Research Notes

Disruption of Drosophila melanogaster dmbrca2 (CG30169) affects rates of female meiotic crossing over.

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Introduction

The breast cancer susceptibility loci BRCA1 and BRCA2 have been the most intensively studied human cancer susceptibility genes, and both are implicated in the repair of double-strand breaks in DNA by homologous recombination (e.g., Venkitaraman, 2002; Gudmundsdottir and Ashworth, 2006; Nagaraju and Scully, 2007). BRCA2 has sequence motifs, termed "BRC repeats" (Bork et al., 1996), interspersed within it that bind to RAD51 recombinase (Pellegrini et al., 2002), the eukaryotic ortholog of RecA which promotes DNA repair and homologous recombination. As a result of its interaction with Rad51, mutants for BRCA2 exhibit lower recombination in human cell lines, and the specific interaction of BRCA2 with Rad51 appears critical for the regulation of homologous recombination (Xia et al., 2001). However, researchers still struggle to determine how BRCA2 coordinates its Rad51- and ssDNA-binding activities to facilitate the orderly transfer of Rad51 onto DNA (but see Shivji et al., 2006). To this end, Pelligrini and Venkitaraman (2004) suggested that "primitive organisms harbouring a simpler version of the BRCA2 protein will provide useful model systems."

Studies in Drosophila melanogaster have contributed greatly to our understanding of homologous recombination (see e.g., Champion and Hawley, 2002; Blanton and Sekelsky, 2004), and this species may be useful for examining the function of BRCA2. Early attempts to identify a homolog for BRCA2 in D. melanogaster were unsuccessful (Fortini et al., 2000; Rabinow, 2002; Warren et al., 2002). However, when sequence fingerprints representing key residues for BRCA2–RAD51 interactions were examined, a putative homolog for BRCA2 in D. melanogaster was identified: the transcript corresponding to CG30169 (Lo et al., 2003). Unlike human BRCA2, D. melanogaster CG30169 bears only three BRC repeats, suggesting it harbors a simpler version of the protein. No Gene Ontology (GO) data are available for CG30169, though an insertion putatively upstream of it seems to result in high ovulation (Ejima et al., 2004), and CG30169 was found to interact with spindle-B in a yeast-two-hybrid assay (Giot et al., 2003). As spnB encodes a component of the RAD52 DNA repair pathway (Ghabrial et al., 1998), and given the known interaction of RAD51 and RAD52, this finding further implicates D. melanogaster CG30169 as a potentially useful model system for studying BRCA2 and the BRC-RAD51 interaction in particular.

Recently, Brough et al. (2008) and Klovstad et al. (2008) reported detailed functional analyses of CG30169 (dubbed "dmbrca2") in DNA repair in cell lines, in the male pre-meiotic germline and in the female meiotic germline (the last via association with an eggshell patterning defect). They observed that mutation of dmbrca2 disrupts double-strand break repair by homologous recombination gene conversion, causing a relative increase in single-strand annealing repair in the male pre-meiotic germline. Additionally, cells deficient for dmbrca2 were less viable after irradiation than control cells, suggesting that the deficiency increases sensitivity to DNA damage. An examination of eggshell patterning defects suggested that dmbrca2 is associated with meiotic double-
strand break repair and activation of the meiotic recombination checkpoint. Finally, the studies noted that the product of dmbrca2 physically interacts with both Rad9 and spnA, the D. melanogaster Rad51, and the proteins are recruited to nuclear foci after DNA damage. Many of these observations match the role of BRCA2 in human double-strand break repair (see review in Wang, 2007).

In this study, we tested for a role of dmbrca2 in determining the rate of D. melanogaster female meiotic crossing over (there is no meiotic crossing over in Drosophila males). Such a role is predicted given the proposed effects of this gene on meiotic DNA repair (Klovstad et al., 2008), but this effect was not directly tested. We used progeny from a cross between a line in which dmbrca2 transcription was disrupted via P-element insertion to examine the differences in crossover rate associated with having 0, 1, or 2 functional copies of this gene.

Methods

Strains used in this study were D. melanogaster Zimbabwe 30, stock number 13272 bearing a P-element disruption in region immediately upstream of the coding region of dmbrca2 (y[1] w[67c23]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}CG30169[KG03961]), and stock number 9068 bearing a deletion of the CG30169 region (w[1118]; Df(2R)ED4061, P{w[+mW.Scer\FRT.hs3]=3'.RS5+3.3'}ED4061/SM6a (deletion 60C8;60D13)). For simplicity, we refer to these stocks as "Z30", "P-dmbrca2", and "del".

We first crossed P-dmbrca2 females to Z30 males. Offspring females were backcrossed to P-dmbrca2 males. The offspring of this backcross possessed one of four genotypes at dmbrca2: A) homozygous for the P-element insertion, B) heterozygous for the P-element insertion and the Cy balancer, C) heterozygous for the P-element insertion and the Z30-derived region, and D) heterozygous for the Cy balancer and the Z30-derived region (see Table 1). We sequenced and identified a nonsynonymous SNP distinguishing Z30 from the other lines within dmbrca2 (GenBank accessions EU541213-EU541214), and this SNP was used in conjunction with the curly wing phenotype to identify which of the four genotypes at dmbrca2 each female bore. Type A putatively bore no functional copies of dmbrca2, types B and C bore one functional copy, and type D bore two functional copies. Additionally, we performed some single-fly RNA preparations (Bertucci and Noor, 2001) and used reverse transcription and PCR to examine expression of dmbrca2 in a subset of adult females from the four classes above (primer sequences available in GenBank accessions).

