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Dosage compensation of the sex chromosomes and autosomes

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Abstract

Males are XY and females are XX in most mammalian species. Other species such as birds have a different sex chromosome make-up: ZZ in males and ZW in females. In both types of organisms one of the sex chromosomes, Y or W, has degenerated due to lack of recombination with its respective homolog X or Z. Since autosomes are present in two copies in diploid organisms the heterogametic sex has become a natural "aneuploid" with haploinsufficiency for X- or Z-linked genes. Specific mechanisms have evolved to restore a balance between critical gene products throughout the genome and between males and females. Some of these mechanisms were co-opted from and/or added to compensatory processes that alleviate autosomal aneuploidy. Surprisingly, several modes of dosage compensation have evolved. In this review we will consider the evidence for dosage compensation and the molecular mechanisms implicated.

Keywords

Dosage compensation; X chromosome; aneuploidy; sex chromosome evolution; sex differences

Introduction

The adaptive advantages of recombination favor sexual reproduction [1], which is often accompanied by differentiation of sex chromosomes. In mammals, males are XY and females XX, while in birds, males are ZZ and females ZW. These systems evolved because sex is genetically determined [2, 3]. Other vertebrates such as reptiles rely on temperature-sensitive systems for sex determination. Muller hypothesized that differentiation of the sex chromosomes would inevitably arise from lack of recombination due to the appearance of a sex-determining gene on the Y or W chromosome [4]. Ohno expanded these ideas by proposing the concept of ancestral sex chromosomes (protosex chromosomes) that progressively evolved to the present-day sex chromosomes by degeneration of the Y or W. [5].

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Sex chromosomes have evolved independently multiple times: for example, mammalian and avian sex chromosomes derive from different ancestral autosomes. It has been proposed that some chromosomes may be better suited to become sex chromosomes based on their gene content [3, 6]. Both Muller and Ohno predicted that sex chromosome divergence would lead to dosage compensation of the natural type of aneuploidy caused by degeneration of one chromosome in the heterogametic sex. Indeed, a variety of dosage compensation mechanisms that regulate the sex chromosomes have evolved, resulting in a dazzling array of systems throughout the plant and animal kingdoms. The evolution of vertebrate sex chromosomes and of dosage compensation were recently comprehensively reviewed by us and by others [3, 7–9]. In addition, X chromosome inactivation, one of the main form of X regulation in mammals is discussed in detail by others in this issue.

Here, we summarize salient features of dosage compensation of sex-linked and autosomal genes with a focus on molecular mechanisms of dosage regulation. Two major types of sex chromosome dosage compensation, often confounded in the literature, can be recognized; one type balances gene expression throughout the genome by changing the relative expression of X-linked or Z-linked genes versus autosomal genes or vice versa, and the other equalizes sex-linked gene expression between homogametic and heterogametic sexes. The former type of dosage regulation is critical to maintain fitness. Finally, a narrower definition of dosage compensation has been proposed as representing evolutionary adaptive changes in expression of ancestral autosomal genes that evolved into sex-linked genes [10]. Such definition is necessarily based on a restricted number of conserved genes in different species.

Dosage regulation of the sex chromosomes can be viewed as either global, i.e. employing mechanisms that modify most - but not all - genes on an entire chromosome, or local, i.e. acting on individual genes. This distinction is somewhat fluid as the number of dosage-compensated genes on a given sex chromosome varies between tissues, and also depends on methods of analysis. Intuitively, not all genes need to be regulated by either type of dosage compensation mentioned above. Indeed, balanced expression throughout the genome may be critical only for dosage-sensitive genes implicated in protein complexes and functional networks, but may not apply to dosage-insensitive genes unless they are swept in a global regulatory system. Deleterious effects of copy-number changes may be subtle at the individual gene level, but cumulative effects of large chromosomal imbalance are often severe and yet to be fully understood. Conversely, patchy dosage compensation may be advantageous and selected for in terms of sex-specific traits important in male/female conflicts. This is particularly relevant for testis- or ovary-specific genes abundant on the sex chromosomes.

Differentiation of the sex chromosomes

Dosage compensation of sex-linked genes should be considered in light of sex chromosome evolution. One of the best studied systems in which progressive evolutionary steps have been deciphered is represented by the human sex chromosomes that have evolved for about 300 million years [11–14]. Based on DNA sequence analyses of genes retained on the human sex chromosomes it is apparent that the Y underwent large inversions that progressively

prevented large regions from undergoing X/Y recombination. This may have been helped by a gradual spread of regions with reduced recombination [15]. Detailed sequence analyses led to the definition of six evolutionary strata on the X chromosome, each containing genes that diverged from their Y paralogs for a similar length of time [13]. Evolutionary strata have also been found in other mammals and in birds [11, 14, 16].

