A STUDY OF bobbed MUTANTS INDUCED BY ETHYL-METHANE-SULFONATE IN DROSOPHILA MELANOGASTER

M. Marrakechi and N. Prud'homme
Centre de Génétique Moléculaire du C.N.R.S.
GIF-SUR-YVETTE (91) - France

Received February 24, 1971

SUMMARY :Bobbed mutants induced by Ethyl-Methane-Sulfonate (EMS) fall into two classes :

- One has less DNA capable of hybridizing with wild type ribosomal RNA.
- The other class of mutants retains the same hybridizing potential as the wild-type reference strain.

Two hypothesis are proposed to explain the latter class of mutants.

INTRODUCTION

By using the DNA-RNA hybridizing technique, RITOSSA, ATWOOD and SPIEGELMAN (1) have shown that the region corresponding to the bobbed locus in Drosophila melanogaster is composed of a series of cistrons governing the synthesis of both ribosomal RNA species : 18s and 28s. In all cases examined so far bobbed mutants have shown a diminished amount of DNA capable of hybridizing with wild-type ribosomal RNA. We shall refer to this particular DNA as rDNA. In one case, however, RITOSSA (6) has shown that the limited loss of rDNA suffered by a bobbed mutant (strain Y^{-bb}) was not sufficient to account for it's phenotype ; this mutation behaved phenotypicaly as a total deletion of the bobbed region.

The present work gives the DNA-RNA hybridization results for a number of bobbed mutants induced by EMS.

These results clearly indicate the existance of at least two classes of bobbed mutants :

- mutants of the first are classical in that they show a diminished amount of rDNA.
- mutants of the other contain a number of rDNA cistrons comparable to that of the wild-type reference strain.

MATERIAL AND METHODS

- Origin of strains :
  - Wild-type strain oregon R : Gif collection
  - Strains M-5 ; yvbb ; In Am : Urbana collection
  - Strain In (1) sc^{4L} sc^{8R} : Pasadena collection

Mutants bb^{P1} and bb^{P2} were obtained by N. PRUD'HOMME in Urbana during her stay at Dr. SPIEGELMAN's laboratory.

They were induced by EMS on the double inversion M-5.

- Selection of bobbed mutants

The experimental technique is that described by LIM and SNYDER (2). EMS was used in a 1 % saccharose solution at a concentration of 0,025 M. $+/Y^+$ or M-5 B^+/Y^+ male flies were treated.

- RNA labeling :

Labeled RNAs were extracted solely from wild-type Oregon R larvae. Growth medium composition is as follows :

- 0.8 g Corn flour
- 0.6 g Saccharose
- 0.1 g Agar
- 27 mg methyl-p-hydroxybenzoate
- aprox. 0.5 g of yeast

in 10 ml of water.

The yeast strain used is a uracil requiring strain previously grown on a minimal medium containing 10 mCi of H^3 uracil per liter. In addition to the labelled yeast 4 mCi of H^3 uridine are added per 10 ml of the drosophila growth medium. Purified ribosomal RNA has a specific radioactivity of about 30.000 c.p.m. µg. All counts are made in a low yield (15 to 20 %) scintillation spectrometer (Nuclear Chicago Scintillation Spectometer).

- RNA extraction and purification :

The techniques used are those discribed previously by RITOSSA and SPIEGELMAN (3).

- DNA extraction and purification :

DNA is extracted from adults. The methods are those discribed by RITOSSA and al. (3) with the following modifications: after being submitted to the action of pancreatic ribonuclease and α amylase , the extract is treated overnight with pronase. This treatment is followed by two successive deproteinisations

with chloroform-isoamyl alcohol (24/1 v/v). After precipitation by alcohol the DNA is resuspended in a 0.1 M phosphate at pH 6,8 and run through a hydroxyapatite column (4).

The final solution at a concentration of 50 to 150 Y/ml is dialysed against 0,1 SSC for 24 hours at 4°C.

- DNA-RNA hybridization :

All hybridization experiments performed on nitrocellulose filters (5). Denatured DNA (3) is dissolved in 6,6 SSC at a concentration varying from 8 to 15 Yper ml. Fixation on the filter was insured by incubating at 60°C for twelve hours. Hybridization was performed at 65°C during twelve hours in a final volume of 1 ml of 2 SSC.

RESULTS

The following table shows the phenotype of differents EMS induced bobbed mutants and their respective hybridization potential expressed as the percent of DNA hybridized with wild-type rRNA at saturation. Each value is the mean of results obtained on 4 to 7 filters ; all expériments were repeated twice.

The mutants fall into two classes :

- bb^{P5} and bb^{P6}, the phenotypes of which are correlated with the amount of hybridizable rDNA, constitute one class.

