FEBS Letters 567 (2004) 8–14 FEBS 28368

Minireview

Lifting a chromosome: dosage compensation in *Drosophila melanogaster*

Gregor D. Gilfillan[1], Ina K. Dahlsveen[1], Peter B. Becker*

Adolf-Butenandt-Institut, Molekularbiologie Schillerstrasse 44, 80336 Munich, Germany

Received 11 March 2004; accepted 14 March 2004

Available online 22 April 2004

Edited by Horst Feldmann

Abstract Twofold differences in gene expression levels can be vital for an organism. This is beautifully illustrated by the process of 'dosage compensation' in *Drosophila*, which doubles transcription from the single male X chromosome to equal the mRNA levels originating from the two X chromosomes in female cells. Failure of the process leads to male-specific lethality. A number of recent publications have furthered our understanding of the ribonucleoprotein complex, which mediates dosage compensation and how it targets the male X chromosome. Deciphering the principles of X chromosome recognition and the nature of the chromatin configuration, that allows fine-tuning of transcription, remain the most interesting challenges.

Keywords: Chromatin; Histone acetylation; MSL; Non-coding RNA; Transcription

1. Dosage compensation and epigenetic gene regulation

In diploid cells, proteins are commonly expressed from both alleles and their expression levels are adjusted accordingly. Exceptions from this rule are imprinted genes, which are not considered here, and X chromosomal genes in species with heteromorphic sex chromosomes, like humans and fruit flies. In these cases the female genotype features two X chromosomes, whereas male cells carry only one X chromosome in addition to the gene-poor Y chromosome. Fine-tuning of protein expression levels involves compensation of the different doses of X-linked genes in the two sexes. During evolution, several parallel dosage compensation strategies have arisen [1]. Inactivation of one female X chromosome in humans leaves both sexes with one active allele, which is regulated to meet the needs of the cell. More complicated regulatory schemes are in place in the worm, *Caenorhabditis elegans*, where both female X chromosomes are dampened to half activity, and in fruit flies, where the single male X chromosome is activated twofold. The twofold adjustments in transcription implemented in the latter two cases are vital: failure is lethal for male flies and worms. In all three systems mentioned, dosage compensation involves changing the transcription rates of relevant genes through modulation of their chromatin organization. This is most obvious in the human case, where one of the two female X chromosomes is globally inactivated by conversion of the chromatin into facultative heterochromatin [2,3].

The folding of the nucleosomal fibre into higher-order structures may lead to states that are either repressive or permissive for transcription. The precise nature of these configurations is not known, let alone the specifics that allow fine tuning of transcription in a twofold range. However, it is clear that chromatin folding correlates with patterns of post-translational histone modifications and corresponding non-histone proteins involved in setting and interpreting these modification marks [4,5]. Site-specific, post-translational modifications of the N-terminal domains of histones are hallmarks of chromatin configurations with functional consequences. Among them, histone phosphorylation and acetylation are suited to constitute quick, reversible switches between functional states, whereas methylation appears to be most suited to implement a lasting chromatin configuration. The observation of a wealth of different modifications has led to the concept of a 'histone code' according to which patterns of modifications determine the functional state of chromatin [5].

Inactivation of one of the human X chromosomes involves methylation of both histone and DNA components of chromatin [3]. By contrast, activation of the single male X chromosome in *Drosophila* is at least in part due to acetylation of histone H4 at lysine 16 (H4K16) [6,7]. The mechanistic relationship between certain modifications and functional states is not known. Methylated histones can be bound by non-histone proteins involved in chromatin organization, such as the heterochromatin protein 1 or the developmental repressor polycomb [8,9]. So far no ligand for acetylated H4K16 has been identified. In any case it is assumed that modification of histone N-terminal tails will affect the tightness and type of chromatin folding by modifying the tails' molecular interactions. Setting up a particular chromatin configuration is a multi-step process coordinated by complex assemblies of proteins. Interestingly, in both human X inactivation and fly X activation, long non-coding RNAs are crucial for the process [10,11]. 'Coating' of the chromatin with these regulatory RNAs provides a visual impression of their involvement in altering chromatin structure.

The rich phenomenology of dosage compensation in human cells, worms and flies provides excellent opportunities for exploring the intricacies of gene regulation through chromatin organization. Mainly due to its potential for genetic analysis, our understanding of the process in *Drosophila* is most

* Corresponding author. Fax: +49-89-5996-425.
E-mail address: pbecker@med.uni-muenchen.de (P.B. Becker).

[1] These authors contributed equally to this work.

0014-5793/$22.00
doi:10.1016/j.febslet.2004.03.110

advanced. In what follows we will review the most recent progress in this area and summarize the state of knowledge in a speculative model.

