Dosage compensation: do birds do it as well?

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In birds males carry ZZ and females ZW sex chromosomes, and it has been proposed that there is no dosage compensation in the expression of sex-linked genes. However, recent data suggest the opposite, indicating that male and female birds might demonstrate similar levels of expression of Z-linked genes. If they do, the equalization between the sexes is probably not achieved by inactivation of one of the male Z chromosomes. Other possible mechanisms include the transcription of Z-linked genes being upregulated in females or downregulated in males, or equalization at the translation stage in either sex. A recently identified hypermethylated region on the Z chromosome, with similarities to the X inactivation centre on the mammalian X chromosome, might play a part in this process or have a role in avian sex determination.

In birds, the sex chromosomes are ZZ (male) and ZW (female). It is almost 20 years since the first Z-linked gene in birds was identified – encoding the iron responsive element binding protein, IREBP, also known as aconitase. Analysis of IREBP enzyme activity in liver from adult birds showed that ZZ males expressed twice as much gene product as ZW females [1]. This suggested the lack of a mechanism to compensate for the males' double dose of genes on the Z chromosome. In other organisms with non-homologous sex chromosomes, the expression of sex-linked genes is equalized by some form of up- or downregulation, including virtual inactivation of one sex chromosome [2]. The apparent absence of dosage compensation in birds might relate to avian sex determination: the double dose of Z-linked genes expressed by males could trigger their sexual differentiation to the male phenotype [3]. However, it is widely recognized that an imbalance between males and females in the expression of a substantial number of genes would impose problems in development and other life processes.

Birds probably do it…
A recent report by McQueen *et al.* [4] challenges the traditional view of how birds deal with the expression of Z-linked genes. They describe the first systematic study of the expression levels of sex-linked genes in a bird. Real-time quantitative reverse transcriptase polymerase chain reaction (RT–PCR) was used to monitor RNA transcription of sex-linked genes in chicken embryos. The expression levels of eight out of nine Z-linked genes were similar in males and females. These eight genes are distributed along most of the Z chromosome (short and long arm; Fig. 1a) and represent a variety of functional categories. The most straightforward interpretation of these data is that dosage compensation does occur in birds.

IREBP was among the genes shown in the above study to have sex-equal expression levels. This result contrasts with that of the earlier enzyme study, but could be explained by the use of different tissues for the assays [4]. By choosing to use day-three and day-four embryos McQueen *et al.* [4] avoided the secondary sex-related effects that in the chicken start to develop at day seven, following formation of the genital ridge and sexual differentiation. Moreover, this experimental design suggests that dosage compensation is established early in avian development.

…but at what stage?
The data presented by McQueen and colleagues seem solid and convincing, but a recent report by Kuroda *et al.* [5] might at first glance be taken as evidence against dosage compensation. Kuroda and colleagues applied fluorescence *in situ* hybridization (FISH) to nascent transcripts of Z-linked genes in male chicken, to determine whether transcription took place on a single Z chromosome or on both Z chromosomes. For five out of five different genes, including three also studied by McQueen *et al.* (*IREBP*, *ALDOB* and *CHRNB3*), transcription from both Z chromosomes was detected. Therefore, if dosage compensation occurs in birds, it occurs at a different stage from that in mammals, where there is virtual inactivation of one of the X chromosomes.

A possible synthesis of these two new observations is that male and female birds express similar levels of Z-linked genes or gene products – that is, dosage compensation occurs – but that equalization is achieved by a means other than Z chromosome inactivation. Alternative mechanisms include differences between the sexes in the regulation of transcription or in post-transcriptional or translational processes. The observation of equalized levels of mRNA expression [4], for example, would support regulation at the transcription stage. If any of these mechanisms is valid, birds could achieve dosage compensation in the same way as nematodes or flies. In *Caenorhabditis elegans*, both X chromosomes of hermaphrodites are downregulated, whereas in *Drosophila* the single X chromosome of males is hypertranscribed [2].

