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Review

X chromosome inactivation and active X upregulation in therian mammals: facts, questions, and hypotheses

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X chromosome inactivation is a mechanism that modulates the expression of X-linked genes in eutherian females (XX). Ohno proposed that to achieve a proper balance between X-linked and autosomal genes, those on the active X should also undergo a 2-fold upregulation. Although some support for Ohno's hypothesis has been provided through the years, recent genomic studies testing this hypothesis have brought contradictory results and fueled debate. Thus far, there are as many results in favor as against Ohno's hypothesis, depending on the nature of the datasets and the various assumptions and thresholds involved in the analyses. However, they have confirmed the importance of dosage balance between X-linked and autosomal genes involved in stoichiometric relationships. These facts as well as questions and hypotheses are discussed below.

Keywords: X inactivation, X chromosome upregulation, monoallelic expression, imprinting, dosage balance

Rise and fall of sex chromosomes

Sex chromosomes underlie sex determination and subsequent differentiation. By convention, we name them X–Y when the male is the heterogametic sex (as is the case in mammals, *Drosophila*, and papaya) and Z–W when the female is the heterogametic sex (as in birds, butterflies, snakes, and poplar). The X (or Z) chromosomes are generally large and gene-rich, whereas the Y (or W) are smaller, mostly heterochromatic and carry few active genes. Although the sex chromosomes have been shaped by convergent evolution, comparative gene-mapping studies have revealed that they evolved independently in the different organisms mentioned above (Matsubara et al., 2006; Bachtrog, 2013).

Therian mammals such as human, mouse, and kangaroo share the same sex chromosome system with a common evolutionary origin. The X and Y emerged around 166–180 MYA (million years ago) from a pair of autosomes when one of the chromosomes acquired a sex-determining gene, namely *Sry* (for sex-determining region on the Y), following a mutational change of the *Sox3* gene (Veyrunes et al., 2008; Cortez et al., 2014). The chromosome bearing this sex-determining gene became *de facto* the proto-Y. Once male-specific, the Y chromosome accumulated, around the newly formed sex-determining region, other male-beneficial alleles that can be neutral or even disadvantageous for females (Rice, 1987). Then, selection kept this cluster of sexually antagonistic genes as a unit by suppressing recombination between the nascent sex chromosomes. This scenario is reminiscent of the concept of supergene, i.e. a group of neighboring genes transmitted together because of their tight genetic linkage due for instance to a chromosomal inversion (Joron et al., 2006). The non-recombining region of the Y progressively extended to include many more genes, to leave one or two small segment(s) that still recombine(s) with the X, called Pseudo-Autosomal Region(s) (PAR), which ensure(s) a proper segregation of the sex chromosomes (Wilson and Makova, 2009). The abundance of inversions on the Y chromosome suggests that they played a role in recombination suppression (Ross et al., 2005; Wimmer et al., 2005). However, other mechanisms may have also been involved, such as genetic modification of recombination rates. In the absence of recombination, the sex chromosomes started to differentiate. The Y chromosome became vulnerable to the accumulation of deleterious mutations, deletions and invasion of retrotransposons, which cannot be expunged or repaired and contributes to its degradation (Charlesworth and Charlesworth, 2000; Ellegren, 2011; Bachtrog,

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2013). The X chromosome, however, still recombines with its homologue in females and is thus sheltered from degeneration. After 166–180 MY, the X chromosome retains more than a thousand of genes (Ross et al., 2005), whereas the Y has lost >95% of its original gene content (Skaletsky et al., 2003). Hence, since early work by Ohno (1967) until recently, it was assumed that the X had undergone very few changes, whereas the Y chromosome was a passive player that inexorably degenerated, keeping only a few crucial genes. However, recent genomic advances reveal a quite different picture. In fact, the gene contents of the X and the Y have profoundly diverged from that of the original autosomes. Both sex chromosomes have recruited or lost many genes (Bachtrog, 2006; Graves, 2006). For example, the human X chromosome has been enriched in genes involved in sex, reproduction, and cognition (Saifi and Chandra, 1999; Graves et al., 2002; Vallender and Lahn, 2004) and the Y accumulated genes involved in sex determination and spermatogenesis that have often been duplicated (Lahn and Page, 1997). These biased gene contents are essentially due to sex-related selection and other evolutionary forces, linked to the sex-biased transmission of the X and Y and their hemizygosity in the heterogametic sex (Graves et al., 2006; Vicoso and Charlesworth, 2006; Gurbich and Bachtrog, 2008; Wilson and Makova, 2009; Bachtrog et al., 2011; Ellegren, 2011). For example, the patrilineal transmission of the Y chromosome makes male-specific genes more likely to become fixed. Indeed, although losing most of its genes, the Y chromosome has also gained genes that were transposed from the autosomes such as *DAZ*, a spermatogenesis gene (Saxena et al., 1996), or from ubiquitously expressed X-linked genes that acquired fertility functions on the Y copies, such as *RBMY* (Skaletsky et al., 2003). Recent comparative studies on gene content of Y chromosomes of different mammalian species confirmed that gene survival was non-random and that the Y became specialized through selection to preserve homologous dosage-sensitive X–Y gene pairs involved in regulatory functions (Bellott et al., 2014; Cortez et al., 2014). With regard to the X, theoretical studies show that recessive male-beneficial and dominant female-beneficial mutations are more likely to accumulate on it because of its hemizygous state in males and because it spends 'twice as much time' in females than in males, respectively (Rice, 1984). Thus, the X chromosome may represent a preferred location for both male- and female-beneficial genes. For example, the X is indeed enriched in genes preferentially expressed in spermatogonia and in the placenta (Wang et al., 2001; Khil et al., 2004). A recent analysis showed that the evolution of human and mouse X chromosomes was bimodal: most (94%–95%) X-linked single-copy genes are shared by humans and mice and are expressed in both sexes. However, only 31% of human and 22% of mouse X-ampliconic genes (i.e. forming multigenic families) had orthologs in the other species and are expressed predominantly in testicular germ cells. Moreover, many of them were independently acquired since the divergence from the common ancestor of humans and mice (Mueller et al., 2013). Another property that can affect X gene content is meiotic sex chromosome inactivation (MSCI), in which the unpaired parts of the X and Y chromosomes (i.e. most of their lengths except the PARs) are silenced during male meiosis (Turner, 2007). It has thus been shown that genes expressed in early spermatogenesis (pre-MSCI) are enriched on the X, whereas those expressed in late spermatogenesis (during and post-MSCI) are depleted, having been relocated to autosomes (Khil et al., 2004). However, this does not apply to multicopy gene families (18% of mouse X-linked genes) whose expression occurs predominantly during post-meiosis (Mueller et al., 2008). Not surprisingly, many functional retrogenes have been selectively exported out of the X chromosome (to autosomes) to compensate for the silencing of their X-linked 'ancestral' genes during late spermatogenesis (Emerson et al., 2004; Potrzebowski et al., 2010). However, an excess of recruitment of retrogenes with sex-biased expression from the autosomes to the X has also been noted (Emerson et al., 2004; Potrzebowski et al., 2010). Hence, contrary to what was previously thought, during their evolution, the human sex chromosomes have experienced extensive in and out traffic of retrogenes (Emerson et al., 2004), genes (Skaletsky et al., 2003; Ross et al., 2005), and even of entire chromosomal regions (Waters et al., 2001).

