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An overview of factors influencing sex determination and gonadal development in birds

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Abstract. The morphological development of the embryonic gonads is very similar in birds and mammals, and recent evidence suggests that the genes involved in this process are conserved between these classes of vertebrates. The genetic mechanism by which sex is determined in birds remains to be elucidated, although recent studies have reinforced the contention that steroids may play an important role in the structural development of the testes and ovaries in birds. So far, few genes have been assigned to the avian sex chromosomes, but it is known that the Z and W chromosomes do not share significant homology with the mammalian X and Y chromosomes. The commercial importance of poultry breeding has motivated considerable investment in developing physical and genetic maps of the chicken genome. These efforts, in combination with modern molecular approaches to analyzing gene expression, should help to elucidate the sex-determining mechanism in birds in the near future.

Key words. Birds; gonads; sex determination; sex chromosomes; estrogen; estrogen receptor.

Introduction

For many years the accessibility and resilience of the chick embryo made this system the preferred model for studies on vertebrate development. However, since the advent of modern molecular techniques, the paucity of genetic resources and the difficulties in adapting transgenic technologies to birds has seen the mouse supersede the chick as the model system of choice. This legacy has meant that the morphological development and endocrine control of secondary sexual differentiation is as well understood in birds as in mammals. Unfortunately, it has also meant that the understanding of the genetic control of sex determination and gonadal differentiation in birds lags far behind that in mammals. In recent years there has been renewed interest in investigating these aspects of avian development, and while the primary motivation has been commercial, the impetus has derived from advances in the mammalian field, particularly the identification of the testis-determining gene. This article will review recent progress in sex determination and gonadal development in birds.

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General gonadal development

There are many shared features between gonadogenesis in birds and in mammals. In both, the gonads arise from a ridge of tissue, known as the genital ridge, which appears on the surface of the developing mesonephros [1]. The cells which contribute to the developing gonad are derived from the mesenchymal blastema of the genital ridge, from the overlying coelomic epithelium and from the mesonephros; however, the exact contribution from each is under debate [2–4]. During the initial stages of gonadal development there are no obvious differences between males and females, and this is designated the indifferent period. At a particular point in gonadogenesis, development in the male and female diverges: the male gonad displays testicular characteristics, whereas the female gonad displays ovarian characteristics. While the fully developed organs appear dramatically different in structure, the testis and ovary are essentially similar in function and at a cellular level. In both males and females the cells of the indifferent gonad differentiate into the specialized supporting and steroidogenic cells which form tissue cords around the germ cells [2]. It is the localization of these cords within

the gonads that leads to the distinctive structural differences between the ovary and testis. In generalized terms, the chick gonads are considered to be composed of two major components, an inner medulla and an outer cortex [2, 5, 6]. In the testis, the cords of cells form in the medullary region, which continues to develop, and the cortex regresses, whereas in the ovary the early medullary cords degenerate and secondary cords develop in an expanded cortex (fig. 1). At a molecular level, this process can be considered to be under the control of two distinct genetic programs: (i) an underlying gene cascade which regulates the differentiation of the specialized cells of the testis and ovary, and (ii) superimposed on this a sex-determining mechanism which controls the decision to follow the pathway of testicular or ovarian development.As previously noted, the development of individual gonads is similar in birds and mammals at a morphological level. However, a major difference in gonadogenesis between these classes is that only the left ovary fully develops in most birds (fig. 2). The right ovary differentiates to the stage where it produces a female pattern of hormones and then ceases to develop further. Instances where the left ovary is removed or damaged result in the development of the right gonad as a testis or ovo-testis [7, 8], and It has been suggested that the right female gonad only has the capacity to develop as a testis. However, if the left ovary is removed early in embryonic development, the right gonad will develop as a normal ovary, demonstrating an inherent capacity to form an ovary, if only for a restricted period [9, 10].

The right-left asymmetry seen in ovarian development also extends to the distribution of the germ cells, with approximately 70% being found in the left gonad in both sexes [11, 12].