…and what is the mechanism?
So what might be the mechanism that underlies any dosage compensation in birds? Teranishi *et al.* [6] recently identified a tandem repetitive region, consisting of more than 200 copies of a 2.2-kb sequence, on the short arm of the Z chromosome of chicken and other birds. The region is hypermethylated, at CpG sites, on the two Z chromosomes of male embryos but is hypomethylated on the single Z chromosome of females; hence the term male hypermethylated [MHM] region has been coined. Transcription of

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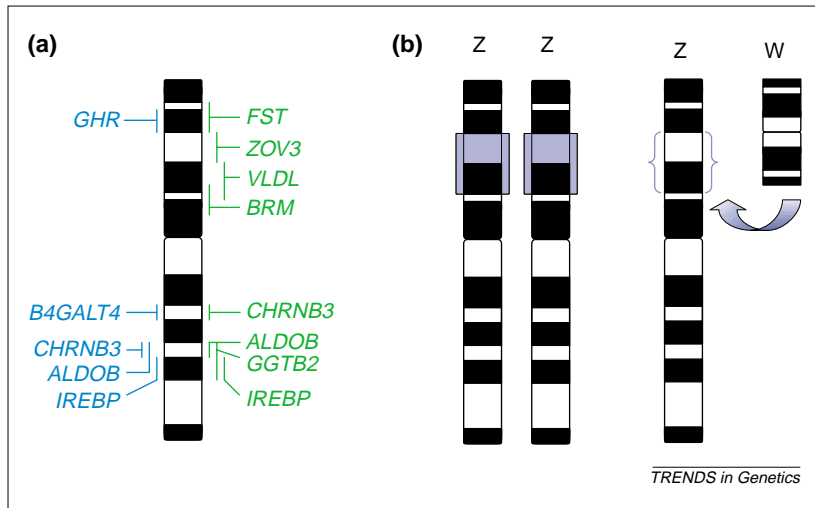e-mail:
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Fig. 1. Genes of the avian sex chromosomes (male, ZZ; female ZW). (a) Genes and their location on the chicken Z chromosome that have been analysed for dosage compensation. Labels on the left indicate genes for which inactivation of one of two Z chromosomes in chicken males could not be shown [5]; labels on the right indicate genes for which equal expression levels in male and female chicken were found [4]. (b) A possible model whereby a W-encoded factor inhibits methylation of the male hypermethylated (MHM) region on the single Z chromosome of female birds, thus enabling MHM transcription. In the absence of this factor, methylation of the MHM region (shaded area) in the two Z chromosomes of male birds prevents transcription.

heterogeneous, high molecular mass poly(A)⁻ RNA from the MHM region occurs only in females – in males, no transcription can be detected. The absence of long open-reading frames suggests that MHM RNA is noncoding, and it accumulates in the nucleus at the site of transcription on the female's single Z chromosome. As demethylation of male DNA results in MHM transcription, transcriptional silencing in males is probably governed by hypermethylation, although indirect effects cannot be excluded.

Sex linkage, methylation-sensitive expression of noncoding RNA and RNA accumulation at the site of transcription are features of the MHM region that resemble to some extent the *Xist* gene within the X-chromosome inactivation centre (Xic) of mammals. *Xist* produces heterogeneous, high molecular-mass RNA that is noncoding and coats the X chromosome [7,8]. After being inactivated, the X chromosome becomes methylated. Is it possible that the shared properties of MHM and *Xist* are coincidental, or do these similarities indicate a common function? Molecular data from the three lineages best characterized with respect to dosage compensation – *C. elegans*, *Drosophila* and mammals – show that genes taking part in dosage compensation mechanisms have evolved independently in the different lineages [2]. And in each case the problem of an imbalance in gene dosage between the sexes has been solved in a distinctly different way (downregulation, upregulation and gene silencing, respectively). In this context it might be important that a closer comparison of MHM and *Xist* reveals not only similarities but also differences: *Xist* is spliced and polyadenylated, *Xist* RNA coats genes on more or less the entire X chromosome (MHM accumulation is

site-specific), and methylation seems to be a consequence of *Xist* transcription, not a factor regulating it [7,8].

