Ectopic histone H3S10 phosphorylation causes chromatin structure remodeling in Drosophila

Huai Deng
Xiaomin Bao
Weili Cai
Melissa J. Blacketer
Andrew S. Belmont

See next page for additional authors
Ectopic histone H3S10 phosphorylation causes chromatin structure remodeling in Drosophila

Abstract
Histones are subject to numerous post-translational modifications that correlate with the state of higher-order chromatin structure and gene expression. However, it is not clear whether changes in these epigenetic marks are causative regulatory factors in chromatin structure changes or whether they play a mainly reinforcing or maintenance role. In Drosophila phosphorylation of histone H3S10 in euchromatic chromatin regions by the JIL-1 tandem kinase has been implicated in counteracting heterochromatization and gene silencing. Here we show, using a LacI-tethering system, that JIL-1 mediated ectopic histone H3S10 phosphorylation is sufficient to induce a change in higher-order chromatin structure from a condensed heterochromatin-like state to a more open euchromatic state. This effect was absent when a 'kinase dead' LacI-JIL-1 construct without histone H3S10 phosphorylation activity was expressed. Instead, the 'kinase dead' construct had a dominant-negative effect, leading to a disruption of chromatin structure that was associated with a global repression of histone H3S10 phosphorylation levels. These findings provide direct evidence that the epigenetic histone tail modification of H3S10 phosphorylation at interphase can function as a causative regulator of higher-order chromatin structure in Drosophila in vivo.

Keywords
Histone H3S10 phosphorylation, Chromatin structure remodeling, JIL-1 kinase, Drosophila

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

Comments

Authors
Huai Deng, Xiaomin Bao, Weili Cai, Melissa J. Blacketer, Andrew S. Belmont, Jack Girton, Jørgen Johansen, and Kristen M. Johansen
Ectopic histone H3S10 phosphorylation causes chromatin structure remodeling in Drosophila

Huai Deng¹, Xiaomin Bao¹, Weili Cai¹, Melissa J. Blacketer¹, Andrew S. Belmont², Jack Girton¹, Jørgen Johansen¹ and Kristen M. Johansen¹,*

INTRODUCTION

Almost all known histone modifications correlate with activating or repressive functions dependent on which histone variant or amino acid residue is modified (Allis et al., 2007). However, these histone modifications do not occur in isolation but rather in a combinatorial manner, leading to both synergistic and antagonistic pathways (Allis et al., 2007) in which the same mark may participate (Berger, 2007). This has made it difficult to establish a defined causative biological effect of the addition or removal of a single mark in vivo. In Drosophila it has recently been demonstrated that histone H3S10 phosphorylation by the JIL-1 kinase is important for maintaining chromatin structure and gene expression (Wang et al., 2001; Ebert et al., 2004; Deng et al., 2005; Zhang et al., 2006; Bao et al., 2007). The JIL-1 histone H3S10 tandem kinase localizes specifically to euchromatic interband regions of polytene chromosomes (Jin et al., 1999), and analysis of a JIL-1 null allele, JIL-1z2, has shown that JIL-1 is essential for viability (Wang et al., 2001; Zhang et al., 2003). Furthermore, mutational analysis has demonstrated that a reduction in JIL-1 kinase activity leads to a global disruption of chromatin structure and that maintaining histone H3S10 phosphorylation levels at the euchromatic regions is necessary to counteract heterochromatization and gene silencing (Wang et al., 2001; Ebert et al., 2004; Zhang et al., 2006; Bao et al., 2007). However, we were interested in determining whether phosphorylation of the histone H3S10 residue by the JIL-1 kinase in addition may serve as an epigenetic mark that can play a causative role in establishing euchromatin chromatin regions. To test this hypothesis we applied a LacI-tethering system that has previously been used to study the effects of transcriptional activators on large-scale chromatin structure in mammalian cells (Tumbar et al., 1999; Carpenter et al., 2005) as well as the effects of ectopic HP1 on chromatin structure and gene silencing in Drosophila (Li et al., 2003; Danzer and Wallrath, 2004). Using this approach we show that JIL-1 mediated ectopic histone H3S10 phosphorylation is sufficient to induce a change in higher-order chromatin structure from a condensed heterochromatin-like state to a more open euchromatic state. This effect was absent when a ‘kinase dead’ LacI-JIL-1 construct without histone H3S10 phosphorylation activity was expressed. Instead, the ‘kinase dead’ construct had a dominant-negative effect, leading to a disruption of chromatin structure that was associated with a global repression of histone H3S10 phosphorylation levels. These findings provide direct evidence that the epigenetic histone tail modification of H3S10 phosphorylation at interphase can function as a causative regulator of higher-order chromatin structure in Drosophila in vivo.

