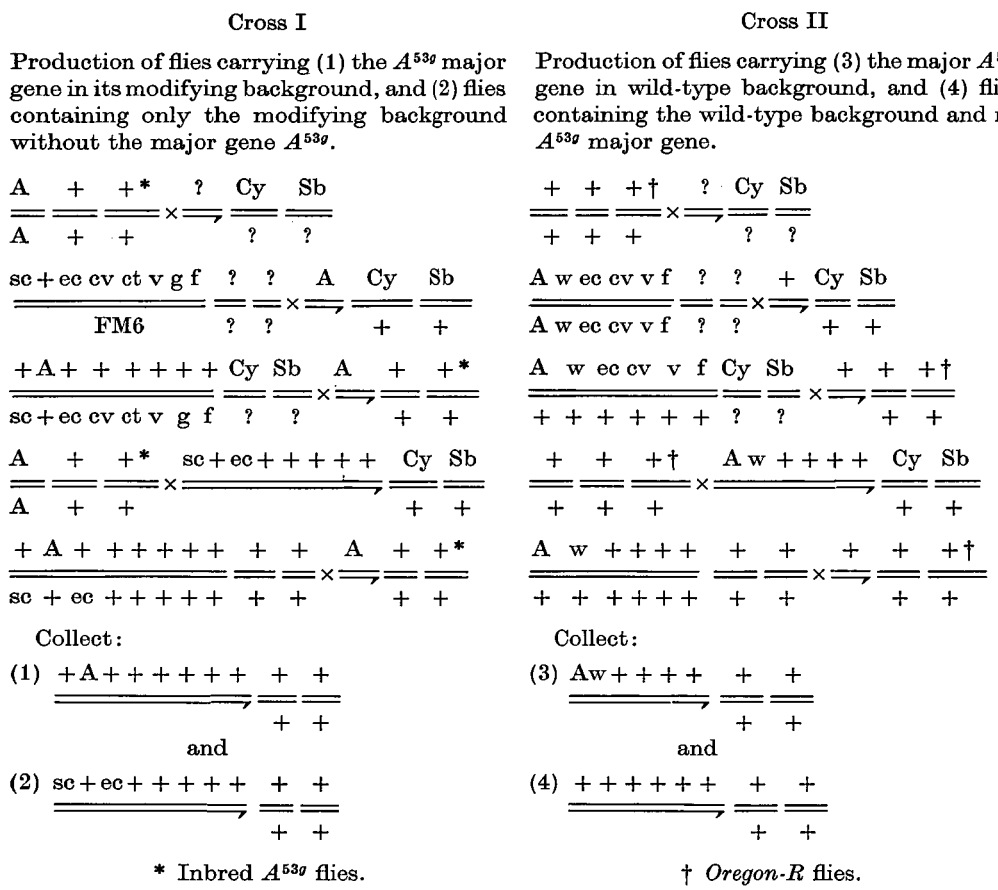

Fig. 1

2. MATERIALS AND METHODS

The A^{53g} stock and the *Oregon-R* (*Ore-R*) wild-type stock used in these experiments have been selected and maintained in the laboratory for several years. All stocks were bred and maintained on a standard corn-meal–karo agar medium in half-pint milk bottles at 25 °C.

The post-microsomal supernatant fraction, which served as a source of tRNA aminoacylating enzymes, and the *Drosophila* tRNA were prepared according to Rose & Hillman (1969). Stripped *E. coli* tRNA was purchased from General Biochemicals, Chagrin Falls, Ohio. *In vitro* aminoacylations were performed according to the method of Rose & Hillman (1969).

To obtain the heterozygote genotypes for study of *in vitro* tRNA aminoacylation, reciprocal crosses were made between the inbred A^{53g} and *Ore-R* stocks. The F_1 generation was collected and separated into groups of males and females which were stored at −50 °C until used as a source of post-microsomal supernatant fluid. Isolation of the A^{53g} region of the X chromosome and the modifying genes on the X chromosome and the autosomes was accomplished through a series of

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crosses with stocks of known marker genes (Fig. 1). The genotypes produced by this series of genetic manipulations were the A^{53g} region in an *Ore-R* background without modifiers, and A^{53g+} in an A^{53g} background which contains the modifying system. Males with known genotypes were collected, stored at -50 °C, and used for study.

Comparisons were made for each amino acid using enzyme preparations from flies which were the same age but which were of different genotypes. Supernatant fractions from each genotype had been prepared concurrently, and comparisons were made using only these concurrently prepared fractions. In all cases absolute values varied but relative activites of the respective fractions were constant. Each comparative result reported was repeated at least three times.

