Binding of Trithorax and Polycomb proteins to the bithorax complex: dynamic changes during early *Drosophila* embryogenesis

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In *Drosophila*, the maintenance of developmentally important transcription patterns is controlled at the level of chromatin structure. The *Polycomb* group (PcG) and *trithorax* group (trxG) genes encode proteins involved in chromatin remodelling. PcG genes have been proposed to act by packaging transcriptionally repressed chromosomal domains into condensed heterochromatin-like structures. Some of the trxG proteins characterized so far are members of chromatin opening complexes (e.g. SWI/SNF and GAGA/NURF) which facilitate binding of transcription factors and components of the basal transcriptional machinery. Genetic and biochemical data suggest that these two groups of regulatory factors may act through a common set of DNA elements. In the present study, we have investigated the binding of Trithorax (TRX) and Polycomb (PC) protein in the bithorax complex (BX-C) during embryogenesis. In addition, we have identified the minimal fragments from the Ultrabithorax (Ubx) regulatory region that are capable of recruiting TRX to chromosomal sites containing them. Comparative analysis of the binding of the two proteins shows that TRX and PC bind target sequences (PcG-regulated elements, PREs) by cellular blastoderm, when BX-C transcription begins. At the same stage, TRX but not PC is strongly associated with core promoters. Later, at germ band extension, the time of derepression in Polycomb mutants, PC binding is also detected outside core PREs and additionally binds to the fragments containing promoters.

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Introduction

In *Drosophila*, the basic body plan is laid down in the early embryo through the action of transiently expressed transcription factors that establish the spatially restricted patterns of homeotic gene expression. These patterns and the determined cell fates they implement are faithfully maintained throughout the rest of development by the action of another set of factors encoded by the Polycomb group (PcG) and trithorax group (trxG) genes. The PcG proteins are required to maintain the transcriptionally inactive state, whereas the trxG proteins are necessary to counteract silencing and maintain the transcriptionally active state. Both PcG and trxG proteins are thought to function by establishing closed or open chromatin configurations at their target genes (for reviews see Paro and Harte, 1996; Pirrotta, 1997).

Mutations in the trithorax (*trx*) gene result in transformations of body structures reminiscent of loss-of-function mutations in homoeotic genes (Ingham, 1985). After disappearance of the transiently acting patterning factors like those encoded by the segmentation genes, maintenance of the initial transcriptional patterns of homoeotic genes requires the trithorax (TRX) protein. *trx* is expressed both maternally and zygotically, and TRX protein is detectable in very early embryos (E.P.Jane and P.J.Harte, unpublished data). Genetic analyses indicate that *trx* expression is required continuously throughout development, consistent with its maintenance function, but there also appears to be a critical early requirement, which if compromised cannot be compensated by subsequent continuous expression (Ingham and Whittle, 1980). Comparatively little is known about the molecular environment in which the TRX protein is integrated. However, an elucidation of this issue could be particularly rewarding as chromosomal aberrations involving the human homologue of TRX (MLL, ALL-1, HRX) is one of the most frequent genetic changes in infant leukemias of myeloid and lymphoid lineage and in treatment-induced secondary leukemias (for review see Look, 1997).

Additional trxG genes have also been identified as suppressors of the derepression phenotypes caused by the breakdown of silencing in PcG mutants (Kennison and Tamkun, 1988). However, this does not necessarily mean that alltrxG proteins play a specific role in countering or modulating PcG repression. While some trxG proteins may function as specific ‘anti-repressors’, directly modulating PcG-mediated repression, others are likely to be involved in more general aspects of transcriptional activation. Indeed, several of the trxG proteins appear to be likely to influence PcG repression indirectly through a more general role in transcription. For example, Moira encodes the SWI2-related TAFII170 protein, which appears to be an integral component of the basal transcription machinery (van der Knaap et al., 1997). Brahma and ISWI (SWI2 homologues; Tamkun et al., 1992), and also the trithorax-like GAGA factor (Farkas et al., 1994) are associated with chromatin remodelling complexes possessing ATP-dependent nucleosome displacement activities, which facilitate the binding of other transcription factors and clearly have more global roles in transcriptional activation (reviewed by Kingston et al., 1996).