KEY WORDS: Histone H3S10 phosphorylation, Chromatin structure remodeling, JIL-1 kinase, Drosophila

MATERIALS AND METHODS

LacI fusion constructs

The DNA-binding domain of the lac repressor from Escherichia coli was fused to the NH2-terminus of the full-length JIL-1 cDNA (Wang et al., 2001) inserted in the pUAST vector. A ‘kinase dead’ lacI-JIL-1 was generated using the Transformer™ Site-Directed Mutagenesis kit (Clon-Tech) to introduce K293A and K652A substitutions in the ATP-binding loops for each kinase domain. The fidelity of the constructs was verified by sequencing at the Iowa State University Sequencing Facility.

Drosophila melanogaster stocks

Fly stocks were maintained according to standard protocols (Roberts, 1998). Lac operator insertion lines and the GFP-lacI fusion line are described in Li et al. (Li et al., 2003) and Danzer and Wallrath (Danzer and Wallrath, 2004). LacI-JIL-1 and LacI-JIL-1 kinase dead pUAST lines were generated by standard P-element transformation (BestGene, Inc.) and driven using the tub-GAL4 (P(tub>CD2>Gal4)) or Sgs3-GAL4 drivers (obtained from the Bloomington Stock Center) introduced by standard genetic crosses.

Immunohistochemistry

Polytene chromosome squash preparations were performed as in Kelley et al. (Kelley et al., 1999) using either 1 or 5 minute fixation protocols and labeled with antibody as described in Jin et al. (Jin et al., 1999). Primary antibodies include chicken anti-GFP (Aves Labs, Tigard, OR), rabbit anti-H3S10ph (Epitomics), mouse anti-Pol II0ser5 (Covance), mouse anti-Pol II0ser2 (Covance), rabbit anti-H4K16ac (Upstate Biotechnology), rabbit anti-BRM (gift from Dr J. Tamkun), mouse anti-lacI (Upstate Biotechnology), rabbit anti-JIL-1 (Jin et al., 1999), chicken anti-JIL-1 (Jin et al., 2003) and anti-JIL-1 mAb S9 (Jin et al., 2000). DNA was visualized by staining with Hoechst 33258 or with propidium iodide (Molecular Probes) in PBS. The final preparations were mounted in 90% glycerol containing 0.5% s-propyl gallate and examined using epifluorescence optics (40× Plan-Neofluar 1.30

¹Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA 50011, USA. ²Department of Cell and Structural Biology, University of Illinois, Urbana, IL 61801, USA.

*Author for correspondence (e-mail: kristen@iastate.edu)

Accepted 4 December 2007
Immunoblot analysis

Immunoblot analysis was performed as described by Wang et al. (2001) using extracts from third-instar salivary glands of the specified genotype. For the quantification of immunolabeling, digital images of exposures of immunoblots on blue X-ray film (Phenix) were analyzed using the ImageJ software as previously described (Zhang et al., 2006).

RESULTS

Within the eukaryotic nucleus, genomic DNA is organized into distinct chromosomal domains consisting of condensed, silent chromatin interspersed with regions of decondensed, transcriptionally active chromatin (Khorasanizadeh, 2004). A clear example of this is found within the band-interband regions observed in Drosophila larval polytene chromosomes (Ananiev and Barsky, 1985; Zhimulev et al., 2004), where gene-active interband regions are made up of parallel 10 nm nucleosome fibrils loosely aligned, whereas in the transcriptionally repressed banded regions the nucleosome fibrils are folded into 30 nm chromosome fibrils that are further compacted into dense higher-order chromatin structures (Ananiev and Barsky, 1985; Zhimulev et al., 2004). The two states of chromatin can be readily distinguished in polytene squash preparations by labeling with the fluorescent DNA dyes Hoechst 33258 or propidium iodide, which bind stoichiometrically to DNA (Haugland, 2002). In order to determine whether ectopic histone H3S10 phosphorylation would be sufficient to induce a change in higher-order chromatin structure and turn a condensed banded chromatin region into an interband region with a more open euchromatic chromatin structure, we applied a LacI tethering system (Tumbar et al., 1999; Li et al., 2003; Danzer and Wallrath, 2004; Carpenter et al., 2005). The tethering system has two components: a reporter transgene containing 256 repetitive binding sites (lacO repeats) for the lac repressor DNA-binding domain (LacI) and a transgene expressing a LacI domain fused to the protein of interest under UAS-GAL4 promoter control. We generated expression stocks containing two transgenes, one encoding the LacI-binding domain fused to full-length JIL-1, and one encoding a ‘kinase dead’ version of JIL-1 in which the crucial lysine for catalytic activity (Bjørbæk et al., 1995) in each of the two kinase domains (K293 and K 652, respectively) was changed to alanine (Fig. 1). In addition, an expression construct containing a transgene encoding green fluorescent protein (GFP) was used as a control (Li et al., 2003).