2. The dosage compensation complex of *Drosophila*

Dosage compensation in *Drosophila* is mediated by a ribonucleoprotein complex known as the dosage compensation complex (DCC), which forms in male cells and coats most of the X chromosome. Five protein components of the DCC are generally known as the MSL (male-specific lethal) proteins: MSL1, -2 and -3, MLE (maleless; a helicase) and MOF (males absent on the first; a histone acetyltransferase (HAT)) (Fig. 1). Much of what is known about these proteins has been reviewed recently elsewhere [12]. A sixth protein member of the DCC, the JIL1 histone H3 kinase, is also enriched on the X chromosome and interacts with the MSL proteins [13,14]. No MSL phenotype has yet been identified for JIL1 as it is essential in other processes [15], but hypomorphic JIL1 alleles produce a lower male to female ratio [14]. The example of JIL1 reminds us that factors which have additional, more general functions, besides their roles in dosage compensation, will not have been identified by screens for male-specific lethality. In addition to the protein factors, two non-coding RNAs, *roX1* and *roX2* (RNA on the X) are essential, albeit redundant, components of the DCC [10,16–18].

Targeting of the MOF HAT results in X-specific acetylation of H4K16, which correlates with reduced chromosome compaction relative to the autosomes and the female X chromosomes [6,19]. H4K16 acetylation by MOF has been shown to cause de-repression of chromatin transcription in vitro and in vivo [7].

3. Assembly of the DCC

In early development, the mechanism of sex determination detects gender and directs the appropriate male or female development pathways, including the appropriate expression of the DCC in males. The master regulator of the sex determination pathway is *sex lethal* (SXL) (for review see [20]). Gender determination by SXL involves a number of numerator (X-linked) and denominator (autosomal) loci used to somehow sense the X to autosome ratio [21]. SXL expression is thereby activated and maintained by positive feedback in female cells, triggering the female development pathway. SXL concomitantly inhibits translation of the MSL2 mRNA by blocking its interaction with the ribosome [22]. Suppression of MSL2 prevents DCC formation in females, as MSL1 and MSL3 require MSL2 protein for sustained expression [23,24] (see Fig. 1). Initial doses of the other MSL proteins are maternally provided [25], but MSL2 expression in male embryos at blastoderm is necessary and sufficient to trigger DCC formation. Accordingly, ectopic expression of an MSL2 transgene in females is sufficient to induce complex formation on both female X chromosomes, accompanied by a drastic reduction in female viability [24]. Initial transcription of *roX1* RNA is independent of the MSLs and occurs also in females, although the RNA soon requires MLE for stability [26]. *roX2* expression is male-specific and occurs slightly later in embryogenesis. However, continued expression and stability of the *roX* RNAs depends on the MSL proteins [26]. Complete DCC is first observed binding to the male X chromosome approximately 3 h after egg laying, just prior to detection of the acetylation of H4 lysine 16 [26].

A number of biochemical studies have documented the protein–protein interactions within the DCC. Briefly, MSL1 is able to interact with MSL2, MSL3 and MOF and hence coordinate their assembly into a stable, soluble complex with robust and specific HAT activity in the absence of RNA [14,24,27,28]. A host of genetic studies imply an interdependence of the complex components for assembly which have been used to suggest a pathway for the stepwise assembly of the DCC (for review, see [29]). Accordingly, MSL1 and MSL2 are at the base of DCC assembly, since they are interdependent for X chromosome binding and will do so in the absence of any of the other sub-units in vivo [30]. However, the recent study of Meller [26] highlighted the maternal provision of all MSLs

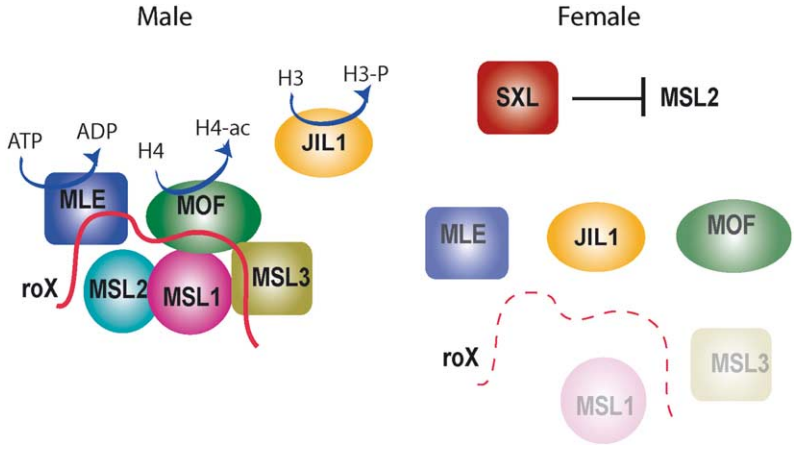

Fig. 1. Assembly of the DCC. In males, expression of MSL2 mediates stable expression of the other protein and RNA sub-units, and coordinates their assembly into the DCC. The complex possesses helicase/ATPase (MLE), HAT (MOF) and histone kinase (JIL1) activities. In females, SXL is expressed and blocks MSL2 production. The resulting deficiency for MSL2 prevents DCC formation and results in lower expression and/or instability of MSL1, MSL3 and both roX RNAs (shown here by their transparency). In contrast, MLE and MOF may also have yet unidentified roles in females, as both proteins are expressed.

save MSL2, raising the possibility that zygotic transcription of MSL2 may simply complete an already organized complex.

