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Perspective

Do the BEAF insulator proteins regulate genes involved in cell polarity and neoplastic growth?

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ABSTRACT

It was reported that a chromosome with the *BEAF*^{NP6377} (*NP6377*) allele leads to a loss of cell polarity and neoplastic growth in *Drosophila melanogaster* when homozygous (Gurudatta et al., 2012). We had previously generated the *BEAF*$^{AB\text{-}KO}$ (*AB-KO*) allele by homologous recombination and did not note these phenotypes (Roy et al., 2007). Both alleles are null mutations. It was unclear why two null alleles of the same gene would give different phenotypes. To resolve this, we performed genetic tests to explore the possibility that the chromosome with the *NP6377* allele contained other, second site mutations that might account for the different phenotypes. We found that the chromosome with *NP6377* has at least two additional mutations. At least one of these, possibly in combination with the *NP6377* allele, is presumably responsible for the reported effects on gene expression, cell polarity and neoplastic growth.

Results and discussion

The evidence that both *AB-KO* and *NP6377* are null alleles of *BEAF* is strong. The *BEAF* gene encodes two 32 kDa proteins, BEAF-32A and BEAF-32B (Hart et al., 1997). These two proteins are encoded by unique 5′ exons, and share a 3′ exon encoding the C-terminal two-thirds of the proteins. The *AB-KO* allele was made by targeted gene replacement and has point mutations eliminating the AUG start codons for 32A and 32B and placing two tandem stop codons in the shared exon. The presence of these mutations was confirmed, and neither 32A nor 32B protein are detectable on Western blots of *AB-KO* animals (Roy et al., 2007). *NP6377* is a null allele caused by insertion of a P[w^+] transposon into the 5′ end of the coding sequences of the shared exon (FlyBase, 2008; Hayashi et al., 2002). BEAF protein cannot be detected on Western blots of *NP6377* animals (Gurudatta et al., 2012).

Two types of genetic tests are commonly used to determine if phenotypes are due to a particular mutation when homozygous. These tests are important because when a chromosome with an allele of interest is homozygous, any second site mutations that are present would also be homozygous. One type of test uses transgenes to see if the phenotypes can be rescued. For instance, phenotypes associated with the *AB-KO* allele can be rescued by a *BEAF* transgene (Roy et al., 2007). The other type of test is to place the allele of interest over an independently derived mutation in the same gene (or a chromosomal deficiency that deletes the gene). If the mutations were independently derived, the chromosomes they are on are unlikely to share the same second site mutations. Results of these tests on the chromosome with the *NP6377* allele are reported below.

First, we attempted to rescue the chromosome with the *NP6377* allele with two different *BEAF* transgenes. Neither attempt was successful, as shown in Table 1. The chromosome with the *NP6377* allele is recessive lethal at the larval stage. Larval lethality was not rescued by either a 5 kb genomic fragment encompassing *BEAF* or a transgene encoding GFP-tagged 32A and 32B proteins; no *NP6377*/*NP6377* flies were obtained. In line with previous results (Roy et al., 2007), parallel crosses using the *AB-KO* allele with the same transgenes resulted in rescue. This provides evidence that the lethality reported by Gurudatta et al. (Gurudatta et al., 2012) is due to one or more second site mutations on the *NP6377* chromosome.

Next, we tested for viability when the chromosome with the *NP6377* allele was over the chromosome with the *AB-KO* allele. Results presented in Table 2 provide further evidence that the chromosome with the *NP6377* allele has at least one recessive lethal mutation that is not *NP6377*. It is possible to obtain flies homozygous for the *AB-KO* allele. Homozygous males are fertile, while homozygous females are nearly sterile (Roy et al., 2007). When *AB-KO*/*CyO* females were crossed with *AB-KO*/*AB-KO* males, 37% of the progeny had the *AB-KO*/*AB-KO* genotype (straight wings). In line with our previously reported results, the *AB-KO*/*AB-KO* females laid very few eggs and no adults were obtained from crosses to *AB-KO*/*AB-KO* males. In contrast, all flies from *inter se* crosses of *NP6377*/*CyO GFP* flies had curly wings, indicating recessive lethality. When *NP6377*/*CyO GFP* virgin females were

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Table 1
Test for rescue of chromosomes with *BEAF* mutations by *BEAF* transgenes.

