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Role and control of X chromosome dosage in mammalian development

Edda G Schulz and Edith Heard

Many species have evolved sex chromosomes with highly divergent gene content, such as the X and Y chromosomes in mammals. As most non sex-specific genes probably need to be expressed at similar levels in males and females, dosage compensation mechanisms are in place to equalize the gene dosage between the sexes, and possibly also between sex chromosomes and autosomes. In mammals, one out of two X chromosomes is inactivated early during development in a process called X-chromosome inactivation that has been investigated intensively in the 50 years since it was discovered. Less is known about the potential functional roles of X-linked gene dosage, for example in controlling development in a sex-specific manner. In this review, we discuss the evolution of dosage compensation and how it is controlled during embryogenesis of mammals. In addition we will summarize evidence on the potential role of X chromosome number during early development.

Address
Mammalian Developmental Epigenetics Group, Institut Curie, CNRS UMR 3215, INSERM U934, Paris 75248, France

Corresponding author: Heard, Edith (Edith.Heard@curie.fr)

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Introduction

In many species, sex is determined by sex chromosome composition, such as XY and XX in male and female mammals. The X and Y chromosomes presumably evolved from a pair of autosomes, one of which (the proto-Y) acquired a sex determining gene and subsequently accumulated male beneficial genes. To permanently link these genes to the male sex, the Y chromosome had to cease recombining with the X during meiosis, and this facilitated a progressive loss of Y-linked genes (reviewed in [1]). Therefore for most X-linked genes, only one copy remains, on the X, and this creates a dosage imbalance, both with respect to interacting autosomal genes and between the sexes (XX versus XY). To resolve this dosage imbalance, a variety of dosage compensation mechanisms have evolved (reviewed in [2]). The first step in compensating the loss of Y-linked genes is probably the upregulation of their X-linked homologs (Ohno's hypothesis [3]). In the well-studied example of Drosophila this upregulation is restricted to males, thereby restoring both dosage equality between the X chromosome and autosomes, as well as between the sexes. In contrast, worms (*Caenorhabditis elegans*) and mammals are thought to upregulate the X chromosome in both sexes and therefore require additional mechanisms to balance X-linked gene dosage between males and females. To achieve this, worms downregulate both X's in hermaphrodites (XX), while mammals, on which we focus in this review, silence one of the two X chromosomes in each female cell in a process called X-chromosome inactivation (XCI). Albeit widespread, the extent of dosage compensation between the sexes varies considerably among species. In birds for example, dosage compensation of Z-linked genes is only partial, as males (ZZ) express 1.2–1.4-fold higher levels compared to females (ZW) [2].

Although in mammals, dosage compensation for most genes on the X is probably vital, dosage differences might also play a functional role at several levels. In particular, genes that escape X inactivation are thought to contribute to the phenotypic differences between the sexes [4]. In addition, before initiation of XCI during early development all genes on the X should be present as a double dose in females, but it is not known whether this results in sex-specific developmental differences and so far, this question has received little attention. In this review, we will discuss recent advances in our understanding of the evolution of dosage compensation in mammals, between the X chromosome and autosomes, as well as between the sexes. We will also discuss how dosage compensation is achieved and how X-chromosome dosage might contribute to sex differences, during mammalian development.

Evolution of sex chromosome dosage compensation in mammals

In recent years the question of whether the X chromosome was indeed upregulated during the evolution of the sex chromosomes (Ohno's hypothesis) has received much attention. Initial studies comparing median expression levels of present day chromosomes, that is, X versus autosomes, lead to conflicting conclusions: when analyzing all expressed genes, the levels of X-linked genes were found to be indistinguishable from those of autosomal genes, while an analysis that excluded lowly expressed

genes showed some degree of upregulation of the X chromosome [5–8]. As expression can vary significantly even between different autosomes, it has been difficult to draw definitive conclusions from these X-to-autosome comparisons [9••]. To try and circumvent this problem, two groups recently compared X-linked genes with orthologous genes in species, where these genes have remained autosomal, because their sex chromosomes evolved from a different pair of autosomes (e.g. birds) [9••,10]. These studies did not find any obvious upregulation of the X chromosome. However, there are indications that instead of chromosome-wide upregulation, initial dosage compensation was restricted to a subset of particularly dose-sensitive genes. For example, components of large protein complexes, whose stoichiometry probably needs to be maintained for proper function, show a ~2-fold higher median expression when they are located on the X chromosome compared to autosomal protein complex genes [11•]. Moreover, the comparison of X-linked genes with autosomal orthologs in other species has suggested that *autosomal* genes, the protein products of which physically interact with those of X-linked genes, might have actually been *down*regulated to achieve dosage compensation [9••]. Therefore, the available evidence suggests that the X chromosome has not necessarily been upregulated in a chromosome-wide fashion. Instead, dosage compensation in relation to autosomal genes might have been restricted mainly to certain dosage sensitive genes. However, even the compensation of a subset of genes makes it necessary to equalize X-linked gene dosage between XY males and XX females, which, in mammals, is ensured by X inactivation.