The Y chromosome in mammals and the W chromosome in birds are gene-poor, having lost many functional genes [17]. It is estimated that the human Y retains about 3% of the genes originally located on the proto-sex chromosomes, whereas the X retains about 98% of genes [14]. The human Y is rich in palindromic duplicated sequences that may help retention of specific Y-linked genes important in male fertility, but also facilitate deletions and gene loss [18]. Such deletions are often associated with male infertility [19, 20], in which case they would not be transmitted, thus preserving some integrity of the Y chromosome.

When comparing sex chromosomes in divergent mammalian species such as marsupials (metatherian) it is evident that the eutherian sex chromosomes acquired a large piece of chromosome that is autosomal in marsupials [21]. Graves proposed that successive cycles of addition and attrition have shaped the sex chromosomes [3]. Small regions have also been added or deleted more recently to the eutherian sex chromosomes, reshaping the pseudoautosomal region (PAR) but rarely changing the content of the rest of the X [22, 23]. Altogether, the X chromosome in eutherian mammals is highly conserved, probably because it is subjected to complex mechanisms of dosage regulation [5].

Specialized gene content of the sex chromosomes

When studying dosage regulation of the sex chromosomes one must consider their gene content. For example, male-biased genes often expressed in testis are abundant on the Y chromosome, a location predicted to be favorable to the accumulation of sexually antagonistic genes with a male benefit [24]. Interestingly, the X chromosome is also highly enriched in male-biased genes [25]. Hemizygosity in males favors the accumulation of male-advantageous mutations at both X and Y locations. In addition to being enriched in male-biased genes the X chromosome is also enriched in femalebiased genes expressed in ovary [26]. Of special interest is the accumulation of brain expressed genes on the X chromosome, possibly a by-product of sexual reproduction [27–29].

Some of the male-biased genes located on the sex chromosomes were recently and independently acquired in different clades [30]. The convergent evolution of the bird Z and mammalian X chromosomes, both of which demonstrate massive enrichment in multi-copy genes expressed in testis, shows striking similarities between the two types of heterogametic systems [31]. The mouse Y represents an extreme case of specialization with accumulation of hundreds of copies of fertility genes. In this case, both Y- and X-linked paralogs are amplified, suggesting meiotic driver and suppressor pairs with cycles of amplification in response to interchromosomal X/Y conflict [32].

The mammalian sex chromosomes also carry genes essential for survival of human embryos. Over 95% of 45,X embryos die during development and those that survive are often mosaic

for a normal XX or XY line [33]. A subset of highly conserved dosage-sensitive X/Y paralogs with essential functions are the main candidates in the context of embryo survival [11, 14]. These genes usually escape X inactivation in females and thus are bi-allelically expressed in both sexes [34–36]. Note that even though many of the X/Y paralogs retain apparently similar functions their sequence has in some cases diverged, suggesting that the Y-linked paralog may be acquiring a male-specific function. An early example of concerted divergence between X/Y paralogs and acquisition of X inactivation is represented by the gene pairs ZFX/ZFY and Zfx/Zfy1-2 in human and mouse. In human, which would represent the more primitive condition, both ZFX and ZFY are ubiquitously expressed and ZFX escapes X inactivation, ensuring similar expression between sexes. In mouse, Zfy1-2 have acquired a testis-specific function, while the ubiquitously expressed Zfx is subject to X inactivation [37–39].

The specialized gene content of the sex chromosomes results in phenotypic sex differences manifested in health and disease susceptibility. Specific mouse breeding schemes including the four-core genotype to generate sex-reversed animals have helped sort out the roles of X- and Y-linked genes versus those of sex hormones [40, 41]. The complicated dosage regulation of the sex chromosomes, for example escape from X inactivation, may have evolved in part to enhance such sex-specific differences [7] (see sex differences below).

Dosage compensation responses to aneuploidy

The heterogametic sex would have had to survive a natural form of aneuploidy. How do organisms respond to any aneuploidy, whether autosomal or sex-linked? Aneuploidy causes significant phenotypic abnormalities and loss of fitness [42]. Duplications are generally better tolerated than deletions. In *Drosophila melanogaster*, deletions that affect 1% of the genome reduce viability [43], but of course, this very much depends on the gene content of the deleted region (see dosage-sensitive genes below). Usually, the larger the deletion the more lethal it is, indicating a clear cumulative effect. The effects of deletions and duplications in mammals are less well-studied than in model organisms such as yeast and fly, and no systematic series of deletions have been generated yet. Thus, most information comes from spontaneously occurring copy-number changes in human. Overall, copy-number changes of ≥1Mb are associated with abnormal phenotypes and rare in normal individuals [44]. Monosomy for a whole human chromosome is lethal but trisomy is better tolerated. However, even trisomy for the smallest human chromosome, trisomy 21, causes widespread gene dysregulation [45]. Interestingly, not all genes located on chromosome 21 show the expected 1.5 expression increase, indicating dampening. Furthermore, the expression and chromatin features of genes located elsewhere in the genome is also altered [46]. In human trisomy 8 and 21, differentially methylated regions are found not only along the trisomic chromosome but also genome-wide, suggesting that DNA methylation plays important roles in compensatory adjustments of gene expression [47, 48].

