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Regulation of chromatin structure by histone H3S10 phosphorylation

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Abstract

The epigenetic phospho-serine 10 modification of histone H3 has been a puzzle due to its association with two apparently opposed chromatin states. It is found at elevated levels on the highly condensed, transcriptionally inactive mitotic chromosomes yet is also correlated with the more extended chromatin configuration of active genes, euchromatic interband regions, and activated heat shock puffs of *Drosophila* polytene chromosomes. In addition, phosphorylation of histone H3S10 is up-regulated on the hypertranscribed male X chromosome. Here we review the cellular effects of histone H3S10 phosphorylation and discuss a model for its involvement in regulating chromatin organization and heterochromatization that would be applicable to both interphase and mitotic chromosomes.

Introduction

T. H. Morgan's early experiments in *Drosophila* are heralded for their significance in uncovering the linear arrangement of genes on chromosomes. The choice of *Drosophila* as his experimental organism was deliberate: the organism's short life cycle, high fecundity, and ease and minimal expense of culturing all contributed to its attraction. As it so happened, the first of the tractable mutations that Morgan discovered and focused his attention upon was *white,* a mutation that results in the fly having white eyes instead of the normal red eyes. The selection of this gene proved particularly significant as Morgan's study of this allele led to his discovery of sex-linked inheritance and established a special significance to the X chromosome that was unexpected at the time. To this day we are still learning surprising new things about the X chromosome in terms of its organization within the nucleus and its epigenetic regulation. In order to achieve equal levels of X-linked gene products in male and female flies, the male X chromosome is epigenetically marked with high levels of acetylation of the lysine 16 residue of histone H4 (Turner *et al*. 1992), a modification that is correlated with gene activation (reviewed in Turner 2000, Strahl & Allis 2000). However, in *Drosophila* the male X is also epigenetically marked with high levels of histone H3 serine 10 phosphorylation (Wang *et al*. 2001) (Figure 1C), an epigenetic mark that is much less well understood. Phosphorylation of the histone H3S10 residue was first described to occur at high levels in mitotic cells and to be associated with chromosomal condensation (Hendzel *et al*. 1997, Wei *et al*. 1998, 1999) (Figure 1A). However, phosphorylation of H3S10 also has been found to occur in mitogenically stimulated cells (Mahadevan *et al*. 1991, Barratt *et al*. 1994) and at transcriptionally activated heat shock loci (Nowak & Corces 2000) (Figure 1B) implying a role for phosphorylation of histone H3S10 in regulation of gene expression in addition to its role in chromosome condensation (Mizzen *et al*. 1998, Thomson *et al*. 1999). Furthermore, it has been

demonstrated that MSK1/2 kinase activity and histone H3 phosphorylation have roles in chromatin remodeling and gene transcription in mammals (reviewed in Dunn *et al*. 2005). Thus, histone H3S10 phosphorylation is associated with two apparently opposed chromatin states, namely the highly condensed mitotic chromosomes and the relaxed chromatin of certain activated genes during interphase (reviewed in Prigent & Dimitrov 2003). This apparent contradiction in function has sparked speculation that the effect of the H3S10 modification on chromatin structure might be context-dependent and influenced by other histone epigenetic marks such as acetylation or S28 phosphorylation (Turner 2000, Strahl & Allis 2000) but the actual cellular effect of H3S10 phosphorylation remains enigmatic. In this review we will discuss experiments and ideas related to the modification of chromatin structure and function by histone H3S10 phosphorylation.

Phosphorylation of histone H3S10 during mitosis

Phosphorylation of S10 of histone H3 was first noted as a distinctive marker for dividing cells since the highly condensed, metaphase chromosomes are heavily phosphorylated at this site in all organisms analyzed thus far including *Drosophila* (Hans & Dimitrov 2001, Giet & Glover 2001, Adams *et al*. 2001, Wang *et al*. 2001). A strong correlation between initial chromatin condensation and S10 phosphorylation was observed in a number of different model systems (Hendzel *et al*. 1997, van Hooser *et al*. 1998) and in their early studies in *Tetrahymena*, Wei *et al*. (1998, 1999) suggested a model whereby H3S10 phosphorylation promotes recruitment of chromosomal condensation factors required for normal mitotic chromosome assembly and segregation. In *Drosophila* the Barren condensin protein is recruited to the chromosomes with the same timing and dynamics as phosphorylation of H3S10 (Giet & Glover 2001). However the requirement for H3S10 phosphorylation for normal chromosome condensation and segregation does not appear to be universal: in budding yeast a histone H3S10A mutation does not impair chromosome transmission or cell cycle progression (Hsu *et al*. 2000), in maize phosphorylation of S10 is found at pericentromeric regions but not on the condensed mitotic chromosomal arms and most of the meiotic

