Temperature-dependent sex determination in the American alligator: expression of SF1, WT1 and DAX1 during gonadogenesis

Patrick S. Western a,b, Jenny L. Harry c, Jennifer A. Marshall Graves b, Andrew H. Sinclair a,*

a Department of Paediatrics and Centre for Hormone Research, University of Melbourne, Royal Children’s Hospital, Melbourne, Victoria 3052, Australia
b Department of Biochemistry and Genetics, La Trobe University, Melbourne, Victoria 3083, Australia
c Proteome Systems Limited, Unit 35–41 Waterloo Road, North Ryde, NSW 2113, Australia

Received 13 July 1999; received in revised form 21 September 1999; accepted 12 October 1999

Received by E. Boncinelli

Abstract

Sex determination in mammals and birds is chromosomal, while in many reptiles sex determination is temperature dependent. Morphological development of the gonads in these systems is conserved, suggesting that many of the genes involved in gonad development are also conserved. The genes SF1, WT1 and DAX1 play various roles in the mammalian testis-determining pathway. SF1 and WT1 are thought to interact to cause male-specific gene expression during testis development, while DAX1 is believed to inhibit this male-specific gene expression. We have cloned SF1 and DAX1 from the American alligator, a species with temperature-dependent sex determination (TSD).

SF1, DAX1 and WT1 are expressed in the urogenital system/gonad throughout the period of alligator gonadogenesis which is temperature sensitive. SF1 appears to be expressed at a higher level in females than in males. This SF1 expression pattern is concordant with the observed pattern during chicken gonadogenesis, but opposite to that observed during mouse gonadogenesis. Although the observed sexual dimorphism of gonadal SF1 expression in alligators and chickens is opposite that observed in the mouse, it is probable that SF1 is involved in control of gonadal steroidogenesis in all these vertebrates. DAX1 and WT1 are both expressed during stages 22–25 of both males and females. However, there appear to be no sex differences in the expression patterns of these genes. We conclude that DAX1, WT1 and SF1 may be involved in gonadal development of the alligator. These genes may form part of a gonadal-development pathway which has been conserved through vertebrate evolution. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Alligator, DAX1; Gonadogenesis; SF1; Temperature-dependent sex determination; WT1

1. Introduction

In mice and humans, expression of SRY (sex region Y-chromosome gene) is required for differentiation of Sertoli cells in the developing testis (Sinclair et al., 1990; Koopman et al., 1991). Females lack SRY and therefore Sertoli cells fail to develop. By an unknown mechanism, SRY initiates a cascade of genetic events leading to the activation of male-specific genes such as anti-Mullerian hormone (AMH) and the SRY-related gene, SOX-9 (SRY like box containing gene). Three proteins which may have roles in activating or repressing this male-specific gene activation are the orphan nuclear receptors, steroidogenic factor 1 (SF1), and DAX1 (Dosage Sensitive Sex Reversing-Adrenal Hypoplasia Congenita).
family (for review see Parker and Schimmer, 1997). Mice with homozygous null mutations in Sf1 lack gonads and adrenals (Luo et al., 1994). Consistent with the phenotype exhibited by Sf1 null mice, a heterozygous mutation has recently been detected in the Sf1 gene of a phenotypically female XY human who exhibited failure of gonadal and adrenal gland development. These data confirm that Sf1 is also required for normal testis and adrenal differentiation in humans (Achnermann et al., 1999) in addition to its roles in regulating steroidogenesis.

In humans, the distinct conditions Denys Drash syndrome (DDS), Frazier syndrome (FS) and WAGR syndrome (Wilms’ tumour, Aniridia, Genital abnormalities and mental Retardation) are all caused by mutations in various regions of the Wilms’ tumour suppressor gene (Wt1) (for examples see Pelletier et al., 1991; Barbaux et al., 1997). Significantly, XY patients with these syndromes share the characteristics of abnormal urogenital system and dysgenic gonads. Mice homozygous null for Wt1 exhibit complete failure of urogenital development (Pelletier et al., 1991; Hastie, 1992). Therefore it appears that Wt1 functions early during development of the urogenital system and later during gonad development.