Dosage compensation: the heritage of Lyon and Ohno

One consequence of the massive gene loss of the Y chromosome is the unequal dosage of X-borne genes between XX females (two doses) and XY males (one dose). This dosage imbalance, equivalent to a monosomy would have been deleterious and various mechanisms have evolved to alleviate this potential problem. Ohno and Lyon proposed X chromosome inactivation (XCI) as a dosage compensation mechanism equalizing expression of X-linked genes in eutherian females (XX) and males (XY) (Ohno et al., 1959; Lyon, 1961) (Figure 1). In marsupials, it is invariably the paternal X that is inactivated (imprinted XCI or pXCI). In placental mammals, however, the inactivated X is randomly chosen in each embryonic cell and is then somatically inherited by the daughter cells (random XCI or rXCI). rXCI implies the existence of a counting mechanism sensing how many X chromosomes are present. The existence of this mechanism explains why individuals with supernumerary X chromosomes have only one active X (Augui et al., 2011). These imprinted and random inactivation patterns lead to different expression patterns of deleterious mutations. For instance, marsupial females (pXCI) are functionally hemizygous for the X chromosome and a mutation on the maternal X will be

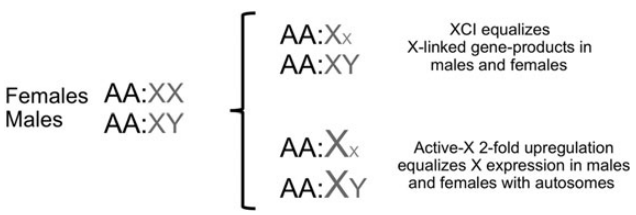

Figure 1 X chromosome inactivation and active X upregulation. X chromosome inactivation (XCI) equalizes expression of X-linked genes in eutherian females (XX) and males (XY). Active X (Xa) upregulation should ensure a balance between autosome (AA)- and X-encoded gene products.

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expressed. The tissues of eutherian females (rXCI) are mosaics in which the cells express one allele or the other in similar proportions. In the case of mutations, a proportion of 50% of cells expressing the normal allele is often compatible with a normal phenotype, as illustrated by X-linked recessive conditions in human. The evolutionary processes behind rXCI and pXCI remain unclear. A recent theoretical population genetic analysis shows that the interaction between allelic dominance and sex-differential selection can generate a broad and continuous range of XCI scenarios, including unequal rates of inactivation between maternal and paternal X chromosomes. rXCI is favored over strict pXCI when alleles deleterious to females are sufficiently recessive (Connallon and Clark, 2013).

In eutherian mammals, the silencing of the inactivated X is established through epigenetic factors initiated by the X Inactivation Center (XIC) involving, in particular, the *Xist* gene that encodes a long non-coding RNA, which is transcribed exclusively from the chromosome to be inactivated. The *Xist* transcript then spreads in *cis* from the XIC to coat the whole X, provoking along the way a series of chromatin changes that trigger inactivation and heterochromatinization of the chromosome (Augui et al., 2011; Gribnau and Grootegoed, 2012). It seems that the simpler paternal XCI (pXCI) has evolved first. It does not require a counting mechanism because the paternal X is always inactivated. Moreover, pXCI seems to require fewer *cis* and *trans* factors than random XCI. For instance, a 250 kb *Xist*-transgene recapitulates pXCI, whereas rXCI requires a segment almost twice as long (Pessia et al., 2013; Schulz and Heard, 2013 and references therein). It is interesting to note that several epigenetic modifications associated with XCI do not cover the inactive X homogeneously, showing the existence of regional differences (Chadwick and Willard, 2004; Prothero et al., 2009). About 15% of human X-linked genes escape XCI and such genes display a non-random chromosomal distribution. Most of them map to the youngest segment of the short arm of the X, as most of the X-Y homologous gene pairs (Carrel and Willard, 2005; Ross et al., 2005). It has been proposed that specific X sequences might act as 'way stations' or 'boosters' that propagate the XCI signal (Gartler and Riggs, 1983; Lyon, 1998). One candidate is the LINE-1 repeat which is enriched on the X and depleted within the regions escaping XCI (Lyon, 1998; Prothero et al., 2009). However, any sequence rapidly accumulating on the X might in principle play the role of booster (Jegalian and Page, 1998). The accumulation of such sequences in the older X chromosome regions might have been facilitated by time and by the fact that sequence insertions in the X can be less well removed by repair than on the autosomes because the X recombines only in the female.

XCI in itself cannot correct the expression imbalance between X-linked and autosomal genes. That is why Ohno later proposed that genes on the active X (hereafter called Xa) should also undergo a 2-fold upregulation. This is the so-called 'Ohno's hypothesis' (Ohno, 1967). This idea is grounded on previous work on the negative effects of aneuploidy. In diploid organisms, there is a spectrum of aneuploid lesions ranging from monosomy (loss of one chromosome) to trisomy (gain of a chromosome) or even more complex cases. The underlying idea of chromosome balance can be tracked back to the early days of genetics with the work on the flowering plant, *Datura stramonium* (Blakeslee et al., 1920). Even then, it was known that the addition of a single chromosome to a genotype was highly detrimental whereas the addition of a whole genome (which makes a polyploid) was viable and resulted in lesser phenotypic effects.

In modern genetic terms, it is known that a dominant phenotype can result from the loss of function of one allele at a given locus. That is, half of the normal amount of active gene-product is not sufficient to ensure a normal phenotype. This phenomenon is called haploinsufficiency (HI) (Veitia, 2002). HI of genes the products of which are involved in macromolecular complexes has been frequently reported and, in such cases, dominance often arises from interactions among several genes. This realization has engendered the idea of gene dosage balance. Loosely speaking, the various products involved in the complex should respect some degree of stoichiometric balance to avoid malfunctioning. This can easily be depicted with the trimer A-B-A (where B is a bridge). If the trimer assembles via the intermediates subcomplexes AB and BA, a decrease in the concentration of A can lead to a disproportionate reduction of trimer. This also works in the case of the overexpression of a subunit of a complex. An excess of B can titrate A and lead to inactive subcomplexes (AB and BA) compromising the amount of active trimer. Thus, both A and B should be balanced to avoid a decrease of trimer concentration with potential phenotypic consequences (Birchler and Veitia, 2012). The idea of dosage balance is also applicable to signaling pathways involving, for instance, enzymes with opposing activities such as a kinase and a phosphatase with a common substrate (Veitia, 2004). All these elements are the core of the 'gene balance hypothesis'.

The need for dosage compensation has also been studied in the context of metabolic control analysis. This classical theory of genetic dominance defines, loosely speaking, the control coefficient C of a particular step as the change of metabolic flux following a change in enzyme concentration. The sum of the values of C over a metabolic pathway is 1 (Kacser and Burns, 1981). Hence, during the process of Y degeneration, genes encoding enzymes involved in longer metabolic pathways should have been lost first, as halving these genes should not drastically reduce flux (and fitness) because the C values of each step are small. However, the increasing cost of hemizygosity would have driven selection for an increased gene expression, as expected also for the complexes and signaling pathways mentioned above. The emerging dosage compensation would have facilitated the loss of genes involved in short metabolic pathways (Hall and Wayne, 2013).