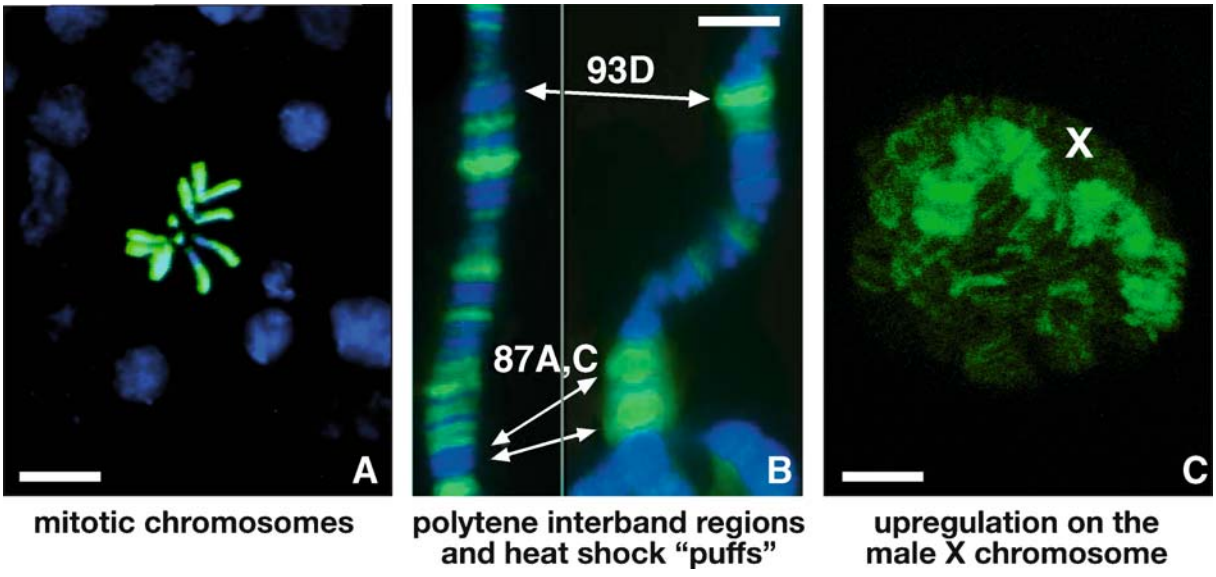

Figure 1. Histone H3S10 phosphorylation in *Drosophila*. **A:** H3S10ph antibody labeling (in green) of mitotic chromosomes in a larval neuroblast. Labeling of DNA by Hoechst is shown in blue. **B:** Distribution of phosphorylated histone H3S10 in polytene chromosomes before and after heat shock. The preparations were double-labeled with H3S10ph antibody (in green) and with Hoechst (in blue). The images show the change in staining of three heat shock loci (87A, 87C, and 93D) on a section of chromosome 3R. The heat shocked chromosome is to the right. The figure is modified from Nowak & Corces (2000). **C:** Confocal image from a whole-mount preparation of a salivary gland polytene nuclei from a male third-instar larvae labeled with H3S10ph antibody. The labeling of phosphorylated histone H3S10 is up-regulated on the male X chromosome (X). Scale bar equals 5 μm in (**A**) and (**C**) and 2 μm in (**B**).

chromosome condensation occurs prior to H3S10 phosphorylation (Kaszas & Cande 2000). In *Drosophila* S2 cells only a weak correlation between H3S10 phosphorylation and the degree of chromatin condensation was observed (Adams *et al*. 2001). In addition, analysis in different mutant lines revealed that H3S10 phosphorylation was apparently normal in undercondensed chromosomes in *gwl* mutants (Yu *et al*. 2004) while a strong reduction in H3S10 levels was observed in *borr* mutants that had essentially undetectable condensation defects (Hanson *et al*. 2005). While in most cases there seems to be a correlation between chromosome condensation and histone H3S10 phosphorylation, the connection is not absolute and thus the precise cellular effect of this modification during mitosis is not clear.

In *Drosophila* the gene responsible for phosphorylation of H3S10 at mitosis is known as *ial* (*Ipl1-aurora-like kinase)* or, more commonly, *aurora B*. No mutations have yet been identified in the *aurora B* locus so in order to address its cellular function, RNAi treatment to deplete Aurora B in S2 cells was performed in two separate studies (Giet & Glover 2001, Adams *et al*. 2001). RNAi depletion of *aurora B* kinase leads to decreased phosphorylation of H3S10 during mitosis and failure to recruit condensin to the chromosomes. Whereas the decreased level of phosphorylated H3S10 measured in different experiments did not correlate with the extent of condensation defects observed, decrease in phosphorylated H3S10 levels did correlate well with the level of defects observed in mitotic chromosome morphology. Low levels of phosphorylated H3S10 resulted in 'dumpy' mitotic chromosomes in which sister chromatids did not appear to resolve (Adams *et al*. 2001). Although progression of mitosis was not blocked, the chromosomes failed to segregate normally and many defects were observed including lack of sister kinetechore disjunction, lagging chromatids, and extensive chromatin bridging at anaphase (Giet & Glover 2001, Adams *et al*. 2001). Depletion of *Aurora B* also led to increased levels of polyploidy, possibly as a consequence of failure of the centromeric regions to organize properly. In *aurB* RNAi cells immunolocalization of Prod, which normally localizes to the centromeres of mitotic chromosomes, showed a punctate staining throughout the poorly condensed chromatin mass consistent with a failure of centromeric regions to reach the spindle poles (Giet & Glover 2001). Thus, *aurB* RNAi leads to an array of defects including decreased histone H3S10 phosphorylation, aberrant mitotic chromosome structure, and errors in metaphase chromosome alignment supporting the hypothesis that phosphorylation of histone H3S10 plays an essential role in regulation of mitotic chromosome remodeling, sister chromatid and kinetechore disjunction, and mitotic spindle architecture (Giet & Glover 2001, Adams *et al*. 2001).

