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Balancing up and downregulation of the *C. elegans* X chromosomes

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Abstract

In *Caenorhabditis elegans*, males have one X chromosome and hermaphrodites have two. Emerging evidence indicates that the male X is transcriptionally more active than autosomes to balance the single X to two sets of autosomes. Because upregulation is not limited to males, hermaphrodites need to strike back and downregulate expression from the two X chromosomes to balance gene expression in their genome. Hermaphrodite-specific downregulation involves binding of the dosage compensation complex to both Xs. Advances in recent years revealed that the action of the dosage compensation complex results in compaction of the X chromosomes, changes in the distribution of histone modifications, and ultimately limiting RNA Polymerase II loading to achieve chromosome-wide gene repression.

Introduction

Dosage compensation balances the gene expression of the X and autosomes while equalizing sex chromosome-linked gene expression levels between the sexes. Recent genomic analyses of gene expression uncovered evidence for the presence of dosage compensation in some organisms and evidence for the absence, or incomplete nature, of dosage compensation in others [1]. The three mechanistically best understood examples of chromosome-wide dosage compensation include the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and mammals (especially mice and humans).

In *Caenorhabditis elegans*, males have one X chromosome, and hermaphrodites have two. The ratio of X chromosomes to autosomes (X:A ratio), regulates both sexual fate and dosage compensation through a complex molecular chromosome counting mechanism [2]. Dosage compensation in this organism is believed to involve two mechanisms. First, upregulation of the X chromosome balances X-linked gene expression levels to autosomes in males [3,4].

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However, unlike in the fruit fly, where upregulation is limited to males, in *C. elegans* the X chromosomes appear to be upregulated also in hermaphrodites. This upregulation leads to overexpression of the Xs, necessitating the second mechanism: downregulation of both X chromosomes by a factor of two [5] (Figure 1). Research in the past few years focused on elucidating the mechanism of the hermaphrodite-specific X-downregulation, mediated by the dosage compensation complex (DCC). However, some studies also provided hints as to the mechanism of X-upregulation.

The dosage compensation complex

The DCC binds along the length of both hermaphrodite X chromosomes to downregulate gene expression [5]. Most components of the DCC were originally identified in genetic screens for sex-specific phenotypes [6–12]. Since X-repression is hermaphrodite-specific, but X-upregulation is thought to occur in both sexes, by design, these screens would identify components of the DCC, but not the X-upregulation machinery. Additional subunits were identified by nature of their biochemical interaction with known DCC components [13,14].

The DCC contains a five-subunit condensin complex, condensin I^{DC} [13–18], and five associated proteins [19–23] (Figure 2). Condensin complexes function to compact and segregate chromosomes during mitosis and meiosis [24]. Condensin I^{DC} appears to be a bona fide condensin. In fact it shares several subunits with mitotic condensins (Figure 2) [13,17]. Yet it differs from canonical condensins in both chromosome-specificity and cell cycle specificity of function [13,17]. Additional non-condensin DCC members, notably SDC-2, DPY-30, and SDC-3, dictate chromosome specificity of DCC binding [19,21,22]. Some additional DCC members also function outside dosage compensation. DPY-30 functions as part of the histone methyltransferase complex COMPASS/MLL [22], and DPY-21 regulates growth and metabolism downstream of TOR complex 2 both in the context of the DCC and independently of it [*25] (Figure 2).

Robust X-chromosome specific binding of the DCC also requires sumoylation of several subunits, SDC-3, DPY-27 and DPY-28 [*26]. DPY-28 also functions in the context of condensin I, but it is only sumoylated when part of the DCC (Figure 2). Depletion of SUMO reduced X chromosome binding of condensin I^{DC}, but not binding of SDC-2 and DPY-30, indicating that sumoylation is needed for stable interaction between X targeting proteins and other DCC members, rather than initial X recognition [*26]. Interestingly, SUMO depletion also leads to the appearance of novel DCC binding sites on autosomes [*26], suggesting that while SUMO may be required to restrict DCC binding to the X chromosome. This latter interpretation is similar to a previous finding, where restriction of DCC binding to the X chromosome required the histone variant HTZ-1 [27]. Whether DCC restriction to the X is through sumoylation of a DCC subunit or some other protein is not known.