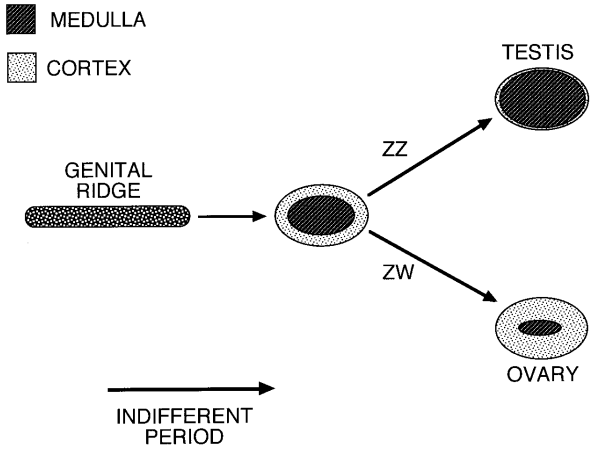

Figure 1. Relative contribution of the medullary and cortical components to the developing gonads.

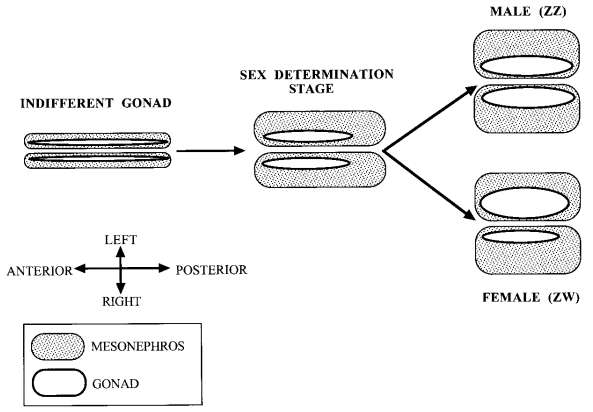

Figure 2. Schematic illustrating the development of the male and female avian gonads. Prior to gonad differentiation male and female embryos are indistinguishable. After gonad differentiation individual embryos can be sexed on the basis of the asymmetrical development of the female gonads.

Sex chromosomes

With the exception of the primitive family of ratites, male and female birds have clearly distinguishable sex chromosomes, indicating that the mechanism which controls the developmental decision to form an ovary or testis is chromosomal. The avian karyotype is composed of both macrochromosomes and microchromosomes, the latter being very small in size and cytologically indistinguishable from one another. The chicken has 39 pairs of chromosomes consisting of 10 pairs of macrochromosomes and 29 pairs of microchromosomes [13]. The sex chromosomes of birds have been designated the Z chromosome and the W chromosome; males are homogametic (ZZ) and females are heterogametic (ZW) [14, 15]. In the chicken, the Z chromosome is the fifth largest of the chromosomes and comprises approximately 7% of the genome, whereas the W chromosome is the size of a microchromosome and comprises approximately 1.5% of the genome [16, 17]. There is no significant homology between the avian Z and the mammalian X chromosomes, suggesting that the sex chromosomes of birds and mammals evolved from different pairs of autosomes [18, 19]. The W chromosome does show some superficial similarity to the mammalian Y chromosome, being composed largely of heterochromatic DNA, which is made up mainly of two families of repeat sequences (fig. 3) [20, 21]. In addition, during meiosis, a small region at the tip of the