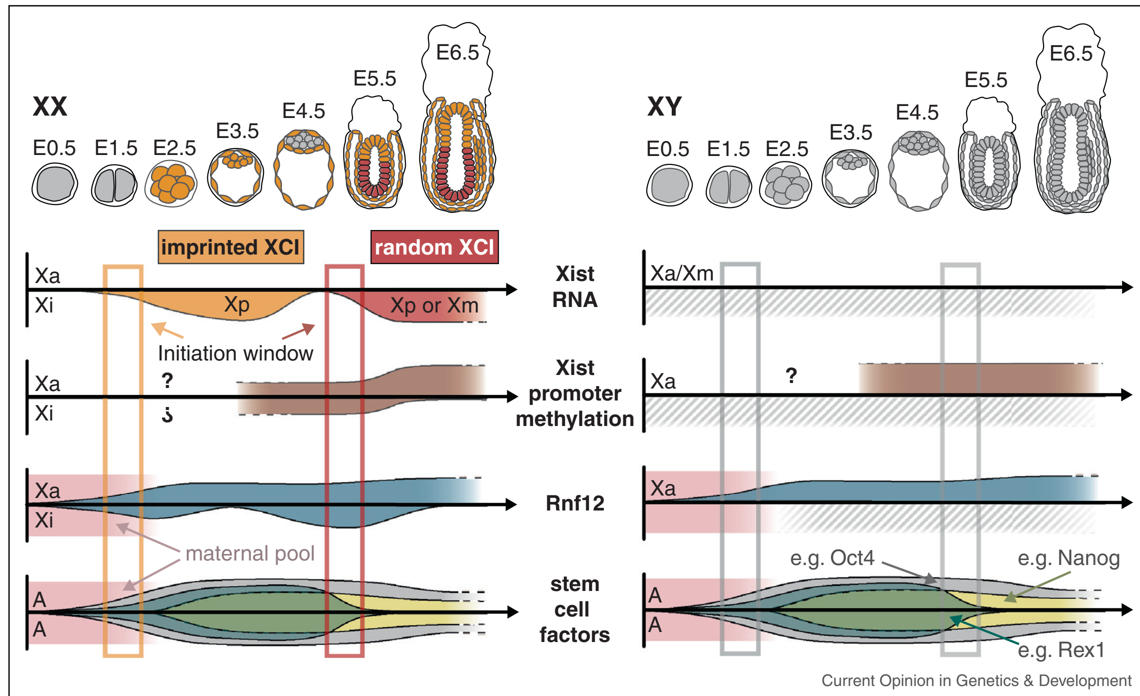
X inactivation is found in all mammals with the exception of the most ancestral monotreme lineage, which, similarly to birds, do not appear to show global dosage compensation between the sexes [9••]. In marsupials, X inactivation is imprinted so that the paternal X is inactive in all cells, while placental mammals inactivate one randomly chosen X chromosome in each cell. Mice, which show random XCI in somatic tissues, have, in addition, evolved an imprinted form of X inactivation, which does not appear to be present in humans or rabbits, on the basis of existing data [12]. In mice, the paternal X is inactivated in the pre-implantation embryo and remains silent in extra-embryonic tissues, while it is reactivated in the embryo proper, before the onset of random XCI in the epiblast (Figure 1). In placental mammals XCI is controlled by the long non-coding RNA Xist, which is expressed from the inactive X and mediates gene silencing *in cis* [13]. In marsupials, Xist is not present, although another, apparently unrelated, non-coding RNA, called Rsx, seems to be involved in XCI. The exact role and mechanism of action of Rsx in marsupial dosage compensation remain to be defined [14•].

X-chromosomal dosage defects

The importance of X-linked gene dosage in mammals is illustrated by the symptoms associated with certain X aneuploidies. X monosomy (XO) for example, which leads to Turner syndrome in humans, is associated with congenital malformations and infertility [15]. Why should XO and XX females differ, when both have only one active X chromosome in theory? In fact, the observed defects are attributed to a subset of genes that escape X inactivation [4]. Some of these genes have a homolog on the Y (e.g. in the pseudoautosomal region), and are therefore present at reduced levels in Turner patients compared to normal males and females. Interestingly, XO mice show no phenotype except for being infertile [16]. This is probably due to the fact that far fewer genes escape XCI in mice (~3%) compared to humans (~15%) and also because the human X carries more pseudoautosomal genes than the mouse X chromosome [17,18]. Some behavioral symptoms of Turner patients are specific to cases where the paternal or the maternal X is present, suggesting a role of imprinted genes on the X. While Turner syndrome is associated with reduced levels of escape genes, the XXY Klinefelter syndrome presumably results from increased levels and manifests itself by tall stature, infertility and behavioral abnormalities [16]. Surprisingly, symptoms due to increased X-chromosome copy number are more severe in the male XXY context. Triple-X females (XXX) are often not even diagnosed because of their very mild phenotype [19]. This suggests a sex-specific function of escape genes.