Phosphorylation of histone H3S10 during interphase

Meanwhile other studies found that phosphorylation of histone H3S10 occurred also during interphase but in a much smaller fraction of nucleosomes than found in mitotic chromosomes. Examples of this phosphorylation correlated with transcriptional activation of immediate early-response genes such as *c-fos* and *c-jun* during the G_0–G_1 transition (Barratt *et al*. 1994) or in response to early differentiative signaling such as that mediated in ovarian follicles by FSH (DeManno *et al*. 1999) suggesting a linkage between H3S10 phosphorylation and gene expression. Light-induced phosphorylation of histone H3S10 is correlated with gene expression in certain neuronal cells, suggesting its involvement in a circadian regulatory mechanism (Crosio *et al*. 2000) and H3S10 phosphorylation was also observed to occur during up-regulation of IFNγ after viral infection (Agalioti *et al*. 2002) and in NFκB pathway induction (Anest *et al*. 2003, Yamamoto *et al*. 2003). In yeast where gene pathways have been comprehensively characterized by transcriptome analysis (Holstege *et al*. 1998), phosphorylation of H3S10 is essential for the expression of some genes but not for others, demonstrating that histone H3S10 phosphorylation is not a general requirement for transcription at all promoters but may play a distinct role tailored for specific promoters (Lo *et al*. 2000, 2005).

The heat shock response of polytene chromosomes in *Drosophila* larval salivary glands provides a particularly useful model system to examine regulated gene expression in conjunction with observation of chromosomal morphological changes that occur during both gene induction and recovery. Because recurrent S phases occur without subsequent mitotic cycles, and since the resulting approximately 1024 copies of each chromosome are organized in

parallel alignment in these cells, a reproducible pattern of bands, where the chromatin is more compact, and interbands, where the chromatin is more extended, can easily be observed by microscopy. Active genes are generally in the more extended interband regions and in the case of induction of extremely high levels of transcription as occurs during the heat shock response, the relevant polytene chromosome regions undergo a distinctive morphological change known as 'puffing' (Ashburner 1970). At the same time that heat shock activates a specific set of stress-response genes, a global down-regulation of transcription at other loci occurs (reviewed in Lindquist 1986).

Using this system the correlation of H3S10 phosphorylation with gene transcription received strong support when Nowak & Corces (2000) reported a dramatic increase in phosphoserine 10-antibody labeling at actively transcribing *Drosophila* larval salivary chromosome heat shock puffs during the heat shock response (Figure 1B). Whereas prior to heat shock phosphorylated H3S10 labeling was distributed in a large number of bands throughout the chromosomes with higher levels at ecdysone-induced transcriptional puffs, a 20-minute heat shock resulted in signal redistribution to a much smaller number of discrete sites that predominantly correlated with the heat shock loci and showed the characteristic puffing phenotype. Analysis of these regions before heat shock did not reveal any obvious phosphorylated H3S10 signal. The redistribution of phosphorylated H3S10 in the genome was dynamic with changes already noticeable after only 1 minute of heat shock, and nearly complete redistribution of phosphorylated H3S10 to heat shock puffs by 10 minutes. During recovery after heat shock, the re-establishment of the normal H3S10ph genomic pattern followed a similar time-course as has been previously reported for the restoration of normal gene expression. Surprisingly Nowak & Corces (2000) did not observe changes in acetylation patterns, suggesting that it was the phosphoepitope that correlated with transcriptional activation. However, whether histone H3S10 phosphorylation is part of the transcriptional activation mechanism appears to vary with different promoters. Using transgenes whose expression could be driven using the Gal4-transcriptional activator, Labrador & Corces (2003) observed that, when transcription was driven from an *Hsp70* promoter, H3S10 was hyperphosphorylated, but when transcription was driven from a P element transposase promoter, phosphorylated H3S10 was not detected. Thus, as appears to be the case in yeast (Lo *et al*. 2005), histone H3S10 phosphorylation is not a general requirement for transcription at all promoters but may play a distinct role tailored for specific promoters.

Histone H3S10 phosphorylation – an effect on chromosomal architecture?

