A REVIEW OF SEX DETERMINATION AND DIFFERENTIATION IN POULTRY

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Summary

The genetic basis of avian sex determination has not been elucidated. It has been suggested that sex in birds is determined by a genetic balance in the ratio of Z chromosomes to autosomes. Yet evidence based on the phenotypic observation of individuals with aberrant sex chromosomes suggests that the W chromosome contains a major female (ovary) sex determining gene, the effect of which is reversed by more than one dose of a male (testis) sex determining gene on the Z chromosome. Molecular genetic studies are anticipated to provide major breakthroughs in the understanding of avian sex determination as the genes that regulate gonadal differentiation are identified.

I. INTRODUCTION

The question of how genetic sex determination influences primary sexual differentiation in birds is unclear. Avian species have a ZZ/ZW sex chromosome system. In normal fowls, the male is the homogametic sex, ZZ, and the female is heterogametic, ZW. Both embryonic gonads in genetic males develop to form two identical testes. In genetic females, only the left gonad develops as a functional ovary. The right gonad develops only marginally during incubation and regresses to a microscopic vestige.

While sex determination in birds is an event of genetic programming, sex differentiation involves the development of observable phenotypic changes indicative of the specific sex. Sex differentiation is divided into two stages. The primary event is the development of the gonad which encompasses the differentiation of germ cells and somatic elements within the gonad. The secondary event involves the development of the accessory sex organ which is influenced by hormones produced by the somatic cells of the gonad. The endocrine control of secondary sexual differentiation in birds is well-understood (McCarrey and Abbott, 1979).

During early embryonic development, the gonads are sexually indifferent and can differentiate into either a testis or an ovary depending on the genotypic sex of the embryo. The period of indifferent sexual development extends from about day three to day five of incubation in the chicken, and morphological differentiation begins between day five and six of incubation (Romanoff, 1960). The early gonad is typically composed of a medulla and a cortex, and sexual differentiation results from the development of only one of these gonadal components. In birds, a testis forms from a proliferation of the medulla and regression of the cortex, while the reverse occurs for development of the ovary. Detailed descriptions of the differentiation of male and female gonads of the fowl may be found in Romanoff (1960), Gilbert (1979) and Van Krey (1990).

II. DISCUSSION

The genetic mechanism by which sex is determined in birds has been difficult to elucidate. As mentioned, avian species have a ZZ/ZW sex chromosome system. This is the opposite situation to mammals where the male is the heterogametic sex, XY, and the female

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is the homogametic sex, XX. In the chicken, the Z chromosome comprises about 7.6% of the haploid genome (Fechheimer, 1990), while the much smaller W chromosome comprises only 1.4% of the total genomic DNA in the female (Tone *et al.*, 1984). During female meiosis, a small homologous pairing region is observed between the short arm of the W chromosome and a terminal segment of the short arm of the Z chromosome (Solari *et al.*, 1988). In somatic cells the Z chromosomes are euchromatic and replicate with the autosomes, but the W chromosome is late-replicating (Schmid, 1962) and heterochromatic with a highly repetitive DNA content (Tone *et al.*, 1984). In effect, most of the avian W chromosome is genetically isolated from the rest of the genome. A similar situation exists with the mammalian Y chromosome which is also small, late-replicating and heterochromatic (Graves and Schmidt, 1992).

In mammals, sex is known to be determined by the Y chromosome which is dominant and leads to testis formation (Kent *et al.*, 1966). It has been suggested that avian sex determination is similar to mammals and that the W chromosome is dominant, containing a major sex determining gene that initiates development of the ovary in females (Bitgood and Shoffner, 1990). Alternatively, it has been proposed that avian sex determination is more closely related to the Drosophila mechanism involving a genic balance in which the ratio of Z chromosomes to autosomes is sex determining, rather than the presence or absence of the W chromosome (McCarrey and Abbott, 1979; Sittmann, 1984; Halverson and Dvorak, 1993).

Traditionally, most knowledge on sex determination has come from observation of the sexual phenotype of individuals with aberrant sex chromosomes. Diploid fowls with ZO and ZZW sex chromosomes would be the most informative, but critical evidence from these genotypes is lacking. ZO chickens have never been detected and evidence suggests that this genotype may be lethal (Kagami *et al.*, 1995). Only one report of the putative ZZW diploid fowl is known describing a male bird with sex-linked plumage traits indicative of a female (Crew, 1933). The male was proposed to be ZZW on the basis of the phenotype and presumptive cytology of the bird's progeny, but the Z chromosome was incorrectly identified in this early work. Further, as modern cytological techniques were unavailable to positively substantiate the presence of the W chromosome, it is not possible to assign a male phenotype with any certainty to the ZZW diploid karyotype from this single report. Other factors could also have accounted for the atypical plumage pattern of the male bird such as partial sex linkage, chimerism or Z-autosome translocation.