All PcG proteins analysed so far have been found to co-localize at many specific chromosomal sites (De Camillis
et al., 1992; Rastelli et al., 1993; Lonie et al., 1994; Carrington et al., 1996), indicating that they interact directly with their target genes to silence them. Some PcG proteins have been further shown to be associated in high molecular weight complexes in vivo (Franke et al., 1992; Jones et al., 1998; Kyba and Brock, 1998), suggesting that silencing requires the assembly of large multiprotein complexes at target genes. So far, none of the characterized PcG proteins has been shown to bind DNA in a sequence-specific manner. However, genetic and biochemical studies clearly identified specific DNA elements responsive to PcG proteins (PcG response elements, PREs) (Castelli-Gair and Garcia-Bellido, 1990; Gyurkovics et al., 1990; Zink et al., 1991; Simon et al., 1993; Chan et al., 1994; Chang et al., 1995; Gindhart and Kaufmann, 1995). A general feature of PREs when analysed in transgenic flies is that a large PRE works better than a minimal PRE. In addition, the latter performs best in combination with regulatory elements taken from its natural gene (Müller and Bienz, 1991; Chan et al., 1994; Müller, 1995).

Co-localization of TRX and PcG proteins on chromosomes suggests that PREs may be closely associated with trxG response elements (TREs). Transgene studies have shown that DNA fragments containing the PRE identified in the 5′ regulatory region of the Ultrabithorax (Ubx) gene also contain a TRE (Chan et al., 1994; Chang et al., 1995; Chinwalla et al., 1995). PREs and TREs have been identified within the same 11 kb fragment of the homeotic gene, Scr (Gindhart and Kaufman, 1995). GAGA factor is intimately associated with PREs and despite its general function in transcriptional activation, also appears to be required for PcG repression, probably by facilitating binding of PcG proteins (Hagstrom et al., 1997; Strutt et al., 1997). So far, a large proportion of members of the trxG and PcG that have been analysed constitutively co-localize at common chromosomal elements, independent of the current transcriptional activity that the element is sustaining. This finding eliminates the simple view that mutually competing activating and repressing complexes maintain the expression status as binding alone appears not to be a hallmark of function. As such it will be important to define the molecular interplay between the various members of the trxG and PcG at the common target sites, in order to assess their respective roles in establishing and maintaining closed versus open chromatin conformations.

We have utilized the formaldehyde crosslinking chromatin-IP methodology (X-CHIP) to map TRX- and PC-binding sites within the BX-C of embryonic chromatin at high resolution. This allowed us to compare the temporal dynamics of TRX- and PC-binding at PREs/TREs during subsequent stages of embryogenesis. Additionally, both proteins were found to interact with promoters of homeotic genes. We find that PC and TRX first bind at their primary sites around cellular blastoderm. Interestingly, at that stage PC is not present on chromosomal regions flanking PREs as is seen later when implementation of PcG silencing is completed. In contrast, by germ band extension high levels of PC are found on fragments encompassing core PREs as well as on flanking regions.

Fig. 1. Mapping of TRX binding sites within the large 5′ regulatory region of the Ubx gene on polytene chromosomes. A schematic view of the Ubx 5′ regulatory region is shown at the top. The co-ordinates are in relation to the numbering of the BX-C sequence (DDBJ/EMBL/GenBank accession No. U31961; Martin et al., 1995). The arrow indicates the start of transcription. The thick line indicates the beginning of the coding sequence. The major PRE (Chan et al., 1994; Strutt et al., 1997) is indicated below the line as a black bar and selected enhancers are indicated as white blocks (Chan et al., 1994) The PRE region is expanded above, corresponding to the clone p338 used in this study. The fragments of the Ubx regulatory region contained in P element constructs analysed are shown below, with the restriction sites defining their ends indicated (S, Stul; K, KpnI; Sa, Sau3AI; E, EcoRI; P, PstI; N, NdeI; St, StyI). Whether each construct creates new TRX binding sites at their insertion sites is indicated (+/-) to the right of each construct. The constructs used have been previously described (see Materials and methods). Note that only constructs containing the principle PRE bind TRX in this assay. 19UZ has an internal 2 kb deletion generated by removal of two Sau3AI fragments that span the PRE.