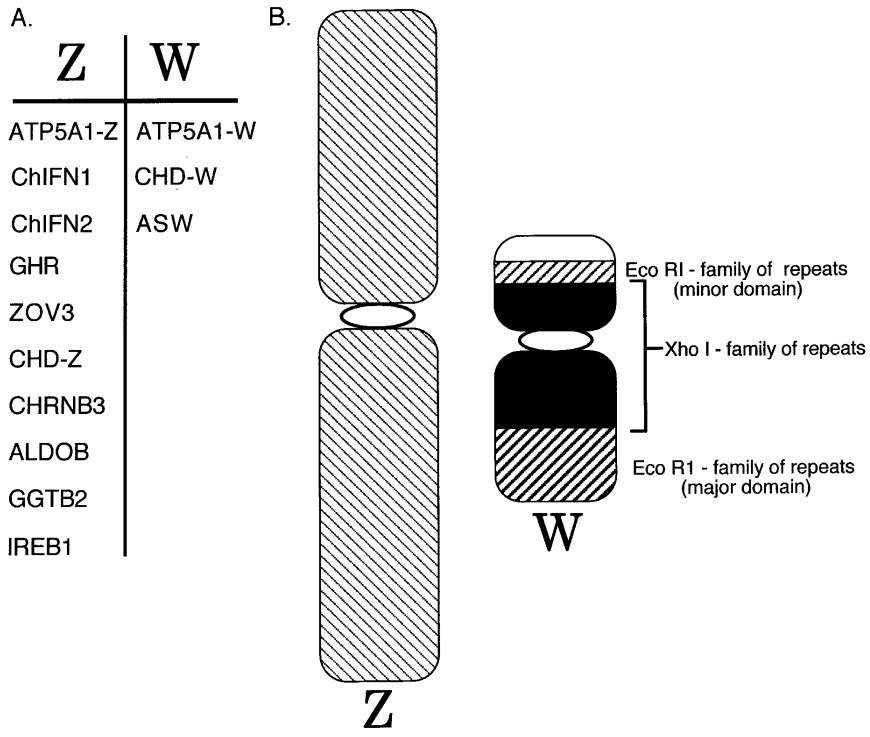

Figure 3. Sex chromosomes of the chicken. (*A*) Genes assigned to the Z and W chromosomes of the chicken. ChIFN1, interferon 1; ChIFN2, interferon 2; ATP5A1, F1 ATP synthase α; ZOV3, Z chromosome-linked immunoglobulin superfamily; GHR, growth hormone receptor; CHD, chromodomain helicase DNA-binding protein; CHRNB3, nicotinic acetylcholine receptor; ALDOB, aldolase B; GGTB2, glycoprotein-4-β galactosyl transferase; IREBP, iron-responsive element-binding protein; ASW, avian sex-specific gene W chromosome [27, 28]. (*B*) Relative size of chicken sex chromosomes and distribution of W chromosome repeat sequences.

short arm of the W chromosome is able to pair with a similar region on the Z chromosome, reminiscent of the mammalian psuedo-autosomal region (PAR) [22–24]. So far, only three genes, *CHD-W*, *DWM1/ATP5A1-W* and *ASW* have been localized to the nonrepeat regions of the W chromosome. CHD-W is a chromohelicase-DNA-binding protein [25], DMW1/ATP5A1-W is an avian homologue of the adenosine 5′-triphosphate (ADP) synthase α subunit [19, 26], and both of these genes have related sequences on the Z chromosome. ASW is reported to be specific to the W chromosome and is of unknown function [27]. The avian Z chromosome has been described as composed of three discernible regions. There is a PAR at the tip of the short arm, although no crossing over occurs in this region between the Z and W chromosomes. The second region contains a single recombination nodule located near the pairing end of the ZW bivalent, and the third region comprises the remainder of the short arm and the entire long arm, which is specific to the Z chromosome. Only a small number of genes and anonymous DNA markers have so far been assigned to the chick Z chromosome; however, a concentrated mapping effort has recently been initiated, and a chicken genome database established at the Roslin Institute [28]. Figure 3 illustrates the relative sizes of the chicken Z and W chromosomes and lists the genes so far assigned to these.

In mammals where the female has two X chromosomes and the male only one, the imbalance of the copy number of X-linked genes is equalized by regulation at the level of gene expression in the female [29]. This dosage compensation is achieved by inactivating one of the female X chromosomes, which results in the cytological feature known as the Barr body [30]. In birds, it has been widely accepted that there is no dosage compensation system in operation and that the level of expression of Z-linked genes in males will be double that in females [13, 31]. Evidence cited in support of this theory includes a failure to detect the presence of a Barr body in males, no asynchronous replication of the Z chromosomes and a report of higher levels of the Z-linked aconitase/IREB gene product in males than females [32–34]. However, individually none of these data would be conclusive proof that birds have no system of

dosage compensation, and even in combination the evidence is mostly circumstantial. It is still quite possible that a dosage-compensation system, not based on Z chromosome inactivation, exists in birds.

Sex-determining mechanism

The mechanism which regulates the developmental decision to form a testis or an ovary in birds is unknown, but there is wide support for two different proposed mechanisms of sex determination. These are based on models established in other organisms with heteromorphic sex chromosomes and propose that the sex-determining mechanism in birds is regulated by either a dominant genetic switch as employed by mammals or a dosage-based mechanism as typified by the systems in operation in *Drosophila* and *Caenorhabditis elegans* [6, 15, 35, 36]. In mammals, the male-specific gene Sry, located on the Y chromosome, has been identified as the testis-determining gene [37, 38]. It has been demonstrated that expression of this gene in the gonads at the appropriate time in development is necessary and sufficient to induce testis differentiation [39, 40]. In birds with homogametic males (ZZ) and heterogametic females (ZW) a male-specific testis-determining gene is inconceivable. Under the proposed 'dominant gene' model, the female-specific W chromosome would carry an ovary-determining gene that would operate in a fashion analogous to the mammalian *Sry* gene. The alternative dosage mechanism would depend on the ratio of Z chromosome number to the number of sets of autosomes. Under this model, the male with two Z chromosomes and a diploid set of autosomes would have a ratio of 1.0, whereas the female with one Z chromosome would have a ratio of 0.5. Here the interaction of an autosomal factor with a single dose (ZW) or a double dose (ZZ) of a Z-linked gene product would decide the fate of the developing gonad.