While the expression of many genes varies in direct proportion to their dose, expression of some genes is compensated, presumably to reduce deleterious dosage imbalance [9, 49, 50]. Oliver defined three types of compensatory responses: (1) buffering or passive absorption of gene dose perturbation by inherent system properties, (2) feedback or gene-specific sensing

and adjustment of levels, which can result in overexpression, and (3) feedforward responses representing systems that apply only to special cases, for example the male X chromosome in *Drosophila* [51]. In a diploid *Drosophila* cell line with multiple copy-number changes, monosomic regions show 0.75 expression and trisomic regions 1.33, relative to a value of 1.0 in disomic regions [52]. By comparing multiple cell lines it is evident that even for the same deletion compensatory responses may differ [52]. Such compensation of aneuploidy has also been observed in yeast aneuploids in which different mechanisms adjust mRNA abundance, splicing, stability, or translation in response to gene amplification [53]. Alterations and potential adjustments in levels of long non-coding RNAs (lncRNA) and miRNAs are less well documented. Other mechanisms operate at the protein level: abnormal protein levels and macromolecular interactions such as folding, aggregation, and interactions can be alleviated by induction of proteolysis [42, 54]. Figure 1 summarizes various types of adjustments in response to dosage imbalance.

In addition to compensatory mechanisms that control gene expression and amounts of protein products other systems may rely on adjustments in copy number for the restoration of a balanced genome, for example in the case of multi-copy ribosomal genes that encode the 5S and 45S rRNA in human cells (Fig. 1). Although the multi-copy arrays are on different human chromosomes they display rapid and concerted copy number adjustment to fulfill their stoichiometric requirements [55]. Rapid co-variation of rDNA gene copy number has been observed not only between siblings but also in response to environment changes, underlying the need to efficiently maintain a balance between ribosomal components. Correlated copy number changes have also been observed in *Drosophila* cell lines [52]. Interestingly, of 142 protein-protein interaction networks identified, 84 had a greater than 90% co-occurrence of copy number changes in the same direction.

Therapeutic interventions may ultimately be developed to artificially correct deleterious effects of aneuploidy. In the case of trisomy 21 amelioration of neural cell growth was obtained by insertion of a highly expressed copy of the lncRNA XIST within one of the trisomic chromosome 21 in a cell line. This approach took advantage of the property of XIST, which is essential for the onset of X inactivation, in cis-induction of chromosome-wide gene silencing [56]. The timing of intervention in terms of dosage compensation may be critical. This conundrum is exemplified in a recent report of a zebrafish with a mutation in an extracellular matrix protein, which shows no phenotypes, whereas a morpholino-induced knockdown causes severely abnormal phenotypes [57]. This observation is attributed to adaptive compensatory mechanisms that upregulate other extracellular proteins during development, whereas the rapid effects of the knockdown fail to be compensated.

Finally, it is important to point out that aneuploidy can be neutral or advantageous. Adaptive aneuploidy has been reported in yeast exposed to oxidative stress [58]. In human, Grompe's group has reported frequent aneuploidy together with genomic deletions in hepatocytes [59]. Frequent copy-number changes have also been observed in human neuronal cells, but it is unclear whether these have any advantage [60]. Overall, somatic mosaicism for copy-number changes is widespread and much work needs to be done to determine whether this is simply tolerated or selected for. Thus, there may be a subtle equilibrium between maintenance of dosage by compensation systems and selection of specific imbalances

advantageous to specific cell types. Clearly, copy-number changes in cancer cells such as amplification of oncogenes or deletion of tumor suppressor genes provide growth advantage to these cells [61]. In contrast, germ cells would be expected to maintain an intact genome with sporadic copy-number changes strongly selected against or compensated during development.

Allele-specific expression

Some genes are normally expressed from a single allele. Do these genes get compensated? While a majority of these genes are X-linked in mammals and will be discussed below, others are autosomal. One category of such autosomal genes is represented by imprinted genes. It should be noted that there are interesting parallels between mechanisms that silence imprinted genes and those that silence genes regulated by X inactivation [62], both types of genes being regulated by cis-acting lncRNAs, histone modifications, DNA methylation, and specific nuclear positioning [63]. One study has addressed the question of dosage compensation of imprinted genes by measuring expression of 59 such genes in mice using expression arrays and quantitative RT-PCR [64]. While inherently limited due to the paucity of imprinted genes this study concludes that imprinted genes are partially upregulated. Increased expression could help alleviate deleterious effects due to transcriptional noise at mono-allelically expressed genes.