XCI and gene balance reflected in evolution

Most eukaryotic lineages including yeast, vertebrates, protozoa and particularly in plants have experienced cycles of whole genome duplication (WGD) followed by reduction in gene number to near the diploid level (reviewed in Birchler and Veitia, 2012). As genes are deleted in the progression to the near diploid state, the types of genes that are preferentially retained are not random (Freeling, 2009). As a general rule, potentially HI genes are retained in the

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lineages for longer periods of time than other classes of genes. In contrast, they are underrepresented in small scale duplications (Freeling, 2009). An explanation for these observations is that functionally interacting genes have negative fitness consequences if part of the complex is changed in stoichiometry whereas there is less of an impact when all of the interacting genes are varied in dosage as in a WGD. Eventually, however, new balances are apparently found as only a few duplicates with diverged functions are retained over deep evolutionary time. In some sense, the degeneration of a chromosome to form a Y or W might be expected to follow similar rules: genes that are typically lost early following WGD would also be lost easily from a degenerating sex chromosome. Hence HI genes would be resistant to loss unless there are accommodating changes on the intact sex homologue (i.e. dosage compensation or adjustments in the autosomal interactors).

If we return to Ohno's own words he said that 'In the case of placental mammals, however, the Y has shed all the Mendelian genes which were allelic to the genes on the X. As a result, most, if not all, of the X-linked genes exist in the hemizygous state in the male. Each X-linked gene must have accommodated itself to this hemizygous state by doubling the rate of product output. Once this doubling in efficiency was accomplished, the genetic disparity between the male with one X and the female with two Xs became very great. A need arose to adjust the dosage effect of X-linked genes between the two sexes. In mammals, the dosage compensation is accomplished by random inactivation of one or the other X in individual female somatic cells' (Figure 1). These statements recognized the biological problem with which Ohno should be credited; however, they do not explain the interplay between X upregulation-X inactivation achieved through evolution to avoid aneuploid effects.

Dosage effects are often viewed as dramatic but a careful analysis of developmental regulators in mice reveals that as mutant heterozygotes, they can also provide subtle changes on the phenotype (Boell et al., 2013). It is the sum of the imbalance effects over a whole chromosome that explains why most aneuploidies are deleterious or lethal. This points toward a need of stepwise X-Y divergence. This has been recorded as evolutionary strata in the X chromosome (Lahn and Page, 1999). Thus, X-Y homologs mapping to the older strata have diverged more than those in younger strata. In the original paper of Lahn and Page, four evolutionary strata for the human X and Y chromosomes were identified. However, subsequent analyses redefined the stratum 3 boundary and proposed the existence of a fifth stratum and even more recent studies have described up to nine (Pandey et al., 2013). As previously stated, chromosomal inversions may underlie recombination suppression between the proto-XY leading to the different strata. At least for strata 4 and 5, inversions seem to be the most likely mechanism of recombination suppression (Ross et al., 2005; Lemaitre et al., 2009).

Despite the appeal of Ohno's idea, other possibilities have been proposed such that XCI evolved first and was subsequently recruited for dosage compensation. This idea is supported by a population genetic model showing that XCI can evolve under Ohno's hypothesis with somewhat restricted conditions (Engelstädter and Haig, 2008). However, an exhaustive analysis of such alternative theories is beyond the scope of this paper.

Gene expression of the functionally hemizygous X chromosome

Although some anecdotal support for Ohno's hypothesis has been provided through the years in therian mammals, only the advent of genome-wide approaches fueled genomic tests. Unfortunately, microarray and RNAseq data have brought contradictory results and fostered debate. Early comparisons of the global expression of X-linked genes to that of autosomal (AA) ones using microarrays yielded Xa:AA expression ratios close to 1, in agreement with Ohno's hypothesis (see for instance Nguyen and Disteche, 2006). However, recent studies using RNAseq data have reported Xa:AA ratios near 0.5, challenging Ohno's idea (see for instance Xiong et al., 2010). Many other papers (reviewed in Pessia et al., 2013) have claimed and counter-claimed Xa upregulation. The conclusions in favor or against Ohno's hypothesis (almost 50:50 thus far) depend on the nature of the datasets and the various assumptions and thresholds involved in the analyses (Castagné et al., 2011). This is further strengthened by an analysis of RNA-seq data from X-monosomic female human and mouse tissues (which are uncomplicated by genes escaping X-inactivation) as well as of published RNA-seq data. The parameters of short-read mapping programs, the choice of reference genome annotation, expression data distribution, tissue source for RNA and RNA-seq library construction methods have profound effects on the results. Moreover, the high number of paralogous gene families on the X relative to autosomes contributes to the ambiguity. Thus, an analysis considering that single- and multi-copy genes are compensated differently suggests that, in many somatic tissues, there is Xa up-regulation (Jue et al., 2013). One of the latest papers to date, compatible with Ohno's idea, shows that active mouse X-linked promoters are enriched in the initiation form of RNA polymerase II and in specific histone marks associated with active chromatin. However, ancestral X-linked genes and newly acquired ones are upregulated to different extents, as if they were differentially upregulated depending on their evolutionary history (Deng et al., 2013).

Two papers published in 2012 failed to detect whole Xa upregulation but provided concordant transcriptomic and proteomic evidence for the upregulation of X-linked genes encoding members of large protein complexes (Lin et al., 2012; Pessia et al., 2012). These findings are in agreement with the gene balance hypothesis and, beyond the debate on the chromosome-wide validity of Ohno's hypothesis, it is clear that the latter holds for dosage-sensitive genes (i.e. those whose products are involved in stoichiometric relationships with autosome-encoded factors; Lin et al., 2012; Pessia et al., 2012). Another paper has explored the possibility of achieving dosage balance through downregulation of autosomal genes functionally interacting with dosage-sensitive X-linked genes (to compensate for the decrease of their expression due to chromosome Y degradation) (Julien et al., 2012). This process can take place on a one-by-one gene basis and can thus parallel the decay of the Y chromosome. Another type of monoallelic expression deserves mention here. It concerns hundreds of

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genes undergoing random allelic exclusion (Gendrel et al., 2014). As previously mentioned for rXCI, such random monoallelic expression also leads to functional hemizygosity of many loci throughout the genome, which may unmask deleterious mutations. Thus, it is puzzling why evolution favored this mechanism instead of decreasing the expression of both alleles. On the positive side, random monoallelic expression (and also rXCI) may increase phenotypic variability for the same genotypes (owing to stochastic biases in the selection of alleles expressed in small cell populations, see Veitia, 2005), which can be advantageous under certain circumstances. It is not clear whether there is any connection between random monoallelically expressed (downregulated) autosomal genes and compensation of X-linked genes. If there is such a link, it can be predicted that monoallelically expressed autosomal genes functionally interacting with X-linked genes in eutherians should be biallelically expressed in marsupials given the rather consistent Xa-upregulation in the later group of mammals (Julien et al., 2012).

HI genes play an important role in human disease and have an impact on genome organization. As a Y chromosome degenerates and its homologue emerges in the hemizygous state, HI regulators and their fate are an important consideration of how the process takes place. If an HI regulator becomes hemizygous, then there will likely be consequences across the genome. If these autosomal effects are detrimental, the HI regulator would be maintained in two copies, transposed to the autosomes or undergo expression upregulation. Thus, 'dosage compensation' is basically a way to counteract global effects of the hemizygous chromosome for which the effective dosage of pontentially HI genes is likely to be critical.