Fig. 1. Diagrams of the LacI-fusion constructs used for tethering to lacO repeat transgenic insertion lines. The JIL-1 histone H3S10 kinase is a 1207 amino acid protein with two kinase domains, KDI and KDII.

Fig. 2. Ectopic tethering of lacI-JIL-1 fusion protein to a polytene band induces histone H3S10 phosphorylation and chromatin decondensation. (A-C) Triple labelings of polytene squash preparations from third instar Drosophila larvae homozygous for the lacO repeat line P11.3, which is inserted into the middle of a polytene band in region 96C1-2. GFP-LacI was tethered to the lacO repeats in A and LacI-JIL-1 in B, C. GFP-, LacI- and JIL-1-antibody labeling is shown in green, H3S10ph-antibody labeling in red and Hoechst 33258 labeling of DNA in blue or gray. The white arrows indicate the lacO repeat insertion site. The polytene chromosomes from the three preparations are aligned to show the ‘split’ in the polytene bands, reflecting decondensation of the chromatin when lacI-JIL-1 fusion protein is tethered to the band, in contrast to its wild-type morphology, when GFP-Lac is tethered and there is no ectopic upregulation of histone H3S10 phosphorylation. Note: the endogenous JIL-1 and H3S10ph antibody labeling is too weak relative to the LacI-JIL-1 signal and the induced hyperphosphorylation of H3S10 to be clearly visible at this exposure level. (D) Without a GAL4-driver line there is no LacI expression or changes to the band/interband structure. Double labelings with Lac antibody (in green) and Hoechst 33258 (in blue or gray) of polytene squash preparations from third instar larvae homozygous for the lacO repeat line P11.3 and containing a LacI-JIL-1 transgene but without a GAL-4 driver. Arrows indicate the approximate lacO repeat insertion sites.
Ectopic tethering of LacI-JIL-1 induces histone H3S10 phosphorylation and chromatin decondensation

We first analyzed the effect of tethering JIL-1 using line P11.3 and a tub-GAL4 driver for LacI fusion protein expression. The lacO-repeat-containing P-element in P11.3 is inserted into the middle of a polytene band in region 96C1-2 (Li et al., 2003), as verified by PCR analysis (Li et al., 2003) as well as by light and electron microscopy (Novikof et al., 2007). When LacI-GFP was tethered as detected by GFP-antibody (Fig. 2A) condensed morphology of the band in polytene squash preparations from third instar larval salivary glands remained without any obvious subdivisions as labeled by Hoechst 33258. However, when LacI-JIL-1 was tethered to this location as detected by either LacI (Fig. 2B) or JIL-1 antibody (Fig. 2C), the chromatin attained an interband morphology and the band appeared ‘split in two’, reflecting a decondensation of the chromatin. This phenotype was robust and found in at least 75% of the chromosomes examined from more than 30 independent squash preparations, and was not present in the absence of tub-GAL4 induction (Fig. 2D). Furthermore, the tethering of the LacI-JIL-1 fusion protein was clearly associated with distinct hyperphosphorylation of the histone H3S10 residue, as demonstrated by double labeling with H3S10ph antibody. This is in contrast to the tethering of LacI-GFP, which did not result in ectopic or upregulated H3S10 phosphorylation (Fig. 2A). Thus, these experiments strongly suggest that histone H3S10 phosphorylation by the JIL-1 kinase is sufficient to promote striking changes in chromosomal packaging into a more open euchromatic state in an otherwise condensed banded chromatin region that is normally without histone H3S10 phosphorylation.