Of considerable interest are the interactions between *roX* RNAs and the MSL proteins as well as the role of these RNAs in DCC function. MLE, an RNA helicase, may act as an RNA chaperone required to integrate *roX* RNA into the DCC. Deletion of MLE limits the localization of both *roX* RNAs to their sites of synthesis [31]. Besides MLE, DCC sub-units MSL3 and MOF are also able to bind RNA. Their interaction with the X chromosome is sensitive to RNA degradation in isolated nuclei [6,32–35], suggesting that they contact *roX* RNA in vivo. MSL3 requires MOF for X chromosome binding, and both MSL3 and MOF require MLE [36,37]. Interaction with RNA (and hence chromosomal association of the RNA binding sub-units) may be a regulated event. Akhtar and colleagues [35] found that MOF is capable of acetylating MSL3 and that this acetylation led to a loss of MSL3's RNA binding and X chromosome association. Acetylation of MSL3 may therefore represent a regulatory modification controlling association with the X chromosome. MOF is also capable of acetylating MSL1 and of self-acetylation [28,35], opening further possible avenues for regulation.

A complex consisting of only DCC proteins (and no *roX* RNA) is partially functional and is able to support survival if MSL1 and MSL2 are overexpressed in flies [34]. This indicates that *roX* RNA function is more prominent at limiting physiological MSL protein levels. Under normal conditions, incorporation of *roX* RNA is necessary for dosage compensation: simultaneous deletion of *roX1* and *roX2* is lethal for males. Although *roX1* and *roX2* show almost no sequence similarity and are expressed at different times during embryonic development, they have redundant functions as either can be singly deleted without loss of dosage compensation [18,26,31]. This suggests that different complexes can exist with either RNA, but it is not clear if any complexes contain both RNAs. Likewise, the stoichiometry of the MSL proteins in a functional DCC is not known.

4. DNA determinants of DCC association with the X chromosome: entry sites

Mutations in any MSL protein sub-unit abolish coating of the X chromosome by the DCC and dosage compensation. However, mutations in MLE, MSL3 or MOF reveal a drastically reduced, yet reproducible set of binding sites for partial complexes on the X chromosome (Fig. 2). These are proposed to be high affinity 'entry sites', that may serve as nucleation sites from which the complex can spread in *cis* to coat adjacent regions of the chromosome [38–40]. Interestingly, two of these entry sites are found within the *roX1* and *roX2* genes [31,38]. The discovery that the *roX* genes not only contained high affinity sites, but could also mediate 'spreading' of the DCC from an autosomal transgene into flanking chromatin [31,38,41] led to the proposal of the 'two step' model of DCC recruitment. In this model, complexes first assemble at one of the 35–55 high affinity 'entry sites', then 'diffuse' preferentially in *cis* to coat the remainder of the X chromosome (spreading).

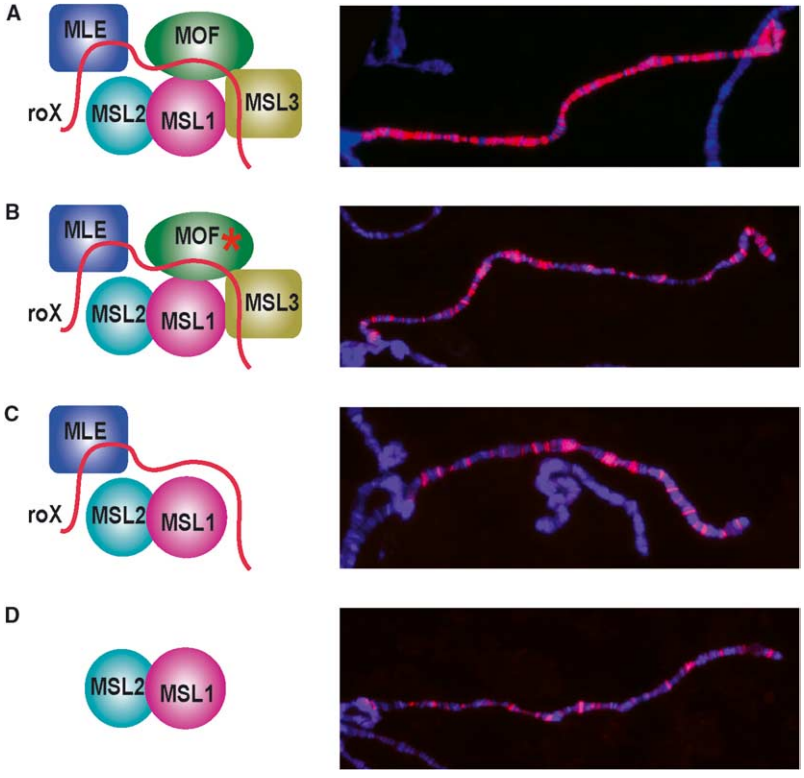

Fig. 2. A hierarchy of binding sites for the DCC. All images are of polytene chromosomes stained for MSL1 in red and DNA in blue. In the wild-type male (A), the DCC binds to hundreds of sites on the X chromosome. In a *mof1* mutant (B), the DCC is complete, but not functional and binds to about 60–70 sites. In the absence of MSL3 and/or MOF (C), a partial complex binds to about 55 of these sites. Finally, in the absence of MLE (D), the MSL1 and MSL2 minimal complex binds reproducibly to about half of these sites and weakly to the rest.

