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BRIEF REPORT

The lamin Dm_0 allele *Ari3* acts as an enhancer of position effect variegation of the w^{m4} allele in *Drosophila*

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Abstract The association of lamin and lamin binding proteins with peripheral heterochromatin suggests the possibility that lamins may influence gene expression by participating in the epigenetic regulation of chromatin stucture. To test this hypothesis we have examined the effect of a recently generated partial loss-of-function lamin Dm_0 allele *Ari3* on PEV of the w^{m4} allele in the *Drosophila* eye. The Lam^{Ari3} allele is characterized by a truncation of the COOH-terminal domain and lacks the CaaX box that localizes lamin to the inner nuclear membrane. We show that the Lam^{Ari3} allele strongly increased silencing of w^{m4} expression, thus acting as an enhancer of PEV. These results indicate that lamins may be involved in regulating gene silencing and heterochromatic spreading at the w^{m4} locus and provide evidence that lamins may contribute to the regulation of higher-order chromatin organization.

Keywords Lamin Dm_0 · Eye · w^{m4} inversion · Position effect variegation · *Drosophila*

Introduction

Recently it has become clear that lamins are involved in several nuclear activities in addition to providing a barrier between the nucleoplasm and the cytoplasm (reviewed in Goldberg et al. 1999; Gotzmann and Foisner 1999; Wilson et al. 2001). One of these functions of the nuclear lamins is to serve as scaffold proteins that provide attachment sites for interphase chromatin directly or indirectly regulating chromatin organization as well as DNA replication and transcription (reviewed in Mattout-Drubezki and Gruenbaum 2003). Futhermore, heterochromatin including centromeres, telomeres, and repetitive DNA is preferentially positioned near the nuclear envelope and its interaction with lamin and lamin-binding proteins has been suggested to be important for regulating the higher-order organization of the peripheral heterochromatin (Gotzmann and Foisner 1999; Mattout-Drubezki and Gruenbaum 2003). Interestingly in this context the lamin associated protein, LBR, has been shown to directly interact with the heterochromatin binding protein HP1 (Ye et al. 1997). HP1 is a highly evolutionarily conserved chromodomain protein that was originally identified in *Drosophila* as a suppressor of position effect variegation (PEV) (Eissenberg and Elgin 2000; Mattout-Drubezki and Gruenbaum 2003). PEV is the transcriptional silencing of euchromatic genes as a result of their placement near heterochromatin by chromosomal translocations (reviewed by Wallrath 1998; Schotta et al. 2003). Repression typically occurs in only a subset of cells and is heritable leading to mosaic patterns of gene expression (Schotta et al. 2003). Studies of this effect suggest that the gene silencing may be due to spreading of heterochromatic factors from the heterochromatin and that the degree of spreading depends on the organization of chromatin at the breakpoint (reviewed in Weiler and Wakimoto 1995). PEV in *Drosophila* has served as a major paradigm for the identification of evolutionarily conserved determinants of epigenetic regulation of chromatin

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structure through the isolation of mutations that act as suppressors (*Su(var)*) or enhancers (*E(var)*) of variegation (Schotta et al. 2003). The association of lamin and lamin binding proteins with peripheral heterochromatin suggests the possibility that lamins may similarly play a role in regulating PEV. In order to test this hypothesis we have in this study examined the effect of a recently generated loss-of-function lamin Dm_0 allele on PEV of the w^{m4} allele in the *Drosophila* eye.

Materials and methods

Fly stocks were maintained according to standard protocols (Roberts 1998). Canton-S was used for wild-type preparations. The Lam^{Ari3} allele is described in Patterson et al. (2004) and was the generous gift of Dr. J.A. Fischer. The Lam^{4643} is described in Guillemin et al. (2001) and together with the $In(1)w^{m4}$ stock were obtained from the Bloomington Stock Center. Balancer chromosomes and markers are described in Lindsley and Zimm (1992).

Strains containing the $In(1)w^{m4}$ X chromosome and the partial loss-of-function allele Lam^{Ari3} as well as heteroallelic combinations of Lam^{Ari3} with the strong lamin Dm_0 allele Lam^{4643} were produced by standard crossing. As a control, w^{m4} PEV was analyzed in flies homozygous for a Canton S wild-type second chromosome. To quantify the variegated phenotype newly enclosed adults were collected, aged for 4–5 days at 25°C and were then sorted into different classes based on the percentage of the eye that was red. Eyes from representative individuals from these crosses were photographed using an Olympus Stereo Microscope and a Spot digital camera (Diagnostic Instruments).