To verify that these results were not an artifact of the polytene squash method or limited to the P11.3 lacO insertion line, we examined heterozygous preparations of two other insertion lines, P19.9 and 4D5, in addition to P11.3. The lacO repeat line P19.9 is inserted into a band-interband junction in polytene region 63C5 (Li et al., 2003), whereas line 4D5 is inserted into an interband at region 4D5 (Danzer and Wallrath, 2004). In the heterozygous condition only one of the paired chromatids of the polytene chromosomes will carry the lacO repeats, with the other chromatid being wild type. As illustrated in Fig. 3, when LacI-JIL-1 was targeted to such heterozygous polytene chromosome preparations morphological changes and chromatin decondensation predominantly occurred in the chromatid where LacI-JIL-1 was tethered. In the case of the interband and the band-interband junction insertions, the increased accumulation of LacI-JIL-1 at the tethering site nucleated spreading of high levels of LacI-JIL-1 and histone H3S10 phosphorylation to surrounding chromosome regions, in some cases leading to chromatin decondensation of adjacent bands (Fig. 3B-D). Such spreading was also observed in P11.3/+ preparations; however, from this insertion site the spreading was often discontinuous, resulting in small ectopic patches of upregulated LacI-JIL-1 (Fig. 3A). In some preparations the two paired chromatids of the polytene chromosomes were slightly separated at the insertion site, making the LacI-JIL-1-induced changes in chromatin structure especially evident (Fig. 3C,D). This separation may be due to alterations in chromatin alignment caused by the changes in chromatin structure in only one of the chromatids.

Tethering of LacI-JIL-1 ‘kinase dead’ to lacO repeat insertion sites has a dominant-negative effect

To verify that the observed chromatin structure changes depended on JIL-1 kinase-mediated histone H3S10 phosphorylation and not on the tethering of the LacI-JIL-1 construct itself, we examined the effects of tethering a LacI-JIL-1 ‘kinase dead’ construct (LacI-JIL-1-kd). When this construct was expressed in the lacO repeat line P11.3 it accumulated at higher concentrations in the target area (Fig. 4A); however, instead of opening up the compacted chromatin, as in the case when wild-type LacI-JIL-1 was targeted, it induced severe chromatin structure perturbations as well as numerous ectopic contacts between non-homologous chromatin regions (Fig. 4B,C). This phenotype was observed at every target site examined in more than 50 chromosome squash preparations. As illustrated in Fig. 4B, the accumulation of LacI-JIL-1-kd was not associated with any detectable upregulation of histone H3S10 phosphorylation. Furthermore, immunoblot analysis of protein extracts from third instar larval salivary glands (Fig. 4D) showed that endogenous JIL-1 (0.70±0.13 of wild-type levels, n=6), as well as histone H3S10 phosphorylation levels (0.24±0.18 of wild-type levels, n=8), were reduced when LacI-JIL-1-kd was expressed. These results suggest that expression of LacI-JIL-1-kd had a dominant-negative effect and
that it reduced global histone H3S10 phosphorylation levels by displacing native JIL-1 from the normal binding sites of JIL-1. That LacI-JIL-1-kd localized to euchromatic interband regions is illustrated by LacI antibody labeling in Fig. 4A. Furthermore, Fig. 4A shows that this localization often led to chromatin disruption and ectopic chromatin associations at additional chromosome sites to that of the lacO repeat insertion site. The chromatin structure disruption caused by LacI-JIL-1-kd recruitment in homozygous insertion lines was too extensive to allow for the determination of the exact location of the target site (Fig. 4B). However, in some cases remnants of the polytene band-interband organization was sometimes still discernible, as shown in Fig. 4C, which further illustrates that the ectopic chromatin connections between non-homologous chromatin regions near the target sites were associated with high levels of LacI-JIL-1-kd.

**Chromatin remodeling induced by Lac-JIL-1 tethering is not due to a stage-specific developmental program**

The tub-GAL4 driver is active throughout salivary gland polytene chromosome formation. Therefore, in order to test whether LacI-JIL-1-induced histone H3S10 phosphorylation can also effect changes after polytene chromosome band/interband structure has been well established at second and early third instar larval stages (Ananiev and Barsky, 1985; Zhimulev et al., 2004), we performed tethering experiments with the salivary-gland-specific Sgs3-GAL4 driver line. The onset of expression of this driver is not before the mid-third instar larval transition midway through the third larval instar (Cherbas et al., 2003). As illustrated in Fig. 5A,B we observed similar changes in chromatin structure to those induced using the tub-GAL4 driver line.
The LacI-JIL-1 tethering induced chromatin changes are not associated with enhanced transcriptional activity

