Screen the modifier for the expression of eyg
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Abstract

Eye gene (eyg) is a gene expressing at the equatorial region of the eye in Drosophila. Therefore, there is supposed to exist some regulatory genes that can suppress eyg expression in the other region of eye, and also some genes enhancing eyg expression in the equatorial region. We used the chemical mutagens, EMS (ethyl methanesulfonate), to induce point mutation in the genome of fly, and then screen for the repressor and the activator of eyg expression. Fifteen lines were collected. These were divided to three groups. The first group showed the reduction of eye size. The second group showed black scars on the eye. The third group were homozygous lethal in female. In addition, most of the lines were homozygous lethal.

Introduction

Regulation of gene expression is an important mechanism in development biology. In general, the expression of a gene can be regulated by several repressor and activator proteins. eyg is a gene on the third chromosome and expresses at the equatorial region of fly eye, shows spatially regulated expression.

In order to screen the modifiers of the expression of eyg, we used the fly stock y w, eyFLP, FRT-40A, eyg⁰. Males carrying an FRT site at position 40A were mutagenized with 25mM EMS and crossed to females carrying the same FRT site, and FLP recombinase driven by the eyg¹ promoter on X chromosome. Flies were screened in the next generation for different patterns of eye pigmentation.

If one of the repressors of eyg is mutated by EMS, a deepened red color in the equatorial region, or a red spot in the non-equatorial region of eye may be found. Likewise, if one of the activators is mutated, a lightened red color of the equatorial region may be found. Because the FRT is at position 40A, only the mutations on the left arm of the second chromosome were screened.

Fifteen mutant lines were collected after screening about 12,360 flies. These were divided into three phenotype groups.

Materials and Methods

Genetics

FLP recombinase is an enzyme that can recognize FRT, a length of DNA sequence, and make recombination at the FRT site. eyFLP is the FLP driven by eyg¹ promoter on the X chromosome. Because the fly stock y w, eyFLP, FRT-40A, eyg⁰ is used, so if any mutations exist on the left arm of the second chromosome, the mutation will be homozygous in the eyes and heterozygous in the other region. Therefore, we can see the mutant phenotype of eyes, while the phenotype of body is normal.

Screening

y w, eyFLP, FRT-40A, eyg⁰ males were treated by 25mM EMS over night and then crossed to virgin carrying the same genotype. To amplify the number of flies, we set 30 crossed and transferred them everyday for 10 days so that we can screen 300 vials, about 12,360 flies. In the F1 generation, 33 mutant flies were found, and 15 of them were germ-line transmission.

Screening Scheme

After screening about 12,360 flies, we set 15 lines. The descriptions of the phenotype are shown in this form.

Fig.1 eyg gene expresses at equatorial region of eye. (A-B) Normal phenotype of Eqg(yig)⁰ (C-F) 22, shows the lightened Eq phenotype

Fig.2 The mutations cause the reduction of eye size in this group, and the reductions are different from each other. (A-B) 3-31 (C) 2-39 (D) 3-47 (E) 1-58 (F) 1-79

Fig.3 The mutations cause the black scar on eyes, but not all of eyes have the scar. The size and position of the scars are variable. (A-C) 20 (D-F) 20

Fig.4 The females in this group are homozygous lethal, and males shows lightened Eq or normal phenotype. (A-B) 2-41 (C) 2-37 (D) 3-63 (E) F 6-3 (All males)

Result

Mating Scheme

<table>
<thead>
<tr>
<th>Mother</th>
<th>Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>eyg¹</td>
<td>eyFLP</td>
</tr>
<tr>
<td>yw FRT-40A eyg⁰</td>
<td>yw FRT-40A eyg⁰</td>
</tr>
<tr>
<td>eyg¹</td>
<td>eyFLP</td>
</tr>
</tbody>
</table>

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Summary

1. Do the complementation test to see if the lines in the same group are the mutations of the same gene.
2. Use the deficiency kit to confirm the position of the mutations.
3. Check the interaction of the modifiers of eyg and find out the regulation system of eyg gene in fly development.

Future Work

1. Do the complementation test to see if the lines in the same group
2. Use the deficiency kit to confirm the position of the mutations.
3. Check the interaction of the modifiers of eyg and find out the regulation system of eyg gene in fly development.

References