In cases where X-chromosome dosage is disturbed due to the absence of XCI, more severe defects are seen. For example, ring X chromosomes that cannot undergo XCI because they lack the *Xist* locus cause multiple malformations and mental retardation due to a double dose of some of the (usually rather few) genes present on the ring [15]. The consequences of an abnormal dose of the entire X chromosome have been studied extensively in mice in different situations where XCI is not properly triggered. Defective imprinted XCI in the early embryo, leads to severe abnormalities in the extra-embryonic tissues and the embryos die around day 7 of gestation [20–22], while defective XCI in the embryo *proper* results in death around day 10 d.p.c. [23]. It is therefore clear that several developmental processes are sensitive to abnormal X dosage, but which genes actually require this stringent control? In general, genes participating in protein complexes and regulatory networks are thought to be especially dosage sensitive [24]. This is supported by the observation that most haplo-insufficiencies in humans involve transcription factors and protein complex genes, but rarely metabolic enzymes [25]. In the case of the X chromosome, however, the genes that need to be tightly controlled for normal development to occur remain to be identified.

Figure 1



Regulation of X-chromosome inactivation during early mouse development. Imprinted XCI is initiated by *Xist* upregulation (yellow) from the paternal X at the 2–4-cell stage, when the *Xist* activator Rnf12 is present as a large maternal pool (rose). Imprinted XCI is reversed in the inner cell mass of the blastocyst (~E4.5), where *Xist* becomes downregulated. Subsequently, *Xist* (red) is upregulated from one randomly chosen X (Xm or Xp) in each cell, at least in part triggered by the downregulation of mostly autosomal (A), repressive stem cell factors (green). Female specific upregulation of *Xist* is partly ensured by a double dose of Rnf12 (blue) and maybe by reduced methylation levels of the *Xist* promoter (brown). On the inactive X (Xi) X-linked genes like *Rnf12* are silenced and the *Xist* promoter loses methylation, while the *Xist* promoter on the active X (Xa) becomes fully methylated. The methylation state of the *Xist* promoter (brown) in male and female embryos is unknown, and was estimated from ES cell data. The depicted dynamics of stem cell factors are on the basis of [44,59]. Boxes indicate the time window when imprinted and random XCI are initiated.

Developmental regulation of X inactivation

In mice, imprinted XCI is initiated on the paternal X at the 2–4-cell stage [26]. In the inner cell mass (ICM) of the blastocyst the paternal X is then reactivated and random XCI occurs during the transition from the late blastocyst (E4.5) to the epiblast (E5.5) (Figure 1) [26,27]. The *Xist* imprint is established in the female germ line and prevents *Xist* upregulation [28,29]. Its exact nature is still unknown, but it is located within a 210 kb *Xist*-containing transgene that was shown to be sufficient to recapitulate imprinted XCI [30]. All X chromosomes (and *Xist* transgenes so far) that are not protected by the imprint are inactivated during imprinted XCI, independently of the number of X chromosomes in the cell [29,31]. Random XCI, by contrast, is regulated by more complex mechanisms than the imprinted version and cannot even be recapitulated by a large 460 kb *Xist*-containing transgene [32]. As the imprint on *Xist* is lost or overridden before the onset of random XCI, alternative mechanisms must ensure *Xist* expression from exactly one out of two *identical* chromosomes in a female-specific (i.e. X dose sensitive) fashion. Female specificity is in part ensured by the X-linked *Xist* activator Rnf12 (Rlim) present as a double dose in female XX cells (Figure 1) [33]. Rnf12 is a E3 ubiquitin ligase that might function by targeting the *Xist* repressor Rex1 (Zfp42) (see below) for degradation [34••]. During imprinted XCI, Rnf12 is provided as a large maternal pool independently of the sex of the embryo and allows the paternal *Xist* gene to be upregulated right after zygotic genome activation [35•]. The maternal *Xist* gene resists this due to its repressive imprint. Although additional regulators as well as trans-interactions have been proposed to contribute to female-specific *Xist* expression during random XCI (reviewed in [36]), Rnf12 remains the only factor whose overexpression leads to ectopic Xist upregulation in male cells. However, additional X-linked activators must exist, since even with a single dose of Rnf12 (in $Rnf12^{+/-}$ heterozygous ESCs or mice), females can still initiate XCI [33,35•].

With regard to the mechanisms that control the onset of random XCI, many insights have come from the use of embryonic stem cells (ESCs), which are derived from the ICM of the blastocyst and undergo XCI upon differentiation *in vitro*. These studies have led to a model whereby *Xist* is repressed by stem cell factors, which