In mammals, the nature of the sex-determining mechanism was elucidated as a direct result of identifying individuals with sex chromosome abnormalities. Such individuals can arise due to the nondisjunction of the sex chromosomes during oogenesis or spermatogenesis [41]. During oogenesis, failure of the members of the X chromosome pair to separate and move into one daughter cell will produce oocytes with either two X chromosomes or none. Fertilization of such oocytes by normal spermatozoa will result in zygotes with either XXX, XO, XXY or YO sex chromosomes. Alternatively, nondisjunction during spermatogenesis can give rise to sperm with no sex chromosomes, with both an X and a Y, or with two Y sex chromosomes. Fertilization of a normal oocyte could then result in zygotes with either XO, XXY or XYY sex chromosome complements. Klinefelter's syndrome (XXY or XXXY) and Turner's syndrome (XO) are relatively common examples of such occurrences [42]. The observation that in such cases individuals with a normal Y chromosome were always male and individuals lacking a Y chromosome were always female led to the conclusion that the Y chromosome carried a testis-determining gene. The identification of individuals with sex chromosome translocations and deletions finally led to the identification of the Sry gene as the testis-determining gene (reviewed in [43]).

The identification of birds with equivalent sex chromosome abnormalities would help to resolve which of the proposed models of sex determination was most likely correct. Table 1 list a number of potential avian genotypes and the predicted sex of each under both of the proposed models. Unfortunately, karyotype analysis has so far failed to reliably identify individuals with the truly informative genotypes such as ZO and ZZW. While a single report of a male ZZW aneuploid would appear to support the Z chromosome:autosome ratio model [44], others have convincingly argued that cytological techniques available at that time were not sufficiently reliable to assign a credible karyotype [45]. The most detailed karyotyping studies available in birds have resulted from the analysis of a triploid line of

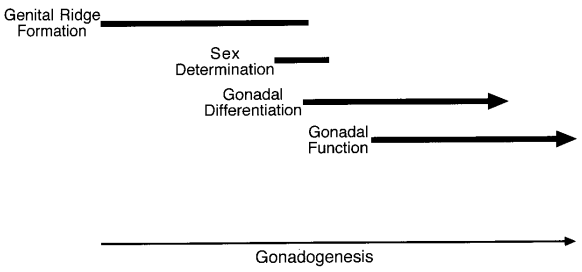

Figure 4. Phases of embryonic gonadal development.

Table 1. Theoretical sex chromosome genotypes and predicted sex under the 'dominant gene' and 'Z:A ratio' models of sex determination.

Sex chromosomes	Predicted sex	
	dominant gene	Z:A ratio
ZO	male	female
ZZ	male	male
ZZZ	male	male
ZZW	female	male
ZW	female	female
WO	female	?

Table 2. Karyotypes reported for the chicken and assigned phenotypes.

Genotype	Phenotype	Predicted by model	
		dominant gene	Z:A ratio
2A:ZW	female	yes	yes
2A:ZZ	male	yes	yes
3A:ZZZ	male	yes	yes
3A:ZZW	intersex	no	yes
3AZWW	female	yes	yes

Agreement of individual karyotypes with the 'dominant gene' and 'Z:A ratio' models of sex determination is indicated. (3AZWW karyotype was reported for a single individual sexed as female at day 16 of embryonic development.)

chickens by Thorne et al. [45–50]. In these studies, triploid birds with a ZZZ sex chromosome complement developed testes but were infertile, whereas ZZW birds were classified as intersexes with two developed gonads. In the ZZW triploids, the right gonad initially developed as a testis, whereas the left gonad resembled an ovary; however, both gradually became masculinized, and eventually both gonads produced abnormal spermatozoa. Table 2 summarizes the reliable aberrant karyotype information that is available for the chicken. While instances of individual karyotypes can be selected which support either of the proposed sex-determining models, overall there is insufficient evidence presently available to form a definite conclusion.