Nucleosomal remodeling by chromatin-remodeling complexes plays a critical role in chromosome dynamics (reviewed in Becker & Horz 2002). Actively transcribed regions are characterized by nucleosome-free, or 'DNase-hypersensitive' sites whereas silenced or heterochromatic regions are characterized by tightly packed, ordered nucleosomal arrays (Elgin 1988, Wallrath & Elgin 1995). Besides the local remodeling of a small number of nucleosomes in the promoter region, larger-scale nucleosomal remodeling is also implicated in the unfolding of large chromatin domains (Tumbar *et al*. 1999, Dietzel *et al*. 2004, reviewed in Peterson 2003). Current models suggest that within the nucleus there exist regions of condensed, silent chromatin interspersed with regions of decondensed, active chromatin. A clear example of this is found within the band–interband pattern observed in *Drosophila* larval polytene chromosomes (Labrador & Corces 2002, Zhimulev *et al*. 2003). That such an organization is not restricted to 'unusual' cells such as polyploid salivary gland cells is supported by increasing evidence of clustering or looping of gene-active regions within the nucleus (de Laat & Grosveld 2003, Chambeyron & Bickmore 2004, Kato & Sasaki 2005). The challenge is to identify the molecules and molecular mechanisms that determine how this organization is established and maintained and the signal transduction events that regulate this process.

The JIL-1 histone H3S10 kinase

With the goal of identifying such molecules Jin *et al*. (1999) characterized a novel tandem kinase in *Drosophila*, JIL-1, that localizes specifically to euchromatic interband regions of polytene chromo-

somes (Figure 2A) and which is the predominant kinase regulating histone H3S10 phosphorylation at interphase (Wang *et al*. 2001). Histone H3S10 phosphorylation levels are severely reduced in *JIL-1* hypomorphic or null mutants; however, the histone H3S10 levels are restored by introduction of transgenic JIL-1 activity. Furthermore, 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 misalignment of the interband polytene chromatin fibrils that is further associated with coiling of the chromosomes and an increase of ectopic contacts between non-homologous regions (Jin *et al*. 2000, Wang *et al*. 2001, Zhang *et al*. 2003, Deng *et al*. 2005) (Figure 2B,D). This results in a shortening and folding of the chromosomes with a non-orderly intermixing of euchromatin and the compacted chromatin characteristic of banded regions (Deng *et al*. 2005). The intermingling of non-homologous regions can be so extensive that these regions become fused and confluent, further shortening the chromosome arms. Based on these findings a model was proposed in which JIL-1 functions to establish or maintain euchromatic chromatin regions by repressing the formation of contacts and intermingling of non-homologous chromatid regions (Deng *et al*. 2005).

In order to further study the mechanisms of perturbations to chromatin structure in the absence of JIL-1 activity and histone H3S10 phosphorylation Zhang *et al*. (2006) studied the distribution of the heterochromatin markers H3K9me2 and HP1 in *JIL-1* null mutant backgrounds. In *Drosophila* formation of heterochromatin and repression of transcription involves covalent modifications of histone tails and/or the exchange of histone variants (Swaminathan *et al*. 2005). Current evidence suggests that a major pathway in the establishment of heterochromatin is initiated by the RNAi machinery, which marks prospective heterochromatic regions (Volpe *et al*. 2002, Pal-Bhadra *et al*. 2004, Verdel *et al*. 2004). This leads to deacetylation of histone H3K9 followed by dimethylation of this residue and recruitment of HP1 (Lachner *et al*. 2001, Nakayama *et al*. 2001, Ebert *et al*. 2004). Thus, dimethylation of histone H3K9 and the presence of HP1 serve as major chromatin modification marks for the presence of transcriptionally silenced chromatin (Fischle *et al*. 2003a, Swaminathan *et al*. 2005). The studies of Zhang *et al*. (2006) demonstrated that a reduction in the levels of the JIL-1 histone H3S10 kinase resulted in the spreading of the major heterochromatin markers H3K9me2 and HP1 to ectopic locations on the chromosome arms with the most pronounced increase on the X chromosomes. However, overall levels of the H3K9me2 mark and HP1 were unchanged, suggesting that the spreading is accompanied by a reduction in the levels of pericentromeric heterochromatin in *JIL-1* hypomorphic mutant backgrounds. Genetic interaction assays demonstrated that *JIL-1* functioned *in vivo* in a pathway with *Su(var)3-9* which is a major catalyst for dimethylation of the histone H3K9 residue, HP1 recruitment, and formation of silenced heterochromatin (Schotta *et al*. 2002). Zhang *et al*. (2006) provided further evidence that JIL-1 activity and localization were not affected by the absence of Su(var)3-9 activity, suggesting that *JIL-1* was upstream to *Su(var)3-9* in this pathway. Taken together these findings suggested that JIL-1 functions in a novel pathway to establish or maintain euchromatic regions by antagonizing Su(var)3-9 mediated heterochromatization (Zhang *et al*. 2006). According to the histone code (Strahl & Allis 2000) and the recently proposed binary switch model (Fischle *et al*. 2003b) phosphorylation of a site adjacent to a methyl mark that engages an effector molecule may regulate its binding. JIL-1 phosphorylates the histone H3S10 residue in euchromatic regions of polytene chromosomes (Jin *et al*. 1999, Wang *et al*. 2001), raising the possibility that this phosphorylation at interphase prevents ectopic recruitment and/or spreading of the heterochromatin-promoting factors HP1 and Su(var)3-9, thus antagonizing the formation of silenced heterochromatin at interbands.