are present at high levels in the ICM and ESCs. *Xist* upregulation is then triggered by the downregulation of these repressors, in addition to the expression of X-dosage sensitive factors such as Rnf12. A series of stem cell factors have been proposed to be implicated in *Xist* regulation (summarized in Table 1), as their depletion in ESCs results in upregulation of *Xist* [34••,37–39]. It is however difficult to identify the factor(s) that directly regulate *Xist* and are required for its repression, because the stem cell network is highly inter-connected and depletion of any one factor affects the expression of many others [40]. Often, binding of a candidate regulator to a locus is taken as an indication for direct regulation. In the case of *Xist*, however, nearly all stem cell factors tested so far, bind to one or more sites within or around the *Xist* gene (Figure 2, Table 1) [34••,37–39,41,42]. One of these binding sites (in *Xist* intron 1) has recently been tested for functionality by deletion and was found to have only a minor effect, if any, on *Xist* expression during differentiation [43]. This suggests either that only a subset of binding sites are functional, or that the sites and therefore also the factors that occupy them, act in a redundant fashion. To narrow down the list of potential relevant repressors of *Xist*, their expression kinetics are clearly important. Apart from binding to the locus and *Xist* upregulation upon deletion or depletion of the factor, a relevant *Xist* repressor should be downregulated before or concomitantly with *Xist* upregulation *in vivo*. While the core pluripotency factors Oct4, Nanog and Sox2 are still expressed in the E5.75 epiblast, some other stem cell factors such as Rex1 and Prdm14 are strongly downregulated [44] (Table 1, Figure 1). Rex1 is indeed a likely *Xist* repressor both as a target of the Rnf12 ubiquitin ligase and as an activator of *Tsix*, the antisense repressor of *Xist* [34••,38]. Also a potential role of Prdm14 in *Xist* regulation merits further investigation, as its ectopic expression in combination with Klf2, increases the efficiency of *Xist* reactivation during reprogramming of epiblast stem cells [45••]. Taken together, the emerging picture is that the core pluripotency factors may not directly control the initiation of XCI but rather stem cell factors, such as Rex1 or Prdm14 may play important roles in *Xist* regulation, the precise nature of which needs to be explored further.

Sex-specific functions of X-chromosome dosage in development

The two time windows during mouse development when X-chromosome number would be predicted to have the strongest effect, are in the ICM and in the female germ line, when both X's are active. At least in ES cells, X-linked genes show on average a 2-fold higher expression in female cells compared to males [6]. Interestingly, X dosage also affects global levels of DNA methylation, such that XX cells are hypomethylated at repeats and imprinted regions compared to XY and XO cells [46,47]. The fact that female primordial germ cells, which also have two active X chromosomes, are similarly hypomethylated, supports a role of X-chromosomal dosage in controlling global DNA methylation levels [48]. The X-linked gene(s) responsible for this effect remain to be found however. These sex-specific differences in methylation levels might also contribute to ensuring the female-specificity of random XCI. Mirroring global methylation differences, the *Xist* promoter is fully methylated in undifferentiated male ES cells, but only partially methylated in females [49,50]. During differentiation of female ESCs, the promoter gains methylation on the active X (where *Xist* is silenced) and loses methylation on the inactive X from which *Xist* is expressed [51]. *Xist* promoter methylation on the active X is important to maintain its silent state during differentiation, since a loss of methylation in embryonic fibroblasts results in *Xist* derepression, and male ES cells deficient for DNA methyltransferases erroneously upregulate *Xist* at later stages of differentiation [52–54]. Given these sex-specific differences in ESC DNA methylation levels, it is interesting to speculate that DNA hypomethylation in XX cells might be required to allow X inactivation in females. This hypothesis however remains to be investigated.

A hint that X-chromosome dosage, and the transient double dose of X-linked genes in the ICM of the female blastocyst (see above) might indeed affect development