Results

Mapping TRX binding sites on Ubx regulatory sequences in larval polytene chromosomes

The TRX protein binds to at least 75 discrete sites on the polytene chromosomes of the larval salivary glands, including sites of known target genes; the BX-C, the Antennapedia complex (ANT-C; Kuzin et al., 1994; Chinwalla et al., 1995) and the engrailed gene (Breen et al., 1995). PcG proteins co-localize with TRX at more than half of these sites. For comparison with sites identified by the X-CHIP method (see below), we have also used this chromosome binding assay to map TRX binding sites within the Ubx gene, which is transcriptionally inactive in the salivary gland and has no prior history of transcriptional activity in the progenitors of this tissue. We previously showed that a P-element construct containing 14 kb of the 5′ regulatory region of Ubx produces a novel TRX binding site at its chromosomal insertion site, indicating that a TRX binding site lies within this 14 kb (Chinwalla et al., 1995).

Figure 1 summarizes results of further analyses of constructs covering the 36 kb of 5′ sequence flanking the Ubx coding sequence. The only detectable TRX binding in this entire region occurs within a single small 670 bp fragment corresponding to the core of the major Ubx PRE.
Developmental profile of PC and TRX binding

Fig. 2. TRX binding to transgenes containing Ubx regulatory regions. On polytene chromosomes, TRX binds to a single region within the large 5′ regulatory region of the Ubx gene, corresponding to the principle Ubx PRE. (A) Chromosome arm 2L from a wild-type larva. (B) The same arm from a transformant containing the 670 bp PstI–NdeI fragment of the PRE inserted at 65B. Note the new TRX binding site at 65B. The same result was obtained using HRP-linked secondaries.

We detect no TRX binding to other fragments that do not contain the core PRE. To allow for the possibility that there might be some weak TRX binding sites, whose occupancy might depend on the presence of other neighbouring or even distant sites, we tested two large fragments flanking the PRE that together cover the entire region analysed minus the PRE: the 22 kb KpnI–StuI fragment extending from the PRE to within the Ubx coding sequence (including the promoter, transcription start site and 1 kb of 5′ untranslated sequence) and the adjacent 14 kb KpnI–XbaI fragment extending further 5′ towards the abd-A gene and containing an internal deletion of the PRE. The binding of several PcG proteins to these constructs was also monitored (data not shown) and parallels the results seen with TRX: no TRX- or PcG-binding is detected on fragments that do not contain the PRE (Figure 1; Chan et al., 1994). The failure to detect TRX binding to these large fragments in salivary gland nuclei suggests that if additional TRX binding sites do exist outside the PRE, binding to them either does not occur in this tissue, or is dependent on the presence of the PRE on the same fragment for their stable binding, or is not detectable in this assay. As we suggested previously (Chinwalla et al., 1995), given the transcriptional inactivity of Ubx in the salivary gland, binding of TRX to the PRE is likely to be constitutive or depend on other factors that bind constitutively in this region.

Co-localization of trithorax and Polycomb proteins in the BX-C analysed by chromatin immunoprecipitation

We have adjusted the X-CHIP methodology to be suitable for the analysis of material derived from embryos. The choice of the DNA targets of the BX-C to be analysed (Figure 3) is based on our previous detection of PC, GAGA factor and TRX distributions in SL2 tissue culture cells (Strutt et al., 1997; V.Orlando and R.Paro, unpublished data).