Genes in gonadal development

The embryonic development of the gonad can be considered as a series of sequential but overlapping phases, as depicted in figure 4. In mammals, a number of the genes associated with the development of these phases have been identified, and these are listed in table 3 [37, 38, 51–57].

We and others have attempted to isolate chick homologues to the genes involved in mammalian gonadal development and to study the expression of these genes during gonadal development in the chick. Attempts to identify a direct homologue to the mammalian testis-determining gene (Sry) have so far proved unsuccessful, although a large number of related SOX (Sry box) genes have been identified [58–60]. Given that there is no male-specific sex chromosome in birds, the failure to detect a Sry homologue may be unsurprising. However, the possibility that a closely related SOX gene on the W chromosome may act as an ovary-determining gene cannot be excluded, and we have demonstrated that a number of SOX genes are expressed in the genital ridge at the appropriate time in development [58]. With the exception of Sry, and to date Dax-1, chick homologues to the remaining genes listed in table 3 have been isolated [57, 61–64]. The expression of the majority of these genes during gonadal development has been analyzed by various groups [57, 63, 65–69]. While these results indicate similar patterns of expression in birds and mammals, the piecemeal nature of these reports makes it difficult to ascertain the exact temporal relationships of the expression patterns. We have prepared RNA populations from pools of male gonads and pools of female gonads isolated from embryos incubated for 4.5, 5.5, 6.5, 7.5 and 8.5 days. We have analyzed the expression of the chick, WT-1, SF-1, Sox9, AMH and aromatase genes in these RNA samples by Northern analysis and in isolated gonads by in situ hybridization (unpublished data). Our data reveal that WT-1, Sox9 and SF-1 are expressed in the developing gonad of both sexes throughout this period, with Sox9 and SF-1 being expressed at higher levels in males than females from day 6.5 onwards. AMH expression is first detectable at very low levels in male gonads at day 5.5 of development and at increasing levels thereafter (fig. 5 shows examples of SF-1 and AMH expression).

While the embryonic expression of AMH in mammals is male-specific, in birds, AMH is required to effect the regression of the right Mullerian duct, and consequently a low level of AMH expression is detectable in both right and left female gonads from day 6.5 onwards. Aromatase is required for the conversion of androgens to estrogen, and the expression of the chick aromatase gene is detectable from day 6.5 only in female gonads. The expression patterns that we observed in this study are in general agreement with patterns reported for

Table 3. Association of previously identified genes with specific phases of gonadal development.

Genital ridge formation	Sex determination	Gonadal differentiation	Gonadal function
WT 1	Sry	*SF 1*	AMH
SF 1		*Sox9*	steroid-metabolizing enzymes (including aromatase)
		Dax 1	

WT-1, Wilms's tumor gene-1; *SF-1*, steroidogenic factor-1; *Sox9*, Sry box-containing gene 9; Sry, sex-determining region Y chromosome; *Dax-1*, DSS-AHC critical region on the X chromosome, gene 1; AMH, anti-Müllerian hormone [38–40, 51–55, 57].

Figure 5. Expression of SF-1 and AMH in developing chick gonads by whole-mount in situ hybridization analysis. (*A*) Expression of SF-1 in the chick embryo at 3.5 days of development. The embryo has been decapitated and the ventral surface and viscera removed. Hybridization was performed using a digoxygenin-labeled antisense SF-1 probe. The signal is indicated by purple deposits. The genital ridge is indicated by arrowheads. (*B*) Expression of AMH in the isolated gonads and mesonephroi of the chick embryo at day 8 of incubation. Hybridization was performed using a digoxygenin-labeled antisense AMH probe. g, gonad; m, mesonephros.

these individual genes by others [57, 63, 65–69]. The expression profiles of WT-1, SF-1 and Sox9 suggest a high degree of conservation of the molecular mechanisms regulating gonadal development in the chick and mammal. The temporal relationships between the expression profiles of these genes and those of *AMH* and *aromatase* would indicate that sex determination in chickens occurs no later than day 5.5 of embryonic development. A comparison of the expression profiles of the major genes involved in gonadal development in birds and mammals is shown in table 4.

