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Proposed role of W chromosome inactivation and the absence of dosage compensation in avian sex determination

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SUMMARY

Three features of avian sex chromosomes – female heterogamety (ZZ male, ZW female), the apparently inactive state of the W chromosome, and dose-dependent expression of Z-linked genes – are examined in regard to their possible relation to sex determination. It is proposed that the W chromosome is facultatively heterochromatic and that the Z and W chromosomes carry one or more homologous sex-determination genes. The absence of dosage compensation in ZZ embryos, and W inactivation in ZW embryos, would then bring about a $2n$(ZZ)–n(ZW) inequality in the effective copy number of such genes. The absence of dosage compensation of Z-linked genes in ZZ embryos is viewed as a means by which two copies of Z-W homologous sex determination genes are kept active to meet the requirements of testis determination. W inactivation may promote ovarian development by reducing the effective copy number of these genes from $2n$ to n. If there is a W-specific gene for femaleness, spread of heterochromatization to this gene in cells forming the right gonadal primordium may explain the latter's normally undifferentiated state; reversal of heterochromatization may similarly explain the development of the right gonad into a testis following left ovariectomy.

Nearly all bird species so far studied appear to be female heterogametic, with males having two copies of a relatively large sex chromosome, the Z chromosome, and females having one Z and a smaller sex chromosome, the W (Ohno 1967). In chickens, the short arms of Z and W chromosomes pair during meiosis suggesting that, as in the case of the mammalian X and Y, they share a homologous, pseudoautosomal region (Burgoyne 1982; Evans *et al.* 1982; Rahn & Solari 1986; Solari *et al.* 1988). The presence of a recombination nodule in a region of the paired ZW element suggests that, as has been observed in the XY bivalent in humans (Weissenbach 1987), there may be a single obligatory crossover between the short arms of the Z and W chromosomes. In somatic cells, the Z chromosomes are euchromatic and replicate along with the autosomes in both sexes; the W chromosome, however, is late replicating, and forms a chromocentre similar in appearance to, but smaller than, the X-chromatin of mammals (Kosin & Ishizaki 1959; Schmid 1962; Galton & Bredbury 1966). Thus in many, perhaps most, somatic cells the W chromosome exhibits the characteristics of an inactive chromosome. In birds, and several other female-heterogametic species, there is evidence of a curious absence of dosage compensation of Z-linked genes (Cock 1964; Baverstock *et al.* 1982; reviewed in Chandra 1991). The regulation of Z-linked genes in birds is thus in contrast to that of X-linked genes in *Drosophila melanogaster*, *Caenorhabditis elegans*, and mammals: in these male-heterogametic species, X-linked genes are subject to dosage compensation (Lucchesi & Manning 1987; Meyer 1988; Lyon 1989). The absence of dosage compensation of Z-linked genes in birds is of particular interest because the Z chromosome makes up nearly 10% of the haploid genome (Ohno 1967). What function does the absence of dosage compensation serve? I propose that in birds the absence of dosage compensation complements W-chromosome inactivation, and that, together, they regulate sex determination.

Spontaneous sex inversion of gonads is known from several bird species, mostly in the female; such inversion is rare in males. Normally, in female birds the right gonad remains undifferentiated and the left gonad functions as an ovary. However, in young chicks, following ovariectomy of the left gonad, or its atrophy due to disease, the right gonad differentiates into a testis (Crew 1923; McCarrey & Abbot 1979). This leads to masculinization of the bird. In avian interspecific hybrids, masculinization of chromosomally female individuals is common, whereas feminization of chromosomally male hybrids is not (Gomot 1975). Such observations are consistent with the view that genes for testis differentiation can become activated in ZW individuals under abnormal conditions. Cytogenetic analysis of avian sex determination is hampered by the presence, in the normal chromosome

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complement, of many minichromosomes, which makes identification of deleted W chromosomes difficult. This feature of the avian karyotype, and the absence of suitable sex chromosome-specific probes, has precluded the definitive identification of several potentially informative types of structural anomalies involving the W chromosome. In addition, data on diploid ZZW and ZO birds are few (McCarrey & Abbott 1979) and, as a result, it has not been possible to decide whether sex determination in chickens bears a similarity to that in mammals. Thorne *et al.* (1988) developed a line of chicken which produces a high incidence of viable triploids (3A-ZZW and 3A-ZZZ, where A is one set of autosomes). ZZW triploids are sexed as females at hatching but have an adult intersex phenotype; viable ZZZ triploids have a normal male phenotype. Unlike ZW diploid females, 3A-ZZW birds have two gonads, typically a left ovotestis and right testis. A normally developed left oviduct is present; the right reproductive duct is also an oviduct, but small and undifferentiated. The onset of spermatogenesis corresponds with the external maleness of such 3A-ZZW birds. Development of testis in 3A-ZZZ triploids is the same as in 2A-ZZ males; meiosis, however, is abnormal in triploid males (Thorne *et al.* 1988). 2A-ZZW and 3A-ZZW triploids appear to have different sexual phenotypes, the former being apparently male and the latter hermaphrodite, suggesting the possibility of an effect of autosomes on sex determination. This has led to the suggestion that avian sex determination may be closer to the *Drosophila* type (i.e. based on Z:A ratio measurement) rather than the mammalian type (i.e. based on a dominant W chromosome) (Abdel-Hameed & Shoffner 1971; McCarrey & Abbott 1979).