De Clare et al. (2011) showed that the human X chromosome is depleted of HI genes. These authors consider that the exclusion of HI genes from the X took place in a therian ancestor and ensured protection against a selective disadvantage implied by X inactivation (under a tacit scenario without global Xa-upregulation). Indeed, the presence of HI genes on the X chromosome would have been detrimental for both the heterogametic male, and the female with an inactive X unless Xa upregulation is invoked. Another possibility is that the ancestral autosome that became the X already contained few HI genes and that situation was preserved through evolution. This is a logical idea and might explain why this ancestral chromosome and not another one became the X. However, the authors propose that there might have (also) been active depletion. Indeed, mapping orthologues of human X-linked genes on the zebrafish genome shows the existence of many rearrangements that may have provided the possibility for depleting the X chromosome of HI genes, although this is less obvious when comparing the eutherian X with the chicken genome (i.e. a large portion of the X is orthologous with chicken chromosomes 1 and 4, Figure 2). The depletion of HI genes on the X obviates the need of global Xa upregulation.

Considering whole genomic effects rather than just the sex chromosome

The focus of dosage compensation thought in the past has been on the expression of the sex chromosome itself. We suggest that this focus be shifted to consider the whole genomic effects given the evidence that dosage-sensitive genes involved in regulatory processes such as transcription factors and signal transduction components will modulate their many target genes when changed in dosage (Birchler et al., 2001). Global genomic effects have the potential to obscure or skew the magnitude of determinations of whether there is dosage compensation of a hemizygous sex chromosome and might contribute to the difficulty in making such determinations as described above. This complication is particularly acute if the global effects are greater in one or the other direction from the norm. Particularly troublesome is a tendency to assume that the autosomes are equivalent in expression between the sexes and

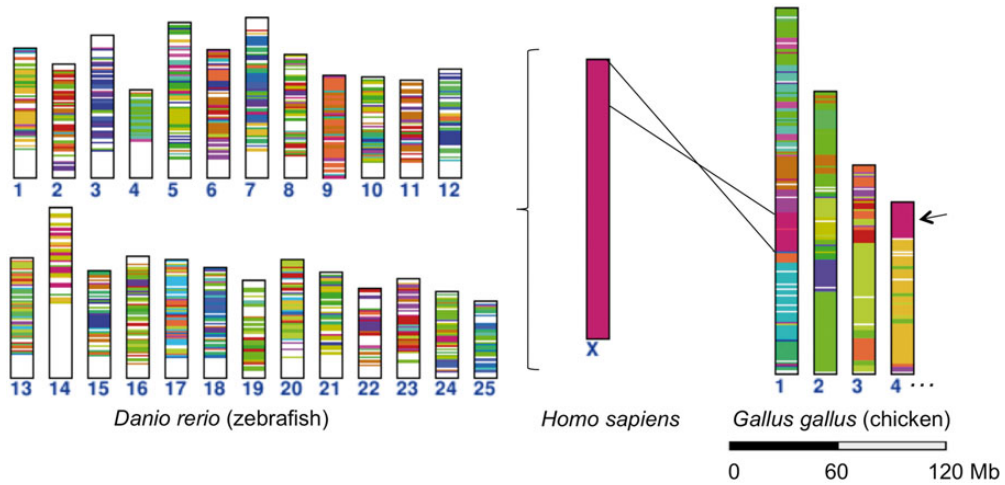

Figure 2 Mapping homologues of human X-linked genes on the zebrafish and chicken genomes using the Cinteny server with default parameters (http://cinteny.cchmc.org/). The orthologs of human genes (in dark pink) are scattered throughout the zebrafish genome. On the contrary, a substantial portion of the X is orthologous with chicken's chromosomes 1 and 4. The other colors represent orthologous segments of the rest of the human chromosomes. Only four chicken chromosomes are represented for simplicity.

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are used as a normalization standard for the sex chromosomes. Indeed, by the very nature of gene expression determinations, if there are unidirectional global effects on the autosomes, which constitute a much larger fraction of the genome than the sex chromosomes, there will be an unwitting normalization that could alter the apparent result of dosage compensation. As examples, one can consider the global upregulation caused by c-Myc that is obscured in RNA-seq data when the number of sequence reads for each sample are corrected by the total reads in a sequencing lane (Lovén et al., 2012). As a second example, when comparing gene expression in diploids and tetraploids, the numerical output per gene is usually very similar between ploidies but needs to be interpreted in light of the fact that twice as many genes are contributing to the RNA pool in a tetraploid and that the cells are larger in the polyploid (Coate and Doyle, 2010).

The importance of considering global effects is illustrated by studies that examine gene expression in aneuploids that have attempted to circumvent these normalization issues. Varying large fractions of the genome in maize and *Drosophila* will modulate expression of genes on and off of the varied chromosome. There are both positive and negative effects of the varied chromosome but an inverse effect is the most common (Birchler, 1979; Birchler and Newton, 1981; Devlin et al., 1988; Guo and Birchler, 1994; Malone et al., 2012; Sun et al., 2013a, b, c). Indeed, many target loci on aneuploid chromosomal segments exhibit dosage compensation (i.e. total expression does not change with segmental dosage). The inverse effect is of a magnitude that it will cause the responsive genes on the varied chromosome to be dosage compensated but at the same time will modulate responsive genes on the unvaried chromosomes. Unlike these experimental conditions, sex chromosomes arrive at the hemizygous state over evolutionary time and thus might either use the inverse effect as a mechanism of dosage compensation or must circumvent the global effects by modulating HI regulators or their autosomal interactors. Indeed, compensation mechanisms are often framed in terms of equalization of gene expression between the sexes but the considerations should really concern the within genome balance issues. In fact, gender differences in expression that produce sexual dimorphisms might actually capitalize upon HI regulators. A study in *Drosophila* showed that genes with sex-biased expression for both males and females are likely modulated by dosage-sensitive effectors (Sun et al., 2013c). Thus, as a sex chromosome evolves to be hemizygous in one sex, any change in dosage of HI regulators might serve to produce gender differences if generalized detrimental genomic effects are minimized.

As already noted, X inactivation without upregulation of HI genes would have mimicked a deletion and their negative fitness effects. However, it is worth noting that a deletion does not always equate to a diminution of the amount of gene product, even without invoking an active mechanism of upregulation. For instance, in some *Drosophila* lines containing heterozygous chromosomal deletions, a negative correlation between gene expression and the degree of dosage compensation has been reported (Malone et al., 2012). That is, the deletions have less effect on the levels of poorly expressed genes than on highly expressed ones. This might also be applicable to some genes on the X (at the beginning of its evolution or even now), which would imply that all the observed X-upregulation is not necessarily due to an active process. We have previously argued that some degree of apparent transcriptional compensation in cases of monosomy can be envisioned for genes activated by limiting amounts of transcription factors (TFs). In such cases, a deletion (or inactivation) of a chromosomal fragment can lead to stronger transcription of the alleles in the homologous segment by a collective effect of reallocation of available limiting TFs. This effect is more likely to appear for poorly expressed genes, which often undergo stochastic expression owing to the low concentrations of both DNA and the effector TFs (Veitia et al., 2013). This idea is also applicable to post-transcriptional events if we postulate that the splicing machinery is limiting. In normal conditions this would lead to the production of some 'mispliced degradation-bound' transcripts. Chromosomal deletions (or inactivation) would relieve the splicing machinery, rendering it less limiting, which would reduce the amount of aberrantly spliced transcripts (now becoming normal mRNAs) and leading to buffering of the deletion/inactivation. However, these mechanisms bear also genome-wide consequences, as sketched in Figure 3.