DCC-mediated changes in chromatin structure and compaction

The similarity of condensin I^{DC} to mitotic condensin suggests that repression of the X chromosome may be mediated by a mechanism similar to mitotic chromosome condensation. Although this was suggested decades ago [15], experimental evidence only emerged recently. Chromosome volume measurements demonstrated that the DCC

compacts the X chromosomes (Figure 3) [28]. While the X chromosome represents 18% of the genome in hermaphrodites, physically it occupies only about 10% of the nuclear volume. When the DCC is not present, the X decompacts to its expected size. These results are consistent with the general idea that more compact chromatin represses transcription.

The DCC modifies the X chromatin at the level of nucleosome structure as well. Interestingly, the modifications acquired by the dosage compensated X mirror changes that occur at mitosis. The dosage compensated X is enriched for monomethylation of H4 lysine 20 (H4K20me1) [29,30] and is depleted of H4 lysine 16 acetylation (H4K16Ac) compared to autosomes [30] (Figure 3). These changes are also seen on mitotic chromosomes [31–33]. X-chromosome enrichment of H4K20me1 requires not only the activity of the DCC, but also the activities of SET-1 (homolog of H4K20 monomethylase Pr-Set7) and SET-4 (homolog of H4K20 di- and trimethylase Suv4-20h1/h2) [29,30]. In mammals, levels of both Pr-Set7 and H4K20me1 increase in G2, remain high in mitosis, then decline in G1, suggesting that this modification functions in mitosis [31,32]. Two condensin II subunits can directly bind to an H4 tail peptide monomethylated at K20, suggesting a role for this modification in condensin II loading [34]. Thus, a link between condensin and H4K20me1 is suggested both in mitosis and in dosage compensation, although in mitosis H4K20me1 is proposed to recruit condensin (II), while in dosage compensation, a condensin I-like complex is required for the X-enrichment of H4K20me1.

Interestingly, dosage compensation appears disrupted both in *set-1* mutants (which lack H4K20me1) and in *set-4* mutants (which have higher than normal level of H4K20me1 evenly distributed across the nucleus) [25,30]. These results suggest that it is not H4K20me1 per se that is required for dosage compensation, but rather a difference in H4K20me1 levels between the X and the autosomes. This interpretation is also consistent with earlier gene expression studies which showed that defects in DCC activity lead to an increase in X-linked gene expression and a decrease in autosomal gene expression [35]. These observations evoke a balancing act between the X and the autosomes, rather than a regulatory process limited to the X chromosomes.

Enrichment of H4K20me1 on the X chromosomes can be achieved by a DCC-dependent increase in SET-1 activity or reduction SET-4 activity on the X, or a combination of both. These different models make different predictions as to the changes in global H4K20me1 levels in DCC mutants. Two studies that addressed this question obtained conflicting results [25,29], and therefore the question of how the DCC influences SET-1 and SET-4 activities remains open. Another unexplored possibility is that an H4K20 demethylase activity [34] also contributes to the process.

H4K20me1 antagonizes H4K16ac [36]. It is therefore not surprising that H4K16ac is depleted on dosage compensated X chromosomes [30]. This depletion depends not only on the DCC and the histone deacetylase SIR-2.1, but also on the activities of SET-1 and SET-4. Thus, H4K20me1 levels do in fact influence H4K16ac levels in the context of dosage compensation. However, the reverse is not true; changes in H4K16ac do not result in perturbation of the X-enrichment of H4K20me1, suggesting that H4K20me1 acts upstream of H4K16ac [30]. Both enrichment of H4K20me1 and depletion of H4K16ac appear to

contribute to compaction of the dosage compensated X chromosomes, since the X appears decompacted in *set-1*, *set-4* and *sir-2.1* mutants [**28]. However, while *set-1* and *set-4* depletion is sufficient to rescue males that inappropriately activate the DCC (and consequently die of insufficient X chromosome expression), *sir-2.1* is not [*30], indicating that the gene expression changes caused by the lack of *set-1* or *set-4* are more significant than the changes caused by lack of *sir-2.1*. One possible explanation is that low levels of H4K16ac may be a consequence, rather than a cause, of differences in gene expression. In addition, H4K20me1 influences other aspects of chromosome dynamics. For example, L3MBTL1 was shown to drive chromatin compaction via interactions with H4K20me1 [37].