The effect of JIL-1 mutations on position effect variegation

Higher-order chromatin structure is important for epigenetic regulation and control of gene activation and silencing. In *Drosophila* euchromatic genes can be transcriptionally silenced as a result of their placement in or near heterochromatin, a phenomenon known as position effect variegation (PEV) (reviewed by Wallrath 1998, Henikoff 2000, Schotta *et al*. 2003). Repression typically occurs in only a subset of cells and can be heritable leading to mosaic patterns of gene expression (Schotta *et al*. 2003, Delattre

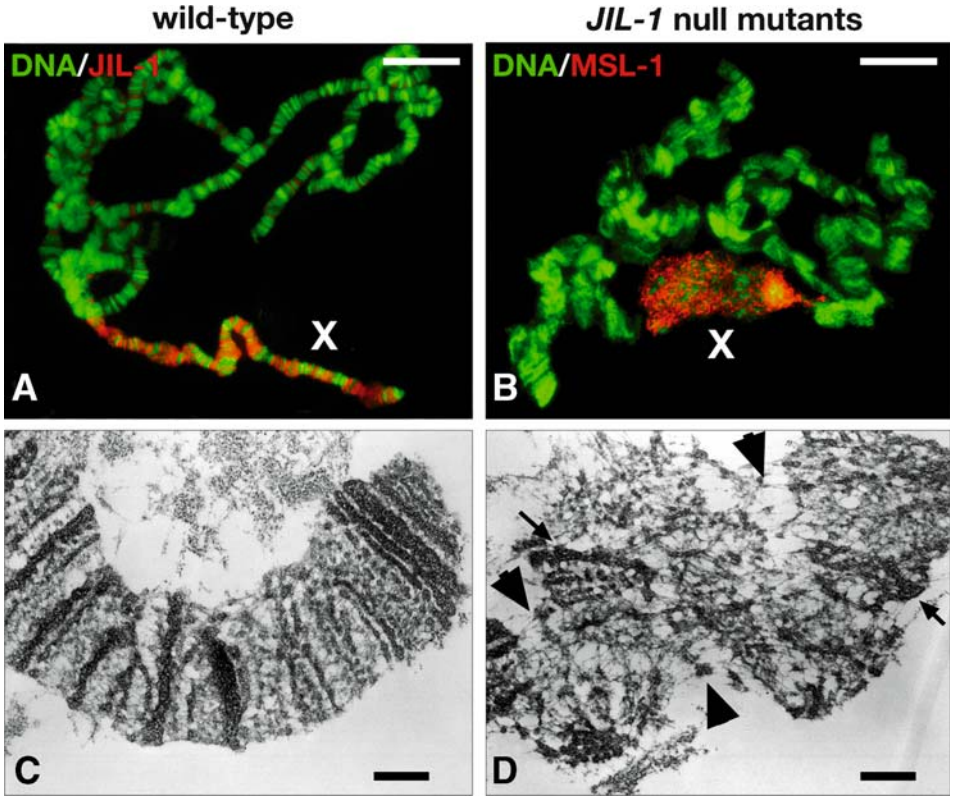

Figure 2. Reduced levels of the JIL-1 histone H3S10 kinase have a severe effect on the structure and organization of larval polytene chromosomes. Preparations are shown from wild-type (**A**, **C**) and homozygous *JIL-1* null (**B**, **D**) larvae. In (**A**) and (**B**) polytene chromosome squashes from male third-instar larvae were labeled with Hoechst to visualize the chromatin (green) and with antibodies to JIL-1 and MSL-1 (in red), respectively. The micrograph in (**A**) shows that the JIL-1 histone H3S10 kinase is up-regulated about two-fold on the male X chromosome. In (**B**) note the misalignment and intermixing of interband and banded regions and the extensive coiling and folding of the chromosome arms in *JIL-1* null mutant chromosomes. The male X chromosome (X) identified by MSL-1 antibody labeling is particularly affected and no remnants of banded regions are discernable (**B**). (**C**, **D**) show TEM micrographs of the ultrastructure of polytene autosomes. The micrograph in (**C**) shows the orderly segregation into bands and interbands and the parallel alignment of euchromatic chromatid fibrils in wild-type polytene chromosomes. In contrast the micrograph in (**D**) shows a coiled autosome with extensive ectopic contacts (arrowheads) between the folds from a *JIL-1* homozygous null larvae. The euchromatic chromatids are misaligned and intermixed with scattered patches of compacted chromatin. A few remnants of recognizable banded regions are still present (arrows). Scale bar equals 20 μm in (**A**) and (**B**) and 1 μm in (**C**) and (**D**).