Lessons about global effects from other species

There is emerging evidence that the different doses of the sex chromosome in the two sexes has an effect on the autosomes or that any such potential effects have been muted. Classically, it was assumed that the male-specific lethal (MSL) complex that accumulates on the male X in *Drosophila* was the primary mediator of its upregulation. However, several studies have shown that when the normalization concerns mentioned above are taken into consideration, there is little change in expression of the X but an increase in expression of the autosomes upon disruption of the MSL complex (Hiebert and Birchler, 1994; Bhadra et al., 2005) and that the sex chromosome compensation extends to triple X females but with a reduced autosomal expression (Sun et al., 2013b). The compensation is apparently mediated by the inverse dosage effect caused by the monosomic X but the MSL complex sequesters a histone acetylase from the autosomes to the X. This sequestration mutes the autosomal inverse effect, which becomes apparent with the disruption of the MSL complex. The regulatory genes that produce the inverse dosage effect must themselves not be compensated or at least be in an altered stoichiometric relationship with interactors in males relative to females. On the X, the MSL complex constrains the effect of increased acetylation (Sun et al., 2013a). Global studies of MSL disruption are subject to the caveats noted above because they all involve X by autosomal normalization, which gives the false impression of reduced X compensation.

In nematodes, there are gene products that are associated with the X or the autosomes in the soma or germline. In the germline, the maternal effect sterile MES-4 protein is preferentially present on the autosomes. MES-2, MES-3 and MES-6 exclude MES-4 from the X (Fong et al., 2002; Bender et al., 2006). When MES-4 is mutated, interestingly, the X shows an apparent increased expression (Bender et al., 2006). In somatic cells, a different system

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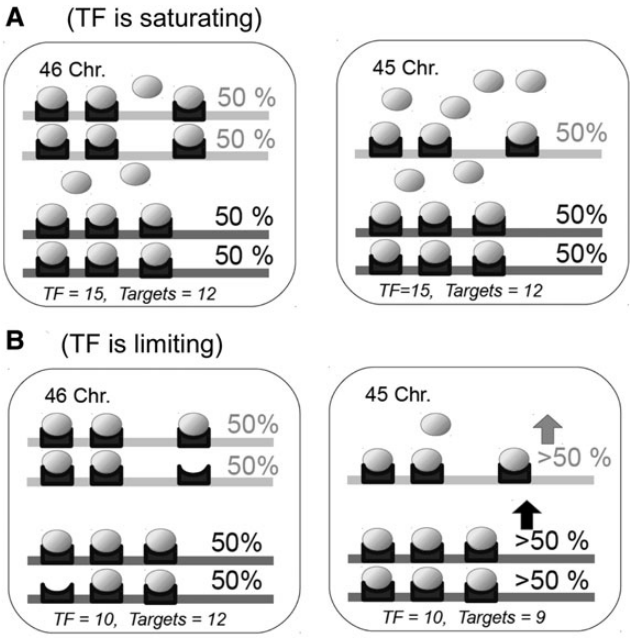

Figure 3 Expression buffering of gene dosage changes and genome-wide effects. Heuristic example showing the genome-wide occupancy of promoters recognized by a transcription factor (TF) in the case of a normal genome (left panels) and with a monosomic chromosome (right panels). Light gray lines represent the monosomic chromosome. The effect of aneuploidy is represented for promoters/genes (black boxes) regulated by a TF (ovals) not encoded by the monosomic chromosome. For simplicity, the available TF is always bound to available promoters. Gene expression is assumed to be proportional to promoter occupancy and the percentages indicate the per-allele expression levels compared with the normal genome (i.e. 50% is the normal per-allele expression). (**A**) When the TF is saturating (e.g. 15 TF molecules for 12 target promoters), the expression level is determined by gene/chromosome copy number. That is, monosomy translates into halving gene expression of the relevant genes. (**B**) When the TF is limiting (e.g. 10 TF molecules for 12 target promoters), the expression level of any gene depends on the occupancy of the other promoters genome-wide. Since binding reactions are reversible, at different times or in different cells, the available TF molecules will be recognizing different promoters (only one possible 'configuration' is illustrated). In the monosomic case, since there is a lower number of targets available, the promoters are on average more occupied by the TF. In the figure, the TF (assumed to be constant in number because of being encoded outside the monosomic chromosome) saturates all the promoters. Hence, the monosomic target genes are expressed at a higher level than in the normal diploid. This leads to an apparent buffering of the monosomic targets but also to genome-wide effects (i.e. increase of the expression of targets outside the monosomic chromosome).

operates. A condensin complex is present on the two X chromosomes in hermaphrodites. When this complex is disrupted, an apparent increased expression of X-linked genes occurs but there is also a reduction of autosomal gene expression (Jans et al., 2009; Kruesi et al., 2013). The single X chromosome in males is apparently upregulated (Gupta et al., 2006; Kruesi et al., 2013). The possibility exists that the mechanism that upregulates the male X is muted in the remainder of the genome by an autosomal specific repressive effect. In hermaphrodites, this repressor might be sequestered from the autosomes to the two X chromosomes to keep the autosomes from being downregulated because there is no X/A imbalance that might cause them to be increased. When released upon disruption of the condensin complex, the X expression might increase and the autosomes would then be reduced in expression, as the apparent experimental results suggest, when the repressor is released from the X and now shows autosomal association. Alternatively, the condensin complex might down regulate in hermaphrodites the process involved in X upregulation in males. When the condensin complex is dissociated, a repressive effect of increased expression of dosage-sensitive regulators would become genome-wide.

Sex-reversed mice have been used to test whether the dosage of the X chromosome will affect autosomal gene expression (Wijchers et al., 2010). Hundreds of genes were found to be modulated by the differential complement of the sex chromosomes but in the same sex. The major trend was that the affected autosomal genes were expressed at a higher level in XY females than in XX females, which might reflect an effect of X dosage or the presence of the Y. A comparison of XX*Sry*+ males versus XY males showed a major trend of higher expression in the former, suggesting that it is the number of X chromosomes that influences the expression of hundreds of autosomal genes.

The above examples illustrate that when attention is paid to the autosomes, there is evidence that global effects in the genome should be considered in understanding the changes that occur for the sex chromosomes.

Open questions

A bit of speculation suggests that if most X-linked genes are dosage-insensitive, upregulation of only dosage-sensitive ones to achieve the balance with autosomes would be advantageous. This would avoid paying the cost of producing an excess of dosage-insensitive gene products (implied by the scenario of whole X-upregulation). This simple costs-benefits idea argues against a chromosome-wide necessity of upregulation. On mechanistic grounds, there are also constraints to transcriptional upregulation. Indeed, for X-linked genes with already high expression levels a further increase might have been difficult because the strength of a nearly saturated promoter cannot be increased.