DCC-mediated changes in transcription

Repression by the DCC ultimately leads to a decrease in X-linked transcript levels [9]. In dosage compensation mutants, the increase in X-linked mRNA levels is accompanied by a decrease in autosome-linked mRNA levels [35]. Consistent with that, ChIP-chip experiments in these mutants revealed an increased level of RNA Polymerase II (Pol II) occupancy on X-linked [22]. To determine which step in transcription is regulated, a recent study mapped transcriptionally engaged Pol II using global run-on sequencing (GRO-seq) and defined transcription start sites using a modified form of GRO-seq to enrich for RNAs with a 5'-cap (GRO-cap) [**38]. GRO-seq revealed a uniform increase in transcriptionally engaged Pol II along the gene bodies of X-linked genes in dosage compensation mutants, suggesting that dosage compensation restricts either recruitment or initiation of Pol II. An increase in hypophosphorylated (uninitiated) Pol II at transcription start sites further suggested that the predominant step of regulation is recruitment [**38] (Figure 3).

Evidence for X-upregulation

Ohno argued in 1967 that the X chromosome in monogametic males needs to be upregulated to create a genomic balance between the X and autosomes [39]. It was not until decades later that experimental evidence emerged to support this hypothesis in *C. elegans* [3,4]. These studies showed that the average gene expression level from the single male X appears comparable to the average gene expression level from autosomes. A recent study found evidence both for and against X-upregulation in three additional nematode species [*40]. When they limited analysis to highly expressed genes (to exclude germline-repressed genes), all chromosomes, including the X, showed comparable levels of gene expression in both males and females/hermaphrodites, supporting the X-upregulation hypothesis. However, analysis of a limited number of one-to-one orthologs located on the X in one species and on an autosome in another indicated that the autosomal ortholog is expressed at a higher level, arguing against X-upregulation [*40]. Perhaps X-upregulation is not complete and is limited to a subset of X-linked genes.

The GRO-seq studies described above confirmed that the X chromosomes in DCC mutant hermaphrodites are more transcriptionally active than the autosomes [**38]. In wild type hermaphrodites, levels of transcriptionally engaged Pol II on the X chromosomes (postulated to be subjected to both upregulation and downregulation) are equivalent to levels on autosomes. Furthermore, in dosage compensation mutant hermaphrodites, levels of

engaged Pol II on the X (subject to upregulation only) are elevated compared to autosomes [**38]. Males were not analyzed in this study.

Consistent with X-upregulation in males, chromosome volume measurements suggest that the male X is unexpectedly decondensed [**28]. The model summarized in Fig. 1 predicts that the volume of the single male X should be half of the two Xs in DCC mutant hermaphrodites. However, measurements showed that the male X occupies an even larger area of the nucleus, suggesting that the male X is different from the Xs in DCC mutant hermaphrodites [**28]. One possibility is that DCC depletions were incomplete in this study, leading to less than maximal decondensation in hermaphrodites. However, levels of decondensation were remarkably consistent between different DCC depletions and mutants, arguing against this possibility [**28]. A similar difference between the male X and the DCC-depleted hermaphrodite X was also observed with respect to H4K16ac levels [*30]. While wild type hermaphrodite X chromosomes are depleted of H4K16ac, DCC mutant hermaphrodite X chromosomes are enriched for this modification, as observed by fluorescent microscopy. High levels of H4K16ac are consistent with an upregulated state. However, this enrichment was not observed on the male X chromosome [*30]. An alternative possibility is that DCC activity is not the only difference between male and hermaphrodite X chromosomes, and additional sex- or karyotype-specific disparities exist, perhaps regulated by a feedback loop between sex determination and dosage compensation [6,41–43].

The onset of dosage compensation

Establishment of dosage compensation is a multistep process. Interestingly, there is no evidence of X-upregulation temporarily preceding X-downregulation, and thus representing a “first” step prior to the “second” downregulation step. In fact, the X in early embryos, both male and hermaphrodite, appears underacetylated (suggesting that it is hypotranscribed) compared to autosomes [*44]. The X chromosome is enriched for genes which are not turned on until the 40–60 cell stage in development [45], the same time when the DCC assembles on the X chromosomes [21]. Thus, there does not appear to be a time in wild type hermaphrodite development when the X is hypertranscribed and needs to be downregulated. Instead, the two processes may be initiated simultaneously. Consistently, the X in male embryos appear to transition from a hypoacetylated state to a presumably hypertranscribed state at about this time in development, although the exact timing is hard to pinpoint without better molecular markers [*44].