Sittmann (1984) analysed the inheritance of a Z-linked marker in data collected by Durham (1926) on the progeny of two matroclinous female canaries, and favoured the view that the two females were more likely to be ZO than ZZW. These data, and those on an abnormal rooster which Crew (1933) thought was ZZW, led Sittmann to suggest that sex determination in birds may be similar to that in *Drosophila*. However, it is difficult to evaluate this possibility critically because the data on chromosome constitution were obtained at a time when modern cytological techniques were not available.

There is a close connection between sex determination and dosage compensation in both *D. melanogaster* and *C. elegans*. In the latter, sex determination may in fact be under feedback control by dosage compensation (DeLong *et al.* 1993). It has been argued that mammalian X chromosome inactivation may have, in addition to its role in dosage compensation, a sex-determining function (Chandra 1985, 1986). X inactivation, by rendering 'regulatory as well as structural genes non-functional', could bring about a 'significant reduction in the effective copy number of X chromosome-linked DNA sequences concerned with sex determination' (Chandra 1985). If among X-Y homologous DNA sequences there are some with sex-determining functions, X inactivation might introduce a sex-determining inequality between XX and XY embryos (Chandra 1985, 1986). In marsupials, X-chromosome imprinting and X inactivation (dosage compensation) appear to have a role in sexual differentiation (Graves & Short 1990; Cooper *et al.* 1993). In humans, two active copies of a distal segment of Xp interfere with the development of maleness in individuals with apparently normal Y chromosomes that include an intact *SRY*, the sex-determination gene on the Y short arm (Stern *et al.* 1990; Ogata *et al.* 1992; Arn *et al.* 1993). Since 47, XXY individuals are male, these results suggest that one or more dose-sensitive, sex-determining genes, normally inactive in the inactive X, may be present on distal Xp and that sex reversal may result when they are present in two active copies. Nearly half the human XX males studied (McElreavy *et al.* 1993) and most human XX true hermaphrodites (McElreavy *et al.* 1992) are SRY^{-}. This observation, and data from families in which SRY^{-}XX maleness and SRY^{-}XX true hermaphroditism coexist (Kuhnle *et al.* 1993), suggests the presence of additional sex-determination genes, some of which may be on the sex chromosomes. A gene closely related to *SRY* is present in the Xq 26–27 region (Stevanovic *et al.* 1993). This gene, *SOX-3*, is a candidate gene for Borjeson-Forssman-Lehmann, an X-linked mental retardation syndrome (Stevanovic *et al.* 1993). Affected XY individuals also show poor testicular and genital development and postpubertal gynecomastia (Weber *et al.* 1978; Turner *et al.* 1989).

SRY has been detected in southern blots of avian genomic DNA but, unlike in mammals, it is present in both sexes (Tiersch *et al.* 1991). Therefore, if *SRY* has a role in avian sex determination, the manner in which it functions must be different in the two groups. Extension of the gene dosage hypothesis (Chandra 1985) from mammals to birds would imply that testis development in birds might also require twice ($2n$) as many copies of particular sex chromosome-linked sex determination genes as might ovarian development (n). Inactivation of one of the Z chromosomes in ZZ embryos (i.e. dosage compensation of the mammalian type) would then be inappropriate for avian sex determination. However, the hypothetical gene copy number requirement (n) for ovarian development could be met by inactivation of one of the sex chromosomes in the heterogametic female. This may be the significance of the apparently inactive state of the W chromosome in most somatic cells. By reducing the effective copy number of those sex-determination genes that may be present on both Z and W chromosomes, W inactivation may complement the absence of dosage compensation and bring about a $2n$(ZZ)-n(ZW) type of inequality for the purposes of sex determination. The W chromosome is usually thought of as being constitutively heterochromatic, but it is a requirement of this hypothesis that it be facultatively heterochromatic (Brown 1966), wholly or in part.

If there is a W chromosome-specific gene for ovarian development, spread of heterochromatization into W-chromosomal domains that include such a gene in cells forming the right gonadal primordium may explain the undifferentiated state of the right gonad. Differentiation of the right gonad into a testis following

ovariectomy may be similarly explained on the basis of reactivation of such hitherto heterochromatic domains. Sex reversal attributable to spread of inactivation has been observed in T(X; 16)H/X*Sxr* mice (McLaren & Monk 1982).

It has been suggested that sex determination in diploid chickens may be dependent simply on the number of active Z copies (ZZ, male; ZO, female) (Witschi 1961; Galton & Bredbury 1966). This hypothesis would lead one to expect a higher frequency of sex reversal in ZZ birds than in ZW birds because of occasional mutation in, or loss of, Z chromosomes, but contrary to this expectation sex reversal is most often observed in females and rarely in males. However, if the W carries Z-homologous sex-determining genes in a segment of the chromosome that is facultatively heterochromatic, abnormal reactivation of such genes may provide a basis for understanding the masculinization of 2A-ZW and 3A-ZZW birds. Variability in the degree of masculinization of ZW birds may be related to the extent to which such W-linked genes are activated as well as the number and type of cells in which such reactivation might occur. Reactivation of previously inactive genes in facultatively heterochromatic chromosomes is known to occur in coccid insects (Chandra 1963; Nur 1967; Bregman 1968) and in mammals (Cattanach 1974; Wareham *et al*. 1987).

Two predictions follow from this outline. The first is that homologous, dose-sensitive sex-determining genes be present on the Z and W chromosomes; and second, that at least some W-linked sex-determining genes occur in a segment that is facultatively heterochromatic, and be subject to inactivation during normal development. This further suggests that appropriate treatment of ZW embryos with compounds which decondense chromatin and facilitate transcription may cause sex reversal.

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