A large number of non-imprinted autosomal genes show consistent heritable allelic biases in terms of chromatin structure and levels of gene expression in human [65]. Such allelic biases reflect close proximity of enhancers and promoters, or strong interactions at longer distances [65]. Pervasive allelic imbalance has been reported in mouse crosses where 80% genes show cis-regulatory variation [66], and in humans in relation to specific haplotypes [67]. The role of allelic differences in causing subtle deficiency or overexpression is not well understood and it is unclear whether specific compensatory mechanisms adjust allelic expression. Random allelic silencing of autosomal genes was initially illustrated in clonal cell lines [68], but subsequent studies in single cells have confirmed this phenomenon [69]. There are again parallels between molecular signatures of randomly silenced autosomal and X-linked alleles. For example, SMCHD1, a protein implicated in X inactivation in part for the establishment of DNA methylation, plays a role in mono-allelic repression of autosomal genes, e.g. protocadherin genes [70, 71]. Common epigenetic features such as H3K27me3 also decorate both silenced X-linked genes and mono-allelically expressed autosomal genes [70–73].

Dosage compensation between sex chromosomes and autosomes

Balanced expression between X/Z-linked and autosomal genes can be attained by increasing X/Z expression or by decreasing autosomal expression in the heterogametic sex. X upregulation is best documented in *Drosophila* males to increase expression of a large portion of genes [74]. In organisms where dosage adjustment is sex-specific - as in *Drosophila* - there would be no need for adjustment in the homogametic sex. However, in systems in which X/Z expression is increased relative to autosomal expression in both sexes, there would be a need to reduce expression in the homogametic sex. This is achieved by X inactivation in mammals, or X repression in *Caenorhabditis elegans*. These repressive

systems have been extensively reviewed [9, 75]. Here, we will limit our discussion to the balance in the heterogametic sex.

Ratios of gene expression between autosomes and sex chromosomes could result from modulations of either sex-linked or autosomal genes [76]. However, it is inherently difficult to measure absolute amounts of transcripts in cells. Global studies of gene expression make specific assumptions about normalization to the total transcriptome that may not always be correct and lead to aberrant conclusions when comparing samples [77, 78]. Co-isolation of RNA and genomic DNA together with counting the number of cells from which the nucleic acids are extracted are helpful, as well as spiking reactions with exogenous RNA. Almost no studies of dosage compensation include such controls. We performed one study in human triploid lines where correlated changes in X expression and number of active X chromosomes, but no detectable autosomal gene expression changes, were observed [79].

As measured by X:autosome expression ratios mammalian X upregulation is controversial and more studies are needed [80]. Initial expression microarray studies supported the existence of increased expression of X-linked versus autosomal genes in multiple mammalian species and tissues [81, 82]. Subsequent RNA-seq studies carried out in multiple somatic tissues either disproved or confirmed X upregulation [83, 84]. Presence of X upregulation is based on finding average X:autosome expression ratios between 0.8 and 1.0, while absence of X upregulation is based on finding low (around 0.5) median X:autosome ratios. Clearly, X upregulation only operates on expressed genes. As we have shown, low median X:autosome ratios reported by some [83] are obtained in somatic tissues when all X-linked genes whose expression distribution comprises a long tail of non-expressed testis-specific genes, are included. When testis-specific genes are excluded, X:autosome expression ratios increase [84, 85]. Furthermore, both the depth of sequencing and the methodologies used to analyze RNA-seq data can lead to low expression medians [86]. A recent analysis in multiple mouse strains confirmed similar expression between X-linked and autosomal genes [66]. However, analyses of protein levels suggest absence of X upregulation [87].

One study that supports X upregulation addresses expression changes during ES cell differentiation when X inactivation takes place [88]. Undifferentiated male and female ES cells show X:autosome expression ratios of 1.4–1.6. After 2–3 weeks of differentiating male ES cells X upregulation results in equal X-linked and autosomal expression throughout the genome. In differentiating female ES cells gene-by-gene silencing on one allele results in a progressive decrease in X expression to achieve a balanced expression [88]. Another study done in haploid cells shows that a high proportion (35%) of genes upregulated (in comparison to diploid cells) are X-linked [89]. In bovine blastocysts there is also evidence of increased X expression in females [90]. However, a recent study in human and mouse mature oocytes reports low X:autosome expression ratios [91].