Several studies have recently shed light on the question of how the DCC is targeted to the X chromosome, including the nature of the entry sites, the role of the RNAs, as well as the importance of tight regulation of DCC concentration (see below). As more phenomena are being revealed, the simple 'spreading model' has to be revised [42].

Over 50 entry sites of varying intensities have been mapped cytologically on polytene chromosomes [30,40]. This is illustrated in Fig. 2, which also summarizes the binding patterns of partial and non-functional complexes in other mutant backgrounds. A minimal complex consisting of only MSL1 and MSL2 can only bind reproducibly to about half of the full set of sites, but when MLE is present, the affinity for most of the sites is increased. The same set of sites were also observed to recruit complete DCC under conditions of low complex concentrations achieved by reducing the amount of MSL2 protein [40]. These genetic studies therefore suggest that there exists a hierarchy of entry sites to which the DCC has different affinities and that partial complexes may only be able to bind to the highest affinity sites (see Fig. 3). Curiously, none of the DCC components have so far been shown to have sequence-specific DNA binding activity.

The nature of the 'entry sites' is obviously of great interest. They can be studied in vivo by integration of X-derived DNA into autosomes [38]. If these sequences can attract the DCC to the autosomal insertion, particularly in the absence of MSL3, it contains an 'entry site'. This assay, combined with chromatin immuno-precipitation and DNase I hypersensitive site analysis, allowed mapping of the *roX1* and *roX2* entry sites to 200–300-bp fragments within the genes [31,38,39,43]. Comparison of these sequences to the corresponding *roX* sequences from other *Drosophila* species highlighted several short islands of sequence conservation within a 110-bp region [43]. Mutation of these sequences produced a variable reduction of DCC binding in wild-type larvae. Although these analyses suggested the first sequence determinants of DCC binding in vivo, related sequences from the X chromosome identified by homology in silico did not recruit the DCC.

Characteristically, insertion of the *roX* entry sites on autosomes not only leads to recruitment of both complete and partial DCCs, but in addition complexes can be seen to spread out from the insertion site to label distinct interbands on polytene chromosomes [31,38,41]. This 'spreading' into flanking chromatin is highly dependent on the levels of DCC present and whether the endogenous *roX1* and *roX2* sites are still present on the X chromosome ([34,41,43] and see below). Interaction of the DCC with the *roX* entry sites is particularly dependent on the presence of at least one *roX* RNA and MLE [39,43] but independent of transcription through the site [18,39].

Obviously, a more general 'entry site' definition requires comparison of more than just two sequences. However, the recent characterization of a third entry site, the first outside of a *roX* gene, did not allow further refinement of the definition. The 'entry site' within the 18D region of the X chromosome does not share any obvious sequence similarity with the *roX* sites and is also clearly different in other respects [42]. Unlike the *roX* entry sites, the 18D site does not seem to be transcribed, nor does it require *roX* RNA to attract the DCC. Binding of partial DCC complex in *msl3* mutants could not be demonstrated to a distinct sequence element of less than 510 bp unless multimerized. Finally, autosomal spreading from the 18D site was very limited.

The operational definition of 'entry sites' appears, therefore, to subsume diverse sites of MSL binding, which may vary in sequence, structure and in affinity for complete or partial DCC