An important issue is whether the observed changes in chromatin structure are associated with transcriptional activation or whether the changes occur independently of transcription. We therefore labeled the LacI-JIL-1 tethering site in preparations homozygous for the $\text{lacO}$ repeat line P11.3 with antibody to the elongating form of RNA polymerase II (Pol II$^{\text{ser}2}$), which is phosphorylated at serine 2 in the COOH-terminal domain (Weeks et al., 1993; Boehm et al., 2003). As illustrated in Fig. 6A, there is no upregulation of Pol II$^{\text{ser}2}$ labeling at the tethering site and the labeling is several fold less than at adjacent transcriptionally active regions, as indicated by the robust levels of Pol II$^{\text{ser}2}$ at these sites. We also labeled the LacI-JIL-1 tethering site with antibody to the paused form of RNA polymerase II (Pol II$^{\text{ser}5}$), which is phosphorylated at serine 5 in the COOH-terminal domain (Weeks et al., 1993; Boehm et al., 2003). As shown in Fig. 6B, the labeling was absent or at very low levels at the tethering site compared with adjacent interband regions. These data indicate that the chromatin structure changes are likely to be independent of enhanced transcriptional activity.

JIL-1 is associated with the male specific lethal (MSL) dosage compensation complex (Jin et al., 1999; Jin et al., 2000) and therefore potentially could recruit dosage compensation proteins, leading to local acetylation of histone H4K16. However, as illustrated in Fig. 6C, tethering of LacI-JIL-1 does not lead to enhanced histone H4K16 acetylation at the $\text{lacO}$ tethering site. Moreover, the chromatin structure changes resulting from LacI-JIL-1 tethering occur in both male and females, suggesting that it is highly unlikely that the MSL dosage compensation complex contributes to these changes. Another candidate complex to mediate the chromatin structure changes if recruited to the LacI-JIL-1 tethering sites is the Brahma (BRM) chromatin remodeling complex, which is associated with nearly all transcriptionally active chromatin at chromosome puffs and interband polytene chromosome regions (Armstrong et al., 2002). However, as shown in Fig. 6D, levels of the BRM protein are considerably lower at the LacI-JIL-1 tethering site in the homozygous P11.3 $\text{lacO}$ insertion line compared with the levels at adjacent interband regions.

Fig. 5. Tethering of LacI-JIL-1 to $\text{lacO}$ repeat insertion lines using a late-onset $\text{5gs3-GAL4}$ driver. The figure shows polytene squash preparations double labeled with LacI antibody (green) and propidium iodide (red or gray). (A) Homozygous preparation for the band insertion line P11.3. (B) Heterozygous preparation for the interband insertion line 4D5. Arrows in A,B point to areas of compacted chromatin that were decondensed where LacI-JIL-1 was targeted.