One way championed by Kaessman to evaluate the existence of X upregulation is to compare expression of X-linked genes in mammals to that of “ancestral” genes in chicken [10]. This study concludes that there is no or very little evolutionary upregulation of X-linked genes in mammals. The authors propose that genome balance is maintained in

eutherians by an overall expression reduction in a subset of autosomal genes to compensate for a decrease in X-linked expression. X inactivation then would serve to reduce X expression in females to compensate for the inverse effect on autosomes. While this comparative study may address evolutionary aspects of X expression it remains unclear whether contemporary mammals can be directly compared to contemporary birds with different physiology. In addition, the number of genes that can be compared is restricted and it has not been shown that haploinsufficiency of the genes in question would be harmless if deleted in chicken. Indeed, it would be interesting to produce monosomy for chicken chromosomes 1 and 4 to evaluate the consequences of such large chromosomal defects.

Surprisingly, marsupial but not monotreme X-linked genes were fully upregulated compared to ancestral genes in chicken [10, 92]. It will be informative to determine whether the autosomal genes with reduced expression in eutherians have increased expression in marsupials. Birds only have partial dosage compensation, leading to sex differences in levels of Z-linked gene products [93]. This was confirmed in multiple somatic tissues in chicken where the Z:autosome ratios for protein and RNA were 1.0 and 0.8 in males, and 0.8 and 0.6 in females, respectively [94, 95]. A potentially useful avenue of research into evolution of dosage regulation would be to compare X- or Z-linked ohnologs to their autosomal copy. Ohnologs are genes that result from evolutionary whole genome duplications hypothesized by Ohno [96]. A subset of ohnologs are thought to have been retained because of balance constraints. In human, persistent ohnologs are refractory to copy-number changes and cause abnormal phenotypes [97].

Species with newly evolved sex chromosomes have been tested to determine whether dosage compensation can rapidly evolve. In sticklebacks recently evolved sex chromosomes allowed the definition of two main strata along the X, with the older strata showing evidence of upregulation in males and overexpression in females at least for a subset of genes [98]. Complete dosage compensation by upregulation of the male X chromosome evolved independently in *Drosophila melanogaster* and in a mosquito *Anopheles stephensi* [99], while in another insect, *Heliconius* butterfly, there is only partial dosage compensation [100]. In a plant (*Silene latifola*) with newly evolved sex chromosomes no global dosage compensation between sexes was detected in one study [101], while two others showed increased X expression [102, 103], further demonstrating the difficulty of reaching a consensus. In *C. elegans* an initial study that showed absence of X upregulation [83] was subsequently shown to be flawed because it did not take into account the progressive accumulation of germ cells in which the X chromosome is silenced [84]. A more recent study confirmed balanced expression of X-linked and autosomal genes in *C. elegans*, but argued against evolutionary X upregulation by measuring ancestral gene expression in another species [104].

We and others have proposed that mammalian X upregulation would probably operate gene-by-gene once the Y-linked homolog is lost by recruiting a number of different molecular mechanisms of gene regulation (see below and Fig. 1) [7, 84]. Expression of multiple genes in large X regions could also have been concomitantly adjusted if several adjacent Y-linked genes degenerated together [105]. In this case deleterious effects of haploinsufficiency would likely have been cumulative, in a manner analogous to loss of a whole chromosome

or a large copy-number change. Upregulation is expected to only regulate expressed genes [7, 106], and to especially target dosage-sensitive genes that need to remain in stoichiometric equilibrium in large macromolecular complexes [107]. A study of dosage-sensitive genes defined as those included in large protein complexes is consistent with upregulation of X-linked genes, with no downregulation of autosomal genes [108].

A recent study has proposed an alternative novel mechanism to compensate for loss of a Y paralog, which is to move a copy of that gene to another genomic location (Fig. 1) [109]. This rescue of dosage-sensitive Y-linked genes by translocation to the X or to an autosome was initially discovered by studying the spiny rat where the Y chromosome has disappeared altogether [110, 111]. This rescue mechanism was subsequently shown to have happened multiple times in mammalian lineages [109].

Molecular evidence of X upregulation

Precise adjustment of X-linked gene expression requires a combination of systems for enhancement and suppression of expression. In *Drosophila*, upregulation of the male X chromosome is achieved by recruitment of the MSL complex to increase levels of H4K16 acetylation and open chromatin, which results in increased transcription initiation and elongation (Fig. 1) [74]. Recruitment of the MSL complex to the male X is mediated by a 2–4 fold enrichment in specific binding motifs. Heterochromatin elements counteract chromatin unfolding to achieve doubling in gene expression [112]. An alternative hypothesis proposed by Birchler who examined MSL mutants is that the X:autosome balance in *Drosophila* depends genome-wide adjustments of gene expression. This is the so-called inverse-hypothesis [113, 114]. In *C. elegans*, X upregulation is associated with visible de-condensation of the active X together with increased levels of H4K16 acetylation [115].