Immunoblot analysis was performed as described in Wang et al. (2001) and Zhang et al. (2003) using extracts from adult flies of the specified genotype. The immunoblots were labeled with the lamin Dm_0 mAb HL1203 (Gruenbaum et al. 1988) or with α-tubulin antibody (Sigma) as a loading control.

Results and discussion

The variegated w^{m4} expression is a classic example of PEV. The $In(1)w^{m4}$ X chromosome contains an inversion that juxtaposes the euchromatic *white* gene and heterochromatic sequences adjacent to the centromere (Muller 1930; Schultz 1936). The resulting somatic variegation of w^{m4} expression occurs in clonal patches in the eye (Fig. 1A) reflecting heterochromatic spreading from the inversion breakpoint that silences w^{m4} expression in the white patches and euchromatic packaging of the *w* gene in those patches that appear red (reviewed in Grewal and Elgin 2002). In the present experiments the $In(1)w^{m4}$ chromosome was crossed into hetero- or homozygous Lam^{Ari3} mutant backgrounds. In order to control for possible second site modifiers in the Lam^{Ari3} allele a heteroallelic combination of Lam^{Ari3} with the strong lamin Dm_0 allele Lam^{4643} was also analyzed. The Lam^{Ari3} allele is an EMS induced point mutation that introduces a premature stop codon resulting in a truncated protein that lacks part of the α-helical rod domain and the entire COOH-terminal domain including the NLS and the CaaX box which localizes lamin to the inner nuclear membrane (Patterson et al. 2004). *Lam* null alleles including Lam^{4643} are homozygous lethal; however, the Lam^{Ari3} allele is homozygous viable and acts as a partial loss-of-function mutation (Patterson et al. 2004). The Lam^{4643} allele contains a recessive lethal P element insertion 258 bp upstream of the translation initiation site that results in low to undetectable lamin Dm_0 protein levels (Guillemin et al. 2001; Patterson et al. 2004).

$w^{m4}/(Y)$ flies with the $+/Lam^{Ari3}$, Lam^{Ari3}/Lam^{Ari3}, and Lam^{Ari3}/Lam^{4643} genotypes were scored based on the percentage of the variegated eyes that was red with $w^{m4}/(Y)$; +/+ flies serving as controls. As documented in Fig. 2 and Table 1 the distribution of the proportion of red ommatidia in the variegated eyes was strikingly different in wild-type and Lam^{Ari3}/Lam^{Ari3} and Lam^{Ari3}/Lam^{4643} mutant backgrounds. Since there is a sex-specific difference in PEV of the w^{m4} locus the distribution of red ommatidia in male and female flies has been indicated separately in addition to the distribution for the total population of flies. In wild-type lamin Dm_0 backgrounds almost half of the flies (48.3%) had at least 50% red ommatidia compared to 0% in Lam^{Ari3}/Lam^{Ari3} and Lam^{Ari3}/Lam^{4643} flies. This is concomitant with a dramatic increase in nearly completely white eyes. In Lam^{Ari3}/Lam^{Ari3} flies more than 90% of the flies had less than 10% red ommatidia (Fig. 1B) compared to only about 11% of flies with a wild-type lamin Dm_0 background (Fig. 2 and Table 1). This difference was statistically significant ($P < 0.001$, χ^2-test). In hemizygous $+/Lam^{Ari3}$ flies there was also a shift towards an increasing proportion of white ommatidia although this shift was less pronounced (Fig. 2 and Table 1). By immunoblot analysis we verified that wild-type levels of lamin Dm_0 protein was reduced to about half in $+/Lam^{Ari3}$ flies and was below detectable levels in Lam^{Ari3}/Lam^{Ari3} homozygous flies (Fig. 3). The enhancement of PEV was also

