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Expression profile analysis of *menin1* mutants.

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The absence of the tumor suppressor Menin results in multiple endocrine neoplasia type I (MEN1) in humans (reviewed by Agarwal *et al.*, 2004). *Drosophila* Menin is encoded by *mnn1* (Guru *et al.*, 2001). We assayed the effect of *mnn1* loss-of-function and *mnn1* over expression on adult flies and embryos by microarray experiments and found remarkably little change in expression correlated with *mnn1* genotype, suggesting that *mnn1* has very little effect on gene expression under the tested conditions.

Mammalian Menin is a classic tumor suppression protein, where tumors occur in individuals heterozygous for loss-of-function alleles.   Additionally Menin is required for embryonic development, as homozygous embryos die showing hemorrhages and defective neural tube closure (reviewed by Agarwal *et al.*, 2004). In contrast, the *Drosophila Menin1* gene (*mnn1*) is not required for viability, but appears to alter response to stress according to two somewhat incongruent reports (Papaconstantinou *et al.*, 2005; Cerrato *et al.*, 2006). Flies mutant for *mnn1* are also sensitive to mutagens, suggesting that *Drosophila* Menin plays a role in DNA repair (Busygina *et al.*, 2004; Busygina *et al.*, 2006). There is abundant evidence suggesting that Menin interacts with transcription factors (reviewed by Agarwal *et al.*, 2004), including AP1 in both *Drosophila* and mammals (Cerrato *et al.*, 2006). In *Drosophila*, thoracic closure and eye development are particularly susceptible to lowered or raised *mnn1* expression in the context of AP1 mis-expression (Cerrato *et al.*, 2006). In mammals, Menin is a subunit of a Trithorax Complex that methylates Histone H3 at Lysine 4 (Hughes *et al.*, 2004; Yokoyama *et al.*, 2004; Chen *et al.*, 2006; Scacheri *et al.*, 2006). Trithorax complexes typically regulate large batteries of genes, and have especially well studied roles in the regulation of HOX genes (Schuettengruber *et al.*, 2007). Indeed, mammalian Menin is a regulator of at least one HOX gene (Hughes *et al.*, 2004; Yokoyama *et al.*, 2004). Interestingly, even though Menin is bound at many active promoters in mammalian cells, the loss of Menin has very little effect on transcription (Scacheri *et al.*, 2006). To determine if Menin has a general role in transcription in *Drosophila*, we assayed global gene expression in flies lacking or over expressing relative to wild type flies.

We isolated mRNA from *y w; mnn1*$^{\Delta46}$ and *y w; mnn1*$^{\Delta79}$ (both are protein null alleles on *mnn1*) adult flies, as well as mRNA from the isogenic *y w; mnn1*$^{+84}$ and *y w; mnn1*$^{+113}$ flies (precise excision lines of the P-element used to generate the null alleles), another *y w* line, and mRNA from heterozygous combinations of *mnn1* alleles.   These samples were labeled and hybridized to Affymetrix DrosGenome1 arrays. Gene expression profiles for both females and males were performed, for a total of eleven hybridizations were performed and are available from the Gene

Expression Omnibus (GEO) (Edgar *et al.*, 2002). Replicate hybridizations from different samples showed very good reproducibility ($R^2 \geq 0.98$).

We observed very few significant differences in expression between the *mnn1*⁻ flies and the remaining *mnn1*⁺ or *mnn1*⁺/ *mnn1*⁻ flies. There was a greater effect of *mnn1*⁻ loss-of-function on males. Following false discovery rate correction after Benjamini-Hochberg (Hochberg and Benjamini, 1990), two genes are differentially expressed in *mnn1*⁻ mutants relative to wild type at an adjusted p-value cutoff < 0.01. Both of these genes (*Act88F* and *Acp26Aa*) show lower expression in *mnn1*⁻ males based on array data. No statistically significant differential expression was found between *mnn1*⁻ and *mnn1*⁺ females.

At lower confidence, there are more changes in differential expression. One must be more cautious in using these changes as a basis for further experiments, but several genes might be targets for follow-up work. For example, when we simply observed fold changes, and selected genes at least 2-fold differentially expressed relative to the isogenic control, thirty genes showed 2-fold differences in one of the sexes. Among these genes, Gene Ontology annotation (Harris *et al.*, 2004) for immune response and antibacterial response were significantly enriched ($p << 0.01$). We did not determine the response of *mnn1* mutants on pathogen challenge. Even if all of the genes showing 2-fold differential expression are true direct or indirect targets of Menin, these data suggest that Menin has a limited role in transcriptional regulation in adult flies.

Consistency of the response is also a valuable criterion for follow-up work. Only four genes showed 2-fold differences between *mnn1*⁻ and *mnn1*⁺ in both females and males. The *CG11909* gene showed greater expression in *mnn1*⁻ flies, while the *CG9682*, *Jon99Fi*, and *CG18404* genes showed poorer expression in *mnn1*⁻ flies. While we have no reason to suspect a sex-biased role for *mnn1*⁺, sex-biased expression in *Drosophila* is known to be extensive (Zhang *et al.*, 2007). However, these results do not clearly identify a particular non-sex-biased process that responds to Menin.