To determine when TRX binds to the BX-C and whether its binding exhibits temporal changes during early development when PcG silencing is established, in vivo fixed chromatin was prepared from embryos of three different stages; 0–2, 2–5 and 5–8 h after egg laying. It should be noted that chromatin collected from 0–2 h embryos will largely represent the late embryonic stages of this window as the very early zygotes will only have a few nuclei. The hybridization signals obtained with the TRX-IP-enriched DNAs are shown in Figure 4. The corresponding signal intensities were quantitated and plotted according to their position on the tested fragments (Figures 5 and 6). At pre-blastoderm stages (0–2 h) TRX is not associated with any BX-C sequences. Between 2 and 5 h (early blastoderm to extended germ band) TRX becomes associated with the Fab7-PRE (p4344; Mihaly et al., 1997), the PRE in Mcp (p5989; Busturia et al., 1997), and the Ubx core PREs in the bxd and bx regions (p3384 and p2571) (Figure 5). In addition, TRX is also found at the transcription start site of the Abd-B γ-promoter (p8106; see also note in legend to Figure 6), the abd-A
promoter (p1894) and the Ubx promoter (p3901). This early binding of TRX to the BX-C sequences coincides with the previously described early temperature-sensitive period of \( \text{trx}^1 \) (Ingham and Whittle, 1980). Interestingly, within these sites substantial changes in the TRX binding patterns occur between 2 and 5, and 5 and 8 h and coincide with changes in PC binding at these same sites during this period (Figures 5 and 6; see below).

**Binding profile of PC during embryo development**

In PcG mutants, the breakdown of silencing and consequent ectopic expression of the AN-C and BX-C homeotic genes is first detectable at the beginning of germ band extension. This indicates that PC is not required for establishing the initial boundaries of homeotic gene expression and suggests that it might not associate with the BX-C and AN-T-C genes until just before derepression is detected in PcG mutants. However, PC protein is present during oogenesis and a substantial amount of maternally synthesized PC is present in the early embryo, and may also have a role at earlier stages of embryogenesis (Denell, 1982; Haynie, 1983; Sato and Denell, 1985; Paro and Zink, 1993). In addition, early roles of certain PcG proteins in repression of segmentation genes have been documented (McKeon et al., 1994; Pelegri and Lehmann, 1994).

To determine when PC first associates with the BX-C and whether its pattern of binding changes with time, we have studied PC binding to the BX-C during the same stages of early embryogenesis examined for TRX binding. The same blots used for studying TRX distribution were stripped and reprobed with DNA obtained from PC-IPs (Figure 4 and corresponding plots in Figures 5 and 6). At pre-blastoderm stage (0–2 h) PC is present in significant amounts on sites in the \( iab-2 \) (p3870) and \( iab-4 \) (p7652) regions, on the \( \text{abd-A} \) promoter (p1894) and to a minor extent in the \( \text{bxd} \) region (p3384). This PC must be of maternal origin since zygotic transcription has not yet begun at this stage. Interestingly, a number of early noncoding transcripts, whose functions, if any, remain elusive, originate from the \( \text{bxd} \) and \( iab-4 \) regulatory regions where PC is bound at this stage (Lipshitz et al., 1987; Zavortink and Sakonju, 1989). After 2–5 h, PC is found on most core PREs.

In comparison with previously reported PC binding in SL2 cells, several features of PC binding in embryos at pre-blastoderm stage are worth noting. The first is the general absence or low-level binding to regions adjacent to PREs. This suggests that PC binding to target sites in the BX-C is restricted to small chromosomal elements at this stage. We do not believe that this is due to unequal
PCR amplification, since the input DNA in the immunoprecipitation and the quantity of PCR products were the same in all samples.