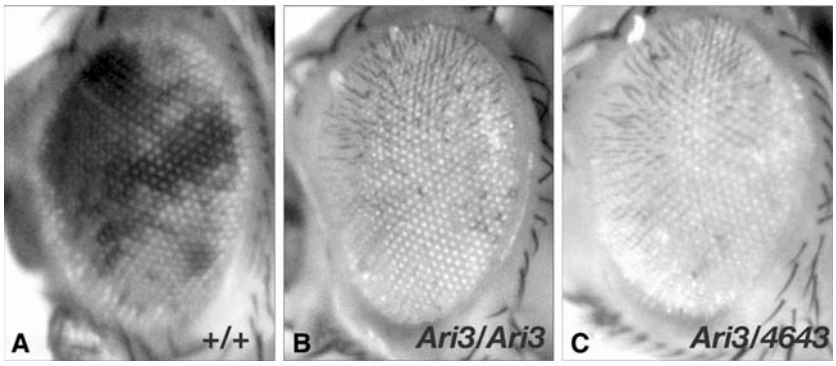

Fig. 1 The effect of the Lam^{Ari3} allele on w^{m4} PEV. (**A**) Typical variegated eye of a *ln(1)*w^{m4} fly in a wild-type (+/+) lamin Dm_0 background. (**B**) Strong enhancement of w^{m4} PEV in a Lam^{Ari3}/Lam^{Ari3} (*Ari3/Ari3*) homozygous mutant background as indicated by a nearly completely white eye phenotype. (**C**) Strong enhancement of w^{m4} PEV in a Lam^{Ari3}/Lam^{4643} (*Ari3/4643*) heteroallelic mutant background as indicated by a nearly completely white eye phenotype. In addition, Lam^{Ari3} flies are characterized by having rough eyes (Patterson et al. 2004)

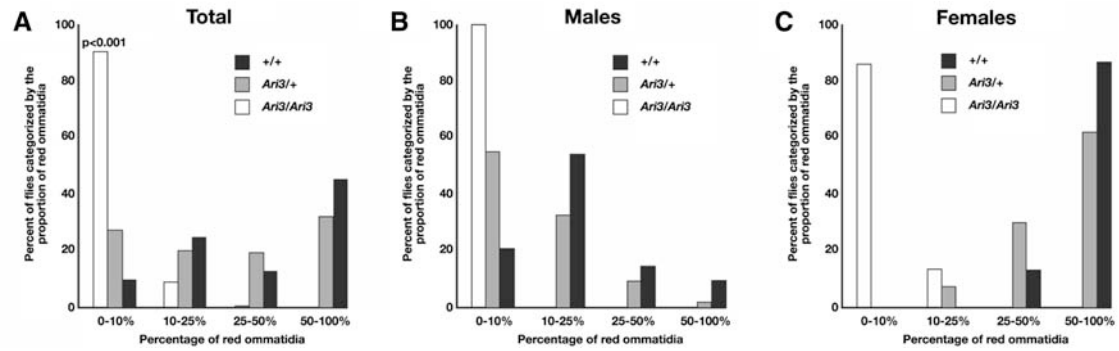

Fig. 2 The lamin Dm_0 *Ari3* allele affects the distribution of the percentage of red ommatidia in w^{m4} flies. (**A**) In the histogram the eyes from wild-type (+/+, *n*=1670) and homozygous Lam^{Ari3} (*Ari3/Ari3*, *n*=203) flies were sorted into different classes based on the percentage of the eye that was red. The data suggest that w^{m4} PEV is enhanced in homozygous Lam^{Ari3} mutant flies as indicated by an increased proportion of white ommatidia. The difference between wild-type lamin Dm_0 and homozygous Lam^{Ari3} flies with less than 10% red ommatidia was compared using a χ^2-test. (**B**) The effect of the lamin Dm_0 *Ari3* allele on the distribution of the percentage of red ommatidia in male w^{m4} flies. (**C**) The effect of the lamin Dm_0 *Ari3* allele on the distribution of the percentage of red ommatidia in female w^{m4} flies

Table 1 The Lam^{Ari3} allele enhances PEV of w^{m4}

Genotype[a]	*n*	Percent of flies categorized by the proportion of red ommatidia			
		0–10% red	10–25% red	25–50% red	50–100% red
+/+	1670	10.8	26.8	14.1	48.3
Males	828	21.8	54.0	14.9	9.4
Females	842	0.0	0.1	13.4	86.5
Ari3/+	801	27.6	20.6	19.5	32.3
Males	400	55.0	33.5	9.3	2.3
Females	401	0.2	7.7	29.7	62.3
Ari3/Ari3	203	90.1	9.1	0.5	0.0
Males	64	100.0	0.0	0.0	0.0
Females	139	85.6	13.7	0.7	0.0
Ari3/Lam^{4643}	153	95.4	4.6	0.0	0.0
Males	71	100.0	0.0	0.0	0.0
Females	82	91.5	8.5	0.0	0.0

[a] Genotype of the third chromosome. In addition, all flies are homozygous (females) or hemizygous (males) for w^{m4} on the X chromosome