Parents		Progeny		N flies
Female	Male	% Cy	% Straight	
NP6377/CyO; gBF.3C/gBF.3C	*NP6377/CyO; gBF.3C/gBF.3C*	100.0	0.0	288
AB-KO/CyO; gBF.3C/gBF.3C	*AB-KO/CyO; gBF.3C/gBF.3C*	63.7	34.6[a]	214
NP6377/CyO; GFBF.3C/+	*NP6377/CyO; GFBF.3C/+*	100.0	0.0	376
AB-KO/CyO; GFBF.3C/+	*AB-KO/CyO; GFBF.3C/+*	63.7	36.3[b]	193

The *gBF.3C* transposon is on the third chromosome and contains a 5 kb genomic fragment encompassing *BEAF*.
The *GFBF.3C* transposon is on the third chromosome and contains a transgene encoding GFP-tagged BEAF-32A and BEAF-32B proteins.

[a] *AB-KO/AB-KO; gBF.3C/gBF.3C* flies were maintained as a viable stock.
[b] *AB-KO/AB-KO; GFBF.3C/+* flies were maintained as a viable stock, but lost viability if the *GFBF.3C* chromosome was lost.

Table 2
Test for viability of chromosomes with the *NP6377* allele and the *AB-KO* allele.

Parents		Progeny		Number of flies
Female	Male	% Cy	% Straight	
NP6377/CyO GFP	*NP6377/CyO GFP*	100.0	0.0	210
AB-KO/CyO	*AB-KO/AB-KO*	63.1	36.9[a]	260
NP6377/CyO GFP	*AB-KO/AB-KO*	52.5	47.5[b]	217

[a] *AB-KO/AB-KO* females laid very few eggs, none of which gave rise to adults.
[b] *NP6377/AB-KO* flies are viable. They have survived over 25 generations, and appear robust.