et al. 2004). PEV in *Drosophila* has served as a major paradigm for the identification and genetic analysis of evolutionarily conserved determinants of epigenetic regulation of chromatin structure through the isolation of mutations that act as suppressors (*Su(var)*) or enhancers (*E(var)*) of variegation (Schotta *et al*. 2003). Thus, if the histone H3S10 kinase JIL-1 functions to maintain euchromatic regions and antagonize heterochromatization and gene silencing it would be predicted that mutations in JIL-1 would affect PEV. Interestingly, some of the strongest suppressors of PEV described, *Su(var)3-1* mutations, were recently identified to be alleles of the *JIL-1* locus that generate proteins with COOH-terminal deletions (Ebert *et al*. 2004). Furthermore, Lerach *et al*. (2006) demonstrated that *JIL-1* hypomorphic loss-of-function mutations also act as strong suppressors of PEV at the w^{m4} locus. However, an important difference between *Su(var)3-1* and *JIL-1* hypomorphic alleles is that while the amount of heterochromatic factors is constant in both mutant backgrounds (Ebert *et al*. 2004, Zhang *et al*. 2006) there is a

marked redistribution of the heterochromatic markers H3K9me2 and HP1 in *JIL-1* hypomorphic mutants (Zhang *et al*. 2006). Therefore, the underlying molecular mechanism of suppression of PEV in Su(var)3-1 mutants is likely to be different from that occurring in loss-of-function null and hypomorphic JIL-1 alleles. To account for these differences Lerach et al. (2006) proposed a model in which suppression of PEV at the w^{m4} locus in *JIL-1* hypomorphic backgrounds is due to a reduction in the level of heterochromatic factors at the pericentromeric heterochromatin near the inversion breakpoint site that reduces its potential for heterochromatic spreading and silencing. In contrast, $JIL\text{-}1^{Su(var)3\text{-}1}$ alleles are characterized by deletions of COOH-terminal sequences that do not affect JIL-1 kinase activity but are required for proper chromosomal localization and lead to $\text{JIL-1}^{\text{Su(var)3-1}}$ protein mislocalization to ectopic chromosome sites (Ebert *et al*. 2004, Zhang *et al*. 2006). Thus, the dominant gain-of-function effect of the $JIL\text{-}1^{Su(var)3\text{-}1}$ alleles may be attributable to JIL-1 kinase activity at ectopic locations either by mis-regulated localization of the phosphorylated histone H3S10 mark or possibly through phosphorylation of novel target proteins (Ebert *et al*. 2004, Zhang *et al*. 2006).

Thus, it appears that a major function of the JIL-1 histone H3S10 kinase is to restrict the formation of heterochromatin at inappropriate sites. When ectopic redistribution of these markers occurs, the effect is a titration of heterochromatic factors away from the pericentromeric region, which could explain the Su(var) effect observed in *JIL-1* hypomorphic mutants. In this model the decrease in concentration of centromeric heterochromatic factors diminishes the ability of heterochromatin to spread long distances, resulting in a decreased capacity for w^{m4} silencing. In addition, the extensive mislocalization of heterochromatic factors would also be consistent with the ultrastructural findings of chromatin structure defects by Deng *et al*. (2005). Rather than functioning to directly specify a euchromatic environment, JIL-1 may instead protect the maintenance of euchromatic domains via a mechanism that antagonizes a default tendency towards heterochromatic silencing at certain genomic sites. Indeed, current evidence suggests that the default pathway for cellular memory modules is to be silenced in the absence of alternative signals (reviewed in Ringrose & Paro 2004). In support of this hypothesis the lethality normally associated with a severely hypomorphic *JIL-1* heteroallelic combination (Zhang *et al*. 2003) was almost completely rescued by reduction of dimethyl H3K9 levels as a consequence of the introduction of a loss-of-function allele for the methyltransferase Su(var)3-9 (Zhang *et al*. 2006). In addition, such a reduction in the levels of H3K9 dimethylation results in a significant improvement in chromosome morphology (Zhang *et al*. 2006). These results suggest that the JIL-1 histone H3S10 kinase is essential due to its regulation of heterochromatin behavior rather than due to a direct role in transcriptional activation of essential genes. One possible model is that, just as phosphorylation of H3S10 serves to displace HP1 binding to methylated H3K9 during mitosis, the phosphorylation of H3S10 during interphase is necessary to displace or prevent ectopic euchromatic HP1 binding. This would prevent the kind of heterochromatic spreading that might occur if the normal euchromatic HP1 were to bind Su(var)3-9 leading in turn to methylation of neighboring H3K9 residues and creation of additional HP1 binding sites in a self-propagating process as has been proposed to occur at the centromeric heterochromatin (Grewal & Elgin 2002).

Enhanced histone H3S10 phosphorylation on the male X chromosome correlates with dosage compensation mechanisms

Just as the X chromosome provided new insight into gene organization in Morgan's time, the X chromosome has been instrumental in advancing our understanding of epigenetic regulation. In organisms utilizing an XY heterogametic system, females have two copies of the X chromosome while males have only one, despite the fact that many of the X-linked genes are required equally by both sexes. Thus different dosage compensation mechanisms have evolved to equalize levels of X-linked gene products in males and females, and although the strategy differs in various organisms, a common theme is epigenetic modification of the X. However, the approach taken by different organisms can vary: in humans, one of the two X chromosomes in females is inactivated by condensation of the

chromosome into a Barr body while in *Caenorhabditis elegans*, association of condensin-like molecules with the two hermaphroditic X chromosomes reduces transcription levels of each to about half (reviewed in Lucchesi *et al*. 2005). However, in *Drosophila* both female X chromosomes are actively expressed and so in order to achieve equal levels of X-linked genes, the male shows a two-fold hypertranscription from the single X chromosome (Straub *et al*. 2005, Hamada *et al*. 2005). Whereas the inactive mammalian Barr body is highly condensed and heterochromatic, the hyperactive *Drosophila* male X shows a more diffuse chromosome structure such that, despite the fact that it contains half the DNA content, the male X chromosome appears to be of the same width as the paired female X chromosomes and the autosomes indicating expansion of the typical chromatin structure (reviewed in Gorman & Baker 1994).

