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piRNA-mediated transposon regulation and the germ-line mutation rate in *Drosophila melanogaster* males

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ABSTRACT

Transposons, especially retrotransposons, are abundant in the genome of *Drosophila melanogaster*. These mobile elements are regulated by small RNAs that interact with the Piwi family of proteins—the piwi-interacting or piRNAs. The Piwi proteins are encoded by the genes *argonaute3* (*ago3*), *aubergine* (*aub*), and *piwi*. Heterochromatin Protein 1 (HP1), a chromatin-organizing protein encoded by the *Suppressor of variegation 205* [*Su(var)205*] gene, also plays a role in this regulation. To assess the mutational impact of weakening the system for transposon regulation, we measured the frequency of recessive X-linked lethal mutations occurring in the germ lines of males from stocks that were heterozygous for mutant alleles of the *ago3*, *aub*, *piwi*, or *Su(var)205* genes. These mutant alleles are expected to deplete the wild-type proteins encoded by these genes by as much as 50%. The mutant alleles of *piwi* and *Su(var)205* significantly increased the X-linked lethal mutation frequency, whereas the mutant alleles of *ago3* did not. An increased mutation frequency was also observed in males from one of two mutant *aub* stocks, but this increase may not have been due to the *aub* mutant. The increased mutation frequency caused by depleting Piwi or HP1suggests that chromatin-organizing proteins play important roles in minimizing the germ-line mutation rate, possibly by stabilizing the structure of the heterochromatin in which many transposons are situated.

1. Introduction

It is a truism that mutation is the source of genetic variation for evolutionary change. Yet, because many mutations are harmful, too much mutation can impose an intolerable genetic load on a population [1–3]. It is therefore not surprising that organisms possess a plethora of mechanisms to minimize mutational damage: proofreading polymerases, biochemical pathways to detoxify or eliminate potential mutagens, and an assortment of systems to repair damaged DNA or to prevent its propagation [4]. In addition, some organisms have developed ways of repressing the potentially mutagenic activity of transposable genetic elements. In *Drosophila melanogaster*, this repression is mediated by small RNA molecules derived from these elements [5–7]. The small RNAs target transposon mRNAs and either bring about their destruction or prevent them from functioning as templates for polypeptide or cDNA synthesis. They may also help to reorganize chromatin in a way that represses transposon activity [8].

The small RNAs involved in transposon regulation have been called repeat-associated (ras) RNAs or Piwi-interacting (pi) RNAs [6,7,9,10]. The latter term derives from the fact that these RNAs are found in complexes that contain members of the Piwi family of proteins. In *D. melanogaster*, these proteins are encoded by the autosomal genes *agonaute3* (*ago3*), *aubergine* (*aub*) and *piwi*. Flies carrying mutant alleles of these genes might be expected to have a reduced ability to regulate transposable elements. Indeed, mutant alleles of *aub* and *piwi* have been shown to disrupt the regulation of one type of transposon, the *P* element [8,11–13,22]. Unregulated *P*-element activity leads to a syndrome of germ-line abnormalities called hybrid dysgenesis [14]. This syndrome includes frequent mutation, chromosome breakage and a temperature-dependent form of sterility that arises from the failure of the germ line to develop.

Mutant alleles of another autosomal gene, *Suppressor of variegation 205* [*Su(var)205*], also disrupt *P*-element regulation [12,15,16]. However, the product of this gene, a protein known as

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Heterochromatin Protein 1 (HP1), has not been directly implicated in piRNA synthesis or transport. Rather, HP1 plays an important role in chromatin organization. This protein is found in the pericentric heterochromatin, at some euchromatic loci, and at telomeres where it appears to cap the ends of chromosomes [17,18]. Cytological evidence indicates that mutant alleles of *aub* and *piwi* alter the location of HP1 and other chromatin-organizing proteins in the chromosomes of somatic cells [19], and biochemical evidence indicates that the Piwi protein interacts physically with HP1 [20]. Chromatin-organizing proteins such as HP1 may influence transposon behavior by regulating the expression of piRNA loci or by regulating the expression or transposition of the transposons themselves, possibly in collaboration with piRNAs. Thus, HP1 may act either upstream or downstream of the piRNA pathway [8].

Given the de-repressing effect of mutant *aub*, *piwi* and *Su(var)205* alleles on *P* elements, we might expect that these alleles would de-repress other transposons as well, increasing the amount of transposon activity in the genome. The genetic consequence of this activity would be an increase in the germ-line mutation rate. We test this idea by estimating the frequency of new recessive lethal mutations occurring on X chromosomes from mutant *aub*, *piwi* and *Su(var)205* stocks. We also estimate the frequency of lethals occurring on X chromosomes from mutant *ago3* stocks. The *Drosophila* genome contains about 100 different families of transposable elements, most of them retrotransposons. This paper is the first attempt to assess the mutational impact of weakening the system that keeps these elements in check.

The basic experimental technique derives from H.J. Muller's classic study of X-linked lethal mutations [21]. Individual X chromosomes are extracted from males from the various mutant stocks and a control stock, and then manipulated by using a marked balancer X chromosome to determine how often the extracted chromosomes have acquired new, recessive lethal mutations. The advantage of the technique is that it permits an investigator to detect mutations in any of the large number of vital loci on the X chromosome. Comparisons among the frequencies of new lethals should reveal if the mutation rate varies among the stocks tested. Since its inception, this method has been used to test various factors such as radiations, chemicals, and genotypes for mutagenicity. Here we use it to determine if mutant *ago3*, *aub*, *piwi*, and *Su(var)205* alleles increase the germ-line mutation rate in males.