Fig. 6. Tethering of LacI-JIL-1 is not associated with upregulation of either Pol II$^{\text{ser}2}$, Pol II$^{\text{ser}5}$, histone H4K16 acetylation or of the BRM complex at the $\text{lacO}$ insertion site. Triple labelings with JIL-1 antibody (in green), Pol II$^{\text{ser}2}$ antibody (A) or Pol II$^{\text{ser}5}$ antibody (B) (in red), and Hoechst 33258 (in blue or gray) of polytene squash preparations from larvae homozygous for the $\text{lacO}$ repeat line P11.3. (C) Triple labeling with JIL-1 antibody (in green), histone H4K16ac antibody (in red) and Hoechst (in blue or gray) of a polytene squash preparation from a male third instar larvae homozygous for the $\text{lacO}$ repeat line P19.9. The upregulation of histone H4K16 acetylation on the male X chromosome (X) is clearly evident in comparison to the normal autosomal level at the tethering site (arrows). (D) Triple labelings with LacI antibody (in green), Brahma antibody (in red) and Hoechst 33258 (in blue or gray) of polytene squash preparations from larvae homozygous for the $\text{lacO}$ repeat line P11.3.
DISCUSSION
A large number of histone modifications, such as acetylation, methylation and phosphorylation, have been correlated with changes in chromatin structure and gene transcription (Allis et al., 2007). The modifications have been broadly classified as either repressing or activating; however, it has become clear that many of these marks may have several complex and seemingly conflicting roles (Berger, 2007). For this reason it has been difficult to assign clear mechanistic functions to these histone marks and to determine whether they represent a cause or an effect. For example, in previous studies of histone H3S10 phosphorylation in Drosophila using mutational analysis (Wang et al., 2001; Zhang et al., 2006) it could not be resolved whether the H3S10ph mark had the capacity to induce chromatin changes or whether it played only a reinforcing or maintenance role. Here we demonstrate using a LacI tethering system that ectopic histone H3S10 phosphorylation by the JIL-1 kinase is sufficient to cause striking changes in chromatin packaging from a condensed to an open state. This effect was absent when a ‘kinase dead’ LacI-JIL-1 construct without histone H3S10 phosphorylation activity was expressed. This indicates that the observed chromatin structure changes depended on JIL-1-kinase-mediated histone H3S10 phosphorylation and not on the tethering of the LacI-JIL-1 construct itself. Instead, the kinase dead construct had a dominant-negative effect, leading to a disruption of chromatin structure that was associated with a global repression of histone H3S10 phosphorylation and not on the tethering observed chromatin structure changes depended on JIL-1-kinase-mediated histone H3S10 phosphorylation levels. Interestingly, these dominant-negative effects of LacI-JIL-1-kd on chromatin structure phenocopy those observed in JIL-1 loss-of-function null mutants (Wang et al., 2001; Deng et al., 2005). Furthermore, using a late-onset driver we show that LacI-JIL-1-induced histone H3S10 phosphorylation can also affect changes after polytene chromosome band/interband structure has been well established at second and early third instar larval stages (Ananiev and Barsky, 1985; Zhimulev et al., 2004). Thus, the changes in chromatin packaging are not likely to depend on constitutive histone H3S10 phosphorylation or a stage-specific developmental program.

Phosphorylation of histone H3S10 has been linked to heat-shock-induced chromatin puffs and transcriptional activation in Drosophila (Nowak and Corces, 2004; Ivaldi et al., 2007), suggesting the possibility that the chromatin changes resulting from ectopic LacI-JIL-1 tethering could be associated with activation of the RNA polymerase II machinery. However, several studies have indicated that the expanded chromatin state during puffing in many cases precedes and/or is separable from gene activation (Meyerowitz et al., 1985; Tulin and Spradling, 2003). Using antibody to the elongating form of RNA polymerase II we did not detect any indications of increased transcriptional activity at the LacI-JIL-1 tethering sites. Similarly, there was no upregulation compared to adjacent interband regions of the BRM chromatin remodeling complex, which has been shown to play a general role in facilitating transcription by RNA polymerase II in Drosophila (Armstrong et al., 2002). Taken together, these results indicate that the histone H3S10-phosphorylation-induced changes observed in this study are not likely to be a consequence of enhanced transcriptional activity. However, it should be emphasized that a role for the BRM complex or other chromatin remodeling complexes in mediating these changes in chromatin structure cannot be ruled out based on the present experiments but will require more comprehensive studies.

The above described observations suggest a model for how targeting of LacI-JIL-1 can establish euchromatic domains in otherwise banded polytene regions with condensed higher-order chromatin (Fig. 7). The presence of an extended region of lacO repeats recruits a high level of LacI-JIL-1, which in turn hyperphosphorylates histone H3S10 at the target site as well as at adjacent chromatin regions (Fig. 7B). The ectopic phosphorylation of histone H3S10 subsequently induces the release of condensing factors and/or recruits euchromatic remodeling factors, resulting in an euchromatic chromatin state (Fig. 7).

Fig. 7. Model for the establishment of an euchromatic chromatin state by ectopic H3S10 phosphorylation. (A) lacO repeats (in red) inserted into a polytene band region with condensed chromatin in the absence of LacI-JIL-1 expression. The region has normal band-interband morphology. (B) When LacI-JIL-1 is expressed, the extended region of lacO repeats recruits high levels of LacI-JIL-1, which in turn hyperphosphorylates histone H3S10 at the target site as well as at adjacent chromatin regions. The ectopic phosphorylation of histone H3S10 subsequently induces the release of condensing factors and/or recruits chromatin remodeling factors, resulting in an euchromatic chromatin state at and near the insertion site.

Development 135 (4)
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. We especially thank Dr L. Wallrath for providing the GFP-lacI transgenic stock 128.1 and the lac operator repeats transgenic stock 4DS and Dr J. Tamkun for the Brahma antibody. This work was supported by National Institutes of Health grant GM62916 to K.M.J. and grant GM58460 to A.S.B.

References