In an effort to determine the molecular basis for dosage compensation in *Drosophila*, a number of genetic screens were performed which have identified several genes necessary for achieving equal levels of most X-linked transcription (Fukunaga *et al*. 1975, Belote & Lucchesi 1980). The products of these genes assemble into a complex termed MSL (male specific lethal) that targets a histone acetyltransferase that acetylates histone H4 (H4K16ac) to the up-regulated male X chromosome (Hilfiker *et al*. 1997, Smith *et al*. 2000). Absence of any of the MSL complex subunits prevents both MSL complex assembly as well as the enhanced H4K16ac modification on the male X chromosome (Bone *et al*. 1994). Acetylation of the H4K16 residue is correlated with transcriptional activity in a wide variety of systems (Turner 2000, Strahl & Allis 2000) and the discovery of its up-regulation on the *Drosophila* male X was an early indicator that dosage compensation mechanisms may involve epigenetic regulation of chromatin, in this case to facilitate higher levels of expression (reviewed in Akhtar 2003).

The finding that the JIL-1 kinase is associated with the MSL complex and that histone H3S10 phosphorylation is up-regulated on the male X chromosome in a pattern similar to that of the JIL-kinase suggests that regulation of histone H3S10 phosphorylation may also play a role in dosage compensation mechanisms (Jin *et al*. 1999, 2000, Wang *et al*. 2001). This is underscored by the observation that male eclosion rates were significantly reduced as compared to female rates in *JIL-1* hypomorphic mutant backgrounds with reduced levels of histone H3S10 phosphorylation (Wang *et al*. 2001, Zhang *et al*. 2003). This male to female sex ratio can be rescued to near wild-type ratios by introduction of a JIL-1-GFP transgene. Furthermore, to directly test whether JIL-1 may play a role in dosage compensation Lerach *et al*. (2005) measured eye pigment levels of mutants in the X-linked *white* gene in an allelic series of *JIL-1* hypomorphic mutants. The experiments showed that dosage compensation of w^a alleles that normally do exhibit dosage compensation (Zachar & Bingham 1982) was severely impaired in the *JIL-1* mutant backgrounds. As a control a hypomorphic *white* allele w^e that fails to dosage compensate in males due to a *pogo* element insertion (Smith & Lucchesi 1969, O'Hare *et al*. 1991) was also examined. 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 indicated that proper dosage compensation of eye pigment levels in males controlled by X-linked *white* alleles requires normal JIL-1 function.

These changes in dosage compensation in *JIL-1* hypomorphs are directly correlated with a reduction in the levels of histone H3S10 phosphorylation. However, it should be noted that the experiments do not address whether JIL-1 may mediate phosphorylation of other histone residues in conjunction with histone H3S10 or regulate other proteins. Nonetheless, the wild-type interphase polytene male X chromosome showed a striking enhancement of H3S10ph levels that was absent in *JIL-1* mutant animals and this same pattern also was observed using antibodies specific for the double modification of phosphoS10 and acetylK14 residues of histone H3 (Wang *et al*. 2001). It has been proposed that, whereas phosphorylation of H3S10 may signal mitosis, phosphorylation of H3S10 in the context of acetylation would instead be an indicator for gene activity (Strahl & Allis 2000, Turner 2000). Thus, the male X is epigenetically modified with chromatin marks that are associated with higher levels of transcriptional activity that includes phosphorylated histone H3S10. Interestingly, the recent demonstration that conditional depletion of HP1 in transgenic

flies results in increased male lethality suggests that modulation of transcription in a sex-specific manner may also involve epigenetic regulation of HP1 activity (Liu *et al*. 2005) and raises the prospect that the mechanism underlying this regulation might involve a methyl/phos binary switch.