Each backcross female was paired singly with a P-dmbrca2 male and allowed to lay eggs for approximately 7 days prior to genotyping to assign to the four categories above. To assay rates of meiotic crossover, the offspring of the genotyped females were genotyped for two microsatellite loci (DMC30B8 and X8312980gt: see http://i122server.vu-wien.ac.at/ Microsatellite%20Loci/X.xls) that distinguished the Z30 and P-dmbrca2 lines. These loci are X-linked and separated by over 6.25 megabases.

Finally, to confirm that our results were not an artifact of another factor on the P-dmbrca2 chromosome, we crossed P-dmbrca2 to the del line. Wild-type female offspring from this cross putatively bore no functional copies of dmbrca2 (like category A above, also confirmed by RT-PCR). These females were then crossed to P-dmbrca2 males, and offspring were again genotyped for the two microsatellite loci to assay rates of meiotic crossover.

Results and Discussion

We were unable to amplify dmbrca2 transcript from females putatively bearing no functional copies of dmbrca2, but successfully amplified this transcript from all other classes of flies. This difference suggests that the P-element in P-dmbrca2 disrupts normal expression of dmbrca2 and
supports our assignments on the number of functional copies of the gene borne by females from each category.

Table 1 presents the crossover rates measured from females of each of the four categories. Most females of genotype A produced no progeny, consistent with previous reports of female sterility associated with disruptions of this gene (Klovstad et al., 2008). However, a handful of females produced a very small number of surviving offspring. Crossover rates were significantly lower in category A females than in B, C, or D females (Fisher’s exact test p < 0.01 in each case). However, crossover rates between B, C, and D females did not differ significantly (p > 0.5 in each case).

To confirm that the reduced recombination was related to the dmbrca2 gene region, we also assayed rates of crossing over in females heterozygous for the P-element in dmbrca2 and a deletion of this region. Again, most such females failed to produce offspring, but 12 females produced some progeny and were scored. Crossover rates in P/del females were significantly lower than those observed in category B, C, and D females from the previous study (Fisher’s exact test p < 0.0001 in each case) but not significantly different from recombination in females homozygous for the P-element insertion (group A, p > 0.5).

We conclude that crossing over is severely reduced in females bearing no functional copies of dmbrca2 relative to females bearing one or two functional copies. Consistent with results from studies of mitotic and meiotic DNA repair functions of dmbrca2, our results further suggest that dmbrca2 is required for normal female meiotic crossing over in Drosophila melanogaster. We cannot exclude the possibility that the P-element insertion line bore polymorphism at another nearby locus that reduced recombination, but this seems a less parsimonious explanation for the results observed, since a recombinational role was also documented in studies of human BRCA2 (see Introduction). In sum, our findings further demonstrate the potential utility of this system as a simple model for uncovering details of the interaction of Rad51 and the BRC-repeats of BRCA2 in mediating homologous recombination, and more generally, novel roles for BRCA2 during development.

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Caffeine supplementation increases mortality in female *Drosophila melanogaster* without reproductive or metabolic impairment.

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Caffeine (1,3,7-trimethylxanthine) is a naturally occurring stimulant substance that is extracted from over 60 sources and used in common comestibles. It is mainly found in substances such as coffee, tea, and energy drinks, but also may be found in cold medications, chocolate, and analgesics (Benowitz, 1990).

Caffeine antagonizes adenosine receptors that affect cellular concentration of cyclic AMP and triggers the release of norepinephrine, dopamine, and serotonin in the nervous system (Fredholm, 1985). Additionally, caffeine has been shown to stimulate thermogenesis by enhancing noradrenaline release, therefore, increasing metabolic rate in rodents and humans (Acheson *et al.*, 1980; Bukowiecki *et al.*, 1983; Cheung *et al.*, 1988; Astrup *et al.*, 1991). Furthermore, caffeine consumption results in an increased heart rate and blood pressure (Belza *et al.*, 2007). Caffeine has also been shown to possess antioxidant activity (Devasagayam *et al.*, 1996) along with ergogenic properties (Keisler and Armsey, 2006).

In a previous study, *Drosophila prosaltans* treated with caffeine demonstrated a dose-dependent decrease in fecundity (Itoyama and Bicudo, 1992). Furthermore, caffeine has been shown to inhibit mating frequency and copulation duration in the same model organism (Itoyama *et al.*, 1995). Additionally, after ten generations of caffeine treatment, longevity was significantly reduced in male and female *Drosophila prosaltans* (Itoyama *et al.*, 1998).

The main objective of this study was to evaluate the effect of caffeine on mortality, fecundity, and metabolic rate in *Drosophila melanogaster*.

**Materials and Methods**

**Caffeine**

Stock solution for each dose was prepared and mixed into yeast paste. The calculated doses reflect the final concentration of caffeine in yeast paste that flies consumed during the experiment. Caffeine was obtained from the Sigma-Aldrich manufacturer. For each assay, three doses of caffeine were compared to a control. We evaluated caffeine at the following doses: low (0.008mg/ml), medium (0.08 mg/ml) and high (0.8mg /ml). Adult flies, starting at day 1 were supplemented with caffeine at these doses.