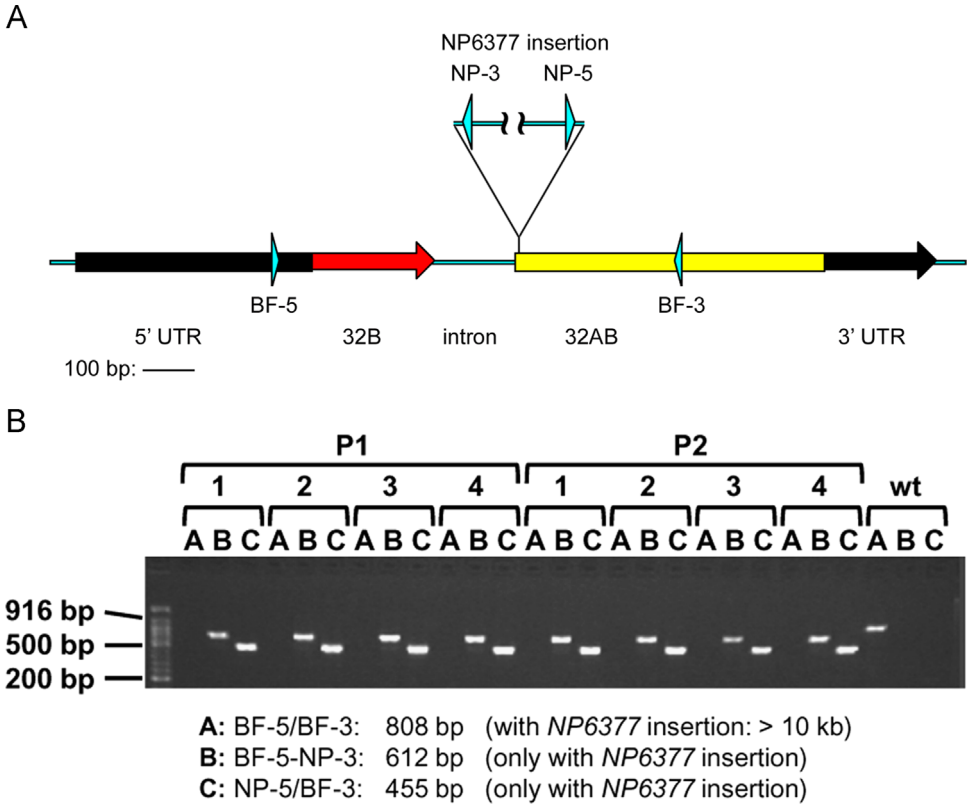

Fig. 1. *Adult flies homozygous for the NP6377 allele can be obtained after meiotic recombination with the chromosome with the AB-KO allele.* (A) Schematic of a 1800 bp region of the *BEAF* gene showing the locations of the *NP6377* transposon insertion and the primers used for PCR. (B) Single fly PCR demonstrates that the *NP6377* allele is homozygous. Two parental males (P1 and P2) from a *NP6377/AB-KO* stock were individually crossed to *CyO/Sp[1]* females. In both cases, all progeny had pigmented eyes indicating that P1 and P2 were homozygous for the *NP6377* allele. *NP6377/CyO* males and virgin females from each were crossed to establish fly lines. DNA was isolated from four individual flies that lacked the *Cy* phenotype from both stocks, as well as from a fly with a wild-type *BEAF* gene. All four flies from P1 and P2 gave the expected PCR products with the BF-5/NP-3 and NP-5/BF-3 primer pairs, but no product with the BF-5/BF-3 primer pair (note that a product > 10 kb would not be produced under the PCR conditions used). The opposite was found for the wild-type fly. This demonstrates that the *NP6377* allele can be separated from the recessive lethal allele originally present on the chromosome.

crossed to *AB-KO*/*AB-KO* males, nearly 50% of the progeny had an *NP6377*/*AB-KO* genotype (straight wings). Since both *AB-KO* and *NP6377* are null alleles, the recessive lethality of the chromosome with the *NP6377* allele must be due to a mutation other than *NP6377*.

Unexpectedly, *NP6377*/*AB-KO* flies are viable. These flies have survived over 25 generations and appear healthy. This suggested that the *NP6377* allele might be a hypomorph. However, as previously reported (Gurudatta et al., 2012), we were unable to detect even truncated forms of BEAF on Western blots of homozygous *NP6377* larvae (or *NP6377*/*AB-KO* flies). Subsequent results suggest that, in addition to the recessive lethal mutation, the chromosome with the *NP6377* allele has a dominant mutation that suppresses the female fertility defect caused by a lack of BEAF protein. By meiotic recombination between the chromosomes with the *NP6377* allele and the *AB-KO* allele, fertile male and female *AB-KO*/*AB-KO* flies have been obtained. It should be noted that the *AB-KO* and *NP6377* alleles can be distinguished by eye color since the *NP6377* insertion is P[w^+]. PCR followed by restriction digestion was used to confirm that the *AB-KO* allele is homozygous (data not shown), as previously described (Roy et al., 2007). These flies have been maintained for over 20 generations, and appear healthy. This is in contrast to the original chromosome with the *AB-KO* allele, which resulted in nearly sterile females when homozygous. As mentioned above, the female sterility is rescued by *BEAF* transgenes, indicating that the sterility is due to the *AB-KO* allele.

In addition, *NP6377*/*NP6377* flies have been obtained. This has been confirmed by PCR (Fig. 1). One line, established from a single fly as described in the legend to Fig. 1, also gives rise to fertile homozygous females and has survived for over 20 generations. The homozygous *NP6377* larvae do not exhibit the phenotypes described by Gurudatta et al. (Gurudatta et al., 2012). This supports our conclusion that there is a dominant mutation that suppresses the fertility defect, and confirms that the recessive mutation that causes larval lethality can be separated from the *NP6377* mutation.

It does not appear that the same mutation is responsible for recessive lethality and dominant suppression of the fertility defect of mutant *BEAF* flies. If that were the case, then fertile females and at least some males in the homozygous *AB-KO* and *NP6377* stocks would be heterozygous for the second mutation. As a result, some larvae in these stocks would be homozygous for the second mutation. They would not pupate and would exhibit neoplastic growths. This is not observed, all larvae look normal, pupate and eclose.

Taken together, our results indicate that the original chromosome with the *NP6377* allele contains at least two additional mutations. Surprisingly, one is a dominant mutation that suppresses the female fertility defect caused by a lack of BEAF. After meiotic recombination with the chromosome with the *AB-KO* allele, flies homozygous for either *AB-KO* or *NP6377* can be maintained as viable stocks. Larvae from the homozygous *NP6377* stock do not have the phenotypes described by Gurudatta et al. The other mutation is recessive lethal. The original chromosome with the *NP6377* allele cannot be rescued with *BEAF* transgenes capable of rescuing the *AB-KO* allele, and it is viable over the chromosome with the *AB-KO* allele. Therefore the *BEAF* mutation is not the primary cause of the altered gene expression, larval lethality, defects in cell polarity, and neoplastic growth reported by Gurudatta et al. (Gurudatta et al., 2012). While the *BEAF* mutation might contribute, the primary cause is one or more second site mutations on the same chromosome.

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