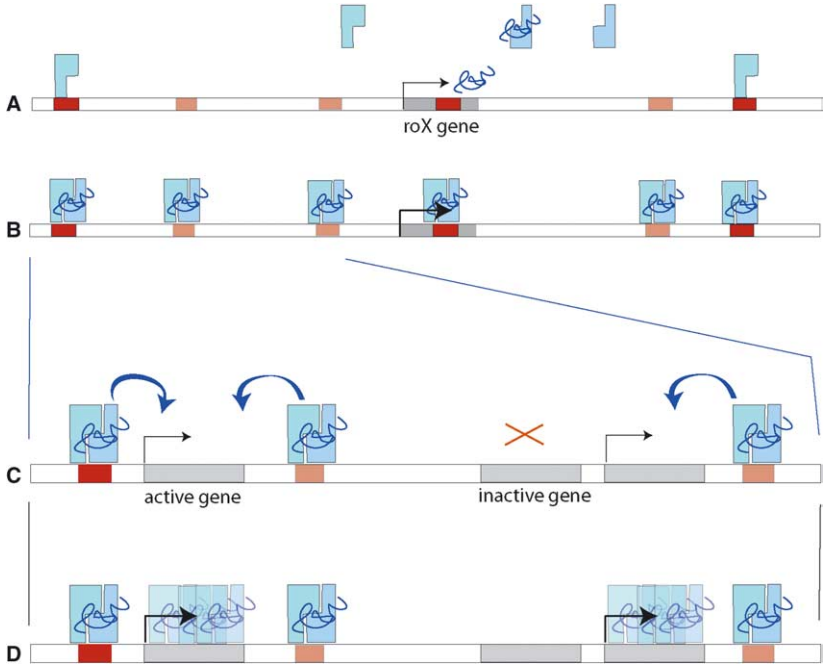

Fig. 3. Speculative model of how dosage compensation may be achieved. (A) Initially, only DC sub-complexes bind to high affinity 'entry' sites (dark red). *roX* RNA is bound by MSL proteins as it is being transcribed from the X chromosome. (B) Complete DCC is able to interact with secondary (light red) binding sites. (C) 'Spreading' of DCC involves association with unknown features of transcriptionally active chromatin (arrows symbolize transcription), but is not propagated to inactive genes (red X). (D) The presence of the DCC at sites of active transcription leads to doubling of RNA production.

complexes. Furthermore, many X chromosome-derived sequences are able to recruit the DCC when inserted into autosomes in wild-type males even if they do not contain a mapped entry site [42]. This suggests that in addition to the entry sites, which attract DCC sub-complexes (e.g., those lacking MSL3) under 'stringent' conditions, other DNA sequence determinants exist on the X chromosome, able to attract intact DCC. The X chromosome, therefore, appears to contain a much higher number of specific binding sites for targeting than previously thought.

5. The role of roX RNA in targeting

Available data are consistent with a role of *roX* RNA not for assembly of the DCC, but for its proper binding to and distribution over the X chromosome. In the absence of *roX* RNA, low levels of DCC can be found ectopically bound to autosomal sites indicating that the RNAs may assist in targeting the DCC specifically to the X chromosome [31]. The RNA seems to affect targeting in multiple ways. First, proper targeting and distribution of the DCC on the X chromosome apparently requires well-balanced levels of protein and RNA components. Unbalancing these levels by genetic trickery leads to interesting phenomenology, which may suggest targeting rules. For example, increasing the levels of MSL1 and MSL2 in flies lacking one *roX* gene leads to accumulation of these proteins around the remaining *roX* gene [34]. This has been interpreted to mean that increased levels of protein components lead to very efficient, but premature, completion of complex assembly at the site of *roX* transcription. The result is that the DCC is less able to move to other sites. The underlying assumption here is that sub-complexes may be more 'mobile' than complete complexes and, hence, that the chromosome interactions are highly dynamic.

A number of studies indicate that the *roX* genes compete for a limiting pool of MSL proteins, which they direct preferentially to the chromosome in which they reside. These results suggest that the transcription of the *roX* RNAs indeed has a role in targeting or sequestering the complex to the X chromosome, possibly by ensuring high local concentrations of the complex [34,41,43]. On the other hand, *roX* RNAs can also function in *trans*, suggesting that they also have a functional role in the recognition of the X chromosome, conceivably through interaction with the protein components. The lethality caused by lack of both *roX* genes on the X chromosome can be rescued by an ectopic autosomal *roX* transcript [18,44]. This indicates that the *roX* RNA can explore the nucleoplasm in search of high affinity ligands and that X chromosomal targeting determinants are sufficiently strong to attract the complete DCC, even without the concentration effect of local transcription. The entry sites found within the two *roX* genes are also not strictly required for the initiation of faithful targeting of the DCC, since *roX1* or *roX2* RNAs lacking their entry sites can rescue a double *roX* mutant [43]. The functional elements of *roX* RNA, which may correspond to binding sites for MSL proteins and perhaps other, yet unknown factors, are still mysterious. A recent detailed study indicated that *roX1* RNA contains several redundant functional elements, although the 3′ 600 bp appeared to be most important for processing and localization [44]. *roX1* may thus contain multiple functional elements, which could either contribute to targeting of the complex or serve as a scaffold for the arrangement of MSL proteins within the DCC, as has been suggested for the *Xist* RNA involved in mammalian dosage compensation [11].

6. 'Spreading' of the DCC over the X chromosome

In support of the two-step targeting and spreading model [38], the ATPase and HAT activities of MLE and MOF, respectively, are not required for initial entry site recognition but are essential for spreading [45] (Fig. 2). The *roX* RNAs are also required for spreading [18,41]. However, considering the multitude of DCC binding sites with different properties, the model is clearly an over-simplification. Accordingly, the model has since been modified to incorporate a significant amount of spreading by soluble complex in *trans* [34,41].

