12-2005

JIL-1 kinase, a member of the male-specific lethal (MSL) complex, is necessary for proper dosage compensation of eye pigmentation in Drosophila

Stephanie Lerach
Iowa State University

Weiguo Zhang
Iowa State University

Huai Deng
Iowa State University

Xiaomin Bao
Iowa State University

Jack Girton
Iowa State University, bpatch@iastate.edu

See next page for additional authors

JIL-1 kinase, a member of the male-specific lethal (MSL) complex, is necessary for proper dosage compensation of eye pigmentation in Drosophila

Abstract
The upregulation of the JIL-1 kinase on the male X chromosome and its association with the MSL complex suggest that JIL-1 may play a role in regulating dosage compensation. To directly test this hypothesis we measured eye pigment levels of mutants in the X-linked white gene in an allelic series of JIL-1 hypomorphic mutants. We show that dosage compensation of wa alleles that normally do exhibit dosage compensation was severely impaired in the JIL-1 mutant backgrounds. As a control we also examined a hypomorphic white allele we that fails to dosage compensate in males due to a pogo element insertion. In this case the relative pigment level measured in males as compared to females remained approximately the same even in the most severe JIL-1 hypomorphic background. These results indicate that proper dosage compensation of eye pigment levels in males controlled by X-linked white alleles requires normal JIL-1 function.

Keywords
JIL-1 kinase, dosage compensation, eye pigmentation, MSL complex, Drosophila

Disciplines
Biochemistry, Biophysics, and Structural Biology | Genetics | Molecular Genetics

Authors
Stephanie Lerach, Weiguo Zhang, Huai Deng, Xiaomin Bao, Jack Girton, Jorgen Johansen, and Kristen M. Johansen

Published in final edited form as:
Genesis. 2005 December ; 43(4): 213–215. doi:10.1002/gene.20172.

THE JIL-1 KINASE, A MEMBER OF THE MSL COMPLEX, IS NECESSARY FOR PROPER DOSAGE COMPENSATION OF EYE PIGMENTATION IN *DROSOPHILA*

Stephanie Lerach, **Weiguo Zhang**, **Huai Deng**, **Xiaomin Bao**, **Jack Girton**, **Jørgen Johansen**, and **Kristen M. Johansen**
Department of Biochemistry, Biophysics, and Molecular Biology Iowa State University Ames, Iowa 50011

Abstract

The upregulation of the JIL-1 kinase on the male X chromosome and its association with the MSL complex suggest that JIL-1 may play a role in regulating dosage compensation. To directly test this hypothesis we measured eye pigment levels of mutants in the X-linked *white* gene in an allelic series of *JIL-1* hypomorphic mutants. We show that dosage compensation of w^a alleles that normally do exhibit dosage compensation was severely impaired in the *JIL-1* mutant backgrounds. As a control we also examined a hypomorphic *white* allele w^e that fails to dosage compensate in males due to a *pogo* element insertion. In this case the relative pigment level measured in males as compared to females remained approximately the same even in the most severe *JIL-1* hypomorphic background. These results indicate that proper dosage compensation of eye pigment levels in males controlled by X-linked *white* alleles requires normal JIL-1 function.

Keywords

JIL-1 kinase; dosage compensation; eye pigmentation; MSL complex; *Drosophila*

We have recently characterized a novel tandem kinase in *Drosophila*, JIL-1, that associates with the chromosomes throughout the cell cycle, localizes specifically to the gene-active interband regions of larval polytene chromosomes, and phosphorylates histone H3S10 (Jin et al., 1999; Wang et al., 2001; Zhang et al., 2003). Analysis of *JIL-1* null and hypomorphic alleles showed that *JIL-1* is essential for viability and that reduced levels of JIL-1 protein lead to a global change in chromosome structure (Wang et al., 2001; Deng et al., 2005). However, JIL-1 is also found at two-fold higher levels on the male X chromosome and associates with the male specific lethal (MSL) dosage compensation complex (Jin et al., 2000). The MSL complex is required for the necessary hypertranscription of genes on the male X chromosome for dosage compensation in flies (reviewed in Meller & Kuroda, 2002). This enhanced transcription is thought to arise from MSL complex-induced histone H4 acetylation generating a more open chromatin structure (Smith et al., 2000). The upregulation of JIL-1 protein on the male X chromosome, JIL-1's association with the MSL complex, and the higher level of male lethality associated with hypomorphic *JIL-1* alleles (Wang et al., 2001) all suggest that JIL-1 may play a role in regulating dosage compensation. However, so far evidence for this hypothesis has only been correlative. Therefore, to directly examine whether JIL-1 is involved in male upregulation of genes whose increased expression levels depend upon the dosage compensation complex we have

CORRESPONDENCE: Kristen M. Johansen Department of Biochemistry, Biophysics, & Molecular Biology 3154 Molecular Biology Building Iowa State University Ames, Iowa 50011 ph. (515) 294-7959; fax. (515) 294-4858; kristen@iastate.edu.

compared the eye pigment levels controlled by X-linked *white* alleles in males and females in different *JIL-1* hypomorphic mutant backgrounds.