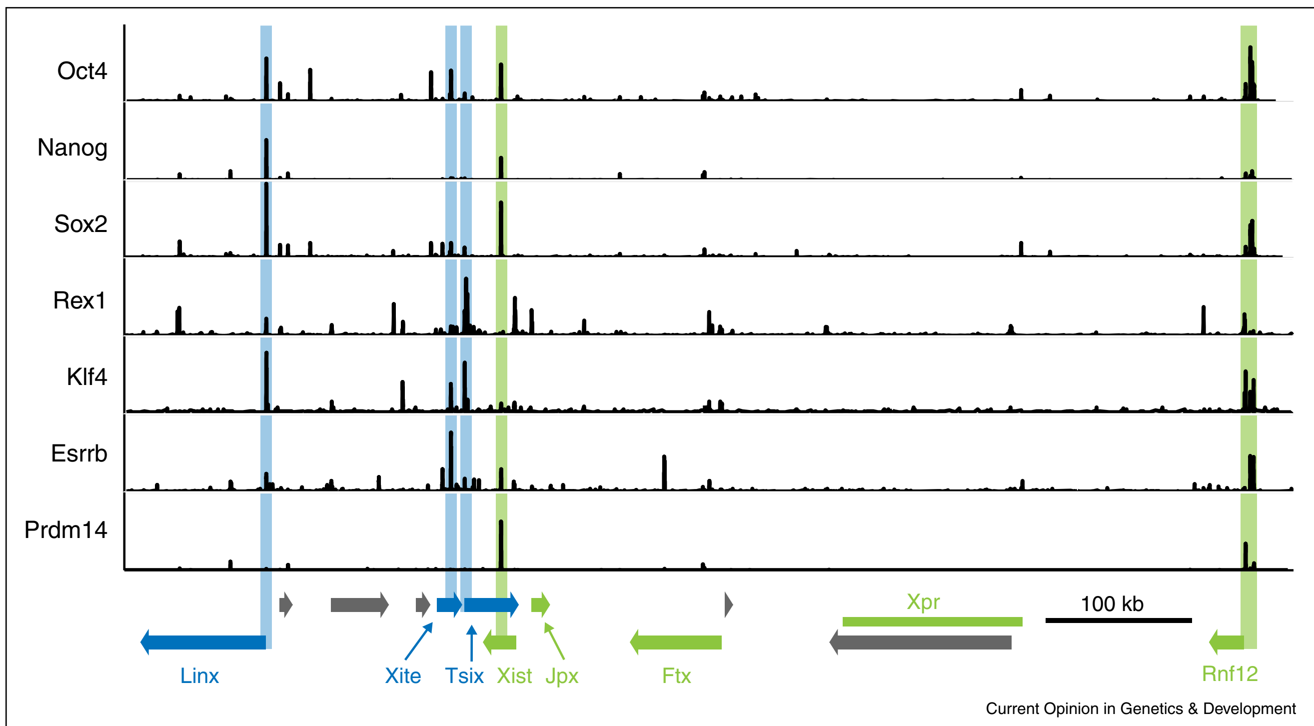
Table 1

Potential *Xist* repressors in stem cells

	Xist upregulation upon depletion	Binding to the *Xist*/*Tsix* locus	Fold downregulation in the epiblast [44]
Oct4/Pou5f1	+ [39,37]	+ [60,37,39]	1×
Sox2	− [37]	+ [60,37,39]	4×
Nanog	+ [39]	+ [60,39]	16×
Rex1/Zfp42	+ [34••,38]	+ [34••,38]	>64×
Klf4	n.d.	+ [41,38]	64×
Esrrb	− [61]	+ [41]	32×
Prdm14	+ [42]	+ [42]	>64×
Klf2	n.d.	n.d.	64×

For each stem cell factor it is indicated, whether knockdown or knockout of the respective factor result in *Xist* upregulation (first column, n.d.: not determined). The second column summarizes the available ChIP-Seq data shown in Figure 2, and the third column indicates the fold downregulation of the respective factors in the E5.75 epiblast compared to ES cells.

Figure 2



Binding profiles of stem cell factors around the *Xist* locus. ChIP-Seq profiles of several stem cell factors are aligned to a genomic region of 800 kb surrounding the *Xist* gene [34••,41,42,60]. Gene locations are indicated by thick arrows below the binding profiles. Putative repressive regulators of XCI, such as the Linx, Xite and Tsix non-coding RNAs are shown in blue, while putative positive regulators, such as Xist, Rnf12, the non-coding transcripts Jpx and Ftx and the *Xpr* locus are colored green (reviewed in [36]). Several regions that bind multiple stem cell factors are highlighted, such as the Linx promoter, Xite, the Tsix promoter, Xist intron 1 and the Rnf12 promoter (from left to right).

of the epiblast, came from a series of studies performed by Paul Burgoyne in the 1990s. It was previously known that in several mammalian species male embryos grow faster during early development [55]. By comparing the growth rates of various XX, XO and XY karyotypes, Burgoyne and colleagues could show that the Y chromosome (of most mouse strains) accelerates embryonic development before implantation (<E3.5), while the presence of two X chromosomes slows down embryonic growth just after implantation into the uterus [56–58]. As a consequence it could be hypothesized that either sex-specific DNA methylation differences and/or the double dose of X-linked genes might slow down development of XX embryos compared to XY or XO embryos, shortly after the time window when both X's are active in the ICM of the blastocyst and when random XCI is first initiated.

Conclusions

Questions surrounding the extent and mechanisms of X-chromosome dosage compensation relative to autosomal genes, as well as to differences between the sexes, have been debated for decades. Since the advent of genome-wide approaches, several efforts have been recently undertaken to test Ohno's hypothesis. As so often however, the devil seems to be in the detail and different groups come to opposite conclusions depending on the specificities of their analysis. The degree to which X chromosome-wide dosage compensation is necessary seems to depend on how many genes actually require such compensation. Indeed, whether the well-known phenotypes associated with dosage defects are caused by a few highly dose sensitive genes or whether they are rather a cumulative result from the dosage imbalance of many genes remains an open question. The identification of the genes involved represents an important challenge for the future.

The molecular mechanisms underlying dosage compensation between the X and autosomes, as well as between the sexes remain areas of active investigation. In the case of XCI, although the role of X-linked gene dosage in regulating X inactivation is better understood, even here the genes involved (apart from *Rnf12*) remain to be identified and the mechanisms of such fine tuned regulation elucidated. It also remains a challenge to disentangle the complex links between the stem cell network and XCI, and to define the different actors that ensure female-specific, monoallelic *Xist* expression. Apart from XCI, the only developmental sex difference that has been attributed to X dosage *in vivo* so far is differential growth

during post-implantation development. Sex differences at the molecular level (DNA methylation, transcription) have only been analyzed in the ESC *in vitro* system, but it is now becoming technically possible to test their *in vivo* relevance and hopefully to elucidate their potential impact on development and female versus male growth rates. Hopefully, our knowledge about the impact of X-chromosomal gene dosage on different processes will increase rapidly in coming years.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Vicoso B, Bachtrog D: **Progress and prospects toward our understanding of the evolution of dosage compensation**. *Chromosome Res* 2009, **17**:585-602.