In mammals, genes on the active X chromosome are distinguishable from autosomal genes in several respects. We and others have shown an average of 30% enrichment in RNA polymerase II at the 5’end of expressed X-linked genes compared to autosomal genes, suggesting increased initiation of transcription (Fig. 1) [84, 106, 116]. In addition, a subset of X-linked genes is sensitive to MOF, the acetyltransferase that modifies H4K16 [106]. Reduced RNA decay at X-linked versus autosomal transcripts is also evident and X-linked transcripts have a longer half-life (Fig. 1) [117]. A new analysis further confirms increased mRNA stability and also demonstrates a greater number of ribosomes on X-linked versus autosomal genes, indicating a higher rate of translation (Fig. 1) [118]. Manipulation of mRNA stability causes a greater decrease in X than autosomal expression, but the increased stability of X-linked transcripts does not appear to be related to poly-A tail length, GC and GC3 contents, or any detected 3’UTR sequence features [118]. Control of ribosome translocation for polypeptide elongation, which is also influenced by relative abundance of tRNAs, is another potential regulatory mechanism that has not been fully evaluated in terms of X chromosome regulation [119].

Thus, several mechanisms have been demonstrated that increase transcription, RNA stability, and translation, for X-linked genes (Fig. 1). Additional studies are needed to fully characterize these processes, for example in terms of regulatory motifs to help understand

targeting of molecular changes to the X chromosome. In contrast to mammals, *Drosophila* X-linked genes have fewer ribosomes and lower translational rates than autosomal genes in males [120]. It may be that mammals employ compensatory mechanisms that act on RNA, while *Drosophila* mechanisms are more specifically targeted to the rate of transcription. In mammals, both ancestral genes defined as those located on the X in marsupials and acquired genes that are autosomal in marsupials have similar characteristics, suggesting that the molecular changes observed rapidly occurred during differentiation of the sex chromosomes [7].

An emerging feature of dosage regulation of chromosomes concerns their nuclear position and structure. In mammals, the heterochromatic inactive X is often located near the nuclear membrane or the nucleolus, possibly helped by specific elements including the lncRNA Firre [121]. In addition, the inactive X forms a bipartite structure with two superdomains of condensation separated by a hinge that binds the lncRNA DXZ4/Dxz4 in both human and mouse [122, 123]. Genes that escape X inactivation tend to be located at the periphery of the condensed structure, suggesting that location is important in terms of expression [122, 124]. Factors involved in location of the active X, which is often located near the nuclear membrane in mammals, remain to be determined. In *Drosophila* and *C. elegans*, nuclear pore complexes associate with the active X chromosome, consistent with a specific nuclear location [125, 126].

Sex differences due to dosage

In mammals, gene expression in somatic tissues is fairly similar between sexes except in sex organs. Indeed, the Y chromosome contains few genes except for those implicated in male fertility, and X inactivation effectively silences most genes on one allele in females, except for genes that escape X inactivation [127, 128]. Escape from X inactivation may enhance sex-specific differences via female bias. Such effects can be indirect, as shown for the reproduction-related Hox genes Rhox6/9 expressed highly in ovary and regulated by the histone demethylase KDM6A encoded by a female-biased escape gene [129]. Sexual dimorphisms in gene expression arise in early development prior to hormonal influence [130], for example, in male and female ES cells also shown to differ in epigenetic features such as DNA methylation and histone modifications [129, 131]. In addition, the testis-determining gene Sry acts as a repressor in somatic tissues and plays a role in sexual dimorphisms throughout the genome [132]. X-linked imprinted genes could also lead to sexual dimorphisms since males only inherit a maternal X, and females both a maternal and paternal X [133]. An interesting hypothesis is that X inactivation evolved not to reduce overexpression in females, but rather as a type of imprinting mechanism to silence growth-inhibiting genes in embryos [134].

Both the inactive X and the Y chromosomes are heterochromatic and this structural feature is probably important to consider in terms of sexual dimorphisms in the epigenetic landscape of the genome in males and females. These large heterochromatic structures represent sinks that sequester specific factors otherwise important for epigenetic modifications. Since the Y chromosome is smaller than the inactive X such effects may differ between sexes [135]. In

cases of abnormal sex chromosome complements sink effects are multiplied and could influence the entire genome by altering the overall nuclear structure.

In contrast to mammals, birds have extensive sex-bias in gene expression due to incomplete dosage compensation [10]. This is also the case for snakes and some fish (reviewed in [3]). In birds, Z-linked genes have about 30% higher expression in males than in females, and thus may play important roles in sexual dimorphisms. The male-tofemale expression ratio ranges from 1 to 2, suggesting a continuum in the level of piecemeal compensation. Protein analyses are consistent with a sex ratio of 1.32, similar to that obtained by RNA-seq (1.29) [94].