In these studies we have taken advantage of the properties of two alleles of the *white* locus, *white[apricot]* (w^a) and *white[eosin]* (w^e). The w^a allele was created by the insertion of a *copia* transposon that results in an overall reduction of red pigment in the eyes of both male and female flies (Zachar & Bingham, 1982). Males carrying the w^a allele retain the ability to dosage compensate for the *white* gene, and they show roughly equivalent expression to that of the female carrying two copies of w^a (Zachar & Bingham, 1982; this study). This is in contrast to the hypomorphic w^e allele which has lost the ability to dosage compensate in males (Smith & Lucchesi, 1969). This mutation is due to insertion of a *pogo* element into the *Doc* element present in the w^1 allele (O'Hare et al., 1991). The w^a and w^e alleles were chosen for this study because they are both hypomorphic alleles with comparable pigment levels and because their eye pigments have maximum absorbance at the same wavelength. In the experiments the w^a and w^e alleles were crossed into different *JIL-1* mutant backgrounds that combined hypomorphic and null *JIL-1* alleles ($JIL\text{-}1^{z28}$, $JIL\text{-}1^{z60}$, and $JIL\text{-}1^{z2}$) in order to generate progeny producing different amounts of JIL-1 protein. The $JIL\text{-}1^{z28}$ allele is a weak hypomorph producing 45% the normal level of wild-type JIL-1 protein, the $JIL\text{-}1^{z60}$ allele is a strong hypomorph producing only 0.3% of wild-type JIL-1 protein levels, whereas the $JIL\text{-}1^{z2}$ allele is a true null and homozygous animals do not survive to adulthood (Wang et al., 2001; Zhang et al., 2003). The $JIL\text{-}1^{h9}$ allele expresses a truncated JIL-1 protein that lacks part of the second kinase domain and the entire COOH-terminal domain and acts as a strong hypomorph (Zhang et al., 2003). The $JIL\text{-}1^{z60}/JIL\text{-}1^{z60}$ and $JIL\text{-}1^{z2}/JIL\text{-}1^{z60}$ allelic combinations are semi-lethal and only a few eclosed animals from large scale crosses could be analyzed. To compare eye pigment levels between male and female flies we performed pigment assays essentially as in Ashburner (1989) using sets of 10 pooled fly heads of each genotype. It should be noted that the *z* series of *JIL-1* mutations carry the ry^{506} allele which modifies pigment color (Rørth et al., 1998) wherefore the optical density measurements cannot be directly compared between these alleles, wild-type, and heteroallelic combinations with $JIL\text{-}1^{h9}$. However, this difference does not affect the determination of the relative optical density of pigment levels in males and females of the same genotype.

The results show that as JIL-1 levels decrease in an allelic series of *JIL-1* mutants the male to female pigment ratio of w^a eye pigment decreases (Figure 1A and Table 1). In a wild-type *JIL-1* background the pigment ratio was 1.00; however, this ratio was only 0.64 in the most severe hypomorphic *JIL-1* heteroallelic combination ($JIL\text{-}1^{z2}/JIL\text{-}1^{h9}$). This difference was statistically significant ($p<0.005$, Student's t-test). Thus, these results indicate that males were unable to properly dosage compensate in the absence of normal levels of JIL-1 protein. However, that the ratio was not reduced to 50% suggests that partial dosage compensation can still occur possibly due to the low level of JIL-1 activity that was necessary to allow eclosion or the presence of a parallel pathway independent of JIL-1. Interestingly, consistent with previous observations of a clear maternal effect of *JIL-1* (Zhang et al., 2003) dosage compensation was most affected in *JIL-1* mutant heteroallelic combinations where the most severely hypomorphic allele was maternally provided (Table 1).