3. RESULTS

To determine if the *in vitro* effect of the A^{53g} genotype at the translation level is due to the *X* chromosome carrying the major gene, the autosomal modifying systems, or the interaction of both, crosses were made between A^{53g} and the *Ore-R* stock which had been shown to have a lower aminoacylating capacity (Rose & Hillman, 1969). The F_1 males were then used to prepare supernatant enzymes. Table 1 shows the results of the reciprocal cross of A^{53g} and *Ore-R* wild-type. In the heterozygous males both leucine and lysine show charging levels intermediate between the values for homozygous *Ore-R* and A^{53g} males. In each case the supernatant enzymes from the heterozygotes with the *X* chromosome from the *Ore-R* parent charge tRNA at a lower level than do the enzymes from the corresponding heterozygotes with the *X* chromosome carrying A^{53g}. The difference between the two heterozygote values gives some indication as to the effect of the mutant *X* chromosome, while the difference between the homozygous values indicates the effect of the mutant *X* chromosome plus the mutant autosomal system. In addition, comparison of values from the *Ore-R* homozygote ($+ \Vert^{+}, + \vert\vert +$), the *Ore-R*:$A^{53g}F_1$ heterozygotes ($A^{53g} \Vert^{+}, + \vert\vert +(A)$ and $+ \Vert^{+}, + \vert\vert +(A)$), and the A^{53g} homozygote ($A^{53g} \Vert^{+}, +(A) \vert\vert +(A)$), indicates that even in the heterozygous condition, the autosomal modifying genes have a stimulatory effect on *in vitro* aminoacylation.

Evidence for an effect of the A^{53g} genotype on the aminoacylating system in

Table 1. *Aminoacylation of* E. coli *tRNA by post-microsomal supernatant fluid prepared from* F_1 *males of the reciprocal cross of* $A^{53g} \times$ Ore-R (*cpm/mg tRNA* $\times 10^{-3}$)

	Genotype			
	+		A^{53g}	
	+	+	+	+(A)
[^{14}C]amino acid	+	+(A)	+(A)	+(A)
Phe	119·5	122·0	117·4	120·1
Lys	167·9	178·3	189·8	259·7
Leu	222·4	227·9	252·7	280·0

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Table 2. *Aminoacylation of* E. coli *tRNA by post-microsomal supernatant fluid prepared from* F_1 *flies of the cross of* $A^{53g} \times$ Ore-R (*cpm/mg tRNA* $\times 10^{-3}$)

[^{14}C]-amino acid	Wild-type ♀	Wild-type ♂	Heterozygote ♀	Heterozygote ♂	A^{53g} ♀	A^{53g} ♂
Glu	8·7	7·4	9·1	10·4	11·6	10·5
Val	55·5	74·5	53·1	72·5	96·3	77·1
Phe	65·7	115·7	88·0	107·8	124·0	125·7
Lys	83·4	161·4	204·1	199·3	432·1	268·0
Leu	2·8	192·1	4·9	213·6	289·8	298·3

A^{53g} females may be found in Table 2. In the cross of A^{53g} females with *Oregon-R* males, supernatant from the homozygous A^{53g} females aminoacylates *E. coli* valyl, phenylalanyl and lysyl tRNA's to a higher level than does that from either the homozygous or heterozygous wild-type. There is an additive effect of the A^{53g} genotype on the aminoacylation of *E. coli* lysyl-tRNA which is also reflected in the action of supernatant derived from males. That this additive effect might in part be an *X* chromosome dosage effect is supported by the aminoacylating lysyl-tRNA ability of supernatant derived from heterozygous males with and without the A^{53g} *X* chromosome (Table 1). Of interest also is the inhibitory effect of the *Oregon-R* homozygous and heterozygous *X* chromosome on the aminoacylation of *E. coli* leucyl-tRNA (Table 2). This inhibition of the aminoacylation of heterologous tRNA is suggestive of an inhibitory action of the wild-type *X* chromosome which is overcome by the presence of the *Y* chromosome. The hypothesis of inhibition by the *X* chromosome is supported by the fact that, excluding glutamic acid, seven of the eight tests of aminoacylating capacity from supernatants of wild-type and heterozygous flies (Table 2) show a reduction in aminoacylating ability of no less than 18·5% by those supernatants obtained from females as compared to those from males. The only exception is the control of lysyl-tRNA charging in the heterozygote where the gene responsible for aminoacylation is hypothesized to be sex-linked.

It is unclear, however, whether the *in vitro* stimulation of aminoacylation by the mutant genotype is due to the sex-linked major gene, to the modifying system, autosomal as well as sex-linked, or to both. To answer this question, genetic crosses were made to put both the mutant major gene and its wild-type allele into the genetic backgrounds of the *Ore-R* and the A^{53g} stocks (Fig. 1). The four classes of male flies collected and tested had the following genomes: A^{53g} in the A^{53g} modifying background; A^{53g} in the *Ore-R* background; A^{53g+} in the *Ore-R* background; and A^{53g+} in the A^{53g} background.

These results (Table 3) show that there is *in vitro* stimulation of aminoacylation by A^{53g} supernatant enzymes utilizing tRNA from *E. coli*, from the A^{53g} flies, and from *Ore-R* flies. In all cases the stimulation is found only in the systems using supernatant fluid prepared from flies containing the A^{53g} modifier genes. The increased aminoacylation therefore is not a function of the major gene, A^{53g}, but rather of the residual genotype which contains the A^{53g} modifier gene system.