We have no direct evidence that the MHM region has a role in governing dosage compensation among birds. There are no functional data to implicate the MHM region in any biological process – only a correlation between chromosomal sex and MHM mRNA accumulation. However, the locus and its peculiar features clearly merit further investigation. The feature of potentially most consequence is production of large noncoding RNA. In this respect the MHM region is similar not only to *Xist* but also to the X-linked *roX1* and *roX2* genes in *Drosophila*, which produce nontranslated transcripts that are incorporated into the dosage compensation–ribonucleoprotein complex [9]. This shared characteristic of *Xist* and *roX1*/*roX2* might point to an ancient, conserved mechanism of dosage compensation, or convergence constrained by a limited set of solutions to a common problem [2]. The MHM region in birds could be a third member of this group.

Z-linked genes might not all be subject to dosage compensation

The avian Z chromosome is large (an estimated 100 Mb or 8% of the chicken genome [10]) and obviously contains many genes. For one of the nine genes analysed by quantitative RT–PCR, *ScII*, expression levels were consistently twice as high in males as in females [4]. This might suggest that some avian Z-linked genes escape dosage compensation in a similar manner to about 10% of X-linked human genes [11]. Genes not subject to transcriptional (epigenetic) silencing are nonrandomly distributed on the human X chromosome, being located mainly on the p arm and, in particular, close to the pseudoautosomal region (PAR) on the terminal part of Xp [11]. The PAR is the small region on X that still recombines with homologous sequences on the terminal part of Yp.

Originally, the X and Y sex chromosomes were a normal pair of autosomes but early during vertebrate evolution they began to differentiate, as a result of gradual or stepwise suppression of recombination between the two chromosomes [12]. One way to explain the cluster of genes on the X chromosome that escape dosage compensation is that compensation has not yet evolved fully among genes in the region of the chromosome that most recently recombined with the Y chromosome. Taking this view, one would expect avian genes that escape dosage compensation to be close to the PAR on Zp. However, *ScII* maps to Zq. As McQueen *et al.* [4] found that other genes close to *ScII* on Zq were compensated, the significance of this observation remains unclear. A speculative possibility is that *ScII* escapes compensation for adaptive reasons. McQueen *et al.* [4] noted that the orthologue of *ScII* in *C. elegans*, *MIX-1*, is associated with dosage compensation-specific proteins in XX hermaphrodites [13]. Perhaps *ScII* has a similar role in birds.

Box 1. Definition of the term gametologue (gametologous genes)

As the evolution of homologous genes can follow several possible paths, the terms paralogues (paralogous genes) and orthologues (orthologous genes) are often used to distinguish between two main processes of gene origin and differentiation. Orthologous genes in different taxa evolve after lineage splitting (Fig. Ia), that is, gene A′ in species 1 is orthologous to gene A″ in species 2. Paralogous genes arise after gene duplication (Fig. Ib). For example, after an ancestral duplication of gene A into genes A_1 and A_2. Thus in species 1, gene A_1′ is paralogous to gene A_2′. In this case, gene A_1′ is also orthologous to gene A_1″ in species 2, and paralogous to gene A_2″.

(a) Orthologous genes **(b)** Paralogous genes

Fig. I

TRENDS in Genetics
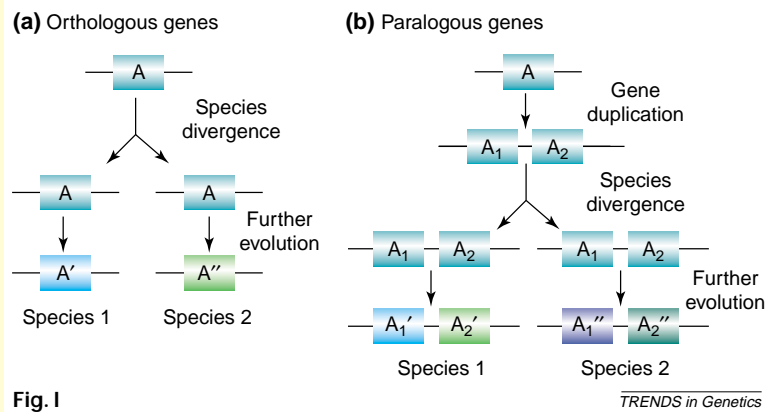
Making reference to genes shared between the opposite sex chromosomes, yet representing independently evolving entities, is not a simple matter. Given the common ancestry of the genes, that is, they were represented by a single gene before the sex chromosomes started to diverge, they are obviously homologous and can therefore be said to represent homologues. But what if we want to be more specific about their origin? As they have not arisen by duplication, they are not paralogous according to the usual definition of this term. To recognize this previously unnamed type of homology, Garcia-Moreno and Mindell [29] coined the term gametologues (gametologous genes) to denote homologous genes on the opposite sex chromosomes that have arisen by cessation of recombination between an ancestral pair of autosomes. As an example, chicken *PKCIW* is gametologous to chicken *PKCIZ*, and orthologous to turkey *PKCIW*. Similarly, human *ZFY* is gametologous to human *ZFX*, and orthologous to chimpanzee *ZFY*.