The time window in which dosage compensation initiates [21] is also coincides with the onset of gastrulation [46]. Prior to this stage, embryonic blastomeres can be forced to adapt alternate cell fates by expression of a developmental regulator, but after this transition they cannot [47–52]. Timing of this transition from plasticity to differentiation is under the control of the Polycomb-like complex made up of MES-2, MES-3 and MES-6 [53]. In *mes* mutants, both the transition from plasticity to differentiation [53] and the onset of dosage compensation are delayed [*44]. The onset of dosage compensation is linked to differentiation in mammals as well [54], indicating that this link might be universal.

Another similarity between the various dosage compensation systems is the stepwise nature of the appearance of the different chromatin features associated with dosage compensation. In *C. elegans*, the DCC assembles on the X chromosomes at about the 30-cell stage [21], but H4K20me1 enrichment does not appear until much later, around the comma stage [*29,*44]. By this time, the *C. elegans* embryo consists of about 600 cells, and most cells have stopped cycling [46]. What exactly triggers the timing of DCC localization and the timing of H4K20me1 accumulation on X remains to be determined.

Using temperature sensitive alleles of DCC components, the critical window of DCC action was defined as a 4–5 hour time window centered around the comma stage [11]—significantly, the time window when DCC mediated chromatin marks are being established [*29,*44]. The observation that temperature shifts after this time period have less of an effect on viability suggests that once dosage compensation is established, the DCC becomes dispensable. However, recent studies established postembryonic roles for the DCC in the regulation of dauer arrest [*55], indicating that DCC-mediated regulation of X-linked genes continues to be important for normal development post-embryogenesis.

Conclusions and future directions

Despite recent advances, many questions remain. It is not known how the described changes in chromatin structure influence RNA Pol II activity. Additionally, the severity of chromatin changes does not correlate with the severity of dosage compensation defects in the various mutants [*30], indicating that the DCC likely contributes to dosage compensation in other ways. It is interesting to note that condensin II subunits in *Drosophila* and mammalian cells were found to colocalize with other architectural proteins at boundaries of topologically associating domains (TADs), suggesting that these proteins may cooperate in shaping the interphase genome at the level of TAD organization [56]. In *C. elegans* interphase nuclei, condensin II sites showed a significant overlap with condensin I^{DC} sites on the X chromosomes [57], raising the possibility that condensing I^{DC} and condensin II might cooperate in shaping the interphase structure of the dosage compensated X chromosome.

The main question of how DCC-mediated changes in the structure of the X chromosomes lead to changes in RNA Polymerase recruitment remains unresolved. DCC binding to a gene is neither necessary, nor sufficient, for transcriptional regulation. DCC binding correlates with Pol II occupancy, but does not predict dosage compensation status [35,**38]. These results suggest that the DCC is not directly regulating bound genes, but rather is acting at a distance. The rules of which genes are to be regulated and which ones are not remain to be deciphered.

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Figure 1. Regulation of the X chromosomes in *Caenorhabditis elegans*

Although the mechanism of upregulation is not known, it is believed that the X chromosomes in *C. elegans* are transcriptionally more active than the autosomes (green X chromosomes). X-upregulation balances the single X to two sets of autosomes in males, but creates X overexpression in hermaphrodites. A second, and better understood, mechanism then downregulates both X chromosomes by half, to restore genomic balance in hermaphrodites (red X chromosomes).

Figure 2. The dosage compensation complex (DCC)

The DCC consists of a five subunit condensin complex, as well as at least five additional subunits. Several of its subunits are also members of other complexes and perform DCC-independent functions as well. Condensin I^{DC} shares four of its five subunits with condensin I, and one subunit with condensin II. Several DCC subunits need to be sumoylated. In case of dual function subunits, such as DPY-28, the protein is only sumoylated when part of the DCC. DPY-30, in addition to interacting with several DCC members, is also part of the COMPASS/MLL histone methyltransferase complex. DPY-21 has both DCC-dependent and DCC-independent functions, although the context of its DCC-independent function is not known.

Figure 3. Summary of DCC-mediated changes in chromatin and chromosome compaction

As a result of DCC function, the X chromosomes (shown in red) in hermaphrodites compact to take up a smaller portion of the nucleus than what would be predicted by DNA content. Compaction is accompanied by enrichment of H4K20me1 (gray flags) and depletion of H4K16ac (green flags). These changes are consistent with a mechanism related to mitotic chromosome condensation.