We over-expressed Menin using the UAS-Gal4 system with the *How24-GAL4* driver (Brand and Perrimon, 1993). This results in defective thoracic closure (Cerrato *et al.*, 2006). An over-expression of Menin in *How24>mnn1*⁺ was detected in embryos by western blotting (not shown). We therefore collected and aged embryos to assay expression at 2-4, 4-6, 6-8, and 2-16 hrs post egg deposition. As controls, samples from two embryos having only the *How24-GAL4* driver or only the *UAS-mnn1*⁺ were also collected for each. Expression was assayed using 2-color FlyGEM arrays (Johnston *et al.*, 2004) with dye-flip replicates between each *How24>mnn1*⁺ and control at each time point. As was observed in adults, there was little differential expression observed that correlated with the *mnn1* genotype in embryos. The only gene showing a time-course change in gene expression consistent with Menin over expression (beginning in 4-6 hr embryos) and the sole significantly differentially expressed gene (FDR adjusted $p < 0.01$) was *white*. Given that Gal4 expression might be expected to increase the expression of the *white*⁺ marker in the *UAS-mnn1*⁺ construct, it is quite likely that this is simply a surrogate marker for *mnn1* expression (We did not detect increased expression of *mnn1* transcripts, which we suggest is due to poor performance of the array element for *mnn1*). Briefly, over expression of Menin during embryogenesis does not appear to have a major effect on gene expression.

As previously reported in mammalian cells (Scacheri *et al.*, 2006), our data suggest that *mnn1* has little effect on transcription under standard conditions. This is somewhat enigmatic for a subunit of a major transcriptional regulation complex.

Materials and Methods

Drosophila mutants, and driver UAS pairs, and standard growth conditions have been previously described (Cerrato *et al.*, 2006). Labeling and hybridization were performed with

standard Affymetrix (Santa Clara, California) protocols by the NIDDK microarray core facility (http://genomics.niddk.nih.gov/array.shtml), or performed as previously described (Johnston *et al.*, 2004).

For Affymetrix experiments, intensities were extracted, normalized, and summarized by robust multi-array average (RMA) (Irizarry, 2003) using the Bioconductor affy package (Irizarry *et al.*, 2003; Gentleman *et al.*, 2004). Differential expression in *mnn1⁻* mutants compared to wild type was then calculated using Linear modeling using the limma package (Smyth, 2004), with p-values adjusted by the Benjamini and Hochberg method (Hochberg and Benjamini, 1990). Fold-change analyses are from comparisons between mutant strains and isogenic controls.

For FlyGEM experiments, array data were normalized within slides by print-tip loess followed by quantile between-array normalization using the limma package (Smyth, 2004). Fold-change comparisons were performed at each time-point and for each control. Significance measures were calculated using linear modeling as with the Affymetrix arrays, and p-values extracted for mutant vs. control contrasts for each sex separately.

All expression data are available in GEO at these accession numbers: GSM239567, GSM239568, GSM239569, GSM239570, GSM239571, GSM239572, GSM239573, GSM239574, GSM239575, GSM239577, GSM239578, GSM238950, GSM238954, GSM238956, GSM238957, GSM238958, GSM238959, GSM238960, GSM238961, GSM238962, GSM238964, GSM238965, GSM238966, GSM238968, GSM238969, GSM238970, GSM238971, GSM238972, GSM238973.

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Quantitative courtship acts of 3LA inversion homo- and heterokaryotypes of *Drosophila ananassae*.

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The relationship between inversions, morphological traits and fitness characters has been well documented in *Drosophila*. However, the relationship between inversion and behavioural traits has not been studied. Therefore, the present study has been carried out to find out the effect of inversions on courtship acts in *Drosophila ananassae*. Homozygous 3LA inversion stock of *D. ananassae* was established from the females collected at a semi-domestic locality of Mysore, India. This stock was maintained at 22ºC ±1ºC and relative humidity of 70% for ten generations. Before starting the experiments, the inversion heterokaryotypes were generated by crossing males with homokaryotic inversion with normal female or vice versa. When the progeny appeared, the virgin females and bachelor males were isolated, kept separately, aged for five days and used for observation of courtship behaviour. The courtship behaviour of males and females was observed by confining one male and a female of a given type in an Elens-Wattiaux mating chamber. A total of fifteen pairs of the following combinations were studied: a. both male and female heterokaryotypic; b, male homokaryotypic and female heterokaryotypic; c, both male and female homokaryotypic; and d, male heterokaryotypic and female homokaryotypic. The courtship elements were quantified following the procedure of Hegde and Krishna (1997). Following courtship elements such as tapping, scissoring, vibration, circling, licking, ignoring, extruding and decamping were analyzed. The data gathered were subjected to one way ANOVA.

In the present study, it is noticed that, except tapping and wing vibration, courtship acts such as scissoring, circling, licking, ignoring, extruding and decamping were lesser in crosses involving homokaryotypic male and female than in crosses involving heterokaryotypic male and females with 3LA inversion. This suggests that the inversion heterokaryotypes perform greater courtship acts than homokaryotypes. Studies have shown that flies which perform greater courtship acts during courtship