A genic balance sex determining mechanism has been favoured by some (McCarrey and Abbott, 1979; Halverson and Dvorak, 1993) on the basis of observations of gynandromorphs in chickens. Gynandromorphs are bilateral sex chimeras with one half of the body being male and the other half female (Hutt, 1949 for review; Cock, 1954; Abbott and Yee, 1975). In has been concluded that the chromosome constitution of the gynandromorphs was ZZ male/ ZO female based on the assumption that their origin was due to loss of a Z chromosome at the first cleavage division (Hutt, 1949; Halverson and Dvorak, 1993). The assumption that the ZO half of the gynandromorph was female is argued to be consistent with the hypothesis that sex is determined by the autosome to Z chromosome ratio. However, the ZZ/ZO chromosome complement was never verified in any of these reports. Thorne (1995) reported a cytogenetic study of a gynandromorph chicken that was found to be a ZZ/ZW diploid. The presence of the W chromosome was positively verified by C-banding which stains heterochromatin intensely. Varying proportions of ZZ and ZW sex chromosomes were found in a variety of somatic tissues analysed from both the male and female side of the body. The origin of this gynandromorph may be explained by either fertilisation of a regular gamete and the first polar body,

fertilisation of a binucleated oocyte, or fusion of two blastoderms. Autopsy revealed that the bird had an atretic left ovary, a right testis, and left and right oviducts. In this case, a dominant effect of the W chromosome on avian sex determination cannot be dismissed in favour of a genic balance system.

Observations of chimeric and polyploid chickens have provided further insights on avian sex determination. The sexual phenotype of a number of chimeric chickens with varying ploidy levels suggests that the W chromosome does have a major influence on determining femaleness in birds (Thorne *et al.*, 1987; Thorne and Sheldon, 1993). For example, viable haploid-diploid chickens with Z/ZW and Z/ZZ sex chromosome complements have female and male phenotypes, respectively, and*d* despite the additional dose of Z-bearing haploid cells, the Z/ZW chimeras have normal female reproductive organs (Thorne *et al.*, 1987). A diploid-triploid chimera with ZZ/ZZW sex chromosomes and a low proportion of triploid cells, was observed to have a normal male phenotype. Its gonads, though, consisted of a left ovotestis and a right testis indicating that the presence of a W chromosome in only a small proportion of ZZW triploid cells was sufficient to cause some ovarian development of the left gonad (Thorne *et al.*, 1987; Thorne and Sheldon, 1993).

Studies of a large number of ZZW triploid intersex birds from a unique selected strain of chickens (Thorne and Sheldon, 1991; 1993; Thorne *et al.*,1991) have provided significant knowledge on avian sex determination. The ZZW triploids are females at hatching and they maintain an external female phenotype until sexual maturity after which time masculinization occurs. Their adult reproductive organs consist typically of a left ovotestis, right testis, and left and right oviducts (Thorne *et al.*, 1988; Lin *et al.*, 1995a; 1995b). The initial determination and differentiation of the ZZW triploid embryo as a female is observed to be normal and complete, but masculinization of the left ovary and development of a right testis in the place of a regressed right ovary starts before hatching. The masculinization of the gonads of the ZZW triploid embryo is reversible (at least temporarily) by administering estrogen during the indifferent sexual period (Thorne *et al.*, 1992). The observations on the intersex ZZW triploids, together with those of the chimeric chickens, support the hypothesis that the W chromosome contains a major female (ovary) determining gene, the effect of which is able to be inhibited by more than one dose of a male (testis) determining gene on the Z chromosome.

(a) Absence of dosage compensation

In contrast to mammals, an interesting property of the avian Z chromosome is the apparent absence of dosage compensation for Z-linked genes in the homogametic ZZ male. No evidence for dosage compensation has been found for Z-linked plumage colour and pattern genes in chickens, pigeons and canaries (Cock, 1964), or for the activity of the Z-linked cytoplasmic aconitase gene in liver cells of guinea fowl (Baverstock *et al.*, 1982),or for a Z-linked recessive white skin mutation affecting blood plasma colouration in chickens (Lasher and Bitgood, 1982).

The apparent absence of dosage compensation in birds has been proposed as a sex determining mechanism itself by Chandra (1993). Chandra has suggested that both the Z and W chromosomes carry one or more homologous sex determining genes. W-inactivation would halve the effective copy number of such genes in the ZW zygote, enabling ovarian development to occur. The absence of inactivation of Z-linked genes in ZZ embryos is viewed as a means by which two copies of W-homologous sex determination genes are kept active to meet the requirements of testis determination. Thus a complementary mechanism

of W-chromosome inactivation and absence of dosage compensation of Z-linked genes would regulate the sex determining mechanism. A prerequisite of this theory is that the sex-determining region of the W chromosome must consist of facultative heterochromatin rather than constitutive heterochromatin. Additionally, the apparent absence of dosage compensation in birds requires further verification, because only a relatively small number of genes have been examined for Z-chromosome inactivation. In mammals, it is known that a number of X chromosome genes escape inactivation and show greater levels of expression in females than males indicating that balancing dosage is not critical for some genes (Jones *et al.*, 1989; Fisher *et al.*, 1990).