An interesting question is the significance of genes escaping XCI. They have been thought to be at an intermediate stage of the dosage compensation process (Jegalian and Page, 1998). Dosage effects of genes escaping XCI may explain phenotypes in individuals aneuploid for the X chromosome, such as patients with Turner (generally 45,X0) and Klinefelter (XXY) syndromes (Prothero et al., 2009). Thus, supernumerary X chromosomes, even if mostly inactivated, lead to abnormal phenotypes. Under the hypothesis of whole-Xa upregulation it is difficult to understand why X monosomy impacts development, fertility and longevity in humans and some other species (Bondy and Cheng, 2009). Increased dosage of genes located in PAR cannot fully account

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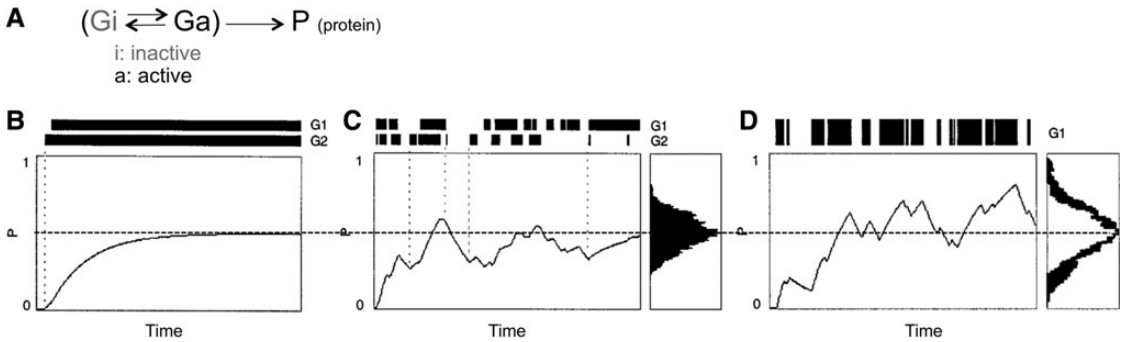

Figure 4 Stochastic gene expression and X inactivation. (**A**) Minimalist model of gene (G) expression with two states: transcriptionally inactive or active + translation. Depending on the activation/inactivation kinetics, a stable gene expression is achieved (**B**) or fluctuations of the protein P appear. In panels **C** and **D**, the same average level of P must be achieved by intermittently activating (with the same kinetics) two alleles (i.e. G1 and G2) or only one allele (but with doubling its per-allele expression). The expression variance is wider in **D**, i.e. compare the black histogram for the single-allele case (**D**) with the white histogram for the two-allele case (**C**). Black stripes over the graphs represent the times when transcription is ON for each allele. Panels **B**, **C**, **D** are reproduced and modified from Cook et al. (1998), with permission 'Copyright (1998) National Academy of Sciences, USA'.

for these phenotypes, because 47,XYY males have 3 PAR copies, but do not share most of these features (save the tall stature, probably related to an excess of SHOX/Y (Prothero et al., 2009 and references therein)).

One can also ask why a complex XCI process evolved in females, rather than leaving two Xa's and capitalizing on a male-specific X-upregulating mechanism. The existence of stochasticity in gene expression is relevant to this discussion. Indeed, the two alleles of lowly expressed genes experience on and off periods, which result in fluctuations of the amount of gene product over time (Cook et al., 1998). Stochastic models show that the net result of intermittent expression of two alleles is not equivalent to doubling the per-allele expression of only one allele (i.e. on Xa). Indeed, when the same total expression is elicited from one or two alleles, the expression variance is wider in the first case (Figure 4). Thus, inactivating one X in females along with upregulation of Xa (chromosome-wide or not) avoids differences in the levels of X-linked gene expression variance between XX and XY eutherians. Selection must have adjusted the variance of X-encoded genes with that of their interacting partners on the autosomes. In this respect, it would be interesting to compare the expression variance of X-linked genes in eutherian mammals and the variance levels of their autosomal orthologs in other species, for instance on chicken's chromosome 4 (for comparable expression levels). Genes that escape X inactivation should also display lower variance levels than X inactivated genes (for similar expression levels) as well.

It is also worth noting that leaving two Xa in females with X-upregulation in males (where the Y is largely heterochromatic) might have brought a 'nucleotypic' disparity, because of the potentially different euchromatic contents. Indeed, an extra Xa in females might have increased nuclear and cell volumes compared to males, leading to sex-specific changes in the concentrations of many factors. Indeed, it is known that trichostatin A (histone deacetylase inhibitor) treatment, which increases the euchromatic DNA fraction, is paralleled by an increase of nuclear volume (Rao et al., 2007). This can also modify transport processes. Finally, it is worth noting that DNA-binding proteins can establish non-functional interactions with DNA/chromatin (Veitia et al, 2013 and references therein). Thus, two euchromatic copies of the X would have increased the number of available sites for non-functional binding, which might have (slightly but notably) altered the genome-wide distribution of productive binding in XX compared to XY individuals. All in all, further studies, both experimental and theoretical are required to fully understand the 'whys and hows' of XCI.

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References

Augui, S., Nora, E.P., and Heard, E. (2011). Regulation of X-chromosome inactivation by the X-inactivation centre. Nat. Rev. Genet. *12*, 429–442.

Bachtrog, D. (2006). A dynamic view of sex chromosome evolution. Curr. Opin. Genet. Dev. *16*, 578–585.

Bachtrog, D. (2013). Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. Nat. Rev. Genet. *14*, 113–124.

Bachtrog, D., Kirkpatrick, M., Mank, J.E., et al. (2011). Are all sex chromosomes created equal? Trends Genet. *27*, 350–357.

Bellott, D.W., Hughes, J.F., Skaletsky, H., et al. (2014). Mammalian Y chromosomes retain widely expressed dosage-sensitive regulators. Nature *508*, 494–499.

Bender, L.B., Suh, J., Carroll, C.R., et al. (2006). MES-4: an autosome-associated histone methyltransferase that participates in silencing the X chromosomes in the C. elegans germ line. Development *133*, 3907–3917.

Bhadra, M.P., Bhadra, U., Kundu, J., et al. (2005). Gene expression analysis of the function of the male-specific lethal complex in Drosophila. Genetics *169*, 2061–2074.

Birchler, J.A. (1979). A study of enzyme activities in a dosage series of the long arm of chromosome one in maize. Genetics *92*, 1211–1229.

Birchler, J.A., and Newton, K.J. (1981). Modulation of protein levels in chromosomal dosage series of maize: the biochemical basis of aneuploid syndromes. Genetics *99*, 247–266.

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Birchler, J.A., and Veitia, R.A. (2012). Gene balance hypothesis: connecting issues of dosage sensitivity across biological disciplines. Proc. Natl Acad. Sci. USA *109*, 14746–14753.

Birchler, J.A., Bhadra, U., Bhadra, M.P., et al. (2001). Dosage-dependent gene regulation in multicellular eukaryotes: implications for dosage compensation, aneuploid syndromes, and quantitative traits. Dev. Biol. *234*, 275–288.

Blakeslee, A.F., Belling, J., and Farnham, M.E. (1920). Chromosomal duplication and mendelian phenomena in datura mutants. Science *52*, 388–390.