Duplication of the X-chromosome located DAX1 gene is thought to cause dosage-sensitive sex reversal in XY human patients (Zanaria et al., 1994). This is supported by experiments in mice where the product of a Dax1 transgene has been shown to weakly antagonise Sry, thus causing XY sex reversal (Swain et al., 1998). DAX1 is expressed in the undifferentiated mouse gonad of males and females at 11 dpc and continues to be expressed in the developing ovary, but is apparently down-regulated in the developing testis at 12.5 dpc (reviewed by Swain and Lovell-Badge, 1997). In the mouse, DAX1 and Sf1 expression co-localise to the steroidogenic cells of the developing gonads, adrenals, hypothalamus and anterior pituitary, indicating that these proteins function in common endocrine systems (Ikeda et al., 1996). Although DAX1 has been proposed to act as an ovary-determining factor (Swain and Lovell-Badge, 1997), ovarian development appears to proceed normally (although multiple germ cells were noted in some follicles) in XX mice with homozygous DAX1 mutations (Yu et al., 1998). In XY mice with the same DAX1 mutations, somatic development of the testis appeared normal although germ cell development was disrupted (Yu et al., 1998). The function of DAX1 during sexual development remains unclear, although it appears to play an important role in germ cell development.

In contrast with the chromosomal sex-determining mechanisms evident in mammals and birds, egg incubation temperature determines sex in many reptiles (for examples see Lang and Andrews, 1994; Pieau, 1996). Despite the apparent difference in the primary mechanism of chromosomal and temperature-dependent sex determination (TSD), morphological development of the gonads in these groups is conserved, suggesting that many of the genes leading to gonad morphogenesis are conserved.

In the American alligator (Alligator mississippiensis), incubation of eggs at a constant temperature of 33°C yields 100% male offspring, while incubation at 30°C or 34.5°C yields 100% or 95% female offspring, respectively (Lang and Andrews, 1994). Commitment to male or female development occurs within a 10 day temperature-sensitive period (TSP) of gonadogenesis during developmental stages 21–24 of alligator embryogenesis (Lang and Andrews, 1994). Throughout the TSP the indifferent alligator gonad undergoes ovarian or testicular morphogenesis. At the male temperature (33°C) the gonad is undifferentiated at stage 21. During stages 21–23 supporting cells in the medulla proliferate substantially, and during stage 23–24 these cells arrange into the seminiferous tubules of the testis (Smith and Joss, 1993). We have previously shown that male-specific gene expression occurs in the developing testis during this time (Western et al., 1999). AMH expression is upregulated from stage 22 and Sf1 expression from stage 23.5, indicating that the proliferating supporting cells are differentiating as immature Sertoli cells during stages 22–23.5 (Western et al., 1999). At the female temperature of 30°C, proliferation of the supporting cells in the medulla does not occur. Rather there is a proliferation of germ cells in the cortex and fragmentation of the medulla through stages 21–23. By stage 23, germ cell proliferation has given rise to oogonial nests (Smith and Joss, 1993). To date, genes expressed during ovarian differentiation have yet to be identified.

Sf1 has been implicated in the activation of AMH expression during testicular development (Giuli et al., 1997 and references therein). Recent data indicate that the activation of AMH expression is enhanced by a synergistic interaction between Sf1 and Wt1 on the AMH proximal promoter. In addition, it was shown that DAX1 could compete with this activation, thus inhibiting AMH expression in this system (Nachitgal et al., 1998). Since Sf1, Wt1 and DAX1 are thought to be involved in mammalian sex determination, we decided to examine the expression of these genes in developing alligator gonads during temperature-dependent sex determination. To address this question we isolated American alligator orthologues of Sf1, DAX1 and Wt1 and examined their expression patterns in developing male (33°C) and female (30°C and 34.5°C) alligator gonads.

2. Materials and methods

2.1. Egg collection, incubation and tissue preparation

Alligator eggs were collected within 2 days of laying from nests of a wild American alligator population.
located on the Rockefeller Wildlife Refuge, Grand Chenier, LA, USA. The eggs were incubated at constant temperatures of 33 °C (male determining) and 30 °C or 34.5 °C (female determining). At developmental stages 20, 21, 22, 23, 24, 25 and 27 (according to the staging protocol in Ferguson, 1987) embryos were removed from their eggs, decapitated and the gonadal and urogenital tissues removed by dissection. Tissues were snap-frozen in liquid nitrogen and stored for RNA and DNA preparation. During the earlier stages of alligator development, the gonad cannot be clearly separated from the surrounding tissues. Consequently, tissue from stages 20 to 23 analysed by RT–PCR included: gonad and adrenal and a small amount of mesonephros (GAM). However, for samples at stages 24–27 every effort was made to ensure that only gonadal tissue was used.