The role of steroids in avian gonadal development

Shortly after the onset of gonadal differentiation, the embryonic gonads of both birds and mammals are considered to have the capacity to produce steroids [70, 71]. In mammals, steroids are thought to be responsible for the development of accessory sex organs and secondary sex characteristics and to play no role in the development of the gonads [72]. However, in birds it appears that steroids produced by the embryonic gonad may play a major role in the development of the gonads themselves. This evidence comes from a large body of work focused on sex reversal which has been carried out in the chicken. In addition to documenting cases of spontaneous sex reversal, experimental approaches have included castration, transplantation, administration of various compounds including estrogens and androgens, antiestrogens and antiandrogens, and aromatase inhibitors (a nonexhaustive list includes [73–79]). From these data it would appear that both male and female sex reversal can be induced under certain experimental conditions, although in cases of spontaneous sex reversal these are most commonly female to male. Experiments have shown that if the synthesis of estrogen is blocked in early genetically female embryos by the administration of an aromatase inhibitor, approximately 50% of the embryos develop testis [80]. Similarly, embryonic testicular implants can cause testis development in genetically female embryos, and this reversal is preventable by the simultaneous administration of estrogens [81]. Conversely, the administration of exogenous estrogen can feminize the gonads of male embryos, whereas estrogen antagonists are able to disrupt the normal development of the ovary [49, 82, 83]. From these reports estrogens would appear to be an absolute requirement for the development of the normal ovary. This ability of steroids to sex-reverse the embryonic gonads implies a certain 'plasticity' in the development of the avian gonads which is not apparent in the development of the mammalian gonad. Reports of sex reversal of adult animals would also suggest that this

plasticity is not confined to the embryonic stages. However, the majority of these cases seem to be the result of either ovarian disease, which presumably alters the steroid profile produced, or the effects of environmental steroids. It seems likely that reports of adult sex reversal are the result of hormonal influence on secondary sexual characteristics rather than gonadal transformation.

The ability of steroids to effect physiological changes is often the result of modifications in the expression patterns of specific genes and is mediated through specific nuclear proteins, the steroid receptors. These receptors belong to a large family of ligand-activated transcription factors which regulate gene expression by interacting either in a protein/DNA manner with cognate DNA sequences called responsive elements [84] or in a protein/protein manner with other transcription factors [85]. Recent reports of the expression of the estrogen receptor gene in chicken have further emphasized the importance of estrogen in chick gonadal development [68, 86, 87]. These reports demonstrated that the expression of the estrogen receptor gene is gonad-specific in the early embryo and detail the expression of this gene throughout gonad development. Using in situ hybridization and reverse transcriptase-polymerase chain reaction (RT-PCR), estrogen receptor expression was detected in the gonads, coincident with the first indications of differentiation. The authors reported estrogen receptor expression in both gonads of male and female embryos with higher levels of expression in the left gonads. The expression of the estrogen receptor in the male gonads was transitory but could be maintained by the administration of estrogen. The expression data on the estrogen receptor coupled with the female-specific ability to synthesize the appropriate ligand strongly supports a pivotal role for estrogen in regulating the development of the ovary. Expression of the estrogen receptor in the developing male gonads would also explain the ability of exogenous estrogen to induce ovary formation in male embryos. Slightly at variance with this suggestion are the results of immunohistochemistry studies by Andrews et al. [86] which detected the presence of the estrogen receptor protein only in female gonads.

While the weight of evidence would point to a crucial role for estrogen in inducing cortex formation in the differentiating gonad, data from Andrews et al. [86] and Smith et al. [68] also raise the possibility that estrogen and/or the estrogen receptor may play an even more central role in the sex-determining process. Although Nakabayashi et al. [87] were unable to detect expression of the estrogen receptor gene until after the point of sex determination, Andrews et al. [86] and Smith et al. [68]

Table 4. Comparison of expression profiles of genes associated with gonadal development in birds and mammals.

Genes	Mammals	Birds
WT-1	Expressed in males and females throughout gonadal development.	Expressed in males and females throughout gonadal development.
SF-1	Expressed in males and females throughout gonadal development. Expressed at higher levels in males during differentiation phase.	Expressed in males and females throughout gonadal development. Expressed at higher levels in males during differentiation phase.
Sox9	Expressed in males and females during sex determination. Male-specific expression during differentiation phase.	Expressed in males and females during sex determination. Expressed at higher levels in males during differentiation phase.
Sry	Male-specific expression during sex-determination phase.	Absent.
Dax-1	Expressed in males and females during differentiation phase. Higher levels of expression in females (*).	Unknown.
AMH	Male-specific expression during differentiation and function phases.	Expressed in both males and females during differentiation and function phases. Higher levels in males.
Aromatase	Expressed in males and females during late differentiation and function phases.	Female-specific expression during late differentiation and function phases.