Boell, L., Pallares, L.F., Brodski, C., et al. (2013). Exploring the effects of gene dosage on mandible shape in mice as a model for studying the genetic basis of natural variation. Dev. Genes Evol. *223*, 279–287.

Bondy, C.A., and Cheng, C. (2009). Monosomy for the X chromosome. Chromosome Res. *17*, 649–658.

Carrel, L., and Willard, H.F. (2005). X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature *434*, 400–404.

Castagné, R., Rotival, M., Zeller, T., et al. (2011). The choice of the filtering method in microarrays affects the inference regarding dosage compensation of the active X-chromosome. PLoS One *6*, e23956.

Chadwick, B.P., and Willard, H.F. (2004). Multiple spatially distinct types of facultative heterochromatin on the human inactive X chromosome. Proc. Natl Acad. Sci. USA *101*, 17450–17455.

Charlesworth, B., and Charlesworth, D. (2000). The degeneration of Y chromosomes. Philos. Trans. R. Soc. Lond. B Biol. Sci. *355*, 1563–1572.

Coate, J.E., and Doyle, J.J. (2010). Quantifying whole transcriptome size, a prerequisite for understanding transcriptome evolution across species: an example from a plant allopolyploid. Genome Biol. Evol. *2*, 534–546.

Connallon, T., and Clark, A.G. (2013). Sex-differential selection and the evolution of X inactivation strategies. PLoS Genet. *9*, e1003440.

Cook, D.L., Gerber, A.N., and Tapscott, S.J. (1998). Modeling stochastic gene expression: implications for haploinsufficiency. Proc. Natl Acad. Sci. USA *95*, 15641–15646.

Cortez, D., Marin, R., Toledo-Flores, D., et al. (2014). Origins and functional evolution of Y chromosomes across mammals. Nature *508*, 488–493.

De Clare, M., Pir, P., and Oliver, S.G. (2011). Haploinsufficiency and the sex chromosomes from yeasts to humans. BMC Biol. *9*, 15.

Deng, X., Berletch, J.B., Ma, W., et al. (2013). Mammalian X upregulation is associated with enhanced transcription initiation, RNA half-life, and MOF-mediated H4K16 acetylation. Dev. Cell *25*, 55–68.

Devlin, R.H., Holm, D.G., and Grigliatti, T.A. (1988). The influence of whole-arm trisomy on gene expression in Drosophila. Genetics *118*, 87–101.

Ellegren, H. (2011). Sex-chromosome evolution: recent progress and the influence of male and female heterogamety. Nat. Rev. Genet. *12*, 157–166.

Emerson, J.J., Kaessmann, H., Betrán, E., et al. (2004). Extensive gene traffic on the mammalian X chromosome. Science *303*, 537–540.

Engelstädter, J., and Haig, D. (2008). Sexual antagonism and the evolution of X chromosome inactivation. Evolution *62*, 2097–2104.

Fong, Y., Bender, L., Wang, W., et al. (2002). Regulation of the different chromatin states of autosomes and X chromosomes in the germ line of C. elegans. Science *296*, 2235–2238.

Freeling, M. (2009). Bias in plant gene content following different sorts of duplication: tandem, whole-genome, segmental, or by transposition. Annu. Rev. Plant Biol. *60*, 433–453.

Gartler, S.M., and Riggs, A.D. (1983). Mammalian X-chromosome inactivation. Annu. Rev. Genet. *17*, 155–190.

Gendrel, A.-V., Attia, M., Chen, C.-J., et al. (2014). Developmental dynamics and disease potential of random monoallelic gene expression. Dev. Cell *28*, 366–380.

Graves, J.A.M. (2006). Sex chromosome specialization and degeneration in mammals. Cell *124*, 901–914.

Graves, J.A.M., Gécz, J., and Hameister, H. (2002). Evolution of the human X—a smart and sexy chromosome that controls speciation and development. Cytogenet. Genome Res. *99*, 141–145.

Graves, J.A.M., Koina, E., and Sankovic, N. (2006). How the gene content of human sex chromosomes evolved. Curr. Opin. Genet. Dev. *16*, 219–224.

Gribnau, J., and Grootegoed, J.A. (2012). Origin and evolution of X chromosome inactivation. Curr. Opin. Cell Biol. *24*, 397–404.

Guo, M., and Birchler, J.A. (1994). Trans-acting dosage effects on the expression of model gene systems in maize aneuploids. Science *266*, 1999–2002.

Gupta, V., Parisi, M., Sturgill, D., et al. (2006). Global analysis of X-chromosome dosage compensation. J. Biol. *5*, 3.

Gurbich, T.A., and Bachtrog, D. (2008). Gene content evolution on the X chromosome. Curr. Opin. Cell Biol. *18*, 493–498.

Hall, D.W., and Wayne, M.L. (2013). Ohno's 'peril of hemizygosity' revisited: gene loss, dosage compensation, and mutation. Genome Biol. Evol. *5*, 1–15.

Hiebert, J.C., and Birchler, J.A. (1994). Effects of the maleless mutation on X and autosomal gene expression in Drosophila melanogaster. Genetics *136*, 913–926.

Jans, J., Gladden, J.M., Ralston, E.J., et al. (2009). A condensin-like dosage compensation complex acts at a distance to control expression throughout the genome. Genes Dev. *23*, 602–618.

Jegalian, K., and Page, D.C. (1998). A proposed path by which genes common to mammalian X and Y chromosomes evolve to become X inactivated. Nature *394*, 776–780.

Joron, M., Papa, R., Beltrán, M., et al. (2006). A conserved supergene locus controls colour pattern diversity in Heliconius butterflies. PLoS Biol. *4*, e303.

Jue, N.K., Murphy, M.B., Kasowitz, S.D., et al. (2013). Determination of dosage compensation of the mammalian X chromosome by RNA-seq is dependent on analytical approach. BMC Genomics *14*, 150.

Julien, P., Brawand, D., Soumillon, M., et al. (2012). Mechanisms and evolutionary patterns of mammalian and avian dosage compensation. PLoS Biol. *10*, e1001328.

Kacser, H., and Burns, J.A. (1981). The molecular basis of dominance. Genetics *97*, 639–666.

Khil, P.P., Smirnova, N.A., Romanienko, P.J., et al. (2004). The mouse X chromosome is enriched for sex-biased genes not subject to selection by meiotic sex chromosome inactivation. Nat. Genet. *36*, 642–646.

Kruesi, W.S., Core, L.J., Waters, C.T., et al. (2013). Condensin controls recruitment of RNA polymerase II to achieve nematode X-chromosome dosage compensation. ELife, *2*, e00808.

Lahn, B.T., and Page, D.C. (1997). Functional coherence of the human Y chromosome. Science *278*, 675–680.

Lahn, B.T., and Page, D.C. (1999). Four evolutionary strata on the human X chromosome. Science *286*, 964–967.

Lemaitre, C., Braga, M.D.V., Gautier, C., et al. (2009). Footprints of inversions at present and past pseudoautosomal boundaries in human sex chromosomes. Genome Biol. Evol. *1*, 56–66.