The *DMRT1* locus is important, but for what?

The possibility that adaptive escape from dosage compensation might occur in birds is also suggested by the sex difference seen in embryonic expression levels for another Z-linked gene, *DMRT1* [6,14–16]. The DM domain of DMRT1 has been recognized as the first protein or protein motif to play a part in animal sex determination across phyla [17], suggesting the evolutionary conservation of an ancient sex-determining gene (other genes directing the process of sexual differentiation are virtually unrelated between phyla). In birds, *DMRT1* is expressed in the genital ridge and Wolffian duct before sexual differentiation, and levels are about twofold higher in ZZ embryos than they are in ZW embryos. The association of hemizygosity (resulting from large deletions) of

human *DMRT1* with XY sex reversal [17], together with the observation that avian *DMRT1* is Z-linked, has prompted speculation that the presence of two copies of *DMRT1* in male birds is directly or indirectly connected to the development of a male phenotype [3]. In this scenario the effect of *DMRT1* would be mediated by dose.

An important observation made by Teranishi *et al.* [6] is that MHM accumulates at a position cytogenetically indistinguishable from that of the *DMRT1* locus. Could it be that MHM is not a factor that governs overall dosage compensation of Z-linked genes, but one that specifically allows *DMRT1* to escape compensation, thereby controlling sex determination? This is an attractive idea, because it would explain what is probably the most significant difference between MHM, and *Xist* and *roX* transcripts, that is, site-restricted versus chromosome-wide accumulation. However, as yet there is no direct evidence to support this view. The MHM and *DMRT1* loci are close to each other on the Z chromosome, so the appearance of MHM RNA at this site might, on the one hand, merely reflect the physical proximity of the genes. On the other hand, the genes might be located close to one another simply to allow MHM transcript accumulation near the *DMRT1* locus.

A possible model

In avian genetics, one of the classic questions is whether it is the presence of the W chromosome or the number of Z chromosomes that determines sexual differentiation [18]. A corresponding question applies to the sex differences seen for the MHM region and their possible connection to sex-related processes. Teranishi *et al.* [6] solved this problem using triploid lines of chicken. In ZZW intersexes they observed hypomethylation and transcription of the MHM region, whereas in ZZZ male birds, they detected hypermethylation but no transcription. These findings indicate that an unknown factor encoded by the W chromosome inhibits MHM methylation in female embryos soon after fertilization, enabling RNA transcription (Fig. 1b). In other words, the presence of two (rather than one) Z chromosomes in the male is not in itself sufficient to trigger hypermethylation, that is, there is not a counting mechanism similar to that found, for example, in mammals. If this line of reasoning is correct, we have here the first evidence for the avian W chromosome having a dominant role in a process associated with sexual differentiation.

The W chromosome is gene poor, with only four genes identified so far in chicken. All four have independently evolving homologues (gametologues, see Box 1) on the Z chromosome and for three of them the Z and W gametologues are similar at the amino acid level (Table 1). It seems unlikely that any of these three W-linked genes could represent the factor inhibiting MHM methylation in females. The only W chromosome-encoded protein clearly specific to female birds is an altered form of the protein kinase C

Table 1. Genes on the avian W chromosome

W-linked gene	Z-linked gametologue	Z–W homology (aa identity)	Refs
CHD1W	*CHD1Z*	97	[23,24]
ATP5A1W	*ATP5A1Z*	'Very high'	[25–27]
PKCIW[a]	*PKCIZ*	65	[19,20]
SPINW	SPINZ	97	[28]