Expression profiles in the chicken may not reflect gonad-specific expression, as profiles are derived from Northern analyses of RNA isolated from genital ridge plus mesonephros (unpublished data). In situ hybridization studies report male-specific expression of Sox9 during the differentiation phase in the chicken [56, 69]. *Recent studies reports higher levels of Dax-1 in males during the differentiation phase [95] and suggest that Dax-1 has a critical role in spermatogenesis rather than ovary determination [96]. *WT-1*, Wilms's tumor gene-1; *SF-1*, steroidogenic factor-1; *Sox9*, Sry box-containing gene 9; Sry, sex-determining region Y chromosome; *Dax-1*, DSS-AHC critical region on the X chromosome, gene 1; AMH, anti-Müllerian hormone.

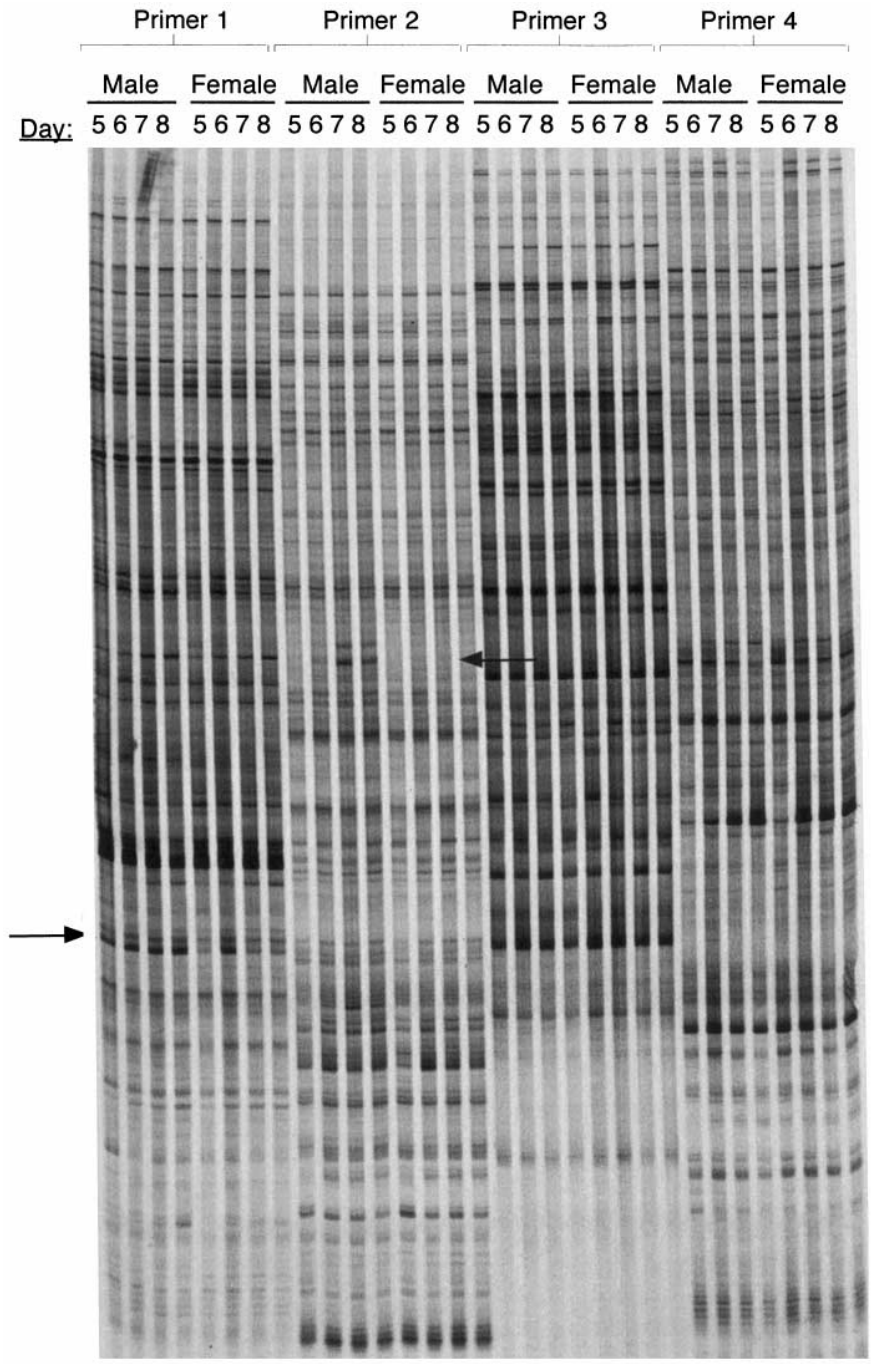

Figure 6. Partial differential display RT-PCR analysis of gene expression in the developing gonads of male and female chick embryos. Gel bands represent individual gene transcripts, and each primer combination displays ~250 transcripts at each stage of development. Examination of display gel allows a comparison of the expression profiles of genes in male and females throughout gonadal development. Genes differentially expressed in the male and female gonads are readily identified as indicated by arrows. Day, day of development.

reported expression of this transcript in both male and female gonads continuously from day 4.5 to day 12.5 of incubation. In addition Smith et al. [68] report a sexually dimorphic pattern of expression of the estrogen receptor in embryos at day 3.5 of incubation. In these embryos, estrogen receptor gene expression was

confined to the genital ridge of female embryos only. This raises the possibility that estrogen and the estrogen receptor could play an important role in the formation of the genital ridge and in very early sex-determining events. Studies reporting the surprising detection of steroids in the genital ridge of very early chick embryos would appear to reinforce this possibility [88, 89]. These studies reported the presence of steroids in day 3.5 embryos with higher levels of estrogens in females and higher levels of androgens in males by day 5.5, providing a mechanism whereby steroids could regulate sex-specific differentiation of the testis and ovary from the time of genital ridge formation. Unfortunately, it is hard to reconcile these reports with evidence on the expression patterns of genes coding for the steroid-metabolizing enzymes [65–68]. The expression of these genes, indicative of the differentiation of the specialized cells of the testis and ovary, cannot be detected until day 6.5 of incubation. In an attempt to clarify this issue, we have used radioimmunoassay to measure estradiol 17β levels in the gonads of male and female chick embryos from day 4.5 to day 12.5 of development (unpublished data). In contrast to the earlier studies, we