2. Disteche CM: **Dosage compensation of the sex chromosomes**. *Annu Rev Genet* 2012 http://dx.doi.org/10.1146/annurev-genet-110711-155454.

3. Ohno S: *Sex Chromosomes and Sex-linked Genes*. Springer; 1967.

4. Berletch JB, Yang F, Disteche CM: **Escape from X inactivation in mice and humans**. *Genome Biol* 2010, **11**:213.

5. Deng X, Hiatt JB, Nguyen DK, Ercan S, Sturgill D, Hillier LW, Schlesinger F, Davis CA, Reinke VJ, Gingeras TR *et al.*: **Evidence for compensatory upregulation of expressed X-linked genes in mammals, *Caenorhabditis elegans* and *Drosophila melanogaster***. *Nature* 2011, **43**:1179-1185.

6. Lin H, Gupta V, VerMilyea MD, Falciani F, Lee JT, O'Neill LP, Turner BM: **Dosage compensation in the mouse balances up-regulation and silencing of X-linked genes**. *PLoS Biol* 2007, **5**:e326.

7. Xiong Y, Chen X, Chen Z, Wang X, Shi S, Wang X, Zhang J, He X: **RNA sequencing shows no dosage compensation of the active X-chromosome**. *Nature* 2010, **42**:1043-1047.

8. Nguyen DK, Disteche CM: **Dosage compensation of the active X chromosome in mammals**. *Nat Genet* 2005, **38**:47-53.

9. •• Julien P, Brawand D, Soumillon M, Necsulea A, Liechti A, Schütz F, Daish T, Grützner F, Kaessmann H: **Mechanisms and evolutionary patterns of mammalian and avian dosage compensation**. *PLoS Biol* 2012, **10**:e1001328.

In this comprehensive study, the authors use RNA-sequencing data from several tissues of different species to gain better insight into dosage compensation mechanisms. Among other results they show that, in higher mammals, X-linked genes have not been upregulated compared to their autosomal orthologs in other species.

10. Lin F, Xing K, Zhang J, He X: **Expression reduction in mammalian X chromosome evolution refutes Ohno's hypothesis of dosage compensation**. *Proc Natl Acad Sci U S A* 2012, **109**:11752-11757.

11. • Pessia E, Makino T, Bailly-Bechet M, McLysaght A, Marais GAB: **Mammalian X chromosome inactivation evolved as a dosage-compensation mechanism for dosage-sensitive genes on the X chromosome**. *Proc Natl Acad Sci U S A* 2012, **109**:5346-5351.

This study shows that X-linked genes, whose protein products participate in larger protein complexes and which are therefore expected to be dosage sensitive, are dosage compensated relative to autosomal genes.

12. Okamoto I, Patrat C, Thépot D, Peynot N, Fauque P, Daniel N, Diabangouaya P, Wolf J-P, Renard J-P, Duranthon V *et al.*: **Eutherian mammals use diverse strategies to initiate X-chromosome inactivation during development**. *Nature* 2011, **472**:370-374.

13. Chow J, Heard E: **X inactivation and the complexities of silencing a sex chromosome**. *Curr Opin Cell Biol* 2009, **21**:359-366.

14. • Grant J, Mahadevaiah SK, Khil P, Sangrithi MN, Royo H, Duckworth J, McCarrey JR, VandeBerg JL, Renfree MB, Taylor W *et al.*: **Rsx is a metatherian RNA with Xist-like properties in X-chromosome inactivation**. *Nature* 2012 http://dx.doi.org/10.1038/nature11171.

This study identifies an X-linked gene called *Rsx* in the marsupial *Monodelphis domestica*, which exhibits a similar expression pattern to the Xist RNA in placental mammals and can initiate gene silencing *in cis*.

15. Leppig KA, Disteche CM: **Ring X and other structural X chromosome abnormalities: X inactivation and phenotype**. *Semin Reprod Med* 2001, **19**:147-157.

16. Heard E, Turner J: **Function of the sex chromosomes in mammalian fertility**. *Cold Spring Harb Perspect Biol* 2011, **3**:a002675.

17. Carrel L, Willard HF: **X-inactivation profile reveals extensive variability in X-linked gene expression in females**. *Nature* 2005, **434**:400-404.

18. Yang F, Babak T, Shendure J, Disteche CM: **Global survey of escape from X inactivation by RNA-sequencing in mouse**. *Genome Res* 2010, **20**:614-622.

19. Ogata T, Matsuo N: **Turner syndrome and female sex chromosome aberrations: deduction of the principal factors involved in the development of clinical features**. *Hum Genet* 1995, **95**:607-629.