- the other is made up of mutants bb^{P2}, bb^{P3} and bb^{P4} that have retained an amount of rDNA comparable to that of the wild-type reference strain, and of mutant bb^{P1} the extreme phenotype of which cannot be explained simply in terms of rDNA defficiency. These mutations are lethal in association with Y^{-bb}. Homozygous females are also lethal with the exception of bb^{P2}/bb^{P2} homozygotes which have a viability of about 5 % and are sterile. The additive effect of mutations bb^{P3} and bb^{P4} is slight or non-existent in association with yvbb. This is also the case for bb^{P1} in association with either yvbb, uco$_3$bb or Y^{B^S} bb. Mutation bb^{P2}, however, has a notable additive effect in association with these same bobbed mutations.

DISCUSSION

Mutations bb^{P5} and bb^{P6} confirm the hypothesis that bobbed mutants may be due to a partial deficiency of rDNA. (RITOSSA et al) (1).

Mutations bb^{P1} shows a slight deficiency when compared to the wild-type. This defficiency is not sufficiently large however to account for the severe phenotype. This mutation behaves like that of one of the Y^{-bb} strains studied by RITOSSA (6) ; it's rDNA appears to be functionally inert.

Mutations bb^{P2} bb^{P3} and bb^{P4} retain the same amount of rDNA as the reference strain. The bobbed phenotype could be due in these cases to the production of a qualitatively or quantitatively modified rRNA.

In the case of a qualitative modification we can imagine that a few cistrons only are modified, which are essential for a given developmental stage. Such a mutation should complement with most of the classical bobbed mutants (defficient in rDNA) yielding wild-type or near wild-type. However all these mutants tested in association with yvbb (0,069 % of hybridization) gave no complementation response or only a slight one ; this rules out our hypothesis, unless we suppose that the bobbed mutation (yvbb) partialy damages these essential cistrons.

On the other hand a qualitative modification of rRNA might involve an alteration of the majority of cistrons minute enough not to hinder their hybridization with wild-type rRNA. That EMS in itself could have produced such mutationnal events as those required in this hypothesis seems improbable but the hypothesis of a Master-Slave gene organisation put forward by CALLAN (7) may offer an explanation : mutations bb^{P5} and bb^{P6} would correspond to a decrease in the number of slave cistrons and mutations bb^{P2}, bb^{P3} and bb^{P4} to a modification of the master. As for mutation bb^{P1} it could be considered as corresponding to a modification of the master gene accompanied by a decreased number of slaves.

In the case of a quantitative modification of rRNA the effect of mutations bb^{P1}, bb^{P2}, bb^{P3} and bb^{P4} could be explained by assuming that all rRNA coding cistrons have a common linked regulator and that the mutations have affected this region.

A more detailed analysis of these different mutants and in particular the study of the rRNA produced by the viable bb^{P2} homozygotes might allow us to discriminate between these hypothesis and may serve as a first step in the understanding of the mecanisms governing gene expression at the bobbed locus in D. melanogaster.

Estimate of rDNA content of EMS induced mutants

Genotype	Phenotype	rDNA x 100 / total DNA	haploid Genotype	haploid** Phenotype	rDNA x 100 / total DNA
In (1) sc^{4L} sc^{8R}/Y^{Bs}	(+)	0,145	Y^{Bs}	(+)	0,145
In Am/In Am	(+)	0,337	In Am	(+)	0,168
M-5 B^{+}/Y^{Bs}	(+)	0,276	M-5 B^{+}	(+)	0,126
bb^{P5}/Y^{Bs}	(+)	0,200	bb^{P5}	severe bb^{a}	0,055
bb^{P6}/Y^{Bs}	(+)	0,23	bb^{P6}	light bb^{b}	0,085
bb^{P2}/In Am	(+)	0,310	bb^{P2}	lethal	0,142
bb^{P2}/bb^{P2}	Sublethal	0,290	bb^{P2}	lethal	0,145
bb^{P1}/In Am	(+)	0,265	bb^{P1}	lethal	0,097
bb^{P3}/In Am	(+)	0,295	bb^{P3}	lethal	0,127
bb^{P4}/In Am	(+)	0,316	bb^{P4}	lethal	0,148

* Hybridizable DNA per haploid genotype as deduced from values in colum 1 and 3.

** $X^{bb}/0$ and X^{bb}/Y^{-bb} male phenotype.

a : light bb = short bristles.

b : severe bb = short bristles, abnormal abdomen, low fertility.

Financial assistance from the "Commissariat à l'Energie Atomique" is gratefully acknowledged.

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