The second feature is a series of temporal changes in the PC-binding pattern that occur concomitantly with changes in the TRX binding pattern. For example, at 2–5 h the Fab7-PRE is poorly enriched for PC, whereas TRX is present prominently present. PC is present on a distal EcoRI–XhoI 1482 bp fragment (p4344). After 5–8 h the situation has changed: PC is strongly present on the 1482 bp fragment, adjacent fragments (XhoI–XbaI 884 and 778 bp in p4344) and the core PRE, while remarkably, TRX is removed from the core PRE. We also analysed later stages of embryogenesis and did not observe any further changes in the distribution of PC and TRX (not shown), suggesting that the pattern of binding of both proteins becomes mature and stable during germ band extension (5–8 h).

The PC binding seen in early embryogenesis (0–2 h) in the iab-2, iab-4, bxd and abd-A promoter regions is probably not related to the inactive state of the BX-C genes at this time. It is more likely to represent constitutive binding to certain ‘core’ sites, some of which probably play important roles in organizing the mature pattern that emerges after 5–8 h. However, the strong and dynamic association of TRX and PC with transcription start sites is intriguing and may represent their ultimate sites of action. Promoter association also seems very likely to be dependent on the transcriptional status of each gene in a particular cell and may involve the recruitment of PC
Fig. 6. Binding of PC and TRX to Ubx, abd-A and Abd-B promoters during different embryonic stages. Each panel is a schematic view of PC (upper)- and TRX (lower)-binding to particular restriction fragments of BX-C promoter-containing clones (see Figure 3). Transcription start sites are indicated by an arrow. Note that in the case of the Abd-B-γ promoter the TRX peak is in the AccI–AccI fragment 5’ to the published site of the promoter located 153 bp downstream. However, the beginning of the Abd-B-γ transcript was deduced from cDNA analysis and not from 5’ extension analysis (Kuziora and McGinnis, 1988). As such, the real 5’ end of the transcript might be located within the adjacent AccI fragment encompassing the TRX peak. Images were processed as in Figure 5.

and/or TRX from PREs by specific activators or components of the general transcriptional machinery assembled at the promoter.

Discussion

This study investigates the binding of TRX and PC proteins to the BX-C and their dynamic changes during embryogenesis. By using the X-CHIP method we show that binding of PC and TRX occurs much earlier than previously thought, and that the binding profile changes significantly during the first several hours of embryogenesis involving PREs, flanking regions and core promoters.

However, the interpretation of this set of data has to take into account the intrinsic heterogeneity of the material analysed. Unlike the previously analysed material from SL2 tissue culture cells, embryonic material contains a mixture of cells expressing various patterns of BX-C genes. In fact, at cellular blastoderm the Ubx and abd-A genes show rather broad transcription domains that only later become spatially restricted (Beachy, 1990; Karch et al., 1990). The Abd-B gene shows a fairly restricted pattern already at cellular blastoderm (Kuziora and McGinnis, 1988). At the same stages another large untranslated transcript located upstream the Ubx promoter (bsd; Lipschitz et al., 1987) and including the bsd-PRE is produced in PS 6–13 (Irish et al., 1989). Therefore, concerning BX-C gene transcriptional activity, a certain degree of heterogeneity is also intrinsic in some cases at the single-cell level. Nevertheless, we appear to observe defined binding profiles at the different PREs and promoters, despite the different ratios of cells in the mixture expressing specific subsets of BX-C genes. As such, the X-CHIP results certainly forbid an extrapolation of the found PC–TRX interactions to the single-cell level. However, the observed developmental profiles still appear to allow an assessment of how the two proteins bind to the majority of their target sites.