20. Burgoyne PS, Tam PP, Evans EP: **Retarded development of XO conceptuses during early pregnancy in the mouse**. *J Reprod Fertil* 1983, **68**:387-393.

21. Tada T, Takagi N, Adler ID: **Parental imprinting on the mouse X chromosome: effects on the early development of X0, XXY and XXX embryos**. *Genet Res* 1993, **62**:139-148.

22. Marahrens Y, Panning B, Dausman J, Strauss W, Jaenisch R: **Xist-deficient mice are defective in dosage compensation but not spermatogenesis**. *Genes Dev* 1997, **11**:156-166.

23. Takagi N, Abe K: **Detrimental effects of two active X chromosomes on early mouse development**. *Development* 1990, **109**:189-201.

24. Birchler JA, Veitia RA: **The gene balance hypothesis: implications for gene regulation, quantitative traits and evolution**. *New Phytol* 2010, **186**:54-62.

25. Veitia RA: **Exploring the etiology of haploin sufficiency**. *Bioessays* 2002, **24**:175-184.

26. Okamoto I, Otte AP, Allis CD, Reinberg D, Heard E: **Epigenetic dynamics of imprinted X inactivation during early mouse development**. *Science* 2004, **303**:644-649.

27. Mak W, Nesterova TB, de Napoles M, Appanah R, Yamanaka S, Otte AP, Brockdorff N: **Reactivation of the paternal X chromosome in early mouse embryos**. *Science* 2004, **303**:666-669.

28. Tada T, Obata Y, Tada M, Goto Y, Nakatsuji N, Tan S, Kono T, Takagi N: **Imprint switching for non-random X-chromosome inactivation during mouse oocyte growth**. *Development* 2000, **127**:3101-3105.

29. Kay GF, Barton SC, Surani MA, Rastan S: **Imprinting and X chromosome counting mechanisms determine Xist expression in early mouse development**. *Cell* 1994, **77**:639-650.

30. Okamoto I, Arnaud D, Le Baccon P, Otte AP, Disteche CM, Avner P, Heard E: **Evidence for de novo imprinted X-chromosome inactivation independent of meiotic inactivation in mice**. *Nature* 2005, **438**:369-373.

31. Okamoto I, Tan S, Takagi N: **X-chromosome inactivation in XX androgenetic mouse embryos surviving implantation**. *Development* 2000, **127**:4137-4145.

32. Heard E, Kress C, Mongelard F, Courtier B, Rougeulle C, Ashworth A, Vourc'h C, Babinet C, Avner P: **Transgenic mice carrying an Xist-containing YAC**. *Hum Mol Genet* 1996, **5**:441-450.

33. Jonkers I, Barakat TS, Achame EM, Monkhorst K, Kenter A, Rentmeester E, Grosveld F, Grootegoed JA, Gribnau J: **RNF12 is an X-encoded dose-dependent activator of X chromosome inactivation**. *Cell* 2009, **139**:999-1011.

34. •• Gontan C, Achame EM, Demmers J, Barakat TS, Rentmeester E, van IJcken W, Grootegoed JA, Gribnau J: **RNF12 initiates X-chromosome inactivation by targeting REX1 for degradation**. *Nature* 2012, **485**:386-390.

This study identifies the Xist repressor Rex1 as the main target of the X-linked Xist activator Rnf12 in ESCs.

35. • Shin J, Bossenz M, Chung Y, Ma H, Byron M, Taniguchi-Ishigaki N, Zhu X, Jiao B, Hall LL, Green MR *et al.*: **Maternal Rnf12/RLIM is required for imprinted X-chromosome inactivation in mice**. *Nature* 2010, **467**:977-981.

This study shows that Rnf12, provided as a large maternal pool, is also crucial for imprinted XCI, since the maternal transmission of a mutant allele prevents Xist upregulation in the early embryo.

36. Pollex T, Heard E: **Recent advances in X-chromosome inactivation research**. *Curr Opin Cell Biol* 2012 http://dx.doi.org/10.1016/j.ceb.2012.10.007.

37. Donohoe ME, Silva SS, Pinter SF, Xu N, Lee JT: **The pluripotency factor Oct4 interacts with Ctcf and also controls X-chromosome pairing and counting**. *Nature* 2009, **460**:128-132.

38. Navarro P, Oldfield A, Legoupi J, Festuccia N, Dubois A, Attia M, Schoorlemmer J, Rougeulle C, Chambers I, Avner P: **Molecular coupling of Tsix regulation and pluripotency**. *Nature* 2010, **468**:457-460.

39. Navarro P, Chambers I, Karwacki-Neisius V, Chureau C, Morey C, Rougeulle C, Avner P: **Molecular coupling of Xist regulation and pluripotency**. *Science* 2008, **321**:1693-1695.

40. Young RA: **Control of the embryonic stem cell state**. *Cell* 2011, **144**:940-954.

41. Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, Wong E, Orlov YL, Zhang W, Jiang J *et al.*: **Integration of external signaling pathways with the core transcriptional network in embryonic stem cells**. *Cell* 2008, **133**:1106-1117.