[a]This gene has recently been isolated from chicken by two independent groups [19,20]. O'Neill *et al.* [19], although noting that it is similar to protein kinase C inhibitor (*PKCI*), named it *ASW*, for 'Avian Sex-specific W-linked'. Hori *et al.* [20] called it *Wpkci* but recognized that the gene is an altered form of *PKCI*, lacking an HIT triad motif present in other *PKCI* genes and showing a unique Leu- and Arg-rich region. Hori *et al.* also identified a related gene on the chicken Z chromosome that is similar to *PKCI* in, for example, mammals, and therefore named this gene *PKCI*. A phylogenetic analysis suggests that avian PKCI and *ASW/Wpkci* genes diverged after the bird-mammal split (data not shown). Given their chromosomal location, they could therefore be referred to as gametologues (Box 1). The three previously identified gametologues on the Z and W chromosomes are all named by the appropriate gene abbreviation followed by 'Z' or 'W', to specify the copy (see table). Similarly, all but one of some 20 gametologues on the mammalian X and Y chromosomes are named by gene abbreviation followed by 'X' or 'Y' (e.g. *ZFX/ZFY*; the only exception is the gametologous *SRY/SOX3* gene pair). I therefore find it reasonable that *ASW/Wpkci* should be named *PKCIW*. The *PKCI* gene on the avian Z chromosome should analogously be named *PKCIZ*. This nomenclature was also used in a recent review of avian sex determination [18].

inhibitor (PKCIW), which is distinctly different from its gametologue PKCIZ [19,20]. It would seem worthwhile to test whether the gene product of *PKCIW* could be interacting with the MHM region.

Prospects for better understanding of avian dosage compensation

It is generally held that the evolution of dosage compensation is intimately connected with the evolution of sex chromosomes [21]. The decay of genes in the sex-limited chromosome, which occurs as a result of suppression of sex chromosome recombination, exerts strong pressure for the evolution of compensatory mechanisms to equalize the levels of transcription of sex-linked genes between the sexes. As sex chromosomes have evolved independently in many different lineages, including birds [22], it is not surprising that dosage compensation also seems to have done so. From studies of *C. elegans*, *Drosophila* and mammals we have insights into how the problem of dosage can be dealt with. However, we know little about how compensatory systems evolve when proto-sex chromosomes start to differentiate from an ancestral pair of autosomes. Similarly, we know little about how the dosage compensation mechanisms gradually adapt to new demands encountered in the course of sex chromosome evolution.

Further studies of dosage compensation in birds might shed some light on general aspects of the evolution of compensating systems. The heteromorphic sex chromosomes of birds are of relatively recent origin when compared with the mammalian X and Y chromosomes [22]. In one extant avian lineage, the ratites (Palaeognathae), the Z and W sex chromosomes are almost indistinguishable or only moderately differentiated. Thus, among ostriches and their allies, dosage compensation is perhaps just about to evolve or has only recently done so. The next step in the clarification of dosage compensation in birds, therefore, is to screen for the presence of the MHM region and MHM transcript in ratites. Studies in this group of birds could also help to determine whether the male or the female expression level represents the avian default state.

References

1 Baverstock, P.R. *et al.* (1982) A sex-linked enzyme in birds – Z chromosome conservation but no dosage compensation. *Nature* 296, 763–766
2 Marin, I. *et al.* (2000) The evolution of dosage-compensation mechanisms. *BioEssays* 22, 1106–1114
3 Nanda, I. *et al.* (2000) Conserved synteny between the chicken Z sex chromosome and human chromosome 9 includes the male regulatory gene *DMRT1*: a comparative (re)view on avian sex determination. *Cytogenet. Cell Genet.* 89, 67–78
4 McQueen, H.A. *et al.* (2001) Dosage compensation in birds. *Curr. Biol.* 11, 253–257
5 Kuroda, Y. *et al.* (2001) Absence of Z-chromosome inactivation for five genes in male chickens. *Chromosome Res.* 9, 457–468
6 Teranishi, M. *et al.* (2001) Transcripts of the MHM region on the chicken Z chromosome accumulate as non-coding RNA in the nucleus of female cells adjacent to the *DMRT1* locus. *Chromosome Res.* 9, 147–165
7 Brockdorff, N. (1998) The role of Xist in X-inactivation. *Curr. Opin. Genet. Dev.* 8, 328–333
8 Meller, V.H. (2000) Dosage compensation: making 1X equal 2X. *Trends Cell Biol.* 10, 54–59
9 Franke, A. and Baker, B.S. (1999) The *rox1* and *rox2* RNAs are essential components of the compensasome, which mediates dosage compensation in *Drosophila. Mol. Cell* 4, 117–122
10 Smith, J. and Burt, D.W. (1998) Parameters of the chicken genome (*Gallus gallus*). *Anim. Genet.* 29, 290–294