2.2. Gene cloning

A mixed-sex stage 22–23 alligator urogenital lambda ZAP cDNA (Stratagene) library was screened separately with a chicken DAX1 probe and a zebra finch SF1 probe (kindly supplied by Prof. Arthur Arnold) using standard protocols. Lambda phage which reacted with the probes were isolated, and the Bluescript vectors containing inserts were purified according to standard protocols (Stratagene). The inserts contained in each clone were sequenced using primer-walking strategies and automated sequencing (Perkin Elmer). Sequences were analysed using Macvector software.

2.3. RT–PCR

Total RNA was isolated from frozen tissue and reverse transcribed using d(T)$_{12}$ and d(N)$_{12}$ primers according to standard protocols (Perkin Elmer). Two independent sets of RNA samples (each set containing RNA extracted from GAM or gonad tissues of stage 20, 21, 22, 23, 24, 25 and 27 alligator embryos incubated at the 33 °C male and 30 °C female temperatures or stage 20, 21, 22, 23 and 25 alligator embryos incubated at the 34.5 °C female temperature) were analysed by RT–PCR. At each stage analysed, RNA was extracted from the pooled GAM or gonad tissue of three to four alligator embryos. cDNA derived from each reverse transcription reaction was analysed by PCR. 200 ng of template was added to a master mix containing PCR buffer, 1.5 mM MgCl$_2$, 1 unit/50 µl AmpliTaq Gold (Perkin Elmer) and 0.2 mM dNTPs. This PCR mix was then divided between four PCR reaction tubes, each containing an aliquot of DAX1, WT1, SF1 or β actin PCR primers. Therefore, for each tissue sample, the PCR reactions for each gene were identical apart from the inclusion of equal amounts of the appropriate gene-specific primers. The PCR reactions were cycled through the following reaction profile: 94 °C for 10 min, 61 °C for 50 s for 1 cycle; followed by 94 °C for 30 s, 61 °C for 50 s for 25 cycles. The actin PCR was stopped after 23 cycles as this gene is expressed at a very high level. If actin was amplified for a greater number of cycles, the PCR would reach the linear amplification phase and become less quantitative. The primer pairs used in the RT–PCR reactions were as follows:

<table>
<thead>
<tr>
<th>Probe</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF1</td>
<td>5'-CGGCCCTCCATCTGGACAACAC-3'</td>
<td>5'-GCTTGGGCTGGAGGACATTCCG-3'</td>
</tr>
<tr>
<td>DAX1</td>
<td>5'-AGTTCGAGCACTGAGCATGG-3'</td>
<td>5'-ACTCCTCCAGAGCGCATGTCCG-3'</td>
</tr>
<tr>
<td>WT1</td>
<td>5'-CCAGCTTGAAGCTATGGACATGG-3'</td>
<td>5'-GAGGACGGGAGCATGAG-3'</td>
</tr>
<tr>
<td>β actin</td>
<td>5'-GGGTTGTGCTGGTGGGACATTG-3'</td>
<td>5'-AACAGAAGATGGGCTGGAAAAGAGG-3'</td>
</tr>
</tbody>
</table>

The PCR reaction products were separated on a 1.5% agarose gel containing 1 µg/ml ethidium bromide and visualised under ultra-violet illumination. Each RT–PCR analysis was repeated at least three times for each set of RNA samples.

3. Results

Since the alligator WT1 gene sequence has been previously published (Kent et al., 1995), primers were designed for use in PCR amplification of alligator WT1. Partial cDNA sequences of the alligator SF1 and DAX1 genes were cloned from an alligator mixed-sex urogenital cDNA library. Screening the alligator cDNA library with a zebra finch SF1 probe resulted in isolation of a single putative alligator SF1 clone. The insert was sequenced and the sequence used to screen the GenBank sequence database. This revealed that the putative alligator SF1 clone shared 80% and 78% DNA sequence homology with human and mouse SF1, respectively. The alligator sequence was translated and found to contain a continuous amino acid reading frame of 131 amino acids (Fig. 1; accession number AF180296) which was 91%, 74% and 78% identical and 97%, 90% and 92% similar to the amino acid sequence of chicken, human and mouse SF1, exons 7 and 8 (Fig 2).