Considering the two steps of the model, 'recruitment' to entry sites and 'spreading' into neighbouring chromatin, interesting analogies to the dosage compensation processes in humans and *C. elegans* can be observed. The process of X inactivation in humans is a multi-step process involving several chromatin modifications. It is initiated by the stabilization of a non-coding RNA, *Xist*, transcribed from a tightly regulated X inactivation centre (Xic) on the chromosome to be inactivated [2,11]. Through unknown principles, the Xist RNA is then directed to selectively 'coat' the X chromosome to be inactivated, which in turn leads to implementation of heterochromatin through successive histone deacetylation, histone methylation, incorporation of histone variants, and, finally, DNA methylation [2,11]. Most recently, evidence for a 'two-step' model of dosage compensation involving the recruitment of a DCC to defined sites on the X chromosomes, followed by 'spreading' into the neighbouring chromatin, has also been obtained in *C. elegans* [46].

But what does 'spreading' really mean? The term suggests a continuous propagation from an initiation point (e.g., an entry site) into the neighbouring chromatin, but does not indicate what the 'unit size' of chromatin interaction might be. Conceptually, DCCs could make contacts with each individual nucleosome of the chromosome as they move along a nucleosomal array, or just with the outside of a 30-nm chromatin fibre. Alternatively, in a scenario of different extremes, the DCC may just concentrate in specialized locus control regions dedicated to specifying the activity of a large chromosomal domain [47]. Spreading of heterochromatin is a generally accepted phenomenon in position effect variegation, where propagation of constitutively heterochromatic regions (such as pericentromeric and sub-telomeric regions) can be seen into neighbouring euchromatin if heterochromatin proteins are overexpressed [8]. Similarly, spreading of the DCC is facilitated when MSL1 and MSL2 are overexpressed, leading to promiscuous binding to many autosomal sites including the heterochromatin of the fourth chromosome and the chromocentre [40]. A careful regulation of MSL and *roX* RNA concentrations is therefore important to limit DCC activity to appropriate targets [34,40,41].

However, if 'spreading' of the DCC involves a continuous propagation of complexes, there must be principles that restrict this propagation in analogy to boundary elements, which limit the action of heterochromatin components [40,45]. This is because the binding pattern of the DCC in wild-type males is quite discontinuous and includes many gaps [40]. These sites

where the DCC is absent may correspond to the condensed, gene-poor polytene bands, blocks of inactive genes, or to areas filled with genes whose activity is compensated by an alternative strategy (for example, by down-regulation in females by SXL [24]). Spreading from autosomal *roX* inserts is similarly discontinuous and may 'jump' over several hundreds of kilobases [41]. This suggests that the process does not involve a continuous wrapping of the chromatin fibre, but rather that the DCC travels in a saltatory fashion. The question, then, becomes as to what the determinants of the distributed (non-entry) binding sites might be. The observation that the DCC was recruited to actively transcribed genes [48] may suggest that some aspect of transcription, such as a specific chromatin configuration or even the polymerase machinery itself, could be recognized. However, this type of targeting to active genes would have to be discriminative as well, as there are several transcriptionally active areas on the X chromosome which are devoid of DCC (I.K.D., unpublished).

Several of the above-mentioned observations challenge the previous assumption that DCC distribution may mainly be due to regulated *cis* and/or *trans* spreading originating from just a few entry sites. In addition, the fact that about half of the gaps in DCC staining on the X chromosome reside immediately adjacent to entry sites [40] suggests that bidirectional spreading in *cis* from these sites does not occur, at least at the level of resolution characteristic of polytene chromosome analysis. Furthermore, part of the third chromosome transposed to the X chromosome did not attract DCC binding despite being flanked by regions of bound complex and the presence of an entry site nearby, arguing against indiscriminate spreading in *cis* and *trans* [42].

Considering these findings, a revised model may be proposed (Fig. 3). Accordingly, transcription of MSL2 and stabilization of MSL1 lead to formation of partial DCCs complexes able to bind to high affinity sites on the X chromosome (Fig. 3(a)). Transcription of the *roX* RNAs from the X chromosome and their incorporation into close-by complexes leads to formation of complete complexes, with enhanced affinity for high and moderate affinity sites, which they reach by diffusion (Fig. 3(b)). As the complex levels rise, lower affinity sites are increasingly recognized, which reinforces the accumulation of the complex throughout the X chromosomal territory. Once a sufficiently high level of complex has been reached, the DCC is attracted locally to active genes in order to compensate transcription (Fig. 3(c) and (d)). According to this model, the closer a gene is to high affinity sites (in space, not necessarily on a linear chromatin fibre!) the more likely its encounter with DCCs would be. This model explains variable- and concentration-dependent spreading from *roX* genes on autosomes, as these could potentially contain low affinity sites for binding that are recognized at high complex concentration. It also explains that overexpression of MSL1 and MSL2 leads to increased autosomal binding, as once complex levels are sufficiently high, the DCC can spread outside the X chromosomal territory and locate moderate or low affinity sites on autosomes.