It has been demonstrated that the effect of loss of JIL-1 on chromatin structure of the male X chromosome is qualitatively different from that of the autosomes and the female X chromosome (Deng et al., 2005). Therefore, to control for effects of the *JIL-1* hypomorphic mutations specific to the male X chromosome, but unrelated to dosage compensation mechanisms, we examined the male/female pigment ratio in the eyes of w^e flies. As shown in Figure 1B and Table 2 w^e males do not dosage compensate and have only about half (46%) the pigment level of w^e females (see also Smith & Lucchesi, 1969). This ratio was not

significantly changed in hypomorphic *JIL-1* mutant backgrounds (Figure 1B and Table 2) suggesting that *JIL-1* did not have a differential effect on *white* expression in males as compared to females unrelated to the function of the dosage compensation machinery. Furthermore, these data suggest that while the optical density measurements of pigment expression levels varied somewhat in the different mutant backgrounds this did not affect the determination of the male/female pigmentation ratios in flies of the same genotype. Thus, our results strongly indicate that JIL-1 function is required for proper dosage compensation at the *white* locus in *Drosophila*. In future experiments it will be informative to further explore how the interaction between JIL-1 and the MSL complex may contribute to regulation of the dosage compensation machinery.

MATERIALS AND METHODS

Drosophila stocks and crosses

Fly stocks were maintained according to standard protocols (Roberts, 1998). Oregon-R was used for wild-type preparations. The $JIL\text{-}1^{z2}$, $JIL\text{-}1^{z28}$, $JIL\text{-}1^{z60}$, and $JIL\text{-}1^{h9}$ alleles are described in Wang et al. (2001) and in Zhang et al. (2003). The *z* series of *JIL-1* mutations carry the ry^{506} allele (Rørth et al., 1998). Balancer chromosomes and markers are described in Lindsley & Zimm (1992). Strains containing either the w^a or the w^e allele on the X chromosome and a loss-of-function *JIL-1* allele (either $JIL\text{-}1^{z2}$, $JIL\text{-}1^{z60}$, $JIL\text{-}1^{z28}$, or $JIL\text{-}1^{h9}$) heterozygous with the *TM6 Sb Tb e* third chromosome balancer were produced by standard crossing. Subsequent crosses between these strains generated flies with different *JIL-1* allelic combinations in w^a or w^e backgrounds.

Eye pigment assays

For pigment level comparisons adult flies were collected from the respective crosses at eclosion, aged 6 days at 25°C, frozen in liquid nitrogen, and stored at −80°C until assayed. The pigment assays were performed essentially as in Ashburner (1989) using sets of 10 fly heads of each genotype collected from males and females, respectively. For each sample the heads from the 10 flies were homogenized in 125 μl of chloroform and 125 μl 0.1% ammonium hydroxide, centrifuged, and the optical density (OD) of the supernatant spectrophotometrically measured at a wavelength of 365 nm. In order to obtain a measure of the relative pigments levels in male and female eyes the OD determined for male flies was divided by the OD determined for female flies of the same genotype.

Acknowledgments

We thank members of the laboratory for discussion, advice, and critical reading of the manuscript. We also wish to acknowledge Ms. V. Lephart for maintenance of fly stocks and Mr. Laurence Woodruff for technical assistance. This work was supported by NIH Grant GM62916 (KMJ).

REFERENCES

Ashburner, M. *Drosophila*. A Laboratory Manual. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, New York: 1989.

Deng H, Zhang W, Bao X, Martin JN, Girton J, Johansen J, Johansen KM. The JIL-1 kinase regulates the structure of *Drosophila* polytene chromosomes. Chromosoma. 2005; 114:173–182. [PubMed: 15986206]

Jin Y, Wang Y, Walker DL, Dong H, Conley C, Johansen J, Johansen KM. JIL-1: A novel chromosomal tandem kinase implicated in transcriptional regulation in *Drosophila*. Mol Cell. 1999; 4:129–135. [PubMed: 10445035]

Jin Y, Wang Y, Johansen J, Johansen KM. JIL-1, a chromosomal kinase implicated in regulation of chromatin structure, associates with the male specific lethal (MSL) dosage compensation complex. J Cell Biol. 2000; 149:1005–1010. [PubMed: 10831604]

Lindsley, DL.; Zimm, GG. The Genome of *Drosophila melanogaster*. Academic Press; New York: 1992.

Meller VH, Kuroda MI. Sex and the single chromosome. Adv Genet. 2002; 46:1–24. [PubMed: 11931222]

O'Hare K, Aalley MR, Cullingford TE, Driver A, Sanderson MJ. DNA sequence of the *Doc* retrotransposon in the *white-1* mutant of *Drosophila melanogaster* and of secondary insertions in the phenotypically altered derivatives *white-honey* and *white-eosin*. Mol Gen Genet. 1991; 225:17–24. [PubMed: 1705654]

Roberts, DB. *Drosophila*: A Practical Approach. IRL Press; Oxford: 1998.