Binding of PC and TRX to the BX-C regulatory regions becomes evident at cellular blastoderm. PC is already associated with all the BX-C PREs at this stage, but not significantly with flanking regions and promoters as is evident at later stages. TRX is also associated with all the BX-C PREs at this stage, before reductions in BX-C transcription become evident in trx mutants (Breen and Harte, 1991; Sedkov et al., 1994). This suggests that PREs/TREs are pre-loaded with both trxG and PcG components poised for maintenance of both active and repressed transcriptional states before either are apparently required. In addition to PREs/TREs, both TRX and PC show strong association with transcription start sites in the BX-C. In gastrulating stages TRX is strongly associated with the Ubx, abd-A and Abd-B-γ core promoters (Figure 6), whereas PC is virtually absent at this stage. At later stages, as the expression domains of the homeotic genes become fixed, TRX is much reduced at promoters and at most PREs/TREs, though this reduction might also reflect the increasing complexity of the material used. These dynamic changes in the binding of PC and TRX to PREs/TREs and promoters of target genes may point to important transitions necessary for the establishment of the cellular memory mechanism supported by the PcG and trxG.

Extension of PC-binding profile over developmental time

Our results show an increase in binding sites of PC during embryonic development. While in early embryos,
fragments containing core PREs are co-precipitated with PC, in material from 5–8 h embryos PC is also bound in adjacent fragments, extending in some cases over several thousand base pairs. This late pattern, which according to the genetic data should represent the fully functional state, resembles the results we obtained with SL2 tissue culture cells (Orlando and Paro, 1993; Strutt et al., 1997). However, such an extended binding pattern differs substantially from what we observe with a sequence-specific protein like the GAGA factor, where only single fragments encompassing the consensus binding sites are immunoprecipitated (Strutt et al., 1997). Also, TRX depicts a much more restricted binding profile in late stages. Unfortunately, the underlying structures connected to the individual binding profiles remain elusive. PcG proteins were proposed to keep their target genes inactive by using heterochromatin-like structures (Paro, 1990). However, our current knowledge of the structure of heterochromatin is limited to yeast telomeres, where a spreading by an interaction of the regulating SIR protein complexes with the nucleosomal backbone has been demonstrated, utilizing different means including a X-CHIP analysis (Hecht et al., 1995; Strahl-Bolsinger et al., 1997; Boscheron et al., 1996). In the case of Drosophila it remains undetermined whether the extended binding profile seen for PC uncovers a similar path. Alternative explanations that suggest an interaction of PcG proteins with weak PREs scattered along the BX-C (Pirrotta, 1997) are also supported by our data as there is insufficient resolution to distinguish between the two possibilities. On similar lines, the binding of PC and TRX to promoter regions found here might indicate a direct interaction of PREs/TREs with promoters (Bienz and Müller, 1995). However, whether this is achieved by looping, as has been suggested for enhancer–promoter interactions, is an issue we cannot resolve on the basis of our X-CHIP results presented here. Clearly, additional methodology and analyses are needed to increase the resolution and uncover the molecular interactions involved in generating the higher order chromatin structures regulated by the PcG and trxG.

**PRE and TRE: common elements involved in repression and anti-repression**

It appears that the early transcriptional state of a promoter at the time PcG silencing is implemented is decisive in conferring subsequent repressive or activating function to PREs/TREs in a particular cell (Poux et al., 1996). In this light, the communication of PREs/TREs with specific subsets of enhancers is likely to be important for their function (Sigrist et al., 1997; Zhou et al., 1997). This suggests a direct interference with some aspects of enhancer-mediated activation in chromatin. In mammalian cells enhancers have been shown to directly counteract repressive chromatin (Walters et al., 1996). In yeast, UAS-regulated transcription requires SWI/SNF gene products. Interestingly, promoters with a deleted UAS are capable of directing stable high levels of transcription when combined with mutations in repressing chromatin components (Prelich and Winston, 1993). Therefore, at least UAS-dependent activated transcription may be by-passed if chromatin-mediated repression is defective. This suggests an intriguing interdependence between a stable dominant effect of repressing chromatin and specific activation pathways that involve remodelling of repressive chromatin.