Screening of the alligator cDNA library with a chicken DAX1 probe resulted in isolation of a single putative alligator DAX1 clone. The insert was sequenced and the sequence used to screen the GenBank sequence database. This sequence was used to screen the GenBank database, resulting in a strong match with the human and mouse DAX1 nucleotide sequences. Translation of the alligator DAX1 cDNA sequence revealed a continuous reading frame which ended with a TAA stop codon at a position which was conserved in the human and mouse DAX1 protein sequences. The amino acid sequences of DAX1, chicken, human and mouse DAX1 were compared. Within the ligand binding domain, the alligator sequence showed 77%, 57% and
Fig. 1. Translation of the alligator SF1 partial cDNA sequence. The boundary of exons 7 and 8 in the mouse SF1 gene is indicated by the asterisks above base pairs 148 and 149 in the alligator SF1 sequence. The position of the PCR primers used in the analysis of SF1 expression is indicated by underlined bold type.

Fig. 2. Alignment of the alligator, chicken, mouse and human SF-1 amino acid sequences. Identical amino acids are indicated by the dark grey shading and similar in light grey shading.

53% identity and 87%, 69% and 65% similarity with the equivalent region in the chicken, human and mouse DAX1 amino acid sequences (Fig. 4a). Comparison of the human and mouse DAX1 sequence, which includes the three and one half repeat elements representing the putative DNA binding domain (Bae et al., 1996), with the alligator sequence suggested that a single, poorly conserved copy of the repeat unit is present in the predicted alligator DAX1 protein. Since the alligator DAX1 cDNA clone did not include a potential methionine start codon, it was not possible to conclude whether a more extensive putative DNA binding domain is coded for by the alligator DAX1 homologue. However, comparison of the alligator DAX1 amino acid sequence with chicken DAX1 revealed strong overall identity within the ligand binding domain and the apparently truncated DNA binding domain (Fig. 4b).

A partial cDNA sequence of the alligator WTI gene
Fig. 3. Translation of the partial alligator DAX1 DNA sequence. The position of the intron in mouse and chicken DAX1 is indicated by the asterisks above base pairs 493–494 in the alligator sequence. The position of the PCR primers used in the analysis of DAX1 expression is indicated by underlined bold type.

has been previously published ('Kent et al., 1995). Using this sequence PCR primers were designed which span the exon 4 to exon 6 boundary. These primers should amplify the sequence between nucleotides 255 and 503 of the published alligator WT1 sequence (GenBank accession number X85734). The alligator WT1 PCR primers were used to produce an RT-PCR product of the expected size (249 bp) from alligator gonadal RNA. Sequencing of the resulting RT-PCR product showed that it matched the published alligator WT1 sequence (data not shown).

PCR primers were also designed for alligator SF1 and DAX1. Each primer pair was designed to span the positions of introns present in the human and mouse
WT1, SF1 and DAXI genes. RT-PCR was used to examine the expression pattern of alligator SF1, DAXI and WT1 in urogenital systems of embryos incubated at the female (30°C and 34.5°C) and male (33°C) temperatures.

At the male (33°C) and female (30°C and 34.5°C) temperatures, DAXI and WT1 were expressed during stages 20, 21, 22 and 23 in the developing GAM tissues and during stages 24, 25 and 27 in the developing testis and ovary. Throughout stages 20-27 it appeared that the expression of DAXI increased from a low level during stages 20-22 to higher levels through stages 23-27. There were no observable differences in the male and female expression patterns for DAXI and WT1 (Fig. 5a,b). SF1 was expressed in the developing GAM/gonad tissues of both males and females through stages 20-27. The amount of SF1 expression in female samples (30°C and 34.5°C) remained constant during stages 20-27 (Fig. 5c). However, apparently this was not the case in the male samples, where SF1 expression levels appeared to be equivalent to the female samples during stages 20-22 but lower in the male than the female samples during stages 23-25. By stage 27 the amount of SF1 expressed in the testis seemed to have increased again (Fig. 5c).