Our application of Muller's method has several important qualifications. First, because mutant alleles of *ago3*, *aub*, *piwi*, and *Su(var)205* are either lethal or sterile in homozygous condition, we could only test these alleles for an effect on the X-linked mutation rate in males that were heterozygous for them. However, previous work has shown that some of these alleles disrupt *P*-element regulation through heterozygous effects [11,13,15,16,22,23]. This disruption is apparently due to a reduction in the amount of wild-type protein caused by mutating one copy of the relevant gene. Thus, it seemed possible that mutational depletion of these wild-type proteins might also disrupt the regulation of other transposons. Second, to focus on these other transposons, we used stocks that did not have any transposase-encoding *P* elements. Without the transposase, *P* elements are quiescent. Thus, any new X-linked lethal mutations would not be due to *P*-element activity. Third, although we screened for lethal mutations occurring in the germ lines of males, it is possible that these mutations were influenced by the effects of heterozygous mutant alleles in the mothers of these males—that is, by maternal effects. Indeed, some of the mutant alleles we studied have been shown to enhance *P*-element activity through maternal effects [11,13,16,23]. Fourth, not all X-linked lethal mutations that occur in males will be detected because some may jeopardize the survival or function of the hemizygous cells that carry them. This caveat is especially relevant for mutations that occur during the development of the germ line, when transposons are likely to be active. Selection against these lethals might make it difficult to detect an increase in the mutation rate arising from a weakening of transposon regulation. However, using Muller's method it has been possible to document an increased X-linked lethal mutation rate in the male germ line when regulation of one transposon family—the *P* elements—was compromised [24,25].

The data we present demonstrate that heterozygous mutant alleles of *piwi* and *Su(var)205* increase the X-linked lethal mutation rate, whereas mutant alleles of *ago3* and *aub* do not. These findings indicate that the chromatin-organizing protein HP1 and its interacting partner Piwi play important roles in minimizing the mutation rate.

2. Materials and methods

2.1. Drosophila stocks and husbandry

The genetic markers and balancer chromosomes are described in Lindsley and Zimm [26], the Flybase website, or references cited in the text. The X chromosome that was screened for new recessive lethal mutations—that is, the target X chromosome—carried an incomplete *P* element, called *TP5*, which is situated in the telomere associated sequences (TAS) at the chromosome's left end, and a null allele of the *white* eyes (*w*) gene. Because of these two features we symbolize the target X chromosome as *TP5 w*. The *TP5* element evokes a state that represses *P*-element movement; however, because no transposase-producing *P* elements were present in any of the stocks used in these experiments, the repressing function of *TP5* is irrelevant.

The *TP5 w* target X chromosome was crossed into stocks heterozygous for mutations in the *ago3* (alleles *t1* and *t3*), *aub* (alleles *QC42* and *ΔP-3a*), *piwi* (alleles *1* and *2*), or *Su(var)205* (allele *04*) genes, all of which are either lethal or sterilizing when homozygous. The *aub*, *piwi*, and *Su(var)205* mutations, located on chromosome II, were balanced with *CyO*, and the *ago3* mutations, located on chromosome III, were balanced with *TM3*, *Sb Ser*. The *TP5 w* target X chromosome was also crossed into a stock heterozygous for the II-linked mutation *Gla*, which has not been implicated in any aspect of transposon regulation; this stock therefore served as a control.

Stock and experimental cultures were reared on a cornmeal-molasses-yeast-agar medium in 95 mm × 25 mm flat-bottomed shell vials at 25 °C. X-linked lethal test cultures were reared on a sugar-yeast-agar medium [24] in 100 mm × 13 mm round-bottom tubes on a split temperature regime (7 days at 21 °C followed by 8 days at 25 °C) to enhance the efficiency of the screening process.

2.2. Scheme to detect X-linked, recessive lethal mutations

The mutation detection scheme (Fig. 1) employed the *FM7* balancer X chromosome to keep the *TP5 w* target X chromosome intact—that is, free of recombination with another X chromosome. This balancer carries *B* (*Bar*-shaped eyes), a dominant marker that permits easy tracking of the chromosome through crosses, as well as y^{31d}, a recessive allele of the *yellow* body gene, and sn^{x2}, a recessive female-sterilizing allele of the *singed* bristle gene. Because of the latter, the *FM7* balancer was maintained in heterozygous condition with *Ins(1)*sc^7 *+AM,* sc^7 w^a ptg^4 *l(1)78 h* [24], an X chromosome with two inversions, the recessive lethal mutation *l(1)78 h*, and three recessive visible mutations, sc^7 (*scute* bristles), w^a (*white-apricot* eyes) and ptg^2 (*pentagon*, affecting body pigmentation). For simplicity, we denote this partner of the *FM7* balancer chromosome as w^a.

In the P generation of the mutation detection scheme, single *TP5 w* males from stocks heterozygous for a mutant allele of the *ago*, *aub*, *Gla*, *piwi*, or *Su(var)205* genes—denoted, in general, as