11 Carrel, L. *et al.* (1999) A first-generation X-inactivation profile of the human X chromosome. *Proc. Natl. Acad. Sci. U. S. A.* 96, 14440–14444
12 Lahn, B.T. and Page, D.P. (1999) Four evolutionary strata on the human X chromosome. *Science* 286, 964–967
13 Lieb, J.D. *et al.* (1998) MIX-1: an essential component of the *C. elegans* mitotic machinery executes X chromosome dosage compensation. *Cell* 92, 265–277
14 Raymond, C.S. (1999) Expression of Dmrt1 in the genital ridge of mouse and chicken embryos suggests a role in vertebrate sexual development. *Dev. Biol.* 215, 208–220
15 Smith, C.A. *et al.* (1999) Conservation of a sex-determining gene. *Nature* 402, 601–602
16 Shan, Z. *et al.* (2000) Sex-specific expression of an evolutionarily conserved male regulatory gene, DMRT1, in birds. *Cytogenet. Cell Genet.* 89, 252–257
17 Raymond, C.S. *et al.* (1998) Evidence for evolutionary conservation of sex-determining genes. *Nature* 391, 691–695
18 Ellegren, H. (2001) Hens, cocks and avian sex determination – a quest for genes on Z or W? *EMBO Reports* 2, 1–5
19 O'Neill, M. *et al.* (2000) *ASW*: a gene with conserved avian W-linkage and female specific expression in chick embryonic gonad. *Dev. Genes Evol.* 210, 243–249
20 Hori, T. *et al.* (2000) Wpkci, encoding an altered form of PKCI, is conserved widely on the avian W chromosome and expressed in early female embryos: implication of its role in female sex determination. *Mol. Biol. Cell* 11, 3535–3660

21 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
22 Ellegren, H. (2000) Evolution of the avian sex chromosomes and their role in sex determination. *Trends Ecol. Evol.* 15, 188–192
23 Ellegren, H. (1996) First gene on the avian W chromosome provides a tag for universal sexing of non-ratite birds. *Proc. R. Soc. Lond. B* 263, 1635–1641
24 Griffiths, R. *et al.* (1996) Sex identification in birds using two CHD genes. *Proc. R. Soc. Lond. B* 263, 1251–1256
25 Dvorak, J. *et al.* (1992) cDNA cloning of a Z- and W-linked gene in Gallinaceous birds. *J. Hered.* 83, 22–25.
26 Fridolfsson, A.K. *et al.* (1998) Evolution of the avian sex chromosomes from an ancestral pair of autosomes. *Proc. Natl. Acad. Sci. U. S. A.* 95, 8147–8152
27 Carmichael, A.N. *et al.* (2000) Male-biased mutation rates revealed from Z and W chromosome-linked ATP synthase alpha-subunit (*ATP5A1*) sequences in birds. *J. Mol. Evol.* 50, 443–447
28 Itoh, Y. *et al.* (2001) Chicken *spindlin* genes on W and Z chromosomes: transcriptional expression of both genes and dynamic behavior of spindlin in interphase and mitotic cells. *Chromosome Res.* 9, 283–299
29 Garcia-Moreno, J. and Mindell, D.P. (2000) Rooting a phylogeny with homologous genes on opposite sex chromosomes (gametologues): a case study using avian *CHD. Mol. Biol. Evol.* 17, 1826–1832