Partial rather than complete dosage compensation between the sexes is common in insects other than *Drosophila,* as well as in other organisms, leading to the suggestion that efficient mechanisms to equalize X or Z expression between males and females is in fact rare [136]. Overall, this type of dosage compensation appears to be more frequent in XX/XY than in ZZ/ZW systems, which may be due to stronger selection on X-linked genes in males or to faster Y chromosome decay due to a greater number of cell divisions in spermatogenesis than oogenesis [137]. Alternatively, there may be an insufficient number of organisms examined so far to draw a meaningful conclusion about the prevalence of sex bias due to sex-linked genes.

Dosage-sensitive genes

In human, dosage-sensitive genes have been identified as those causing disease if mutated, deleted, or present in supernumerary copies [138]. A comprehensive list of DNA sequences intolerant to alterations was compiled by considering coding as well as non-coding regions to include regulatory features [139]. Gene expression analysis using expression microarrays in a very large number of tumors has shown that many genes are dosage-sensitive, with their expression usually correlated to copy number changes [140]. Furthermore, abundantly expressed genes are more dosage-sensitive. Conversely, some genes are apparently dosage-insensitive and heterozygous deletions do not cause anomalies.

Copy-number aberrations of the human X chromosome are frequently associated with abnormal transcript levels, which lead to abnormal phenotypes in males, while females are often protected by mosaicism or skewing of X inactivation. Numerous examples of such effects have been reported, suggesting a greater abundance of dosage-sensitive genes on the X chromosome than previously suspected. One interesting example is a 0.3Mb copy-number (2–5 copies) change at band Xq38 [141]. The expression level of genes (including GDI1, a gene associated with intellectual disability) in the region was proportional to the number of copies, as was the severity of phenotypes in males and the degree of skewing in females.

Two important studies published in 2014 have shown that a conserved set of genes with paralogs on the X/Y chromosomes in mammals and on the Z/W chromosomes in birds have persisted in many species [11, 31]. In mammals, persistence of such genes in multiple species indicate that despite widespread Y degeneration, the Y copy could not get lost because of dosage-sensitivity. Interestingly, these intolerant genes are all engaged in critical

functions such as transcription, translation, chromatin structure, splicing and ubiquitination [11, 31]. The X paralogs usually escape X inactivation, thus preserving bi-allelic expression in both sexes. Consistent with being dosage-sensitive genes escape genes are under greater purifying selection than genes subject to X inactivation [142, 143].

Thus, dosage of the broadly expressed X/Y genes is important and preserved. The mouse has fewer of these genes compared to human, which may explain the difference in severity between the near-lethal Turner syndrome versus X0 mice that have a much milder phenotype [127]. Women with a single X chromosome are often mosaic, suggesting that the presence of two copies of the X/Y dosage-sensitive genes at least in some tissues may be necessary for survival [33]. Two of the X paralogs of preserved X/Y gene pairs are KDM5C and KDM6A both included in the list of intolerant genes known to cause disease if present in an abnormal number of copies [139]. Note that for X/Y gene pairs Y-linked expression is often lower than X-linked expression, consistent with X upregulation and suggesting Y decay or evolution of new male function.

Are dosage-sensitive genes excluded from the X compared to autosomes? Chen and Oliver measured the effects of X-linked and autosomal deletions in terms of gene expression alterations in *Drosophila* and concluded that the X chromosome is neither more robust nor sensitive to dosage change [144]. They measured a 1.1 response to these deletions, which suggests that even with the contribution of the MSL complex estimated to be around 1.4 there must be an additional, yet to be discovered, mechanism to reach a two-fold adjustment.

Emerging view

Conflicting statements about the existence of dosage compensation in haploid chromosomes such as the sex chromosomes in part result from unclear definition of the term “dosage compensation”. Any adjustment of dosage, partial or global, chromosome-specific or genome-wide, indicates some form of dosage regulation. For sex-linked genes such regulation is in part co-opted from responses to any form of autosomal aneuploidy, with added mechanisms to help restore genome balance. Findings of molecular differences between sex chromosomes and autosomes are a strong indicator that organisms respond to haploinsufficiency due to differentiation of the sex chromosomes. The overall picture that is emerging about any form of so-called dosage compensation of the sex chromosomes is that not all genes are dosage compensated in an organism and that mechanisms widely differ between species. There is clear a continuum between small and large adaptive changes in gene expression. Additional studies are needed to understand the importance of this regulation in relation to sex differences.