(b) Sex reversal

Another unusual feature of avian sex determination is that it appears to be partially labile and reversible. Spontaneous sex reversal occurs, usually in the adult female, but very rarely in the adult male (Hutt, 1949; Van Krey, 1990). Female sex reversal arises following atrophy of the left ovary due to disease, or after ovariectomy. In the absence of ovarian estrogen secretion, medullary tissue in the rudimentary right gonad is induced to differentiate into a testis-like gonad, secreting androgens that masculinize the female bird. If sex determination in birds involves only a Z chromosome dosage mechanism, a higher frequency of spontaneous sex reversal would be expected in males as a result of mutation, or loss of a Z chromosome. The fact that this is not observed further implicates the W chromosome with a primary role in avian sex determination.

It has also been well-established in birds that sex steroid treatment of the early embryo can cause sex-reversal. For example, administration of estradiol to the male embryo during the indifferent sexual period results in the formation of a left ovotestis (Van Krey, 1990). The estradiol apparently induces the germinal epithelium of the embryonic male left gonad to develop an ovarian cortex. Administration of testosterone, however, to genetic female embryos during the indifferent sexual period does not modify the female gonads (Romanoff, 1960; Thorne *et al.*, 1992), but grafts of embryonic testes will induce varying degrees of male gonadal differentiation in the female embryo which may remain permanently after hatching (Maraud *et al.*, 1986). In the latter case, the testis graft is thought to secrete anti-Müllerian hormone which decreases ovarian estrogen secretion and causes male gonadal development. The importance of gonadal steroid hormones in influencing the initial differentiation of the avian gonad is apparent from the cited studies.

(c) Molecular genetic studies of avian gonadal hormones

The timing and expression of genes involved in sex steroid synthesis in the early chicken embryo has been studied by Mizuno *et al.* (1993). The genes analysed encode the steroid 17-hydroxylase/17,20-lyase (P-450c17), which is a key enzyme in the conversion of cholesterol to testosterone, and aromatase (P-450arom) which is essential in the conversion of testosterone to estradiol-17. Transcription of the P-450c17 gene appears to begin as early as the second day of incubation in both sexes, whereas transcription of the P-450arom gene begins at day five to six of incubation in the female embryo only. The latter coincides with the morphological differentiation of the gonads, which is evident at approximately day five and a half of incubation. Elbrecht and Smith (1992) found that administering an aromatase inhibitor (which blocks the synthesis of estrogen from testosterone) to chicken embryos at a stage when their gonads were bipotential, caused genetic females to develop a permanent male phenotype. These sex reversed female chickens had the physical appearance of males

and developed bilateral testes that were capable of spermatogenesis. The results suggest that exposure to estrogen during the early phase of gonadal development is crucial for the normal development of the ovary. Regulation of aromatase enzyme activity is therefore a key element in the control of gonadal differentiation and sex determination in chickens. Aromatase presumably lies in the sex determining pathway downstream from a major avian sex determining gene or genes.

(d) Molecular genetic studies in search of avian sex determining genes

In recent years, the identification of a number of genes involved in mammalian sex determination and differentiation, has led to an increased interest in avian sex determination. The testis-determining gene on the mammalian Y chromosome, *SRY,* which was isolated by genetic analysis of sex-reversed individuals (Sinclair *et al.,* 1990) has been examined in birds. The SRY gene, however, was not expressed in a sex-specific manner (Griffiths, 1991; Tiersch *et al.*, 1991) indicating that it does not have a role in avian sex determination. Recently, an SRY-related gene, SOX9, has been shown to have male-specific expression during the sex determination period of both the mouse and chicken (Kent *et al.,* 1996). SOX9 is thus the first reported testis-specific gene in birds. SOX9 is not, however, located on either the Z or W sex chromosome, and it is expressed after the first signs of sexual dimorphism in the chicken indicating that it is not a major switch gene in the sex determining pathway, but its expression is consistent with an important role in testis and genitourinary development. Studies of its regulation may yield significant knowledge on avian sex determination.

III. CONCLUSIONS

Studies based on the phenotypic observation of individuals with aberrant sex chromosomes implicate the W sex chromosome with a major role in avian sex determination, rather than a genic balance sex determining mechanism. Evidence suggests that the W chromosome contains a major female sex determining gene that directs development of the ovary, but its effect is reversed by more than one dose of a male sex determining gene on the Z chromosome. Detailed molecular genetic studies are expected eventually to resolve the question of avian sex determination. Identification of the sex determining genes in birds will provide greater insight into the factors regulating gonadal differentiation and will allow important evolutionary comparisons at the molecular level of female and male heterogametic sex determining systems.

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