Histone H3S10 phosphorylation and polytene structure of the male X chromosome

The JIL-1 kinase and phosphorylated histone H3S10 are up-regulated on the male X chromosome (Figures 1C and 2A) and the polytene chromosome structure of the male X chromosome is differentially affected from the autosomes in *JIL-1* mutants (Jin *et al*. 1999, Wang *et al*. 2001) (Figure 2B). In *JIL-1* mutant backgrounds coiling and folding of the male X polytene chromosome in contrast to autosomes is not observed (Deng *et al*. 2005). Furthermore, no banded regions were discernible in *JIL-1* null polytene chromosomes as only small patches of electron-dense compacted chromatin were left of the banded regions, and these patches were scattered among a widely dispersed and loosely connected network of euchromatic chromatin fibrils (Deng *et al*. 2005). Therefore, the shortening of the male X chromosome appears not to be caused by coiling and fusion of non-homologous regions but rather by increased dispersal of the chromatin into a diffuse and progressively widening network. These results are likely to reflect an inherent difference in the structure of the male X chromosome as compared to autosomes and the female X chromosome. One possibility is that the difference in chromosome structure may be linked to the increased transcriptional activity of the male X which correlates with a more open chromosome architecture, such that, despite the fact that it contains half the DNA content, the normal male X chromosome has the same width as the paired female X chromosome and the autosomes (Gorman & Baker 1994). This more open chromatin structure is likely to be maintained by the activity of the MSL dosage compensation complex (Bone *et al*. 1994, Hilfiker *et al*. 1997, reviewed in Akhtar 2003). This leads to hyperacetylation of histone H4 and hyperphosphorylation of histone H3, and these particular chromatin modifications thus have the potential to provide a basis for the different chromatin structure of the male X chromosome as compared to the female X and the autosomes.

Phosphorylation of H3S10 – a shared function at interphase and mitosis?

Paradoxically, two chromosome states that exhibit high levels of H3S10 phosphorylation show opposite chromatin packaging: the euchromatic interband regions of polytene chromosomes adopt a special expanded, chromatin structure while the mitotic chromosome is highly compacted. This raises the question whether phosphorylation of histone H3S10 has any direct effect on nucleosome organization into higher-order structures. Studies analyzing the folding patterns of synthetic, *in vitro*-assembled nucleosomal arrays have demonstrated that formation of higher-order folded structures can be directly influenced by histone composition: removal of the histone amino-terminal tails or alternatively histone hyperacetylation results in nucleosomal arrays that are defective in their ability to assemble into higher-order folded fibers (reviewed in Fry *et al*. 2004). However, arrays assembled with homogeneously phosphorylated H3S10 do not behave differently from unmodified H3S10, suggesting that H3S10 phosphorylation does not have any direct effect on internucleosomal tail interactions important for higher-order folding events (Fry *et al*. 2004). In addition, SWI/SNF-remodeling of nucleosomal arrays was not observed to be sensitive to the phosphorylation state of histone H3 serine 10 (Shogren-Knaak *et al*. 2003). Thus the available data argue against a model in which H3S10 phosphorylation directly alters biophysical properties important in regulating chromatin structure.

The ‘histone code’ hypothesis, however, proposes that an important consequence of histone tail modification is the generation of specific ‘signaling platforms’ containing multiple binding sites recognized by different modules that in turn regulate chromatin structure. This model has found strong support with the demonstration that acetylated tail residues are bound by bromodomains present in an assortment of histone-modifying enzyme complexes while a methylated lysine residue is recognized by the chromodomain of HP1 (reviewed in Strahl &

Allis 2000, Turner 2000, Fischle *et al*. 2003a). However, to date no motif has been identified that specifically recognizes a phosphorylated tail residue and thus there is no current indication that a chromatin complex is directly recruited to the H3S10ph residue. Instead phosphorylation of the H3S10 residue has more recently been proposed to play a role in regulating a binary methyl/phos switch that serves to prevent HP1 binding to the methylated H3K9 residue (Fischle *et al*. 2003b). This would provide a rapid means of regulating the activity of the relatively stable methyl mark; indeed, recent studies have indicated that phosphorylation of H3S10 is responsible for the dissociation of the methyl K9-binding protein HP1 during mitosis (Fischle *et al*. 2005, Hirota *et al*. 2005).

Taken together, these observations may provide a working hypothesis to explain the conundrum of why phosphorylation of H3S10 is found at such high levels on transcriptionally inactive, condensed chromosomes at mitosis yet is also correlated with an active, extended chromatin state at interphase. One plausible scenario is that phosphorylation of H3S10 plays a regulatory role in mediating release of chromosomal attachment to a scaffold that promotes heterochromatic packaging of DNA. For example, phosphorylation of a methyl/phos binary switch prevents HP1 binding (Fischle *et al*. 2005, Hirota *et al*. 2005) which would also be an effective means at interphase to regulate heterochromatization of a region. Thus, an interphase kinase that phosphorylated H3S10 would promote detachment of specific regions from the heterochromatic scaffold to allow decondensation and gene expression whereas phosphatase activity would restore heterochromatization, promoting condensation and silencing in a regulated fashion. The regulation of chromatin packaging with such a methyl/phos binary switch would provide an especially flexible mechanism to regulate genes that respond to developmental or environmental cues and are characterized by periods of induction followed by periods of silencing. On the other hand, during metaphase the entire chromosome must detach from its interphase scaffolding, and the ejection of methyl binding proteins by uniform high levels of H3S10 phosphorylation would be an efficient mechanism to promote this detachment, thus enabling the chromosomal remodeling that is necessary for proper mitotic chromosome interaction with the mitotic spindle. In future experiments the *Drosophila* model system promises to be especially useful for determining the precise mechanisms of how histone H3S10 phosphorylation mediates these diverse effects on the regulation of chromatin structure, gene expression, and chromosome organization, as well as to characterize the signal transduction pathways involved.

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