Rørth P, Szabo K, Bailey A, Laverty T, Rehm J, et al. Systematic gain-of-function genetics in *Drosophila*. Development. 1998; 125:1049–1057. [PubMed: 9463351]

Smith PD, Lucchesi JC. The role of sexuality in dosage compensation in Drosophila. Genetics. 1969; 61:607–618. [PubMed: 17248430]

Smith ER, Pannuti A, Gu W, Seurnagel A, Cook RG, Allis CD, Lucchesi JC. The *Drosophila* MSL complex acetylates histone H4 at lysine 16, a chromatin modification linked to dosage compensation. Mol Cell Biol. 2000; 20:312–318. [PubMed: 10594033]

Wang Y, Zhang W, Jin Y, Johansen J, Johansen KM. The JIL-1 tandem kinase mediates histone H3 phosphorylation and is required for maintenance of chromatin structure in *Drosophila*. Cell. 2001; 105:433–443. [PubMed: 11371341]

Zachar Z, Bingham PM. Regulation of *white* locus expression: the structure of mutant alleles at the *white* locus of *Drosophila melanogaster*. Cell. 1982; 30:529–541. [PubMed: 6291773]

Zhang W, Jin Y, Ji Y, Girton J, Johansen J, Johansen KM. Genetic and phenotypic analysis of alleles of the Drosophila chromosomal JIL-1 kinase reveals a functional requirement at multiple developmental stages. Genetics. 2003; 165:1341–1354. [PubMed: 14668387]

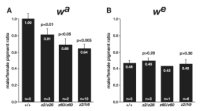

Figure 1.

JIL-1 regulates dosage compensation at the *white* locus in males. (A) The ratio of male/female eye pigment levels in an allelic series of *JIL-1* hypomorphs (comprised of the alleles $JIL\text{-}1^{z28}$, $JIL\text{-}1^{z60}$, $JIL\text{-}1^{h9}$, $JIL\text{-}1^{z2}$) in a w^a mutant background. In w^a flies a *copia* retrotransposon causes a general reduction of pigment level in both sexes without interfering with the dosage compensation process. The decrease in male/female eye pigment ratios correlates directly with the severity of the *JIL-1* hypomorphic alleles. (B) In w^e mutant flies there is no dosage compensation due to a *pogo* element insertion. In this mutant background there is no significant effect of *JIL-1* hypomorphic alleles on male/female eye pigment ratios. Each histogram represents the average male/female pigment ratio from sets of OD_{365} measurements from 10 pooled fly heads with S.D. The average male/female pigment ratio in *JIL-1* mutants were compared to that in a wild-type *JIL-1* background using a Student's t-test.

Table 1

Dosage compensation of eye pigment in w^a flies in *JIL-1* mutant backgrounds

genotype[a]	sex	n[b]	OD_{365} with S.D	male/female pigment ratio	Student's t-test
+/+	female	5	0.416±0.033	1.00	
+/+	male	5	0.414±0.022		
z28/z28	female	3	0.220±0.018	0.98	p>0.35
z28/z28	male	3	0.216±0.028		
z2/z28	female	3	0.254±0.041	0.81	p<0.01
z2/z28	male	3	0.205±0.016		
h9/z2	female	6	0.441±0.019	0.71	p<0.005
h9/z2	male	6	0.315±0.020		
z60/z60	female	2	0.224±0.046	0.69	p<0.05
z60/z60	male	2	0.154±0.016		
z60/z2	female	4	0.312±0.027	0.68	p<0.005
z60/z2	male	4	0.212±0.012		
z2/h9	female	10	0.438±0.020	0.64	p<0.005
z2/h9	male	10	0.279±0.019		

[a] Genotype of the third chromosome with maternal contribution listed first. In addition, all flies are homozygous (females) or hemizygous (males) for w^a on the X chromosome. The *z* series of *JIL-1* mutations also carry the ry^{506} allele.

[b] Each pigment concentration was determined from 10 pooled fly heads.

Table 2

Dosage compensation of eye pigment in w^e flies in *JIL-1* mutant backgrounds

genotype[a]	sex	n[b]	OD_{365} with S.D.	male/female pigment ratio	Student's t-test
+/+	female	5	0.738±0.080	0.46	
+/+	male	5	0.340±0.143		
z2/z28	female	5	0.361±0.030	0.49	p>0.20
z2/z28	male	5	0.176±0.009		
z60/z60	female	1	0.241	0.43	
z60/z60	male	1	0.104		
z2/h9	female	6	0.376±0.030	0.45	p>0.30
z2/h9	male	6	0.168±0.029		

[a] Genotype of the third chromosome. In addition, all flies are homozygous (females) or hemizygous (males) for w^e on the X chromosome. The *z* series of *JIL-1* mutations also carry the ry^{506} allele.

[b] Each pigment concentration was determined from 10 pooled fly heads.