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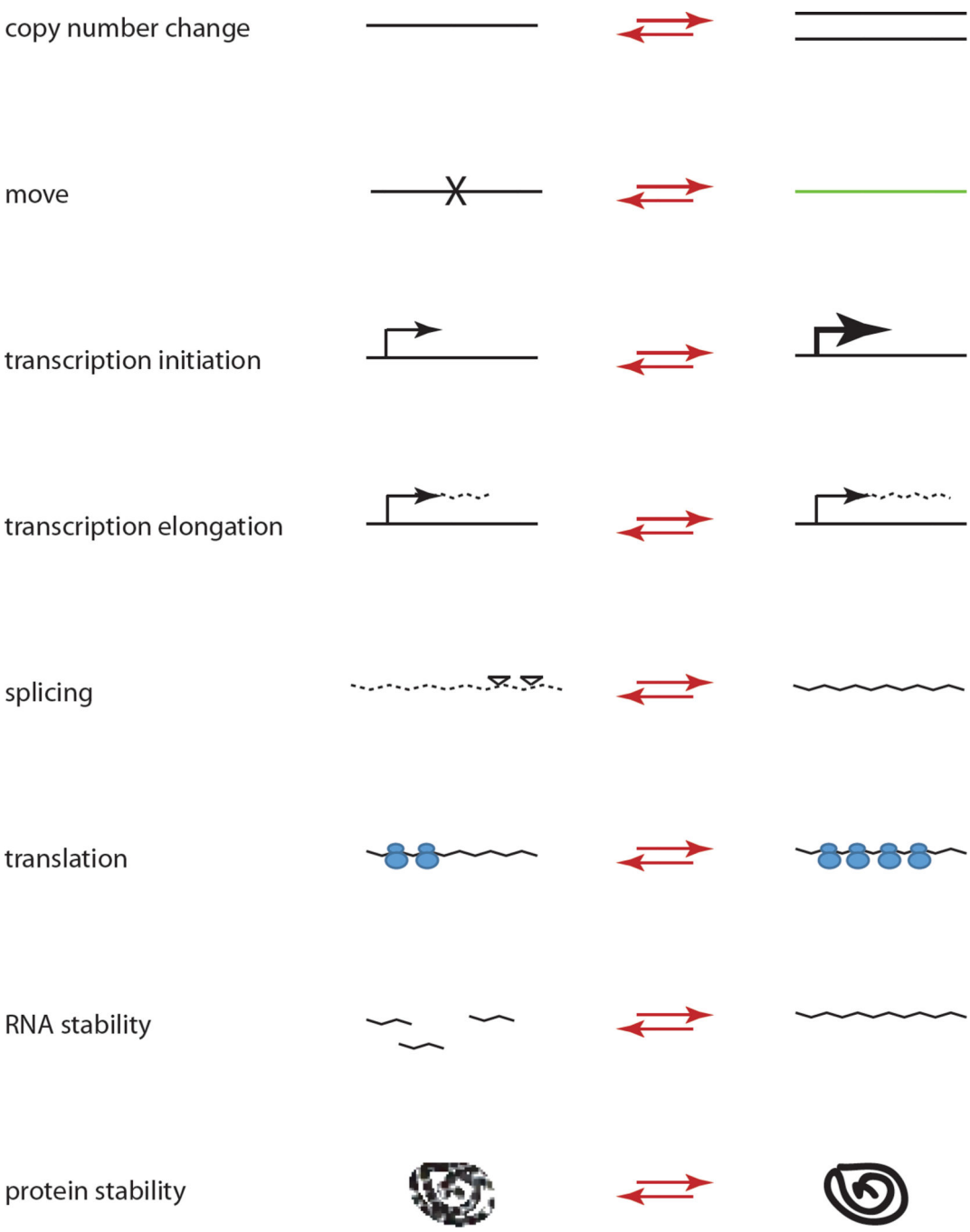

Figure 1. Types of mechanisms that can modulate dosage responses to imbalance

From top: 1. Copy-number adjustments occur, for example, in the case of multi-copy ribosomal genes or mouse sex-linked genes engaged in a meiotic conflict; 2. Move to a different genomic location occurs in the case of genes lost from the mammalian Y but translocated to the X or to an autosome to preserve function; 3. Increased initiation of transcription can represent a feedback mechanism or a feedforward mechanism, as in mammalian and *Drosophila* X upregulation; 4. Increased elongation of transcription represents a feedforward mechanism in *Drosophila* X upregulation; 5. Increased efficiency

of splicing have been observed in aneuploid yeast; 6. Increased number of ribosomes on RNA to enhance translation occurs in mammalian X upregulation; 7. Increased RNA stability has been associated with mammalian X upregulation; 8. Adjustment at the protein level, for example, by changing stability has been reported in *Drosophila* aneuploidy. Note that all types of adjustments can work in reverse to decrease gene/protein products, for example to adjust genome-wide expression in response to a deficiency (inverse effects). The mechanisms included here have been implicated in dosage responses, but this does not exclude other mechanisms known to change gene expression (e.g. miRNA, lncRNA) and protein production. Epigenetic mechanisms including chromatin and nuclear organization associated with enhancement or repression of gene expression are not included in this figure. See text for further details and references.