Table 3. *Stimulation of* in vitro *tRNA aminoacylation by post-microsomal fluid prepared from* Drosophila melanogaster *males carrying the modifier genes of* A^{53g} (*cpm/mg tRNA* $\times 10^{-3}$)

tRNA source	[^{14}C]amino acid	(A^{53g}, no modifiers)	(A^{53g} plus modifiers)	(A^{53g+}, no modifiers)	(A^{53g+} plus modifiers)
E. coli	Phe	507·9	631·5	550·6	609·2
	Lys	948·7	991·3	946·0	1049·8
	Leu	1348·7	1268·2	1076·7	1202·3
Ore-R	Val	261·7	292·6	260·2	310·8
	Phe	293·9	333·8	302·9	337·0
	Lys	324·9	340·9	296·2	387·3
	Leu	268·5	310·7	251·8	321·1
	Glu	91·6	115·2	85·3	106·7
A^{53g}	Val	296·7	344·9	300·4	362·6
	Phe	294·6	347·5	304·7	352·7
	Lys	279·1	319·2	255·7	306·3
	Leu	302·1	345·5	291·7	358·4
	Glu	117·6	161·5	118·4	137·4

4. DISCUSSION

The investigation of the relationship between modifying genes and the penetrance and expressivity of a particular inherited characteristic is often very complicated. In *Drosophila*, which has been studied extensively with regard to this relationship, Hunt (1969) and Hunt & Burnet (1969) have shown that the properties of the genetic background play a major role in the control of the gene–environment interactions of the *eye-gone* (*eyg*) mutation. Spofford (1956) has studied the role of modifiers in the expression of *eyeless*, and environmental modification of the penetrance and expression of this gene have been reported by Sang & Burnet (1963). In addition, Fraser (1967) has described the systems of modifiers of the scutellar bristles in this insect. In the mouse, Wittman & Hamburgh (1968) have studied the *brachyury* mutation and have suggested that the genetic background of the genotype consists of several factors which independently control the expression of some aspect of the *T* gene.

The genotype being studied here is complex with reference to both the morphological and biochemical phenotypes which it controls. The major gene, A^{53g}, is located distally on the *X* chromosome and a series of modifying genes are located on the *X* chromosome as well as on the autosomes. Essentially, there are two main parts of the genome – the major gene and the array of modifiers which enhance the morphological penetrance and expressivity of A^{53g}. The modifiers alone produce no visible phenotypic effect, and A^{53g} alone produces only a slight abnormality (Hillman, 1973).

The effect of the A^{53g} genome being studied here, that of increased *in vitro* aminoacylation of tRNA, might have been accounted for as a function of the major gene, a property of the modifying system of genes, or a combination of both of these. If the observed effect is due to the influence of the major gene alone and

not to the modifiers, it implies that the A^{53g} gene produces a product which is responsible for the observed *in vitro* results as well as the gross phenotypic alteration. If the *in vitro* observation and the phenotype are causally related (which would be implied if they are controlled by the same genetic unit), the enhancer modifier genes would therefore exert their ultimate influence at a point other than this aspect of gene action; e.g. would cause an increase in the expression and penetrance of A^{53g} at a level other than tRNA aminoacylation.

However, if the modifier genes alone are responsible for the increased aminoacylation of tRNA, the theory could then state that the A^{53g} gene increases its expression and penetrance in the presence of genes which alter the translation process. This would suggest that A^{53g} is a mutant structural gene which is transcribed into a mutant messenger RNA for a specific 'hypodermal' protein which, in the presence of mistranslating machinery, is expressed as a changed or inactive gene product. The translating inefficiency would in this case magnify the error of the mutant messenger RNA. Since the organism is normal phenotypically in every other respect, the implication is that the translation error is small and normally has no phenotypic effect. A phenotypic alteration is seen only when a vast amount of identical protein must be made and used very rapidly. In the case of a protein which is produced in hypodermal cells undergoing a massive period of proliferation, a low level of error would then be magnified and a phenotypic change would ensue.

In the initial experiments which involved testing males from reciprocal wild-type and A^{53g} crosses, both the mutant X chromosome and heterozygous autosomal modifiers stimulated charging of tRNA. This observation indicates that the increased aminoacylation of tRNA in the A^{53g} flies is under the control of a multiple gene system with components located on both the X chromosome and the autosomes. The elimination of the major gene, A^{53g}, from the genetic system responsible for the stimulation of tRNA aminoacylation is clearly indicated by the final experiment in which the major gene and modifier components of the genotype were separated and tested independently of each other. The data show clearly that the increase in tRNA aminoacylation is a function of that portion of the genome which contains the modifier genes. The residual genotype is responsible for either slightly altered aminoacyl tRNA synthetase enzymes or for a substance which causes increased aminoacylation by the synthetase enzymes. This increase in aminoacylation may then be magnified by an alteration in the structure or function of the gene product produced under the direction of the A^{53g} major gene. The combination would finally result in greater penetrance and expressivity of the phenotypically observable mutation.

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