4. Discussion

Comparison of the SF1 exon 7 and 8 amino acid sequences between alligator, chicken, human and mouse strongly suggests that it is the alligator orthologue of SF1. Examination of the expression pattern of SF1 during male and female alligator gonadogenesis revealed an important difference when compared with SF1 expression patterns in the developing gonads of mammals. SF1 appeared to be expressed at a higher level during stages 22-23, 24 and 25 at 30°C and at 33°C in the developing ovary than in the testis of the alligator. This contrasts with the pattern of SF1 expression during mouse and rat gonad development where, after the initial stages of gonadal differentiation, SF1 is strongly expressed in the testis but down-regulated in the ovary (Hatano et al., 1994; Parker and Schimmer, 1997). The pattern of SF1 expression observed in the alligator ovary and testis is very similar to that observed in the chicken. In the chicken embryo, SF1 displays a higher expression level in the differentiating ovary than
Fig. 5. SF1, DAX1 and WT1 expression during alligator gonadogenesis. (a) DAX1 expression appeared to be equivalent between male and female stages at all stages examined. In male and female GAM tissues, DAX1 was expressed at a relatively low level during stages 20, 21 and 22 and had increased by stage 23. The level of DAX1 expression remained high in stage 24, 25 and 27 gonadal tissues. (b) WT1 was expressed in a pattern very similar to that of DAX1, although the level of WT1 in stage 20–22 GAM tissues appeared higher than DAX1. WT1 expression appeared to be equivalent between male and female stages at all stages examined. In male and female GAM gonadal tissues, WT1 expression appeared to increase during stages 20–23 and remain high until stage 27. (c) SF1 was expressed at an equivalent level in male and female GAM samples during stages 20, 21 and 22. However, during stages 23, 24 and 25 the level of SF1 in the male appeared to decrease to a low level before increasing again by stage 27. In contrast, in the female samples SF1 expression appeared to be maintained at a constant level during stages 23, 24, 25 and 27.

The expression patterns were consistent in three RT–PCR experiments performed on RNA extracted from two separate tissue samples (see Materials and Methods). A water control was performed for each RT–PCR experiment and in each case no product was amplified (not shown).

the testis (Smith et al., 1999a,b). However, in a turtle with TSD (Trachemys scripta) gonadal SF1 expression is upregulated in males and downregulated in females (Fleming et al., 1999), opposite to the alligator and chicken patterns.

In addition to playing a developmental role in gonadogenesis, SF1 is an important regulator of steroidogenesis. SF1 has been implicated in the transcriptional control of the aromatase enzyme, which catalyses the conversion of androgens to oestrogen, a process which appears to be necessary for ovary formation in birds and reptiles (Elbrecht and Smith, 1992; Crews et al., 1994; Lance and Bogart, 1994; Pieau, 1996). Aromatase activity is upregulated during gonadal differentiation of at least two turtle species at the female temperature but not at the male (Jeyasuria and Place, 1997; reviewed by Pieau, 1996). Higher SF1 expression in the developing alligator ovary than in the testis may reflect a role for SF1 in upregulating enzymes involved in oestrogen production in the ovary. Lower levels of SF1 expression in the testis may be necessary to prevent the stimulation of oestrogen production, a process normally involved in ovary formation. Whether aromatase is differentially expressed in the developing alligator gonad during the TSP is unclear, although higher levels of aromatase enzyme activity were observed from the end of the TSP in female but not male alligators (Smith et al., 1995). Clearly, an examination of female and male aromatase transcription and protein production during alligator gonadogenesis would resolve this question.

Conversely, since SF1 production is not completely abolished in the developing alligator testes, it is probable that SF1 protein also functions during testis differentiation. This suggests that the relative level of SF1 production in developing testes and ovaries is important for normal gonadal differentiation. In mammalian embryos, SF1 is required for transcription of AMH during testis differentiation (Giulii et al., 1997; Nachtigal...
et al., 1998). In the alligator, AMH upregulation occurs from stage 22 (during the TSP) in the developing testis but not in the ovary (Western et al., 1999). Therefore, initiation of AMH transcription precedes the apparent downregulation of SF1 in the urogenital system of male alligators. Although SF1 would be expected to be strongly expressed in the developing testis if it is required for upregulation of AMH, it is possible that the lower level of SF1 expression is sufficient to promote testicular AMH production. Alternatively, after its initiation, AMH expression may become independent of SF1, via autoregulation or another mechanism. Indeed, since SF1 is expressed in both sexes, different specificities of SF1 action in males and females over development may rely on its interaction with other unknown sex-specific proteins.