7. More questions than answers

Unresolved questions revolve around the role of the critical acetylation of H4K16 in the compensation process. In vitro and in vivo experiments show that this single modification can lead to de-repression of transcription on chromatin templates [7]. Is the dosage compensation machinery then solely a complex device to assure proper targeting of MOF activity? Dosage compensation in flies does not involve derepression of a chromatin template, but a twofold enhancement of whatever transcriptional activity would be characteristic for a given gene, which may require feed-back principles yet to be uncovered. Other chromatin features yet to be described may also contribute to proper gene regulation by the DCC. For example, we currently know nothing of the role of JIL1 in dosage compensation; since H3 phosphorylation at serine 10 is a hallmark of immediate-early gene activation, particularly if combined with H3 acetylation [49] this modification may play a role as well. Currently, the best proposal of how the altered chromatin configuration might be translated into a twofold enhancement of gene expression is that it affects transcription at the level of elongation [48]. Recently, with the identification of new factors dedicated to regulating RNA polymerase elongation in the chromatin fibre, the complexities of this process have been further appreciated [50]. Inheritance of gene expression patterns through epigenetic principles requires that a stable chromatin configuration is propagated from one chromosome generation to the next, independent of the cues that originally defined the pattern. Whether this is the case for dosage compensation in *Drosophila* can only be evaluated once the principles are known by which the X chromosomal domains are identified. Among the pertinent questions to be answered is whether this identification happens only once during early embryonic development (in which case the maintenance of the pattern will rely on purely epigenetic principles), or after each round of DNA replication. The MSL proteins remain associated with the male X chromosome throughout the cell cycle ([51]; T. Straub, I.K.D. and P.B.B, unpublished observations) and may therefore be directly involved in the definition of the hyperactive nuclear domain. Repressive, heritable chromatin configurations commonly rely on stable methylation of DNA and histones, which is reinforced by interacting proteins and tethering of methylases. Certain methylation marks, such as methylation of histone H3 lysine 4, are correlated with active chromatin [52]. It will be interesting to find out whether stable modifications of this kind are involved in permanent marking of the hyperactive X chromosome, or whether H4K16 acetylation (perhaps in combination with histone H3 phosphorylation) may fill the role of epigenetic mark for the activation needed in *Drosophila* dosage compensation. Alternatively, perhaps the DCC requires the flexibility to rapidly compensate activated X-linked genes, yet also to be rapidly removed when these genes are turned off again, as part of a developmental program. Relatively stable histone methylation would therefore be inappropriate and a modification with a rapid turnover desirable.

The concerted modulation of transcriptional activity of large chromosomal domains is a requirement for dosage compensation in species as different as flies, worms and humans. Because several key RNA and protein components involved in dosage compensation in *Drosophila* are known, the system appears well suited to unravel basic principles that assure discrimination between the chromosomes and the assembly and inheritance of chromatin configurations that permit the fine-tuning of transcription within a twofold range. It is likely

that these basic principles will turn into recurring themes once we know more about dosage compensation in diverse species.

Acknowledgements: Research on dosage compensation in the laboratory of the authors is supported by the Deutsche Forschungsgemeinschaft through 'Transregio 5' and the BMBF Verbundprojekt PTJ-Bio/0313030B. We thank M. Chioda and V. Maier for critical reading of the manuscript.