were unable to detect estrogens in the male gonads at any stage and only from day 6.5 onwards in the female gonad. Our observations on estrogen synthesis are in complete agreement with the coincident expression of the aromatase gene reported by ourselves and others [65–68]. Despite the apparent conflict between the presence of the receptor and the availability of the ligand, the possibility remains that the estrogen receptor may regulate gene expression via an as yet unidentified alternative ligand or by ligand-free transactivation, and could still play a central role in formation of the genital ridge and sex determination in birds.

Future prospects

Recent studies suggest that many of the genes involved in the differentiation of the specialized cells of the mammalian gonad are likely to play similar roles during avian gonadogenesis. In addition, these studies have also helped to understand some of the tremendous legacy of data on sex reversal in birds. Despite these advances, the nature of the chromosomal mechanism by which sex is determined in birds remains unknown. While shadowing advances in the mammalian field has so far proved fruitful, it is unlikely that the approaches used to elucidate the sex-determining mechanism and identify the testis-determining gene in mammals will succeed in birds. This is principally due to the difficulties in identifying individuals with chromosomal abnormalities and the lack of detailed information on the sex chromosomes. In addition, it appears as if the process by which the sex-determining mechanism is implemented in birds may be more akin to that in fish and reptiles where estrogen plays a central role [90, 91] than to that in mammals. For these reasons, a number of groups have invested considerable effort in alternative approaches such as differential display–RT-PCR [92, 93] and representational difference analysis [94]. These approaches compare gene expression in the developing male and female gonads, with the aim of identifying transcripts which exhibit sex-specific or sexually dimorphic expression. A typical example of a differential display analysis is shown in figure 6. A notable success using this approach was the identification of the female-specific *ASW* gene [27], and we have recently isolated a Z-linked gene which is expressed in the developing gonads with higher levels in males than in females (unpublished data).

The rapidly expanding Chickmap program and recent improvements in techniques such as differential display should help to identify the genes involved in avian sex determination and gonadal development. However, to fully understand these processes and to assess the significance of novel genes, a concentrated effort is required to identify individuals with informative sex chromosome genotypes and to resolve the issue of dosage compensation.

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