The C terminal 192 amino acids of the alligator DAX1 homologous protein showed high identity with the ligand binding domain contained in the human and mouse DAX1 protein sequences; however, the level of identity decreased significantly in the N-terminal region which, in the human and mouse DAX1 proteins, contains a putative DNA binding motif. This motif comprises three and a half copies of a 65-67 amino acid repeat sequence (Zanaria et al., 1994). The alligator DAX1 homologue showed a high degree of overall identity with chicken DAX1, both of which lack the N-terminal repeat motif found in the mammalian DAX1 protein. Thus, while the DAX1 ligand binding domain is highly conserved in the reptiles, birds and mammals, the putative DAX1 DNA binding motif appears to be poorly conserved. The alligator DAX1 N-terminal domain shows a small region with weak homology to a single copy of the repeat sequence found in the mammalian DAX1 protein. This may function as a DNA binding domain. The alternative possibility is that the alligator and chicken DAX1 proteins are not capable of binding DNA. Further analysis of DAX1 DNA binding capacity in alligators and chickens is required to resolve this question.

Current evidence in humans and mice suggests that extra copies of DAX1 act antagonistically toward SRY and cause male to female sex reversal (Zanaria et al., 1994; Swain et al., 1998). Since SRY does not appear to exist in non-mammalian vertebrates (Coriat et al., 1993), DAX1 cannot act to antagonise it during TSD in alligators. However, it has also been suggested that WT1 and SF1 interact to activate testis-specific gene expression in mammals and that DAX1 inhibits this activation (Nachigal et al., 1998). This hypothesis relies on the interaction of these factors within the nucleus and therefore their expression in the same cell. Since WT1, SF1 and DAX1 were expressed at the same time during urogenital and gonad development in the alligator, it is possible that these proteins interact during alligator sex determination.

WT1 and DAX1 are both expressed in the developing testis and ovary during chicken gonadogenesis (Smith et al., 1999b). Although DAX1 expression is upregulated in the developing mouse ovary and downregulated in the testis (Swain et al., 1996), this may not be the case in the rat where DAX1 expression appears to be maintained in the developing testis (Nachigal et al., 1998). At present, the role of DAX1 in mammalian sex determination is unclear. In Dax1 homozygous knockout mice, which lack functional Dax1 protein expression, the ovaries and testes nevertheless differentiate (although spermatogenesis is disrupted). This shows that Dax1 is not required for murine sex determination (Yu et al., 1998). By contrast, WT1 is required for development of the gonadal system in mice. WT1 is expressed at equal levels in the developing mouse testes and ovaries (Nachigal et al., 1998). This is consistent with results obtained for WT1 expression in alligators, chickens (Smith et al., 1999b) and the turtle, Testudo scripta (Spotila et al., 1998).

The expression data presented in this study are preliminary. Although it is clear that SFI, SF1 and WT1 are expressed during gonadogenesis in the alligator, the relative levels of SFI, WT1 and DAX1 expression in the developing alligator ovary and testis require further examination. The expression patterns of SFI, WT1 and DAX1 in both male and female developing gonads of alligators suggest that the control of the male- and female-specific gonad development pathways is complex. It appears that if WT1 and DAX1 act sex-specifically during alligator gonadogenesis, the actions of these factors may rely on their interaction with other unknown factors which confer a sex-specific effect.

The expression profiles of SFI, DAX1 and WT1 during alligator gonadogenesis are very similar to those observed during testis and ovary differentiation in chickens (Smith et al., 1999b) and the alligator, T. scripta (Spotila et al., 1998). Together these data support the conclusion that the role of SFI, DAX1 and WT1 in bird and reptile gonadogenesis has been conserved through evolution. Although the gonadal expression patterns of SFI appear to differ between mammals, birds, crocodilians and turtles, it is clear that SFI, DAX1 and WT1 are all expressed during male and female gonadogenesis in mammals, birds and reptiles. Such evidence suggests that an underlying genetic pathway directing gonadal development has been conserved through evolution, despite the apparent differences in the primary chromosomal and temperature controlled sex-determining mechanisms present in these vertebrates.

Acknowledgements

We thank Ruth Elsey, Guthrie Perry, James Manning and the staff from Rockefeller Wildlife Refuge,
Louisiana for access to alligator eggs. We also thank Jeff Lang for helpful advice on egg incubation. Craig Smith is thanked for suggestions on the manuscript.

This work was supported by an Australian Research Council grant awarded to A.H.S.

References