References

[1] Pannuti, A. and Lucchesi, J.C. (2000) Curr. Opin. Genet. Dev. 10, 644–650.
[2] Cohen, D.E. and Lee, J.T. (2002) Curr. Opin. Genet. Dev. 12, 219–224.
[3] Avner, P. and Heard, E. (2001) Nat. Rev. Genet. 2, 59–67.
[4] Jenuwein, T. and Allis, C.D. (2001) Science 293, 1074–1080.
[5] Fischle, W., Wang, Y. and Allis, C.D. (2003) Curr. Opin. Cell Biol. 15, 172–183.
[6] Smith, E.R., Pannuti, A., Gu, W., Steurnagel, A., Cook, R.G., Allis, C.D. and Lucchesi, J.C. (2000) Mol. Cell. Biol. 20, 312–318.
[7] Akhtar, A. and Becker, P.B. (2000) Mol. Cell 5, 367–375.
[8] Richards, E.J. and Elgin, S.C. (2002) Cell, 489–500.
[9] Orlando, V. (2003) Cell 112, 599–606.
[10] Kelley, R.L. and Kuroda, M.I. (2000) Cell 103, 9–12.
[11] Wutz, A. (2003) Bioessays 25, 434–442.
[12] Akhtar, A. (2003) Curr. Opin. Genet. Dev. 13, 161–169.
[13] Jin, Y., Wang, Y., Johansen, J. and Johansen, K.M. (2000) J. Cell Biol. 149, 1005–1010.
[14] Wang, Y., Zhang, W., Jin, Y.H., Johansen, J. and Johansen, K.M. (2001) Cell 105, 433–443.
[15] Zhang, W., Jin, Y., Ji, Y., Girton, J., Johansen, J. and Johansen, K.M. (2003) Genetics 165, 1341–1354.
[16] Meller, V.H., Wu, K.H., Roman, G., Kuroda, M.I. and Davis, R.L. (1997) Cell 88, 445–457.
[17] Amrein, H. and Axel, R. (1997) Cell 88, 459–469.
[18] Meller, V.H. and Rattner, B.P. (2002) EMBO J. 21, 1084–1091.
[19] Bone, J.R., Lavender, J., Richman, R., Palmer, M.J., Turner, B.M. and Kuroda, M.I. (1994) Genes Dev. 8, 96–104.
[20] Penalva, L.O. and Sanchez, L. (2003) Microbiol. Mol. Biol. Rev. 67, 343–359, table of contents.
[21] Cline, T.W. and Meyer, B.J. (1996) Annu. Rev. Genet. 30, 637–702.
[22] Gebauer, F., Grskovic, M. and Hentze, M.W. (2003) Mol. Cell 11, 1397–1404.
[23] Palmer, M.J., Richman, R., Richter, L. and Kuroda, M.I. (1994) Genes Dev. 8, 698–706.
[24] Kelley, R.L., Solovyeva, I., Lyman, L.M., Richman, R., Solovyev, V. and Kuroda, M.I. (1995) Cell 81, 867–877.
[25] Rastelli, L., Richman, R. and Kuroda, M.I. (1995) Mech. Dev. 53, 223–233.
[26] Meller, V.H. (2003) Mech. Dev. 120, 759–767.
[27] Scott, M.J., Pan, L.L., Cleland, S.B., Knox, A.L. and Heinrich, J. (2000) EMBO J. 19, 144–155.
[28] Morales, V., Straub, T., Neumann, M., Mengus, G., Akhtar, A. and Becker, P.B. (2004) EMBO J., in press.
[29] Amrein, H. (2000) Genome Biol. 1, Reviews 1030.
[30] Lyman, L.M., Copps, K., Rastelli, L., Kelley, R.L. and Kuroda, M.I. (1997) Genetics 147, 1743–1753.
[31] Meller, V.H., Gordadze, P.R., Park, Y., Chu, X., Stuckenholz, C., Kelley, R.L. and Kuroda, M.I. (2000) Curr. Biol. 10, 136–143.
[32] Richter, L., Bone, J.R. and Kuroda, M.I. (1996) Genes Cells 1, 325–336.
[33] Akhtar, A., Zink, D. and Becker, P.B. (2000) Nature 407, 405–409.
[34] Oh, H., Park, Y. and Kuroda, M.I. (2003) Genes Dev. 17, 1334–1339.
[35] Buscaino, A., Kocher, T., Kind, J.H., Holz, H., Taipale, M., Wagner, K., Wilm, M. and Akhtar, A. (2003) Mol. Cell 11, 1265–1277.
[36] Gorman, M., Franke, A. and Baker, B.S. (1995) Development 121, 463–475.
[37] Gu, W., Szauter, P. and Lucchesi, J.C. (1998) Dev. Genet. 22, 56–64.
[38] Kelley, R.L., Meller, V.H., Gordadze, P.R., Roman, G., Davis, R.L. and Kuroda, M.I. (1999) Cell 98, 513–522.
[39] Kageyama, Y., Mengus, G., Gilfillan, G., Kennedy, H.G., Stuckenholz, C., Kelley, R.L., Becker, P.B. and Kuroda, M.I. (2001) EMBO J. 20, 2236–2245.
[40] Demakova, O.V., Kotlikova, I.V., Gordadze, P.R., Alekseyenko, A.A., Kuroda, M.I. and Zhimulev, I.F. (2003) Chromosoma 112, 103–115.
[41] Park, Y., Kelley, R.L., Oh, H., Kuroda, M.I. and Meller, V.H. (2002) Science 298, 1620–1623.
[42] Oh, H., Bone J.R. and Kuroda, M.I. (2004). Curr. Biol., 14, 481–487.
[43] Park, Y., Mengus, G., Bai, X., Kageyama, Y., Meller, V.H., Becker, P.B. and Kuroda, M.I. (2003) Mol. Cell 11, 977–986.
[44] Stuckenholz, C., Meller, V.H. and Kuroda, M.I. (2003) Genetics 164, 1003–1014.
[45] Gu, W., Wei, X., Pannuti, A. and Lucchesi, J.C. (2000) EMBO J. 19, 5202–5211.
[46] Csankovszki, G., McDonel, P. and Meyer, B.J. (2004) Science 303, 1182–1185.
[47] Bulger, M., Sawado, T., Schubeler, D. and Groudine, M. (2002) Curr. Opin. Genet. Dev. 12, 170–177.
[48] Sass, G.L., Pannuti, A. and Lucchesi, J.C. (2003) Proc. Natl. Acad. Sci. USA 100, 8287–8291.
[49] Thomson, S., Clayton, A.L. and Mahadevan, L.C. (2001) Mol. Cell 8, 1231–1241.
[50] Svejstrup, J.Q. (2002) Curr. Opin. Genet. Dev. 12, 156–161.
[51] Lavender, J.S., Birley, A.J., Palmer, M.J., Kuroda, M.I. and Turner, B.M. (1994) Chromosome Res. 2, 398–404.
[52] Kouzarides, T. (2002) Curr. Opin. Genet. Dev. 12, 198–209.