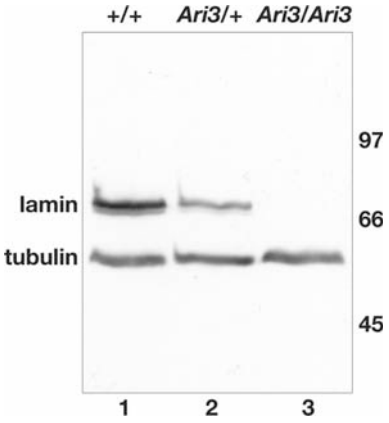

Fig. 3 Lamin Dm_0 expression in Lam^{Ari3} hetero- and homozygous larvae compared to wild-type larvae. The immunoblots were labeled with Lamin Dm_0 mAb HL1203 and with antibody to α-tubulin as a loading control. mAb HL1203 recognizes a carboxyl-terminal epitope that is deleted in the Lam^{Ari3} allele. The immunoblot indicates that the level of wild-type lamin Dm_0 protein in Lam^{Ari3}/Lam^{Ari3} larvae was greatly reduced. The relative migration of molecular weight markers is indicated to the right

pronounced in the $w^{m4}/(Y)$; Lam^{Ari3}/Lam^{4643} heteroallelic combination suggesting that the effect of the Lam^{Ari3} allele on PEV was not the result of second site modifiers. Consequently, these experiments provide evidence that the Lam^{Ari3} allele increases silencing of w^{m4} expression and acts as an enhancer of PEV. The Lam^{Ari3} allele is characterized by a truncation of the COOH-terminal domain and lacks the CaaX box that localizes lamin to the inner nuclear membrane (Patterson et al. 2004). Thus, these results are consistent with a model where impaired lamin function leads to misregulation of the association between peripheral heterochromatin and proteins in the inner nuclear membrane leading to a change in chromatin structure and the spreading of heterochromatic factors that may result in increased gene silencing at adjacent loci such as the w^{m4} locus.

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References

Eissenberg JC, Elgin SC (2000) The HP1 protein family: getting a grip on chromatin. Curr Opin Genet Dev 10:204–210

Goldberg M, Harel A, Gruenbaum Y (1999) The nuclear lamina: molecular organization and interaction with chromatin. Crit Rev Eukaryot Gene Express 9:285–293

Gotzmann J, Foisner R (1999) Lamins and lamin-binding proteins in functional chromatin organization. Crit Rev Eukaryot Gene Express 9:257–265

Grewal SI, Elgin SC (2002) Heterochromatin: new possibilities for the inheritance of structure. Curr Opin Genet Dev 12:178–187

Gruenbaum Y, Landesman Y, Drees B, Bare JW, Saumweber H, Paddy MR, Sedat JW, Smith DE, Benton BM, Fisher PA (1988) *Drosophila* nuclear lamin precursor Dm_0 is translated from either of two developmentally regulated mRNA species apparently encoded by a single gene. J Cell Biol 106:585–596

Guillemin K, Williams T, Krasnow MA (2001) A nuclear lamin is required for cytoplasmic organization and egg polarity in *Drosophila*. Nat Cell Biol 3:848–851

Lindsley DL, Zimm GG (1992) The genome of *Drosophila melanogaster*. Academic Press, New York

Mattout-Drubezki A, Gruenbaum Y (2003) Dynamic interactions of nuclear lamina proteins with chromatin and transcriptional machinery. Cell Mol Life Sci 60:2053–2063

Muller HJ (1930) Types of visible variegations induced by X-rays in *Drosophila*. J Genet 22:299–335

Patterson K, Molofsky AB, Robinson C, Acosta S, Cater C, Fischer JA (2004) The functions of Klarsicht and nuclear lamin in developmentally regulated nuclear migrations of photoreceptor cells in the *Drosophila* eye. Mol Biol Cell 15:600–610

Roberts DB (1998) *Drosophila*: a practical approach. IRL Press, Oxford

Schotta G, Ebert A, Dorn R, Reuter G (2003) Position-effect variegation and the genetic dissection of chromatin regulation in *Drosophila*. Semin Cell Dev Biol 14:67–75

Schultz J (1936) Variegation in *Drosophila* and the inert chromosome regions. Proc Natl Acad Sci USA 22:27–33

Wallrath LL (1998) Unfolding the mysteries of heterochromatin. Curr Opin Genet Dev 8:147–153

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

Weiler KS, Wakimoto BT (1995) Heterochromatin and gene expression in *Drosophila*. Annu Rev Genet 29:577–605

Wilson KL, Zastrow MS, Lee KK (2001) Lamins and disease: insights into nuclear infrastructure. Cell 104:647–650

Ye QA, Callebaut I, Pezhman A, Courvalin JC, Worman HJ (1997) Domain specific interactions of human hp1 type chromodomain proteins and inner nuclear membrane protein LBR. J Biol Chem 272:14983–14989

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