Lin, F., Xing, K., Zhang, J., et al. (2012). Expression reduction in mammalian X chromosome evolution refutes Ohno's hypothesis of dosage compensation. Proc. Natl Acad. Sci. USA *109*, 11752–11757.

Lovén, J., Orlando, D.A., Sigova, A.A., et al. (2012). Revisiting global gene expression analysis. Cell *151*, 476–482.

Lyon, M.F. (1961). Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature *190*, 372–373.

Lyon, M.F. (1998). X-chromosome inactivation: a repeat hypothesis. Cytogenet. Cell Genet. *80*, 133–137.

Malone, J.H., Cho, D.-Y., Mattiuzzo, N.R., et al. (2012). Mediation of Drosophila autosomal dosage effects and compensation by network interactions. Genome Biol. *13*, r28.

Matsubara, K., Tarui, H., Toriba, M., et al. (2006). Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. Proc. Natl Acad. Sci. USA *103*, 18190–18195.

Mueller, J.L., Mahadevaiah, S.K., Park, P.J., et al. (2008). The mouse X chromosome is enriched for multicopy testis genes showing postmeiotic expression. Nat. Genet. *40*, 794–799.

Mueller, J.L., Skaletsky, H., Brown, L.G., et al. (2013). Independent specialization of the human and mouse X chromosomes for the male germ line. Nat. Genet. *45*, 1083–1087.

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Nguyen, D.K., and Disteche, C.M. (2006). Dosage compensation of the active X chromosome in mammals. Nat. Genet. *38*, 47–53.

Ohno, S. (1967). Sex Chromosomes and Sex-Linked Genes. Berlin: Springer.

Ohno, S., Kaplan, W.D., and Kinosita, R. (1959). Formation of the sex chromatin by a single X-chromosome in liver cells of Rattus norvegicus. Exp. Cell Res. *18*, 415–418.

Pandey, R.S., Wilson Sayres, M.A., and Azad, R.K. (2013). Detecting evolutionary strata on the human X chromosome in the absence of gametologous y-linked sequences. Genome Biol. Evol. *5*, 1863–1871.

Pessia, E., Makino, T., Bailly-Bechet, M., et al. (2012). Mammalian X chromosome inactivation evolved as a dosage-compensation mechanism for dosage-sensitive genes on the X chromosome. Proc. Natl Acad. Sci. USA *109*, 5346–5351.

Pessia, E., Engelstädter, J., and Marais, G.A.B. (2013). The evolution of X chromosome inactivation in mammals: the demise of Ohno's hypothesis? Cell. Mol. Life Sci. *71*, 1383–1394.

Potrzebowski, L., Vinckenbosch, N., and Kaessmann, H. (2010). The emergence of new genes on the young therian X. Trends Genet. *26*, 1–4.

Prothero, K.E., Stahl, J.M., and Carrel, L. (2009). Dosage compensation and gene expression on the mammalian X chromosome: one plus one does not always equal two. Chromosome Res. *17*, 637–648.

Rao, J., Bhattacharya, D., Banerjee, B., et al. (2007). Trichostatin-A induces differential changes in histone protein dynamics and expression in HeLa cells. Biochem. Biophys. Res. Commun. *363*, 263–268.

Rice, W.R. (1984). Sex chromosomes and the evolution of sexual dimorphism. Evolution *38*, 735–742.

Rice, W.R. (1987). Genetic hitchhiking and the evolution of reduced genetic activity of the Y sex chromosome. Genetics *116*, 161–167.

Ross, M.T., Grafham, D.V., Coffey, A.J., et al. (2005). The DNA sequence of the human X chromosome. Nature *434*, 325–337.

Saifi, G.M., and Chandra, H.S. (1999). An apparent excess of sex- and reproduction-related genes on the human X chromosome. Proc. Biol. Sci. *266*, 203–209.

Saxena, R., Brown, L.G., Hawkins, T., et al. (1996). The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. Nat. Genet. *14*, 292–299.

Schulz, E.G., and Heard, E. (2013). Role and control of X chromosome dosage in mammalian development. Curr. Opin. Genet. Dev. *23*, 109–115.

Skaletsky, H., Kuroda-Kawaguchi, T., Minx, P.J., et al. (2003). The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature *423*, 825–837.

Sun, L., Fernandez, H.R., Donohue, R.C., et al. (2013a). Male-specific lethal complex in Drosophila counteracts histone acetylation and does not mediate dosage compensation. Proc. Natl Acad. Sci. USA *110*, E808–E817.

Sun, L., Johnson, A.F., Donohue, R.C., et al. (2013b). Dosage compensation and inverse effects in triple X metafemales of Drosophila. Proc. Natl Acad. Sci. USA *110*, 7383–7388.

Sun, L., Johnson, A.F., Li, J., et al. (2013c). Differential effect of aneuploidy on the X chromosome and genes with sex-biased expression in Drosophila. Proc. Natl Acad. Sci. USA *110*, 16514–16519.

Turner, J.M.A. (2007). Meiotic sex chromosome inactivation. Development *134*, 1823–1831.

Vallender, E.J., and Lahn, B.T. (2004). How mammalian sex chromosomes acquired their peculiar gene content. Bioessays *26*, 159–169.

Veitia, R.A. (2002). Exploring the etiology of haploinsufficiency. Bioessays *24*, 175–184.

Veitia, R.A. (2004). Gene dosage balance in cellular pathways: implications for dominance and gene duplicability. Genetics *168*, 569–574.

Veitia, R.A. (2005). Stochasticity or the fatal 'imperfection' of cloning. J. Biosci. *30*, 21–30.

Veitia, R.A., Bottani, S., and Birchler, J.A. (2013). Gene dosage effects: nonlinearities, genetic interactions, and dosage compensation. Trends Genet. *29*, 385–393.

Veyrunes, F., Waters, P.D., Miethke, P., et al. (2008). Bird-like sex chromosomes of platypus imply recent origin of mammal sex chromosomes. Genome Res. *18*, 965–973.

Vicoso, B., and Charlesworth, B. (2006). Evolution on the X chromosome: unusual patterns and processes. Nat. Rev. Genet. *7*, 645–653.

Wang, P.J., McCarrey, J.R., Yang, F., et al. (2001). An abundance of X-linked genes expressed in spermatogonia. Nat. Genet. *27*, 422–426.

Waters, P.D., Duffy, B., Frost, C.J., et al. (2001). The human Y chromosome derives largely from a single autosomal region added to the sex chromosomes 80–130 million years ago. Cytogenet. Cell Genet. *92*, 74–79.

Wijchers, P.J., Yandim, C., Panousopoulou, E., et al. (2010). Sexual dimorphism in mammalian autosomal gene regulation is determined not only by Sry but by sex chromosome complement as well. Dev. Cell *19*, 477–484.

Wilson, M.A., and Makova, K.D. (2009). Genomic analyses of sex chromosome evolution. Annu. Rev. Genomics Hum. Genet. *10*, 333–354.

Wimmer, R., Kirsch, S., Rappold, G.A., et al. (2005). Evolutionary breakpoint analysis on Y chromosomes of higher primates provides insight into human Y evolution. Cytogenet. Genome Res. *108*, 204–210.

Xiong, Y., Chen, X., Chen, Z., et al. (2010). RNA sequencing shows no dosage compensation of the active X-chromosome. Nat. Genet. *42*, 1043–1047.

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