By definition, PREs are responsible for the maintenance of the inactive state of homeotic genes in the absence of the early trans-acting repressors. PcG defects are suppressed by trxG mutations by preventing the ectopic overexpression of homeotic genes. One possibility is that PcG loss of function could be suppressed by loss of enhancer function involving general transcription activators. Another possibility is that the dominant-negative action of the PRE on the enhancer could be counteracted specifically by trxG proteins at TREs, when these have to act to maintain the active state. This would imply the action of more specialized activators, like TRX, at TREs. Genetic analyses of deletions of the BX-C Fab-7 boundary/PRE element shows that trxG are required for preventing the PRE-PcG repression. When boundary and PRE elements are both deleted trxG alleles show no effect (Mihaly et al., 1997). Therefore, the TRE-function in the PRE could be specifically responsible for counteracting PcG repression.

An anti-repression model would explain why the dose of trxG and PcG proteins at a certain time and place in the embryo plays an important role in stabilizing early determined states in chromatin. It was previously shown that increasing amounts of a transcriptional activator can displace proportional amounts of Pc at an ectopic PC binding site (PRE) on polyten chromosomes created by insertion of an inducible reporter-flanked PRE (Zink and Paro, 1995). TRX may exert or mediate an analogous competitive effect on PcG silencing. Consistent with this possibility, the dominant homeotic derepression phenotypes seen in Pc mutants are enhanced by extra copies of trx+ and suppressed in flies carrying only one copy of the trx gene (Capdevila and Garcia-Bellido, 1981; Capdevila et al., 1986; Kennison and Russell, 1987). Elimination of the maternally provided component of PC has been shown to increase the Pc phenotype (Denell, 1982). Finally, co-transfection experiments with the Ubx PRE/TRE carrying reporter constructs have shown dose-dependent competitive interactions between PC and TRX in the activation and repression of the reporter (Chang et al., 1995). This suggests that the relative amounts of PC and TRX proteins at early stages of embryogenesis may be crucial. While TRX is required continuously throughout development (Ingham, 1985), the early temperature-sensitive period (~0–4 h) determined using the trx1 allele (Ingham and Whittle, 1981) very probably reflects a critical early requirement for TRX, which cannot be compensated by providing it later (Ingham, 1981). We start to see a co-localization of PC and TRX during this time, suggesting that its role involves preventing implementation of the beginning of PcG silencing.

**Interaction of PC and TRX with core promoters**

The interaction of TRX and PC with both PREs/TREs and promoters seems to be an important issue of PcG function. In transgenic flies, it has been shown that the major Ubx PRE (bxd) works more efficiently when assayed together with the Ubx promoter (Chan et al., 1994; Müller, 1995), which is also found in coin-transfection experiments using tissue culture cells (Chang et al., 1995). Also, the transcriptional activity of a gene at early stages
of embryogenesis interferes with establishment of PcG silencing (Poux et al., 1996). Our finding that in early embryogenesis PC and TRX are dynamically associated with core promoters suggests that these proteins somehow interfere with enhancer–promoter interactions.

The mechanism of enhancer action is not fully understood. In particular, typical enhancer binding proteins like the segmentation gene products have been shown to act by direct interference with distinct elements of the basal transcription apparatus (Sauer et al., 1995). In addition, tissue-specific TAFs have been described to support the action of developmentally regulated genes (Sauer et al., 1996). It could be that in the initial transition phase PC stabilizes a repressed conformation of enhancer–promoter by contacting some repressive components of the basal transcriptional machinery. In a subsequent step the inactive expression state might become stabilized by extending PcG complexes over the respective cis-regulatory elements. Such structures might be accompanied by specific chromatin modifications inducing changes in the acetylation patterns of histones and other associated chromosomal proteins (reviewed by Pazin and Kadonaga, 1997). In such a scenario maintenance of the repressed state would imply stable association of histone de-acetylase factors specifically targeted by the PcG/trxG proteins to these regulatory regions.

Materials and methods

Mapping TRX binding sites on polytene chromosomes


References


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