42. Ma Z, Swigut T, Valouev A, Rada-Iglesias A, Wysocka J: **Sequence-specific regulator Prdm14 safeguards mouse ESCs from entering extraembryonic endoderm fates**. *Nat Struct Mol Biol* 2011, **18**:120-127.

43. Barakat TS, Gunhanlar N, Gontan Pardo C, Achame EM, Ghazvini M, Boers R, Kenter A, Rentmeester E, Grootegoed JA, Gribnau J: **RNF12 activates Xist and is essential for X chromosome inactivation**. *PLoS Genet* 2011, **7**:e1002001.

44. Hayashi K, Ohta H, Kurimoto K, Aramaki S, Saitou M: **Reconstitution of the mouse germ cell specification pathway in culture by pluripotent stem cells**. *Cell* 2011, **146**:519-532.

45. •• Gillich A, Bao S, Grabole N, Hayashi K, Trotter MWB, Pasque V, Magnúsdóttir E, Surani MA: **Epiblast stem cell-based system reveals reprogramming synergy of germline factors**. *Cell Stem Cell* 2012, **10**:425-439.

In this study a variety of stem cell factors are tested for their ability to promote X reactivation during reprogramming of epiblast stem cells. The authors show that the combined ectopic expression of Prdm14 and Klf2 strongly increases the reprogramming efficiency.

46. Ooi SK, Wolf D, Hartung O, Agarwal S, Daley GQ, Goff SP, Bestor TH: **Dynamic instability of genomic methylation patterns in pluripotent stem cells**. *Epigenet Chromatin* 2010, **3**:17.

47. Zvetkova I, Apedaile A, Ramsahoye B, Mermoud JE, Crompton LA, John R, Feil R, Brockdorff N: **Global hypomethylation of the genome in XX embryonic stem cells**. *Nat Genet* 2005, **37**:1274-1279.

48. Popp C, Dean W, Feng S, Cokus SJ, Andrews S, Pellegrini M, Jacobsen SE, Reik W: **Genome-wide erasure of DNA methylation in mouse primordial germ cells is affected by AID deficiency**. *Nature* 2010, **463**:1101-1105.

49. Norris DP, Patel D, Kay GF, Penny GD, Brockdorff N, Sheardown SA, Rastan S: **Evidence that random and imprinted Xist expression is controlled by preemptive methylation**. *Cell* 1994, **77**:41-51.

50. Sado T, Tada T, Takagi N: **Mosaic methylation of Xist gene before chromosome inactivation in undifferentiated female mouse embryonic stem and embryonic germ cells**. *Dev Dyn* 1996, **205**:421-434.

51. Sun BK, Deaton AM, Lee JT: **A transient heterochromatic state in Xist preempts X inactivation choice without RNA stabilization**. *Mol Cell* 2006, **21**:617-628.

52. Beard C, Li E, Jaenisch R: **Loss of methylation activates Xist in somatic but not in embryonic cells**. *Genes Dev* 1995, **9**:2325-2334.

53. Panning B, Jaenisch R: **DNA hypomethylation can activate Xist expression and silence X-linked genes**. *Genes Dev* 1996, **10**:1991-2002.

54. Sado T: **De novo DNA methylation is dispensable for the initiation and propagation of X chromosome inactivation**. *Development* 2004, **131**:975-982.

55. Tsunoda Y, Tokunaga T, Sugie T: **Altered sex ratio of live young after transfer of fast- and slow-developing mouse embryos**. *Gamete Res* 1985, **12**:301-304.

56. Thornhill AR, Burgoyne PS: **A paternally imprinted X chromosome retards the development of the early mouse embryo**. *Development* 1993, **118**:171-174.

57. Burgoyne PS: **A Y-chromosomal effect on blastocyst cell number in mice**. *Development* 1993, **117**:341-345.

58. Burgoyne PS, Thornhill AR, Boudrean SK, Darling SM, Bishop CE, Evans EP, Capel B, Mittwoch U: **The genetic basis of XX-XY differences present before gonadal sex differentiation in the mouse [and discussion]**. *Philos Trans R Soc B: Biol Sci* 1995, **350**:253-261.

59. Hamatani T, Carter MG, Sharov AA, Ko MSH: **Dynamics of global gene expression changes during mouse preimplantation development**. *Dev Cell* 2004, **6**:117-131.

60. Marson A, Levine SS, Cole MF, Frampton GM, Brambrink T, Johnstone S, Guenther MG, Johnston WK, Wernig M, Newman J *et al.*: **Connecting microRNA genes to the core transcriptional regulatory circuitry of embryonic stem cells**. *Cell* 2008, **134**:521-533.

61. Feng B, Jiang J, Kraus P, Ng J-H, Heng J.-C.D., Chan Y-S, Yaw L-P, Zhang W, Loh Y-H, Han J *et al.*: **Reprogramming of fibroblasts into induced pluripotent stem cells with orphan nuclear receptor Esrrb**. *Nat Cell Biol* 2009, **11**:197-203.